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Genome-Wide Transcription And Dna Methylation Profiling In An App Mouse Model Of Alzheimer'S Disease

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GENOME-WIDE TRANSCRIPTION AND DNA METHYLATION PROFILING IN AN APP MOUSE MODEL OF ALZHEIMER'S DISEASE

by

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NEUROSCIENCE

ABSTRACT

The ability to encode information for long-term behavioral adaptation relies on experience-dependent alterations in neuronal plasticity. Neuronal plasticity encompasses the cellular and molecular changes that modulate synaptic communication between neurons as well as intrinsic electrophysiological properties within neurons. Epigenetic mechanisms, including DNA cytosine methylation and histone post-translational modifications, are powerful regulators of neuronal gene expression, allowing for dynamic, bidirectional regulation of transcriptional signatures necessary for neuronal plasticity. Emerging evidence using candidate-gene and microarray-based approaches suggest that the deficits in neuronal plasticity and cognitive impairment observed in Alzheimer's disease (AD) is attributable, in part, to aberrant cytosine methylation and transcription of genes involved in cell signaling, inflammation, and neurotransmission. The work presented in this dissertation goes beyond previous attempts by using cutting-edge, next-generation sequencing technologies to systematically characterize genome-wide alterations in gene expression and DNA methylation in hAPP(J20) mice, an amyloid-beta (Aβ) over-expressing mouse model of AD. Hippocampal Aβ-deposition was associated with widespread transcriptional dysregulation, targeting, in particular, genes implicated in extracellular matrix restructuring and immune function as well as chromatin biology and neuronal plasticity. In contrast, Aβ-deposition was associated with fewer alterations in DNA methylation, enriched, however, at genes linked to transcriptional regulation and

neuronal differentiation. Most notably, the work in this dissertation utilizes an integrative transcriptomic meta-analysis in combination with network analyses to identify the histone deacetylase, HDAC2, as a conserved therapeutic target of interest and validates the use of anti-sense oligonucleotide mediated knockdown of HDAC2 as a viable treatment for AD-related cognitive impairment. Thus, the findings presented here provide additional evidence in support of AD-related transcriptional and epigenetic dysregulation and provide a new framework by which to investigate and treat Aβ-associated cognitive impairment.

DEDICATION

I dedicate this dissertation to my parents, Miguel Guzman and Gunilla Karlsson, who instilled in me the principles of hard work, patience and perseverance. Your sacrifices and life decisions have allowed me the fortunate opportunity to follow my curiosity and pursue a career filled with lifelong learning. Most importantly, thank you for always being so generous with your love and support.

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TABLE OF CONTENTS

Page

LIST OF FIGURES

Figure *Page*

LIST OF TABLES

Table *Page*

GENOME-WIDE TRANSCRIPTION AND DNA METHYLATION PROFILING IN AN APP MOUSE MODEL OF ALZHEIMER'S DISEASE

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by worsening cognitive decline, progressing from mild cognitive impairment (MCI) to dementia and ultimately death (*1*). Early clinical symptoms are characterized by episodic memory loss, apathy, and depression whereas later symptoms include impaired communication, confusion, and behavior changes. Additionally, AD is associated with an increased risk of seizures and epileptiform activity, particularly in the early stages of MCI (*2, 3*). Histopathologically, AD is characterized by three major hallmarks: (1) neurodegeneration in selected brain regions, including the hippocampus and the neocortex, (2) extracellular plaques, comprised of amyloid-β (Aβ), and (3) neurofibrillary tangles (NFTs), which are intracellular inclusions of hyperphosphorylated microtuble-associated tau.

As the most common form of dementia, AD places a significant medical and economic burden upon society. Currently, dementia affects 46.8 million individuals worldwide, with the total yearly treatment and care costs estimated to be ~$604 billion (*4*). In the United States, 5.4 million Americans are afflicted and direct medical costs total an estimated $818 billion (*5*). Increasing our understanding of the pathogenic mechanisms underlying AD is thus of great public health importance, particularly given the aging trajectory of the population and an expected worldwide prevalence of 131.5 million by 2050 (*4*). Although, the U.S. Food and Drug Administration (FDA) have approved

cholinesterase inhibitors (*e.g.* Donepezil, Galantamine, Rivastigmine, Tacrine) and the NMDA receptor antagonist memantine to temporarily improve AD symptomology, the benefit from their use is limited as none are disease-modifying (*6*).

Genetic Basis of Alzheimer's Disease

While all AD patients share the hallmarks of neurodegeneration, Aβ plaques, and NFTs, they can be divided into two distinct subtypes depending on the presence or absence of hereditary aggregation, namely familial and sporadic. Individuals with familial AD (FAD) or early onset AD (EAOD), typically present with symptoms early in life (30-60 years of life) and by definition have a high degree of the familial clustering (7). In contrast, individuals with sporadic AD, or late onset AD (LOAD), typically develop the disease later in life (> 60 years of age) and instead exhibit a complex pattern of genetic inheritance. Epidemiological studies estimate that the majority of AD cases are in fact sporadic, with 1-5% of patients exhibiting monogenic forms of the disease that result in FAD (8).

Early genetic linkage screens, followed by positional cloning have successfully identified a catalog of dominant mutations in the amyloid-beta precursor protein (*APP*), presenilin (*PSEN1*), and presenilin 2 (*PSEN2*) genes (9) (http://www.molgen.ua.ac.be/ADMutations/). APP is a membrane-bound protein that when cleaved and secreted by neurons produces Aβ (*10*). PSEN1 and PSEN2 are gamma-secretases involved in the enzymatic cleave of APP to produce an Aβ peptide of 40 (Aβ40) or 42 (Aβ42) amino acids in length (*10*). Currently, there are 511 documented mutations, with the 219 localized to *PSEN1*, 51 to *APP*, and 16 to *PSEN2* (9). The vast majority of mutations alter secretase activity, effectively increasing the ratio of Aβ42/Aβ40, with Aβ42

being more prone to oligomerization and neurotoxicity (11). However, certain *APP* mutations result in an increased production of both Aβ40 and Aβ42 as well as an increase in the amyloidgenicity of Aβ42/Aβ40 (9, 12).

Although not present in the majority of LOAD, the identification of the aforementioned genes and their associated mutations have formed much of the foundation underlying the biomedical investigation of amyloid biology and its role in AD pathogenesis. Currently there are a variety of transgenic mouse models for AD that express one or more of AD-associated genes with known mutations (13). Each mouse model exhibits varying phenotypes as it relates to the degree of cognitive impairment, Aβ load, NFTs, gliosis, synapse loss, and neurodegeneration. This variability is not only dictated by the specific transgene/mutation expressed but also by the efficiency and cell-type selectivity of the driving promoter as well as the animal's overall genetic background. It is for this reason that certain AD mouse models are better suited to test certain hypothesis over others. Frequently, however, experimental evidence obtained across several models is necessary to arrive at a convergent biological conclusion with generalizability to human AD.

In addition to genetic linkage screens and autosomal dominant mutations in AD-associated genes, a large number of genome-wide association studies (GWAS) have identified more than 20 loci that contain common risk alleles, including *APOE*, *ABCA7*, *BIN1*, *CD33*, *CLU*, *CR1*, *CD2AP*, *EPHA1*, *MS4A6A-MS4A4E*, PICALM, *HLA-DRB5–DRB1*, *SORL1*, *PTK2B*, *SLC24A4*, *RIN3*, *ZCWPW1*, *CELF1*, *INPP5D*, *NME8*, *MEF2C*, *CELF1*, *FERMT2*, and *CASS4* (14-18). Currently the strongest risk factor gene is *APOE*, accounting for 40-65 % of AD cases (19). The *APOE* gene encodes apolipoprotein E (Apo-

E) and exists as three polymorphic alleles in the general population: Apoε2 is protective and delays the age of onset about 10 years, Apoε3 is the most common, and Apoε4 is associated with increased risk and earlier age of onset (20). Functionally, Apo-E displays broad involvement in several biological processes involving Aβ clearance, neuroinflammation, and mitochondrial function (21, 22). Importantly, Apoε4 heterozygosity increases AD risk 4-fold over non-carriers (*i.e.* those carrying ε3/ε3) while homozygosity increases the risk up to 9- to 15-fold (23).

In contrast to *APOE*, all the other GWAS-identified variants unfortunately have fairly small effect sizes (with odds ratios less than 2), making their use in disease prognosis challenging even if the variants are considered in aggregate (24). Likely a contributing factor is the fact that GWAS are better fitted to identify disease-associated genetic regions as opposed to specific genes. For example, identifying the specific gene or variant driving a disease association may be close to impossible in instances where the risk locus is located on a gene-rich region with a high number of genetic variants in high linkage disequilibrium (25). As a result of these intrinsic problems, recent efforts have begun to incorporate whole exome sequencing (WES) and whole genome sequencing (WGS) to identify low frequency and rare variants with a large effect size for disease risk. Indeed, this was the approach used recently to identify additional novel coding variants with large effect sizes in *TREM2*, *PLD3*, *UNC5C* and *AKAP9* (26-28). Currently, the only consistently replicated gene is *TREM2* (29), which codes for a type 1 transmembrane receptor protein expressed on myeloid cells including microglia and is necessary for regulation of phagocytosis and suppression of inflammatory reactivity (30, 31).

In summary, during the past two and a half decades, AD genetic research has

undergone incredible growth in an attempt to understand a clinically heterogeneous neurodegenerative disease with a strong genetic component. While early efforts focused on identifying causal mutations with high penetrance in a small subpopulation of afflicted patients, more recent efforts are directed towards dissecting the moderate and mild genetic risk factors that affect the predominant population of patients with sporadic LOAD. The use of WES and WGS in combination with large-scale genetic and functional data mining (24, 25, 32) shows great promise in identify large effect risk variants. It should be noted that although common variants identified by GWAS have small effect sizes and hence are unlikely to become the basis of new AD models, collectively the identified variants have had marked influence in highlighting novel research areas of focus. For example, GWAS underscored the importance of the immune system and inflammation prior to identification of *TREM2* (15, 16, 33). Indeed, many of the genes identified by GWAS (*e.g. CR1*, *EPHA1*, *CLU*, *MS4A6A*, *CD33*, *HLA*, and *INPP5D*) all have clear functional roles in immunity. With that said, the future of AD genetic research will only benefit by relying on a multi-dimensional approach to integrating genetic and other pathobiological evidence. As such, the next section will examine the role of epigenetic mechanisms in AD pathogenesis.

Epigenetic Dysregulation in Alzheimer's Disease

Although the molecular and cellular basis of AD is complex, it is well accepted that AD pathogenesis is initiated by Aβ, whose accumulation and oligomerization triggers neuron-wide dysfunction including altered intracellular signaling, axonal transport failure, oxidative damage, impaired synaptic transmission, aberrant transcription, and more recently dysregulated epigenetic mechanisms (*7, 8*). Epigenetic mechanisms primarily act

through chemical modifications of DNA and histone proteins, reversibly regulating genetic readout without altering the underlying DNA sequence. Given that sporadic AD occurs largely in the absence of genetic mutation implies a potential role for epigenetic dysregulation of amyloid processing genes as the inciting event upstream of Aβ accumulation (*9*). Although supported by some evidence, this hypothesis remains controversial and predominantly unsubstantiated (*7*). Alternatively, epigenetic mechanisms are dynamically regulated in response to a variety of well-conserved intracellular transduction cascades (*10, 11*) many of which are altered in AD (*12, 13*) suggesting that epigenetic dysfunction might also occur downstream of Aβ-induced toxicity.

In the absence of pathology, epigenetic mechanisms are regulated in response to experience, allowing for dynamic, bidirectional regulation of gene expression profiles necessary for neuronal plasticity and long-term behavioral memory (*10, 14*). In this dissertation, the section entitled EPIGENETIC REGULATION OF MEMORY FORMATION AND MAINTENANCE provides an extensive review of epigenetic mechanisms in the nervous system and their involvement in long-term behavioral memory across different behavioral tasks and distinct brain regions. Meanwhile, the section entitled TRANSCRIPTIONAL AND EPIGENETIC REGULATION OF HEBBIAN AND NON-HEBBIAN PLASTICITY examines how neuroepigenetic mechanisms influence neuronal function at the electrophysiological level, focusing on synaptic plasticity, intrinsic excitability, and homeostatic synaptic synaptic scaling. Interestingly, alterations in all of these forms of neuronal plasticity are thought to contribute to the pathophysiology of Aβ–induced cognitive impairment (*15-18*) suggesting that epigenetic dysregulation may be at

the epicenter of neuron-wide dysfunction in AD. With the role of epigenetic mechanisms in learning and memory and neuronal plasticity discussed in subsequent sections, the remainder of this introduction will examine the role of epigenetic dysfunction in AD. Specifically, I will concentrate on histone acetylation and cytosine DNA methylation, as these are the most widely studied epigenetic modifications and the main focus of this dissertation.

Histone Modifications

The structural unit of a eukaryotic chromosome is the nucleosome, which consists of a 146 bp length of DNA coiled around an octamer core of histone proteins. These octamers consist of two pairs of histone H2A-H2B dimers and an H3-H4 tetramer. Nucleosomes are primarily regulated by post-translational modification of the N-terminal histone tails, which functionally modulate the electrostatic affinity between DNA and histones. The functional modifications that can be added to histone tails include, but is not limited to, acetylation, phosphorylation, methylation, ubiquitination, ADP-ribosylation, sumolyation, and O-GLNAcylation. Additionally, specific N-terminal amino acids can be differentially modified. For example, lysines can be acetylated, methylated, or polyubiquitylated whereas serines and threonies can be phosphorylated and O-GlcNAcylated. Each modification has a family of antagonistic enzymes that are able to catalyze both the forward and reverse reaction. Importantly, histone modifications can modulate gene expression through direct effects on chromatin or through indirect recruitment of other chromatin-modifying proteins.

Histone Acetylation

Histone acetylation is catalyzed by three families of histone acetyltransferases (HATs): GNAT, MYST, and CBP/p300 (*19*). In contrast, the reverse reaction is catalyzed by histone deacetylase enzymes (HDACs), for which there are 18 distinct mammalian isoforms that are classified into four classes based on sequence homology to yeast and catalytic mechanism utilized (*20*). These classes include the zinc-dependent class I, II, and IV HDACS and the nicotinamide adenine dinucleotide (NAD)-dependent class III HDACS (also known as sirtuins). HDAC1, 2, 3, and 8 make up class I HDACs. Class II HDACs further separate into two classes, class IIA containing HDAC4, 5, 7, 9 and class IIb containing HDAC6 and 10. Class III contains SirT1-7. Class IV only contains HDAC11 (*21*). Histone acetylation is largely permissive of gene expression, in part because it diminishes the basic charge of lysine residues thereby reducing the electrostatic affinity between the histones and negatively charged DNA (*22, 23*). The subsequent reduction in steric hindrance allows access for other chromatin-remodeling factors, transcription factors, and RNA polymerase II (*24*).

Histone Acetylation and Alzheimer's Disease

Investigations into the role of histone acetylation in AD pathogenesis have primarily focused on the use of AD mouse models as opposed to post-mortem human tissue. Over the last decade, substantial accumulated evidence suggests that amyloid-deposition is associated with reductions in global and locus-specific histone acetylation, which in some cases are associated with repressed memory-associated gene expression profiles (*25-29*). It should be noted though that there are reports that do not document

changes in basal histone acetylation in both post-mortem AD human samples (*30, 31*) as well as the Tg2576 and 3xTg-AD mouse models (*32, 33*).

Furthermore, inhibition of HDAC enzymes, a manipulation that effectively increases histone acetylation, ameliorates deficits in gene expression, synaptic plasticity, and hippocampus-dependent memory in a variety of AD mouse models (*25-28, 32, 34-38*). However, the mechanism by which HDAC inhibitors produce such effects is still not well understood. Some studies suggest that HDAC inhibition reduces amyloid plaque deposition (*37*) as well as tau pathology (*28, 36*), while others others attribute the neuroprotective effects to decreased inflammation (*25, 37*). It is reasonable to assume that HDAC inhibitors operate by fundamentally increasing histone acetylation-dependent transcription. However, most studies have explicitly examined this hypothesis, focusing on global changes in histone acetylation (*26-28, 38*) as well as locus-specific changes in acetylation and transcription of a small set of candidate genes (*26-28*). One recent study using APP/PS1-21 mice in combination with genome-wide profiling tools showed oral administration of the HDAC inhibitor SAHA restored spatial memory deficits by exerting anti-inflammatory action and reinstating genome-wide epigenetic balance (specifically at acetylated H4K12) and transcriptional homeostasis (*25*). These findings in combination with the observation that certain HDAC isoforms, such as HDAC6, are known to act on non-histone substrates like α-tubulin and HSP90 suggest HDAC inhibitors likely operate via both transcription-dependent and -independent mechanisms (*39, 40*).

It is believed that one of the primary targets of HDAC inhibitors that accounts for their precognitive effects is HDAC2. Given that age is the strongest rick factor for AD, it's notable that HDAC2 is documented to be elevated with age in mice and humans (*41, 42*).

A variety of AD mouse models, including APPswe/PSEN1dE9, CK-p25, and 5x-FAD mice, also exhibit elevated HDAC2 levels (*27, 43*), which is in line with observed elevations of HDAC2 in the hippocampus and entorhinal cortex of human AD samples (*27*). However, the most direct evidence implicating HDAC2 in cognitive function is based on the ability of viral-mediation depletion and pharmacological inhibition of HDAC2 to restore AD-related synaptic and cognitive deficits (*27, 44*). Similarly, embryonic deletion of HDAC2 in excitatory neurons produces precognitive effects in wild-type animals (*45*). Overall, the available research on histone acetylation in AD paints a picture of HDAC-driven repression of chromatin plasticity that negatively effects learning and memory-related transcriptional output.

DNA Methylation

DNA methylation involves the covalent attachment of a methyl group to the 5' carbon within the cytosine-pyrimidine ring (5-methylcytosine, 5mC) and is catalyzed by a family of methyltransferase (DNMTs) that use S-adenosyl methionine (SAM) as a methyl-donating cofactor (*46, 47*). There are three identified DNMTs in mammals: DNMT1 – maintains DNA methylation patters by recognizing hemimethylated DNA and methylating the symmetrically located cytosine on the opposite strand – and DNMT3A and DNMT3B, both of which are involved in establish *de novo* methylation patterns on a single DNA strand (*48, 49*). Typically, DNA methylation occurs at CpG dinucleotides. Although distributed throughout the genome, there are locations in the genome, primarily around gene promoters, which are enriched for CpGs and as such are referred to as CpG islands (CGIs). Traditionally, CGIs are unmethylated whereas CpGs in a non-CGI context are fully

methylated.

Historically, DNA methylation at gene promoters is often associated with transcriptional suppression via direct steric hindrance of transcription factor binding sites, or through the recruitment of transcriptional co-repression complexes. These complexes often include proteins with methyl-binding domains (*e.g.* methyl-CpG-binding protein 2), HDACs, as well as other chromatin-modifying enzymes (*50-52*). However, over the past decade the functional consequence of cytosine methylation has become more nuanced than had been previously suspected, with its influence on transcription depending on developmental time point, cell type, and genomic region (*53-55*). In addition to promoter methylation, intragenic and intergenic methylation are also involved in active regulation of physiological and disease-associated gene expression (*56*). Epigenome-wide analyses indicate that actively transcribed genes are associated with decreased promoter methylation and increased intragenic methylation, whereas repressed genes contain the opposite pattern (*57, 58*). In addition, existing evidence suggests that histone acetylation shares extensive cross-talk with other epigenetic modifications including DNA methylation (*59*), raising the possibility that altered DNA methylation may contribute to AD-related hypoacetylation or even the beneficial effects of HDAC inhibitors previously described.

DNA Methylation in Alzheimer's Disease

Like histone acetylation, DNA methylation alterations in AD have been documented at different levels of investigation: global, locus-specific, and genome-wide (*60*). However, in contrast to histone acetylation, studies have primarily focused on post-mortem human tissue and as opposed to mouse models. Global or bulk assessment of 5mc levels has often relied on immunofluorescence-based approaches like western blotting,

immunohistochemistry, and enzyme-linked immunosorbent assays. The first emerging data suggested that the AD brain might be characterized by global hypomethylation in the entorhinal cortex (*61, 62*) and hippocampus (*63*). However, recent studies utilizing the same approaches and investigating the same brain regions report contradictory results, with either a lack of differential methylation in AD cases compared to control subjects (*64*) or even hypermethylation (*65, 66*).

In addition to assessing global changes in 5mc, researchers have also assessed gene- or locus-specific DNA methylation beginning first with targeted analysis of genes involved tau and amyloid processing pathways (*7*). Given that LOAD occurs largely in the absence of genetic mutation, a reasonable hypothesis was put forth suggesting a potential role for epigenetic dysregulation, namely hypomethylation, of amyloid processing genes as the inciting event upstream of Aβ accumulation (*9*). Although *APP* promoter hypomethylation was supported by some early evidence (*67, 68*), recent studies using larger sample sizes demonstrate no correlation between cortical or hippocampal APP methylation status and AD pathology (*69-72*). Similarly, neither *PSEN1* nor *TAU* exhibit differential methylation in post-mortem AD tissue (*69, 71*). Candidate-gene approaches have identified, however, differential methylation outside of well-established AD genetic risk loci. Rao *et al.* reported promoter hypomethylation of inflammatory genes *NFKB* and *COX2* and hypermethylation of neuronal genes *BDNF* and *SYP* in the frontal cortex of human patients (*31*). Hypermethylation and decreased DNMT1/3A binding of the *Insulin-Like Growth Factor Binding Protein 7 (IGFBP7)* promoter have been documented in APPPS1-21 model mice and human frontal cortex samples (*73*). Both of these changes were further associated with increased *IGFBP7* transcript levels and protein abundance. Lastly, methylation-induced silencing of *neuroligin-1* (*Nlgn1*) was responsible for dendritic atrophy, impaired synaptic

plasticity, and memory deficits in mice that received hippocampal Aβ1–40 fibrils microinjections (*74*).

Recently, the advent of genome-wide DNA methylation profiling tools has dramatically increased the throughput for detecting AD-related differential DNA methylation. All the work thus far has predominately relied on the use of promoter methylation-microarrays as whole genome bisulfite sequencing (WGBS) continues to be relatively cost prohibitive. However, the technological landscape surrounding epigenetic investigation in the nervous system is rapidly progressing; an exciting reality exemplified by new emerging datasets utilizing WGBS that reveal shared DNA methylome profiles across a variety of neurodegenerative diseases including AD, Parkinson's disease, dementia with Lewy bodies, and the Alzheimer-like neurodegeneration profile associated with Down's syndrome (*75*). In the context of AD mouse models, Cong *et al.* used promoter-methylation microarrays to identify 2,346 hypermethylated CpG nucleotides (representing 485 unique genes) associated with amyloid-deposition in APPswe/PS1dE9 mice (*76*). Surprisingly, no hypomethylated genes were reported and a majority of the methylation changes occurred at genes implicated in inflammation and tissue injury. In contrast, Sanchez-Mut *et al.* profiled 12-well defined mouse brain regions and used this information to identify DNA hypermethylation-associated inactivation of *thromboxane A2 receptor* (*Tbxa2r*), *coagulation factor II (thrombin) receptor-like 2* (*F2rl2*), *sorbin and SH3 domain containing 3* (*Sorbs3*) and *spectrin beta 4* (*Spnb4*) in the frontal cortex of APPswe/PS1dE9 and 3xTg-AD mice (*77*). Further validating their AD model findings, pyrosequencing of human AD cortical tissue confirmed promoter hypermethylation in three out of the four human gene counterparts (*TBXA2R*, *SORBS3* and *SPTBN4*). Of note, hypermethylation of *SORB3* was also independently confirmed by Siegmund *et al*. using

an age-dependent DNA-methylation study that included AD patient-control comparisons (*78*). Besides *SORB3*, the only other independently validated, differentially methylated gene is *Ankyrin1* (*ANK1*), which was recently identified be the two largest epigenome-wide association studies (EGWAS) performed to date (*79, 80*). Overall, the research on DNA methylation in AD paints a picture of locus-specific hypermethylation. However, the extent of these alterations and how they exactly influence gene transcription as it relates to AD pathobiology remains unclear.

Goals and Experimental Approach

Although the previously mentioned evidence supports the idea of dysregulated transcription and epigenetic mechanisms in AD, several studies provide conflicting results likely due to differences in animal model used, brain region studied, and methodology employed. Additionally, most studies typically do not investigate alterations in both epigenetic modifications and transcriptional alterations in an attempt to understand how the two interact to contribute to AD-related pathology. To address these issues, this dissertation goes beyond previous attempts by using cutting-edge, next-generation sequencing technologies to systematically characterize genome-wide alterations in gene expression and cytosine DNA methylation in the dentate gyrus of hAPP(J20) mice, an Aβ overexpressing mouse model of AD. The overarching hypothesis is that cognitive impairment in hAPP(J20) mice results from Aβ-induced aberrant expression of DNA methylation/demethylation machinery that in turn alters DNA methylation of memory-associated genes thereby leading to the transcriptional silencing of memory-activating genes and transcriptional activation of memory-suppressing genes.

The use of hAPP(J20) mice over other models provides several advantages

including neuron-specific expression of a single mutated human APP transgene that contains both the Swedish K670N/M671L and the Indiana (V717F) mutations (*81*). Expression of a single transgene allows for a reductionist approach to studying transcriptional and epigenetic dysregulation downstream of Aβ accumulation (*82*). In addition, hAPP(J20) exhibit immunoreactive amyloid deposits in hippocampal and cortical regions (*83*) as well as a wide variety of behavioral abnormalities, including spatial memory deficits (*84*), at 5 months of age, which is considerably earlier than other AD mouse models (*82*). Examination of dentate gyrus-specific changes in gene expression and DNA methylation reduces the incidence of false-negative results that can occur when examining whole hippocampal tissue, an important point considering hAPP(J20) mice exhibit differential levels of cellular and molecular pathology throughout the hippocampus (*85-87*). Finally, in contrast to PCR- or microarray-based methods, RNA sequencing (RNA-seq) allows for unbiased, quantitative genome-wide analysis that benefits from both low background noise and frequency of false positive signals as well as high dynamic range and reproducibility (*88*). Similarly, methyl-CpG binding domain (MBD) protein-enriched genome sequencing (MBD-seq) permits cost-effective, genome-wide methylation profiling of CpG dinucleotides (*89, 90*).

EPIGENETIC REGULATION OF MEMORY FORMATION AND MAINTENANCE

by

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Abstract

Understanding the cellular and molecular mechanisms underlying the formation and maintenance of memories is a central goal of the neuroscience community. It is well regarded that an organism's ability to lastingly adapt its behavior in response to a transient environmental stimulus relies on the central nervous system's capability for structural and functional plasticity. This plasticity is dependent on a well-regulated program of neurotransmitter release, post-synaptic receptor activation, intracellular signaling cascades, gene transcription, and subsequent protein synthesis. In the last decade, epigenetic markers like DNA methylation and post-translational modifications of histone tails have emerged as important regulators of the memory process. Their ability to regulate gene transcription dynamically in response to neuronal activation supports the consolidation of long-term memory. Furthermore, the persistent and self-propagating nature of these mechanisms, particularly DNA methylation, suggests a molecular mechanism for memory maintenance. In this review, we will examine the evidence that supports a role of epigenetic mechanisms in learning and memory. In doing so, we hope to emphasize (1) the widespread involvement of these mechanisms across different behavioral paradigms and distinct brain regions, (2) the temporal and genetic specificity of these mechanisms in response to upstream signaling cascades, and (3) the functional outcome these mechanisms may have on structural and functional plasticity. Finally, we consider the future directions of neuroepigenetic research as it relates to neuronal storage of information.

Introduction

Learning and memory can be broadly defined as lasting alterations of a behavioral output produced in response to a transient environmental input (Sweatt 2010). In order for a transient stimulus to induce a lasting change in behavior, cells must undergo a complex set of stimulus-specific cellular and molecular changes that will consolidate a memory into an everlasting trace. Since the 1960s, memory researchers have recognized the importance of gene transcription and protein synthesis in long-term memory formation in a variety of experimental memory paradigms (Agranoff 1965; Agranoff et al. 1965, 1966; Squire et al. 1980). However, given that most proteins turn over on a timescale of hours, these findings raised important conceptual questions regarding the molecular basis for lifelong memory maintenance. It soon became evident that a self-perpetuating biochemical reaction would be required to preserve the molecular changes induced by short-lived environmental stimuli. With this necessity in mind, Crick (1984) and Holliday (1999) put forth the proposition that epigenetic mechanisms, particularly DNA methylation, possess the biochemical properties necessary to propagate memories over a lifetime. DNA methylation has long been appreciated as a stable and self-perpetuating regulator of cellular identity through the establishment and propagation of persistent, heritable changes in gene expression across cell divisions (Bird 2002). This mnemogenic quality suggested that epigenetic mechanisms may provide a suitable molecular basis for memory formation and maintenance.

As a result, the last decade has seen a series of studies demonstrating that epigenetic markers are actively and transiently regulated in post-mitotic neurons of adult rodents, honeybees, aplysia, and drosophila during the normal process of learning and

memory (e.g., Levenson et al. 2004; Chwang et al. 2006; Lubin et al. 2008; Miller et al. 2008; Gupta et al. 2010; Lockett et al. 2010; Miller et al. 2010; Kramer et al. 2011; Maddox and Schafe 2011; Monsey et al. 2011; Biergans et al. 2012). Considering the stable nature of DNA methylation during development, it was surprising to find both dynamic and activity-induced changes in DNA methylation in the adult central nervous system. This unconventional operation of epigenetic mechanisms led to the formulation of a subfield of epigenetics, termed neuroepigenetics (also referred to as behavioral epigenetics [Lester et al. 2011]). Neuroepigenetics encompasses "the unique mechanisms and processes allowing dynamic experience-dependent regulation of the epigenome in nondividing cells of the nervous system" (Day and Sweatt 2011). A growing number of studies under this umbrella have demonstrated a critical role for epigenetic mechanisms in a wide range of learning and memory tasks and across diverse brain regions. Thus, epigenetic mechanisms seem to play a ubiquitous role in the establishment of lasting neural and behavioral modifications in response to environmental stimuli.

In this review, we will primarily discuss the role of epigenetic mechanisms in the formation and maintenance of fear memory. We also hope to demonstrate the universal role these mechanisms have in other learning and memory behavioral paradigms. In doing so, we will review the evidence that transient epigenetic modifications mediate memory consolidation by regulating gene expression within the first few hours after learning, whereas sustained changes in epigenetic modifications in cortical brain regions underlie memory maintenance over prolonged periods of time. Finally, we will examine the current understanding of the basic principles that regulate the establishment of specific patterns of epigenetic modifications and speculate on some possible mechanisms

through which these modifications translate into cellular changes that support memory formation and maintenance.

Defining Epigenetics

Epigenetic mechanisms are key regulators of DNA compaction and transcription. To allow long stretches of DNA to fit inside the cell nucleus, 146 bp sections of DNA are coiled around an octamer of histone proteins, which contains two pairs of histone H2A-H2B dimers and an H3-H4 histone tetramer (Quina et al. 2006). Adjacent nucleosomes join with one another via the linker histone H1 to form chromatin (Happel and Doenecke 2009), which can exist either as heterochromatin, characterized by a closed, highly compacted state restrictive to transcription, or as euchromatin, characterized by an open state amenable to transcription (Arney and Fisher 2004). The switching between the opposing chromatin states and the assembly of transcriptional machinery at gene promoters is mediated by epigenetic modifications, primarily DNA methylation and post-translational modifications of histones (see Fig. 1). DNA methylation preferentially occurs on cytosines positioned adjacent to guanine nucleobases (CpG) and is established via DNA methyltransferase (DNMT) enzymes, which catalyze the covalent binding of a methyl group from the methyl donor S-adenosyl-methionine (SAM) to the 5′ position on the cytosine-pyrimidine ring (Chiang et al. 1996; Turker 1999; Bird 2002; Price 2010). Distinct subgroups of DNMTs carry out distinct functions, whereby de novo DNMTs 3a and 3b establish novel methylation marks and the maintenance DNMT1 maintains previously established methylation marks (Cheng et al. 2010). Methylation of a cytosine on one strand prompts maintenance DNMTs to methylate the corresponding cytosine on

the opposite strand, which allows for the self-perpetuation and persistence of this mark throughout cell division and in the face of DNA damage (Santos et al. 2005). Although DNA methylation primarily represses transcription by interfering with the binding of transcriptional machinery to regulatory sites on DNA (Iguchi-Ariga and Schaffner 1989) and by promoting closed chromatin states via the recruitment of transcriptional repressors (Karymov et al. 2001; Drewell et al. 2002; Fuks et al. 2003), recent evidence suggests that methyl-CpG-binding protein 2 (MeCP2) can also activate transcription through interactions with CREB (Chahrour et al. 2008). Such duality of function was recently reported for de novo DNMTs as well, whereby DNMT3a1 and DNMT3a2 isoforms are associated with heterochromatin and euchromatin, respectively (Chen et al. 2002; Kotini et al. 2011).

Post-translational modifications (PTMs) of histones are another critically important regulator of chromatin compaction and gene expression. The positive charge of unmodified histone proteins facilitates interactions with negatively charged DNA and promotes closed chromatin states (Muhlbacher et al. 2006). Histones can undergo a number of modifications, including acetylation, phosphorylation, and methylation, which alter their charge and binding properties (Muhlbacher et al. 2006; Sanchez Mde and Gutierrez 2009). Histone acetylation is the most widely studied modification and involves the transfer of an acetyl group from acetyl coenzyme A to lysine residues of histone tails via histone acetyltransferase (HAT) enzymes (Hebbes et al. 1988). In contrast to DNA methylation, histone acetylation is associated with transcriptional activation, which is largely attributed to acetylated histones acting as recognition sites for chromatin-remodeling proteins, transcriptional regulators, and RNA polymerase II (Mujtaba et al.

2007). Whereas acetylation and phosphorylation are primarily associated with transcriptional activation, histone methylation can either promote or repress transcription, depending on which residue is modified and with how many methyl groups (Nakayama et al. 2001; Peters and Schubeler 2005). For example, methylation of H3 lysine 4 (H3K4) is associated with transcriptional activation regardless of the number of methyl groups, whereas di- or tri-methylation of H3K9 is associated with transcriptional repression (Binda et al. 2010). Different types of modifications are not independent, in that specific modifications tend to co-occur, based largely on their role as transcriptional activators or repressors (Strahl and Allis 2000). In addition, DNA methylation interferes with histone acetylation through the recruitment of complexes that include histone deacetylase (HDAC) enzymes, which remove acetyl groups from histones (Wade 2001a,b). The opposite may also hold true, as a recent study in plants has implicated HAT enzymes in active DNA demethylation though a yet unknown mechanism (Qian et al. 2012). It is important to note that the roles of specific modifications in either activation or repression of transcription are based on generalizations of predominant instead of absolute associations with transcriptional outcomes. For example, bidirectional promoters are silenced on one side and active on the other in spite of similar chromatin states (Lin et al. 2007; Vastenhouw and Schier 2012), and histone H3K9me2 is considered to be a repressive mark even though it does not strictly correlate with transcriptional activation or repression on a genome-wide scale (He and Lehming 2003; Barski et al. 2007). Thus, caution is warranted when extrapolating transcriptional outcomes from observations of histone modifications in isolation. Even more important, the imperfect congruence between individual epigenetic modifications and transcription emphasizes the importance

of investigating the overall pattern of epigenetic modifications on transcriptional outcomes, particularly when drawing inferences regarding complex outcomes, such as learning and memory.

There is now growing evidence that DNA methylation is dynamically and bidirectionally regulated in response to a variety of experience-induced events, including neural activity in the brain, estrogen treatment in human cells, and exercise in muscle (Kangaspeska et al. 2008; Metivier et al. 2008; Guo et al. 2011a,b; Barres et al. 2012). Tremendous gains have been rapidly made in identifying the mechanisms of active DNA demethylation, which include base excision repair in response to deamination of a methylated cytosine by Gadd45 or oxidation by TET proteins, which convert methylated cytosines (5mC) into hydroxyl-methyl-cytosines (5hmC) (Gehring et al. 2009; Kriaucionis and Heintz 2009; Ma et al. 2009; Tahiliani et al. 2009; Wu and Sun 2009; Guo et al. 2011b; Niehrs and Schafer 2012). In addition, the same DNMT enzymes that methylate DNA have been implicated in DNA demethylation (Metivier et al. 2008), but this mechanism has not yet been investigated in the brain. Thus, active regulation of epigenetic marks is a critical regulator of gene expression in a variety of tissue types.

Achieving Signaling- and Task-Specificity at the Level of Epigenetic Regulation

For epigenetic mechanisms to support the formation of distinct and diverse memories, epigenetic modifications must be responsive to signaling cascades induced by environmental stimuli. Resulting modifications of chromatin structure must then regulate the expression of memory-associated genes within the appropriate neural networks. In other words, epigenetic modifications must be actively and selectively induced by

specific signaling cascades at specific genes in the cells and brain regions that support specific types of memory. Indeed, many studies have now shown that epigenetic changes that support memory formation and maintenance involve task-, region-, gene-, time-, and signaling-cascade specific changes in the epigenetic regulation of gene expression.

Epigenetic Mechanisms Regulate Memory Across Tasks and Brain Regions

The majority of evidence supporting an epigenetic basis for memory formation and maintenance has been found using hippocampus-dependent tasks that include contextual fear conditioning, Morris water maze (MWM), novel object recognition (NOR), and object-location memory. In general, hippocampus-dependent tasks have been associated with global increases of euchromatin-related post-translational modifications of histones and with positive regulation of gene expression. For example, contextual fear conditioning produced increased levels of acetylation at H3 lysine 14 (H3K14), phosphorylation at H3 serine 10 (H3S10), and trimethylation at H3 lysine 4 (H3K4me3) in the hippocampus (Levenson et al. 2004; Chwang et al. 2006), although a recent study also found increased levels of the heterochromatin-related dimethylation at H3 lysine 9 (H3K9me2) (Gupta et al. 2010; Gupta-Agarwal et al. 2012). Similarly, training on the Morris water maze induced increased acetylation at H4 lysine 12 (H4K12) and pan-acetylation of H2B (tetra-acetylated-H2BK5K12K15K20) (Bousiges et al. 2010).

Memory consolidation for a particular task involves the establishment of distinct epigenetic modifications in individual components of memory-supportive networks. For example, contextual fear conditioning induced distinct H3K9me2 and H3K4me3 patterns in the hippocampus compared with those of the entorhinal cortex, and the inhibition of

H3K9me2 in the entorhinal cortex, but not in the hippocampus, enhanced memory formation (Gupta-Agarwal et al. 2012). Similarly, cued fear conditioning as well as BDNF-induced plasticity in cell culture resulted in different patterns of histone PTMs at the homer1 promoter in hippocampal and amygdala neurons (Mahan et al. 2012), indicating that the same gene can be differentially regulated by identical stimuli in different regions of the brain. Epigenetic modifications can be further localized to discrete subregions of the hippocampus. For example, Castellano and colleagues (2012) found that training in a one-day redundant place/cue version of the MWM induced increased pan-acetylation of H3 and H4 and decreased acetylation of H3 lysine 9 (H3K9) in the CA1, whereas only H3 pan-acetylation was increased in area CA3. In addition to increased pan-acetylation of H3, the dentate gyrus (DG) was also characterized by sparse immunolabeling for H3S10. These data highlight the subregion specificity of histone modifications and exemplify the need to further refine our analysis to distinct subregions and, ideally, to specific genes and cell populations to better understand the sort of information encoded by these modifications.

In contrast to studies involving contextual fear conditioning and MWM that directly measured histone PTMs induced by learning, the role of histone modifications in object-recognition and object-location memory have been substantiated primarily via pharmacological and genetic manipulations of HATs and HDACs (Vecsey et al. 2007; Stefanko et al. 2009; Roozendaal et al. 2010; Haettig et al. 2011; McQuown et al. 2011). Given the role of histone acetylation in long-term memory formation, many groups have targeted different HATs such as CREB-binding protein (CBP), E1A-binding protein (p300), and p300/CBP-associated factor (PCAF) (Oike et al. 1999; Bourtchouladze et al.

2003; Alarcon et al. 2004; Korzus et al. 2004; Wood et al. 2005, 2006; Maurice et al. 2008; Chen et al. 2010; Barrett et al. 2011; Oliveira et al. 2011; Valor et al. 2011). In a comprehensive review, Barrett and Wood (2008) examined the numerous memory deficits associated with mouse models containing mutations in one of the three previously mentioned HATs. Interestingly, a deficit in NOR memory was the most common impairment among all the different genetically modified mice, suggesting the relative importance of HAT activity and histone acetylation for NOR memory.

Studies using a variety of behavioral paradigms in brain regions outside of the hippocampus, particularly in the amygdala, the prefrontal cortex, the insular cortex, and the striatum, have begun to investigate the role of epigenetic mechanisms in relation to learning and memory paradigms associated with those brain regions, including cued fear conditioning, memory extinction, conditioned taste aversion, and reward learning, respectively (Swank and Sweatt 2001; Bredy et al. 2007; Kwon and Houpt 2010; e.g., Barros et al. 2011; Bayerlein et al. 2011; Monsey et al. 2011). For example, increased expression of euchromatin-associated modifications of histones was reported during the consolidation of cued fear conditioning in the amygdala (Monsey et al. 2011). In addition, histone modifications may be important for the consolidation of conditioned taste aversion (CTA) memories. Studies in the mollusk Helix lucorum show unilateral increases in H3K14 acetylation in premotor interneurons that initiate withdrawal behavior in response to a food item previously paired with an aversive electric shock (Danilova et al. 2010). In rodents, a role for histone acetylation in CTA memory is indirectly supported by increased levels of lysine-HAT activity in the insular cortex after training (Swank and Sweatt 2001). There is also a rapidly growing literature on the role of

epigenetic modifications in the striatum in the development of drug addiction and a number of excellent reviews are available on the subject (e.g., Maze and Nestler 2011; Robison and Nestler 2011). The breadth of epigenetic involvement in various learning and memory tasks is indicative of the seemingly universal requirement for epigenetic mechanisms in producing lasting changes in neural function and behavior in response to a variety of transient environmental stimuli.

Fewer studies have been conducted on the role of DNA methylation in learning and memory and this role is best characterized for fear conditioning. In 2007, Miller and Sweatt reported the first evidence for increased de novo DNMT expression in the hippocampus in response to contextual fear conditioning. This change in enzyme levels was accompanied by increased DNA methylation at the promoter of the memory-suppressor gene protein phosphatase I (PP1) and decreased methylation at the promoter of the plasticity-associated gene reelin. Later studies identified learning-induced changes in DNA methylation of BDNF, arc, and calcineurin genes (Lubin et al. 2008; Miller et al. 2010; Munoz et al. 2010; Penner et al. 2011), which play critical roles in memory formation and maintenance. Recent studies from Glen Schafe's group demonstrated that DNA methylation is important for the consolidation and re-consolidation of cued fear conditioning in the amygdala using DNMT inhibitors to block DNA methylation (Maddox and Schafe 2011; Monsey et al. 2011), although that group has not directly investigated DNA methylation changes at specific genes. A number of studies have also found DNA methylation to be associated with drug responses and drug-related reward learning in striatal structures (e.g., Barros et al. 2011; Bayerlein et al. 2011; Nielsen et al. 2012) and with the establishment of lasting behavioral modification across species,

including Aplysia and the honey bee (Lockett et al. 2010; Biergans et al. 2012; Rajasethupathy et al. 2012). Although the specific nature of the link between DNA methylation and behavioral modification is not clear, the available evidence suggests that DNA methylation is critical for coordinating appropriate patterns of gene silencing and activation.

DNA Methylation and the Balance Between Memory Activators and Memory Suppressors

An interesting feature of DNA methylation is the gene-specificity and the directionality of observed changes, with some genes exhibiting increased and others exhibiting decreased DNA methylation after learning (Miller and Sweatt 2007; Lubin et al. 2008; Feng et al. 2010; Gupta et al. 2010; Miller et al. 2010). Given this observation, it is not clear whether memory deficits observed after DNMT inhibition reflect a greater functional relevance of methylation over demethylation, or a disrupted balance between these opposing modifications. There is ample evidence to support the need for a balance between memory activators and inhibitors, including the opposing actions of proteases and phosphatases in the cytoplasm and of transcriptional activators and repressors at gene promoters (Blitzer et al. 1995; Wang and Kelly 1997; Wang et al. 1997; Koshibu et al. 2009; Lee and Silva 2009; Rajasethupathy et al. 2012). In fact, it has been suggested that a similar need for balance may be required at the epigenetic level (Koshibu et al. 2009), where DNA methylation must remain in balance with demethylation, and histone acetylation must be balanced with histone deacetylation, and so on. A requirement for an epigenetic balance is consistent with the observation that increased expression of

plasticity genes reelin and BDNF in response to DNMT inhibition is not sufficient to support memory consolidation in the face of increased expression of PP1 (Miller and Sweatt 2007; Lubin et al. 2008).

The available evidence suggests that DNA methylation may tip the balance to favor the expression of plasticity-associated genes by inhibiting the activity of memory-suppressor genes (Miller and Sweatt 2007; Miller et al. 2010; Rajasethupathy et al. 2012). Indeed, such a mechanism has been described for SIRT1, a class III HDAC that promotes memory formation by inhibiting microRNA134-mediated degradation of CREB (Gao et al. 2010). Memory-suppressor genes PP1 (protein phosphatase 1) and calcineurin (a.k.a. Ca2+/calmodulin dependent protein phosphatase, PP2) in rodents, and CREB2 in Aplysia provide a powerful constraint on memory, such that the silencing of any one of these genes reduces the threshold for memory formation and improves memory retention (Bartsch et al. 1995; Malleret et al. 2001; Genoux et al. 2002; Koshibu et al. 2009). Cytoplasmic PP1 promotes memory suppression through dephosphorylation of signaling molecules critical for memory formation, including CaMKII and GluR1 (Genoux et al. 2002), whereas nuclear PP1 promotes memory suppression through dephosphorylation of serine 10 on histone H3 (Koshibu et al. 2009, 2011). In addition, PP1 interacts with HDACs and histone demethylases to increase their activity, thus promoting transcriptional silencing through histone deacetylation and demethylation (Koshibu et al. 2009). Calcineurin enhances PP1 activity by dephosphorylating a key PP1 inhibitor (Malleret et al. 2001), whereas CREB2 mediates memory suppression through inhibition of CREB1, a transcriptional activator critical for memory formation in Aplysia (Bartsch et al. 1995). DNA methylation relieves the repressive effects of these genes to allow for

memory consolidation and the expression of plasticity-promoting genes in rodents and in Aplysia. As mentioned previously, fear conditioning is associated with increased methylation and decreased expression of hippocampal PP1 1 h after training (Miller and Sweatt 2007) and with increased methylation and decreased expression of cortical calcineurin 30 d after training (Miller et al. 2010). Similarly, treatment with the memory modulator serotonin induces DNA methylation and transcriptional repression of CREB2, the major memory suppressor in Aplysia (Rajasethupathy et al. 2012). These studies provide correlational evidence to support the hypothesis that methylation of memory-suppressor genes may prove to be a key mechanism for supporting memory consolidation, but additional studies are required to test this hypothesis directly.

A somewhat peculiar observation regarding the balance between opposing epigenetic modifications is that memory deficits produced by DNMT inhibitors can be reversed by treatment with HDAC inhibitors (Miller et al. 2008; Maddox and Schafe 2011; Monsey et al. 2011). In other models, including cancer, DNMT and HDAC inhibitors tend to have synergistic effects (Zhu and Otterson 2003; Fraczek et al. 2012), an outcome that is consistent with the transcriptionally repressive role of both enzymes. We speculate that the opposing action of DNMT and HDAC inhibitors in the hippocampus may be at least partly explained by increased expression of PP1 in response to DNMT inhibition (Miller and Sweatt 2007). Inhibition of nuclear PP1 reduces HDAC activity (Koshibu et al. 2009) and HDAC inhibitors disrupt the interaction between HDAC and PP1 complexes (Brush et al. 2004). Thus, by increasing PP1 expression, DNMT inhibitors may also result in increased HDAC activity, which would account for the reversal of memory deficits by treatment with HDAC inhibitors. Although this

hypothesis has not been investigated directly, a recent study has shown that intra-cortical administration of DNMT inhibitors reduced histone acetyltransferase expression and produced a concomitant decrease in H3 and H4 acetylation (Sui et al. 2012). Based on these observations, we hypothesize that epigenetic modifications may regulate a fine balance between the activity of memory suppressors and promoters at the level of individual genes in a fashion consistent with the required balance between memory suppressors and activators at all levels of signaling (Blitzer et al. 1995; Wang and Kelly 1997; Wang et al. 1997; Koshibu et al. 2009; Lee and Silva 2009; Rajasethupathy et al. 2012). Direct tests of this hypothesis will provide a critical contribution to our understanding of epigenetic mechanisms in memory formation.

Additional explanations for memory impairment produced by DNMT inhibitors are also possible and are not mutually exclusive. For example, some evidence suggests that DNA methylation may not always repress transcription. In fact, the methyl CpG binding protein MeCP2 is capable of acting as a transcriptional repressor when bound to HDACs, or an activator when bound to CREB (Chahrour et al. 2008). Such mechanisms appear relevant in the brain, as regulation of the gene encoding the norepinephrine transporter was associated with changes in MeCP2 binding, but not with changes in DNA methylation in mouse cortical cells (Harikrishnan et al. 2010). Nonrepressive methylation mechanisms may also be relevant for contextual fear conditioning, as zif268 expression was enhanced 30 min after training even though promoter methylation was increased at that time point (Gupta et al. 2010). Another possibility is that DNMTs may be involved in both DNA methylation and demethylation (Metivier et al. 2008), such that blanket inhibition of these enzymes with DNMT inhibitors would disrupt both processes,

although this hypothesis has not been tested in neural tissue. However, a recent study showed that a novel DNMT isoform, DNMT3a2, is associated with transcriptional activation rather than repression (Chen et al. 2002) and is positively associated with memory formation (Oliveira et al. 2012), although the mechanism that mediates this positive association is not clear. Currently, there are no techniques available to manipulate epigenetic modifications at individual genes selectively, but the available techniques can, nevertheless, provide important insights into potential ways in which these mechanisms interact to regulate memory formation and maintenance.

Time-Course Specificity

Temporal specificity of gene expression and protein synthesis in appropriate brain regions is essential for the formation and persistence of a lasting memory trace (e.g., Katche et al. 2010). Numerous studies show that learning induces temporally distinct waves of gene expression and protein synthesis in the hippocampus (Igaz et al. 2002, 2004a,b; Bekinschtein et al. 2007; Katche et al. 2010; Lonergan et al. 2010). Specifically, early changes occurring within 3 h of training are critical for the initial memory formation, whereas delayed changes occurring between 12 and 24 h after training are required for memory persistence over time (Bekinschtein et al. 2007). Changes in protein expression that support memory persistence at later time points are driven by transcriptional events at earlier time points. This is illustrated in a study by Bekinschtein and colleagues (2007), who found that blocking hippocampal BDNF 12 h after training impaired c-fos expression at 24 h and impaired memory recall at 7 d (Bekinschtein et al. 2007; Katche et al. 2010), indicating that the timing of gene expression within the

hippocampus is a critical regulator of memory formation and stabilization. The delayed changes in hippocampal gene expression may reflect the process of systems consolidation, in which the hippocampus has a temporally restricted role in memory formation and undergoes a process of "downloading" the memory to the cortex for maintenance over prolonged periods of time (Frankland et al. 2004, 2006; Frankland and Bontempi 2005; Teixeira et al. 2006; Ding et al. 2008; Wang et al. 2009; Lesburgueres et al. 2011).

The majority of available studies on epigenetic mechanisms in memory have found that epigenetic markers are dynamically and specifically regulated during the initial consolidation window in the hippocampus. Using fear conditioning as a model of associative learning, Miller and Sweatt (2007) found that DNA methylation was rapidly altered 1 h after training and that the changes in DNA methylation returned to baseline within 24 h. Also using fear conditioning, it was found that histone acetylation, phosphorylation, and methylation followed a similar temporal pattern (Levenson et al. 2004; Chwang et al. 2006; Miller et al. 2008; Gupta et al. 2010; Gupta-Agarwal et al. 2012). Consistent with a role for epigenetic mechanisms in initial memory consolidation, the administration of DNMT inhibitors impaired fear memory and HDAC inhibitors improved fear memory (Miller and Sweatt 2007; Lubin et al. 2008; Miller et al. 2008; Monsey et al. 2011; Fass et al. 2013) only if administered during the restricted consolidation window shortly after training. Neither drug was effective at reversing memory if administered 6 h after training (Miller et al. 2008; Monsey et al. 2011). However, more recent studies have reported protracted epigenetic changes during the hippocampus-dependent process of consolidation. For example, the transcriptionally

repressive H3K9me2 mark was increased 1 h and reduced 24 h after fear conditioning in the hippocampus (Gupta et al. 2010), whereas the transcriptionally permissive H3K4me3 mark was increased 1 h and decreased 24 h after fear conditioning in the entorhinal cortex (Gupta-Agarwal et al. 2012) compared to untrained controls. These results indicate that epigenetic changes may also occur in waves that contribute to temporally distinct patterns of gene expression that are involved in establishing transient and persistent memory traces. However, temporal dynamics of epigenetic changes in the hippocampus have not been extensively studied and much more work is required to test directly a potential epigenetic basis for regulating distinct waves of gene expression at different stages of consolidation.

The finding that epigenetic modifications in the hippocampus are transient challenged the initial hypothesis that persistent changes in DNA methylation support long-lasting memories (Miller and Sweatt 2007). The systems consolidation theory of memory maintenance (Frankland and Bontempi 2005; Frankland et al. 2006) suggests that the transient changes in epigenetic markers in the hippocampus parallel the transient involvement of the hippocampus in memory formation. According to the theory, memory consolidation is dependent on the hippocampus immediately after and for ~7 d after training, whereas older memories (≥7 d, approximately) are downloaded to the cortex for maintenance over prolonged periods of time. Accordingly, a number of studies have shown that newly acquired memories are associated with transiently increased gene expression and spine density in the hippocampus, but that older memories are associated with altered gene expression and increased spine density in the cortex (Maviel et al. 2004; Restivo et al. 2009). Indeed, in contrast to transient changes (<24 h) in DNA

methylation observed in the hippocampus (Miller and Sweatt 2007; Gupta et al. 2010), increased DNA methylation in the medial prefrontal cortex becomes evident 1 d after training, increases thereafter, and persists for at least 30 d (Miller et al. 2010). Altered DNA methylation at 30 d is critical for memory maintenance, as interference with DNA methylation by the administration of DNMT inhibitors into the medial prefrontal cortex blocks the recall of memory, suggesting that stable changes in cortical DNA methylation may support the maintenance of memory over time. Gräff et al. (2012) recently implicated dynamic histone modifications during systems-wide consolidation of hippocampus-dependent memories. As with DNA methylation, hippocampal H3S10 phosphorylation and H3K14 and H4K5 acetylation were transiently induced after object-recognition learning, whereas modifications in the cortex were delayed and persisted for up to 7 d. These biochemical changes were measured after memory recall and may thus reflect retrieval-induced epigenetic modifications in the cortex, a hypothesis that is indirectly supported by the absence of hippocampal histone modifications at 24 h in the absence of recall. Although fascinating in its own right, additional studies are required to confirm the spatiotemporal dynamics of histone modifications during memory consolidation and in the absence of memory recall.

Studies of cortical DNA methylation in remote memory indicate that distinct memory-associated genes exhibit temporally distinct patterns of methylation (Miller et al. 2010). Of the candidate genes examined, only calcineurin was persistently methylated, whereas reelin was transiently methylated and downregulated during the period of transition (approximately encompassing the first 7 d after training) from the hippocampus to the medial prefrontal cortex (Miller et al. 2010). We speculate that cortical DNA

methylation at earlier time points (1–7 d) may direct sustained changes in DNA methylation at later time points (≥7 d) to regulate the timing and the duration of gene expression required for different stages of memory formation and maintenance. Although this notion is largely speculative at this point, there is some evidence to suggest that DNA methylation and histone modifications may delay or prolong the inhibition of memory-suppressor genes to allow for sufficient relief from the mechanisms that promote forgetting. For example, CREB2, a memory suppressor that inhibits activation of the memory promoter CREB1 in Aplysia (Bartsch et al. 1995), exhibits the highest levels of methylation and inhibition between 12 and 24 h after serotonin application, which corresponds with the period of increased activity of CREB1 and memory consolidation (Rajasethupathy et al. 2012). Importantly, these data point to a dual role of epigenetic modifications as dynamic regulators of gene expression in the hippocampus and as persistent maintainers of remote memories in the cortex. More work is required to investigate the role of temporally and region-specific changes in DNA methylation in memory formation and maintenance, as well as the distinct ways in which these temporally distinct epigenetic modifications translate into functional memories.

Epigenetic Modifications are Regulated in Response to Specific Signaling Cascades

Intertwined with the requirement for temporal specificity is the need for epigenetic marks to be laid down in a regulated fashion to allow for precise changes in gene expression by appropriate upstream signaling events. For example, NMDA receptor activation initiates the extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) signaling cascade, which, via nuclear kinases such as mitogen- and

stress-activated protein kinase 1 (MSK1), is involved in downstream histone H3 acetylation and phosphorylation in the hippocampus (Levenson et al. 2004; Chwang et al. 2006, 2007). Importantly, stimulation of ERK signaling (Levenson et al. 2004) and treatment with HDAC inhibitors (Graff and Tsai 2011; Graff et al. 2011) produced gene- and histone-specific changes in PTMs, indicating that distinct signaling cascades may establish precise histone codes that correspond to particular types of memory (Graff et al. 2011). Moreover, BDNF activity regulates the consolidation of contextual fear conditioning through alterations of histone- and residue-specific post-translational modifications at the homer1 promoter in the hippocampus and the amygdala (Mahan et al. 2012). In addition, nitric oxide (NO) has been implicated in histone acetylation by regulating the dissociation of HDAC2 from CREB-regulated gene promoters (Nott et al. 2008; Nott and Riccio 2009) and, most recently, HDAC inhibitors were shown to reverse fear conditioning deficits in NO knockout mice through increased H3 acetylation in the hippocampus and the amygdala (Itzhak et al. 2012). Similarly, the enhancement of object-recognition memory by HDAC inhibitors can be blocked with antagonism of the glucocorticoid receptor and the downstream activation of PKA (Roozendaal et al. 2010). DNA methylation and histone acetylation also appear to be regulated by overlapping signaling cascades, as evidenced by impaired DNA methylation in response to NMDA receptor antagonist treatment in the hippocampus (Lubin et al. 2008; Miller et al. 2010) and reduced DNMT3a expression in response to ERK/MAPK inhibition in the amygdala (Monsey et al. 2011). Similarly, DNA methylation of the memory-suppressor gene CREB2 in Aplysia is dependent on serotonin signaling, in that DNMT inhibitors blocked serotonin-induced CREB2 silencing and the associated enhancement of cellular

activation, and the inhibition of serotonin signaling blocked DNMT-inhibitor mediated alterations of cell activity (Rajasethupathy et al. 2012).

Many of the post-translational modifications of histones discussed above are specifically induced at particular residues on specific histones, while others are not affected (e.g., Chwang et al. 2006; Gupta et al. 2010; Gupta-Agarwal et al. 2012; Mahan et al. 2012), indicating that upstream signaling cascades may differentially regulate the activity of multiple HATs, HDACs, histone methylases, and histone demethylases that regulate specific modifications at distinct residues. For example, specific HATs, including HPA2 and Gcn, specifically acetylate H3K14 (Angus-Hill et al. 1999; P Cheung et al. 2000a,b; WL Cheung et al. 2000), and the histone methyltransferase Mll specifically methylates H3K4 (Milne et al. 2002), whereas the G9a/G9a-like protein (GLP) lysine dimethyltransferase complex catalyzes methylation of K3K9 (Kubicek et al. 2007; Leung et al. 2011; Shinkai and Tachibana 2011).

Different enzymes that catalyze the addition and removal of post-translational modifications appear to have at least partially independent effects on memory formation. For example, knocking out different HATs, CBP or p300, produces distinct patterns of memory deficits in mice (Korzus et al. 2004; Wood et al. 2005), and HDAC2 (and not HDAC1) is negatively associated with spatial memory (Guan et al. 2009). In contrast, class III HDACs (also called sirtuins) are positively associated with memory formation (Kim et al. 2007; Gao et al. 2010), supporting the idea that different HATs and HDACs may regulate different types of memory (Graff and Tsai 2011). However, it is not entirely clear whether relevant modifications occur only at specific residues, or whether general enhancement or reduction of a particular modification is critical for memory formation.

The best example of residue-specific modifications in memory has been described in a recent study of H3K9 methylation in Drosophila. Kramer et al. (2011) isolated H3K9me by knocking out an H3K9-specific euchromatin histone methyltransferase (EHMT) that resulted in deficient learning and memory, and impaired dendrite development. The deficits were associated with changes in H3K9 dimethylation on neural plasticity-related genes and were reversed with the induction of EHMT expression in adulthood. This study provides an excellent example of isolating a single modification that is dynamically regulated on a subset of genes, many of which are involved in memory formation, although the modification remained stable on other genes. This observation provides further evidence for the dual role of epigenetic modifications in the maintenance of stable patterns of gene expression and in regulating dynamic changes in gene expression in response to environmental signals.

In addition to subcategories of methyltransferases, HDACs, HATs, and histone methylases, DNMTs can also be classified into either de novo or maintenance subcategories and recent studies have identified different subtypes of DNMT3a, wherein DNMT3a1 is associated with gene repression and DNMT3a2 is associated with gene activation (Chen et al. 2002; Kotini et al. 2011). The latter is selectively and positively associated with memory for trace fear conditioning and novel object recognition (Oliveira et al. 2012). Overall, these findings indicate that a precise pattern of chromatin modifications in the nucleus is established in response to upstream signaling cascades, although the role of specific enzymes and modifications of specific sites in learning and memory need to be better elucidated. Based on their position downstream of environmental stimuli and the associated signaling cascades, epigenetic mechanisms are

well suited to integrate the upstream signaling information and translate it into gene-specific transcriptional regulation.

Translation of Epigenetic Mechanisms into a Functional Memory Trace

Traditionally, studies of memory formation have focused on activity-dependent changes at the synapse, particularly long-term potentiation (LTP) and the formation of new synaptic contacts indexed by changes in spine density (Bliss and Coolingridge 1993; Restivo et al. 2009). However, it has been difficult to reconcile such a synapse-specific basis of memory with the presumably cell-wide changes produced by epigenetic modifications in the nucleus. One of the most obvious links between epigenetic mechanisms and synaptic function is the epigenetic regulation of genes that have a known role in the establishment of LTP and memory formation. Reelin and BDNF are epigenetically regulated (Miller and Sweatt 2007; Lubin et al. 2008) and have an established role in LTP induction, synapse maturation, and spine development (Weeber et al. 2002; Beffert et al. 2005; Qiu and Weeber 2007; Niu et al. 2008; Mei et al. 2011; Amaral and Pozzo-Miller 2012; Vigers et al. 2012). A number of studies have found evidence supporting a role for HDACs in synapse formation and plasticity (Kim et al. 2007; Guan et al. 2009; Gao et al. 2010; Calfa et al. 2012). Effects appear to be HDAC specific, wherein HDAC2 is associated with reduced synaptic plasticity, synapse number, and spine density (Guan et al. 2009), and the HDAC SIRT1 is associated with enhanced synaptic plasticity (Gao et al. 2010) and greater dendritic complexity (Michan et al. 2010). Moreover, DNMT inhibitors reduce synaptic plasticity and impair LTP induction (Levenson et al. 2006; Nelson et al. 2008), thus highlighting the importance of epigenetic

modifications in regulating traditional mechanisms of memory. Although the mechanism underlying the effect of epigenetic modifications on synaptic structure and function is not clear, recent studies have found that homer1 and TrkB (a receptor for BDNF) may serve as activity-regulated synaptic tags that could localize BDNF and other plasticity-associated proteins to recently activated synapses (Okada et al. 2009; Lu et al. 2011), implicating synaptic tagging as a potential mechanism for targeting epigenetically regulated genes to appropriate synaptic sites. Eric Kandel's group recently put forth another interesting idea regarding the role of DNA methylation as a regulator of memory allocation (Rajasethupathy et al. 2012). Memory formation occurs in a subset of cells that exhibit higher levels of CREB1, such that the memory trace is preferentially "allocated" to neurons expressing higher CREB1 levels (Han et al. 2009; Zhou et al. 2009). Kandel's group observed widespread inter-cell variation in CREB2 methylation, which is positively associated with CREB1 expression. This led the group to propose that CREB2 inhibition may distinguish neurons that are currently involved in memory formation from those that are not, thereby implicating DNA methylation in regulating the sequence of cellular involvement in particular forms of memory.

Euchromatin-Associated Modifications Enhance Memory

So far in our discussion, we have focused primarily on the ability of epigenetic mechanisms to promote memory by selectively responding to specific stimuli in the environment. However, many studies have found that, in addition to the specific epigenetic modifications that occur only in response to associative learning (e.g., Levenson et al. 2004; Chwang et al. 2006; Miller et al. 2008; Gupta et al. 2010; Gupta-

Agarwal et al. 2012), exposure to nonassociative control treatments also induces epigenetic modifications. In fact, exposure to a novel environment produced increased levels of ERK1/2 dependent H3 phosphorylation and acetylation that was abolished by environmental habituation (Sarantis et al. 2012). Similarly, context exposure alone produced increased H3K9me2 in the CA1 (Gupta-Agarwal et al. 2012) and exposure to a visible-platform control condition produced similar changes in H3 acetylation to those seen after MWM training (Castellano et al. 2012). With respect to DNA methylation, the specificity of the changes observed in response to the context and shock pairing is dependent on the gene of interest, with some genes, including egr1/zif268 and bdnf exon 1, exhibiting similar modifications when shock and context are presented individually as when they are presented in combination (Lubin et al. 2008; Miller et al. 2010). Divergent patterns of specificity can even be observed at the single cytosine level, as evidenced by a training-specific methylation at only one cytosine in the promoter region of bdnf exon IV among the multiple modifications that occurred in response to nonassociative context exposure (Lubin et al. 2008). Such findings suggest that certain epigenetic modifications may be induced by specific sets of environmental stimuli, some of which reflect associative memory whereas others are induced by novel environmental inputs that are independent of associative learning.

The nonspecific sensitivity of epigenetic mechanisms to diverse inputs from the environment suggests that exposure to new stimuli can promote neural plasticity irrespective of associative learning. By extension, the nonspecific epigenetic modifications produced by environmental exploration and novelty may serve a function that alters the epigenome in a way that may either promote or impair future learning. This

is exactly the argument that has been made to account for the effects of environmental enrichment on learning and memory, as evidenced by heightened levels of histone acetylation and open chromatin states in rodents exposed to enriched environments (for review, see Graff and Tsai 2011). Indeed, Graff and Tsai (2011) have argued for beneficial effects of such an "increased dose" of euchromatin-associated epigenetic modifications based on evidence that manipulations that increase euchromatin-associated PTMs, such as PP1 inhibition, estrogen treatment, or the activation of glucocorticoid receptors (Genoux et al. 2002; Koshibu et al. 2009; Roozendaal et al. 2010; Zhao et al. 2010; Koshibu et al. 2011) also enhance memory formation. This link is further supported by evidence that HDAC inhibitors enhance learning in response to weak stimuli that do not induce memory on their own (e.g., Fass et al. 2012; Stafford et al. 2012). In addition, variations in maternal behavior in early life, which result in different patterns of hippocampal and cortical DNA methylation in adult offspring, are associated with altered patterns of learning and memory (Caldji et al. 1998; Bredy et al. 2003; Champagne et al. 2008; Roth et al. 2009), indicating that preexisting differences in epigenetic modifications can reduce the threshold for learning and memory. Similarly, one study has found that post-training individual differences in DNA methylation of the BDNF gene in the hippocampus correlate with performance on a spontaneous object-recognition task (Munoz et al. 2010), implying that preexisting changes in epigenetic marks may mediate the responsivity of epigenetic marks to memory-inducing stimuli. Ultimately, these findings point to a bidirectional relationship between epigenetic mechanisms and learning and memory, whereby learning induces the formation of novel epigenetic marks and preexisting levels of epigenetic marks regulate the threshold for

learning and memory.

General Considerations and Limitations

Within the last decade, increased interest in neuroepigenetics has resulted in exciting methodological and conceptual advances in the field. As with any new field of study, however, it is important to consider the inherent technical limitations and their impact on the interpretation of data. For example, many studies of DNA methylation used bisulfite sequencing to obtain single-nucleotide resolution of cytosine methylation, which is unable to discriminate between 5mc and 5hmc (Huang et al. 2010; Jin et al. 2010; Nestor et al. 2010), suggesting that the observed changes in DNA methylation in previous studies will have to be reexamined for the presence of hydroxyl methylation. Recently, several variants of bisulfite sequencing, including oxidative bisulfite sequencing (oxBS-Seq) and Tet-assisted bisulfite sequencing (TAB-Seq), have been developed in order to address such limitations (Booth et al. 2012; Yu et al. 2012). Furthermore, for mapping studies that do not require single base resolution, immunoprecipitation techniques that employ 5mc and 5hmc specific antibodies or proteins (e.g., hydroxylated/methylated DNA immunoprecipitation [h/MeDIP] and methylated-CpG island recovery assay [MIRA]) are available as reasonable alternatives if a DNA fragment does not contain both modified cytosines (Jin et al. 2010). As the field continues to grow, it is imperative that such techniques become the standard in the field to ensure accurate 5mc and 5hmc measurements.

On a similar note, certain commercially available antibodies used to evaluate histone PTMs may, in fact, also lack the necessary specificity to distinguish one

modification from another. Using peptide array technology, Castellano et al. (2012) showed that several of their purchased antibodies recognized various histone modifications in addition to the ones supposedly targeted by their antibodies. This lack of antibody specificity has important implications for both past and future studies that examine how combinatorial patterns of histone modifications work together to regulate gene expression. Furthermore, a lack of studies that examine antibody cross-reactivity complicates cross-study comparisons and the proper resolution of potential discrepancies (Castellano et al. 2012). Use of pharmacological approaches also has inherent limitations. For example, a causal link between histone modifications and behavior is often inferred on the basis of pharmacological enhancement of memory with HDAC inhibitors, many of which have widespread effects that are not specific to histones, HDAC subtypes, or to modifications of specific histone residues (for review, see Zovkic and Sweatt 2013), although important advances have been made in understanding the role of specific HATs and HDACs from studies that used genomic tools to interfere with subtype-specific expression (e.g., Wood et al. 2005, 2006; McQuown et al. 2011; Gräff et al. 2012). Although the results of the latter studies are generally in agreement with those obtained with pharmacological HDAC inhibitors, they nevertheless point to a need to develop additional HDAC-specific compounds that mimic effects observed with genetic manipulations, as reported for the pharmacological and genetic inhibition of HDAC3 (e.g., McQuown et al. 2011).

Conclusions and Future Directions

In this review, we hope to have underscored the notion that epigenetic regulation of gene expression is a ubiquitous mechanism across learning paradigms and brain regions. It is now evident that integration and regulation of epigenetic modifications allows for complex control of gene expression necessary for long-term memory formation and maintenance. Dynamic changes in DNA methylation and chromatin structure are the result of well-established intracellular signaling cascades that converge on the nucleus to adjust the precise equilibrium of gene repression and activation. It is through this altered transcriptional profile that cells are then able to modulate the plasticity that underlies memory formation, as depicted in Figure 2. Together, these findings usher an exciting era of neuroepigenetics that will certainly continue to grow.

As the field expands, several mechanistic questions remain to be answered. Specifically, although tremendous progress has been made in recent years, more research is required to better understand the mechanisms by which epigenetic modifications are generated, maintained, and removed. For example, the processes that direct DNA methylation to specific sequences are largely unknown. Some studies indicate that transcription factors may act as "docking stations" for DNMT enzymes, which exhibit minimal sequence specificity on their own (Brenner et al. 2005; Cheng et al. 2010). Other evidence suggests that factors intrinsic to the DNA sequence are relevant, including spacing of CpGs in CpG islands (Cokus et al. 2008; Zhang et al. 2009; Cheng et al. 2010). More work is also needed to investigate how the epigenetic code manifests functional change within specific cells and neural circuits. It is clear that epigenetic modifications alter LTP and synaptic plasticity (Levenson et al. 2006; Guan et al. 2009;

Gao et al. 2010), but the mechanism through which this relationship occurs is not clear. In addition, a potential role for epigenetic modifications in regulating different types of plasticity is not well defined. For example, little is known about the involvement of epigenetic mechanisms in regulating intrinsic plasticity, defined as the efficiency of coupling between excitatory potentials and spikes in the post-synaptic neuron, compared to synaptic plasticity, defined as the efficiency of synaptic connections between neurons (Benito and Barco 2010). Although indirect evidence implicates epigenetic mechanisms in both cell excitability and synaptic plasticity (Guan et al. 2002; Levenson et al. 2004, 2006; Yeh et al. 2004; Miller et al. 2008; Nelson et al. 2008; Feng et al. 2010), the nature of this link is still not clear. Further, a potential role for epigenetic mechanisms in memory allocation and the related concept of metaplasticity also remain unexplored, although some studies suggest a possible involvement of DNA methylation in both. As mentioned earlier, memory allocation refers to the distribution of memory to specific cells (Silva et al. 2009), whereas metaplasticity refers to the ability of a neuron's activation history to prime it for future encoding (Abraham 2008). The ability of epigenetic modifications to be selectively induced and to persist under appropriate conditions makes them perfectly positioned to regulate the likelihood of a particular cell to be activated in the future. Indeed, just such a mechanism was recently proposed by Kandel's group, as discussed above (Rajasethupathy et al. 2012).

To address these questions, future studies will benefit from methodologies that increase the cellular and molecular resolution of our investigations. A higher degree of cellular resolution will allow researchers to restrict their analysis to specific cell populations, ideally focusing on cells that make up the memory trace for a particular

behavioral paradigm. To fulfill this need, techniques that allocate memories to specific cell populations within a neural circuit (Han et al. 2009; Zhou et al. 2009) or that tag cells activated during memory acquisition (Reijmers et al. 2007; Tayler et al. 2011) could be combined with techniques such as laser capture microscopy and fluorescence-associated cell sorting. Additionally, these techniques in combination with reporter rodent models could also be used to address how epigenetic mechanisms may be differentially regulated in distinct cell populations (excitatory neurons vs. inhibitory neurons vs. glia) and how these mechanisms may integrate to regulate the function of an entire memory circuit. Recent evidence suggests that there is cell-type specific expression of different HDAC isoforms (Baltan et al. 2011), again underscoring the importance of developing and using targeted manipulations of epigenetic modifying enzymes.

In addition to increased cellular resolution, a higher degree of molecular resolution will allow researchers to better understand how signaling cascades and nuclear protein complexes interact to generate specific epigenetic modifications and how these modifications integrate to regulate overall neuronal function and synaptic plasticity. The advent of high-throughput sequencing has already increased the molecular resolution available to researchers by allowing genome-wide analysis of DNA methylation and post-translational modifications of histones. Although the studies conducted thus far have focused on candidate genes that have a known role in memory, genome-wide studies may identify additional epigenetically regulated genes that are critical for memory formation and maintenance. Determining whether these genes regulate synaptic or intrinsic plasticity will help elucidate how exactly these epigenetic marks contribute to learning and memory. Presumably, regulation of certain genes will lead to consolidation of

synaptic plasticity via the regulation of synaptic effector molecules, whereas other genes might be better positioned to regulate the intrinsic excitability of a cell via modulation of Na+ and K+ channel functions. With these considerations in mind, future research will undoubtedly enrich our understanding of the cellular and molecular underpinnings of learning and memory at large, as well as further elucidate the role epigenetic mechanisms have in this process.

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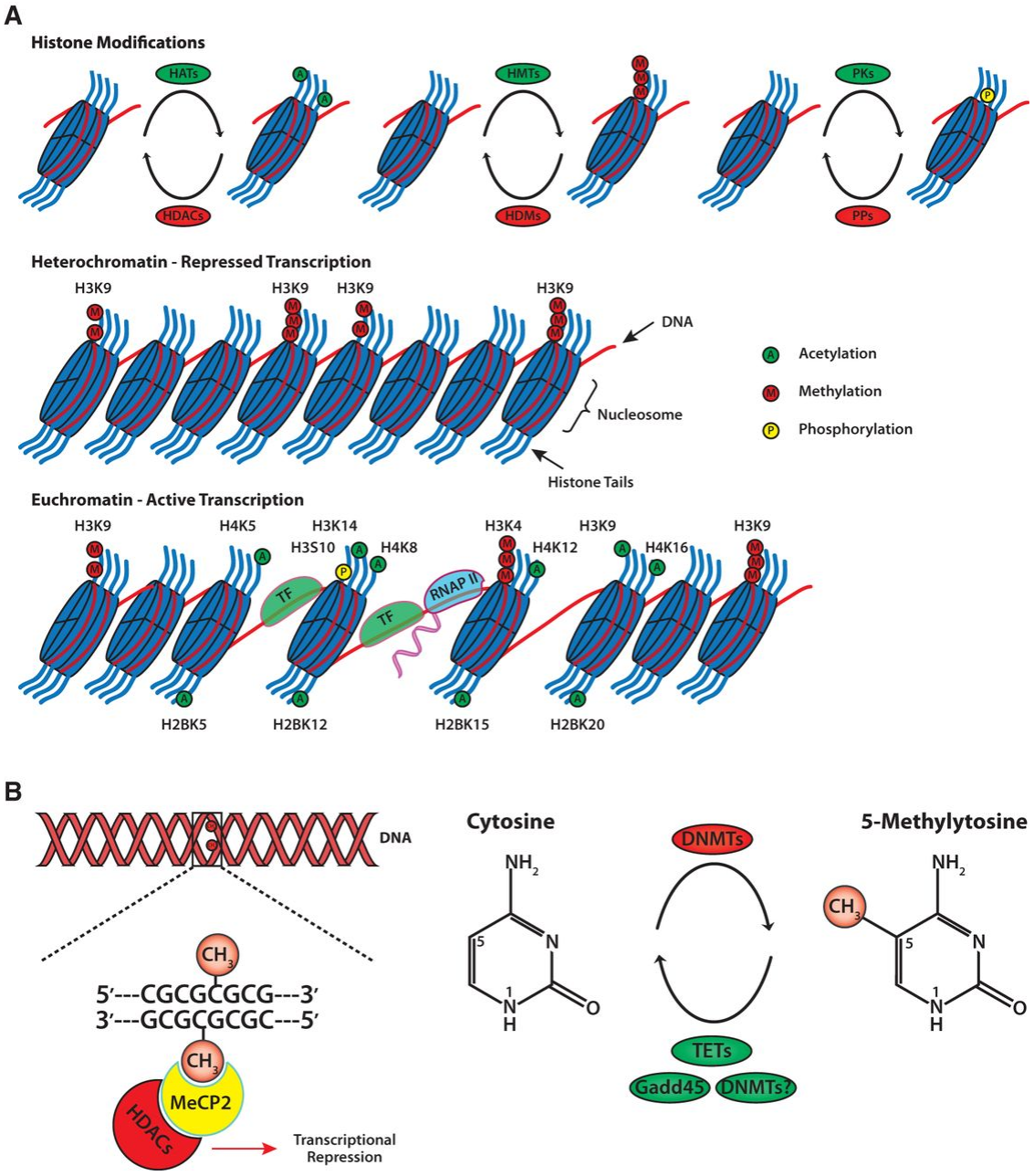
Figure 1


A
Histone Modifications
HATs
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Heterochromatin - Repressed Transcription
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DNA
Nucleosome
Histone Tails
Acetylation
Methylation
Phosphorylation
Euchromatin - Active Transcription
H3K9
H4K5
H3K14
H3S10
H4K8
H3K4
H4K12
H3K9
H4K16
H3K9
TF
TF
RNAP II
H2BK5
H2BK12
H2BK15
H2BK20
B
DNA
CH3
5′---CGCGCGCG---3′
3′---GCGCGCGC---5′
CH3
MeCP2
HDACs
Transcriptional Repression
Cytosine
5-Methylytosine
DNMTs
TETs
Gadd45
DNMTs?

Figure 1. General schematic of epigenetic modifications. (A) Packaging of DNA into chromatin is achieved through the wrapping of 146 bp of DNA around octamers of histone proteins. Chromatin-modifying enzymes dynamically regulate the addition and removal of post-translational modifications on histone N-terminal tails. Modifications associated with learning and memory include histone acetylation, phosphorylation, and methylation. The specific combination of histone tail modifications dictate whether or not the chromatin exists as heterochromatin or euchromatin. Heterochromatin is characterized by condensed chromatin and subsequent transcriptional repression. Euchromatin is characterized by a relaxed chromatin state that allows transcriptional machinery access to DNA for gene expression. (B) Methylation of DNA involves covalent addition of a methyl group to the 5′ position of the cytosine pyrimidine ring by DNMTs. DNA methylation commonly occurs at genes enriched with cytosine-guanine nucleotides (CpG islands). Proteins with methyl-binding domains, like MeCP2, bind to methylated DNA and recruit repressor complexes containing HDACs. Recent evidence suggests that active DNA demethylation can occur via several mechanisms involving members of the Gadd45 family, TET family, and DNMTs themselves. (DNMTs) DNA methyltransferases, (Gadd45) growth arrest and DNA damage 45, (HATs) histone acetyltransferases, (HDACs) histone deacetylases, (HDMs) histone demethylases, (HMTs) histone methyltransferases, (MeCP2) methyl CpG binding protein 2, (PKs) protein kinases, (PPs) protein phosphatases, (TETs) ten eleven translocation, (TF) transcription factor, (RNAP II) RNA polymerase II.

Figure 2.

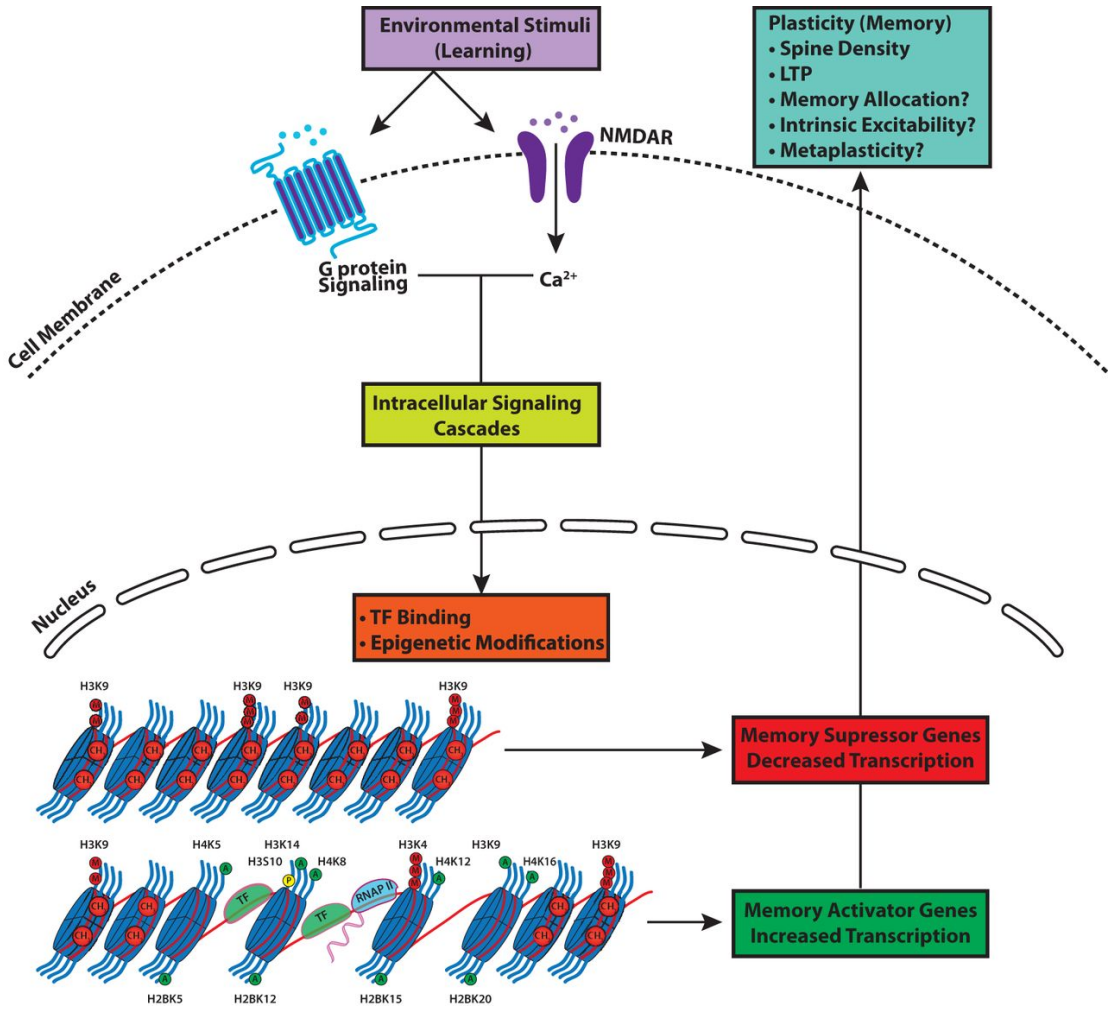

Figure 2. A model depicting the role of epigenetic mechanisms in memory formation and maintenance. Environmental stimuli, which consist primarily of associative learning tasks in animal models, initiate cellular communication by activating specific post-synaptic receptors. Receptor activation stimulates specific intracellular signaling cascades that lead to particular patterns of epigenetic modifications, which in turn regulate the access of transcription factors (TF) and RNA polymerase II (RNA P II) to gene promoters. These regulatory processes result in an increased transcription of memory activator genes and decreased transcription of memory-suppressor genes, which ultimately promote memory formation and maintenance through effects on long-term potentiation (LTP), spine density, memory allocation, cell excitability, and metaplasticity.

TRANSCRIPTIONAL AND EPIGENETIC REGULATION OF HEBBIAN AND NON-HEBBIAN PLASTICITY

by

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Abstract

The epigenome is uniquely positioned as a point of convergence, integrating multiple intracellular signaling cascades into a cohesive gene expression profile necessary for long-term behavioral change. The last decade of neuroepigenetic research has primarily focused on learning-induced changes in DNA methylation and chromatin modifications. Numerous studies have independently demonstrated the importance of epigenetic modifications in memory formation and retention as well as Hebbian plasticity. However, how these mechanisms operate in the context of other forms of plasticity is largely unknown. In this review, we examine evidence for epigenetic regulation of Hebbian plasticity. We then discuss how non-Hebbian forms of plasticity, such as intrinsic plasticity and synaptic scaling, may also be involved in producing the cellular adaptations necessary for learning-related behavioral change. Furthermore, we consider the likely roles for transcriptional and epigenetic mechanisms in the regulation of these plasticities. In doing so, we aim to expand upon the idea that epigenetic mechanisms are critical regulators of both Hebbian and non-Hebbian forms of plasticity that ultimately drive learning and memory.

Keywords: Epigenetics, DNA methylation, Hebbian, histone modifications, homeostatic, intrinsic, metaplasticity, non-Hebbian, synaptic, synaptic scaling

1. Introduction

Long-term changes in neuronal function underlying learning and memory are driven by changes in gene expression with corresponding modifications in protein synthesis and neuronal connectivity (Barondes and Jarvik, 1964; Cohen and Barondes, 1966; Kim and Linden, 2007; Martin et al., 2000). Specifically, changes in the expression of growth factors, ion channels, ligand-gated receptors, and structural proteins are necessary to support long-lasting functional and structural changes within a neuronal circuit (Baker-Andresen et al., 2013a; McClung and Nestler, 2008). Recent evidence suggests epigenetic modifications that remodel chromatin, including DNA methylation and post-translational modifications (PTMs) of histones, likely serve as molecular mechanisms for bi-directional regulation of necessary gene expression (Chen et al., 2003a; 2003b; Levenson and Sweatt, 2005; Martinowich et al., 2003; Nelson and Turrigiano, 2008). This is supported by experimental evidence demonstrating the pathways upstream and downstream of chromatin remodeling are necessary components in synaptic plasticity and long-term behavioral memory (Day and Sweatt, 2011; Levenson et al., 2004a; 2006; Lipsky, 2013; Roberson and Sweatt, 1999; Roberson et al., 1999; Selcher et al., 2002; Sweatt, 2010).

At present, there are several broad questions that remain unanswered. What is the complete transcriptional profile necessary for acquisition and consolidation of long-term memory? How is the epigenome dynamically regulated to subserve these changes in gene expression? More importantly, how do the resulting gene products interact concordantly to produce neuronal plasticity and long-term behavioral adaptation? Historically, the field has focused on how epigenetic mechanisms modulate Hebbian plasticity. However, it is

becoming increasingly evident that memory is also reliant on non-Hebbian forms of plasticity, such as intrinsic plasticity and synaptic scaling (Figure 1) (Baker-Andresen et al., 2013b; Nelson and Turrigiano, 2008). We propose that a thorough examination of how epigenetic mechanisms drive Hebbian and non-Hebbian forms of plasticity will allow for a more comprehensive understanding of the global transcriptional and epigenetic changes necessary for long-term behavioral memory. This review will examine a role for epigenetic regulation first in Hebbian plasticity, and later, in two forms of non-Hebbian plasticity – intrinsic plasticity and synaptic scaling. Additionally, we discuss each form of plasticity in the process of memory formation and explore how each is driven by transcriptional and epigenetic mechanisms.

2. Hebbian Plasticity

2.1 Relevance to Learning and Memory

Hebbian plasticity is defined as synapse-specific changes in strength driven by the coordination of pre-synaptic input and post-synaptic depolarization (see Figure 1A). Long-term potentiation (LTP) is a form of Hebbian plasticity characterized by long-lasting enhancement in synapse-specific neurotransmission in response to repetitive, high frequency stimulation. LTP is a widely accepted cellular mechanism underlying long-term memory formation (Bauer et al., 2001; Blair et al., 2001; Bliss and Collingridge, 1993; Lynch et al., 1988; 2008; 2007; 2013; Malenka and Bear, 2004; Maren, 2005). Potentiation of excitatory synaptic transmission can be induced in various regions of the mammalian brain, including the hippocampus, amygdala, striatum, and cortex (Fourcaudot et al., 2009; Huang and Kandel, 1998; Huang et al., 2000; Iriki et al., 1989; Lee and Kirkwood, 2011;

Maren, 1999; Rex et al., 2010; Weisskopf et al., 1999). Enhancements and deficits in memory are often correlated with increases or decreases in LTP, respectively, across many behavioral tasks and corresponding brain regions mediating the behaviors (Izquierdo and Medina, 1995; Lynch, 2002; Martin et al., 2000; Rodrigues et al., 2004; Staubli et al., 1994). In addition, LTP induction mechanisms are similar to those necessary for long-term memory formation (Klann et al., 2004; Pittenger and Kandel, 2003). Classical LTP of hippocampal cornu ammonis (CA)1 excitatory synapses is driven by N-Methyl-D-Aspartate (NMDA)-dependent Ca2+ influx, which subsequently activates, directly or indirectly, signaling cascades that modify the strength of targeted synapses (Malenka and Nicoll, 1993). The blockade of these receptors, or their downstream effectors, inhibits both LTP in vitro and memory in vivo. Furthermore, pharmacological and genetic manipulations of epigenetic targets affect the induction of LTP and memory formation (Levenson and Sweatt, 2006).

It should be noted that for subsequent discussions we have chosen to group together the two topics of transcriptional and epigenetic regulation as we believe that both processes are required to achieve a coordinated orchestration of gene expression and nuclear output that in turn effects cellular physiology and animal behavior. However, we readily acknowledge that although intimately coupled, each process likey possesses specific functions and limitations. We define transcriptional regulation as those mechanisms that are directly involved in the synthesis of RNA (either coding or non-coding) like transcription factor activation/binding and RNA polymerase association/activity. As such, their functionality is dependent on their ability to act as singaling relays between cystolic and nuclear mechanisms in order to set in motion precise gene expression profiles that are

specific to a particular transcription factor and its associated upstream signaling cascades. In contrast, we find epigenetic mechanisms to act as powerful modulators of the aformentioned transcriptional machinery with their strength inherent in their capacity to serve as molecular tags of present and past neuronal activity and behavioral experience. The capability of epigenetic mechanisms to produce long-lasting cellular change provides a platform with extensive computational power that integrates stimuli across time to more appropriately fine-tune the transcriptional potential of the genome.

2.2 Transcriptional and Epigenetic Regulation

Eukaryotic DNA is tightly packaged into a DNA-protein complex known as chromatin. Positively-charged histones serve as a core around which negatively-charged DNA is tightly coiled. Conventionally, transcription is repressed by spatial restrictions caused by interactions of DNA with histones, which occludes RNA polymerase II/DNA interaction. Initiation of transcription requires the disruption of chromatin's tightly compacted structure through the PTMs of histones (Roth and Sweatt, 2009; Varga-Weisz and Becker, 1998). At present, the most frequently characterized PTMs of histones are acetylation, methylation, ubiquitination, and phosphorylation; each modification serves as a distinct functional epigenetic tag (Rea et al., 2000; Strahl and Allis, 2000). The most extensively studied histone modification in the context of learning and memory is the acetylation of lysine residues on histone tails through the activity of histone acetyltransferases (HATs) (Lau et al., 2000; Tanner et al., 2000a; 2000b; 1999), an effect reversed by histone deacetylase (HDAC) activity (Fischle et al., 2003; Saha and Pahan, 2006; Varga-Weisz et al., 1999).

Recent reports demonstrate that histone-modifying enzymes and histone acetylation are necessary for mammalian associative learning and Hebbian plasticity (for a review of these mechanism in invertebrates please see Rahn et al., 2013) (Alarcon et al., 2004; Chen et al., 2003a; Chwang et al., 2007; Guan et al., 2009; Gupta et al., 2010; Koshibu et al., 2009; Levenson et al., 2004b; Vecsey et al., 2007). For example, mice with genetic mutations in the HAT cyclic adenosine monophosphate (cAMP)/Ca2+-response element binding protein (CREB) binding protein (CBP), have decreased histone acetylation and deficits in transcription-dependent LTP (Alarcon et al., 2004). Interestingly, those deficits were ameliorated by administration of the HDAC inhibitor (HDACi) suberoylanilide hydroxamic acid. In contrast, mice with deletion of HDAC2, displayed enhanced hippocampal LTP, whereas overexpression in the hippocampus blunted LTP (Guan et al., 2009). Moreover, LTP induction resulted in increased histone H3 and H4 acetylation and the enhancement of histone acetylation and LTP induction were both facilitated by HDACi application (Levenson et al., 2004b; Miller et al., 2008; Sui et al., 2012; Vecsey et al., 2007; Yeh et al., 2004; Zeng et al., 2011). Furthermore, LTP specifically increased changes in histone acetylation at the promoter regions of *Bdnf* and *Reln*, genes involved in synaptic transmission (Sui et al., 2012). Collectively, these studies argue for an intimate relationship between levels of histone acetylation and LTP.

In addition to histone modifications, DNA methylation is a canonical regulator of gene transcription. Methylation is the most common covalent modification occurring in eukaryotic DNA and has been studied extensively in development as a static process following cell differentiation (Rakyan et al., 2001). Recent reports have challenged the established dogma by demonstrating that DNA methylation is dynamically regulated in the

adult nervous system and that this cellular mechanism is a crucial step in memory formation (Day et al., 2013; Feng et al., 2010; Lubin et al., 2008; Miller and Sweatt, 2007; Miller et al., 2010). Importantly, both DNA methylation and DNA methyl-binding proteins have been implicated in the induction of long-term synaptic plasticity (Cortés-Mendoza et al., 2013).

DNA methylation is a reaction catalyzed by DNA methyltransferase (DNMT) enzymes, during which a methyl group is added to the carbon at the 5′ position of the pyrimidine ring (Chen et al., 1991). Methylation was thought to only occur at cytosine bases followed by a guanine base, however this notion has recently been challenged (Lister et al., 2013; Varley et al., 2013; Xie et al., 2012). This dinucleotide sequence (designated CpG, with p corresponding to a phosphate group) is highly underrepresented in the genome and often found in both high-density clusters called CpG islands and low-density regions near CpG islands called CpG shores (Bird, 1978; Deaton and Bird, 2011; Guo et al., 2011b). Activity-induced changes in methylation occur predominantly in low-density regions in both inter- and intra-genic locations (Guo et al., 2011b). Although much attention has been paid to changes in promoter methylation, recent findings highlight changes in intragenic methylation observed with memory formation (Day et al., 2013).

There are two classes of DNMTs: maintenance and de novo DNMTs. The de novo DNMTs (DNMT3a and DNMT3b) methylate sites lacking methyl-cytosine on either DNA strand, while the maintenance DNMT isoform, DNMT1, methylates hemi-methylated DNA (Goll and Bestor, 2005). It should be noted that DNMT1 can also regulate de novo methylation under certain circumstances (Fatemi et al., 2002; Hsieh, 2005). Maintenance DNMTs perpetuate methylation after cell division by regenerating the methyl-cytosine

marks on the newly synthesized complementary DNA strand that arises with DNA replication (Feng and Fan, 2009; Feng et al., 2010; Okano et al., 1999a; 1999b). Although there are examples of DNA methylation associated with increased gene transcription (Chahrour et al., 2008; Day et al., 2013; Uchida et al., 2011), it is commonly accepted that methylation of DNA suppresses gene transcription, and in specific circumstances extensive DNA methylation triggers complete silencing of the associated gene (Sweatt et al., 2012). Methylation can repress gene expression by directly interfering with binding of transcription factors to regulatory elements or by actively recruiting methyl-CpG-binding proteins. These methyl-CpG binding proteins repress transcription by recruiting other chromatin-remodeling enzymes such as HDACs, repressor element 1 (RE1) silencing transcription factor/ neuron-restrictive silencing factor (REST/NRSF), and CoREST (an associated HDAC), among others (Ballas and Mandel, 2005; Ballas et al., 2005; Klose et al., 2005; Levenson and Sweatt, 2005).

The link between DNA methylation and Hebbian plasticity represents a molecular mechanism of memory storage, and investigating this relationship has been approached via pharmacological and genetic methods. Blocking DNA methylation prior to LTP induction with the DNMT inhibitors zebularine or 5-aza-2-deoxycytidine disrupted hippocampal LTP and resulted in significant demethylation of Reln and Bdnf promoters (Levenson et al., 2006). Both genes are associated with synaptic plasticity and undergo comparable changes in methylation following fear conditioning (Lubin et al., 2008; Miller and Sweatt, 2007). A subsequent study demonstrated that the LTP deficit produced by DNMT inhibition could be reversed by pretreatment with the HDACi trichostatin A (TSA), suggesting cross-talk between histone acetylation and DNA methylation during plasticity

(Miller et al., 2008). In support of these pharmacological studies, Feng and colleagues (2010) recently reported that mice with a double knockout of DNMT1 and DNMT3a in forebrain post-mitotic neurons have impaired hippocampal LTP and enhanced LTD. The effects on synaptic function were further correlated with a reduction in global DNA methylation and deregulation of specific genes. Furthermore, neurons lacking only one DNMT isoform had normal hippocampal plasticity, indicating that DNMT1 and DNMT3a may have overlapping roles in adult neurons, and that at least one form is required to maintain normal hippocampal LTP (Feng et al., 2010).

Though passive DNA demethylation is a largely accepted mechanism in dividing cells, the presence of active DNA demethylation (*i.e.,* demethylation that occurs in the absence of DNA replication) in neurons has been controversial (Ooi and Bestor, 2008; Wu and Zhang, 2010). However, mounting evidence suggests that active demethylation does occur in the adult nervous system and regulates synaptic plasticity (Guo et al., 2011a; 2011b; Li et al., 2013; Sultan et al., 2012). One recent report provides evidence for activity-dependent DNA demethylation of *Reln* and *Bdnf* genes following induction of LTP in the medial pre-frontal cortex (Sui et al., 2012). Additionally, a landmark study by Ma and colleagues (2009) demonstrated that GADD45B (a member of the growth arrest and DNA damage inducible 45 family and an activity-induced immediate early gene) is necessary for the demethylation and transcriptional activation of both *Bdnf* promoter IX and *Fgf1* promoter B following electroconvulsive stimulation of the dentate gyrus (Ma et al., 2009). A subsequent study by Sultan and colleagues (2012) reported that GADD45B regulated hippocampal LTP and memory formation. Extracellular recordings from hippocampal slices showed genetic deletion of *Gadd45b* resulted in a selective enhancement of late-

phase LTP despite using a near-threshold stimulus. Additionally, mutant mice exhibited enhanced memory in tasks including motor performance, aversive conditioning, and spatial navigation (Sultan et al., 2012). Further studies are needed to elucidate detailed demethylation mechanisms, but these data highlight the importance of GADD45B and other modulators of DNA demethylation in plasticity and memory.

The studies presented here clearly implicate PTMs of histones and DNA methylation as necessary epigenetic mechanisms subserving Hebbian plasticity and long-term memory. Owing to space restrictions, we are unable to explore the topic of synaptogenesis in the context of synaptic plasticity and long-term behavioral memory. However, there is growing evidence for a role of epigenetic mechanisms in the regulation of spine and synapse formation both in the context of activity-dependent processes as well as aging and disease states with deficits in learning and memory (for reviews on the topics please see Kavalali et al., 2011; McEwen et al., 2012; Na et al., 2013). It should be noted that epigenetic regulation of structural plasticity at large warrants further consideration and integration with the topics covered in this review. In the subsequent sections we will examine how transcriptional and epigenetic mechanisms may subserve modulation of non-Hebbian forms of plasticity. We will focus our discussion on intrinsic plasticity and a form of homeostatic plasticity known as synaptic scaling.

3. Intrinsic Plasticity

The involvement of enduring, synapse-specific, Hebbian modifications in memory formation and storage is readily evident and well-documented. However, emerging evidence suggests that activity-dependent alterations in intrinsic neuronal excitability,

termed intrinsic plasticity, may also be a necessary component of the cellular processes underlying learning and memory (Daoudal, 2003; Frick and Johnston, 2005; Sehgal et al., 2013; Zhang and Linden, 2003), in addition to regulating network function and informational processing at large (Nelson and Turrigiano, 2008; Remme and Wadman, 2012). Intrinsic plasticity involves the attunement of passive and/or active membrane properties as to modulate the input/output relationships that govern action potential (AP) firing rates. Modification of a neuron's intrinsic properties can be mediated by regulating the expression or the biophysical properties of voltage- and calcium-gated ion channels (see Figure 2). In addition, the specific location on the neuron where these adaptations take place also determines how intrinsic plasticity manifests itself (see Figure 1B). For example, alterations in active and passive membrane properties can occur locally, targeting specific dendrites and influencing synaptic throughput of a small set of synapses. Some of the relevant currents involved in this process include the afterhyperpolarization (AHP) current (generated by Ca2+-activated K+ channels) (Storm, 1990), the IA current (subserved by rapidly inactivating A-type K+ channels) (Storm, 1990), and the Ih current (produced by hyperpolarization-activated cyclic nucleotide-gated (HCN) channels) (Biel et al., 2009); together these currents modify the degree of summation and propagation of synaptic input to the soma, as well as the amplitude and duration of back propagating APs. Intrinsic alterations can also occur globally, impacting the axo-somatic membrane as well as larger portions of proximal dendrites as to modify throughput for all synapses. Global alterations largely involve modulation of Na+ and K+ currents to regulate AP initiation (which depends on parameters like AP threshold and resting membrane potential), spike frequency adaptation or accommodation, and AP properties (*e.g.,* amplitude and duration). Together,

these local and global alterations ultimately dictate how information flows within and between neurons (for a more in depth examination of these topics see Daoudal, 2003; Frick and Johnston, 2005; Sehgal et al., 2013; Zhang and Linden, 2003).

3.1 Relevance to Learning and Memory

Experimental evidence for learning-induced changes in intrinsic plasticity stems from a variety of model systems and behavioral paradigms. We will focus our discussion on studies performed on mammals (for additional information on invertebrates see Mozzachiodi and Byrne, 2010). One of the first reported studies examining learning-induced changes in intrinsic plasticity involved a feline associative conditioning task where a cat associated an auditory click (conditioned stimulus) with a tap between the eyebrows (unconditioned stimulus), such that future clicks elicited both an eyeblink and a nose twitch (conditioned responses) (Brons and Woody, 1980). Intracellular recordings from the pericruciate sensorimotor cortex of conditioned animals revealed an increase in neuronal excitability evidenced by a reduction in the threshold current needed for spike initiation. Similarly, whole-cell electrophysiological recordings from rabbit hippocampal slices following acquisition of trace eyeblink conditioning (EBC) revealed increased excitability in approximately 50% of CA1 and CA3 pyramidal neurons (Coulter et al., 1989; Disterhoft et al., 1986; 1988; Thompson et al., 1996). This hyperexcitability was characterized by an increased number of spikes elicited by a sustained depolarizing current injection, also termed reduced spike-frequency adaptation or accommodation, and a marked reduction in the AHP amplitude evoked by a spike burst.

Learning-related changes in intrinsic plasticity have also been observed in other

species and additional behavioral paradigms such as Morris water maze (MWM) (Oh et al., 2003; Ohno et al., 2006), odor fear conditioning (Motanis et al., 2012; Rosenkranz and Grace, 2002), rule learning on odor discrimination tasks (Motanis et al., 2012; Saar et al., 1998; Zelcer et al., 2006), auditory fear conditioning (both delay and trace versions) (Motanis et al., 2012; Santini et al., 2008), and contextual fear conditioning (Kaczorowski and Disterhoft, 2009; McKay et al., 2009). In most cases, learning is associated with increased intrinsic excitability, although exceptions to this rule have been found in the infralimbic prefrontal cortex with tone fear conditioning (Santini et al., 2008) and the basolateral amygdala (BLA) with odor fear conditioning (Motanis et al., 2012). Overall, reductions in spike threshold, spike accommodation, and amplitude of burst-evoked AHPs are the electrophysiological changes most often observed following learning.

It is clear from the previously outlined studies that changes in intrinsic excitability appear to be evolutionarily conserved across species and evidenced in a variety of behavioral tasks. However, many questions remain regarding the exact mechanisms underlying the induction, expression, and maintenance of intrinsic plasticity in the context of animal behavior. Moreover, determining the functional role of intrinsic plasticity is an active area of ongoing research. As previously suggested (Sehgal et al., 2013; Zhang and Linden, 2003), growing evidence indicates that intrinsic plasticity may in fact serve three distinct, yet overlapping, functions: as part of the memory engram itself, as a modulator of behavioral memory and Hebbian plasticity, and as a component in the overall repertoire of homeostatic adaptations (for a review on this last topic see Nelson and Turrigiano, 2008).

Although intrinsic plasticity is associated with learning, a mnemonic function for intrinsic plasticity seems unlikely given that changes in excitability are short-lived in

comparison to memory of the behavioral task (Motanis et al., 2012; Moyer et al., 1996; Saar et al., 1998; Thompson et al., 1996; Zelcer et al., 2006). For example, changes in intrinsic excitability of rabbit CA1 and CA3 pyramidal neurons with EBC are present for 1–3 days after training before returning to baseline values by 5–7 days, even though the behavioral memory is present for at least 6 months (Moyer et al., 1996; Thompson et al., 1996). Such data suggests that intrinsic plasticity might not comprise the actual memory engram itself, but may instead serve an early role during the acquisition and consolidation processes. However, the transient nature of hippocampal excitability changes may also reflect the time-limited involvement of the hippocampus in remote memory storage (Frankland and Bontempi, 2005; Kim et al., 1995; Wiltgen et al., 2004). Additionally, there are reports of persistent changes in intrinsic plasticity with learning that can last as long as one month (Brons and Woody, 1980; Schreurs et al., 1998) suggesting there may be instances in which intrinsic plasticity indeed possesses a mnemonic function.

Instead, the majority of existing data make a stronger case for intrinsic plasticity as a modulator of behavioral memory and Hebbian plasticity (a prime example of metaplasticity, which will be covered in more depth in section 4). At the behavioral level, learning-induced increases in excitability are associated with enhanced learning of the same or different behavioral tasks (Saar et al., 1998; Zelcer et al., 2006). For example, training rats in the MWM soon after olfactory learning takes place (during the time point where hyperexcitability is observed in CA1 pyramidal neurons) results in enhanced acquisition of the spatial task (Zelcer et al., 2006). Furthermore, the enhanced spatial learning capability of olfactory-trained rats is no longer observed once excitability levels revert to baseline. These observations suggest that by rendering neurons more excitable,

the circuit may be primed to readily acquire future information. Indeed, this interpretation is supported by pharmacological and genetic interventions that increase neuronal excitability which in turn increase learning rate and/or capability (Disterhoft and Oh, 2006; Han et al., 2007; Zhou et al., 2009). At the electrophysiological level, manipulations of intrinsic excitability can also influence Hebbian plasticity, often facilitating the induction of LTP (Chen et al., 2006; Cohen and Abraham, 1996; Cohen et al., 1999; Kramár et al., 2004; Sah and Bekkers, 1996).

3.2 Transcriptional and Epigenetic Regulation

Given the involvement of intrinsic plasticity in learning, memory, and Hebbian plasticity, investigations have shifted towards understanding the molecular substrates that underlie this process. Mechanistic investigations have revealed intrinsic plasticity requires many of the same molecular mechanisms implicated in Hebbian plasticity; roles for Ca2+ signaling and intracellular signaling cascades like protein kinases C (PKC), cAMP-dependent protein kinase (PKA), cyclic guanosine monophosphate (cGMP)-dependent protein kinase (PKG), and Ca2+/calmodulin-dependent protein kinase II (CaMKII) are well documented (Daoudal, 2003; Zhang and Linden, 2003). Similar to Hebbian plasticity, the long-term maintenance of intrinsic plasticity is also protein-synthesis dependent (Cohen-Matsliah et al., 2010; Xu et al., 2005). However, only recently has transcriptional and epigenetic regulation of intrinsic plasticity begun to be examined in more depth.

Transcriptional involvement in intrinsic plasticity is strongly evidenced by a series of studies in which manipulations of the level or activity of CREB bidirectionally modulated neuronal intrinsic excitability (for review see Benito and Barco, 2010). Dong

and colleagues were the first to demonstrate a positive correlation between intrinsic excitability and levels of CREB (Dong et al., 2006). Overexpression of constitutively active CREB increased the firing rate of medium spiny neurons (MSN) in the nucleus accumbens (NA) as well as decreased the threshold to elicit an AP and the minimal current needed to fire a spike. In contrast, overexpression of dominant-negative CREB revealed the opposite effect: decreased firing rate and increased threshold for eliciting an AP.

Several laboratories have recapitulated similar findings in the locus coeruleus (Han et al., 2006), CA1 region of the hippocampus (Lopez de Armentia et al., 2007), and the BLA (Viosca et al., 2009; Zhou et al., 2009) using recombinant neurotropic viral vectors, transgenic mice, and gene-targeting techniques to manipulate CREB levels and activity (for a thorough review on these methodologies see Barco and Marie, 2011). Overall, gain-of-function manipulations of CREB were associated with increased neuronal excitability, whereas loss-of-function interventions decreased excitability. Electrophysiological recordings revealed CREB modulated AP threshold, firing rate, AHP amplitude, input resistance, and resting membrane potential, although differences were observed depending on cell-type examined and methodology used to manipulate CREB. It is worth mentioning many of these studies found concurrent changes in Hebbian plasticity, which is not surprising considering CREB's well-documented role in regulating long-term memory processes as well as underlying changes in Hebbian plasticity (Alberini, 2009; Josselyn and Nguyen, 2005; Sakamoto et al., 2011; Silva et al., 1998). Together these data demonstrate CREB-mediated gene transcription is capable of modulating intrinsic plasticity in addition to Hebbian plasticity.

However, the complete transcriptional profile underlying these observed changes

in excitability is only beginning to be characterized. As previously mentioned, modulation of intrinsic neuronal properties occurs by regulating expression level or biophysical properties of voltage- and calcium-gated ion channels. As suggested by others (Benito and Barco, 2010; Won and Silva, 2008), CREB-mediated changes in intrinsic excitability likely involve direct and indirect modulation of positive and negative regulators of intrinsic excitability. Specifically, modifications of positive regulators could involve direct regulation of ion channel mRNA levels (including specific splice variants) as well as alteration of secondary messenger systems (kinases and phosphatases) that modulate ion channel function. Given that CREB is a transcriptional activator, inhibition of negative regulators would likely require activation of transcriptional repressors and small non-coding RNAs, like microRNAs and piwi-interacting RNAs, whose activation would inhibit downstream effectors of intrinsic plasticity (see Figure 2). CREB-mediated transcription is known to rely on the recruitment of co-activator complexes, like p300 (another HAT) and CBP, which subsequently restructure chromatin to influence gene expression (Alarcon et al., 2004; Barrett et al., 2011; Bourtchouladze et al., 2003; Chen et al., 2010; Korzus et al., 2004; Maurice et al., 2008; Oike et al., 1999; Oliveira et al., 2011; Valor et al., 2011; Wood et al., 2005). Therefore, one would expect to detect changes in epigenetic modifications, including histone acetylation, at CREB target genes that are positive and negative regulators of intrinsic excitability. This is in fact the case for genes implicated in Hebbian plasticity, like *Bdnf*, *Egr1*, and *Pp1*, which are not only known CREB targets but also genes that undergo epigenetic regulation during the acquisition and consolidation of long-term memories (Day et al., 2013; Lubin et al., 2008; Miller and Sweatt, 2007).

Efforts to characterize the transcriptional profile underlying changes in excitability

have primarily focused on the level or function of voltage-gated Na+ and K+ channels, as well as potentiation of the adenylyl cyclase (AC)/cAMP/PKA pathway. Microarray analysis of the NA from inducible transgenic animals overexpressing CREB revealed upregulation of the voltage-dependent Na+ channel 1β subunit (Scn1b) and downregulation of the voltage-dependent K+ channel KV1.4 subunit (Kcna4) (McClung and Nestler, 2003). Likewise, current-clamp recordings from MSN neurons overexpressing CREB showed a potentiation of Na+ conductances and an inhibition of K+ conductances, effects reversed by expression of a dominant-negative CREB (Dong et al., 2006).

Converging evidence suggests CREB may regulate intrinsic plasticity via positive feedback onto the AC/cAMP/PKA pathway. Interestingly, viral-mediated overexpression of wild-type CREB in the locus coeruleus enhanced the excitatory effect of forskolin (an activator of AC) on these neurons (Han et al., 2006). The increased basal firing rate observed with forskolin could occur via CREB-mediated induction of AC8. Adcy8, which encodes AC8, is a direct target for CREB (Lane-Ladd et al., 1997), and its expression is regulated by CREB in vitro and in vivo (Chao et al., 2002). Furthermore, activation of the cAMP pathway has been shown to increase neuronal excitability in noradrenergic neurons of the locus coeruleus (Alreja and Aghajanian, 1995; Ivanov and Aston-Jones, 2001; Wang and Aghajanian, 1987). More specifically, the AC/cAMP/PKA pathway regulates many of the previously mentioned currents involved in modulating intrinsic plasticity, including the AHP current (Haug and Storm, 2000; Oh et al., 2009; Pedarzani and Storm, 1995; 1993), the Ia current (Hoffman and Johnston, 1998), and the Ih current (Pape, 1996). Together these observations support the hypothesis that CREB-mediated changes in intrinsic plasticity are mediated via potentiation of the AC/cAMP/PKA pathway. It is evident more

research is necessary to completely understand the transcriptional changes underlying CREB-mediated increases in excitability; and to differentiate these changes from those underlying Hebbian plasticity.

It is worth noting that although CREB-mediated modulation of intrinsic plasticity is well supported, there is a shortage of direct evidence confirming epigenetic regulation of intrinsic plasticity in the context of learning and memory. However, recent evidence from the pain and epilepsy fields demonstrate epigenetic mechanisms underlie the characteristic neuronal hyperexcitability in these disease states (Beck and Yaari, 2008). As with long-term memory formation and synaptic plasticity, there is growing evidence that epigenetic mechanisms also play an important in role in the development and maintenance of different pain states and epileptogenesis (for pain-relevant reviews see: Denk and McMahon, 2012; Géranton, 2012; Rahn et al., 2013; for epileptogenesis-relevant reviews see: Lubin, 2012; Qureshi and Mehler, 2010; Roopra et al., 2012).

In animal models of neuropathic pain, sustained downregulation of genes encoding for sodium channel Nav1.8, the μ-opiod receptor, and potassium channel Kv4.3, occurred in the dorsal root ganglion (DRG) following nerve injury (Uchida et al., 2010a; 2010b). These decreased transcript levels were associated with enhanced binding of NRSF and hypoacetylation of histone H3 and H4 at neuron-restrictive silencer elements (NRSEs, also known as RE1s) in the promoter regions of these genes. NRSF is an activity-regulated transcription factor that targets genes containing NRSE sites and silences their expression by actively recruiting chromatin modifying and remodeling complexes that can include proteins like methyl CpG binding protein 2 (MeCP2), Co-REST, Sin3a, HDACs, histone methyltransferases, and histone demethylases (Roopra et al., 2001; 2012). Interestingly,

nerve injury resulted in a chronic elevation of NRSF transcript and protein levels that were further correlated with increased acetyl H4 enrichment at the NSRF promoter II (Uchida et al., 2010a; 2010b).

NRSF-mediated repression of ion channels is also implicated in epileptogenesis. Seizure activity resulted in NRSF binding to the *Hcn1* promoter in the hippocampus of kainite-treated animals (McClelland et al., 2011); decreased Hcn1 transcript levels as well as attenuation of Ih were observed. As mentioned previously, HCN channels dampen dendritic excitability in hippocampal cortical neurons and modify overall synaptic integration and somatic-dendritic coupling (Magee, 1999; Poolos et al., 2002; Santoro et al., 2000). Pharmacological blockade of HCN channels causes neuronal and network hyperexcitability (Albertson et al., 2013; 2011). Furthermore, animals lacking Hcn1 have more excitable neurons, are more prone to seizures, and have higher seizure-induced mortality (Huang et al., 2009; Santoro et al., 2010). Administration of oligonucleotides targeting the Hcn1-NRSE blocked REST binding of *Hcn1*, thereby restoring HCN1 protein levels and Ih current amplitudes as well as producing fewer spontaneous seizures (McClelland et al., 2011).

In addition to ion channels, there is evidence of epigenetic regulation of secondary messenger systems that modulate ion channel function. Following neuronal injury, p300 and cyclooxygenase 2 (COX-2) were upregulated in the lumbar spinal cord of rats (Zhu et al., 2012). More importantly, the degree of p300 binding to the COX-2 promoter dictated subsequent COX-2 transcript and protein level. COX-2 regulates the production of several prostaglandins that contribute to the development and maintenance of spinal cord hyperexcitability (Latremoliere and Woolf, 2009; Willingale et al., 1997). Similar gene-

specific regulation was also observed in a model of inflammatory pain where peripheral infusion of an inflammatory agent resulted in demethylation of the cystathionine-β-synthase (*Cbs*) gene promoter with subsequent upregulation of *Cbs* mRNA and protein in the DRG (Qi et al., 2013). Like COX-2, CBS synthesizes an endogenous molecule, in this case hydrogen sulfide (H2S), whose activity was necessary and sufficient to elicit enhanced excitability of DRG neurons (Qi et al., 2013; Xu et al., 2009). More specifically, in vitro addition of NaHS (an H2S donor) significantly depolarized the resting membrane of DRG neurons, reduced rheobase and AP threshold, and increased firing frequency. This effect was mediated, in part, via potentiation of tetrodotoxin-resistant sodium channel currents, an effect dependent on the PKA pathway. These data are consistent with previous in vitro studies showing H2S modulates the AC/cAMP/PKA pathway (Muzaffar et al., 2009; Shao et al., 2011; Smith, 2009). That the AC/cAMP/PKA pathway mediates changes in excitability in both pain and memory circuits suggests shared homologous cellular and molecular mechanisms and underscores the fundamental importance of this pathway in regulating intrinsic neuronal properties (Rahn et al., 2013). Hence, it is conceivable that, in learning and memory, epigenetic mechanisms may impinge on intrinsic excitability directly via modulation of signaling proteins in the AC/cAMP/PKA pathway or indirectly via intermediary effectors as is seen in the pain system.

These studies collectively demonstrate the existence of epigenetic regulation of ion channels and associated signaling pathways involved in intrinsic plasticity. More specifically, REST appears to play an essential function in this regulation, which is not surprising given that many genes involved in neuronal excitability contain NRSE consensus sequences (Roopra et al., 2001). These and other mechanisms should be

examined more closely in the context of learning and memory.

Are there learning-associated changes in epigenetic regulators or modifications at genes implicated in intrinsic plasticity? Besides CREB, are there additional transcription factors that may also mediate the necessary changes in gene expression contributing to intrinsic plasticity? Based on the pain and epilepsy literature, both of these scenarios seem likely. REST appears to be an excellent candidate transcription factor that may orchestrate specialized epigenetic machinery to relevant genes of interest. Other potential candidates include nuclear factor-κB (NF-κB) as it too is known to associate with epigenome-modifying complexes (Chen et al., 2011; Lanzillotta et al., 2010). In fact, hippocampal neurons from mice with mutations in the IκBα promoter, the primary inhibitor of NF-κB, exhibit spontaneous burst firing and hyperexcitability (Shim et al., 2011) that could be explained via modulation of voltage-dependent calcium channels and ionotropic glutamate receptor channels (Furukawa and Mattson, 2002). Finally, do genetic or pharmacological manipulations of epigenetic enzymes modulate neuronal intrinsic excitability? To our knowledge this last question remains completely unanswered, as it has not been directly investigated in the learning and memory, pain, or epilepsy fields.

4. Synaptic Scaling

Homeostatic plasticity refers to the cellular changes, both synaptic (Turrigiano and Nelson, 2004) and intrinsic (Zhang and Linden, 2003), that allow neurons to maintain relatively stable firing rates; thus mediating one of the most salient and paradoxical characteristics of neuronal networks: robust stability in the face of remarkable plasticity (Nelson and Turrigiano, 2008). While functionally distinct, synaptic and intrinsic

homeostatic mechanisms are not completely independent and can influence one another in a manner directed from membrane-to-synapse (Ibata et al., 2008) or synapse-to-membrane (Ishikawa et al., 2009). A well-characterized form of homeostatic synaptic plasticity, synaptic scaling, involves bidirectional compensatory changes in post-synaptic receptor density in response to chronically elevated or depressed activity levels (Kilman et al., 2002; Rannals and Kapur, 2011; Shin et al., 2012; Turrigiano et al., 1998; Wierenga et al., 2005). Importantly, although homeostatic synaptic plasticity has been shown to operate at local synaptic inputs (Hou et al., 2011; Lee et al., 2013; 2010; Pozo and Goda, 2010) and even at the individual synapse level (Hou et al., 2011; Lee et al., 2010), synaptic scaling occurs via a highly coordinated, cell-wide program that multiplicatively adjusts post-synaptic weights across all synapses (see Figure 1C) (Turrigiano et al., 1998; Turrigiano, 2008). This program is initiated in a cell-autonomous manner, as neurons respond robustly to fluctuations in their own spiking rates by sensing concomitant changes in intracellular Ca2+ (Blackman et al., 2012; Goold and Nicoll, 2010; Ibata et al., 2008; Peng et al., 2013). Additionally, soluble factors such as brain-derived neurotrophic factor (BDNF) provide higher order control to scaling processes, through the coordination of transcription-dependent and independent processes (see section 3.2).

4.1 Relevance to Learning and Memory

Theoretical arguments detailing the role of synaptic scaling in learning and memory have been discussed extensively (Nelson and Turrigiano, 2008; Pozo and Goda, 2010; Queenan et al., 2012). The significance of global, multiplicative adjustments in post-synaptic strength lies in the fact that this mechanism has been posited to maintain relative

synaptic weights in the context of a highly active and plastic neural network. It is hypothesized that scaling allows for preservation of information acquired through experience-dependent, Hebbian plasticity. As synapse-specific changes are thought to underlie memory storage, synaptic scaling allows for relative preservation of these changes and thus preservation of the memory trace itself. Furthermore, feedforward processes such as LTP and LTD have the potential to create positive feedback loops, driving gain to infinity or zero, respectively. Synaptic scaling is thought to act as negative feedback to these processes, therefore providing a cohesive solution to this theoretical problem. Supporting evidence comes from computational models that suggest within networks utilizing Hebbian plasticity, synaptic scaling, in cooperation with other homeostatic mechanisms such as the dynamic regulation of intrinsic excitability (Remme and Wadman, 2012), robustly increases network stability and information storage capacity (Tetzlaff et al., 2011; 2012).

While theoretical and computational models aid our understanding of how synaptic scaling may operate within in vivo circuitry, there is currently a need for evidence demonstrating a direct biological role for synaptic scaling in learning and memory. However, indirect evidence comes from studies in hippocampal slice cultures. These studies demonstrate that scaling, alongside other homeostatic adaptations, occurs within the intact hippocampal circuitry, setting it within proper anatomical context to participate in memory formation, consolidation, and storage. For example, in response to chronic inactivity, throughput circuits such as dentate gyrus (DG)-CA3 and CA3-CA1 scale up post-synaptic excitatory strength, while in the recurrent CA3-CA3 circuit, excitatory strength scales down (Kim and Tsien, 2008). Even within the CA3 region itself,

homeostatic adaptions to inactivity modulate connectivity and synaptic strength in a complex manner, whereby certain contacts between pairs of pyramidal neurons are strengthened while others are silenced (Mitra et al., 2012). An interesting hypothesis put forward by Kim and Tsien (2008) is that homeostatic adaptions within the hippocampal circuit, including synaptic scaling, are necessary for maintaining proper directionality of information flow while concomitantly keeping potentially destabilizing reverberations in check.

4.2 Transcriptional and Epigenetic Regulation

Even as the studies discussed in the previous section move us closer to understanding the biological roles synaptic scaling may play within the context of learning and memory, the precise molecular mechanisms underlying its induction, maintenance, and expression are only beginning to be understood. For example, scaling up and down are not simply regulated in an inverse manner, but are mediated by non-overlapping molecular pathways (Pozo and Goda, 2010; Seeburg et al., 2008; Siddoway et al., 2013; Sun and Turrigiano, 2011). However, with rare exceptions (Aoto et al., 2008; Soden and Chen, 2010; Wang et al., 2011), the coordinated, cell-wide expression of synaptic scaling suggests an equally coordinated and integral role for transcriptional regulation. Indeed, the induction of bidirectional scaling across a range of modalities employed to manipulate activity can be inhibited by the transcription inhibitor, actinomycin-D (Goold and Nicoll, 2010; Han and Stevens, 2009; Ibata et al., 2008; Seeburg et al., 2008). In this section, we will examine salient studies that are beginning to dissect the transcriptional mechanisms at play, with a focused discussion of their place within the context of transcriptional

regulation of synaptic plasticity in general. Furthermore, as epigenetic mechanisms are now being recognized as key regulators of gene expression and neuronal function in general, we will discuss how synaptic scaling may be driven through epigenetic modifications and suggest areas for further investigation.

As opposed to Hebbian plasticity, where coordinating pre-synaptic input with post-synaptic depolarization drives changes in synaptic strength, synaptic scaling occurs in a cell-autonomous manner. This is important as it suggests the initial wave of transcriptional changes are mediated not via cell-to-cell signaling, but that a given neuron adjusts its own synaptic weights by responding to fluctuations in Ca2+ entry through voltage-gated channels. Of particular interest here are those studies demonstrating a role for CaMKIV in regulating synaptic scaling in response to both increased (Goold and Nicoll, 2010) and decreased (Ibata et al., 2008) activity. CaMKIV activity itself is regulated via changes in Ca2+ flux through L-type voltage-gated Ca2+ channels (LTCCs) (see Figure 2) (Deisseroth et al., 1998); increased Ca2+ entry activated CaMKIV and led to downscaling, whereas decreased Ca2+ entry decreased CaMKIV activity and caused upscaling (Goold and Nicoll, 2010; Ibata et al., 2008). CaMKIV-regulated gene expression has been well-described in the context of Hebbian plasticity (Kang et al., 2001); however, besides a requirement for its activity, very little else is known about its role in synaptic scaling or how it interacts with other signaling events to regulate transcription.

How do prolonged changes in CaMKIV activity lead to scaling of excitatory synaptic weights? When activity is increased acutely, a rise in intracellular Ca2+ through LTCCs leads to nuclear translocation of Ca2+/calmodulin and increased CaMKIV acitivity (Deisseroth et al., 1998). Active CaMKIV may phosphorylate CREB and CBP, increasing

CREB/CBP-dependent gene transcription and leading to histone acetylation via CBP's HAT activity (Deisseroth et al., 1998; Vo and Goodman, 2001). There is a clear role for CREB/CBP-dependent transcription in promoting memory (Korzus et al., 2004; Silva et al., 1998; Tully et al., 2003). CBP+/- mice exhibit disrupted chromatin acetylation, impaired memory, and decreased Hebbian plasticity (Alarcon et al., 2004). The memory promoting effects of the broad-spectrum HDACi, TSA, have been attributed to specifically promoting CREB/CBP-dependent transcription (Levenson et al., 2004b; Vecsey et al., 2007). However, there is no established role for these players in synaptic scaling. Experiments to determine if neuronal cultures from CBP+/- mice have deficits in synaptic scaling and if these deficits may be rescued with HDAC inhibitors may provide an interesting initial approach. Furthermore, in light of the memory- and LTP-promoting effects of activated CaMKIV, it is interesting to note that the scaling down of excitatory synaptic weights is driven by a chronic increase in CaMKIV activity (Goold and Nicoll, 2010). Clearly, time course effects are playing an important role and need to be worked out in future experiments. The findings suggest CaMKIV signaling within an initial time window promotes the potentiation of synaptic strength, as in LTP, but if activity remains continually elevated, CaMKIV signaling promotes scaling down. As synaptic scaling is proposed to provide negative feedback to positive feedback processes such as LTP, this order of events fits the role of scaling processes within the current learning and memory model.

In addition to elucidating the mechanisms downstream of CaMKIV activity, investigations into the effect of mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK)-mediated signaling and its interactions with CaMKIV will

likely provide further insight into the transcriptional and epigenetic regulation of synaptic scaling. The prolonged time course to induce scaling fits with a time frame involving an influential role for MAPK/ERK (Wu et al., 2001). Although there is overwhelming evidence demonstrating a clear role for MAPK/ERK-signaling in mediating the long-term changes in gene expression and epigenetic modifications necessary for Hebbian plasticity and learning and memory (Chwang et al., 2006; Impey et al., 1998; Levenson et al., 2004b; Roberson et al., 1999), evidence for this pathway in synaptic scaling is scant. Ca2+/calmodulin-stimulated AC activity and the subsequent activation of PKA may provide an exciting focal point for initial studies, especially as this pathway has been shown to be critical for the nuclear translocation of ERK, the induction of CREB-dependent transcription, and the induction of histone PTMs (Chwang et al., 2006; Ferguson and Storm, 2004; Impey et al., 1998; Levenson et al., 2004b; Wang and Zhang, 2012). Indeed, the activation of AC1 via Ca2+ entry through LTCCs has been implicated in synaptic scaling (Gong et al., 2007). Furthermore, a recent study using a kinase-dead knock-in mutation of mitogen- and stress-activated protein kinase-1 (MSK1), a component of the MAPK/ERK pathway critical for mediating histone H3 and CREB Ser133 phosphorylation (Arthur et al., 2004; Soloaga et al., 2003), showed its kinase activity to be necessary for the scaling up of excitatory strength in response to activity deprivation (Corrêa et al., 2012). However, one caveat is that although MSK1 can be activated through the activity of Ca2+ -stimulated ACs (Sindreu et al., 2007), the authors of this study focused on its activation via BDNF signaling (see below for further discussion).

Although somatic Ca2+ fluctuations are currently thought to serve as the initial induction locus, leading to the first wave of signaling pathways impinging on the nucleus

to regulate the transcriptional and epigenetic programs necessary for the expression of synaptic scaling, the release of the neurotrophin BDNF has been implicated as well (see Figure 2). As one of the most widely studied and influential regulators of synaptic transmission (Elmariah et al., 2004; Marty et al., 2000; Nelson et al., 2008), plasticity (Bramham and Messaoudi, 2005; Figurov et al., 1996), and behavior (Lubin et al., 2008; Mizuno et al., 2012; Rattiner et al., 2005), it is no surprise that BDNF regulates synaptic scaling. However, BDNF operates in an incredibly complex manner, and its precise role in the context of synaptic scaling is unclear. In networks homeostatically adapting to inactivity, BDNF signaling can produce state-dependent, pre-synaptic effects (Jakawich et al., 2010; Lindskog et al., 2010), and its effects on synaptic scaling are cell-type specific (pyramidal vs. interneuron; (Rutherford et al., 1998; Wenner, 2011)) and receptor-specific (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA) vs. γ-Aminobutyric acid ligand gated receptors (GABAA)); (Bolton et al., 2000; Peng et al., 2010; Swanwick et al., 2006)). Further complicating the story, BDNF may act through transcriptionally-dependent (Calfa et al., 2012; Corrêa et al., 2012) or independent (Fortin et al., 2012; Jakawich et al., 2010) mechanisms.

From a transcriptional and epigenetic standpoint, two broad questions remain regarding BDNF and synaptic scaling. First, how do the chronic, cell-autonomous changes in somatic Ca2+ entry required to induce synaptic scaling affect the transcription of the Bdnf gene, and are these changes mediated via epigenetic modifications? Indeed, studies have found that prolonged elevations in AP firing lead to increased Bdnf expression mediated via decreased promoter methylation (Nelson et al., 2008). Chronic inhibition of DNMT activity seemed to mimic this decrease in methylation (Nelson et al., 2008), a

finding especially relevant in light of evidence showing DNMT inhibition in vivo also leads to promoter demethylation and altered expression of the Bdnf gene (Lubin et al., 2008). In both cases, it was argued these changes were regulated through NMDA receptor-mediated signaling, leaving the role of prolonged changes in somatic Ca2+ undefined. Yet, there is likely a capacity for chronic changes in somatic Ca2+ to mediate epigenetic changes at the *Bdnf* gene as acute, strongly depolarizing stimuli can lead to changes in *Bdnf* promoter methylation, and these changes are partly mediated through the Ca2+-dependent phosphorylation and unbinding of a repressive MeCP2 complex (Chen et al., 2003a; Martinowich et al., 2003). Although there is an abundance of evidence for the transcriptional regulation of *Bdnf* by MeCP2 (for review see Li and Pozzo-Miller, 2013), their relationship in the context of synaptic scaling is unclear. Interestingly, MeCP2 itself has been shown to be necessary to scale up (Blackman et al., 2012) and scale down (Qiu et al., 2012) post-synaptic strength, and it does so in a cell-autonomous manner (Blackman et al., 2012). Furthermore, as the *Bdnf* gene is known to contain multiple promoter regions (Aid et al., 2007; Liu et al., 2006; Timmusk et al., 1993), and the methylation status of these regions may be specifically regulated by Hebbian plasticity (Sui et al., 2012) and learning (Lubin et al., 2008; Mizuno et al., 2012), it may be helpful to determine the promoter-specific methylation changes induced during synaptic scaling.

The second broad question: how does BDNF signaling interact with the Ca2+-mediated pathways discussed above to further modify the epigenetic landscape during synaptic scaling? Clearly, this is a complex question, especially given the heterogeneous data regarding BDNF's effects on scaling processes. However, there are certainly targets to be investigated. For instance, we should continue to dissect the interaction between

BDNF-mediated ERK signaling and Ca2+/calmodulin-mediated signaling. As both BDNF signaling and Ca2+/calmodulin-stimulated AC are known to activate MSK1 (Alonso et al., 2004; Arthur et al., 2004; Corrêa et al., 2012; Sindreu et al., 2007; Soloaga et al., 2003), it is particularly interesting to consider how these pathways converge on this kinase and cooperate with CaMKIV to control CREB/CBP-dependent transcription and PTMs of histones. Moreover, how might these pathways antagonize each other? For example, how is synaptic scaling influenced by the competition of HAT/HDAC activity? BDNF has been shown to affect quantal neurotransmission via the activity of HDACs (Calfa et al., 2012), and HDACs themselves have been shown to affect neurotransmission in a class specific manner (Akhtar et al., 2009; Hanson et al., 2013; Kim et al., 2012). As synaptic scaling is a modulation of baseline neurotransmission in response to chronic changes in activity, there is likely a role for these mechanisms in its induction, maintenance, or expression. Therefore, it may be beneficial to determine the time course of global histone modifications during synaptic scaling and how these changes are influenced via BDNF/TrkB antagonization or HDAC inhibition.

5. Concluding Remarks

In the last decade, the field of neuroepigenetics has made tremendous progress in recognizing the importance of epigenetic mechanisms in the memory process. It is now evident that, in order to generate a lasting effect on behavior, neuronal circuits must modify their function in a persistent yet flexible manner. Currently the field has focused on examining how individual genes and epigenetic modifications drive these necessary long-lasting changes in neuronal function. However, technological advancements offered by the

"-omics" revolution are making it increasingly possible to understand how entire programs of genes are epigenetically regulated to impact overall neuronal function and behavior. Advances in next-generation sequencing will allow investigators not only to fully characterize genome-wide changes in gene expression associated with a particular learning experience but also to define the accompanying epigenetic modifications regulating these changes. For example, bisulfite sequencing (BS-seq) in combination with oxidative bisulfite sequencing (oxBS-seq) or Tet-assisted bisulfite sequencing (TAB-seq) will allow for single-nucleotide resolution of cytosine methylation and 5-hydroxy-methylation (Booth et al., 2012; Yu et al., 2012). Similarly, chromatin immunoprecipitation followed by sequencing (ChIP-Seq) will allow for large-scale mapping of transcription factor binding and histone PTMs as has recently been done following fear memory acquisition (Park et al., 2013). Characterizing the memory transcriptome and epigenome will undoubtedly further our understanding of the molecular underpinnings of long-term memory, identifying new gene products that can be further targeted and explored.

Additional technological innovations are also making it increasingly possible to determine the precise functional role of individual DNA or histone modifications. Although genetic and pharmacological manipulations of epigenetic machinery have revealed the necessity of epigenetic mechanisms in learning and memory, dissecting the causal role of individual modifications has been challenging due to the correlative nature of existing chromatin-based approaches. Utilization of customizable zinc-finger arrays and transcription activator–like effector (TALE) proteins will allow investigators to activate or repress specific genes (Joung and Sander, 2013), to catalyze locus-specific DNA demethylation (Maeder et al., 2013) and to direct the addition or removal of specific histone

modifications (Konermann et al., 2013; Mendenhall et al., 2013). These systems are further amenable to optogenetic modulation allowing for sophisticated manipulation of the epigenome in an inducible and reversible manner (Konermann et al., 2013).

However, as with all new technological developments, the newly acquired information will be hard to make sense of if we are unable to extract functional relevance from the data as it relates to neuronal function and plasticity. To obtain a better understanding of what these genome-wide expression and epigenetic changes mean for neuronal plasticity and behavior overall, two goals should be undertaken: (1) understanding how different forms of plasticity (Hebbian vs. non-Hebbian) are epigenetically regulated on an individual level, and (2) understanding how these forms of plasticity interact and modulate each other at level of the epigenome.

In this review, we hope to have broadened the functional relevance of epigenetic mechanisms to include regulation of both Hebbian and non-Hebbian forms of plasticity. In doing so, we provide a series of experimental starting points that will hopefully spur further exploration of these topics. Interestingly, our examination revealed these distinct plasticities rely on overlapping induction mechanisms like intracellular Ca2+ signaling and subsequent activation of several second-messenger pathways (see Figure 2). It is possible that once the upstream initiating signal is propagated to the nucleus, the epigenome acts as a point of convergence and divergence integrating upstream signals into a particular epigenetic and transcriptional signature that is plasticity-specific (e.g., LTP or synaptic scaling). Understanding how these different forms of plasticity are epigenetically regulated on an individual level will help us compartmentalize network topologies when analyzing whole-genome studies. Therefore, when examining learning-induced genome-wide

changes in expression and epigenetic modifications, there will be a subset of changes that are inherently mnemonic in function. However, as previously mentioned there will be concurrent changes underlying other forms of plasticity (e.g., intrinsic plasticity and synaptic scaling) that may not constitute part of the molecular memory engram but are nonetheless critical to the overall memory process.

This latter point is becoming increasingly salient considering these different forms of plasticity are able to interact with one another in a metaplastic manner. Both intrinsic plasticity and synaptic scaling are able to modulate LTP (Arendt et al., 2013; Chen et al., 2006; Cohen and Abraham, 1996; Cohen et al., 1999; Kramár et al., 2004; Roth-Alpermann et al., 2006; Sah and Bekkers, 1996; Thiagarajan et al., 2007). These plasticities also interact at the level of the behaving animal (Lambo and Turrigiano, 2013; Maffei and Turrigiano, 2008; Nataraj et al., 2010; Saar et al., 1998; Zelcer et al., 2006). These observations are interesting given that HDAC inhibitors are able to facilitate both LTP induction and learning (Fass et al., 2013; Levenson et al., 2004b; Miller et al., 2008; Stafford et al., 2012; Sui et al., 2012; Vecsey et al., 2007; Yeh et al., 2004; Zeng et al., 2011), suggesting the underlying mechanism of action for these drugs may involve alterations in intrinsic and homeostastic processes as well as synapse-specific changes. Furthermore, intrinsic plasticity and homeostatic plasticity are intimately tied as chronic changes in neuronal activity can dynamically regulate intrinsic excitability thereby modulating the whole neuron's response to incoming stimuli (Desai et al., 1999). CREB-mediated changes in intrinsic excitability have also been shown to influence which neurons get recruited to a given memory trace (Han et al., 2007; Kim et al., 2013; Zhou et al., 2009), a process termed memory allocation (Silva et al., 2009; Won and Silva, 2008). Although it

is evident these different forms of plasticity interact at the level of the cell membrane to influence behavior, it is conceivable they also interact at the level of epigenome where a given neuron's epigenetic "state" may ultimately dictate a neuron's cellular "state" via the simultaneous regulation of Hebbian and non-Hebbian plasticity. In such a case, the epigenome would be a prime candidate for metaplasticity at large, which is an idea recently put forth in the literature (Baker-Andresen et al., 2013b).

As the field of neuroepigenetics expands into exciting and new territories, these topics amongst others will need to be addressed to obtain a comprehensive understanding of the global transcriptional and epigenetic changes necessary for long-term behavioral memory. Using the previously outlined ideas as foundational points, we propose the following simplified operational model to better understand and integrate such changes. A given learning experience drives nuclear change by impinging on a variety of well-conserved signaling cascades. Once at the nucleus, these signaling cascades engage a parallel set of chromatin remodeling processes that produce several overlapping, yet independent, gene expression profiles that ultimately regulate the levels/activity of ion channels, receptors, trafficking proteins, and signaling molecules. These cell-wide changes in transcriptional output ultimately modulate long-term behavioral memory by regulating synapse-specific Hebbian plasticity either directly or indirectly. Direct regulation may be accomplished by targeted transport of necessary gene products to tagged synapses, whereas indirect regulation may occur through non-Hebbian cell-wide functional changes. As a result, the combined output of transcriptional and epigenetic mechanisms may serve multifaceted functions including encoding of mnemonic information, adaptation to plasticity-inducing experiences, and subsequent modulation of future plasticity.

Research Highlights

- Hebbian and non-Hebbian forms of plasticity are critical to the memory process.
- We review the data supporting epigenetic mechanisms in Hebbian plasticity (LTP).
- Non-hebbian plasticity includes intrinsic plasticity and synaptic scaling.
- Transcriptional and epigenetic regulation of non-Hebbian plasticity is examined

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Abbreviations

AC	adenylyl cyclase
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AP	action potential
BDNF	brain-derived neurotrophic factor
BLA	basolateral amygdala
CA	cornus ammonis

CaMKII/IV	Ca2+/calmodulin-dependent protein kinase II/IV
CBP	CREB binding protein
CREB	cAMP/Ca2+-response element binding protein
CNS	central nervous system
cAMP	cyclic adenosine monophosphate
cGMP	cyclic guanosine monophosphate
DG	dentate gyrus
ERK	extracellular-signal regulated kinase
GABAA	γ-Aminobutyric acid ligand gated receptors
H2S	hydrogen sulfide
HATs	histone acetyltransferases
HCN	hyperpolarization-activated cyclic nucleotide-gated
HDAC	histone deacetylase
HDACi	HDAC inhibitor
DRG	dorsal root ganglion
LA	lateral amygdala
LTCCs	L-type voltage-gated Ca2+ channels
MAPK	mitogen-activated protein kinase
MeCP2	methyl CpG binding protein 2
MSK1	mitogen- and stress-activated protein kinase-1
MSN	medium spiny neurons
NMDA	N-Methyl-D-Aspartate
NRSE	neuron-restrictive silencer elements

NRSF	neuron-restrictive silencing factor
NA	nucleus accumbens
PKA	cAMP-dependent protein kinase
PKC	protein kinase C
PKG	cGMP-dependent protein kinase
PTMs	post-translational modifications
RE1	repressor element 1
REST	RE1 silencing transcription factor
TSA	trichostatin A

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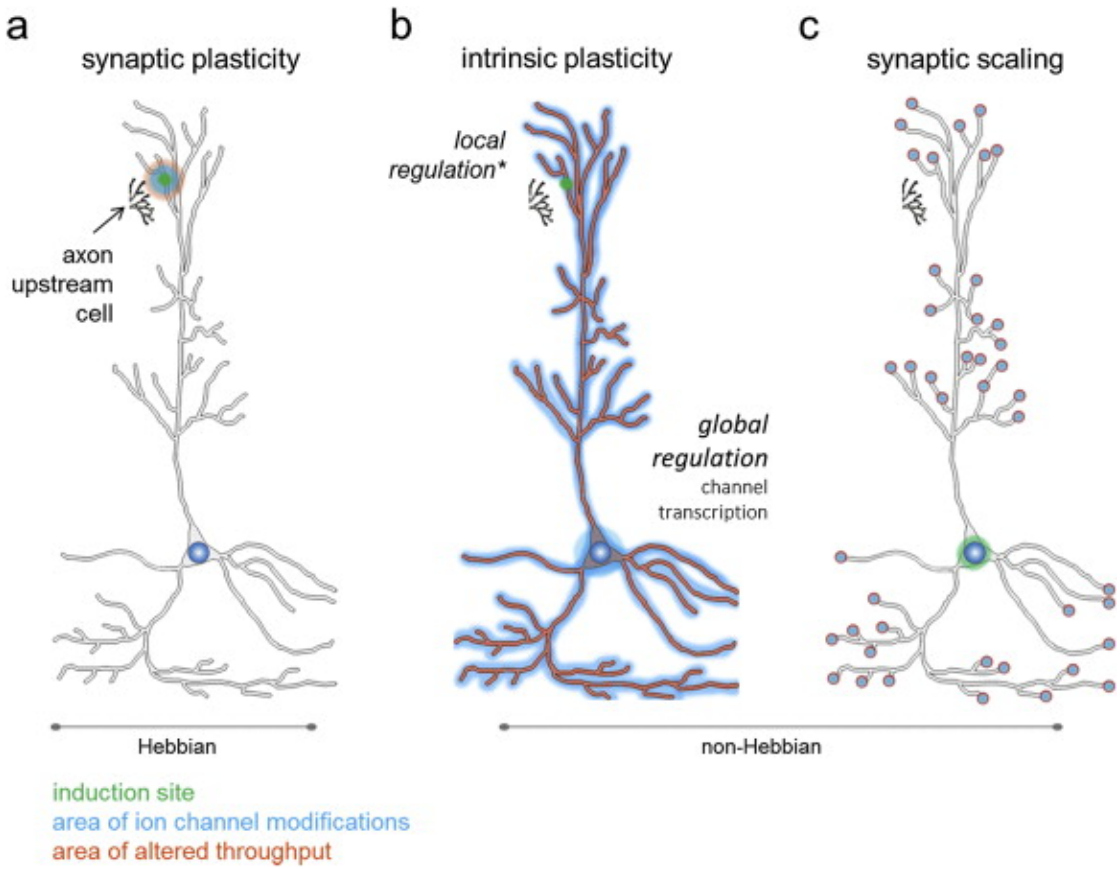
Figure 1


a
synaptic plasticity
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regulation*
global
regulation
channel
transcription
non-Hebbian
c
synaptic scaling
induction site
area of ion channel modifications
area of altered throughput

Figure 1. Induction and Expression Sites for Hebbian and non-Hebbian Forms of Plasticity. Hebbian plasticity involves the modulation of synaptic efficacy due to precise coordination of pre- and post-synaptic activity. In contrast, non-Hebbian forms of plasticity are not dependent on coincident activity. Hebbian and non-Hebbian plasticities are induced and expressed differently, suggesting each possesses specific functions in the memory process. (a) The modulation of synaptic efficacy in Hebbian plasticity is synapse-specific; as a result, the sites of induction (green) and expression (blue) are co-localized. Classically, expression is thought to occur post-synaptically via trafficking of ligand-gated ionotropic receptors (e.g., AMPARs), although there is evidence for the involvement of presynaptic modifications. The throughput (red), or the ability of synaptic activity to elicit an action potential, is altered only at those synapses expressing changes in synaptic efficacy. (b) Intrinsic plasticity is a form of non-Hebbian plasticity where modulation of voltage- and calcium-gated ion channels regulates synaptic integration and action potential generation. There is evidence that changes in intrinsic plasticity can be induced by local synaptic activity (as shown) as well as global changes in action potential firing. Similarly, intrinsic plasticity can be expressed locally (restricted to a subset of distal dendrites) or globally (as shown; involving broader changes along the dendritic tree and/or the axo-somatic membrane). In the setting of global changes, there is potential for throughput of all synapses to be altered. (c) Synaptic scaling is a form of non-Hebbian plasticity involving the multiplicative modification of postsynaptic ligand-gated ionotropic receptor (e.g., AMPAR) density across all synapses. Such changes occur in response to a given neuron sensing chronic alterations in its own firing rate through variations in Ca2+ influx at the soma. Since modifications occur at all synapses the throughput of all synapses are changed. However, relative weights of preexisting synaptic changes are maintained since scaling occurs in a multiplicative fashion. Adapted from Zhang and Linden (2003).

Figure 2

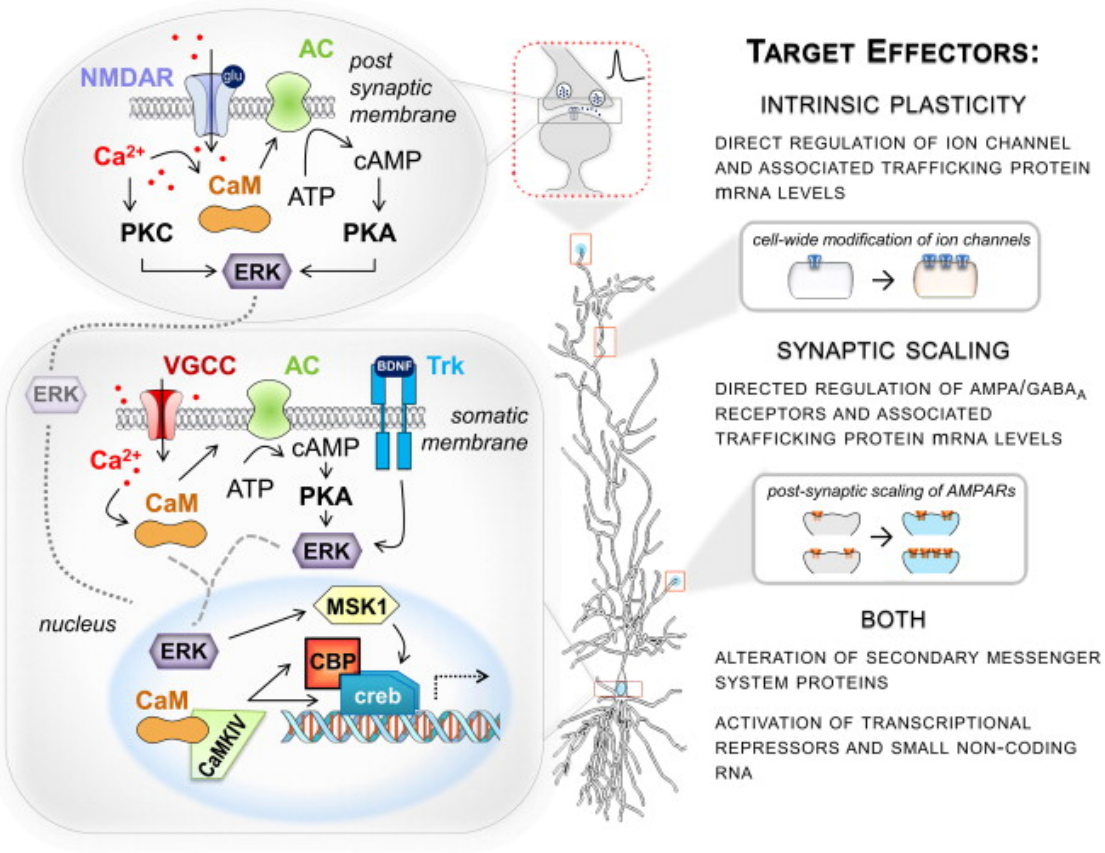
NMDAR
glu
AC
post synaptic membrane
Ca2+
CaM
ATP
cAMP
PKC
PKA
ERK
VGCC
AC
BDNF
Trk
ERK
somatic membrane
Ca2+
cAMP
ATP
CaM
PKA
ERK
nucleus
MSK1
ERK
CBP
creb
CaM
CaMKIV
TARGET EFFECTORS:
INTRINSIC PLASTICITY
DIRECT REGULATION OF ION CHANNEL AND ASSOCIATED TRAFFICKING PROTEIN mRNA LEVELS
cell-wide modification of ion channels
SYNAPTIC SCALING
DIRECTED REGULATION OF AMPA/GABAA RECEPTORS AND ASSOCIATED TRAFFICKING PROTEIN mRNA LEVELS
post-synaptic scaling of AMPARs
BOTH
ALTERATION OF SECONDARY MESSENGER SYSTEM PROTEINS
ACTIVATION OF TRANSCRIPTIONAL REPRESSORS AND SMALL NON-CODING RNA

Figure 2. Potential Shared Molecular Mechanisms of Transcriptional Regulation between Intrinsic Plasticity and Synaptic Scaling. Although clear distinctions exist in the induction and expression of intrinsic plasticity (IP) and synaptic scaling (SS), accumulating evidence suggests both plasticities rely on conserved molecular mechanisms also known to be involved in the long-term changes in gene expression necessary for Hebbian plasticity. Here we present a simplified model intended to demonstrate likely points of molecular convergence between IP and SS that require further experimental confirmation and elucidation. Despite differences in induction site (synaptic as in IP and somatic as in SS), there is a clear role for transcriptional regulation via Ca2+-mediated signaling. Ca2+ entry either through synaptic NMDA receptors or somatic voltage-gated calcium channels (VGGCs) directly and/or indirectly activates protein kinases like protein kinase C (PKC) and cAMP-dependent protein kinase (PKA) which converge on extracellular receptor kinase (ERK) and lead to its nuclear translocation. In SS, brain-derived neurotrophic factor (BDNF) binding of TrkB receptors likely serves as a level of higher-order control in the regulation of ERK nuclear translocation. Nuclear ERK may engage cAMP/Ca2+-response element binding protein (CREB)-mediated gene transcription through activation of downstream kinases such as mitogen- and stress-activated protein-kinase 1 (MSK1). Additionally, nuclear translocation of Ca2+/calmodulin (CaM) regulates Ca2+/calmodulin-dependent kinase IV (CaMKIV) activity, a key mediator of both CREB and CREB-binding protein (CBP) phosphorylation and activation. Furthermore, it is likely both IP and SS engage transcriptional repressors and small non-coding RNAs along with transcriptional activators like CREB. Coordinated expression of specific ion channels/receptors, associated trafficking proteins, and secondary messenger proteins will dictate how each form of plasticity manifests at the level of the cell membrane.

GENOME-WIDE TRANSCRIPTION AND DNA METHYLATION PROFILING IN AN APP MOUSE MODEL OF ALZHEIMER'S DISEASE

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Abstract

Neuronal plasticity and long-term memory rely on dynamic, bidirectional regulation of transcription. Recent evidence suggests that dysregulated transcription and epigenetic mechanisms, like histone modifications and DNA cytosine methylation, may contribute to neuronal dysfunction and cognitive impairment in Alzheimer's disease (AD). Previous research in human patients and AD mouse models have utilized gene-candidate and microarray-based methods to reveal aberrant cytosine methylation and transcription of genes involved in cell signaling, inflammation, and neurotransmission. However, a systematic characterization of genome-wide alterations is lacking. To address this we utilized next-generation sequencing technologies to study transcriptome and methylome plasticity in the dentate gyrus of naïve hAPP(J20) mice, an amyloid-beta (Aβ) over-expressing mouse model of AD. RNA-seq analysis revealed widespread bidirectional alterations in gene expression with upregulated genes involved in steroid biosynthesis and extracellular matrix restructuring and downregulated transcripts implicated in ion channel activity, transcriptional regulation, chromatin binding and calcium signaling. In contrast, MBD-seq analysis revealed surprisingly few alterations in CpG dimethylation. However, the few differentially methylated genes were predominantly transcription factors whose hypermethylation status was associated with decreased transcript levels suggesting that these changes might drive the secondary, more widespread transcriptional alterations. Additionally, using a transcriptome meta-analysis, we identified HDAC2 as a conserved therapeutic target and demonstrate that antisense-oligonucleotide mediated knockdown of HDAC2 improves AD-related cognitive impairment. Together, our data provides a molecular framework by which integrative

transcriptomic and epigenomic analyses can be used to identify novel therapeutic, plasticity-promoting gene targets that may ameliorate AD-related cognitive impairment.

Introduction

Epigenetic modifications, including post-translational modifications of histones and DNA methylation, have emerged as critical regulators of neuronal gene expression. In the absence of pathology, changes in epigenetic modifications are necessary for long-term behavioral memory and neuronal plasticity, implicating their dysregulation in Alzheimer's disease (AD)-related transcriptional alterations, neuronal dysfunction, and cognitive decline (*1, 2*). Indeed, substantial evidence from human patients and AD mouse models supports the hypothesis of amyloid-beta (Aβ)-induced transcriptional and epigenetic dysfunction in AD-related cognitive impairment. Transcriptionally, Aβ deposition is associated with aberrant hippocampal expression of genes involved in cell signaling, metabolism, and inflammation (*3-7*). As expected, genes related to neurotransmission, synaptic plasticity, and memory formation are also disrupted (*3, 5, 6, 8, 9*). Epigenetically, work in AD mouse models implicate histone hypoacetylation as well as increased levels/activity of histone deacetylase (HDAC) enzymes as critical players in Aβ pathobiology (*8, 10, 11*). Interestingly, HDAC inhibitors have emerged as promising therapeutic options, ameliorating deficits in gene expression, synaptic plasticity and long-term memory in a variety of AD mouse models (*3, 8, 10-17*).

In contrast to histone acetylation, the role of DNA methylation in AD is less understood. DNA methylation involves the 5' modification of cytosine nucleotides by DNA methyltransferase (DNMT) enzymes. Methylation can repress gene expression by

directly interfering with binding of transcription factors to regulatory elements or increase expression by blocking insulators or repressors (*18*). Additionally, binding of 5-methylcytosine by proteins with methyl binding domains can recruit other chromatin-remodeling enzymes, allowing for complex regulation of gene transcription (*19, 20*). Currently, the majority of studies have investigated DNA methylation in post-mortem tissue from AD patients (*21*). Generally speaking, AD patient brains are characterized by locus-specific hypermethylation (*22*). In contrast, relatively few studies have used AD mouse models to isolate Aβ-induced alterations in DNA methylation, often relying on candidate gene or microarray-based approaches (*23-25*). Although promising, these studies are limited in scope, as they exclusively focus on promoter methylation and examinations of the associated transcriptional changes are restricted to a small set of candidate genes.

Therefore, in the present study we utilize next-generation sequencing technology to systematically characterize genome-wide alterations in gene expression and DNA methylation in the dentate gyrus of hAPP(J20) mice, an Aβ over-expressing mouse model of AD. We also perform integrative transcriptomic analysis, incorporating recently published RNA-sequencing data from two additional AD mouse models, to create an improved map of hippocampal transcriptional signatures and networks in response to Aβ deposition. In doing so, we identify HDAC2 as a conserved therapeutic target and demonstrate the use of an antisense-oligonucleotide based interventions as potential treatments of AD-related cognitive impairment.

Results

Differential gene expression analysis of hAPP(J20) vs non-transgenic mice

The current cost of sequencing limits the experimental sample size traditionally used for rodent-based biochemical studies, which is additionally problematic in cases of high inter-animal variability as observed with AD mouse models including hAPP(J20) mice (Figure 2B-C). To circumvent this issue, we used a "selective genotyping" (*26*) approach in which mice displaying behavioral abnormalities predictive of subsequent memory impairment (*i.e*. a behavioral biomarker) were targeted for analysis. To do so, mice were screened using the open and elevated plus maze behavioral paradigms as mice with observed hyperactivity and increased open-arm time on these tasks are more likely to display greater cellular and molecular pathology as well as more severer spatial memory deficits (*27, 28*). Based on performance in the open field and elevated plus maze, we then selected the most behaviorally impaired hAPPJ20 mice (n=6) for subsequent sequencing analysis (Figure 1C). For controls (n=6), we used age- and strain-matched non-transgenic (NTG) mice with a behavioral performance closest to the median of the population. As an additional layer of screening prior to transcriptomic analysis, we confirmed previously documented transcriptional changes in *Fos*, *Egr1*, and *Penk*, whose altered expression levels are known to correlate well with memory-impairments in hAPP(J20) mice (Figure 1D) (*27, 29, 30*).

We performed poly(A)+ RNA sequencing on hippocampal dentate gyrus (DG) tissue from naïve hAPP(J20) and NTG mice. Using DESeq2 analysis (*31*) we identified widespread, bidirectional transcriptional alterations, with 959 upregulated genes and 1003 downregulated genes (false discovery rate (FDR)= 0.1) (Figure 1A and

Supplementary Table 1-2). The replicates for the hAPP(J20) mice clustered together and revealed greater uniformity than the NTG mice, indicating that our selective genotyping approach enriched for a homogenous population (Figure 1A and Supplementary Figure 1). Of note, differentially expressed genes (DEGs) in hAPP(J20) mice were significantly enriched with genome wide association-identified AD-risk genes containing single-nucleotide polymorphisms (*32, 33*) as well as genes with associated DNA methylation changes (*34*) (Figure 1B). Additionally, we observed significant overlap with hippocampal CA1 DEGs identified between AD patients and age-matched, non-demented controls (*4*) (Figure 1B). In line with our previous qRT-PCR findings, *Fos*, *Egr1*, and *Penk* were also identified as differentially expressed via RNA-seq (Figure 1B-C) Together, these findings, confirms the reliability of our methods and corroborates the biological relevance and external validity of the hAPP(J20) mouse model to study the pathogenesis and/or treatment of human AD.

To further understand the functional consequence of these transcriptional alterations, we performed gene set enrichment analysis. Amyloid-associated decreases in gene expression were linked to biochemical pathways canonically associated with neuronal plasticity and long-term behavioral memory, including the MAPK signaling pathway, regulation of actin cytoskeleton, long-term potentiation, calcium signaling, and neuro-active ligand-receptor interactions (Figure 1D, Supplementary Figure 2, Supplementary Tables 3-9). Consistent with the hypothesis of nuclear dysfunction in AD, downregulated genes were enriched for transcription factor activity, DNA binding, and chromatin binding (Figure 1D). Indeed, this family of DEGs encompassed numerous immediate-early genes (IEGs), including *Fos*, *Egr1*, *Egr2*, *Egr3*, *Egr4*, *Junb*, *Nr4a1*, and

Npas4, which have a well-documented role in neuronal plasticity associated with learning and memory (*35, 36*). Additionally, enrichment for genes involved in poly(A) RNA binding and the spliceosome pathway were intriguing considering emerging data implicating abnormal splicing as a mediator of age- and amyloid-associated cognitive impairment (*3, 37*). Closer examination of chromatin binding genes revealed broad involvement of epigenetic enzymes (Supplementary Figure 3), notably DNA demethylases (*e.g. Tet3*), histone deacetylases (*e.g. Hdac1*), histone acetyltransferases (*e.g. Kat6a* and *Kat6b*), histone methyltransferases (*e.g. Kmt2e*, *Setd8*, *Suv39H2*, *Prdm2*, *Prmt8*, and *Ash1l*), and histone demethylases (*e.g. Kdm4c*, *Kdm7a*). Furthermore, integration of downregulated genes with a library of protein-protein interactions (*38-40*) highlighted *Egr1*, *Fos*, *Tcf4*, *Hdac1*, and *Nova1* as top protein nodes with a high degree of interconnectivity within the list of DEGs (Supplementary Figure 5A). Taken together, these results suggest that the broad gene expression changes observed in hAPP(J20) mice might be in part due to the dysregulation of transcriptional and epigenetic enzymes.

Amongst a variety of differentially expressed voltage- and ligand-gated ion channels, voltage-gated K^+ channels emerged as an enriched group of dysregulated gene products. This family included A-type K^+ (*e.g. Kcnc3* and *Kcnd2*) and delayed-rectifier (*e.g. Kcnnb1* and *Kcnq3*) subfamilies, as well as their associated auxiliary Beta subunits (*e.g. Kcnab2*, *Kcnip2*, and *Kcnip4*), which together are known to be critical regulators of neuronal intrinsic excitability (*41*). Neuronal hyperexcitability plays an important role in the pathogenesis of AD (*42, 43*), a feature replicated in a variety of mouse models including hAPP(J20) mice (*44*). Our results combined with emerging data of transcriptional and epigenetic regulation of intrinsic excitability (*45*) provides a

transcriptional context for the hyperexcitability and network dysfunction observed in AD. In contrast, genes upregulated in hAPP(J20) mice were linked to extracellular matrix (ECM) binding, steroid biosynthesis, growth-factor binding, focal adhesion, and immune function (Figure 1D, Supplemental Figure 4, Supplemental Tables 10-15). Notably, a number of genes categorized as ECM constituents comprised a variety of collagen proteins including *Col1a1*, *Col3a1*, *Col4a1*, *Col5a2*, *Col6a1*, and *Col13a1*. Associated changes in collagen have previously been documented both in AD mouse models as well as human AD disease tissue (*46-48*), with specific collagen alterations exhibiting both a negative (*48, 49*) and positive role (*46, 50*) in AD pathogenesis. The enrichment of both steroid biosynthesis and immune function was especially interesting considering that, within AD and Parkinson's disease (PD), both of these pathways have been highlighted in recent pathway-based analyses of GWA-implicated genetic loci (*51-53*). In regards to the immune system, the hAPP(J20) transcriptional profile suggests a predominant involvement of the innate immunity including genes involved in the classical complement system (*e.g. C1qa*, *C1qb*, *C1qc*, *C1ra*, *C2*, *C4b*), chemokine/cytokine signaling (*e.g. Ccr2*, *Tgfbr2*, *Smad2*, *Cxcl5*, *Cxcl10*) and microglial/monocyte activation (*e.g. Tlr4*, *Cd68*). Indeed, a recent report found that upregulation of complement and microglial activation mediates early synapse loss, prior to overt plaque deposition, in hAPP(J20) mice (*54*). A network analysis of protein-protein interactions between and within the list of upregulated genes identified top nodes related to amyloid trafficking (*e.g. App*, *Grb2*), insulin signaling (*e.g. Pik3r1*), immune function (*e.g. Tlr4*, *Smad2*, *Kit*) (Supplementary Figure 5B). Surprisingly, as observed with amyloid-associated downregulated genes, upregulated genes were also comprised of

numerous voltage- and ligand-gated ion channels, including those involved in glutamatergic signaling (*e.g. Grin2d*, *Grik1*, *Grik4*) and serotonergic signaling (*e.g. Htr2a*, *Htr3a*, *Htr5a*, *Htr7*) as well as voltage-gated K^+ channels (*e.g. Kcnc2*, *Kcnd3*, *Kcnq5*) and hyperpolarization-activated cyclic nucleotide-gated (HCN) channels (*e.g. Hcn3*). Electrophysiologically, the upregulation of voltage-gated K^+ channels and HCN channels would seem to counter the previously observed downregulation of voltage-gated K^+ channels presumably in an attempt to main network homeostasis (*55, 56*). Together, these observations not only pinpoint ECM binding, steroid biosynthesis, and immune function as key pathways modified by amyloid-deposition but also suggests a heterogeneous gene expression profile consisting of both pathological and potentially compensatory transcriptional responses.

hAPP(J20) DEGs correlate with hippocampal learning-associated transcriptional signatures.

The ability for memory formation relies on long-lasting, experience-dependent adaptations that affect neuronal structure and function (*57, 58*). These persistent changes are dependent on altered transcription and translation of growth factors, ion channels, ligand-gated receptors, structural proteins, and intracellular signaling proteins (*45, 59*). This coordinated orchestration of gene expression involves bidirectional modulation, with activation of positive regulators (*i.e.* memory-promoting genes) and removal of inhibitory constraints *(i.e.* memory-suppressing genes) (*1, 60*). Therefore, one strategy to distinguish which transcriptional alterations in hAPP(J20) mice are pathological (*i.e.* memory-suppressing) from compensatory (*i.e.* memory-promoting) would involve comparing amyloid- and learning-associated transcriptional profiles. To this end, we first

performed RNA-seq on hippocampal tissue from NTG mice trained in contextual fear conditioning, a hippocampus-dependent behavioral task, to generate a learning-associated transcriptional signature. Then we cross-compared hAPP(J20) and learning-associated DEG lists with four pairwise comparisons between the up- and down-regulated genes in each dataset (Figure 3). Given existing data suggesting transcriptional repression of memory-promoting genes in AD models (*3, 5, 6, 8, 9*), we hypothesized the two datasets would exhibit an inverse correlation, with downregulated hAPP(J20) DEGs displaying upregulation following learning, and upregulated hAPP(J20) DEGs displaying downregulation following learning. Surprisingly, the datasets were positively correlated, exhibiting significant overlap between genes that were either upregulated or downregulated in both datasets (Figure 3A-B). Conversely, there was no significant enrichment when comparing genes downregulated in hAPP(J20) mice and upregulated following learning as well as genes upregulated in hAPP(J20) mice and downregulated following learning. Interestingly, the 351 DEGs upregulated in both datasets ($p = 1.08\ e^{-55}$) were enriched for voltage-gated K^+ activity, calcium/calmodulin binding, and Rho guanyl-nucleotide exchange factor activity (Figure 3C), while the 231 DEGs downregulated in both datasets ($p = 7.25\ e^{-115}$) favored ECM-receptor interaction, terpenoid backbone biosynthesis, and focal adhesion (Figure 3D). Although not significantly enriched, we also examined the genes inversely correlated between the two datasets. The 81 DEGs upregulated in hAPP(J20) but downregulated following learning were associated with insulin and neurotrophin signaling pathways (Figure 3E), while the 57 DEGs downregulated in hAPP(J20) but upregulated following learning favored transcription factors, including *Egr1*, *Egr2*, *Egr4*, *Fos*, *Junb*, and *Nr4a1* (Figure 3F).

Together, these results suggest that ~ 30% of the transcriptional alterations in hAPP(J20) mice may in fact serve a compensatory role, preserving circuit plasticity and counterbalancing the downregulation of key memory-associated IEGs, which likely underlie the impaired plasticity and cognitive impairment in hAPP(J20) mice.

Differential DNA methylation analysis of hAPP(J20) vs non-transgenic mice

To determine the potential dysregulation of genome-wide CpG methylation in response to amyloid deposition, another cohort of hAPP(J20) mice underwent behavioral screening to select animals for further sequencing (data not shown). DNA was then extracted from area DG of the hippocampus, fragmented to lengths between 200-300bp by sonication, and incubated with MethylCpG-binding domain protein 2 (MBD2) in order to enrich for CpG dimethylation, but not hemi-methylation or oxidized methylcytosine species (*61*). These CpG methylated DNA fragments (between 6-9% of all fragments) were then sequenced and mapped to the mouse genome. Surprisingly, when we used published methods (*62, 63*) (see methods section) in order to identify specific differentially methylated regions (DMRs), we only identified a single DMR in the 3' untranslated region of the *App* gene. This part of the murine *App* gene shares 85-95% homology with the human *APP* gene likely reflecting increased mapping of the hAPP(J20) transgene to the murine *App* locus. However, an alternative explanation we cannot exclude is the possibility that this reflects a compensatory mechanism to reduce *App*/*APP* gene expression.

Given these results, we then divided the genome based on different features of interest (*e.g*. promoters, CpG islands, gene bodies, *etc.*) in order to increase our power of

detection by decreasing the amount of multiple testing correction required. In doing so, we identified 30 differentially methylated genes (DMGs) (Figure 4A and Supplementary Table 18). Interestingly, compared to NTG controls, all these genes exhibited hypermethylation in hAPP(J20) mice and were predominantly enriched for transcription factors (*e.g. Tcf4*, *Gtf2ird1*, *Zhx2*, *Bach2*, *Prox1*, *Nfia*, *Zfp536*, and *Zbtb20*) as well as genes involved in dendritic self-avoidance (*e.g. Dscam*, *Dscaml1*) and regulation of neuron projection (*e.g. Ephb2*, *Sema4d*, *Ptprg*, *Tnik*). When we compared the list of DMGs to the list of previously identified DEGs, we found a significant overrepresentation, with all overlapping genes (except *Ptprg*) exhibiting decreased expression via RNA-seq. This observation is line with the notion of overall gene methylation correlating with decrease gene expression (*64*). Overall, our results suggest a surprising lack of dysregulated CpG dimethylation in hAPP(J20) mice. However, the notable enrichment of transcription factors and neuron-specific genes suggests that these few alterations may play a critical role in amyloid-associated pathophysiology.

Identification of a set of Aβ-responsive genes using RNA-seq meta-analysis

It is widely accepted that AD mouse models exhibit varying phenotypes as it relates to the degree of cognitive impairment, Aβ load, NFTs, gliosis, synapse loss, and neurodegeneration (*65*). More importantly, no model is able to perfectly capture the complexities of human AD pathology. Therefore, in order to better define a robust set of genes associated with AD-related amyloid deposition we accessed three publicly available hippocampal RNA-seq datasets from two recent studies utilizing CK-p25 (*6*) and APP/PS1-21(*3*) model mice and compared them to the hAPP(J20) transcriptome

(Figure 5A). CK-p25 mice, harboring the Cdk5 activator p25 transgene, display DNA damage, aberrant gene expression, and increased Aβ levels at early stages (2 weeks post transgene induction) (*66*), followed by neuronal and synaptic loss and cognitive impairment at late stages (6 weeks post transgene induction) (*67, 68*). In contrast, the APP/PS1-21 mice, harboring the Swedish *APP* (K670M/N671L) and the L166P *PSEN1* mutations, display severe amyloid deposition, phosphorylated tau positive neurites, robust gliosis, and cognitive impairment but with modest neuron loss restricted to the dentate gyrus (*10, 69*).

While each dataset had relative equal proportions of upregulated and downregulated genes, CK-p25 and APP/PS1-21 mice exhibited more DEGs than hAPP(J20) mice, with APP/PS1-21 having four times as many (Figure 5B). Similarly, the distribution of effect sizes was larger for CK-p25 and APP/PS1-21 (Figure 5C). However, this was only evident for upregulated genes, as downregulated genes shared similar distributions across all datasets. Cross-comparison of the upregulated genes (Figure 5D) and downregulated genes (Figure 5E) for all datasets revealed that hAPP(J20) DEGs were significantly enriched for CK-p25 and APP/PS1-21 DEGs. However, the overlap between the two CK-p25 datasets and APP/PS1-21 was markedly higher suggesting that although hAPP(J20) DEGs exhibit significant global similarity with the other AD models, CK-p25 and APP/PS1-21 transcriptional signatures are more similar to each other than they are with hAPP(J20) mice. In line with this interpretation, pairwise correlations based on fold changes across all genes showed inferior correlations between hAPP(J20) mice and the other models (Figure 5E). It should be noted though that the chord diagrams also revealed distinct sets of genes that were differentially shared

across the different AD models. Additionally, ~50% of DEGs in both hAPP(J20) and APP/PS1-21 were unique to their respective dataset whereas only ~20% of CK-p25 DEGs were not shared with any other AD model. Overall, the observations of significant enrichment of all four datasets with one another in spite of individual differences suggest a conserved and Aβ-dependent dysregulated transcriptome.

Given the transcriptional heterogeneity across models, we performed a meta-analysis using Fisher's sum of logs method, which sums the logarithm of the q-values across all studies for a given gene (*70*), and then filtered the corresponding gene list for shared unidirectional alterations. In doing so, we increased our statistical power and defined a more confident list of 3,404 genes (FDR = 0.05) associated with AD-related amyloid deposition (Figure 6A). Indeed, many of the gene ontologies and biochemical pathways dysregulated in hAPP(J20) mice (Figure 1) were enriched for again in the meta-analysis gene list (Figure 6A), with upregulated DEGs primarily being linked to ECM structural components and immune function (Figure 6B) and downregulated DEGs favoring ligand- and voltage-gated ion channels, synaptic plasticity signaling pathways, and chromatin binding (Figure 6C). It should be noted that for several of the categories, especially upregulated DEGs, the overall enrichment ratio and –log p-value was amplified compared to what was observed with hAPP(J20) DEGs alone. A network analysis of protein-protein interactions identified top interconnected nodes (Figure 6D), with upregulated nodes associated with immune function and downregulated nodes including genes with synaptic and nuclear function. Notably, *Tcf4* was again identified as a highly interconnected node and was one of the few genes exhibiting altered methylation status.

Hdac2 knockdown is sufficient to rescue cognitive impairments in hAPP(J20) and APPswe/PS1dE9

Interestingly, our meta-analysis identified *Hdac2* as a top downregulated node, highlighting its potential as a conserved druggable target. Prior work in AD mouse models suggest that inhibition of class I HDACs (which includes HDAC2) as well as HDAC6 ameliorates deficits in histone acetylation, gene expression, synaptic plasticity, and hippocampus-dependent memory (*3, 8, 10-17*). Indeed, treating hAPP(J20) mice sub-chronically with the FDA-approved drug, suberoylanilide hydroxamic acid (SAHA, Vorinostat, Zolinza), was sufficient to rescue spatial-memory deficits in the object location memory (OLM) behavioral task (Supplementary Figure 6). Given the shared sequence homology of Class I HDACs, various research groups have been actively developing isoform-specific HDAC inhibitors with the goal of maximizing procognitive benefits and minimizing undesirable side effects. Although this effort has made progress (*13, 71*), an alternative strategy entails the use of modified anti-sense oligonucleotides (ASOs) to elicit isoform-selective and sustained RNA knockdown. Inherent benefits to an ASO-based approach include a high-degree of brain penetrance as they do not require viral-mediated delivery and can instead be administered intracerebroventricularly or intrathecally for a CNS-restricted distribution (*72, 73*). Additionally, phosphorothiate and 2′-O-methoxyethyl (2′-MOE) modified ASOs are water soluble and show enhanced nuclease resistance (*74, 75*) and prolonged half-life, remaining active in the CNS for months (*72, 73*). Given the ideal therapeutic properties of modified ASOs to potentially treat chronic CNS diseases like AD, we sought to determine whether ASO-mediated

knockdown of HDAC2 was sufficient to rescue AD-related cognitive impairment in hAPP(J20) mice as well as another model, APPswe/PS1dE9.

To this end, an HDAC2-targeting ASO or a scrambled control ASO was administered via intracerebroventricular (ICV) injections into 6-9 month old hAPP(J20) mice and 12-13 month old APPswe/PS1dE9 mice, along with respective age- and strain-matched NTG control mice (Figure 7A). Three weeks following ASO administration, both cohorts of mice underwent a behavioral battery including open field, elevated plus maze, and OLM. Molecular analysis after behavioral testing confirmed HDAC2-ASO treatment significantly decreased HDAC2 protein abundance (Supplementary Figure 7). HDAC2 knockdown did not influence basal activity levels as measured in the open field task (Figure 7B and E). However, compared to control-ASO treated NTG mice, HDAC2-ASO treated hAPP(J20) mice exhibited a significant reduction in the percent time spent in the open arms of the elevated plus maze (Figure 7C). Interestingly, the same effect was seen in APPswe/PS1dE9 mice, albeit also in the corresponding NTG group (Figure 7D). More importantly, in the OLM task HDAC2-ASO treated hAPP(J20) and APPswe/PS1dE9 mice showed significantly improved discrimination for the object placed in the novel location, comparable to levels observed for NTG mice (Figure 7D and E). Notably, the HDAC2 ASO also improved the memory performance for NTG mice, suggesting that the procognitive benefits of ASO-mediated knockdown of HDAC2 are generalizable outside the context of amyloid-deposition. This interpretation is in line with evidence that genetic or embryonic deletion of HDAC2 in NTG mice enhances hippocampus-dependent memory (*76*) and that viral-mediated depletion of HDAC2 in the hippocampal CA1 region of CK-p25 mice is sufficient to rescue memory deficits (*8*).

Together, these results highlight the therapeutic potential of using an ASO-mediated approach for AD-related cognitive enhancement.

Discussion

In this study, our goal was to better understand the role of transcriptional and epigenetic dysregulation in relation to Aβ deposition and AD-related cognitive impairment. Our approach utilized next-generation sequencing to characterize transcriptional and DNA methylation alterations in hAPP(J20) mice, followed by integrative genomic and network analyses to provide better insight to the biological context for our results. In doing so, we produced numerous novel findings that should inform future research on the role of transcriptional and epigenetic mechanisms in AD pathogenesis.

Our findings established profound transcriptional and epigenetic dysregulation associated with Aβ deposition. Our RNA-seq analysis of hAPP(J20) mice revealed broad dysregulation of transcription factors and epigenetic enzymes, some of which have been implicated in AD pathobiology while others have been less studied. The majority of genes identified as differentially methylated by our MBD-Seq analysis, albeit few in total number, were also enriched for transcription factors. Downregulated genes identified in our transcriptome meta-analysis were also enriched of genes linked to chromatin binding function, including those specifically classified as histone demethylases (*e.g*. *Kdm4c*, *Kdm7a*, and *Phf8*). Given that most epigenetic research in the context of AD has focused on histone acetylation and DNA methylation, our results provide a series of new experimental starting points that will hopefully spur further exploration. One of these

starting points includes *transcription factor 4* (*Tcf4*), a member of the basic helix-loop-helix (bHLH) transcription factor family, which is known to interact with histone acetyltransferases (HATs) such a p300 (*77, 78*). *Tcf4* is thought to play a critical role in neurodevelopment and cognition, being associated with both schizophrenia and autism-spectrum intellectual disability (*79, 80*). However, the role of *Tcf4* in AD is largely unexplored. A second starting point includes examining the role of histone methylation. Only a few studies have examined AD-related alterations of histone methylation, focusing on H3K9 methylation in particular and exhibiting contradictory results (*6, 81, 82*). However, emerging data have now implicated histone methylation and the associated enzymes that catalyze the addition and removal of methyl marks in memory formation (*83-88*) as well as brain disorders with cognitive impairment (*89-92*). Finally, a third starting point involves the examination of *neuro-oncological ventral antigen 1* (*Nova1*) and alternative splicing. Although recent studies have documented differential splicing in postmortem brain tissue from AD patients (*7, 93*) and in AD mouse models (*3, 37*), only one study has documented decreased levels of NOVA1 protein level in postmortem AD tissue (*94*). Interestingly, NOVA1 co-regulates alternative splicing of RNAs encoding synaptic proteins many of which are involved in excitatory and inhibitory synaptic signaling (*95*).

Our results also implicate a degree of transcriptional compensation in hAPP(J20) mice as reflected by the positive correlation of hAPP(J20) DEGs and learning-associated DEGs. However, we cannot exclude the possibility that the hAPP(J20) transcriptional signature may also reflect a transcriptional output of an overall hyperexcitable hippocampal network. As previously mentioned, neuronal hyperexcitability plays an

important role in the pathogenesis of AD (*42, 43*). Interestingly, functional magnetic resonance imaging (fMRI) studies show hippocampal hyperactivity during episodic memory tasks in patients in the mild cognitive impairment (MCI) stage of AD (*96-98*) whereas patients in later stages of AD will show decreased activity and connectivity of the hippocampus and cortex hippocampal hyperactivity (*99*). In line with our conclusion, many interpret the early increase in hippocampal activity in MCI patients as a compensatory mechanism needed to overcome neural dysfunction in order to preserve the ability for memory encoding and retrieval (*98, 100*). This would also suggest the transcriptome of hAPP(J20) mice reflect more MCI-like states of pathology whereas the transcriptional profiles of CK-p25 and APP/PS1-21 mice model later stages disease where inflammation and neurodegeneration are more prevalent. Indeed, microarray studies in control and AD brains at different ages reveal upregulation of genes associated with synaptic signaling and structure in the hippocampus of MCI patients whereas downregulation of these genes predominated in moderate and severe stages of AD (*101, 102*).

It should be noted though, that there is evidence to support the notion that the hyperxcitability observed in human patients as well as mouse models actively contributes to cognitive impairment (*43*). In support of this idea, treatment with levetiracetam, an antiepileptic drug that reduces hippocampal activity, improves cognitive abilities in both MCI individuals (*103*) as well as hAPP(J20) mice (*104*). Additionally, enhanced dendritic excitability as a result of reduced $K_v4.2$, an A-type voltage-gated K+ channel, has been shown to contribute to neural dysfunction and cognitive impairment in hAPP(J20) mice (*44*). Both our hAPP(J20) RNA-seq and meta-analysis corroborated the

previously observed decrease in Kv4.2 (*Kcnd2*) transcript level and further highlighted a large variety of additional downregulated voltage-gated K^+ channels, including other A-type channels as well as delayed-rectifier K^+ channels. Thus, our results suggest that in addition to dendritic simplification (*105*), impairment of inhibitory interneuron activity (*106, 107*), and increased presynaptic vesicle release (*108*), transcriptional dysregulation of voltage-gated and calcium-activated ion channels may also be a potential mechanism contributing to the hyperexcitability observed in AD mouse models and human patients.

Another significant finding of our work involved the use of a meta-analysis and network analysis to identify *Hdac2* as a therapeutic target, for which ASO-mediated knockdown rescued hippocampus-dependent spatial memory in two mouse models of AD. Although HDAC2 has been previously identified as a high priority target of AD-related cognitive impairment (*8, 71*), the fact that our unbiased integrative genomic analysis independently identified HDAC2 as a therapeutic target of interest validates the use of our overall approach and implicates the other identified gene targets as viable therapeutic targets. It was surprising that *Hdac2* was downregulated in our meta-analysis given that several AD models have documented elevated HDAC2 protein levels (*8, 109*). However, work by Gonzalez-Zuñiiga *et al.* suggests that as opposed to a transcriptionally mediated mechanism, amyloid-dependent elevated HDAC2 may rely on tyrosine kinase c-Abl-mediated phosphorylation of HDAC2 to stabilize protein levels from degradation (*109*).

The high interconnectivity of HDAC2 with other proteins dysregulated in response to amyloid-deposition suggests that interventions that decrease HDAC2 levels, as opposed to pharmacological agents that solely inhibit deacetylase activity, could yield

superior procognitive effects, presumably via modulating both direct protein-protein interactions as well as indirect effects on chromatin plasticity as a result of decreasing HDAC2's histone deacetylase activity. Such an interpretation might explain the ability of HDAC2 ASO treatment to enhance memory performance in both NTG and AD model mice. Additionally, the ability of an ASO-mediated approach to rescue AD-related cognitive impairment is noteworthy. As there are currently no disease-modifying treatments for AD, the development of new valid therapeutic targets is a pressing and compelling societal and biomedical issue. Rapid progress in oligonucleotide (*110*) and epigenetic-based therapeutics (*111*) make it increasingly possible to target specific RNAs and epigenetic marks in the central nervous system for the prolonged treatment of neuropsychiatric diseases. Indeed, ASO-mediated approaches are actively being investigated for a variety of neurodegenerative diseases, including spinal muscular atrophy (SMA) (*112, 113*), amyotrophic lateral sclerosis (ALS) (*114-116*), and Huntington's disease (HD) (*72, 117*). In the context of AD, ASOs have been used to target *APP* (*118, 119*), *TAU* (*120*), and *GSK3B* (*121*). Thus far, completed phase 1 clinical trials of ASO-based therapies in ALS and SMA patients have been successful with no major adverse events reported (*114, 122*). Overall, the ease of delivery, long-term efficacy, and preliminary safety profiles make the use of ASO-based therapies a promising therapeutic for the treatment of AD-related cognitive impairment.

Finally, one surprising finding from our work was the relative lack of genome-wide alterations in CpG dimethylation. Given that our enrichment-approach preferentially enriches for CpG dimethylation, it is possible that amyloid-associated changes in DNA methylation involve alterations in CpG hemi-methylation, non-CpG methylation, or

oxidized methylcytosine species (*e.g.* 5-hydroxylmethylcytosine), all of which are unique features of the CNS methylome (*123, 124*). Additionally, cellular heterogeneity may be another factor impacting our molecular resolution as alterations in DNA methylation in one cell type may oppose or dilute those in another (*21*), an important point considering the mixture of neurons, astrocytes, and microglia in the AD brain. Extensive methylome reconfiguration also occurs during neurodevelopment (*124*), perhaps decreasing our signal-to-noise ratio as we sequenced dentate gyrus tissue, which of course is a primary region for neurogenesis. Undoubtedly, future investigation will be required to explore these topics amongst others in order to obtain a comprehensive understanding on how DNA methylation contributes to Aβ-induced transcriptional dysregulation and AD-related cognitive impairment.

Materials and Methods

Animals

For all sequencing studies, male hAPP(J20) transgenic mice were used. hAPP(J20) express a mutant form of the human amyloid protein precursor (hAPP) bearing both the Swedish (K670N/M671L) and the Indiana (V717F) mutations under the control of the PDGF promoter (*125*). These mice were on a congenic C57BL/6J background (>15 backcrosses). For SAHA and HDAC2-ASO experiments, male and female hAPP(J20) and APPswe/PS1dE9 double transgenic mice were used. APPswe/PS1dE9 mice (*126*) were obtained from The Jackson Laboratory with strain name B6C3-Tg (APPswe,PSEN1dE9)85Dbo/J; stock number 004462). These mice express a chimeric mouse/human APP transgene that contains the Swedish mutations (K595N/M596L) as well as a mutant human *PSEN1* transgene carrying the deleted exon

9 variant under control of mouse prion promoter elements. Mice were maintained as double hemizygotes by crossing transgene positive males with wild-type females on a B6C3F1/J background strain (Jackson Laboratories: stock number 100010). All mice were genotyped through tail clips and subsequent PCR analysis of genomic DNA. All mice were kept on a 12 h light/12 h dark cycle and had ad libitum access to food and water. The studies were approved by the Institutional Animal Care and Use Committees (IACUC) of the University of Alabama at Birmingham and conducted in full compliance with the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and the National Institutes of Health's Guide for the Care and Use of Laboratory Animals.

Open Field

Mice were placed in an open-field apparatus (43.2 cm x 43.2 cm x 30.5 cm) for two hours or 10 min, as specified, to assess horizontal activity, thigmotaxis (a measure of anxiety), and habituation to a novel context. Movement was tracked by an automatic video tracking system (Med Associates).

Elevated Plus Maze

Mice were placed animals in an apparatus with two open and two closed arms for 5 minutes. Entries and time spent in the open and closed arms were measured by an automatic video tracking system (Med Associates). As mice have an innate aversion to brightly lit areas, the amount of time spent in the closed arms (dimly light) versus the open arms (brightly lit) serves as an index of basal anxiety.

Object Location Memory

Mice were trained with two 50 mL beakers and one black line spatial cue in a 10 x 10 x 12 in (x,y,z) opaque polyurethane open box containing autoclaved bedding for 10 min. Prior to training, mice were habituated to the box and bedding without objects for 4 days, 5 min each day. 24 h after training, one beaker was moved to a novel location and the mice were tested for 5 min. Videos were scored by hand and blind to subject identity, and object interaction was scored as previously described (*127*).

Drugs

SAHA (suberoylanilide hydroxamic acid, Vorinostat, Zolinza) was purchased from TCI America, dissolved in vehicle (10% DMSO, 0.9% saline), and administered at 50 mg/kg once per day intraperitoneally for 6 weeks prior to behavioral assessment. On days of behavior, SAHA was administered 2 h prior to behavioral testing.

Isolation of hippocampus

Following behavioral screening, the animals will be left to rest in the vivarium for 2 weeks prior to euthanization via rapid decapitation, allowing for any activity-induced changes in gene expression incurred during screening to return to baseline. Brains were submerged in oxygenated (95%/5% O_2/CO_2) ice-cold cutting solution (125 mM NaCl, 3 mM KCl, 1.25 mM NaH_2PO_4, 25 mM $NaHCO_3$, 0.5 mM $CaCl_2$, 7 mM$MgCl_2$, 10 mM glucose, 0.6mM ascorbate) immediately after rapid decapitation and during gross dissection of the dentate gyrus.

Immunoblots

Mouse tissue samples, stored at -80°C, were homogenized in RIPA supplemented with protease inhibitors (Sigma), DTT, and sodium butyrate. Protein samples were placed in Laemmli sample buffer and 2-mercaptoethanol and were heated for 10 min at 95°C, then separated by on 4-20% Mini-TGX Protean Gel (Bio-Rad) and transferred to Immobilon-FL PVDF membranes (Millipore) using the TransTurbo semidry transfer system (Bio-Rad). The membrane was blocked in LI-COR Odyssey blocking buffer for 1 h at room temperature and incubated with the appropriate primary antibody. The specific antibody treatment parameters were as follows: HDAC2 (Abcam, ab12169, 1:1000, overnight 4°C room- temperature), and Actin (Abcam, ab3280, 1:1000, 1 h room-temperature). After primary antibody treatment, membranes were washed 3 times in 0.1% TBS-T, followed by incubation for 1 h in goat anti-mouse, AlexaFluor 800 (1:20,000, LI-COR. Membranes were then washed 3 times in 0.1% TBS-T, followed by a single wash in TBS, imaged on the LI-COR Odyssey fluorescent imaging system, and quantified using LiCor Image Studio.

HDAC2 ASO

HDAC2-ASO (CToCoAoCTTTTCGAGGTToCoCTA) and control-ASO that targets no mouse or human genes (GToToToTCAAATACACCToToCAT) were generated by ISIS Pharmaceuticals using the phosphorothiate and 2′-O-methoxyethyl (2′-MOE) modified ASO platform (*72, 74*). C, T, A, G indicate 5-methylcytosine, thymine, adenine, and guanine nucleosides, respectively. Underlined residues are deoxynculeosides, all others are 2’-methoxyethyl nucleosides. All linkages are

phosphorothioate except those indicate by o between residues, which are phosphodiester. Mice were anesthetized with 2% isoflurane and secured in a stereotaxic apparatus (David Kopf Instruments). ASOs (300ug at 60ug/ul) were injected ICV with the following coordinates: (AP -0.2; ML -1.0; DV -2.4) at a rate of 1 uL/min, with 3 weeks allowed for recovery.

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted (Qiagen, miRNeasy), DNase-treated (Qiagen), quantified spectrophotometrically (Thermo Scientific, NanoDrop 200c), and reverse-transcribed (Bio-Rad, iScript cDNA Synthesis Kit). qRT-PCR was then carried out using a CFX96 touch real-time PCR detection system (Bio-Rad) with either SSO Advanced Universal SYBR Green Supermix (Bio-Rad) and 500 nM of intron-spanning primers (Table S19). PCR amplifications were performed in triplicate with the following cycling conditions: 95 °C for 30 s, followed by 40 cycles of 95 °C for 10 s and 60 °C for 30 s, followed by real-time melt analysis to verify product specificity. Differential gene expression between samples was determined by the comparative Ct (ΔΔCt) method using using *hypoxanthine phosphoribosyltransferase 1* (*Hprt1*) or *glyceraldehyde-3-phosphate dehydrogenase* (*Gapdh*) as an internal control (*128, 129*).

RNA Sequencing

Total mRNA was extracted (Qiagen, miRNeasy), DNase-treated (Qiagen), quality controlled (Bioanalyzer, Agilent), poly(A) selected and sequenced (Genomic Services Lab at HudsonAlpha) on the Illumina platform (HiSeqv4, paired-end, 50 bp, 25 million

reads). Raw paired-end sequenced reads were quality controlled (FASTQC v0.63, Galaxy), filtered for read quality (FASTX toolkit, Galaxy, 90% of all bases were required to have a quality score >20) and aligned to the mouse genome (mm10 assembly) in Galaxy (*130*) using TopHat v2.0.14 (with custom settings --read-realign-edit-dist 0 –r 131 --mate-std-dev 64 --no-coverage-search --microexon-search --no-novel-juncs –G iGenomesmm10UCSCRefSeq.GTF --b2-very-sensitive) (*131*). Read counts for all genes and all exons (mm10 assembly) were obtained (FeaturesCount (*132*), GalaxEast) and gene expression differences between groups calculated with the DESeq2 (*31*) algorithm available in the SARTools (*133*) package for R v3.3.0 (with custom settings: alpha = 0.1, pAdjustMethod = BH, typeTrans= rlog). Gene ontology overrepresentation of significantly altered genes was conducted with PANTHER (*134, 135*) (v10.0, http://www.pantherdb.org/), using the GO Ontology database (2015-08-06 release) and the Benjamini–Hocheberg multiple-testing correction (*136*). KEGG pathway, Wikipathway and phenotypic analysis were performed using the free online WebGestalt software (http://bioinfo.vanderbilt.edu/webgestalt/) (*137, 138*).

CpG Methylomic Analysis

For CpG dimethylation sequencing (MBDseq), DNA was extracted (Qiagen, ALLprep) and sonicated to an average length of 300 bp as determined by gel electrophoreses. Methylated DNA fragments were then sequestered using the MethylMiner Methylated DNA Enrichment Kit (Life Technologies). The methylated DNA was then sized selected between 200 and 400 bp, amplified by PCR, and sequenced (HudsonAlpha) on the Illumina platform (HiSeq v4, single-end, 50 bp, 25 million reads).

Reads were trimmed (2 nt from the 5′ end), PCR duplicates removed (10.7 – 22.6 million novel reads per sample) and mapped with Bowtie for Illumina to the mm10 genome (Galaxy(*130*)). To determine differentially methylated regions, we used the published MEDIPS package (*62*) in R v3.3.0 (ws = 300, extend = 300, shift= 0, uniq = T, quantile normalized, FDR = 0.1). To determine differentially methyaled genes, we used SeqMonk v0.34, in conjunction with the DESeq2 (*31*) algorithm. An FDR = 0.1 was used to account for multiple comparisons.

Meta-analysis

To identify transcriptomic changes that are conserved across a variety of AD models we used publicly available RNA-sequencing data sets from APP/PS1-21(*3*) and CK-p25 (*6*) mice. The APP/PS1-21 dataset consisted of CA1 hippocampal samples from a cohort of 10-month-old WT (n=11) and APP/PS1-21 (n=9) vehicle treated mice. The CK-p25 dataset was collected from a cohort of 3-month-old double-transgenic CK-p25 mice and aged-matched controls. Data were collected at two time points: 2 weeks after p25 induction and 6 weeks after p25 induction. Raw sequencing reads were downloaded and processed as previously outlined making adjustments for single-end versus paired-end sequencing. Results from the differential expression analysis were then parsed using Matlab v9.0 to generate lists of unique and overlapping genes. Gene overlap and log2 fold changes in gene expression were visualized using the OmicCircos (*139*) package and the UpSetR package (*140*) in R v3.3.0. Correlation matrices of log2 fold changes in gene expression were then generated using the Corrplot v0.77 R package. Meta-analysis was performed using the metaRNASeq R package (*70*). Within this package, Fisher's p-value

combination was performed in order to generate a comprehensive list of differentially expressed genes (FDR = 0.05). This list was then filtered to determine genes with a common direction of alteration across all studies.

Statistical and graphical analyses

Statistical analyses were performed with a two-way analysis of variance (ANOVA), followed by Tukey's posthoc analyses, using GraphPad Prism version 7. The threshold for statistical significance was set at a $p < 0.05$ and a FDR = 0.1 for DESeq2 analyses. Results are expressed as mean ± SEM. Protein-protein network analyses were performed with NetworkAnalyst (*38-40*).

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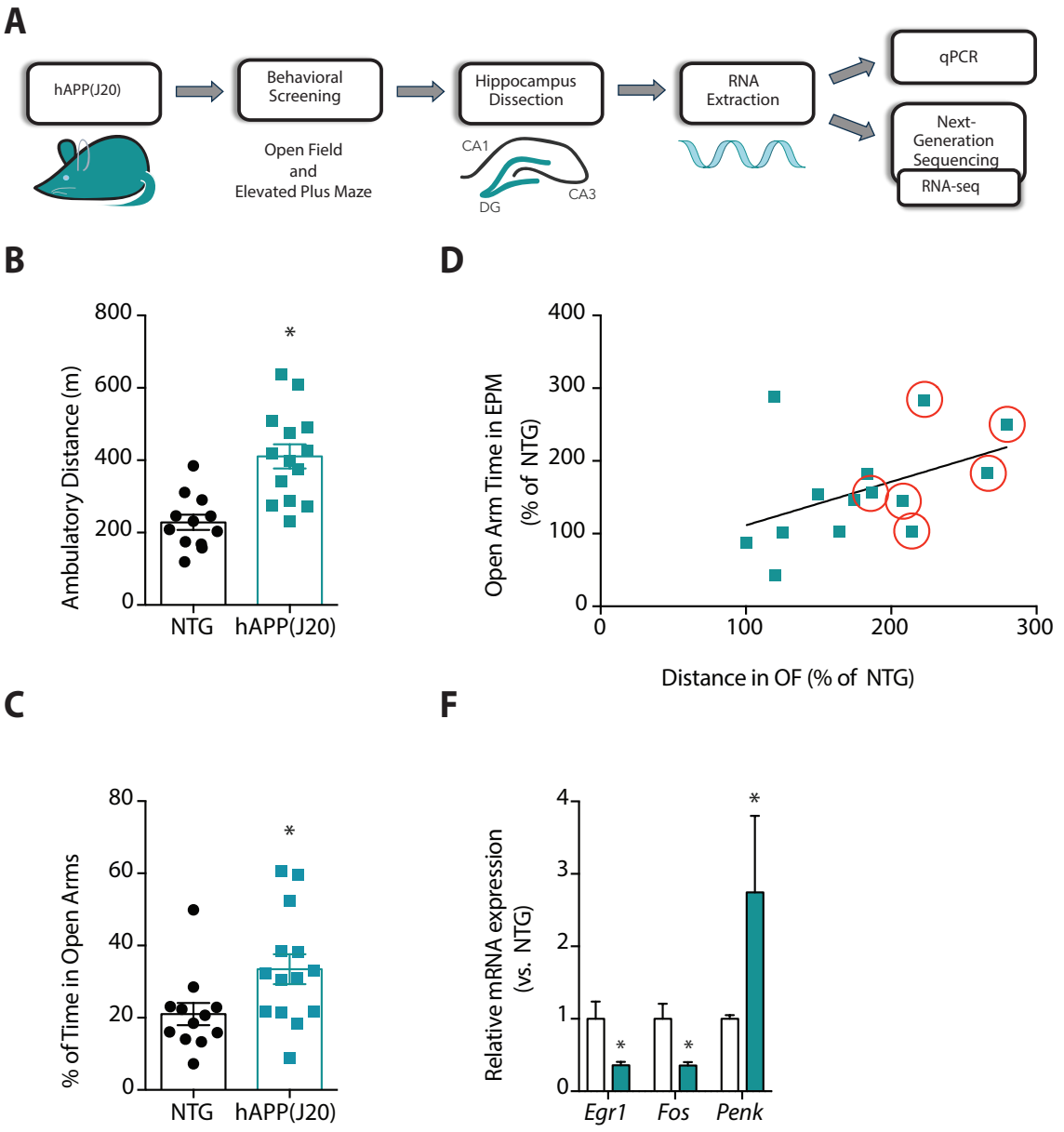


Figure 1. Selective genotyping targets behaviorally impaired hAPP(J20) mice for transcriptomic analysis. (**A**) Schematic overview of experimental design. (**B**) Total distance travelled during a 2-hour trial of the open field (n = 12 to 14 mice per genotype; age 6 to 7 months; **P* < 0.05, Student's unpaired *t* test). **(C)** Percent of time spent in open arms during a 10-min exploration of the elevated plus maze (n = 12 to 14 mice per genotype; age 6 to 7 months; **P* < 0.05, Student's unpaired *t* test). **(D)** Red circles denote the hAPP(J20) mice with the most impaired open field and elevated plus maze behavior selected for transcriptomic analysis via RNA-seq. (**E**) qRT-PCR of selected genes known to be differentially expressed in cognitively impaired hAPP(J20) mice (*27, 29, 30*) (n = 6 mice per genotype; age 6 to 7 months; **P* < 0.05, Student's unpaired *t* test). Bar graphs shows mean ± SEM.

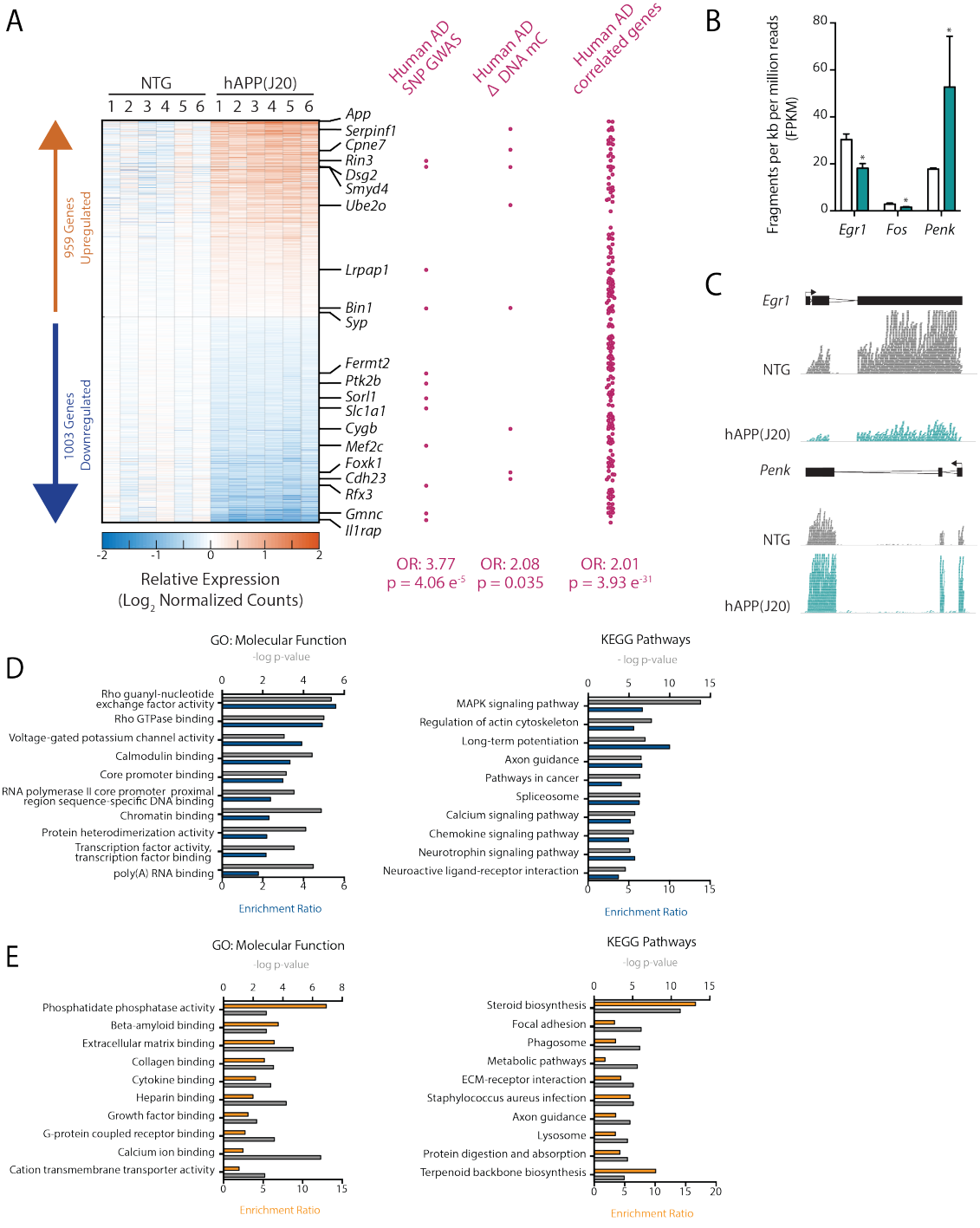

A
NTG
hAPP(J20)
1 2 3 4 5 6 1 2 3 4 5 6
959 Genes Upregulated
1003 Genes Downregulated
App
Serpinf1
Cpne7
Rin3
Dsg2
Smyd4
Ube2o
Lrpap1
Bin1
Syp
Fermt2
Ptk2b
Sorl1
Slc1a1
Cygb
Mef2c
Foxk1
Cdh23
Rfx3
Gmnc
Il1rap
-2 -1 0 1 2
Relative Expression
(Log2 Normalized Counts)
Human AD SNP GWAS
Human AD Δ DNA mC
Human AD correlated genes
OR: 3.77
p = 4.06 e-5
OR: 2.08
p = 0.035
OR: 2.01
p = 3.93 e-31
B
Fragments per kb per million reads (FPKM)
Egr1
Fos
Penk
C
Egr1
NTG
hAPP(J20)
Penk
NTG
hAPP(J20)
D
GO: Molecular Function
-log p-value
Rho guanyl-nucleotide exchange factor activity
Rho GTPase binding
Voltage-gated potassium channel activity
Calmodulin binding
Core promoter binding
RNA polymerase II core promoter proximal region sequence-specific DNA binding
Chromatin binding
Protein heterodimerization activity
Transcription factor activity, transcription factor binding
poly(A) RNA binding
Enrichment Ratio
KEGG Pathways
- log p-value
MAPK signaling pathway
Regulation of actin cytoskeleton
Long-term potentiation
Axon guidance
Pathways in cancer
Spliceosome
Calcium signaling pathway
Chemokine signaling pathway
Neurotrophin signaling pathway
Neuroactive ligand-receptor interaction
Enrichment Ratio
E
GO: Molecular Function
-log p-value
Phosphatidate phosphatase activity
Beta-amyloid binding
Extracellular matrix binding
Collagen binding
Cytokine binding
Heparin binding
Growth factor binding
G-protein coupled receptor binding
Calcium ion binding
Cation transmembrane transporter activity
Enrichment Ratio
KEGG Pathways
-log p-value
Steroid biosynthesis
Focal adhesion
Phagosome
Metabolic pathways
ECM-receptor interaction
Staphylococcus aureus infection
Axon guidance
Lysosome
Protein digestion and absorption
Terpenoid backbone biosynthesis
Enrichment Ratio

Figure 2. Gene expression patterns in the hippocampus of hAPP(J20) mice. (A) Heatmap of poly(A) RNAseq differentially expressed genes in the hAPP(J20) dentate gyrus showing individual replicates (FDR = 0.1). The degree of enrichment with human genes with AD-associated SNPs (*32, 33*), differentially methylated DNA (*34*), and differential expression (*4*) is shown alongside the associated odds ratios and hypergeometric p-values. **(B)** Quantitative gene expression based on RNAseq computed fragments per kilobase per million sequencing reads (FPKM; n = 6 mice per genotype; age 6 to 7 months; * $P_{adj} < 0.10$). **(C)** RNAseq tracks showing aligned reads at the *Egr1* and *Penk* loci. **(E** and **F)** Main molecular function Gene Ontology (GO; left) categories and KEGG Pathways (right) enriched in genes upregulated **(E)** and downregulated **(F)** in hAPP(J20) mice. All data shown are from 6 mice per genotype
at 6-7 months of age. Error bars indicate ± SEM.

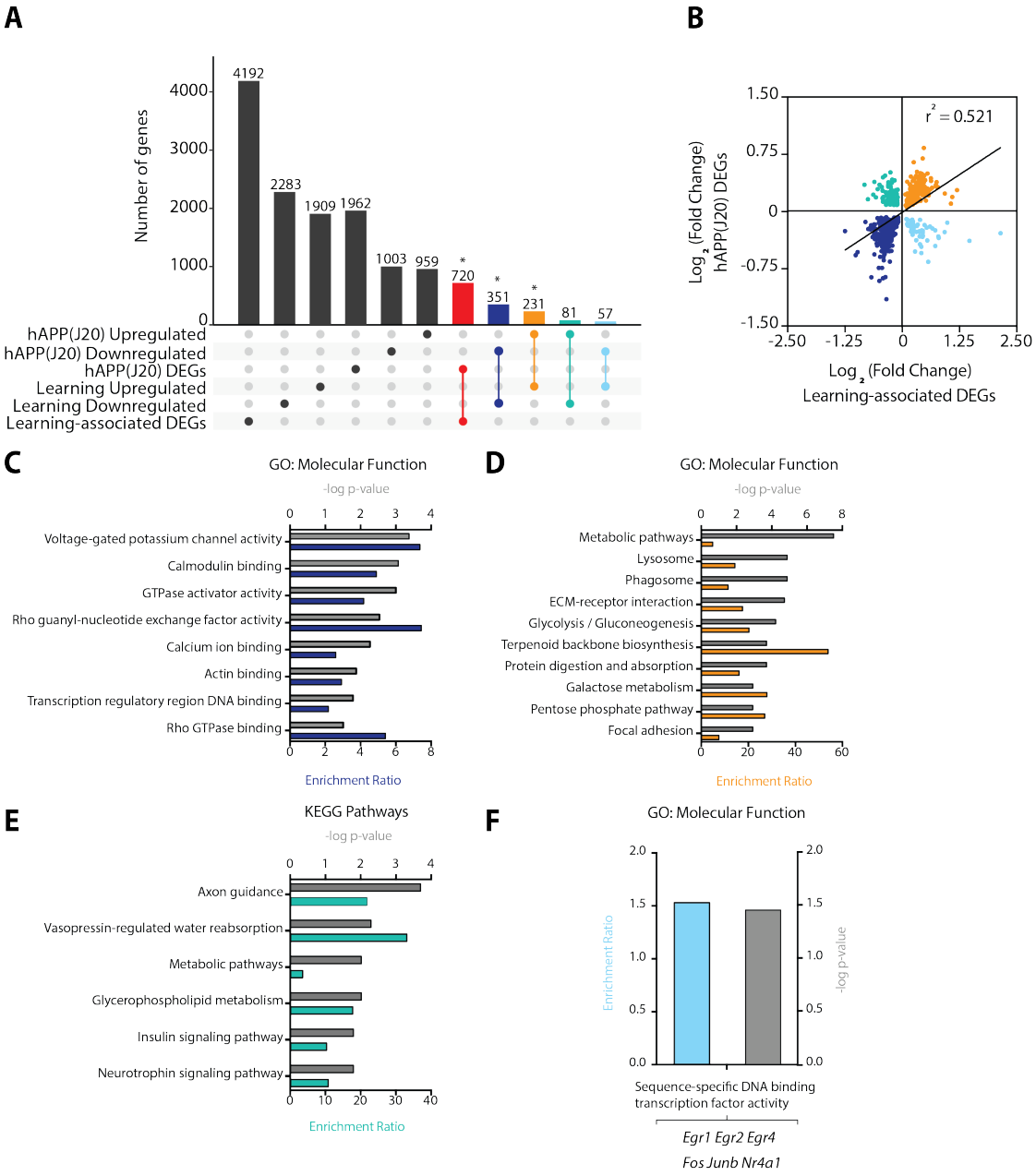

A
Number of genes
4192
2283
1909
1962
1003
959
720
351
231
81
57
hAPP(J20) Upregulated
hAPP(J20) Downregulated
hAPP(J20) DEGs
Learning Upregulated
Learning Downregulated
Learning-associated DEGs
B
r² = 0.521
Log₂ (Fold Change) hAPP(J20) DEGs
Log₂ (Fold Change) Learning-associated DEGs
C
GO: Molecular Function
-log p-value
Voltage-gated potassium channel activity
Calmodulin binding
GTPase activator activity
Rho guanyl-nucleotide exchange factor activity
Calcium ion binding
Actin binding
Transcription regulatory region DNA binding
Rho GTPase binding
Enrichment Ratio
D
GO: Molecular Function
-log p-value
Metabolic pathways
Lysosome
Phagosome
ECM-receptor interaction
Glycolysis / Gluconeogenesis
Terpenoid backbone biosynthesis
Protein digestion and absorption
Galactose metabolism
Pentose phosphate pathway
Focal adhesion
Enrichment Ratio
E
KEGG Pathways
-log p-value
Axon guidance
Vasopressin-regulated water reabsorption
Metabolic pathways
Glycerophospholipid metabolism
Insulin signaling pathway
Neurotrophin signaling pathway
Enrichment Ratio
F
GO: Molecular Function
Enrichment Ratio
-log p-value
Sequence-specific DNA binding transcription factor activity
Egr1 Egr2 Egr4
Fos Junb Nr4a1

Figure 3. Enrichment of hAPP(J20) DEGs with hippocampal learning-associated transcriptional signatures. (**A**) Intersection plot showing the number of overlapping genes shared between hAPP(J20) mice and NTG mice that have undergone hippocampal-dependent, contextual fear learning (data not shown). Pairwise comparisons between upregulated and downregulated genes are shown and color-coded. (**B**) Positive correlation of $\log_2$ (Fold Change) between hAPP(J20) DEGs and learning-associated DEGs (Pearson's correlation coefficient, r = 0.521, $P < 0.0001$). (**C-F**) Main GO categories or KEGG Pathways significantly enriched in genes downregulated in both datasets **(C)**, upregulated in both datasets **(D)**, upregulated in hAPP(J20) mice but downregulated during learning **(E)** and downregulated in hAPP(J20) mice but upregulated during learning. *$P < 0.05$,
hypergeometric test.

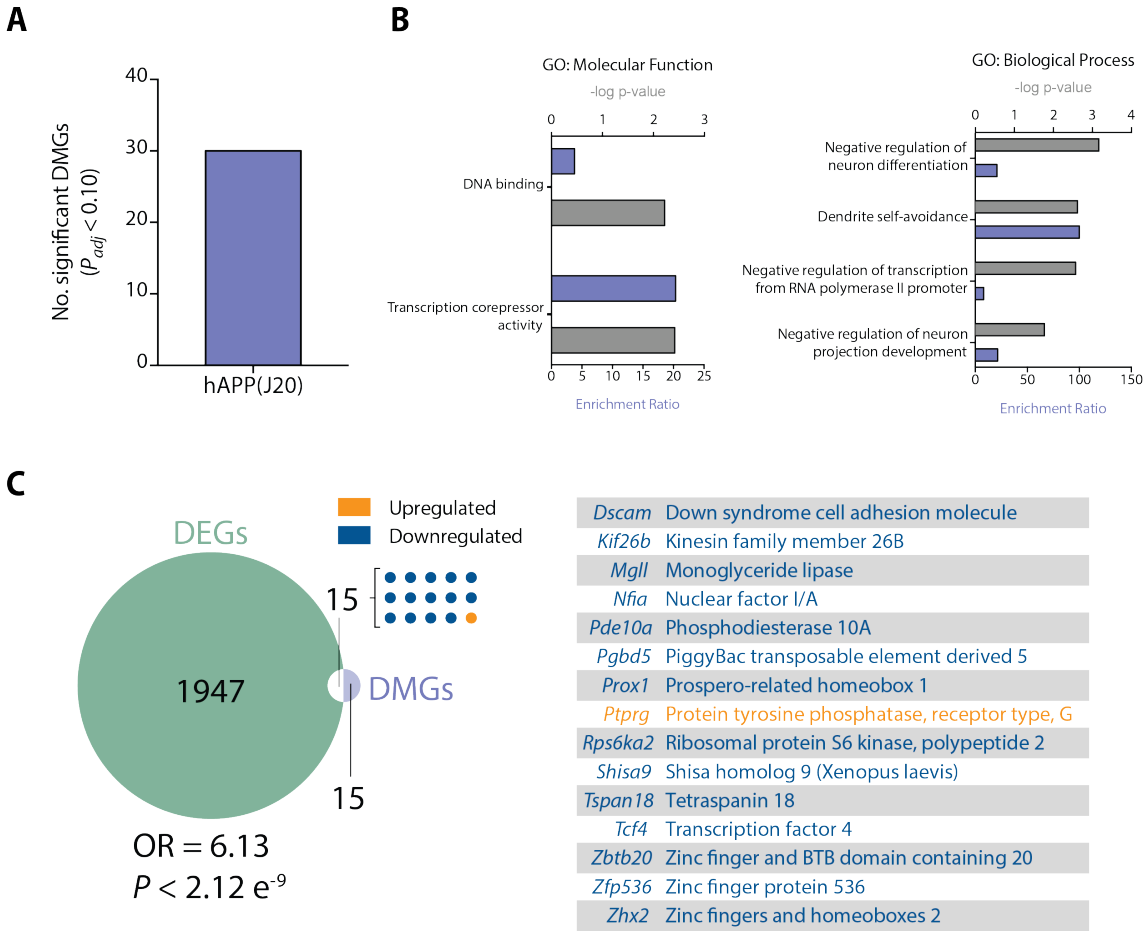


Figure 4. Differentially methylated genes in the hippocampus of hAPP(J20) mice. **(A)**. Number of differentially methylated genes (DMGs) identified by MBD-Seq (FDR= 0.10). (**B**) Main GO categories enriched in DMGs. **(C)** Venn diagram (left) and table (right) showing enrichment of DMGs with DEGs in hAPP(J20) mice.

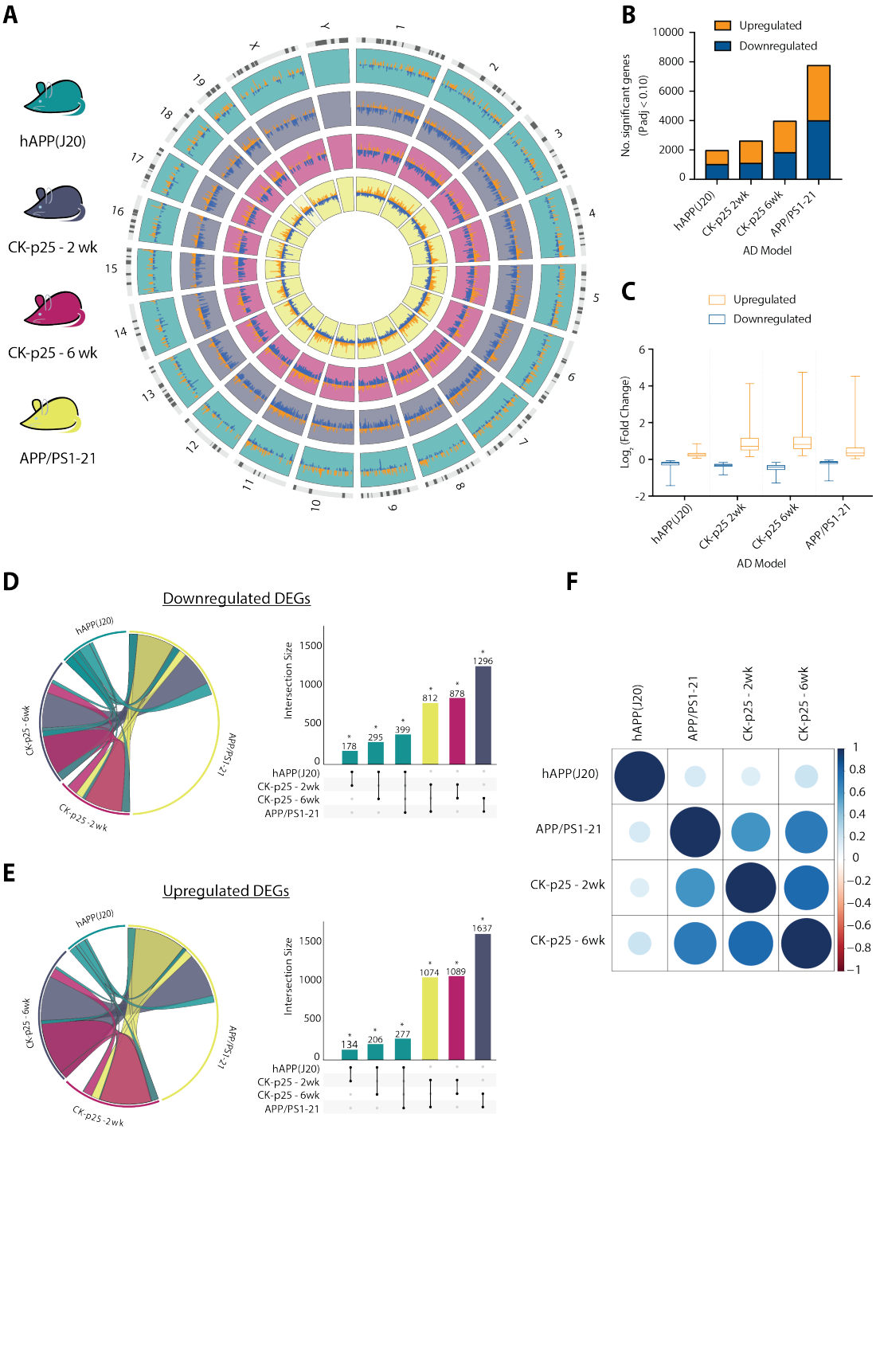

A
hAPP(J20)
CK-p25 - 2 wk
CK-p25 - 6 wk
APP/PS1-21
B
Upregulated
Downregulated
No. significant genes (Padj < 0.10)
10000
8000
6000
4000
2000
0
hAPP(J20)
CK-p25 2wk
CK-p25 6wk
APP/PS1-21
AD Model
C
Upregulated
Downregulated
Log2 (Fold Change)
6
4
2
0
-2
hAPP(J20)
CK-p25 2wk
CK-p25 6wk
APP/PS1-21
AD Model
D
Downregulated DEGs
hAPP(J20)
CK-p25 - 6wk
APP/PS1-21
CK-p25 -2wk
Intersection Size
1500
1000
500
0
178
295
399
812
878
1296
hAPP(J20)
CK-p25 - 2wk
CK-p25 - 6wk
APP/PS1-21
E
Upregulated DEGs
hAPP(J20)
CK-p25 - 6wk
APP/PS1-21
CK-p25 -2wk
Intersection Size
1500
1000
500
0
134
206
277
1074
1089
1637
hAPP(J20)
CK-p25 - 2wk
CK-p25 - 6wk
APP/PS1-21
F
hAPP(J20)
APP/PS1-21
CK-p25 - 2wk
CK-p25 - 6wk
1
0.8
0.6
0.4
0.2
0
−0.2
−0.4
−0.6
−0.8
−1

Figure 5. Comparison of amyloid-associated DEGs across different AD mouse models. (**A**) Publically available hippocampal RNA-seq datasets for CK-p25 (*6*) and APP/PS1-21 (*3*) were downloaded and analyzed with the same RNA-seq pipeline. Circos plot showing chromosomal distribution of DEGs and associated $\log_2$ (Fold Change) for each dataset. The outer perimeter represents the cytogenic bands of each chromosome with the subsequent smaller, colored circles representing the data as follows: hAPP(J20) (green), CK-p25 – 2wk (violet), CK-p25 – 6wk (magenta), and APP/PS1-21 (yellow). Upregulated and downregulated genes are depicted in orange and blue ticks, respectively. (**B**) Number of significantly upregulated and downregulated genes for each dataset. (**B**) Box plots showing distribution of $\log_2$ (Fold Change) for each dataset. **(C-D)** Chord diagrams (left) and intersection plots (right) showing the number of overlapping downregulated **(C)** and upregulated **(D)** genes shared between the datasets. **(F)** Correlation matrix showing Pearson's correlation coefficient (r) for all pairwise comparisons based on $\log_2$ (Fold Change) across all genes. All correlations were found to be significant, $P < 0.05$. Box plots represent the minimum and maximum of all the data.

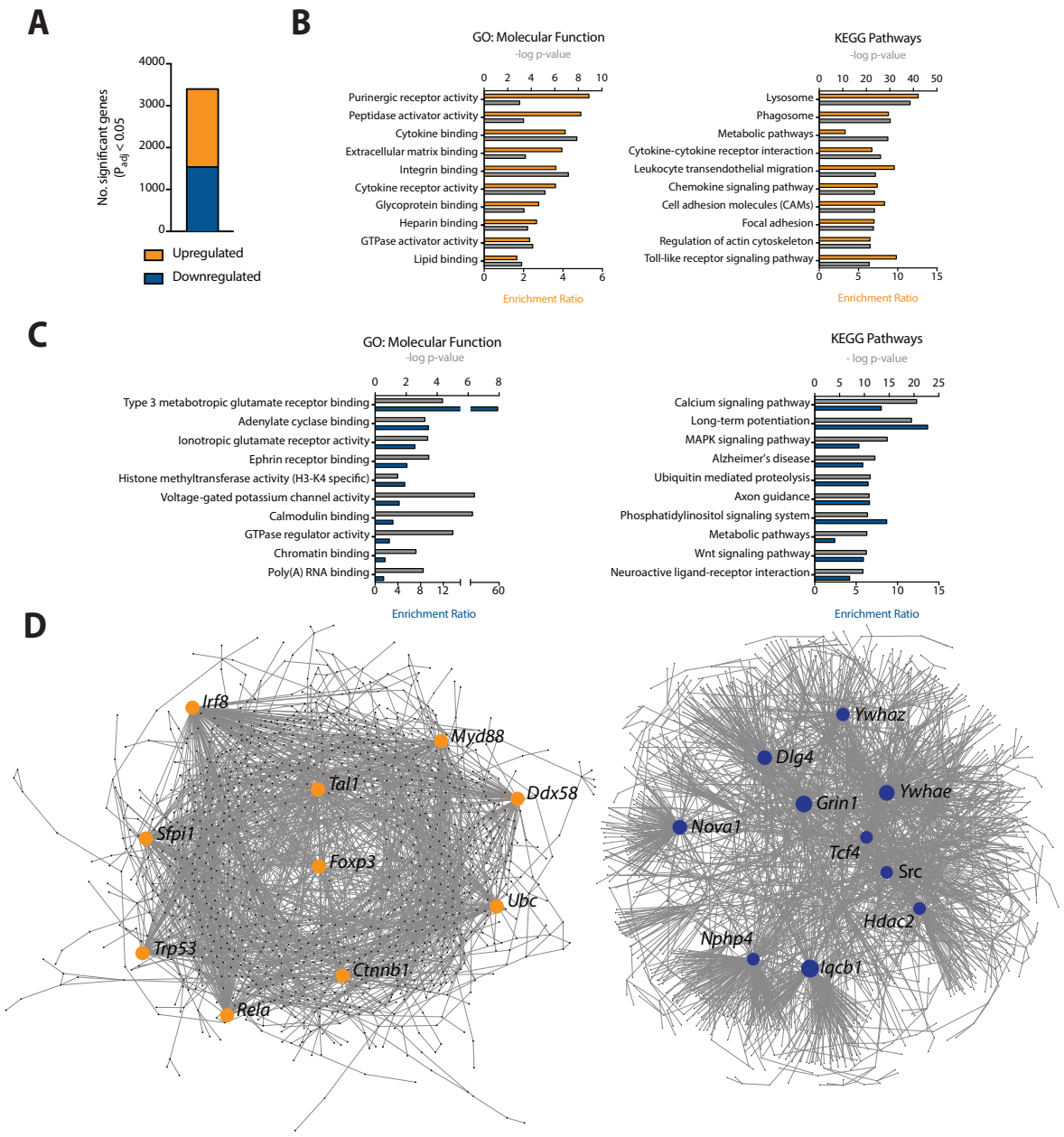


Figure 6. Meta-analysis of hAPP(J20), CK-p25, APP/PS1-21 models identifies shared AD-related transcriptome. (**A**). Number of significantly upregulated and downregulated genes determined by Fischer's p-value combination meta-analysis. (**B-C**) Main molecular function GO categories (left) and KEGG Pathways (right) enriched in upregulated **(B)** and downregulated **(C)** genes. **(D)** Protein-protein interaction network analysis for upregulated (left) and downregulated (right) genes. The top ten protein nodes with highest degree of centrality are highlighted for each gene set.

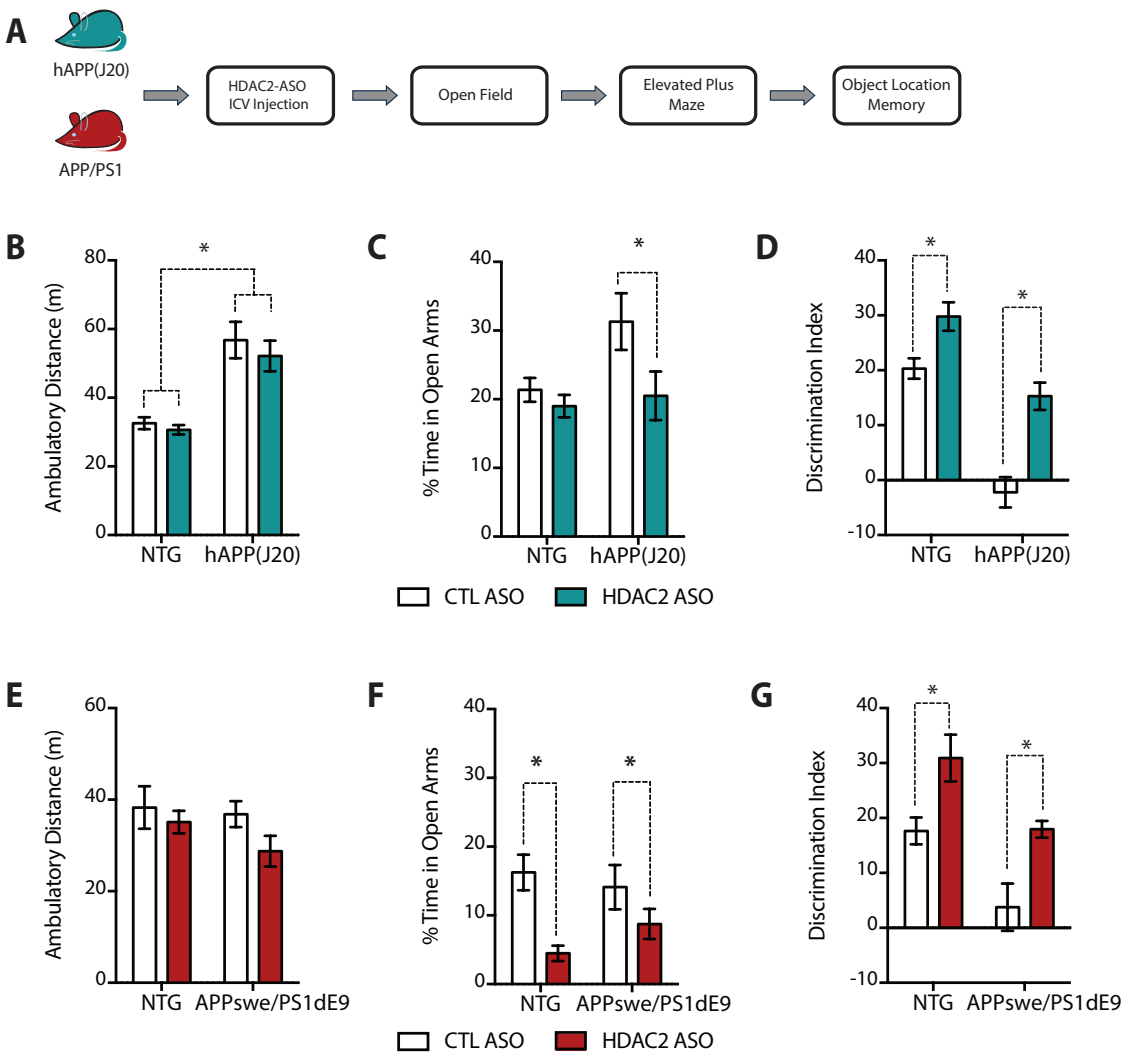


Figure 7. HDAC2 knockdown rescues hippocampal-dependent spatial memory in hAPP(J20) and APP/PS1 mice. (**A**). Schematic of surgical and behavioral analysis. Three weeks following ICV injection with either HDAC2-targeting ASO or a scramble control, hAPP(J20) **(B-D)** and APPswe/PS1dE9 mice **(E-G)** underwent a behavioral battery including open field, elevated plus maze, and object location memory. (**B)** Total distance travelled during a 10-minute trial of the open field (n = 18 to 29 mice per group; age 6 to 9 months; two-way ANOVA: genotype effect, $F_{1,90} = 53.13$, $P < 0.0001$; Tukey *post hoc* tests, $^*P < 0.05$). (**C)** Percent of time spent in open arms during a 5-min exploration of the elevated plus maze (n = 18 to 27 mice per group; age 6 to 9 months; two-way ANOVA: genotype effect, $F_{1,87} = 4.555$, $P < 0.05$; treatment effect, $F_{1,87} = 6.063$, $P < 0.05$; Tukey *post hoc* tests, $^*P < 0.05$). (**D**) Discrimination index for the novel location versus the familiar location during a 10-minute memory test (n = 18 to 29 mice per group; age 6 to 9 months; two-way ANOVA: genotype effect, $F_{1,90} = 55.69$, $P < 0.0001$; treatment effect, $F_{1,90} = 29.46$, $P < 0.0001$; Tukey *post hoc* tests, $^*P < 0.05$). (**E**) Total distance travelled during a 10-minute trial of the open field (n = 11 to 14 mice per

group; age 12 to 13 months; two-way ANOVA: no significant effect of genotype nor treatment). (**F)** Percent of time spent in open arms during a 5-min exploration of the elevated plus maze (n = 11 to 13 mice per group; age 12 to 13 months; two-way ANOVA: treatment effect, $F_{1,87} = 4.555$, $P < 0.05$; treatment effect, $F_{1,87} = 6.063$, $P < 0.05$; Tukey *post hoc* tests, $^{*}P < 0.05$). (**G**) Discrimination index for the novel location versus the familiar location durg a 10-minute memory test (n = 11 to 14 mice per group; age 12 to 13 months; two-way ANOVA: genotype effect, $F_{1,44} = 15.46$, $P < 0.05$; treatment effect, $F_{1,44} = 29.46$, $P < 0.5$; Tukey *post hoc* tests, $^{*}P < 0.05$). Error bars indicate ± SEM.

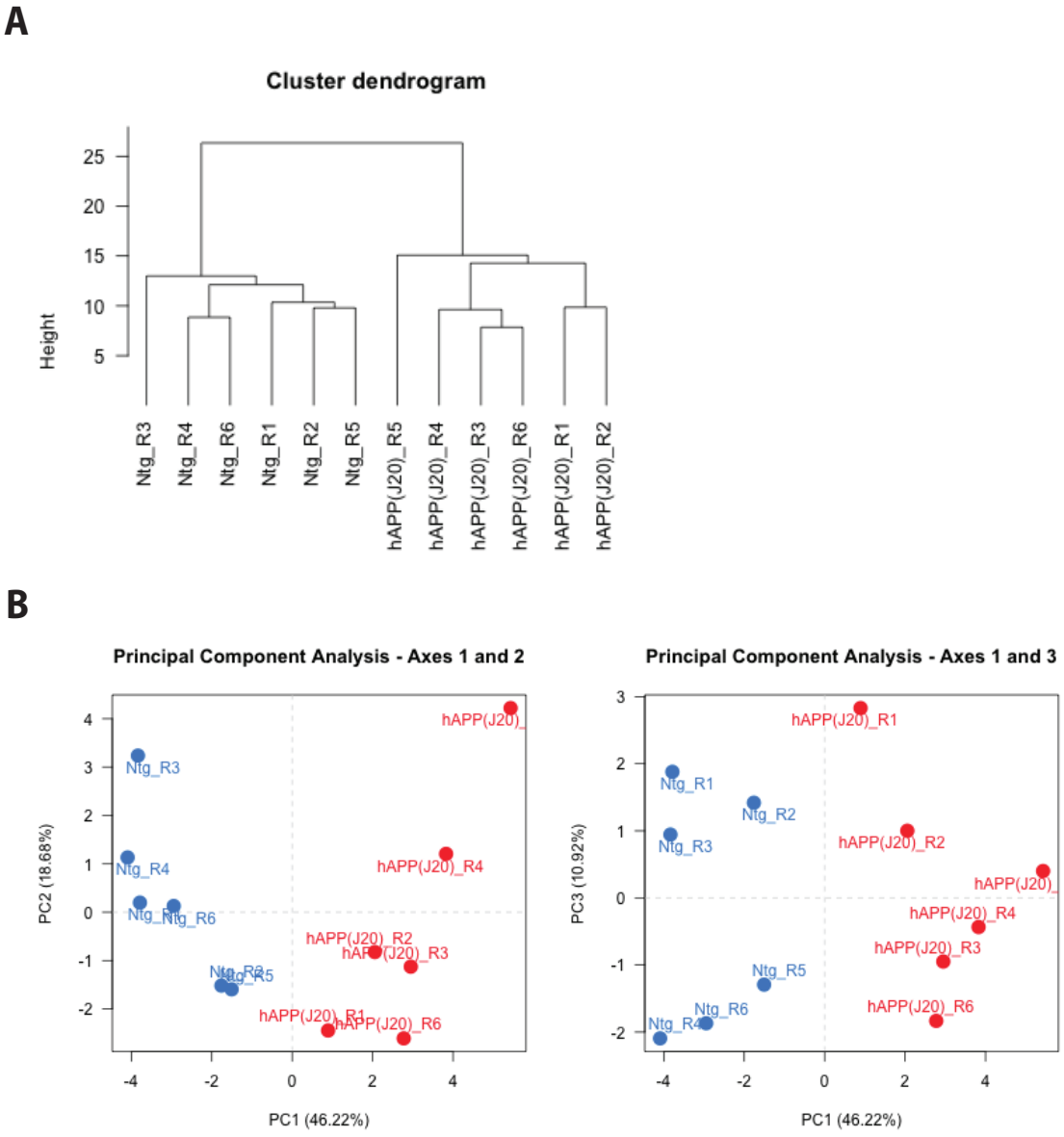


Figure S1. RNAseq quality control. (**A**) Dendrogram obtained from regularized log (rlog) transformed RNAseq count data. An euclidean distance was computed between samples, and the dendrogram built upon the Ward criterion. The samples are separated by genotype and the replicates grouped together. **(B)** Principal Component Analysis (PCA) from regularized log (rlog) transformed RNAseq count data. The first principal component (PC1) separated hAPP(J20) from NTG samples, meaning that the biological variability due to genotype is the main source of variance in the data.

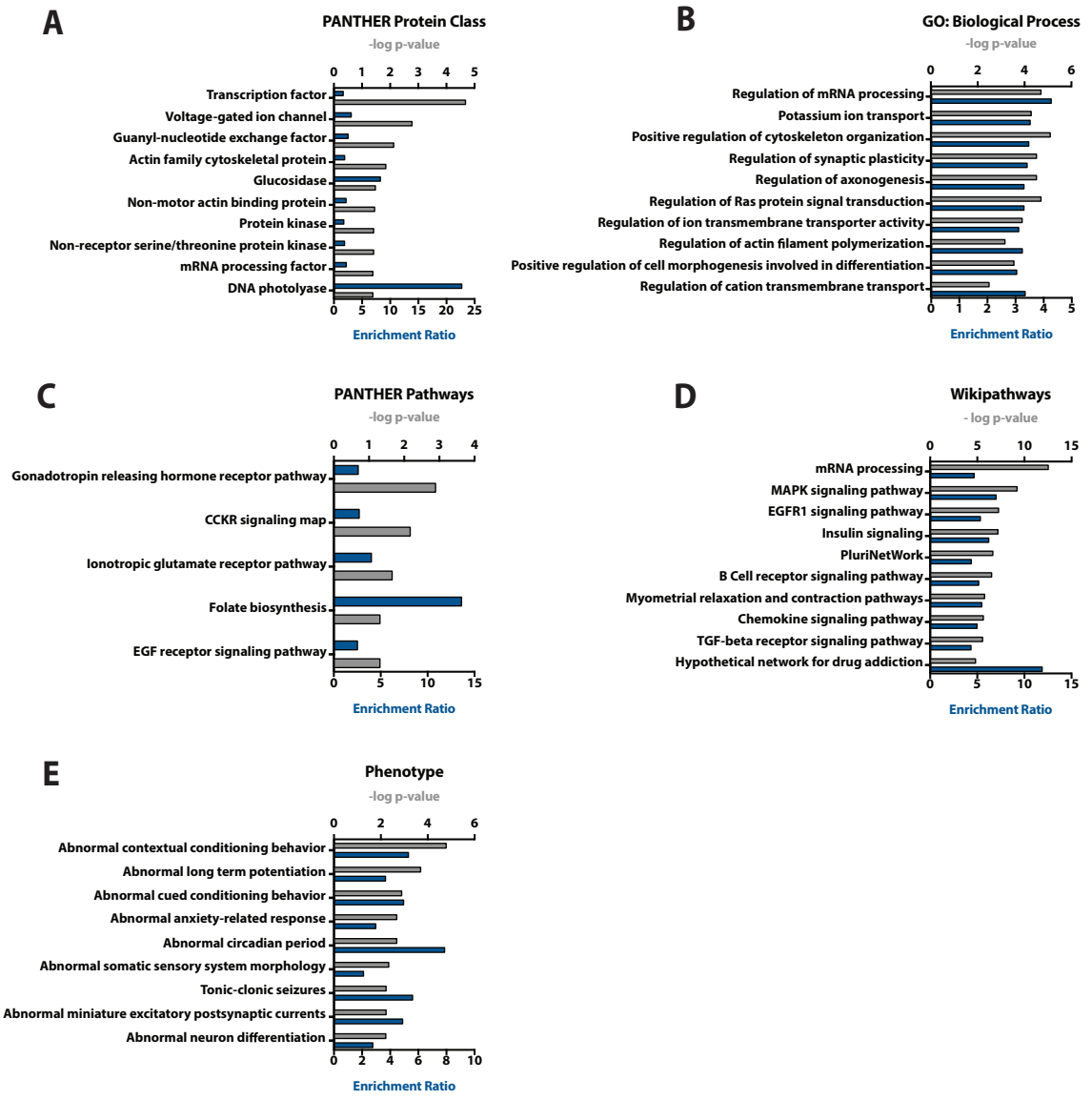
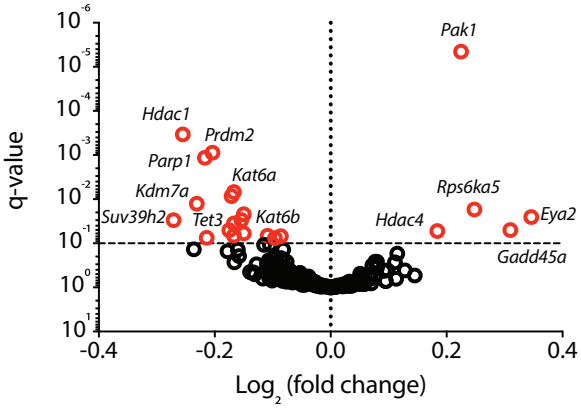


Figure S2. Additional gene ontology and pathway enrichment analysis of genes downregulated in hAPP(J20) mice. (**A-E**) Main PANTHER protein classes **(A)**, biological process Gene Ontology (GO) categories **(B)**, PANTHER pathways **(C)**, Wikipathways **(D)** and Mammalian Phenotypes **(E)** associated with amyloid-induced downregulated genes.

A



B

Downregulated		Upregulated
Tet3	DNA demethylase	*Gadd45a*
Hdac1	Histone deacetylase	*Hdac4*
Cdyl *Kat6a* *Kat6b*	Histone acetyltransferases	
Ash1l *Kmt2e* *Setd8* *Suv39h2* *Prmd2*	Histone lysine methyltransferases	
Kdm4c *Kdm7a*	Histone lysine demethylases	
Pmrt8	Histone arginine methyltransferases	
Parp1	Histone lysine ribosylase	
	Histone kinase	*Rps6ka5 (MSK1)*
Ppp1cc	Histone phosphatase	*Eya2*
Rnf20	Histone ubiquitinase	
Usp16	Histone deubiquitinase	

Figure S3. hAPP(J20) mice exhibit broad dysregulation of epigenetic enzymes. (A) Volcano plot showing log2 (fold change) and q-value for all epigenetic enzymes. Circles in red denote significant DEGs. **(B)** Table displaying differentially expressed epigenetic enzymes and their associated functional categorization.

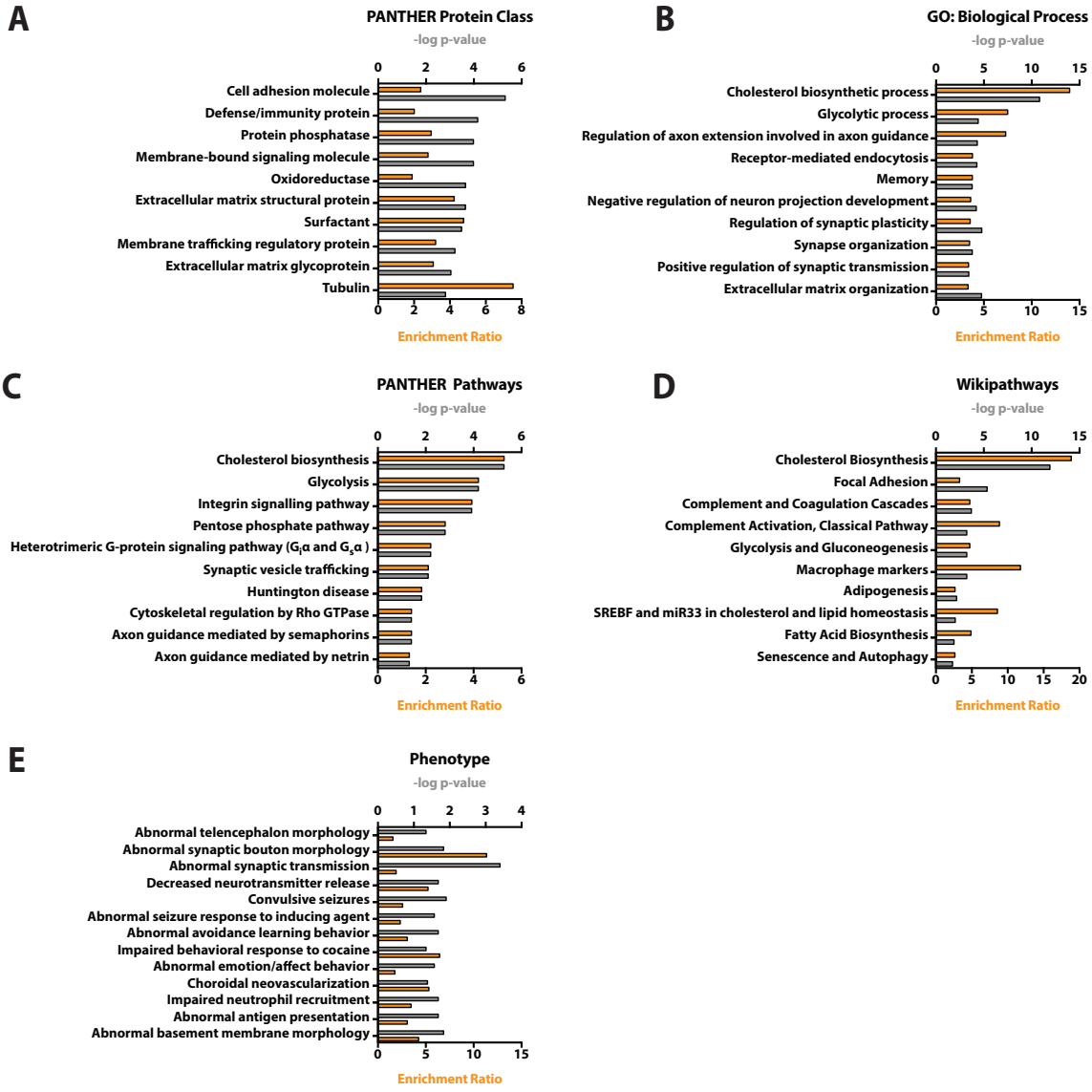


Figure S4. Additional gene ontology and pathway enrichment analysis of genes upregulated in hAPP(J20) mice. (**A-E**) Main PANTHER protein classes **(A)**, biological process Gene Ontology (GO) categories **(B)**, PANTHER pathways **(C)**, Wikipathways **(D)** and Mammalian Phenotypes **(E)** associated with amyloid-induced upregulated genes.

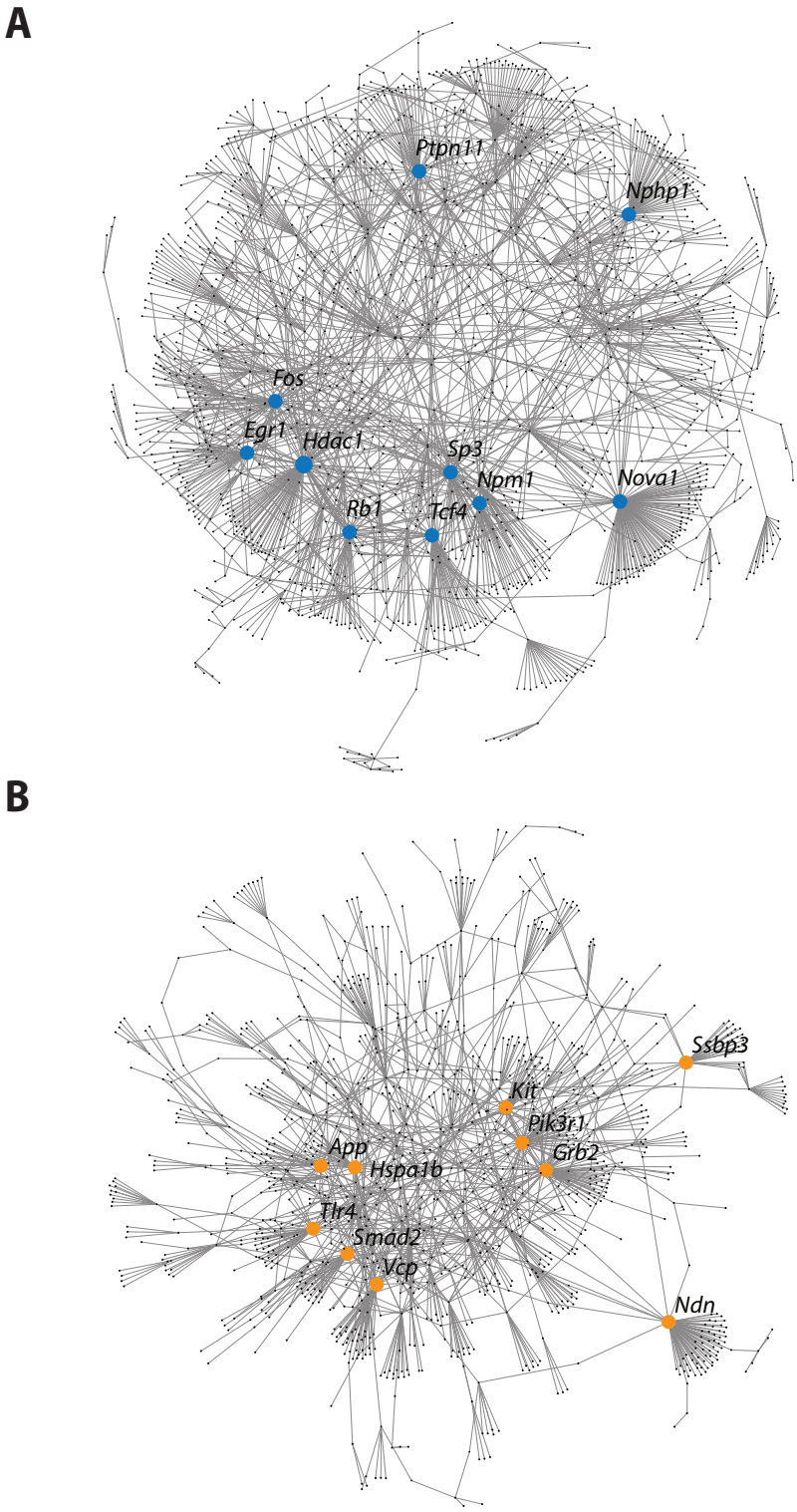


Figure S5. Interactome networks for hAPP(J20) mice DEGs. (**A-B**) Protein-protein interaction network analysis for upregulated **(A)** and downregulated **(B)** genes. The top ten protein nodes with highest degree of centrality are highlighted for each gene set.

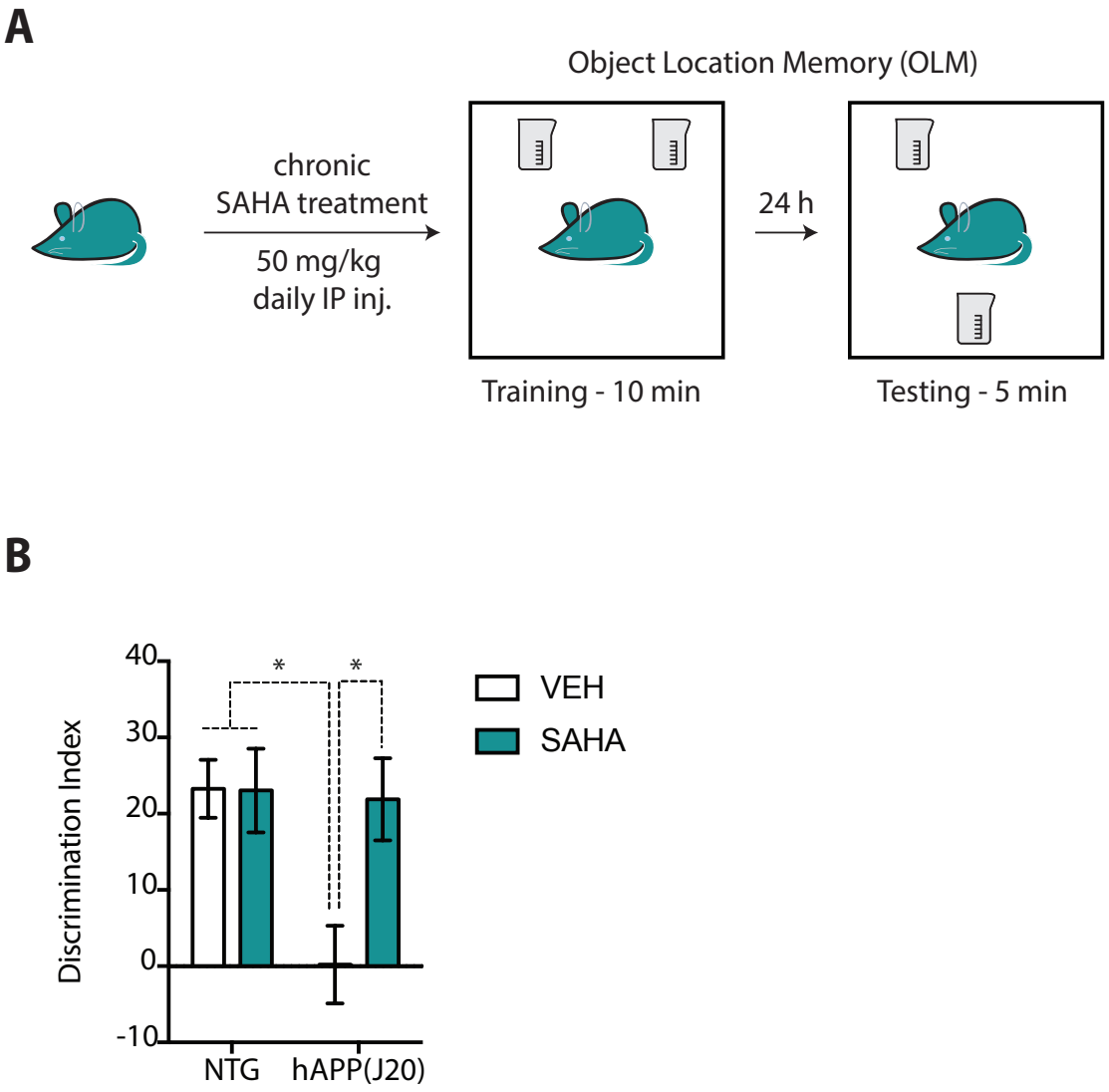


Figure S6. Pharmacological HDAC inhibition rescues hippocampal-dependent spatial memory in h(APP)J20 and APP/PS1 mice. (A) Schematic of experiment. hAPP(J20) mice received daily intraperitoneal (IP) injections of suberoylanilide hydroxamic acid (SAHA) for 6 weeks prior to behavioral training and testing in the object location memory task. **(B)** Discrimination index for the novel location versus the familiar location during a 10-minute memory test (n = 9 to 10 mice per group; age 5 to 7 months; two-way ANOVA: genotype effect, $F_{1,34} = 5.925$, $P < 0.05$; treatment effect, $F_{1,34} = 4.653$, $P < 0.05$; interaction effect, $F_{1,34} = 4.855$, $P < 0.05$; Tukey *post hoc* tests, *$P < 0.05$).

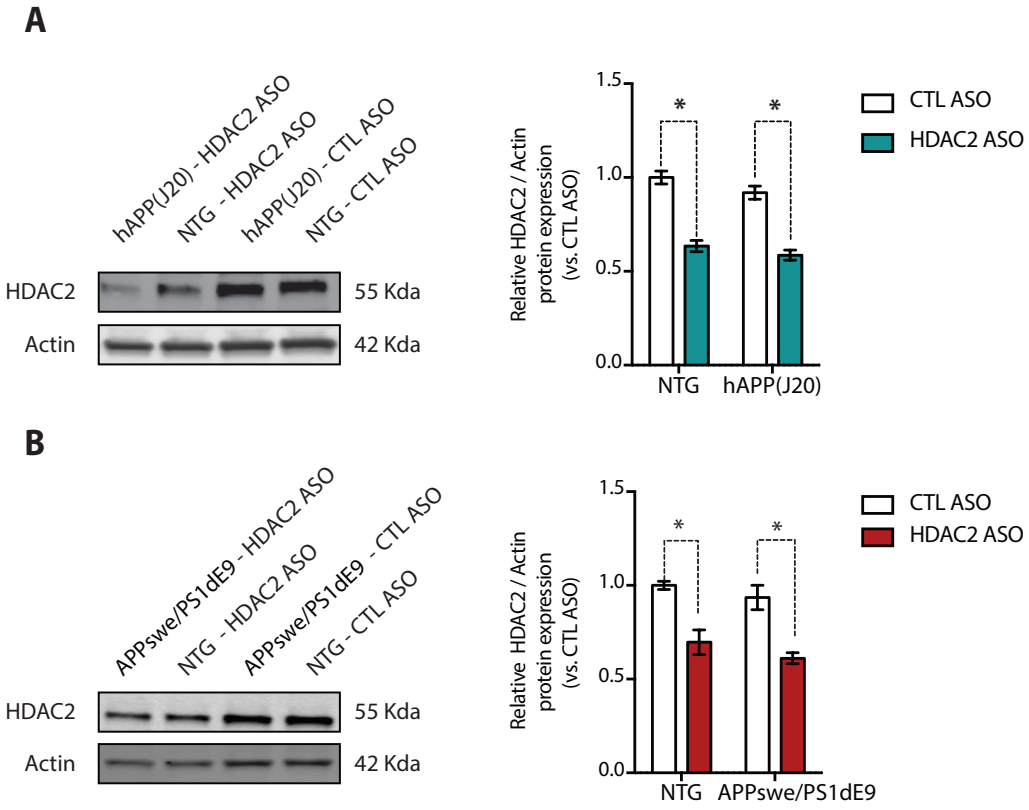


Figure S7. HDAC2 knockdown in hAPP(J20) and APPswe/PS1dE9 mice. (A) Immunoblots showing HDAC2 knockdown in hAPP(J20) mice (n = 18 to 27 mice per group; age 6 to 9 months; two-way ANOVA: treatment effect, $F_{1,88} = 113.8$, $P < 0.0001$; Tukey *post hoc* tests, $^*P < 0.05$). **(B)** Immunoblots showing HDAC2 knockdown in APPswe/PS1dE9 mice (n = 8 to 11 mice per group; age 12 to 13 months; two-way ANOVA: treatment effect, $F_{1,34} = 38.34$, $P < 0.0001$; Tukey *post hoc* tests, $^*P < 0.05$).

Table S1. Downregulated genes in hAPP(J20) mice.

Gene Name	NTG	hAPP(J20)	log2FoldChange	pvalue	padj
Gm16551	53	6	-1.431	6.61E-35	3.39E-31
Dsp	2550	693	-1.297	1.01E-33	3.89E-30
Onecut1	44	9	-1.150	4.01E-23	8.80E-20
Pdyn	367	158	-0.950	3.03E-23	7.77E-20
Spata13	438	204	-0.909	2.06E-25	6.34E-22
Adcy1	35150	18287	-0.875	4.31E-48	3.31E-44
Ptchd4	286	139	-0.861	6.46E-23	1.24E-19
Ryr1	820	359	-0.859	1.43E-16	1.22E-13
Pla2g2f	98	45	-0.750	4.29E-12	1.22E-09
Mkx	430	222	-0.739	3.39E-14	1.58E-11
Slc26a10	321	143	-0.739	3.72E-11	8.30E-09
Npnt	1014	520	-0.738	5.19E-14	2.33E-11
Cacng5	124	61	-0.735	2.89E-12	8.55E-10
Fam160a1	189	81	-0.727	1.59E-10	2.99E-08
Tnnt2	24	7	-0.660	7.32E-09	8.79E-07
Il1rap	1416	844	-0.656	5.80E-19	7.44E-16
Gfra2	605	272	-0.640	3.03E-08	3.10E-06
Il1r1	551	324	-0.639	1.07E-13	4.55E-11
Asic4	171	92	-0.638	1.88E-09	2.67E-07
Fam84b	756	421	-0.638	1.71E-10	3.17E-08
Zcchc5	106	22	-0.612	7.11E-09	8.61E-07
C630031E19Rik	87	40	-0.611	1.34E-07	1.05E-05
Adamts17	204	117	-0.602	1.20E-09	1.76E-07
Gabrd	499	312	-0.598	2.03E-15	1.36E-12
Ralgapa2	824	520	-0.593	1.55E-16	1.26E-13
Tbc1d8b	772	462	-0.592	1.01E-10	1.99E-08
Asb11	32	13	-0.587	3.75E-07	2.55E-05
Hectd2	1074	684	-0.573	2.36E-14	1.17E-11
Stc2	55	25	-0.573	8.01E-07	5.07E-05
Mcm6	384	238	-0.572	2.10E-11	5.09E-09
Card6	106	60	-0.571	4.52E-08	4.43E-06
Kcng3	104	52	-0.571	6.37E-07	4.17E-05
Upk1b	34	14	-0.569	8.33E-07	5.23E-05
1500017E21Rik	427	272	-0.562	1.50E-12	4.61E-10
Dgkh	1651	1047	-0.562	5.61E-12	1.51E-09
Lct	1565	866	-0.561	3.47E-07	2.40E-05

Fam83d	16	4	-0.558	2.44E-07	1.76E-05
Fam163b	4105	2634	-0.556	1.29E-12	4.15E-10
Cort	46	20	-0.550	2.10E-06	1.16E-04
Tac1	94	57	-0.543	4.93E-08	4.68E-06
Prox1	2124	1366	-0.539	1.11E-10	2.16E-08
Ramp3	47	22	-0.533	4.42E-06	2.14E-04
Zbtb20	1250	832	-0.532	1.61E-15	1.17E-12
Flt3	163	98	-0.529	5.14E-07	3.45E-05
Jph1	1697	1099	-0.527	5.67E-10	9.08E-08
Kitl	611	388	-0.524	1.58E-08	1.73E-06
Pcp4	1600	1050	-0.524	3.59E-11	8.11E-09
Osbpl3	297	195	-0.523	1.83E-11	4.69E-09
Gmnc	126	38	-0.521	1.55E-06	8.91E-05
Fat4	1572	979	-0.520	1.46E-07	1.13E-05
Plekhh2	286	187	-0.520	8.43E-10	1.31E-07
Stard13	493	324	-0.519	1.27E-10	2.44E-08
Bcl6	897	596	-0.516	3.94E-11	8.60E-09
Egfem1	214	138	-0.515	5.12E-09	6.40E-07
Vav3	327	213	-0.514	2.44E-09	3.29E-07
Rbm24	360	230	-0.513	6.38E-08	5.84E-06
Spry1	174	109	-0.508	3.47E-07	2.40E-05
Adamts1	405	264	-0.507	5.70E-09	7.01E-07
Calb1	2939	1803	-0.505	1.45E-06	8.39E-05
Wnt9a	223	93	-0.504	1.02E-05	4.15E-04
Scgn	31	10	-0.503	4.91E-06	2.29E-04
Capn3	270	158	-0.500	6.42E-06	2.82E-04
Dock11	409	274	-0.499	7.64E-10	1.20E-07
Pip5k1b	827	562	-0.493	3.97E-11	8.60E-09
Kcng2	356	241	-0.490	1.80E-10	3.29E-08
Frzb	498	310	-0.483	5.02E-06	2.32E-04
Upp1	40	22	-0.479	3.20E-05	1.02E-03
Tmem144	278	186	-0.478	2.49E-08	2.61E-06
Grb14	374	254	-0.477	4.40E-09	5.64E-07
Itga7	474	322	-0.476	9.06E-09	1.06E-06
Pde7b	621	413	-0.475	2.24E-07	1.64E-05
Pqlc1	373	260	-0.475	2.59E-13	9.95E-11
Magi1	1580	1107	-0.471	1.76E-14	9.00E-12
Ptgr1	62	36	-0.470	2.89E-05	9.33E-04
Fst	33	9	-0.469	5.89E-06	2.63E-04

Ahcyl2	5889	4122	-0.466	4.74E-12	1.30E-09
Dbpht2	1063	740	-0.462	3.19E-10	5.34E-08
Moxd1	128	84	-0.462	6.87E-06	2.97E-04
Arhgap20	2078	1468	-0.460	4.06E-13	1.49E-10
Tgfa	590	414	-0.454	3.43E-10	5.67E-08
Abcc8	651	453	-0.453	1.90E-08	2.01E-06
Dgat2	1628	1155	-0.452	1.35E-12	4.25E-10
Egr1	1352	801	-0.452	7.23E-05	1.96E-03
Ppp1r16b	4295	3085	-0.450	1.33E-16	1.20E-13
Sh3bgr	36	21	-0.450	9.61E-05	2.49E-03
Lrrtm4	1420	1006	-0.447	9.44E-11	1.89E-08
Mn1	525	370	-0.447	3.67E-09	4.78E-07
9930014A18Rik	235	161	-0.446	6.41E-07	4.17E-05
Isoc1	416	288	-0.445	2.64E-07	1.89E-05
Scn3b	5884	4227	-0.434	8.76E-11	1.77E-08
Ccdc85a	2852	2043	-0.432	1.11E-09	1.64E-07
Fam13a	556	395	-0.430	9.69E-09	1.12E-06
Rasgrf2	2123	1531	-0.430	8.61E-11	1.77E-08
Arl4d	91	58	-0.428	7.34E-05	1.98E-03
Palm2	1292	945	-0.428	1.45E-18	1.59E-15
Ntf3	162	95	-0.427	2.19E-04	4.84E-03
Cblb	1054	769	-0.425	5.29E-14	2.33E-11
Frmpd1	244	172	-0.423	3.10E-07	2.20E-05
Pgm5	219	156	-0.423	1.28E-07	1.01E-05
B3galt5	1474	1056	-0.420	7.16E-08	6.33E-06
Gm19522	53	33	-0.420	2.11E-04	4.68E-03
Asxl3	291	204	-0.419	1.71E-06	9.65E-05
Kcnh5	93	64	-0.419	1.14E-05	4.46E-04
Cenpa	20	9	-0.417	2.25E-04	4.96E-03
Cttnbp2	5166	3847	-0.408	8.49E-20	1.31E-16
Wipf3	9653	7057	-0.407	4.39E-09	5.64E-07
Gypc	38	23	-0.405	4.71E-04	8.85E-03
Etv4	125	87	-0.404	2.60E-05	8.58E-04
Slc9a5	235	165	-0.404	1.46E-05	5.38E-04
Zbtb1	407	298	-0.404	1.00E-09	1.51E-07
Sypl2	79	53	-0.403	1.61E-04	3.76E-03
Il27ra	38	19	-0.402	4.12E-04	8.01E-03
Pter	3385	2502	-0.402	5.92E-11	1.23E-08
Rgs13	23	12	-0.402	4.50E-04	8.54E-03

Zfp607	66	45	-0.401	2.28E-04	5.00E-03
Fam19a2	1571	1146	-0.400	1.03E-07	8.48E-06
Umodl1	47	29	-0.397	5.68E-04	1.02E-02
Egfl6	75	52	-0.396	1.40E-04	3.35E-03
Fam150b	54	36	-0.395	4.12E-04	8.01E-03
Lrrc16a	561	413	-0.395	3.43E-08	3.47E-06
Pcdh20	3532	2596	-0.394	7.20E-08	6.33E-06
Trdn	9	2	-0.394	2.39E-05	7.97E-04
Cecr2	374	262	-0.391	7.67E-05	2.05E-03
Slc35g1	50	33	-0.391	4.81E-04	8.96E-03
Cdh8	1357	1008	-0.390	2.44E-09	3.29E-07
Gch1	29	16	-0.390	7.39E-04	1.25E-02
Il20rb	88	59	-0.390	2.73E-04	5.82E-03
Lpar4	118	84	-0.389	1.74E-05	6.19E-04
Tdo2	208	65	-0.387	1.02E-04	2.62E-03
Wnk4	137	94	-0.387	2.10E-04	4.67E-03
Trim71	37	20	-0.386	7.98E-04	1.30E-02
Dhx33	1792	1313	-0.385	2.20E-06	1.19E-04
Fos	89	47	-0.385	7.77E-04	1.28E-02
Hlf	3095	2234	-0.384	2.12E-05	7.25E-04
Foxq1	63	42	-0.383	3.77E-04	7.44E-03
Plcl1	878	643	-0.383	1.92E-06	1.08E-04
Rasal2	3322	2450	-0.382	1.05E-06	6.55E-05
Sema5b	218	158	-0.382	3.52E-06	1.76E-04
Arhgap20os	61	42	-0.381	4.24E-04	8.19E-03
C1ql3	6207	4661	-0.381	9.41E-10	1.43E-07
D16Ertd472e	374	268	-0.381	6.07E-05	1.71E-03
Btbd3	8807	6587	-0.380	1.45E-08	1.60E-06
Kalrn	15017	11444	-0.379	3.46E-20	5.91E-17
Npas4	86	59	-0.379	4.13E-04	8.02E-03
Tmem38b	363	268	-0.379	3.28E-06	1.66E-04
Fhad1	123	84	-0.377	3.07E-04	6.41E-03
Dusp4	370	267	-0.376	4.44E-05	1.34E-03
Igsf3	649	480	-0.376	1.30E-06	7.75E-05
Sertad4	422	313	-0.375	7.08E-07	4.54E-05
Tspan18	604	420	-0.374	3.65E-04	7.27E-03
Inf2	1705	1239	-0.372	4.56E-05	1.36E-03
Srl	239	174	-0.372	2.04E-05	7.05E-04
Sycp2	48	32	-0.372	7.74E-04	1.28E-02

Rasd1	326	226	-0.371	5.30E-04	9.64E-03
Cap2	4313	3306	-0.370	5.70E-18	5.84E-15
Maml2	708	497	-0.370	3.44E-04	6.93E-03
Stc1	78	53	-0.370	7.78E-04	1.28E-02
Hsd11b1	439	330	-0.369	1.28E-07	1.01E-05
Cry1	215	159	-0.368	1.13E-05	4.46E-04
Slc30a3	1456	1103	-0.365	3.07E-08	3.13E-06
Ttn	119	74	-0.365	1.66E-03	2.29E-02
Ablim3	758	561	-0.364	2.39E-05	7.97E-04
Mir568	56	38	-0.361	9.34E-04	1.47E-02
2010300C02Rik	8060	6181	-0.360	4.25E-11	9.08E-09
Btg2	333	241	-0.360	1.62E-04	3.77E-03
Cartpt	15	7	-0.360	1.14E-03	1.71E-02
Acot5	61	39	-0.359	1.76E-03	2.40E-02
Kcnj14	26	16	-0.359	2.02E-03	2.68E-02
Tenm1	811	614	-0.359	3.60E-07	2.46E-05
Abca8a	266	195	-0.358	1.64E-04	3.81E-03
Marcksl1	1079	813	-0.358	2.29E-06	1.23E-04
Orai2	3228	2443	-0.358	6.91E-07	4.47E-05
Kif26b	412	298	-0.353	3.68E-04	7.31E-03
Sema5a	5555	4105	-0.353	1.05E-04	2.68E-03
Kbtbd11	7160	5515	-0.352	1.10E-09	1.64E-07
Dock10	3265	2328	-0.351	7.49E-04	1.26E-02
F730043M19Rik	313	228	-0.351	3.88E-04	7.63E-03
Smim3	140	103	-0.350	1.46E-04	3.46E-03
4933427G17Rik	38	24	-0.349	2.46E-03	3.06E-02
Clvs2	874	670	-0.349	1.58E-07	1.19E-05
Cngb1	15	7	-0.349	1.37E-03	1.97E-02
Nrarp	241	182	-0.349	1.98E-05	6.91E-04
Zdhhc23	171	127	-0.349	6.83E-05	1.87E-03
Clspn	35	22	-0.348	2.72E-03	3.29E-02
Jag1	254	185	-0.348	5.06E-04	9.27E-03
Mlip	161	121	-0.348	3.83E-05	1.18E-03
Rfx3	4845	3645	-0.348	2.76E-05	8.98E-04
Egr4	153	106	-0.346	1.77E-03	2.41E-02
Mfap3	770	589	-0.345	2.12E-06	1.16E-04
Sipa1l2	1776	1351	-0.344	1.19E-05	4.60E-04
Nhsl2	2636	2043	-0.343	3.12E-09	4.13E-07
Npy1r	739	562	-0.343	8.77E-06	3.63E-04

Pdzrn4	142	102	-0.343	8.39E-04	1.35E-02
Galnt6	102	76	-0.342	2.55E-04	5.52E-03
BC048546	60	42	-0.341	2.25E-03	2.89E-02
Amer3	929	716	-0.340	6.94E-07	4.47E-05
Trabd2b	63	40	-0.340	3.46E-03	3.90E-02
Epha7	5368	4044	-0.338	1.21E-04	2.99E-03
Wdhd1	206	153	-0.338	2.77E-04	5.91E-03
Rnf19a	1112	863	-0.337	5.43E-08	5.12E-06
Apln	239	181	-0.336	2.28E-05	7.73E-04
Kirrel2	130	96	-0.336	1.07E-03	1.64E-02
Cdc42ep4	596	459	-0.335	7.84E-06	3.31E-04
Ddit4l	138	102	-0.335	7.85E-04	1.29E-02
1700086L19Rik	300	229	-0.334	2.54E-05	8.44E-04
Fam114a2	638	498	-0.334	6.85E-09	8.36E-07
Foxn2	760	588	-0.334	1.03E-06	6.47E-05
Pm20d2	472	360	-0.334	5.43E-05	1.57E-03
Zfhx4	516	398	-0.334	3.91E-06	1.92E-04
2900052N01Rik	956	716	-0.332	3.62E-04	7.23E-03
9830166K06Rik	64	44	-0.331	3.61E-03	4.02E-02
Rps6ka2	787	611	-0.331	5.70E-07	3.78E-05
Cdh23	37	24	-0.330	4.47E-03	4.74E-02
Doc2b	2724	2091	-0.330	2.97E-05	9.55E-04
Prdm8	1311	993	-0.330	2.04E-04	4.56E-03
Vwa5b1	25	11	-0.330	1.94E-03	2.59E-02
Tnxb	411	307	-0.329	6.39E-04	1.11E-02
Chrdl1	107	79	-0.328	1.38E-03	1.98E-02
Pwwp2a	796	623	-0.328	3.28E-07	2.30E-05
Cacna1h	2809	2124	-0.327	3.11E-04	6.47E-03
Dmp1	108	80	-0.327	7.89E-04	1.29E-02
Gdf10	200	154	-0.327	5.26E-05	1.53E-03
Nr4a1	617	416	-0.327	4.48E-03	4.75E-02
Stard10	193	146	-0.327	3.07E-04	6.42E-03
A630089N07Rik	71	52	-0.326	1.87E-03	2.51E-02
Cntnap5b	343	265	-0.326	1.14E-05	4.46E-04
Zfp947	25	15	-0.326	4.21E-03	4.53E-02
Npy5r	181	136	-0.325	1.73E-04	3.98E-03
Zbtb16	1657	1300	-0.325	4.27E-07	2.89E-05
Bhlhe22	2069	1606	-0.324	1.14E-05	4.47E-04
Fbn1	363	277	-0.322	2.76E-04	5.89E-03

Ptprj	3390	2667	-0.322	4.60E-08	4.45E-06
Bcas1	1034	798	-0.321	5.44E-05	1.57E-03
Camkk2	2251	1771	-0.321	2.18E-07	1.60E-05
Mc4r	80	57	-0.321	3.80E-03	4.19E-02
Ngef	1492	1182	-0.321	1.86E-10	3.36E-08
Tiam1	3430	2668	-0.321	1.39E-05	5.20E-04
Sowaha	3157	2475	-0.320	1.15E-06	7.01E-05
Sik1	175	128	-0.319	2.56E-03	3.15E-02
Add2	9684	7706	-0.318	2.89E-13	1.08E-10
Ippk	643	506	-0.318	2.63E-06	1.37E-04
Rras2	490	380	-0.317	3.10E-05	9.90E-04
Hnrnpa3	1227	974	-0.316	2.03E-09	2.87E-07
Lysmd3	194	151	-0.315	1.38E-04	3.31E-03
Foxk1	2105	1670	-0.314	9.97E-09	1.14E-06
Il16	624	477	-0.314	4.49E-04	8.53E-03
Kcnd2	2724	2172	-0.314	2.01E-11	5.08E-09
Zfp882	261	206	-0.314	1.74E-05	6.19E-04
Rcor2	186	142	-0.313	5.18E-04	9.43E-03
Rph3a	5878	4620	-0.313	6.23E-06	2.75E-04
Slc16a10	145	105	-0.312	3.67E-03	4.07E-02
Arhgef3	1449	1148	-0.311	3.31E-07	2.32E-05
Hhip	91	68	-0.310	2.58E-03	3.17E-02
Igfbp4	899	704	-0.310	5.66E-05	1.62E-03
Pdp1	1295	1034	-0.310	2.23E-10	3.94E-08
Rai2	127	96	-0.310	1.03E-03	1.58E-02
Atp10d	77	58	-0.309	3.06E-03	3.57E-02
Cenpp	45	31	-0.309	6.07E-03	5.88E-02
Cxcl12	1450	1136	-0.308	6.24E-05	1.74E-03
Lamp5	540	422	-0.308	1.17E-04	2.92E-03
Nfxl1	261	207	-0.307	1.52E-05	5.58E-04
Zhx2	534	423	-0.307	1.42E-05	5.27E-04
Fat3	1770	1402	-0.306	2.62E-06	1.37E-04
Dnajb5	2016	1596	-0.305	5.54E-06	2.51E-04
LOC102634101	22	14	-0.305	8.37E-03	7.46E-02
Iqgap2	2939	2352	-0.304	1.24E-08	1.39E-06
Ntrk3	2055	1641	-0.304	2.74E-08	2.85E-06
Pdzd2	1584	1028	-0.303	9.21E-03	8.01E-02
Kcnk9	254	199	-0.302	4.30E-04	8.26E-03
4930455H04Rik	20	12	-0.299	9.32E-03	8.08E-02

Acvr2a	1550	1230	-0.299	1.28E-05	4.88E-04
Cyth4	314	244	-0.298	5.78E-04	1.03E-02
Gm5415	40	28	-0.298	8.56E-03	7.59E-02
Kcnt2	618	496	-0.298	2.04E-07	1.51E-05
Klf13	4912	3948	-0.298	1.78E-08	1.90E-06
Rapgef5	2119	1690	-0.298	2.53E-06	1.33E-04
Bcl11b	4680	3760	-0.297	8.71E-08	7.45E-06
Ccnjl	161	120	-0.297	5.20E-03	5.24E-02
Syt10	143	106	-0.297	5.20E-03	5.24E-02
Zfp189	524	415	-0.297	1.91E-04	4.31E-03
A330050F15Rik	251	195	-0.296	7.94E-04	1.30E-02
Map3k5	467	376	-0.296	5.38E-07	3.59E-05
Mkl2	6583	5276	-0.296	7.45E-07	4.74E-05
Slc4a4	5474	4414	-0.296	1.41E-09	2.03E-07
St8sia6	51	36	-0.295	9.40E-03	8.12E-02
2810025M15Rik	187	145	-0.294	2.06E-03	2.71E-02
B4galt4	540	428	-0.294	5.31E-05	1.54E-03
Cys1	135	102	-0.294	4.65E-03	4.87E-02
Klf9	2828	2278	-0.293	8.87E-08	7.51E-06
Mtrr	267	212	-0.293	2.47E-04	5.35E-03
Nhlh1	44	10	-0.293	4.53E-04	8.55E-03
Syt7	8387	6697	-0.293	1.99E-05	6.91E-04
Jdp2	352	282	-0.292	1.84E-05	6.46E-04
Arpc2	3987	3231	-0.291	3.83E-10	6.27E-08
Spns2	1110	888	-0.291	3.73E-05	1.16E-03
Arsi	17	10	-0.290	1.03E-02	8.59E-02
Cdh6	43	28	-0.290	1.23E-02	9.78E-02
Nme9	15	8	-0.290	9.51E-03	8.16E-02
Cbx2	42	29	-0.289	1.17E-02	9.44E-02
Fnip2	631	501	-0.289	1.99E-04	4.48E-03
Grik3	955	755	-0.289	3.24E-04	6.67E-03
Pls1	160	126	-0.289	8.68E-04	1.39E-02
Gpr61	409	329	-0.287	9.71E-06	3.96E-04
Igsf1	215	165	-0.287	4.65E-03	4.87E-02
Dio2	1306	1058	-0.286	1.66E-07	1.25E-05
Plcg2	126	97	-0.286	3.54E-03	3.96E-02
Drd5	298	228	-0.285	5.13E-03	5.22E-02
Elmo2	3077	2489	-0.285	1.22E-06	7.32E-05
Tgfb2	978	787	-0.285	1.68E-05	6.02E-04

Zcchc7	703	570	-0.285	1.63E-08	1.75E-06
1810010H24Rik	43	31	-0.284	1.14E-02	9.23E-02
Ano3	4775	3828	-0.284	8.57E-05	2.26E-03
Egr2	29	12	-0.284	3.88E-03	4.25E-02
Nek10	100	72	-0.284	1.22E-02	9.73E-02
Pgbd5	3655	2954	-0.284	3.85E-06	1.90E-04
Palmd	431	349	-0.283	4.84E-06	2.27E-04
Tbr1	646	509	-0.283	1.31E-03	1.90E-02
Vps37b	555	446	-0.283	2.29E-05	7.76E-04
Bora	55	41	-0.282	9.19E-03	8.00E-02
Echdc2	194	146	-0.281	1.01E-02	8.50E-02
Gls2	180	144	-0.279	1.02E-03	1.57E-02
Slc25a37	354	286	-0.279	1.24E-04	3.05E-03
Sphkap	6451	5229	-0.279	6.87E-06	2.97E-04
Dcxr	58	43	-0.278	9.50E-03	8.15E-02
Rtp1	11	5	-0.278	4.65E-03	4.87E-02
Sh3kbp1	557	447	-0.278	9.33E-05	2.43E-03
Tunar	406	316	-0.278	4.14E-03	4.47E-02
Camk2b	26852	21881	-0.277	7.41E-07	4.73E-05
Neurod2	2387	1937	-0.276	1.89E-05	6.60E-04
Il33	477	382	-0.274	3.83E-04	7.54E-03
Ankrd13a	589	481	-0.273	3.14E-06	1.60E-04
Zfp536	353	284	-0.272	1.80E-04	4.10E-03
Scn3a	2200	1798	-0.271	2.37E-06	1.26E-04
Suv39h2	119	95	-0.271	2.38E-03	3.00E-02
Tmem81	90	71	-0.270	6.37E-03	6.07E-02
Mertk	675	555	-0.269	4.82E-06	2.27E-04
2610018G03Rik	158	129	-0.268	7.55E-04	1.26E-02
Dbndd2	1434	1183	-0.268	7.23E-12	1.92E-09
Junb	436	354	-0.268	6.89E-04	1.18E-02
Cep152	114	89	-0.267	5.99E-03	5.82E-02
Cyp4f15	186	148	-0.267	1.77E-03	2.40E-02
Kcnip2	2850	2333	-0.267	1.14E-05	4.46E-04
Rpusd3	142	114	-0.267	1.73E-03	2.37E-02
Trpm3	1838	1490	-0.267	2.88E-04	6.08E-03
Ddit4	920	745	-0.266	6.02E-04	1.06E-02
Dapk1	3010	2472	-0.265	8.88E-06	3.66E-04
Gjc3	480	393	-0.265	2.38E-05	7.97E-04
Mthfd2l	62	48	-0.265	1.26E-02	9.92E-02

Tbc1d16	1143	935	-0.265	3.91E-05	1.20E-03
Ankrd45	1054	862	-0.264	5.71E-05	1.63E-03
Dab1	2568	2130	-0.264	1.62E-13	6.56E-11
Gm15880	9	4	-0.264	7.81E-03	7.07E-02
Grm2	1219	961	-0.264	7.04E-03	6.57E-02
Qtrtd1	364	298	-0.264	1.46E-04	3.45E-03
Scarb1	407	334	-0.264	1.52E-05	5.58E-04
Vrk1	328	266	-0.264	1.36E-04	3.30E-03
Cacng3	785	646	-0.263	1.25E-07	1.01E-05
Parvb	397	321	-0.262	8.24E-04	1.34E-02
Chd7	535	434	-0.261	1.16E-03	1.74E-02
Lefty1	116	91	-0.261	1.12E-02	9.14E-02
Stxbp6	4181	3259	-0.261	1.19E-02	9.52E-02
Dusp6	717	584	-0.260	1.00E-03	1.55E-02
Gpr146	284	232	-0.260	4.80E-04	8.96E-03
Sox8	476	390	-0.260	3.20E-04	6.60E-03
Akap7	731	603	-0.259	1.24E-05	4.75E-04
C630043F03Rik	317	258	-0.259	1.34E-03	1.94E-02
Fzd2	112	89	-0.258	9.79E-03	8.31E-02
Gm5069	179	142	-0.258	7.92E-03	7.15E-02
Ipw	508	415	-0.257	1.07E-03	1.63E-02
Boc	200	165	-0.255	8.89E-04	1.41E-02
Ddn	43204	35380	-0.255	5.46E-04	9.83E-03
Gpr12	321	259	-0.255	3.88E-03	4.25E-02
Hdac1	464	384	-0.255	8.14E-06	3.41E-04
Lrrn1	1800	1493	-0.255	6.28E-07	4.13E-05
D3Bwg0562e	10588	8814	-0.254	7.83E-09	9.20E-07
Kansl1l	399	328	-0.254	6.76E-04	1.16E-02
Pxdc1	130	106	-0.254	5.94E-03	5.78E-02
Rasgrf1	9873	8238	-0.254	2.66E-12	8.01E-10
Adcy8	365	299	-0.253	8.21E-04	1.33E-02
Dok4	306	252	-0.253	8.51E-04	1.37E-02
Fam131a	6855	5694	-0.253	2.43E-06	1.29E-04
Mef2c	3480	2871	-0.253	1.12E-04	2.83E-03
Adamts20	477	387	-0.252	3.83E-03	4.21E-02
Nox1	135	110	-0.252	3.82E-03	4.21E-02
Obscn	106	84	-0.251	1.02E-02	8.53E-02
Lonrf3	958	794	-0.250	1.10E-04	2.78E-03
Sec14l1	4026	3378	-0.250	4.58E-15	2.71E-12

Diap1	1286	1073	-0.249	4.25E-06	2.07E-04
Rmdn1	203	166	-0.249	6.10E-04	1.07E-02
Slc6a15	1581	1307	-0.249	2.46E-04	5.35E-03
1700010K23Rik	262	216	-0.248	1.15E-03	1.73E-02
Fam222b	570	476	-0.248	1.65E-06	9.38E-05
Kctd4	1946	1583	-0.248	4.22E-03	4.53E-02
Nedd4l	4703	3900	-0.248	1.03E-04	2.64E-03
Ssbp2	1635	1362	-0.248	7.52E-06	3.22E-04
Trim59	172	137	-0.248	9.65E-03	8.22E-02
Cyth1	1788	1482	-0.247	2.10E-04	4.67E-03
Luzp2	2881	2379	-0.247	4.85E-04	8.98E-03
Matn4	136	111	-0.247	4.59E-03	4.83E-02
Vps13c	4176	3452	-0.247	4.48E-04	8.51E-03
9130019P16Rik	338	279	-0.246	4.92E-04	9.09E-03
Arhgap39	2403	1998	-0.246	1.10E-04	2.78E-03
Fam151a	197	160	-0.246	3.34E-03	3.79E-02
Gabpb2	1068	879	-0.246	1.68E-03	2.32E-02
Glul	27979	23350	-0.246	5.93E-06	2.63E-04
Lrrc1	87	70	-0.246	1.05E-02	8.72E-02
Etv5	797	652	-0.245	3.92E-03	4.27E-02
Lurap1	140	115	-0.245	3.07E-03	3.57E-02
Pou3f2	267	221	-0.245	1.17E-03	1.75E-02
Tpd52l1	403	336	-0.245	2.74E-05	8.94E-04
Acot11	725	604	-0.244	2.99E-06	1.53E-04
AI464131	534	444	-0.244	4.55E-05	1.36E-03
Hs3st1	154	125	-0.244	5.78E-03	5.68E-02
Gse1	2260	1873	-0.243	5.78E-04	1.03E-02
Neurod1	655	539	-0.243	2.51E-03	3.11E-02
Slc27a2	96	77	-0.243	1.17E-02	9.42E-02
Dtna	2892	2417	-0.242	1.54E-05	5.60E-04
Fam163a	251	206	-0.242	2.35E-03	2.97E-02
Kbtbd8	194	159	-0.242	2.70E-03	3.27E-02
Cry2	2114	1778	-0.241	1.20E-07	9.74E-06
Emx2os	258	214	-0.241	2.31E-03	2.95E-02
Abhd10	310	256	-0.240	1.41E-03	2.02E-02
Bhlhe40	1492	1225	-0.240	4.81E-03	4.98E-02
C030046E11Rik	1335	1123	-0.240	6.22E-08	5.76E-06
Prkd1	325	270	-0.239	7.37E-04	1.25E-02
Siah2	141	116	-0.239	5.53E-03	5.49E-02

Smarcd2	345	286	-0.239	3.40E-03	3.84E-02
Susd1	300	249	-0.239	2.92E-03	3.45E-02
Zbtb18	10706	8924	-0.239	3.63E-04	7.23E-03
Gpd1	1868	1566	-0.238	1.04E-05	4.19E-04
Irak1bp1	325	272	-0.238	2.83E-04	5.98E-03
Plekhh3	132	109	-0.238	4.39E-03	4.67E-02
Kcnj11	589	493	-0.237	1.13E-04	2.86E-03
Them6	381	317	-0.237	1.68E-04	3.91E-03
Fam188a	1009	850	-0.236	1.43E-06	8.29E-05
Klhdc7a	336	280	-0.236	8.34E-04	1.35E-02
Nt5c1a	103	84	-0.236	1.18E-02	9.45E-02
Plxna4	6472	5425	-0.236	7.35E-05	1.98E-03
Rgs7bp	6455	5425	-0.236	1.22E-05	4.71E-04
Tub	5300	4413	-0.236	1.02E-03	1.57E-02
Grin2c	819	686	-0.235	2.31E-04	5.05E-03
Mast4	1254	1054	-0.235	1.08E-05	4.31E-04
Nfia	2056	1733	-0.235	5.99E-07	3.96E-05
Onecut2	399	334	-0.235	5.85E-04	1.04E-02
Elovl2	320	266	-0.234	4.71E-04	8.85E-03
Rasgrp4	107	88	-0.234	1.25E-02	9.86E-02
St8sia4	451	377	-0.234	1.78E-04	4.07E-03
Gm9962	411	344	-0.233	5.37E-04	9.73E-03
Mars2	184	152	-0.233	1.98E-03	2.63E-02
2700069I18Rik	8	3	-0.232	8.83E-03	7.75E-02
Gria2	26103	22126	-0.232	4.62E-10	7.48E-08
AI593442	4423	3649	-0.231	7.67E-03	6.97E-02
Fam181b	154	126	-0.231	8.22E-03	7.37E-02
Kdm7a	2667	2234	-0.231	7.58E-04	1.26E-02
Mctp1	1496	1258	-0.231	2.03E-04	4.54E-03
Zfp867	237	196	-0.231	2.82E-03	3.38E-02
Cdr2l	374	312	-0.229	3.28E-03	3.75E-02
Cygb	857	705	-0.229	1.08E-02	8.87E-02
Gstm7	340	283	-0.229	2.30E-03	2.94E-02
Kcnq3	1003	846	-0.229	2.46E-06	1.31E-04
Tmtc1	1538	1293	-0.229	3.45E-04	6.93E-03
Klf10	478	398	-0.228	6.14E-03	5.93E-02
Lca5	312	262	-0.228	7.39E-04	1.25E-02
Limd2	1320	1110	-0.228	4.94E-04	9.11E-03
Mmp16	764	636	-0.228	3.64E-03	4.04E-02

Map3k4	1144	959	-0.227	2.44E-03	3.04E-02
Map6d1	734	621	-0.227	7.80E-06	3.30E-04
Elmsan1	826	690	-0.226	3.36E-03	3.81E-02
Samd4	366	310	-0.226	2.17E-04	4.80E-03
Tiam2	586	490	-0.226	2.74E-03	3.30E-02
Eepd1	239	201	-0.225	1.27E-03	1.86E-02
Gabra5	3399	2888	-0.225	2.31E-06	1.24E-04
Nova1	770	654	-0.225	1.06E-05	4.28E-04
Ccdc28b	213	180	-0.224	5.00E-03	5.12E-02
Cdh19	126	105	-0.223	9.34E-03	8.09E-02
Mast3	6477	5501	-0.223	2.14E-05	7.29E-04
Ppfia2	5473	4614	-0.223	9.17E-04	1.45E-02
Axin2	495	416	-0.222	2.02E-03	2.68E-02
Chst12	200	167	-0.222	7.41E-03	6.80E-02
Cdr1	392	327	-0.221	6.32E-03	6.04E-02
Egr3	1989	1680	-0.221	1.33E-03	1.93E-02
Extl2	1552	1320	-0.221	2.01E-05	6.96E-04
Gpr39	824	692	-0.221	4.31E-03	4.60E-02
Lgi1	3465	2941	-0.221	7.54E-05	2.02E-03
Mobp	1060	888	-0.221	4.37E-03	4.65E-02
Ppp3ca	28133	23793	-0.221	7.52E-04	1.26E-02
Sidt1	1094	928	-0.221	3.69E-04	7.31E-03
Wwc1	1196	1015	-0.221	6.08E-05	1.71E-03
4833424O15Rik	1342	1141	-0.220	4.66E-05	1.38E-03
Ago1	2284	1950	-0.220	5.71E-08	5.36E-06
Bcor	372	313	-0.220	7.48E-03	6.85E-02
Slc9a3r1	604	515	-0.219	4.76E-05	1.40E-03
Lrrn3	1374	1160	-0.218	1.87E-03	2.52E-02
Shisa7	3866	3292	-0.218	1.17E-04	2.92E-03
Zswim5	437	370	-0.218	8.77E-04	1.40E-02
Parp1	1840	1570	-0.217	3.69E-05	1.15E-03
Sgk3	161	134	-0.217	5.94E-03	5.78E-02
Zfp61	250	212	-0.217	2.76E-03	3.32E-02
Pard3	366	310	-0.216	1.63E-03	2.26E-02
Twistnb	216	181	-0.216	5.20E-03	5.24E-02
Ip6k2	1638	1402	-0.215	1.37E-05	5.18E-04
Tmem74	204	170	-0.215	1.20E-02	9.57E-02
Cdyl	149	125	-0.214	8.43E-03	7.50E-02
Kcnip4	976	836	-0.214	6.95E-05	1.89E-03

Pitpnm2	6877	5762	-0.214	1.22E-02	9.73E-02
Kcnc3	3498	2964	-0.213	3.50E-03	3.93E-02
Runx2	296	252	-0.213	3.06E-03	3.57E-02
Acot13	645	549	-0.212	3.41E-04	6.89E-03
Cbfb	611	522	-0.212	1.78E-04	4.08E-03
Fam76b	527	449	-0.212	1.17E-04	2.92E-03
Otud7b	1094	937	-0.212	5.31E-06	2.44E-04
Slc24a5	680	582	-0.212	4.21E-04	8.16E-03
4930570G19Rik	285	240	-0.211	6.37E-03	6.07E-02
Mgat5b	752	641	-0.211	1.40E-03	2.01E-02
Cadm2	7525	6436	-0.210	4.38E-04	8.40E-03
Ccdc12	278	236	-0.210	1.23E-03	1.81E-02
Fut8	1457	1248	-0.210	2.28E-04	5.00E-03
Pde10a	1649	1415	-0.210	4.06E-05	1.24E-03
Sbk1	1158	988	-0.210	1.48E-03	2.10E-02
Zfp667	469	400	-0.210	1.82E-03	2.46E-02
Btbd9	3016	2585	-0.209	1.83E-04	4.16E-03
Mbp	12394	10556	-0.209	2.55E-03	3.15E-02
Nwd1	1547	1332	-0.209	5.89E-08	5.49E-06
Trim9	4921	4228	-0.209	5.43E-06	2.47E-04
Cdh12	307	259	-0.208	8.57E-03	7.59E-02
Daam2	1592	1364	-0.208	9.98E-05	2.57E-03
Lypd1	1002	850	-0.208	6.24E-03	5.99E-02
Nr3c2	2736	2340	-0.208	6.31E-04	1.10E-02
Apcdd1	464	394	-0.207	4.72E-03	4.92E-02
Cd34	712	602	-0.207	1.20E-02	9.60E-02
Fkbp5	1260	1070	-0.207	6.30E-03	6.02E-02
Ncdn	39398	33738	-0.207	7.46E-04	1.25E-02
Cbs	451	385	-0.206	6.95E-04	1.19E-02
Eml4	1064	915	-0.206	1.16E-05	4.53E-04
Nrn1	4803	4086	-0.206	5.73E-03	5.64E-02
Slc8a2	9272	7888	-0.206	5.08E-03	5.18E-02
Taf8	227	195	-0.206	3.67E-03	4.07E-02
A830018L16Rik	2029	1743	-0.205	4.51E-04	8.54E-03
Emx2	327	281	-0.205	1.10E-03	1.67E-02
Mxd1	379	327	-0.205	3.13E-03	3.63E-02
Ddx21	613	527	-0.204	8.87E-05	2.33E-03
Gpc4	1340	1137	-0.204	1.04E-02	8.67E-02
Pde1b	2501	2144	-0.204	1.22E-03	1.80E-02

Prdm2	1545	1332	-0.204	2.71E-05	8.86E-04
Slc2a13	3112	2682	-0.204	2.58E-05	8.54E-04
Eif4ebp2	339	289	-0.203	2.21E-03	2.84E-02
Mios	512	439	-0.203	3.36E-04	6.84E-03
Sema7a	2071	1772	-0.203	3.02E-03	3.53E-02
Dscam	1227	1057	-0.202	1.77E-05	6.26E-04
Lmo3	2097	1798	-0.202	1.99E-03	2.65E-02
Ptma	2774	2391	-0.202	1.30E-04	3.18E-03
Acap2	3968	3406	-0.201	2.42E-03	3.03E-02
Afap1l2	196	166	-0.201	1.19E-02	9.53E-02
Fbxl18	298	255	-0.201	2.21E-03	2.84E-02
Heca	540	463	-0.201	5.18E-03	5.24E-02
Ptbp3	984	848	-0.201	1.72E-03	2.36E-02
Auts2	2549	2187	-0.199	3.57E-03	3.99E-02
Ezr	936	808	-0.199	1.11E-04	2.79E-03
Irs2	504	432	-0.199	4.71E-03	4.91E-02
Sfpq	4012	3482	-0.199	1.21E-06	7.28E-05
Igsf11	697	601	-0.198	2.07E-04	4.63E-03
Slc1a1	3214	2792	-0.198	1.13E-07	9.21E-06
Brpf1	779	674	-0.197	3.13E-04	6.50E-03
Chn1os3	1856	1599	-0.197	1.90E-03	2.55E-02
Gpr37	657	565	-0.197	1.63E-03	2.26E-02
Lgr4	882	765	-0.197	1.40E-05	5.24E-04
Nab2	611	523	-0.197	9.65E-03	8.22E-02
Ppp2r1b	374	322	-0.197	2.06E-03	2.72E-02
Slc12a4	279	241	-0.197	3.13E-03	3.63E-02
Wee1	365	314	-0.197	3.30E-03	3.75E-02
Ccdc43	485	422	-0.196	8.59E-04	1.38E-02
Kcnj3	1230	1064	-0.196	1.50E-04	3.54E-03
Notch1	634	551	-0.196	1.80E-04	4.10E-03
Rlf	940	814	-0.196	3.45E-05	1.09E-03
Syt16	1090	942	-0.196	2.71E-03	3.28E-02
Adcyap1r1	4610	3962	-0.195	7.61E-03	6.93E-02
Chsy1	285	247	-0.195	3.22E-03	3.71E-02
Rab40b	956	824	-0.195	3.32E-03	3.77E-02
Sema6d	1526	1318	-0.195	1.19E-03	1.76E-02
Tlr3	290	249	-0.195	8.66E-03	7.64E-02
Arhgef2	2756	2387	-0.194	9.61E-04	1.50E-02
Dcakd	512	441	-0.194	1.07E-03	1.63E-02

Erf	744	643	-0.194	2.24E-03	2.89E-02
Foxo4	358	309	-0.194	6.64E-03	6.28E-02
Tle1	1526	1314	-0.194	3.99E-03	4.34E-02
Dagla	3134	2720	-0.193	2.96E-04	6.22E-03
Ipmk	920	802	-0.193	1.38E-05	5.19E-04
Nasp	373	323	-0.193	2.62E-03	3.20E-02
P4ha1	1003	864	-0.192	8.12E-03	7.31E-02
Ppp1r13b	1601	1390	-0.192	5.45E-04	9.83E-03
Rbfox1	10514	9081	-0.192	4.60E-03	4.83E-02
Tmcc3	911	789	-0.192	8.27E-04	1.34E-02
Ybx3	378	329	-0.192	1.63E-03	2.26E-02
Dpp6	6933	6046	-0.191	5.21E-07	3.48E-05
Pou3f3	1000	870	-0.191	4.75E-04	8.88E-03
Sri	928	808	-0.191	4.55E-06	2.19E-04
B3gat1	4033	3520	-0.190	1.04E-05	4.19E-04
Plekhb1	2320	2014	-0.190	9.06E-04	1.43E-02
Ppp1r3c	832	718	-0.190	7.12E-03	6.62E-02
Dclk1	12963	11256	-0.189	1.69E-03	2.32E-02
Dgkz	7227	6298	-0.189	1.72E-04	3.96E-03
Gatsl2	1336	1167	-0.189	1.38E-05	5.19E-04
Itpka	3179	2765	-0.189	1.16E-03	1.74E-02
Mmp17	3105	2698	-0.189	9.82E-04	1.53E-02
Zfp146	602	522	-0.189	3.09E-03	3.59E-02
Camk2a	88651	77556	-0.188	1.72E-07	1.29E-05
Mdga2	950	828	-0.188	6.43E-04	1.11E-02
Zbtb26	258	224	-0.188	4.21E-03	4.53E-02
Prkg1	533	465	-0.187	1.06E-03	1.63E-02
Abcb7	1128	982	-0.186	2.16E-03	2.80E-02
Abhd6	796	695	-0.186	2.52E-05	8.38E-04
Dbt	431	375	-0.186	6.57E-04	1.13E-02
Gldc	800	700	-0.186	8.18E-05	2.17E-03
Smc4	258	225	-0.186	5.81E-03	5.69E-02
Sorl1	4624	4030	-0.186	8.07E-04	1.31E-02
Arhgef25	4612	4029	-0.185	4.53E-04	8.55E-03
Cldn12	623	541	-0.185	2.51E-03	3.11E-02
G2e3	473	414	-0.185	2.49E-03	3.09E-02
Nkain2	1310	1143	-0.185	4.41E-04	8.42E-03
S100pbp	266	230	-0.185	1.27E-02	9.99E-02
Camk2n1	12134	10629	-0.184	3.81E-05	1.18E-03

Dhx29	582	511	-0.184	5.04E-04	9.27E-03
Rbfox3	4411	3861	-0.184	6.53E-05	1.81E-03
Rbm15	223	194	-0.184	9.70E-03	8.25E-02
Arsb	2408	2109	-0.183	4.95E-05	1.45E-03
N6amt2	198	171	-0.183	1.11E-02	9.04E-02
Pnrc2	739	646	-0.183	3.67E-04	7.30E-03
Dixdc1	1784	1565	-0.182	7.76E-06	3.30E-04
Fndc5	971	851	-0.182	7.05E-04	1.20E-02
Sema4f	851	743	-0.182	4.25E-03	4.55E-02
Spry2	706	612	-0.182	8.15E-03	7.32E-02
Sptb	6217	5458	-0.182	4.24E-06	2.07E-04
Dkc1	476	416	-0.181	3.40E-03	3.84E-02
Gpc5	806	703	-0.181	6.87E-04	1.18E-02
Smad7	286	249	-0.181	1.05E-02	8.72E-02
Ttc14	5388	4728	-0.181	1.36E-04	3.30E-03
Dnah9	428	373	-0.180	5.79E-03	5.68E-02
Rasa1	1920	1678	-0.180	1.71E-03	2.34E-02
Carhsp1	473	414	-0.179	2.81E-03	3.36E-02
Ppp1r1b	525	459	-0.179	7.35E-03	6.77E-02
Rhobtb2	1074	941	-0.179	1.66E-03	2.29E-02
Shisa9	1352	1182	-0.179	3.97E-03	4.32E-02
Ccnl1	672	592	-0.178	2.65E-04	5.70E-03
Htra1	1973	1730	-0.178	1.28E-03	1.88E-02
Zbtb43	600	524	-0.178	4.87E-03	5.02E-02
Fam204a	331	287	-0.177	1.13E-02	9.20E-02
Fam212b	1715	1502	-0.177	2.98E-03	3.49E-02
Snrpb	623	545	-0.177	5.12E-03	5.22E-02
Tm7sf3	792	696	-0.177	4.84E-04	8.98E-03
Zfp120	335	292	-0.177	7.67E-03	6.97E-02
1700113A16Rik	325	285	-0.176	2.83E-03	3.38E-02
B3galnt2	555	484	-0.176	1.26E-02	9.90E-02
Grid1	870	760	-0.176	6.51E-03	6.18E-02
Ikzf5	561	494	-0.176	4.29E-04	8.25E-03
Nmt1	2057	1812	-0.176	1.43E-04	3.41E-03
Paqr7	912	798	-0.176	9.31E-03	8.08E-02
Rogdi	2142	1880	-0.176	2.87E-03	3.41E-02
Slc25a25	823	726	-0.176	5.38E-04	9.74E-03
Dusp7	1150	1010	-0.175	2.25E-03	2.89E-02
Mtf2	1345	1186	-0.175	4.71E-05	1.39E-03

Rbbp5	1176	1036	-0.175	7.71E-05	2.05E-03
Setd8	717	630	-0.175	4.91E-03	5.04E-02
Soga1	1350	1185	-0.175	1.67E-03	2.29E-02
BC030336	1490	1310	-0.174	6.20E-04	1.09E-02
Jarid2	669	590	-0.174	1.37E-04	3.30E-03
Map3k13	466	409	-0.174	2.61E-03	3.19E-02
Slain1	534	468	-0.174	6.10E-03	5.90E-02
Srf	1005	884	-0.174	1.29E-03	1.88E-02
Bccip	726	641	-0.173	8.96E-05	2.35E-03
Crot	584	512	-0.173	2.47E-03	3.06E-02
Egln1	1346	1189	-0.173	2.33E-05	7.82E-04
Synpo	3469	3056	-0.173	9.70E-04	1.51E-02
Zfp488	775	682	-0.173	6.26E-04	1.09E-02
Atf5	348	306	-0.172	9.42E-03	8.12E-02
Gucy1b3	2839	2514	-0.172	2.15E-06	1.17E-04
Cpeb4	4495	3978	-0.171	1.30E-05	4.93E-04
Ddx26b	1168	1032	-0.171	2.12E-03	2.77E-02
Paqr8	2905	2566	-0.171	7.30E-04	1.24E-02
Tnfrsf19	1119	989	-0.171	1.53E-05	5.58E-04
Usp16	621	547	-0.171	4.47E-04	8.51E-03
Zfp760	266	235	-0.171	1.21E-02	9.67E-02
Alkbh5	1164	1031	-0.170	3.78E-05	1.17E-03
Ccdc39	889	786	-0.170	6.31E-04	1.10E-02
Mak16	518	455	-0.170	4.51E-03	4.78E-02
Ptk2b	15466	13650	-0.170	1.16E-03	1.74E-02
Abhd17c	825	730	-0.169	4.23E-04	8.18E-03
Gm608	1935	1714	-0.169	1.03E-07	8.48E-06
Hip1	867	768	-0.169	2.15E-04	4.76E-03
Khdrbs1	2134	1891	-0.169	1.18E-06	7.14E-05
Wasl	3220	2853	-0.169	1.09E-04	2.76E-03
1700052N19Rik	334	294	-0.168	5.87E-03	5.73E-02
Akt2	959	846	-0.168	5.40E-04	9.75E-03
Lrp1b	1985	1750	-0.168	3.67E-03	4.07E-02
Srek1	1777	1571	-0.168	1.28E-03	1.88E-02
Abhd13	928	822	-0.167	5.41E-04	9.76E-03
Asphd2	1294	1144	-0.167	1.42E-03	2.03E-02
Dclre1c	333	294	-0.167	7.29E-03	6.73E-02
Ing1	384	338	-0.167	1.22E-02	9.73E-02
Kat6a	1711	1517	-0.167	3.41E-04	6.89E-03

Med12l	1366	1207	-0.167	3.01E-03	3.52E-02
Nphp1	328	288	-0.167	1.24E-02	9.78E-02
Prmt8	1188	1047	-0.167	7.27E-03	6.73E-02
Rb1	833	735	-0.167	2.87E-03	3.41E-02
Srsf9	688	606	-0.167	9.64E-03	8.22E-02
Tet3	1365	1206	-0.167	3.05E-03	3.56E-02
Vegfa	884	778	-0.167	1.17E-02	9.42E-02
Zfp275	414	364	-0.167	1.06E-02	8.77E-02
Cox20	440	388	-0.166	5.76E-03	5.66E-02
Eml6	1007	891	-0.166	1.77E-04	4.05E-03
Pabpc1	3852	3418	-0.166	5.90E-06	2.63E-04
Ptpn1	771	685	-0.166	2.30E-04	5.05E-03
8030462N17Rik	458	406	-0.165	3.42E-03	3.85E-02
Bcan	3436	3042	-0.165	3.75E-03	4.15E-02
Gba2	1323	1172	-0.165	2.97E-03	3.49E-02
Hmgn1	696	616	-0.165	4.85E-04	8.98E-03
Mettl14	379	336	-0.165	1.01E-02	8.48E-02
Negr1	3765	3335	-0.165	2.21E-03	2.84E-02
Pknox2	580	512	-0.165	1.11E-02	9.07E-02
Rps25	1581	1398	-0.165	4.50E-03	4.77E-02
Tor1b	631	561	-0.165	5.28E-03	5.29E-02
Erbb2ip	1560	1383	-0.164	9.22E-04	1.45E-02
Fam208a	1646	1456	-0.164	6.33E-03	6.04E-02
Tubgcp2	735	650	-0.164	2.86E-03	3.41E-02
Bmi1	1251	1111	-0.163	5.05E-04	9.27E-03
Dlg3	4462	3960	-0.163	1.58E-03	2.21E-02
Hnrnpm	2717	2408	-0.163	3.26E-03	3.74E-02
Nfyc	304	270	-0.163	9.63E-03	8.22E-02
Atp1b2	6624	5896	-0.162	9.23E-05	2.41E-03
Cdkn1b	689	614	-0.162	2.39E-03	3.00E-02
Fam220a	694	615	-0.162	2.54E-03	3.14E-02
Fermt2	1449	1284	-0.162	2.47E-03	3.07E-02
Fkbp4	1412	1256	-0.162	2.91E-04	6.12E-03
Hipk3	2074	1847	-0.162	6.62E-05	1.82E-03
Zfp191	637	567	-0.162	1.25E-03	1.83E-02
2210018M11Rik	862	768	-0.161	1.94E-03	2.59E-02
Gng7	3800	3378	-0.161	1.36E-03	1.97E-02
Gtf2h2	416	368	-0.161	1.17E-02	9.42E-02
Rnf185	491	435	-0.161	7.88E-03	7.12E-02

Usp1	638	568	-0.161	1.64E-03	2.27E-02
Farp1	968	861	-0.160	4.14E-03	4.47E-02
Megf10	542	482	-0.160	5.65E-03	5.58E-02
Micall1	903	802	-0.160	5.16E-03	5.24E-02
Peli1	1012	899	-0.160	3.20E-03	3.69E-02
Pop5	412	366	-0.160	5.84E-03	5.70E-02
Trpm7	1587	1414	-0.160	1.24E-03	1.82E-02
Ythdc1	1623	1446	-0.160	4.08E-04	7.96E-03
4933431E20Rik	2699	2408	-0.159	6.66E-05	1.83E-03
Cpsf6	2972	2658	-0.159	3.25E-07	2.29E-05
Crtc3	402	356	-0.159	8.36E-03	7.46E-02
Gm14420	353	315	-0.159	1.01E-02	8.50E-02
Gria1	23892	21297	-0.159	6.53E-04	1.13E-02
Hspa2	1374	1224	-0.159	4.45E-03	4.72E-02
Nckap1	14864	13267	-0.159	2.60E-05	8.58E-04
Nhsl1	1076	957	-0.159	1.12E-02	9.10E-02
Osbp2	1370	1218	-0.159	1.05E-02	8.70E-02
Rcc2	1655	1476	-0.159	1.75E-04	4.03E-03
1810041L15Rik	1510	1340	-0.158	1.02E-02	8.54E-02
Iffo2	512	454	-0.158	1.23E-02	9.78E-02
Pcf11	903	804	-0.158	5.35E-03	5.34E-02
Pde8b	1421	1266	-0.158	3.40E-03	3.84E-02
Ppif	296	263	-0.158	1.10E-02	8.98E-02
Ctdspl	525	469	-0.157	1.22E-02	9.73E-02
Pogz	1403	1254	-0.157	3.26E-04	6.69E-03
Smox	441	390	-0.157	1.16E-02	9.36E-02
Srsf11	2423	2162	-0.157	9.04E-04	1.43E-02
Zfp365	6652	5931	-0.157	2.93E-03	3.46E-02
Arhgap21	5396	4813	-0.156	2.67E-03	3.25E-02
Lrrc4	2266	2020	-0.156	2.78E-03	3.33E-02
U2surp	2383	2130	-0.156	9.47E-06	3.88E-04
Phlpp2	1201	1070	-0.155	5.81E-03	5.69E-02
Tbc1d15	811	722	-0.155	1.19E-02	9.54E-02
Chn1	19107	17081	-0.154	2.39E-03	3.00E-02
Diap2	822	732	-0.154	4.68E-03	4.89E-02
Fam174a	763	682	-0.154	1.85E-03	2.49E-02
Kdm4c	729	650	-0.154	2.34E-03	2.97E-02
Mbnl2	4733	4249	-0.154	1.10E-06	6.78E-05
Slco1c1	954	850	-0.154	3.99E-03	4.34E-02

Kif1c	2005	1796	-0.153	1.04E-04	2.65E-03
Nupl1	624	558	-0.153	1.20E-03	1.77E-02
Sipa1l3	3202	2860	-0.153	6.39E-03	6.08E-02
Tmem132b	3999	3583	-0.153	7.23E-05	1.96E-03
Atrnl1	1996	1785	-0.152	2.14E-03	2.79E-02
Fbrsl1	772	692	-0.152	2.30E-03	2.94E-02
Gstm5	1611	1446	-0.152	3.38E-04	6.86E-03
Herc3	3514	3148	-0.152	1.43E-03	2.04E-02
Map3k3	487	435	-0.152	5.64E-03	5.57E-02
Rbm12	512	456	-0.152	3.17E-03	3.66E-02
Spata2	1113	997	-0.152	2.20E-03	2.84E-02
Zfp281	1204	1077	-0.152	1.32E-03	1.91E-02
Zranb1	669	598	-0.152	4.68E-03	4.89E-02
Jakmip3	768	685	-0.151	1.13E-02	9.20E-02
Med13	2730	2445	-0.151	1.20E-03	1.77E-02
Pnisr	5272	4720	-0.151	4.77E-03	4.96E-02
Rrs1	337	300	-0.151	9.31E-03	8.08E-02
Tial1	2159	1938	-0.151	8.62E-04	1.38E-02
Amigo1	1883	1688	-0.150	2.99E-03	3.51E-02
Esco1	440	393	-0.150	7.97E-03	7.18E-02
Kat6b	1436	1285	-0.150	6.18E-03	5.95E-02
Ppp1cc	781	702	-0.150	1.57E-03	2.19E-02
Snx25	870	778	-0.150	1.14E-02	9.25E-02
Zfp654	546	490	-0.150	7.41E-03	6.80E-02
Desi2	490	438	-0.149	1.27E-02	1.00E-01
Gm17066	856	767	-0.149	6.24E-03	5.99E-02
Gng2	5386	4827	-0.149	5.52E-03	5.49E-02
Jmjd4	646	579	-0.149	3.61E-03	4.02E-02
Rfxap	366	329	-0.149	1.13E-02	9.15E-02
Sp3	1226	1101	-0.149	2.95E-03	3.48E-02
Tmem161b	460	414	-0.149	3.04E-03	3.55E-02
Cdc42bpa	7023	6318	-0.148	1.51E-05	5.57E-04
Mat2b	1527	1370	-0.148	4.58E-03	4.82E-02
Atg3	630	565	-0.147	8.44E-03	7.50E-02
Ppig	2464	2221	-0.147	2.50E-05	8.32E-04
Cntnap1	3455	3104	-0.146	6.92E-03	6.50E-02
Epas1	2521	2270	-0.146	1.12E-03	1.70E-02
Epn2	2898	2614	-0.146	1.30E-04	3.17E-03
Eps8l3	582	520	-0.146	6.21E-03	5.97E-02

Wipf2	1791	1611	-0.146	2.20E-03	2.84E-02
Cacnb4	3166	2848	-0.145	2.74E-03	3.30E-02
Gpr155	1441	1300	-0.145	2.37E-03	3.00E-02
Grhl1	697	625	-0.145	7.71E-03	6.98E-02
Nob1	368	332	-0.145	1.08E-02	8.87E-02
Sltm	1709	1547	-0.145	4.59E-05	1.37E-03
Snx30	1173	1051	-0.145	1.02E-02	8.54E-02
Tmem167	1404	1267	-0.144	1.41E-03	2.02E-02
Atp1a1	12695	11440	-0.143	3.86E-03	4.24E-02
Chrm1	4581	4140	-0.143	4.96E-06	2.31E-04
Brms1l	1133	1022	-0.142	3.38E-04	6.86E-03
Map1b	27662	24987	-0.142	6.78E-04	1.16E-02
Prpf39	1714	1550	-0.142	2.78E-04	5.92E-03
Sept3	9770	8834	-0.142	1.12E-06	6.89E-05
Akap17b	483	436	-0.141	5.18E-03	5.24E-02
Fbxl14	430	387	-0.141	1.06E-02	8.74E-02
Krr1	775	697	-0.141	6.61E-03	6.26E-02
Mcu	610	550	-0.141	7.15E-03	6.64E-02
Stag2	1605	1447	-0.141	5.72E-03	5.63E-02
Top1	2416	2185	-0.141	1.71E-04	3.94E-03
Vps29	991	895	-0.141	5.42E-03	5.41E-02
Ybx1	1726	1559	-0.141	3.41E-04	6.89E-03
Ctdsp2	1681	1522	-0.140	2.14E-03	2.79E-02
Hnrnpdl	4080	3691	-0.140	8.95E-04	1.42E-02
Mgll	2924	2639	-0.140	6.62E-03	6.27E-02
Rapgefl1	4104	3705	-0.140	6.18E-03	5.95E-02
Rprd1a	1606	1451	-0.140	3.62E-03	4.03E-02
Rsbn1l	937	848	-0.140	9.43E-03	8.12E-02
Ss18l1	1166	1057	-0.140	1.99E-03	2.65E-02
Uhrf2	968	874	-0.140	1.11E-02	9.05E-02
Actr3	4521	4087	-0.139	2.76E-03	3.32E-02
Ahi1	5320	4807	-0.139	7.09E-03	6.61E-02
Als2	1194	1079	-0.139	5.65E-03	5.58E-02
Msl2	1370	1239	-0.139	4.34E-03	4.62E-02
Robo2	2443	2204	-0.139	1.18E-02	9.46E-02
Arpc3	1346	1216	-0.138	1.20E-02	9.60E-02
Chpf	976	881	-0.138	6.62E-03	6.27E-02
Gatm	976	884	-0.138	1.95E-03	2.60E-02
Igip	1270	1148	-0.138	1.16E-03	1.74E-02

Myef2	1979	1792	-0.138	3.12E-03	3.62E-02
Rev1	720	654	-0.138	7.10E-03	6.61E-02
Atxn1	2036	1852	-0.137	1.53E-05	5.58E-04
Ccdc6	1185	1075	-0.137	4.61E-03	4.83E-02
Hivep2	5899	5348	-0.137	9.30E-04	1.46E-02
Polr3f	567	514	-0.137	8.28E-03	7.41E-02
Qk	7053	6396	-0.136	2.03E-03	2.68E-02
Rsrc2	1602	1450	-0.136	1.05E-02	8.72E-02
Vash1	1403	1268	-0.136	1.26E-02	9.91E-02
Cipc	1482	1345	-0.135	7.02E-04	1.20E-02
Eftud2	1694	1539	-0.135	1.82E-03	2.46E-02
Hmgn2	604	547	-0.135	5.49E-03	5.46E-02
Prkrir	1394	1265	-0.135	3.79E-03	4.18E-02
Sec23ip	1135	1030	-0.135	6.65E-03	6.28E-02
Capzb	2826	2566	-0.134	4.23E-03	4.54E-02
Peg13	3732	3390	-0.134	2.59E-03	3.17E-02
Csrp1	2185	1983	-0.133	9.89E-03	8.37E-02
Dcaf6	1774	1612	-0.133	1.91E-03	2.57E-02
Med14	1558	1413	-0.133	1.75E-03	2.39E-02
Ube2g1	984	891	-0.133	4.03E-03	4.37E-02
Zfp655	586	532	-0.133	1.01E-02	8.49E-02
Camta2	6769	6157	-0.132	3.25E-03	3.72E-02
Eif1b	1165	1061	-0.132	1.45E-03	2.06E-02
Mzt1	1397	1268	-0.132	5.67E-03	5.59E-02
Zcchc14	1977	1800	-0.132	1.77E-03	2.41E-02
Naa60	1050	953	-0.131	2.70E-03	3.27E-02
Rock1	1023	934	-0.131	2.54E-03	3.14E-02
Cplx2	39263	35767	-0.130	2.43E-03	3.04E-02
Eif4a1	2957	2693	-0.130	4.73E-03	4.92E-02
Zfp932	548	498	-0.130	9.49E-03	8.15E-02
Aagab	1250	1138	-0.129	1.26E-02	9.89E-02
Cttn	2756	2512	-0.129	3.28E-03	3.75E-02
Mat2a	2757	2516	-0.129	2.38E-03	3.00E-02
Sept11	1433	1306	-0.129	3.94E-03	4.29E-02
Srsf10	1242	1134	-0.129	6.16E-03	5.94E-02
Yipf6	980	891	-0.129	5.54E-03	5.49E-02
Zfp827	852	778	-0.129	1.70E-03	2.33E-02
Sf3b1	6162	5636	-0.128	2.30E-07	1.67E-05
Socs5	2102	1913	-0.128	7.28E-03	6.73E-02

Cnot6l	1624	1478	-0.127	1.17E-02	9.44E-02
Sptbn2	19568	17884	-0.127	2.63E-04	5.66E-03
Zfp664	1964	1792	-0.127	4.79E-03	4.97E-02
Aldh5a1	2280	2083	-0.126	2.77E-05	8.98E-04
Smek1	900	825	-0.126	1.82E-03	2.47E-02
Unc80	8575	7845	-0.126	7.76E-06	3.30E-04
Zfp84	560	513	-0.126	1.24E-02	9.80E-02
Gabrb3	8575	7844	-0.125	4.34E-04	8.33E-03
Ythdf1	1001	918	-0.125	2.35E-03	2.97E-02
Crebzf	1232	1129	-0.124	2.85E-03	3.39E-02
Ctcf	1373	1258	-0.124	4.23E-03	4.54E-02
Fam102a	1507	1377	-0.124	3.26E-03	3.73E-02
Mark3	1262	1155	-0.124	3.13E-03	3.63E-02
Nup153	992	908	-0.124	8.58E-03	7.60E-02
Bex2	2915	2670	-0.122	1.47E-03	2.07E-02
Cx3cl1	7082	6491	-0.122	3.28E-04	6.73E-03
Dek	1117	1024	-0.122	1.08E-02	8.87E-02
Dlgap1	12125	11127	-0.122	3.39E-05	1.07E-03
Gstm1	1999	1826	-0.122	5.11E-03	5.20E-02
Zfp329	1055	962	-0.122	1.00E-02	8.45E-02
Ppm1g	1160	1064	-0.121	2.34E-03	2.97E-02
Tmem170b	2125	1948	-0.121	2.16E-03	2.81E-02
Zfp445	5166	4734	-0.121	8.46E-03	7.52E-02
6030458C11Rik	835	767	-0.120	9.45E-03	8.12E-02
Fmr1	1551	1425	-0.120	5.57E-04	1.00E-02
Kcnab2	11217	10280	-0.120	1.27E-02	9.96E-02
Mtf1	719	658	-0.120	7.40E-03	6.80E-02
Nol4	1494	1371	-0.120	7.63E-03	6.94E-02
Ptms	6675	6121	-0.120	6.24E-03	5.99E-02
Tnks2	4452	4083	-0.120	4.29E-03	4.58E-02
Zcchc11	846	777	-0.120	7.59E-03	6.93E-02
Rab28	785	720	-0.119	1.16E-02	9.41E-02
Ralgapa1	3527	3239	-0.119	9.00E-04	1.42E-02
Synj2bp	1443	1331	-0.119	6.05E-04	1.07E-02
Zfp131	718	660	-0.119	1.24E-02	9.82E-02
Atp2b3	5360	4926	-0.118	1.76E-03	2.40E-02
Spire1	2623	2415	-0.118	6.91E-05	1.88E-03
Luc7l3	4693	4316	-0.117	5.81E-03	5.69E-02
Fam135a	829	763	-0.116	4.57E-03	4.81E-02

Npm1	2116	1946	-0.116	9.03E-03	7.90E-02
Pdpk1	3237	2977	-0.116	5.27E-03	5.29E-02
Raf1	1044	960	-0.116	8.03E-03	7.24E-02
Actb	21675	19967	-0.115	2.96E-03	3.48E-02
Ddx46	1989	1832	-0.115	4.19E-03	4.52E-02
Hepacam	1191	1097	-0.115	7.20E-03	6.68E-02
Nipbl	1786	1646	-0.114	3.83E-03	4.21E-02
Synrg	1745	1609	-0.113	4.59E-03	4.83E-02
Ap1ar	1297	1196	-0.112	4.73E-03	4.92E-02
Papola	2196	2030	-0.112	2.44E-03	3.04E-02
Slc12a5	7632	7054	-0.112	8.25E-04	1.34E-02
Zfc3h1	1540	1420	-0.112	1.23E-02	9.75E-02
Anks1b	6409	5922	-0.111	3.28E-03	3.75E-02
Arid4b	1534	1416	-0.111	7.26E-03	6.73E-02
S1pr1	2142	1983	-0.111	1.67E-03	2.30E-02
Mt3	2562	2364	-0.110	1.13E-02	9.20E-02
Ndrg3	7065	6528	-0.110	7.62E-03	6.94E-02
Brwd1	2631	2436	-0.109	6.63E-04	1.14E-02
Cisd2	914	846	-0.109	6.74E-03	6.35E-02
Kmt2e	2856	2643	-0.109	7.35E-03	6.77E-02
Thoc2	1850	1710	-0.109	3.93E-03	4.29E-02
Ccpg1	2649	2452	-0.108	5.34E-03	5.34E-02
Kcnb1	3603	3333	-0.108	1.25E-02	9.84E-02
Slit3	1244	1149	-0.108	9.06E-03	7.91E-02
Hnrnpa2b1	6797	6302	-0.107	1.30E-03	1.89E-02
Lgalsl	1268	1174	-0.107	1.05E-02	8.72E-02
Aldoc	9483	8791	-0.106	5.18E-03	5.24E-02
Bai3	2579	2388	-0.106	1.04E-02	8.68E-02
Nufip2	2250	2089	-0.106	7.12E-03	6.62E-02
Orc4	1041	964	-0.106	4.79E-03	4.97E-02
Bod1l	2896	2690	-0.105	1.11E-03	1.68E-02
Ddx5	17002	15780	-0.105	2.86E-03	3.40E-02
Fbxl17	1950	1811	-0.105	3.76E-03	4.16E-02
Hnrnpu	9016	8378	-0.105	4.75E-05	1.40E-03
Strn3	1875	1740	-0.105	9.18E-03	7.99E-02
Otud5	1233	1144	-0.104	1.27E-02	1.00E-01
Riok3	1211	1128	-0.104	1.05E-02	8.70E-02
Dcp2	1908	1773	-0.103	2.78E-03	3.33E-02
Eif5b	2635	2449	-0.103	7.42E-03	6.80E-02

Ppp1r21	1089	1013	-0.102	1.08E-02	8.90E-02
Rai1	1555	1444	-0.102	1.00E-02	8.46E-02
Fam120a	3243	3019	-0.101	9.85E-03	8.34E-02
Scn8a	7872	7326	-0.101	2.42E-03	3.03E-02
Srsf3	1917	1789	-0.100	7.69E-03	6.97E-02
Dennd4a	1312	1223	-0.099	9.08E-03	7.92E-02
Abi1	3510	3273	-0.098	8.48E-03	7.53E-02
Hnrnpl	2591	2417	-0.098	1.19E-02	9.53E-02
Itsn2	1270	1186	-0.097	6.09E-03	5.89E-02
Zfp704	1986	1853	-0.097	8.78E-03	7.72E-02
Epb4.1l1	9150	8548	-0.096	7.74E-04	1.28E-02
Rnf20	1689	1580	-0.096	8.63E-03	7.62E-02
R3hdm4	3820	3572	-0.095	6.89E-03	6.47E-02
Trak1	2832	2650	-0.095	4.53E-03	4.79E-02
Enah	4435	4150	-0.094	5.83E-03	5.70E-02
Med13l	2808	2629	-0.094	8.32E-03	7.43E-02
Tcf4	12324	11536	-0.093	4.69E-03	4.89E-02
Socs7	2876	2694	-0.092	1.97E-03	2.62E-02
Nbea	9438	8849	-0.091	7.60E-03	6.93E-02
Psat1	1277	1196	-0.090	9.62E-03	8.21E-02
Tspan7	16407	15408	-0.089	7.84E-03	7.09E-02
Ccdc47	3043	2860	-0.087	6.99E-03	6.54E-02
Ash1l	4441	4178	-0.086	7.55E-03	6.89E-02
Braf	3911	3694	-0.082	3.97E-03	4.32E-02
Map4k4	2350	2221	-0.081	8.17E-03	7.33E-02
Rbm39	4313	4078	-0.080	6.27E-03	6.00E-02
Ptpn11	3278	3102	-0.078	8.74E-03	7.69E-02
Usp7	3248	3076	-0.077	6.73E-03	6.34E-02
Ahcyl1	7077	6706	-0.076	2.38E-03	3.00E-02
Arf3	23228	22041	-0.075	4.82E-03	4.98E-02
Pcm1	2669	2532	-0.074	1.07E-02	8.83E-02

Table S2. Upregulated genes in hAPP(J20) mice.

Gene Name	NTG	hAPP(J20)	log2FoldChange	pvalue	padj
Arhgap36	8	58	0.854	6.99E-15	3.78E-12
Igfbp6	93	240	0.834	1.66E-13	6.56E-11
App	20694	37433	0.813	3.63E-59	5.58E-55
Rxfp3	72	162	0.777	1.05E-12	3.42E-10
Megf6	96	218	0.741	3.16E-11	7.40E-09
Scn7a	80	161	0.724	3.46E-12	1.00E-09
Col6a1	338	2197	0.697	3.30E-11	7.59E-09
Pde11a	116	220	0.688	1.14E-11	2.97E-09
Stard4	192	323	0.646	2.86E-15	1.84E-12
Sstr1	432	729	0.640	2.58E-14	1.24E-11
Serpina3g	8	33	0.638	5.37E-09	6.66E-07
AW551984	45	113	0.626	6.97E-08	6.24E-06
Clec18a	38	83	0.624	7.06E-08	6.28E-06
Dach2	22	47	0.621	6.25E-08	5.76E-06
Lipg	20	43	0.620	6.53E-08	5.95E-06
Spint1	26	52	0.618	3.82E-08	3.80E-06
Tmc6	59	259	0.606	1.41E-08	1.57E-06
Lyz2	73	136	0.604	2.82E-08	2.91E-06
Trpc7	35	67	0.597	7.63E-08	6.67E-06
Cntn4	269	437	0.574	2.16E-10	3.86E-08
Bmp3	29	70	0.569	8.16E-07	5.15E-05
Scn9a	101	179	0.555	3.36E-07	2.34E-05
Col18a1	79	151	0.548	1.64E-06	9.38E-05
Hmgcs1	3565	5300	0.525	1.23E-16	1.18E-13
Ldlr	356	536	0.525	7.13E-15	3.78E-12
A2m	58	100	0.522	1.71E-06	9.65E-05
Htr3a	118	191	0.522	1.33E-07	1.05E-05
Gfap	6953	11923	0.514	3.02E-06	1.54E-04
Cd68	82	128	0.513	9.91E-08	8.24E-06
Sqle	737	1076	0.502	1.67E-15	1.17E-12
Cd44	65	122	0.501	1.45E-05	5.38E-04
Nsdhl	289	421	0.500	1.36E-15	1.05E-12
Flnc	27	52	0.499	1.68E-05	6.02E-04
Necab1	1227	1774	0.499	1.79E-19	2.50E-16
Serinc2	90	167	0.499	1.53E-05	5.58E-04
Dpp10	496	756	0.493	6.66E-08	6.03E-06

Slc7a4	831	1220	0.492	2.05E-11	5.08E-09
A330040F15Rik	21	39	0.491	2.13E-05	7.27E-04
Medag	84	136	0.490	3.34E-06	1.68E-04
P4ha3	24	43	0.490	1.73E-05	6.19E-04
Gjb2	119	208	0.488	1.67E-05	6.00E-04
Sdk1	76	122	0.488	2.59E-06	1.36E-04
Tenm4	1256	1812	0.488	6.22E-15	3.54E-12
Serpinf1	121	209	0.486	1.56E-05	5.64E-04
Cntn6	75	120	0.477	5.39E-06	2.46E-04
Osr1	20	44	0.476	3.45E-05	1.09E-03
Aspg	44	71	0.473	7.46E-06	3.20E-04
D8Ertd82e	232	335	0.472	5.63E-11	1.19E-08
Hspa1b	104	160	0.472	1.35E-06	7.95E-05
BC005764	452	657	0.470	1.29E-09	1.87E-07
Col6a2	98	284	0.469	1.45E-05	5.38E-04
Adamts18	14	32	0.468	3.51E-05	1.10E-03
Cxcl5	8	28	0.468	7.96E-06	3.35E-04
Nr2f2	576	877	0.467	1.70E-06	9.64E-05
Plcxd3	79	126	0.467	1.30E-05	4.93E-04
Adamts15	66	124	0.464	6.41E-05	1.78E-03
Cdh13	373	537	0.464	6.83E-10	1.08E-07
Slc9a4	237	351	0.464	1.27E-07	1.01E-05
Sytl4	40	65	0.464	2.20E-05	7.48E-04
Gpr83	109	194	0.462	6.38E-05	1.77E-03
Piezo2	15	38	0.462	3.76E-05	1.17E-03
Efemp1	230	356	0.461	5.67E-06	2.56E-04
Tnfaip8l3	376	531	0.461	5.95E-13	2.08E-10
Fdft1	991	1386	0.459	1.27E-18	1.50E-15
Pafah1b3	56	85	0.459	3.73E-06	1.85E-04
Ap1s1	2183	3084	0.458	8.35E-13	2.86E-10
Glra3	22	41	0.454	8.94E-05	2.35E-03
Gm11549	45	90	0.453	9.22E-05	2.41E-03
Rprm	394	1087	0.450	3.10E-05	9.90E-04
Adamts9	160	233	0.449	9.10E-08	7.61E-06
H2-Eb1	12	33	0.447	4.32E-05	1.30E-03
Magel2	14	30	0.445	9.92E-05	2.56E-03
Sel1l3	573	802	0.445	4.36E-13	1.56E-10
Col5a2	153	286	0.443	1.36E-04	3.29E-03
Serpinb8	51	91	0.442	1.38E-04	3.33E-03

Megf11	254	379	0.441	8.15E-06	3.41E-04
2310002F09Rik	38	58	0.439	4.52E-05	1.35E-03
Camk2d	599	855	0.437	1.80E-07	1.34E-05
Cgref1	96	139	0.437	1.10E-06	6.78E-05
Pcolce	73	134	0.437	1.71E-04	3.94E-03
Tuba8	67	103	0.436	6.21E-05	1.74E-03
Socs2	146	209	0.435	8.88E-08	7.51E-06
Epb4.1l4b	101	150	0.433	1.33E-05	5.02E-04
Rspo3	207	318	0.432	5.33E-05	1.54E-03
Cobl	204	314	0.431	5.10E-05	1.49E-03
Rcn3	85	135	0.427	1.19E-04	2.95E-03
Msmo1	1028	1410	0.426	1.50E-13	6.23E-11
Slit2	613	871	0.426	6.42E-07	4.17E-05
Wisp1	22	47	0.426	1.69E-04	3.91E-03
Prss35	70	104	0.423	2.74E-05	8.94E-04
Tmem159	138	192	0.423	4.69E-08	4.51E-06
Bok	547	808	0.421	2.96E-05	9.53E-04
Gsn	433	611	0.421	2.65E-07	1.89E-05
Stac2	544	758	0.421	1.28E-08	1.43E-06
Gpr165	73	104	0.419	1.07E-05	4.28E-04
Sema3e	1302	1797	0.419	2.06E-09	2.88E-07
Dgkb	3815	5279	0.417	9.75E-09	1.12E-06
Pde4b	954	1322	0.417	1.60E-08	1.74E-06
Ppapdc1a	53	100	0.416	3.26E-04	6.69E-03
Pvrl3	468	677	0.414	1.83E-05	6.43E-04
Thbs2	95	138	0.414	6.97E-06	3.01E-04
Trnp1	728	1030	0.414	2.90E-06	1.50E-04
Fam26e	94	132	0.413	3.33E-06	1.68E-04
C2cd4c	324	454	0.412	1.41E-06	8.24E-05
Cd74	20	54	0.411	1.28E-04	3.13E-03
Gm16702	60	92	0.410	2.08E-04	4.64E-03
Plxnd1	384	555	0.410	2.30E-05	7.78E-04
C4b	899	1353	0.408	1.46E-04	3.45E-03
Cnr1	3608	4880	0.408	9.19E-13	3.07E-10
Reln	1088	1498	0.407	8.34E-08	7.21E-06
Fstl5	302	425	0.406	3.79E-06	1.88E-04
Tmsb10	451	640	0.406	1.13E-05	4.46E-04
Ccdc162	11	24	0.405	3.13E-04	6.50E-03
Thsd7b	206	293	0.405	1.26E-05	4.82E-04

Tmem255a	577	835	0.405	4.53E-05	1.36E-03
Itgbl1	134	191	0.404	2.08E-05	7.16E-04
Pcbd1	34	52	0.404	2.69E-04	5.76E-03
Plp2	16	30	0.404	4.30E-04	8.26E-03
Cxcr4	32	50	0.402	3.44E-04	6.93E-03
S100a6	87	126	0.402	7.20E-05	1.95E-03
Lamc2	16	36	0.401	2.98E-04	6.25E-03
Vim	542	776	0.401	2.87E-05	9.30E-04
5730559C18Rik	31	47	0.400	3.56E-04	7.13E-03
Ifltd1	3	14	0.400	3.08E-05	9.86E-04
Kcnmb2	56	81	0.400	5.70E-05	1.63E-03
Evpl	31	53	0.399	5.93E-04	1.05E-02
Ctsh	111	155	0.397	2.11E-06	1.16E-04
Emilin1	28	46	0.394	5.91E-04	1.05E-02
Ggta1	77	111	0.394	8.73E-05	2.30E-03
Hebp2	22	35	0.392	5.92E-04	1.05E-02
Pde1a	3040	4171	0.391	1.55E-06	8.91E-05
D630023F18Rik	84	119	0.390	1.40E-04	3.35E-03
Sh2d5	889	1375	0.390	5.34E-04	9.68E-03
Abi3bp	23	43	0.389	7.61E-04	1.27E-02
Myoc	222	381	0.388	8.50E-04	1.37E-02
C3ar1	36	54	0.387	6.00E-04	1.06E-02
Dhcr24	1156	1526	0.385	3.35E-15	2.06E-12
Oxtr	98	134	0.385	1.09E-05	4.34E-04
Popdc3	54	79	0.385	3.16E-04	6.54E-03
Dusp26	217	297	0.384	6.64E-06	2.89E-04
Fras1	468	742	0.384	8.19E-04	1.33E-02
Dpysl3	719	957	0.383	2.66E-10	4.59E-08
Pkhd1l1	14	28	0.383	7.51E-04	1.26E-02
Tgfbr2	263	362	0.383	8.42E-06	3.50E-04
Cpne7	2470	3682	0.382	5.08E-04	9.28E-03
Htr7	89	132	0.382	3.53E-04	7.08E-03
Papl	16	31	0.382	8.00E-04	1.31E-02
1600029O15Rik	37	55	0.381	5.88E-04	1.04E-02
Slc20a1	1932	2577	0.380	4.65E-09	5.91E-07
Stmn2	2946	3918	0.380	8.55E-10	1.32E-07
Idh1	581	773	0.379	3.50E-09	4.60E-07
Pdk1	1067	1412	0.379	3.17E-11	7.40E-09
Rspo2	252	367	0.378	3.98E-04	7.79E-03

Scml4	89	126	0.378	1.88E-04	4.28E-03
Lum	88	136	0.377	7.88E-04	1.29E-02
C1qtnf1	36	57	0.376	9.86E-04	1.53E-02
Kcnq5	1022	1395	0.375	1.43E-05	5.30E-04
Sorcs1	146	296	0.374	8.56E-04	1.37E-02
Galnt14	322	423	0.373	2.08E-09	2.88E-07
Lsp1	23	37	0.373	1.30E-03	1.89E-02
H2-Ab1	9	22	0.372	5.79E-04	1.03E-02
Klhdc8b	210	302	0.372	4.02E-04	7.86E-03
Wnt5a	159	219	0.372	1.01E-05	4.10E-04
Clec7a	5	20	0.371	6.60E-05	1.82E-03
Id3	290	391	0.369	2.09E-06	1.16E-04
Sv2b	5526	7419	0.369	2.51E-06	1.32E-04
Timp2	3076	4220	0.368	6.01E-05	1.70E-03
Col9a2	54	96	0.367	1.53E-03	2.14E-02
Col1a1	89	210	0.366	5.87E-04	1.04E-02
H2-Aa	16	33	0.366	1.06E-03	1.63E-02
Htr5a	175	233	0.366	1.16E-06	7.05E-05
Phlda3	71	99	0.365	1.61E-04	3.76E-03
Samd9l	67	98	0.364	7.15E-04	1.21E-02
Sema4g	254	341	0.364	8.44E-06	3.50E-04
Cadm3	5540	7301	0.363	4.60E-08	4.45E-06
Hk2	127	169	0.363	7.46E-06	3.20E-04
Pcdh7	1105	1489	0.363	1.81E-05	6.37E-04
Anxa2	94	141	0.362	1.12E-03	1.70E-02
Calb2	643	971	0.361	1.50E-03	2.11E-02
Gm16998	19	35	0.361	1.61E-03	2.24E-02
Kcnh2	238	315	0.361	8.54E-08	7.34E-06
Comtd1	76	103	0.360	9.58E-05	2.49E-03
Gm14446	32	46	0.360	1.02E-03	1.57E-02
Ifit3	63	96	0.360	1.38E-03	1.99E-02
Knstrn	17	29	0.360	1.96E-03	2.61E-02
Parp3	47	69	0.360	1.23E-03	1.80E-02
Tgfbi	33	52	0.360	1.66E-03	2.29E-02
Kif6	31	46	0.359	1.46E-03	2.06E-02
Wbscr27	73	99	0.359	1.15E-04	2.89E-03
Gpr150	34	52	0.357	1.44E-03	2.05E-02
Cd52	6	13	0.356	8.96E-04	1.42E-02
Fdps	432	570	0.356	2.01E-06	1.12E-04

Gdpd5	142	189	0.356	1.94E-05	6.78E-04
Igsf9	40	65	0.356	2.19E-03	2.83E-02
Tmem164	364	488	0.356	1.73E-05	6.19E-04
Rin3	26	38	0.355	1.70E-03	2.34E-02
Nhlh2	23	36	0.354	2.15E-03	2.80E-02
Plce1	253	335	0.354	5.95E-06	2.63E-04
Arhgap29	419	548	0.353	3.49E-08	3.51E-06
Serping1	97	154	0.352	2.32E-03	2.96E-02
Crtac1	1156	1493	0.351	2.12E-11	5.09E-09
Plau	24	38	0.351	2.42E-03	3.03E-02
Prr16	73	102	0.351	6.36E-04	1.11E-02
Cntnap5c	293	384	0.350	1.90E-07	1.41E-05
Gprin3	71	102	0.350	1.19E-03	1.76E-02
Col6a5	12	22	0.349	2.10E-03	2.76E-02
Figf	55	87	0.348	2.74E-03	3.30E-02
Igf1	152	200	0.347	9.00E-06	3.70E-04
Ecm1	62	101	0.346	2.94E-03	3.46E-02
Eya2	22	43	0.346	1.90E-03	2.55E-02
Wdr6	2142	2816	0.346	5.36E-06	2.45E-04
Acss3	14	24	0.345	2.85E-03	3.40E-02
Caln1	900	1168	0.345	1.50E-07	1.15E-05
Cybrd1	78	107	0.345	1.02E-03	1.58E-02
Nmbr	19	30	0.345	2.92E-03	3.46E-02
Rxrg	44	65	0.345	2.30E-03	2.94E-02
Grik4	884	1172	0.344	4.49E-05	1.35E-03
Hapln4	1008	1320	0.344	4.69E-06	2.23E-04
Nell1	580	756	0.344	1.36E-07	1.06E-05
Tnc	97	140	0.343	1.52E-03	2.14E-02
Maats1	8	15	0.340	2.06E-03	2.71E-02
Cited2	314	410	0.339	4.65E-06	2.22E-04
Ptpn5	1394	1822	0.339	8.42E-06	3.50E-04
Rassf8	133	188	0.339	1.75E-03	2.39E-02
Dsg2	82	118	0.338	2.27E-03	2.91E-02
Loxl1	50	71	0.338	2.09E-03	2.74E-02
Plagl1	206	371	0.338	2.98E-03	3.50E-02
Cntnap3	69	103	0.337	3.30E-03	3.75E-02
Smyd4	238	309	0.337	9.42E-06	3.86E-04
Tacr3	16	42	0.337	8.41E-04	1.35E-02
Pcdha2	34	50	0.336	2.73E-03	3.30E-02

Cul7	342	450	0.335	3.26E-05	1.04E-03
S100a11	27	43	0.335	3.77E-03	4.16E-02
Efr3b	2574	3357	0.334	1.85E-05	6.49E-04
Fmo1	74	104	0.334	1.50E-03	2.12E-02
Unc5d	648	953	0.334	3.29E-03	3.75E-02
Nptxr	37	55	0.333	3.49E-03	3.92E-02
Cdh18	99	135	0.332	1.08E-03	1.64E-02
Fmo2	33	61	0.332	3.41E-03	3.84E-02
Gpr179	8	15	0.332	2.93E-03	3.46E-02
Ccl6	22	37	0.331	4.10E-03	4.44E-02
Cxcl10	3	9	0.331	6.34E-04	1.11E-02
Krt5	11	21	0.331	3.23E-03	3.71E-02
Psmb8	36	51	0.331	2.38E-03	3.00E-02
Tspan9	458	597	0.330	4.79E-05	1.41E-03
4930404N11Rik	178	237	0.329	4.41E-04	8.42E-03
Gpr101	105	313	0.329	6.24E-04	1.09E-02
Paqr6	74	99	0.329	3.91E-04	7.68E-03
Rgs4	1768	2457	0.329	2.13E-03	2.78E-02
Cpne2	399	598	0.328	4.42E-03	4.70E-02
Oit3	3	12	0.328	2.00E-04	4.49E-03
Sema4a	332	433	0.328	6.84E-05	1.87E-03
3632451O06Rik	294	378	0.327	2.99E-06	1.53E-04
Lacc1	106	147	0.327	2.57E-03	3.15E-02
Sdk2	134	178	0.327	2.79E-04	5.92E-03
Ccdc109b	42	59	0.326	2.54E-03	3.14E-02
Ltbp3	316	431	0.326	1.44E-03	2.05E-02
Mylk	418	556	0.326	4.66E-04	8.77E-03
Cd274	44	61	0.325	2.93E-03	3.46E-02
Il34	390	501	0.325	1.52E-07	1.16E-05
Hcn3	112	146	0.324	1.19E-04	2.95E-03
Nnmt	12	20	0.324	4.61E-03	4.83E-02
Mrc1	58	96	0.323	5.36E-03	5.35E-02
Arhgef15	119	169	0.322	3.89E-03	4.26E-02
Arhgef26	860	1094	0.322	7.46E-09	8.90E-07
Blnk	42	61	0.322	4.92E-03	5.05E-02
Ephb6	926	1206	0.322	1.39E-04	3.33E-03
St8sia2	37	54	0.322	4.67E-03	4.89E-02
Edil3	2081	2627	0.321	3.17E-10	5.34E-08
Ankrd55	41	57	0.320	2.96E-03	3.48E-02

C1ra	19	32	0.320	5.24E-03	5.27E-02
Thsd4	741	961	0.320	7.61E-05	2.03E-03
Zfp941	378	477	0.320	7.85E-08	6.82E-06
Cachd1	502	642	0.319	2.98E-06	1.53E-04
Grp	180	378	0.319	2.79E-03	3.35E-02
Nkd2	93	137	0.319	5.45E-03	5.43E-02
Prdx4	81	108	0.319	4.83E-04	8.98E-03
Stra6	220	301	0.319	2.40E-03	3.02E-02
Col16a1	174	242	0.318	3.48E-03	3.91E-02
Dcn	473	1042	0.318	2.42E-03	3.03E-02
Hs3st4	1044	1351	0.318	9.54E-05	2.48E-03
Ppp1r26	230	297	0.318	3.49E-05	1.10E-03
Slc5a5	164	223	0.318	2.64E-03	3.21E-02
Slc6a7	829	1058	0.318	4.51E-06	2.18E-04
Adra2c	229	298	0.317	1.20E-04	2.98E-03
Gcnt1	63	87	0.317	3.62E-03	4.03E-02
Cdh7	33	51	0.316	6.61E-03	6.26E-02
Mettl24	31	44	0.316	5.65E-03	5.58E-02
Sema3a	64	98	0.316	6.28E-03	6.01E-02
Zan	6	13	0.316	2.56E-03	3.15E-02
Agpat9	24	35	0.315	6.17E-03	5.95E-02
Col12a1	257	344	0.314	2.12E-03	2.77E-02
Cp	318	436	0.314	3.22E-03	3.71E-02
Fcgr3	107	138	0.313	3.14E-04	6.52E-03
Angpt1	108	148	0.312	3.05E-03	3.56E-02
Gm5468	16	24	0.312	7.32E-03	6.75E-02
Itgax	4	12	0.312	8.01E-04	1.31E-02
Tmem132e	130	172	0.312	1.16E-03	1.74E-02
Chst7	40	60	0.310	7.62E-03	6.94E-02
Gadd45a	50	70	0.310	4.87E-03	5.02E-02
Cela1	14	24	0.309	7.48E-03	6.85E-02
Cst7	2	20	0.309	8.09E-05	2.15E-03
Myo16	236	305	0.309	5.49E-04	9.87E-03
Rwdd2a	196	252	0.309	7.47E-05	2.01E-03
Sh2d3c	125	162	0.309	1.72E-04	3.96E-03
Srebf2	2619	3271	0.309	4.50E-12	1.26E-09
Themis2	7	15	0.309	4.81E-03	4.98E-02
Sh3bp2	43	58	0.308	3.61E-03	4.03E-02
March3	14	23	0.307	8.08E-03	7.27E-02

Maf	251	326	0.306	3.61E-04	7.22E-03
Mxra8	77	106	0.306	4.88E-03	5.02E-02
Oasl2	44	61	0.306	5.30E-03	5.31E-02
Tapbpl	66	87	0.306	1.85E-03	2.49E-02
Abca4	66	104	0.305	8.53E-03	7.57E-02
Aox3	46	76	0.305	8.31E-03	7.43E-02
Slc7a3	70	98	0.305	6.65E-03	6.28E-02
Hgf	39	54	0.304	6.25E-03	5.99E-02
Cacna2d2	246	338	0.303	5.91E-03	5.76E-02
Il3ra	15	24	0.303	8.32E-03	7.43E-02
Me1	633	793	0.303	7.70E-09	9.11E-07
Mfsd12	184	241	0.303	1.49E-03	2.10E-02
P2rx6	97	124	0.303	5.02E-04	9.23E-03
Adcy5	1448	1805	0.302	2.10E-09	2.88E-07
Cd163	28	44	0.302	9.32E-03	8.08E-02
Gab2	314	400	0.302	2.83E-04	5.98E-03
Sulf2	1063	1336	0.302	3.41E-06	1.71E-04
C2	34	55	0.301	9.10E-03	7.94E-02
Ccdc110	5	11	0.301	2.99E-03	3.50E-02
Fam110a	42	58	0.301	5.19E-03	5.24E-02
Wnt4	162	218	0.301	4.14E-03	4.48E-02
Plxdc1	79	105	0.300	4.22E-03	4.53E-02
Klhl13	383	482	0.299	2.93E-05	9.46E-04
Rem2	116	160	0.299	6.59E-03	6.25E-02
Akr1b8	7	14	0.298	5.90E-03	5.76E-02
Tlr4	26	37	0.298	8.34E-03	7.44E-02
Ackr1	375	480	0.297	5.91E-04	1.05E-02
Phex	9	16	0.297	8.56E-03	7.59E-02
Rnasel	131	172	0.297	2.28E-03	2.93E-02
Ssc5d	41	60	0.297	1.04E-02	8.67E-02
Bgn	229	314	0.296	7.24E-03	6.72E-02
Dock8	104	132	0.296	1.13E-03	1.70E-02
Fstl1	577	723	0.296	5.44E-06	2.47E-04
Glp1r	16	25	0.295	1.05E-02	8.72E-02
Etl4	800	1008	0.294	7.05E-05	1.92E-03
Gpnmb	140	243	0.294	8.83E-03	7.75E-02
Prune2	1242	1535	0.294	2.66E-10	4.59E-08
Slc1a6	110	144	0.294	2.88E-03	3.42E-02
Vav2	100	129	0.294	9.83E-04	1.53E-02

F930015N05Rik	9	16	0.293	8.98E-03	7.86E-02
Fzd7	132	177	0.292	5.01E-03	5.13E-02
Slc15a3	23	34	0.292	1.20E-02	9.57E-02
Uchl1	4192	5192	0.292	2.15E-08	2.27E-06
Gm19757	63	82	0.291	3.71E-03	4.11E-02
Lgals3bp	174	223	0.291	1.15E-03	1.73E-02
Serpina3n	730	991	0.291	8.24E-03	7.39E-02
A330070K13Rik	6	12	0.290	5.31E-03	5.32E-02
Fgl2	42	68	0.290	1.19E-02	9.54E-02
Rgs6	90	119	0.290	5.32E-03	5.32E-02
Shisa4	683	865	0.290	4.71E-04	8.85E-03
Spsb2	20	30	0.290	1.24E-02	9.78E-02
Trim30d	28	41	0.290	1.22E-02	9.70E-02
Gad2	2026	2508	0.289	3.52E-07	2.42E-05
Nnat	2663	3726	0.289	1.12E-02	9.12E-02
Ppap2c	22	31	0.289	1.24E-02	9.78E-02
Slc41a3	30	42	0.289	1.04E-02	8.68E-02
Tmem41a	94	120	0.289	1.06E-03	1.63E-02
Asl	145	181	0.288	1.21E-04	2.99E-03
Cradd	44	59	0.288	7.39E-03	6.80E-02
Fxyd1	211	265	0.288	1.44E-04	3.43E-03
Hsd17b7	377	466	0.288	1.46E-07	1.13E-05
Lhfp	244	320	0.288	4.99E-03	5.11E-02
Sox11	530	732	0.288	1.09E-02	8.93E-02
Vwa1	345	434	0.288	1.61E-04	3.76E-03
Fbxo36	10	17	0.287	1.18E-02	9.46E-02
Lpin2	1653	2084	0.287	3.87E-04	7.61E-03
Trim36	104	134	0.287	2.30E-03	2.94E-02
Dhcr7	382	472	0.286	9.00E-08	7.57E-06
Prom2	4	9	0.286	3.27E-03	3.75E-02
Adamts12	6	13	0.285	6.67E-03	6.29E-02
Ddr2	273	358	0.284	4.82E-03	4.98E-02
Ndnf	282	354	0.284	2.37E-04	5.16E-03
Prkar1b	5368	6656	0.284	1.20E-05	4.63E-04
Rasl12	38	53	0.284	1.08E-02	8.86E-02
Rbms3	377	466	0.284	4.85E-06	2.27E-04
Tnfrsf8	11	19	0.284	1.03E-02	8.64E-02
Adra2a	137	178	0.283	5.65E-03	5.58E-02
Insig1	860	1062	0.283	6.48E-06	2.83E-04

Npas1	133	167	0.283	3.38E-04	6.86E-03
Arpp21	2408	3022	0.282	4.91E-04	9.09E-03
H2-D1	111	143	0.281	3.55E-03	3.97E-02
Spock1	2370	2969	0.281	3.94E-04	7.71E-03
Uchl1os	1070	1320	0.281	2.07E-06	1.16E-04
C530008M17Rik	1411	1742	0.280	6.27E-06	2.76E-04
Kcne1l	54	72	0.280	7.08E-03	6.60E-02
Mr1	72	94	0.280	4.24E-03	4.54E-02
Pcyt1b	741	914	0.280	1.24E-05	4.75E-04
Serpinb9	155	195	0.280	2.33E-04	5.07E-03
Arhgdig	214	269	0.279	3.16E-04	6.54E-03
Epb4.1l4a	64	82	0.279	4.57E-03	4.81E-02
Kif17	325	428	0.279	9.01E-03	7.88E-02
Pdzrn3	128	168	0.278	7.26E-03	6.73E-02
Sema3d	112	151	0.278	1.07E-02	8.83E-02
Amz1	113	145	0.277	4.87E-03	5.02E-02
Ap2a2	4478	5486	0.277	1.50E-07	1.15E-05
C1qa	410	508	0.277	2.91E-04	6.12E-03
Efna1	72	95	0.277	8.92E-03	7.82E-02
Arrdc1	58	76	0.276	9.24E-03	8.03E-02
Igfbp3	143	185	0.276	7.03E-03	6.57E-02
Mvd	184	229	0.276	1.12E-03	1.70E-02
Nynrin	129	164	0.276	2.32E-03	2.95E-02
Sh3gl2	4449	5425	0.276	1.42E-10	2.71E-08
Clec10a	3	8	0.275	2.83E-03	3.38E-02
Col13a1	20	37	0.275	1.18E-02	9.45E-02
Idi1	171	212	0.275	4.42E-04	8.43E-03
Kndc1	1491	1903	0.275	4.26E-03	4.55E-02
Plxna1	2445	2995	0.275	2.84E-07	2.02E-05
Ifngr1	188	232	0.274	1.34E-04	3.26E-03
Pgam2	90	113	0.274	3.15E-03	3.65E-02
Resp18	278	362	0.274	8.96E-03	7.85E-02
Kcnc2	1588	1946	0.273	1.39E-06	8.12E-05
Mgat5	472	577	0.273	3.59E-06	1.79E-04
Tppp3	128	160	0.273	1.01E-03	1.57E-02
Camk2n2	448	554	0.272	1.17E-04	2.92E-03
Col3a1	58	162	0.272	2.95E-03	3.48E-02
Tspan17	238	297	0.272	1.53E-03	2.14E-02
Col4a1	776	971	0.271	1.53E-03	2.15E-02

Fam196a	82	105	0.271	7.15E-03	6.64E-02
Plat	574	713	0.271	5.08E-04	9.28E-03
Acat2	331	407	0.270	2.58E-04	5.57E-03
Efnb2	538	680	0.270	3.40E-03	3.84E-02
Gpx1	339	417	0.270	4.46E-06	2.16E-04
Klk8	122	155	0.270	3.63E-03	4.04E-02
Pik3r1	1931	2396	0.270	6.25E-04	1.09E-02
Plch2	1372	1707	0.270	8.16E-04	1.33E-02
Rab7l1	46	61	0.270	1.16E-02	9.36E-02
Rac3	139	175	0.270	2.19E-03	2.84E-02
Rabgap1l	3966	4862	0.269	3.37E-05	1.07E-03
Smoc1	211	266	0.269	2.41E-03	3.02E-02
Kit	1326	1670	0.268	3.47E-03	3.91E-02
Ache	429	540	0.267	3.56E-03	3.98E-02
Fgfbp1	7	18	0.266	4.80E-03	4.97E-02
Kiss1r	64	82	0.266	7.68E-03	6.97E-02
Podxl2	1234	1517	0.266	2.27E-04	5.00E-03
Slc41a2	331	410	0.266	1.47E-03	2.08E-02
Agt	217	275	0.265	5.58E-03	5.53E-02
6430548M08Rik	3084	3819	0.264	1.36E-03	1.96E-02
Grik1	133	165	0.264	3.82E-03	4.21E-02
Schip1	80	100	0.264	5.17E-03	5.24E-02
Tapbp	324	399	0.264	1.92E-04	4.33E-03
Elavl2	1139	1402	0.263	4.29E-04	8.25E-03
Itih3	229	285	0.263	2.63E-03	3.21E-02
Ltbr	70	88	0.263	5.82E-03	5.69E-02
Psma8	3	8	0.263	4.15E-03	4.48E-02
Slbp	220	270	0.263	2.66E-04	5.71E-03
Dchs1	287	352	0.262	3.21E-04	6.62E-03
Hrh3	404	517	0.262	1.06E-02	8.80E-02
Copz2	57	74	0.261	1.08E-02	8.87E-02
Cyb5r1	257	316	0.261	4.64E-04	8.74E-03
Nos1	814	998	0.261	4.51E-04	8.54E-03
Zcchc12	591	739	0.261	3.91E-03	4.27E-02
Fcrls	338	413	0.260	7.67E-04	1.27E-02
Gm11837	170	208	0.260	3.36E-04	6.84E-03
Mgst1	79	98	0.260	6.27E-03	6.01E-02
Abca9	176	220	0.259	5.18E-03	5.24E-02
Cav1	254	314	0.259	1.16E-03	1.74E-02

Efr3a	2626	3194	0.259	5.71E-05	1.63E-03
Fxyd6	864	1081	0.259	5.19E-03	5.24E-02
Gm16982	279	338	0.259	1.16E-04	2.90E-03
Gpr123	593	734	0.259	2.67E-03	3.25E-02
Ntng2	454	557	0.259	6.60E-04	1.14E-02
Peg10	344	441	0.259	1.26E-02	9.93E-02
Adrbk2	2053	2493	0.258	3.95E-05	1.21E-03
Cyb5r3	759	918	0.258	2.29E-07	1.67E-05
Olfml3	250	304	0.258	9.09E-05	2.38E-03
Hpgd	77	98	0.257	1.03E-02	8.64E-02
L1cam	1512	1842	0.257	1.79E-04	4.09E-03
Lag3	71	88	0.257	1.05E-02	8.72E-02
Penk	365	1078	0.257	3.51E-03	3.94E-02
Prtn3	5	10	0.257	9.62E-03	8.21E-02
Zfp367	227	280	0.256	7.96E-04	1.30E-02
Arfgap3	222	274	0.255	1.45E-03	2.06E-02
Ndc1	188	230	0.255	1.45E-03	2.05E-02
Peak1	1378	1668	0.255	3.69E-05	1.15E-03
Scpep1	165	201	0.255	9.54E-04	1.49E-02
Tuba1a	5278	6333	0.255	3.11E-10	5.32E-08
Dennd3	88	110	0.254	9.90E-03	8.37E-02
Mfsd9	110	136	0.254	2.69E-03	3.27E-02
Rtn2	624	751	0.254	3.26E-06	1.66E-04
Grem2	252	307	0.253	1.22E-03	1.80E-02
Hopx	762	915	0.253	1.04E-07	8.51E-06
Ifitm3	95	122	0.253	1.25E-02	9.86E-02
Steap3	98	124	0.253	9.31E-03	8.08E-02
Mgat4c	140	172	0.252	2.79E-03	3.35E-02
Il12a	4	9	0.251	9.36E-03	8.10E-02
Akap13	1434	1725	0.250	4.85E-06	2.27E-04
C230029M16	3	7	0.250	7.41E-03	6.80E-02
Gm14204	71	88	0.250	9.52E-03	8.16E-02
Ptpn14	320	400	0.250	7.62E-03	6.94E-02
Vtn	839	1052	0.250	1.01E-02	8.51E-02
Cyp4v3	144	178	0.249	2.08E-03	2.74E-02
Fads3	299	363	0.249	4.86E-05	1.42E-03
Bcat1	1072	1289	0.248	2.02E-05	6.99E-04
Itgav	1532	1893	0.248	5.90E-03	5.76E-02
Mmab	318	380	0.248	4.08E-05	1.24E-03

Neto1	1636	1975	0.248	2.55E-04	5.52E-03
Rps6ka5	378	461	0.248	1.13E-03	1.71E-02
Tep1	95	118	0.248	4.57E-03	4.81E-02
6330403K07Rik	2135	2558	0.247	2.10E-06	1.16E-04
Flrt3	792	962	0.247	7.99E-04	1.31E-02
Icosl	178	218	0.246	1.81E-03	2.46E-02
Syt12	397	479	0.245	4.75E-04	8.88E-03
Tm4sf1	140	174	0.245	6.02E-03	5.84E-02
Tfr2	104	130	0.244	1.09E-02	8.95E-02
Ccdc148	295	352	0.243	1.06E-04	2.70E-03
Htr2a	111	136	0.243	6.66E-03	6.28E-02
Isyna1	234	292	0.243	1.17E-02	9.43E-02
Optn	197	239	0.243	1.07E-03	1.63E-02
Stab1	266	327	0.243	5.19E-03	5.24E-02
Aqp4	3078	3688	0.242	6.08E-05	1.71E-03
Itgb2	77	94	0.242	1.08E-02	8.86E-02
Lrrc48	83	102	0.242	8.71E-03	7.67E-02
A130010J15Rik	116	143	0.241	9.71E-03	8.25E-02
E330009J07Rik	121	150	0.241	5.70E-03	5.61E-02
Grip1	409	490	0.241	1.35E-04	3.29E-03
Slc14a1	425	512	0.241	1.18E-03	1.75E-02
Evc2	524	626	0.240	5.93E-06	2.63E-04
Col4a2	914	1122	0.239	9.35E-03	8.09E-02
Nedd9	161	196	0.239	2.44E-03	3.04E-02
Cav2	501	598	0.238	4.77E-05	1.40E-03
Rtn4rl1	1532	1839	0.238	7.11E-04	1.21E-02
Cmip	4573	5414	0.237	4.68E-09	5.91E-07
Lmf2	153	183	0.237	1.58E-03	2.20E-02
Oprl1	520	621	0.237	7.52E-05	2.02E-03
Slc29a1	233	285	0.237	8.15E-03	7.32E-02
C1qc	395	472	0.236	8.61E-04	1.38E-02
Gm15612	406	491	0.236	3.23E-03	3.71E-02
Tcta	296	352	0.236	6.57E-05	1.82E-03
Cntnap4	274	331	0.235	5.61E-03	5.55E-02
Klf6	465	555	0.235	1.59E-04	3.73E-03
Rab11fip5	931	1110	0.235	4.31E-05	1.30E-03
Arpc1b	139	167	0.234	2.19E-03	2.84E-02
Bid	113	139	0.234	6.92E-03	6.50E-02
Lrsam1	328	392	0.234	2.83E-04	5.99E-03

Mdm1	100	121	0.234	8.39E-03	7.47E-02
C1qb	449	540	0.233	5.13E-03	5.22E-02
Prrg3	372	446	0.233	1.94E-03	2.59E-02
Tmeff2	1253	1506	0.233	2.29E-03	2.94E-02
2010107G23Rik	284	344	0.232	1.06E-03	1.63E-02
Dak	120	146	0.232	8.69E-03	7.66E-02
Dcaf4	209	250	0.232	1.10E-03	1.67E-02
Slc9a2	432	527	0.232	1.01E-02	8.50E-02
Wls	385	466	0.232	2.82E-03	3.37E-02
Arl10	123	149	0.231	8.34E-03	7.44E-02
Dpy19l3	1187	1418	0.231	8.31E-04	1.34E-02
Iqgap1	540	650	0.231	2.57E-03	3.16E-02
Nefh	602	724	0.231	3.90E-03	4.27E-02
Pros1	242	290	0.231	3.24E-03	3.71E-02
Ppp2r2b	1214	1455	0.230	2.33E-03	2.96E-02
Lmo4	2582	3039	0.229	2.61E-09	3.49E-07
Osbpl5	183	222	0.229	8.16E-03	7.32E-02
Tead1	884	1057	0.229	1.44E-03	2.05E-02
Jak1	3298	3884	0.228	4.06E-08	4.00E-06
Lig1	103	125	0.227	1.06E-02	8.79E-02
Acsl5	1022	1212	0.226	6.19E-04	1.09E-02
Ifit2	132	158	0.226	6.02E-03	5.84E-02
Prr13	353	417	0.226	7.53E-05	2.02E-03
Ccr2	3	8	0.225	1.17E-02	9.42E-02
Dcx	375	444	0.225	1.17E-03	1.74E-02
Pak1	3874	4552	0.225	4.72E-08	4.51E-06
Samd14	542	650	0.225	4.26E-03	4.56E-02
Unc5c	1308	1566	0.225	4.79E-03	4.97E-02
Ctsd	3771	4446	0.224	6.25E-05	1.75E-03
Fam210b	541	649	0.223	8.85E-03	7.76E-02
Arhgap33	1278	1523	0.222	3.23E-03	3.71E-02
Drd1a	100	246	0.222	1.23E-02	9.73E-02
Fam214b	407	481	0.222	4.83E-04	8.98E-03
Pgap1	548	651	0.222	2.31E-03	2.95E-02
Ptprf	813	971	0.222	5.25E-03	5.28E-02
Rgmb	766	899	0.222	5.08E-06	2.34E-04
Scd1	2220	2610	0.222	1.24E-05	4.75E-04
Dner	1609	1896	0.221	1.38E-04	3.31E-03
Pcsk5	414	495	0.221	6.87E-03	6.46E-02

Cyp51	994	1165	0.220	8.02E-06	3.37E-04
Gng3	1520	1788	0.220	6.73E-05	1.85E-03
Ociad2	1522	1816	0.220	5.11E-03	5.20E-02
Parp8	540	637	0.220	1.67E-04	3.88E-03
Pold2	110	130	0.220	9.04E-03	7.90E-02
Prkab2	674	807	0.220	7.89E-03	7.13E-02
Tbc1d9	1407	1647	0.220	1.36E-06	8.01E-05
Tmem178b	471	557	0.220	1.56E-03	2.18E-02
Itm2a	664	789	0.219	2.17E-03	2.82E-02
Magi3	827	970	0.219	1.65E-05	5.97E-04
Skap2	186	221	0.219	4.44E-03	4.72E-02
Man1c1	522	619	0.218	1.22E-03	1.80E-02
Arhgef6	415	492	0.217	1.41E-03	2.02E-02
Gm5607	202	238	0.217	2.03E-03	2.68E-02
Gm9866	239	284	0.217	4.57E-03	4.81E-02
Hspb8	197	234	0.217	3.34E-03	3.79E-02
Ankrd29	309	364	0.216	4.79E-03	4.97E-02
Apaf1	463	553	0.216	7.57E-03	6.91E-02
Syn2	11134	13033	0.216	2.33E-05	7.82E-04
Fbxo2	754	884	0.215	1.19E-05	4.60E-04
Ptprn	5976	7036	0.215	1.20E-03	1.78E-02
Pcnxl2	1191	1400	0.214	8.97E-04	1.42E-02
Plcxd2	440	522	0.214	5.57E-03	5.53E-02
Ppap2a	190	222	0.214	1.94E-03	2.59E-02
Slc9a1	827	968	0.214	4.19E-05	1.27E-03
Tecr	2691	3136	0.213	1.10E-06	6.78E-05
Tex9	140	168	0.213	1.15E-02	9.34E-02
Tpm1	2450	2863	0.213	5.46E-05	1.57E-03
Ankrd42	165	196	0.212	8.11E-03	7.30E-02
Ak4	971	1144	0.211	2.59E-03	3.17E-02
Kcnip1	148	176	0.211	1.20E-02	9.55E-02
Lrrc8d	675	788	0.211	1.45E-04	3.44E-03
Pias3	283	338	0.211	7.54E-03	6.89E-02
1700034P13Rik	122	144	0.210	8.43E-03	7.50E-02
Ctps2	690	808	0.210	3.30E-04	6.74E-03
Fam134b	588	689	0.210	2.45E-03	3.04E-02
Fam185a	225	264	0.210	1.43E-03	2.04E-02
Ppp4r4	1049	1224	0.210	5.74E-05	1.63E-03
Ptprt	1724	2018	0.210	8.80E-04	1.40E-02

Rab15	2576	3008	0.210	1.41E-04	3.36E-03
Rassf5	247	291	0.210	1.48E-03	2.09E-02
Fam171a2	689	804	0.209	1.08E-04	2.75E-03
Ifi27	388	457	0.209	6.52E-03	6.18E-02
Grin2d	137	164	0.208	1.09E-02	8.92E-02
Nipsnap3b	143	169	0.208	9.67E-03	8.23E-02
Oxr1	3906	4548	0.208	5.99E-05	1.69E-03
Lss	280	328	0.207	1.17E-03	1.75E-02
Tm7sf2	170	202	0.207	9.99E-03	8.44E-02
Cmya5	158	186	0.206	9.55E-03	8.17E-02
Ggact	225	265	0.206	2.74E-03	3.30E-02
Glrx	217	255	0.206	4.56E-03	4.81E-02
Ndn	1347	1573	0.206	1.33E-03	1.93E-02
Ppp2r3a	916	1064	0.206	5.46E-05	1.57E-03
Slc4a10	5323	6187	0.206	4.69E-05	1.39E-03
Tmem63c	601	701	0.206	2.50E-04	5.42E-03
Cdh2	1959	2263	0.205	6.96E-08	6.24E-06
H2-K1	321	380	0.205	7.06E-03	6.59E-02
Decr2	434	504	0.204	2.80E-04	5.94E-03
Gpc1	1518	1778	0.203	5.47E-03	5.45E-02
Tuba1b	1249	1448	0.202	2.38E-04	5.19E-03
Gkap1	171	201	0.201	8.59E-03	7.60E-02
St6gal1	290	341	0.201	1.24E-02	9.82E-02
St8sia5	397	466	0.201	9.54E-03	8.16E-02
Vasp	149	176	0.201	9.86E-03	8.35E-02
Zwint	4014	4633	0.201	1.24E-06	7.39E-05
Hmgcr	1684	1948	0.200	2.27E-04	5.00E-03
Map6	1320	1526	0.200	4.85E-07	3.27E-05
Mmd	7512	8712	0.200	6.39E-04	1.11E-02
R74862	214	252	0.200	1.08E-02	8.88E-02
Slc45a1	454	531	0.200	4.38E-03	4.66E-02
Anxa7	1060	1230	0.199	3.74E-04	7.39E-03
Basp1	6528	7531	0.199	4.58E-06	2.19E-04
Cd63	656	761	0.199	5.19E-04	9.45E-03
Tubb3	1728	2001	0.199	3.17E-04	6.55E-03
Tubb4b	1557	1797	0.199	5.85E-06	2.62E-04
Aacs	592	685	0.198	5.90E-05	1.67E-03
Phactr2	430	502	0.198	7.28E-03	6.73E-02
Snap91	5729	6615	0.198	4.04E-05	1.23E-03

Acaca	972	1124	0.197	4.72E-04	8.85E-03
Acox3	356	414	0.197	7.51E-04	1.26E-02
Eno1b	288	337	0.197	1.20E-02	9.57E-02
Acot7	1999	2312	0.196	7.67E-04	1.27E-02
Ebp	155	182	0.196	9.50E-03	8.15E-02
Fbxo44	794	914	0.196	6.60E-06	2.88E-04
Pfkp	2673	3101	0.196	2.63E-03	3.21E-02
Unc119	158	184	0.196	9.81E-03	8.32E-02
Cabp7	635	734	0.195	6.10E-04	1.07E-02
Syngr1	5274	6065	0.195	4.78E-06	2.26E-04
Parva	1233	1418	0.194	5.92E-05	1.68E-03
2210013O21Rik	196	226	0.193	5.07E-03	5.17E-02
Gprasp2	1796	2069	0.193	1.91E-04	4.31E-03
Rian	6204	7160	0.193	7.52E-04	1.26E-02
Sfxn3	1129	1301	0.193	5.07E-04	9.27E-03
Trim3	1169	1349	0.193	3.13E-04	6.50E-03
Amph	3688	4236	0.192	2.11E-05	7.25E-04
Lifr	1392	1604	0.192	3.96E-05	1.21E-03
Slc19a2	235	275	0.192	9.43E-03	8.12E-02
Slc7a8	1163	1342	0.192	7.41E-04	1.25E-02
Trpm2	410	477	0.192	9.43E-03	8.12E-02
Endod1	1256	1445	0.191	3.57E-04	7.15E-03
Ifitm7	562	646	0.190	3.31E-04	6.76E-03
Shoc2	974	1119	0.190	3.94E-05	1.21E-03
Dzank1	3008	3445	0.189	1.76E-05	6.25E-04
Enox2	250	290	0.189	6.79E-03	6.39E-02
Ttc27	236	271	0.189	3.73E-03	4.13E-02
6530402F18Rik	336	388	0.188	8.85E-03	7.76E-02
Kcnd3	2278	2615	0.188	4.21E-04	8.17E-03
Snph	5804	6632	0.188	1.24E-08	1.39E-06
Bsn	8662	9944	0.187	7.60E-04	1.26E-02
Gmps	907	1038	0.186	3.71E-04	7.34E-03
Kdelr2	273	316	0.186	6.95E-03	6.52E-02
Rgs17	1351	1557	0.186	5.20E-03	5.24E-02
2310022B05Rik	891	1020	0.185	9.29E-04	1.46E-02
Clstn1	12384	14133	0.185	1.31E-06	7.78E-05
Pcdhga4	169	196	0.185	1.12E-02	9.10E-02
Hdac4	349	402	0.184	5.26E-03	5.28E-02
Unc13b	885	1021	0.184	9.10E-03	7.94E-02

Cyp46a1	1699	1954	0.183	6.14E-03	5.93E-02
Rin1	1354	1553	0.183	3.19E-03	3.68E-02
Slc45a4	539	614	0.183	1.16E-04	2.91E-03
Abca8b	745	858	0.182	1.25E-02	9.84E-02
BC037034	614	702	0.182	7.81E-04	1.29E-02
Acly	2158	2456	0.181	2.70E-05	8.85E-04
Arl4a	573	656	0.181	2.93E-04	6.17E-03
Gpsm1	405	466	0.181	9.43E-03	8.12E-02
Dgkg	4690	5335	0.180	1.14E-05	4.47E-04
Fgf12	1635	1874	0.180	5.43E-03	5.42E-02
Ints7	319	366	0.180	9.99E-03	8.44E-02
Pde2a	4892	5580	0.179	1.19E-03	1.76E-02
Atp8a2	266	303	0.178	5.46E-03	5.44E-02
Napb	7288	8300	0.178	3.73E-04	7.38E-03
Wrb	743	847	0.178	1.58E-04	3.71E-03
Lrrtm2	1354	1544	0.177	4.22E-03	4.53E-02
Med16	655	744	0.177	3.62E-04	7.23E-03
Acsl4	2022	2300	0.176	8.84E-04	1.40E-02
Sgsm1	902	1033	0.176	9.38E-03	8.10E-02
Cic	2244	2552	0.175	7.45E-04	1.25E-02
Myadm	822	934	0.175	2.11E-03	2.77E-02
Psd3	12776	14519	0.175	7.63E-04	1.27E-02
Sc5d	959	1088	0.175	1.10E-04	2.78E-03
Ypel5	1602	1830	0.175	9.58E-03	8.19E-02
Bscl2	768	872	0.174	1.43E-04	3.41E-03
Prep	349	397	0.174	3.48E-03	3.91E-02
Rab8b	1354	1544	0.174	6.38E-03	6.08E-02
Lrpap1	2349	2670	0.173	2.17E-03	2.82E-02
Pgd	381	435	0.173	3.56E-03	3.98E-02
Raph1	951	1079	0.173	6.67E-04	1.15E-02
Fhl1	859	979	0.172	8.44E-03	7.50E-02
Ly6e	1561	1772	0.172	2.08E-03	2.74E-02
Fam199x	396	452	0.171	6.96E-03	6.52E-02
Npc2	744	840	0.171	2.33E-03	2.96E-02
Opcml	4813	5441	0.171	4.15E-05	1.26E-03
Ptprn2	4471	5074	0.171	2.03E-03	2.68E-02
Aldh3a2	681	770	0.170	1.08E-03	1.64E-02
Rcan3	415	470	0.170	4.34E-03	4.62E-02
Tmem237	310	352	0.170	4.24E-03	4.54E-02

B4galt2	644	731	0.169	1.08E-03	1.64E-02
Ddr1	721	814	0.169	6.44E-04	1.11E-02
Epdr1	1706	1930	0.169	1.60E-03	2.23E-02
Smad2	585	662	0.169	2.31E-03	2.94E-02
Stmn3	3422	3873	0.169	1.85E-03	2.49E-02
Tmx2	1534	1728	0.169	2.32E-05	7.82E-04
Rnh1	410	464	0.168	9.42E-03	8.12E-02
Slc3a2	1245	1410	0.168	8.35E-04	1.35E-02
Fkbp9	628	715	0.167	7.46E-03	6.84E-02
Flot1	995	1126	0.167	7.70E-04	1.27E-02
Gnaz	628	710	0.167	1.63E-03	2.26E-02
Hspa4l	1651	1866	0.167	1.30E-03	1.89E-02
Lipa	275	312	0.167	8.16E-03	7.32E-02
Ptpdc1	460	520	0.167	3.32E-03	3.77E-02
Rabac1	894	1010	0.167	4.41E-04	8.42E-03
Ap1b1	2335	2627	0.166	5.71E-06	2.57E-04
Rps6kc1	604	685	0.166	1.29E-03	1.89E-02
Sdr39u1	424	478	0.166	1.40E-03	2.01E-02
Slc32a1	673	758	0.166	7.67E-04	1.27E-02
Bbs2	376	425	0.165	5.80E-03	5.69E-02
Hsd17b10	265	300	0.165	7.40E-03	6.80E-02
Ssbp3	1585	1784	0.165	6.90E-04	1.18E-02
Sv2a	5934	6666	0.165	3.60E-08	3.60E-06
Kctd6	682	773	0.164	1.17E-02	9.42E-02
Dnajc6	4990	5619	0.163	1.06E-03	1.63E-02
Orai3	314	357	0.163	1.19E-02	9.54E-02
Pign	380	429	0.163	6.30E-03	6.02E-02
Ap2m1	3199	3587	0.162	3.28E-05	1.04E-03
Ap3b2	2464	2765	0.162	2.65E-05	8.71E-04
Gaa	3855	4331	0.162	5.72E-05	1.63E-03
Gdap1l1	629	708	0.162	6.41E-04	1.11E-02
Lnx1	321	362	0.162	5.15E-03	5.24E-02
Tmem245	688	778	0.162	1.27E-02	9.99E-02
Mrps27	268	303	0.161	9.52E-03	8.16E-02
Anxa5	514	578	0.160	3.80E-03	4.19E-02
Gcnt2	401	452	0.160	3.08E-03	3.59E-02
Dact3	1468	1648	0.159	2.02E-03	2.68E-02
Reep5	5250	5886	0.159	9.81E-05	2.54E-03
Prkag2	2091	2346	0.158	1.90E-04	4.31E-03

Slc6a17	7023	7913	0.158	1.23E-02	9.75E-02
Atp6v1b2	8282	9274	0.157	7.29E-04	1.24E-02
Gnai1	2825	3173	0.157	6.42E-03	6.11E-02
Pygb	4185	4679	0.157	2.86E-06	1.49E-04
Uhrf1bp1l	1663	1862	0.157	8.64E-04	1.38E-02
Abca3	1432	1603	0.156	2.10E-03	2.76E-02
Rab4b	467	523	0.156	1.03E-02	8.59E-02
Snca	5024	5618	0.156	4.78E-04	8.93E-03
Spock2	8659	9677	0.156	8.12E-05	2.16E-03
Degs1	853	954	0.155	5.61E-04	1.00E-02
Ppp1r16a	726	815	0.155	1.13E-02	9.15E-02
Ptprg	2000	2243	0.155	7.03E-03	6.57E-02
Rab2b	516	578	0.155	1.52E-03	2.13E-02
Tkt	1400	1562	0.155	1.53E-04	3.61E-03
Adck3	850	951	0.154	2.41E-03	3.02E-02
Gpr137	854	954	0.154	4.82E-03	4.98E-02
Krt222	538	603	0.154	2.18E-03	2.82E-02
Cacna2d3	1372	1539	0.153	7.86E-03	7.11E-02
Capns1	1318	1471	0.153	8.28E-05	2.19E-03
Dzip1	1304	1460	0.153	6.42E-03	6.11E-02
Mark4	710	796	0.153	1.20E-03	1.77E-02
Pgam1	868	970	0.153	2.71E-03	3.28E-02
Stxbp1	9872	11021	0.153	2.69E-04	5.76E-03
Gdi1	7794	8677	0.152	2.16E-06	1.17E-04
Megf8	2853	3180	0.152	1.03E-04	2.64E-03
2310067B10Rik	769	857	0.151	2.80E-03	3.35E-02
Il6st	1245	1393	0.151	2.08E-03	2.73E-02
Rusc1	1422	1593	0.151	1.01E-02	8.50E-02
Cep170b	2612	2909	0.150	4.23E-04	8.18E-03
Bcap31	597	664	0.149	3.38E-03	3.83E-02
Dbnl	805	894	0.149	3.06E-04	6.40E-03
Klc1	7121	7914	0.149	1.11E-05	4.42E-04
Reps2	5051	5625	0.149	9.44E-04	1.48E-02
Slc27a4	862	962	0.149	2.16E-03	2.80E-02
Slc39a10	2540	2824	0.149	4.96E-04	9.13E-03
Cpeb2	1201	1340	0.148	5.24E-03	5.27E-02
Napg	2880	3195	0.148	1.34E-06	7.94E-05
Syn1	6448	7176	0.148	8.74E-04	1.39E-02
Aldoa	18777	20858	0.147	5.61E-04	1.00E-02

Ptprs	8292	9218	0.147	5.49E-04	9.87E-03
Ywhag	18427	20440	0.147	5.01E-06	2.32E-04
Atp6ap2	3140	3495	0.146	6.07E-03	5.88E-02
Ids	9307	10322	0.146	1.10E-05	4.40E-04
Lpgat1	3364	3732	0.145	1.39E-03	2.00E-02
Rhof	404	450	0.145	8.68E-03	7.66E-02
Scd2	20129	22348	0.145	1.11E-03	1.67E-02
Disp2	6661	7372	0.144	4.24E-06	2.07E-04
Plxna2	2581	2865	0.144	9.97E-04	1.55E-02
Praf2	533	591	0.144	6.19E-03	5.96E-02
Stx1a	1035	1153	0.144	1.09E-02	8.92E-02
Tmem130	1739	1933	0.144	7.67E-03	6.97E-02
Usp46	1691	1874	0.143	1.60E-03	2.22E-02
BC004004	524	578	0.142	5.05E-03	5.16E-02
Dbi	950	1050	0.142	6.42E-04	1.11E-02
Gpr162	2150	2376	0.142	1.22E-04	3.00E-03
Slc20a2	782	864	0.142	2.76E-03	3.32E-02
Fam13c	1977	2194	0.141	9.35E-03	8.09E-02
Gas2l1	658	730	0.141	6.73E-03	6.34E-02
Rangap1	2443	2704	0.141	9.96E-04	1.55E-02
Tmem184b	714	794	0.141	6.81E-03	6.41E-02
Mapk8ip1	3801	4203	0.140	1.32E-03	1.92E-02
Spryd3	2020	2232	0.140	2.17E-06	1.18E-04
Apba2	3076	3391	0.139	3.77E-05	1.17E-03
Rab3gap2	1834	2030	0.139	3.93E-03	4.29E-02
Gls	7629	8430	0.138	3.19E-03	3.68E-02
Grn	692	763	0.138	4.09E-03	4.43E-02
Hexa	613	676	0.138	1.05E-02	8.72E-02
Lztr1	2309	2549	0.138	1.17E-04	2.92E-03
Tmem175	616	680	0.138	6.18E-03	5.95E-02
Tubb5	3993	4404	0.138	4.75E-05	1.40E-03
Cadps	4450	4911	0.137	2.55E-03	3.15E-02
Plekhb2	3352	3692	0.137	2.01E-04	4.52E-03
Zc2hc1a	850	938	0.137	7.75E-03	7.02E-02
Fkbp8	3078	3386	0.136	1.54E-04	3.61E-03
Nomo1	1901	2087	0.136	2.31E-04	5.05E-03
Rnf150	1972	2178	0.136	7.47E-03	6.84E-02
Usp20	1218	1341	0.136	7.07E-04	1.20E-02
A830010M20Rik	2386	2628	0.135	2.56E-03	3.15E-02

Rrbp1	851	939	0.135	6.36E-03	6.06E-02
2900011O08Rik	2838	3125	0.134	2.87E-03	3.41E-02
Gnas	12839	14132	0.134	7.54E-04	1.26E-02
Cd200	1057	1165	0.133	9.71E-03	8.25E-02
Mroh1	628	691	0.133	1.02E-02	8.55E-02
Pip5k1c	4723	5207	0.133	1.05E-02	8.73E-02
Por	991	1093	0.133	3.41E-03	3.84E-02
Tpi1	5725	6294	0.133	1.60E-03	2.23E-02
Nfe2l1	4264	4682	0.132	1.35E-04	3.29E-03
Rapgef6	1099	1212	0.132	2.44E-03	3.04E-02
Rusc2	1397	1537	0.131	1.03E-03	1.59E-02
Ttl	744	820	0.131	1.17E-02	9.42E-02
Ak1	1194	1308	0.130	1.86E-03	2.51E-02
Fam115a	2720	2981	0.130	4.09E-04	7.97E-03
Scrn1	1040	1142	0.130	4.87E-03	5.02E-02
Apba1	3203	3512	0.129	1.66E-03	2.29E-02
Arhgap1	734	808	0.129	5.32E-03	5.32E-02
Lmbrd2	771	847	0.129	9.17E-03	7.99E-02
Gnptab	1277	1400	0.127	6.27E-03	6.00E-02
Hn1	807	886	0.127	8.85E-03	7.76E-02
Sema6b	1712	1874	0.127	5.06E-04	9.27E-03
Tbcb	544	594	0.127	9.71E-03	8.25E-02
Ankrd28	1083	1184	0.126	5.06E-03	5.17E-02
Hpcal4	16218	17750	0.126	1.92E-03	2.57E-02
Copg1	4486	4897	0.125	4.61E-06	2.20E-04
Cplx1	5491	6012	0.125	1.04E-02	8.67E-02
Hsp90aa1	9239	10116	0.125	1.07E-02	8.84E-02
Cd81	3562	3891	0.124	3.29E-04	6.73E-03
Clcn6	796	870	0.124	3.16E-03	3.65E-02
Enpp5	2669	2910	0.124	2.61E-04	5.62E-03
Atp13a2	1693	1851	0.123	1.17E-02	9.43E-02
Atxn7l3	2913	3180	0.123	1.57E-03	2.19E-02
Snx10	1855	2027	0.123	1.16E-02	9.39E-02
Anapc2	973	1062	0.122	1.37E-03	1.97E-02
Grb2	2252	2461	0.122	1.11E-02	9.07E-02
Prepl	5624	6133	0.122	5.31E-04	9.65E-03
Synj1	9066	9889	0.121	4.18E-03	4.51E-02
Tmem135	922	1004	0.121	5.53E-03	5.49E-02
Eno2	8715	9500	0.120	3.89E-03	4.26E-02

Pgrmc1	4190	4569	0.120	7.30E-03	6.74E-02
Scaper	829	906	0.120	3.32E-03	3.77E-02
Celf4	8281	9009	0.119	9.57E-04	1.50E-02
Slc25a22	2718	2962	0.119	9.49E-03	8.15E-02
Apc2	2022	2200	0.118	2.70E-03	3.27E-02
Ctsl	915	996	0.117	8.64E-03	7.63E-02
Magee1	2222	2415	0.117	2.00E-03	2.65E-02
Ppp2r5b	1199	1305	0.117	1.93E-03	2.58E-02
Chst2	2917	3173	0.116	1.01E-02	8.50E-02
Nsg1	3710	4028	0.116	2.06E-04	4.61E-03
Tmem132a	2185	2376	0.116	9.91E-03	8.38E-02
Armcx1	965	1050	0.115	3.49E-03	3.92E-02
Clip2	1942	2111	0.115	4.72E-03	4.92E-02
Fasn	4355	4726	0.115	6.86E-05	1.88E-03
Tmem8b	1590	1729	0.115	7.07E-03	6.59E-02
Rnf14	4355	4720	0.113	7.28E-03	6.73E-02
Cpeb3	1162	1260	0.112	4.84E-03	5.00E-02
Faxc	1922	2082	0.112	8.71E-03	7.67E-02
Gprasp1	10084	10914	0.112	6.29E-05	1.75E-03
Ube2o	1902	2061	0.112	1.04E-03	1.60E-02
Bin1	3074	3329	0.111	5.92E-03	5.76E-02
Apbb1	4153	4491	0.110	4.89E-03	5.03E-02
Dync1h1	11927	12891	0.110	1.22E-03	1.79E-02
Wdtc1	1233	1331	0.109	3.21E-03	3.70E-02
Gapdh	11570	12491	0.108	4.94E-03	5.07E-02
Sae1	996	1075	0.108	6.05E-03	5.86E-02
Sec23a	2363	2551	0.108	2.60E-03	3.18E-02
Ttc3	16484	17805	0.108	5.97E-03	5.80E-02
Atp1a3	32253	34800	0.107	1.78E-03	2.42E-02
Hars	1097	1184	0.106	5.80E-03	5.69E-02
Srcin1	4325	4662	0.106	1.09E-03	1.65E-02
Usp5	2394	2580	0.106	2.54E-03	3.14E-02
Zcchc18	3846	4148	0.106	1.03E-02	8.60E-02
Fbxo38	894	964	0.105	5.04E-03	5.15E-02
Vcp	3049	3285	0.105	3.88E-03	4.25E-02
Katnal1	1516	1632	0.104	5.59E-03	5.53E-02
Rcn2	1538	1654	0.104	9.37E-03	8.10E-02
Slc3a1	1081	1165	0.104	8.28E-03	7.41E-02
Tnpo2	3016	3246	0.104	1.25E-04	3.07E-03

Cep170	1723	1855	0.103	1.11E-02	9.07E-02
Gpi1	6431	6914	0.103	9.65E-04	1.51E-02
Syp	18992	20418	0.103	7.16E-04	1.21E-02
Aars	1994	2143	0.101	3.06E-03	3.57E-02
Dnm1	14250	15285	0.099	3.89E-03	4.26E-02
Ctsb	5864	6285	0.098	9.85E-03	8.34E-02
Lrpprc	1309	1402	0.097	5.25E-03	5.27E-02
Ttbk2	2008	2151	0.097	1.07E-02	8.83E-02
Armcx2	1326	1418	0.096	4.54E-03	4.79E-02
Slc38a1	2413	2587	0.096	9.75E-03	8.28E-02
Trpc4ap	2111	2258	0.096	4.20E-03	4.52E-02
Ric3	1610	1724	0.095	1.13E-02	9.20E-02
Klhdc2	1927	2060	0.093	1.07E-02	8.84E-02
Acox1	3195	3409	0.092	1.19E-02	9.53E-02
Atp6v0b	1986	2122	0.092	4.09E-03	4.43E-02
Wdfy3	4146	4418	0.091	8.79E-03	7.73E-02
Ap3m2	2056	2190	0.090	1.19E-02	9.53E-02
Herc1	6193	6594	0.089	7.02E-03	6.57E-02
Mtor	1960	2085	0.087	1.03E-02	8.60E-02
Rundc3a	3470	3689	0.087	7.12E-03	6.62E-02
Gnl3l	4196	4453	0.085	1.07E-02	8.84E-02
Uba1	5368	5699	0.085	8.63E-03	7.62E-02
Atp6v0e2	4633	4904	0.081	9.60E-03	8.21E-02
Sirpa	5528	5846	0.079	7.50E-03	6.86E-02
Tmcc2	3606	3804	0.077	1.10E-02	8.99E-02
Psap	21573	22492	0.060	2.87E-03	3.41E-02

Table S3. Gene Ontology: Molecular function for downregulated genes in hAPP(J20) mice.

GO: Molecular Function	# of Reference Genes	# of Observed Genes	# of Expected Genes	Ratio of Enrichment	rawP	adjP
binding (GO:0005488)	12136	726	534.48	1.36	1.96E-36	9.51E-33
protein binding (GO:0005515)	7764	522	341.94	1.53	6.19E-32	1.50E-28
organic cyclic compound binding (GO:0097159)	5171	344	227.74	1.51	3.54E-17	4.30E-14
heterocyclic compound binding (GO:1901363)	5083	337	223.86	1.51	1.56E-16	1.51E-13
nucleic acid binding (GO:0003676)	3317	241	146.08	1.65	1.56E-15	1.26E-12
ion binding (GO:0043167)	5182	333	228.22	1.46	2.29E-14	1.59E-11
molecular_function (GO:0003674)	20461	958	901.13	1.06	2.93E-14	1.78E-11
metal ion binding (GO:0046872)	3328	233	146.57	1.59	2.82E-13	1.37E-10
DNA binding (GO:0003677)	1946	156	85.7	1.82	3.66E-13	1.59E-10
cation binding (GO:0043169)	3414	237	150.36	1.58	3.93E-13	1.59E-10
enzyme binding (GO:0019899)	1823	144	80.29	1.79	1.00E-11	3.73E-09
regulatory region nucleic acid binding (GO:0001067)	800	80	35.23	2.27	2.41E-11	8.36E-09
transcription regulatory region DNA binding (GO:0044212)	794	79	34.97	2.26	4.08E-11	1.32E-08
regulatory region DNA binding (GO:0000975)	797	79	35.1	2.25	4.86E-11	1.47E-08
macromolecular complex binding (GO:0044877)	1544	122	68	1.79	4.75E-10	1.36E-07
sequence-specific DNA binding (GO:0043565)	977	87	43.03	2.02	9.14E-10	2.46E-07
sequence-specific double-stranded DNA binding (GO:1990837)	669	67	29.46	2.27	1.01E-09	2.58E-07
transcription regulatory region sequence-specific DNA binding (GO:0000976)	646	65	28.45	2.28	1.51E-09	3.56E-07
double-stranded DNA binding (GO:0003690)	743	70	32.72	2.14	4.94E-09	1.09E-06

transcription factor activity, sequence-specific DNA binding (GO:0003700)	1056	89	46.51	1.91	7.59E-09	1.60E-06
nucleic acid binding transcription factor activity (GO:0001071)	1056	89	46.51	1.91	7.59E-09	1.60E-06
RNA polymerase II transcription factor activity, sequence-specific DNA binding (GO:0000981)	647	62	28.49	2.18	2.10E-08	4.08E-06
Rho guanyl-nucleotide exchange factor activity (GO:0005089)	69	17	3.04	5.59	2.36E-08	4.41E-06
ion channel activity (GO:0005216)	383	43	16.87	2.55	5.14E-08	8.91E-06
RNA polymerase II regulatory region sequence-specific DNA binding (GO:0000977)	572	56	25.19	2.22	5.14E-08	8.91E-06
Rho GTPase binding (GO:0017048)	83	18	3.66	4.92	6.13E-08	9.92E-06
RNA polymerase II regulatory region DNA binding (GO:0001012)	578	56	25.46	2.2	7.20E-08	1.13E-05
chromatin binding (GO:0003682)	492	50	21.67	2.31	8.97E-08	1.36E-05
transcription factor activity, RNA polymerase II core promoter proximal region sequence-specific binding (GO:0000982)	365	41	16.08	2.55	1.03E-07	1.52E-05
substrate-specific channel activity (GO:0022838)	394	43	17.35	2.48	1.10E-07	1.57E-05
potassium ion transmembrane transporter activity (GO:0015079)	141	23	6.21	3.7	1.58E-07	2.19E-05
poly(A) RNA binding (GO:0044822)	1110	87	48.89	1.78	2.55E-07	3.35E-05
passive transmembrane transporter activity (GO:0022803)	422	44	18.59	2.37	2.69E-07	3.44E-05
channel activity (GO:0015267)	422	44	18.59	2.37	2.69E-07	3.44E-05
calmodulin binding (GO:0005516)	170	25	7.49	3.34	3.12E-07	3.79E-05
cation channel activity (GO:0005261)	286	34	12.6	2.7	3.60E-07	4.25E-05
metal ion transmembrane transporter activity (GO:0046873)	398	42	17.53	2.4	3.68E-07	4.25E-05

Ras guanyl-nucleotide exchange factor activity (GO:0005088)	117	20	5.15	3.88	4.90E-07	5.53E-05
gated channel activity (GO:0022836)	292	34	12.86	2.64	5.70E-07	6.29E-05
protein heterodimerization activity (GO:0046982)	498	48	21.93	2.19	7.12E-07	7.68E-05
voltage-gated cation channel activity (GO:0022843)	136	21	5.99	3.51	1.27E-06	1.34E-04
voltage-gated channel activity (GO:0022832)	185	25	8.15	3.07	1.40E-06	1.45E-04
voltage-gated ion channel activity (GO:0005244)	185	25	8.15	3.07	1.40E-06	1.45E-04
transcription factor activity, transcription factor binding (GO:0000989)	463	44	20.39	2.16	2.92E-06	2.88E-04
RNA polymerase II core promoter proximal region sequence-specific DNA binding (GO:0000978)	344	36	15.15	2.38	2.97E-06	2.88E-04
transcription factor activity, protein binding (GO:0000988)	467	44	20.57	2.14	3.62E-06	3.38E-04
core promoter proximal region sequence-specific DNA binding (GO:0000987)	363	37	15.99	2.31	3.90E-06	3.57E-04
core promoter proximal region DNA binding (GO:0001159)	365	37	16.08	2.3	4.40E-06	3.96E-04
molecular function regulator (GO:0098772)	1168	85	51.44	1.65	6.15E-06	5.43E-04
protein serine/threonine kinase activity (GO:0004674)	436	41	19.2	2.14	8.05E-06	6.98E-04
potassium channel activity (GO:0005267)	118	18	5.2	3.46	8.34E-06	7.10E-04
GTPase binding (GO:0051020)	303	32	13.34	2.4	8.65E-06	7.12E-04
core promoter binding (GO:0001047)	167	22	7.35	2.99	8.66E-06	7.12E-04
small GTPase binding (GO:0031267)	277	30	12.2	2.46	1.03E-05	8.33E-04
kinase binding (GO:0019900)	650	54	28.63	1.89	1.08E-05	8.59E-04
guanyl-nucleotide exchange factor activity (GO:0005085)	183	23	8.06	2.85	1.14E-05	8.86E-04
voltage-gated potassium channel activity (GO:0005249)	87	15	3.83	3.91	1.15E-05	8.86E-04

protein kinase binding (GO:0019901)	587	49	25.85	1.9	2.43E-05	1.78E-03
mRNA binding (GO:0003729)	166	21	7.31	2.87	2.46E-05	1.78E-03
RNA binding (GO:0003723)	1511	101	66.55	1.52	2.61E-05	1.86E-03
protein dimerization activity (GO:0046983)	1182	83	52.06	1.59	2.77E-05	1.95E-03
Rac guanyl-nucleotide exchange factor activity (GO:0030676)	13	6	0.57	10.48	2.97E-05	2.06E-03
transcriptional activator activity, RNA polymerase II core promoter proximal region sequence-specific binding (GO:0001077)	253	27	11.14	2.42	3.56E-05	2.43E-03
phosphotransferase activity, alcohol group as acceptor (GO:0016773)	689	54	30.34	1.78	5.01E-05	3.38E-03
transcriptional activator activity, RNA polymerase II transcription regulatory region sequence-specific binding (GO:0001228)	348	33	15.33	2.15	5.11E-05	3.40E-03
nucleoside phosphate binding (GO:1901265)	2136	132	94.07	1.4	5.55E-05	3.58E-03
nucleotide binding (GO:0000166)	2136	132	94.07	1.4	5.55E-05	3.58E-03
transcription cofactor activity (GO:0003712)	412	37	18.14	2.04	5.61E-05	3.58E-03
inorganic cation transmembrane transporter activity (GO:0022890)	494	42	21.76	1.93	6.12E-05	3.86E-03
Ras GTPase binding (GO:0017016)	263	27	11.58	2.33	6.74E-05	4.19E-03
protein kinase activity (GO:0004672)	583	47	25.68	1.83	7.99E-05	4.91E-03
anion binding (GO:0043168)	2490	149	109.66	1.36	8.16E-05	4.95E-03
monovalent inorganic cation transmembrane transporter activity (GO:0015077)	342	32	15.06	2.12	8.39E-05	5.03E-03
transcription factor binding (GO:0008134)	535	44	23.56	1.87	8.65E-05	5.12E-03
Rac GTPase binding (GO:0048365)	40	9	1.76	5.11	9.13E-05	5.34E-03
protein complex binding (GO:0032403)	1009	71	44.44	1.6	9.96E-05	5.76E-03
cation transmembrane transporter activity (GO:0008324)	583	46	25.68	1.79	1.53E-04	8.74E-03

small molecule binding (GO:0036094)	2454	145	108.08	1.34	1.81E-04	1.02E-02
actin binding (GO:0003779)	374	33	16.47	2	1.89E-04	1.05E-02
kinase activity (GO:0016301)	746	55	32.85	1.67	1.98E-04	1.09E-02

Table S4. Gene Ontology: Biological process for downregulated genes in hAPP(J20) mice.

GO: Biological Process	# of Reference Genes	# of Observed Genes	# of Expected Genes	Ratio of Enrichment	rawP	adjP
regulation of cellular process (GO:0050794)	9949	622	438.17	1.42	3.85E-32	5.56E-28
biological regulation (GO:0065007)	10873	653	478.86	1.36	3.04E-29	2.19E-25
regulation of biological process (GO:0050789)	10353	630	455.96	1.38	4.95E-29	2.38E-25
regulation of metabolic process (GO:0019222)	5462	394	240.55	1.64	3.13E-27	1.13E-23
regulation of cellular metabolic process (GO:0031323)	5180	379	228.13	1.66	4.33E-27	1.25E-23
regulation of macromolecule metabolic process (GO:0060255)	5096	372	224.43	1.66	2.97E-26	7.14E-23
regulation of primary metabolic process (GO:0080090)	5108	371	224.96	1.65	9.60E-26	1.98E-22
regulation of nitrogen compound metabolic process (GO:0051171)	3685	292	162.29	1.8	7.17E-25	1.29E-21
regulation of nucleobase-containing compound metabolic process (GO:0019219)	3459	278	152.34	1.82	2.08E-24	3.34E-21
regulation of gene expression (GO:0010468)	3614	286	159.16	1.8	3.43E-24	4.95E-21
regulation of RNA metabolic process (GO:0051252)	3132	256	137.94	1.86	3.10E-23	4.07E-20
single-multicellular organism process (GO:0044707)	4913	351	216.37	1.62	9.70E-23	1.17E-19
cellular process (GO:0009987)	13456	738	592.62	1.25	1.05E-22	1.17E-19
regulation of biosynthetic process (GO:0009889)	3671	281	161.68	1.74	1.39E-21	1.43E-18
regulation of transcription, DNA-templated (GO:0006355)	3001	243	132.17	1.84	2.13E-21	1.96E-18
regulation of cellular macromolecule biosynthetic process (GO:2000112)	3339	262	147.05	1.78	2.22E-21	1.96E-18
regulation of cellular biosynthetic process (GO:0031326)	3611	277	159.03	1.74	2.31E-21	1.96E-18

regulation of nucleic acid-templated transcription (GO:1903506)	3022	243	133.09	1.83	5.23E-21	4.19E-18
regulation of RNA biosynthetic process (GO:2001141)	3029	243	133.4	1.82	7.03E-21	5.34E-18
regulation of signaling (GO:0023051)	2647	220	116.58	1.89	1.58E-20	1.14E-17
regulation of macromolecule biosynthetic process (GO:0010556)	3449	265	151.9	1.74	2.07E-20	1.42E-17
negative regulation of cellular process (GO:0048523)	3982	293	175.37	1.67	4.07E-20	2.67E-17
negative regulation of biological process (GO:0048519)	4267	308	187.92	1.64	4.57E-20	2.87E-17
single-organism developmental process (GO:0044767)	4886	339	215.19	1.58	1.05E-19	6.31E-17
developmental process (GO:0032502)	4917	340	216.55	1.57	1.53E-19	8.83E-17
regulation of cell communication (GO:0010646)	2691	219	118.51	1.85	2.30E-19	1.28E-16
multicellular organism development (GO:0007275)	4262	305	187.7	1.62	2.76E-19	1.48E-16
anatomical structure development (GO:0048856)	4634	323	204.09	1.58	6.97E-19	3.59E-16
positive regulation of biological process (GO:0048518)	4866	332	214.3	1.55	4.35E-18	2.09E-15
cellular macromolecule metabolic process (GO:0044260)	5272	352	232.19	1.52	6.37E-18	2.87E-15
RNA metabolic process (GO:0016070)	2604	209	114.68	1.82	9.04E-18	3.95E-15
nervous system development (GO:0007399)	1919	167	84.52	1.98	3.33E-17	1.41E-14
regulation of developmental process (GO:0050793)	2242	186	98.74	1.88	3.97E-17	1.64E-14
system development (GO:0048731)	3702	267	163.04	1.64	5.55E-17	2.23E-14
nucleic acid metabolic process (GO:0090304)	3014	229	132.74	1.73	7.88E-17	3.07E-14
neurogenesis (GO:0022008)	1493	139	65.75	2.11	1.33E-16	5.05E-14
cellular aromatic compound metabolic process (GO:0006725)	3625	261	159.65	1.63	1.82E-16	6.74E-14
single-organism cellular process (GO:0044763)	10981	611	483.62	1.26	2.16E-16	7.79E-14
nucleobase-containing compound metabolic process	3449	251	151.9	1.65	2.55E-16	8.80E-14

(GO:0006139)						
cellular metabolic process (GO:0044237)	6852	423	301.77	1.4	2.56E-16	8.80E-14
organic cyclic compound metabolic process (GO:1901360)	3829	271	168.63	1.61	3.13E-16	1.05E-13
heterocycle metabolic process (GO:0046483)	3586	258	157.93	1.63	3.23E-16	1.06E-13
positive regulation of cellular process (GO:0048522)	4515	306	198.85	1.54	5.11E-16	1.64E-13
regulation of transcription from RNA polymerase II promoter (GO:0006357)	1698	150	74.78	2.01	5.50E-16	1.73E-13
transcription, DNA-templated (GO:0006351)	1881	161	82.84	1.94	5.91E-16	1.81E-13
nucleic acid-templated transcription (GO:0097659)	1882	161	82.89	1.94	6.19E-16	1.86E-13
generation of neurons (GO:0048699)	1400	131	61.66	2.12	7.84E-16	2.31E-13
RNA biosynthetic process (GO:0032774)	1890	161	83.24	1.93	8.91E-16	2.54E-13
single-organism process (GO:0044699)	12383	668	545.36	1.22	8.99E-16	2.54E-13
regulation of biological quality (GO:0065008)	3123	231	137.54	1.68	1.10E-15	3.05E-13
cell differentiation (GO:0030154)	3222	236	141.9	1.66	1.42E-15	3.87E-13
regulation of signal transduction (GO:0009966)	2318	184	102.09	1.8	4.01E-15	1.07E-12
biological_process (GO:0008150)	20544	961	904.78	1.06	1.06E-14	2.78E-12
nucleobase-containing compound biosynthetic process (GO:0034654)	2129	171	93.76	1.82	1.74E-14	4.41E-12
aromatic compound biosynthetic process (GO:0019438)	2202	175	96.98	1.8	2.02E-14	5.03E-12
cell development (GO:0048468)	1513	134	66.63	2.01	2.13E-14	5.21E-12
organic substance metabolic process (GO:0071704)	7441	442	327.71	1.35	2.59E-14	6.23E-12
cellular developmental process (GO:0048869)	3422	242	150.71	1.61	2.70E-14	6.36E-12
regulation of cellular component organization (GO:0051128)	2227	176	98.08	1.79	2.73E-14	6.36E-12
macromolecule metabolic process (GO:0043170)	5896	367	259.67	1.41	4.41E-14	1.01E-11
heterocycle biosynthetic process (GO:0018130)	2194	173	96.63	1.79	5.82E-14	1.31E-11
organic cyclic compound biosynthetic process (GO:1901362)	2304	179	101.47	1.76	7.03E-14	1.56E-11

cellular component organization (GO:0016043)	4498	296	198.1	1.49	8.76E-14	1.92E-11
primary metabolic process (GO:0044238)	7142	424	314.54	1.35	1.78E-13	3.83E-11
neuron differentiation (GO:0030182)	883	91	38.89	2.34	1.81E-13	3.84E-11
negative regulation of cellular metabolic process (GO:0031324)	2207	172	97.2	1.77	1.89E-13	3.95E-11
metabolic process (GO:0008152)	7916	460	348.63	1.32	1.97E-13	4.06E-11
regulation of cell differentiation (GO:0045595)	1570	134	69.14	1.94	3.05E-13	6.20E-11
regulation of multicellular organismal process (GO:0051239)	2596	193	114.33	1.69	3.30E-13	6.56E-11
negative regulation of nitrogen compound metabolic process (GO:0051172)	1424	125	62.71	1.99	3.32E-13	6.56E-11
cellular component organization or biogenesis (GO:0071840)	4651	301	204.84	1.47	3.83E-13	7.47E-11
neuron development (GO:0048666)	711	78	31.31	2.49	5.36E-13	1.03E-10
negative regulation of metabolic process (GO:0009892)	2357	178	103.81	1.71	9.53E-13	1.81E-10
regulation of response to stimulus (GO:0048583)	3185	223	140.27	1.59	1.11E-12	2.08E-10
negative regulation of macromolecule metabolic process (GO:0010605)	2185	168	96.23	1.75	1.16E-12	2.15E-10
nitrogen compound metabolic process (GO:0006807)	4340	283	191.14	1.48	1.23E-12	2.25E-10
gene expression (GO:0010467)	3003	213	132.26	1.61	1.27E-12	2.29E-10
negative regulation of gene expression (GO:0010629)	1389	121	61.17	1.98	1.37E-12	2.44E-10
negative regulation of signaling (GO:0023057)	1105	103	48.67	2.12	1.69E-12	2.97E-10
cellular nitrogen compound metabolic process (GO:0034641)	4056	267	178.63	1.49	2.66E-12	4.63E-10
negative regulation of RNA metabolic process (GO:0051253)	1179	107	51.92	2.06	2.93E-12	5.03E-10
cellular macromolecule biosynthetic process (GO:0034645)	2682	194	118.12	1.64	3.24E-12	5.50E-10
negative regulation of cell communication	1120	103	49.33	2.09	3.67E-12	6.10E-10

(GO:0010648)						
regulation of intracellular signal transduction (GO:1902531)	1427	122	62.85	1.94	3.68E-12	6.10E-10
anatomical structure morphogenesis (GO:0009653)	2066	159	90.99	1.75	4.97E-12	8.15E-10
macromolecule biosynthetic process (GO:0009059)	2710	194	119.35	1.63	8.00E-12	1.30E-09
cell projection organization (GO:0030030)	912	88	40.17	2.19	1.42E-11	2.27E-09
negative regulation of nucleobase-containing compound metabolic process (GO:0045934)	1309	113	57.65	1.96	1.43E-11	2.27E-09
negative regulation of cellular macromolecule biosynthetic process (GO:2000113)	1261	110	55.54	1.98	1.49E-11	2.34E-09
negative regulation of cellular biosynthetic process (GO:0031327)	1382	117	60.86	1.92	1.96E-11	3.04E-09
regulation of localization (GO:0032879)	2424	176	106.76	1.65	3.21E-11	4.93E-09
regulation of multicellular organismal development (GO:2000026)	1733	137	76.32	1.79	3.30E-11	5.01E-09
regulation of neurogenesis (GO:0050767)	761	77	33.52	2.3	3.40E-11	5.11E-09
negative regulation of biosynthetic process (GO:0009890)	1412	118	62.19	1.9	3.51E-11	5.22E-09
positive regulation of metabolic process (GO:0009893)	2890	201	127.28	1.58	3.77E-11	5.55E-09
negative regulation of transcription, DNA-templated (GO:0045892)	1098	98	48.36	2.03	6.42E-11	9.36E-09
negative regulation of macromolecule biosynthetic process (GO:0010558)	1329	112	58.53	1.91	7.25E-11	1.05E-08
positive regulation of macromolecule metabolic process (GO:0010604)	2671	188	117.63	1.6	7.48E-11	1.07E-08
positive regulation of cellular metabolic process (GO:0031325)	2715	190	119.57	1.59	9.22E-11	1.30E-08
localization (GO:0051179)	4254	270	187.35	1.44	9.54E-11	1.34E-08
negative regulation of nucleic acid-templated	1123	99	49.46	2	9.84E-11	1.37E-08

transcription (GO:1903507)						
regulation of nervous system development (GO:0051960)	857	82	37.74	2.17	1.09E-10	1.50E-08
regulation of cell morphogenesis (GO:0022604)	536	60	23.61	2.54	1.32E-10	1.80E-08
negative regulation of RNA biosynthetic process (GO:1902679)	1136	99	50.03	1.98	1.81E-10	2.44E-08
regulation of cell development (GO:0060284)	950	87	41.84	2.08	2.42E-10	3.23E-08
regulation of ion transmembrane transport (GO:0034765)	398	49	17.53	2.8	3.24E-10	4.29E-08
cellular nitrogen compound biosynthetic process (GO:0044271)	2609	182	114.9	1.58	3.29E-10	4.29E-08
positive regulation of gene expression (GO:0010628)	1690	131	74.43	1.76	3.30E-10	4.29E-08
regulation of anatomical structure morphogenesis (GO:0022603)	964	87	42.46	2.05	4.86E-10	6.26E-08
regulation of neuron differentiation (GO:0045664)	628	65	27.66	2.35	5.07E-10	6.48E-08
cellular biosynthetic process (GO:0044249)	3390	222	149.3	1.49	6.21E-10	7.86E-08
regulation of molecular function (GO:0065009)	2234	160	98.39	1.63	8.17E-10	1.03E-07
regulation of small GTPase mediated signal transduction (GO:0051056)	200	32	8.81	3.63	1.02E-09	1.27E-07
regulation of cell projection organization (GO:0031344)	597	62	26.29	2.36	1.13E-09	1.39E-07
regulation of transmembrane transport (GO:0034762)	415	49	18.28	2.68	1.25E-09	1.53E-07
positive regulation of nucleic acid-templated transcription (GO:1903508)	1402	112	61.75	1.81	1.47E-09	1.78E-07
positive regulation of transcription, DNA-templated (GO:0045893)	1402	112	61.75	1.81	1.47E-09	1.78E-07
positive regulation of RNA biosynthetic process (GO:1902680)	1405	112	61.88	1.81	1.65E-09	1.97E-07
positive regulation of macromolecule biosynthetic process (GO:0010557)	1618	124	71.26	1.74	2.04E-09	2.41E-07

organic substance biosynthetic process (GO:1901576)	3479	224	153.22	1.46	2.20E-09	2.58E-07
neuron projection development (GO:0031175)	550	58	24.22	2.39	2.30E-09	2.68E-07
positive regulation of transcription from RNA polymerase II promoter (GO:0045944)	1016	88	44.75	1.97	2.66E-09	3.07E-07
biosynthetic process (GO:0009058)	3547	227	156.21	1.45	2.78E-09	3.18E-07
positive regulation of RNA metabolic process (GO:0051254)	1454	114	64.04	1.78	2.90E-09	3.30E-07
positive regulation of biosynthetic process (GO:0009891)	1772	132	78.04	1.69	3.21E-09	3.62E-07
positive regulation of cellular component organization (GO:0051130)	1152	96	50.74	1.89	3.24E-09	3.63E-07
regulation of ion transport (GO:0043269)	600	61	26.42	2.31	3.36E-09	3.73E-07
positive regulation of cellular biosynthetic process (GO:0031328)	1738	129	76.54	1.69	6.15E-09	6.78E-07
metal ion transport (GO:0030001)	509	54	22.42	2.41	6.78E-09	7.41E-07
positive regulation of nitrogen compound metabolic process (GO:0051173)	1765	130	77.73	1.67	8.34E-09	9.05E-07
negative regulation of signal transduction (GO:0009968)	982	84	43.25	1.94	1.08E-08	1.16E-06
animal organ development (GO:0048513)	2677	179	117.9	1.52	1.08E-08	1.16E-06
regulation of transport (GO:0051049)	1756	129	77.34	1.67	1.12E-08	1.19E-06
positive regulation of nucleobase-containing compound metabolic process (GO:0045935)	1670	124	73.55	1.69	1.24E-08	1.31E-06
negative regulation of transcription from RNA polymerase II promoter (GO:0000122)	731	68	32.19	2.11	1.34E-08	1.40E-06
positive regulation of nervous system development (GO:0051962)	520	54	22.9	2.36	1.36E-08	1.41E-06
regulation of synapse structure or activity (GO:0050803)	263	35	11.58	3.02	1.72E-08	1.77E-06
multicellular organismal process (GO:0032501)	6561	370	288.95	1.28	1.76E-08	1.80E-06

cell morphogenesis involved in neuron differentiation (GO:0048667)	369	43	16.25	2.65	1.85E-08	1.88E-06
positive regulation of neurogenesis (GO:0050769)	454	49	19.99	2.45	2.02E-08	2.04E-06
regulation of neuron projection development (GO:0010975)	472	50	20.79	2.41	2.57E-08	2.58E-06
tissue development (GO:0009888)	1516	114	66.77	1.71	2.68E-08	2.67E-06
regulation of cell morphogenesis involved in differentiation (GO:0010769)	347	41	15.28	2.68	2.73E-08	2.70E-06
positive regulation of multicellular organismal process (GO:0051240)	1503	113	66.19	1.71	3.14E-08	3.08E-06
actin filament-based process (GO:0030029)	419	46	18.45	2.49	3.37E-08	3.29E-06
positive regulation of cell differentiation (GO:0045597)	910	78	40.08	1.95	3.42E-08	3.31E-06
cell surface receptor signaling pathway (GO:0007166)	1615	119	71.13	1.67	3.80E-08	3.66E-06
regulation of actin filament-based process (GO:0032970)	313	38	13.78	2.76	4.45E-08	4.23E-06
cell morphogenesis involved in differentiation (GO:0000904)	525	53	23.12	2.29	4.54E-08	4.28E-06
cell morphogenesis (GO:0000902)	836	73	36.82	1.98	4.71E-08	4.41E-06
positive regulation of developmental process (GO:0051094)	1254	98	55.23	1.77	4.83E-08	4.50E-06
negative regulation of response to stimulus (GO:0048585)	1301	100	57.3	1.75	7.60E-08	7.03E-06
positive regulation of cytoskeleton organization (GO:0051495)	170	26	7.49	3.47	8.70E-08	8.00E-06
movement of cell or subcellular component (GO:0006928)	1099	88	48.4	1.82	8.77E-08	8.01E-06
cellular component morphogenesis (GO:0032989)	917	77	40.39	1.91	9.48E-08	8.61E-06
cellular response to chemical stimulus (GO:0070887)	1704	122	75.05	1.63	1.16E-07	1.05E-05
positive regulation of cell development (GO:0010720)	557	54	24.53	2.2	1.21E-07	1.08E-05

neuron projection morphogenesis (GO:0048812)	397	43	17.48	2.46	1.35E-07	1.20E-05
response to nitrogen compound (GO:1901698)	606	57	26.69	2.14	1.43E-07	1.27E-05
regulation of cellular component size (GO:0032535)	345	39	15.19	2.57	1.80E-07	1.58E-05
locomotion (GO:0040011)	984	80	43.34	1.85	1.92E-07	1.68E-05
modulation of synaptic transmission (GO:0050804)	334	38	14.71	2.58	2.21E-07	1.92E-05
muscle structure development (GO:0061061)	434	45	19.11	2.35	2.29E-07	1.98E-05
regulation of mRNA processing (GO:0050684)	101	19	4.45	4.27	2.30E-07	1.98E-05
regulation of Ras protein signal transduction (GO:0046578)	179	26	7.88	3.3	2.30E-07	1.98E-05
positive regulation of molecular function (GO:0044093)	1316	99	57.96	1.71	2.36E-07	2.00E-05
positive regulation of neuron differentiation (GO:0045666)	363	40	15.99	2.5	2.39E-07	2.02E-05
cell migration (GO:0016477)	680	61	29.95	2.04	2.55E-07	2.14E-05
regulation of organelle organization (GO:0033043)	1061	84	46.73	1.8	2.77E-07	2.31E-05
regulation of phosphate metabolic process (GO:0019220)	1570	113	69.14	1.63	2.78E-07	2.31E-05
negative regulation of multicellular organismal process (GO:0051241)	1047	83	46.11	1.8	3.11E-07	2.56E-05
regulation of phosphorus metabolic process (GO:0051174)	1575	113	69.36	1.63	3.24E-07	2.66E-05
regulation of synaptic plasticity (GO:0048167)	160	24	7.05	3.41	3.78E-07	3.08E-05
regulation of cytoskeleton organization (GO:0051493)	413	43	18.19	2.36	3.81E-07	3.09E-05
actin cytoskeleton organization (GO:0030036)	370	40	16.3	2.45	3.84E-07	3.09E-05
regulation of axonogenesis (GO:0050770)	172	25	7.58	3.3	3.85E-07	3.09E-05
synaptic transmission (GO:0007268)	316	36	13.92	2.59	4.43E-07	3.53E-05
trans-synaptic signaling (GO:0099537)	316	36	13.92	2.59	4.43E-07	3.53E-05
anterograde trans-synaptic signaling (GO:0098916)	316	36	13.92	2.59	4.43E-07	3.53E-05
regulation of protein modification process (GO:0031399)	1595	113	70.25	1.61	5.93E-07	4.65E-05

synaptic signaling (GO:0099536)	321	36	14.14	2.55	6.34E-07	4.95E-05
potassium ion transport (GO:0006813)	142	22	6.25	3.52	6.77E-07	5.25E-05
macromolecule modification (GO:0043412)	2254	148	99.27	1.49	7.45E-07	5.75E-05
cellular protein modification process (GO:0006464)	2122	141	93.46	1.51	7.52E-07	5.77E-05
protein modification process (GO:0036211)	2122	141	93.46	1.51	7.52E-07	5.77E-05
muscle organ development (GO:0007517)	256	31	11.27	2.75	8.01E-07	6.08E-05
response to endogenous stimulus (GO:0009719)	1025	80	45.14	1.77	9.28E-07	7.01E-05
regulation of metal ion transport (GO:0010959)	341	37	15.02	2.46	9.56E-07	7.19E-05
regulation of phosphorylation (GO:0042325)	1348	98	59.37	1.65	1.20E-06	8.97E-05
regulation of cellular component biogenesis (GO:0044087)	730	62	32.15	1.93	1.21E-06	9.00E-05
regulation of actin cytoskeleton organization (GO:0032956)	275	32	12.11	2.64	1.23E-06	9.10E-05
cell projection morphogenesis (GO:0048858)	605	54	26.64	2.03	1.44E-06	1.06E-04
axonogenesis (GO:0007409)	305	34	13.43	2.53	1.47E-06	1.08E-04
positive regulation of cell projection organization (GO:0031346)	348	37	15.33	2.41	1.52E-06	1.11E-04
cation transport (GO:0006812)	687	59	30.26	1.95	1.56E-06	1.13E-04
regulation of growth (GO:0040008)	641	56	28.23	1.98	1.75E-06	1.26E-04
regulation of ion transmembrane transporter activity (GO:0032412)	175	24	7.71	3.11	1.76E-06	1.26E-04
regulation of cation transmembrane transport (GO:1904062)	214	27	9.42	2.86	1.92E-06	1.37E-04
regulation of anatomical structure size (GO:0090066)	502	47	22.11	2.13	2.01E-06	1.43E-04
mRNA metabolic process (GO:0016071)	442	43	19.47	2.21	2.13E-06	1.51E-04
regulation of mRNA metabolic process (GO:1903311)	119	19	5.24	3.63	2.49E-06	1.75E-04
regulation of protein phosphorylation (GO:0001932)	1249	91	55.01	1.65	2.72E-06	1.91E-04
localization of cell (GO:0051674)	766	63	33.74	1.87	2.76E-06	1.91E-04
cell motility (GO:0048870)	766	63	33.74	1.87	2.76E-06	1.91E-04
regulation of catalytic activity (GO:0050790)	1723	117	75.88	1.54	2.77E-06	1.91E-04

regulation of transmembrane transporter activity (GO:0022898)	180	24	7.93	3.03	2.82E-06	1.94E-04
regulation of transporter activity (GO:0032409)	193	25	8.5	2.94	2.90E-06	1.98E-04
positive regulation of organelle organization (GO:0010638)	542	49	23.87	2.05	3.14E-06	2.14E-04
regulation of protein polymerization (GO:0032271)	169	23	7.44	3.09	3.24E-06	2.19E-04
mRNA processing (GO:0006397)	360	37	15.85	2.33	3.25E-06	2.19E-04
positive regulation of neuron projection development (GO:0010976)	275	31	12.11	2.56	3.37E-06	2.26E-04
striated muscle tissue development (GO:0014706)	289	32	12.73	2.51	3.39E-06	2.27E-04
cell part morphogenesis (GO:0032990)	626	54	27.57	1.96	3.82E-06	2.54E-04
regulation of developmental growth (GO:0048638)	334	35	14.71	2.38	3.94E-06	2.61E-04
positive regulation of cell morphogenesis involved in differentiation (GO:0010770)	172	23	7.58	3.04	4.30E-06	2.83E-04
central nervous system development (GO:0007417)	694	58	30.56	1.9	4.31E-06	2.83E-04
regulation of cellular localization (GO:0060341)	1264	91	55.67	1.63	4.34E-06	2.83E-04
axon development (GO:0061564)	324	34	14.27	2.38	5.23E-06	3.40E-04
cellular response to organic substance (GO:0071310)	1343	95	59.15	1.61	5.35E-06	3.46E-04
heart morphogenesis (GO:0003007)	227	27	10	2.7	5.53E-06	3.56E-04
head development (GO:0060322)	570	50	25.1	1.99	5.59E-06	3.59E-04
cell communication (GO:0007154)	4841	272	213.2	1.28	5.74E-06	3.67E-04
regulation of GTPase activity (GO:0043087)	341	35	15.02	2.33	6.10E-06	3.87E-04
organelle organization (GO:0006996)	2773	170	122.13	1.39	6.11E-06	3.87E-04
regulation of actin polymerization or depolymerization (GO:0008064)	152	21	6.69	3.14	6.83E-06	4.30E-04
macromolecular complex subunit organization (GO:0043933)	1852	122	81.56	1.5	6.91E-06	4.34E-04
regulation of cellular protein metabolic process (GO:0032268)	2216	141	97.6	1.44	7.23E-06	4.52E-04
brain development (GO:0007420)	528	47	23.25	2.02	7.35E-06	4.57E-04

regulation of actin filament length (GO:0030832)	153	21	6.74	3.12	7.53E-06	4.66E-04
single organism signaling (GO:0044700)	4738	266	208.67	1.27	8.17E-06	5.04E-04
signaling (GO:0023052)	4741	266	208.8	1.27	8.57E-06	5.26E-04
regulation of protein kinase activity (GO:0045859)	612	52	26.95	1.93	8.65E-06	5.29E-04
chromatin modification (GO:0016568)	469	43	20.66	2.08	8.93E-06	5.44E-04
muscle tissue development (GO:0060537)	304	32	13.39	2.39	9.22E-06	5.59E-04
regulation of mRNA splicing, via spliceosome (GO:0048024)	65	13	2.86	4.54	9.48E-06	5.71E-04
macromolecule localization (GO:0033036)	1865	122	82.14	1.49	9.50E-06	5.71E-04
enzyme linked receptor protein signaling pathway (GO:0007167)	503	45	22.15	2.03	1.01E-05	6.02E-04
response to chemical (GO:0042221)	3018	181	132.92	1.36	1.04E-05	6.18E-04
cytoskeleton organization (GO:0007010)	801	63	35.28	1.79	1.08E-05	6.39E-04
single-organism organelle organization (GO:1902589)	2005	129	88.3	1.46	1.12E-05	6.60E-04
regulation of actin filament polymerization (GO:0030833)	133	19	5.86	3.24	1.17E-05	6.86E-04
response to organonitrogen compound (GO:0010243)	507	45	22.33	2.02	1.23E-05	7.19E-04
cellular response to nitrogen compound (GO:1901699)	353	35	15.55	2.25	1.25E-05	7.27E-04
regulation of protein complex assembly (GO:0043254)	353	35	15.55	2.25	1.25E-05	7.27E-04
regulation of membrane potential (GO:0042391)	368	36	16.21	2.22	1.25E-05	7.27E-04
negative regulation of developmental process (GO:0051093)	841	65	37.04	1.75	1.34E-05	7.71E-04
behavior (GO:0007610)	606	51	26.69	1.91	1.35E-05	7.73E-04
RNA processing (GO:0006396)	656	54	28.89	1.87	1.38E-05	7.87E-04
negative regulation of protein modification process (GO:0031400)	558	48	24.58	1.95	1.40E-05	7.96E-04
cognition (GO:0050890)	269	29	11.85	2.45	1.55E-05	8.77E-04
regulation of Rho protein signal transduction	101	16	4.45	3.6	1.66E-05	9.36E-04

(GO:0035023)						
RNA splicing (GO:0008380)	286	30	12.6	2.38	1.85E-05	1.04E-03
activation of GTPase activity (GO:0090630)	70	13	3.08	4.22	2.04E-05	1.14E-03
skeletal muscle cell differentiation (GO:0035914)	60	12	2.64	4.54	2.07E-05	1.15E-03
response to hormone (GO:0009725)	551	47	24.27	1.94	2.11E-05	1.17E-03
cell-cell signaling (GO:0007267)	520	45	22.9	1.96	2.24E-05	1.24E-03
lamellipodium organization (GO:0097581)	42	10	1.85	5.41	2.36E-05	1.30E-03
regulation of kinase activity (GO:0043549)	670	54	29.51	1.83	2.43E-05	1.33E-03
intracellular signal transduction (GO:0035556)	1215	85	53.51	1.59	2.52E-05	1.37E-03
regulation of cellular component movement (GO:0051270)	790	61	34.79	1.75	2.53E-05	1.37E-03
cell proliferation (GO:0008283)	539	46	23.74	1.94	2.54E-05	1.37E-03
cellular protein complex localization (GO:0034629)	26	8	1.15	6.99	2.61E-05	1.41E-03
single-organism behavior (GO:0044708)	444	40	19.55	2.05	2.66E-05	1.43E-03
rhythmic process (GO:0048511)	249	27	10.97	2.46	2.73E-05	1.46E-03
regulation of cell death (GO:0010941)	1460	98	64.3	1.52	2.96E-05	1.58E-03
negative regulation of phosphorus metabolic process (GO:0010563)	545	46	24	1.92	3.30E-05	1.74E-03
negative regulation of phosphate metabolic process (GO:0045936)	545	46	24	1.92	3.30E-05	1.74E-03
single-organism localization (GO:1902578)	2760	165	121.55	1.36	3.31E-05	1.74E-03
telencephalon development (GO:0021537)	210	24	9.25	2.59	3.35E-05	1.76E-03
ossification (GO:0001503)	224	25	9.87	2.53	3.39E-05	1.77E-03
positive regulation of protein polymerization (GO:0032273)	85	14	3.74	3.74	3.63E-05	1.89E-03
monovalent inorganic cation transport (GO:0015672)	357	34	15.72	2.16	3.64E-05	1.89E-03
ionotropic glutamate receptor signaling pathway (GO:0035235)	20	7	0.88	7.95	3.73E-05	1.93E-03
regulation of protein metabolic process (GO:0051246)	2391	146	105.3	1.39	3.78E-05	1.95E-03
regulation of RNA stability (GO:0043487)	64	12	2.82	4.26	3.83E-05	1.97E-03

positive regulation of cellular component biogenesis (GO:0044089)	389	36	17.13	2.1	3.88E-05	1.99E-03
response to organic substance (GO:0010033)	2003	126	88.21	1.43	3.91E-05	1.99E-03
negative regulation of intracellular signal transduction (GO:1902532)	437	39	19.25	2.03	4.08E-05	2.07E-03
positive regulation of axonogenesis (GO:0050772)	75	13	3.3	3.94	4.09E-05	2.07E-03
inorganic cation transmembrane transport (GO:0098662)	360	34	15.85	2.14	4.28E-05	2.16E-03
chromosome organization (GO:0051276)	805	61	35.45	1.72	4.29E-05	2.16E-03
ion transport (GO:0006811)	1073	76	47.26	1.61	4.62E-05	2.32E-03
glutamate receptor signaling pathway (GO:0007215)	37	9	1.63	5.52	5.08E-05	2.54E-03
negative regulation of phosphorylation (GO:0042326)	426	38	18.76	2.03	5.14E-05	2.56E-03
positive regulation of ion transport (GO:0043270)	259	27	11.41	2.37	5.25E-05	2.60E-03
cellular response to endogenous stimulus (GO:0071495)	743	57	32.72	1.74	5.51E-05	2.72E-03
potassium ion transmembrane transport (GO:0071805)	112	16	4.93	3.24	5.57E-05	2.74E-03
chromatin organization (GO:0006325)	558	46	24.58	1.87	5.71E-05	2.80E-03
transmembrane receptor protein tyrosine kinase signaling pathway (GO:0007169)	320	31	14.09	2.2	5.83E-05	2.85E-03
positive regulation of developmental growth (GO:0048639)	177	21	7.8	2.69	6.06E-05	2.95E-03
negative regulation of cellular protein metabolic process (GO:0032269)	939	68	41.35	1.64	6.13E-05	2.98E-03
cellular potassium ion transport (GO:0071804)	113	16	4.98	3.22	6.17E-05	2.99E-03
negative regulation of secretion (GO:0051048)	219	24	9.65	2.49	6.35E-05	3.07E-03
positive regulation of catalytic activity (GO:0043085)	1030	73	45.36	1.61	6.41E-05	3.08E-03
positive regulation of cation transmembrane transport (GO:1904064)	102	15	4.49	3.34	6.86E-05	3.28E-03
long-term memory (GO:0007616)	30	8	1.32	6.05	7.04E-05	3.35E-03

negative regulation of secretion by cell (GO:1903531)	193	22	8.5	2.59	7.25E-05	3.44E-03
response to peptide (GO:1901652)	250	26	11.01	2.36	7.44E-05	3.52E-03
regulation of MAPK cascade (GO:0043408)	632	50	27.83	1.8	7.53E-05	3.55E-03
cation transmembrane transport (GO:0098655)	403	36	17.75	2.03	7.80E-05	3.67E-03
negative regulation of protein phosphorylation (GO:0001933)	389	35	17.13	2.04	8.50E-05	3.98E-03
regulation of glutamate receptor signaling pathway (GO:1900449)	31	8	1.37	5.86	8.81E-05	4.12E-03
regulation of chemotaxis (GO:0050920)	182	21	8.02	2.62	8.87E-05	4.13E-03
muscle cell differentiation (GO:0042692)	253	26	11.14	2.33	8.97E-05	4.16E-03
regulation of axon guidance (GO:1902667)	40	9	1.76	5.11	9.13E-05	4.22E-03
cellular response to stimulus (GO:0051716)	5771	307	254.16	1.21	9.15E-05	4.22E-03
skeletal muscle tissue development (GO:0007519)	130	17	5.73	2.97	9.55E-05	4.39E-03
regulation of mRNA stability (GO:0043488)	60	11	2.64	4.16	9.66E-05	4.43E-03
regulation of MAP kinase activity (GO:0043405)	269	27	11.85	2.28	9.70E-05	4.43E-03
positive regulation of hydrolase activity (GO:0051345)	539	44	23.74	1.85	1.02E-04	4.64E-03
negative regulation of cell death (GO:0060548)	903	65	39.77	1.63	1.05E-04	4.77E-03
learning or memory (GO:0007611)	241	25	10.61	2.36	1.05E-04	4.77E-03
regulation of transferase activity (GO:0051338)	762	57	33.56	1.7	1.06E-04	4.78E-03
regulation of neurotransmitter receptor activity (GO:0099601)	32	8	1.41	5.68	1.09E-04	4.90E-03
ameboidal-type cell migration (GO:0001667)	158	19	6.96	2.73	1.12E-04	5.02E-03
regulation of receptor activity (GO:0010469)	107	15	4.71	3.18	1.15E-04	5.14E-03
organ morphogenesis (GO:0009887)	889	64	39.15	1.63	1.18E-04	5.26E-03
forebrain development (GO:0030900)	334	31	14.71	2.11	1.24E-04	5.51E-03
positive regulation of ion transmembrane transport (GO:0034767)	133	17	5.86	2.9	1.25E-04	5.53E-03
inorganic ion transmembrane transport (GO:0098660)	413	36	18.19	1.98	1.25E-04	5.53E-03
negative regulation of cell differentiation	629	49	27.7	1.77	1.27E-04	5.59E-03

(GO:0045596)						
tissue morphogenesis (GO:0048729)	612	48	26.95	1.78	1.27E-04	5.59E-03
long term synaptic depression (GO:0060292)	17	6	0.75	8.01	1.28E-04	5.60E-03
positive regulation of GTPase activity (GO:0043547)	289	28	12.73	2.2	1.30E-04	5.67E-03
lamellipodium assembly (GO:0030032)	33	8	1.45	5.5	1.35E-04	5.87E-03
myelination (GO:0042552)	85	13	3.74	3.47	1.40E-04	6.07E-03
protein phosphorylation (GO:0006468)	736	55	32.41	1.7	1.43E-04	6.18E-03
positive regulation of actin filament polymerization (GO:0030838)	63	11	2.77	3.96	1.47E-04	6.33E-03
positive regulation of positive chemotaxis (GO:0050927)	25	7	1.1	6.36	1.47E-04	6.33E-03
regulation of axon extension (GO:0030516)	98	14	4.32	3.24	1.59E-04	6.81E-03
regulation of sequence-specific DNA binding transcription factor activity (GO:0051090)	339	31	14.93	2.08	1.60E-04	6.83E-03
actin filament organization (GO:0007015)	163	19	7.18	2.65	1.66E-04	7.07E-03
positive regulation of transport (GO:0051050)	955	67	42.06	1.59	1.69E-04	7.17E-03
cellular localization (GO:0051641)	1705	107	75.09	1.42	1.71E-04	7.24E-03
transcription from RNA polymerase II promoter (GO:0006366)	294	28	12.95	2.16	1.71E-04	7.24E-03
establishment of localization (GO:0051234)	3385	191	149.08	1.28	1.80E-04	7.57E-03
regulation of positive chemotaxis (GO:0050926)	26	7	1.15	6.11	1.87E-04	7.85E-03
regulation of extent of cell growth (GO:0061387)	112	15	4.93	3.04	1.87E-04	7.85E-03
skeletal muscle organ development (GO:0060538)	138	17	6.08	2.8	1.91E-04	7.97E-03
regulation of RNA splicing (GO:0043484)	100	14	4.4	3.18	1.94E-04	8.07E-03
ensheathment of neurons (GO:0007272)	88	13	3.88	3.35	1.95E-04	8.09E-03
axon ensheathment (GO:0008366)	88	13	3.88	3.35	1.95E-04	8.09E-03
negative regulation of transport (GO:0051051)	473	39	20.83	1.87	2.03E-04	8.37E-03
regulation of locomotion (GO:0040012)	800	58	35.23	1.65	2.04E-04	8.39E-03
regulation of ion homeostasis (GO:2000021)	194	21	8.54	2.46	2.09E-04	8.57E-03
cellular chemical homeostasis (GO:0055082)	525	42	23.12	1.82	2.20E-04	9.00E-03

single-organism transport (GO:0044765)	2548	149	112.22	1.33	2.32E-04	9.46E-03
regulation of ossification (GO:0030278)	196	21	8.63	2.43	2.39E-04	9.72E-03
regulation of axon extension involved in axon guidance (GO:0048841)	36	8	1.59	5.05	2.41E-04	9.77E-03
positive regulation of transmembrane transport (GO:0034764)	141	17	6.21	2.74	2.44E-04	9.86E-03
positive regulation of growth (GO:0045927)	255	25	11.23	2.23	2.44E-04	9.86E-03
protein complex localization (GO:0031503)	67	11	2.95	3.73	2.47E-04	9.93E-03
negative regulation of programmed cell death (GO:0043069)	825	59	36.33	1.62	2.55E-04	1.02E-02
epithelium development (GO:0060429)	971	67	42.76	1.57	2.66E-04	1.06E-02
regulation of hydrolase activity (GO:0051336)	935	65	41.18	1.58	2.69E-04	1.07E-02
negative regulation of protein metabolic process (GO:0051248)	1008	69	44.39	1.55	2.69E-04	1.07E-02
cardiac chamber morphogenesis (GO:0003206)	116	15	5.11	2.94	2.70E-04	1.07E-02
negative regulation of apoptotic process (GO:0043066)	811	58	35.72	1.62	2.87E-04	1.13E-02
positive regulation of axon guidance (GO:1902669)	7	4	0.31	12.97	2.93E-04	1.16E-02
positive regulation of axon extension involved in axon guidance (GO:0048842)	7	4	0.31	12.97	2.93E-04	1.16E-02
positive regulation of receptor-mediated endocytosis (GO:0048260)	47	9	2.07	4.35	2.98E-04	1.17E-02
negative regulation of cellular component organization (GO:0051129)	602	46	26.51	1.74	3.05E-04	1.19E-02
peptidyl-amino acid modification (GO:0018193)	620	47	27.31	1.72	3.13E-04	1.22E-02
S-adenosylmethionine metabolic process (GO:0046500)	13	5	0.57	8.73	3.17E-04	1.23E-02
regulation of programmed cell death (GO:0043067)	1351	87	59.5	1.46	3.21E-04	1.25E-02
protein localization (GO:0008104)	1600	100	70.47	1.42	3.23E-04	1.25E-02
calcium ion transport (GO:0006816)	201	21	8.85	2.37	3.31E-04	1.28E-02

regulation of apoptotic process (GO:0042981)	1335	86	58.8	1.46	3.43E-04	1.32E-02
negative regulation of glucocorticoid secretion (GO:2000850)	3	3	0.13	22.71	3.47E-04	1.33E-02
negative regulation of corticosteroid hormone secretion (GO:2000847)	3	3	0.13	22.71	3.47E-04	1.33E-02
negative regulation of steroid hormone secretion (GO:2000832)	3	3	0.13	22.71	3.47E-04	1.33E-02
regulation of cell migration (GO:0030334)	693	51	30.52	1.67	3.48E-04	1.33E-02
response to stimulus (GO:0050896)	7225	369	318.2	1.16	3.49E-04	1.33E-02
positive regulation of cellular protein metabolic process (GO:0032270)	1281	83	56.42	1.47	3.66E-04	1.39E-02
positive regulation of phosphorus metabolic process (GO:0010562)	1003	68	44.17	1.54	3.84E-04	1.45E-02
positive regulation of phosphate metabolic process (GO:0045937)	1003	68	44.17	1.54	3.84E-04	1.45E-02
response to metal ion (GO:0010038)	204	21	8.98	2.34	4.00E-04	1.50E-02
transmission of nerve impulse (GO:0019226)	60	10	2.64	3.78	4.15E-04	1.56E-02
ventricular septum morphogenesis (GO:0060412)	30	7	1.32	5.3	4.38E-04	1.63E-02
negative regulation of RNA splicing (GO:0033119)	30	7	1.32	5.3	4.38E-04	1.63E-02
muscle cell development (GO:0055001)	135	16	5.95	2.69	4.40E-04	1.63E-02
cellular response to organonitrogen compound (GO:0071417)	297	27	13.08	2.06	4.48E-04	1.66E-02
regulation of hormone secretion (GO:0046883)	266	25	11.71	2.13	4.49E-04	1.66E-02
positive regulation of protein modification process (GO:0031401)	1028	69	45.27	1.52	4.56E-04	1.68E-02
regulation of insulin secretion (GO:0050796)	163	18	7.18	2.51	4.56E-04	1.68E-02
peripheral nervous system development (GO:0007422)	61	10	2.69	3.72	4.71E-04	1.73E-02
positive regulation of gliogenesis (GO:0014015)	61	10	2.69	3.72	4.71E-04	1.73E-02
response to inorganic substance (GO:0010035)	314	28	13.83	2.02	4.77E-04	1.74E-02

transport (GO:0006810)	3245	181	142.91	1.27	4.83E-04	1.76E-02
regulation of protein serine/threonine kinase activity (GO:0071900)	395	33	17.4	1.9	4.86E-04	1.76E-02
posttranscriptional regulation of gene expression (GO:0010608)	331	29	14.58	1.99	5.03E-04	1.82E-02
positive regulation of phosphorylation (GO:0042327)	885	61	38.98	1.57	5.07E-04	1.83E-02
establishment or maintenance of cell polarity (GO:0007163)	137	16	6.03	2.65	5.14E-04	1.85E-02
phosphorus metabolic process (GO:0006793)	1624	100	71.52	1.4	5.30E-04	1.90E-02
response to peptide hormone (GO:0043434)	224	22	9.87	2.23	5.47E-04	1.96E-02
pallium development (GO:0021543)	138	16	6.08	2.63	5.55E-04	1.98E-02
negative regulation of hormone secretion (GO:0046888)	86	12	3.79	3.17	5.57E-04	1.98E-02
regulation of release of sequestered calcium ion into cytosol (GO:0051279)	74	11	3.26	3.38	5.61E-04	1.99E-02
negative regulation of mRNA metabolic process (GO:1903312)	41	8	1.81	4.43	5.63E-04	2.00E-02
circulatory system development (GO:0072359)	818	57	36.03	1.58	5.96E-04	2.10E-02
cardiovascular system development (GO:0072358)	818	57	36.03	1.58	5.96E-04	2.10E-02
regulation of cell motility (GO:2000145)	728	52	32.06	1.62	5.96E-04	2.10E-02
response to abiotic stimulus (GO:0009628)	782	55	34.44	1.6	5.98E-04	2.10E-02
response to insulin (GO:0032868)	139	16	6.12	2.61	5.99E-04	2.10E-02
regulation of N-methyl-D-aspartate selective glutamate receptor activity (GO:2000310)	15	5	0.66	7.57	6.03E-04	2.11E-02
stem cell differentiation (GO:0048863)	196	20	8.63	2.32	6.04E-04	2.11E-02
response to organic cyclic compound (GO:0014070)	605	45	26.64	1.69	6.12E-04	2.13E-02
signal transduction (GO:0007165)	4474	239	197.04	1.21	6.15E-04	2.13E-02
mechanoreceptor differentiation (GO:0042490)	75	11	3.3	3.33	6.25E-04	2.16E-02
actin nucleation (GO:0045010)	23	6	1.01	5.92	6.28E-04	2.17E-02
circadian rhythm (GO:0007623)	100	13	4.4	2.95	6.41E-04	2.21E-02

blood vessel remodeling (GO:0001974)	42	8	1.85	4.32	6.58E-04	2.26E-02
neuron projection guidance (GO:0097485)	183	19	8.06	2.36	6.68E-04	2.29E-02
morphogenesis of an epithelium (GO:0002009)	470	37	20.7	1.79	6.76E-04	2.31E-02
regulation of calcium ion transport into cytosol (GO:0010522)	88	12	3.88	3.1	6.79E-04	2.32E-02
positive regulation of transporter activity (GO:0032411)	76	11	3.35	3.29	6.96E-04	2.37E-02
insulin receptor signaling pathway (GO:0008286)	53	9	2.33	3.86	6.97E-04	2.37E-02
regulation of homeostatic process (GO:0032844)	454	36	19.99	1.8	7.00E-04	2.37E-02
associative learning (GO:0008306)	89	12	3.92	3.06	7.48E-04	2.53E-02
phosphate-containing compound metabolic process (GO:0006796)	1583	97	69.72	1.39	7.51E-04	2.53E-02
protein complex subunit organization (GO:0071822)	1199	77	52.81	1.46	7.59E-04	2.55E-02
positive regulation of axon extension (GO:0045773)	43	8	1.89	4.22	7.64E-04	2.56E-02
regulation of peptide hormone secretion (GO:0090276)	200	20	8.81	2.27	7.70E-04	2.58E-02
negative regulation of protein kinase activity (GO:0006469)	215	21	9.47	2.22	7.73E-04	2.58E-02
positive regulation of receptor internalization (GO:0002092)	24	6	1.06	5.68	7.82E-04	2.61E-02
divalent metal ion transport (GO:0070838)	246	23	10.83	2.12	7.96E-04	2.64E-02
S-adenosylmethionine cycle (GO:0033353)	4	3	0.18	17.03	7.97E-04	2.64E-02
negative regulation of circadian rhythm (GO:0042754)	16	5	0.7	7.1	8.03E-04	2.66E-02
gliogenesis (GO:0042063)	186	19	8.19	2.32	8.06E-04	2.66E-02
regulation of sequestering of calcium ion (GO:0051282)	103	13	4.54	2.87	8.36E-04	2.75E-02
cellular modified amino acid metabolic process (GO:0006575)	172	18	7.58	2.38	8.36E-04	2.75E-02
positive regulation of protein kinase activity	375	31	16.52	1.88	8.37E-04	2.75E-02

(GO:0045860)						
cellular ion homeostasis (GO:0006873)	459	36	20.21	1.78	8.46E-04	2.77E-02
inner ear development (GO:0048839)	187	19	8.24	2.31	8.58E-04	2.80E-02
divalent inorganic cation transport (GO:0072511)	248	23	10.92	2.11	8.85E-04	2.88E-02
positive regulation of protein complex assembly (GO:0031334)	173	18	7.62	2.36	8.92E-04	2.90E-02
cellular protein localization (GO:0034613)	1055	69	46.46	1.49	8.92E-04	2.90E-02
regulation of calcium ion transmembrane transport (GO:1903169)	117	14	5.15	2.72	8.92E-04	2.90E-02
cardiac septum morphogenesis (GO:0060411)	55	9	2.42	3.72	9.01E-04	2.91E-02
negative regulation of kinase activity (GO:0033673)	233	22	10.26	2.14	9.04E-04	2.91E-02
regulation of cell growth (GO:0001558)	377	31	16.6	1.87	9.09E-04	2.92E-02
positive regulation of protein metabolic process (GO:0051247)	1380	86	60.78	1.42	9.22E-04	2.96E-02
positive regulation of ion transmembrane transporter activity (GO:0032414)	67	10	2.95	3.39	9.54E-04	3.05E-02
regulation of secretion (GO:0051046)	726	51	31.97	1.6	9.56E-04	3.05E-02
blood vessel endothelial cell migration (GO:0043534)	25	6	1.1	5.45	9.63E-04	3.06E-02
negative regulation of mRNA splicing, via spliceosome (GO:0048025)	25	6	1.1	5.45	9.63E-04	3.06E-02
regulation of neuron death (GO:1901214)	297	26	13.08	1.99	9.66E-04	3.06E-02
positive regulation of signaling (GO:0023056)	1402	87	61.75	1.41	9.76E-04	3.09E-02
kidney epithelium development (GO:0072073)	92	12	4.05	2.96	9.90E-04	3.13E-02
circadian regulation of gene expression (GO:0032922)	56	9	2.47	3.65	1.02E-03	3.21E-02
regulation of peptide secretion (GO:0002791)	205	20	9.03	2.22	1.03E-03	3.24E-02
response to oxygen-containing compound (GO:1901700)	1005	66	44.26	1.49	1.04E-03	3.26E-02
positive regulation of histone deacetylation (GO:0031065)	17	5	0.75	6.68	1.05E-03	3.29E-02
regulation of cell shape (GO:0008360)	133	15	5.86	2.56	1.07E-03	3.34E-02

negative regulation of mRNA processing (GO:0050686)	35	7	1.54	4.54	1.07E-03	3.34E-02
cellular macromolecule localization (GO:0070727)	1063	69	46.82	1.47	1.08E-03	3.36E-02
epithelial cell proliferation (GO:0050673)	93	12	4.1	2.93	1.08E-03	3.36E-02
cellular protein metabolic process (GO:0044267)	2664	150	117.33	1.28	1.09E-03	3.38E-02
regulation of glucocorticoid secretion (GO:2000849)	10	4	0.44	9.08	1.10E-03	3.40E-02
regulation of secretion by cell (GO:1903530)	677	48	29.82	1.61	1.11E-03	3.42E-02
inorganic ion homeostasis (GO:0098771)	536	40	23.61	1.69	1.13E-03	3.48E-02
positive regulation of chemotaxis (GO:0050921)	120	14	5.28	2.65	1.13E-03	3.48E-02
striated muscle cell development (GO:0055002)	120	14	5.28	2.65	1.13E-03	3.48E-02
cellular component assembly (GO:0022607)	1683	101	74.12	1.36	1.14E-03	3.49E-02
positive regulation of protein phosphorylation (GO:0001934)	842	57	37.08	1.54	1.15E-03	3.51E-02
regulation of neuronal synaptic plasticity (GO:0048168)	57	9	2.51	3.59	1.15E-03	3.51E-02
sprouting angiogenesis (GO:0002040)	46	8	2.03	3.95	1.17E-03	3.55E-02
regulation of vesicle-mediated transport (GO:0060627)	451	35	19.86	1.76	1.18E-03	3.57E-02
spindle localization (GO:0051653)	36	7	1.59	4.42	1.25E-03	3.77E-02
regulation of multicellular organism growth (GO:0040014)	82	11	3.61	3.05	1.27E-03	3.83E-02
multicellular organismal signaling (GO:0035637)	95	12	4.18	2.87	1.29E-03	3.88E-02
negative regulation of peptide hormone secretion (GO:0090278)	58	9	2.55	3.52	1.30E-03	3.90E-02
histone lysine methylation (GO:0034968)	58	9	2.55	3.52	1.30E-03	3.90E-02
negative regulation of cell development (GO:0010721)	336	28	14.8	1.89	1.30E-03	3.90E-02
negative regulation of cellular amide metabolic process (GO:0034249)	122	14	5.37	2.61	1.32E-03	3.94E-02
cardiac ventricle morphogenesis (GO:0003208)	70	10	3.08	3.24	1.32E-03	3.94E-02

pattern specification process (GO:0007389)	437	34	19.25	1.77	1.32E-03	3.94E-02
regulation of system process (GO:0044057)	472	36	20.79	1.73	1.36E-03	4.03E-02
small GTPase mediated signal transduction (GO:0007264)	305	26	13.43	1.94	1.39E-03	4.11E-02
ion homeostasis (GO:0050801)	560	41	24.66	1.66	1.39E-03	4.11E-02
regulation of potassium ion transport (GO:0043266)	83	11	3.66	3.01	1.40E-03	4.12E-02
negative regulation of transmembrane receptor protein serine/threonine kinase signaling pathway (GO:0090101)	96	12	4.23	2.84	1.41E-03	4.14E-02
regulation of synapse organization (GO:0050807)	123	14	5.42	2.58	1.42E-03	4.17E-02
positive regulation of filopodium assembly (GO:0051491)	27	6	1.19	5.05	1.42E-03	4.17E-02
heart trabecula morphogenesis (GO:0061384)	27	6	1.19	5.05	1.42E-03	4.17E-02
sensory organ development (GO:0007423)	526	39	23.17	1.68	1.45E-03	4.23E-02
cellular response to hormone stimulus (GO:0032870)	372	30	16.38	1.83	1.46E-03	4.24E-02
muscle tissue morphogenesis (GO:0060415)	71	10	3.13	3.2	1.46E-03	4.24E-02
axon guidance (GO:0007411)	181	18	7.97	2.26	1.46E-03	4.24E-02
positive regulation of cation channel activity (GO:2001259)	37	7	1.63	4.3	1.46E-03	4.24E-02
negative regulation of neurogenesis (GO:0050768)	274	24	12.07	1.99	1.47E-03	4.24E-02
positive regulation of protein localization to early endosome (GO:1902966)	5	3	0.22	13.62	1.51E-03	4.35E-02
regulation of protein localization to early endosome (GO:1902965)	5	3	0.22	13.62	1.51E-03	4.35E-02
positive regulation of kinase activity (GO:0033674)	407	32	17.92	1.79	1.54E-03	4.42E-02
osteoblast differentiation (GO:0001649)	111	13	4.89	2.66	1.61E-03	4.61E-02
regulation of potassium ion transmembrane transport (GO:1901379)	60	9	2.64	3.41	1.63E-03	4.66E-02
negative regulation of peptide secretion (GO:0002792)	60	9	2.64	3.41	1.63E-03	4.66E-02

negative regulation of transferase activity (GO:0051348)	261	23	11.49	2	1.69E-03	4.81E-02
stem cell division (GO:0017145)	38	7	1.67	4.18	1.70E-03	4.83E-02
ATP-dependent chromatin remodeling (GO:0043044)	38	7	1.67	4.18	1.70E-03	4.83E-02
endothelial cell proliferation (GO:0001935)	19	5	0.84	5.98	1.70E-03	4.83E-02
cellular cation homeostasis (GO:0030003)	444	34	19.55	1.74	1.71E-03	4.83E-02
homeostatic process (GO:0042592)	1275	79	56.15	1.41	1.72E-03	4.85E-02
muscle fiber development (GO:0048747)	49	8	2.16	3.71	1.73E-03	4.86E-02
negative regulation of insulin secretion (GO:0046676)	49	8	2.16	3.71	1.73E-03	4.86E-02
cardiac muscle tissue development (GO:0048738)	169	17	7.44	2.28	1.73E-03	4.86E-02
regulation of cell-substrate adhesion (GO:0010810)	169	17	7.44	2.28	1.73E-03	4.86E-02
regulation of gliogenesis (GO:0014013)	112	13	4.93	2.64	1.74E-03	4.86E-02
cellular component biogenesis (GO:0044085)	1866	109	82.18	1.33	1.76E-03	4.90E-02
negative regulation of cellular component movement (GO:0051271)	246	22	10.83	2.03	1.77E-03	4.92E-02
regulation of protein depolymerization (GO:1901879)	73	10	3.22	3.11	1.78E-03	4.94E-02

Table S5. Panther protein class for downregulated genes in hAPP(J20) mice.

PANTHER Protein Class	# of Reference Genes	# of Observed Genes	# of Expected Genes	Ratio of Enrichment	rawP	adjP
transcription factor (PC00218)	1523	111	67.07	1.65	1.96E-07	2.10E-05
zinc finger transcription factor (PC00244)	451	41	19.86	2.06	1.73E-05	1.23E-03
G-protein modulator (PC00022)	445	40	19.6	2.04	2.80E-05	1.50E-03
voltage-gated ion channel (PC00241)	133	18	5.86	3.07	3.92E-05	1.68E-03
guanyl-nucleotide exchange factor (PC00113)	180	20	7.93	2.52	2.10E-04	7.49E-03
actin family cytoskeletal protein (PC00041)	394	33	17.35	1.9	4.65E-04	1.42E-02
voltage-gated potassium channel (PC00242)	83	11	3.66	3.01	1.40E-03	3.34E-02
potassium channel (PC00188)	83	11	3.66	3.01	1.40E-03	3.34E-02
glucosidase (PC00108)	11	4	0.48	8.26	1.56E-03	3.34E-02
non-motor actin binding protein (PC00165)	200	19	8.81	2.16	1.83E-03	3.56E-02
basic helix-loop-helix transcription factor (PC00055)	89	11	3.92	2.81	2.38E-03	3.85E-02
KRAB box transcription factor (PC00029)	286	24	12.6	1.91	2.53E-03	3.85E-02
kinase (PC00137)	526	38	23.17	1.64	2.55E-03	3.85E-02
protein kinase (PC00193)	389	30	17.13	1.75	2.79E-03	3.85E-02
non-receptor serine/threonine protein kinase (PC00167)	289	24	12.73	1.89	2.88E-03	3.85E-02
nucleic acid binding (PC00171)	2347	131	103.36	1.27	3.11E-03	3.91E-02
mRNA processing factor (PC00147)	166	16	7.31	2.19	3.50E-03	4.12E-02
DNA photolyase (PC00014)	2	2	0.09	22.71	3.66E-03	4.12E-02
ion channel (PC00133)	435	32	19.16	1.67	4.12E-03	4.41E-02

Table S6. Panther pathways for downregulated genes in hAPP(J20) mice.

PANTHER Pathway	# of Reference Genes	# of Observed Genes	# of Expected Genes	Ratio of Enrichment	rawP	adjP
Gonadotropin releasing hormone receptor pathway (P06664)	227	26	10	2.6	1.55E-05	2.57E-03
CCKR signaling map (P06959)	159	19	7	2.71	1.22E-04	1.01E-02
Ionotropic glutamate receptor pathway (P00037)	51	9	2.25	4.01	5.32E-04	2.94E-02
Folate biosynthesis (P02742)	5	3	0.22	13.62	1.51E-03	5.84E-02
EGF receptor signaling pathway (P00018)	126	14	5.55	2.52	1.76E-03	5.84E-02

Table S7. KEGG pathways for downregulated genes in hAPP(J20) mice.

KEGG Pathway	# of Reference Genes	# of Observed Genes	# of Expected Genes	Ratio of Enrichment	rawP	adjP
MAPK signaling pathway	268	31	4.64	6.68	1.20E-16	1.54E-14
Regulation of actin cytoskeleton	216	21	3.74	5.61	2.62E-10	1.68E-08
Long-term potentiation	69	12	1.19	10.04	2.43E-09	1.04E-07
Axon guidance	131	15	2.27	6.61	1.01E-08	3.23E-07
Pathways in cancer	325	23	5.63	4.09	1.78E-08	4.37E-07
Spliceosome	138	15	2.39	6.28	2.05E-08	4.37E-07
Calcium signaling pathway	178	16	3.08	5.19	1.04E-07	1.90E-06
Chemokine signaling pathway	185	16	3.2	4.99	1.76E-07	2.82E-06
Neurotrophin signaling pathway	131	13	2.27	5.73	5.17E-07	7.35E-06
Neuroactive ligand-receptor interaction	277	18	4.8	3.75	2.10E-06	2.69E-05
Dilated cardiomyopathy	89	10	1.54	6.49	3.43E-06	3.99E-05
Renal cell carcinoma	71	9	1.23	7.32	3.85E-06	4.11E-05
Long-term depression	72	9	1.25	7.22	4.34E-06	4.27E-05
Chronic myeloid leukemia	74	9	1.28	7.02	5.46E-06	4.99E-05
Metabolic pathways	1184	43	20.5	2.1	6.04E-06	5.15E-05
Aldosterone-regulated sodium reabsorption	44	7	0.76	9.19	1.00E-05	8.00E-05
Focal adhesion	200	14	3.46	4.04	1.21E-05	9.11E-05
Glioma	66	8	1.14	7	1.86E-05	1.00E-04
ErbB signaling pathway	87	9	1.51	5.97	2.07E-05	1.00E-04
Gastric acid secretion	73	8	1.26	6.33	3.91E-05	2.00E-04
Fc gamma R-mediated phagocytosis	90	9	1.56	5.77	2.73E-05	2.00E-04
GnRH signaling pathway	99	9	1.71	5.25	5.82E-05	3.00E-04
Non-small cell lung cancer	55	7	0.95	7.35	4.50E-05	3.00E-04

Vascular smooth muscle contraction	123	10	2.13	4.69	5.97E-05	3.00E-04
Hypertrophic cardiomyopathy (HCM)	83	8	1.44	5.57	9.87E-05	5.00E-04
Tight junction	137	10	2.37	4.22	1.00E-04	5.00E-04
Pancreatic cancer	71	7	1.23	5.69	2.00E-04	9.00E-04
mTOR signaling pathway	53	6	0.92	6.54	3.00E-04	1.30E-03
Proximal tubule bicarbonate reclamation	20	4	0.35	11.55	3.00E-04	1.30E-03
Leukocyte transendothelial migration	120	9	2.08	4.33	3.00E-04	1.30E-03
Melanogenesis	100	8	1.73	4.62	4.00E-04	1.50E-03
Amyotrophic lateral sclerosis (ALS)	56	6	0.97	6.19	4.00E-04	1.50E-03
Wnt signaling pathway	154	10	2.67	3.75	4.00E-04	1.50E-03
Salivary secretion	77	7	1.33	5.25	4.00E-04	1.50E-03
Cysteine and methionine metabolism	39	5	0.68	7.4	5.00E-04	1.80E-03
Glycosaminoglycan biosynthesis - chondroitin sulfate	22	4	0.38	10.5	5.00E-04	1.80E-03
Insulin signaling pathway	137	9	2.37	3.79	7.00E-04	2.40E-03
TGF-beta signaling pathway	85	7	1.47	4.76	7.00E-04	2.40E-03
Oocyte meiosis	113	8	1.96	4.09	8.00E-04	2.60E-03
Bladder cancer	43	5	0.74	6.71	9.00E-04	2.70E-03
Prostate cancer	89	7	1.54	4.54	9.00E-04	2.70E-03
Colorectal cancer	65	6	1.13	5.33	9.00E-04	2.70E-03
Osteoclast differentiation	118	8	2.04	3.91	1.10E-03	3.30E-03
Bile secretion	71	6	1.23	4.88	1.50E-03	4.30E-03
Bacterial invasion of epithelial cells	71	6	1.23	4.88	1.50E-03	4.30E-03
Cell cycle	127	8	2.2	3.64	1.70E-03	4.60E-03
Endocytosis	220	11	3.81	2.89	1.70E-03	4.60E-03
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	74	6	1.28	4.68	1.80E-03	4.80E-03
Endometrial cancer	52	5	0.9	5.55	2.00E-03	5.20E-03

VEGF signaling pathway	76	6	1.32	4.56	2.10E-03	5.30E-03
B cell receptor signaling pathway	76	6	1.32	4.56	2.10E-03	5.30E-03
Pancreatic secretion	104	7	1.8	3.89	2.30E-03	5.70E-03
Phosphatidylinositol signaling system	78	6	1.35	4.44	2.40E-03	5.80E-03
Glycine, serine and threonine metabolism	34	4	0.59	6.79	2.70E-03	6.40E-03
Purine metabolism	168	9	2.91	3.09	2.80E-03	6.40E-03
RNA transport	168	9	2.91	3.09	2.80E-03	6.40E-03
Cardiac muscle contraction	81	6	1.4	4.28	2.90E-03	6.50E-03
Inositol phosphate metabolism	57	5	0.99	5.07	3.10E-03	6.70E-03
Acute myeloid leukemia	57	5	0.99	5.07	3.10E-03	6.70E-03
Cytokine-cytokine receptor interaction	245	11	4.24	2.59	3.90E-03	8.30E-03
Progesterone-mediated oocyte maturation	88	6	1.52	3.94	4.30E-03	9.00E-03
Carbohydrate digestion and absorption	39	4	0.68	5.92	4.50E-03	9.30E-03
Jak-STAT signaling pathway	153	8	2.65	3.02	5.40E-03	1.10E-02
Circadian rhythm - mammal	22	3	0.38	7.87	6.20E-03	1.24E-02
Natural killer cell mediated cytotoxicity	125	7	2.16	3.23	6.30E-03	1.24E-02
Adherens junction	75	5	1.3	3.85	9.80E-03	1.90E-02
Glycosaminoglycan biosynthesis - heparan sulfate	26	3	0.45	6.66	1.00E-02	1.91E-02
RNA degradation	76	5	1.32	3.8	1.04E-02	1.96E-02
Notch signaling pathway	50	4	0.87	4.62	1.10E-02	2.04E-02
Protein digestion and absorption	78	5	1.35	3.7	1.15E-02	2.10E-02
Glycerolipid metabolism	51	4	0.88	4.53	1.17E-02	2.11E-02
T cell receptor signaling pathway	110	6	1.9	3.15	1.25E-02	2.22E-02
Fc epsilon RI signaling pathway	80	5	1.39	3.61	1.28E-02	2.24E-02
Arginine and proline metabolism	54	4	0.94	4.28	1.43E-02	2.47E-02
Basal cell carcinoma	55	4	0.95	4.2	1.52E-02	2.59E-02
Gap junction	88	5	1.52	3.28	1.86E-02	3.13E-02

Alanine, aspartate and glutamate metabolism	33	3	0.57	5.25	1.92E-02	3.19E-02
mRNA surveillance pathway	93	5	1.61	3.1	2.30E-02	3.77E-02
Glycosaminoglycan biosynthesis - keratan sulfate	15	2	0.26	7.7	2.71E-02	4.39E-02
Adipocytokine signaling pathway	68	4	1.18	3.4	3.04E-02	4.80E-02
Chagas disease (American trypanosomiasis)	100	5	1.73	2.89	3.02E-02	4.80E-02

Table S8. Wikipathways for downregulated genes in hAPP(J20) mice.

Wikipathway	# of Reference Genes	# of Observed Genes	# of Expected Genes	Ratio of Enrichment	rawP	adjP
mRNA processing	483	39	8.36	4.66	3.16E-15	3.00E-13
MAPK signaling pathway	165	20	2.86	7	1.28E-11	6.08E-10
EGFR1 Signaling Pathway	217	20	3.76	5.32	1.77E-09	5.60E-08
Insulin Signaling	158	17	2.74	6.21	2.78E-09	6.60E-08
PluriNetWork	292	22	5.06	4.35	1.18E-08	2.24E-07
B Cell Receptor Signaling Pathway	202	18	3.5	5.15	1.92E-08	3.04E-07
Myometrial Relaxation and Contraction Pathways	158	15	2.74	5.48	1.26E-07	1.71E-06
Chemokine signaling pathway	186	16	3.22	4.97	1.90E-07	2.26E-06
TGF-beta Receptor Signaling Pathway	240	18	4.16	4.33	2.63E-07	2.78E-06
Hypothetical Network for Drug Addiction	34	7	0.59	11.89	1.64E-06	1.56E-05
Calcium Regulation in the Cardiac Cell	152	13	2.63	4.94	2.79E-06	2.41E-05
G Protein Signaling Pathways	94	10	1.63	6.14	5.64E-06	4.47E-05
Diurnally regulated genes with circadian orthologs	48	7	0.83	8.42	1.81E-05	1.00E-04
IL-6 signaling Pathway	117	10	2.03	4.94	3.90E-05	3.00E-04
T Cell Receptor Signaling Pathway	143	11	2.48	4.44	4.32E-05	3.00E-04
Regulation of Actin Cytoskeleton	160	11	2.77	3.97	1.00E-04	6.00E-04
Kit Receptor Signaling Pathway	70	7	1.21	5.77	2.00E-04	1.00E-03
Circadian Exercise	49	6	0.85	7.07	2.00E-04	1.00E-03
serotonin and anxiety	18	4	0.31	12.83	2.00E-04	1.00E-03

Focal Adhesion	186	11	3.22	3.42	4.00E-04	1.70E-03
MicroRNAs in cardiomyocyte hypertrophy	102	8	1.77	4.53	4.00E-04	1.70E-03
Integrin-mediated cell adhesion	100	8	1.73	4.62	4.00E-04	1.70E-03
Signaling of Hepatocyte Growth Factor Receptor	38	5	0.66	7.6	5.00E-04	2.00E-03
G13 Signaling Pathway	38	5	0.66	7.6	5.00E-04	2.00E-03
Heart Development	61	6	1.06	5.68	7.00E-04	2.70E-03
IL-7 Signaling Pathway	45	5	0.78	6.42	1.10E-03	3.90E-03
Endochondral Ossification	67	6	1.16	5.17	1.10E-03	3.90E-03
MAPK Cascade	30	4	0.52	7.7	1.70E-03	5.80E-03
Glutathione and one carbon metabolism	34	4	0.59	6.79	2.70E-03	8.80E-03
Senescence and Autophagy	109	7	1.89	3.71	3.00E-03	9.50E-03
p38 MAPK Signaling Pathway (BioCarta)	36	4	0.62	6.42	3.40E-03	1.01E-02
Wnt Signaling Pathway NetPath	141	8	2.44	3.28	3.30E-03	1.01E-02
Mitochondrial Gene Expression	19	3	0.33	9.12	4.10E-03	1.18E-02
G1 to S cell cycle control	65	5	1.13	4.44	5.40E-03	1.51E-02
Methylation Pathways	8	2	0.14	14.44	7.80E-03	2.11E-02
Non-odorant GPCRs	270	11	4.68	2.35	8.00E-03	2.11E-02
Adipogenesis	133	7	2.3	3.04	8.70E-03	2.23E-02
methylation	9	2	0.16	12.83	9.90E-03	2.48E-02
Delta-Notch Signaling Pathway	111	6	1.92	3.12	1.30E-02	3.13E-02
TNF-alpha NF-kB Signaling Pathway	215	9	3.72	2.42	1.32E-02	3.13E-02
Cell Differentiation - Index	12	2	0.21	9.62	1.76E-02	3.89E-02
MaxYvesSuperCombo	12	2	0.21	9.62	1.76E-02	3.89E-02
Ptf1a related regulatory pathway	12	2	0.21	9.62	1.76E-02	3.89E-02

TGF Beta Signaling Pathway	59	4	1.02	3.91	1.92E-02	4.15E-02
miRs in Muscle Cell Differentiation	13	2	0.23	8.88	2.06E-02	4.25E-02
ESC Pluripotency Pathways	123	6	2.13	2.82	2.05E-02	4.25E-02
EPO Receptor Signaling	35	3	0.61	4.95	2.25E-02	4.42E-02
Androgen Receptor Signaling Pathway	126	6	2.18	2.75	2.28E-02	4.42E-02
IL-2 Signaling Pathway	92	5	1.59	3.14	2.21E-02	4.42E-02

Table S9. Phenotypes for downregulated genes in hAPP(J20) mice.

Phenotype	# of Reference Genes	# of Observed Genes	# of Expected Genes	Ratio of Enrichment	rawP	adjP
Abnormal nervous system electrophysiology	227	34	11.15	3.05	4.61E-09	2.33E-06
Abnormal neuron morphology	991	86	48.7	1.77	6.28E-08	1.76E-05
Abnormal nervous system development	824	75	40.49	1.85	7.57E-08	1.91E-05
Abnormal locomotor activation	665	64	32.68	1.96	1.06E-07	2.43E-05
Abnormal long term potentiation	163	25	8.01	3.12	3.37E-07	5.32E-05
Abnormal hippocampus morphology	201	27	9.88	2.73	1.70E-06	2.00E-04
Decreased body weight	1194	94	58.67	1.6	1.32E-06	2.00E-04
Abnormal sarcoplasmic reticulum morphology	11	6	0.54	11.1	5.10E-06	4.00E-04
Decreased anxiety-related response	79	15	3.88	3.86	5.51E-06	4.00E-04
Lethality during fetal growth through weaning	1593	115	78.28	1.47	5.16E-06	4.00E-04
Convulsive seizures	116	18	5.7	3.16	1.28E-05	9.00E-04
Abnormal contextual conditioning behavior	95	16	4.67	3.43	1.35E-05	9.00E-04
Abnormal synaptic plasticity	40	10	1.97	5.09	1.66E-05	1.00E-03
Abnormal craniofacial morphology	795	66	39.06	1.69	1.26E-05	9.00E-04

Table S10. Gene Ontology: Molecular function for upregulated genes in hAPP(J20) mice.

GO: Molecular Function	# of Reference Genes	# of Observed Genes	# of Expected Genes	Ratio of Enrichment	rawP	adjP
protein binding (GO:0005515)	7764	490	325.93	1.5	4.32E-28	2.10E-24
binding (GO:0005488)	12136	657	509.47	1.29	4.60E-23	1.12E-19
receptor binding (GO:0005102)	1489	125	62.51	2	2.36E-13	2.86E-10
ion binding (GO:0043167)	5182	304	217.54	1.4	7.64E-11	6.18E-08
calcium ion binding (GO:0005509)	568	59	23.84	2.47	4.91E-10	2.80E-07
glycosaminoglycan binding (GO:0005539)	182	30	7.64	3.93	5.74E-10	2.80E-07
anion binding (GO:0043168)	2490	168	104.53	1.61	5.76E-10	2.80E-07
molecular_function (GO:0003674)	20461	905	858.96	1.05	8.53E-10	3.76E-07
sulfur compound binding (GO:1901681)	234	33	9.82	3.36	3.65E-09	1.36E-06
peptide binding (GO:0042277)	262	34	11	3.09	1.58E-08	5.11E-06
molecular function regulator (GO:0098772)	1168	90	49.03	1.84	3.89E-08	1.16E-05
carbohydrate derivative binding (GO:0097367)	2079	139	87.28	1.59	4.08E-08	1.16E-05
amide binding (GO:0033218)	287	35	12.05	2.9	4.35E-08	1.17E-05
extracellular matrix binding (GO:0050840)	52	14	2.18	6.41	7.86E-08	2.01E-05
catalytic activity (GO:0003824)	5427	298	227.83	1.31	1.28E-07	3.11E-05
enzyme binding (GO:0019899)	1823	123	76.53	1.61	1.74E-07	4.02E-05
transmembrane transporter activity (GO:0022857)	929	74	39	1.9	1.97E-07	4.35E-05
heparin binding (GO:0008201)	141	22	5.92	3.72	2.74E-07	5.78E-05
protein complex binding (GO:0032403)	1009	78	42.36	1.84	2.92E-07	5.91E-05
transporter activity (GO:0005215)	1164	86	48.87	1.76	4.52E-07	8.78E-05
G-protein coupled receptor binding (GO:0001664)	253	29	10.62	2.73	2.03E-06	3.65E-04
collagen binding (GO:0005518)	60	13	2.52	5.16	2.45E-06	4.25E-04
cytokine binding (GO:0019955)	94	16	3.95	4.05	3.85E-06	6.44E-04

substrate-specific transmembrane transporter activity (GO:0022891)	849	65	35.64	1.82	3.99E-06	6.46E-04
substrate-specific transporter activity (GO:0022892)	1008	73	42.32	1.73	6.82E-06	1.00E-03
beta-amyloid binding (GO:0001540)	31	9	1.3	6.92	8.96E-06	1.28E-03
phosphatidate phosphatase activity (GO:0008195)	11	6	0.46	12.99	8.96E-06	1.28E-03
ion transmembrane transporter activity (GO:0015075)	788	60	33.08	1.81	1.10E-05	1.48E-03
cation transmembrane transporter activity (GO:0008324)	583	48	24.47	1.96	1.25E-05	1.64E-03
binding, bridging (GO:0060090)	116	17	4.87	3.49	1.33E-05	1.70E-03
oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, NAD(P)H as one donor, and incorporation of one atom of oxygen (GO:0016709)	42	10	1.76	5.67	1.58E-05	1.97E-03
enzyme regulator activity (GO:0030234)	878	64	36.86	1.74	2.09E-05	2.54E-03
chemorepellent activity (GO:0045499)	27	8	1.13	7.06	2.43E-05	2.88E-03
protein binding, bridging (GO:0030674)	99	15	4.16	3.61	2.89E-05	3.34E-03
protein dimerization activity (GO:0046983)	1182	79	49.62	1.59	4.44E-05	5.01E-03
structural constituent of cytoskeleton (GO:0005200)	58	11	2.43	4.52	4.72E-05	5.21E-03
coenzyme binding (GO:0050662)	183	21	7.68	2.73	4.92E-05	5.29E-03
dopamine receptor binding (GO:0050780)	22	7	0.92	7.58	5.01E-05	5.29E-03
NADP binding (GO:0050661)	39	9	1.64	5.5	5.26E-05	5.43E-03
cation binding (GO:0043169)	3414	188	143.32	1.31	5.42E-05	5.48E-03
identical protein binding (GO:0042802)	1268	83	53.23	1.56	5.71E-05	5.66E-03
growth factor binding (GO:0019838)	131	17	5.5	3.09	5.90E-05	5.73E-03
small molecule binding (GO:0036094)	2454	141	103.02	1.37	8.97E-05	8.54E-03
receptor tyrosine kinase binding (GO:0030971)	54	10	2.27	4.41	1.25E-04	1.16E-02

oxidoreductase activity (GO:0016491)	751	54	31.53	1.71	1.28E-04	1.16E-02
phosphoric ester hydrolase activity (GO:0042578)	387	33	16.25	2.03	1.47E-04	1.27E-02
kinase binding (GO:0019900)	650	48	27.29	1.76	1.66E-04	1.39E-02
cofactor binding (GO:0048037)	261	25	10.96	2.28	1.69E-04	1.39E-02
substrate-specific channel activity (GO:0022838)	394	33	16.54	2	2.02E-04	1.61E-02
metal ion binding (GO:0046872)	3328	180	139.71	1.29	2.06E-04	1.61E-02
protein kinase binding (GO:0019901)	587	44	24.64	1.79	2.24E-04	1.70E-02
semaphorin receptor binding (GO:0030215)	20	6	0.84	7.15	2.35E-04	1.75E-02
insulin-like growth factor receptor binding (GO:0005159)	13	5	0.55	9.16	2.55E-04	1.83E-02
neurotransmitter receptor activity (GO:0030594)	60	10	2.52	3.97	2.86E-04	2.01E-02
channel regulator activity (GO:0016247)	123	15	5.16	2.9	3.01E-04	2.09E-02
protein tyrosine kinase binding (GO:1990782)	61	10	2.56	3.91	3.25E-04	2.14E-02
passive transmembrane transporter activity (GO:0022803)	422	34	17.72	1.92	3.26E-04	2.14E-02
channel activity (GO:0015267)	422	34	17.72	1.92	3.26E-04	2.14E-02
cell adhesion molecule binding (GO:0050839)	181	19	7.6	2.5	3.31E-04	2.14E-02
active transmembrane transporter activity (GO:0022804)	356	30	14.94	2.01	3.49E-04	2.23E-02
cytoskeletal protein binding (GO:0008092)	804	55	33.75	1.63	3.71E-04	2.31E-02
sodium ion transmembrane transporter activity (GO:0015081)	128	15	5.37	2.79	4.52E-04	2.78E-02
calcium-dependent protein binding (GO:0048306)	64	10	2.69	3.72	4.71E-04	2.86E-02
ion channel binding (GO:0044325)	116	14	4.87	2.87	5.21E-04	3.12E-02
ion channel activity (GO:0005216)	383	31	16.08	1.93	5.44E-04	3.22E-02
lipid phosphatase activity (GO:0042577)	9	4	0.38	10.59	6.26E-04	3.60E-02
cation channel activity (GO:0005261)	286	25	12.01	2.08	6.31E-04	3.60E-02
amino acid transmembrane transporter activity	79	11	3.32	3.32	6.45E-04	3.63E-02

(GO:0015171)						
signaling adaptor activity (GO:0035591)	44	8	1.85	4.33	6.51E-04	3.63E-02
TPR domain binding (GO:0030911)	4	3	0.17	17.87	6.94E-04	3.79E-02
kininogen binding (GO:0030984)	4	3	0.17	17.87	6.94E-04	3.79E-02
nucleoside phosphate binding (GO:1901265)	2136	120	89.67	1.34	7.30E-04	3.82E-02
nucleotide binding (GO:0000166)	2136	120	89.67	1.34	7.30E-04	3.82E-02
L-amino acid transmembrane transporter activity (GO:0015179)	56	9	2.35	3.83	7.32E-04	3.82E-02
protein heterodimerization activity (GO:0046982)	498	37	20.91	1.77	8.00E-04	4.13E-02
glucose-6-phosphate isomerase activity (GO:0004347)	1	2	0.04	47.64	8.56E-04	4.37E-02
sterol 14-demethylase activity (GO:0008398)	1	2	0.04	47.64	8.56E-04	4.37E-02
oxidoreductase activity, acting on paired donors, with oxidation of a pair of donors resulting in the reduction of molecular oxygen to two molecules of water (GO:0016717)	10	4	0.42	9.53	9.22E-04	4.61E-02
metal ion transmembrane transporter activity (GO:0046873)	398	31	16.71	1.86	9.98E-04	4.94E-02

Table S11. Gene Ontology: Biological processes for upregulated genes in hAPP(J20) mice.

GO: Biological Process	# of Reference Genes	# of Observed Genes	# of Expected Genes	Ratio of Enrichment	rawP	adjP
single-organism process (GO:0044699)	12383	690	519.84	1.33	1.23E-30	1.78E-26
localization (GO:0051179)	4254	304	178.58	1.7	1.56E-22	1.13E-18
single-organism cellular process (GO:0044763)	10981	602	460.99	1.31	1.27E-20	6.11E-17
regulation of localization (GO:0032879)	2424	196	101.76	1.93	3.16E-19	7.60E-16
establishment of localization (GO:0051234)	3385	246	142.1	1.73	1.50E-18	3.09E-15
regulation of multicellular organismal process (GO:0051239)	2596	203	108.98	1.86	2.28E-18	4.11E-15
transport (GO:0006810)	3245	237	136.23	1.74	4.66E-18	7.47E-15
system development (GO:0048731)	3702	257	155.41	1.65	5.79E-17	8.36E-14
single-multicellular organism process (GO:0044707)	4913	315	206.25	1.53	2.41E-16	3.16E-13
regulation of biological quality (GO:0065008)	3123	223	131.1	1.7	7.93E-16	9.54E-13
nervous system development (GO:0007399)	1919	157	80.56	1.95	9.68E-16	1.07E-12
positive regulation of biological process (GO:0048518)	4866	310	204.28	1.52	1.19E-15	1.23E-12
biological regulation (GO:0065007)	10873	577	456.45	1.26	1.74E-15	1.67E-12
sterol biosynthetic process (GO:0016126)	35	19	1.47	12.93	2.61E-15	2.35E-12
multicellular organism development (GO:0007275)	4262	278	178.92	1.55	4.87E-15	4.13E-12
single-organism localization (GO:1902578)	2760	200	115.87	1.73	1.00E-14	8.02E-12
neurogenesis (GO:0022008)	1493	129	62.68	2.06	1.19E-14	9.04E-12
regulation of locomotion (GO:0040012)	800	85	33.58	2.53	1.81E-14	1.31E-11
secondary alcohol biosynthetic process (GO:1902653)	29	17	1.22	13.96	2.23E-14	1.53E-11

cholesterol biosynthetic process (GO:0006695)	29	17	1.22	13.96	2.23E-14	1.53E-11
regulation of cellular component movement (GO:0051270)	790	84	33.16	2.53	2.50E-14	1.57E-11
cellular process (GO:0009987)	13456	674	564.89	1.19	6.18E-14	3.72E-11
response to organic substance (GO:0010033)	2003	156	84.09	1.86	6.91E-14	3.99E-11
regulation of cell motility (GO:2000145)	728	78	30.56	2.55	1.53E-13	8.49E-11
regulation of cellular component organization (GO:0051128)	2227	167	93.49	1.79	1.79E-13	9.23E-11
generation of neurons (GO:0048699)	1400	120	58.77	2.04	1.95E-13	9.70E-11
regulation of multicellular organismal development (GO:2000026)	1733	139	72.75	1.91	2.52E-13	1.21E-10
anatomical structure development (GO:0048856)	4634	289	194.54	1.49	2.80E-13	1.30E-10
response to chemical (GO:0042221)	3018	208	126.7	1.64	3.38E-13	1.52E-10
single-organism transport (GO:0044765)	2548	183	106.97	1.71	4.20E-13	1.84E-10
regulation of cell migration (GO:0030334)	693	74	29.09	2.54	7.77E-13	3.30E-10
regulation of cell differentiation (GO:0045595)	1570	128	65.91	1.94	8.68E-13	3.58E-10
lipid metabolic process (GO:0006629)	974	92	40.89	2.25	1.05E-12	4.21E-10
cellular response to chemical stimulus (GO:0070887)	1704	135	71.53	1.89	1.40E-12	5.46E-10
regulation of cell development (GO:0060284)	950	90	39.88	2.26	1.62E-12	6.15E-10
regulation of nervous system development (GO:0051960)	857	84	35.98	2.33	1.72E-12	6.37E-10
sterol metabolic process (GO:0016125)	106	26	4.45	5.84	1.96E-12	7.07E-10
single-organism developmental process (GO:0044767)	4886	297	205.12	1.45	2.43E-12	8.55E-10
response to stimulus (GO:0050896)	7225	405	303.31	1.34	2.52E-12	8.66E-10
developmental process (GO:0032502)	4917	298	206.42	1.44	3.10E-12	1.04E-09
biological_process (GO:0008150)	20544	912	862.44	1.06	6.35E-12	2.04E-09

positive regulation of cellular process (GO:0048522)	4515	277	189.54	1.46	7.53E-12	2.31E-09
regulation of developmental process (GO:0050793)	2242	162	94.12	1.72	8.25E-12	2.48E-09
regulation of neurogenesis (GO:0050767)	761	76	31.95	2.38	8.86E-12	2.61E-09
cell communication (GO:0007154)	4841	292	203.23	1.44	1.03E-11	2.97E-09
cell adhesion (GO:0007155)	889	84	37.32	2.25	1.07E-11	3.03E-09
regulation of biological process (GO:0050789)	10353	537	434.62	1.24	1.32E-11	3.66E-09
cellular response to organic substance (GO:0071310)	1343	111	56.38	1.97	1.56E-11	4.25E-09
biological adhesion (GO:0022610)	899	84	37.74	2.23	1.86E-11	4.97E-09
small molecule metabolic process (GO:0044281)	1458	117	61.21	1.91	2.52E-11	6.61E-09
regulation of neuron differentiation (GO:0045664)	628	66	26.36	2.5	2.75E-11	7.09E-09
single organism signaling (GO:0044700)	4738	285	198.9	1.43	2.90E-11	7.34E-09
signaling (GO:0023052)	4741	285	199.03	1.43	3.12E-11	7.76E-09
cell-cell signaling (GO:0007267)	520	58	21.83	2.66	5.02E-11	1.23E-08
vesicle-mediated transport (GO:0016192)	888	82	37.28	2.2	5.76E-11	1.39E-08
regulation of transport (GO:0051049)	1756	132	73.72	1.79	8.44E-11	1.96E-08
cholesterol metabolic process (GO:0008203)	99	23	4.16	5.53	1.03E-10	2.36E-08
regulation of response to stimulus (GO:0048583)	3185	206	133.71	1.54	1.41E-10	3.18E-08
negative regulation of biological process (GO:0048519)	4267	259	179.13	1.45	1.56E-10	3.46E-08
secondary alcohol metabolic process (GO:1902652)	102	23	4.28	5.37	1.83E-10	4.00E-08
small molecule biosynthetic process (GO:0044283)	346	44	14.53	3.03	2.32E-10	4.97E-08
negative regulation of multicellular organismal process (GO:0051241)	1047	90	43.95	2.05	2.34E-10	4.97E-08
alcohol metabolic process (GO:0006066)	246	36	10.33	3.49	2.73E-10	5.71E-08
positive regulation of multicellular organismal	1503	116	63.1	1.84	3.08E-10	6.35E-08

process (GO:0051240)						
regulation of signaling (GO:0023051)	2647	177	111.12	1.59	3.48E-10	7.07E-08
cell surface receptor signaling pathway (GO:0007166)	1615	122	67.8	1.8	3.65E-10	7.32E-08
regulation of cell communication (GO:0010646)	2691	179	112.97	1.58	4.02E-10	7.95E-08
positive regulation of response to stimulus (GO:0048584)	1697	126	71.24	1.77	5.14E-10	1.00E-07
synaptic transmission (GO:0007268)	316	41	13.27	3.09	5.37E-10	1.02E-07
trans-synaptic signaling (GO:0099537)	316	41	13.27	3.09	5.37E-10	1.02E-07
anterograde trans-synaptic signaling (GO:0098916)	316	41	13.27	3.09	5.37E-10	1.02E-07
lipid biosynthetic process (GO:0008610)	397	47	16.67	2.82	5.53E-10	1.02E-07
steroid biosynthetic process (GO:0006694)	90	21	3.78	5.56	6.15E-10	1.12E-07
regulation of neuron projection development (GO:0010975)	472	52	19.81	2.62	7.71E-10	1.39E-07
positive regulation of cell communication (GO:0010647)	1422	110	59.7	1.84	8.29E-10	1.46E-07
regulation of molecular function (GO:0065009)	2234	154	93.78	1.64	8.38E-10	1.46E-07
synaptic signaling (GO:0099536)	321	41	13.48	3.04	8.40E-10	1.46E-07
neuron projection development (GO:0031175)	550	57	23.09	2.47	1.05E-09	1.80E-07
alcohol biosynthetic process (GO:0046165)	84	20	3.53	5.67	1.12E-09	1.90E-07
regulation of cellular process (GO:0050794)	9949	509	417.66	1.22	1.36E-09	2.28E-07
positive regulation of signaling (GO:0023056)	1402	108	58.86	1.83	1.51E-09	2.51E-07
response to endogenous stimulus (GO:0009719)	1025	86	43.03	2	1.86E-09	3.05E-07
regulation of cell projection organization (GO:0031344)	597	59	25.06	2.35	2.97E-09	4.82E-07
steroid metabolic process (GO:0008202)	209	31	8.77	3.53	3.45E-09	5.53E-07
organic substance transport (GO:0071702)	1648	120	69.18	1.73	4.27E-09	6.77E-07
cellular lipid metabolic process (GO:0044255)	731	67	30.69	2.18	4.74E-09	7.44E-07

regulation of synapse structure or activity (GO:0050803)	263	35	11.04	3.17	5.25E-09	8.15E-07
positive regulation of cell adhesion (GO:0045785)	344	41	14.44	2.84	5.80E-09	8.91E-07
phosphorus metabolic process (GO:0006793)	1624	118	68.18	1.73	6.58E-09	9.89E-07
cell differentiation (GO:0030154)	3222	200	135.26	1.48	7.79E-09	1.16E-06
negative regulation of cellular process (GO:0048523)	3982	237	167.17	1.42	7.79E-09	1.16E-06
positive regulation of cell migration (GO:0030335)	419	46	17.59	2.62	8.19E-09	1.19E-06
positive regulation of locomotion (GO:0040017)	452	48	18.98	2.53	1.07E-08	1.54E-06
behavior (GO:0007610)	606	58	25.44	2.28	1.23E-08	1.76E-06
positive regulation of cellular component movement (GO:0051272)	442	47	18.56	2.53	1.46E-08	2.07E-06
response to oxygen-containing compound (GO:1901700)	1005	82	42.19	1.94	1.52E-08	2.13E-06
cellular response to stimulus (GO:0051716)	5771	319	242.27	1.32	1.62E-08	2.25E-06
phosphate-containing compound metabolic process (GO:0006796)	1583	114	66.45	1.72	1.96E-08	2.67E-06
positive regulation of cell motility (GO:2000147)	432	46	18.14	2.54	2.00E-08	2.70E-06
macromolecule localization (GO:0033036)	1865	129	78.29	1.65	2.08E-08	2.78E-06
positive regulation of cellular component organization (GO:0051130)	1152	90	48.36	1.86	2.10E-08	2.78E-06
cell projection organization (GO:0030030)	912	76	38.29	1.99	2.26E-08	2.97E-06
modulation of synaptic transmission (GO:0050804)	334	39	14.02	2.78	2.32E-08	3.02E-06
tissue development (GO:0009888)	1516	110	63.64	1.73	2.45E-08	3.16E-06
regulation of cell adhesion (GO:0030155)	603	57	25.31	2.25	2.49E-08	3.18E-06
positive regulation of cell development (GO:0010720)	557	54	23.38	2.31	2.61E-08	3.30E-06
ion transport (GO:0006811)	1073	85	45.04	1.89	2.95E-08	3.70E-06
axon development (GO:0061564)	324	38	13.6	2.79	3.13E-08	3.86E-06

positive regulation of developmental process (GO:0051094)	1254	95	52.64	1.8	3.48E-08	4.22E-06
positive regulation of cell differentiation (GO:0045597)	910	75	38.2	1.96	4.35E-08	5.15E-06
regulation of catalytic activity (GO:0050790)	1723	120	72.33	1.66	4.83E-08	5.62E-06
cellular developmental process (GO:0048869)	3422	205	143.66	1.43	7.31E-08	8.31E-06
animal organ development (GO:0048513)	2677	168	112.38	1.49	8.50E-08	9.58E-06
neuron development (GO:0048666)	711	62	29.85	2.08	1.02E-07	1.14E-05
positive regulation of nervous system development (GO:0051962)	520	50	21.83	2.29	1.09E-07	1.21E-05
single-organism behavior (GO:0044708)	444	45	18.64	2.41	1.13E-07	1.24E-05
endocytosis (GO:0006897)	370	40	15.53	2.58	1.14E-07	1.25E-05
neuron differentiation (GO:0030182)	883	72	37.07	1.94	1.22E-07	1.32E-05
circulatory system development (GO:0072359)	818	68	34.34	1.98	1.40E-07	1.50E-05
cardiovascular system development (GO:0072358)	818	68	34.34	1.98	1.40E-07	1.50E-05
anatomical structure morphogenesis (GO:0009653)	2066	136	86.73	1.57	1.41E-07	1.50E-05
single-organism metabolic process (GO:0044710)	3558	210	149.37	1.41	1.46E-07	1.54E-05
organic hydroxy compound metabolic process (GO:1901615)	375	40	15.74	2.54	1.60E-07	1.67E-05
regulation of synaptic plasticity (GO:0048167)	160	24	6.72	3.57	1.62E-07	1.68E-05
single-organism biosynthetic process (GO:0044711)	960	76	40.3	1.89	1.70E-07	1.75E-05
extracellular matrix organization (GO:0030198)	185	26	7.77	3.35	1.72E-07	1.76E-05
regulation of vesicle-mediated transport (GO:0060627)	451	45	18.93	2.38	1.74E-07	1.77E-05
signal transduction (GO:0007165)	4474	253	187.82	1.35	1.79E-07	1.81E-05
extracellular structure organization (GO:0043062)	186	26	7.81	3.33	1.91E-07	1.91E-05
establishment of protein localization (GO:0045184)	1176	88	49.37	1.78	1.94E-07	1.93E-05

organophosphate metabolic process (GO:0019637)	660	58	27.71	2.09	2.07E-07	2.05E-05
regulation of axon guidance (GO:1902667)	40	12	1.68	7.15	2.13E-07	2.09E-05
vasculature development (GO:0001944)	502	48	21.07	2.28	2.30E-07	2.24E-05
regulation of membrane potential (GO:0042391)	368	39	15.45	2.52	2.67E-07	2.59E-05
cellular localization (GO:0051641)	1705	116	71.58	1.62	2.70E-07	2.60E-05
regulation of endocytosis (GO:0030100)	204	27	8.56	3.15	3.19E-07	3.05E-05
organic acid metabolic process (GO:0006082)	806	66	33.84	1.95	3.60E-07	3.40E-05
cellular component organization (GO:0016043)	4498	252	188.83	1.33	4.13E-07	3.82E-05
nucleoside diphosphate phosphorylation (GO:0006165)	51	13	2.14	6.07	4.17E-07	3.83E-05
glycolytic process (GO:0006096)	35	11	1.47	7.49	4.32E-07	3.95E-05
cellular response to endogenous stimulus (GO:0071495)	743	62	31.19	1.99	4.44E-07	4.03E-05
ADP metabolic process (GO:0046031)	43	12	1.81	6.65	4.53E-07	4.09E-05
chemical homeostasis (GO:0048878)	829	67	34.8	1.93	4.61E-07	4.13E-05
positive regulation of neurogenesis (GO:0050769)	454	44	19.06	2.31	5.14E-07	4.58E-05
cell migration (GO:0016477)	680	58	28.55	2.03	5.32E-07	4.71E-05
positive regulation of transport (GO:0051050)	955	74	40.09	1.85	5.47E-07	4.81E-05
ATP generation from ADP (GO:0006757)	36	11	1.51	7.28	5.68E-07	4.97E-05
regulation of axon extension involved in axon guidance (GO:0048841)	36	11	1.51	7.28	5.68E-07	4.97E-05
regulation of system process (GO:0044057)	472	45	19.81	2.27	5.91E-07	5.11E-05
protein localization (GO:0008104)	1600	109	67.17	1.62	6.08E-07	5.22E-05
receptor-mediated endocytosis (GO:0006898)	125	20	5.25	3.81	6.43E-07	5.49E-05
response to organonitrogen compound (GO:0010243)	507	47	21.28	2.21	7.16E-07	6.08E-05
cation transport (GO:0006812)	687	58	28.84	2.01	7.32E-07	6.18E-05
blood vessel development (GO:0001568)	476	45	19.98	2.25	7.38E-07	6.19E-05

negative regulation of neuron projection development (GO:0010977)	138	21	5.79	3.62	7.51E-07	6.27E-05
regulation of cell proliferation (GO:0042127)	1457	101	61.17	1.65	7.68E-07	6.37E-05
response to nitrogen compound (GO:1901698)	606	53	25.44	2.08	7.99E-07	6.59E-05
cellular homeostasis (GO:0019725)	623	54	26.15	2.06	8.20E-07	6.72E-05
locomotion (GO:0040011)	984	75	41.31	1.82	8.37E-07	6.79E-05
oxoacid metabolic process (GO:0043436)	792	64	33.25	1.92	8.43E-07	6.80E-05
nucleotide phosphorylation (GO:0046939)	56	13	2.35	5.53	1.16E-06	9.30E-05
cellular response to oxygen-containing compound (GO:1901701)	598	52	25.1	2.07	1.20E-06	9.57E-05
intracellular signal transduction (GO:0035556)	1215	87	51.01	1.71	1.35E-06	1.07E-04
purine ribonucleoside diphosphate metabolic process (GO:0009179)	48	12	2.02	5.96	1.40E-06	1.10E-04
purine nucleoside diphosphate metabolic process (GO:0009135)	48	12	2.02	5.96	1.40E-06	1.10E-04
axonogenesis (GO:0007409)	305	33	12.8	2.58	1.41E-06	1.10E-04
chemotaxis (GO:0006935)	410	40	17.21	2.32	1.43E-06	1.11E-04
organic hydroxy compound biosynthetic process (GO:1901617)	144	21	6.05	3.47	1.45E-06	1.12E-04
regulation of cell-substrate adhesion (GO:0010810)	169	23	7.09	3.24	1.48E-06	1.14E-04
oxidoreduction coenzyme metabolic process (GO:0006733)	109	18	4.58	3.93	1.49E-06	1.14E-04
negative regulation of cell projection organization (GO:0031345)	157	22	6.59	3.34	1.56E-06	1.19E-04
homeostatic process (GO:0042592)	1275	90	53.52	1.68	1.57E-06	1.19E-04
positive regulation of signal transduction (GO:0009967)	1238	88	51.97	1.69	1.57E-06	1.19E-04
taxis (GO:0042330)	412	40	17.3	2.31	1.61E-06	1.20E-04
neuron projection morphogenesis (GO:0048812)	397	39	16.67	2.34	1.65E-06	1.23E-04

cellular chemical homeostasis (GO:0055082)	525	47	22.04	2.13	1.82E-06	1.35E-04
regulation of hydrolase activity (GO:0051336)	935	71	39.25	1.81	1.88E-06	1.38E-04
monocarboxylic acid metabolic process (GO:0032787)	462	43	19.39	2.22	1.91E-06	1.40E-04
carbohydrate derivative metabolic process (GO:1901135)	762	61	31.99	1.91	2.05E-06	1.49E-04
synapse organization (GO:0050808)	136	20	5.71	3.5	2.27E-06	1.64E-04
cell development (GO:0048468)	1513	102	63.52	1.61	2.27E-06	1.64E-04
protein oligomerization (GO:0051259)	450	42	18.89	2.22	2.35E-06	1.68E-04
learning or memory (GO:0007611)	241	28	10.12	2.77	2.36E-06	1.68E-04
response to cytokine (GO:0034097)	482	44	20.23	2.17	2.38E-06	1.68E-04
regulation of signal transduction (GO:0009966)	2318	143	97.31	1.47	2.39E-06	1.68E-04
protein transport (GO:0015031)	1068	78	44.83	1.74	2.42E-06	1.70E-04
memory (GO:0007613)	113	18	4.74	3.79	2.44E-06	1.70E-04
negative regulation of neuron differentiation (GO:0045665)	214	26	8.98	2.89	2.45E-06	1.70E-04
response to wounding (GO:0009611)	299	32	12.55	2.55	2.53E-06	1.75E-04
cellular component organization or biogenesis (GO:0071840)	4651	254	195.25	1.3	2.78E-06	1.90E-04
regulation of anatomical structure morphogenesis (GO:0022603)	964	72	40.47	1.78	2.81E-06	1.91E-04
cellular protein localization (GO:0034613)	1055	77	44.29	1.74	2.88E-06	1.95E-04
positive regulation of peptidyl-tyrosine phosphorylation (GO:0050731)	164	22	6.88	3.2	3.11E-06	2.10E-04
regulation of cell morphogenesis involved in differentiation (GO:0010769)	347	35	14.57	2.4	3.17E-06	2.13E-04
regulation of protein metabolic process (GO:0051246)	2391	146	100.37	1.45	3.17E-06	2.13E-04
single-organism carbohydrate catabolic process	62	13	2.6	4.99	3.47E-06	2.30E-04

(GO:0044724)						
movement of cell or subcellular component (GO:0006928)	1099	79	46.14	1.71	3.69E-06	2.43E-04
cellular macromolecule localization (GO:0070727)	1063	77	44.63	1.73	3.76E-06	2.46E-04
ribonucleoside diphosphate metabolic process (GO:0009185)	53	12	2.22	5.39	3.81E-06	2.48E-04
nicotinamide nucleotide metabolic process (GO:0046496)	94	16	3.95	4.05	3.85E-06	2.49E-04
regulation of phosphorus metabolic process (GO:0051174)	1575	104	66.12	1.57	4.36E-06	2.81E-04
regulation of peptidyl-tyrosine phosphorylation (GO:0050730)	222	26	9.32	2.79	4.65E-06	2.98E-04
regulation of chemotaxis (GO:0050920)	182	23	7.64	3.01	4.91E-06	3.14E-04
pyridine nucleotide metabolic process (GO:0019362)	96	16	4.03	3.97	4.99E-06	3.17E-04
regulation of peptidase activity (GO:0052547)	356	35	14.94	2.34	5.45E-06	3.45E-04
transmembrane transport (GO:0055085)	789	61	33.12	1.84	5.92E-06	3.73E-04
positive regulation of synaptic transmission (GO:0050806)	133	19	5.58	3.4	6.03E-06	3.78E-04
regulation of phosphate metabolic process (GO:0019220)	1570	103	65.91	1.56	6.37E-06	3.98E-04
cognition (GO:0050890)	269	29	11.29	2.57	6.44E-06	4.01E-04
negative regulation of cellular component organization (GO:0051129)	602	50	25.27	1.98	6.57E-06	4.07E-04
cation homeostasis (GO:0055080)	520	45	21.83	2.06	6.99E-06	4.31E-04
ion transmembrane transport (GO:0034220)	554	47	23.26	2.02	7.27E-06	4.47E-04
homophilic cell adhesion via plasma membrane adhesion molecules (GO:0007156)	88	15	3.69	4.06	7.52E-06	4.60E-04
neurotransmitter transport (GO:0006836)	123	18	5.16	3.49	7.63E-06	4.65E-04

response to stress (GO:0006950)	2657	157	111.54	1.41	7.73E-06	4.69E-04
blood circulation (GO:0008015)	363	35	15.24	2.3	8.19E-06	4.94E-04
pyridine-containing compound metabolic process (GO:0072524)	100	16	4.2	3.81	8.22E-06	4.94E-04
blood vessel morphogenesis (GO:0048514)	379	36	15.91	2.26	8.40E-06	5.03E-04
response to cocaine (GO:0042220)	31	9	1.3	6.92	8.96E-06	5.34E-04
carbohydrate catabolic process (GO:0016052)	68	13	2.85	4.55	9.19E-06	5.46E-04
positive regulation of molecular function (GO:0044093)	1316	89	55.25	1.61	9.32E-06	5.51E-04
tissue morphogenesis (GO:0048729)	612	50	25.69	1.95	1.01E-05	5.95E-04
circulatory system process (GO:0003013)	367	35	15.41	2.27	1.03E-05	6.04E-04
morphogenesis of a branching epithelium (GO:0061138)	191	23	8.02	2.87	1.05E-05	6.14E-04
positive regulation of phosphorus metabolic process (GO:0010562)	1003	72	42.11	1.71	1.05E-05	6.14E-04
positive regulation of phosphate metabolic process (GO:0045937)	1003	72	42.11	1.71	1.05E-05	6.14E-04
regulation of cellular localization (GO:0060341)	1264	86	53.06	1.62	1.07E-05	6.18E-04
negative regulation of developmental process (GO:0051093)	841	63	35.31	1.78	1.09E-05	6.27E-04
membrane organization (GO:0061024)	683	54	28.67	1.88	1.11E-05	6.36E-04
muscle contraction (GO:0006936)	165	21	6.93	3.03	1.12E-05	6.39E-04
cell morphogenesis involved in neuron differentiation (GO:0048667)	369	35	15.49	2.26	1.15E-05	6.53E-04
metal ion homeostasis (GO:0055065)	450	40	18.89	2.12	1.23E-05	6.96E-04
negative regulation of catalytic activity (GO:0043086)	669	53	28.08	1.89	1.27E-05	7.16E-04
developmental growth (GO:0048589)	371	35	15.57	2.25	1.28E-05	7.19E-04
secretion (GO:0046903)	451	40	18.93	2.11	1.30E-05	7.27E-04

cation transmembrane transport (GO:0098655)	403	37	16.92	2.19	1.31E-05	7.30E-04
regulation of phosphorylation (GO:0042325)	1348	90	56.59	1.59	1.34E-05	7.44E-04
regulation of anatomical structure size (GO:0090066)	502	43	21.07	2.04	1.41E-05	7.77E-04
cellular response to catecholamine stimulus (GO:0071870)	18	7	0.76	9.26	1.42E-05	7.79E-04
positive regulation of phosphorylation (GO:0042327)	885	65	37.15	1.75	1.44E-05	7.87E-04
positive regulation of cellular component biogenesis (GO:0044089)	389	36	16.33	2.2	1.45E-05	7.90E-04
inorganic ion homeostasis (GO:0098771)	536	45	22.5	2	1.46E-05	7.92E-04
regulation of long-term neuronal synaptic plasticity (GO:0048169)	33	9	1.39	6.5	1.46E-05	7.92E-04
cellular response to organonitrogen compound (GO:0071417)	297	30	12.47	2.41	1.52E-05	8.19E-04
nucleoside diphosphate metabolic process (GO:0009132)	72	13	3.02	4.3	1.66E-05	8.87E-04
growth (GO:0040007)	440	39	18.47	2.11	1.68E-05	8.95E-04
negative regulation of transport (GO:0051051)	473	41	19.86	2.06	1.70E-05	9.02E-04
regulation of cell growth (GO:0001558)	377	35	15.83	2.21	1.78E-05	9.34E-04
localization of cell (GO:0051674)	766	58	32.16	1.8	1.80E-05	9.41E-04
cell motility (GO:0048870)	766	58	32.16	1.8	1.80E-05	9.41E-04
negative regulation of locomotion (GO:0040013)	270	28	11.33	2.47	1.83E-05	9.50E-04
cell morphogenesis involved in differentiation (GO:0000904)	525	44	22.04	2	1.88E-05	9.73E-04
positive regulation of protein metabolic process (GO:0051247)	1380	91	57.93	1.57	1.89E-05	9.74E-04
ion homeostasis (GO:0050801)	560	46	23.51	1.96	1.99E-05	1.02E-03
response to catecholamine (GO:0071869)	19	7	0.8	8.78	2.00E-05	1.02E-03

cellular response to monoamine stimulus (GO:0071868)	19	7	0.8	8.78	2.00E-05	1.02E-03
positive regulation of cell projection organization (GO:0031346)	348	33	14.61	2.26	2.03E-05	1.03E-03
regulation of protein phosphorylation (GO:0001932)	1249	84	52.43	1.6	2.04E-05	1.03E-03
muscle system process (GO:0003012)	214	24	8.98	2.67	2.12E-05	1.07E-03
morphogenesis of a branching structure (GO:0001763)	200	23	8.4	2.74	2.13E-05	1.07E-03
regulation of receptor-mediated endocytosis (GO:0048259)	74	13	3.11	4.18	2.20E-05	1.10E-03
calcium ion homeostasis (GO:0055074)	319	31	13.39	2.31	2.28E-05	1.14E-03
semaphorin-plexin signaling pathway (GO:0071526)	35	9	1.47	6.13	2.30E-05	1.14E-03
negative regulation of neurogenesis (GO:0050768)	274	28	11.5	2.43	2.37E-05	1.18E-03
negative regulation of axon guidance (GO:1902668)	27	8	1.13	7.06	2.43E-05	1.20E-03
cellular calcium ion homeostasis (GO:0006874)	305	30	12.8	2.34	2.47E-05	1.22E-03
single-organism catabolic process (GO:0044712)	740	56	31.07	1.8	2.55E-05	1.25E-03
regulation of axon extension (GO:0030516)	98	15	4.11	3.65	2.58E-05	1.26E-03
regulation of response to external stimulus (GO:0032101)	812	60	34.09	1.76	2.59E-05	1.26E-03
regulation of kinase activity (GO:0043549)	670	52	28.13	1.85	2.60E-05	1.26E-03
cellular response to nitrogen compound (GO:1901699)	353	33	14.82	2.23	2.68E-05	1.30E-03
cellular divalent inorganic cation homeostasis (GO:0072503)	322	31	13.52	2.29	2.71E-05	1.31E-03
response to monoamine (GO:0071867)	20	7	0.84	8.34	2.76E-05	1.33E-03
multicellular organismal process (GO:0032501)	6561	333	275.43	1.21	2.87E-05	1.38E-03

regeneration (GO:0031099)	76	13	3.19	4.07	2.88E-05	1.38E-03
secretion by cell (GO:0032940)	356	33	14.94	2.21	3.15E-05	1.50E-03
cellular metal ion homeostasis (GO:0006875)	388	35	16.29	2.15	3.16E-05	1.50E-03
regulation of cellular protein metabolic process (GO:0032268)	2216	132	93.03	1.42	3.22E-05	1.52E-03
female pregnancy (GO:0007565)	100	15	4.2	3.57	3.23E-05	1.52E-03
regulation of growth (GO:0040008)	641	50	26.91	1.86	3.27E-05	1.54E-03
central nervous system development (GO:0007417)	694	53	29.13	1.82	3.31E-05	1.55E-03
regulation of cytosolic calcium ion concentration (GO:0051480)	206	23	8.65	2.66	3.34E-05	1.56E-03
regulation of blood circulation (GO:1903522)	235	25	9.87	2.53	3.37E-05	1.57E-03
phospholipid metabolic process (GO:0006644)	250	26	10.5	2.48	3.43E-05	1.59E-03
negative regulation of nervous system development (GO:0051961)	296	29	12.43	2.33	3.59E-05	1.66E-03
negative regulation of peptidase activity (GO:0010466)	222	24	9.32	2.58	3.75E-05	1.73E-03
response to acid chemical (GO:0001101)	208	23	8.73	2.63	3.85E-05	1.77E-03
positive regulation of protein phosphorylation (GO:0001934)	842	61	35.35	1.73	3.87E-05	1.77E-03
fatty acid metabolic process (GO:0006631)	298	29	12.51	2.32	4.04E-05	1.85E-03
divalent inorganic cation homeostasis (GO:0072507)	345	32	14.48	2.21	4.08E-05	1.86E-03
regulation of protein kinase activity (GO:0045859)	612	48	25.69	1.87	4.10E-05	1.86E-03
protein homooligomerization (GO:0051260)	268	27	11.25	2.4	4.16E-05	1.88E-03
protein complex subunit organization (GO:0071822)	1199	80	50.33	1.59	4.20E-05	1.89E-03
regulation of cell size (GO:0008361)	181	21	7.6	2.76	4.22E-05	1.89E-03
neuron-neuron synaptic transmission (GO:0007270)	68	12	2.85	4.2	4.31E-05	1.93E-03

positive regulation of catalytic activity (GO:0043085)	1030	71	43.24	1.64	4.31E-05	1.93E-03
cellular cation homeostasis (GO:0030003)	444	38	18.64	2.04	4.45E-05	1.98E-03
carboxylic acid metabolic process (GO:0019752)	738	55	30.98	1.78	4.48E-05	1.98E-03
positive regulation of neuron differentiation (GO:0045666)	363	33	15.24	2.17	4.55E-05	2.01E-03
establishment of localization in cell (GO:0051649)	1279	84	53.69	1.56	4.60E-05	2.02E-03
regulation of vasculature development (GO:1901342)	240	25	10.08	2.48	4.69E-05	2.06E-03
pyruvate metabolic process (GO:0006090)	58	11	2.43	4.52	4.72E-05	2.06E-03
signal release (GO:0023061)	155	19	6.51	2.92	4.73E-05	2.06E-03
developmental cell growth (GO:0048588)	80	13	3.36	3.87	4.82E-05	2.10E-03
positive regulation of cellular protein metabolic process (GO:0032270)	1281	84	53.78	1.56	4.85E-05	2.10E-03
cell growth (GO:0016049)	129	17	5.42	3.14	4.91E-05	2.11E-03
angiogenesis (GO:0001525)	286	28	12.01	2.33	4.92E-05	2.11E-03
fatty acid biosynthetic process (GO:0006633)	104	15	4.37	3.44	5.00E-05	2.14E-03
negative chemotaxis (GO:0050919)	30	8	1.26	6.35	5.06E-05	2.16E-03
regulation of neurotransmitter levels (GO:0001505)	171	20	7.18	2.79	5.72E-05	2.42E-03
regulation of endopeptidase activity (GO:0052548)	274	27	11.5	2.35	5.98E-05	2.52E-03
cellular response to cytokine stimulus (GO:0071345)	385	34	16.16	2.1	6.12E-05	2.56E-03
cell projection morphogenesis (GO:0048858)	605	47	25.4	1.85	6.14E-05	2.56E-03
neurotransmitter secretion (GO:0007269)	82	13	3.44	3.78	6.16E-05	2.56E-03
signal release from synapse (GO:0099643)	82	13	3.44	3.78	6.16E-05	2.56E-03
regulation of axonogenesis (GO:0050770)	172	20	7.22	2.77	6.18E-05	2.56E-03
enzyme linked receptor protein signaling pathway (GO:0007167)	503	41	21.12	1.94	6.53E-05	2.70E-03

regulation of lipid metabolic process (GO:0019216)	291	28	12.22	2.29	6.58E-05	2.71E-03
negative regulation of endopeptidase activity (GO:0010951)	146	18	6.13	2.94	6.89E-05	2.83E-03
behavioral response to cocaine (GO:0048148)	16	6	0.67	8.93	7.11E-05	2.92E-03
oxidation-reduction process (GO:0055114)	806	58	33.84	1.71	7.15E-05	2.92E-03
ribose phosphate metabolic process (GO:0019693)	262	26	11	2.36	7.27E-05	2.96E-03
coenzyme metabolic process (GO:0006732)	232	24	9.74	2.46	7.32E-05	2.98E-03
wound healing (GO:0042060)	247	25	10.37	2.41	7.34E-05	2.98E-03
cell-substrate adhesion (GO:0031589)	147	18	6.17	2.92	7.50E-05	3.02E-03
negative regulation of cell differentiation (GO:0045596)	629	48	26.41	1.82	7.82E-05	3.14E-03
synaptic vesicle transport (GO:0048489)	96	14	4.03	3.47	7.85E-05	3.15E-03
establishment of synaptic vesicle localization (GO:0097480)	96	14	4.03	3.47	7.85E-05	3.15E-03
protein complex assembly (GO:0006461)	902	63	37.87	1.66	8.11E-05	3.23E-03
protein complex biogenesis (GO:0070271)	902	63	37.87	1.66	8.11E-05	3.23E-03
G-protein coupled receptor signaling pathway, coupled to cyclic nucleotide second messenger (GO:0007187)	148	18	6.21	2.9	8.15E-05	3.23E-03
regulation of wound healing (GO:0061041)	122	16	5.12	3.12	8.53E-05	3.37E-03
positive regulation of cell junction assembly (GO:1901890)	24	7	1.01	6.95	8.57E-05	3.38E-03
synaptic vesicle localization (GO:0097479)	97	14	4.07	3.44	8.73E-05	3.43E-03
cellular ion homeostasis (GO:0006873)	459	38	19.27	1.97	8.74E-05	3.43E-03
presynaptic process involved in synaptic transmission (GO:0099531)	85	13	3.57	3.64	8.78E-05	3.43E-03
regulation of fatty acid metabolic process (GO:0019217)	74	12	3.11	3.86	9.48E-05	3.70E-03
learning (GO:0007612)	150	18	6.3	2.86	9.61E-05	3.74E-03

negative regulation of molecular function (GO:0044092)	927	64	38.92	1.64	9.83E-05	3.81E-03
synaptic vesicle endocytosis (GO:0048488)	17	6	0.71	8.41	9.87E-05	3.82E-03
regulation of ion transport (GO:0043269)	600	46	25.19	1.83	9.87E-05	3.82E-03
regulation of extent of cell growth (GO:0061387)	112	15	4.7	3.19	1.12E-04	4.31E-03
epithelium development (GO:0060429)	971	66	40.76	1.62	1.19E-04	4.57E-03
head development (GO:0060322)	570	44	23.93	1.84	1.20E-04	4.59E-03
positive regulation of protein modification process (GO:0031401)	1028	69	43.16	1.6	1.20E-04	4.59E-03
axon guidance (GO:0007411)	181	20	7.6	2.63	1.21E-04	4.61E-03
regulation of developmental growth (GO:0048638)	334	30	14.02	2.14	1.22E-04	4.63E-03
regulation of coenzyme metabolic process (GO:0051196)	44	9	1.85	4.87	1.30E-04	4.92E-03
cell part morphogenesis (GO:0032990)	626	47	26.28	1.79	1.34E-04	5.06E-03
purine nucleotide metabolic process (GO:0006163)	257	25	10.79	2.32	1.34E-04	5.06E-03
negative regulation of cell development (GO:0010721)	336	30	14.11	2.13	1.34E-04	5.06E-03
non-canonical Wnt signaling pathway via MAPK cascade (GO:0038030)	6	4	0.25	15.88	1.36E-04	5.10E-03
striated muscle contraction (GO:0006941)	89	13	3.74	3.48	1.37E-04	5.12E-03
associative learning (GO:0008306)	89	13	3.74	3.48	1.37E-04	5.12E-03
neuron projection guidance (GO:0097485)	183	20	7.68	2.6	1.40E-04	5.21E-03
negative regulation of axon extension involved in axon guidance (GO:0048843)	26	7	1.09	6.41	1.40E-04	5.21E-03
positive regulation of cardiac muscle hypertrophy (GO:0010613)	26	7	1.09	6.41	1.40E-04	5.21E-03
positive regulation of muscle hypertrophy (GO:0014742)	26	7	1.09	6.41	1.40E-04	5.21E-03
forebrain neuron development (GO:0021884)	35	8	1.47	5.44	1.45E-04	5.34E-03

purine ribonucleotide metabolic process (GO:0009150)	243	24	10.2	2.35	1.45E-04	5.34E-03
positive regulation of cell-substrate adhesion (GO:0010811)	102	14	4.28	3.27	1.46E-04	5.35E-03
regulation of cofactor metabolic process (GO:0051193)	45	9	1.89	4.76	1.53E-04	5.59E-03
positive regulation of neuron projection development (GO:0010976)	275	26	11.54	2.25	1.55E-04	5.65E-03
regulation of cellular response to growth factor stimulus (GO:0090287)	229	23	9.61	2.39	1.56E-04	5.67E-03
intracellular transport (GO:0046907)	1153	75	48.4	1.55	1.57E-04	5.69E-03
glycoprotein metabolic process (GO:0009100)	260	25	10.91	2.29	1.60E-04	5.79E-03
cytokine-mediated signaling pathway (GO:0019221)	245	24	10.29	2.33	1.64E-04	5.92E-03
negative regulation of cellular component movement (GO:0051271)	246	24	10.33	2.32	1.73E-04	6.21E-03
antigen processing and presentation of exogenous peptide antigen (GO:0002478)	27	7	1.13	6.18	1.76E-04	6.30E-03
axonal fasciculation (GO:0007413)	19	6	0.8	7.52	1.79E-04	6.39E-03
long-term synaptic potentiation (GO:0060291)	46	9	1.93	4.66	1.80E-04	6.41E-03
ameboidal-type cell migration (GO:0001667)	158	18	6.63	2.71	1.80E-04	6.41E-03
single-organism cellular localization (GO:1902580)	725	52	30.44	1.71	1.81E-04	6.42E-03
cell morphogenesis (GO:0000902)	836	58	35.1	1.65	1.83E-04	6.47E-03
regulation of muscle adaptation (GO:0043502)	68	11	2.85	3.85	1.87E-04	6.60E-03
regulation of angiogenesis (GO:0045765)	217	22	9.11	2.42	1.89E-04	6.65E-03
ATP metabolic process (GO:0046034)	131	16	5.5	2.91	1.89E-04	6.65E-03
regulation of neuronal synaptic plasticity (GO:0048168)	57	10	2.39	4.18	1.91E-04	6.69E-03
synaptic vesicle cycle (GO:0099504)	80	12	3.36	3.57	1.93E-04	6.74E-03

regulation of protein modification process (GO:0031399)	1595	97	66.96	1.45	1.94E-04	6.76E-03
branching morphogenesis of an epithelial tube (GO:0048754)	160	18	6.72	2.68	2.09E-04	7.27E-03
positive regulation of intracellular signal transduction (GO:1902533)	822	57	34.51	1.65	2.10E-04	7.29E-03
negative regulation of response to stimulus (GO:0048585)	1301	82	54.62	1.5	2.10E-04	7.29E-03
response to organic cyclic compound (GO:0014070)	605	45	25.4	1.77	2.25E-04	7.75E-03
regulation of response to wounding (GO:1903034)	397	33	16.67	1.98	2.31E-04	7.92E-03
ribonucleotide metabolic process (GO:0009259)	251	24	10.54	2.28	2.32E-04	7.93E-03
regulation of smooth muscle cell migration (GO:0014910)	70	11	2.94	3.74	2.38E-04	8.12E-03
regulation of cell adhesion molecule production (GO:0060353)	7	4	0.29	13.61	2.45E-04	8.34E-03
negative regulation of chemotaxis (GO:0050922)	48	9	2.02	4.47	2.45E-04	8.34E-03
negative regulation of hydrolase activity (GO:0051346)	365	31	15.32	2.02	2.47E-04	8.37E-03
response to lipid (GO:0033993)	572	43	24.01	1.79	2.47E-04	8.37E-03
carbohydrate metabolic process (GO:0005975)	537	41	22.54	1.82	2.51E-04	8.46E-03
multicellular organismal response to stress (GO:0033555)	83	12	3.48	3.44	2.68E-04	9.00E-03
clathrin-mediated endocytosis (GO:0072583)	29	7	1.22	5.75	2.70E-04	9.04E-03
morphogenesis of an epithelium (GO:0002009)	470	37	19.73	1.88	2.81E-04	9.39E-03
nucleotide metabolic process (GO:0009117)	368	31	15.45	2.01	2.83E-04	9.43E-03
synapse assembly (GO:0007416)	49	9	2.06	4.38	2.84E-04	9.44E-03
epithelial tube morphogenesis (GO:0060562)	335	29	14.06	2.06	2.84E-04	9.44E-03
negative regulation of response to external stimulus	287	26	12.05	2.16	2.94E-04	9.71E-03

(GO:0032102)						
regulation of behavior (GO:0050795)	84	12	3.53	3.4	2.98E-04	9.82E-03
regulation of heart contraction (GO:0008016)	151	17	6.34	2.68	3.07E-04	1.01E-02
amino acid transport (GO:0006865)	110	14	4.62	3.03	3.11E-04	1.02E-02
regulation of transferase activity (GO:0051338)	762	53	31.99	1.66	3.23E-04	1.06E-02
positive regulation of gliogenesis (GO:0014015)	61	10	2.56	3.91	3.25E-04	1.06E-02
visual learning (GO:0008542)	61	10	2.56	3.91	3.25E-04	1.06E-02
multi-multicellular organism process (GO:0044706)	124	15	5.21	2.88	3.27E-04	1.06E-02
anatomical structure formation involved in morphogenesis (GO:0048646)	970	64	40.72	1.57	3.29E-04	1.07E-02
neuron recognition (GO:0008038)	30	7	1.26	5.56	3.30E-04	1.07E-02
phosphorylation (GO:0016310)	951	63	39.92	1.58	3.30E-04	1.07E-02
response to external stimulus (GO:0009605)	1500	91	62.97	1.45	3.33E-04	1.07E-02
positive regulation of cell proliferation (GO:0008284)	819	56	34.38	1.63	3.33E-04	1.07E-02
neutral lipid metabolic process (GO:0006638)	73	11	3.06	3.59	3.38E-04	1.08E-02
response to fatty acid (GO:0070542)	40	8	1.68	4.76	3.51E-04	1.12E-02
negative regulation of axon extension (GO:0030517)	40	8	1.68	4.76	3.51E-04	1.12E-02
negative regulation of endocytosis (GO:0045806)	40	8	1.68	4.76	3.51E-04	1.12E-02
regulation of smooth muscle cell proliferation (GO:0048660)	125	15	5.25	2.86	3.55E-04	1.12E-02
cell-cell adhesion via plasma-membrane adhesion molecules (GO:0098742)	125	15	5.25	2.86	3.55E-04	1.12E-02
regulation of nucleotide metabolic process (GO:0006140)	197	20	8.27	2.42	3.56E-04	1.12E-02
purine ribonucleoside monophosphate metabolic process (GO:0009167)	153	17	6.42	2.65	3.56E-04	1.12E-02

positive regulation of membrane potential (GO:0045838)	14	5	0.59	8.51	3.56E-04	1.12E-02
regulation of metal ion transport (GO:0010959)	341	29	14.32	2.03	3.77E-04	1.18E-02
organic substance catabolic process (GO:1901575)	1169	74	49.07	1.51	3.79E-04	1.18E-02
purine nucleoside monophosphate metabolic process (GO:0009126)	154	17	6.46	2.63	3.83E-04	1.19E-02
astrocyte development (GO:0014002)	22	6	0.92	6.5	3.89E-04	1.20E-02
regulation of long-term synaptic potentiation (GO:1900271)	22	6	0.92	6.5	3.89E-04	1.20E-02
peptide cross-linking via chondroitin 4-sulfate glycosaminoglycan (GO:0019800)	8	4	0.34	11.91	4.04E-04	1.25E-02
smooth muscle cell migration (GO:0014909)	8	4	0.34	11.91	4.04E-04	1.25E-02
defense response (GO:0006952)	1056	68	44.33	1.53	4.15E-04	1.27E-02
proteoglycan metabolic process (GO:0006029)	63	10	2.64	3.78	4.17E-04	1.28E-02
second-messenger-mediated signaling (GO:0019932)	127	15	5.33	2.81	4.18E-04	1.28E-02
cell recognition (GO:0008037)	127	15	5.33	2.81	4.18E-04	1.28E-02
inorganic cation transmembrane transport (GO:0098662)	360	30	15.11	1.99	4.18E-04	1.28E-02
positive regulation of cell-cell adhesion (GO:0022409)	200	20	8.4	2.38	4.29E-04	1.30E-02
intracellular protein transport (GO:0006886)	588	43	24.68	1.74	4.33E-04	1.31E-02
tube morphogenesis (GO:0035239)	378	31	15.87	1.95	4.39E-04	1.32E-02
ribonucleoside monophosphate metabolic process (GO:0009161)	156	17	6.55	2.6	4.42E-04	1.33E-02
regulation of cellular component size (GO:0032535)	345	29	14.48	2	4.52E-04	1.36E-02
regulation of cell morphogenesis (GO:0022604)	536	40	22.5	1.78	4.61E-04	1.38E-02
visual behavior (GO:0007632)	64	10	2.69	3.72	4.71E-04	1.41E-02

negative regulation of proteolysis (GO:0045861)	313	27	13.14	2.05	4.77E-04	1.42E-02
nucleoside phosphate metabolic process (GO:0006753)	380	31	15.95	1.94	4.79E-04	1.43E-02
protein secretion (GO:0009306)	115	14	4.83	2.9	4.79E-04	1.43E-02
regulation of cardiac muscle hypertrophy (GO:0010611)	42	8	1.76	4.54	4.83E-04	1.43E-02
positive regulation of long-term synaptic potentiation (GO:1900273)	15	5	0.63	7.94	4.86E-04	1.44E-02
synaptic vesicle recycling (GO:0036465)	23	6	0.97	6.21	4.90E-04	1.45E-02
response to alkaloid (GO:0043279)	89	12	3.74	3.21	4.95E-04	1.45E-02
tube development (GO:0035295)	592	43	24.85	1.73	4.95E-04	1.45E-02
negative regulation of wound healing (GO:0061045)	53	9	2.22	4.05	4.97E-04	1.45E-02
regulation of ion transmembrane transport (GO:0034765)	398	32	16.71	1.92	4.98E-04	1.45E-02
protein localization to membrane (GO:0072657)	298	26	12.51	2.08	5.11E-04	1.49E-02
regulation of muscle system process (GO:0090257)	188	19	7.89	2.41	5.21E-04	1.52E-02
regulation of muscle cell apoptotic process (GO:0010660)	65	10	2.73	3.66	5.30E-04	1.54E-02
negative regulation of protein metabolic process (GO:0051248)	1008	65	42.32	1.54	5.35E-04	1.55E-02
nitrogen compound transport (GO:0071705)	417	33	17.51	1.89	5.36E-04	1.55E-02
peptidyl-tyrosine dephosphorylation (GO:0035335)	43	8	1.81	4.43	5.62E-04	1.62E-02
regulation of synaptic transmission, GABAergic (GO:0032228)	33	7	1.39	5.05	5.77E-04	1.66E-02
antigen processing and presentation of exogenous antigen (GO:0019884)	33	7	1.39	5.05	5.77E-04	1.66E-02
monosaccharide biosynthetic process (GO:0046364)	33	7	1.39	5.05	5.77E-04	1.66E-02

feeding behavior (GO:0007631)	104	13	4.37	2.98	5.91E-04	1.69E-02
positive regulation of cell death (GO:0010942)	598	43	25.1	1.71	6.04E-04	1.72E-02
gluconeogenesis (GO:0006094)	24	6	1.01	5.96	6.11E-04	1.74E-02
cellular component morphogenesis (GO:0032989)	917	60	38.5	1.56	6.13E-04	1.74E-02
neuromuscular process (GO:0050905)	118	14	4.95	2.83	6.13E-04	1.74E-02
regulation of synaptic vesicle priming (GO:0010807)	9	4	0.38	10.59	6.26E-04	1.77E-02
metal ion transport (GO:0030001)	509	38	21.37	1.78	6.28E-04	1.77E-02
single-organism carbohydrate metabolic process (GO:0044723)	456	35	19.14	1.83	6.30E-04	1.78E-02
regulation of cytoplasmic transport (GO:1903649)	456	35	19.14	1.83	6.30E-04	1.78E-02
single-organism membrane organization (GO:0044802)	600	43	25.19	1.71	6.44E-04	1.81E-02
glucose 6-phosphate metabolic process (GO:0051156)	16	5	0.67	7.44	6.49E-04	1.82E-02
membrane raft organization (GO:0031579)	16	5	0.67	7.44	6.49E-04	1.82E-02
regulation of muscle hypertrophy (GO:0014743)	44	8	1.85	4.33	6.51E-04	1.82E-02
brain development (GO:0007420)	528	39	22.17	1.76	6.53E-04	1.82E-02
regulation of cellular component biogenesis (GO:0044087)	730	50	30.65	1.63	6.57E-04	1.83E-02
negative regulation of cellular protein metabolic process (GO:0032269)	939	61	39.42	1.55	6.58E-04	1.83E-02
cellular response to acid chemical (GO:0071229)	133	15	5.58	2.69	6.65E-04	1.84E-02
positive regulation of ion transmembrane transport (GO:0034767)	133	15	5.58	2.69	6.65E-04	1.84E-02
response to amino acid (GO:0043200)	67	10	2.81	3.56	6.66E-04	1.84E-02
regulation of protein binding (GO:0043393)	177	18	7.43	2.42	6.72E-04	1.85E-02
muscle tissue development (GO:0060537)	304	26	12.76	2.04	6.80E-04	1.87E-02
neutral amino acid transport (GO:0015804)	34	7	1.43	4.9	6.86E-04	1.88E-02

regulation of cysteine-type endopeptidase activity involved in apoptotic process (GO:0043281)	193	19	8.1	2.35	7.08E-04	1.94E-02
adult behavior (GO:0030534)	163	17	6.84	2.48	7.13E-04	1.95E-02
regulation of neurological system process (GO:0031644)	80	11	3.36	3.28	7.14E-04	1.95E-02
positive regulation of kinase activity (GO:0033674)	407	32	17.09	1.87	7.18E-04	1.96E-02
response to hormone (GO:0009725)	551	40	23.13	1.73	7.73E-04	2.10E-02
purine ribonucleoside triphosphate metabolic process (GO:0009205)	150	16	6.3	2.54	8.01E-04	2.17E-02
catabolic process (GO:0009056)	1362	82	57.18	1.43	8.15E-04	2.20E-02
purine-containing compound metabolic process (GO:0072521)	308	26	12.93	2.01	8.18E-04	2.21E-02
triglyceride metabolic process (GO:0006641)	57	9	2.39	3.76	8.27E-04	2.23E-02
adenylate cyclase-modulating G-protein coupled receptor signaling pathway (GO:0007188)	136	15	5.71	2.63	8.29E-04	2.23E-02
positive regulation of ion transport (GO:0043270)	259	23	10.87	2.12	8.31E-04	2.23E-02
methylglyoxal biosynthetic process (GO:0019242)	1	2	0.04	47.64	8.56E-04	2.29E-02
cholesterol biosynthetic process via 24,25-dihydrolanosterol (GO:0033488)	1	2	0.04	47.64	8.56E-04	2.29E-02
regulation of epithelial cell migration (GO:0010632)	166	17	6.97	2.44	8.68E-04	2.32E-02
isoprenoid biosynthetic process (GO:0008299)	26	6	1.09	5.5	9.21E-04	2.44E-02
branchiomotor neuron axon guidance (GO:0021785)	10	4	0.42	9.53	9.22E-04	2.44E-02
aldehyde biosynthetic process (GO:0046184)	10	4	0.42	9.53	9.22E-04	2.44E-02
negative regulation of axonogenesis (GO:0050771)	58	9	2.43	3.7	9.33E-04	2.46E-02
negative regulation of blood vessel morphogenesis (GO:2000181)	83	11	3.48	3.16	9.57E-04	2.52E-02
cofactor metabolic process (GO:0051186)	295	25	12.38	2.02	9.67E-04	2.54E-02

regulation of cation transmembrane transport (GO:1904062)	214	20	8.98	2.23	9.70E-04	2.55E-02
regulation of transmembrane transport (GO:0034762)	415	32	17.42	1.84	9.80E-04	2.56E-02
cell-cell adhesion (GO:0098609)	540	39	22.67	1.72	9.81E-04	2.56E-02
ribonucleoside triphosphate metabolic process (GO:0009199)	153	16	6.42	2.49	9.81E-04	2.56E-02
nucleoside monophosphate metabolic process (GO:0009123)	168	17	7.05	2.41	9.86E-04	2.57E-02
smooth muscle contraction (GO:0006939)	47	8	1.97	4.05	9.90E-04	2.57E-02
nucleobase-containing small molecule metabolic process (GO:0055086)	433	33	18.18	1.82	9.95E-04	2.58E-02
acylglycerol metabolic process (GO:0006639)	71	10	2.98	3.36	1.03E-03	2.67E-02
regulation of protein localization (GO:0032880)	940	60	39.46	1.52	1.10E-03	2.84E-02
regulation of receptor internalization (GO:0002090)	37	7	1.55	4.51	1.11E-03	2.86E-02
hexose biosynthetic process (GO:0019319)	27	6	1.13	5.29	1.11E-03	2.86E-02
receptor internalization (GO:0031623)	48	8	2.02	3.97	1.13E-03	2.90E-02
positive regulation of endocytosis (GO:0045807)	126	14	5.29	2.65	1.14E-03	2.92E-02
generation of precursor metabolites and energy (GO:0006091)	217	20	9.11	2.2	1.14E-03	2.92E-02
regulation of gliogenesis (GO:0014013)	112	13	4.7	2.76	1.15E-03	2.94E-02
regulation of cell death (GO:0010941)	1460	86	61.29	1.4	1.15E-03	2.94E-02
negative regulation of cysteine-type endopeptidase activity involved in apoptotic process (GO:0043154)	85	11	3.57	3.08	1.15E-03	2.94E-02
gliogenesis (GO:0042063)	186	18	7.81	2.31	1.16E-03	2.95E-02
positive regulation of transmembrane transport (GO:0034764)	141	15	5.92	2.53	1.18E-03	2.99E-02

protein localization to plasma membrane (GO:0072659)	156	16	6.55	2.44	1.19E-03	3.01E-02
positive regulation of MAPK cascade (GO:0043410)	438	33	18.39	1.79	1.20E-03	3.03E-02
negative regulation of developmental growth (GO:0048640)	99	12	4.16	2.89	1.22E-03	3.08E-02
positive regulation of cytosolic calcium ion concentration (GO:0007204)	172	17	7.22	2.35	1.26E-03	3.17E-02
positive regulation of smooth muscle cell migration (GO:0014911)	49	8	2.06	3.89	1.29E-03	3.24E-02
adenylate cyclase-activating dopamine receptor signaling pathway (GO:0007191)	11	4	0.46	8.66	1.31E-03	3.28E-02
membrane raft assembly (GO:0001765)	11	4	0.46	8.66	1.31E-03	3.28E-02
regulation of uterine smooth muscle contraction (GO:0070472)	11	4	0.46	8.66	1.31E-03	3.28E-02
glycolytic process through fructose-6-phosphate (GO:0061615)	11	4	0.46	8.66	1.31E-03	3.28E-02
tonic smooth muscle contraction (GO:0014820)	11	4	0.46	8.66	1.31E-03	3.28E-02
dichotomous subdivision of an epithelial terminal unit (GO:0060600)	11	4	0.46	8.66	1.31E-03	3.28E-02
positive regulation of cell adhesion molecule production (GO:0060355)	5	3	0.21	14.29	1.31E-03	3.28E-02
plasma membrane raft organization (GO:0044857)	5	3	0.21	14.29	1.31E-03	3.28E-02
plasma membrane raft assembly (GO:0044854)	5	3	0.21	14.29	1.31E-03	3.28E-02
non-canonical Wnt signaling pathway via JNK cascade (GO:0038031)	5	3	0.21	14.29	1.31E-03	3.28E-02
myoblast proliferation (GO:0051450)	5	3	0.21	14.29	1.31E-03	3.28E-02
dichotomous subdivision of terminal units involved in salivary gland branching (GO:0060666)	5	3	0.21	14.29	1.31E-03	3.28E-02
regulation of intracellular signal transduction	1427	84	59.91	1.4	1.34E-03	3.29E-02

(GO:1902531)						
chondroitin sulfate proteoglycan metabolic process (GO:0050654)	28	6	1.18	5.1	1.34E-03	3.29E-02
regulation of synaptic vesicle transport (GO:1902803)	28	6	1.18	5.1	1.34E-03	3.29E-02
developmental growth involved in morphogenesis (GO:0060560)	114	13	4.79	2.72	1.34E-03	3.29E-02
purine nucleoside triphosphate metabolic process (GO:0009144)	158	16	6.63	2.41	1.36E-03	3.32E-02
negative regulation of cysteine-type endopeptidase activity (GO:2000117)	87	11	3.65	3.01	1.39E-03	3.38E-02
regulation of intracellular transport (GO:0032386)	570	40	23.93	1.67	1.43E-03	3.47E-02
cell-matrix adhesion (GO:0007160)	101	12	4.24	2.83	1.44E-03	3.49E-02
regulation of anion transport (GO:0044070)	144	15	6.05	2.48	1.44E-03	3.49E-02
regulation of cellular ketone metabolic process (GO:0010565)	115	13	4.83	2.69	1.45E-03	3.51E-02
protein localization to cell periphery (GO:1990778)	159	16	6.67	2.4	1.45E-03	3.51E-02
cellular glucose homeostasis (GO:0001678)	50	8	2.1	3.81	1.46E-03	3.52E-02
regulation of purine nucleotide metabolic process (GO:1900542)	190	18	7.98	2.26	1.47E-03	3.54E-02
regulation of cysteine-type endopeptidase activity (GO:2000116)	206	19	8.65	2.2	1.49E-03	3.58E-02
positive regulation of stress fiber assembly (GO:0051496)	39	7	1.64	4.28	1.50E-03	3.60E-02
negative regulation of vasculature development (GO:1901343)	88	11	3.69	2.98	1.51E-03	3.61E-02
monocarboxylic acid biosynthetic process (GO:0072330)	145	15	6.09	2.46	1.54E-03	3.68E-02
positive regulation of cation transmembrane transport (GO:1904064)	102	12	4.28	2.8	1.56E-03	3.72E-02

locomotory behavior (GO:0007626)	223	20	9.36	2.14	1.57E-03	3.74E-02
anion transport (GO:0006820)	410	31	17.21	1.8	1.57E-03	3.74E-02
positive regulation of protein kinase activity (GO:0045860)	375	29	15.74	1.84	1.58E-03	3.75E-02
regulation of cell-cell adhesion (GO:0022407)	358	28	15.03	1.86	1.61E-03	3.81E-02
positive regulation of metabolic process (GO:0009893)	2890	153	121.32	1.26	1.61E-03	3.81E-02
negative regulation of ion transport (GO:0043271)	131	14	5.5	2.55	1.62E-03	3.82E-02
regulation of GTPase activity (GO:0043087)	341	27	14.32	1.89	1.63E-03	3.84E-02
antigen processing and presentation of peptide antigen (GO:0048002)	63	9	2.64	3.4	1.64E-03	3.86E-02
glial cell differentiation (GO:0010001)	146	15	6.13	2.45	1.65E-03	3.87E-02
mucopolysaccharide metabolic process (GO:1903510)	51	8	2.14	3.74	1.65E-03	3.87E-02
regulation of hormone levels (GO:0010817)	465	34	19.52	1.74	1.65E-03	3.87E-02
vesicle organization (GO:0016050)	208	19	8.73	2.18	1.65E-03	3.87E-02
positive regulation of lipid biosynthetic process (GO:0046889)	76	10	3.19	3.13	1.69E-03	3.94E-02
regulation of secretion (GO:0051046)	726	48	30.48	1.57	1.69E-03	3.94E-02
negative regulation of hemostasis (GO:1900047)	40	7	1.68	4.17	1.73E-03	4.02E-02
negative regulation of blood coagulation (GO:0030195)	40	7	1.68	4.17	1.73E-03	4.02E-02
inorganic ion transmembrane transport (GO:0098660)	413	31	17.34	1.79	1.75E-03	4.05E-02
regulation of cellular protein localization (GO:1903827)	522	37	21.91	1.69	1.79E-03	4.13E-02
semaphorin-plexin signaling pathway involved in axon guidance (GO:1902287)	12	4	0.5	7.94	1.79E-03	4.13E-02
cAMP catabolic process (GO:0006198)	12	4	0.5	7.94	1.79E-03	4.13E-02

positive regulation of reactive oxygen species metabolic process (GO:2000379)	90	11	3.78	2.91	1.80E-03	4.14E-02
hexose metabolic process (GO:0019318)	118	13	4.95	2.62	1.81E-03	4.15E-02
regulation of proteolysis (GO:0030162)	615	42	25.82	1.63	1.81E-03	4.15E-02
plasma membrane organization (GO:0007009)	210	19	8.82	2.16	1.84E-03	4.21E-02
cellular response to amino acid stimulus (GO:0071230)	52	8	2.18	3.66	1.86E-03	4.25E-02
neuron projection extension (GO:1990138)	52	8	2.18	3.66	1.86E-03	4.25E-02
adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains (GO:0002460)	148	15	6.21	2.41	1.87E-03	4.25E-02
synaptic transmission, glutamatergic (GO:0035249)	30	6	1.26	4.76	1.89E-03	4.29E-02
cytosolic transport (GO:0016482)	195	18	8.19	2.2	1.93E-03	4.37E-02
negative regulation of anion transport (GO:1903792)	41	7	1.72	4.07	1.98E-03	4.48E-02
negative regulation of synaptic transmission (GO:0050805)	65	9	2.73	3.3	2.01E-03	4.54E-02
purine ribonucleoside metabolic process (GO:0046128)	212	19	8.9	2.13	2.04E-03	4.60E-02
negative regulation of cellular response to growth factor stimulus (GO:0090288)	120	13	5.04	2.58	2.08E-03	4.68E-02
protein tetramerization (GO:0051262)	120	13	5.04	2.58	2.08E-03	4.68E-02
regulation of cellular catabolic process (GO:0031329)	509	36	21.37	1.68	2.12E-03	4.76E-02
establishment of protein localization to plasma membrane (GO:0090002)	92	11	3.86	2.85	2.13E-03	4.77E-02
antigen processing and presentation of exogenous peptide antigen via MHC class I (GO:0042590)	6	3	0.25	11.91	2.20E-03	4.92E-02

positive regulation of synaptic vesicle transport (GO:1902805)	6	3	0.25	11.91	2.20E-03	4.92E-02
positive regulation of steroid metabolic process (GO:0045940)	31	6	1.3	4.61	2.22E-03	4.94E-02
negative regulation of cell adhesion (GO:0007162)	230	20	9.66	2.07	2.22E-03	4.94E-02
glycosaminoglycan metabolic process (GO:0030203)	79	10	3.32	3.02	2.22E-03	4.94E-02

Table S12. Panther protein class for upregulated genes in hAPP(J20) mice.

PANTHER Protein Class	# of Reference Genes	# of Observed Genes	# of Expected Genes	Ratio of Enrichment	rawP	adjP
receptor (PC00197)	1770	138	74.31	1.86	2.35E-12	1.68E-10
extracellular matrix protein (PC00102)	375	49	15.74	3.11	8.69E-12	4.65E-10
cell adhesion molecule (PC00069)	463	46	19.44	2.37	1.43E-07	4.89E-06
membrane traffic protein (PC00150)	346	38	14.53	2.62	1.60E-07	4.89E-06
transporter (PC00227)	1159	85	48.66	1.75	7.10E-07	1.69E-05
defense/immunity protein (PC00090)	605	51	25.4	2.01	3.56E-06	6.93E-05
signaling molecule (PC00207)	1084	78	45.51	1.71	4.12E-06	7.35E-05
enzyme modulator (PC00095)	1415	95	59.4	1.6	6.11E-06	1.01E-04
protein phosphatase (PC00195)	186	23	7.81	2.95	6.92E-06	1.05E-04
membrane-bound signaling molecule (PC00152)	214	25	8.98	2.78	7.34E-06	1.05E-04
hydrolase (PC00121)	1566	102	65.74	1.55	9.52E-06	1.27E-04
oxidoreductase (PC00176)	643	51	26.99	1.89	1.78E-05	2.24E-04
extracellular matrix structural protein (PC00103)	73	13	3.06	4.24	1.91E-05	2.27E-04
surfactant (PC00212)	55	11	2.31	4.76	2.94E-05	3.31E-04
phosphatase (PC00181)	303	29	12.72	2.28	5.38E-05	5.48E-04
membrane trafficking regulatory protein (PC00151)	119	16	5	3.2	6.43E-05	6.25E-04
extracellular matrix glycoprotein (PC00100)	124	16	5.21	3.07	1.03E-04	9.18E-04
tubulin (PC00228)	19	6	0.8	7.52	1.79E-04	1.53E-03
serine protease inhibitor (PC00204)	151	17	6.34	2.68	3.07E-04	2.53E-03
oxidase (PC00175)	145	16	6.09	2.63	5.63E-04	4.46E-03

cytokine receptor (PC00084)	306	26	12.85	2.02	7.46E-04	5.70E-03
G-protein modulator (PC00022)	445	33	18.68	1.77	1.54E-03	1.14E-02
protease (PC00190)	557	38	23.38	1.63	2.96E-03	1.92E-02
antibacterial response protein (PC00051)	142	14	5.96	2.35	3.32E-03	2.03E-02
immunoglobulin superfamily cell adhesion molecule (PC00125)	98	11	4.11	2.67	3.42E-03	2.03E-02
immunoglobulin receptor superfamily (PC00124)	209	18	8.77	2.05	3.96E-03	2.29E-02
guanyl-nucleotide exchange factor (PC00113)	180	16	7.56	2.12	4.76E-03	2.63E-02
serine protease (PC00203)	334	25	14.02	1.78	4.79E-03	2.63E-02
protease inhibitor (PC00191)	308	23	12.93	1.78	6.80E-03	3.64E-02
microtubule family cytoskeletal protein (PC00157)	207	17	8.69	1.96	7.82E-03	3.98E-02

Table S13. Panther pathways for upregulated genes in hAPP(J20) mice.

PANTHER Pathway	# of Reference Genes	# of Observed Genes	# of Expected Genes	Ratio of Enrichment	rawP	adjP
Cholesterol biosynthesis (P00014)	12	8	0.5	15.88	6.41E-08	5.32E-06
Glycolysis (P00024)	31	10	1.3	7.68	1.14E-06	6.31E-05
Integrin signalling pathway (P00034)	176	23	7.39	3.11	2.87E-06	1.19E-04
Pentose phosphate pathway (P02762)	9	5	0.38	13.23	4.65E-05	1.54E-03
Heterotrimeric G-protein signaling pathway-Gi alpha and Gs alpha mediated pathway (P00026)	133	16	5.58	2.87	2.23E-04	6.17E-03
Synaptic_vesicle_trafficking (P05734)	30	7	1.26	5.56	3.30E-04	7.83E-03
Huntington disease (P00029)	163	17	6.84	2.48	7.13E-04	1.48E-02
Cytoskeletal regulation by Rho GTPase (P00016)	94	11	3.95	2.79	2.50E-03	3.92E-02
Axon guidance mediated by semaphorins (P00007)	22	5	0.92	5.41	2.60E-03	3.92E-02
Axon guidance mediated by netrin (P00009)	34	6	1.43	4.2	3.48E-03	4.81E-02

Table S14. KEGG pathways for upregulated genes in hAPP(J20) mice.

KEGG Pathway	# of Reference Genes	# of Observed Genes	# of Expected Genes	Ratio of Enrichment	rawP	adjP
Metabolic pathways	1184	87	19.6	4.44	5.85E-31	9.42E-29
Steroid biosynthesis	18	13	0.3	43.62	5.15E-20	4.15E-18
Focal adhesion	200	28	3.31	8.46	7.52E-18	4.04E-16
Phagosome	176	25	2.91	8.58	3.05E-16	1.23E-14
Axon guidance	131	19	2.17	8.76	7.52E-13	2.42E-11
ECM-receptor interaction	86	16	1.42	11.24	9.25E-13	2.48E-11
Lysosome	123	18	2.04	8.84	2.57E-12	5.91E-11
Staphylococcus aureus infection	50	12	0.83	14.5	2.70E-11	5.43E-10
Protein digestion and absorption	78	14	1.29	10.84	4.07E-11	7.28E-10
Pathways in cancer	325	25	5.38	4.65	3.17E-10	5.10E-09
Neuroactive ligand-receptor interaction	277	22	4.59	4.8	2.00E-09	2.93E-08
Complement and coagulation cascades	76	12	1.26	9.54	4.66E-09	6.25E-08
Glycolysis / Gluconeogenesis	62	11	1.03	10.72	5.70E-09	7.06E-08
Endocytosis	220	19	3.64	5.22	6.37E-09	7.33E-08
Gap junction	88	12	1.46	8.24	2.57E-08	2.76E-07
Fc gamma R-mediated phagocytosis	90	12	1.49	8.05	3.33E-08	3.35E-07
Terpenoid backbone biosynthesis	14	6	0.23	25.88	5.44E-08	5.15E-07
Antigen processing and presentation	78	11	1.29	8.52	6.86E-08	6.14E-07
Rheumatoid arthritis	81	11	1.34	8.2	1.02E-07	8.64E-07
Chagas disease (American trypanosomiasis)	100	12	1.66	7.25	1.10E-07	8.85E-07

Glycerolipid metabolism	51	9	0.84	10.66	1.47E-07	1.13E-06
Toxoplasmosis	127	13	2.1	6.18	2.17E-07	1.53E-06
Cell adhesion molecules (CAMs)	149	14	2.47	5.67	2.18E-07	1.53E-06
Type I diabetes mellitus	59	9	0.98	9.21	5.40E-07	3.48E-06
Insulin signaling pathway	137	13	2.27	5.73	5.25E-07	3.48E-06
Amoebiasis	116	12	1.92	6.25	5.64E-07	3.49E-06
Regulation of actin cytoskeleton	216	16	3.58	4.47	7.90E-07	4.71E-06
PPAR signaling pathway	80	10	1.32	7.55	8.55E-07	4.92E-06
Leishmaniasis	64	9	1.06	8.49	1.10E-06	6.11E-06
Natural killer cell mediated cytotoxicity	125	12	2.07	5.8	1.26E-06	6.76E-06
Calcium signaling pathway	178	14	2.95	4.75	1.88E-06	9.76E-06
Biosynthesis of unsaturated fatty acids	25	6	0.41	14.5	2.74E-06	1.38E-05
Cytokine-cytokine receptor interaction	245	16	4.06	3.94	4.08E-06	1.99E-05
Malaria	46	7	0.76	9.19	1.02E-05	4.83E-05
Viral myocarditis	89	9	1.47	6.11	1.75E-05	8.05E-05
Gastric acid secretion	73	8	1.21	6.62	2.85E-05	1.00E-04
Glycerophospholipid metabolism	80	8	1.32	6.04	5.56E-05	2.00E-04
Galactose metabolism	27	5	0.45	11.18	7.34E-05	3.00E-04
Pentose phosphate pathway	28	5	0.46	10.79	8.82E-05	3.00E-04
Intestinal immune network for IgA production	43	6	0.71	8.43	7.33E-05	3.00E-04
Chemokine signaling pathway	185	12	3.06	3.92	6.78E-05	3.00E-04
Fatty acid metabolism	48	6	0.79	7.55	1.00E-04	4.00E-04
Small cell lung cancer	87	8	1.44	5.55	1.00E-04	4.00E-04
Hepatitis C	137	10	2.27	4.41	1.00E-04	4.00E-04

Allograft rejection	52	6	0.86	6.97	2.00E-04	6.00E-04
p53 signaling pathway	70	7	1.16	6.04	2.00E-04	6.00E-04
Bile secretion	71	7	1.18	5.95	2.00E-04	6.00E-04
Pancreatic cancer	71	7	1.18	5.95	2.00E-04	6.00E-04
Osteoclast differentiation	118	9	1.95	4.61	2.00E-04	6.00E-04
Systemic lupus erythematosus	149	10	2.47	4.05	2.00E-04	6.00E-04
Fructose and mannose metabolism	37	5	0.61	8.16	3.00E-04	9.00E-04
Amyotrophic lateral sclerosis (ALS)	56	6	0.93	6.47	3.00E-04	9.00E-04
Melanogenesis	100	8	1.66	4.83	3.00E-04	9.00E-04
Jak-STAT signaling pathway	153	10	2.53	3.95	3.00E-04	9.00E-04
Hypertrophic cardiomyopathy (HCM)	83	7	1.37	5.09	5.00E-04	1.50E-03
Ubiquitin mediated proteolysis	140	9	2.32	3.88	6.00E-04	1.70E-03
MAPK signaling pathway	268	13	4.44	2.93	6.00E-04	1.70E-03
Pyruvate metabolism	43	5	0.71	7.02	7.00E-04	1.90E-03
Dilated cardiomyopathy	89	7	1.47	4.75	7.00E-04	1.90E-03
Adipocytokine signaling pathway	68	6	1.13	5.33	9.00E-04	2.40E-03
Wnt signaling pathway	154	9	2.55	3.53	1.10E-03	2.90E-03
Amino sugar and nucleotide sugar metabolism	48	5	0.79	6.29	1.20E-03	3.10E-03
Bacterial invasion of epithelial cells	71	6	1.18	5.1	1.20E-03	3.10E-03
Valine, leucine and isoleucine degradation	50	5	0.83	6.04	1.40E-03	3.50E-03
N-Glycan biosynthesis	50	5	0.83	6.04	1.40E-03	3.50E-03
Butanoate metabolism	30	4	0.5	8.05	1.50E-03	3.60E-03
Adherens junction	75	6	1.24	4.83	1.50E-03	3.60E-03
B cell receptor signaling pathway	76	6	1.26	4.77	1.70E-03	4.00E-03
Salivary secretion	77	6	1.27	4.71	1.80E-03	4.20E-03

Phosphatidylinositol signaling system	78	6	1.29	4.65	1.90E-03	4.40E-03
Propanoate metabolism	33	4	0.55	7.32	2.10E-03	4.50E-03
Arginine and proline metabolism	54	5	0.89	5.59	2.00E-03	4.50E-03
Graft-versus-host disease	54	5	0.89	5.59	2.00E-03	4.50E-03
Purine metabolism	168	9	2.78	3.24	2.10E-03	4.50E-03
Protein processing in endoplasmic reticulum	169	9	2.8	3.22	2.10E-03	4.50E-03
Fc epsilon RI signaling pathway	80	6	1.32	4.53	2.20E-03	4.60E-03
Peroxisome	80	6	1.32	4.53	2.20E-03	4.60E-03
Inositol phosphate metabolism	57	5	0.94	5.3	2.50E-03	5.20E-03
Apoptosis	86	6	1.42	4.21	3.10E-03	6.30E-03
Progesterone-mediated oocyte maturation	88	6	1.46	4.12	3.50E-03	7.00E-03
Fatty acid biosynthesis	6	2	0.1	20.13	3.90E-03	7.70E-03
Leukocyte transendothelial migration	120	7	1.99	3.52	3.90E-03	7.70E-03
Alzheimer's disease	188	9	3.11	2.89	4.30E-03	8.30E-03
Colorectal cancer	65	5	1.08	4.65	4.50E-03	8.60E-03
Glioma	66	5	1.09	4.58	4.80E-03	9.10E-03
Autoimmune thyroid disease	67	5	1.11	4.51	5.10E-03	9.50E-03
Asthma	23	3	0.38	7.88	6.30E-03	1.15E-02
Starch and sucrose metabolism	45	4	0.75	5.37	6.50E-03	1.15E-02
Fat digestion and absorption	45	4	0.75	5.37	6.50E-03	1.15E-02
ABC transporters	45	4	0.75	5.37	6.50E-03	1.15E-02
Renal cell carcinoma	71	5	1.18	4.25	6.50E-03	1.15E-02
Long-term depression	72	5	1.19	4.19	6.90E-03	1.21E-02
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	74	5	1.23	4.08	7.70E-03	1.33E-02

Glycosphingolipid biosynthesis - lacto and neolacto series	26	3	0.43	6.97	8.90E-03	1.51E-02
Type II diabetes mellitus	49	4	0.81	4.93	8.80E-03	1.51E-02
Synthesis and degradation of ketone bodies	10	2	0.17	12.08	1.13E-02	1.87E-02
mTOR signaling pathway	53	4	0.88	4.56	1.15E-02	1.87E-02
Cardiac muscle contraction	81	5	1.34	3.73	1.12E-02	1.87E-02
Oocyte meiosis	113	6	1.87	3.21	1.15E-02	1.87E-02
Glutathione metabolism	54	4	0.89	4.47	1.23E-02	1.98E-02
Basal cell carcinoma	55	4	0.91	4.39	1.31E-02	2.07E-02
Non-small cell lung cancer	55	4	0.91	4.39	1.31E-02	2.07E-02
TGF-beta signaling pathway	85	5	1.41	3.55	1.36E-02	2.13E-02
Acute myeloid leukemia	57	4	0.94	4.24	1.47E-02	2.28E-02
ErbB signaling pathway	87	5	1.44	3.47	1.49E-02	2.28E-02
Prostate cancer	89	5	1.47	3.39	1.63E-02	2.48E-02
Alanine, aspartate and glutamate metabolism	33	3	0.55	5.49	1.71E-02	2.57E-02
Prion diseases	35	3	0.58	5.18	2.00E-02	2.98E-02
Ether lipid metabolism	36	3	0.6	5.03	2.15E-02	3.18E-02
Neurotrophin signaling pathway	131	6	2.17	2.77	2.22E-02	3.25E-02
Glycosaminoglycan biosynthesis - keratan sulfate	15	2	0.25	8.05	2.49E-02	3.55E-02
Glycosphingolipid biosynthesis - ganglio series	15	2	0.25	8.05	2.49E-02	3.55E-02
Base excision repair	38	3	0.63	4.77	2.48E-02	3.55E-02
Carbohydrate digestion and absorption	39	3	0.65	4.65	2.66E-02	3.76E-02
Sphingolipid metabolism	41	3	0.68	4.42	3.03E-02	4.24E-02

Melanoma	72	4	1.19	3.36	3.17E-02	4.40E-02
Vasopressin-regulated water reabsorption	43	3	0.71	4.21	3.42E-02	4.71E-02
Chronic myeloid leukemia	74	4	1.23	3.26	3.46E-02	4.72E-02
Aldosterone-regulated sodium reabsorption	44	3	0.73	4.12	3.63E-02	4.90E-02
T cell receptor signaling pathway	110	5	1.82	2.75	3.65E-02	4.90E-02

Table S15. Wikipathways for upregulated genes in hAPP(J20) mice.

Wikipathway	# of Reference Genes	# of Observed Genes	# of Expected Genes	Ratio of Enrichment	rawP	adjP
Cholesterol Biosynthesis	16	13	0.26	49.07	3.47E-21	3.37E-19
Focal Adhesion	186	26	3.08	8.44	1.17E-16	5.67E-15
Complement and Coagulation Cascades	61	12	1.01	11.88	3.28E-10	1.06E-08
Adipogenesis	133	15	2.2	6.81	6.85E-09	1.66E-07
EGFR1 Signaling Pathway	217	18	3.59	5.01	2.96E-08	5.58E-07
Non-odorant GPCRs	270	20	4.47	4.47	3.45E-08	5.58E-07
Glycolysis and Gluconeogenesis	51	9	0.84	10.66	1.47E-07	1.78E-06
T Cell Receptor Signaling Pathway	143	14	2.37	5.91	1.31E-07	1.78E-06
Complement Activation, Classical Pathway	17	6	0.28	21.32	2.15E-07	2.32E-06
Macrophage markers	10	5	0.17	30.2	2.90E-07	2.56E-06
Senescence and Autophagy	109	12	1.8	6.65	2.86E-07	2.56E-06
Myometrial Relaxation and Contraction Pathways	158	14	2.62	5.35	4.49E-07	3.63E-06
Integrin-mediated cell adhesion	100	11	1.66	6.64	8.98E-07	6.70E-06
PPAR signaling pathway	93	10	1.54	6.49	3.46E-06	2.40E-05
G Protein Signaling Pathways	94	10	1.56	6.43	3.81E-06	2.46E-05
Fatty Acid Biosynthesis	29	6	0.48	12.5	6.96E-06	4.22E-05
Calcium Regulation in the Cardiac Cell	152	12	2.52	4.77	9.71E-06	5.54E-05
Monoamine GPCRs	33	6	0.55	10.98	1.53E-05	8.25E-05
SREBF and miR33 in cholesterol and lipid homeostasis	11	4	0.18	21.96	2.25E-05	1.00E-04
Peptide GPCRs	70	8	1.16	6.9	2.09E-05	1.00E-04

GPCRs, Class A Rhodopsin-like	225	14	3.73	3.76	2.75E-05	1.00E-04
Alzheimers Disease	77	8	1.27	6.28	4.21E-05	2.00E-04
MicroRNAs in cardiomyocyte hypertrophy	102	9	1.69	5.33	5.22E-05	2.00E-04
metapathway biotransformation	147	11	2.43	4.52	3.72E-05	2.00E-04
cytochrome P450	44	6	0.73	8.24	8.37E-05	3.00E-04
Endochondral Ossification	67	7	1.11	6.31	1.00E-04	3.00E-04
Regulation of Actin Cytoskeleton	160	11	2.65	4.15	8.03E-05	3.00E-04
Chemokine signaling pathway	186	12	3.08	3.9	7.14E-05	3.00E-04
PluriNetWork	292	15	4.83	3.1	1.00E-04	3.00E-04
Wnt Signaling Pathway and Pluripotency	97	8	1.61	4.98	2.00E-04	6.00E-04
IL-6 signaling Pathway	117	9	1.94	4.65	2.00E-04	6.00E-04
GPCR-orphan-noGO	21	4	0.35	11.5	4.00E-04	1.20E-03
IL-3 Signaling Pathway	111	8	1.84	4.35	5.00E-04	1.50E-03
IL-9 Signaling Pathway	25	4	0.41	9.66	7.00E-04	2.00E-03
Alpha6-Beta4 Integrin Signaling Pathway	90	7	1.49	4.7	8.00E-04	2.20E-03
ESC Pluripotency Pathways	123	8	2.04	3.93	1.10E-03	3.00E-03
Inflammatory Response Pathway	30	4	0.5	8.05	1.50E-03	3.80E-03
GPCRs, Other	161	9	2.67	3.38	1.50E-03	3.80E-03
Prostaglandin Synthesis and Regulation	31	4	0.51	7.79	1.60E-03	4.00E-03
B Cell Receptor Signaling Pathway	202	10	3.34	2.99	2.10E-03	5.10E-03
Glycogen Metabolism	35	4	0.58	6.9	2.60E-03	6.20E-03
Wnt Signaling Pathway	61	5	1.01	4.95	3.40E-03	7.90E-03
IL-2 Signaling Pathway	92	6	1.52	3.94	4.30E-03	9.70E-03
Apoptosis	93	6	1.54	3.9	4.60E-03	1.01E-02
Androgen Receptor Signaling Pathway	126	7	2.09	3.36	5.20E-03	1.07E-02
Insulin Signaling	158	8	2.62	3.06	5.00E-03	1.07E-02
Odorant GPCRs	193	9	3.2	2.82	5.10E-03	1.07E-02

Kit Receptor Signaling Pathway	70	5	1.16	4.31	6.10E-03	1.23E-02
serotonin and anxiety	23	3	0.38	7.88	6.30E-03	1.25E-02
Pentose Phosphate Pathway	8	2	0.13	15.1	7.20E-03	1.40E-02
IL-4 signaling Pathway	75	5	1.24	4.03	8.20E-03	1.56E-02
One carbon metabolism and related pathways	49	4	0.81	4.93	8.80E-03	1.64E-02
TNF-alpha NF-kB Signaling Pathway	215	9	3.56	2.53	1.01E-02	1.85E-02
IL-5 Signaling Pathway	80	5	1.32	3.77	1.07E-02	1.92E-02
Splicing factor NOVA regulated synpatic proteins	58	4	0.96	4.17	1.56E-02	2.75E-02
MaxYvesSuperCombo	12	2	0.2	10.07	1.62E-02	2.81E-02
Alanine and aspartate metabolism	13	2	0.22	9.29	1.89E-02	3.22E-02
Signaling of Hepatocyte Growth Factor Receptor	38	3	0.63	4.77	2.48E-02	4.15E-02

Table S16. Phenotypes for upregulated genes in hAPP(J20) mice.

Phenotype	# of Reference Genes	# of Observed Genes	# of Expected Genes	Ratio of Enrichment	rawP	adjP
Abnormal synaptic transmission	443	58	22.54	2.57	1.59E-11	8.05E-09
Abnormal CNS synaptic transmission	378	48	19.23	2.5	2.94E-09	1.06E-06
Abnormal brain morphology	1119	100	56.94	1.76	5.46E-09	1.73E-06
Seizures	282	39	14.35	2.72	9.13E-09	2.31E-06
Abnormal neuron morphology	991	91	50.43	1.8	8.48E-09	2.31E-06
Abnormal motor capabilities/coordination/movement	1294	107	65.84	1.63	9.65E-08	2.04E-05
Abnormal learning/memory/conditionin	423	48	21.52	2.23	1.09E-07	2.12E-05
Convulsive seizures	116	20	5.9	3.39	1.34E-06	2.00E-04
Mortality/aging	3365	220	171.23	1.28	1.33E-06	2.00E-04
Impaired behavioral response to addictive substance	46	12	2.34	5.13	2.09E-06	3.00E-04
Abnormal emotion/affect behavior	361	40	18.37	2.18	2.45E-06	3.00E-04
Decreased body weight	1194	96	60.76	1.58	1.94E-06	3.00E-04
Abnormal neurotransmitter secretion	48	12	2.44	4.91	3.41E-06	4.00E-04
Abnormal choroid vasculature morphology	21	8	1.07	7.49	4.79E-06	5.00E-04
Abnormal glial cell physiology	51	12	2.6	4.62	6.76E-06	6.00E-04
Abnormal motor coordination/ balance	530	51	26.97	1.89	6.65E-06	6.00E-04
Abnormal homeostasis	2649	178	134.79	1.32	6.11E-06	6.00E-04
Abnormal antigen presentation	61	13	3.1	4.19	9.03E-06	7.00E-04
Abnormal neuron physiology	392	41	19.95	2.06	7.78E-06	7.00E-04
Abnormal basement membrane morphology	30	9	1.53	5.9	1.16E-05	9.00E-04

Abnormal avoidance learning behavior	62	13	3.15	4.12	1.09E-05	9.00E-04
Abnormal limbic system morphology	239	29	12.16	2.38	1.11E-05	9.00E-04
Abnormal voluntary movement	970	79	49.36	1.6	1.21E-05	9.00E-04
Impaired neutrophil recruitment	46	11	2.34	4.7	1.39E-05	1.00E-03
Decreased inflammatory response	205	26	10.43	2.49	1.44E-05	1.00E-03
Abnormal temporal lobe morphology	207	26	10.53	2.47	1.72E-05	1.10E-03
Abnormal synaptic plasticity	40	10	2.04	4.91	2.25E-05	1.40E-03
Abnormal behavioral response to xenobiotic	300	33	15.27	2.16	2.22E-05	1.40E-03

Table S17. Overlapping genes containing DEGs between amyloid- and learning-associated datasets.

Comparison	Genes
Upregulated hAPP(J20) vs. Upregulated Learning	*1700034P13Rik 2010107G23Rik 2310022B05Rik 2900011O08Rik 4930404N11Rik A330070K13Rik Aacs Acot7 Adra2a Agt Ak4 Aldoa Amz1 Anapc2 Angpt1 Anxa5 Anxa7 Aox3 Ap1s1 Ap2m1 Apbb1 Arhgap33 Arhgdig Arpp21 Arrdc1 Asl Aspg Atp6v0b Atp6v0e2 Atp6v1b2 Atxn7l3 B4galt2 BC005764 Basp1 Bcap31 Bscl2 C2 Camk2d Camk2n2 Capns1 Ccdc109b Cd200 Cd44 Cd63 Cd74 Celf4 Cited2 Cmip Col6a1 Col6a2 Col9a2 Comtd1 Cplx1 Cpne7 Crtac1 Ctsd Cul7 Cyb5r3 Cybrd1 Cyp46a1 D630023F18Rik Dact3 Dbi Dner Dnm1 Dpp10 Dusp26 Fads3 Fam185a Fbxo2 Fcgr3 Fdps Fgfbp1 Fhl1 Fkbp8 Fkbp9 Flot1 Fmo1 Fxyd1 Fxyd6 Fzd7 Gaa Gadd45a Gapdh Gdap1l1 Gdi1 Ggact Gm11549 Gm11837 Gm15612 Gm16702 Gnas Gng3 Gpr162 Gpsm1 Gpx1 Grin2d Hars Hcn3 Hebp2 Hexa Hgf Hmgcr Hpgd Hsp90aa1 Htr3a Htr7 Idh1 Ifitm7 Ifngr1 Igfbp3 Igfbp6 Il34 Il3ra Isyna1 Itih3 Klc1 Klhdc8b L1cam Lamc2 Lhfp Lnx1 Lrpap1 Lrsam1 Lsp1 Ltbp3 Ly6e Maf Mapk8ip1 March3 Med16 Megf11 Mmab Mrps27 Mvd Ndn Necab1 Nedd9 Nkd2 Nmbr Nnat Nr2f2 Osr1 Oxr1 Oxtr Pafah1b3 Pde2a Penk Pfkp Pgam1 Pgd Pgrmc1 Plagl1 Plxnd1 Pold2 Popdc3 Por Ppap2a Ppp1r26 Ppp2r2b Prdx4 Prkab2 Prkar1b Prr13 Psap Rab15 Rab4b Rabac1 Rangap1 Rcn3 Rem2 Rhof Rnf14 Rspo3 Rtn2 Rundc3a Rusc1 Rusc2 Rwdd2a S100a6 Sae1 Samd14 Scpep1 Sfxn3 Sgsm1 Sh2d3c Sh2d5 Sirpa Slbp Slc25a22 Slc3a1 Slc3a2 Slc5a5 Smoc1 Snap91 Socs2 Spryd3 Spsb2 Stard4 Stmn2 Stmn3 Stx1a Syn1 Syn2 Syp Tapbpl Tbcb Tfr2 Thbs2 Timp2 Tm4sf1 Tmem130 Tmem132a Tmem132e Tpi1 Tppp3 Trim3 Tubb3 Tubb4b Tubb5 Uchl1 Uchl1os Unc119 Usp5 Vtn Vwa1 Wls Wnt4 Ywhag Zfp367 Zwint*
Upregulated hAPP(J20) vs. Downregulated Learning	*1700113A16Rik 2900052N01Rik Adcy8 Akt2 Aldoc Arl4d Atf5 Bccip Cacng3 Camk2n1 Cdkn1b Chn1os3 Cyp4f15 Ddit4 Dmp1 Dnajb5 Dusp4 Dusp6 Egr1 Egr2 Egr4 Eif1b Eps8l3 Fam151a Fam181b Fkbp4 Fos Fzd2 Gpr39 Hnrnpa2b1 Ing1 Junb Kitl Luzp2 Matn4 Moxd1 Mt3 Nab2 Nox1 Npas4 Nr4a1 P4ha1 Ppp1r1b Ppp1r3c Ppp2r1b Ptms Rbfox3 Rtp1 Sik1 Slc24a5 Slc9a3r1 Smox Snrpb Srsf3 Tac1 Tnnt2 Zfp365*
	1700086L19Rik 2010300C02Rik 2210018M11Rik 4833424O15Rik 8030462N17Rik

Downregulated hAPP(J20) vs. Downregulated Learning	*9930014A18Rik AI593442 Abca8a Abcb7 Abhd13 Ablim3 Acap2 Acot5 Actb Actr3 Adamts20 Adcy1 Add2 Ago1 Ahcyl1 Ahcyl2 Akap7 Aldh5a1 Alkbh5 Amigo1 Ankrd45 Anks1b Ano3 Arhgap20 Arhgap21 Arid4b Ash1l Asxl3 Atp2b3 Atxn1 Auts2 B3galt5 BC030336 Bai3 Bcl11b Bcl6 Bmi1 Bod1l Braf Brwd1 Btbd3 Btbd9 C030046E11Rik Cacna1h Cadm2 Camk2b Cblb Ccdc47 Ccdc6 Cdc42bpa Cdh12 Cdh6 Cdh8 Cdr1 Cecr2 Chd7 Chn1 Cipc Cnot6l Cntnap5b Cpeb4 Crtc3 Ctdsp2 Cttnbp2 D16Ertd472e D3Bwg0562e Dab1 Dagla Dcp2 Ddx46 Dennd4a Desi2 Dgkh Dgkz Dhx33 Diap2 Dixdc1 Dlgap1 Dnah9 Doc2b Dock10 Dock11 Dpp6 Drd5 Dscam Dusp7 Egfem1 Egfl6 Eif4ebp2 Eml4 Eml6 Emx2os Enah Epas1 Epb4.1l1 Epha7 Epn2 Erbb2ip Fam135a Fam163b Fam204a Fam208a Fam84b Fat3 Fat4 Fbxl17 Fbxl18 Fhad1 Fnip2 Foxk1 Foxn2 Gabpb2 Gabrb3 Gm17066 Gm19522 Gm5069 Gm608 Gng7 Gpr12 Gpr155 Gria1 Gria2 Grid1 Herc3 Hhip Hipk3 Hivep2 Hnrnpl Hsd11b1 Igsf3 Il1rap Ipw Iqgap2 Itsn2 Jakmip3 Jarid2 Jmjd4 Kalrn Kansl1l Kat6a Kat6b Kbtbd11 Kbtbd8 Kcnb1 Kcnc3 Kcnd2 Kcng2 Kcng3 Kcnh5 Kcnip2 Kcnip4 Kcnj3 Kcnk9 Kcnq3 Kdm4c Kdm7a Kif1c Kif26b Klf13 Kmt2e Lct Lmo3 Lonrf3 Lrp1b Lrrc16a Lrrc4 Lrrn1 Lurap1 Lypd1 Lysmd3 Maml2 Map1b Map3k13 Map4k4 Map6d1 Mars2 Mast3 Mast4 Mbnl2 Mdga2 Med12l Med13 Med13l Med14 Megf10 Micall1 Mios Mir568 Mkl2 Mkx Mmp16 Mn1 Mtf1 Mxd1 Myef2 Nbea Nckap1 Nedd4l Nfia Nhsl2 Nipbl Nr3c2 Nt5c1a Ntrk3 Nufip2 Nupl1 Nwd1 Obscn Onecut1 Onecut2 Palm2 Pard3 Pcf11 Pcm1 Pde10a Pdpk1 Phlpp2 Plcl1 Plekhh2 Plxna4 Pm20d2 Pogz Ppfia2 Ppp1r16b Ppp1r21 Prdm2 Prkg1 Prkrir Prox1 Ptbp3 Ptchd4 Ptpn11 Ptprj Pwwp2a Qk Qtrtd1 Ralgapa1 Ralgapa2 Rapgef5 Rasa1 Rasal2 Rasgrf1 Rbfox1 Rbm15 Rev1 Rgs7bp Rlf Robo2 Rock1 Rph3a Rprd1a Rsbn1l Runx2 Ryr1 Scarb1 Scgn Scn3a Scn3b Scn8a Sec14l1 Sema6d Sept3 Sertad4 Sf3b1 Shisa7 Sipa1l2 Sipa1l3 Slc1a1 Slc26a10 Slc2a13 Slc4a4 Slit3 Smc4 Snx30 Socs5 Socs7 Soga1 Sorl1 Spata13 Sphkap Sptb Sptbn2 Srl Ss18l1 Stag2 Stard13 Strn3 Susd1 Synj2bp Synrg Sypl2 Syt7 Tbc1d15 Tbc1d8b Tcf4 Tenm1 Tet3 Tgfa Thoc2 Tial1 Tiam1 Tle1 Tmem132b Tmem167 Tmem170b Tmem74 Tmem81 Tmtc1 Tnks2 Trim71 Trpm3 Trpm7 Ttn Tub Twistnb U2surp Unc80 Usp1 Usp7 Vash1 Vps13c Wasl Wdhd1 Wipf2 Wipf3 Yipf6 Zbtb16 Zbtb18 Zbtb20 Zcchc11 Zcchc14 Zdhhc23 Zfc3h1 Zfhx4 Zfp120 Zfp281 Zfp445 Zfp536 Zfp654 Zfp667 Zfp704 Zfp827 Zfp84 Zfp867 Zhx2 Zswim5*
	1700113A16Rik 2900052N01Rik Adcy8 Akt2 Aldoc Arl4d Atf5 Bccip Cacng3 Camk2n1

Downregulated hAPP(J20) vs. Upregulated Learning	*Cdkn1b Chn1os3 Cyp4f15 Ddit4 Dmp1 Dnajb5 Dusp4 Dusp6 Egr1 Egr2 Egr4 Eif1b Eps8l3 Fam151a Fam181b Fkbp4 Fos Fzd2 Gpr39 Hnrnpa2b1 Ing1 Junb Kitl Luzp2 Matn4 Moxd1 Mt3 Nab2 Nox1 Npas4 Nr4a1 P4ha1 Ppp1r1b Ppp1r3c Ppp2r1b Ptms Rbfox3 Rtp1 Sik1 Slc24a5 Slc9a3r1 Smox Snrpb Srsf3 Tac1 Tnnt2 Zfp365*

Table S18. Differentially methylated genes in hAPP(J20) mice.

Gene Symbol	EntrezGene
Ncald	neurocalcin delta
Kirrel3	kin of IRRE like 3 (Drosophila)
Dscaml1	Down syndrome cell adhesion molecule like 1
Rps6ka2	ribosomal protein S6 kinase, polypeptide 2
Zhx2	zinc fingers and homeoboxes 2
Snd1	staphylococcal nuclease and tudor domain containing 1
Pde10a	phosphodiesterase 10A
Tnik	TRAF2 and NCK interacting kinase
Sema4d	sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4D
Prox1	prospero-related homeobox 1
Nfia	nuclear factor I/A
Asap1	ArfGAP with SH3 domain, ankyrin repeat and PH domain1
Pgbd5	piggyBac transposable element derived 5
Dscam	Down syndrome cell adhesion molecule
Tmem108	transmembrane protein 108
Tcf4	transcription factor 4
Sorcs2	sortilin-related VPS10 domain containing receptor 2
Bach2	BTB and CNC homology 2
Tspan18	tetraspanin 18
Shisa9	shisa homolog 9 (Xenopus laevis)
Mgll	monoglyceride lipase
Gtf2ird1	general transcription factor II I repeat domain-containing 1
Ptprg	protein tyrosine phosphatase, receptor type, G
Dpf3	D4, zinc and double PHD fingers, family 3

Kif26b	kinesin family member 26B
Spon1	spondin 1, (f-spondin) extracellular matrix protein
Hspa12a	heat shock protein 12A
Zfp536	zinc finger protein 536
Zbtb20	zinc finger and BTB domain containing 20
Ephb2	Eph receptor B2

Table S19. Primers used in this study.

Gene	Sense primer (5'-> 3')	Antisense Primer (5'-> 3')	Annealing Temp (^{o}C)
Hprt1	GGAGTCCTGTTGATGTTGCCAGTA	GGGACGCAGCAACTGACATTTCTA	60
Gapdh	GTGGAGTCATACTGGAACATGTAG	AATGGTGAAGGTCGGTGTG	60
Fos	AATGGTGAAGACCGTGTCAGGA	TTGATCTGTCTCCGCTTGGAGTGT	60
Egr1	AGCGCCTTCAATCCTCAAG	TTTGGCTGGGATAACTCGTC	60
Penk	GCCTTGTCAATGATGTTTCTTGTC	CAACATAGCCATAAGAGACCAATACTG	60

DISCUSSION

The work presented in this dissertation provides numerous novel findings and insights that should inform future investigation into the role of neuroepigenetic mechanisms in AD. First, it presents a comprehensive overview of the scientific understanding on how epigenetic mechanisms are involved in modulating transcriptional programs necessary for synaptic plasticity and long-term behavioral memory. Second, it expands this framework to include epigenetic regulation of non-Hebbian forms of plasticity, like intrinsic excitability and synaptic scaling. Third, it generates publically available data that adds to the accumulation of other "omic" studies that are important as reference transcriptomes and epigenomes for neurodegenerative disorders. And fourth, provides evidence to support the use of HDAC2-targeted, anti-sense oligonucleotide based therapies for the treatment of AD-related cognitive impairment.

In regards to the specific work presented in this dissertation, there are many possible starting points for future studies. The first involves additional mechanistic studies underlying the procognitive effects associated with ASO-mediated knockdown of HDAC2. Prior work by Gräff *et al.* in CK-p25 mice showed that short-hairpin-RNA-mediated knockdown of HDAC2 unlocks the epigenetic repression of learning and memory-related genes, in turn reinstating structural and synaptic plasticity as well as cognitive abilities (*27*). However, their work only examined a series of learning and memory candidate genes including immediate early genes (IEGs) (*e.g. Arc*, *Egr1*, *Homer1*) as well as genes involved

in glutamatergic signaling (*e.g. Gria1*, *Gria2*, *Grin2a*, *Grin2b*), and synaptic transmission *(e.g. Syp*, *Syt1*) (*27*). How exactly HDAC2 knockdown or inhibition effects other gene families known to be dysregulated in hAPP(J20) mice or other models is unknown. Work by Benito *et al.* suggests that HDAC inhibition in general might provide procogntive benefits via anti-inflammatory action. In their study, they showed that oral administration of SAHA in APP/PS1-21 mice dampened a primarily immune upregulated transcriptional signature (*25*). This might seem a bit counterintuitive, given that HDAC inhibitors by design are considered transcriptional activators. However, the notion of HDAC inhibitor mediated transcriptional repression is supported by a recent study in which HDAC inhibition of wildtype mice upregulated and downregulated an equal number genes, with upregulated genes including transcriptional repressors known to interact with MBD proteins in methylation-induced gene silencing (*91*). Hence, the increased expression of such transcriptional repressors could in turn target and downregulate select gene families, including inflammation and immune-related genes. It should be noted though that SAHA treatment was also associated with reinstating both genome-wide H4K12 acetylation profiles and synapse-associated gene expression from a previously hypoacetylated and downregulated state, respectively. Whether or not similar effects are observed with HDAC2 knockdown could be determined with RNA-seq profiling of HDAC2 ASO treated animals. ChIP-Seq experiments targeting HDAC2 in hAPP(J20) and other AD models would also be invaluable, as they would identify genes directly regulated by HDAC2. This would not only be helpful to dissociate the previously outlined potential direct and indirect mechanisms of HDAC2 knockdown mediated cognitive enhancement but also identify the specific gene targets that could in themselves serve as potential therapeutic targets.

These and other types of profiling experiments should also be performed in the context of activity-dependent learning tasks. One possible explanation that accounts for the discrepancies between many of the previously mentioned studies searching for transcriptional and epigenetic alterations in AD is that instead of alterations in basal levels of epigenetic modifications and gene expression levels, AD might be more related with the inability of dynamically modifying the epigenome and transcriptome in an activity-dependent manner during learning and memory. In support of this view, transcriptional dysregulation is sometimes only evident when AD model mice undergo behavioral learning (*92*). Similarly, the basal levels of acetylated H4K12 in mice remain constant with age; however, when subjected to a learning task aged mice do not exhibit the increase in learning-related H4K12 acetylation exhibited by their younger counterparts(*93*). This same phenomenon has also been documented in Tg2576 model mice in relation to learning-induced changes in global H4 acetylation(*32*). Hence, this may explain the surprisingly few number of differentially methylated genes identified in naïve hAPP(J20) mice. In the context of the presented HDAC2 results, HDAC2 knockdown may not alter gene expression level in and of itself but instead "prime" the levels of histone acetylation in preparation of learning-induced transcriptional change (*94-96*). Overall, it is encouraged that future studies using AD mouse models incorporate an experimental design that allows for the examination of differential learning-induced transcriptional and epigenetic alteration.

With large public research projects like the Encyclopedia of DNA Elements (ENCODE), the NIH Epigenomics Roadmap Project, and NIH Genomics of Gene Regulation project, the last decade has and will continue to see and explosion of large data

sets profiling genetic, epigenetic, and other gene regulatory mechanisms across different disease states, cell-types, and experimental conditions. Although these "omic" maps will be critical to gaining a system's biology perspective underlying disease, there will be a growing need to understanding how genetic variants and epigenetic alterations modulate the expression and activity of proteins, the physiology of neurons and the function of larger brain circuits. In the section TRANSCRIPTIONAL AND EPIGENETIC REGULATION OF HEBBIAN AND NON-HEBBIAN PLASTICITY, a conceptual framework was presented arguing for an augmented view of how epigenetic modifications may regulate neuronal physiology in the context of learning and memory as well as disease. At the time of publication (*14*), much of neuroepigenetic research focused on the role of epigenetic mechanisms in forms of Hebbian synaptic plasticity such as long-term potentiation and long-term depression. Since then, two studies have been published providing evidence implicating DNA cytosine methylation in homeostatic synaptic scaling (*97, 98*) and currently our research group is in the final stages of submitting for publication evidence that supports the involvement of DNA methylation in intrinsic membrane excitability. Together, these findings support the proposed notion that epigenetic mechanisms act as powerful modulators of whole-cell function and highlight the need for continued work to understand the functional implication of altered transcriptional and epigenetic states. It is worth noting that an emerging body of work suggests homeostatic changes in intrinsic excitability and synaptic scaling also contribute to AD-related pathobiology (*15, 16, 99, 100*). Hence, it is advisable that future studies incorporate the proposed framework when interpreting the functional significance of AD-related molecular perturbations.

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APPENDIX

IACUC Approval Form

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM
Institutional Animal Care and Use Committee (IACUC)

MEMORANDUM

DATE: 13-Jan-2016

TO: Sweatt, John David

FROM: Robert A. Kesterson, Ph.D., Chair

Institutional Animal Care and Use Committee (IACUC)

SUBJECT: **NOTICE OF APPROVAL**

The following application was approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee (IACUC) on 13-Jan-2016.

Protocol PI: Sweatt, John David

Title: Molecular Neuropharmacology and Signaling of Histone H2A.Z.

Sponsor: National Institute of Mental Health/NIH/DHHS

Animal Project Number (APN): IACUC-20283

This institution has an Animal Welfare Assurance on file with the Office of Laboratory Animal Welfare (OLAW), is registered as a Research Facility with the USDA, and is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

Institutional Animal Care and Use Committee (IACUC) | Mailing Address:
CH19 Suite 403 | CH19 Suite 403
933 19th Street South | 1530 3rd Ave S
(205) 934-7692 | Birmingham, AL 35294-0019
FAX (205) 934-1188 |

FOR OFFICE USE ONLY		
Date Received:	Date Approved:	Animal Project Number (APN):

PROVIDE A COPY OF THE CORRESPONDING GRANT, CONTRACT APPLICATION, OR STUDY PLAN (PDF format is preferred), which should be consistent with the AUR but do not refer to it instead of responding to the questions below.

SECTION I: GENERAL INFORMATION (Questions 1-4)

1. Principal Investigator

Name	John David Sweatt	**Blazer ID**	dsweattt
Department	Neurobiology	**Division**	SOM
Office Address	SHEL 1010	**Office Phone**	4-4066
Email	dsweatt@uab.edu	**FAX Number**	5-5097
Contact who should receive copies of IACUC correspondence (Optional)			
Name	**Cristin Gavin**	**Email**	**cfgavin@uab.edu**
Phone	**4-6433**	**FAX Number**	**4-6571**

2. Application

Project Title	Molecular Neuropharmacology and Signaling of Histone H2A.Z.
Sponsor	NIH
Project Period (start date & end date)	Jan 2016-Dec 2020
Previous APN	
If part of a Program Project, SCORE, Center, etc:	
Name of PI	
Title	

3. Will personnel other than the PI (e.g., faculty, staff, students, or fellows) be involved in the animal work being proposed? ☐ NO ☒ YES

If the response is YES, provide the name(s) below.

Name	Blazer ID	For Nonhuman Primate Users: Date of Confirmation of Negative TB Status
Cristin Gavin	cfgavin	
Jordan Brown	Jbrown24	
Garrett Kaas	gkaas	
Mikael Guzman Karlsson	mkguzman	
Jarrod Meadows	Jpmeado1	
Andrew Kennedy	ajkenn	
Scott Phillips	scottep	
Sarah Strange	Sarahks1	
Jing Wang	Jingwang	
John Lewis	epiphany	

4. Certification

By submission of this form, I certify that the information provided in this Animal Use Request (AUR) completely and accurately describes the work to be performed and all work proposed in the associated grant application, contract application, or study plan.

I further certify that

- **No personnel working under my direction will perform any animal procedures until their experience and training has been reviewed and approved by the IACUC.**
- **I will submit to the IACUC the names and qualifications of new or additional personnel including students and visiting faculty before they become involved in these studies,**
- **I will ensure that all personnel are enrolled in the institutional Occupational Health Program prior to their contact with animals or their entry into the animal facilities.**
- I will comply with the procedures described in the NIH Guide for the Care and Use of Laboratory Animals, the PHS Policy on Humane Care and Use of Laboratory Animals, the USDA Animal Welfare Act Regulations, applicable UAB policies, and Standard Operating Procedures as described by the Animal Resources Program and IACUC.
- I acknowledge responsibility for this project and all faculty, staff and students who participate in it.

I also understand that I must submit a modification to an approved protocol and obtain IACUC approval before I

- **Use additional animal species, increase the number of animals to be used, or increase the number of procedures performed on individual animals.**
- **Change procedures that in any way increase the pain/distress an animal might experience or that might be considered a significant departure from those described in this AUR.**
- **Perform procedures not described in this AUR.**
- **Use or allow to be used for other studies animals purchased, produced, or otherwise acquired for this project.**