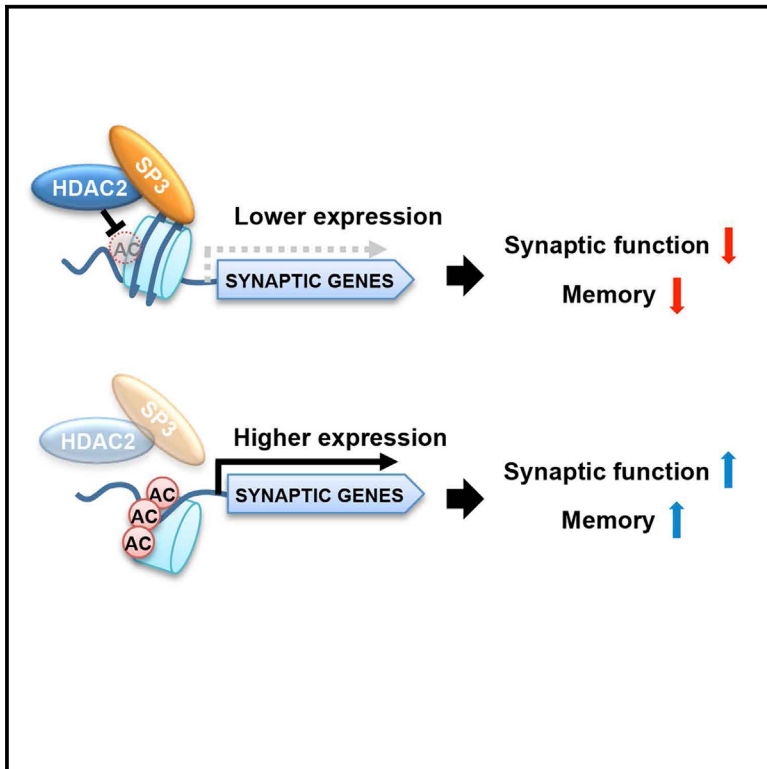


Cell Reports

The Transcription Factor Sp3 Cooperates with HDAC2 to Regulate Synaptic Function and Plasticity in Neurons

Graphical Abstract



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In Brief

Yamakawa et al. demonstrate that Sp3 and HDAC2 interact to repress gene expression and negatively regulate synaptic plasticity. Inhibiting HDAC2-Sp3 binding ameliorates memory impairment in a mouse neurodegeneration model. Targeting the HDAC2-Sp3 complex might be a feasible approach to improve cognitive function without affecting other HDAC-related processes.

Highlights

- Sp3 and HDAC2 form a complex to cooperatively regulate synaptic gene expression
- Sp3 and HDAC2 negatively regulate synaptic function in neurons
- Inhibiting HDAC2-Sp3 interaction ameliorates memory deficit in an AD-like model
- Targeting the HDAC2-Sp3 complex may be a feasible approach for AD therapy

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The Transcription Factor Sp3 Cooperates with HDAC2 to Regulate Synaptic Function and Plasticity in Neurons

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SUMMARY

The histone deacetylase HDAC2, which negatively regulates synaptic gene expression and neuronal plasticity, is upregulated in Alzheimer's disease (AD) patients and mouse models. Therapeutics targeting HDAC2 hold promise for ameliorating AD-related cognitive impairment; however, attempts to generate HDAC2-specific inhibitors have failed. Here, we take an integrative genomics approach to identify proteins that mediate HDAC2 recruitment to synaptic plasticity genes. Functional screening revealed that knockdown of the transcription factor Sp3 phenocopied HDAC2 knockdown and that Sp3 facilitated recruitment of HDAC2 to synaptic genes. Importantly, like HDAC2, Sp3 expression was elevated in AD patients and mouse models, where Sp3 knockdown ameliorated synaptic dysfunction. Furthermore, exogenous expression of an HDAC2 fragment containing the Sp3-binding domain restored synaptic plasticity and memory in a mouse model with severe neurodegeneration. Our findings indicate that targeting the HDAC2-Sp3 complex could enhance cognitive function without affecting HDAC2 function in other processes.

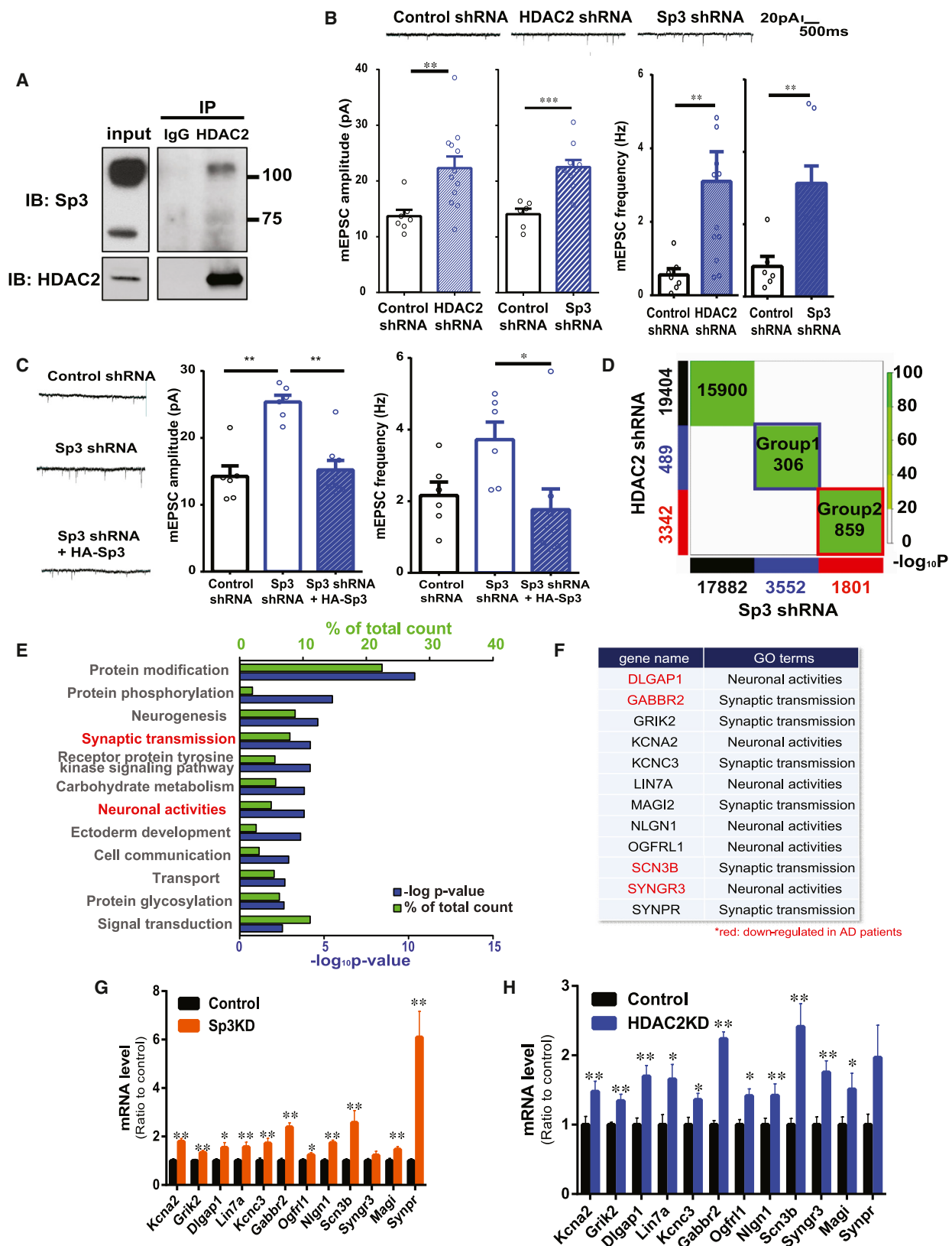
INTRODUCTION

Epigenetic mechanisms, such as DNA and histone modifications, are critical modulators of transcriptional activity regulating diverse biological processes (Jaenisch and Bird, 2003). Histone acetylation favors, while deacetylation antagonizes, gene expression at loci throughout the genome (Eberharter and Becker, 2002). Importantly, dynamic regulation of histone acetylation status is associated with synaptic plasticity and memory, and numerous studies implicate both histone acetyltransferase

(HAT) and histone deacetylase (HDAC) enzymes in various cognitive processes (Alarcón et al., 2004; Fischer et al., 2007; Flavell and Greenberg, 2008; Korzus et al., 2004; Penney and Tsai, 2014). Among histone-modifying enzymes, HDAC2 is a critical negative regulator of structural and functional plasticity in the mammalian nervous system (Guan et al., 2009; Hanson et al., 2013). HDAC2 localizes to the promoters of numerous synaptic-plasticity-associated genes, where it deacetylates histone substrates (Gräff et al., 2012; Guan et al., 2009). Consistently, loss of HDAC2 or HDAC inhibitor treatments promotes synaptic gene expression, long-term synaptic plasticity, and memory processes, while HDAC2 overexpression has opposing effects (Fischer et al., 2007; Gräff et al., 2012, 2014; Guan et al., 2009; Morris et al., 2013).

Altered HDAC function can contribute to numerous pathological states, including cancer, cardiovascular disease, and various forms of neurological dysfunction (Falkenberg and Johnstone, 2014). Notably, HDAC2 levels are elevated in both Alzheimer's disease (AD) patient brains and in multiple mouse models of the disease (Gonzalez-Zuñiga et al., 2014; Gräff et al., 2012). In mouse models of AD, HDAC2 upregulation results from both transcriptional and post-transcriptional mechanisms and demonstrably contributes to cognitive impairment; HDAC inhibitor treatments and direct HDAC2 knockdown both result in striking recovery of impaired cognitive functions (Gonzalez-Zuñiga et al., 2014; Gräff et al., 2012). As such, targeting HDAC2 holds promise as a therapeutic to treat the cognitive symptoms of AD as well as a number of other neurological disorders.

A major hurdle, however, is the lack of specificity of current HDAC inhibitor compounds. These compounds target the deacetylase catalytic domain, and a number of them exhibit selectivity for the class I HDACs (HDACs 1, 2, 3, and 8) over class II, III, and IV enzymes, but HDAC2-specific inhibitors have yet to be reported. This lack of specificity is particularly problematic, given the distinct, and sometimes opposing, functions of the different HDAC enzymes (Dobbin et al., 2013; Kim et al., 2008; Wang et al., 2013). Further complicating matters is the large number of different chromatin-binding complexes that HDAC enzymes can participate in (Bantscheff et al., 2011; Falkenberg and



(legend on next page)

Johnstone, 2014). Indeed, HDAC2 and other HDACs often interact with different binding partners and regulate distinct subsets of genes depending on cell type, developmental stage, and any number of other intrinsic or extrinsic signals (Bantscheff et al., 2011; Falkenberg and Johnstone, 2014). Thus, the essential questions, when targeting HDAC2 for therapeutics, are which HDAC2 complex(es) should be inhibited to enhance cognitive function and whether these are distinguishable from the complexes whose inhibition leads to the adverse side effects of pan-HDAC inhibitors.

In an attempt to address these questions, we have taken an innovative approach to target HDAC2 function: to identify and disrupt its interaction with the DNA-binding proteins(s) responsible for recruitment of HDAC2 to the promoters of synaptic-plasticity-associated genes. Utilizing weighted gene co-expression network analysis (WGCNA), we identified putative HDAC2 co-regulators. Functional screening of the potential co-regulators revealed that knockdown of the transcription factor Sp3 was similar to HDAC2 knockdown in its ability to facilitate synaptic transmission. Consistent with a role in recruitment of HDAC2 to target genes, knockdown of Sp3 was able to reduce HDAC2 occupancy and increase histone acetylation at synaptic gene promoters, as well as facilitating synaptic gene expression. Also, we found that, as with HDAC2, Sp3 expression was elevated in the brain of a mouse model of AD-like neurodegeneration, as well as in AD patients. Importantly, exogenous expression of an HDAC2 fragment containing the Sp3-binding domain was able to counteract the synaptic plasticity and memory defects found in an AD-like mouse model. Together, these findings indicate that HDAC2 and Sp3 cooperate to regulate neuronal plasticity genes and provide proof of principle that disruption of the HDAC2-Sp3 interaction is an effective strategy to disrupt the synaptic-plasticity-suppressing functions of this complex.

RESULTS

Identification of Potential HDAC2 Co-regulators through WGCNA

HDACs, including HDAC2, associate with a number of different chromatin-modifying complexes, each of which regulates multiple processes within cells. To determine which binding partners are essential for HDAC2 recruitment to genes involved in partic-

ular processes, we considered techniques other than classical immunoprecipitation (IP) followed by mass spectrometry (MS). IP-MS would indiscriminately identify all proteins bound to HDAC2 and would be of limited value in pinpointing the specific proteins that mediate recruitment of HDAC2 to genes involved in synaptic plasticity. Due to these caveats, we utilized WGCNA. Under the hypothesis that genes with similar expression patterns often encode for interacting proteins or groups of proteins involved in similar cellular processes, we applied WGCNA to publicly available gene expression data from 187 healthy human postmortem brains. As a pilot study, we first extracted a subset of 28 individuals with “high” *HDAC2* expression (greater than 1 SD above the mean) and 35 with “low” *HDAC2* expression (greater than 1 SD below the mean) and then performed unbiased clustering of global gene expression (Figure S1A). With few exceptions, this analysis reliably distinguished “high” from “low” *HDAC2*-expressing individuals, indicating that a gene expression signature can be associated with *HDAC2* levels.

Next, we tested whether this natural variation in *HDAC2* gene expression could be used to identify the HDAC2-binding partners involved in synaptic plasticity. We, therefore, performed WGCNA on the entire dataset (regardless of *HDAC2* levels) and identified the genes most tightly correlated or anti-correlated with *Hdac2* based on gene expression (Figure S1B). This analysis revealed an *HDAC2*-containing module of 2,282 genes, which included many genes encoding known HDAC2-binding proteins. Based on gene ontology (GO) analysis, the list of potential HDAC2 co-regulators was further narrowed down to transcriptional repressors (as defined by the GO terms “histone deacetylase binding,” “transcription corepressor activity,” “histone deacetylase activity,” and “transcription repressor activity”). Finally, we calculated the pairwise correlation between the transcriptional repressors (including *HDAC2*) and all the genes in the *HDAC2* module to find the putative HDAC2 co-regulators showing the same direction of correlation as HDAC2 (Figure S1C). The consequent list of 22 candidates included several genes encoding HDAC2-binding proteins as previously reported, such as the DNA-binding proteins Sp3, Tdp2, and Sap30 (Madabhushi et al., 2015; Sun et al., 2002; Won et al., 2002; Zhang et al., 1998). The physical interaction of Sp3 and Tdp2 with HDAC2 was confirmed through IP of HDAC2 followed by western blotting using anti-Sp3 and anti-Tdp2 antibodies (Figures 1A and S1D).

Figure 1. Sp3 Regulates Synaptic Function and Synaptic Gene Expression

(A) Representative western blot of co-immunoprecipitation of Sp3 with anti-HDAC2 antibody from mouse cortical tissue.

(B) Representative mEPSC traces (top) and quantifications of mEPSC amplitude and frequency (bottom) from neurons transduced with control shRNA, *Hdac2* shRNA, or Sp3 shRNA (n = 6–12). **p < 0.01; ***p < 0.001 (two-tailed Welch's or Student's t test depending on the result of an F test). Values are means ± SEM. IB, immunoblot; HA, hemagglutinin.

(C) Representative traces of mEPSC amplitude and frequency in neurons transduced with control shRNA, Sp3 shRNA or shRNA-resistant Sp3 combined with Sp3 shRNA (n = 6–8). *p < 0.05; **p < 0.01 (Dunnett's test). Values are means ± SEM.

(D) The matrix is a comparison of differentially expressed genes following *Hdac2* or Sp3 shRNA expression in primary neurons. The p values were calculated using the Fisher's exact test. Genes in black indicate no change in expression, blue indicates decreased expression, and red indicates increased expression following shRNA treatment. *Hdac2* and Sp3 shRNAs both mediate decreased expression of Group 1 genes and increased expression of Group 2 genes.

(E) Gene ontology analysis of genes upregulated by *Hdac2* and Sp3 shRNA using DAVID (Huang et al., 2009a, 2009b).

(F) List of the “synaptic” genes selected for ChIP analysis. Expression of each gene was increased by both *Hdac2* and Sp3 knockdown as well as decreased in CK-p25 mice. The red genes were also decreased in AD patients.

(G and H) qRT-PCR results of the target genes in primary neurons transduced with Sp3 (G) or *Hdac2* (H) shRNAs (n = 3–7). *p < 0.05; **p < 0.01 (one-tailed Student's or Welch's t test). Values are means ± SEM.

See also Figures S2 and S3.

Like HDAC2, Sp3 Negatively Regulates Synaptic Function

HDACs, including HDAC2, cannot directly bind DNA, so we focused our subsequent efforts on HDAC2-interacting proteins that can bind DNA (Sp3, Sap30, and Ttrap/Tdp2). To aid in identifying whether these three proteins could be required for recruitment of HDAC2 to synaptic genes, we next assessed the role of each protein in regulating synaptic function.

We measured miniature excitatory post-synaptic currents (mEPSCs) from cultured mouse primary neurons transduced with shRNA (short hairpin RNA) targeting *Hdac2*, *Sp3*, *Sap30*, or *Ttrap* (transduction with each shRNA resulted in greater than 50% reduction of mRNA; [Figures S2A and S2B](#)). As expected, *Hdac2* knockdown resulted in increased mEPSC amplitude and frequency ([Figure 1B](#)). Interestingly, knockdown of *Sp3* also increased average mEPSC amplitude and frequency ([Figure 1B](#)), while knockdown of *Sap30* or *Ttrap* did not significantly alter either parameter ([Figure S2C](#)). This facilitation of mEPSCs by *Sp3* knockdown was completely reversed by the expression of an shRNA-resistant form of *Sp3*, confirming the specificity of the effect ([Figures 1C and S2D](#)).

Sp3 Represses the Expression of Synaptic Genes via the Recruitment of HDAC2

Since *Sp3* binds to HDAC2, and depletion of *Sp3* from mouse primary neurons recapitulated the effect of HDAC2 knockdown on mEPSCs, we next determined whether *Sp3* and HDAC2 co-regulate synaptic gene expression in neurons. To do so, we performed transcriptomic analysis through RNA sequencing (RNA-seq) from primary neurons transduced with control, *Hdac2*, or *Sp3* shRNA (with >50% reduction of each protein; [Figures S3A–S3D](#)). Supporting our hypothesis that HDAC2 and *Sp3* are functionally similar, we found a statistically significant overlap of genes altered by knockdown of *Hdac2* or *Sp3* ([Figure 1D](#)) (see [Supplemental Experimental Procedures](#)). Intriguingly, genes involved in synaptic transmission and neuronal activities were significantly enriched among the genes upregulated after knockdown of either *Hdac2* or *Sp3* ([Figure 1E](#)). A number of these changes in gene expression were validated by qRT-PCR, including changes in the expression of subunits of potassium channels, sodium channels, and synaptic membrane proteins and receptors ([Figures 1F–1H](#)).

To examine whether the expression of genes co-regulated by HDAC2 and *Sp3* is changed under pathological conditions, we compared the overlapping genes altered by *Hdac2* or *Sp3* knockdown with the genes dysregulated in the CK-p25 mouse model of neurodegeneration, which displays elevated levels of HDAC2 in the hippocampus (Gräff et al., 2012). In addition, these mice exhibit memory deficits and several AD-related pathologies, such as neuronal loss, Tau hyperphosphorylation/aggregation, increased amyloid load, and reduced synaptic density, following a 6-week induction of p25 by withdrawing doxycycline (Cruz et al., 2006; Fischer et al., 2005). p25, a truncated version of p35, is an activator of cyclin-dependent kinase 5 (CDK5) and is implicated in AD (Cruz et al., 2003; Sundaram et al., 2013; Wen et al., 2008). Inhibition of p25 generation was recently shown to prevent the expression of AD phenotypes in AD model mice, supporting the notion that p25 accumulation can be a trigger of AD

(Seo et al., 2014). Accordingly, gene expression and epigenomic signatures of the CK-p25 mouse after p25 induction correlate with those of human AD patients (Gjoneska et al., 2015). Interestingly, genes upregulated by *Hdac2* or *Sp3* knockdown showed significant overlap with genes downregulated in CK-p25 mice (Gjoneska et al., 2015) ([Figure S3E](#)) (see [Supplemental Experimental Procedures](#)), as well as genes downregulated in the brains of AD patients (Liang et al., 2008) ([Table 1](#); [Table S1](#)). Specifically, synaptic genes like *Dlgap1*, *Gabbr2*, *Scn3b*, and *Synpr* are downregulated in both CK-p25 mice and AD patients and negatively co-regulated by HDAC2 and *Sp3*. Overall, our genome-wide expression analysis provides evidence that *Sp3* and HDAC2 negatively regulate the expression of an overlapping set of genes related to synaptic function.

Taken together, our findings support the notion that the DNA-binding protein, *Sp3*, may serve to recruit HDAC2 to the promoters of genes involved in synaptic function. To address this hypothesis, we utilized chromatin immunoprecipitation (ChIP) followed by qPCR (ChIP-qPCR) to determine whether HDAC2 and *Sp3* directly bind to the promoters of synaptic genes that were upregulated after *Hdac2* or *Sp3* knockdown ([Figure 1F](#)). Primer pairs were designed to amplify regions of the promoter both upstream and downstream of the transcription start site (TSS). Additional primers amplify regions roughly 4 kb downstream of the transcriptional start site and serve as negative controls for HDAC2 and *Sp3* enrichment, as these proteins have previously been shown to localize to promoter regions (Wang et al., 2009; Yu et al., 2003). Due to our interest in the role of HDAC2 and *Sp3* at the promoters of synaptic genes and in neuronal function, we sought to isolate and directly probe neurons from the mouse brain. Isolation of neuronal nuclei was achieved through staining for the neuronal marker NeuN, followed by fluorescence-activated cell sorting (FACS) to separate NeuN[−] glial populations from NeuN⁺ neurons ([Figures 2A and S4A](#)). ChIP-qPCR using chromatin derived from cortical neuronal (NeuN⁺) nuclei of wild-type mice with anti-HDAC2 and anti-*Sp3* antibodies demonstrated that HDAC2 and *Sp3* often colocalize at the promoters of synaptic genes, with clear enrichment relative to the immunoglobulin G (IgG) control ([Figures 2B and 2C](#)). Additionally, we determined the enrichment of HDAC2 and *Sp3* across the promoters of control genes defined by RNA-seq. For control genes, we chose *Cd81*, whose expression was increased only by HDAC2 knockdown (KD) but not *Sp3* KD; *Mkln1* and *Fam171b*, which were increased by *Sp3* KD but not HDAC2 KD; and *Tanc2* and *Engase*, which were not changed by either HDAC2 KD or *Sp3* KD. In ChIP-qPCR experiments using NeuN⁺ nuclei derived from hippocampal tissue, the enrichment and distribution of HDAC2 and *Sp3* at synaptic gene promoters were similar to those observed in cortical neurons, suggesting that this phenomenon is conserved across brain regions ([Figures S4B–S4F](#)).

Next, we wanted to test whether *Sp3* mediates HDAC2 recruitment to the promoters of synaptic genes co-regulated by *Sp3* and HDAC2. To address this question, we examined the effect of *Sp3* knockdown on HDAC2 enrichment at synaptic gene promoters in primary neurons. Interestingly, ChIP experiments revealed that knockdown of *Sp3* alone was sufficient to significantly reduce HDAC2 recruitment to the promoters of these

Table 1. Enrichment of Genes Upregulated by HDAC2/Sp3 Knockdown for Terms in the Chemical and Genetic Perturbations (CGP) Database

Gene Set Name	Description	FDR q Value
BLALOCK_ALZHEIMERS_DISEASE_DN	genes downregulated in brain from patients with AD	8.39E-30
GRAESSMANN_APOPTOSIS_BY_DOXORUBICIN_DN	genes downregulated in ME-A cells (breast cancer) undergoing apoptosis in response to doxorubicin (PubChem: 31703).	8.87E-28
GOBERT_OLIGODENDROCYTE_DIFFERENTIATION_DN	genes downregulated during differentiation of Oli-Neu cells (oligodendroglial precursor) in response to PD174265 (PubChem: 4709)	4.83E-26
NUYTEN_EZH2_TARGETS_UP	genes upregulated in PC3 cells (prostate cancer) after knockdown of EZH2 (GeneID: 2146) by RNAi	1.64E-24
WONG_ADULT_TISSUE_STEM_MODULE	the “adult tissue stem” module: genes coordinately upregulated in a compendium of adult tissue stem cells	1.97E-21
SCHAEFFER_PROSTATE_DEVELOPMENT_48HR_DN	genes downregulated in the urogenital sinus (UGS) of E16 (embryonic day 16) females exposed to the androgen dihydrotestosterone (PubChem: 10635) for 48 hr	6.76E-21
GEORGES_TARGETS_OF_MIR192_AND_MIR215	genes downregulated in HCT116 cells (colon cancer) by expression of MIR192 or MIR215 (GeneID: 406967 and 406997) at 24 hr	3.06E-20
BENPORATH_SUZ12_TARGETS	set “Suz12 targets”: genes identified by ChIP on chip as targets of the Polycomb protein SUZ12 (GeneID: 23512) in human embryonic stem cells	9.83E-19
PEREZ_TP53_TARGETS	genes upregulated in the HMECs (primary mammary epithelium) upon expression of TP53 (GeneID: 7157) off the adenoviral vector	2.60E-18
YOSHIMURA_MAPK8_TARGETS_UP	genes upregulated in vascular smooth muscle cells (VSMC) by MAPK8 (JNK1) (GeneID: 5599)	4.12E-18

These data are available at <http://www.broadinstitute.org/gsea/msigdb/annotate.jsp>. FDR, false discovery rate; HMECs, human mammary epithelial cells.

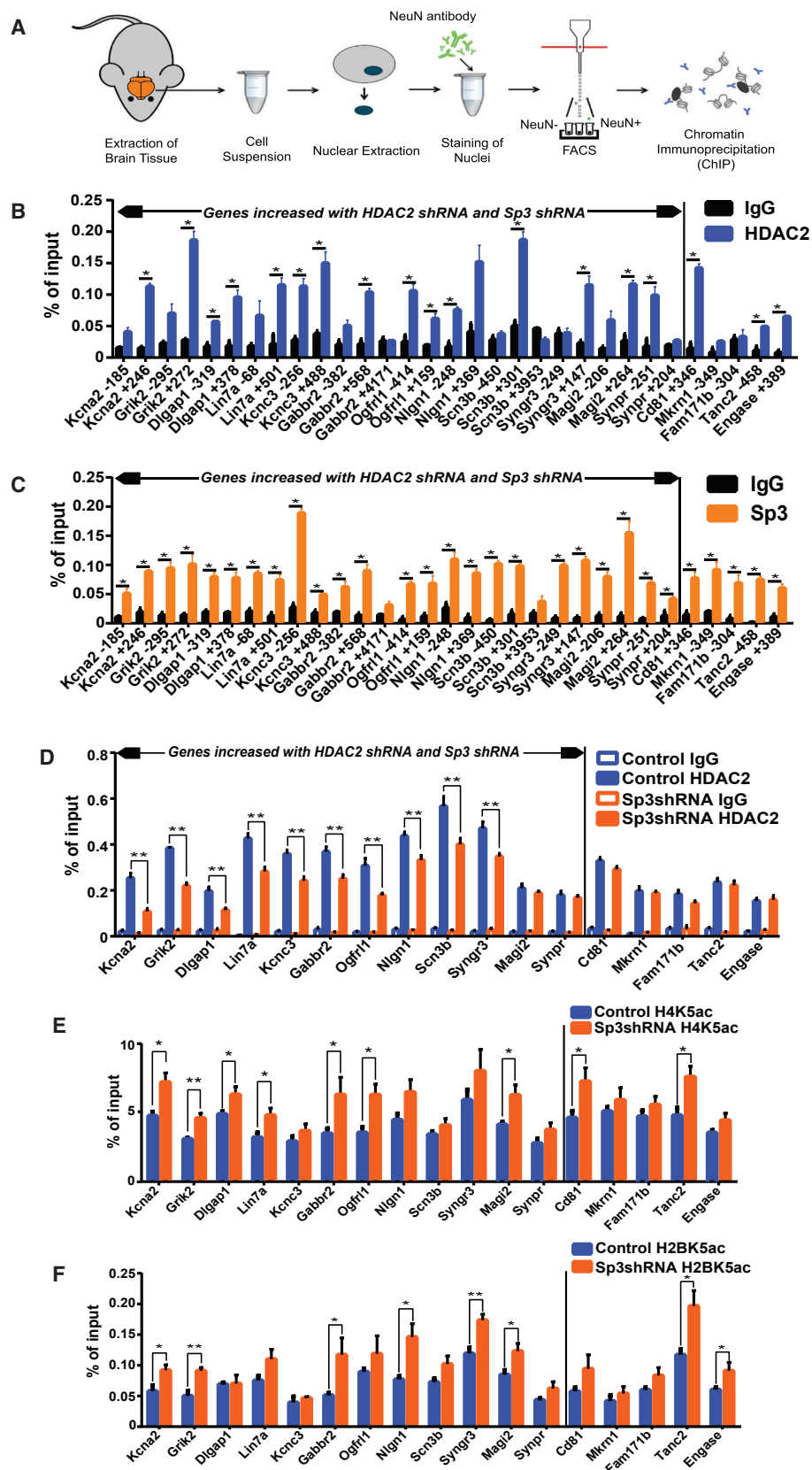
genes (Figure 2D). Importantly, HDAC2 enrichment at control genes (*Cd81*, *Mkln1*, *Fam171b*, *Tanc2*, and *Engase*) was not affected by loss of Sp3 (Figure 2D). We also tested whether histone acetylation at co-regulated synaptic gene promoters was altered by Sp3 knockdown, as would be expected if HDAC2 recruitment to these sites were reduced. Indeed, the decrease in HDAC2 binding due to knockdown of Sp3 was accompanied by increased histone H4 lysine 5 acetylation (H4K5ac) or histone H2B lysine 5 acetylation (H2BK5ac) at the promoters of several genes, including *Kcna2*, *Grik2*, *Dlgap1*, *Lin7a*, *Gabbr2*, *Ogfr1*, *Nlgn1*, *Syngn3*, and *Magi2* (Figures 2E and 2F). These findings are consistent with the idea that Sp3 recruits HDAC2 to the promoters of synaptic genes, where HDAC2 then mediates the deacetylation of histones to regulate gene expression.

Expression of HDAC2 and Sp3 Are Deregulated in AD

Our gene expression profiling indicated that HDAC2 and Sp3 co-regulate a subset of synaptic genes, many of which are also deregulated in the context of AD pathology. These observations, together with our earlier findings that HDAC2 protein levels were increased in AD patients and mouse models of neurodegeneration, prompted us to test whether Sp3 expression might also be upregulated in AD. First, we examined published gene expression data collected from hippocampal CA1 pyramidal neurons from 13 healthy controls and 10 AD patients (Liang et al., 2008) and found significant increases in the expression of both HDAC2 and Sp3 in AD patients (Figures 3A and 3B; see Supplemental Information for the sample information).

Furthermore, we applied WGCNA to the dataset to investigate the alteration of gene expression networks in AD patients. We observed that, even in this dataset combining healthy controls and AD patients, HDAC2 and Sp3 again segregate into the same gene expression module (Figure 3C). Moreover, the expression of genes in the HDAC2/Sp3 module was higher in AD patients, compared with controls, and negatively correlated with the expression of genes in the module most enriched for synaptic function (Figures 3D and 3E).

Next, we examined Sp3 levels in CK-p25 mice. We previously reported, and validate here, that the expression of HDAC2 is elevated in the cortex and the hippocampus of the 6-week-induced CK-p25 mice (Gräff et al., 2012) (Figures S5A and S5B). Interestingly, Sp3 protein levels were also elevated in the cortex (Figure 4A) and hippocampus (Figure S5B) of the 6-week-induced CK-p25 mice. Similarly, the complex of HDAC2 and Sp3, as assessed by co-immunoprecipitation with an anti-HDAC2 antibody, was increased in the CK-p25 mouse (Figures 4B and S5C). Further, we also assessed the levels of HDAC2 and Sp3 bound to the promoters of synaptic genes downregulated in 6-week-induced CK-p25 mice. Consistent with the notion that the HDAC2-Sp3 complex antagonizes synaptic gene expression in these mice, we found increased HDAC2 and Sp3 binding at many of these loci in CK-p25 NeuN+ neuronal nuclei, compared to the CK control (Figures 4C, 4D, and S5D). Importantly, this occurs concomitantly with a decrease in the expression of genes associated with learning, memory, and synaptic plasticity (Figure S5E).



(legend on next page)

To more directly test the importance of elevated Sp3 levels to AD-related pathology, we expressed a shRNA targeting *Sp3* in the hippocampus of CK-p25 mice (Figures S6A–S6C). We previously performed similar experiments to show that expression of an *Hdac2* shRNA to normalize HDAC2 levels in CK-p25 mice was sufficient to reverse deficits in long-term synaptic plasticity (Gräff et al., 2012). While long-term potentiation (LTP) in the CA3-CA1 Schaffer collateral pathway was severely impaired in CK-p25 mice injected with control shRNA, CK-p25 mice injected with Sp3 shRNA showed robust LTP, comparable to that in control mice (Figure 4E). Sp3 knockdown did not significantly affect basal synaptic transmission in CK-p25 mice (Figure S6D). Taken together, we show that both HDAC2 and Sp3 are upregulated in CK-p25 model mice and postmortem AD hippocampal tissue. Further, we find that, like HDAC2, downregulation of Sp3 expression ameliorated deficits in synaptic plasticity in CK-p25 mice.

Inhibiting the HDAC2-Sp3 Complex Enhances Synaptic Function

From the aforementioned data, it appears that Sp3 plays a key role in the recruitment of HDAC2 to the promoters of synaptic genes and that this mechanism is deregulated in AD. Unlike HDAC2, HDAC1 does not repress synaptic gene expression and cognitive function, although the two proteins share 80% amino acid homology, with the greatest divergence at the carboxyl terminus (C terminus) (Guan et al., 2009; Hanson et al., 2013; Morris et al., 2013). Instead, loss of HDAC1 results in double-stranded DNA breaks, aberrant reentry into the cell cycle, and neuronal death, and HDAC1 gain of function is neuroprotective (Dobbin et al., 2013; Kim et al., 2008; Wang et al., 2013). To further characterize the HDAC2-Sp3 interaction, we mapped the region of HDAC2 involved in regulating synaptic functions and binding to Sp3. We generated three chimeras of HDAC2 and the closely related HDAC1, each of which contains the highly conserved HDAC2 catalytic domain and nuclear localization signal (Figure 5A). For chimera A, the amino terminus of HDAC2 (amino acids 1–121) was replaced with that of HDAC1 (amino acids 1–120). A middle domain of HDAC2 (amino acids 227–357) has been replaced with that of HDAC1 (amino acids 226–356) in chimera B. In chimera C, the divergent C terminus of HDAC2 (amino acids 391–488) has been replaced with that of HDAC1 (amino acids 390–482). Each of these chimeras were expressed in cultured primary neurons, and levels of expression were determined using primers against *HDAC2* (blue arrow) and *HDAC1* (red arrow) across the regions annotated in Figure 5A. After the knockdown of *Hdac2* in cultured neurons, we find that only chimera B expresses the middle portion of HDAC1 at the same level as full-length HDAC1 (Figure 5B). Furthermore, chimeras A, B, and C express a region of HDAC2 between amino acids 120 and 226 at similar levels, un-

like full-length HDAC1, suggesting that any differential effects seen in subsequent experiments are not due to variable expression of the constructs (Figures 5B and 5C). In addition, we examined cell viability and histone deacetylase activities attributable to each chimeric protein following overexpression in HEK293 cells and found no significant differences compared to each other or full-length HDAC2 (Figures S7A and S7B). Each construct was then tested for its ability to dampen the increased mEPSC amplitudes caused by *Hdac2* knockdown in cultured primary neurons. Notably, expression of full-length HDAC1 or chimera C (HDAC2 with the C terminus of HDAC1) did not counteract the effect of *Hdac2* knockdown on mEPSCs (Figures 5D and 5E). In contrast, chimeras A and B, as well as full-length HDAC2, did significantly rescue *Hdac2* knockdown (Figures 5D and 5E). These data suggest that the divergent C terminus of HDAC2 is critical for regulating synaptic function.

These results prompted us to test whether the divergent C terminus of HDAC2 alone is capable of binding to Sp3. If so, we could potentially block the HDAC2-Sp3 interaction through overexpression of this domain. To test this, the C-terminal domain of either HDAC2 (termed 2C) or HDAC1 (termed 1C) fused with mCherry, or mCherry alone, was transfected into neuronal N2A cells. We found, using co-immunoprecipitation experiments, that 2C, but not 1C or mCherry alone, robustly bound to endogenous Sp3 (Figure 6A). Importantly, we did not detect binding of 2C to Sin3A, a well-characterized partner of the HDAC1/2 complexes that controls cell-cycle progression, suggesting that Sin3A binds to a different region of HDAC2 (Heideman et al., 2014).

Next, we examined whether synaptic function was affected by the expression of 2C. We found that expression of 2C in cultured primary neurons facilitated mEPSC amplitude and frequency reminiscent of either *Hdac2* or *Sp3* knockdown (Figure 6B). We also tested whether recruitment of HDAC2 to synaptic genes was perturbed by expression of 2C as it was by knockdown of *Sp3* (Figure 2D). Consistently, we found that HDAC2 enrichment at the promoters of genes involved in synaptic transmission was significantly reduced after the expression of 2C (Figure 6C). Further, we observed increased expression of the majority of synaptic genes tested after the expression of 2C (Figure 6D). This increase in gene expression occurred concomitantly with an increase in H4K5ac (Figure 6E) and, to a lesser extent, H2BK5ac at the promoters of these genes (Figure 6F). Together, these data indicate that overexpression of the C-terminal domain of HDAC2 mimics the effects of *Hdac2* and *Sp3* knockdown on synaptic function, gene expression, and HDAC2 localization across DNA, possibly through the eviction of HDAC2 from the relevant genomic loci.

Next, we evaluated whether inhibition of HDAC2 recruitment to the promoters of synaptic genes via overexpression of 2C

Figure 2. Sp3 Knockdown Decreases HDAC2 Recruitment to Target Genes

(A) Schematic of neuronal sorting for ChIP.

(B and C) ChIP-qPCR results of HDAC2 (B) and Sp3 (C) at the promoters of potential target genes and control genes identified by RNA-seq in neurons sorted from mouse cortices (n = 3). The locations of the amplified regions relative to each genes transcription start site are indicated. Values are means ± SEM.

(D–F) ChIP-qPCR results of HDAC2 (D), H4K5ac (E), H2BK5ac (F) at the promoters of the target genes in primary neurons transduced with *Sp3* shRNA or control virus (n = 3). *p < 0.05; **p < 0.01 (Dunnett's test). Values are means ± SEM.

See also Figure S4.

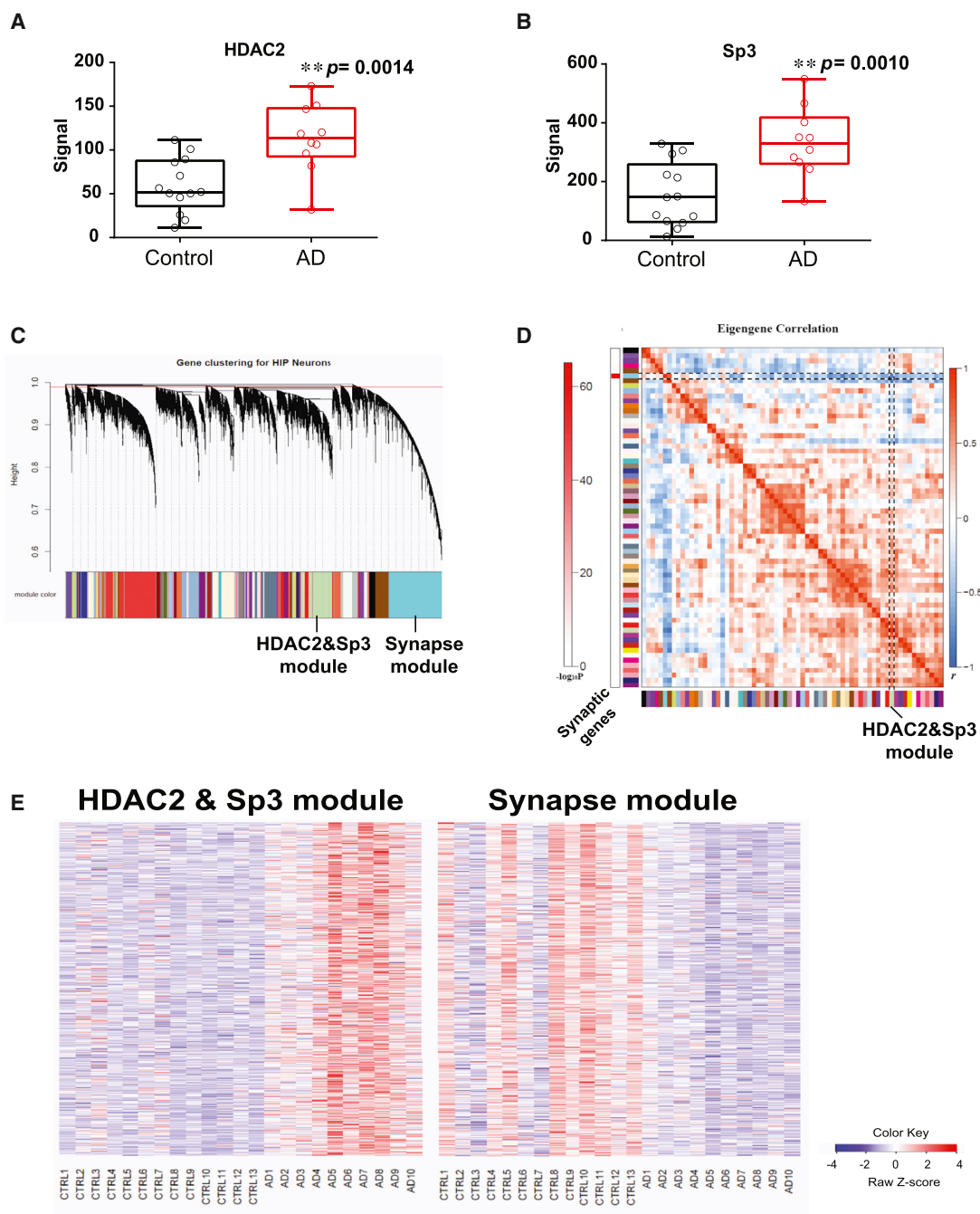


Figure 3. HDAC2 and Sp3 Expression Is Elevated in AD Patients and Anti-correlated with Synaptic Gene Expression

(A and B) mRNA levels of *HDAC2* (A) and *Sp3* (B) in postmortem hippocampal CA1 tissue from 13 healthy controls and 10 AD patients. $**p < 0.01$ (two-tailed Student's *t* test). Values are means $\pm$ SEM.

(C) Gene dendrogram and co-expression modules generated from the dataset of 13 control and 10 AD patients. HIP, hippocampus.

(D) The correlation matrix of the expression of eigengenes from the identified modules to compare relationships between modules. Each eigengene is the gene that best represents the standardized expression data for a given module. The module where synaptic genes are most significantly enriched is considered the "synapse module," while the "HDAC2&Sp3 module" contains both *HDAC2* and *Sp3*. Synaptic genes were defined by SynSysNet. Expression of the eigengene representing the synapse module is anti-correlated with expression of the eigengene representing the HDAC2/Sp3 module (as highlighted with black dotted lines). The left red-white scale indicates the statistical $-\log_{10}p$ value for the enrichment of synaptic genes, which was generated by Fisher's exact test in R. The right red-blue scale indicates the r value, the correlation coefficient between two eigengenes.

(E) Heatmaps of expression levels of genes in the HDAC2&Sp3 module (left) and the synapse module (right). The 13 columns to the left of each heatmap are from control cases; the ten columns to the right are from AD patients.

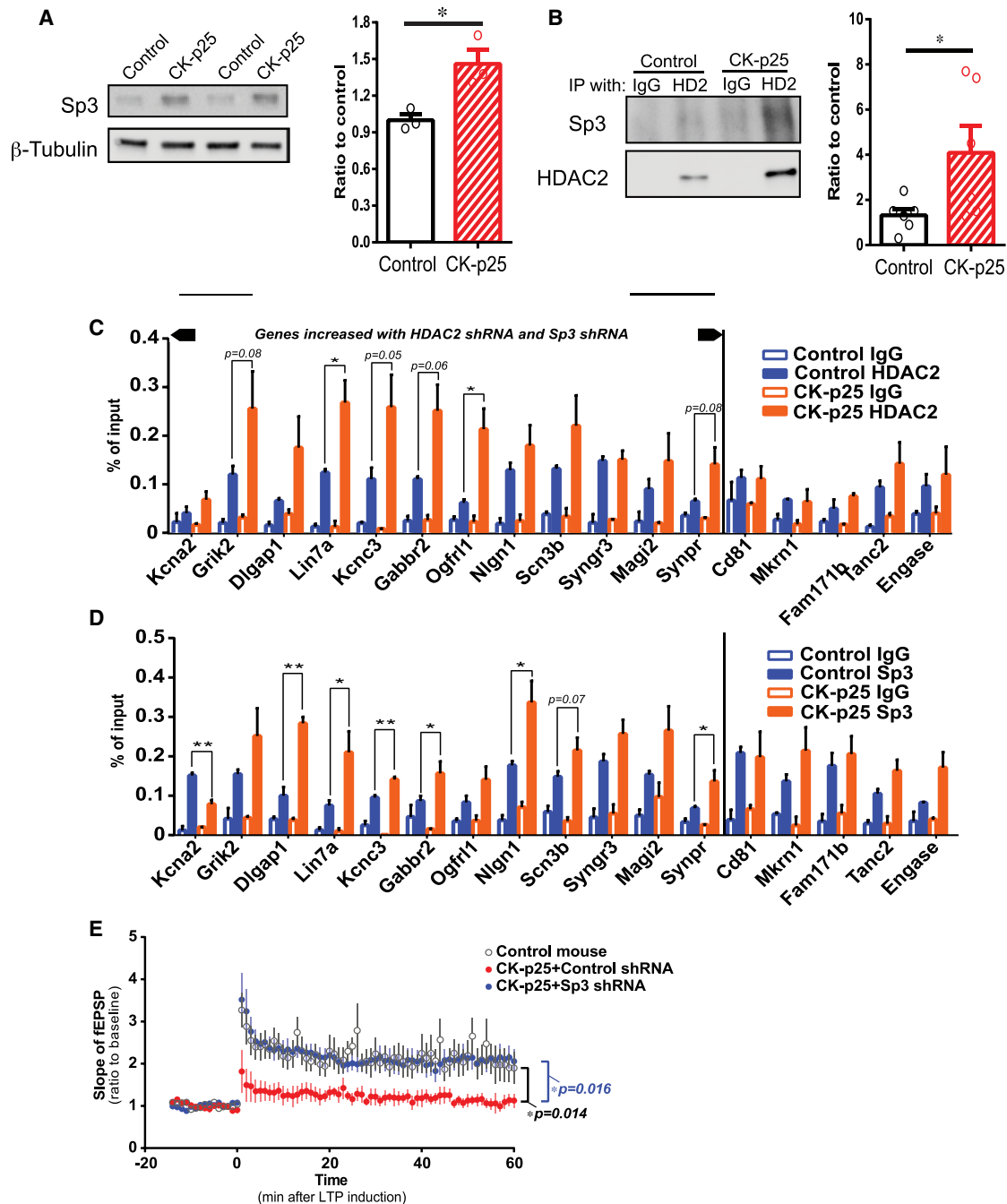


Figure 4. Elevated Levels of Sp3 and HDAC2 Impair Synaptic Plasticity in CK-p25 Mice

(A) Representative western blot images and quantification of Sp3 from the cortex of control and CK-p25 mice ($n = 3$). The quantifications were done after normalizing to β -tubulin. $*p < 0.05$ (two-tailed Student's t test). Values are means $\pm$ SEM.

(B) Representative immunoblots and quantifications of Sp3 co-immunoprecipitated with HDAC2 from cortical tissues from control and CK-p25 mice ($n = 6$). IP was performed with anti-HDAC2 antibody (ab12169) or mouse IgG (negative control). $*p < 0.05$ (one-tailed Student's t test). Values are means $\pm$ SEM.

(C and D) ChIP-qPCR for HDAC2 (C) and Sp3 (D) at the promoters of their target genes and control genes in neurons sorted from cortex of control and CK-p25 mice ($n = 3$). $*p < 0.05$; $**p < 0.01$ (Dunnett's test). Values are means $\pm$ SEM.

(E) Field excitatory postsynaptic potential (fEPSP) slopes in hippocampal area CA1 of control and CK-p25 mice injected with control or Sp3 shRNAs. Slopes were normalized by the average of slopes before $2 \times$ theta-burst stimulation (TBS) ($n = 5$ – 9 slices). $*p < 0.05$ (repeated-measurement two-way ANOVA). Values are means $\pm$ SEM.

See also Figures S5 and S6.

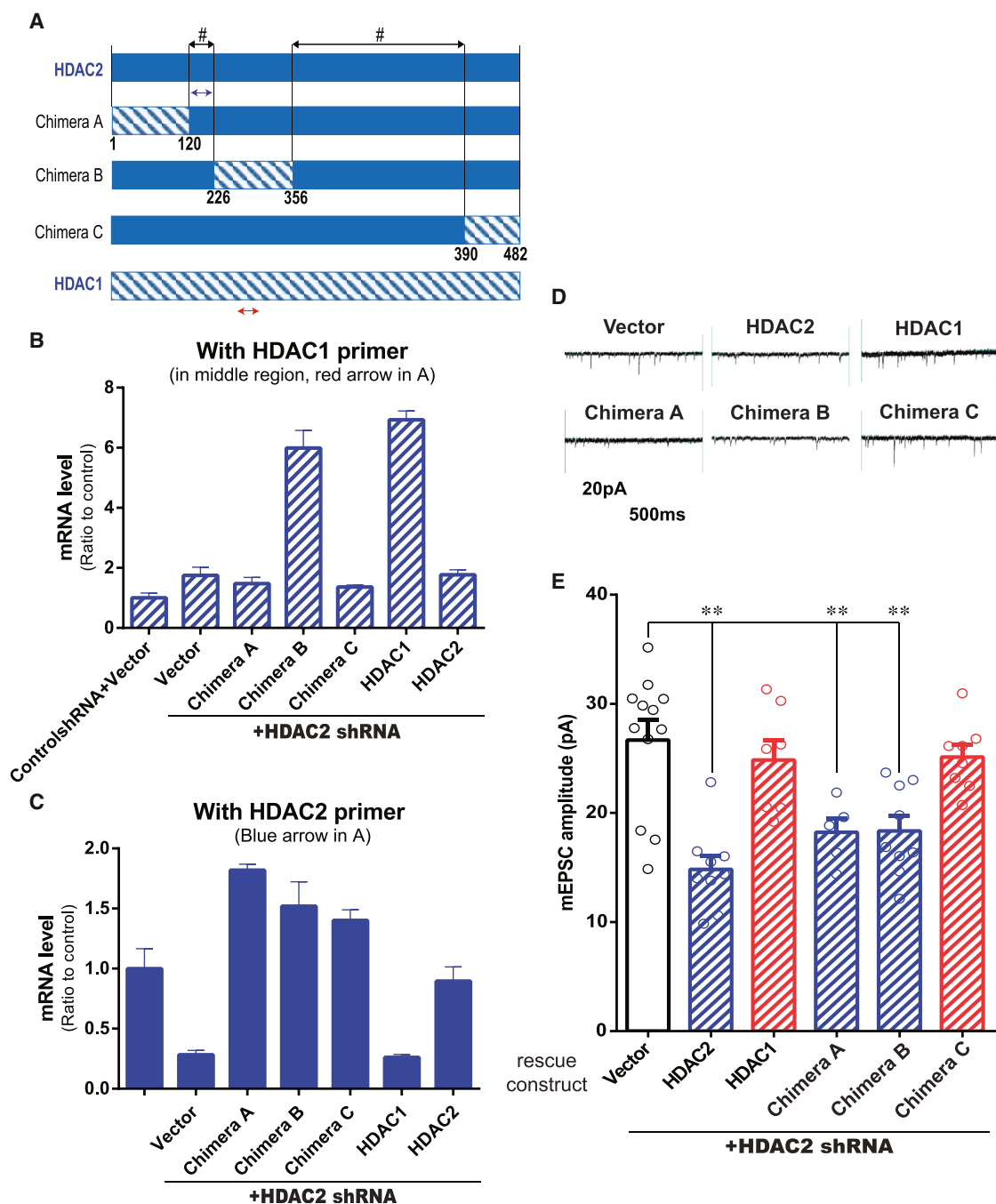


Figure 5. The C-Terminal region of HDAC2 Is Critical for Regulation of Synaptic Function

(A) Diagram of the HDAC2 and HDAC1 chimera constructs. The regions labeled with a pound sign (#) are identical between HDAC1 and HDAC2. The regions filled with light blue are from HDAC2, and the ones with blue stripes are from HDAC1. Red and blue two-way arrows indicate qPCR primer amplicons used in (B) and (C) for HDAC1 and HDAC2, respectively.

(B and C) qRT-PCR using primers detecting HDAC1 (B) or HDAC2 (C) from primary neurons transduced with the indicated constructs.

(D) Representative mEPSC traces corresponding to the conditions shown in (E).

(E) The amplitude of mEPSCs following rescue of HDAC2-knockdown neurons with the indicated constructs ($n = 5-12$). Red-shaded and blue-shaded columns indicate no rescue and significant rescue, respectively. $**p < 0.01$ (Dunnett's test). Values are means $\pm$ SEM.

Values are means $\pm$ SEM.

affects cell proliferation. Currently available pan-HDAC inhibitors block cell-cycle progression, which could elicit undesirable effects (Heideman et al., 2014; Siegel et al., 2009; Wilting et al., 2010). We, therefore, tested whether proliferation of mouse embryonic fibroblasts (MEFs) was affected by overexpression of 2C. While the rate of proliferation in MEFs was significantly decreased by simultaneous knockdown of *Hdac1* and *Hdac2*, we found no effect of 2C expression on proliferation, compared to mCherry controls (Figure S7C). These results suggest that targeting the C-terminal domain of HDAC2 enabled us to selectively manipulate synaptic function while avoiding deleterious effects on cell proliferation.

As a validation of the therapeutic potential of targeting the HDAC2-Sp3 complex through the expression of 2C, we tested the effects of 2C expression on CA3-CA1 Schaffer collateral LTP and memory function, using the CK-p25 model of neurodegeneration. Lentiviral expression of 2C, but not control virus, in the hippocampus of the CK-p25 mouse had no effect on basal synaptic transmission but enhanced LTP in these mice (Figures 6G and S7D). Hippocampus-dependent memory formation, as evaluated by contextual and cued fear-conditioning assays, is also markedly impaired in the 6-week-induced CK-p25 mouse. Importantly, overexpression of 2C in the hippocampus was able to ameliorate both context-dependent and cued fear learning deficits (Figures 6H and S7E). Thus, overexpression of 2C can counteract synaptic and cognitive deficits in a mouse model of neurodegeneration. Finally, we tested whether the behavioral, synaptic functional, and epigenetic changes we describe in response to 2C or *Sp3* shRNA expression in CK-p25 mice are paralleled by gene expression alterations. For this analysis, we extracted hippocampal RNA from control and CK-25 mice as well as from CK-p25 mice infected with 2C or *Sp3* shRNA-expressing viruses. We found that most synaptic genes examined exhibit reduced expression in CK-p25 mice relative to controls and that many of these changes were abrogated by the expression of 2C or *Sp3* shRNA (Figure 6I). Taken together, our findings indicate that targeting the C terminus of HDAC2 constitutes a plausible and specific strategy to inhibit the HDAC2-Sp3 complex and treat neurological disorders associated with memory impairment.

DISCUSSION

Although HDAC2-specific inhibition poses a potential avenue for treating AD, none of the currently available HDAC inhibitors are selective against HDAC2, due to the high conservation of active sites among mammalian HDAC isoforms (West and Johnstone, 2014). This lack of specificity is problematic, considering the diverse functions of HDAC enzymes throughout the body. Indeed, HDAC inhibition can be deleterious, depending on the enzyme, tissue, or specific context. For example, during hematopoiesis, loss or inhibition of HDAC1 and HDAC2 leads to defects in differentiation and thrombocytopenia (Dannenberg et al., 2005; Heideman et al., 2014; Wilting et al., 2010). However, in the context of neuronal function, HDAC2 appears to play a specific and critical role in regulating synaptic gene expression and cognitive processes. Therefore, in this study, we attempted

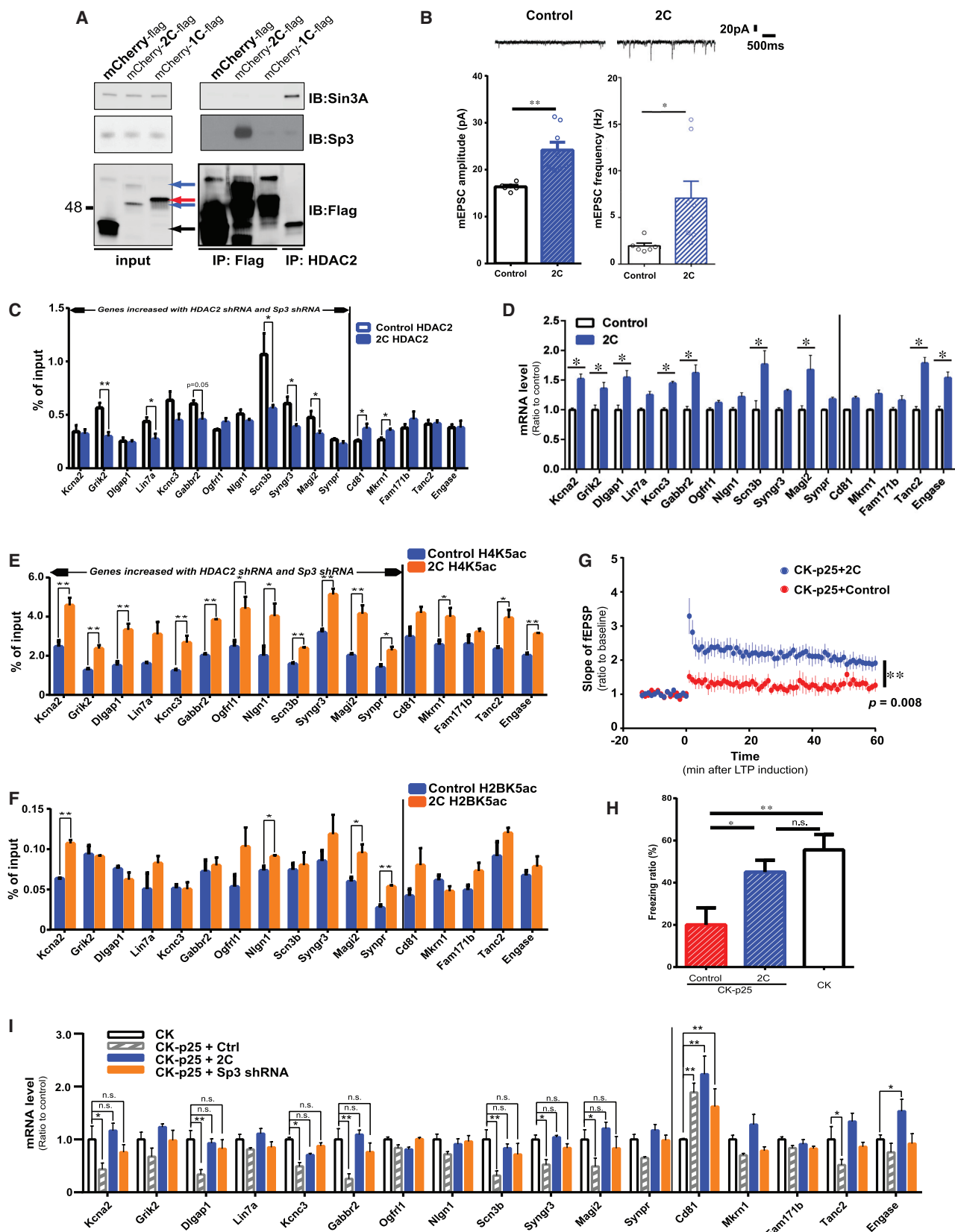
to identify the key members of the HDAC2 complex that control synaptic gene expression. Targeting specific proteins within this complex would provide an avenue for relieving HDAC2-mediated repression of neuronal genes during neurodegeneration while sparing the essential role of HDAC2 in other functions and pathways.

Rather than traditional IP-MS-based proteomic screening methods, we utilized an integrated genomics approach to identify HDAC2 partners essential for regulating synaptic plasticity. This approach incorporated WGCNA, a method previously used to identify signaling pathways altered in disease conditions or following compound treatments (Hawrylycz et al., 2012; Horvath et al., 2006). Here, we succeeded in broadening its application to identify potential co-regulators of HDAC2. The consequent list of genes included several known HDAC2-binding proteins, including TDP2 and Sap30 (Eom et al., 2014; Tong et al., 1998; Wu et al., 2001), as well as Sp3. Importantly, the strongly correlated expression of Sp3 and HDAC2 was clearly observed across datasets, indicating the robustness of the association (Figures 3C, 3E, and S1B, and S1C). Critically, the potential co-regulators identified by our *in silico* analysis were interrogated using biological assays to validate their functional significance. This interplay of bioinformatic network and functional analyses provides a powerful methodology that could be applied to other epigenetic regulators and tissue types.

The roles of Sp3 in regulating synaptic plasticity and cognitive function have been elusive, although Sp3-mediated gene regulation has been previously reported in neurons. In the context of AD, there are a handful of reports addressing altered Sp3 expression in the presence of phosphorylated tau or oxidative stress (Boutillier et al., 2007; Ross et al., 2002; Ryu et al., 2003). Our discovery of the crucial role that Sp3 plays in regulating synaptic plasticity and cognitive function was highly unexpected. Indeed, Sp3 appears to be an important negative regulator of synaptic gene expression and synaptic function that likely also plays an important role in cognitive decline in AD patients.

In addition to increased HDAC2 and Sp3 protein in CK-p25 mice, we also found that mRNA levels of the two genes were increased in postmortem hippocampal tissues of AD patients, indicating their transcriptional upregulation under pathological conditions. HDAC2 is induced by stresses such as A β and H₂O₂ through the activation of glucocorticoid receptor (GR) (Gräff et al., 2012). Likewise, Sp3 was shown to be increased by oxidative stress (Ryu et al., 2003), and, intriguingly, the promoter of Sp3 contains putative glucocorticoid response elements between –875 and –858 from its transcription start site (as predicted by JASPAR; http://jaspar.genereg.net/cgi-bin/jaspar_db.pl?rm=browse&db=core&tax_group=vertebrates), suggesting the possibility that HDAC2 and Sp3 are co-regulated by common stress response pathways. This possibility is consistent with the tight correlation of Sp3 and HDAC2 expression we observed across WGCNA datasets.

Moreover, we identified Sp3 as an important DNA-binding protein for HDAC2 recruitment to “synaptic” gene loci. However, the knockdown of Sp3 did not completely eliminate HDAC2 localization to chromatin. This implies that other HDAC2-interacting proteins are important for HDAC2 recruitment across



(legend on next page)

the genome. Notably, along with an upregulation of gene expression, histone acetylation was substantially increased by the partial reduction of HDAC2 at the promoters of several synaptic genes following Sp3 knockdown. Even at promoters without clear increases in acetyl H2B or H4, acetylation of other histone residues not tested here might be increased. Importantly, Sp3 knockdown reversed the impairment of synaptic plasticity in CK-p25 mice, consistent with our previous finding that partial reduction of HDAC2 levels is sufficient to reverse synaptic and cognitive deficits in these same mice (Gräff et al., 2012).

Lastly, by generating chimeras of HDAC2 and the closely related HDAC1, whose inhibition has no effect on synaptic plasticity (Guan et al., 2009; Hanson et al., 2013; Morris et al., 2013), we demonstrated that 2C binds to Sp3 and is necessary for HDAC2 to negatively regulate synaptic function. Similar to Sp3 knockdown, 2C expression led to a mild reduction of HDAC2 occupancy across the promoters of synaptic genes, which was sufficient to reverse the impairment of synaptic plasticity and cognitive function in CK-p25 mice. Again, this is consistent with our previous finding that the partial knockdown of HDAC2 was able to ameliorate hippocampus-dependent memory impairment (Gräff et al., 2012). In addition, 2C was found to bind to Sp3, but not Sin3A, an HDAC2 complex protein that plays a crucial role in cell-cycle progression in proliferative cells (Heideman et al., 2014). Together with our observation that MEF proliferation was not affected by 2C expression, these findings suggest that HDAC2-Sin3A and HDAC2-Sp3 complexes are distinct and that the latter can be selectively inhibited by administration of the HDAC2 C-terminal domain. We cannot, however, exclude the possibility that 2C also disrupts HDAC2 binding to other proteins or that its expression influences the functions of proteins other than Sp3. While further mapping of the Sp3-binding site within the 2C region may be required to more stringently inhibit only the HDAC2-Sp3 interaction for drug discovery, the mode of inhibiting the HDAC2-Sp3 complex described here could circumvent the toxicity of HDAC inhibitors in clinical use (Ali et al., 2013; Siegel et al., 2009) while still stimulating synaptic gene expression and enhancing neuronal plasticity and cognitive function.

In conclusion, we have identified the HDAC2-Sp3 complex as a critical epigenetic regulator of synaptic function in neurons and

demonstrated the therapeutic potential of selective inhibition of this complex in AD. Growing evidence indicates the involvement of HDAC2 in a number of other neurological disorders, such as depression, post traumatic stress disorder (PTSD), and schizophrenia (Covington et al., 2009; Gräff et al., 2014; Kurita et al., 2012). As these disorders share some common features such as impaired synaptic plasticity, a common underlying etiology could exist (Cominski et al., 2014; Frantseva et al., 2008; Howland and Wang, 2008). Further studies may reveal the therapeutic potential of targeting the HDAC2-Sp3 complex in these disorders as well. Thus, our findings provide alternative avenues for the development of drugs to treat AD, and potentially other neurological disorders, by illustrating a feasible paradigm to selectively target a specific HDAC2 complex to boost memory function without affecting other complexes whose inhibition likely mediates the negative side effects of pan-HDAC inhibitors.

EXPERIMENTAL PROCEDURES

Animal Models

All mouse work was approved by the Committee for Animal Care of the Division of Comparative Medicine at the Massachusetts Institute of Technology. Male CK-p25 mice were crossed with female CK or p25 mice to get WT, CK, p25, and double-transgenic CK-p25 mice. CK or p25 mice were used as negative controls. 2.5- to 3.5-month-old double-transgenic CK-p25 mice (and their littermates) were used to induce p25 expression by changing food pellets containing doxycycline to ones lacking doxycycline. All behavioral experiments and ex vivo LTP recordings were performed between 6 and 8 weeks of p25 induction, the time when cognitive deficits are strongly observed (Fischer et al., 2005).

ChIP

Crosslinking was performed with 1% formaldehyde at room temperature for Sp3 and acetylated histones. For HDAC2 ChIP, additional crosslinking with 2 mM disuccinimidyl glutarate (DSG) was done for 35 min, followed by the addition of formaldehyde (final 1%) and another 10-min incubation. The reaction was stopped with 125 mM glycine. For primary cultured neurons, cell pellets were lysed with 50 mM HEPES-KOH (pH 7.4), 140 mM NaCl, 1 mM EDTA, 10% glycerol, 0.5% NP-40, 0.25% Triton X-100, protease inhibitor cocktail for 10 min. Nuclei were pelleted by spinning at 1,000 rpm for 5 min at 4°C. The pellets were resuspended with 10 mM Tris-HCl (pH 8.0), 0.5 mM EGTA, 1 mM EDTA, 200 mM NaCl and rocked for 10 min at room temperature followed by centrifugation at 1,000 rpm for 5 min at 4°C. The

Figure 6. Exogenous Expression of HDAC2 C-Terminal Domain Ameliorates Synaptic and Cognitive Dysfunction in CK-p25 Mice

- (A) Representative western blot images of co-immunoprecipitation of Sp3 or Sin3A with HDAC2, FLAG-tagged mCherry, 1C, and 2C in Neuro2A cells. Red, blue, and black arrows indicate the bands of mCherry-1C, mCherry-2C, and mCherry, respectively.
- (B) Representative traces and quantifications of the amplitude and frequency of mEPSCs from primary neurons transduced with control (mCherry) or 2C-expressing virus (n = 5–8). *p < 0.05; **p < 0.01 (two-tailed Welch's t test). Values are means ± SEM.
- (C) ChIP-qPCR results of HDAC2 at the promoters of target genes and control genes in primary neurons transduced with control (mCherry) or 2C-expressing virus (n = 3). *p < 0.05; **p < 0.01 (one-tailed Student's t test). Values are means ± SEM.
- (D) qRT-PCR results of the target genes and control genes in primary neurons transduced with 2C (n = 4). Values are means ± SEM. *p < 0.05 (unpaired t test corrected by Holm-Sidak method).
- (E and F) ChIP-qPCR results of H4K5ac (E) and H2BK5ac (F) in primary neurons transduced with control (mCherry) or 2C-expressing virus (n = 3). *p < 0.05; **p < 0.01 (one-tailed Student's t test). Values are means ± SEM.
- (G) fEPSP slopes from hippocampal area CA1 of CK-p25 mice injected with control or 2C-expressing virus. Slopes were normalized to baseline for each slice before 2× TBS (n = 5–6 slices). **p < 0.01 (repeated-measurement two-way ANOVA). Values are means ± SEM.
- (H) Freezing responses of CK (control) and CK-p25 mice injected with control or 2C-expressing virus, 24 hr after contextual fear conditioning (n = 10 CK-p25 mice each; n = 8 CK mice). *p < 0.05; **p < 0.01 (Tukey's test); n.s., not significant. Values are means ± SEM.
- (I) qRT-PCR results of the target and control genes in hippocampi of CK or CK-p25 mice transduced with 2C or Sp3 shRNA (n = 4). Values are means ± SEM. *p < 0.05; **p < 0.01 (two-way ANOVA followed by Dunnett's test); n.s., not significant.
- In (C)–(F), the vertical line separates synaptic from control genes. See also Figure S7.

resultant pellets were nuclear fractions for ChIP experiments. For brain tissues, isolation of neuronal nuclei was conducted after crosslinking, as described previously (Lu et al., 2014). Isolated nuclei were subjected to FACS after staining with Alexa 488-conjugated anti-NeuN antibody (Millipore, MAB 477X). Purified NeuN-positive nuclei or nuclear fractions of primary neurons were sonicated in 10 mM Tris-HCl (pH 8.0), 0.5 mM EGTA, 1 mM EDTA, 0.5% (w/v) N-lauroylsarcosine sodium salt using Bioruptor (setting high, 40 cycles of 30 s ON and 30 s OFF). Sheared chromatin was immunoprecipitated with antibodies against HDAC2 (Abcam; ab12169), Sp3 (Santa Cruz Biotechnology; sc-644 X), H4K5ac (Abcam; ab51997), or H2BK5ac (Cell Signaling Technology; 2574S). Immunoprecipitated DNA was extracted by phenol/chloroform/isoamyl alcohol, purified by ethanol precipitation, and subjected to qPCR using primers specific to the promoter regions of the genes assayed (see [Supplemental Information](#) for primer sequences). The fluorescent signal of the amplified DNA (SYBR Green, BioRad) was normalized to input.

Electrophysiology

Acute hippocampal slices were prepared from the mice injected with lentivirus 4 weeks after viral injection. The mice were anesthetized with isoflurane and decapitated. The experimenter was blinded to which virus was injected. Transverse hippocampal slices (400 μ m thick) were prepared in ice-cold dissection buffer (in millimolar: 211 sucrose, 3.3 KCl, 1.3 NaH₂PO₄, 0.5 CaCl₂, 10 MgCl₂, 26 NaHCO₃, and 11 glucose) using a Leica VT1000S vibratome (Leica). Slices were recovered in a submerged chamber with 95% O₂/5% CO₂-saturated artificial cerebrospinal fluid (ACSF) consisting of (in millimolar) 124 NaCl, 3.3 KCl, 1.3 NaH₂PO₄, 2.5 CaCl₂, 1.5 MgCl₂, 26 NaHCO₃, and 11 glucose for 1 hr at 28°C–30°C. To ensure that an equivalent number of virus-transduced cells was present in each slice, the number of GFP/mCherry-expressing cells was quantified. For extracellular recording, CA1 field potentials evoked by Schaffer collateral stimulation with bipolar electrode was measured every 30 s. After recording the baseline for 15 min, LTP was induced by repeated (2 $\times$) theta-burst stimulations (TBSs, containing 10 brief bursts, which consisted of four pulses at 100 Hz). The slopes of field excitatory postsynaptic potentials (fEPSPs) were measured to quantify the strength of synaptic transmission. A HEKA instrument (EPC10) was used for data acquisition, and data were analyzed with pClamp10 (Axon Instruments). The input-output curve was obtained by plotting the slopes of fEPSPs against stimulation intensity (in milliamperes). For mEPSC recordings of primary cortical neurons (days in vitro [DIV]17–22), the external solution consisted of (in millimolar) 140 NaCl, 4 KCl, 2 CaCl₂, 2 MgCl₂, 10 HEPES, and 10 glucose ([pH 7.3] with NaOH), and 315 mOsm. The internal solution contained (in millimolar): 145 CsCl, 5 NaCl, 10 HEPES, 10 EGTA, 4 MgATP, and 0.3 Na₂GTP (pH 7.3 with CsOH), 305 mOsm. The external solution also contained 1 μ M tetrodotoxin (TTX) and 10 μ M bicuculline. Series resistance was compensated. The membrane potential of each cell was patched at -70 mV during recording. Recordings were obtained at room temperature. Data were acquired using the Axopatch 200B amplifier and analyzed with pClamp10 software (Molecular Devices).

Statistics

Student's or Welch's t test was used for the statistical comparison of two groups, following an F test. Multiple comparisons were carried out with Dunnett's test, unless otherwise noted. To examine the significance of overlaps in RNA-seq data, Fisher's exact test was used.

ACCESSION NUMBERS

The accession number for the RNA-seq data reported in this paper is GEO: GSE87441 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE87441>).

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, seven figures, and one table and can be found with this article online at <http://dx.doi.org/10.1016/j.celrep.2017.07.044>.

AUTHOR CONTRIBUTIONS

This study was designed, directed, and coordinated by H.Y. and L.-H.T. H.Y. generated all the constructs and performed biochemical experiments and quantification of gene expression. J.C. conducted FACS and ChIP experiments. J.P. contributed to biochemical and gene expression analyses. F.G. generated RNA-seq libraries and performed bioinformatics and RNA-seq analyses. R.R. prepared primary neurons and performed stereotaxic injections and immunohistochemical experiments. J.W. conducted all the electrophysiological experiments. S.Y. performed qPCR and ChIP experiments and prepared primary neurons. O.K. set up transgenic mice and performed behavioral experiments. E.G. contributed to the identification of genes co-regulated by HDAC2-Sp3 and downregulated in CK-p25. The manuscript was written by H.Y., J.C., J.P., and L.-H.T. and commented on by all authors.

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