MeCP2 and histone deacetylases 1 and 2 in dorsal striatum collectively suppress repetitive behaviors

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Class I histone deacetylases (HDACs) Hdac1 and Hdac2 can associate together in protein complexes with transcriptional factors such as methyl-CpG-binding protein 2 (MeCP2). Given their high degree of sequence identity, we examined whether Hdac1 and Hdac2 were functionally redundant in mature mouse brain. We demonstrate that postnatal forebrain-specific deletion of both Hdac1 and Hdac2 in mice impacts neuronal survival and results in an excessive grooming phenotype caused by dysregulation of Sap90/Psd95-associated protein 3 (Sapap3; also known as Dlgap3) in striatum. Moreover, Hdac1- and Hdac2-dependent regulation of Sapap3 expression requires MECP2, the gene involved in the pathophysiology of Rett syndrome. We show that postnatal forebrain-specific deletion of Mecp2 causes excessive grooming, which is rescued by restoring striatal Sapap3 expression. Our results provide new insight into the upstream regulation of Sapap3 and establish the essential role of striatal Hdac1, Hdac2 and MeCP2 for suppression of repetitive behaviors.

Histone deacetylases (HDACs) are a class of enzymes that remove acetyl groups from histone tails and promote chromatin remodeling. The role of individual HDACs in the brain is an active area of investigation with recent data suggesting that Hdac2 is a negative regulator of learning and memory. Hdac2 shares 85% sequence identity with another Class I family member, Hdc1, however loss of Hdc1 in the brain does not impact learning and memory. The target genes of Hdc1 and Hdc2 in brain are unclear and distinguishing their functional roles is complicated by the fact that Hdc1 and Hdc2 can also associate to form a complex with other proteins, such as methyl-CpG-binding protein 2 (MeCP2). Given their high degree of sequence identity, we examined whether Hdc1 and Hdc2 were functionally redundant.

MeCP2 is a transcription factor known to play important roles in mediating complex behavior and synaptic function. Loss-of-function mutations in MECP2 lead to the neurological disorder Rett syndrome (RTT), and genomic duplications spanning MECP2 also result in neurodevelopmental abnormalities with autistic features and behaviors. RTT patients display a range of phenotypes including repetitive behaviors such as stereotypical hand movements, similar to other phenotypes observed in patients with obsessive-compulsive disorder (OCD). Previous studies have shown that mice lacking MeCP2 recapitulate several of the behavioral aspects of RTT, including social and motor defects. In the present study we examined whether postnatal deletion of both Hdc1 and Hdc2 selectively in forebrain would mimic the phenotypes observed in mice with a brain-specific deletion of Hdc2. Unexpectedly, we identified a functional redundancy between Hdc1 and Hdc2 in neuronal survival that impacted the lifespan of the Hdc1;Hdc2 double-knockout mice. We also observed exacerbated grooming behavior that was due to dysregulation of Sapap3 in the striatum, similar to the phenotype and altered expression of Sapap3 we identified in conditional MeCP2-knockout mice. We were able to rescue the grooming phenotype in conditional MeCP2-knockout mice by expression of Sapap3 in the striatum, suggesting that Sapap3 is a putative target gene of MeCP2 in association with Hdc1 and Hdc2. Collectively, our data reveal unexpected negative effects of HDAC inhibition in postnatal brain as well as uncover a role for Hdc1, Hdc2 and MeCP2 in regulation of Sapap3.

RESULTS

Postnatal loss of Hdc1 and Hdc2 results in behavioral abnormalities and premature death

To examine the role of Hdc1 and Hdc2 in mature brain, we generated mice with forebrain-specific deletions of both genes during postnatal development. Homozygous Hdc1loxP/loxP;Hdc2loxP/loxP mice were crossed to calcium/calmodulin-dependent protein kinase II (CaMKII)-Cre93 mice to generate the conditional deletion of Hdc1 and Hdc2 (referred to as cDKO) in forebrain regions during postnatal development. To determine whether potential phenotypes were due to the loss of both Hdc1 and Hdc2 and not the result of the deletion of either individual HDAC, conditional Hdc1-loxP;Hdc2loxP mice as well as conditional Hdc2-loxP (Hdc2 cKO) mice were generated using CaMKII-Cre93. The cDKO mice were born at normal Mendelian ratios and appeared indistinguishable from littermate controls at birth. Immunohistochemistry and western blot analysis of the cDKO mice at 8 weeks of age confirmed the deletion of Hdc1 and Hdc2 in forebrain regions including frontal cortex, hippocampus and striatum, with no change in expression in cerebellum (Fig. 1a–d), consistent with the original characterization

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of the CaMKII-Cre93 line\textsuperscript{13}. Individual Hdac1 cKO or Hdac2 cKO mice showed a similar pattern of deletion of the gene of interest\textsuperscript{8}. The cDKO mice were indistinguishable in body weight from littermate CTLs for the first few weeks of life, but at 6 weeks of age the cDKO mice began to lose weight (Fig. 2a) and all cDKO mice died at \textasciitilde9 weeks of age. Mice with a single copy of either allele did not show alterations in weight or early postnatal lethality (data not shown). The Hdac1 cKO or Hdac2 cKO mice were also indistinguishable from littermate CTLs in weight and had a normal lifespan (Supplementary Fig. 1a,b). Collectively, these data are consistent with the loss of both Hdac1 and Hdac2 in postnatal forebrain regions impacting the viability of the mice.

Necroscopic analysis of cDKO mice did not reveal peripheral abnormalities (data not shown). However, there was a significant reduction in overall brain size and weight in 8 week old cDKO mice compared to age-matched CTLs, which appeared to be due to a decrease in the size of the cortical areas, consistent with the regional deletion of Hdac1 and Hdac2 (Fig. 2b and Supplementary Fig. 1c). The CaMKII-Cre93 line expresses Cre recombinase at postnatal days 10–14; therefore, we examined the brain mass of cDKO mice at a time-point coinciding with early expression of Cre recombinase\textsuperscript{13}, postnatal day 16, and found no difference compared to CTLs (Fig. 2c), demonstrating that the reduction in brain size occurred after the deletion of Hdac1 and Hdac2. We also observed no differences in brain weight in 8-week-old Hdac1 cKO or Hdac2 cKO mice, further supporting the hypothesis that deletion of both Hdac1 and Hdac2 led to the reduction in brain mass (Supplementary Fig. 1c,d). Compared to CTLs (Fig. 2d), hematoxylin and eosin staining revealed aberrant cellular patterns and layering in cortex (Fig. 2e) and hippocampus (Fig. 2f) of cDKO mice. Rather unexpectedly, we found no cell morphology abnormalities in the striatum of cDKOs compared to CTLs (Fig. 2f,g) although Hdac1 and Hdac2 expression was significantly reduced in this brain region (Fig. 1a–k), as CaMKII–Cre93 is known to express Cre recombinase in medium spiny neurons of the striatum\textsuperscript{14}. There were also no detectable differences in the cerebellar structure between cDKO and CTL mice, consistent with the forebrain-specific deletion (Fig. 2j,k). Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) revealed increased apoptosis in the cortex and hippocampus of cDKOs compared to CTLs but not in the striatum or cerebellum (Supplementary Fig. 2), consistent with the regional morphological changes observed in hematoxylin and

![Image](https://example.com/image1.png)

**Figure 1** Characterization of Hdac1:Hdac2 cDKO mice. Fluorescent immunohistochemistry confirmed a selective loss of (a) Hdac1 and (b) Hdac2 in forebrain regions (frontal cortex, FC; striatum, STR; hippocampus, HC) but not in cerebellum (CBL) of cDKO mice. Scale bar represents 100 µm. Presented are representative images from a cohort of cDKO mice and respective CTLs, and results were replicated in a second cohort of mice. (c,d) Western blot (WB) analysis confirmed over 50% reduction of (c) Hdac1 and (d) Hdac2 normalized to Gapdh in forebrain regions of cDKO mice with no alterations in CBL. Full length blots are presented in Supplementary Figure 8. (n = 5 mice per group for Hdac1; two-tailed t-test; t\textsubscript{(8)} = 4.408; P < 0.0018 for FC CTL vs. cDKO; t\textsubscript{(8)} = 6.832; P < 0.0001 for STR CTL vs. cDKO; t\textsubscript{(8)} = 0.8062; P = 0.2536 for CBL CTL vs. cDKO). Data in c and d are shown as median, 25th and 75th percentile, and min and max value. *P < 0.05.
Hdac1;Hdac2 cDKO mice developed facial lesions and displayed excessive grooming

We observed that every cDKO mouse developed a severe lesion on the face and neck regions at ~7 weeks of age (Fig. 3a). The fur lesion could not be attributed to fighting with cage mates or social grooming, as the lesion still appeared in each of the cDKO mice that were singly housed after weaning (starting at 3 weeks of age prior to the appearance of the fur lesion; data not shown), and not in any CTL or Hdac1;Hdac2 double-heterozygous mice. We also did not observe facial or neck lesions on any of the Hdac1 cKO or Hdac2 cKO mice, demonstrating that the lesion was the result of loss of both Hdac1 and Hdac2. The lesion did not appear to be due to any form of dermatitis, so we investigated whether the fur lesion was due to excessive grooming in cDKO mice. We monitored the behavior of 3- and 6-week-old cDKO mice for a 30 min period (between 8 a.m. and 12 p.m.) and scored the total amount of time the mouse spent self-grooming. As most studies only score the initial 10 min, we assessed this time interval and found the cDKO mice spent approximately twice the amount of time grooming as CTL mice (Fig. 3b and Supplementary Fig. 3c). The phenotype was consistent at the 20 min and 30 min time intervals, revealing a significant—more than twofold—increase in grooming of cDKO mice compared to CTLs (P = 0.0267 for 0–10 min CTL versus cDKO; P = 0.0003 for 0–20 min CTL versus cDKO; P = 0.0001 for 0–30 min). In contrast, Hdac1 cKO or Hdac2 cKO mice showed normal grooming behavior compared to their respective littermate CTLs (Supplementary Fig. 4a,b), demonstrating that the fur lesion as well as the excessive grooming phenotype was due to concurrent loss of Hdac1 and Hdac2.
Hdac1;Hdac2 cDKO mice have altered Sapap3 expression

Mice lacking Sapap3 display an excessive grooming phenotype resulting in a facial lesion25, similar to the phenotype displayed by cDKO mice. We therefore assessed whether Sapap3 expression was altered by the loss of Hdac1 and Hdac2. Quantitative real time (qRT)-PCR analysis revealed a significant decrease in Sapap3 expression in frontal cortex and striatum, with no alterations in hippocampus and cerebellum (Fig. 3d), in cDKO mice. This result is in agreement with previous work showing that the grooming phenotype of Sapap3-knockout mice is mediated through the corticostriatal pathway25. Sapap3 expression was not altered in Hdac1 cKO or Hdac2 cKO mice, consistent with the premise that loss of both Hdac1 and Hdac2 was necessary to dysregulate its expression (Supplementary Fig. 4d,e). We also examined the expression of Slitrk5, another gene that has been linked to excessive grooming behavior in mice26, and found no change in its expression in the striatum or cortex, showing specificity for the altered Sapap3 expression in the cDKO mice (Supplementary Fig. 4f).

Striatal specific deletion of Hdac1;Hdac2 recapitulates excessive grooming seen in cDKO mice

We next examined whether deletion of both Hdac1 and Hdac2 selectively in the striatum is sufficient to disrupt Sapap3 expression and elicit the excessive grooming behavior. We used stereotoxic methods to bilaterally inject into the dorsal striatum of adult Hdac1\textsuperscript{lox\textsubscript{P}/lox\textsubscript{P}};Hdac2\textsuperscript{lox\textsubscript{P}/lox\textsubscript{P}} mice an adeno-associated viral (AAV) vector expressing GFP tagged to Cre recombinase (GFP-Cre) or GFP alone as a control (Fig. 4a). Mice were behaviorally tested three weeks after surgery, a time-point sufficient for Cre mediated recombination with AAV-GFP-Cre (ref. 31) and killed for analysis to confirm viral placements using laser microscopy (Fig. 4b), with off-target-injected animals eliminated from further analysis. qRT-PCR showed an approximate 50% reduction in Hdac1 and Hdac2 expression in dorsal striatum of AAV-GFP-Cre compared to AAV-GFP injected mice (Fig. 4c). The striatum-specific deletion of Hdac1 and Hdac2 did not result in any obvious structural alterations or cell death within the striatum (Supplementary Fig. 5d) and did not impact lifespan, weight, locomotor activity, open field behavior or motor coordination compared to AAV-GFP injected mice (Supplementary Fig. 5a–c). However, deletion of Hdac1 and Hdac2 selectively in the striatum resulted in a significant increase in the time spent grooming (Fig. 4d) with a significant decrease in Sapap3 expression in the striatum compared to AAV-GFP injected mice (Fig. 4e) similar to data from the cDKO mice.

Postnatal conditional deletion of MeCP2 recapitulates grooming phenotype of cDKO mice

Our data demonstrate that loss of both Hdac1 and Hdac2 in the striatum downregulated Sapap3 expression and resulted in an excessive grooming phenotype. Hdac1 and Hdac2 do not bind DNA directly1,9,32 but rather participate in protein complexes to impact gene transcription. MeCP2 is a transcription factor that has been shown to interact with Hdac1 and Hdac2 in a co-repressor complex and regulate gene expression10,11. We confirmed that this interaction also occurs in the striatum by crossing MeCP2\textsuperscript{lox\textsubscript{P}/lox\textsubscript{P}} mice with CaMKII-Cre93 mice to generate conditional MeCP2-knockout mice (MeCP2 cKO) and performing immunoprecipitation with an antibody against Hdac2 on striatal samples from CTL and MeCP2 cKO mice (Supplementary Fig. 6). The function of MeCP2 in the central nervous system is complex, as it has been shown to activate and repress transcription11,33,34. To investigate whether MeCP2 binds the Sapap3 promoter, we performed chromatin immunoprecipitation (ChIP) from the striatum of MeCP2 cKO mice and CTLS using an antibody to MeCP2. qPCR analysis of the ChIP DNA revealed specific MeCP2 binding to the Sapap3

Chronic administration of fluoxetine attenuates excessive grooming

Increased grooming in mice has been suggested to model aspects of OCD, with previous data establishing that the serotonin selective reuptake inhibitor (SSRI) fluoxetine can alleviate excessive grooming of OCD, with previous data establishing that the serotonin selective reuptake inhibitor (SSRI) fluoxetine can alleviate excessive grooming

**Figure 3** Hdac1;Hdac2 cDKO mice have increased grooming behavior and dysregulation of Sapap3. (a) Representative images of the facial lesion that occurs in cDKO mice at approximately 7 weeks of age. Results were replicated in every cDKO mouse generated in this study. (b) Grooming behavior was assessed by quantifying the total time spent grooming over a 30-min period. cDKO mice spend significantly more time grooming at 6 weeks of age compared to littermate CTL mice (CTL n = 7; cDKO n = 6; two-tailed t-tests; t\textsubscript{11} = 2.556, P = 0.0267 for 0–10 min CTL vs. cDKO; t\textsubscript{11} = 5.129, P = 0.0003 for 20–30 min CTL vs. cDKO; t\textsubscript{11} = 6.787, P = 0.0001 for 0–30 min CTL vs. cDKO). (c) Fluoxetine (Fix) administration for 21 days attenuated the grooming phenotype in cDKOs to levels comparable to that seen in CTL mice. (d) qRT-PCR analysis showed that Sapap3 mRNA levels were significantly downregulated in the frontal cortex (FC) and striatum (STR) of cDKO mice compared to CTLS, with no change in the hippocampus (HC) and cerebellum (CBL); (FC CTL n = 7; cDKO n = 6; STR CTL n = 6, cDKO = 7; HC CTL n = 8, cDKO n = 7; CBL CTL n = 7, cDKO = 4; two-tailed t-tests; t\textsubscript{11} = 2.769, P = 0.0183 for FC CTL vs. cDKO; t\textsubscript{11} = 2.278, P = 0.0437 for STR CTL vs. cDKO; t\textsubscript{13} = 0.9407, P = 0.3640 for HC CTL vs. cDKO; t\textsubscript{8} = 1.181, P = 0.2677 for CBL CTL vs. cDKO). Data in b–d are shown as median, 25th and 75th percentiles, and min and max value. *P < 0.05.
A representative diagram of the approximate virus injection site in the dorsal striatum (Fig. 4a) and a coronal section indicating the GFP-infected neurons at the injection site. Results were replicated in 7 additional mice. Scale bar represents 100 μm.

c) qRT-PCR confirmed a significant knockdown of approximately 50% of both of Hdac1 and Hdac2 mRNA in the striatum of Hdac1loxP/loxP;Hdac2loxP/loxP mice that received AAV-GFP-Cre compared to mice that received AAV-GFP (Hdac1 AAV-GFP n = 7; AAV-GFP-Cre n = 6; two-tailed t-test; t11 = 2.211, P = 0.0491 for AAV-GFP vs. AAV-GFP-Cre; Hdac2 AAV-GFP n = 7; AAV-GFP-Cre n = 8; two-tailed t-test; t13 = 4.084, P = 0.0013 for AAV-GFP vs. AAV-GFP-Cre). (d) Hdac1loxP/loxP;Hdac2loxP/loxP mice that received AAV-GFP-Cre spent significantly more time grooming compared to Hdac1loxP/loxP;Hdac2loxP/loxP mice injected with AAV-GFP, recapitulating a similar phenotype observed in the cDKO mice (AAV-GFP n = 9; AAV-GFP-Cre n = 8, two-tailed t-tests; t15 = 2.134, P = 0.0497 for 0–10 min AAV-GFP vs. AAV-GFP-Cre; t15 = 2.863, P = 0.0119 for 0–20 min AAV-GFP vs. AAV-GFP-Cre; t15 = 3.709, P = 0.0021 for 0–30 min AAV-GFP-Cre). Data in c–e are shown as median, 25th and 75th percentile, and min and max values. *P < 0.05.

Restoring Sapap3 expression in the striatum of MeCP2 cKO mice rescues excessive grooming phenotype

To establish a direct link between MeCP2 and Sapap3 in mediating the grooming phenotype, we generated adeno-associated DJ serotype viruses expressing either GFP only (control) or a human influenza hemagglutinin (HA)-tagged Sapap3 (AAV-Sapap3) and stereotaxically injected them bilaterally into the dorsal striatum of adult MeCP2 cKO and CTL mice to test whether this would rescue the grooming phenotype (Fig. 5a). We confirmed the localized expression of Sapap3 in the striatum by immunohistochemistry (Fig. 5b). We also corroborated the rescue of Sapap3 expression in the striatum of MeCP2 cKO mice injected with the AAV-Sapap3 virus by qRT-PCR analysis (Fig. 5c). We found that AAV-mediated expression of Sapap3 in the striatum of the MeCP2 cKO mice rescued the excessive grooming behavior to a level comparable to CTL mice injected with GFP alone (Fig. 5d), indicating that this deficit was the result of decreased expression of Sapap3. The AAV expression of Sapap3 in the dorsal striatum did not alter locomotor activity, and in contrast to the reversal of the grooming phenotype, the previously reported impaired performance of the MeCP2 cKO mice on the rotarod was still present (Supplementary Fig. 7a,b), suggesting that this deficit is mediated through a different mechanism.
DISCUSSION

Our results reveal that postnatal deletion of both Hdac1 and Hdac2 in the brain results in several adverse effects, including neuronal apoptosis in cortical and hippocampal regions as well as early postnatal lethality, demonstrating functionally redundant roles in neuronal survival past embryogenesis. Given the recent efforts in the development of HDAC2 inhibitors for the treatment of neurodegeneration and cognitive enhancement, our results suggest caution with compounds targeting both HDAC1 and HDAC2 and emphasize the importance of subtype specific inhibitors. We also observed that the concurrent loss of Hdac1 and Hdac2 results in an increase in striatum-dependent mRNA expression of Sapap3 compared to CTLs (n = 4 mice per group; two-tailed t-test; t(4) = 4.018; P = 0.00105 for interaction). Data in a–d are shown as median, 25th and 75th percentile, and min and max value. *P < 0.05.
Developmental abnormalities, has been shown to interact with HDAC1 of the paper. The behavior of between the behavior of through transcription-dependent processes. Given the high similarity with MeCP2 within the striatum, regulate repetitive behaviors and suggest that HDAC1 and HDAC2, in concert data provide insight into the neurobiological mechanisms underlying promoter, that loss of regions of the mouse resulted in increased repetitive behaviors, similar to the phenotype observed in mice with loss of MeCP2 in inhibitory neurons, as well as decreased Sapap3 expression in the dorsal striatum. We then showed that the grooming phenotype in the MeCP2cko mice can be rescued by reintroducing Sapap3 expression in the dorsal striatum. Our results showing that MeCP2 binds to the Sapap3 promoter, that loss of MeCP2 attenuates Sapap3 expression and that restoring Sapap3 in the striatum rescues the grooming phenotype, suggest that the repetitive behaviors observed in Rett syndrome may be due to dysregulation of SAPAP3 in the striatum. Collectively, these data provide insight into the neurobiological mechanisms underlying repetitive behaviors and suggest that HDAC1 and HDAC2, in concert with MeCP2 within the striatum, regulate repetitive behaviors through transcription-dependent processes. Given the high similarity between the behavior of Hda1c/Hdca2 cDKO mice and some aspects of OCD, our results emphasize the need for large-scale genetic studies to determine whether variants in SAPAP3 are associated with OCD in patients.

METHODS
Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS
M.M. and M.A. performed the behavioral experiments. M.M., M.A., K.S., M.H.C. and X.L. contributed to the molecular experiments. M.M. performed the statistical analyses. M.M. made the figures and wrote the corresponding sections of the paper. L.M.M. and E.T.K. designed the study, supervised the experiments and wrote the paper.

COMPETING FINANCIAL INTERESTS
The authors declare competing financial interests: details are available in the online version of the paper.

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ONLINE METHODS

Overview. Animals were housed and maintained as previously described. Molecular studies consisted of western blot analysis, quantitative PCR or chromatin immunoprecipitation on tissue lysates from frontal cortex, hippocampus, striatum or cerebellum. Histological analysis consisted of immunohistochemistry, terminal deoxynucleotidyl transferase-mediated UTP end labeling, and H&E staining and performed as previously described. Behavioral studies and stereotaxic surgeries were performed using male mice as previously described. Obsessive-compulsive-like behavior was assessed by scoring grooming behavior. Briefly, mice were placed in a home-cage environment and total time spent grooming was measured for 30 min. All drugs were administered via intraperitoneal injection. All experiments were performed and analyzed blind to test variable. Sample sizes were estimated based on our previous experience performing similar experiments.

Mice. The conditional Hdac1 knockout (Hdac1 cKO), Hdac2 knockout (Hdac2 cKO) and Hdac1:Hdac2 double knockout (cDKO) mice were generated by breeding transgenic mice expressing Cre recombinase under the control of the calcium/calmodulin-dependent kinase II promoter (CaMKII-Cre59) line with Hdac1loxP/loxP, Hdac2loxP/loxP, or Hdac1loxPloxP;Hdac2loxPloxP mice. Previous work has demonstrated that the CaMKII-Cre59 mice express Cre recombinase at postnatal days 10–14, selectively in forebrain neurons. The Hdac1loxPloxP, Hdac2loxPloxP and CaMKII-Cre59 lines were on a mixed 129/SvBALBc background and were backcrossed to a C57BL/6 line for at least 10 generations. The Hdac1 cKO, Hdac2 cKO and cDKO mice were genotyped using PCR analysis from genomic DNA isolated from tails as previously described. Littermates not carrying the Cre recombinase transgene regardless of loxp alleles were used as control mice in all experiments. Conditional Mecp2 knockout (Mecp2 cKO) mice are previously reported. Representative images of cDKO and CTI brains (taken at 8 weeks old and at P16) and the facial lesion in cDKO mice were captured using a Nikon D5100 camera. Mice were maintained on a 12-h light/dark cycle with ad libitum access to food and water. Mice were housed 3–5 mice per cage, with the exception of when mice were singly housed for analyzing the lesion in cDKO mice. All animal protocols were approved by the Institutional Animal Care and Use Committee at The University of Texas Southwestern Medical Center.

Drug injections. All injections were delivered intraperitoneally on naïve male mice. The cDKO mouse control littermates received a once-daily injection of either 0.9% saline or fluoxetine (Eli Lilly) at a concentration of 10 mg kg\(^{-1}\) in 0.9% saline, during the morning hours of the day (8 am–12 pm) for 7 or 21 d.

Protein quantification. Brain regions were dissected out and homogenized in a lysis buffer containing 25 mM HEPES at pH 7.9, 150 mM NaCl, 1 mM PMSF, 20 mM NaF, 1 mM DTT, 0.1% NP40, and proteinase inhibitor cocktails (Sigma), and spun down to isolate the lysate. Protein concentrations were determined by Bradford assays, and 20 µg of the protein was loaded on 10% SDS-PAGE gels, electrophoresed, transferred to nitrocellulose membranes and then blocked with 5% nonfat milk before overnight incubation with primary antibodies. Dilutions of primary antibodies were 1:2,000 for both rabbit anti-HDAC1 (Abcam, ab19845) and rabbit anti-HDAC2 antibodies (Abcam, ab32117). 1:1,000 for MeCP2 antibody (ThermoFisher, PA1-8887) and 1:5,000 for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody (Cell Signaling Technology, 21185). The following day the membranes were washed and then incubated with peroxidase-labeled anti-rabbit secondary antibody (Vector, P-1000) at 1:5,000 for Hdac1, Hdad2 and MeCP2, and 1:10,000 for Gapdh. Protein bands were detected using enhanced chemiluminescence (ECL) and exposed to film. Immunoreactivity was quantitated by the NIH ImageJ analysis software. Hdad1 and Hdad2 were normalized to Gapdh bands. For Hdad1 and Hdad2 westerns, following the transfer, each membrane was cut below the 50 kDa marker in order to individually probe for either Hdad1 or Hdad2, and Gapdh on the same blot. Separate gels were run for FC, HC, STR and CBL, and all blots were processed in parallel. For western blot following immunoprecipitation, primary antibodies were 1:1,000 for MeCP2 (ThermoFisher, PA1-8888) and 1:6,000 for Actin (Abcam, ab2676). Secondary antibodies were 1:5,000 for IRDye 680RD donkey anti-mouse (LI-COR, 926-68072) and 1:5,000 for IRDye 800CW donkey anti-rabbit (LI-COR, 926-32213). Blots were imaged using a LICOIR Odyssey CI X Imaging System (LI-COR).

Immunohistochemistry. Mice were transcardially perfused with 4% paraformaldehyde (PFA) in 0.1 M phosphate-buffered saline (PBS) and brains were removed from the skull. Following postfixation in 4% PFA overnight, the brains were cryo-protected in 30% sucrose in 0.1 M PBS before sectioning on a freezing microscope. The brains were coronally sectioned at 30 µm and subjected to immunohistochemistry. Briefly, free-floating sections were incubated overnight in primary antibody solution composed of 3% normal goat serum and 0.3% Triton X-100 in PBS. Dilutions for the primary antibodies were 1:250 for rabbit anti-HDAC1 (Abcam, ab19845), 1:2,000 for rabbit anti-HDAC2 (Abcam, ab32117), 1:1,600 for anti-HA (Cell Signaling, 3274S), and 1:200 for anti-GFP (Cell Signaling, 2956S). For Hdad1 staining, the sections were treated in 10 mM citric acid (pH 6) for 15 min at 95 °C for antigen retrieval before primary antibody incubation. Immunoreactivity was visualized by secondary antibodies conjugated with either Alexa Fluor 594 (HDAC1, Invitrogen, A11012) or Alexa Fluor 488 (HDAC2, HA, GFP, Invitrogen, A11008). The sections were incubated at a 1:200 dilution at room temperature for 2 h, counterstained with 4',6-diamidino-2-phenylindole (DAPI), and then mounted on Superfrost plus slides in Vectashield mounting media (Vector Laboratories). Images were captured using an Olympus BX51 epifluorescence microscope and Olympus DP70 software.

Hematoxylin and eosin (H&E) staining. H&E staining was carried out as previously described. Briefly, paraffin sections made from formalin fixed tissue were affixed to microscope slides through sequential room temperature and heated air-drying. Dried sections were deparaffinized and stained with hematoxylin, then destained using 70% ethanol. Sections were then stained with eosin, destained, and dehydrated in ascending ethanol solutions. Sections were rinsed in xylene then coverslipped with synthetic mounting media. Images were captured using an Olympus BX51 brightfield microscope and Olympus DP70 software.

TUNEL. Terminal deoxynucleotidyl transferase-mediated UTP end labeling (TUNEL) staining for apoptotic cells was done according to the manufacturer's protocol (Promega DeadEnd Fluorometric TUNEL System, Madison, WI). Apoptotic cells were labeled with fluorescein and the sections were counterstained with propidium iodide.

Vector construction and AAV preparation. A lentiviral vector containing the initial gfp-Sapap3 construct was received from the lab of Dr. G. Feng (Massachusetts Institute of Technology). Two AAV vectors were constructed from this plasmid. The eGFP and Sapap3 were PCR-amplified independently and cloned using standard methods into an expression cassette containing the human synapsin promoter and growth hormone polyadenylation signal surrounded by AAV-2 inverted tandem repeats. A human influenza hemagglutinin (HA) tag was attached to the N-terminus of Sapap3 during PCR amplification before cloning. Primers used were:

- **GFP-EcoRV:** 5′-ATA TGA TAT CCT ACT TCT GAT ACA GCT CGT CGT CG-3′;
- **GFP:** Sapap3.SpeI.F: 5′-ATA TAC TAG TAC CAA TGG TGA GCA AGG AGG AGG AGC-3′;
- **Sapap3.SpeI:** 5′-ATA TAC TAG TAC CAA TGG TGA GCA AGG AGG AGC-3′;
- **Sapap3.EcoRV:** 5′-ATA TGA TAT CCT ACA GCC TGG GTC GGT CGG-3′;
- **pAAV-GFP** or **pAAV-HA-Sapap3** were cotransfected with pAAV-DJ and pHelper into HEK-293 cells. Seventy-two hours after transfection, cells were harvested, lysed by freeze–thaw and incubated with Benzonase (Sigma) at 37 °C. To isolate the AAV vector, iodixanol gradient was performed. Cell lysate was transferred to the iodixanol gradient in quick-seal centrifugation tubes and centrifuged at 48,000 rpm in a Beckman Type 70Ti rotor at 18 °C for 2 h 10 min. After centrifugation, 40% gradient fraction was collected. The collected fraction was applied onto an Amicon Ultra 100K (Millipore), washed with PBS (−) and concentrated. Viral titers were determined by RT-PCR using primers for HGGolyA, and were 1.27 × 10^{12} GC ml\(^{-1}\) for AAV-GFP and 4.9 × 10^{12} GC ml\(^{-1}\) for AAV-Sapap3.

Adeno-associated virus injection. The adeno-associated virus-green fluorescent protein (AAV-GFP) or adeno-associated virus expressing Cre recombinase tagged
with GFP (AAV-GFP-Cre) were obtained from Penn Vector Core; AAV-GFP and AAV-GFP-Cre were AAV-1.CMV.P.IgeP.WPRE.BGH (viral titer of 3.18 × 10^{13} GC ml^{-1}) and AAV-1.CMV.HL.P.GFP.Cre.SV-40 (viral titer of 7.88 × 10^{12} GC ml^{-1}), respectively. Previous work demonstrated that GFP-tagged Cre recombines possess normal enzymatic activity. To inject the AAV-GFP, AAV-GFP-Cre or AAV-Sapap3 in striatum, 3–5-month-old naive Hdaclfloxed/Hdac2floxed/P600 mice were anaesthetized and placed into the stereotaxic frame. A 1 μl of AAV-GFP or AAV-GFP-Cre, or 0.5 μl of AAV-Sapap3 bilaterally at the following coordinates relative to bregma, at a 10° angle: anteroposterior +1.2 mm, lateral +2.5 mm and dorsoventral −3.0 mm. Mice were allowed to recover for 3 weeks before the commencement of behavioral testing.

RNA extraction and qRT-PCR. To determine relative expression of Hdac1 and Hdac2 mRNA after stereotaxic AAV injection, we performed qRT-PCR as described previously. Briefly, the animals were killed by rapid decapitation and the brains were sectioned at 14 μm. The dorsal striatum expressing GFP or Cre-GFP was laser microdissected from each section using an AS LMD (Leica) system. Eight sections were pooled to extract RNA using a PicoPure RNA isolation kit (Arcturus). Each section was 140 μm thick. Eight sections were pooled to extract RNA using a PicoPure RNA isolation kit (Arcturus). Each section was 140 μm thick. Eight sections were pooled to extract RNA using a PicoPure RNA isolation kit (Arcturus). Each section was 140 μm thick.

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test grooming behavior. A distinct cohort of cDKOs and CTLs was used to test grooming behavior following 1 week of fluoxetine treatment, and the same cohort was scored for grooming behavior following 3 weeks of fluoxetine treatment. A distinct cohort of Hda1loxP/loxP;Hda2loxP/loxP mice was used for stereotaxic injections with AAV-GFP and AAV-GFP-Cre, and after recovery this same cohort of mice was tested in multiple behavioral tasks in the following order: locomotor activity, open field, grooming and rotarod. A distinct cohort of mice was used to assess grooming in Mecp2 cKO and CTL mice. For rescue experiments using AAV-GFP and AAV-Sapap3, a distinct cohort of Mecp2 cKO and CTL mice was used for stereotaxic injections, and following recovery this same cohort of mice was tested in multiple behavioral tasks in the following order: locomotor activity, grooming and rotarod.

Locomotor activity. Mice were placed individually in a standard mouse home cage (18 cm × 28 cm) with fresh bedding, and activity was monitored over 2 h by five horizontal photobeams linked to data acquisition software (Photobeam Activity System, San Diego Instruments, San Diego, CA). Ambulatory activity was measured by counting the number of consecutive beam breaks in 5-min increments.

Open field. Mice were placed in the periphery of a novel open field environment (44 cm × 44 cm, walls 30 cm high) in a dimly lit room and allowed to explore for 10 min. The animals were monitored from above by a video camera connected to a computer running video tracking software (Ethovision 3.0, Noldus, Leesburg, Virginia) to determine the total time spent in the periphery (5 cm from the walls), center (14 cm × 14 cm), and complete center (34 cm × 34 cm). The open field areas were cleaned between mice. The amount of locomotor activity was also determined and in all experiments was consistent with locomotor activity data using standard mouse home cages.

Rotarod. Each mouse was placed on the rotarod (IITC Life Science), which accelerated from 0 to 45 rpm over the course of 60 s. Each session ended when the mouse fell off the rod and the total time spent on the rotarod before falling was measured. The mouse was returned to its original cage for 1 h before starting the next trial. The test was repeated for a total of 8 trials over the course of 2 d (4 trials per d).

Grooming. Grooming behavior was assessed as previously described, with modifications25. Mice were placed individually into a fresh cage and allowed to move freely for 30 min. Test sessions were recorded under red light by a video camera directly in front of the mice located inside the testing room. An observer blind to group and genotype analyzed the video tape and scored self-grooming of any part of the body including the face, head and ears, as well as full-body grooming. The total amount of time spent grooming (duration) was measured at 10-min intervals. Continuous grooming for greater than one second was recorded as a grooming bout, and sessions separated by ≥2 s constituted a new bout (modified from Welch et al.25).

Statistical analysis. Data are presented either as dot plots with error bars representing mean ± s.e.m., or box-and-whisker plots with interquartile ranges represented as the following: whiskers (error bars) are min and max of data and box is 25th, median and 75th percentile, as calculated in Prism GraphPad software. Statistical differences were calculated in Prism GraphPad software using unpaired, two-tailed t-tests when comparing two groups, or one-way or two-way ANOVA with multiple comparisons when appropriate for comparing three or more groups. Tukey, Bonferroni or Holm-Sidak post hoc tests were used following one-way or two-way ANOVA as appropriate. Statistical significance was defined as *P < 0.05. For all experiments requiring statistical analysis, the statistical test used, exact P values, sample size (n), t values, ANOVA F values and degrees of freedom for each experiment are indicated in the figure legend. No statistical methods were used to predetermine sample sizes; however, sample sizes were estimated based on similar experiments reported in previous publications from our lab3,7,41,45. Data distribution was assumed to be normal with similar variance between groups, but this was not formally tested. The Grubbs test was used when appropriate to identify and remove significant outliers.

A Supplementary Methods Checklist is available.

Data availability. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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