Histone deacetylase knockouts modify transcription, CAG instability and nuclear pathology in Huntington disease mice

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Abstract
Somatic expansion of the Huntington’s disease (HD) CAG repeat drives the rate of a pathogenic process ultimately resulting in neuronal cell death. Although mechanisms of toxicity are poorly delineated, transcriptional dysregulation is a likely contributor. To identify modifiers that act at the level of CAG expansion and/or downstream pathogenic processes, we tested the impact of genetic knockout, in HttQ111 mice, of Hdac2 or Hdac3 in medium-spiny striatal neurons that exhibit extensive CAG expansion and exquisite disease vulnerability. Both knockouts moderately attenuated CAG expansion, with Hdac2 knockout decreasing nuclear huntingtin pathology. Hdac2 knockout resulted in a substantial transcriptional response that included modification of transcriptional dysregulation elicited by the HttQ111 allele, likely via mechanisms unrelated to instability suppression. Our results identify novel modifiers of different aspects of HD pathogenesis in medium-spiny neurons and highlight a complex relationship between the expanded Htt allele and Hdac2 with implications for targeting transcriptional dysregulation in HD.

Introduction
Huntington’s disease (HD) is a dominantly inherited neurodegenerative disorder typically manifesting in midlife with motor, cognitive, and psychiatric symptoms, leading to death after 15–20 years (Vonsattel et al., 1985; Harper, 1999). HD is caused by the inheritance of an expansion >35 repeats of a polymorphic CAG repeat tract in the HTT gene (Macdonald et al., 1993), ultimately resulting in cellular dysfunction and death, with medium-spiny neurons (MSNs) of the striatum being exquisitely sensitive to this mutation (Vonsattel et al., 1985). The expanded HTT CAG repeat undergoes further time-dependent, CAG length-dependent and tissue/cell-type-dependent expansion (Wheeler et al., 1999; Kennedy and Shelbourne, 2000; Kennedy et al., 2003; Veitch et al., 2007; Gonitel et al., 2008; Swami et al., 2009; Lee et al., 2010; Lee et al., 2011; Kovalenko et al., 2012; Larson et al., 2015; Geraerts et al., 2016; Ament et al., 2017; Mouro Pinto et al., 2020). The repeat is highly unstable in the brain, particularly in MSNs (Kovalenko et al., 2012), with individual-specific differences in the extent of somatic CAG expansion in HD postmortem brain associated with age of onset (Swami et al., 2009). Recent genome-wide association studies (GWAS) for modifiers of HD onset highlight somatic CAG expansion as a key driver of the rate of disease onset (Genetic Modifiers of Huntington’s Disease (GeM-HD)
Results

**MSN-specific knockout of Hdac2 and Hdac3 in Htt\textsuperscript{Q111} knock-in mice**

To investigate the role of HDACs in Htt\textsuperscript{Q111} mice we took a genetic approach, focusing on two Class I HDACs, HDAC2 and HDAC3, due to previous interest in therapeutic targeting of this class of HDACs in HD (Grajich and Sadri-Vakili, 2015) and the relatively high expression levels of these HDACs in the striatum and in neurons (Broide et al., 2007). As constitutive genetic knockout of Hdac2 can result in perinatal lethality (Trivedi et al., 2007; Montgomery et al., 2007), and knockout of Hdac3 results in early embryonic lethality (Montgomery et al., 2008), we took advantage of conditional ‘floxed’ Hdac2 and Hdac3 alleles (Montgomery et al., 2007; Montgomery et al., 2008), and deleted each of these genes in striatal MSNs of heterozygous Htt\textsuperscript{Q111/+) mice in crosses together with ‘DARPP-32 Cre’ (D9-Cre) transgenic mice expressing Cre recombinase under the control of the Ppp1r1b gene promoter and regulatory elements from 5 to 6 weeks of age (Bogush et al., 2005; Figure 1A; Figure 1—figure supplement 1). We previously used these mice in a similar strategy to delete the Msh2 gene in MSNs (Kovalenko et al., 2012). We generated Htt\textsuperscript{Q111/+} or Htt\textsuperscript{+/-} mice harboring two floxed Hdac2 or Hdac3 alleles together with the D9-Cre transgene, resulting in
Figure 1. MSN-specific deletion of Hdac2 or Hdac3. (A) D9-Cre mice, expressing Cre recombinase from the Ppp1r1b promoter from 5 to 6 weeks of age, were crossed with floxed Hdac2 or Hdac3 mice (schematic illustrates exons flanked by loxP sites), and with Htt111/+ mice to obtain mice with or without a single Htt111 allele either expressing HDAC2/3 (WT) or with Hdac2 or Hdac3 allele deleted in MSNs (KO). See Figure 1—figure supplement 1 for a detailed breeding scheme. (B) Fluorescent micrographs of 10 week Htt111/+ Hdac2 KO (left) and Htt111/+ Hdac3 KO (right) striata co-stained with DARPP-32/HDAC2 and DARPP-32/HDAC3 antibodies, respectively.

The online version of this article includes the following source data and figure supplement(s) for figure 1:

Figure supplement 1. Detailed mating scheme.
Figure supplement 2. Upregulation of HDAC1 in Hdac2 KO striata.
Figure supplement 2—source data 1. HDAC2 and HDAC1 protein levels in Hdac2 KO mice.
Figure supplement 3. Hdac2 or Hdac3 knockout in striatal MSNs does not affect HDAC2 or HDAC3 levels in cortex.
deletion of the Hdac2 or Hdac3 alleles in MSNs (Hdac2 or Hdac3 knockout [KO]) and their littermates expressing both Hdac2 or Hdac3 alleles (Hdac2 WT or Hdac3 WT). HDAC2 or HDAC3 protein are no longer detectable in the majority of the striatal cells in Hdac2 KO or Hdac3 KO mice (Figure 1B). The remaining HDAC2- or HDAC3-immunopositive cells are DARPP-32 negative, reflecting continued expression in cell types other than MSNs in the striatum (Figure 1B). Western blots also confirmed the loss of HDAC2 protein in striata from Hdac2 KO mice and revealed a compensatory increase in HDAC1 protein levels as seen in other systems with long-term loss of HDAC2 (Figure 1—figure supplement 2). As expected, HDAC2 and HDAC3 expression were not altered in the cortex (Figure 1—figure supplement 3). Whereas Hdac2 KO mice appeared indistinguishable from their littermates, Hdac3 KO mice were significantly smaller in size and had lower body weight, with more than half of the total number of Hdac3 KO pups not surviving to five months of age, precluding some aspects of this study; notably, we were unable to generate sufficient numbers of Hdac3 KO mice to perform RNA-seq. We hypothesize that this may be the result of Ppp1r1b-driven Cre expression in cell types outside MSNs during development and/or in the periphery that are sensitive to levels of HDAC3, for example in the gut or liver (Alenghat et al., 2013; Knutson et al., 2008). Nonetheless, this observation highlights the non-redundant functions of HDAC2 and HDAC3 within the mammalian body.

**Impact of MSN-specific Hdac2 knockout and of the HttQ111 allele on the transcriptome**

Due to the central role of HDAC2 in regulating transcription (Kelly and Cowley, 2013) and previous evidence for a beneficial effect of HDAC inhibitors in HD mouse models, we first performed RNA-seq in the Hdac2 KO cohort to assess both the global impact of MSN-specific Hdac2 KO and the extent to which genes dysregulated by the expression of the HttQ111 allele might be reversed. Transcriptional dysregulation occurs in HttQ111/+ mice by 2–6 months of age (Ament et al., 2017;}

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**Figure 2.** RNA-seq analyses in HttQ111 striata with MSN-specific Hdac2 knockout. Results of differential gene expression analyses showing the number of differentially expressed genes (DEGs) for each of five contrasts. Number and % of DEGs at nominal p<0.05 or FDR adjusted p<0.05 are indicated. Total number of genes analyzed: contrast 1 = 17,132; contrast 2 = 17,175; contrast 3 = 17,139; contrast 4 = 17,116; contrast 5 = 17,094. The number of male (M) and female (F) mice in each group is indicated.

The online version of this article includes the following source data and figure supplement(s) for figure 2:

**Figure supplement 1.** Expression of Hdac genes and Cre recombinase.

**Figure supplement 2.** Principal Components Analyses (PCA).

**Figure supplement 3.** Global H3K9 and H4K12 acetylation levels.

**Figure supplement 3—source data 1.** AchH3K9 and AchH4K12 levels in Hdac2 KO mice.

**Figure supplement 4.** Cross-study comparison of HttQ111-differentially expressed genes.
Langfelder et al., 2016; Bragg et al., 2017). We performed RNA-seq in striata from 5-month Htt+/+
Hdac2 WT, HttQ111/+ Hdac2 WT, Htt+/+ Hdac2 KO and HttQ111/+ Hdac2 KO mice (Figure 2). HttQ111/+ mice with Hdac2 WT and Hdac2 KO genotypes were matched for constitutive CAG repeat
lengths (Source data 1). Analyses of exon-specific Hdac2 transcripts revealed marked depletion of
reads mapping to exons 2, 3, and 4 as well as the presence of exon 1-exon 5 splice junction reads in
the Hdac2 KO mice, consistent with Cre-mediated deletion of exons 2–4 (Montgomery et al.,
2007), and further supported by detection of Cre-specific transcripts in these mice (Figure 2—figure
supplement 1). Analyses of transcript levels of other Hdac genes revealed a compensatory increase
in Hdac1 transcripts in Hdac2 KO striata, consistent with western blots from the same mice (Figure 1—figure
supplement 2), but no major impact on the expression level of any other Hdac gene (Figure 2—figure
supplement 1). The HttQ111 allele did not alter expression of Hdac genes (Figure 2—figure
supplement 1), consistent with previous observations that the HTT mutation does not influence HDAC expression in primary neurons, or in CAG140 knock-in mice, although expression of
some HDACs was altered in R6/2 exon 1 transgenic mice (Hoshino et al., 2003; Quinti et al.,
2010). Principal component analysis (PCA) of the full dataset revealed that the greatest variance
between samples was attributable to Hdac2 genotype, with the second principal component largely
separating males and females. There was no clear distinction between samples based on Htt geno-
type (Figure 2—figure supplement 2). Global levels of striatal H3K9 and H4K12 acetylation, previ-
ously shown to be sensitive to HDAC2 levels in the brain (Guan et al., 2009), revealed overall fairly
subtle alterations that appeared to depend on age (Figure 2—figure supplement 3), consistent with
cell-type-specific knockout of Hdac2 and previous observations that the effects of HDAC inhibi-
tion on histone acetylation are restricted to specific genomic loci (Lopez-Atalaya et al., 2013).

To assess the impact of the two genetic mutations (Hdac2 KO and HttQ111) on the transcriptome we
compared differential gene expression analyses to test the effects of the MSN-specific Hdac2 KO
in the Htt+/− background (contrast 1), or the HttQ111/+ background (contrast 2), and the effects
of the HttQ111 allele in a Hdac2 WT background (contrast 3) or the Hdac2 KO background (contrast
4). We also compared HttQ111/+ Hdac2 KO and Htt+/+ Hdac2 WT striata (contrast 5) (Figure 2;
Source data 2). Figure 2 displays the number of differentially expressed genes (DEGs) in each of
the differential gene expression contrasts. Strikingly, knockout of Hdac2 in MSNs elicited a substi-
tional transcriptional response, roughly comparable in Htt+/+ or HttQ111/+ mice, with ~11–12% of genes
statistically significantly dysregulated (FDR adjusted p<0.05) and being both up- and down-regu-
lated in approximately equal proportion. In line with previous studies (Ament et al., 2017; Langfelder et al., 2016; Bragg et al., 2017), HttQ111/+ striata exhibited a large number of genes
that were both up- and down-regulated, although only a moderate number reached statistical signif-
icance when accounting for multiple testing. Interestingly, the transcriptional dysregulation mediated
by the HttQ111 allele appeared to be somewhat muted in the Hdac2 knockout background (Figure 2—compare contrasts 3 and 4). The comparison of HttQ111/+ Hdac2 KO and Htt+/+ Hdac2 WT striata
indicated an effect similar in magnitude to that of the Hdac2 KO mutation alone.

We compared the HttQ111-DEGs in this study with those in striata of heterozygous HttQ111/+ mice
in a previous study by Langfelder et al., 2016. In the Langfelder study, heterozygous HttQ111/+ mice
were analyzed as part of a larger allelic series of Htt CAG knock-in mice; here, we reanalyzed the
Langfelder data comparing HttQ111/+ to wild-type striata using the same analysis method used in the
current study (Figure 2—figure supplement 4; Materials and methods). A large proportion of gene
expression changes in the current study overlapped with those in the Langfelder dataset, although
many more DEGs were identified in the latter (Figure 2—figure supplement 4A, B). Differences
may be due to the age of the mice - 5 months in current study and 6 months in the Langfelder study
– especially considering the dramatic increase in transcriptional dysregulation that occurs between 2
and 6 months of age (Langfelder et al., 2016). In addition, factors such as genetic heterogeneity
(Materials and methods) and disproportionate male:female ratios of mice in our study may contrib-
ute to reduced power to detect HttQ111-driven transcriptional changes. Importantly, the vast major-
ity of the common genes were dysregulated in the same direction (Figure 2—figure supplement
4B); at a nominal p value of < 0.05, 721 genes were coordinately dysregulated (298 up-regulated in
both studies and 433 down-regulated in both studies). Correcting for multiple testing (FDR adjusted
p<0.05), 29 genes were coordinately dysregulated (11 up-regulated in both studies and 18 down-
regulated in both studies). These 29 genes are displayed in Figure 2—figure supplement 4C,
highlighting several genes including Ppp1r1b, Penk and Pdeo10a also found to be dysregulated in
Htt\textsuperscript{Q111/+} mice in additional previous studies (Ament et al., 2017; Bragg et al., 2017). We refer to these 29 genes as ‘commonly dysregulated genes’ in downstream analyses.

Overlaps in gene dysregulation elicited by the Hdac2 KO and the Htt\textsuperscript{Q111} allele

To gain insight into the transcriptionally dysregulated genes in each of the four contrasts we determined the enrichment of the DEGs in biological pathways (Figure 3; Source data 3). These analyses revealed that the Hdac2 KO and Htt\textsuperscript{Q111} mutations altered a number of shared processes and pathways. For example, pathways related to cell adhesion, plasma membrane, calcium ion binding and morphine addiction were amongst the most significantly up-regulated in response to either the Hdac2 KO mutation (Figure 3, contrast 1) or the Htt\textsuperscript{Q111} allele (Figure 3, contrast 3). Broadly, pathways related to neuronal function were down-regulated in both in Hdac2 KO striata and Htt\textsuperscript{Q111/+} striata, although the nature of these pathways was somewhat different, for example an enrichment for signaling pathways in Htt\textsuperscript{Q111/+} striata and for pathways related to neuronal and synapse structure in the Hdac2 KO. To gain further insight into commonalities between the impacts of Hdac2 KO and Htt\textsuperscript{Q111} mutations on transcriptional dysregulation we determined the overlaps in the DEGs and pathways enriched in DEGs elicited by the two mutations (i.e. overlap between contrasts 1 and 3) (Figure 3—figure supplement 1, yellow highlighted cells). There was a highly significant overlap in the DEGs and enriched pathways due to the Hdac2 KO or the Htt\textsuperscript{Q111} allele, largely reflecting overlaps in DEGs and enriched pathways altered in the same direction, i.e. up- or down-regulated in both conditions (statistical tests of enrichment were performed using one-sided Fisher exact tests – see Materials and methods, and results are shown in Figure 3—figure supplement 1C). We also compared the effect of the Hdac2 KO in the presence and absence of the Htt\textsuperscript{Q111} allele (Figure 3, contrasts 1 and 2). There were highly significant overlaps in the DEGs and enriched pathways altered in the same direction between two contrasts (Figure 3—figure supplement 1; pink highlighted cells). Finally, we compared the effect of the Htt\textsuperscript{Q111} allele in the presence and absence of the Hdac2 KO mutation (Figure 3, contrasts 3 and 4). The pathways downregulated by the Htt\textsuperscript{Q111} allele in the Hdac2 KO mice again largely encompassed those involved in neuronal function, whilst the most significantly upregulated pathways highlighted the extracellular matrix rather than cell adhesion pathways that were predominantly upregulated by the Htt\textsuperscript{Q111} allele on the Hdac2 WT background. Again, there were significant overlaps in DEGs and enriched pathways that were altered in the same direction by the Htt\textsuperscript{Q111} allele in Hdac2 WT and KO conditions (Figure 3—figure supplement 1, blue highlighted cells).

It should be noted that a direct comparison of gene dysregulation elicited by the Htt\textsuperscript{Q111} allele and Hdac2 KO is complicated by the fact that the Htt\textsuperscript{Q111} allele is constitutively expressed whereas the Hdac2 KO mutation is specific to striatal MSNs. Nevertheless, taken together, the results of our differential gene expression analyses, pathway enrichment and overlap analyses indicate that: (1) Genes and pathways can be dysregulated in the same direction by either the Htt\textsuperscript{Q111} allele or the Hdac2 KO mutation; (2) There is substantial overlap in gene dysregulation by the Hdac2 KO mutation in the presence or absence of the Htt\textsuperscript{Q111} allele, and vice versa. (3) The impact of one mutation depends in part on the presence of the other.

A subset of genes dysregulated in Htt\textsuperscript{Q111/+} striata is reversed by knockout of Hdac2 in MSNs

We were interested to determine the extent to which Htt\textsuperscript{Q111} -dysregulated genes might be reversed by the MSN-specific Hdac2 KO. However, given the overlap in gene dysregulation elicited by either the Htt\textsuperscript{Q111} allele or the Hdac2 KO (Figure 3; Figure 3—figure supplement 1) we wondered whether Htt\textsuperscript{Q111} -dysregulated genes might in fact be further changed in the same direction by the Hdac2 KO. To assess this, we first determined the set of genes that was dysregulated both in the comparison of Htt\textsuperscript{Q111/+} vs. Htt\textsuperscript{+/+} striata in the Hdac2 WT background (contrast 3) and in the comparison of Hdac2 KO vs. Hdac2 WT striata in the Htt\textsuperscript{Q111/+} background (contrast 2) (Figure 4A, left table; Figure 3—figure supplement 1, green highlighted cells, Source data 4). We identified 520 DEGs common to both comparisons applying a nominal p value < 0.05 to each differential gene expression analysis, while 17 were common to both comparisons applying an FDR-adjusted p value<0.05. The overlapping genes included both those that changed in opposite directions, i.e. up-
regulated by HttQ111 and then down-regulated by Hdac2 KO and vice versa, and those that changed in the same direction. The former (69 up-regulated by HttQ111 and down-regulated by Hdac2 KO plus 144 down-regulated by HttQ111 and up-regulated by Hdac2 KO) comprise 17% of all HttQ111-dysregulated genes (p<0.05), indicating possible rescue. The latter (158 up-regulated and 149 down-regulated by HttQ111 and by Hdac2 KO in HttQ111 striata) comprise ~24% of all HttQ111-dysregulated genes (p<0.05), indicating a sizeable proportion of genes for which the HttQ111-mediated dysregulation is exacerbated by the Hdac2 KO. Therefore, it appears that gene dysregulation elicited by the HttQ111 allele can be both exacerbated and reversed by Hdac2 KO.

Figure 3. Pathway enrichment of differentially expressed genes. The most significantly enriched pathways that are up- or down-regulated in each of four differential gene expression contrasts are shown. Numbers at the end of each bar show the number of genes represented in each pathway. For contrasts 1 and 2, DEGs used in the pathway analyses met the FDR < 0.05 threshold; for contrasts 3 and 4 DEG genes had a nominal p<0.05. The full set of pathways analyses is provided in Source data 2.

The online version of this article includes the following figure supplement(s) for figure 3:

Figure supplement 1. Overlaps of differentially expressed genes and enriched pathways in each of the four contrasts.
Figure 4. Rescue by Hdac2 KO of gene expression levels of a subset of genes dysregulated by the HttQ111 allele. (A) To identify genes dysregulated by the HttQ111 allele and by Hdac2 KO in HttQ111/+ mice, the overlapping genes in contrasts 3 and 2 were identified (left table). This shows the numbers of genes (p<0.05) up-regulated by HttQ111 and either further up-regulated, or down-regulated by Hdac2 KO in HttQ111/+ mice, and the numbers of genes down-regulated by HttQ111 and either further down-regulated, or up-regulated by Hdac2 KO in HttQ111/+ mice. To identify HttQ111-dysregulated genes whose expression level was normalized by Hdac2 KO, genes from the contrast 3/2 overlap whose expression level did not differ significantly (p>0.05) between HttQ111/+ Hdac2 WT and HttQ111/+ Hdac2 KO striata were identified (right table). (B) Heat map of the 97 genes down-regulated by HttQ111 and up-regulated by Hdac2 KO in HttQ111/+ striata and of the 55 genes up-regulated by HttQ111 and down-regulated by Hdac2 KO in HttQ111/+ striata, Figure 4 continued on next page
Figure 4 continued

whose expression levels did not differ significantly between Htt<sup>+/+</sup> Hdac2 WT and Htt<sup>Q111/+</sup> Hdac2 KO. (C) Examples of four such genes, two down-regulated by Htt<sup>Q111</sup> and two up-regulated by Htt<sup>Q111</sup> are displayed as box-plots.

The online version of this article includes the following figure supplement(s) for figure 4:

**Figure supplement 1.** Impact of Hdac2 KO on the most significantly dysregulated genes common to this study and Langfelder et al.

To investigate reversal of Htt<sup>Q111</sup>-mediated dysregulation by Hdac2 KO we performed t-tests on the 520 overlapping genes to define genes whose expression level did not differ significantly (p>0.05) between Htt<sup>+/+</sup> Hdac2 WT and Htt<sup>Q111/+</sup> Hdac2 KO striata, i.e. reversion of gene expression to wild-type in the presence of both mutant alleles (**Figure 4A**, right table). 152 genes had expression levels that did not differ significantly between these two genotypes, of which 97 were down-regulated by Htt<sup>Q111</sup> and up-regulated by Hdac2 KO and 55 were up-regulated by Htt<sup>Q111</sup> and down-regulated by Hdac2 KO (**Figure 4B**, lower table, *Source data 4*). These genes represent ~12% of the total number of Htt<sup>Q111</sup>-dysregulated genes (p<0.05). Note that a far greater number of genes did show significant differences in expression between Htt<sup>+/+</sup> Hdac2 WT and Htt<sup>Q111/+</sup> Hdac2 KO striata (**Figure 2**, contrast 5). Gene expression values for these 152 genes across all four genotypes are displayed as a heat map in **Figure 4B**, highlighting the comparable gene expression levels in Htt<sup>+/+</sup> Hdac2 WT and Htt<sup>Q111/+</sup> Hdac2 KO striata (middle two panels) for both Htt<sup>Q111/+</sup> down-regulated genes (left hand heat map) and Htt<sup>Q111/+</sup> up-regulated genes (right hand heat map). Also apparent are effects of the Hdac2 KO on these genes in Htt<sup>+/+</sup> striata (compare second and fourth panels), though to a lesser degree than in Htt<sup>Q111/+</sup> striata. Examples of two of these Htt<sup>Q111</sup> down-regulated genes and two Htt<sup>Q111</sup>-up-regulated genes are displayed as box-plots in **Figure 4C**. Of interest, one of these genes is Tcerg, a candidate human onset modifier (Genetic Modifiers of Huntington’s Disease (GeM-HD) Consortium, 2019).

We further specifically examined the gene expression of Hdac2 KO on the 29 genes (**Figure 2**—figure supplement 4) commonly dysregulated (FDR adjusted p<0.05) in the current study and in the Langfelder study (**Figure 4**—figure supplement 1). Two of these 29 genes (Ppp1r1b and Slc29a1) overlapped with the set of 152 genes whose expression level did not differ between Htt<sup>+/+</sup> Hdac2 WT and Htt<sup>Q111/+</sup> Hdac2 KO (**Figure 4D**; **Figure 4**—figure supplement 1A). An additional 13 (Cd200, Dmkn, Dsg, Gpd1, Innpsj, Myo5c, Pdp1, Ppp4r4, Ptprh, Ptprv, Sbsn, Syp, and Vill) overlapped with the set of 520 genes whose expression level was altered by Htt<sup>Q111</sup> and by Hdac2 KO in Htt<sup>Q111</sup> mice, but for which the Htt<sup>Q111</sup>-mediated gene dysregulation was not normalized by Hdac2 KO in Htt<sup>Q111</sup> mice. For the majority of these genes, Hdac2 KO altered gene expression in the same direction as the Htt<sup>Q111</sup> allele, indicating an exacerbation of the Htt<sup>Q111</sup>-mediated dysregulation (**Figure 4**—figure supplement 1B). For 14 of the 29 commonly Htt<sup>Q111</sup>-dysregulated genes (B3gnt2, C4a, Foxp4, Gpr6, Homer1, Lrrtm4, Myadn, Ndst3, Olfm3, Pde10a, Penk, Rgs9, Trpv2, Vrk1), their expression level was not further altered by the Hdac2 KO (**Figure 4**—figure supplement 1C).

Interestingly, we noticed that in these 29 commonly dysregulated genes, the relative impact of the Htt<sup>Q111</sup> allele was reduced in Hdac2 KO mice, i.e. the difference in gene expression between Htt<sup>Q111/+</sup> and Htt<sup>+/+</sup> is less in Hdac2 KO than in Hdac2 WT striata (**Figure 4**—figure supplement 1). To better visualize this, we plotted the absolute log 2-fold change (i.e. log2 fold-change regardless of the direction of change) between Htt<sup>Q111/+</sup> and Htt<sup>+/+</sup> for each of these genes in Hdac2 WT mice and in Hdac2 KO mice (**Figure 5**), highlighting the reduced fold-change for each of these genes in the Hdac2 KO, though to different degrees. Permutation (Materials and methods) showed a statistically significant enrichment for a reduced relative impact (absolute log2 fold-change) of the Htt<sup>Q111</sup> allele in the Hdac2 KO in these 29 genes (p<1e-5).

We therefore observe the following: (1) The absolute expression levels of a subset of Htt<sup>Q111</sup>-dysregulated genes are effectively normalized in Htt<sup>Q111</sup> mice harboring the Hdac2 KO mutation to levels present in wild-type mice, suggesting rescue of these gene expression changes; (2) The normalization of absolute expression levels of a subset of Htt<sup>Q111</sup>-dysregulated genes occurs in a substantially larger background of gene dysregulation elicited by the Hdac2 KO; (3) In addition, and apparent in a set of the most significantly Htt<sup>Q111</sup>-dysregulated genes, Hdac2 KO lessens the relative impact of the Htt<sup>Q111</sup> mutation, regardless of the absolute effect of the Hdac2 KO on the level
of gene expression. Taken together, our results indicate a potentially complex relationship between Hdac2 KO and the HttQ111 mutation.

Hdac2 and Hdac3 modify CAG expansion in HttQ111 knock-in mice

We next tested the impact of MSN-specific Hdac2 or Hdac3 KO on CAG expansion to determine whether these genes might be involved in the mechanism that drives the rate of phenotypic onset in this vulnerable cell population. We analyzed and quantified CAG striatal expansions in ABI GeneMapper traces of HTT CAG repeat-containing PCR products from cohorts of HttQ111/+ Hdac2 KO mice at 2.5, 5, and 10 months of age and from HttQ111/+ Hdac3 KO mice at 5 months of age (Figure 6). This analysis provides a measure of repeat length distribution across the entire population of striatal cells, and as MSNs contribute to the majority of the unstable alleles at the ages analyzed, it is sensitive to changes in repeat length that are confined to MSNs as shown previously (Kovalenko et al., 2012). Shown are representative raw GeneMapper traces (Figure 6A) and normalized GeneMapper data, averaged per genotype, showing the height of each peak and the change in CAG length relative to those of the main (modal) allele (Figure 6B). We determined an expansion index (Lee et al., 2010) from the GeneMapper data, a measure incorporating both the relative height and distance from the main allele of the expansion peaks representing the mean repeat expansion in the cell population (Figure 6C). Mean CAG lengths were matched between genotypes for each cohort (Source data 1). At 2.5 months of age, we observed a subtle decrease in expansion index in Hdac2 KO striata that was not statistically significant (Hdac2 WT: mean 5.76, 95%
CI \([4.64, 6.88]\); \(Hdac2\) KO: mean 5.29, 95\% CI \([4.23, 6.35]\); two-tailed unpaired t-test \(p=0.42\). At 5 months of age the expansion index was significantly reduced in \(Hdac2\) KO striata (\(Hdac2\) WT: mean 15.22, 95\% CI \([14.16, 16.28]\); \(Hdac2\) KO: mean 12.69, 95\% CI \([10.91, 14.47]\); two-tailed unpaired t-test \(p=0.0094\)), while at 10 months of age, we again observed a small reduction in expansion in \(Hdac2\) KO striata; this was most apparent from the GeneMapper traces of closely CAG repeat-matched mice (panel A), though this did not reach statistical significance (\(Hdac2\) WT: mean 21.55, 95\% CI \([19.62–23.48]\); \(Hdac2\) KO: mean 20.02, 95\% CI \([17.1–22.94]\); two-tailed unpaired t-test \(p=0.26\)) across this cohort of mice with a wide range of CAG lengths (Source data 1). Knockout of \(Hdac3\) in MSNs also decreased the expansion index in the striata of 5 month mice (\(Hdac3\) WT: mean

Source data 1. Expansion Indices in \(Hdac2\) and \(Hdac3\) KO mice.

Figure supplement 1. Expression of Cre transgene does not affect \(HTT\) CAG repeat instability and nuclear huntingtin accumulation in MSNs.

Figure supplement 1—source data 1. Expansion Indices and mAb5374 nuclear huntingtin immunostaining intensities in Cre-expressing HttQ111 mice.

Figure supplement 2. Effect of \(Hdac2\) or \(Hdac3\) deletion in striatal MSNs on number of DARPP-32-positive cells and DARPP-32 levels.

Figure supplement 2—source data 1. DARPP32-positive cells and DARP32 levels in \(Hdac2\) and \(Hdac3\) KO mice.

Figure supplement 3. DNA repair gene expression.

Figure supplement 3—source data 1. MSH3 protein levels in \(Hdac2\) KO mice.
13.94, 95% CI [12.81–15.07]; Hdac3 KO: mean 10.43, 95% CI [7.62–13.24]; two-tailed unpaired t-test p=0.0026).

Note that the expression of Cre recombinase itself did not impact CAG expansion (Figure 6—figure supplement 1); therefore, the observed impacts on striatal instability are attributable to the Hdac2 or Hdac3 genotype of the mice. Further, as neither Hdac2 KO nor Hdac3 KO resulted in any significant loss of MSNs as determined by the number of DARPP-32-positive cells (Figure 6—figure supplement 2), reduced striatal expansions cannot be readily explained by an altered cellular population biased toward stable alleles. Of note, HDAC3 is required for the long-term maintenance of cerebellar purkinje cells (Venkatraman et al., 2014), although its knock-out in the CA1 region of the hippocampus did not have a detrimental effect on cell survival (McQuown et al., 2011) in line with the lack of obviousMSN degeneration in our Hdac3 knockout mice. Thus, HDAC3’s role in neuronal survival may depend on cell-type and/or stage of neuronal development or differentiation.

Overall, our results support the conclusion that Hdac2 and Hdac3 are moderate enhancers of CAG expansion in MSNs of HttQ111/– mice, with the impact of Hdac2 KO appearing to depend on the age of the mice. To explore a possible mechanism for instability modification, we investigated the RNA-seq data for expression levels of DNA repair genes that modify repeat instability in mice (Kovalenko et al., 2012; Dragileva et al., 2009; Pinto et al., 2013; Kovtun et al., 2007; Mollersen et al., 2012; Zhao and Usdin, 2018; Miller et al., 2020). Of these, only Msh3 mRNA levels were modestly decreased in HttQ111/– Hdac2 KO striata relative to HttQ111/+ Hdac2 KO striata (nominal p<0.01; adjusted p=0.07) (Figure 6—figure supplement 3A, B). However, MSH3 protein levels were not reduced in Hdac2 KO striata from these mice (Figure 6—figure supplement 3C, D). Therefore, dysregulation of DNA repair gene expression does not provide an obvious explanation for the impact of Hdac2 KO on CAG expansion.

Pharmacological HDAC inhibition promotes CAG contraction in a cell-based assay

Previous studies have found that Class I HDAC inhibitors suppress CAG expansion (Jung and Bonini, 2007; Suelves et al., 2017; Debacker et al., 2012). To test further the impact of HDAC inhibition on CAG instability, we took advantage of a well-established, sensitive human cell-based assay for repeat contractions (Figure 7A). In this system, a CAG95 repeat is inserted in an intron of an HPRT mini-gene construct. Contractions below a threshold of ~38–41 CAGs restore HPRT activity allowing cell growth in selective media. Although the system is blind to expansions, there is relatively good consistency between genetic modifiers identified in this assay and those that modify CAG expansion in mouse models, although the direction of effect can be different (consistency between genetic modifiers identified in this assay and those that modify CAG expansion).

Impact of MSN-specific Hdac2 and Hdac3 knockout on nuclear huntingtin pathology

HttQ111 mice exhibit both early (~2.5 months) diffusely immunostaining nuclear mutant huntingtin, detected with the anti-huntingtin antibody EM48 (or mAb5374), followed at ~6–12 months by the...
appearance of discreet intra-nuclear inclusions (Wheeler et al., 2000; Wheeler et al., 2002; Lloret et al., 2006). These cellular phenotypes, which occur selectively in MSNs in HttQ111 mice, are highly CAG repeat length-dependent and are modified by genes that alter somatic expansion (Kovalenko et al., 2012; Pinto et al., 2013). To assess the impact of MSN-specific Hdac2 and Hdac3 KO on these phenotypes we immunostained striatal sections from cohorts of mice (Hdac2 KO at 2.5, 5 and 10 months of age; Hdac3 KO at 5 months of age) matched for constitutive CAG repeat length (Source data 1) with mAb5374 (Figure 8). With the exception of the 2.5-month Hdac2 KO cohort, mAb5374 immunostaining was performed on the contralateral striata of the same mice analyzed for somatic CAG expansion above.

In 2.5- and 5-month mice, we quantified the intensity of the diffuse nuclear signal. Hdac2 KO in MSNs reduced the intensity of diffusely immunostaining nuclear mutant huntingtin at both 2.5 months (Hdac2 WT: mean 153.9, 95% CI [127.9, 179.9]; Hdac2 KO: mean 96.35, 95% CI [5.63, 187.1]; two-tailed unpaired t-test p=0.025) and 5 months of age (Hdac2 WT: mean 226.8, 95% CI [159.9, 293.8]; Hdac2 KO: mean 120.9, 95% CI [77.34, 164.5]; two-tailed unpaired t-test p=0.0062), with a stronger impact at 5 months (Figure 8A, B). Diffuse nuclear huntingtin immunostaining was not altered by Cre expression alone (Figure 6—figure supplement 1). In 10-month mice, nuclei contained distinct inclusions. As the immunofluorescent signal is saturated in these dense inclusions we quantified their number rather than their intensity. This phenotype was variable between mice of the same genotype, largely due to constitutive CAG length. We therefore separated the mice into ‘high’ and ‘low’ CAG groups that reflected different breeding cohorts for comparison between Hdac2
Figure 8. Impact of MSN-specific Hdac2 or Hdac3 knockout on striatal nuclear mutant huntingtin immunostaining phenotypes. (A) Fluorescent micrographs of Htt<sup>Q111/+</sup> Hdac2 KO striata immunostained with anti-huntingtin mAb5374 and histone H3 antibodies. Mice were matched by CAG repeat length within each age group. Images of 2.5-month and 5-month striata were taken at 40x; images of 10-month striata taken at 20x with 2x digital zoom. The inserts in the 5-month and 10-month Hdac2 WT mAb5374 images and merged images below highlight the overlap of nuclear huntingtin immunostaining over the entire H3-positive nucleus in the 5-month mice, in contrast to the discrete nuclear huntingtin inclusion immunostaining intensity within the nucleus at 10 months. (B) Mean integrated intensity of mAb5374 staining (intensity normalized by the total number of nuclei as determined by the number of H3-positive nuclei) is shown for 2.5-month and 5-month cohorts of Htt<sup>Q111/+</sup> Hdac2 KO mice. For 10-month mice, the number of nuclei containing an inclusion (we have not observed more than one inclusion per nucleus) was counted as a percentage of the total number of nuclei, determined by histone H3 immunostaining. The 10-month group of mice was divided into two subgroups based on CAG repeat length: low CAG repeat subgroup represented by the first two columns in the graph (Hdac2 WT CAG 117, 119, 120; Hdac2 KO CAG 110, 116) and high CAG repeat subgroup represented by the last two columns in the graph (Hdac2 WT CAG 138, 141, 146; Hdac2 KO CAG 132, 140, 140). (C) Fluorescent micrographs (20x) of 5 month Hdac3 KO from CAG length-matched mice stained with mAb5374 and histone H3 antibodies (left) and quantified mean integrated intensity of mAb5374 staining (right). 5-months. Numbers of mice for each group are indicated on the bar graphs.

Source data 1. mAb5474 nuclear intensity and number of nuclear inclusions in Hdac2 and Hdaac3 KO mice.

Figure supplement 1. Huntingtin expression.

Figure supplement 1—source data 1. Zip file: RNAseq_codes_and_files.
the low CAG group that did not reach statistical significance (Hdac2 WT: mean 8.76, 95% CI [5.29, 22.81]; Hdac2 KO: mean 0.66, 95% CI [-7.74, 9.06]; 2-tailed unpaired t-test p=0.153), and a statistically significant decrease in the number of cells containing inclusions in the high CAG group (Hdac2 WT: mean 37.8, 95% CI [21.26, 54.31]; Hdac2 KO: mean 23.44, 95% CI [16.58, 30.31]; 2-tailed unpaired t-test p=0.0261). In the 5-month Hdac3 KO cohort, we observed a non-statistically significant trend towards decreased diffuse nuclear mutant huntingtin in a small number of immunostained 5-month Hdac3 WT striata (Hdac3 WT: mean 234, 95% CI [91.13, 376.9]; Hdac3 KO: mean 181.2, 95% CI [72.92, 289.5]; two-tailed unpaired t-test p=0.21). (Figure 8C). To examine the relationship between CAG expansion and nuclear huntingtin pathology, we compared directly expansion indices and mAb5374 intensity in the 5-month Hdac2 KO cohort for which we had both measures from the same mice with a reasonable number (N = 5) of each genotype. There was a strong correlation (Pearson r = 0.87, p=0.0011) between mAb5374 intensity and expansion index (Figure 8D), consistent with somatic CAG expansion as a modifier of this cellular phenotype. Overall, the fairly moderate suppression of the mAb5374 nuclear intensity and nuclear inclusion phenotypes appears in line with the subtly reduced somatic expansion elicited by the Hdac2 or Hdac3 gene knockouts.

As altered huntingtin levels could contribute to the reduced nuclear immunostaining phenotypes, we investigated huntingtin mRNA and protein levels in the Hdac2 KO mice. In the RNA-seq data, Hdac2 KO decreased total huntingtin mRNA levels in Htt+/+ striata by 5.9% (nominal p=0.06; adjusted p=0.25) and in HttQ111/+ striata by 11% (nominal p<0.001; adjusted p<0.05) (Figure 8—figure supplement 1). However, western blot analyses on the contralateral striata from a subset of the mice used for RNA-seq did not provide evidence for an impact of Hdac2 KO on steady state levels of wild-type or mutant huntingtin protein (Figure 8—figure supplement 1C, D). We also investigated the RNA-seq data for reads spanning exon 1 and intron 1, reflecting aberrantly spliced transcripts identified in a number of other HD models and predicted to encode exon1-containing N-terminal fragment of mutant huntingtin (Sathasivam et al., 2013). In support of previous data (Sathasivam et al., 2013), we identified exon1-intron1-spanning transcripts in HttQ111/+ striata. However, Hdac2 KO had no impact on the number of these sequence reads (Figure 8—figure supplement 1B). It appears therefore that altered levels of mutant huntingtin or exon1 mutant huntingtin expressed from mis-spliced exon1-intron1 transcripts do not readily explain suppression of nuclear huntingtin pathology elicited by Hdac2 KO. This is consistent with modification of these highly CAG length-dependent cellular phenotypes occurring as a consequence of reduced CAG repeat lengths in striatal MSNs. However, analyses of larger cohorts of mice as well as analyses of rare expansions not captured using GeneMapper would be needed to rigorously discern Hdac2 and Hdac3 genotype effects on nuclear huntingtin pathology phenotypes that are dependent on or independent of somatic CAG expansion.

Discussion

Identification of genetic modifiers of disease phenotypes in HD mouse models provides an important route to dissecting underlying mechanisms of pathogenesis and ultimately for identifying therapeutic targets. Here, we have taken a cell type-specific genetic knockout approach to investigate the roles of Hdac2 and Hdac3 genes in as modifiers of molecular or cellular phenotypes elicited in MSNs by an expanded CAG repeat in heterozygous HttQ111 knock-in mice.

We restricted our analyses to previously described robust molecular and cellular changes in this model, specifically somatic instability, thought to drive the rate of disease onset in patients, transcriptional dysregulation and nuclear huntingtin pathology. We did not quantify neuronal cell loss and altered astrocytic or microglial cell density as these phenotypes are extremely subtle or not observed in this model. Similarly, we have not examined behavioral endpoints that are also subtle, as such studies would need significantly larger numbers of mice to be sufficiently powered.

Our results show that MSN-specific knockout of Hdac2 reversed the transcriptional dysregulation of a subset of genes and resulted in a moderate reduction in somatic CAG expansion and nuclear huntingtin pathology. MSN-specific knockout of Hdac3 resulted in a comparable reduction in somatic CAG expansion to the Hdac2 knockout. The impact of genetic knock-out or reduction of several Hdac genes (Hdac3, Hdac4, Hdac6, Hdac7) has previously been investigated in the R6/2 exon 1 mouse model of HD and in HdhQ150 knock-in mice with the goal of understanding which HDAC targets mediated the beneficial effects of broad-acting HDAC inhibitors (Benn et al., 2009;
Bobrowska et al., 2011; Moumné et al., 2012; Mielcarek et al., 2013). With the exception of Hdac6, the impact of the full knock-out could not be studied due to embryonic or early postnatal lethality. Of the genes tested, only Hdac4 was found to modify mouse phenotypes, uncovering a novel role for this gene in modulating mutant huntingtin aggregation (Mielcarek et al., 2013). In R6/2 mice, the lack of any modifying effect of Hdac3 may be due to the modest reduction in HDAC3 protein level (to 80% of wild-type overall, or to 60% of wild-type in the nucleus) in the Hdac3+/− mice (Moumné et al., 2012).

Strikingly, knockout of Hdac2 in MSNs caused substantial transcriptional dysregulation, resulting in both up- and down-regulation of a large number of genes. These findings are consistent with data showing that HDAC1/2 can both positively and negatively regulate gene expression (Kelly et al., 2018). It is notable that the majority of mouse tissue-specific deletions of either Hdac2 or Hdac1 exhibited no discernible phenotype, presumably due to functional redundancy of the two highly related HDAC1 and HDAC2 proteins (Kelly et al., 2018). Our data reveal that, despite its up-regulation, HDAC1 is unable to compensate for the lack of HDAC2 in MSNs, providing evidence for a specific role of HDAC2 in MSNs and supporting distinct functions of HDAC1 and HDAC2 in adult neurons (Guan et al., 2009; Kim et al., 2008). The expression level of approximately 12% of genes dysregulated by the HttQ111 allele was normalized by the Hdac2 KO to levels present in wild-type mice. However, this occurs against a much larger background of transcriptional dysregulation elicited by the Hdac2 KO, with evidence for shared pathways and processes being impacted by both the HttQ111 and Hdac2 knockout mutations. Indeed, a large proportion (~24%) of genes dysregulated by the HttQ111 allele was further dysregulated in the same direction by the Hdac2 KO. It should be noted in interpreting these data that the majority of genes dysregulated by the HttQ111 allele did not meet a p value threshold corrected for multiple testing, although a large proportion of genes dysregulated by the Hdac2 KO did. We therefore examined a core set of 29 genes significantly dysregulated (FDR adjusted p<0.05) by HttQ111 in both in the current study and in Langfelder et al., an extensive investigation of transcriptional dysregulation in HD knock-in mice Langfelder et al., 2016. Of these genes, only two met our criteria for rescue (Ppp1r1b and Slc29a1); for the other genes in this set, Hdac2 KO either further altered the absolute gene expression level in the same direction as the HttQ111 allele or had no impact on expression. However, in these significantly dysregulated genes, we found that Hdac2 KO lessened the relative impact of the HttQ111 allele, regardless of the absolute gene expression change elicited by the Hdac2 KO. This genetic interaction indicates a functional relationship between these two mutations that may in part reflect a role of huntingtin in regulating the REST-containing transcriptional repressor complex that includes HDAC2 (Buckley et al., 2010).

Several studies have shown beneficial effects of HDAC inhibitors in HD mouse models, including broad-acting HDAC inhibitors SAHA, phenylbutyrate and parabinoestat (LBH589), as well as compounds RGFP966 and 4b that show moderate selectivity towards HDAC3 (Hockly et al., 2003; Gardian et al., 2005; Thomas et al., 2008; Mielcarek et al., 2011; Jia et al., 2012a; Jia et al., 2012b; Jia et al., 2016; Chopra et al., 2016; Suelves et al., 2017; Siebzehnrübl et al., 2018). The impact on transcriptional dysregulation was investigated in a number of these studies, with most focusing on specific candidate genes. Overall, these studies revealed relatively modest reversal of transcriptional changes elicited by the mutant transgenes, though HDAC inhibitor 4b was found to normalize ~1/3 of genes altered in R6/2 mice harboring 300 CAG repeats (Thomas et al., 2008). Our data indicate that HDAC inhibitors with activity against HDAC2 would have the potential to reverse some of the early transcriptional changes elicited by an expanded CAG mutation. However, our results also indicate a complex relationship between the expanded CAG repeat mutation and Hdac2, with potential implications for therapies directed at HDAC2 inhibition.

We demonstrate here that Hdac2 and Hdac3 are enhancers of CAG expansion in MSNs. It is important to note that neither knockout of Hdac2 nor Hdac3 had a large impact on CAG expansion, in contrast to a number of MMR genes that are fundamental drivers of this process and whose knockouts eliminate expansion (Wheeler et al., 2003; Dragileva et al., 2009; Pinto et al., 2013). Our results are consistent with those of Suelves et al. in which treatment of HttQ111 mice with HDAC inhibitor RGFP966, resulted in a slight reduction in striatal CAG expansion (Suelves et al., 2017). HDAC3 and HDAC5 enhanced CAG expansion in a selectble human astrocyte cell-based assay, whereas HDAC9 suppressed expansions (Debacker et al., 2012; Gannon et al., 2012). A role for HDAC2 was not implicated in this system, based on differential effects of HDAC inhibitors with
selectivity towards HDACs 1 and 2 or HDAC3 (Debacker et al., 2012). Further studies are needed to understand the mechanism(s) by which Hdac2 and Hdac3 enhance CAG expansion in HD mice. There is evidence that CAG instability may depend on the chromatin environment in the vicinity of the repeat (Libby et al., 2003; Libby et al., 2008; Nestor and Monckton, 2011; Neto et al., 2017; Goula et al., 2012; Dion and Wilson, 2009), and both HDAC2 and HDAC3 play roles in regulating genome stability via effects on histone acetylation and recruitment or accessibility of DNA repair proteins (Miller et al., 2010; Bhaskara et al., 2010). Therefore, it is possible that Hdac2 or Hdac3 modify CAG instability by altering chromatin structure close to the CAG repeat tract. We did observe a small reduction in huntingtin mRNA in Hdac2 knockout mice (Figure 8—figure supplement 1) suggesting a possible alteration in transcriptional dynamics at the Htt locus that may reflect an underlying change in chromatin structure. Our data argue, at least for the Hdac2 KO, against altered transcription of genes encoding DNA repair proteins underlying the reduction in somatic expansion. However, it is possible that changes in acetylation of DNA repair proteins could lead to their altered activity (Chatterjee et al., 2012; Choudhary et al., 2009; Zhang et al., 2014; Radhakrishnan et al., 2015; Piekna-Przybylska et al., 2016; Zhang et al., 2019). This was proposed as a plausible mechanism by which HDAC3 and HDAC5 enhance CAG expansion in a selectable human astrocyte cell-based assay, in part based on epistasis experiments showing that HDAC3 and HDAC5 act in the same pathway as MSH2 (Debacker et al., 2012; Gannon et al., 2012). It was recently suggested that HDAC3 might modify CAG instability by altering the subcellular localization of MSH3 (Williams et al., 2020). Understanding the interplay between HDACs and DNA repair proteins controlling CAG repeat dynamics may provide important insight into the regulation of these DNA repair proteins and potentially novel ways in which to intervene in the process of CAG expansion.

MSN-specific Hdac2 knockout also reduced nuclear huntingtin intensity and inclusion phenotypes, consistent with decreased striatal CAG expansions and the known sensitivity of these phenotypes to repeat length and to modification of somatic instability (Kovalenko et al., 2012; Dragileva et al., 2009; Pinto et al., 2013; Wheeler et al., 2002). We did not observe a significant effect of Hdac3 knockout on nuclear huntingtin intensity in the few mice analyzed and would likely need larger numbers of mice to observe a reduction in this phenotype that might be predicted by the lower striatal CAG repeat lengths in Hdac3 KO mice. Most studies of pharmacological or genetic inhibition of class I HDACs in HD mouse models have reported a lack of impact on nuclear inclusions or aggregation (Hockly et al., 2003; Gardian et al., 2005; Jia et al., 2016; Moumne et al., 2012), although HDAC inhibitor 4b reduced aggregates in the N171-82Q model (Jia et al., 2012b). We suggest that Hdac2 knockout modifies nuclear intensity and inclusion phenotypes in HttQ111/+ mice at least in part as a consequence of reduced CAG expansion in MSNs. Other mechanisms are also plausible, however, effects on mutant huntingtin levels themselves are not supported by our data. In contrast, the modest reduction in CAG expansion caused by the Hdac2 knockout is unlikely to explain the reversal of transcriptional dysregulation elicited by the HttQ111 allele, in which gene expression levels that can be distinguished between wild-type and HttQ111/+ mice appear effectively normalized to wild-type in HttQ111/+ mice harboring the Hdac2 knockout mutation.

In summary, we have tested whether Hdac2 and Hdac3 are modifiers of different aspects of the HD pathogenic process in MSNs that are both inherently prone to CAG repeat expansion and specifically vulnerable in the disease process. The results of this study indicate moderate effects of these genes on CAG expansion, which in humans drives the rate of disease onset, and for Hdac2, on downstream molecular and cellular phenotypes that may be important in cellular toxicity mechanisms.

Materials and methods

Mouse lines and breeding

HttQ111 knock-in mice used in this study were on a C57BL/6J background (Lee et al., 2011) and were maintained by breeding heterozygous males to C57BL/6J wild-type females from The Jackson Laboratory (Bar Harbor, ME). D9-Cre transgenic mice contain a genomic fragment, comprising about 2 kb of 5’ regulatory sequence, the endogenous ATG, and some of the introns and exons of the mouse Ppp1r1b gene encoding DARPP-32, driving the expression of Cre recombinase in MSNs after
5–6 weeks of age (Bogush et al., 2005). These mice were maintained on a C57BL/6J background. Mice harboring conditional ‘floxed’ Hdac2 or Hdac3 alleles contained loxp sites upstream of exon2/ downstream of exon 4 of the Hdac2 gene (Montgomery et al., 2007) and upstream of exon 11/ downstream of exon 14 of Hdac3 gene (Montgomery et al., 2008). Both floxed mouse lines were originally on a mixed C57BL/6/129SeEv background and subsequently maintained by breeding heterozygous males or females to C57BL/6J wild-type mice from The Jackson Laboratory.

Detailed breeding schemes are shown in Figure 1—figure supplement 1. Mice carrying conditional floxed Hdac2 or Hdac3 alleles were crossed with HttQ111/ mice and, in a separate cross, with D9-Cre mice. The progeny from the two crosses were bred together to obtain Htt+/+ and HttQ111/+ mice with or without the Hdac2 (or Hdac3) floxed allele and/or Cre transgene. Mice harboring either no floxed Hdac alleles in the presence of Cre or one, two, or no floxed alleles in the absence of Cre were wild-type with respect to HDAC expression and are called Hdac2 WT or Hdac3 WT. Mice homozygous for the floxed Hdac alleles and expressing Cre are called Hdac2 KO or Hdac3 KO. In these mice the Hdac2 or Hdac3 alleles are deleted in MSNs and do not express HDAC2 or HDAC3 in MSNs. In a parallel cross, HttQ111/+ mice were crossed with D9-Cre mice to generate Htt+/+ and HttQ111/+ mice with or without the Cre transgene, with the purpose of testing the effect of Cre expression alone on the phenotypes being measured. Note that mice were not fully backcrossed to C57BL/6J prior to the crosses with B6J.D9-Cre mice and B6J.HttQ111 mice. However, analyses of our RNA-seq data showed that all mice had a B6 haplotype for the Mlh1 gene, where genetic variation between B6 and 129 strains is known to associate with CAG instability (Pinto et al., 2013).

Numbers of mice used in this study were gauged based on extensive prior knowledge of cellular and molecular phenotypes elicited by the HttQ111 allele and their genetic modification (Kovalenko et al., 2012; Pinto et al., 2013; Langfelder et al., 2016), and incorporated controlling for inherited CAG repeat length (Source data 1). In all the mouse experiments, ‘N’=number of mice (biological replicates).

Genotyping

Genomic DNA was extracted from tail or ear biopsies taken at weaning for genotyping, or from adult striatum for the CAG repeat instability assay (Qiagen DNeasy blood and tissue kit). Genotypes were further confirmed in striatal DNA. CAG sizing was carried out as previously described, using human HTT CAG-specific primers (Dragileva et al., 2009). PCR products were resolved using the ABI3730xl automated DNA analyzer (Applied Biosystems). GeneMapper v3.7 with GeneScan 500-LIZ as internal size standard was used to assign repeat size, defined as the highest peak in the Gene-Mapper trace. All runs included the same control DNAs of known HTT CAG repeat size. We incorporated a novel PCR genotyping assay that distinguished knock-in and mouse wild-type alleles using primers flanking the 5’ length of the floxed neomycin resistance cassette used in the original gene targeting (White et al., 1997; Wheeler et al., 1999). The assay included 0.5 μM forward primer (5’TCTGATACAGCCCATGCTGA), 0.5 μM reverse primer (5’CTGAGTTCAA TCCCTGCATT), and 2X Phusion High-Fidelity PCR Master Mix with HF Buffer (New England Biolabs). Cycling conditions were 98°C for 30 s; 30 cycles of 98°C for 5 s, 62°C for 15 s, 72°C for 20 s; 72°C for 10 min, generating a 129 bp product from the wild-type allele and a 240 bp product from the HttQ111 knock-in allele.

D9-Cre mice were genotyped as described (Bogush et al., 2005). For Hdac2 genotyping, wild-type and floxed alleles were detected by a triplex PCR assay. The forward primer upstream of 5’ loxP site (HDAC2WT-F: 5’GGCCAAAGCATATCAACACC) and the reverse primer between the 5’ loxP site and exon 2 (HDAC2-REV: 5’GTCAGCTAGTAGTGCCTTGG) generated a 226 bp product for the wild type Hdac2 allele, while the forward primer within the 5’ loxP site (HDAC2MUT-F: 5’G TCCCTGCAGGCTAGGACC) and the same reverse primer gave a 146 bp PCR product for the floxed allele. The PCR assay contained 0.2 μM each of HDAC2WT-F and HDAC2MUT-F primers, 0.4 μM of HDAC2-REV primer, 0.025 units GoTaq DNA polymerase (Promega), 5X GoTaq buffer (Promega), additional MgCl2 (5 mM), and dNTP mixture (0.05 mM each). Cycling conditions were 94°C for 2 min; 30 cycles of 94°C for 15 s, 60°C for 30 s, 72°C for 40 s; 72°C for 5 min. To detect specifically the deleted Hdac2 allele, a different forward primer upstream of 5’ loxP site (5’GTGGGAAGCA TGCCAGCATG) and reverse primer downstream of the 3’ loxP site, between exons 4 and 5 (5’ GCCTCTAAGAACCCTCAGGGAAC) were used, resulting in a 550 bp product upon Cre-mediated excision. PCR conditions were 0.2 μM of each primer, 0.025 units GoTaq DNA polymerase.
Promega), 5x GoTaq buffer (Promega), additional MgCl₂ (5 mM), and dNTP mixture (0.05 mM each). Cycling conditions were 94°C for 2 min; 30 cycles of 94°C for 15 s, 68°C for 30 s, 72°C for 40 s; 72°C for 5 min. For Hdac3 genotyping, a triplex assay was used to detect all three alleles, with one forward/reverse pair of primers up- and downstream from the 5’ loxP site (HDAC3-5’-loxP-F: 5’ GC TTGTAGCCAGCCAGCTTAG, and HDAC3-5’-loxP-R: 5’ CATGTGACCCCAGACATGACTGG), and the third (reverse) primer downstream of the 3’ loxP site (HDAC3-3’-loxP-R: 5’ CAGTCCATGCCCTTAAATCCAGC). This assay produced a 350 bp product for the wild-type Hdac3 allele, a 450 bp product for the ‘floxed’ allele (both products were from HDAC3-5’-loxP-F and HDAC3-5’-loxP-R primers), and a 650 bp product for the deleted Hdac3 allele after Cre-mediated excision (from HDAC3-5’-loxP-F and HDAC3-3’-loxP-R primers). The PCR assay contained 0.2 μM each of the three primers, 0.025 units GoTaq DNA polymerase (Promega), 5x GoTaq buffer (Promega), additional MgCl₂ (5 mM), and dNTP mixture (0.05 mM each). Cycling conditions were 94°C for 2 min; 30 cycles of 94°C for 15 s, 60°C for 30 s, 72°C for 40 s; 72°C for 5 min.

Measurement of CAG expansion

Expansion indices in striatum were quantified from GeneMapper traces of PCR-amplified HTT CAG repeat, as described previously (Lee et al., 2010), taking into account only expansion peaks to the right of the highest peak (main allele) and without filtering peaks based on relative peak height. Although there is intrinsic PCR bias in analyses of instability from ‘bulk DNA’, this nevertheless allows quantification of subtle differences in repeat instability, and metrics derived from the GeneMapper traces correlate well with repeat length distributions obtained from analyses of single molecules (Lee et al., 2010). Briefly, normalized peak heights were calculated by dividing each expansion peak height by the sum of the heights of the main allele plus all expansion peaks, the change in CAG length of each expansion peak from the main allele was determined, each normalized peak height was multiplied by the CAG change from the main allele, and these values were summed to generate an expansion index that represents the mean positive CAG repeat length change in the population of cells being analyzed.

Immunohistochemistry

Primary antibodies used were: mouse monoclonal anti-huntingtin (mAb5374, Millipore), rabbit polyclonal anti-histone H3 (ab1791, Abcam), rabbit polyclonal anti-HDAC2 (ab7029, Abcam), rabbit monoclonal anti-HDAC3 (clone Y415; formerly available from Millipore), mouse monoclonal anti-DARPP-32 (D32-6a; a kind gift from Dr. Angus Nairn) and rabbit polyclonal anti-DARPP-32 (ab40801, Abcam).

Immunostaining was performed on 8 μm coronal sections of periodate-lysine-paraformaldehyde (PLP)-perfused and -post-fixed, paraffin-embedded brains. Perfusion and tissue processing/embedding methods have been described previously (Wheeler et al., 2000). For most mouse cohorts, one hemisphere of the brain was embedded for sectioning and the other one was used for dissection of striatum for analysis of somatic instability. Sections were dewaxed and subjected to heat-mediated epitope retrieval (Na-citrate buffer pH 6.0) followed by quenching of endogenous peroxidase with 0.3% H₂O₂/methanol for 30 min at room temperature (RT), and blocked in 3% normal horse serum (NHS) in Tris-buffered saline (TBS) for 1 hr at room temperature. Immunostaining for diffuse nuclear huntingtin, huntingtin inclusions and histone H3 was described in detail (Kovalenko et al., 2012). For double staining with mouse anti-DARPP-32/rabbit anti-HDAC3 antibodies sections were incubated with both primary antibodies (D32-6a at 1:200 and Y415 at 1:2000 in 1% NHS/TBS) overnight at 4°C, followed by sequential amplification of HDAC3 and DARPP-32 signal. First, HDAC3 signal was amplified using the TSA Biotin System (Perkin Elmer) according to manufacturer’s instructions and quenching of HRP activity (0.1% sodium azide/0.3% H₂O₂ in TBS for 30 min at RT) was performed before the addition of streptavidin-Alexa Fluor 555. Then, DARPP-32 signal was amplified using TSA kit #2 (Invitrogen/Thermo Fisher Scientific) by incubating with goat anti mouse-HRP at 1:100 for 1 hr at RT and then with Tyramide-Alexa Fluor-488 at 1:100 in the amplification diluent (all provided with the TSA kit #2), for 25 min at RT. For double staining with mouse anti-DARPP-32/rabbit anti-HDAC2 antibodies sections were incubated with both primary antibodies (D32-6a at 1:200 and ab7029 at 1:1000 in 1% NHS/TBS) overnight at 4°C.
DARPP-32 signal was amplified as described above, and HDAC2 signal was detected with donkey anti-rabbit Alexa Fluor 555-conjugated secondary antibody (Invitrogen/Thermo Fisher Scientific, 1:1000) added as the last step after DARPP-32 signal amplification. For staining with rabbit anti-DARPP-32 antibody sections were incubated with ab40801 at 1:750 at 4˚C overnight, followed by donkey anti-rabbit Alexa Fluor 488 secondary antibody.

All slides were mounted in ProLong Gold antifade reagent (Invitrogen/Thermo Fisher Scientific). Fluorescent microscopy was performed with a Zeiss Axioskop two microscope equipped with AxioCamMRm camera and AxioVision 4.6 image acquisition software, using Plan Apochromat 20x/0.8 M27 or Plan Neofluar 40x/0.75 Ph2 objectives. Images that were to be quantified and compared were taken with the same exposure times.

Quantification of immunohistochemical data

Quantification was performed as described previously (Kovalenko et al., 2012), using CellProfiler 3.0.0 image analysis software (Carpenter et al., 2006). Briefly, diffuse nuclear huntingtin immunostaining was quantified in four 40x striatal images per mouse (one medial, located next to the lateral ventricle, and one lateral, located at the end of external capsule; from two consecutive sections). Total (integrated) intensity of mAb5374 staining was measured in all mAb5374-positive nuclei in each image and normalized by the total number of nuclei (as determined by the number of all histone H3-positive nuclei) in the same image. The resulting value, representing the mean intensity of mAb5374 staining per nucleus, was averaged from four images for each mouse. The number of nuclear huntingtin inclusions (total per image) was quantified, blind to genotype, with CellProfiler 3.0.0 in two 20x images per mouse (taken mid-striatum from two consecutive coronal sections), normalized by the total number of histone-H3-positive nuclei in each image (taken as 100%), and resulting percentages were averaged over these two images for each mouse. Number of DARPP-32-positive cells and total integrated intensity of DARPP-32 staining in DARPP-32-positive cells were also quantified with CellProfiler 3.0.0 in four 40x images per mouse, taken in the same striatal locations as for diffuse nuclear huntingtin immunostaining quantification. Total integrated intensity was normalized by the number of DARPP-32-positive cells in each image to obtain mean integrated intensity values. Both mean integrated intensity of DARPP-32 staining and the number of DARPP32-positive cells were averaged from four images for each mouse.

Western blot analysis

Striatal extracts were prepared as described previously (Kovalenko et al., 2012). For detection of huntingtin protein, 90 µg of striatal extracts were resolved by SDS-PAGE in Novex 3–8% Tris-acetate gels (Invitrogen/Thermo Fisher Scientific) and transferred to 0.45 µm nitrocellulose membrane (Bio-Rad) in Transfer Buffer (Boston BioProducts) at 100 V for 72 min. After blocking with 5% non-fat milk, HTT protein was detected using mAb2166 antibody (Millipore) at 1:1000 in 5% non-fat milk/TBS/0.05% Tween-20 and visualized with ECL kit (ThermoScientific).

For HDAC2 and HDAC1, 40 µg of striatal extracts were resolved on 12% Bis-Tris gels (Invitrogen/Thermo Fisher Scientific) and transferred in Transfer Buffer with 10% methanol, 0.025% SDS at 110 V for 75 min. The membranes were blocked with 5% non-fat milk in TBS/0.05% Tween-20 and incubated with anti-HDAC2 H3159 antibody (Sigma) at 1:10000 or anti-HDAC1 ab31263 (Abcam) at 1:2000 in 1% non-fat milk/TBS/0.05% Tween-20 and visualized with ECL kit.

For detection of acetylated histones H3AcK9 and H4AcK12, 35 µg of striatal extracts were resolved on 12% Bis-Tris gels (Invitrogen/Thermo Fisher Scientific) and transferred in Transfer Buffer with 10% methanol, 0.025% SDS at 150 V for 75 min. The membranes were blocked as above and incubated with anti-H3AcK9 ab10812 (Abcam) at 1:500 or anti-H4AcK12 #04–119 (Millipore) at 1:500 in 1% non-fat milk/TBS/0.01% Tween-20 and developed with ECL kit.

Total histone H3 was detected after stripping and re-probing the same membrane with ab1791 (Abcam) at 1:2000 in 1% non-fat milk/TBS/0.05% Tween-20.

For detection of MSH3, SDS-PAGE and transfer was as for HDAC1 and HDAC2 (above). The membranes were blocked as above and incubated with anti-MSH3 mouse monoclonal antibody 2F11 -a kind gift from Glen Morris and Ian Holt (Holt et al., 2011)- at 1:300 in 5% non-fat milk/TBS/0.05% Tween-20.
For quantification of Western blot data the density of protein bands of interest was measured with Quantity One software (Bio-Rad) with local background subtraction and normalized by the total amount of protein per corresponding lane as measured by the density of the entire lane stained by Novex reversible protein stain (Invitrogen/Thermo Fisher Scientific). Normalized values (one per mouse) were averaged over each genotype group.

**Selective cell-based assay for CAG repeat contractions**

The selectable assay for repeat contractions, based on insertion of a CAG repeat into an intron of an HPRT minigene, was performed in FLAH25 cells as described (Hubert et al., 2011). Cell maintenance, inhibitor treatment regimen, and selection for resistant colonies in HAT medium (0.1 mM hypoxanthine, 0.4 μM aminopterin, and 16 μM thymine) were as described (Hubert et al., 2011).-4.5 x 10⁶ cells were plated and the number of HPRT⁺ colonies was adjusted by cell survival ([number of positive colonies] / [number of plated cells multiplied by plating efficiency]). The effect of inhibitors on CAG repeat contractions was expressed as fold change of the adjusted number of positive colonies over DMSO.

**RNA-seq library preparation and sequencing**

RNA was isolated from striatal tissues of 36 5-month mice of four genotype groups: Htt+/+ Hdac2 WT (N = 9: six males, three females), HttQ111/+/+ Hdac2 WT (N = 7: four males, three females), Htt+/+ Hdac2 KO (N = 9: five males, four females) and HttQ111/+/+ Hdac2 KO (N = 11: eight males, three females) lines using TRIzol reagent. Briefly, pelleted cells were resuspended in TRIzol reagent and then extracted with chloroform, followed by isopropanol precipitation of RNA from the aqueous phase and three 70% ethanol washes. RNA pellets were solubilized in 30–50 μl of RNase-free water (Ambion, AM9937). RNA quality was assessed using the Agilent Bioanalyzer TapeStation 2200 (Agilent Technologies, Santa Clara, CA). In total, 36 RNA-seq libraries were prepared using the Illumina TruSeq Stranded mRNA Sample Prep Kit. Each library in this study included 1 ul of a 1:10 dilution of ERCC RNA Control Spike-Ins (Ambion) that were added from one of two mixes, each containing the same 92 synthetic RNA standards of known concentration and sequence. These synthetic RNAs cover a 10⁶ range of concentration, as well as varying in length and GC content to allow for validation of dose response and the fidelity of the procedure in downstream analyses (Jiang et al., 2011). Libraries were multiplexed, pooled, and sequenced on multiple lanes of an Illumina HiSeq2000, generating median 40.7M paired-end reads per library of 75 bp.

**RNA-seq data processing and analysis**

Quality checking of sequence reads was assessed using fastQC (v. 0.10.1) (http://www.bioinformatics.babraham.ac.uk/projects/fastqc), blind to genotype. Sequence reads were aligned to the mouse reference genome (GRCm38, Ensembl build v. 75) using GSNAP (version 2015-06-23) with parameters ‘-a off -N 1 –pairexpect=450 -B 3 –query-unk-mismatch=1’ (Wu and Nacu, 2010). Gene level counts were tabulated using BedTools’s multibamcov algorithm on unique alignments for each library at all Ensembl genes (GRCm38 v.75). Based on quality checking of alignments assessed by custom scripts utilizing PicardTools (https://broadinstitute.github.io/picard/), RNASeqQC (DeLuca et al., 2012), RSeQC (Wang et al., 2012) and SamTools (Li et al., 2009), one male sample with genotype Htt+/+ Hdac2 WT was identified as an outlier and thus excluded from further analysis as this sample showed high intergenic (25%), intronic (23%), duplication rates (57%) and low exonic rates (53%), which indicated DNA contamination in this sample. Analysis of ERCC spike-ins as described (Blumenthal et al., 2014) estimated the expression threshold for detection to be at least six uniquely mapped reads. Genes smaller than 250 nt in length and tRNA and rRNA genes were removed from further analyses. Principal component analysis (PCA) of the remaining 35 samples was performed using a Bioconductor package, DESeq2 (v. 1.18.1) (Love et al., 2014) relying on regularized log-transformed counts after keeping genes expressed in all the samples from at least one genotype group with at least six counts. Differentially expressed genes (DEGs) in five pair-wise comparisons Htt+/+ Hdac2 KO vs. Htt+/+ Hdac2 WT [contrast 1], HttQ111/+/+ Hdac2 KO vs. HttQ111/+/+ Hdac2 WT [contrast 2], HttQ111/+/+ Hdac2 WT vs. Htt+/+ Hdac2 WT [contrast 3], HttQ111/+/+ Hdac2 KO vs. Htt+/+ Hdac2 KO [contrast 4] and HttQ111/+/+ Hdac2 KO vs. Htt+/+ Hdac2 WT [contrast 5], were identified by edgeR’s quasi-likelihood F test (v. 3.12) (Robinson et al., 2010) with surrogate variables (SVs).
accounting for batch effects and unknown factors in data, which were determined by Bioconductor Package SVA (v. 3.18) (Leek et al., 2012; Leek, 2014). To this end, SVA was applied to all 35 RNA-seq samples separately for each comparison of interest where the full model was mod = ~ 0 + genotype, while the null model was mod0 = ~ 1, analyzing genes that passed the expression detection threshold (≥ 6 counts), in at least one genotype group samples in a given comparison. As the number of analyzed genes differed from comparison to comparison, SVA identified a distinct set of SVs for each comparison, although they were highly similar between comparisons (average correlation = 0.99). DEGs were defined at two statistical significance levels: less stringent, using a nominal p < 0.05 cut-off, and more stringent, using a false discovery rate (FDR) cut-off of < 0.05, calculated based on the Benjamini-Hochberg procedure (Benjamini and Hochberg, 1995). KEGG pathway and gene ontology (GO) enrichments were performed using DAVID (v. 6.8) for DEGs at both p < 0.05 and FDR < 0.05 levels (Huang et al., 2009). In doing so, lists of DEGs in either category of direction of dysregulation (upregulated, downregulated and both) for a given comparison were used as a query list, while a list of all the analyzed genes was treated as a background list. Differentially regulated pathways were also defined at less stringent (p<0.05) and more stringent (FDR<0.05) significance levels. We compared our differential expression results from the HttQ111/+ Hdc2 WT vs Htt+/+ Hdc2 WT comparison (contrast 3) with those from a comparison of 6 month HttQ111/+ (4 female, 4 male) vs Htt+/+ (4 female, 4 male) striata using the RNA-Seq dataset described in Langfelder et al., 2016. To this end, we reanalyzed the Langfelder RNA-Seq data employing the same EdgeR + SVA pipeline as in the present study (as above). 16,395 genes that passed our expression threshold (≥ 6 counts) in either all mutant or all control samples were analyzed. We compared DEGs identified in both studies at both p < 0.05 and FDR < 0.05 thresholds as above. To assess the statistical significance of overlaps between DEGs or enriched pathways between pairs of contrasts (Figure 3—figure supplement 1) we performed one-tailed Fisher’s Exact tests, commonly used to assess the statistical significance of overlap of two sets or enrichment of one set in other (overrepresentation). To identify genes where Hdc2 knockout is either protective or exacerbates the effect of the HttQ111 allele, we first compared DEGs (p < 0.05) in contrast 3 (HttQ111/+ vs. Htt+/+ in Hdc2 WT background) with those in contrast 2 (Hdc2 KO vs. WT in HttQ111/+ background). Next, we performed 2-tailed unpaired t-tests on those shared DEGs to identify genes that were not significantly differentially expressed (p > 0.05) between Htt+/+ Hdc2 WT and HttQ111/+ Hdc2 KO. To assess the statistical significance of observing reduced relative expression (fold-change of HttQ111 versus Htt+/+) of the 29 commonly dysregulated genes shared between the study and Langfelder et al. in the Hdc2 KO background compared to the Hdc2 WT background we performed a permutation test. To this end, we randomly selected 29 genes 100,000 times from 15,508 genes analyzed in both this study and Langfelder et al. In each iteration, we compared the absolute value of log2 fold-changes of genes between HttQ111/+ and Htt+/+ genotypes in Hdc2 KO and Hdc2 WT backgrounds and assessed how many times the randomly selected 29 genes showed the observed effect, that is \( \text{abs}(\log_{2}\text{FC}_{\text{Hdc2.WT}}) < \text{abs}(\log_{2}\text{FC}_{\text{Hdc2.KO}}) \). where \( \text{abs}(\log_{2}\text{FC}_{\text{Hdc2.KO}}) \) is absolute value of log2 fold-change of \( i^{th} \) gene between HttQ111/+ and Htt+/+ genotypes in Hdc2 KO background. All analyses were run on R platforms (versions 3.2.2, 3.3.2, 3.4.3, 3.6).

**Statistical analyses**

We performed statistical analyses to compare quantitative instability and mAb5374 phenotypes between genotype groups. As we were not testing the effect of age on these phenotypes, and as our datasets were well controlled for inherited CAG length, we performed two-tailed unpaired t-tests to compare mean values between two genotype groups at any one age. Statistical analyses relevant to the RNA-seq data analyses are described in the section above.

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Additional information

Competing interests
Daniel M Fass: DMF is a member of the scientific advisory board of Psy Therapeutics. Stephen J Haggarty: SJH is a member of the scientific advisory board of Psy Therapeutics, Frequency Therapeutics and Souvien Therapeutics, and former member of the scientific advisory board of Rodin Therapeutics that is focused on HDAC2 inhibitors, none of whom were involved in the present study. SJH has also received speaking or consulting fees from Amgen, AstraZeneca, Biogen, Merck, Regeneron Pharmaceuticals, as well as sponsored research or gift funding from AstraZeneca, JW Pharmaceuticals, and Vesigen unrelated to the content of this manuscript. His financial interests were reviewed and are managed by Massachusetts General Hospital and Partners HealthCare in accordance with their conflict of interest policies. Vanessa C Wheeler: VCW is a scientific advisory board member of Triplet Therapeutics, a company developing new therapeutic approaches to address triplet repeat disorders such Huntington’s disease and Myotonic Dystrophy and of LoQus23 Therapeutics. Her financial interests in Triplet Therapeutics, were reviewed and are managed by Massachusetts General Hospital and Partners HealthCare in accordance with their conflict of interest policies. The other authors declare that no competing interests exist.

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Marina Kovalenko, Conceptualization, Data curation, Formal analysis, Supervision, Validation, Investigation, Visualization, Methodology, Writing - original draft, Writing - review and editing; Serkan Erdin, Conceptualization, Resources, Data curation, Formal analysis, Validation, Investigation, Visualization, Methodology, Writing - original draft; Marissa A Andrew, Leroy Hubert, Formal analysis, Investigation, Writing - review and editing; Jason St Claire, Melissa Shaughnessy, Investigation, Writing - review and editing; João Luís Neto, Investigation, Methodology, Writing - review and editing; Alexei Stortchevoi, Formal analysis, Daniel M Fass, Resources, Writing - review and editing; Ricardo Mouro Pinto, Formal analysis, Funding acquisition, Methodology, Writing - review and editing; Stephen J Haggarty, Conceptualization, Resources, Supervision, Writing - review and editing; John H Wilson, Michael E Talkowski, Conceptualization, Supervision, Writing - review and editing; Vanessa C Wheeler, Conceptualization, Data curation, Formal analysis, Supervision, Funding acquisition, Validation, Visualization, Methodology, Writing - original draft, Project administration, Writing - review and editing

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Ethics

Animal experimentation: This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health under an approved protocol (2009N000216) of the Massachusetts General Hospital Subcommittee on Research Animal Care.

Decision letter and Author response

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Additional files

Supplementary files

- Source code 1. RNA-seq codes and data files.
- Source data 1. CAG lengths of mouse cohorts.
- Source data 2. Differentially expressed genes.
- Source data 3. Pathway analyses.
- Source data 4. Rescue analysis.
- Transparent reporting form

Data availability

RNA-Seq data is deposited in GEO, under the accession number GSE148440.

The following dataset was generated:

| Author(s)                  | Year | Dataset title                                                                 | Dataset URL                                                                 | Database and Identifier                   |
|----------------------------|------|-------------------------------------------------------------------------------|------------------------------------------------------------------------------|-------------------------------------------|
| Kovalenko M, Erdin S, Andrew MA, Claire J, Shaughnessy M, Hubert L, Neto JL, Stortchevoi A, Fass DM, Pinto RM, Haggarty SJ, Wilson JH, Tallowski ME, Wheeler VC | 2020 | Histone deacetylase knockouts modify transcription, CAG instability and nuclear pathology in Huntington disease mice | https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE148440 | NCBI Gene Expression Omnibus, GSE148440 |

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