**Supplementary Information**

# Inhibition of DNA Methyltransferases Blocks Mutant Huntingtin-Induced Neurotoxicity

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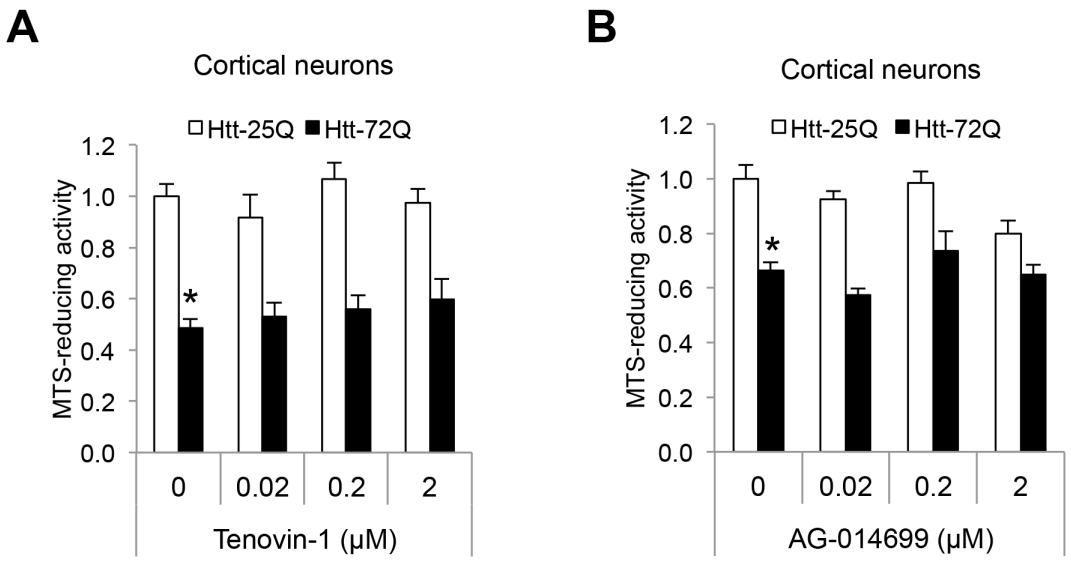


**Figure S1. Validation of hits from epigenetic drug screen.**

(A) Tenovin-1 (p53 activator), one of the top three positive compounds from the cell-based screen (Figure 1A), was subjected to cell viability assay using MTS. Treatments with tenovin-1 did not significantly increase the viability of mutant Htt-expressing neurons (ANOVA, **P* < 0.0001 compared to Htt-25Q (0 μM); *P* = 0.591, Htt-72Q (0.02 μM) vs Htt-72Q (0 μM); *P* = 0.355, Htt-72Q (0.2 μM) vs Htt-72Q (0 μM); *P* = 0.168, Htt-72Q (2 μM) vs Htt-72Q (0 μM); n = 6-12 wells per group).

(B) AG-014699 (PARP1 inhibitor), another screen hit, was subjected to cell viability assay as in (A). There is no significant difference in survival between the AG-014699- and vehicle-treated Htt-72Q neurons (ANOVA, **P* < 0.0001 compared to Htt-25Q (0 μM); *P* = 0.142, Htt-72Q (0.02 μM) vs Htt-72Q (0 μM); *P* = 0.254, Htt-72Q (0.2 μM) vs Htt-72Q (0 μM); *P* = 0.781, Htt-72Q (2 μM) vs Htt-72Q (0 μM); n = 12 wells per groups).

Data are presented as mean + SEM.

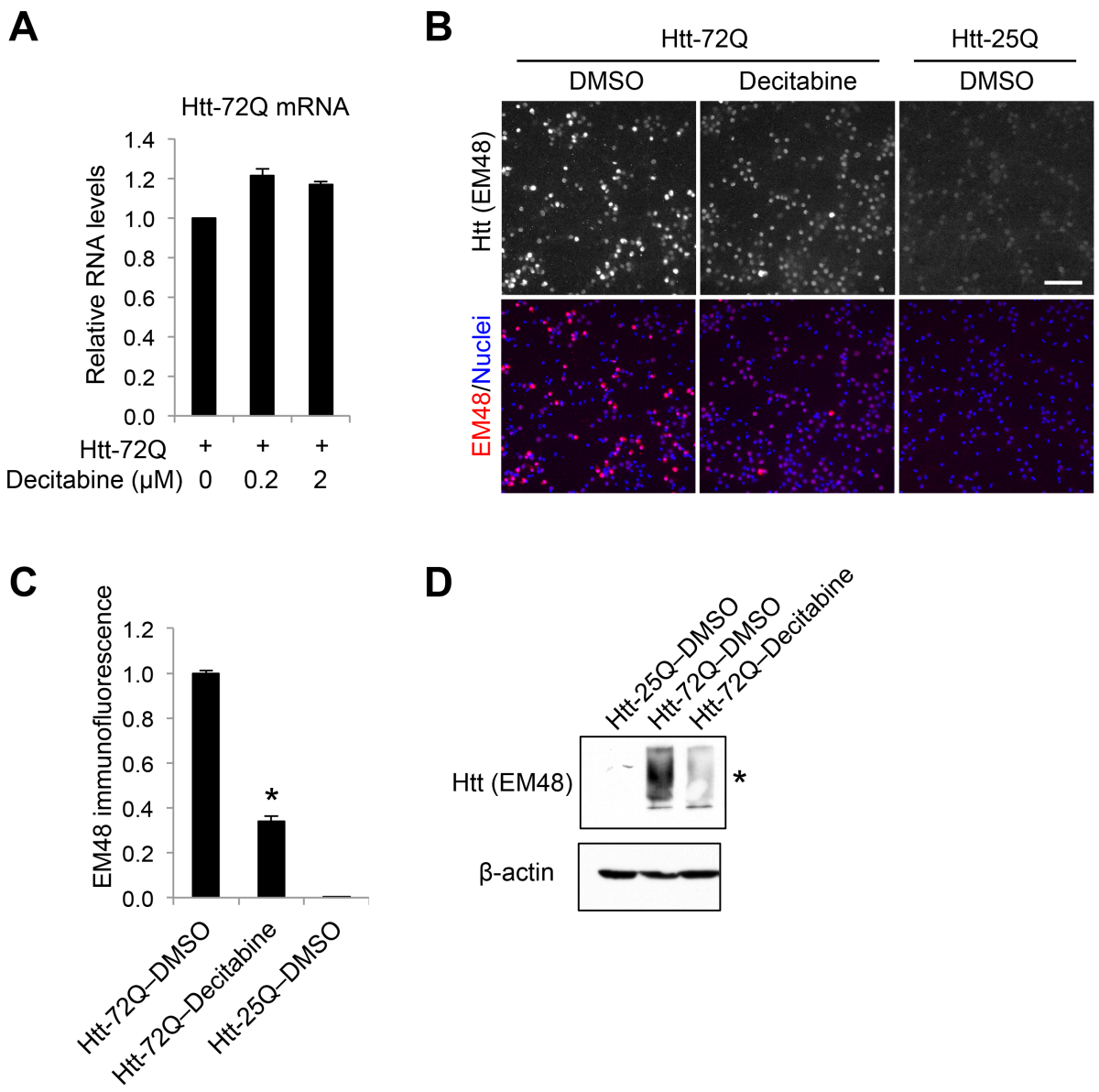


**Figure S2. Inhibition of DNMTs attenuates mutant Htt aggregates in primary cortical neurons**

(A) DIV 5 mouse primary cortical neurons were infected with Htt-72Q lentivirus and treated with decitabine or vehicle (DMSO). RNA was prepared at DIV 10 and subjected to qRT-PCR analysis for Htt-72Q (human). *β-actin* and *Hprt* were used as reference genes. Decitabine did not decrease the levels of Htt-72Q mRNA compared to vehicle (ANOVA, n = 6 independent experiments).

(B) DIV 5 cortical neurons were infected with Htt lentivirus and treated with decitabine (0.2 μM) or DMSO. Neurons were fixed at DIV 11-12 and subjected to indirect immunofluorescence with a specific mouse monoclonal Htt antibody EM48, which preferentially detects aggregated mutant protein. Nuclei were labeled with Hoechst 33342. Images were captured using an Operetta high-content imaging system (PerkinElmer) with a 20× objective lens. Bar, 100 μm.

(C) EM48 immunofluorescence intensity in (B) was quantified using an ImageJ-based macro. Decitabine significantly decreased the levels of mutant Htt aggregates in Htt-72Q-expressing primary cortical neurons (ANOVA, $*P < 0.0001$ vs. Htt-72Q–DMSO, n = 18 wells from 6 independent experiments).

(D) Cortical neurons transduced and processed as in (A) were directly harvested in SDS sample buffer at DIV 10 and subjected to immunoblotting with anti-Htt EM48 monoclonal antibody. Blot was reprobed with anti-β-actin antibody. Representative immunoblot from 3 independent experiments is shown. Decitabine could decrease the levels of aggregated high molecular weight mutant Htt in a stacking gel (*).

Data in (A) and (C) are presented as mean + SEM.

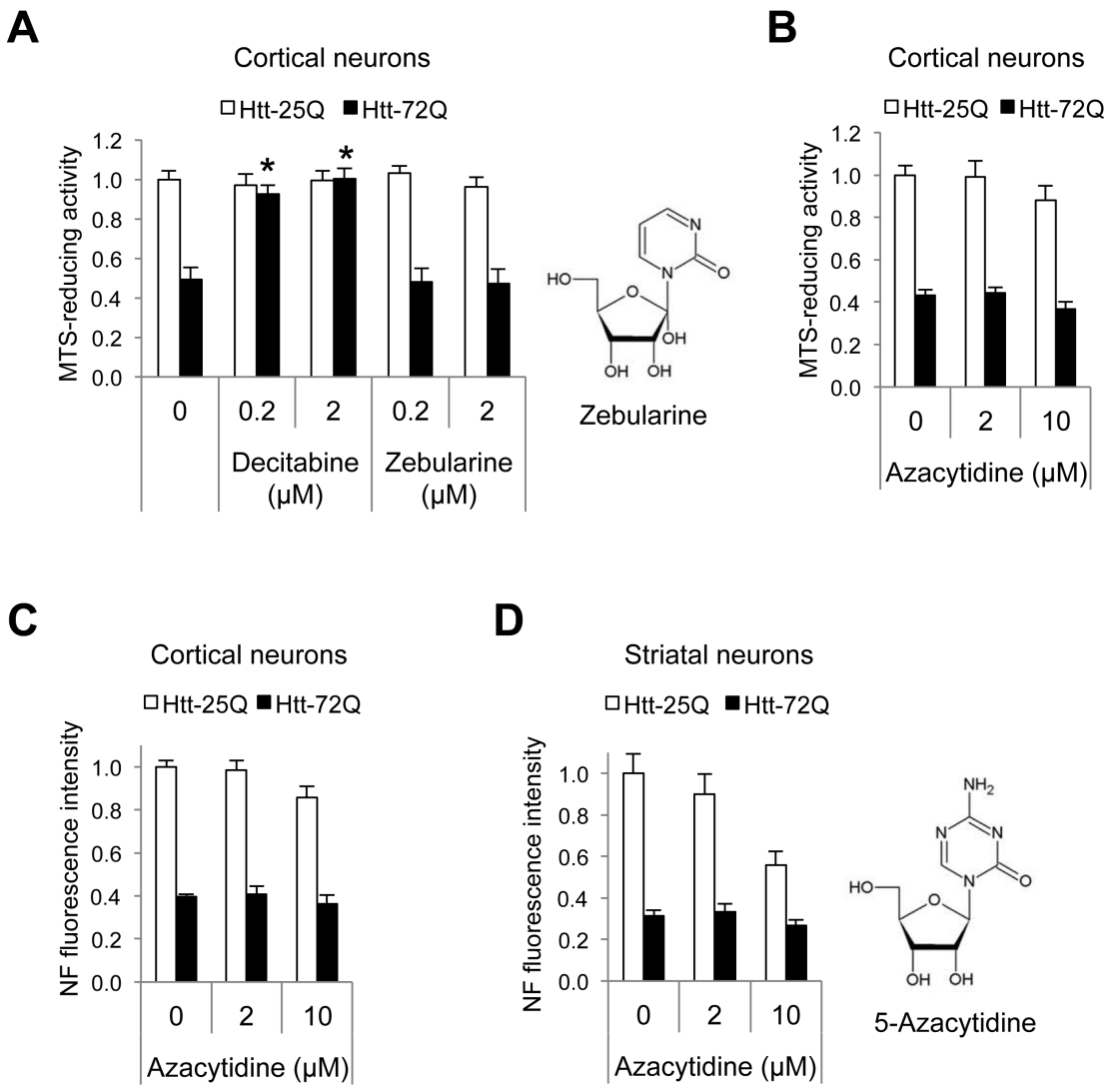


**Figure S3. The effects of nucleoside analog DNMT inhibitors on mutant Htt-induced toxicity in primary neurons.**

(A) DIV 5 cortical neurons transduced with lentivirus expressing Htt-72Q or Htt-25Q fragment were treated with the indicated drugs or DMSO (= 0 μM) and subjected to MTS assay at DIV 14. Decitabine, but not zebularine, increased the viability of mutant Htt-expressing neurons (ANOVA, **P* < 0.0001 compared to Htt-72Q (0 μM); no significant difference in survival between zebularine and vehicle-treated Htt-72Q neurons; n = 8-18 wells per group, 3 independent experiments).

(B) Primary cortical neurons transduced and treated with 5-azacytidine were subjected to MTS assay as in (A). There was no significant difference in survival between 5-azacytidine- and vehicle-treated Htt-72Q neurons (ANOVA, n = 6-15 wells per group, 3 independent experiments).

(C) Primary cortical neurons were transduced and treated as in (A) and subjected to neurofilament (NF) immunofluorescence. There was no significant difference in NF immunofluorescence intensity between 5-azacytidine- and vehicle-treated Htt-72Q cortical neurons (ANOVA, n = 6-15 wells per group, 3 independent experiments)

(D) Primary striatal neurons transduced with Htt-25Q or Htt-72Q lentivirus at DIV 4 were treated with 5-azacytidine or DMSO. Seven days later, neurons were subjected to NF immunofluorescence. There was no significant difference in NF immunofluorescence intensity between 5-azacytidine- and vehicle-treated Htt-72Q striatal neurons (ANOVA, n = 9-17 wells, 3 independent experiments).

Data are presented as mean + SEM.

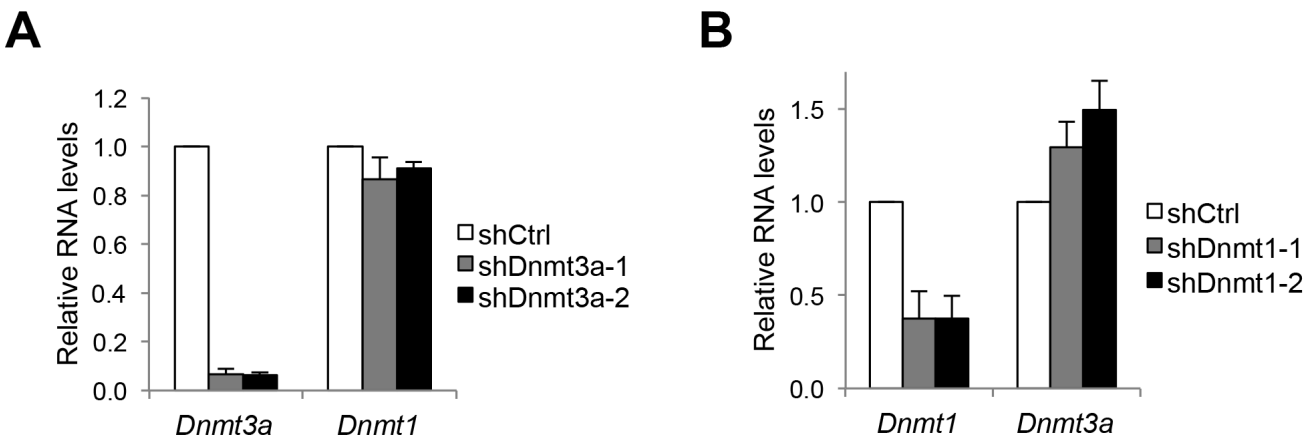


**Figure S4. Knockdown of DNMT3A or DNMT1 did not decrease the expression of the other DNMTs.**

(A) DIV 5 cortical neurons were transduced with lentivirus expressing *Dnmt3a* shRNA (shDnmt3a-1 or -2) or control shRNA along with Htt-72Q virus; 5 days later, RNA was prepared and subjected to qRT-PCR analysis. *Dnmt3a* RNAi did not significantly reduce the levels of *Dnmt1* mRNA in mutant Htt-expressing neurons, showing the specificity of these shRNAs (ANOVA, n = 3 independent experiments).

(B) Cortical neurons were transduced with lentivirus expressing *Dnmt1* shRNA (shDnmt1-1 or -2) or control shRNA along with Htt-72Q virus and processed as in (A). *Dnmt1* RNAi did not decrease the levels of *Dnmt3a* mRNA in mutant Htt-expressing neurons, showing the specificity of these shRNAs (ANOVA, n = 3 independent experiments).

Data are presented as mean + SEM.

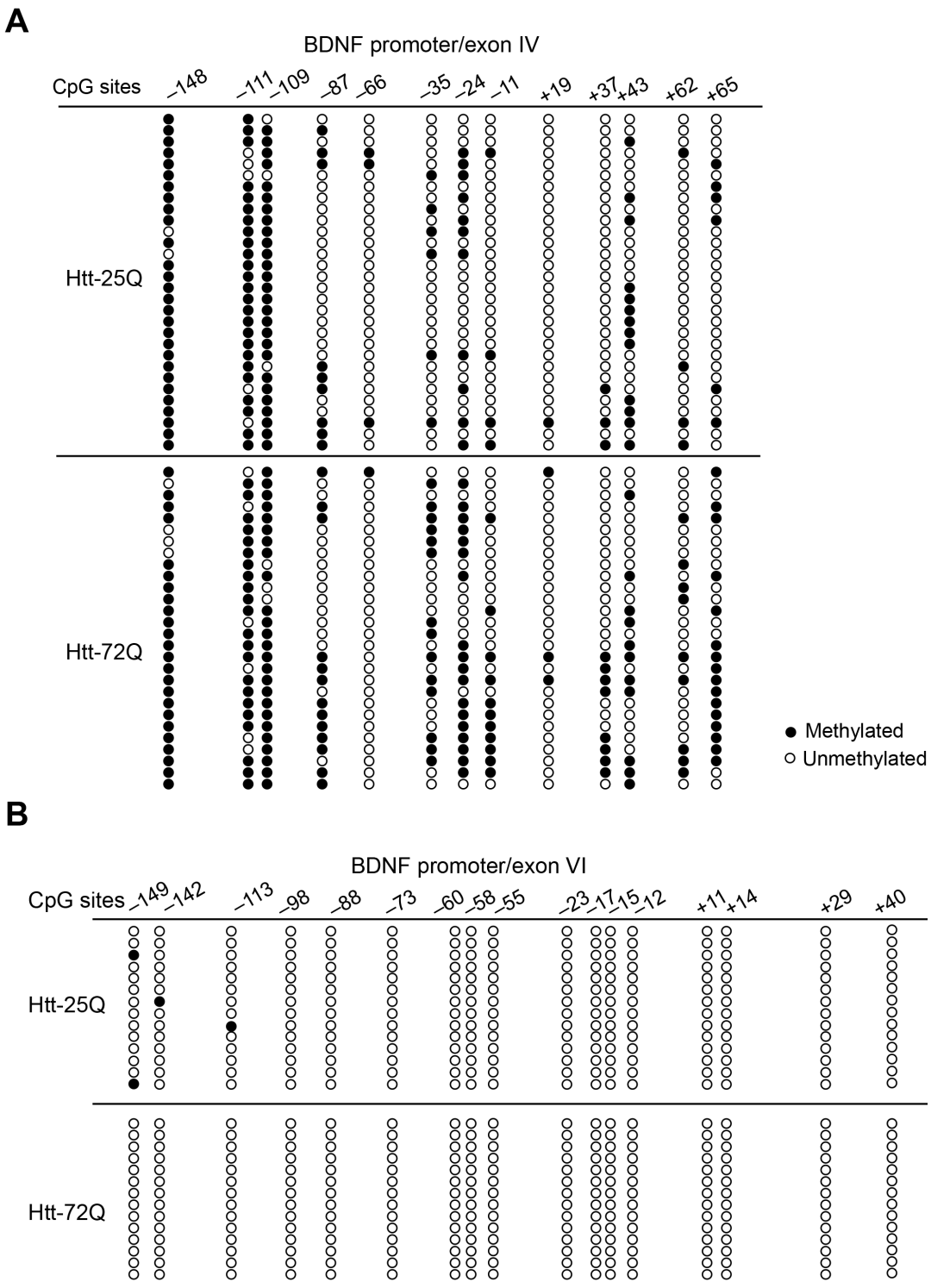


**Figure S5. Analysis of CpG methylation status of *Bdnf* exon IV and VI regulatory regions in mutant Htt-expressing primary cortical neurons.**

(A) Primary cortical neurons were transduced with Htt-25Q or Htt-72Q at DIV 5. Five days later, genomic DNA was prepared and subjected to bisulfite DNA sequencing analysis on the 13 CpG sites in the regulatory region of *Bdnf* exon IV. The positions of CpG sites are indicated relative to the TSS. Data are pooled from 7 independent experiments. Percentage of methylated cytosine residues was calculated and presented in Figure 4B.

(B) Primary cortical neurons were transduced and processed for bisulfite DNA sequencing analysis on the 17 CpG sites in the regulatory region of *Bdnf* exon VI as in (A). Data are pooled from 3 independent experiments. The CpG sites in this region were mostly unmethylated in Htt-25Q- and Htt-72Q-expressing neurons.

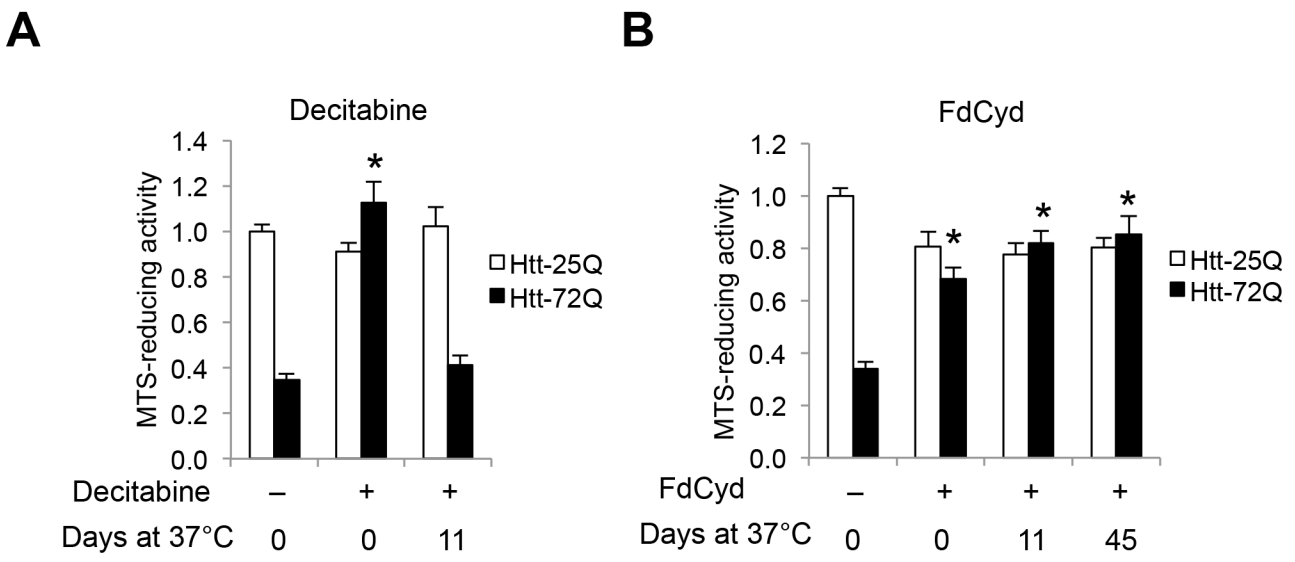


**Figure S6. *In vitro* stability of decitabine and FdCyd.**

(A) Decitabine (0.2 mM in saline) was first incubated at 37°C for indicated number of days *in vitro* and then added to the culture media at a final concentration 0.2 μM of DIV 5 primary cortical neurons transduced with Htt-25Q or Htt-72Q lentivirus. The neuroprotective activity of decitabine was tested using MTS assay at DIV 14. Decitabine lost its activity when tested after 11 days of incubation (ANOVA, **P* < 0.0001 compared to Htt-72Q plus vehicle, no difference between Htt-72Q plus vehicle vs Htt-72Q plus decitabine preincubated for 11 days, n = 9 wells, 3 independent experiments).

(B) Similar experiments were performed with FdCyd as in (A). In contrast to decitabine, FdCyd preincubated for 11 or 45 days showed neuroprotective effects comparable to FdCyd with no preincubation (ANOVA, **P* < 0.0001 compared to Htt-72Q plus vehicle, n = 9 wells, 3 independent experiments).

Data are presented as mean + SEM.

**Table S1. List of compounds used in the epigenetic drug screen**

| | | |
|---|---|---|
| 1 | CAY10433 | HDAC inhibitor |
| 2 | Piceatannol | Resveratrol analog |
| 3 | CAY10591 | SIRT1 activator |
| 4 | EX-527 | SIRT1 inhibitor |
| 5 | SAHA | Calss I and II HDAC inhibitor |
| 6 | 2-PCPA (hydrochloride) | LSD1 inhibitor |
| 7 | 3-amino Benzamide | PARP inhibitor |
| 8 | SB 939 | HDAC inhibitor |
| 9 | PCI 34051 | HDAC8 inhibitor |
| 10 | 4-iodo-SAHA | CalssI and II HDAC inhibitor (SAHA derivative) |
| 11 | Sirtinol | SIRT inhibitor |
| 12 | C646 | HAT p300 inhibitor |
| 13 | Tubastatin A (trifluoroacetate salt) | HDAC6 inhibitor |
| 14 | Garcinol | p300 and PCAF HAT inhibitor |
| 15 | Ellagic Acid | Antioxidant; inhibitor of H3R17 methylation |
| 16 | Suberohydroxamic Acid (SBHA) | HDAC inhibitor |
| 17 | Apicidin | HDAC inhibitor |
| 18 | UNC0321 (trifluoroacetate salt) | G9a HMTase inhibitor |
| 19 | (-)-Neplanocin A | SAH hydrolase inhibitor (SAM-dependent MT inhibitor) |
| 20 | Cl-Amidine | PAD4 deiminase inhibitor |
| 21 | F-Amidine (trifluoroacetate salt) | PAD4 deiminase inhibitor |
| 22 | JGB1741 | SIRT1 inhibitor |
| 23 | UNC0638 | G9a HMTase inhibitor |
| 24 | Isoliquiritigenin | Antioxidant, anti-inflammatory, antitumor activities |
| 25 | CCG-100602 | Inhibitor of Rho pathway-mediated signaling and activation of serum response factor transcription |
| 26 | CAY10669 | pCAF (p300/CREB-binding protein-associated factor) HAT inhibitor |
| 27 | Zebularine | DNMT inhibitor |
| 28 | Delphinidin chloride | p300/CBP HAT inhibitor |
| 29 | Suramin (sodium salt) | SIRT1 inhibitor |
| 30 | Nicotinamide | SIRT inhibitor |
| 31 | 2,4-Pyridinedicarboxylic Acid | Histone demethylase inhibitor |
| 32 | PFI-1 | BET bromodomain inhibitor |
| 33 | 5-Azacytidine | DNMT inhibitor |
| 34 | Decitabine | DNMT inhibitor |
| 35 | (+)-JQ1 | BET bromodomain inhibitor |
| 36 | (-)-JQ1 | Negative control for (+)-JQ1 |
| 37 | BSI-201 | PARP1 inhibitor |
| 38 | 1-Naphthoic Acid | SIRT inhibitor |
| 39 | AG-014699 | PARP-1 inhibitor |
| 40 | IOX1 | inhibitor of 2-oxoglutarate oxygenases |
| 41 | MI-2 (hydrochloride) | Inhibitor of the menin-MLL fusion protein interaction |
| 42 | MI-nc (hydrochloride) | Weak inhibitor of the menin-MLL fusion protein interaction; negative control for MI-2 |
| 43 | Lomeguatrib | $O^6$-Methylguanine-DNA methyltransferase (MGMT) inhibitor |
| 44 | Daminozide | Inhibitor of the human 2-oxoglutarate (JmjC) histone demethylases, KDM2A, PHF8, and KDM7A |
| 45 | GSK-J1 (sodium salt) | JMJD3 selective histone demethylase inhibitor |
| 46 | GSK-J2 (sodium salt) | Poor JMJD3 inhibitor (negative control) |
| 47 | GSK-J4 (hydrochloride) | JMJD3 selective histone demethylase inhibitor |
| 48 | GSK-J5 (hydrochloride) | Weak JMJD3 demethylase inhibitor (inactive control) |

| | | |
|---|---|---|
| 49 | Valproic Acid (sodium salt) | HDAC inhibitor |
| 50 | Tenovin-1 | p53 activator; SIRT1 and SIRT2 inhibitor |
| 51 | Tenovin-6 | p53 activator; SIRT1, SIRT2, and SIRT3 inhibitor |
| 52 | Sodium Butyrate | HDAC inhibitor |
| 53 | Anacardic Acid | HAT (p300 and pCAF) inhibitor; anti-inflammatory, anti-tumor, molluscicidal, and anti-microbial activity |
| 54 | AGK2 | SIRT2 inhibitor |
| 55 | CAY10603 | HDAC6 inhibitor |
| 56 | Chaetocin | HMT inhibitor with selectivity for Lys9-HMTs (SU(VAR)3-9, G9a, DIM5) |
| 57 | Splitomicin | Sir2p HDAC inhibitor |
| 58 | CBHA | HDAC inhibitor, |
| 59 | M 344 | HDAC inhibitor |
| 60 | Oxamflatin | HDAC inhibitor |
| 61 | Salermide | SIRT1 and SIRT2 inhibitor |
| 62 | Mirin | Mre11-Rad50-Nbs1 (MRN) inhibitor |
| 63 | Pimelic Diphenylamide 106 | Calss I HDAC inhibitor |
| 64 | (S)-HDAC-42 | HDAC inhibitor |
| 65 | MS-275 | HDAC (HDAC1) inhibitor |
| 66 | RG-108 | DNMT inhibitor |
| 67 | 2',3',5'-triacetyl-5-Azacytidine | DNMT inhibitor |
| 68 | S-Adenosylhomocysteine (SAH) | Product of SAM-dependent methylation of DNA, RNA, and histones and other proteins |
| 69 | UNC0224 | G9a HMTase inhibitor |
| 70 | Chidamide | HDAC inhibitor |
| 71 | 3-Deazaneplanocin A | S-adenosyl-L-homocysteine hydrolase inhibitor; EZH2 inhibitor |
| 72 | Sinefungin | SET domain-containing methyltransferase inhibitor |
| 73 | N-Oxalylglycine | Inhibitor of α-ketoglutarate-dependent enzymes and prolyl hydroxylase domain-containing proteins PHD1 and PHD2 |
| 74 | AMI-1 (sodium salt) | PRMTs inhibitor |
| 75 | UNC1215 | L3MBTL3 domain inhibitor |
| 76 | trans-Resveratrol | Antioxidant, antiproliferative and anti-inflammatory activity, and cyclooxygenase-1 inhibitor |
| 77 | 2,4-DPD | HIF-PH inhibitor |
| 78 | DMOG | HIF-PH inhibitor |
| 79 | Trichostatin A | HDAC inhibitor |
| 80 | CAY10398 | HDAC1 inhibitor |
| 81 | SGC0946 | DOT1L inhibitor |
| 82 | EPZ5676 | DOT1L inhibitor |
| 83 | EPZ6438 | EZH2 inhibitor |
| 84 | GSK126 | EZH2 inhibitor |

1-80: Epigenetic Screening Library from Cayman Chemical (Item Number 11076)

81-84: Xcessbio Biosciences Inc.

**Table S2. List of primers used for experiments**

**(A) Primer sequences for mRNA analysis**

| Genes | Forward | Reverse |
|---|---|---|
| Dnmt3a | AATAGAGACCCTCGGAGGCA | CCTGCTGCTAGTTGGGTTCT |
| Dnmt1 | AACAGCTCCAGCCCGAGT | TTTTCTGTTAAGCCATCTCTTTCC |
| Bdnf exon IX (protein coding) | GACAAGGCAACTTGGCCTAC | CGTGCTCAAAAGTGTCAGCC |
| Bdnf exon I | CCTGCATCTGTTGGGGAGAC | GCCTTGTCCGTGGACGTTTA |
| Bdnf exon II | CTAGCCACCGGGGTGGTGTAA | CGCCTTCATGCAACCGAAGT |
| Bdnf exon III | GCTTCATTGAGCCCAGTTCC | GCCTTGTCCGTGGACGTTTA |
| Bdnf exon IV | CAGAGCAGCTGCCTTGATGTT | GCCTTGTCCGTGGACGTTTA |
| Bdnf exon VI | TTGGGGCAGACGAGAAAGCGC | AGGATGGTCATCACTCTTCTC |
| 18s | AGTCGGCATCGTTTATGGTC | CGAAAGCATTTGCCAAGAAT |
| Hprt | TTGACACTGGTAAAACAATGCAAAC | GAGAGGTCCTTTTCACCAGCA |
| β-actin (Actb) | AGTGTGACGTTGACATCCGTA | GCCAGAGCAGTAATCTCCTTCT |
| Drd2 | CTGGAGCCAAAAGCAGTCTG | TCCTTCAGGTTTCCGACGCC |
| Ppp1r1b | CCAACCCCTGCCATGCTTT | TTGGGTCTCTTCGACTTTGGG |
| Penk | TGGCGTAGGGCCTGCGTC | TGTAAAGCGGCCGCGTCG |
| Pcp4 | CGACCAACGGAAAAGACAAG | TGTCTCTGGTGCATCCATGT |
| Rasd2 | AACTGCGCCTACTTCGAGG | GGTGAAAAGCATCGCCGTACT |
| Kdm8 | GCTGGACCTCGGTGAGAAG | TCCCAGGAGTAGTCTAGGACG |
| Adora2A | GCCATCCCATTCGCCATCA | GCAATAGCCAAGAGGCTGAAGA |
| human HTT exon1 | TCAACCTCCTCCACAGGCAC | AGGCTCCTCAGCCACAGCT |

**(B) Primer sequences for bisulfite sequencing analysis**

| Genes | Forward | Reverse |
|---|---|---|
| Bdnf IV | GTGAATTTGTTAGGATTGGAAGTGAAAATA | CTAAACAAAAACTAAAAAATTTCATACTAACTC |
| Bdnf VI | GGTAGGTATAGAGTTTTGGGTTTAAGTAG | ACACTAAAATCAAACATTATTTAACTCTTC |

**(C) Primer sequences for MeDIP analysis**

| Genes | Forward | Reverse |
|---|---|---|
| Bdnf IV | GCGCGGAATTCTGATTCTGGTA | CTGCCTTGACGTGAGCTGTC |
| Gapdh | CTCTGCTCCTCCCTGTTCC | TCCCTAGACCCGTACAGTGC |

**(D) Primer sequences for ChIP analysis**

| Genes | Forward | Reverse |
|---|---|---|
| Bdnf IV | CTTCTGTGTGCGTGAATTTGCT | AGTCCACGAGAGGGCTCCA |