SUPPLEMENTARY MATERIAL

The HTT1a protein initiates HTT aggregation in a knock-in mouse model of Huntington's disease

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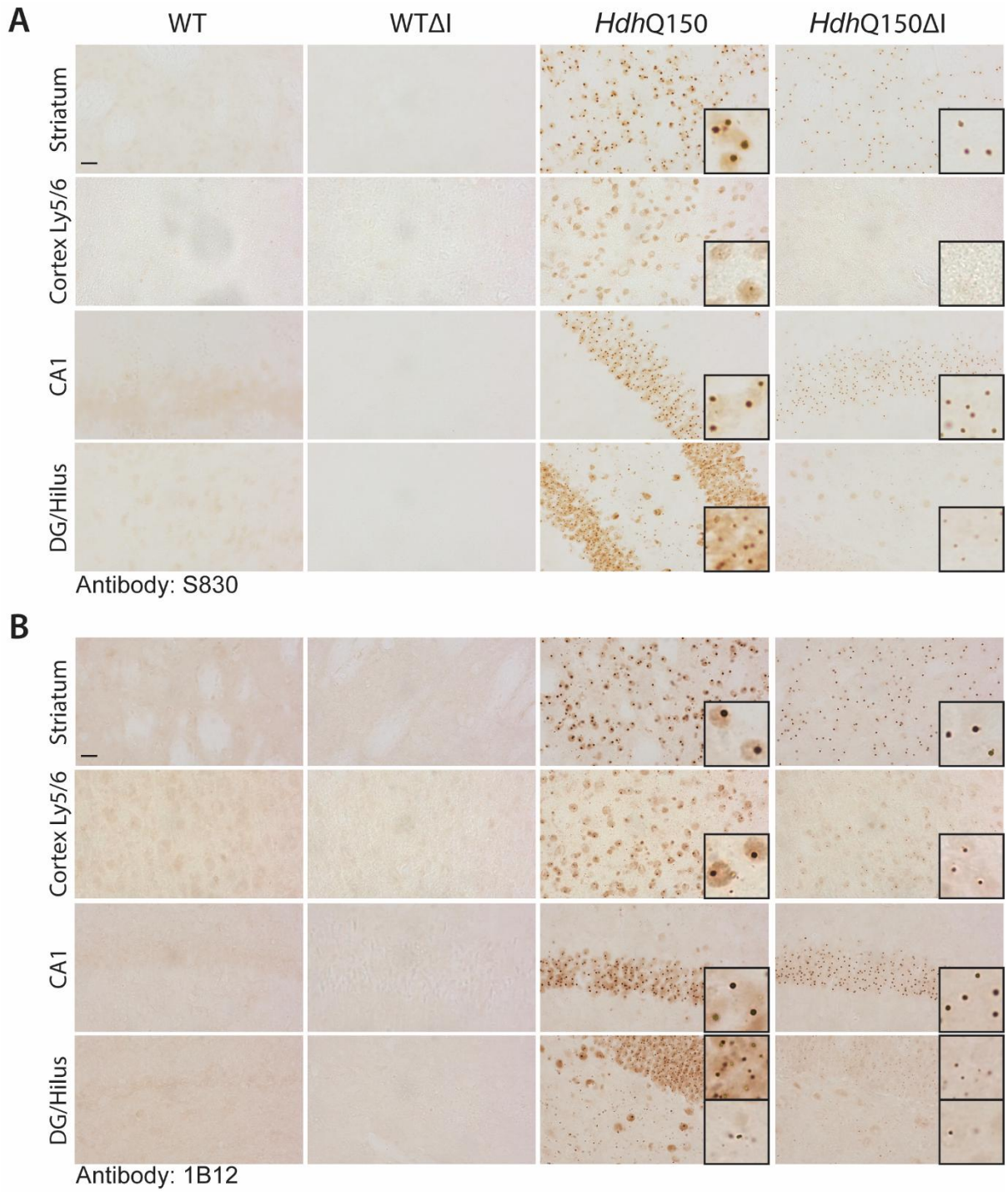
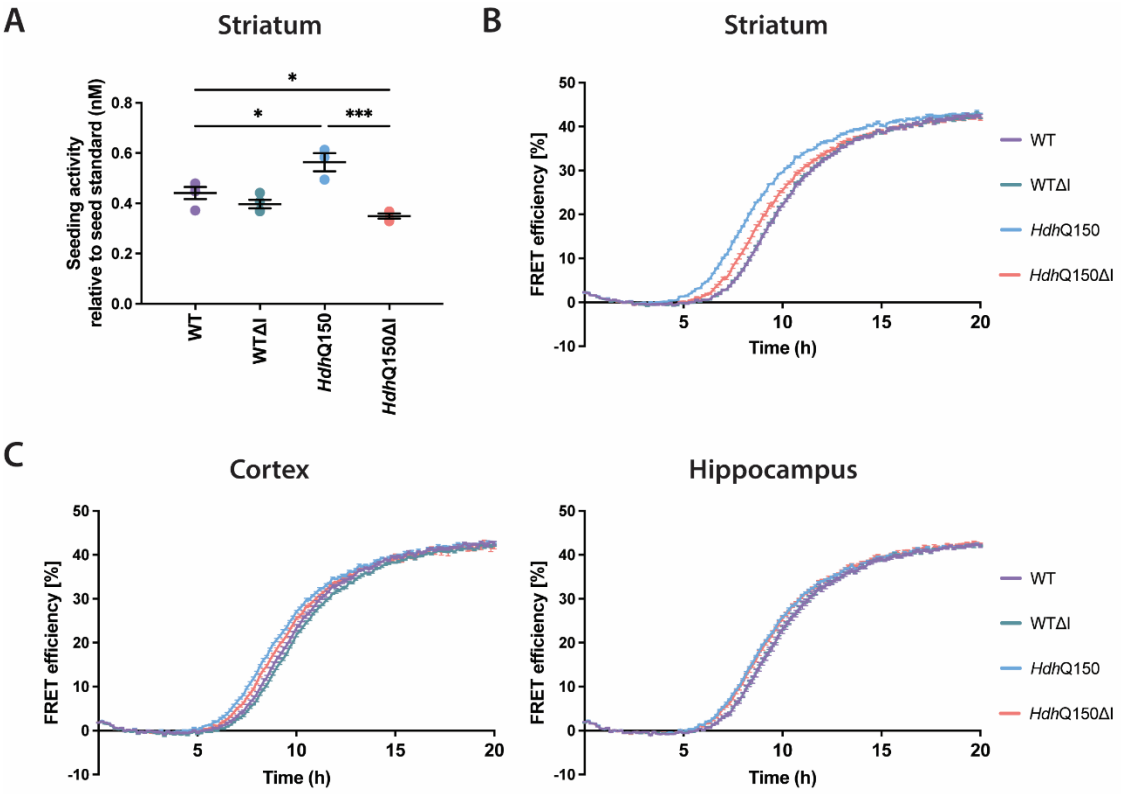
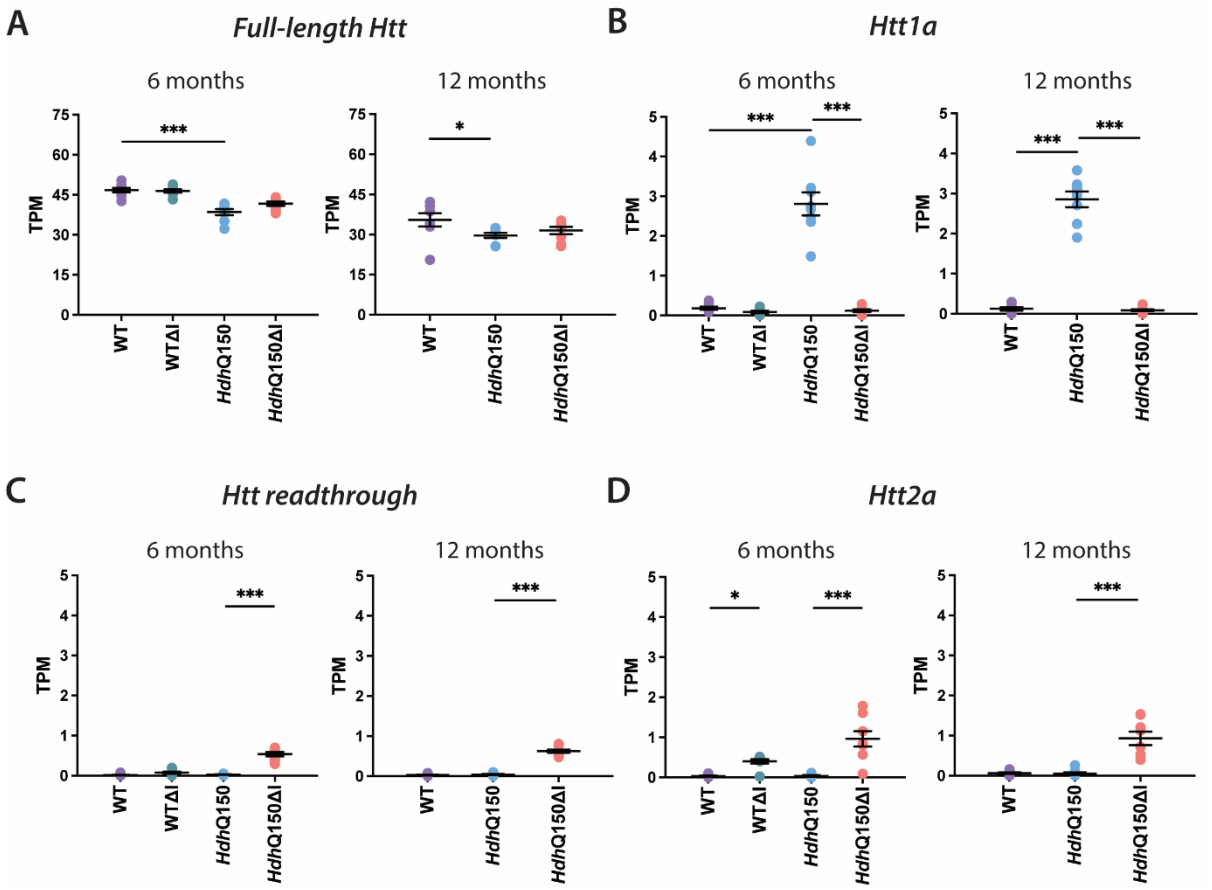


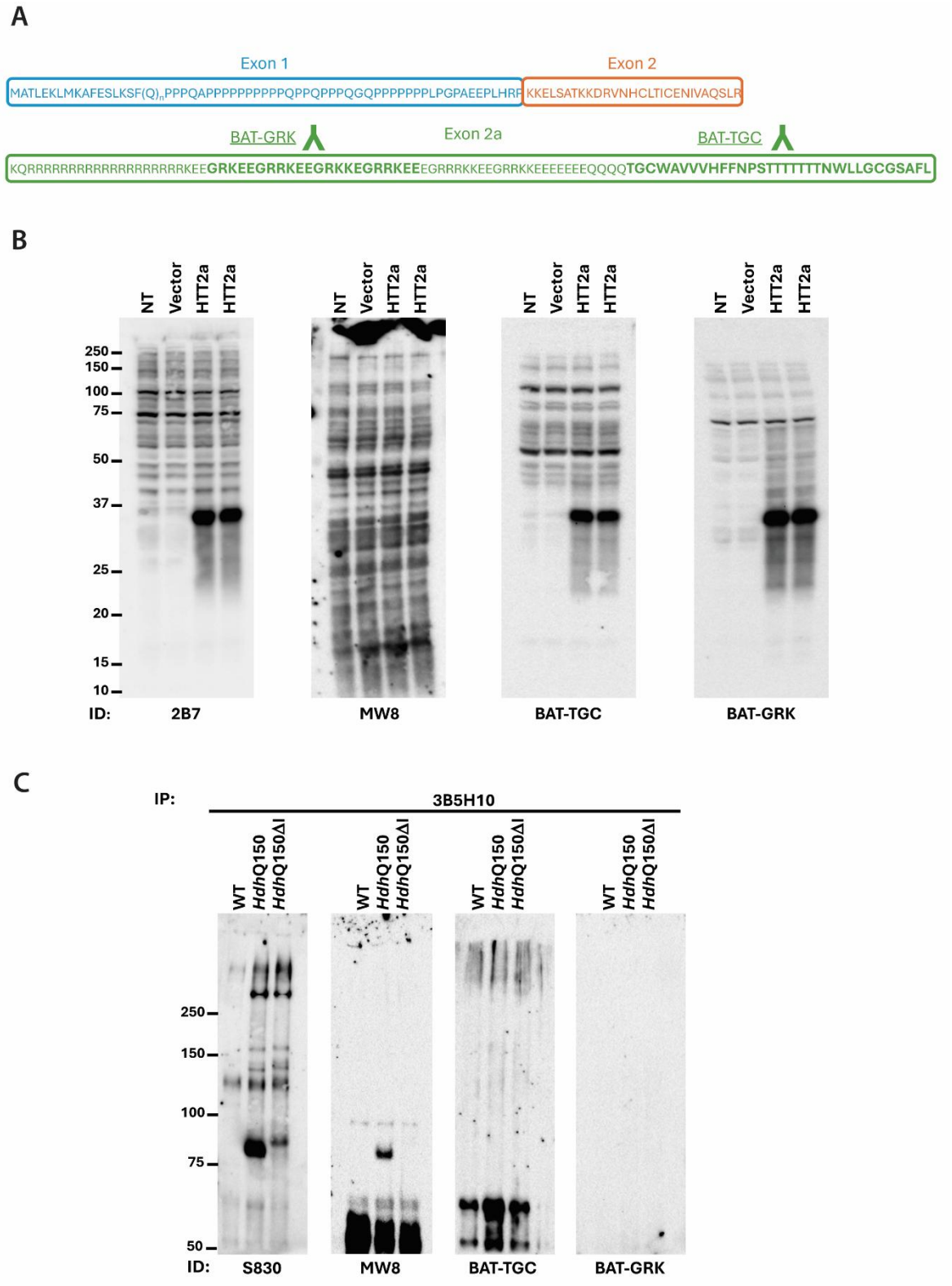
Supplementary Figure 1. The S830 and 1B12 antibodies do not detect an aggregate signal in either WT or WTΔI sections. Immunohistochemistry with the (**A**) S830 and (**B**) 1B12 antibodies to brain sections from WT, WTΔI, *Hdh*Q150 and *Hdh*Q150ΔI mice at 17 months of age. Scale bar = 20 μm, zoomed images are 20 μm^2. DG = dentate gyrus, Ly = layers, WT = wild type.



Supplementary Figure 2. HTT aggregate seeding activity is present in *Hdh*Q150 striatum at 6 months of age. (**A**) Quantification of mutant HTT seeding activity in the striatum of WT, WTΔI, *Hdh*Q150 and *Hdh*Q150ΔI mice at 6 months of age. Only the *Hdh*Q150 striatum gave a signal above background; n = 3-4 / genotype. (**B**) Effect of striatal homogenates from the WT, WTΔI, *Hdh*Q150 and *Hdh*Q150ΔI mice at 6 months of age on the Ex1Q48-CyPet and -YPet (1:1 mixture, 1.2 nM) co-aggregation; n = 4 / genotype. (**C**) Effect of cortical and hippocampal homogenates from the WT, WTΔI, *Hdh*Q150 and *Hdh*Q150ΔI mice at 6 months of age on the Ex1Q48-CyPet and -YPet (1:1 mixture, 1.2 nM) co-aggregation; n = 4 / genotype. Statistical analysis was one-way ANOVA with Tukey's post hoc correction. Error bars: mean ± SEM. *$P \leq 0.05$, ***$P \leq 0.001$. h = hours, WT = wild type.



Supplementary Figure 3. Hippocampal huntingtin transcripts generated from the genetically modified loci. (**A-D**) Quantification of (**A**) the full length *Htt* transcript (**B**) the *Htt1a* transcript (**C**) the *Htt readthrough* transcript and (**D**) the *Htt2a* transcript in WT, WTΔI, *Hdh*Q150 and *Hdh*Q150ΔI hippocampus at 6 months of age and WT, *Hdh*Q150 and *Hdh*Q150ΔI hippocampus at 12 months. $n = 8$ / genotype, equal sexes. Statistical analysis was by DESeq2 with Benjamini-Hochberg correction. Error bars: mean ± SEM. $*P \leq 0.05$, $***P \leq 0.001$. TPM (transcripts per million) were computed from RNA-seq data at the indicated ages. WT = wild type.



Supplementary Figure 4. The HTT2a protein could not be detected in the brains of *Hdh*Q150ΔI mice. (**A**) The predicted sequence of the HTT2a protein and position of the sequences used to raise the BAT-GRK and BAT-TGC antibodies. (**B**) Western blot of lysates from COS-1 cells transfected with a *Htt2a* construct fractionated on a 12% SDS-PAGE gel. The HTT2a protein is detected by the 2B7 (amino acids 7-12), BAT-GRK and BAT-TGC

antibodies, but not by the HTT1a specific antibody, MW8. (**C**) Mutant HTT proteins immunoprecipitated with the 3B5H10 antibody from brain hemispheres of WT, *Hdh*Q150 and *Hdh*Q150ΔI mice at 2 months of age and fractionated on a 10% SDS-PAGE gel. The western blot was immunoprobed with S830, MW8, BAT-GRK and BAT-TGC. S830 detects full-length HTT, its proteolytic fragments and HTT1a and MW8 detects HTT1a. The BAT-GRK and BAT-TGC antibodies do not detect any bands specific to the *Hdh*Q150ΔI brains that could correspond to HTT2a. NT = non-transfected, transfection reagents only, vector = empty vector. Size markers are in kDa.

Founder	**Sex**	**Deletion**	**Genomic coordinates**	**Selected**
#113	F	19998 bp	722-20719	
#123	M	19998 bp	722-20719	x
#130	F	19997 bp	723-20719	
#142	F	19997 bp + 10 bp insertion	723-20719	
#146	M	20002 bp	721-20722	x
#220	F	20010 bp	713-20722	
#242	M	20003 bp + 4 bp insertion aact	721-20723	

Supplementary Table 1. Summary of the nature of the intron 1 deletion on the WT allele from the first genetic modification experiment. Colonies were established from lines #123 and #146 and line #123 was used as the WTΔI line.

Name	Immunogen	Epitope	Species	Source
2B7	Human HTT peptide: aa 1-17[1]	LMKAFE*	Mouse monoclonal	CHDI Foundation
MW1	GST-DRPLA (19Q)[2]	PolyQ	Mouse monoclonal	CHDI Foundation
3B5H10	GST-HTT N171 (66Q)[3]	PolyQ	Mouse monoclonal	Sigma-Aldrich P1874
S830	GST-human exon 1 HTT (53Q)[4]	N/A	Sheep polyclonal	In house
CHDI-90001414	Mouse proline-rich regions of HTT1a	Mouse polyP region	Rabbit polyclonal	CHDI Foundation
MW8	Human exon1 HTT (67Q)[2]	C-terminus of HTT1a[5]	Mouse monoclonal	CHDI Foundation
CHDI-90000148		C-terminus of HTT1a	Rabbit polyclonal	CHDI Foundation
P90 clone: 1B12	AEEPLHRP-OH	C-terminus of HTT1a	Rabbit monoclonal	CHDI Foundation
AB2644	Human exon 2 HTT	N/A	Rabbit polyclonal	This publication
MAB5490	Human HTT: aa 115-129[6]	Within: QSVRNSPEFQKLLGI (mouse L)	Mouse monoclonal	Sigma-Aldrich, MAB5490
MAB2166	HTT fusion protein: aa 181-810[7]	Within aa 443-457 GKVLLGEEEALEDD S [8]	Mouse monoclonal	Sigma-Aldrich, MAB2166
D7F7	HTT peptide	Within: aa 1214-1223* QSDTSGPVTT (mouse A)	Rabbit monoclonal	Cell Signaling Technology #5656
BAT-GRK	GRKEEGRRKEEGRK KEGRRKEE	N/A	Rabbit polyclonal	This publication
BAT-TGC	TGCWAVVVHFFNPS TTTTTTTNWLLGCG SAFL	N/A	Rabbit polyclonal	This publication

*Information provided by the CHDI Foundation

Supplementary Table 2. Summary and source of the antibodies used for western blotting, immunohistochemistry and the HTRF assays. DRPLA = dentatorubral-pallidoluysian atrophy, polyQ = polyglutamine, polyP = polyproline.

Antibody Pairing	HTT isoform	Donor (ng / well)	Acceptor (ng / well)	Lysate Concentration
D7F7-Tb:MAB5490-d2	Full-length HTT	1 ng	40 ng	5%
MAB5490-Tb: MW1-d2	Mutant HTT	1 ng	40 ng	5%
2B7-Tb: MW8-d2	HTT1a	1 ng	40 ng	10%
MW8-Tb: CHDI-1414-d2	HTT aggregation	1 ng	40 ng	10% Crude Lysate

Supplementary Table 3. Summary of the antibody concentrations and lysate dilutions used for the HTRF assays.

Ensembl	Gene	Mean	AbsLog2FC	Log2FC	Stat	P value	AdjP	Log2FCSE
ENSMUSG00000068876	*Cgn*	102.17	0.56	0.56	5.15	2.58E-07	3.17E-03	0.11
ENSMUSG00000062078	*Qk*	7475.94	0.18	-0.18	-5.23	1.65E-07	3.17E-03	0.03
ENSMUSG00000042133	*Ppig*	2315.00	0.19	-0.19	-5.07	3.98E-07	3.27E-03	0.04
ENSMUSG00000020570	*Sypl*	1102.43	0.23	-0.23	-4.88	1.04E-06	6.40E-03	0.05
ENSMUSG00000024411	*Aqp4*	1931.38	0.27	-0.27	-4.77	1.81E-06	8.88E-03	0.06
ENSMUSG00000031578	*Mak16*	555.82	0.22	-0.22	-4.62	3.89E-06	1.59E-02	0.05
ENSMUSG00000019966	*Kitl*	859.38	0.29	-0.29	-4.45	8.45E-06	2.45E-02	0.06
ENSMUSG00000068798	*Rap1a*	1058.84	0.22	-0.22	-4.44	8.95E-06	2.45E-02	0.05
ENSMUSG00000093989	*Rnasek*	1746.65	0.12	0.12	4.45	8.53E-06	2.45E-02	0.03
ENSMUSG00000022558	*Mroh1*	1144.99	0.21	0.21	4.34	1.42E-05	3.49E-02	0.05
ENSMUSG00000063954	*H2ac19*	58.86	7.20	7.20	4.30	1.72E-05	3.52E-02	1.68
ENSMUSG00000022899	*Slc15a2*	410.13	0.35	-0.35	-4.31	1.60E-05	3.52E-02	0.08
ENSMUSG00000028100	*Nudt17*	38.60	0.70	0.70	4.24	2.21E-05	3.87E-02	0.17
ENSMUSG00000024974	*Smc3*	1604.73	0.14	-0.14	-4.25	2.18E-05	3.87E-02	0.03

Supplementary Table 4. List of genes that are differentially expressed between WTΔI and WT striata at 6 months of age. Differential expression between the WTΔI and WT striatal RNA-seq data sets from 6-month-old mice at a significance threshold of an adjusted $P < 0.05$ after multiple-test correction, and fold changes of at least 20% in either direction. AbsLog2FC = absolute $\log^2$ fold change, Log2FC = directional $\log^2$ fold change; a negative value indicates decreased expression in WTΔI mice, Stat = Wald statistic, AdjP = false discovery rate adjusted *P* value, Log2FCSE = standard error of the $\log^2$ fold change.

HD Signature	HD Signature	Reversed	% Reversed	Full Reversal	Partial Reversal	Super Reversal	Exacer-bation	% Exacer-bation
***Hdh*Q150ΔI – 6 m Striatum**	1254	314	25	86	228	0	6	0
***Hdh*Q150ΔI – 6 m Hippocampus**	61	18	30	5	13	0	0	0
***Hdh*Q150ΔI – 12 m Striatum**	2821	1141	40	461	663	17	14	0
***Hdh*Q150ΔI – 12 m Hippocampus**	633	331	52	123	207	1	0	0

Supplementary Table 5. Change in the numbers of dysregulated transcripts in the *Hdh*Q150ΔI striatum as compared to *Hdh*Q150. M = months.

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