

OMTN, Volume 17

Supplemental Information

CRISPR-Cas9-Mediated Genome Editing Increases Lifespan and Improves Motor Deficits in a Huntington's Disease Mouse Model

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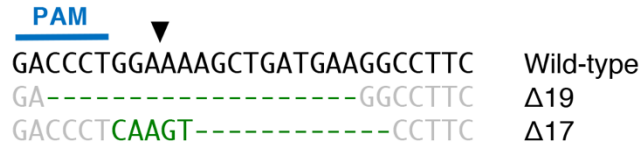


Figure S1. Sanger sequencing of individual HTT transgenes. Human HTT transgenes from the HTT-CFP reporter plasmid were PCR amplified from transfected HEK293T cells and cloned into pcDNA 3.1 for Sanger sequencing. Indels are colored dark green. Wild-type sequence is colored grey. Arrowhead indicates the predicted SaCas9 cleavage site.

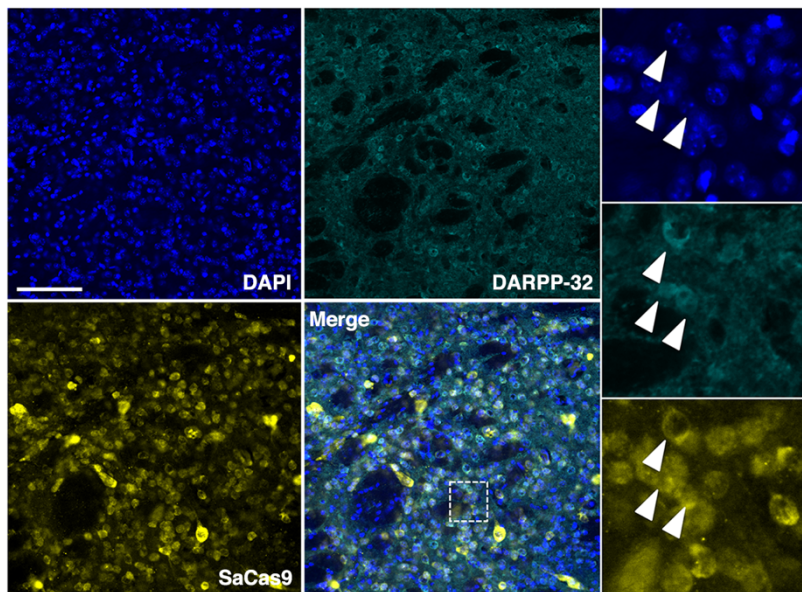


Figure S2. AAV1 can mediate delivery of CRISPR-Cas9 to the striatum. Immunofluorescent staining of striatal sections four weeks after R6/2 mice were injected with 6×10^{10} vector genomes of AAV1-SaCas9-HTT or AAV1-SaCas9-mRosa26. Arrowheads indicate representative SaCas9⁺ and DARPP-32⁺ cells. Inset showed high-magnification image. Scale bar, 100 μ m.

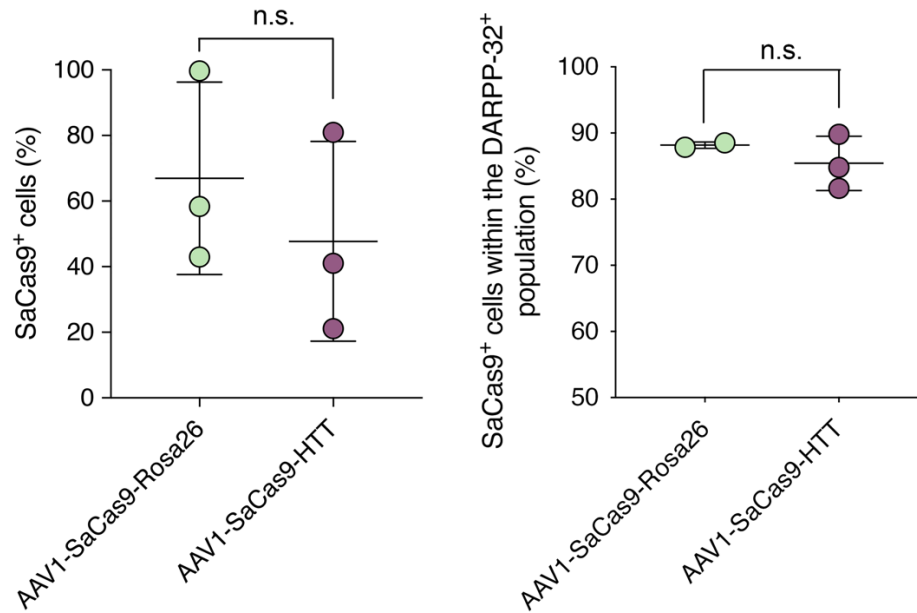


Figure S3. Quantitation of SaCas9 expression in the striatum. Mean number of (left) SaCas9⁺ and (right) dual SaCas9⁺ cells within the DARPP-32⁺ cell population in the striatum four weeks after R6/2 mice were injected with 6×10^{10} vector genomes of AAV1-SaCas9-HTT ($n = 3$) or AAV1-SaCas9-mRosa26 ($n = 3$). Circles represent data from an injected hemisphere. Error bars indicate SD. n.s. indicates not-significant, unpaired t test.

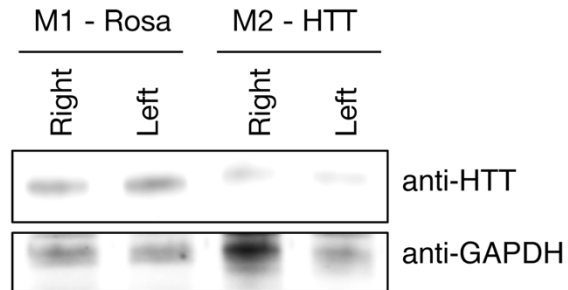


Figure S4. CRISPR-Cas9-mediated gene editing reduced mutant HTT protein in R6/2 mice. Representative western blot of striatal lysate from two R6/2 mice four weeks after a bilateral injection with 6×10^{10} vector genomes of (left) AAV1-SaCas9-mRosa26 or (right) AAV1-SaCas9-HTT. “Left” and “right” denotes the injected hemisphere. Quantitation of western blot results in Fig. 2E.



Figure S5. CRISPR-Cas9 induces indels in the human HTT gene *in vivo*. (A) Representative indels from the human HTT transgene from homogenized striatal tissue harvested from R6/2 mice at 8 weeks following stereotaxic injection with 6×10^{10} vector genomes of AAV1-SaCas9-HTT. Indels are colored dark green. Wild-type sequence is colored grey. Arrowhead indicates predicted SaCas9 cleavage site. The binding site for 3' next-generation sequencing (NGS) primer used for PCR amplification is indicated in light green. (B) Indel frequencies from NGS analysis from mice treated with 6×10^{10} vector genomes of either AAV1-SaCas9-mRosa26 or AAV1-SaCas9-HTT. Indels were quantified using CRISPResso within a 25 bp window around the predicted cleavage site (base substitutions were ignored).

A

hHTT	GAAGGCCTTCATCAGCTTTTC	
mHTT	GAAGGCCTTCATCAGCTTTTC	Chr 5:34761912
OT1	GAAGGgCTaCATCAGCTTGCTTC	Chr 7:93570016
OT2	G--GGCCTTCAaCAGCTTTTC	Chr 3:10135241
OT3	GAAGGC-TTCATaAGCTgTTC	Chr 3:41559057
OT4	GAAGG--TTaATaAGCTTTTC	Chr 3:69106284
OT5	GAAGGgCTTCAT--GgTTTTTC	Chr 3:25571752
OT6	GAAGaCCTTC--CAGCTTTTg	Chr 7:74116528
OT7	GAAGGgCT-CATCAGCTTTcC	Chr 1:124841961
OT8	GAAGGaCTTCAT--GCTgTTC	Chr 6:73244101
OT9	GgAGtCCT--ATCAGCTTTTC	Chr 19:57577634

B

	AAV9-SaCas9-HTT	AAV9-SaCas9-mRosa26
mHTT	0.12% ± 0.01%	0.19% ± 0.05%
OT1	0.04% ± 0.01%	0.11% ± 0.08%
OT2	0.03% ± 0.01%	0.04% ± 0.01%
OT3	0.03% ± 0.01%	0.03% ± 0.01%
OT4	0.05% ± 0.02%	0.05% ± 0.02%
OT5	0.11% ± 0.01%	0.12% ± 0.01%
OT6	0.04% ± 0.02%	0.03% ± 0.02%
OT7	0.08% ± 0.03%	0.07% ± 0.02%
OT8	0.03% ± 0.01%	0.03% ± 0.01%
OT9	0.06% ± 0.02%	0.07% ± 0.01%

Figure S6. Analysis of off-target mutations introduced by CRISPR-Cas9 (A) Sequence and genomic location of the candidate off-target sites in the mm10 mouse reference genome identified using Cas-OFFinder. Target mismatches are colored red. **(B)** Indel frequencies at candidate off-target sites R6/2 mice injected with 6×10^{10} vector genomes of either AAV1-SaCas9-mRosa26 or AAV1-SaCas9-HTT. Indels were quantified within a 25 bp window around the predicted cleavage site (base substitutions were ignored).

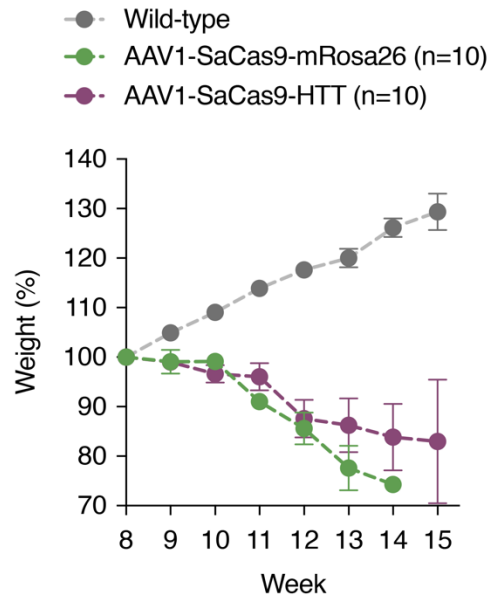


Figure S7. Genome editing did not significantly affect weight loss in R6/2 mice. Weight of R6/2 mice bilaterally injected with 6×10^{10} vector genomes of AAV1-SaCas9-HTT ($n = 10$) and AAV1-SaCas9-mRosa26 ($n = 10$). Wild-type ($n = 11$) are litter-matching B6CBAF1 mice. Values are means and error bars indicate S.E.M. Weight was analyzed using a two-way analysis of variance (ANOVA) followed by Tukey's post hoc analysis.

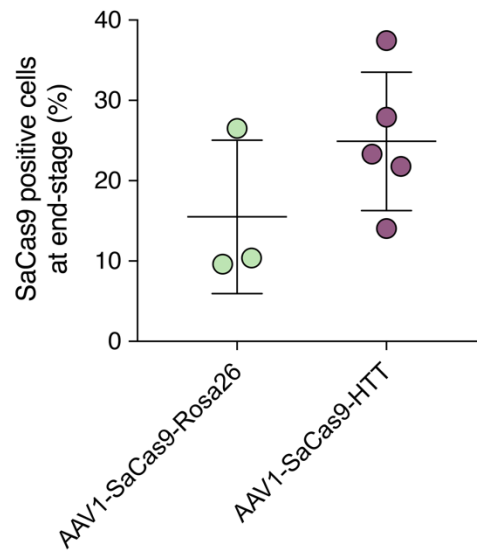


Figure S8. CRISPR-Cas9-mediated gene editing increases neuron survival. Mean number of SaCas9⁺ cells in the striatum at end-stage for R6/2 mice bilaterally injected with 6×10^{10} vector genomes of AAV1-SaCas9-HTT ($n = 3$) or AAV1-SaCas9-mRosa26 ($n = 3$). Circles represent individual mice. Error bars indicate SD. n.s. indicates not-significant, unpaired t test.

Table S1

Oligonucleotides used in this study.	
Name	Sequence (5' to 3')
hSyn-SpeI-Fwd	ATTATTGACTAGTCTGCAGAGGGCCCTGCGTATGA GTGCAAG
hSyn-AgeI-Rev	GGTGGCACCGGTCGACTGCGCTCTCAGGCACGACA CGACTCC
HTT-sgRNA-1-Fwd	CACCGAAGGCCTTCATCAGCTTTTC
HTT-sgRNA-1-Rev	AAACGAAAAGCTGATGAAGGCCTTC
HTT-sgRNA-2-Fwd	CACCGCTGCTGCTGGAAGGACTTGA
HTT-sgRNA-2-Rev	AAACTCAAGTCCTTCCAGCAGCAGC
HTT-sgRNA-3-Fwd	CACCGCAGCGGCTGTGCCTGCGGCG
HTT-sgRNA-3-Rev	AAACCGCCGCAGGCACAGCCGCTGC
EcoRI-HTT-Exon 1-Fwd	GGCTAGGAATTCCCGCTCAGGTTCTGCTTTTA
XbaI-HTT-Exon 1-Rev	GGCTAGTCTAGATGGAAGGACTTGAGGGACTC
qPCR-BGH-Fwd	GCCTTCTAGTTGCCAGCCAT
qPCR BGH Rev	GGCACCTTCCAGGGTCAAG

R6/2-Genotype-Fwd	CCGCTCAGGTTCTGCTTTTA
R6/2-Genotype-Rev	TGGAAGGACTTGAGGGACTC
Off Target mHTT FWD EXT	GCGGAGAGTCTTAAACTAGCAGAGG
Off Target mHTT REV EXT	TGCTGCTGCTGTTGCTGCTGAAACG
Off Target 1 FWD EXT	TATTAGTGGGTGGAGTCTGATGTGT
Off Target 1 REV EXT	TAATCTCTCAACATCAATGGCCTCA
Off Target 2 FWD EXT	GTTGGGTTTATCAGGTATGAAGACAA
Off Target 2 REV EXT	CCGCAGTATAACAATGCTGCATTA
Off Target 3 FWD EXT	ACTATGAAGCAGGGCAGAATGAAGG
Off Target 3 REV EXT	CAAATTAAGAACCATATTCTTCCACATT
Off Target 4 FWD EXT	TGTAAAGTTACATATATGTCCCCAACC
Off Target 4 REV EXT	CCAGGGATCCAACCTAAAGTCCTTAG
Off Target 5 FWD EXT	GGGTGCACATGACATTTTGTAATTTTG
Off Target 5 REV EXT	CGTGAGCTAATTGTTCTTATCACAAC

Off Target 6 FWD EXT	TCGGAGGCTTCATCAGCACTTTCTC
Off Target 6 REV EXT	CATTGAATTTAGGGTACATCCTACATCC
Off Target 7 FWD EXT	AGATATTACCAGAATACAATCTGCC
Off Target 7 REV EXT	GGTGAGTTTACAAATTTAGAAGTTTCC
Off Target 8 FWD EXT	GGTCATTGCTGACAATCTACTTCTC
Off Target 8 REV EXT	CATGTAATGTAATATCATGCTAAAGGC
Off Target 9 FWD EXT	GCTGATAACAAAATCTGGGATGGCC
Off Target 9 REV EXT	GAACTGCTACCATCTTTGAAATGTAAC
On Target hHTT FWD EXT	CCGCTCAGGTTCTGCTTTTA
On Target hHTT REV EXT	CTGCTGCTGCTGGAAGGACT
Off Target mHTT FWD INT	NNNNNGCTCTTCCGATCTTAAGTGGCGCCGCGTAG TGCCAGTA
Off Target mHTT REV INT	NNNNNGCTCTTCCGATCTTGCTGCTGCTGTTGCTGC TGAAACGACTTG
Off Target 1 FWD INT	NNNNNGCTCTTCCGATCTGAAGTGTCTTCCCCATC TCTTGATTAATT
Off Target 1 REV INT	NNNNNGCTCTTCCGATCTTACATAATCTCAGAGCA AAGGGCTGGAATA

Off Target 2 FWD INT	NNNNNGCTCTTCCGATCTCCTATCAGCCAACCTGA AGTGTCTCATTTTC
Off Target 2 REV INT	NNNNNGCTCTTCCGATCTACTCCAGCATGTGTTCTG TAATGCCAGAA
Off Target 3 FWD INT	NNNNNGCTCTTCCGATCTCTGGGTGGGCATTGTTA CTAGTCAGTCTGT
Off Target 3 REV INT	NNNNNGCTCTTCCGATCTAGGAGGAGAGATGGTAG GTTAAGGGATTGG
Off Target 4 FWD INT	NNNNNGCTCTTCCGATCTAGCCTAGCCTACATAAT AAGACTCTATCTC
Off Target 4 REV INT	NNNNNGCTCTTCCGATCTTTAAGGGAATGGATATA CAGTGCATCTGGT
Off Target 5 FWD INT	NNNNNGCTCTTCCGATCTCGCTAGATTAAGAAGCA TGTAATGGCAGC
Off Target 5 REV INT	NNNNNGCTCTTCCGATCTGGACTGTGATGAGAAAA TTAGAGGCTCTAA
Off Target 6 FWD INT	NNNNNGCTCTTCCGATCTCAAGACCCAGCGTATTG TAAAACACAAGAA
Off Target 6 REV INT	NNNNNGCTCTTCCGATCTAGTTAAGACATGGGTCT ATCATCTAGCGGA
Off Target 7 FWD INT	NNNNNGCTCTTCCGATCTACTTTCTCAAGATCTAG ACTCACACTAGAC
Off Target 7 REV INT	NNNNNGCTCTTCCGATCTGTGTTTCAATGTTTATGT GCATATATACCATGTG
Off Target 8 FWD INT	NNNNNGCTCTTCCGATCTGGGTTTTTCATACAATTCT TACATTAAGAGGGC
Off Target 8 REV INT	NNNNNGCTCTTCCGATCTACAGCCACAATGGGAGC CCCTCACA

Off Target 9 FWD INT	NNNNNGCTCTTCCGATCTCATAACTAATAGGAACA AACTGTGACTTTA
Off Target 9 REV INT	NNNNNGCTCTTCCGATCTCAAAGATCTTAGGGTTG CTGCCAAAGGACA
On Target hHTT FWD INT	NNNNNGCTCTTCCGATCTCCCATTCATTGCCCCGGT GCTGAGC
On Target hHTT REV INT	NNNNNGCTCTTCCGATCTCTGCTGCTGCTGGAAGG ACT