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Supplemental information

Liver-directed gene therapy corrects neurologic disease in a murine model of mucopolysaccharidosis type I-Hurler

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Supplemental Figures

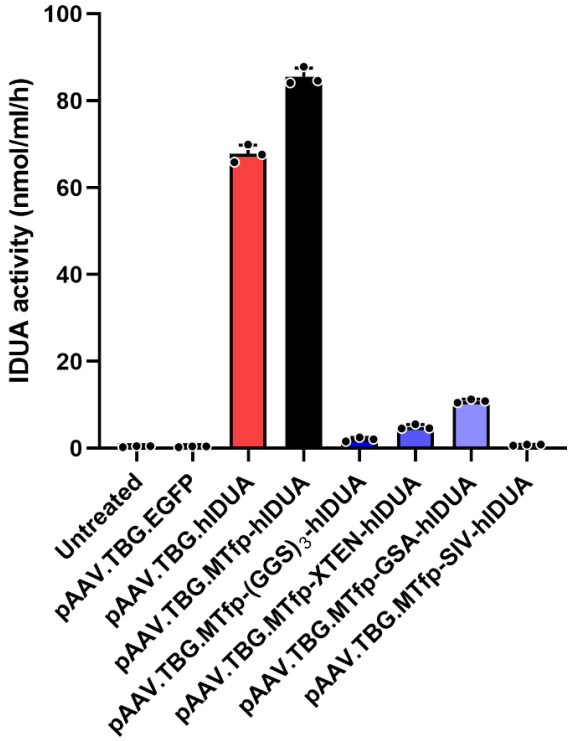

Figure S1. Comparison of IDUA enzyme activity using different linker peptides. In order to compare the effects of linker peptides on IDUA enzyme activity, the rigid $(EAAAK)_3$ sequence in pAAV.TBG.MTfp-hIDUA plasmid was replaced with the flexible linkers to construct following plasmids: pAAV.TBG.MTfp-$(GGS)_3$-hIDUA, pAAV.TBG.MTfp-XTEN-hIDUA, pAAV.TBG.MTfp-GSA-hIDUA and pAAV.TBG.MTfp-SIV-hIDUA. These plasmids, along with pAAV.TBG.hIDUA and pAAV.TBG.MTfp-hIDUA, were transfected into Huh7 cells, respectively. After 48 h, cell culture supernatants were collected to determine IDUA activity. Mock-transfected supernatant is served as a control (untreated). n=3 biological replicates each. Mean ± SD are shown. GSA represents GSAGSAAGSGEF linker and SIV represents SIVAQLSRPDPA linker. The sequences of linkers are shown in Table S1.

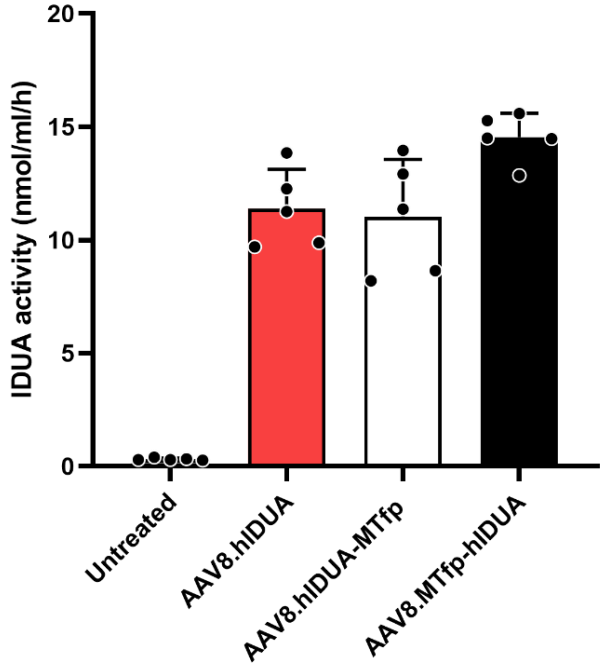

Figure S2. IDUA enzyme activity of AAV8 transduced Huh7 cells. Huh7 cells were transduced with AAV8 vectors for three days. The cell culture supernatant was collected for IDUA enzyme activity analysis. Mock-transduced supernatant is served as a control (untreated). n=5 biological replicates each. Mean ± SD are shown.

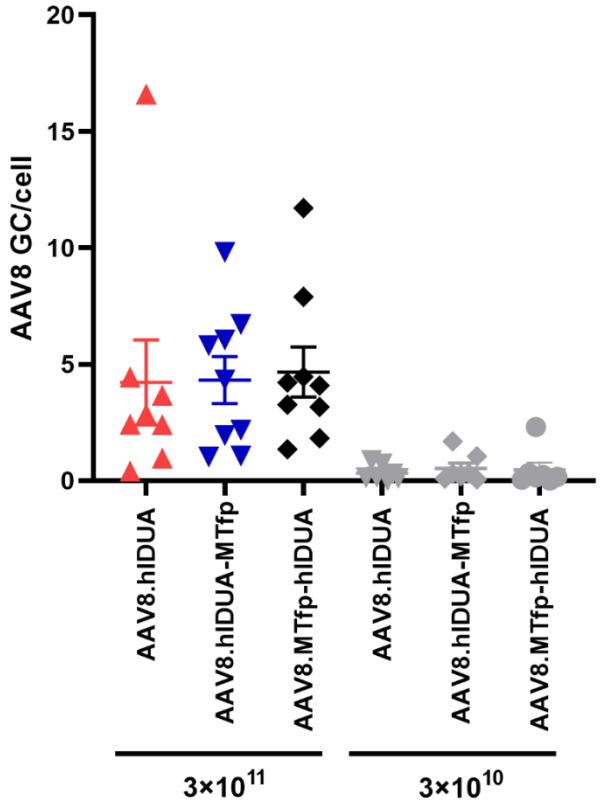

Figure S3. Vector genome copy number in liver. Quantitative analysis of viral genome copy number in liver tissues 16 weeks after injection by qPCR. 3×10^{11} GC/mouse dose group (AAV8.hIDUA treated mice, n=8, AAV8.hIDUA-MTfp treated mice, n=9 and AAV8.MTfp-hIDUA treated mice, n=9); 3×10^{10} GC/mouse dose group, n=7. Mean ± SD are shown.

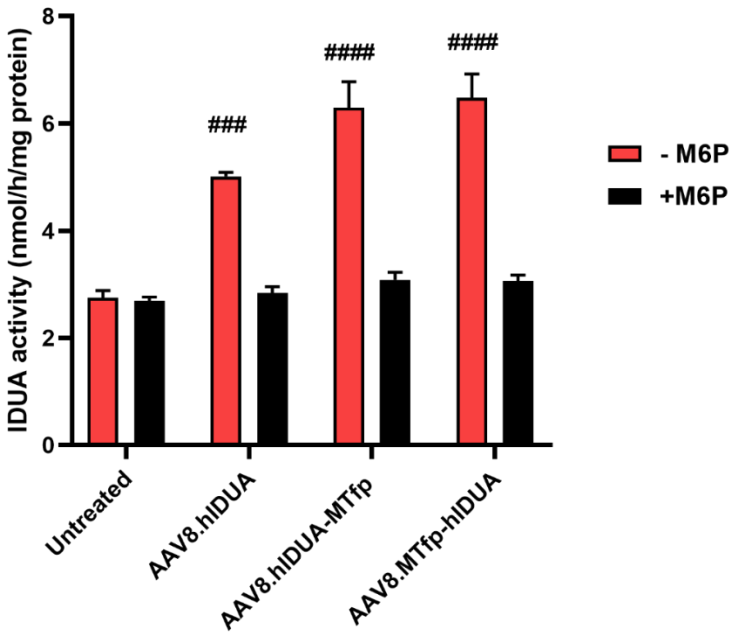

Figure S4. Excess free mannose-6-phosphate (M6P) blocks uptake of IDUA. IDUA enzyme activity in protein extracts from cells incubated with untreated media or IDUA-conditioned media in the presence or absence of 5 mM M6P was detected. The untreated media is the conditioned media without IDUA. n=3 biological replicates each. Mean ± SD are shown. ####$p<0.0001$, when compared to the group incubated in the same IDUA-conditioned media (but with the addition of M6P).

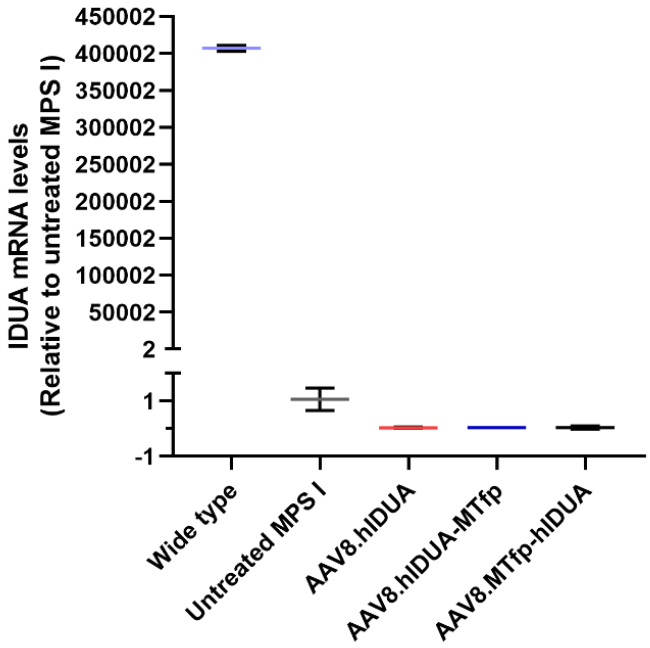

Figure S5. IDUA mRNA levels in the brain. To rule out that the recovered IDUA activity in the brain after intravenous injection of AAV8 was derived from brain cell expression, IDUA mRNA levels in the brain were measured by RT-qPCR 16 weeks after high-dose AAV8 injection. Mean ± SD are shown. Wild-type mice, n=3; untreated MPS I mice, n=3; 3×10^{11} GC/mouse dose group (AAV8.hIDUA treated mice, n=3, AAV8.hIDUA-MTfp treated mice, n=1 and AAV8.MTfp-hIDUA treated mice, n=3). IDUA mRNA levels could not be detected in the brains of treated and untreated MPS I mice.

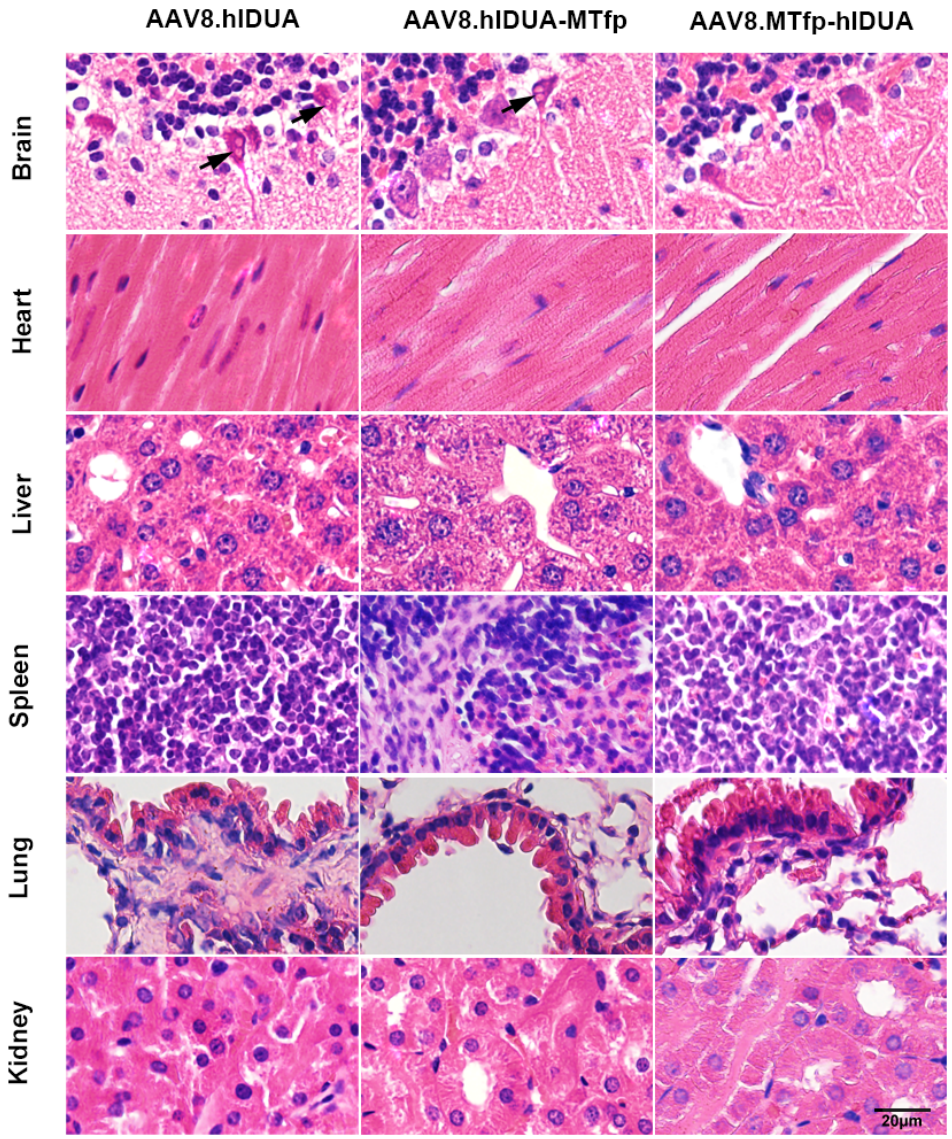

Figure S6. Correction of histological abnormalities in low-dose treated mice. Histological analysis of the brain, heart, liver, spleen, lung and kidney 16 weeks after injection by H&E staining. Scale bar, 20 μm. Black arrows indicate cytoplasmic cellular vacuolation in Purkinje neurons or foamy macrophages in the heart, liver, spleen, lung and kidney due to GAG accumulation. The H&E staining of treated mice were obtained from low-dose groups.

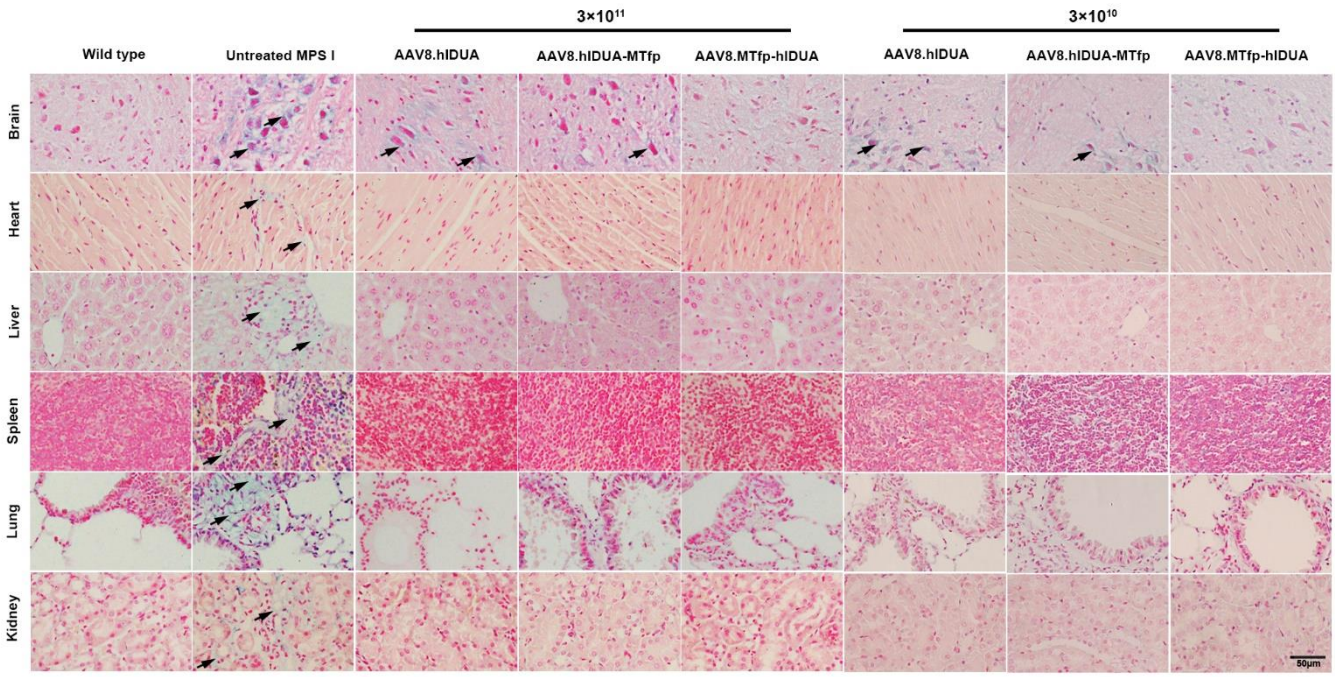

Figure S7. Correction of histological abnormalities in treated mice. The tissues were stained with Alcian blue to detect GAGs storage 16 weeks after injection. Scale bar, 50 μm. Black arrows indicate the GAGs storage in the brain, heart, liver, spleen, lung and kidney.

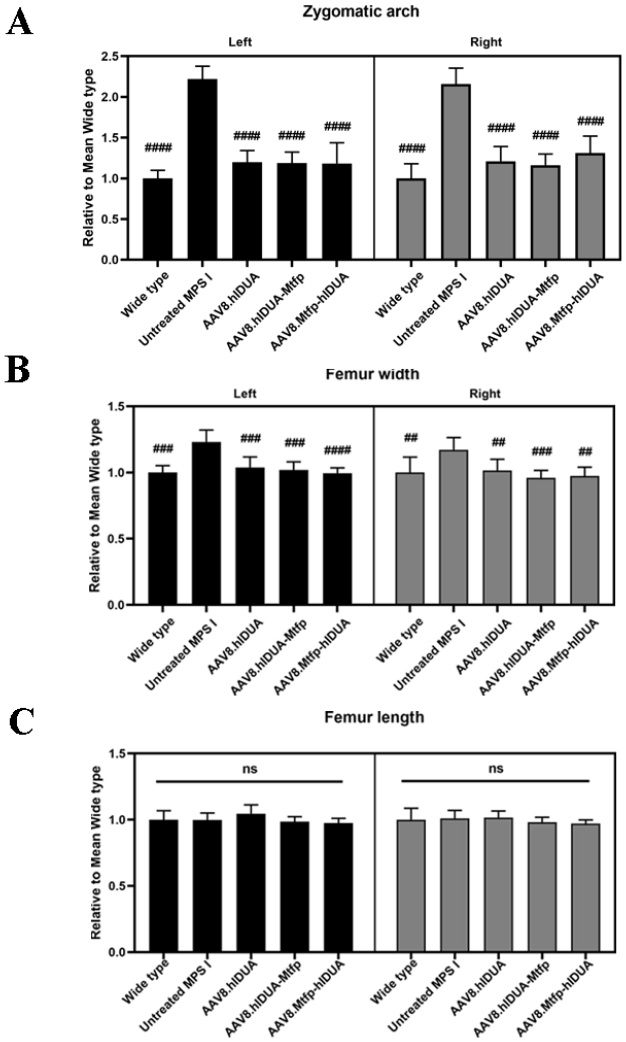

Figure S8. Reversion of bone abnormalities in low-dose treated mice. (A) Quantification of zygomatic arch width. (B) Quantification of femur width. (C) Quantification of femur length. (A-C) Images were analyzed using the ImageJ program. Data represent the relative value to mean of wild-type mice. The data of treated mice were obtained from low-dose groups. Mean ± SD are shown. Wild-type mice, n=7; untreated MPS I mice, n=7; 3×10^{10} GC/mouse dose group, n=7. ns=nonsignificant. ##p<0.01, ###p<0.001, ####p<0.0001, when compared to the untreated MPS I group.

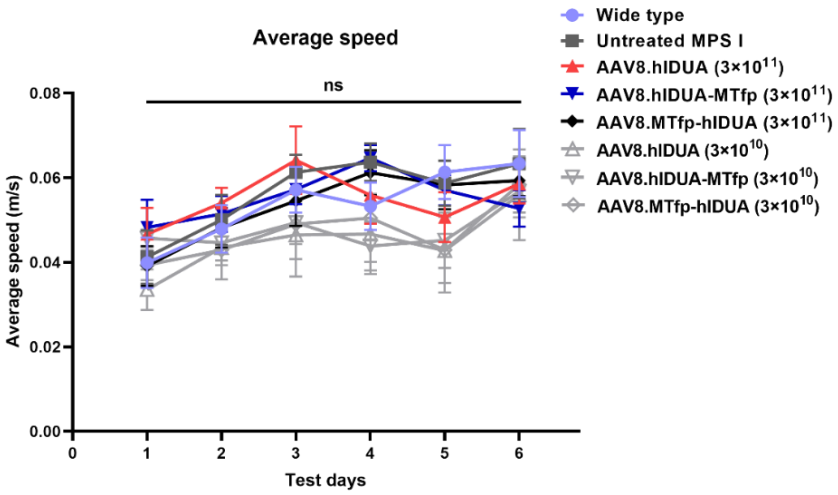

Figure S9. Average running speed for all groups on six-day behavioral testing. To confirm that the deficits displayed by the MPS I mice were not due to motor ability deficits caused by physical illness, statistics on the average running speed of all groups were performed. Wild-type mice, n=7; untreated MPS I mice, n=7; 3×10^{11} GC/mouse dose group (AAV8.hIDUA treated mice, n=8, AAV8.hIDUA-MTfp treated mice, n=9 and AAV8.MTfp-hIDUA treated mice, n=9); 3×10^{10} GC/mouse dose group, n=7. Mean ± SEM are shown. The average speed per day is the average of four trials. ns=nonsignificant. No significant differences were observed in this parameter between any animal groups.

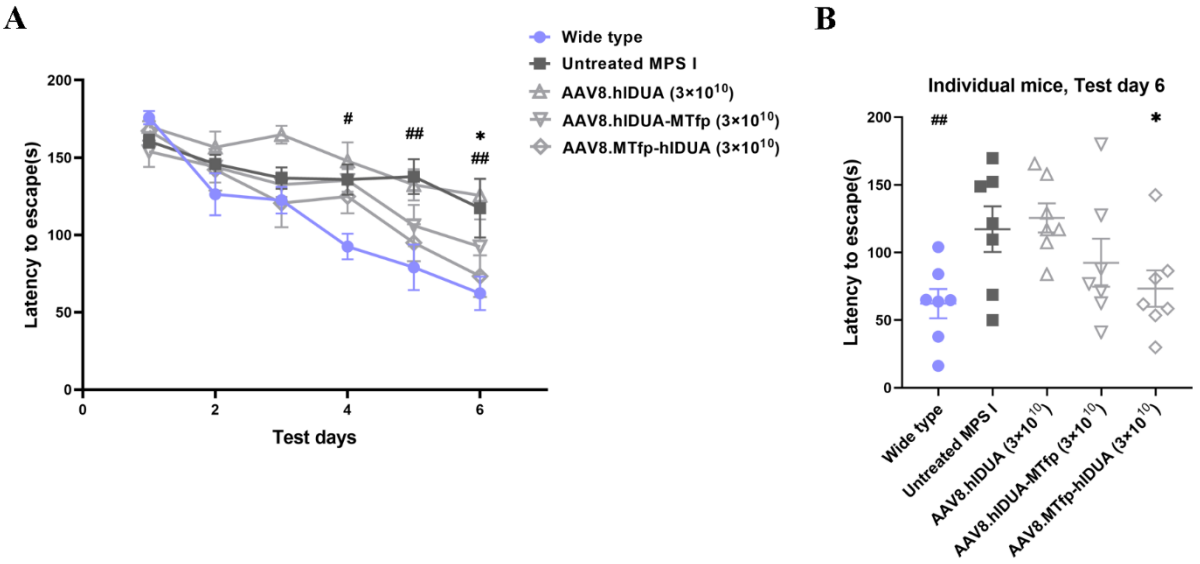

Figure S10. Latency to escape of low-dose treated groups on six-day behavioral testing. (A) Cognitive performance was assessed using the DMP dry maze 12 weeks after treatment. Data represent the time required to escape the platform over 6 days of testing. (B) Data from individual mice from all low-dose groups on day 6 of testing are shown. (A and B) Data are shown as the mean ± SEM. Wild-type mice, n=7; untreated MPS I mice, n=7; 3×10^{10} GC/mouse dose group, n=7. #Wild-type group, *AAV8.MTfp-hIDUA low dose group, #p<0.05, ##p<0.01, *p<0.05, compared to the untreated MPS I group. Throughout the six-day test period, there was no significant difference in latency to escape between the AAV8.hIDUA treated mice and untreated MPS I mice. The latency to escape times of AAV8.hIDUA-MTfp treated mice had a slight improvement, with average escape latency ranging from 154 to 92 s. The average escape latency of mice treated with low-dose AAV8.MTfp-hIDUA ranged from 167 to 81 s, which was not significantly different from wild-type mice on the last test day.

Supplemental Methods

RT-qPCR

Total RNA was extracted from brains with a FastPure Tissue Total RNA Isolation Kit (Vazyme, Nanjing, China) according to the manufacturer's instructions. Then, RNA was reverse transcribed to cDNA using the PrimeScript™ RT reagent Kit with gDNA eraser (perfect real time) (Takara, Kusatsu, Japan) according to the manufacturer's instructions. DNase was used in RNA extraction and transcription to ensure elimination of DNA contamination. Quantitative PCR (qPCR) was performed with TB Green Premix Ex TaqTM II (Takara, Kusatsu, Japan). Primers for hIDUA, mouse IDUA (mIDUA) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes are presented in the supplemental Table S2. qPCRs were run in triplicate for each gene per sample. The specificity of the qPCR was confirmed by detection of a single distinct peak on examination of the dissociation curve profile of the reaction product. The relative gene expression was calculated using the ΔΔCt method[1]. Target gene expression was normalized to the housekeeping reference gene GAPDH and then compared to the control (untreated MPS I).

Total protein determinations

We used the BCA assay for total protein determinations (Pierce BCA protein assay kit, 23225, Thermo). Firstly, we configured BCA working solution by fully mixing A and B solution in the kit (A:B=50:1). In the second step, 2ul of different concentrations of standard BSA and 2ul of protein samples were added to the 96-well plate, respectively, and then each well was supplemented to 20ul with PBS. Each sample was performed in triplicate. In the third step, we added 200ul BCA working solution to each well and mix. After incubation at 37°C for 30 minutes, the absorbance was measured at 562 nm and the total protein concentration was calculated from the standard curve.

Supplemental Tables

Table S1. The nucleotide sequences of linkers.

Name	sequence
$(EAAAK)_3$	GAGGCCGCTGCTAAAGAGGCTGCCGCCAAAGAAGCCGCCGCTAAG
$(GGS)_3$	GGTGGCAGCGGAGGATCTGGTGGATCT
XTEN	TCCGGCAGCGAGACGCCAGGCACCTCCGAGAGCGCTACGCCTGAATCC
GSAGSAAGSGEF	GGCTCTGCCGGATCTGCTGCTGGATCTGGCGAATTT
SIVAQLSRPDPA	TCCATCGTGGCCCAGCTGAGCAGACCTGATCCTGCT

Table S2. qPCR Primers.

Genes	Forward primer	Reverse primer
GAPDH	TGTGAACGGATTTGGCCGTA	ACTGTGCCGTTGAATTTGCC
hIDUA	TGTACGTGACCCGCTATCTG	TGTCCTGGAGGCTTCTCTGG
mIDUA	CACCAGAACCTGCTGTTTGC	CCCAGCCTTTGAGACCTCTG

Supplemental Sequences

Signal peptide sequences are orange, MTfp sequences are pink, Linker sequences are blue.

Amino acid sequences of hIDUA

MRPLRPRAALLALLASLLAAPPVAPAEAPHLVHVDAARALWPLRRFWRSTGFCPPLPHS
QADQYVLSWDQQLNLAYVGAVPHRGIKQVRTHWLLELVTTRGSTGRGLSYNFTHLDG
YLDLLRENQLLPGFELMGSASGHFTDFEDKQQVFEWKDLVSSLARRYIGRYGLAHVSK
WNFETWNEPDHHDFDNVSMTMQGFLNYYDACSEGLRAASPALRLGGPGDSFHTPPRSP
LSWGLLRHCHDGTNFFTGEAGVRLDYISLHRKGARSSISILEQEKVVAQQIRQLFPKFAD
TPIYNDEADPLVGWSLPQPWRADVTYAAMVVKVIAQHQNLLLANTTSAFPYALLSNDN
AFLSYHPHPFAQRTLTARFQVNNTRPPHVQLLRKPVLTAMGLLALLDEEQLWAEVSQA
GTVLDSNHTVGVLASAHRPQGPADAWRAAVLIYASDDTRAHPNRSVAVTLRLRGVPPG
PGLVYVTRYLDNGLCSPDGEWRRLGRPVFPTAEQFRRMRAAEDPVAAAPRPLPAGGRL
TLRPALRLPSLLLVHVCARPEKPPGQVTRLRALPLTQGQLVLVWSDEHVGSKCLWTYEI
QFSQDGKAYTPVSRKPSTFNLFVFSPDTGAVSGSYRVRALDYWARPGPFSDPVPYLEVP
VPRGPPSPGNP*

Amino acid sequences of hIDUA-MTfp

MRPLRPRAALLALLASLLAAPPVAPAEAPHLVHVDAARALWPLRRFWRSTGFCPPLPHS
QADQYVLSWDQQLNLAYVGAVPHRGIKQVRTHWLLELVTTRGSTGRGLSYNFTHLDG
YLDLLRENQLLPGFELMGSASGHFTDFEDKQQVFEWKDLVSSLARRYIGRYGLAHVSK
WNFETWNEPDHHDFDNVSMTMQGFLNYYDACSEGLRAASPALRLGGPGDSFHTPPRSP
LSWGLLRHCHDGTNFFTGEAGVRLDYISLHRKGARSSISILEQEKVVAQQIRQLFPKFAD
TPIYNDEADPLVGWSLPQPWRADVTYAAMVVKVIAQHQNLLLANTTSAFPYALLSNDN
AFLSYHPHPFAQRTLTARFQVNNTRPPHVQLLRKPVLTAMGLLALLDEEQLWAEVSQA

GTVLDSNHTVGVLASAHRPQGPADAWRAAVLIYASDDTRAHPNRSVAVTLRLRGVPPG

PGLVYVTRYLDNGLCSPDGEWRRLGRPVFPTAEQFRRMRAAEDPVAAAPRPLPAGGRL

TLRPALRLPSLLLVHVCARPEKPPGQVTRLRALPLTQGQLVLVWSDEHVGSKCLWTYEI

QFSQDGKAYTPVSRKPSTFNLFVFSPDTGAVSGSYRVRALDYWARPGPFSDPVPYLEVP

VPRGPPSPGNPEAAAKEAAAKEAAAKDSSHAFTLDELR*

Amino acid sequences of MTfp-hIDUA

MRPLRPRAALLALLASLLAAPPVAPAEDSSHAFTLDELREAAAKEAAAKEAAAKAPHL

VHVDAARALWPLRRFWRSTGFCPPLPHSQADQYVLSWDQQLNLAYVGAVPHRGIKQV

RTHWLLELVTTRGSTGRGLSYNFTHLDGYLDLLRENQLLPGFELMGSASGHFTDFEDKQ

QVFEWKDLVSSLARRYIGRYGLAHVSKWNFETWNEPDHHDFDNVSMTMQGFLNYYD

ACSEGLRAASPALRLGGPGDSFHTPPRSPLSWGLLRHCHDGTNFFTGEAGVRLDYISLHR

KGARSSISILEQEKVVAQQIRQLFPKFADTPIYNDEADPLVGWSLPQPWRADVTYAAMV

VKVIAQHQNLLLANTTSAFPYALLSNDNAFLSYHPHPFAQRTLTARFQVNNTRPPHVQL

LRKPVLTAMGLLALLDEEQLWAEVSQAGTVLDSNHTVGVLASAHRPQGPADAWRAAV

LIYASDDTRAHPNRSVAVTLRLRGVPPGPGLVYVTRYLDNGLCSPDGEWRRLGRPVFPT

AEQFRRMRAAEDPVAAAPRPLPAGGRLTLRPALRLPSLLLVHVCARPEKPPGQVTRLRA

LPLTQGQLVLVWSDEHVGSKCLWTYEIQFSQDGKAYTPVSRKPSTFNLFVFSPDTGAVS

GSYRVRALDYWARPGPFSDPVPYLEVPVPRGPPSPGNP*

Reference

1. Pfaffl, M.W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res *29*, e45. 10.1093/nar/29.9.e45.