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Supplemental Information

A Highly Efficacious PS Gene Editing System Corrects Metabolic and Neurological Complications of Mucopolysaccharidosis Type I

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Supplemental Figures

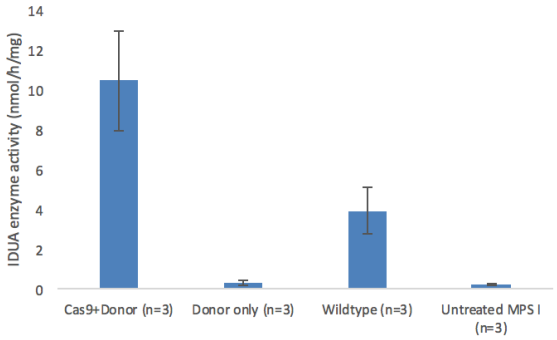

Fig.S1. Hydrodynamic injection of plasmids encoding the PS system into adult MPS I mice. The two plasmids were administered into adult MPS I mice through hydrodynamic injection. All mice were euthanized 2 days post injection. The enzyme activities in mice receiving Cas9 and donor plasmids were significantly higher than those in untreated or mice receiving the donor plasmid only.

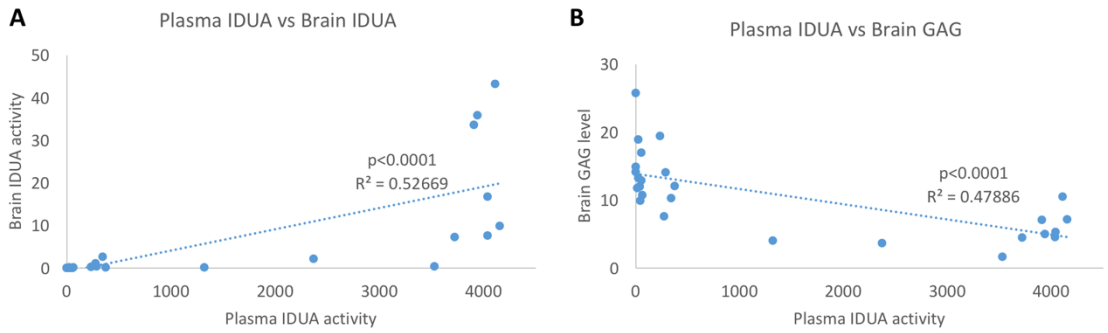

Fig.S2. Correlation between plasma IDUA enzyme activity and IDUA enzyme activity and GAG level in the brain. (A) Significant correlation between plasma IDUA enzyme activity at month 10 and brain IDUA enzyme activity in treated MPS I mice (all dose groups) was observed. **(B)** Significant correlation between plasma IDUA enzyme activity at month 10 and brain GAG level in treated MPS I mice (all dose groups) was observed. Pearson correlation analysis was performed.

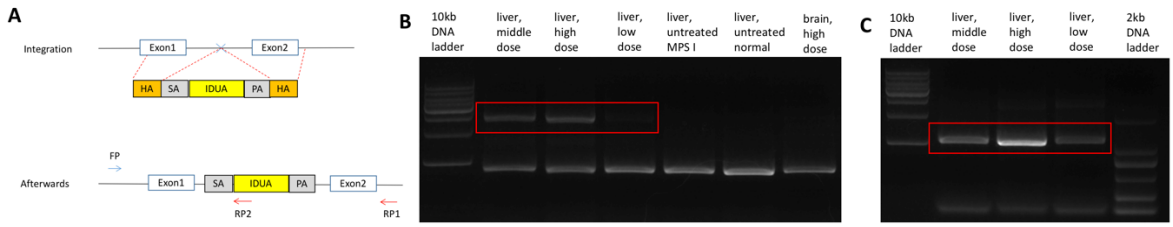

Fig.S3. Nested PCR to confirm integration. (A) Two sets of primers were designed to detect insertion of the donor sequence. FP: forward primer; RP1&2: reverse primer 1&2. In the first round of PCR, FP and RP1 generate an amplicon of 3,137 bp if there is an integration, and generate an amplicon of 924 bp in untreated controls. In the second round of PCR using the amplicon from the first round as the template, FP and RP2 generate an amplicon of 1,133 bp if there is an integration. **(B)** The gel image of the first round PCR was shown. Liver samples from all three treated groups had the band at the expected size of 3,137 bp, albeit the band from the low dose being faint. Liver samples from untreated MPS I and normal mice, as well as brain samples from the high dose group had no bands indicating insertion. **(C)** The gel image of the first round PCR was shown. Liver samples from all three treated groups had the band at the expected size of 1,133 bp. The amplicons are sequenced for further confirmation.

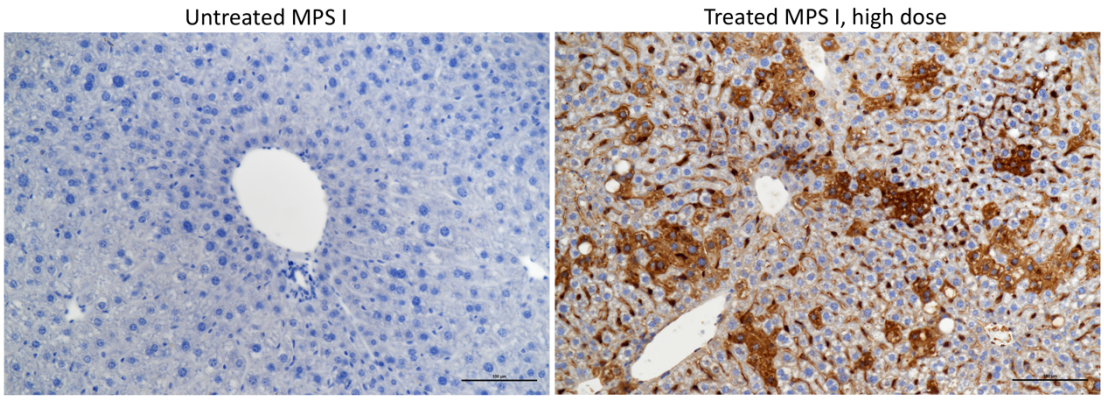

Fig.S4. Immunohistochemistry for IDUA in the liver of MPS I mice. IDUA-expressing cells are those with very strong signal (dark color). The number of IDUA-expressing cells constitute ~24.1% of all hepatocytes in MPS I mice neonatally treated with the high dose.

Supplemental Tables

	Plasma IDUA enzyme activity at 1 month post-dosing (nmol/h/mL)
MPS I, donor only, high dose (n=6)	**1.5±0.4**
MPS I, donor only, middle dose (n=6)	**0.03±0.02**
MPS I, donor only, low dose (n=6)	**0**

Table S1. Plasma IDUA enzyme activity in neonatal MPS I mice treated with the donor vector only. High dose, $3x10^{14}$ vg/kg; middle dose, $3x10^{13}$ vg/kg; low dose, $3x10^{12}$ vg/kg.

	MPS I, untreated (n=9)	Normal (n=3)	MPS I, high dose (n=8)	MPS I, middle dose (n=8)	MPS I, low dose (n=14)
Liver, foam cells	7/9*	0/3	0/8	0/8	1/14
Purkinje cell vacuolation	7/8*	0/3	1/7	8/8	14/14

Table S2. Incidence of characteristic lesions associated with MPS I disease. *= number of evaluable tissues.

Supplemental sequencing results

1. Sequencing the amplicon at the human albumin locus

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2. Sequencing the amplicon at the mouse albumin locus

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