OMTN, Volume *32*

Supplemental information

In vivo genome editing for hemophilia B therapy by the combination of rebalancing and therapeutic gene knockin using a viral and non-viral vector

Jeong Hyeon Lee, Jeong Pil Han, Dong Woo Song, Geon Seong Lee, Beom Seok Choi, MinJeong Kim, Yeji Lee, Seokjoong Kim, Hyukjin Lee, and Su Cheong Yeom

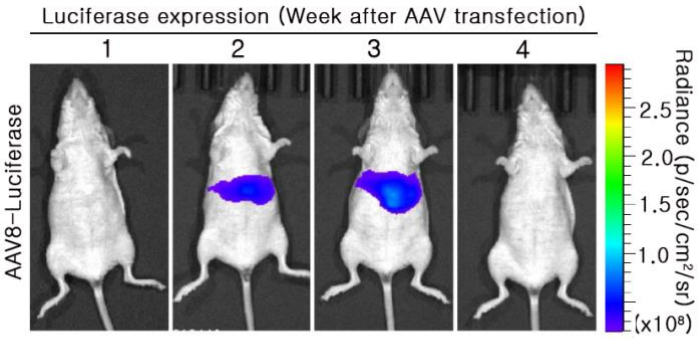

Figure S1. 1×10^{12} vg/kg of AAV8 -Luciferase was injected into a hairless mouse via tail vein, and time course *in vivo* imaging for luciferase was conducted. Images were acquired every week and normalized using the same exposure conditions. Luciferase intensity is shown as radiance ($p/sec/cm^2/sr$).

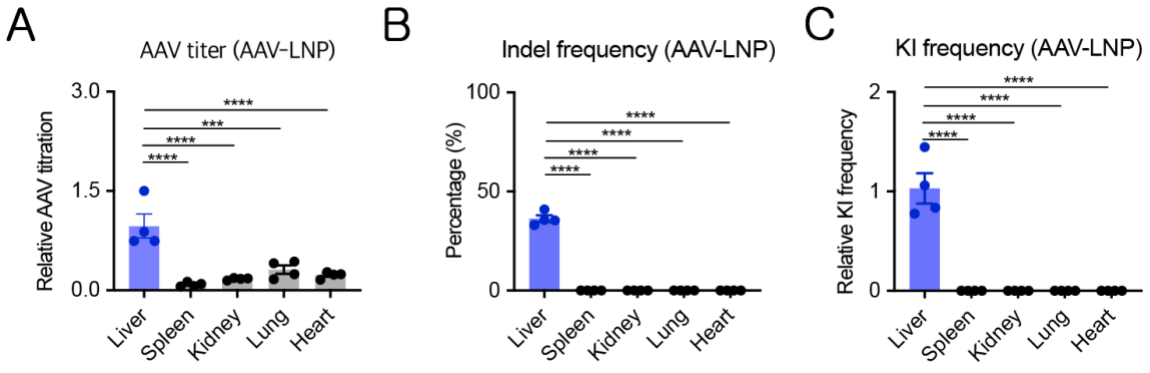

Figure S2. Measuring AAV copy number, indel rate, and KI frequency in mice from the AAV-LNP group. **A**. Relative AAV titer was calculated using qPCR for the ITR of AAVs. AAV titration of each organ was normalized to that of the liver. **B**. Indel analysis was conducted using T7E1 and image analysis. **C**. Relative KI frequency was calculated using qPCR in the 3' site of the expected KI locus. KI frequency of each organ was normalized to that of the liver. (n = 4 per group). Data are presented as mean ± standard error of mean, calculated using one-way ANOVA. (***: $p < 0.001$ and ****: $p < 0.0001$.)

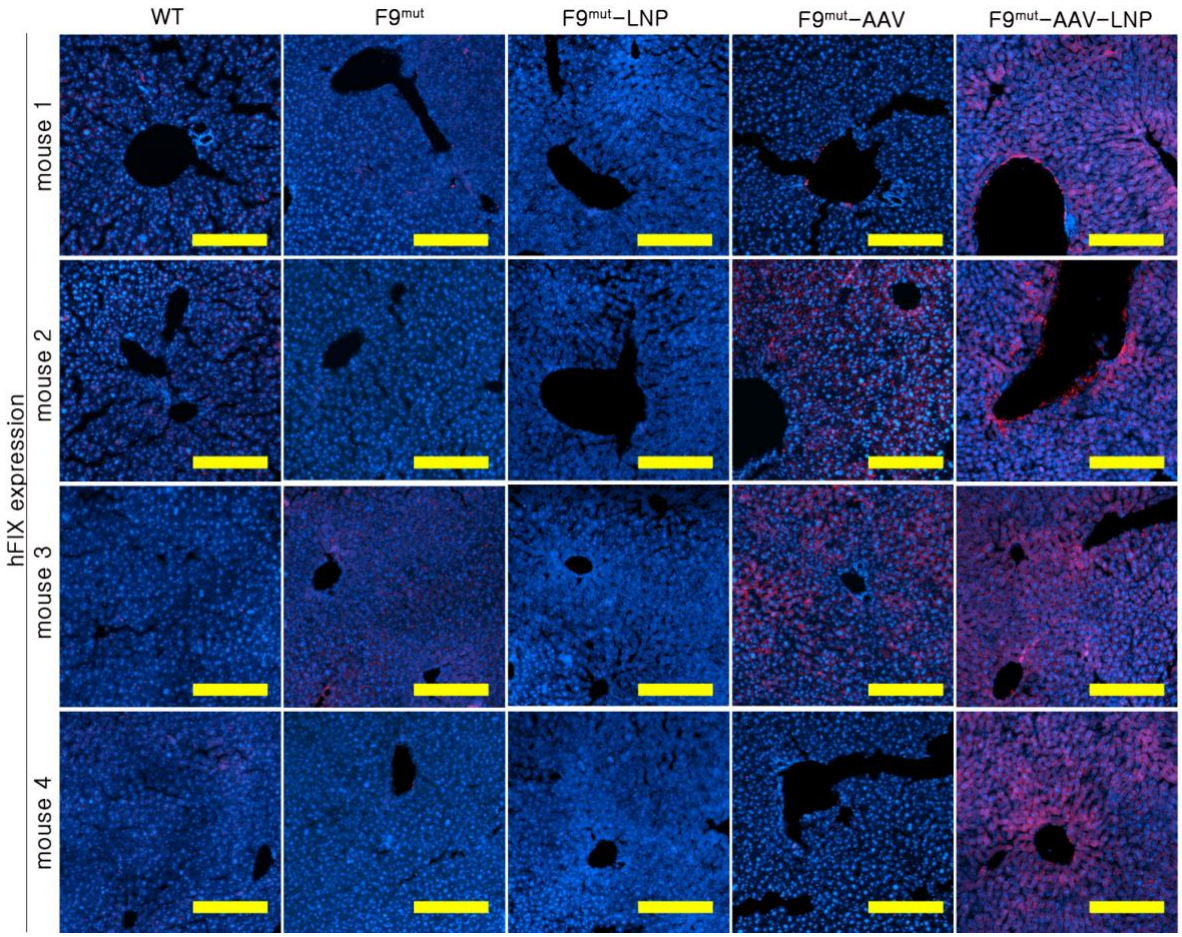

Figure S3. FIX detection after in vivo KI. The images confirm hFIX expression in different groups of mice, as detected using immunofluorescence. hFIX expression image of mouse 1 of each group is same as those shown in Figure 3E. Red color: hFIX, blue color: DAPI, yellow scale bar: 200 μm (100x).

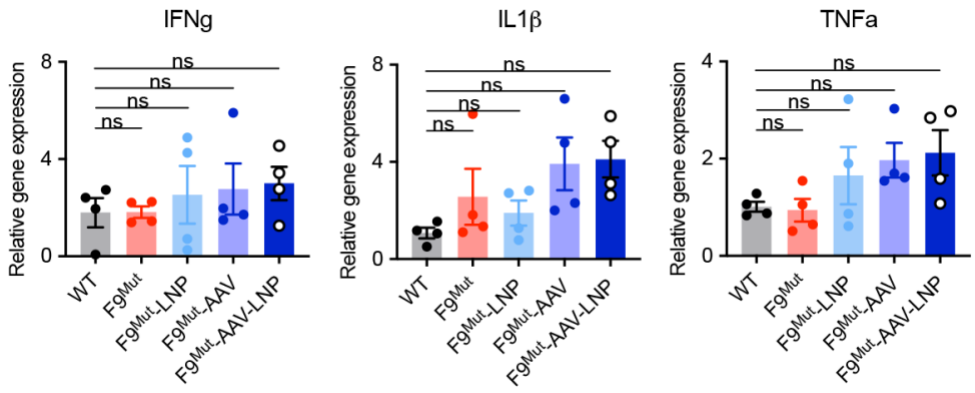

Figure S4. Relative gene expressions of Interferon- γ (IFNg), Tumor necrosis factor-α (TNFa), and interleukin 1β (IL1β) in liver tissue were calculated using qPCR. Gene expressions were normalized to that of the WT. (n = 4 per group). Data are presented as mean ± standard error of mean, calculated using one-way ANOVA. NS: Not significant

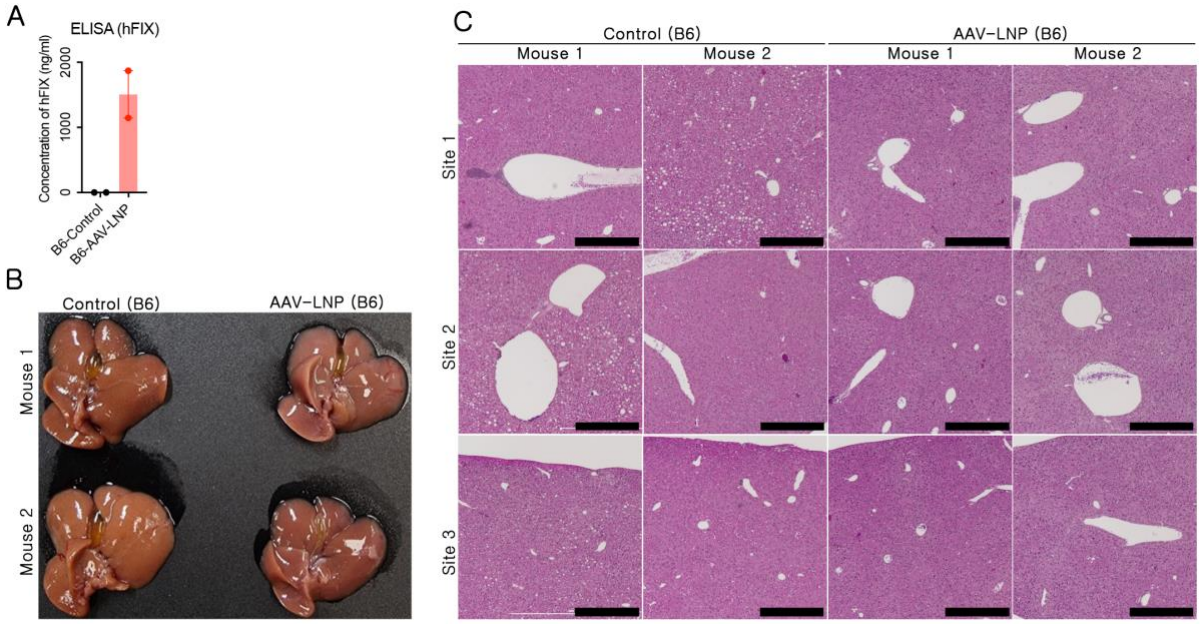

Figure S5. Screening of hepatocellular carcinoma. **A**. Mice after 26 months of AAV and LNP treatment were sacrificed, and blood hFIX concentration was analyzed for confirming *in vivo* h*F9* KI. **B**. Comparison of the appearance of the liver of the control and AAV-LNP group. **C**. Hematoxylin-eosin staining image of 3 different sites from each mouse of control and AAV-LNP group. Black scale bar: 500 μm (40x)

Table S1. Brief information regarding the whole genome sequencing and alignment

Sequencing result

Sample ID	Total reads[a]	Total bases[b]	GC base (%)[c]	Q20 (%)[d]	Q30 (%)[e]
Control_1	686,331,116	103,635,998,516	42.49%	96.79%	92.46%
h*F9*_KI_1	669,210,436	101,050,775,836	41.78%	96.73%	92.42%
h*F9*_KI_2	787,081,650	118,849,329,150	41.87%	96.61%	92.08%
h*F9*_KI_3	810,478,736	122,382,289,136	44.24%	96.64%	92.29%

Alignment result

Sample ID	Mapped reads (%)	Average depth	1X (%)	10X (%)	20X (%)	30X (%)
Control_1	580,937,079 (99.39%)	34.67	99.29%	98.61%	93.89%	69.93%
h*F9*_KI_1	572,393,399 (99.45%)	33.42	99.29%	98.92%	95.94%	67.31%
h*F9*_KI_2	668,750,747 (99.45%)	39.04	99.30%	99.05%	97.67%	87.06%
h*F9*_KI_3	666,038,794 (99.31%)	41.36	99.00%	92.63%	82.07%	68.96%

Table S2. On-target and off-target indel analysis

	Targets	Chr.	Location (mm39)	Sequence (5' to 3')	Related gene	Indel (WGS)
On-target	On	1	160817065	TGTGCATTTACCGCTCCCCTGGG	*Serpinc1*	20.12%
Unbiased off-target candidate site	Di-Off1	4	115780188	TGTACATTCACCTCTCCCCTTGGG	Intron (*Dmbx1*)	ND
	Di-Off2	9	56131003	GGCTCTCCGCACCGGACCCGGTC CCGACGGG	Intron (Peak1)	ND
	Di-Off3	10	69762079	TATGCAAATACCCCTCCCCTTGG	Intergenic (Ank3)	ND
In silico off-target candidate site	Off1	4	115780189	TGT**a**CATT**c**ACC**t**CTCCCCTTGG	Intron (*Dmbx1*)	ND
	Off2	7	79730396	TGTGCA**c**TTACCG**aa**CCCCTGGG	Intron (*Zfp710*)	ND
	Off3	9	42703562	T**a**TGCATTTAC**t**GCTC**a**CCTGGG	Intron (*Grik4*)	ND
	Off4	10	53628857	TGTGCATTT**t**C**t**GCTCCC**t**TAAG	Intergenic	ND
	Off5	14	60055947	TGTGCATTTA**at**GCTCCCC**a**TAG	Intron (*Atp8a2*)	ND
	Off6	18	53613104	TG**a**GCATTTACCGC**ct**CCCTCAG	Intron (*Prdm6*)	ND
	Off7	18	86877140	TGTGCATTTAC**a**G**t**TCCC**a**TGGG	Intergenic	ND

Off-target candidate sites were selected by *in silico* design (www.rgenome.net/cas-offinder). Indel frequencies on the on- and off-target candidate sites were calculated based on the WGS experiment. (Bold lowercase letters: mismatch sequences with respect to the on-target sequence, ND: not detected).

Table S3. Random integration analysis

Targets	Chr.	Location (mm39)	CpG island	Sequence (5' to 3') Predicted-**KI sequence**-Soft-clipped read	Related gene
Off1	18	88880155	CpG island	AAGTAGTGAC-**h*F9***-AGCATACTAG	Intergenic
Off2	19	10282372	CpG island	GCCGTCGCCA-**Fragmented h*F9***-TGGTCACCGC	Intergenic
Off3	19	123564247		ACATACAAGG-**Fragmented h*F9***-GGAGAATTTC	Intergenic

As sequencing reads could not cover the whole KI region, opposite sequences from detected clipped reads were predicted based on the h*F9* integration site (blue letters).

Table S4 Sequences of primers used in this study

	Target gene		Sequence (5'-3')	Product size	
qPCR	*Serpinc1*	F	5'- GGCTGCTGGTGAGAGGAAG-3'	129 bp	Fig. 1B
		R	5'- GGATTCACGGGGATGTCTCG-3'		
	Protein C	F	5'- CCACCTGGGGAATATCTAGCA-3'	101 bp	Fig. 1B
		R	5'- GAAGCTGTTGGCACGTCTG-3'		
	Tfpi	F	5'- CAGGCGTCGGGATTATCGTG-3'	140 bp	Fig. 1B
		R	5'- TTCCCCCACATCCAGTGTAGT-3'		
	ITR for titration	F	5'- GGAACCCCTAGTGATGGAGTT-3'	62 bp	Fig. S2
		R	5'- CGGCCTCAGTGAGCGA-3'		
	Tnfα	F	5'- CCCTCACACTCAGATCATCTTCT-3'	61 bp	Fig. S4
		R	5'- GCTACGACGTGGGCTACAG-3'		
	Ifn γ	F	5'- ATGAACGCTACACACTGCATC-3'	182 bp	Fig. S4
		R	5'- CCATCCTTTTGCCAGTTCCTC-3'		
	Il1β	F	5'- GAAATGCCACCTTTTGACAG-3'	116 bp	Fig. S4
		R	5'- TGGATGCTCTCATCAGGACA-3'		
	Gapdh	F	5'- AGGTCGGTGTGAACGGATTTG -3'	231 bp	Fig. 1B, S2,
		R	5'- TGTAGACCATGTAGTTGAGGTCA -3'		S4
PCR	*Serpinc1*	F	5'- CATTCTCTTACCCATTTTCGCC -3'	952 bp	Fig. 2D
	(Indel analysis)	R	5'- CTGTCTCTAACCCCACTTCC -3'		
PCR	*KI-Left*	F	5'- GGATGGGGAGTCATGGTT -3'	987 bp	Fig. 3A, 3B
		R	5'- GGTGCTCTGGGTGATGTT-3'		
	KI-Right	F	5'- AAGCCAAAGGGACACCAA-3'	1040 bp	Fig. 3A,3B
		R	5'- CTGTCTCTAACCCCACTTCC -3'		

F: Forward; R: Reverse

Table S5. Character information of LNP used in this study

	Encapsulation efficiency (%)	Size (nm)	PDI	Zeta potential (mV)
LNP	82.3	93.24	0.0095	-2.48

PDI: polydispersity index

Table S6. Information on the ELISA kits used in this study

	Name	Sample type	Sample dilution	Company (Cat number)
ELISA kit	Human Factor IX	Cell protein	7.5μg protein per well loaded(PBS)	Abcam(ab108831)
		Plasma	1:400 (Dilution buffer)	
	Mouse Antithrombin	Plasma	1:16000 (Dilution buffer)	Abcam(ab108800)
Assay kit	ALT Activity Colorimetric/Fluorometric	Plasma	1:3 (Dilution buffer)	APExBIO (K2170-100)
	AST Activity Colorimetric	Plasma	1:3 (Dilution buffer)	APExBIO (K2171-100)
	Factor IXa Activity (Fluorometric)	Plasma	1:5 (Assay buffer)	Abcam(ab204727)

Table S7. List of antibodies used in this study

	Target	Clone	Host	Dilution	Company (Cat number)
Primary antibodies	FIX/PTC	Polyclonal	Rabbit	1:150 (IF)	Abcam(ab97619)
	Ki67	Monoclonal	Rat	1:100 (IF)	eBioscience(14-5698-82)
Secondary antibodies	Anti-Rabbit IgG (Alexa Fluor™ 594)		Goat	1:400 (IF)	Invitrogen(A11012)
	Anti-Rat IgG (Alexa Fluor™ 488)		Goat	1:200 (IF)	Invitrogen(A11006)