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Supplemental Information

CRISPR/Cas9-Mediated Gene Correction in Newborn Rabbits with Hereditary Tyrosinemia Type I

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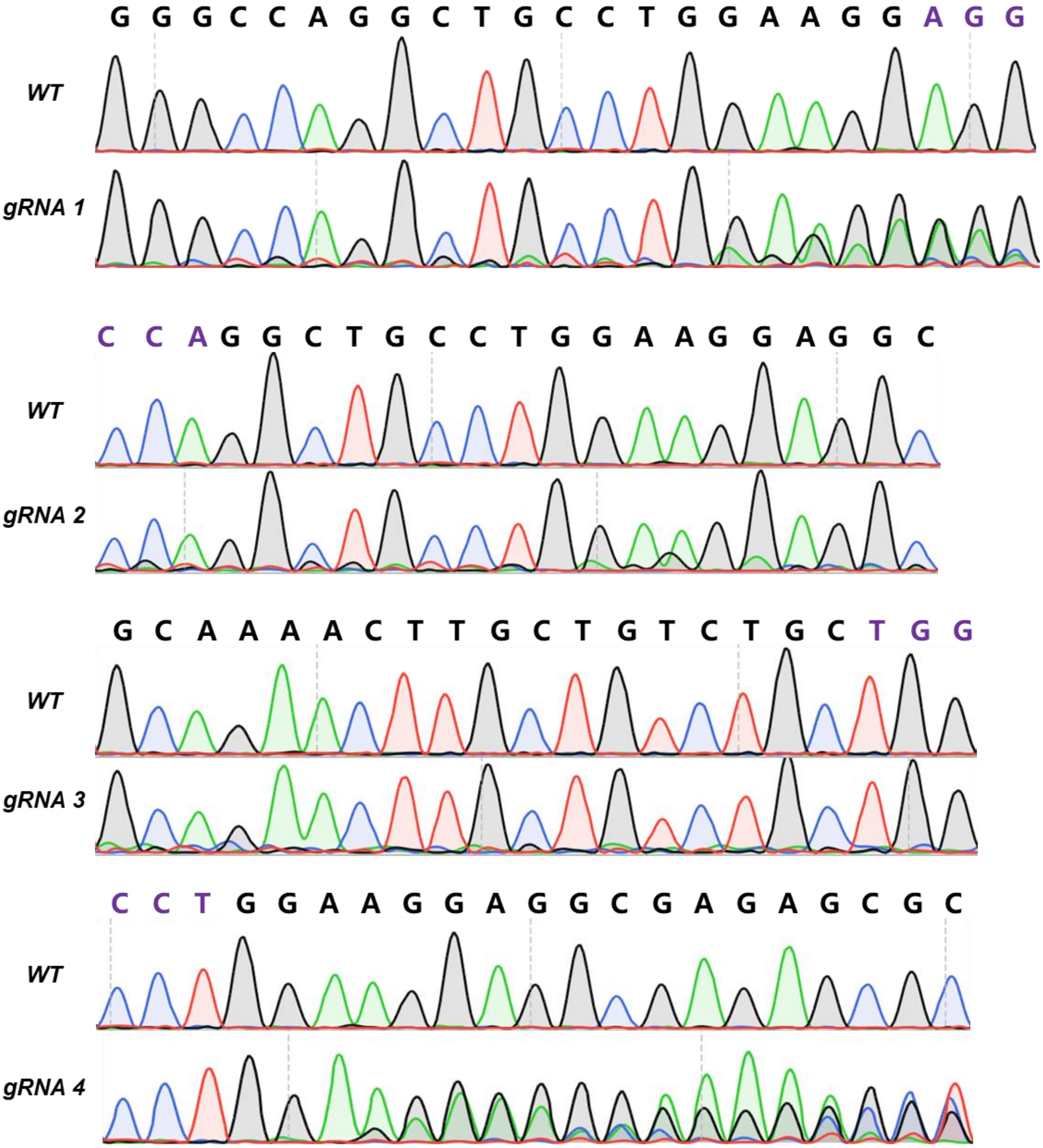
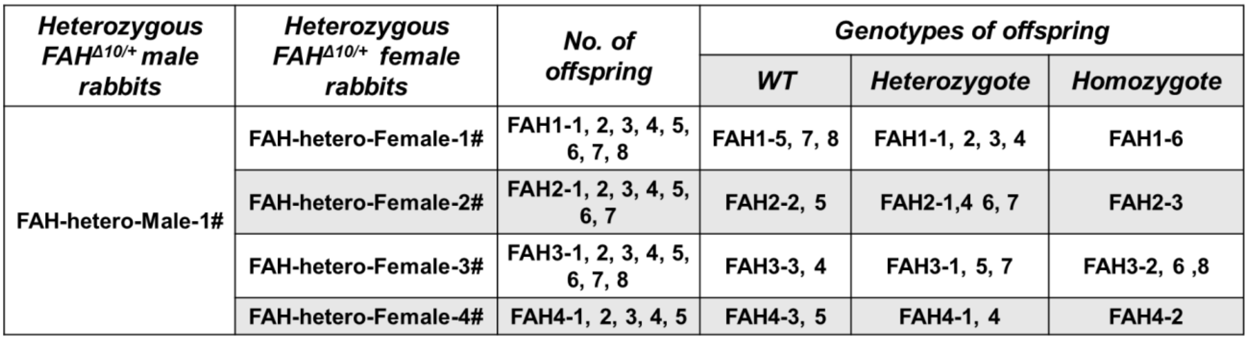


Figure S1. Sanger sequencing chromatograms of RFFs electroporated with sgRNA1–4 and SpCas9 expressing vectors.



Heterozygous $FAH^{\Delta 10/+}$ male rabbits	*Heterozygous $FAH^{\Delta 10/+}$ female rabbits*	*No. of offspring*	*Genotypes of offspring*		
			WT	*Heterozygote*	*Homozygote*
FAH-hetero-Male-1#	FAH-hetero-Female-1#	FAH1-1, 2, 3, 4, 5, 6, 7, 8	FAH1-5, 7, 8	FAH1-1, 2, 3, 4	FAH1-6
	FAH-hetero-Female-2#	FAH2-1, 2, 3, 4, 5, 6, 7	FAH2-2, 5	FAH2-1,4 6, 7	FAH2-3
	FAH-hetero-Female-3#	FAH3-1, 2, 3, 4, 5, 6, 7, 8	FAH3-3, 4	FAH3-1, 5, 7	FAH3-2, 6 ,8
	FAH-hetero-Female-4#	FAH4-1, 2, 3, 4, 5	FAH4-3, 5	FAH4-1, 4	FAH4-2

Figure S2. Summary of one heterozygous *FAH*$^{\Delta 10/+}$ male rabbit mating with four *FAH*$^{\Delta 10/+}$ female rabbits.

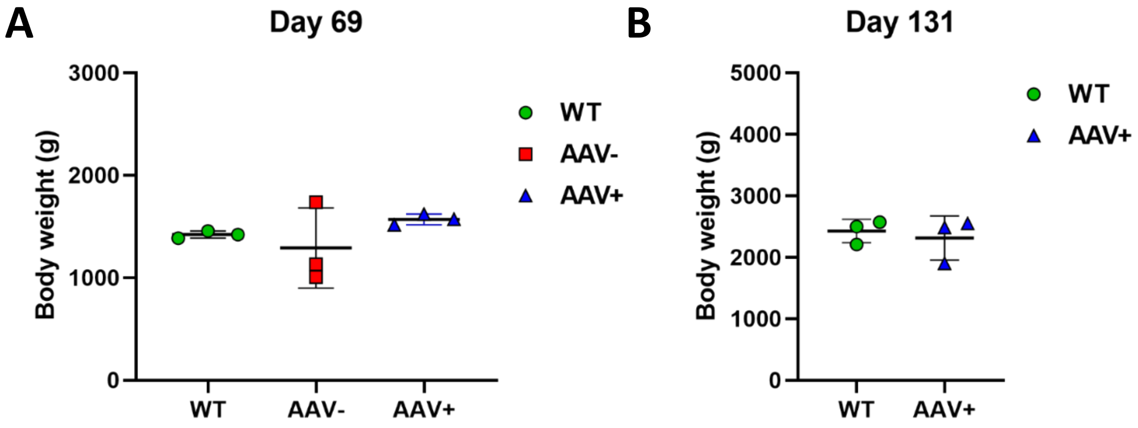


Figure S3. Body weight analysis. (**A**) Body weight of wild-type, HT1 rabbits after injection with AAV or PBS 69 days. (**B**) Body weight of HT1 rabbits after injection with AAV or PBS 131 days.

A

	WT			AAV-			AAV+		
	WT1	WT2	WT3	FK1	FK2	FK3	FA1	FA2	FA3
ALT (IU/L)	23.00	20.00	22.00	46.00	32.00	50.00	22.00	20.00	23.00
AST (IU/L)	16.00	17.00	14.00	144.00	459.00	630.00	10.00	12.00	16.00
SA (μmol/L)	0.50	0.50	0.50	10.00	5.60	6.20	0.50	0.50	0.50
Tyr (μmol/L)	83.23	57.75	75.73	85.05	2600.54	2587.87	89.76	94.40	83.23
TBIL (g/L)	1.00	0.80	0.90	30.20	1.80	2.50	1.80	1.70	1.00
D-BIL (g/L)	0.30	0.30	0.10	5.90	1.00	1.20	0.70	0.70	0.30
BUN (mmol/L)	7.83	8.16	5.17	26.11	27.70	27.52	8.23	9.83	7.83
CREA (μmol/L)	59.00	65.00	40.00	279.00	269.00	276.00	71.00	77.00	59.00
TG (μmol/L)	1.64	0.85	1.46	14.93	1.91	1.90	3.73	2.58	1.64
CHOL (mmol/l)	4.01	3.40	3.22	11.48	5.77	5.33	2.19	2.04	4.01

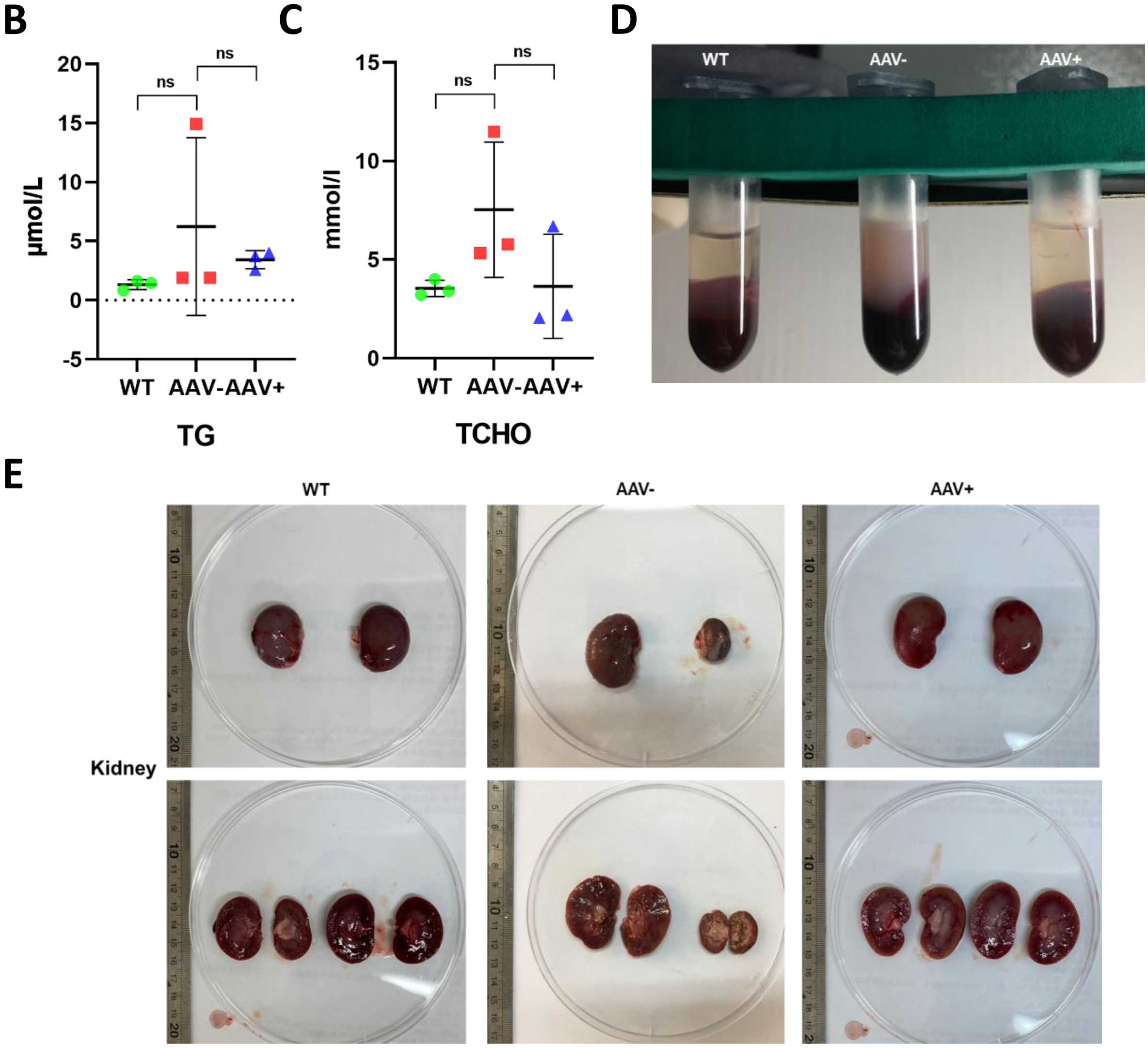


Figure S4. The efficacy analysis of CRISPR/Cas9-mediated gene therapy of HT1 rabbits. (**A**) Detailed information of blood biochemical indexes, including ALT, AST, SA, Tyr, TBIL, D-BIL, BUN, CREA, TG, and CHOL. (**B**) and (**C**) TG (**B**) and CHOL (**C**) analysis of wild-type, AAV-treated and untreated HT1 rabbits. Error bars, mean ± SD. ns, not significant. (**D**) Picture of blood samples from wild-type, AAV-treated and untreated HT1 rabbits. (**E**) The intact (top) and dissected (bottom) kidneys of wild-type, AAV-treated and untreated HT1 rabbits.

	Target (Copies/100ng DNA)		Editing efficiency
	A2-Cas9	A3-gRNA-donor	
FA1#-L1	1360	3320	6.74%
FA1#-L2	496	4260	9.13%
FA1#-L3	976	3130	11.80%
FA1#-L4	712	7840	7.95%
FA1#-L5	4320	7040	9.17%
FA1#-L6	2600	5200	8.20%
FA2#-L1	2306	5920	10.85%
FA2#-L2	1292	2316	7.42%
FA2#-L3	1648	14820	8.06%
FA2#-L4	3080	7400	8.65%
FA2#-L5	1408	9240	6.47%
FA2#-L6	856	11400	5.81%
FA3#-L1	7580	2212	13.92%
FA3#-L2	17300	4000	12.76%
FA3#-L3	7240	13380	15.04%
FA3#-L4	7460	1852	13.41%
FA3#-L5	6100	2386	10.97%
FA3#-L6	15220	5360	13.40%

Figure S5. The detailed information of copy numbers of AAV vectors in 100ng whole DNAs extracted from FA1#-L1–L6, FA2#L1–L6, and FA3#L1–L6.

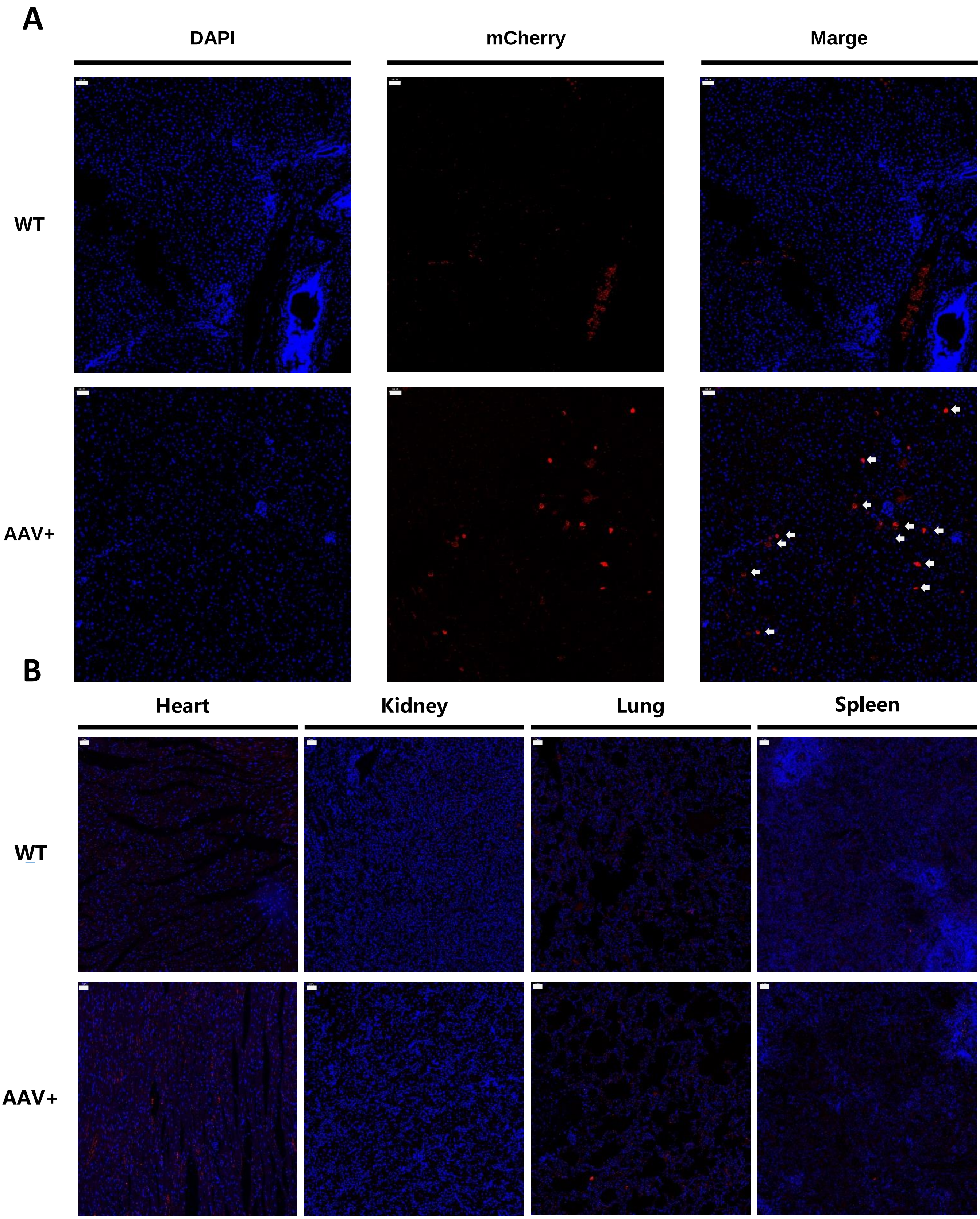


Figure S6. Fluorescence staining by anti-mCherry antibodies in the liver (A), heart, kidney, lung, and spleen (B) of AAV-treated HT1 rabbits. Scale bar, 50 µm.

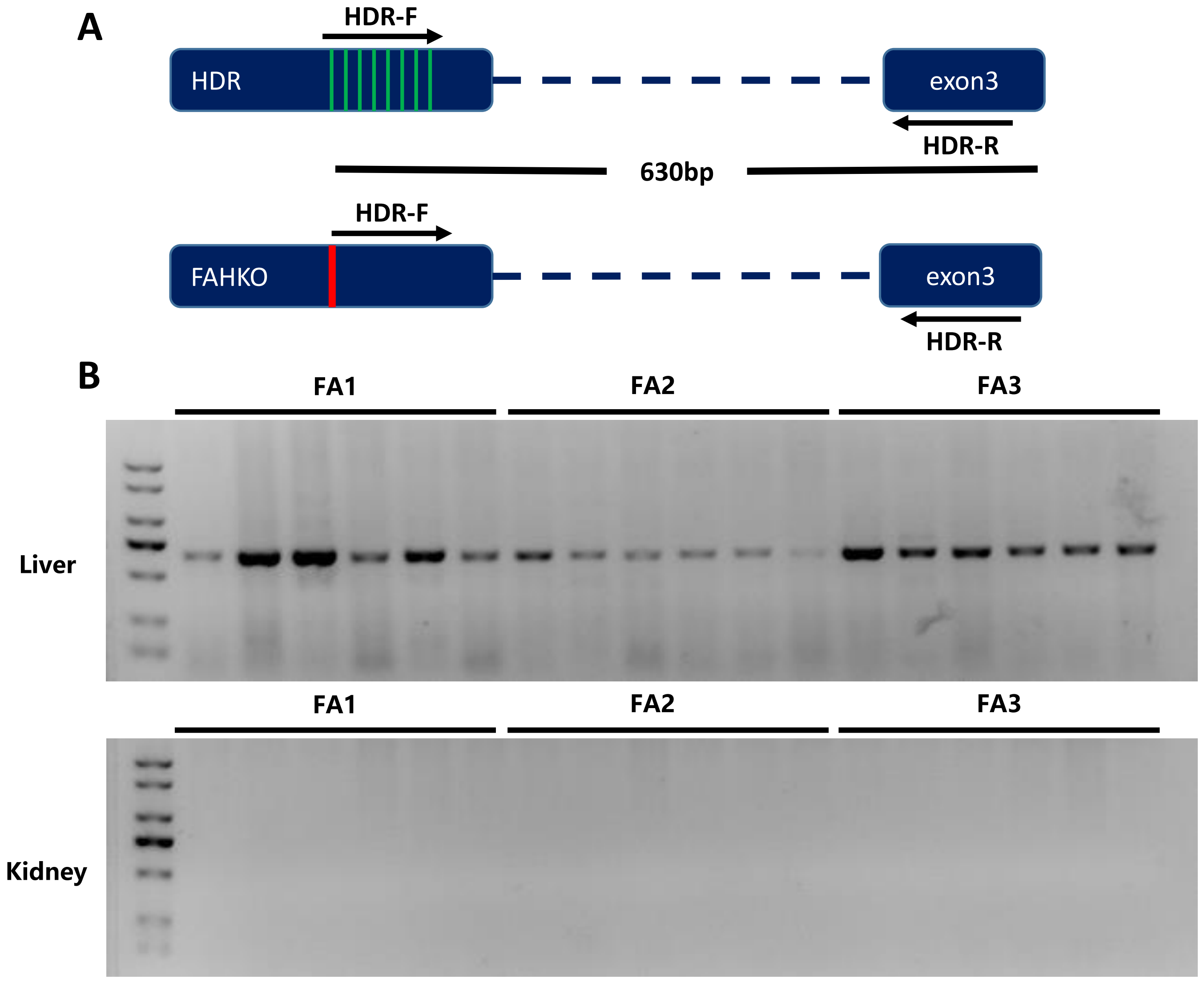


Figure S7. Schematic views (A) and pictures of agarose gel electrophoresis (B) of the PCR products with specific HDR-F and HDR-R primers in the livers and kidneys of AAV-treated HT1 rabbits.

A

	KO	SNP (In gRNA)	I3N	I3N+1	I3N+2	D3N	D3N+1	D3N+2	HDR
FA1#-L1	93.26%	0.14%	0.08%	2.28%	0.14%	0.36%	2.16%	0.29%	1.29%
FA1#-L2	90.87%	0.18%	0.07%	3.98%	0.16%	0.33%	1.66%	0.41%	2.34%
FA1#-L3	88.20%	0.13%	0.06%	4.83%	0.21%	0.52%	2.31%	0.68%	3.07%
FA1#-L4	92.05%	0.13%	0.05%	3.20%	0.16%	0.32%	2.04%	0.37%	1.68%
FA1#-L5	90.83%	0.13%	0.07%	3.82%	0.13%	0.33%	1.56%	0.41%	2.71%
FA1#-L6	91.80%	0.13%	0.07%	3.46%	0.17%	0.30%	1.55%	0.43%	2.09%
FA2#-L1	89.15%	0.13%	0.03%	4.40%	0.28%	0.31%	2.46%	0.57%	2.67%
FA2#-L2	92.58%	0.13%	0.00%	2.81%	0.23%	0.37%	2.15%	0.13%	1.59%
FA2#-L3	91.94%	0.14%	0.06%	3.16%	0.09%	0.30%	1.77%	0.47%	2.08%
FA2#-L4	91.35%	0.42%	0.14%	3.41%	0.01%	0.41%	1.72%	0.56%	1.98%
FA2#-L5	93.53%	0.17%	0.05%	2.38%	0.07%	0.30%	1.61%	0.46%	1.43%
FA2#-L6	94.19%	0.14%	0.01%	2.02%	0.16%	0.49%	1.70%	0.37%	0.90%
FA3#-L1	86.08%	1.44%	0.08%	5.26%	0.18%	0.51%	2.65%	0.47%	3.33%
FA3#-L2	87.24%	1.40%	0.08%	4.54%	0.27%	0.58%	2.91%	0.68%	2.30%
FA3#-L3	84.96%	1.41%	0.11%	5.72%	0.24%	0.53%	2.69%	0.63%	3.71%
FA3#-L4	86.59%	2.20%	0.07%	4.20%	0.15%	0.60%	2.57%	0.75%	2.87%
FA3#-L5	89.03%	1.39%	0.08%	3.92%	0.22%	0.52%	2.13%	0.46%	2.26%
FA3#-L6	86.60%	1.85%	0.06%	5.01%	0.15%	0.58%	2.02%	0.46%	3.28%

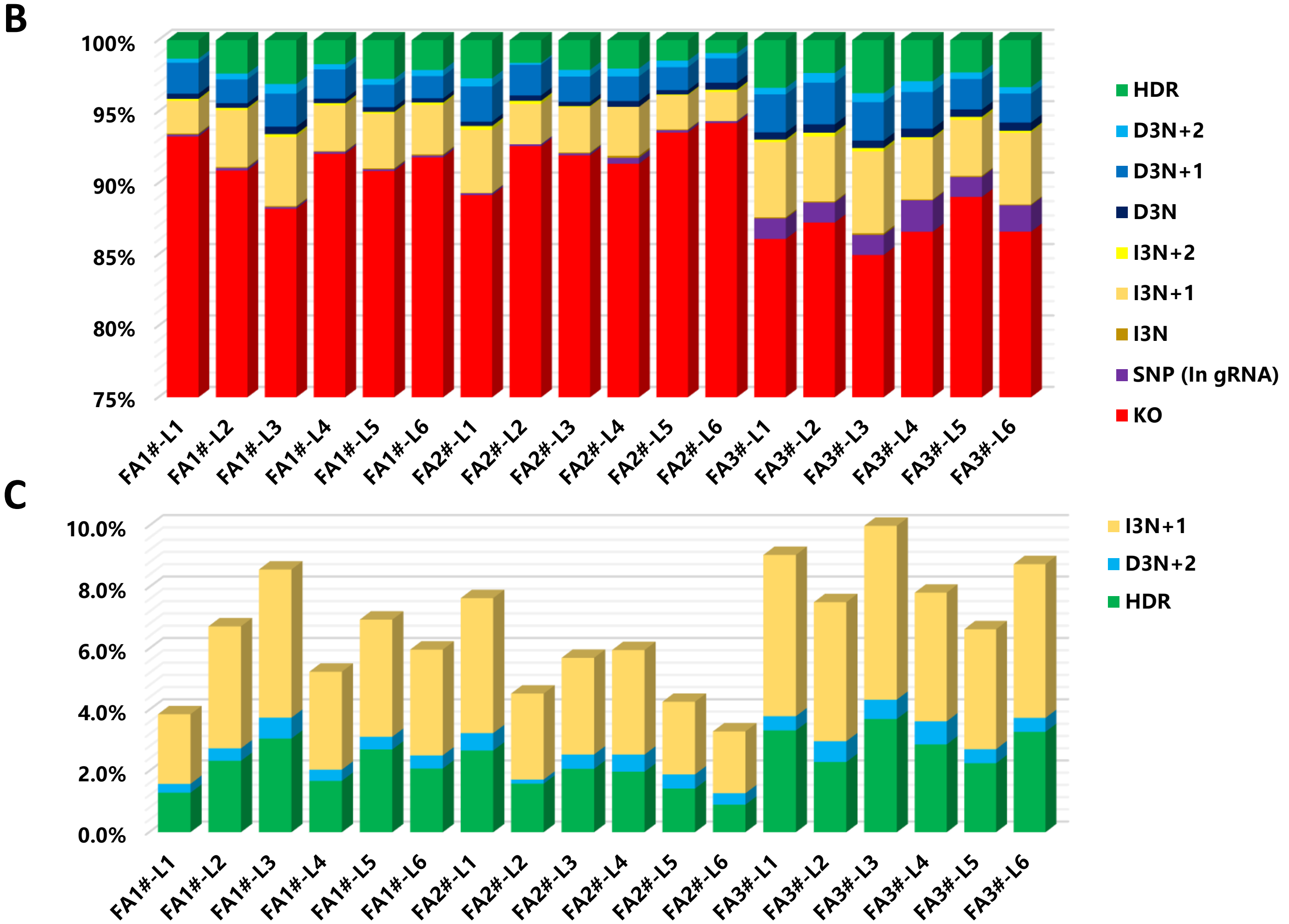


Figure S8. The results of amplicons deep sequencing of AAV-treated adult HT1 rabbits. (**A**) The detailed efficiencies of different mutation patterns of each detected tissues of AAV-treated adult rabbits. (**B**) Histogram of the deep sequencing results analysis, including 3N+1 bp insertion (I3N+1), 3N+2 bp insertion (I3N+2), 3N bp insertion (I3N), 3N+1 bp deletion (D3N+1), 3N+2 bp deletion (D3N+2), 3N bp deletion (D3N), SNP (In the sgRNA) and HDR. (**C**) Histogram of efficiencies of HDR-mediated precise gene correction and I3N+1 and D3N+2-mediated out-of-frame to in-frame gene correction in each detected tissues.

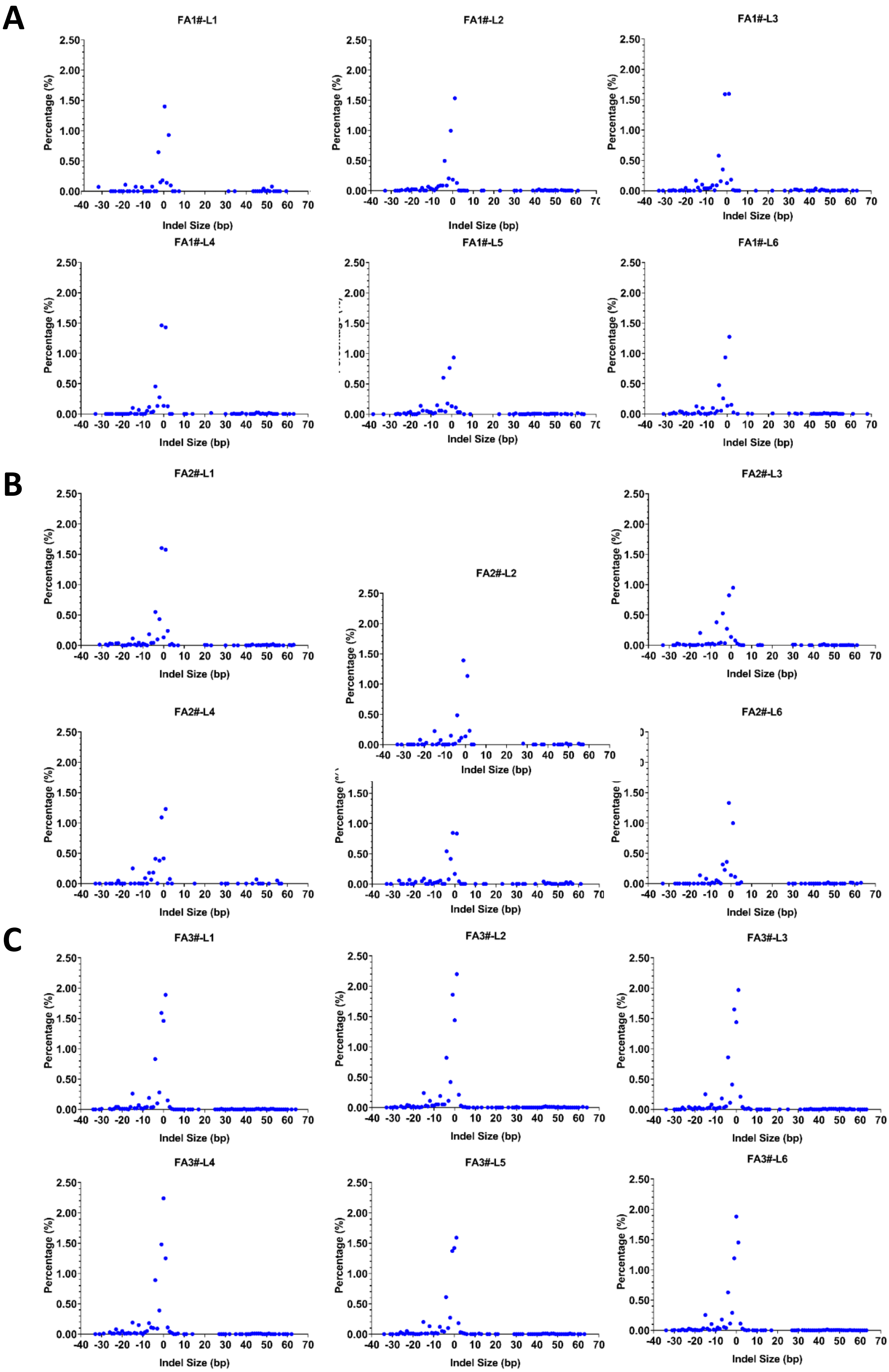
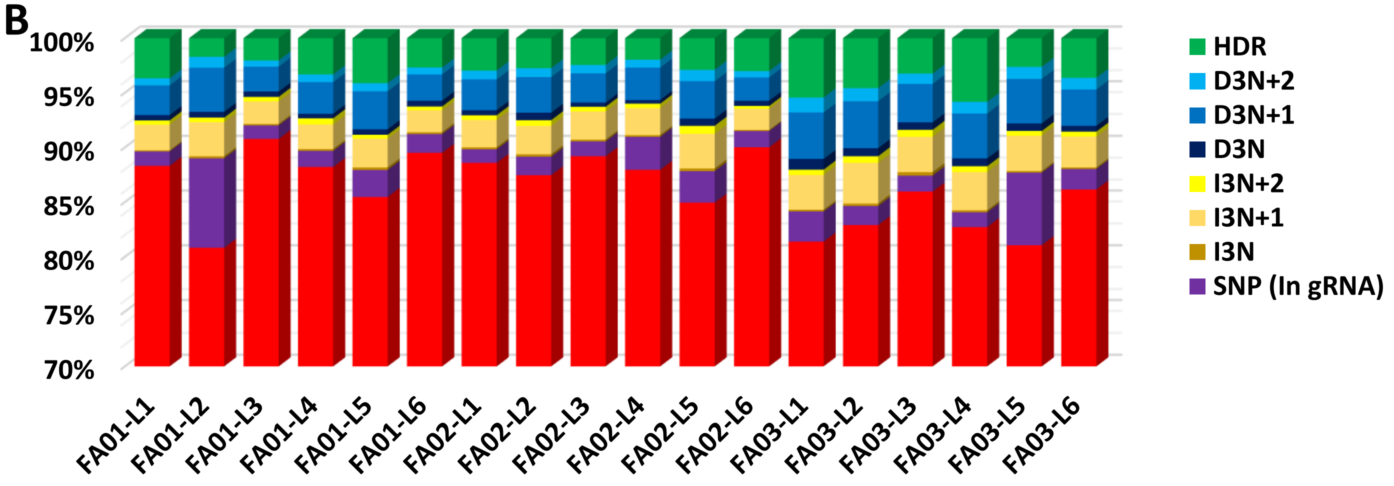


Figure S9. Diagrams analyzed indels length distribution of FA1#-L1–L6, FA2#L1–L6, and FA3#L1–L6 at sgRNA4 targeting site.

A

	KO	SNP (In gRNA)	I3N	I3N+1	I3N+2	D3N	D3N+1	D3N+2	HDR
FA01-L1	88.31%	1.32%	0.11%	2.35%	0.39%	0.49%	2.71%	0.66%	3.67%
FA01-L2	80.83%	8.18%	0.18%	3.11%	0.43%	0.53%	4.03%	1.00%	1.71%
FA01-L3	90.78%	1.24%	0.13%	2.06%	0.42%	0.48%	2.28%	0.56%	2.04%
FA01-L4	88.22%	1.47%	0.17%	2.25%	0.57%	0.41%	2.90%	0.71%	3.32%
FA01-L5	85.47%	2.47%	0.23%	2.63%	0.36%	0.50%	3.48%	0.74%	4.13%
FA01-L6	89.52%	1.71%	0.16%	2.01%	0.34%	0.53%	2.40%	0.63%	2.69%
FA02-L1	88.59%	1.24%	0.18%	2.51%	0.43%	0.46%	2.81%	0.81%	2.97%
FA02-L2	87.46%	1.70%	0.21%	2.60%	0.52%	0.69%	3.26%	0.81%	2.75%
FA02-L3	89.20%	1.36%	0.16%	2.57%	0.43%	0.38%	2.67%	0.77%	2.46%
FA02-L4	87.95%	3.02%	0.16%	2.47%	0.41%	0.34%	2.96%	0.72%	1.97%
FA02-L5	84.95%	2.88%	0.25%	3.17%	0.73%	0.65%	3.42%	1.04%	2.90%
FA02-L6	90.02%	1.49%	0.10%	1.93%	0.27%	0.47%	2.14%	0.58%	3.01%
FA03-L1	81.39%	2.76%	0.15%	3.18%	0.49%	0.98%	4.26%	1.35%	5.44%
FA03-L2	82.90%	1.75%	0.22%	3.72%	0.61%	0.74%	4.30%	1.20%	4.57%
FA03-L3	85.96%	1.46%	0.32%	3.22%	0.64%	0.69%	3.52%	0.94%	3.24%
FA03-L4	82.72%	1.36%	0.18%	3.50%	0.54%	0.71%	4.11%	1.07%	5.83%
FA03-L5	81.07%	6.64%	0.14%	3.24%	0.43%	0.69%	4.07%	1.10%	2.64%
FA03-L6	86.15%	1.88%	0.15%	2.81%	0.46%	0.52%	3.33%	1.06%	3.63%



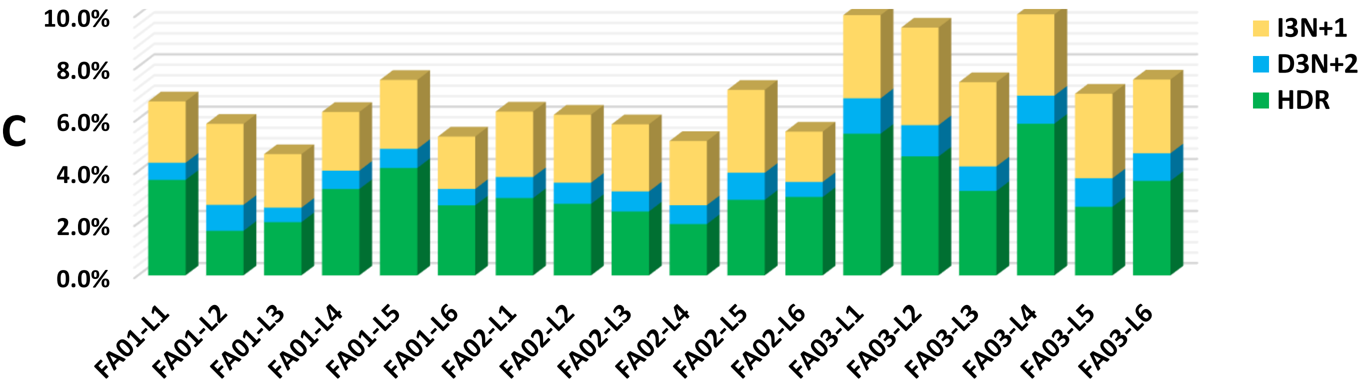


Figure S10. The results of amplicons deep sequencing of newborn HT1 rabbits with one week AAV treatment. (**A**) The detailed initial efficiencies of different mutation patterns of each detected tissues of newborn HT1 rabbits with one week AAV treatment. (**B**) Histogram of initial efficiencies by analysis of the deep sequencing data. (**C**) Histogram of initial efficiencies of HDR-mediated precise gene correction and I3N+1 and D3N+2-mediated out-of-frame to in-frame gene correction.

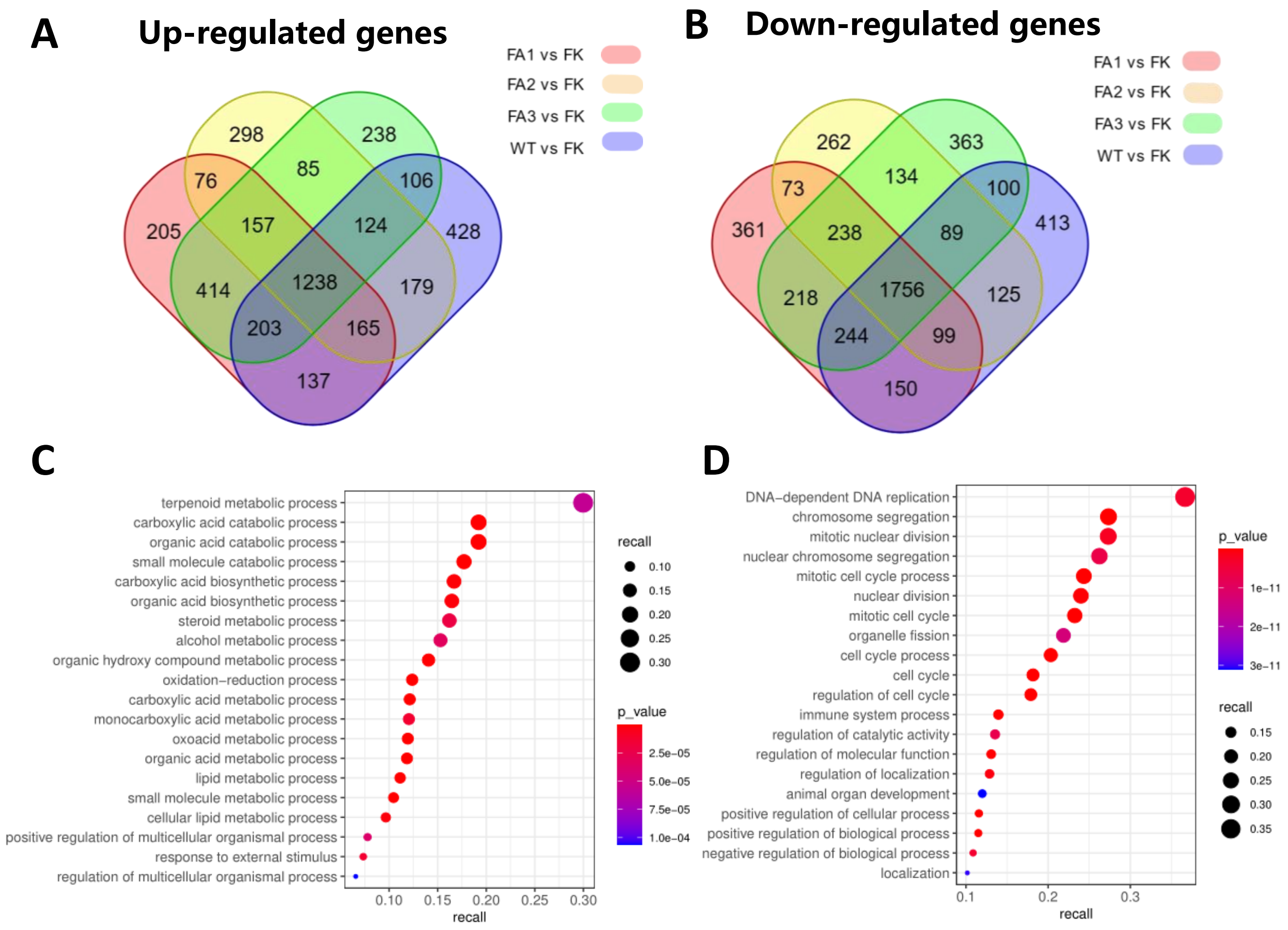


Figure S11. Analysis of up-regulated and down-regulated genes. (**A**) and (**B**) The number genes of up-regulated (**A**) and down-regulated (**B**) in the wild-type and AAV-treated (FA1, FA2, and FA3) rabbits (Venn diagram). The overlap corresponds to the genes commonly upregulated or downregulated in the wild-type and AAV-treated rabbits. (**C**) and (**D**) Gene ontology (GO) analysis of commonly up-regulated (**C**) and down-regulated (**D**) differentially genes in wild-type (*n*=3) and AAV-treated (*n*=3) rabbits vs PBS-treated (*n*=3) HT1 rabbits.

Primer	5'-3'
FAH-gRNA1-F	CACCGGGCCAGGCTGCCTGGAAGG
FAH-gRNA1-R	AAACCCTTCCAGGCAGCCTGGCCC
FAH-gRNA2-F	CACCGCCTCCTTCCAGGCAGCC
FAH-gRNA2-R	AAACGGCTGCCTGGAAGGAGGC
FAH-gRNA3-F	CACCGCAAAACTTGCTGTCTGC
FAH-gRNA3-R	AAACGCAGACAGCAAGTTTTGC
FAH-gRNA4-F	CACCGCGCTCTCGCCTCCTTCC
FAH-gRNA4-R	AAACGGAAGGAGGCGAGAGCGC
FAH-HDR-F1	GGGGACCCCTCTAGCATAGGA
FAH-HDR-R1	CGCGTCTTTCCACGCTGCTTGCCCGAGGCCCATGAAGCTGTTGAGAG
FAH-HDR-F2	CCTCGGGCAAGCAGCGTGGAAAGACGCGAGAGCGCTGCTGCAAAACT
FAH-HDR-R2	GCAGGATCCATGCAGACCATC
FAH-F	CAGGTCTCAGGTTACAGAGC
FAH-R	AGGTGCATCGTGGCAACAGC
HDR-F	CGGGCAAGCAGCGTGGAAAG
HDR-R	TCGTGGCAACAGCCTGAGAG
OT1-F	GCACCTGCACCTCTAATGCT
OT1-R	GGCCAGGCCTTAAGAGTCTG
OT2-F	ATGCAACCAATGTGCAAACA
OT2-R	CACTTCTGTGTCGCCTGTGA
OT3-F	GAGGGAAGGAGAAAGGCTCG
OT3-R	GCTCGGTACTCCACGCTC
OT4-F	AGTTCTCACTCGCATGCACA
OT4-R	AGCCGTTTAAGGAGCTGCTT
OT5-F	CAAGGCCTCTCACTGGACTG
OT5-R	ATAGACCCTTCACGCCTCCT
OT6-F	TCTCCATCCGTCCGGTAGAG
OT6-R	GCTTTAGGTTTGCCTTGGCC
OT7-F	GGTTTCGTAGTCGCACGGTA
OT7-R	CAGGACCCTGCTCAGCTTC
OT8-F	CTGCGAGTGTAAGACGGAGG
OT8-R	TGGATTGGATATGGAGCCGC
OT9-F	GAAAGGGAGGGAGACAGGGA
OT9-R	TGTAGTGCAGCAGGTTAGGC
OT10-F	CAGAGCCTGCCTGGGATTAG

Table S1. Primers used in this study