

Supplementary Table 1. Summary of the frequencies of indels, correction of *OTC spf^{ash}* mutation, and HDR in animals treated with the dual AAV gene-targeting vectors.

ID	Treatment	Time after treatment (week)	Indel (%)	OTC reads with a 'G' (%)	Reads with Perfect HDR (%)	Reads with Partial HDR (%)
4001	Neonatal, sgRNA1	3	26.5	20.1	17.2	7.6
4003	Neonatal, sgRNA1	3	35.5	7.2	6.4	2.7
307	Neonatal, sgRNA1	3	30.5	8.9	7.2	2.9
835	Neonatal, sgRNA1	8	26.7	6.8	5.5	2.2
836	Neonatal, sgRNA1	8	29.6	10.8	8.9	3.3
844	Neonatal, sgRNA1	8	34.4	6.7	5.4	2.4
630	Adult, untargeted, low dose	3	0.3	0.02	0.01	0.01
637	Adult, sgRNA1, low dose	3	50.3	0.3	0.2	0.1
640	Adult, sgRNA1, low dose	3	45.0	0.3	0.3	0.1
641	Adult, sgRNA1, low dose	3	38.5	0.2	0.1	0.04
658	Adult, untargeted, high dose	2	0.1	0.03	0.02	0.01
648	Adult, sgRNA1, high dose	2	43.6	1.8	1.5	0.3
649	Adult, sgRNA1, high dose	2	48.5	2.1	1.7	0.4
653	Adult, sgRNA1, high dose	2	34.0	1.3	1.1	0.2
	Untreated <i>spf^{ash}</i>	n/a	0.04	0.002	0.001	0.004

Supplementary Table 2. A full list of simple indels (insertions or deletions) detected by deep sequencing on the *OTC* target region in the liver of two *spf^{ash}* mice (#4001 and #4003) 3 weeks after neonatal injection of the sgRNA1 dual AAV vectors, and two adult *spf^{ash}* mice (#648 and #653) 2 weeks after injection of high-dose sgRNA1 dual AAV vectors. The rates of complex indels (insertions + deletions) are also indicated. Please see attached Excel file.

Supplementary Table 3. Deletion sizes identified by on-target deep sequencing and their distribution in neonatal-treated and adult-treated animals.

Size range	Neonatal (3w)		Adult (high-dose, 2w)	
	#4001	#4003	#648	#653
0-10 bp	82.12	89.78	86.10	86.55
11-20 bp	7.45	4.99	2.29	2.41
21-30 bp	5.55	2.84	0.90	0.79
31-40 bp	2.00	1.07	0.50	0.31
41-50 bp	0.73	0.22	0.23	0.59
51-60 bp	0.37	0.17	0.06	0.24
61-70 bp	0.35	0.11	0.21	0.24
71-80 bp	0.18	0.09	0.12	0.22
81-90 bp	0.09	0.03	0.14	0.15
91-100 bp	0.09	0.04	0.21	0.12
>100 bp	1.08	0.66	9.24	8.38
Indel overlap with Exon 4	1.36	0.72	7.76	5.18

Supplementary Table 4. Off-target analysis. Potential off-target sequences for sgRNA1 identified and scored by Benchling's off-target analysis.

ID	Sequence	PAM	Score	Chromosome	Strand	Position	Mismatches	On-target	Indel (treated)	Indel (untreated ctl)
sgRNA1	TCTCTTTTAAACTAACCCAT	CAGAG	100	X	-	10276774	0	TRUE		
OT1	TATCTTTTCAACTAACCCAA	TAGGA	1.144	17	-	58464514	3	FALSE	0.00034	0.00038
OT2	TTTCTTTTACTAGCCCAT	AGGGG	0.864	X	-	10299926	3	FALSE	0.00040	0.00033
OT3	TCTTTTTTAAATAACCCAT	TAGAA	0.861	8	-	113731689	3	FALSE	0.0011	0.0011
OT4	TGGCATTAAACTAACCCAA	CTGGG	0.847	5	-	30737756	4	FALSE	0.00020	0.00017
OT5	TCTTCATGAAACTAACCCAT	CTGAA	0.832	19	+	14415840	4	FALSE	0.00019	0.00023
OT6	ATTTTTTAAACAACCCAT	CTGGA	0.66	2	-	102196549	4	FALSE	0.0010	0.0011
OT7	GATCTTGAATCTAACCCAT	GTGAA	0.628	6	+	87522107	4	FALSE	0.00018	0.00018
OT8	CCTTTATAATCTAACCCAT	GTGAA	0.617	9	-	91587078	4	FALSE	0.00034	0.00032
OT9	TCTGTTTTAAAGTAACCCTT	CAGGG	0.595	1	-	88987170	3	FALSE	0.00020	0.00022
OT10	TTGCTTTTCAACTAACCCAA	GTGGG	0.542	8	-	108010534	4	FALSE	0.00025	0.00024
OT11	AATATTTTAAACTATCCCAT	GTGAG	0.485	12	-	4955834	4	FALSE	0.00021	0.00020
OT12	TTACTTTAAACTATCCCAT	CTGAA	0.478	X	-	145956518	4	FALSE	0.00022	0.00022
OT13	ACACTTTTAAAATAACCCAG	ATGAA	0.471	8	-	28090672	4	FALSE	0.00033	0.00033
OT14	AATCTTCTAAACCAACCCAT	TGGAG	0.464	6	+	44013659	4	FALSE	0.00023	0.00017
OT15	TCTTTATTAGACTAACCCAG	CTGAG	0.457	X	-	152025104	4	FALSE	0.00035	0.00036
OT16	TTTCATTTAACATAACCCAT	ATGGA	0.456	15	+	35702518	4	FALSE	0.00014	0.00018

Supplementary Table 5. Primers and sequences for construction and analysis of the donor template and sgRNA plasmids.

Name	Sequence (5'->3')	Note
WT sequence	GAAAGTCTCACAGACACCGCTC GG TTTGAAAACTTTTCTTCCTTCCAAAGTTT----- ATTTCAAACCTGTGATGGGTTAGTTTAAAAGAGAAGATG	Part of <u>OTC exon 4</u> and intron 4
<i>spf^{esh}</i> sequence	GAAAGTCTCACAGACACCGCTC A GGTTTGAAAACTTTTCTTCCTTCCAAAGTTT----- ATTTCAAACCTGTGATGGGTTAGTTTAAAAGAGAAGATG	<i>spf^{esh}</i> G->A mutation
OTC donor sequence for sgRNA1 (892-989 bp)	GAAAGTCTCACAGACACCGCTC GG TTTGAAAACTTTTCTTCCTTCCAAAGTTT ACCGGT ATTTCAAT TGGT GATGGGTTAGTTTAAAAGAGAAGATG	PAM mutation; AgeI Restriction site insertion
OTC sgRNA1_Fwd	CACC GTCTCTTTTAAACTAACCCAT	OTC target sequence 1
OTC sgRNA1_Rev	AAAC ATGGGTTAGTTTAAAAGAGAC	
OTC sgRNA2_Fwd	CACC GCACAAGACATTCACCTGGGT	OTC target sequence 2
OTC sgRNA2_Rev	AAAC ACCCAAGTGAATGTCTTGTGC	
OTC sgRNA3_Fwd	CACC GAAAGTTTATTTCAAACCTCG	OTC target sequence 3
OTC sgRNA3_Rev	AAAC CAGAGTTTGAATAAACTTTC	
HDR-Fwd	TGGAGCAATTCTGCACATGGA	OTC PCR for RFLP analysis
HDR-Rev	CTTACTGAACATGGCAGTTTCCC	
OTC_PointM_F	GGCTATGCTTGGGAATGTCCT	OTC PCR for Surveyor assay
OTC_PointM_R	GCTACAGAATGAAAGAGAGGCG	

Supplementary Table 6. PCR primer sequences for detecting potential off-target effects by deep sequencing.

Primer Name	Sequence (5'->3')	Note
OTC_OT1F1	CTGGTGCCTTTTTCTATCGCC	Primers for OT1
OTC_OT1R1	CCAAGAGCAACTACAATGGCTT	
OTC_OT1F2	GCATTTTCATGAGCATTCCA	
OTC_OT1R2	CATGTTGTGCCTGCATCTCT	
OTC_OT2F1	GCAGACTCCAAGATGCAAGAC	Primers for OT2
OTC_OT2R1	GATGTTGTTCCACCCGCATCT	
OTC_OT2F2	CACTGAGCCAAGTCACTGGA	
OTC_OT2R2	AGGGACAAAACCAAACAGCA	
OTC_OT3F1	TGGCCTTCTAAAGCAACCAA	Primers for OT3
OTC_OT3R1	CCGTCTCCAGATCACATGAC	
OTC_OT3F2	ATAACTCATAATCTATGCATGGCACAA	
OTC_OT3R2	TTTGATCATGGTGTTTATCAGAGC	
OTC_OT4F1	TTGAGACCTAGCTCATGCC	Primers for OT4
OTC_OT4R1	TAACGCAGAAGTGGCACAGG	
OTC_OT4F2	TCAGCTTCGAATCACACCAG	
OTC_OT4R2	GAATGTGGCATTGGCTTTTT	
OTC_OT5F1	GTCCCCGACAAACCAAGCTA	Primers for OT5
OTC_OT5R1	TGAACTGGCAGTATGCAGGG	
OTC_OT5F2	AACATGGTTTCTGCCCTCAG	
OTC_OT5R2	GGACCATGCCGAACCTTAC	
OTC_OT6F1	GAGAGAGCCAATCTGCCCAT	Primers for OT6
OTC_OT6R1	CACCGGAAACGTGTGAGAGA	
OTC_OT6F2	ACTTCCCATGATCCCATTGA	
OTC_OT6R2	AGCTTCCCTCCAAGTGCCT	
OTC_OT7F1	GATGGGCATAAGCCCGAAGTA	Primers for OT7
OTC_OT7R1	TAAGGCCAGTGTTGTTGTGT	
OTC_OT7F2	GTAGCAGGGGCTCTGTGAAG	
OTC_OT7R2	TGGCCTGAAATACCCAGAAC	
OTC_OT8F1	GTTGAATTCGCGTGTCCAGG	Primers for OT8
OTC_OT8R1	TCCCATGGCGAGAATGTCAC	
OTC_OT8F2	CCCTGTAGGAAACACAGAGGA	
OTC_OT8R2	TGCTTTGGATGTTGATTCTAAA	
OTC_OT9F1	GGCAAAGGACTAGCTTGCAC	Primers for OT9
OTC_OT9R1	GGGTGCTATGAGGACCAGTG	
OTC_OT9F2	CAGTGGTGTGTGGAGAGCTG	
OTC_OT9R2	AGAGAGAGCGCTTGA	

Primer Name	Sequence (5'->3')	Note
OTC_OT10F1	CTTTGACTCCCGGCGAAAGA	
OTC_OT10R1	TTGTCCATCCGGGTCATTGC	Primers for OT10
OTC_OT10F2	AAGTCCTTCTTGCCCAACTT	
OTC_OT10R2	CAGCCCAATGCATTTTT	
OTC_OT11F1	ATTACAGGTCCTGGTTGGGC	
OTC_OT11R1	ACTGAGCCTGGTAGAGCCTT	Primers for OT11
OTC_OT11F2	GGAAGGTGAAGGAAGGAAGG	
OTC_OT11R2	TTTTCTAGGAATTCAGGACATACA	
OTC_OT12F	TCATGGTCCTTAAAAATTTTGC	
OTC_OT12R	TCCAGGTATGCAAAGTGGAT	Primers for OT12
OTC_OT13F1	TTCAGTTGTACTTTGGATGCTCTGA	
OTC_OT13R1	CATCTGAATAGCAGCAGGCG	Primers for OT13
OTC_OT13F2	TAGCACAGCCCAATGACTT	
OTC_OT13R2	TCATGAAACCCATAATCAGAA	
OTC_OT14F1	AGTGGGTCATCCTTTGTTACCC	
OTC_OT14R1	TGCCAGTTATCAGCCAAGCA	Primers for OT14
OTC_OT14F2	CCCAGGAACTTAACTCAGGTG	
OTC_OT14R2	TGCCATTTGACCTCATAAGTCT	
OTC_OT15F1	TTCAGCCCCCTTGAGTGTTTA	
OTC_OT15R1	GTCTCTGAGCACAAAGAGACGA	Primers for OT15
OTC_OT15F2	TTGCCTGTCCCAACTAGAGC	
OTC_OT15R2	GGCCCAAGAATGCACATTTA	
OTC_OT16F1	CCACACACTGGCTAGGACTG	
OTC_OT16R1	ACTGGCAGCACTTGAGACAA	Primers for OT16
OTC_OT16F2	GATGGCATGCTGTGGTTTTT	
OTC_OT16R2	CAATGCTTCCACACAGAACC	