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Supplementary

An Exon-Specific Small Nuclear U1 RNA (ExSpeU1) Improves Hepatic OTC Expression in a Splicing-Defective *spf/ash* Mouse Model of Ornithine Transcarbamylase Deficiency

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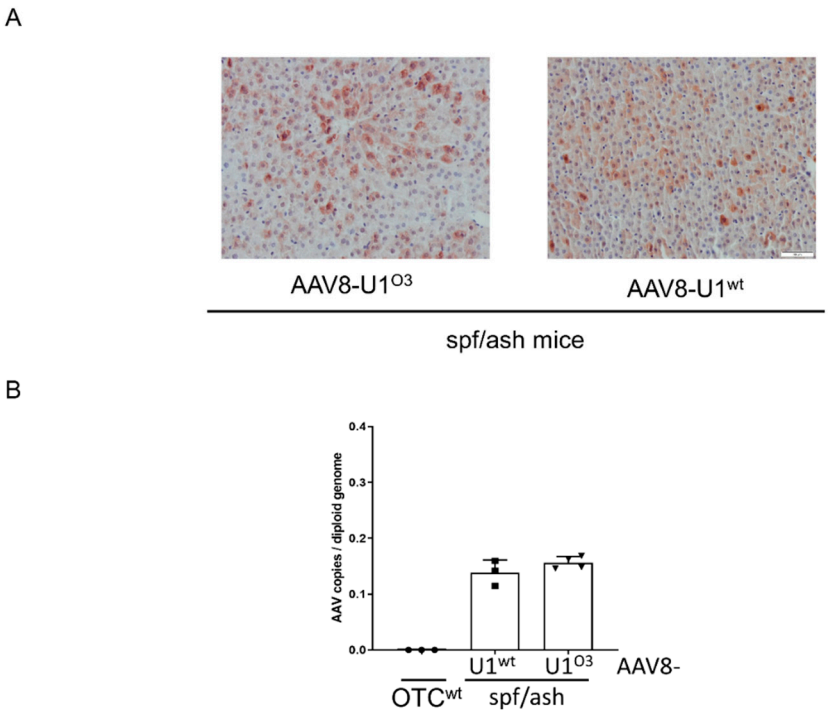

Supplementary Figure S1. (A) eGFP expression in livers of spf/ash mice injected with the AAV8-U1^{wt} or AAV8-U1^{O3}. Representative examples of liver sections for both animal models are shown. Images are taken at 20× magnification. Scale bar, 50 μm. **(B)** eGFP gene copy number in liver expressed as AAV copies per diploid mouse genome.

Supplementary Table S1. Sequences of oligonucleotides.

Creation of mouse OTC minigene		
4F	forward	tttaaa<u>catatg</u>ctgtgtaacctggaagctctatgat
4R	reverse	tttaaa<u>catatg</u>ccttcaacattgtccttgttatttc
Mutant mouse OTC minigene		
OTC mut F	forward	ctcacagacaccgctc**a**gtttgtaaaacttttc
OTC mut R	reverse	gaaaagttttacaaact**g**agcggtgtctgtgag
Engineered U1snRNA variants for mouse OTC 5′ss		
U1^{O} F	forward	aggcccaagatctgatACAAACCGAgcaggggagataccat
U1^{O1} F	forward	aggcccaagatctgatTTTACAAACgcaggggagataccat
U1^{O2} F	forward	aggcccaagatctgatAAGTTTTACgcaggggagataccatgatca
U1^{O3} F	forward	aggcccaagatctgatAGAAAAGTTgcaggggagataccatgatca
Engineered U7snRNA variant for mouse OTC 5′ss		
U7^{O}	forward	acagaggcctttccgcacttttaaactaacccatcagagaatttttggag
Plasmid-specific primers used to analyze splicing pattern		
alfa	forward	caacttcaagctcctaagccactgc
bra	reverse	taggatccggtcaccaggaagttggttaaatca
Primers used to assess in vivo splicing		
mOTCex3	forward	aagaagtactcgaacaagactgtcc
Primers used to evaluate the correctly spliced transcripts		
mOTCwtex4	reverse	catctgtcatgctagataagacacgag
Primers used to evaluate the titer of AAV vectors		
eGFP	forward	GCGGGGCAAGTGACCGTGTG
	reverse	TGCGCAAACCCAGGGCTGCC
Primers used to evaluate U1 expression		
U1^{wt}Ex	forward	agatctcatacttacctg
U1^{O3}Ex	forward	agatctgatagaaaagtt
U1Ex	reverse	gaacgcagtcccccactaccac
Primers used to evaluate the AAV8 gene copy number		
GFP	forward	catggtcctgctggagttc
	reverse	gtcaggtcagcttggtcttc

The NdeI restriction site is underlined; mutated nucleotides are in bold; the replaced 9 nucleotides of 5′ tail of U1snRNA variants are indicated in upper case.