Supplementary Figures and Table

Figure S1

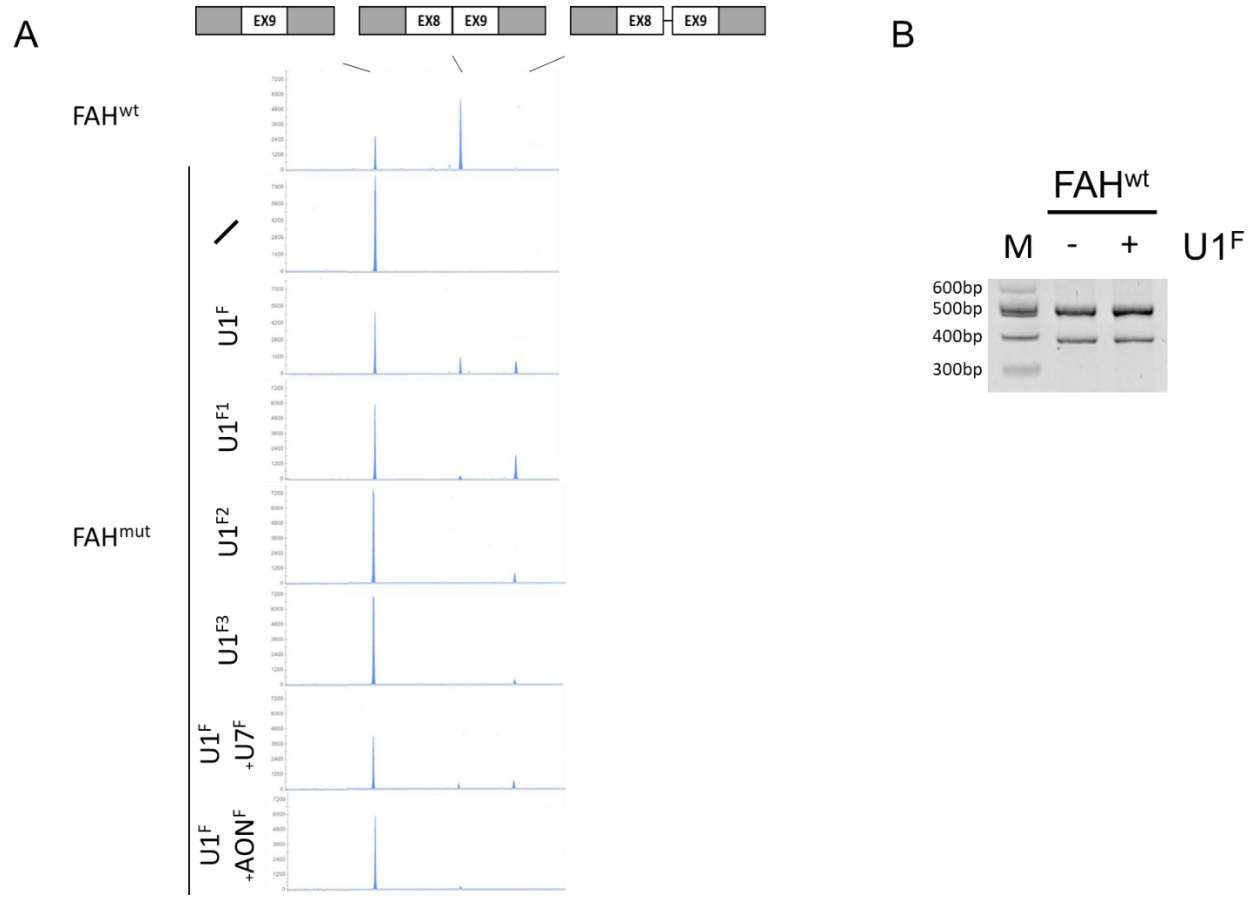

Figure S1. (A) Evaluation of *FAH* minigene splicing patterns by denaturing capillary electrophoresis of fluorescently-labelled PCR products. The amount of transcripts is represented by the area under each peak, and the analysis is shown in figure 1B. The scheme of transcripts is reported on top. RFU: Relative Fluorescence Units **(B)** FAH splicing patterns in Hepa1-6 cells transiently transfected with the wild-type (FAH^{wt}) minigene, alone or in combination with $U1^F$. Amplified products were separated on 2% agarose gel. M, 100 bp molecular weight marker.

Figure S2

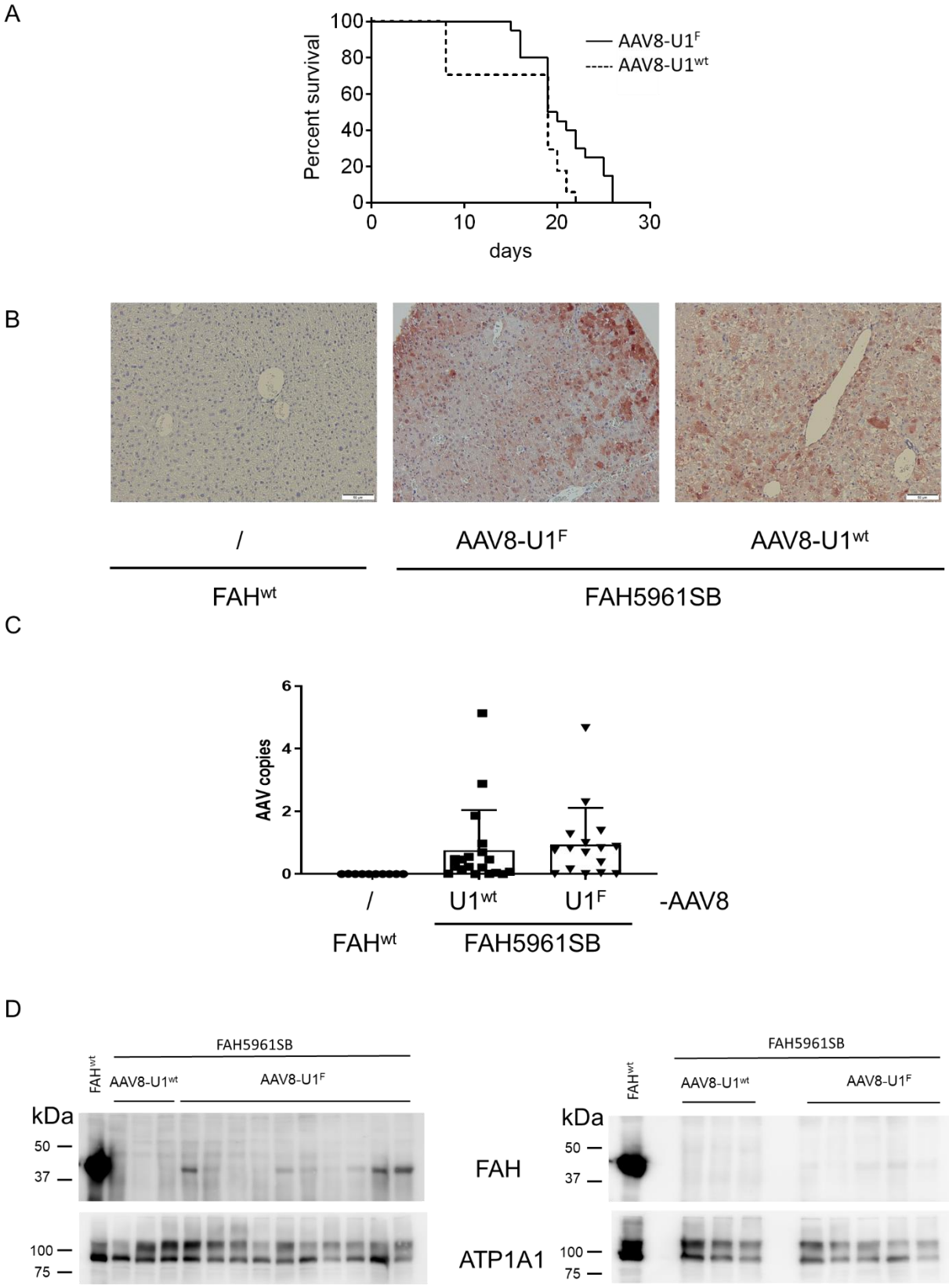

Figure S2. (A) Survival curve of *FAH5961SB* mice injected with the AAV8-U1^{wt} (dotted line) or AAV8-U1^{F} (continuous line) and kept on normal drinking water (-NTBC). The time until the mice reached the humane

endpoint is shown. **(B)** Examples of liver sections from *FAH5961SB* mice injected with the AAV8-U1^{wt} or AAV8-U1^{F}, upon staining with an anti-GFP antibody (brown). Images are taken at 20× magnification. Scale bar, 50 μm. **(C)** Evaluation of liver transduction efficiency by evaluation of AAV8 gene copy number. **(D)** Western blotting analysis through a specific anti-FAH antibody in liver homogenates of wild type (FAH^{wt}) and *FAH5961SB* mice treated with AAV8-U1^{wt} or AAV8-U1^{F}. The mouse ATPase Na^+/K^+ Transporting Subunit Alpha 1 (*ATP1A1*) has been exploited as load control. The protein marker, reporting the molecular size of bands, is reported on the left.

Table S1

Oligonucleotide		Sequence (5′→3′)
Creation of mouse *FAH* minigene		
7F	forward	tgaccatatgagggaagtaatgccaggt
9R	reverse	tacgcatatgcttatagcctctattccaa
Mutant mouse *FAH* minigene		
FAH mut F	forward	gaacgactggagcagtaatgcctggtg
FAH mut R	reverse	caccaggcattactgctccagtcgttc
Engineered U1snRNA variants for mouse *FAH* 5′ss		
U1^{F} F	forward	aggcccaagatctgatCATTACTGCgcaggggagataccat
U1^{F1} F	forward	aggcccaagatctgatAGGCATTACgcaggggagataccat
U1^{F2} F	forward	aggcccaagatctgatCCAGGCATTgcaggggagataccat
U1^{F3} F	forward	aggcccaagatctgatCCACCAGGCgcaggggagataccat
Engineered U7snRNA variant for mouse *FAH* 5′ss		
U7F	forward	acagaggcctttccgcagtcccatacccaactcaatttttggag
Plasmid-specific primers used to analyze splicing pattern		
alfa	forward	caacttcaagctcctaagccactgc
bra	reverse	taggatccggtcaccaggaagttggttaaatca
Primers used to assess in vivo splicing		
mFAHex7	forward	actcttagacatggagttggaaatg
mFAHex9	reverse	gtactcccattgctggatgtct
Primers used to evaluate the correctly spliced transcripts		
mFAHwtex8	reverse	ttgctggatgtctcgtgcgc
Primers used to evaluate U1 expression		
U1^{wt}Ex	forward	agatctcatacttacctg
U1^{F}Ex	forward	agatctgatcattactgc
U1Ex	reverse	gaacgcagtcccccactaccac
Primers used to evaluated RNAseq data		
Nnmt	forward	atattctgcctgggtgctgt
	reverse	gtcaaaggctcctggttcct
Dio1	forward	gggatttcattcaaggcagca
	reverse	acgttgttcttaaaagcccagc
GAPDH	forward	cgaccactttgtcaagctcat
	reverse	ccctgttgctgtagccaaatt
Primers used to evaluate the titer of AAV vectors		
eGFP	forward	gcggggcaagtgaccgtgtg
	reverse	tgcgcaaacccagggctgcc
Primers used to evaluate the AAV8 gene copy number		
GFP	forward	catggtcctgctggagttc
	reverse	gtcaggtcagcttggtcttc

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