

**Supplementary materials online for:**

**Rescue of a severe mouse model for Spinal Muscular Atrophy by U7 snRNA-mediated splicing modulation**

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## Supplementary Table S1

ID	Sex	mSmn	hSMN2	SMA Status	LV copy number	GFP MFI	father	mother	Data shown in Figs.
epfl-4	f	+/+	-/-	wt	27.8	n.d.	?	?	
epfl-9	f	+/+	-/-	wt	3.6	n.d.	?	?	
520	f	+/-	+/-	hz	6.0	555	504	epfl-4	
523	f	+/-	+/-	hz	14.8	1006	504	epfl-4	
558	f	+/-	+/-	hz	6.9	159	504	epfl-9	
567	f	+/+	+/+	wt	6.6	370	507	523	3, 4
568	f	-/-	+/+	SMA	5.5	625	507	523	2, 3, 4
569	f	+/-	+/-	hz	7.4	67	507	523	3, 4
570	f	+/+	+/+	wt	4.1	76	507	523	3, 4
571	m	+/-	+/+	hz	15.4	529	507	523	3, 4
572	f	+/+	+/+	wt	5.1	345	507	523	3, 4
573	f	-/-	+/+	SMA	12.3	757	507	523	2, 3, 4
574	m	+/-	+/-	hz	7.3	924	507	523	3, 4
575	f	-/-	+/+	SMA	7.3	690	507	523	2, 3, 4
558-1-4	n.d.	-/-	+/+	SMA	0.5	n.d.	538	558	2, 3
558-1-5	n.d.	-/-	+/+	SMA	1.0	n.d.	538	558	2, 3
576	f	+/+	+/+	wt	n.d.	122	538	558	3, 4
577	m	+/+	+/-	wt	1.8	38	538	558	3, 4
578	f	+/-	+/-	hz	0.5	4	538	558	3, 4
580	f	-/-	+/+	SMA	1.3	n.d.	538	558	2, 3, 4
581	m	+/-	+/-	hz	0.5	4	538	558	3, 4
582	m	+/+	+/+	wt	1.9	142	538	558	3, 4
642	m	-/-	+/+	SMA	1.9	n.d.	571	539	5
539-4-1	m	+/+	+/+	wt	4.1	221	571	539	5
539-4-2	f	+/-	+/+	hz	5.0	17	571	539	5
539-4-9	m	-/-	+/+	SMA	1.6	64	571	539	5
539-4-12	m	-/-	+/+	SMA	1.9	441	571	539	5
523-4-7	m	-/-	+/+	SMA	6.0	117	562	523	5
503-3-4	m	-/-	+/+	SMA	-	-	504	503	2, 5, 7
608-2-1	n.d.	-/-	+/+	SMA	-	-	624	608	2, 6
520-4-2	n.d.	-/-	+/+	SMA	5.3	n.d.	571	520	6
520-4-9	n.d.	+/+	+/+	wt	2.5	n.d.	571	520	6
634	f	+/+	+/+	wt	0.0	485	571	539	7
635	f	+/-	+/+	hz	5.7	137	571	539	7
636	m	+/+	+/+	wt	1.4	499	571	539	7
637	m	-/-	+/+	SMA	1.2	356	571	539	2, 7
638	m	+/+	+/+	wt	4.4	278	571	539	7
639	m	-/-	+/+	SMA	1.7	n.d.	571	539	2, 7
640	m	+/-	+/+	hz	1.6	130	571	539	7
641	f	+/-	+/+	hz	0.2	489	571	539	7
642	m	-/-	+/+	SMA	1.3	n.d.	571	539	2, 7
503-4-4	m	-/-	+/+	SMA	1.6	n.d.	571	503	2
628	f	-/-	+/+	SMA	2.4	n.d.	571	503	2
649	f	-/-	+/+	SMA	7.1	n.d.	571	503	2
650	m	-/-	+/+	SMA	n.d.	n.d.	571	503	2
558-3-1	f	-/-	+/+	SMA	n.d.	n.d.	538	558	2
558-3-2	m	-/-	+/+	SMA	2.8	n.d.	538	558	2

**Supplementary Table S1.** Genotypes of mice used for experiments described in this paper. Columns: ID, identification number; Sex, gender (m=male, f=female); mSmn, genotype with respect to mouse *smn* (-/-, homozygous for insertional neomycin mutant form; +/-, heterozygous with one functional *smn* copy and one mutant allele; +/+, homozygous for the mouse *smn* gene); hSMN2, presence of human *SMN2* (-/-, none; +/-, 1 copy or heterozygous; +/+, 2 copies or homozygous); SMA status summarises the consequence of the *smn* genotype, i.e. whether the mice are wild type (wt, 2 *smn* copies), heterozygous SMA carriers (hz, 1 *smn1* copy) or theoretically SMA-affected (SMA, no functioning *smn* gene); LV copy number, integration number of the lentiviral vector carrying the therapeutic U7-ESE-B cassette measured by Q-PCR; GFP MFI, mean fluorescence intensity of GFP measured in white blood cells; father, mother, IDs of parents, yellow and white cases indicating SMA carrier mice and parents transmitting the U7-ESE-B gene, respectively. n.d., not determined.

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**Supplementary Table S2.** Oligonucleotide sequences.

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**Regular oligonucleotides**

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U7-5end-XhoI	5' -TA <u>ACTCGAG</u> CCACATCGCCTGC-3'
U7-3end-EcoRV	5' -CTAGATAT <u>CCCCC</u> CAGAGGAGGC-3'
mSmn Fw	5' -TCCGGGATATTGGGATTGTA-3'
mSmn Re	5' -CAGGTCCCACCACCTAAGAA-3'
Neo border	5' -GCAGCTGTGCTCGACGTTGTC-3'
Tg89-Grm7-Fw	5' -CTGACCTACCAGGGATGAGG-3'
Tg89-Grm7-Neg	5' -CCCAGGTGGTTTATAGACTCAGA-3'
Tg89-SMN-Re	5' -GGTCTGTTCTACAGCCACAGC-3'
pWPTS-Sequencing	5' -GAACGGATCTCGACGGTATCGG-3'
GFP10b	5' -GAACTTGTGGCCGTTTACGTC-3'
Sry 5'	5' -AGCTCTTACACTTTAAGTTTTGACTTC-3'
Sry 3'	5' -GCAGCTCTACTCCAGTCTTGCC-3'

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**TaqMan oligonucleotides for quantitative PCR**

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mTtn Probe	5' -CF-TGCACGGAAGCGTCTCGTCTCAGTC-TMR-3'
mTtn Forward	5' -AAAACGAGCAGTGACGTGAGC-3'
mTtn Reverse	5' -TTCAGTCATGCTGCTAGCGC-3'
WPRE Probe	5' -CF-ACGTCCTTTCCATGGCTGCTCGC-TMR-3'
WPRE Forward	5' -GGCACTGACAATTCCGTGGT-3'
WPRE Reverse	5' -AGGGACGTAGCAGAAGGACG-3'
Gag Probe	5' -CF-ACAGCCTTCTGATGTTTCTAACAGGCCAGG-TMR-3'
Gag Forward	5' -GGAGCTAGAACGATTCGCAGTTA-3'
Gag Reverse	5' -GGTGTAGCTGTCCCAGTATTTGTC-3'

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**Oligonucleotides for reverse transcription PCR**

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SMN-Ex6-Fw	5' -GCTGATGCTTTGGGAAGTATGTTA-3'
SMN-Ex6-Fw (CF)	5' -CF-GCTGATGCTTTGGGAAGTATGTTA-3'
SMN-Ex8-Re	5' -ATTCCAGATCTGTCTGATCG-3'

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**Primers for primer extension analysis**

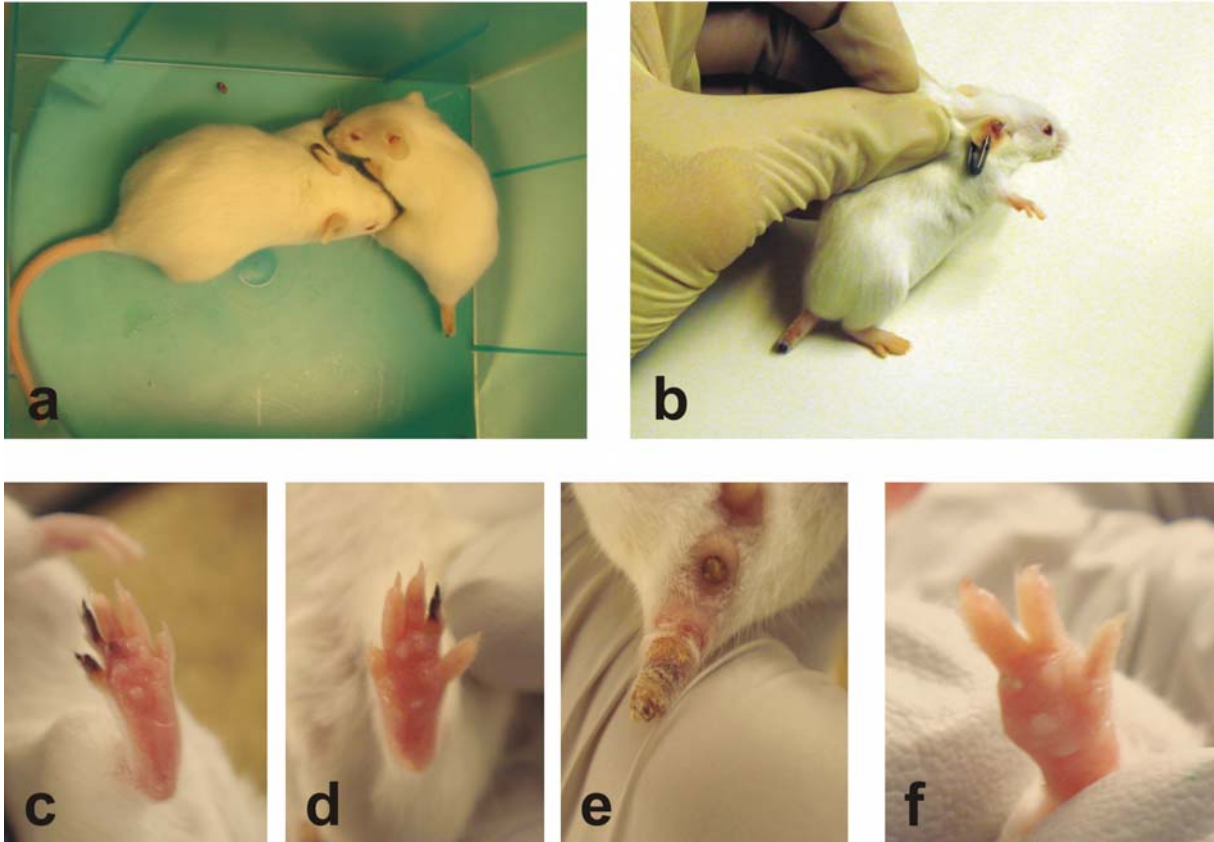
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Pex-U7-Universal	5' -GTCAGAAAACCTGCTCC-3'
Pex-U2-WT	5' -CTGATAAGAACAGATAC-3'

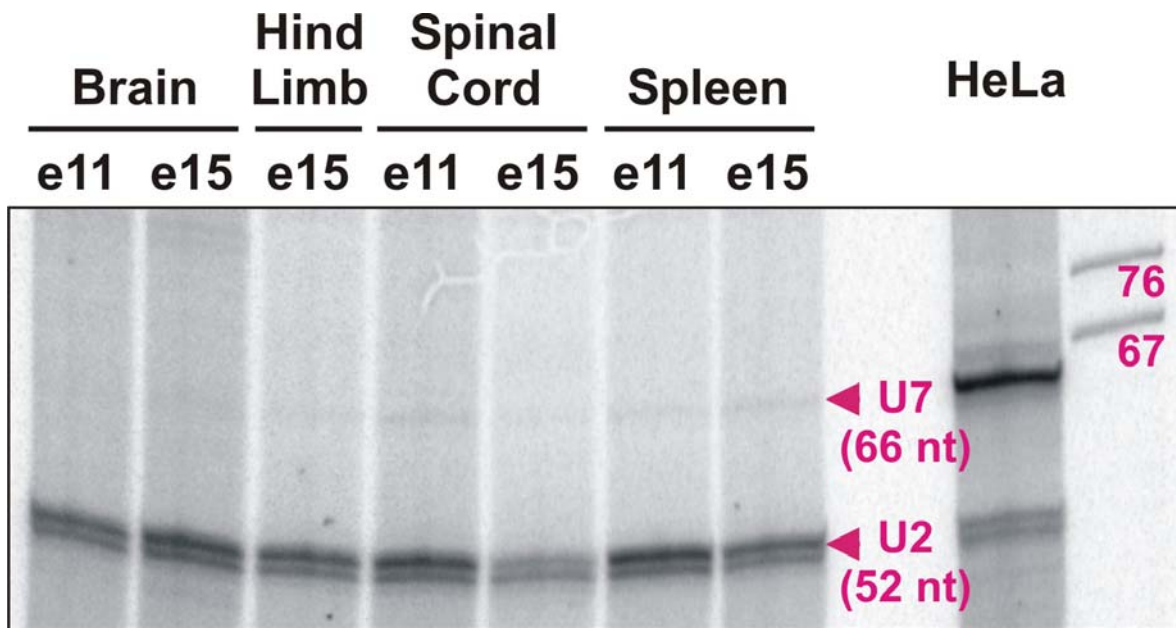
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Abbreviations: CF, 6-carboxyfluorescein (dye); TMR, 6-carboxy-tetramethyl-rhodamine (quencher).

## Supplementary Figures



**Supplementary Figure S1.** Pictures of mouse 580 (SMA with low U7 copy number) taken on days P40 (a-b), P57 (c-e) and P77 (f). **(a)** Mouse 580 (right) shown with one of its littermates (left) for size comparison (note that the littermate is male, whereas mouse 580 is female). **(c-e)** Details showing necrotic tissue on right (c) and left hind paws (d) and tail (e). **(f)** Detail of right hind paw after healing.



**Supplementary Figure S2.** The U7-ESE-B corrective cassette is expressed at a very low level in transgenic mice. Total RNA was extracted from various tissues and the amount of U7-ESE-B snRNA quantified by primer extension. For comparison, a sample from HeLa cells stably transduced with the U7-ESE-B corrective cassette (15 integrated copies) was run in parallel. The endogenous U2 snRNA, also detected by primer extension in the same reaction is used as loading control. Mice epf111 (e11) and epf115 (e15) were first generation transgenics for the U7-ESE-B cassette which were not used for further breeding. They contained approx. 7 and 19 lentiviral vector integrations, respectively.