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**Supplemental information**

**Rescue of a familial dysautonomia mouse**

**model by AAV9-Exon-specific U1 snRNA**

**Giulia Romano, Federico Riccardi, Erica Bussani, Simone Vodret, Danilo Licastro, Isabella Ragone, Giuseppe Ronzitti, Elisabetta Morini, Susan A. Slaugenhaupt, and Franco Pagani**

## Supplemental Information

### Supplemental Methods

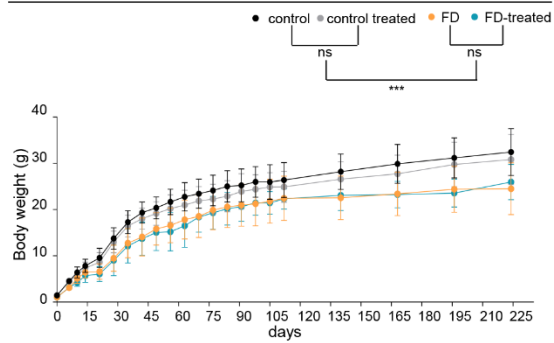
#### Primer list

<i>mIkbkap</i> (genotyping)	For	5'-TGATTGACACAGACTCTGGCCA-3'
	Rev	5'-CTTCACTCTGAAATTACAGGAA-3'
<i>hIkbkap</i> (genotyping)	For	5'-GCCATTGTA CTGTTTGC GACT-3'
	Rev	5'-TGAGTGTCACGATTCTTTCTGC-3'
<i>hELP1</i>	For	5'-GGCCGGCCTGAGCAGCAATCATGTGTCC-3'
	Rev	5'-GATTCTCAGCTTTCTCATGCATTC-3'
<i>ExSpeU1-FD</i>	For	5'-ATAGCAAACAGTACAATGC-3'
	Rev	5'-CACTACCACAAATTATGCA-3'
<i>UI</i>	For	5'-ATACTTACCTGGCAGGGGAGAT-3'
	Rev	5'-GGAAAGCGCGAACGCAGTCCCCCAC-3'
<i>mGAPDH</i>	For	5'-ATGGTGAAGGTCGGTGTGAA-3'
	Rev	5'-GTTGATGGCAACAATCTCCA-3'

### Supplemental Figures

Figure S1

Body Weight



**Figure S1. Measurements of body weights in FD mice and littermate controls treated with AAV9-U1-FD or saline only.**

Weekly measurements were reported in the graph as mean  $\pm$ SD; color code: littermate controls + saline in black; littermate controls + AAV9-U1-FD in gray; FD + saline in orange; FD-treated with AAV9-U1-FD in blue. ns=not significant, \*\*\*  $p < 0.001$ .  $n > 15$  mice for each group.

Figure S2

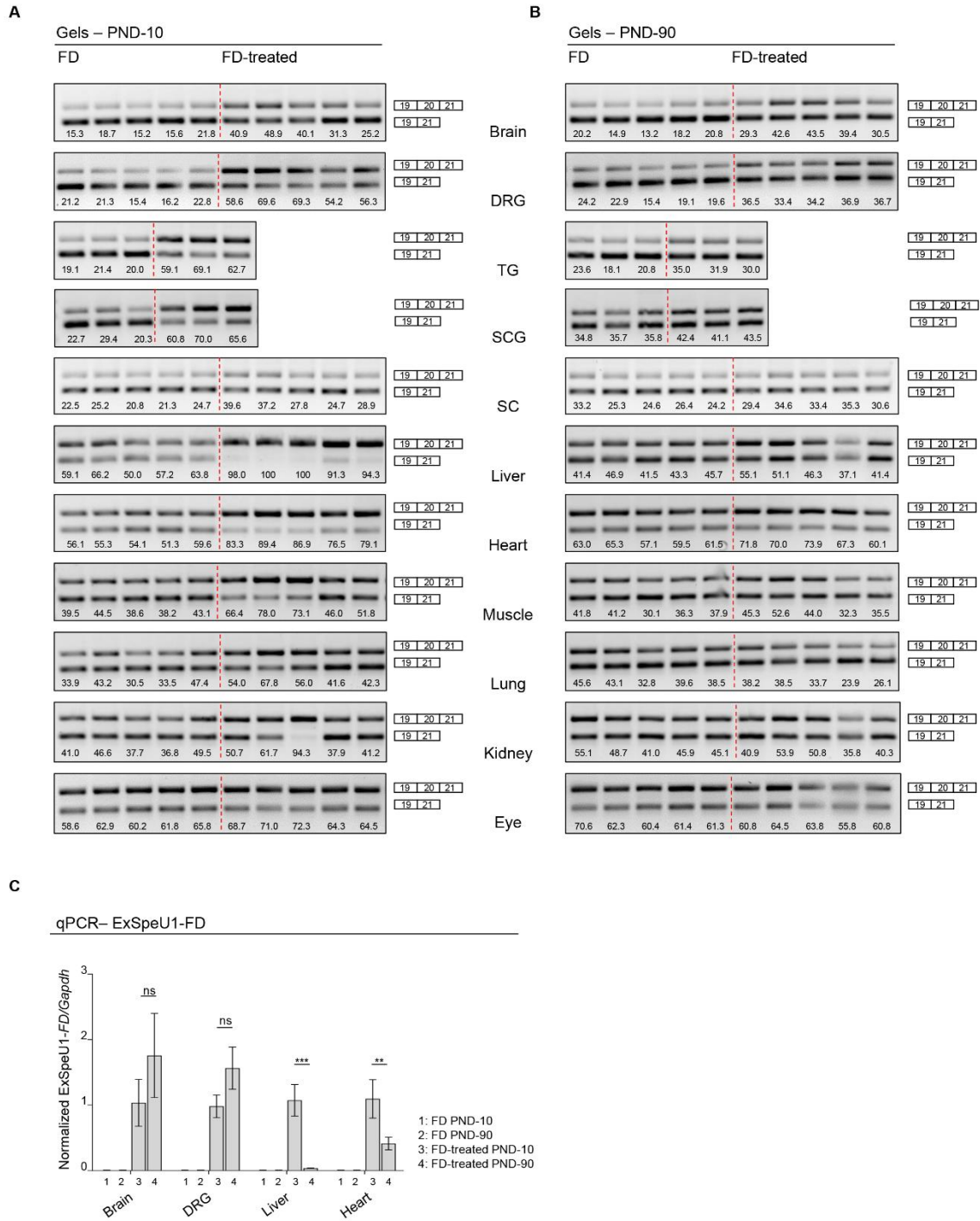


Figure S2. Tissue specific *ELP1* splicing rescue in FD mice.

(A-B) Agarose gels of *ELP1* splicing isoforms in FD animals (*Ikkkap<sup>Δ20</sup>/Ikkkap<sup>fllox</sup>*; h-TgFD9/+) treated with saline and AAV9-U1-FD at PND-10 and PND-90 respectively in (A) and in (B). The upper band of 202 bp represents the *ELP1* isoform in which exon 20 is included, whereas the lower band of 128 bp represents the isoform in which exon 20 is skipped. DRG=dorsal root ganglia; TG= trigeminal ganglia; SCG=superior cervical ganglia; SC=spinal cord. The percentage of exon 20 inclusion is reported below each lane, for all tissues the 5 analyzed animals are shown (except for SCG and TG in which 3 animals per group were tested). (C) qPCR quantification of ExSpeU1-FD in FD animals treated with saline and AAV9-U1-FD at PND-10 and PND-90. Expression levels were normalized on *Gapdh* and one FD-treated PND-10 mouse was set to 1. ns=not significant, \*\*\*  $p < 0.001$  and \*\* $p < 0.01$ . Data are expressed as mean $\pm$ SEM of two technical replicates on 4 mice per group.

Figure S3

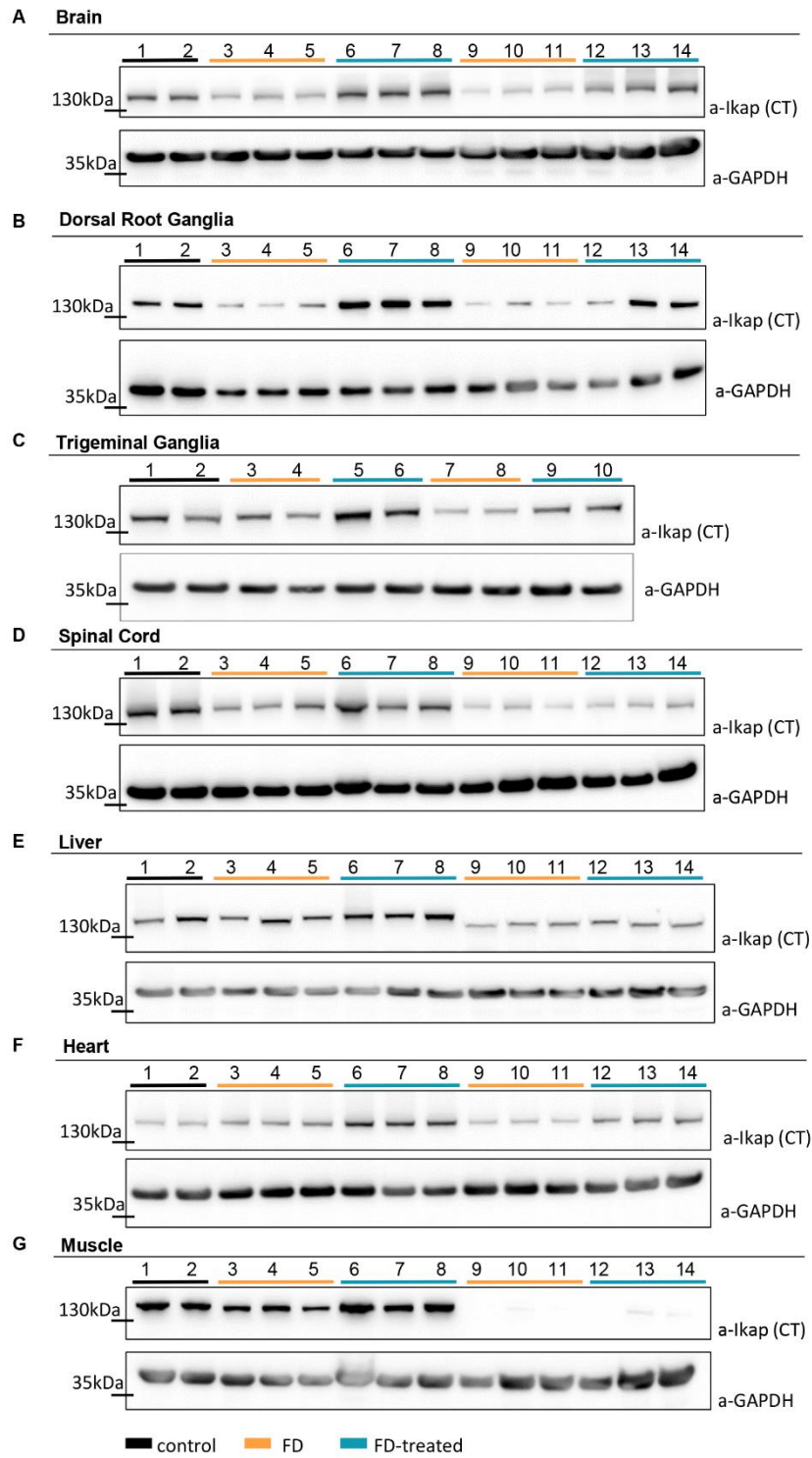
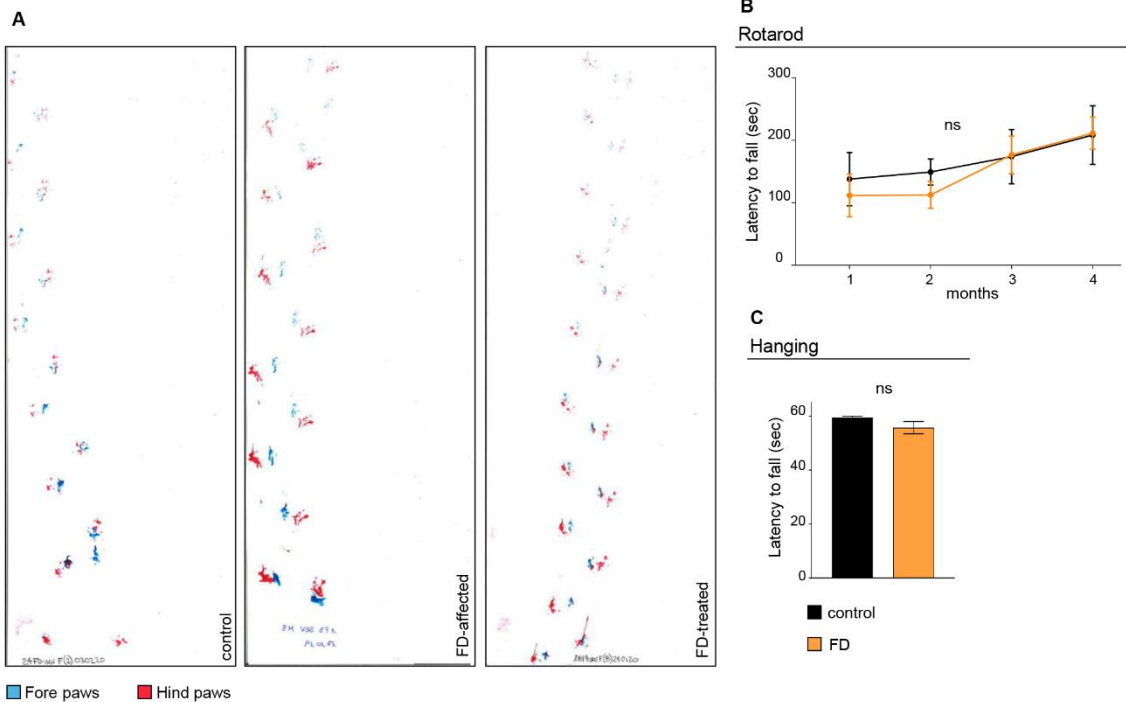


Figure S3. AAV9-U1-FD treatment rescues protein levels in FD mice.

(A-G) Western blot membranes probed with anti-Ikap and anti-GAPDH in different tissues of control mice at PND-10 (*Ikkap<sup>fllox/+</sup>*; h-TgFD9/+; lanes 1 and 2), FD (*Ikkap<sup>A20/Ikkap<sup>fllox</sup></sup>*; h-TgFD9/+; lanes 3-5 at PND-10 and lanes 9-11 at PND-90) and FD-treated (*Ikkap<sup>A20/Ikkap<sup>fllox</sup></sup>*; h-TgFD9/+; lanes 6-8 at PND-10 and lanes 12-14 at PND-90). 3 mice for each group were tested, except for trigeminal ganglia group in which 2 animals were analyzed: (C) control mice at PND-10 lanes 1 and 2, FD + saline lanes 3-4 at PND-10 and lanes 7-8 at PND-90 and FD-treated lanes 5-6 at PND-10 and lanes 9-10 at PND-90.

Figure S4



**Figure S4. AAV9-U1-FD treatment in FD mice rescues some of the pathological traits of FD.**

Several tests and analyses were performed on littermate controls (*Ikkap<sup>fllox/+</sup>*; h-TgFD9/+) and FD mice (*Ikkap<sup>A20/Ikkap<sup>fllox</sup></sup>*; h-TgFD9/+) treated with one intracerebral ventricular injection (ICV) at PND-0 and a second intraperitoneal injection (IP) at PND-2 of AAV9-U1-FD ( $1.6 \times 10^{11}$  VG/mouse) or saline only. Littermate controls + saline in black; FD + saline in orange; FD-treated with AAV9-U1-FD in blue. (A) Gait assay pattern. A representative footprint pattern is shown for each group, fore paws are stained in blue and hind paws are stained in red. (B) Rotarod test in control and FD mice ( $n=10$  per group) at 1, 2, 3 and 4 months. (C) Hanging test in control and FD mice ( $n=10$

per group) at 2 months. Statistical analysis was performed using one-way ANOVA with Bonferroni correction. Values reported in (B and C) are means  $\pm$  SEM. ns=not significant.

Figure S5

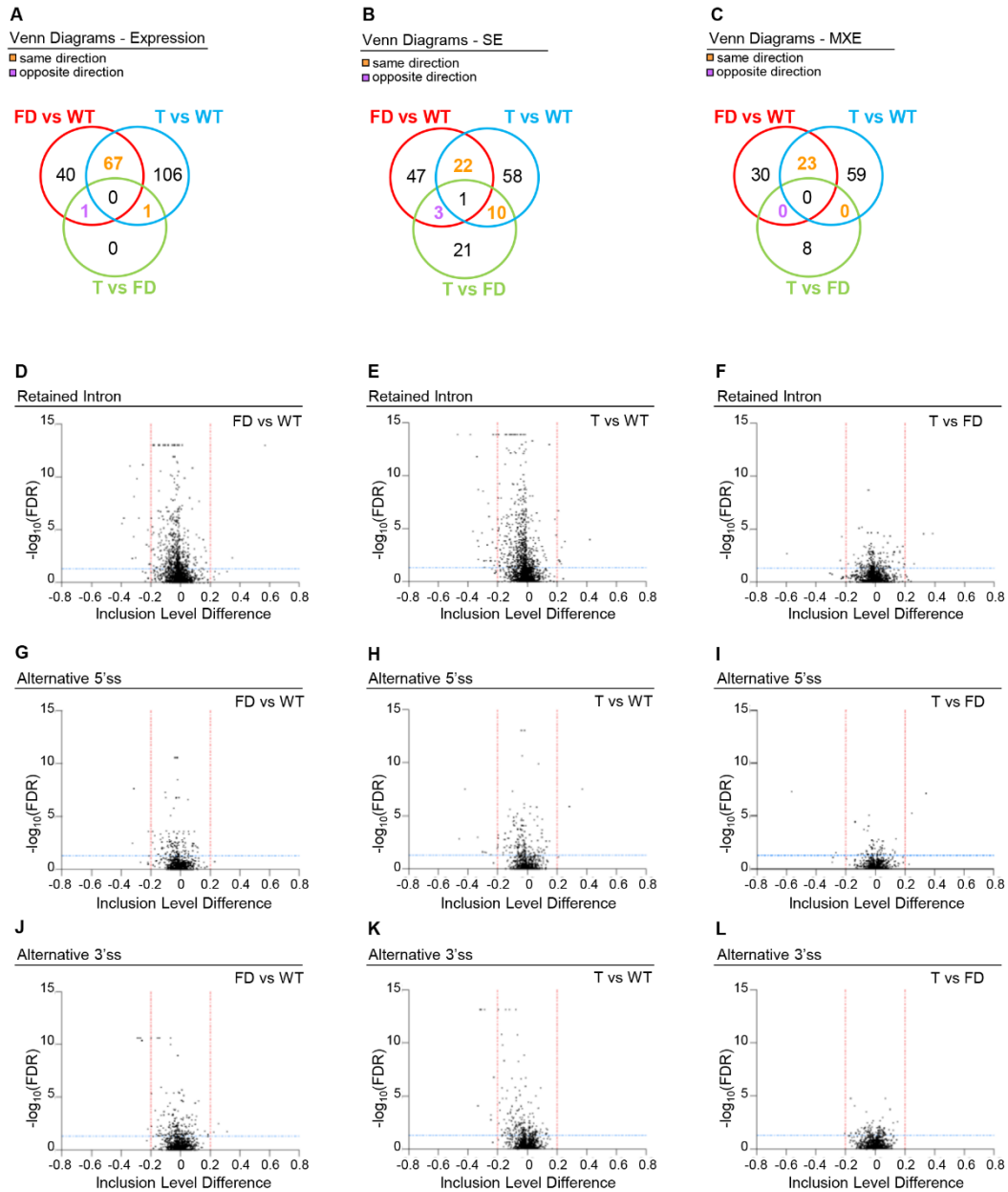
Group	Alternative Splicing Events	n of Analyzed Events		Significant Different Events	
		$\Sigma$ mean counts $\geq 20$ (SE-MXE-RI) $\geq 40$ (A5SS-A3SS)	%	$-0.2 \leq$ Inclusion Level Difference $\leq 0.2$ FDR $\leq 0.05$	down/up
ExSpeU1-FD vs Empty	SE	101,518	0.05%	51	26/25
	MXE	37,465	0.5%	197	105/92
	RI	2,604	0.2%	5	1/4
	A5SS	2,757	0.04%	1	0/1
	A3SS	4,039	0.05%	2	1/1

**Figure S5. Summary table of alternative splicing analysis in HEK-293 Flp-In T-REx stable clone overexpressing ExSpeU1-FD.**

The total number of analyzed events and the percentage of the significant ones are reported for each category (SE=skipped exon; MXE=mutually exclusive exon; RI=retained intron; A5SS=alternative 5' splice site; A3SS=alternative 3' splice site). Total number of analyzed events = sum ( $\Sigma$ ) of the counts means  $\geq 20$  (SE-MXE-RI) or  $\geq 40$  (A5SS-A3SS). Significant events: FDR  $\leq 0.05$  and Inclusion Level Difference  $\leq -0.2$  or  $\geq 0.2$ .



Figure S6



**Figure S6. Expression and alternative splicing events in dorsal root ganglia in FD mice.**

(A-C) Non-quantitative Venn diagram showing the significantly differentially expressed genes (A), skipped exons (B) and mutually exclusive exons (C) shared across FD vs WT (red circle), T vs WT (blue circle) and T vs FD (green circle) comparisons at PND-10. Common genes are labeled in orange to indicate events that go in the same direction or in violet to indicate those that go in the opposite direction. (D-L) Volcano plots showing global alternative splicing

changes in (D-F) retained introns (RI), (G-I) alternative 5' splice sites (A5SS) and (J-L) alternative 3' splice sites (A3SS) at PND-10. In panels (D), (G) and (J) FD mice are compared to WT mice (FD vs WT); in panels (E), (H) and (K) FD-treated mice are compared to WT (T vs WT); in panels (F), (I) and (L) FD-treated mice are compared to FD mice (T vs FD). Horizontal blue line ( $FDR \leq 0.05$ ) and vertical red lines (Inclusion Level Difference  $\leq -0.2$  or  $\geq 0.2$ ) indicate cut-off values and determine significant events in each category.

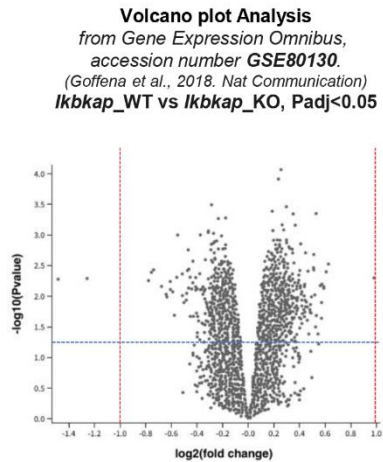
Figure S7

Group	Alternative Splicing Events	n of Analyzed Events $\Sigma$ mean counts $\geq 20$ (SE-MXE-RI) $\geq 40$ (A5SS-A3SS)	Significant Different Events $-0.2 \leq$ Inclusion Level Difference $\leq 0.2$ FDR $\leq 0.05$		
			%	n tot	down/up
FD vs WT	SE	48,783	0.15%	73	45/28
	MXE	8,890	0.60%	53	30/23
	RI	3,171	0.50%	17	15/2
	A5SS	2,246	0.13%	3	3/0
	A3SS	3,740	0.13%	8	5/3
T vs WT	SE	43,708	0.21%	91	55/36
	MXE	7,615	0.81%	62	31/31
	RI	3,171	0.82%	33	26/7
	A5SS	2,087	0.38%	10	8/2
	A3SS	3,470	0.20%	7	7/0
T vs FD	SE	47,804	0.07%	35	18/17
	MXE	8,672	0.06%	8	5/3
	RI	3,233	0.12%	7	4/3
	A5SS	2,198	0.09%	4	2/2
	A3SS	3,634	0.03%	1	1/0

**Figure S7. Summary table of alternative splicing analysis in dorsal root ganglia in FD mice.**

The total number of analyzed events and the percentage of the significant ones are reported in each group (FD vs WT, T vs WT and T vs FD) for each category (SE=skipped exon; MXE=mutually exclusive exon; RI=retained intron; A5SS=alternative 5' splice site; A3SS=alternative 3' splice site). Total number of analyzed events = sum ( $\Sigma$ ) of the counts means  $\geq 20$  (SE-MXE-RI) or  $\geq 40$  (A5SS-A3SS). Significant events:  $FDR \leq 0.05$  and Inclusion Level Difference  $\leq -0.2$  or  $\geq 0.2$ .

Figure S8



**Figure S8. Gene expression analysis in a different FD model.**

Volcano plots showing differential gene expression changes in the FD model published by *Goffena et al., 2018*<sup>1</sup>, in which there is a selective ablation of the *ELP1* gene in DRG. In this mouse *Ikkap* is deleted from the neural crest resulting in embryonic lethality and dorsal root ganglia were analyzed at E17-18, comparing *Ikkap*\_WT (*Ikkap*<sup>+/*LoxP*</sup>) versus *Ikkap*\_KO (*Wnt1-Cre; Ikkap*<sup>*LoxP/LoxP*</sup>). Transcriptome data deposited at Gene Expression Omnibus, accession number GSE80130. The horizontal blue line ( $\text{padj} \leq 0.05$ , corresponding to a  $-\log_{10} = 1.3$ ) and vertical red lines ( $\log_2\text{FoldChange} \leq -1$  or  $\geq 1$ ) indicate cut-off values. Only three genes are significant differentially expressed with these cut-off values.

## Supplemental Tables

**Table S1. RNA-seq analysis in HEK Flp-In T-Rex cell line, ExSpeU1-FD versus control.** Summary (sheet1); list of the significant candidates of the differential gene expression (sheet 2) and Ingenuity Pathway analysis (sheet 3). Alternative splicing events analysis (Skipped Exon, sheet 4; Retained Intron, sheet 5; Mutually Exclusive Exons, sheet 6; Alternative 5' splice site, sheet 7; Alternative 3' splice site, sheet 8) in HEK293 Flp-In-T-Rex cell line. ExSpeU1-FD vs control clones.

**Table S2. RNA-seq analysis in DRG of PND-10 mice.** Summary (sheet 1); list of the significant candidates of the differential gene expression in dorsal root ganglia of FD mice vs control (WT) (sheet 2), treated (T) vs WT (sheet 3) and T vs FD (sheet 4). Ingenuity Pathway analysis on DGE of FD vs WT (sheet 5) and T vs WT (sheet 6). Mice at PND 10.

**Table S3. List of the significant alternative splicing events in dorsal root ganglia of FD mice vs control (WT) at PND-10.** Summary, sheet 1; Skipped Exon, sheet 2; Retained Intron, sheet 3; Mutually Exclusive Exons, sheet 4; Alternative 5' splice site, sheet 5; Alternative 3' splice site, sheet 6.

**Table S4. List of the significant alternative splicing events in dorsal root ganglia of treated (T) mice vs control (WT) at PND-10.** Summary, sheet 1; Skipped Exon, sheet 2; Retained Intron, sheet 3; Mutually Exclusive Exons, sheet 4; Alternative 5' splice site, sheet 5; Alternative 3' splice site, sheet 6.

**Table S5. List of the significant alternative splicing events in dorsal root ganglia of treated (T) mice vs FD at PND-10.** Summary, sheet 1; Skipped Exon, sheet 2; Retained Intron, sheet 3; Mutually Exclusive Exons, sheet 4; Alternative 5' splice site, sheet 5; Alternative 3' splice site, sheet 6.

## **Supplemental References**

1. Goffena, J., Lefcort, F., Zhang, Y., Lehrmann, E., Chaverra, M., Felig, J., Walters, J., Buksch, R., Becker, K.G., and George, L. (2018). Elongator and codon bias regulate protein levels in mammalian peripheral neurons. *Nat. Commun.* 9, 1–10.