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Supplemental Information

Rapid and Complete Reversal of Sensory Ataxia by Gene Therapy in a Novel Model of Friedreich Ataxia

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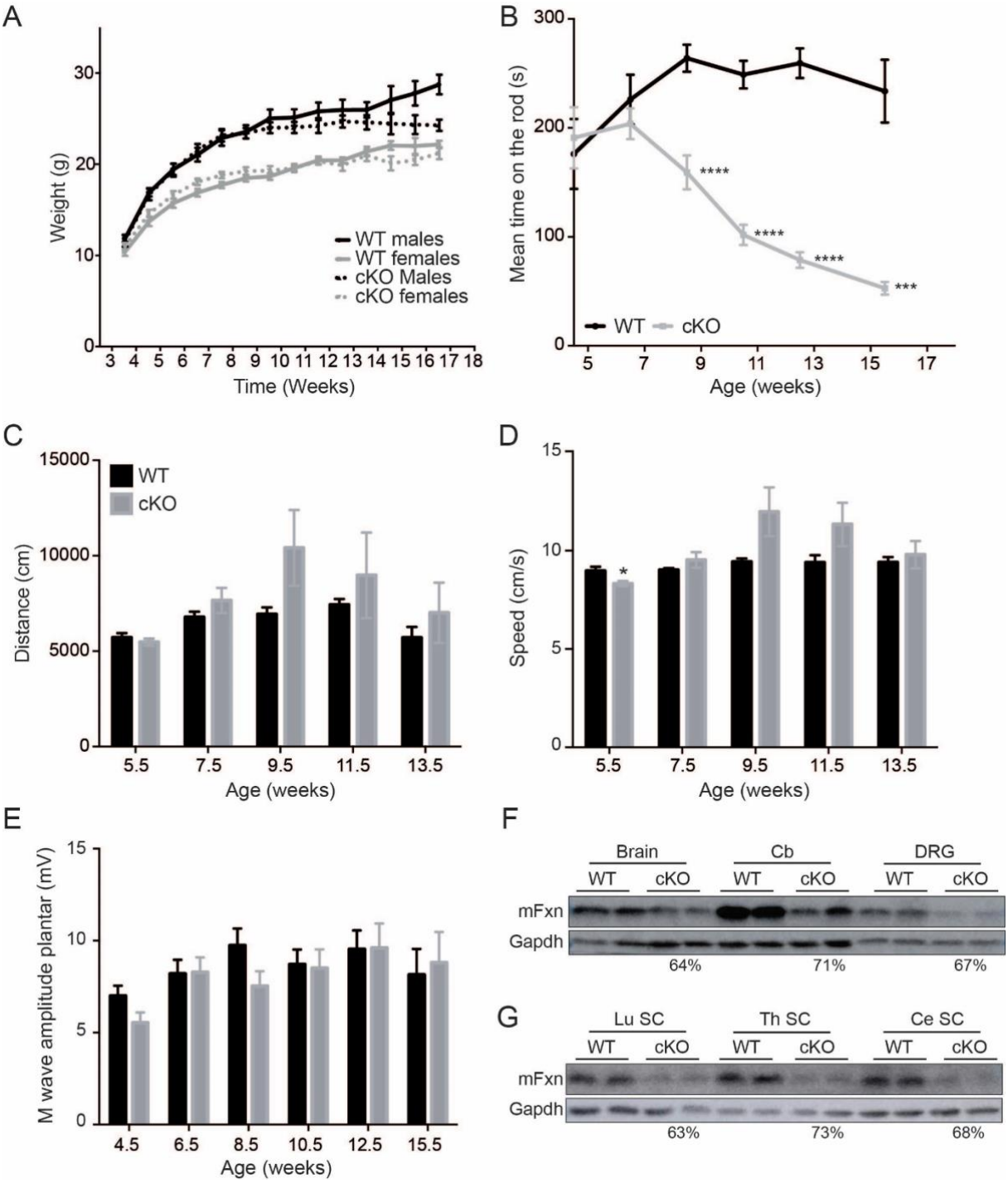

Figure S1: *Pvalb* cKO mice characterization

(**A**) Growth curve, N= 10 WT and N= 11 *Pvalb* cKO. (**B**) Evaluation of rotarod performances, N= 10 WT and N= 11 *Pvalb* cKO. (**C**-**D**) Openfield analysis with measurement of the distance (**C**) and mean speed in the arena (**D**) in a 20 min activity session, N= 6 WT and N= 7 *Pvalb* cKO**.** (**E**) Amplitude of motor wave (M-Wave) were recorded after plantar sciatic nerve stimulation, N= 6 WT and N= 7 *Pvalb* cKO. *Pvalb* cKO animals did not differ from control animals. (**F**-**G**) Expression of mouse Frataxin (mFXN) protein in brain, cerebellum, total DRG (**F**) lumbar, thoracic and cervical spinal cord (LuSC, Th SC, Ce SC, respectively) (**G**) from WT and *Pvalb* cKO mice at 7.5 weeks of age. Percentage of depletion in each tissue is indicated under the western blot. Data are represented as mean +/- SEM. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

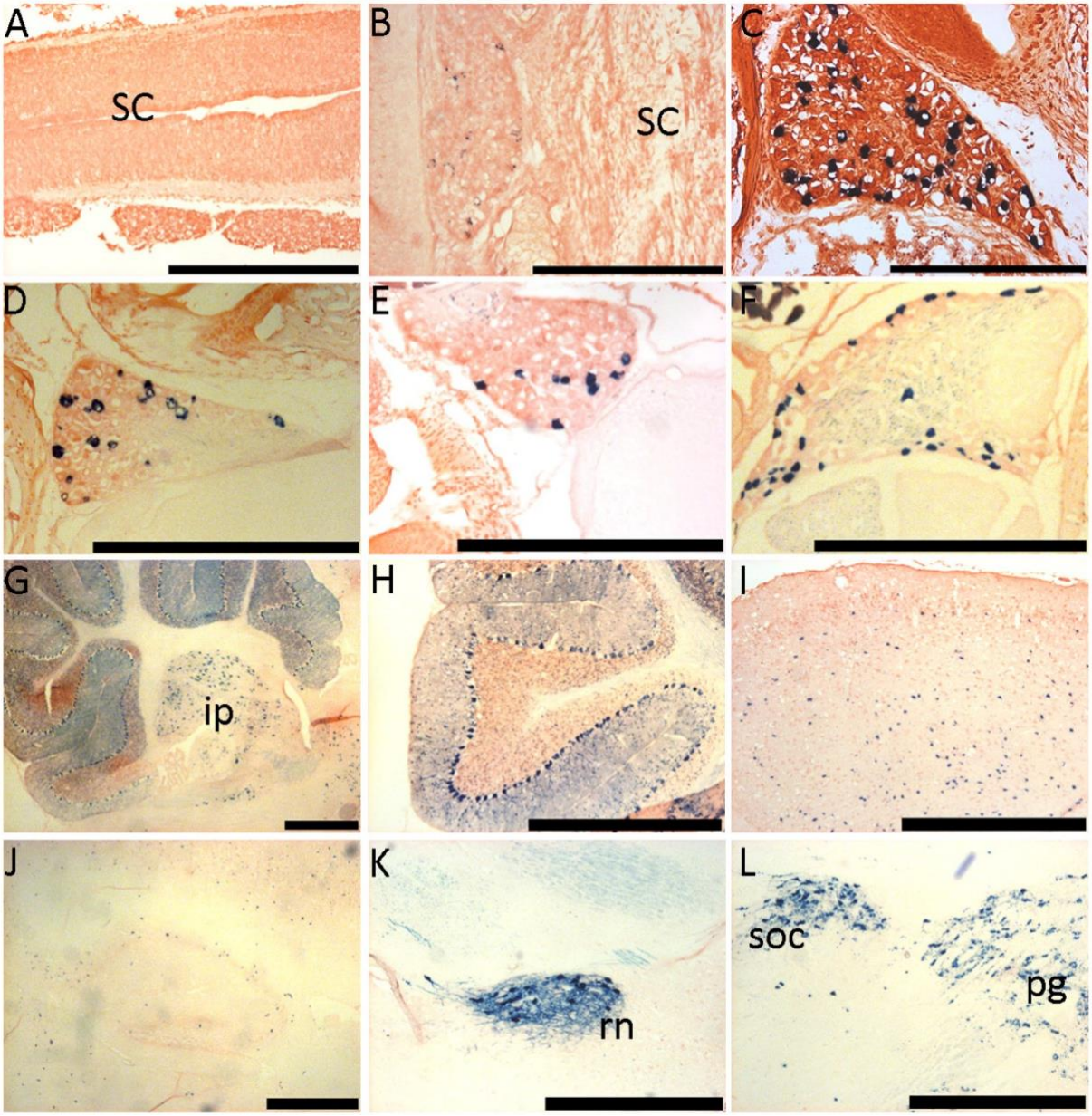

Figure S2: *Pvalb*-Cre expression in DRG and brain overtime.
LacZ staining on frozen section of DRG at E14.5 (**A**), E17.5 (**B**) and p8 (**C**) and at 21.5 weeks in cervical (**D**), thoracic (**E**) and lumbar (**F**) DRG. Enlargements of cerebellum (**G-H**), cortex (**I**), hippocampus (**J**), midbrain (**K**) and pons (**L**) at 21.5 weeks; ip: interposed nucleus, rn: red nucleus, soc: superior olive complex, pg: pontine grey. Scale bars, 500µm**.**

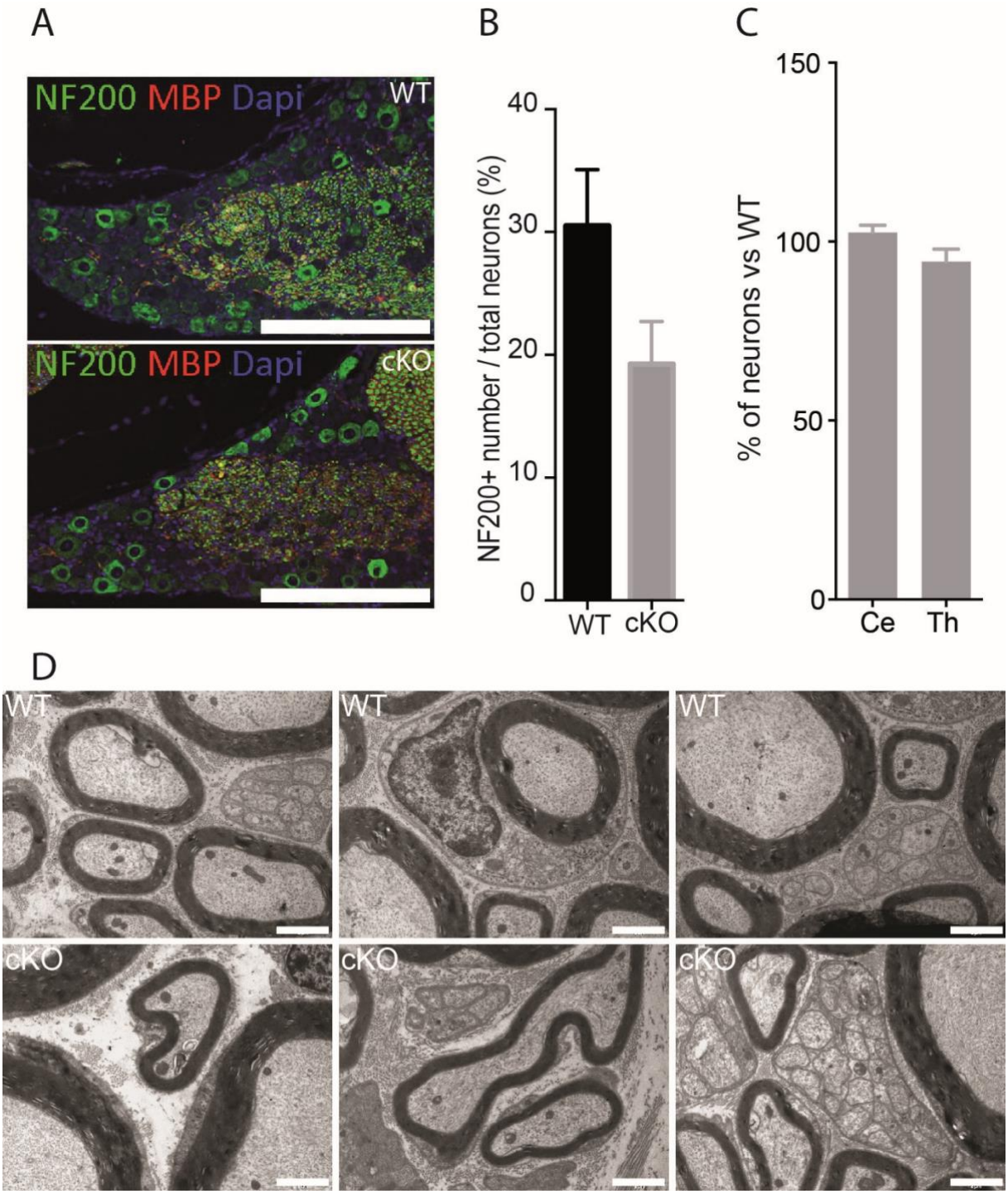

Figure S3: Histological evaluation of *Pvalb* cKO animals.
(**A**) Representative images of NF200 and MBP co-staining in 21.5 weeks DRG. Scale bars, 200µm. (**B**) Scoring of neurons co-stained with NF200 and MBP showed a 30% loss of large neurons within DRG, N= 4 WT and N= 4 *Pvalb* cKO scored. (**C**) Mean number of neuron per DRG of WT and *Pvalb* cKO was evaluated at the cervical and thoracic level of the spinal cord, N= 3-4 mice analyzed per group for the cervical and thoracic portions. (**D**) Ultrathin sections of radial and median nerves at 18.5 weeks. Scale bars, 2µm. Data are represented as mean +/- SEM.

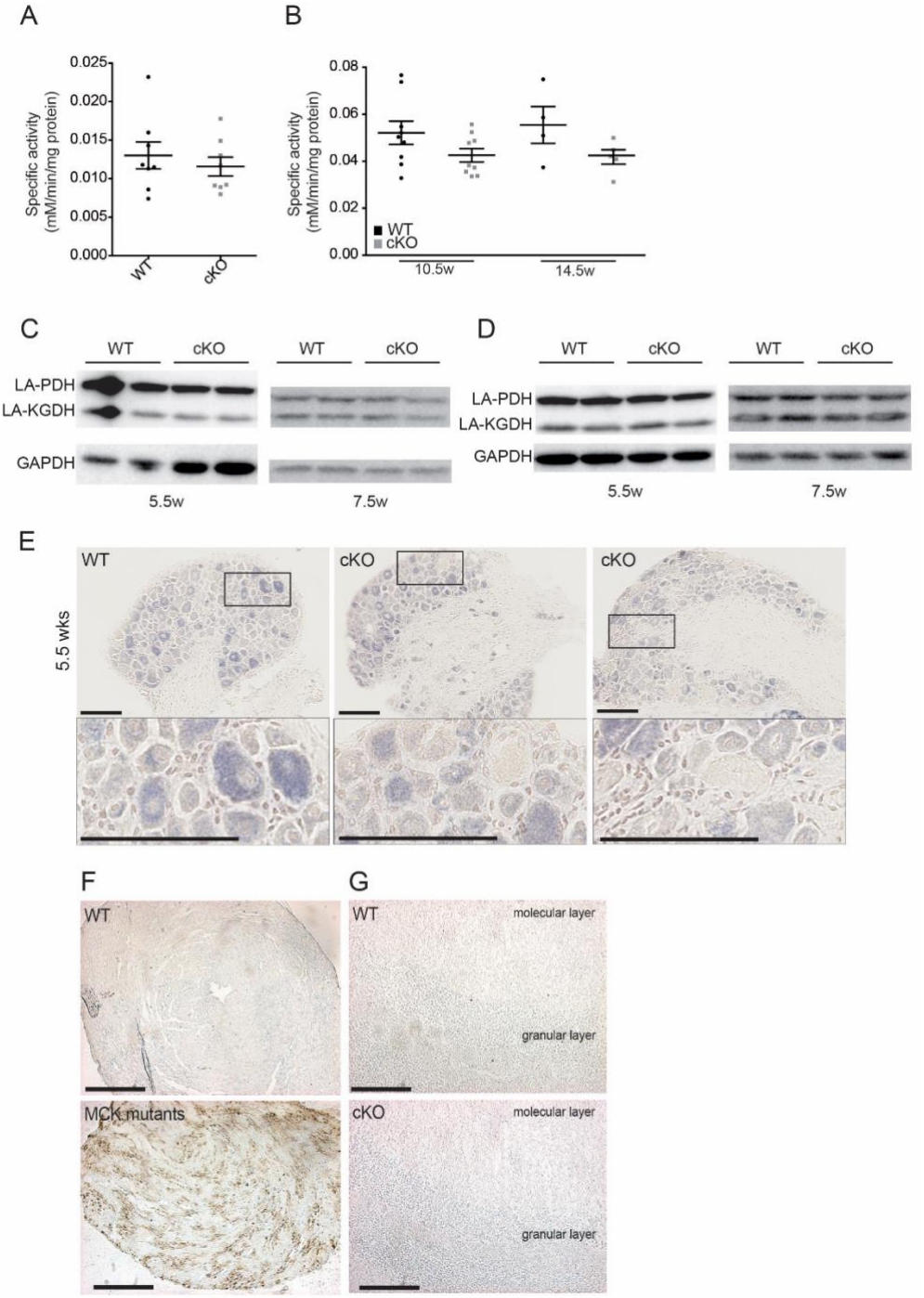

Figure S4: Fe-S cluster protein deficit is not detected on total protein extracted samples.
(**A**) SDH specific enzymatic activity on lumbar DRG at 7.5 weeks of age, N = 8 per group. (**B**) SDH specific enzymatic activity on cerebellum samples at 10.5 and 14.5 weeks of age, N= 9 per group at 10.5 weeks, N= 4 per group at 14.5 weeks. (**C-D**) Expression of Fe-S cluster apoprotein in DRG (**C**) and cerebellum (**D**) samples from WT and *Pvalb* cKO mice at 5.5 and 7.5 weeks of age. LA-PDH: lipoic acid bound PDH, LA-KGDH: lipoic acid bound KGDH. (**E**) Representative SDH histoenzymatic activity staining in lumbar DRG at 5.5 weeks of age WT and *Pvalb* cKO mice. Scale bars, 100µm. (**F-G**) Heart cryosections (apex) from 9 weeks WT and MCK mutant mice[19] (**F**) and cerebellum cryosections from 13.5 weeks WT and *Pvalb* cKO mice (**G**) stained with Perl's DAB. Scale bars, 500µm.

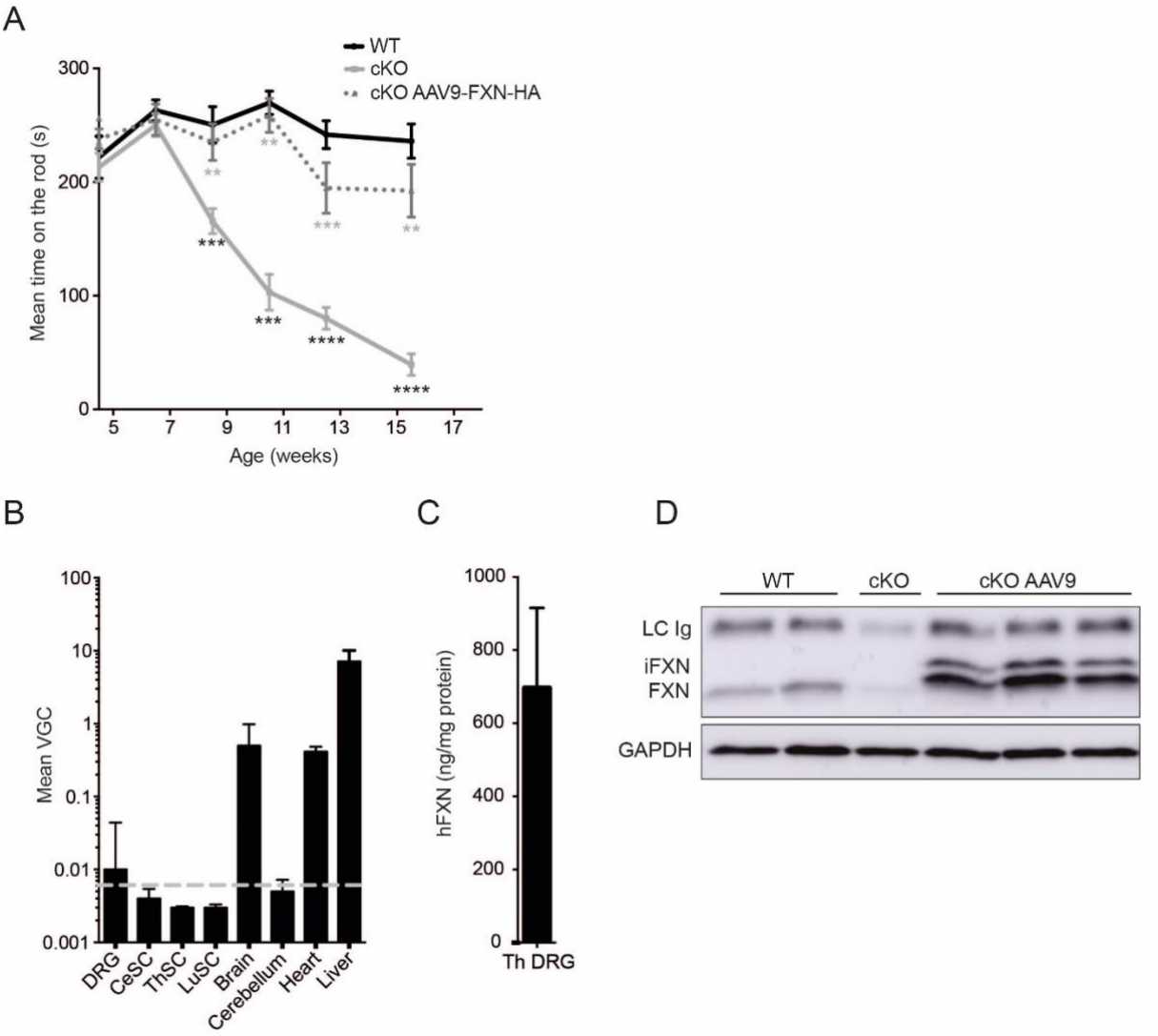

Figure S5: Early symptomatic treatment in *Pvalb* cKO animals

(**A**) Motor performances after early symptomatic treatment on the rotarod N= 11 WT, N= 9 *Pvalb* cKO and N= 9 *Pvalb* cKOAAV. (**B**) Biodistribution of AAV9-CAG-hFXN-HA after IV delivery and (**C**) quantification of human frataxin expression in thoracic DRG by ELISA, N= 3 animals per group. (**D**) Expression of mouse and human frataxin protein in thoracic DRG from WT, *Pvalb* cKO and *Pvalb* cKOAAV mice at 10.5weeks of age. LC Ig: Immunoglobulins light chains; iFXN: intermediate frataxin; FXN: mature frataxin. Data are represented as mean +/- SEM. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

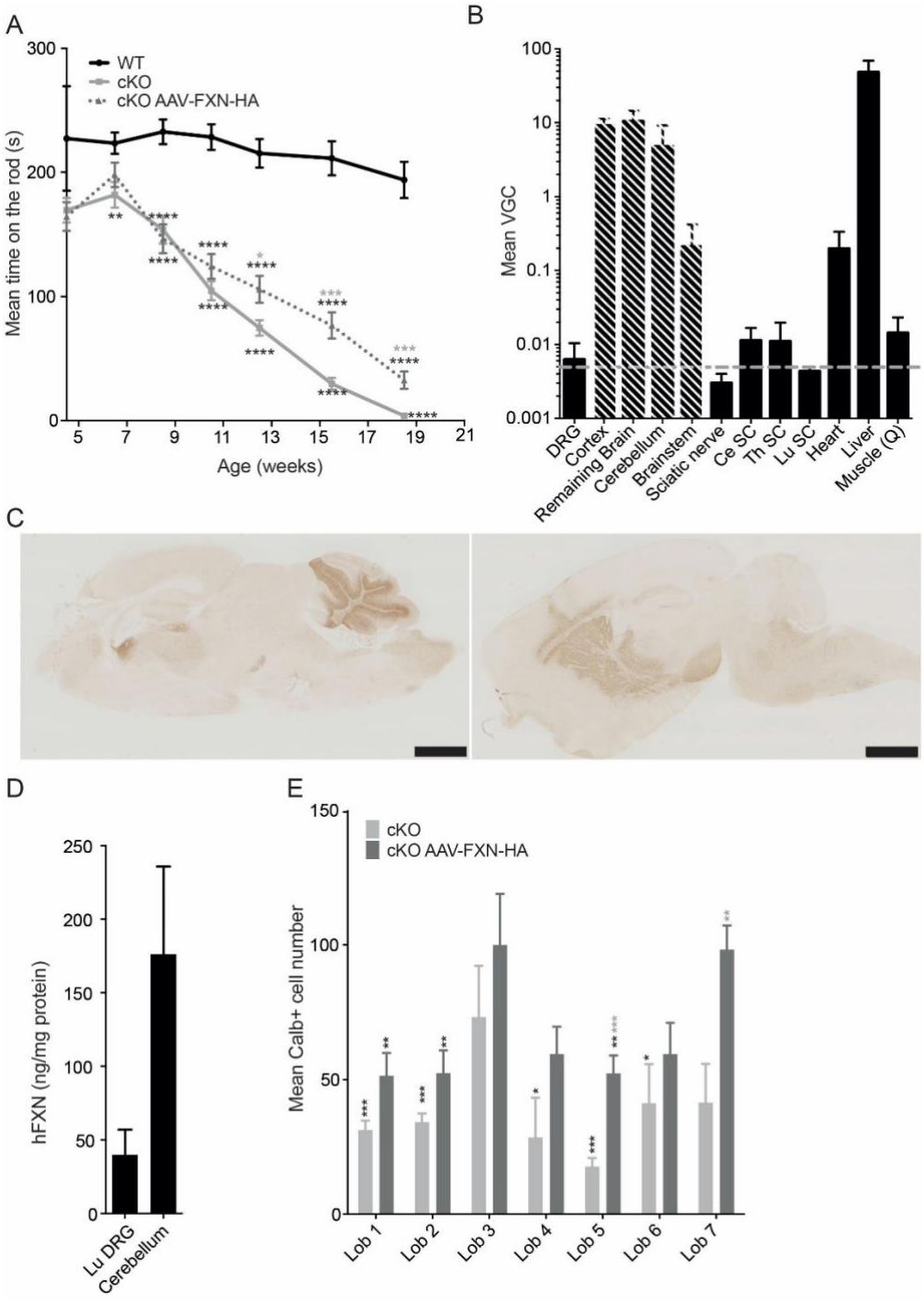

Figure S6: Late symptomatic treatment in *Pvalb* cKO animals

(A) Motor performances after late symptomatic treatment on the rotarod N= 26 WT, N= 29 *Pvalb* cKO and N= 32 *Pvalb* cKOAAV. (**B**) Biodistribution of AAV-CAG-hFXN-HA after IV and IC delivery, dashed bars correspond to VGC coming mainly from AAVrh10 IC delivery, N= 3. (**C**) Immunostaining for Frataxin HA expression in brain, Scale bars, 100µm. (**D**) Quantification of human frataxin (hFXN) expression in lumbar DRG and cerebellum, N= 3 animals per group. (**E**) Mean Purkinje cell number in different lobules of the cerebellum of WT, *Pvalb* cKO treated and untreated mice, N= 6 mice analyzed per group. Black stars correspond to p-value versus WT and grey stars versus KO. Data are represented as mean +/- SEM. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

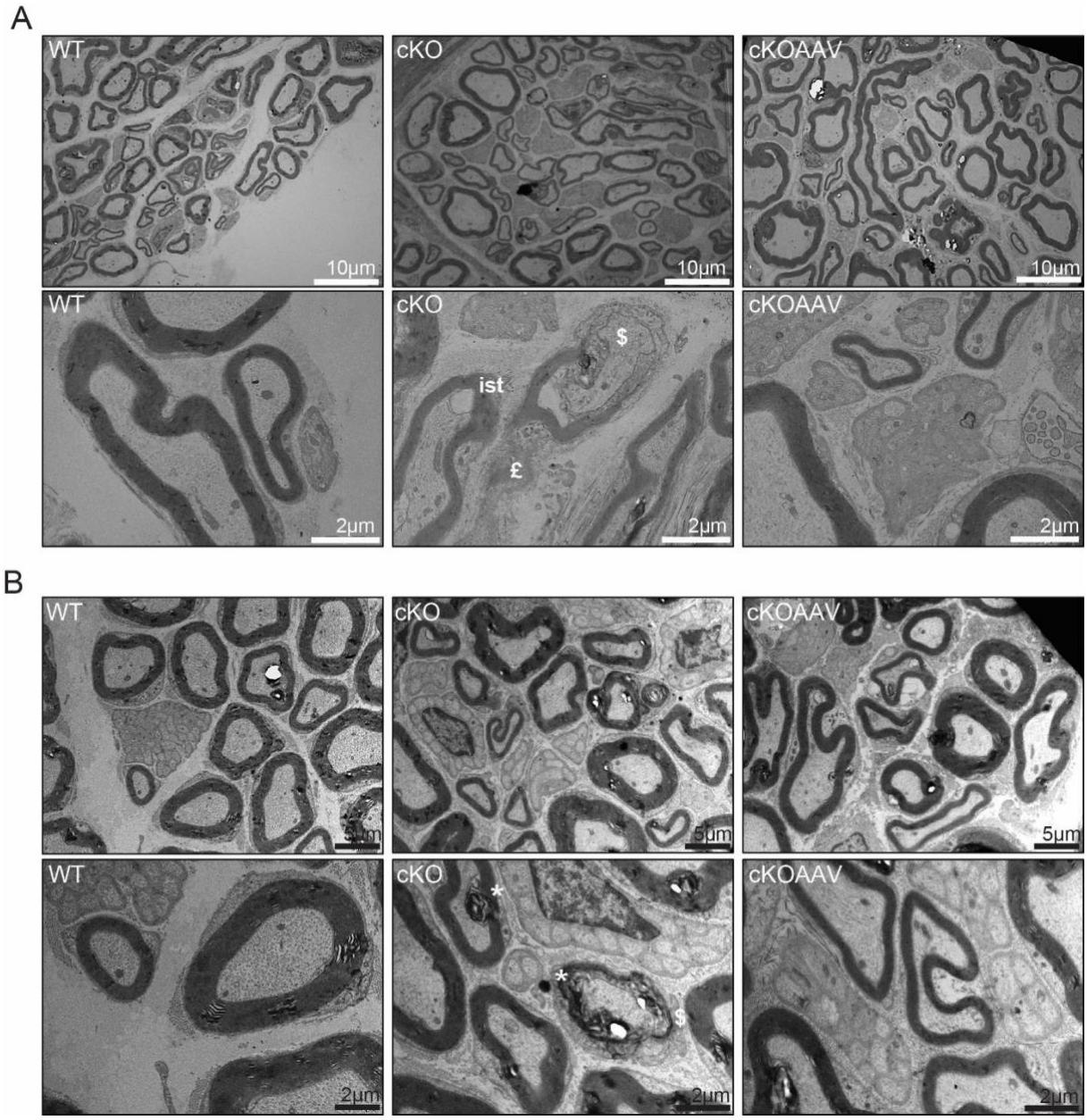

Figure S7: **Regeneration process in the sciatic nerve of *Pvalb* cKO animals treated at 7.5 weeks of age.** Ultrathin sections of sciatic nerves of WT, *Pvalb* cKO untreated and treated at 7.5 weeks and analyzed 1 week (**A**) or 2 weeks (**B**) post injection; ist: inner swelling tongue; £: fibrosis; $: degeneration; * : myelin debris. Scale bars, indicated sizes.

Table S1: Individual EMG data on characterization cohort

Mice	Sex	Genotype	6.5 weeks		8.5 weeks		10.5 weeks		12.5 weeks	
			Amp M wave	**Amp H wave**	Amp M wave	**Amp H wave**	Amp M wave	**Amp H wave**	Amp M wave	**Amp H wave**
84	F	WT	3,9	**0,4**	9,6	**0,5**	8,4	**0,6**	6	**0,9**
86	F	WT	6,9	**0,7**	3,9	**0,5**	6,8	**1,2**	5,7	**0,6**
87	F	WT	7	**1,3**	5	**1**	3,9	**0,4**	17,7	**1,5**
97	M	WT	11,8	**2,9**	9,2	**0,8**	11,7	**0,9**	4,8	**0,7**
98	M	WT	10,9	**1,1**	8,1	**0,6**	8,8	**0,6**	4,6	**0,7**
110	M	WT	4	**0,4**	5,3	**1**	7,7	**0,6**	7,2	**0,4**
88	F	KO	8,6	**0**	9,1	**0**	10,4	**0**	3,8	**0**
89	F	KO	8,4	**0,9**	3,7	**0**	4,2	**0**	12,3	**0**
90	F	KO	7,7	**1,2**	4	**0**	4,4	**0**	10,5	**0**
93	M	KO	7,5	**0,1**	5,4	**0**	5,4	**0**	3,5	**0**
95	M	KO	15,4	**1**	6,3	**0**	5,9	**0**	15	**0**
109	M	KO	4	**0**	12,3	**0**	4	**0**	11,2	**0**
111	M	KO	6	**0,3**	18,7	**0**	13,3	**0**	7,3	**0**

Table S2: ELISA assay on FXN expression

	Mean quantity of frataxin per mg of protein +/- SEM		% of FXN quantity in *Pvalb* cKO mice compare to WT		FXN level decrease in *Pvalb* cKO
	WT	***Pvalb* cKO**	**WT**	***Pvalb* cKO**	
Ce DRG	12.2 +/- 0.6 ng	2.1 +/- 0.2 ng	100%	17.2%	82.80%
Th DRG	25.8 +/- 2.3 ng	7.2 +/- 0.4 ng	100%	27.9%	72.10%
Lu DRG	25.6 +/- 2.1 ng	7.0 +/- 0.6 ng	100%	27.3%	72.70%
Cerebellum	27.8 +/- 0.7 ng	4.6 +/- 0.2 ng	100%	16.5%	83.50%

Table S3: Calculation for determining percentage of FXN expression

	Experimental value	Expected value		Experimental value		Difference between expected value and experimental value	
	WT ($Fxn^{+/L3}$)	Heterozygous without *Pvalb* Cre expression ($Fxn^{L3/L-}$)		*Pvalb* cKO ($Fxn^{L3/L-}$ *Pvalb* Cre)			
	Measured value (in ng of frataxin / mg prot)	Value WT/2 (in ng / mg prot)	Considered % of frataxin in heterozygous tissues	Quantity of frataxin expressed by the tissues (in ng / mg prot)	Equivalent % (vs heterozygotes) expressed by the tissues	Difference between expected value and experimental value (in ng / mg prot)	Equivalent % (vs heterozygotes) expressed by Cre positive cells
Ce DRG	12.2	6.1	100%	2.1	34.4 %	4	65.6 %
Th DRG	25.8	12.9	100%	7.2	55.8 %	5.7	44.2 %
Lu DRG	25.6	12.8	100%	7.0	54.7 %	5.8	45.3%
Cerebellum	27.8	13.9	100%	4.6	33.1 %	9.3	67%

Table S4: Individual EMG data on early-symptomatic treatment cohort

Mice	Sex	Genotype	6.5 weeks		8.5 weeks		10.5weeks		12.5weeks		15.5weeks	
			Amp M wave	**Amp H wave**	Amp M wave	**Amp H wave**	Amp M wave	**Amp H wave**	Amp M wave	**Amp H wave**	Amp M wave	**Amp H wave**
32	F	Fxn +/L3	6,4	**1,6**	7	**1,1**	5,3	**0,9**	8,4	**1**	ND	**ND**
145	F	Fxn +/L3	3,8	**0,4**	14,5	**0,4**	5,4	**1,1**	5,2	**0,3**	5,5	**1**
146	F	Fxn +/L3	7,6	**1,6**	7,3	**1,4**	7	**0,3**	10,4	**2,1**	7,7	**1**
141	F	Fxn +/L3	3	**0,2**	10,5	**0,7**	10	**0,6**	16,2	**0,7**	6,3	**0,6**
158	M	Fxn L3/L-	5,7	**0,2**	8,4	**1,9**	6,8	**0,5**	4,9	**0,3**	10,3	**0,6**
155	M	Fxn +/L3	4	**0,3**	7,7	**0,6**	11,3	**0,5**	3,4	**0,5**	15,5	**0,3**
152	M	Fxn +/L3 Pvalb +	7	**0,8**	14,5	**0,2**	10,9	**0,7**	4,9	**0,3**	2,9	**0,2**
5	F	Fxn L3/L-	5,1	**1**	13,2	**0,5**	14,8	**3,1**	13,3	**0,4**	ND	**ND**
7	F	Fxn L3/L-	11	**0,4**	10,5	**2,3**	11,1	**0,6**	8,1	**1,2**	ND	**ND**
16	M	Fxn +/L3	2,7	**0**	14,1	**1,1**	5,8	**0,4**	11,5	**0,3**	11,1	**1,2**
25	M	Fxn +/L3	3,9	**0,6**	4,5	**0,6**	6,7	**1,7**	12,2	**0,6**	5,8	**0,8**
30	F	Fxn L3/L- Pvalb +	15,4	**1**	9,6	**0,6**	15,5	**0**	5,4	**0**	ND	**ND**
34	M	Fxn L3/L- Pvalb +	8,1	**0,5**	7,6	**0,1**	4,9	**0**	9,9	**0**	ND	**ND**
151	F	Fxn L3/L- Pvalb +	6,2	**0**	5,8	**0**	5,7	**0**	2,6	**0**	16,1	**0**
143	F	Fxn L3/L- Pvalb +	17,5	**0**	10	**0,4**	11,7	**0**	10,2	**0**	6,4	**0**
153	M	Fxn L3/L- Pvalb +	10	**0**	1,5	**0**	1,6	**0**	7,1	**0**	10,6	**0**
154	M	Fxn L3/L- Pvalb +	12	**0,3**	8	**0**	7,2	**0**	7	**0**	4,2	**0**
15	M	Fxn L3/L- Pvalb +	9,8	**0,5**	11,1	**0**	12,5	**0**	12,5	**0**	6,2	**0**
37	M	Fxn L3/L- Pvalb +	10	**0**	3,9	**0**	3,6	**0**	ND	**ND**	12,7	**0**
58	F	Fxn L3/L- Pvalb +	9,7	**1,2**	12,9	**0,6**	11,6	**0,7**	ND	**ND**	5,4	**0**
142	F	Fxn L3/L- Pvalb + AAV	11,5	**0,9**	9,3	**0,5**	7,4	**0,4**	12,5	**2,9**	3	**0,7**
149	F	Fxn L3/L- Pvalb + AAV	4,8	**1,1**	8,8	**0,4**	6,1	**0,5**	10,4	**1,1**	13,3	**0,7**
156	M	Fxn L3/L- Pvalb + AAV	9,1	**0,6**	4	**0,2**	9,4	**0,9**	4,4	**0,4**	7,5	1
157	M	Fxn L3/L- Pvalb + AAV	4,9	**0,7**	5	**1,3**	3	**0,3**	5	**0,2**	6,6	0,1
159	M	Fxn L3/L- Pvalb + AAV	6,3	**0,8**	8,7	**2,2**	6,4	**0,6**	10,3	**0,7**	4,6	0,6
6	F	Fxn L3/L- Pvalb + AAV	7,4	**0,7**	10,1	**1,2**	12	**3,2**	6,4	**0,6**	ND	ND
8	F	Fxn L3/L- Pvalb + AAV	8,7	**0,6**	11	**0,9**	13,3	**0,6**	9,7	**0,8**	ND	ND
39	M	Fxn L3/L- Pvalb + AAV	2,1	**0,4**	8,2	**2,5**	4,5	**0,9**	ND	ND	4,3	1,1
62	M	Fxn L3/L- Pvalb + AAV	11,6	1,2	9	**2**	14,1	**1,2**	ND	ND	16,1	1

ND: not determined because mouse would not sleep

Table S5: Individual EMG data on post-symptomatic treatment cohort

Mice	Sex	Genotype	6.5 weeks		8.5 weeks		10.5weeks		12.5weeks		15.5weeks		19.5weeks	
			Amp M wave	**Amp H wave**	Amp M wave	**Amp H wave**	Amp M wave	**Amp H wave**	Amp M wave	**Amp H wave**	Amp M wave	**Amp H wave**	Amp M wave	**Amp H wave**
75	M	Fxn +/L3	12,2	**1,2**	6	**1,8**	6,1	**0,3**	11,9	**0,6**			4,5	**0,3**
61	F	Fxn +/L3 Pvalb+	6,9	**0,3**	2	**0,3**	4,8	**0,8**	7	**0,2**	4,1	**0,5**		
78	F	Fxn +/L3	5,8	**0,6**	16,9	**0,7**	8,9	**1,9**	4,7	**0,1**			7,1	**0,4**
83	F	Fxn +/L3	5,7	**0,2**	4,2	**0,3**	14,2	**0,7**	7,6	**0,4**			6,3	**0,5**
84	F	Fxn +/L3	8,2	**1,3**	9,7	**0,6**	5,6	**0,5**	6,2	**1,1**			5,9	**0,6**
103	F	Fxn +/L3	7,2	**2**	13,8	**0,7**	11,5	**1**	9,2	**0,8**	13,3	**1,2**	13,3	**0,5**
108	F	Fxn +/L3	15,6	**0,4**	11,2	**1,1**	4	**0**	3,7	**0,4**	5,6	**0,3**	11,7	**0,5**
112	M	Fxn +/L3	11,6	**0,4**	16,8	**1,4**	9,8	**1,9**	3,7	**1,1**	5,5	**0,1**	5,5	**0,2**
113	M	Fxn +/L3	11,8	**0,4**	5,3	**1,3**	7,4	**0,5**	2,5	**0,3**	4,9	**0,4**	5,1	**0,3**
85	F	Fxn +/L3	2,3	**0,6**	ND	**ND**	8,1	**1,2**	ND	**ND**	7	**0,7**		
143	F	Fxn +/L3	ND	**ND**	ND	**ND**	8	**1,6**	6,5	**0,8**	11,2	**0,8**		
145	M	Fxn +/L3	ND	**ND**	ND	**ND**	6,7	**1,1**	7,8	**0,7**	7,9	**0,1**		
94	F	Fxn +/L3	4,1	**0,3**	2,5	**0,1**	3	**0,5**	5,4	**0,3**	7,4	**1**		
101	M	Fxn +/L3	ND	**ND**	2,6	**0,3**	1,2	**0,3**	4,3	**0,4**	1,7	**0,3**		
109	F	Fxn +/L3 Pvalb +	5,9	**1,2**	9,2	**1,2**	2,6	**1,3**	7,7	**0,9**	5,4	**1,2**		
115	M	Fxn +/L3 Pvalb +	1,9	**0,3**	3	**0,4**	4,9	**0,2**	3,4	**0,5**	4,5	**0,9**		
12	M	Fxn +/L3 Pvalb+	4,4	**0,7**	4,2	**0,3**	4,6	**0,6**	5,2	**0,7**	5,7	**0,5**		
66	F	Fxn +/L3 Pvalb+	5,9	**0,4**	4,3	**0,7**	6,5	**0,3**	3,4	**0,9**	5,7	**0,5**		
68	F	Fxn +/L3 Pvalb+	1,2	**0,4**	6	**0,9**	6,7	**1**	3,2	**0,3**	9,3	**0,4**		
69	F	Fxn +/L3 Pvalb+	3,2	**0,4**	5,2	**0,6**	3,3	**0,3**	4,9	**0,4**	6,9	**0,8**		
71	F	Fxn +/L3 Pvalb+	3,2	**0,5**	5,3	**0,7**	4	**0,6**	4,2	**0,8**	6,6	**0,7**		
52	M	Fxn +/L3 Pvalb+	6,6	**0,3**	7,2	**0,6**	4	**0,4**	ND	**ND**	10,7	**0,8**		
53	M	Fxn +/L3 Pvalb+	4,9	**0,4**	5,7	**0,2**	6,1	**0,9**	ND	**ND**	4,3	**0,6**		
57	F	Fxn +/L3 Pvalb+	ND	**ND**	3,1	**0,3**	7,7	**1,8**	2,5	**0,4**	4,6	**0,8**		
162	F	Fxn +/L3 Pvalb+	4,7	**0,2**	13,2	**0,7**	4,1	**1,1**	3	**0,6**	8,2	**1,3**		
79	M	Fxn +/L3 Pvalb+	4,1	**0,6**	5,3	**0,3**	3	**0,3**	4,8	**0,8**	3,7	**0,3**		
76	M	Fxn L3/L- Pvalb+	7,2	**0**	3,5	**0**	6	**0**	4,7	**0**			15,1	**0**
77	M	Fxn L3/L- Pvalb+	8,5	**0**	5,4	**0**	4,9	**0**	6,6	**0**			4,4	**0**
71	F	Fxn L3/L- Pvalb+	9,9	**0,9**	15,5	**0**	14,9	**0**	3,9	**0**			11,6	**0**
74	F	Fxn L3/L- Pvalb+	5,4	**0,4**	8,9	**0**	6,6	**0**	14,8	**0**			7,1	**0**
79	F	Fxn L3/L- Pvalb+	8,2	**0,4**	7,9	**0**	4,4	**0**	9,7	**0**			8,1	**0**
117	F	Fxn L3/L- Pvalb+	6,9	**0**	4,1	**0**	7,6	**0**	11,6	**0**			5,7	**0**
118	F	Fxn L3/L- Pvalb+	2,1	**0**	2,2	**0**	5	**0**	4,9	**0**	7	**0**	2,3	**0**
124	F	Fxn L3/L- Pvalb+	6,1	**0**	4,4	**0**	7,7	**0**	14,7	**0**	7,8	**0**	9,6	**0**
161	F	Fxn L3/L- Pvalb+	3,5	**0**	3,3	**0**	7	**0**	4,8	**0**	6,4	**0**		
88	M	Fxn L3/L- Pvalb+	3,1	**0,2**	1,1	**0**	2,5	**0**	4,3	**0**	3,2	**0**		
154	M	Fxn L3/L- Pvalb+	ND	**ND**	ND	**ND**	10,6	**0**	2	**0**	4,5	**0**		
144	F	Fxn L3/L- Pvalb+	ND	**ND**	ND	**ND**	5,3	**0**	0,9	**0**	5,4	**0**		
159	M	Fxn L3/L- Pvalb+	ND	**ND**	ND	**ND**	2,3	**0**	10,1	**0**	10,7	**0**		
98	F	Fxn L3/L- Pvalb+	2,4	**0,7**	4,2	**0**	6,5	**0**	8,7	**0,1**	2,3	**0**		
107	F	Fxn L3/L- Pvalb+	2,9	**0,1**	3,2	**0**	1,2	**0**	3,1	**0**	5,1	**0**		
122	F	Fxn L3/L- Pvalb+	1,9	**0,2**	7,8	**0**	1,8	**0**	11,4	**0**	5,2	**0**		
124	M	Fxn L3/L- Pvalb+	4	**0**	6,1	**0**	2,8	**0**	7,2	**0,1**	3,3	**0**		
184	F	Fxn L3/L- Pvalb+	10,2	**0,5**	3,1	**0**	5,7	**0**	5,2	**0**	9	**0**		
188	M	Fxn L3/L- Pvalb+	6,7	**0**	5,9	**0**	3,4	**0**	5,8	**0**	8,6	**0**		
179	M	Fxn L3/L- Pvalb+	6,1	**0,7**	5,5	**0**	2	**0**	4,9	**0**	5,2	**0**		
160	F	Fxn L3/L- Pvalb+	1,4	**0**	2,2	**0**	10,4	**0**	7,3	**0**	6,5	**0**		
163	M	Fxn L3/L- Pvalb+	4	**0**	7,6	**0**	9,5	**0**	3,5	**0**	6,1	**0**		
14	M	Fxn L3/L- Pvalb+	3,7	**0**	6	**0**	4,8	**0**	7,2	**0**	6,5	**0**		
183	F	Fxn L3/L- Pvalb+	4,1	**0,3**	2,6	**0**	7,5	**0**	4,9	**0**	3,7	**0**		
73	F	Fxn L3/L- Pvalb+	4,2	**0,5**	14	**0**	6,3	**0**	4,9	**0**	11,4	**0**		
77	M	Fxn L3/L- Pvalb+	ND	**ND**	2	**0**	3,8	**0**	11,2	**0**	3	**0**		
49	F	Fxn L3/L- Pvalb+	4	**0**	10,2	**0**	10,4	**0**	10,7	**0**	5,2	**0**		
58	F	Fxn L3/L- Pvalb+	4,1	**0**	4,7	**0**	8,4	**0**	11	**0**	2,9	**0**		
64	M	Fxn L3/L- Pvalb+	4,5	**0**	4,5	**0**	8,3	**0**	1,2	**0**	5	**0**		
82	F	Fxn L3/L- Pvalb+ AAV	9,5	**0,9**	5,1	**1,4**	6,1	**0,5**	12	**1**			9,6	**0,9**
85	F	Fxn L3/L- Pvalb+ AAV	2,5	**1,1**	5,5	**0,6**	6,7	**0,6**	2,9	**0,9**			4,2	**0,5**
88	M	Fxn L3/L- Pvalb+ AAV	7,1	**0**	7,1	**0,2**	7,2	**1,5**	6,4	**0,3**			6,7	**0,7**
105	F	Fxn L3/L- Pvalb+ AAV	3,3	**0**	5,9	**0,6**	5,7	**0**	7,4	**0,2**	6,6	**1**	7,7	**0,4**
106	F	Fxn L3/L- Pvalb+ AAV	11,1	**0,5**	2,7	**0,1**	2,5	**1**	6,2	**0,5**	8,7	**0,7**	6,3	**0,3**
110	F	Fxn L3/L- Pvalb+ AAV	8	**0,2**	9,9	**1,3**	6,3	**0,8**	1,4	**0,2**	6,3	**0,3**	5,8	**0,6**
125	M	Fxn L3/L- Pvalb+ AAV	9,9	**1,2**	5	**0,4**	8,4	**0,8**	10,9	**0,2**	11,7	**0,4**	4,4	**0,3**
127	M	Fxn L3/L- Pvalb+ AAV	4,2	**0**	10,5	**0,5**	5,8	**0,3**	7,2	**0,7**	4,6	**0,4**	8,8	**0,3**
128	M	Fxn L3/L- Pvalb+ AAV	2,8	**0**	7,4	**0,3**	11,6	**0,3**	7,9	**0,2**	2,7	**0,7**	5,1	**0,4**
137	F	Fxn L3/L- Pvalb+ AAV	5,3	**0**	4,8	**0**	6,7	**0**	5,4	**0**	10,6	**0**		
160	F	Fxn L3/L- Pvalb+ AAV	9,9	**0**	4,6	**0,5**	9,8	**0,8**	8,2	**0,4**	8,9	**1**		
149	M	Fxn L3/L- Pvalb+ AAV	ND	**ND**	ND	**ND**	5,9	**0,9**	6,4	**0,8**	14,1	**0,4**		
156	F	Fxn L3/L- Pvalb+ AAV	ND	**ND**	ND	**ND**	4,1	**1**	2	**0,6**	4,1	**0,1**		
160	M	Fxn L3/L- Pvalb+ AAV	ND	**ND**	ND	**ND**	2	**0**	3,6	**0,5**	8,6	**0,4**		
95	F	Fxn L3/L- Pvalb+ AAV	8	**0,3**	14,5	**0,5**	3,7	**0,3**	6,8	**0,2**	6,8	**0,4**		
96	M	Fxn L3/L- Pvalb+ AAV	2,7	**0**	10,7	**0,4**	4	**0,1**	7,9	**0,3**	3,1	**0,2**		
100	M	Fxn L3/L- Pvalb+ AAV	2,3	**0**	5,5	**0,2**	1,6	**0,5**	ND	**ND**	6,4	**0,6**		
106	F	Fxn L3/L- Pvalb+ AAV	ND	**ND**	10,4	**1,6**	5,8	**0,2**	7,3	**0**	ND	**ND**		
111	F	Fxn L3/L- Pvalb+ AAV	12,5	**0,5**	4,5	**0,3**	2,3	**0,2**	8,4	**0,3**	8,2	**0,8**		
118	M	Fxn L3/L- Pvalb+ AAV	6,3	**0,1**	9,7	**0,1**	8,7	**0,2**	5,6	**0,8**	3,7	**0,8**		
190	M	Fxn L3/L- Pvalb+ AAV	5,2	**0,3**	7,1	**0,7**	2,8	**0,4**	2,8	**0,2**	4,7	**0,3**		
178	F	Fxn L3/L- Pvalb+ AAV	2,5	**0,1**	4,4	**0,4**	5,7	**0,9**	2,2	**0,3**	ND	**ND**		
180	M	Fxn L3/L- Pvalb+ AAV	2,4	**0**	2,9	**0,4**	4,4	**0,2**	3,2	**0,3**	2,9	**0,3**		
174	F	Fxn L3/L- Pvalb+ AAV	10	**0**	5,4	**0,3**	6,5	**0,7**	3,2	**0,3**	5,1	**0,4**		
161	F	Fxn L3/L- Pvalb+ AAV	4,4	**0**	5,1	**0,3**	3,4	**0,6**	7,1	**0,5**	2,2	**0,8**		
164	M	Fxn L3/L- Pvalb+ AAV	0,5	**0**	7,2	**0,2**	3,3	**0,4**	3,7	**0,5**	5,1	**0,4**		
165	M	Fxn L3/L- Pvalb+ AAV	4,8	**0**	3,2	**0,3**	ND		7,4	**0,5**	4,8	**0,5**		
5	F	Fxn L3/L- Pvalb+ AAV	5,1	**0**	3,6	**1,3**	8,7	**0,4**	2,8	**0,6**	3,2	**0,4**		
181	F	Fxn L3/L- Pvalb+ AAV	3,2	**0,4**	5,8	**0,2**	2,7	**0,5**	4,5	**0,2**	3,2	**0,7**		
60	F	Fxn L3/L- Pvalb+ AAV	5,4	**0**	12	**0,7**	3,8	**0,6**	7,5	**0,4**	7,8	**0,5**		
50	M	Fxn L3/L- Pvalb+ AAV	5,4	**0**	9,3	**0,9**	7,6	**1**	3,3	**0,4**	5,6	**0,4**		
51	M	Fxn L3/L- Pvalb+ AAV	5,6	**0**	8,7	**0,6**	2	**0,4**	8,2	**0,5**	1,2	**0,5**		

ND: not determined because mouse would not sleep

Hatched: not measured

Table S6: Stereotaxic coordinates for injection

	Stereotaxic coordinates from the Bregma		
Injection site	Antero-posterior (AP)	Medio-lateral (ML)	Dorso-ventral (DV)
Striatum 1st injection site	+0.5mm	+2.2mm	-3.3mm
Striatum 2nd injection site	+0.5mm	-2.2mm	-3.3mm
White matter of the cerebellum	-6.48mm	0mm	-2.5mm

Table S7: List of antibodies used

	Antibodies	Application	Used Concentration	Species	Reference	Company
primary antibodies	Anti HA-TAG	IF	1:250	rabbit	C29F4	Cell Signalling Technology
	Anti Calbindin	IF	1:500	rabbit	CB38-A	Swant swiss Antibodies
	Anti MBP	IF	1:200	mouse	SMI-94	Calbiochem
	Anti NF-200	IF	1:200	chicken	ab4680	Abcam
	Anti TfR1	IF	1:500	rabbit	ab84036	Abcam
	Anti GFAP	IF	1:500	guinea pig	173004	Synaptic systems
	Anti frataxin polyclonal	WB	1:1,000	rabbit	R1250	IGBMC
	Anti frataxin monoclonal	WB	1:1,000	mouse	4F9	IGBMC
	Anti GAPDH	WB	1:20,000	mouse		IGBMC
	Anti lipoic acid	WB	1:5,000	rabbit	437695	Calbiochem
secondary antibodies	Biotinylated Anti-Rabbit IgG	IF	1:500	goat	BA-1000	Vector laboratories
	Anti-Rabbit IgG 488	IF	1:1,000	goat	A-11008	Life Technologies
	Anti-Mouse IgG 594	IF	1:1,000	goat	A-11005	Life Technologies
	Anti-Chicken IgG 488	IF	1:1,000	donkey	703-545-155	Interchim SA
	Anti-Rabbit IgG 594	IF	1:1,000	goat	A-11037	Life Technologies
	Anti-Guinea pig IgG 488	IF	1:1,000	goat	A-11073	Life Technologies
	Peroxydase Anti-mouse IgG	WB	1:5,000	goat	115-035-046	Jackson ImmunoResearch
	Peroxydase Anti-rabbit IgG	WB	1:5,000	goat	115-035-146	Jackson ImmunoResearch

Supplemental results:

Validation of expression of Pvalb-Cre transgene

As endogenous parvalbumin expression is not restricted to the proprioceptive sensory neurons (http://mouse.brain-map.org/gene/show/19056) and behavioral analysis clearly showed that the *Pvalb* cKO develop a cerebellar/cortical phenotype, in addition to the sensory neuropathy, we evaluated the tissue specificity and the temporal expression of the *Pvalb-Cre* transgene in *Pvalb*$^{tm1(Cre)Arbr/J}$ transgenics using the ROSA26/LacZ reporter stain. LacZ expression was analyzed at several time points: E14.5, E17.5, P8, P40 and 21.5 weeks. While no LacZ staining was detected at E14.5, positive LacZ in DRG was observed at E17.5 (Figure S2A-B). By p8, approximately 15% of DRG neurons were positive for LacZ (Figure S2C) and in a similar manner in cervical, thoracic and lumbar DRG (Figure S2D-F respectively). Therefore, the *Pvalb*-Cre expression in the DRG starts between E14 and E17.5 as described previously[21] and is fully expressed by p8. No LacZ staining was detect in peripheral nerves. In the central nervous sytem, no LacZ staining was seen at p8. At p40, LacZ staining was specifically detected in Purkinje cells (Figure S2G-H). At 21.5 weeks, positive LacZ staining was detected in the interposed nucleus, a deep central grey nucleus (Figure S2G), in interneurons in the cortex (Figure S2I), few positive cells in the hippocampus (Figure S2J), strong staining in the majority of the red nucleus (Figure S2K), superior olivary complex and the pontine grey (Figure S2L). No LacZ positive staining was detected in any peripheral tissues at any time point analyzed.

Quantification of frataxin expression

WT animals are +/L3 for the frataxin allele, meaning that they express 100% of frataxin in all tissues. *Pvalb* cKO animals are heterozygous for the frataxin locus (*Fxn*$^{L3/L-}$) in all cells except the cells expressing the Cre recombinase which are homozygous for the depletion (*Fxn*$^{L-/L-}$). Thus, we expect 50% of frataxin expression in the heterozygous cells (*Fxn*$^{L3/L-}$) and no expression in the knocked-out cells (*Fxn*$^{L-/L-}$). By measuring frataxin levels in the heterogeneous tissues of interest (DRG and cerebellum), we can determine the amount/percentage of frataxin expressed by the Cre positive cells (Expected values in heterozygotes animals – experimental values in *Pvalb* cKO animals).

Example for calculation in lumbar DRG (other tissues are in table S2 and S3):

25.6ng of frataxin per mg of protein were experimentally measured in WT lumbar DRG. Therefore, we expect 12.8 ng of frataxin per mg of protein in heterozygous lumbar DRG without *Pvalb*-Cre expression (considered as 100%). Upon *Pvalb*-Cre expression 7.0 ng of frataxin were measured (~55%). Thus, the Cre positive cells express 5.8 ng of frataxin per mg of protein (12.8 – 7.0 = 5.8), approximately 45%.