

AAV1.NT-3 gene therapy for X-linked Charcot-Marie-Tooth Neuropathy type 1

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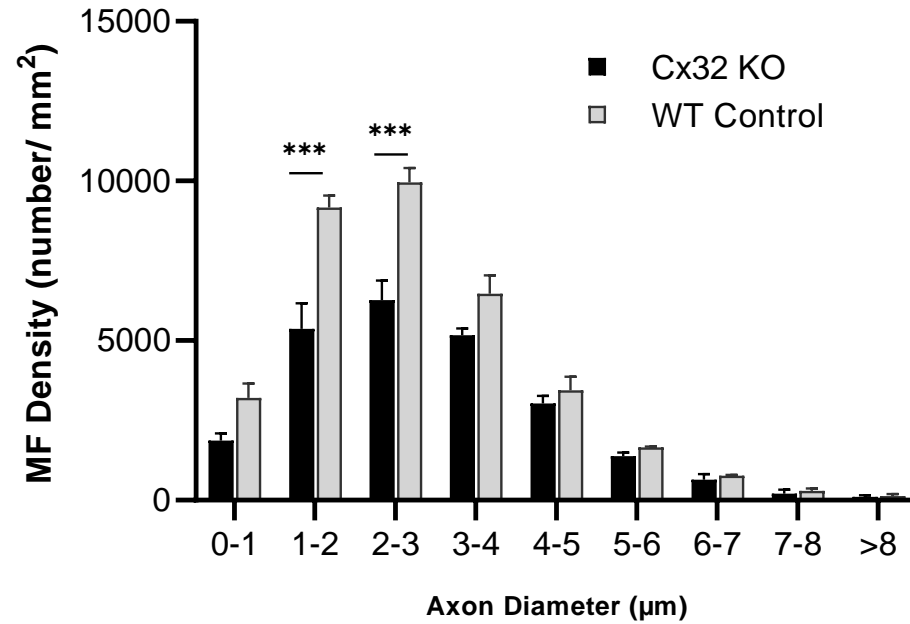
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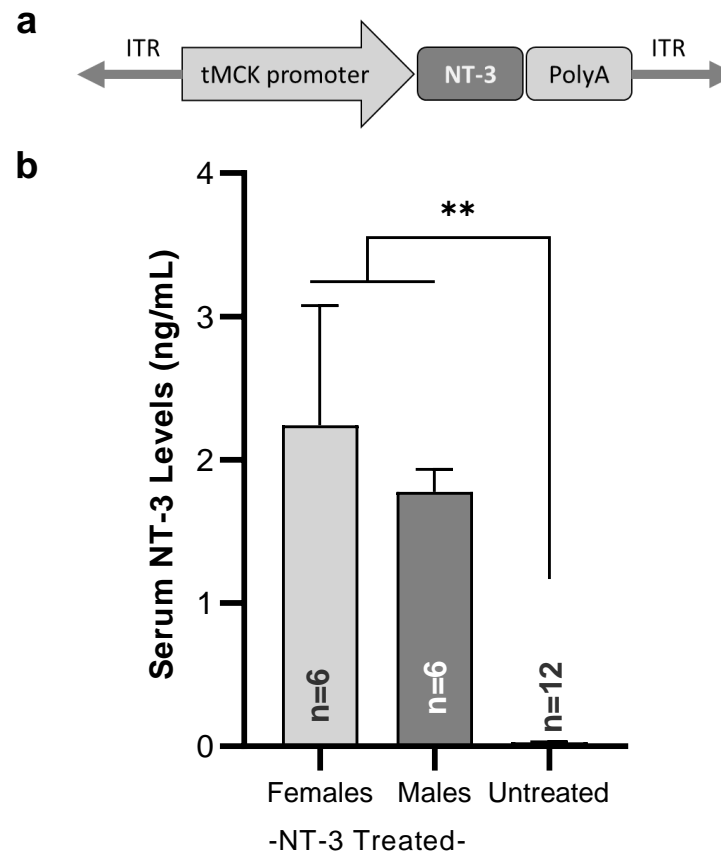
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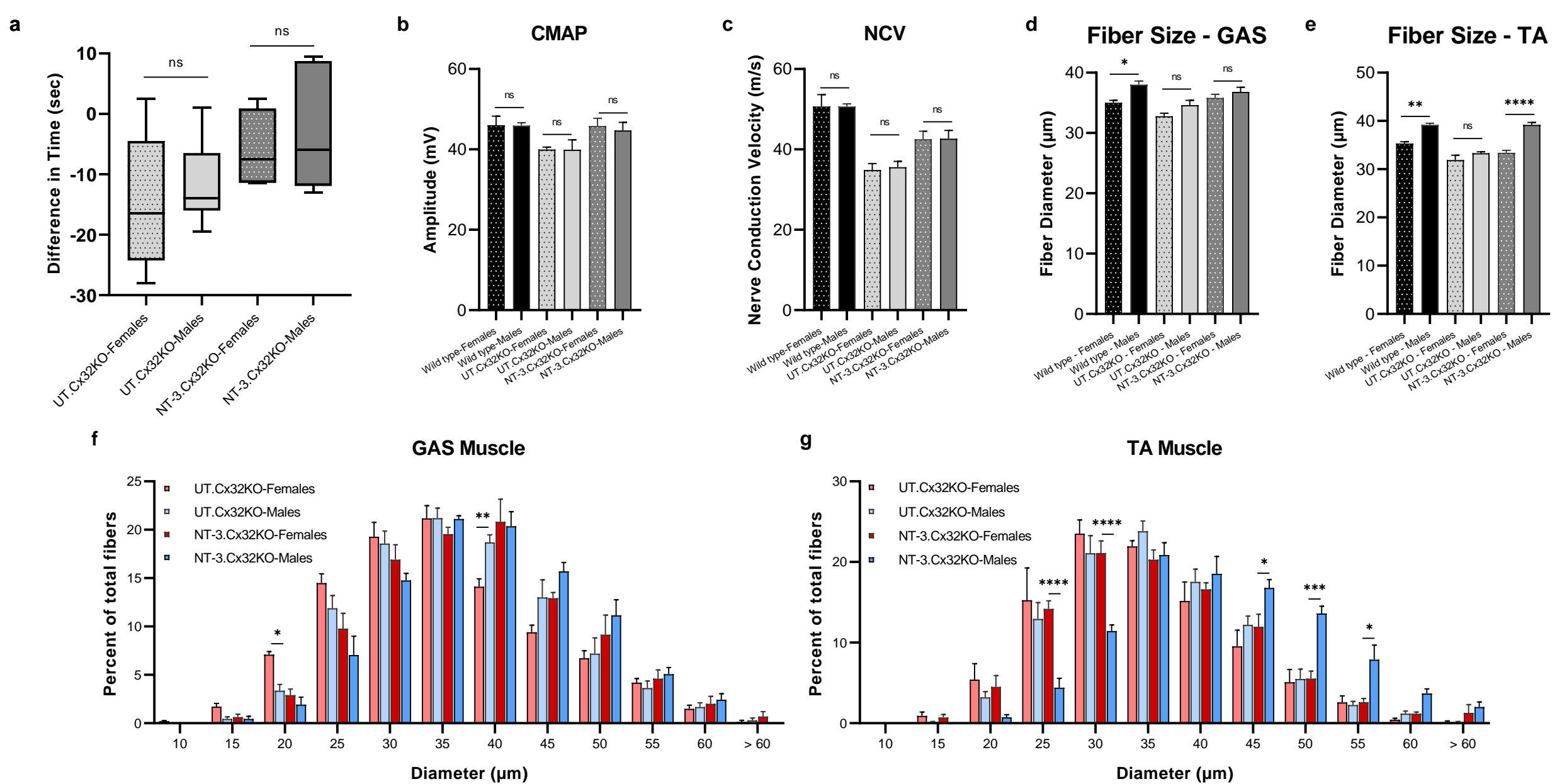
Supplementary Data



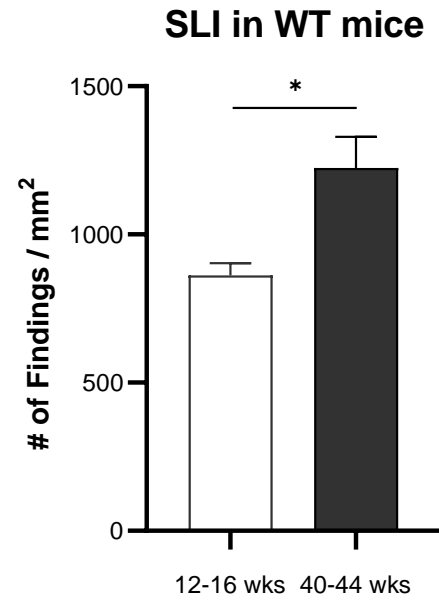
Supplementary Figure 1. Myelinated fiber-axon size distribution in the regenerating sciatic nerves, 5 mm distal to crush site from Cx32 KO and wild type control mice at 2 months post-crush. Small myelinated fiber subpopulation with axon size within 1-2 µm and 2-3 in diameter range was significantly less the Cx32 KO regenerating nerves. The total number of myelinated fiber density (Cx32 KO: 24026.8 ± 1548.1 vs. WT: 35095.1 ± 1283.3 ; $p < 0.0001$) is markedly reduced in Cx32 KO compared to WT regenerating nerves. Error bars represent \pm SEM; *** $p \leq 0.001$, **** $p \leq 0.0001$, $n = 3$ per group; Bonferroni's multiple comparisons test.



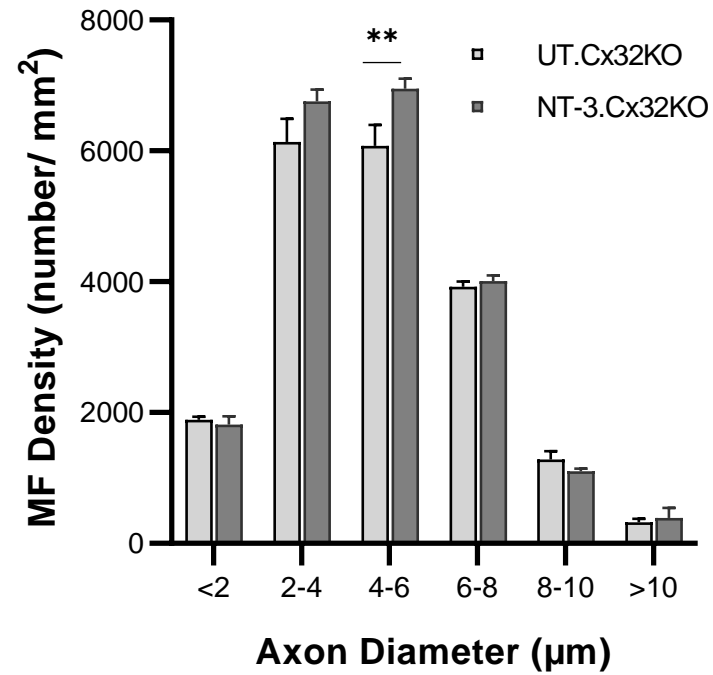
Supplementary Figure 2. scAAV1.tMCK.NT-3 vector and NT-3 serum levels. (a) NT-3 expression is driven by a tMCK in the scAAV vector backbone. The diagram shows the cassette composed of a tMCK enhancer/ promoter region (714 bp), the full-length NT-3 cDNA (774 bp) and the SV40 polyA tail (211 bp). (b) Cx32 KO mice were injected with 1×10^{11} vg of scAAV1.tMCK.NT-3 into the right gastrocnemius muscles or Ringer's lactate as control. Serum samples were obtained from treated and untreated Cx32 KO mice via cardiac puncture at six months post-treatment/endpoint, and NT-3 levels were determined by using enzyme-linked immunosorbent assay; shown separately in females and males. Error bars are \pm SEM; n=12 (NT-3 treated), n=12 (UT), **P< 0.01; unpaired t test.



Supplementary Figure 3. The graphs showing analyses on rotarod (a), electrophysiology (b,c), muscle fiber size (d,e) and muscle fiber size distribution (f,g) separated by sex. Error bars represent \pm SEM; ns $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$, Tukey's multiple comparisons test.



Supplementary Figure 4. Histopathological quantitative analysis of Schmidt-Lanterman incisures (SLIs) performed on sciatic nerves of young (12-16 wks) and old (40-44 wks) wild type mice. Old cohort shows an increase in the number of SLI compared to young mice. Error bars represent \pm SEM; * $p=0.031$, $n=4$ per group; unpaired t test.



Supplementary Figure 5. Myelinated fiber distribution in the sciatic nerves from NT-3 treated and untreated Cx32 mice. An increase in the subpopulation of axons 2-6 µm in diameter was seen with treatment. Error bars represent ± SEM; **p=0.0046, n=6 per group; Bonferroni's multiple comparisons test.

Supplementary Table 1 Average G ratios of the sciatic nerves from Cx32KO mice

Cohorts	<i>n</i>	Number of measurements	Mean \pm SEM
Untreated	5	2858	0.761 \pm 0.001
NT-3 Treated	4	2167	0.663 \pm 0.002****
Untreated-Female	3	1503	0.752 \pm 0.002
NT-3-Female	2	1040	0.658 \pm 0.002****
Untreated-Male	2	1355	0.771 \pm 0.002
NT-3-Male	2	1127	0.667 \pm 0.002****

**** $p \leq 0.0001$ between NT-3 treated and untreated, unpaired t test

Supplementary Table 2 Myelinated fiber density (number/mm²) in the sciatic nerves from Cx32KO and wild type mice

Cohorts	<i>n</i>	Mean ± SEM
Untreated	6	19,636 ± 609
NT-3	6	21,030 ± 197
WT (age-matched)	4	20,249 ± 356
WT (young/12-16 wks)	3	21,071 ± 267

Supplementary Table 3 Mean muscle fiber diameter in gastrocnemius and tibialis anterior samples from Cx32 KO and WT mice

Fiber Type	Gastrocnemius					
	Wild type (n=8)		Untreated (n=8)		NT-3 Treated (n=8)	
	Number	Diameter (μm)	Number	Diameter (μm)	Number	Diameter (μm)
STO	101.9 \pm 6.8	30.0 \pm 0.7 ^{††}	79.1 \pm 6.1	26.4 \pm 0.6	75.9 \pm 6.2	29.3 \pm 0.8*
FTO	48.5 \pm 2.6	34.1 \pm 0.7 ^{††}	91.3 \pm 4.5	30.7 \pm 0.6	77.5 \pm 6.2	34.0 \pm 1.0**
FTG	170.5 \pm 6.3	41.2 \pm 1.0 [†]	178.8 \pm 8.8	38.5 \pm 0.6	182.6 \pm 8.6	40.2 \pm 0.6
All fiber	320.9 \pm 9.7	36.5 \pm 0.8 [†]	349.1 \pm 6.0	33.7 \pm 0.6	336.0 \pm 10.4	36.3 \pm 0.5*

Fiber Type	Tibialis anterior					
	Wild type (n=8)		Untreated (n=8)		NT-3 Treated (n=8)	
	Number	Diameter (μm)	Number	Diameter (μm)	Number	Diameter (μm)
STO	91.6 \pm 10.1	30.5 \pm 1.0 ^{††}	90.6 \pm 7.2	25.7 \pm 0.6	88.6 \pm 11.0	27.9 \pm 1.2
FTO	66.3 \pm 3.5	36.3 \pm 1.1 ^{††}	95.6 \pm 7.5	32.1 \pm 0.9	92.5 \pm 7.8	35.2 \pm 1.2
FTG	162.5 \pm 5.7	41.5 \pm 0.7 ^{††}	167.5 \pm 6.3	36.6 \pm 0.7	160.4 \pm 9.6	41.2 \pm 1.0**
All fiber	320.4 \pm 13.6	37.2 \pm 0.9 ^{††}	353.8 \pm 14.7	32.6 \pm 0.8	341.5 \pm 19.2	36.3 \pm 1.2*

Data represented as mean \pm SEM

*p \leq 0.05, **p \leq 0.01 between NT-3 treated and untreated group at the end point

[†]p \leq 0.05, ^{††}p \leq 0.01, ^{†††}p \leq 0.001 between WT and untreated group at the end point, Tukey's multiple comparisons test.