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Supplemental information

Gene replacement therapy in two Golgi-retained CMT1X mutants before and after the onset of demyelinating neuropathy

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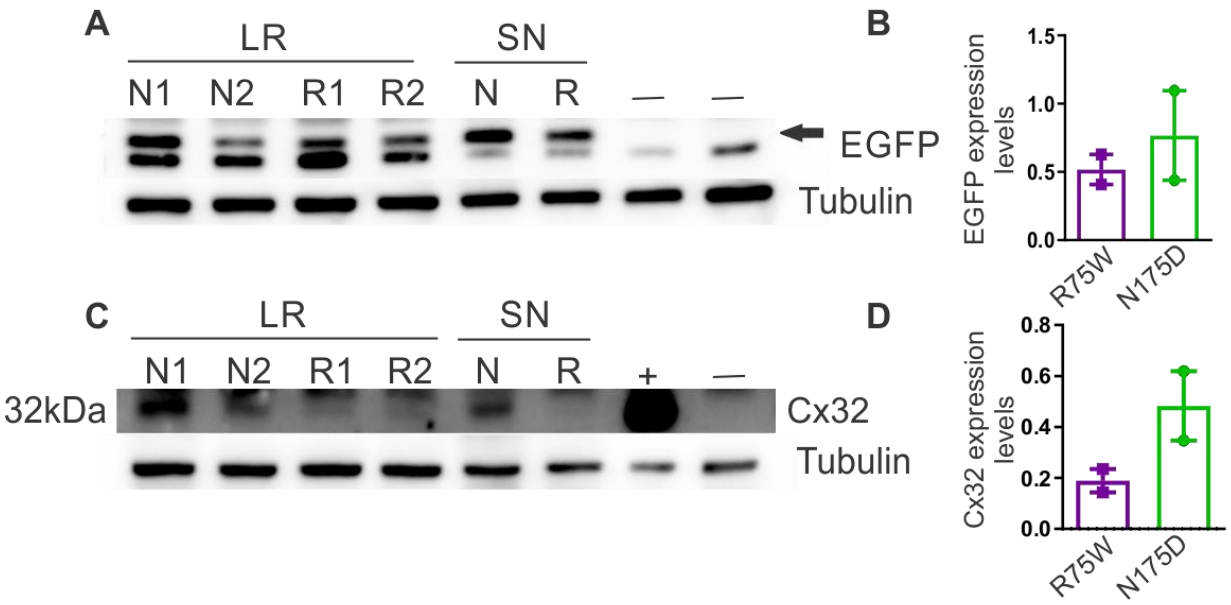

Figure S1: EGFP and Cx32 expression levels in R75W/*Gjb1*-null and N175D/*Gjb1*-null mice.

A: Immunoblot analysis of lumbar spinal root (LR) and sciatic nerve (SN) lysates from N175D/*Gjb1*-null (N) or R75W/*Gjb1*-null (R) mice, as indicated, confirms the presence of the specific EGFP band as opposed to the corresponding WT and *Gjb1*-null samples run as negative controls (−). β-Tubulin serves as a loading control. **B:** Quantification of EGFP expression levels normalized for tubulin levels in lumbar roots from R75W/*Gjb1*-null and N175D/*Gjb1*-null mice shows a trend for higher EGFP expression levels in the N175D/*Gjb1*-null but not significant. **C:** Immunoblot analysis of Cx32 levels in LR and SN of R75W/*Gjb1*-null and N175D/*Gjb1*-null tissues confirms mutant Cx32 expression in both CMT1X mouse models but at lower levels compared to the WT positive control (+), while Cx32 is absent from *Gjb1*-null tissues used here as a negative control (-). **D:** Quantification of Cx32 expression levels normalized for tubulin levels in lumbar roots from R75W/*Gjb1*-null and N175D/*Gjb1*-null mice shows higher expression levels of Cx32 in the N175D/*Gjb1*-null mice although with no statistical difference. Values represent mean±SEM.

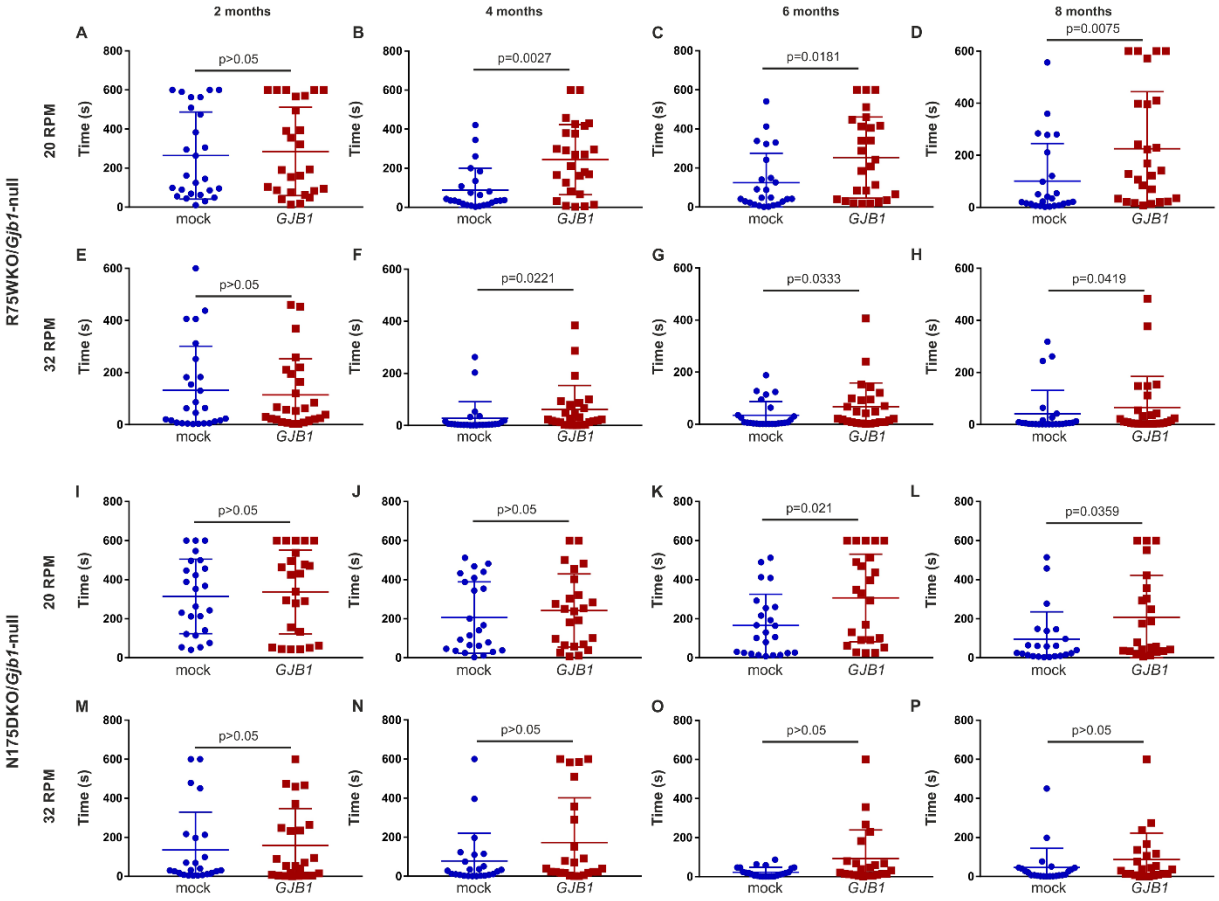

Figure S2: Rotarod analysis of pre-onset treated AAV9.*Mpz-GJB1*-injected compared with mock-injected R75W/*Gjb1*-null and N175D/*Gjb1*-null mice. Rotarod results show improved motor performance in pre-onset treated R75W/*Gjb1*-null mice at 2, 4, and 6 months post-injection (at 4, 6, and 8 months of age, as indicated), at both speeds examined (**A-H**). Motor performance of pre-onset treated N175D/*Gjb1*-null mice was improved only at the lowest speed examined and only after 4 and 6 months following treatment (**I-L**). No differences were observed between treated and mock-treated mice at the high speed at all timepoints (**M-P**).

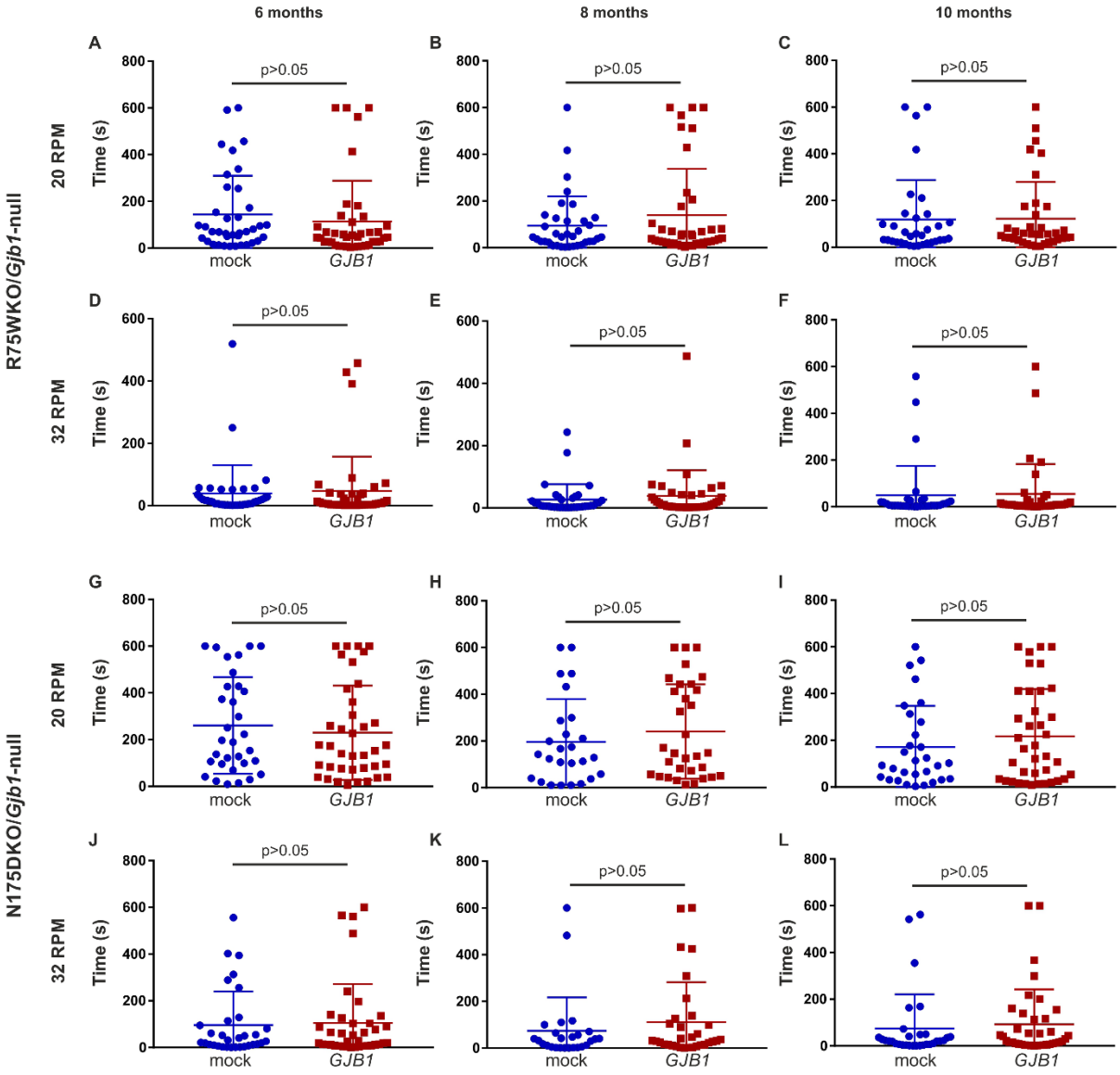

Figure S3: Rotarod analysis of post-onset treated AAV9.*Mpz-GJB1*-injected compared with mock injected R75W/*Gjb1*-null and N175D/*Gjb1*-null mice. Rotarod results show no differences between the mock-treated and treated R75W/*Gjb1*-null mice at 2 and 4 months post-injection (at 8 and 10 months of age, as indicated), at both speeds examined (**A-F**) when treated after the onset of the neuropathy. Likewise, the motor performance of N175D/*Gjb1*-null post-onset treated mice was also not improved at both speeds examined at all timepoints (**G-L**).

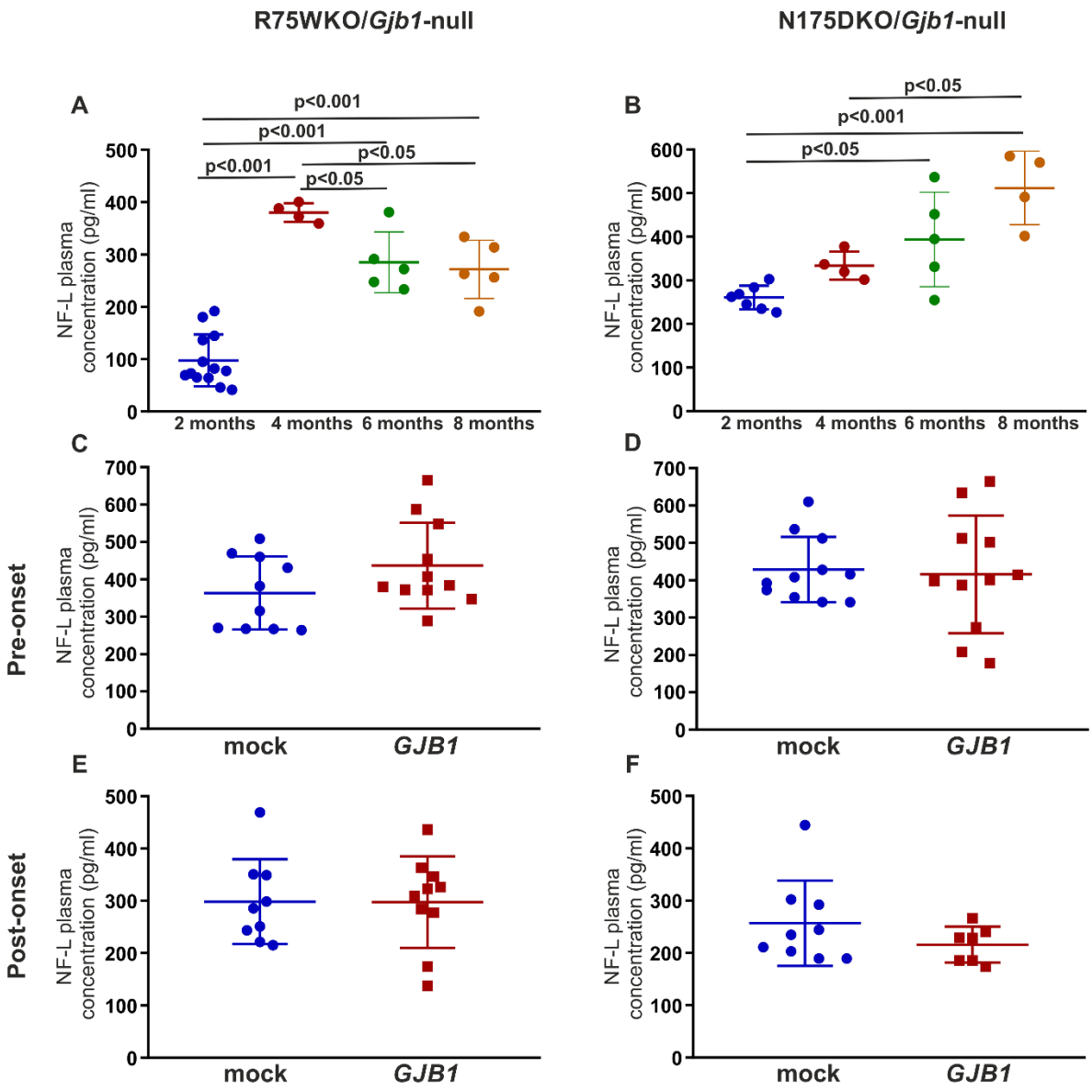

Figure S4: NF-L plasma concentrations in untreated R75W/*Gjb1*-null and N175D/*Gjb1*-null mice and in AAV9.*Mpz-GJB1* pre- and post-onset treated R75W/*Gjb1*-null and N175D/*Gjb1*-null mice. Analysis of NF-L blood levels in R75W/*Gjb1*-null mice showed increase in the NF-L concentration at 4 months of age followed by a significant decrease at 6 and 8 months of age. Despite the decrease, the NF-L concentration remained higher at 6 and 8 months of age when compared to 2 months (**A**). In the N175D/*Gjb1*-null model NF-L levels significantly increased over time from 2 to 8 months of age with the higher values observed at 8 months of age (**B**). NF-

L were similar between the mock-treated and treated mice for both genotypes when treated either before (**C-D; 8 months of age**) or after the onset of the neuropathy (**E-F; 10 months of age**). Statistical analysis was performed using Mann-Whitney or one-way ANOVA.

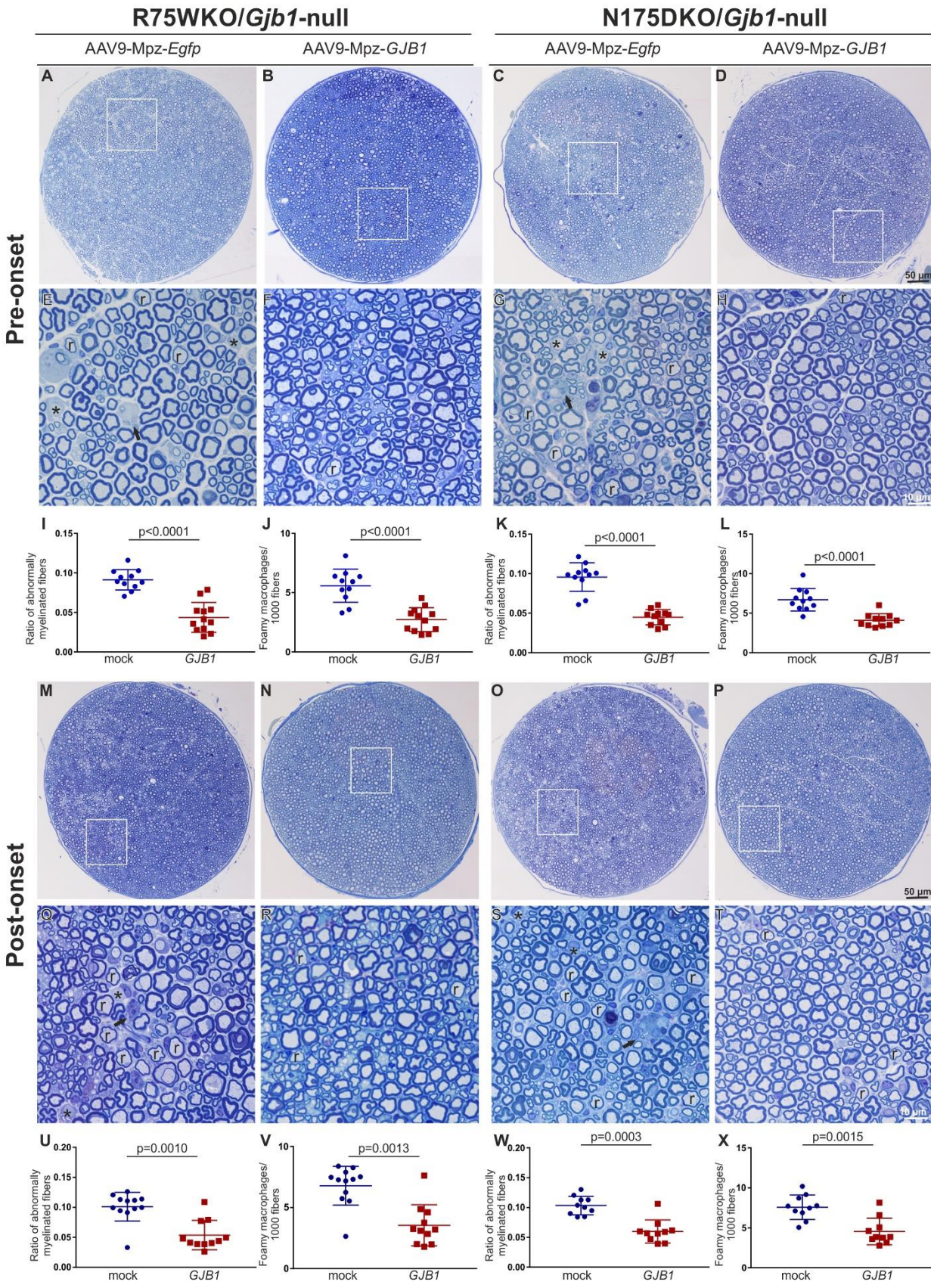
R75WKO/Gjb1-null
N175DKO/Gjb1-null
AAV9-Mpz-Egfp
AAV9-Mpz-GJB1
AAV9-Mpz-Egfp
AAV9-Mpz-GJB1
Pre-onset
Post-onset
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
S
T
U
V
W
X
50 μm
10 μm
Ratio of abnormally myelinated fibers
Foamy macrophages/ 1000 fibers
p<0.0001
p<0.0001
p<0.0001
p<0.0001
p=0.0010
p=0.0013
p=0.0003
p=0.0015
mock
GJB1

Figure S5: Morphological analysis of sciatic nerves of R75W/ and N175D/*Gjb1*-null mice following pre- and post-onset intrathecal delivery of the AAV9-*Mpz.GJB1* or mock (AAV9-*Mpz.EGFP*) vector. Representative images of semithin sections of pre-onset treated R75W/*Gjb1*-null mid-sciatic nerves at low (**A**-**B**) and higher (**E-F**) magnification, with morphometric analysis results (**I-J**). Representative images of semithin sections of pre-onset N175D/*Gjb1*-null mid-sciatic nerves at low (**C**-**D**) and higher (**G-H**) magnification, with morphometric analysis results (**K-L**). Semithin sections of mid sciatic nerves of post-onset treated R75W/*Gjb1*-null at low (**M-N**) and higher (**Q**-**R**) magnification and corresponding morphometric analysis results (**U-V**). Semithin sections of mid sciatic nerves of post-onset treated N175D/*Gjb1*-null at low (**O-P**) and higher (**S-T**) magnification and corresponding morphometric analysis results (**W-X**), from mock and full vector treated mice as indicated, at 10 months of age (4 months after treatment). Compared to mock-treated littermates, AAV9-*Mpz.GJB1* injected mice show improved myelination in mid-sciatic nerves with fewer demyelinated (*) and remyelinated (r) fibers. Quantification of the ratios of abnormally myelinated fibers confirms significant improvement in the numbers of abnormally myelinated fibers (**I, K, U, W**), as well as reduction in the numbers of foamy macrophages (**J, L, V, X**) in the treated compared with mock treated littermates. Statistical analysis was performed using Mann-Whitney test.

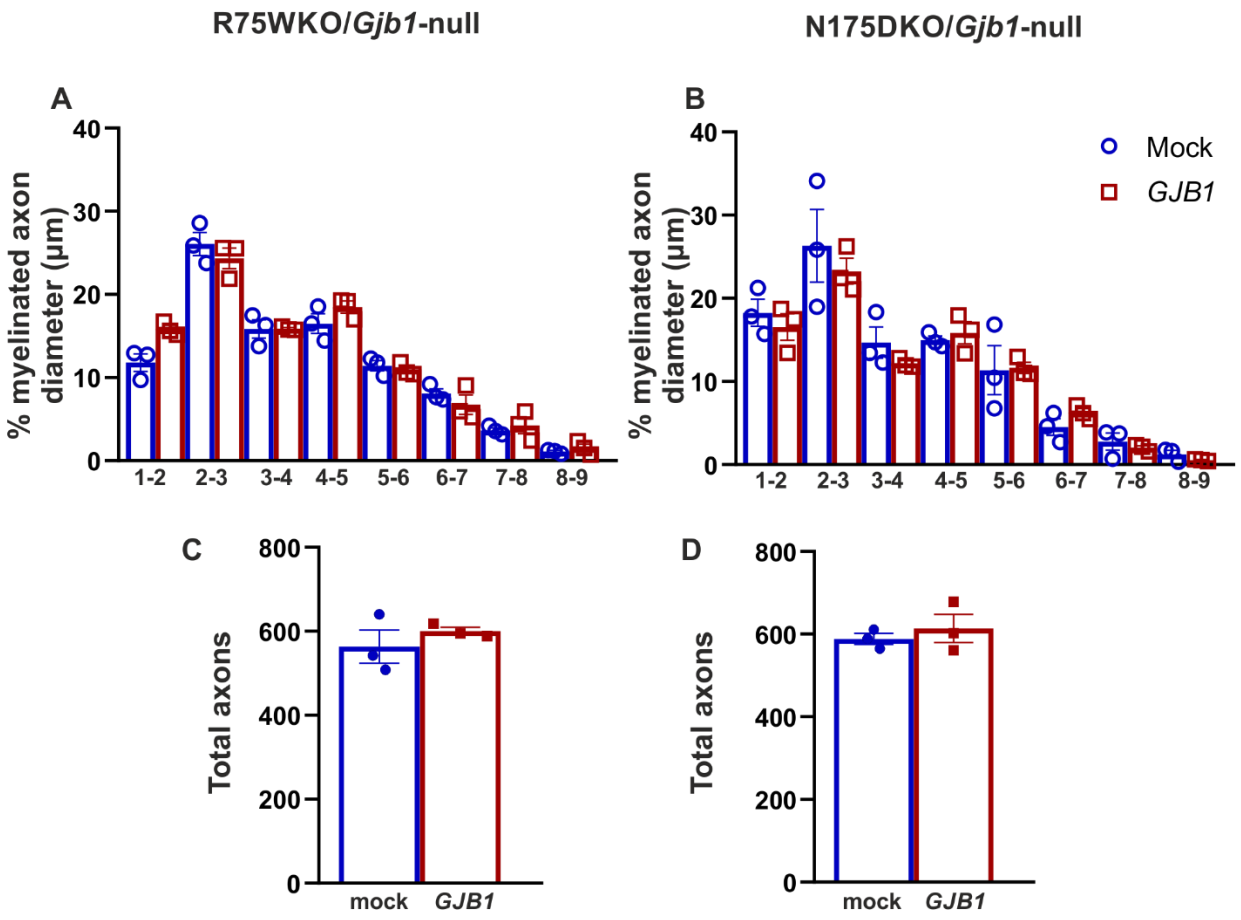

Figure S6: Axonal profiling of R75W/*Gjb1*-null and N175D/*Gjb1*-null mice following post-onset intrathecal delivery of the AAV9-*Mpz.GJB1* (full) or mock (AAV9-*Mpz.EGFP*) vector. Quantitative analysis of axon diameters in post-onset treated R75W/*Gjb1*-null **(A)** and N175D/*Gjb1*-null mice **(B)** femoral nerves following intrathecal delivery of the AAV9-*Mpz.GJB1* (full) or AAV9-*Mpz.Egfp (mock)* vector. Axon diameter measurements did not show any statistical difference between mice injected with AAV9-*Mpz.GJB1* (n=3 mice) or AAV9-*Mpz.Egfp* (n=3 mice) in either of the two transgenic lines. The total number of axons is not significantly changed in nerves from treated as opposed to mock treated mice (**C-D**).