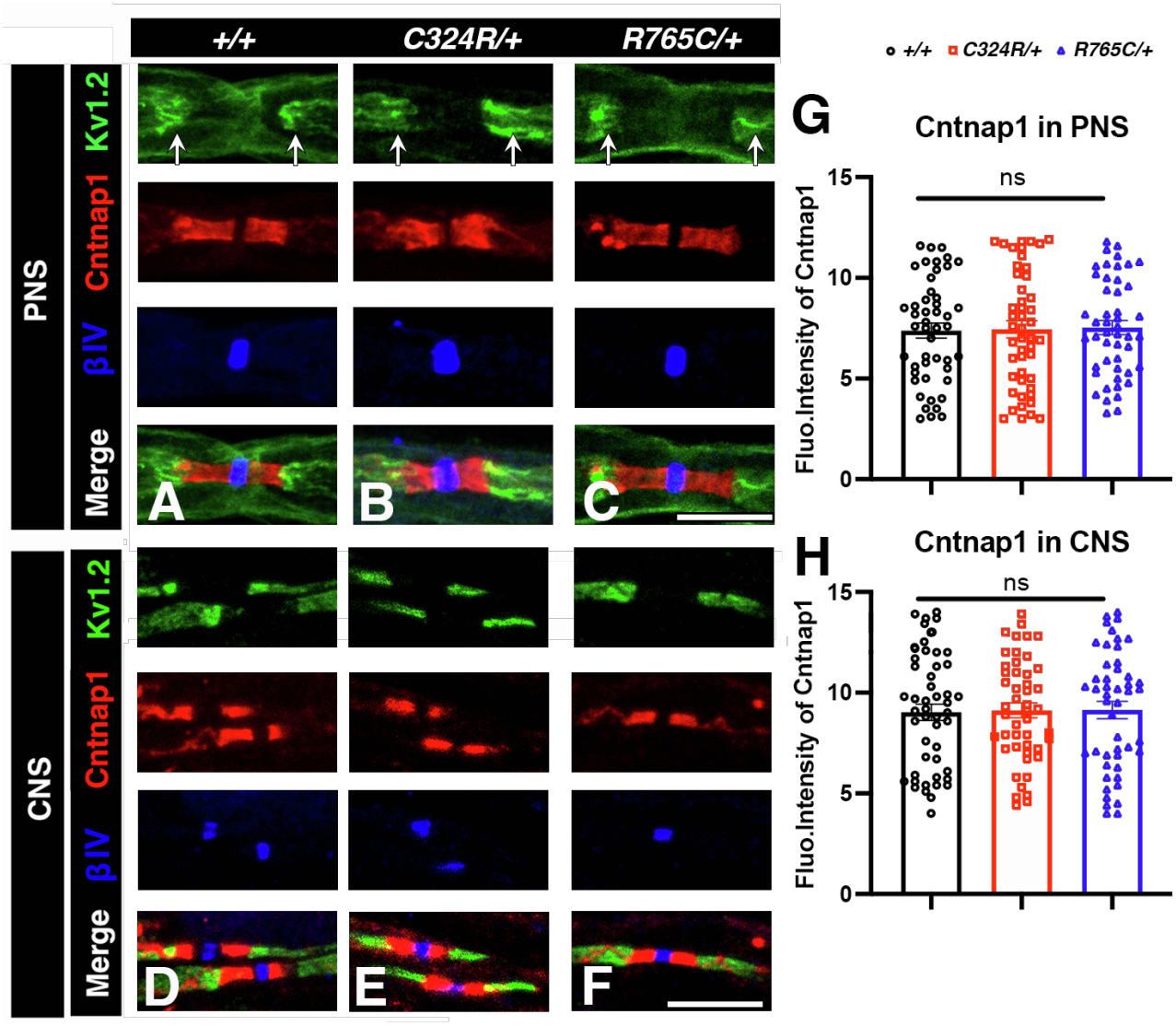
Cell Reports, Volume *42*

Supplemental information

Mouse models of human *CNTNAP1*-associated congenital hypomyelinating neuropathy and genetic restoration of murine neurological deficits

Cheng Chang, Lacey B. Sell, Qian Shi, and Manzoor A. Bhat

Supplementary Figure 1



Supplementary Figure 1. Cntnap1 heterozygous mice (*Cntnap1*$^{C324R/+}$ and *Cntnap1*$^{R765C/+}$) do not exhibit differences in intensity and distribution of Cntnap1 in paranodal regions.

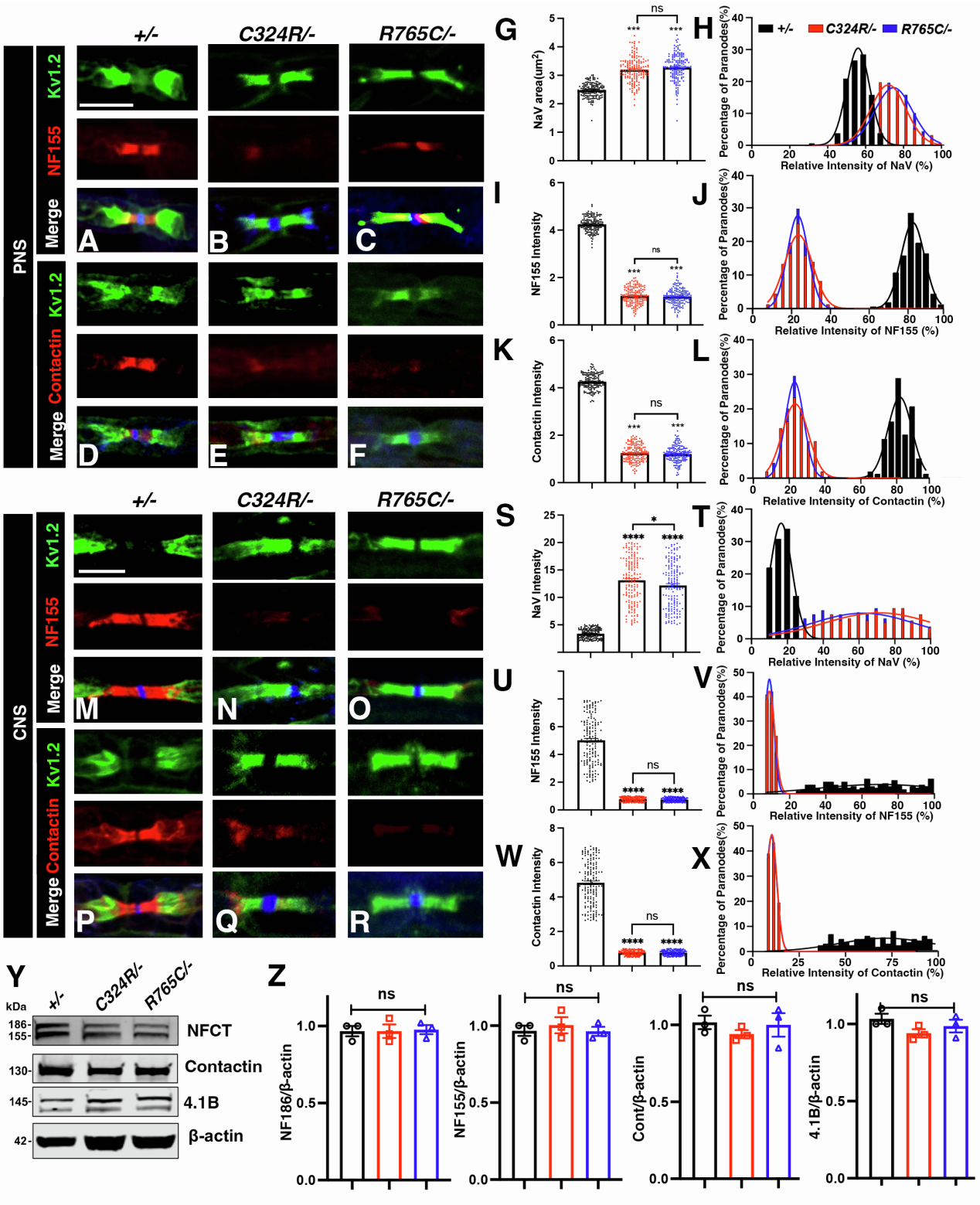
(**A-C**) Immunostaining of sciatic nerves from +/+ (**A**), *C324R/+*(**B**) *and R765C/+* (**C**) mice using antibodies against K_v1.2 (juxtaparanodes, green), Cntnap1 (paranodes, red) and βIV Spectrin (nodes, blue). Scale Bar: 5 µm.

(**D-F**) Immunostaining of spinal cord from +/+ (**D**), *C324R/+*(**E**) *and R765C/+* (**F**) mice using antibodies against K_v1.2 (juxtaparanodes, green), Cntnap1 (paranodes, red) and βIV Spectrin (nodes, blue). Scale Bar: 5 µm.

(**G**) The measurement of relative fluorescence intensity of Cntnap1 at the paranodes in the sciatic nerves in +/+, *C324R/+, R765C/+* mice.

(**H**) The measurement of relative fluorescence intensity of Cntnap1 at the paranodes in the spinal cords in +/+, *C324R/+* and *R765C/+* mice.

Supplementary Figure 2



Supplementary Figure 2. Key Paranodal Proteins Fail to Localize Properly in Cntnap1C324R/- and Cntnap1R765C/- Mouse Mutants

(**A-F**) Immunostaining of sciatic nerves. Scale Bar: 5 μm.

(**G-L**) Quantification of the relative fluorescence intensity and distribution of nodal NaV, paranodal NF155 and Contactin from +/-, C324R/- and R765C/- mice.

(M-R) Immunostaining of spinal cords from +/-, C324R/- and R765C/- mice. Scale Bar: 5 μm.

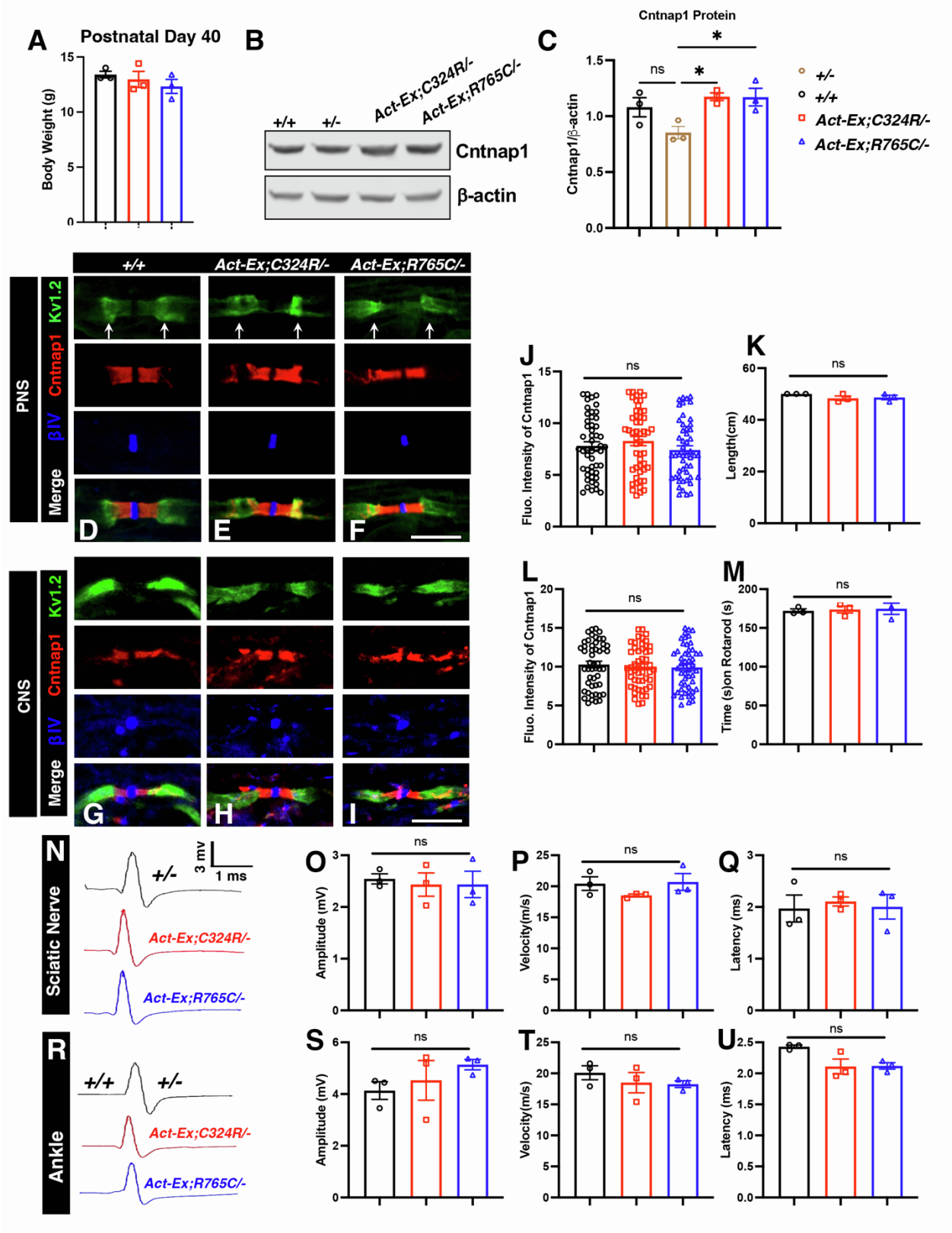
(**S-X**) Quantification of the relative fluorescence intensity and distribution of nodal NaV, paranodal NF155 and Contactin from +/-, C324R/- and R765C/- mice.

(**Y**) Representative immunoblots showing protein expression of total Neurofascin (NF186 and NF155) (using NF-CT antibody), Contactin, 4.1B in spinal cords.

(**Z**) Quantification of relative band intensities of the proteins immunoblotted in Y.

Data are represented as the mean ± SEM of 3-6 biological replicates.

Supplementary Figure 3



Supplementary Figure 3. Global Expression of the Wild-type Cntnap1 Gene Completely Restores the Axonal Domain Organization, motor function and nerve conduction velocity in Cntnap1C324R/- and Cntnap1R765C/- Mutants.

(**A**) Body weights of wild-type (+/+), *Act-Ex;C324R/- and Act-Ex;R765C/-* mice at postnatal day 40.

(**B-C**) Representative immunoblots showing the protein expression of Cntnap1 in the CNS (spinal cords) from wild-type (+/+), *Act-Ex;C324R/- and Act-Ex;R765C/-* mice; and the relative band intensity was quantified in **C**.

(**D-F**) Immunostaining of sciatic nerves from wild-type (+/+), *Act-Ex;C324R/- and Act-Ex;R765C/-* mice using antibodies against $K_v1.2$ (juxtaparanodes, green), Cntnap1 (paranodes, red) and βIV Spectrin (nodes, blue). Scale Bar: 5 μm.

(**G-I**) Immunostaining of spinal cord from wild-type (+/+), *Act-Ex;C324R/- and Act-Ex;R765C/-* mice using antibodies against $K_v1.2$ (juxtaparanodes, green), Cntnap1 (paranodes, red) and βIV Spectrin (nodes, blue). Scale Bar: 5 μm.

(**J**) The measurement of relative fluorescence intensity of Cntnap1 at the paranodes in the sciatic nerves in wild-type (+/+), *Act-Ex;C324R/- and Act-Ex;R765C/-* mice.

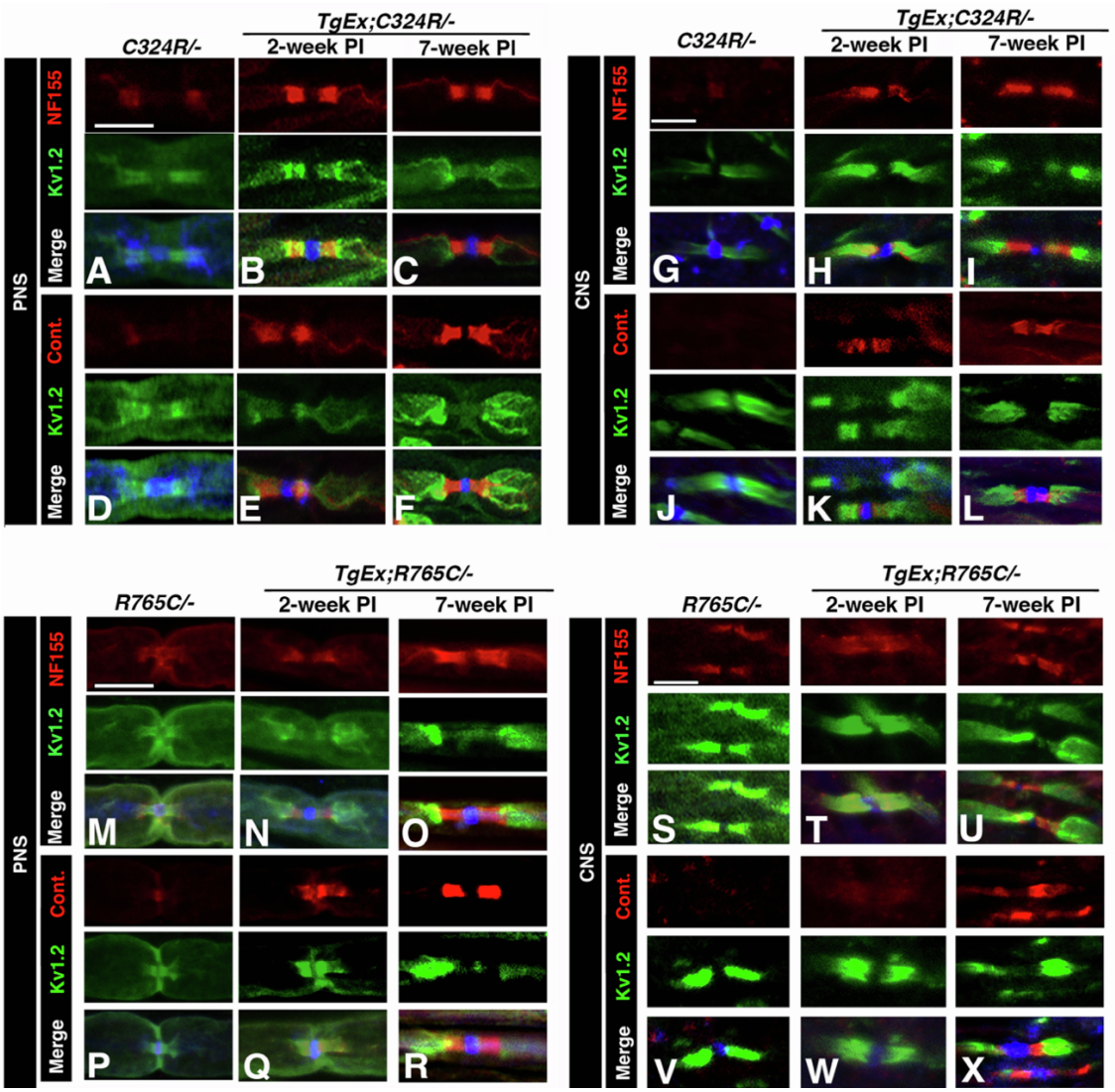
(**K**) Beam walking motor coordination performance of wild-type (+/+), *Act-Ex;C324R/- and Act-Ex;R765C/-* mice. Walking distances traveled by each mouse (the full length of the beam is 50 cm).

(**L**) The measurement of relative fluorescence intensity of Cntnap1 at the paranodes in the spinal cords in wild-type (+/+), *Act-Ex;C324R/- and Act-Ex;R765C/-* mice.

(**M**) Motor learning ability of +/+, *Act-Ex;C324R/- and Act-Ex;R765C/-* mice as measured by Rotarod test. The total time spent on the rotarod for each trial is quantified here.

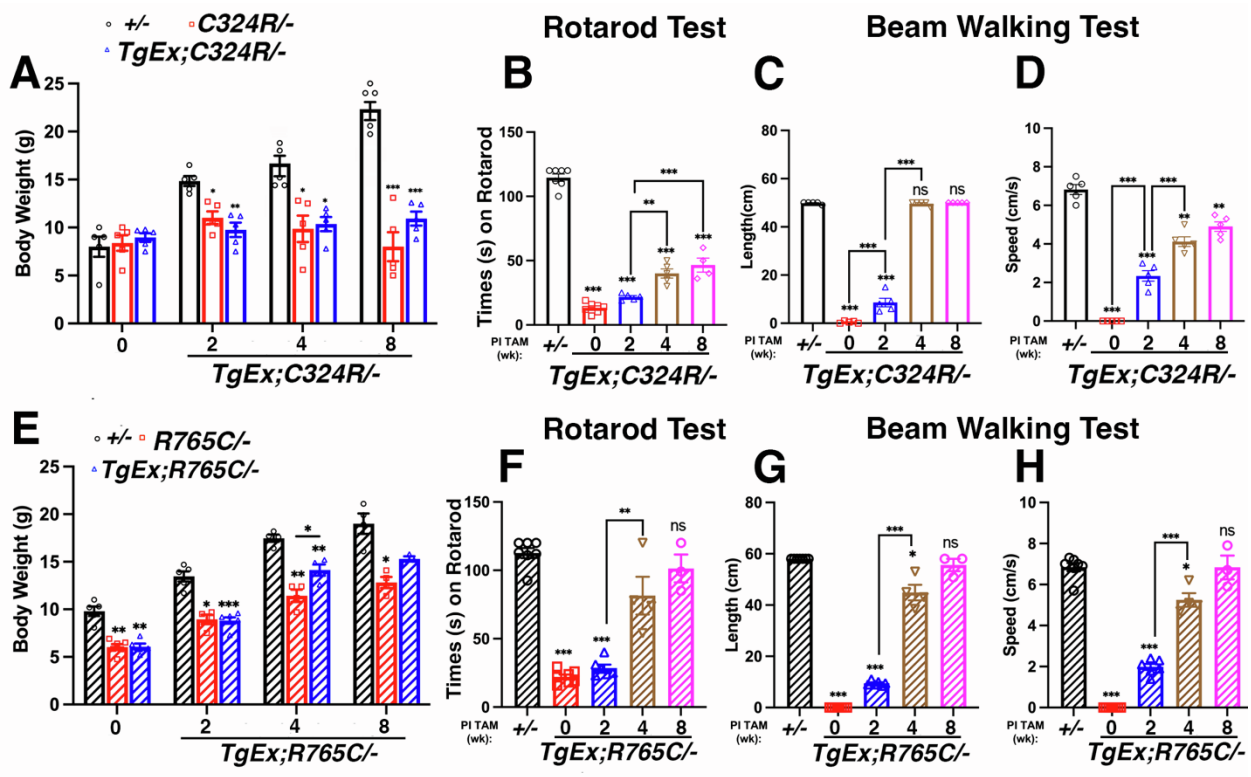
(N-U) Representative electrical impulse traces from the ankle (**N**) and sciatic nerves (**R**) from +/+, *Act-Ex;C324R/-* and *Act-Ex;R765C/-* (all at P40). Measurement and quantification of amplitude (**O, S**), nerve conduction velocity (NCV) (**P, T**) and latency (**Q, U**).

Supplementary Figure 4



Supplementary Figure 4. Neuronal Expression of the Wild-type Cntnap1 Gene Progressively Restores the Axonal Domain Organization and Expression of Contactin and Neurofasin155 in Cntnap1C324R/- and Cntnap1R765C/- Mutant Myelinated Axons. **(A-L)** Immunostaining of sciatic nerves (**A-F**) or spinal cords (**G-L**) from *Cntnap1*$^{C324R/-}$ (**A, D, G, J**), and *LSL-Cntnap1;Slick-H-Cre;Cntnap1*$^{C324R/-}$ (*TgEx;C324R/-*) mice at 2 weeks (**B, E, H, K**) or 7 weeks (**C, F, I, L**) after tamoxifen injection using antibodies against K_v1.2 (juxtaparanodes, green), Neurofasin^{155} or Contactin (paranodes, red) and Na_v channels (nodes, blue). Scale Bar: 5 μm.
(M-X) Immunostaining of sciatic nerves (**M-R**) or spinal cords (**S-X**) from *Cntnap1*$^{R765C/-}$ (**M, P, S, V**), and *LSL-Cntnap1;Slick-H-Cre;Cntnap1*$^{R765C/-}$ (*TgEx;R765C/-*) mice at 2 weeks (**N, Q, T, W**) or 7 weeks (**O, R, U, X**) after tamoxifen injection using antibodies against K_v1.2 (juxtaparanodes, green), Cntnap1 or Flag (paranodes, red) and Na_v channels (nodes, blue). Scale Bar: 5 μm.

Supplementary Figure 5



Supplementary Figure 5. Neuronal overexpression of wild-type Cntnap1 restoration of body weight and motor functions.

(**A**) The body weight of age-matched +/- (control), *C324R/-*, and *TgEx;C324R/-* at various stages after tamoxifen injection.

(**B**) Rotarod test showing motor performance by +/- (control), *C324R/-*, and *TgEx;C324R/-* at various stages after tamoxifen injection.

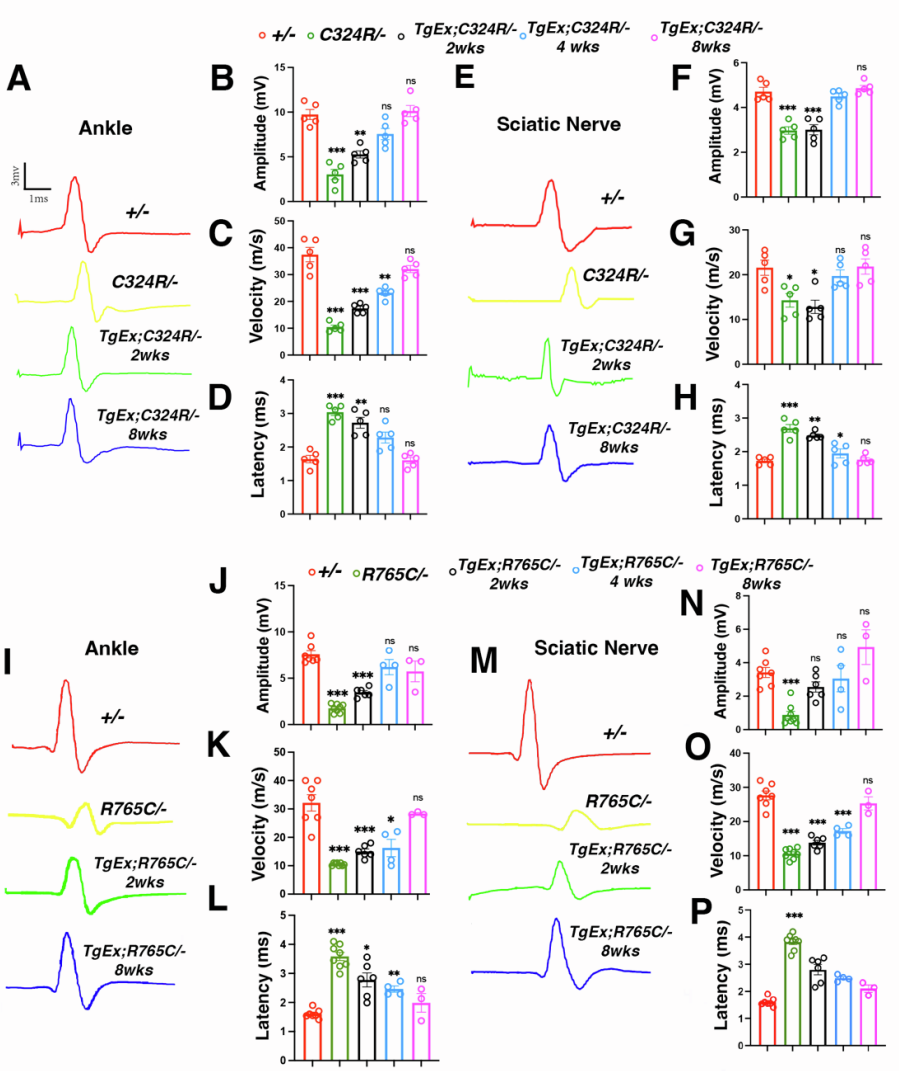
(**C, D**) Motor coordination performance in beam walking tests measured as walking distances (**C**) and walking speed (**D**) by +/- (control), *C324R/-*, and *TgEx;C324R/-* at various stages after tamoxifen injection.

(**E**) The body weight of age-matched +/-, *R765C/-*, and *TgEx;R765C/-* at various stages after tamoxifen injection.

(**F**) Rotarod test showing motor performance by +/-, *R765C/-*, and *TgEx;R765C/-* mutants at various stages after tamoxifen injection.

(**G, H**) Motor coordination performance in beam walking tests measured as walking distances (**G**) and walking speed (**H**) by +/-, *R765C/-*, and *TgEx;R765C/-* mutants at various stages after tamoxifen injection.

Supplementary Figure 6



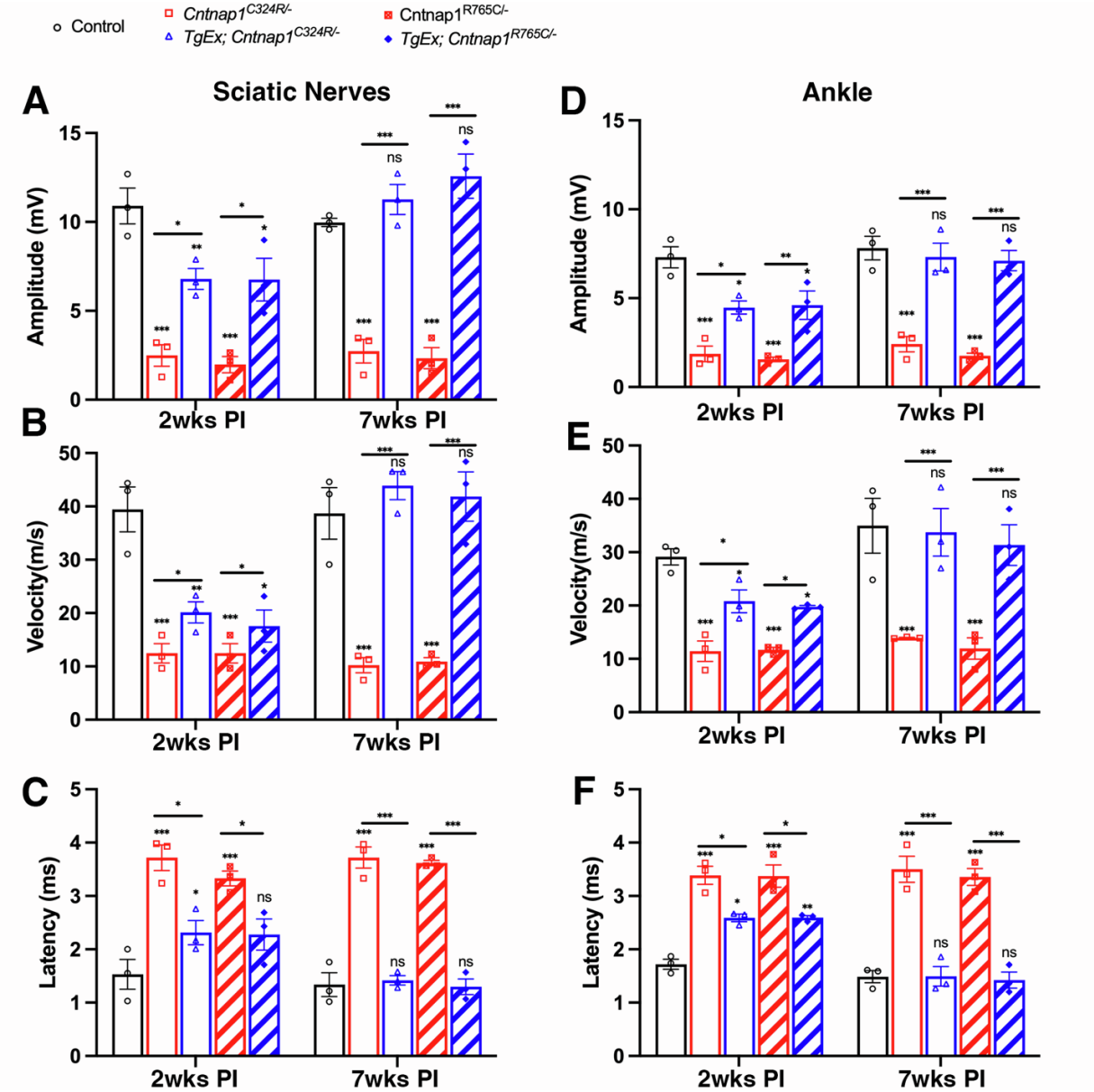
Supplementary Figure 6. P21 Neuronal Expression of the Wild-type Cntnap1 Gene Progressively Restores Nerve Conduction Properties in *Cntnap1*$^{C324R/-}$ and *Cntnap1*$^{R765C/-}$ Mutant Mice.

(A-H) Representative electrical impulse traces from the ankle (**A**) and sciatic nerves (**E**) from *Cntnap1*$^{+/-}$ (control, P40), *Cntnap1*$^{C324R/-}$ (P40), *TgEx; C324R/-* mice 2 weeks or 8 weeks after tamoxifen injection. Measurement and quantification of amplitude (**B, F**), nerve conduction velocity (NCV) (**C, G**) and latency (**D, H**).

(I-P) Representative electrical impulse traces from the ankle (**I**) and sciatic nerves (**M**) from *Cntnap1*$^{+/-}$ (control, P40), *Cntnap1*$^{R765C/-}$ (P40), *TgEx;R765C/-* mice 2 weeks or 8 weeks after tamoxifen injection. Measurement and quantification of amplitude (**J, N**), nerve conduction velocity (NCV) (**K, O**) and latency (**L, P**).

Comparisons between genotypes were performed by one-way ANOVA with Tukey post test (all quantification data panel). ** $p<0.01$, *** $p<0.001$ as compared with control. All data are presented as Mean ± S.E.M.

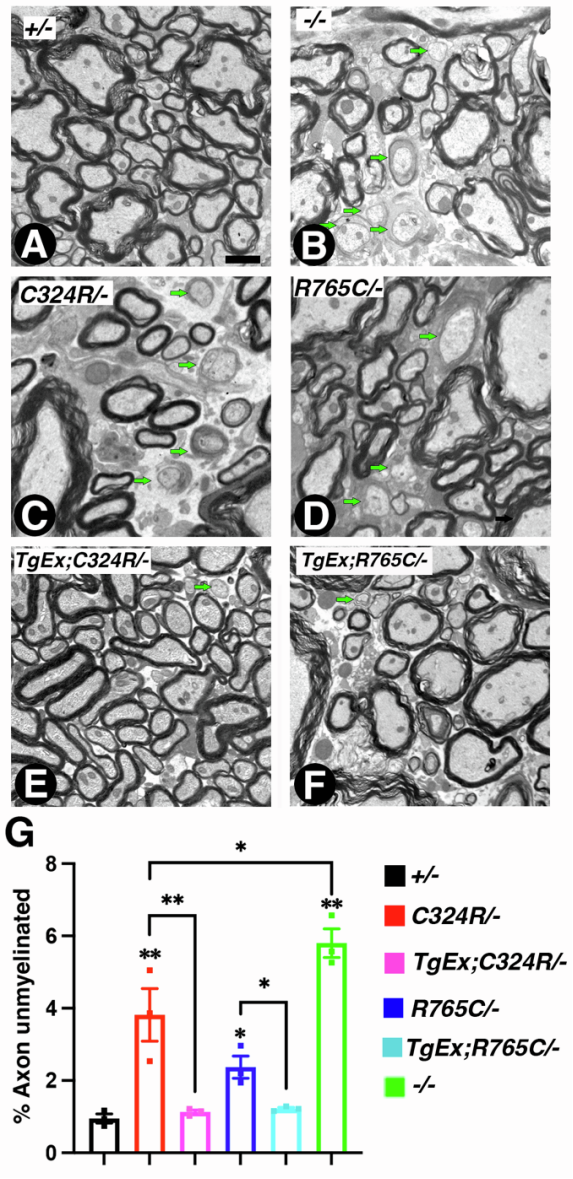
Supplementary Figure 7



Supplementary Figure 7. Earlier Neuronal Expression (P5) of the Wild-type Cntnap1 Gene Completely Restores Nerve Conduction Properties in *Cntnap1*$^{C324R/-}$ and *Cntnap1*$^{R765C/-}$ Mutant Mice.

Measurement and quantification of nerve conduction from the sciatic nerves (**A-C**) and ankle (**D-F**) from *Cntnap1*$^{+/-}$ (control, age matched), *Cntnap1*$^{R765C/-}$, *TgEx;R765C/-* mice 2 weeks or 7 weeks after tamoxifen injection at P5-P7. Measurement and quantification of amplitude (**A, D**), nerve conduction velocity (NCV) (**B, E**) and latency (**C, F**). Comparisons between genotypes within the same injection group were performed by one-way ANOVA with Tukey post test (all quantification data panel). n.s., non-significant; * p<0.01, ** p<0.01, *** p<0.001 as compared with control. All data are presented as Mean ± S.E.M.

Supplementary Figure 8



Supplementary Figure 8. Neuronal Expression of Cntnap1 Rescues Reduced Myelination in C324R/- and R765C/- Mutants

(A–F) 7 weeks after tamoxifen injection, TEM of cross sections from the spinal cords of 2 months old +/- (control) (**A),** -/- **(B),** *C324R/-(**C**), R765C/-(**D**), TgEx;C324R/-* (**E**), and *TgEx;R765C/-* (**F**) mice. Unmyelinated axons were indicated with green arrows. Scale bar= 2 μm.

(**G**) Morphometric analysis showing percentage of unmyelinated axons of total axons in spinal cord from all genotypes mentioned above. Comparisons between genotypes within the groups were performed by one-way ANOVA with Tukey post test (all quantification data panel). * $p<0.01$, ** $p<0.01$, *** $p<0.001$ as compared with +/- control. All data are presented as Mean ± S.E.M.

Supplementary Table 1
List of DNA sequences for generating the transgenic mutant mice

Mice line	gRNA	Donor DNA
Cntnap1$^{C324R/-}$	GCCUACC GCCAUAA UUUUCG	CCTAAGCCCATGGAAATGTCTCTTCATCTGCTTCC TACAGATATTCATCGGGGGTCTAGTGGGCGCAGC CCGTAAGAACCTGGCCTACCGCCATAATTTTCGC GGCCGCATAGAAAACGTGATCTACAACCGGATCA ACATTGCAGAAATGGCAGTGATGCGCCATTCGCG GATCACCTTTGAGGCCAGTGGGCAGGG
Cntnap1$^{R765C/-}$	CUCAAAU UCUGAAG CUCAGU	GAGTCACCGAATCCTATATTCCCACAGGAGAACA GACAAGGGGCTCCTGACCTTTGTAGACCATCTGC CTGTCACTCAGGTAGTGGTAGGTGATACAAACtGC TCAAATTCTGAAGCTCAGTTCTTCCTGAGGCCTCT GCGCTGCTATGGTGACCGTGAGTGGCAGACTCCT TTGGTGTGCCTGTCCAGTACTGTTTC
LSL-Cntnap1^{Flag}	ACUCCAG UCUUUCU AGAAGA	Linearized *LSL-GAG-Cntnap1*^{Flag} construct

Supplementary Table 2
List of DNA primers for generating Cntnap1 mutant plasmid

DNA mutant	Forward primers	Reverse Primers
Cntnap1^{C324R}	GCCATAACTTCCGTGGCCG CATAGAAAACGTGATCTAC	GTAGATCACGTTTTCTATGCGGC CACGGAAGTTATGGC
Cntnap1^{R765C}	GGTAGGTGATACAAACTGC TCAAATTCTGAAGC	GCTTCAGAATTTGAGCAGTTTGT ATCACCTACC

* The underline indicates the codon for substituting amino acids.