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Supplementary Information for

Cell-autonomous requirement of TDP-43, an ALS/FTD signature protein, for oligodendrocyte survival and myelination

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Supplementary Information

SI Material and Methods

Mouse models

All studies were carried out under protocols approved by the Institutional Animal Care and Use Committee (IACUC) of the National University of Singapore, and were in compliance with Association for Assessment of Laboratory Animal Care (AAALAC) guidelines for animal use. All mice used in this study were maintained on a C57BL/6J background and were housed in groups in individually ventilated cages under a 12:12-hour light/dark cycle with access to food and water *ad libitum*. Because there is no apparent gender bias to the overserved phenotypes and pathology, female and male mice were included and randomly allocated to experimental groups according to age and genotype. No animals or samples were excluded in any of the experiments.

Conditional TDP-43 (*Tardbp*$^{fl/fl}$) mice (stock number 017591) and reporter *Rosa26*-GNZ mice (stock number 008606) were purchased from the Jackson Laboratory. *Cnp*-Cre mice were described previously (1). For genotyping, genomic DNA was isolated from tail biopsies using salt extraction methods and subjected to routine PCR methods using the following primers:

Tardbp$^{fl/fl}$: 5'-ccctggctcatcaagaactg-3' and 5'-tccaggacagccaggactac-3'

Rosa26-GNZ: 5'-taagcctgcccagaagactc-3', 5'-aaagtcgctctgagttgttat-3' and 5'-tccagttcaacatcagccgctaca-3'

Cnp-Cre: 5'-ggggattcctcaactgacaa-3' and 5'-catgtttagctggcccaaat-3'

Behavioral testing and phenotype scoring

Mice from both *Cnp-Cre*;*Tardbp*$^{fl/+}$ and *Cnp-Cre*;*Tardbp*$^{fl/fl}$ groups were scored in aspects of tremor, ledge test and gait, following the previously described protocol (2). The mice were scored on a scale from 0 to 3, where 0 representing no certain phenotype shown, while 3 is given for a severe phenotype. Longevity of mice was plotted as a survival curve using Prism software (GraphPad Software, Inc, La Jolla, CA, USA).

For all behavioral testing, at least 5 mice from each genotype were examined. Mice were habituated in the behavioral facility room at least 1 hour before the test.

Balance beam. Mice were placed at one end of the beam, and time taken to cross the beam was measured and recorded. Each mouse crossed a 12-mm width and 6-mm width beam 3 times respectively. They were given 15 seconds to rest before they started the next trial.

Grip strength. Age-matched transgenic mice and control mice at 5 weeks and 7 weeks were tested for grip strength using grip strength meter. Mice were positioned parallel to a wire grid with either fore paws alone or four limbs gripping the grid, they were pulled back gently with steady force until the grid was released. Peak force (*g*) was recorded for three consecutive trials.

Immunohistochemistry, FluoroMyelin Red, LacZ staining, Nissl and Black-Gold II staining

Tissue preparation for immunohistochemistry was described previously (3). In brief, mice were anesthetized with isoflurane and perfused transcardially with phosphate buffered saline (PBS), followed by 4% paraformaldehyde (PFA) in phosphate buffer for fixation. Spinal cord and gastrocnemius muscles were dissected and post-fixed in 4% PFA in PBS for 2 hours. Tissues were cryopreserved in 30% sucrose for over 24 hours and embedded in Tissue-Tek before sectioning. Lumbar spinal cords were sectioned at 30 μm and gastrocnemius muscles were sectioned longitudinally at 40 μm using a cryostat or microtome.

For spinal cord section staining, after washing 3 times with 1xPBS, sections were permeabilized in 0.3% Triton X-100 in 1xPBS for 5 minutes. The permeabilization steps were repeated twice. Primary blocking buffer containing 5% BSA and 0.5% Tween-20 in 1xPBS was used for blocking and for the primary antibody incubation. After blocking at room temperature for 1 hour, the sections were incubated with primary antibodies at 4 degrees overnight. The antibodies used in this study include: mouse monoclonal APC(CC1) (1:200, Millipore, OP80), in-house mouse monoclonal TDP-43 FL4 (1:500, in-house (4)), rabbit polyclonal TDP-43 (1:500, Proteintech, 10782-2-AP), goat polyclonal choline acetyltransferase (ChAT) (1:200, Millipore, AB144P), rabbit polyclonal NeuN (D3S3I) (1:1000, Cell Signaling Technology, 12943S), rabbit polyclonal GFAP (D1F4Q) (1:1000, CST, 12389S), mouse monoclonal GFP (1:200, Wako, 012-20461), rabbit

polyclonal GFP (1:200, Proteintech, 50430-2-AP), rabbit polyclonal Olig2 (1:500, Millipore, AB9610), rabbit polyclonal GST-Pi (1:1000, Proteintech, 15902-1-AP), mouse monoclonal RIPK1 (1:500, BD Biosciences, 610459), rabbit polyclonal NG2 (1:500, Millipore, AB5320) and mouse monoclonal Ki67, clone MIB-5 (1:500, Dako, M7248). Sections were carefully washed with 1xPBS 3 times, 5 minutes each. Secondary antibodies conjugated with Alexa Fluor 488, 568, 643 (1:1000, Thermo Fisher Scientific) and 1 μg/ml DAPI were diluted in secondary blocking buffer containing 2% BSA and 0.5% Tween-20 in 1xPBS, and sections were incubated at room temperature for 1h in the dark. For RIPK1 and CC1 co-labeling, anti-mouse IgG2a cross-absorbed secondary antibody Alexa Fluor 488 (Thermo Fisher Scientific, A-21131) and anti-mouse IgG2b cross-absorbed secondary antibody Alexa Fluor 568 (Thermo Fisher Scientific, A-21144) were used at 1:1000 dilution. After incubation, sections were washed and mounted onto slides with Prolong Gold anti-fade reagent (Thermo Fisher Scientific, P36930). The details of primary and secondary antibodies used in this study were listed in Supplemental Table 4 and 5, respectively.

Neuromuscular junction (NMJs) staining. Gastrocnemius muscle sections were washed 3 times with 1 x PBS, followed by permeabilization in 0.3% Triton X-100 in 1x PBS. Sections were blocked in primary blocking buffer for 3-4 hours at room temperature before incubating with primary antibodies at room temperature overnight. Rabbit polyclonal synaptophysin (1:300, Thermo Fisher Scientific, PA1-1043), rabbit monoclonal neurofilament-L (C28E10) (1:2000, Cell Signaling Technology, 2837). After washing, secondary antibodies and α-Bungarotoxin, Alexa Fluor 488 conjugate (1:2000, Thermo Fisher Scientific, B13422) were diluted and incubated with sections at room temperature for 1 hour in the dark. After incubation, sections were washed and mounted onto slides with Prolong Gold anti-fade reagent.

FluoroMyelin Red staining. Free-floating spinal cord sections were washed 3 times with 1x PBS before incubating with FluoroMyelin Red (1:300, Thermo Fisher Scientific, F34652) at room temperature for 20min. Sections were mounted onto slides after washing with 1xPBS.

LacZ (β-galactosidase) staining. Free floating lumbar spinal cord sections were washed with 1xPBS, before mounting on gelatin-coated slides. After the slides were completely

dry, they were washed in Detergent Rinse Solution containing 0.01% sodium deoxycholate (Sigma) and 2mM $MgCl_2$ (Sigma) in 1x PBS at room temperature for 10min. X-gal powder (Thermo Fisher Scientific) was diluted into X-gal staining solution containing 0.01% sodium deoxycholate, 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide and 2 mM $MgCl_2$ in 1x PBS to reach final concentration of 1mg/ml. Slides were immersed into staining solution and kept at 37 degrees overnight in the dark. On the following day, slides were post-fixed in 4% paraformaldehyde at room temperature for 10 minutes. Slides were washed with 1xPBS for 10 minutes, followed by washing in MilliQ water twice. Dehydration process was continued by immersing the slides into 75%, 95% and absolute ethanol, then into xylene. Permount was added to coverslips and slides were dried in a fume hood before visualizing under a light microscope.

Nissl staining. Brain and spinal cord sections were washed with 1x PBS and mounted on the adhesive slides. After drying, the sections were rehydrated in 0.1% PBS for 10 minutes, followed by distilled water for 3 minutes. Sections were incubated with 0.1% cresyl violet for 10 minutes followed by a 3-minutes wash in distill water, and 1-minute in 70% ethanol/0.01% glacial acetic acid incubation. Sections were dehydrated in 80%, 90%, 95% ethanol for 3 minutes each followed by 3 times of 100% ethanol and 3 times of xylene incubation before mounting.

Black-Gold II staining. Myelin of brain and spinal cord sections was stained with Black-Gold II aurohalophosphate complex (Millipore, AG105) according to manufacturer's manual.

TUNEL assay

Spinal cord sections were mounted on coated slides and air dried before performing the staining. DeadEnd™ Fluorometric TUNEL system kit from Promega was used according to manufacturer's instruction with minor modification. Briefly, slides were washed in 1x PBS followed by immersing in PBST (0.5% Triton X-100 in 1x PBS) at 85 degrees for 20 minutes. After incubation, slides were rinsed 3 times in 1x PBS. As a positive control, which used DNase to induce DNA fragmentation, slides were incubated with DNase buffer (40 mM Tris, pH 7.9, 10 mM NaCl, 6 mM $MgCl_2$, 10 mM $CaCl_2$) for 5 minutes at room temperature. Then liquid was removed and sections were incubated with DNase

buffer containing 20 units/ml of DNase for 15 minutes at room temperature. After incubation, sections were washed 3-4 times in deionized water. All slides were then equilibrated with equilibration buffer for 5 minutes at room temperature. During equilibration time, reaction mix (200 µl/slide) was prepared as follows: equilibration buffer 180 µl, nucleotide mix 20 µl, rTdT enzyme 4 µl. For negative control, rTdT enzyme was replaced with deionized water. Slides were incubated in reaction mix buffer for 1 hour in a humid chamber at 37 degrees. Reaction was stopped by immersing in 2 x SSC buffer for 10 minutes at room temperature. Slides were washed in PBST for 3 times. After this point, slides had to be kept in dark. To combine with immunofluorescent staining of cell markers, sections were blocked in blocking buffer (5% BSA, 0.5% Tween-20 in 1 x PBS) for 1 hour at room temperature before applying primary antibodies. The following steps are the same as normal immunofluorescent staining. TUNEL signal was visualized using green fluorescence at 488 nm.

EdU (5-ethynyl-2'deoxyuridine) injection and staining

EdU powder was dissolved in sterile 1x PBS at the stock concentration of 2.5 mg/ml. It was further diluted to 1 mg/ml in sterile 1x PBS as working concentration. Mice at P20 were injected with 100 µl of EdU at 1 mg/ml intraperitoneally for 5 consecutive days. One day after the last injection, mice were perfused with 1x PBS, followed by 4% PFA in phosphate buffer, brain and spinal cord were dissected for further tissue sectioning. Spinal cords were sectioned at 30 µm thickness for EdU staining. EdU staining was performed on spinal cord sections using Click-iT™ EdU Alexa Fluor™ 594 Imaging Kit (Invitrogen, C10339) according to the manufacturer's instruction. Briefly, Click-iT reaction cocktail containing 1 x Click-iT reaction buffer, Copper protectant, Alexa Fluor 594 picolyl azide and reaction buffer additive were mixed in order. Spinal cord sections were incubated in reaction cocktail for 1 hour at room temperature in dark, followed by 3 times wash with 1x PBS. To combine with cell markers staining, sections were permeabilized with 0.3% PBST for 15 minutes, followed by blocking with blocking buffer (5% BSA, 0.5% Tween-20 in 1x PBS) for 1 hour at room temperature, before applying primary antibody overnight in dark. The following steps are the same as normal immunofluorescent staining. EdU signal was visualized using red fluorescence at 594 nm and cell markers were visualized using green fluorescence at 488 nm.

Tissue protein extraction and Immunoblotting

Spinal cords and brains were harvested and snap frozen in liquid nitrogen before protein extraction. RIPA buffer containing 15 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris, pH8.0 was prepared and protease inhibitors (Thermo Fisher Scientific, 88266) were added before use. Tissues were homogenized in RIPA buffer and incubated at 4 degrees for 2 hours with gentle agitation. After incubation, lysed samples were centrifuged at 4 ^{0}C for 30 minutes at 20,000 *xg* using a desktop centrifuge and the supernatants were collected for BCA protein concentration measurement (Thermo Fisher Scientific, 23225). 30 µg of total protein for each sample was loaded for 10% SDS-PAGE. Proteins were transferred to PVDF membrane using 1x transfer buffer containing 1x Tris-Glycine (25 mM Tris base and 192 mM glycine) and 20% methanol at 100V for 90min. Membranes were blocked using 5% milk in 1x TBST (50 mM Tris, 150 mM NaCl, 0.1% Tween 20, pH 7.4) at room temperature for 1 hour. After blocking, membranes were incubated with primary antibodies at 4 degrees overnight. Mouse monoclonal CNP (1:2000, Sigma, C5922), Mouse monoclonal MOG (1:2000, Millipore, MAB5680), rat monoclonal MBP (1:2000, Millipore, MAB386), rabbit polyclonal TDP-43 (1:1000, Proteintech, 10782-2-AP), rabbit monoclonal RIPK1 (D94C12) (1:1000, Cell Signaling Technology, 3493)， rabbit polyclonal MLKL (1:1000, Abcam, ab172868) and mouse monoclonal GAPDH (1:5000, Millipore, MAB374). Membranes were washed with 1XTBST and incubated with appropriate HRP-conjugated secondary antibodies (1:1000, Thermo Fisher Scientific) at room temperature for 1 hour. After washing with 1xTBST extensively, target proteins were probed using SuperSignal^{TM} West Pico Chemiluminescent Substrate (Thermo Fisher Scientific). ImageStudioLite software was used for quantification. The details of primary and secondary antibodies used in this study were listed in Supplemental Table 4 and 5, respectively.

Electron microscopy (EM) and Toluidine blue staining

Mice were anaesthetized and perfused transcardially with 1xPBS, followed by 4% paraformaldehyde and 2.5% glutaraldehyde in 1xPBS. Spinal cord was dissected and stored in the fixative at least 1 week before further process. Lumbar spinal cord was sliced into small pieces and washed in 1xPBS. Samples were treated with 1% OsO_4 in 1xPBS, pH7.4 at room temperature for 1 hour and dehydrated using 25%, 50%, 75%, 95%, 100% ethanol and 100% acetone. Infiltration was carried on by immersing samples

in 100% acetone: resin (1:1 vol/vol) at room temperature for 30 minutes, followed by 100% acetone: resin (1:6 vol/vol) at room temperature overnight. The next day, samples were embedded in pure resin. For toluidine blue staining, semi-thin sections were obtained from the resin-embedded tissues. After sections were completely dry, they were stained by toluidine blue staining solution containing 1% toluidine blue and 2% sodium borate in distilled water. After rinsing off excess staining with distilled water, slides were dried and applied with normal mounting medium. Ultra-thin sections (90 nm) were obtained using microtome and stained with citrate lead.

Electron microscopy images were visualized using a JEM.1010 electron microscope equipped with Erlangshen ES500W camera using Gatan DigitalMicrograph software. G-ratio was measured using Image J. The inner and outer boundary of axons in white matter were selected using polygon selection tool. The perimeters were measured and converted into diameters, assuming they were circles. G-ratio equaled inner diameter divided by outer diameter. 50 axons were measured and calculated for each mouse with 3 animals per genotype.

Image acquisition and quantification

Confocal images were acquired with a Zeiss LSM700 inverted confocal microscope with 4 laser lines (405/488/555/639 nm) with either a 20x/0.8 N.A. air or 63x/1.15 N.A. oil immersion objectives. Images were captured using a AxioCam MRm monochromatic CCD camera (Zeiss) run by Zeiss Zen software. For the quantification of CC1-, NG2- and Olig2-imuunopositive cells, all confocal images were taken at 20x/0.8 N.A. objective and original files were imported into ImageJ for analysis detailed below. The boundary of the grey and white matter of the spinal cord was defined manually prior to quantification. The total numbers of CC1- or Olig2-positive cells were counted from the whole spinal cord section by adjusting threshold with particles function (at least 6 sections per animal, with 3 animals per genotype). Subsequently, the CC1- and Olig2-positive cell density of grey and white matter is calculated using total cell numbers counted of the grey and white matter divided by the area of grey and white matter, respectively. Because of elaborated morphology of NG2-positive cells, 2 areas with a 0.2 mm^2 square grid within the grey and white matter of each spinal cord slice were randomly selected and NG2-immunopositive cells were counted manually (at least 6 sections per animal, with 3 animals per genotype). For NG2 cell body measurement and Sholl analysis, 10 cells

were measured for each animal with 3 animals per genotype. For NG2 cell body size quantification, the area of the cell body was traced and measured using polygon selection in the ImageJ. For Sholl analysis, images were converted into grayscale. The center of the cell body was defined as the starting point, and the parameters were set as follows: starting radius: 0 μm, ending radius: 50 μm, radius step size: 10 μm. For NG2 cell tracing, images were converted into grayscale and analyzed using simple neurite tracer under segmentation plugin in ImageJ. Basically, the cell body was defined as the starting point, and the path was completed by clicking on the other end of the branch. After finishing tracing all the branches, images were saved by making line stack and exported as TIF format.

RNA extraction and quantitative RT-PCR

Total RNAs were extracted from spinal cord tissues using Trizol reagent (Thermo Fisher Scientific) according to manufacturer's instruction. After DNase treatment using RQ1 RNase-Free DNase (Promega), 1μg RNA were reverse transcribed using Maxima First Strand cDNA Synthesis Kit for RT-qPCR (Thermo Fisher Scientific). mRNA levels were determined using Maxima SYBR Green qPCR master mix (Thermo Fisher Scientific). Primers for genes of interest were listed in Supplemental Table 6.

Primary oligodendrocyte precursor cell culture, oligodendrocyte differentiation and immunofluorescence

Primary oligodendrocyte precursor cell culture from mouse spinal cords were isolated from postnatal day 7 (P7) mouse pups based on the immunopanning protocol as described (5) with the following modifications. In brief, spinal cord tissues were diced with a sterile scalpel blade and incubated at 37^0C for 20 minutes in papain solution (20 U/ml; Sigma). The tissue was then triturated sequentially in a solution containing ovomucoid (Worthington). Cell suspension was filtered through a nylon cell strainer (70 μm pore size). The filtered cell suspension was first placed on a lectin (Bandeiraea Simplificolia Lectin 1, BSL1, Vector Laboratories)-coated plate for 15 minutes at room temperature. The nonadherent cells were transferred to the second BSL1 plate for 15 minutes. After which the nonadherent cells were transferred to the anti-Mouse CD140a (BD Pharmingen)-coated plate for 45 minutes, with brief and gentle agitation every 15 minutes. The plate containing $CD140a^+$ oligodendrocyte precursor cells (OPCs) was washed 8 times with 10 ml of DPBS with Mg^{2+} and Ca^{2+} (Gibco) with agitation to remove

all antigen-negative nonadherent cells. Adherent cells were incubated in 5 ml of Trypsin/EBSS (1000 U/ml; Sigma) for 5 minutes at 37^0C in 5% CO_2 incubator. The cells were then gently dislodged using DMEM high glucose (Thermo Fisher Scientific) and centrifuged ate 1200 rpm for 10 minutes. The pelleted cells were then resuspended in SATO medium containing bovine insulin (50 μg/ml; Sigma), N-acetyl-cysteine (50 μg/ml; Sigma), forskolin (5 nM; Sigma), trace element B (Cellgro), penicillin-streptomycin (Biowest), L-glutamine (Invitrogen), sodium pyruvate (Invitrogen), B27 supplement (Invitrogen) and SATO stock (Apo-transferrin, progesterone, sodium selenite) in DMEM, and cultured on poly-D-lysine (0.1 mg/ml; Sigma)-coated glass coverslips in 24-well plates. Human PDGF-AA (10 ng/ml; Peprotech) and Human bFGF (10 ng/ml; Peprotech) were added to the culture every day for proliferation for 7 days. PDGF and bFGF were replaced with T3 (40 ng/ml; Peprotech) for oligodendrocyte differentiation for 3 days.

Cells were fixed on the third day in 4% paraformaldehyde supplemented with 4% Sucrose in 1x PBS. Fixed cells were washed once with PBS followed by 20 mM glycine (Sigma). Cells were permeablised in 0.3% PBS-T then blocked in 5% donkey serum in 0.3% PBS-T. Primary antibodies were prepared in 1% donkey serum in PBS and incubated overnight at 4C. The primary antibodies used in this study were: MBP (Millipore, MAB386, 1:500), and TDP43 (FL4, in-house, 1:500). Cell were then washed with PBS three times and incubated in Secondary antibodies at room temperature for 1 hour. The secondary antibodies used were donkey anti-mouse IgG Alexa Fluor 568 conjugate (Thermo Fisher Scientific) and donkey Anti-rat IgG Alexa Fluor 488 conjugate (Thermo Fisher Scientific). After which cells were washed three times in PBS, then mounted with ProLong Gold antifade reagent (Thermo Fisher Scientific). A Zeiss LSM700 inverted confocal microscope and a 63×/1.15 N.A. oil immersion objective was used for visualization and image acquisition.

Statistical analysis

For datasets with no repeated measures, ANOVA was used to test the effect of the various variables on the respective responses. When repeated measures from individual mice were present, we employed linear mixed-effects models to partition fixed effects (e.g. genotype, tissue type) from the random effect of the individual mouse variation (6). Model selection was performed using the AICc metric, a sample size corrected version of the Akaike information criterion. Here, a set of a priori models, as well as an intercept-

only null model were scored, and the best scoring (lowest AICc) was chosen. Post-hoc analyses of ANOVA and linear mixed model results were performed using Tukey's HSD test using the emmeans R package with Kenward-Roger approximations of degrees of freedom.

SI Figures

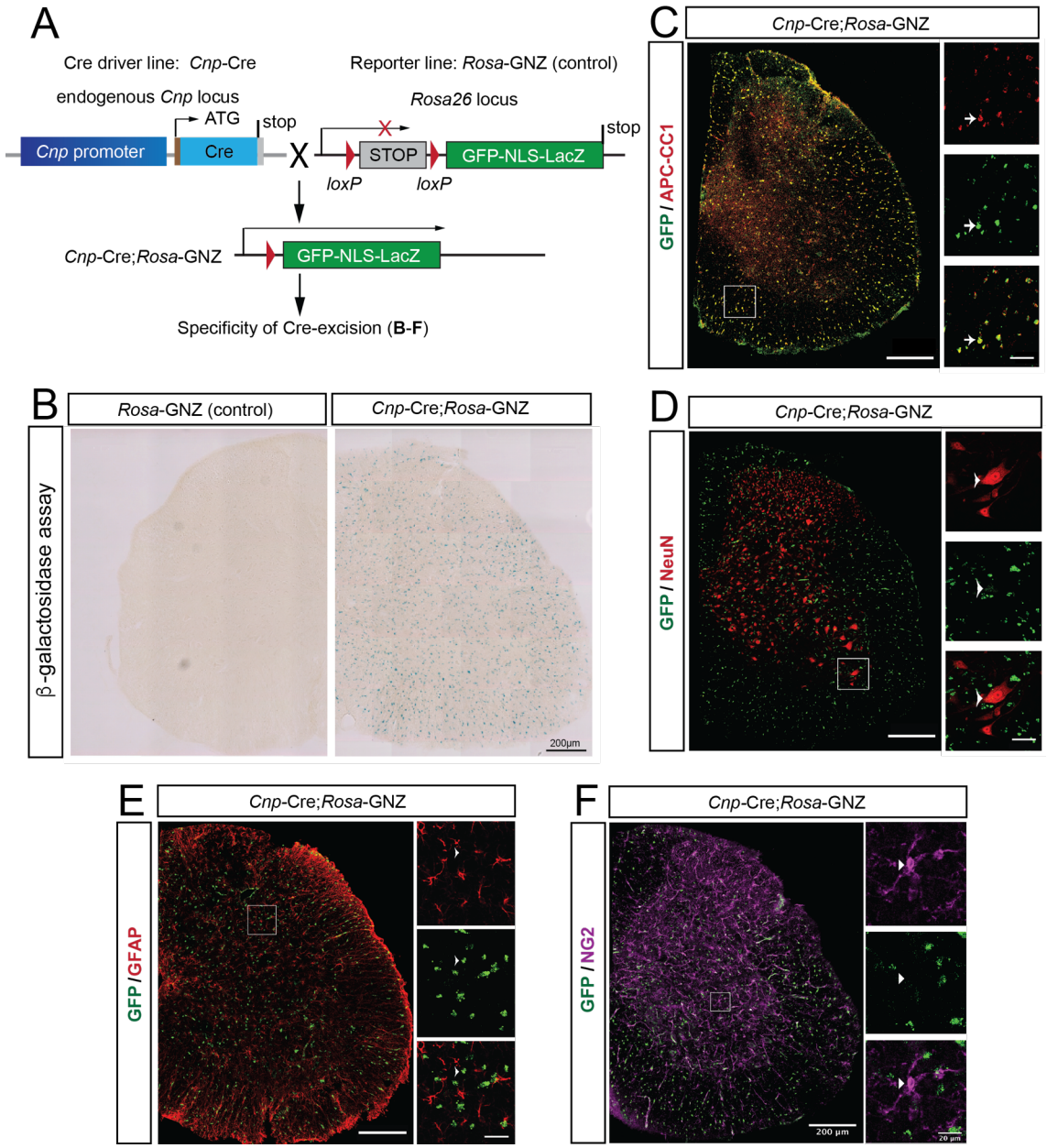

Fig. S1. Cre-mediated recombination is restricted to mature oligodendrocytes in the spinal cord of *Cnp*-Cre mice. (A) Assessment of *Cnp*-Cre-specificity by crossing the *Cnp*-Cre driver line with a *Rosa26*-GNZ reporter line. Schematics outlining the mating strategy used to obtain *Cnp-Cre*;*Rosa26*-GNZ mice and subsequent analysis. (B) β-galactosidase (LacZ) staining is ubiquitous in the transverse section of *Cnp-Cre*;*Rosa26*-GNZ lumbar spinal cord (30 μm), while in the *Rosa26*-GNZ control, no positive staining is seen. Scale bar = 200 μm. (C-F) GFP double labeling with cell type-specific markers. GFP signals co-localized with mature oligodendrocyte marker APC-CC1 (C), but not with neuronal marker NeuN (D), astrocyte marker GFAP (E) or OPC marker NG2 (F) in *Cnp-Cre*;*Rosa26*-GNZ spinal cord. Scale bar = 200 μm.

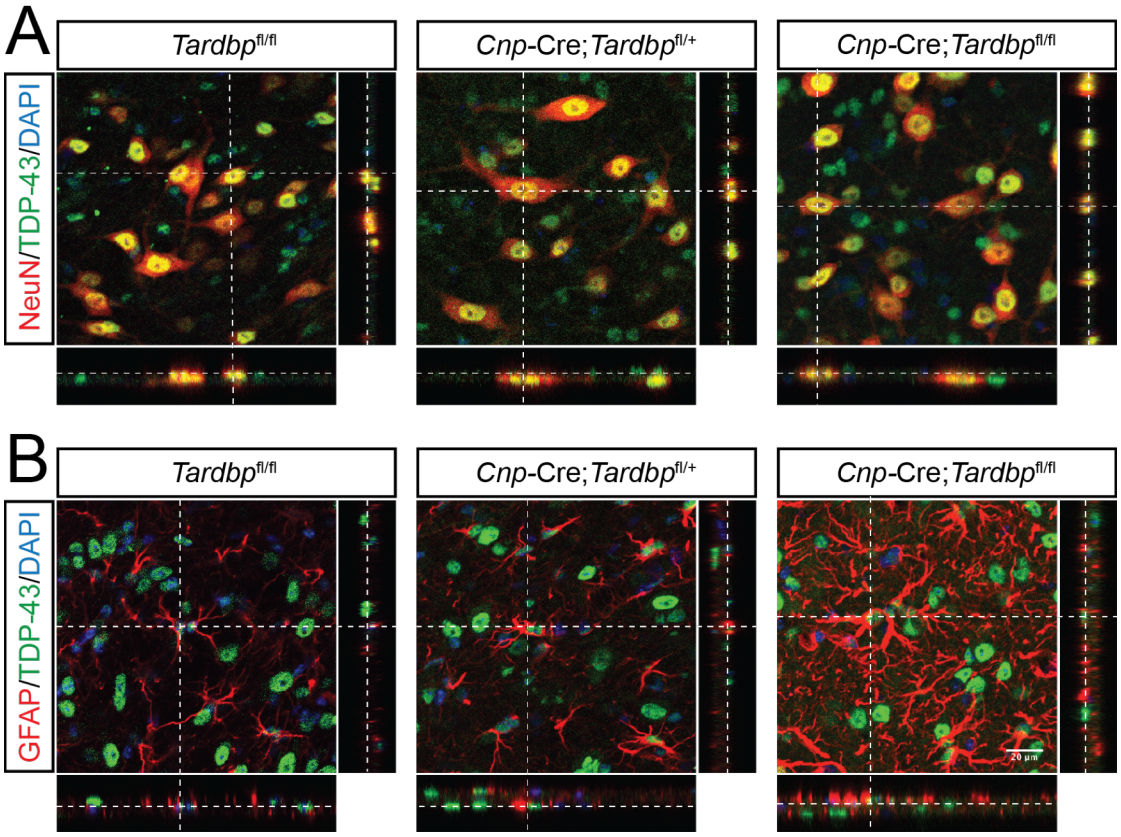

Fig. S2. TDP-43 expression is retained at neurons and astrocytes in the spinal cord of *Cnp-Cre*;*Tardbp*$^{fl/fl}$ mice. (A) Orthogonal view of neurons (NeuN^{+}, red) and TDP-43 (green) co-staining in spinal cord sections of three genotypes at 60 days of age. (B) Orthogonal view of astrocytes (GFAP^{+}, red) and TDP-43 (green) co-staining in 60days spinal cord sections of three genotypes. Astrocyte activation is evident in *Cnp-Cre*; *Tardbp*$^{fl/fl}$ mice. Scale bar = 20 μm.

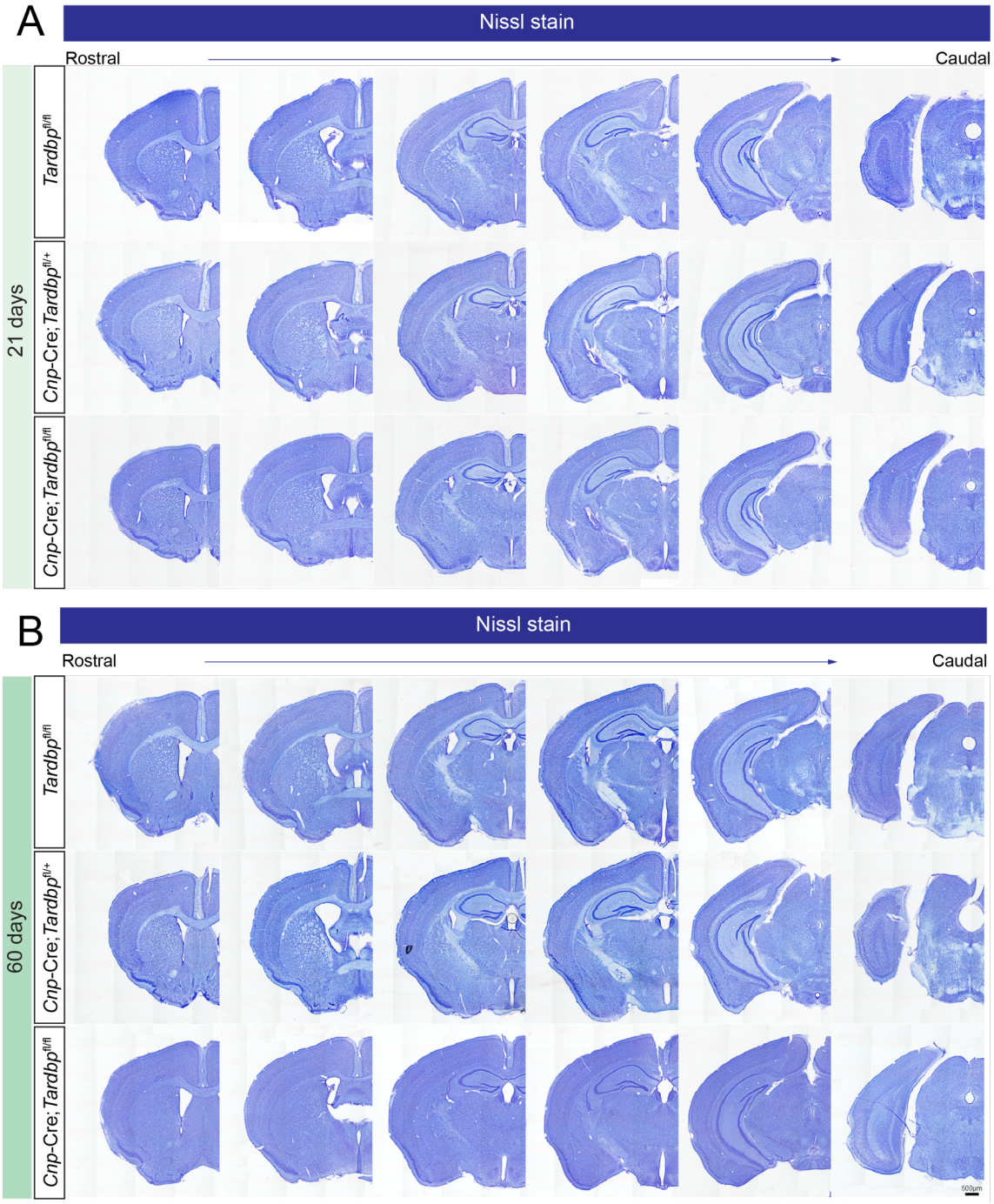

Fig. S3. No gross neuroanatomical changes in the brains of *Cnp-Cre*;*Tardbp*$^{fl/fl}$ mice. (A) Nissl staining of coronal brain sections from 21-day-old *Tardbp*$^{fl/fl}$, *Cnp*-Cre;*Tardbp*$^{fl/+}$ and *Cnp*-Cre;*Tardbp*$^{fl/fl}$ mice. (B) Nissl staining of coronal brain sections from 60-day-old *Tardbp*$^{fl/fl}$, *Cnp*-Cre;*Tardbp*$^{fl/+}$ and *Cnp*-Cre;*Tardbp*$^{fl/fl}$ mice. Scale bar=500 μm.

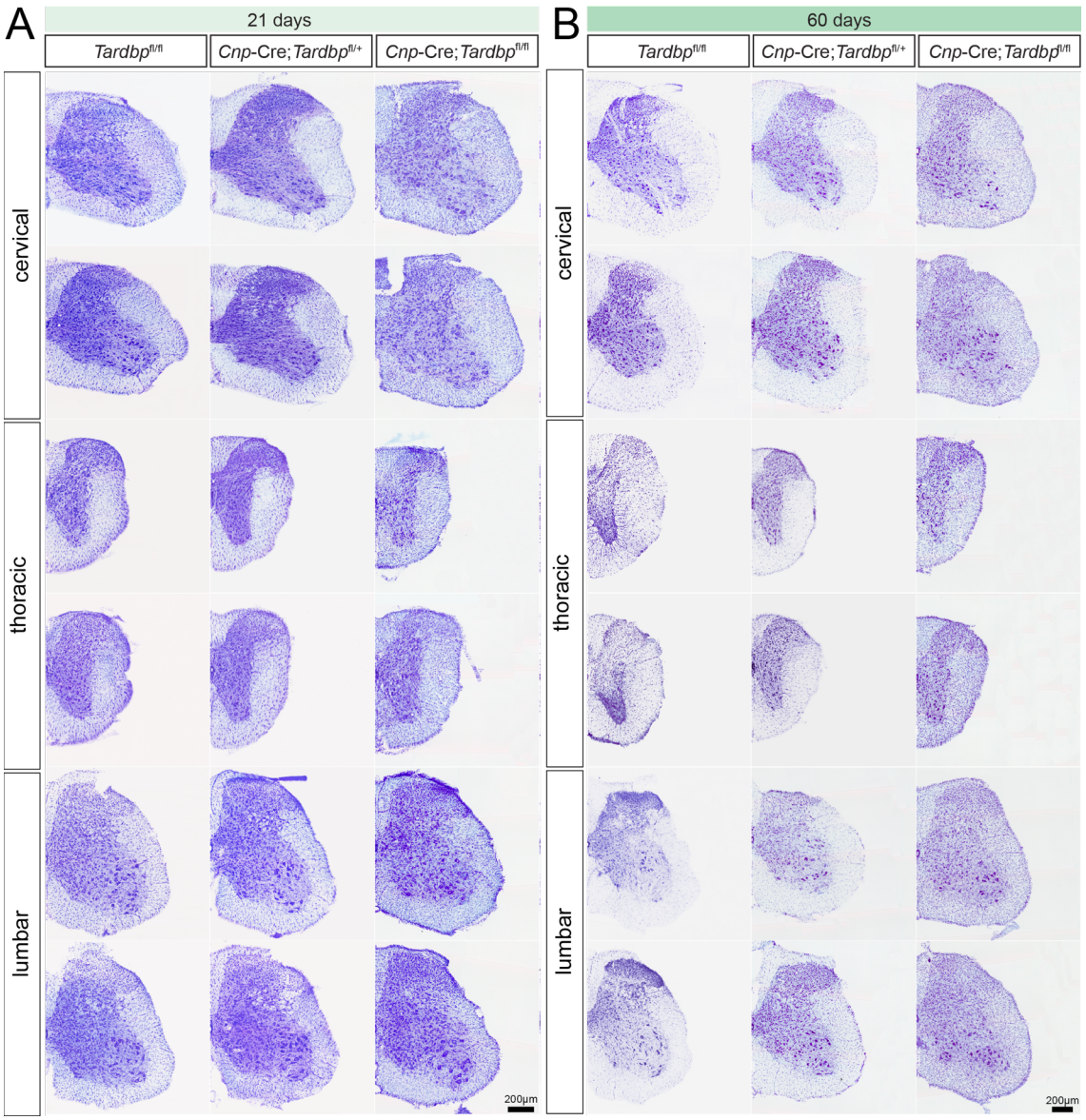

Fig. S4. **No gross neuroanatomical changes in all levels of spinal cord of *Cnp-Cre*;*Tardbp*$^{fl/fl}$ mice.** (A) Nissl staining of transverse cervical, thoracic and lumbar sections of spinal cords from 21-day-old *Tardbp*$^{fl/fl}$, *Cnp*-Cre;*Tardbp*$^{fl/+}$ and *Cnp*-Cre;*Tardbp*$^{fl/fl}$ mice. (B) Nissl staining of transverse cervical, thoracic and lumbar sections of spinal cords from 60-day-old *Tardbp*$^{fl/fl}$, *Cnp*-Cre;*Tardbp*$^{fl/+}$ and *Cnp*-Cre;*Tardbp*$^{fl/fl}$ mice. Scale bar=200 μm.

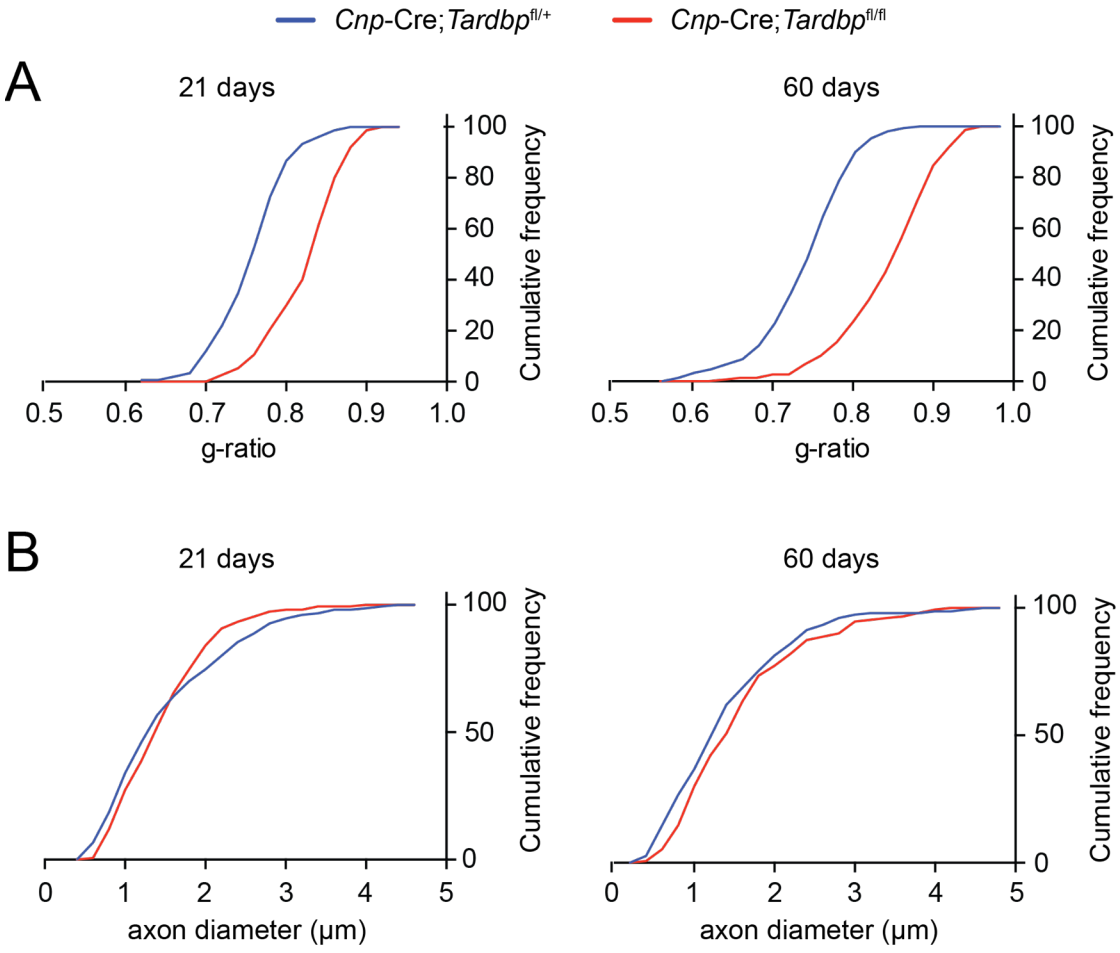

Fig. S5. **Normal axon diameter in the white matter of spinal cord.** (A) Cumulative frequency of g-ratio of the ventral corticospinal axons in the spinal cord white matter of *Cnp*-Cre;*Tardbp*$^{fl/+}$ and *Cnp*-Cre;*Tardbp*$^{fl/fl}$ mice. (B) Cumulative frequency of inner axon diameters of the ventral corticospinal axons of the *Cnp*-Cre;*Tardbp*$^{fl/+}$ and *Cnp*-Cre;*Tardbp*$^{fl/fl}$ mice.

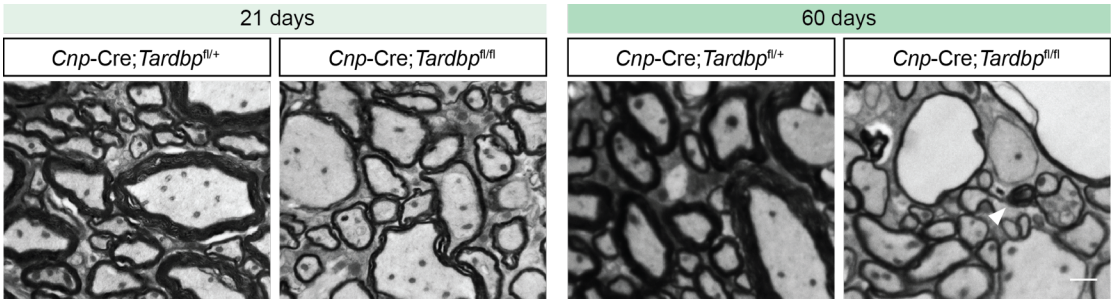

Fig. S6. Reduced myelination and signs of axonal degeneration in the lateral column of 60-day-old *Cnp-Cre*;*Tardbp*$^{fl/fl}$ mice. Electron microscopy images of the lateral column axons in the spinal cord of *Cnp*-Cre;*Tardbp*$^{fl/+}$ and *Cnp*-Cre;*Tardbp*$^{fl/fl}$ mice at both 21 days and 60 days of age. Signs of axonal degeneration was observed in 60-day-old *Cnp*-Cre;*Tardbp*$^{fl/fl}$ mice (arrowhead). Scale bar = 1 μm.

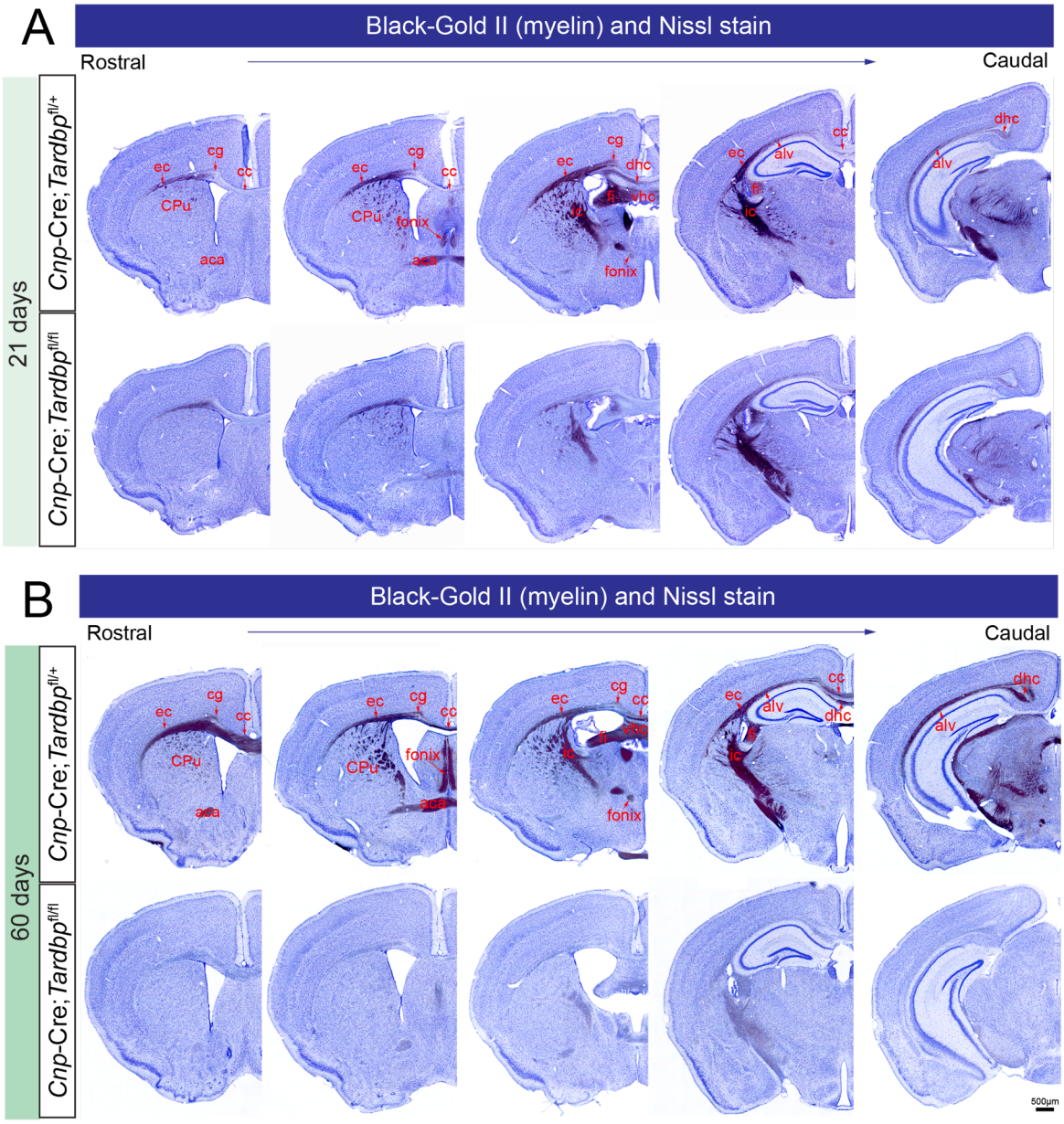

Fig. S7. **Progressive loss of major white matter tracks without gross neuroanatomical changes in oligodedroglial TDP-43 deleted mice.** Black-Gold II myelin stain and Nissl stain of coronal brain sections from *Tardbp*$^{fl/fl}$, *Cnp*-Cre;*Tardbp*$^{fl/+}$, and *Cnp*-Cre;*Tardbp*$^{fl/fl}$ mice at 21 days (A) and 60 days (B) of age. cc: corpus callosum, cg: cingulum, ec: external capsule, cpu: caudate putamen (striatum), aca: anterior commissure, ic: internal capsule, fi: fimbria hippocampus, vhc: ventral hippocampus commissure, dhc: dorsal hippocampus commissure, alv: alveus hippocampus. Scale bar=500 µm.

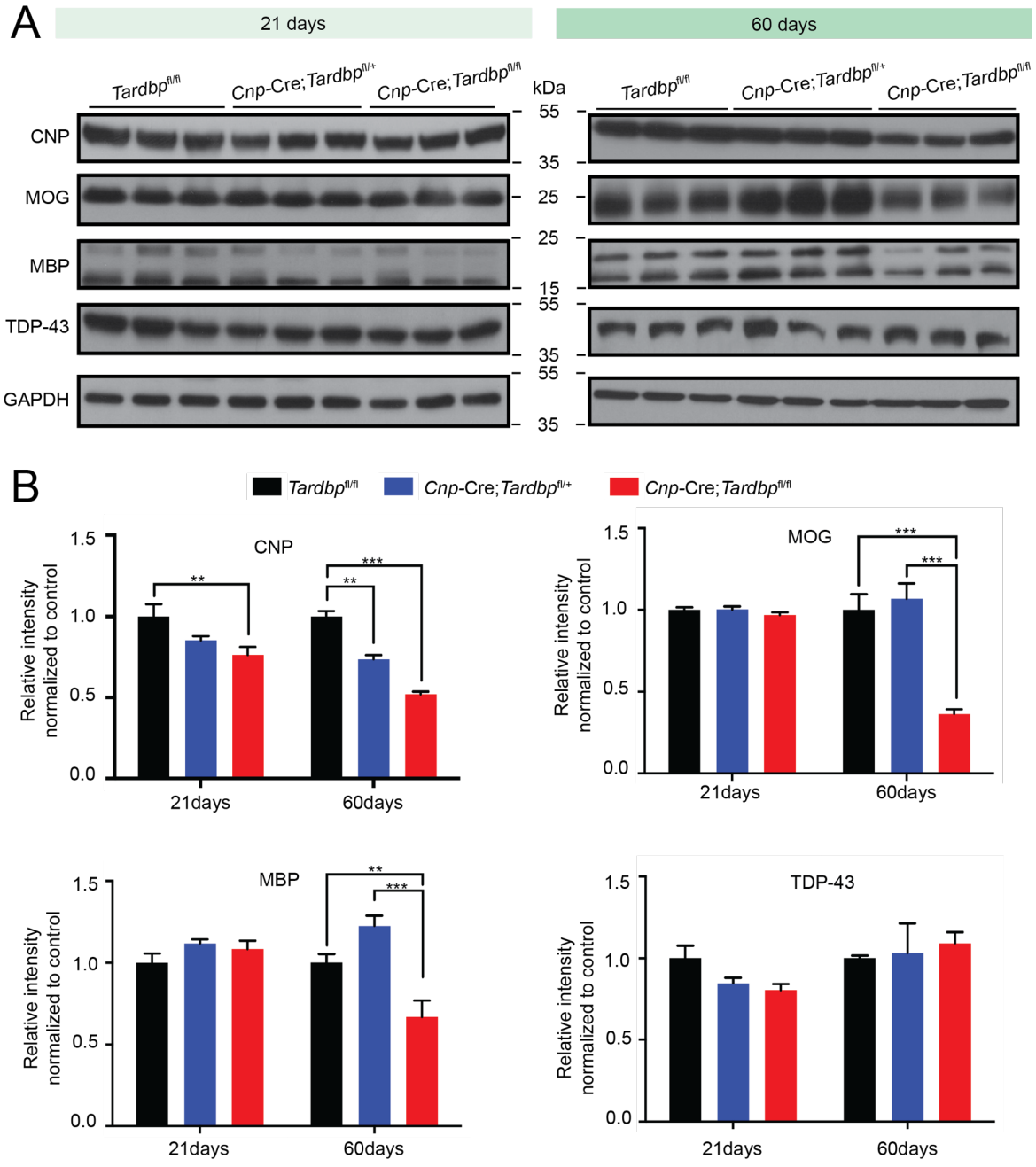

Fig. S8. **Progressive reduction of myelin proteins in the spinal cord of *Cnp*-Cre;*Tardbp*$^{fl/fl}$ mice.** (A) Western blot analysis of myelin proteins CNP, MOG and MBP, as well as TDP-43, using whole spinal cord lysate from 21 day- and 60-day old mice (n=3 per genotype). GAPDH was probed as internal control. (B) Quantifications of CNP, MOG, MBP and TDP-43 normalized to control. **: p <0.01, ***: p <0.001, One-way ANOVA.

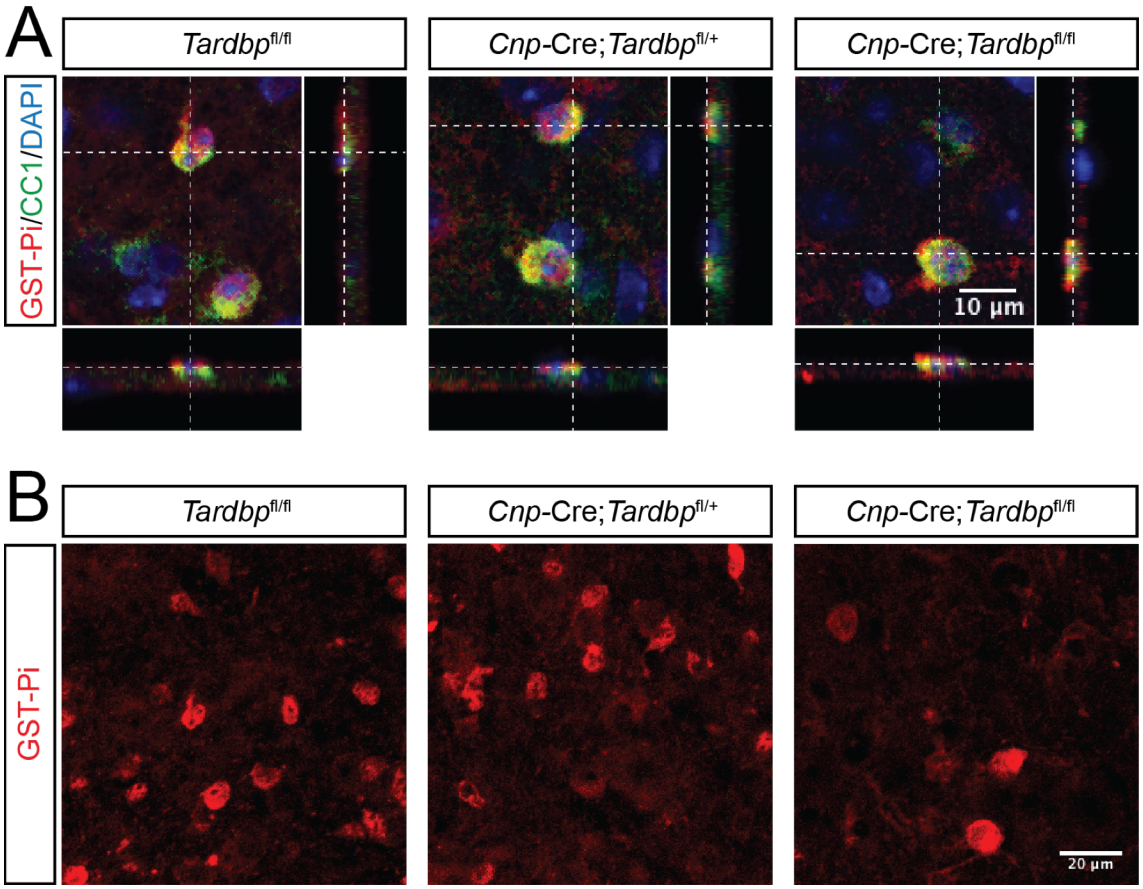

Fig. S9. Cell-autonomous degeneration of mature oligodendrocytes in the grey matter of the spinal cords of *Cnp-Cre*;*Tardbp*$^{fl/fl}$ mice. (A) Co-labelling of APC-CC1 and GST-Pi. Scale bar = 10 µm. (B) Reduced numbers of GST-pi, a mature oligodendrocyte marker, in the grey matter of *Cnp*-Cre;*Tardbp*$^{fl/fl}$ mice spinal cord. Scale bar = 20 µm.

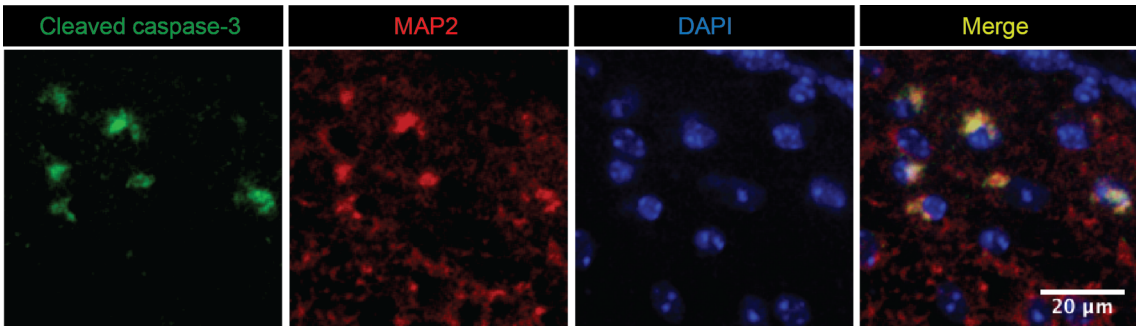

Fig. S10. Detection of cleaved caspase-3 following middle cerebral artery occlusion (MCAO). Confocal images of activated caspase-3 (green) co-labelled with MAP2 (red) on brain sections of mice with ischemic stroke caused by middle cerebral artery occlusion. Scale bar = 20 µm.

Supplemental Table 1. Quantification of CC1-positive cells

Age	Genotype	CC1-postivie cells (per mm^2)	
		Grey matter	White matter
21 days	*Tardbp*$^{fl/fl}$	1173 ± 56	1265 ± 89
	Cnp-Cre; *Tardbp*$^{fl/+}$	1164 ± 95	1196 ± 76
	Cnp-Cre; *Tardbp*$^{fl/fl}$	998 ± 5	1287 ± 34
60 days	*Tardbp*$^{fl/fl}$	1292 ± 17	1229 ± 66
	Cnp-Cre; *Tardbp*$^{fl/+}$	1291 ± 16	1225 ± 87
	Cnp-Cre; *Tardbp*$^{fl/fl}$	372 ± 37	1236 ± 102

Supplemental Table 2. Quantification of NG2-positive cells

Age	Genotype	NG2-postivie cells (per mm^2)	
		Grey matter	White matter
21 days	*Tardbp*$^{fl/fl}$	527 ± 13	590 ± 14
	Cnp-Cre; *Tardbp*$^{fl/+}$	563 ± 35	654 ± 12
	Cnp-Cre; *Tardbp*$^{fl/fl}$	525 ± 24	896 ± 18
60 days	*Tardbp*$^{fl/fl}$	320 ± 12	314 ± 4
	Cnp-Cre; *Tardbp*$^{fl/+}$	306 ± 1	360 ± 7
	Cnp-Cre; *Tardbp*$^{fl/fl}$	398 ± 15	875 ± 33

Supplemental Table 3. Quantification of Olig2-positive cells

Age	Genotype	Olig2-postivie cells (per mm^2)	
		Grey matter	White matter
21 days	*Tardbp*$^{fl/fl}$	1732 ± 56	1692 ± 120
	Cnp-Cre; *Tardbp*$^{fl/+}$	1737 ± 166	1696 ± 79
	Cnp-Cre; *Tardbp*$^{fl/fl}$	1512 ± 25	2059 ± 74
60 days	*Tardbp*$^{fl/fl}$	1824 ± 34	1751 ± 25
	Cnp-Cre; *Tardbp*$^{fl/+}$	1751 ± 12	1661 ± 8
	Cnp-Cre; *Tardbp*$^{fl/fl}$	744 ± 57	2273 ± 32

Supplemental Table 4: Primary antibodies used in this study

Name	Source	Catalog number	Concentration
Mouse anti-GFP	Wako	012-20461	1:200 (IF)
Rabbit anti-GFP	ProteinTech	50430-2-AP	1:200 (IF)
Rabbit anti-TDP-43	ProteinTech	10782-2-AP	1:500 (IF), 1:1,000 (WB)
Rabbit anti-GFAP (D1F4Q)	Cell Signaling Technology	12389S	1:1,000 (IF)
Rabbit anti-NeuN (D3S3I)	Cell Signaling Technology	12943S	1:1,000 (IF)
Mouse anti-APC (CC1)	Millipore	OP80	1:200 (IF)
Rabbit anti-NG2	Millipore	AB5320	1:500 (IF)
Rabbit anti-Olig2	Millipore	AB9610	1:500 (IF)
Mouse anti-Ki67	Dako	M7248	1:500 (IF)
Rat anti-MBP	Millipore	MAB386	1:500 (IF), 1:2,000 (WB)
Mouse anti-RIPK1	BD Biosciences	610459	1:500 (IF)
Mouse anti-CNP	Sigma	C5922	1:2,000 (WB)
Mouse anti-MOG	Millipore	MAB5680	1:2,000 (WB)
Mouse anti-GAPDH	Millipore	MAB374	1:5,000 (WB)
Rabbit anti-RIPK1 (D94C12)	Cell Signaling Technology	3493	1:1,000 (WB)
Rabbit anti-MLKL	Abcam	ab172868	1:1,000 (WB)
Mouse anti-GAPDH	ProteinTech	60004-1-Ig	1:10,000 (WB)
Mouse anti-TDP-43	In-house	Ling et al., PNAS 2010	1:1,000 (IF) 1:1,000 (WB)

Supplemental Table 5: Secondary antibodies used in this study

Name	Source	Catalog number	Concentration
Alexa Fluor™ 488 Donkey anti-mouse IgG (H+L)	Thermo Fisher Scientific	A21202	1:1,000 (IF)
Alexa Fluor™ 568 Donkey anti-mouse IgG (H+L)	Thermo Fisher Scientific	A10037	1:1,000 (IF)
Alexa Fluor™ 647 Donkey anti-mouse IgG (H+L)	Thermo Fisher Scientific	A31571	1:1,000 (IF)
Alexa Fluor™ 488 Donkey anti-rabbit IgG (H+L)	Thermo Fisher Scientific	A21206	1:1,000 (IF)
Alexa Fluor™ 568 Donkey anti-rabbit IgG (H+L)	Thermo Fisher Scientific	A10042	1:1,000 (IF)
Alexa Fluor™ 647 Donkey anti-rabbit IgG (H+L)	Thermo Fisher Scientific	A31573	1:1,000 (IF)
Alexa Fluor™ 488 Donkey anti-rat IgG (H+L)	Thermo Fisher Scientific	A21208	1:1,000 (IF)
Anti-mouse IgG2a cross-absorbed secondary antibody Alexa Fluor 488	Thermo Fisher Scientific	A21131	1:1,000 (IF)
Anti-mouse IgG2b cross-absorbed secondary antibody Alexa Fluor 568	Thermo Fisher Scientific	A21144	1:1,000 (IF)
Goat anti-rabbit IgG (H+L) secondary antibody, HRP	Thermo Fisher Scientific	31460	1:10,000 (WB)
Goat anti-mouse IgG (H+L) secondary antibody, HRP	Thermo Fisher Scientific	31430	1:10,000 (WB)
Goat anti-rat IgG (H+L) secondary antibody, HRP	Thermo Fisher Scientific	31470	1:10,000 (WB)

Supplemental Table 6: qRT-PCR primer sequences

Target gene	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
Mouse ***Ripk1***	GAAGACAGACCTAGACAGCGG	CCAGTAGCTTCACCACTCGAC
Mouse ***Ripk3***	TCTGTCAAGTTATGGCCTACTGG	GGAACACGACTCCGAACCC
Mouse ***Plp1***	CACTTACAACTTCGCCGTCCT	GGGAGTTTCTATGGGAGCTCAGA
Mouse ***Mag***	CTGCCGCTGTTTTGGATAATGA	CATCGGGGAAGTCGAAACGG
Mouse ***Mbp***	GCTCCCTGCCCCAGAAGT	TGTCACAATGTTCTTGAAGAAATGG
Mouse ***Mog***	AGCTGCTTCCTCTCCCTTCTC	ACTAAAGCCCGGATGGGATAC
Mouse ***Gapdh***	TCACCACCATGGAGAAGGC	GCTAAGCAGTTGGTGGTGCA

Supplemental table 7. Summary of statistical analysis

(1) Figure 1C: Phenotype scoring for Ledge, Tremor, Gait and Body Weight tests

Ledge

Contrasts				ANOVA		
Contrast	**Age (weeks)**	**Estimate**	**P**	**Param**	**F value**	**P**
het - homo	4	-0.150	0.233	genotype	921.70	<0.001
het - homo	5	-0.559	<0.001	week	36.52	<0.001
het - homo	6	-1.438	<0.001	genotype:week	40.06	<0.001
het - homo	7	-1.656	<0.001			
het - homo	8	-1.868	<0.001			
het - homo	9	-2.313	<0.001			
het - homo	10	-2.000	<0.001			

Tremor

Contrasts				ANOVA		
Contrast	**Age (weeks)**	**Estimate**	**P**	**Param**	**F value**	**P**
het - homo	4	-0.600	<0.001	genotype	1086.49	<0.001
het - homo	5	-0.588	<0.001	week	39.03	<0.001
het - homo	6	-1.125	<0.001	genotype:week	42.82	<0.001
het - homo	7	-2.132	<0.001			
het - homo	8	-2.395	<0.001			
het - homo	9	-1.313	<0.001			
het - homo	10	-1.500	<0.001			

Gait

Contrasts				ANOVA		
Contrast	**Age (weeks)**	**Estimate**	**P**	**Param**	**F value**	**P**
het - homo	4	-0.100	0.430	genotype	137.45	<0.001
het - homo	5	-0.088	0.388	week	10.92	<0.001
het - homo	6	-0.125	0.346	genotype:week	12.91	<0.001
het - homo	7	-0.579	<0.001			
het - homo	8	-0.947	<0.001			
het - homo	9	-1.125	<0.001			
het - homo	10	-1.000	<0.001			

Body Weight

Contrasts				ANOVA		
Contrast	**Age (weeks)**	**Estimate**	**P**	**Param**	**F value**	**P**
het - homo	4	2.966	0.006	genotype	47.01	<0.001
het - homo	5	2.454	0.005	week	21.73	<0.001
het - homo	6	2.106	0.059	genotype:week	0.46	0.8369
het - homo	7	2.634	0.002			
het - homo	8	2.442	0.004			
het - homo	9	3.700	0.004			
het - homo	10	5.000	0.006			

(2) Figure 1D: Grip strength test

Contrasts					Linear mixed models		
contrast	**test**	**timepoint**	**estimate**	**P**	**Param**	**Coeff**	**P**
het - homo	Full Body	21	17.775	0.0248	Week 7 (W7)	11.201	0.116
het - homo	Full Body	60	35.267	<0.001	Fore Paws(FP)	-82.268	<0.001
het - homo	Fore Paws	21	5.797	0.450	Homozygous (H)	-17.775	0.020
het - homo	Fore Paws	60	20.525	0.010	W7:FP	8.401	0.404
					W7:H	-17.492	0.054
					FP:H	11.978	0.187
					W7:FP:H	2.764	0.830

(3) Figure 1E: Balance beam test

6mm

Contrasts				Linear mixed models		
Contrast	**Week**	**Estimate**	**P**	**Param**	**Coeff**	**P**
het - homo	5	-0.015	0.996	Homozygous (Ho)	0.015	0.996
het - homo	7	-13.353	<0.001	Week 7 (W7)	-0.628	0.725
				Ho:W7	13.338	<0.001

12mm

Contrasts				Linear mixed models		
Contrast	**Week**	**Estimate**	**P**	**Param**	**Coeff**	**P**
het - homo	5	0.180	0.954	Homozygous (Ho)	-0.180	0.945
het - homo	7	-13.244	<0.001	Week 7 (W7)	-0.371	0.824
				Ho:W7	13.425	<0.001

(4) Figure 2C: Quantification of g-ratio

Contrasts			Linear mixed models		
contrast	**estimate**	**P**	**Param**	**Coeff**	**P**
het – homo, 21d	-0.067	< 0.001	log(axonal_diameter) (AD)	0.025	<0.001
het – homo, 60d	-0.105	< 0.001	Homozygous (Ho)	0.044	0.003
			Time-60d (T60)	-0.023	0.113
			AD:Ho	0.061	<0.001
			AD:T60	0.025	0.002
			Ho:T60	0.032	0.116

(5) Figure 3B: Quantification of CC1-positive cells

Contrasts					Linear mixed models		
Contrast	**Tissue**	**Age**	**Estimate**	**P**	**Param**	**Coeff**	**P**
control - het	grey	21	9.333	0.994	Heterozygous (He)	4.717	0.415
control - homo	grey	21	175.333	0.165	Homozygous (Ho)	0.701	0.904
het - homo	grey	21	166.000	0.196	White matter (W)	-21.178	<0.001
control - het	white	21	68.667	0.742	Time-60d (T60)	-0.530	0.927
control - homo	white	21	-22.667	0.968	He:W	-10.141	0.064
het - homo	white	21	-91.333	0.593	Ho:W	20.040	<0.001
control - het	grey	60	1.333	1.00	He:T60	-3.496	0.670
control - homo	grey	60	920.667	< 0.001	Ho:T60	5.126	0.531
het - homo	grey	60	919.333	< 0.001	W:T60	-4.800	0.380
control - het	white	60	3.667	0.999	He:W:T60	8.109	0.295
control - homo	white	60	-7.333	0.997	Ho:W:T60	22.992	0.003
het - homo	white	60	-11.000	0.992			

(6) Figure 3C: Quantification of TUNEL-positive cells

Day 21

Contrasts				Linear mixed models		
Contrast	**Tissue**	**Estimate**	**P**	**Param**	**Coeff**	**P**
control - het	grey	-1.000	0.963	Heterozygous (He)	1.000	0.795
control - homo	grey	-29.917	<0.001	Homozygous (Ho)	29.917	<0.001
het - homo	grey	-28.917	<0.001	White matter (W)	5.583	0.012
control - het	white	-0.083	1.000	He:W	-0.917	0.770
control - homo	white	-25.500	<0.001	Ho:W	-4.417	0.158
het - homo	white	-25.417	<0.001			

Day 60

Contrasts				Linear mixed models		
Contrast	**Tissue**	**Estimate**	**P**	**Param**	**Coeff**	0.444
control - het	grey	-0.889	0.727	Heterozygous (He)	1.000	<0.001
control - homo	grey	-11.889	<0.001	Homozygous (Ho)	29.917	0.849
het - homo	grey	-11.000	<0.001	White matter (W)	5.583	0.787
control - het	white	-0.444	0.923	He:W	-0.917	<0.001
control - homo	white	-4.556	0.002	Ho:W	-4.417	0.444
het - homo	white	-4.111	0.005			

(7) Figure 3E: RIPK1 and RIPK3 expression

RIPK1

Contrasts				ANOVA		
Contrast	**Age**	**Estimate**	**P**	**Param**	**F value**	**P**
control - het	21 days	-0.003	1	timepoint	2.41	0.1337
control - homo	21 days	-0.703	<0.001	condition	30.27	< 0.001
het - homo	21 days	-0.699	<0.001	timepoint:condition	0.45	0.6409
control - het	60 days	-0.195	0.439			
control - homo	60 days	-0.874	<0.001			
het - homo	60 days	-0.680	<0.001			

RIPK3

Contrasts				ANOVA		
Contrast	**Age**	**Estimate**	**P**	**Param**	**F value**	**P**
control - het	21 days	-0.058	0.98	timepoint	6.27	0.0195
control - homo	21 days	-3.599	<0.001	condition	96.17	<0.001
het - homo	21 days	-3.541	<0.001	timepoint:condition	5.21	0.0132
control - het	60 days	0.072	0.976			
control - homo	60 days	-2.190	<0.001			
het - homo	60 days	-2.262	<0.001			

(8) Figure 4A: Quantification of NG2-positive cells

Contrasts					Linear mixed models		
contrast	**tissue**	**timepoint**	**estimate**	**P**	**Param**	**Coeff**	**P**
control - het	grey	21	-35.333	0.388	Heterozygous (Het)	35.333	0.181
control - homo	grey	21	2.000	0.997	Homozygous (Homo)	-2.000	0.940
het - homo	grey	21	37.333	0.350	White matter (WM)	62.667	0.018
control - het	white	21	-64.333	0.057	Time-60 (T60)	-207.333	<0.001
control - homo	white	21	-306.000	<0.001	Het:WM	29.000	0.438
het - homo	white	21	-241.667	<0.001	Homo:WM	308.000	<0.001
control - het	grey	60	13.667	0.863	Het:T60	-49.000	0.190
control - homo	grey	60	-78.333	0.018	Homo:T60	80.333	0.032
het - homo	grey	60	-92.000	0.005	WM:T60	-69.000	0.065
control - het	white	60	-46.667	0.202	Het:WM:T60	31.333	0.553
control - homo	white	60	-561.000	<0.001	Homo:WM:T60	174.667	0.001
het - homo	white	60	-478.500	<0.001			

(9) Figure 4B: Quantification of Ki67^{+} NG2-positive cells

Contrasts				Linear mixed models		
contrast	**tissue**	**estimate**	**P**	**Param**	**Coeff**	**P**
control - het	Grey	-4.333	0.9116	Heterozygous (He)	4.333	0.628
control - homo	Grey	-39.556	0.020	Homozygous (Ho)	39.556	<0.001
het – homo	Grey	-35.222	0.033	White matter (Wm)	4.889	0.206
control – het	White	-7.833	0.757	He:Wm	3.500	0.522
control – homo	White	-75.556	<0.001	Ho:Wm	36.000	<0.001
het – homo	White	-67.722	0.0011			

(10) Figure 4D: Quantification of cell body size of NG2$^+$ OPCs

Contrasts					Linear mixed models		
contrast	**tissue**	**timepoint**	**estimate**	**P**	**Param**	**Coeff**	**P**
control - het	grey	21	-4.717	0.698	Heterozygous (He)	4.717	0.415
control - homo	grey	21	-0.701	0.992	Homozygous (Ho)	0.701	0.904
het - homo	grey	21	4.015	0.770	White matter (W)	-21.178	<0.001
control - het	white	21	5.424	0.623	Time-60d (T60)	-0.530	0.927
control - homo	white	21	-20.742	0.005	He:W	-10.141	0.064
het - homo	white	21	-26.166	<0.001	Ho:W	20.040	<0.001
control - het	grey	60	-1.220	0.976	He:T60	-3.496	0.670
control - homo	grey	60	-5.828	0.581	Ho:T60	5.126	0.531
het - homo	grey	60	-4.607	0.709	W:T60	-4.800	0.380
control - het	white	60	0.812	0.989	He:W:T60	8.109	0.295
control - homo	white	60	-48.861	<0.001	Ho:W:T60	22.992	0.003
het - homo	white	60	-49.673	<0.001			

(11) Figure 4F: Sholl analysis of NG2$^+$ cell branches in lumbar spinal cord

Day 21 Grey matter

Contrasts				Linear mixed models		
contrast	**Distance from cell body**	**estimate**	**P**	**Param**	**Coeff**	**P**
control - het	10	1.867	0.650	Distance-20 (D20)	10.033	<0.001
control - homo	10	2.033	0.601	Distance-30 (D30)	-4.333	0.013
het - homo	10	0.167	0.997	Distance-40 (D40)	-17.867	<0.001
control - het	20	3.200	0.293	Distance-50 (D50)	-20.267	<0.001
control - homo	20	1.567	0.737	Heterozygous (He)	-1.867	0.373
het - homo	20	-1.633	0.718	Homozygous (Ho)	-2.033	0.332
control - het	30	-4.800	0.073	D20:He	-1.333	0.587
control - homo	30	-3.700	0.199	D30:He	6.667	0.007
het - homo	30	1.100	0.860	D40:He	3.467	0.157
control - het	40	-1.600	0.728	D50:He	2.300	0.348
control - homo	40	-4.167	0.133	D20:Ho	0.467	0.849
het - homo	40	-2.567	0.448	D30:Ho	5.733	0.020
control - het	50	-0.433	0.977	D40:Ho	6.200	0.012
control - homo	50	-0.633	0.951	D50:Ho	2.667	0.277
het - homo	50	-0.200	0.995			

Day 21 White matter

Contrasts				Linear mixed models		
contrast	**Distance from cell body**	**estimate**	**P**	**Param**	**Coeff**	**P**
control - het	10	1.833	0.639	Distance-20 (D20)	0.700	0.581
control - homo	10	2.333	0.491	Distance-30 (D30)	-11.100	<0.001
het - homo	10	0.500	0.966	Distance-40 (D40)	-15.333	<0.001
control - het	20	-1.467	0.748	Distance-50 (D50)	-16.067	<0.001
control - homo	20	8.500	0.002	Heterozygous (He)	-1.833	0.359
het - homo	20	9.967	0.001	Homozygous (Ho)	-2.333	0.243
control - het	30	-1.367	0.776	D20:He	3.300	0.066
control - homo	30	3.767	0.181	D30:He	3.200	0.075
het - homo	30	5.133	0.056	D40:He	2.133	0.235
control - het	40	-0.300	0.988	D50:He	1.933	0.282
control - homo	40	0.667	0.941	D20:Ho	-6.167	<0.001
het - homo	40	0.967	0.880	D30:Ho	-1.433	0.425
control - het	50	-0.100	0.999	D40:Ho	1.667	0.353
control - homo	50	0.000	1.000	D50:Ho	2.333	0.194
het - homo	50	0.100	0.999			

Day 60 Grey matter

Contrasts				Linear mixed models		
contrast	**distance_from_cell_body**	**estimate**	**P**	**Param**	**Coeff**	**P**
control - het	10	3.533	0.555	Distance-20 (D20)	14.167	<0.001
control - homo	10	7.800	0.081	Distance-30 (D30)	12.633	<0.001
het - homo	10	4.267	0.430	Distance-40 (D40)	-4.700	0.045
control - het	20	3.400	0.579	Distance-50 (D50)	-18.133	<0.001
control - homo	20	12.000	0.007	Heterozygous (He)	-3.533	0.292
het - homo	20	8.600	0.051	Homozygous (Ho)	-7.800	0.021
control - het	30	-1.767	0.859	D20:He	0.133	0.968
control - homo	30	9.467	0.031	D30:He	5.300	0.109
het - homo	30	11.233	0.011	D40:He	7.033	0.034
control - het	40	-3.500	0.561	D50:He	5.800	0.080
control - homo	40	5.233	0.291	D20:Ho	-4.200	0.204
het - homo	40	8.733	0.048	D30:Ho	-1.667	0.615
control - het	50	-2.267	0.781	D40:Ho	2.567	0.438
control - homo	50	2.067	0.813	D50:Ho	5.733	0.083
het - homo	50	4.333	0.420			

Day 60 White matter

Contrasts				Linear mixed models		
contrast	**distance_from_cell_body**	**estimate**	**P**	**Param**	**Coeff**	**P**
control - het	10	3.467	0.258	Distance-20 (D20)	7.433	<0.001
control - homo	10	5.433	0.048	Distance-30 (D30)	-1.933	0.230
het - homo	10	1.967	0.632	Distance-40 (D40)	-15.667	<0.001
control - het	20	3.333	0.284	Distance-50 (D50)	-19.133	<0.001
control - homo	20	10.433	<0.001	Heterozygous (He)	-3.467	0.104
het - homo	20	7.100	0.009	Homozygous (Ho)	-5.433	0.011
control - het	30	-3.500	0.251	D20:He	0.133	0.954
control - homo	30	12.267	<0.001	D30:He	6.967	0.003
het - homo	30	15.767	<0.001	D40:He	7.167	0.002
control - het	40	-3.700	0.216	D50:He	4.300	0.059
control - homo	40	4.000	0.171	D20:Ho	-5.000	0.028
het - homo	40	7.700	0.005	D30:Ho	-6.833	0.003
control - het	50	-0.833	0.919	D40:Ho	1.433	0.529
control - homo	50	0.933	0.900	D50:Ho	4.500	0.048
het - homo	50	1.767	0.690			

(12) Figure 5B: Quantification of Edu$^+$NG2$^+$ cells

White matter

Contrasts			Linear mixed models		
Contrast	**estimate**	**P**	**Param**	**Coeff**	**P**
control - het	7.756	0.666	Heterozygous	-7.756	0.375
control - homo	-145.083	<0.001	Homozygous	145.083	<0.001
het - homo	-152.839	<0.001			

Grey matter

Contrasts			Linear mixed models		
Contrast	**estimate**	**P**	**Param**	**Coeff**	**P**
control - het	-6.375	0.749	Heterozygous	6.375	0.459
control - homo	-61.431	<0.001	Homozygous	61.431	<0.001
het - homo	-55.056	0.002			

(13) Figure 5D: Quantification of Edu$^+$CC1$^+$ cells

White matter

Contrasts			Linear mixed models		
Contrast	**estimate**	**P**	**Param**	**Coeff**	**P**
control - het	-8.786	0.745	Heterozygous	8.786	0.454
control - homo	-225.84	<0.001	Homozygous	225.840	<0.001
het - homo	-217.054	<0.001			

Grey matter

Contrasts			Linear mixed models		
Contrast	**estimate**	**P**	**Param**	**Coeff**	**P**
control - het	-1.715	0.651	Heterozygous	1.715	0.360
control - homo	-18.183	<0.001	Homozygous	18.183	<0.001
het - homo	-16.468	<0.001			

(14) Figure 5E: Quantification of Olig2-positive cells

Contrasts					Linear mixed models		
contrast	**tissue**	**timepoint**	**estimate**	**P**	**Param**	**Coeff**	**P**
control - het	grey	21	-5.000	0.999	White matter (W)	-39.667	0.614
control - homo	grey	21	219.667	0.108	Heterozygous (He)	5.000	0.962
het - homo	grey	21	224.667	0.099	Homozygous (Ho)	-219.667	0.034
control - het	white	21	-3.667	0.999	Time-60d	92.000	0.373
control - homo	white	21	-366.667	0.005	W:He	-1.333	0.991
het - homo	white	21	-363.000	0.005	W:Ho	586.333	<0.001
control - het	grey	60	72.667	0.763	W:T60	-33.333	0.765
control - homo	grey	60	1080.333	<0.001	He:T60	-77.667	0.595
het - homo	grey	60	1007.667	<0.001	Ho:T60	-860.667	<0.001
control - het	white	60	90.000	0.663	W:He:T60	-16.000	0.919
control - homo	white	60	-522.333	<0.001	W:Ho:T60	1,016.333	<0.001
het - homo	white	60	-612.333	<0.001			

(15) Figure 6B: Quantification of qRT-PCR

Plp1 21days

Contrasts			ANOVA		
Contrast	**Estimate**	**P**	**Param**	**F value**	**P**
control - het	-0.094	0.524	condition	8.155	0.006
control - homo	0.238	0.039			
het - homo	0.332	0.005			

Plp1 60 days

Contrasts			ANOVA		
Contrast	**Estimate**	**P**	**Param**	**F value**	**P**
control - het	-0.244	0.111	condition	11.394	0.002
control - homo	0.284	0.060			
het - homo	0.528	0.001			

Mbp 21days

Contrasts			ANOVA		
Contrast	**Estimate**	**P**	**Param**	**F value**	**P**
control - het	-0.081	0.700	condition	3.305	0.072
control - homo	0.168	0.244			
het - homo	0.248	0.006			

Mbp 60 days

Contrasts			ANOVA		
Contrast	**Estimate**	**P**	**Param**	**F value**	**P**
control - het	-0.168	0.233	condition	11.921	<0.001
control - homo	0.300	0.024			
het - homo	0.468	0.001			

Mag 21days

Contrasts			ANOVA		
Contrast	**Estimate**	**P**	**Param**	**F value**	**P**
control - het	-0.069	0.807	condition	0.544	0.594
control - homo	0.044	0.951			
het - homo	0.114	0.570			

Mag 60 days

Contrasts			ANOVA		
Contrast	**Estimate**	**P**	**Param**	**F value**	**P**
control - het	-0.149	0.297	condition	13.117	<0.001
control - homo	0.327	0.013			
het - homo	0.476	<0.001			

Mog 21days

Contrasts			ANOVA		
Contrast	**Estimate**	**P**	**Param**	**F value**	**P**
control - het	-0.092	0.565	condition	0.906	0.430
control - homo	-0.111	0.444			
het - homo	-0.019	0.975			

Mog 60 days

Contrasts			ANOVA		
Contrast	**Estimate**	**P**	**Param**	**F value**	**P**
control - het	-0.191	0.167	condition	8.973	0.004
control - homo	0.223	0.097			
het - homo	0.414	0.003			

Supplemental Video 1. Locomotor activites of 7-week-old *Cnp-Cre*;*Tardbp*$^{fl/+}$ and *Cnp-Cre*;*Tardbp*$^{fl/fl}$ mice. *Cnp-Cre*;*Tardbp*$^{fl/fl}$ mice showed tremor phenotype.

Supplemental Video 2. Balance beam test for 5-week-old *Cnp-Cre*;*Tardbp*$^{fl/+}$ mice.

Supplemental Video 3. Balance beam test for 5-week-old *Cnp-Cre*;*Tardbp*$^{fl/fl}$ mice.

Supplemental Video 4. Balance beam test for 7-week-old *Cnp-Cre*;*Tardbp*$^{fl/+}$ mice.

Supplemental Video 5. Balance beam test for 7-week-old *Cnp-Cre*;*Tardbp*$^{fl/fl}$ mice.

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