Additional file 4 (Supplemental figures)

ALS-linked mutant TDP-43 in oligodendrocytes induces oligodendrocyte damage and exacerbates motor dysfunction in mice

Mai Horiuchi, Seiji Watanabe, Okiru Komine, Eiki Takahashi, Kumi Kaneko, Shigeyoshi Itohara, Mayuko Shimada, Tomoo Ogi and Koji Yamanaka

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Supplementary Methods

Motor neuron				
	Reference	**Animal model**	**Promoter**	**Phenotype and Pathology**
Loss-of-function	Iguchi et al. (2013) [28]	*Tardbp* ^{floxed} conditional knockout mouse	*VAChT-Cre*	Progressive motor dysfunction and atrophy of motor neurons
	Donde et al. (2019) [12]	*Tardbp* ^{floxed} conditional knockout mouse (Jax stock: 017591)	*ChAT-IRES-Cre*	Progressive motor dysfunction and motor neuron death
Gain-of-toxicity	Huang et al. (2012) [24]	TDP-43^{M337V} conditional transgenic rat	*ChAT* (Tet-On system)	Progressive paralysis and motor neuron death

Oligodendrocyte				
	Reference	**Animal model**	**Promoter**	**Phenotype and Pathology**
Loss-of-function	Wang et al. (2018) [67]	*Tardbp* ^{floxed} conditional knockout mouse (Jax stock: 017591)	*Cnp-Cre*	Progressive motor dysfunction and early lethality accompanied with myelin reduction
	Heo et al. (2022) [22]	*Tardbp* ^{floxed} conditional knockout mouse	*Pdgfra-iCreER*	Progressive loss of oligodendrocyte precursor cells
			Mobp-iCre	Seizures and early lethality accompanied with morphological abnormalities in oligodendrocytes
			Mog-iCre	No obvious motor dysfunction Myelin thinner and loss of myelinated axon
			Mobp-iCreER	Hindlimb paralysis and hypertrophy of oligodendrocytes
Gain-of-toxicity	This study	TDP-43^{M337V} conditional transgenic mouse	*Mbp-Cre*	Mild motor dysfunction accompanied with oligodendrocyte dysfunction

Figure S1. Summary of TDP-43-based rodent models focusing on motor neurons or oligodendrocytes

A list of the TDP-43-based rodent models focusing on gain-of-toxicity or loss-of-function in motor neurons or oligodendrocytes with the literature review.

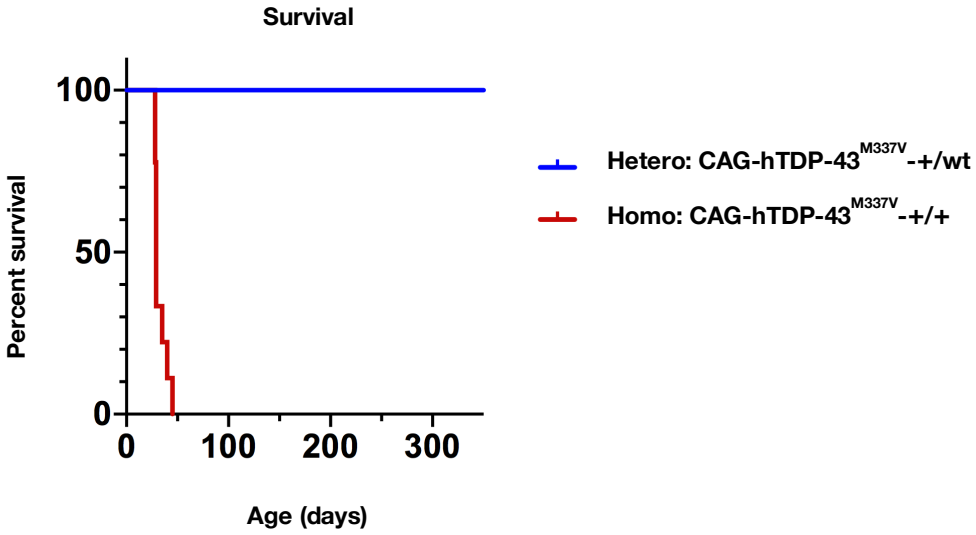

Figure S2. Ubiquitous overexpression of TDP-43^{M337V} causes early lethality in mice.

Survival curves for CAG-hTDP-43^{M337V}-+/wt (n = 11) and CAG-hTDP-43^{M337V}-+/+ (n = 9) mice. CAG-hTDP-43^{M337V}-+/+ mice with ubiquitous overexpression of TDP-43^{M337V} exhibited early lethality.

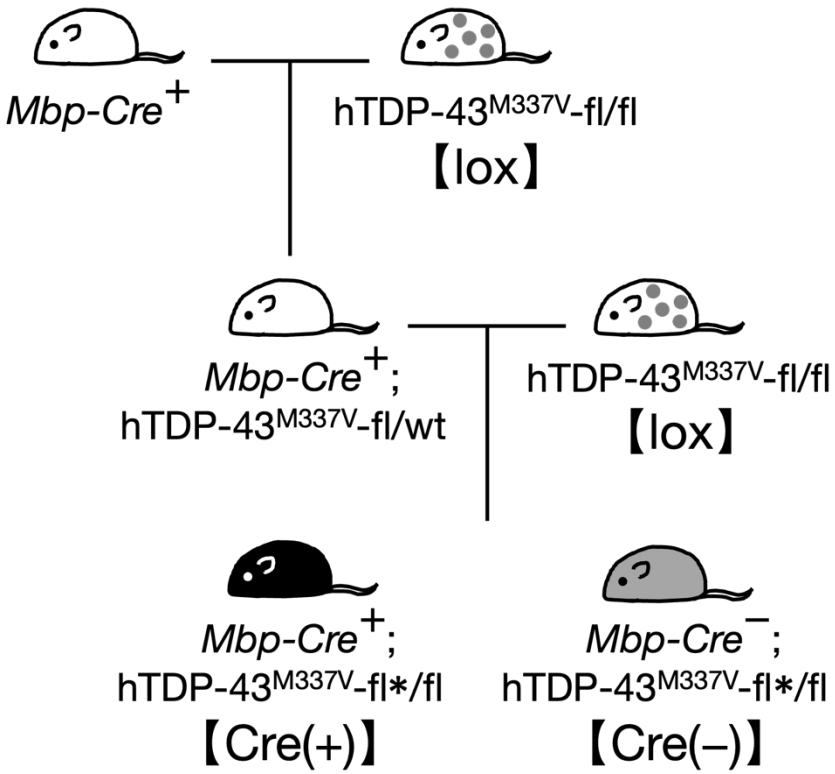

Figure S3. Schematic of crossbreeding to generate *Mbp-Cre*; hTDP-43^{M337V}-fl*/fl mice

Mbp-Cre^{+} mice were crossbred with homozygous hTDP-43^{M337V}-fl/fl mice to generate *Mbp-Cre*$^{+}$; hTDP-43^{M337V}-fl/wt mice, which were further crossed with hTDP-43^{M337V}-fl/fl mice to generate Cre(+) and Cre(–) mice. Asterisks indicate recombinant alleles.

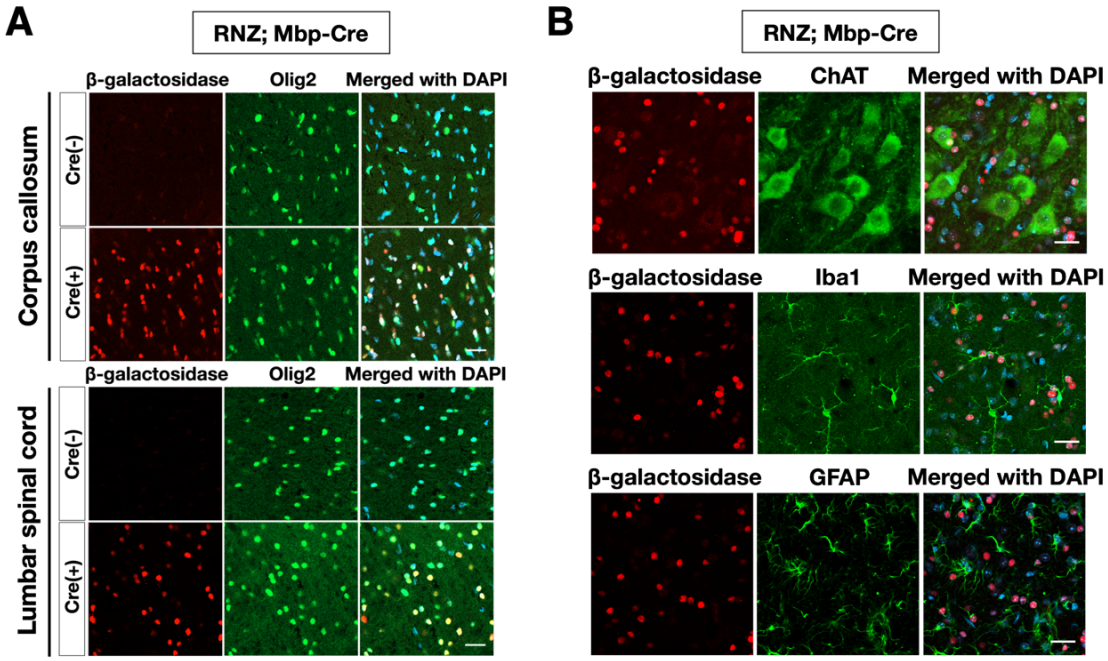

Figure S4. Cre-dependent recombination occurs in the oligodendrocytes of *Mbp-Cre*$^{+}$; *Rosa*-NLS-LacZ-fl/wt mice.

(A) Representative immunofluorescence images for β-galactosidase (red) and oligodendrocytes (Olig2, green) in the corpus callosum and lumbar spinal cord of *Mbp-Cre*$^{-}$; *Rosa*-NLS-LacZ-fl/wt mice and *Mbp-Cre*$^{+}$; *Rosa*-NLS-LacZ-fl/wt mice at one month of age. β-galactosidase signals were positive in the Olig2^{+} cells of Cre-positive mice. (B) Representative immunofluorescence images of motor neurons (ChAT), microglia (Iba1), and astrocytes (GFAP) with β-galactosidase in the lumbar spinal cord of *Mbp-Cre*$^{+}$; *Rosa*-NLS-LacZ-fl/wt mice at one month of age. β-galactosidase immunoreactivity was negative in these cell types. Scale bars: 25 μm (A, B).

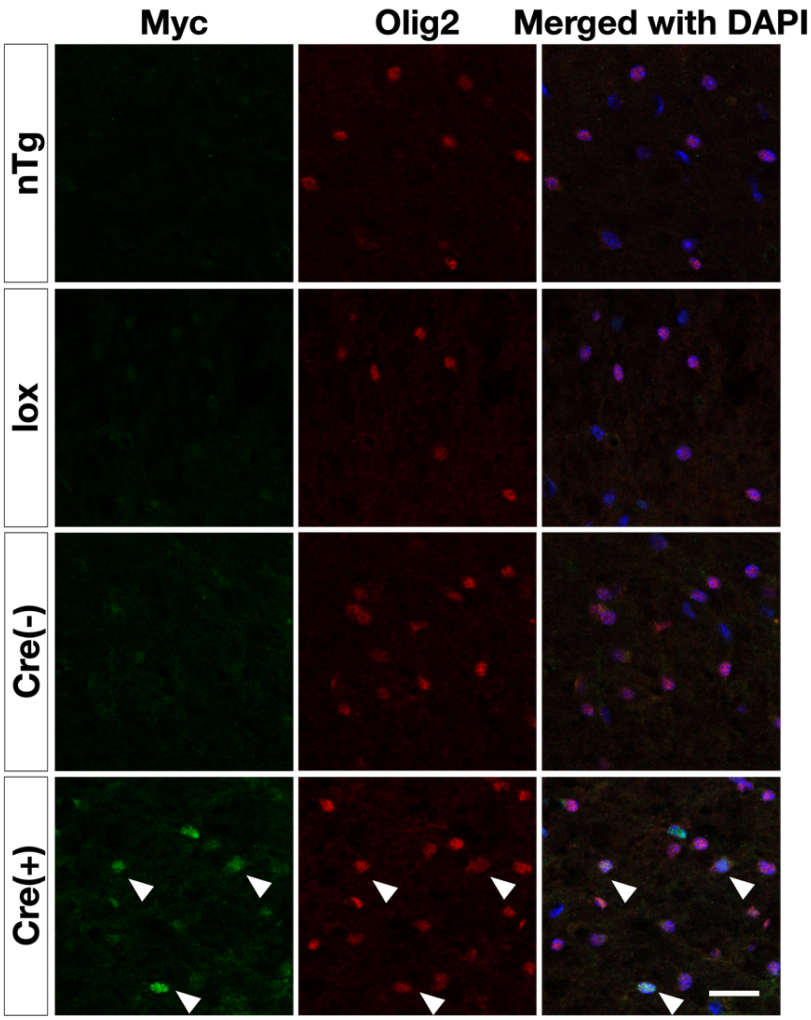

Figure S5. Oligodendrocytes overexpress TDP-43^{M337V} in *Mbp-Cre*$^{+}$; hTDP-43^{M337V}-fl*/fl mice.

Representative images showing immunofluorescence staining for Myc (green) and oligodendrocytes (Olig2, red) in the lumbar spinal cord of Cre(−) and Cre(+) mice at 6 months of age. Arrowheads indicate colocalization of Myc- and Olig2-positive oligodendrocytes. Scale bar: 25 μm.

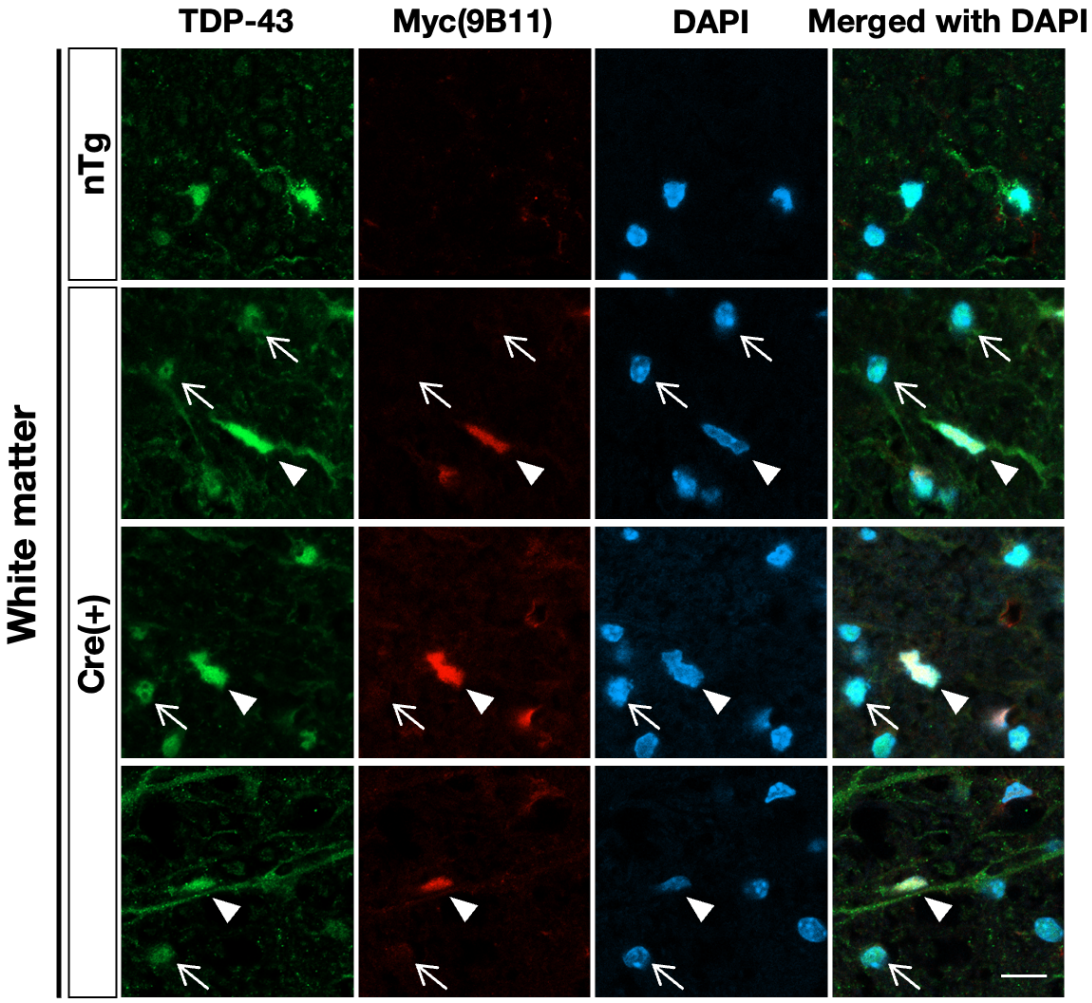

Figure S6. Endogenous TDP-43 localizes in the nucleus of *Mbp-Cre*$^{+}$; hTDP-43^{M337V}-fl*/fl mice overexpressing TDP-43^{M337V}.

Representative images showing immunofluorescence staining for endogenous and mutant TDP-43 (green) and mutant TDP-43 (Myc, red) in the lumbar spinal cord at 12 months of age. Arrowheads indicate Myc-positive cells; arrows indicate Myc-negative cells. TDP-43 signal (green) colocalized with the nuclei (DAPI) in both cells. Scale bar: 10 μm.

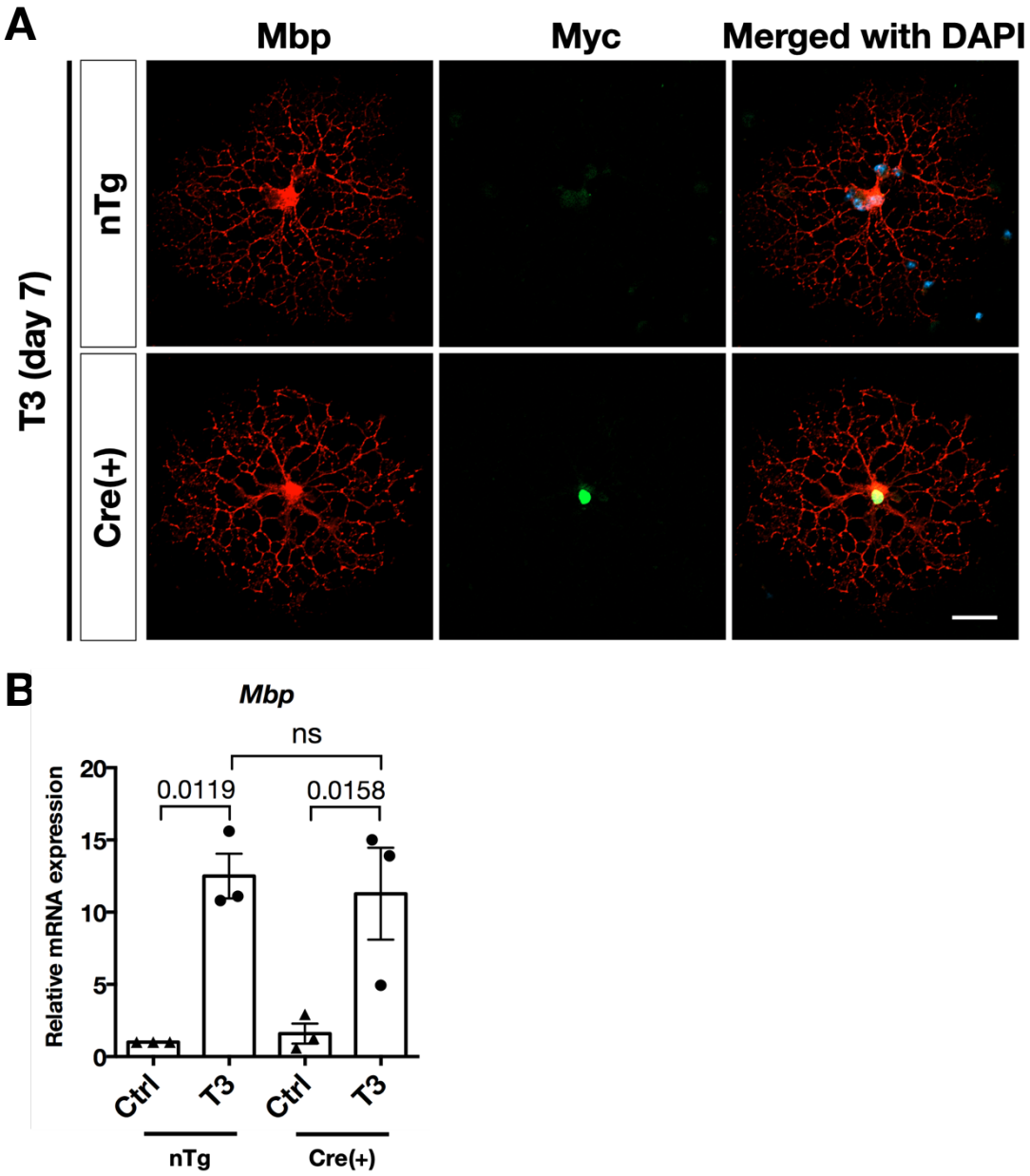

Figure S7. Differentiation is not impaired in oligodendrocyte precursor cells of *Mbp-Cre*$^{+}$; hTDP-43^{M337V}-fl*/fl mice.

(A) Representative immunofluorescence images for differentiated oligodendrocytes from nTg and Cre(+) mice cultured with differentiation medium for 7 days. The morphology of oligodendrocytes (Mbp, red) expressing mutant TDP-43 (Myc, green) was unaffected (bottom). (B) mRNA levels of *Mbp* in oligodendrocyte precursor cells cultured with proliferation medium (Ctrl) or differentiation medium (T3) for 3 days. Scale bar: 25 μm.

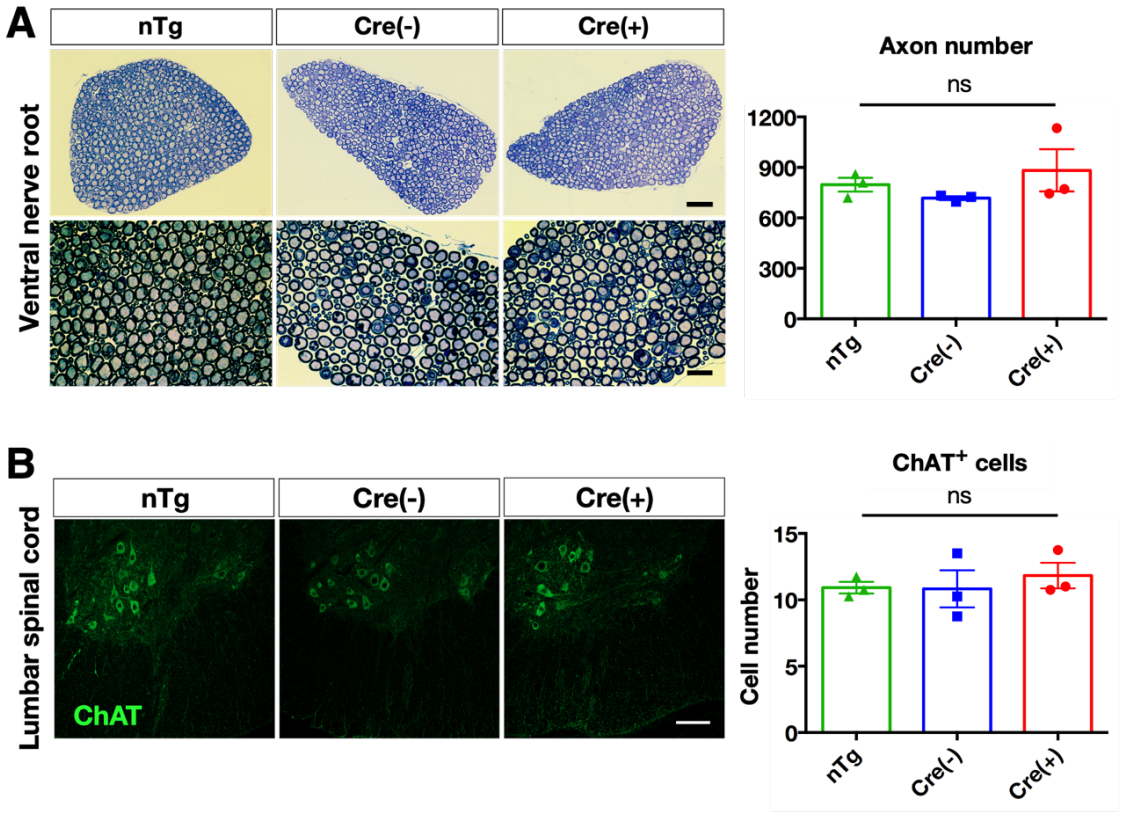

Figure S8. Motor neuronal loss in the lumbar spinal cord is not observed in *Mbp-Cre*$^{+}$; hTDP-43^{M337V}-fl*/fl mice.

(A) Representative images for toluidine blue staining of the L4 ventral nerve roots of Cre(－) and Cre(+) mice at 12 months of age. No axonal degeneration was observed in Cre(+) mice. (B) Representative images showing immunofluorescence staining for motor neurons (ChAT, green) in the ventral horns of the lumbar spinal cords at 12 months of age. No neuronal degeneration was observed in Cre(+) mice. Quantification of axon numbers and ChAT-positive motor neurons are shown in (A) and (B), respectively (n = 4 sections per mouse). Data are presented as means ± SEMs; *p*-values were determined using one-way ANOVA followed by Tukey-Kramer multiple comparison tests. Scale bars: 50 μm (A, upper panel), 25 μm (A, lower panel), and 100 μm (B).

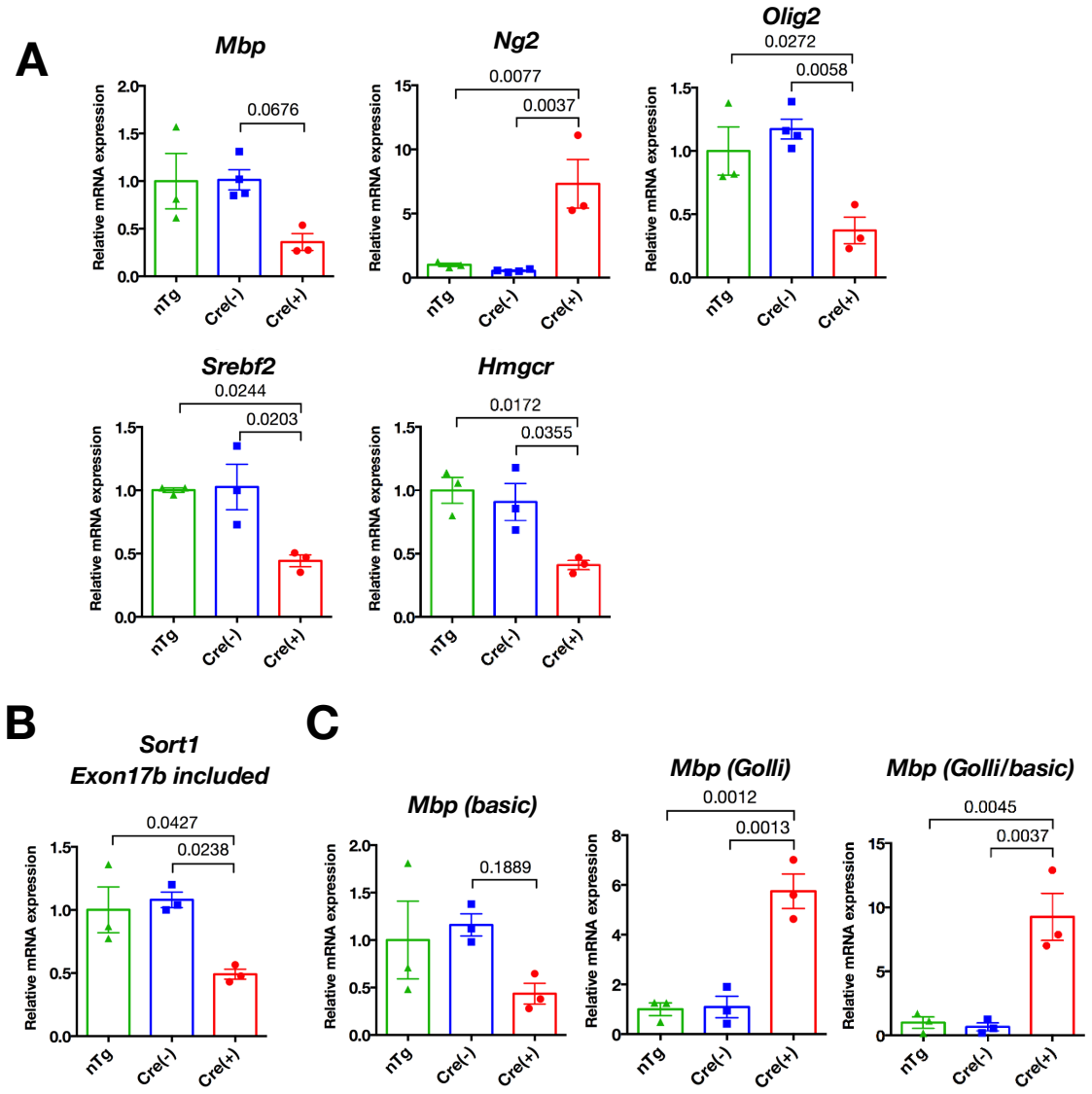

Figure S9. The genes involved in myelination are downregulated in oligodendrocyte lineage cells of *Mbp-Cre*$^{+}$; hTDP-43^{M337V}-fl*/fl mice.

mRNA levels of representative markers of oligodendrocyte lineage cells, cholesterol-related genes (A), and genes regulated by TDP-43 (B, C) in oligodendrocyte lineage cells isolated by MACS from whole brains of 12-month-old nTg, Cre(–), and Cre(+) mice were determined via quantitative PCR. Data are presented as means ± SEMs; *p*-values were determined using one-way ANOVA followed by Tukey–Kramer multiple comparison tests.

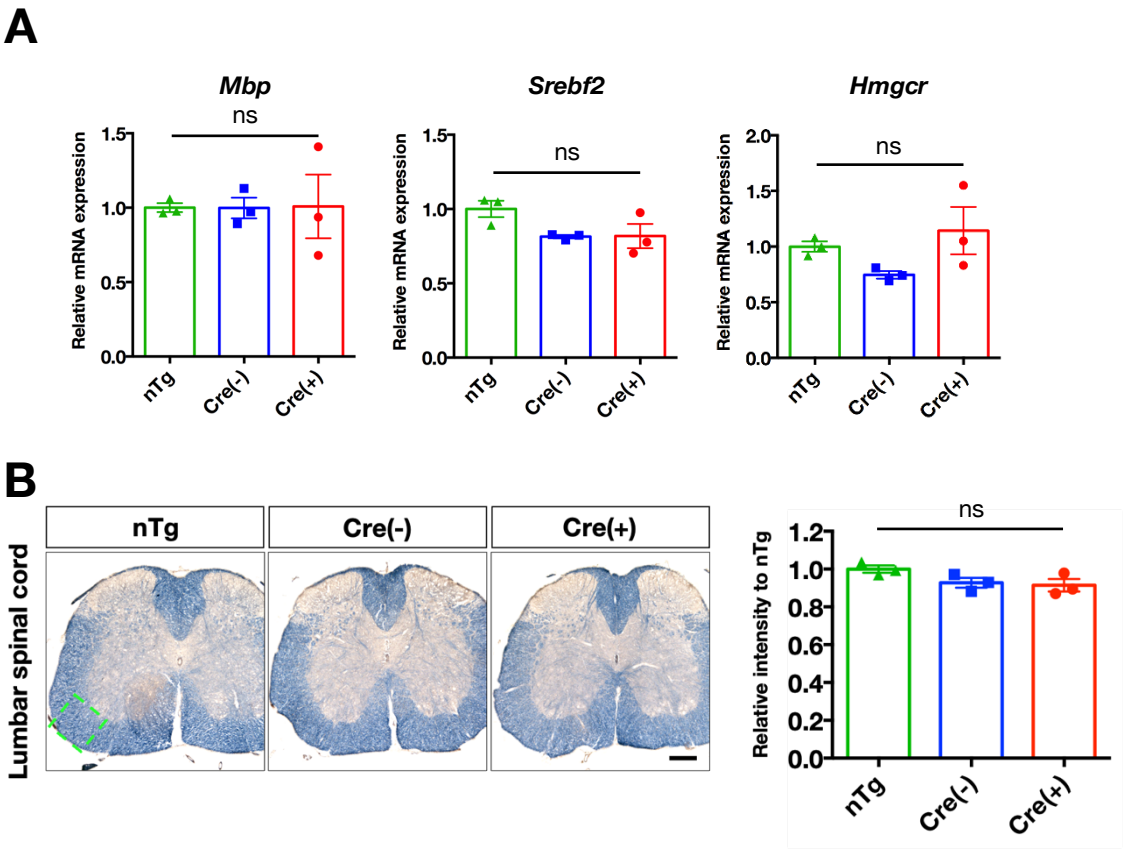

Figure S10. Oligodendrocyte dysfunction is not observed in *Mbp-Cre*$^{+}$; hTDP-43^{M337V}-fl*/fl mice at 1 month of age.

(A) mRNA levels of a representative oligodendrocyte marker and cholesterol-related genes in O1- and/or O4-positive oligodendrocyte lineage cells isolated by MACS from whole brains of 1-month-old mice were determined by quantitative PCR. (B) Representative images of Woelcke staining and signal intensity relative to nTg in the regions indicated by dashed squares in the white matter of the lumbar spinal cord in 1-month-old mice (n = 4 sections per mouse). Data are presented as means ± SEMs; *p*-values were determined using one-way ANOVA followed by Tukey-Kramer multiple comparison tests. Scale bar: 20 μm.

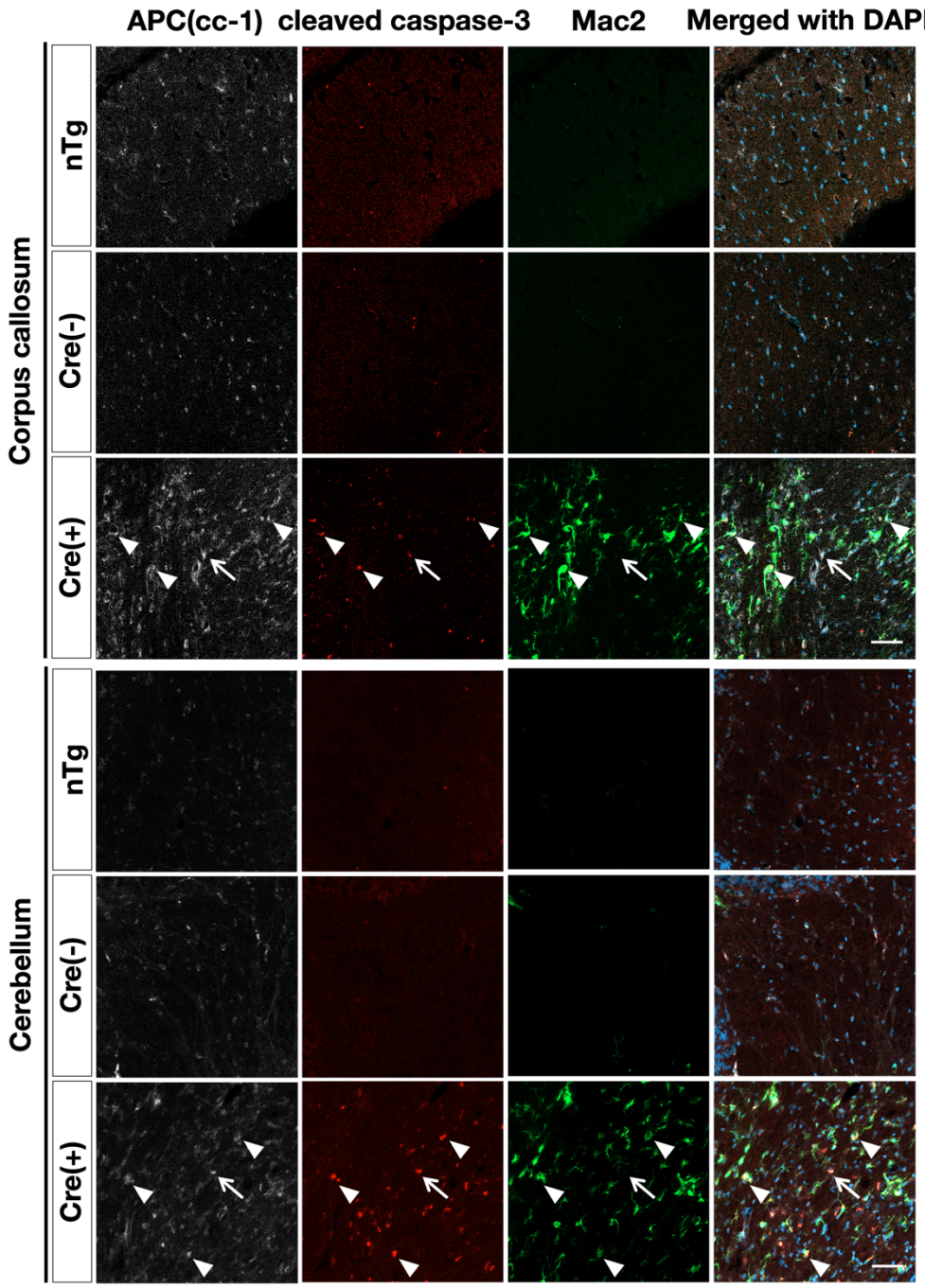

Figure S11. Activation of caspase-3 with gliosis in the corpus callosum and the cerebellum of *Mbp-Cre*$^{+}$; hTDP-43^{M337V}-fl*/fl mice

Representative immunofluorescence images of the corpus callosum and the cerebellar white matter from 12-month-old mice stained for APC, cleaved caspase-3, and Mac2. Arrowheads indicate apoptotic oligodendrocytes and activated microglial colocalization; arrows indicate apoptotic oligodendrocytes without microglial colocalization. Scale bars: 50 μm.

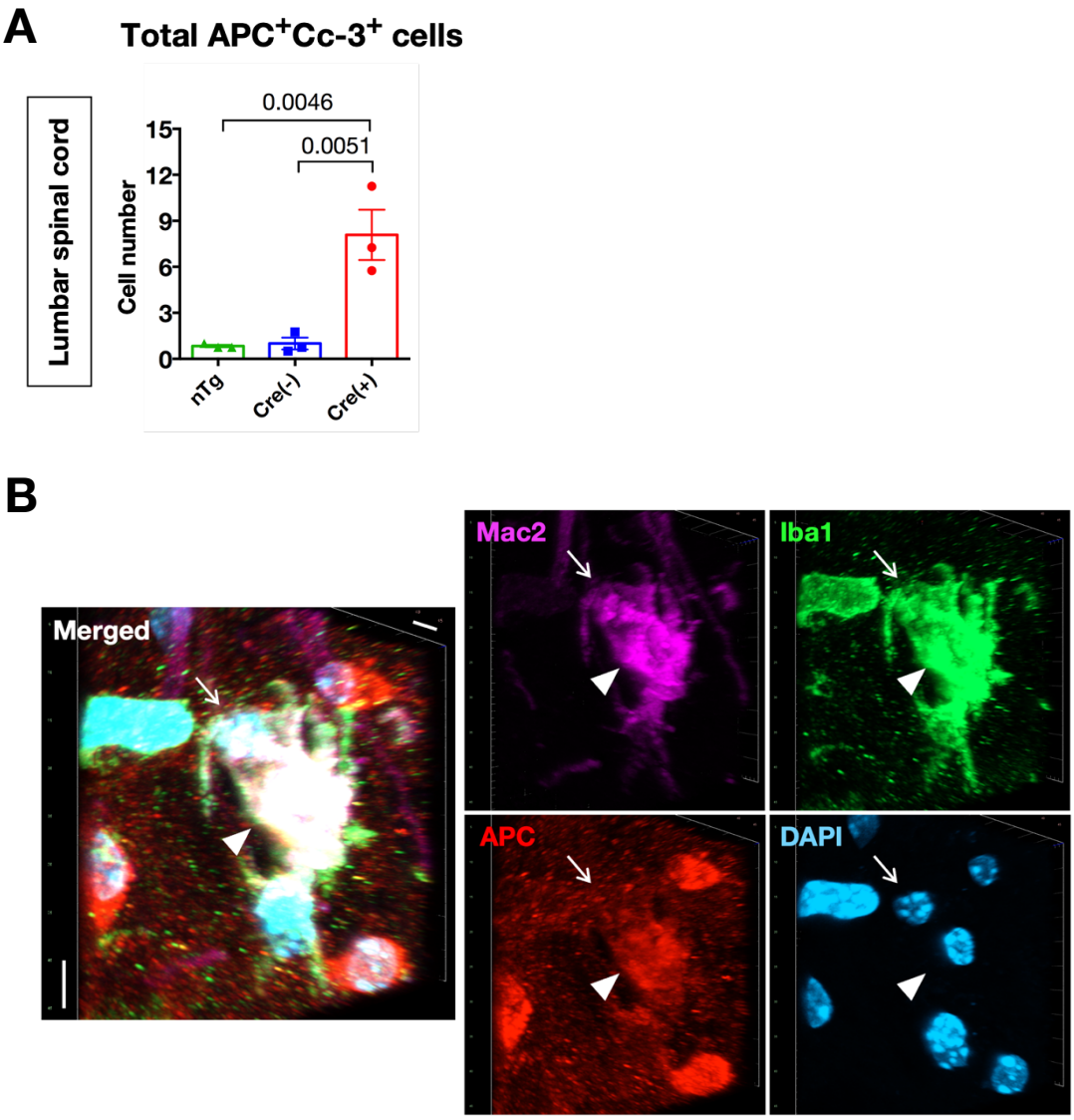

Figure S12. Activated microglia engulf oligodendrocytes in the white matter of *Mbp-Cre*$^{+}$; hTDP-43^{M337V}-fl*/fl mice.

(A) The total number of APC^{+}Cc-3^{+}(Cleaved caspase-3^{+}) cells per 0.1 mm^{2} in the white matter of the lumbar spinal cord was quantified and plotted (n = 4 sections per mouse). (B) Representative Z-stack images taken from immunofluorescence staining of the white matter in the lumbar spinal cord of a 12-month-old Cre(+) mouse stained for Mac2, Iba1, and APC. Arrowheads indicate an oligodendrocyte; arrows indicate activated microglia. Scale bar: 5 μm.

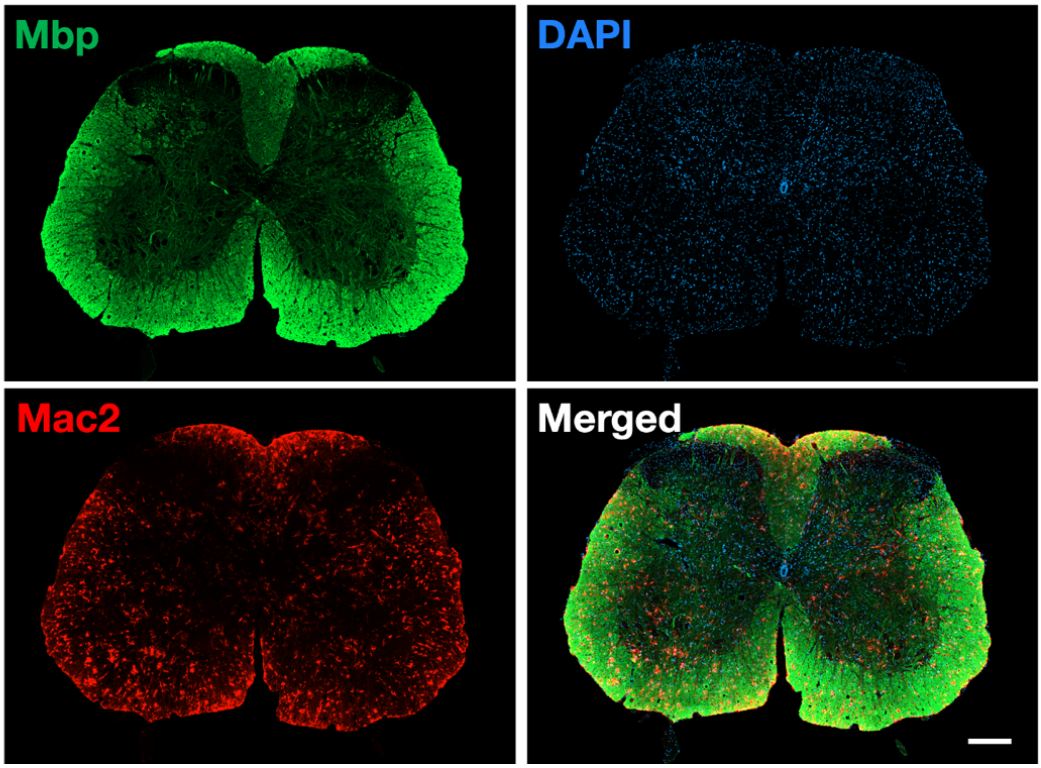

Figure S13. Gliosis in the white matter of the lumbar spinal cord from *Mbp-Cre*$^{+}$; hTDP-43^{M337V}-fl*/fl mice

Representative images showing immunofluorescence staining for the myelin basic protein (Mbp, green) and activated microglia (Mac2, red) in the lumbar spinal cord of Cre(+) mice at 12 months of age. Scale bar: 250 μm.

Supplementary Methods

Primary oligodendrocyte precursor cell culture

Primary OPCs were prepared as previously described with modifications [1]. Briefly, brains were isolated from mice on postnatal day 0–1, and the meninges were carefully removed in PBS. The tissue was minced into small pieces using a pipette, digested by incubation with 0.25% trypsin and 0.01% DNase at 37ºC for 10 min, and mechanically dissociated by gentle pipetting. Debris was removed by passing the samples through a 70 μm pore cell strainer. The resulting cells were plated on poly-L-lysine (Sigma-Aldrich)-coated flasks (25 cm^2), and cultured in glial medium [Dulbecco's Modified Eagle's Medium (DMEM; Sigma-Aldrich) containing 10% (v/v) fetal bovine serum (Gibco, Gaithersburg, MD, USA), 1% (v/v) L-glutamine solution (Wako) and 1% (v/v) Penicillin-Streptomycin solution (Wako)]. The culture medium was changed every 2–3 days. After the resulting cells were cultured for 14–21 days, the cells were detached using 0.25% trypsin EDTA (Wako) and incubated on Petri dishes at 37ºC for 30 min. The non-adherent cells were collected and resuspended in BSA/PBS (80 μL per 10^7 cells). The cell suspensions were incubated with Fc receptor blocking reagent (10 μL per 10^7 cells; #130-101-502, Miltenyi Biotec) and CD140a (PDGFRα) MicroBead Kit (10 μL per 10^7 cells; #130-101-502, Miltenyi Biotec) at 4ºC for 10 and 15 min, respectively. OPCs bound to magnetic beads were isolated via MACS using the autoMACS Pro Separator (Miltenyi Biotec). The cells were resuspended in proliferation medium [DMEM (Sigma-Aldrich) containing sodium 1 mM pyruvate (Sigma-Aldrich), 0.1% (w/v) BSA (Sigma-Aldrich), 30 nM sodium selenite (Kanto Chemical), 10 nM biotin (Sigma-Aldrich), 10 nM hydrocortisone (Sigma-Aldrich), 5 μg/mL insulin (Sigma-Aldrich), 50 μg/mL apo-transferrin (Sigma-Aldrich), 10 ng/mL platelet-derived growth factor-AA (PeproTech), and 10 ng/mL basic fibroblast growth factor (PeproTech) and 1% (v/v) Penicillin-Streptomycin solution (Wako)]. The cells were plated on a 12-well plate at 1 x 10^5 cells per well for quantitative PCR or on a 4-well chamber slide at 6 x 10^4 cells per well for immunocytochemistry. The medium was changed after 24 hours. To differentiate OPCs, proliferation medium was replaced with differentiation medium [DMEM containing 1 mM sodium pyruvate, 0.1% (w/v) BSA, 30 nM sodium selenite, 10 nM biotin, 10 nM hydrocortisone, 5 μg/mL insulin, 50 μg/mL apo-transferrin, 40 ng/mL 3,3',5-triiodo-L-thyronine sodium salt (T3; Sigma-Aldrich) and 1% (v/v) Penicillin-Streptomycin solution (Wako)]. The cells were cultured at 37 °C, 5% CO_2 in air, and 95% humidity.

Immunocytochemistry

The cells were fixed with 4% (w/v) paraformaldehyde for 15 min at room temperature, permeabilized with 0.5% (w/v) Triton X-100 in PBS for 30 min and incubated in blocking buffer [5% (v/v) normal serum and 0.3% (w/v) Triton X-100 in PBS] for 60 min. Following blocking, cells were incubated for 60 min with primary antibodies at room temperature, followed by incubation with secondary antibodies. Primary and secondary antibodies were diluted with blocking buffer. Slides were mounted using Fluoromount/Plus (Diagnostic BioSystems), and images were acquired using confocal laser scanning microscopy (LSM-700; Carl Zeiss AG) with Zen software (Carl Zeiss).

Toluidine blue staining

L4 dorsal root ganglia with dorsal and ventral nerve roots from mice fixed with 4% (w/v) paraformaldehyde in 0.1 M phosphate buffer were dissected, and the nerve roots were postfixed in osmium solution [2% (v/v) osmium tetroxide (Nacalai Tesque) and 30 mM HEPES-NaCl buffer (pH 7.4)] for 2 h. After washing in distilled water, samples were embedded in water-soluble resin using the JB-4 Mini Embedding Kit (Polysciences, PA, USA) following the manufacturer's instructions. Nerve root blocks were sectioned at 1 μm using a microtome (Leica), and the sections were placed on microscope slides, which were stained with 1% (w/v) toluidine blue in distilled water for 30 s and washed with distilled water. The sections were mounted using Permount (FALMA).

Quantification of mRNA levels via quantitative PCR

Total RNA of oligodendrocyte lineage cells isolated by MACS was extracted using RNeasy Micro Kit according to the manufacturer's instructions. cDNA was prepared and amplified from 8 ng of oligodendrocyte lineage cell total RNA using the PrimeScript™ RT reagent Kit. One-fifth of the resulting cDNA yield was amplified with TB Green Premix Ex Taq™ II using Thermal Cycler Dice Real Time System II. The thermal cycler protocol was the same as described above. 18S ribosomal RNA was used as an internal control.

Total RNA of cultured OPCs was extracted using RNeasy Micro Kit according to the manufacturer's instructions. cDNA was prepared and amplified from 120 ng of cultured OPC total RNA using the PrimeScript™ RT reagent Kit. One-fifteenth of the resulting cDNA yield was amplified with TB Green Premix Ex Taq™ II using Thermal

Cycler Dice Real Time System II. The thermal cycler protocol was the same as described above. β-actin was used as an internal control.

References

1 Hamaguchi M, Muramatsu R, Fujimura H, Mochizuki H, Kataoka H, Yamashita T (2019) Circulating transforming growth factor-β1 facilitates remyelination in the adult central nervous system. Elife 8: Doi 10.7554/eLife.41869