

Supplementary Files for Xin et al., 2023 (bioRxiv)

A NG2CreER:tau-mGFP visual cortex

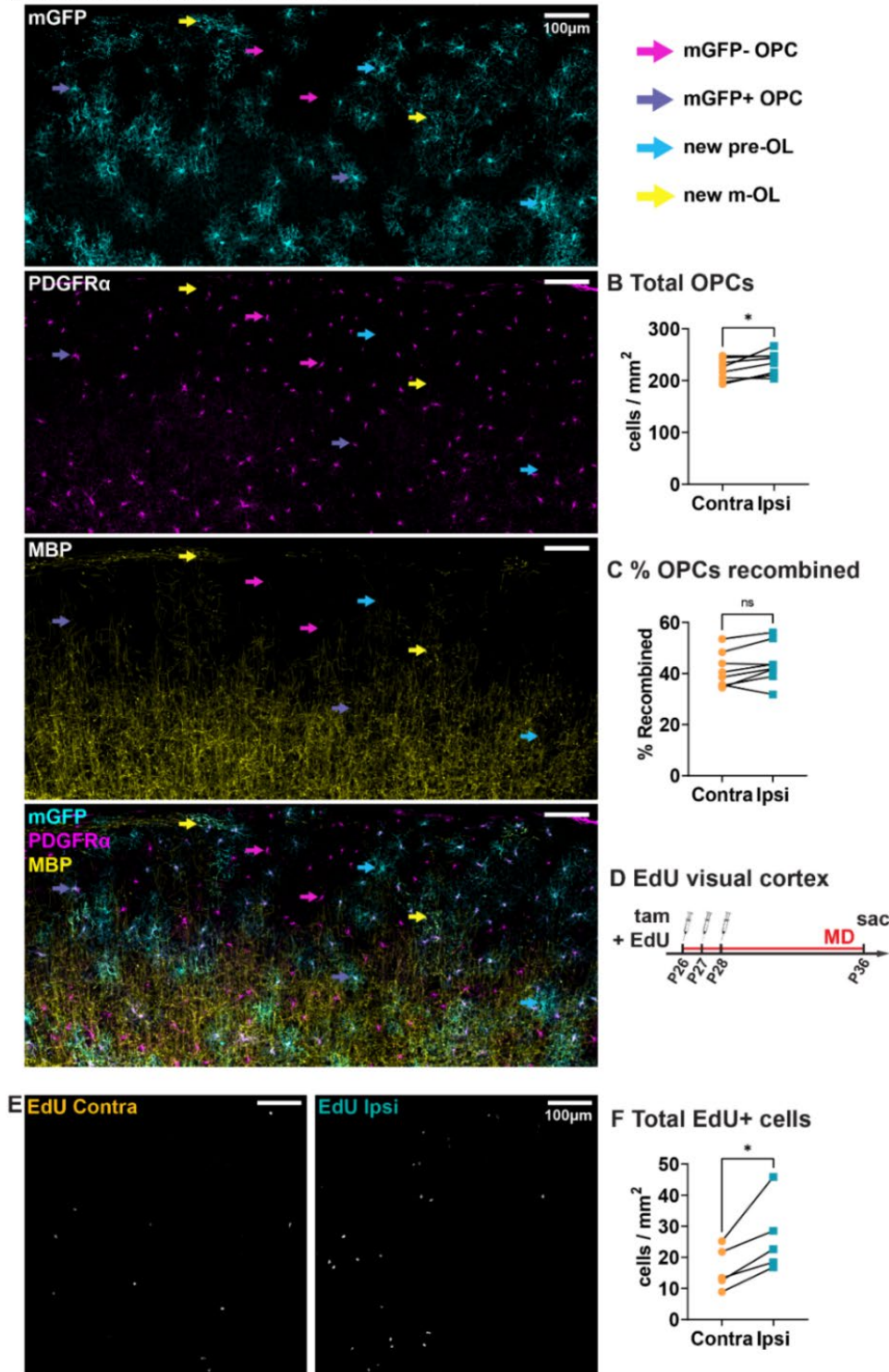


Fig. S1.

**Tracking adolescent oligodendroglial dynamics using NG2CreER:tau-mGFP mice.** (A) Example image from visual cortex. OPC = oligodendrocyte precursor cell, pre-OL = pre-myelinating oligodendrocyte, m-OL = mature oligodendrocyte. (B) Total PDGFR $\alpha$ <sup>+</sup> OPCs per hemisphere. Contra = hemisphere contralateral to the deprived eye, ipsi = hemisphere ipsilateral to the deprived eye. (C) Percentage of PDGFR $\alpha$ <sup>+</sup> OPCs that were mGFP<sup>+</sup>. (D) Timeline of EdU injections. (E, F) Example images and quantification of total EdU<sup>+</sup> cells in both hemispheres of visual cortex. Statistical details in Table S1. \* $p < 0.05$ , ns = not significant.

## Myrf cKO visual cortex

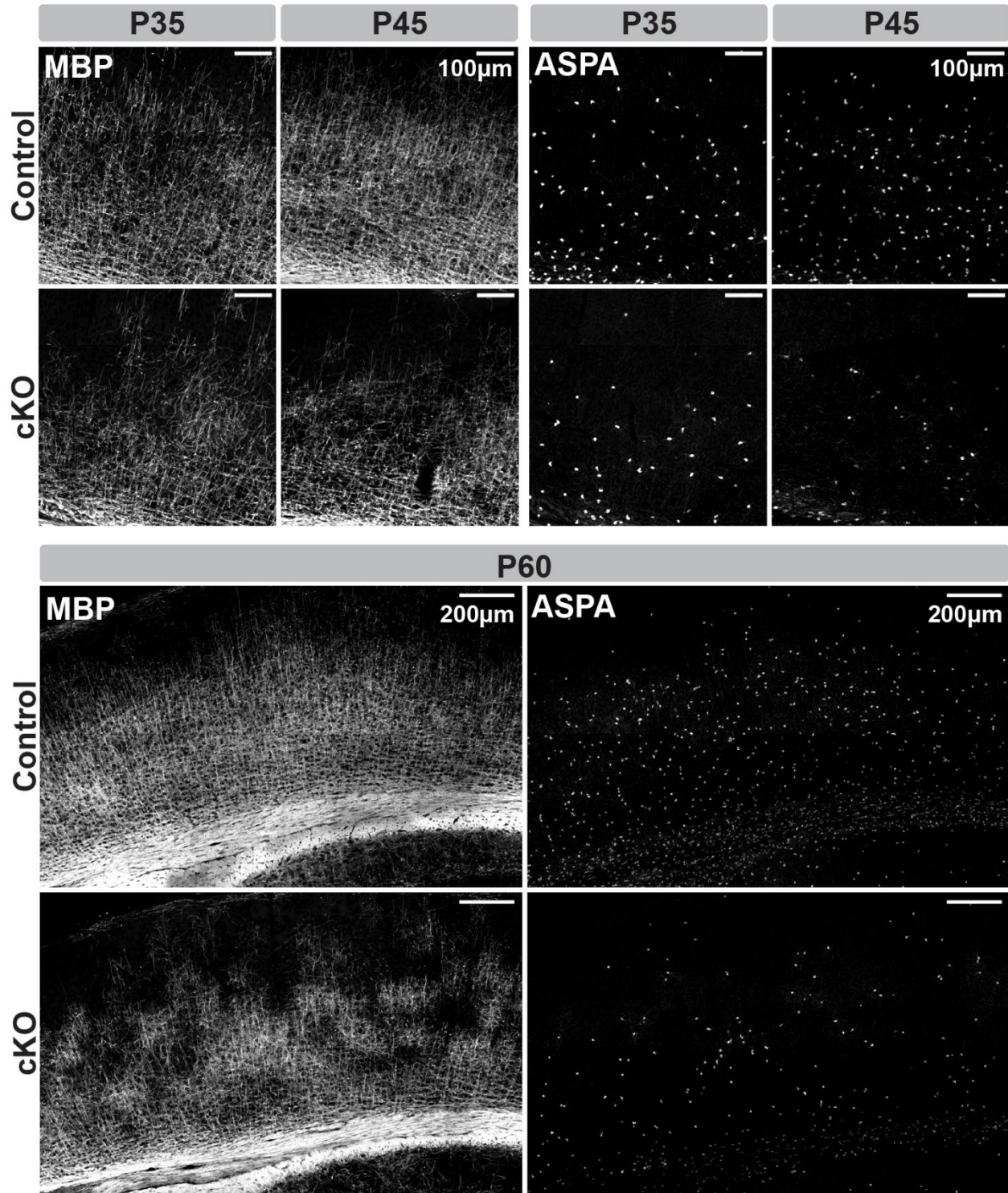
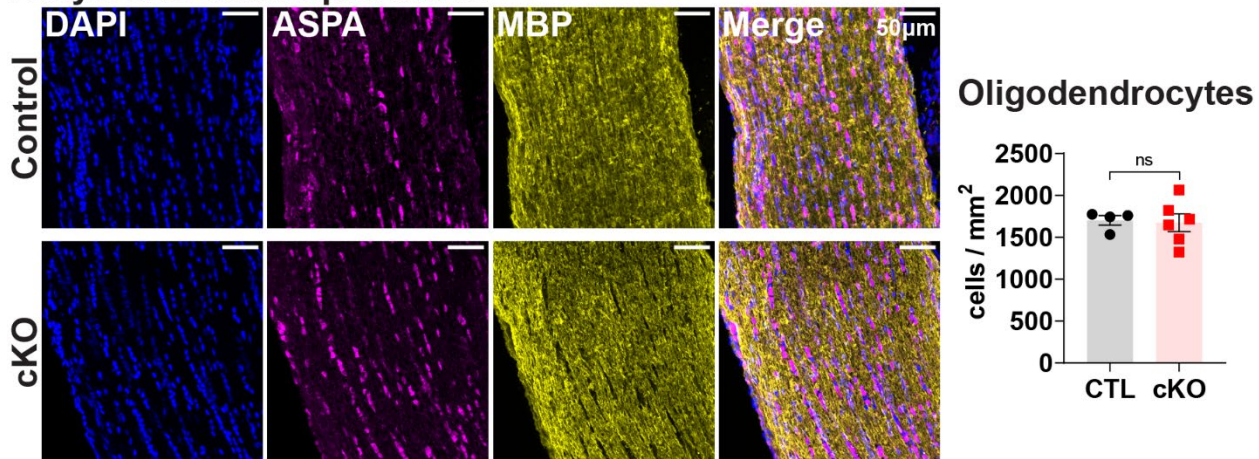


Fig. S2.

**OPC-specific deletion of Myrf in adolescence impairs oligodendrogenesis and myelination in visual cortex.** Example images of myelin (MBP) and mature oligodendrocytes (ASPA) in visual cortex of control and Myrf cKO mice at P35, P45, and P60.



### A Myrf cKO adult optic nerve



### B Myrf cKO adult lateral geniculate nucleus

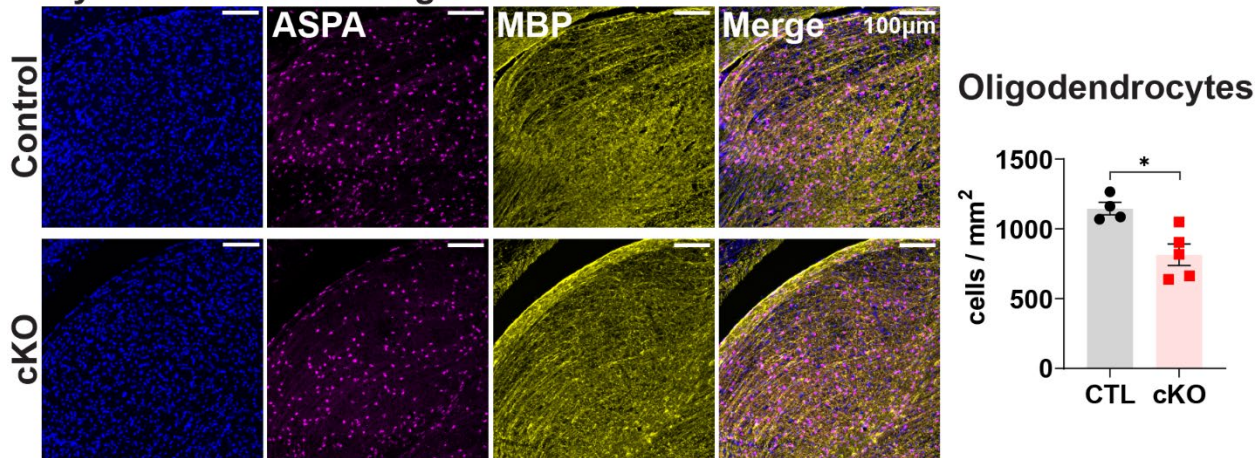
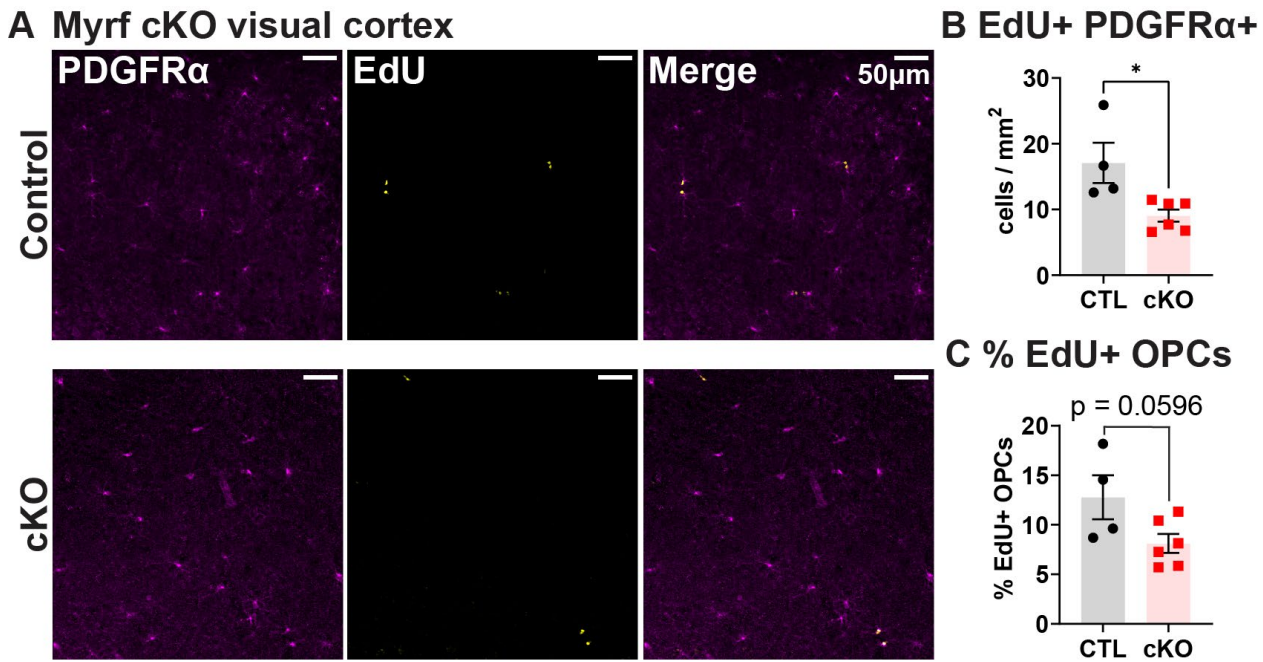


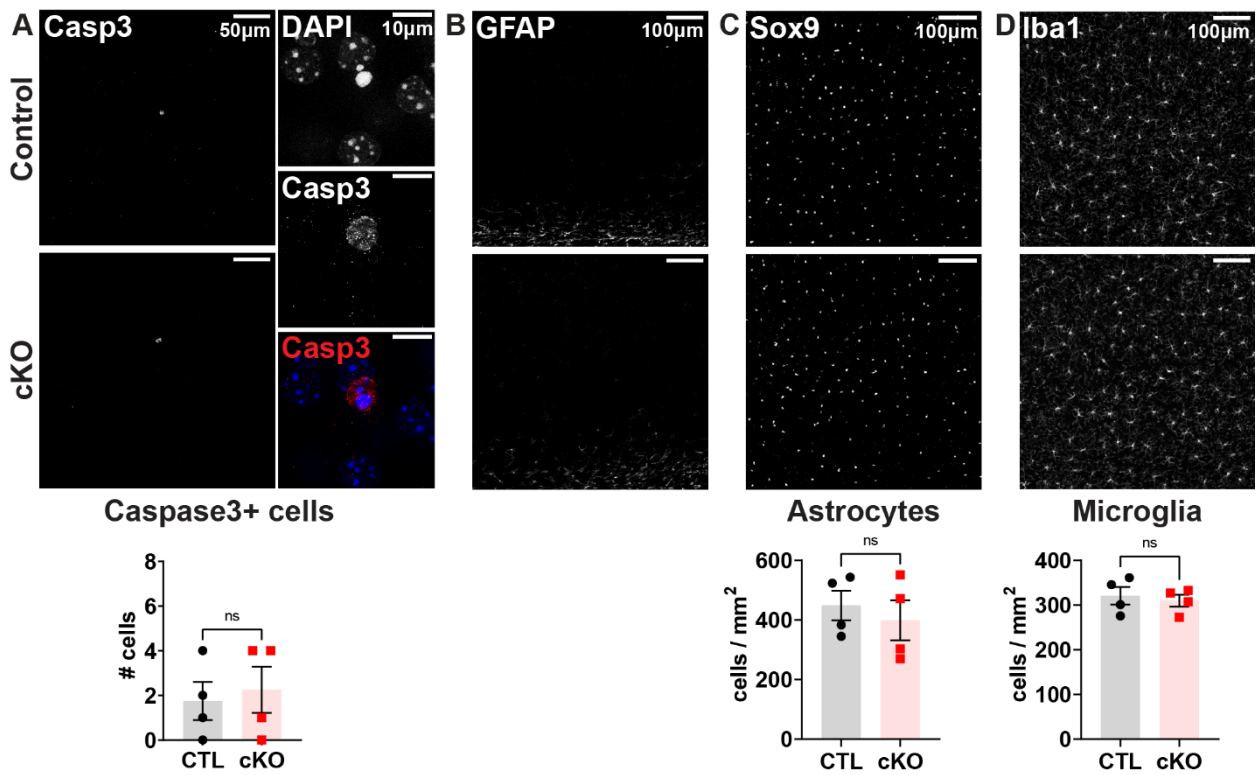
Fig. S3.

**Oligodendrogenesis and myelination in the optic nerve and lateral geniculate nucleus of adult control and Myrf cKO mice.** (A) Example images of myelin (MBP) and example images/quantification of mature oligodendrocytes (ASPA) in optic nerve. (B) Example images of myelin and and example images/quantification of mature oligodendrocytes in lateral geniculate nucleus. Statistical details in Table S1. \* $p < 0.05$ , ns = not significant.



**Fig. S4.**

**OPC proliferation in visual cortex of adult control and Myrf cKO mice.** (A) Example images of OPCs (PDGFRα) and EdU labeling in visual cortex. (B) Quantification of PDGFRα<sup>+</sup> EdU<sup>+</sup> OPCs in visual cortex. (C) Quantification of percentage of PDGFRα<sup>+</sup> OPCs that were EdU<sup>+</sup> in visual cortex. Statistical details in Table S1. \*p<0.05.

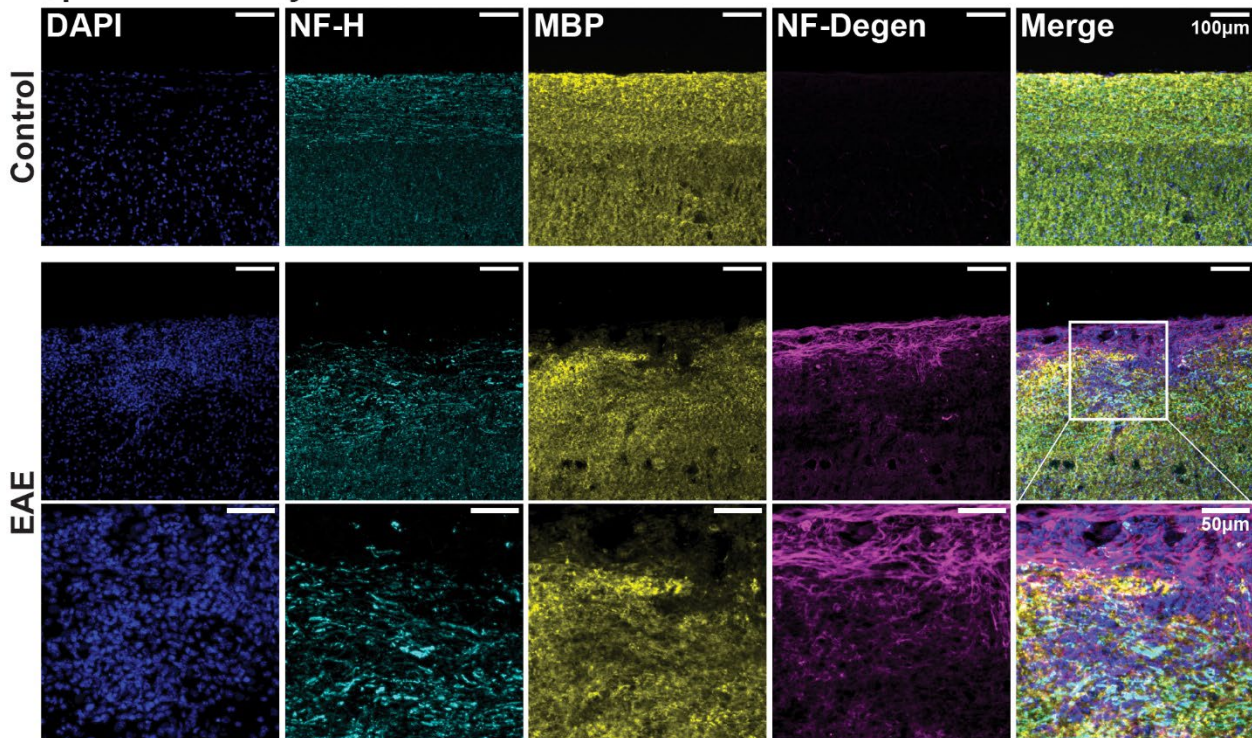


**Fig. S5.**

**Cell death, astrocyte density, and microglia density in adult control and Myrf cKO mice. (A)** Example images and quantification of Caspase<sup>+</sup> cells in visual cortex. **(B)** Example images of GFAP immunostaining in visual cortex. **(C)** Example images and quantification of Sox9<sup>+</sup> astrocytes in visual cortex. **(D)** Example images and quantification of Iba1<sup>+</sup> microglia in visual cortex. Statistical details in Table S1. ns = not significant.



### A Spinal cord demyelination



### B Visual cortex Myrf cKO

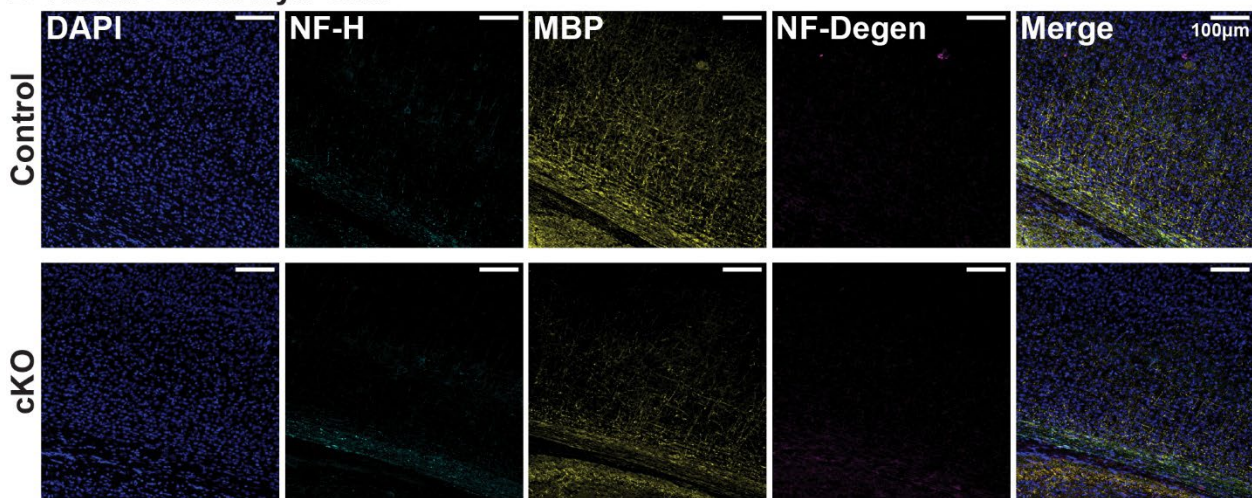
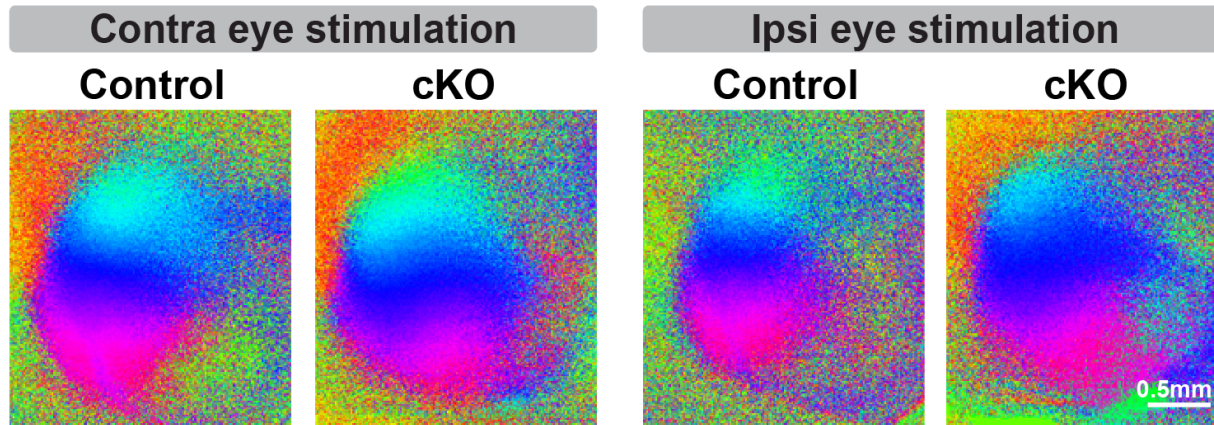


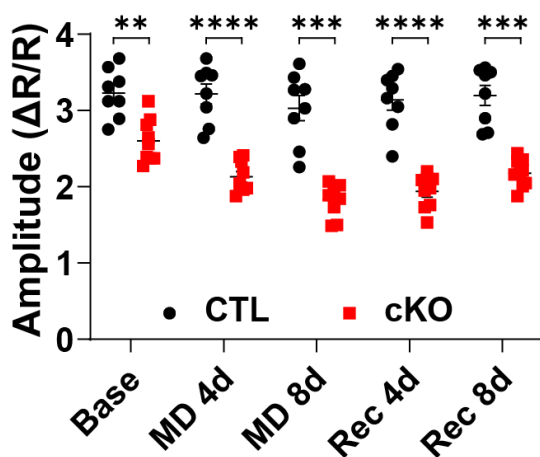
Fig. S6.

**Assessing neurodegeneration in demyelination and in Myrf cKO mice.** (A) Immunostaining for neurofilament H (NF-H), myelin basic protein (MBP), and neurofilament light chain DegenTag (NF-Degen) in spinal cords of control mice and mice that underwent experimental autoimmune encephalomyelitis (EAE). Disordered NF-H and prominent NF-Degen signal can be detected in the spinal cord of EAE mice, most notably in regions of demyelination. (B) Immunostaining for NF-H, MBP, and NF-Degen in visual cortex of control and Myrf cKO mice.

## A Retinotopic organization in primary visual cortex



## B Contra eye response



## C Ipsi eye response

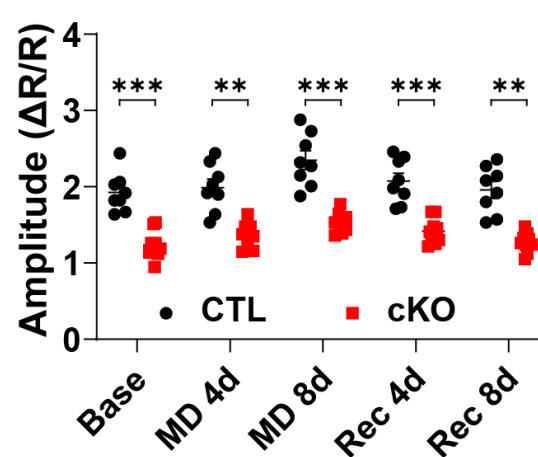
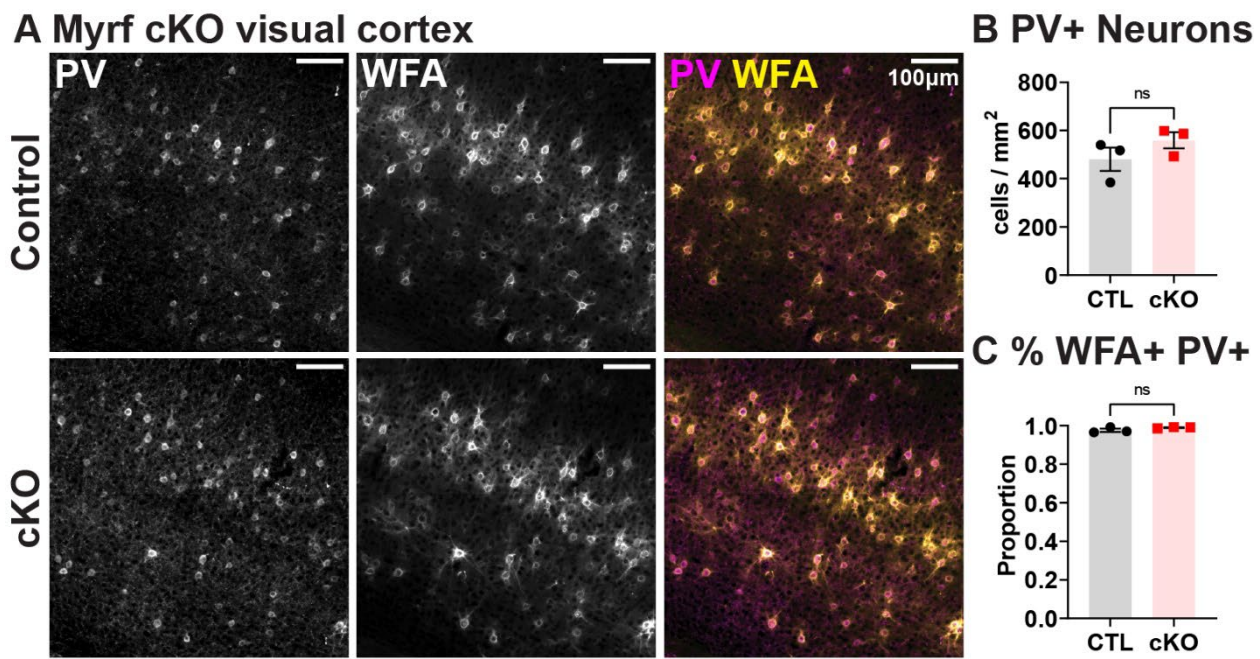


Fig. S7.

**Retinotopic organization and amplitude of visual cortex responses to visual stimulation in adult control and *Myrf* cKO mice.** (A) Example intrinsic signal images of retinotopy in primary visual cortex. (B, C) Amplitude of intrinsic signal imaging responses to stimulation of the contralateral deprived eye (contra) or ipsilateral non-deprived eye (ipsi) in binocular visual cortex of control (CTL) and cKO mice. Statistical details in Table S1. \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .

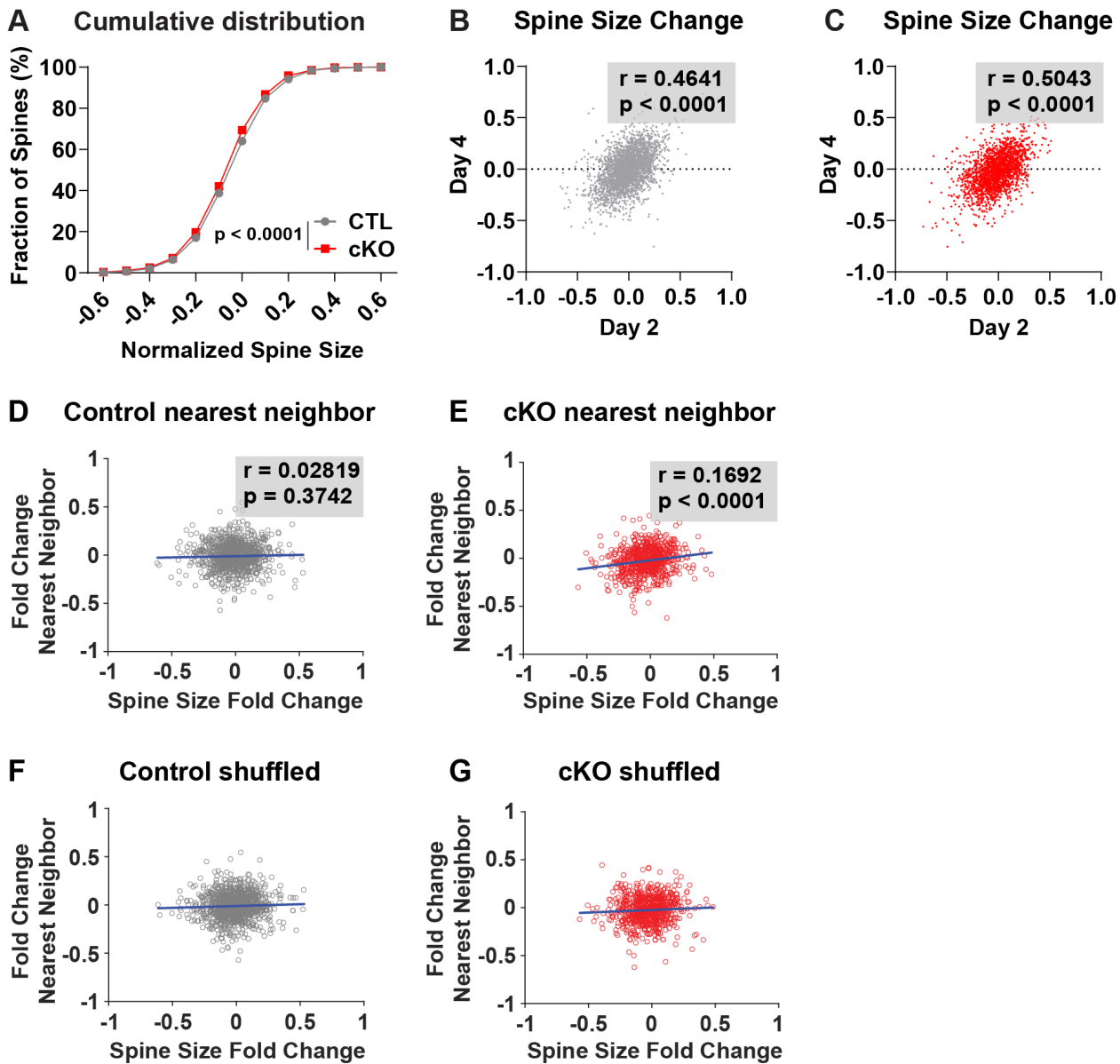




**Fig. S8.**

**Parvalbumin neuron density and perineuronal net coverage in adult control and Myrf cKO mice. (A)** Example images and quantification (**B, C**) of immunostaining for parvalbumin (PV) and perineuronal nets (WFA) in adult visual cortex of control (CTL) and cKO mice. Statistical details in Table S1. ns = not significant.





**Fig. S9.**

**Spine size changes following monocular deprivation in adult control and Myrf cKO mice.** (A) Cumulative distribution plot of spine size changes in control (CTL) and cKO mice after four days of monocular deprivation. (B, C) Correlation of spine size changes after two days of monocular deprivation with spine size changes after four days of monocular deprivation in control and cKO mice. (D, E) Correlation of average size change following monocular deprivation for a given spine and size change of its nearest neighbor in control and cKO mice. (F, G) Example correlation of nearest neighbor spine changes in one set of shuffled spine pairings for control and cKO mice. Statistical details in Table S1.

**Table S1.****Statistical analysis**

<b>Figure</b>	<b>Sample size</b>	<b>Statistical test</b>	<b>Values</b>
1H	8 mice	Paired t-test (two-tailed)	t = 5.494, p = 0.0009
1I	8 mice	Paired t-test (two-tailed)	t = 3.06, p = 0.0183
1J	8 mice	Paired t-test (two-tailed)	t = 0.3199, p = 0.7584
1K	8 mice	Paired t-test (two-tailed)	t = 3.84, p = 0.0064
1L	5 mice	Paired t-test (two-tailed)	t = 3.867, p = 0.018
1M	5 mice	Paired t-test (two-tailed)	t = 1.64, p = 0.1763
2B	3-6 mice per age, per genotype	Two-way ANOVA followed by Sidak's multiple comparisons test	Genotype: F = 165.9, p < 0.0001 Interaction: F = 9.55, p = 0.0002  CTL – cKO P28: p = 0.1808 P35: p < 0.0001 P45: p < 0.0001 P60: p < 0.0001
2C	3-4 mice per age, per genotype	Two-way ANOVA followed by Sidak's multiple comparisons test	Genotype: F = 0.7504, p = 0.7504 Interaction: F = 0.0979, p = 0.7608  CTL vs cKO P28: p > 0.9999 P60: p = 0.8672
2I	8-9 mice per genotype	Two-way ANOVA followed by Sidak's multiple comparisons test	Genotype: F = 30.64, p < 0.0001 Interaction: F = 0.4264, p = 0.5237
2J	8 mice	Two one-way repeated measures ANOVAs (one for contra and one for ipsi) followed by Tukey's multiple comparisons test	Contra F = 1.733, p = 0.1804 Baseline vs MD4: p = 0.9863 Baseline vs MD8: p = 0.1922 MD4 vs MD8: p = 0.1814  Ipsi F = 30.17, p < 0.0001 Baseline vs MD4: p = 0.5364 Baseline vs MD8: p = 0.0004 MD4 vs MD8: p = 0.0015
2K	9 mice	Two one-way repeated measures ANOVAs (one for contra and one for ipsi) followed by Tukey's multiple comparisons test	Contra F = 66.55, p < 0.0001 Baseline vs MD4: p = 0.0002 Baseline vs MD8: p < 0.0001 MD4 vs MD8: p = 0.0015  Ipsi F = 20.66, p < 0.0001 Baseline vs MD4: p = 0.0575 Baseline vs MD8: p = 0.0013 MD4 vs MD8: p = 0.0053
2L	8-9 mice per genotype	Unpaired t-test (two-tailed)	t = 5.082, p = 0.0001



2M	8-9 mice per genotype	Two-way ANOVA followed by Sidak's multiple comparisons test	Genotype: F = 36.22, p < 0.0001 Interaction: F = 15.62, p < 0.0001  CTL vs cKO MD 4d: p = 0.0003 MD 8d: p = 0.0007 Rec 4d: p = 0.0002 Rec 8d: p = 0.0013
2N	8-9 mice per genotype	Two-way ANOVA followed by Sidak's multiple comparisons test	Genotype: F = 1.247, p = 0.3008 Interaction: F = 1.922, p = 0.1859  CTL vs cKO MD 4d: p = 0.5115 MD 8d: p = 0.9725 Rec 4d: p = 0.2595 Rec 8d: p = 0.9961
3D	10 control mice (111 dendrites)  10 cKO mice (97 dendrites)	Two-way repeated measures ANOVA followed by Holm-Sidak's multiple comparisons test	Genotype: F = 7.394, p = 0.0071 Interaction: F = 2.115, p = 0.0972  CTL vs cKO Day -2: p = 0.0311 Day 0: p = 0.0103 Day 2: p = 0.0095 Day 4: p = 0.0391
3E	10 control mice (111 dendrites)  10 cKO mice (97 dendrites)	Mixed-effects analysis (REML) followed by Holm-Sidak's multiple comparisons test	Genotype: F = 8.82, p = 0.0033 Interaction: F = 0.6344, p = 0.5931  CTL vs cKO Day -2: p = 0.3037 Day 0: p = 0.0103 Day 2: p = 0.0178 Day 4: p = 0.0586
3F	10 control mice (111 dendrites)  10 cKO mice (97 dendrites)	Mixed-effects analysis (REML) followed by Holm-Sidak's multiple comparisons test	Genotype: F = 13.9, p = 0.0002 Interaction: F = 1.101, p = 0.3484  CTL vs cKO Day -2: p = 0.0288 Day 0: p = 0.0048 Day 2: p = 0.0521 Day 4: p = 0.192
3G	10 control mice (111 dendrites)  10 cKO mice (97 dendrites)	Mixed-effects analysis (REML) followed by Holm-Sidak's multiple comparisons test	Genotype: F = 3.483, p = 0.0634 Interaction: F = 0.9613, p = 0.4107

			CTL vs cKO Day -2: $p = 0.7148$ Day 0: $p = 0.2422$ Day 2: $p = 0.1721$ Day 4: $p = 0.1721$
3H	10 control mice (111 dendrites)  10 cKO mice (97 dendrites)	Two-way repeated measures ANOVA followed by Holm-Sidak's multiple comparisons test	Genotype: $F = 2.471$ , $p = 0.1175$ Interaction: $F = 2.64$ , $p = 0.0486$  CTL vs cKO Day -2: $p = 0.7333$ Day 0: $p = 0.7241$ Day 2: $p = 0.9743$ Day 4: $p = 0.0110$
4C	10 control mice (110 dendrites)  10 cKO mice (96 dendrites)	Unpaired t-test (two-tailed)	$t = 0.3287$ , $p = 0.7427$
4D	10 control mice (110 dendrites)  10 cKO mice (96 dendrites)	Unpaired t-test (two-tailed)	$t = 2.826$ , $p = 0.0052$
4E	10 control mice (110 dendrites)  10 cKO mice (96 dendrites)	Unpaired t-test (two-tailed)	$t = 2.56$ , $p = 0.0112$
4F	10 control mice (110 dendrites)  10 cKO mice (96 dendrites)	Unpaired t-test (two-tailed)	$t = 1.627$ , $p = 0.1052$
4G	10 control mice (996 spine pairs) 10 cKO mice (704 spine pairs)	Monte Carlo p value calculated by summing the tail of the histogram from 10000 shuffled spine pairings	$p = 0.686$
4H	10 control mice (996 spine pairs) 10 cKO mice (704 spine pairs)	Monte Carlo p value calculated by summing the tail of the histogram from 10000 shuffled spine pairings	$p = 0.939$
4I	10 control mice (996 spine pairs) 10 cKO mice (704 spine pairs)	Monte Carlo p value calculated by summing the tail of the histogram from 10000 shuffled spine pairings	$p = 0.913$
4J	10 control mice (996 spine pairs) 10 cKO mice (704 spine pairs)	Monte Carlo p value calculated by summing the tail of the histogram from 10000 shuffled spine pairings	$p = 0.66$



4K	10 control mice (996 spine pairs) 10 cKO mice (704 spine pairs)	Monte Carlo p value calculated by summing the tail of the histogram from 10000 shuffled spine pairings	p = 0.862
4L	10 control mice (996 spine pairs) 10 cKO mice (704 spine pairs)	Monte Carlo p value calculated by summing the tail of the histogram from 10000 shuffled spine pairings	p = 0.001
4M	10 control mice (996 spine pairs) 10 cKO mice (704 spine pairs)	Monte Carlo p value calculated by summing the tail of the histogram from 10000 shuffled spine pairings	p = 0.002
4N	10 control mice (996 spine pairs) 10 cKO mice (704 spine pairs)	Monte Carlo p value calculated by summing the tail of the histogram from 10000 shuffled spine pairings	p = 0.022
S1B	8 mice	Paired t-test (two-tailed)	t = 2.565, p = 0.0373
S1C	8 mice	Paired t-test (two-tailed)	t = 2.176, p = 0.066
S1F	5 mice	Paired t-test (two-tailed)	t = 3.637, p = 0.022
S3A	4-6 mice per genotype	Unpaired t-test (two-tailed)	t = 0.1984, p = 0.8477
S3B	4-5 mice per genotype	Unpaired t-test (two-tailed)	t = 3.461, p = 0.0105
S4B	4-6 mice per genotype	Unpaired t-test (two-tailed)	t = 2.982, p = 0.0176
S4C	4-6 mice per genotype	Unpaired t-test (two-tailed)	t = 2.195, p = 0.0594
S5A	4 mice per genotype	Unpaired t-test (two-tailed)	t = 0.3735, p = 0.7216
S5C	4 mice per genotype	Unpaired t-test (two-tailed)	t = 0.595, p = 0.5736
S5D	4 mice per genotype	Unpaired t-test (two-tailed)	t = 0.4586, p = 0.6627
S7B	8-9 mice per genotype	Two-way ANOVA followed by Sidak's multiple comparisons test	Genotype: F = 55.3, p < 0.0001 Interaction: F = 11.73, p < 0.0001  CTL vs cKO Base: p = 0.0041 MD 4d: p < 0.0001 MD 8d: p = 0.0004 Rec 4d: p < 0.0001 Rec 8d: p = 0.0002
S7C	8-9 mice per genotype	Two-way ANOVA followed by Sidak's multiple comparisons test	Genotype: F = 42.31, p < 0.0001 Interaction: F = 2.183, p = 0.0816  CTL vs cKO Base: p = 0.0002

			MD 4d: $p = 0.0022$ MD 8d: $p = 0.0008$ Rec 4d: $p = 0.0009$ Rec 8d: $p = 0.0011$
S8B	3 mice per genotype	Unpaired t-test (two-tailed)	$t = 1.331$ , $t = 0.2538$
S8C	3 mice per genotype	Unpaired t-test (two-tailed)	$t = 1.635$ , $t = 0.1773$
S9A	10 control mice (3484 spines) 10 cKO mice (2438 spines)	Kolmogorov-Smirnov test	$p < 0.0001$
S9B	10 control mice (3809 spines)	Pearson r correlation	$r = 0.4641$ , $p < 0.0001$
S9C	10 cKO mice (3484 spines)	Pearson r correlation	$r = 0.5043$ , $p < 0.0001$
S9D	10 control mice (996 spine pairs)	Pearson r correlation	$r = 0.02819$ , $p = 0.3742$
S9E	10 cKO mice (704 spine pairs)	Pearson r correlation	$r = 0.1692$ , $p < 0.0001$
S9F, G	N/A	Example of one shuffled spine pairing as part of Monte Carlo simulation; no statistics involved	N/A



**Table S2.****Primary antibodies**

<b>Antibody</b>	<b>Source</b>	<b>Identifier</b>	<b>Concentration</b>
Rabbit anti-ASPA	GeneTex	Cat# GTX113389; RRID AB_2036283	1:1000
Chicken anti-GFP	Rockland	Cat# 600-901-215; RRID AB_1537403	1:1000
Rat anti-MBP	Millipore	Cat# MAB386; RRID AB_94975	1:200
Rabbit anti-PDGFR $\alpha$	W.B. Stallcup	N/A	1:200
Rabbit anti-cleaved Caspase3	Cell Signaling	Cat# 9661S; RRID AB_2341188	1:200
Mouse anti-GFAP	Millipore	Cat# MAB360; RRID AB_11212597	1:1000
Goat anti-Sox9	R&D Systems	Cat# AF3075; RRID AB_2194160	1:2000
Rabbit anti-Iba1	Wako	Cat# 019-19741; RRID AB_839504	1:1000
Mouse anti-NF-L Degenotag	Encor	Cat# MCA-1D44; RRID AB_2923483	1:1000
Rabbit anti-NF-H	Abcam	Cat# ab8135; RRID AB_306298	1:1000
Mouse anti-PV	Swant	Cat# 235; RRID AB_10000343	1:1000
Biotinylated WFA	Vector Labs	Cat# B-1355; RRID AB_2336874	1:400