Activation of oligodendroglial Stat3 is required for efficient remyelination

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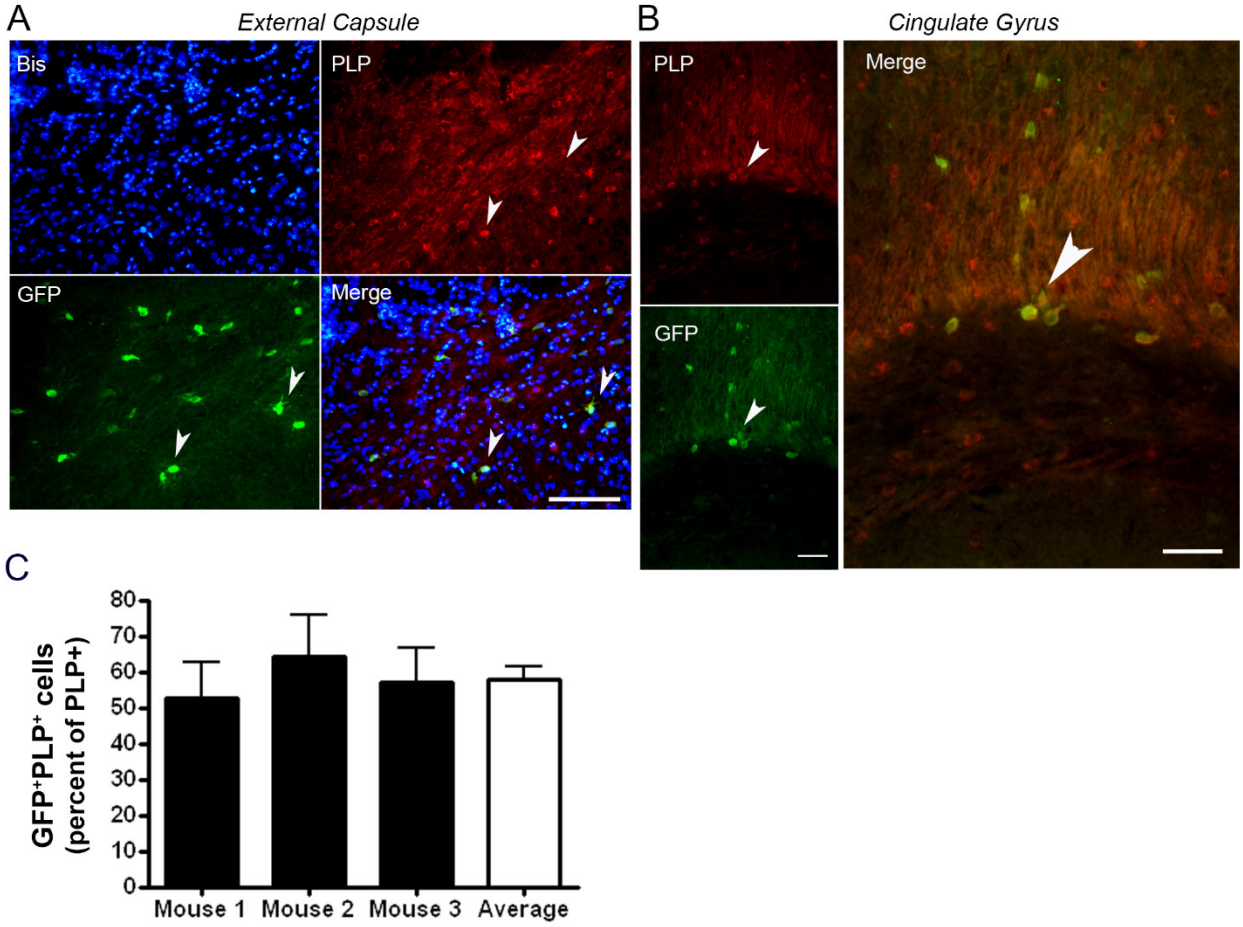
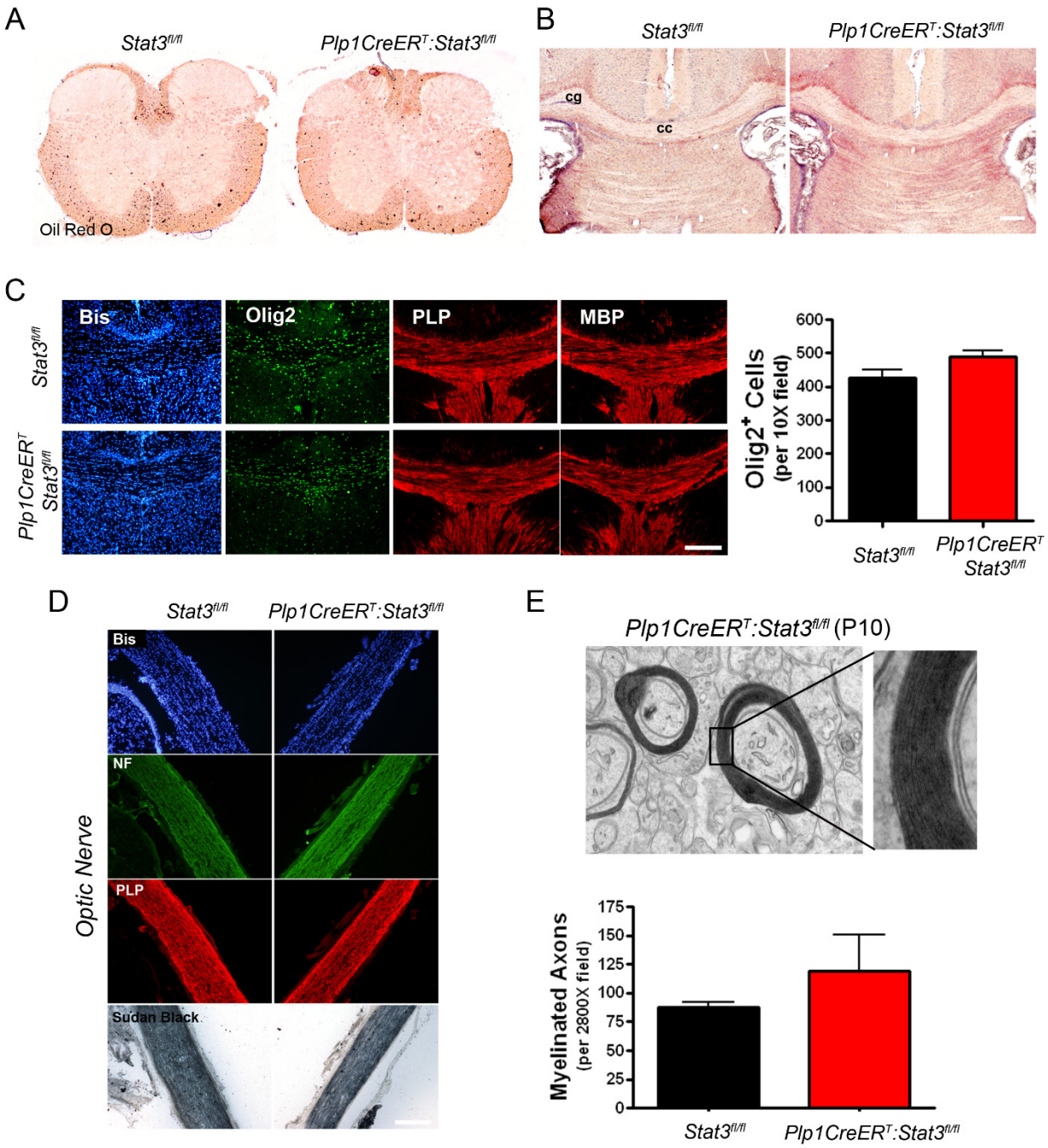
Appendix A. Supplementary data

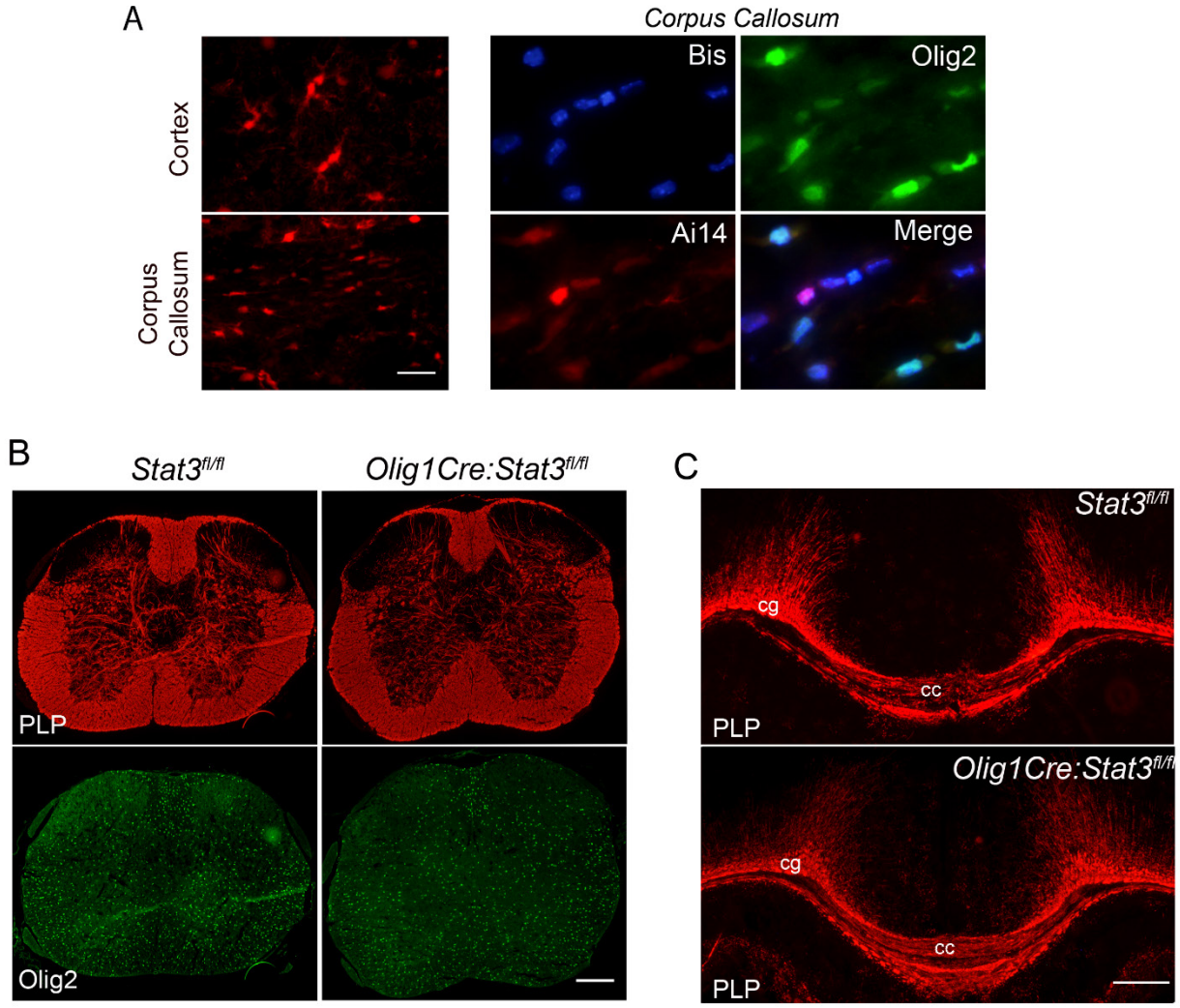


Supplementary Figure 1: Recombination efficiency in *Plp1*-driven inducible Cre mice. To evaluate the efficiency of tamoxifen-induced Cre-LoxP recombination, *Plp1CreER*^{T} mice were bred with *Rosa26EYFP* reporter mice. Perinatal tamoxifen-induced recombination in offspring was carried out by daily treatment of lactating dams with tamoxifen (i.p., 300µg dissolved in sunflower seed oil) during postnatal day 0 to 12, and the pups were collected at P18. ***A-B,*** Representative immunohistochemistry images showing GFP^{+} PLP^{+} cells in the external capsule and cingulate gyrus of a *Plp1CreER*^{T}*: Rosa26EYFP* reporter mouse at P18. Results are representative of 3 mice. Scale bars: 100 µm in **A**, 50 µm in **B**. ***C,*** Quantification of the percentage of GFP^{+}PLP^{+} OLs in the cingulate gyrus. Results are means ± s.e.m. of 4-5 20x pictures per mouse, and average is the combined means ± s.e.m. from 3 mice.

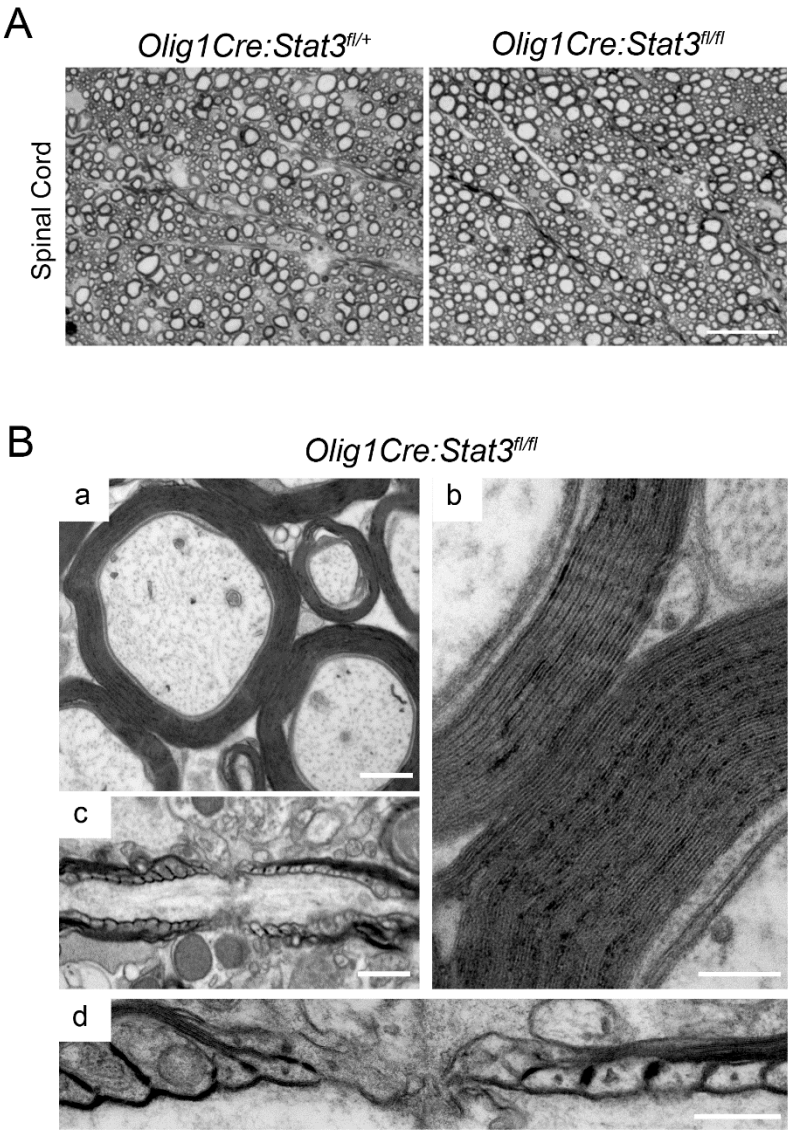


Supplementary Figure 2: ***Plp1*** **driven conditional deletion of Stat3 does not affect CNS myelination during development.** ***A-C,*** *Plp1CreER*^{T}*:Stat3*$^{fl/fl}$ mice were mated with *Stat3*$^{fl/fl}$. Dams were injected daily with tamoxifen (300µg) dissolved in sunflower seed oil from P0-P12. Pups were sacrificed at P14 and CNS myelination was examined between control (*Stat3*$^{fl/fl}$) and conditional knockout (*Plp1CreER*^{T}*:Stat3*$^{fl/fl}$) mice. Myelination in the spinal cord (**A**) and the brain (**B**) as determined by Oil red O staining was similar between genotypes. ***C,*** Immunochemistry analysis of the oligodendrocyte specific transcription factor Olig2 and myelin proteins PLP and MBP in the corpus callosum (*left*), and quantification of Olig2^{+} cells in the corpus callosum (*right*). Scale bars, 200µm. Cg, cingulate gyrus; cc, corpus callosum. Results are means ± s.e.m. of 3 mice per group. ***D-E***, Dams were injected with tamoxifen as above. Myelination of the optic nerves of P10 pups was assessed. ***D,*** Representative pictographs of optic nerves from control or conditional knockout mice stained for neurofilament (green) and

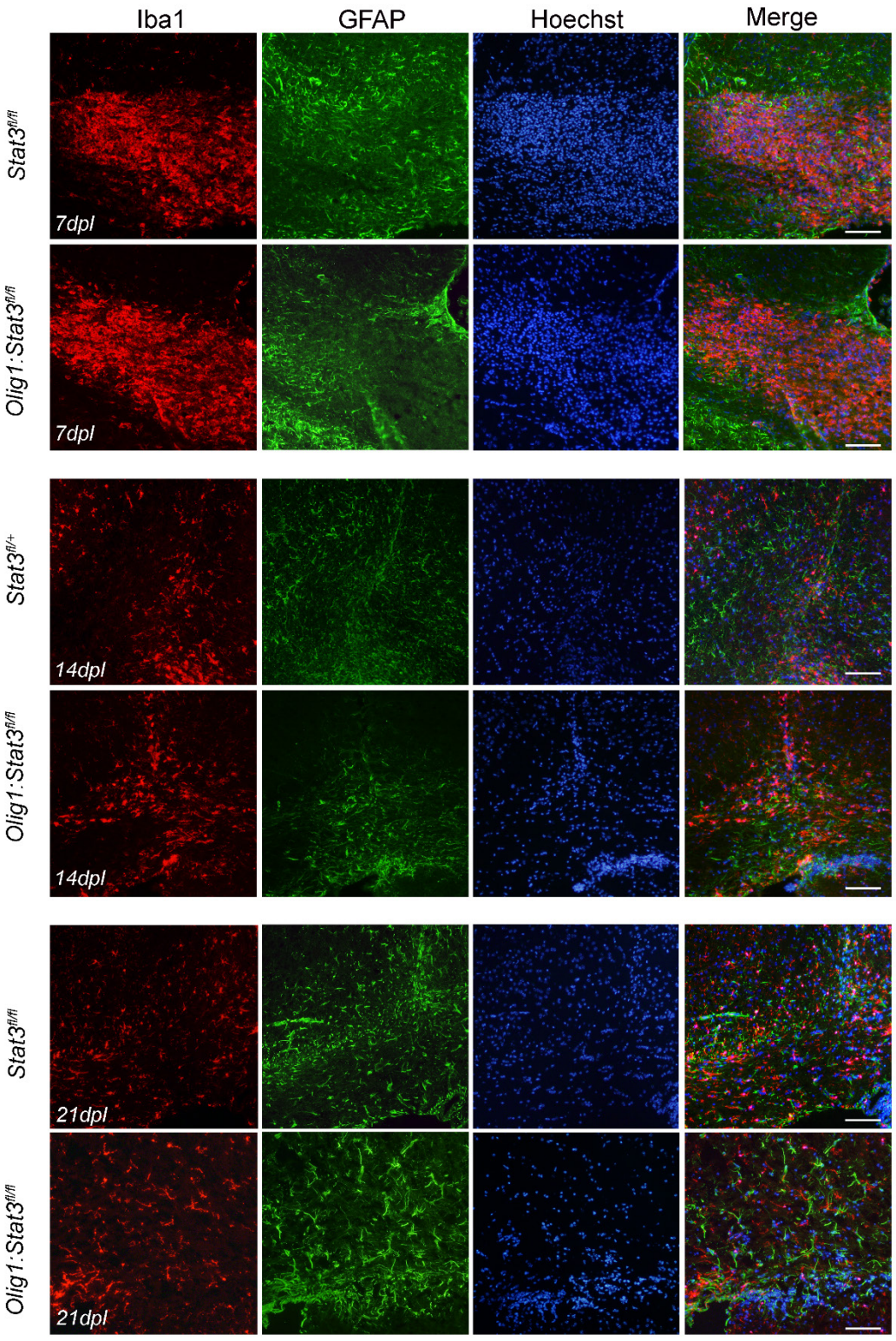
PLP (red). Myelin was stained with Sudan black. Scale bar, 200µm. ***E*,** Transmission electron microscopy analysis of myelination of the optic nerve in P10 mice. Boxed area shows normal compact myelin at higher magnification. The number of myelinated axons did not differ between genotypes. Data are mean ± s.e.m. from 7-12 microscopic fields (2800x) per mouse and represent 2-3 mice per genotype.



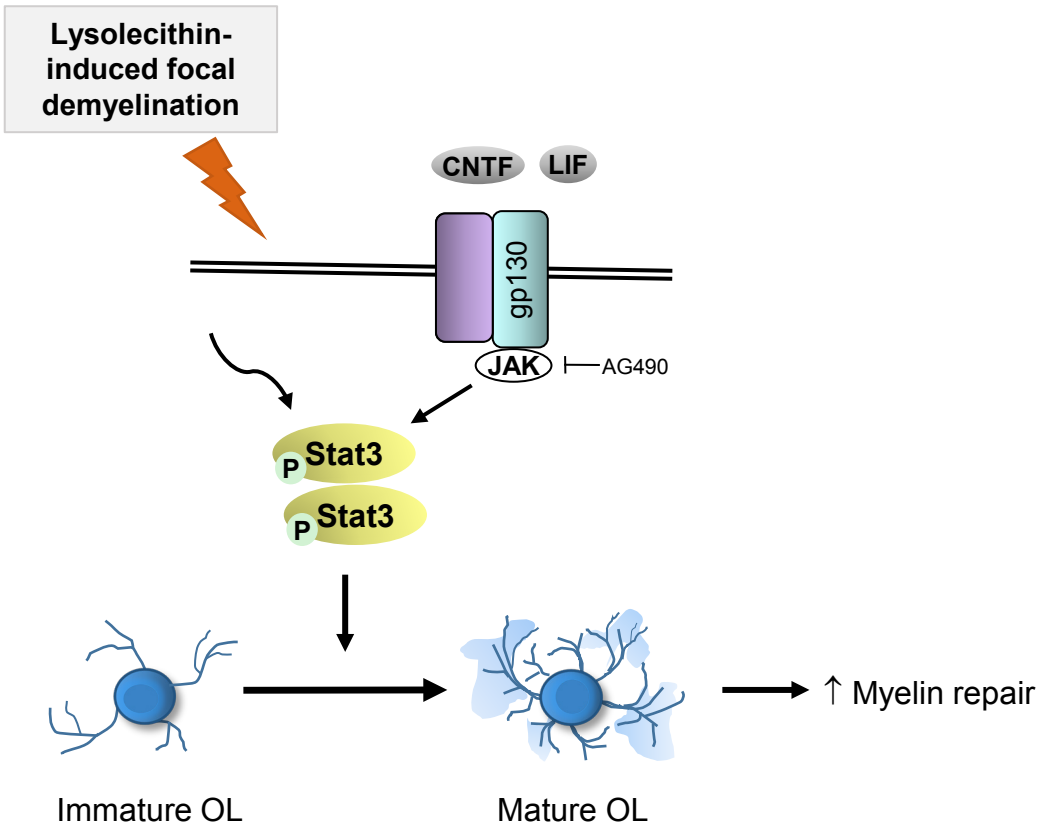
Supplementary Figure 3: *Olig1* promoter driven conditional deletion of Stat3 does not affect myelination during normal CNS development. ***A.*** *Olig1Cre* mice were mated to Rosa26-tdTomato (Ai14) reporter mice. Representative pictographs showing colocalization of Ai14 with Olig2 in the corpus callosum at P21. Scale bar, 20 µm. ***B-C*,** the spinal cords and brains from P14 *Stat3*$^{fl/fl}$ and *Olig1Cre:Stat3*$^{fl/fl}$ mice were examined by immunostaining for PLP and Olig2. Results are representative of 2-3 mice per genotype. Cg, cingulate gyrus; cc, corpus callosum. Scale bars, 200µm.



Supplementary Figure 4: Normal myelin integrity in the spinal cord of 12 month old *Olig1Cre:Stat3$^{fl/fl}$* mice. ***A,*** Histological examination of semi-thin sections stained with Richardson's epoxy stain showed no differences in myelinated axons between *Olig1Cre:Stat3$^{fl/+}$* and *Olig1Cre:Stat3$^{fl/fl}$* mice. ***B,*** Electron micrographs showing structural integrity of myelin in 12 month old *Olig1Cre:Stat3$^{fl/fl}$* mice in the white matter (a, b) and the gray matter of the spinal cord (c, d). Scale bars, 20μm in A; 500nm in B a&c; 100nm in b; 250nm in d.



Supplementary Figure 5: Activation of microglia/macrophages and astrocytes after focal LPC injury. Activation of microglia/macrophages (Iba-1^{+}) and astrocytes (GFAP^{+}) in responses to focally injected LPC at 7, 14 and 21 days post lesion was evaluated by double immunohistochemistry. Reactive astrocytes in lesioned mutant mice appear to be hypertrophic at 21 dpl with respect to lesioned control mice. Representative images of the lesion region of wildtype controls (*Stat3*$^{fl/fl}$ or *Stat3*$^{fl/+}$) and OL lineage specific-*Stat3* mutant (*Olig1Cre:Stat3*$^{fl/fl}$) mice are shown. Data represent 3-4 mice per group. Scale bars, 100 µm.



Supplementary Figure 6: Schematic overview of oligodendroglial Stat3 signaling in promoting mature OL generation after focal demyelinating injury.