Cell Stem Cell, Volume *6*

Supplemental Information

CNS-Resident Glial Progenitor/Stem Cells Produce Schwann Cells as well as Oligodendrocytes during Repair of CNS Demyelination

Malgorzata Zawadzka, Leanne E. Rivers, Stephen P.J. Fancy, Chao Zhao, Richa Tripathi, Françoise Jamen, Kaylene Young, Alexander Goncharevich, Hartmut Pohl, Matteo Rizzi, David H. Rowitch, Nicoletta Kessaris, Ueli Suter, William D. Richardson, and Robin J.M. Franklin

Inventory of Supplemental Information

Figure S1:

Describing an experimental scheme, tamoxifen treatment regimen, efficiency of tamoxifen-induced Cre recombination in *Pdgfra-creER^{T2}* : *Rosa26-YFP* mice and labeling of cells defined as OLP as shown in the Figure 1.

Figure S2:

Showing the cellular composition of reconstructed CNS lesions. In addition to data shown in Figure 2 and Figure 3, the phenotype of YFP$^+$/CC1$^-$/OLIG2$^+$ cells was examined and identified as undifferentiated precursors and the phenotype YFP$^+$/CC1$^-$/OLIG2$^-$ as Schwann cells.

Figure S3:

Showing that some SCIP$^+$ cells also expressed Nkx2.2, oligodendrocyte lineage marker, normally expressed by oligodendrocytes but not Schwann cells. This transitional co-expression supports the data shown in Figure 3.

Figure S4:

In addition to data showing that most reactive astrocytes in demyelinated lesions do not derive from OLPs (Figure 7) we also exclude the possibility that *Fgfr3*$^+$ cells generate remyelinating oligodendrocytes – double immunostaining revealed no expression of OLIG2 in the YFP-expressing cells in white matter of *Fgfr3-icreER^{T2}:Rosa26-YFP* mice.

Movie S1 (available online):

Movie demonstrating the process of filling the cell (OLP – derived oligogodendrocyte) with Alexa Fluor 488 by electroporation in addition to image shown in Figure 2E.

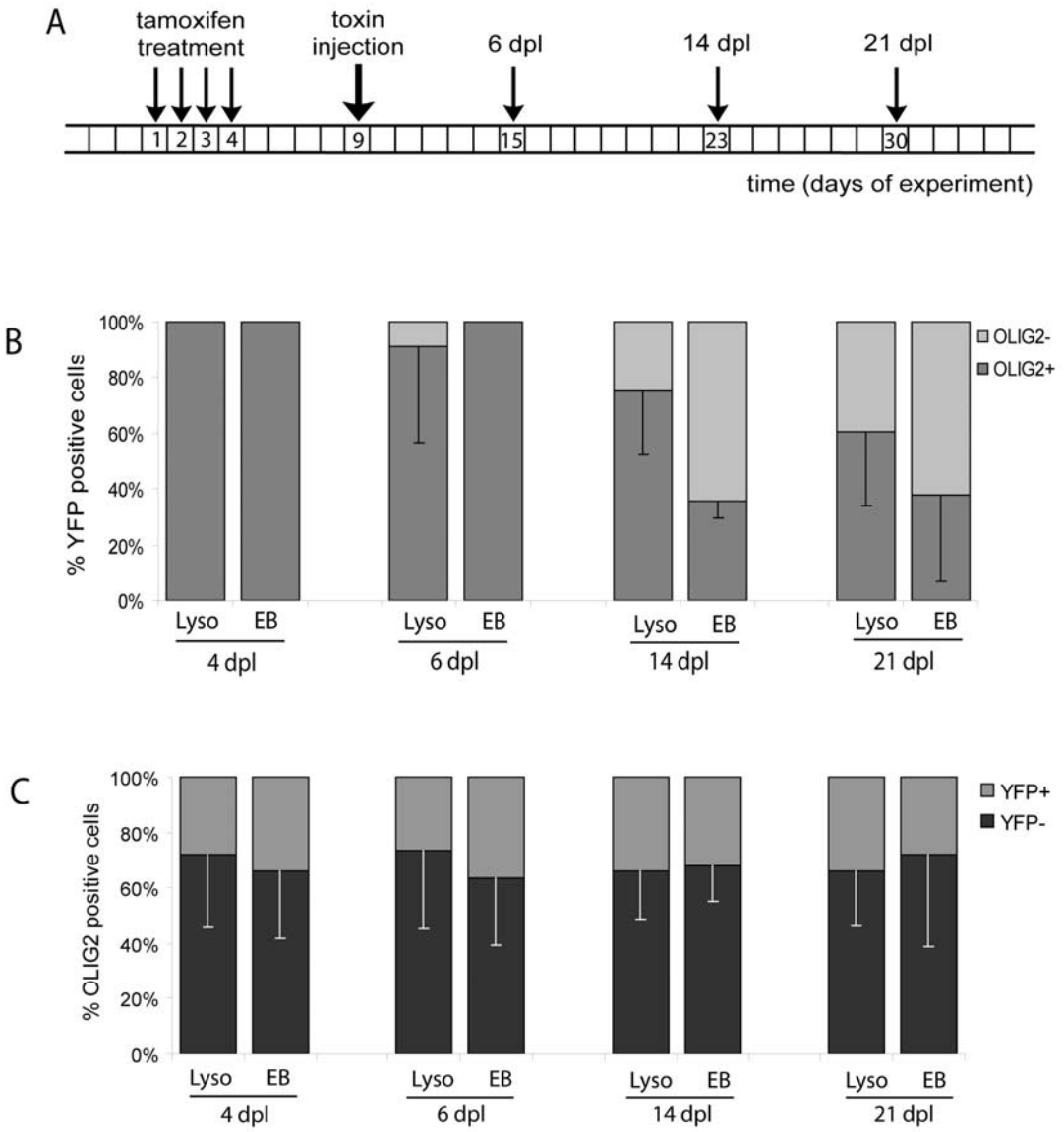

Figure S1, related to Figure 1. (A) Experimental scheme. Cre recombination was induced by administering tamoxifen on each of four consecutive days (experimental days 1-4). Four days after tamoxifen induction, focal demyelination was induced in spinal cord white matter by injection of a demyelinating toxin (day 9). Immunohistochemistry was performed 6 days post-lesion induction (day 15) to examine OLPs' response to injury and 14 or 21 days post-lesion to identify the fate of YFP^+ cells in the reconstructed white matter. (B) The proportion of $OLIG2^+$ within the YFP^+ cell population at each survival time (4, 6 dpl n=10, 14, 21 dpl n=6). (C) The proportion of YFP^+ cells within the $OLIG2^+$ cell population was similar at each survival time examined in both lysolecithin (lyso) and ethidium bromide (EB)-induced lesions, indicating a recombination efficiency in these experiments of between 30-40% in *Pdgfra-creER*^{T2} mice treated with tamoxifen.

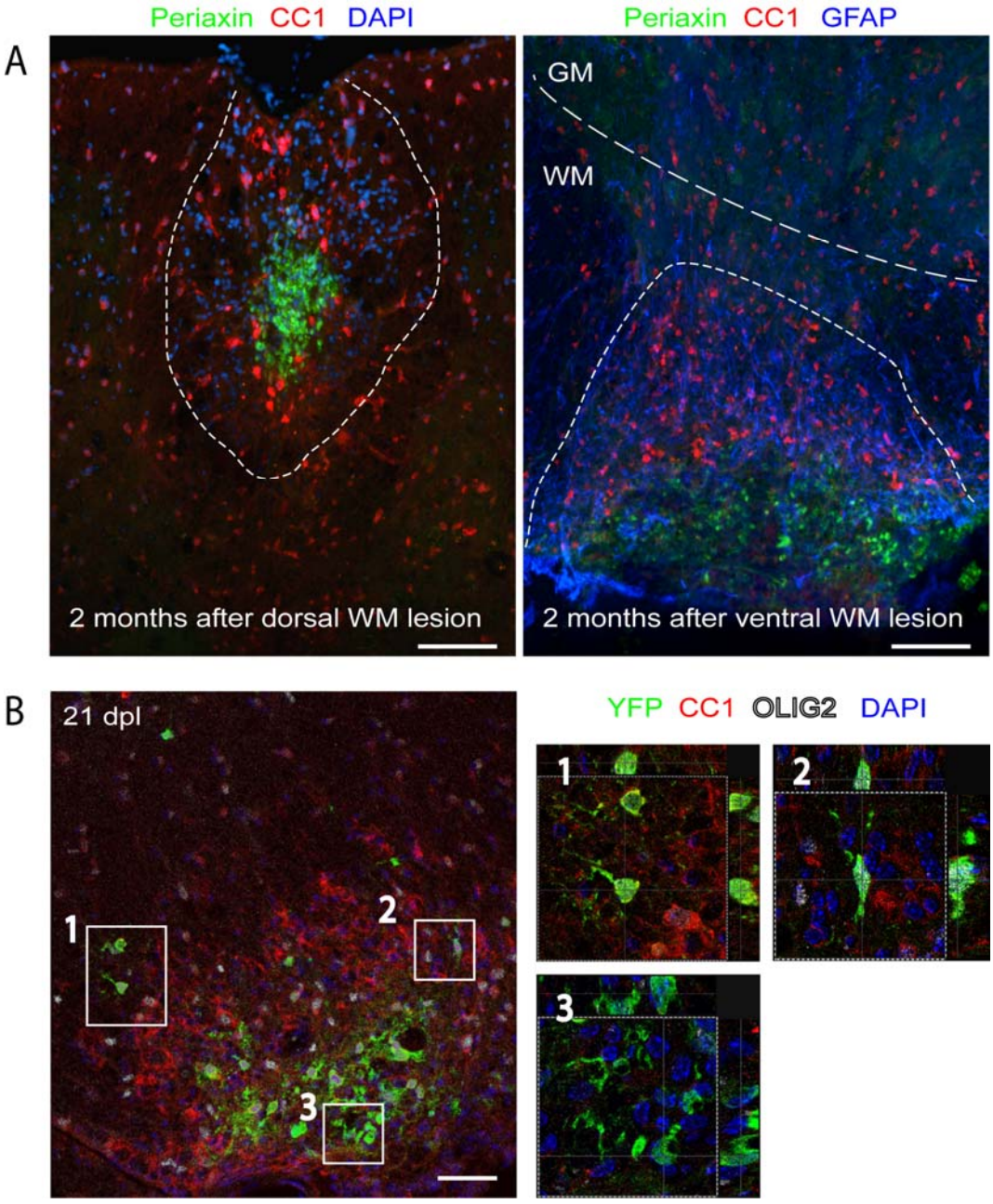

Figure S2, related to Figure 2 and to Figure 3. Contribution of oligodendrocytes and Schwann cells to remyelination of dorsal and ventral white matter lesions two months after lysolecithin injection (A). Note the increased number of newly produced $CC1^+$ oligodendrocytes within the lesion area (bordered by a dashed line) compared with intact white matter. Also note the $Periaxin^+$ Schwann cells in remyelinated areas that are deficient in $GFAP^+$ astrocytes (scale bar, 50 μm). (B) Cellular composition of repaired ventral white matter lesion at 21 dpl. Triple labeling revealed that in regions populated by $YFP^+/CC1^+$ oligodendrocytes (strongly or weakly expressing OLIG2 – box 1) there are some cells of $YFP^+/CC1^-$ phenotype existing, which we identified as undifferentiated precursors as they express $OLIG2^+$ (box 2). In some areas there are cells of $YFP^+/CC1^-/OLIG2^-$ phenotype which posses Schwann cell morphology (box 3, scale bar, 50 μm).

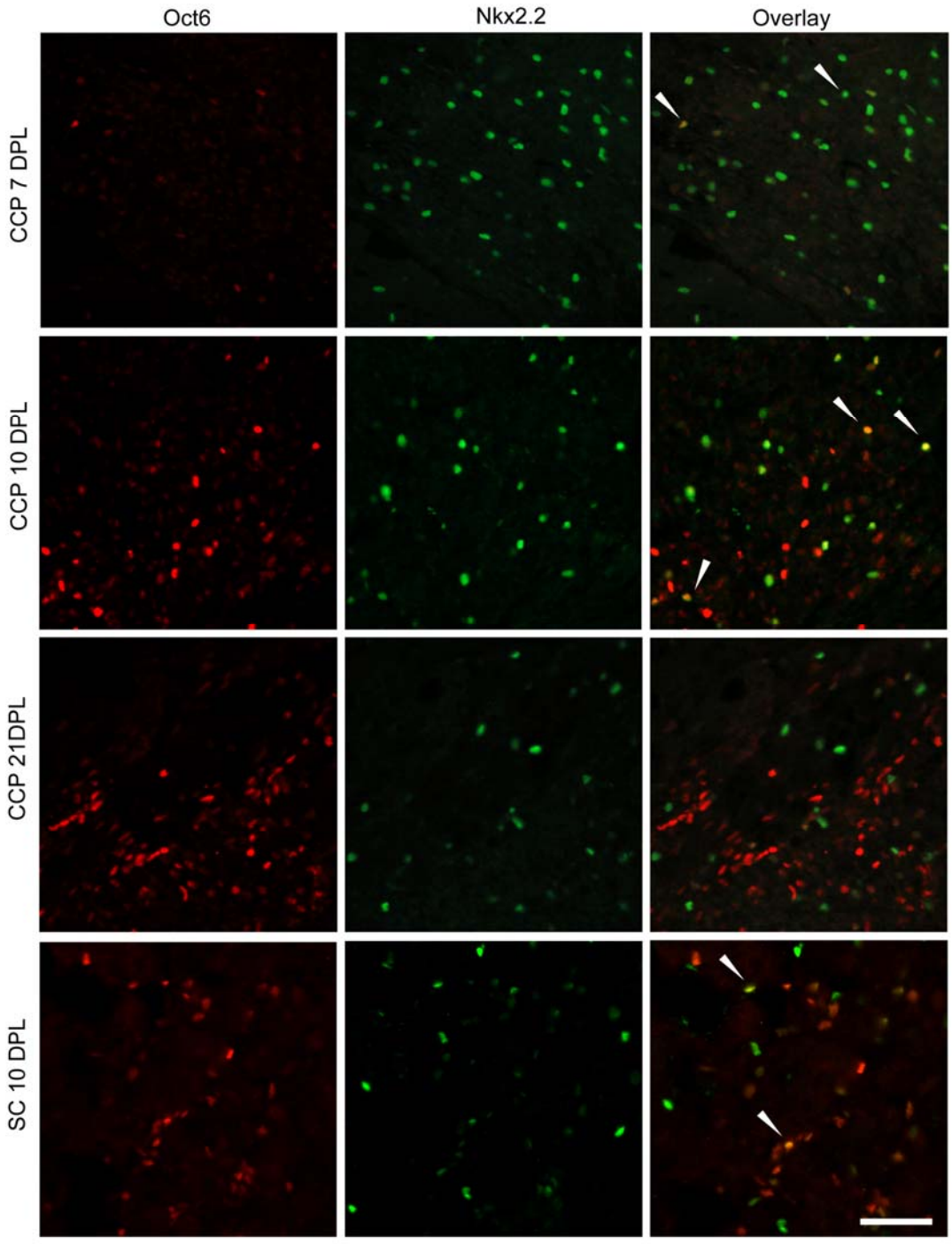

Figure S3, related to Figure 4. A transitional stage of OLP differentiation into the Schwann cell lineage is revealed by co-expression of markers of both OLPs and immature Schwann cells in individual YFP^+ cells. Double immuno-labelling was performed on ethidium bromide-induced demyelinated lesions in the adult rat caudal cerebellar peduncle. $SCIP^+$ cells were occasionally seen in lesions at 7 days post lesion induction (scale bar, 50 μm).

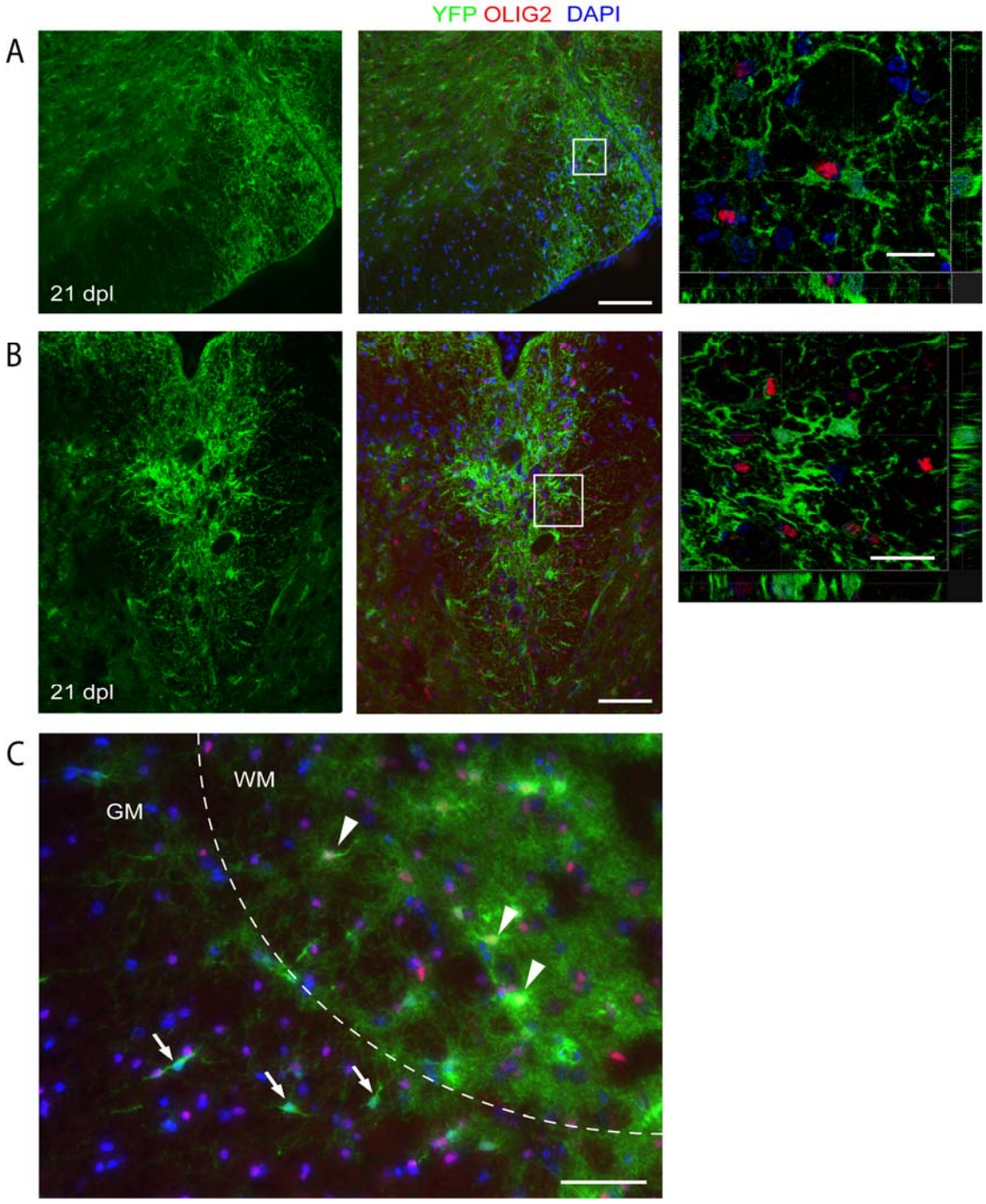

Figure S4, related to Figure 7. Ventral (A) and dorsal (B) lysolecithin-induced demyelinating lesions in FGFR3-Cre mice. There is no expression of OLIG2 in the YFP-expressing cells within the lesion or in the white matter outside the lesion (scale bars, 100 μm in main images, 20 μm in higher-magnification images of areas indicated by boxes). (C) $YFP^+/OLIG2^+$ cells were detected in the gray matter (arrowheads) while there were no such cells in normal appear white matter (arrows, scale bar, 50 μm).