

F. BrdU<sup>+</sup> cell population breakdown. EGFP<sup>+</sup>CC1<sup>+</sup> oligodendrocytes, EGFP<sup>neg</sup>GFAP<sup>+</sup> astrocytes and NG2<sup>+</sup> cells comprise about half of all BrdU<sup>+</sup> cells in the chronic animal.

**Figure 6. *In vivo* treatment with glial growth factor 2 and basic fibroblast growth factor spinal cord injury results in increased numbers of EGFP<sup>+</sup>CC1<sup>+</sup> oligodendrocytes.**

SCI Mice were injected subcutaneously with 0.02mg/kg FGF2 and 0.8mg/kg GGF2 for 8 days, beginning at 1 DPI, and sacrificed on day 9. NG2 immunoreactivity was increased in GGF2 + FGF2 mice (A), compared to saline-injected controls (B). EGFP<sup>+</sup>CC1<sup>+</sup> oligodendrocytes were also increased in GGF2 + FGF2 treated mice (C). D. Quantification of NG2<sup>+</sup> cells indicating a significant increase in EGFP<sup>neg</sup>NG2<sup>+</sup> cells. E. Quantification of CC1<sup>+</sup> cells indicating a significant increase in EGFP<sup>+</sup>CC1<sup>+</sup> oligodendrocytes; bars represent mean ± SEM. Two-way ANOVA was performed with Bonferroni's post-hoc analysis.

## SUPPLEMENTAL MATERIAL

**Supplemental Figure 1. Histopathology and functional deficits after spinal cord injury in the CNP-EGFP mouse**

A. Representative sections depicting eriochrome (myelin, blue), hemotoxylin (nuclei, purple) and eosin/phyloxine (cytoplasm, pink) stain showing an uninjured control spinal cord section (top) and sections from 1, 3, and 7 days post-injury (top to bottom, respectively), every 1mm from 2mm rostral to 2mm caudal. Scale bar = 500 μm.

**B.** Total Spared White Matter, measured as a percentage of the total cross-sectional area of the spinal cord at the epicenter, decreases with time after injury, and is significantly decreased at the injury epicenter as early as 1 day. Results represented as mean  $\pm$  SEM. One-way ANOVA with Tukey's post-hoc analysis, \*  $p < 0.05$ ; \*\*  $p < 0.01$  vs. Control.  $p < 0.05$ ; †  $p < 0.01$  vs. 1 day, #  $p < 0.05$  vs. 3 days.  $N = 8$  animals for all timepoints.

**C.** Hindlimb functional recovery after spinal cord injury. Animals exhibit significant hindlimb paralysis, slight movement at the ankle joint, no plantar placement (BMS score 1) at 1 day post-injury, and recover to weight supported plantar stepping with little coordination (BMS score 5-6) by 7 days. Continued recovery occurs with further significant increase at 35-42 days. Symbols represent mean  $\pm$  SEM.  $N = 8$  animals for acute timepoints,  $N = 6$  for chronic timepoints. Two-way ANOVA with Bonferroni's post-hoc analysis was performed. \* =  $p < 0.01$  vs. 1 day, # =  $p < 0.05$  vs. 7 days.

**D.** CNP-EGFP cells (green) are distributed in both grey and white matter in uninjured control tissue. The white dotted line delineates grey matter. By 1 day at the injury epicenter, EGFP cells are visibly lost dorso-centrally. This loss continues at 7 days and is maintained at 42 days post-injury. GM = gray matter, WM = white matter. Sections are  $10\mu\text{m}$  coronal sections from thoracic level 8 of CNP-EGFP transgenic mice. Scale bar =  $250\mu\text{m}$ .

**E.** Upper panel:  $\text{CC1}^+$  oligodendrocytes and  $\text{GFAP}^+$  astrocytes (asterisks) in the uninjured ventral-lateral white matter. Inset shows a  $\text{GFAP}^+$  cell neighboring a  $\text{CC1}^+$  cell.  $\text{CC1}^+$  cells are always  $\text{EGFP}^+$ .  $\text{GFAP}^+$  cells are always  $\text{EGFP}^-$ . Scale bars =  $10\mu\text{m}$ . Lower panel:  $\text{EGFP}^+\text{CC1}^+$  oligodendrocytes and  $\text{EGFP}^-\text{GFAP}^+$  astrocytes (asterisks) at 1

day post-injury. After injury,  $CC1^+$  cells are always  $EGFP^+$  and  $GFAP^+$  cells are always  $EGFP^-$ . Scale bars = 10  $\mu\text{m}$ .

**F-G.** Quantification of  $DAPI^+$  nuclei, and of  $EGFP^+CC1^+$  oligodendrocytes and  $EGFP^{neg}GFAP^+$  astrocytes after SCI. Most oligodendrocyte and astrocyte loss occurs at the epicenter by 1 day after injury; loss at the epicenter is significant at all time points. Represented as Mean  $\pm$  SEM. One-Way ANOVA. \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$  vs. Control. However, this loss is not reflected in the density of  $DAPI^+$  nuclei. Black boxes in the ventral-lateral white matter indicate where cell counts were performed.

**Supplemental Figure 2. Nestin and S100beta are expressed in subsets of  $EGFP^+NG2^+$  cells in the spinal cord and after spinal cord injury.**

**A.** Example of a nestin (blue) $^+$   $EGFP^+$  (green)  $NG2^+$  (red) cell in the ventrolateral white matter in injured tissue at 3 DPI. Scale bar = 10 $\mu\text{m}$ .

**B.** Quantitative analysis for  $EGFP^+NG2^+$  nestin $^+$  cells in the control animal and in the injured animal at 1, 3 and 7 DPI, at 1.5mm rostral, epicenter, and 1.5mm caudal. Cell counts were performed in the ventral-lateral white matter. A side-by-side comparison for  $EGFP^+NG2^+$  and  $EGFP^+NG2^+$  nestin $^+$  cells is shown for each timepoint. A subset of  $EGFP^+NG2^+$  cells express nestin. Immunoreactive cells were quantified for 5 animals every 0.5 mm from the injury epicenter, and at the injury epicenter in the spared ventral-lateral white matter. For each distance at each timepoint, three 10  $\mu\text{m}$ -thick sections were counted, left and right hemispheres, for a total of six fields per animal. Therefore, thirty

200  $\mu\text{m}^2$  fields of view were quantified for each. One-Way ANOVA with Tukey's post-hoc analysis for variance. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  vs. control.

C. Example of  $\text{S100}\beta^+$  (blue)  $\text{EGFP}^+$  (green)  $\text{NG2}^+$  (red) cells in the ventrolateral white matter in injured tissue at 3 DPI. Scale bar =  $10\mu\text{m}$ .

D. Quantitative analysis for  $\text{EGFP}^+\text{NG2}^+\text{S100}\beta^+$  cells in the control animal and in the injured animal at 1, 3 and 7 DPI, at 1.5mm rostral, epicenter, and 1.5mm caudal. Cell counts were performed in the ventral-lateral white matter. A side-by-side comparison for  $\text{EGFP}^+\text{NG2}^+$  and  $\text{EGFP}^+\text{NG2}^+\text{S100}\beta^+$  cells is shown for each timepoint. A subset of  $\text{EGFP}^+\text{NG2}^+$  cells express  $\text{S100}\beta$ . Immunoreactive cells were quantified for 5 animals every 0.5 mm from the injury epicenter, and at the injury epicenter in the spared ventral-lateral white matter. For each distance at each timepoint, three  $10\mu\text{m}$ -thick sections were counted, left and right hemispheres, for a total of six fields per animal. Therefore, thirty  $200\mu\text{m}^2$  fields of view were quantified for each. One-Way ANOVA with Tukey's post-hoc analysis for variance. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  vs. control.

**Supplemental Figure 3. Olig2, Sox10 and Sox17 expression in  $\text{EGFP}^+\text{NG2}^+$  cells is altered after spinal cord injury.**

A. Examples of  $\text{EGFP}^+\text{NG2}^+$  cells (blue) expressing Olig2 (red) in acutely injured (top panels) and chronically injured (bottom panels) white matter. DAPI (white) labels nuclei. Scale bar =  $10\mu\text{m}$ .

B-C. Cell counts of  $\text{EGFP}^+\text{NG2}^+\text{Olig2}^+$  cells in acutely injured (B; 1, 3 and 7 DPI) and chronically injured (C; 6 weeks DPI) at 1.5mm rostral, at the injury epicenter, and at

1.5mm caudal. Cell counts were performed as described above. One-Way ANOVA with Tukey's post-hoc test for variance. \*  $p < 0.05$ ; \*\*  $p < 0.01$  vs. control.

**D.** Examples of EGFP<sup>+</sup>NG2<sup>+</sup> cells (blue) expressing Sox10 (red, top panels) and Sox17 (red, bottom panels) in acutely injured animals. DAPI (white) labels nuclei. Scale bar = 10 $\mu$ m.

**E-F.** Cell counts of Sox10<sup>+</sup>EGFP<sup>+</sup>NG2<sup>+</sup> (E) and Sox17<sup>+</sup>EGFP<sup>+</sup>NG2<sup>+</sup> (F) cells in acutely injured (1, 3 and 7 DPI) at 1.5mm rostral, at the injury epicenter, and at 1.5mm caudal. Cell counts were performed as described above. One-Way ANOVA with Tukey's post-hoc test for variance. \*  $p < 0.05$ ; \*\*  $p < 0.01$  vs. control; \*\*\*  $p < 0.001$ .