

Supplementary figure legends

Supplementary Figure 1: Co-staining of either astrocytes (GFAP, red) and Schwann cell-associated myelin (P0, green, **A,C**), or of axons (NF200, red) and oligodendrocyte-associated myelin (PLP, green, **B,D**) in uninjured spinal cord sections from tamoxifen control (Tx control, **A,B**) and Nrg1-ablated mice (conNrg1, **C,D**). Nrg1-ablation does not alter any of these markers in the absence of injury. Scale bar, 250 μ m.

Supplementary Figure 2: Images depicting separately the individual GFAP and P0 staining at the injury epicentre (from middle panels in Fig. 1) for vehicle control (Vh control, **A**), Tamoxifen control (Tx control, **B**) and Nrg1-ablated (conNrg1, **C**) mouse spinal cords. Boxed areas in the middle panels indicate the dorsal column region where P0 expression was quantified. Scale bar, 250 μ m.

Supplementary Figure 3: (**A-C**) Sections from the injury epicentre stained with hematoxylin and eosin (H&E) show that GFAP-devoid region apparent at the injury epicentre in contused mice (in Supplementary 1) is cellular and not a cavity. The H&E stain shows the core of the lesion filled with cellular and tissue matrix in vehicle control (Vh control, **A**), Tamoxifen control (Tx control, **B**) and Nrg1-ablated (conNrg1, **C**) mouse spinal cords at 10 weeks post-injury. (**D-F**) High power images are shown of the lesion core and the bordering dorsal column area stained for DAPI and P0 in injured vehicle control (Vh control, **D**), Tamoxifen control (Tx control, **E**) and Nrg1-ablated (conNrg1, **F**) mouse spinal cords. This confirmed the filled area of the lesion core to be cellular (indicated by DAPI positive cell nuclei), but not to contain Schwann cell myelin (P0 immunoreactivity shows Schwann cell myelin in the dorsal column region but not in the lesion core in Vh and Tx controls, and again confirms the absence of Schwann cell myelin in conNrg1 mice). Scale bars, 250 μ m (**C**); 100 μ m (**F**).

Supplementary Figure 4: Ablation of Nrg1 does not affect central myelination after spinal cord injury. (A-C) Co-staining of axons (NF200, red) and oligodendrocyte-associated myelin (PLP, green) in serial sections of the spinal cord that span the rostrocaudal axis of the injury epicentre in vehicle control (Vh control, **A**), tamoxifen control (Tx control, **B**) and Nrg1-ablated (conNrg1, **C**) contused mouse spinal cords. At 10 weeks post-injury, a significant reduction of oligodendrocyte-associated myelin (PLP) is observed in the spinal dorsal columns around the site of injury, especially in the lesion epicentre, with a similar distribution of PLP in all groups (**A-C,D**). (A'-C') High magnification of boxed areas indicated in panels **A-C**. (**D**) Quantification of PLP staining in the dorsal column after injury on sections that span the rostrocaudal axis of the injury site reveals no difference between the groups, indicating that Nrg1 ablation that prevents Schwann cell-mediated remyelination does not trigger compensatory oligodendrocyte-mediated remyelination (^{ns}p > 0.05, one-way ANOVA, post hoc Tukey's, n=4-5 animals/group). Scale bars, 250 µm (**C**); 50 µm (**C'**).

Supplementary Figure 5: Ablation of Nrg1 does not affect oligodendrocyte abundance at the injury site. Expression of Olig2 in transverse sections of the contused spinal cord in vehicle control (Vh control, **A**) and Nrg1-ablated (conNrg1, **B**) mice reveals no significant change (**C**) in the number of oligodendrocytes after ablation of Nrg1 at 4 weeks post-injury (the time point at which Schwann cell-mediated remyelination is normally apparent after spinal contusion injury). Scale bar, 250 µm.

Supplementary Figure 6: (A-F) Toluidine blue stained semi-thin section of a spinal cord at 10 weeks post contusion showing the zone analysed for quantitative ultrastructural assessment in injured control spinal cords (**a-c**) and in injured spinal cords from mice lacking Nrg1 (**D-F**). (**A,D**) Semi-thin sections taken from the lesion epicentre showing the analysed zone. The area indicated by the box was cut for ultrathin sections. The area boxed in (**B**) and (**E**) shows a typical area in an injured control spinal cord containing Schwann cell-myelinated

axons (C) that are absent in the animals lacking Nrg1 (conNrg1; F). (G) Example electronmicrograph showing normal uninjured oligodendrocyte-myelinated axons. (H,I) Electron micrographs showing criteria to distinguish between Schwann cell and oligodendrocyte mediated remyelination. (H) Electron micrograph of axons undergoing remyelination mediated by Schwann cells (SC) within the dorsal column identified by signet ring-like appearance of Schwann cell myelin (SCM), thicker and more compact myelin appearance, and SC basal lamina (arrows) within an area containing collagen fibres. Note the presence of SC-nuclei (asterisk) in SC processes surrounding myelinated axons. (I) Electronmicrograph showing naked axons (N, non-myelinated axons) and axons with a thin myelin sheath provided by oligodendrocytes (Op, oligodendrocyte process; ODM, oligodendrocyte myelin) identified by absence of basal lamina, absence of nuclei in the cytoplasm process surrounding the myelin sheath and the intimate contact between two adjacent myelin sheaths. Scale bar, 250 μm (A); 2 μm (I).

Supplementary Figure 7: Time course of the expression of Nrg1 isoforms and of ErbB in the spinal cord after injury. (A) The relative mRNA expression levels of Nrg1 types I, II and III were determined by qPCR at 1 and 4 weeks after spinal cord injury in wild type mice, examined in the spinal cord lesion epicentre and compared to expression in naïve (uninjured) controls. (B) The relative mRNA expression levels of ErbB2, 3 and 4 receptors were determined by qPCR at 1 and 4 weeks after spinal cord injury in wild type mice, examined in the spinal cord lesion epicentre and compared to naïve controls. Data are shown as mean \pm SEM. ([#]p<0.05 One-way ANOVA on ranks post hoc Dunn's method; *p<0.05, **p<0.01, ***p<0.001 One-way ANOVA, posthoc Tukey's; n = 6-8 animals/group).

Supplementary Figure 8: Time course of the expression of Nrg1 isoforms in the peripheral dorsal root ganglia after spinal cord injury. The relative mRNA expression levels of Nrg1 types I, II and III were determined by qPCR at 1 and 4 weeks after spinal cord

injury in wild type mice, examined in the L4 and 5 dorsal root ganglia and compared to expression in naïve (uninjured) controls. Data are shown as mean \pm SEM. (* $p < 0.05$ One-way ANOVA, posthoc Tukey's; $n = 6-8$ animals/group).

Supplementary Figure 9: Quantification of P0-positive area in the dorsal columns of the rat spinal cord after spinal contusion injury in the presence (A) or absence (B) of dorsal roots. Removal of multiple dorsal roots resulted in a reduced level of P0-positive areas which, however, did not reach statistical significance. Data are presented as mean \pm SEM. (^{ns} $p < 0.05$, one-way ANOVA, post hoc Tukey's, $n=4-5$ animals/group). Scale bar, 100 μm .

Supplementary Figure 10: (A-B) Quantification by qPCR of the transcript encoding IgNrg1 isoforms in thoracic spinal cord (A) and dorsal root ganglia (B). Primers used contain the sequence of exon 4 which is flanked by loxP sites in IgNrg1^{fl/fl} mice and encodes for one of the subunits of the Ig-like domain. **(C-D)** Quantification by qPCR of the transcript encoding Nrg1 type III isoform in thoracic spinal cord (SC, C) and dorsal root ganglia (D). Data are presented as mean \pm SEM. (** $p < 0.01$, student *t*-test, $n=5-6$ group).

Supplementary Table 1: RT-qPCR primers. Primers highlighted with the asterisk were described previously in Makinodan *et al.* (2012). The remaining primers were designed with Primer Blast software (<http://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi>) and submitted for BLAST analysis to ensure specificity.

Supplementary Movie 1: Video showing an injured control mouse during BMS scoring at 8 weeks after injury.

Supplementary Movie 2: Video showing an injured mouse lacking Nrg1 (conNrg1) during BMS scoring at 8 weeks after injury.