Supplemental Information

Injured Axons Instruct Schwann Cells to Build Constricting Actin Spheres to Accelerate Axonal Disintegration

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SUPPLEMENTAL INFORMATION

Supplemental Figures

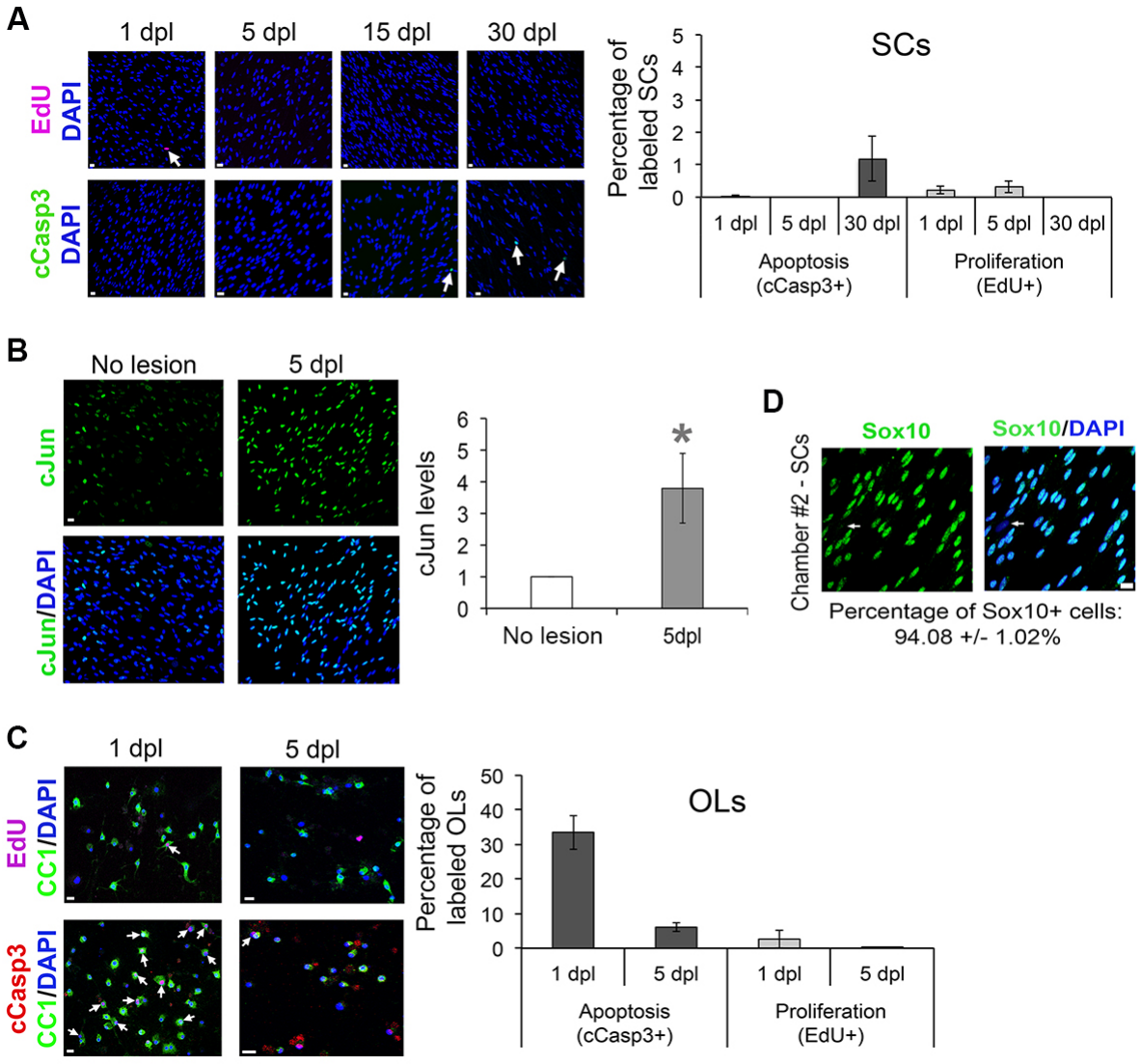

Figure S1. Further characterization of lesion models, Related to Figure 2

(A,C) cleaved Caspase 3 (cCasp3, apoptosis marker) immunofluorescence or EdU incorporation (proliferation marker) and DAPI labeling (nuclei) in (A) SCs or (C) CC1-positive OLs (mature OLs) in chamber#2 at different time-points after lesion, and quantification.

(B) immunofluorescence of cJun (major SC dedifferentiation marker) and DAPI labeling and quantification by CellProfiler 2.0 of cJun levels at 5dpl compared to unlesioned cultures.

(D) Immunofluorescence images of Sox10 (SC marker) and DAPI labeling and percentage of Sox10-positive cells.

For each image, the single-optical section displaying the highest signal intensity is shown. Three to four chambers (at least 300 cells counted per chamber) were used per time-point for each quantification. Paired one-tailed Student's *t*-test, p value: *<0.05 (B), values=mean, error bars=s.e.m. Scale bars, 10 µm.

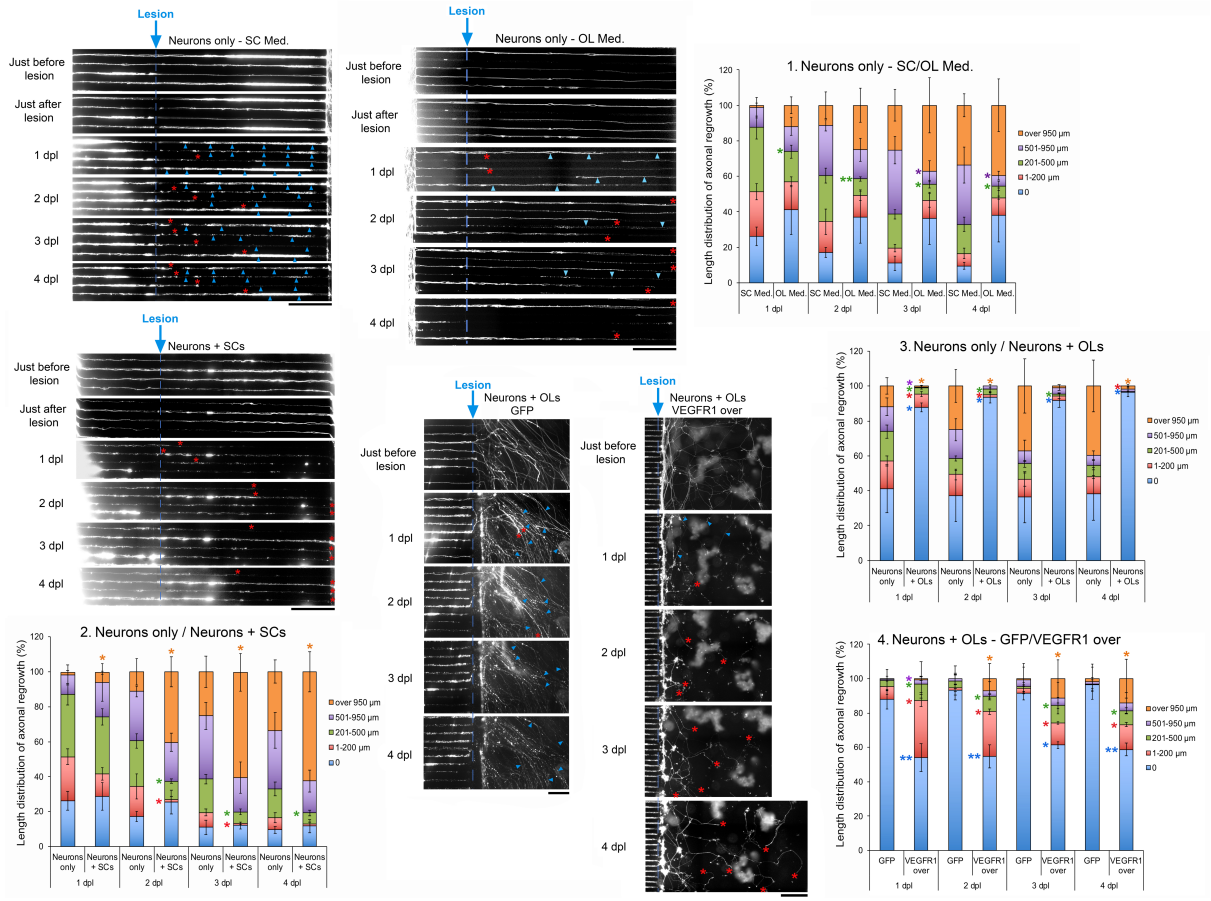

Figure S2. SCs and OLs expressing VEGFR1 promote whereas control OLs inhibit axonal regrowth after lesion in microfluidic chambers, Related to Figure 3

Time-lapse imaging (wide-field) before lesion and at different time-points after lesion of DsRed-labeled axons (white) in microgrooves of Neurons only cultures (cultured either with the Neurons+SCs or the Neurons+OLs culture medium) and of Neurons+SCs cultures, or at the exit of microgrooves of Neurons+OLs cultures where OLs express either VEGFR1 or GFP. Red asterisks in the images indicate growth cone position of axons that have regrown further than the lesion site (indicated by a fine blue dashed line). Note that lesions were carried out at the exit of microgrooves for Neurons+OLs cultures to measure axonal regrowth in chamber#2 where OLs are present at high density. Blue arrowheads indicate non-disintegrated distal cut axons. The graphs show the length distribution of axonal regrowth after lesion in microgrooves of Neurons only and Neurons+SCs cultures and at the exit of microgrooves in Neurons+OLs cultures at different time-points after lesion. Graph 1. compares axonal regrowth in Neurons only cultured either with the Neurons+SCs or the Neurons+OLs media. Graph 2. compares axonal regrowth in Neurons+SCs and Neurons only cultures (both cultured with the same medium). Graph 3. compares axonal regrowth in Neurons+OLs and Neurons only cultures (both cultured with the same medium). Graph 4. compares axonal regrowth in Neurons+OLs cultures where OLs express either VEGFR1 or GFP. Twenty-five to 100 microgrooves were quantified per chamber per day, n=3 to 4 chambers per group. Unpaired one-tailed or two-tailed Student's *t*-test, p value: *<0.05, **<0.01, values=mean, error bars=s.e.m. Scale bars, 250 µm.

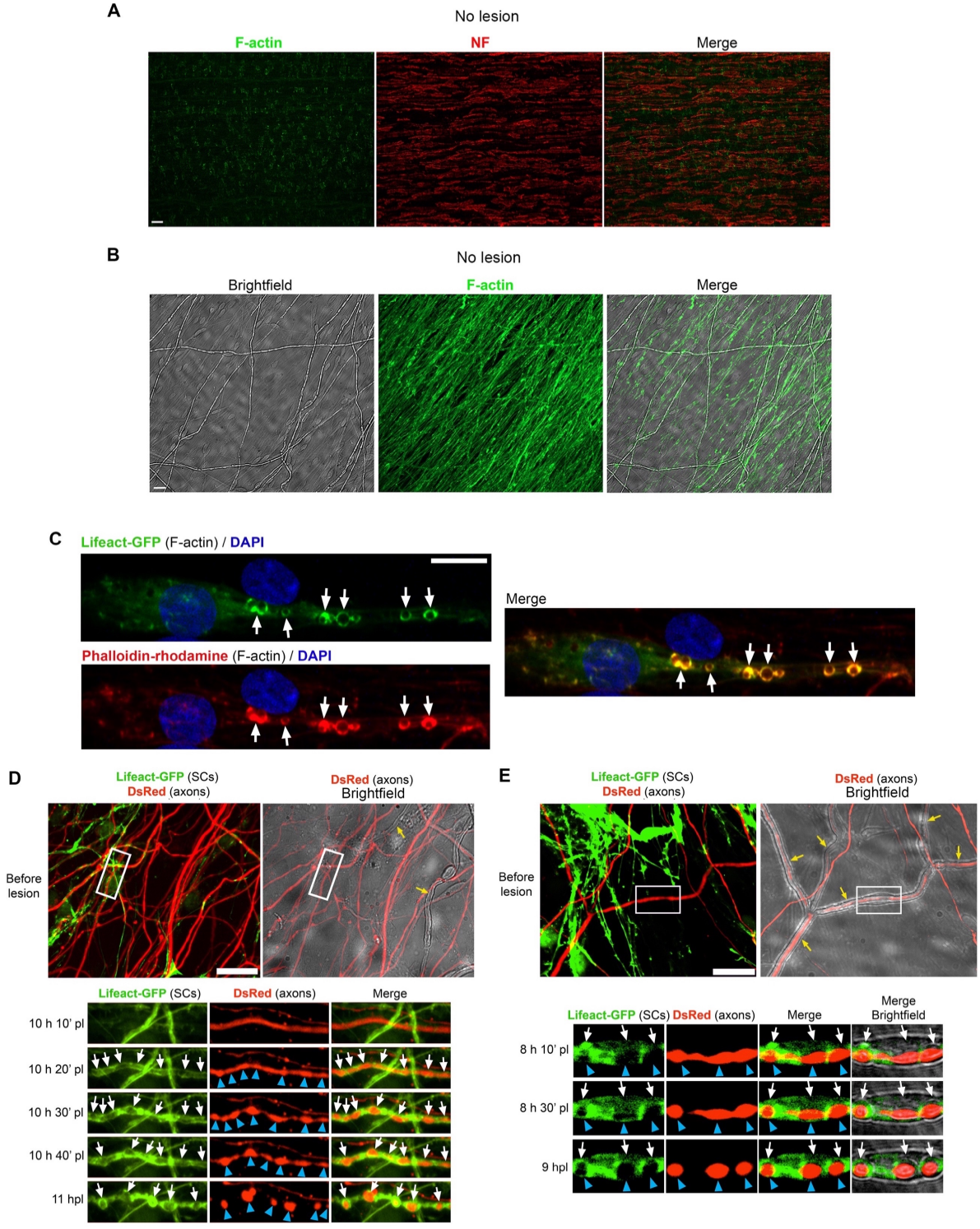

Figure S3. Actin spheres are formed only upon lesion by myeliating and non-myelinating SCs, Related to Figure 4

(A) Co-labeling of F-actin (Phalloidin) with Neurofilament (NF) in 60-µm thick cryosection of unlesioned adult mouse sciatic nerve. Of note, F-actin is known to be localized in Schmidt-Lanterman incisures (**Jung et al., 2011**, **Catenaccio et al., 2017**) in unlesioned sciatic nerves. (B) Co-labeling of F-actin (Phalloidin) with brightfield in chamber#2 of unlesioned neurons/SCs model.

(C) Co-labeling of F-actin by Lifeact-GFP and Phalloidin-rhodamine in SC 6 h after lesion in chamber #2 of neurons/SCs lesion model.
(D,E) Live-cell imaging of DsRed-labelled axons with Lifeact-GFP-labelled SCs together with brightfield images showing myelinating SCs (yellow arrows indicate myelin sheaths) and non-myelinating SCs before lesion. The lower images show magnified time lapses of the regions highlighted by white boxes on the upper images. In (E), the formation of actin spheres by a non-myelinating SC is shown and in (D), actin spheres formed by a myelinating SC are shown. White arrows indicate the position of actin spheres and blue arrowheads show expanded axons within actin spheres. Scale bars, 10 μm (A,B,C), 25 μm (D,E). A representative region of the sciatic nerve of three animals or of three chambers is shown. Images are z-series projections.

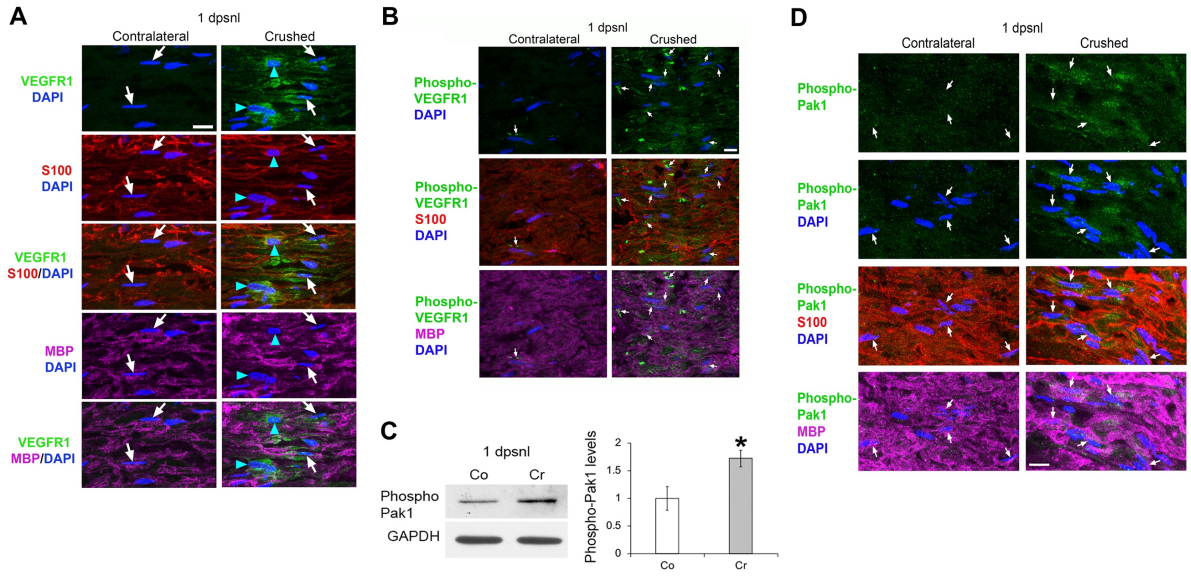

Figure S4. Levels of VEGFR1, phospho-VEGFR1 and phospho-Pak1 are increased in SCs in vivo 1 day after sciatic nerve crush lesion, Related to Figure 5

(A,B,D) Co-immunofluorescence of VEGFR1 (A), phospho-VEGFR1 (B) or phospho-Pak1 (D) with S100 (SC marker), MBP (myelinating SC marker) and DAPI labeling in adult mouse sciatic nerves at 1dpsnl in uninjured contralateral and crushed sciatic nerves. Scale bars, 10 µm. Representative images of crushed and contralateral nerves of 3 animals.

(C) Western blot of phospho-Pak1 in contralateral (Co) and crushed (Cr) mouse sciatic nerves at 1dpsnl and quantification normalized to GAPDH. Unpaired two-tailed Student's *t*-test, p value: * < 0.05, n=3 animals.

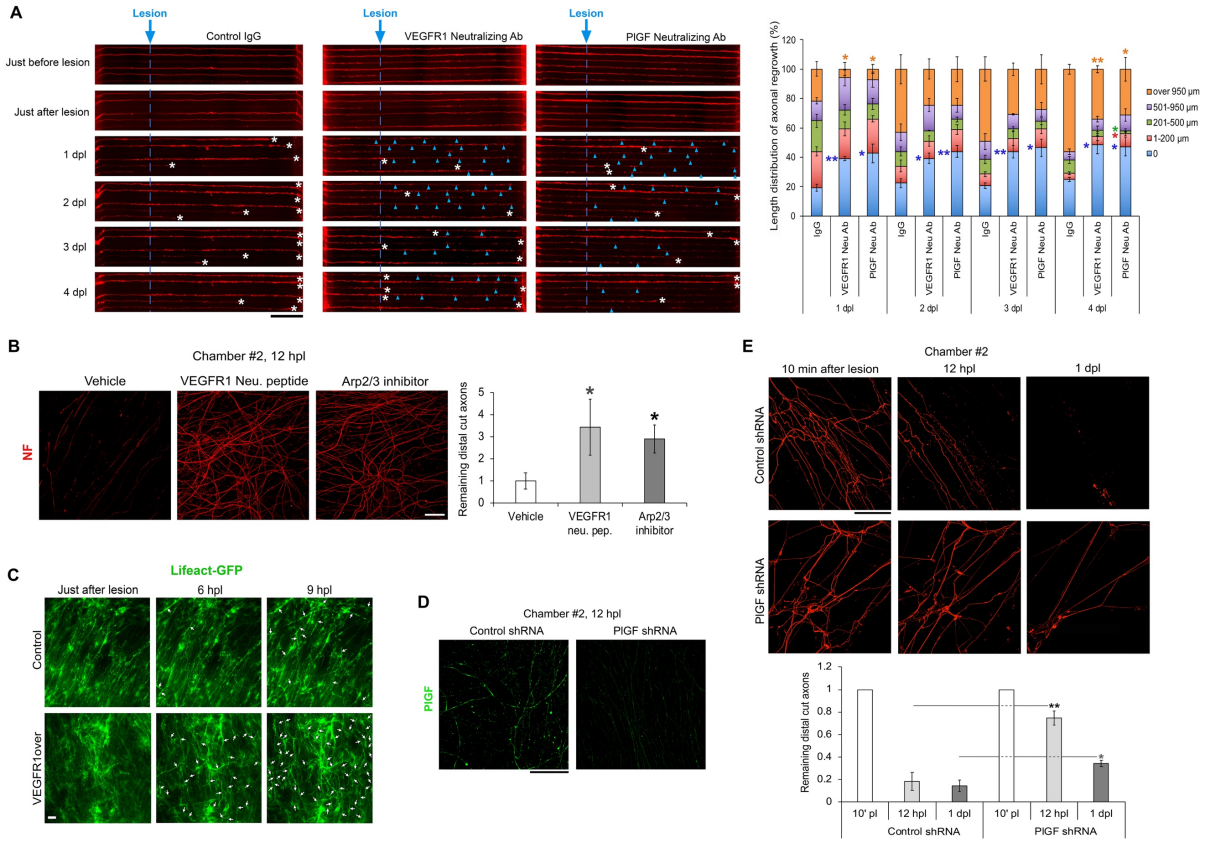

Figure S5. VEGFR1 overexpression accelerates and increases the formation of strings of constricting actin spheres in SCs, Related to Figure 5

(A) Time-lapse imaging (wide-field) of DsRed-labeled axons before lesion and at different time-points after lesion in microgrooves of Neurons+SCs cultured in the presence of a VEGFR1 neutralizing antibody or a PlGF neutralizing antibody or a control IgG. Red asterisks in the images indicate growth cone position of axons that have regrown further than the lesion site (indicated by a fine blue dashed line). Blue arrowheads indicate non-disintegrated distal cut axons. The graph shows the length distribution of axonal regrowth after lesion in microgrooves. Twenty-one to 48 axons were measured per chamber, 3 to 4 chambers were used per condition.

(B) NF immunofluorescence at 12hpl in chamber #2 of Neurons+SCs cultures treated 1 h before lesion with the VEGFR1 neutralizing peptide or with the Arp2/3 inhibitor (100 µM CK666) or their vehicle, and quantification of remaining distal cut axons using NF signal and Cell Profiler 2.0. n=5 chambers for Vehicle, 3 chambers for VEGFR1 neu. peptide and 4 chambers for Arp2/3 inhibitor.

(C) Time-lapse imaging in chamber #2 of Lifeact-GFP-labeled SCs overexpressing VEGFR1 (VEGFR1over) or expressing endogenous levels of VEGFR1 (Control). Arrows indicate strings of actin formed after lesion. Representative photos of 3 videos per condition are shown.

(D) PlGF immunofluorescence at 12 hpl in chamber#2 of Neurons only cultures transduced with PlGF shRNA or control shRNA lentiviral vectors.

(E) Time-lapse imaging (wide-field) of DsRed-labeled axons at different time-points after lesion in chamber #2 of Neurons+SCs cultures where neurons have been transduced with PlGF shRNA or control shRNA lentiviral vectors, and quantification of remaining distal cut axons using DsRed signal and Cell Profiler 2.0. n=3 chambers per group.

Unpaired one-tailed (grey and colored asterisks) or two-tailed (black and colored asterisks) Student's *t*-test, p value: * < 0.05, ** < 0.01, n=3 to 5 chambers per condition for each panel. Scale bars, 250 µm (A), 100 µm (D,E), 50 µm (B), 10 µm (C).

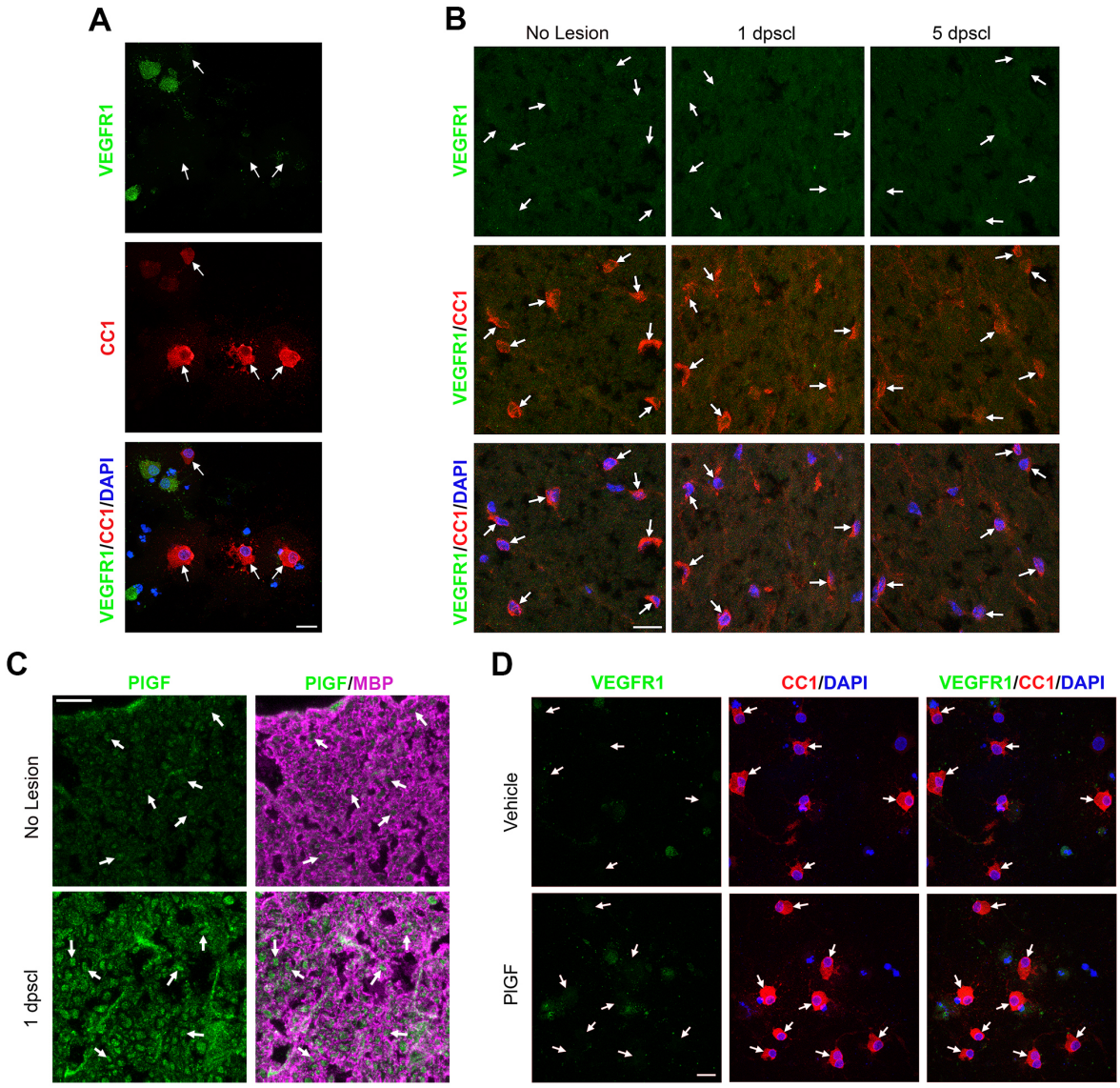

Figure S6. Mature OLs do not express and do not upregulate VEGFR1 after lesion or in the presence of PlGF, Related to Figure 7

(A,B) Co-immunofluorescence of VEGFR1 and CC1 (marker of mature OLs) and DAPI (nuclei) labeling in (A) primary OLs cultured under differentiating conditions for 7 days, or in (B) unlesioned (No Lesion) spinal cords or at 1dpscl or 5dpscl. In (B), images distal to the lesion site and on the side of the hemisection are shown.

(C) Co-immunofluorescence of PlGF and MBP in cross sections of unlesioned spinal cord or at 1dpscl. Arrows indicate axons surrounded by myelin marked by MBP.

(D) Co-immunofluorescence of VEGFR1 and CC1 and DAPI labeling in primary OLs cultured under differentiating conditions for 7 days and either treated with 250 ng/ml PlGF or its vehicle for an additional day.

In (A,B,D) arrows point to mature (CC1+) OLs. Note that while some CC1-negative cells express VEGFR1, mature OLs do not express VEGFR1, even after PlGF treatment.

Scale bars, 10 µm. Representative images of 3 independent experiments or of 3 animals per time-point are shown.