Supporting Information

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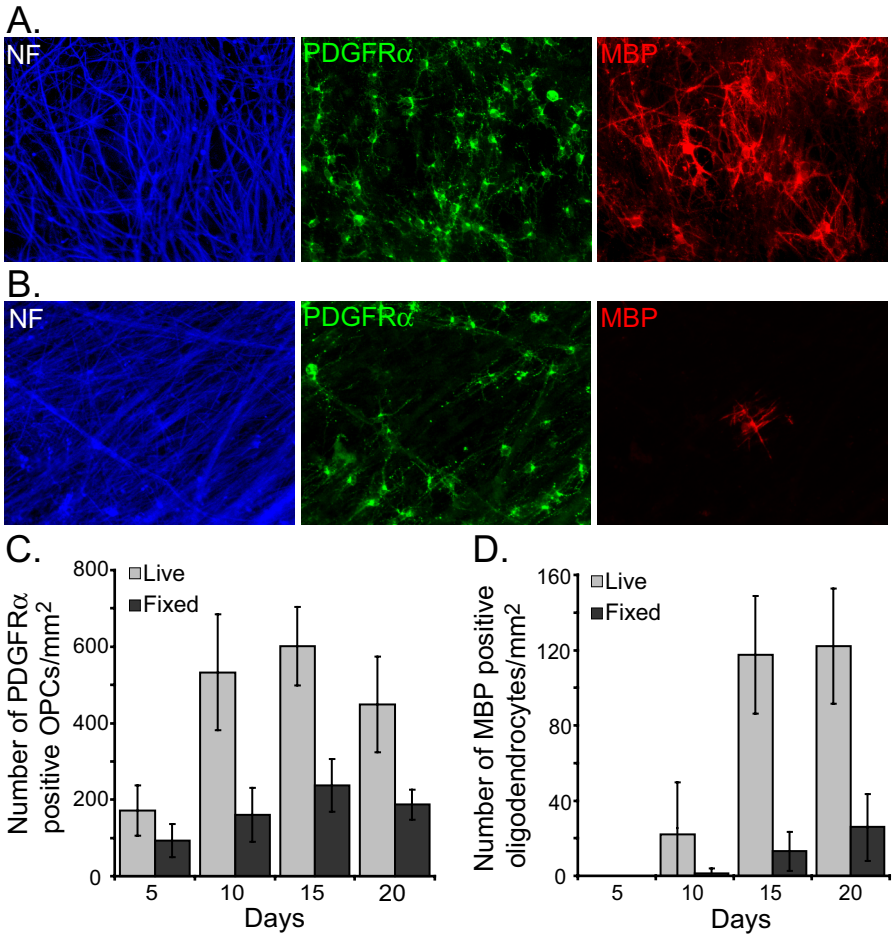

Fig. S1. The inhibition of proliferation is not sufficient to induce differentiation. (*A* and *B*) Immunostaining of cocultures 15 days after seeding a standard density of 200,000 OPCs onto DRG neurons. Axons (blue) are identified by immunostaining for neurofilament (NF). (*A*) Live axons. (*B*) Axons fixed with paraformaldehyde to eliminate dynamic axonal signaling. Oligodendrocyte precursor cells (OPCs) (green) are identified by immunostaining for platelet-derived growth factor receptor α (PDGFRα). The number of OPCs is reduced on fixed axons as compared to live axons. Differentiated oligodendrocytes (red), identified by immunostaining for myelin basic protein (MBP), are largely absent from fixed axon cocultures. (*C*) Quantification of changes over time in the number of PDGFRα^+ OPCs per millimeter squared on live and fixed axons. Compared to live axon cocultures, the proliferation of OPCs is greatly reduced on fixed axons. (*D*) Quantification of changes over time in the number of MBP$^+$ oligodendrocytes per millimeter squared on live and fixed axons. Compared to live axon cocultures, the extent of differentiation on fixed axons is greatly reduced, suggesting that an inhibition of proliferation is not sufficient to induce differentiation. Error bars represent standard deviation.

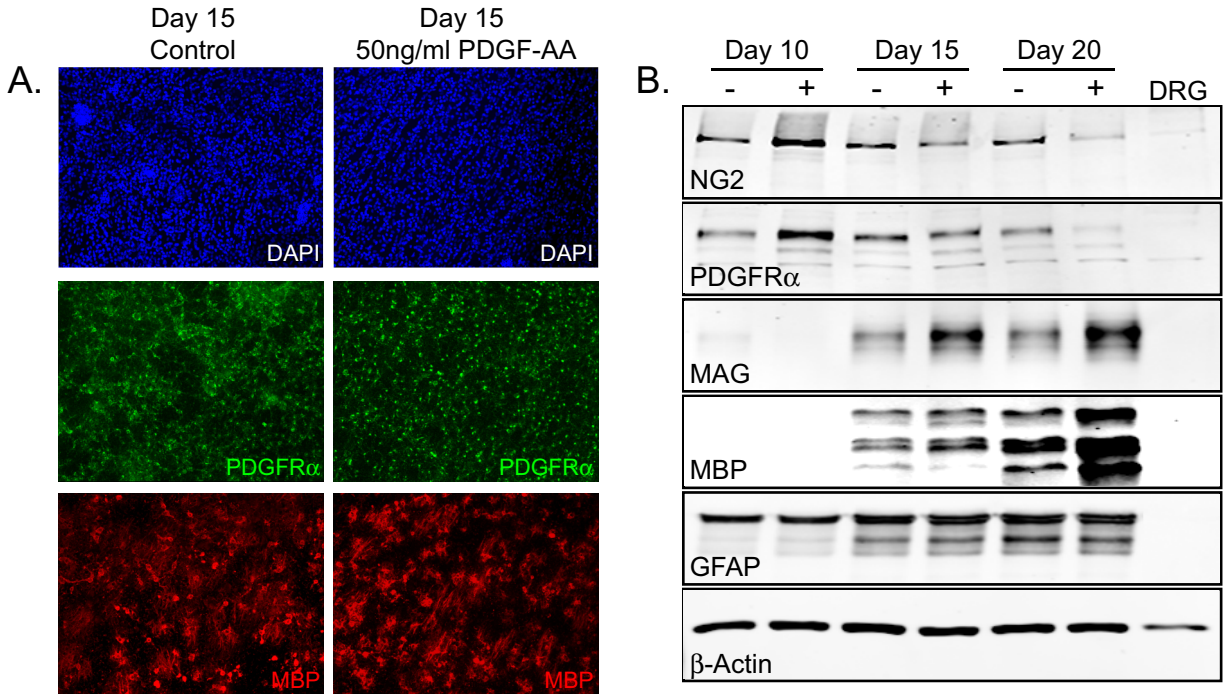

Fig. S2. An increase in available mitogens is not sufficient to delay differentiation. (*A*) Immunostaining of live axon cocultures 15 days after seeding a standard density of 200,000 OPCs. Oligodendrocytes (red) are identified by immunostaining for MBP. OPCs are identified by immunostaining for platelet-derived growth factor receptor α (PDGFRα). Nuclei are stained using DAPI (blue). The addition of 50 ng/ml of platelet-derived growth factor AA (PDGF-AA) does not delay the onset of differentiation as compared to control cultures. (*B*) Western blot analysis of live axon cocultures seeded with 200,000 OPCs. Cocultures with (+) and without (−) the addition of 50 ng/ml of exogenous PDGF-AA, as well as sensory dorsal root ganglion (DRG) neurons cultured alone, are probed for proteins expressed by OPCs (NG2 and PDGFRα), oligodendrocytes [myelin-associated glycoprotein (MAG) and MBP], and astrocytes [glial fibrillary acidic protein (GFAP)]. Actin serves as a loading control. These results suggest that the addition of excess mitogen does not delay the onset of differentiation and may actually accelerate the initiation of this process.

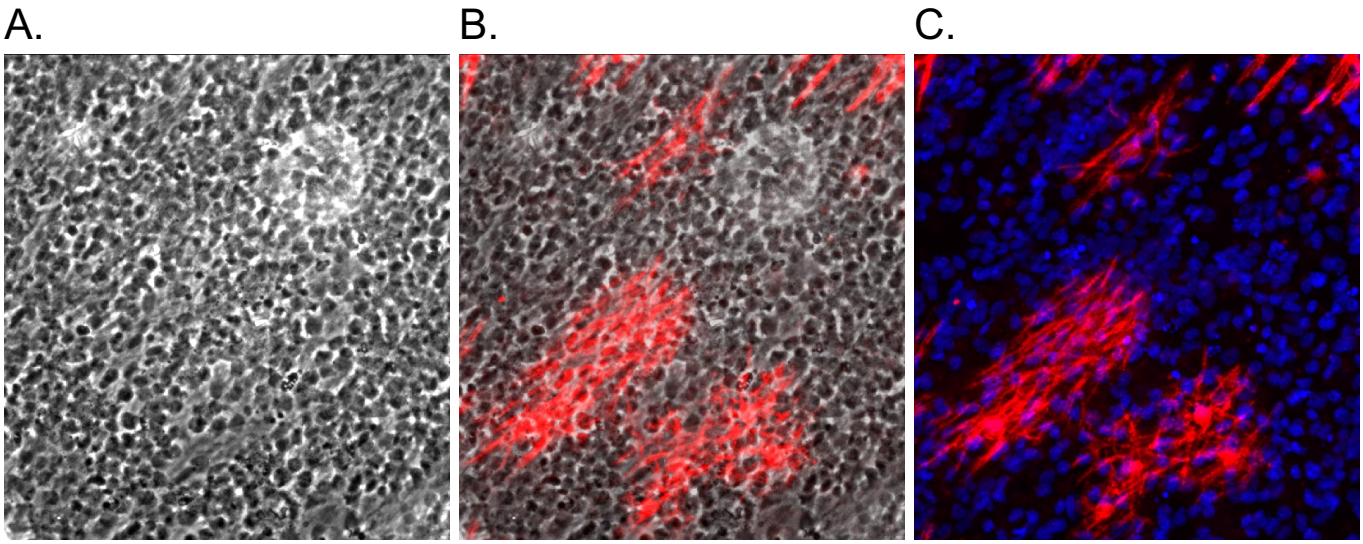

Fig. S3. Secreted factors are not responsible for the induction of oligodendrocyte differentiation by Schwann cells. High density Schwann cells (2 million) were fixed with 4% paraformaldehyde and seeded onto live axons. Cocultures were then seeded with a standard density of OPCs (200, 000). (*A* and *B*) Phase contrast microscopic images of cocultures five days after seeding OPCs. (*B* and *C*) Oligodendrocytes (red) are identified by immunostaining for MBP. (*C*) Nuclei are stained by DAPI (blue). These results suggest that the induction of differentiation is dependent on a contact-mediated interaction between neighboring cells.

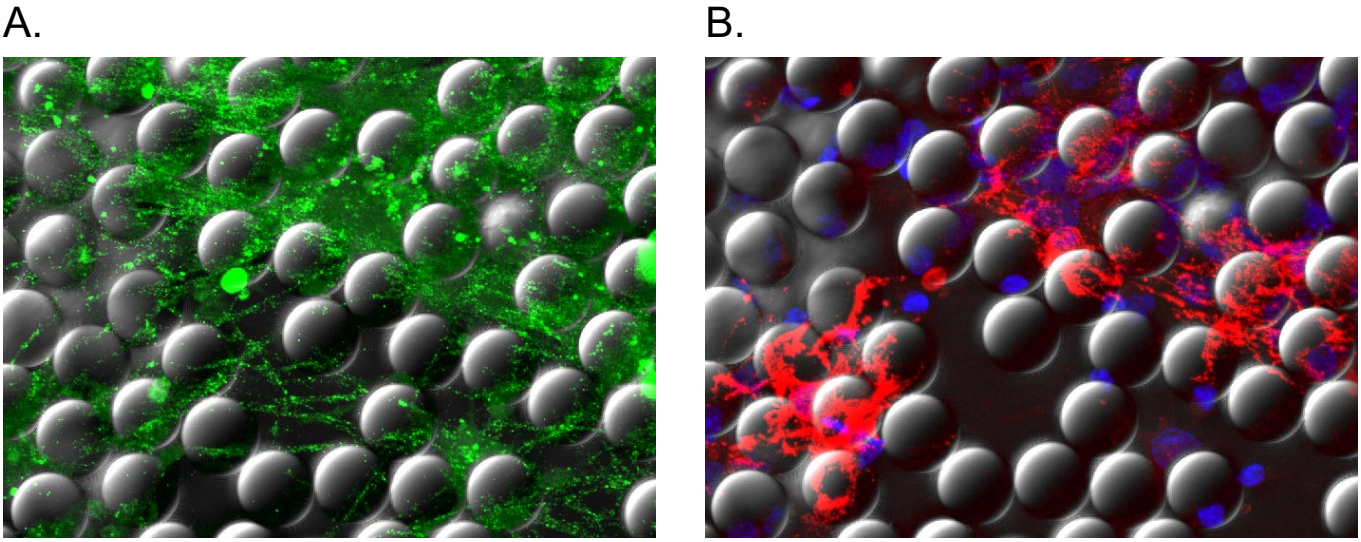

Fig. S4. Dynamic axonal signaling is not required for the induction of differentiation by spatial and geometric constraints. (*A* and *B*) Axons were fixed with 4% paraformaldehyde; 20 μm polystyrene beads were then coated with an antibody to the $p75^{NTR}$ and conjugated at high density to the fixed axons. A standard density of OPCs was seeded onto the axons and immunostained after 5 days. Polystyrene beads were visualized by using DIC microscopy. (*A*) Axons are identified by immunostaining for neurofilament (green). (*B*) Oligodendrocytes (red) are identified by immunostaining for MBP. Nuclei are stained using DAPI (blue).