SUPPLEMENTAL DATA

MATERIALS AND METHODS (extended version)

Mice

Five to 8 week-old C57/Bl6 mice were purchased from Janvier (France). All animal manipulations were performed after a period of adaptation to the animal house of at least one week. The experiments were performed according to the European Union regulations and approved by the ethical committee for animal use (approval number 18471). Mice were housed under standard conditions and given ad libitum access to dry food and water.

Isolation and culture of mouse hematopoietic stem/progenitor cells

Six-8 weeks-old female mice were sacrificed by cervical dislocation. Tibias and femurs were dissected and bone marrow flushed using a syringe. Differentiated white blood cells were then removed using Direct Lineage Cell Depletion Kit (Miltenyi Biotec). The resulting mixture of hematopoietic stem and progenitor cells was then kept in culture in IMDM (Sigma) supplemented with FBS (Sigma), and mSCF, hIL6, and mIL3 (all from Peprotech) in 96-well plates for 16-24 hours at 37°C.

Lentiviral vector generation

DNA sequence encoding GFP alone or GFP and human Semaphorin 3F[23], separated by a self-cleaving T2A sequence, were synthesised and inserted into the self-inactivated vector (SIN) backbone containing the WPRE element and the murine ubiquitous phosphoglycerate kinase 1 promoter (PGK). Lentiviral vectors were generated at the Molecular biology and vector production platform, MIRCEN, François Jacob Institute of Biology, CEA, (Fontenay-aux-Roses, France), as described previously[24]. The SIN vectors were pseudotyped with VSVg glycoprotein G. Viral particles were produced in HEK-293 T cells by a four plasmid transient transfection system. The supernatant was collected 48 h later and filtered, and the particle content of the viral batches was determined by ELISA for the p24 antigen (Gentaur, Paris, France). High-titre stocks were obtained by ultracentrifugation. The pellet was re-suspended in 1% BSA in PBS, frozen and stored at −80 °C.

Transduction of HEK cells and supernatant collection for migration assays

Human embryonic kidney (HEK) 293T cells were grown in DMEM-Glutamax™ medium (Gibco) supplemented with 10% foetal calf serum at 37°C and 5% CO_2. Eighty per cent confluent cells were transduced with PGK-GFP-T2A-Sema3F or PGK-GFP lentiviral vectors at a Multiplicity of Infection (MOI) of 10-20 for 24h. Medium was replaced and transduced cells were grown for 3-5 days. Supernatant was collected and stored at −80 °C.

Lentiviral transduction

Lentiviral transductions were performed in IMDM medium supplemented with FBS, mSCF, hIL6, mIL3, and protamine sulfate (Sigma). PGK-GFP and PGK-GFP-T2A-Semaphorin 3F vectors were applied at an MOI of 50, and cells were incubated at 37°C for 24 hours. Control cells were incubated without lentiviral vectors.

In vitro expansion, transduction efficiency and viability test

Transduced cells were collected, washed, counted, and resuspended in IMDM medium with FBS (Sigma), and mSCF, hIL6, and mIL3 (all from Peprotech) in 6-well plates at initial density of 10^5 cells/mL. Every other day, fresh medium was added to the well, and cells were passaged to new wells as needed. After 5 days of culture, cells were collected, washed, counted and resuspended in PBS. GFP expression (endogenous fluorescence) and viability (propidium iodide (PI) and Viobility 405/520 Fixable Dye (Miltenyi Biotec) staining) were investigated using flow cytometry *(*LSR Fortressa, Benton Dickinson*)*

Colony-forming unit assay

Transduced cells were collected, washed, and resuspended in MethoCult™ GF M3434 medium (Stemcell) at a density of 1000 cells/mL and grown in 6-well plates (1000 cells/well) for 10-11 days. Numbers of erythroid, myeloid, and GFP+ colonies were counted and compared between the conditions.

Western Blot

Following transduction and 5-day expansion, cells were collected by centrifugation and the supernatants were collected. Cell pellets were lysed in a lysis buffer containing protease inhibitor cocktail (Sigma or Thermofisher). Both the protein extracts from cell pellets and the supernatants from cultured cells were subjected to protein quantification using BCA Protein Assay Kit (Thermofisher). Samples were boiled at 99 °C for 2 min, size-separated by SDS-PAGE in 4–20% Criterion TGX Precast gels and transferred using Trans-Blot Turbo Midi Nitrocellulose Transfer Packs (Bio-Rad). Membranes were blocked with 5% BSA in Tris-buffered saline/0. % Tween-20 (TBS-T) for 1h at RT. Primary antibodies (Goat anti-GFP biotin conjugated, Vector Labs; sheep polyclonal anti-human-sema3f antibody, R&D systems; mouse anti-GADPH,Abcam) were diluted in the blocking buffer, and the membranes were incubated with antibody solution under gentle shaking overnight at 4 °C. Following 3 washes in TBS Tween-20 0,1%, Biotin-conjugated polyclonal anti-sheep IgG (Vector Labs) in the case of anti Sema3F antibody was applied for 1 h at RT. HRP-conjugated streptavidin (Vector Labs) was applied for 1 h. For anti GADPH labelling, HRP-conjugated Donkey anti-Mouse IgG (Thermofisher) was applied. Bands were visualized using enhanced chemiluminescence detection kit (BioRad). Images were acquired using a Fusion FX imaging chamber (Vilber).

In vitro migration assay

Purified OPCs were resuspended in a modified Bottenstein-Sato (BS) medium and plated at the upper face of the porous membrane of the transwell chamber (Corning Costar Co, USA)

positioned over the well within the 24-well plate (Corning) at a density of 10000 cells per membrane. Five hundred microliters containing a 1:1 mixture of the BS medium and supernatant from either non-transduced (NT)-, PGK-GFP transduced-, or PGK-GFP-T2A-Sema3F-transduced HEK cells were added to the well. Following a 24-hour incubation period, the cells present at the upper surface of the membrane were scraped off and the membrane fixed using 4% paraformaldehyde solution. Nuclei were labelled with Hoechst and cells present on the lower surface of the membrane counted. The assays were performed as duplicates or triplicates in 4 independent experiments. The statistical analyses were performed on log-normalized data using two-way ANOVA and presented as fold change in the number of cells crossing the membrane insert relative to the control (NT) for each independent experiment.

Preconditioning and cell transplantation

Recipient mice (10-12 week old males) were injected (intraperitoneally) with cyclophosphamide (Sigma) at 200mg/kg for 2 consecutive days (total of 400 mg/kg), and then with busulfan (Sigma) at 25 mg/kg for 4 consecutive days (total of 100 mg/kg).

The day following the last injection of busulfan, transduced cells were collected, washed, and resuspended in alphaMEM (Fisher Scientific) at a density of 3000 cells/µL. Pre-conditioned mice were anaesthetized with isofluorane, and 200µL of cell suspension was injected retro-orbitally. Mice were treated with Baytril for 1 month following preconditioning and transplantation, and their weight was recorded weekly.

Detection of blood chimerism for transplanted cells

Presence of donor cells was investigated in the blood of transplanted mice using a qPCR for sex determining region of the chromosome Y (SRY). Briefly, at 8 weeks post-transplantation, blood was isolated from transplanted mice by submandibular bleed, and gDNA was extracted. qPCR was performed using primers specific for actin (Sigma) and SRY (Thermofisher). Quantification was performed using the $2^{-\Delta\Delta Ct}$ algorithm.

Detection and characterization of transduced cells in the blood of transplanted mice

To detect presence of cells transduced with lentiviral vectors in the blood of transplanted mice, blood was isolated at 8 weeks following transplantation. Red blood cells were lysed using a Red Blood Cell Lysis Buffer (Thermofisher), the cells were washed and the proportion of GFP+ cells was investigated using flow cytometry.

To characterize transduced cells in the blood, we performed labelings with antibodies specific for different hematopoietic lineages: CD11b (BD Bioscience; monocyte marker), CD3 (BD Bioscience; T cell marker), CD19 (Bd Bioscience; B cells marker), Sca1 (Miltenyi; hematopoietic stem/progenitor marker), CD117 (Miltenyi; hematopoietic stem cell marker). The viability was investigated using Viobility 405/520 Fixable Dye (Life Technologies). All markers were applied at 1:10 dilution. Cells were analyzed using the cytometer LSR Fortessa (Benton Dickinson).

Induction of demyelinating lesions

Demyelinating lesions were induced in the spinal cord by a stereotaxic injection of 0.5µl of 1% lysophosphatidylcholine (LPC, Sigma Aldrich) in sterile 0.9% NaCl solution. Prior to the surgery

mice were anaesthetized by intraperitoneal injection of ketamine (90 mg/kg) and xylazine (20 mg/kg) cocktail. Two longitudinal incisions into longissimus dorsi at each side of the vertebral column were performed and the muscle tissue covering the column was removed. Animals were placed in a stereotaxic frame, the 13th thoracic vertebra fixed in between the bars designed for manipulations on mouse spinal cord (Stoelting, USA) and intravertebral space exposed by removing the connective tissue. An incision into dura mater was performed using a 30G needle and LPC injected using a glass micropipette attached via a connector to Hamilton's syringe and mounted on a stereotaxic micromanipulator. The lesion site was marked with sterile charcoal. Following LPC injection, the muscle sheaths were sutured with 3/0 monocryl, and the skin incision closed with 4/0 silk.

Perfusion and tissue processing

Mice were euthanized with Imalgene and perfused with a 4% paraformaldehyde (PFA; Sigma) solution in phosphate buffered saline (PBS; pH 7.4) for immunohistochemical (IHC) analysis or 4% glutaraldehyde (Electron Microscopy Sciences) solution supplemented with calcium chloride in 0.1 M phosphate buffer (PB) for electron microscopy (EM). For IHC analysis, spinal cords were re-equilibrated in 15% sucrose solution and frozen in 15% sucrose-7% gelatine solution in PBS. Twelve-micron coronal sections were cut using cryostat (Leica). For EM analyses, spinal cords were post-fixed overnight, washed in 0.1M PB and cut into four 1mm-thick transverse blocks surrounding the injection area. The next day, the tissue was post-fixed in 1% osmium for 2 hrs, rinsed, dehydrated, and embedded in Epon-812 resin (EMS).

Antibodies and immunohistochemistry

Primary antibodies used for immunohistochemistry were: 1) Millipore antibodies: chicken anti-MBP (AB9348, 1:200), mouse anti-Olig2 (MABN50; 1:200), rabbit anti-GFP (AB3080, 1:500), mouse anti-MOG (MAB5680, 1:200); BD Biosciences antibodies: rat anti-PDGFRα (558774, 1:200), and rat anti CD45R/B220 (557390, 1:200); rabbit anti-cleaved caspase 3 (9661, Cell Signalling, 1:400); mouse anti-APC (OP80, Calbiochem; 1:200); guinea pig anti-Iba1 (Synaptic Systems; 234004, 1:300); Serotec antibodies: rat anti-CD68 (MCA19575, 1:200) rat anti-CD11b (MCA711; 1:200), and rat-anti CD3 (MCA1477, 1:50). Secondary antibodies used were Alexa-conjugated goat antibodies (Invitrogen) and were used at a 1:1000 dilution.

Sections were air-dried for 1h and re-hydrated in TBS. For all stainings but that for PDGFRα, antigen retrieval was performed by heating the sections in a low pH retrieval buffer (Vector Labs) at high power using a microwave for 45 seconds. After washing in TBS, slides were incubated in absolute ethanol solution for 15 minutes at -20°C. After washing, blocking buffer (TBS, 5% NGS, 1%BSA, 0.1% Triton) was applied for 30 min, followed by primary antibody incubation overnight at 4°C. In the case of PDGFRα, triton was not used for overnight incubation. Sections were washed and secondary antibodies applied for 1 hr. After washing, nuclei were counterstained with DAPI. Slides were mounted in Fluoromount-G (Thermofisher Scientific).

Cell type quantification in demyelinating lesions

Images were collected from 2-3 consecutive sections per mouse 144 µm apart containing the central part of the demyelinating lesion using a TCS STED CW SP8 super-resolution microscope

(Leica) and imported into NIH ImageJ software. The area lacking MBP staining (lesion) was delimited on a corresponding channel and imported onto the other channels to regionally restrict the analyses. For each image, the area of demyelination was measured and cells positive for the marker(s) of interest counted. Results are presented as the total number of positive cells per lesion area measured or the percentage of the total.

Quantification of remyelination

Images were taken within the demyelinated area using a transmission electron microscope JEOL JEM 1400 Plus (JEOL) connected to a digital camera (sCMOS), and imported into ImageJ. Remyelinated and demyelinated axons were counted. Results are presented as the percentage of the total axons counted.

Supplemental Figures

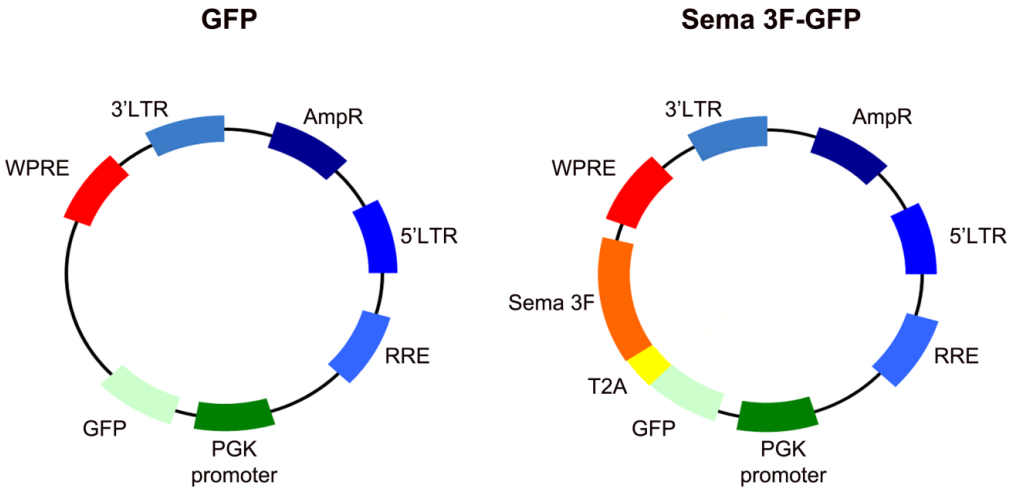

Figure S1. Scheme illustrating lentiviral vector constructions used in the study. WPRE-Woodchuck hepatitis virus Posttranscriptional Regulatory Element; LTR-Long Terminal Repeat; AmpR-Ampicillin Resistance; RRE- Rev Response Element; PGK-PhosphoGlycerate Kinase; GFP-Green Fluorescent protein; Sema-Semaphorin.

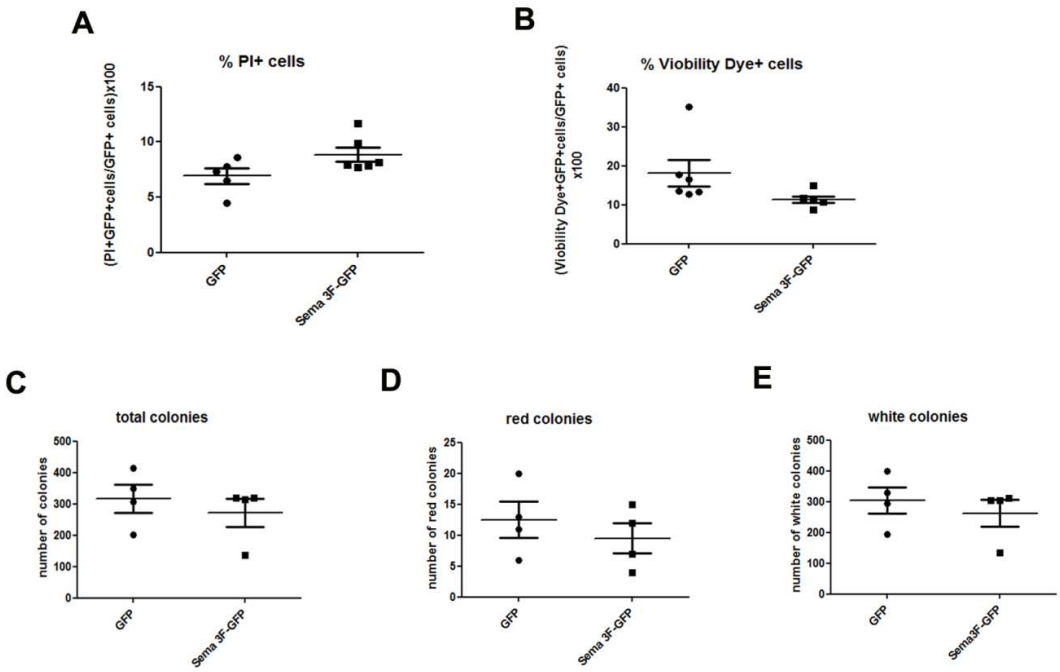

Fig S2. Viability and proliferation/differentiation following Sema3F transduction in vitro. A-B. Flow cytometry analyses of cell death. Hematopoietic stem/progenitor cell preparation was transduced in vitro using PGK-GFP and PGK-Sema3F-GFP lentiviral vectors and expanded for 5 days. Dead/apoptotic cells were labeled using Propidium Iodide (PI) and Viobility 405/520 Fixable Dye. A. PI labelling. B. Viobility Dye labelling. C-E. Colony assay. C.Total colonies formed by transduced cell preparations. D. Red colonies formed by transduced cell preparations. E. White colonies formed by transduced cell preparations.

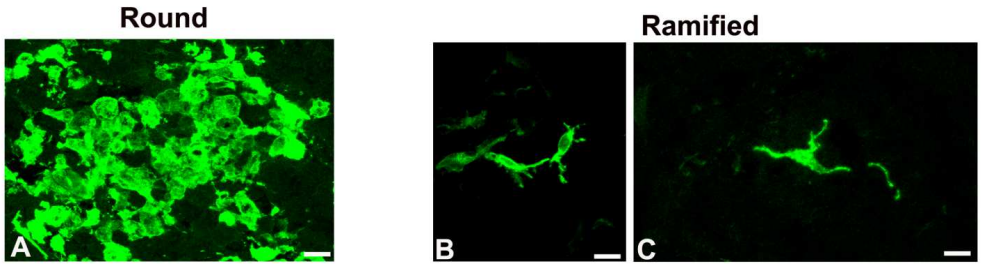

Figure S3. Morphology of GFP+ cells in the spinal cord. A. Large, round cells observed in the demyelinating lesions at all time points. This particular image is of a lesion at 7 dpl. B-C. Ramified cells observed in the lesion-neighbouring tissue at 7 and 10 dpl, but also in the lesions at 60 dpl. Scale bars A=20 µm, B=10 µm, and C=10 µm.