Supporting Information

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SI Methods

Contusion Spinal Cord Injury. Female mice aged 3 mo were anesthetized via i.p. injection of ketamine (100 mg/kg; Fort Dodge Animal Health) and xylazine (20 mg/kg; Lloyd Laboratories). The skin of the back was shaved and scrubbed with Betadine and ethanol. s.c. local anesthetic agent (lidocaine and bupivacaine 1 mg/kg each; Hospira) was injected, the skin was opened, and a dorsal laminectomy was performed at thoracic segment (T) 9 or T10. An impact probe was lowered onto the dural surface and displaced by 0.43 mm (i.e., moderate injury) by using The Ohio State University contusion device. Gelfoam was placed on the dural surface, and muscle and skin were closed in layers. Animals were kept on a heating pad (38 °C) until fully awake. s.c. warm Ringer solution was given for hydration, and gentamicin (4.8 mg/kg; APP) and twice-daily buprenorphine (0.05 mg/kg; Reckitt Benckiser) were administered for 7 and 2 d, respectively. Manual bladder expression was performed twice daily until voluntary control returned.

Virus Microinjection. Six days postcontusion, animals were anesthetized as described earlier, and the laminectomy site was reexposed. Two 2-μL injections of virus were made into the dorsal columns 0.5 mm rostral and 0.5 mm caudal to the lesion epicenter at a rate of 0.5 μL/min. The micropipette was slowly extracted after 2 min. Wound closure and postoperative care were as described earlier. Animals received s.c. warm Ringer solution and buprenorphine and i.p. BrdU (10 mg/mL).

Uninjured control animals (n = 3) received a 1-μL spinal microinjection of virus to label developmentally generated myelin. Postnatal day 4 to 5 pups were anesthetized on ice for 5 min and maintained on an ice pack during injection. Skin of the back was sterilized as described earlier, and a hemilaminectomy was performed at T9 or T10. Virus was injected just lateral of the dorsal spinal artery at a rate of 0.5 μL/min at 0.1 to 0.2 mm below the dural surface, and the micropipette was extracted after a 2-min wait. Gelfoam was placed on the dural surface, and the skin was closed with Vetbond. Pups were kept on a heating pad (38 °C) until fully awake. Pups received s.c. buprenorphine (0.03 mg/kg) and were returned to the nest. They were allowed to mature until euthanasia at age 3 to 4 mo.

Tissue Preparation. Mice were given an overdose of Beuthanasia-D (Schering-Plough) and intracardiac perfusion with Ringer solution to exsanguinate, then with 4% paraformaldehyde. Injured mice were killed at 5 d post virus injection (DPV; $n = 4$), 14 DPV ($n = 4$), 1 MPV ($n = 5$), 3 MPV ($n = 5$), or 6 MPV ($n = 5$). Spinal cords were postfixed in 4% paraformaldehyde overnight, equilibrated in 30% sucrose for cryoprotection, frozen in Optimum Cutting Temperature medium (Ted Pella), and sectioned longitudinally onto slides at 20 to 40 μm.

Immunohistochemistry. Slides were rinsed with PBS solution for 10 min and blocked for 60 min in PBS solution with 5% donkey serum (Jackson ImmunoResearch) and 0.5% Triton X-100 (Amresco). Sections were incubated for 24 to 48 h at 4 °C in block with primary antibodies (as detailed later), then at 4 °C overnight in block with appropriate secondary antibodies: anti-mouse, anti-chicken, anti-rat, or anti-rabbit 647 or 594 (1:300; Invitrogen). Tissue was rinsed five times for 10 min each in PBS solution. The last rinse included the nuclear marker DAPI (1:1,000). Slides were coverslipped with Gelvatol. Primary antibodies included rabbit anti-neural/glial antigen 2 (1:500; gift of W. Stallcup, Sanford–Burnham Medical Research Institute, La Jolla, CA), rat anti-BrdU (1:500; Novus Biologicals), pan-axonal neurofilament mouse anti–SMI-312R and -311R (1:500; Covance), rabbit anti–contactin-associated protein (1:100; gift of S. Trimmer, University of California, Davis, CA), mouse anti–APC-CC1 (1:200; Calbiochem/EMD Biosciences), rabbit anti-myelin basic protein (Millipore), and chicken anti-P0 (1:100; AvesLabs) or rabbit anti-periaxin (1:2,000; gift of P. Brophy, University of Edinburgh, Edinburgh, United Kingdom).

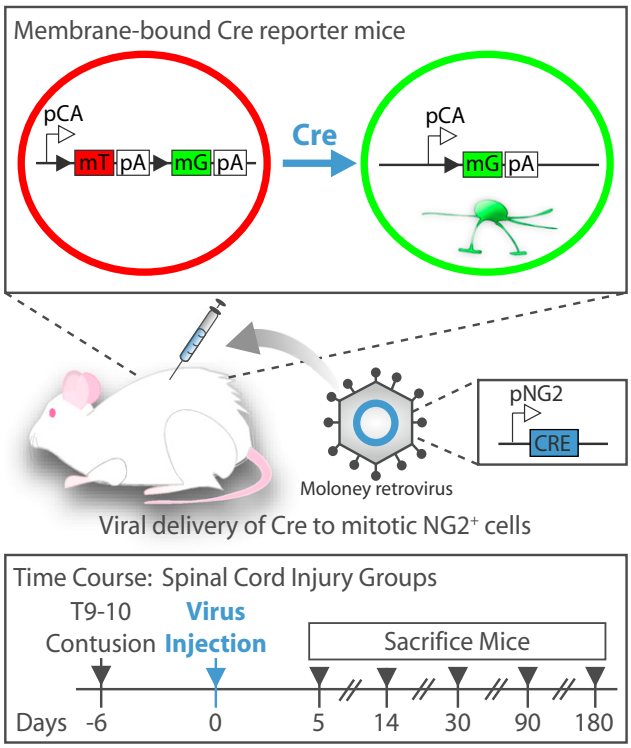

Fig. S1. Experimental design. (*Upper*) Double-fluorescent Cre-reporter mice ubiquitously express membrane-targeted tandem dimer Tomato (mT) before Cre-mediated excision, after which cells express membrane-targeted GFP (mG). CMV enhancer/chicken β-actin core promoter (pCA) drives expression of the loxP-flanked mT sequence followed by a polyadenylation signal (pA). An mG sequence is distal to the second loxP, followed by pA. Cre excision removes the mT cassette and permits expression of mG [schematic adapted from Muzumdar et al. (1)]. (*Lower*) Mice received thoracic contusion followed 6 d later by perilesional injection of high-titer retrovirus to deliver a neural/glial antigen 2 (NG2) promoter-driven Cre sequence to dividing cells. Mice were killed at five time points after injury and cords examined for mG^+ myelin sheaths.

1. Muzumdar MD, Tasic B, Miyamichi K, Li L, Luo L (2007) A global double-fluorescent Cre reporter mouse. *Genesis* 45(9):593–605.