Supporting Information

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SI Materials and Methods

Generation of Tropomyosin Receptor Kinase B and Brain Derived Neurotrophic Factor Knockout Mice. Mice made null for the gene for tropomyosin receptor kinase B (trkB) in all cells die as neonates (1), so they could not be used here. Instead we used the Cre-lox system to generate conditional trkB knockout mice. For host mice, we used a mouse called SLICK (Single neuron Labeling In Cre-mediated Knockout) (2). In SLICK mice, YFP and a tamoxifen-inducible Cre are expressed under the control of the same *thy-1* promoter as *thy-1-YFP-H* mice. The YFP is expressed in neurons in a manner similar to that in the *thy-1-YFP-H* mice (3). In untreated SLICK mice, YFP is expressed in a subset of motor and sensory neurons, and after tamoxifen treatment the YFP^+ neurons also express Cre recombinase. We bred SLICK mice with mice in which the coding region of the trkB gene was flanked by loxP sites (a generous gift of Dr. Luis Parada, Department of Developmental Biology, UT Southwestern Medical Center, Dallas, TX) (4). In the resulting *SLICK::trkB*$^{f/f}$ mice, Cre expression was initiated in YFP-expressing neurons after oral tamoxifen treatment (3), resulting in a knockout of trkB expression in YFP^+ neurons. Examples of immunoreactivity to the full-length trkB receptor in YFP^+ and YFP^- motoneurons in tamoxifen-treated *SLICK::trkB*$^{f/f}$ mice are shown in Fig. S1.

Mice null for the brain derived neurotrophic factor (BDNF) gene also die as neonates (5–7) and thus could not be used in our experiments. Using the Cre-lox system, we generated mice in which the gene for BDNF is knocked out systemically in adults after the administration of oral tamoxifen (*Tam-Cre::BDNF*$^{f/f}$), as described previously (3). By crossing these mice with *thy-1-YFP-H* mice, we obtained mice in which axons in peripheral nerves were marked with YFP and the BDNF gene could be knocked out conditionally. In four mice, the grafts used to repair nerves were obtained from Schwann cell-specific BDNF knockout mice. Mice containing a floxed BDNF allele were crossed with mice in which Cre was expressed under the control of the promoter for ciliary neurotrophic factor (CNTF), a generous gift of Michael Sendtner, Institute for Clinical Neurobiology, University of Würzburg, Würzburg, Germany. In the resulting *CNTF-Cre:: BDNF*$^{f/f}$ mice, we have shown that BDNF is eliminated from Schwann cells in the grafts (3).

Drug Treatments in Fibrin Glue. Fibrin glue was produced by combining fibrinogen, fibronectin, and thrombin solutions, in a ratio of 1:1:2, and then applying this final preparation to the connection between the nerve graft and the proximal segment of the cut nerve (8). The combination rapidly forms a clot and, when applied to repaired nerves, provides a mechanically secure connection between the nerve graft and the proximal stump of the cut nerve. To include 7,8 dihydroxyflavone (7,8 DHF) or deoxygedunin in the glue, we prepared the fibronectin portion of the fibrin glue in solution containing a sufficient amount of the small-molecule trkB agonist to make a final glue solution of either 50 or 500 nM. We chose the concentration of 500 nM because that concentration had been shown to be effective in in vitro experiments (9, 10). The two concentrations used thus represent a 1:1 or 1:10 dilution of the effective concentration. In all cases, 5 μL of the final glue preparation were used to secure the grafts in place.

Tissue Harvesting and Confocal Microscopy. In anatomical experiments, mice were killed 2 weeks after nerve repair surgery with an overdose of pentobarbital (150 mg/kg, i.p.) and perfused with periodate-lysate-paraformaldehyde fixative (11). The cut and repaired nerves, with complete grafts attached, were removed and placed onto a microscope slide and coverslipped using Vectashield (Vector Laboratories). Edges of the coverslip were sealed with clear nail polish. These whole mounts were then imaged using a laser scanning confocal microscope (Zeiss LSM 510). Stacks of optical sections were obtained at 10-μm intervals through the entire depth of the nerve at 10× magnification. Stacks were obtained from contiguous and overlapping microscope fields encompassing the entire extent of the graft and the distal 1 mm of the proximal nerve segment. Stacks were stitched together using Adobe Photoshop, with the result being a large stack of optical sections of the entire nerve graft. In all of the mice used as hosts, a subset of sensory and motor axons contains YFP, and these axons fluoresce brightly against the tissues used in the grafts.

Electrophysiology. The time course of muscle reinnervation was studied in 16 mice using nerve conduction methods similar to those used by Navarro and colleagues (12). In ketamine–xylazine-anesthetized animals, the sciatic nerve was stimulated, as it emerged from the pelvis, using a needle electrode acting as an anode, with a cathode placed in the lumbar axial muscles. Evoked EMG activity in the gastrocnemius muscle was recorded from bipolar fine wire electrodes inserted into the muscles using a 25 G hypodermic needle (13). Short (0.5 ms) constant voltage stimulus pulses were applied to the needle electrode to evoke a compound muscle action potential or M response in the two muscles. Stimulus intensity was graded in small increments from subthreshold to supramaximal for each muscle, and responses were recorded to disk. Maximum amplitude M responses were measured as the average rectified voltage in the M response window, typically 0.5–4.0 ms after stimulus application (14). All recordings were made bilaterally.

Once baseline recordings were obtained, right sciatic nerves were cut in anesthetized mice and repaired by end-to-end anastomosis and fibrin glue. Four mice were untreated, four were treated with 5 mg/kg of 7,8 DHF, four were treated with 5 mg/kg of deoxygedunin, and four were treated with vehicle (1% DMSO). All animals were injected i.p. every day for 2 wk. One of the deoxygedunin-treated mice died before all data could be collected, leaving $n = 3$ for that group. These electrophysiological recordings were then repeated at 2, 4, 6, and 8 wk after nerve transection and repair. For each muscle in each recording session, the average voltage in the M response time window was obtained from multiple stimulus presentations at the intensity evoking the maximal response. These maximum M responses from the right (operated) side of the animal were scaled to the average of the pretransection recordings and the recordings obtained from the left (intact) side of each animal. Data collected were then subjected to multiple linear regression analysis (Statistica, StatSoft) using the treatment groups and scaled M responses as dependent variables and posttransection time as an independent variable. This method evaluates the significance of differences in regression coefficients in the different groups.

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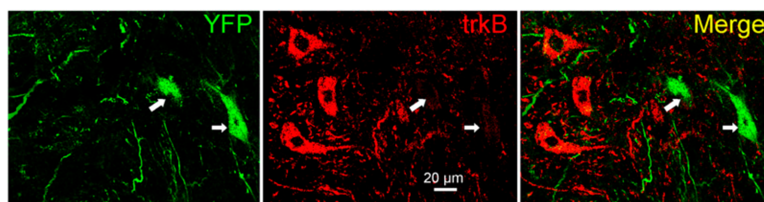

Fig. S1. Cryostat sections of lumbar spinal cord of *SLICK::trkB*$^{f/f}$ mice that had been treated with tamoxifen were reacted with an antibody to the full-length trkB protein [Santa Cruz Biotechnology; TrkB (H-181), catalog no. sc-8316], overnight at 4 °C, followed by a secondary antibody conjugated to Alexafluor 594 (Invitrogen). In this image, taken from lamina IX of the L4 spinal segment of one of these mice, the two motoneurons to the right in each panel express YFP (arrows) and also express cre, so that the trkB gene is knocked out. Immunoreactivity to trkB protein is absent in these neurons. In three adjacent YFP^{-} motoneurons (left side of each panel) immunoreactivity to trkB protein (red fluorescence) is strong.