**Supporting Information**

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**SI Methods**

**Lentiviral Vector Cloning and Production.** Wild-type, full-length trkB rat cDNA (a gift from L. F. Reichardt) was used to generate a Gateway entry vector in pEnter-2B (Invitrogen) with a C-terminal HA tag (pEnter-trkBHA). This construct was then recombined with a lentiviral expression cassette behind a CMV/β-actin hybrid (CAG) promoter (1). Following the trkB-HA transgene was a second promoter, EF1-α, driving expression of the reporter gene copepod-derived GFP (copGFP) with a 3′ woodchuck posttranscriptional regulatory element (lenti-trkB-copGFP). Control vectors expressed copGFP behind the EF1-α promoter (lenti-copGFP). Vascular stromatits virus glycoprotein pseudotyped, self-inactivating lentivirus was generated by transient transfection of 293T cells as described previously (2, 3).

**Infectious titers were determined through infection of 293T cells with serial dilutions of concentrated lentivirus; all viral stocks were diluted to 1 × 10^6 infectious units based on copGFP expression 48 h after infection.**

**Mutant trkB Generation.** The PDGFR-β PI3K signaling domain YYVM was inserted at trkB residue L645 via 3-way ligation at a site homologous to the trkA residue L605 used by Aschcroft et al. (4). pEnter-trkBHA was double-digested with EcoRI and XhoI and ligated to pEnter-trkBHA PCR products generated by using the primer set 5′-GCCAATTTGTACAAAAAGGC-3′ with 5′-CAACATCTGGATCACTAACAATCTCATCAGCC-ATCAGGACTTCATGGGCCC-3′ (double-digested with EcoRI and SnaBI), and the set 5′-ATGTTTGAATCGTACCAATGTGGATGAGGTTAACCAGGCCC-3′ with 5′-GAAATGCTTTTTATTAATGCCC-3′ (double-digested with XhoI and SnaBI).

The juxtamembrane KFG domain was deleted by using the Phusion Site Directed Mutagenesis kit (New England Biolabs). pEnter-trkBHA was amplified by using phosphorylated primers 5′-AGAAAGCGCCAGCTTCCG-3′ and 5′-GGATGTCCAGGAAGACGG-3′. pEnter-trkBHA PCR products generated by using primers 5′-CAACATCTGGATCACTAACAATCTCATCAGCC-ATCAGGACTTCATGGGCCC-3′ (double-digested with EcoRI and SnaBI), and the set 5′-ATGTTTGAATCGTACCAATGTGGATGAGGTTAACCAGGCCC-3′ with 5′-GAAATGCTTTTTATTAATGCCC-3′ (double-digested with XhoI and SnaBI).

**Tissue culture dishes.** To determine Erk phosphorylation by trkB mutant receptors, 20,000 transduced PC12 cells per well were plated in collagen I-coated, 96-well tissue culture plates overnight. Cells were treated with NGF or BDNF (50 ng/mL) or PBS control for 40 min and fixed in 4% formaldehyde solution for 20 min. A CASE kit (SA Biosciences) was used to determine Erk phosphorylation levels per the manufacturer's instructions. For Western blot determination of mutant trkB activity in vitro, 5 million transduced PC12 cells per well were plated in PC12 media on 6-well tissue culture dishes. The next day, media were changed to low-serum RPMI and cultured overnight. Cells were stimulated for either 5 or 40 min with NGF or BDNF (50 ng/mL) or PBS control, washed twice with ice-cold PBS, and collected in 0.5 mL of RIPA buffer with protease and phosphatase inhibitors.

**PCR Cell Culture.** PC12 cells were grown to confluency in T25 flasks in RPMI (Invitrogen) with 10% horse serum, 5% FBS, and Pen/Strep/Glu (PC12 media), infected with lentiviral supernatants from transfected 293T cell cultures, and FAC sorted 5 days after viral infection on a FacStar (BD Biosciences). For neurite outgrowth assay, 5,000 transduced PC12 cells per well were plated on type I collagen-coated, 24-well tissue culture plates in 1 mL of low-serum RPMI (2% horse serum and 1% FBS) and treated with NGF or BDNF (50 ng/mL) or PBS control (n = 3 wells per condition). Growth factors were replenished every other day with a 0.5-mL media change. After 8 days, random fields of PC12 cells were counted, and cells were examined for neurites with a length greater than 2 times the cell diameter.

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**Protein concentrations were calculated by using Bradford assay (Bio-Rad), normalized, and 20 μg of protein run per lane. Primary antibodies were used to detect Erk1/2, phosphorylated Erk1/2 (pErk1/2), CREB, pCREB, trkBY515F, pShc (70 kDa), pFRS-2, pPLC-γ, and GAPDH (Cell Signaling Technology Inc.). Phosphorylated and nonphosphorylated controls were run in parallel gels. GAPDH was used as a loading control for all Western blottings.**

**DRG Neuron Culture.** Animal use in this research was approved by the VA Medical Center Institutional Animal Use and Safety Committee. Adult (150–165 g) female Fischer 344 rats were deeply anesthetized with 2 mL/kg of a 25 mg/mL ketamine, 1.3 mg/mL xylazine, and 0.25 mg/mL acepromazine mixture (ketamine mixture) and decapitated, and the spinal columns were removed and submerged in ice-cold DMEM/F-12 (Invitrogen). DRGs were dissected from the capsule, cut in half, and collected in Hibernate A (Brain Bits LLC). DRGs were washed twice in DMEM/F-12 and then digested for 1 h at 37 °C in collagenase type XI (1:1; DMEM/F-12 to 0.5% collagenase XI in L15 medium; Worthington Biochemical Corp.) with gentle agitation every 15 min. Cells were centrifuged 2 min at 735 × g and collagenase solution was removed and substituted with ice-cold DMEM/F-12 (Invitrogen), DRGs were dissected from the capsule, cut in half, and collected in Hibernate A (Brain Bits LLC). DRGs were washed twice in DMEM/F-12 and then digested for 1 h at 37 °C in collagenase type XI (1:1; DMEM/F-12 to 0.5% collagenase XI in L15 medium; Worthington Biochemical Corp.) with gentle agitation every 15 min. Cells were centrifuged 2 min at 735 × g, and collagenase solution was removed and gently replaced with DMEM/F-12 plus 10% FBS so as not to disturb the cell pellet. Cells were then washed twice by gently applying DRG culture medium (DMEM/F-12 with B-27 supplement and Pen/Strep/ Glu) so as not to disturb the pellet. Cells were resuspended in 1 mL of DRG culture medium and allowed to settle for 45 s, and then 0.5-mL of cell suspension was added to 3.5 mL of DRG culture medium. A total of 0.5 mL of final cell suspension was added to 0.5 mL of culture medium in poly-d-lysine-coated, 35-mm cell culture dishes. DRGs isolated from individual animals were plated separately.

Four hours after plating, DRG cultures were infected with 5 × 10^5 units of lentivirus per 35-mm culture dish. Cultures were either stimulated with rh-BDNF (20 ng/mL; Peprotech) or treated with equal amounts of PBS every 24 h. After 72 h of...
culture at 37 °C in 5% CO₂, cells were fixed with 4% paraformaldehyde (PFA) for 30 min, washed 3 times with TBS, blocked, and permeabilized with TBST (TBS plus 0.25% Triton X-100) with 5% serum for 1 h and then incubated overnight at 4 °C in TBS and 1% serum with primary antibodies: mouse anti-βIII-tubulin (1:1,000; Promega) and rabbit anti-CGRP (1:2,500; Millipore). Cells were washed 3 times with TBS, incubated at room temperature for 2.5 h with secondary antibodies donkey anti-rabbit 594 and donkey anti-mouse 647 (1:250; Invitrogen) and DAPI (1 μg/mL; Sigma–Aldrich), and then washed 3 times in TBS.

DRGs virally transduced to express trkB were treated with inhibitors of trkB downstream signaling kinases for 72 h at 37 °C in 5% CO₂ with rh-BDNF (20 ng/mL). Inhibitors were added every 24 h: Erk inhibitor (3-(2-aminoethyl)-5-((4-ethoxyphenyl)methylene)-2,4-thiazolidinedione, HCl; 100 μM, 0.5% DMSO), PI3K inhibitor (LY294002; 10 μM, 0.5% DMSO), Akt inhibitor (1,6-hydroxymethyl-chiro-inositol-2-(R)-2-O-methyl-3-O-octadecyl-sn-glycerocarbonate; 10 μM, 0.5% DMSO; EMD), and control 0.5% DMSO. Immunocytochemistry was performed as described above. Neurite length and Sholl quantification: monochrome images of virally transduced, C8PEP-immunoreactive DRG neurons were acquired with PictureFrame software for a MicroFire digital camera (Optronics) mounted on an upright fluorescence microscope (Olympus). The NeuronJ plugin for ImageJ (National Institutes of Health) was used for longest neurite tracing and quantification on acquired images (5). Sholl analysis was performed on images inverted and thresholded in ImageJ with the Sholl ImageJ plugin version 1.0 (Ghosh laboratory, University of California at San Diego, La Jolla, CA). DRG longest neurite length data were analyzed by Kruskal–Wallis non-parametric analysis. Sholl data were analyzed by repeated-measures ANOVA statistical analysis.

**Lentiviral Transduction of Motor Cortex.** Sixteen adult (150–165 g) female Fischer 344 rats were anesthetized with ketamine mixture, and bone overlying primary motor cortex was shaved to an extent adequate to allow access to the thin bone layer (to avoid cortical injury) into 21 sites, 500 nL per site, of the right motor cortex by using a beveled 36-gauge nanoliter syringe (World Precision Instruments Inc.) at a depth of 1.2 mm and a rate of 200 nL/min by using a steretacutically mounted syringe pump (Kent Scientific Corp.). Nineteen additional animals were used in the examination of trkB signaling domains in vivo. First, layer V corticospinal neurons were selectively labeled with the retrograde tracer CTB (LIST Biological Laboratories). A total of 2 μL of a 1% CTB solution was injected into the dorsal columns at C4 bilaterally (±0.2 mm lateral to midline, 0.8 mm ventral to the dorsal spinal cord surface). Then, lentivirus-trkB-copGFP (n = 5), lentivirus-trkB-VSVGf–PI3KcopGFP (n = 5), lentivirus-trkB1KFG–Y316F-copGFP (n = 4), or lenti-copGFP (n = 5) (0.5 × 10¹⁰ units/mL) was delivered to 21 sites as described above.

**Syngeneic Fibroblast Culture and Transduction.** Rat fibroblasts were generated from skin biopsies and transduced with BDNF-encoding retrovirus as described previously (6). Two-site BDNF-secreting fibroblasts (6). Cells were cultured in standard medium (DMEM with 10% FBS and Pen/Strep/Glu) with G418. Transduced fibroblasts (6). Cells were cultured in standard medium (DMEM with 10% FBS and Pen/Strep/Glu) with G418. Transduced fibroblasts (6). Cells were cultured in standard medium (DMEM with 10% FBS and Pen/Strep/Glu) with G418. A total of 2 μL of scAAV6-eGFP (1 × 10¹⁰ viral particles per milliliter; University of North Carolina Vector Core, Chapel Hill, NC) was injected with a pulled glass micropipette (OD, 40 μm) in the lateral gray matter (0.6 mm lateral to midline, 1.0 and 0.5 mm ventral to the dura) at 6 points along the length of the cervical enlargement by using a Picospritzer II (General Valve). During the same surgical session, 7 of these rats were injected with lenti-trkB-copGFP, and 6 were injected with control lenti-copGFP as described above. Two weeks later, all animals underwent subcortical aspiration lesions and grafting of BDNF-secreting fibroblasts in collagen I as described above. Animals were transcardially perfused 2 wk after subcortical lesion with ice-cold saline, followed by 4% PFA fixation.

**EBr Injection to Enhance Vector Uptake.** Twenty-three animals were anesthetized with ketamine mixture, and a C3 laminectomy was performed. All animals were injected with 1 μL of 0.1% EBr in 4 sites: 0.2 mm lateral to midline, 0.6 and 1.0 mm ventral from the dura, and 1.0 mm. Two weeks later, animals were injected with 1 μL of scAAV6-eGFP (1 × 10¹⁰ viral particles) into the demyelinated cervical spinal cord. At the same time as retrograde infection with scAAV6-eGFP, 13 animals were injected with lenti-trkB-copGFP, and 10 animals were injected with control lenti-copGFP as described above. Two weeks later, all animals underwent subcortical aspiration lesions and grafting of BDNF-secreting fibroblasts in type I collagen as described above. Animals were transcardially perfused 2 wk after subcortical lesion with ice-cold saline, followed by 4% PFA fixation.

**Sectioning and Histology.** Brains were removed from the skull and sectioned transversely on a cryostat set at 40-μm intervals (Leica). For light-level immunohistochemistry, sections were washed 3 times in TBS, and endogenous peroxidases were quenched for 15’ at room temperature in 0.6% H₂O₂ in TBS, washed 2 more times, and then blocked for 1 h in TBS (TBS plus 0.25% TRITON X-100) with 5% serum at room temperature before overnight incubation at 4 °C in TBS with 5% serum and primary antibodies. On the second day, sections were washed and then incubated in biotinylated secondary antibody generated in either donkey or horse (1:250; Jackson ImmunoResearch Laboratories) at room temperature for 1 h, washed again, and then incubated in avidin-biotinylated enzyme complex (Vector Laboratories) for 1 h at room temperature before development in 3,3’-diaminobenzidine (DAB). For fluorescent immunohistochemistry, sections were washed in TBS, then blocked in TBS with 5% serum for 1 h at room temperature, incubated overnight.
at 4 °C in TBST with 5% serum and primary antibodies, washed, and incubated for 2.5 h in TBS with Alexa Fluor-conjugated secondary antibodies generated in donkey (1:250; Invitrogen) with DAPI (1 mg/mL; Sigma–Aldrich). For HA immunohistochemistry, sections were first incubated in 0.01 M Tris-HCl, pH 9.0, for 6 h at 65 °C before peroxidase quenching or blocking. The following primary antibodies were used in this study: rabbit anti-copGFP (light level, 1:10,000; fluorescent, 1:2,000; Wako Chemicals USA Inc.), mouse anti-HA (1:500; Sigma–Aldrich), rabbit anti-NF200 (1:500), mouse anti-GAP-43 (1:1,000), rabbit anti-eGFP (light level, 1:6,000; fluorescent, 1:1,500), mouse anti-parvalbumin (1:2,500), mouse anti-GFAP (1:1,000) (Millipore), mouse anti-nonphosphorylated neurofilament (SMI-32; 1:1,000; Covance Research Products Inc.), goat anti-CTB (3-day primary incubation; 1:10,000; LIST Biological Laboratories), and goat anti-5-HT (1:500; ImmunoStar Inc.).

**Quantification and Analysis.** For quantification of NF200 immunoreactivity in subcortical grafts, fluorescent images were inverted and thresholded, and pixel density was quantified by using ImageJ (National Institutes of Health). To quantify growth into the graft of NF200-, SMI-32-, parvalbumin-, 5-HT-, GAP-43-, and GFP-immunoreactive axons, all continuous segments of axons within grafts were quantified under brightfield at 400×. Total numbers of axon profiles per graft were quantified in every seventh section for NF200 and GAP-43 and in every 14th section for SMI-32, parvalbumin, and 5-HT labeling, then multiplied by 7 or 14, respectively, to estimate total axonal profiles per subject. When quantifying the number of corticospinal motor neurons retrogradely infected with scAAV6-eGFP, cells with distinct GFP immunoreactivity within soma, dendritic, and axonal processes were counted. GFP-immunoreactive corticospinal motor neurons and corticospinal axons were counted in every fourth transverse section through the motor cortex and multiplied by 4 to estimate the total number of axons per subject. For quantification of surviving corticospinal motor neurons after subcortical axotomy, the total number of CTB-labeled corticospinal motor neurons was quantified in the lesioned and contralateral intact hemispheres in every seventh transverse section. The number of surviving corticospinal motor neurons on the lesioned side was divided by the number of corticospinal motor neurons on the intact side to determine the proportion of neurons surviving axotomy. NF200 pixel density and SMI-32, parvalbumin, and GFP axonal profile data were analyzed by Kruskal–Wallis nonparametric statistics. Statistical analysis of NF200 and GAP-43 axonal profile data was done by ANOVA. Serotonin axon profile data were analyzed by Student’s t test. Statistical comparison of retrogradely infected corticospinal motor neurons quantified in animals with and without EtBr was performed by using Student’s t test, as was the ratio of eGFP-immunoreactive regenerating axons to retrogradely infected corticospinal motor neurons. All quantification was performed by an experimenter blinded to the subject group.

Fig. S1. Lenti-trkB-EF1α-copGFP construct encodes full-length, HA-tagged trkB behind the CAG promoter with the reporter gene copGFP expressed by the EF1α promoter. Signaling domains within trkB involved in Erk1/2-mediated neurite outgrowth include a juxtamembrane KFG domain (required for FRS-2 activation in trkA), an Sho/FRS2 activation site at tyrosine 515, and a PLCγ activation site at tyrosine 816.
Fig. S2. Lentiviral trkB expression induces regeneration of heavy-chain neurofilament-immunoreactive axons into a BDNF-secreting graft. Dashed lines indicate host-graft interface. (Scale bar: 100 µm.)
Fig. S3. Morphology of corticospinal axons. (A) TrkB-infected corticospinal axons rarely branch as they regenerate into BDNF-secreting cell grafts. The example shown is typical of most axons in BDNF-secreting grafts in the lesion site, with nonbranching profiles. (B) In contrast, BDA-labeled corticospinal axons exhibit readily detectable axonal branching in the spinal cord, observable both in intact subjects and in subjects that have undergone partial transections of corticospinal tract projections (shown is a subject that underwent a cervical dorsal column lesion, sparing the ventral corticospinal tract). Arrowhead indicates branching BDA-labeled axon in gray matter caudal to the lesion site. (Scale bar: 25 μm.)
Fig. S4. Lentiviral trkB expression induces regeneration of GFP-expressing corticospinal axons (green) beyond the GFAP-immunoreactive (red) astroglial host–graft interface and into a BDNF-secreting graft. Dashed lines indicate host-graft interface. (Scale bar: 100 μm.)
Fig. S5. Neurite outgrowth of transduced PC12 cells after 8 days of NGF stimulation was independent of trkB or mutated trkB expression.
Fig. S6.  The percentage of CTB retrogradely labeled corticospinal motor neurons surviving 2 wk after lesion did not differ between groups as measured relative to copGFP controls.