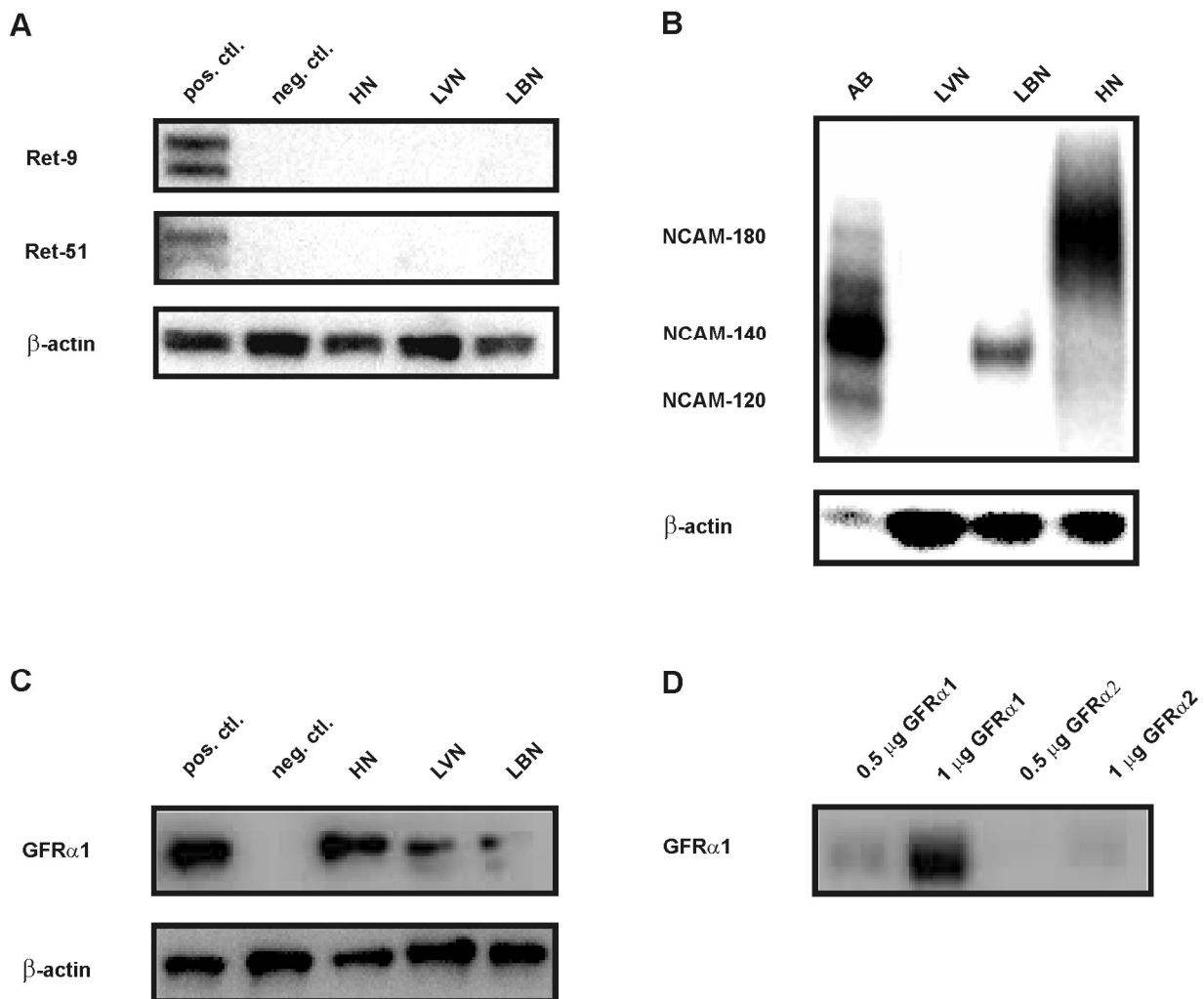
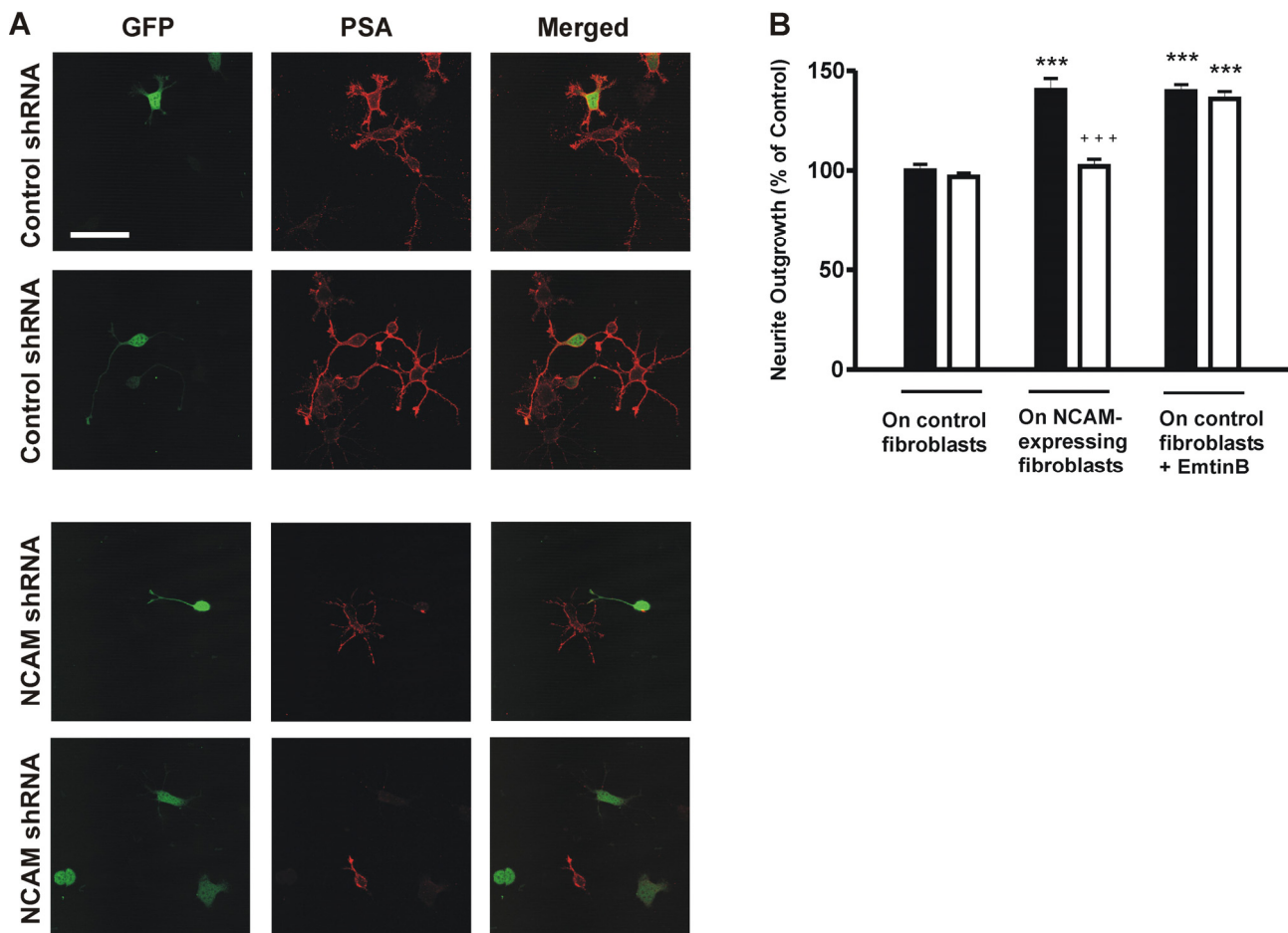
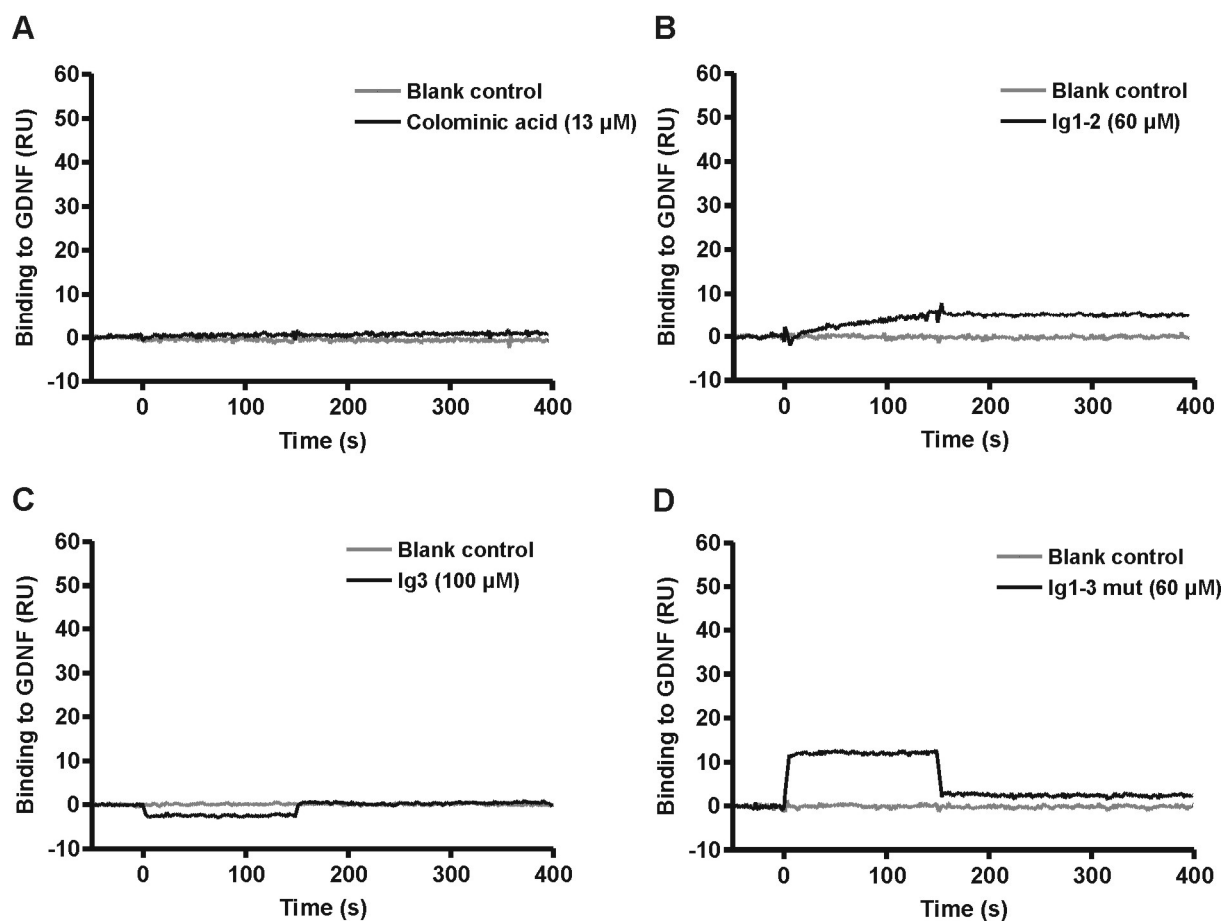




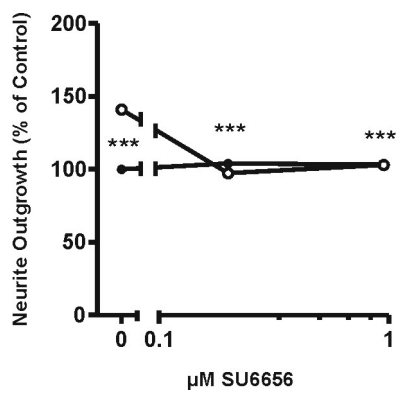
Supplemental Figure S1. Receptor expression profiling of Ret, NCAM, and GFR α 1. All cells and tissue homogenates were lysed in RIPA buffer (150 mM NaCl, 10 mM Tris-HCl [pH 7.4], 1 mM ethylenediaminetetraacetic acid [EDTA], 1 mM ethyleneglycoltetraacetic acid [EGTA], 0.5% NP-40, 1% Triton X-100) supplemented with Complete™ EDTA-free protease inhibitor cocktail (Roche, Basel, Switzerland). Proteins were separated by SDS-PAGE, transferred to Immobilon-P membranes (Millipore, Copenhagen, Denmark), and stained using anti-Ret antibodies (C-19 and C-20 from Santa Cruz Biotechnology, Heidelberg, Germany), rabbit anti-NCAM antibody (Protein laboratory, Copenhagen, Denmark), anti-GFR α antibody (1135 from ProSci, Poway, CA, USA) or anti- β -actin antibody (Cell Signaling Technology, Danvers, MA, USA; for verification of loading) followed by incubation with appropriate horseradish peroxidase (HRP)-conjugated antibodies (DAKO, Glostrup, Denmark). Blots were visualized by chemiluminescence (SuperSignal West Dura Extended Duration Substrate; Pierce Biotechnology, Rockford, IL, USA) using a GeneGnome (Syngene, Cambridge, UK) and the accompanying GenTools software. **A**, Representative immunoblots of Ret expression in rat hippocampal neurons (HN) and LVN and LBN cells. Antibodies recognizing the short isoform (Ret-9) or the long isoform (Ret-51) were used. Lysates of NG108-15 (American Type Culture Collection; ATCC) and SVEC4-10 (ATCC) cells served as positive and negative control, respectively. **B**, Representative immunoblot of NCAM expression in adult rat brain (AB), LVN and LBN cells, and rat hippocampal neurons (HN). Antibodies recognizing the extracellular part of NCAM were used. All three main isoforms of NCAM as well as PSA-NCAM (seen as a high molecular-weight smear in the last lane) are stained. **C**, Representative immunoblot of GFR α 1 expression in rat hippocampal neurons (HN) and LVN and LBN cells. Antibodies recognizing GFR α 1 were used. Homogenates of embryonic rat and adult rat heart served as positive and negative control, respectively. **D**, Representative immunoblot showing that the anti-GFR α antibody specifically recognizes recombinant human GFR α 1/Fc chimera (R&D Systems, Abingdon, UK), but not recombinant human GFR α 2/Fc chimera (R&D Systems).



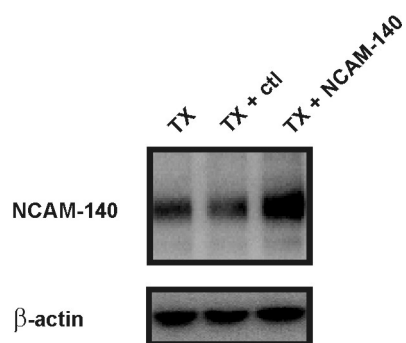
Supplemental Figure S2. Expression of NCAM shRNA reduces NCAM expression but does not affect NCAM-independent neurite outgrowth from hippocampal neurons. **A**, Confocal micrographs of hippocampal neurons transfected with control shRNA or NCAM shRNA, and stained for PSA (red). The staining for PSA was used to assay NCAM expression, since the anti-PSA antibody (AbCys, Paris, France) was specific as shown in Fig. 2A. Because most NCAM on embryonic hippocampal neurons is polysialylated (Supplemental Fig. S1B) and PSA is found almost exclusively on NCAM, assuming that a reduction in PSA expression reflects a reduction in NCAM expression appears reasonable. Transfected neurons are identified by GFP-expression. Scale bar = 25 μ m. **B**, Hippocampal neurons transfected with either control shRNA plasmid (black bars) or NCAM shRNA (white bars) were grown on monolayers of control fibroblasts or NCAM-expressing fibroblasts for 24 h in the absence or presence of 4.5 μ M EmtinB (SAGSCKCKESKSTS; synthesized as a tetrameric dendrimer coupled to a lysine backbone by Schafer-N, Copenhagen, Denmark) and neurite outgrowth was quantified. *, compared with control neurons (control shRNA, control fibroblasts, without EmtinB; $29.67 \mu\text{m} \pm 0.91 \mu\text{m}$, $n = 4$); +, compared with NCAM-stimulated neurons (control shRNA, NCAM-expressing fibroblasts, without EmtinB).



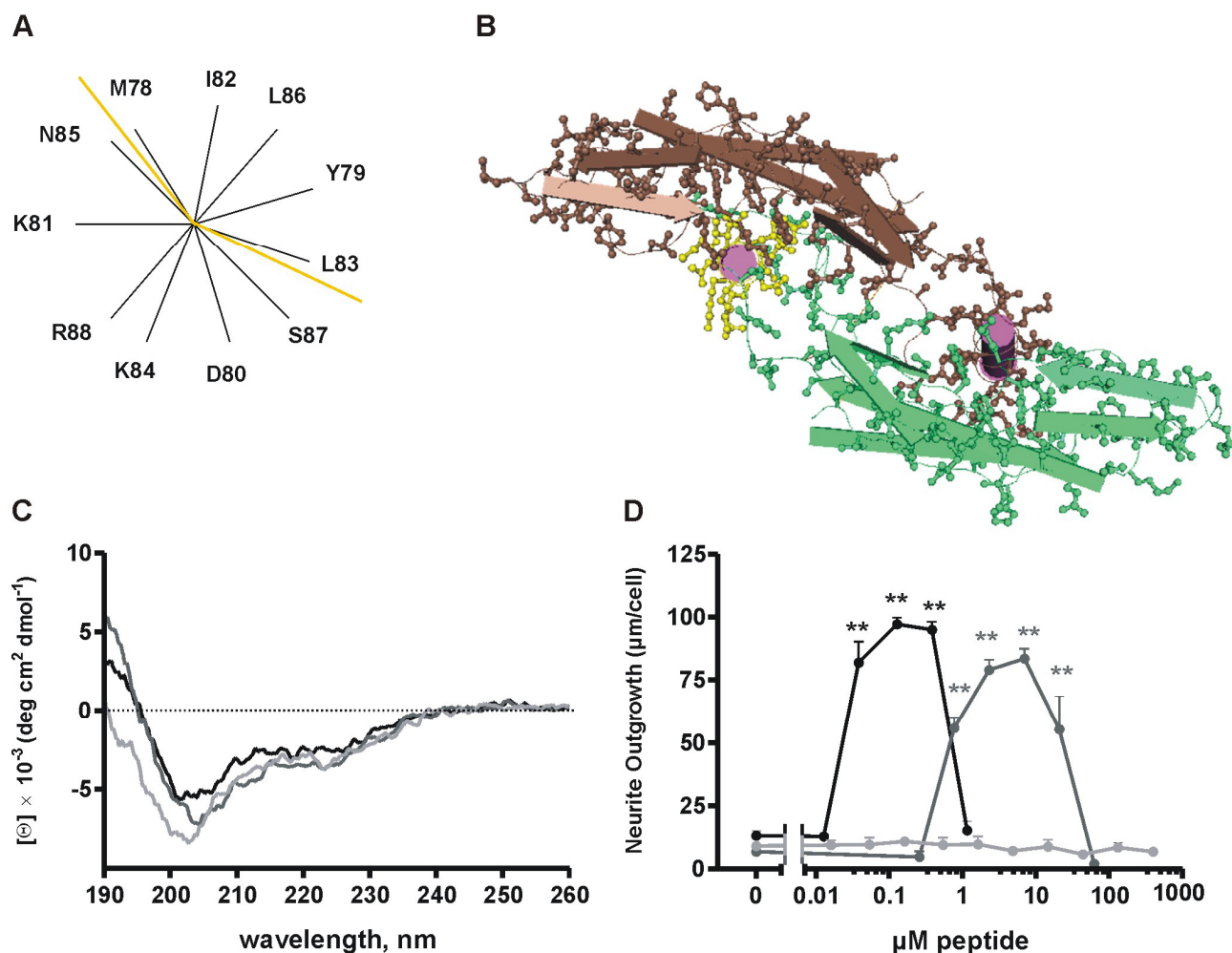
Supplemental Figure S3. Binding analysis of GDNF. Approximately 1000 RU GDNF were immobilized on a CM4 sensorchip, and various recombinant proteins in HBS-EP were injected into the chip at a flow rate of 30 μ l/min. **A-D**, Representative binding curves for the binding of **A**, colominic acid; **B**, NCAM Ig1-2; **C**, NCAM Ig3; **D**, NCAM Ig1-3 mut to GDNF. Injections were initiated at time 0 and lasted for 150 s. Concentrations of injected proteins are indicated in each panel. Recombinant rat NCAM Ig1-2 (residues 20-208; Swiss-Prot # P13596) was produced as previously described (Jensen et al. [1999] Nat Struct Biol 6:486-493). Recombinant rat NCAM Ig1-3 mut (residues 20-308; Swiss-Prot # P13596; similar to Ig1-3 but with the following substitutions: E30A, E16A, and K18A), and Ig3 (residues 210-308; Swiss-Prot # P13596) proteins were produced as previously described (Soroka et al. [2003] Structure 11:1291-1301.)



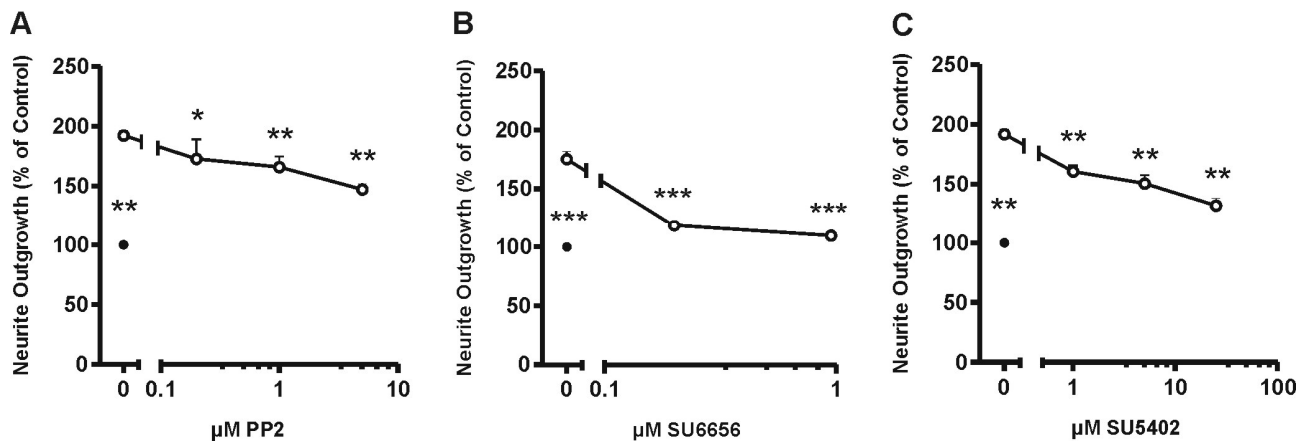
Supplemental Figure S4. GDNF-induced neurite outgrowth involves a Src family kinase. Hippocampal neurons were grown on monolayers of control fibroblasts for 24 h in the absence (filled circles) or presence (open circles) of 0.3 nM GDNF and treated with the Src family kinase inhibitor SU6656 (Calbiochem, La Jolla, CA, USA; 0.2, and 1 μ M). Neurons grown without SU6656 served as controls. *, compared with GDNF-stimulated neurons (with GDNF, without SU6656: $56.28 \mu\text{m} \pm 2.25 \mu\text{m}$, $n = 4$).



Supplemental Figure S5. T-REx-293 cells express NCAM-140. Representative immunoblot of NCAM expression in T-REx-293 cells (TX), T-REx-293 cells transfected with an empty control vector (TX + ctrl), and T-REx-293 cells transfected with a vector encoding NCAM-140 (TX + NCAM-140). Antibodies recognizing the extracellular part of NCAM were used and blots were performed as described for supplemental Fig. S1. It is seen that T-REx-293 cells express one NCAM isoforms, which judged from apparent molecular weight is NCAM-140. Transfection of T-REx-293 cells with a vector encoding NCAM-140 resulted in an increase of the presumed NCAM-140 band, substantiating that T-REx-293 cells express NCAM-140.



Supplemental Figure S6. The Gliafin sequence forms an amphipathic α -helix within GDNF and the Gliafin peptide is folded as an α -helix. **A**, The Gliafin sequence forms an amphipathic α -helix. An amphipathic α -helix enriched in hydrophobic side chains on one of its sides, and in hydrophilic side chains on the other is revealed by the displaying the residues of the Gliafin peptide on a helical wheel. Numbering of residues according to the crystal structure of rat GDNF (PDB # 1AGQ). **B**, Position of the Gliafin sequence within rat GDNF. The viewing angle is the same as in **A**. One GDNF monomer is shown in brown, the other in green. The Gliafin sequence of the green monomer is marked in yellow. **C**, Circular dichroism spectra for the tetrameric (black), dimeric (dark gray; produced as a dimer coupled to a lysine backbone; Schafer-N, Copenhagen, Denmark), and monomeric (light gray; Schafer-N) forms of Gliafin. All three forms are predominantly α -helical. Circular dichroism spectra were recorded on a Jasco J-810 spectropolarimeter using 10 μM peptide samples at 25°C. Peptide samples were prepared by diluting peptide stock solutions (0.7-2.4 mM in 10 mM tris-HCl, pH 8) with H_2O . A 1 mm cuvette was used and the width of the slit was set to 1 mm. Each spectrum was recorded from 260 nm to 190 nm as an average of five scans. The recording speed was 100 nm/min. **D**, Oligomeric, but not monomeric forms of Gliafin induces neurite outgrowth. Hippocampal neurons were grown for 24 h on permaxon plastic in the presence of tetrameric Gliafin (0, 0.01, 0.04, 0.13, 0.4, and 1.2 μM ; $n = 4$; black circles), dimeric Gliafin (0, 0.3, 0.8, 2.3, 6.9, 20.8, and 62.5 μM ; $n = 3-5$; dark gray circles), or monomeric Gliafin (0, 0.02, 0.05, 0.16, 0.5, 1.6, 4.9, 14.6, 43.8, 131, and 394 μM ; $n = 3$; light gray circles). *, compared with untreated controls.



Supplemental Figure S7. Gliafin-induced neurite outgrowth involves a Src family kinase and FGFR. **A-C**, Hippocampal neurons were grown on monolayers of control fibroblasts for 24 h in the absence (filled circles) or presence (open circles) of 0.4 μM Gliafin and treated with, **A**, the Src family kinase inhibitor PP2 (0.2, 1, and 5 μM), **B**, the Src family kinase inhibitor SU6656 (Calbiochem, La Jolla, CA, USA; 0.2 and 1 μM) or, **C**, the FGFR inhibitor SU5402 (1, 5, and 25 μM). Neurons grown without PP2, SU6656 or SU5402 served as controls. *, compared with Gliafin-stimulated neurons (**A**, with Gliafin, without PP2: $75.53 \mu\text{m} \pm 0.60 \mu\text{m}$, $n = 4-8$; **B**, with Gliafin, without SU6656: $73.54 \mu\text{m} \pm 2.17 \mu\text{m}$, $n = 4$; **C**, with Gliafin, without SU5402: $75.43 \mu\text{m} \pm 0.66 \mu\text{m}$, $n = 5-8$).