Supporting Information

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SI Text

Reagents. All cell culture media and supplement components were from Invitrogen. Mouse anti-Fyn mAb and mouse anti-Cas mAb were from BD Bioscience. Rabbit anti-Fyn polyclonal antibody and protein A/G agarose were from Santa Cruz Biotechnology. Rabbit anti-phospho-Src family (Y416) antibody and anti-phospho-Cas (Y410) antibody were from Cell Signaling. Mouse anti-Tau-1 mAb, anti-actin mAb, and ReBlot plus antibody stripping solution were from Chemicon. Src family kinase inhibitor PP2 and its analog PP3, protein kinase A inhibitor Rp-cAMP, MAPK inhibitor PD98059, and actin polymerization inhibitor cytochalasin D were from Calbiochem. Proteinase inhibitor mixture was from Roche. Halt phosphatase inhibitors mixture was from Pierce. Rat neuron nucleofector kit and the Nucleofector device were from Amaxa. Ethanol and all of the other reagents were obtained from Sigma–Aldrich.

Culture of CGNs. The cerebellum was excised from 6- to 7-day-old rats. The cerebella were cut into small pieces, incubated in 1% trypsin/0.05% DNase for 15 min at room temperature, washed with HBSS, and resuspended in a 0.05% DNase solution. Cells were dissociated by mechanical trituration, placed on the top of a 15% FCS cushion, and centrifuged at 300×g to isolate CGNs. Cells were washed sequentially with HBSS and culture medium before plating. Cells were plated on poly-L-lysine (PLL)-coated culture plates and maintained in Neurobasal serum-free medium with 2 mM L-glutamine, 1 mg/ml BSA, 12.5 μg/ml insulin, 4 nM thyroxine, 100 μg/ml transferrin, and 30 nM sodium selenite.

Cell Lysate Preparation and Immunoprecipitation. CGNs were incubated for various durations in the absence or presence of NAP (10^{-12} M), ethanol (100 mM), PP2, or PP3. Cells were washed with ice-cold PBS and lysed on ice for 30 min in 20 mM Tris·HCl (pH 7.5), 150 mM NaCl, 5 mm EDTA, and 1% Triton X-100 with cocktails of proteinase inhibitors and phosphatase inhibitors, and centrifuged at 16,000×g for 20 min. The protein concentration in the supernatant was determined by the bicinchinonic acid method. For immunoprecipitation of Fyn kinase, 300 μg of cell lysates was incubated overnight at 4 °C with rabbit anti-Fyn antibody and protein A/G-agarose beads. The immunoprecipitates were boiled in SDS sample buffer for 5 min and processed for immunoblot analysis of levels of phosphorylated Fyn kinase, total Fyn kinase, and phosphorylated Cas.

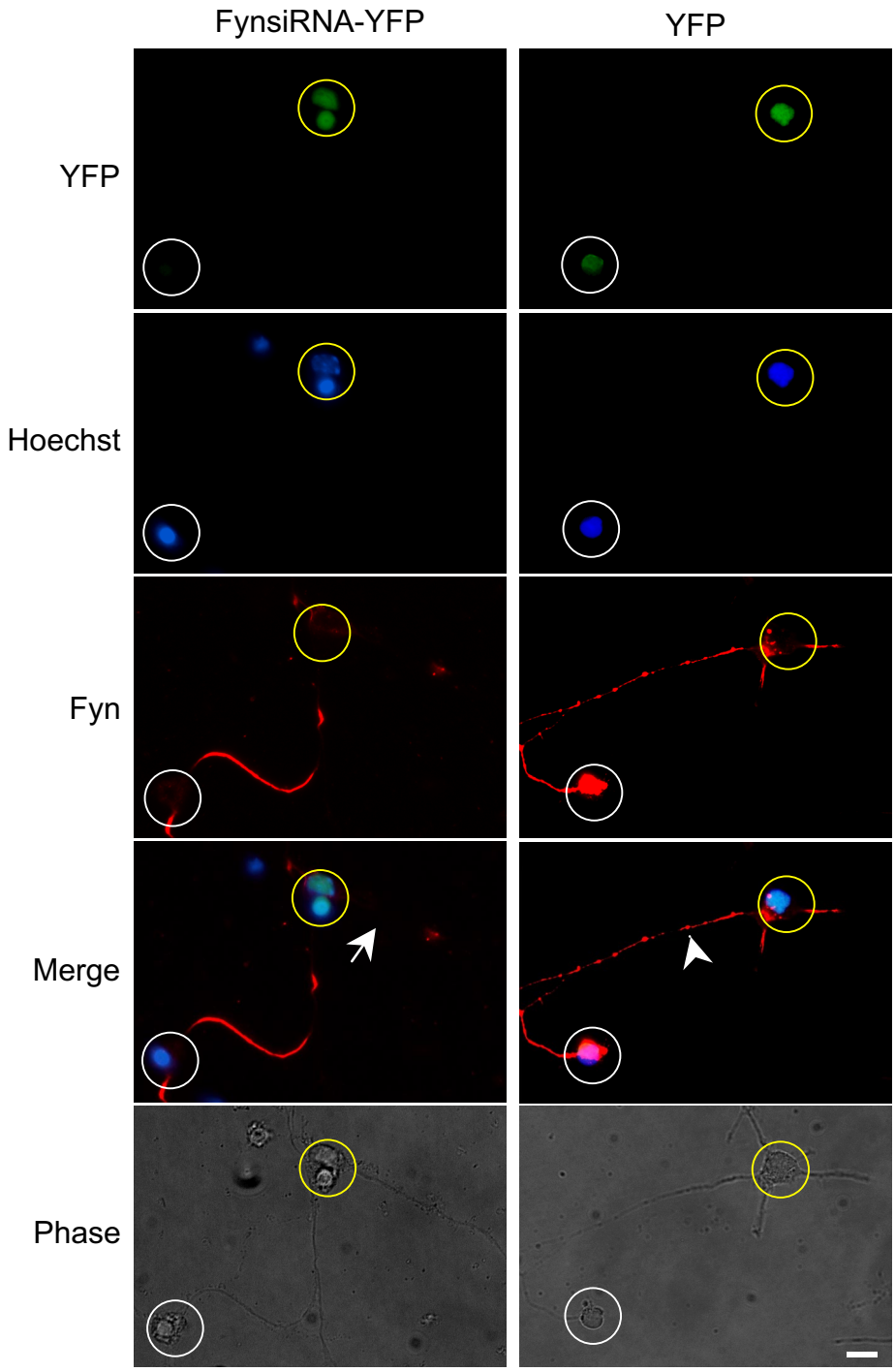

Fig. S1. Knockdown of Fyn kinase expression in CGNs. Dissociated CGNs were transfected with YFP or Fyn siRNA-YFP plasmids by using nucleofection. Cells were fixed 48 h after transfection and processed for immunostaining with anti-Fyn kinase antibody. Micrographs depict Fyn siRNA-YFP-transfected (yellow circles), YFP-transfected (yellow circles) and nontransfected (white circles) cells. From top to bottom: YFP- or Fyn siRNA-YFP-transfected cells show green fluorescent nuclei; Hoechst stain identifies nuclei of transfected and nontransfected cells; Fyn immunostaining of axons is present in YFP-transfected (arrowhead) and nontransfected cells, but not in Fyn siRNA-YFP-transfected cells (arrow); merged images allow visualization of Fyn-expressing and nonexpressing axons; phase-contrast images of the same cells. (Scale bar: 5 μm.)