Supporting Information

Qin et al. 10.1073/pnas.1008652107

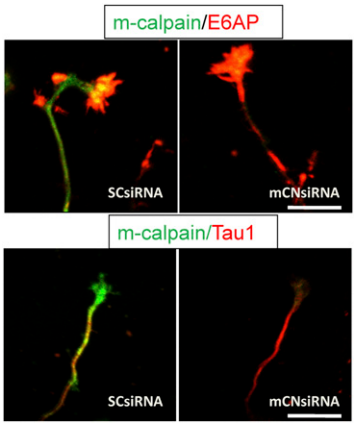

Fig. S1. siRNA suppresses m-calpain expression in axons and growth cones. Cultured hippocampal neurons were transfected on DIV 3 with siRNA against m-calpain (mCNsiRNA) or scrambled siRNA (SCsiRNA) and treated on DIV 4 with DMSO for 5 min before being fixed and processed for immunostaining with anti–m-calpain (green) and anti-E6AP (red) antibodies (*Upper*) or anti–m-calpain (green) and Tau1 (red, an axon marker) antibodies (*Lower*) as described in *Materials and Methods*. (Scale bar, 20 μm.)

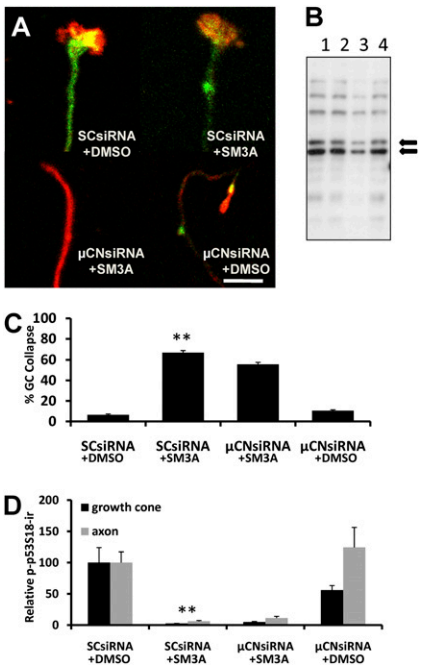

Fig. S2. Suppression of μ-calpain with siRNA did not block semaphorin 3A–induced growth cone collapse. (*A*) Cultured hippocampal neurons were transfected on DIV 3 with siRNA against μ-calpain (μCNsiRNA) or scrambled siRNA (SCsiRNA) and treated on DIV 4 with DMSO or semaphorin 3A (SM3A) for 5 min before being fixed and processed for immunostaining with anti–p-p53 (green) and anti-E6AP (red) antibodies. Results are representatives of three or four culture dishes from three independent experiments. (Scale bar, 20 μm.) (*B*) Immunoblotting analysis of m-calpain in cultured cortical neurons. Cortical neurons were transfected on DIV 3 without siRNA (lane 1), with scrambled siRNA (lane 2), or siRNA against μ-calpain (lane 3) or m-calpain (lane 4) and processed on DIV 4 for immunoblotting with anti–μ-calpain. Arrows indicate that μ-calpain was decreased only by siRNA against μ-calpain. (*C*) Percentage of collapsed growth cones (GC) in hippocampal neurons in experiments shown in Fig. S1*A* ($n = 100$ growth cones from three independent experiments; $^{**}P < 0.001$ vs. DMSO-treated). (*D*) Levels of p-p53S18-ir in axons and growth cones of hippocampal neurons in experiments shown in Fig. S1*A* (data are means ± SEM percent of DMSO-treated neurons; $n = 25$–30 growth cones; $^{**}P < 0.01$ vs. DMSO group).

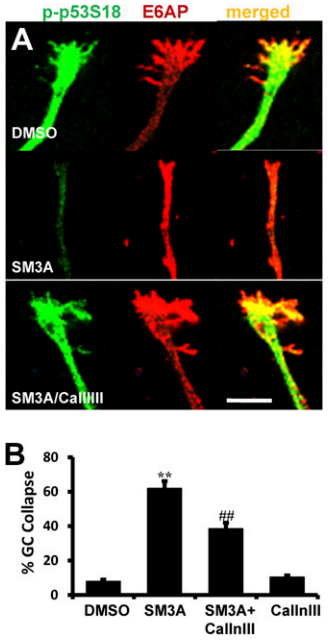

Fig. S3. Semaphorin 3A-induced growth cone collapse is reduced by calpain inhibitor III. (*A*) Cultured hippocampal neurons were treated with semaphorin 3A (2 nM; SM3A) for 5 min in the presence or absence of pretreatment with calpain inhibitor III (CalInIII, 5 μM) for 3 h. They were fixed and immunostained with anti–p-p53 (green) or anti–E6AP antibodies (red). (*B*) Percentage of collapsed growth cones (GC) in hippocampal neurons in experiments shown in Fig. S2*A*. (**$P < 0.01$ vs. controls; $^{\#\#}P < 0.01$ vs. Sema3A group; $n = 90$ from three independent experiments.) (Scale bar, 20 μm.)

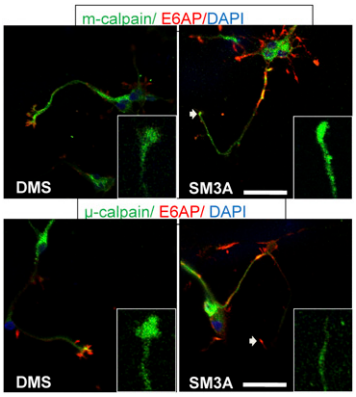

Fig. S4. Distribution of m-calpain and μ-calpain in cultured hippocampal neurons. Immunofluorescence analysis of m-calpain and μ-calpain (green) was performed in hippocampal neurons treated with DMSO or semaphorin 3A (SM3A) at DIV 4. E6AP (red) was used to label growth cones and DAPI (blue) was used to label nuclei. *Insets*: High-magnification images of growth cones. Note that semaphorin 3A treatment induced a decrease in μ-calpain, but not in m-calpain, immunoreactivity in growth cones. Arrows in "SM3A" panels indicate collapsed growth cones. (Scale bar, 50 μm.)

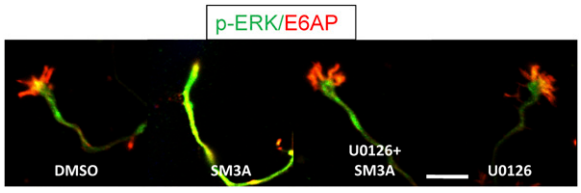

Fig. S5. Distribution of p-ERK in axons and growth cones. Immunofluorescence analysis of phosphorylated/active ERK (p-ERK, green) distribution was performed in hippocampal neurons pretreated with U0126 or DMSO for 3 h followed by 5 min semaphorin 3A (SM3A) treatment. E6AP (red) was used to label growth cones. Note that semaphorin 3A treatment induced a marked increase in p-ERK in axons and collapsed growth cones, and these effects were blocked by pretreatment with U0126. (Scale bar, 20 μm.)