Supplemental Figures

Supplemental Figure 1: Sh3rf2 binds proteins associated with the apoptotic JNK signaling pathway. A, The indicated constructs were co-expressed in HEK293 cells. Cells were collected 20 hours after transfection. An aliquot of total lysate was reserved and the remaining sample was immunoprecipitated with anti-Flag beads and the immunoprecpitates were subjected to western immunoblotting and probed with anti-HA. B, Myc-tagged Sh3rf2 was co-expressed with Flag-tagged JIPs as shown. 20 hours later cells were collected and an aliquot reserved as "lysate". The remaining sample was immunoprecipitated with anti-Myc beads and subjected to immunoblot with anti-Flag.

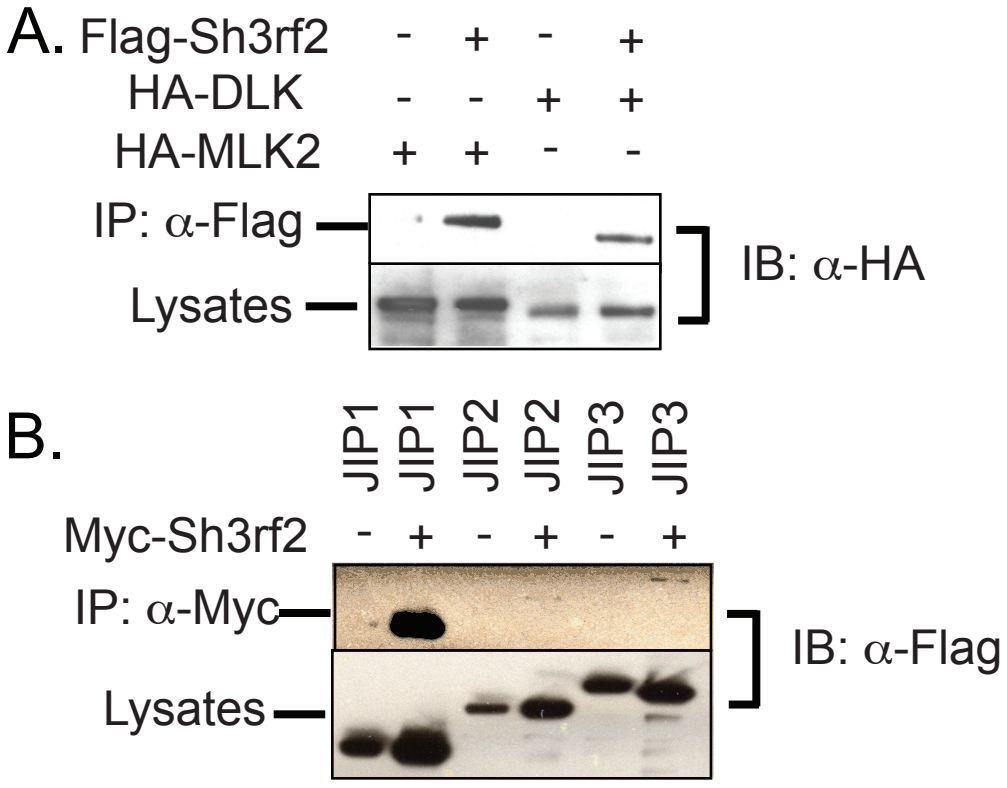
Supplemental Figure 2: siSh3rf2 increases cell death following NGF deprivation. PC12 cells were transfected with GFP 48 hours after priming and treated with siSh3rf2 or control siRNA as indicated. Cells were then subjected to NGF deprivation 24 hours later as in Materials and Methods. Strips of cells were counted each day starting immediately after NGF deprivation ("Day 1"). * $P<0.05$ versus cells in NGF. The experiment was performed twice with similar results.

Supplemental Figure 3: sihuSh3rf2 effectively knocks down Sh3rf2. HEK293 cells were transfected with pCMS.EGFP-Sh3rf2 and the indicated siRNA. SihuSh3rf2 targets both the rat cDNA sequence and the predicted human sequence. Lysates were prepared 20 hours later and subjected to immunoblot for Sh3rf2. The membrane was reprobed for GFP to confirm equal transfection efficiency and loading. Note the decreased Sh3rf2 band in cells expressing sihuSh3rf2, which likely reflects reduction of endogenous Sh3rf2 relative to cells expressing siSh3rf2.

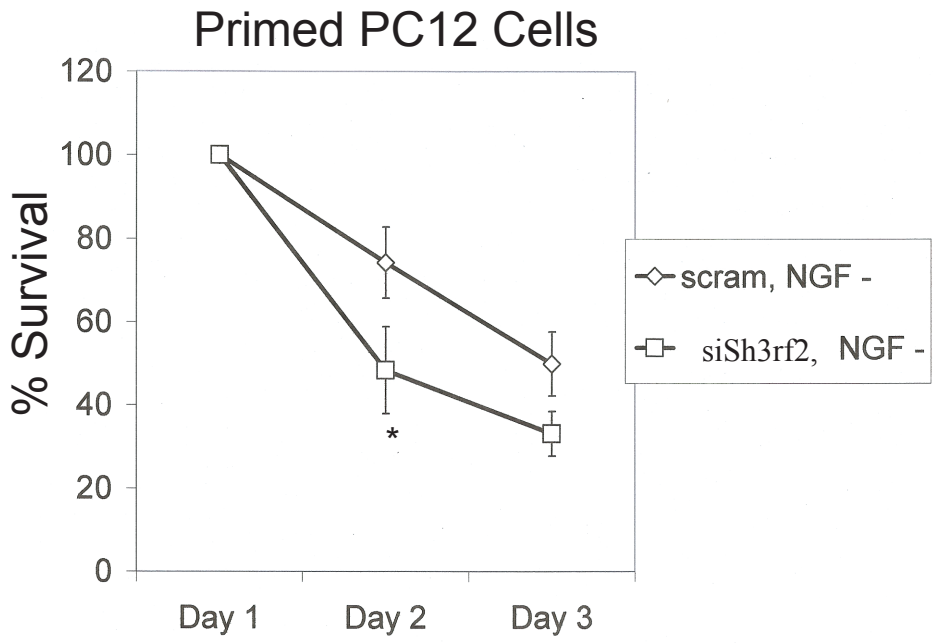
Supplemental Figure 4: Apoptosis induced by siSh3rf2 is prevented by JNK inhibition. Neuronal PC12 cells were transfected with siSh3rf2 or scrambled siRNA ("scram") and pCMS.EGFP. Cells were treated with a cell-permeable JNK inhibitor ("JI", DJNKi1 (5μM), see Materials and Methods) as indicated. Transfected cells were counted starting 24 hours after transfection ("Day 1"). Survival was normalized to the scrambled siRNA for each condition. Shown is one experiment performed in triplicate. The experiment was performed three times with similar results. (* $p<0.05$ for the same time-point scrambled siRNA, # $p<0.05$ for the same time point siSh3rf2)

Supplemental Figure 5: Sh3rf2 is degraded in cortical neurons following camptothecin treatment. Cortical neurons were treated with either camptothecin (10 μM) or an equal quantity of DMSO for the indicated time. Cells were collected and subjected to immunoblot as indicated. Two separate experiments are shown.

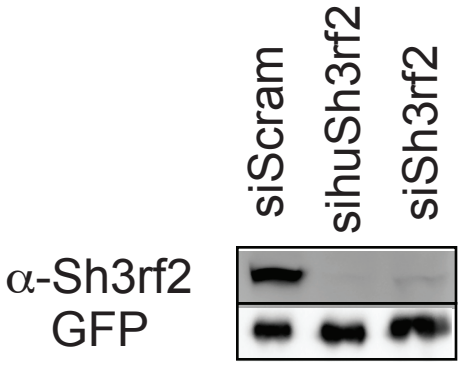
Supplemental Figure 6: Adenoviral-mediated knockdown of Sh3rf2 promotes JNK activation and apoptosis in cortical neurons. A. Primary murine cortical neurons were transduced with adenovirus expressing the indicated shRNAs. 96 hours later cells were collected and subjected to immunoblot for the indicated proteins. The same blot was reprobed sequentially. Shown is a representative example from a set of three experiments. The grap shows the results from three independent experiments (*, $p < 0.1$)**B.** Primary cortical cultures were treated with the same adenoviral constructs as in A. At 120 hours after transduction, cells were fixed and TUNEL labeling examined as in Materials and Methods. Shown is a representative image from one experiment, which was performed in triplicate. At least fifty fields were examined for each condition. DNAse treatment was used as a positive control (not shown). Grey-scale images are shown for DNA staining using Hoechst 33342 and for TUNEL (Alexa-488) and the merged image is shown in color. Note the marked increase in TUNEL positivity in the shSh3rf2-treated neurons.



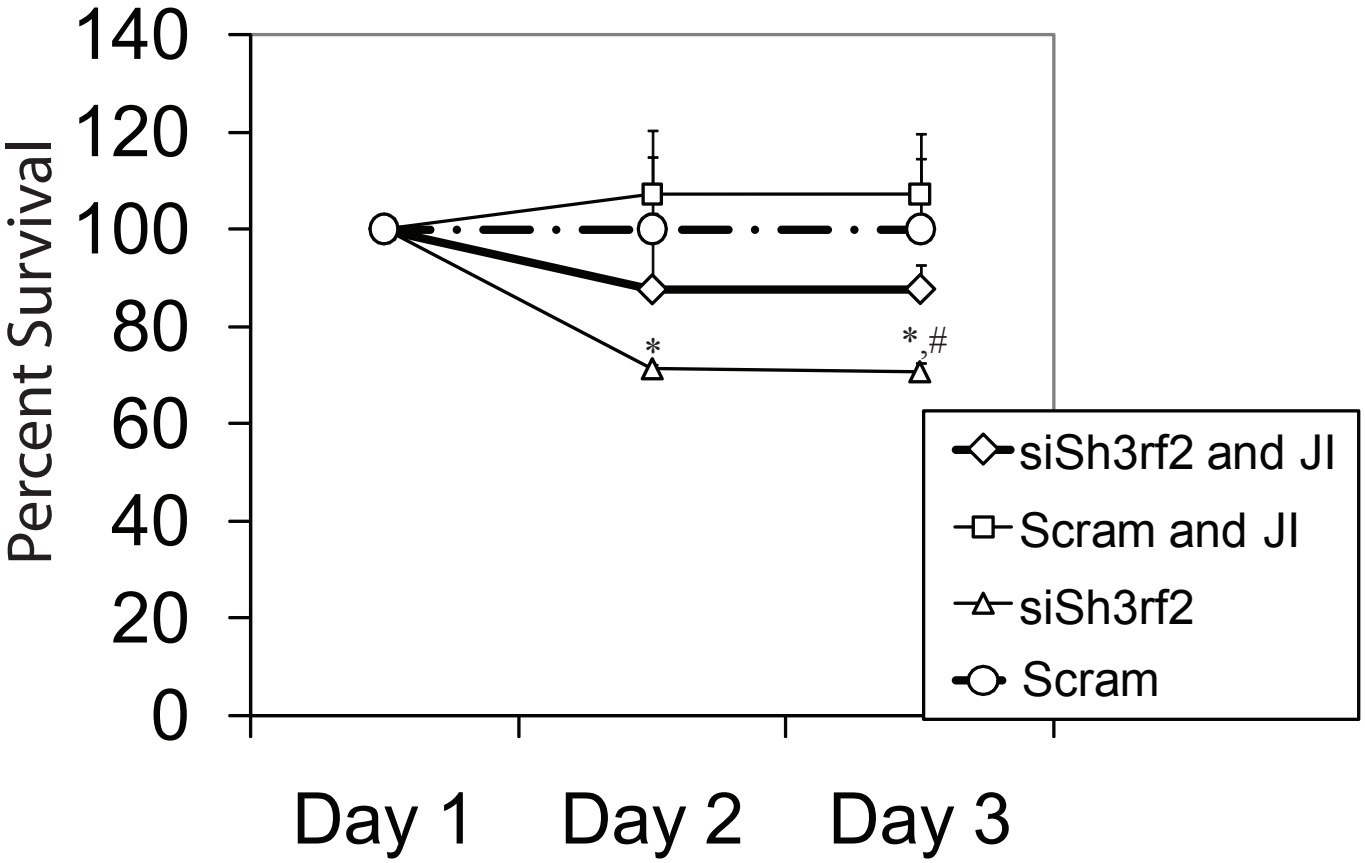
Supplemental Figure 1



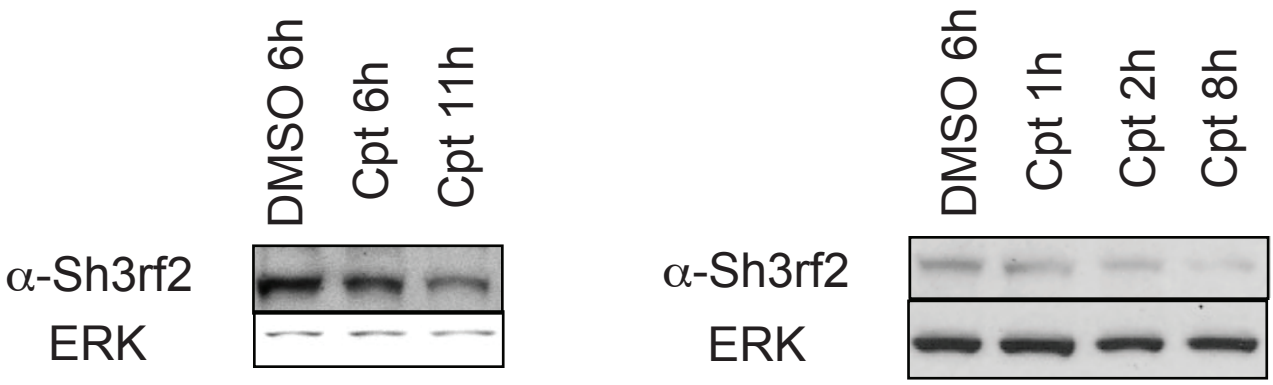
Supplemental Figure 2



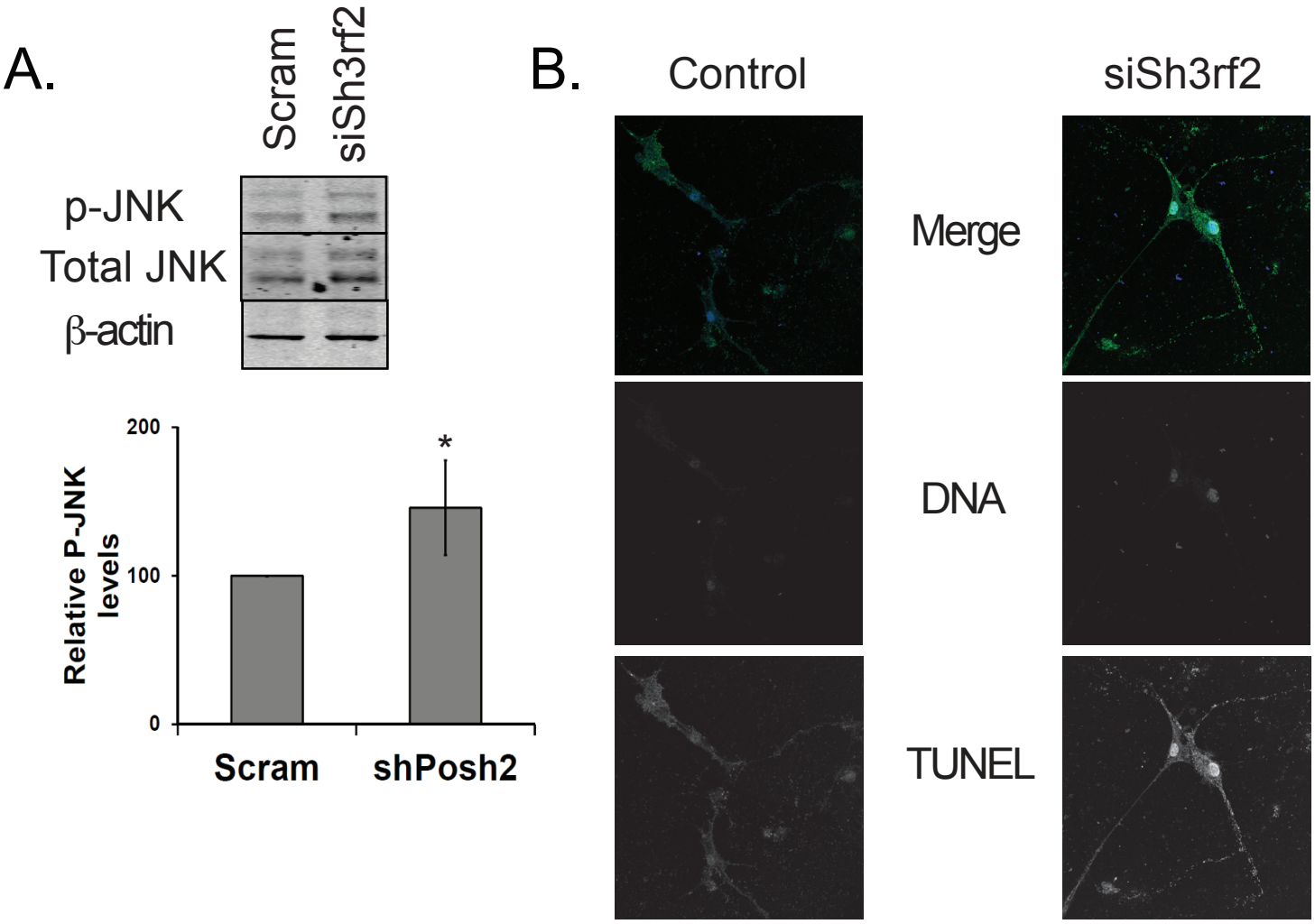
Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6