LeClaire et al., http://www.jcb.org/cgi/content/full/jcb.201404095/DC1

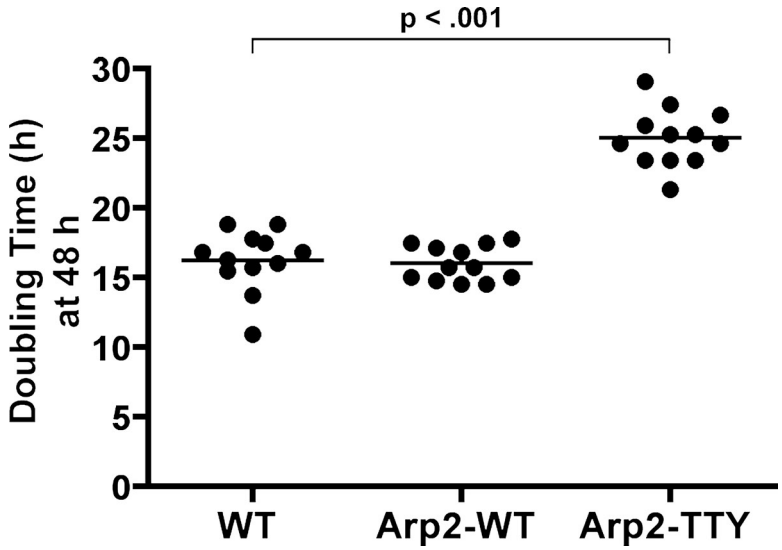

Figure S1. **Arp2-TTY/A decreases the cell proliferation rate.** Doubling time of MTLn3 cells in growth medium determined 48 h after plating is similar for WT cells and cells expressing Arp2 WT but significantly greater for cells expressing Arp2-TTY. Data show values of individual wells from four separate cell plating preparations (WT, *n* = 12; Arp2-WT, *n* = 12; Arp2-TTY, *n* = 12).

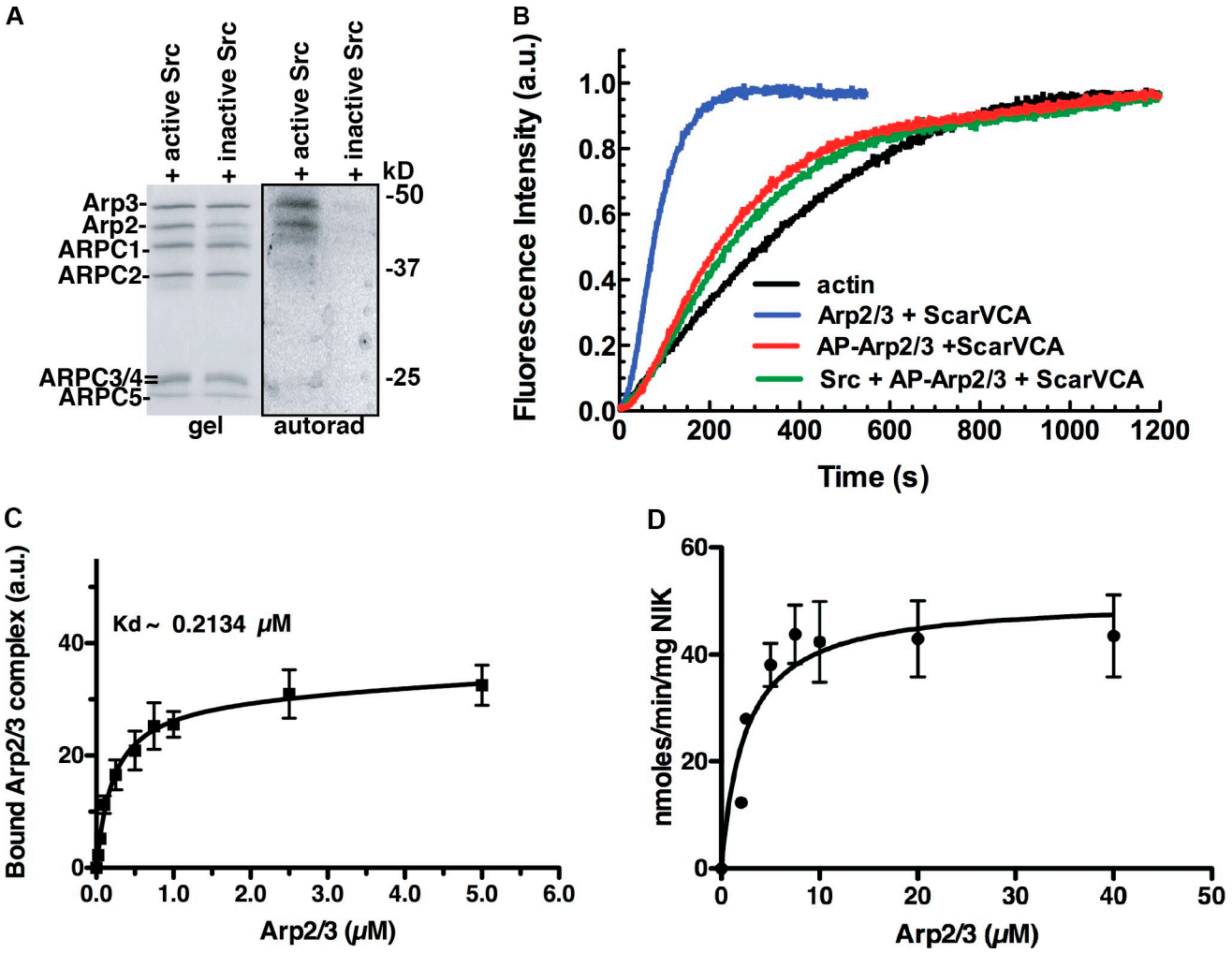

Figure S2. **Arp2/3 complex phosphorylation and binding.** (A and B) In vitro, WT Src but not kinase-inactive Src phosphorylates the Arp3 and Arp2 subunits of the Arp2/3 complex purified from *A. castellanii* (A) but has no effect on Arp2/3 complex–nucleating activity (B). (C) Binding affinity for Arp2/3 complex with recombinant, purified NIK 340–500 immobilized on CH-Sepharose. Data are taken from five separate experiments. (D) In vitro phosphorylation of Arp2/3 complex purified from *A. castellanii* by NIK immunopreciptated from HEK293 cells expressing HA-tagged WT, full-length NIK. The data shown for pyrene actin traces are from a single representative experiment out of three repeats. Error bars indicate standard deviation from the mean.

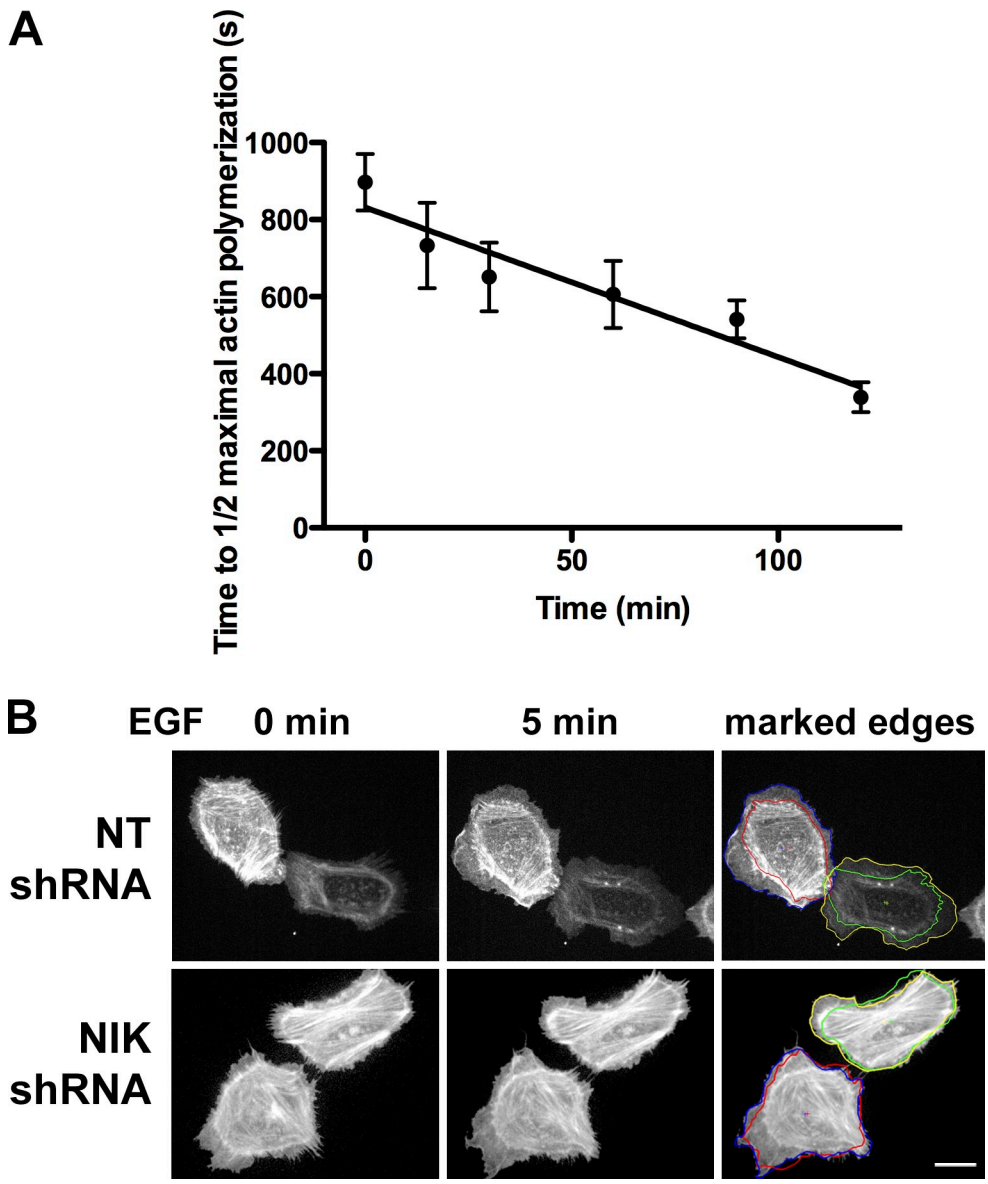

Figure S3. **NIK regulates the Arp2/3 actin nucleation rate and membrane protrusion.** (A) Arp2/3 complex was incubated with WT NIK for 0-, 15-, 30-, 60-, 90-, and 120-min time points. The T1/2 of actin polymerization decreased with longer Arp2/3-NIK incubation times. Each data point is the mean of three separate polymerization assays. (B) Time-lapse images of MTLn3 cells expressing Lifeact-GFP in the absence (0 min) and presence (5 min) of EGF show differences in morphology and changes in plasma membrane protrusions for cells infected with nontargeting (NT) or NIK shRNA. Also shown are overlapped tracings of the cell periphery in the absence and presence of EGF that were used to quantify cell area shown in Fig. 5 E.