SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1: pMLC increases at the CF in p190-depleted cells and decreases in p190 overexpressing cells and does not affect the level of total Rho and localization of Actin, Aurora B, or Microtubules.

A-B: GFP blots of cells depleted of endogenous p190 and rescued by exogenous GFP-p190 (A) or GAP mutant of p190 (p190 R1283A) (B) to demonstrate that the exogenous proteins are expressed. **C:** Quantification of pMLC II at CF. Using Volocity®5.5 (Perkin Elmer) software (Parkin Elmer) total sum intensity of pMLC was quantified by drawing regions of interest (ROIs) around CF and outside of furrow for individual cells with the laso tool. Scale bars, 3.5 μm. **D:** Confocal images of MLC II and pMLC II in HeLa cells during cytokinesis 48 hrs after luciferase or p190RhoGAP siRNA treated. Fixed cells were stained with anti-MLC II (red) and anti-pMLC II (far red) antibodies, and images were merged with those of DAPI-stained DNA (blue). Images shown are representative of n>30. The area outlined in red defines the CF (region of interest - ROI), which was analyzed for pixel intensity. Scale bars, 5 μm. **D`:** Quantitation of pMLC II at the CF. HeLa cells were treated with Luciferase siRNA and p190RhoGAP siRNA for 48 hours, fixed, stained with MLCII and pMLC II (Thr18/Ser19) antibodies. Pixel intensity was determined as described in Experimental Procedures. The ratio of pMLC II to MLC II was determined and expressed as the mean pixel intensity ratio +/- standard error of the mean (SEM). Values for Luc siRNA treated cells were set to 1. n>3 with >10 cells/experiment. * =p<0.005 (t test) as compared to Luc siRNA treated cells. **E:** Quantitation of specific pMLC II. HeLa cells were transiently transfected with GFP-empty plasmid or GFP-p190 plasmid, treated with Anillin siRNA and C3 toxin, and pMLC II levels. Results are expressed as the mean fold ratio over that of Vector (Mock)-transfected cells +/- SEM, n>3. *=p<0.05 as compared to Mock (t test). **F:** Western blot of total Rho levels. Western blot shown is representative of n>3. **G:** Images of RhoA (A), Aurora B (B), microtubules (C) or actin (A-C) localization during cytokinesis. HeLa cells were either Mock-treated or transiently transfected with GFP-empty plasmid or GFP-p190 plasmid, synchronized with Nocodazole, fixed, and immuno-stained for the indicated proteins and DNA as described in Experimental Procedures. Mock and vector-only transfected cells gave similar results. Images shown are representative of n>30. Scale bars = 5 μm.

Supplemental Figure 2: A: The multinucleation phenotype generated by depleting p190 was rescued by partial inhibition of ROCK kinase using the ROCK inhibitor Y27632. Failed cytokinesis events were scored as the percentage of bi- or multinucleated interphase cells relative to the total population of cells ($n \geq 300$). Error bars represent standard error of mean from multiple independent experiments. **B:** Recombinant 6His-p190(1-763) and MBP-Anillin(608-1087) proteins migrate as monomers. Size exclusion chromatography profile of the 6His-p190(1-763) and MBP-anillin(608-1087) proteins stained with Coommassie. Arrows (top) indicate the migration of standards and bottom arrows indicate the fraction that was used for pull down assay. **C.** GFP blots of cells depleted of endogenous p190 and rescued by exogenous p190 ΔS1C to demonstrate that the exogenous protein is expressed.

Supplemental Figure 3: Anillin is required for p190 and Rho localization and MLC phosphorylation but not actin or MLC localization.

A: Effect of anillin silencing on p190 localization at the CF. Images of HeLa cells stained for anillin (red), p190 (green), and DNA (blue), after 48 hrs treatments with either control or anillin siRNA. Images shown are representative of n>30. Scale bars, 5 μm. Quantitative effects of anillin silencing on p190 localization at the CF. Results of microscopic examination are expressed as the mean percent of total cells in cytokinesis with p190 accumulation at the CF +/- SEM, n>3, with >15 cells/experiment. *=p<0.001 as compared to control siRNA. Inset: Immunoblot of HeLa cells treated with Luciferase or p190 siRNA. **B:** Quantitation of percent HeLa cells exhibiting Rho, actin, or MLC accumulation, and MLC phosphorylation at the CF during cytokinesis. HeLa cells were treated with either luciferase or anillin siRNA for 48 hrs and stained for MLC, pMLC, actin, and Rho as described in Experimental Procedures. Confocal images were analyzed for accumulation of each protein at the CF, and results are expressed as the mean percent of total cells in cytokinesis with accumulation of the indicated protein at the CF +/- SEM, n>3, with >15 cells/experiment. *=p<0.001 as compared to luciferase siRNA. **C:** Densitometric quantitation of pMLC, MLC, Rho and Actin at the CF in Anillin-depleted cells during cytokinesis.

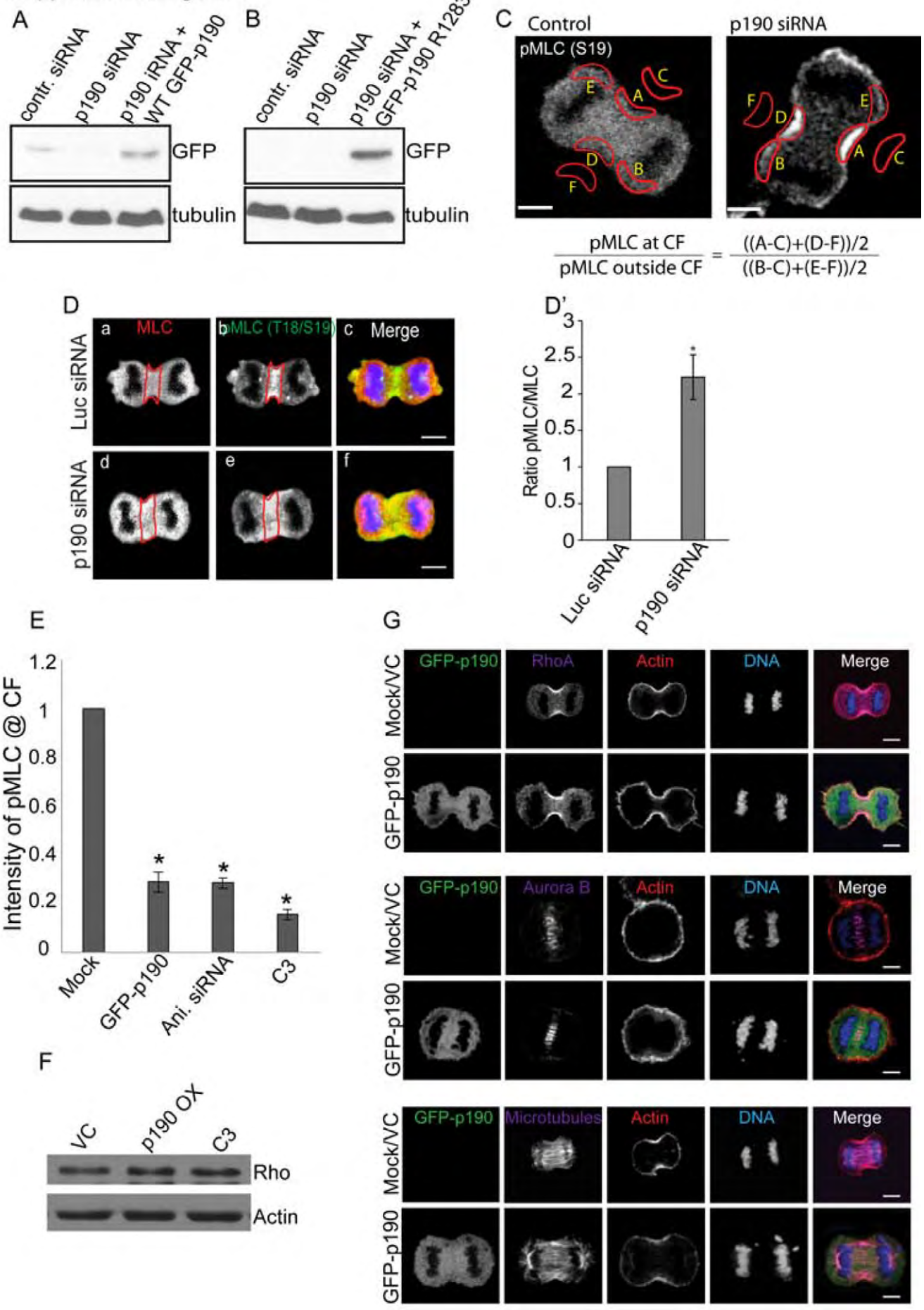
Supplemental Figure 4: Anillin binds section 1 of p190 middle domain.

A: Diagram of the p190RhoGAP mutants: Full-length, GBD/S1 and middle domain (MD), and S1 alone of p190 are depicted. **B:** Co-immunoprecipitation of anillin with full length HA-p190 and the isolated GBD/S1 and middle domains of p190 from cytokinetic HeLa cells. HeLa cells were transfected with various HA-p190 plasmids or empty vector as indicated and synchronized with nocodazole. IPs were performed with anillin antibody, and precipitated proteins were subjected to Western blotting analysis with anti-HA- or anti-anillin to detect association of exogenous HA-p190 variants with anillin. Western blots shown are representative of n>3. WCL=Whole cell lysate, with tubulin as a loading control. **C:** Co-IP of anillin with the isolated HA-S1 domain of p190 in cytokinetic HeLa cells. Western blots shown are representative of n>3. WCL=Whole cell lysate with tubulin as a loading control.

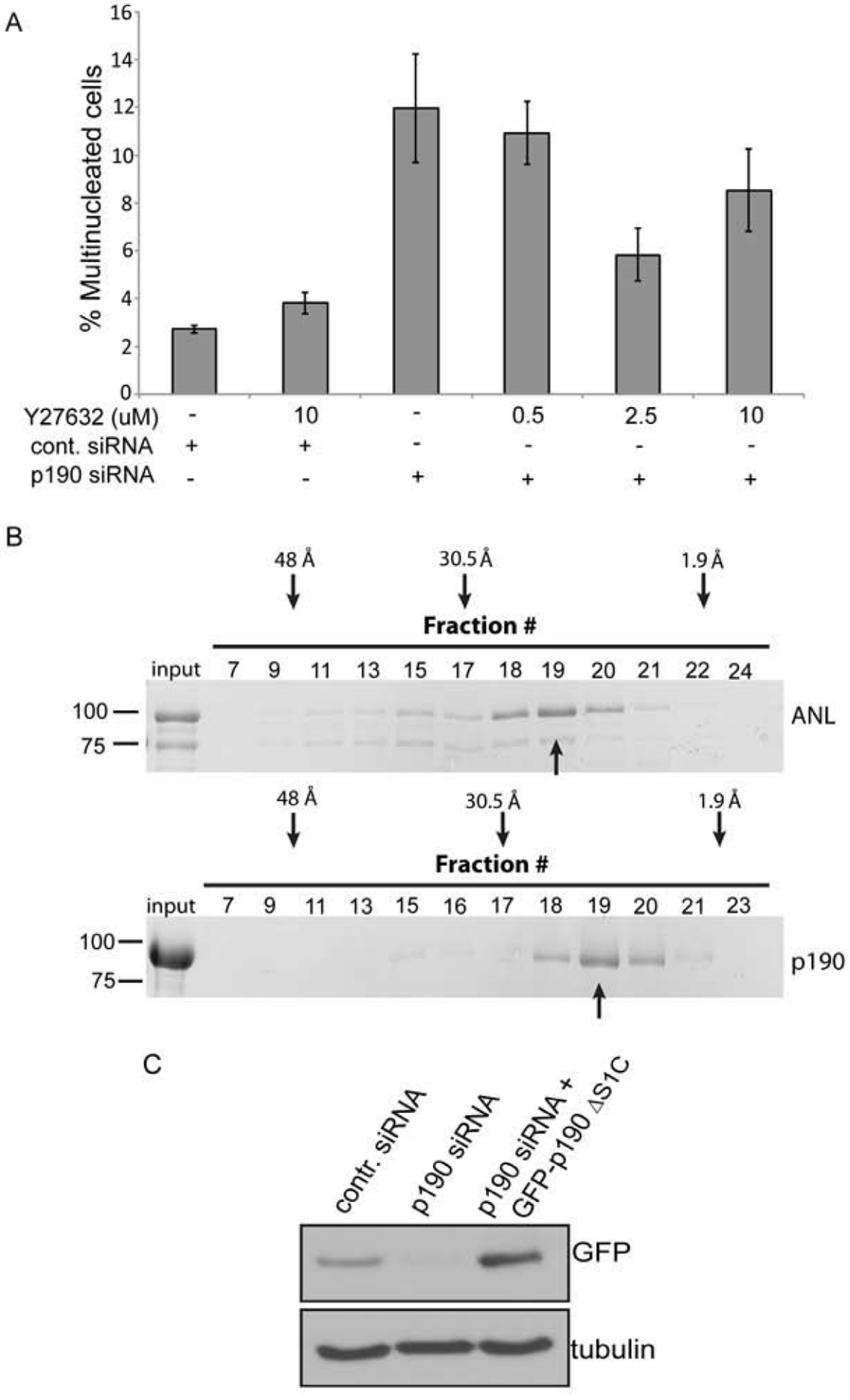
Supplemental Video 1 and 2: P190-depleted cells failed in abscission.

Control (video1) and p190 depleted (video2) HeLa cells were follow\ed from interphase into cytokinesis. Brightfield images were acquired every 3 min. Playback is 5 frames/s.

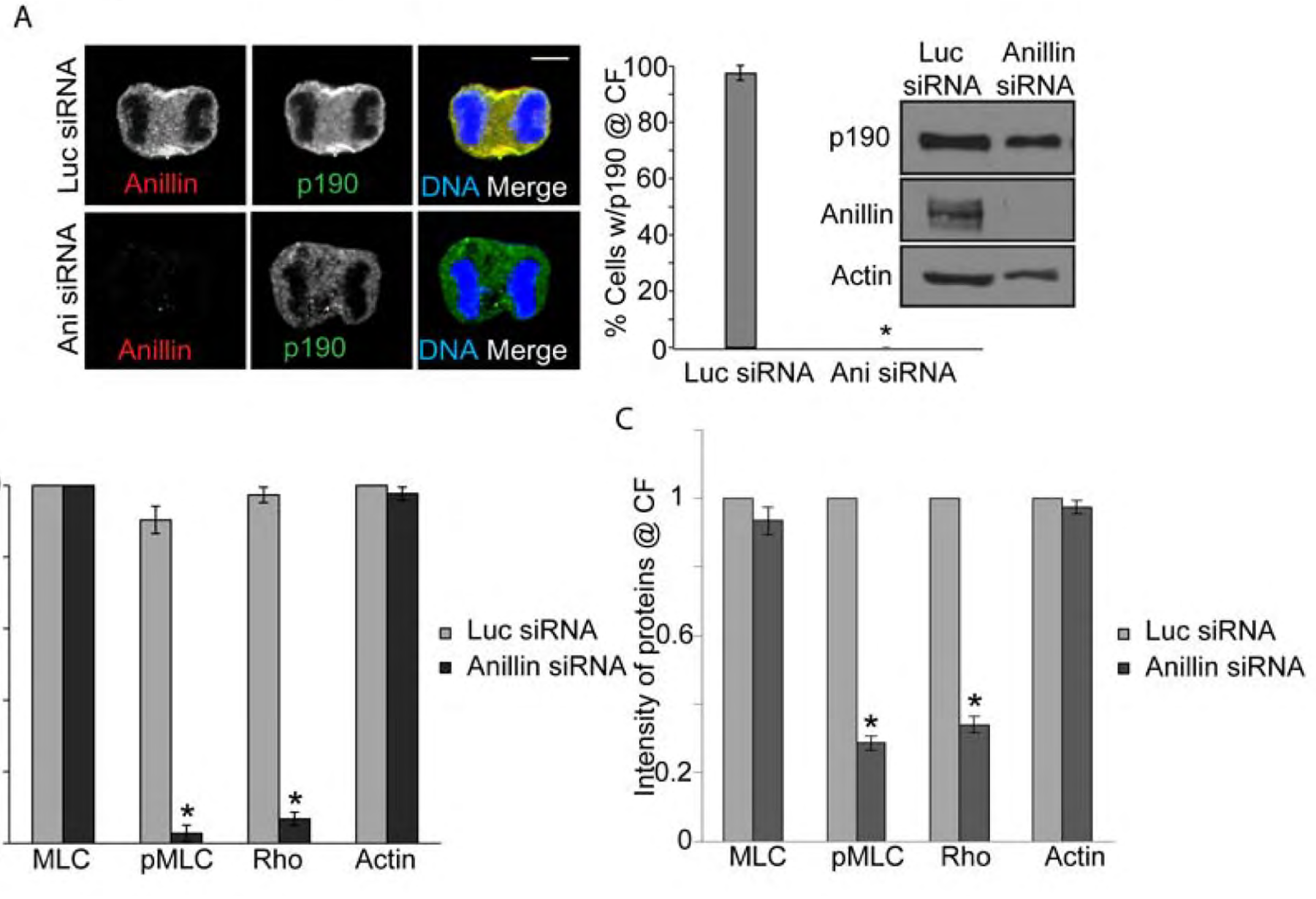
Supplemental Figure 1



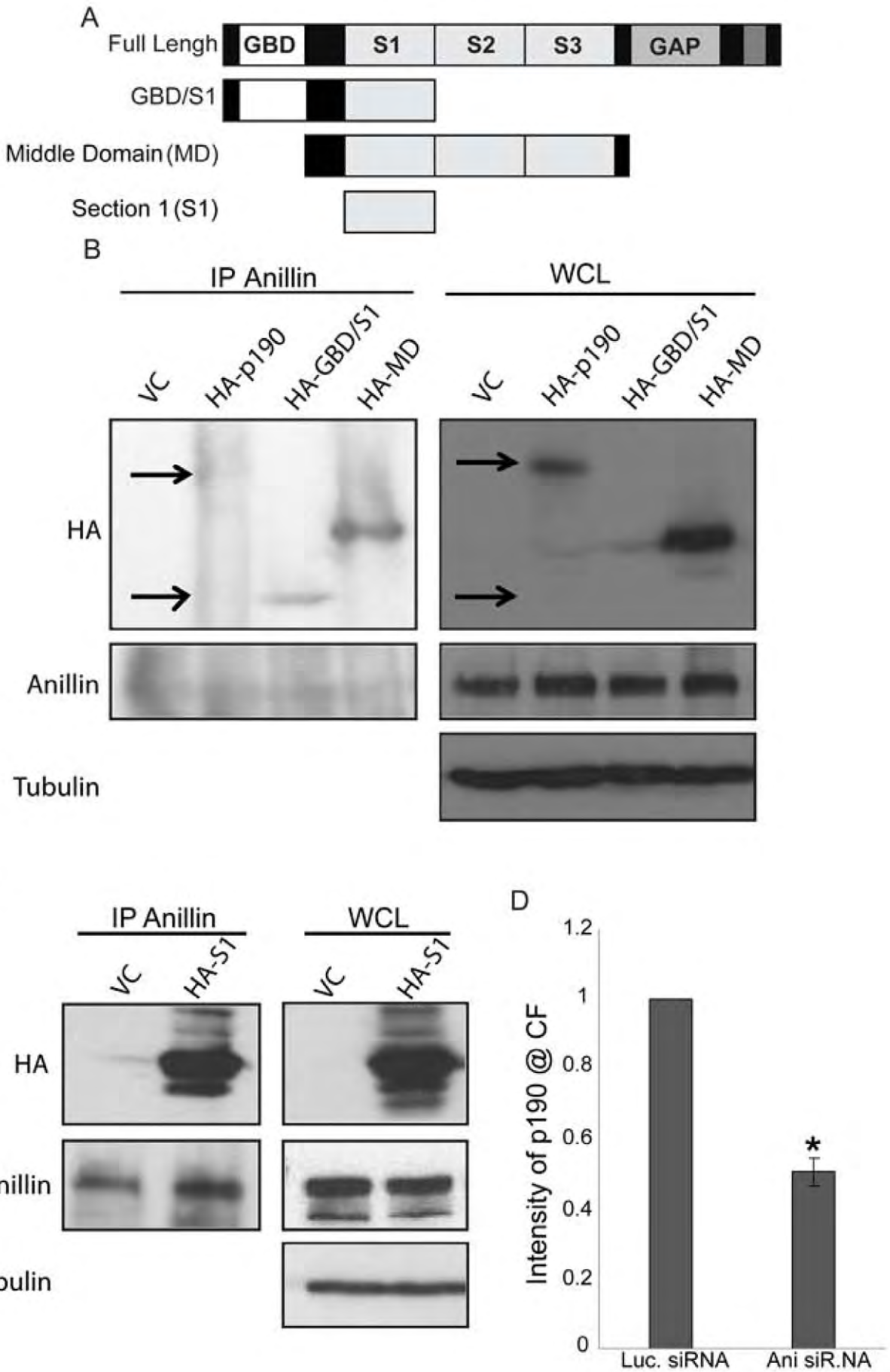
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



Movie 1.

Movie 2.