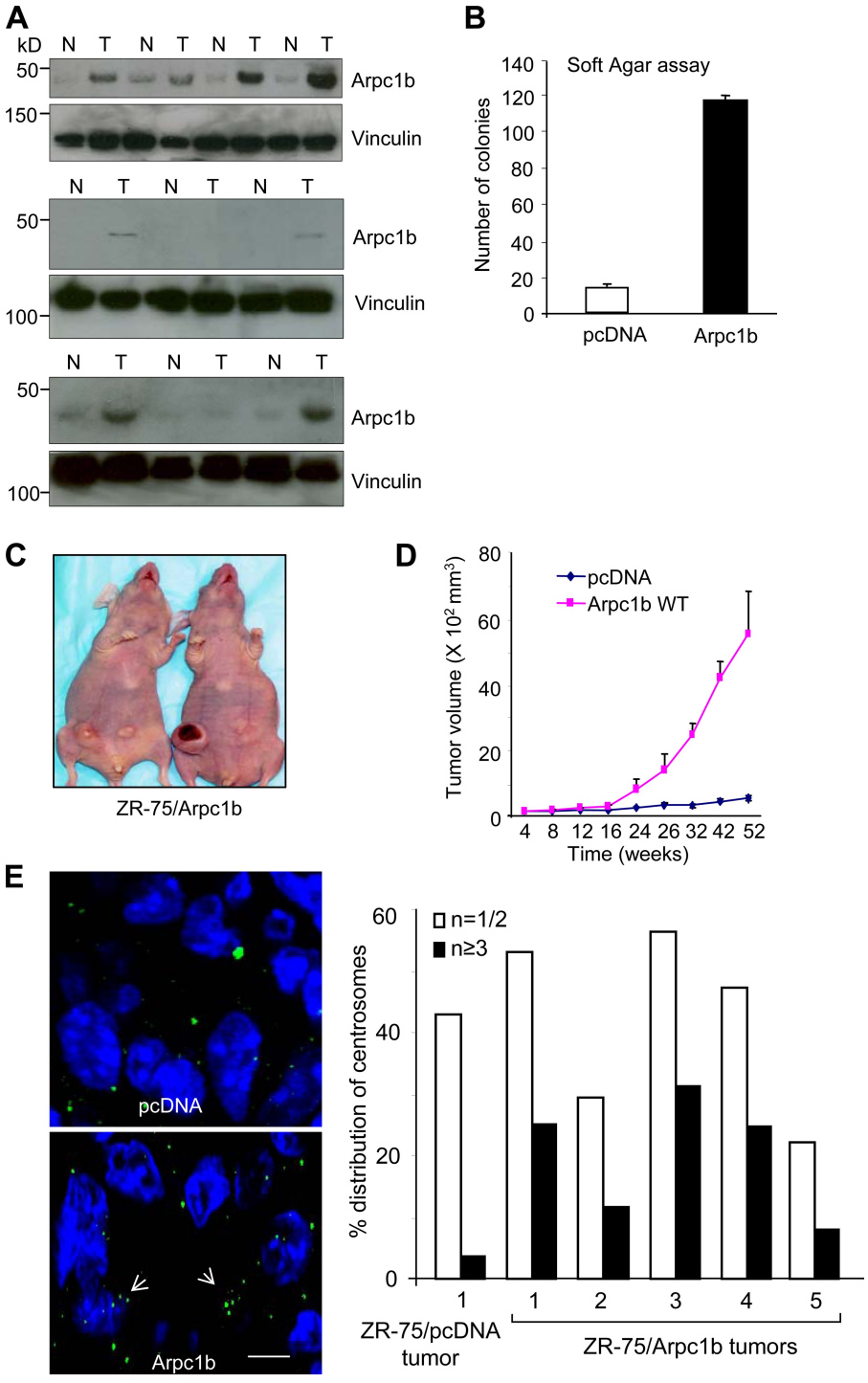
Molli et al., http://www.jcb.org/cgi/content/full/jcb.200908050/DC1

Figure S1. **Initial exploratory experiments leading to this study.** (A) Western blot analyses of Arpc1b expression in human normal and breast cancer tissue samples. (B) Anchorage-independent colony formation assays using ZR-75/pcDNA, ZR-75/Arpc1b cells were plated in soft agar. Columns, number of colonies; bars, SD. (C) Representative image showing tumors in nude mice injected with ZR-75/Arpc1b cells. (D) ZR-75/Arpc1b cells have a higher tumor incidence rate than ZR-75/pcDNA cells. Tumor size was measured every 4 wk. Points, tumor volume; bars, SD. (E) Representative images for centrosome staining using an antibody to centrin in tumor section from nude mice. Arrows mark cells with multiple centrosomes. A total of 100 DAPI-positive cells were counted for each tumor (DAPI stains DNA). Bar plot shows percent distribution of cells with two or more spots for centrin staining. Bar, 5 µm. kD, kilodaltons.



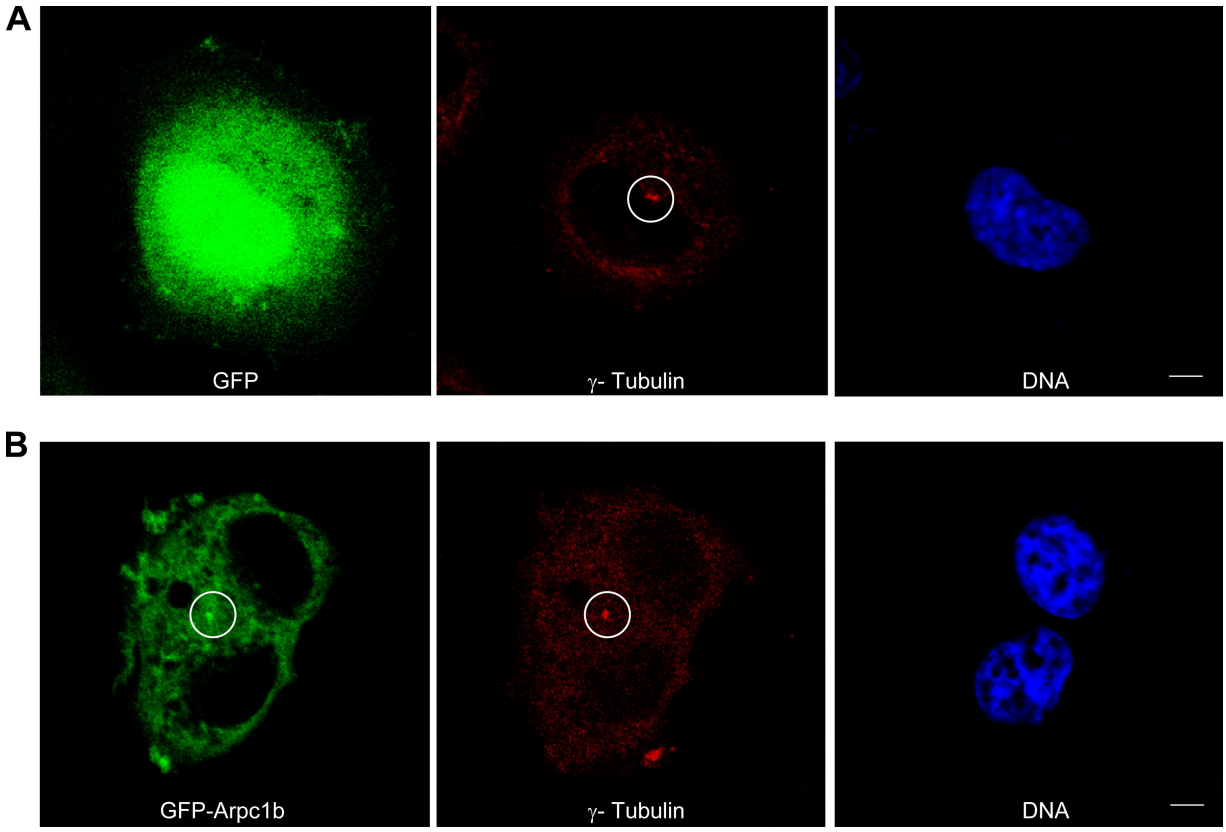


Figure S2. **Localization of GFP-Arpc1b.** ZR-75 cells were transfected with either GFP (A) or GFP-Arpc1b (B) and fixed with paraformaldehyde (4%) after 24 h and immunostained for γ-tubulin (red) and DNA (blue). Circle marks centrosome location. Bars, 5 μm.

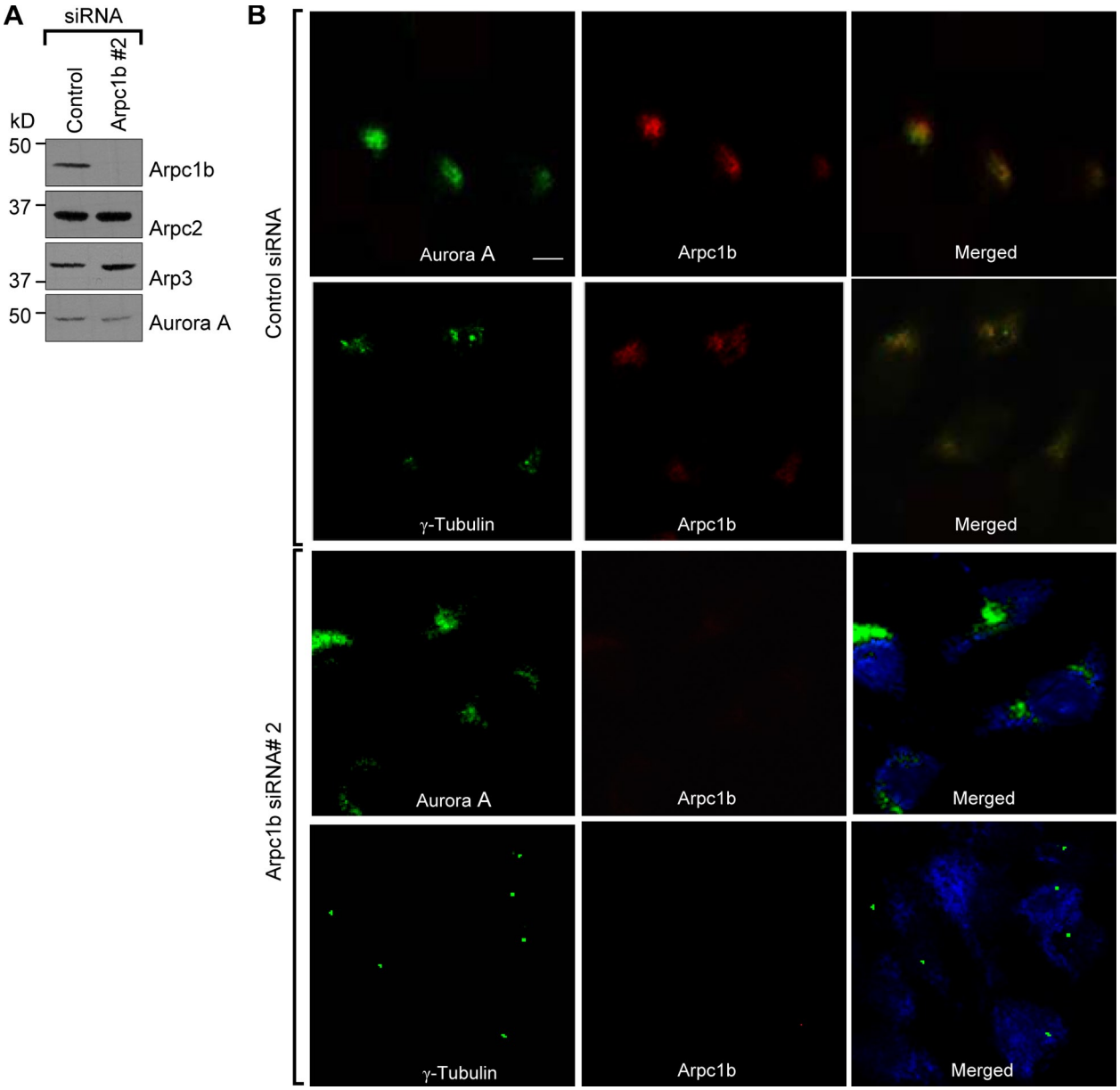


Figure S3. **Verification of the specificity of Arpc1b siRNA.** (A) Western blot analysis shows effective knockdown of endogenous Arpc1b upon transfection with siRNA to Arpc1b in the ZR-75 cells. Arpc2, Arp3, and Aurora A were used as controls. (B) Synchronized ZR-75 cells transfected with control or Arpc1b siRNA were released for 6 h and analyzed for endogenous Arpc1b colocalization with centrosomal proteins Aurora A and γ-tubulin. Aurora A and γ-tubulin (green); endogenous Arpc1b (red) and DNA (blue). Circle marks centrosome location. Bar, 5 μm. kD, kilodaltons.

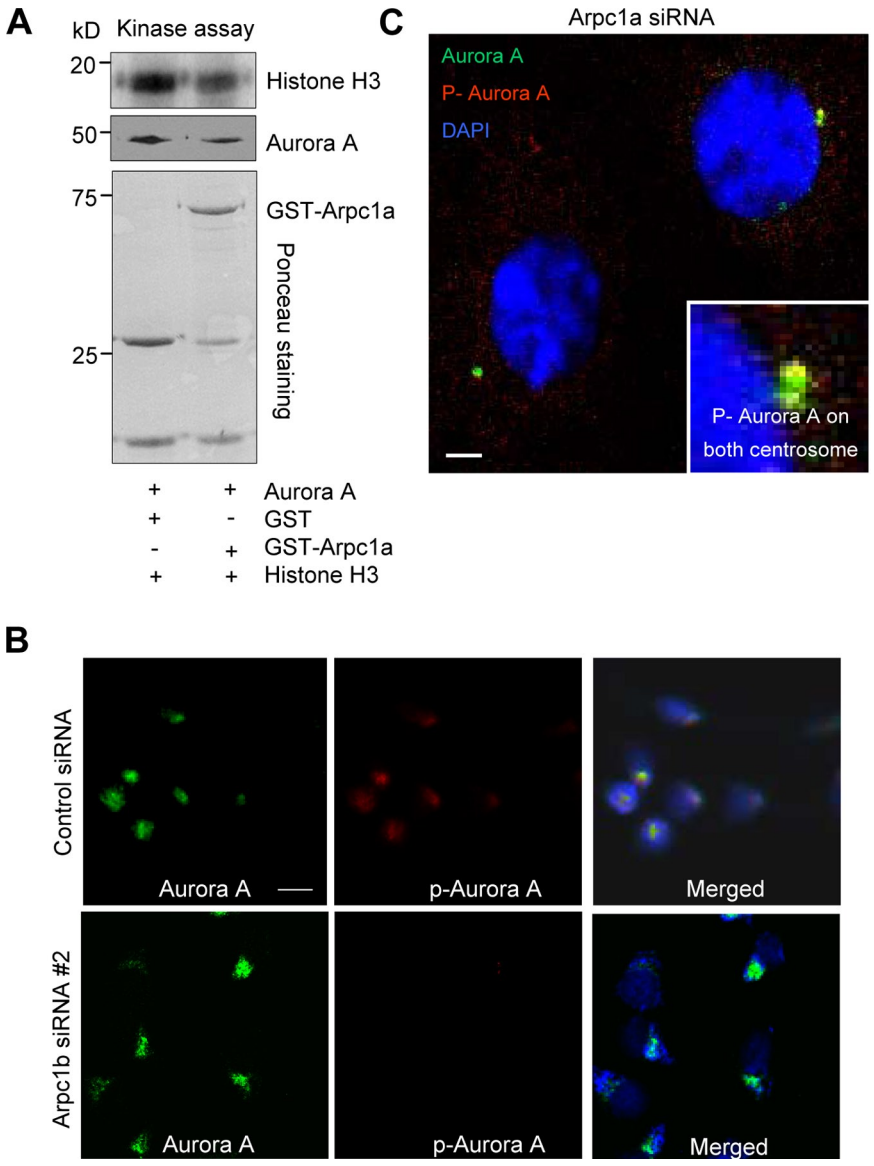


Figure S4. **Arpc1a fails to stimulate Aurora A activity.** (A) Kinase assay for Aurora A in presence of either GST or GST-Arpc1a proteins. Aurora A in the second panel indicates comparable amounts of protein loading. Ponceau-stained blot shows equal quantity of GST-tagged proteins used in the study. (B) ZR-75 cells were transfected with control, Arpc1b-siRNA#2 and immunostained with antibodies to Aurora A (green), phospho-Aurora A (Thr288) (red), and DNA (blue) as indicated. (C) Synchronized ZR-75 cells transfected with either control or Arpc1a siRNA and released for 7 h after G1-S arrest were immunostained with antibodies to Aurora A (green) or phospho-Aurora A T288 (red) and DNA (blue) as indicated. Insets represent higher magnification images of centrosomes. Bar, 5 µm. kD, kilodaltons.

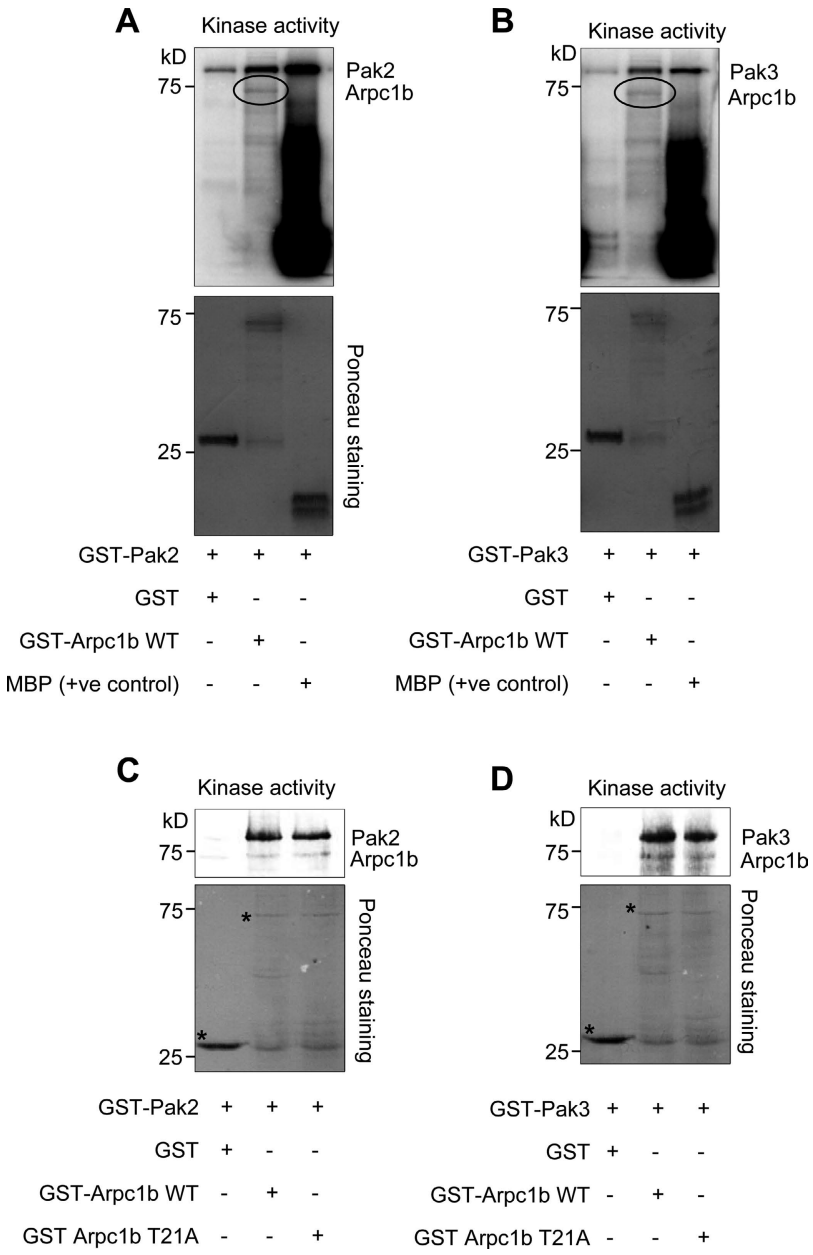


Figure S5. **Arpc1b phosphorylation by Pak2 and Pak3.** GST alone and GST- Arpc1b was used in an in vitro kinase assay with either GST-Pak2 (A) or GST-Pak3 (B). Myelin basic protein (MBP) was used as a positive control. GST alone, GST-Arpc1bWT, and GST-Arpc1bT21A were used in an in vitro kinase assay with either GST-Pak2 (C) or GST-Pak3 (D). Ponceau-stained blot shows equal amount of GST proteins used for all the reactions. WT, wild type; +ve, positive control; kD, kilodaltons. Circle marks Arpc1b specific band; Asterisk denotes the GST-fused protein of interest.