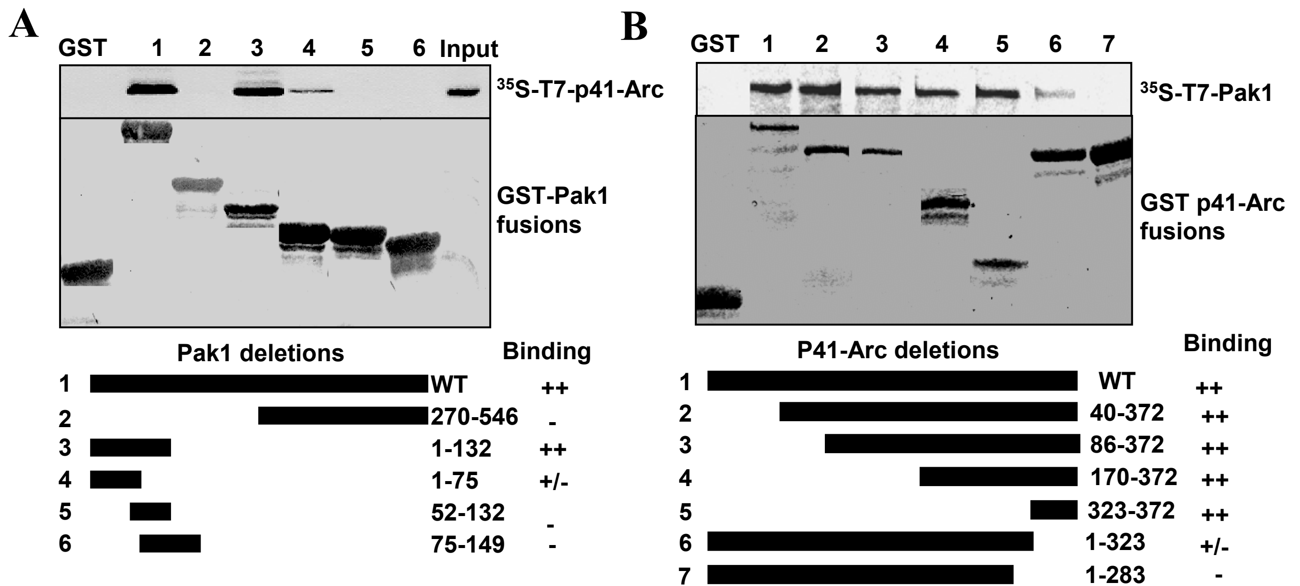
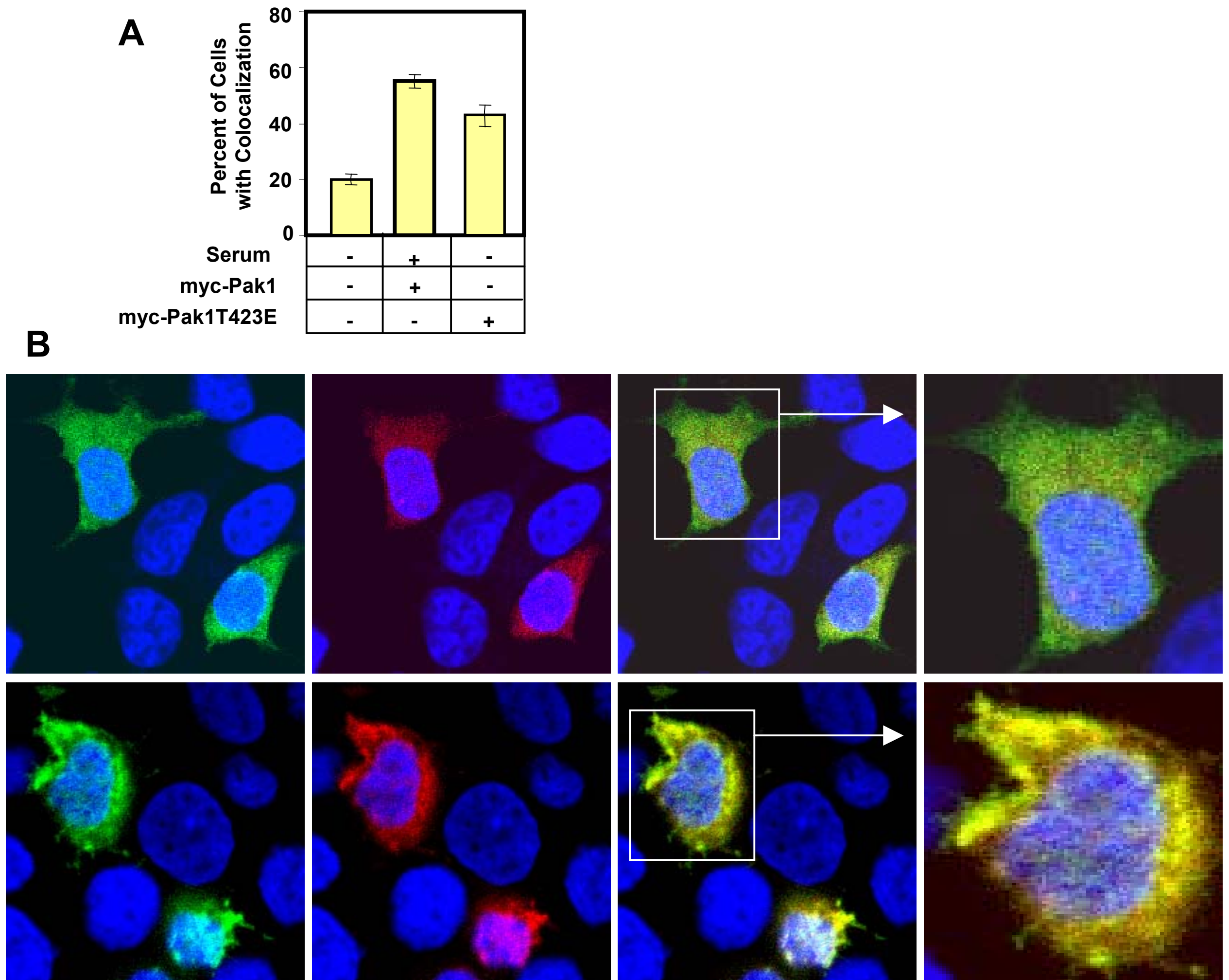
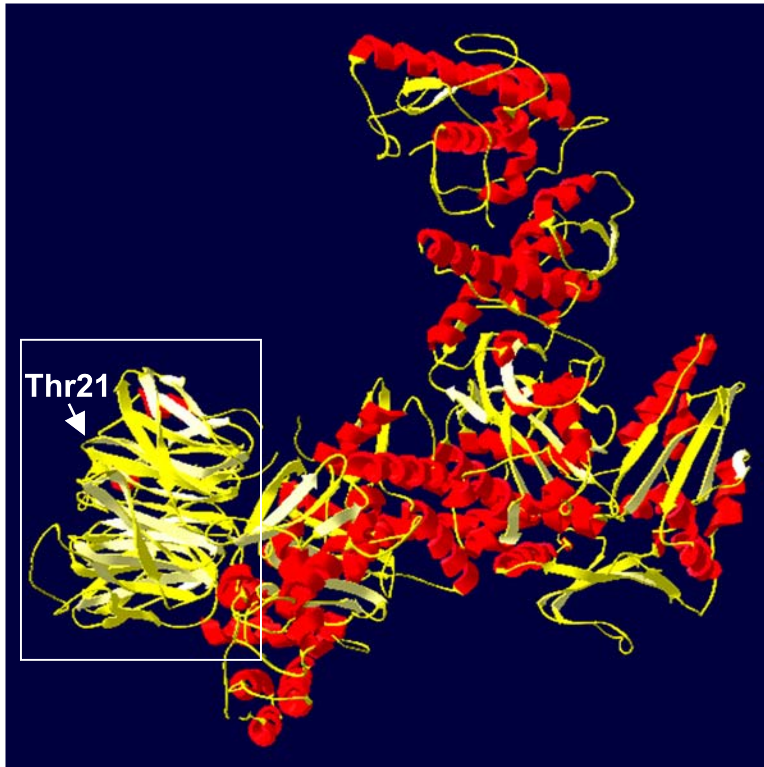
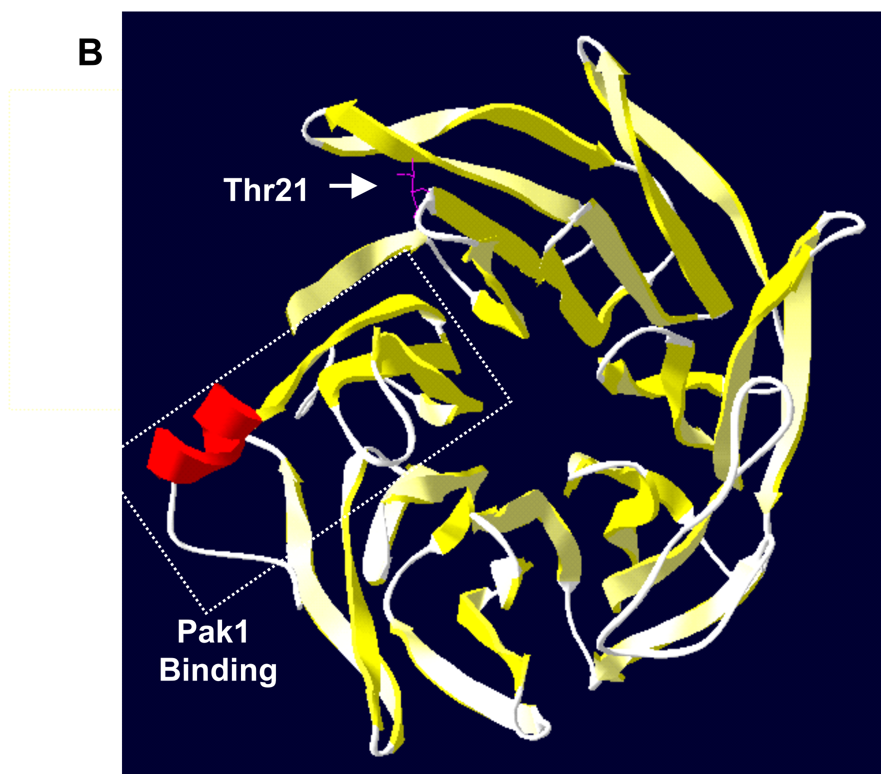


Supplemental Figure S1. (A) Mapping the minimal binding domain of Pak1 for p41-Arc using a series of GST fusion proteins expressing different Pak1 domains. GST-Pak1 fusion proteins were incubated with *in vitro* translated p41-Arc, and binding was analyzed by GST pull-down assay. Pak1 kinase domain did not interact with p41-Arc, while N-terminal 1-132 amino acid interacted strongly with p41-Arc. Further analysis of this region suggested the presence of a p41-Arc binding region in the N-terminal 1-75 amino acids. However, the observed low-affinity of binding of N-terminal amino acids 1-75 fragment suggest that the CRIB domain may indirectly contribute to the noticed high-affinity interaction of p41-Arc binding with the N terminal amino acids 1-132 region. (B) To identify the minimal region of p41-Arc required for its interaction with Pak1, we also serially deleted the WD repeats of p41-Arc. GST-p41-Arc fusion proteins were incubated with *in vitro* translated Pak1, and binding was analyzed by GST pull-down assay. Results suggested that amino acids 283-372 of p41-Arc, which contain the seventh WD domain, constitute the minimal binding region for binding to Pak1.

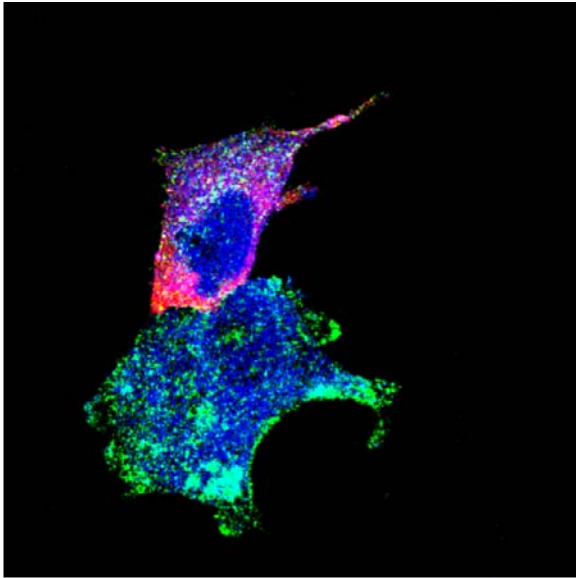


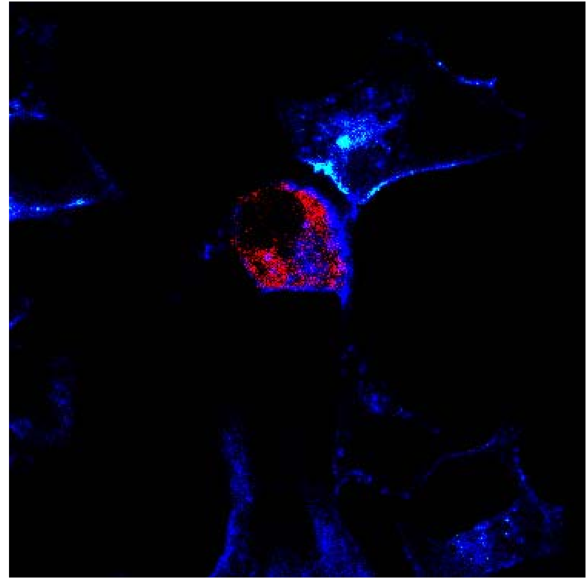
Supplementary Figure S2. Pak1 regulation of p41-Arc interaction with Arp2/3 complex in vivo. (A) MCF-7 cells were transfected with T7-tagged p41-Arc and either myc-tagged wild-type Pak1 or Pak1 T423E (DA-Pak1, then serum starved prior to treatment with 10% fetal calf serum for 30 min and processing for immunofluorescence (green, anti-T7; red, anti-myc; blue, DNA) and confocal microscopy. Diffuse and specific co localization of transfected proteins were counted and graphed as a percentage of total cells counted (n=250 for each Pak1 construct and treatment condition). Data are means +/- S.D. of 3 experiments. (B) Representative examples of diffused and colocalization of p41-Arc and Pak1 are shown. To explore the functional significance of Pak1 and p41-Arc interactions, we examined the effects of myc-tagged wild-type Pak1, a catalytically active Pak1 T423E mutant (DA-Pak1) (Vadlamudi *et al.*, 2002), on the localization of T7-tagged p41-Arc at the cell membrane and/or in intracellular nucleation spots. Stimulation of wild type Pak1 expressing cells with serum increased the colocalization of Pak1 with P41-Arc. Similarly expression of kinase-active Pak1 (T423E) increased the percentage of cells with p41-Arc and Pak1 colocalization under serum starved conditions compared to wild type expressing serum starved cells. These results suggest that signals, which induce actin reorganization/polymerization signals, induce colocalization of p41-Arc and Pak1 and activation of Pak1 may have a role in its colocalization with p41-Arc.





Supplementary Figure S3. Localization of Pak1 binding and phosphorylation sites in the Arp 2/3 complex. **A**. Crystal structure of the Arp 2/3 complex (PDB 1K8K) with the p41 subunit outlined. **B**. View of the beta-propeller-like structure of p41-Arc, with the high affinity Pak1 binding region outlined and the Thr21 phosphorylation site is indicated. Figures were created using SWISS Prot PDB Viewer Software.





Control siRNA **Pak1 siRNA**

Supplemental Figure S4. MCF7 breast cancer cells were transiently transfected with T7-tagged p41-Arc, then 24 hours later transfected with Pak1-specific siRNA (Qiagen) as previously described (Wang et al., EMBO J., 21, 5437-5447, 2002 and Barnes et al., NSB, 10, 662-628, 2003). Cells were grown in serum-free media for forty-eight hours then treated with epidermal growth factor for 30 minutes. Pak1-specific siRNA severely attenuated endogenous Pak1 protein expression (green) and reduced transfected p41-Arc (red) colocalization with phalloidin-stained F-actin (blue).