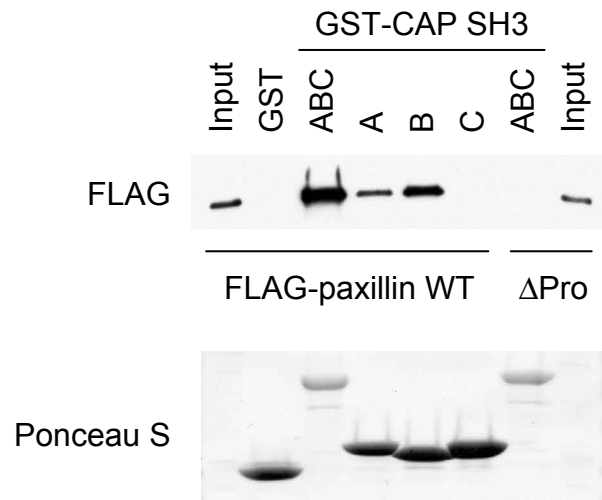
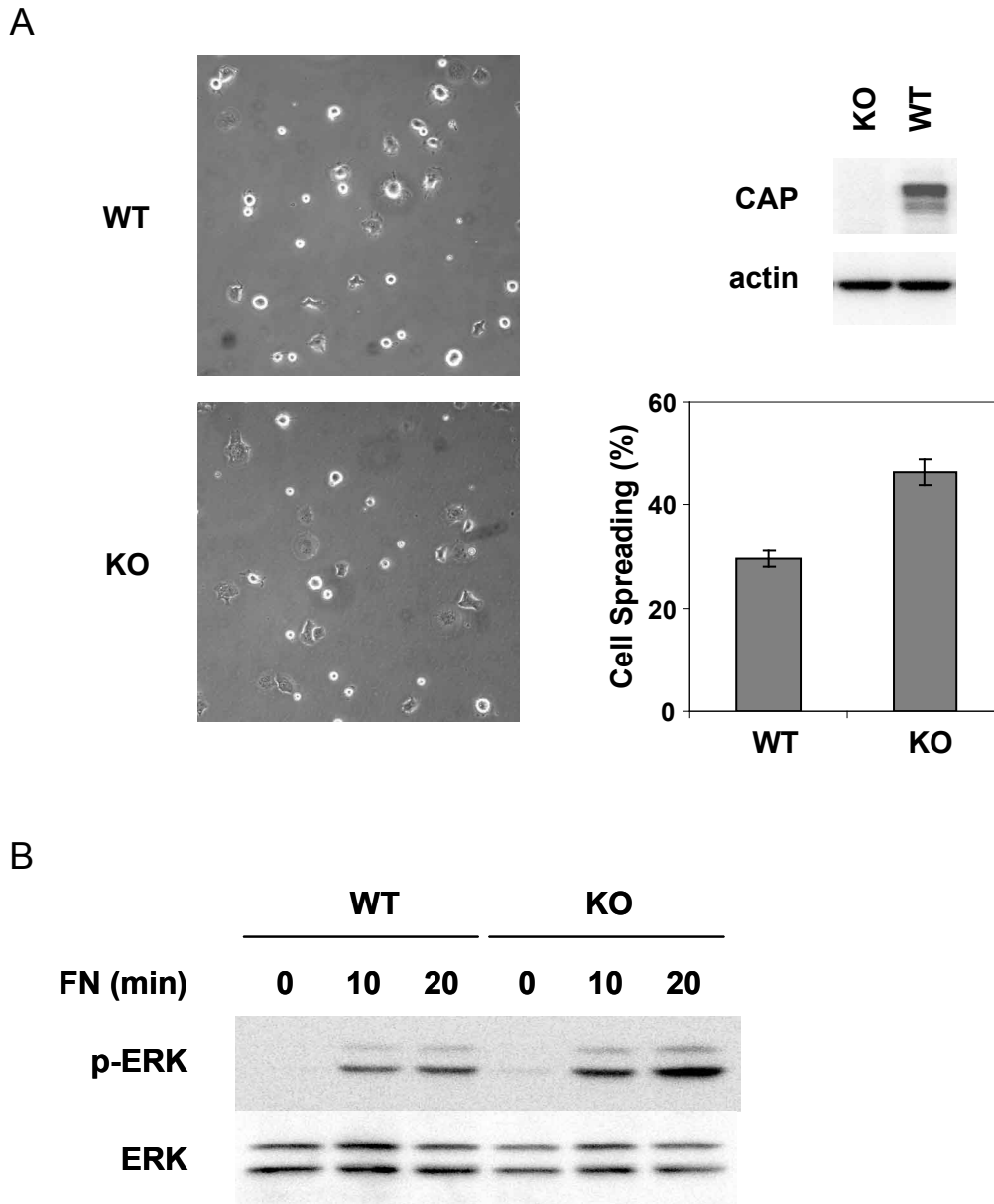


## Supplementary Figure S1



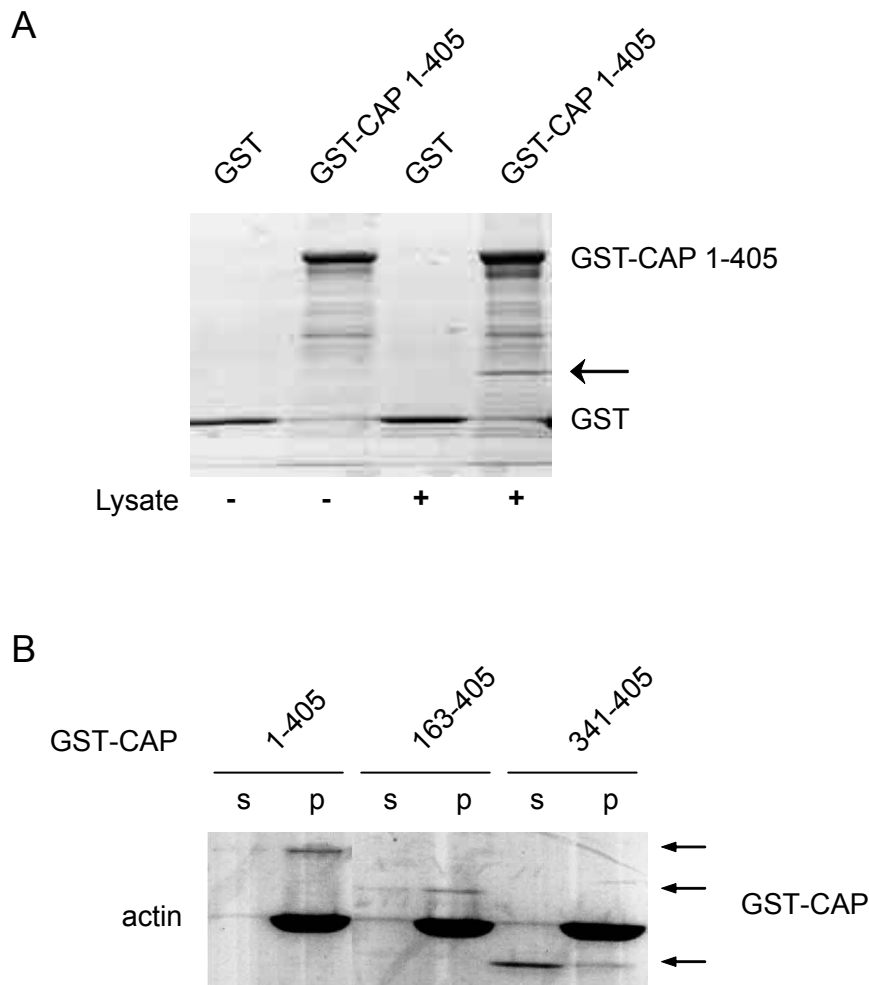
**S1. Paxillin binds to the first two SH3 domains of CAP.** GST fusion proteins of CAP SH3 domains were used to pull down in vitro translated FLAG-paxillin WT or the  $\Delta$ Pro mutant. The precipitates were subjected to SDS-PAGE and western blot analysis. The amount of GST fusion proteins used is shown by Ponceau S staining.

## Supplementary Figure S2



**S2. CAP-null MEFs exhibit enhanced spreading on fibronectin.** (A) Wild-type (WT) and CAP-null (KO) MEF cells were plated on fibronectin-coated plates for 10 min, photographed (left) and counted (right). The data represent mean  $\pm$  S.E. (B) WT and KO MEF cells were kept in suspension or plated on fibronectin for 10 or 20 minutes, Lysates were separated by SDS-PAGE and subjected to western blot analysis. KO MEFs exhibited enhanced adhesion-induced ERK phosphorylation.

## Supplementary Figure S3



**S3. CAP interacts with F-actin.** (A) GST-CAP 1-405 was used to affinity purify associating proteins from fibroblast lysates. Cell lysates were subjected to pull down by GST or GST-CAP 1-405, followed by SDS-PAGE, and stained with Coomassie blue. Bands were cut out and analyzed by Mass Spectrometry. The arrow indicates the presence of actin specifically in GST-CAP 1-405 pull down (lane 4). (B) CAP binds to F-actin in actin co-sedimentation assays. GST-CAP fragments (as indicated) were incubated with skeletal muscle F-actin, followed by centrifugation. The supernatant (S) and pellet (P) fractions were analyzed by SDS-PAGE. The proteins were transferred to nitrocellulose membrane and stained with Ponceau S.