Supplementary Figure Legends

Figure S1. Arp2/3 functions downstream of Rac in insulin signal pathway and functions independent of Akt.

A) L6GLUT4myc myoblasts transiently expressing CA-Rac-GFP were transfected with NR or Arp3 siRNA. Following 3 h serum starvation, unstimulated cells were labelled with rhodamine-phalloidin. Representative images of 3 independent experiments are shown. Bars, 20 μm. **B)** L6GLUT4myc myoblasts were transfected with NR, p34, or Arp3 siRNA. Insulin (100 nM) was applied for 10 min after 3 h of serum starvation. Total cell lysates were then collected and immunoblotted for **A)** p34, P-Akt, actinin-1 and **B)** Arp3, P-Akt, actinin-1. Representative blots of 3 independent experiments are shown.

Figure S2. Expression of *Dictyostellium* Arp3-GFP restores actin remodelling and GLUT4 translocation in Arp3 knockdown myoblasts.

A) *Dictyostellium* Arp3-GFP was transiently expressed in L6GLUT4myc myoblasts transfected with NR or Arp3 siRNA. After 3 h serum starvation, cells were challenged with 100 nM insulin for 10 min. F-actin was stained with rhodamine phalloidin to indicate actin remodelling. Representative images of 4 independent experiments are shown. Bars, 20 μm. **B)** Quantification of changes in insulin-stimulated dorsal actin remodelling relative to siNR control (mean ± S.E.). **C)** Myoblasts with NR or Arp3 siRNA were transfected with/without *Dictyostellium* Arp3-GFP. Following serum depletion, cells were stimulated with insulin for 10 min. Surface GLUT4myc content was measured via single cell detection of surface myc fluorescent intensity and quantified as fold increases over siNR basal (mean ± S.E., n=6, $^{\#}p<0.05$).

Figure S3. Down-regulation of LIMK1 does not affect insulin-induced cofilin dephosphorylation.

Lysates from myoblasts treated with LIMK1 siRNA were prepared to determine the extent of LIMK knockdown and its contribution towards insulin-induced cofilin dephosphorylation by immunoblotting for P-cofilin. Representative blots of 3 independent experiments are shown.

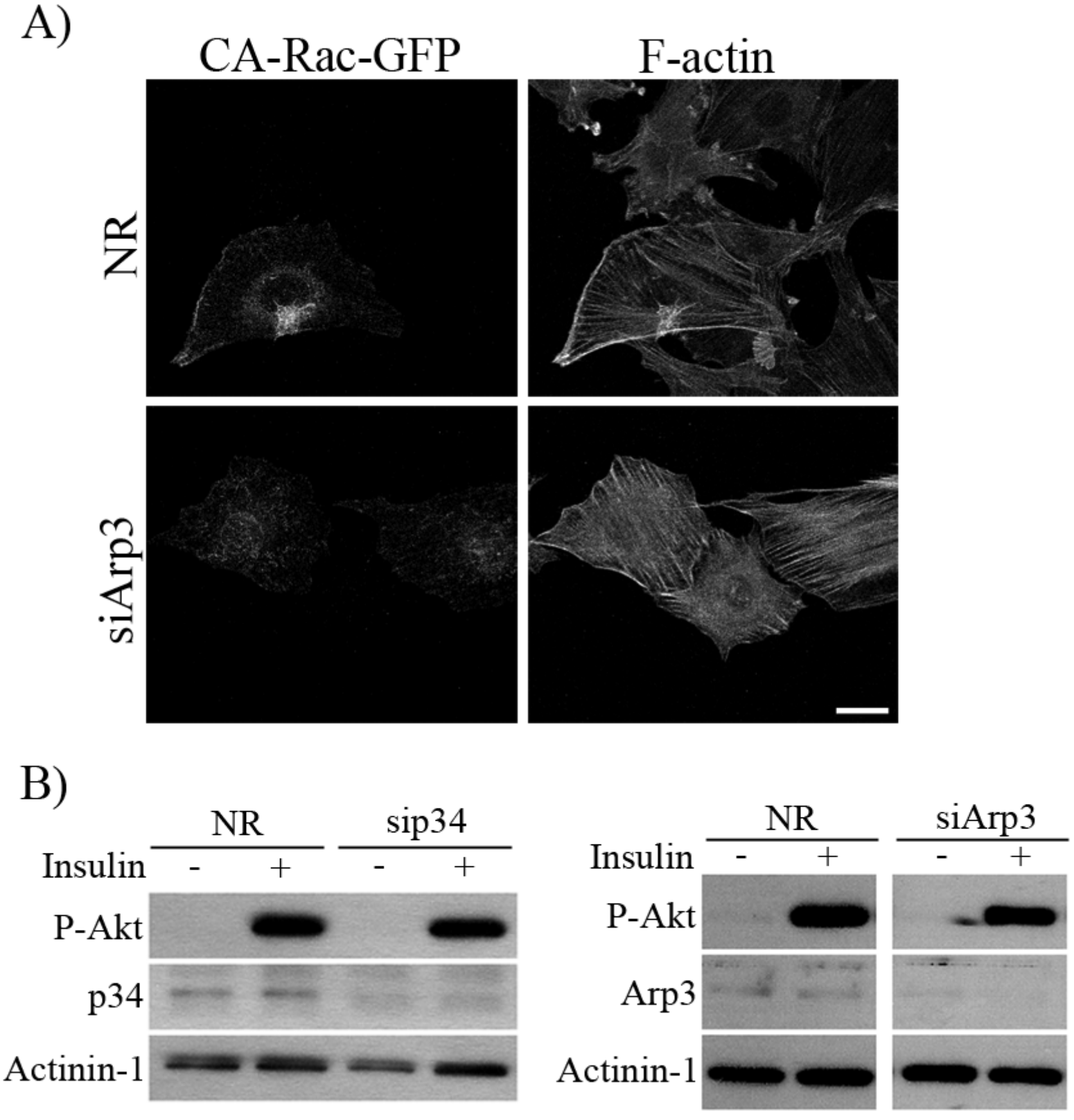
Figure S4. SSH1 redistributes to the zone of actin remodelling upon insulin stimulation.

L6 myoblasts were transiently transfected with SSH1 cDNA, serum-deprived cells and stimulated with 100 nM insulin for 10 min, followed by fixation and permeabilization, to allow detection of transfected SSH1 and F-actin. Representative images of 3 independent experiments are shown. Bar: 10 μm.

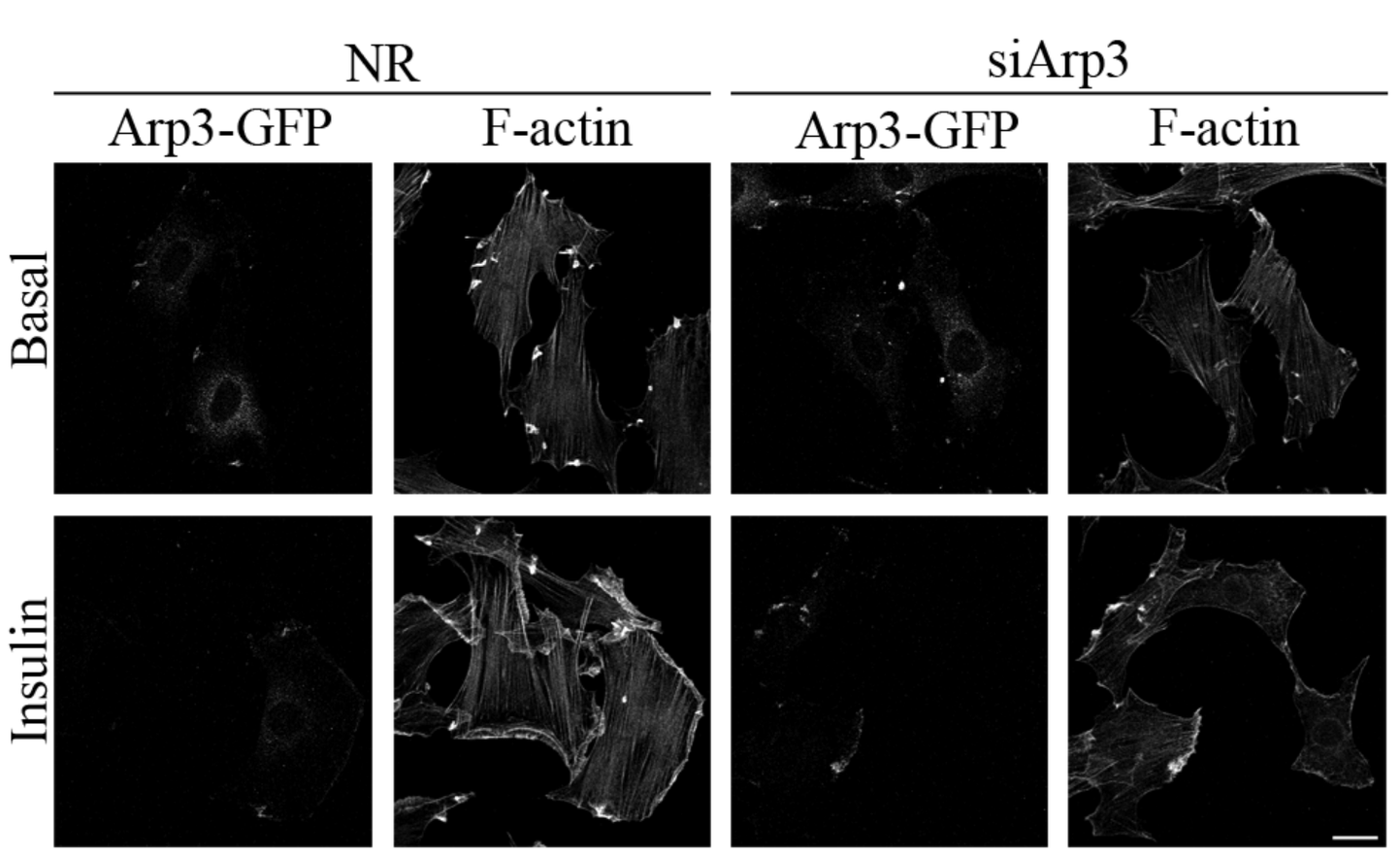
Figure S5. The P-AC/cofilin ratio at the cell periphery decreases with insulin stimulation.

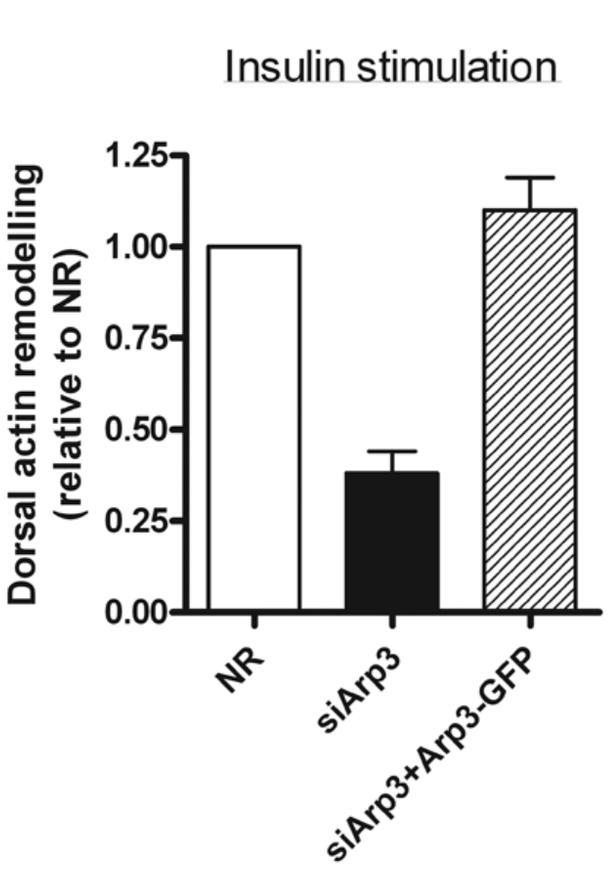
A) Serum-starved L6 myoblasts were stimulated with or without 100 nM insulin for 10 min, followed by fixation and permeabilization. The cells were labelled for phosphorylated AC and total cofilin using specific affinity-purified antibodies. Bars, 10 μm. **B)** Areas of similar size of the cell periphery containing remodelled actin, and background areas that did not contain any cells, were selected. The fluorescence intensity in these regions from 50 basal-state and 43 insulin-stimulated cells were determined, averaged and plotted as ratio of P-AC/cofilin signal (mean ± S.E., $^{\#}p<0.05$).

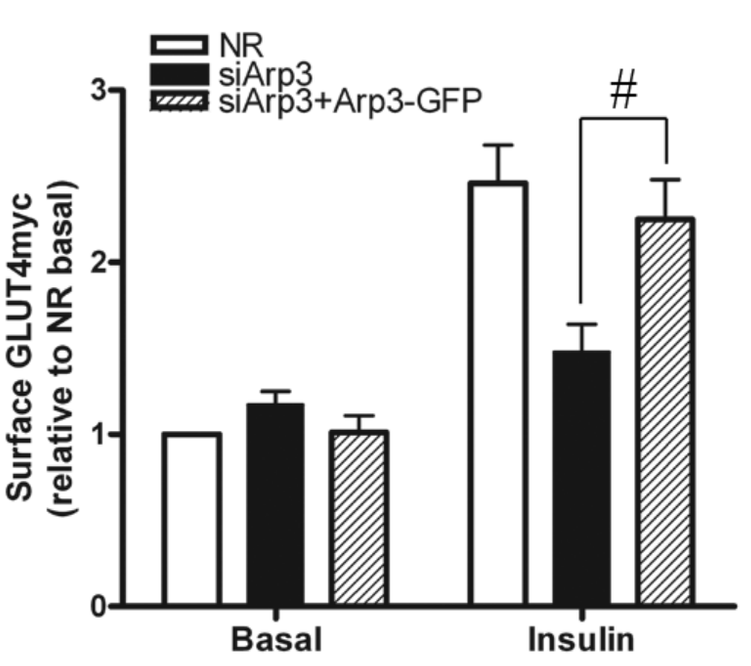
Supplementary Figure 1



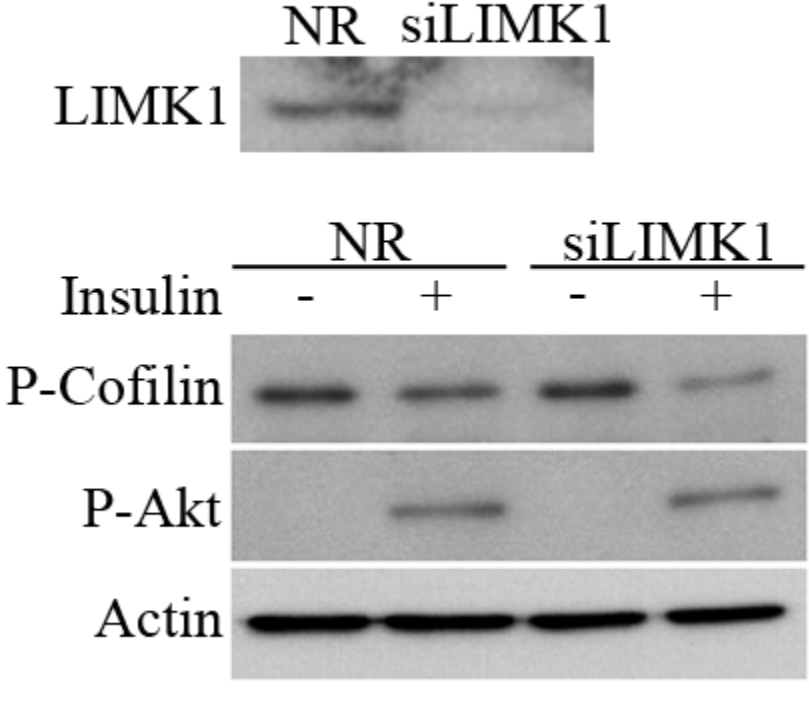
Supplementary Figure 2



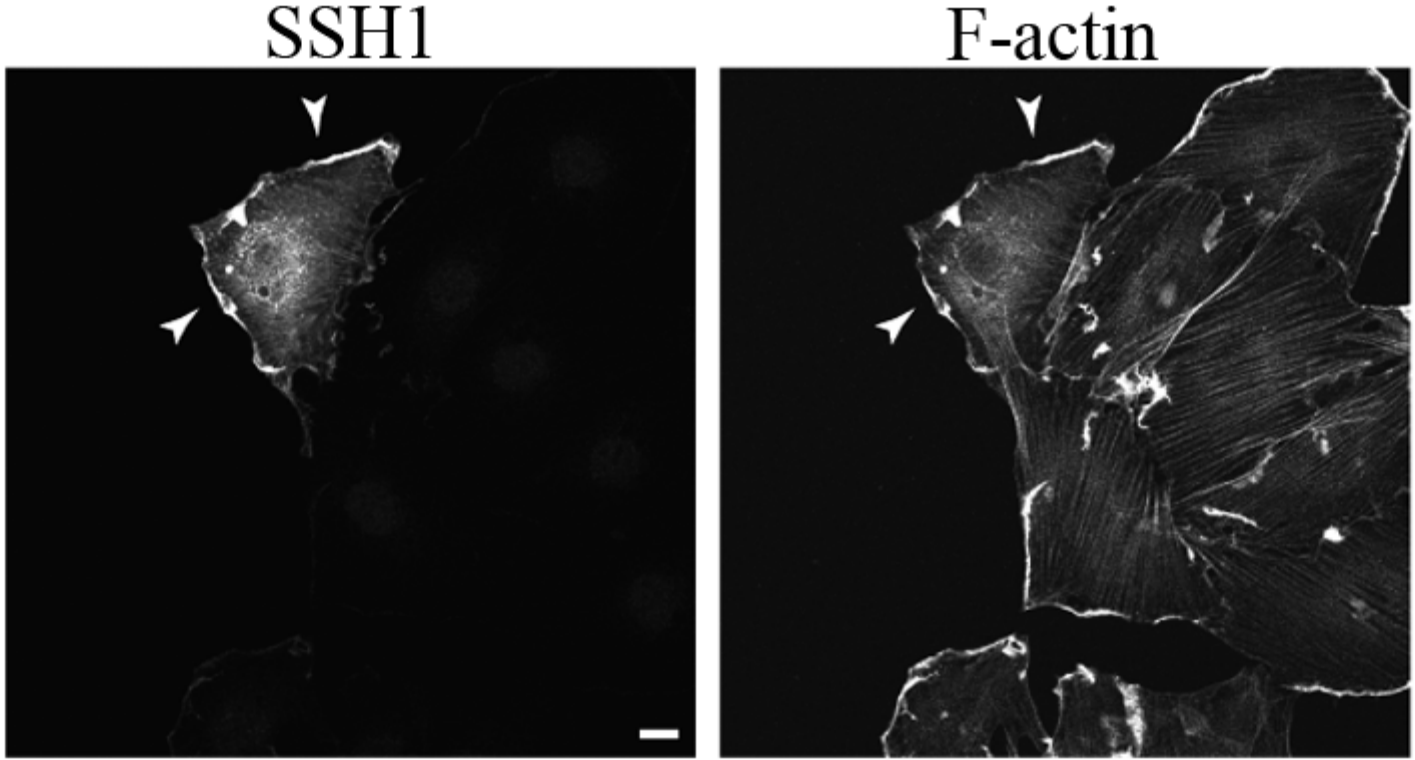




Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5