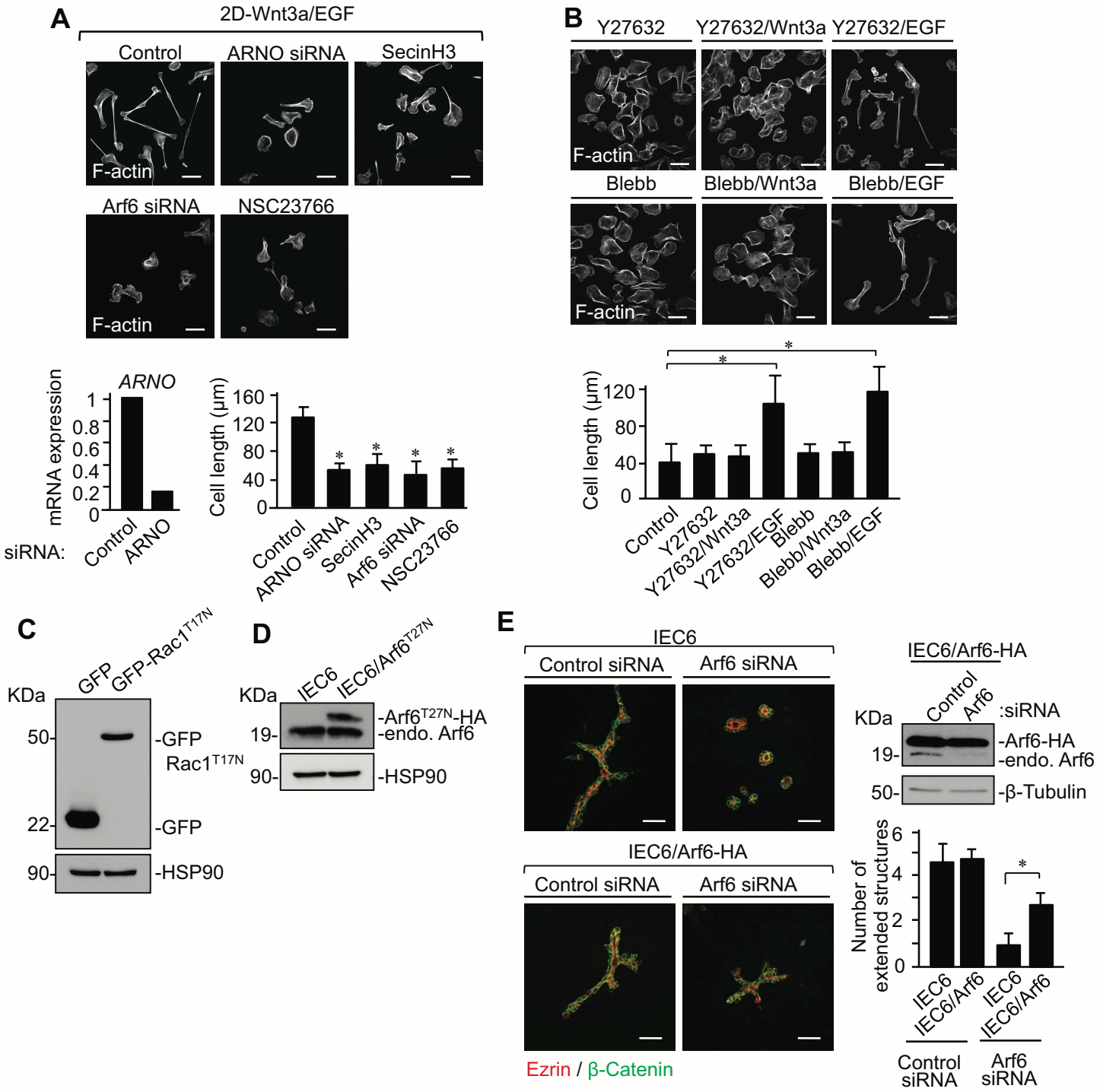
Matsumoto et al., Figure S7



Supplementary Figure S7 The Arf6-Rac-Rho axis is required for Wnt3a/EGF-dependent changes in cell morphology and tube formation. (**A**) IEC6 cells treated with SecinH3 or NSC23766 or transfected with indicated siRNAs were stimulated with Wnt3a/EGF for 18 h in 2D culture and stained with phalloidin. Real-time PCR analyses for *ARNO* mRNA expression were performed. The length of the long axis of cells was measured (n=50). (**B**) IEC6 cells were stimulated with Y27632 (2 μM), Y27632/Wnt3a, Y27632/EGF, blebbistatin (2 μM), blebbistatin/Wnt3a, or blebbistatin/EGF for 18 h in 2D culture and stained with phalloidin. Blebb, blebbistatin. The length of the long axis of cells was measured (n=50). (**C**) Lysates of IEC6 cells stably expressing GFP or GFP-Rac1^{T17N} were probed with anti-GFP and anti-HSP90 antibodies. (**D**) Lysates of IEC6 cells stably expressing Arf6^{T27N}-HA were probed with anti-Arf6 and anti-HSP90 antibodies. (**E**) Control IEC6 cells or IEC6 cells stably expressing Arf6-HA were transfected with control or Arf6 siRNA and treated with Wnt3a/EGF for 60 h in 3D culture. Cells were stained with anti-β-catenin and anti-ezrin antibodies. Lysates were probed with anti-Arf6 and anti-β-tubulin antibodies. The number of extended structures from multicellular trunks were counted (n=30). Results are shown as the mean ± SE from three independent experiments. Scale bars in (**A**, **B**, and **E**), 50 μm.