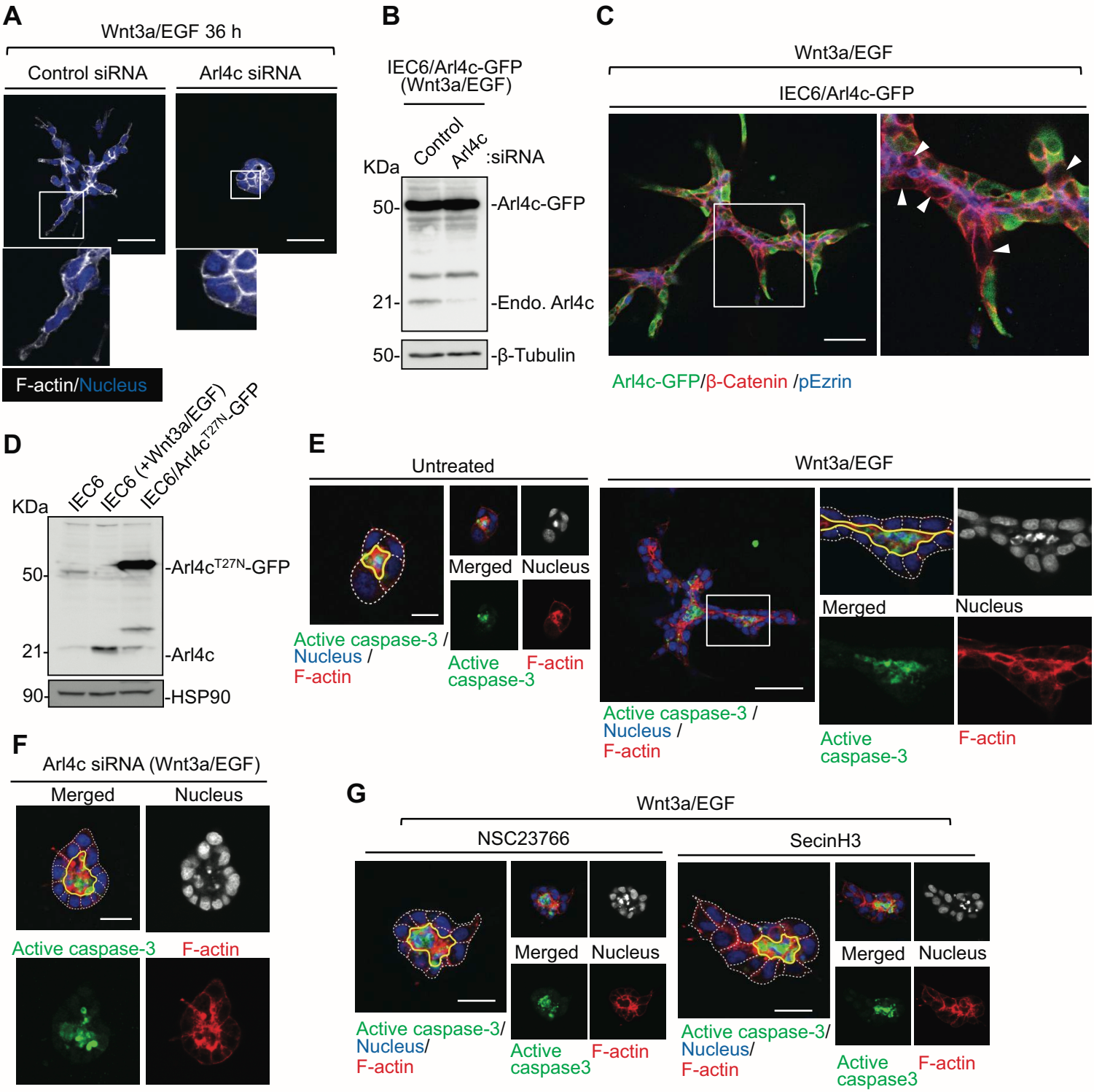
Matsumoto et al., Figure S4



Supplementary Figure S4 Knockdown of Arl4c or treatment with NSC23766 or SecinH3 do not affect cell viability. (**A**) IEC6 cells transfected with Arl4c siRNA were cultured with Wnt3a/EGF for 36 h in 3D culture and stained with phalloidin and DRAQ5. White boxes show enlarged images. (**B**) IEC6 cells stably expressing Arl4c-GFP, which were transfected with control or Arl4c siRNA, were treated with Wnt3a/EGF for 12 h. Lysates were probed with anti-Arl4c, anti-β-tubulin antibodies. (**C**) IEC6 cells stably expressing Arl4c-GFP (IEC6/Arl4c-GFP) were cultured with Wnt3a/EGF for 60 h and stained with anti-GFP, anti-phospho-ezrin, and anti-β-catenin antibodies. GFP was positive in approximately 80% of IEC6/Arl4c-GFP cells based on assessment with an anti-GFP antibody. White arrowheads indicate cells which do not express Arl4c-GFP. (**D**) IEC6 cells treated with or without Wnt3a/EGF for 12 h and IEC6 cells stably expressing Arl4c^{T27N}-GFP were lysed. Lysates were probed with anti-Arl4c and anti-HSP90 antibodies. (**E**) IEC6 cells were cultured with or without Wnt3a/EGF for 60 h in 3D culture and stained with anti-active caspase-3 antibody, phalloidin, and DRAQ5. White dashed lines and yellow solid lines indicate outlines of basolateral and apical membranes, respectively. (**F**) IEC6 cells transfected with Arl4c siRNA were cultured with Wnt3a/EGF for 60 h and stained with anti-active caspase-3 antibody, phallodin, and DRAQ5. (**G**) IEC6 cells were treated with Wnt3a/EGF in the presence of SecinH3 or NSC23766 for 48 h and stained with anti-active caspase-3 antibody, phalloidin, and DRAQ5. Scale bars in (**A** and **C**), 50 μm; in (**E**), 10 μm (left panel) and 50 μm (right panel); in (**F** and **G**), 20 μm.