



Supplementary Figure S3 Arl4c expression is required for changes in cell morphology in 2D culture. (A) IEC6 cells were treated with Wnt3a/EGF for 12 h in 2D culture in the presence or absence of actinomycin D and stained with phalloidin. (B) IEC6 cells were treated with Wnt3a for 12 h in 2D culture in the presence or absence of actinomycin D and real-time PCR analyses for *Axin2* mRNA expression were performed. (C) IEC6 cells were treated with Wnt3a/EGF for 18 h in 2D culture and stained with anti-Arl4c antibody and phalloidin. White boxes show enlarged images. (D, E) IEC6 cells transfected with control or Arl4c siRNA (D) and IEC6 cells stably expressing GFP or Arl4c-GFP (E) were treated with EGF for 18 h in 2D culture. Cells were stained with phalloidin, and the length of the long axis of cells was measured (n = 50). (F) IEC6 cells transfected with Arl4c siRNA and treated with Wnt3a/EGF for 12 h (left panels) or IEC6 cells treated with or without Wnt3a/EGF for 12 h and IEC6 cells stably expressing Arl4c-GFP (right panels) were lysed. Lysates were probed with anti-Arl4c, anti- β -tubulin, and anti-HSP90 antibodies. Results are shown as the mean \pm SE from three independent experiments. *, $P < 0.01$. Scale bars in (A, C, D, and E), 50 μm .