



Supplementary Figure S2 IEC6 cells respond to Wnt3a/EGF after they formed cysts in 3D culture. **(A)** IEC6 cells were cultured for 36 h in 3D culture to form cysts and then the cysts were treated with the indicated conditions for 72 h (left six panels). As a control, IEC6 cells were cultured with Wnt3a/EGF for 72 h in 3D culture (right panel). Cells were stained with anti-β-catenin and anti-phospho-ezrin antibodies. **(B)** IEC6 cells were cultured with Wnt3a/EGF for 72 h (0-72 h), or IEC6 cells were cultured for 36 h in 3D culture and treated with Wnt3a/EGF for 72 h (36-108 h). Cells were stained with anti-β-catenin and anti-phospho-ezrin antibodies. The number of extended structures from multicellular trunks were counted (n=30). Results are shown as the mean ± SE from three independent experiments. *, $P < 0.01$. Scale bars in (A), 20 μm (upper left five panels) and 50 μm (right two panels).