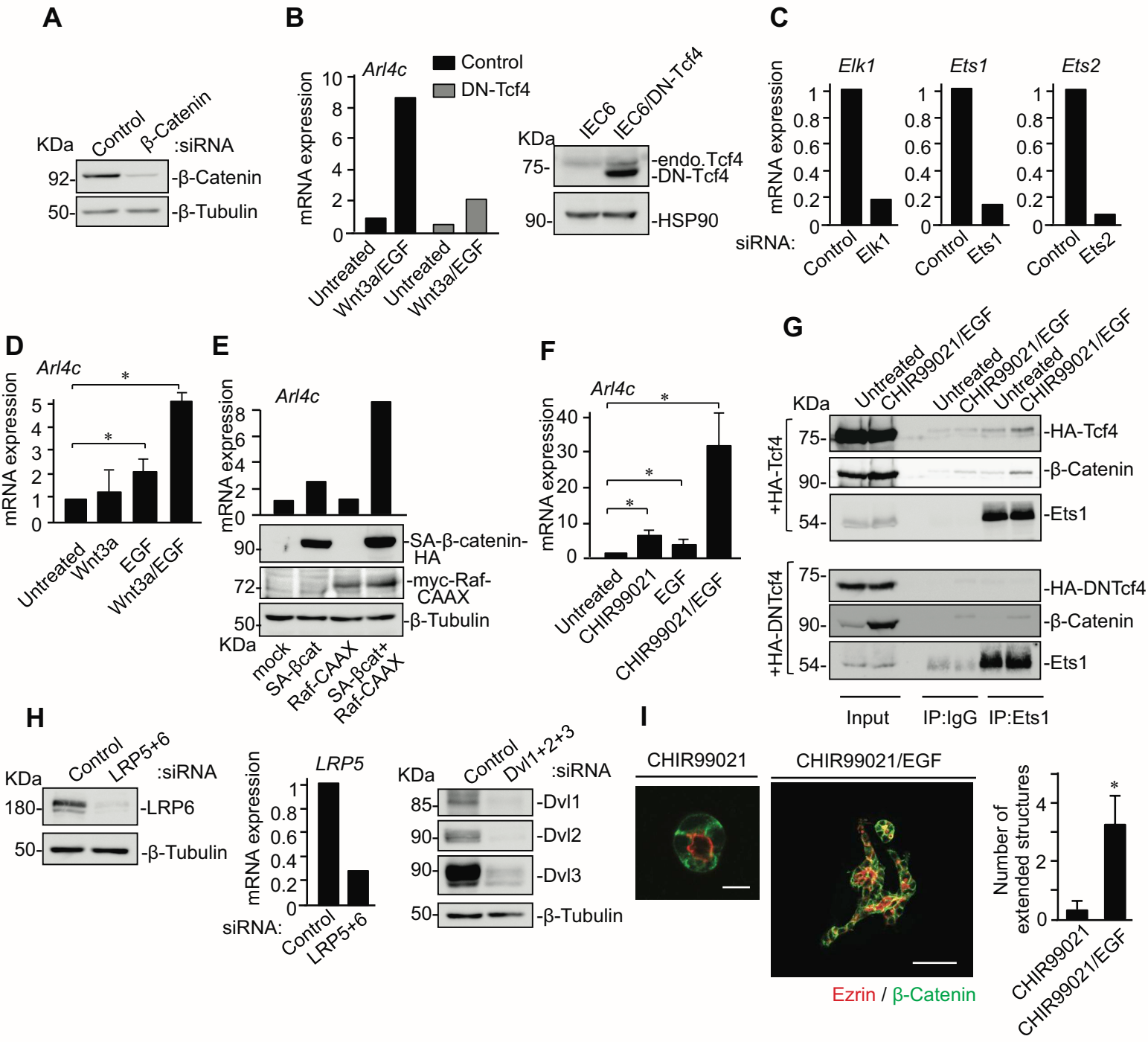
Matsumoto et al., Figure S5



Supplementary Figure S5 The β-catenin and MAP kinase pathways are involved in Wnt3a/EGF-dependent Arl4c expression and tube formation. (**A**) Lysates of IEC6 cells transfected with β-catenin siRNA were probed with anti-β-catenin and anti-β-tubulin antibodies. (**B**) IEC6 cells stably expressing DN-Tcf4 were stimulated with Wnt3a and Wnt3a/EGF for 8 h. Real-time PCR analyses for *Arl4c* mRNA expression were performed. Lysates of IEC6 cells and IEC6 cells stably expressing DN-Tcf4 were probed with anti-Tcf4 and anti-HSP90 antibodies. (**C**) IEC6 cells were transfected with indicated siRNAs and real-time PCR analyses for *Elk1*, *Ets1*, and *Ets2* mRNA expression were performed. (**D**) HeLaS3 cells were stimulated with Wnt3a, EGF, or Wnt3a/EGF for 8 h to measure *Arl4c* mRNA levels. (**E**) HeLaS3 cells were transiently transfected with indicated plasmids and cultured for 24 h to measure *Arl4c* mRNA levels. Lysates were probed with anti-HA, anti-myc, and anti-β-tubulin antibodies. (**F**) IEC6 cells were treated with or without CHIR99021, EGF, or CHIR99021/EGF for 6 h to measure *Arl4c* mRNA levels. (**G**) HeLaS3 cells expressing HA-Tcf4 or HA-DN-Tcf4 were treated with CHIR99021/EGF for 3 h. Lysates were immunoprecipitated with anti-Ets1 antibody and the immunoprecipitates were probed with the indicated antibodies. (**H**) Lysates of IEC6 cells transfected with the indicated siRNAs were probed with anti-LRP6, anti-Dvl1, anti-Dvl2, anti-Dvl3, and anti-β-tubulin antibodies. Real-time PCR analyses for *LRP5* mRNA expression were performed. (**I**) IEC6 cells were cultured with either CHIR99021 alone or CHIR99021/EGF for 60 h and stained with anti-β-catenin and anti-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 (**I**), 10 μm (left panel) and 50 μm (right panel).