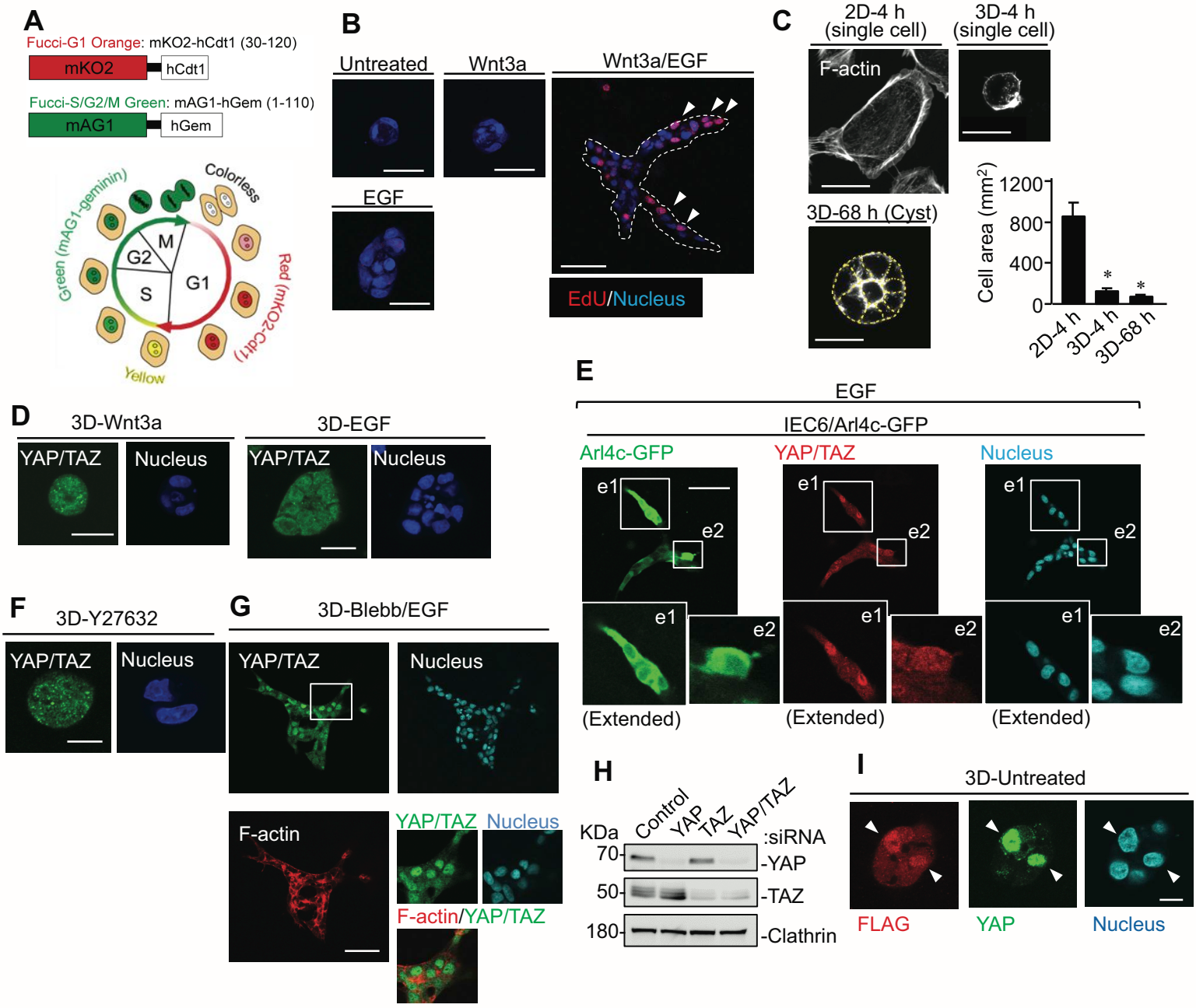
Matsumoto et al., Figure S8



Supplementary Figure S8 Cell growth is promoted in the extended region of tubular structures through nuclear localization of YAP/TAZ. (**A**) Schematic diagram of two-color cell cycle mapping using the Fucci constructs. Cells transfected with the Fucci construct showed cdt1 expression (red), a ubiquitin E3 ligase, in G1 phase, and geminin expression (green), another ubiquitin E3 ligase, in S/G2/M phase. (**B**) IEC6 cells were cultured with or without Wnt3a, EGF, or Wnt3a/EGF for 60 h in 3D culture and incubated with EdU for 20 min before fixation. The cells were stained with *propidium* iodide. White dashed lines indicate the outline of basal membranes of IEC6 tubes and white arrowheads indicate EdU-incorporated cells. (**C**) IEC6 cells were grown for 4 h in 2D culture or for 4 h or 68 h in 3D Matrigel culture and stained with phalloidin. Cell adhesive areas were measured (n=30). Yellow dashed lines indicate the outline of basolateral and apical membranes of IEC6 cysts. (**D**) IEC6 cells were treated with Wnt3a or EGF for 60 h in 3D culture and stained with anti-YAP/TAZ antibody, DRAQ5, and phalloidin. (**E**) IEC6 cells stably expressing Arl4c-GFP were cultured with EGF for 60 h in 3D culture and stained with anti-YAP/TAZ, anti-GFP antibodies and DRAQ5. White boxes show enlarged images. (**F, G**) IEC6 cells were treated with Y27632 (2 μM) (F) or blebbistatin (2 μM)/EGF (G) for 60 h in 3D culture and stained with anti-YAP/TAZ antibody, DRAQ5, and phalloidin. (**H**) IEC6 cells were transfected with YAP and/or TAZ siRNAs and lysates were probed with anti-YAP/TAZ and anti-Clathrin antibodies. (**I**) IEC6 cells expressing FLAG-YAP^{5SA} (IEC6/FLAG-YAP^{5SA}) grown for 3 h in 3D culture were stained with anti-FLAG and anti-YAP/TAZ antibodies and DRAQ5. White arrowheads indicate FLAG-YAP^{5SA} –expressing cells. Scale bars in (**B**), 20 μm (left three panels) and 50 μm (right panel); in (**C** and **D**), 20 μm; in (**E** and **G**), 50 μm; in (**F** and **I**), 10 μm.