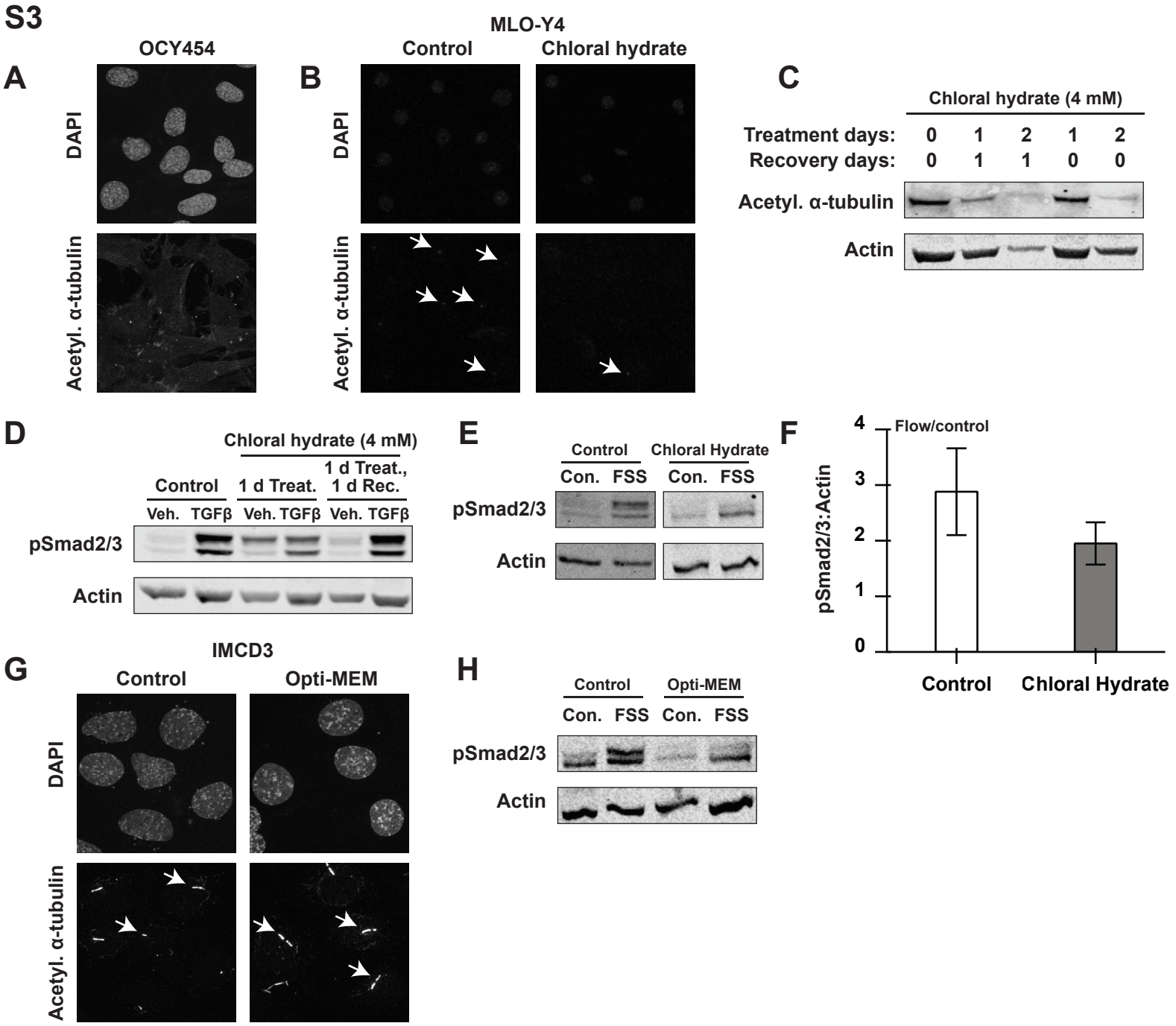

Supplemental Figure S3: Role of primary cilium length in regulating FSS activation of TGFβ signaling. (A) OCY454 cells appear to not possess clear primary cilia that are positive for acetylated-α-tubulin. (B) Most MLO-Y4 cells treated with 4 mM chloral hydrate for 24 h followed by 24 h recovery lose their primary cilia, shown using immunofluorescence for acetylated α-tubulin. (C) Treatment of MLO-Y4 with chloral hydrate for longer than 24 h has detrimental effects on cell viability, and lack of a recovery period after treatment reduces deciliation effectiveness. (D) A 24 h treatment, 24 h recovery regimen of chloral hydrate does not affect baseline TGFβ signaling nor TGFβ sensitivity in MLO-Y4 cells. (E, F) Stimulation of chloral hydrate-treated MLO-Y4 cells with FSS (0.1 Pa, 30 minutes) still induces Smad2/3 phosphorylation. Although FSS-induction of pSmad2/3 levels appears lower in chloral hydrate treated cells than in control cells, these differences are not statistically significant. (G) IMCD3 kidney epithelial cells natively possess long primary cilia that increase in length ollowing treatment with Opti-MEM reduced serum media (24 h). (H) IMCD3 cells with lengthened primary cilia still show FSS-inducible Smad2/3 phosphorylation, but may be less responsive to FSS stimulation than control cells.