Expanded View Figures

Figure EV1. Morphology distinguishes wound-associated epithelial cell and stem cell spheroids.

A Quantification of the average expression ± s.e.m. of Cldn4 mRNA in mouse jejunal spheroids cultured in stem cell or in differentiation medium with the indicated supplements relative to the DMSO group (n = 3 independent experiments). ****P < 0.0001 by one-way ANOVA and Tukey’s post-test.

B Representative bright-field images of stem or dmPGE2-treated spheroids. Stem spheroids have a smooth appearance, whereas dmPGE2-treated spheroids have a dimpled “golf ball-like” appearance. Scale bars, 200 μm.

Figure EV2. Expression of additional wound-associated epithelial cell and enterocyte markers in human ileal and mouse colonic spheroids.

A, B Human ileal spheroids were cultured in stem cell medium or in differentiation medium containing dmPGE2 or EP4i. Quantification of the average mRNA expression ± s.e.m. of the WAE cell markers DPCR1 and CD55 (A) and the enterocyte markers ACE2 and MAOA (B) relative to the stem group (n = 3 independent human lines). **P < 0.01, ***P < 0.001, ****P < 0.0001 by one-way ANOVA and Tukey's post-test.

C, D Mouse colon spheroids were cultured as indicated. Quantification of the average mRNA expression ± s.e.m. of the WAE marker Dpcr1 (C) and the colonocyte marker Car4 (D) relative to stem group (n = 3 independent experiments). *P < 0.05, **P < 0.01 as determined by one-way ANOVA and Tukey’s post-test.
Figure EV3. Limited presence of secretory cell populations in EP4i-treated spheroids.

A-C Mouse jejunal spheroids were cultured in stem cell medium or in differentiation medium containing dmpGPE2 or EP4i following assessment of intestinal secretory lineages. *P < 0.05, **P < 0.01 by one-way ANOVA and Tukey’s post-test. (A) Quantification of the average mRNA expression ± s.e.m. of the endocrine marker chromogranin A (ChgA), the goblet cell marker mucin 2 (Muc2), and the Paneth cell marker lysozyme (Lyz) relative to the stem group (n = 3 independent experiments). (B) Representative fluorescent images of histological sections of spheroids stained with ChgA (red; arrowheads) or co-stained for Muc2 (red; arrowheads) and Lyz (green; arrows) as indicated. Asterisks indicate cells that co-expressed Muc2 and Lyz. Nuclei are visualized with bisbenzimide (blue). Scale bars, 20 μm. (C) Quantification of the cells expressing the indicated protein markers as a percentage ± s.e.m. of the total nuclei counted per group (n = minimum of three images counted from each treatment group; two independent experiments).
Figure EV4. β-catenin localizes to the nucleus of wound-associated epithelial cells in vitro.
Representative histological sections of mouse jejunal spheroids cultured in stem, enterocyte (EP4i), or WAE (dmPGE2) media or in enterocyte medium containing 10 μM CHIR 99021 or an equivalent volume of DMSO were immunostained for β-catenin (brown). Nuclear localization of β-catenin was observed in the positive control samples (Stem, EP4i + CHIR 99021) and in the PGE2-treated spheroids, but not in the negative control samples (EP4i, EP4i + DMSO). Scale bars, 20 μm. Insets show a high magnification view of the boxed regions in the low magnification images.
Figure EV5. Nuclear β-catenin but not canonical Wnt signaling targets are present in wound-associated epithelial cells in vivo.

A, B Representative high power, bright-field images of control mice wound tissue sections immunostained for β-catenin (brown). Scale bars, 20 μm. (A) WAE cells covering wound bed. (B) Surface colonocytes from an uninjured area. Examples of cells with accumulation of nuclear β-catenin are indicated by arrows, whereas examples of cells with strong membrane staining but low nuclear β-catenin staining are indicated with arrowheads.

C Representative in situ hybridization result for Axin2 mRNA on day 6 post-biopsy showing accumulation of signal at the crypt bases where stem cells reside (arrows), but no signal in WAE cells (arrowheads). Scale bar, 50 μm.

D Representative whole-mount fluorescent image of colonic wound on day 6 post-biopsy. Endogenous GFP expression is driven by the Lgr5 promoter (this genetic mouse model has mosaic GFP expression). The wounded area is outlined in a dashed white line. Scale bar, 50 μm.

E Representative image of control mouse wound tissue section on day 4 post-biopsy stained for Ki67 (green). Nuclei are visualized with bisbenzimide (blue). The white dashed line separates the non-proliferative WAE cells from the highly proliferative cells of the wound bed. Scale bar, 50 μm.