SUPPLEMENTARY INFORMATION

Supplementary Methods

Reagents. Human colonic crypt culture media and supplements: Advanced F12/DMEM, B27 and N2 were all from Invitrogen. Small molecule inhibitors were: A83-01 (Tocris), DMH-1 (Tocris) and IWP2 (Sigma). Growth factor-reduced Matrigel was purchased from BD Bioscience. Human recombinant growth factors used for colonic crypt culture experiments were: IGF-1 (Sigma); R-spondin-1 (Sino Biological or R&D Systems); Wnt 3A (R&D systems), Gremlin-1 (R&D Systems); Noggin (Peprotech); TGFβ-1 (R&D systems), BMP4 (Peprotech), EGF (Sigma) and DKK-1 (R&D Systems).

Immunolabelling of native and cultured colonic crypts was performed using the following antibodies: mouse anti-β-catenin (BD Bioscience), mouse anti-dephospho-beta catenin (Upstate), goat anti-E-cadherin (R&D Systems), mouse anti-LGR5 (Origene), mouse anti-OLFM4 [1], rabbit anti-OLFM4 (Abcam), rabbit anti-Axin-2 (Abcam), anti-active-caspase 3 (Cell Signalling), mouse anti-Ki67 antibody (Dako), rat anti-BrdU (Abcam), anti-phospho-SMAD1,5,8 (Cell Signalling), anti-phospho-SMAD2,3 (Cell Signalling), rabbit anti-Wnt-3A (Abcam), goat anti-GFP (Abcam) and goat anti-FITC (Abcam).

Human colonic crypt isolation and culture. Colonic crypts were isolated as described previously [2 3]. Fresh mucosal tissue samples were collected in ice cold PBS, transported to the laboratory and incubated in Hepes-buffered saline (HBS): (mM) NaCl 140, KCl 5, Hepes (N-2-hydroxyethylpiperazine-N2-ethanesulphonic acid) 10, d-glucose 5.5, Na2HPO4 1, MgCl2 0.5, CaCl2 1, and placed in HBS, which was devoid of both Ca^{2+} and Mg^{2+}, and supplemented with EDTA (diaminoethanetetraacetic acid disodium salt) (1mM), for 1 h at room temperature. Crypts were liberated by serial rounds of vigorous shaking, crypt sedimentation and collection. Sedimented crypts were collected and mixed in Matrigel and a 20 μl droplet containing 50-100 crypts was placed onto no. 0 glass coverslips (VWR) contained within a 12 well plate. After polymerisation at 37^0C for 5-10 mins, crypts were flooded with 0.5 mls of human colonic crypt culture medium (hCCCM), a variant of that described recently for human intestinal stem cell/organoid culture[4 5]: advanced F12/DMEM containing B27, N2, n-acetylcysteine (1 mM), hepes (10 mM), penicillin/ streptomycin (100 U/ml), L-Glutamine (2mM), Wnt-3A (100 ng/ml), IGF-1 (50 ng/ml), Noggin (100 ng/ml) or Gremlin-1 (200 ng/ml), RSPO-1 (500 ng/ml), and the ALK 4/5/7 inhibitor A83-01-01 (0.5 μM). hCCCM was changed every two days and was modified further according to the stated experimental conditions. BrdU (10 μM) was added to the experimental crypt culture media as described to monitor crypt cell proliferation and migration. Propidium iodide (1 μg/ml) and calcein (5 μM) were added to the medium for visualisation of live/dead crypt cells.

Whole mount immunohistochemistry. Following embedding in Matrigel, microdissected-native crypts or cultured-crypts were fixed with 4% PFA for 1 hour and permeabilized with either SDS (1%) or Triton X-100 (0.5% w/v PBS, 30 min). Non-specific binding sites were blocked with 10% goat and/or donkey serum and 1% bovine serum albumin for 2 h and washed with PBS. Crypts were incubated with primary antibodies (1:100-200 dilution) overnight at 4^oC. Immmunolabelling was visualised by using an appropriate combination of species-specific Alexafluor-conjugated secondary antibodies (488, 568, and 647 nm) raised in donkey and/or goat (Invitrogen). Crypts were mounted on glass slides with Vectashield containing DAPI (Vector labs).

Dual whole mount mRNA *in situ* hybridisation and immunohistochemistry. High affinity ~20'mer locked nucleic acid (LNA), FAM-conjugated probes were used in combination with high stringent hybridisation and washing to label *LGR5* mRNA. The processing for *in situ* hybridisation was modified from that described previously [6]. Briefly, microdissected-native crypts embedded in Matrigel were fixed with 4% PFA and washed with PBS-T (0.1% Tween-20). Crypts were then treated with HCl-PBST (0.2 N) followed by Proteinase K (10 μg/ml, Sigma) and post-fixed with PFA (4%) for 10 mins. After a PBST wash, crypts were incubated in a mix of probe (40 nM), denatured salmon sperm (50 μl/ml, Sigma) and hybridisation buffer (formamide,50%; 1.3xSSC; CHAPS, 0.5%; Tween-20, 0.2%; EDTA, 5 mM; in DEPC H_20) for 14 hours at the hybridisation temperature specified for the probe (Exiqon). Crypts were treated with x5 SSC for 10 mins followed by 0.2% SSC for 1 hour. A 1 hour block with BBM (Roche) preceded addition of the primary antibodies: goat anti-FITC, mouse anti-OLFM4 and rabbit anti-E-cadherin (all at 1:100 dilution). The next day, secondary antibodies were added as described for the immunohistochemistry procedure. The custom LNA probe sequence used for *LGR5* was*:* /5'-FAM/ATGAGGAAGCAAAGGGAATTGAGC /3'-FAM/. Hybridisation specificity was confirmed by use of a positive control β-actin LNA-probe and a negative control scrambled LNA-probe (both Exiqon).

RNA isolation Freshly isolated or cultured crypts were pelleted and placed in RNA later (Ambion). RNA was extracted using the RNeasy mini kit (Qiagen). cDNA was generated using the Superscript (II) RT kit (Invitrogen).

RT-PCR was performed using the BioTAQ PCR kit (Bioline) with a G-Storm thermal cycler (GRI). After 30 cycles the PCR products were run on a 1% agarose gel visualised by ethidium bromide staining. The forward and reverse primers for Wnt3A were:

Gene	Forward Primer	Reverse Primer	Product (base pairs)
Wnt3A	ctgctcagctgcgccccttcttt	ttcagcggcctccccattcattcc	404

Measurement of gene expression by quantitative RT-PCR. qRTPCR was performed using KAPA Universal Probe qPCR kit (Primer Design) following manufacturer's instructions. YWHAZ was used to normalize expression. ΔcT values were generated for each sample and relative ΔΔcT values are presented. Primers are available on request.

Supplementary References

45. **Oue N,** Sentani K, Noguchi T, et al. Serum olfactomedin 4 (GW112, hGC-1) in combination with Reg IV is a highly sensitive biomarker for gastric cancer patients. *Int J Cancer* 2009;**125**:2383-92.
46. **Sweetman D,** Goljanek K, Rathjen T, et al. Specific requirements of MRFs for the expression of muscle specific microRNAs, miR-1, miR-206 and miR-133. *Dev Biol* 2008;**321**:491-9.

Supplementary Figure 1: Intestinal stem/progenitor cell status in native human colonic crypts. (A) Schematic representation of immunofluoresence images of human colonic crypt-base; crypt cell types were identified according to congruent expression of stem cell markers OLFM4 and LGR5, crypt cell (nuclear) morphology and comparison with dual immuno/*in situ* hybridisation fluorescence labelling of muc2/lgr5-mRNA, as shown in (B). (C) Double immunofluorescence labelling of OLFM4 and Ki-67 to assess the proliferative status of intestinal stem/progenitor cells, accompanying analysis is presented in the main Figure 1F. (D) Spatial correlation of mitogenic Wnt signals (i.e. nuclear beta catenin) and target gene expression (i.e. c-MYC) in the stem cell niche located at the human colonic crypt-base; images complement data presented in the main Figure 2. Scale bar = (A, D) 50 mm, (C) 20 mm; DIC – differential interference contrast; E-CAD – e-cadherin.

Supplementary Figure 2: **Dual fluorescence mRNA *in situ* hybridisation and immunolabelling along the native human colonic crypt-axis.** (A) example of congruent expression for lgr5-mRNA and OLFM4-protein by slender cells interspersed between goblet-like cells at the native human colonic crypt-base (*cf.* main Figure 1C and supplementary Figure 1B). (B) a corresponding negative control for fluorescence *in situ* hybridisation using a scrambled oligonucleotide probe in conjunction with OLFM4-immunolabelling; the crypt was obtained from the same sample as in (A) and the processing conditions and acquisition settings were identical. (D) positive control for fluorescence *in situ* hybridisation using an antisense oligonucleotide probe specific for beta actin-mRNA, note labelling along the entire crypt axis compared to lgr5-mRNA which is only located at the crypt-base (*cf.* main Figure 1C). DIC – differential interference contrast; E-CAD – e-cadherin.

Supplementary Figure 3: Immunolabelling of distinct cell types within native human colonic crypts. (A) The enterocyte marker FABP1 is predominantly expressed by columnar enterocytes located towards the top of the crypt. A monoclonal MUC-2 antibody marks perinuclear nascent protein in cells with a goblet-like morphology (A), while labelling with a MUC-2 polyclonal antibody is more prominent throughout the cytoplasm of goblet cells (B), which are interspersed with distinct, slender OLFM4-positive cells. (C) Relatively rare chromogranin A (CGA)-positive enteroendocrine cells intermingle between goblet-like cells and slender OLFM4-positive cells. (D) Recently characterised tuft cells are visualised by immunolabelling COX-1 and are typically elliptical with a nucleus indented from the basal pole. These immunolabelling patterns are reproduced in cultured human colonic crypts (see main Figure 6H). Scale bar = (A) 50 μm, (B) 30 μm.

Supplementary Figure 4: Comparative histology of native versus cultured human colonic crypts.

Fixed biopsy samples (A) and cultured human colonic crypts (day 4) (B) were processed for haematoxylin and eosin staining. Cultured colonic crypts exhibit similar topology, polarity and morphology to native colonic crypts, but are not surrounded by sub-epithelial mesenchymal cell types. (C) Microdissected (i.e. native) and (D) cultured human colonic crypts were processed for immunolabelling of smooth muscle actin, a marker of sub-epithelial myofibroblasts. Scale bar = 50 μm

Supplementary Figure 5: Inhibition of Wnt signals or activation of TGFβ/BMP signals compromises cultured human colonic crypt morphology

(A) The contribution of RSPO1/Wnt3A to the maintenance of crypt length (*cf.* main Figure 3C) is suppressed by the canonical Wnt pathway inhibitor DKK-1 (800 ng/ml). (B) BMP (100 ng/ml) treatment compromises crypt length and morphology and this is abrogated by noggin (100/ng/ml); similarly the adverse effects of TGF beta (20 ng/ml) are prevented by the ALK4/5/7 inhibitor, A83-01 (0.5 μM). Crypts were cultured for three days under the indicated conditions. In each experiment, crypts were derived from N≥2 subjects, with n ≥ 4 crypts in each experimental group. The control culture conditions were: (A) = ADF12/B27/N2/nAC/IGF1 (50 ng/ml)/Gremlin1 (200 ng/ml);
(B) = ADF12/B27/N2/nAC/IGF1 (50 ng/ml)/Wnt3A (100 ng/ml)/RSPO1 (500 ng/ml) /A83-01 (0.5 μM);
(C) = ADF12/B27/N2/nAC/IGF1 (50 ng/ml)/Wnt3A (100 ng/ml)/RSPO1 (500 ng/ml)/Noggin (100 ng/ml). Significant differences between pairs of mean values are indicated by linked blue lines ($p<0.05$); ADF12 – advanced DMEM/F12; nAC – n-acetylcysteine

Supplementary Figure 6: Effects of EGF on cultured human colonic crypt morphology

Replacement of IGF-1 with EGF (50 ng/ml) in the colonic crypt culture media induced re-modelling of human colonic crypt morphology into a typical budding organoid configuration over a 6-day culture period. D1, D3 and D6 indicate number of days in culture; dashed white lines indicate lumen of original crypt; dashed black lines indicate zoomed regions containing a crypt domain budding from the original isolated crypt; scale bar – 50 μm

Supplementary Figure 7: Wnt, TGFβ and BMP signalling gradients are maintained along the cultured human colonic crypt-axis.

Crypts were cultured for 4 days and processed for axin-2, p-SMAD2,3 or p-SMAD1,5,8 immunolabelling. Confocal microscopy using optimised acquisition settings for each label revealed an immunofluorescence gradient that predominated at the crypt-base for axin2 and at the crypt-top for p-SMAD1,5,8, while the labelling was highest in the mid-crypt region for p-SMAD2,3. These patterns recapitulated those observed in native crypts (*c.f.* Figure 2 in the main paper). Colonic crypt culture: Wnt 3A (100 ng/m), IGF1 (50 ng/ml), Noggin (100 ng/ml), R-spondin-1 (500 ng/ml), A83-01 (0.5 μM).

Supplementary Figure 8: Expression profile for several marker genes is similar for native and cultured human colonic crypts crypts.

RNA was extracted from freshly isolated human colonic crypts and from crypts cultured for 3 days. Expression of stem cell-, proliferation- and differentiation-markers was analysed by qRT-PCR. Data are presented as relative changes in gene expression between freshly isolated and colonic crypts cultured in the presence and absence of noggin. The presence of noggin maintains LGR5 and OLFM4 expression albeit at apparently reduced levels, while ID2 gene expression is downregulated. One

caveat to bear in mind when making this comparison is that mRNA levels in freshly isolated crypts may be subject to stress induced change during the isolation procedure. Colonic crypt culture: Wnt 3A (100 ng/m), IGF1 (50 ng/ml), Noggin (100 ng/ml), R-spondin-1 (500 ng/ml), A83-01 (0.5 μM).

Supplementary Table 1: Comparative characteristics/parameters of native versus cultured human colonic crypts

Characteristic/Parameter	**Native human colonic crypts**	**Cultured human colonic crypts**	**Data source**
% Stem/Progenitor cells at crypt-base (% proliferating SP cells)	50 (50)	40 (40)	Figs 1E and 6F (Figs 1F and 6G)
Crypt cell proliferation (% $Ki67^+$) Base-,Mid-,Upper-crypt	 55,45,20	 55;30;10	 Figs 1B and 6A, 7B, 7D
% stem:% goblet:% other cell types Crypt-base Mid-crypt Upper-crypt	 50:30:20 15:45:40 5:40:55	 40:30:30 15:30:55 5:30:65	 Figs 1E and 6D, 6F, 6I
Presence of enterocytes, enteroendocrine and tuft cells	YES	YES	Supplementary Fig. 3 and main Fig. 6H
Wnt signalling gradient (Nuclear beta catenin & nuclear axin2)	High-to-low from crypt-base	High-to-low from crypt-base	Figs 2A, 2B and Figs 4A-D
BMP/p-SMAD 1,5,8 gradient	High-to-low from crypt surface	High-to-low from crypt surface	Figs 2D and Supplementary Fig. 5
TGFβ/p-SMAD 2,3 profile	Higher in mid-crypt	Higher in mid-crypt	Figs 2C and Supplementary Fig. 7
Cell apoptosis/death/shedding Crypt base Crypt top/surface epithelium	 <0.5% (caspase 3^+) Cell shedding	 <0.5% (caspase $3^+/PI^+$) Cell Shedding	 Data not shown; Fig. 8D Data not shown; Fig. 8D

Supplementary Movie 1

3D reconstruction of confocal image stack of LGR5/OLFM4/E-CAD immunolabelling

Supplementary Movie 2

Timelapse movie of colonic crypt proliferation and migration (x20 objective)

Supplementary Movie 3

Timelapse movie of colonic crypt proliferation (x63 objective)

Supplementary Movie 4

Timelapse movie of detachment-induced cell death following shedding from the surface epithelium. Loss of cell viability was visualised by intense red bursts of fluorescence associated with propidium iodide binding to cell nuclei following rupture of cell membranes.