SUPPLEMENTARY MATERIALS AND METHODS

Intestinal crypt isolation

In order to isolate crypt units, 6-8 biopsies or 1-2 cm^2 surgical specimens from non-IBD controls, patients with UC (Patient Group 1, Supplementary Table 1A) or patients with CD (Patient Group 1, Supplementary Table 1B) were used. For UC and CD patients, only tissue samples with no or mild disease activity were used, since crypt isolation efficiency was considerably reduced in moderate and severely inflamed mucosa. Crypts were isolated from intestinal tissue as previously described[1]. For colectomy surgical samples, the muscle and sub-mucosa layers were carefully removed. The mucosa was cut into small pieces and washed for 20 min at room temperature (RT) in phosphate buffer solution (PBS, Gibco, Grand Island, NY) containing a mixture of antibiotics: Normocin (InvivoGen, San Diego, CA), Gentamycin (Lonza, Walkersville, MD) and Fungizone (Gibco). Next, tissue fragments were washed twice with 10 mM DTT (Sigma, Saint Louis, MO) in PBS for 5 min at RT. Samples were then transferred to 8 mM EDTA (Promega, Madison, WI) in PBS and incubated under rotation for 45 min at 4 ºC. The EDTA buffer was replaced by fresh cold PBS, and vigorous shaking of the sample yielded supernatants enriched in single crypts. Fetal bovine serum (FBS, Biosera, Nuaille, France) was added to a final concentration of 5% and fractions were centrifuged at 150 x *g* for 3 min. Crypts were then washed with Advanced DMEM/F12 (ADF) medium (Invitrogen. Carlsbad, CA) supplemented with 2 mM GlutaMax (Invitrogen), 10 mM HEPES (Sigma), and 5% FBS (Washing buffer: WB).

Biopsies were washed in PBS and incubated with the mixture of antibiotics as described above. Next, biopsies were transferred to 8 mM EDTA, 0.5 mM DTT in PBS and incubated for 45 min at 4 ºC under rotation. Supernatant was replaced by fresh PBS,

and single crypt units were released after vigorous shaking. FBS was added to a final concentration of 5% and fractions were centrifuged 200 x *g* for 3 min. An additional wash with WB was performed as for the surgical samples.

Epithelial organoid cultures

In order to obtain 3-D colonic epithelial organoid cultures (EpOCs) and small intestinal epithelial organoid cultures (SInt-EpOCs), 30-60 purified human crypts/well embedded in 25μL Matrigel were overlaid with 250 μL "stem medium" containing: Wnt3a-conditioned medium produced using an L-Wnt3a cell line (ATCC CRL-2647) and ADF 50:50, 2mM Glutamax, 10 mM HEPES, N-2 (1×) (Gibco), B-27 without retinoic acid (1×) (Gibco), 10 mM nicotinamide (Sigma), 1 mM N-Acetyl-L cysteine (Sigma), 500 ng/mL R-spondin-1 (RSPO1, Sino Biologicals, Beijing, China), 50 ng/mL human epidermal growth factor (EGF) (Invitrogen), 100 ng/mL human Noggin (Peprotech, Rocky Hill, NJ), 1 μg/mL Gastrin (Tocris Bioscience, Bristol, UK), 500 nM LY2157299 (Axon MedChem, Groningen, The Netherlands), 10 μM SB202190 (Sigma), and 0.01 μM prostaglandin E2 (PGE2; Sigma). Medium was replaced with fresh stem medium every other day. For passaging, Matrigel-embedded organoids were released using Cell Recovery Solution (BD Biosciences, San Jose, CA). After re-suspension in HEPES-buffered ADF medium containing GlutaMax and 5% FBS, single cells and debris were removed by centrifugation at 400 × *g* for 3 min. Organoids were then incubated in Disaggregation Medium (ADF, Glutamax, 10 mM HEPES, N-2 (1x), B-27 (1x) without retinoic acid, 10 mM nicotinamide, 1 mM N-Acetyl-L-cysteine, 10 μM Y-27632 (Calbiochem, San Diego, CA), 2.5 μM PGE2, 0.1-0.5 mg/mL Dispase (BD Biosciences) for 15 min at 37 °C in a water bath. Afterwards, the cell suspension was syringed using a 1.2 mm G20 needle. After re-plating at the usual 1:3 dilution in fresh Matrigel, the culture was overlaid with stem medium along with 10 μM Y-27632

for the first 2-3 days after passaging. Medium was changed every other day. On the fifth day the supernatants from EpOCs were harvested, centrifuged and stored at -20 ˚C until assayed. Matrigel-embedded organoids were washed with cold PBS and re-suspended in 500 µL Trizol (Ambion, Foster City, CA) for RNA extraction. To induce EpOC differentiation into differentiated EpOCs (d-EpOCs), nicotinamide, SB202190 and Wnt3a-conditioned medium were removed from the culture medium. RSPO1 was reduced to 250 ng/mL during the first 4 days and then completely removed. On the fifth day the supernatants from the d-EpOCs were harvested, centrifuged and stored at -20 ˚C until assayed. Matrigel-embedded organoids were washed with cold PBS and re-suspended in 500 µL Trizol for RNA extraction.

RNA isolation

Biopsies from non-IBD subjects and UC patients (Patient Group 2, Supplementary Table 1A) were placed in RNAlater RNA Stabilization Reagent (Qiagen) and stored at -80 °C until RNA extraction. Total RNA from biopsies, EpOCs/d-EpOCs and SInt-EpOCs/SInt-d-EpOCs was isolated using the RNeasy Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Total RNA purity and integrity were assessed with the 2100 Bioanalyzer (Agilent, Santa Clara, CA), and the concentration was determined using a NanoDrop spectrophotometer (Nanodrop Technologies, Wilmington, DE). Only samples with an RNA integrity number (RIN) greater than 7.0 were used.

Microarray analysis

RNA isolated from EpOCs and d-EpOCs generated after the first passage (Patient Group 1, Supplementary Table 1A) were used for the microarray analysis. Hybridization of samples was performed in a single batch to avoid data bias.

Microarray raw data was analyzed using Bioconductor tools in R (version 3.1.0). The data was normalized using the RMA method and probes were summarized using the revised entrez-based probe annotation of Dai (package hug219hsentrezgcdf, version 18.0.0). We then employed a probe-filtering step, excluding those probes that showed a lower coefficient of variation or which were non-informative (not annotated). Principal component analysis (PCA) of the given log2 microarray expression data matrix was carried out using basic tools in R (prcomp). Differential expression analysis was assessed using linear models for microarray data (LIMMA), based on empirical Bayes moderated t-statistics for all filtered probe sets. In addition, F-statistics were used to test for any change in expression over comparisons. To correct for multiple testing, the false discovery rate (FDR) was estimated from p-values derived from the moderated t-statistics using the method of Benjamini and Hochberg. Expression values were Z-transformed and empirical Bayesian statistics and fold-change were stored in an AFM-macro Excel file. Genes were considered significantly up- or down-regulated if they had a $P \leq .05$ and at least a 1.5-fold variation in mean expression ($|\log2\ fc| > 0.5849$).

Quantitative real-time RT-PCR (qPCR)

Total RNA (250 ng for EpOCs/d-EpOCs and SInt-EpOCs/SInt-d-EpOCs (Patient Group 1, Supplementary Table 1A; Patient Group 1, Supplementary Table 1B); 500 ng for biopsy samples (Group 2, Supplementary Table 1)), was transcribed to cDNA using reverse transcriptase (High Capacity cDNA Archive RT kit, Applied Biosystems, Carlsbad, CA). qPCR was performed in TaqMan Universal PCR Master Mix PCR (Applied Biosystems) according to the manufacturer's instructions. The following TaqMan probes (Applied Biosystems) were used: *LGR5*, *ASCL2*, *AXIN2*, *MKI67*, *MYC*, *ANPEP*, *CA1*, *MUC2*, *TFF3*, *APOA4*, *DEFA5*, *LYZ*, *CLCA4*, *MUC5B*, *CLDN18*, *ANXA10*, *HYAL1*, *SERPINB2*, *AQP8*, *ZG16*, *CLCA1*, *MUC12* and *PLA2G2A*. In order

to normalize the Ct values, the mRNA transcriptional levels were standardized to *ACTB* endogenous control gene. Fluorescence was detected using an ABI PRISM 7500 Fast RT-PCR System (Applied Biosystems).

External Datasets

In order to inspect the expression profile identified in UC versus Ctrl EpOCs and d-EpOCs in biopsy tissue samples, publicly available microarray data from the Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) was used. Two datasets were assessed: 1) GSE38713, including gene expression data from colonic biopsies of involved (n=15) and uninvolved (n=7) areas of patients with active UC, remission areas of inactive patients with quiescent UC (n=8) and non-IBD controls[2]; 2) GSE10867, including gene expression data from different human gastrointestinal (GI) tract regions: antrum (n=4), duodenum (n=3), jejunum (n=3), ileum (n=4) and transverse colon (n=4)[3]. The raw data (CEL files) was downloaded and analyzed using Bioconductor, as previously described.

Immunostaining

Biopsies and surgical samples from non-IBD controls and UC patients (Group 3, Supplementary Table 1A) were fixed for 2 or 24 hours in 10% neutral buffered formalin (NBF; Sigma), respectively, and paraffin embedded.

EpOCs and d-EpOCs (Group 1, Supplementary Table 1A) released from Matrigel using Cell Recovery Solution, or isolated crypt units were washed with 0.5% bovine serum albumin (BSA; Sigma) in PBS and fixed for 30 min in NBF. After a wash in 0.5% BSA in PBS, the released organoids were gently re-suspended in 1% agarose (Sigma) in PBS and placed in biopsy cryomolds. Additional NBF fixation of the agarose block was performed overnight at 4 °C followed by conventional paraffin embedding.

Paraffin-embedded sections (2µm) from colonic samples and organoid or crypt/agarose blocks were stained with H&E, or were used for immunostaining. For immunostaining, sections were pre-treated for de-paraffinization, rehydration, and epitope retrieval using Dako EnVision Flex Target Retrieval Solution in conjunction with PT Link (Dako, Carpinteria, CA). A warming step of 20 min at 95 ºC was used. Sections were blocked with 1% BSA for 30 min. Samples were incubated overnight at 4 ºC using the following commercially available antibodies: mouse anti-antigen KI-67 (1:100; Leica Biosystems, Wetzlar, Germany), rabbit anti-L-FABP (1:500; Sigma), rabbit anti-mucin-2 (1:250; Santa Cruz Biotechnology, Dallas, TX), rabbit anti-lysozyme C (1:4000; Dako), rabbit anti-claudin-18 (1:500; Sigma), rabbit anti-hyal-1 (1:100; Sigma), rabbit anti-hZG16 (1:500; Sigma), rabbit anti-hCLCA1 (1:500; Sigma), rabbit anti-GIIC sPLA2 (1:10000; Abcam, Cambridge, UK), mouse anti-Ep-CAM (1:100; Dako), rabbit anti-E-Cadherin (1:100; Cell Signaling, Danvers, MA), mouse anti-vimentin (1:100; Leica Biosystems), rabbit anti-CD3 (1:200; Vector Laboratories, Burlingame, AL) and mouse anti-CD45 (1:50; BD Pharmingen, San Jose, CA).

For immunohistochemical staining, signal detection was determined using the immunoperoxidase detection system (Vector Laboratories). In dual immunofluorescent staining, goat anti-rabbit Alexa 488 (Jackson Immunoresearch, West Grove, PA) and goat anti-mouse Cy3 (Jackson Immunoresearch) were used as secondary antibodies. Sections were then mounted with Vectashield Mounting Medium with DAPI (Vector Laboratories). Image acquisition was performed on an Olympus (Tokio, Japan) BX51 microscope using CellF Software. Negative controls were processed under the same conditions in the absence of the corresponding primary antibodies.

RNA chromogenic *in situ* hybridization

The RNAscope 2.5 assay was performed according to the supplier's instructions (Advanced Cell Diagnostics, Hayward, CA). Briefly, 5 μm paraffin-embedded sections from EpOCs and d-EpOCs (Group 1, Supplementary Table 1A) were de-paraffinized through 100% xylene and ethanol washes. Tissues were then treated serially with: RNAscope Hydrogen Peroxide solution for 10 min at RT; RNAscope Target Retrieval Reagents solution for 15 min at 98-102 ºC; RNAscope Protease for 30 min at 40 ºC in the HybEZ Oven (Advanced Cell Diagnostics). Tissues were then hybridized with *LGR5* mRNA probe (Advanced Cell Diagnostics) at 40 ºC for 2 h in the HybEZ Oven. An Hs-PPIB probe as a positive control and a DapB probe as a negative control were included. After rinsing with wash buffer, amplification of the hybridized probe signal was obtained by the serial application of Amp 1, Amp 2, Amp 3, Amp 4, Amp 5 and Amp 6. Rinses with wash buffer were performed after each Amp step. HRP activity was then demonstrated by the application of DAB chromogen for 10 min. Sections were then counterstained with hematoxylin (50% in distilled water), dehydrated through graded ethanol steps (70% and 95%) and xylene, and then mounted with DPX Mountant for histology (Fluka). The *LGR5* mRNA signal was evaluated by the presence of punctuate dots.

Enzyme-linked immunosorbent assay (ELISA)

Supernatants from EpOCs and d-EpOCs (Group 1, Supplementary Table 1A) were used for the detection of soluble proteins using commercially available ELISA. The following proteins were analyzed: lysozyme C (Abcam, Cambridge, UK) and hZG16 (BioVendor, Brno, Czech Republic).

Lysozyme activity quantification

Lysozyme activity in the supernatants from EpOCs and d-EpOCs (Group 1, Supplementary Table 1A) was measured using the EnzChek Lysozyme Assay Kit (Molecular Probes, Eugene, OR) following the manufacturer's instructions.

SUPPLEMENTARY REFERENCES

1. Jung P, Sato T, Merlos-Suarez A et al. Isolation and in vitro expansion of human colonic stem cells. Nat Med 2011; 17: 1225-1227.

2. Planell N, Lozano JJ, Mora-Buch R et al. Transcriptional analysis of the intestinal mucosa of patients with ulcerative colitis in remission reveals lasting epithelial cell alterations. Gut 2013; 62: 967-976.

3. Comelli EM, Lariani S, Zwahlen MC et al. Biomarkers of human gastrointestinal tract regions. Mamm Genome 2009; 20: 516-527.