Supplementary Materials and Methods

Passaging of mucosoid cultures

After 13 days cells could be passaged. Top and bottom of inserts were washed twice with PBS, followed by 30-60 min incubation with 0.05% trypsin/EDTA (Thermo Scientific 25300) on both sides of the filter. Cells were harvested, washed, and reseeded at 200,000 cells per new filter.

Antibacterial activity of mucus

Mucosoid cultures (+W+ R) were either mock infected or infected at MOI 100 with *H. pylori* P12. After 3 days, the mucus layer was collected in microcentrifuge tubes. In parallel, *H. pylori* P12-GFP (kan^R) were grown on GC agar plates with vancomycin and kanamycin. Bacteria were collected and washed once with PBS, and a suspension containing $\sim 2\times10^7$ CFU/ml was prepared in PBS. Aliquots of 5 µl ($\sim 10^5$ CFU) were mixed with 20 µl of fresh mucus collected from ALI cultures. After incubation at 37°C, surviving bacteria were enumerated following culture on GC agar plates with vancomycin. To kill wild type bacteria used for cell infection, GC plates also contained kanamycin. Results are expressed as the percentage of bacteria surviving from the initial input.

Transmission electron microscopy

For fine structural analysis, mucosoid cultures were fixed in 2.5% glutaraldehyde and postfixed with 0.5% osmium-tetroxide, contrasted with uranyl-acetate and tannic acid, dehydrated in a graded ethanol series, and infiltrated in Polybed (Polysciences). Excised pieces of filter were stacked in flat embedding molds with Polybed. After polymerization, specimens were cut at 60 nm and contrasted with lead citrate. Specimens were analyzed in a Leo 906E transmission electron microscope (Zeiss, Oberkochen, DE) equipped with a sidemounted digital camera (Morada, SIS-Olympus, Münster, DE).

Test of WNT3A and RSPO1 conditioned media

The amount of WNT3A and RSPO1 conditioned media added to culture medium was 50% and 25%, respectively. WNT3A and RSPO conditioned supernatant was obtained from the producing cell lines LWNT3a and 293T HA Rspo1 Fc 3/3, respectively. To standardize the amount of WNT3A and RSPO1 used both conditioned supernatants were added for 24 h to 239T test cells transfected with a 7TCF/LEF promoter-binding site driving the expression of GFP and seeded on a poly-L-lysine coated 48-well plate. Cells were then fixed for 20 min with PFA 4% and stained with Hoechst. The number of green cells divided by the number of nuclei representing the "activated cells" was determined automatically from images acquired with an automated microscope (Olympus Soft Imaging Solutions).

Different lots of WNT3a conditioned media were used only if 20% to 25% of the conditioned medium diluted in DMEM activated 50% of the test cells. Similarly, lots of RSPO1 were used only if 5% to 10% of 293T HA Respo1 Fc 3/3 conditioned medium activated 50% of the test cells (5% of LWNT3A conditioned medium was used as a co-activator in the RSPO1 test).

Isolation and culture of gastric stromal cells of the *lamina propria* (GSCs)

A 2x2 cm piece of human gastric antrum was excised directly adjacent to the one used for isolating epithelial glands. The mucosa was placed with the glands facing up and the *lamina propria* gently scraped off with a scalpel, without disturbing the *muscularis mucosa*. The scraped off cells containing epithelium and stroma were incubated in 2.6 mM DDT and 50 mM EDTA in PBS (GIBCO, without calcium and magnesium) for 20 min at 37 °C in a shaking incubator, centrifuged, supernatant removed and cells incubated in 0.05% trypsin for 20 min at 37 °C. Trypsin was inactivated with 10x the volume of ADF/10% fetal calf serum (Biochrom S0115) and cell aggregates left to settle by gravity. The supernatant was centrifuged and resuspended in 4 ml ADF/10% FCS/7.5 µM Y-27632 in one well of a 6-well plate for one week

until colonies of fibroblastic GSCs appeared. GSCs can be propagated indefinitely in ADF/10% FCS by passaging onto fresh plates once cells reach confluence, using digestion with 0.05% trypsin as before.

WNT activation reporter assay.

239T cells were transfected with a vector containing 7 TCF/LEF binding sites driving the expression of GFP. Cells were seeded on a poly-L-lysine-coated 48-well plate and exposed to different media to test for the presence of WNT activators or inhibitors derived from the gastric stromal cells. The percentage of GFP positive cells was normalized against the total number of nuclei (Olympus Soft Imaging Solutions).

DNA, RNA Isolation and qPCR Analysis

Filters were excised from the insert and directly transferred to a tube containing 1 ml Trizol (Thermo). After vortexing, samples were incubated for 10 min at RT, vortexed again and frozen at -80 ºC for long-term storage. 500 µl chloroform was added to thawed samples before vortexing, incubation for 2 min at RT and centrifugation for 15min at 4 ^{0}C. The aqueous phase was mixed with isopropanol, inverted 6 x and incubated for 10 min at RT. The sample was transferred to a Total RNA isolation Kit column (Thermo) and processed according to the manufacturer's instructions. Total RNA was measured using a NanoDrop and reverse transcription carried out using the Tetro cDNA synthesis Kit (Bioline). DNA was extracted using AllPrep DNA/RNA Mini Kit (Qiagen) according to the manufacturer's instructions; Total DNA was measured using a NanoDrop. qPCR (Step One, Applied Biosystem) was performed using the SensiMix™ SYBR® hi-ROX Kit (Bioline, 3-step cycling according to the manufacturer). Primers are listed in Supplementary Table 5.

Histology

Filters were fixed overnight in 4% paraformaldehyde at 4 °C, washed, embedded orthogonally in Histogel (HG-4000-144) inside a casting mould and paraffinized overnight in a Leica TP1020 tissue processor. The paraffin blocks are generated inside a casting mould on a Paraffin console (Microm). 5 µm sections are cut with a paraffin rotation microtome (Microm). For de-waxing and antigen retrieval, sample slides were washed twice with xylene (10 min) followed by a descending series of alcohols (20 sec each), followed by two washes with water and 30 min in target retrieval solution (Dako) at 95 °C, 20 min at room temperature (RT) and 5 min under running water. For whole mount samples, the filters were fixed for 20 min in 4% PFA at 37 °C and washed with PBS. A 10 min cold (-20 °C) methanol shock was use to permeabilize samples. Rehydrated samples (whole mount or sections) were washed twice with PBS and incubated with blocking solution (PBS, 1% bovine serum albumin, 2% FCS) for 1 h followed by primary antibody (in blocking solution) for 90 min at RT. After 3 washes with PBS, samples were incubated with fluorescently labeled secondary antibodies and Draq5 (1:1000; Cell Signaling) for 90 min in the dark at RT. The antibodies are listed in Supplementary Table 4. Samples were washed three times with PBS, mounted in Mowiol and analyzed by confocal microscopy using a Leica TCS SP-8 microscope. Images were processed, analyzed with FIJI and imported into Adobe Illustrator.

Protein Lysates and Immunoblot Analysis

100 µl 2x Laemmli buffer (4% SDS, 20% glycerol, 120 mM Tris-HCl (pH 6.8) and 0.02% bromphenol blue) was added to the filters. Cells were gently scraped with a pipette tip and the lysate transferred to a tube. After addition of 5 µl β-mercapto-ethanol, samples were boiled for 5 min at 95 °C, separated on a 8% SDS-polyacrylamide gel and transferred to a polyvinylidene difluoride membrane by Western blotting. Membranes were blocked with TBS buffer containing 0.1% Tween-20 and 3% BSA for 2 h and incubated with primary antibodies

overnight at 4°C, followed by HRP-conjugated secondary antibodies for 2 h. Membranes were covered with Hyperfilm ECL (Amersham) and signals detected with X-ray films. Antibodies are listed in Supplementary Table 4.

Microarray Analysis

Microarrays were hybridized for mucosoid cultures derived from 3 different patients cultured in +W+R or -W-R medium for 5 days followed by infection for three days in the same condition. Filters with mucosoid cultures were dissolved in 1 ml Trizol (Life Technologies) and RNA isolated as per the manufacturer's protocol. Quantity of RNA was measured using NanoDrop 1000 UV-Vis spectrophotometer (Kisker) and quality was assessed by Agilent 2100 Bioanalyzer with an RNA Nano 6000 microfluidics kit (Agilent Technologies). Microarray experiments were performed as single-color hybridizations on custom whole genome human 8x60k Agilent arrays (Design ID 048908) according to the manufacturer's instructions and Agilent Feature Extraction software used to obtain probe intensities. The extracted single-color raw data files were background corrected, quantile normalized and further analyzed for differential gene expression using R [1] and the associated BioConductor package LIMMA [2]. Microarray gene expression comparisons between groups were performed using paired test between conditions. Microarray data have been deposited in the Gene Expression Omnibus (GEO; www.ncbi.nlm.nih.gov/geo/) of the National Center for Biotechnology Information and can be accessed with the GEO accession number GSE94032.

Mass spectrometry sample preparation

The mucus samples were prepared according to the FASP method [3] and following the modification published by Rodriguez-Pineiro et al., (2013) [4]. Samples were diluted with 200 µl 6 M guanidinium hydrochloride in 0.1 M Tris/HCl pH 8.5 (GuHCl) according to a previously published protocol and transferred into MRCF0R030 Microcon-30 kDa centrifugal filters. Cysteines were reduced by adding 100 µl 0.1 M dithiothreitol (Sigma-Aldrich D0632) at 60 °C

for 15 min and alkylated with 100 µl 0.05 M iodoacetamide (Sigma-Aldrich I6125) at RT for 20 min in the dark. After washing two times with 100 µl GuHCl followed by two times 100 µl 50 mM ammonium bicarbonate/5% acetonitrile the proteins were digested with 0.2 µg sequencing-grade modified trypsin (Promega V5111) in 40 µl overnight at 37 °C. After digestion, peptide mixtures were acidified with TFA to 0.5% (vol/vol), desalted using ZipTip C18 (Millipore, 0.6 µl bed volume) and then lyophilized

LC-MS/MS Analysis

The peptides were analyzed using a QExactive Plus mass spectrometer (Thermo Fisher Scientific) coupled online to a Dionex UltiMate 3000 RSLC nano system (Thermo Fisher Scientific). After solubilization in 13 µl 2:98 (v/v) acetonitrile/water containing 0.1% TFA, 10 µl of each sample was loaded on a C18 PepMap 100 trap column (300 µm x 5mm; 5 µm particle size 100 Å pore size; Thermo Fisher Scientific) at a flow rate of 20 µl/min 2:98 (v/v) acetonitrile/water containing 0.1% TFA for pre-concentration and desalting. Separation was performed using an Acclaim C18 PepMap RSLC column (75µm x 250 mm; 2 µm particle size 100 Å pore size; Thermo Fisher Scientific) at a flow rate of 300 nl/min. HPLC solvent A was 0.1% (v/v) FA and peptides were eluted from the column using HPLC solvent B 80:20 (v/v) acetonitrile/water containing 0.1% FA starting from 3%, increasing to 40 % in 45 minutes, and to 98% in 5 minutes. The peptides were analyzed in data-dependent acquisition mode that alternated between one MS scan and 10 MS/MS scans for the most abundant precursor ions. MS scans were acquired over a mass range of m/z 350–1600 and resolution was set to 70,000. Peptides were fragmented using HCD at 27 % normalized collision energy and measured in the orbitrap at a resolution of 17500.

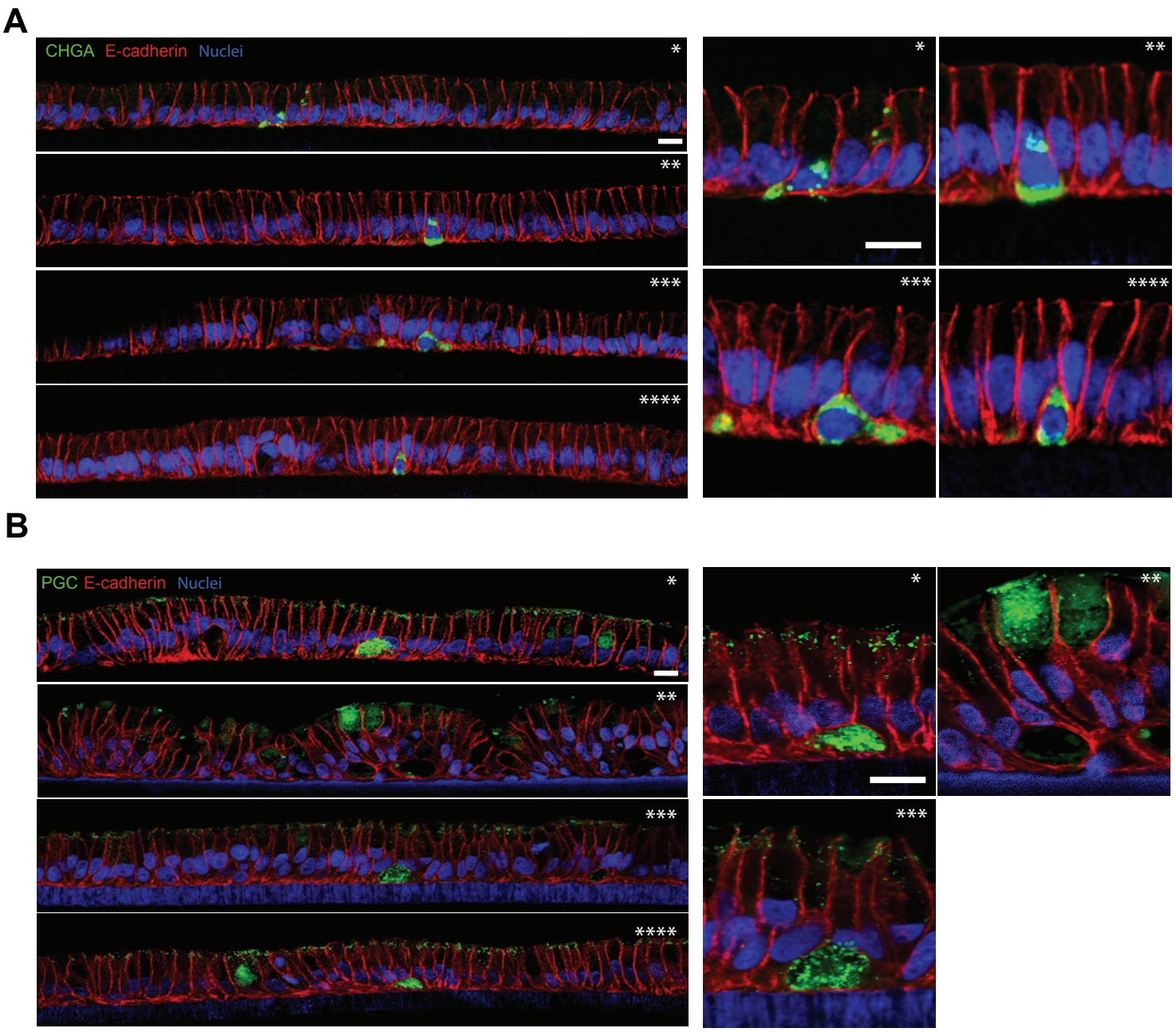
Protein identification

Proteins were identified and quantified using the MaxQuant software (Version 1.601) [5] [6] searching against the SwissProt human sequence database (released July 11, 2017, 20214

entries). Searches were performed using the following parameters: max. missed cleavages 2; variable modifications Oxidation (M); Acetyl (Protein N-term); pyro-Glu (Gln) and carbamidomethylation of cysteines as fixed modification. The false discovery rate was set to 0.01 for proteins, peptides and modified sites.

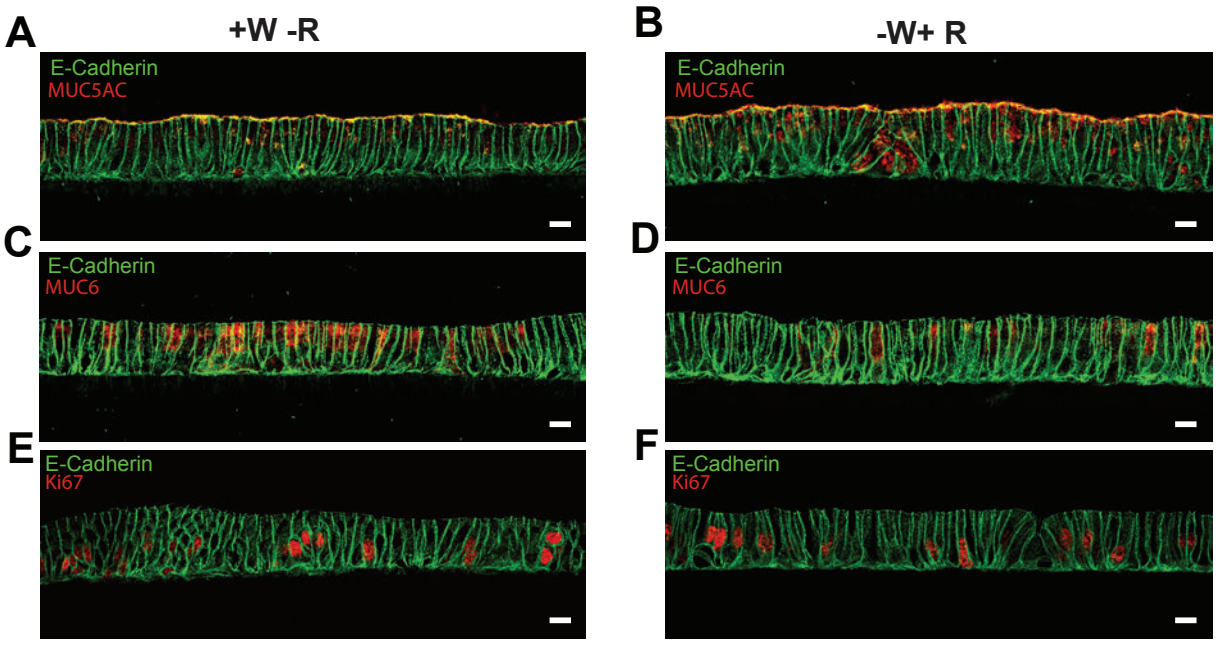
Supplementary References

1. R Development Core Team. R: a language and environment for statistical computing. URL: http://www.gbif.org/resource/81287
2. Ritchie, M.E., B. Phipson, D. Wu, Y. Hu, C.W. Law, W. Shi, and G.K. Smyth (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res *43*, e47.
3. Wisniewski, J.R., A. Zougman, N. Nagaraj, and M. Mann (2009). Universal sample preparation method for proteome analysis. Nat Methods *6*, 359-62.
4. Rodriguez-Pineiro, A.M., J.H. Bergstrom, A. Ermund, J.K. Gustafsson, A. Schutte, M.E. Johansson, and G.C. Hansson (2013). Studies of mucus in mouse stomach, small intestine, and colon. II. Gastrointestinal mucus proteome reveals Muc2 and Muc5ac accompanied by a set of core proteins. Am J Physiol Gastrointest Liver Physiol *305*, G348-56.
5. Cox, J. and M. Mann (2008). MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. Nat Biotechnol *26*, 1367-72.
6. Tyanova, S., T. Temu, and J. Cox (2016). The MaxQuant computational platform for mass spectrometry-based shotgun proteomics. Nat Protoc *11*, 2301-2319.



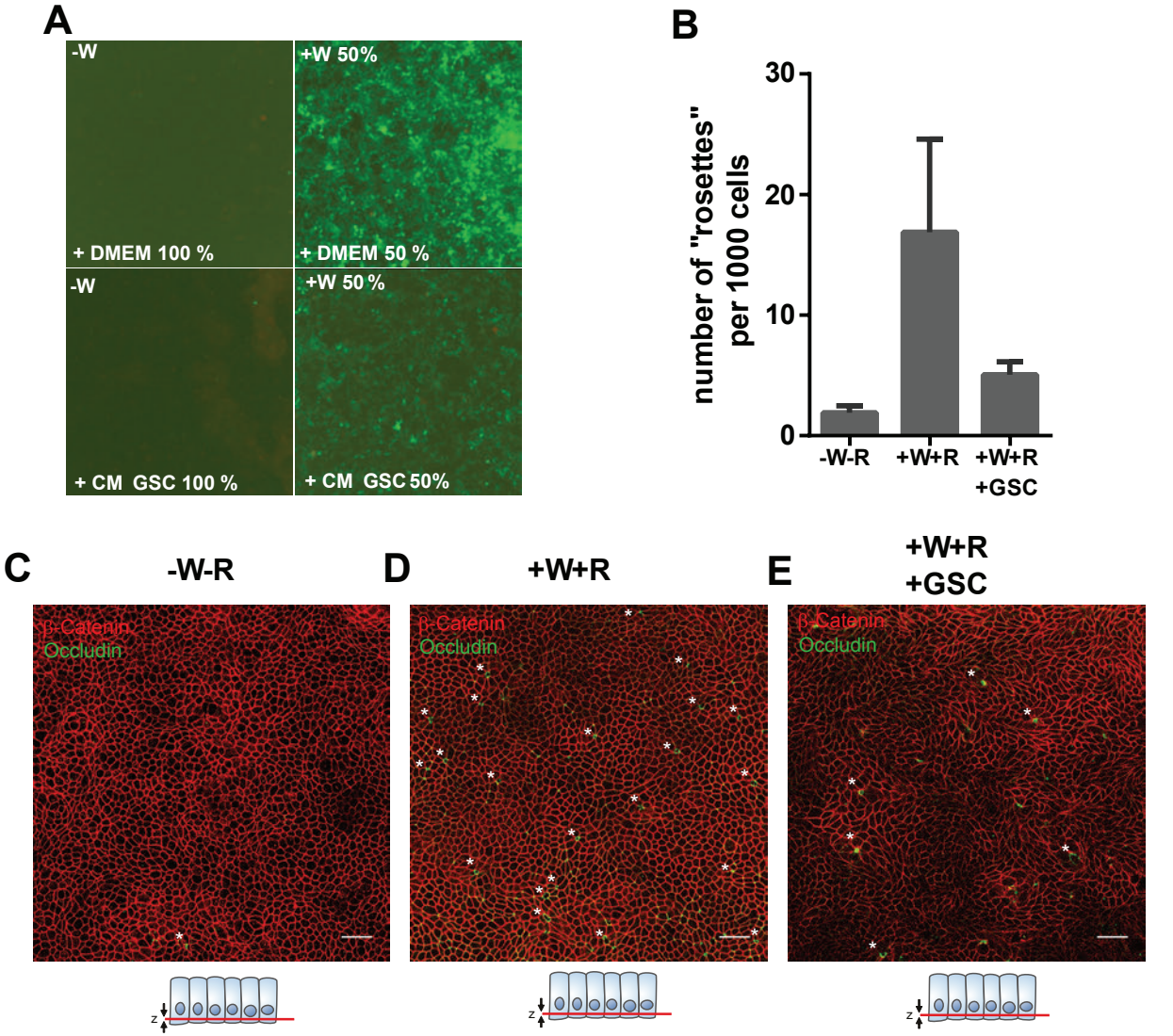
Supplementary Figure 1

(A) Multiple IF labelling against chromogranin A showing positive granules on the basal side of hormone-producing enteroendocrine cells of an antral mucosoid culture. (B) IF labelling against pepsinogen CII showing pepsinogen II-producing chief cells in an antrum-derived mucosoid culture. Scale bars: 10 µm. * indicates respective enlargements.



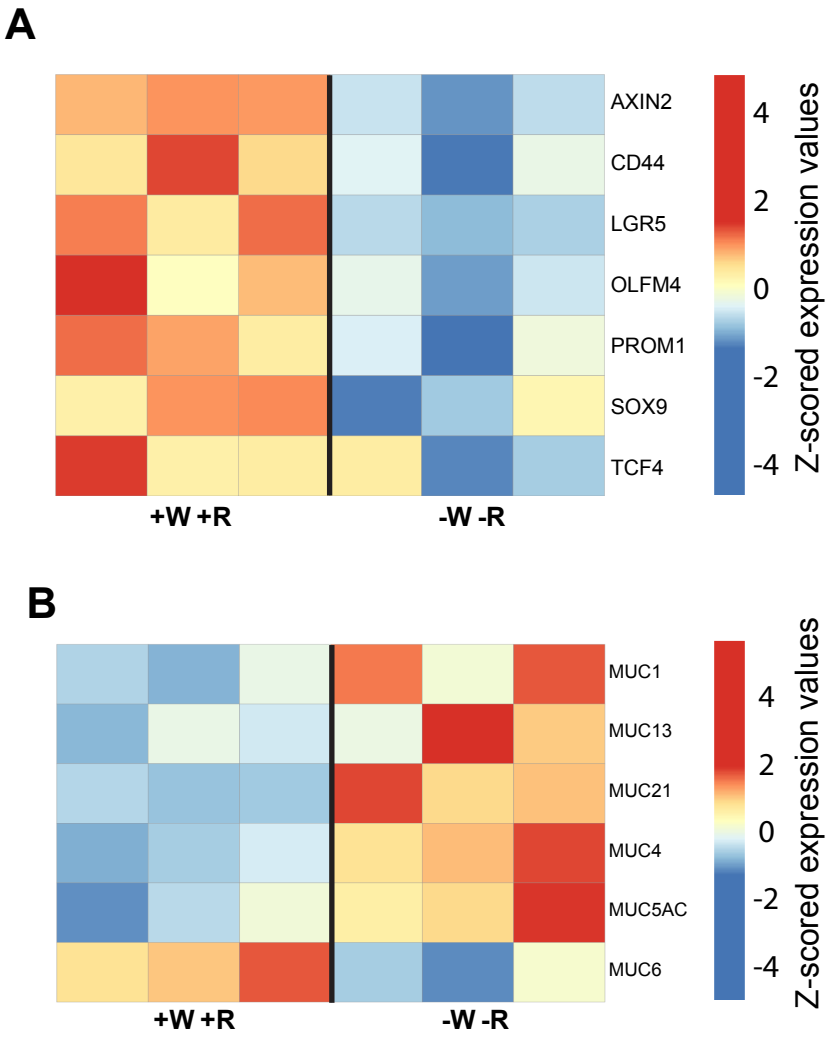
Supplementary Figure 2

Gastric mucosoid cultures cultured for 6 days in the absence of either RSPO3 or Wnt3A to assess their effect on differentiation via fluorescent labelling with antibodies against (A,B) MUC5AC (C,D) MUC6 or (E,F) Ki67.



Supplementary Figure 3

(A) 293T cells transfected with a 7xbinding site for TCF driving GFP expression were used to test Wnt pathway activation via activation of a TCF dependent promoter. Addition of 50% conditioned medium from Wnt3A producing LW3NTA cells strongly activate GFP signal while GSC-conditioned medium reduces it. (B-E) Mucosoid cultures were cultured in +W+R medium and (C) Wnt3A and RSPO1 discontinued for 6 days (D) never discontinued or (E) GSCs were co-cultured in the lower compartment for the same time. Immunofluorescence labelling with antibodies against occludin and β-catenin in whole mount preparations. Images show occludin labelling around which the cells are organized in rosette-like structures (see also Figure 2D). (B) Those structures were counted and normalized for the number of cells in three independent experiments. Scale bars: 30 µm



Supplementary Figure 4

(A) Heatmap of the sample described in Figure 6C showing the Z-scored expression values from the array of stem cell related genes (*LGR5*, *TCF4*, *OLFM4*, *SOX9*, *CD44*, *AXIN2*, *PROM1*) after removing Wnt3A and RSPO1 for 8 days in three independent experiments. (B) Expression of gel-forming and surface-associated mucins detectable in +W+R or -W-R samples.

Supplementary table 1

MaqQuant results of the most abundant proteins in the mucus

Protein IDs	MUC5A	LYSC	TRFE	MUC6	TFF2	FCGBP	TRY2	TRY1	NGAL	LG3BP	MUC5B	MUC1	A1AT	QSOX1	EZRI	RNAS1	TRFL	TFF1	TFF3	PROM1	GKN1
Score	323,3	323,3	323,3	323,3	323,3	323,3	323,3	323,3	323,3	323,3	323,3	323,3	323,3	323,3	323,3	323,3	323,3	323,3	223,6	323,3	263,9
LFQ intensity GAT24 +WR	1,55E+11	7,27E+10	2,92E+10	1,15E+11	2,90E+10	5,70E+10	3,48E+10	6,41E+10	1,35E+11	2,47E+10	7,20E+09	3,57E+10	2,15E+10	2,47E+10	1,84E+10	1,46E+10	2,81E+09	3,90E+08	2,44E+09	1,20E+10	0,00E+00
LFQ intensity GAT24 –WR	3,12E+11	7,22E+10	1,43E+11	2,17E+10	1,98E+10	1,15E+11	6,45E+10	7,28E+10	4,10E+10	4,31E+10	6,05E+09	3,09E+10	7,42E+09	7,33E+09	3,24E+10	6,51E+09	5,48E+08	5,46E+07	1,65E+09	3,84E+09	2,28E+09
LFQ intensity GAT26 +WR	3,64E+11	2,69E+11	1,53E+10	1,56E+11	9,74E+10	3,31E+10	3,89E+10	4,29E+10	1,95E+10	2,81E+10	2,41E+10	3,53E+09	2,60E+10	2,36E+10	2,59E+09	6,22E+09	2,84E+08	4,57E+09	9,75E+09	2,69E+09	0,00E+00
LFQ intensity GAT26 -WR	1,81E+12	9,12E+10	1,97E+10	1,00E+10	8,38E+10	3,46E+10	5,62E+10	3,78E+10	6,88E+09	1,02E+10	6,32E+09	2,28E+09	1,59E+09	6,92E+09	2,87E+09	2,41E+09	9,78E+07	1,59E+10	2,16E+09	3,76E+08	2,36E+09
LFQ intensity GAT27 +WR	5,14E+10	2,37E+11	2,38E+11	1,01E+11	7,51E+10	1,53E+10	1,60E+10	1,69E+10	3,42E+09	1,69E+10	1,87E+10	1,43E+09	4,22E+09	4,45E+09	1,28E+09	8,17E+09	4,14E+09	1,51E+08	9,84E+09	8,86E+08	0,00E+00
LFQ intensity GAT27 -WR	6,68E+11	5,43E+10	1,46E+11	1,59E+10	6,46E+10	3,99E+10	1,49E+10	1,58E+10	3,66E+09	1,18E+10	1,40E+10	3,77E+09	6,61E+08	3,78E+09	5,16E+09	1,72E+09	1,76E+08	5,32E+09	2,21E+09	5,41E+08	1,04E+09
LFQ intensity GAT28 +WR	9,03E+10	9,14E+10	2,49E+11	6,75E+10	1,18E+10	2,56E+09	1,88E+10	3,56E+09	6,20E+09	7,49E+09	2,87E+10	2,44E+08	5,69E+09	8,75E+08	1,43E+09	4,99E+09	2,70E+10	2,44E+07	3,20E+08	8,22E+08	0,00E+00
LFQ intensity GAT28 -WR	8,80E+11	1,47E+11	5,74E+10	4,16E+10	7,62E+10	2,67E+10	4,60E+10	2,87E+10	1,30E+10	9,91E+09	1,26E+10	2,21E+09	1,16E+10	5,70E+09	3,44E+09	3,95E+09	5,21E+09	6,48E+09	5,17E+08	4,96E+08	1,67E+08
LFQ AVERAGE intensity +WR	1,65E+11	1,68E+11	1,33E+11	1,10E+11	5,33E+10	2,70E+10	2,71E+10	3,19E+10	4,10E+10	1,93E+10	1,97E+10	1,02E+10	1,43E+10	1,34E+10	5,93E+09	8,48E+09	8,56E+09	1,28E+09	5,59E+09	4,11E+09	0,00E+00
LFQ AVERAGE intensity -WR	9,17E+11	9,12E+10	9,15E+10	2,23E+10	6,11E+10	5,41E+10	4,54E+10	3,88E+10	1,61E+10	1,87E+10	9,74E+09	9,78E+09	5,31E+09	5,93E+09	1,10E+10	3,65E+09	1,51E+09	6,93E+09	1,64E+09	1,31E+09	1,46E+09
LFQ AVERAGE intensity	5,41E+11	1,29E+11	1,12E+11	6,61E+10	5,72E+10	4,05E+10	3,62E+10	3,53E+10	2,86E+10	1,90E+10	1,47E+10	1,00E+10	9,83E+09	9,68E+09	8,45E+09	6,07E+09	5,04E+09	4,11E+09	3,61E+09	2,71E+09	7,31E+08
Log2 LFQ AVERAGE fold change +WR/-WR	-2,47	0,88	0,54	2,30	-0,20	-1,00	-0,74	-0,28	1,35	0,04	1,01	0,06	1,44	1,18	-0,89	1,22	2,50	-2,43	1,77	1,65	#NUM!

Protein IDs	MUC5A	LYSC	TRFE	MUC6	TFF2	FCGBP	TRY2	TRY1	NGAL	LG3BP	MUC5B	MUC1	A1AT	QSOX1	EZRI	RNAS1	TRFL	TFF1	TFF3	PROM1	GKN1
Razor + unique peptides GAT24 +WR	129	26	61	55	9	117	9	17	25	27	49	10	36	37	50	6	34	4	3	35	0
Razor + unique peptides GAT24 -WR	134	16	71	18	10	131	10	15	22	22	31	10	34	32	61	5	27	4	3	30	5
Razor + unique peptides GAT26 +WR	194	31	68	79	13	118	12	21	24	40	81	9	49	54	39	11	2	6	8	25	0
Razor + unique peptides GAT26 -WR	234	25	55	43	14	111	10	18	18	37	62	7	18	40	32	14	3	6	7	11	13
Razor + unique peptides GAT27 +WR	72	23	93	55	13	48	8	13	10	24	50	4	18	20	14	9	4	5	7	6	0
Razor + unique peptides GAT27 -WR	181	23	89	41	14	88	7	12	13	21	55	5	7	21	33	9	3	6	5	8	10
Razor + unique peptides GAT28 +WR	98	23	85	49	10	20	4	7	11	14	57	5	19	8	10	11	38	3	3	6	0
Razor + unique peptides GAT28 -WR	210	26	74	53	14	87	9	17	18	22	65	5	30	28	26	14	30	5	3	9	2
Unique peptides GAT24 +WR	122	26	61	55	9	117	6	14	25	27	49	10	36	37	36	6	34	4	3	35	0
Unique peptides GAT24 -WR	127	16	71	18	10	131	7	13	22	22	31	10	34	32	45	5	27	4	3	30	5
Unique peptides GAT26 +WR	187	31	68	79	13	118	8	17	24	40	81	9	49	54	30	11	2	6	8	25	0
Unique peptides GAT26 -WR	227	25	55	43	14	111	7	15	18	37	62	7	18	40	25	14	3	6	7	11	13
Unique peptides GAT27 +WR	69	23	93	55	13	48	5	11	10	24	50	4	18	20	9	9	4	5	7	6	0
Unique peptides GAT27 -WR	175	23	89	41	14	88	4	11	13	21	55	5	7	21	26	9	3	6	5	8	10
Unique peptides GAT28 +WR	93	23	85	49	10	20	2	6	11	14	57	5	19	8	8	11	38	3	3	6	0
Unique peptides GAT28 -WR	204	26	74	53	14	87	5	14	18	22	65	5	30	28	19	14	30	5	3	9	2
Sequence coverage GAT24 +WR [%]	33,3	84,5	68,1	27,4	69,8	44,8	49,8	66,8	81,8	49,4	12,4	8,9	60,8	48,3	62,5	59	53,8	57,1	38,8	42,1	0
Sequence coverage GAT24 -WR [%]	34,5	68,9	78,1	10,3	69,8	47,9	48,2	65,2	77,3	46,2	10,2	8,9	59,8	43,4	76,3	43,6	46,6	57,1	38,8	35,7	20,1
Sequence coverage GAT26 +WR [%]	38,6	87,2	77,7	29,8	69,8	41	49,8	60,3	81,8	59,7	18,9	8	73,2	56,1	58,2	71,8	3	57,1	53,8	32,6	0
Sequence coverage GAT26 -WR [%]	41,3	81,1	65,9	18,7	69,8	34,8	49,8	60,3	76,3	60,2	16,2	6,1	31,1	50,6	54,6	73,7	5,1	57,1	53,8	17,8	34,7
Sequence coverage GAT27 +WR [%]	23,5	81,1	83	22,6	69,8	16,3	37,7	51	49,5	49,9	14,8	2,9	37,1	31,6	26,6	70,5	6,6	57,1	53,8	9	0
Sequence coverage GAT27 -WR [%]	36,1	81,1	83	19,4	69,8	30,5	26,7	49,4	66,7	46,2	16,2	2,9	18,7	33,3	58,2	70,5	4,5	57,1	53,8	14,7	34,7
Sequence coverage GAT28 +WR [%]	25,3	84,5	83	20,9	64,3	6,4	25,1	42,9	57,6	37,4	15,4	2,9	36,8	10,8	18,8	70,5	55,6	54,8	45	10,2	0
Sequence coverage GAT28 -WR [%]	37,8	84,5	80,5	21,5	69,8	26,3	31,6	51	76,3	50,8	16,9	4,8	54,1	36,5	45,1	73,7	49,3	57,1	45	15,1	10,6
Intensity	4,74E+12	1,28E+12	7,20E+11	5,85E+11	5,21E+11	3,59E+11	3,46E+11	3,24E+11	2,49E+11	1,80E+11	1,17E+11	9,76E+10	9,99E+10	9,82E+10	7,30E+10	5,18E+10	2,73E+10	4,05E+10	3,66E+10	2,33E+10	6,46E+09
Intensity GAT24 +WR	1,63E+11	4,35E+11	1,53E+10	1,09E+11	2,83E+10	5,77E+10	4,83E+10	7,89E+10	1,49E+11	7,22E+10	5,95E+09	3,73E+10	2,48E+10	2,63E+10	1,74E+10	1,60E+10	2,97E+09	8,22E+08	1,96E+09	1,28E+10	0,00E+00
Intensity GAT24 -WR	4,43E+11	8,60E+10	1,32E+11	1,89E+10	3,85E+10	1,31E+11	9,39E+10	8,57E+10	4,61E+10	5,23E+10	3,48E+09	3,91E+10	8,29E+09	6,13E+09	4,03E+10	4,29E+09	7,91E+08	2,24E+08	1,22E+09	4,40E+09	2,79E+09
Intensity GAT26 +WR	6,61E+11	4,21E+11	1,93E+10	2,61E+11	1,79E+11	5,48E+10	1,05E+11	7,49E+10	2,98E+10	3,73E+10	4,68E+10	1,21E+10	4,96E+10	4,56E+10	4,85E+09	1,48E+10	7,23E+08	9,06E+09	2,17E+10	4,18E+09	0,00E+00
Intensity GAT26 -WR	1,97E+12	7,13E+10	1,74E+10	9,68E+09	9,20E+10	4,30E+10	4,17E+10	3,61E+10	5,97E+09	6,51E+09	7,36E+09	1,90E+09	1,41E+09	8,10E+09	2,66E+09	3,55E+09	1,06E+08	1,50E+10	2,58E+09	3,63E+08	2,55E+09
Intensity GAT27 +WR	2,11E+10	1,13E+11	1,83E+11	8,86E+10	4,48E+10	8,42E+09	1,37E+10	1,02E+10	1,96E+09	4,14E+09	1,32E+10	1,87E+09	2,27E+09	3,08E+09	5,74E+08	4,48E+09	3,83E+09	2,74E+08	6,63E+09	4,78E+08	0,00E+00
Intensity GAT27 -WR	5,59E+11	3,35E+10	1,34E+11	1,31E+10	5,73E+10	3,58E+10	1,27E+10	1,37E+10	2,42E+09	3,40E+09	1,30E+10	3,79E+09	3,44E+08	3,53E+09	3,93E+09	1,57E+09	2,26E+08	6,09E+09	2,08E+09	3,64E+08	1,08E+09
Intensity GAT28 +WR	3,44E+10	3,38E+10	1,57E+11	4,13E+10	5,96E+09	1,20E+09	4,79E+09	1,50E+09	1,12E+09	1,35E+09	1,58E+10	9,18E+07	3,06E+09	4,21E+08	3,90E+08	3,49E+09	1,45E+10	1,65E+07	1,22E+08	3,30E+08	0,00E+00
Intensity GAT28 -WR	8,87E+11	8,30E+10	6,07E+10	4,31E+10	7,50E+10	2,72E+10	2,62E+10	2,26E+10	1,18E+10	2,75E+09	1,18E+10	1,37E+09	1,02E+10	5,09E+09	2,80E+09	3,60E+09	4,18E+09	9,03E+09	3,23E+08	3,61E+08	4,43E+07

Supplementary Table 2

Patients

Code	Date of isolation	Age	Gender	Comments
GAT11	2014-11-12	31	Female	BMI: 68, *H.pylori* negative
GAT15	2015-05-06	47	Male	BMI: 52, *H.pylori* negative, diabetes
GAT16	2015-07-14	34	Male	BMI: 56, *H.pylori* negative
GAT18	2015-11-11	50	Female	BMI: 43, *H.pylori* negative
GAT19	2015-11-18	57	Male	BMI: 48, *H.pylori* negative, bladder cancer, no chemotherapy, no radiotherapy
GAT20	2015-01-15	45	Female	BMI: 45, *H.pylori* negative
GAT23	2016-04-15	55	Female	BMI: 45, *H.pylori* negative
GAT24	2016-09-30	47	Male	BMI: 36, *H.pylori* negative
GAT26	2017-02-17	69	Female	BMI: 50, *H.pylori* negative
GAT27	2017-05-10	36	Female	BMI: 69, *H.pylori* negative, type 2 diabetes
GAT28	2017-05-16	32	Male	BMI: 43, *H.pylori* negative
GAT29	2017-05-16	43	Female	BMI: 48, *H.pylori* negative

Supplementary Table 3

Medium Composition

Name	Concentration	Manufacturer	Code
ADF	18.45% V/V	Thermo Fischer	12634
conditioned Wnt3A-medium (as in Willert et al), 25%	50% V/V		
conditioned R-spondin1 medium	25% V/V		
4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid	10 mM	Thermo Fischer	15630-056
glutamax	1% V/V	Thermo Fischer	35050-087
B27	2% V/V	Thermo Fischer	17504044
N2	1% V/V	Thermo Fischer	17502048
human epidermal growth factor (EGF)	20 ng/ml	Thermo Fischer	PHG0311
human noggin (Peprotech)	150 ng/ml	Peprotech	120-10C-1000
human fibroblast growth factor (FGF)-10	150 ng /ml	Peprotech	100-26-1000
nicotinamide	10 mM	Sigma	N0636
human gastrin	10 nM	Sigma	G9145
A83–01	1 µM	Calbiochem	616454
Y-27632*	7.5 µM	Sigma	Y0503

*reduced to 1.5 µM after 3rd day

Supplementary Table 4

Histology

Antibody	Dilution	Manufacturer	Code
Occludin	1:200	Invitrogen	331500
Pepsinogen II	1:100	Abcam	ab9013
CagA-b300	1:200	SCBT	sc-25760
E-Cadherin	1:100	BD Bioscience	610181
Ki67	1:100	Cell Signaling	9027
MUC6	1:100	Abcam	ab49462
Cromogranin A	1:100	Abcam	ab15160
MUC5AC	1:100	Abcam	ab3649
β-Catenin	1:100	Sigma-Aldrich	C2206

Western Blot

Antibody	Dilution	Manufacturer	Code
CagA-b300	1:200	SCBT	sc-25760
pTyr PY99	1:500	SCBT	sc-7020
β-actin	1:5000	Sigma	A5441

Supplementary Table 5

Primers

CXCL1-3	Sequence (5' -> 3')
Forward Primer	CGCCCAAACCGAAGTCATAG
Reverse Primer	GCTCCCCTTGTTCAGTATCTTTT
CCL20	Sequence (5' -> 3')
Forward Primer	TGCTGTACCAAGAGTTTGCTC
Reverse Primer	CGCACACAGACAACTTTTTCTTT
LTB	Sequence (5' -> 3')
Forward Primer	GTACGGGCCTCTCTGGTACA
Reverse Primer	GTCCACCATATCGGGGTGAC
IL23A	Sequence (5' -> 3')
Forward Primer	CTCAGGGACAACAGTCAGTTC
Reverse Primer	ACAGGGCTATCAGGGAGCA
KRT19	Sequence (5' -> 3')
Forward Primer	GTCACAGCTGAGCATGAAAGC
Reverse Primer	AGCTGGGCTTCAATACCGC
KRT18	Sequence (5' -> 3')
Forward Primer	TTCTGGGGGCATGAGCTTCAC
Reverse Primer	GCGCCTGCATAGACGCTG
KRT8	Sequence (5' -> 3')
Forward Primer	GCTGGCCGTAAACTGCTTTG
Reverse Primer	ACATTTGGCAGCCAGCTTTG
EPCAM	Sequence (5' -> 3')
Forward Primer	GCTGGCCGTAAACTGCTTTG
Reverse Primer	ACATTTGGCAGCCAGCTTTG
CDH1	Sequence (5' -> 3')
Forward Primer	TACCCTGGTGGTTCAAGCTG
Reverse Primer	CCTGACCCTTGTACGTGGTG
LGR5	Sequence (5' -> 3')
Forward Primer	CTCCCAGGTCTGGTGTGTTG
Reverse Primer	GCTCGCAATGACAGTGTGTG
CTNNB	Sequence (5' -> 3')
Forward Primer	AGCAATTTGTGGAGGGGGTC
Reverse Primer	AGCAGCTGCACAAACAATGG
CD44	Sequence (5' -> 3')
Forward Primer	AGCACCATTTCAACCACACC
Reverse Primer	GCAGTGGTGCCATTTCTGTC
PGC	Sequence (5' -> 3')
Forward Primer	TGTCTTTGGGGGTGTGGATAG
Reverse Primer	ATGAGGAACTCTTCAATGCCAATC
MUC6	Sequence (5' -> 3')
Forward Primer	CAGCTCAACAAGGTGTGTGC
Reverse Primer	TGGGGAAAGGTCTCCTCGTA
MUC5AC	Sequence (5' -> 3')
Forward Primer	GGAGGTGCCCACTTCTCAAC
Reverse Primer	CTTCAGGCAGGTCTCGCTG
CHGA	Sequence (5' -> 3')
Forward Primer	CCAAGGAGAGGGCACATCAG
Reverse Primer	TCTTCCACCGCCTCTTTCAG
ATP4b	Sequence (5' -> 3')
Forward Primer	TGGGTGTGGATCAGCCTGTA
Reverse Primer	CTGGTCTTGGTAGTCCGGTG
IL-8	Sequence (5' -> 3')
Forward Primer	ACACTGCGCCAACACAGAAAT
Reverse Primer	ATTGCATCTGGCAACCCTACA
TNF	Sequence (5' -> 3')
Forward Primer	TCCCCAGGGACCTCTCTCTA
Reverse Primer	GAGGGTTTGCTACAACATGGG

sFRP1	Sequence (5' -> 3')
Forward Primer	ACGTGGGCTACAAGAAGATGG
Reverse Primer	CAGCGACACGGGTAGATGG
sFRP2	Sequence (5' -> 3')
Forward Primer 1	ACGTGGGCTACAAGAAGATGG
Reverse Primer 1	CAGCGACACGGGTAGATGG
sFRP2	Sequence (5' -> 3')
Forward Primer 2	CTGGCCCGACATGCTTGAG
Reverse Primer 2	GCTTCACATACCTTTGGAGCTT
sFRP3	Sequence (5' -> 3')
Forward Primer 2	ACACAGACTTACAGGGCTTGAT
Reverse Primer 2	GAGCCCATACTCATCAAGTACCG
sFRP4	Sequence (5' -> 3')
Forward Primer	CCTGGAACATCACGCGGAT
Reverse Primer	CGGCTTGATAGGGTCGTGC
sFRP5	Sequence (5' -> 3')
Forward Primer	AGGAGTACGACTACTATGGCTG
Reverse Primer	GGTCGGCAGGGATGTCAAG
DKK1	Sequence (5' -> 3')
Forward Primer	CCTTGAACTCGGTTCTCAATTCC
Reverse Primer	CAATGGTCTGGTACTTATTCCCG
DKK2	Sequence (5' -> 3')
Forward Primer	CTCACAGATCGGCAGTTCG
Reverse Primer	ATGCCAGTCCTTGGTACATGC
DKK3	Sequence (5' -> 3')
Forward Primer 1	AGGACACGCAGCACAAATTG
Reverse Primer 1	CCAGTCTGGTTGTTGGTTATCTT
DKK3	Sequence (5' -> 3')
Forward Primer 2	ACGAGTGCATCATCGACGAG
Reverse Primer 2	GCAGTCCCTCTGGTTGTCAC
DKK4	Sequence (5' -> 3')
Forward Primer	ACGGACTGCAATACCAGAAAG
Reverse Primer	CGTTCACACAGAGTGTCCCAG
DKKL1	Sequence (5' -> 3')
Forward Primer	CTCTACCCTGGTGATCCCCTC
Reverse Primer	CGAAGCAGGTTACCTTTCAGGA
USAG1	Sequence (5' -> 3')
Forward Primer	GCCATCAGAGATGTATTTGGTGG
Reverse Primer	GTGCTCCCTAACTGGATTGGA
WIF1	Sequence (5' -> 3')
Forward Primer	TCTCCAAACACCTCAAAATGCT
Reverse Primer	GACACTCGCAGATGCGTCT
BARX1	Sequence (5' -> 3')
Forward Primer	TTCCACGCCGGACAGAATAGA
Reverse Primer	AGTAAGCTGCTCGCTCGTTG
TFF2	Sequence (5' -> 3')
Forward Primer	CGGGGAGTGAGAAACCCTC
Reverse Primer	CACTGGAGTCGAAACAGCATC
GAPDH	Sequence (5' -> 3')
Forward Primer	GGTATCGTGGAAGGACTCATGAC
Reverse Primer	ATGCCAGTGAGCTTCCCGTTCAG
rDNA 16s H.pylor	Sequence (5' -> 3')
Forward Primer	TTTGTTAGAGAAGATAATGACGGTATCTAAC
Reverse Primer	CATAGGATTTCACACCTGACTGACTAT C
gDNA hGAPDH	Sequence (5' -> 3')
Forward Primer	GACTTCAACAGCGACACC C
Reverse Primer	AGAAGATGAAAAGAGTTGTCAGGGC