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METHODOLOGY

Animal models

To generate specific deletion of *Pggt1b* and *Rac1* genes in intestinal epithelium, *Pggt1b*^{flx} [87] and *Rac1*^{flx} [88] mice were crossbred with VillinCreERT2[89] mice. Ethanolic tamoxifen (Sigma Aldrich) solution was emulsified in sunflower oil, and administered for three consecutive days by intraperitoneal (i.p) injection (1 mg/mouse/day). Littermates carrying loxp-flanked target gene but not the Cre-recombinase were used as control. Genotyping was performed by PCR in ear genomic DNA. Animal studies were conducted in a gender and age-matched manner using littermates for each experiment. Both male and female animals were used at the age of 8-12 weeks. All mice were kept under specific pathogen–free conditions. Mice were routinely screened for pathogens according to FELASA guidelines. Animals were euthanized by cervical dislocation and biological materials were immediately snap-frozen in liquid nitrogen and kept at -80°C until usage. Animal tissues were also, either formalin-fixed to be further embedded in Paraffin or embedded in Cryo molds with Tissue Tek for cryostat sectioning.

DSS-induced colitis

Mice were fed *ad libitum* with 2% DSS (MP Biologicals) in drinking water for 7 days. On day 7, DSS was withdrawn and substituted by water. Animals were sacrificed on day 10.

Adoptive lymphocyte transfer colitis

Immunodeficient Rag1$^{-/-}$ mice received 1 million of CD4^{+}CD25^{-} T cells via i.p route. Mononuclear cells were isolated from the spleen of C57/BL6 donor mice and purified using MACS technology. Animals were sacrificed three weeks upon cell transfer.

Mouse mini-endoscopy

In the case of colon, gut status of mice was monitored by high-resolution mouse video endoscopy[90]. On the other hand, ileum and duodenum tissue from *Pggt1b*$^{i\Delta IEC}$ and *Rac1*$^{i\Delta IEC}$ mice was exteriorized and imaged *ex vivo*.

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Intestinal epithelial permeability assessment (FITC-Dextran assay)

Intestinal permeability was assessed *in vivo*, measured by transmucosal passage of orally administered FITC-Dextran (Sigma-Aldrich; 4000 g/mol average molecular weight), as previously described[91]. In brief, mice were deprived of food and water for 4 hours. Afterwards, 60 mg FITC-Dextran/100 g body weight was given by oral gavage. Serum was collected 4 hours after the gavage, and FITC concentration was determined spectrophotometrically using FITC-Dextran solution as standard; the measurements were performed with the NOVOstar (BMG-LabTech) microplate reader at a wavelength of 485 nm and 510nm.

Intravital microscopy assessment of cell shedding

Briefly, mice were anaesthetized by ketamin/xylazin i.p injection, and the intestine was exteriorized and opened. The mucosa was topically stained with acriflavine (1 mg/ml), and Rhodamine-dextran (10,000 g/mol; 2 mg/ml) was used as luminal dye[51]. Surgical preparation was pinned up on a coverslide and mounted in a chamber for perfusion of saline solution. Time sequential Z-stacks images of the villus tip were taken with a confocal microscope (Leica, SP8).

Quantification of epithelial cell shedding

Epithelial cell shedding was quantified by several techniques. On one side, cell shedding rate was quantified by counting of cell shedding events detected by intravital microscopy. The number of cell shedding events was normalized by the length of the basal membrane on the individual pictures and the time of image acquisition. On the other hand, we have also counted the number of leakage and so-called “permeable cells” in two single in vivo images from each specimen[50]. Finally, a similar method was performed in order to quantify the number of arrested versus completed cell shedding events. However, in this case we took advantage of actin staining with AlexaFluor488-Phalloidin, which allowed us to detect the cytoskeleton protein redistribution occurring in early stages of the cell shedding process. We thus quantified the overall cell shedding rate, the distribution of different stages of cell shedding and the appearance of dextran-permeable cells.

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Real-time fluorescence deformability cytometry

Real-time fluorescence deformability cytometry (RT-FDC) measurements were performed as previously described[53], using an AcCellerator instrument (Zellmechanik Dresden GmbH). Briefly, the intestinal tissue was mechanically dissociated into a single cell suspension and incubated for 20 minutes at room temperature with the following antibodies: anti-mouse CD326-Alexa Fluor® 488 (1:200) and anti-mouse CD45-Alexa Fluor® 700 (1:1000) (BioLegend) diluted in PBS, 2% FBS. Cells were washed with PBS, 2% FBS and resuspended in the measurement buffer (0.6% (w/v) Methyl Cellulose in Phosphate Saline Buffer without Mg2+ or Ca2+); with adjusted viscosity of 25mPa·s using a HAAKE Falling Ball Viscometer type C (ThermoFischer Scientific). The cell suspension was drawn into a 1 ml Luer-Lok syringe (BD Biosciences) attached to a syringe pump and connected by PEEK-tubing (IDEX Health & Science LLC) to a microfluidic chip made of PDMS bonded on cover glass. A second syringe filled with pure measurement buffer was attached to the chip and used to hydrodynamically focus the cells inside the constriction channel. The microfluidic chip consists of a sample inlet, a sheath inlet and an outlet connected by a central channel constriction of a 20 x 20 µm square cross-section and a length of 300 µm. The total flow rate is 0.06 µl/s, of which the sheath flow rate was 0.045 µl/s and the sample flow rate was 0.015 µl/s. The chip was mounted on the stage of an inverted high-speed microscope equipped with a CMOS camera. The laser power for each fluorophore was adjusted accordingly, based on single stain controls and an unstained sample. An image of every cell was captured in a region of interest of 250 x 80 pixels at a frame rate of 2,000 fps. Morphological, mechanical and fluorescent parameters were acquired in real-time.

RT-FDC Data analysis

Cell images were analysed using ShapeOut software[92] and OriginPro 2019. For each animal, the data were filtered for an area ratio of 1.00-1.06, which is defined as the ratio between the area of the convex hull of the contour and the area of the contour of the cell. Manual gating was applied to select a pure population of EPCAM positive/CD45 negative cells and to exclude

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debris, small and out of focus cells. The threshold of the positive fluorescent signal was determined by an unstained control sample. The calculation of deformation, a measure of how much the cell shape deviates from circularity, was obtained from the image using the projected area (A) and cell contour length calculated from the convex hull (l):

$$Deformation = \frac{1 - 2\sqrt{\pi A}}{l} \qquad \text{Eq. 1}$$

The calculation of the Young's modulus was done using a look-up table derived from simulations based on the finite elements method[93] and the analytical solution[94]. Statistical analysis was performed using linear mixed models[95].

3D Traction force microscopy

3D Traction Force microscopy is performed as previously described[56], with minor modifications. Grown organoids are splitted as described above, and embedded in collagen 1 hydrogels (1.2 mg/ml final concentration). The hydrogels consist of acid-dissolved rat tail (R) and bovine skin (G1) collagen (Matrix Bioscience, Germany), mixed at a mass ratio of 1:2 and dissolved in a solution of 1 vol part $NaHCO_3$, 1 vol part 10 × DMEM and 8 vol parts H_2O. Silica beads (4 µm diameter, Kisker Biotech, Germany) are added to the ice-cold solution at a concentration of $4 \cdot 10^6$ beads/ml.

1500 µl of the collagen/bead mixture is transferred to each well of a 6-well plate (Corning, USA) on ice, and polymerization of the collagen solution is initiated by increasing the pH of the solution to 9 by adding NaOH (1 M), and increasing the temperature to 37° C after. The mixture is allowed to polymerize for at least 15 min in an incubator while organoids are centrifuged (300 rcf for 5 min), excess media is aspirated away, and organoids are gently resuspended in another 1500 µl of freshly prepared ice-cold collagen/bead mixture. The mixture, complete with suspended organoids, is then transferred drop-by-drop to the 6-well plate using a cut-off pipette tip. After organoid seeding, the gel is incubated at 37 °C and 5% CO_2 for 1 h. Afterwards, 2 ml of pre-warmed cell media containing tamoxifen (0.5 mg/ml) is added to the dish.

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The dish is then transferred to a microscope (Applied Scientific Instrumentation, USA) placed inside a tissue culture incubator to allow for long-term imaging (72 h). Prior to imaging, the x,y,z positions of each of the organoids are stored. Depending on the total number of (typically 100 - 200) organoids in the 6-well plate, searching for the organoids and storing their positions takes approximately 1 h. Afterwards, time-lapse imaging is performed using a 4x magnification 0.13 NA objective (Olympus, Japan) and a CCD camera (Lumenera Infinity 3-6UR, Canada), with a time interval of 20 min between images.

From the time-lapse images, the deformation of the collagen gel around each organoid is measured from the silica beads displacement using particle image velocimetry[56 96 97]. From the deformation field, we calculated the contractile force of the organoid as previously described[56]. This calculation is based on a look-up table that predicts the collagen deformation as a function of distance from the organoid surface and as a function of the total contractile force of the organoid. The look-up table is generated using a finite-element simulation. This simulation approximates the organoid as a spheroid. The simulation furthermore requires knowledge of the non-linear stress-strain material properties of the collagen, which we measure in a cone-plate rheometer (TA Instruments Discovery HR-3, USA, strain rate = 1 %/s; geometry 20 mm; angle 2°; truncation gap 54 μm; polymerization at 37°C for 30 min) and in a custom-made uniaxial stretcher[98] as previously described in[56 99 100] **(Supplementary. Fig. 6)**. The non-linear material properties of the 1.2 mg/ml collagen gels are: linear stiffness (K0) = 1449 Pa, linear strain range (λs) = 0.032, exponential stiffening exponent (ds) = 0.055 and exponential buckling exponent (d0) = 0.00215.

From the look-up table, we obtain a force estimate for every position and every radial distance around each organoid (typically 3000 positions). Ideally, the force estimates at each position are identical, but due to measurement noise and deviations of the organoids from a spherical shape, the force estimates are subject to fluctuations and systematic errors especially very close to and very far from the organoid surface. We have previously shown that the lowest noise of the reconstructed forces and the best agreement between the measured and expected

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deformation field is in the so-called far-field[56]. Here, we average the forces at locations around the organoid that are between 5 and 10 effective organoid radii away from the organoid center. The effective organoid radius is computed from the cross-section area of the organoids at the equatorial plane at the first image of the time series. We exclude organoids for which the pressure values in this range vary excessively (with a coefficient of variation greater than 40%, mostly this is due to force interferences from neighboring organoids). This leaves between 5-36 organoids per well. We performed measurements for 4 biological replicates per condition.

Overcrowding

Murine gut cross-sections were stained with AlexaFluor488-Phalloidin. Using ImageJ, we measured cell length (maximum length perpendicular to the basal membrane) and cell diameter (perpendicular to the middle point of the cell length). Five pictures/sample, and a minimum of 10 cells/picture were quantified. Afterwards we calculated the ratio between cell length and diameter. Electron Microscopy pictures were quantified using a similar method.

IEC isolation

Murine gut tissues and human surgical specimens were freshly used for IEC isolation. Therefore, tissue was incubated in a solution containing DTT and EDTA[101]. Purification of IECs was performed by Percoll gradient.

Histology, immunohistochemistry and electron microscopy

Formalin-fixed paraffin-embedded samples were used for H&E staining and histopathological analysis. Scoring was performed in a blinded fashion. We established a score of the histological damage in ileum and duodenum. This histological damage score took into account three parameters “inflammatory cell infiltrate”, “epithelial changes” and “mucosal architecture”. Each parameter was scored (none=0, severe=5) and this results were afterwards summed up (maximum score of 15).

Immunohistochemistry was performed in cryo-sections. Depending on the primary antibody used, a TSA Cy3 amplification Kit (Perkin Elmer) was applied. Images were acquired with an up-right fluorescence microscopy (Leica DMI6000), or by confocal microscopy (Leica TCS SP8

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or Leica Stellaris 8). The following primary antibodies were used overnight at 4°C: anti-BrdU 1:200 (ab6326, Abcam); anti-β-catenin 1:500 (8480, Cell Signaling); anti-Claudin-1 1:500 (71-7800, ThermoFisher Scientific); anti-Claudin-2 1:500 (28530, Cell Signaling); anti-Claudin-8 1:200 (40-0700Z, ThermoFisher Scientific); anti-Claudin-18 1:500 (700178, ThermoFisher Scientific); anti-Cleaved caspase-3 1:300 (9661, Cell Signaling); anti-E-cadherin 1:500 (147301, Biolegend); anti-EpCAM (mouse) 1:200 (118207, Biolegend); anti-EpCAM (human) 1:250 (324201, Biolegend); anti-GGTase-Iβ, 1:100 (sc-18996, Santa Cruz); anti-Ki67 1:500 (ab16667, Abcam); anti-MPO, 1:250 (ab9535, Abcam); anti-MyosinIIA 1:500 (3403, Cell Signaling); anti-MyosinIIB 1:500 (ab24761, Abcam); anti-Rac1 1:500 (ARC03, Cytoskeleton); anti-RhoA 1:500 (2117, Cell Signaling); anti-Wave1 1:500 (PA578273, ThermoFisher Scientific); anti-Wave2 1:500 (PA560975, ThermoFisher Scientific); anti-ZO-1 1:500 (617300, Invitrogen). Biotinylated-Streptavidin antibody pairs were used for detection (1:100-1:200, 30 minutes at RT). In some cases, direct dye-labelled secondary antibodies were used (1:100, 30 minutes at RT). Actin fiber staining was performed using Alexa Fluor 488-labelled Phalloidin (Jackson Immunology). In situ cell death detection kit (Roche) was used for detection of TUNEL positive cells.

For electron microscopy, glutaraldehyde-fixed material was used. After embedding in Epon Araldite, ultrathin sections were cut and analyzed using a Zeiss EM 912 using standard protocol[102].

Quantification of immunohistochemistry

MPO, cleaved caspase 3-TUNEL: number of positive cells/ field was counted using ImageJ software. A total of three different images/sample (20x objective) were counted and the average cell number was calculated, accordingly.

RNA isolation and qPCR analysis

Total RNA was isolated from tissues or IECs, using the Macherey Nagel NucleoSpin RNA kit or peqGOLD Total RNA Kit. Synthesis of cDNA was performed using reverse transcriptase. Gene expression was measured by real time PCR (Bio-Rad) with SyberGreen and Quantitect

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primers (Qiagen). Gene expression was normalized to the housekeeping gene HPRT expression.

RNA Sequencing

Eukaryotic RNA Sequencing including quality control, library preparation, sequencing and bioinformatics analysis, was performed by Novogene. A 250-300 bp insert cDNA library was used; Hisat2 and HTSeq were used for Mapping to Reference Genome and Quantification, respectively. Differential Expression Analysis was performed using DEGseq/DESeq2/edgeR; while Enrichement Analysis of differentially expressed coding genes was performed based on GO and KEGG, taking advantage of GOSeq, topGo, hmmscan and KOBAS softwares.

Immunoblotting

Protein extracts were obtained by suspension and incubation in mammalian protein extraction reagent (ThermoFisher Scientific) supplemented with protease and phosphatase inhibitor tablets (Roche). For membrane/cytosol fraction separation, lysis buffer (RIPA) with and without detergents, and high speed centrifugation steps were used. Protein extract was cleared by centrifugation, protein concentration was measured by Bradford or BCA assays and denaturation was performed by boiling in LDS sample buffer (ThermoFisher Scientific). Afterwards, proteins were separated by SDS-PAGE gels, blotted onto nitrocellulose or PVDF membranes and blocked with 5% non-fat milk. Membranes were incubated overnight at 4°C with the following primary antibodies (dilution, 1:1000-1:2000): anti-β-Actin (4967, Cell Signaling); anti-Caspase-1 (Casper-1, Adipogen Life Science); anti-β-catenin (8480, Cell Signaling); anti-β-Tubulin (2146, Cell Signaling); anti-Claudin-1 (71-7800, ThermoFisher Scientific); anti-Claudin2 (28530, Cell Signaling); anti-Claudin-8 (40-0700Z, ThermoFisher Scientific); anti-Claudin-18 (700178, ThermoFisher Scientific); anti-Cleaved caspase-3 (9661, Cell Signaling); anti-E-cadherin (147301, Biolegend); anti-GAPDH (607902, Biolegend); anti-Gasdermin D (ab209845, Abcam); anti-GGTase-Iβ, (WH0005229M2, Sigma-Aldrich); anti-MLKL (orb32399, Biorbyt); anti-Na/K-ATPase (3010, Cell Signaling); anti-Rac1 (ARC03, Cytoskeleton); anti-Rap1A (sc-1482, Santa Cruz Biotechnology); anti-Villin (PA5-22072,

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ThermoFisher Scientific); anti-ZO-1 (617300, Invitrogen). Corresponding HRP-linked secondary antibodies were used (incubation for 90 minutes at RT), and chemo luminescence was detected by using ECL Western blotting substrate (Thermo Scientific). Protein expression was compared to the level of Actin (4967, Cell Signaling).

Proteomic assay

20 µg of total protein from purified IECs were digested using a modified Filter Aided Sample Preparation (FASP) method. Three biological replicates were included. Reversed-phase nano-UPLC separation of tryptic peptides, mass spectrometric analysis using an ion-mobility enhanced data-independent analysis workflow was performed as previously described[103]. All samples were analyzed in three technical replicates.

Intestinal crypt isolation and organoid culture

Small intestine was aseptically isolated, washed and cut. Intestinal fragments were washed, incubated in chelation buffer (containing EDTA) and vigorously resuspended in order to isolate intestinal crypts. Crypts were embedded in Matrigel on ice and seeded in pre-warmed surface cell culture plates. After polymerization of Matrigel, basal culture medium was added to the culture[104]. The entire medium was changed every 3 days and organoids passaged weekly. Organoids were treated in vitro with 0.5 µg/ml tamoxifen.

Two-dimensional organoid culture

Intestinal crypts are isolated from mouse small intestine or human tissue and grown in matrigel (see above). A 8- or 12-well chamber slide (ibidi) is coated with a thin layer of diluted matrigel and incubated at 37°C for minimum 20 minutes. Sufficient organoids (approximately 200) are collected in a 15-mL conical tube and washed with cold PBS. Pelleted organoids are resuspended with ice-cold 5mM EDTA/ PBS buffer, pipetted thoroughly to release single crypts and incubate at 4°C for 1 hour with rotation. Cell suspensions are centrifuged and washed and resuspended in human intesticult (Stem cell®) supplemented with Y-27362 (Enzo Life Sciences), Primocin (Invitrogen) and Wnt3a conditioned medium or in mouse basal culture medium. Medium containing organoids are placed in the pre-coated chamber slide and allowed

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to firmly attach to the matrigel thin layer until reaching a monolayer in 37°C, 5% CO2 incubator (7 – 10 days, after seeding).

Apical-Out small intestine organoids culture

Mouse small intestinal organoids are cultivated in mouse intesticult (stem cell) supplemented with 25% of Wnt3a medium for 5 days to induce cystic organoids. On the day of experiment, sufficient amount of organoids are collected and incubated in ice-cold gentle cell dissociation buffer (stem cell) to dislodge the matrigel. Afterward, cell suspensions are spun down, washed, resuspended in mouse intesticult medium and seeded in standard cell culture plates.

Trans-epithelial ressitance measurements

Trans epithelial resistance (TER) of intestinal epithelial cell lines (HT29 and Caco2 cells) was measured by using transwell inserts (0.4µm PCF, 12mm diameter, Merck Millipore). Inserts were hydrated for 30 min at room temperature with DMEM (Dulbecco's Modified Eagle Medium) prior to seeding of 150,000 cells/insert in 300µl. Growth culture medium was added to the receiver plate well i.e., 600µl. Outer wells were filled with PBS to avoid evaporation. Cells were incubated for 72h at 37°C until a monolayer was formed, followed by the corresponding treatments for three more days. Resistance was analyzed by Epithelial volt-ohm meter from Millicell ERS2. Each experiment was performed in Triplicate.

Genomic DNA PCR

Tissue samples/isolated cells were digested/lysed using a buffer containing 0.2% SDS and supplemented with proteinase-K. Samples were incubated for at least 6 h at 56°C with gentle shaking. After inactivation of the proteinase at 95°C for five minutes, samples containing genomic DNA were used for routine PCR using Taq polymerase master mix (Jena Biosciences).

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BrdU cell migration assay

Mice received an intravenous injection of BrdU (50 mg/kg body weight) 18-24 hours before euthanize them. Paraffin sections were then stained with anti-BrdU antibody and counterstained with hematoxyilin.

Study Approval

Animal protocols were approved by the Institutional Animal Care and Use Committee of the University of Erlangen and the Regierung Mittelfranken (Würzburg, Germany). Collection of human samples was approved by Ethics Committee of the Department of Medicine of the University of Erlangen-Nuremberg (Erlangen, Germany); Reference Number 440_20 B.

It was not appropriate or possible to involve patients or the public in the design, or conduct, or reporting, or dissemination plans of our research.

Statistics

Statistical analysis and number of biological/technical replicates are described in the corresponding figure legends.

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SUPPLEMENTAL FIGURE LEGENDS

Suppl. Figure 1: Time course phenotype in GGTase conditional KO mice, *Pggt1b*$^{i\Delta IEC}$ mice.
A. Genomic DNA PCR detecting floxed and tamoxifen-induced deleted (Delta) *Pggt1b* gene in ileum (left) and duodenum (right) tissue. Representative pictures, seven independent experiments. **B.** *Pggt1b* gene expression (RT-qPCR) in ileum tissue (left; n = 6/group) and IECs (right; n = 3/group). **C.** Western blot analysis of GGTase-IB and non-prenylated Rap1A (accumulation of non prenylated proteins) in ileum (left; n = 6/group) and isolated IECs (right, n = 3/group). Representative pictures (top), and quantification (bottom). β-actin has been used as loading control. **D.** Histology analysis of colon and duodenum tissue using H&E staining. Representative pictures (top), and corresponding score (bottom). Six and four independent experiments, colon and duodenum, respectively. **E.** Gene expression of IL-6 and TNF-α in colon and duodenum tissue (RT-qPCR; 4 independent experiments). **F.** MPO immunofluorescence staining in cross-sections from colon and duodenum. Representative pictures (left), and corresponding quantification (right); (3 independent experiments, colon and duodenum). Data are expressed as mean ± SEM. One-way ANOVA, Dunnett´s multiple comparisons test. * P value ≤ 0.050; ** P value ≤ 0.001; *** P value ≤ 0.0001.

Suppl. Figure 2: Cell shedding
A. Identification of funnel-like structures using Actin staining (Alexa-Fluor488 phalloidin) in combination with E-cadherin or ZO-1 (red signal). Representative pictures from ileum in WT mice. White arrows indicate funnel-like structures. **B-D.** F-actin fiber staining using AlexaFluor488-phalloidin (green). **B.** Exemplary pictures showing funnel-like structures, early and late cell shedding events in mouse (left) and human (right) small intestine tissue. High resolution conofocal microscopy (Stellaris). **C-D.** Quantification out ot 6 independent experiments. **C. E**arly (top) and completed (bottom) cell shedding events. Multiple paired t-test. **D. C**ell length and diameter, and calculated length/diameter ratio. Mixed-effects analysis. Data are expressed as mean ± SEM. * P value ≤ 0.050.

Suppl. Figure 3: Cytoskeleton rearrangement
A. *S1p2r* gene expression in ileum tissue, RT-qPCR analysis. Four independent experiments. **B.** RT-FDC. Gating strategy (top) and quantification of cell size and deformation (bottom). **C-E.** Gene expression analysis of AJC proteins in Ileum from control and *Pggt1b*$^{i\Delta IEC}$ mice at different time points after tamoxifen treatment (RT-qPCR). Four independent experiments. **C.** Tight junctions: Claudin-1, -2, -3, -4, -5, 7, -8, -18; Occludin-1; JAM-1, -2, -3; ZO-1, ZO-2, ZO-3. **D.** Adherens junctions: E-cadherin; β-catenin; catenin delta-1. **E.** Desmosomes: Desmocolin-2; Desmoglein-2; Desmoplakin; Plakophlin-2, -

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3, -4; Plakoglobin. **F-J.** Expression and redistribution of selected candidate AJC proteins in ileum tissue; immunostaining (left, red signal) and western blot (right). Minimum three independent experiments. **F.** Claudin-8. Three independent experiments. **G.** Claudin-18. Six independent experiments. **H.** ZO-1. Five independent experiments. **I.** β-catenin. Five independent experiments. One-way ANOVA, Dunnett´s multiple comparisons test **(B-D)**, or unpaired t-test **(E)**. * P value ≤ 0.050.

Suppl. Figure 4: Epithelial cell turnover

A. TUNEL (green) and cleaved caspase-3 (red) staining in ileum cross-sections from control and *Pggt1b*$^{i\Delta IEC}$ mice at different time points. Quantification of apoptotic (TUNEL^{+}Cl.Casp3^{+}) and non apoptotic (TUNEL^{+} Cl. Casp-3^{-}) dead cells. 6 independent experiments. **B.** *Mlkl* gene expression measured by RT-qPCR in ileum tissue (left; n = 6) and isolated IECs (right; n = 3). **C.** Gsdm and Dfna5 gene expression in ileum tissue (RT-qPCR analysis). Four independent experiments. **D.** *Ki67* gene expression (RT-qPCR, 6 independent experiments) (left); and representative pictures from immunostaining in ileum tissue (minimum, n=5/group) (right). **E.** Cell migration along the villus-crypt axis, detected by BrdU incorportation assay. Representative pictures (n = 2/group). Data are expressed as mean ± SEM. One-way ANOVA, Dunnett´s multiple comparisons test, except where indicated. ** P value ≤ 0.001.

Suppl. Figure 5: Generation, validation and phenotype of *Rac1*$^{i\Delta IEC}$ mice

A. Expression of RAC1 and RHOA within small intestine IECs from control and *Pggt1b*$^{i\Delta IEC}$ mice at Day 3 upon tamoxifen treatment. Representative blots (left), and band densitometry quantification (right). Three experiments; (n = 6, control; n = 9, *Pggt1b*$^{i\Delta IEC}$). **B.** *Rac1* gene expression (RT-qPCR) in ileum tissue (left; n = 4, Control; n = 5, *Rac1*$^{i\Delta IEC}$) and IECs (right; n = 4, Control; n = 3, *Rac1*$^{i\Delta IEC}$). **C.** Western blot analysis of RAC1 in ileum (left) and isolated IECs (right) (n = 5, Control; n = 7, *Rac1*$^{i\Delta IEC}$). Representative pictures (top), and quantification (bottom). **D.** WAVE 1 and WAVE 2 expression in ileum tissue from *Rac1*$^{i\Delta IEC}$ mice over time upon tamoxifen treatment. Three (WAVE1) and two (WAVE2) independent experiments. **E.** Body weight of control and *Rac1*$^{i\Delta IEC}$ mice over time upon tamoxifen treatment (% of original weight) (n = 8, Control; n = 7, *Rac1*$^{i\Delta IEC}$). **F.** Endsocopy pictures from small and large intestine. **G.** Histology analysis of colon and duodenum ileum tissue using H&E staining; three independent experiments. **H.** Gene expression of TNF-a in colon, ileum and duodenum tissue (Day 6); three independent experiments (RT-qPCR) (n = 13/group). **I.** TUNEL (green) and cleaved caspase-3 (red) staining in ileum cross-sections from control and *Rac1*$^{i\Delta IEC}$ mice (Day 6 upon tamoxifen). Representative pictures (left) and quantification (right) of total TUNEL^{+} (left) and Cl. Casp-3^{+} (right) (n =

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6, Control; n = 5, *Rac1*$^{i\Delta IEC}$). **J.** *Ki67* gene expression (RT-qPCR) and immunostaining in ileum tissue (Day 6) (RT-qPCR) (n = 8, Control; n = 9, *Rac1*$^{i\Delta IEC}$). Immunostaining (Day 7) (n=5/group). **K.** Cell migration along the villus-crypt axis, detected by BrdU incorportation assay. Representative pictures (n = 2/group). **L.** Western blot analysis of cleaved caspase-3 (n = 4, Control; n = 5, *Rac1*$^{i\Delta IEC}$) and MLKL (n = 5, Control; n = 7, *Rac1*$^{i\Delta IEC}$) in ileum tissue and isolated IECs. Representative blots, and corresponding quantification. **M**. Gasdm and Dfna5 gene expression in ileum tissue (RT-qPCR analysis). Three independent experiments. **N.** Detection of Caspase-1 and Gasdermin-D cleavage in small intestine IECs (western blot analysis). Representative blots (top), and band densitometry quantification (bottom). Four independent experiments. **O.** Time course. F-actin fiber staining using AlexaFluor488-phalloidin (green). 3 independent experiments. Quantification of early (left) and late (right) cell shedding events. **P.** Representative pictures of Myosin IIA staining (red) in ileum tissue. **Q.** Detection of selected candidate AJC proteins in ileum tissue. Immunostaining (red signal). Three independent experiments.Claudin-8; Claudin-18; ZO-1; β-catenin. Data are expressed as mean ± SEM. Unpaired t-test, except for **J.** One-way ANOVA, Dunnett´s multiple comparisons test. * P value ≤ 0.050.

Suppl. Figure 6: Organoids

A-D. Validation of tamoxifen-induced abolished expression of GGTase1B **(A-B)** and RAC1 **(C-D)**. **A and C.** Gene expression (RT-qPCR) (n=4/group, *Pggt1b*; n≥6/group, *Rac1*). **B and D.** Protein expression, western blot (n=4/group, *Pggt1b*; n=4, control, n=5, *Rac1*). **E.** Morphology of GGTase- and RAC1-deficient small intestine organoids treated with different cell death pathway inhibitors (Z-VAD 20 μM, apoptosis; Necrostatin-1 30 μM, necroptosis; Disulfiram 20 μM, pyroptosis). Representative pictures; Day 6, *Pggt1b*$^{i\Delta IEC}$; Day 4, *Rac1*$^{i\Delta IEC}$. **F.** Myosin IIA immunostaining (red). (Pggt1b, four experiments; Rac1, three experiments). **G.** Apical-out organoids, representative pictures of Phalloidin staining showing completed/late and arrested/early cell shedding events. **H-K.** 3D Traction Force microscopy. **H.** Stress-strain relationship of a 1.2 mg/ml collagen gel measured in a cone-plate rheometer (mean±sd from 8 samples). The dashed line is the fit of the semi-affine finite-element network model to the data. **I.** Vertical contraction of a 1.2 mg/ml collagen gel (reduction in thickness) in response to a uniaxial horizontal stretch (mean±sd from 3 samples). The dashed line is the fit of the semi-affine finite-element network model to the data, with the same parameters as specified in the methods section. **J.** Inward-directed matrix deformations around a contractile organoid at different time points. **K.** Force reconstruction using a lookup-table of matrix deformations as a function of radial distance and contractile pressure. Deformations and distances are normalized by the effective organoid radius. Black lines show

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the predictions of a non-linear finite-element model for different contractile pressure values. Circles show the measured matrix deformations at different distances from the spheroid surface and for different time points. The best agreement between the measured and expected deformation field is in the far-field region between 5 and 10 effective organoid radii away from the organoid center (red dashed lines). **L.** Tamoxifen-induced deletion of RAC1 in small intestine organoids; time course. **M-S.** RNA Sequencing from RAC1-deficient small intestine organoids (day 3 upon tamoxifen induction). **M.** Pearson correlation between samples. **N.** Venn diagram of sequenced genes. **O.** Heat map of differentially expressed genes (left); top ten up- and down-regulated genes. **P-Q.** qPCR analysis of gene expression from candidate genes identified in the RNASeq analysis (*Adm2*, *hif3a*, *CXCL10*, *Klf2*) (n=4/group;). **P.** RAC1-deficient organoids. **Q.** GGTase-deficient organoids. **R-S.** Gene Set enrichment Analysis (GSEA). Top 20 regulated pathways and Endplots for selected pathways. **R.** Gene Ontology (GO). **S.** KEGG. Data are expressed as box-plots (Min. to Max). Adm2 expression was not detected in 3 out of 4 GGTase-deficient organoids. Paired t-test. * P value ≤ 0.050; ** P value ≤ 0.001; *** P value ≤ 0.0001.

Suppl. Figure 7: Cell shedding in IBD

A. F-actin fiber staining using AlexaFluor488-phalloidin (green) (n = 13, total; n = 5, Control; n = 8, CD). Quantification of early and late cell shedding events (% of total cells). **B.** FABP2 concentration in serum samples from the blood of IBD patients (n = 55; n = 9, Control; n = 24, CD; n = 22 UC; not detected FABP2, 1 Control, 1 CD, 2 UC samples). **C.** Correlation study between FABP2 serum concentration (ELISA), and funnel-like structures (phalloidin staining) and E-cadherin expression (immunostaining, red) in gut tissue (n = 6). Representive pictures (top). Correlation graphs, linear regression fit (bottom). **D.** Expression and rearrangement of selected AJC proteins in 2D organoids generated from small intestine crypts from human patients and treated with a cyotkine cocktail (IL-1β 10 ng/ml, IL-6 10 ng/ml and TNF-α; 20 ng/ml). Representative pictures. **E.** Cytoskeleton rearrangement and epithelial barrier function in HT29 and Caco2 cells treated with NSC-23766 (100 μM), GGTI-298 (5 μM) or a cytokines cocktail (IL-1β 10 ng/ml, IL-6 10 ng/ml and TNF-α; 20 ng/ml). Trans-epithelial resistance measurement in transwell inserts using volt-ohm meter from Millicell ERS2 (left). Representative pictures of actin fiber distribution using phalloidin staining (right). * P value ≤ 0.050.

Suppl. Figure 8: Human relevance of epithelial RAC1 function in IBD

A-B. *Wasf1* and *Wasf2* expression in IECs isolated from mice subjected to mouse experimental colitis (RT-qPCR). **B.** DSS-induced colitis (n=5, Control; n=4, DSS). **C.** Adoptive lymphocyte transfer colitis. (n=5, Control; n=7, DSS). **C.** *Wasf1* and *Wasf2* expression in small intestine organoids developed from

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crypts isolated from *Pggt1b*$^{i\Delta IEC}$ and *Rac1*$^{i\Delta IEC}$ mice (RT-qPCR) (n = 4/group). Unpaired **(A, B)**, and paired **(C)** t-test.