Supplement to

Structural weakening of the colonic mucus barrier is an early event in ulcerative colitis pathogenesis

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Content

Supplementary Methods

Collection of mucus

Biopsies obtained from the sigmoid colon and intended for mucus collection were transported in oxygenated Krebs buffer on ice and directly mounted in our in-house developed horizontal mucus measurement chambers.[1] Two biopsies were mounted in parallel. The exact composition of the Krebs buffer was (in mM): 116 NaCl, 1.3 $CaCl_2$, 3.6 KCl, 1.4 KH_2PO_4, 23 $NaHCO_3$, and 1.2 $MgSO_4$ (Merck). Via an opening of 1.5 mm diameter on the apical side of the biopsy, the mucus can be visualized by light microscopy through the sprinkling of charcoal on its surface. Biopsies were left for one hour in the chamber with continuous apical and basolateral perfusion with physiologically composed buffers containing oxygen and nutrients (10mM D-mannitol on the apical side; 10 mM D-glucose on the serosal side). The pH of all buffers was 7.4. After an initial step-wise increase from room temperature over 10 min., the temperature of the chamber was kept at 37ºC throughout the experiment. After one hour the mucus was harvested through gentle scraping and stored at -80°C with protease inhibitors (1x cOMPLETE, Sigma-Aldrich). For absolute quantification of MUC2, the mucus thickness was measured at the start and end of the experiment, using a micropipette as previously described [1].

Preparation of mucus for mass spectrometry

Mucus from two biopsies was pooled together for the experiments. Mucus samples were mixed 1:1 (vol/vol) in 5 mM EDTA, 100 mM dithiothreitol, 6 M GuHCl (pH 8); solubilized and reduced overnight at 37°C on a thermomixer (Eppendorf). The mucus was digested using a modified version of the filter aided sample preparation method (FASP).[2] Briefly, solubilized mucus samples were transferred to

molecular mass cut-off filter units (10 kDa, Nanosep, Pall Lifescience) and centrifuged at 7,500 x *g* for 10 min. (5415R, Eppendorf). Following a washing step using 200 µl 6 M GuHCl and disposal of the flow through, proteins were alkylated on-filter for 20 min. by the addition of 100 µl 50 mM iodoacetamide in 6 M GuHCl (pH 8), and centrifuged. Buffer exchange was achieved by the addition of 200 µl 50 mM ammonium bicarbonate (pH 8), followed by centrifugation (repeated twice). Proteins were digested overnight at 37°C using 200 ng of trypsin (Promega) in 50 µl of 50 mM ammonium bicarbonate, pH 8. Tryptic digests were transferred to clean tubes by centrifugation at 10,000 x *g* and the filters were eluted once more by the addition of 100 µl 250 mM NaCl. The samples were acidified and cleaned using micro scale C18 stage tips, dried under vacuum and re-dissolved in 15 µl 0.1% TFA prior to mass spectrometry analysis.[3]

Nano-liquid chromatography-tandem mass spectrometry

Liquid chromatography and tandem mass spectrometry (MS/MS) were performed as previously described.[4] Briefly, 3 µL of each sample were trapped on a fritted pre-column (4 cm x 100 µm i.d) connected via a divert valve to an analytical column (15 cm x 75 µm i.d. tip 10 µm, (New Objective) both packed with Reprosil-Pur 3 µm C18-AQ particles (Dr. Maisch). Following loading in 0.2% formic acid, the peptides were eluted using a 80 min gradient from 5-35% of 80% acetonitrile in 0.2% formic acid at a flow rate of ~300 nL/min. Mass spectrometry analysis was performed on a LTQ-Orbitrap XL (Thermo Fisher Scientific) operated in a data dependent mode automatically switching between scan modes, collecting full MS survey scans at high resolution in the Orbitrap analyzer and performing collision induced dissociation MS/MS on the 8 most intense ions per scan in the ion-trap. All raw data are available through the ProteomeXchange repository with the identifier PXD012632.[5]

Mass spectrometry data processing and bioinformatics

MaxQuant version 1.3.0.5 was used for data processing and peptide quantification, and proteins were identified by Andromeda.[6, 7] Database searches were performed against the human SwissProt protein database (v2014_1, 20,181 entries) with the following settings: 1) one missed cleavage allowed; 2) precursor tolerance: 7 ppm; 3) fragment ion tolerance: 0.5 Da; 4) fixed modification: carbamidomethyl on cysteine; 5) variable modifications: oxidized methionine and acetylated protein N-terminal. The false discovery rate (FDR) for both peptide and protein identifications was set to 1% based on a minimum of one unique peptide per protein, and were grouped when based on the same set of peptides. The peptide match between runs option was limited to a retention time window of 2 min. Additional available protein information (i.e., subcellular location, signal peptide and transmembrane domains) was retrieved through the mapping feature of UniProt.[8] The comparative abundances of proteins in the mucus proteome in relation to each other were estimated by dividing the summed peptide intensities for each protein by the number of theoretically observable tryptic peptides between 700 and 2,500 Da; while missed cleavages were neglected.[9] For the comparison of the mucus levels of proteins between patient groups, protein abundance factors were calculated by normalizing individual peptide intensities against the total intensity of each patient sample. Protein-protein interaction data were retrieved for selected proteins from the STRING database (version 8),

and network analyzes were visualized using Cytoscape (version 3).[10, 11] Interaction networks were combined with gene ontology annotation for biological function to identify functional clusters using the ClueGO plugin.[12] Data analysis and statistics were performed using GraphPad Prism version 6 (GraphPad Software) and R language and environment for statistical computing (R Development Core Team, 2013).

Absolute quantification of MUC2 by Parallel Reaction Monitoring

The mucus sample preparation for targeted proteomic analysis was performed as described above. Heavy peptides (JPT Peptide Technologies) for MUC2 absolute quantification (10 peptides, 100 fmol each; Table S8) were added before tryptic digestion. The MUC2 peptides (heavy and light) were quantified by parallel reaction monitoring on a nano-EASY Q-Exactive instrument (Thermo Fisher Scientific), as previously described.[13, 14] Briefly, precursors and fragments of the heavy and corresponding light peptides were scanned over 10 min. retention time windows with a resolution of 35,000.[13] Absolute quantification of MUC2 was performed with the Skyline program (version 3.6.0.1).[15]

Stimulation of colon biopsies with TLR1/2 ligand P_3CSK_4

The sentinel goblet cell dependent secretory response was assessed in ex vivo human biopsies, as previously described for mouse tissue.[16] Biopsies were mounted in a heated, perfused chamber with a 1.5 mm diameter aperture, and the mucus surface visualized using charcoal. Mucus thickness was measured using a glass micropipette at t0 and t30 minutes to establish the baseline mucus growth rate.[1, 16] Biopsies were then treated with the sentinel goblet cell activating TLR1/2 ligand P_3CSK_4 (50 µg/ml; Invivogen), and mucus thickness measured at t45 min to obtain the post-treatment mucus growth rate.

Quantification of sentinel goblet cells in colonic biopsies *ex vivo*

The mean number of sentinel goblet cells per crypt was determined by preparing biopsy whole mounts, and quantifying goblet cells that had endocytosed fluorescent P_3CSK_4 (P_3CSK_4-rhodamine; 50 µg/ml; Invivogen), as previously described.[16]

Immunohistochemistry

Paraffin-embedded Methanol-Carnoy-fixed sections from the sigmoid colon were dewaxed and stained with the primary antibodies listed in Table S1. Anti-rabbit/antigoat IgG conjugated with fluorophores Alexa Fluor 488 or 555 (Thermo Fisher Scientific) were used as secondary antibodies (1:1,000 dilution). DNA was counter-stained with Hoechst 34580 (Thermo Fisher Scientific; 1 µg/ml). Sections were imaged with a fluorescence Eclipse E-1000 microscope (Nikon). The intensity of SLC26A3 staining of the apical membrane and cytoplasm was quantified using the ImageJ software.[17]

Analysis of mucus penetrability by confocal microscopy

The method for the assessment of mucus penetrability has previously been described in detail. [1] Sigmoid biopsies were incubated in a perfusion chamber for 20 min; a suspension of 2 µm green, 1 µm purple and 0.5 µm red beads (Fluospheres, Thermo Fisher Scientific) was added onto the apical surface, and left to sediment through the mucus for 40 min. The tissue was stained by Calcein Violet Blue (1 µl/ml in the serosal perfusate; Invitrogen). Subsequently, confocal z-stack images were acquired using an upright LSM 700 Axio Examiner Z.1 confocal imaging system with a Plan-Apochromat ×20/1.0DIC water objective (Zeiss). The mucus was considered penetrable if there was an accumulation of beads at the epithelial surface, semi-penetrable if there was a scattering of beads throughout the inner mucus layer, and impenetrable in the case of a clear separation between the beads and the epithelium. To quantify the thickness of the impenetrable mucus layer, the mean distance to the 20 most penetrating 2 µm fluorescent beads was also quantified using the Volocity software (v. 6.1.1; Perkin-Elmer), as previously described.[18]

Tables

Table S1 *Primary antibodies used for immunofluorescence*

Antigen	Host	Dilution	Source	Reference
Apomucin 2	Rabbit	1:500	In-house	Asker N, et al. J Biol Chem. 1998; 273(30):18857-18863
SLC26A3	Goat	1:100	Santa Cruz Biotechnologies, Santa Cruz, CA	

Table S2 *Indications for colonoscopy for the control group (n=47 individuals).*

Indications	Number (%)
Anemia/blood in stool	18 (38)
Diarrhea	11 (23)
Altered bowel habits (other than diarrhea) or abdominal pain	7 (15)
Polyps	3 (6)
Check-up post-diverticulitis	2 (4)
Colon cancer heredity	2 (4)
Other*	4 (9)

* Included primary sclerosing cholangitis, weight loss, elevated sedimentation rate and suspicious findings on CT abdomen.

Table S3 *Demographics and clinical characteristics for patients whose biopsies were used for targeted quantification of mucus levels of MUC2, sentinel goblet cell quantification and assessment of mucus growth pre- and post-stimulation with TLR1/2 ligand P_3CSK_4*

	Controls[3]	Active UC	UC in remission
Patients	5	5	5
Females: # (%)	2 (40%)	1 (20%)	2 (40%)
Age: median; range	52 (28-75)	44 (26-71)	35 (27-65)
Active smokers: # (%)	1 (20%)	0 (0%)	0 (0%)
PSC: # (%)	0 (0%)	0 (0%)	3 (60%)
Years since UC diagnosis: median; range	NA	8 (5-43)	15 (13-41)
Mayo Endoscopy Score[1] 0: # (%)	NA	0 (0%)	5 (100%)
Mayo Endoscopy Score[1] 1: # (%)	NA	3 (60%)	0 (0%)
Mayo Endoscopy Score[1] 2: # (%)	NA	1 (20%)	0 (0%)
Mayo Endoscopy Score[1] 3: # (%)	NA	1 (20%)	0 (0%)
Sandborn Histological Score[2] 0: # (%)	NA	0 (0%)	4 (80%)
Sandborn Histological Score[2] 1: # (%)	NA	0 (0%)	1 (20%)
Sandborn Histological Score[2] 2: # (%)	NA	2 (67%)	0 (0%)
Sandborn Histological Score[2] 3: # (%)	NA	0 (0%)	0 (0%)
Sandborn Histological Score[2] 4: # (%)	NA	1 (33%)	0 (0%)
Medication: 5-ASA: # (%)	NA	4 (80%)	5 (100%)
Medication: AZA: # (%)	NA	0 (0%)	0 (0%)
Medication: TNFα-inh: # (%)	NA	0 (0%)	0 (0%)

[1]Refers to the sigmoid colon. [2] Available for 3/5 active UC. Refers to the sigmoid colon.[3] Controls were referred for colonoscopy due to either anemia or blood in stool. Colonoscopy was macroscopically normal in all cases, except for minor polyps. None of these individuals had diarrhoea or other symptoms from the intestines/abdomen.

Table S4 *Demographics and clinical characteristics for patients from whom ileum biopsies were obtained for analysis of the mucus proteome by nano-liquid chromatography-tandem mass spectrometry*

Patient #	Gender	Age	Indication for ileocolonoscopy	Macroscopic findings[1]
1	M	25	Constipation	Normal
2	M	55	Pathological finding at PET-imaging	Normal
3	M	86	Constipation	Normal
4	F	67	Blood in stool	Normal
5	M	63	Blood in stool	Angiodysplasia in colon. Ileum: Normal findings
6	M	65	Check-up after polypectomy in colon	Normal

[1] No biopsies were taken for histology for these patients

Table S5 *Relative abundances of all proteins detected in at least 50% of colonic mucus samples by nano-liquid chromatography-tandem mass spectrometry*.

Results are stratified by patient group: Clinically active UC (UCA), clinical remission (UCR) and controls. Clinical activity was defined as at least two of the following: Typical symptoms (clinical Mayo score ≥3), macroscopic (Mayo endoscopy score ≥1) or microscopic (Sandborn scale ≥2) inflammation. Abundance factors refer to raw intensity values, normalized to the summed intensities for all proteins in the sample. *See separate Excel document.*

Table S6 *Relative abundances of all proteins detected in ileal mucus by nano-liquid chromatography tandem mass spectrometry for six individuals without intestinal pathology.*

Abundance factors refer to raw intensity values, normalized to the summed intensities for all proteins in the sample. *See separate Excel document.*

Table S7 *Secreted proteins altered in UC mucus, in terms of number of identifications*

			Differentially regulated (p-value)	
Protein name	**Gene**	**Suggested role in the mucus layer**	**UCA vs. CTRL***	**UCR vs CTRL***
Myeloperoxidase	*MPO*	Neutrophil granule peroxidase	↑**0.0008 [<0.0001]**	↑**0.01 [0.04]**
Cathepsin G	*CTSG*	Neutrophil serine protease	↑**0.0007 [0.0001]**	0.60 [1.0]
Non-secretory ribonuclease	*RNASE2*	Eosinophil-derived. Dendritic cell chemotaxis.	↑**0.04** [0.07]	↑0.09 [0.29]
Eosinophil cationic protein	*RNASE3/ECP*	Eosinophil granule protein; antibacterial	↑**0.02** [0.11]	↑**0.02** [0.05]
Galectin 10	*CLC*	Eosinophil granule protein; immunoregulatory	↑**0.02 [0.02]**	↑**0.0004 [0.0005]**
Kallikrein 1	*KLK1*	Serine protease found in mucus granules	↓**0.01** [0.07] §	0.28 [0.41]
Mucin-12	*MUC12*	Transmembrane mucin	↓**0.0002 [0.03]** §	0.29 [0.30]
Meprin alpha	*MEP1A*	Metalloprotease	↓**0.0008** [0.07] §	0.61 [0.28]
Protein FAM3D	*FAM3D*	Chemotaxis	↓**0.002** [0.49] §	0.19[0.55]
ProSAAS	*PCSK1N*	Neuropeptide signalling	↓**0.007 [0.02]**	0.31 [0.23]

*Arrows denote a decrease/increase of proteins in UCA or UCR vs. CTRL, the numbers p-values, obtained by Fisher Exact test. P-values outside the [square brackets] refer to the clinical stratification of UC patients based on a synthesis of clinical Mayo score, endoscopy Mayo score and histology. Values [in brackets] refer to patients that were active/in remission based on sigmoid histology (Sandborn scale, cut-off for inflammation ≥2), and exclude patients that were clinically active but did not have inflammation of the sigmoid colon (n=18).

§ Significantly altered in UC patients with clinical activity *without* local, active inflammation in the sigmoid colon (Sandborn scale ≤1), as compared to the control group.

Table S8 *Stable isotope labelled peptides used for absolute quantification of MUC2 by parallel reaction monitoring mass spectrometry.*

Peptide	Mass (Da)
TFDGDVFR	965.95
TVVLLADK	865.98
TETPFGR	816.79
GLYLEAGDVVVR	1300.40
DEGHHVAYTTR	1295.25
SNNDFTTR	963.88
DRPIYEEDLK	1285.34
HETQEVLIK	1104.17
QAVALPYK	897.01
EYQAC^{+IAM}GPAEEPTC^{+IAM}K	1533.60

Table S9 *Comparison of clinical and demographic characteristics of patients with active UC with and without inflammation of the sigmoid colon.*

	No local inflammation	Local inflammation
Number	18	15
Females: # (%)	6 (33)	6 (40)
Age (median; range)	41; 21-70	35; 18-66
PSC: # (%)	3 (17)	1 (7)
Clinical Mayo score (median; range)	2; 1-8	3; 1-9
Sandborn 0 in sigmoid: # (%)	9 (50)	0 (0)
Sandborn 1 in sigmoid: # (%)	9 (50)	0 (0)
Sandborn 2 in sigmoid: # (%)	0 (0)	12 (80)
Sandborn 3 in sigmoid: # (%)	0 (0)	3 (20)
Peroral cortisone: # (%)	2 (11)	2 (13)
Local cortisone: # (%)	1 (6)	1 (7)
Peroral 5-ASA: # (%)	15 (83)	14 (93)
Azathioprine: # (%)	3 (17)	2 (13)
TNFα-inh: # (%)	1 (6)	1 (7)

Table S10 *Relative abundances of all proteins detected in colonic mucus by nano-liquid chromatography tandem mass spectrometry*.

Results are stratified by patient group, as defined by clinical activity and histology of the sampled segment, into the following categories: 1) active UC without local inflammation (UCA, noninflamed), 2) active UC with local inflammation (UCA, inflamed), 3) UC in clinical and histological remission (UCR, histologically confirmed) and 4) controls (C). Inflammation was defined as a Sandborn histological score ≥2. Abundance factors refer to raw intensity values, normalized to the summed intensities for all proteins in the sample. *See separate Excel document.*

Table S11 *Correlations between mucus proteins altered in UC and lamina propria immune cell populations.*

Immune cell counts are based on the mean number of cells/3 high power fields, at 600 times magnification. All immune cells except mast cells were quantified in hematoxylin-eosin stained sigmoid colon sections. Mast cells were quantified in toluidine blue stained sections. Differential counting of immune cells was performed in 26 UC patients (17 with active disease and 9 in remission). The table shows Spearman rank correlation coefficients; p-values are displayed in brackets. IC, immune cells.

	IC total	Eosinophils	Lymphocytes	Macrophages	Mast cells	Neutrophils	Plasma cells
MUC2	-0.13 (0.52)	0.14 (0.49)	0.03 (0.88)	0.15 (0.47)	-0.07 (0.73)	-0.08 (0.72)	-0.29 (0.15)
FCGBP	-0.17 (0.40)	0.18 (0.39)	0.11 (0.59)	0.11 (0.59)	-0.25 (0.24)	-0.01 (0.96)	-0.21 (0.31)
CHGA	-0.17 (0.93)	-0.04 (0.85)	0.20 (0.32)	0.03 (0.88)	-0.01 (0.96)	0.12 (0.57)	-0.17 (0.42)
CLCA1	-0.22 (0.27)	0.16 (0.43)	0.11 (0.60)	0.21 (0.31)	-0.10 (0.64)	0.18 (0.38)	**-0.45 (0.02)**
CTSZ	0.25 (0.24)	0.29 (0.18)	-0.12 (0.56)	0.16 (0.47)	-0.01 (0.98)	**0.58 (0.003)**	0.17 (0.42)
EPX	0.21 (0.47)	-0.26 (0.38)	0.11 (0.72)	0.40 (0.16)	0.22 (0.47)	0.26 (0.38)	0.44 (0.12)
GPA33	-0.19 (0.46)	0.33 (0.20)	0.26 (0.32)	0.24 (0.36)	0.03 (0.91)	0.01 (0.99)	**-0.66 (0.004)**
RNASE3	0.25 (0.24)	0.29 (0.18)	-0.12 (0.56)	0.16 (0.47)	-0.006 (0.98)	**0.58 (0.003)**	0.17 (0.42)
ZG16	**-0.50 (0.02)**	-0.06 (0.79)	0.00 (1.00)	-0.09 (0.71)	-0.35 (0.12)	-0.18 (0.42)	**-0.69 (<0.001)**

Figures

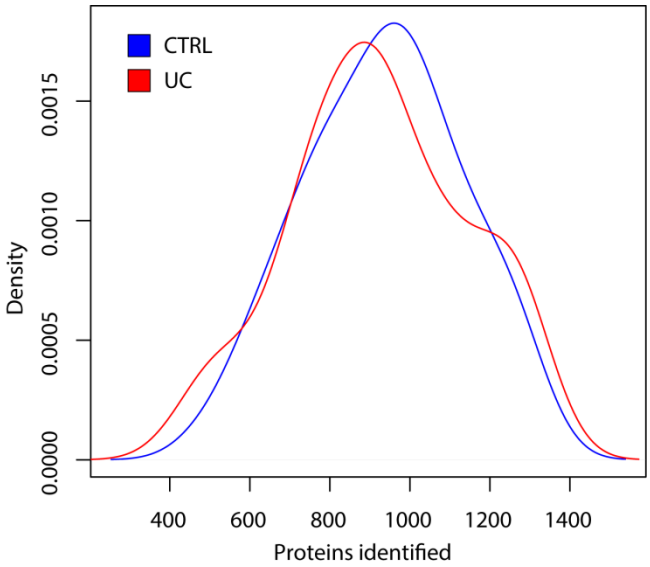

Figure S1 *Distribution plot of protein identifications in UC patients and controls*

The red line illustrates the distribution curve of protein identifications for UC patients (n=64), the blue line the corresponding distribution for individuals without colonic disease (n=47).

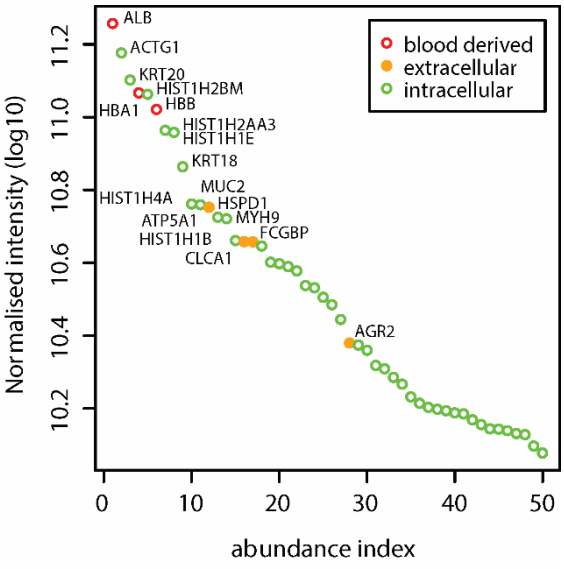

Figure S2 *Abundance curve for the top 50 components in mucus from the sigmoid colon.*

These include the known mucus constituents MUC2, FCGBP, CLCA1 and AGR2, but also a number of intracellular proteins with high copy numbers. Selected proteins are annotated with their gene name. Extracellular proteins are shown as closed orange circles, intracellular proteins as open green circles, and blood contaminants as open red circles.

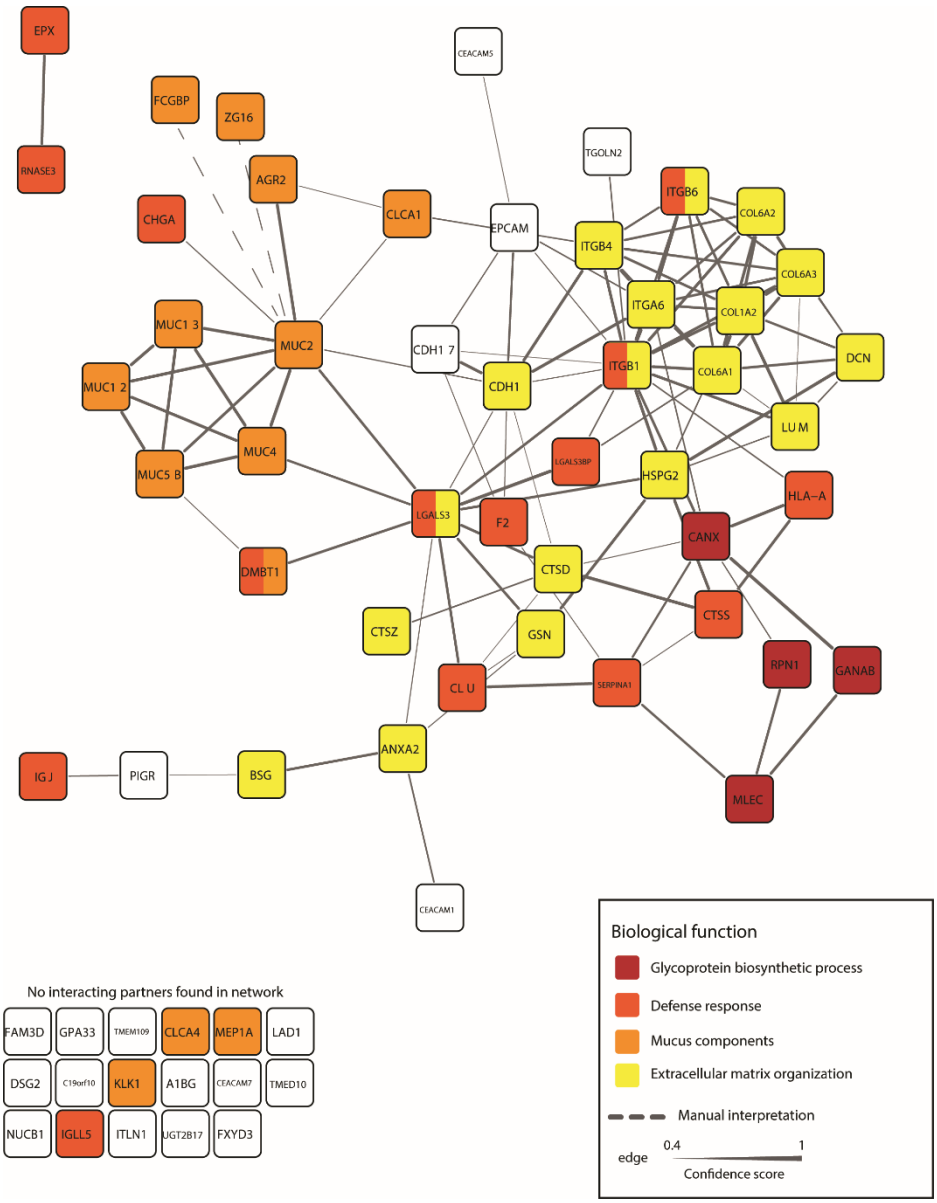

Figure S3 *Interaction network of the control mucus proteome based on data derived from the STRING database combined with GO annotation for biological function.*

Protein-protein interaction data were retrieved from the STRING database (version 8), and network analyses were visualized using Cytoscape (version 3).[10-12]

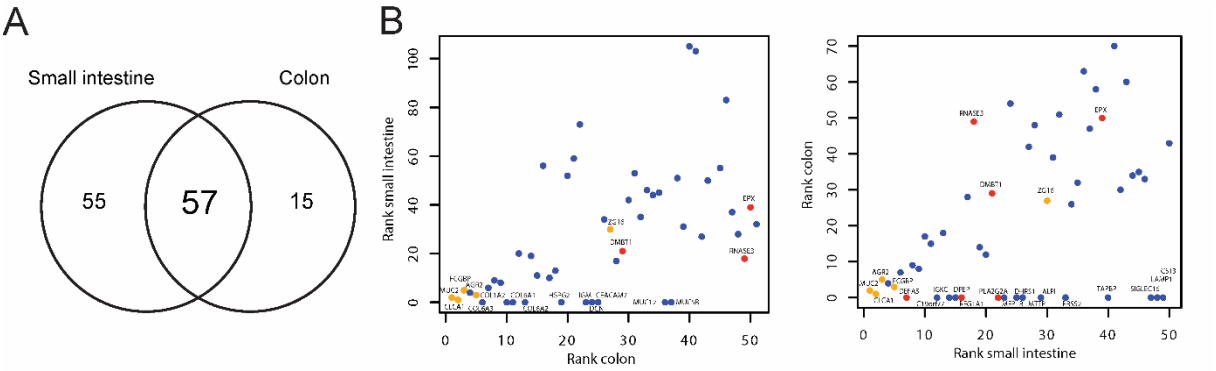

Figure S4 *Comparison of the mucus proteome between small intestine and colon.*

(A) Venn diagram showing the overlap in protein identifications between the secreted proteins in small intestine (ileum) and colon (sigmoid). (B) Rank based comparison of the mucus proteome between small intestine and colon based on normalized protein abundances. Orange circles represent known mucus components; red circles antimicrobial proteins.

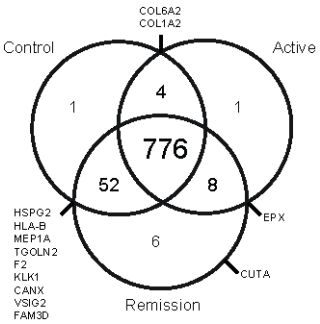

Figure S5 *Venn diagram representing the overlap in protein identifications between the control, UC in remission and active UC datasets.*

Proteins were included if identified in 50% of the patients for each subgroup. Gene names are shown for each secreted protein that was not identified in all patient groups.

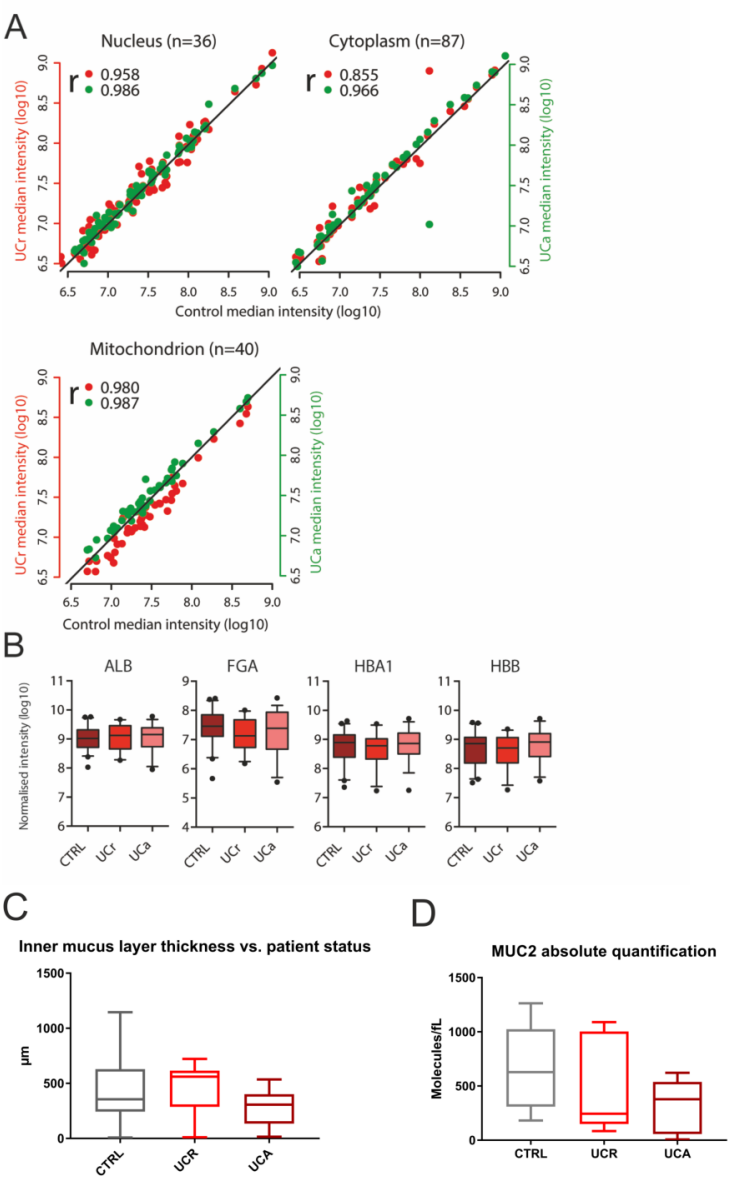

Figure S6 *Cell shedding and bleeding did not skew the comparison of the abundances of mucus proteins between patient groups*.

(A) Correlation of the protein abundances for selected intracellular proteins derived from exfoliated cells retained in the mucus. The abundance of proteins originating from the nucleus, cytoplasm and mitochondrion was compared between controls, UC patients with active disease (UCa) and UC patients in remission (UCr). A strong correlation was found between controls and UC patients regarding all three organelles, indicating similar rates of cell shedding between the patient groups. For UC in remission the correlation is plotted in red on the primary y-axis, and in green on the secondary y-axis for patients with active UC. R-values refer to the Pearson correlation coefficient. (B) Normalized intensities for the 4 most abundant blood proteins in controls, UC patients in remission, and active UC patients. The box represents the interquartile range around the median value and the whiskers the 5th and 95th percentile; data points outside this range are indicated as outliers. (C) Quantification of inner mucus layer thickness in controls (n=9), UC patients in remission (n=14) and patients with active UC (n=21) by confocal microscopy analysis of ex vivo colonic biopsies, using fluorescent beads (2 µm diameter) to assess mucus penetrability. (D) Absolute quantification of MUC2 in mucus from UC patients (active: 5, remission: 5) and individuals without colonic disease (n=5). For (C) and (D) the line represents the median, the box the interquartile range and the whiskers the range.

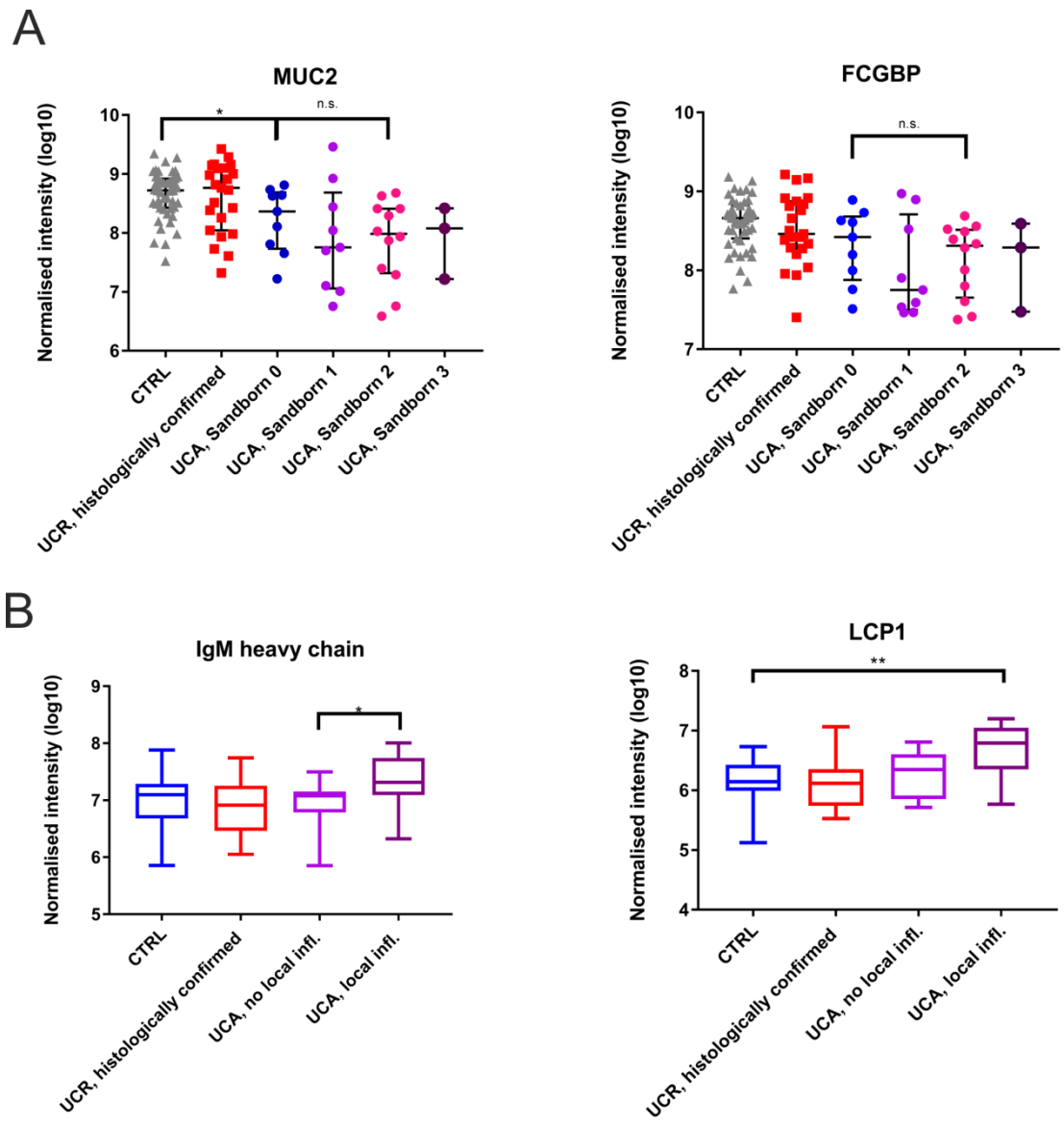

Figure S7 *The reduction in mucus core proteins is not caused by inflammation/tissue damage.*

(A) The abundance of the mucus core components FCGBP and MUC2 in patients with clinically active UC, stratified by sigmoid histology according to the Sandborn score of inflammatory activity. Sandborn score 0 represents normal histology, including a normocellular lamina propria. Data is overlaid by median and interquartile range. Comparisons were performed using the Mann-Whitney U-test, with Bonferroni correction for multiple comparisons. (B) Comparisons of mucus levels of the immune-cell derived proteins IGHM (Immunoglobulin heavy constant mu) and LCP1 (Plastin-2) show a selective increase in patients with locally active inflammation (Sandborn score ≥ 2). The line represents the median, the box the interquartile range and the whiskers the range of values. Comparisons were performed using the Mann-Whitney U-test, with Bonferroni correction for multiple comparisons.

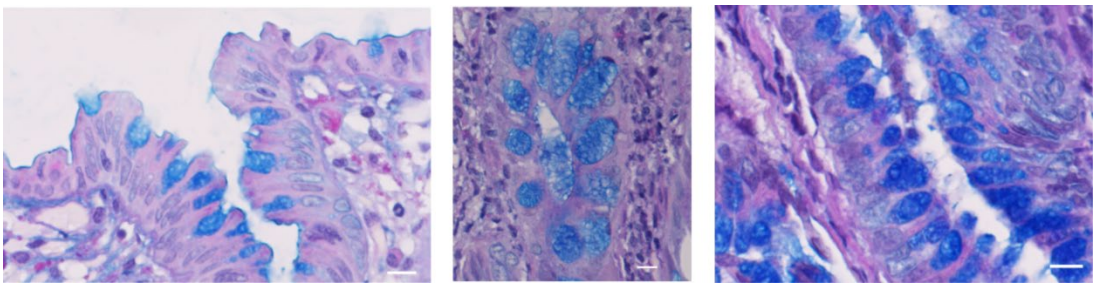

Figure S8 *The attenuation of the goblet cell response to TLR stimulation in active UC is not explained by prior emptying of the mucus content*

Representative images of Alcian blue/PAS-stained sections from sigmoid colon biopsies from active UC patients, obtained after *ex vivo* assessment of mucus growth. The biopsies are from the same study population where quantification of the secretory response to the TLR1/2 ligand P_3CSK_4 was performed. Here, sections from unstimulated biopsies are shown. Each panel represents a different patient. Scale bars: 10 µm.

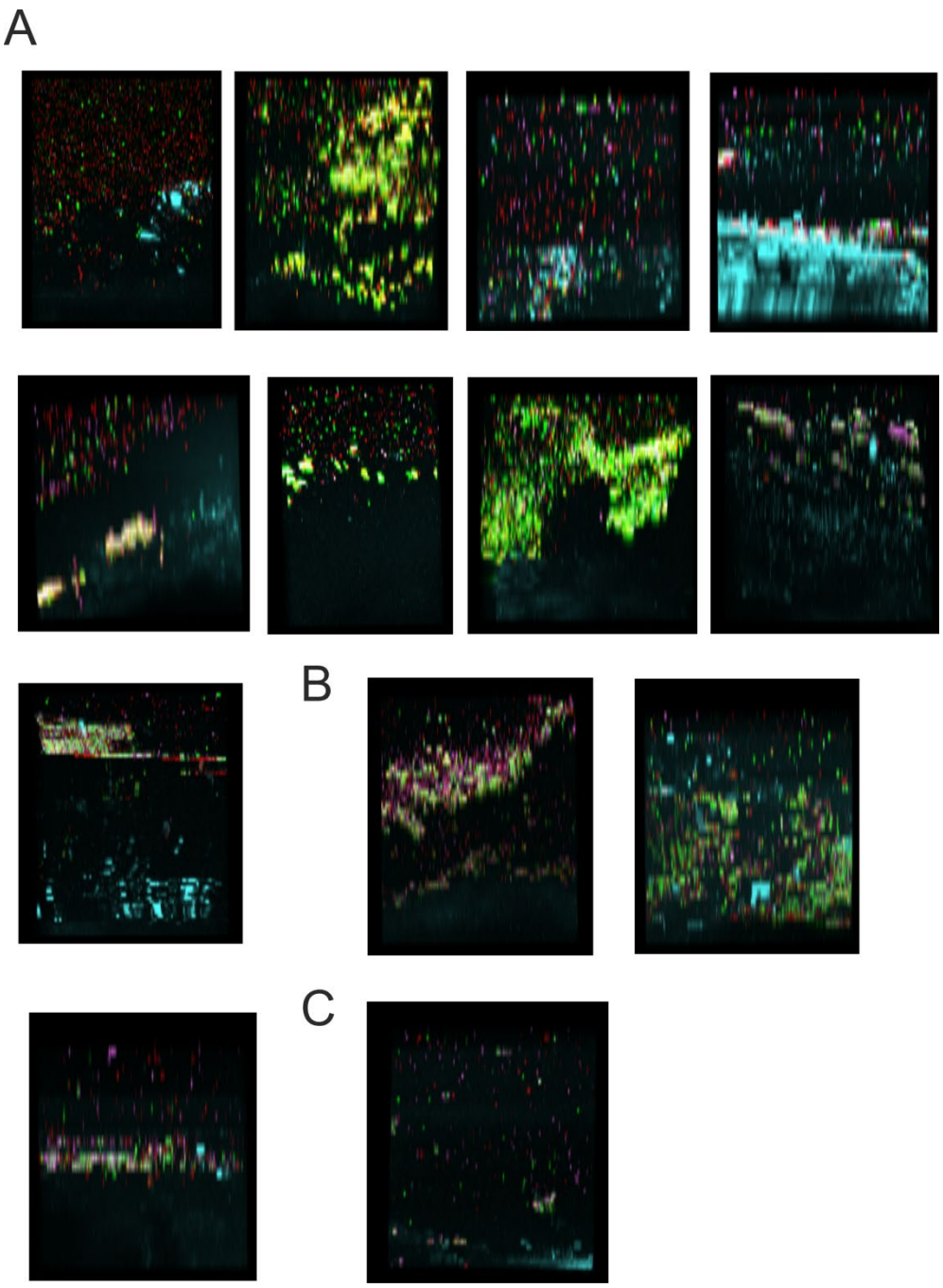

Figure S9 *Representative z-stack images from all biopsies with (semi-)penetrable mucus, acquired by confocal microscopy.*

The blue colour represents the epithelium; red, purple and green dots fluorescent beads with diameters of 0.5 µm, 1 µm and 2 µm, respectively. All images were acquired after the biopsies had been allowed to secrete mucus for one hour in a perfusion chamber with continuous apical and serosal supply of oxygen and nutrients. Fluorescent beads were added on top of the mucus after 20 minutes; i.e., 40 minutes before the images were acquired. (A) Penetrable and semi-penetrable sigmoid colon mucus samples from patients with active UC. (B) Penetrable and semi-penetrable sigmoid colon mucus samples from UC patients in remission. (C) Individual from the control group with penetrable mucus in the sigmoid colon

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