SUPPLEMENTARY MATERIALS AND METHODS

Gene expression analysis

The microarray extraction and analyses are previously described [1, 2].

Human samples

Total RNA was extracted using Ambion *mir*Vana™ miRNA Isolation Kit (Applied Biosystems, CA). RNA quality and integrity was determined using NanoDrop™ Spectrophotometer (Thermo Scientific, DE) and Bioanalyzer (Agilent Technologies, CA). Only samples with high quality and RIN value > 7 were used in microarray analysis. Microarray was done using Illumina human HT-12 expression Bead Chips (Illumina, CA) and the Illumina Bead Station.

For data analysis the R software and Bioconductor package were used [3]. The data were log2 transformed and quantile normalized. Differential expression was identified using limma linear regression [4]. The Benjamin-Hochberg correction for multiple comparisons *P*-values was used with a significance level of < 0.05.

Rat samples

Three endoscopic biopsies from each animal at every time point (2 days before, and 3, 7 and 12 days after colitis induction) were pooled and RNA was extracted using the RNeasy Mini RNA extraction Kit (cat.no. 74106, Qiagen, Hilden, Germany) according to the manufacturer's protocol. Quality of extracted RNA was controlled using NanoDrop™Spectrophotometer and Bioanalyzer. All samples had RIN values > 7 and were deemed suitable for further processing. Biotinylated cRNA was prepared from 400 ng RNA for each sample using the Illumina TotalPrep RNA Amplification kit (Applied Biosystems/Ambion, Austin, TX). Sample cRNA was subsequently hybridized on Illumina human RatRef-12 v1 expression BeadChips (Illumina, San Diego, CA) and scanned on an Illumina BeadStation. Raw data was exported from the Illumina GenomeStudio software and normalized using the *lumi* package for Bioconductor suite [5]. The data were quantile normalized and log2 transformed. Time course differential gene expression analysis was performed using the Bayesian Estimation of Temporal Regulation (BETR) package for R statistical environment [6]. Pairwise group comparisons were performed using a Student *t* test. The fold change (FC) was used to express the changes in average gene expressions between studied groups.

Immunohistochemistry (IHC) and *in situ* hybridization (ISH)

Paraffin embedded sections for IHC and ISH were cut in 4 µm thick sections and mounted on glass slides. The slides had been baked 1 hour at 60 ºC to ensure adherence to the slide and again ½ hour at 60 ºC the day of use before deparaffinization in Neo-Clear® (Merck KGaA, Darmstadt, Germany).

When performing IHC, subsequent immersion in ethanol baths with descending concentration
before blocking of endogenous peroxidase in 3% hydrogen peroxide for 10 minutes, was done. Antigen retrieval was done with TRIS buffer with EDTA at pH 9 with boiling in a microwave oven for 15 minutes. Incubation time with primary antibodies, anti-GN (HPA018215, Atlas Antibodies AB, Stockholm, Sweden; dilution 1:100) and anti-GC-C (HPA037655, Atlas Antibodies AB; dilution 1:50), was 1 hour in room temperature. Secondary antibody (Dako REAL™ EnVision™/HRP, Rabbit Mouse, Dako Denmark A/S, Glostrup, Denmark) was added for 30 minutes. DAB (Dako REAL™ Substate Buffer and Dako REAL™ DAB+ Chromogen, Dako Denmark A/S) reaction time was interrupted with immersion in dH2O after 1 min and 50 seconds for GN and 1 min and 30 seconds for GC-C, respectively, to make in group comparison of immunoreactivity possible. Slides were counterstained with hematoxylin.

ISH was done with the following human target probes GUCA2A (NM_033553, probe region 12 – 507), GUCA2B (NM_007102, probe region 88 - 492) and GUCY2C (NM_004963, probe region 118 – 1104) and rat target probes Guca2a (NM_013118, probe region 2 – 548), Guca2b (NM_022284, probe region 98 - 522) and Gucy2c (NM_013170, probe region 225 – 1606). The biopsies were fixated for 5 days in 4% formaldehyde and embedded in paraffin. We used the RNAscope® 2.0 assay kit (Advanced Cell Diagnostics, Hayward, CA) ISH technology. Here, 20 pairs (depending on design feasibility) of oligoprobes must bind in pairs on the target mRNA transcript to make signal amplification possible, ensuring high sensitivity.

In the ISH procedure, the slides were dehydrated in 100% ethanol and air dried after deparaffinization. Thereafter, peroxidase blocker (Pretreat 1), gentle boiling in Pretreat 2 for 15 min and protease (Pretreat 3) for 30 min at 40 °C was applied. After pretreatment steps, the target probe was applied and hybridized for 2 hours at 40 °C. Subsequently, the amplification steps (Amp 1 – 6) including application of a horseradish peroxidase (HRP)-linked labeling probe were performed prior to DAB-visualization and counterstaining with

hematoxylin. Picture capture of IHC and ISH specimens

Pictures were captured using the Nikon Eclipse E400 microscope and Nikon's NISElements BR 3.00 Imaging Software. Automatic white balance was used and the pictures were stored as TIFF-files.

SUPPLEMENTARY RESULTS

Gene expression of inflammatory cytokines

FC and *P*-values of inflammatory cytokines in IBD (UC and CD) are shown in S1.

Immunoreactivity score (IRS)

Details with sample number, IRS from examiner 1 (ØB) and 2 (MWF) and patient characteristics are shown in S3.