

## **Supplemental Material and Methods**

### **Patients**

Inflammation severity was scored as 0 (no inflammation), 1 (mild inflammation), 2 (medium inflammation) or 3 (severe inflammation) based on previously published criteria.(1-4)  
Responders were defined according to established parameters.(4-6)

### **Mice**

The *Il20<sup>-/-</sup>* (*Il20<sup>tm1(KOMP)Vlcg</sup>*) and *Il20ra<sup>-/-</sup>* (*Il20ra<sup>tm1(KOMP)Vlcg</sup>*) mouse strains were created from embryonic stem cell clones 10036b-C3 and *Il20ra\_AC2* (C57BL/6NTac) generated by Regeneron Pharmaceuticals, Inc.,(7) and made into live mice by the KOMP Repository (<https://www.komp.org> now at <https://www.mmrrc.org>) and the Mouse Biology Program (<https://mbp.mousebiology.org>) at the University of California Davis. We purchased sperm from KOMP and generated live mice by *in vitro* fertilization in the Transgenic Mouse Facility of the Department of Biology, University of Erlangen-Nuremberg. The *Il20rb<sup>-/-</sup>* (*Il20rb<sup>tm1b(KOMP)Wtsi</sup>*) mouse strain was created from embryonic stem cell clone EPD0096\_2\_A08\_M33 (C57BL/6N) generated by the Wellcome Trust Sanger Institute,(8) and made into live mice by the KOMP Repository and the Mouse Biology Program at the University of California Davis. *Il20rb<sup>-/-</sup>* mice were purchased as livestock from KOMP.

The *Stat2<sup>fl/fl</sup>* (conditional knockout mouse model *Stat2-CKO*) on the C57BL/6 background was generated from scratch using CRISPR/Cas9 technology by Applied StemCell, Inc. (Milpitas, CA). Accordingly, two LoxP sequences were inserted into intron 3 and intron 6 of the *Stat2* gene. Qualified gRNA and ssODN along with the Cas9 protein were used for microinjection. For that, a mixture containing *in vitro* transcribed (IVT) active guide RNA molecules (sgRNA), two single-stranded oligo deoxynucleotide (ssODN) donors and the Cas-9 protein was injected into C57BL/6 mice. Tissue was collected from all new born mice and the target region was amplified through PCR. Next generation sequencing libraries were prepared and subsequently sequenced to identify the *Stat2-CKO* mutation. Among the ten tested pups, one mouse had a

DNA sequence with the correct 5'LoxP and the correct 3'LoxP sites. To test for the germline transmission of the Stat2-CKO genotype to F1 mice, this F0 male was bred with C57BL/6 female mice. Genotyping results of the newborn F1 mice indicated that two of the pups were suitable to serve as founders of the colony. We bred these mice over several generations to C57BL/6 mice before crossing them to Villin-Cre transgenic mice,(9) in order to generate mice lacking STAT2 in IECs (*Stat2<sup>ΔIEC</sup>*) on the C57BL/6 background.

*Stat2<sup>-/-</sup>* mice (10) on the C57BL/6 background were purchased from the Jackson Laboratory (JAX stock #023309) and control C57BL/6 WT mice were purchased from Janvier-Labs.

Mice of all strains had normal development and weight gain and did not show any overt phenotype for up to 12 months.

### **Experimental colitis models**

We employed previously reviewer criteria to ensure scientific rigor in colitis experiments,(11) as follows: for Stat2-floxed mice, Cre+ and Cre- littermates were co-housed together; for other strains, we used C57BL/6 mice as the most appropriate wildtype controls. Since a co-housing period of four weeks was sufficient for microbiome equalisation in mice,(12) we mixed the bedding of all cages together four weeks prior to starting a new colitis model, and redistributed the bedding back to the cages every week. Dextran sulfate sodium (DSS)-induced colitis in mice was modeled by the administration of 1.5% or 2% (w/v) DSS (colitis grade, 36-50kDa, MP Biomedicals) in the drinking water for 7 days followed by normal water w/o DSS.(12-13) Oxazolone colitis was induced by intrarectal administration of 0.5% oxazolone (Sigma-Aldrich) in 50% ethanol via a 3.5 F catheter.(12 and 14) Mice were closely evaluated for survival, general condition, body weight, stool consistency and rectal bleeding, and disease activity was scored based on previously described criteria.(12-13) The scoring of inflammation on endoscopy recordings was assessed by two investigators in a blinded manner.

### **Cytokine expression vectors**

For *in vivo* expression of IL-20, a cDNA codon-optimized fragment encoding for murine full-length *IL20* cDNA was synthesized (Eurofins Genomics, Munich, Germany) and cloned into a vector containing hepatocyte specific regulatory elements as described previously.<sup>(15)</sup> DNA was isolated with Qiagen plasmid maxi kits including endotoxin removal and further purified using the MiraClean endotoxin removal kit (Mirus Bio, Madison, WI). 10 µg/mouse of the construct was administered in Krebs–Ringer solution via hydrodynamic tail vein injection. Empty vectors without *IL20* were used as controls.

### **High-resolution mini-endoscopy**

Colitis development was monitored using the COLOVIEW high-resolution video mini-endoscopic system (Karl Storz). Intestinal inflammation was assessed based on criteria previously defined for the murine endoscopic index of colitis severity which scores the following five parameters from 0 to 3: fibrin, granularity, stool, translucency and vascularity.<sup>(16-17)</sup>

### ***In vivo* imaging of neutrophil activity**

Neutrophil infiltration was assessed *in vivo* in real-time using the IVIS Spectrum Preclinical *In Vivo* Imaging System (PerkinElmer). Mice were injected intraperitoneally with 100 µl/25 gram body weight of the myeloperoxidase tracer L-012 (Wako Chemical) dissolved at 20 mmol in H<sub>2</sub>O. Luminescence was measured after 20 minutes in the regions of interest.<sup>(12, 18-19)</sup>

### **Histologic assessment of inflammation**

Inflammation was graded semi-quantitatively according to the degree of inflammatory cell infiltration (subscore 1) and architectural tissue damage (subscore 2) in a blinded fashion.<sup>(20-21)</sup> Zero points were attributed for the lack of inflammatory cell infiltrates as well as for a normal appearance of the colon architecture. The presence of scattered inflammatory cells in the lamina propria was scored as 1; clustered inflammatory cells in the submucosa as 2; a score of 3 was given for transmural infiltrations. Isolated focal epithelial damage was scored as 1; the presence of mucosal erosions/ulcerations was scored as 2; extensive mucosal damage/extension through deeper layers of the bowel wall was scored as 3. Adding up the

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two subscores resulted in a total score ranging from 0, i.e. normal appearance to 6, i.e. widespread cellular infiltrations and extensive architectural tissue damage. The inflammation score was assessed by two investigators in a blinded manner.

### **Immunofluorescence staining, RNAScope and imaging**

Primary antibodies to human CD4 (BioLegend), CD326/EpCAM (BioLegend, clone 9C4), IL-20 (Bio-Techne or PeproTech), IL-20RA (SantaCruz, clone EE09), IL20-RB (Thermo Fisher clone 20RNTC or Sigma-Aldrich), Ki67 (Bio-Techne), pSTAT2 (Cell Signaling Technology or Millipore), pSTAT3 (Cell Signaling Technology), and to mouse CD11c (BioLegend, clone N418), CD45 (BioLegend), CD326/EpCAM (eBioscience, clone G8.8), F4/80 (BioLegend, clone BM8), IL-20 (Bio-Techne), Ki67 (Dako, clone MIB-1), MPO (Abcam), Ulex Europaeus-I (UEA-I, Vector Laboratories), vimentin (Cell Signaling Technology) and glial fibrillary acidic protein/GFAP (Cell Signaling Technology) were used as indicated by the manufacturers. Dye-coupled secondary antibodies were used as recommended by the manufacturer (Cell Signaling Technology or Invitrogen). Primary isotype antibodies or secondary antibody alone served as staining controls. The DeadEnd Fluorometric TUNEL System (Promega) was used to stain late apoptotic cells. Nuclei were counterstained with 4',6-diamidino-2-phenylindole DAPI (BioLegend) or with Hoechst 33342 (Thermo Fisher). RNAScope hybridization using *IL20*-specific human and ACDbio-custom-designed 17ZZ long probes targeting the 2-965 region of murine *Il20* (NM\_001311091.1) were used as indicated by the manufacturer (ACDbio/Bio-Techne) to identify IL-20 in human and mouse tissue, respectively. Images were acquired using a Confocal Laser Scanning Microscope (TCS SP5 II) or a standard immunofluorescence microscope (both from Leica). Positive cells for the target of interest were counted in 2-4 high power fields/staining.

### **Flow cytometry**

Epithelial cells were stained with anti-CD326/EpCAM Alexa Fluor 488 (BioLegend, clone 9C4). Cells were then fixed and permeabilized using the Cytfix Buffer and Phosflow Perm Buffer III

(both from BD) and stained with antibodies against pSTAT3 (Cell Signaling Technology). Data was visualised with FlowJo 10 (BD).

### **Quantitative real-time polymerase chain reaction**

Total RNA was isolated using RNA extraction kits following manufacturer's instructions. (Peqlab/VWR or Macherey-Nagel). Up to 1 µg was reverse-transcribed into cDNA using the Omniscript reverse transcriptase (Qiagen) or the SCRIPT reverse transcriptase (JenaBioscience). Quantitative polymerase chain reaction was performed in duplicates or triplicates on a real-time thermal cycler (CFX or CFX96, both Bio-Rad) using the SsoAdvanced Universal SYBR Green Supermix (Bio-Rad) or the SensiFAST SYBR Kit (Bioline) and QuantiTect primers (Qiagen) specific for human *ACTB*, *IFNG*, *IL20*, *TNF*, *GAPDH* or mouse *Cxcl10*, *Gapdh*, *Mx1*, respectively. Relative mRNA expression was calculated as (Potency (efficiency target gene; - [CT target gene])/(Potency (efficiency reference gene; - [CT reference gene]))).

### **Generation and analysis of intestinal organoids**

Murine small intestine crypts were isolated and cultured as previously described with minor modifications.<sup>(22)</sup> Briefly, small intestines of mice were flushed of feces using ice-cold PBS, cut into pieces of 4-5 mm in length and incubated for 30 minutes at 4°C in 2 mM EDTA/PBS. After being extensively washed with ice-cold PBS, released crypts were passed through a 70-µm cell strainer. Crypts were transferred to DMEM/F-12 (Sigma or Thermo Fisher), counted, pelleted and re-suspended at 100–250 crypts per 50 µl ice-cold Matrigel Growth Factor Reduced Basement Membrane Matrix, Phenol Red-free, LDEV-free (Corning) or Cultrex Reduced Growth Factor Basement Membrane Extract, Type 2, Pathclear (Bio-Techne) and transferred in a pre-warmed 24-well plate (Nunc or Greiner). The plate was incubated for 15 to 20 minutes at 37°C with 5% CO<sub>2</sub> to allow for the gel to solidify, and 450 µl of DMEM/F-12 supplemented with murine EGF (Thermo Fisher), Noggin (Bio-Techne or PeproTech), R-Spondin-1 (Bio-Techne or PeproTech)/R-spondin conditioned medium (produced as indicated

by Bio-Techne). Crypts were passaged after 2-3 days into 48 well plates and allowed to grow in supplemented medium which was freshly added every 2-4 days. Between day 7 and 10, small intestine organoids were used for assays.

Murine colon crypts were isolated and cultured as previously described with minor modifications.<sup>(23)</sup> Briefly, large intestines were flushed of feces using ice-cold PBS, cut into pieces of 4-5 mm in length and incubated for 45 minutes at 4°C in 10 mM EDTA/PBS. After being extensively washed with ice-cold PBS, released crypts were passed through a 100-µm cell strainer. Crypts were transferred to DMEM/F-12 (Sigma or Thermo Fisher), counted, pelleted and re-suspended at 100–250 crypts per 50 µl ice-cold Matrigel (Corning) or Cultrex (Bio-Techne) and transferred in a pre-warmed 24-well plate (Nunc or Greiner). The plate was incubated for 15 to 20 minutes at 37°C with 5% CO<sub>2</sub> to allow for the gel to solidify, and 450 µl of DMEM/F-12 supplemented with B27 (Invitrogen), N-Acetylcystein (Sigma-Aldrich), EGF, Noggin, R-Spondin-1, Wnt Surrogate-Fc Fusion protein (IPA, ImmunoPrecise Antibodies), A83-01 (Tocris), Nicotinamide (Sigma-Aldrich), Y-27632 (Sigma-Aldrich), SB202190 (Sigma-Aldrich) was added. Crypts were passaged after 4-6 days and allowed to grow in DMEM/F-12 supplemented with B27, N-Acetylcysteine, EGF, Noggin, R-Spondin-1/R-spondin conditioned medium (produced as indicated by Bio-Techne), Wnt Surrogate-Fc Fusion protein, A83-01 freshly added every 3-4 days. Colon organoids were used for experiments on days 10-14.

Human colon crypts were isolated and cultured as previously described, with minor modifications.<sup>(24)</sup> Briefly, samples collected from the colons of patients with IBD were minced into pieces of 1mm<sup>3</sup> and digested for 30-45 minutes at 37°C and 140 rpm in 5 ml digestion buffer consisting of DMEM/F-12 (Sigma-Aldrich or Thermo Fisher) supplemented with 10 mM HEPES, 1x GlutaMAX Supplement, 100 U/ml penicillin & 100 µg/ml Streptomycin (all from Thermo Fisher), 100 µg/ml Primocin (Invivogen), 10 µM Rho kinase inhibitor (STEMCELL Technologies) and 5mg/ml collagenase type II (Sigma-Aldrich). Digested tissues was passed through a 100 µm cell strainer, centrifuged (5 minutes at 4°C and 450 x g) and washed 3 times with DMEM/F-12 before being mixed with Cultrex Pathclear or Cultrex Ultimatrix (Bio-Techne)

and plated in multiple 15  $\mu$ l droplets onto a prewarmed 24 well plate (Greiner). The plate was then placed upside-down in a 37°C, 5% CO<sub>2</sub> cell culture incubator to allow droplets to solidify for 20 minutes. The expansion medium consisted of the DMEM/F-12 mixture including the supplements described above (w/o collagenase type II) plus the following reagents: 50 ng/ml human EGF (PeproTech), 100 ng/ml Noggin, 1000 ng/ml R-Spondin-1/R-spondin conditioned medium (produced as indicated by Bio-Techne), 0.5 nM Wnt surrogate (U-Protein Express/ImmunoPrecise Antibodies), 1  $\mu$ M prostaglandin E2 (Bio-Techne), 0.5  $\mu$ M ALK5 inhibitor A83-01 (Bio-Techne), 1x B-27 Supplement (Thermo Fisher), 1.25 mM N-acetylcysteine (Sigma-Aldrich), 10 mM nicotinamide (Sigma-Aldrich) and 10  $\mu$ M p38 inhibitor SB202190 (Sigma-Aldrich). This expansion medium was added to the plate and organoids were grown for 5-7 days before being split by mechanical disruption using a 1ml syringe (B. Braun). After being transferred to 48 well plates, medium (w/o Primocin and w/o Rho kinase inhibitor) was exchanged every 3-4 days and organoids were cultured for another 7-10 days before being used for experiments.

#### Stimulations and analysis of organoids

Organoids were stimulated with IL-10, IL-20 or IFN- $\beta$  (Bio-Techne or PeproTech), or a combination thereof (concentrations and time points are indicated in Figure legends). Control wells received only the vehicle used to dissolve cytokines (i.e. PBS with 0.1% FCS). Light microscopy was used to document the effects of cytokines on organoid growth and bud numbers were observed in 5-10 organoids/well.

Immunofluorescence staining of organoids was performed in suspension as recently described, with minor modifications.<sup>(24)</sup> Briefly, organoids were washed twice with ice cold PBS, fixed for 30 minutes with 4% PFA, permeabilized with 0.2% Triton-X and stained with primary antibodies (IL-20RA, IL-20RB, Ki67) as described above for the immune-stainings. Nuclei were counterstained with Hoechst 33342 and organoids were mounted onto microscopy slides and imaged by confocal microscopy.

### Quantitative assessment of organoid growth and the IFN- $\beta$ induced cell death

Organoid growth was assessed by quantifying the number of buds at different time points as previously described.<sup>(25)</sup> Cell death was investigated in experiments involving the stimulation of organoids with IFN based on a previously published protocol.<sup>(26)</sup> Briefly, after cytokine stimulation and its modulation by Necrostatin-1/Necrosulfonamide, 10 $\mu$ g/mL Hoechst and 10 $\mu$ g/mL Propidium Iodide were added to the wells and the plates were incubated for 30 minutes at 37°C. Afterwards, the medium was exchanged with phenol-red free DMEM/F-12 medium (Thermo Fisher) and the fluorescence of stained organoids was measured from the top, first for propidium iodide (excitation, 535 nm; emission, 617 nm) and after a 30 seconds delay for Hoechst (excitation 361nm; emission 486 nm) using a multiplate reader (Tecan M200). Values of propidium iodide were divided by values of Hoechst and the results are presented as relative fluorescence intensity (RFU) of propidium iodide/Hoechst.

### Determination of organoid viability using MTT-formazan assay

Organoids lost vitality as evaluated by mitochondrial respiration in an assay involving the reduction of yellow-coloured, water-soluble, MTT to dark purple-coloured, water-insoluble, MTT-formazan which is catalysed by mitochondrial dehydrogenase and only occurs in viable cells.<sup>(26-27)</sup> Briefly, after cell death induction, MTT solution was added to the organoid culture to a final concentration of 500  $\mu$ g/ml and incubated for one hour at 37°C. Then, medium was discarded and 20  $\mu$ l of 2% SDS solution in H<sub>2</sub>O was added to each well to solubilize the matrix for one hour at 37°C. Subsequently, 80  $\mu$ l of DMSO was added and incubated for one hour at 37°C to solubilize the reduced MTT. The optical density was then measured at 562 nm in a plate reader (Tecan M200).

Isolation of proteins, co-immunoprecipitation and Western blot analysis. After cytokine stimulations, organoids were washed with ice-cold PBS and then resuspended in Corning Cell Recovery Solution or Cultrex Organoid Harvesting Solution and incubated for 30 minutes on ice under continuous agitation. Proteins were then isolated using RIPA or M-Per extraction



buffers (both Thermo Fisher) supplemented with proteinase inhibitor and phosphatase inhibitors (Roche or Cell Signaling Technology) and PMSF (Cell Signaling Technology). Proteins were denatured in NuPAGE LDS sample buffer (Invitrogen) or Laemmli Sample Buffer (Bio-Rad) with 50 mM DTT (Bio-Rad). The steps described below for the co-immunoprecipitation were performed using materials from Cell Signaling Technology as indicated by the manufacturer. Briefly, cells were first lysed in Cell Lysis Buffer 10x supplemented with Protease/Phosphatase Inhibitor Cocktail (100x) and PMSF and sonicated on ice 3 times with 10 second pulses at 30% amplitude using a Branson Digital Sonifier 450. Lysates were incubated with pre-cleared Protein A Magnetic Beads using the Tube Magnetic Separation Rack before being incubated overnight with either phospho-Stat1 (58D6) rabbit mAb or phospho-Stat3 (D3A7) rabbit mAb or with rabbit (DA1E) mAb IgG Isotype Control (#9167, #9145, #3900 all from Cell Signaling Technology). Lysates that were not subjected to co-immunoprecipitation served as inputs for the western blot analysis. Samples were loaded using the Red Loading Buffer with DTT (Cell Signaling Technology).

Proteins (from lysates of direct isolations for WB or after magnetic isolation for the co-immunoprecipitation) were resolved on 4-15% Mini-PROTEAN TGX gels (Bio-Rad) and transferred on nitrocellulose membranes (Trans-Blot Turbo RTA Mini NC Kit) using the Trans-Blot Turbo Transfer System (all Bio-Rad). Non-specific binding was blocked by incubating membranes in 5% nonfat dry milk (Cell Signaling Technology) in TBS-Tween-20 0.1% for 60 minutes at room temperature. Primary antibodies to pSTAT2 (Millipore or Cell Signaling Technology), STAT2, pSTAT3, MLKL, pMLKL,  $\beta$ -actin or GAPDH (all from Cell Signaling Technology) and secondary horse reddish peroxidase-conjugated antibodies (Cell Signaling Technology) were used as recommended by the manufacturer. Supersignal West Pico Plus, Femto Max or Atto Ultimate Sensitivity Substrate (all Thermo Fisher) were used to detect chemiluminescent signals on a ChemiDoc (Bio-Rad) or an ImageQuant 800 (Amersham/Cytiva) imaging system.

### **RNA-Seq of mouse samples**

Total RNA was extracted from colons of mice and from murine organoids using the peqGOLD total RNA kit (Peqlab/VWR). The quantity and integrity of the isolated RNA was determined using Nanodrop 1000 (Thermo Fisher), Qubit 2.0 Fluorometer (Life Technologies) and Bioanalyzer 2100 (Agilent Technologies). Whole-genome transcriptomic patterns were analyzed on RNA-Seq data (Novogene, UK). Either the Novogene pipeline processing protocol for library preparation, sequencing and analysis (see “Method” as the end of the Supplemental Material and Methods) or our in house steps (described below) were used for further processing of the data.

Trimming was used to clean data by removing reads containing adapter and poly-N sequences and reads with low quality from the raw data. At the same time, Q20, Q30 and GC content of the clean data were calculated. All downstream analyses were based on clean data with high quality.

Mapping of the clean reads to the reference genome *Mus musculus* GRCm38 (genome and gene model annotation files were downloaded from the genome browser website National Center for Biotechnology Information, University of California Santa Cruz or Ensembl) was performed using STAR software. STAR uses multiple seed-based alignment to the maximal mappable length against the reference genome.

FeatureCounts, from the subread package (<https://subread.sourceforge.net/>), was used to count the read numbers mapped of each gene. Differential expression analysis between two conditions or groups (three to five biological replicates per condition) was performed using the DESeq2 R package. DESeq2 provides statistical routines for determining differential expression in digital gene-expression data using a model based on the negative binomial distribution. The data introduced in the algorithm is automatically normalized using the median of ratios method.<sup>(28)</sup> The resulting p values were adjusted using the Benjamini and Hochberg approach for controlling the false discovery rate.<sup>(29)</sup> Genes with an adjusted p value <0.05 found by DESeq2 were assigned as differentially expressed.

Gene-enrichment and functional annotation analysis of differentially expressed genes were performed either with DAVID Bioinformatics Resources (<https://david.ncifcrf.gov/home.jsp>), (30-31) or with the GOstats (<https://bioconductor.org/packages/release/bioc/html/GOstats.html>) R package, in which gene-length bias was corrected. GO terms with adjusted p value < 0.05 were considered significantly enriched by differentially expressed genes. We used the R package ClusterProfiler (<https://bioconductor.org/packages/release/bioc/html/clusterProfiler.html>) to test the statistical enrichment of differential expression of genes in KEGG pathways.

In some figures, Morpheus, <https://software.broadinstitute.org/morpheus> was used to generate heatmaps.

RNA-Seq data available from ArrayExpress (<https://www.ebi.ac.uk/biostudies/arrayexpress>).

#### **RNA-seq of the human IBDome cohort**

Sequence reads were preprocessed and mapped to the human genome GRCh38 and GENCODE v38 annotations using the nf-core RNAseq pipeline version 3.9 [10.5281/zenodo.1400710], (32) with the "star\_salmon" quantification route.

As part of this workflow, reads were trimmed using TrimGalore v0.6.7[[https://www.bioinformatics.babraham.ac.uk/projects/trim\\_galore/](https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/)], a wrapper around cutadapt v3.4 [DOI:10.14806/ej.17.1.200]. Trimmed reads were aligned to the reference genome using STAR version 2.7.10a.(33) Raw counts and transcripts per million (TPM) were quantified with Salmon v1.5.2,(34) using the aligned BAM files as input. Quality control was performed with DupRadar v1.18.0c,(35) FastQC v0.11.9, RSeQC v3.0.1,(36) QualiMap v2.2.2-dev,(37) and Preseq v3.1.1.(38) Quality metrics were aggregated into a MultiQC report.(39) The required software dependencies were obtained as Singularity containers,(40) from the Biocontainers registry.(41)

We tested for outliers and tissue heterogeneity using principal component analysis (PCA) and BioQC.(42) Based on the results, 13 samples were excluded because of wrong tissue labels or an exceptionally high blood content.

We performed differential gene expression analysis between Crohn's disease, Ulcerative colitis and control patients with DESeq2 v1.30.0.(43) False-discovery-rates were calculated using IHW v1.18.0.(44) Genes were considered differentially expressed with an absolute fold change  $> 2$  and a FDR  $< 0.1$ .

Based on the differentially expressed we performed an over-representation test (ORA) of KEGG pathways using the clusterProfiler R package.(45) The over-representation test checks if the list of differentially expressed genes contains more genes from a KEGG pathway than what would be expected by chance.

Sequencing of the human IBDome cohort was performed at the NCCT (NGS Competence Center Tübingen), an initiative of the German Research Foundation (DFG). The RNA-Seq data of human samples have not yet been made publically available but could be provided upon request.

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