**SUPPLEMENTARY METHODS**

**Animal protocols**

Experiments were carried out in both Berlin, Germany at the ECRC as well as the Bergen, Norway at the University of Bergen. Experiments carried out at the ECRC and University of Bergen were approved by the respective local ethics committees and were compliant with the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes. Mice and rats were provided unlimited access to food and water, and maintained on a 12:12, light: dark cycle throughout the experiment. SD rats were aged 19-22 weeks and included both males and females. All mouse experiments were carried out using male C57BL/6J mice aged 8-13 weeks. Specificities regarding the use of animals for experimentation is described in detail below. Wherever applicable, we followed the *ARRIVE* guidelines.

**LPS experiment**

To induce endotoxemia, 4 mg/kg LPS (Escherichia coli 0127:B8, Sigma-Aldrich) dissolved in PBS with 2% bovine serum albumin was injected in the tail vein of anesthetized (2% isoflurane in 100% O2) female SD rats (n =6). A control group (n=6) received a sham injection except for omitting LPS from the injected solution. The rats were kept under continuous isoflurane anesthesia on a servo-controlled heating pad before the experiment was terminated 180 min after injection by a terminal cardiac blood sample and excision of the heart. Immediately thereafter, the rats were transferred to a humidity chamber (100% relative humidity) and duodenum, ileum and colon segments harvested and eluted in 0.9% saline for 24 hours at 4 °C as detailed in the elution method section. The eluates were aliquoted and stored at -80° C for further analysis.

**DOCA-salt experiment**

To induce salt-sensitive hypertension, male (n=3) and female (n=3) WT rats on a Sprague-Dawley background were implanted with a subcutaneous deoxycorticosterone (DOCA) tablet (2 x 150 mg, 50 mg release/week, Innovative Research of America) in combination with 1% saline to drink for 6 weeks. Regular chow was available ad libitum. A control group (female WT SD rats, n=4) without implantation received tap water and regular chow. The experiment was terminated with a cardiac plasma sample and excision of the heart under isoflurane anesthesia. Immediately thereafter the gut was removed, and duodenum, ileum and colon segments harvested in a humidity chamber (100% relative humidity), prepared and eluted as described below. The eluates were aliquoted and stored at -80° C for further analysis.

**Human protocols**

Written informed consent was obtained from all patients and healthy volunteers. All experiments were approved by the institutional board of the Charité-Universitätsmedizin Berlin (EA1/200/17) and conducted in accordance with the principles set out in the declaration of Helsinki. Wherever applicable, we followed the *STROBE* guidelines.

**Centrifugation method**

The centrifugation method is outlined in Wiig et al(3). Modifications to the method were made based on expert opinion to create a method for GI tissue centrifugation for use with mouse or rat specimens. To avoid evaporation, these experiments were performed in a humid environment. An out-of-use incubator provided by the University Clinic in Erlangen was used as a humid chamber. The humid chamber was brought to 100% humidity at room air temperature and was lined with a water repellant surface for sample preparation. The schematic in Fig. 1A illustrates the centrifugation technique as it is described here, though the incubator is not shown. Prior to experimentation one 5 mL Safety cap reaction vessel (Ratiolab) per specimen was weighed with a cut piece of nylon weaved mesh netting (pore size ∼15 × 20 μm, Burmeister AS). The size of the netting was adjusted depending on the animal model used to account for the size difference of their respective GI tissues. A 9 x 9 cm piece of netting was used for rat tissues and 7 x 7 cm for mice tissues. The mesh netting suspends the tissue above the apex of the 5 mL tube while the IF descends into the bottom of the tube during centrifugation. To prevent puncturing delicate GI tissue during preparation, small pieces of 0.5 mm ROTILABO ® silicone hose (Carl Roth) were used to cover sharp surgical tools.

All samples of GI tissue were collected promptly after the sacrifice of each mouse or rat. The distal esophagus to the distal colon was removed, placed immediately in a closed petri dish, and placed inside the humid chamber to avoid evaporation. The sectioning approach varied based on the goal of the experiment and the animal model used and is listed alongside each figure as appropriate. In general, the five segments were collected from the colon, cecum, ileum, jejunum, and duodenum in mice. From rats as the GI tract yields significantly more tissue, non-cecal segments were split into multiple segments for practical reasons. Samples from these segments were not pooled but used separately for analyses. Segments were ligated and cut between the ligations, placed onto mesh netting, then gently positioned inside the 5 mL tube with the netting tucked under the tube cap to suspend the tissue above the tube apex. During preparation, pieces of tissue not yet prepared were kept inside a closed petri dish within the humid chamber. Once all tubes were filled, they were centrifuged together for 10 minutes at 400 x g. After centrifugation, GI tissue and mesh was removed and set aside. Tubes containing centrifuged IF were weighed and ddH20 was added 1:10 as diluent. Diluted IF was then aliquoted and frozen at -80°C.

**Elution method**

The elution approach is described by Wiig et al(3), and was modified for use with GI tissue as shown in Fig. 3A. The full GI tract was removed from each mouse or rat as described above and promptly placed in the humid chamber. As described above, surgical tools were sheathed in silicon hose to minimize damage to the gut. Approximately 2-4 cm sections from the colon, cecum, ileum, jejunum, and ileum were excised and separated (unless otherwise stated). The inner surface of each GI segment was flushed with isosmotic elution buffer to remove any remaining fecal matter using a 1.7 x 50 mm Vasofix® Safety IV catheter (B. Braun Medical Industries) without a needle, which was attached to a 5 mL syringe (B. Braun Medical Industries). Segments were then gently manipulated to remove the remaining elution buffer, ligated, and positioned in a pre-weighed 15 mL tube. Each segment was weighed, and isosmotic elution buffer was added in a 1:10 ratio to the weight. Segments were then held at 4°C and rocked gently on a rocker for a defined time (2-48 hours). After the elution was complete GI segments were removed, and the elution IF was aliquoted and frozen at -80°C.

The isosmotic elution buffer used was dependent on intended use of IF and is listed as such, though for the most part isosmotic saline was used. Mannitol elution buffer was required when ion concentrations were to be measured from elution IF. Due to a matrix-effect, saline elution buffer was required for measurement of SCFA from resultant IF. Prepared isotonic 0.9 % sodium chloride solution for injection (B. Braun Medical Industries) was used as saline elution buffer. Mannitol elution buffer was formulated to an osmolarity of between 285-295 mOsm/kg by adding 5 % pure (99.9999% identity) D-mannitol (Sigma-Aldrich) to double-distilled water (18.2 Ωm). Knauer Semi-Micro Osmometer – Type ML was used to test buffers to ensure the correct physiological osmolarity.

For the elution procedure for human biopsies, two biopsies from respective locations were collected during colonoscopy and immediately placed into a pre-weighed tube containing ice-cold isotonic 0.9 % sodium chloride solution. Samples were incubated on a rocking device at 4 °C for 2 hours. Prior to incubation, biopsy weights were determined. After incubation, biopsies were retrieved and transferred into formalin and the interstitial fluid solution was aliquoted and frozen in liquid nitrogen and stored at -80 °C for further analysis.

**Ion Chromatography**

Sodium and potassium in the eluted tissue solutions and serum were baseline separated in a 10 minutes 7.5-60 mmol/LMSA gradient at a flow rate of 0.2 mL/min by a Dionex IonPac CS 16-4µm RFIC analytical column (2 × 250 mm, P/N 088582) and guard (2x50 mm, P/N 088583) using a Dionex Integrion HPIC System equipped with a CDRS 600 (2 mm) Cation Electrolytic Suppressor and a high pressure EGC 500 methane sulfonic acid eluent generator cartridge. Thereafter ion content was related to tissue wet weight, dry weight, and water content.

**51Cr-EDTAtracer experiments**

*Centrifugation -* Experiments were performed at the University of Bergen (approval ID #10508 and #13922). The radiolabeled isotope 51Cr-EDTA was used as an extracellular tracer. The relative level of the tracer in IF compared with serum was used to assess whether obtained fluid has been diluted by intracellular contents or other compartments. 2 % isoflurane in 100 % O2 was used for anesthesia. During the experiment a servo-controlled heating pad at 37°C was used to maintain body temperature. Male C57BL/6 mice (n=7) were anesthetized and both kidney pedicles ligated to prevent tracer escape during the experiment before 51Cr-EDTA (~ 6 million counts in 100 µL isotonic saline) was injected into the tail vein using an insulin syringe. Mice were kept under continuous anesthesia as 51Cr-EDTA equilibrated within the extracellular fluid phase for 1 hour. After the equilibration period a blood sample was obtained by cardiac puncture and mice were sacrificed by cervical dislocation. Mice were immediately transferred to a humidity chamber with 100 % humidity at room temperature for the rest of the harvesting procedure. The centrifugation protocol was then performed as described above. A small piece of skin was also removed and centrifuged as described previously(6). 51Cr-EDTA from isolated GI IF, skin IF, and serum samples were counted in a gamma counter 1h after collection. Retrieval of 51Cr-EDTA in IF was calculated and expressed as the IF/serum count ratio for each sample from each mouse, respectively. This procedure was performed with minimal modifications in SD rats (n=8) in accordance with centrifugation methods section above. Due to larger body size, rats received a higher dose of chromium (~15 million counts in 100 µL isotonic saline) and the chromium was left to equilibrate for 2 hours (compared to 1hr in mice) prior to sacrifice.

*Elution-* Experiments were performed at the University of Bergen (approval ID #10508 and #13922). 51Cr-EDTA was used to assess the rapidity and repeatability inherent to the elution method as it was applied for use with GI tissue in mice. Mice were continuously anesthetized with 2 % isoflurane in 100 % O2 for the duration of experimentation until sacrifice and body temperature was maintained at 37°C with the aid of a servo-controlled heating pad. After ligation of both kidney pedicles 51Cr-EDTA (~ 14 million counts in 100 µL isotonic saline) was injected into the tail vein with an insulin syringe of male C57BL/6J mice (n=6). The 51Cr-EDTA was equilibrated in the extracellular fluid phase for 1 hour, after which, a blood sample was obtained by cardiac puncture. Mice were then sacrificed by neck dislocation and transferred to a humidity chamber as described above for the rest of the procedure. Elution IF samples were collected as described in the method above using a mannitol-based elution buffer. The samples were placed in a cold room (4°C) on a rocker and after 2, 4, 6, 24 and 48 hours 100 µL from each sample was removed for gamma counting. After 48 hours, all GI segments were removed from the elution buffer and counted. The fraction of 51Cr-EDTA eluted was determined by dividing the count within each elution IF sample at each time point with total counts in each corresponding gut segment prior to elution. Extracellular fluid volume was determined for each gut segment as the plasma equivalent space of 51Cr-EDTA.

**Total tissue water determination**

Total tissue water (ECV and ICV) of a given GI segment was characterized from C57BL/6J mice (n=7) by removing the GI tract as described above. GI tissue was segmented into segments of colon, cecum, ileum, jejunum, and duodenum that were then cut lengthwise to remove fecal contents. Segments were lightly rinsed with 1x DPBS (Gibco), and lightly blotted to remove excess fluid. Cleaned segments were weighed and dried in ceramic dishes in the UF450 drying oven (Memmert GmbH) to dry at 70°C. Tissue segments were weighed after 36 and 100 hours to ensure that all fluid had left the tissue. Weights did not change beyond 36 hours, as each tissue segment had been dried completely. The GI segment dry weight was divided by the respective wet weight to yield the fractional contribution of fluid compartments to the overall tissue weight.

**Sample preparation and protein digestion for proteomics**

*Mouse and rat IF samples*

A quantity of 20 µg of mouse or rat IF was lysed in a 2x SDC-buffer containing 2% Sodium Deoxycholate (Sigma-Aldrich), 20 mM Dithiothreitol (Sigma-Aldrich), 80 mM Chloroacetamide (Sigma-Aldrich), and 200 mM Tris-HCl (pH 8). The lysates were heated for 10 minutes at 95°C and then subjected to digestion with endopeptidase LysC (Wako) and sequence grade trypsin (Promega) at a protein:enzyme ratio of 50:1. Digestion took place overnight at 37°C.

*Human tissue samples*

For human IF samples, the same 20 µg amount was lysed in the 2x SDC-buffer as described. Protein digestion was carried out using trypsin (Promega) at an enzyme-to-protein ratio of 1:20, with the digestion conducted overnight at 37°C.

**Proteomics LC-MS**

*Rat and mouse samples*

The resulting peptides were desalted using stage-tips (24) and then subjected to reversed phase liquid chromatography coupled with mass spectrometry (LC-MS). About 2 µg of peptides were injected into an EASY-nLC 1200 system (Thermo Fisher Scientific) for separation. For rat experiments, a Q Exactive HF-X orbitrap mass spectrometer (HFX, Thermo Fisher Scientific) was utilized, while a Q Exactive Plus (QE+, Thermo Fisher Scientific) mass spectrometer was used for mouse experiments.

Both mass spectrometers were operated in a data-dependent acquisition (DDA) mode. Full scans were conducted at 60K resolution (HFX) or 70K resolution (QE+), followed by data dependent MS2 scans of the top 20 precursors. The MS2 scans were performed at 15K resolution (HFX) or 17.5K resolution (QE+), with ion count targets of 1e5 or 5e4 and isolation windows of 1.3 m/z or 1.6 m/z for HFX or QE+, respectively. A dynamic exclusion of 30 seconds was applied for both setups.

*Human samples*

For human samples, peptides were desalted and separated using the EASY-nLC 1200 system, followed by analysis on an Exploris 480 orbitrap mass spectrometer (Thermo Fisher Scientific) operating in data-independent acquisition (DIA) mode. Full scans were conducted at 120K resolution, followed by MS2 scans with variable window widths. Stepped normalized collision energy settings (26, 29, 32) were used, and the MS2 resolution was set to 30K.

**Proteomics data analysis**

*Rat and mouse samples*

Raw data were processed using the MaxQuant software package (v1.6.10.43 for mouse samples, v1.6.3.4 for rat samples). (25) MS2 spectra were searched against a mouse or rat decoy UniProt database (MOUSE.2019-07; RAT.2019-07) using the Andromeda search engine. (26) Variable modifications included oxidation (M), N-terminal acetylation, and deamidation (N and Q), while carbamidomethylated cysteine was considered a fixed modification. Peptide length was restricted to a minimum of 7 amino acids, with a maximum of three missed cleavages allowed. The false discovery rate (FDR) was set to 1% for peptide and protein identifications. The integrated label-free quantification algorithm was activated. (27) Unique and razor peptides were considered for quantification and the match-between-runs algorithm was turned on. For further data analysis the Perseus software package (v1.6.2.1) was consulted. (28) MaxLFQ intensity values were used for quantification. (27) Reverse hits, contaminants and proteins only identified by site were filtered out. A minimum of three valid values was required in at least one experimental group. Where appropriate, missing values were imputed by random draw from Gaussian distribution with 0.3\*SD and downshift of 1.8\*SD of the observed values per sample. Euclidean distances were used for all cluster-based analyses along an axis. Pearson’s correlation was used to assess the similarities between given samples either individually or by the group mean. Principal component analysis (PCA) with Euclidean distances was used to assess similarities between samples. An FDR correction of 5% was applied for statistical testing unless otherwise stated.

*Human samples*

Raw data for human samples were analyzed using DIA-NN version 1.8.1 (29) in library-free mode. The analysis involved searching against a human Uniprot fasta file with isoforms (HUMAN.2022-03). Subsequent data analysis was carried out in R using the report.tsv output. Precursors were filtered based on Q-values (Q.value ≤ 0.01, Protein.Q.value ≤ 0.01, Lib.Q.Value ≤ 0.01), and LFQ intensities were averaged across precursors and collapsed to protein group level. Protein groups were filtered for a minimum of 75% valid values across all conditions before applying downshift imputation. Differential expression analysis employed the paired Welsh test, and the Benjamini-Hochberg correction was used for multiple hypothesis testing.

**Measurement of SCFA using GC-MS**

SCFA from serum, and IF samples were measured as per the method described previously with minor modifications.(30) Briefly, 90 µL of murine serum or IF were extracted by shaking samples in 100 µL diethyl ether and 10 µL HCl at 25°C for 30 min. To account for low sample volume, predilution of samples with diluent of ddH2O was used where appropriate. Samples were then centrifuged for 5 min at 1500 x g, after which time, 50 µL of the ether phase was transferred into GC-MS glass vials. 10 µL MTBSTFA was added for derivatization and samples were held at 80°C for 30 min, and subsequently held overnight incubation at room temperature. Human serum samples were handled equally. For human IF samples, 45 µL of sample were extracted with 45 µL diethyl ether, 5 µL HCl and 25 µL of 60% (w/v) NaHSO4. Derivatization was performed on 20 µL ether phase with 4 µL MTBSTFA. Spike in of 100 µM crotonic acid was used as an internal control for all samples. 1000 µM sodium acetate was added to murine serum samples to increase the signal above background, hence 1000 µM was subtracted after quantitation from these samples. A dilution series of Volatile Free Acid Mix (CRM46975, Sigma Aldrich) and pure ddH2O were prepared in parallel and measured with each run for absolute quantification. SCFA calibration curve in a saline-based elution buffer was similar to in ddH20, therefore this buffer was used for all experiments.  GC-MS analysis was performed on a Thermo Scientific™ Q Exactive™ hybrid quadrupole Orbitrap mass spectrometer, coupled to a Thermo Scientific™ TRACE 1300 Series gas chromatograph and a Thermo Scientific™ TriPlus RSH Autosampler. For murine samples, 1 µL and for human samples, 4 µL were injected, each with a split of 1:10. Gas-chromatographic separation was performed with an initial temperature of 68°C, held for 2 minutes, followed by a 7 °C/minute ramp until 150 °C and a final 50 °C/minute ramp until 300 °C, held for 2 minutes. Full MS was acquired at a resolution of 60,000 and a scan range of 65 to 600 m/z. Thermo Scientific™ Xcalibur™ Quan Browser Software was used for data analysis. If extraction was not successful or internal standards were not measurable these samples were removed from the analysis.

**Multiplex cytokine analysis of IF**

To characterize the intestinal cytokine profile, IF isolated from human biopsies as described above were analyzed using the human V-Plex Plus Human Cytokine kit (IL-1, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-16, IL-17, TNFα, TNFβ, IP-10, INF) from Meso Scale Diagnostics according the manufacturer’s instructions. Analysis was performed at the Immunological Study Lab of CheckImmune GmbH on a Mesoscale Discovery platform. For each sample, the respective value of the electrochemiluminescence (RFU-Signal) of the analyte concentration was calculated based on a calibration curve. Measurements of the Meso Scale Diagnostics assay were performed and evaluated in accordance with the ICH-GCP Guideline ‘Validation of analytical procedures’.

**Cytokine and SCFA calculations for tissue fluid space of biopsies**

To determine the expected concentration of respective cytokine or SCFA within the total tissue fluid space of eluted biopsies, the following calculations were performed. First, the concentration of cytokine or propionate per gram of tissue (μM/g or pg/g) was calculated using the following formula:

*(Concentration (μM or pg/mL) determined in the elution IF x total elution fluid volume (mL)) / tissue weight (g)*.

Next, the concentration of cytokine or propionate in the total tissue water (μM/mL or pg/mL) was calculated using the following formula:

*Concentration of cytokine or propionate in the tissue (μM/g or pg/g) / 0.79 (mL/g).*

**Targeted microbiome PCR assays**

QPCR gene targeting assays were used to quantify propionate (C3) production potential in different sections of the rat gut. Bacterial gDNA was isolated from content extracted from the colon, ileum, jejunum and duodenum of mice using the ZymoBIOMICS DNA miniprep kit. Primers for the 16s rRNA (V4) gene of Bacteroidetes (GAAGGTCCCCCACATTG and CTTTGAGTTTCACCGTTGCCGG) were used for phylum level amplification. Primers designed against the *mmdA* enzyme in *Bacteroides vulgatus*/*Bacteroides ovatus*/*Bacteroides thetataiotaomicron*/*Bacteroides fragilis* (GTTTCTGCGATGCGTTCAATA and CGGAAGGAATCCCGGTACAT) were used to amplify the *mmdA* enzyme in the main bacterial C3 producers. Universal 16s primers (ACTCCTACGGGAGGCAGCAGT and GTATTACCGCGGCTGCTGGCAC) were used to amplify the overall bacterial content. Amplication was performed with 30 ng template DNA in a Quantstudio 5 Real-Time PCR system (Thermo Fisher) using the PowerUp Sybr green master mix (Thermo Fisher) with 50oC for 2 min, 95oC for 3 min followed by 40 cycles of 95oC for 2 s and 60oC for 30 s. Gene copies were determined using the standard curve method.

**Statistical analysis**

Statistical methods applied were dependent on the experimental design and are listed appropriately alongside each figure. All analyses were performed in a blinded manner. Data were examined for outliers using the ROUT outlier test. Outliers were removed when appropriate. Normal distribution was tested with Shapiro-Wilk test. Where when sequential measurements were taken from one biological sample, repeated-measures one-way ANOVA with Tukey’s multiple comparisons test was used given that these data are considered paired. To assess differences between segments or measurements that were normally distributed and not paired, ordinary one-way ANOVA with Tukey’s multiple comparisons test was used. When the data were not normally distributed the Kruskal-Wallis test corrected for multiple comparison by controlling FDR (Benjamini, Krieger, Yekutieli) was used. Ordinary one-way ANOVA with Dunnett’s post-hoc test was applied when comparing multiple groups to a reference value. Unpaired two-tailed Student’s t-test and two-way ANOVA testing were used depending on the variables being interrogated. P values of less than or equal to0.05 were considered statistically significant. Statistics particular to the proteomics methods are listed within the respective methods section above. All other statistical analyses were performed using GraphPad Prism 9. The analysis plan was developed before the start of the study. All study data are available on request.

**SUPPLEMENTARY LEGENDS**

**Figure S1. Elution-based extracellular fluid isolation within the GI tract of C57BL6/J mice.** A) Schematic of the tissue segments harvested for analysis from C57BL/6J mice (the cecum was used only in (C)). B) Extracellular fluid volume was determined by counting 51Cr-EDTA for each intestinal segment compared to the plasma equilibration of the tracer (n=6). In (C) the total tissue water is shown from C57BL/6J mice (n=7). Significance was tested using an ordinary one-way ANOVA with Tukey’s multiple comparison test. \*p≤0.05, \*\*p≤0.01, \*\*\*p≤0.001.

**Figure S2. 51Cr-EDTA elution indicates the rapid equilibration of this extracellular tracer with the surrounding elution buffer.** A)The extracellular tracer 51Cr-EDTA was used to determine the rate at which the elution fluid equilibrates with the IF from C57BL/6J tissue. In (A-D), at each time point, 100 µL of sample was taken from the eluted solution for gamma counting. The eluted fraction for each segment was determined by dividing the counts in the eluted sample at each time point by the total counts in the corresponding intestinal sample prior to elution. Data (n=6 for all conditions) were tested using repeated measures one-way ANOVA with Tukey’s multiple comparisons test and significant post hoc comparisons are shown. \*p≤0.05, \*\*p≤0.01, \*\*\*p≤0.001, \*\*\*\*p≤0.0001.

**Figure S3.** Bacteroidetes is highly abundant in the colon compared to the other gut sections (A), likewise the *mmdA* enzyme for C3 production expressed in the Bacteroides species is only detectable in the colon (B). The relative abundances were measured using primers for the 16s rRNA V4 for Bacteroidetes and the *mmdA* enzyme in Bacteroides for the C3 production. The abundance is expressed as a percentage of the overall bacterial load, determined using universal 16s rRNA primers. N=4 samples for each of the segments. One value in duodenum panel B was excluded after testing for outliers using the ROUT outlier test. Normal distribution was tested with Shapiro-Wilk test. Data in (A-B) were tested by ordinary one-way ANOVA with Tukey’s multiple comparison test; \*p≤0.05, \*\*p≤0.01.

**Figure S4.** LPS plasma samples were significantly different from all other groups. N=4-6 plasma samples per group were analyzed after testing for outliers using the ROUT outlier test. Normal distribution was tested with Shapiro-Wilk test. Significance in (A) was tested using a two-tailed Mann-Whitney U test and in (B-C) using a two-tailed unpaired *t*-test. \*p≤0.05, \*\*p≤0.01, \*\*\*\*p≤0.0001.