**Supplementary data to the article “Microbiota-related Changes in Bile Acid & Tryptophan Metabolism are Associated with Gastrointestinal Dysfunction in a Mouse Model of Autism” by Golubeva A.V. et al.**

**Supplemental Methods**

***Study design*** Three independent cohorts were used in this study (Fig S1). Animals were randomly assigned to different cohorts using a random number generator. In **cohort 1**, mice at 8 weeks of age were subjected to a battery of behavioral tests over 6 weeks (n=10 in each group). Behavioral tests were performed in the least stressful-to-the most stressful order. At least 2 days of recovery were allowed between tests. In addition to behavioral screening, we measured plasma corticosterone levels in the forced swim test to assess the HPA axis response to an acute stressor, and whole intestinal transit *in vivo*. Three days following the last test, animals were sacrificed; body and organ weights were recorded; blood and tissues samples were harvested for the analysis. Intestinal permeability was measured in distal ileum in Ussing chambers *ex vivo*. Whole mount preparations of distal colon segments were fixed for the morphological analysis of the myenteric plexus. Serotonin levels and gene expression in the gut, as well as caecal microbiota composition and short chain fatty acids (SCFAs) levels were further analyzed in these animals. In **cohort 2**, colonic transit was assessed *ex vivo* in naïve mice at 8 weeks of age (n=8 in C57BL/6 and n=6 in BTBR group). Plasma and feces were collected for the analysis of bile acids; liver samples were harvested for the gene expression analysis. In **cohort 3**, intestinal permeability was measured *ex vivo* in the colonic samples of naïve animals at 14 weeks of age (n=10 in each group). Plasma and gut tissue samples were taken for the analysis of tryptophan and kynurenine levels. Initially, the sample size of the study was calculated using G\*Power software (independent two-sided t-test, 0.05% significance level). Later, the size of the groups was adjusted to the efficacy of breeding.

***Three-chamber social approach task*** Sociability and preference for social novelty were analyzed in the three-chamber test as previously described ([Desbonnet et al., 2014](#_ENREF_4)), with minor modifications. A test mouse was placed into a rectangular apparatus (36×19×30 cm, L×W×H) divided into 3 inter-connected chambers. The test procedure was comprised of three 10 min exploration trials, each starting from the middle chamber. 1) Habituation trial: the arena had two empty mesh wire cages symmetrically positioned in side chambers. 2) “Mouse vs. Object” trial: an object and an age-, sex- and strain-matched unfamiliar mouse were placed into the wire cages in either the left or right side chamber. 3) “Novel vs. Familiar Mouse”: an object was replaced with an unfamiliar mouse serving as a novel mouse. The test was conducted under normal room lighting (~60 lux). Behaviors were video recorded, and the amount of time spent exploring an object or a mouse in each chamber was blindly scored in Ethovision XT software (Noldus, Nottingham, UK). Preference in exploration shown by a test mouse was assessed in % according to the formulas: t [mouse] / (t [mouse] + t [object]) and t [novel mouse] / (t [novel mouse] + t [familiar mouse]).

***Social transmission of food preference test (STFP)*** The STFP test was performed as described previously ([Desbonnet et al., 2014](#_ENREF_4)). 18 h prior to testing, mice were deprived of food, whereas water was given *ad libitum*. A demonstrator mouse was randomly selected from each cage and individually exposed for 1 h to food choices consisting of either 1% ground cinnamon or 2% powdered cocoa (Drinking Chocolate, Cadbury Ltd.) mixed with ground mouse chow. After that, a demonstrator mouse was placed back into the home cage for a 20 min interaction session with cage-mates. Subsequently, cage-mates were individually tested for the preference of cued food (transmitted by the demonstrator) over novel food for 20 min. The choice session was repeated 24 h later. Food containers were weighed before and after each choice session to estimate the food consumption. The test was conducted under normal room lighting (~60 lux). Preference for cued food was calculated in % according to the formula: g [cued food] / (g [cued food] + g [novel food]).

***Resident-intruder test*** This test allows to evaluate the manifestation of aggression as an important aspect of social behavior in rodents (Burokas et al., 2012). An age-, sex-, weight- and strain-matched intruder mouse was placed into the resident's home cage for 10 min. The test was conducted under normal room lighting (~60 lux). Interaction behaviors were blindly scored from video recordings. The latency to first approach, aggressive (attack or menace) interactions, as well as non-aggressive (general sniffing, anogenital sniffing, grooming and touching) interactions of the resident mouse with its counterpart were quantified (in sec). Neither BTBR nor C57BL/6 mice demonstrated aggressive behavior in this test; as such, only non-aggressive interaction was analyzed. Data were calculated in % according to the formula: t [interaction]/ t [total].

***Marble burying test*** This test assesses compulsive and anxious behavior ([Savignac et al., 2014](#_ENREF_20)). A test mouse was placed for 30 min in a novel cage (38×55×18 cm, L×W×H) with 20 marbles equally distributed on top of a 4 cm deep bed of sawdust. The test was conducted under normal room lighting (~60 lux). The number of buried marbles (covered by bedding 2/3 or more) was blindly scored.

***Self-grooming test*** Mice were scored for spontaneous grooming behavior as described earlier ([Yang et al., 2007](#_ENREF_23)). A test mouse was placed into a novel cage (33×15×13 cm, L×W×H) under normal room lighting (~60 lux). Following a 5 min habituation period, the mouse was blindly scored for total time spent grooming all body regions for 15 min (in sec).

***Open field (OF) and elevated plus maze (EPM) tests*** To assess locomotor activity and the response to a novel stressful environment, mice were subjected the OF and EPM tests as described ([Savignac et al., 2014](#_ENREF_20)), with minor modifications. In the EPM test, a test mouse was placed in the center of the maze facing an open arm, and behavior was video recorded for 5 min in dim red light (~7 lux). Numbers of open and closed arms entries were blindly scored in Ethovision XT; open arm entries were presented as % of total number of entries. The first day of habituation in the novel object recognition test arena (see below) was used as the OF test: a test mouse was allowed to explore the arena for 10 min. Behavior was video recorded, and the following parameters were blindly scored in Ethovision XT: total distance travelled (cm); latency to enter the center (sec), number of entries into the center and time spent in the center (sec). In both tests, number of faecal pellets was recorded to estimate stress-induced defecation.

***Forced swim test (FST)*** To assess depressive-like behavior, mice were subjected to the FST as described ([Savignac et al., 2014](#_ENREF_20)). A test mouse was placed in a transparent plexiglas cylinder, containing 15-cm-depth water (25°C) under normal room lighting (~60 lux). Behavior was video typed for 6 min; time spent immobile (in sec) was blindly scored during the last 4 min of the test.

***Novel object recognition (NOR) test*** This test assesses the ability of an animal to discriminate between familiar and novel objects. The NOR protocol was adapted from ([Burokas et al., 2014](#_ENREF_1); [Desbonnet et al., 2015](#_ENREF_5)). Following habituation to the open-topped, rectangular arena (40×32×23 cm, L×W×H) on day 1, a test mouse was exposed for 10 min to two identical objects placed in the corners of the arena (acquisition phase). Following 24 h, one of the familiar objects was substituted with a novel object, and the animal was allowed to explore the objects for 10 min (retention phase). The test was conducted under normal room lighting (~60 lux). Animal behavior was video recorded; time spent in exploration of the objects was blindly scored in Ethovision XT. Exploration behavior was defined as orienting the nose towards the object at a distance < 2 cm, or direct contact with the object. Discrimination index was calculated according to the formula: (t [novel] − t [familiar]) / (t [novel] + t [familiar]). Number of faecal pellets was recorded to estimate stress-induced defecation.

***Plasma corticosterone in response to an acute stressor*** Blood samples were collected via tail incision prior to (baseline) and after the onset of the forced swim test. Plasma corticosterone was measured by ELISA (Enzo Life Sciences, NY).

***Plasma oxytocin levels*** Plasma oxytocin was measured by radioimmunoassay (RIAgnosis, Germany).

***Faecal water content*** Faecal samples were weighed before and after desiccation at 60°C for 16 h ([Lomasney et al., 2014](#_ENREF_14)). Water content was calculated as % from the difference in weight.

***Whole intestinal transit*** The assay is based on the propulsion of non-absorbable colored dye through the intestinal tract ([Nagakura et al., 1996](#_ENREF_18)). A test mouse was administered with 200 μL of 6% carmine dye in 0.5% methylcellulose by oral gavage. The latency (min) for the excretion of the first red-colored faecal pellet was blindly scored.

***Colonic transit ex vivo*** The assay is based on the *ex vivo* analysis of spontaneous faecal pellet propagation along the murine colon ([Heredia et al., 2013](#_ENREF_11)). In cohort 2, whole colon was carefully excised, emptied, and loosely pinned onto a Sylgard-coated organ bath. The bath was perfused with Krebs buffer (1.2mM NaH2PO4, 117mM NaCl, 4.8mM KCl, 1.2mM MgCl2, 25mM NaHCO3, 11mM CaCl2 and 10mM glucose) at 6.0 mL/min rate, and heated to +35**°**C. Artificial pellet (3.0 mm in diameter and 8.0 mm in length, made in-house) was inserted into the proximal end of the colon, and spontaneous propagation of the pellet in proximal-to-distal direction was video recorded. The following parameters were blindly scored: latency to initiate propagation (min) and anterograde propulsive activity of the colon (distance travelled (mm) / time (min)).

***Permeability of intestinal epithelium in Ussing chambers ex vivo*** Distal ileum (a 2.0 cm segment taken ~2.0 cm proximally to the caecum, cohort 1) and middle colon (a 2.0 cm segment, cohort 3) samples were mounted in Ussing chambers with exposed tissue area of 0.12 cm2. No seromuscular stripping was performed. Short-circuit current (*Isc*, µA/cm2) was recorded in zero voltage clamp mode; transepithelial electrical resistance (TEER, Ω•cm2) was measured by discharging a 2 mV pulse across the tissue and measuring the change in *Isc* ([Golubeva et al., 2015](#_ENREF_9)). Permeability of epithelial layer to macromolecules was measured via paracellular flux of 4 kDa FITC-dextran (FD4, Sigma, Ireland). FITC was added to the mucosal chamber at a final concentration of 2.5 mg/mL. To assess serosal-to-mucosal FITC flux across the epithelial layer, 200 μL samples were collected from the serosal chamber every 30 min for the following 3 h. FITC was measured at 485 nm excitation/535 nm emission wavelengths. FITC flux was calculated as an increment in fluorescence intensity vs. baseline fluorescence in the serosal compartment, and presented in ng/mL.

***Myenteric plexus morphology*** The morphology of colonic myenteric plexus was described in whole mount preparations of distal colon in cohort 1. A 1.5 cm segment of distal colon was pinned to the bottom of Sylgard-coated Petri dish, opened along the mesenteric line and stretched to a 1.0 x 2.0 cm (W x L) rectangle. Tissue was fixed with 4% PFA and stored in PBS with 0.02% NaN3 at +4°C. Six animals from each group were randomly chosen for morphological analysis. Whole mounts were stripped of mucosa/submucosa, and the remaining muscular layers with myenteric plexus between them were subjected to immunoflourescent staining. The proximal 1.0 x 1.0 cm segment was co-stained against pan-neuronal marker HuC/HuD (A-21271, ThermoFisher/Molecular Probes, RRID: AB\_221448) and neuronal nitric oxide synthase (nNOS, sc-648, Santa Cruz, RRID: AB\_630935). The distal 1.0 x 1.0 cm segment was stained against choline acetyltransferase (ChAT, AB144P, Millipore, RRID: AB\_2079751), and co-stained with DAPI to label cellular nuclei. Multi-layered stack images of whole mount preparations were acquired on the Olympus FV1000 confocal laser scanning microscope. For each tissue specimen, four visual fields (1270 x 1270 μm) were blindly chosen within the areas where myenteric plexus was intact, and acquired using a 10x objective (for anti-ChAT staining) and a 20x objective (for anti-HuC/D and nNOS staining). The number of Z-planes varied depending on the thickness of the selected region: 20 ± 4 for ChAT and 25 ± 3 for HuC/D and nNOS.

About 80% of myenteric neurons express ChAT ([Furness, 2000](#_ENREF_7)); therefore, we used this staining to describe the general structure of the myenteric plexus. For this, the Z-stacks containing myenteric ganglia and interconnecting nerve tracts were processed with ImageJ-FijI software package (NIH). The images were flattened with Z-Project function and filtered with Median Filter to remove noise and small interganglionic fibres, while preserving the overall geometry of the myenteric plexus. After that they were segmented using Auto Threshold or Auto Local Threshold function and smoothed again with Close function. Numerical metrics for quantification of the myenteric plexus were adopted from J. Gao et al. with modifications ([Gao et al., 2013](#_ENREF_8)). The relative area (% of visual field area) and contact ratio (area (μm)/perimeter (μm)) of myenteric plexus, as well as the mean thickness of myenteric ganglia (μm) and the mean diameter of interganglionic spaces (μm) were assessed. For measuring the mean thickness of myenteric ganglia BoneJ ImageJ plugin was employed.

For HuC/HuD- and nNOS-labelled specimens, the Z-planes containing myenteric neurons were flattened, and the contrast was enhanced. For aiding cell quantification, the look-up table of the HuC/HuD containing channel was inverted and turned greyscale. HuC/HuD- and nNOS-positive neurons were blindly quantified, and expressed as neuronal count per visual field.

***Tryptophan, kynurenine and serotonin measures*** Serotonin was measured by HPLC coupled to electrochemical detection ([Julio-Pieper et al., 2012](#_ENREF_13)). Serotonin HPLC mobile phase consisted of 0.1M citric acid, 0.1M sodium dihydrogen phosphate monohydrate, 5.6mM octane sulphonic acid, 10µm EDTA in 10% methanol solution adjusted to pH 2.8 using 4N sodium hydroxide. Mobile phase was filtered through Millipore 0.45 µm HV Durapore membrane filters. Full thickness colon and ileum tissue were weighed and placed in ice-cold mobile phase spiked with N-methyl-5-hydroxytryptamine (0.1 ng/µL) as an internal standard. Each sample was sonicated (Sonopuls HD 2070, Bandelin, Germany), centrifuged at 14000g for 15 minutes at +8°C, and the supernatant was stored at -80°C until the analysis.

Sample supernatants were diluted 1:10 in mobile phase immediately prior to analysis on the HPLC system. 20 µL of the diluted supernatant was injected onto the HPLC system, which consisted of a SCL-10Avp system controller, LC-10AS pump, SIL-10A auto-injecter, CTO-10A oven and an LECD 6A electrochemical detector. A reverse phase column (Kinetex, 2.6u C18 100 x 4.6 mm, Phenomenex, UK) was utilized to facilitate separation with a flow rate set at 0.9 mL/min. Chromatograms were generated using Class-VP 5 software. Serotonin was identified by its characteristic retention time as determined by standard injections, which were run at regular intervals during the sample analysis. Analyte:internal standard peak height ratios were measured and compared with standard injections, and results were expressed as µg of neurotransmitter per gram of tissue.

Kynurenine and tryptophan concentrations were determined in plasma by HPLC coupled to ultraviolet and fluorescent detection, accordingly ([Clarke et al., 2009](#_ENREF_3)). Kynurenine/tryptophan HPLC mobile phase consisted of 50 mM acetic acid, 100 mM zinc acetate with 3% acetonitrile. Mobile phase was filtered through Millipore 0.45 µm HV Durapore membrane filters. Plasma was spiked with the internal standard 3-nitro-L-tyrosine, along with 6% perchloric acid to deproteinate samples. Samples were centrifuged at 10000g for 15 minutes at +4°C, and the supernatant was stored at -80°C until the analysis.

20 µL of the supernatant was injected on the HPLC system, which consisted of a Waters 510 pump, 717plus cooled autosampler, a 996 PDA detector, a Hewlett Packard 1046A fluorescent detector (Waters Ireland, Dublin, Ireland), a Waters bus SAT/IN module and a croco-cil column oven. Samples were injected onto a reverse phase Luna 3µ C18(2) 150 x 2 mm column (Phenomenex, UK), which was shielded by disposable Krudkatcher pre-column filters and security guard cartridges (Phenomenex, UK). Separations were achieved by isocratic elution at 0.3 mL/min. The PDA detector start wavelength was set at 210 nm and end wavelength at 400 nm, with chromatogram extraction at 330 nm. The fluorescent detector was set to an excitation wavelength of 254 nm and an emission wavelength of 404 nm. Analytes were identified based upon their characteristic retention times as determined by standard injections. The concentration of tryptophan and kynurenine were determined using analyte:internal standard peak height ratios which were then compared to standard injections which were run at regular intervals during the sample analysis. Results were expressed as ng or µg of analyte per mL of plasma.

***Gene expression analysis with real-time RT-PCR*** Distal ileum (a 1.0 cm segment taken ~4.0 cm proximally to the caecum), middle colon (a 1.0 cm segment), the front lobe of the liver and the right hippocampus were used for gene expression analysis in cohort 1. RNeasy Plus Universal Mini kit (Qiagen, Manchester, UK) and *mir*Vana™ miRNA Isolation Kit (Ambion, Life technologies, Waltham, MA, US) were used to extract total RNA from intestinal/liver and brain tissues, respectively. Reverse transcription and real‐time PCR were performed as described ([Golubeva et al., 2014](#_ENREF_10)). Genes of interest were amplified with TaqMan probes designed by Applied Biosystems (Waltham, MA, US) and Integrated DNA Technologies (Coralville, IA, US). Primers’ IDs are listed in Table S1. PCR was run in triplicates on the LightCycler® 480 System (Roche, Basel, Switzerland). Data were analyzed with the comparative cycle threshold (Ct) method. First, the averaged Ct values for each target gene were normalized to the endogenous housekeeping gene (*Actb* in brain/liver samples and *Gapdh* in intestinal samples), and transformed to the relative number of copies with 2−ΔCt equation. Second, relative gene expression levels in BTBR group were normalized to C57BL/6 group, and presented as a fold change.

***Bile acids levels in plasma and faecal material*** Bile acids were extracted according to Joyce et al. ([Joyce et al., 2014](#_ENREF_12)). For the faeces, 80 mg of faecal material was added to dynabeads (Roche) with 300 μl of ice cold 50% methanol containing deuterated internal standards of both Cholic acid and Chenodeoxycholic acid, then subjected to five 30 second rounds of extraction in a dyna-lyser machine (Roche) at 6000 rpm. For plasma extractions, 100 μl was mixed with 300 μl of ice cold 50% methanol also containing deuterated internal standards. Each mixture was vortexed and then centrifuged for 10 mins at 10000 g and the supernatant transferred to a fresh tube. Ice cold acetonitrile (ACN) with formic acid measuring 2 ml was added to each tube, vortexed and agitated at room temperature for 1 hour. Samples were centrifuged again to pellet the debris and the supernatant was added to fresh tubes containing 1 ml of ice cold 100 % ACN. The samples were vortexed and dried under vacuum at +4°C. The dried extracted acids were re-suspended in 150 μl of ice cold 50 % methanol.

***Chemicals*** Standard Conjugated bile salts and free bile acids were purchased from Sigma Aldrich and from Steraloids, Inc. (Newport, Rhode Island). HPLC-grade methanol, acetonitrile, water, ammonium acetate, ammonium formate, ammonium hydroxide, formic acid, and acetic acid and water were obtained from Fisher Scientific (Fair Lawn, NJ). Deuterated cholic acid (D-2452) and deuterated chenodeoxycholic acid (D-2772) were purchased from CDN Isotopes Inc. Standards were constructed as 1mg/ml stock solutions of individual sulfated BAs were prepared in water:MeOH (1:1). They were subsequently combined to a final volume of 1.0 ml in water to give a stock concentration of 40 mg/ml for each. Subsequent dilutions were made to which the same volume of deuterated standards was added. Fatty acids were treated similarly but were resuspended in 100% methanol. These standards were utilized to create standard curves for each analyte examined (Table S4).

***Ultra Performance Liquid Chromatography Tandem Mass Spectrometry*** UPLC-MS was performed using a modified method of Joyce et al. ([Joyce et al., 2014](#_ENREF_12)). Briefly, 5μL from each sample were injected onto a C18 Acquity column (Waters Corp.). Each sample was run in triplicate. Extracts were eluted using a 25-min gradient of 100 % A to 100 % B (A, water, 0.1 % formic acid; B, acetonitrile, 0.1 % formic acid) at a flow rate of 500 μL/min and column temperature of 40°C. Samples were analyzed using an Acquity system (Waters Ltd.) coupled online to an LCT Premier mass spectrometer (Waters MS Technologies, Ltd.) in negative electrospray mode with a scan range of 50–1,000 m/z. Bile acids ionize strongly in negative mode, producing a prominent [M-H]− ion. Capillary voltage was 2.4 Kv, sample cone was 35 V, desolvation temperature was 350°C, source temperature was 120°C, and desolvation gas flow was 900 L/h. Analysis was performed using Waters software Targetlynx for exact quantification against a standard curve for each analyte and Markerlynx for non- biased PCA analysis.

***Caecal microbiota composition and short chain fatty acids (SCFAs) analysis*** Caecum was harvested in cohort 1, snap frozen and stored at -80ºC prior to the analysis. Caecal content was further split into halves for the 16S sequencing and SCFAs analysis.

***Caecal content DNA extraction*** DNA extraction was performed using the QIAmp Fast DNA Stool Mini Kit (Qiagen, Sussex, UK) coupled with an initial bead-beating step. Briefly, 200 mg of each caecal sample were vortex-mixed in a 2 ml screw-cap tubes (Sarstedt, Wexford, Ireland) containing 0.25 g of a 1:1 mix of 0.1 mm and 1.0 mm sterile zirconia beads plus a single 3.5 mm diameter bead (BioSpec Products, Bartlesville, USA) with 1 ml of Qiagen InhibitEX® buffer. Following steps were according to manufacturer’s instructions. DNA was quantified using the QubitTM 3.0 Fluorometer (Bio-Sciences, Dublin, Ireland) and the Qubit® dsDNA HS Assay Kit (Life Technologies, Oregon, USA). Extracted DNA was kept frozen at -20ºC until further analysis.

***16S rRNA Gene Sequence-based microbiota analysis*** The V3-V4 hypervariable region of the 16S rRNA gene were amplified and prepared for sequencing as outlined in the Illumina 16S Metagenomic Sequencing Library Protocol (http://www.illumina.com/content/dam/illumina-support/documents/documentation/chemistry\_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf). Briefly, first PCR was done using forward primer (5’- TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3') and reverse primer (5’- GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3'). Each 25 μl PCR reaction contained 5 ng/μl microbial genomic DNA, 1 μM of each primer and 12.5 μl 2X Kapa HiFi Hotstart ReadyMix (Kapa Biosystems Ltd., UK). The PCR conditions follow as: initial denaturation at 95 ºC x 3 min; 25 cycles of 95 ºC x 30 s, 55 ºC x 30 s, 72 ºC x 30 s; and 72 ºC x 5 min for final extension. PCR products were purified with Agencourt AMPure XP system (Beckman Coulter Genomics, Takeley, UK). In the next step, dual indices and Illumina sequencing adapters were attached to PCR products using the Nextera XT Index Kit (Illumina, San Diego, CA). Each 50 μl PCR reaction contained 5 μl purified DNA, 5 μl index primer 1 (N7xx), 5 μl index primer 2 (S5xx), 25 μl 2x Kapa HiFi Hot Start Ready mix and 10 μl PCR grade water. PCR amplification was completed using the previous program but with only 8 amplification cycles instead of 25. Following this, a second clean-up step with the Agencourt AMPure XP system was done. PCR products were quantified, normalized and pooled in an equimolar fashion using the Qubit® dsDNA HS Assay Kit (Life Technologies, Oregon, USA). Next steps in the library preparation were carried out by Clinical-Microbiomics (Copenhagen, Denmark) prior to 2 × 300 (bp) paired-end sequencing on the Illumina MiSeq platform, using standard Illumina sequencing protocols.

***Bioinformatic sequence analysis*** Bioinformatic sequence analysis was performed as previously described ([Murphy et al., 2017](#_ENREF_17)). Briefly, paired-end sequences were assembled using FLASH ([Magoč and Salzberg, 2011](#_ENREF_16)) and analyzed using QIIME v1.8.0 (Quantitative Insights Into Microbial Ecology) ([Caporaso et al., 2010](#_ENREF_2)). Sequences were quality checked and the remaining sequences were clustered into operational taxonomic units using USEARCH (v7-64bit) ([Edgar, 2010](#_ENREF_6)). Taxonomic ranks were assigned with a BLAST search against the SILVA SSURef database release 116 ([Quast et al., 2013](#_ENREF_19)). Alpha and beta diversities were generated in QIIME and calculated based on weighted Unifrac distance matrices ([Lozupone et al., 2011](#_ENREF_15)). Principal coordinate analysis (PCoA) plots were visualized using EMPeror v0.9.3-dev ([Vázquez-Baeza et al., 2013](#_ENREF_21)). Relative abundance of bacterial taxa was expressed as % of identified sequences.

***Heatmap construction*** Log2 ratios were calculated from group means of highly abundant bacteria at the phylum and genus level using R (version 3.3.2), R Studio (version 1.0.136), and the gtools package (version 3.5.0). Heatmaps for genus and phylum were created in R and imported into Photoshop (version 7.0.1) to draw grey boxes connecting phylum to genus.

***Short chain fatty acids concentration analysis from cecum content*** Analysis of the main SCFA (acetate, propionate and butyrate) and a branched chain fatty acid (BCFA) (iso-butyrate) were carried out in supernatants of homogenized caecal content by gas chromatography (GC), as previously described ([Wall et al., 2012](#_ENREF_22)). Briefly, at least 100 mg of each caecal sample were vortex-mixed with MilliQ water and incubated at room temperature for 10 min. Following this, the supernatant was obtained by centrifugation (10 000 g, 5 min, 4°C), filtered through 0.2μm filters and mixed with 2-Ethylbutyric acid (Sigma Aldrich Ireland Ld, Wicklow, Ireland) as an internal standard. A gas chromatograph Varian 3500 GC flame-ionization system, fitted with a Zebron ZB-FFAP column (30 m x 0.32 mm x 0.25 µm) (Phenomenex, Macclesfield, UK) was used for quantification of SCFA. Chromatographic conditions were as follow: GC oven temperature was held initially at 50 °C for 0.5 min, then raised stepwise, by 10 °C/min, until it reached 140 °C. It was then raised by 20 °C/min up to 240 °C, and held for 5 min. The temperature of the injector and the detector were set at 240°C and 250°C, respectively. Helium was used as the carrier gas at a flow rate of 1.3 ml min-1. A standard curve was built with increasing concentrations of a standard mix containing the SCFAs and BCFA analyzed (Sigma Aldrich Ireland Ld, Wicklow, Ireland). Peaks were integrated by using the Varian Star Chromatography Workstation v6.0 software. The concentration of each SCFA was calculated using the linear regression equations (R2 ≥ 0.999) from the corresponding standard curves. Standards were included in each run to check calibration. Data were presented as µmol / wet caecum weight (g).

***Statistical analysis*** Statistical analysis was done in SPSS and R software environment. All datasets were checked for the normality with Shapiro-Wilk test. Normally distributed data were presented as mean ± SEM; unpaired two-tailed t-test was applied to analyze the difference between groups. These included behavioral scorings, organ weights, hormone levels, gene expression data, serotonin/kynurenine/tryptophan and SCFAs levels, transit scorings, and permeability measures. The corticosterone response to a stressor and FITC permeability data were analyzed with mixed-design ANOVA (Time as a repeated-measured factor and Genotype as an independent factor), followed by unpaired t-test comparisons in each time point. Datasets, in which the condition of normality was violated, as well as discrete data were presented as median (IQR); non-parametric Mann-Whitney test was used for between-group comparisons. These included relative abundances of bacterial taxa, alpha-diversity indices, bile acids levels, the quantitative parameters of the ENS morphology, and stress-induced defecation. Spearman’s rank correlation coefficient was employed for the analysis of associations between physiological/behavioral parameters and bacterial taxa abundance. Benjamini-Hochberg adjustment with a FDR of 0.05 was used to correct a p value for multiple testing. In normally distributed datasets, values outside the mean ± 2SD range were excluded as outliers. A p value <0.05 was deemed significant in all cases.

**Supplemental References**

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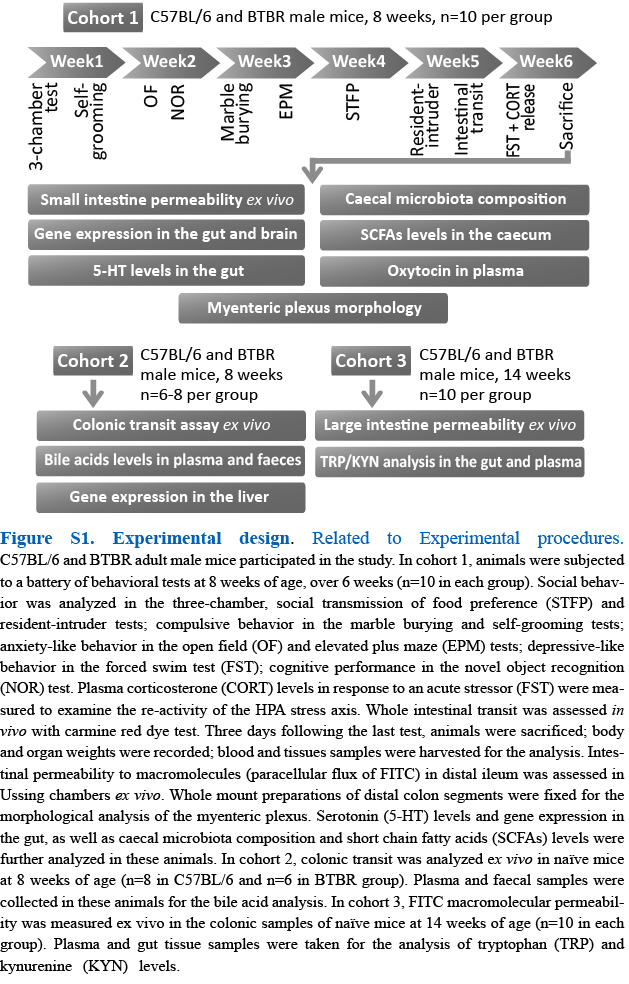
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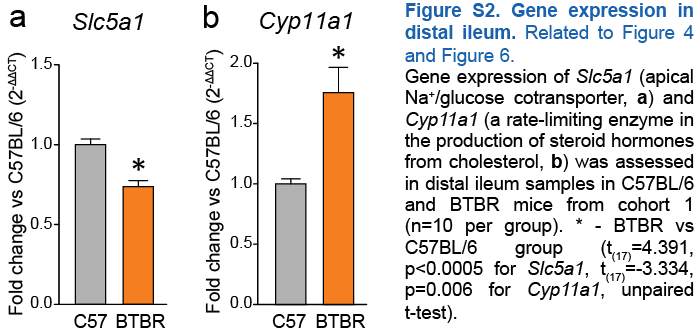
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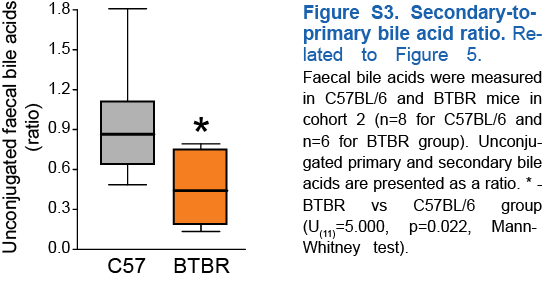
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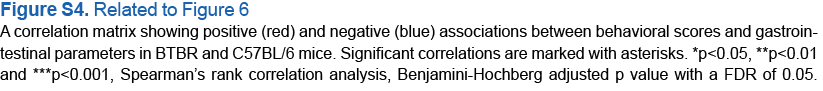
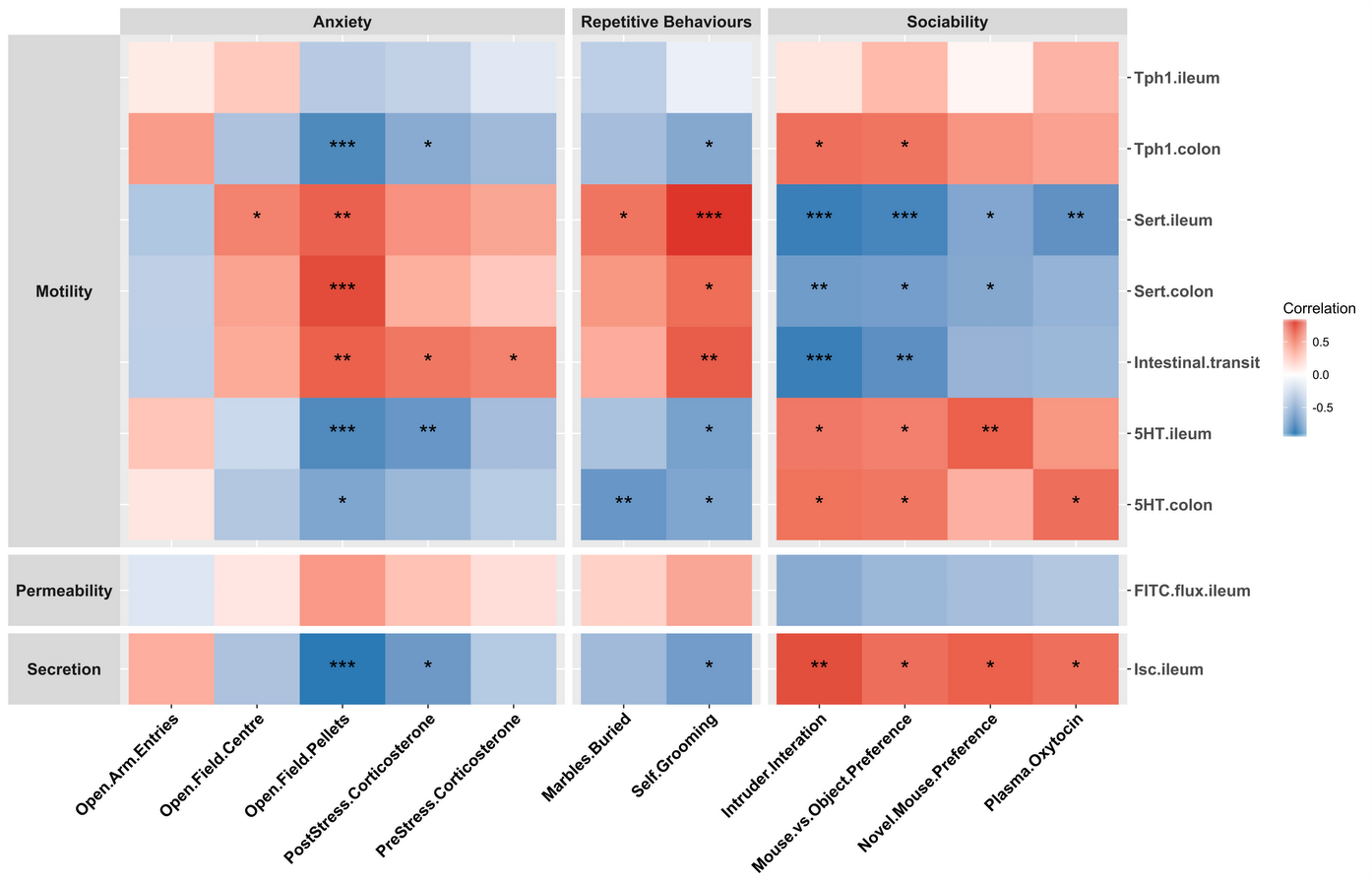
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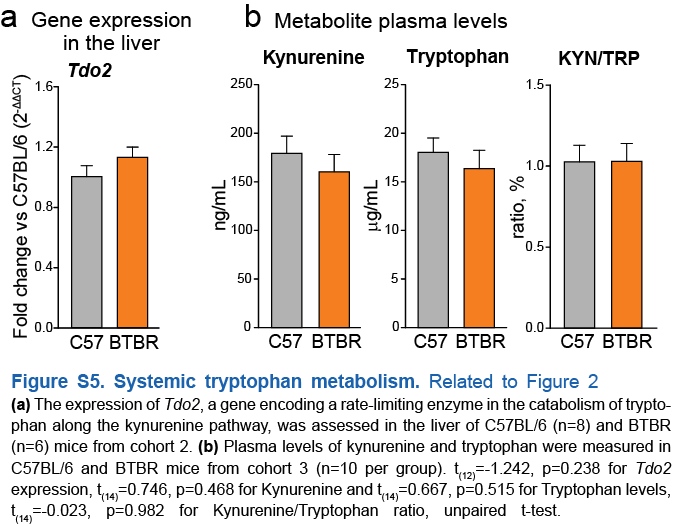
**Supplemental Figures**











**Supplemental Tables**

**Table S1. List of TaqMan probes used in the study.**

Related to Experimental procedures

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene Symbol** | **Common Gene Name** | **Probe ID** | **Manufacturer** |
| ***Abcb11/*** ***Bsep*** | Bile salt export pump | Mm.PT.58.9240423 | Integrated DNA Technologies |
| ***Actb*** | β-actin | 4352341E | Applied Biosystems |
| ***Ckb*** | Creatine kinase, brain type | Mm.PT.58.10065560 | Integrated DNA Technologies |
| ***Ckm*** | Creatine kinase, muscle type | Mm.PT.58.12048190 | Integrated DNA Technologies |
| ***Cldn1*** | Claudin-1 | Mm.PT.58.6163880 | Integrated DNA Technologies |
| ***Cyp7a1*** | Cholesterol 7 alpha-hydroxylase | Mm.PT.58.41588826 | Integrated DNA Technologies |
| ***Cyp7b1*** | 25-hydroxycholesterol 7-alpha-hydroxylase | Mm.PT.58.31179802 | Integrated DNA Technologies |
| ***Epcam*** | Epithelial cell adhesion molecule | Mm.PT.58.11851150 | Integrated DNA Technologies |
| ***Fgf15*** | Fibroblast growth factor 15 | Mm00433278\_m1 | Applied Biosystems |
| ***Gapdh*** | Glyceraldehyde-3-phosphate dehydrogenase | Mm99999915\_g1 | Applied Biosystems |
| ***Ido1*** | Indoleamine 2,3-dioxygenase 1 | Mm00492586\_m1 | Applied Biosystems |
| ***Muc2*** | Mucin 2 | Mm00458299\_m1 | Applied Biosystems |
| ***Mylk*** | Myosin light chain kinase, smooth muscle | Mm.PT.58.7656221 | Integrated DNA Technologies |
| ***Nos2*** | Nitric oxide synthase 2, inducible | Mm00440485\_m1 | Applied Biosystems |
| ***Nr1h4/Fxr*** | Farnesoid X receptor | Mm00436425\_m1 | Applied Biosystems |
| ***Nr2a1/Hnf4a*** | Hepatocyte nuclear factor 4 alpha | Mm.PT.58.6428917 | Integrated DNA Technologies |
| ***Nr3c1/Gr*** | Glucocorticoid receptor | Mm00433832\_m1 | Applied Biosystems |
| ***Nr3c2/Mr*** | Mineralocorticoid receptor | Mm01241596\_m1 | Applied Biosystems |
| ***Ocln*** | Occludin | Mm.PT.58.30118962 | Integrated DNA Technologies |
| ***Osta*** | Organic solute transporter alpha | Mm.PT.58.11977706 | Integrated DNA Technologies |
| ***Rxra*** | Retinoid X receptor alpha | Mm.PT.58.9642216 | Integrated DNA Technologies |
| ***S100a10/P11*** | S100 calcium binding protein A10 (calpactin, p11) | Mm00501457\_m1 | Applied Biosystems |
| ***Slc5a1*** | Solute carrier family 5 member 1 (sodium/glucose cotransporter 1) | Mm.PT.58.16227652 | Integrated DNA Technologies |
| ***Slc6a4/Sert*** | Solute carrier family 6 member 4 (sodium-dependent serotonin transporter) | Mm00439391\_m1 | Applied Biosystems |
| ***Slc10a1/Ntcp*** | Na+/taurocholate cotransporting polypeptide/Sodium/bile acid cotransporter | Mm.PT.58.43000030 | Integrated DNA Technologies |
| ***Slc10a2/Asbt/***  ***Ibat*** | Apical sodium/bile acid transporter/Ileal bile acid transporter | Mm.PT.58.11439836 | Integrated DNA Technologies |
| ***Tjp1*** | Tight junction protein ZO-1 | Mm.PT.58.12952721 | Integrated DNA Technologies |
| ***Tph1*** | Tryptophan hydroxylase 1 | Mm00493794\_m1 | Applied Biosystems |

**Table S2. Relative abundance (%) of bacterial PHYLA in the caecum of C57BL/6 and BTBR mice.** Related to Figure 1

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| ***Phylum*** | ***Median (IQR)*** | | ***U(17) value*** | ***p value*** | ***FDR adjusted p value*** |
| **C57BL/6** | **BTBR** |
| **Actinobacteria** | 0.154 (0.150) | 0.317 (0.249) | 69.000 | 0.053 | 0.089 |
| **Bacteroidetes** | 37.954 (11.813) | 56.546 (13.264) | 88.000 | ***<0.0005\**** | ***<0.0005\**** |
| **Candidate division TM7** | 0.104 (0.086) | 0.015 (0.188) | 24.000 | 0.095 | 0.118 |
| **Cyanobacteria** | 0.038 (0.029) | 0.000 (0.001) | 0.000 | ***<0.0005\**** | ***0.001\**** |
| **Deferribacteres** | 0.963 (0.515) | 0.192 (1.650) | 29.000 | 0.211 | 0.234 |
| **Firmicutes** | 59.238 (11.340) | 40.151 (13.061) | 2.000 | ***<0.0005\**** | ***<0.0005\**** |
| **Proteobacteria** | 1.350 (0.994) | 0.659 (0.263) | 7.000 | ***0.001\**** | ***0.002\**** |
| **Tenericutes** | 0.354 (0.273) | 0.094 (0.224) | 23.000 | 0.079 | 0.113 |
| **Verrucomicrobia** | 0.013 (0.021) | 0.179 (0.334) | 84.500 | ***<0.0005\**** | ***<0.0005\**** |
| **Other** | 0.000 (0.002) | 0.000 (0.004) | 49.500 | 0.720 | 0.720 |

\**p*<0.05, Mann-Whitney U test, Benjamini-Hochberg adjusted *p* value with a FDR of 0.05. n=9 in C57BL/6 and n=10 in BTBR group.

Red color indicates an increase; blue color indicates a decrease in the relative abundance of bacterial taxa in BTBR mice.

**Table S2 (continued). Relative abundance (%) of bacterial FAMILIES in the caecum of C57BL/6 and BTBR mice.** Related to Figure 1

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| ***Family*** | ***Median (IQR)*** | | ***U(17) value*** | ***p value*** | ***FDR adjusted p value*** |
| **C57BL/6** | **BTBR** |
| **Actinobacteria** | | | | | |
| **Bifidobacteriaceae** (Bifidobacteriales) | 0.013 (0.038) | 0.000 (0.000) | 10.000 | ***0.003\**** | ***0.012\**** |
| **Coriobacteriaceae** (Coriobacteriales) | 0.154 (0.137) | 0.317 (0.246) | 73.500 | ***0.017\**** | ***0.046\**** |
| **Bacteroidetes** | | | | | |
| **Bacteroidaceae** (Bacteroidales) | 0.771 (0.633) | 2.240 (2.586) | 77.000 | ***0.008\**** | ***0.026\**** |
| **Porphyromonadaceae** (Bacteroidales) | 1.142 (0.683) | 0.069 (0.275) | 1.000 | ***<0.0005\**** | ***0.001\**** |
| **Prevotellaceae** (Bacteroidales) | 0.208 (0.131) | 0.127 (0.698) | 35.000 | 0.447 | 0.670 |
| **Rikenellaceae** (Bacteroidales) | 6.850 (2.890) | 6.652 (8.597) | 55.000 | 0.447 | 0.635 |
| **S24-7** (Bacteroidales) | 29.679 (12.767) | 45.033 (10.529) | 85.000 | ***<0.0005\**** | ***0.004\**** |
| **Deferribacteres** | | | | | |
| **Deferribacteraceae** (Deferribacterales) | 0.963 (0.515) | 0.192 (1.650) | 29.000 | 0.211 | 0.356 |
| **Firmicutes** | | | | | |
| **Enterococcaceae** (Lactobacillales) | 0.000 (0.004) | 0.000 (0.000) | 29.500 | 0.211 | 0.335 |
| **Lactobacillaceae** (Lactobacillales) | 0.596 (1.271) | 0.313 (0.344) | 26.000 | 0.133 | 0.240 |
| **Caldicoprobacteraceae** (Clostridiales) | 0.000 (0.008) | 0.000 (0.000) | 25.000 | 0.113 | 0.217 |
| **Christensenellaceae** (Clostridiales) | 0.004 (0.004) | 0.004 (0.005) | 49.000 | 0.780 | 0.810 |
| **Clostridiaceae 1** (Clostridiales) | 0.008 (0.019) | 0.006 (0.020) | 45.000 | 1.000 | 1.000 |
| **Defluviitaleaceae** (Clostridiales) | 0.192 (0.129) | 0.638 (0.710) | 71.000 | ***0.035\**** | ***0.086*** |
| **Family XIII** (Clostridiales) | 0.117 (0.040) | 0.060 (0.073) | 15.500 | ***0.013\**** | ***0.040\**** |
| **Lachnospiraceae** (Clostridiales) | 44.708 (11.981) | 20.738 (17.060) | 4.000 | ***<0.0005\**** | ***0.004\**** |
| **Peptococcaceae** (Clostridiales) | 0.467 (0.179) | 0.348 (0.404) | 24.000 | 0.095 | 0.213 |
| **Ruminococcaceae** (Clostridiales) | 11.783 (4.850) | 12.671 (6.125) | 50.000 | 0.720 | 0.845 |
| **Erysipelotrichaceae** (Erysipelotrichales) | 0.033 (0.062) | 0.213 (0.205) | 84.000 | ***0.001\**** | ***0.004\**** |
| **Proteobacteria** | | | | | |
| **Rhodospirillaceae** (Rhodospirillales) | 0.000 (0.002) | 0.000 (0.005) | 50.500 | 0.661 | 0.892 |
| **Desulfovibrionaceae** (Desulfovibrionales) | 1.350 (0.996) | 0.656 (0.258) | 7.000 | ***0.001\**** | ***0.004\**** |
| **Tenericutes** | | | | | |
| **Anaeroplasmataceae** (Anaeroplasmatales) | 0.242 (0.254) | 0.073 (0.224) | 25.500 | 0.113 | 0.234 |
| **Verrucomicrobia** | | | | | |
| **Verrucomicrobiaceae** (Verrucomicrobiales) | 0.013 (0.021) | 0.179 (0.334) | 84.500 | ***<0.0005\**** | ***0.003\**** |

\**p*<0.05, Mann-Whitney U test, Benjamini-Hochberg adjusted *p* value with a FDR of 0.05. n=9 in C57BL/6 and n=10 in BTBR group.

Red color indicates an increase; blue color indicates a decrease in the relative abundance of bacterial taxa in BTBR mice.

**Table S2 (continued). Relative abundance (%) of bacterial GENERA in the caecum of C57BL/6 and BTBR mice.** Related to Figure 1

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| ***Genus*** | ***Median (IQR)*** | | ***U(17) value*** | ***p value*** | ***FDR adjusted p value*** |
| **C57BL/6** | **BTBR** |
| Bifidobacteriaceae(Bifidobacteriales, Actinobacteria) | | | | | |
| ***Bifidobacterium*** | 0.013 (0.038) | 0.000 (0.000) | 10.000 | ***0.003\**** | ***0.012\**** |
| Coriobacteriaceae (Coriobacteriales, Actinobacteria) | | | | | |
| ***Enterorhabdus*** | 0.142 (0.131) | 0.285 (0.243) | 73.000 | ***0.022\**** | ***0.063*** |
| ***Parvibacter*** | 0.008 (0.019) | 0.023 (0.031) | 63.500 | 0.133 | 0.245 |
| Bacteroidaceae (Bacteroidales, Bacteroidetes) | | | | | |
| ***Bacteroides*** | 0.771 (0.633) | 2.240 (2.586) | 77.000 | ***0.008\**** | ***0.029\**** |
| Porphyromonadaceae (Bacteroidales, Bacteroidetes) | | | | | |
| ***Odoribacter*** | 1.117 (0.692) | 0.069 (0.275) | 1.000 | ***<0.0005\**** | ***<0.0005\**** |
| ***Parabacteroides*** | 0.038 (0.042) | 0.000 (0.000) | 0.000 | ***<0.0005\**** | ***<0.0005\**** |
| Prevotellaceae (Bacteroidales, Bacteroidetes) | | | | | |
| ***Prevotella*** | 0.208 (0.131) | 0.127 (0.704) | 35.000 | 0.447 | 0.605 |
| Rikenellaceae (Bacteroidales, Bacteroidetes) | | | | | |
| ***Alistipes*** | 4.629 (1.881) | 6.165 (9.330) | 64.000 | 0.133 | 0.279 |
| ***RC9 gut group*** | 1.092 (1.746) | 0.292 (1.605) | 19.000 | ***0.035\**** | ***0.089*** |
| ***Rikenella*** | 0.129 (0.144) | 0.000 (0.005) | 0.000 | ***<0.0005\**** | ***<0.0005\**** |
| S24-7 (Bacteroidales, Bacteroidetes) | | | | | |
| ***S24-7 Uncultured bacterium*** | 29.679 (12.767) | 45.033 (10.505) | 85.000 | ***<0.0005\**** | ***0.002\**** |
| Deferribacteraceae (Deferribacterales, Deferribacteres) | | | | | |
| ***Mucispirillum*** | 0.963 (0.515) | 0.192 (1.650) | 29.000 | 0.211 | 0.335 |
| Lactobacillaceae (Lactobacillales, Firmicutes) | | | | | |
| ***Lactobacillus*** | 0.596 (1.271) | 0.313 (0.344) | 26.000 | 0.133 | 0.267 |
| Caldicoprobacteraceae (Clostridiales, Firmicutes) | | | | | |
| ***Caldicoprobacter*** | 0.000 (0.008) | 0.000 (0.000) | 25.000 | 0.113 | 0.247 |
| Christensenellaceae (Clostridiales, Firmicutes) | | | | | |
| ***Christensenella*** | 0.004 (0.004) | 0.004 (0.005) | 49.000 | 0.780 | 0.854 |
| Clostridiaceae 1 (Clostridiales, Firmicutes) | | | | | |
| ***Candidatus Arthromitus*** | 0.008 (0.019) | 0.004 (0.014) | 42.000 | 0.842 | 0.880 |
| Lachnospiraceae (Clostridiales, Firmicutes) | | | | | |
| ***Acetatifactor*** | 0.121 (0.150) | 0.140 (0.048) | 50.500 | 0.661 | 0.844 |
| ***Acetitomaculum*** | 0.004 (0.015) | 0.000 (0.000) | 15.000 | ***0.013\**** | ***0.044\**** |
| ***Blautia*** | 26.508 (7.500) | 6.721 (10.242) | 1.000 | ***<0.0005\**** | ***<0.0005\**** |
| ***Butyrivibrio*** | 0.000 (0.079) | 0.000 (0.000) | 25.000 | 0.113 | 0.259 |
| ***Coprococcus*** | 0.029 (0.035) | 0.008 (0.012) | 8.000 | ***0.001\**** | ***0.007\**** |
| ***Lachnospiraceae Incertae Sedis*** | 4.129 (2.485) | 0.785 (1.210) | 7.000 | ***0.001\**** | ***0.005\**** |
| ***Marvinbryantia*** | 0.025 (0.038) | 0.004 (0.008) | 26.000 | 0.133 | 0.255 |
| ***Natranaerovirga*** | 0.000 (0.006) | 0.000 (0.000) | 30.000 | 0.243 | 0.360 |
| ***Roseburia*** | 0.796 (0.456) | 1.535 (2.823) | 56.000 | 0.400 | 0.558 |
| ***Syntrophococcus*** | 0.000 (0.004) | 0.000 (0.000) | 30.000 | 0.243 | 0.349 |
| ***Lachnospiraceae Uncultured*** | 12.417 (8.721) | 11.267 (7.220) | 42.000 | 0.842 | 0.861 |

**Table S2 (continued). Relative abundance (%) of bacterial GENERA in the caecum of C57BL/6 and BTBR mice.** Related to Figure 1

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| ***Genus*** | ***Median (IQR)*** | | ***U(17) value*** | ***p value*** | ***FDR adjusted p value*** |
| **C57BL/6** | **BTBR** |
| Peptococcaceae (Clostridiales, Firmicutes) | | | | | |
| ***Peptococcus*** | 0.054 (0.058) | 0.027 (0.051) | 26.000 | 0.133 | 0.236 |
| Ruminococcaceae (Clostridiales, Firmicutes) | | | | | |
| ***Anaerotruncus*** | 1.021 (0.717) | 0.956 (0.732) | 47.500 | 0.842 | 0.901 |
| ***Ruminococcaceae Incertae Sedis*** | 3.346 (1.229) | 1.752 (1.778) | 15.000 | ***0.013\**** | ***0.041\**** |
| ***Intestinimonas*** | 0.438 (0.596) | 1.102 (0.999) | 72.000 | ***0.028\**** | ***0.076*** |
| ***Oscillibacter*** | 0.996 (0.760) | 0.979 (0.835) | 36.000 | 0.497 | 0.653 |
| ***Ruminococcus*** | 0.363 (0.569) | 0.144 (0.124) | 13.000 | ***0.008\**** | ***0.027\**** |
| ***Ruminococcaceae Uncultured*** | 5.513 (3.452) | 7.021 (4.639) | 61.000 | 0.211 | 0.324 |
| Erysipelotrichaceae (Erysipelotrichales, Firmicutes) | | | | | |
| ***Allobaculum*** | 0.000 (0.044) | 0.000 (0.000) | 27.500 | 0.156 | 0.266 |
| Rhodospirillaceae (Rhodospirillales, Proteobacteria) | | | | | |
| ***Thalassospira*** | 0.000 (0.002) | 0.000 (0.005) | 50.500 | 0.661 | 0.661 |
| Desulfovibrionaceae (Desulfovibrionales, Proteobacteria) | | | | | |
| ***Bilophila*** | 0.188 (0.250) | 0.654 (0.258) | 86.000 | ***<0.0005\**** | ***0.002\**** |
| ***Desulfovibrio*** | 1.142 (0.938) | 0.000 (0.001) | 0.000 | ***<0.0005\**** | ***0.001\**** |
| Anaeroplasmataceae (Anaeroplasmatales, Tenericutes) | | | | | |
| ***Anaeroplasma*** | 0.242 (0.254) | 0.073 (0.224) | 25.500 | 0.113 | 0.273 |
| Verrucomicrobiaceae (Verrucomicrobiales, Verrucomicrobia) | | | | | |
| ***Akkermansia*** | 0.013 (0.021) | 0.179 (0.334) | 84.500 | ***<0.0005\**** | ***0.003\**** |

\**p*<0.05, Mann-Whitney U test, Benjamini-Hochberg adjusted *p* value with a FDR of 0.05. n=9 in C57BL/6 and n=10 in BTBR group.

Red color indicates an increase; blue color indicates a decrease in the relative abundance of bacterial taxa in BTBR mice.

**Table S3. Correlation matrix for caecal microbiota composition and motility-associated data.**

Related to Figure 2

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Relative abundance of bacterial genera (%) | **Intestinal transit** (min) | **Colon length**  (mm) | **5-HT** levels in the **colon**  (μg/g tissue) | **5-HT** levels in the **iluem**  (μg/g tissue) | ***Tph1*** gene  Expression in the **colon** | ***Tph1*** gene  Expression in the **ileum** | ***Sert*** gene  Expression in the **colon** | ***Sert*** gene  Expression in the **ileum** | ***Ido1*** gene  Expression in the **colon** | ***Ido1*** gene  Expression in the **ileum** |
| **Actinobacteria** |  | | | | | | | | | |
| ***Bifidobacterium*** | **-0.604\*\*** | **-0.723\*\*** | **0.601\*\*** | **0.553\*** | 0.445 | 0.382 | **-0.642\*\*** | **-0.759\*\*** | **0.718\*\*** | **0.547\*** |
| ***0.006*** | ***0.000*** | ***0.006*** | ***0.017*** | *0.064* | *0.106* | ***0.003*** | ***0.000*** | ***0.001*** | ***0.019*** |
| ***Enterorhabdus*** | **0.547\*** | 0.414 | -0.262 | -0.351 | **-0.480\*** | 0.007 | 0.215 | **0.515\*** | -0.409 | -0.370 |
| ***0.015*** | *0.078* | *0.279* | *0.153* | ***0.044*** | *0.977* | *0.377* | ***0.024*** | *0.103* | *0.131* |
| ***Parvibacter*** | 0.265 | 0.437 | **-0.554\*** | -0.021 | -0.277 | -0.200 | 0.389 | **0.558\*** | -0.438 | -0.110 |
| *0.273* | *0.061* | ***0.014*** | *0.935* | *0.265* | *0.412* | *0.100* | ***0.013*** | *0.079* | *0.663* |
| **Bacteroidetes** |  | | | | | | | | | |
| ***Bacteroides*** | 0.430 | 0.400 | -0.372 | **-0.606\*\*** | **-0.672\*\*** | -0.426 | 0.400 | 0.342 | -0.333 | **-0.624\*\*** |
| *0.066* | *0.090* | *0.117* | ***0.008*** | ***0.002*** | *0.069* | *0.090* | *0.151* | *0.191* | ***0.006*** |
| ***Odoribacter*** | **-0.734\*\*** | **-0.649\*\*** | **0.652\*\*** | **0.607\*\*** | **0.756\*\*** | **0.457\*** | **-0.702\*\*** | **-0.715\*\*** | **0.683\*\*** | **0.735\*\*** |
| ***0.000*** | ***0.003*** | ***0.002*** | ***0.008*** | ***0.000*** | ***0.049*** | ***0.001*** | ***0.001*** | ***0.003*** | ***0.001*** |
| ***Parabacteroides*** | **-0.728\*\*** | **-0.801\*\*** | **0.635\*\*** | **0.639\*\*** | **0.690\*\*** | **0.493\*** | **-0.759\*\*** | **-0.825\*\*** | **0.768\*\*** | **0.822\*\*** |
| ***0.000*** | ***0.000*** | ***0.003*** | ***0.004*** | ***0.002*** | ***0.032*** | ***0.000*** | ***0.000*** | ***0.000*** | ***0.000*** |
| ***Prevotella*** | -0.316 | -0.239 | 0.112 | -0.217 | -0.092 | -0.097 | -0.125 | -0.381 | 0.228 | 0.202 |
| 0.187 | 0.325 | 0.647 | 0.387 | 0.717 | 0.694 | 0.611 | 0.107 | 0.378 | 0.420 |
| ***Alistipes*** | 0.144 | **0.496\*** | -0.274 | -0.110 | -0.179 | -0.093 | **0.496\*** | **0.499\*** | -0.468 | -0.137 |
| *0.556* | ***0.031*** | *0.257* | *0.663* | *0.478* | *0.705* | ***0.031*** | ***0.030*** | *0.058* | *0.587* |
| ***RC9 gut group*** | -0.353 | **-0.487\*** | **0.758\*\*** | **0.511\*** | **0.684\*\*** | 0.450 | **-0.621\*\*** | **-0.467\*** | 0.409 | 0.231 |
| *0.138* | ***0.034*** | ***0.000*** | ***0.030*** | ***0.002*** | *0.053* | ***0.005*** | ***0.044*** | *0.103* | *0.356* |
| ***Rikenella*** | **-0.629\*\*** | **-0.778\*\*** | **0.598\*\*** | **0.657\*\*** | **0.718\*\*** | **0.482\*** | **-0.751\*\*** | **-0.756\*\*** | **0.672\*\*** | **0.750\*\*** |
| ***0.004*** | ***0.000*** | ***0.007*** | ***0.003*** | ***0.001*** | ***0.036*** | ***0.000*** | ***0.000*** | ***0.003*** | ***0.000*** |
| ***S24-7 Uncultured bacterium*** | **0.741\*\*** | **0.521\*** | -0.447 | **-0.651\*\*** | **-0.628\*\*** | -0.326 | **0.537\*** | **0.535\*** | **-0.613\*\*** | **-0.736\*\*** |
| ***0.000*** | ***0.022*** | 0.055 | ***0.003*** | ***0.005*** | 0.173 | ***0.018*** | ***0.018*** | ***0.009*** | ***0.001*** |
| **Deferribacteres** |  | | | | | | | | | |
| ***Mucispirillum*** | -0.363 | -0.116 | 0.319 | 0.271 | 0.318 | 0.270 | -0.283 | -0.244 | 0.437 | 0.277 |
| *0.127* | *0.637* | *0.183* | *0.277* | *0.198* | *0.263* | *0.241* | *0.314* | *0.080* | *0.266* |
| **Firmicutes** |  | | | | | | | | | |
| ***Lactobacillus*** | -0.292 | **-0.467\*** | 0.319 | 0.187 | 0.020 | 0.211 | **-0.561\*** | **-0.550\*** | 0.429 | 0.172 |
| *0.226* | ***0.044*** | *0.183* | *0.458* | *0.938* | *0.387* | ***0.012*** | ***0.015*** | *0.086* | *0.494* |
| ***Caldicoprobacter*** | **-0.456\*** | **-0.469\*** | 0.400 | 0.053 | 0.390 | 0.227 | -0.417 | **-0.622\*\*** | **0.536\*** | 0.401 |
| ***0.050*** | ***0.043*** | *0.090* | *0.836* | *0.110* | *0.350* | *0.076* | ***0.004*** | ***0.027*** | *0.099* |
| ***Christensenella*** | 0.130 | 0.022 | 0.310 | -0.292 | -0.050 | 0.305 | -0.129 | -0.160 | -0.027 | -0.179 |
| *0.595* | *0.927* | *0.197* | *0.240* | *0.843* | *0.204* | *0.598* | *0.514* | *0.918* | *0.478* |
| ***Candidatus Arthromitus*** | -0.065 | -0.168 | -0.078 | 0.043 | -0.180 | -0.443 | 0.129 | -0.089 | -0.303 | -0.011 |
| *0.791* | *0.491* | *0.751* | *0.865* | *0.475* | *0.057* | *0.598* | *0.716* | *0.236* | *0.967* |
| ***Acetatifactor*** | -0.172 | 0.286 | -0.001 | -0.033 | 0.075 | -0.170 | -0.085 | 0.053 | 0.299 | -0.102 |
| *0.481* | *0.235* | *0.997* | *0.896* | *0.769* | *0.487* | *0.728* | *0.829* | *0.244* | *0.686* |
| ***Acetitomaculum*** | **-0.539\*** | **-0.667\*\*** | 0.358 | **0.496\*** | 0.392 | 0.102 | **-0.552\*** | **-0.655\*\*** | 0.437 | **0.515\*** |
| ***0.017*** | ***0.002*** | *0.132* | ***0.036*** | *0.108* | *0.677* | ***0.014*** | ***0.002*** | *0.079* | ***0.029*** |
| ***Blautia*** | **-0.684\*\*** | **-0.714\*\*** | 0.428 | **0.746\*\*** | **0.674\*\*** | 0.388 | **-0.519\*** | **-0.600\*\*** | **0.615\*\*** | **0.822\*\*** |
| ***0.001*** | ***0.001*** | *0.067* | ***0.000*** | ***0.002*** | *0.101* | ***0.023*** | ***0.007*** | ***0.009*** | ***0.000*** |
| ***Butyrivibrio*** | **-0.475\*** | **-0.479\*** | **0.540\*** | 0.433 | **0.506\*** | **0.480\*** | -0.289 | **-0.467\*** | **0.576\*** | **0.488\*** |
| ***0.040*** | ***0.038*** | ***0.017*** | *0.073* | ***0.032*** | ***0.038*** | *0.230* | ***0.044*** | ***0.016*** | ***0.040*** |
| ***Coprococcus*** | **-0.655\*\*** | **-0.542\*** | 0.359 | **0.494\*** | 0.327 | 0.245 | -0.382 | **-0.598\*\*** | **0.654\*\*** | **0.771\*\*** |
| ***0.002*** | ***0.016*** | *0.131* | ***0.037*** | *0.185* | *0.312* | *0.107* | ***0.007*** | ***0.004*** | ***0.000*** |
| ***Lachnospiraceae***  ***Incertae Sedis***  ***Sedis*** | **-0.698\*\*** | **-0.590\*\*** | 0.311 | **0.492\*** | **0.474\*** | 0.147 | -0.419 | **-0.667\*\*** | **0.610\*\*** | **0.604\*\*** |
| ***0.001*** | ***0.008*** | *0.196* | ***0.038*** | ***0.047*** | *0.547* | *0.074* | *0.002* | *0.009* | *0.008* |
| ***Marvinbryantia*** | -0.237 | -0.382 | 0.339 | **0.530\*** | 0.353 | 0.230 | **-0.506\*** | -0.315 | 0.181 | 0.232 |
| *0.329* | *0.106* | *0.156* | ***0.024*** | *0.151* | *0.343* | ***0.027*** | *0.188* | *0.487* | *0.355* |
| ***Roseburia*** | 0.207 | 0.192 | -0.453 | -0.176 | -0.176 | -0.311 | 0.379 | 0.405 | -0.365 | -0.098 |
| *0.394* | *0.432* | *0.052* | *0.484* | *0.484* | *0.196* | *0.110* | *0.085* | *0.149* | *0.699* |
| ***Lachnospiraceae***  ***Uncultured*** | 0.021 | 0.094 | 0.216 | 0.224 | 0.243 | 0.381 | -0.254 | 0.061 | 0.047 | 0.179 |
| *0.932* | *0.701* | *0.375* | *0.372* | *0.332* | *0.108* | *0.293* | *0.803* | *0.859* | *0.478* |
| ***Peptococcus*** | **-0.586\*\*** | -0.312 | 0.317 | -0.086 | 0.326 | 0.101 | -0.228 | **-0.536\*** | **0.500\*** | 0.279 |
| ***0.008*** | *0.193* | *0.186* | *0.733* | *0.187* | *0.682* | *0.348* | ***0.018*** | ***0.041*** | *0.262* |
| ***Anaerotruncus*** | -0.082 | 0.151 | -0.297 | -0.140 | -0.193 | -0.140 | -0.019 | 0.109 | -0.077 | 0.182 |
| *0.738* | *0.536* | *0.218* | *0.578* | *0.443* | *0.569* | *0.937* | *0.657* | *0.768* | *0.470* |
| ***Ruminococcaceae***  ***Incertae Sedis*** | **-0.786\*\*** | -0.342 | 0.458\* | 0.311 | 0.476\* | 0.305 | **-0.481\*** | **-0.597\*\*** | 0.777\*\* | 0.507\* |
| ***0.000*** | *0.151* | ***0.049*** | *0.210* | ***0.046*** | *0.204* | ***0.037*** | ***0.007*** | ***0.000*** | ***0.032*** |
| ***Intestinimonas*** | 0.190 | **0.662\*\*** | -0.163 | **-0.606\*\*** | -0.102 | -0.214 | 0.265 | 0.348 | -0.206 | **-0.476\*** |
| *0.437* | ***0.002*** | *0.505* | ***0.008*** | *0.687* | *0.379* | *0.273* | *0.144* | *0.428* | ***0.046*** |
| ***Oscillibacter*** | -0.110 | -0.211 | 0.075 | 0.354 | 0.214 | 0.168 | -0.014 | -0.117 | 0.436 | 0.187 |
| *0.655* | *0.386* | *0.759* | *0.150* | *0.395* | *0.491* | *0.955* | *0.634* | *0.080* | *0.458* |
| ***Ruminococcus*** | -0.318 | **-0.622\*\*** | **0.505\*** | **0.515\*** | 0.382 | 0.450 | **-0.567\*** | **-0.515\*** | 0.385 | **0.498\*** |
| *0.184* | ***0.004*** | ***0.027*** | ***0.029*** | *0.118* | *0.053* | ***0.011*** | ***0.024*** | *0.127* | ***0.036*** |
| ***Ruminococcaceae***  ***Uncultured*** | 0.091 | 0.326 | -0.198 | -0.437 | -0.222 | -0.195 | 0.398 | 0.212 | 0.081 | -0.199 |
| *0.710* | *0.174* | *0.416* | *0.070* | *0.376* | *0.424* | *0.091* | *0.383* | *0.758* | *0.428* |
| ***Allobaculum*** | -0.431 | -0.364 | **0.515\*** | 0.461 | 0.456 | **0.457\*** | -0.377 | -0.422 | **0.681\*\*** | 0.255 |
| *0.065* | *0.125* | ***0.024*** | *0.054* | *0.057* | ***0.049*** | *0.112* | *0.072* | ***0.003*** | *0.307* |
| **Proteobacteria** |  | | | | | | | | | |
| ***Thalassospira*** | 0.165 | 0.026 | **0.464\*** | -0.013 | 0.035 | 0.264 | -0.162 | -0.074 | 0.000 | -0.182 |
| *0.500* | *0.917* | ***0.045*** | *0.960* | *0.889* | *0.274* | *0.507* | *0.762* | *1.000* | *0.469* |
| ***Bilophila*** | **0.757\*\*** | **0.617\*\*** | **-0.554\*** | **-0.639\*\*** | -0.414 | -0.435 | **0.572\*** | **0.711\*\*** | **-0.740\*\*** | **-0.775\*\*** |
| ***0.000*** | ***0.005*** | ***0.014*** | ***0.004*** | *0.088* | *0.063* | ***0.011*** | ***0.001*** | ***0.001*** | ***0.000*** |
| ***Desulfovibrio*** | **-0.673\*\*** | **-0.855\*\*** | **0.689\*\*** | **0.737\*\*** | **0.681\*\*** | 0.343 | **-0.785\*\*** | **-0.825\*\*** | **0.648\*\*** | **0.648\*\*** |
| ***0.002*** | ***0.000*** | ***0.001*** | ***0.000*** | ***0.002*** | *0.151* | ***0.000*** | ***0.000*** | ***0.005*** | ***0.004*** |
| **Tenericutes** |  | | | | | | | | | |
| ***Anaeroplasma*** | -0.261 | -0.315 | 0.274 | 0.371 | 0.259 | 0.119 | -0.219 | -0.276 | 0.264 | 0.259 |
| *0.281* | *0.189* | *0.256* | *0.129* | *0.299* | *0.628* | *0.369* | *0.253* | *0.305* | *0.298* |
| **Verrucomicrobia** |  | | | | | | | | | |
| ***Akkermansia*** | **0.599\*\*** | **0.745\*\*** | -0.295 | **-0.641\*\*** | **-0.517\*** | -0.220 | 0.454 | **0.530\*** | **-0.522\*** | **-0.749\*\*** |
| ***0.007*** | ***0.000*** | *0.221* | ***0.004*** | ***0.028*** | *0.366* | *0.051* | ***0.020*** | ***0.032*** | ***0.000*** |

The table presents nonparametric Spearman’s rho and *p* values for each correlation; \*\*correlation is significant at the 0.01 level (2-tailed); \*correlation is significant at the 0.05 level (2-tailed). Red color indicates significant positive and blue color indicates significant negative correlations. N=9-10 in either group.

**Table S3 (continued). Correlation matrix for caecal microbiota composition and intestinal permeability and secretion data.** Related to Figure 4

|  |  |  |  |
| --- | --- | --- | --- |
| Relative abundance of bacterial genera (%) | **Serosal FITC in the ileum** (**60min,** ng/mL) | **Serosal FITC in the ileum** (**180min,** ng/mL) | ***Isc* in the leum** (**60 min,** μA/cm2) |
| **Actinobacteria** |  | | |
| ***Bifidobacterium*** | -0.368 | -0.232 | **0.638\*** |
| *0.177* | *0.406* | ***0.011*** |
| ***Enterorhabdus*** | 0.002 | -0.075 | **-0.552\*** |
| *0.995* | *0.790* | ***0.033*** |
| ***Parvibacter*** | 0.020 | -0.065 | -0.180 |
| *0.944* | *0.819* | *0.522* |
| **Bacteroidetes** |  | | |
| ***Bacteroides*** | **0.543\*** | **0.514\*** | **-0.796\*\*** |
| ***0.037*** | ***0.050*** | ***0.000*** |
| ***Odoribacter*** | -0.445 | -0.441 | **0.631\*** |
| *0.096* | *0.099* | ***0.012*** |
| ***Parabacteroides*** | -0.498 | -0.376 | **0.766\*\*** |
| *0.059* | *0.167* | ***0.001*** |
| ***Prevotella*** | -0.038 | 0.100 | -0.238 |
| *0.894* | *0.723* | *0.394* |
| ***Alistipes*** | 0.161 | 0.021 | -0.361 |
| *0.567* | *0.940* | *0.187* |
| ***RC9 gut group*** | -0.106 | -0.171 | 0.339 |
| *0.706* | *0.542* | *0.217* |
| ***Rikenella*** | -0.495 | -0.474 | **0.744\*\*** |
| *0.061* | *0.074* | ***0.001*** |
| ***S24-7 Uncultured bacterium*** | 0.725\*\* | 0.732\*\* | **-0.729\*\*** |
| *0.002* | *0.002* | ***0.002*** |
| **Deferribacteres** |  | | |
| ***Mucispirillum*** | -0.323 | -0.355 | 0.119 |
| *0.241* | *0.194* | *0.673* |
| **Firmicutes** |  | | |
| ***Lactobacillus*** | -0.146 | -0.061 | 0.132 |
| *0.603* | *0.830* | *0.639* |
| ***Caldicoprobacter*** | -0.039 | 0.116 | 0.270 |
| *0.891* | *0.681* | *0.330* |
| ***Christensenella*** | -0.028 | 0.070 | -0.155 |
| *0.921* | *0.803* | *0.581* |
| ***Candidatus Arthromitus*** | -0.123 | -0.084 | -0.200 |
| *0.663* | *0.765* | *0.475* |
| ***Acetatifactor*** | -0.302 | -0.263 | 0.113 |
| *0.274* | *0.344* | *0.689* |
| ***Acetitomaculum*** | **-0.691\*\*** | **-0.728\*\*** | **0.693\*\*** |
| ***0.004*** | ***0.002*** | ***0.004*** |
| ***Blautia*** | **-0.514\*** | -0.468 | **0.850\*\*** |
| ***0.050*** | *0.079* | ***0.000*** |
| ***Butyrivibrio*** | -0.213 | -0.046 | 0.448 |
| *0.445* | *0.871* | *0.094* |
| ***Coprococcus*** | **-0.633\*** | -0.473 | **0.615\*** |
| ***0.011*** | *0.075* | ***0.015*** |
| ***Lachnospiraceae***  ***Incertae Sedis***  ***Sedis*** | -0.382 | -0.307 | **0.746\*\*** |
| *0.160* | *0.265* | ***0.001*** |
| ***Marvinbryantia*** | -0.428 | **-0.524\*** | 0.369 |
| *0.111* | ***0.045*** | *0.176* |
| ***Roseburia*** | -0.114 | -0.232 | -0.061 |
| *0.685* | *0.405* | *0.830* |
| ***Lachnospiraceae***  ***Uncultured*** | -0.339 | -0.407 | 0.332 |
| *0.216* | *0.132* | *0.226* |
| ***Peptococcus*** | -0.493 | -0.455 | 0.153 |
| *0.062* | *0.088* | *0.586* |
| ***Anaerotruncus*** | -0.279 | -0.375 | -0.221 |
| *0.315* | *0.168* | *0.428* |
| ***Ruminococcaceae***  ***Incertae Sedis*** | **-0.775\*\*** | **-0.700\*\*** | 0.414 |
| ***0.001*** | ***0.004*** | *0.125* |
| ***Intestinimonas*** | 0.161 | 0.132 | **-0.529\*** |
| *0.567* | *0.639* | ***0.043*** |
| ***Oscillibacter*** | 0.100 | 0.196 | 0.146 |
| *0.723* | *0.483* | *0.603* |
| ***Ruminococcus*** | -0.429 | -0.347 | **0.651\*\*** |
| *0.111* | *0.205* | ***0.009*** |
| ***Ruminococcaceae***  ***Uncultured*** | -0.032 | 0.071 | -0.286 |
| *0.909* | *0.800* | *0.302* |
| ***Allobaculum*** | -0.240 | -0.089 | 0.408 |
| *0.388* | *0.752* | *0.131* |
| **Proteobacteria** |  | | |
| ***Thalassospira*** | 0.087 | 0.202 | -0.238 |
| *0.758* | *0.470* | *0.393* |
| ***Bilophila*** | **0.546\*** | 0.436 | **-0.646\*\*** |
| ***0.035*** | *0.104* | ***0.009*** |
| ***Desulfovibrio*** | **-0.572\*** | **-0.559\*** | **0.812\*\*** |
| ***0.026*** | ***0.030*** | ***0.000*** |
| **Tenericutes** |  | | |
| ***Anaeroplasma*** | -0.189 | -0.117 | 0.386 |
| *0.499* | *0.678* | *0.156* |
| **Verrucomicrobia** |  | | |
| ***Akkermansia*** | **0.631\*** | **0.631\*** | **-0.647\*\*** |
| ***0.012*** | ***0.012*** | ***0.009*** |

The table presents nonparametric Spearman’s rho and *p* values for each correlation; \*\*correlation is significant at the 0.01 level (2-tailed); \*correlation is significant at the 0.05 level (2-tailed). Red color indicates significant positive and blue color indicates significant negative correlations. N=9-10 in either group.

**Table S4. Bile acids levels in the plasma of C57BL/6 and BTBR mice (ng/mL).**

Related to Figure 5

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Bile acid (full name)** | **Abbreviation** | ***Median (IQR)*** | | **U(11) value** | ***p value*** |
| **C57BL/6** | **BTBR** |
| **Unconjugated bile acids** | | | | | |
| α/ω-Muricholic acid | αMCA/ ωMCA | 168.3 (139.3) | 69.6 (15.5) | 9.000 | 0.101 |
| β-Muricholic acid | βMCA | 109.3 (210.3) | 104.5 (79.1) | 19.000 | 0.836 |
| **Chenodeoxycholic acid** | **CDCA** | **47.9 (67.5)** | **15.2 (2.6)** | 2.000 | ***0.005\**** |
| **Cholic acid** | **CA** | **96.2 (152.3)** | **32.3 (19.5)** | 7.000 | ***0.051*** |
| Dehydrocholic acid | DHCA | 9.9 (19.3) | 11.0 (12.6) | 18.500 | 0.876 |
| **Deoxycholic acid** | **DCA** | **191.5 (104.9)** | **68.3 (16.0)** | 1.500 | ***0.005\**** |
| **Hyocholic acid** | **HCA** | **9.2 (5.1)** | **5.2 (2.1)** | 3.000 | ***0.008\**** |
| **Hyodeoxycholic acid** | **HDCA** | **14.3 (36.7)** | **5.4 (2.4)** | 3.000 | ***0.008\**** |
| Lithocholic acid | LCA | 44.5 (23.5) | 51.3 (23.0) | 25.000 | 0.628 |
| **Ursodeoxycholic acid** | **UDCA** | **20.4 (19.6)** | **7.5 (3.3)** | 0.000 | ***0.001\**** |
| **Taurine-conjugated bile acids** | | | | | |
| **Taurochenodeoxycholic acid** | **TCDCA** | **37.6 (16.8)** | **21.6 (4.2)** | 5.000 | ***0.048\**** |
| Taurocholic acid | TCA | 224.6 (286.0) | 208.1 (74.9) | 13.000 | 0.530 |
| Taurodeoxycholic acid | TDCA | 27.9 (68.8) | 17.6 (4.9) | 10.000 | 0.268 |
| Taurohyocholic acid | THCA | 7.1 (3.7) | 5.5 (1.2) | 10.000 | 0.268 |
| **Taurohyodeoxycholic acid** | **THDCA** | **10.1 (8.0)** | **5.5 (1.5)** | 3.500 | ***0.018\**** |
| Taurolithocholic acid | TLCA | 4.7 (2.4) | 6.0 (2.2) | 27.000 | 0.149 |
| Tauromuricholic acid | TMCA | 242.5 (178.6) | 213.2 (54.3) | 8.000 | 0.149 |
| **Tauroursodeoxycholic acid** | **TUDCA** | **21.0 (31.1)** | **15.0 (3.5)** | 2.000 | ***0.010\**** |
| **Glycine-conjugated bile acids** | | | | | |
| Glycochenodeoxycholic acid | GCDCA | 5.4 (3.0) | 4.3 (2.9) | 9.500 | 0.202 |
| Glycocholic acid | GCA | 13.4 (8.7) | 9.6 (5.6) | 9.000 | 0.202 |
| Glycodeoxycholic acid | GDCA | 5.7 (4.7) | 5.0 (2.3) | 8.500 | 0.149 |
| Glycohyocholic acid | GHCA | 8.6 (5.7) | 7.9 (1.7) | 13.000 | 0.530 |
| Glycolithocholic acid | GLCA | 4.6 (3.2) | 4.6 (1.6) | 17.000 | 1.000 |
| Glycoursodeoxycholic acid | GUDCA | 5.4 (3.8) | 6.0 (1.3) | 21.000 | 0.639 |
|  | | | | | |
| Taurine (ng/mL) |  | 4830 (1187) | 4631 (1351) | 15.000 | 0.445 |

\*p<0.05, Mann-Whitney U test, n=7 in C57BL/6 and n=6 in BTBR group.

Blue color indicates a significant decrease in plasma bile acids levels in BTBR mice.

**Table S4 (continued). Bile acids levels in the faeces of C57BL/6 and BTBR mice (μg/g faeces).**

Related to Figure 5

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Bile acid (full name)** | **Abbreviation** | ***Median (IQR)*** | | **U(11) value** | ***p value*** |
| **C57BL/6** | **BTBR** |
| **Unconjugated bile acids** | | | | | |
| α/ω-Muricholic acid | αMCA/ ωMCA | 257.4 (251.6) | 191.9 (161.9) | 19.000 | 0.836 |
| β-Muricholic acid | βMCA | 98.5 (178.5) | 206.5 (120.8) | 34.000 | 0.073 |
| Chenodeoxycholic acid | CDCA | 20.2 (25.2) | 13.6 (16.1) | 17.000 | 0.628 |
| Cholic acid | CA | 41.6 (122.4) | 68.7 (116.9) | 25.000 | 0.628 |
| Dehydrocholic acid | DHCA | 0.2 (0.2) | 0.1 (0.2) | 15.000 | 0.445 |
| Deoxycholic acid | DCA | 347.6 (256.5) | 160.1 (198.1) | 10.000 | 0.138 |
| Hyocholic acid | HCA | 7.1 (6.8) | 3.7 (7.3) | 18.000 | 0.731 |
| Hyodeoxycholic acid | HDCA | 1.5 (3.7) | 4.1 (3.2) | 33.000 | 0.101 |
| **Lithocholic acid** | **LCA** | **15.5 (5.1)** | **8.5 (10.1)** | 6.000 | ***0.035\**** |
| Ursodeoxycholic acid | UDCA | 33.9 (16.3) | 16.8 (23.2) | 10.000 | 0.138 |
| **Taurine-conjugated bile acids** | | | | | |
| **Taurochenodeoxycholic acid** | **TCDCA** | **0.27 (0.60)** | **1.44 (4.10)** | 37.000 | ***0.022\**** |
| **Taurocholic acid** | **TCA** | **0.77 (3.81)** | **15.23 (43.75)** | 35.000 | ***0.051*** |
| **Taurodeoxycholic acid** | **TDCA** | **0.27 (0.81)** | **1.44 (2.55)** | 40.000 | ***0.005\**** |
| Taurohyocholic acid | THCA | 0.15 (0.16) | 0.40 (0.52) | 31.500 | 0.138 |
| Taurohyodeoxycholic acid | THDCA | 0.06 (0.21) | 0.25 (0.51) | 30.000 | 0.234 |
| **Taurolithocholic acid** | **TLCA** | **0.04 (0.04)** | **0.14 (0.10)** | 39.000 | ***0.008\**** |
| **Tauromuricholic acid** | **TMCA** | **2.82 (4.96)** | **14.72 (71.86)** | 38.000 | ***0.014\**** |
| **Tauroursodeoxycholic acid** | **TUDCA** | **0.22 (0.46)** | **1.74 (2.52)** | 42.000 | ***0.001\**** |
| **Glycine-conjugated bile acids** | | | | | |
| Glycochenodeoxycholic acid | GCDCA | 0.00 (0.01) | 0.01 (0.02) | 27.000 | 0.445 |
| Glycocholic acid | GCA | 0.13 (0.11) | 0.34 (0.63) | 29.000 | 0.295 |
| Glycodeoxycholic acid | GDCA | 0.10 (0.09) | 0.08 (0.09) | 20.500 | 0.945 |
| Glycohyocholic acid | GHCA | 0.26 (0.17) | 0.22 (0.11) | 17.000 | 0.628 |
| **Glycolithocholic acid** | **GLCA** | **0.03 (0.03)** | **0.10 (0.13)** | 40.500 | ***0.002\**** |
| **Glycoursodeoxycholic acid** | **GUDCA** | **0.24 (0.10)** | **0.12 (0.10)** | 4.500 | ***0.014\**** |
|  | | | | | |
| **Taurine (μg/g faeces)** |  | **15.7 (20.8)** | **47.2 (22.6)** | 38.000 | ***0.014\**** |

\**p*<0.05, Mann-Whitney U test, n=7 in C57BL/6 and n=6 in BTBR group.

Red color indicates an increase, while blue color indicates a decrease in faecal bile acids levels in BTBR group.

**Table S4 (continued). List of bile acids detected and applied for constructing standard curves for exact quantity determinations in this study.**

Related to Experimental Procedures

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Bile acid (full name)** | **[MW]-H** | **Neutral Formula** | **Retention Time min** | **R2 value** |
| Taurine | 124.0068 | C2H7NO3S | 00.75 | 0.983446 |
| Lithocholic Acid | 375.2899 | C24H40O3 | 21.95 | 0.987488 |
| Hyodeoxycholic Acid | 391.2848 | C24H40O4 | 10.21 | 0.908498 |
| Chenodeoxycholic Acid | 391.2848 | C24H40O4 | 19.62 | 0.999107 |
| Ursodeoxycholic aAid | 391.2848 | C24H40O4 | 12.99 | 0.998688 |
| Deoxycholic Acid | 391.2848 | C24H40O4 | 20.55 | 0.944038 |
| Murocholic Acid | 391.2848 | C24H40O4 | 08.16 | 0.998300 |
| Dehydrocholic Acid | 401.2328 | C24H34O5 | 02.12 | 0.975614 |
| Cholic Acid | 407.2797 | C24H40O5 | 14.02 | 0.998456 |
| Hyocholic Acid | 407.2797 | C24H40O5 | 10.75 | 0.956138 |
| α-Muricholic Acid | 407.2797 | C24H40O5 | 07.09 | 0.999866 |
| β-Muricholic Acid | 407.2797 | C24H40O5 | 07.53 | 0.999866 |
| ω-Muricholic Acid | 407.2797 | C24H40O5 | 07.09 | 0.999866 |
| Taurolithocholic Acid | 482.2940 | C26H45NO5S | 15.23 | 0.863135 |
| Taurochenodeoxycholic Acid | 498.2889 | C26H45NO6S | 08.90 | 0.951160 |
| Tauroursodeoxycholic Acid | 498.2889 | C26H45NO6S | 03.16 | 0.995872 |
| Taurodeoxycholic Acid | 498.2889 | C26H45NO6S | 10.32 | 0.973088 |
| Taurohyodeoxycholic Acid | 498.2889 | C26H45NO6S | 03.74 | 0.978165 |
| Taurocholic Acid | 514.2838 | C26H45NO7S | 05.20 | 0.972362 |
| Taurohyocholic Acid | 514.2838 | C26H45NO7S | 03.00 | 0.981646 |
| Tauro α-Muricholic Acid | 514.2838 | C26H45NO7S | 02.03 | 0.998457 |
| Tauro β-Muricholic Acid | 514.2838 | C26H45NO7S | 02.03 | 0.998457 |
| Tauro ω-Muricholic Acid | 514.2838 | C26H45NO7S | 02.03 | 0.998457 |
| Cholic Acid d4 | 411.3049 | C24H36D4O5 | 14.02 | NA |
| Chenodeoxycholic Acid d4 | 395.3099 | C24H36D4O4 | 19.62 | NA |

**Table S4 (continued). Preparation of bile acids for standard curve construction.**

Related to Experimental Procedures

|  |  |  |  |
| --- | --- | --- | --- |
| **Level** | **Concentration**  **(µg/mL)** | **IS volume**  **(µL)** | **Solvent volume**  **(µL)** |
| Std1\_1 | 0.64 | 15 | 132.6 |
| Std1\_2 | 1.28 | 15 | 130.2 |
| Std1\_3 | 2.56 | 15 | 125.4 |
| Std1\_4 | 3.76 | 15 | 120.9 |
| Std1\_5 | 4.96 | 15 | 116.4 |
| Std1\_6 | 6.24 | 15 | 111.6 |
| Std1\_7 | 7.52 | 15 | 106.8 |
| Std1\_8 | 8.72 | 15 | 102.3 |
| Std1\_9 | 10.00 | 15 | 97.5 |
| Std1\_10 | 20.00 | 15 | 60.0 |