Supplementary Materials

Organoid complete growth medium (CGM)

Complete growth medium (CGM) was comprised of Advanced DMEM (Gibco) including 1X penicillin/streptomycin, 2 mM glutamax, 10 mM HEPES (Life Technologies) supplemented with 40 ng ml−1 EGF (R&D), 100 ng/mL Noggin (Peprotech or GoldBio), 1 mM N-acetyl-l-cysteine (Sigma-Aldrich), 10 µM Y-27632 dihydrochloride monohydrate (STEMCELL technologies) and 5% R-Spondin conditioned-media (v/v).

16S rRNA Gene Sequencing and Analysis.

Nucleic acids were extracted from 84 fecal samples and 14 cecal content samples (PowerViral® Environmental RNA/DNA Isolation Kit, MoBio Laboratories, Inc.) and further treated with RNAse A/T1 (30 min at 37°C). gDNA was cleaned (NucleoSpin® gDNA Clean-up columns, Machery-Nagel) and quantified (Nanodrop, Thermo Fisher Scientific, Inc.). Illumina protocols were used to prepare V4 amplicons of the 16S rRNA gene for sequencing on a MiSeq system. Briefly 1st stage PCR was performed in 96 well plates with primers 16S-V4_FWD (5'-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG **GTG CCA GCM GCC GCG GTA A**-3') and 16S-V4_REV (5'-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA **GGA CTA CHV GGG TAT CTA ATC C**-3') using 5 ng microbial gDNA with thermocycler protocol: 95°C for 3 min and 25 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, final extension 72°C for 5 min. Amplification was confirmed by 1.5 % gel electrophoresis and amplicons were cleaned with the Agencourt AMPure XP kit (Beckman Coulter). Nextera XT Index Kit (v2 Set D 96 indexes, FC-131-2004) was used for index PCR to attach dual indices and Illumina sequencing adapters using 5 µL of DNA from 1st stage PCR and thermocycler protocol: 95°C for 3 min and 6 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, final extension 72°C for 5 min. Amplification was confirmed

by 1.5 % gel electrophoresis and amplicons were cleaned with the Agencourt AMPure XP kit. Barcoded and indexed amplicons were quantified (Quant-iT Picogreen dsDNA Assay Kit, Invitrogen), normalized to 4nM using 10mM Tris buffer (pH 8.5), and pooled for sequencing on one lane of an Illumina MiSeq (run type 250/150 paired-end run) resulting in >1.099x10^7 reads.

Paired-end sequenced reads obtained for all 98 samples were formatted as Casava 1.8 FASTQ files. Demultiplexed paired-end reads were imported into QIIME (Quantitative Insights Into Microbial Ecology) v2.0.1 (1), and subsequently summarized as interactive quality plots to determine truncation and trimming parameters for denoising and clustering. Based on quality scores, forward reads were truncated at 243 bp, reverse reads were truncated at 175 bp and initial 10 bp of all reads were trimmed. Denoising and clustering were conducted using DADA2 algorithm to differentiate sequences by as little as 1 bp into amplicon sequence variants (ASVs) for downstream analysis (2). Feature table and representative sequences for ASVs were generated using QIIME 2.0. Phylogenetic diversity tree was processed using representative sequences and fasttree plugin coupled with mafft alignment from QIIME 2. To maximize coverage of sequenced ASVs without losing samples, feature table was rarefied to 15,100 sequences per sample and then used for microbiota α diversity (Richness, Shannon index, and Faith phylogenetic diversity) in addition to β diversity analyses of distance matrices (Bray-Curtis, Unweighted UniFrac, Weighted UniFrac, and Jaccard) with QIIME 2.0. Taxonomies of ASVs representative sequences were assigned using a Naive Bayes classifier trained on Greengenes 13_8 99% OTUs for more accurate classification (1,3). Bray-Curtis distance matrices generated in QIIME 2.0 were imported into Prism 8.0.2 (GraphPad Software, La Jolla, CA). and summarized as 2D Principle Coordinate Analyses (PCoA) plots to show broad changes between bacterial relatedness. ADONIS tests were performed to assess the differential clustering of bacterial communities using the vegan R package v2.5-5 (https://cran.r-project.org/web/packages/vegan/index.html)

The non-rarefied ASV feature table was aggregated at phylum and genus levels to discover features altered by GP supplementation. Repeated-measures two-way ANOVA using Dunnett's multiple comparisons test was utilized to detect change within group from baseline effect while a repeated measures two-way ANOVA followed by a Holm-Sidak correction for multiple comparisons was used to compare relative abundances of bacterial phyla between diet groups across time, as specified in figure legends (**Supplementary Fig. 3**). For groups with same diet base, genera that were present in less than 50% of samples in GP-supplemented group and control group, and genera with average relative abundances lower than 0.05% in both groups were filtered out of a summarized feature table of ASVs classified at the genera-level. For fecal samples at each time point and cecal samples at the endpoint, statistical comparison of genera was done by 12,000 times of permutation (**Supplementary Fig. 5**); p values represent fraction of times that permuted differences assessed by Welch's t-test were greater than or equal to real difference, and were adjusted (q values) using the Benjamini-Hochberg false discovery rate (FDR) with a significance level of 0.05.

LC-MS analysis of serum bile acids.

Serum samples (40 µL) from each mouse was mixed with internal standards (100 µg each of GCA, GCDCA, and TLCA) followed by addition of 110 µL acetonitrile. Samples were incubated at -20 °C for 1 h to precipitate proteins then centrifuged at 16,000x *g* at 4 °C for 10 min. Supernatants were passed through preconditioned Oasis Prime HLB Cartridge (Waters, Milford MA) to remove phospholipids. Both the wash step (5% acetonitrile/95% water (v/v)) and elution step (90% acetonitrile/10% water (v/v)) were collected into separate, labeled tubes for LC-MS analyses. BAs were quantified by external calibration curves (0.00125-10 µg/mL) using >95% pure standards (**Supplementary Table 5**). BA recovery after column-purification was >90% (**Supplementary Table 6**). BAs were analyzed on a Water's Alliance e2695 HPLC system (Waters Milford, MA, USA) coupled to a Water's Acquity QDA mass spectrometer

equipped with an electrospray interphase (ESI; Waters Milford, MA, USA). Retention time, limit of detection, and quantification of analytes are shown in **Supplementary Table 6**. Details of instrument settings are provided in supplementary materials.

Bile acids were analyzed on a Water's Alliance e2695 HPLC system coupled to a Water's Acquity QDA detector mass spectrometer equipped with an electrospray interphase (ESI). Analytes were separated in a Cortecs C18+ column (4.6 x 150 mm, 2.7 µm; Waters Milford, MA, USA) and gradient elution with 0.1 % formic acid in water (solvent A) and 0.1 % formic acid in acetonitrile (solvent B) at flow rate of 1.2 mL/min as follows: 0–30 min linear gradient from 35 to 50% B; 30-31 min isocratic at 50% B; 31–31.10 step gradient to 35% B and 31.10–40 min isocratic at 35% B returning to the initial conditions for the next analysis. The temperature of the column was maintained at 40 °C and the injection volume was 10 µL. Bile acids were detected in full scan mode (ESI+/-, scan range 100-1200 m/z) and selected ion monitoring. BAs were quantified by external calibration curves (0.00125-10 µg/mL) with authentic standards (purity >95%). Average peak area of individual BAs detected in wash and eluent fractions of serum samples, and external calibration curves were calculated from duplicate injections using selective ion recordings (SIR) preprogrammed to discriminate specific masses corresponding to each specific BA species. Standard curves were injected in duplicate at the beginning and end of the sample set. Reference samples (one of the calibration curves) was injected in-between diet groups for quality control. BAs quantified in eluent and wash fractions were pooled The limit of detection (LOD) and limit of quantification (LOQ) were determined from the linear regression analysis using the following equations: LOD= (3.3 x SD/AS) and LOQ= (10 x SD/AS) where SD and AS are the standard deviation of the intercept and the average slope of the linear regressions, respectively. Retention time, limit of detection, and quantification of analytes are presented in **Supplementary table 6**.

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