Supplementary Information for

Mechanisms by which sialylated milk oligosaccharides impact bone biology in a gnotobiotic mouse model of infant undernutrition

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Supplementary Materials and Methods

Human Study

A total of 343 Bangladeshi children, age 6 to 59 months old, with severe acute malnutrition [SAM, weight-for-height Z (WHZ) score <-3 and/or bipedal edema, mid upper arm circumference (MUAC) <11.5 cm] were enrolled in a randomized, double-blind study to compare the efficacy of two locally-produced ready-to-use therapeutic foods (RUTFs) and a commercially available RUTF on the rate of weight gain after initial stabilization using a hospital-based, standardized management protocol (1) (ClinicalTrials.gov identifier: NCT01889329). The study was approved by the Ethical Review Committee at the icddr,b and written informed consent was obtained from the parents or guardians of the study participants. Recruitment occurred at three study locations: Dhaka Hospital of icddr,b, RADDA MCH FP Center Mirpur, Dhaka and TDH, Kurigram. 54 of the children (aged 6-24 months) were enrolled in a sub-study that involved longitudinal fecal and blood sampling over a one-year period following enrollment. After acute stabilization, children were randomized to one of the three treatment arms; 20 children received a commercial RUTF, Plumpy'Nut (Nutriset), 19 were treated with a rice-lentil RUTF and 15 were given a chickpea-containing RUTF, each providing ~200kcal/kg/day. The rice-lentil and chickpea prototypes were designed to (i) to contain affordable, locally available ingredients (2), (ii) meet the macro- and micronutrient specifications for RUTF, and (iii) have acceptable organoleptic properties. Children were discharged after successful treatment, which was defined as having an edema-free WHZ score ≥ -2. The study protocol included three blood draws; one at enrollment, another at the end of the therapeutic intervention, and a final sample at 6-months post-intervention.

Plasma was rapidly prepared from each blood sample and frozen immediately at -80ºC. Coded biospecimens were shipped to Washington University on dry ice where they were stored at -80ºC along with associated clinical metadata (with approval by the Washington University IRB). Plasma CTX-I and P1NP were measured using ELISA kits from Immunodiagnostic Systems (East Boldon, UK). C-reactive Protein was also quantified by ELISA (R&D Systems Quantikine kit). ELISA data were collected using a BioTek Synergy plate reader. The Milliplex MAP Human Bone Magnetic Bead Panel (Millipore Sigma) was used to measure IL-6 and TNFα. Milliplex bead assays was performed on a Luminex MagPix instrument.

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Studies involving gnotobiotic mice

All mouse experiments were performed using protocols approved by the Washington University Animal Studies Committee.

Bacterial culture - Frozen stocks of each sequenced bacterial strain in a culture collection generated from the 6-month old severely stunted Malawian infant (3) were thawed and placed in an anaerobic Coy anaerobic chamber (atmosphere; 75% N_2, 20% CO_2, 5% H_2). An aliquot was removed and diluted 1:50 in LY-BHI medium and the monoculture was grown overnight at 37ºC with growth monitored at OD_{600} using a BioTek Synergy plate reader. Strains were normalized with respect to optical density and pooled in pre-reduced PBS containing 15% glycerol/0.05% cysteine-HCl so that an equal mixture of strains was obtained. The pool was aliquoted into 1.8 mL glass vials (E-Z vials, Wheaton); the tubes were crimped with covers containing a PTFE/grey butyl liner (Wheaton) and stored at -80°C.

Colonization – Germ-free C57BL/6J mice were housed in plastic flexible film gnotobiotic isolators (Class Biologically Clean Ltd., Madison, WI) at 23ºC under a strict 12-hour light cycle (lights on at 0600h and off at 1800h). Animals were initially weaned onto an autoclaved mouse chow (B&K Universal, East Yorkshire, UK; diet 7378000). Mice, aged 5 weeks, were weighed and distributed across treatment groups to balance initial body size. Three days prior to colonization, animals were switched to the M8 diet or M8 supplemented with S-BMO (Hilmar Cheese Company, CA) yielding a dose in the supplemented chow of 1.5% w/w sialyllactose (note that these diets are identical to those used in ref. 3). In separate experiments, mice were given the unsupplemented M8 diet and their drinking water was supplemented with 6.9 mg/mL 2'-FL (Glycosyn). Based on a pilot experiment conducted in age- and gender-matched mice where average daily water and chow consumption were measured, this dose was designed to provide the equivalent level of 2'-FL consumption that would have been delivered by incorporating 1.5% (w/w) of this oligosaccharide into the M8 diet. All diets were sterilized by gamma-irradiation (>20 Gy); sterility was confirmed by culturing the diet in rich media under aerobic and anaerobic conditions (3). For studies in which mice were maintained as germ-free and not colonized, sterility was confirmed by surveillance of their feces (culturing as with chow and by 16S rDNA amplicon sequencing).

Mice were gavaged with 200 μL of the pool of bacterial strains. Weights were measured every 3 days. Body composition was characterized at dpg 1 and dpg 38 using an EchoMRI 3 in1 instrument (EchoMRI, Houston, TX). All animals were euthanized by cervical dislocation without prior fasting.

Community profiling by sequencing (*COPRO-Seq*) - Protocols for quantifying bacterial community composition in cecal samples are described in ref. (4). Briefly, DNA was isolated by subjecting cecal contents to bead-beading for 4 minutes (Biospec Mini-Beadbeater-96, Bartlesville, OK) in a mixture containing 500 μL Buffer A (200 mM NaCl, 200 mM Tris, 20 mM EDTA), 210 μL 20% SDS, 500 μL of phenol:chloroform:isoamyl alcohol (25:24:1, pH 7.9), 250 μL of 0.1 mm diameter zirconia/silica beads (Biospec; catalog number 11079101z) and a 3.97 mm diameter steel ball (McMaster-Carr; catalog number 96455K73). The aqueous phase was collected after centrifugation at room temperature for 4 minutes at 3,200 x g. DNA was purified from a fraction of the aqueous phase using QIAquick-96 plates (Qiagen) and eluted with Buffer EB (Qiagen). Libraries for DNA sequencing were prepared using the Nextera DNA Library Prep kit (Illumina), 0.75ng of genomic DNA was tagmented in a 2.5 μL reaction and tagments were amplified by PCR using barcoded primer pairs. Each library was purified (AMPure XP beads, Beckman Coulter), quantified (Quant-iT dsDNA HS kit, Thermo Fisher Scientific), pooled and subjected to multiplex sequencing [Illumina NextSeq instrument; unidirectional 75 nt reads; $1.3 \times 10^6 \pm 6.1 \times 10^4$ reads/sample (mean ± SD)]. Sequencing reads were processed and mapped to the draft genomes of members of the culture collection. Normalized counts for each bacterial strain in each sample were used to produce a relative abundance table.

Gas Chromatography Mass Spectrometry (GC-MS) of short chain fatty acids – Cecal contents were weighed in 2mL glass screw cap vials. 10 μL of an internal standard mix (20mM of formic acid-^{13}C, acetic acid-^{13}C2,d4, propionic acid-d6, butyric acid-^{13}C4, lactic acid-3,3,3-d3 and succinic acid-^{13}C4) was added to each vial, followed by 20 μL 33% HCl. Diethyl ether (1mL) was introduced and the solution

vortexed vigorously for 10 minutes. The two phases were separated by centrifugation (4,000 x g for 5 minutes). The upper organic layer was transferred into another vial and a second 1 mL diethyl ether extraction was performed. After combining the two ether extracts, a 60 µL aliquot was mixed with 20 µL N-tert.-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) in a GC auto-sampler vial with a 100 µL glass insert and the mixture was incubated for 2 hours at room temperature. Derivatized samples (1 µL aliquots) were injected with 100:1 split into an Agilent 7890A gas chromatography system coupled with a 5977B mass spectrometer detector. Analyses were carried on a HP-5MS capillary column (30 m x 0.25 mm, 0.25 µm film thickness, Agilent J & W Scientific, Folsom, CA) using electronic impact (70 eV) as the ionization mode. Helium was used as a carrier gas at a constant flow rate of 1 mL/minute and the solvent delay time was set to 3.5 minutes. The column head-pressure was 10 p.s.i. The temperatures of the injector, transfer line, and quadrupole were 270 °C, 280 °C and 150 °C, respectively. The GC oven was programmed as follows: 45 °C held for 2.25 minutes, increased to 200 °C at a rate of 20 °C/minute, then to 300 °C at a rate of 100 °C/minute, and finally held at 300 °C for 3 minutes.

Liquid Chromatography Quadrupole Time-of-Flight Mass spectrometry (LC-QTOF-MS) - Cecal contents were homogenized in 20 vol/wt of methanol. After centrifugation (8,000 x g, 4 ºC), a 200 µL aliquot of the supernatant was dried in a centrifugal evaporator. Samples were resuspended in 80 µL of 95% methanol, centrifuged (8,000 x g, 4ºC for 5 minutes) to clarify, and transferred to auto-sampler vials with 100 µL glass inserts. Untargeted metabolomics was performed using an Agilent 1290 LC system coupled to an Agilent 6545 Q-TOF mass spectrometer (Santa Clara, CA). A 5 µL aliquot of each sample prepared for positive ESI ionization was injected onto a BEH C18 column (2.1 × 150 mm, 1.7 µm; Waters Corp., Milford, MA), which was heated to 35 °C. The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). A flow rate of 0.3 mL/minute was applied and the following gradient program was employed; 0 to 14 minutes, mobile phase B eluted from 5% to 100% and then kept for 3 minutes at 100% of B. An equilibration time of 3 minutes was used. To provide accurate mass measurements, reference compounds with m/z 121.0509 and 922.0098 were automatically delivered using a dual ESI source during analyses. Mass accuracy was generally better than 4 ppm. N-acetyl-2,3-dehydro-2-deoxyneuraminic acid was identified in the cecal contents of colonized mice fed the S-BMO supplemented M8 diet based on MS/MS analysis and co-migration with a standard (Sigma).

Micro-computed tomography (µCT) of bone - The femur and tibia were harvested from the right rear leg of each animal and cleaned of muscle and connective tissue. Femurs were fixed for ≥48 hours in 70% ethanol, then embedded in 2% agarose and subsequently scanned with a µCT 40 desktop cone beam instrument (ScanCO Medical, Brüttisellen, Switzerland). For analyses of cortical bone, 100 slices were taken for each sample in the transverse plane, with a 6 µm voxel size (high resolution). For all cortical scans, slices began at the midpoint of the femur and extended toward the distal femur. Boundaries of, and thresholds for bone were drawn manually using µCT 40 software. Volumetric parameters were quantified using software associated with the ScanCO instrument.

Histochemical and dynamic histomorphometric analyses of bone - Nine days prior to sacrifice, mice received Calcein green dye (10 mg/kg) via intraperitoneal injection. Seven days later, mice were given Alizarin red dye (30 mg/kg) intraperitoneally. At the time of euthanasia, tibia were isolated and muscle and tissue were removed prior to fixation in 70% ethanol. Fixed tibias were embedded in methylmethacrylate and cut into 5 µm thick longitudinal sections and stained for tartrate resistant acid phosphatase (TRAP) and with hematoxylin, or left unstained for imaging of calcein and alizarin fluorescence. The longitudinal sections were imaged at 100x magnification with a NanoZoomer 2.0-HT system (Hamamatsu Photonics, Shizuoka, Japan). The resulting images were analyzed using BioQuant OSTEO software (BIOQUANT Image Analysis Co., Nashville, TN). For TRAP-stained sections, the region of interest for analysis of trabecular bone was defined as the 2-dimensional area beginning 750 µm proximal to the distal growth plate, extending to the very proximal edge of the growth plate, and spanning the full width of the bone. Osteoclasts were identified as pink TRAP-positive cells. Regions located adjacent to the growth plate where osteoclast density was too high to accurately count cells were excluded from the analysis. For dynamic histomorphometry, the distance between calcein- and alizarin-stained

bone surfaces was measured as the mineral apposition rate. Total mineralizing bone surface was also assessed, and bone formation rate was calculated as the mineral apposition rate divided by mineralizing bone surface.

Flow Cytometry – Lamina propria cell populations were recovered from the colon by thoroughly rinsing the tissue in Hank's Balanced Salt Solution (HBSS) supplemented with 5% v/v fetal bovine serum (FBS, Gibco) and 25 mM HEPES. Epithelial cells were removed by gentle shaking for 40 minutes at 37ºC in HBSS supplemented with 15 mM HEPES, 5 mM EDTA, 10% FBS and 1 mM dithiothreitol. Halfway through the incubation, colonic samples were transferred to fresh buffer. At the conclusion of the epithelial dissociation, samples were finely chopped with scissors and then digested for 40 minutes at 37ºC with gentle shaking in RPMI 1640 containing 0.17 mg/mL Liberase TL (Roche) and 30 μg/mL DNase (Sigma). Samples were centrifuged at 300 x *g* for 5 minutes at 4 ºC. The resulting pellet was resuspended in HBSS with 5% FBS and 25 mM HEPES and passed through a 100 μm pore diameter cell strainer followed by a 40 μm pore diameter cell strainer (Fisher Scientific). Cells that passed through the strainers were collected by centrifugation (300 x *g* for 5 minutes at 4 ºC) and resuspended in 300 μL of HBSS/5% FBS/25 mM HEPES. 100 μL aliquots of the cell suspension prepared from each colon was placed in wells of a 96-well round bottom plate for antibody staining.

For flow cytometry of bone marrow cells, the femur and tibia were harvested from the left rear leg of each animal and cleaned of all muscle and connective tissue. Bones were carefully cut just below the knee (tibia) and just above the knee (femur) and placed cut side down in a 0.5 mL tube with an added hole from a 22g needle. This tube was then placed inside a 1.5 mL tube which was centrifuged (8,000 x g at room temperature for 10 seconds). Pelleted bone marrow cells were resuspended in Phosphate Buffered Saline (PBS) containing 2% v/v FBS and aliquoted into wells of a 96-well plate.

Colonic cells were initially blocked with TruStain fcX (anti-mouse CD16/32 antibody, BioLegend) for 10 minutes at room temperature. Bone marrow cells were not treated with Fc block since CD16 and CD32 antigens were used to characterize progenitor cell populations. Cells were centrifuged (300 x g for 5 min at 4 ºC) and resuspended in 100 μL of LIVE/DEAD Fixable Aqua solution (Life Technologies) for 20 minutes at room temperature. Cells were subsequently washed twice with 1X PBS/2% FBS and stained with fluorochrome-conjugated antibodies before fixation in 4% paraformaldehyde.

Antibodies used for flow cytometry and their sources were as follows: CD11c (clone N418, Biolegend); Ly6C (clone AL-21, BD Biosciences); SiglecF (Clone E50-2440, BD Biosciences); CD11b (clone M1/70, Biolegend); Ly6G (clone 1A8, Biolegend); CD16/32 (clone 93, Biolegend); cKit (clone ACK2, Biolegend); CD127 (clone A7R34, Biolegend); CD34 (clone RAM34, BD Biosciences); Sca-1 (clone D7, Biolegend); CD135 (clone A2F10, Biolegend); TCRβ (clone H57-597, BD Biosciences); CD3e (17A2, BD Biosciences); CD49b (clone DX5, Biolegend); B220 (clone RA3-6B2, BD Biosciences); Gr1 (clone RB6-8C5, BD Biosciences); Ter119 (clone TER-119, Biolegend); FcεRIα (clone Mar-1, Biolegend); CD115 (clone AFS98, Biolegend); CD19 (clone 6D5, Biolegend); NK1.1 (clone PK136, Biolegend); CD45 (clone 30-F11, Biolegend); CD4 (clone RM4-5, Biolegend); IL-17A (clone TC11-18H10.1, Biolegend); CD8α (clone 53-6.7, Biolegend); Thy1 (clone 53-2.1, eBioscience); and FoxP3 (clone FJK-16s, eBioscience).

Flow cytometry was performed on a BD FACSAria III cytometer (BD Biosciences). All cell populations were initially gated on single cells that were LIVE/DEAD$^-$. LT-HSCs were identified as Lineage (Lin)$^-$Sca-1$^+$ cKit$^+$ CD34$^-$ CD135$^-$ cells, ST-HSC as Lin$^-$ Sca-1$^+$ cKit$^+$ CD34$^+$ CD135$^-$ cells, MPPs as Lin$^-$ Sca-1$^+$ cKit$^+$ CD34$^+$ CD135$^+$ cells, MEPs as Lin$^-$ IL7Rα$^-$ cKit$^+$ Sca-1$^-$ CD34$^-$ CD16/32$^-$ cells, CLPs as Lin$^-$ IL7Rα$^+$ cKit$^+$ Sca-1$^+$ cells, CMPs as Lin$^-$ IL7Rα$^-$ cKit$^+$ Sca-1$^-$ CD34$^+$CD16/32$^-$ cells, GMPs as Lin$^-$ IL7Rα$^-$ cKit$^+$ Sca-1$^-$ CD34$^+$ CD16/32$^+$ cells, MDPs as Lin$^-$ CD115$^+$ cKit$^+$ CD135$^+$ Ly6C$^-$ CD11b$^-$ cells, and CMoPs as Lin$^-$ CD115$^+$ cKit$^+$ CD135$^-$ Ly6C^{hi} cells. For LT-HSCs, ST-HSCs, MPPs, MEPs, CLPs, CMPs and GMPs, the lineage cocktail included TCRβ, CD3e, CD49b, B220, Gr1, CD11c, CD11b, Ter119 and FceRIα. For MDPs, CMoPs and bone marrow monocytes the cocktail included CD3, CD19, NK1.1 and Ly6G (5,6). Neutrophils were identified as

$CD45^+CD11b^+CD11c^-Ly6G^+$ cells, monocytes as $CD45^+CD11b^+$ $CD11c^-$ $Ly6G^-$ $Ly6C^{hi/mid}$, eosinophils as $CD45^+$ $CD11b^+$ $CD11c^-$ $SiglecF^+$ and T cells as $CD45^+$ $TCR\beta^+$. Data were analyzed using FlowJo (version 10; Tree Star, Inc.).

RNA-Seq of distal small intestinal gene expression - Total RNA was isolated from the distal third of the small intestines of mice using the RNeasy Mini Kit and the manufacturer's instructions (Qiagen, Hilden, Germany). RNA quality and concentration were determined using a BioAnalyzer Pico kit (Agilent Genomics). Ribosomal RNA was depleted prior to cDNA synthesis and barcoding using the TruSeq Stranded RNA kit (Illumina). cDNA libraries generated from each sample were pooled and sequenced [Illumina NextSeq instrument; 75 nucleotide unidirectional reads; $3.48 \times 10^7 \pm 2.47 \times 10^6$ reads/sample (mean ± SD)]. Adapter sequences were removed from the resulting reads and quality trimming was performed using trim_galore (v0.4.1). Release 92 of the mouse genome was downloaded from Ensembl and a STAR-compatible database was generated (STAR v2.5.3a). Quality-controlled reads were mapped to the mouse genome database using STAR with default settings aside from "--outFilterMatchNmin 25" to generate bam-format alignment files. Finally, featureCounts (subread v1.5.3) was used to generate gene counts for downstream statistical analysis. Count tables were read into R (v3.3.1) and analyzed using DESeq2 (v1.14.1) to identify differentially-expressed genes. The resulting tables were filtered to only include genes with an adjusted p-value <0.05 and a fold-difference in expression between the two diet conditions (unsupplemented M8 versus M8 containing S-BMO) of greater than 2-fold (increased or decreased). The analysis produced a list of 164 genes.

Immunohistochemical studies of intestinal sections - The entire length of the small intestine was removed immediately following euthanasia. The intestine was quickly divided into proximal, middle, and distal thirds, and each segment was subdivided into thirds. The proximal third of each segment was fixed in Carnoy's solution (18 h at room temperature), transferred to 70% ethanol, then embedded in paraffin, and 5 μm-thick sections were prepared. Unstained sections were rehydrated and antigen unmasking was performed by boiling in 50 mM sodium citrate buffer (pH 6) for 20 minutes. Tuft cells were stained with DCLK1/DCAMKL1(D2U3L) antibody (Cell Signaling Technology, catalog number 62257) diluted 1:300 in a solution containing 20 mM Tris, 130 mM NaCl and 0.1% (v/v) Tween-20. Antigen-antibody complexes were visualized using the EXPOSE rabbit-specific HRP/DAB Detection IHC Kit (Abcam) and betazoid 3, 3' diaminobenzidine (DAB) chromogen. Stained sections were washed with Tris/NaCl/Tween-20 and nuclei were visualized with hematoxylin (Leica). A 20X field of view containing the greatest number of DCLK1-positive cells in a given section of a given small intestinal segment was identified by a pathologist blinded to mouse treatment group. An image was captured (Olympus IX70 microscope), converted to greyscale, and digital image analysis was performed using standard ImageJ 1.51h program features along with the IHC Image Analysis Toolbox and the Trainable Segmentation plugins.

Protein quantification –The cecum was removed from each mouse and after harvest of luminal contents the remaining tissue was rinsed gently with PBS. The proximal third of the cecum was combined with 1.6 mm diameter aluminum oxide particles and 1.6 mm diameter silicon particles (Lysing Matrix F; MP Biomedicals) and 400 μL lysis buffer I [1x HALT Protease Inhibitor (Pierce), 5 mM HEPES]. The tissue was disrupted using a Mini-Beadbeater (BioSpec Products) for 2 minutes at room temperature. 400 μL of Lysis Buffer II [1x HALT Protease Inhibitor (Pierce), 5 mM HEPES, 2% Triton X-100] was added to the homogenate, which was incubated on ice for 30 minutes, followed by centrifugation (13,000 x *g* for 5 minutes at 4°C). The concentration of total protein in the resulting supernatant was determined by a bicinchoninic acid (BCA) assay according to manufacturer's instructions (Pierce). The mouse serum and plasma proteins described in **Dataset S2** were quantified using the Milliplex MAP Mouse Bone Magnetic Bead Panel and Milliplex MAP Mouse 32-plex Chemokine/Cytokine Panel. A Luminex BioPlex instrument was used for the bead-based Milliplex assays while a BioTek Synergy instrument was used for ELISA. Levels of tissue proteins were normalized to total protein concentration.

Supplementary Figures

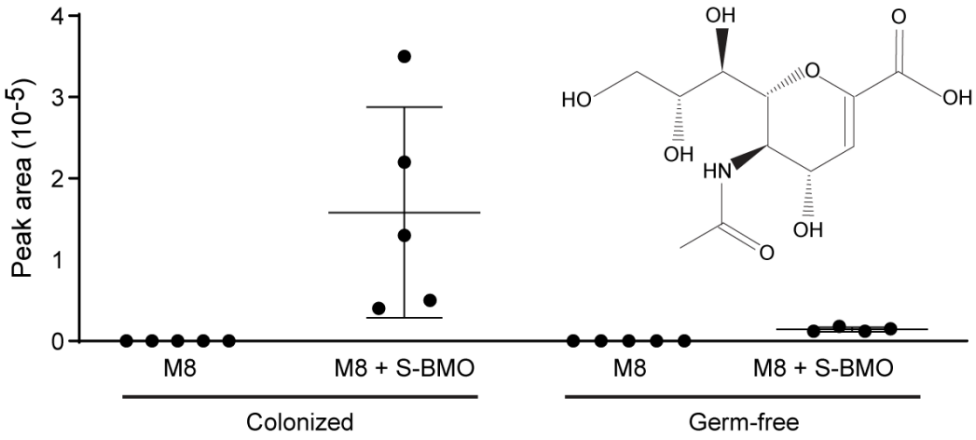

Fig. S1 – Detection of a product of bacterial S-BMO metabolism in cecal contents using LC-QTOF-MS. Colonization- and S-BMO-dependent accumulation of a metabolite (m/z 292.1033) identified as N-Acetyl-2,3-dehydro-2-deoxyneuraminic acid. Each dot represents an individual animal in the indicated treatment group. Horizontal lines denote mean values.

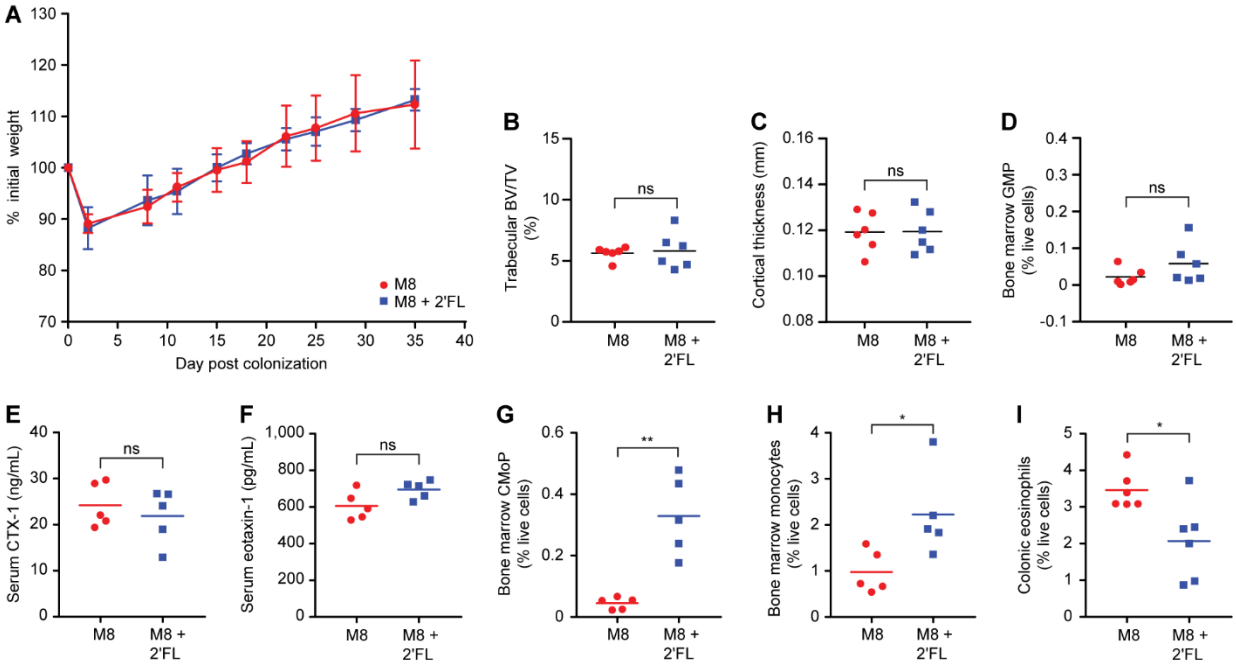

Fig. S2 - The impact of 2'-FL on bone biology and immune function in colonized mice. (A) Weight gain. (**B,C**) Microcomputed tomography of femurs with quantification of trabecular bone volume (panel B) and cortical bone thickness (panel C). (**D**) Representation of bone marrow granulocyte monocyte progenitors. (**E,F**). Serum levels of CTX-1 (panel E) and eotaxin-1 (panel F) determined at the time of euthaniasia on dpg 39. (**G,H**) Representation of bone marrow common monocyte progenitors (CMoPs; panel G) and mature monocytes (panel H). Each dot represents an individual animal. Mean values are indicated by horizontal lines. ns, not significant; *, p <0.05, **, p<0.01 (Mann-Whitney *U* test).

Supplemental References

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Supplementary Datasets

Dataset S1. Consortium of cultured bacterial strains obtained from a severely stunted Malawian infant. (**A**) Effects of S-BMO and 2'-FL on the relative abundances of bacterial strains as measured by COPRO-Seq analysis of cecal contents harvested from gnotobiotic mice at the time of euthanasia. **(B)** Members of the consortium and list of genes encoding known or putative sialidases and fucosidases that are encoded by their genomes.

Dataset S2. Serum and cecal tissue cytokines, chemokines and growth factors from colonized and germ-free mice consuming the M8 diet with or without S-BMO. (A) Serum and cecal cytokines, chemokines and growth factors from colonized mice. (**B**) Serum and cecal cytokines, chemokines and growth factors from germ-free mice.

Dataset S3. Flow cytometry of bone marrow and intestinal cell populations obtained from colonized and germ-free mice fed the M8 diet with or without S-BMO supplementation. **(A)** Cell populations in colonized mice. (**B**) Cell populations in germ-free mice.

Dataset S4. RNA-Seq analysis of the effects of S-BMO on gene expression in the distal third of the small intestine. (A) 164 genes identified as showing significant differences in expression in response to S-BMO administration, as defined by DESeq2 analysis. (**B**) Differentially expressed genes classified under the biological themes 'Eosinophils/Neutrophils,' 'Tuft Cell' and 'Citric/Succinate' as defined by COMPBIO (https://percayai.com).

Dataset S5. Gas chromatography-mass spectrometry of short chain fatty acids in the cecal contents of colonized and germ-free mice fed the M8 diet with or without S-BMO, or with or without 2'-FL supplementation of their drinking water.

Dataset S6. Serum levels of cytokines, chemokines and growth factors in colonized mice with or without 2-FL supplementation