Supplementary information

The composition of the gut microbiota shapes the colon mucus barrier
by Jakobsson *et al.*

Experimental procedures

Animals

Wild type C57BL/6 mice were bred for several generations in two different environments (Room 1 and Room 2) in the animal facility at Gothenburg University. The animals from Room 1 originate from C57BL/6N from Taconic bred in the animal facility for less than 5 generations. The C57BL/6 animals from Room 2 originate from Jackson laboratory but have been bred for more than 10 generations in the animal facility. The animals were housed in standardized conditions of temperature (21-22°C) and illumination (12h light and 12 dark) with food and water provided ad libitum. The animals in each room were fed either a standard chow diet (Food A: R34, Labfor, Lactamin, Stockholm, Sweden) with a Metabolizable Energy of 12.55 kJ/g (composition:16.5% protein, 4.0% fat, 58.0% carbohydrates (nitrogen free extracts), 3.5% fibers and 6.0% minerals (ash) or an autoclaved diet (Food B: 5021 Labdiet®, IPS, UK via Opend, Herfolge, Denmark) with a Metabolizable Energy of 14.07 kJ/g (composition:21.5% protein, 9% fat, 50.4% carbohydrates (nitrogen free extracts), 3.5% fibers and 5.5% minerals (ash). The food was autoclaved at 135°C for 35 min and the sterility controlled. Health reports from sentinel animals screened from the two rooms using FELASA annual serology, FELASA EU annual microbiology, Pinworm and fur Mite panel and Protozoal PCR panel (IDEXX Bioresearch, Ludwigsberg, Germany) were negative for all results except positive for Murine Norovirus and sentinels from Room 2 were positive for *Helicobacter spp./Helicobacter hepaticus* (not identified in the 16SrDNA sequencing results of any animals in the study). Pooled fecal material from several rooms including either room 1 or 2 was positive for Entamoeba muris (cysts were however not detected in fecal histology

samples of analyzed animals). Female mice from the two locations (8-12 weeks of age) were euthanized by isoflurane and cervical dislocation for all experiments. Germ free mice bred in contamination controlled flexible film isolators and were colonized with ceacal flora from mice in the two rooms and treated as conventional animals onwards [1]. All animal experimental procedures were done in full compliance with Swedish animal welfare legislation and were approved by the Swedish Laboratory Animal Ethical Committee in Gothenburg. Free-living house mice (*Mus musculus*) were caught alive in in typical habitats like domestic animal houses and grain storage facilities in southeastern Norway using Ugglan traps (Grahnab, Gnosjö, Sweden), approved by the Norwegian Environment Agency, and transported directly to the University of Gothenburg, approved by the Swedish Board of Agriculture. These mice were caged individually for maximum 4 days prior to analysis, with food and water provided ad libitum. Care was taken to minimize stress prior to euthanasia, and only clinically healthy and behaviorally normal mice were included in the experiments. For this exploratory study animal numbers were chosen with regards to significant differences observed in previous analysis. Groups were selected from preset conditions and not randomized as no treatment was applied.

Explant Tissue

Gastrointestinal tissue was dissected, flushed with ice-cold oxygenated (95% O_2, 5% CO_2) Krebs transport and kept on ice and the longitudinal muscle layer removed by blunt dissection. The intestinal explants were mounted in the horizontal perfusion chamber as described previously [2].

Mucus thickness measurements

The thickness of the intestinal mucus was measured as described previously [2,3]. Briefly, the upper surface of the mucus, secreted by the mounted tissue, was visualized by addition of charcoal particles. The mucus thickness was determined by the distance between the epithelial surface and the mucus surface by a micropipette viewed through a stereomicroscope over time. The mucus that is not attached to the epithelial surface was removed by suctioning with a micropipette and the remaining (small intestine) or inner mucus (colon) was determined. Data is presented as mean ± SEM.

Mucus penetrability assay

Mucus penetrability was measured as described previously [2,4]. Briefly, mouse colonic explants were mounted in the perfusion chamber and incubated for 20 min followed by addition of a suspension of 2 µm green beads, 1 µm far red beads and 0.5 µm red beads (Fluospheres, Life technologies) to the apical surface The beads were left to sediment through the mucus for 40 min after which the position of the beads in relation to the epithelium was analyzed by taking confocal images in XY stacks with an LSM 700 Axio Examiner Z1 confocal imaging system with a Plan- Apochromat 20x/1.0DIC water objective (Zeiss). Images were acquired using the ZEN 2010 software (Zeiss). The impenetrable mucus was determined as the distance to the 20 most penetrating beads. Distances from region of interest at the tissue surface to points indicating beads was measured using the Volocity 6.1.1 software (Perkin-Elmer). Data is presented as boxplots with the median indicated and min-max whiskers.

Tissue fixation and immunostaining

Pieces of ileum or colon with fecal material were fixed in water free Carnoy (methanol) and paraffin embedded. Slide with sections were dewaxed and stained with H&E, Alcian

blue/PAS or hybridized with 10 ng/µl of a general bacterial 16S rRNA probe (EUB 338) and immunostained for Muc2 using the MUC2C3 antisera and DNA by Hoechst 34580 (Life technologies) as previously described [5]. Immunostaining without FISH was performed after citric buffer antigen retrieval using the apoMuc2 antisera [6] or Anti-Ki67 antibody (an16667, Abcam). Images were obtained using a fluorescence microscope, Eclipse E1000 with a Plan-Fluor 40x/0.75 DIC objective (Nikon) or an Axio Examiner Z1 LSM 700 confocal microscope and the ZEN 2010 software (Zeiss).

Longitudinally cut crypts were selected on the stained sections for all measurements. Number of cells in lamina propria was counted on H&E stained sections between two crypts as shown in Fig S1A for 10 locations in 10 mice per group. The crypt length was measured in 6 crypts on 4 mice per group. Ki67 positive cells were counted in 9 crypts per section and in 4 mice per group. Number of goblet cells per crypt was counted on Muc2 stained sections for 15 crypts in 10 mice per group. All measurements were performed in a blinded fashion. Data is presented as boxplots with the median indicated and min-max whiskers.

Proteomic analysis of mucus samples

The mucus samples removed after thickness measurements were solubilized in a guanidinium hydrochloride-based buffer, following the filter-aided sample preparation (FASP) method as described before [7]. Proteins were during this procedure digested with 10 ng/µL porcine trypsin (Promega), and the resulting peptides were eluted and cleaned in StageTips [8]. These samples were then analyzed by nano-reversed phase liquid chromatography (nRPLC) coupled to electrospray ionization-tandem mass spectrometry (ESI-MS/MS) in an LTQ-Orbitrap XL (Thermo Scientific), following the settings previously described for mucus analysis [7].

Data from the MS/MS experiments were analyzed with the MaxQuant 1.2.2.5 software [9]. The databases used were the MaxQuant contaminant database, and the UniProt-SwissProt

mouse database (version of March 2012, only reviewed sequences) completed with our public mucin database (http://www.medkem.gu.se/mucinbiology/databases/). For the searches, we set Met oxidation and N-terminal acetylation as variable modifications, and Cys carbamidomethylation as fixed. The main search error was limited to 6 ppm, with a false discovery rate threshold of 0.01. Relative protein amounts were quantified in ppm by intensity-based absolute quantification (iBAQ) [10] in a total of 3 biological and 2 technical replicates per room and food studied. Data is presented as boxplots with median and whiskers of one standard deviation. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) via the PRIDE partner repository [11] with the dataset identifier PXD001479.

Sample collection for microbiota analysis

Using clean, sterile dissection tools, the ileum (Si8 only), distal colon and caecum were removed. The lumen content from Si8 and distal colon was removed by gently squeezing out the intestinal content in a collection tube. The intestinal segment was then gently flushed with 2x1 ml sterile PBS. The tissue was considered the mucus sample. The samples were immediately flash freezed in liquid nitrogen and later stored at −80°C until analysis.

DNA extraction

DNA was extracted from 80 mg of ceacal sample from each mice, 55-100 mg ileum mucosal tissue and 10-40 mg luminal content from ileum (Si8) and 26-65 mg of mucosal distal colon 30-60 mg luminal content from distal colon. The samples were thawed on ice and transferred to Lysing Matrix E tubes (MP Biomedicals). Lysis buffer (50 mM Tris-HCI, pH 8.0, 500 mM NaCl, 50 mM EDTA, and 4% SDS) was added and the samples were homogenized with a FastPrep®-24 Instrument (MP Biomedicals) for 45 s at speed 5.5. Lysozyme was added to the

sample (100 mg/ml, Sigma Aldrich, Stockholm, Sweden) and the samples were vortexed and incubated first at 37C for 30 min and then at 80C for 15 min. The tubes were inverted every 5 min. Then the samples were centrifuged at full speed for 5 min at 4C. The supernatant was collected and stored on ice. 0.5 mL fresh lysis buffer was added to the lysis tube and all steps without the lysozyme step were repeated and the supernatant from the second centrifugation was pooled with the previous. Then 260 µL of 10M NH4OAc was added the tubes were inverted and incubate on ice for 5 min. The samples were centrifuge at 4C for 10 min at full speed and the supernatant was split into and transferred into two DNA-free 1.5 mL tubes. To each tube one volume of cold isopropanol was added and the samples were then incubated at 4C for 30 min. The samples were then centrifuged at 4C for 20 min at full speed. The supernatant was removed and the pellet washed with 500 µL of cold 70% ethanol. The samples were then centrifuged at 4C for 5 min, the supernatant was removed and the pellet was let to dry at room temperature for approximately 60 min. The pellet was then dissolved 100µL TE buffer and the two aliquots were pooled. The samples were then treated with 2 µL of RNAse A (Qiagen) for 15 min at 37C. 15 µL of proteinase K and 200 µL of Buffer AL (Qiagen) were added and the mixture was incubated at 70°C for 10 min followed by DNA extraction using QIAamp DNA mini kit (Qiagen). The DNA concentration was quantified using a NanoDrop instrument and the quality was assessed on a 1% agarose gel. The DNA samples were stored at -20°C.

16S rRNA gene tag pyrosequencing

Bacterial 16S rRNA gene sequences were amplified from each sample using the primers 27F (5’ AGAGTTTGATCCTGGCTCAG 3’) with Titanium Adaptor B and 338R (5’ TGCTGCCTCCCGTAGGAGT 3’) with Titanium Adaptor A and a sample-specific barcode sequence consisting of twelve nucleotides targeting the V1-V2 hypervariable region of the

16S rRNA gene. Primers included unique error-correcting 12-base barcodes used to tag PCR products from different samples (Fierer et al., 2008). The 16S rRNA genes were amplified in a 50µl reaction containing 1X PCR Buffer, 0.2 uM of each primer, 200 uM dNTP (PurePeak DNA polymerase Mix (Pierce Nucleic Acid Technologies), 1.5U of FastStart Taq DNA Polymerase (Roche) and 10 ng of DNA template for luminal and caecum samples and 1 ul directly from the DNA extraction for the mucus samples. The PCR conditions used were 95ºC for 3 min, 25 cycles of 95ºC for 20 sec, 52ºC for 30 sec and 72ºC for 1 min followed by 72ºC for 10 min. The distal colon samples were run for 28 cycles. Triplicate PCR reactions were performed for each sample. A negative PCR reaction without template was also included in each reaction. The triplicate PCR reaction were pooled and purified with AMPure beads (Becton Dickinson) using a Magnet Particle Separator (Invitrogen) The concentrations of the purified products were measured by Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen). The quality was assessed on a 1% agarose gel. The samples were amplified in PCR-mixture-in-oil emulsions and sequenced from the 338r primer using Roche 454 FLX and Titanium chemistry (Roche) at the Science for Life Laboratories (Solna, Sweden).

16S rRNA Gene Sequence Analysis

Post processing of pyrosequencing data was done using QIIME software 1.7.0 package [12]. For quality filtering the raw data we discarded sequences < 200 bp or > 1,000 bp, and sequences containing primer mismatches, uncorrectable barcodes, ambiguous bases, or homopolymer runs in excess of 6 bases. The sequences were checked for chimeras using ChimeraSlayer [13] and assigned to operational taxonomic units (OTUs) using UCLUST [14] with a 97% threshold of pairwise identity, and then classified taxonomically using Greengenes (GG) reference database (The GGs Databasefile version 13_5). The reads were aligned to the Greengenes Core reference alignment using PyNAST [15]. The GG taxonomies were used to generate summaries of the taxonomic distributions of OTUs across different

levels (phylum, order, family, genus, and species). All OTUs that were observed fewer than 2 times were discarded. To standardize sequence counts across samples with uneven sampling, we randomly selected 1000 sequences per sample (rarefaction) and used this as a basis to compare abundances of OTUs across samples. For phylogenetic tree-based analyses, each OTU was represented by a single sequence that was aligned using PyNAST [15]. A phylogenetic tree was built with FastTree [16] and used for estimates of a-diversity (Rarefraction curves, Chao1 [17], and Shannon diversity [18]) and b-diversity (using unweighted UniFrac [19]. For all diversity measurements, means and standard errors for given categories were calculated from 100 iterations using a rarefaction of 1000 sequences per sample.

Statistical analysis

For all mucus, histology and immunostaining measurements data was analyzed using a two-tailed Mann-Whitney U test with small sample sizes and normal distribution was not in all cases not verified. A P-value <0.05 (*) was regarded as statistically significant (P <0.01 is indicated by **). Only significant differences are marked in graphs. The proteomics data was analyzed for significant differences by ANOVA with 250 randomizations and FDR correction at 1%, no significant difference between rooms of any mucus related protein was observed. Multiscatter between all samples and correlation calculations using Pearson's correlation coefficient was performed.

For the microbiota analysis, statistical significance testing over- and under-representation of the bacterial lineages was made at phylum, class and genus (3% dissimilarity) levels. Significant differences were conducted using the Wilcoxon rank-sum test, and p-values were converted to False Discovery Rate values (q-values) to correct for multiple testing in the R software (http://www.r-project.org/).

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Table S1. The mean of the relative abundance of dominant phyla (bold), classes (bold) and genera in ileum, distal colon, and caecum samples obtained from mice bred in Room 1 or 2 and received either Food A (R34 food) or Food B (AC food). Statistical tests of over or under-representation of bacterial lineages among sample groups (Food A versus Food B) at each sample location and in each room were made at the phylum, class and genus levels using Wilcoxon rank-sum test. To correct for multiple testing, the P-values were converted to False Discovery Rate values (Q-values).
(-) = no sequences present. For values <1 the exact value is only given if significantly different.*p-value <0.01, q-value <0.05, **p-value <0.05, q-value <0.05.

	Ileum lumen Room 1		**Ileum lumen Room 2**		**Ileum mucus Room 1**		**Ileum mucus Room 2**		**Distal colon lumen Room 1**		**Distal colon lumen Room 2**	
	Food A	**Food B**	**Food A**	**Food B**	**Food A**	**Food B**	**Food A**	**Food B**	**Food A**	**Food B**	**Food A**	**Food B**
	n=5 mean % (SD)	n=5 mean % (SD)	n=5 mean % (SD)	n=6 mean % (SD)	n=5 mean % (SD)	n=5 mean % (SD)	n=5 mean % (SD)	n=4 mean % (SD)	n=5 mean % (SD)	n=5 mean % (SD)	n=5 mean % (SD)	n=6 mean % (SD)
Firmicutes	84(4)	91(4)	93(5)*	73(10)*	83(3)	87(4)	94(3)	86(14)	88(8)**	62 (11)**	71(12)	52(16)
Bacilli	51(8)**	22(11)**	75(16)	59(16)	66(9)	18(6)	39(33)	12(9)	36(13)**	12 (4)**	26(19)	11(13)
Lactobacillus	50(9)**	22(11)**	75(16)	59(16)	66(10)	18(6)	38(33)	12(9)	35(12)	12 (4)	26(19)	11(13)
Streptococcus	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Clostridia	2(1)	2(2)	10(13)	8(5)	4(1)	34(31)	51(39)	73(17)	31(21)	11 (2)	42(25)	39(26)
Unknown genus (family unknown)	0.41(0.2)**	0.14(0.1)**	<1	1(1)	<1	<1	<1	<1	14(13)	3 (1)	13(18)	12(8)
Other	0.3(0.1)**	0.1(0.03)**	<1	<1	1(1)	<1	<1	<1	7(5)	2 (1)	13(5)	9(7)
Unknown genus (family Lachnospiraceae	0.1(0.04)**	0.01(0.01)**	2(3)	1(1)	<1	<1	<1	<1	1(1)	<1	7(4)	5(5)
Ruminococcus	0.1(0.04)**	0.01(0.02)**	<1	<1	<1	<1	<1	<1	<1	<1	<1	1(1)
Candidatus arthromitus (Segmented filamentous bacteria)	<1	2(2)	2(2)	2(1)	<1	32(31)	49(38)	70(20)	-	<1	<1	<1
Anaerostipes	0.1(0.1)*	-*	-	-	<1	-	-	-	4(1)	<1	-	-
Dehalobacterium	<1	-	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Oscillospira	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	3(2)	2(1)
Erysipelotrichi	30(5)**	65(9)**	8(9)	6(3)	12(6)	35(24)	4(4)	<1	20(12)**	39 (7)**	2(2)	1(1)
Allobaculum	29(4)**	65(9)**	8(9)	6(3)	12(6)	35(24)	4(4)	<1	19(11)	39 (8)	2(2)	1(1)
Other	2(1)**	0.1(0.1)**	-	-	<1	<1	-	-	1(1)	<1	-	<1
Bacteriodetes	15(4)	6(3)	4(4)*	22(9)*	16(3)**	9(3)**	5(3)	12(13)	11(8)**	33 (11)**	27(11)	44(16)
Bacteroidia	15(4)	6(3)	4(4)	22(9)	16(3)	9(3)	5(3)	12(13)	11(8)**	33 (11)**	27(11)	44(16)
Unknown genus (family S24-7)	15(4)	6(3)	4(4)	22(9)	15(3)	9(3)	5(3)	12(12)	9(6)	13 (1)	11(4)	27(15)
Bacteroides	<1*	<1*	<1	<1	<1	<1	<1	<1	<1	15 (9)	5(3)	3(1)
Parabacteroides	<1	<1	<1	<1	<1	-	<1	<1	<1	1 (1)	6(5)	3(2)
Prevotella	-	-	<1	-	<1	-	-	-	<1	<1	<1	<1
Odoribacter	-	-	-	<1	<1	-	-	<1	<1	<1	<1	1(1)
Unknown genus (family Rikenellaceae)	<1	<1	<1	<1	<1	<1	<1	<1	1(1)	3 (1)	3(1)	8(2)

Proteobacteria	0.2(0.2)**	2(1)**	1(1)	2(1)	1(0.3)**	3(1)**	<1	1(1)	0.6(0.4)**	3(0.4)**	0.3(0.4)*	1(0.3)*
Alphaproteobacteria	-	-	<1	<1	-	<1	<1	<1	-	<1	<1	<1
Betaproteobacteria	0.2(0.2)**	2(0.9)**	<1	<1	<1	3(1)	<1	<1	<1**	2 (1)**	<1	<1
Sutterella	0.2(0.2)**	1(1)**	<1	<1	<1	3(1)	<1	<1	<1	2 (1)	<1	<1
Deltaproteobacteria	0.4(0.2)*	-*	1(1)	1(1)	<1	<1	<1	<1	<1	<1	<1	<1
Desulfovibrio	0.4(0.2)*	-*	1(1)	1(1)	<1	<1	<1	<1	-	<1	<1	<1
Gammaproteobacteria	-	<1	<1	<1	<1	<1	<1	<1	-	<1	-	-
Epsilonbacteria	-	-	-	-	-	-	-	-	-	-	-	-
Actinobacteria	<1	1 (1)	<1	1(1)	<1	<1	<1	<1	0.4(0.1)**	0.8(0.1)**	<1	<1
TM7	<1	<1	0.14(0.21)**	1(0.6)**	<1	-	<1	<1	-*	0.3(0.1)*	0.2(0.1)*	1(0.5)*
Deferribacteres	<1	<1	<1	<1	<1	-	<1	<1	<1	<1	<1	<1
Deferribacteres	-	-	<1	<1	<1	-	<1	<1	<1	<1	<1	<1
Mucispirillum	-	-	<1	<1	<1	-	<1	<1	<1	<1	<1	<1

	Distal colon mucus Room 1		**Distal colon mucus Room 2**		**Caecum Room 1**		**Caecum Room 2**	
	Food A	**Food B**	**Food A**	**Food B**	**Food A**	**Food B**	**Food A**	**Food B**
	n=4 mean % (SD)	n=4 mean % (SD)	n=4 mean % (SD)	n=6 mean % (SD)	n=5 mean % (SD)	n=5 mean % (SD)	n=5 mean % (SD)	n=7 mean % (SD)
Firmicutes	75(12)	55(15)	78(10)	59(16)	88(1)**	73(3)**	83(4)*	73(5)*
Bacilli	20(12)	8(4)	12(8)	8(10)	12(1)**	6(2)**	2(3)	4(3)
Lactobacillus	20(12)	7(4)	12(8)	7(10)	11(1)**	6(2)**	4(3)	4(3)
Streptococcus	<1	<1	<1	<1	<1	<1	<1	<1
Clostridia	49(24)	32(5)	65(13)	50(24)	71(4)**	47(5)**	78(4)*	67(5)*
Unknown genus (family unknown)	22(13)	10(3)	21(21)	18(10)	38(10)**	14(1)**	20(16)	27(9)
Other	13(8)	6(1)	22(14)	12(10)	17(5)**	9(2)**	29(17)	15(5)
Unknown genus (family Lachnospiraceae)	3(2)	3(1)	9(5)	5(4)	3(2)	6(1)	12(5)	6(2)
Ruminococcus	<1	1(1)	1(1)	1(1)	<1	<1	1(1)	1(1)
Candidatus arthromitus (Segmented filamentous bacteria)	<1	<1	1(1)	<1	-	<1	<1	<1
Anaerostipes	1(1)	<1	-	-	1(1)**	<1**	-	-
Dehalobacterium	<1	<1	<1	<1	1.3(0.3)**	0.2(0.2)**	<1	<1
Oscillospira	2(1)	2(1)	4(3)	5(3)	3(1)	2(1)	7(2)	5(2)
Erysipelotrichi	6(4)	15(7)	1(1)	<1	6(3)**	19(4)**	<1	<1
Allobaculum	5(4)	15(7)	1(1)	<1	5(3)**	19(4)**	<1	<1
Other	<1	<1	-	-	-	-	-	-
Bacteriodetes	10(6)	22(11)	10(7)	20(13)	9(1)**	22(4)**	13(3)*	23(5)*
Bacteroidia	10(6)	22(11)	10(7)	20(13)	9(1)**	22(4)**	13(3)*	23(5)*

Unknown genus (family S24-7)	9(4)	8(5)	4(2)	10(8)	6(1)	7(1)	4(1)	10(2)
Bacteroides	<1	10(7)	3(2)	1(1)	0.4(0.3)**	10(3)**	2(1)	1(1)
Parabacteroides	<1	<1	2(2)	1(1)	<1	<1	3(2)	2(1)
Prevotella	<1	-	<1	<1	-*	0.03(0.02)*	<1	<1
Odoribacter	<1	-	1(1)	<1	<1	<1	2(1)	<1
Unknown genus (family Rikenellaceae)	2(1)	2(1)	1(1)	5(3)	2(1)	3(1)	3(2)	8(3)
Proteobacteria	2(1)	19(15)	2(2)	8(5)	1(1)**	3(1)**	<1	1(1)
Alphaproteobacteria	<1	3(2)	<1	1(1)	-	<1	<1	<1
Betaproteobacteria	<1	15(11)	<1	6(4)	0.3(0.2)**	2(0.3)**	0.1(0.02)*	0.3(0.1)*
Sutterella	<1	1(1)	<1	<1	0.3(0.1)**	2(0.3))**	<1	<1
Deltaproteobacteria	<1	<1	<1	<1	<1	1(1)	<1	<1
Desulfovibrio	<1	<1	<1	<1	-*	1(1)*	<1	<1
Gammaproteobacteria	<1	1(1)	<1	<1	-	<1	<1	<1
Actinobacteria	<1	<1	<1	<1	0.3(0.1)**	0.7(0.1)**	<1	<1
TM7	<1	<1	<1	<1	-*	0.2(0.1)*	0.1(0.03)*	0.7(0.4)*
Deferribacteres	13(14)	2(3)	9(9)	11(16)	1(1)	<1	1(1)	2(1)
Deferribacteres	13(14)	2(3)	9(9)	11(16)	1(1)	<1	1(1)	2(1)
Mucispirillum	13(14)	2(3)	9(9)	11(16)	1(1)	<1	1(1)	2(1)

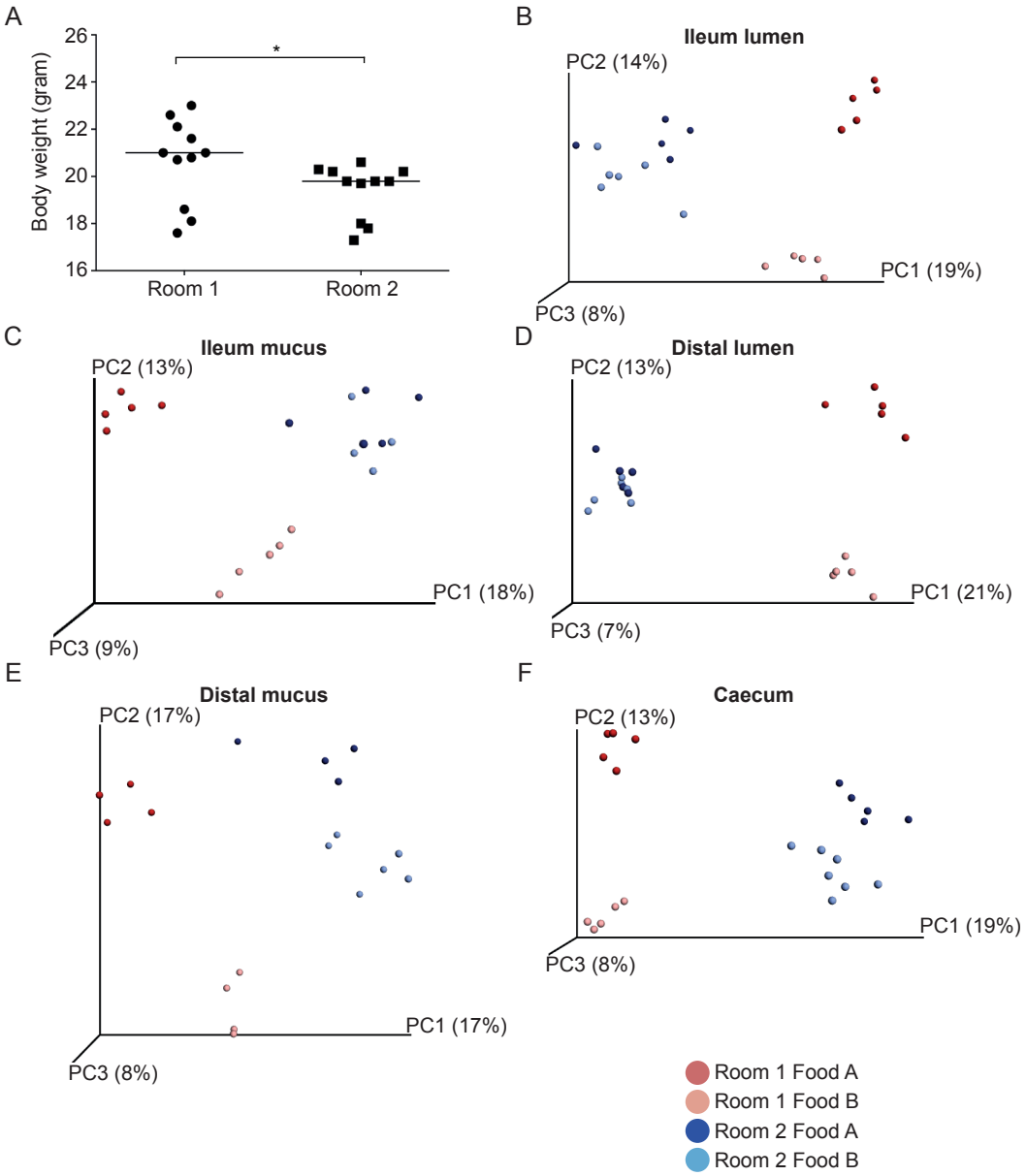

Figure S1. (A) Weight of mice housed in the different rooms (n=11, P=0.022). The median is shown as a straight line. (B-F) Unweighted UniFrac-Based PCoA plot of mice gut microbial communities at different gut segments in the two rooms and with the two foods. B = ileum lumen, C = ileum mucus, D = distal colon lumen, E = distal colon mucus, F = caecum. Dark red = Room 1 and Food A, light red = Room 1 and Food B, Dark blue = Room 2 and Food A, light blue = Room 2 Food B.

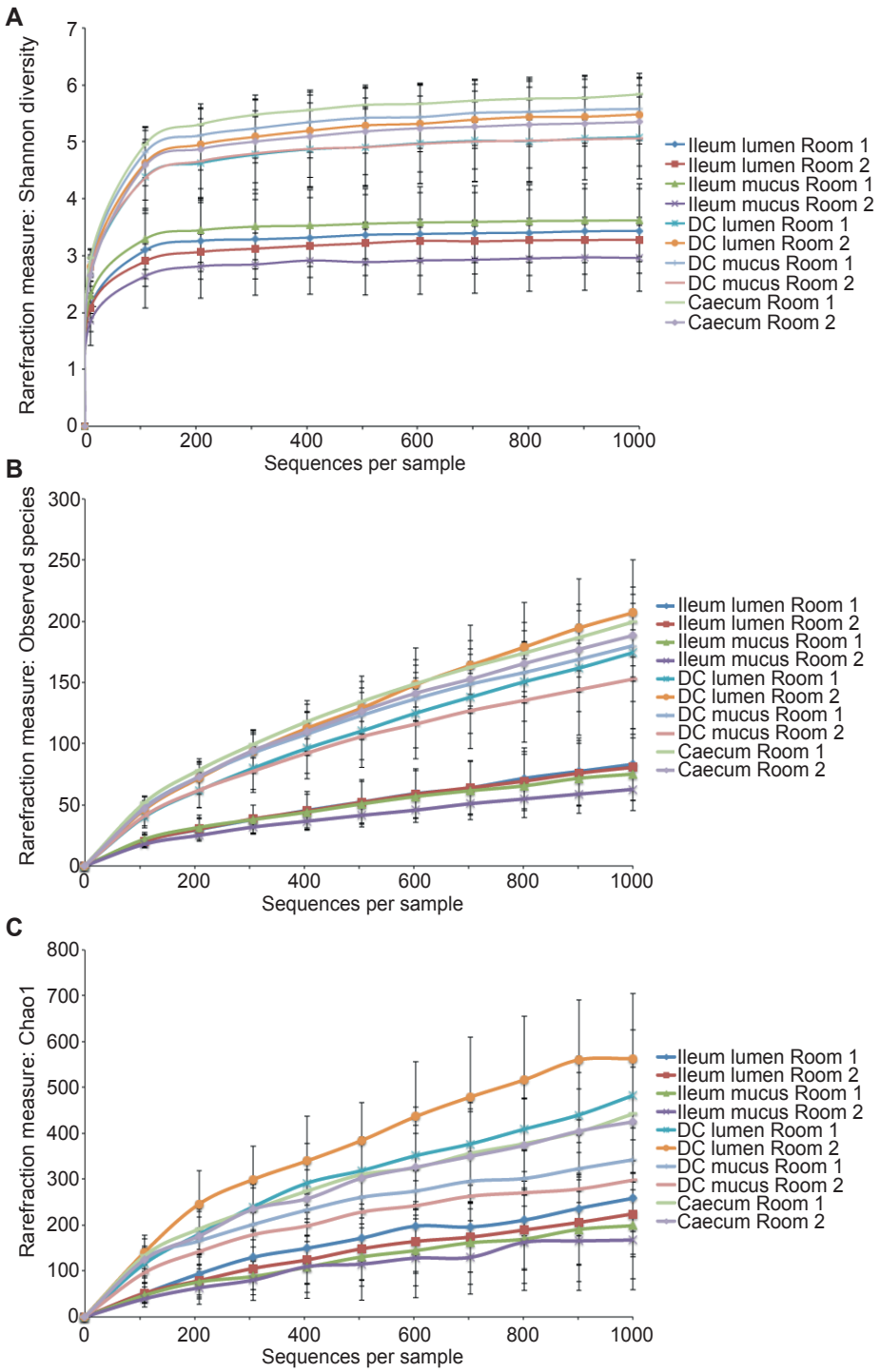

Figure S2. Alpha diversity measurements in Room 1 and 2 at different locations. Rarefraction curves using (A) Shannon diversity index (B) observed species (unique OTUs), and (C) Chao1 as the rarefraction measures from ileum lumen Room 1 (n=11) and Room 2 (n=11), ileum mucus Room 1 (n=11) and Room 2 (n=11), distal colon lumen Room 1 (n=11) and Room 2 (n=11), distal colon mucus Room 1 (n=9) and Room 2 (n=7), and caecum samples Room 1 (n=11) and Room 2 (n=11). Shannon diversity index revealed no significant differences in diversity between the two rooms on Food A, except a higher diversity in ileum mucus samples from mice in Room 1 (A, Mann-Whitney U test, $P = 0.0398$). When using different alpha diversity measurements and rare fraction curves to illustrate diversity and sequencing depth within different gut segments, ileum samples displayed the lowest diversity compared to distal colon and caecum (B and C). The highest diversity was found in distal colon lumen samples from Room 2 (B and C). Data presented as mean±SE.

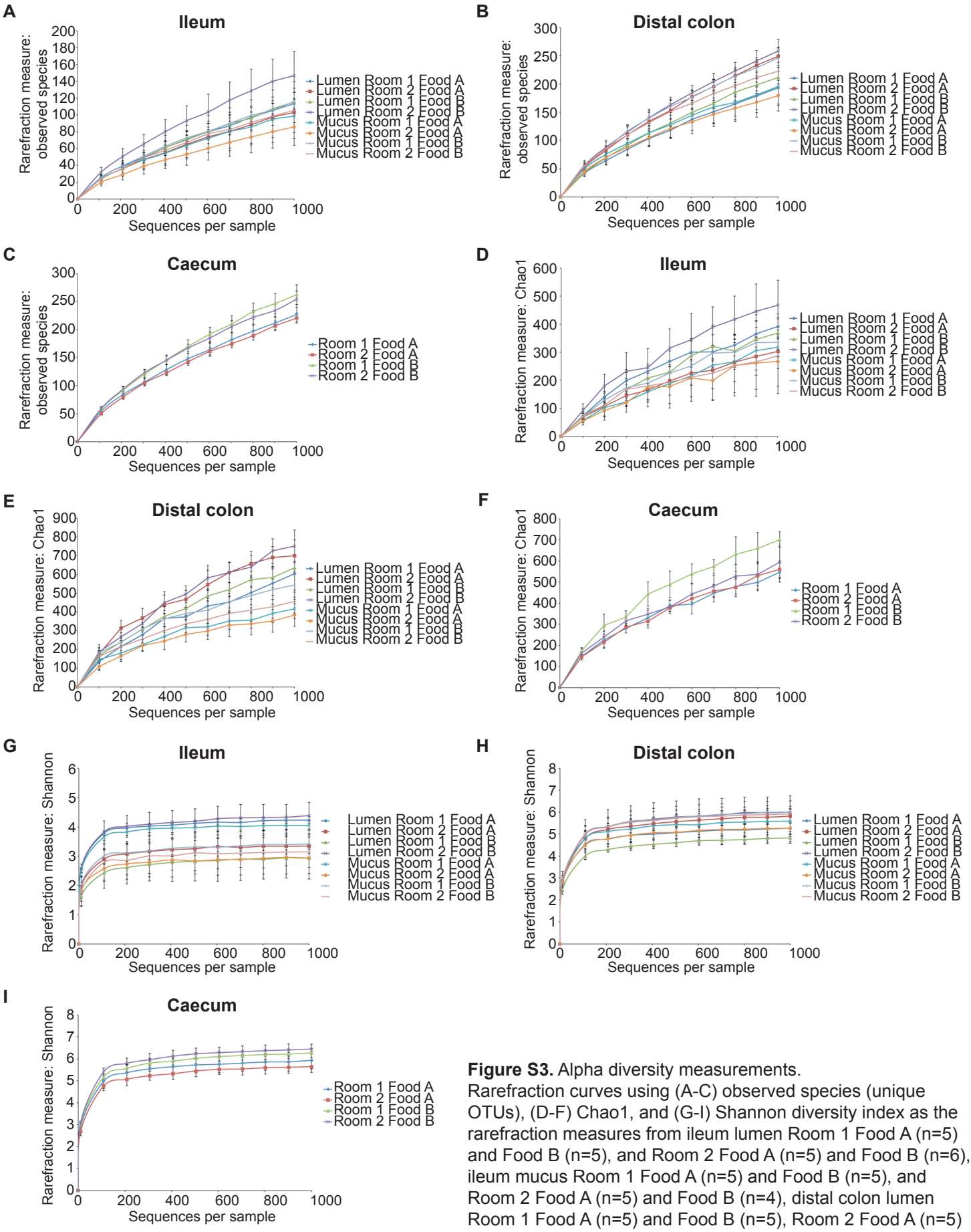

Figure S3. Alpha diversity measurements. Rarefraction curves using (A-C) observed species (unique OTUs), (D-F) Chao1, and (G-I) Shannon diversity index as the rarefraction measures from ileum lumen Room 1 Food A (n=5) and Food B (n=5), and Room 2 Food A (n=5) and Food B (n=6), ileum mucus Room 1 Food A (n=5) and Food B (n=5), and Room 2 Food A (n=5) and Food B (n=4), distal colon lumen Room 1 Food A (n=5) and Food B (n=5), Room 2 Food A (n=5) and Food B (n=6) and distal colon mucus Room 1 Food A (n=4) and Food B (n=4) and Room 2 Food A (n=4) and Food B (n=6), ceacum Room 1 Food A (n=5) and Food B (n=5), Room 2 Food A (n=5) and Food B (n=7). Comparing mice on different foods using different alpha diversity measurements and rare fraction curves it showed that the diversity was highest in lumen samples from mice fed Food B in Room 2 and lowest in mucus samples from mice from the same room fed Food A (A-B and D-E). This indicated that mice from Room 2 were to a larger extent affected by the altered diet. In caecum, the diversity was highest in mice from Room 1 fed Food B and lowest in mice from Room 2 fed Food A (C and F). Shannon diversity index indicated that mice in Room 1 fed Food A had a higher diversity in ileum (lumen $P = 0.0079$, mucus $P = 0.0159$) and mice in Room2 fed Food B had a higher diversity in ileum lumen and caecum ($P = 0.0303$ and $P = 0.0051$ respectively) (G-I). Data presented as mean±SE.

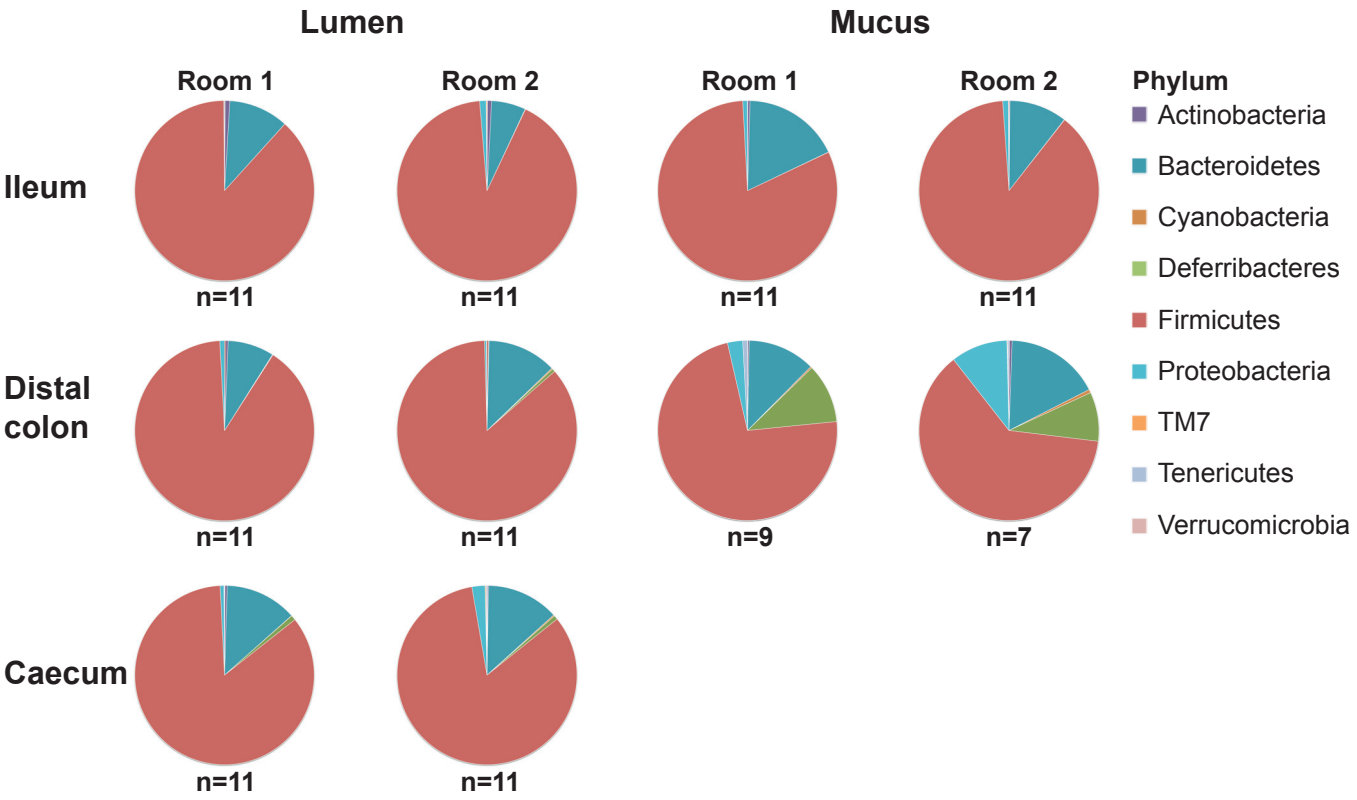

Figure S4. Phylum level microbiota composition in mice from Room 1 and Room 2 (Related to Figure 2). The mean relative abundance (%) of the most abundant bacterial phyla in ileum lumen Room 1 (n=11) and Room 2 (n=11), ileum mucus Room 1 (n=11) and Room 2 (n=11), distal colon lumen Room 1 (n=11) and Room 2 (n=11), distal colon mucus Room 1 (n=9) and Room 2 (n=7), and caecum in Room 1 (n=11) and Room 2 (n=11).

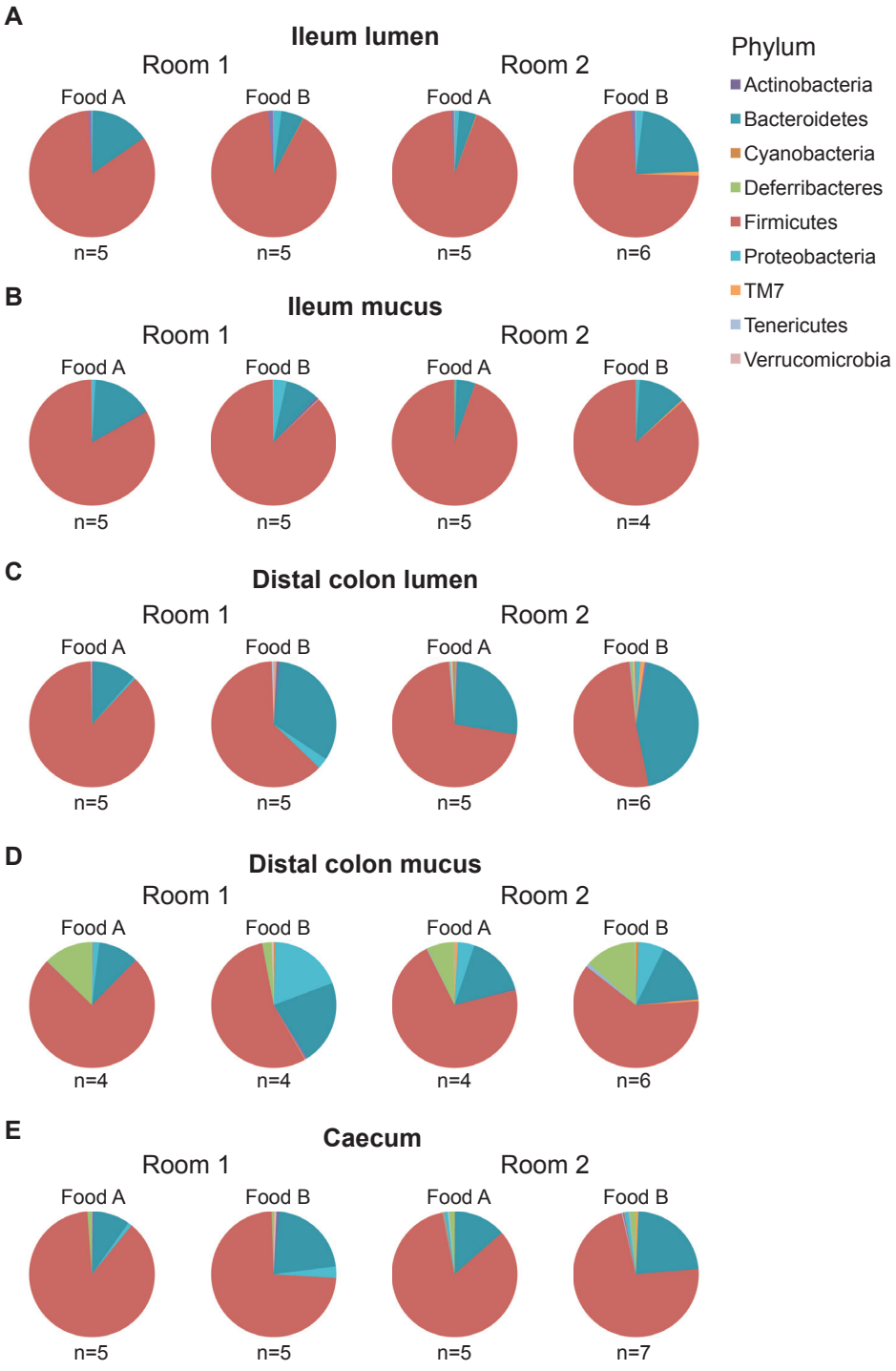

Figure S5. Phylum level microbiota composition in mice fed either Food A or Food B in Room 1 and Room 2. The mean relative abundances (%) of the most abundant bacterial phyla in (A) ileum lumen Room 1 Food A (n=5) and Food B (n=5), and Room 2 Food A (n=5) and Food B (n=6), (B) ileum mucus Room 1 Food A (n=5) and Food B (n=5), and Room 2 Food A (n=5) and Food B (n=4), (C) distal colon lumen Room 1 Food A (n=5) and Food B (n=5), Room 2 Food A (n=5) and Food B (n=6) and (D) distal colon mucus Room 1 Food A (n=4) and Food B (n=4) and Room 2 Food A (n=4) and Food B (n=6), and (E) caecum Room 1 Food A (n=5) and Food B (n=5), Room 2 Food A (n=5) and Food B (n=7).

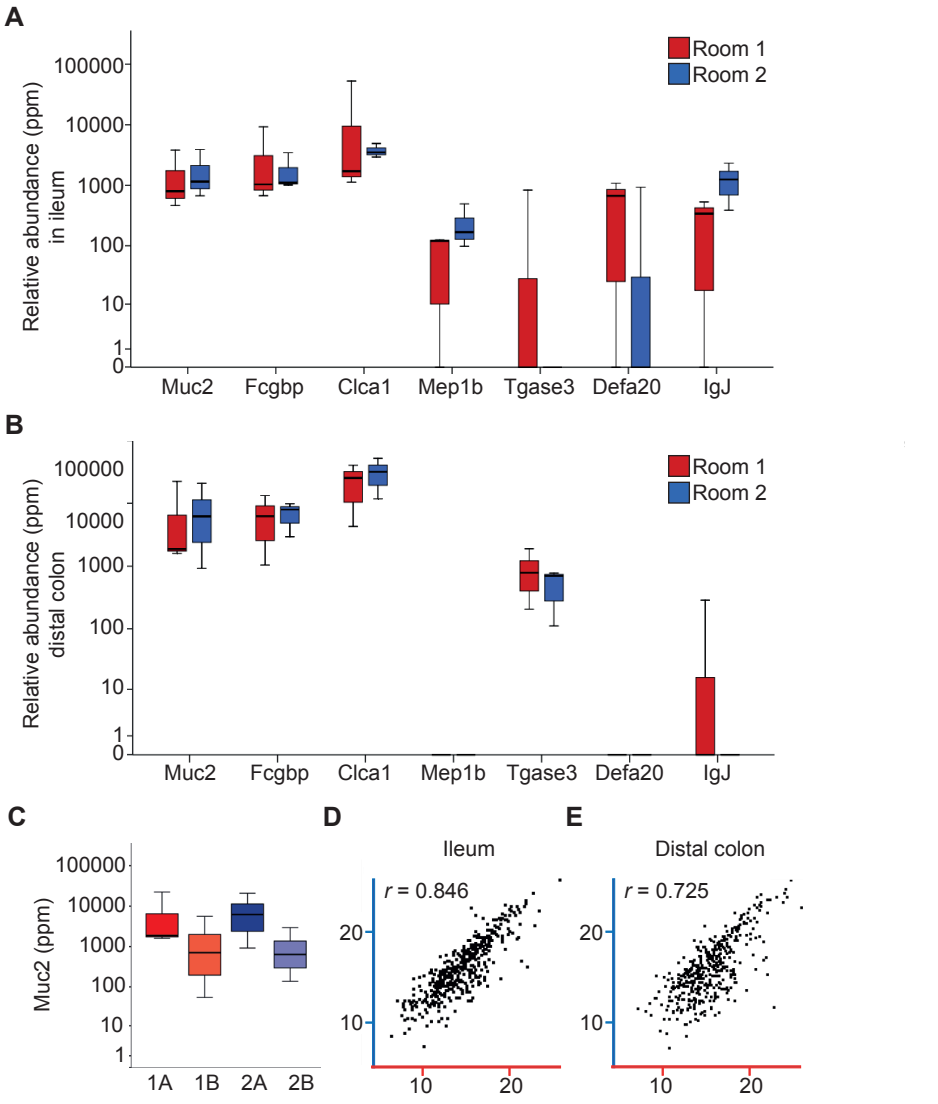

Figure S6. Proteomic analysis of the mucus in ileum and distal colon of mice housed in the two different rooms (Room 1 and Room 2).
The relative abundance (in ppm) is shown for some of the proteins detected in the mucus in (A) ileum and (B) distal colon with Food A (n=3, data is presented as boxplots indicating median and 1SD whiskers). (C) Relative abundance of Muc2 (in ppm) in the distal colon mucus of mice housedin two different rooms and fed the different foods. No significant difference was detected (n=5, data is presented as indicating median and 1SD whiskers). Correlation between the two rooms (Room 1=red, Room 2=blue) of all proteins identified at each location is shown in (C) ileum and (D) distal colon (r= Pearson's correlation coefficient).

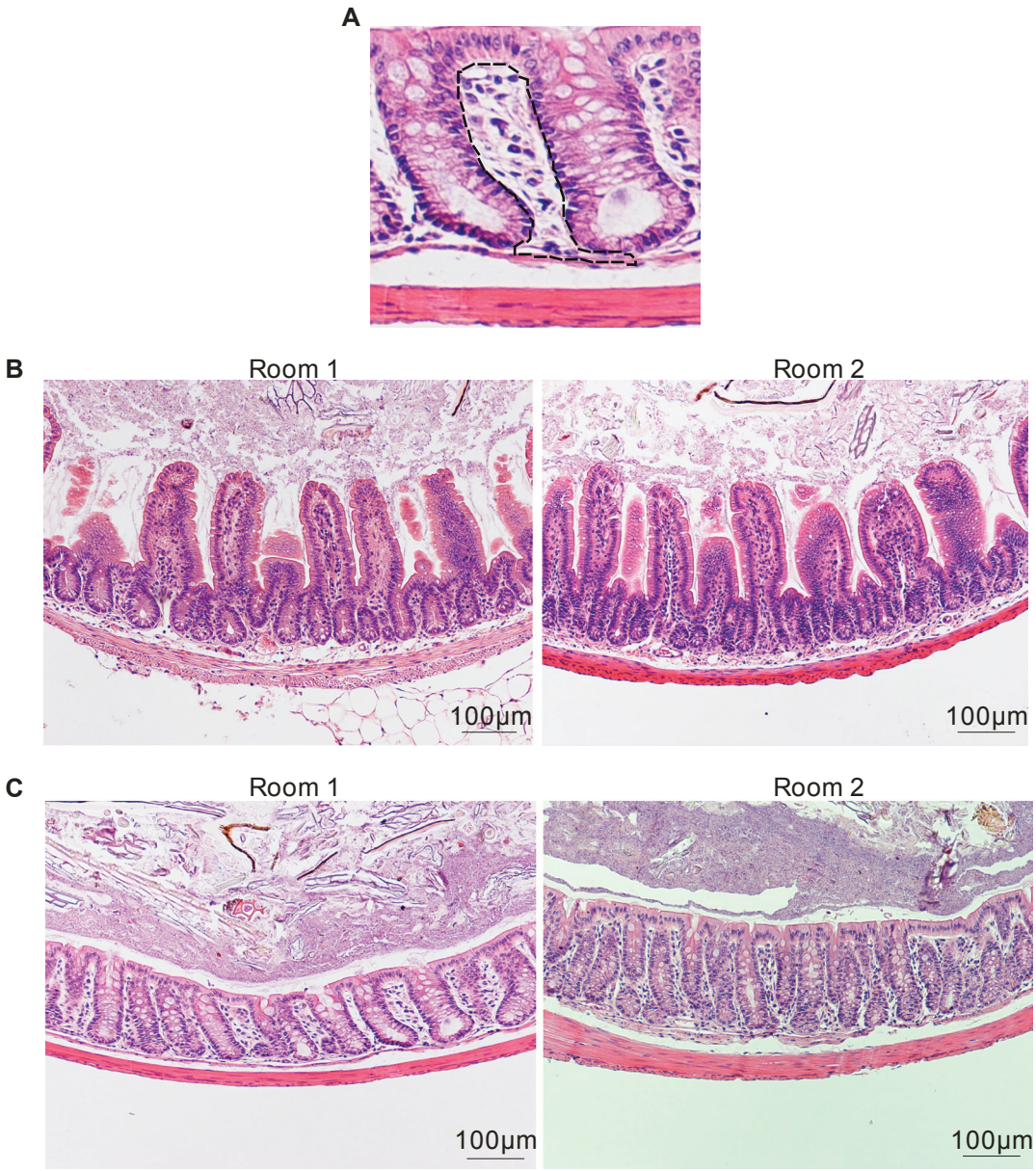

Figure S7. Histology of mice in the two husbandries**.**
A) H&E stained colon section illustrating the area used to count lamina propria cells between crypts to generate data presented in Figure 4G. Histology with H&E stained distal small intestinal (B) or colonic (C) samples from Room 1 and Room 2 on food A.