Supplementary Figure Legends

Supplementary Fig S1. Effect of RB and FRB-intake on AOM/DSS-induced colonic macroscopic lesions in gut microbiota-intact^{spf} (specific pathogen-free) Balb/c mice. (A) Experimental study design; six-week old male Balb/c mice^{spf} fed a control AIN-93 pellet diet for one-week acclimatization period and then administered a single intraperitoneal (i.p) injection of AOM 10 mg/kg body weight in saline followed by exposure to 2% DSS in drinking water for five days. After DSS treatment mice were switched to rice bran (RB, n=20), *B. longum*-fermented rice bran (FRB, n=20) supplemented diets or maintained on a control AIN-93M diet (n=20) for 15 weeks and sacrificed to harvest tissues. Non-AOM/DSS exposed mice (on control AIN-93M diet) served as overall negative controls (NC, n=5) in the study; non-AOM/DSS exposed but fed-with RB (n=5) or FRB (n=4) diets were also included as negative controls. Effect of RB and FRB-supplemented diets on **(B)** colon length, **(C)** total macroscopic lesions/colon, and **(D)** size distribution of macroscopic lesions in proximal, middle, and distal colon (lesion size were categorized as <1mm, 1-2mm, >2-3mm, and >3mm). Quantified data is represented as columns (mean for each group); ***P ≤0 .001, **P ≤ 0.01, and *P ≤ 0.05. RB, rice bran; FRB, *Bifidobacterium longum*-fermented RB; AOM, azoxymethane; DSS, dextran sodium sulfate; mice^{spf}, specific pathogen-free mice.

Supplementary Fig S2. Proteome profiler-based plasma cytokine/chemokine dot-blot array depicting changes in their expression after intake of RB and FRB-supplemented diets during AOM/DSS-induced colon tumorigenesis in gut microbiota-intact^{spf} mice. Plasma collected from untreated (negative controls) and AOM/DSS mice^{spf} (with and without RB and FRB-supplemented diets) was evaluated for the presence of various cytokines/chemokines and other inflammatory-mediators involved in immune responses, and CRC growth and progression.

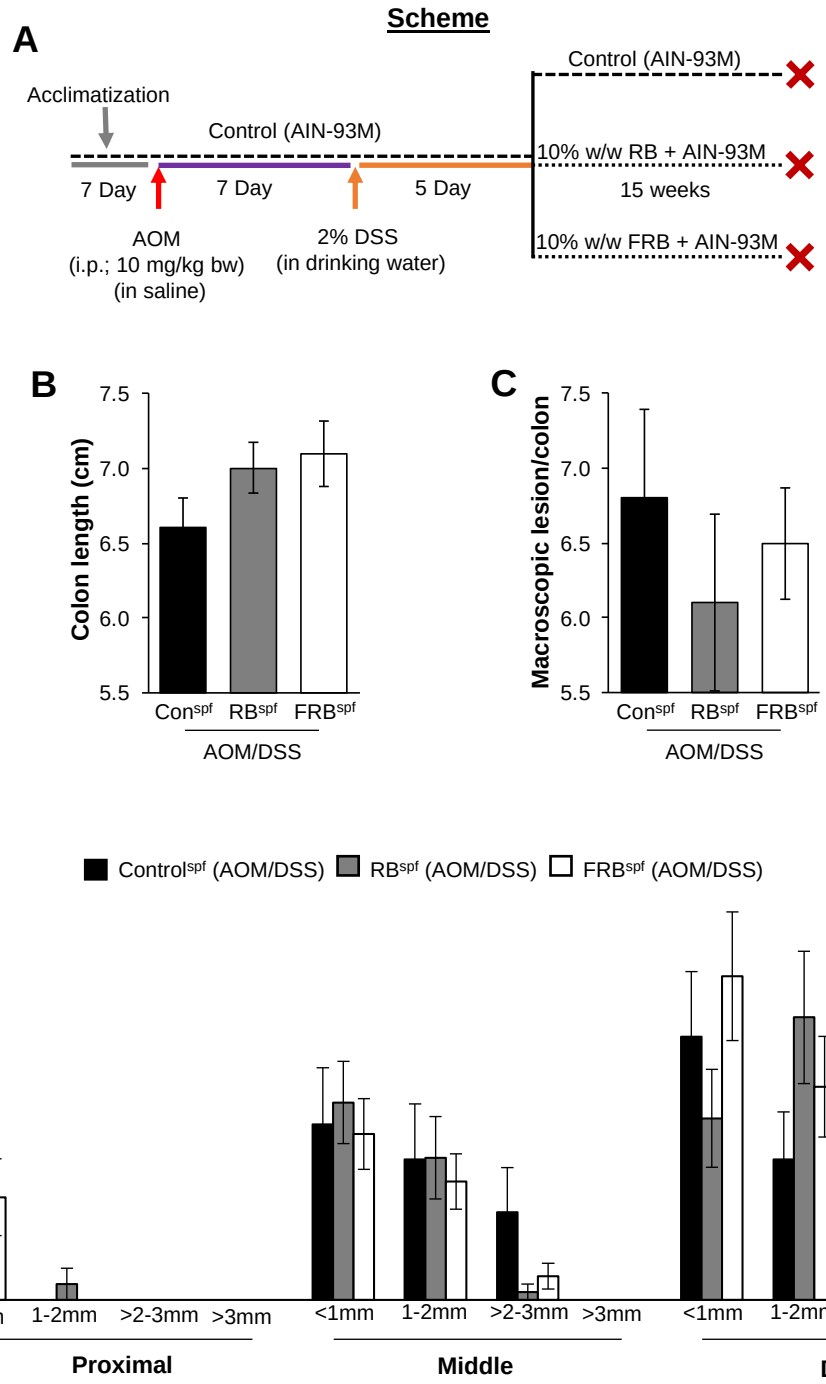
using a mouse-Proteome profiler membrane array containing antibodies to 111 different molecules. Densitometric analysis of dot blots from pooled samples in each group are shown as fold changes relative to negative controls (NC). Quantified data is represented as Columns (mean for each group); bars represent SEM. *P ≤ 0.05. RB, rice bran; FRB, *Bifidobacterium longum*-fermented RB; AOM, azoxymethane; DSS, dextran sodium sulfate; mice^{spf}, specific pathogen-free mice.

Supplementary Fig S3. Gender-specific effects of RB and FRB-intake on AOM/DSS-induced colonic macroscopic lesions in the absence of gut microbiota (germ-free mice^{gf}). **(A)** Experimental study design: germ-free mice^{gf} (C57Bl/6 mice-inbred in gnotobiotic facility) were administered a single intraperitoneal (i.p) injection of 10 mg/kg AOM in saline. Seven days after AOM injection, mice were exposed to 2% DSS in drinking water for five days; this was then followed by 14 days of normal drinking water (resting phase). This cycle (DSS exposure + resting phase) was repeated twice to yield a total of three DSS cycles. After 1st cycle of DSS exposure, Mice^{gf} (male or female) were randomized into three groups according to their diet: Control^{gf} (M = 10, F = 3); RB^{gf} (M = 8, F = 7) and FRB^{gf} (M = 10, F = 5) for 18 weeks and then were sacrificed to harvest tissues. Effect of RB and FRB-supplemented diets on **(B)** colon length, **(C)** total macroscopic lesions/colon, and **(D)** size distribution of macroscopic lesions in proximal, middle, and distal colon (lesion size were categorized as <1mm, 1-2mm, and >2-3mm). Quantified data is represented as columns (mean for each group).***P ≤0 .001, **P ≤ 0.01, and *P ≤ 0.05. RB, rice bran; FRB, *Bifidobacterium longum*-fermented RB; AOM, azoxymethane; DSS, dextran sodium sulfate; mice^{gf}, germ free mice

Supplementary Fig S4. Representative pictographs of immunohistochemical staining for the expression of Ki-67 positive cells, and immunoreactivity scores for β-catenin, Cox-2, NF-κB (p65), ZO-1, and claudin-2 expression levels during AOM/DSS-induced colon tumorigenesis in gut microbiota-intact^{spf} mice. Gender specific effects are shown as male (*left-panel*), and female (*right-panel*). Images were acquired at x400 with digitally magnified insets. % Positive cells were quantified as the number of brown-stained cells x100 per total number of cells counted under ×400 magnification in 5-8 randomly selected fields in each sample. Immunoreactivity (represented by intensity of brown staining) was scored as 0 (no staining), +1 (weak), +2 (moderate), +3 (strong) and +4 (very strong). RB, rice bran; FRB, *Bifidobacterium longum*-fermented RB; AOM, azoxymethane; DSS, dextran sodium sulfate; mice^{gf}, germ free mice.

Supplementary Fig S5. Gender-specific effect of RB and FRB-intake on plasma metabolites, during AOM/DSS-induced colon tumorigenesis in the absence of gut microbiota (germ-free mice^{gf}). Relative abundance of metabolites in plasma of **(A)** male and female (RB^{spf}-AOM/DSS) mice; **(B)** male and female (FRB^{spf}-AOM/DSS) mice. Differential metabolite abundance is reported relative to values obtained in control diet fed AOM/DSS treated mice^{gf}. **(C)** Relative abundance of plasma metabolites (male vs. female) in RB and FRB-fed AOM/DSS^{gf} mice. Quantified data is represented as Columns (relative fold change); *P ≤ 0.05. RB, rice bran; FRB, *Bifidobacterium longum*-fermented RB; AOM, azoxymethane; DSS, dextran sodium sulfate; mice^{gf}, germ free mice.

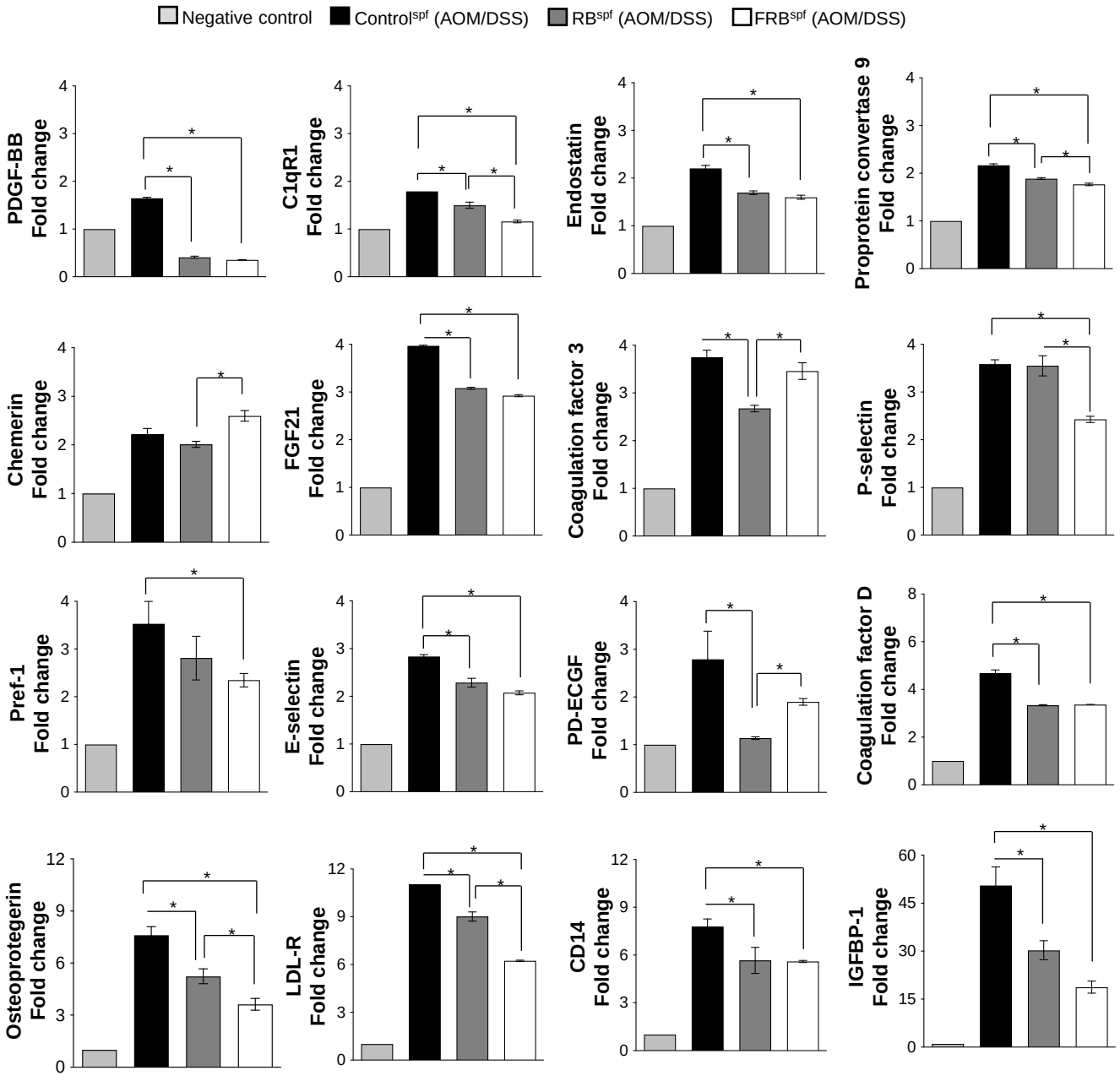
Specific pathogen free mice (Balb/c)



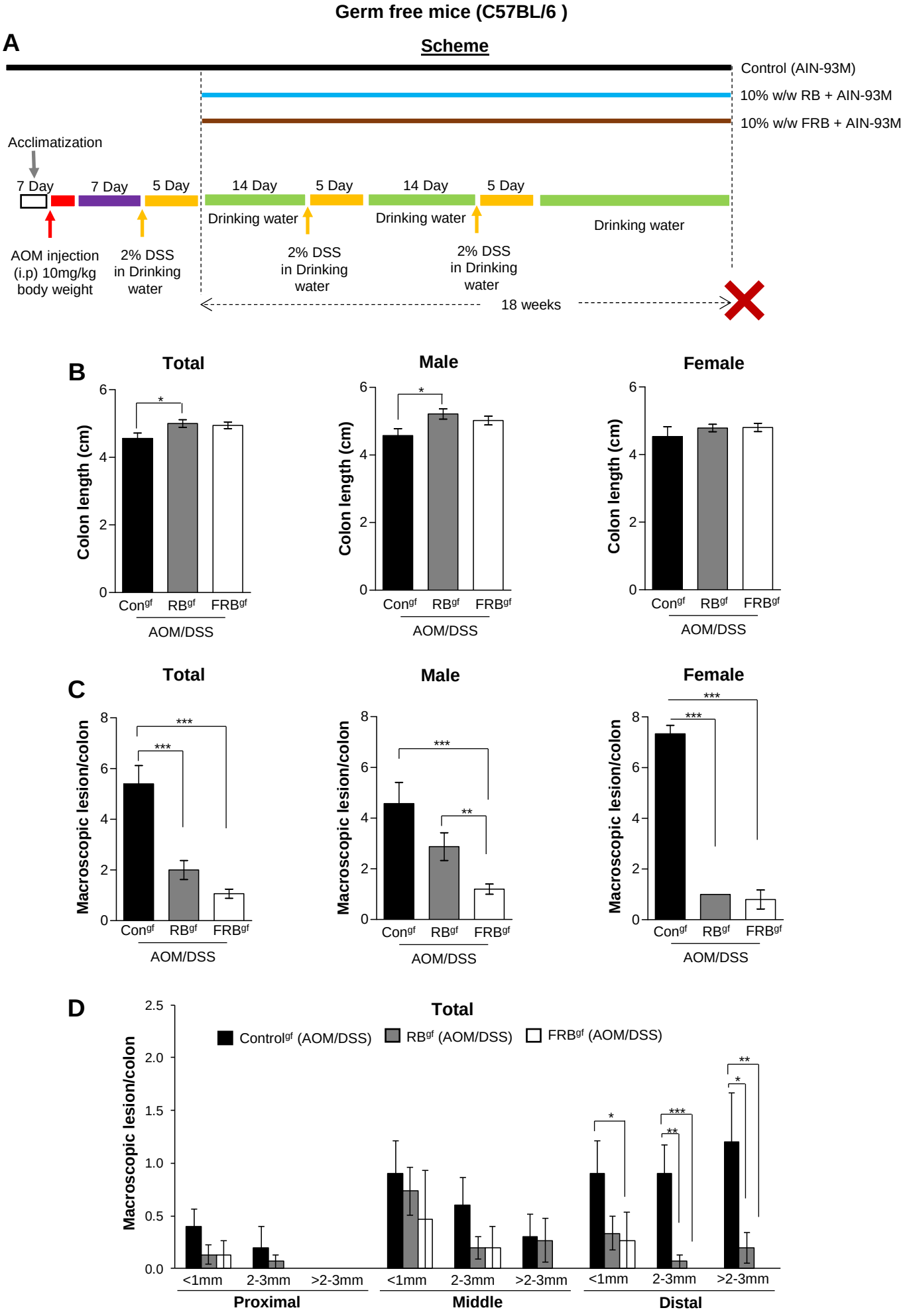
Supplementary Figure S1

Specific pathogen free mice (Balb/c)

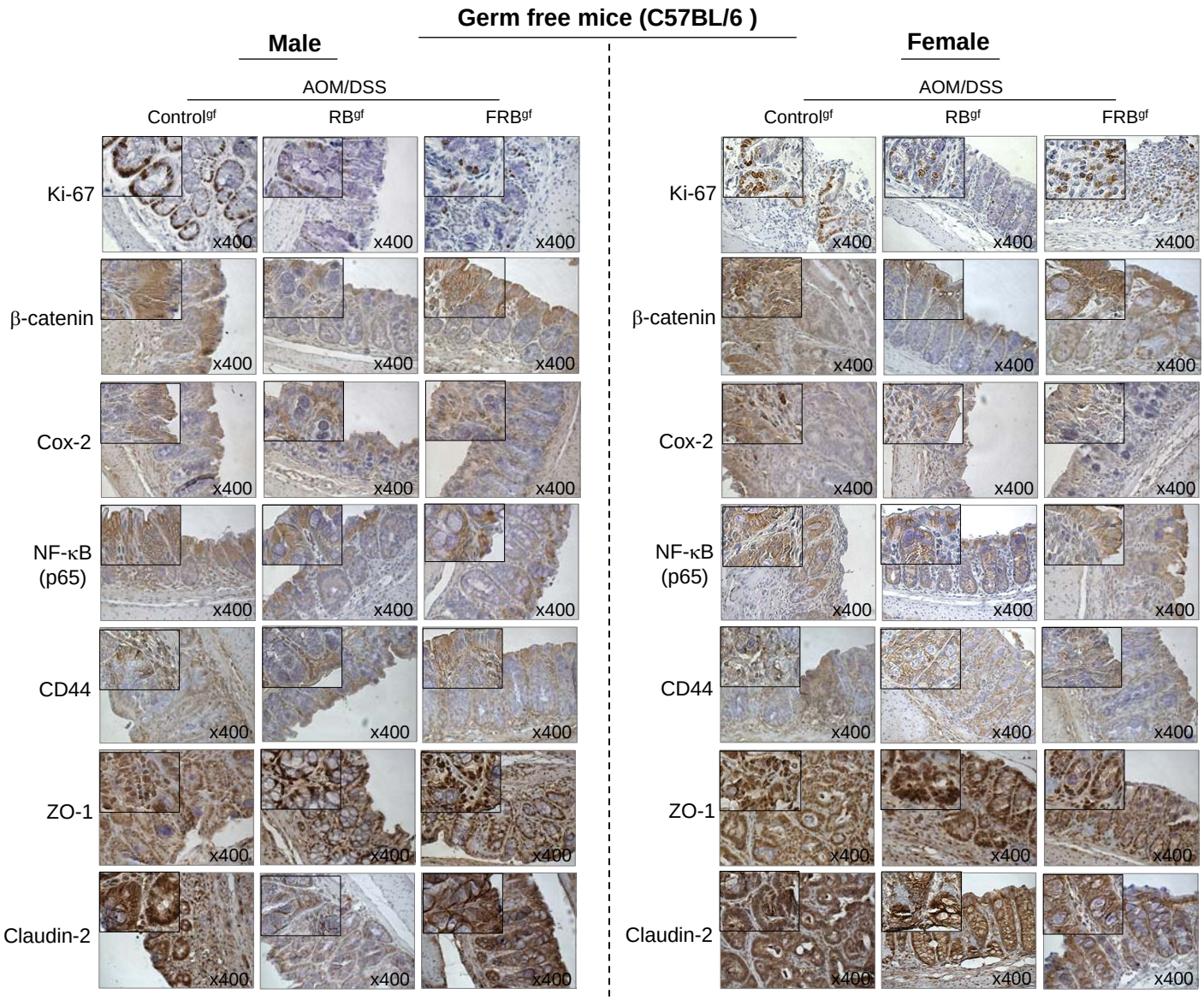
Plasma cytokine profile



Supplementary Figure S2

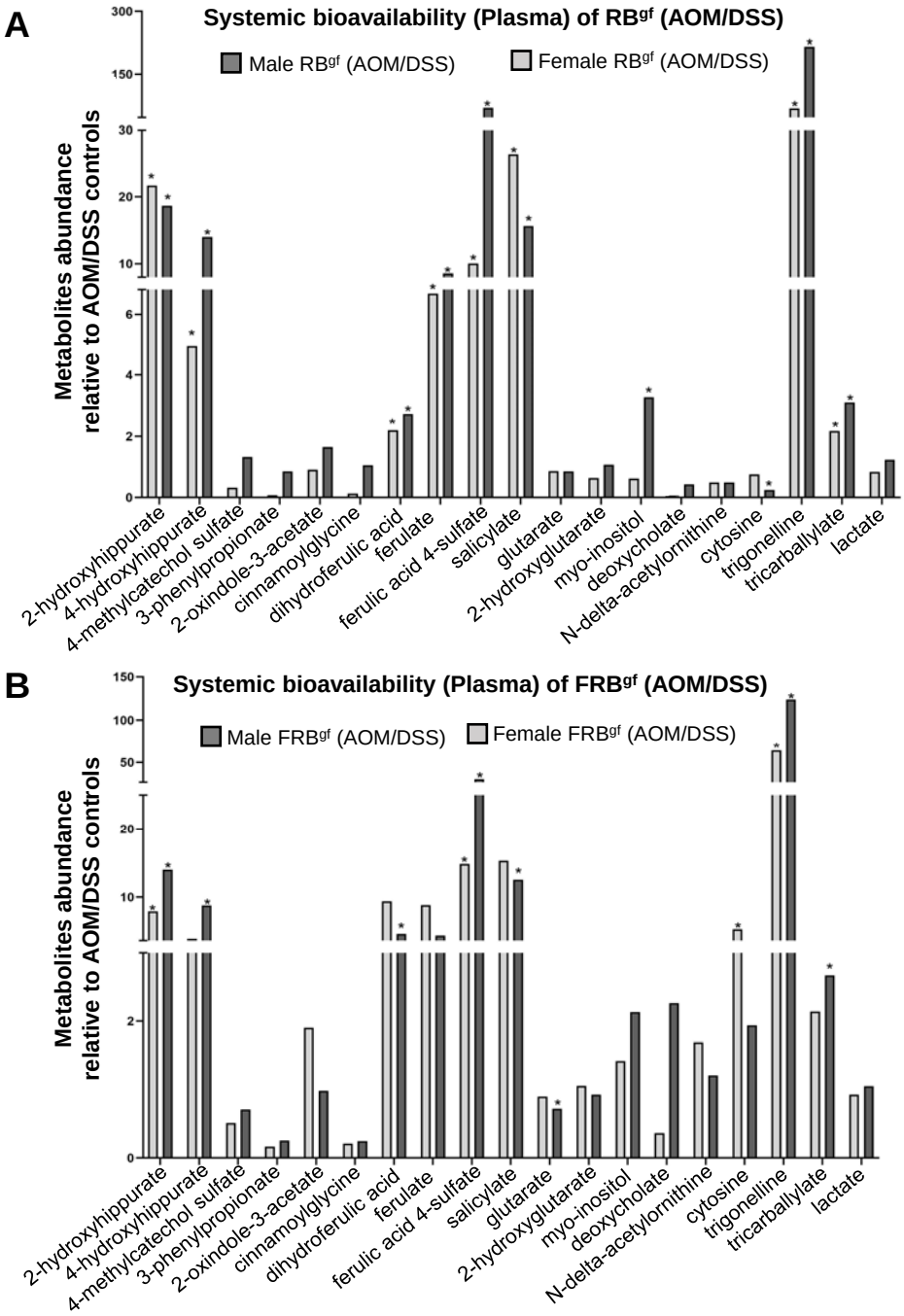


Supplementary Figure S3



Supplementary Figure S4

Germ free mice (C57BL/6)



Supplementary Figure S5

Supplementary Table S1: Pathology scoring criteria used to grade different histopathological changes in the colon tissue of mice exposed to AOM/DSS treatments to induce inflammation-associated colorectal cancer in murine models. *(Note: Adapted and modified from published criteria: PJ Koelink et al., Journal of Crohn's and Colitis, 2018, 794–803, doi:10.1093/ecco-jcc/jjy035)*

Adenoma/Tumor	Dysplasia (Grade)	Crypt Abscess/ Ulceration	Epithelial Erosion
Number of lesions	Low	Absent	Absent
Size range < 1mm, 1-2 mm, >2-3 mm, >3mm	Moderate	Present	Present
	High		Type (Focal, Mucosal-sub-mucosal, and Transmural)

Pathological Score-Colon	Inflammatory Infiltrate	Sub-mucosal inflammation	Loss of Goblet cells	Decrease in Crypt density	Crypt Hyperplasia	Muscle thickening
0	Minimal **[<10%]**	None	None	Normal	None	None
1	Increased presence of inflammatory cells in mucosa **[10%]**	Individual cells	**[<10%]**	**[<10%]**	Slight increase in crypt length	Slight
2	Infiltrates in sub-mucosa **[26-50%]**	Infiltrates	**[10-50%]**	**[<10-50%]**	~2-3 folds increase in crypt length	Strong
3	Transmural infiltration **[> 50%]**	Large infiltrates	**[> 50%]**	**[> 50%]**	> 3 folds increase in crypt length	Excessive

Supplementary Materials and Methods

Animal study design, necropsy, and sample collection

Six-week old male Balb/c mice^{spf} (Charles River Laboratories, Wilmington, MA, USA) were housed in specific pathogen free animal facility at UC Denver-AMC, and fed a control AIN-93M pellet diet for one-week (acclimatization period) and then administered a single intraperitoneal (i.p) injection of AOM 10 mg/kg body weight in saline followed by exposure to 2% DSS in drinking water for five days with one water change once after 2 days providing fresh DSS. After completion of DSS exposure, mice were switched to supplemented diets with rice bran (RB, n=20), *B. longum*-fermented rice bran (FRB, n=20) or maintained on a control AIN-93M diet (n=20) for 15 weeks. An overview of the study design is depicted in Supplementary Figure S1. Non-AOM/DSS exposed mice (on control AIN-93M diet) served as overall negative controls (NC, n=5) in the study; non-AOM/DSS exposed but fed-with RB (n=5) or FRB (n=4) diets were also included as negative controls. Weekly body weight, diet consumption, and general health (stool consistency, incidents of bloody diarrhea/loose stools, and rectal-prolapse were monitored closely for confirmation of colitis events). Aseptic condition was maintained to avoid cross contamination of microbiota between different groups throughout the study. At the end of 15-week feeding phase, all groups were euthanized via CO_2 asphyxiation followed by exsanguination.

Whole blood was collected either in BD vacutainer K2 EDTA coated tubes (for metabolomics studies) or in BD vacutainer Heparin coated tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) (for molecular analysis) and stored at −80 °C. Caecum and its contents were collected, snap frozen, and stored at −80 °C until later use. The entire colon was excised from the caecum onwards to the distal end and cut open longitudinally along its main axis. Swab samples were collected from the proximal and lower portion of the distal colon and used for microbiome

analysis. Next, colons were gently flushed with ice cold saline solution and cleaned with a fine brush to remove remnants of colonic contents as described previously (Nealon et al 2019). Total macroscopic adenomas/ tumors were counted and their size measured with digital calipers under dissecting microscope. Approximately 2.0– 3.0 mm slivers of clean colon tissue from proximal and distal ends were cut, snap frozen, and stored at −80 °C until later use for metabolomics analysis. Colon tissue was then spread flat in ice-cold 4% paraformaldehyde for 2h and then wrapped as swiss rolls and kept for 24h fixation in 4% formaldehyde. In some mice [n=3-4] colon tissue was divided in to three equal portions (proximal, middle and distal)], vertically half portion of each tissue fixed flat in formalin and other frozen in liquid nitrogen (for molecular analysis as relevant).

C57BL/6 germ free mice^{gf} breeding colony and germ-free litters were housed in sterile vinyl isolators in the gnotobiotic facility at UC Denver-AMC. Mice^{gf} were maintained in isolators and provided with ad libitum access to autoclaved water and gamma-irradiated AIN-93M pellet diet. All mice^{gf} were administered a single intraperitoneal (i.p) injection of 10 mg/kg AOM in saline. Seven days after AOM injection, mice were exposed to 2% DSS in drinking water for five days with one water change once after 2 days providing fresh DSS. This was then followed by 14 days of normal drinking water (resting phase). This cycle (DSS exposure + resting phase) was repeated twice to yield a total of three DSS cycles as the C57BL/6 strain is more resistant to AOM/DSS induced pathological manifestations. Mice^{gf} (male or female) were randomized into three groups according to their diet: Control^{gf} (M = 10, F = 3); RB^{gf} (M = 8, F = 7) and FRB^{gf} (M = 10, F = 5) mice. After 1st cycle of DSS exposure, mice were switched to RB, FRB or maintained on control diets for 18 weeks. Body weight, food consumption, and general health of mice was recorded weekly over the course of the study. At the end of the study, animals

were euthanized, and tissue/samples were collected as detailed under in specific pathogen free mice treatment protocol. An overview of the study design is depicted in Supplementary Figure S3.

Metataxonomics sample analysis

Colon and cecum tissue samples from all animal groups were homogenized prior to sample processing. 50 mg/sample were used for DNA extraction performed with the MoBio PowerSoil Kit (MoBio Laboratories Inc., Solana Beach, CA, USA). The extracted DNA samples were stored at -20 °C and concentration and quality checking was performed using NanoDrop 2000 (Thermo-Fisher Scientific). Amplification of the V4 hypervariable region of the 16S rRNA gene and amplicon sequencing on the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) was performed. Full details of this, including PCR conditions, purification, library pooling and primer sequences has been previously published (Nealon et al. 2019).

Raw single-end FASTQ forward sequence reads were imported into the Quantitative Insights Into Microbial Ecology 2 (QIIME 2; https://qiime2.org/) framework. Amplicon sequence variants (ASV) feature tables were constructed, and absolute abundance for each sample was performed using Divisive Amplicon Denoising Algorithm 2 pipeline (DADA2). The feature-classifier plugin was employed for training taxonomic classifiers against 99% operational taxonomic unit (OUT) reference collections from Greengenes 13_8 and SILVA 132, and for assigning taxonomy to each ASV representative sequence. Further processing was performed in R. Microbiome analyses were performed on MacOS Mojave 10.14.3, running versions: biom-format 2.1.7, conda 4.5.12, QIIME 2 2018.11.0, Python 3.6.5, R 3.5.3 ‘Great Truth’, R Studio 1.1.463, and R package versions: ALDEx2 1.14.1, BiocParallel 1.16.6, BiocManager 1.30.4, dplyr 0.8.1.1, ggbiplot 0.55, ggplot2 3.1.1, ggpubr 0.2, grid 3.5.3, reshape2 1.4.3, zCompositions 1.2.0.

Metabolomic analysis

Clean (devoid of any colon fecal contents) proximal and distal colon tissue (50 mg/sample) and plasma (1 mL/sample) from mice were sent to Metabolon Inc for metabolite extraction and identification as previously described (Nealon et al. 2019). Briefly, for each sample 80% methanol was used for extraction and divided into five equal parts for chromatographic extraction including two rounds of reverse-phase ultra-high performance liquid chromatography tandem mass-spectrometry (UPLC-MS/MS) with positive ion mode electrospray ionisation, one round of reverse-phase UPLC-MS/MS with negative ion mode ESI, one round of hydrophilic-interaction (HILIC)/UPLC-MS/MS with negative ion mode ESI and one backup sample.

Statistical Analysis

Metabolite abundances were median scale normalized by dividing the raw abundance of a metabolite by the median raw abundance of that metabolite across the entire dataset. Median scaling was performed individually for each matrix colon. For samples lacking a metabolite, the minimum median-scaled abundance of that metabolite across the given dataset was used as the input value. Metabolite composition explored through PCA ordination followed the compositional data analysis framework described above for metataxonomic data analysis. For a given differentially abundant metabolite, fold differences between groups were transformed using log base 2 prior to visualization using packages dplyr, ggplot2, and ggpubr.