Supplemental Material and Methods:

Liver Histology and Immunohistochemistry Analysis:

H&E, Sirius red and trichrome-stained liver sections were scored by a board-certified veterinary pathologist blinded to experimental groups who assessed fibrosis and periportal/biliary inflammation in a minimum of 12 portal systems within at least two liver lobes. Fibrosis was scored by modified Ludwig criteria [36]: 0, no fibrosis; 1, focal portal/periportal fibrosis, minimal extension into septa; 2, multifocal fibrosis, most (>50%) of portal areas displaying septal fibrosis; 3, fibrosis with most (≥ 50%) portal areas expanded with occasional portal-portal bridging; 4, expanded portal areas with marked (>50%) portal-portal or portal-central bridging; 5, marked bridging with occasional nodules (incomplete cirrhosis); 6, multilobular nodular fibrosis. Periportal/biliary inflammation (0-5) scoring: 0, no inflammation; 1, increased individual inflammatory cells, with rare, loose aggregates (<3 cells); 2, inflammatory cells piling up ≥3 cells deep, +/- rare individual cells in the biliary epithelium; 3, greater aggregation of inflammatory cells expanding/infiltrating along the limiting plate (but not fully connecting to adjacent portal triad) +/- early focal disruption of parenchymal or ductal architecture; 4, more severe than 3, with multifocal single duct architectural disruption, focal areas with more severe architectural disruption along the entire limiting plate between two adjacent portal systems, +/- inflammation with hepatobiliary cell death; 5, severe portal inflammatory infiltrate (loss of lobular architecture with >50% parenchymal inflammatory cell disruption), inflammatory periportal involvement extending to zone 3. We measured hepatic ductular reaction by CK-19, macrophages by F4/80, and neutrophils by MPO staining performed by immunohistochemistry and

semiquantification analysis with Image Pro (Media Cybernetics Inc., Rockville, MD) as previously described. [37,38] Briefly, liver sections (4μm thickness) were deparaffinized and antigen epitope was unmasked with citrate-based heat antigen retrieval following manufacturers protocol (Vector Laboratories, Burlingame, CA). Endogenous peroxidase activity was blocked with hydrogen peroxide and non-specific binding was blocked with 5% BSA prior to overnight incubation with primary antibody at 4°C. Secondary antibody was incubated for 1 hour at room temperature and positive staining was detected via 3,3′-Diaminobenzidine (DAB) following the manufacturer's protocol (Vector Laboratories, Burlingame, CA). Modified Harris hematoxylin provided regressive counterstaining for general nuclear and cytoplasmic staining. Slides were imaged at 100X or 200X magnification with three to five representative sections per mouse with ImageScope 12.1 (Leica, Wetzlar, Germany).

Permeability studies in vivo:

We have optimized FITC-dextran (44kDa, Sigma Aldrich in vivo permeability for distal small intestine/colon (verified by Wood's lamp). Mice were fasted overnight followed by oral gavage of FITC-dextran. Serum collected and read on read on a CLARIOstar Plate Reader (BMG LABTECH in Offenburg, Germany).

Assessing of Bacterial Liver Translocation:

Left lobe (40mg) was sterilely homogenized in 200ml of PBS. 100ml was plated and serially diluted homogenously and spread onto UTI ChromoSelect Agar (Millipore/Sigma 16636) after initially plating on non-selective BHI plates. Colonies were counted after overnight incubation (37ºC). Red-pink and blue colonies representing *E. coli* and

Enterococcus spp (β-D-galactosidase and ß-glucosidase enzyme activity, respectively), were counted and multiplied by the dilution factor. Sanger sequencing verified colonies as *E. faecalis* or *E. coli*.

Primary Sclerosing Cholangitis (PSC) Patient Fecal Analysis:

We analyzed shotgun metagenomic data of fecal samples from 136 patients with PSC and 158 healthy controls. PSC antibiotic (Abx) exposure was defined between 6 weeks and 6 months before study inclusion (+Abx=24, no Abx=112), Abx users within 6 weeks before study inclusion were excluded. Processing and biostatistical analysis were as described.[4]

Bile Acid Analysis:

Serum TBA concentration was determined using the Diazyme Total BA Universal (Diazyme Laboratories, Poway, CA, (DZ042-A-K01)) read on a CLARIOstar Plate Reader (BMG LABTECH in Offenburg, Germany).

Fecal and liver bile acid underwent methanol and acetonitrile tissue processing and isolation prior to injection for LC-MS/MS analysis (conducted by the Zhou laboratory at Virginia Commonwealth University). Quantification using standard a combination of deuterium-labeled and unlabeled internal standards. LC–MS/MS analysis was carried out using a Shimadzu CBM-20A CL communications bus module. The MS analysis was done using an LCMS-8060 CL triple quadrupole instrument with an electrospray ionization source (Shimadzu, Japan). Data acquisition and analysis was performed by

Labsolutions insight.The 7α-hydroxy-4-cholesten-3-one (C4) assay performed by the Zhou laboratory based on HPLC/tandem mass spectrometry. HPLC-MS/MS for bile acid compounds and C4 was optimized in the ESI negative and positive modes, respectively. The total BA (TBA) value is calculated by summing the values of all evaluated BA forms.

ASBT inhibition assay

GSK23306(Linerixibat, a specific ASBT inhibitor) was synthesized by Cayman chemical (23843, Ann Arbor, MI). Three-four week old *mdr2*$^{-/-}$ mice undergoing accelerated 7-day antibiotic pretreatment protocol with GSK23306 (10mg/kg in sterile H_2O) or no GSK23306 for 2 weeks animal weight, BA, molecular and histological data analysis were conducted with tissue samples at time of euthanasia.

Bacterial inoculation/fecal transplant experiments and SCFA administration:

mdr2$^{-/-}$ mouse liver-derived *E. fecalis* and *E. coli,* 17-strain Clostridia (Atarashi Nature 2013), human *E. fecalis* cytolysin active/inactive strains (gift from Dr. Schnable lab), & 23-strain *Lachnospiraceae*[23] were orally gavaged with 10^8 CFU of bacteria/200ul sterile PBS in combination or alone in antibiotic-treated or GF *mdr2*$^{-/-}$ and littermate controls. Bacterial culture media consisted of brain-heart infusion broth (BHI) supplemented with 5% FBS, 0.01% L-cysteine and 1% corn starch twice a week for 2-4 weeks before harvest. Plain BHI medium was used as the vehicle control. Mice were inoculated twice weekly for 2 weeks. For antibiotic SCFA experiments, SPF MDR2-/- mice exposed to *ad libitum* drinking of mixture of vancomycin, (1mg/ml) alone or with SCFA cocktail of 67.5

mM acetate, 40 mM butyrate, 25.9 mM propionate (Sigma-Aldrich) were given to mice in DI H20 [36].

For fecal contents transplant experiments, we transplanted feces collected from SPF *mdr2*$^{-/-}$ C57BL/6 mice diluted in PBS at a ratio of 100mg of pre-reduced anaerobic feces/ml that was homogenized in a bead beater. The insoluble particles were removed by passing the mixture through a laboratory stainless sieve. Total bacterial protein concentration was determined by BCA assay to normalize the amount of bacterial cells. The feces mixtures were stored in -80C until needed for use. We orally gavaged with 200ul of fecal mixture twice every 3 days during the first week.

E.faecalis Gelatinase and Cytolysin Agar Assays:

We performed an adapted cytolysin hemolytic assay from previous studies [37] to identify *E.faecalis* cytolysin activity. Human Gilmore *E. Faecalis* cytolysin active and inactive strains [24] (kind gift from Dr. Schnabl laboratory) were compared to isolated *mdr2*$^{-/-}$ liver colonized *E.fecalis* and *E.coli* strains from 8 different mice following antibiotic administration and plated on sheep blood agar plates (BD BBL Agar) incubated overnight at 37°C. Twenty-four hour plates were assessed for -b-hemolytic activity compared to control strains (S5F).

E. faecalis V583 (originally isolated from infected human blood) and DgelE isogenic mutant was generated as described previously [38]and a kind gift from Dr. Carroll's laboratory was used in our study for positive and negative gelatinase activity controls. The abilities of *E. faecalis* V583 and DgelE isogenic mutant to degrade casein were tested using BHI agar containing 2.5% skim milk with a methodology similar to that of

previously described work[38] compared to isolated *mdr2*$^{-/-}$ liver colonized *E.fecalis* strains from 8 different mice following antibiotic administration (S5E). Additionally, 30ul (1e8/ml) aforementioned bacterial group suspensions were placed in a linear line onto skim milk agar plates and was incubated overnight at 37°C. A zone of clearing surrounding E. faecalis cells indicated caseinolytic/gelatinase activity (S5G).

Biochemical Assessment of Fibrosis:

Hydroxyproline (HYP) was determined biochemically as previously described [39]. Briefly, two snap-frozen liver pieces from the left and right liver lobe (100-150 mg additive total) were hydrolyzed in 5 ml 6 N HCl at 110C for 16 h. Based on relative hepatic HYP (per 100 mg of wet liver), total hepatic HYP were calculated (per total liver, as obtained by multiplying liver weights with relative hepatic HYP).

Real-time qPCR:

Consistent snap frozen right hepatic lobes were stored in RNAlater (Thermo Fisher) at -80C. A small proportion was removed from storage solution and homogenized and total RNA isolation via RNeasay Mini Kit (QIAGEN) according to the manufacturer's recommendations. cDNA was obtained by reverse transcription of 250ng of total RNA using iScript cDNA Synthesis Kit (Biorad) according to the instructions of the manufacturer. Relative mRNA transcript levels were quantified using TaqMan Universal PCR Master Mix (Applied Biosystems) applying the TaqMan methodology or iTaq Universal SYBR Green Supermix (Biorad) for SYBR green probes (3.75 pmol of each primer and an estimated 6.25ng of

complementary DNA (cDNA) were set up with each sample in duplicate-triplicates on 96-well plates (Sarstedt, Numbrecht, Germany). The housekeeping gene 18s was amplified in a parallel reaction for normalization. Primers for the following genes were used: Monocyte Chemoattractant Protein-1 (MCP-1), Lipocalin-2 (Lcn-2), Collagen 1a1 (Col1a1), Tissue inhibitor of metalloproteinases (Timp)-1, inducible nitric oxide (iNos)-2, Interleukin-(IL)-6, Tumor Necrosis Factor (TNF)-alpha. Primer sequences are detailed summarized in Table S2. All TaqMan probes are positioned on exon–exon boundaries of corresponding genes to exclude co-amplification of genomic DNA. Sense and antisense primer (each at 0.5 mM) and 0.125 mM50-phoshorylated probe, labeled at its 50-end with the reporter dye 6-carboxyfluorescein (6-FAMe) and at the 30end with the quencher dye 6-carboxy-tetramethyl-rhodamine (TAMRA), were synthesized by ThermoFisher. The relative expression of each sample was first normalized to the expression of the reference gene 18s, and then normalised to the average expression in samples from *mdr2*$^{-/-}$ mice, and the data were analysed according to the 2^{-DDCT} method.

Serum collection

Blood was collected by cardiac puncture at the time of mouse necropsy, and serum was separated using BD serum separator additive microtainer tubes and centrifugation at 4000 x g rcf for 15 minutes. The recovered serum was stored at -80ºC.

Liver Function Tests:

Serum from fresh blood was made by using BD Microtainer SST tubes (Ref: 365967), and saved in -20C in less than a week before measurement in Clinical Chemistry Core of University of North Carolina in Chapel Hill. Each chemistry test was calibrated and

QCed right before measuring using GEMCAL reference serum(S1-33) and LEVEL1 Chemistry Control(Ref: C1-4) and Level 2 Chemistry Control(Ref:C1-5). They are all from Alfawassermann Diagnostic Technologies, LLC.The serum levels of ALT, ALP, Tbil were measured using the kits of ALT, ALP, and Tbil from Alfawassermann with references of SA1052, SA2002, and SA1008, respectively.

Bacterial DNA Isolation/16s rRNA Sequencing/ Analysis Methods:

Fecal samples were collected from live mice, snap-frozen and stored at -80C. DNA was isolated by incubating fecal material at 65^{o} C for 30min in Lysing Matrix E tubes (MP Biomedicals) containing 200mM NaCl, 100mM Tris, 20mM EDTA (pH 8.0), SDS and proteinase K (QIAGEN). Phenol:Chloroform:Isoamyl alcohol (25:24:1) (Invitrogen) was added, and the samples were homogenized at 4C for 3min using a bead beater homogenizer. The samples were centrifuged at 8000 rpm for 3min at 4C, and the supernatant was incubated with equal volume of Phenol:Chloroform:Isoamyl alcohol for 10 min at room temperature. The samples were centrifuged at 13,000rpms for 5min at 4^{o} C, and the aqueous phase was incubated with isopropanol and 3M sodium acetate, pH 5.2, at -20^{o} C for 15hrs to precipitate DNA. The precipitated DNA was collected by centrifugation at 13,000rpm at 4^{o} C for 20min, washed twice with 100% cold ethanol and resuspended in TE buffer. The DNA was further purified using a DNeasy Blood and Tissue Kit (QIAGEN) according to the manufacturer's protocol.

Fecal DNA samples were amplified by PCR and amplified the 16S V4 hypervariable region using 515F and 806R universal primers. We multiplexed (128/120) samples using 12 nt golay barcodes and were sequenced on an Illumina MiSeq to produce (27/67) million paired-end 250bp reads for a total of (6.9/16.7) Gbp. Primers and

barcodes were trimmed from reads to an average of 220bp. Exp1 had a minimum of 8,291 reads per sample with an average of 71,369. Exp2 had a minimum of 37,544 reads/sample and an average of 154,578 reads/sample.

We used the DADA2 module in QIIME2 to merge, denoise, and produce amplicon sequence variants (ASVs). ASVs were assigned to representative taxa with the QIIME2 tool classify-sklearn using the Greengenes database (gg-13-8). All alpha and beta diversity, differential abundance, PCoA, and group significance analyses were performed using the respective QIIME2 tools. Phylogenetic diversity was computed for ASVs by aligning sequences with MAFFT and building a phylogenetic tree with FastTree. Differential abundance was computed among taxa collapsed at several taxonomic levels using ANCOM, corrected for multiple testing using the Holm-Bonferroni method. Functional predictions to the KEGG ontology were made using the PICRUSt 2 custom tree pipeline in QIIME2.

Metabolomic Analysis:

All analyses of metabolites in serum and cecal content were performed at UNC Chemistry and Analytic Core. A UHPLC/Q-TOF method was used for metabolomic analysis of both serum and cecal content samples. The system consisted of an Agilent 1290 Infinity II LC system (Agilent Technologies, Santa Clara, CA) with a pump (G7120A), a column chamber (G7116B), a multisampler (G7167B), and an Agilent 6550 iFunnel Q-TOF mass spectrometer (G6550A). Extracted metabolites were separated on an Acquity UPLC HSS T3 Column (1.8 µm×2.1 mm, 150 mm) (Waters, Milford, MA). B. Injection volume was 2 µL for each cecal content sample and 5 µL for each serum

sample, both stored at 10 ºC. QTOF analysis was operated in electrospray ionization (ESI) in positive mode, with the following settings: capillary voltage, 3.5 kV; drying gas (nitrogen, 200 ºC) at a flow rate of 14 L/min; nebulizer gas (nitrogen), 40 psig; sheath gas (nitrogen, 300 ºC) at a flow rate of 12 L/min; MS1 mass range, m/z 60-1000; collision energy, 20 eV; acquisition rate of MS1, 2 spectra/sec. Vitamers were analyzed by liquid chromatography-tandem mass spectroscopy (LCMS/MS) whereas short chain fatty acids (SCFA) were analyzed by gas chromatography-tandem mass spectroscopy (GC-MS/MS)

SFCAs in feces or cecal content were quantified using a GC-MS based assay as described in Zheng et al (doi: 10.1007/s11306-013-0500-6) with slight modifications. In brief, for each feces sample, 40 mg was aliquot and was added with 0.005 M sodium hydroxide solution containing deuterated SCFAs as internal standards, namely 50 µg/mL d4-acetate, 10 µg/mL d2-propionate, and 10 µg/mL d2-butyrate. The sample was homogenized on a TissueLyzer (Qiagen, Hilden, Germany) and then centrifuged at 13,200 rpm for 20 min. The supernatant layer was transferred to glass tubes and derivatized by subsequently adding water, 1-propanol:pyridine (3:2, v/v), and propyl chloroformate, followed by 2-min sonication. The propyl derivatives were extracted twice with hexane and the combined extracts were transferred to anhydrous sodium sulfate-containing autosampler vial upon GC-MS analysis. The instrumental analysis was conducted using an Agilent 7820A GC-5977B single quadrupole MSD system installed with an electron ionization source (Santa Clara, CA, USA). One µL of extracted was injected under 1:10 split mode, vaporized, and the analytes were separated by an

Agilent DB-5 column (30 m x 0.25 mm x 0.25 µm) (Santa Clara, CA, USA) with helium flowing through at 1 mL/min as the carrier gas. The following temperature program was used: initial oven temperature held at 50 ºC for 2 min, ramped to 70 ºC by 10 ºC/min, to 85 ºC by 3 ºC/min, to 110 ºC by 5 ºC/min, to 290 ºC by 30 ºC/min, and finally maintained at 290 ºC for 8 min.