**SUPPLEMENTAL MATERIALS AND METHODS for**

**M. Jiang et al.,**

**Probiotic-derived NanoparticlesInhibit ALD through Intestinal miR194 Suppression and Subsequent FXR Activation**

**Patient Demographic Information and Sample Collection**

This study was approved by the University of Louisville Institutional Review Board, and patients’ demographic information are detailed in another publication (1). All study participants provided informed consent before participation in the study, including appropriate authorization for data and sample collection. Healthy participants did not have any clinically diagnosed liver or organ system (or inflammation) disease that could cause altered laboratory values for comparison analyses. Inclusion criteria comprised individuals who were 21 years or older and enrolled in the University of Louisville Hospital System. Individuals who were unwilling or unable to provide

informed consent, had significant comorbid conditions (liver, heart, kidney, lung, neurologic or psychiatric illnesses, sepsis) and active drug abuse, pregnant and lactating women, and prisoners or other vulnerable subjects were excluded from the study.

All patients with AH met the diagnosis for AH based on the clinical and laboratory guidelines published by the NIAAA consortium on AH (2) including both moderate and severe AH (**Table S1**). Detailed eligibility for patients with AH from this trial can be found in our previous publications (1)

All participants’ specimens were collected along with the other morning routine and research samples for the study participants. Stool samples were gathered in the 30 ml leak-proof vial Para-Pak kit (CS 120 - Meridian Biosciences, Inc.), transported to the research laboratory, aliquoted into smaller portions (volume by the requirements of the assigned study projects) and frozen at -80°C. Stool samples were collected from 3 healthy controls (HCs) and 29 patients with AH and analyzed as described in following sections. All study participants had a complete history, physical examination, and laboratory evaluation on study enrollment.

**Chemical and biological reagents**

Antibody against CYP7A1, FXR, β-actin and Histone H3 were purchased from Abcam (Cambridge, MA). SHP and SREBP-1c antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Reagents used for Real-time PCR were purchased from Thermo Fisher Scientific (Waltham, MA). ALT, AST and TG assay Kits were purchased from Thermo Fisher Scientific. Bile acid assay kit was purchased from Diazyme (Poway, CA).

**Serum biochemical analysis**

Serum was collected by centrifugation from whole blood sample at 4000 *g* for 30 min at room temperature. Serum ALT, AST and liver TG were measured by using standard laboratory assays.

**LGG Culture and LDNP Isolation**

LGG was purchased from American Type Culture Collection (ATCC 53103, Rockville, MD) and cultured in autoclaved deMan, Rogosa and Sharpe (MRS) broth at 37°C for 40 hours. The culture density was measured with a spectrophotometer at OD600. The culture suspension (2 × 109 CFU/mL) was centrifuged at 2,000g for 10 minutes, at 5,000g for 20 minutes, and then at 10,000g for 30 minutes to eliminate debris including dead cells and other waste materials. The obtained supernatant was filtered and ultracentrifuged at 150,000g for 70 minutes (Optima L-100XP Ultra Centrifuge; Bechman Coulter, Atlanta, GA). After ultracentrifugation, the supernatants were collected and stored, and the pellet containing LDNPs was washed in phosphate-buffered saline (PBS), ultracentrifuged, and resuspended in PBS, and stored at −80°C for later use.

**miRNA isolation and quantification**

miRNA was isolated using the mirVana™ miRNA Isolation Kit (Ambion, Austin, TX, USA) according to the manufacturer's instructions. The purity and quantity of RNA were assessed using the NanoDrop ND-2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). All the samples were diluted to a final concentration of 10 ng/μl. The samples were used immediately or stored at −80 °C for future use.

**TaqMan® Low-Density Array miRNA qRT-PCR**

The RNA (about 50 ng) was reverse transcribed using the TaqMan® MiRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA), and the TaqMan® MiRNA Multiplex RT Assays, Rodent pool A (v2.0) (Applied Biosystems). 3 µl of RNA was added to each reaction and RT-PCR was carried out on the ABI Veriti™ Thermal cycler (Applied Biosystems). 2.5 µl of the product from each reverse transcription reaction was preamplified per the manufacturer's protocol with the Megaplex™ PreAmp Primers (10×), Rodent pool A (v2.0), and TaqMan® PreAmp Master Mix (2×) using the ABI Veriti™ (Applied Biosystems). Following preamplification the miRNA expression was profiled with TaqMan® Rodent MicroRNA array card A v2.0, performed on a 7900HT Fast Real-Time PCR System (Applied Biosystems), using the manufacturer's recommended protocol.

**Cell culture**

Human intestinal epithelial cells Caco-2 were maintained in DMEM-high glucose. The medium was supplemented with 10% fetal bovine serum, 1X penicillin-streptomycin solution (100U/ ml penicillin, and 100µg/ml streptomycin; Sigma Aldrich) in a humidiﬁed atmosphere (5% CO2, 95% air, 37°C). Cells were utilized for experimentation at 70-80% confluence. Caco-2 cells were treated with LDNPs (0.2 ug/ml) and 100 nM miR194 mimic or mimic control for 24 hours.

**3D organoids culture**

Small intestines of C57BL6 mice were dissected and washed several times in cold PBS. Intestinal fragments were incubated in 25 mL of Gentle Cell Dissociation Reagent (GCDR) [STEMCELL Technologies Inc. (STI)] on a rocking platform (20 rpm) at room temperature for 20 minutes, after which the fragments were allowed to settle by gravity and supernatants removed. Intestinal pieces were suspended in 10 mL cold PBS + 0.1% BSA by pipetting up and down several times. The intestinal pieces were again allowed to settle by gravity and the supernatants were collected, passed through 70 µm filters and kept on ice (fraction 1). This process was repeated three times to generate fractions 2 - 4. The quality of each fraction was assessed using an inverted microscope and selected fractions were centrifuged. Crypts were then resuspended in cold DMEM/F-12 (STI), counted, aliquoted into 15 mL tubes in volumes containing 500 - 3000 crypts and centrifuged. Crypts were resuspended in a 50:50 mixture of complete IntestiCultTM Organoid Growth Medium (Supplement 1, Supplement 2 and 100 units/100 µg per mL penicillin/streptomycin) (STI) and Corning Matrigel® at 1,667 - 10,000 crypts/mL, and 50 µL/well of the suspension was pipetted into pre-warmed 24-well plates to form domes containing 80 - 500 crypts. The domes were solidified at 37 oC for 10 minutes before complete IntesticultTM Organoid Growth Medium was added (750 µL/well). Cultures were incubated at 37 oC, 5% CO2, for 7 - 10 days before passaging, with medium changes 2 - 3 times per week. In some instances, organoids cryopreserved using CryoStor® CS10 were thawed and used to initiate cultures as described above.

***Ex vivo* ileal culture**

C57BL6 mice were sacrificed, and the ileum tissues were collected and placed in a sterile petri dish containing ice-cold PBS. Then, the contents of the lumen were removed, and the ileum was cut into 3–5 mm pieces and placed on a cell strainer which is set in 35 mm-wells then partially submerged in growth medium. After incubation for 2 hr at 37 °C in an incubator with 5% CO2 and 95% air, 5 ml fresh media without any antibiotics was added. The ileum pieces were then transferred from a single cell strainer into a single well of a sterile 12-well cell culture plate. DMEM/F12 medium containing 5% FBS (without any antibiotics) was added. The pieces were incubated for 12 h at 37 °C in an incubator injecting 5% CO2 and 95% air. For mRNA assessment, ileal pieces received either 100 nM hsa-mirVANA miRNA mimics/inhibitors or negative, as previously described. miRNA complexes were formed using RNAiMAX (Invitrogen) in DMEM (Gibco) containing either 10% normal/dialyzed FBS (Gibco). In all conditions, antibiotics were omitted as recommended by the manufacturer to preserve viability during the transfection process. At 48 h after transfection, total mRNA was isolated by use of TRIzol reagent (Life Technologies). Fxr and Fgf15 mRNA were quantified in three independently performed experiments.

**Serum exosome isolation**

Exosomes were isolated by the using ExoQuick-TC® (System Biosciences Inc., Palo Alto, CA, USA) according to the manufacturer’s instructions. In brief, the serum was centrifuged at 2000 *g* for 30 min and supernatant was collected. One-fifth of ExoQuick-TC Exosome Precipitation Solution was added to the supernatant and their suspension was incubated overnight at 4 °C. The suspension was centrifuged at 1500 g for 30 min for total exosome isolation. The pellet was resuspended with PBS. Exosome protein content was qualified using the BCA protein assay kit (Thermo Fisher Scientific) before further experiments.

**Isolation of intestinal mucus and crypt derived exosomes**

Mice were euthanized and the small intestine tissues were removed. The luminal contents of the intestine were removed by gently flushing the intestine with 10 ml of ice-cold PBS. The intestine was then opened longitudinally, the mucus was collected by mild physical separation using round forceps and soaked in PBS before agitating on a rotator at 500 g/min for 15 min. The mucus-PBS mixture was processed by differential centrifugation for exosome isolation. For crypt-derived exosome isolation, the intestinal segments were transferred to a clean dish containing 15 mL of fresh, cold (2 - 8°C) PBS. The intestinal segments were rinsed in PBS buffer thoroughly and cut into 2 mm pieces. 15 mL cold PBS was added to a 50 mL conical tube. Then, scissors were used to cut the intestine tissues into 2 mm pieces. Next, we collected these pieces falling into the tube and centriguging at 200 g for three minutes at 2 - 8°C and the pelleted intestinal crypts were harvested. The crypts were cultured in the DMEM overnight and the exosomes in the culture media were isolated by ultracentrifugation.

**Real-time polymerase chain reaction (Real-time PCR)**

Total RNA was isolated by Trizol according to the manufacturer’s protocol. Total RNA was used for reverse transcription with the cDNA cycle kit (Invitrogen). Primers used in the experiments are listed in **Tables S2 and S3**. 18S and GAPDH were used as internal controls. Real-time PCR was performed by using SYBR green reaction mixture in the ABI 7300 fast real-time PCR system (Applied Biosystems). The relative gene expression was determined by the ΔΔCT method. TaqMan® MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) was used for preparation of cDNA from miRNA. RT reactions were performed in a volume of 15 μl, and each reaction contained 10 ng of total RNA. RT reactions were performed on a GeneAmpPCR System 9600 (Applied Biosystems) with the following conditions: 16 °C for 30 min, 42 °C for 30 min, 85 °C for 5 min, and 4 °C on hold.. 1 μl of RT reaction product was added in the 20 μl qPCR reaction. All TaqMan assays were run in triplicate on an ABI Prism 7500 using TaqMan® Universal PCR Master Mix II without UNG (Applied Biosystems). Real-time PCR cycling conditions consisted of 95 °C for 10 min, followed by 50 cycles of 95 °C for 15 s and 60 °C for 1 min. The Ct is defined as the PCR cycle at which the fluorescent signal of the reporter dye crosses an arbitrarily placed threshold in the exponential phase. For our assays, the constant threshold value was set to 0.200 for easy comparison of results within and between assays. The sample with a Ct value over 40 or an amplification point not reaching the threshold was considered as invalid and was not useful for further analysis. U6B small nuclear RNA (RNU6B, Applied Biosystem) was used as endogenous control for miRNA expression studies.

**Histological analysis**

Liver sections from paraffin-embedded tissues were prepared at 5-μm thickness. Liver pathology was examined by haematoxylin and eosin staining (H&E).

**Western blotting analysis**

Liver tissues were homogenized, and then western blot analysis was performed. Membranes were probed by using related antibodies. The blots were developed by using ECL Western blotting reagents and quantified by using optical densitometry. The intensity of each signal was corrected by the values obtained from the immune-detection of β-actin.

**FXR reporter assay**

HEK293 cells (ATCC) were maintained in DMEM containing 10% FBS and 100U penicillin/streptomycin. Cells were seeded into 24 well plates at a density of 1x105 cells per well, and transfected at 40-60% confluence. For FXR transactivation assay, the transfection mix per well contained 10 ng β-galactosidase expression plasmid (pCMV-β, Stratagene, CA) as a transfection control, 50 ng FXR expression plasmid, and 100 ng FXR reporter plasmid. All cells were co-transfected by lipofection using Lipofectamine reagent according to the manufacturer’s instructions using Opti-MEM (reduced serum medium) as the transfecting medium. After 4 h incubation, the medium was changed to DMEM supplemented with 10% FBS and 1% antimycotic/antibiotic solution and cells were allowed to recover, overnight. 100 mg of fecal samples were resuspended in 500 µL of sterile 1x Dulbecco's Phosphate-Buffered Saline (DPBS), centrifuged at 1,500 x g for 5 min at RT and the fecal supernatant was collected. The cells were stimulated for 6 h with CDCA (20 μM) alone or in combination with serum (10ul) and fecal supernatant (20ul). Luciferase activity was assayed in an Amersham Pharmacia Biotech luminometer by using Reporter Assay System (Promega, Madison, WI) according to the manufacturer's directions. Firefly luciferase and β-galactosidase in same sample simplifies normalization of transfection efficiency.

**Bile acid analysis**

BAs in samples were extracted by solid phase extraction (SPE) as described previously (3). Specifically, serum was first mixed with acetonitrile (ACN) in a ratio of 1:4 (v:v). Then the mixture was centrifuged at 14,000 g for 20 min at 4 oC after being vortexed for 2-3 min. 200 µL of supernatant was transferred into a fresh tube and lyophilized overnight. Each dried sample was reconstituted in 200 µL water (pH ≥ 8.5) and then loaded onto an OASIS HLB cartridge (Waters Corp., Milford, MA, USA) that had been activated and equilibrated with methanol and water (pH ≥ 8.5) following the manufacturer’s instructions. The cartridge was washed with 1 mL of dH2O (pH ≥ 8.5) three times and then eluted three times with 100 µL 70% of ACN (pH ≥ 8.5). The eluate was combined and lyophilized overnight. The residue was then reconstructed in 50% ACN. The sample was then centrifuged at 14,000 g for 20 min at 4 °C. The clear upper solution was transferred to an LC vial for LC-MS analysis. Group-based pooled samples were also prepared by mixing a small portion of the supernatant of each sample in the same group.

To extract BAs from liver samples, the liver tissue was first homogenized in dH2O in a ratio of 1:10 (mg: µL). 100 µL of the homogenized liver sample was mixed with 400 µL of ACN. After a vigorous vortex, the mixture was centrifuged at 14,000 g for 20 min at 4 °C. Then, 400 µL of supernatant was transferred to a fresh tube and lyophilized overnight. The remaining steps of SPE are identical to those of processing serum samples, as described above.

Fecal samples were homogenized in 80% ACN in water in the ratio of 1:10 (mg:µL). The remaining steps were the same as those for liver samples, except 200 µL of supernatant was used for freez drying.

The standards of 46 bile acids were purchased from Cayman Chemical Company (Ann Arbor, MI, USA) and Steraloids Inc. Company (Newport, RI, USA). A stock solution of each bile acid standard was prepared at a concentration of 5-10 mmol/L in methanol. The stock solutions were kept in the dark at -80 °C until use. A total of 12 calibration solutions were then prepared using the stock solutions for each bile acid with the following concentrations: 0.0075, 0.0157, 0.0315, 0.0625, 0.125, 0.25, 0.5, 1.0, 2.5, 5.0, 10, and 20 µmol/L. All of the calibration solutions were prepared in 50% acetonitrile.

A Thermo Q Exactive HF Hybrid Quadrupole-Orbitrap Mass Spectrometer coupled with a Thermo DIONEX UltiMate 3000 UHPLC system (Thermo Fisher Scientific, Waltham, MA, USA) was used in this study. The UHPLC system was equipped with a Cortecs T3 column (100 × 2.1 mm i.d., 1.8 µm) purchased from Waters (Milford, MA, USA). The temperatures of the column and autosampler were set to 60°C and 12°C, respectively. LC-MS methods for analyses of the solutions of bile acid standards and BA extracts were the same as described previously (3). All BA samples were analyzed by LC-MS in random order under positive mode to obtain full MS data for their quantification. The group-based pooled samples were analyzed by LC–MS/MS in negative mode to acquire MS/MS spectra at three collision energies (20, 40, and 60 eV) for BA identification.

The LC-MS/MS data of the pooled samples were used to identify BAs by matching their experiment data with the corresponding information of the 46 BA standards recorded in our in-house database that contains parent ion m/z, MS/MS spectra, and retention time. The MS/MS spectrum similarity threshold was set as ≥ 0.4, and the thresholds of the retention time difference and m/z variation window were set as ≤ 0.15 min and ≤ 5 ppm, respectively. Thermo Scientific Xcalibur instrument control software Quan (2.2 SP1.48) was used to process the LC–MS data for peak picking, standard curve construction, and BA quantification. The signal-to-noise ratio (S/N) was set to S/N ≥ 3. The concentration of each BAs in a biological sample was calculated using the calibration curve constructed from the LC–MS data of a corresponding BA standard.

**Fecal taurine measurement**

Taurine Assay Kit (ab241040) was used to measure the fecal taurine concentration according to the manufacturer’s instructions. Briefly, the fecal sample was vortexed continuously for 5 min until thoroughly homogenized. Then, the sample was centrifuged at 1500 g for 30 min and the supernatant was obtained. Next, we prepared the standard curve, enzyme mix and added it to all wells except background control (added assay buffer instead). Sulfite probe was then added to the sample, background control and standard wells and incubated for 5 mins. At the end, absorbance was measured at 415 nm using microplate reader, and taurine concentration was calculated according to the protocol

**Fecal BSH activity**

BSH activity was measured by determining the amount of amino acids liberated from conjugated bile salts as previously described with several modifications (4). Bacterial BSH activity was measured based on the generation of CA from TCA in the feces. Brieﬂy, fecal protein extract was prepared from 0.5 g of fecal samples in 1 mL of PBS (pH 7.4) and then homogenized throughly. Then, the sample was centrifuged and 1mL of supernatant was obtained. Incubation was carried out by adding 1.8 mL PBS and 0.1 mL 0.1mol/L TCA. After a 30 min incubation at 37°C, reactions were stopped by adding 0.1 mL CCl3COOH for 1min. Then, the mixture was centrifuged and 1mL of supernatant obtained was added to 1mL of 2mol/L trichloroacetic acid buffer and 1mL of ninhydrin reagent (0.5 mL of 1% ninhydrin in 0.5M citrate buffer pH 5.5, 1.2mL of 30% glycerol, 0.2mL of 0.5M citrate buffer pH 5.5). The preparation was vortexed and boiled for 15 min. After subsequent cooling, 3 ml potassium iodate (KIO3) was added, and the absorbance at 570 nm was determined using taurine as the standard. One unit of BSH activity was defined as the amount of enzyme that liberated 1 mmol of amino acid from the substrate per minute.

**Transient transfection with miR194.**

To investigate the effect of miR194 on NR1H4 mRNA expression, Caco-2 cells were seeded in 12-well plates (8×104 and 4×105 cells/ml, respectively). After 24 h, cells were transfected with 100 nM hsa-mirVANA miRNA mimics or anti-miR miRNA inhibitors (hsa-miR-194-5p and corresponding negative controls, Life Technologies) by using Lipofectamine RNAiMax diluted in Opti-MEM I (both purchased from Invitrogen) at a final concentration of 3 mM. At 24 h after transfection, total mRNA was isolated by use of TRIzol reagent (Life Technologies). *FXR* and *FGF19* mRNA were quantified in three independently performed experiments. The effect of miR194 on NR1H4 mRNA expression in 3D organoids was measured as previously described with several modifications (5). For mRNA assessment, organoids received either 100 nM hsa-mirVANA miRNA mimics/inhibitors ornegative, as previously described. miRNA complexes were formed using RNAiMAX (Invitrogen) in DMEM (Gibco) containing either 10% normal/dialyzed FBS (Gibco). In all conditions, antibiotics were omitted as recommended by the manufacturer to preserve viability during the transfection process. 500 μl of formed miRNA complex medium was then bathed over 50 μl matrigel contained within a single well of a 24-well plate overnight and replaced the following morning with normal organoid medium as noted above. At 48 h after transfection, total mRNA was isolated by use of TRIzol reagent (Life Technologies). *Fxr* and *Fgf15* mRNA were quantified in three independently performed experiments.

**16S ribosomal RNA (16S rRNA) gene library preparation and sequencing on the Illumina MiSeq**

Fecal pellets were collected into sterile tubes at the end of the experiment and stored at −80°C. Microbial genomic DNA was extracted from frozen fecal samples using DNeasy PowerSoil kit (Cat#:12888-100, Qiagen, Germantown, MD, USA) according to the manufacturer’s instructions. The composition of fecal microbiota was analyzed using Illumina MiSeq technology targeting the variable V3 and V4 regions of 16S ribosomal RNA. 16S variable regions were amplified using 12.5ng microbial genomic DNA. PCR conditions are as follows: 95°C for 3 min; 25 cycles of 95°C for 30s, 55°C for 30s, and then 72°C for 30s; and 72°C for 5 min. The primers used for 16S Amplicon PCR are as follows: Forward: 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG; Reverse: 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC. Index PCR was performed to attach dual indices and Illumina sequencing adapters using the Nextera Index Kit (Cat#: FC-121-1012, Illumina, San Diego, CA, USA). Each step was followed by the PCR clean-up, using AMPure XP beads to obtain a purified library. After libraries were normalized, pooled, and denatured, sequencing was done using Illumina MiSeq Reagents kit v3 (600 cycles, read lengths up to 2 × 300 bp) (Cat#: MS-102-3003, Illumina, San Diego, CA, USA) on an Illumina MiSeq instrument.

**16S Metagenomic analysis**

Quality control of raw sequence files was performed using FastQC and further analyzed using QIIME 2 (version 2019.04) (6). Briefly, the paired-end files per sample were merged and imported into a QIIME 2 artifact. The sequence reads were then demultiplexed and denoised into amplicon sequence variants (ASVs) using DADA2 in QIIME 2, which can identify more real variants and output fewer spurious sequences than other methods. The resulting feature table and representative sequences were used for the downstream analysis. The rarefaction curve using the observed operational taxonomy unit (OTU) and Shannon index generated by QIIME 2 were used as metrics of α-diversity (7). Principal coordinate analysis (PCoA) was performed to compare microbial community structure between groups (β-diversity), using both weighted and unweighted UniFrac (8). Heat map analysis of OTU abundance was performed using R software (<https://www.r-project.org/>) and the figure was generated by using Microsoft Excel.

**Study approval**

Patients with alcohol associated hepatitis (AH) and healthy controls (HCs) were included in this clinical study. This investigation is part of large national multisite clinical trials (clinicaltrials.gov: NCT01809132 and NCT01922895) supported by the National Institute on Alcohol Abuse and Alcoholism (NIAAA) at the National Institutes of Health, Bethesda MD. This study was approved by the institutional review board (protocol No.12.0427) of the University of Louisville.

**Table S1. AH paptien’s clinical data**

|  |  |  |
| --- | --- | --- |
| **Liver Test** | **Moderate AH** | **Severe AH** |
| **AST (IU/L)** | 94±34.1 | 142.04±51.7 |
| **ALT (IU/L)** | 49.9±24.9 | 51.63±24.8 |
| **Total bilirubin (mg/dl)** | 5.06±2.1 | 20.21±9.2 |
| **Albumin (g/L)** | 2.43±0.3 | 2.52±0.5 |
| **Meld Score** | 16.2±2.5 | 25.83±4.4 |
| **Maddrey Df** | 34.2±19.6 | 62.30±20.2 |

**Table S2: Primer sequences used for Real-time PCR studies in mice**

|  |  |  |
| --- | --- | --- |
| Target genes | Sequences |  |
| *Cyp7a1* | Forward | 5`- TGGAATAAGGAGAAGGAAAGTA -3` |
| *Cyp27a1* | Reverse  Forward  Reverse | 5`- TGTGTCCAAATGCCTTCGCAGA -3`  5`- TGCCTGGGTCGGAGGAT-3`  5`-GAGCCAGGGCAATCTCATACTT -3` |
| *Cyp8b1* | Forward | 5`- CCTCTGGACAAGGGTTTTGTG -3` |
|  | Reverse | 5`- GCACCGTGAAGACATCCCC -3` |
| *Cyp7b1* | Forward | 5`- GGAGCCACGACCCTAGATG -3` |
| *Fxr* | Reverse  Forward  Reverse | 5`- GCCATGCCAAGATAAGGAAGC-3`  5`- TGTGAGGGCTGCAAAGGTTT-3`  5`- ACATCCCCATCTCTCTGCAC -3` |
| *Fgf15* | Forward | 5`- GAGGACCAAAACGAACGAAATT -3` |
| *Shp*    *Oatp4*  *Mrp2*  *Mrp3*  *Mrp4*  *Ost-α*    *Ost-β*  *Asbt* | Reverse  Forward  Reverse  Forward  Reverse  Forward  Reverse  Forward  Reverse  Forward  Reverse  Forward  Reverse  Forward  Reverse  Forward  Reverse | 5`- ACGTCCTTGATGGCAATCG -3`  5`-TCTGCAGGTCGTCCGACTAT -3`  5`-CAGGCAGTGGCTGTGAGAT -3`  5`-GATCCTTCACTTACCTGTTCAA -3`  5`-CCTAAAAACATTCCACTTGCCATA -3`  5`-GCTTCCCATGGTGATCTCTT -3`  5`-ATCATCGCTTCCCAGGTACT -3`  5`-TGAGATCGTCATTGATGGGC -3`  5`-AGCTGAGAGCGCAGGTCG -3`  5`-TTAGATGGGCCTCTGGTTCT -3`  5`-GCCCACAATTCCAACCTTT -3`  5`-TGTTCCAGGTGCTTGTCATCC-3`  5`-CCACTGTTAGCCAAGATGGAGAA -3`  5`-GATGCGGCTCCTTGGAATTA -3`  5`-GGAGGAACATGCTTGTCATGAC -3`  5`-ACCACTTGCTCCACACTGCTT -3`  5`-CGTTCCTGAGTCAACCCACAT -3` |
| *Bsep* | Forward  Reverse | 5`- GCTGCCAAGGATGCTAATGC -3`  5`-GGAGGAACATGCTTGTCATGAC -3` |
| *18S* | Forward | 5`- CTAACCCGTTGAACCCCATT-3` |
|  | Reverse | 5`- CCATCCAATCGGTAGTAGCG-3` |

**Table S3: Primer sequences used for Real-time PCR studies in Caco-2 cells**

|  |  |  |
| --- | --- | --- |
| Target genes  *FGF-19*  *SHP*  *GAPDH* | Sequences  Forward  Reverse  Forward  Reverse  Forward  Reverse | 5`-AGATCAAGGCAGTCGCTCTG -3`  5`- CGGATCTCCTCCTCGAAAGC-3`  5`-TCAAGTCCATTCCGACCAGC -3`  5`- AAGAAGGCCAGCGATGTCAA-3`  5`- CCAGCAAGAGCACAAGAGGA-3`  5`- GAGATTCAGTGTGGTGGGGG-3` |

**Fig. S1**



C

B

A

**Fig. S1. Disruption of bile acid homeostasis in alcoholic liver disease.** (A) Fecal BA levels.(B) Hepatic LCA level. (C) Serum 12α-Hydroxylated BA level. Data are expressed as mean ± SEM (n = 5-8 mice/group). \*P < 0.05.

**Fig. S2**

A



B

D

C

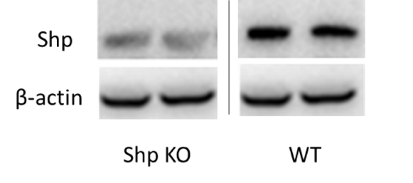
**Fig. S2. Effects of LDNP on liver Cyp8b1, Cyp27a1 and bile acid transporter expressions.** (A) Hepatic mRNA expressions of Cyp8b1and Cyp27a1*.* (B) Hepatic mRNA expression of *Cyp7b1*. (C) Hepatic mRNA expression of *Ost-α, Mrp2, Bsep* and *Ntcp*. (D) Ileal mRNA expression of *Mrp2*, *Ost-α*, *Ost-β* and *Asbt*. Data are expressed as mean ± SEM (n = 5-8 mice/group). \*P < 0.05, \*\*P < 0.01.

**Fig. S3**



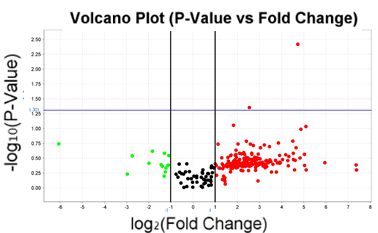
A

B

**

**Fig. S3. Selected liver BAs and Western Blot of Shp in WT and Shp KO mice**. (A) Percentage of selected hepatic BAs. (B) WB of the liver tissues of Shp KO and WT mice. Data are expressed as mean ± SEM (n = 5 mice/group). \*\*P < 0.01, \*\*\*P < 0.001.

**Fig. S4**



**Fig. S4. Volcano plot of ileal miRNA in AF mice compared with PF mice by a microRNA array analysis.** The horizontal axis represents the log2 ratio of the fold changes and the vertical axis represents −log10 of P values (t-test). The red highlighted dots indicate that the significantly increased (more than 2 folds) miRNA by alcohol exposure and the green dots indicate the significantly decreased miRNA. n = 3 mice/group

**Fig. S5**

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A

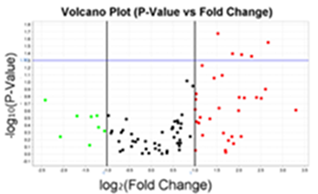
B

**Fig. S5. *miR194* expression in intestinal exosomes.** (A) Mucus exosomal *miR194* expressions. (B) Crypt exosomal *miR194* expressions. Data are expressed as mean ± SEM (n = 3 mice/group). \*P < 0.05.

**Fig. S6**

B

A



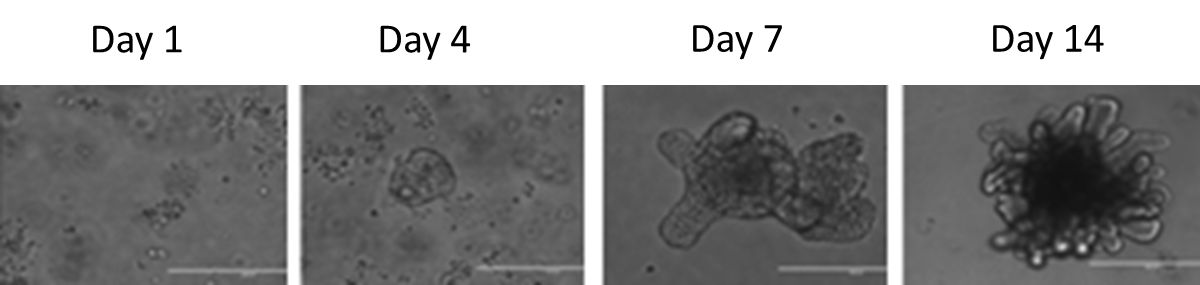


miR194

C

C

****

****

D

**Fig. S6. Effects of alcohol on fecal microRNA expression and 3D organoids development.** (A) Heat map of the most changed fecal miRNAs. (B) Volcano plot of fecal miRNA in AF mice compared with PF mice by a microRNA array analysis. (C) Validation of *miR194* in fecal samples by RT-qPCR. (D) Organoid culture of isolated mouse small intestinal epithelium in Matrigel matrix and ENR media (conventionally differentiated) for 14 days. Isolated crypts form 3D cysts which bud after 7 days of culture to form crypt-and villus-like domains. Data are expressed as mean ± SEM (n = 3/group). \*\*P < 0.01.

**Fig. S7**



**Fig. S7 Validation of miR194 regulation of FXR in *Ex vivo* ileam** Data are expressed as mean ± SEM (n = 3/group). \*\*P < 0.01, \*P < 0.05

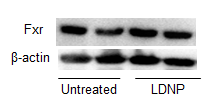
******Fig. S8**

A

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B



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C

**Fig. S8. Alcohol and LDNP did not change miR194, FXR and FGF15 levels in Caco-2 cells.** (A) Relative *FXR*, *FGF19* and *miR194* mRNA expressions in Caco-2 cells treated with different dose of EtOH for 24 hours. (B) *FXR* mRNA (left panel) and protein (right panel) levels in Caco-2 cells treated with LDNP for 24 hours. (C) Relative mRNA expressions of *FGF19* (left panel) and *miR194* (right panel) in Caco-2 cells treated with LDNP for 24 hours. Data shown represent the mean ± SEM of at least three independent experiments performed in triplicate for cell culture studies. \*P < 0.05. Data are expressed as mean ± SEM (n = 3).

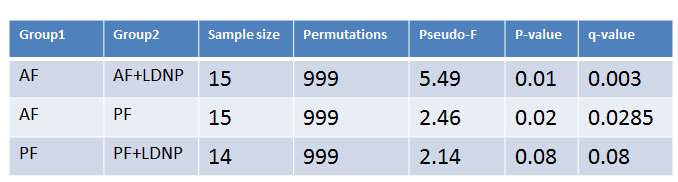
**Fig. S9**

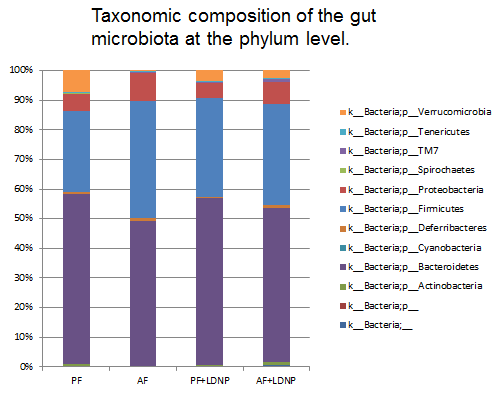
A

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B

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C

**Fig. S9 Alterations of taurine-related microbiome.** (A) The alpha rarefaction curves for the number of observed OTUs. (B) The matrix for the Bray-Curtis (upper panel) and comparison of the mean distance between two condition groups (lower panel). (C) Taxonomic composition of the gut microbiota.

**Fig. S10**

A



B

B

****

**Fig. S10 LDNP reduced conjugated BAs in feces and serum.** (A) Fecal BA classes of mice. (B) Serum BA classes and BA profile of mice. Data are expressed as mean ± SEM (n = 3-5 mice/group). \*P < 0.05.

**Fig. S11**

A

A picture containing text, dark

Description automatically generated

B



**Fig. S11.** **Validation of intestinal Fxr and Fgf15 knock out efficeincy.**(A) Liver and ileum Fxr mRNA and protein levels in WT, *Fxrfl/fl and FxrΔIEC mice.* (B) Ileum Fgf15 mRNA levels in WT and Fgf15 KO mice.  Data are expressed as mean ± SEM (n = 3 mice/group). \*\*P < 0.01.

**Fig. S12**



**Fig. S12** **Serum Fgf15 level in *Fxrfl/fl and FxrΔIEC mice*.**Data are expressed as mean ± SEM (n = 3mice/group). \*P < 0.05, \*\*P < 0.01.

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