CYP8B1 downregulation mediates the metabolic effects of vertical sleeve gastrectomy in mice

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Supplementary Information:

SUPPLEMENTARY METHODS

Animals

C57BL/6 mice were purchased from Charles River. *Cyp8b1*$^{-/-}$ (KO) mice(1) (JAX stock #018771) was purchased from the Jackson Laboratory (Bar Harbor, ME). It's known that mice showed vigorous coprophagous activity, baby mice eat their mother's or littermates' feces before weaning, and self-reinoculation with fecal flora changes microbiota density and composition leading to an altered bile-acid profile in the mouse small intestine(2), therefore, in this research, we crossbred the KO mice (Strain #: 018771) with wild-type (WT) C57B6/J mouse and obtained heterozygous *Cyp8b1*$^{+/-}$ mice to generate KO and WT littermates. We then bred the homozygous KO or WT mice, respectively, to obtain homozygous KO and WT control mice for the experiments, to avoid the GM inoculation through coprophagy. All animal procedures were approved by the City of Hope Institutional Animal Care and Use Committee (IACUC). Mice (6-8 weeks of age) were fed a high-fat diet (HFD, 60 kcal% fat D12492, Research Diets Inc., New Brunswick, NJ) for 14 weeks and divided into body weight-matched groups to receive VSG or sham surgery. Body weight and food intake were measured weekly after surgery. At the end of the study, mice were fasted overnight, then euthanized by carbon dioxide asphyxiation for tissue collection. Blood was collected by cardiac puncture. Liver and intestine tissues were collected and stored immediately in liquid nitrogen for RNA and protein extraction, in 4% paraformaldehyde and formalin for histology.

Surgery

Prior to surgery, mice were fasted overnight to reduce gastric content. For both VSG and sham surgery, mice were anesthetized using isoflurane, and heat support was provided throughout the procedure. Fur in the abdominal area was shaved, and the skin was sanitized using three alternating ethanol and betadine scrubs. Carprofen (5 mg/kg) and gentamicin (8 mg/kg) were administered subcutaneously prior to making incisions. For VSG, about 80% of the stomach was resected, leaving a tubular gastric remnant in continuity with the esophagus and duodenum. For sham surgery, 4-5 suture knots were placed on the gastric wall in a simple interrupted pattern using 6-0 PDS sutures. The abdominal muscle layer was closed with 4-0 Vicryl in a simple continuous pattern, and the skin layer was closed using wound clips that were removed 10-14 d later. Buprenorphine SR (1 mg/kg) was administered subcutaneously immediately after skin closure. For 4 d after surgery, DietGel® Recovery was supplied in place of HFD, and carprofen was administered once daily for 2 d. After 3 d, HFD was supplied to the mice again until euthanasia. We performed a VSG in ~40 min and adjusted a sham surgery to the same length of time. All the surgeries in one experiment were finished within one week. Surgery operated mice were checked every day to ensure their health.

Preparation of recombinant Adeno-associated virus (AAV) virus

For AAV-mediated genetic manipulations, pAAV2/8 constructs were generated by cloning a cDNA or shRNA expression cassette into the plasmid backbone. The AAV gene delivery vector for shRNA (AAV-shRNA-ctrl) was a gift from Hongjun Song (Addgene plasmid #85741). The shRNAs against *Cyp8b1* were designed to target mouse sequences (*Cyp8b1* shRNA1: GGGTGGTACAGGAGGATTATG, shRNA2: CGGCATAAGCTGTTGGTTA). pAAV.TBG.PI.Null.bGH, a gift from James M. Wilson (Addgene plasmid #105536), served as the vector plasmid for packaging pAAV vectors encoding CYP8B1. cDNA fragments encoding CYP8B1 with a 3×HA Tag sequence were generated by PCR amplification and cloned into the pAAV backbone using BamHI and BgIII. pDP8.ape plasmids encoding AAV2 rep and 8 cap genes (PlasmidFactory, Bielefeld, Germany) were used for pAAV vector production. All pAAV vectors were produced in HEK293 cells using the helper virus-free, two-plasmid-based production method. Briefly, subconfluent cells were co-transfected using the polyethyleneimine (PEI) method, with equimolar amounts of a rep/cap/helper plasmid (pDP8.ape) and a vector plasmid. At 72 h post-transfection, cells were lysed, treated with benzonase, and further purified by caesium chloride (CsCl) density gradient centrifugation. Fractions from the gradient were collected, peak fractions were pooled, dialyzed against 1× DPBS containing 10% glycerol, concentrated (Amicon Ultra-15, Millipore, Schwalbach, Germany), filter-sterilized, and stored at −80°C. Genomic particle titers were determined by real-time qPCR using transgene- or promoter-specific primers and probes. All reactions were performed in triplicate. The virus was introduced into C57BL/6 mice (Charles River) using the tail vein injection technique.

Antimicrobial-induced microbiota-depletion (AIMD) model

4−6-week-old male C57Bl/6 mice were fed HFD for about 10−12 weeks. Then, a freshly dissolved cocktail of four antibiotics (400 mg each of ampicillin, metronidazole, and neomycin, and 200 mg of vancomycin, dissolved in sterile water) was administrated to mice at 200 µl per mouse by oral gavage once every other day for 2 weeks to deplete their GM.

Fecal microbiota transplantation (FMT)

Fresh fecal samples of each group of mice were collected in the afternoon (from 13:00-15:00) to minimize possible circadian effects. Samples were collected into sterile microtubes and stored on ice. Collected feces were homogenized by vortexing in phosphate-buffered saline (PBS) at 100 mg/mL for 2 min. Next, the mixtures were centrifuged for 2 min at 270 g. The fecal slurry was passed through a 100-µm cell strainer. Recipient were fed HFD and fasted overnight prior to receiving their first fecal transplant. Each recipient mouse received 200 µL of fecal slurry by oral gavage once every other day for two weeks. Mice in the control group received 200 µl of fecal slurry from sham-operated mice with same genotype.

Gut microbiota analysis

GM analysis was performed on fecal samples collected from individual mice. Frozen fecal samples were shipped to TGen North, the Pathogen and Microbiome Division of the Translational Genomics Research Institute (Flagstaff, AZ) or GENEWIZ from Azenta Life Sciences (Suzhou, China), for DNA extraction and analysis. For DNA extraction, the fecal samples were homogenized by bead beating on a TissueLyser (Qiagen), then extraction was performed using a MagMax Microbiome Ultra Nucleic Acid Isolation Kit (A42358, ThermoFisher Scientific, Waltham, MA) on a KingFisher Magnetic Extraction Instrument (ThermoFisher). Bacterial DNA was quantified using the BactQuant assay (3). For 16S rRNA sequencing, 16S rRNA libraries were built using modified primers (4, 5) that amplified the 16S rRNA gene variable region 4 (V4) of bacteria and archaea. Analysis was performed using QIIME2 (6). Reads were denoised using DADA2, a rooted phylogenetic tree was constructed, and α diversity metrics were calculated using the q2-diversity plugin. Taxonomic classification was performed using the q2-feature-classifier plugin trained on the V4 region of the SILVA database.

Succinate assay

Succinate levels in the small intestinal contents and serum samples of mice were quantified following the guidelines provided by the Succinate Colorimetric Assay Kit (Merck, Darmstadt, Germany).

Glucose tolerance test (GTT)

Mice were fasted for 14 h prior to GTT. An AlphaTRAK 2 Blood Glucose Monitoring System was used to measure blood glucose in blood collected by tail prick. After the initial blood glucose reading (0 min), mice were intraperitoneally injected with glucose solution at 2 g/kg of body weight. Then, blood glucose was measured at 15, 30, 60, and 120 min after glucose injection.

Insulin tolerance test (ITT)

Mice were fasted for 6 h prior to ITT. An AlphaTRAK 2 Blood Glucose Monitoring System was used to measure blood glucose in blood collected by tail prick. After the initial blood glucose reading (0 min), mice were injected with insulin intraperitoneally at 0.75 U/kg of body weight. Then, blood glucose was measured at 15, 30, 60, and 120 min after insulin injection.

Lipid absorption test

Mice were fasted between 7:00 and 11:00 h prior to the lipid absorption test. On the day of the test, blood was collected via the tail vein for baseline measurements. Then, tyloxapol was injected intraperitoneally (500 mg/kg body weight). 15−30 min later, 200 µl of corn oil was administered via oral gavage. Blood was collected at 2, 4, and 6 h after gavage. About 20 µl of blood was collected at each time point. 2 µl of EDTA solution (0.5 M in distilled water) was pipetted into each blood collection tube to prevent coagulation. The plasma was separated,

then triglyceride levels were measured using an L-Type Triglyceride M Assay (Wako Diagnostics, Mountain View, CA).

To study the intestinal absorption of lipids, mice were fasted overnight and then orally gavaged with 200 μL of corn oil or saline as a control. Mice were euthanized before or 2 or 4 h after gavage. The duodenum was collected and thoroughly washed with PBS to remove external lipids. The tissue was then directly fixed in 4% paraformaldehyde for 24−48 h at 4°C and dehydrated in 30% sucrose for 12 h before being embedded in Tissue-Tek O.C.T. Compound (Sakura Finetek, Torrance, CA, USA). The tissues were then stained with Oil red O.

Bile acid (BA) analysis

The composition and concentration of BAs were analyzed using ultra-high performance liquid chromatography-mass spectrometry with modifications (7). To extract BAs from serum, 50 μL of serum was first mixed with 10 μL of internal standards. Then, 250 μL of cold methanol was added, and the mixed solution was vortexed 3× for 10 s and maintained at −20°C for 20 min. Next, the solution was centrifuged at 10,000 *g* for 10 min at 4°C. The supernatant was transferred to an autosampler vial and blown dry with nitrogen. The dry residue was reconstituted by adding 200 μL methanol:water (1:1). Finally, the solution was centrifuged at 10,000 *g* for 5 min at 4°C, and the supernatant was transferred into autosampler vials for LC-MS/MS analysis.

To extract BAs from feces, 20 mg of dry feces was mixed with 10 μL of internal standards. Then, 450 μL of cold methanol was added, and the mixed solution was homogenized for 60 s. The solution was centrifuged at 10,000 *g* for 10 min at 4°C, and the supernatant was transferred to an autosampler vial. Another 450 μL of cold methanol was added for the second extraction, followed by another centrifugation. The supernatant from two extractions were combined and then diluted 10× with methanol:water (1:1) for LC-MS/MS analysis.

An Agilent 1290 Infinity II ultra-high performance lipid chromatography system coupled with a 6490 Triple Quadrupole mass spectrometer (Agilent Technologies Inc., CA, USA) was used for the quantitative analysis of BAs. An ACQUITY UPLC BEH C18 column (1.7 μm, 2.1×100 mm, Waters) was used to separate 30 BAs, with column temperature held at 65°C. The mobile phase consisted of 0.1% formic acid aqueous solution (A) and 0.1% formic acid in acetonitrile (B) at a flow rate of 0.5 mL/min. The elution gradient was performed as follows: for the first 0.5 min, the eluent composition was set at 95% A and 5% B, which was linearly changed to 85% A and 15% B over 4.5 min; then, the proportion of B was increased to 25% B over 4 min and further increased to 40% over the next 10.5 min. Next, the proportion of B was linearly increased to 95% over 2 min, and this ratio was maintained for 1.5 min. Finally, the initial composition was recovered and maintained for 1 min for column conditioning. For MS analysis, an ESI source in negative-ion mode was used for the ionization of the BAs, and

multiple reaction monitoring (MRM) was used to collect the m/z signal of the BAs. Quantitative data analysis was performed using MassHunter software (Agilent).

Dual-Luciferase Reporter Assay

BAs were extracted from serum as described above without adding internal BA standards. Luciferase assays were performed as described (8). HEK 293 cells (ATCC) maintained in DMEM containing 10% (wt./vol.) fetal bovine serum were transiently transfected using polyethylenimine (PEI, Promega, Madison, WI) and allocated to 24-well plates 16 hours before transfection. Cells were transfected with plasmids encoding full-length TGR5 and pCRE-luc reporter, or full-length FXR and EcRE-luc reporter, along with pCMV-Renilla as internal control. Five hours after transfection, cells were treated with BAs extracted from serum. Cells were harvested 18 hours later for the luciferase assay using the Luciferase Assay System (Promega, Madison, WI). Luciferase activity was normalized to Renilla activity.

Serum triglyceride measurements

Triglyceride levels in the serum were quantified using the L-Type Triglyceride M Microtiter Procedure and assay kit (L-type Triglyceride M Enzyme Color A [R1]: 994-02891/998-02992; L-Type Triglyceride M Enzyme Color B [R2]: 990-02991/998-0299; Multi-lipid Calibrator: 464-01601; Mountain View, CA). 4 µl of each serum sample was used with the kit.

Quantitative real-time PCR (qPCR)

Total RNA was extracted from frozen tissues using TRI Reagent® according to the manufacturer's instructions. Reverse transcription of the RNA was performed using 5X All-in-One RT MasterMix (Bioland Scientific LLC). Real-time PCR was carried out using an Applied Biosystems 7300 Real-Time PCR System (Applied Biosystems, Forest City, CA) with PowerUp SYBR Green Master Mix (Invitrogen) and gene-specific primers. The sequence and GenBank accession numbers of the forward and reverse primers used to quantify mRNA are listed in **Supplementary Table 1**. The following conditions were used for real-time PCR: 95°C for 10 min, then 95°C for 15 s and 60°C for 1 min in 45 cycles. The $2^{-\Delta\Delta CT}$ method was used to analyze relative changes in gene expression, normalized against 36B4 mRNA expression.

Hematoxylin and eosin (H&E) and Oil red O staining

After mice were euthanized, their tissues were immediately removed, immersed in cold PBS, and fixed in 10% formalin solution for 24−48 h at 4°C. For H&E staining, the samples were then incubated in 70% ethanol for 12 h, embedded in paraffin, and sectioned into 5-µm sections. For Oil red O staining of liver sections, the tissues were fixed in 4% paraformaldehyde, then incubated in 30% sucrose for 12 h and embedded in Tissue-Tek® O.C.T.

Compound (Sakura Finetek, Torrance, CA, USA). Serial sections (10 μM) were made and stained with 0.5% Oil red O for 10 min and counter-stained with hematoxylin. The red lipid droplets were visualized by microscopy.

Statistical Analysis

All quantitative data are summarized as mean ± SEM. Prism 8.0 or SAS 9.4 was used for statistical analyses. Student's t test was used to compare two independent groups. Two-way ANOVA models are used for the two-factor (genotype x treatment) analysis and post-hoc between-group comparisons. Linear mixed models or one-way ANOVA model with repeated measures were used to account for the variance-covariance structure due to over-time repeated measures within each animal such as weight, blood glucose and serum triglyceride. P values were adjusted for multiple comparisons by Holm-Sidak method. A p value of 0.05 or less was considered statistically significant.

Methods References:

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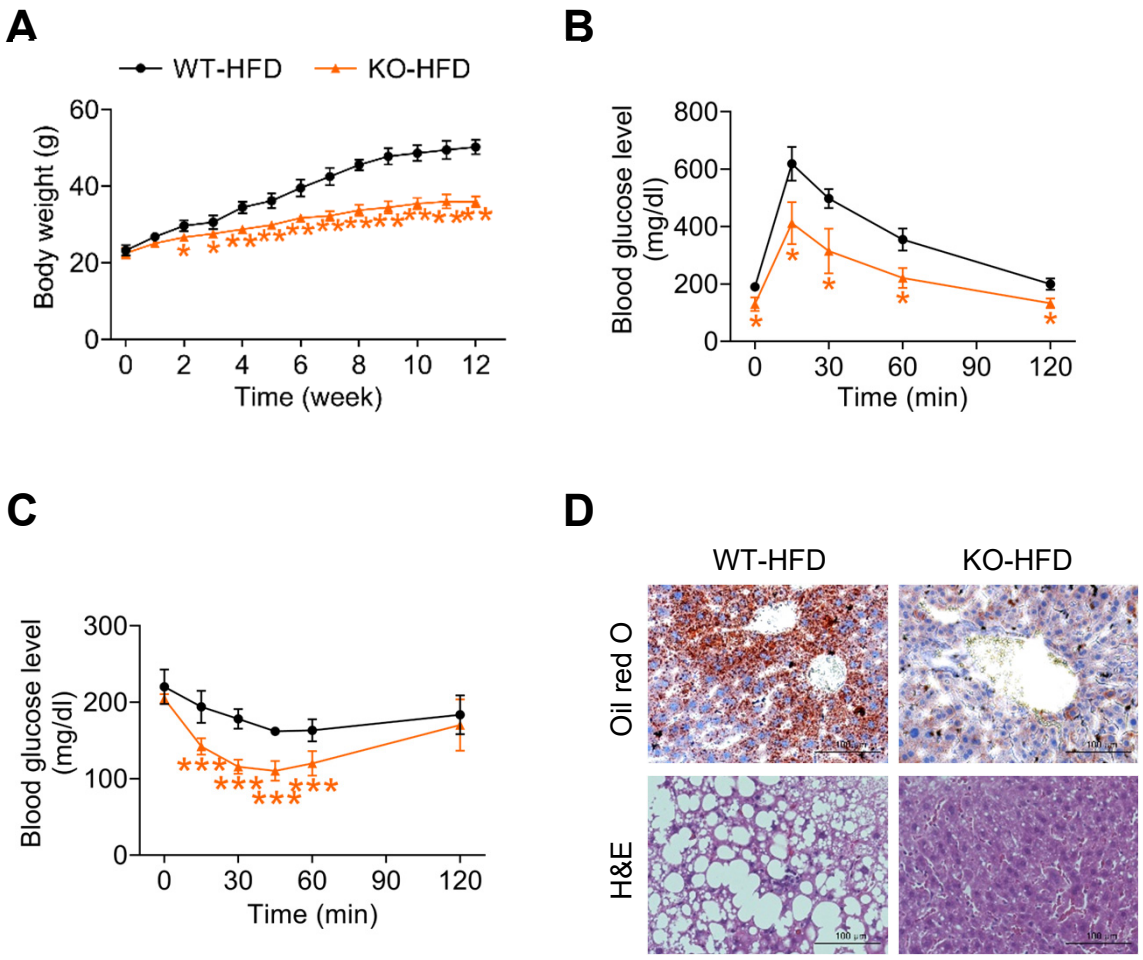
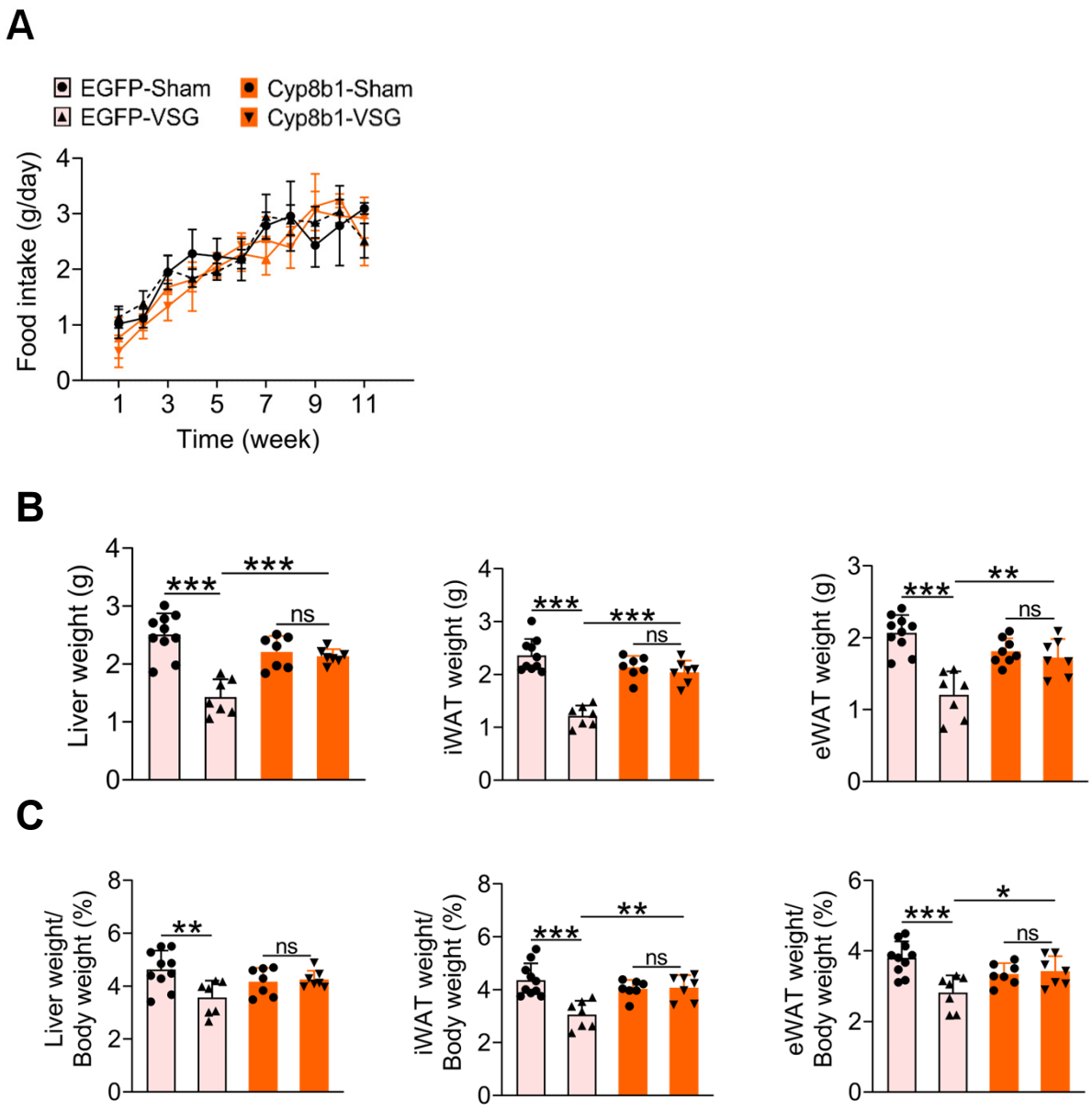


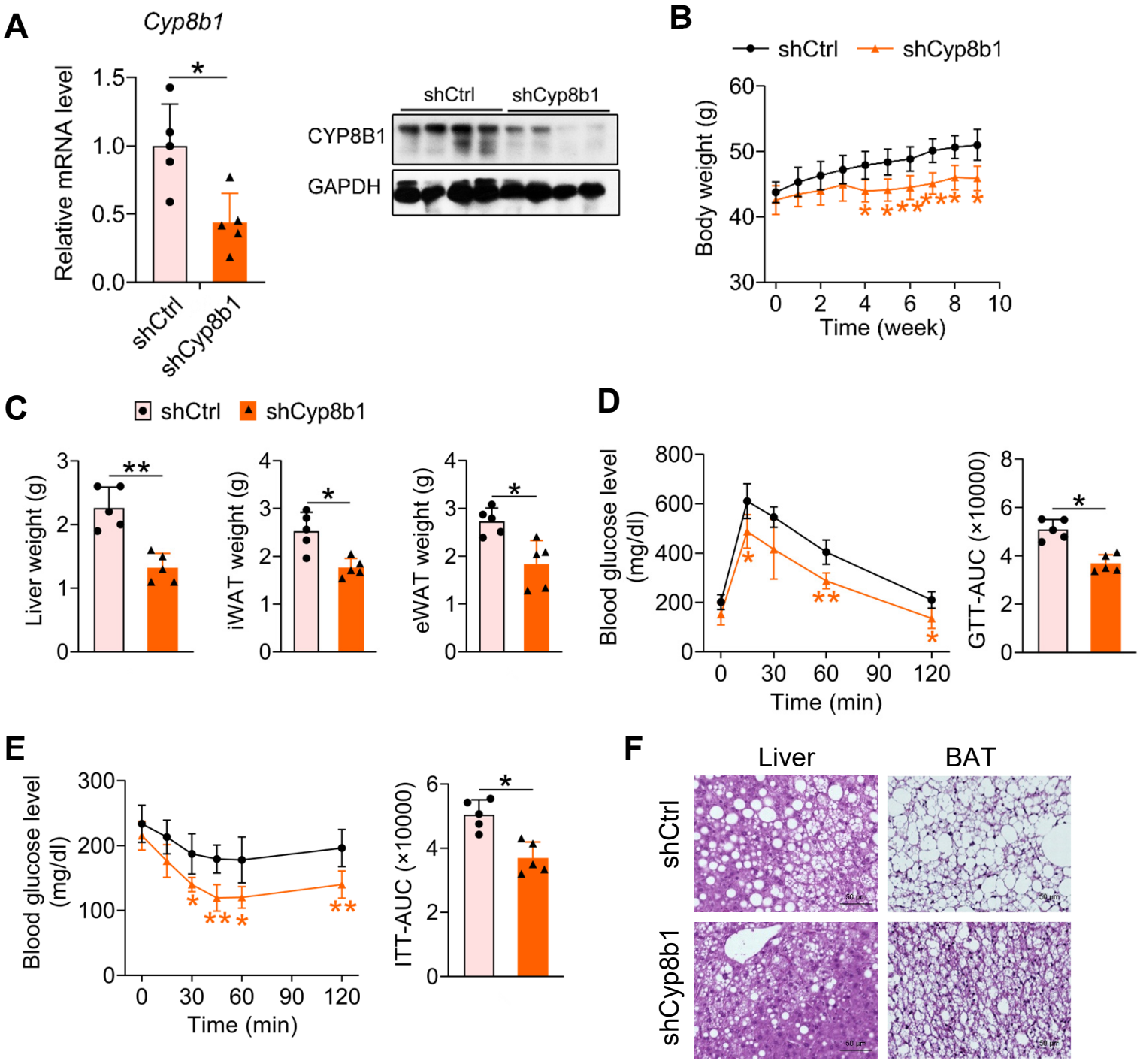
Supplementary Fig. 1 Metabolic phenotypes of *Cyp8b1*-KO mice fed HFD.

WT and *Cyp8b1*-KO mice were fed HFD for 12 weeks. (**A**) Body weight; (**B**) GTT at 11 weeks on HFD; (**C**) ITT at 10 weeks on HFD; (**D**) Representative images of Oil red O-stained and H&E-stained liver sections (Scale bars, 100 μm). n = 6 mice per group. Data are mean ± SEM. *p<0.05, **p<0.01, and ***p<0.001 and ns, no significance, by two-tailed Student's *t* test.



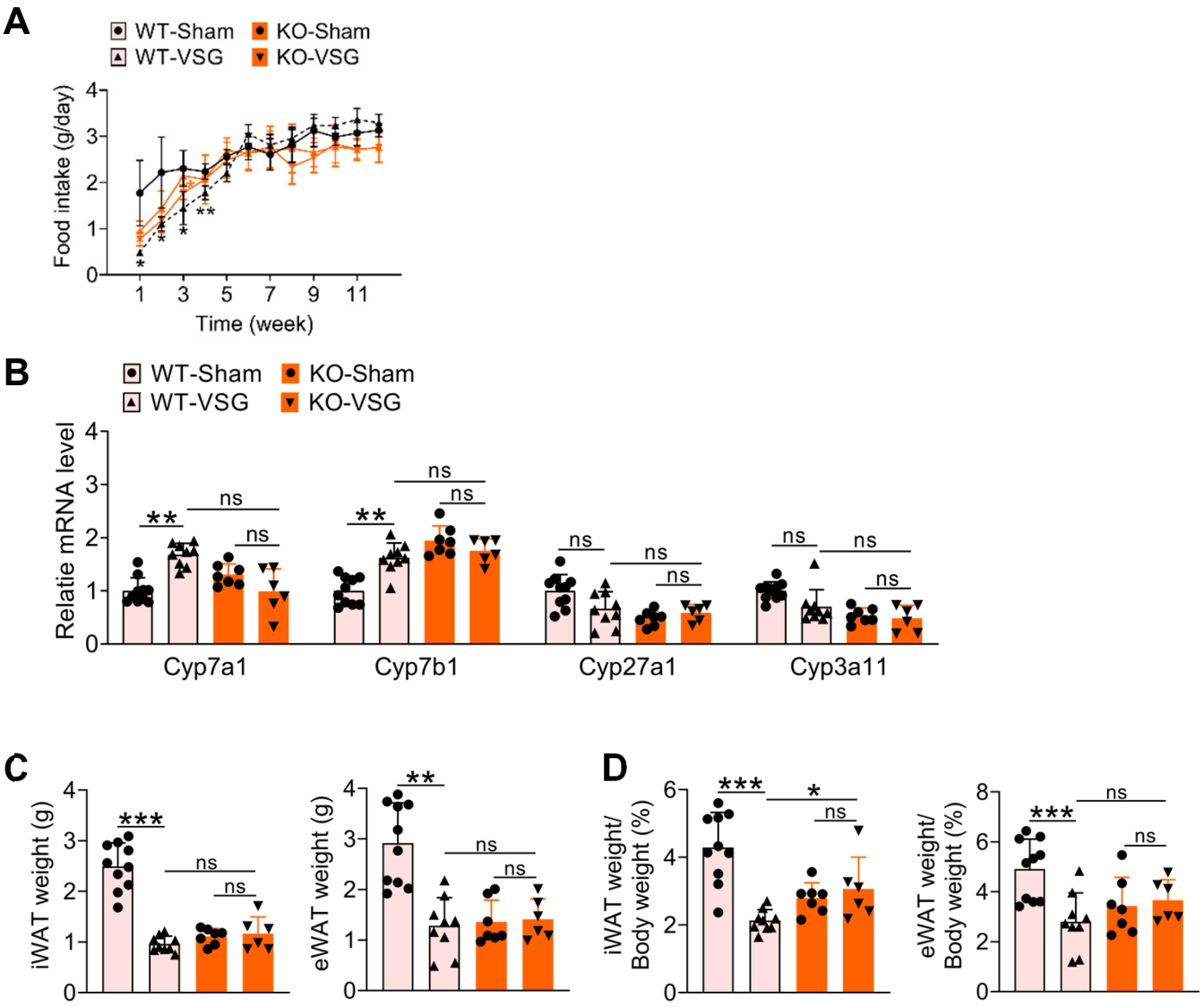
Supplementary Fig. 2 Overexpression of CYP8B1 diminishes the metabolic effects of VSG.

(**A**) Food intake. (**B**) Weights of liver, iWAT and eWAT. (**C**) Weight ratios of liver, iWAT and eWAT to body weight. n = 10 mice in EGFP-Sham group, n = 7 mice in EGFP-VSG group, CYP8B1-Sham group and CYP8B1-VSG group. Data are mean ± SEM. *p<0.05, **p<0.01, and ***p<0.001 and ns, no significance, by two-way ANOVA. Holm-Sidak method was used for p value adjustment for multiple comparisons.



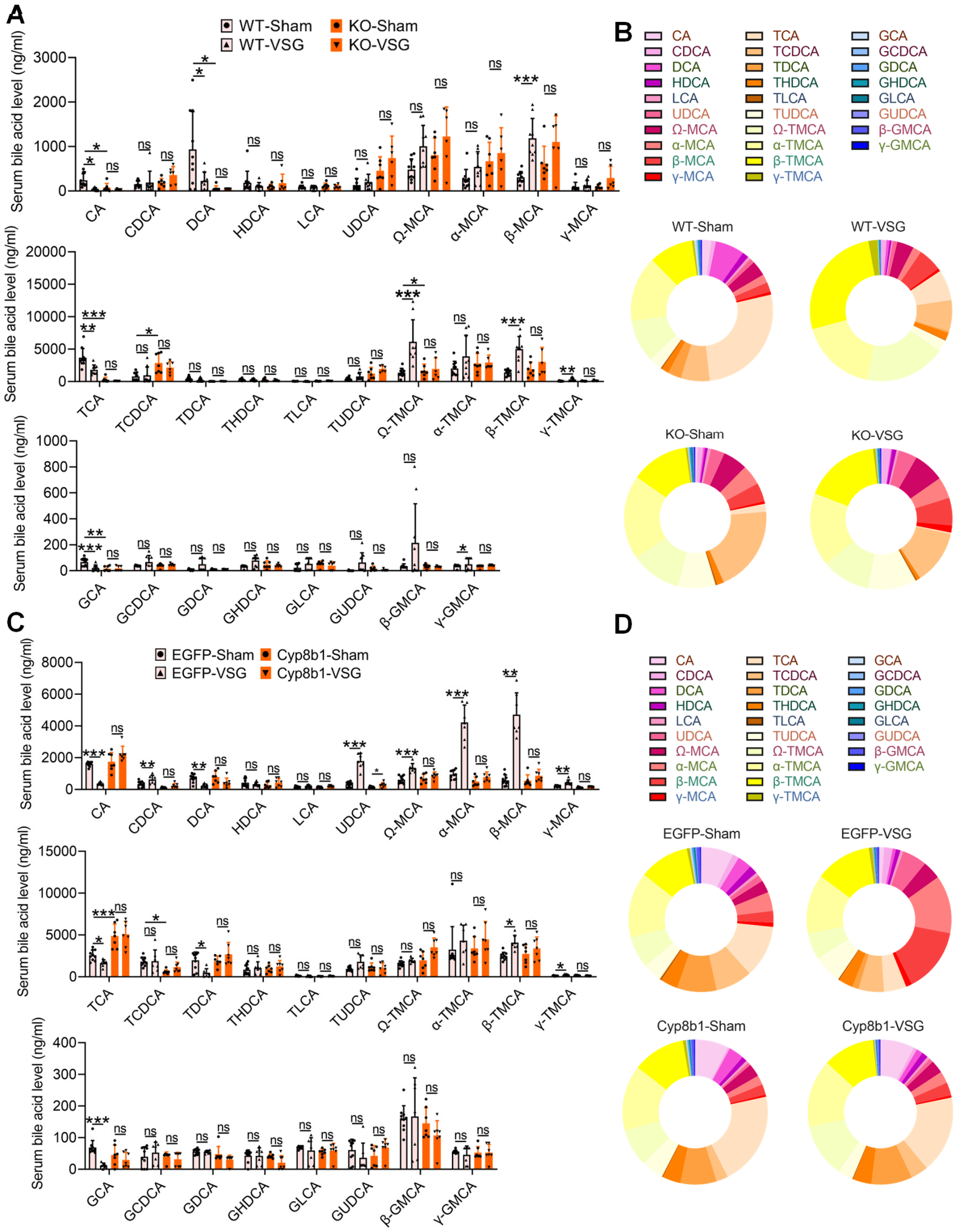
Supplementary Fig. 3 Knockdown of CYP8B1 improves metabolism in mice.

WT mice were injected via the tail vein with an AAV containing control or Cyp8b1-targeting shRNA and fed HFD for 10 weeks. (**A**) Relative mRNA level and Western blot analysis of hepatic CYP8B1. (**B**) Body weight. (**C**) Weights of the liver, iWAT, and eWAT. (**D**) GTT and AUC at 10 weeks on HFD. (**E**) ITT and AUC at 9 weeks on HFD. (**F**) Representative images of H&E-stained liver and BAT sections (scale bar, 50 μm). Data are mean ± SEM. n = 5 mice per group. *p<0.05 and **p<0.01 by two-tailed Student's t test.



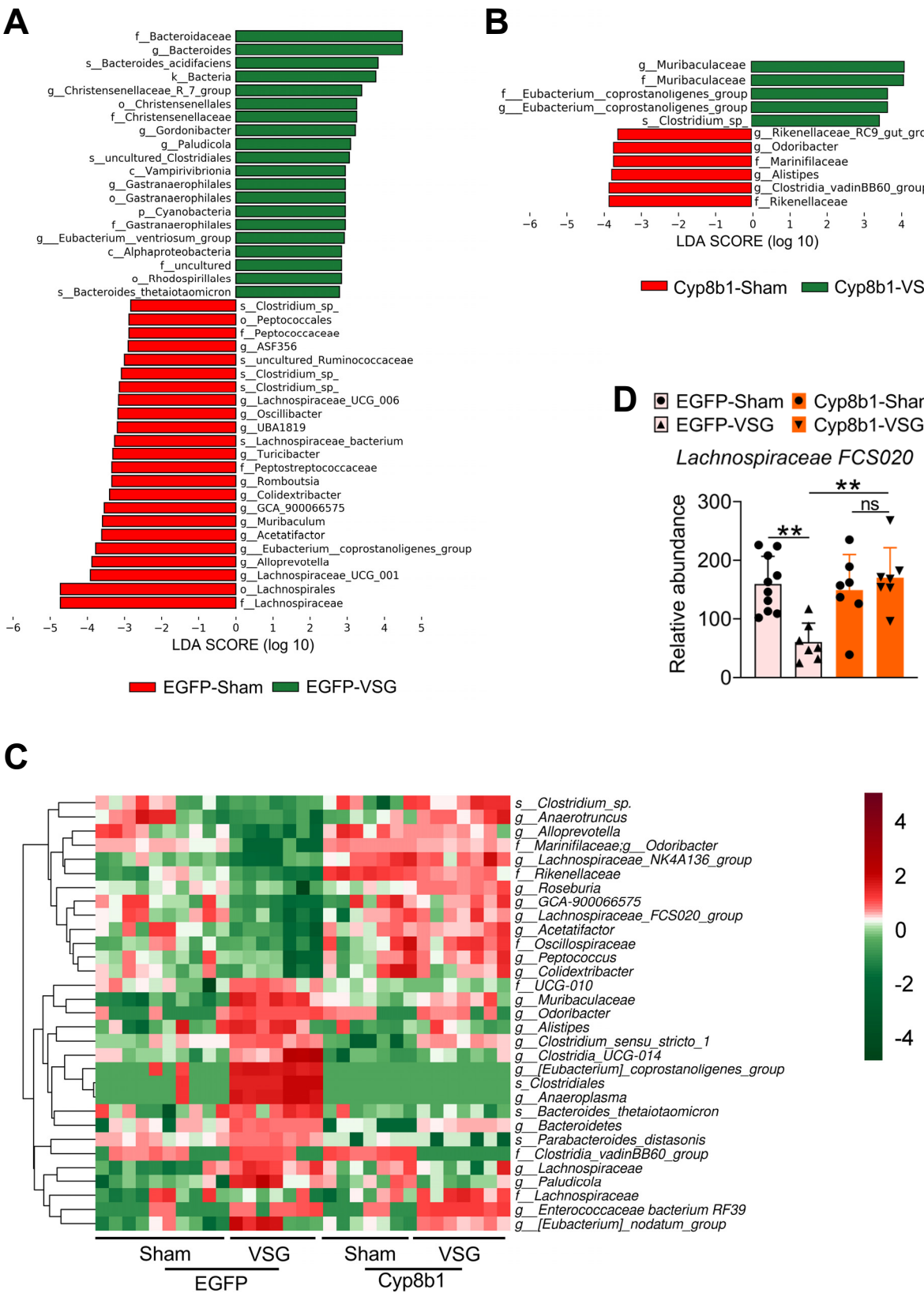
Supplementary Fig. 4 Effect of VSG on HFD-fed *Cyp8b1*-KO mice.

(**A**) Food intake. (**B**) Relative mRNA levels of BA synthesis genes in the liver of WT and *Cyp8b1*-KO mice that received VSG or sham surgery. (**C**) Weights of iWAT and eWAT. (**D**) Weight ratios of iWAT and eWAT to body weight. n=10 mice in WT-Sham group, n=9 mice in WT-VSG group, n=7 mice in KO-Sham group, and n=6 in KO-VSG group. One-way ANOVA with repeated measure (**A**) or two-way ANOVA (**B-D**) were used for group comparisons. Holm-Sidak method was used for p value adjustment for multiple comparisons. Data are mean ± SEM. ns, no significant difference, *p<0.05, **p<0.01.

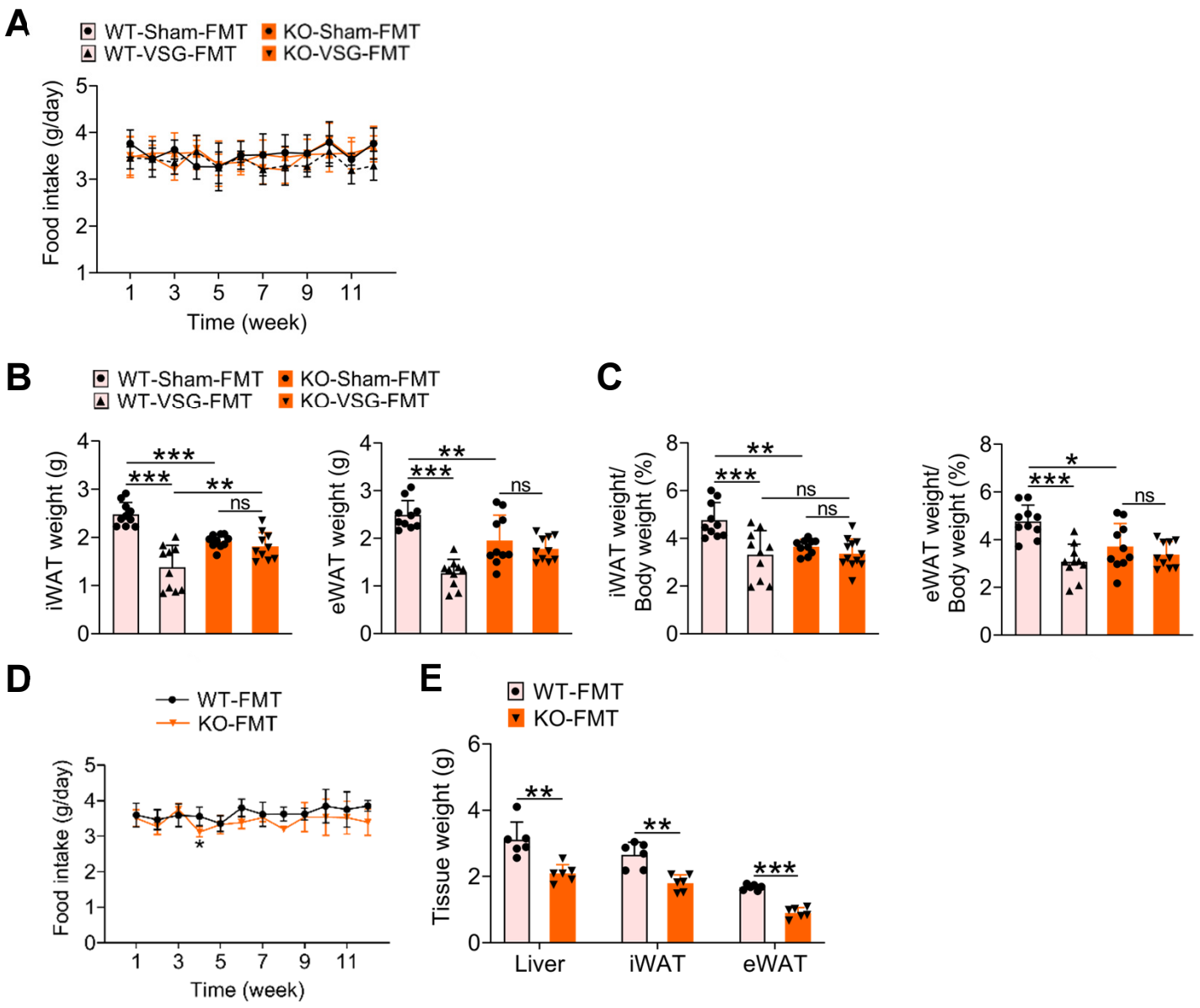

A
WT-Sham
KO-Sham
WT-VSG
KO-VSG
Serum bile acid level (ng/ml)
CA
CDCA
DCA
HDCA
LCA
UDCA
Ω-MCA
α-MCA
β-MCA
γ-MCA
TCA
TCDCA
TDCA
THDCA
TLCA
TUDCA
Ω-TMCA
α-TMCA
β-TMCA
γ-TMCA
GCA
GCDCA
GDCA
GHDCA
GLCA
GUDCA
β-GMCA
γ-GMCA
B
WT-Sham
WT-VSG
KO-Sham
KO-VSG
C
EGFP-Sham
EGFP-VSG
Cyp8b1-Sham
Cyp8b1-VSG
D
EGFP-Sham
EGFP-VSG
Cyp8b1-Sham
Cyp8b1-VSG

Supplementary Fig. 5 BA composition in CYP8B1 overexpressing mice and *Cyp8b1*-KO mice after VSG.

(**A-B**) Serum BA levels (**A**) and BA composition presented by a pie chart (**B**) in WT and *Cyp8b1*-KO mice received VSG or sham surgery. n=10 mice in WT-Sham group; n=9 mice in WT-VSG group, n=7 mice in KO-Sham group, and n=6 in KO-VSG group. (**C-D**) Serum BA levels (**C**) and BA composition presented by a pie chart (**D**) in EGFP or CYP8B1 overexpressing mice received VSG or sham surgery; n=10 mice in EGFP-Sham group, n=7 mice in EGFP-VSG group, CYP8B1-Sham group, and CYP8B1-VSG group. Data are mean ± SEM. ns, no significant difference, *p<0.05, **p<0.01, and ***p<0.001. Two-way ANOVA was used for group comparisons in (**A, C**). Holm-Sidak method was used for p value adjustment for multiple comparisons.

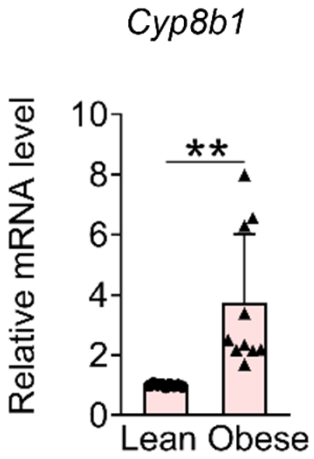


Supplementary Fig. 6 The GM profiles of CYP8B1-overexpressing mice after VSG and sham surgery. (**A-B**) LDA comparing the GM composition of EGFP-overexpressing mice (**A**) and CYP8B1-overexpressing mice (**B**) after VSG and sham surgery. (**C**) Heat map of microbiota composition at the family and genus level. (**D**) Relative abundance of *Lachnospiraceae* FS020 in fecal samples. n=10 mice in EGFP-Sham group, n=7 mice in EGFP-VSG group, CYP8B1-Sham group, and CYP8B1-VSG group. Two-way ANOVA were used for group comparisons. Holm-Sidak method was used for p value adjustment for multiple comparisons. Data are mean ± SEM. ns, no significant difference, **p<0.01 and ***p<0.001.



Supplementary Fig. 7 Phenotypes of FMT recipients from WT or *Cyp8b1*-KO mice after VSG or HFD feeding.

(**A-C**) The fecal microbiota of mice from WT-Sham, WT-VSG mice, KO-Sham, KO-VSG mice were transplanted into DIO WT mice. n=10 recipients per group. (**A**) Food intake. (**B**) Weights of iWAT and eWAT. (**C**) Weight ratios of iWAT and eWAT to body weight. ns, no significance, **p<0.01 by two-way ANOVA. (**D-E**) Food intake (**D**) and weights of liver, iWAT and eWAT (**E**) in DIO WT mice received FMT from HFD-fed WT or *Cyp8b1*-KO mice. n=6 recipients per group. One-way ANOVA with repeated measure (**A, D**) or two-way ANOVA (**B, C**) were used for group comparisons, and two-tailed Student's t test was used for (**E**). Holm-Sidak method was used for p value adjustment for multiple comparisons. ns, no significant difference, *p<0.05, **p<0.01, and ***p<0.001.



Supplementary Fig. 8 *Cyp8b1* expression was increased in obese mice. *Cyp8b1* mRNA expression was measured by qPCR. n=9 in lean mice group and n=10 in obese mice group. Data are mean ± SEM. **p<0.01 by two-tailed Student's t test.

Supplementary Table 1. Primer sequences used in qPCR.

Gene name	Forward sequence (5’ to 3’)	Reverse sequence (5’ to 3’)
Cyp7a1	AGCAACTAAACAACCTGCCAGTACTA	GTCCGGATATTCAAGGATGCA
Cyp8b1	GCCTTCAAGTATGATCGGTTCCT	GATCTTCTTGCCCGACTTGTAGA
Cyp7b1	TAGCCCTCTTTCCTCCACTCATA	GAACCGATCGAACCTAAATTCCT
Cyp27a1	GCCTCACCTATGGGATCTTCA	TCAAAGCCTGACGCAGATG
Cyp3a11	CTCAATGGTGTGTATATCCCC	CCGATGTTCTTAGACACTGCC
36B4	CACTGGTCTAGGACCCGAGAAG	GGTGCCTCTGGAGATTTTCG

Supplementary Table 2. Bile acid profiles in the serum of *Cyp8b1* KO mice that received VSG or Sham surgery.

Bile acid (ng/ml)	WT-Sham	WT-VSG	KO-Sham	KO-VSG	[a] p value	[b] p value	[c] p value
CA	239.93 ± 147.83	44.91 ± 30.07	68.69 ± 75.90	30.53 ± 12.58	0.005	0.846	0.003
CDCA	151.18 ± 71.18	188.94 ± 351.95	164.18 ± 81.90	356.34 ± 193.66	0.782	0.453	0.869
DCA	933.17 ± 851.45	223.29 ± 178.54	154.4 ± 182.52	44.73 ± 4.10	0.018	0.907	0.018
HDCA	183.5 ± 260.02	108.06 ± 116.38	100.41 ± 47.88	171.77 ± 206.2	0.885	0.923	0.840
LCA	89.31 ± 39.60	80.13 ± 52.14	106.27 ± 54.90	95.37 ± 42.24	0.708	0.976	0.959
UDCA	134.7 ± 146.70	198.59 ± 246.17	350.09 ± 283.21	738.73 ± 496.79	0.977	0.402	0.283
ω-MCA	480.58 ± 238.55	1002.49 ± 354.72	952.52 ± 475.03	1223.6 ± 665.07	0.515	0.323	0.514
α-MCA	259.6 ± 223.39	542.37 ± 314.52	678.41 ± 380.93	852.74 ± 568.16	0.974	0.451	0.593
β-MCA	307.45 ± 129.86	1183.29 ± 449.58	823.97 ± 500.10	1101.35 ± 589.22	<0.0001	0.434	0.075
γ-MCA	91.76 ± 102.00	128.82 ± 148.90	95.18 ± 41.78	288.86 ± 270.04	0.959	0.137	0.998
GCA	67.49 ± 29.13	21.11 ± 13.90	23.59 ± 22.52	17.97 ± 19.88	<0.0001	0.959	0.001
GCDCA	38.32 ± 3.47	67.31 ± 20.17	67.09 ± 42.31	45.39 ± 8.02	0.898	0.923	0.236
GDCA	8.18 ± 5.20	50.36 ± 1.99	43.35 ± 45.87	9.82 ± 1.28	0.982	0.988	0.881
GHDCA	32.34 ± 9.68	79.38 ± 48.94	65.36 ± 35.44	39.06 ± 10.76	0.194	0.964	0.730
GLCA	22.35 ± 27.73	53.39 ± 23.30	69.13 ± 28.94	38.69 ± 30.49	0.905	0.784	0.304
GUDCA	0.97 ± 2.43	64.01 ± 3.95	59.13 ± 68.25	3.91 ± 7.01	0.679	0.200	0.060
β-GMCA	34.47 ± 18.38	214.87 ± 19.40	190.94 ± 273.79	29.75 ± 4.76	0.328	0.991	0.987
γ-GMCA	35.96 ± 11.06	50.95 ± 14.95	56.65 ± 34.33	40.17 ± 3.70	0.027	0.090	0.074
TCA	3654.47 ± 1429.05	1813.73 ± 437.09	954.56 ± 1121.73	59.06 ± 19.81	0.001	0.183	<0.0001
TCDCA	789.82 ± 623.18	962.75 ± 1494.93	1885.88 ± 1759.86	2142.91 ± 956.83	0.643	0.643	0.025
TDCA	406.35 ± 306.40	254.47 ± 64.81	185.58 ± 261.03	15.59 ± 9.82	0.351	0.351	0.199
THDCA	229.75 ± 147.65	296.76 ± 180.49	207.51 ± 173.44	127.75 ± 119.93	0.503	0.812	0.995
TLCA	43.93 ± 16.17	58.15 ± 17.38	70.71 ± 37.77	66.57 ± 21.20	0.596	0.850	0.877
TUDCA	400.77 ± 240.79	794.39 ± 479.57	1173.31 ± 831.8	1930.61 ± 494.96	0.977	0.190	0.079
ω-TMCA	1360.51 ± 663.90	6131.36 ± 1042.50	4246.68 ± 4151.88	1926.87 ± 1721.68	<0.0001	0.180	0.044
α-TMCA	2022.03 ± 1099.74	3885.65 ± 3739.30	3210.62 ± 2131.49	2926.14 ± 1142.10	0.449	0.999	0.916
β-TMCA	1393.58 ± 442.81	4989.59 ± 1884.99	2673.46 ± 1722.55	3012.62 ± 2241.75	<0.0001	0.708	0.224
γ-TMCA	63.15 ± 46.81	313.36 ± 258.32	90.81 ± 48.10	97.01 ± 75.69	<0.0001	0.972	>0.9999

Note: Data show the mean ± SEM (ng/ml). a, WT-Sham vs. WT-VSG; b, KO-Sham vs. KO-VSG; c, WT-Sham vs. KO-Sham. Two-way ANOVA was used for group comparisons, and Holm-Sidak method was used for p value adjustment for multiple comparisons.

Supplementary Table 3. Bile acid profiles in the intestinal contents of *Cyp8b1* KO mice that received VSG or sham surgery.

Bile acid (ng/mg)	WT-Sham	WT-VSG	KO-Sham	KO-VSG	[a] p value	[b] p value	[c] p value
CA	99.27 ± 33.51	38.85 ± 17.48	1.60 ± 1.49	2.22 ± 1.29	0.027	0.999	0.007
CDCA	1.97 ± 0.95	12.75 ± 13.46	24.19 ± 7.84	28.75 ± 4.31	0.285	0.425	0.007
DCA	10.02 ± 17.42	5.25 ± 7.32	2.87 ± 3.95	3.42 ± 2.02	0.948	0.989	0.845
HDCA	0.06 ± 0.07	0.68 ± 1.06	0.64 ± 0.53	0.76 ± 0.29	0.684	0.957	0.347
LCA	15.40 ± 7.19	13.27 ± 11.91	0.63 ± 0.31	0.70 ± 0.23	0.940	0.902	0.021
UDCA	9.45 ± 3.31	5.72 ± 5.26	11.39 ± 2.89	6.78 ± 3.19	0.569	0.570	0.991
ω-MCA	1.40 ± 0.71	24.18 ± 30.54	52.75 ± 26.99	86.27 ± 34.25	0.318	0.325	0.026
α-MCA	3.75 ± 3.77	52.11 ± 42.41	44.33 ± 32.56	149.42 ± 114.53	0.217	0.343	0.094
β-MCA	12.80 ± 14.03	213.46 ± 163.81	147.57 ± 79.62	284.17 ± 130.58	0.064	0.211	0.035
γ-MCA	0.72 ± 0.02	0.98 ± 0.11	11.90 ± 18.58	16.04 ± 12.59	0.553	0.964	0.510
GCA	2.36 ± 1.70	1.74 ± 1.42	0.54 ± 0.25	0.65 ± 0.20	0.994	0.852	0.334
GCDCA	0.98 ± 0.04	1.3 ± 0.24	1.81 ± 0.49	1.28 ± 0.60	0.611	0.995	0.299
GDCA	0.85 ± 0.02	0.86 ± 0.07	0.48 ± 0.25	0.52 ± 0.25	0.851	0.932	0.122
GHDCA	1.56 ± 0.02	1.57 ± 0.10	1.09 ± 0.50	1.10 ± 0.51	0.717	>0.9999	0.160
GLCA	0.63 ± 0.07	0.58 ± 0.10	0.69 ± 0.24	0.71 ± 0.13	0.298	0.995	0.997
GUDCA	1.30 ± 0.05	1.66 ± 0.17	1.72 ± 0.29	1.37 ± 0.74	0.986	0.994	0.884
ω-GMCA	0.27 ± 0.04	1.20 ± 0.53	1.46 ± 0.72	1.32 ± 0.78	0.050	0.951	0.052
α-GMCA	0.72 ± 0.07	3.63 ± 1.35	1.53 ± 0.76	1.55 ± 0.84	0.024	0.931	0.179
β-GMCA	1.42 ± 0.48	13.49 ± 3.24	16.00 ± 8.92	14.01 ± 7.39	0.002	0.950	0.057
γ-GMCA	1.25 ± 0.02	1.23 ± 0.02	1.13 ± 0.60	1.47 ± 0.51	0.975	0.596	0.880
TCA	6187.42 ± 1753.06	2697.53 ± 317.87	94.99 ± 41.75	110.89 ± 72.35	0.017	0.976	0.003
TCDCA	521.58 ± 176.94	553.24 ± 357.19	392.38 ± 157.19	633.99 ± 416.23	0.998	0.785	0.464
TDCA	200.11 ± 48.41	148.62 ± 122.58	54.64 ± 27.10	111.15 ± 66.88	0.654	0.375	0.001
TLCA	2.33 ± 0.41	2.40 ± 0.72	2.34 ± 1.58	3.26 ± 1.80	>0.9999	0.554	0.980
TUDCA	450.16 ± 112.6	331.18 ± 119.28	273.82 ± 133.86	455.48 ± 184.22	0.346	0.546	0.133
ω-TMCA	266.85 ± 79.15	500.75 ± 237.50	918.46 ± 219.13	1003.34 ± 116.76	0.206	0.559	0.005
α-TMCA	1848.71 ± 619.44	1710.17 ± 503.5	2388.79 ± 809.83	2876.90 ± 605.33	0.905	0.581	0.704
β-TMCA	4952.43 ± 729.84	6839.20 ± 797.26	7248.20 ± 850.98	9590.87 ± 1896.56	0.015	0.307	0.004
γ-TMCA	3.32 ± 1.89	2.91 ± 0.62	6.82 ± 2.96	10.13 ± 3.19	0.994	0.200	0.143
12α/non 12α BAs ratio	**0.785 ± 0.120**	**0.283 ± 0.053**	**0.013 ± 0.005**	**0.015 ± 0.006**	**<0.001**	**0.968**	**<0.001**

Note: Data show the mean ± SEM (ng/mg dry content). a, WT-Sham vs. WT-VSG; b, KO-Sham vs. KO-VSG; c, WT-Sham vs. KO-Sham. Two-way ANOVA was used for group comparisons, and Tukey's test was used for p value adjustment for multiple comparisons.

Supplementary Table 4. Bile acid profiles in the serum of CYP8B1-overexpressing mice that received VSG or sham surgery.

Bile acid (ng/ml)	EGFP-Sham	EGFP-VSG	Cyp8b1-Sham	Cyp8b1-VSG	[a] p value	[b] p value	[c] p value
CA	1598.23 ± 143.82	371.33 ± 81.69	1737.65 ± 582.45	2236.22 ± 484.14	<0.0001	0.051	0.450
CDCA	327.95 ± 183.6	622.56 ± 254.62	107.03 ± 44.10	233.22 ± 135.01	0.010	0.340	0.050
DCA	701.47 ± 247.53	235.59 ± 106.89	783.30 ± 378.93	415.93 ± 297.85	0.010	0.070	0.550
HDCA	424.33 ± 258.82	327.97 ± 112.71	272.34 ± 184.41	420.11 ± 265.79	0.854	0.710	0.678
LCA	153.96 ± 48.36	213.21 ± 80.32	142.42 ± 40.59	188.60 ± 50.08	0.188	0.439	0.679
UDCA	332.34 ± 143.46	1784.57 ± 486.58	147.24 ± 54.46	271.78 ± 158.35	<0.0001	0.600	0.400
ω-MCA	614.72 ± 228.89	1341.98 ± 282.64	678.62 ± 269.33	860.74 ± 233.74	<0.0001	0.340	0.610
α-MCA	938.02 ± 273.08	4215.27 ± 1093.28	491.86 ± 289.34	781.50 ± 320.86	<0.0001	0.580	0.340
β-MCA	576.35 ± 336.06	4706.11 ± 1372.59	545.20 ± 373.57	856.71 ± 410.04	<0.0001	0.810	0.930
γ-MCA	209.29 ± 47.47	444.68 ± 157.68	115.04 ± 39.66	153.50 ± 29.42	<0.0001	0.391	0.082
GCA	69.1 ± 21.88	12.02 ± 7.59	44.91 ± 31.96	29.67 ± 23.79	0.001	0.300	0.120
GCDCA	39.52 ± 31.22	52.85 ± 32.76	40.76 ± 14.06	31.52 ± 21.89	0.860	0.890	0.930
GDCA	56.18 ± 8.42	54.87 ± 5.16	47.98 ± 24.38	31.04 ± 13.74	0.850	0.130	0.580
GHDCA	42.92 ± 18.20	42.63 ± 25.15	41.63 ± 6.75	20.67 ± 22.64	0.999	0.200	0.999
GLCA	69.06 ± 4.44	59.76 ± 40.07	54.38 ± 9.86	58.51 ± 23.56	0.885	0.960	0.733
GUDCA	60.87 ± 34.23	36.42 ± 46.30	42.73 ± 32.28	68.99 ± 27.66	0.618	0.618	0.672
β-GMCA	160.3 ± 40.84	166.76 ± 122.26	145.36 ± 48.88	106.91 ± 46.69	0.921	0.777	0.921
γ-GMCA	55.25 ± 5.17	45.58 ± 28.09	52.64 ± 18.93	52.42 ± 26.34	0.922	0.989	0.989
TCA	2578.08 ± 626.2	1631.83 ± 406.58	4878.18 ± 1424.06	5076.99 ± 1521.7	0.050	0.730	<0.0001
TCDCA	1809.3 ± 527.82	1850.47 ± 1349.16	606.25 ± 246.33	1140.84 ± 614.26	0.910	0.370	0.020
TDCA	1950.97 ± 1015.66	464.72 ± 491.39	1871.23 ± 594.07	2686.32 ± 1417.59	0.020	0.330	0.870
THDCA	847.76 ± 591.96	1052.65 ± 743.68	941.15 ± 460.99	1225.63 ± 716.28	<0.0001	<0.0001	<0.0001
TLCA	99.15 ± 59.85	91.73 ± 80.51	52.50 ± 23.86	50.38 ± 45.70	0.957	0.957	0.442
TUDCA	935.51 ± 242.59	2077.36 ± 1294.21	1143.03 ± 503.48	1017.66 ± 629.3	0.023	0.938	0.920
ω-TMCA	1565.03 ± 355.80	1930.01 ± 280.11	1967.89 ± 1042.08	3508.10 ± 1125.61	0.648	0.003	0.647
α-TMCA	3231.96 ± 2768.42	4317.72 ± 1840.21	3386.99 ± 1422.01	4462.26 ± 2148.97	0.857	0.857	0.987
β-TMCA	2471.78 ± 453.69	4084.79 ± 865.80	2732.14 ± 1094.3	3385.21 ± 1362.35	0.010	0.450	0.590
γ-TMCA	145.22 ± 51.26	264.77 ± 56.12	160.16 ± 38.77	86.06 ± 44.03	<0.0001	0.024	0.536

Note: Data show the mean ± SEM (ng/ml). a, EGFP-Sham vs. EGFP-VSG; b, Cyp8b1-Sham vs. Cyp8b1-VSG; c, EGFP-Sham vs. Cyp8b1-Sham. Two-way ANOVA was used for group comparisons, and Holm-Sidak method was used for p value adjustment for multiple comparisons.

Supplementary Table 5. Bile acid profiles in the intestinal contents of CYP8B1-overexpressing mice that received VSG or sham surgery.

Bile acid (ng/mg)	EGFP-Sham	EGFP-VSG	Cyp8b1-Sham	Cyp8b1-VSG	[a] p value	[b] p value	[c] p value
CA	193.21 ± 87.73	47.60 ± 38.4	307.07 ± 111.45	227.32 ± 111.77	0.002	0.558	0.168
CDCA	12.01 ± 10.74	2.52 ± 1.86	4.94 ± 4.24	5.36 ± 4.50	0.094	0.999	0.235
DCA	11.62 ± 14.09	29.47 ± 13.57	13.19 ± 14.04	12.89 ± 5.94	0.081	0.999	0.998
HDCA	8.98 ± 6.75	5.72 ± 2.45	3.81 ± 1.58	5.88 ± 2.48	0.523	0.302	0.153
LCA	4.06 ± 1.64	5.75 ± 3.95	5.61 ± 4.47	6.00 ± 3.31	0.718	0.997	0.816
UDCA	8.62 ± 6.77	3.44 ± 3.28	9.33 ± 8.38	6.11 ± 4.28	0.204	0.803	0.997
ω-MCA	3.45 ± 4.72	2.48 ± 2.49	2.92 ± 2.08	3.14 ± 3.67	0.978	0.996	>0.9999
α-MCA	15.36 ± 5.02	13.16 ± 2.58	13.31 ± 2.59	13.52 ± 3.16	0.647	0.999	0.696
β-MCA	18.69 ± 9.00	9.70 ± 4.10	9.98 ± 5.08	8.75 ± 2.28	0.065	0.934	0.095
γ-MCA	1.76 ± 2.92	2.28 ± 2.26	2.08 ± 2.43	1.58 ± 1.90	0.988	0.979	0.999
GCA	30.05 ± 16.13	3.78 ± 4.06	16.20 ± 13.58	27.28 ± 16.23	0.002	0.524	0.263
GCDCA	2.91 ± 0.76	3.86 ± 1.59	2.99 ± 1.41	3.54 ± 0.87	0.497	0.818	0.999
GDCA	0.70 ± 0.92	1.34 ± 1.43	0.73 ± 1.43	3.25 ± 2.9	0.916	0.543	0.999
GHDCA	2.08 ± 3.06	1.83 ± 2.25	2.21 ± 0.82	1.78 ± 1.93	0.97	0.845	0.945
GLCA	1.67 ± 2.19	2.91 ± 1.91	1.71 ± 1.71	2.73 ± 2.79	0.64	0.986	0.981
GUDCA	3.44 ± 0.51	4.92 ± 1.13	3.96 ± 0.91	3.33 ± 0.27	0.995	0.641	0.906
TCA	1441.70 ± 759.79	696.8 ± 400.06	1287.55 ± 474.86	1490.08 ± 978.46	0.082	0.958	0.954
TCDCA	81.20 ± 60.21	31.78 ± 19.52	166.56 ± 126.92	135.2 ± 56.18	0.128	0.93	0.404
TDCA	31.38 ± 16.3	37.89 ± 33.83	41.01 ± 30.68	31.27 ± 23.87	0.963	0.908	0.87
THDCA	3.59 ± 3.52	2.67 ± 2.04	2.39 ± 2.04	2.25 ± 1.83	0.953	0.994	0.881
TUDCA	153.29 ± 92.99	55.10 ± 32.35	191.29 ± 149.31	165.17 ± 158.27	0.041	0.988	0.93
ω-TMCA	1037.85 ± 249.72	1120.93 ± 404.91	1045.52 ± 188.85	965.96 ± 262.48	0.961	0.913	0.999
α-TMCA	376.84 ± 322.91	273.77 ± 213.42	103.59 ± 59.37	138.31 ± 110.65	0.856	0.882	0.101
β-TMCA	820.25 ± 454.41	1124.08 ± 985.21	581.64 ± 190.01	798.90 ± 214.99	0.869	0.24	0.473
12α/non-12α BA ratio	**0.67 ± 0.29**	**0.33 ± 0.19**	**0.82 ± 0.37**	**0.77 ± 0.34**	**0.047**	**0.994**	**0.828**

Note: Data show the mean ± SEM (ng/mg dry content). a, EGFP-Sham vs. EGFP-VSG; b, Cyp8b1-Sham vs. Cyp8b1-VSG; c, EGFP-Sham vs. Cyp8b1-Sham. Two-way ANOVA was used for group comparisons, and Tukey's test was used for p value adjustment for multiple comparisons.