Expanding Methods and Materials

Animal experiments

FXR knockdown lentivirus and scrambled RNA-GFP control lentivirus were purchased by WZ bioscience Inc. (Shandong, China). By transfection control lentivirus into hepatocytes, then counted the multiplicity of infection (MOI). Mice were bolus injected lentivirus (10 MOI) by tail vein to knockdown FXR.

For Oral glucose tolerance test (OGTT), mice were fasted for 16 hours and then intragastric administration with 3 g/kg glucose. For insulin tolerance test (ITT), mice were fasted for 6 hours and then injected (intraperitoneal injection, i.p.) with insulin (I8040, Solarbio) at a dose of 1 U/kg. For pyruvate tolerance test (PTT), mice were fasted for 16 hours then injected (i.p.) with sodium pyruvate (P8380, Solarbio) 2 g/Kg, blood (from tail vein) glucose level was measured using a glucose monitor (Contour TS, Bayer), at 0, 15, 30, 60, 90 and 120 minutes after intervention.

Biotin switch assay of S-sulfhydration

The assay was performed as described by Mustafa et al. (1). Cellular or liver tissue homogenates were prepared by HEN buffer [250 mM Hepes-NaOH (pH 7.7), 1 mM EDTA, and 0.1 mM neocuproine, supplemented with 100 μM deferoxamine], then were sonicated and centrifuged at 12,000 rpm (4 ℃) for 10-15 min. Homogenates treated with DTT, NaHS, or GYY4137 for in vitro sulfhydration. For in vivo sulfhydration, cultured cells were treated with DTT, or NaHS or GYY4137, or transfection with adenovirus, then cellular homogenates were collected. Free thiol was blocked with 20 mM MMTS (Final concentration 2.5% SDS in HEN buffer) at 50°C for 20 min by vortex. The proteins were precipitated by acetone at −20°C for 20 min. Resuspended proteins in HENS buffer (HEN buffer adjusted to 1% SDS) and added in 4 mM biotin-HPDP for 3 hours at room temperature, and then incubated overnight with streptavidin magnet beads at 4 ℃. Then biotinylated protein was pulled down and eluted by SDS-PAGE loading buffer for analysis by Western blotting with an anti-FXR antibody.

Bulk RNA-sequencing

Total RNA extracted from liver tissues then purified using spin columns. After preparing the cDNA library, the size and quality was checked by Aglient 2100 bioanalyzer. The library was sequenced on an Illumina NextSeq 500 sequencers. The reads were aligned to the reference genome (hg38 or mm9) using Hisat2 (v2.0.5) and annotated to Ref Seq genes using feature Counts (v1.5.0-p3). Genes with reads less than 20 in all samples were removed. The differential expression analysis was conducted by edge R (v3.22.5). The P values were adjusted using the Benjamini & Hochberg method. Corrected P-value of 0.05 and absolute foldchange of 2 were set as the threshold for significantly differential expression.

Chromosome immunoprecipitation sequencing (ChIP-seq)

Cells were cross-linked by 1% formaldehyde for 10 min, then quenching with 125mM glycine for 5 min at room temperature. After washing, the pellets were resuspended in cell

lysis buffer (10mM Tris-HCl, pH 7.5, 10mM NaCl, 0.5% NP-40) and incubated on ice for 10 min. Then cross-linked chromatin was sonicated into 200 bp to 500 bp fragments. After centrifuging, 5 μg of anti-FXR antibody was added into the clean supernatant, transferred into cold protein A magnet bead, then incubated overnight at 4 ℃. Beads were washed extensively with ChIP buffer, high-salt buffer, LiCl2 buffer, and TE buffer. Bound chromatin was eluted and reverse-crosslinked at 65 ℃ overnight. DNA was recovered by RNase and proteinase K treatment, phenol/chloroform extraction and ethanol precipitation. Then the DNA was purified using spin columns. After preparing chip library, the size and quality of the DNA was confirmed by Aglient 2100 bioanalyzer. Then the Chip library were sequenced on an Illumina NextSeq 500 sequencers (2). The reads were aligned to the reference genome (hg38 or mm9) and call peaks with Sicer2 (v1.0.2, -window size (bp) 200; -gap size (bp) 600; -False discovery rate (Fdr)<0.05). Differential peaks were detected by Sicer_df (Fdr <0.05 and fold change >1.5). BigWig files were created by deepTools (v3.0.2) and viewed in IGV (v2.3.92).

For analysis of Chip-seq results, Raw reads were filtered to obtain high-quality clean reads by removing sequencing adapters, short reads (length <50 bp) and low-quality reads using Cutadapt (v1.9.1) and Trimmomatic (v0.35). Then FastQC is used to ensure high reads quality. The clean reads were mapped to the mouse genome (assembly GRCm38.p6) using the Bowtie2 (v2.2.6) software. Peak detection was performed using the MACS (v2.1.1) peak finding algorithm with 0.01 set as the p-value cutoff. Annotation of peak sites to gene features was performed using the ChIPseeker R package. For functional enrichment analysis, gene annotation file was retrieved from Ensembl genome browser database (http://www.ensembl.org/index.html).

Cell culture

HepG2 cells 293-HEK were purchased from the National Infrastructure of Cell Line Resource (Beijing, China), cultured in DMEM containing 10% FBS. All cell culture media and serum were obtained from Hyclone (Logan, UT, USA). Cells with 80–85% confluence were incubated with NaHS (100 μM) or GYY4137 (100 μM) or DTT (1 mM) for 12 hours.

Primary mouse hepatocytes isolated using collagenase perfusion technique(3). In brief, insert the cannula into portal vein via the vena cava, perfusion liver with buffer I (500 ml sterile phosphate-buffered saline (PBS), 5 ml 1 M HEPES in 5% (w/v) KCl buffer, 2.5 ml 1 M glucose, 0.5 ml 200 mM EDTA. pH=7.4) to clean the residue blood. Then was perfused buffer I containing a calcium chelator (EGTA) to remove calcium ions resulting in the rapid destruction of intercellular junctions with the consequence that the epithelial cell-cell contacts are lost. Changed buffer I for the collagenase IV solution then continuously perfused for 10 min. Remove the entire liver to a dish containing DMEM, Filter the crude hepatocyte preparation through a gauze mesh filter (100 μm in diameter) and transfer the cell suspension into tubes and centrifuge at 50 rcf for 2 min. Washed isolated hepatocytes three times. After counting the cell number, primary hepatocytes were cultured in CO_2 incubator.

FXR mutation plasmid transfection

FXR plasmid pENTER-FXR (NM_005123, WT) with C-terminal flag and 6×His-tag (CH845725), and FXR zinc finger sites-mutation plasmids: mutation 1 (Cys138/141, M1),

mutation 2 (Cys155/158, M2), mutation 3 (Cys174/180, M3), mutation 1 (Cys190/193, M4) and mutation all (M1+M2+M3+M4) were constructed by Shandong Vigene Biosciences. The expression plasmids were transfected into 293-HEK cells with Lipofectamine 3000 reagent (Invitrogen, USA) for protein purification (in vitro zinc ion binding assay), ChIP-seq and ChIP-qPCR analysis.

Triglyceride and cholesterol detection

Blood samples were collected by inner canthus of mice. Liver tissues (50 mg) were homogenized with RIPA lysis (1:40, weight/volume), the homogenate was centrifuged (12000 g) for 10 min at 4 ℃, then the supernatant was collected. Triglyceride (TG) total cholesterol (TC) in the serum and homogenate supernatant were measured with commercial kits. (BioSino Bio-Technology & Science Inc. China) according to the manufacturer's protocol.

Histology

Fresh liver tissues were fixed in 4% paraformaldehyde, then paraffin sections (5 μm) were cut and stained by hematoxylin and eosin (H&E). Fixed liver slices were embedded in optimal cutting temperature compound (OCT), and then frozen sections were prepared and stained with Oil Red O (Sigma–Aldrich, St. Louis, MO). Paraffin sections were also performed immunohistochemistry (IHC) staining for CSE (ab189916; 1:200), CBS (ab140600; 1:200) according to standard protocol. IHC results were evaluated by a semiquantitative approach used to assign an H-score method (4). H-score is assigned using the following formula: [1 × (% cells 1+) + 2 × (% cells 2+) + 3 × (% cells 3+).

Quantitative real-time PCR (qRT-PCR)

Total RNA extracted from cells or liver tissues, was reverse-transcribed for qRT-PCR analysis with SYBR green I dye in the Mx3000 Quantitative PCR System (Stratagene). The relative expression of target genes was normalized to that of 18S rRNA and analyzed by the $2^{-\Delta\Delta CT}$ method. The primers are in supplemental table 3.

ChIP-qPCR

HepG2 cells were treated with NaHS or DTT, or 293-HEK cells were transfected FXR plasmid, mutation FXR plasmids. The cells were cross-linked in 1% formaldehyde, followed by quenching with glycine for 10 min. Cross-linked chromatin were sonicated to 300-500 bp. By FXR antibody immunoprecipitation, DNA was extracted then purified. FXR DNA binding activity were quantitated by quantitative real-time PCR (qRT-PCR) of human BESP promoter (primers: F: GGGTTTCCCAAGCACACTCTGTGTTT; R: GAGGAAGCCAGAGGAAATGGTGG), and then resolution of the PCR bands on gels.

Western blot analysis

Total protein of cells or tissues were quantified by BCA assay, denatured by boiling, then underwent SDS-PAGE. Isolated proteins were transferred to PVDF membranes, which were blocked, then incubated with primary antibody at 4 ℃ overnight, washed then incubated with corresponding secondary antibody for 1 h at room temperature. After washing, ECL was used to acquire images. The gray density of bands was analyzed by using Image J.

Co-immunoprecipitation assay

FXR or M1 mutation, and RXR (CH864432, Shandong Vigene Biosciences) plasmids were co-transfected into 293-HEK cells for 12 h. NaHS (100 μM) was added for 12 h. The cells were washed with cold PBS then lysed with RIPA buffer. After adjusting protein concentration at 500 μg/ml, primary anti-body (IgG as negative control.) was added then rotation incubation at 4 ℃ overnight, then protein G magnetic beads was added and continuously incubation 12 h. Wash magnetic beads with cold Tris-buffer (50 mM, pH=7.4) for 3 times. The pulldown protein was directly resuspending with load buffer and then electrophoresis by SDS-PAGE. By transferring PVDF membrane, corresponding primary antibody incubation, then secondary antibody, the bands were required by chemiluminescence assay.

Zinc ion binding assay in vitro and in vivo(5)

Wild type human FXR and FXR mutation plasmids with C-terminal 6×His-tag transfected into 293-HEK cells in 15 cm dish. His-tagged proteins purified by immobilized metal ion (Ni^{2+}) affinity chromatography. 1μg purified protein in binding buffer (20% Glycerol, 5mM MgCl2, 2.5mM EDTA, 250mM NaCl, 50mM Tris-HCl pH 7.5) containing 15 μM Zinc acetate, whilst NaHS (1 mM) or DTT (5 mM) was added then incubated for 1 hour at 37 ℃. The protein mixture was desalinated by PD MultiTrap™ G-25 column (MERCK). Then, the purified protein was incubated with 25 μM Zinc indicator (FluoZin^{TM}-3) 30min at 37 ℃ in the dark. Re-desalination treatment, the in vitro FXR-Zn^{2+} binding activity was measured by Zn^{2+} fluorescence in 495~510nm by Automatic microplate reader.

Cells were treated with NaHS (1 mM) or DTT (1 mM), and incubating with Zinc indicator for 1 h, then cells were quickly wash with cold PBS and fixed with cold 4% paraformaldehyde for 4 h in the dark. After blocking with 1% BSA, cells incubated with primary anti-FXR antibody at 4 ℃ overnight. Next, response to secondary antibody, images were acquired by confocal microscopy. The intracellular pink dots are as in vivo FXR-Zn^{2+} binding.

Modified biotin switch assay and LC-MS/MS analysis

Liver samples of NAFLD and non-NAFLD patients were homogenized in HEN buffer, supplemented with 100 μM deferoxamine, and the supernatant was collected by centrifugation at 12,000g at 4°C. The protein was quantitated, then blocked with MMTS at 50 ℃ for 20 min. The MMTS was then removed by cold acetone precipitation. The proteins were resuspended in HENS buffer (containing 1% SDS), then added biotin-HPDP (4mM). After incubation for overnight at 4°C, biotinylated proteins were pulled down by streptavidin magnet beads and eluted and subjected to FXR1 sulfhydration. For LC-MS/MS analysis, samples were run on an SDS-PAGE gel for a short distance (~1cm) and stained with Coomassie brilliant blue. Subsequently, Gel lanes were sliced vertically, and proteins in the gel slices of different samples were treated with trypsin respectively and analyzed by LC-MS/MS. Total 240 proteins in non-NAFLD and 160 proteins in NAFLD samples were identified by LC-MS/MS, and 106 proteins were overlapped. The Metascape (6) (https://metascape.org) was used for Gene ontology (GO) pathway network.

Methylene blue assay for H_2S production

The liver tissues were homogenized in ice cold 50 mmol/L potassium phosphate buffer (pH 6.8) at a ratio of 10 μl/μg. The inner ring of conical flask was added with 0.5 ml 5% zinc acetate and a piece of filter paper (2 × 3 cm) to absorb the released H_2S in the form of ZnS. The outer ring of the conical flask was added with incubation buffer containing 100 μl tissue homogenate, 100 mM potassium phosphate buffer (PH 7.4), 1 mM L-cysteine, and 2 mM pyridoxal 5′-phosphate. The conical flasks were sealed and incubated in a 37 ℃ shaking water bath for 90 min. 1 ml 20% trichloroacetic acid (TCA) was added into the outer ring to block the reaction and incubated for another 60 min to make sure that all H_2S produced was absorbed. The filter paper and zinc acetate solution in the inner ring were transferred to a new tube, and 500 μl 0.2% N,N-dimethyl-p-phenylenediamine sulfate and 50 μl 10% ammonium ferric sulfate were added in turn. After 20 min, the absorbance at 670 nm was measured with a microplate reader.

Materials

TRIzol reagent were from Invitrogen (Carlsbad, CA). Chemicals and reagents, DTT (43815), NaHS (161527), GYY4137 (SML0100) were purchased from Sigma Aldrich (USA). EZ-Link HPDP-Biotin (Cat:21341) and Nuclear and Cytoplasmic Extraction Reagents (Cat: 78833, Thermo), Fluorescent indicators for Zinc (FluoZin™-3, F24195, Invitrogen, USA). BeaverBeads™ Streptavidin (22305-1, Beaver Biosciences Inc.China)

Antibody: anti-SREBP1-c (193318), anti-FASN (ab22759), anti-SCD1 (ab19862), anti-ACC (ab45174), anti-CSE (ab151769, ab136604), anti-CBS (ab96252, 140600), anti-3MST (ab154514), anti-FXR1(ab129089, anti-RXR (ab125001) for western blot and IF; ab245624 for ChIP), anti-Histone 3 (ab1791) from Abcam (Waltham, MA, USA).

Reference

1. Mustafa AK, Gadalla MM, Sen N, Kim S, Mu W, Gazi SK, Barrow RK, et al. H2S signals through protein S-sulfhydration. Sci Signal 2009;2:ra72.
2. Visa N, Jordan-Pla A. ChIP and ChIP-Related Techniques: Expanding the Fields of Application and Improving ChIP Performance. Methods Mol Biol 2018;1689:1-7.
3. Li WC, Ralphs KL, Tosh D. Isolation and culture of adult mouse hepatocytes. Methods Mol Biol 2010;633:185-196.
4. Hirsch FR, Varella-Garcia M, Bunn PA, Jr., Di Maria MV, Veve R, Bremmes RM, Baron AE, et al. Epidermal growth factor receptor in non-small-cell lung carcinomas: correlation between gene copy number and protein expression and impact on prognosis. J Clin Oncol 2003;21:3798-3807.
5. Du C, Lin X, Xu W, Zheng F, Cai J, Yang J, Cui Q, et al. Sulfhydrated Sirtuin-1 Increasing Its Deacetylation Activity Is an Essential Epigenetics Mechanism of Anti-Atherogenesis by Hydrogen Sulfide. Antioxid Redox Signal 2019;30:184-197.
6. Zhou Y, Zhou B, Pache L, Chang M, Khodabakhshi AH, Tanaseichuk O, Benner C, et al. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. Nat Commun 2019;10:1523.

Expanding Figures

Figure S1

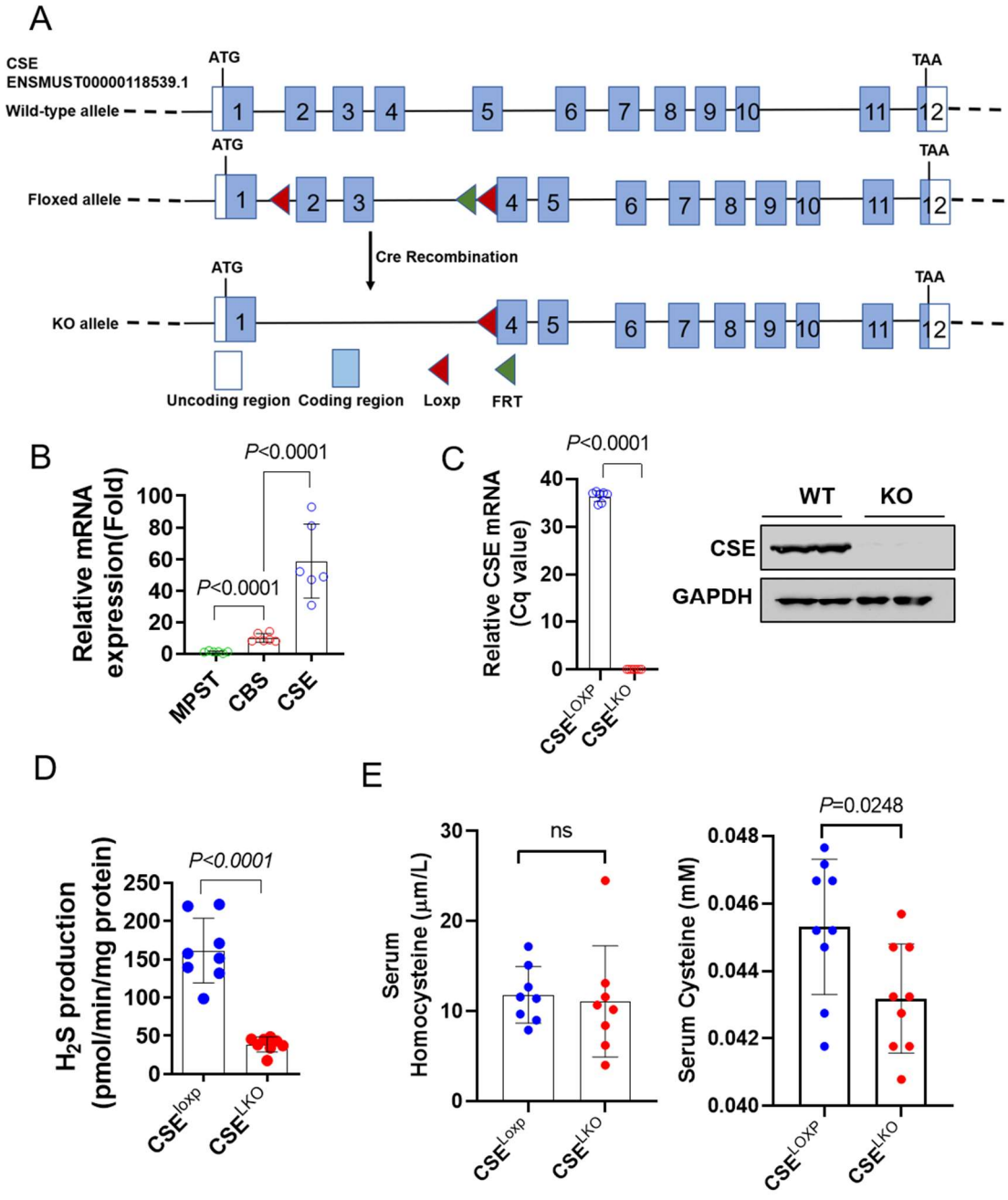

Figure S1 Conditional CSE knockout in hepatocytes protocol and identification. (A) Hepatocyte specific CSE deletion mouse was generated by loxp/cre system. (B) qRT-PCR analyzed relative CBS, CSE and 3MST mRNA expression in mouse liver tissues. (C) CSE protein expression in the kidney and liver of CSE^{LKO} mice. (D) Changes of hepatic ratio in CSE^{LKO} mice. (E) Serum total homocysteine and L-cysteine level between CSE^{Lxop} and CSE^{LKO} mice.

Figure S2

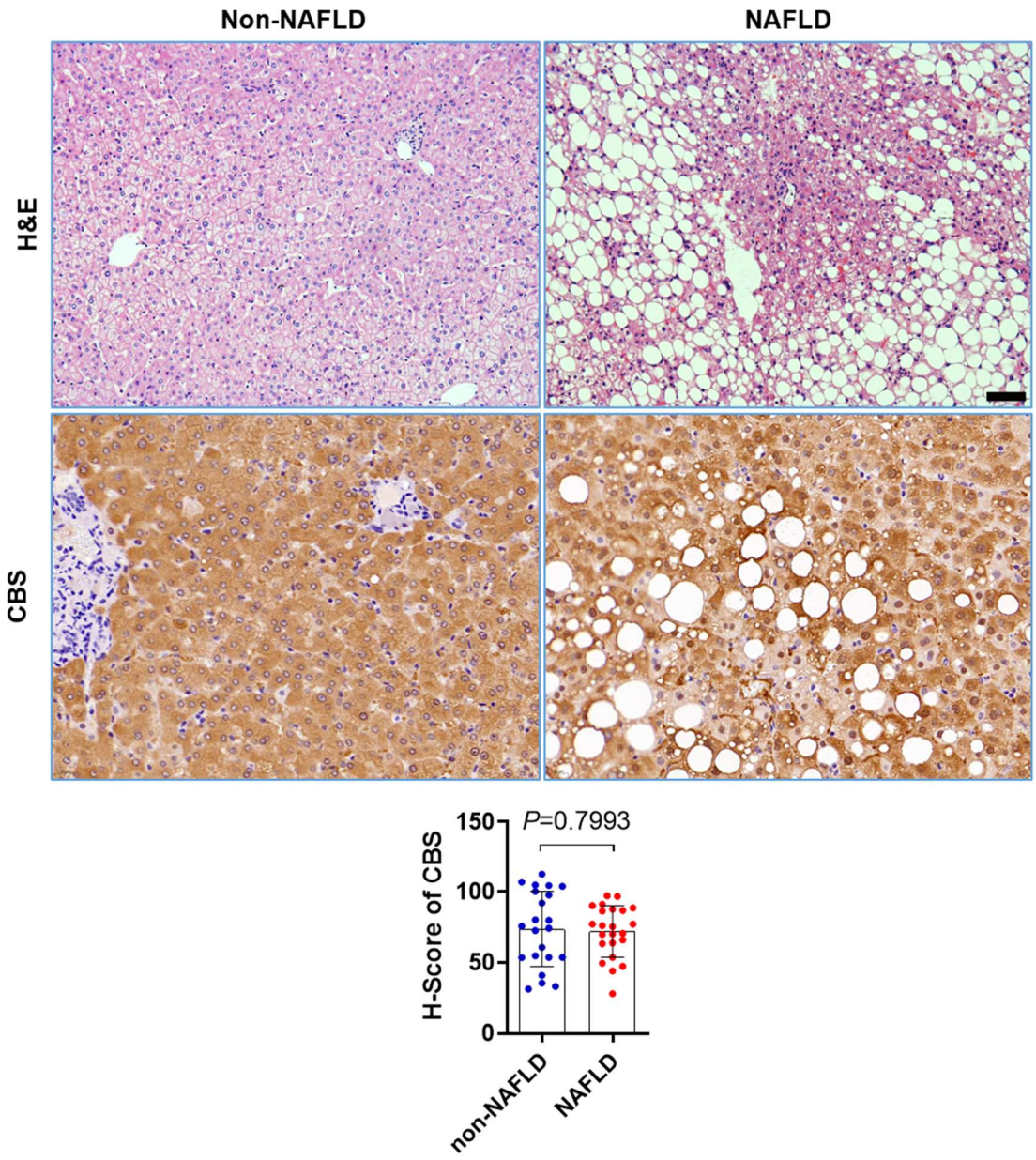

Figure S2 H&E staining and CBS immunohistochemistry staining in NAFLD patient's liver tissues. The CBS relative expression was analyzed and counted by H-score method.

Figure S3

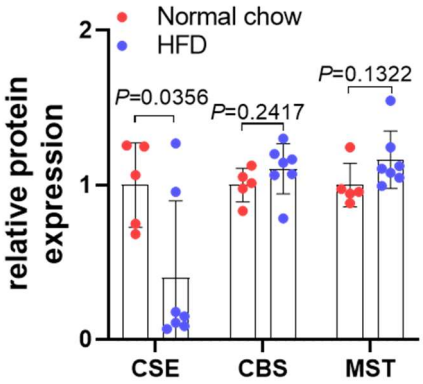

Figure S3 Semi-quantitative analysis of CSE, CBS and 3-MST protein expression in the liver of HFD-induced NAFLD mice.

Figure S4

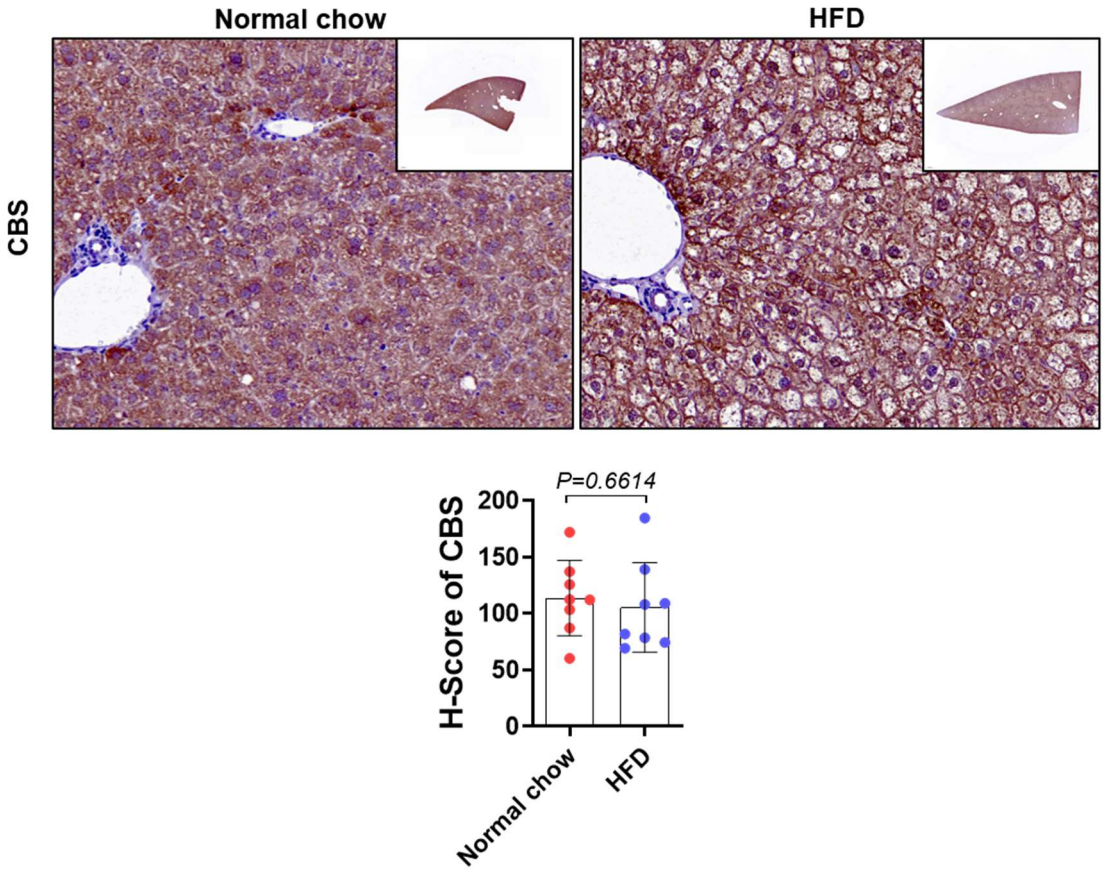

Figure S4 CBS immunohistochemistry staining in the liver of HFD-induced NAFLD mice, and then the relative expression was evaluated by H-score.

Figure S5

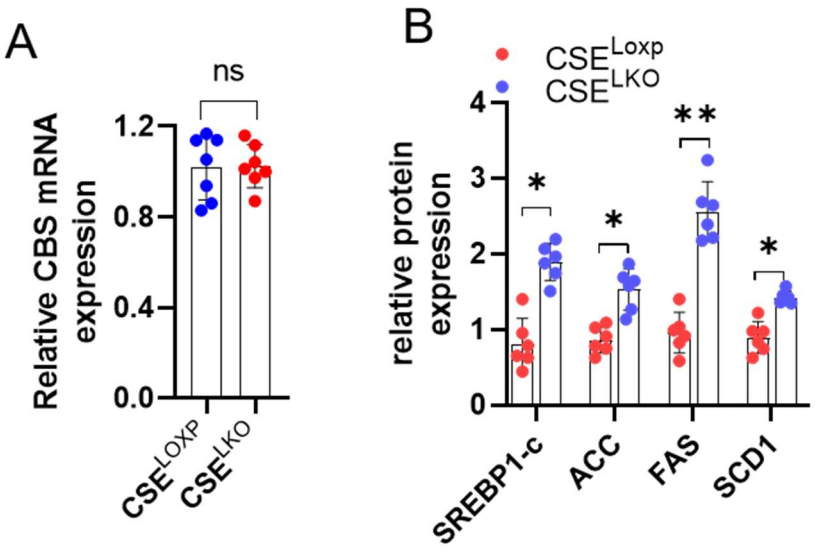

Figure S5 Relative genes expression in CSE^{LKO} liver. (A) qRT-PCR analyzed CBS mRNA expression in hepatocyte deletion CSE. (B) Statistic analysis of SREBP1-c, ACC, FAS, and SCD protein expression. * $P<0.05$, ** $P<0.01$.

Figure S6

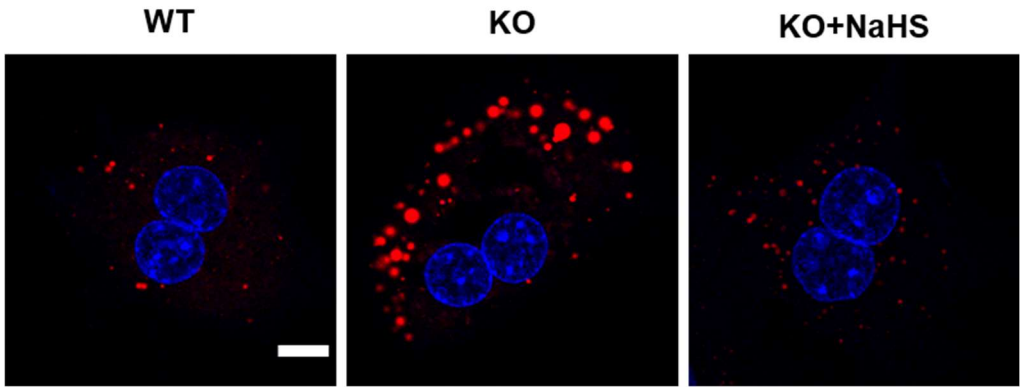

Figure S6 LipidTox staining for lipid deposition in primary hepatocyte of wild type mice, CSE knockout mice, and CSE KO cells treatment with NaSH. Bar=10 μm.

Figure S7

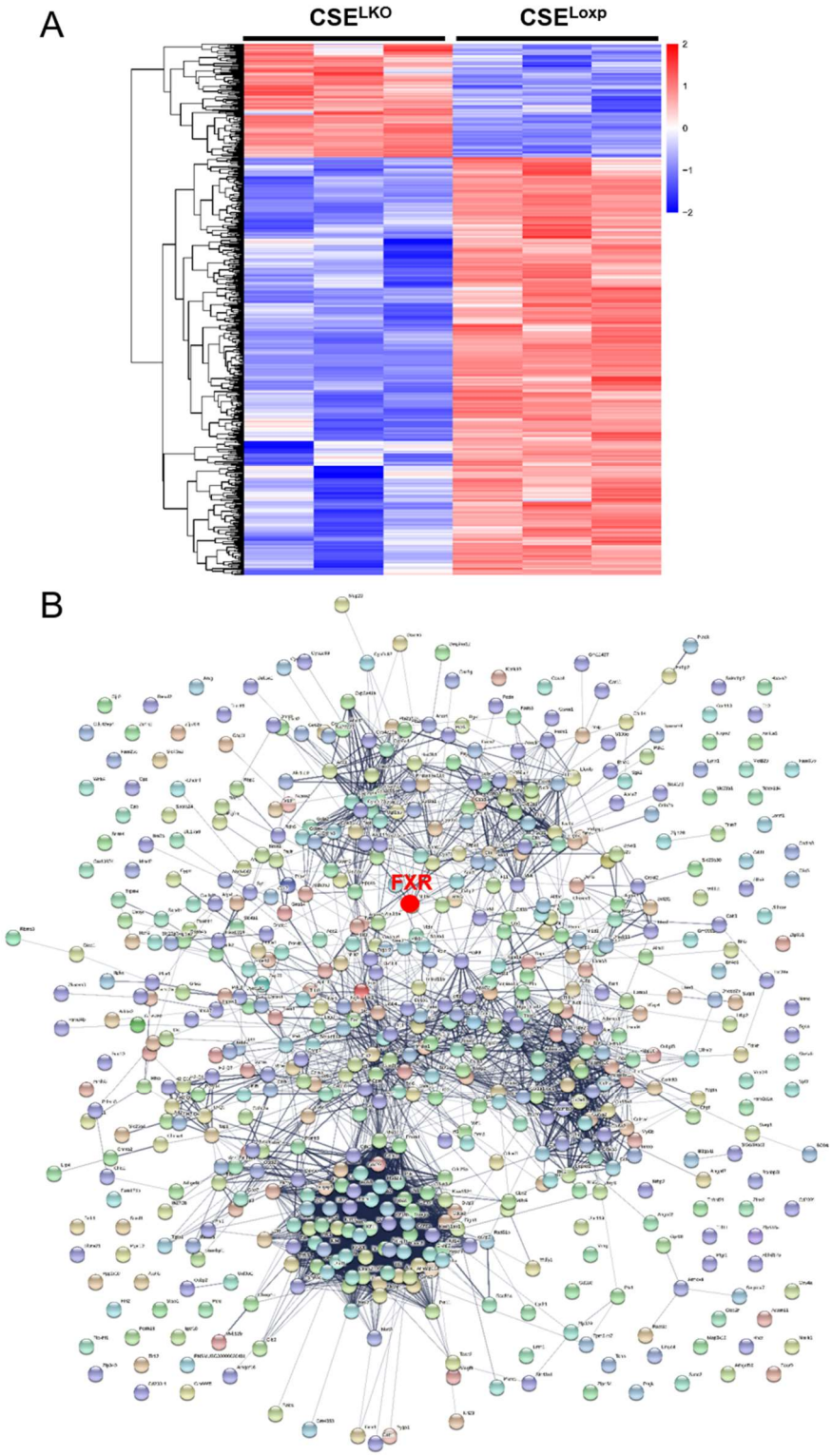

Figure S7 RNA-seq analysis for liver of CSE^{LKO} mice. (A) Heatmap showed the changes genes in the liver of CSE^{LKO} mice. (B) STRING function protein association network analysis of these changes genes.

Figure S8

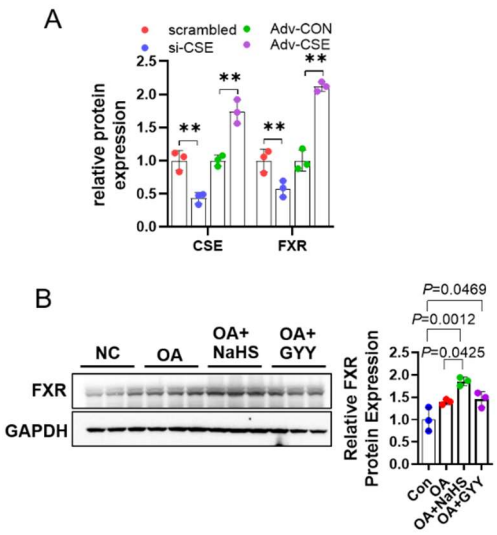

Figure S8 FXR protein expression analysis. (A) Semi-quantitative analysis of CSE and FXR protein expression while knockdown or overexpress CSE in HepG2 cells. ** P<0.01. (B) oleic acid (200 μM) with or without H_2S donor-NaHS and GYY4137, treated primary mouse hepatocytes for 24 h, FXR protein expression was measured by western blot.

Figure S9

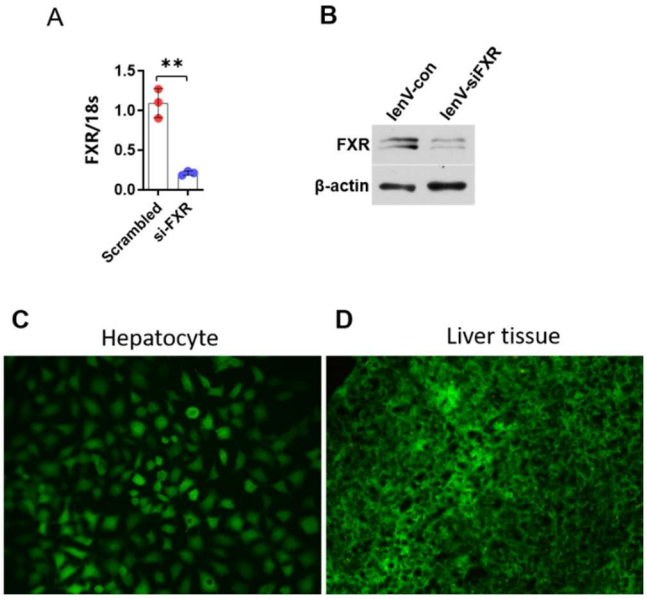

Figure S9 Identification of knockdown FXR. (A) Confirmation siRNA sequence by measurement FXR mRNA expression by siRNA transfection. ** P<0.01. (B) Lentivirus dependent FXR knockdown effect on FXR protein. (C) Control GFP lentivirus transfection into primary mouse hepatocytes and then MOI were counted. (D) 10 MOI lentivirus injection, liver GFP fluorescence was acquired to confirm the transfection.

Figure S10

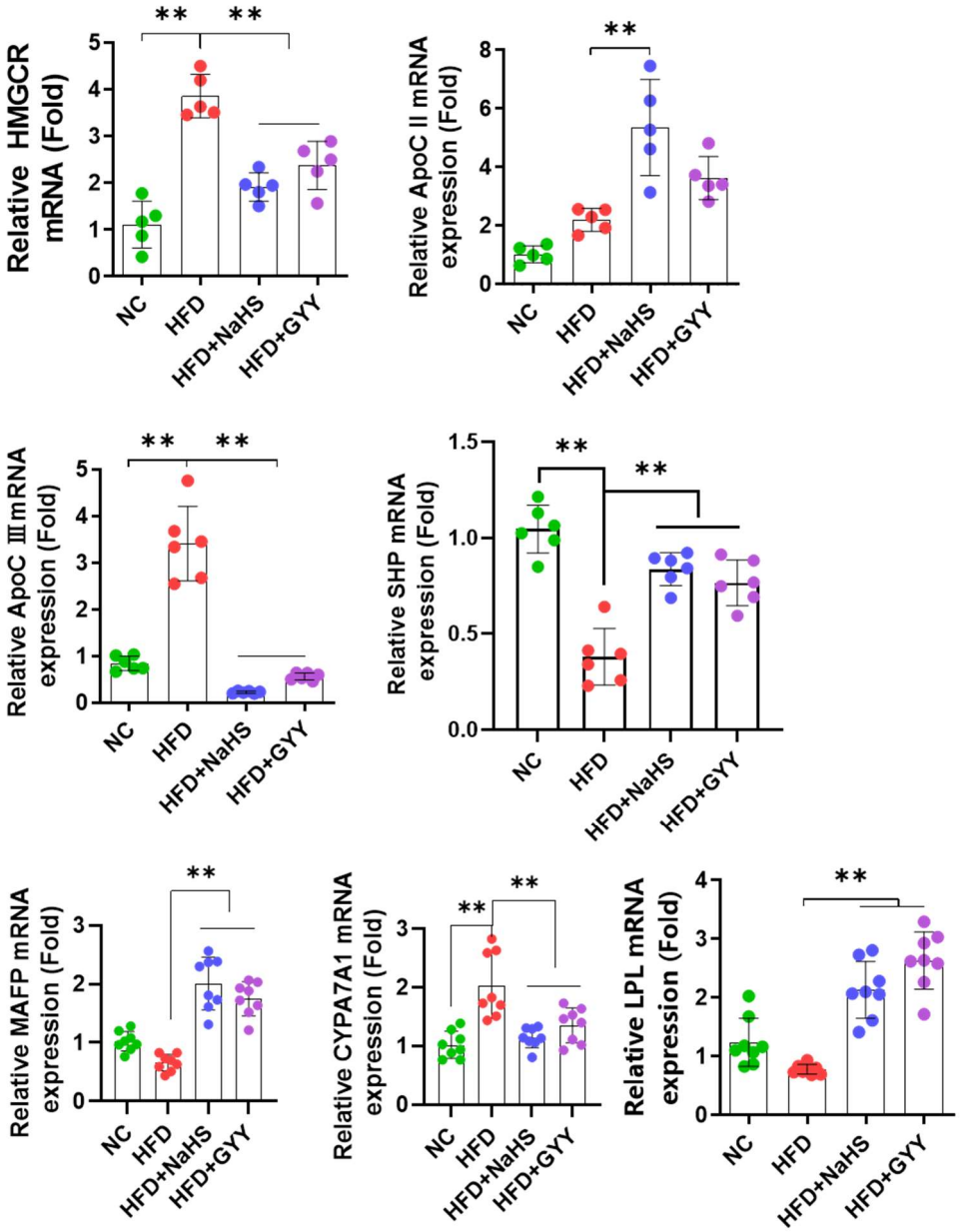

Figure S10 FXR target genes: HMGCR, ApoCII, ApoCIII, SHP, MAFG, CYP7A1, and LPL mRNA expression in liver tissues after H_2S donor's treatment. ** P<0.01

Figure S11

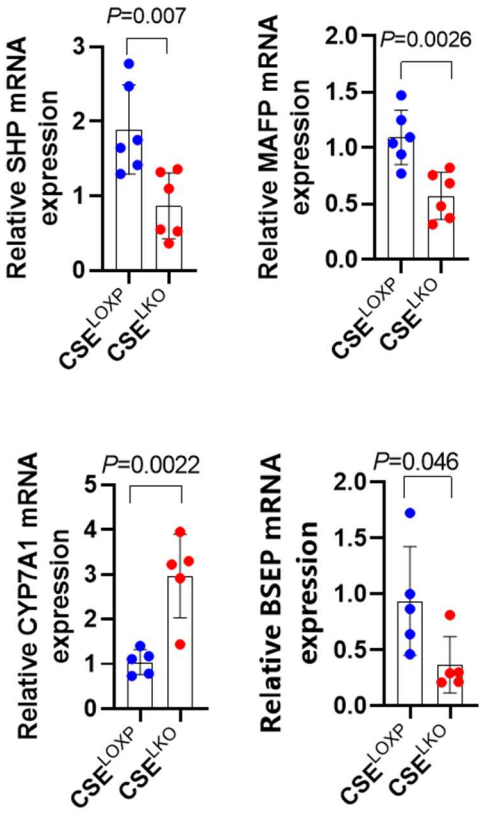

Figure S11 FXR target genes: SHP, MAFG, CYP7A1, and BSEP mRNA expression in CSE^{LKO} mice.

Figure S12

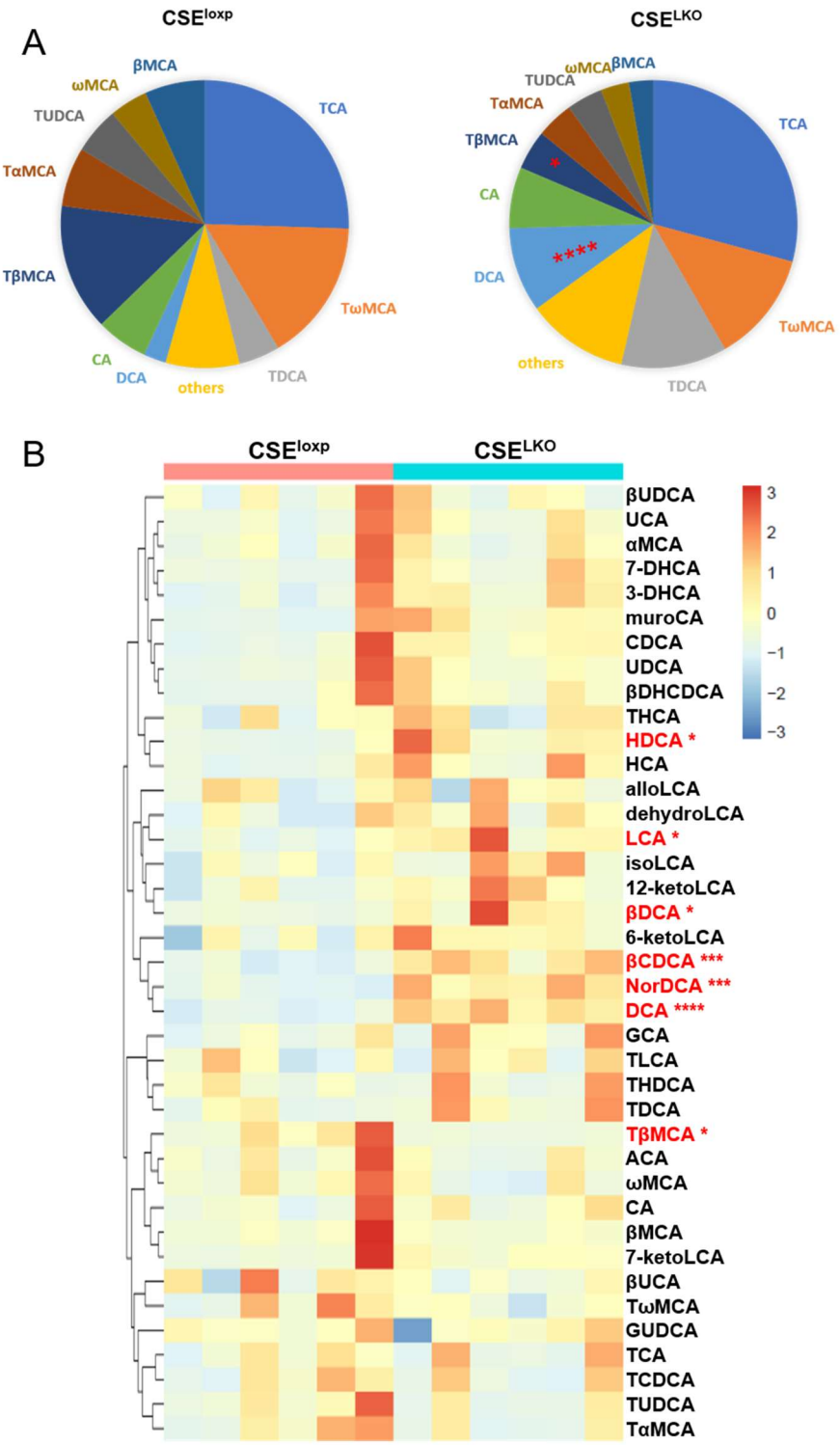

Figure S12 Serum bile acid compositions changes. (A) Serum major bile acid compositions ratio between CSE^{LOXP} and CSE^{LKO} mice. (B) Heatmap showed the serum bile acid compositions. * P<0.05, *** P<0.001, **** P<0.0001 versus CSE^{LOXP}

Figure S13

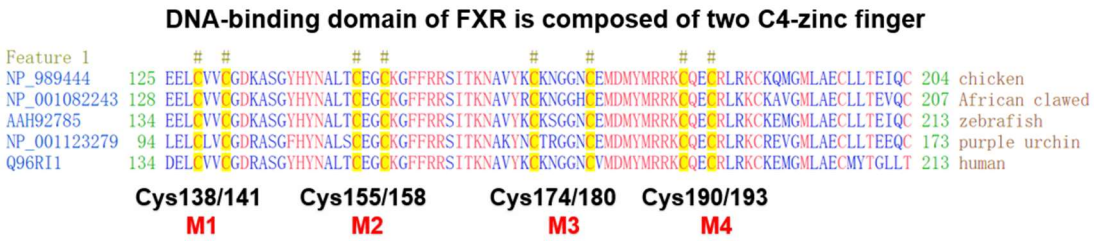

Figure S13 Schematic diagram showed the mutation C4 zinc finger sites of FXR.

Figure S14

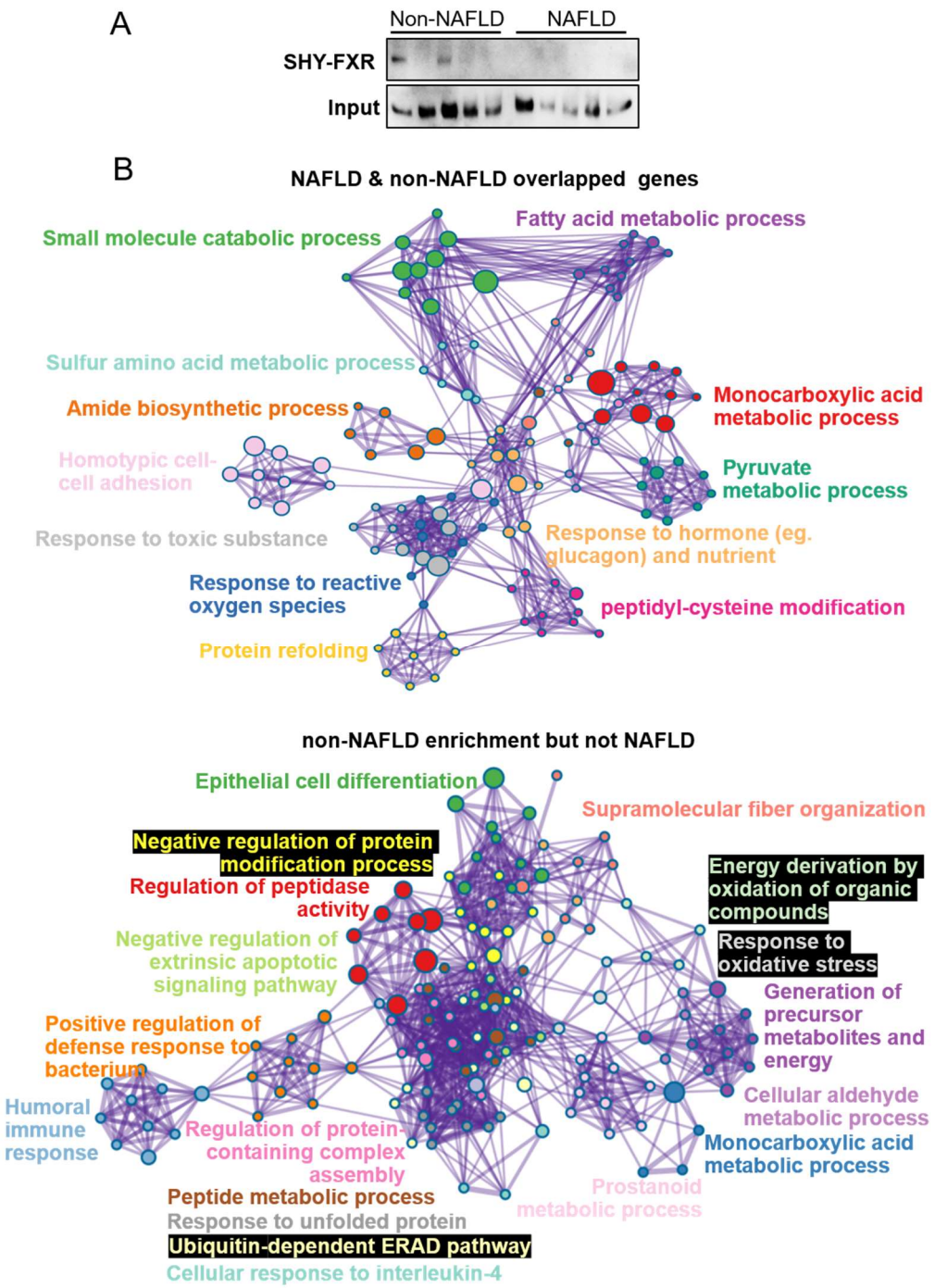

Figure S14 Sulfhydation protein changes in patient's liver tissues. (A) Sulfhydration FXR in non-NAFLD and NAFLD patient's liver tissues. (B) Sulfhydration protein of patients enriched gene GO pathway network by Metascape analysis.

Figure S15

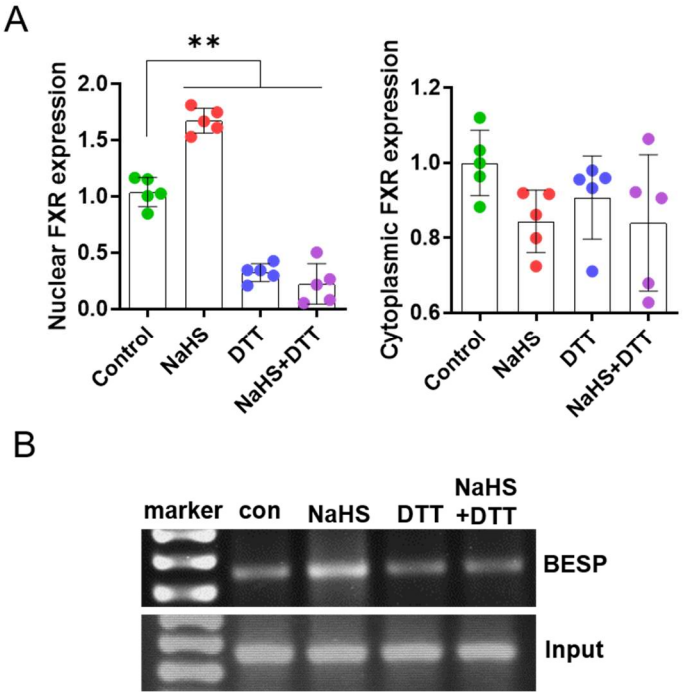

Figure S15 FXR sulfhydration determinates FXR activity. (A) Statistic analysis of FXR nuclear translocation whilst sulfhydration or removing sulfhydration. ** $P<0.01$. (B) ChIP-qPCR end production of FXR binding BSEP promoter was showed by gel resolution.

Figure S16

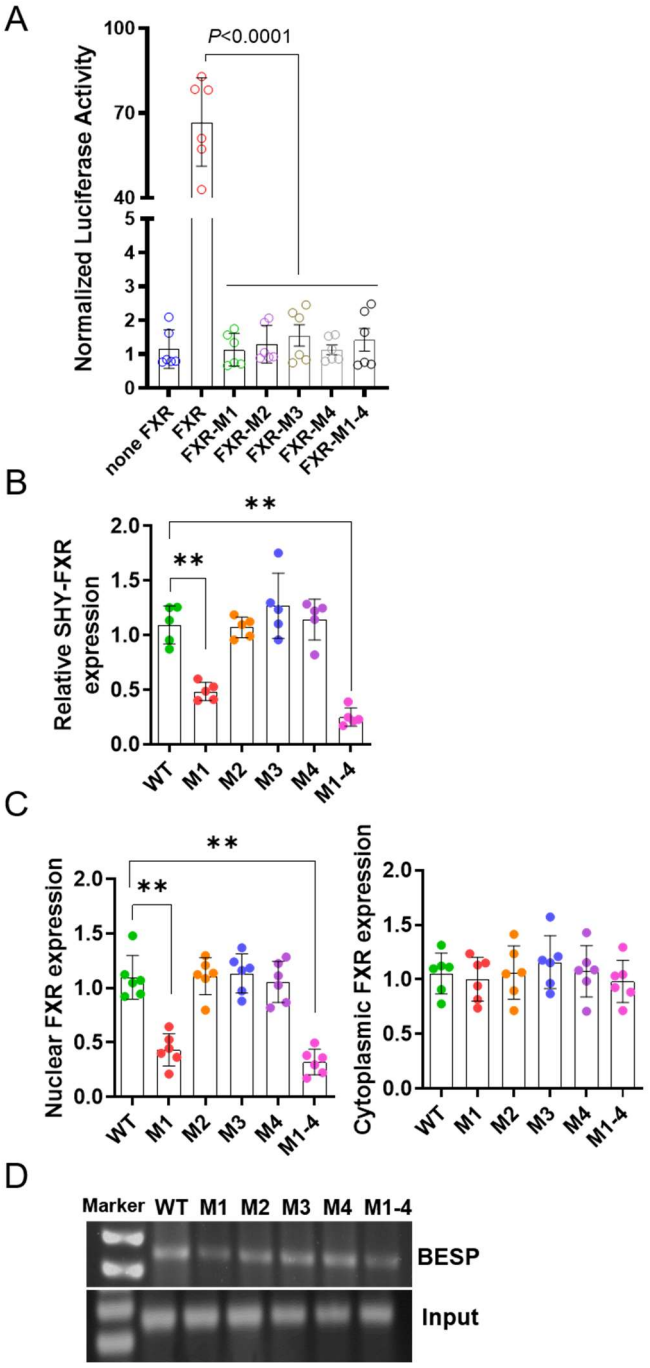

Figure S16 Mutation C4 zinc finger domain on FXR sulfhydration and its nuclear translocation. (A) Co-transfection FXR or mutated FXR, RXR and containing BSEP promoter luciferase report gene plasmids into 293-HEK cells, NaHS treatment for 12 h, the FXR activity was detected by dual luciferase reporter gene assay. (B) Semi-quantitative analysis of FXR sulfhydration (SYH-FXR) after mutation zinc finger sites. (C) Statistic analysis of FXR nuclear translocation while different mutation of FXR. ** P<0.01. (D) ChIP-qPCR end production of FXR binding BSEP promoter was showed by gel resolution.

Figure S17

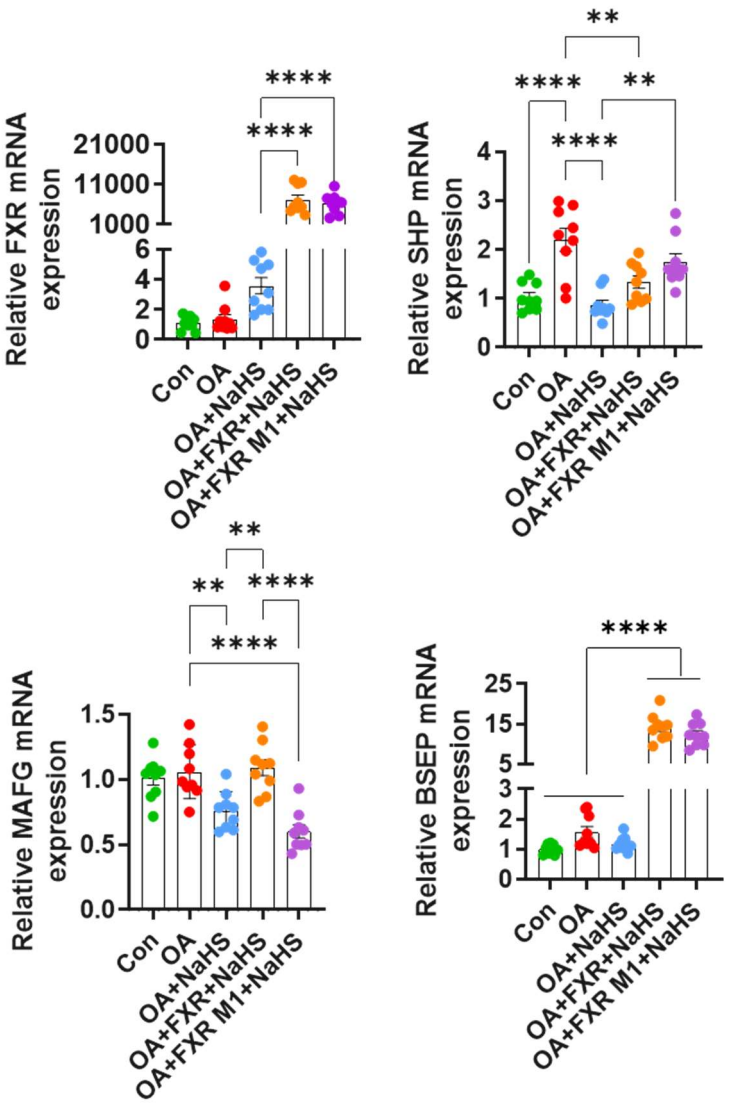

Figure S17 Mutation FXR at Cys138/141 sites, H_2S activated-FXR function activity assay by analysis of target gene: SHP, MAFG and BSEP mRNA expression. ** P<0.01, **** P<0.0001

Figure S18

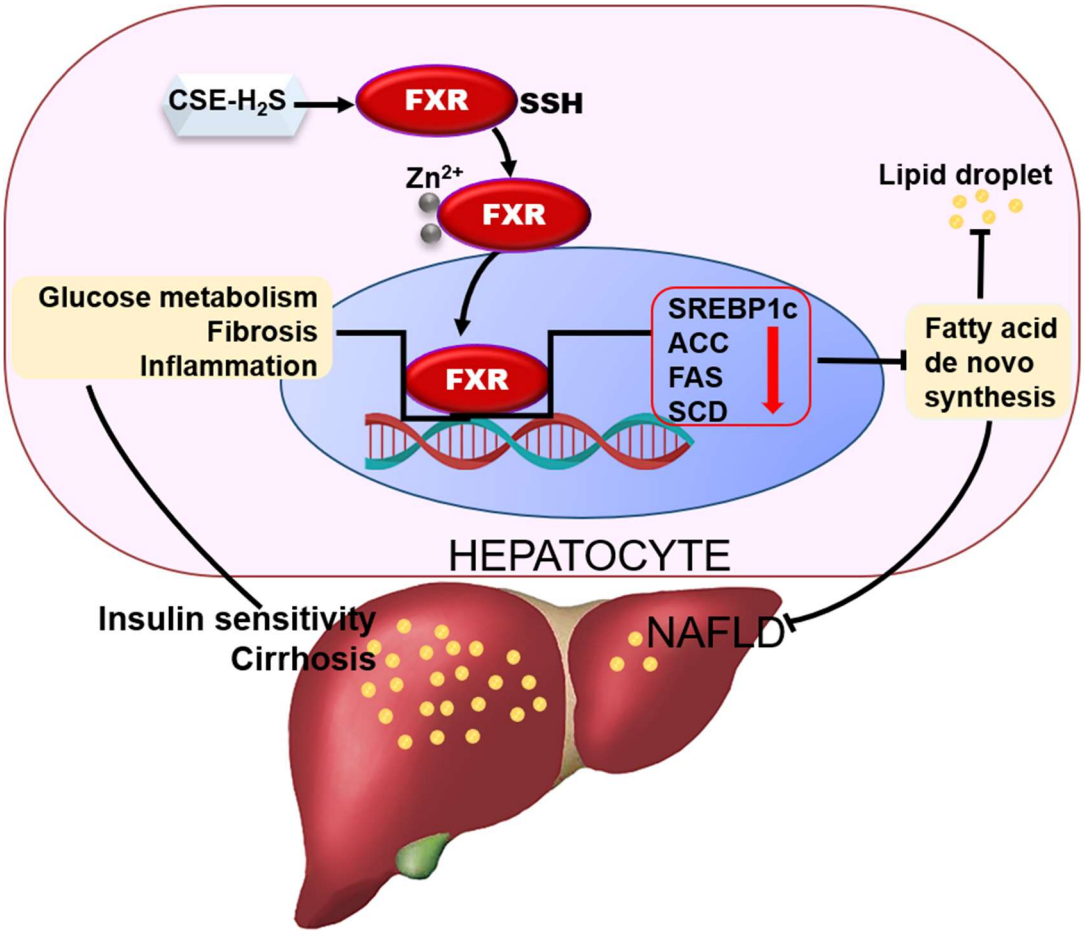

Figure S18 Schematic diagram of present findings. Hepatocytic endogenous CSE/H_2S sulfhydrates FXR at Cys138/141 sites, promotes FXR activity; on the one hand, inhibits fatty acid de novo synthesis related genes transcription, resulting in reduction of NAFLD; the other, modulates lipid and glucose metabolism, fibrosis and inflammation correlated gene's transcription, attenuating insulin resistance and subsequent liver cirrhosis.

Expanding tables

Table S1 The basal clinical characteristics of patients

	Non-NAFLD	NAFLD	P values
Sex (F/M)	8/14	9/14	0.9999
Age	56.45±14.55	49.65±13.82	0.1151
BMI	24.38±5.01	34.14±10.81	0.0004
GLU	6.02±2.68	6.37±2.12	0.6273
TC	4.02±1.23	5.01±1.18	0.0059
TG	1.23±0.47	2.18±1.57	0.0088
HDL	0.90±0.28	0.92±0.25	0.7901
LDL	2.32±0.79	2.88±0.81	0.0209
TBil	10.98±6.63	13.52±8.12	0.2574
DBil	4.71±2.63	4.83±3.20	0.8902
TP	64.43±8.80	70.83±7.42	0.0115
ALB	35.51±7.04	40.68±6.02	0.0112
GLOB	28.92±4.60	30.15±4.18	0.3528
ALT	41.32±35.45	73.74±53.53	0.0216
AST	38.23±27.87	55.00±37.10	0.0947
ALP	114.70±70.71	82.00±25.81	0.0493
GGT	96.30±116.40	68.00±64.87	0.3303
BUN	4.99±1.90	5.84±4.02	0.3721
SCR	63.23±21.99	68.87±18.79	0.3592
eGFR	104.63±10.52	105.54±21.59	0.7968
UA	255.60±113.20	373.50±124.10	0.0018

BMI: body mass index; GLU: glucose; TC: total cholesterol; TG: triglyceride; HDL: high density lipoprotein cholesterol; LDL: low density lipoprotein cholesterol; TBil: total bilirubin; DBil: direct bilirubin; TP: total protein; ALB: albumin; GLOB: globulin; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; GGT: gamma-glutamyltransferase; BUN: blood urea nitrogen; SCR: serum creatinine; eGFR: estimated glomerular filtration rate; UA: uric acid.

Table S2 The pathological characteristics of patients

	steatosis	Ballooning degeneration	lobule hepatitis	Classification of fibrosis
Non-NAFLD1	0	0	1	1
Non-NAFLD2	0	0	1	1
Non-NAFLD3	0	0	1	1
Non-NAFLD4	0	0	1	1
Non-NAFLD5	0	0	1	1
Non-NAFLD6	0	0	3	3
Non-NAFLD7	0	0	3	2
Non-NAFLD8	0	0	2	2
Non-NAFLD9	0	0	2	2
Non-NAFLD10	0	0	1	3
Non-NAFLD11	0	0	1	2
Non-NAFLD12	0	0	2	2
Non-NAFLD13	0	0	2	1
Non-NAFLD14	0	0	2	2
Non-NAFLD15	0	0	2	2
Non-NAFLD16	0	0	2	2
Non-NAFLD17	0	0	1	3
Non-NAFLD18	0	0	1	1
Non-NAFLD19	0	0	1	2
Non-NAFLD20	0	0	2	1
Non-NAFLD21	0	0	1	2
Non-NAFLD22	0	0	1	2
NAFLD1	1	0	1	1
NAFLD2	1	0	1	1
NAFLD3	1	0	2	2
NAFLD4	3	1	1	2
NAFLD5	3	1	2	1
NAFLD6	3	1	2	1
NAFLD7	1	0	1	3
NAFLD8	1	0	1	2
NAFLD9	3	1	2	2
NAFLD10	2	0	1	2
NAFLD11	1	0	2	2
NAFLD12	3	1	2	1
NAFLD13	3	1	1	1
NAFLD14	2	1	3	4
NAFLD15	3	1	1	2
NAFLD16	3	1	2	1

NAFLD17	1	1	1	2
NAFLD18	2	1	1	1
NAFLD19	3	1	2	1
NAFLD20	2	1	3	3
NAFLD21	1	1	2	1
NAFLD22	3	1	3	2
NAFLD23	2	1	3	3

Table S3 the primer sequence

Gene	Forward	Reverse
mCSE	GCAATGGAATTCTCGTGCCG	GCAGCCACTGCTTTTTCCAA
mCBS	TGTGAAGATGGCTCTGCTGG	GGCCCTTCTCCAGTTGTCTC
mMST	GGAGAAGAGCCCAGAGGAGA	ACGTCTGACTTGCCACAGAG
mSREBP1-c	TGACCCGGCTATTCCGTGA	CTGGGCTGAGCAATACAGTTC
mBSEP	TCTGACTCAGTGATTCTTCGCA	CCCATAAACATCAGCCAGTTGT
mACC	CAGGTGCCCTCAACAAAGTC	GGCATGTATGACAGCCAGTG
mFAS	CTAACTACGGCTTCGCCAAC	CCATCGCTTCCAGGACAATG
mSCD	GTTCGTTAGCACCTTCTTGCG	TATCCATAGAGATGCGCGGC
mPPARγ	ACCACTCGCATTCCTTT	CACAGACTCGGCACTCA
mLXRα	GCAGGACCAGCTCCAAGTAG	GGCTCACCAGCTTCATTAGC
mDGAT1	TTCCGCCTCTGGGCATT	AGAATCGGCCCACAATCCA
mDGAT2	AGGCCCTATTTGGCTACGTT	GATGCCTCCAGACATCAGGT
mPPARα	GAGAAGTTGCAGGAGGGGATTGTG	AAGACTACCTGCTACCGAAATGGG
mCPT1	ACAGTGGGACATTCCAGGAG	AGGAATGCAGGTCCACATCA
mMCD	ACTTCTTCTCCCACTGCTCC	TCCTTCACAATGCCCTGGAT
mLCAD	AGGAA GGATG GAGGA AGGAA	TGGAA CCAGA TGGGA AAGAG
mUCP	GCCTCTGGAAAGGGACTTCT	GAAGGCAGAAGTGAAGTGGC
mCD36	ATGGGCTGTGATCGGAACTG	CAGCCAGGACTGCACCAATA
mPPARδ	ACATCGAGACACTGTGGCAG	ATGTTCTTGGCGAACTCGGT
mSREBP2	GAGACCATGGAGACCCTCAC	AAAGTGCAATCCATGGCTCC
mFXR	AGATGGGGATGTTGGCTGAA	TGGTTGTGGAGGTCACTTGT
mHMGCR	AGTGGGAACTATTGCACCGA	ACACCTCTCTCACCACCTTG
mApoC II	GGAAATGAGGTCCAGGGGAA	CCTTGGCAGAGGTCCAGTAA
mApoC III	CACAGAAGGCTTGGGACTCA	GGACTCCTGCACGCTACTTA
mCYP7A1	GGCATCTCAAGCAAACACCA	GCCAGCACTCTGTAATGCTC
mLPL	AGCTGGGCCTAACTTTGAGT	CAGGTGACCCCCTGGTAAAT
m18S	CGCTAGAGGTGAAATTCTTG	GGAACTACGACGGTATCTGA
hSREBP1-c	TGACCGACATCGAAGGTGAA	AAAGTGCAATCCATGGCTCC
hACC	GATGAACCATCTCCCTTGGC	AGGTCCAACTTCACCAGGTT
hFAS	TGAGAGAAGGGGGCTTTCTG	AGAAGGACTTCTTCAGGCCC
hSCD	CTTGCGATATGCTGTGGTGC	CCGGGGGCTAATGTTCTTGT
hFXR	TCAAAGGGGATGAGCTGTGT	TGCCCCCGTTTTTACACTTG
hHMGCR	CTTGCCCGAATTGTGTGTGG	ACAAGATGTCCTGCTGCCAA
hCYP7A1	AATCGCTGAGGCTTTCCAGT	AACCGTCCTCAAGGTGCAAA
hSHP	GTGCCCAGCATACTCAAGAAG	TGGGGTCTGTCTGGCAGTT
hMAFG	GTGGACAGGAAGCAGCTCA	TATTGGGGGTCGTCATAACC

m: mouse; h: human.