

# SREBP2 links ACSS2-dependent acetyl-CoA biosynthesis to metaboloepigenetic activation of hepatic stellate cells and liver fibrosis

Yu Wang<sup>a,1</sup>, Aoqi Kang<sup>a,1</sup>, Shunjie Wang<sup>a</sup>, Zhen Yang<sup>b</sup>, Hong Liu<sup>a</sup>, Linbo Yue<sup>a</sup>, Hao Li<sup>a</sup>, Caiyi Wu<sup>c</sup>, Wenjing Ren<sup>d,e</sup>, Xiulian Miao<sup>f</sup>, Zilong Li<sup>d,e,\*</sup>, Yong Xu<sup>a,f,\*\*</sup>, Zhiwen Fan<sup>g,\*\*\*</sup>

<sup>a</sup> State Key Laboratory of Natural Medicines, Department of Pharmacology, China Pharmaceutical University, Nanjing, China

<sup>b</sup> Graduate School, Dalian Medical University, Dalian, China

<sup>c</sup> Department of Geriatric Nephrology, Jiangsu Province People's Hospital, the First Affiliated Hospital of Nanjing Medical University, Nanjing, China

<sup>d</sup> Department of Endocrinology, Central Hospital Affiliated to Shandong First Medical University, Jinan, China

<sup>e</sup> Institute of Brain Science and Brain-inspired Research, Shandong First Medical University & Shandong Academy of Medical Sciences, Jinan, China

<sup>f</sup> Institute of Biomedical Research and College of Agriculture and Biology, Liaocheng University, Liaocheng, China

<sup>g</sup> Department of Pathology, Nanjing Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School, Nanjing, China

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## ABSTRACT

Aberrant liver fibrosis is frequently observed in and contributes to the pathogenesis of end-stage liver diseases including cirrhosis and hepatocellular carcinoma. Hepatic stellate cells (HSCs) trans-differentiate into ECM-producing myofibroblasts to mediate liver fibrosis. In the present study we investigated the role of sterol response element binding protein 2 (SREBP2) in this process focusing on epigenetic mechanism and translational potential. We report that HSC-specific deletion or myofibroblast-restricted SREBP2 depletion attenuated liver fibrosis in mice. Integrated transcriptomic analysis combining RNA-seq and CUT&Tag-seq identified acyl-coenzyme A synthetase short-chain family member 2 (ACSS2) as a novel SREBP2 target; SREBP2 directly bound to the ACSS2 promoter to activate ACSS2 transcription. Over-expression of ectopic ACSS2 partially rescued the deficiency of HSC-myofibroblast transition when SREBP2 was depleted. In contrast, ACSS2 deletion in HSCs or myofibroblasts ameliorated liver fibrosis in mice. Mechanistically, SREBP2-dependent ACSS2 trans-activation stimulated histone H3K9/H3K27 acetylation necessary for the expression of pro-fibrogenic genes in HSCs. Importantly, a small-molecule ACSS2 inhibitor (ACSS2i) mitigated liver fibrosis in mice. Finally, relevance of the SREBP2-ACSS2 axis was validated in biopsy specimens from patients with chronic liver disease. In conclusion, our data uncover a previously unappreciated role for SREBP2 in HSC activation and provide proof-of-concept for targeting ACSS2 in the intervention of liver fibrosis.

## 1. Introduction

Chronic liver disease (CLD) represents a major health burden with a mortality rate comparable to other forms of chronic diseases (e.g., metabolic disease, respiratory disease, and cardiovascular disease) [1]. Grossly under-diagnosed, CLD affects over 1.5 billion individuals worldwide with approximately 2 million premature deaths annually [2]. CLD prevalence is projected to increase in the next decade largely due to

an aging global population and ongoing epidemics of obesity and diabetes [3–5]. CLD is pathologically characterized by persistent liver parenchymal injury, low-magnitude chronic inflammation, and aberrant liver fibrosis [6]. Widely perceived as host defense mechanism, liver fibrosis, when promptly initiated and timely terminated, serves to safeguard the liver from injuries. However, persistent injurious stimuli, as often associated with CLD, lead to dysregulation of liver fibrosis that ultimately disrupts normal hepatic anatomy and interferes with liver

\* Corresponding author at: Shandong First Medical University, Jinan 250117, China.

\*\* Correspondence to: Y. Xu, China Pharmaceutical University, Nanjing, 211198, China.

\*\*\* Correspondence to: Z. Fan, Nanjing Drum Tower Hospital, Nanjing, 210009, China.

E-mail addresses: [zlli@sdfmu.edu.cn](mailto:zlli@sdfmu.edu.cn) (Z. Li), [yjxu@cpu.edu.cn](mailto:yjxu@cpu.edu.cn) (Y. Xu).

<sup>1</sup> These authors contributed equally to this work.

function accelerating CLD pathogenesis. No curative pharmacotherapy is currently available for liver fibrosis.

Regardless of etiology, myofibroblasts, a pool of specialized cells that are highly migratory, proliferative, contractile, and synthetic (*i.e.*, capable of producing extracellular matrix proteins), represent the *bona fide* mediator of liver fibrosis [7]. The transient nature of myofibroblasts, rapid emergence following liver injury and taciturn exit when liver fibrosis is resolved, fueled the interest of those who work in the field to define their origin(s). It was proposed, over the course of several decades, that epithelial cells, endothelial cells, and myeloid cells, might give rise to the ECM-producing myofibroblasts in liver fibrosis [8]. The discovery by the Schwabe laboratory that hepatic stellate cells (HSCs), the lipid-laden cells tucked between the liver parenchyme and the liver sinusoid under physiological conditions, constitute the predominant, if not exclusive, source from which mature myofibroblasts are derived marks an important milestone [9]. In the same pioneering study it has also been demonstrated that HSCs can be faithfully labeled with and reliably traced by lecithin retinol acyltransferase (*Lrat*); the *Lrat*-Cre driver has since become an essential genetic tool for the investigation of liver fibrosis. When quiescent HSCs trans-differentiate into myofibroblasts simultaneous transcriptomic and metabolic reprogramming has been documented [10]. These two processes are intimately inter-entangled: expression levels of metabolic enzymes are often altered at the transcriptional level whereas metabolites contribute to transcriptional regulation. For instance, a recent study by Rho *et al* has shown that hexokinase 2 (HK2) orchestrates dynamic histone lactylation by catalyzing lactate biosynthesis to promote HSC-myofibroblast transition and liver fibrosis [11].

Sterol regulatory element binding protein (SREBP) family of transcription factors, consisting of SREBP1a, SREBP1c, and SREBP2, are considered master regulator of *de novo* lipogenesis [12]. Although it is abundantly clear that SREBP proteins can potentially contribute to CLD by accelerating lipid synthesis in hepatocytes, whether they are able to regulate HSC activation and hence liver fibrosis remains inconclusive. For instance, global deletion of SREBP1c has been shown to protect the mice from CCl<sub>4</sub>-induced liver fibrosis that can be attributed to down-regulation of lipocalin-2 in hepatocytes [13]. In contrast, hepatocyte-specific deletion of SCAP, a molecular chaperone required for SREBP activation, exacerbates liver fibrosis in PTEN-null mice, which could be rescued by SREBP1a over-expression [14]. Tomika *et al* have previously proposed that SREBP2-dependent cholesterol synthesis sensitizes HSCs to TGF- $\beta$  indicating that SREBP2 might play a pro-fibrogenic role in the liver [15]. According to Kong *et al*, however, SREBP2 knockdown in primary murine HSCs up-regulates the expression of myofibroblast markers [16]. It should be noted that none of these studies have provided compelling *in vivo* evidence that links an HSC-autonomous SREBP role to liver fibrosis. Of interest, Zhao *et al* have recently reported that myofibroblast-specific SREBP2 depletion attenuates myocardial fibrosis in animal models of ischemic and non-ischemic cardiomyopathy [17]. Intrigued by this observation, we asked whether HSC/myofibroblast-restricted manipulation of SREBP2 would influence liver fibrosis. Our data as presented here suggest that HSC-autonomous SREBP2 promotes liver fibrosis by enabling acetyl-CoA biosynthesis.

## 2. Methods

### 2.1. Animals

All animal protocols were reviewed and approved by the intramural China Pharmaceutical University Committee on Ethical Treatment of Experimental Animals (IACUC-2109023). Liver fibrosis was induced in 6–8 week-old male mice as previously described [18] using one of the two following protocols: 1) the mice were subjected to bile duct ligation (BDL) or the sham procedure for 2 weeks; 2) the mice were injected with carbon tetrachloride (CCl<sub>4</sub>, 1.0 ml/kg body weight as 50% vol/vol) or corn oil thrice a week for 4 weeks. *Srebp2*<sup>f/f</sup> mice were generated by

inserting LoxP sites to flank exon 2 and exon 3 of the *Srebp2* gene. *Acss2*<sup>f/f</sup> mice were generated by inserting LoxP sites to flank exon 3 and exon 7 of the *Acss2* gene. HSC conditional SREBP2 and ACSS2 knockout mice were generated by crossing the *Srebp2*<sup>f/f</sup> mice and the *Acss2*<sup>f/f</sup> mice, respectively, to the *Lrat*-Cre mice as previously described [19,20]. Recombinant adeno-associated virus 6 (AAV6) [21] carrying shRNA targeting SREBP2 (CUGGUACGCGUUGUACUCATT) or ACSS2 (UGAUGUGACUAAAGGGAAATT) downstream of the *Postn* promoter [22] was injected into C57BL/6 J mice intravenously (*i.v.*) at a dose of  $1 \times 10^{11}$  vg. In certain experiments, the mice were injected with a small-molecule ACSS2 inhibitor (ACSS2i, TargetMol, 2711039–08-4) peritoneally at a dose of 5 mg/kg every other day.

### 2.2. Cell culture

Human immortalized hepatic stellate cells (LX-2, ATCC) were maintained in DMEM supplemented with 2% FBS. Human primary HSCs (aHSCs) were purchased from Lonza (Cat#: HUCLS-200 K). Primary hepatic stellate cells were isolated and maintained as previously described [23]. Small interfering RNAs were purchased from Dharmacon. ACSS2 promoter-luciferase construct was generated by amplifying genomic DNA spanning the proximal promoter and the first exon of ACSS2 gene (–2000/+120) and ligating into a pGL4-basic vector (Promega). Mutagenesis was performed using a QuikChange kit (Thermo Fisher Scientific, Waltham, MA, United States) and verified by direct sequencing. Transient transfections were performed with Lipofectamine 2000. Luciferase activities were assayed 24–48 h after transfection using a luciferase reporter assay system (Promega) as previously described [24].

### 2.3. RNA isolation and Real-time PCR

RNA was extracted with the RNeasy RNA isolation kit (Qiagen). Reverse transcriptase reactions were performed using a SuperScript First-strand Synthesis System (Invitrogen). Real-time PCR reactions were performed on an ABI Prism 7500 system with the primers listed in supplementary Table 1. Ct values of target genes were normalized to the Ct values of housekeeping control gene (18 s, 5'-CGCGGTTCTATTTTGTGGT-3' and 5'-TCGTCTTCGAACTCCGACT-3' for both human and mouse genes) using the  $\Delta\Delta$ Ct method and expressed as relative mRNA expression levels compared to the control group which is arbitrarily set as 1. All experiments were performed in triplicate wells and repeated three times. One representative experiment was shown in the figures.

### 2.4. Protein extraction and Western blot

Whole cell lysates were obtained by re-suspending cell pellets in RIPA buffer (50 mM Tris pH 7.4, 150 mM NaCl, 1% Triton X-100) with freshly added protease inhibitor (Cat#: 11873580001, Roche) as previously described [25–27]. Antibodies used for Western blotting are listed in the supplementary Table 2.

### 2.5. Statistical analysis

One-way ANOVA with post-hoc Scheffé analyses were performed by SPSS software (IBM SPSS v18.0, Chicago, IL, USA). Unless otherwise specified, values of  $p < 0.05$  were considered statistically significant. The assumptions of normality were checked using Shapiro-Wilks test and equal variance was checked using Levene's test; both were satisfied.

## 3. Results

### 3.1. HSC-specific SREBP2 ablation attenuates liver fibrosis

In LX-2 cells, SREBP2 silencing by small interfering RNA (siRNA)

abolished TGF- $\beta$  induced HSC activation as evidenced by down-regulation of myofibroblast markers, decreased cell proliferation, weakened cell migration, and dampened cell contraction (Fig.S1). Similar observations were made in primary human HSCs (aHSCs, Fig. S2).

In order to define an HSC-specific and autonomous role for SREBP2 in liver fibrosis, the *Srebp2*<sup>f/f</sup> mice were cross-bred with the *Lrat*-Cre to generate a new strain harboring HSC conditional SREBP2 deletion (*Srebp2* <sup>$\Delta$ HSC</sup>). When both the *Srebp2* <sup>$\Delta$ HSC</sup> mice and the control mice were given CCL<sub>4</sub> injection to induce liver fibrosis (Fig. 1A), it was discovered that restricted SREBP2 deletion in HSCs apparently did not influence liver injury because plasma ALT (Fig. 1B) and AST (Fig. 1C) levels were comparable in these mice. Histopathological staining of paraffin sections with PicroSirius Red (PSR) and Masson's Trichrome (MT) revealed a significant reduction in ECM deposition in the *Srebp2* <sup>$\Delta$ HSC</sup> livers compared to the control livers (Fig. 1D) suggesting that SREBP2 might be essential for HSC activation *in vivo*. The essentiality of HSC-derived SREBP2 in liver fibrosis was further verified by hydroxyproline quantification (Fig. 1E) and qPCR measurements of myofibroblast markers in the liver (Fig. 1F). In a second model in which the mice were subjected to the BDL procedure to induce liver fibrosis, it was similarly revealed that HSC-derived SREBP2 was required for liver fibrosis but not for liver injury (Fig.S3).

To determine whether myofibroblast specific SREBP2 depletion would achieve similar effects as described above, C57BL/6 J mice were injected with CCL<sub>4</sub> to induce liver fibrosis followed by intervention via AAV6-mediated delivery of shRNA targeting SREBP2 (AAV-shSrebp2, Fig. 1G). The AAV6 system is known to specific target hepatic myofibroblasts [21]. Indeed, qPCR (Fig.S4) confirmed that SREBP2 was depleted in HSCs but left intact in other hepatic lineages in mice injected with AAV-shSrebp2 compared to those injected with a control vector (AAV-shC). Post-injury SREBP2 depletion in myofibroblasts did not significantly alter plasma ALT (Fig. 1H) or AST (Fig. 1I) levels. H&E staining showed that liver injury was comparable in the AAV-shSrebp2 mice and the AAV-shC mice whereas PSR/MT staining clearly demonstrated that liver fibrosis was less extensive in the AAV-shSrebp2 mice than the AAV-shC mice (Fig. 1J). Hydroxyproline quantification (Fig. 1K) and qPCR measurements of myofibroblast expression levels (Fig. 1L) provided corroborating evidence that SREBP2 silencing in myofibroblasts led to amelioration of liver fibrosis in mice. These observations were replicated in the second model of liver fibrosis induced by BDL surgery (Fig.S5). Taken together, these data suggest that SREBP2 might contribute to liver fibrosis by enabling the acquisition and maintenance of myofibroblast phenotype.

### 3.2. Integrated transcriptomic analysis identifies novel SREBP2 targets in HSCs

In order to explore the mechanism whereby SREBP2 might regulate HSC activation, the following strategy was exploited. When RNA-seq was performed to compare the transcriptome of LX-2 cells with or without SREBP2 depletion, it was discovered that SREBP2 knockdown markedly influenced gene expression (Fig. 2A). Overall, more than 3000 genes were differentially expressed in the absence of SREBP2 (Fig. 2B). GO analysis of the differentially expression genes indicated that these genes were mostly involved in regulating cell behaviors including migration, proliferation, ECM synthesis, and response to external stimulation (Fig. 2C). KEGG analysis confirmed that these genes were predominantly represented by classic pro-fibrogenic signaling pathways (Fig. 2D). Next, genomewide SREBP2 binding patterns in activated HSCs were evaluated by CUT&Tag-seq; as shown in Fig. 2E, strong and specific signals were detected by the anti-SREBP2 antibody. Approximately 25% of the SREBP2 peaks were localized to the promoter regions (Fig. 2F). GO analysis (Fig. 2G) and KEGG (Fig. 2H) suggested that although genes involved in steroid biosynthesis, the best characterized functionality for SREBP2, was indeed enriched genes that regulate cell

behaviors relevant to HSC activation and fibrogenesis were predominantly represented.

In order to identify direct SREBP2 targets that might mediate its pro-fibrogenic effects a number of screening criteria were introduced for the integrated analysis: for any gene to be considered as a *bona fide* pro-fibrogenic SREBP2 target, it must be abundantly expressed in activated HSCs (mean RPKM>50), its expression must be significantly down-regulated by SREBP2 depletion (3x fold change,  $p < 0.05$ ), and its promoter must be occupied by SREBP2. Overall, 35 genes were found to have met these criteria (Fig. 2I, top 10 ranked by  $p$  values shown). We focused on acyl-coenzyme A synthetase short-chain family member 2 (ACSS2) for the remainder of this study because (1) ACSS2 ranked near the top of the list of potential SREBP2 targets and (2) being an enzyme ACSS2 can be potentially manipulated with small-molecule inhibitors and thus would be relatively easier for clinical translation (Fig. 2J).

### 3.3. SREBP2 activates ACSS2 transcription to promote HSC activation

The following experiments were performed to further authenticate ACSS2 as an SREBP2 target and the relevance to HSC activation. ACSS2 expression was up-regulated in LX-2 cells by TGF- $\beta$  treatment but down-regulated by SREBP2 silencing (Fig. 3A, B). SREBP2 knockdown also down-regulated ACSS2 expression in primary human HSCs (Fig. 3C, D). In addition, primary HSCs isolated from the mice displayed lower ACSS2 expression than those from the WT mice (Fig. 3E, F). Lower ACSS2 expression was also detected in the liver tissues of the *Srebp2* <sup>$\Delta$ HSC</sup> mice than the *Srebp2*<sup>f/f</sup> mice following either CCL<sub>4</sub> injection or BDL surgery (Fig.S6). Finally, a decrease in ACSS2 expression was observed in the liver tissues from the mice injected with the AAV-shSrebp2 compared to those injected with AAV-shC following either CCL<sub>4</sub> injection or BDL surgery (Fig.S7). Of note, the effect of SREBP2 deletion on ACSS2 expression was only evident in HSCs but not in hepatocytes in both the *Srebp2* <sup>$\Delta$ HSC</sup> mice (Fig.S6) and in the AAV-shSrebp2 mice (Fig.S7) again confirming that SREBP2 might be specifically deleted in HSCs or myofibroblasts.

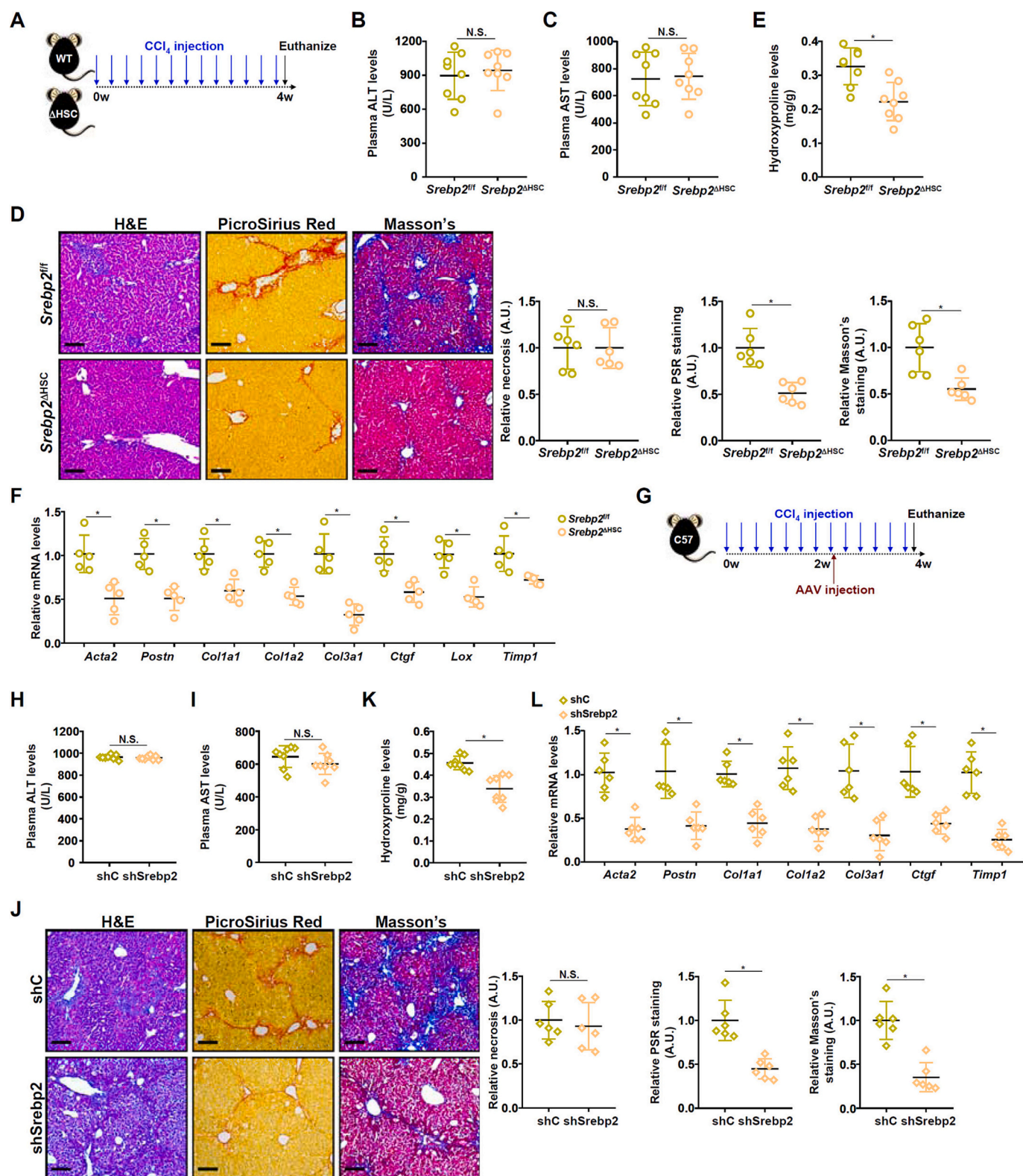
A conserved sterol regulatory element (SRE) was detected within the proximal ACSS2 promoter per CUT&Tag-seq data (Fig. 2J). When a wild type ACSS2 promoter-luciferase construct and one harboring mutated SBE were transfected into LX-2 cells it was found that TGF- $\beta$  augmented the activity of the former but not the latter indicating the SREBP2 binding might be indispensable for ACSS2 trans-activation by TGF- $\beta$  stimulation in HSCs (Fig. 3G). Indeed, ChIP assay confirmed that SREBP2 was recruited to the proximal ACSS2 promoter in response to TGF- $\beta$  treatment in LX-2 cells (Fig. 3H). SREBP2 binding to the *Acss2* promoter was also detected in activated murine HSCs compared to quiescent HSCs and in the fibrotic livers of mice compared to the normal livers (Fig.S8).

To further demonstrate the functional relevance of SREBP2-mediated ACSS2 trans-activation, SREBP2 was depleted in LX-2 cells by siRNA followed by over-expression of an ectopic ACSS2 via adenoviral transduction; ACSS2 supplementation offset SREBP2 deficiency and rescued the expression of myofibroblast markers (Fig. 3I), cell proliferation (Fig. 3J), cell migration (Fig. 3K), and cell contraction (Fig. 3L). Likewise, ACSS2 over-expression rescued the myofibroblast phenotype in primary human HSCs in the absence of SREBP2 (Fig.S9).

### 3.4. Conditional ACSS2 deletion/depletion attenuates liver fibrosis in mice

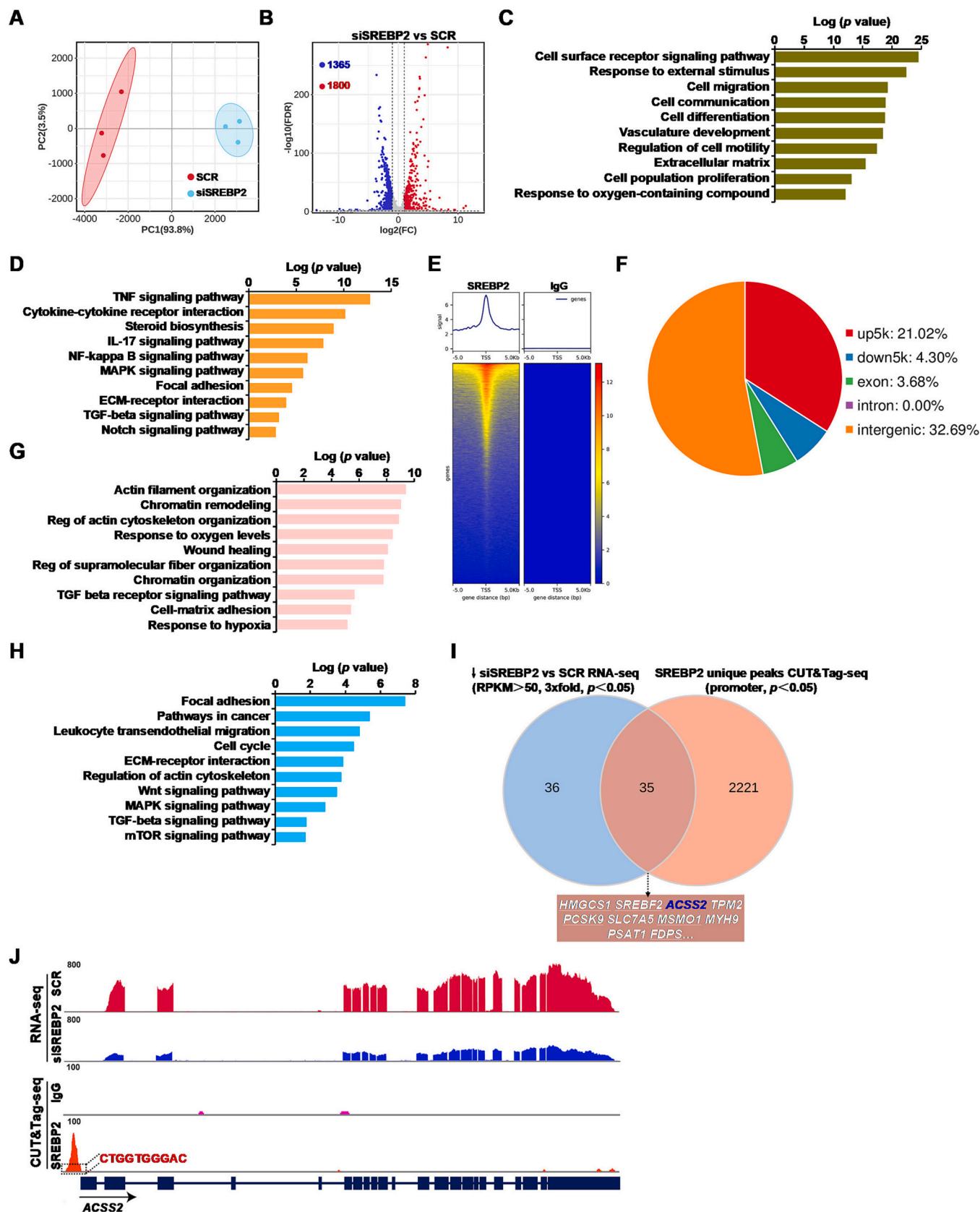
In order to affirm the regulatory role of ACSS2 in liver fibrosis, the *Acss2*<sup>f/f</sup> mice were cross-bred with the *Lrat*-Cre to achieve HSC conditional ACSS2 deletion (*Acss2* <sup>$\Delta$ HSC</sup>); both the *Acss2*<sup>f/f</sup> mice and the *Acss2* <sup>$\Delta$ HSC</sup> mice were then injected with CCL<sub>4</sub> to induce liver fibrosis (Fig. 4A). Compared to the control mice (*Acss2*<sup>f/f</sup>), the *Acss2* <sup>$\Delta$ HSC</sup> mice displayed similar levels of plasma ALT (Fig. 4B) and plasma AST (Fig. 4C) indicating that ACSS2 deficiency in HSCs likely did not



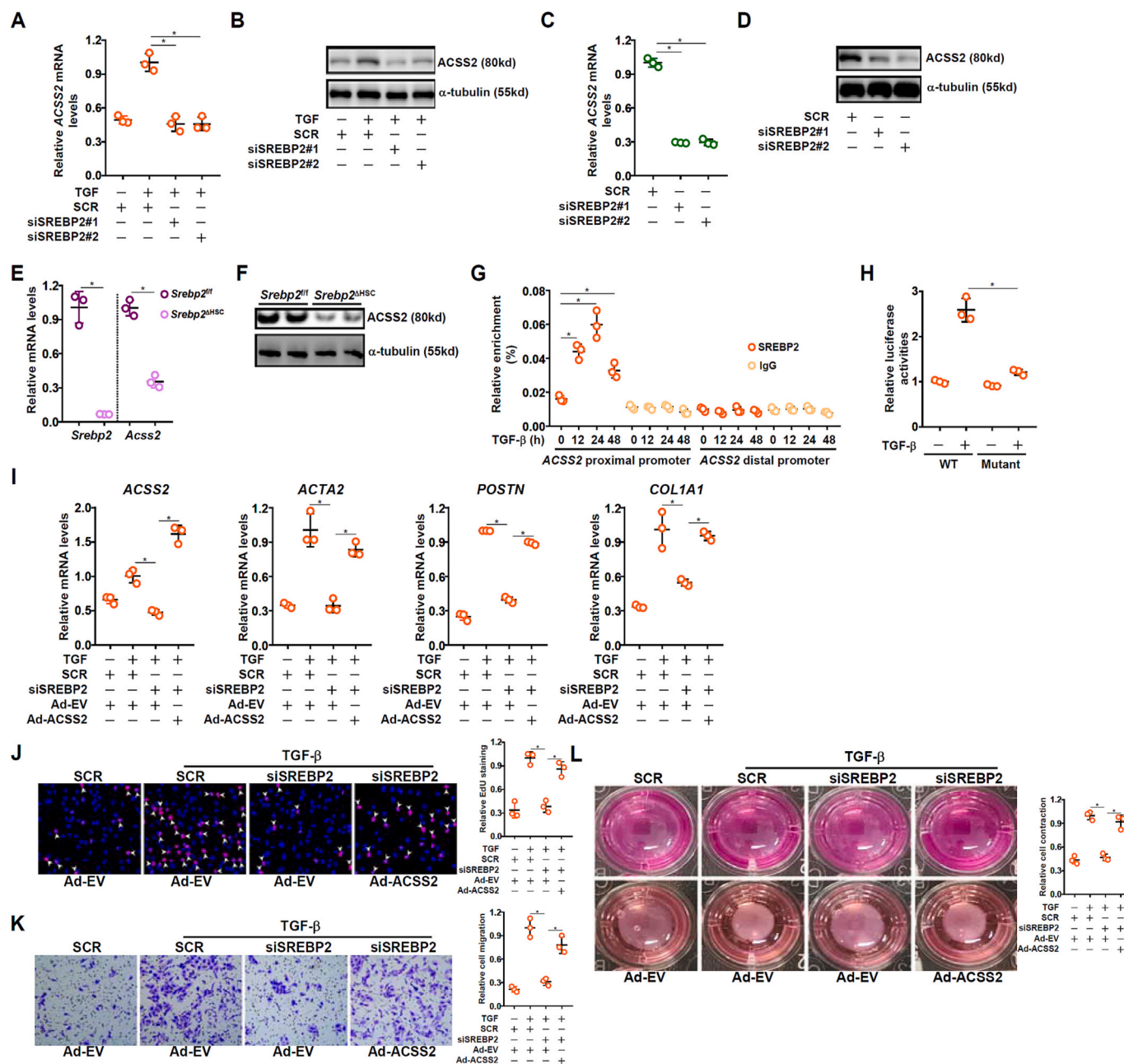


**Fig. 1.** Conditional SREBP2 deletion attenuates liver fibrosis in mice. (A–F) HSC conditional SREBP2 knockout mice and wild type mice were injected with CCl<sub>4</sub> to induce liver fibrosis. Scheme of protocol (A). Plasma ALT levels (B). Plasma AST levels (C). PSR staining and Masson's staining (D). Hydroxyproline levels (E). Myofibroblast markers were examined by qPCR (F). (G–L) C57BL/6 J mice were injected with CCl<sub>4</sub> to induce liver fibrosis followed by injection with AAV-shSrebp2. Scheme of protocol (G). Plasma ALT levels (H). Plasma AST levels (I). PSR staining and Masson's staining (J). Hydroxyproline levels (K). Myofibroblast markers were examined by qPCR (L). *N* = 6–8 mice for each group. Data are expressed as mean ± S.D. \*, *p* < 0.05, two-tailed student's test.





**Fig. 2.** Integrated transcriptomic analysis identifies novel SREBP2 targets in HSCs. (A–D) LX-2 cells were transfected with siRNA targeting SREBP2 or scrambled siRNA (SCR) followed by treatment with TGF- $\beta$  (5 ng/ml) for 24 h. RNA-seq was performed as described in Methods. PCA plot (A). Volcano plot (B). GO analysis (C). KEGG analysis (D). (E–H) LX-2 cells were treated with TGF- $\beta$  (5 ng/ml) for 24 h. CUT&Tag-seq was performed as described in Methods. Heatmap of peaks (E). Pie chart of SREBP2 unique peaks (F). GO analysis (G). KEGG analysis (H). (I) Venn diagram. Known SREBP2 targets are underlined. (J) CUT&Tag tracks of SREBP2 signals and RNA-Seq tracks of the read coverage surrounding the ACSS2 loci.

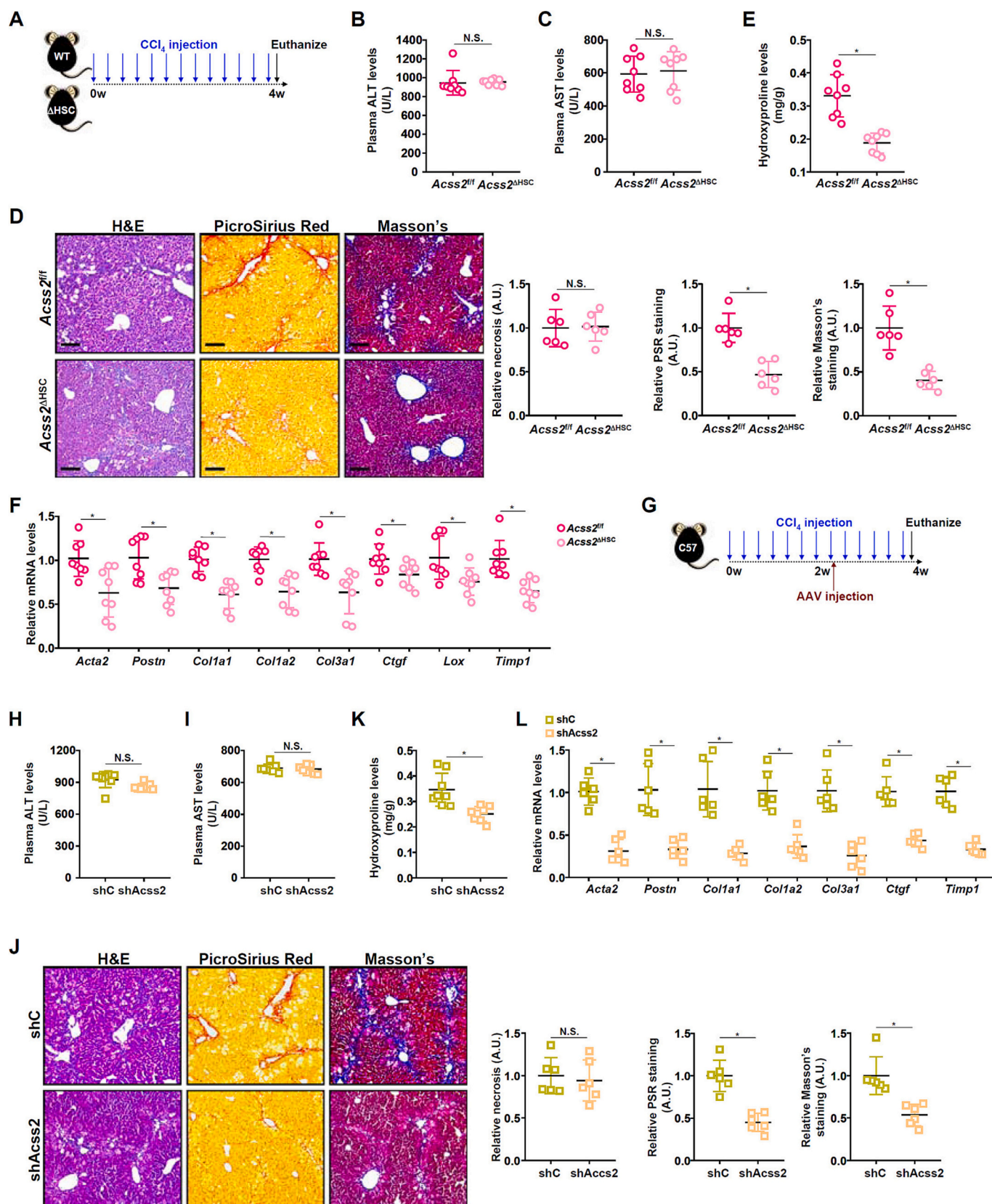


**Fig. 3.** SREBP2 activates ACSS2 transcription to promote HSC activation. (A, B) LX-2 cells were transfected with siRNA targeting SREBP2 or scrambled siRNA (SCR) followed by treatment with TGF- $\beta$  (5 ng/ml) for 24 h. ACSS2 expression was examined by qPCR and Western blotting.  $N = 3$  biological replicates. Data are expressed as mean  $\pm$  S.D. \* $p$  < 0.05, one-way ANOVA with post-hoc Dunnett's test. (C, D) Primary human HSCs were transfected with siRNA targeting SREBP2 or scrambled siRNA (SCR). ACSS2 expression was examined by qPCR and Western blotting.  $N = 3$  biological replicates. Data are expressed as mean  $\pm$  S.D. \* $p$  < 0.05, one-way ANOVA with post-hoc Dunnett's test. (E, F) Primary HSCs isolated from the HSC conditional SREBP2 knockout mice and wild type mice were allowed to undergo spontaneous activation *in vitro* for 7d. ACSS2 expression was examined by qPCR and Western blotting.  $N = 3$  biological replicates. Data are expressed as mean  $\pm$  S.D. \* $p$  < 0.05, one-way ANOVA with post-hoc Scheffe's test. (G) LX-2 cells were treated with TGF- $\beta$  (5 ng/ml) and collected at indicated time points. ChIP assay was performed with anti-SREBP2 or IgG.  $N = 3$  biological replicates. Data are expressed as mean  $\pm$  S.D. \* $p$  < 0.05, one-way ANOVA with post-hoc Dunnett's test. (H) Wild type and mutant ACSS2 promoter-luciferase constructs were transfected into LX-2 cells followed by treatment with TGF- $\beta$  (5 ng/ml) for 24 h. Luciferase activities were normalized by protein concentration and GFP fluorescence.  $N = 3$  biological replicates. Data are expressed as mean  $\pm$  S.D. \* $p$  < 0.05, two-way ANOVA with post-hoc Tukey's test. (I-L) LX-2 cells were transfected with siRNA targeting SREBP2 or scrambled siRNA (SCR) and transduced with adenoviral ACSS2 followed by treatment with TGF- $\beta$  (5 ng/ml) for 24 h. Myofibroblast markers were examined by qPCR (I). Cell proliferation was examined by EdU incorporation (J). Cell migration was examined by Boyden transwell assay (K). Collagen contraction assay (L).  $N = 3$  biological replicates. Data are expressed as mean  $\pm$  S.D. \* $p$  < 0.05, one-way ANOVA with post-hoc Tukey's test.

influence liver injury. Evidently, liver fibrosis was dampened by the lack of ACSS2 expression in HSCs because histological staining (Fig. 4D) and hydroxyproline quantification (Fig. 4E) both detected fewer collagenous tissues in the liver. In addition, myofibroblast marker expression was significantly down-regulated in the *Acss2*<sup>ΔHSC</sup> livers as opposed to the

*Acss2*<sup>f/f</sup> livers (Fig. 4F). In a second model of BDL-induced liver fibrosis, similar observations were made that restricted ACSS2 deletion in HSCs markedly alleviated liver fibrosis but did not impact liver injury (Fig. S10).

In order to tackle the question as to whether ACSS2 deletion in



**Fig. 4.** Conditional ACSS2 deletion/depletion attenuates liver fibrosis in mice. (A-F) HSC conditional ACSS2 knockout mice and wild type mice were injected with CCl<sub>4</sub> to induce liver fibrosis. Scheme of protocol (A). Plasma ALT levels (B). Plasma AST levels (C). PSR staining and Masson's staining (D). Hydroxyproline levels (E). Myofibroblast markers were examined by qPCR (F). (G-L) C57BL/6 J mice were injected with CCl<sub>4</sub> to induce liver fibrosis followed by injection with AAV-Acscs2. Scheme of protocol (G). Plasma ALT levels (H). Plasma AST levels (I). PSR staining and Masson's staining (J). Hydroxyproline levels (K). Myofibroblast markers were examined by qPCR (L). *N* = 6–8 mice for each group. Data are expressed as mean ± S.D. \*, *p* < 0.05, two-tailed student's test.



myofibroblasts would affect liver fibrosis, an AAV6-based gene delivery strategy similar to the one used for SREBP2 as described earlier was employed (Fig. 4G); qPCR data confirmed that ACSS2 expression was specifically silenced in myofibroblasts (aHSCs) but not in hepatocytes (Fig.S11). ACSS2 depletion in myofibroblasts did not result in significant alteration of either plasma ALT levels (Fig. 4H) or AST (Fig. 4I) levels indicating that myofibroblast-derived ACSS2 might be dispensable for liver injury. Consistent with HSC-conditional ACSS2 deletion, ACSS2 deficiency in myofibroblasts lessened liver fibrosis as assessed by PSR staining/Masson's staining (Fig. 4J), hydroxyproline quantification (Fig. 4K), and expression of myofibroblast markers in the liver (Fig. 4L). Again, this conclusion was further supported by data collected from an alternative model of liver fibrosis induced by BDL (Fig.S12).

### 3.5. SREBP2 controls acetyl-CoA synthesis to regulate histone H3K9/H3K27 acetylation

ACSS2 is a rate-limiting enzyme for the synthesis of acetyl co-enzyme A (Ac-CoA). Naturally we hypothesized that SREBP2, via regulating ACSS2 expression, might contribute to the cellular Ac-CoA pool. Indeed, TGF- $\beta$  treatment up-regulated Ac-CoA production in LX-2 cells and primary human HSCs (aHSCs), which was suppressed by SREBP2 knockdown but rescued by ACSS2 over-expression (Fig. 5A). Similarly, the addition of acetate to the culture media also restored cellular Ac-CoA levels in LX-2 cells (Fig.S13A) and human aHSCs (Fig.S14A). Consistently, acetate addition normalized the expression of myofibroblast markers (Fig.S13B, S14B) and cellular proliferative/migratory/contractile behaviors (Fig.S13C-S13E, S14C-S14E).

Ac-CoA contributes to status of histone acetylation, a key epigenetic factor in transcriptional activation, and, by extension, cellular transcriptome. Among the lysine residues of core histones documented to be subject to acetylation H3K9 [28–30] and H3K27 [31–33] are the best characterized to demarcate actively transcribed chromatin. Therefore, we tested the hypothesis that SREBP2-dependent ACSS2 expression fuels HSC activation by rate-limiting Ac-CoA production to promote histone H3K9/H3K27 acetylation. RNA-seq showed that ACSS2 supplementation largely overcame SREBP2 deficiency and normalized cellular transcriptome (Fig. 5B, C). Previously it has been reported that ACSS2 can migrate into the nucleus and interact with transcription factors such as TFEB [34] and HIF2 [35] to coordinately regulate transcription of genes involved in autophagy/lysosomal biogenesis and adaptation to hypoxia, respectively, in a range of cancer cells. Immunofluorescence staining showed that ACSS2 was predominantly located in the cytoplasm in both quiescent and activated HSCs suggesting that the ability of ACSS2 to function as a nuclear transcriptional regulator might be cell type-dependent (Fig.S15).

Western blotting showed that global H3K9/H3K27 acetylation was increased by TGF- $\beta$  treatment, down-regulated by SREBP2 knockdown but restored by ACSS2 supplementation (Fig.S16). Importantly, CUT&Tag-seq performed with anti-H3K9Ac and anti-H3K27Ac antibodies indicated that SREBP2 knockdown significantly diminished genomewide deposition of acetylated H3K9 and acetylated H3K27, which was rescued by ACSS2 supplementation (Fig. 5D, E). GO and KEGG analyses revealed that genes sensitive to H3K9Ac status and H3K27Ac status regulated overlapping but not identical processes relevant to HSC activation (Fig. 5F). Likewise, HOMER analysis indicated that H3K9Ac-dependent genes were most likely regulated by SMAD, TEAD, PU.1, and H3K27Ac-dependent genes were primarily regulated by PBX1, TEAD, SMAD, all of which are well-established pro-fibrogenic transcription factors (Fig. 5G). An attempt to integrate the RNA-seq data and the CUT&Tag-seq data unveiled 167 genes, whose expression levels were sensitive to SREBP2 knockdown and ACSS2 supplementation and whose promoters harbored SREBP2/ACSS2-dependent H3K9Ac/H3K27Ac markers (Fig. 5H). A majority of these genes encoded proteins with a cataloged role in organ fibrosis, which included growth factors (e.g., *TGF $\beta$ 1*), transcription factors (e.g., *TEAD3*), metabolic enzymes (e.g.,

*GLS1*), and extracellular matrix components (e.g., *COL5A1*) thus providing well-buttressed mechanistic insights to explain the mode of action for the SREBP2-ACSS2 axis in regulating HSC activation and liver fibrosis (Fig. 5I).

### 3.6. ACSS2 inhibition attenuates liver fibrosis in mice

ACSS2-IN-1 (Cpmpound 1) is a small-molecule ACSS2 inhibitor (ACSS2i) that has been exploited for the intervention of cancer [36] and chronic kidney disease [37] (Fig. 6A). The translational potential of targeting ACSS2 with ACSS2i was verified in two different scenarios of liver fibrosis. In the first scenario (preventive) ACSS2i was administered shortly after liver injury (1 week after the start of CCl<sub>4</sub> injection) but before full-blown liver fibrosis (Fig. 6B). ACSS2 inhibition by ACSS2i did not significantly impact liver injury as evaluated by plasma ALT (Fig. 6C) and AST (Fig. 6D) levels. PSR staining and Masson's staining revealed a significant reduction in collagenous tissue accumulation in the livers of ACSS2i-injected mice compared to the vehicle-injected mice (Fig. 6E). Indeed, ACSS2i administration decreased hepatic hydroxyproline levels (Fig. 6F) and down-regulated expression of myofibroblast markers (Fig. 6G) in the liver clearly indicative of its effectiveness as an antifibrotic reagent. In the BDL model of liver fibrosis, it was similarly observed that ACSS2i administration immediately following the surgical procedure ameliorated liver fibrosis without affecting liver injury (Fig.S17).

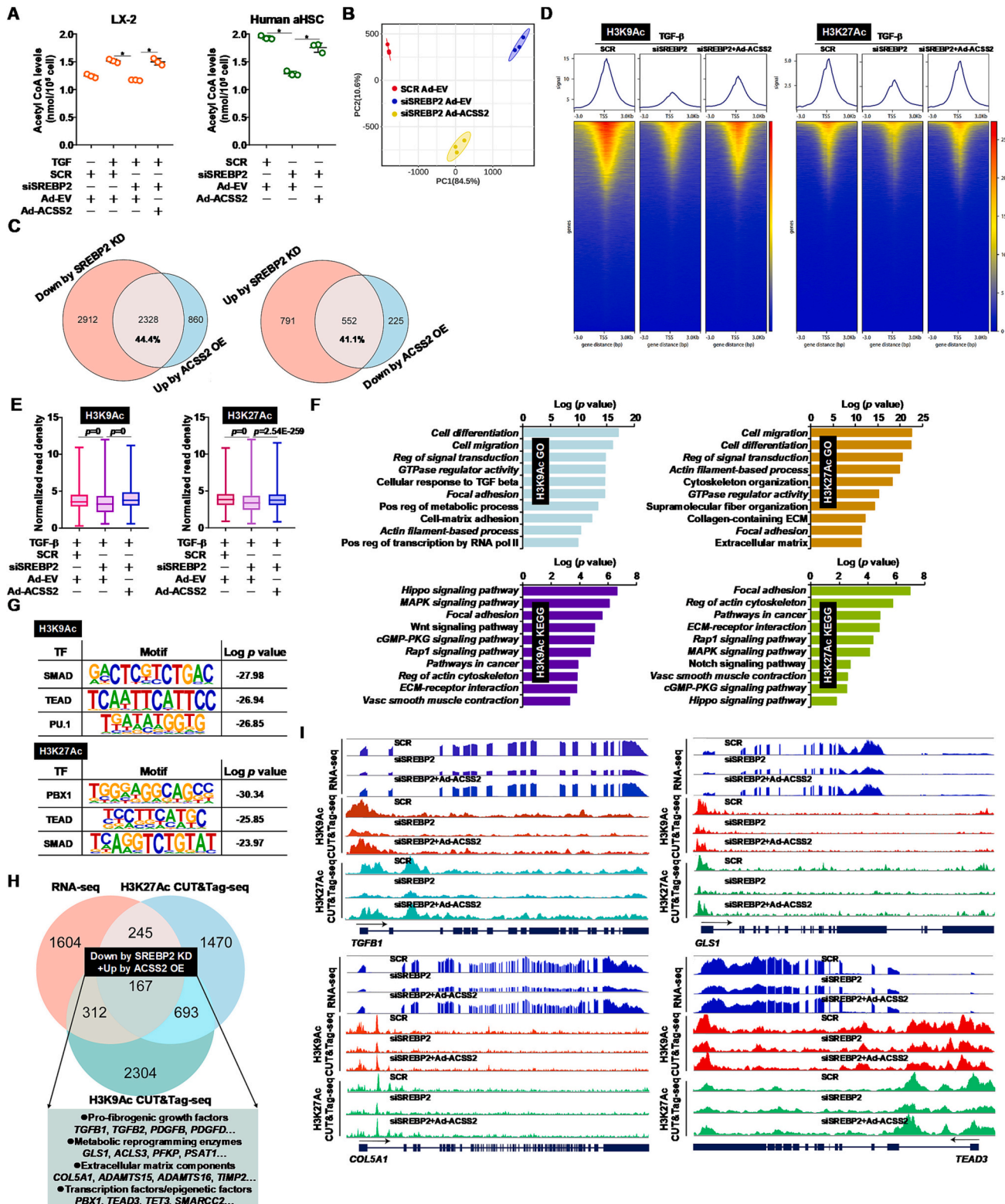
In the second scenario ACSS2i was administered to mice when liver fibrosis was pre-established (Fig. 6H, 3 weeks after the start of CCl<sub>4</sub> injection). It was observed that ACSS2 administration significantly, however slightly, mitigated liver injury as evidenced by a small reduction in both plasma ALT (Fig. 6I) and AST (Fig. 6J) levels. Liver fibrosis was attenuated by a much larger margin in mice receiving ACSS2i administration than those treated with vehicle (Fig. 6K–6M), which could not be fully accounted for the changes in liver injury. In the BDL-induced model of established liver fibrosis, ACSS2i administration at 10d post-surgery reversed liver fibrosis; ACSS2i administration led to a similar trend in liver injury, which did not reach statistical significance (Fig.S18).

### 3.7. Validation of SREBP2-ACSS2 axis in humans

Finally, the relevance of ACSS2-mediated acetyl-CoA production in CLD patients) was verified. As shown in Fig. 7A, ACSS2 levels were significantly elevated in the livers from patients diagnosed with liver fibrosis than those from healthy donors. In addition, a positive correlation between ACSS2 levels and magnitude of liver fibrosis, using myofibroblast marker (*COL1A1*) level and fibrosis grade as proxies, was identified (Fig. 7B). Similarly, ACSS2 activity was higher in CLD patients than in the controls (Fig. 7C) and appeared to correlate with severity of liver fibrosis (Fig. 7D). Finally, higher acetyl Co-A levels were detected in the livers from CLD patients than from the controls (Fig. 7E) and able to predict (more severe) liver fibrosis (Fig. 7F). These data suggest that SREBP2-ACSS2-AcCoA axis might contribute to pathogenesis of cirrhosis in humans.

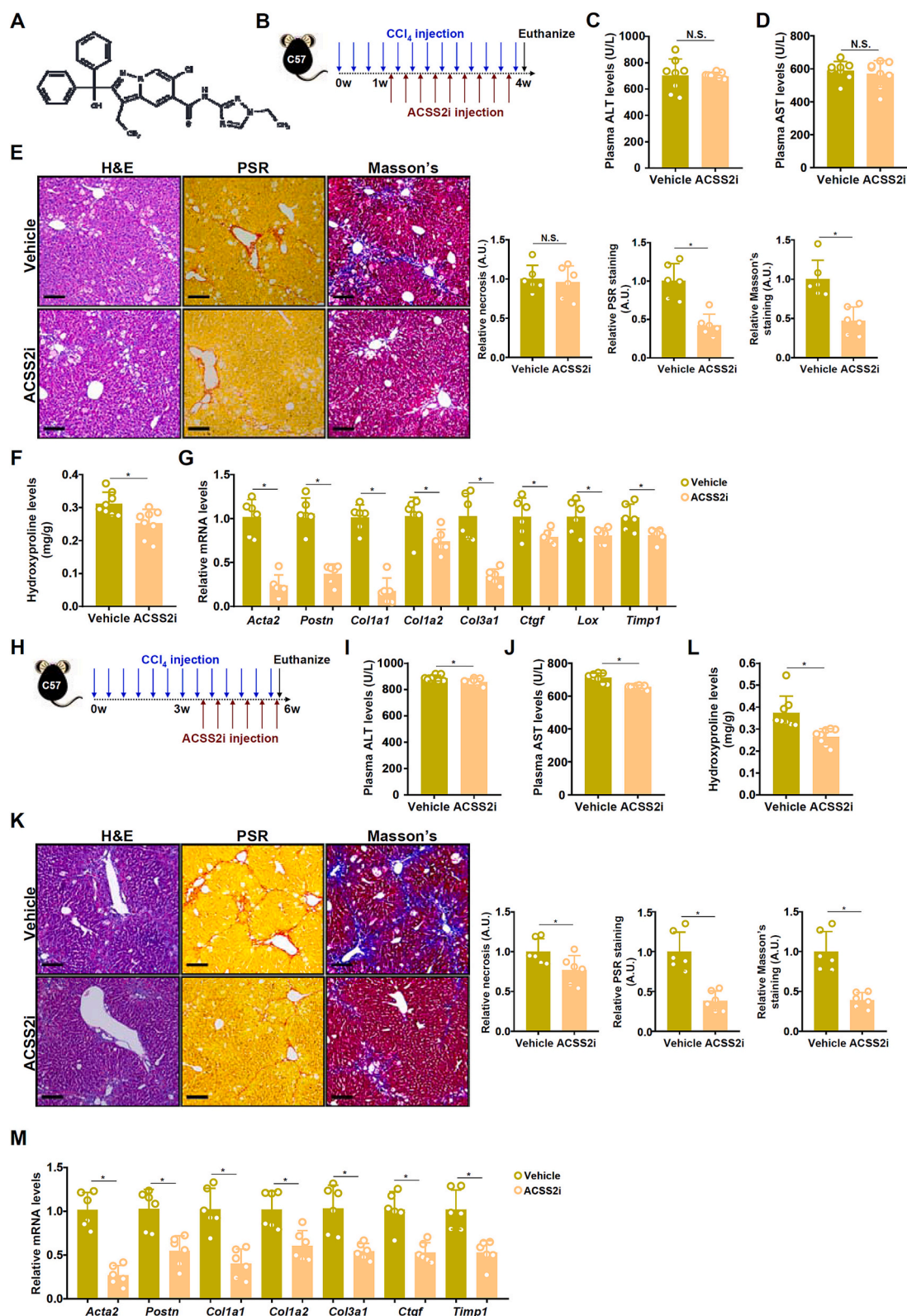
## 4. Discussion

HSC-myofibroblast transition is the paradigm underlying pathogenesis of liver fibrosis and as such represents a perennial theme in hepatology translational research. In this report we detail a previously unrecognized role for HSC-derived SREBP2 in liver fibrosis: SREBP2-dependent trans-activation of ACSS2 fuels acetyl-CoA biosynthesis to catalyze histone H3K9/H3K27 acetylation, which rate-limits the transcription of a panel of different pro-fibrogenic genes cumulatively driving HSC activation (Fig. 7G). Previous studies that focused on a hepatocyte role for the SREBP proteins have produced varying and sometimes conflicting data. For instance, SREBP1c deletion in



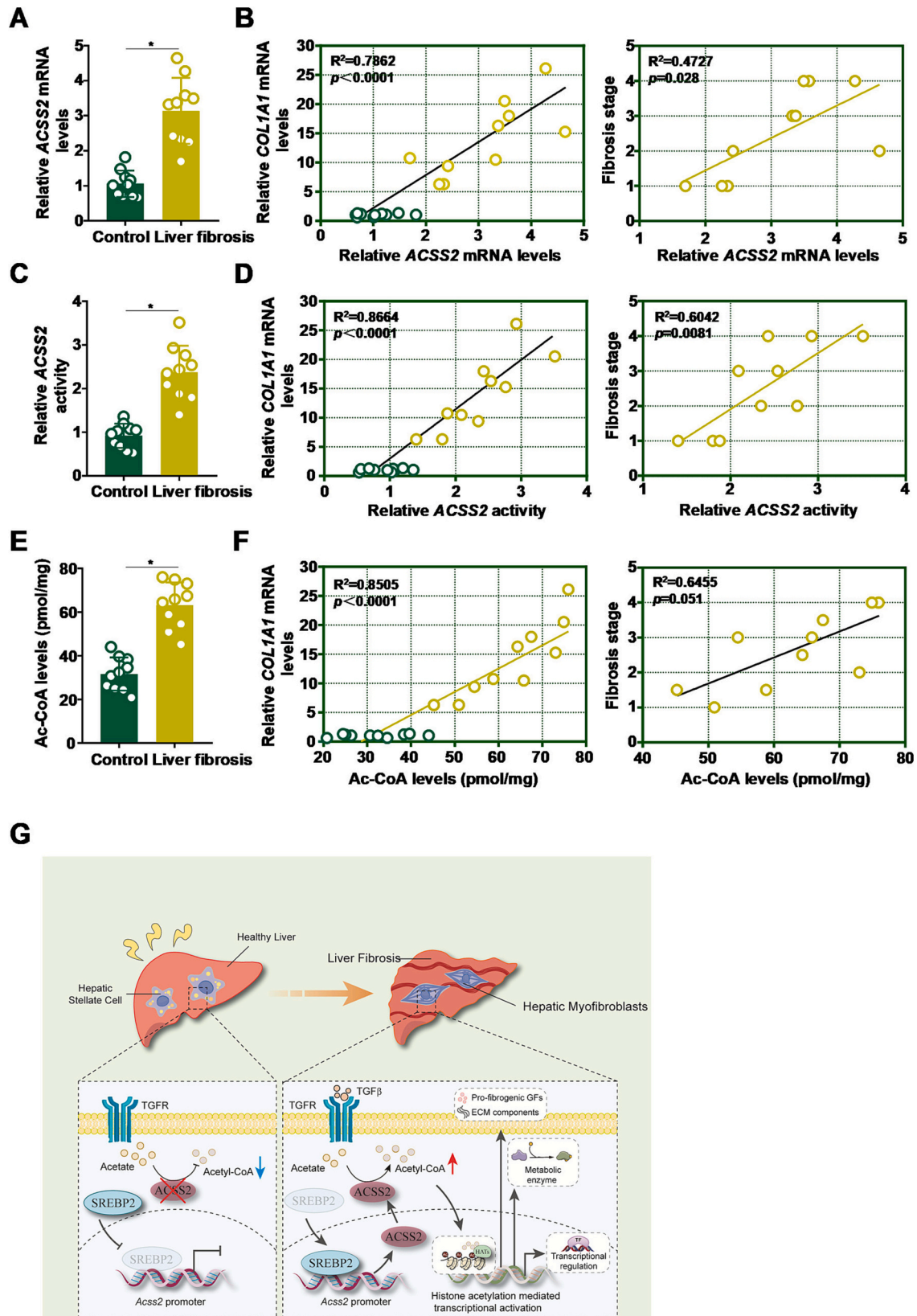
**Fig. 5.** SREBP2 controls acetyl-CoA synthesis to regulate histone H3K9/H3K27 acetylation. (A) LX-2 cells were transfected with siRNA targeting SREBP2 or scrambled siRNA (SCR) and transduced with adenoviral ACSS2 followed by treatment with TGF-β (5 ng/ml) for 24 h. Intracellular acetyl-CoA levels were examined by a colorimetric assay. N = 3 biological replicates. Data are expressed as mean ± S.D. \*,  $p < 0.05$ , two-way ANOVA with post-hoc Tukey's test. (B, C) LX-2 cells or primary human HSCs (aHSCs) were transfected with siRNA targeting SREBP2 or scrambled siRNA (SCR) and transduced with adenoviral ACSS2 followed by treatment with TGF-β (5 ng/ml) for 24 h. RNA-seq was performed as described in Methods. PCA plot (B). Venn diagram (C). (D-G) LX-2 cells or primary human HSCs (aHSCs) were transfected with siRNA targeting SREBP2 or scrambled siRNA (SCR) and transduced with adenoviral ACSS2 followed by treatment with TGF-β (5 ng/ml) for 24 h. CUT&Tag-seq was performed with anti-acetyl H3K9 and anti-acetyl H3K27. Heatmaps (D). Box-Whisker plot (E). GO and KEGG analyses (F). HOMER analysis (G). (H) Venn diagram showing overlap of RNA-seq and CUT&Tag-seq. (I) CUT&Tag tracks of H3K9Ac/H3K27Ac signals and RNA-Seq tracks of the read coverage surrounding the *TGFB1*, *GLS1*, *COL5A1*, and *TEAD3* loci.





**Fig. 6.** ACSS2 inhibition attenuates liver fibrosis in mice. (A) Chemical structure of ACSS2-IN-1 (Compound 1). (B-G) C57BL/6 J mice were injected with CCl<sub>4</sub> to induce liver fibrosis followed by injection with ACSS2i (preventive scheme). Scheme of protocol (B). Plasma ALT levels (C). Plasma AST levels (D). PSR staining and Masson's staining (E). Hydroxyproline levels (F). Myfibroblast markers were examined by qPCR (G). (H-M) C57BL/6 J mice were injected with CCl<sub>4</sub> to induce liver fibrosis followed by injection with ACSS2i (therapeutic scheme). Scheme of protocol (H). Plasma ALT levels (I). Plasma AST levels (J). PSR staining and Masson's staining (K). Hydroxyproline levels (L). Myfibroblast markers were examined by qPCR (M). N = 6–8 mice for each group. Data are expressed as mean ± S.D. \*,  $p < 0.05$ , two-tailed student's test.





**Fig. 7.** Validation of SREBP2-ACSS2 axis in humans. (A, B) Gene expression in human liver biopsy specimens was examined by qPCR. (C, D) ACSS2 activity in human liver biopsy specimens was examined by a colorimetric kit. (E, F) Acetyl CoA levels in human liver biopsy specimens were measured by a colorimetric kit.  $N = 10$  individuals for each group. Data are expressed as mean  $\pm$  S.D. \*,  $p < 0.05$ , two-tailed student's test. Pearson correlation was performed using Graphpad. (G) A schematic model.

hepatocytes seems to shelter the mice from liver fibrosis [13]. In contrast, hepatocyte-specific deletion of SCAP, the chaperone required for SREBP trans-location and activation, exacerbates liver fibrosis probably due to insufficient SREBP1a processing [14]. It should be noted that these observations regarding altered liver fibrosis are likely secondary to liver injury. Our data, relying on HSC/myofibroblast conditional SREBP2 deletion, seem to suggest that regulation of liver fibrosis by SREBP2 is cell-autonomous and can be divorced from liver injury. Global SREBP2 deficiency is incompatible with embryonic development [38]. Mice with hypomorphic SREBP2 expression [38] or mice with hepatocyte-conditional SREBP2 deletion [39] are leaner with lower circulating/hepatic cholesterol levels than wild type littermates but otherwise viable and display minimal adverse phenotype. In addition, it has been demonstrated by the Ivashkiv group that SREBP2 might function as a pro-inflammatory transcription factor, independent of cholesterol synthesis, to mediate TNF-induced macrophage activation [40]. In light of our finding, it is tempting to speculate that targeting SREBP2 might achieve anti-fibrotic and anti-inflammatory benefits without exacerbating liver injury. This possibility certainly deserves further attention in follow-up studies.

Through integrated transcriptomic screening ACSS2 is identified as a *bona fide* SREBP2 target that contributes to HSC activation and liver fibrosis. Liver fibrosis finds most significant relevance in chronic liver diseases (CLD) including metabolic dysfunction associated steatotic liver disease (MASLD) and hepatocellular carcinoma (HCC). Of interest, previous investigations have provided concrete evidence that ACSS2 promotes pathogenesis of both MASLD [41–43] and HCC [44,45] *in vivo*. Therefore, we propose that targeting ACSS2 may be considered as a reasonable strategy for CLD intervention beyond the models described in this study. However, we do not and we cannot rule out the possibility that SREBP2 might contribute to HSC-myofibroblast transition and liver fibrosis *via* downstream targets other than ACSS2. For instance, the simplest explanation accounting for SREBP2-dependent HSC activation might be increased intracellular cholesterol levels likely through key enzymes involved in cholesterol metabolism including HMGCS1, MSMO1, FDPS, and PCSK9. Indeed, cholesterol-rich diets are known to induce liver fibrosis whereas the cholesterol-depleting agent beta cyclodextrin has been shown to antagonize liver fibrosis in mice [46]. Alternatively, SLC7A5 has been detected as one of the most significantly up-regulated genes during HSC activation by a recent study [47]. SLC7A5 regulates trans-membrane trafficking and thus intracellular bioavailability of amino acids, a process acutely linked to the pro-fibrogenic mTOR signaling [48]. Thus, it is plausible that SREBP2 might form a crosstalk with mTOR, possibly *via* SLC7A5, to regulate HSC activation. Finally, the Mutlu laboratory has previously found that phosphoserine aminotransferase 1 (PSAT1) contributes to pulmonary fibrosis by catalyzing *de novo* serine synthesis, which leads to secondary elevation of glycine levels to be used for collagen production in lung fibroblasts [49,50]. More recently, Zhang *et al.* have reported that AdipoRon, an agonist to the lipocalin receptor, dampens HSC activation likely by targeting glutaminolysis-related enzymes including PSAT1 [51]. Because the evidence in the Zhang *et al.* paper [51] that links PSAT1 to HSC activation and liver fibrosis is correlative and circumstantial, it might be of interest to ascertain whether PSAT1, as a downstream target of SREBP2, can directly program HSC activation.

Through genetic and pharmacological approaches we are able to assign a pro-fibrogenic role for ACSS2 in HSCs. However, a few caveats regarding this model, *i.e.*, ACSS2 contributes to liver fibrosis by orchestrating H3K9/H3K27 acetylation-dependent pro-fibrogenic transcription in HSCs need close scrutiny. First, our data do not at all foreclose the possibility that extra-HSC derived ACSS2 might play a role in liver fibrosis. Accumulating evidence suggests that ACSS2 plays a role in shaping the immune niche under physiological/pathophysiological conditions by modulating the phenotype of macrophages, lymphocytes, and dendritic cells [52–54]. Of intrigue, it was observed that ACSS2i, which presumably targets ACSS2 non-selectively, had an effect on liver

injury only when administrated for an extended period of time (the CCL<sub>4</sub> interventional scheme in which ACSS2i was injected for three weeks) suggesting that ACSS2 in different cellular compartments might respond to ACSS2i with different kinetics and/or sensitivity. Because skewed immune microenvironment is considered pivotal in pathogenesis of liver fibrosis, further examination of immune cell-specific ACSS2 in this context might provide additional insights on how ACSS2 contributes to CLD. Second, ACSS2-dependent catalysis of acetyl-CoA can be utilized for not only histone acetylation but non-histone acetylation [55,56]. In addition, a recent study has implicated ACSS2 as a genuine lysine lactyltransferase [57]. Therefore, ACSS2-dependent non-histone lysine acetylation and lysine lactylation should be investigated to complement the current mechanism.

Despite the advances proffered by our study there are limitations that may dampen its translational potential. First, cirrhosis typically develops in humans over the course of years, if not decades, the pathogenesis of which is unlikely recapitulated by the models (CCL<sub>4</sub> or BDL) described in this study. In addition, MASLD has become the most important underlying cause for cirrhosis. Therefore, the proposition that SREBP2-ACSS2 promotes metaboloepigenetic regulation of liver fibrosis should be further validated in animal models with more relevance to the human pathology. Second, we focused on H3K9/H3K27 acetylation as readout of ACSS2-dependent acetyl-CoA production. However, acetylation status of other histone lysine residues including H3K14 [58] and H3K18 [59,60] can and has been shown to contribute to liver fibrosis. In addition, acetylation of non-histone factors including SMAD2 [61], HMGB1 [62], Atg5 [63] and SOD2 [64], subject to the availability of acetyl-CoA levels, can also play a role in HSC activation. These nuances should be carefully investigated and analyzed in future studies. Third, the relative small sample size of human specimens used for validation of our model leaves much room for doubts and alternative interpretation. This caveat was further compounded by the fact that whole liver tissues, instead of purified HSCs, were used for validation thus leaving the model, *i.e.*, an ACSS2-Acetyl-CoA axis regulates HSC activation, without full support. Further authentication of these findings in larger cohorts across different disease spectrums would hopefully provide more information to devise translational strategies to combat chronic liver disease.

#### CRedit authorship contribution statement

**Yu Wang:** Investigation, Methodology, Writing – original draft, Writing – review & editing. **Aoqi Kang:** Investigation, Methodology, Writing – original draft, Writing – review & editing. **Shunjie Wang:** Investigation, Methodology, Writing – original draft, Writing – review & editing. **Zhen Yang:** Investigation, Methodology, Writing – original draft, Writing – review & editing. **Hong Liu:** Investigation, Methodology, Writing – original draft, Writing – review & editing. **Linbo Yue:** Investigation, Methodology, Writing – original draft, Writing – review & editing. **Hao Li:** Investigation, Methodology, Writing – original draft, Writing – review & editing. **Caiyi Wu:** Investigation, Methodology, Writing – original draft, Writing – review & editing. **Wenjing Ren:** Investigation, Methodology, Writing – original draft, Writing – review & editing. **Xiulian Miao:** Investigation, Methodology, Writing – original draft, Writing – review & editing. **Zilong Li:** Conceptualization, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing. **Yong Xu:** Supervision, Writing – original draft, Writing – review & editing. **Zhiwen Fan:** Conceptualization, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

#### Declaration of competing interest

None.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.metabol.2026.156597>.

## Data availability

The data that support the findings of this study are available upon reasonable request.

## References

- Marcellin P, Kutala BK. Liver diseases: a major, neglected global public health problem requiring urgent actions and large-scale screening. *Liver Int* 2018;38 (Suppl. 1):2–6.
- Devarbhavi H, Asrani SK, Arab JP, Nartey YA, Pose E, Kamath PS. Global burden of liver disease: 2023 update. *J Hepatol* 2023;79:516–37.
- Balakrishnan M, Rehm J. A public health perspective on mitigating the global burden of chronic liver disease. *Hepatology* 2024;79:451–9.
- En Li Cho E, Ang CZ, Quek J, Fu CE, Lim LKE, Heng ZEQ, et al. Global prevalence of non-alcoholic fatty liver disease in type 2 diabetes mellitus: an updated systematic review and meta-analysis. *Gut* 2023;72:2138–48.
- Castera L, Cusi K. Diabetes and cirrhosis: current concepts on diagnosis and management. *Hepatology* 2023;77:2128–46.
- Taru V, Szabo G, Mehal W, Reiberger T. Inflammasomes in chronic liver disease: hepatic injury, fibrosis progression and systemic inflammation. *J Hepatol* 2024;81: 895–910.
- Friedman SL. Evolving challenges in hepatic fibrosis. *Nat Rev Gastroenterol Hepatol* 2010;7:425–36.
- Kisseleva T. The origin of fibrogenic myofibroblasts in fibrotic liver. *Hepatology* 2017;65:1039–43.
- Mederacke I, Hsu CC, Troeger JS, Huebener P, Mu X, Dapito DH, et al. Fate tracing reveals hepatic stellate cells as dominant contributors to liver fibrosis independent of its aetiology. *Nat Commun* 2013;4:2823.
- Horn P, Tacke F. Metabolic reprogramming in liver fibrosis. *Cell Metab* 2024;36: 1439–55.
- Rho H, Terry AR, Chronis C, Hay N. Hexokinase 2-mediated gene expression via histone lactylation is required for hepatic stellate cell activation and liver fibrosis. *Cell Metab* 2023;35(1406–23):e8.
- Brown MS, Goldstein JL. Sterol regulatory element binding proteins (SREBPs): controllers of lipid synthesis and cellular uptake. *Nutr Rev* 1998;56: S1–3; discussion S54–75.
- Lee EH, Lee JH, Kim DY, Lee YS, Jo Y, Dao T, et al. Loss of SREBP-1c ameliorates liver-induced liver fibrosis by decreasing lipocalin-2. *Exp Mol Med* 2024;56: 1001–12.
- Kawamura S, Matsushita Y, Kurosaki S, Tange M, Fujiwara N, Hayata Y, et al. Inhibiting SCAP/SREBP exacerbates liver injury and carcinogenesis in murine nonalcoholic steatohepatitis. *J Clin Invest* 2022;132.
- Tomita K, Teratani T, Suzuki T, Shimizu M, Sato H, Narimatsu K, et al. Free cholesterol accumulation in hepatic stellate cells: mechanism of liver fibrosis aggravation in nonalcoholic steatohepatitis in mice. *Hepatology* 2014;59:154–69.
- Kong M, Wu J, Fan Z, Chen B, Wu T, Xu Y. The histone demethylase Kdm4 suppresses activation of hepatic stellate cell by inducing MiR-29 transcription. *Biochem Biophys Res Commun* 2019;514:16–23.
- Zhao Q, Shao T, Huang S, Zhang J, Zong G, Zhuo L, et al. The insulin-like growth factor binding protein-microfibrillar associated protein-sterol regulatory element binding protein axis regulates fibroblast-myofibroblast transition and cardiac fibrosis. *Br J Pharmacol* 2024;181:2492–508.
- Kong M, Dong W, Kang A, Kuai Y, Xu T, Fan Z, et al. Regulatory role and translational potential of CCL11 in liver fibrosis. *Hepatology* 2023;78:120–35.
- Knorr J, Kaufmann B, Inzaugarat ME, Holtmann TM, Geisler L, Hundertmark J, et al. Interleukin-18 signaling promotes activation of hepatic stellate cells in mouse liver fibrosis. *Hepatology* 2023;77:1968–82.
- Kim JW, Tung HC, Ke M, Xu P, Cai X, Xi Y, et al. The de-sulfinylation enzyme sulfiredoxin-1 attenuates hepatic stellate cell activation and liver fibrosis by modulating the PTPN12-NLRP3 axis. *Hepatology* 2025;82:92–109.
- Rezvani M, Espanol-Sunyer R, Malato Y, Dumont L, Grimm AA, Kienle E, et al. In vivo hepatic reprogramming of Myofibroblasts with AAV vectors as a therapeutic strategy for liver fibrosis. *Cell Stem Cell* 2016;18:809–16.
- Piras BA, Tian Y, Xu Y, Thomas NA, O'Connor DM, French BA. Systemic injection of AAV9 carrying a periostin promoter targets gene expression to a myofibroblast-like lineage in mouse hearts after reperfused myocardial infarction. *Gene Ther* 2016;23:469–78.
- Kong M, Hong W, Shao Y, Lv F, Fan Z, Li P, et al. Ablation of serum response factor in hepatic stellate cells attenuates liver fibrosis. *J Mol Med (Berl)* 2019;97: 1521–33.
- Dong W, Kong M, Liu H, Xue Y, Li Z, Wang Y, et al. Myocardin-related transcription factor A drives ROS-fueled expansion of hepatic stellate cells by regulating p38-MAPK signalling. *Clin Transl Med* 2022;12:e688.
- Sun L, Chen B, Wu J, Jiang C, Fan Z, Feng Y, et al. Epigenetic regulation of a disintegrin and metalloproteinase (ADAM) promotes colorectal cancer cell migration and invasion. *Front Cell Dev Biol* 2020;8:581692.
- Mao L, Liu L, Zhang T, Qin H, Wu X, Xu Y. Histone deacetylase 11 contributes to renal fibrosis by repressing KLF15 transcription. *Front Cell Dev Biol* 2020;8:235.
- Yang Y, Liu L, Li M, Cheng X, Fang M, Zeng Q, et al. The chromatin remodeling protein BRG1 links ELOVL3 trans-activation to prostate cancer metastasis. *Biochim Biophys Acta Gene Regul Mech* 2019;1862:834–45.
- Roh TY, Cuddapah S, Zhao K. Active chromatin domains are defined by acetylation islands revealed by genome-wide mapping. *Genes Dev* 2005;19:542–52.
- Gates LA, Shi J, Rohira AD, Feng Q, Zhu B, Bedford MT, et al. Acetylation on histone H3 lysine 9 mediates a switch from transcription initiation to elongation. *J Biol Chem* 2017;292:14456–72.
- Hezroni H, Sailaja BS, Meshorer E. Pluripotency-related, valproic acid (VPA)-induced genome-wide histone H3 lysine 9 (H3K9) acetylation patterns in embryonic stem cells. *J Biol Chem* 2011;286:35977–88.
- Hnisz D, Abraham BJ, Lee TI, Lau A, Saint-Andre V, Sigova AA, et al. Super-enhancers in the control of cell identity and disease. *Cell* 2013;155:934–47.
- Heintzman ND, Hon GC, Hawkins RD, Kheradpour P, Stark A, Harp LE, et al. Histone modifications at human enhancers reflect global cell-type-specific gene expression. *Nature* 2009;459:108–12.
- Sato Y, Hilbert L, Oda H, Wan Y, Heddleston JM, Chew TL, et al. Histone H3K27 acetylation precedes active transcription during zebrafish zygotic genome activation as revealed by live-cell analysis. *Development* 2019;146.
- Li X, Yu W, Qian X, Xia Y, Zheng Y, Lee JH, et al. Nucleus-translocated ACS2 promotes gene transcription for lysosomal biogenesis and autophagy. *Mol Cell* 2017;66:684–97 e9.
- Chen R, Xu M, Nagati J, Garcia JA. Coordinate regulation of stress signaling and epigenetic events by Acss2 and HIF-2 in cancer cells. *PLoS One* 2017;12:e0190241.
- Bacigalupa ZA, Arner EN, Vlach LM, Wolf MM, Brown WA, Krystofiak ES, et al. HIF-2alpha expression and metabolic signaling require ACS2 in clear cell renal cell carcinoma. *J Clin Invest* 2024;134.
- Li L, Xiang T, Guo J, Guo F, Wu Y, Feng H, et al. Inhibition of ACS2-mediated histone crotonylation alleviates kidney fibrosis via IL-1beta-dependent macrophage activation and tubular cell senescence. *Nat Commun* 2024;15:3200.
- Vergnes L, Chin RG, de Aguiar Vallim T, Fong LG, Osborne TF, Young SG, et al. SREBP-2-deficient and hypomorphic mice reveal roles for SREBP-2 in embryonic development and SREBP-1c expression. *J Lipid Res* 2016;57:410–21.
- Rong S, Cortes VA, Rashid S, Anderson NN, McDonald JG, Liang G, et al. Expression of SREBP-1c requires SREBP-2-mediated generation of a sterol ligand for LXR in livers of mice. *Elife* 2017;6.
- Kusnadi A, Park SH, Yuan R, Pannellini T, Giannopoulou E, Oliver D, et al. The cytokine TNF promotes transcription factor SREBP activity and binding to inflammatory genes to activate macrophages and limit tissue repair. *Immunity* 2019;51:241–57 e9.
- Zhao S, Jang C, Liu J, Uehara K, Gilbert M, Izzo L, et al. Dietary fructose feeds hepatic lipogenesis via microbiota-derived acetate. *Nature* 2020;579:586–91.
- Zhang M, Ji J, Lei Y, Qin F, Tao Y, Li N, et al. Dual inhibition of hepatic ACLY and ACS2: a synergistic approach to combat NAFLD through lipogenesis reduction and mitochondrial enhancement. *Pharmacol Res* 2025;215:107706.
- Huang Z, Zhang M, Plec AA, Estill SJ, Cai L, Repa JJ, et al. Acss2 promotes systemic fat storage and utilization through selective regulation of genes involved in lipid metabolism. *Proc Natl Acad Sci USA* 2018;115: E9499–E506.
- Comerford SA, Huang Z, Du X, Wang Y, Cai L, Witkiewicz AK, et al. Acetate dependence of tumors. *Cell* 2014;159:1591–602.
- Gao X, Lin SH, Ren F, Li JT, Chen JJ, Yao CB, et al. Acetate functions as an epigenetic metabolite to promote lipid synthesis under hypoxia. *Nat Commun* 2016;7:11960.
- Ignat SR, Dinescu S, Varadi J, Fenyvesi F, Nguyen TLP, Ciceu A, et al. Complexation with random methyl-beta-cyclodextrin and (2-hydroxypropyl)-beta-cyclodextrin promotes Chrysin effect and potential for liver fibrosis therapy. *Materials (Basel)* 2020;13.
- Zhao Y, Xu X, Cai H, Wu W, Wang Y, Huang C, et al. Identification of potential biomarkers from amino acid transporter in the activation of hepatic stellate cells via bioinformatics. *Front Genet* 2024;15:1499915.
- Marcondes-de-Castro IA, Reis-Barbosa PH, Marinho TS, Aguilu MB, Mandarim-de-Lacerda CA. AMPK/mTOR pathway significance in healthy liver and non-alcoholic fatty liver disease and its progression. *J Gastroenterol Hepatol* 2023;38:1868–76.
- Nigdelioglu R, Hamanaka RB, Meliton AY, O'Leary E, Witt LJ, Cho T, et al. Transforming growth factor (TGF)-beta promotes de novo serine synthesis for collagen production. *J Biol Chem* 2016;291:27239–51.
- Hamanaka RB, O'Leary EM, Witt LJ, Tian Y, Gokalp GA, Meliton AY, et al. Glutamine metabolism is required for collagen protein synthesis in lung fibroblasts. *Am J Respir Cell Mol Biol* 2019;61:597–606.
- Zhang X, Zeng Y, Ying H, Hong Y, Xu J, Lin R, et al. AdipoRon mitigates liver fibrosis by suppressing serine/glycine biosynthesis through ATF4-dependent glutaminolysis. *Ecotoxicol Environ Saf* 2025;289:117511.



- [52] Marquez S, Fernandez JJ, Mancebo C, Herrero-Sanchez C, Alonso S, Sandoval TA, et al. Tricarboxylic acid cycle activity and remodeling of glycerophosphocholine lipids support cytokine induction in response to fungal patterns. *Cell Rep* 2019;27: 525–36 e4.
- [53] Miller KD, O'Connor S, Pniewski KA, Kannan T, Acosta R, Mirji G, et al. Acetate acts as a metabolic immunomodulator by bolstering T-cell effector function and potentiating antitumor immunity in breast cancer. *Nat Can* 2023;4:1491–507.
- [54] Sabari BR, Tang Z, Huang H, Yong-Gonzalez V, Molina H, Kong HE, et al. Intracellular crotonyl-CoA stimulates transcription through p300-catalyzed histone crotonylation. *Mol Cell* 2015;58:203–15.
- [55] Chen N, Zhao M, Wu N, Guo Y, Cao B, Zhan B, et al. ACS2 controls PPARGgamma activity homeostasis to potentiate adipose-tissue plasticity. *Cell Death Differ* 2024; 31:479–96.
- [56] Li Z, Liu H, He J, Wang Z, Yin Z, You G, et al. Acetyl-CoA synthetase 2: a critical linkage in obesity-induced tumorigenesis in myeloma. *Cell Metab* 2021;33:78–93 e7.
- [57] Zhu R, Ye X, Lu X, Xiao L, Yuan M, Zhao H, et al. Acss2 acts as a lactyl-CoA synthetase and couples KAT2A to function as a lactyltransferase for histone lactylation and tumor immune evasion. *Cell Metab* 2025;37:361–76 e7.
- [58] Zhou B, Luo Y, Bi H, Zhang N, Ma M, Dong Z, et al. Amelioration of nonalcoholic fatty liver disease by inhibiting the deubiquitylating enzyme RPN11. *Cell Metab* 2024;36:2228–44 e7.
- [59] Hu F, Zhou X, Peng Q, Ma L. Suppressed histone H3 lysine 18 acetylation is involved in arsenic-induced liver fibrosis in rats by triggering the dedifferentiation of liver sinusoidal endothelial cells. *Toxics* 2023;11.
- [60] Garrido A, Kim E, Teijeiro A, Sanchez Sanchez P, Gallo R, Nair A, et al. Histone acetylation of bile acid transporter genes plays a critical role in cirrhosis. *J Hepatol* 2022;76:850–61.
- [61] Zhang J, Li Y, Liu Q, Huang Y, Li R, Wu T, et al. Sirt6 alleviated liver fibrosis by Deacetylating conserved lysine 54 on Smad2 in hepatic stellate cells. *Hepatology* 2021;73:1140–57.
- [62] Xiao ZH, Xie ZY, Wang Q, Lu H, Cao HW. SIRT7 affects autophagy and activation of hepatic stellate cells by regulating the acetylation level of high mobility group protein 1. *Immunobiology* 2023;228:152323.
- [63] Sun S, Li Z, Huan S, Kai J, Xia S, Su Y, et al. Modification of lysine deacetylation regulates curcumin-induced necroptosis through autophagy in hepatic stellate cells. *Phytother Res PTR* 2022;36:2660–76.
- [64] Lai TL, Park SY, Nguyen G, Pham PTM, Kang SM, Hong J, et al. Irisin attenuates hepatic stellate cell activation and liver fibrosis in bile duct ligation mice model and improves mitochondrial dysfunction. *Endocrinol Metab (Seoul)* 2024;39: 908–20.