SCARNA10, a nuclear-retained long non-coding RNA, promotes liver fibrosis and serves as a potential biomarker

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Supplementary Information

Supplementary Materials and Methods

Histology and immunohistochemistry

The specimens were sequentially fixed in 10% formalin for two days, transferred to ethanol of different concentration and embedded in paraffin in preparation for histopathological analysis. Thin sections (5 μm) were stained with H&E and Sirius red for histopathological study. According to the above results, three sections were chosen from each group for immunohistochemical analysis. Briefly, sections prepared on slides were first submitted to antigen retrieval by incubation in citrate buffer (pH 6.0) for 5min at 108℃ and pretreated with 3% H_2O_2 for 15 min at room temperature followed by washing with PBS. Slides were subsequently incubated in normal goat serum for 20min to block the nonspecific immunoreactivity. Next, the slides were treated with primary antibody α-SMA (1:50), Col1α1 (1:1000), TIMP1(1:50), PCNA (1:800), TGF-β (1:50) or KLF6 (1:50) overnight at 4 °C. In addition, tissue sections were processed omitting the primary antibody as the negative control. The slides were incubated with secondary antibody (1:500) (HRP-conjugated anti-rabbit IgG). And the reaction products were visualized using diaminobenzidine (DAB) and monitored by microscopy.

Liver enzyme measurement

Serum specimens were refrigerated and shipped to a biochemical laboratory for analysis. The laboratory (Tianjin Third Central Hospital, Tianjin, China) used a Hitachi model 704 multichannel analyzer to measure biochemistry profile, including levels of ALT, AST, ALB and GGT.

Hydroxyproline assay

Total collagen content was tested by measuring the amount of hydroxyproline in liver tissue using commercially available hydroxyproline detection kits purchased from Nan Jing Jian Cheng Biochemical Institute (Nanjing, China) according to the manufacturer's instructions.

Cell proliferation assay

Cell growth was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazo-lium bromide (MTT) (Sigma-Aldrich, St. Louis, MO, USA) dye conversiton at 570 nm following manufacturer's instructions. Briefly, cells were seeded onto 96-well culture plats (5×10^3cells per well) in DMEM with 10% FBS and allowed to attach for 24 h, and then treated with various kinds of lentivirus respectively. In all cases the initial number of cells was the same. Approximately 72 hours post lentivirus treatment, the cells were treated with MTT (5 mg/ml) for 4 hours at 37℃ and addition of 100 μl/well DMSO.

Absorbance at 570 nm was read using a plate reader and the experiment was performed triplicately.

EdU incorporation assay

The proliferation of cells with different treatment were assessed using a YF594A Click-iT EdU Flow Cytometry Assay kit (C6021, UE, Suzhou, China) according to the manufacturer's instruction. Briefly, 10 μM EdU were added to the plates 12 h before harvesting the cells. After the incubation, washed the cells once with 1% BSA and collected the cells by centrifugation. Then, resuspended the cells with 100 μl 4% paraformaldehyde followed incubating for 15 min at room temperature in the dark. Next, washed the cells twice with 1% BSA in PBS and resuspended them with 100 μl 0.5% Triton X-100 followed by incubating for 20 min at room temperature. Finally, 1 ml Click-iT reaction mixture was added to each tube incubating for 30 min at room temperature in the dark, and analyzed by flow cytometry.

Apoptosis assay

The apoptosis of cells with different treatment were analyzed using a FITC Annexin V Apoptosis Detection Kit I (BD Biosciences) according to the manufacturer's instruction. Briefly, AML12 cells were scraped and washed twice with ice-cold PBS and then re-suspended in 1 × binding buffer at a concentration of 1×10^6 cells / ml. Next 100 μl of the solution, 5 μl of FITC Annexin V and 5 μl PI were sequentially transferred to a 5 ml culture tube followed by gently vortex and incubating for 15 min at 25°C in the dark. Finally 400 μl of 1 × binding buffer was added to each tube and analyzed by flow cytometry within 1 h.

Confocal microscopy

Freshly isolated primary HSCs and LX-2 cells were re-plated on poly-lysine pre-coated glass coverslips and incubated overnight at 37℃ to reach typical adhesion and spreading. Cells were transfected with siRNA targeting SCARNA10 or siRNA-control for 48h, recombinant TGFβ was then added to cells transfected with siRNA-SCARNA10 or siRNA-control for additional 24 h. Other cells were transfected with LV-SCARNA10 or LV-control for 72 h. And then cells were sequentially fixed with 4% paraformaldehyde in PBS overnight at 4 ℃, permeabilized with 1% Triton X-100 in PBS for 30 min and blocked using 5% bovine serum albumin (BSA) in TBST with 0.1% Tween-20 for 30 min at room temperature. Next, the cells were incubated with primary antibodies against α-SMA (1:300), Col1α1 (1:500), MMP2 (1:250), Smad2/3 (1:125) overnight at 4℃ and an irrelevant isotype rabbit IgG was used as a negative control. After washing in PBS, cells were incubated with FITC-conjugated

secondary antibodies (1:100) in PBS away from light for 1 h at room temperature. And the nuclei were stained with DAPI (5 μg/ml). Finally, the slides were washed with PBS and the coverslips were mounted with an anti-fade Mounting Medium (P0126, Beyotime, Shanghai, China). All immunofluorescence was then visualized by a confocal microscope (LSM 700) or a fluorescence microscope.

Quantitative real-time polymerase chain reaction

Total RNA extracted from liver tissues or cells with Trizol reagent (Takara, Dalian, China), total RNA extracted from serum samples with Trizol LS reagent (Life Technologies, Grand Island, NY, USA), nuclear and cytoplasmic RNA prepared using PARIS™ Kit (Invitrogen, Grand Island, NY, USA) and the RNA isolated using the Magna RIP kit were measured with a NanoDrop ND-2000 spectrophotometer (Life Technologies, Grand Island, NY, USA). All RNA was digested with DNase I (Takara, Dalian, China). Briefly, the 10 μl RT reactions (1 μg RNA, 1 μl Buffer, 1 μl DNase1 and water) were incubated for 15min at 37°C followed by adding 1 μl of EDTA, incubated for 10 min at 65 °C and then maintained at 4 °C. Next, the first-strand cDNA was synthesized using AMV Reverse Transcriptase (Thermo Fisher Scientific, Basingstoke, UK) according to the manufacturer's instructions. For real-time PCR, all reactions were performed in triplicate with SYBR Green master mix (Takara, Dalian, China) under the following conditions: 15 min at 95 °C for initial denaturation, followed by 40 cycles of segments of 95 °C for 30 sec and 60 °C for 30 sec in the Light Cycler®96 Real-Time PCR System (Roche, Mannheim, Germany). The expression level of housekeeping gene GAPDH was used to normalize the expression level of the genes-of-interest. The sequences of primers for real-time PCR are listed in Table S3.

Western blot analysis

Protein extracted from liver tissues, primary HSCs, primary HCs, AML-12 and LX-2 cells with different treatment were analyzed by Western blotting. Briefly, protein was sequentially lysed in ice-cold RIPA buffer containing 1% PMSF and 1% phosphatase inhibitor, transferred to tubes, incubated in ice for 30 min and vortex every 5 min. Next, the lysates were centrifuged at 14,000 rpm for 30 min at 4°C followed by transferring the supernatants to new tubes. Protein concentrations were measured by the BCATM Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA, USA) using BSA as standard. Appropriate amount of protein samples along with 4 × loading buffer and ddH_2O were boiled for 4 min and then subjected to SDS-PAGE. Following by electrophoresis, the separated proteins were

blotted onto PVDF membranes in transfer buffer with constant current of 300 mA for 3 h at 4 °C. Then the PVDF membranes were sequentially washed with TBST containing 0.2% Tween-20, blocked with 5% nonfat milk in TBST and incubated with the interested primary antibodies of α-SMA (1:1000), Col1α1 (1:1000), MMP2 (1:2000), TIMP1 (1:200), PCNA (1:1000), TGF-β (1:2000), Smad2/3 (1:1000), pSmad2/3 (1:1000) and KLF6 (1:200) diluted in TBST containing 0.2% Tween-20 overnight at 4 °C. The levels of GAPDH were severed as control for total protein amount. Next, the membranes were incubated with secondary antibody for 1 h at RT with shaking. Signal was detected using the chemiluminescence (ECL) system (Merck Millipore, Darmstadt, Germany).

RNA interference

For gene knockdown analysis, small interferring (si) RNA targeting the SCARNA10 sequence, SUZ12 sequence, EZH2 sequence and non-targeting siRNA were obtained from GenePharma Biological Technology (Shanghai, China). Cells were transfected with the siRNAs at 50% confluence using lipofectamine MAX according to the manufacturer's instructions (Invitrogen, Grand Island, NY, USA). After culturing for 48 h, cells were analyzed by real-time PCR to determine knockdown efficiency. Target sequences of these siRNA are listed in Table S3.

Supplementary Figures

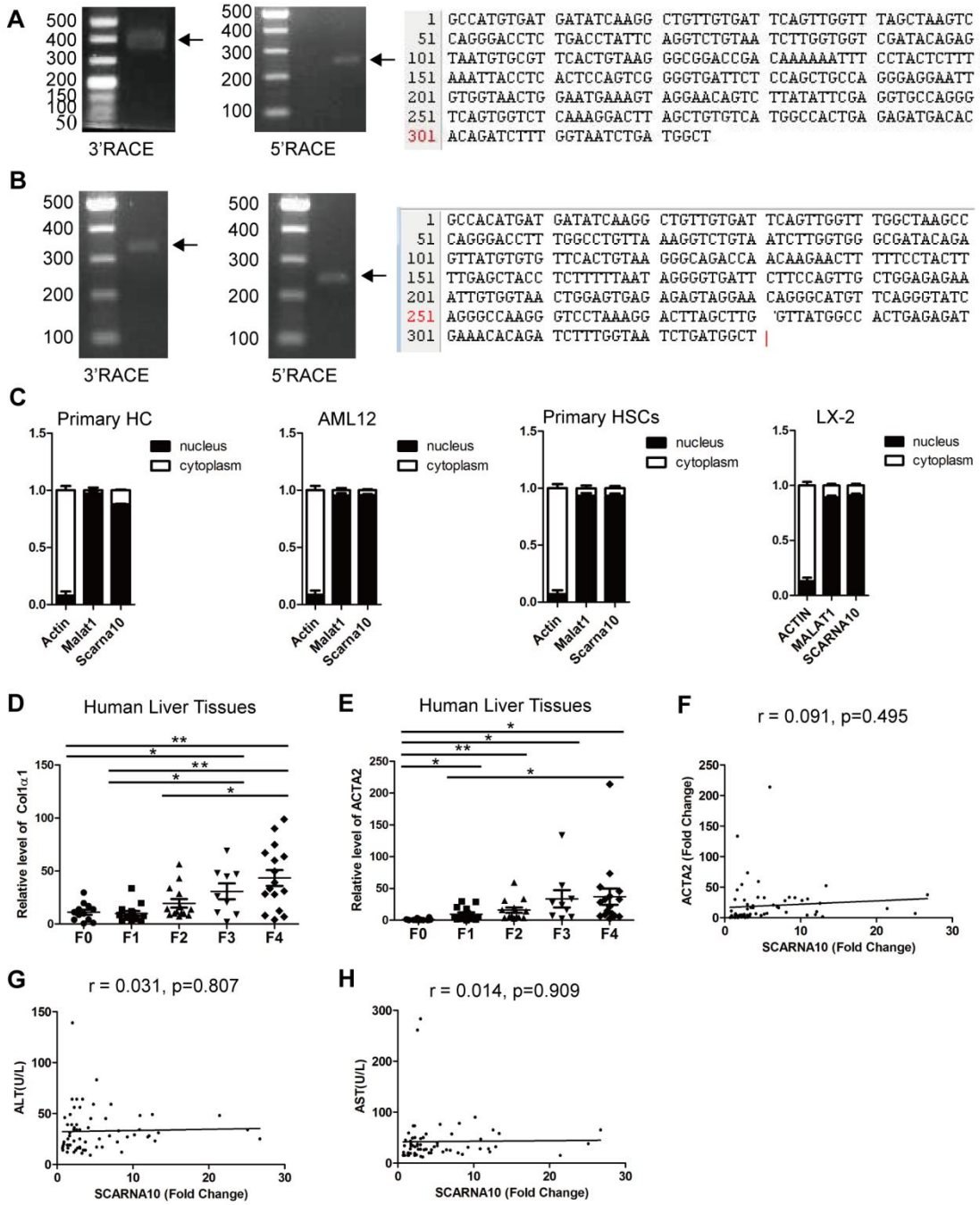

Fig. S1, related to Fig. 1. (A, B) Agarose gel electrophoresis of PCR products from the 5'-RACE procedure and 3'-RACE procedure. The molecular weight markers (base pairs) are indicated on the side. The major PCR product is marked with an arrow. Nucleotide sequence of the full-length lnc-SCARNA10 was confirmed by RACE in mouse liver tissues (A) and LX-2 cells (B). (C) RNA was extracted from the nuclei or cytoplasm of primary HCs, AML12, LX-2 or primary HSCs. 1 μg of RNA was used for the qRT-PCR analysis of *SCARNA10, MALAT1* (nuclear retained), and *β-actin* mRNAs (cytoplasm retained). (D, E) qRT-PCR analysis of the transcript of *Col1α1* and *ACTA2* in liver samples

of healthy, fibrotic and cirrhotic patients. (F-G) The correlation between *SCARNA10* expression level and *ACTA2*, ALT and AST level in patient liver tissues was assessed using Pearson′ correlation analysis, n=65. The data are expressed as the mean ± SD for at least triplicate experiments, **p*< 0.05, ***p*< 0.01.

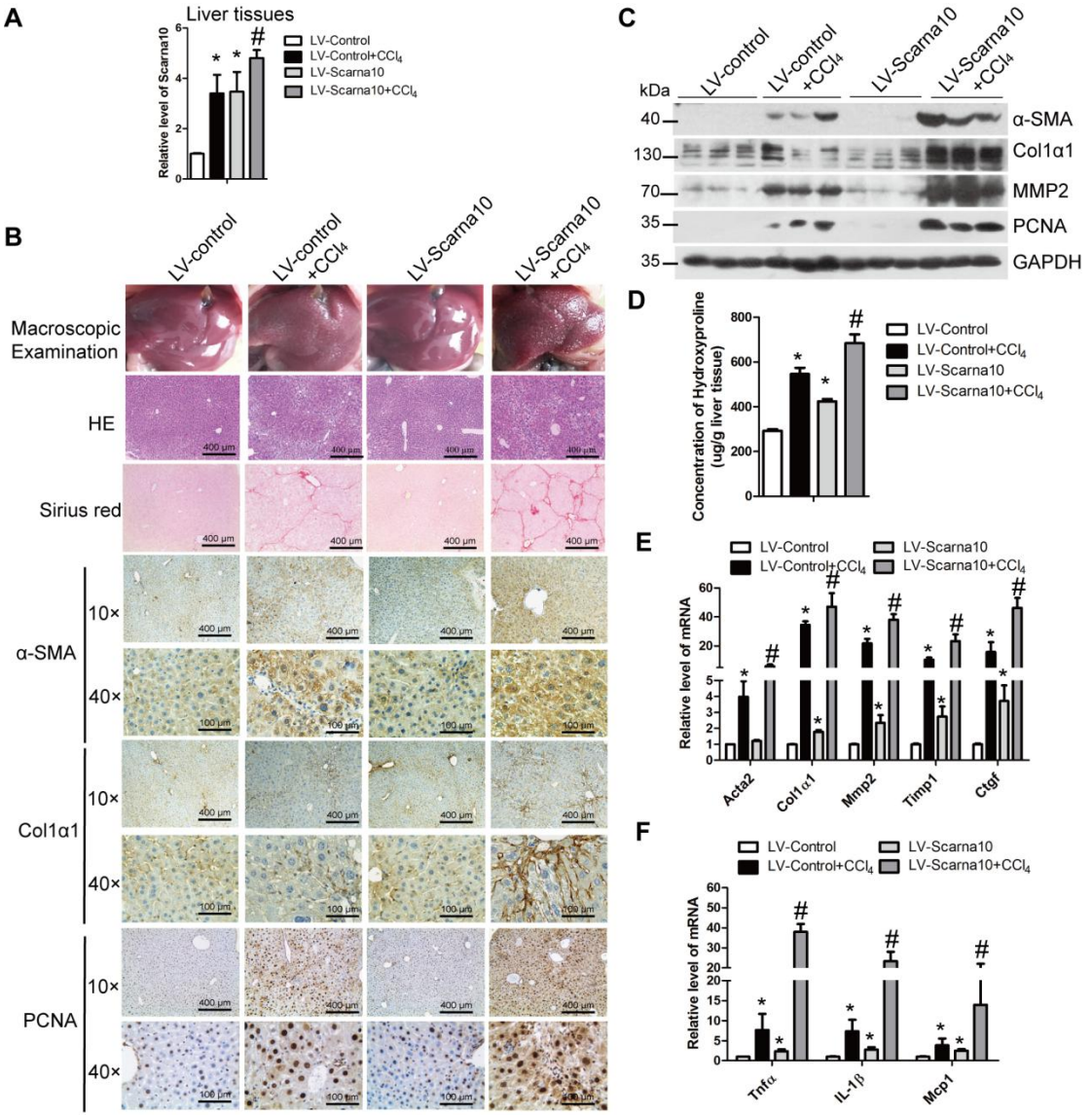

Fig. S2, related to Fig. 2 and 3. Over-expression of Scarna10 *in vivo* aggravates CCl_4-induced liver fibrosis. Mice were treated with oil in combination with injection of lenti-control (LV-control, n = 10), or CCl_4 in combination with injection of lenti-Control (LV-Control + CCl_4, n = 10), or oil in combination with injection of lenti-Scarna10 (LV-Scarna10, n = 10), or CCl_4 in combination with injection of lenti-Scarna10 (LV-Scarna10 + CCl_4, n = 10). (A) The expression of Scarna10 in liver tissues of each group was examined by qRT-PCR. (B) Liver fibrosis was evaluated by macroscopic examination, H&E staining, Sirius red staining and IHC for α-SMA, Collagen1 and PCNA; scale bar = 400 μm for 10× and 100 μm for 40×. (C) The protein level of α-SMA, MMP2, Collagen1 and PCNA was determined by western blot. GAPDH was used as an internal control. (D) Quantification of hepatic hydroxyproline content. The data are expressed as hydroxyproline (μg)/liver wet weight (g). (E, F) The mRNA level of hepatic pro-fibrogenic genes (*Acta2, Col1α1, MMP2, TIMP1* and *CTGF*) (E) and

pro-inflammation genes (*TNFα, IL-1β* and *MCP1*) (F) was determined by qRT-PCR. The data are expressed as the mean ± SD for at least triplicate experiments, */#$p<0.05$. *$p<0.05$ for vs LV-Control. #$p<0.05$ for vs LV-Control + CCl_4.

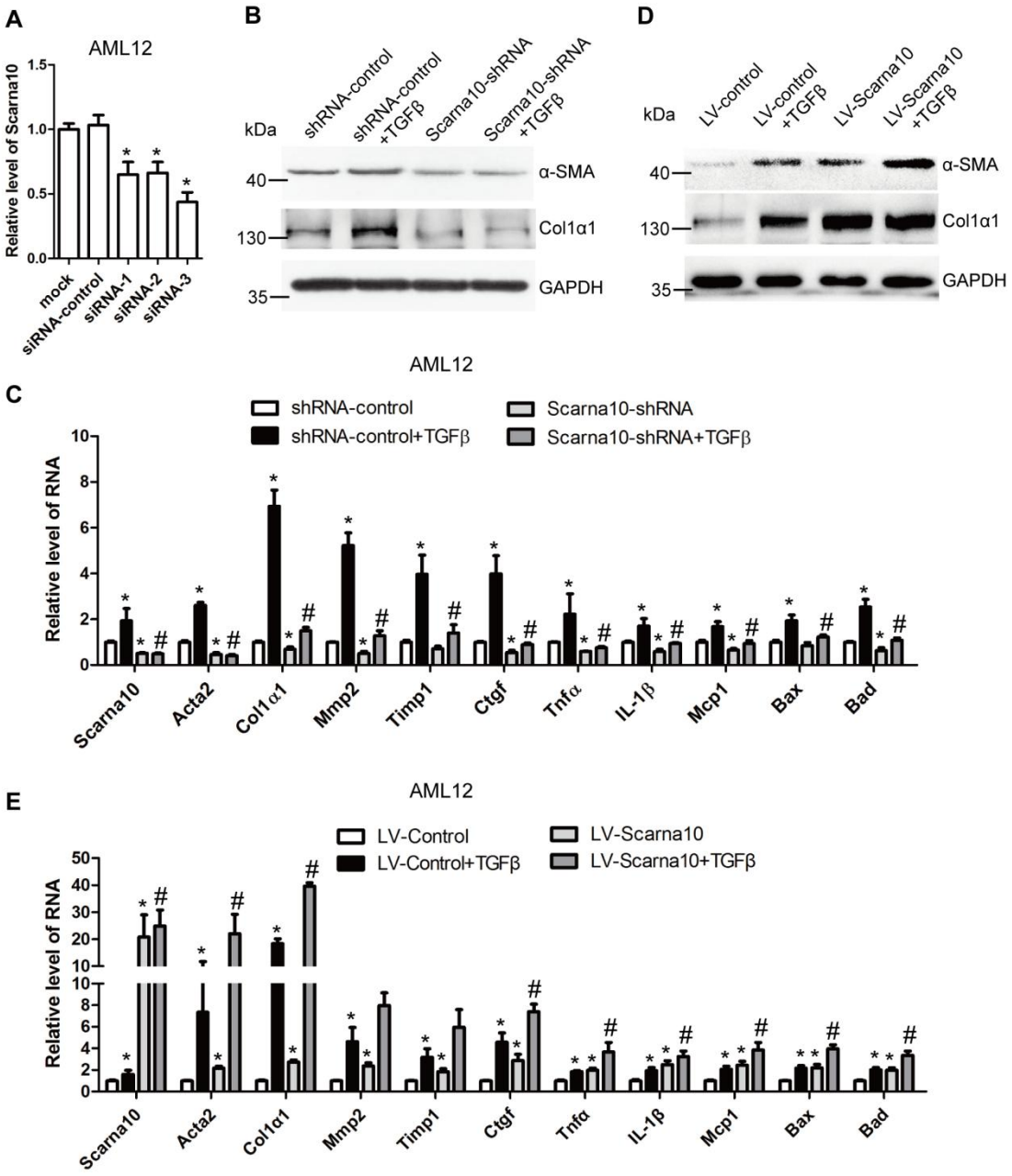

Fig. S3, related to Fig. 4. Scarna10 regulates the expression of extracellular matrix genes in AML12 cells. (A) Targeting of Scarna10 in AML12 cells by three independent siRNAs led to significant depletion of the transcript as measured by qRT-PCR. (B) AML12 cells were infected with lentivirus-mediated shScarna10 for 72 h and further treated with 10 ng/ml TGF-β for additional 24 h. The protein level of α-SMA and Col1α1 was detected by western blot. GAPDH was used as an internal control. (C) The expression of *Scarna10, Acta2, Col1α1, MMP2, TIMP1, CTGF, TNFα, IL-1β, MCP1*, *BAX* and *Bad* was detected by qRT-PCR. (D) The protein level of α-SMA and Col1α1 was detected in Scarna10 up-regulated AML12 cells by western blot. GAPDH was used as an internal control. (E) The RNA level of *Scarna10, Acta2, Col1α1, MMP2, MMP10, TIMP1, TNFα, IL-1β, MCP1*, *BAX* and *Bad*

was detected in AML12 cells infected with lenti-Scarna10 or lenti-Control by qRT-PCR. The data are expressed as the mean ± SD for at least triplicate experiments, */#$p<0.05$. *$p<0.05$ for vs shRNA-control or LV-Control. #$p<0.05$ for vs shRNA-control + TGF-β or LV-Control + TGF-β.

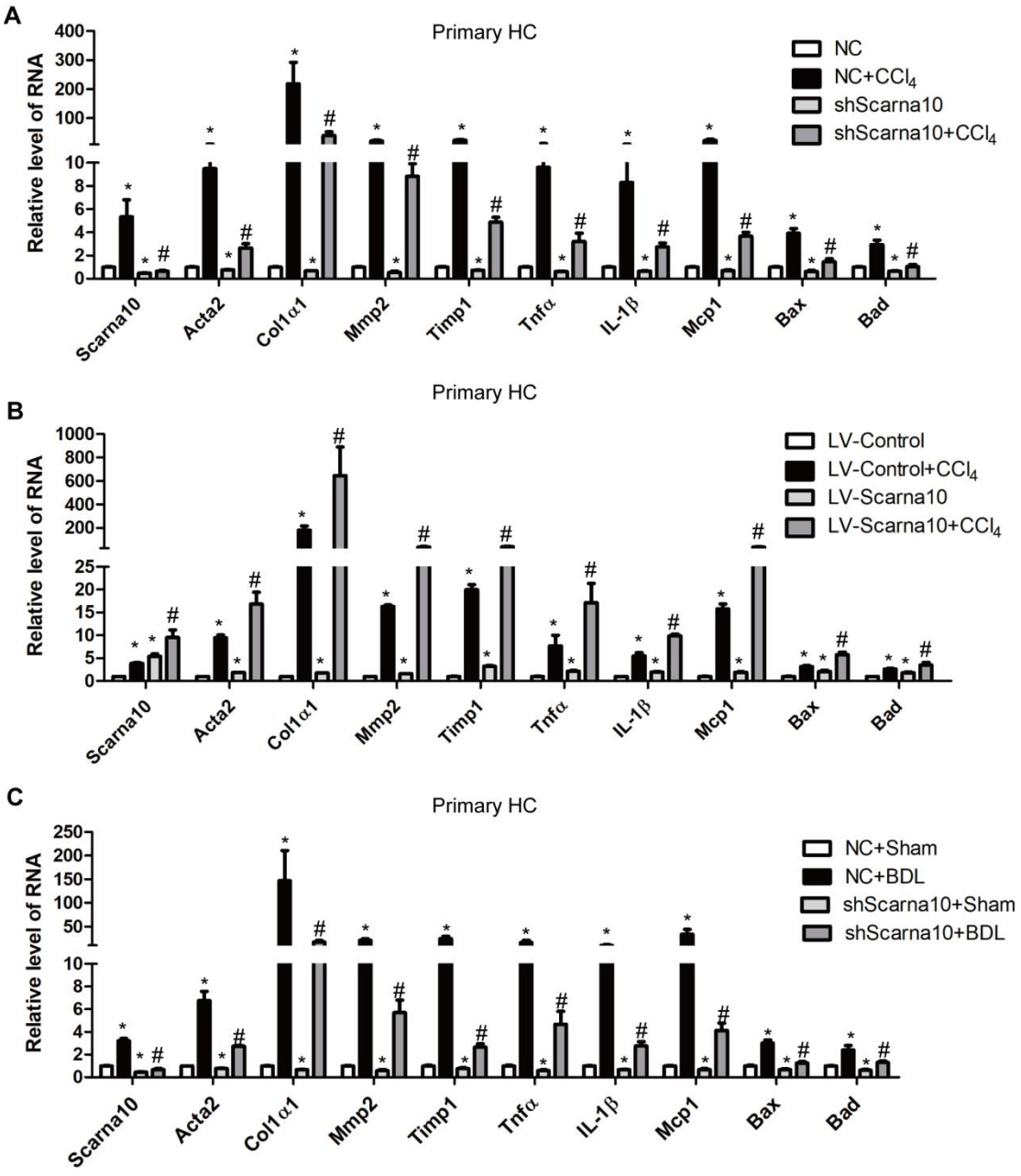

Fig. S4, related to Fig. 4. (A) Mice were treated with oil in combination with injection of lenti-NC (Negative Control, n = 10), or CCl_4 in combination with injection of lenti-NC (NC + CCl_4, n = 10), or oil in combination with injection of lenti-shScarna10 (shScarna10, n = 10), or CCl_4 in combination with injection of lenti-shScarna10 (shScarna10 + CCl_4, n = 10). qRT-PCR analysis of Scarna10, pro-fibrogenic, pro-inflammation and pro-apoptosis genes level in the primary HCs, which were isolated from mice in each group. The data are expressed as the mean ± SD for at least triplicate experiments, */#p<0.05. *p<0.05 for vs NC. #p<0.05 for vs NC+ CCl_4. (B) Mice were treated with oil in combination with injection of lenti-Control (LV-Control, n = 10), or CCl_4 in combination with

injection of lenti-Control (LV-Control+CCl_4, n=10), or oil in combination with injection of lenti-Scarna10 (LV-Scarna10, n=10), or CCl_4 in combination with injection of lenti-Scarna10 (LV-Scarna10+CCl_4, n=10). qRT-PCR analysis of Scarna10, pro-fibrogenic, pro-inflammation and pro-apoptosis genes level in the primary HCs, which were isolated from mice in each group. The data are expressed as the mean ± SD for at least triplicate experiments, The data are expressed as the mean ± SD for at least triplicate experiments, */#p<0.05. *p<0.05 for vs LV-Control. #p<0.05 for vs LV-Control + CCl_4. (C) Mice were treated with sham operation in combination with injection of lenti-NC (NC+Sham, n = 15), BDL operation in combination with injection of lenti-NC (NC+BDL, n = 15), sham operation in combination with injection of lenti-shScarna10 (shScarna10+Sham, n = 15) and BDL operation in combination with injection of lenti-shScarna10 (shScarna10+BDL, n = 15). qRT-PCR analysis of Scarna10, pro-fibrogenic, pro-inflammation and pro-apoptosis genes level in the primary HCs, which were isolated from mice in each group. The data are expressed as the mean ± SD for at least triplicate experiments, */#p<0.05. *p<0.05 for vs NC+Sham. #p<0.05 for vs NC+BDL.

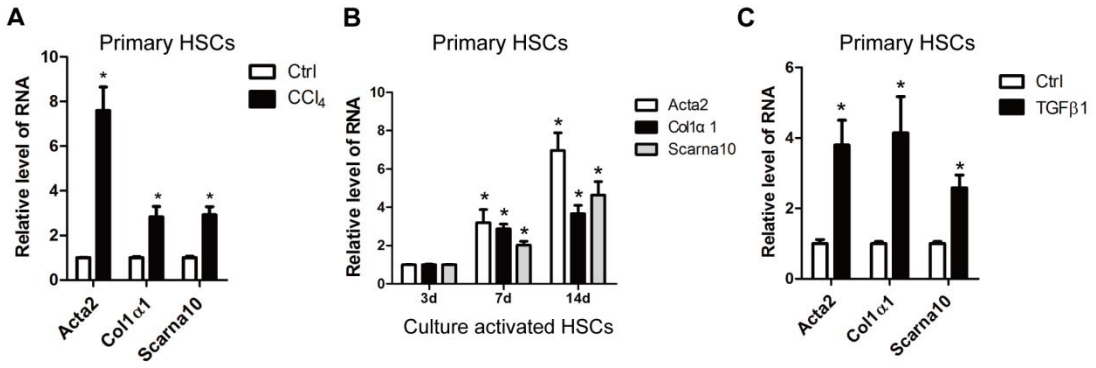

Fig. S5, related to Fig. 5. (A) Primary HSCs were isolated from livers of Balb/c mice treated for 6 weeks with CCl_4 or oil, and the transcripts of *Scarna10* and *Acta2* were determined by qRT-PCR. (B) *Scarna10* and *Acta2* level was measured by qRT-PCR in HSCs after culture-induced activation. (C) Primary HSCs cultured at day 3 were stimulated with TGF-β for 24 h and the transcript of *Scarna10, Col1α1* and *Acta2* was determined by qRT-PCR. The data are expressed as the mean ± SD for at least triplicate experiments, *p< 0.05.

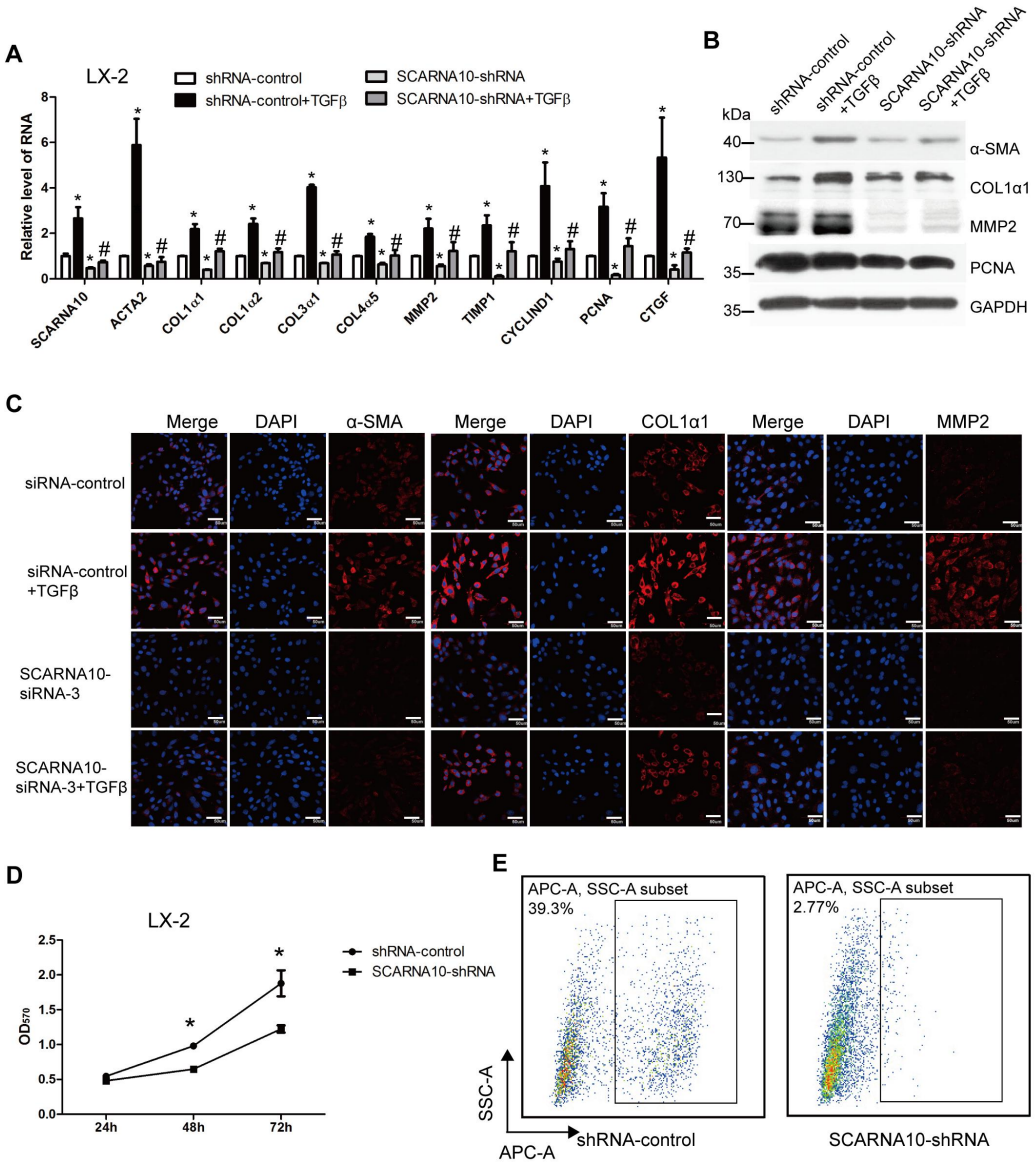

Fig. S6, related to Fig. 5. SCARNA10 promotes LX-2 cells activation and proliferation. (A, B) LX-2 cells were infected with lentivirus-mediated shSCARNA10 for 72 h and further treated with 10 ng/ml TGF-β for additional 24 h. The expression of *Acta2, Col1α1, Col1α2, Col3α1, Col4α5*, *MMP2, TIMP1, CyclinD1, PCNA* and *CTGF* was detected by qRT-PCR (A). The protein level of α-SMA, Col1α1, MMP2 and PCNA was detected by western blot. GAPDH was used as an internal control (B). (C) LX-2 cells were transfected with siRNA for SCARNA10 for 48 h and further treated with 10 ng/ml TGF-β for additional 24 h. The expression of α-SMA, Col1α1 and MMP2 was determined by confocal microscopy. DAPI stained nuclei blue; scale bar = 50 μm. (D, E) LX-2 cells were assessed by an MTT (D) and flow cytometry assays (E) for viability following infected with lenti-shSCARNA10 or lenti-Control. The data are expressed as the mean ± SD for at least triplicate experiments, */#$p<0.05$. *$p<0.05$ for vs shRNA-control. #$p<0.05$ for vs shRNA-control + TGF-β.

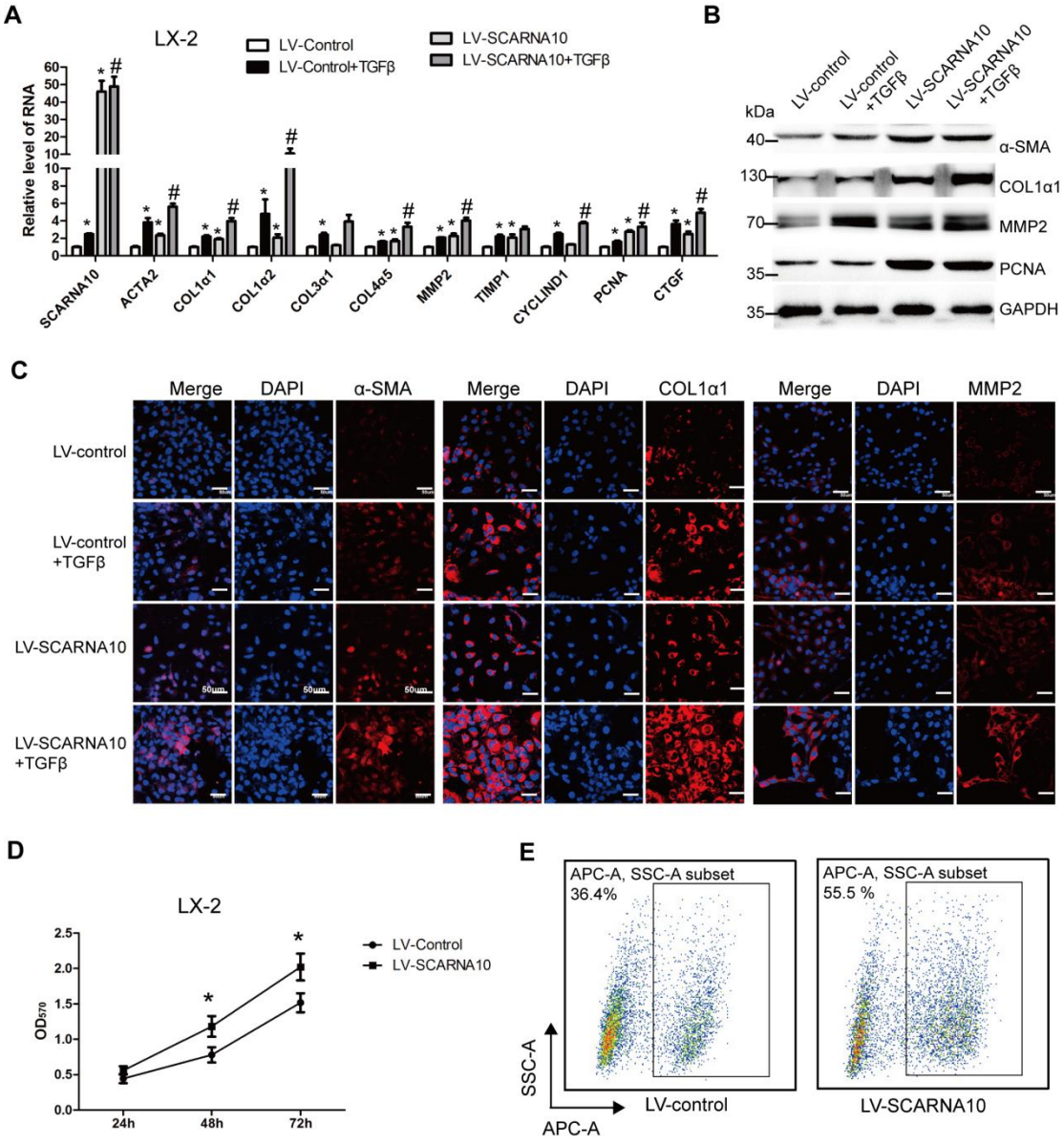

Fig. S7, related to Fig. 5. SCARNA10 promotes LX-2 cells activation and proliferation. (A-C) LX-2 cells were infected with lentivirus-mediated SCARNA10 for 72 h and further treated with 10 ng/ml TGF-β for additional 24 h. The expression of *Acta2, Col1α1, Col1α2, Col3α1, Col4α5*, *MMP2, TIMP1, CyclinD1, PCNA* and *CTGF* was detected by qRT-PCR (A). The protein level of α-SMA, Col1α1, MMP2 and PCNA was detected by western blot. GAPDH was used as an internal control (B). The expression of α-SMA, Col1α1 and MMP2 was determined by confocal microscopy. DAPI stained nuclei blue; scale bar = 50 μm (C). (D, E) LX-2 cells were assessed by an MTT (D) and flow cytometry assays (E) for viability following infected with lenti-SCARNA10 or lenti-Control. The data are expressed as the mean ± SD for at least triplicate experiments, */#p<0.05. *p<0.05 for vs LV-Control. #p<0.05 for vs LV-Control + TGF-β.

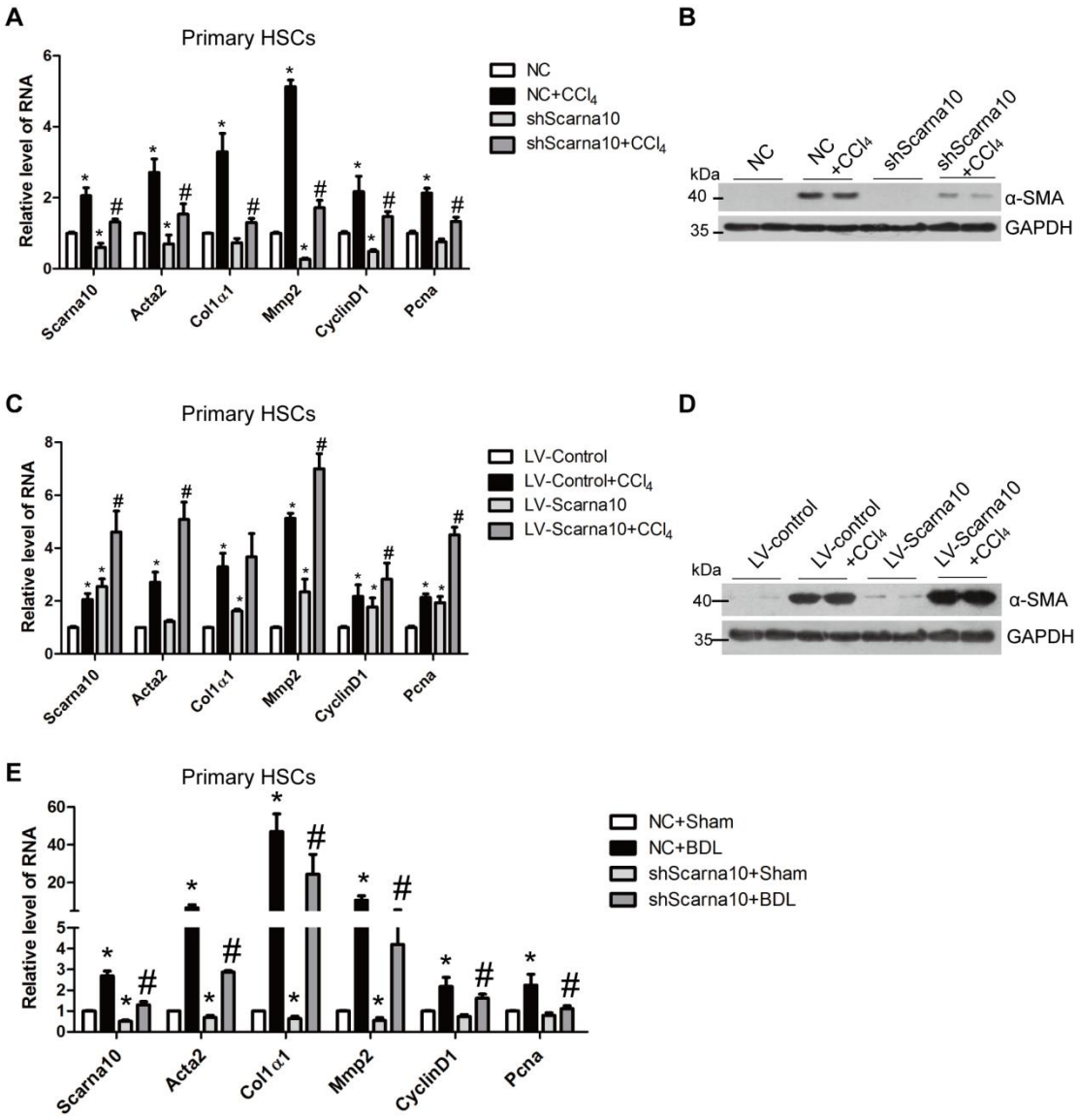

Fig. S8, related to Fig. 5. (A, B) Mice were treated with oil in combination with injection of lenti-NC (Negative Control, n = 10), or CCl_4 in combination with injection of lenti-NC (NC + CCl_4, n = 10), or oil in combination with injection of lenti-shScarna10 (shScarna10, n = 10), or CCl_4 in combination with injection of lenti-shScarna10 (shScarna10 + CCl_4, n = 10). qRT-PCR analysis of Scarna10, pro-fibrogenic and pro-proliferation genes level in the primary HSCs, which were isolated from mice in each group (A). Western blot analysis for α-SMA protein level in primary HSCs that isolated from mice in each group (B). GAPDH was used as an internal control. The data are expressed as the mean ± SD for at least triplicate experiments, */#p<0.05. *p<0.05 for vs NC. #p<0.05 for vs NC + CCl_4. (C, D) Mice were treated with oil in combination with injection of lenti-Control (LV-Control, n=10), or CCl_4 in combination with injection of lenti-Control (LV-Control + CCl_4, n = 10), or oil in combination with injection of lenti-Scarna10 (LV-Scarna10, n = 10), or CCl_4 in combination with injection of

lenti-Scarna10 (LV-Scarna10 + CCl_4, n = 10). qRT-PCR analysis of Scarna10, pro-fibrogenic and pro-proliferation genes level in the primary HSCs, which were isolated from mice in each group (C). Western blot analysis for α-SMA protein level in primary HSCs that isolated from mice in each group (D). GAPDH was used as an internal control. The data are expressed as the mean ± SD for at least triplicate experiments, */#$p<0.05$. *$p<0.05$ for vs LV-Control. #$p<0.05$ for vs LV-Control + CCl_4. (E) Mice were treated with sham operation in combination with injection of lenti-NC (NC + Sham, n = 15), BDL operation in combination with injection of lenti-NC (NC + BDL, n = 15), sham operation in combination with injection of lenti-shScarna10 (shScarna10 + Sham, n = 15) and BDL operation in combination with injection of lenti-shScarna10 (shScarna10 + BDL, n = 15). qRT-PCR analysis of Scarna10, pro-fibrogenic and pro-proliferation genes level in the primary HSCs, which were isolated from mice in each group. The data are expressed as the mean ± SD for at least triplicate experiments, */#$p<0.05$. *$p<0.05$ for vs NC + Sham. #$p<0.05$ for vs NC + BDL.

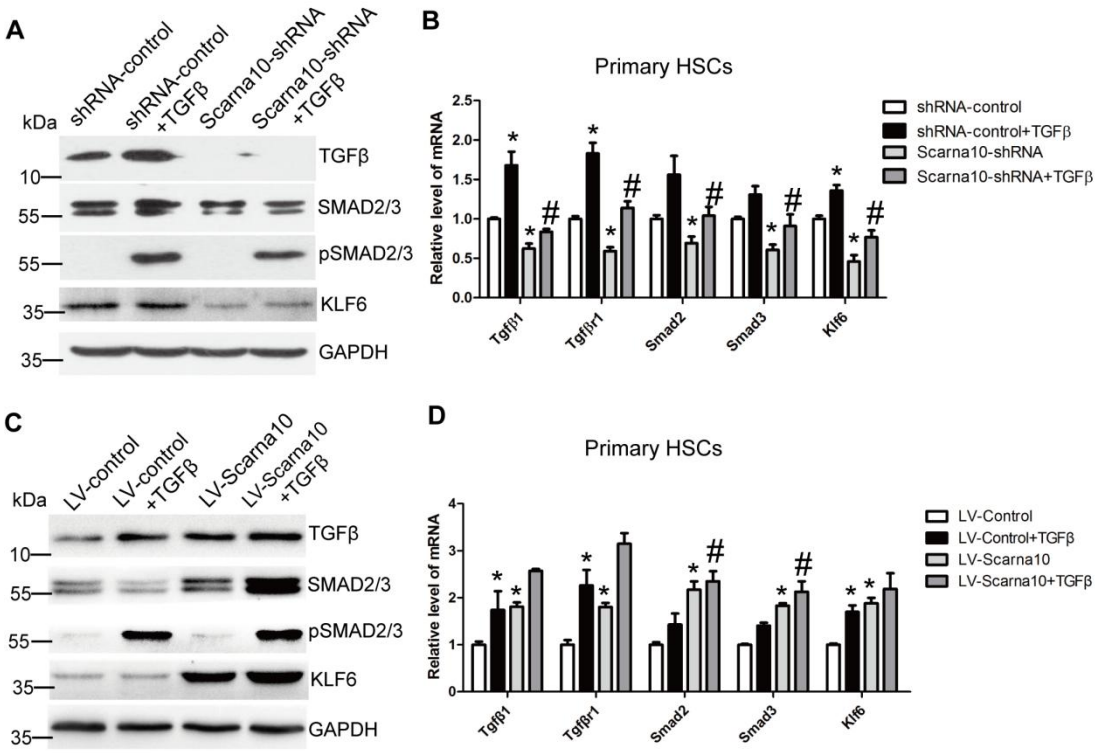

Fig. S9, related to Fig. 6. (A, B) Primary HSCs were infected with lentivirus-mediated shScarna10 for 72 h and further treated with 10 ng/ml TGF-β for additional 24 h. The protein level of pSmad2/3, total Smad2/3 and TGF-β was detected by western blot. GAPDH was used as an internal control (A). The mRNA level of *Tgf-β, Tgf-βr1, Smad2, Smad3* and *Klf6* was determined by qRT-PCR (B). (C, D) Primary HSCs were infected with lentivirus-mediated Scarna10 for 72 h and further treated with 10 ng/ml TGF-β for additional 24 h. The protein level of pSmad2/3, total Smad2/3 and TGF-β was detected by western blot. GAPDH was used as an internal control(C). The mRNA level of *Tgf-β, Tgf-βr1, Smad2, Smad3* and *Klf6* was determined by qRT-PCR(D). The data are expressed as the mean ± SD for at least triplicate experiments, */#$p<0.05$. *$p<0.05$ for vs shRNA-control or LV-Control. #$p<0.05$ for vs shRNA-control + TGF-β or LV-Control + TGF-β.

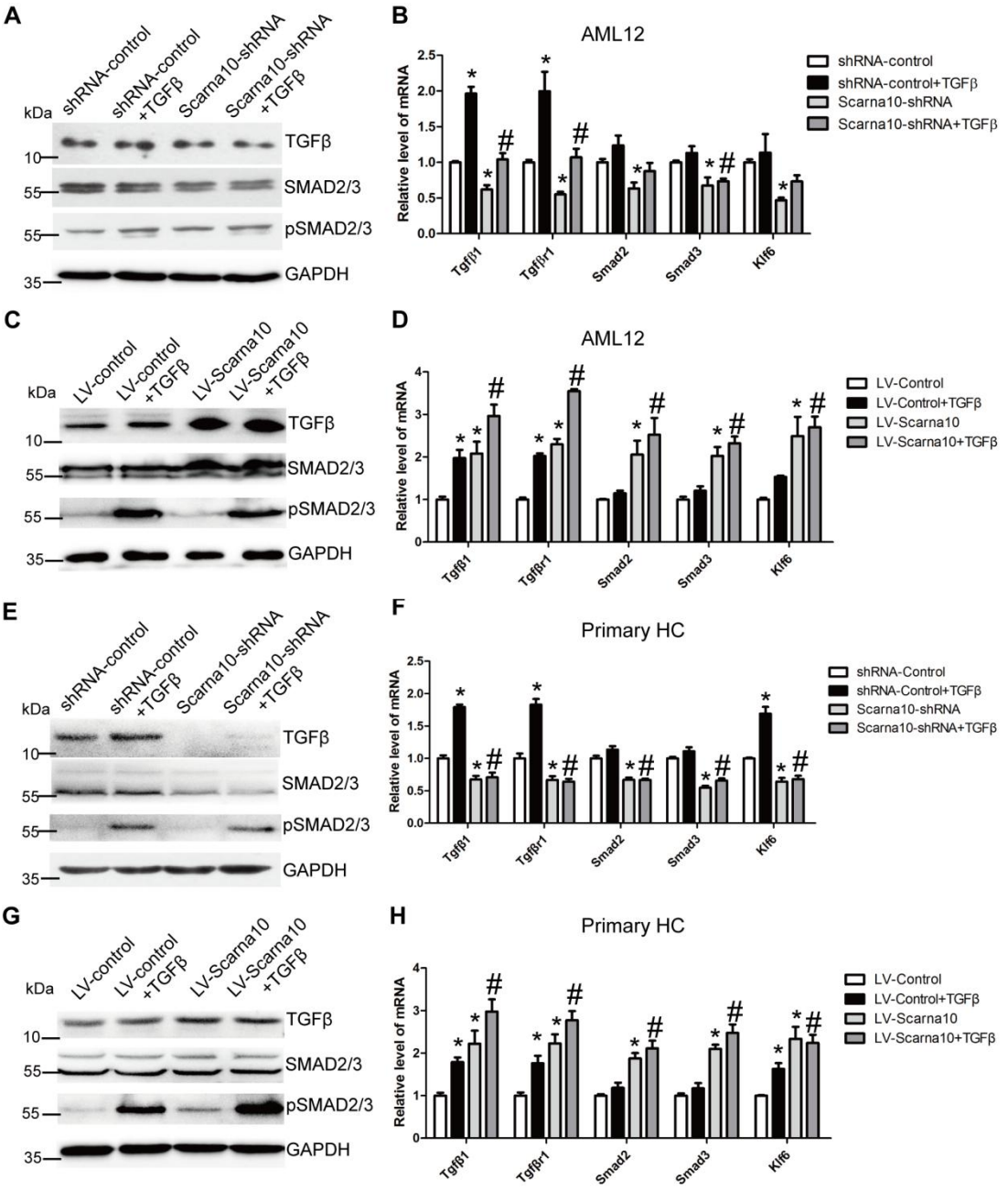

Fig. S10, related to Fig. 6. (A, B) AML12 cells were infected with lentivirus-mediated shScarna10 for 72 h and further treated with 10 ng/ml TGF-β for additional 24 h. The protein level of TGF-β, pSmad2/3 and total Smad2/3 was detected by western blot. GAPDH was used as an internal control (A). The mRNA level of *Tgf-β, Tgf-βr1, Smad2, Smad3* and *Klf6* was determined by qRT-PCR (B). (C, D) AML12 cells were infected with lentivirus-mediated Scarna10 for 72 h and further treated with 10 ng/ml TGF-β for additional 24 h. The protein level of pSmad2/3, total Smad2/3 and TGF-β was detected by western blot. GAPDH was used as an internal control(C) . The mRNA level of *Tgf-β, Tgf-βr1, Smad2, Smad3* and *Klf6* was determined by qRT-PCR (D). (E, F) Primary HCs were infected

with lentivirus-mediated shScarna10 for 72 h and further treated with 10 ng/ml TGF-β for additional 24 h. The protein level of TGF-β, pSmad2/3 and total Smad2/3 was detected by western blot. GAPDH was used as an internal control (E). The mRNA level of *Tgf-β, Tgf-βr1, Smad2, Smad3* and *Klf6* was determined by qRT-PCR (F). (G, H) Primary HCs were infected with lentivirus-mediated Scarna10 for 72 h and further treated with 10 ng/ml TGF-β for additional 24 h. The protein level of pSmad2/3, total Smad2/3 and TGF-β was detected by western blot. GAPDH was used as an internal control(G) . The mRNA level of *Tgf-β, Tgf-βr1, Smad2, Smad3* and *Klf6* was determined by qRT-PCR (H). The data are expressed as the mean ± SD for at least triplicate experiments, */#$p<0.05$. *$p<0.05$ for vs shRNA-control or LV-Control. #$p<0.05$ for vs shRNA-control +TGF-β or LV-Control + TGF-β.

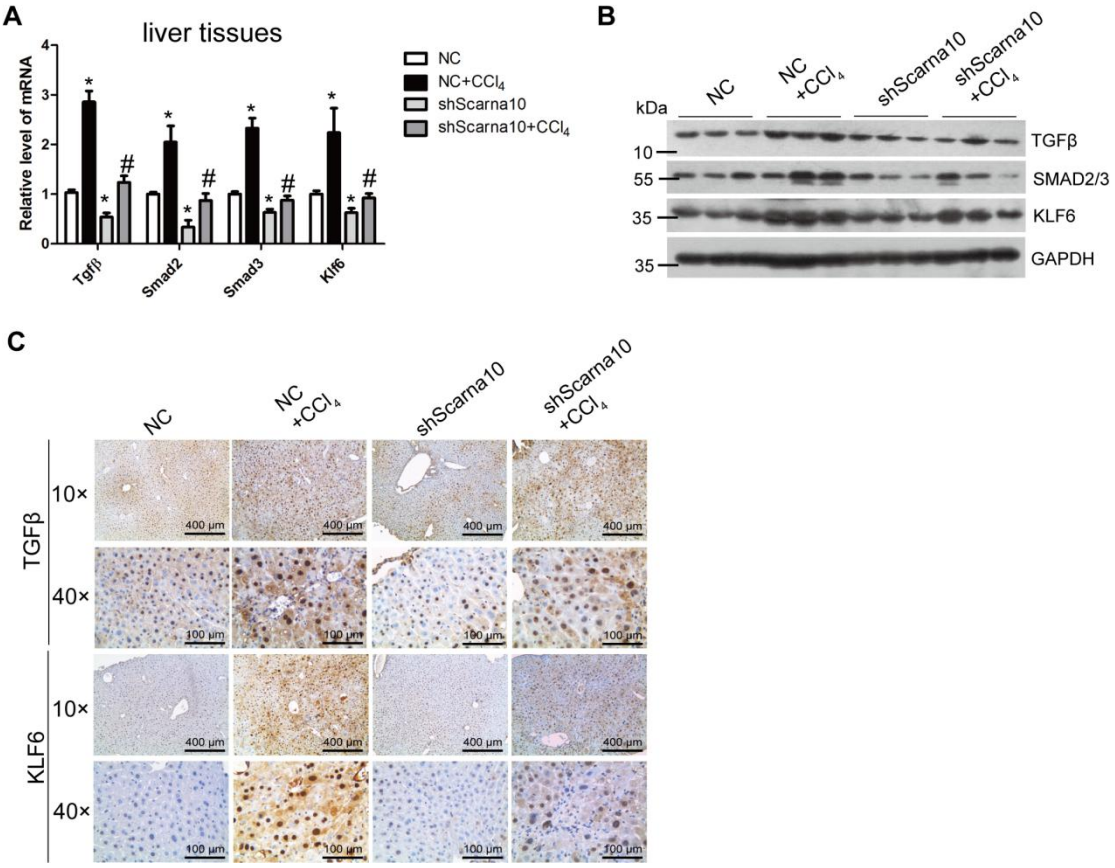

Fig. S11, related to Fig. 6. (A-C) Mice were treated with oil in combination with injection of lenti-NC (Negative Control, n = 10), or CCl_4 in combination with injection of lenti-NC (NC + CCl_4, n = 10), or oil in combination with injection of lenti-shScarna10 (shScarna10, n = 10), or CCl_4 in combination with injection of lenti-shScarna10 (shScarna10 + CCl_4, n = 10). The expression level of TGF-β, KLF6, Smad2 and Smad3 was detected in each group by qRT-PCR(A), western blot (B) and IHC (C). GAPDH was used as an internal control; scale bar = 400 μm for 10× and 100 μm for 40×. The data are expressed as the mean ± SD for at least triplicate experiments, */#p<0.05. *p<0.05 for vs NC. #p<0.05 for vs NC+ CCl_4.

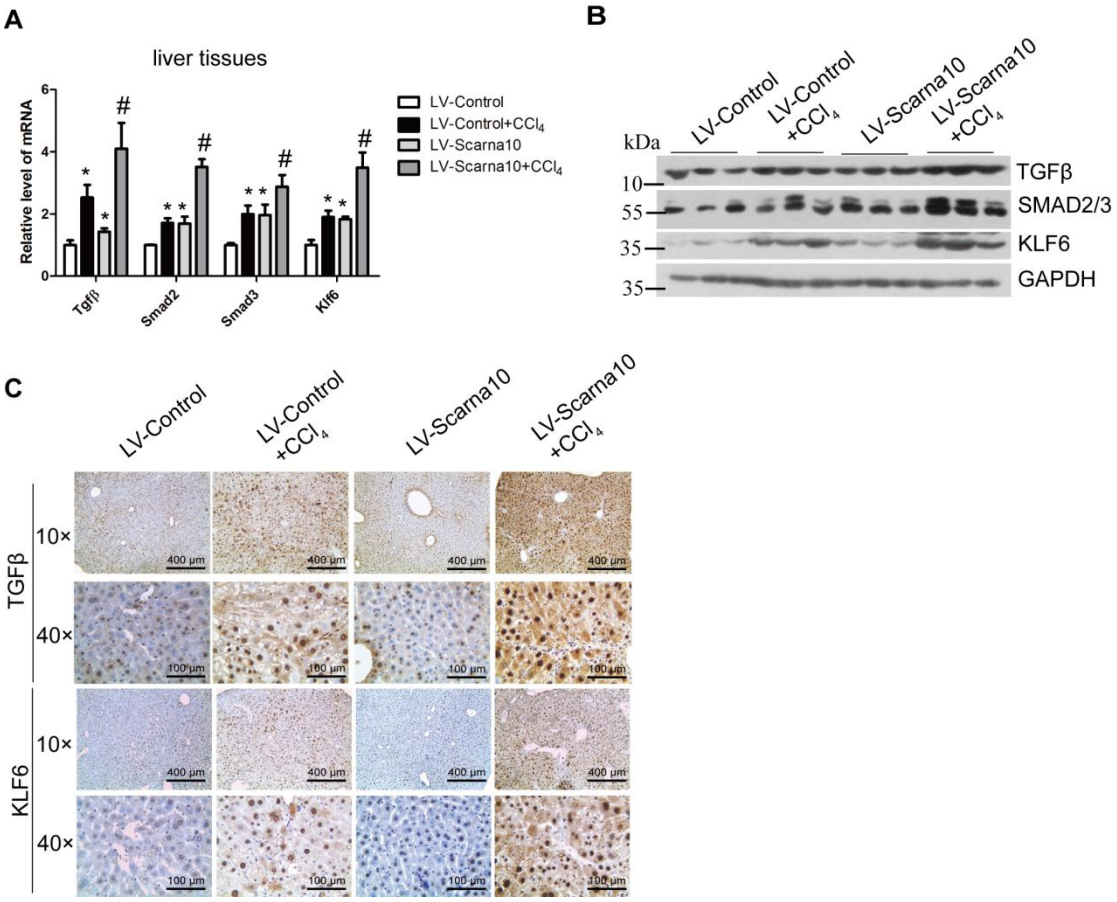

Fig. S12, related to Fig. 6. (A-C) Mice were treated with oil in combination with injection of lenti-Control (LV-Control, n = 10), or CCl_4 in combination with injection of lenti-Control (LV-Control + CCl_4, n = 10), or oil in combination with injection of lenti-Scarna10 (LV-Scarna10, n = 10), or CCl_4 in combination with injection of lenti-Scarna10 (LV-Scarna10 + CCl_4, n = 10). The expression level of TGF-β, KLF6, Smad2 and Smad3 level was detected in each group by qRT-PCR(A), western blot (B) and IHC (C). GAPDH was used as an internal control; scale bar = 400 μm for 10× and 100 μm for 40 ×. The data are expressed as the mean ± SD for at least triplicate experiments, */#p<0.05. *p<0.05 for vs LV-Control. #p<0.05 for vs LV-Control + CCl_4.

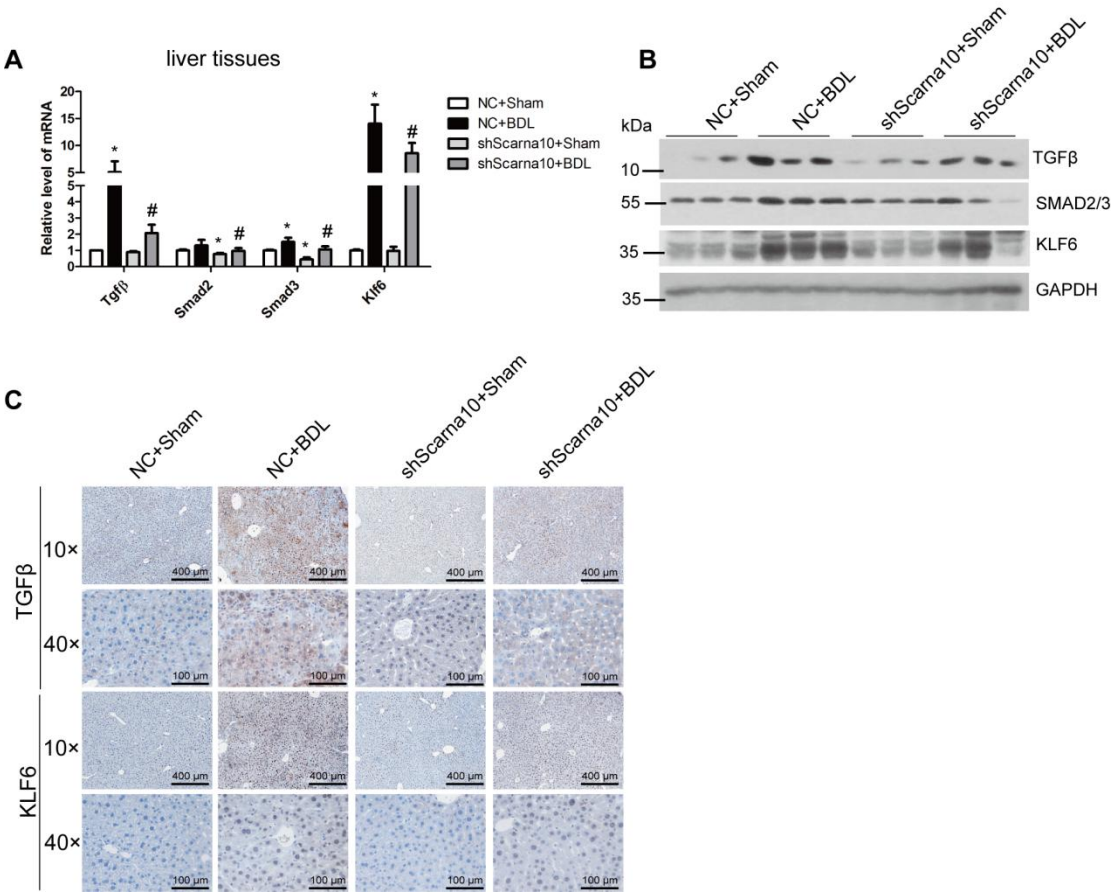

Fig. S13, related to Fig. 6. (A-C) Mice were treated with sham operation in combination with injection of lenti-NC (NC + Sham, n = 15), BDL operation in combination with injection of lenti-NC (NC + BDL, n = 15), sham operation in combination with injection of lenti-shScarna10 (shScarna10 + Sham, n = 15) and BDL operation in combination with injection of lenti-shScarna10 (shScarna10 + BDL, n = 15). The expression level of TGF-β, KLF6, Smad2 and Smad3 level was detected in each group by qRT-PCR(A), western blot (B) and IHC (C). GAPDH was used as an internal control; scale bar = 400 μm for 10× and 100 μm for 40×. The data are expressed as the mean ± SD for at least triplicate experiments, */#$p<0.05$. *$p<0.05$ for vs NC + Sham. #$p<0.05$ for vs NC + BDL.

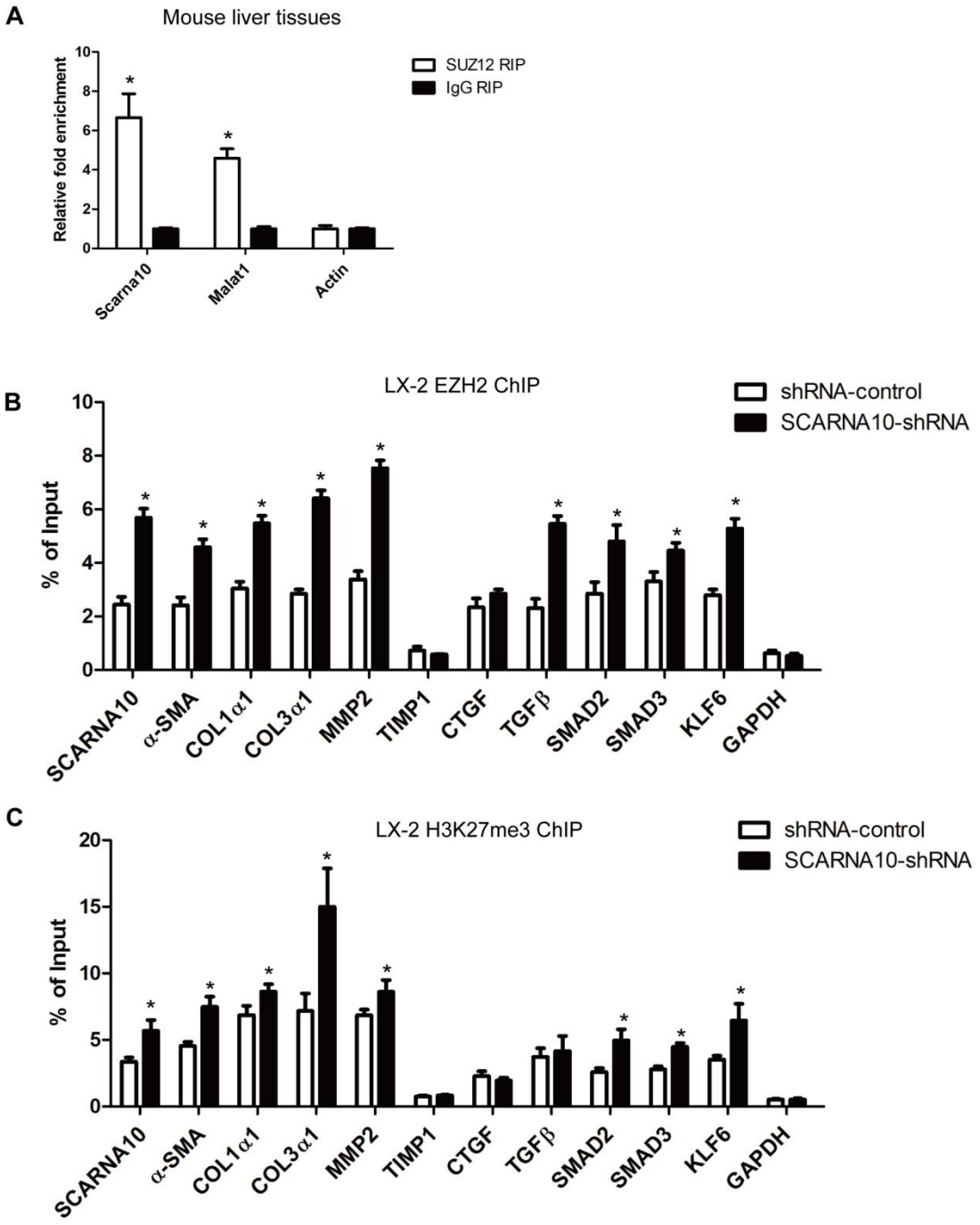

Fig. S14, related to Fig. 7. (A) qRT-PCR detection of *Scarna10* and *Malat1* retrieved by SUZ12-specific antibody compared with IgG in the RIP assay within the single cell suspensions isolated from mouse liver. (B, C) LX-2 cells were infected with lenti-shSCARNA10 or shRNA-control, and ChIP analyses were performed on indicated genes promoter regions using anti-EZH2 and anti-H3K27me3 antibody. Enrichment was shown relative to input. The data are expressed as the mean ± SD for at least triplicate experiments, *$p< 0.05$.

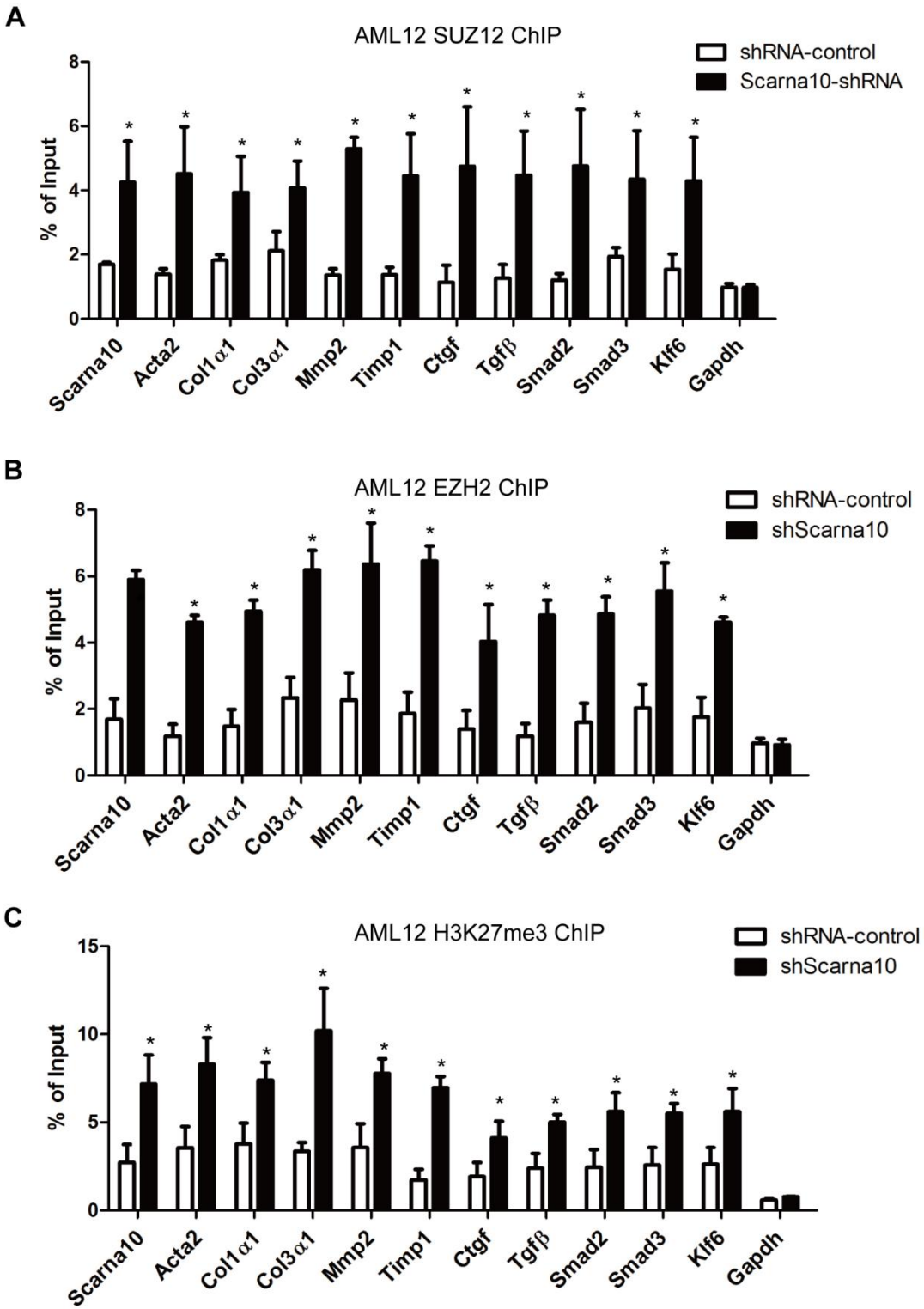

Fig. S15, related to Fig. 7. (A-C) AML12 cells were infected with lenti-shScarna10 or shRNA-control, and ChIP analyses were performed on indicated genes promoter regions using anti-SUZ12, anti-EZH2 and anti-H3K27me3 antibody. Enrichment was shown relative to input. The data are expressed as the mean ± SD for at least triplicate experiments, *$p< 0.05$.

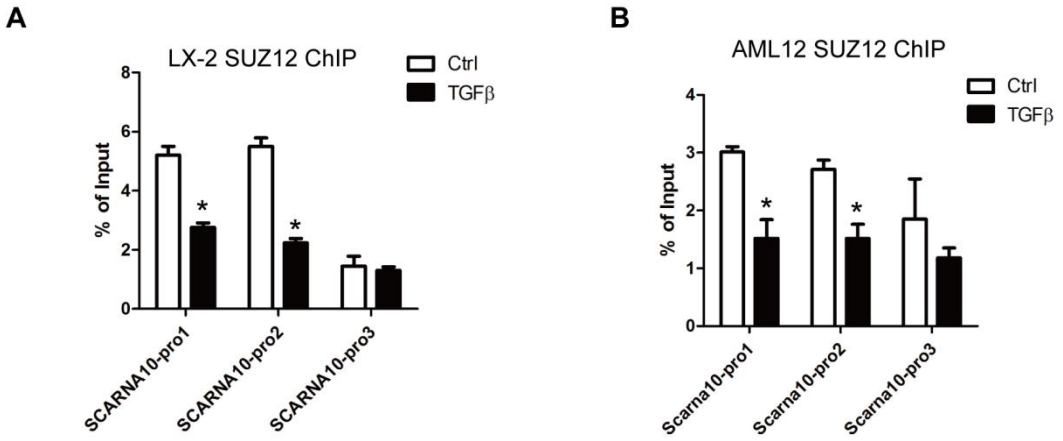

Fig. S16, related to Fig. 7. (A, B) LX-2 and AML12 cells were treated with or without TGF-β, and ChIP analyses were performed on SCARNA10 promoter regions using anti-SUZ12 antibody. Enrichment was shown relative to input. The data are expressed as the mean ± SD for at least triplicate experiments, *$p< 0.05$.

Supplementary Tables

Table S1. Baseline characteristics of patients with liver tissue

Metavir score	F0	F1	F2	F3	F4
Cases(n)	12	14	14	9	16
Age (years)*	55.1±13.9	52.6±10.7	53.7±9.0	48.8±10.2	52.6±8.4
Male sex (*n* (%))	8 (66.7)	6(42.9)	7(50.0)	5(55.6)	10 (62.5)
ALT(U/L)*	19.2±9.1	30.8±17.1	43.5±32.0	34.0±14.6	34.6±17.5
AST(U/L)*	20.8±10.4	46.4±63.5	53.9±67.7	41.1±16.0	45.3±23.0
ALB(g/L)*	43.7±7.9	44.8±12.9	42.1±15.9	46.3±11.5	44.4±7.3
GGT(U/L)*	34.3±22.7	67.7±45.5	76.5±55.0	116.2±94.9	137.9±114.4
Etiology(*n* (%))					
Biliary Obstruction	0(0)	4(28.6)	4(28.6)	3(33.3)	1(6.2)
HBV	0(0)	10(71.4)	8(57.1)	6(66.7)	13(81.3)
HCV	0(0)	0(0)	2(14.3)	0(0)	2(12.5)

*Mean±SD.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, Albumin; GGT, γ-glutamyl transpeptadase; HBV, hepatitis B virus; HCV, hepatitis C virus.

Table S2. Baseline characteristics of patients with serum

Characteristics	Controls	Fibrosis	Cirrhosis
Cases(n)	35	38	45
Age (years)*	51.4±4.6	52.4±11.4	55.0±10.4
Male sex (*n* (%))	22 (62.9)	25 (65.8)	23 (51.1)
ALT(U/L)*	15.7±6.8	50.8±51.2	76.3±62.1
AST(U/L)*	16.3±6.2	69.1±53.8	73.1±50.9
ALB(g/L)*	46.0±5.3	43.1±7.7	37.7±6.4
GGT(U/L)*	25.9±19.6	75.0±65.4	128.8±109.9
Etiology(*n* (%))			
Alcoholic	0(0)	2(5.3)	5(11.1)
HBV	0(0)	34(89.5)	38(84.4)
HCV	0(0)	2(5.3)	2(4.4)

*Mean±SD.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, Albumin; GGT, γ-glutamyltranspeptadase; HBV, hepatitis B virus; HCV, hepatitis C virus.

Table S3. Primers and Oligonucleotides

qRT-PCR primers for analysis of transcript levels

Gene symbol	Forward 5' - 3'	Reverse 5 '- 3'
Mouse Scarna10	CCAGGGAGGAATTGTGGTAA	TCTGTGTGTCATCTCTCAGTGG
Mouse Scarna10	GTCCAGGGACCTCTGACCTA	TTTTTGTCGGTCCGCCCTTA
Mouse Scarna10	TAAGGGCGGACCGACAAAAA	TGACCCTGGCACCTCGAATA
Mouse Malat1	AAATTGATGGCCTTTTCTGG	AGCTGGATCCTTGAGGTCAC
Mouse β-Actin	ATGCCACAGGATTCCATACCCAAGA	CTCTAGACTTCGAGCAGGAGATGG
Mouse Gapdh	GGCATGGACTGTGGTCATGAG	TGCACCACCAACTGCTTAGC
Mouse Col1α1	ATCGGTCATGCTCTCTCCAAACCA	ACTGCAACATGGAGACAGGTCAGA
Mouse Col1α2	CCTTTGTCAGAATACTGAGCAGC	GTAACTTCGTGCCTAGCAACA
Mouse Col3α1	TGCTCCAGTTAGCCCTGCAA	GGTCCTGCAGGCAACAGTGGTTC
Mouse Col4α5	CTCCCTTACCGCCCTTTTCTC	AGGCGAAATGGGTATGATGGG
Mouse Ctgf	ATCCAGGCAAGTGCATTGGTA	GGGCCTCTTCTGCGATTTC
MousePcna	TTTGAGGCACGCCTGATCC	GGAGACGTGAGACGAGTCCAT
Mouse Acta2	TCGGATACTTCAGCGTCAGGA	GTCCCAGACATCAGGGAGTAA
Mouse Timp1	TCCGTCCACAAACAGTGAGTGTCA	GGTGTGCACAGTGTTTCCCTGTTT
Mouse Mmp2	GTGTTCTTCGCAGGGAATGAG	GATGCTTCCAAACTTCACGCT
Mouse Tgfβ1	TGTGTTGGTTGTAGAGGGCAAGGA	TTTGGAGCCTGGACACACAGTACA
MouseTgfβr1	GACAACATCAGGGTCTGGATCA	ACTTCTCCAAACCGACCTTTGC
Mouse Ki67	CATCCATCAGCCGGAGTCA	TGTTTCGCAACTTTCGTTTGTG
Mouse Bax	TTGCTGATGGCAACTTCAAC	GATCAGCTCGGGCACTTTAG
MouseBad	AGAGTATGTTCCAGATCCCAG	GTCCTCGAAAAGGGCTAAGC
MouseTnfα	CATCTTCTCAAAATTCGAGTGACAA	TGGGAGTAGACAAGGTACAACCC
Mouse Il-1β	GTCGCTCAGGGTCACAAGAA	GTGCTGCCTAATGTCCCCTT
MouseMcp1	GTTAACGCCCCACTCACCTG	GGGCCGGGGTATGTAACTCA
MouseCyclinD1	TCAAGTGTGACCCGGACTG	ATGTCCACATCTCGCACGTC
MouseKlf6	CGCACTCACACAGGAGAAAA	GTATGCTTTCGGAAGTGTCT
Mouse Smad2	CTTGGCTGTCCTCATACACGAA	CCGAGTCTCCTGTTCCCGTA

Mouse Smad3	GTTGGACGAGCTGGAGAAG	GTAGTAGGAGATGGAGCAC
Mouse SUZ12	CTCAGGATATACATCGCCAACCT	TGTTCTTTTTGGCCTGCAAAC
Mouse EZH2	TTGTTGGCGGAAGCGTGTAAAATC	TCCCTAGTCCCGCGCAATGAGC
Human SCARNA10	GTTTGGCTAAGCCCAGGGAC	GTTGGTCTGCCCTTACAGTGA
Human SCARNA10	CTTGGTGGGCGATACAGAGT	CTTTAGGACCCTTGGCCCTG
Human SCARNA10	AATCTTGGTGGGCGATACAG	CAGCAACTGGAAGAATCACC
Human MALAT1	GCTCTGTGGTGTGGGATTGA	GTGGCAAAATGGCGGACTTT
Human β-ACTIN	GCCGGGACCTGACTGACTAC	TTCTCCTTAATGTCACGCACGAT
Human GAPDH	ACCCAGAAGACTGTGGATGG	TTCAGCTCAGGGATGACCTT
Human COL1α1	AACCAAGGCTGCAACCTGGA	GGCTGAGTAGGGTACACGCAGG
Human COL1α2	GCAGGAGGTTTCGGCTAAGT	GCAACAAAGTCCGCGTATCC
Human COL3α1	AGCCTTGCGTGTTCGATAT	GAAGATGTCCTTGATGTGC
Human COL4α5	TTCAGCGTTTCTGACTGAGG	AGAGCATCCAGCCATTCATT
Human CTGF	TGCTTTGAACGATCAGACAA	CTTGTGGCAAGTGAATTTCC
Human PCNA	TCCATCCTCAAGAAGGTGTT	GGTAGGTGTCGAAGCCC
Human ACTA2	GCCATGTTCTATCGGGTACTTC	CAGGGCTGTTTTCCCATCCAT
Human TIMP1	GGGGCTTCACCAAGACCTAC	GGAAGCCCTTTTCAGAGCCT
Human MMP2	GTGTTCTTCGCAGGGAATGAG	GATGCTTCCAAACTTCACGCT
Human MMP9	CCTTGTGCTCTTCCCTGGAG	GGCCCCAGAGATTTCGACTC
Human MMP10	GCTCTGCCTATCCTCTGAGTG	CAACGTCAGGAACTCCACACC
Human TGFβ1	TGTTGGACAGCTGCTCCACCT	GGCAGTGGTTGAGCCGTGGA
HumanTGFβR1	TCCAACTACTGTAAAGTCATCACC	AAGCACACTGGTCCAGCAAT
Human CYCLIND1	GTGGCCTCTAAGATGAAGGAGA	GGAAGTGTTCAATGAAATCGTG
Human KLF6	CGGACGCACACAGGAGAAAA	CGGTGTGCTTTCGGAAGTG
Human SMAD2	GCCTTTACAGCTTCTCTGAACAA	ATGTGGCAATCCTTTTCGAT
Human SMAD3	CCCCAGCACATAATAACTTGG	AGGAGATGGAGCACCAGAAG
Human SUZ12	GGGAGACTATTCTTGATGGGAAG	ACTGCAACGTAGGTCCCTGA
Human EZH2	TTGTTGGCGGAAGCGTGTAAAATC	TCCCTAGTCCCGCGCAATGAGC

RACE primers for Mouse-Scarna10

gene specific primer	Sequence 5' - 3'
3' OUTER PRIMER	GATTACGCCAAGCTTCCAGGGACCTCTGACCTATTCAGGTCTG
3' INNER PRIMER	GATTACGCCAAGCTTCCAGGGAGGAATTGTGGTAACTGGAATG
5' OUTER PRIMER	GATTACGCCAAGCTTTGTGTCATCTCTCAGTGGCCATGACACA
5' INNER PRIMER	GATTACGCCAAGCTTGCTGGAGAATCACCCCGACTGGAGTG

RACE primers for Human-SCARNA10

gene specific primer	Sequence 5' - 3'
3' OUTER PRIMER	GATTACGCCAAGCTTGGTCTGTAATCTTGGTGGGCGATACAGA
3' INNER PRIMER	GATTACGCCAAGCTTTGTGTTCACTGTAAGGGCAGACCAAC
5' OUTER PRIMER	GATTACGCCAAGCTTTAGGACCCTTGGCCCTGATACCCTG
5' INNER PRIMER	GATTACGCCAAGCTTTGCCCTGTTCCTACTCTCTCACTCC

Cloning primers for Mouse-Scarna10

Name	Sequence 5' - 3'
Scarna10 5' BamHI F	CGCGGATCCGCCATGTGATGATATCAAGGC
Scarna10 5' BamHI R1	CGCGGATCCAGCCATCAGATTACCAAAGATC
Scarna10 5' BamHI R2	CGCGGATCCAGCCATCAGATTACCAAAG

Cloning primers for Human-SCARNA10

Name	Sequence 5' - 3'
SCARNA10 5' BamHI F	CGCGGATCCGCCACATGATGATATCAAGGC
SCARNA10 5' BamHI R1	CGCGGATCCAGCCATCAGATTACCAAAGATC
SCARNA10 5' BamHI R2	CGCGGATCCAGCCATCAGATTACCAAAG

siRNA sequences

Name	Forward 5' - 3'	Reverse 5' - 3'
Mouse Scarna10-1	GGACCUCUGACCUAUUCAGTT	CUGAAUAGGUCAGAGGUCCTT
Mouse Scarna10-2	CCAGGGAGGAAUUGUGGUATT	UACCACAAUUCCUCCCUGGTT
Mouse Scarna10-3	GAGAUGACACACAGAUCUUTT	AAGAUCUGUGUGUCAUCUCTT
Mouse SUZ12-1	GGAUGUAAGUUGUCCAAUA	UAUUGGACAACUUACAUCC
Mouse SUZ12-2	GGAUGUAAGUUGUCCAAUA	UAUUGGACAACUUACAUCC
Mouse EZH2-1	GUGUAUGAGUUUAGAGUCA	UGACUCUAAACUCAUACAC
Mouse EZH2-2	GCUGACCAUUGGGACAGUA	UACUGUCCCAAUGGUCAGC
Human SCARNA10-1	GGGACCUUUGGCCUGUUAATT	UUAACAGGCCAAAGGUCCCTT
Human SCARNA10-2	CCAAGGGUCCUAAAGGACUTT	AGUCCUUUAGGACCCUUGGTT
Human SCARNA10-3	CACAGAUCUUUGGUAAUCUTT	AGAUUACCAAAGAUCUGUGTT
Human SUZ12-1	GGAUGUAAGUUGUCCAAUA	UAUUGGACAACUUACAUCC
Human SUZ12-2	GGAUGUAAGUUGUCCAAUA	UAUUGGACAACUUACAUCC
Human SUZ12-3	GUCGCAACGGACCAGUUAA	UUAACUGGUCCGUUGCGAC
Human EZH2-1	GUGUAUGAGUUUAGAGUCA	UGACUCUAAACUCAUACAC
Human EZH2-2	GCUGACCAUUGGGACAGUA	UACUGUCCCAAUGGUCAGC
Human EZH2-3	GGCACUUACUAUGACAAUU	AAUUGUCAUAGUAAGUGCC
negative control	GUUCUCCGAACGUGUCACGTT	CGUGACACGUUCGGAGAACTT

shRNA sequences

Name	Sequence 5' - 3'
Mouse sh-Scarna10-1 Forward	GATCCCCGGACCTCTGACCTATTCAGTTCAAGAGACTGAATAGGTCAGAGGTCCTTTTTA
Mouse sh-Scarna10-1 Reverse	AGCTTAAAAAGGACCTCTGACCTATTCAGTCTCTTGAACTGAATAGGTCAGAGGTCCGGG
Mouse sh-Scarna10-2 Forward	GATCCCCCCAGGGAGGAATTGTGGTATTCAAGAGATACCACAATTCCTCCCTGGTTTTTA
Mouse sh-Scarna10-2 Reverse	AGCTTAAAAACCAGGGAGGAATTGTGGTATCTCTTGAATACCACAATTCCTCCCTGGGGG
Mouse sh-Scarna10-3 Forward	GATCCCCGAGATGACACACAGATCTTTTCAAGAGAAAGATCTGTGTGTCATCTCTTTTTA
Mouse sh-Scarna10-3 Reverse	AGCTTAAAAAGAGATGACACACAGATCTTTCTCTTGAAAAGATCTGTGTGTCATCTCGGG

Human sh-SCARNA10-1 Forward	GATCCCCGGGACCTTTGGCCTGTTAATTCAAGAGATTAACAGGCCAAAGGTCCCTTTTTA
Human sh-SCARNA10-1 Reverse	AGCTTAAAAAGGGACCTTTGGCCTGTTAATCTCTTGAATTAACAGGCCAAAGGTCCCGGG
Human sh-SCARNA10-2 Forward	GATCCCCCCAAGGGTCCTAAAGGACTTTCAAGAGAAGTCCTTTAGGACCCTTGGTTTTTA
Human sh-SCARNA10-2 Reverse	AGCTTAAAAACCAAGGGTCCTAAAGGACTTCTCTTGAAAGTCCTTTAGGACCCTTGGGGG
Human sh-SCARNA10-3 Forward	GATCCCCCACAGATCTTTGGTAATCTTTCAAGAGAAGATTACCAAAGATCTGTGTTTTTA
Human sh-SCARNA10-3 Reverse	AGCTTAAAAACACAGATCTTTGGTAATCTTCTCTTGAAAGATTACCAAAGATCTGTGGGG
Negative control Forward	GATCCCCGTTCTCCGAACGTGTCACGTTCAAGAGACGTGACACGTTCGGAGAACTTTTTA
Negative control Reverse	AGCTTAAAAAGTTCTCCGAACGTGTCACGTCTCTTGAACGTGACACGTTCGGAGAACGGG

Primers for Mouse ChIP qRT-PCR

Locus	Forward 5' - 3'	Reverse 5 '- 3'
Scarna10(-370--165)	GCTCTGGAAAGAACTGGTGGC	GCCTACAGAGCCCATTCTCA
Scarna10(-1250--1133)	TAACAGGCGGGTTGCAGAAG	AAATGTCCCGTACGGTTGCT
Scarna10(-1850--1780)	CTCATTTCTGGGGCACCGAT	TATGGACTGTTTCTGGGCCG
Col1α1(-424--253)	CCAGGAGGACCTTTTCCCAA	GTGCTGTCACTGGAGTGTGG
Col1α1(-730--640)	GGATGTCAAAGGTCTCCCCA	GGGTGCCTATCTGTTCTGCC
Col1α1(-1455--1380)	GACTCCCTGCTTCCACGTTT	TTGCAGGGCCCATAGACATC
Col1α1(-1969--1869)	GCTTCGTGGCATTCTACCCT	TTCCAAAGGATGCCCCACTC
Col1α2(-340--230)	AGCCCACGTAGGTGTCCTAA	GCTTTCGAGGGGGAACTCTG
Col1α2(-1343--1243)	TTCCTCACCGGGAAGTCGAA	TCACAGCAGACACAGCATCTT
Acta2(-549--314)	AGGAGAGTGAGCAGGCTTCATT	AGTGAGGATTAACCAGCCTGT
Acta2(-1147--1242)	AACTATGCATGCGCTCAGGT	TAGGGAAACCCCAGGGTGAA
Acta2(-1929--1998)	GAGGAATGTGCAAACCGTGC	CAACTGCTCAAATGCCCAGAC
Ctgf(-39-+81)	GAATGTGAGGAATGTCCCTGTT	CTTGGAGAGAAGAGCTGTGTGA
Ctgf(-1090--1059)	CAACACACGAGCAGGGGATA	AATAGCTTGCAGGCTCGTGG
Tgfβ1(-231--93)	TCACCGGCTTTAGTAGTGCTC	GGGGGCACTGTCTTCATCT
Tgfβ1(-1263--1089)	TGGACTTTGTTCTGTGGCCC	GAAACCACTGGAGACCTCGC

Tgfβ1(-1984--1915)	AGAGTCTCAGAACATAGTCCAGC	GAAGGGTGACATTTTGGCACA
Mmp2(-440--305)	GTTTGGAGAAGGAAGGCTGGT	AGAAACAAGAGGGTCCCAACC
Mmp2(-843-729)	CCAACTCTGTTCAGGCAGGT	CAGGGGCCAGCAAGGATAAT
Mmp2(-1517--1389)	CCCAGCTCAGGTCCTTGTTT	AGGGATTCACGGTTGTCACC
Timp1(-630--440)	TAGGACTCCAGGGTCAGGAAG	AGCCTAGGTACCCCAAACCT
Timp1(-291--132)	AGTTTGTCACCCTCTGACACC	AAGCTTTGTGCCTCTCAGGTT
Timp1(-1909--1837)	GGCTCATAGAAGAGGCGAGAC	GCTGAGTAACTAGGCGGCAG
Smad2(-222--80)	TCAAGGAGCACACGCATAGG	TCCGTGCGGTTGGTATTAGG
Smad2(-1076-1002)	TGGTGGTGCTGGGGTTAAAA	GCAGAGGATAGAGCTTCCCG
Smad2(-1853--1733)	TTCAGCTCGTCTTGACCCAC	AAAGGGAATAGGGGGCAACC
Smad3(-198--7)	GAAGGAAAGTCCAACCCCCA	GCTGCGTGAAACGTAGACTTG
Smad3(-1271--1803)	ACGGATTTGGGGCGTTACAT	ATAGGGCTTCGTAAAGCGCA
Smad3(-1952-1778)	TGCTACTGGCCCTAGAACTGT	GAATCAACACTGGCCTCCACT
Klf6(-330--217)	TTCTGCAACGTTGGGCTGTA	CTGCGCTGATTGGAAAGACG
Klf6(-1049--882)	CCGAGACTGCCTTCTACGAG	AAGATCGCATCCGTGACCTG
Klf6(-1516--1369)	GAGGATCCGAATCCCAGCAC	ATGTATGGGGGCGAAGATGG
Gapdh intron	ATCCTGTAGGCCAGGTGATG	AGGCTCAAGGGCTTTTAAGG

Primers for Human ChIP qRT-PCR

Locus	Forward 5' - 3'	Reverse 5 '- 3'
SCARNA10-pro1(-432—279)	TGCAGTAAGTGAAACACCCAAC	GCAAGCTGGGTACATGCTCA
SCARNA10-pro2(-1190—1013)	ATTGTGGGATCCTGAAGCAG	TGAGCTTCACCACACCTGAG
SCARNA10-pro3(-1962—1817)	CCAGGAGGTCAAGGCTACAG	TTGGTCTCTGCGATCTCCTT
COL1α1(-709--525)	CCTAGGGTTTGGAGGAAGGC	GTCTTCTGGTGTGGCTAGGG
COL1α1(-1263--1086)	GCATAGAGCAATGACCGGGA	GCCCCTTCTCCAGTTGTACC
COL1α1(-1846--1619)	CTGCCACATGGTCGGGATAA	TGGTTTTGTGCAACGAAGGC
COL3α1(-186--47)	TGCATACAAACTCCAGATGTGC	CCTCACTTTCCAGCCCCTTT
COL3α1(-698--478)	TGTCTTTCCCAGGCAGCATA	AGAAATGCCACCGTATGCCC
COL3α1(-1819--1693)	CCCAAGCAGTGACTCTCCAA	TCAGTCACAAGGACACAAACCA

α-SMA(-368--234)	GGAACGAGTACCACCAACCC	CTGTAAGTCGCTGCCTGAGT
α-SMA(-1105--975)	CAGCCTCTGGTAAAGGTGCTA	ACTGCAATGTTGGCTGCTTTG
α-SMA(-1632--1409)	CACCCATCTATGTCCAGCCC	AAATTGGTTTTGCGCTCACGA
CTGF(-155--15)	TGAGTGTCAAGGGGTCAGGA	AGGCTTTTATACGCTCCGGG
CTGF(-858--657)	TCTCCTGGGGCAGATTTCCA	AACCCCCTTTGCATCCCAGT
CTGF(-1737--1640)	CTTCCTGAGCTTCAGAGGGC	TACCTCGTTAAGCCAGCCAC
PCNA(-345--124)	AGCGGCTCACAGTTCCCTTA	TGCCTCCCTGGCGTATTTG
PCNA(-1061--906)	GGACTGGCTCACTGGGTAAC	ACTTAGGCTCTCCGTTCCCT
PCNA(-1626--1426)	GAGCCTGGTGTAAGGCAACA	GGATTGCCCATGAGTGACCT
TGFβ1(-410--298)	CCCCATGTTGACAGACCCTC	GGCTTAATCCGGGGGATGAG
TGFβ1(-1112--863)	TCCCTCCTTTCCCCTCTCTC	GCCCCACTGTAGATGGTGTC
TGFβ1(-1622--1473)	CTGAGGACATGGGCAAAGCTA	CCTTGTCAGGGACACCTCCT
MMP2(-270--161)	GGCCCCTGACTGCTCTATTT	TCCCAGGTTGCTTCCTTACC
MMP2(-975--754)	TGTTCCCTAAAACATTCCCC	GTCTCTGAGGAATGTCTTCT
MMP2(-1821--1573)	TGAAGGGAGTCACATACAAGGC	GGCCTGTGGGCTAAATCCA
TIMP1(-496--335)	ATTTGAGACCCTGGCTTTGG	GCAGCAGTGGAGGGAGATAA
TIMP1(-961--754)	ATCAGAACCCCAGGGAAGGT	TGGTGCGGGTGAATGAATGA
TIMP1(-1619--1426)	CACGCCTGTAATCCCAACAC	CCTCCGGGGTTCAAGAGATT
SMAD2(-375--190)	GCTTCTCCCGAACCCCTTTT	CGCGCATTAAGACGATTCCC
SMAD2(-702--580)	TGTGCAAGTTGGGGCCTAAT	CCATGTCCCGAAGGGTCTTT
SMAD2(-1503--1361)	GTTCGCACGCAACAGAAGAG	GGCAAATGGAACTCACCTGC
SMAD3(-423--289)	ACGACGCTTACTTGCTGCGA	GGAAACACGGCGAGAGGG
SMAD3(-1092--931)	AGTAGCTCCTGAAAACCGCC	AAAGTGGGGCAAGACAGGAG
SMAD3(-1650--1512)	AACGTGAGGCACAGGAACAT	CTTCCCGTCCGTCAAGAGAG
KLF6(-256--169)	CCCGCGCCTTATATACCCTG	CCGCACCATTGGCTCAATTC
KLF6(-856--629)	CTCCCAATTCCAAGGGGAC	CCCCAACTGTCCTCTTCTGC
KLF6(-1355--1272)	GCTCTCAGTTGCTGGGGTAA	GTATGTGGGCGGTGAAGGTT
KLF6(-1720--1564)	ATAAAATGGGACACCCTCACC	TAAGCAGGAGTGTGGAGCAAG
GAPDH(-2813--2524)	GGTAGGGAGTTCGAGACCAG	TCAACGCAGTTCAGTTAGGC

Table S4. Serum levels of ALT, AST in CCl_4-induced liver fibrosis model (mean ± SD, n = 8)

Group	ALT (U/L)	AST (U/L)
NC group	40.3±7.4	65.4±12.5
NC+CCl_4 group	238.5±43.7*	323.6±61.2*
shScarna10 group	37.3±7.2	58.8±8.3
shScarna10+CCl_4 group	118.3±21.5#	151±43.8#

*p<0.05 compared with the NC group. #p<0.05 compared with NC + CCl_4 group. All statistical analyses were performed using SPSS version 13.0 software and p<0.05 indicated statistical significance.

Table S5. Serum levels of ALT, AST in CCl_4-induced liver fibrosis model (mean ± SD, n = 8)

Group	ALT (U/L)	AST (U/L)
LV-Control group	42.5±9.6	53.2±8.2
LV-Control +CCl_4 group	257.5±52.3*	307.4±55.1*
LV-Scarna10 group	44.3±6.2	52.8±11.3
LV-Scarna10+CCl_4 group	359.3±41.5#	389.2±60.3#

*p<0.05 compared with the LV-Control group. #p<0.05 compared with LV-Control + CCl_4 group. All statistical analyses were performed using SPSS version 13.0 software and p<0.05 indicated statistical significance.

Table S6. Serum levels of ALT, AST in BDL-induced liver fibrosis model (mean ± SD, n = 10)

Group	ALT (U/L)	AST (U/L)
NC group	38.6±8.3	50.1±7.3
NC+BDL group	214.2±48.5*	334.8±57.6*
shScarna10 group	35.1±7.2	49.5±8.7
shScarna10+BDL group	102.9±31.6#	136.5±48.3#

*p<0.05 compared with the NC group. #p<0.05 compared with NC + BDL group. All statistical analyses were performed using SPSS version 13.0 software and p<0.05 indicated statistical significance.