SUPPLEMENTARY METHODS

Serum alanine transferase and aspartate transaminase detection

Serum alanine transferase (ALT) and aspartate transaminase (AST) levels were detected according to the instruction of the commercial kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Briefly, serum was mixed with alanine (for ALT)/aspartic acid (for AST) and α-ketoglutaric acid and bathed in water at 37°C. Thirty minutes later, 2, 4-dinitrophenylhydrazine hydrochloric acid solution was added and bathed in water at 37°C for 20 minutes. Sodium hydroxide solution was added and incubated at room temperature for 15 minutes and then detecting absorbance value at 510 nm.

Assay for liver triglyceride

Liver triglyceride (TG) was determined using a commercial kit (APPLYGEN, Beijing, China) according to the manufacturer's instructions. Briefly, 10 mg of liver tissue was cut into pieces, and the lysate was in lysis buffer. Centrifuge hepatic lysate at 14,000 g for 10 minutes, and then transfer the supernatant to a new tube. The supernatant was bathed in water at 70°C for 10 minutes and then centrifuged at 2,000 g for 5 minutes. The supernatant reacts with the working solution (R1:R2=4:1) at 37°C for 15 minutes and then detects an absorbance value at 550 nm. TG content was adjusted for protein concentration per gram.

Serum interleukin 33 measurement

Serum interleukin 33 (IL33) level in mice was measured by enzyme-linked immunosorbent assay (ELISA) (Elabscience, Wuhan, China) according to the manufacturer's instructions.

Histological analysis of liver tissue

Paraffin-embedded liver sections were stained with hematoxylin and eosin, and Sirius Red. The images of the liver tissue were captured by microscope (MshOt, Guangzhou, China). The positive area was calculated using Image Pro Plus software (Media Cybernetics, Rockville, MD, USA).

Quantitative real-time polymerase chain reaction

About 20 mg liver tissue were used to isolate total RNA by using TRIzol (Invitrogen, Waltham, MA, USA), and the concentration of RNA was determined by Nanodrop (Thermo Fisher Scientific, Waltham, MA, USA). cDNA was obtained from 1 μg RNA reverse transcription according to the manufacturer's instructions (Takara, Kusatsu, Japan). Quantitative real-time polymerase chain reaction was performed over 35 cycles on a Quant Studio 7 (Thermo Fisher Scientific) using TB Green® Premix Ex Taq™ II (Takara). The relative amount of each gene was analyzed using the delta-delta CT method, where glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as endogenous control. The following sequences were used: nitric oxide synthase 2 (Nos2) forward 5′-CAGAGGACCCAGAGACAAGC-3′, reverse 5′-TGCTGAAACATTTCCTGTGC-3′; endothelin 1 (Edn-1) forward 5′-AAGTTGGGAAAGAAGTGTAT-3′, reverse 5′-AAGATGCCTTGATGCTATT-3′; nitric oxide synthase 3 (Nos3) forward 5′-TACGCACCCAGAGCTTTTCT-3′, reverse 5′-CTTGGTCAACCGAACGAA-GT-3′; Klf2 forward 5′-TGCCATCTGTGCGATCGT-3′, reverse 5′-GGCTACATGTGCCGTT-TCATG-3′; vascular endothelial growth factor receptor 2 (Vegfr2) forward 5′-TTTGGCAAATACAACCCTTCAGA-3′, reverse 5′-GCAGAAGATACTGTCACCACC-3′; vascular cell adhesion molecule 1 (Vcam1) forward 5′-GGGAAGATGGTCGTGATCCTT-3′, reverse 5′- CAGCACCGTG-AATGTGATCT-3′; intercellular adhesion molecule 1 (Icam1) forward 5′-ATTCGTTTCCGGAGAGTGTG-3′, reverse 5′-CAGCACC-GTGAATGTGATCT-3′; Gapdh forward 5′-AACTTTGGCATTGT-GGAAGG-3′, reverse 5′-ACAC-ATTGGGGGTAGGAACA-3′.

Scanning electron microscopy

Liver tissues were perfused with a fixation solution containing 4% paraformaldehyde and fixed overnight at 4°C. Scanning electron microscopy (SEM) was used to measure hepatic sinusoidal cell capillarization. Liver sections were dehydrated in ethanol after fixed with 1% osmium, and then dried with hexamethyldisilazane. Images were captured by a SU8010 SEM (HITACHI, Tokyo, Japan).

Immunofluorescence

Liver tissue frozen sections were washed with cold phosphate buffer solution for 5 minutes three times, followed by cold 0.5% Triton X-100 incubation for 30 minutes at room temperature. Slides were incubated with 3% bovine serum albumin for 1 hour at room temperature and then washed three times in phosphate buffer solution. Primary antibodies alpha smooth muscle actin (1:200; Proteintech, Rosemont, IL, USA; 14395-1-AP) and lymphatic vessel endothelial hyaluronan receptor 1

(LYVE-1) (1:200; Invitrogen, 14-0440-82) were incubated for overnight, corresponding secondary goat anti-rabbit antibodies (AF488, Thermo Fisher Scientific) and goat anti-mouse (AF555, Thermo Fisher Scientific) were incubated for 1 hour. Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI) (Beyotime, Haimen, China) and analyzed with a fluorescent microscope (Olympus, Tokyo, Japan).

Treatment of hepatic stellate cells with conditioned medium of liver sinusoidal endothelial cells

SK-Hep1 cells or human liver sinusoidal endothelial cells (LSECs) were seeded in 12-well plates and cultured with Dulbecco's Modified Eagle Medium (DMEM) containing 10 % fetal bovine serum and 1% penicillin/streptomycin. SK-Hep1 cells or human LSECs were treated with recombinant IL33 (rIL33) or PD98059 in the presence of palmitic acid plus high glucose (PAHG) for 24 hours, and the culture medium was removed. After the following 24 hours incubation, collecting the conditioned medium (CM) of different groups of SK-Hep1 cells or human LSECs and then centrifuged at 12,000 rpm to remove the cell debris. LX-2 cells were cultured with CM of different groups for 48 hours.

Measurement of nitric oxide

Nitric oxide (NO) levels of liver tissue or cell supernatant were detected using a nitrite oxide detection kit (Beyotime) according to the manufacturer's instructions. NO level of liver tissue was adjusted for protein concentration per gram.

Hydroxyproline assay

Hepatic hydroxyproline concentrations were quantified by Hydroxyproline Assay Kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) following the manufacturer's recommendation.

Transfection of small interfering RNA targeting ST2

Three small interfering RNA targeting ST2 (siST2) were constructed by Tsingke Biotech Co. Ltd. (Beijing, China). The sequences of siST2-1, siST2-2, and siST2-3 are as follows: siST2-1, sense 5′-CACGGUCAAGGAUGAGCAA-3′; siST2-2, 5′-GCACCUCUUGAGUGGUUUA-3′; siST2-3, 5′-GAAAGUUCCAGCAAUGACA-3′; and scramble RNA, 5′-AUGGCUAUGUACCUGCAUG-3′. For siST2 transfection, LSECs were transfected with scramble RNA or siST2 with Lipo3000 (Invitrogen), and detecting the protein level after 48 hour later.

Intracellular reactive oxygen species generation detection

Intracellular reactive oxygen species (ROS) levels were examined with the regent dihydroethidium (DHE) (Beyotime). Briefly, SK-Hep1 cells or human LSECs were seeded into 12-well plates, after 48 hours incubation, they were washed and then incubated with 1 μM DHE for 30 minutes at 37°C in the dark. The fluorescence intensity of the cells was measured with fluorescent microscope (Olympus) and analyzed by Image Pro Plus 8.0.