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Supplementary Materials for

Hyaluronan synthase 2–mediated hyaluronan production mediates Notch1 activation and liver fibrosis

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at stm.sciencemag.org/cgi/content/full/11/496/eaat9284/DC1)

Data file S1 (Microsoft Excel format). Individual subject-level data.

Materials and Methods

Histologic examination

Formalin-fixed, paraffin-embedded liver tissues were cut using a microtome into 5 μm thickness. After being dewaxed and rehydrated, endogenous peroxidase was blocked with 0.3% H_2O_2 for 30 min. A modified citrate buffer, pH 6.1 (Target Retrieval Solution, cat. S1699, Dako) was used for antigen retrieval. Dako serum-free protein block was applied for 30 min, and then tissues were incubated with mouse monoclonal anti-HAS2 antibody (1:100, cat. 140671, Abcam). Secondary antibody (1:200, cat. BA1001, Boster Biological Technology) was used. Slides were developed using diaminobenzidine, followed by a hematoxylin counterstaining. For Sirius Red staining, a solution of saturated picric acid containing 0.1% Fast Green FCF (cat. P6744, cat. F7252, Sigma-Aldrich) and 0.1% Direct Red 80 (cat. 365548, Sirius Red R3B; Sigma-Aldrich) were applied for 1 hour. Sirius red-positive area was quantified from randomly selected 8-10 fields of ×100 magnification per slide by NIH ImageJ software (https://imagej.nih.gov/ij).

Immunofluorescence staining

For α-SMA and HAS2 double staining, liver sections were incubated with anti-HAS2 antibody (1:100, cat. 140671, Abcam) and anti-α-SMA antibody (1:100, cat. 5694, Abcam), followed by Cy3-conjugated goat anti-mouse IgG (1:200, cat. 115-165-062, Jackson ImmunoResearch) and Alexa Fluor 488-conjugated affiniPure Goat anti-Rabbit IgG (1:200, cat no. 111-545-003, Jackson ImmunoResearch). Imaging analyses were performed using a Zeiss LSM800 inverted stand and Axio Imager Z2m confocal system. For HA and cell-specific marker protein double staining, tissue sections were incubated with biotin-HABP and anti-Desmin antibody (cat.

RB9014P0, Thermo Scientific), anti-HNF4α antibody (cat. sc-8987, Santa Cruz Biotechnology), anti-F4/80 antibody (cat. 14-4801, eBioscience), or anti-CD31 antibody (cat. Ab28364, Abcam) at 4°C overnight. After washing with PBS/0.05% Tween 20, tissue sections were incubated with Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed, Alexa Fluor 594 (cat. A21207, Invitrogen), Goat anti-Rat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 568 (cat. A11077, Invitrogen), or Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 568 (cat. A10042, Invitrogen) at room temperature for 1 h, and followed by FITC Streptavidin (cat. 554060, BD Bioscience) for 1 h. Hoechst 33342 (cat. H3570, Invitrogen) was used for nuclear staining. Imaging analyses were performed using a LEICA TCS SP5X. The immunofluorescence staining for expression of CD44, frozen liver sections were analyzed from Col-GFP reporter mice or Col-GFP/*ASMA-HAS2Tg* mice 2 weeks after bile duct ligation (BDL) or 10 times CCl_4 treatments. The specimens were blocked in Dako serum-free protein block at room temperature for 30 minutes and incubated with purified Rat Anti-Mouse CD44 (1:100, cat. 553131, Clone IM7, BD Biosciences) at 4°C overnight. After washing with PBS/0.05% Tween 20, the specimen was incubated with Goat anti-Rat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 568 (1:200, cat. A-11077, Thermo Fisher Scientific) at room temperature for 1 hour. Nuclei were stained with DAPI for 5 minutes. In 4-methylumbelliferone (4-MU, cat. M1508, Sigma-Aldrich) in vitro experiment, primary HSCs isolated from Col-GFP reporter mice were treated with 0.5 mM 4-MU at day2. 4-MU was refreshed every other day. At day8, cells were stained with CellTracker Red CMTPX Fluorescent Probes (cat. C-34552, Molecular Probes) before fluorescent image acquisition. Imaging analyses were performed using an Olympus BX51 florescence microscopy or LEICA DMi8 or LEICA TCS SP5X or KEYENCE BZ-X710.

Primary cell isolation

HSCs, Kupffer cells, and liver sinusoid endothelial cells (LSECs) were isolated from mice by *in situ* liver perfusion with Pronase E (cat. 10165921001, Sigma-Aldrich) and Collagenase D (cat. 11088882001, Sigma-Aldrich), followed by density gradient centrifugation with Nycodenz (cat. AN1002424, Accurate Chemical and Scientific Corporation), as previously described (*11*). Magnetic antibody sorting (MACS; Miltenyi Biotec) using CD11b (cat. 130-097-142, Miltenyi Biotec) were used to deplete contaminated Kupffer cells in HSC fraction or positively select Kupffer cells. The purity of HSCs was evaluated by autofluorescence of Vitamin A under a LEICA DMi8 fluorescent microscope. For activated HSCs, cells were maintained in Dulbecco's modified Eagle's (DMEM) medium supplemented with 10% fetal bovine serum (FBS) and 1% Antibiotic-Antimycotic (cat. 15240-062, Invitrogen) for 7 days. Kupffer cells were maintained in RPMI medium supplemented with 10% FBS and 1% Antibiotic-Antimycotic. LSECs were purified by MACS using anti-CD146 (cat. 130-092-007, Miltenyi Biotec) and anti-CD11b MACS beads. $CD146^+$ and $CD11b^-$ cells were used. Primary hepatocytes were obtained with collagenase perfusion, followed by Percoll (cat. 17-5445-02, GE Healthcare) density gradient centrifugation, as previously described (*48*). More than 90% cell viability were confirmed by trypan blue exclusion assay. Isolated hepatocytes were plated on rat collagen type I-coated dishes and maintained in Media 199 cell culture medium supplemented with 10% FBS and 1% Antibiotic-Antimycotic. Non-passage cells were used for all experiments.

Glucose tolerance test

Wild-type (WT) or $Has2^{\Delta HSC}$ Mice were fed CDHFD for 10 weeks. Mice were fasted overnight. Before glucose administration, fasting glucose was measured from tail vein blood using the

OneTouch ULTRA BLUE Blood Glucose Monitoring System. 2 g/kg glucose were orally given, and blood glucose were monitored at the indicated times.

BrdU cell proliferation assay

Primary HSCs isolated from WT, *Has2*$^{\Delta HSC}$, or *ASMA-HAS2Tg* mice were cultured in DMEM medium supplemented with 10% FBS and 1% Antibiotic-Antimycotic for 6 days, and cells were starved in serum free medium overnight. Then, cells were incorporated with BrdU in the presence of 10% serum for 24 hours. In a separate set of experiments, primary HSCs isolated from WT, *Cd44*$^{-/-}$, *Tlr4*$^{-/-}$, or *Notch1*$^{\Delta HSC}$ mice were cultured for 6 days, and then conditioned media collected from WT-HSC or *ASMA-HAS2Tg*-HSC (day4 to day7) were treated for 36 hours. The media was removed and conditioned media containing BrdU was added and incubated for additional 24 hours. BrdU assay was performed using BrdU Cell Proliferation Assay Kit (cat. 2752, EMD Millipore), according to manufacturer's instruction.

Matrigel invasion assay

Primary HSCs isolated from WT, *Has2*$^{\Delta HSC}$ or *ASMA-HAS2Tg* mice were cultured in DMEM medium supplemented with 10% FBS and 1% Antibiotic-Antimycotic for 7 days or primary HSCs isolated from WT, *Cd44*$^{-/-}$, *Tlr4*$^{-/-}$, or *Notch1*$^{\Delta HSC}$ mice were cultured for 6 days, and then conditioned media collected from WT-HSC or *ASMA-HAS2Tg*-HSC (day4 to day7) were treated for 48 hours. Equal numbers of HSCs were transferred onto the Corning BioCoat Matrigel Invasion Chambers with 8.0μm PET Membrane (cat. 354480, Corning Incorporated). After 12 hours, invaded cells were fixed and stained with hematoxylin and eosin. Non-invading cells were

removed from upper surface of the Matrigel with a cotton swab. The invading cells were counted in randomly selected 6-8 fields of ×100 magnification per slide.

Migration assay

Primary Kupffer cells were isolated from WT, *Cd44*$^{-/-}$, or *Tlr4*$^{-/-}$ mice, and then 10^5 cells were transferred onto the Corning Transwell polycarbonate membrane cell culture inserts (cat. CLS3422-48EA). Conditioned media collected from WT-HSC or *ASMA-HAS2Tg*-HSC (day4 to day7) were placed in the lower chamber. After 3 h, cells were fixed and stained with hematoxylin and eosin. The migrated cells were counted in randomly selected 8 fields of ×100 magnification per slide.

Transient transfection

Human LX-2 or mouse HSCs cells were maintained in DMEM medium containing 10% FBS, 50 U/ml penicillin, and 50 μg/ml streptomycin at 37°C in a humidified atmosphere with 5% CO_2. Transient transfection was carried out using FuGene HD Reagent (cat. E2311, Promega), according to manufacturer's procedure. The plasmid encoding Has2 was suppled from Origene (cat. MR208762). The pCDNA/CD44 plasmid vector was a kind gift of Drs. L. Rodríguez-Rodríguez (Rutgers Cancer Institute of New Jersey) and K.E. Miletti-González (Delaware State University) (*33*). Lipofectamine RNAiMAX Transfection Reagent (cat. 13778150, Invitrogen) was used for transfection of small interfering RNA (siRNA) directed against mouse *Wt1* mRNA and control siRNA (cat. sc-36845, cat. sc-37007, Santa Cruz Biotechnology). Thirty-six hours after transfection, the medium was replaced with DMEM medium containing 10% FBS and

cultured for 24 hours. Subsequently, serum was deprived for 12 hours and cells were treated with 5 ng/ml TGF-β for 16 hours.

Chromatin immunoprecipitation assay

ChIP was carried out using the manufacturer's suggested protocol from EZ-ChIP kit (cat. 17-371, Millipore). Briefly, DNA was immunoprecipitated by CD44 antibody (cat. Ab157107, abcam). The purified DNA was measured by real-time PCR analysis. PCR of the human *NOTCH1* promoter containing the CIRE1 used forward primer 5'-CTGAGCCTCACTAGTGCCTC-3' and reverse primer 5'-CTGATCCCGGGACTCCAGAA-3' and the first intron regions containing the CIRE2 used forward primer 5'-GGCAACGCTTTGGAGAGTAG-3' and reverse primer 5'-TGCTCAGTATCATGGGTTGC-3'.

Alanine aminotransferase (ALT) measurement

The mouse whole blood was collected via cardiac puncture and left at room temperature for 30 minutes. The clot was removed by centrifuging at 5,000 rpm for 15 min. Serum ALT concentrations were determined by Infinity ALT (GPT) liquid stable reagent (cat. TR71121, Thermo Scientific), according to manufacturer's instruction.

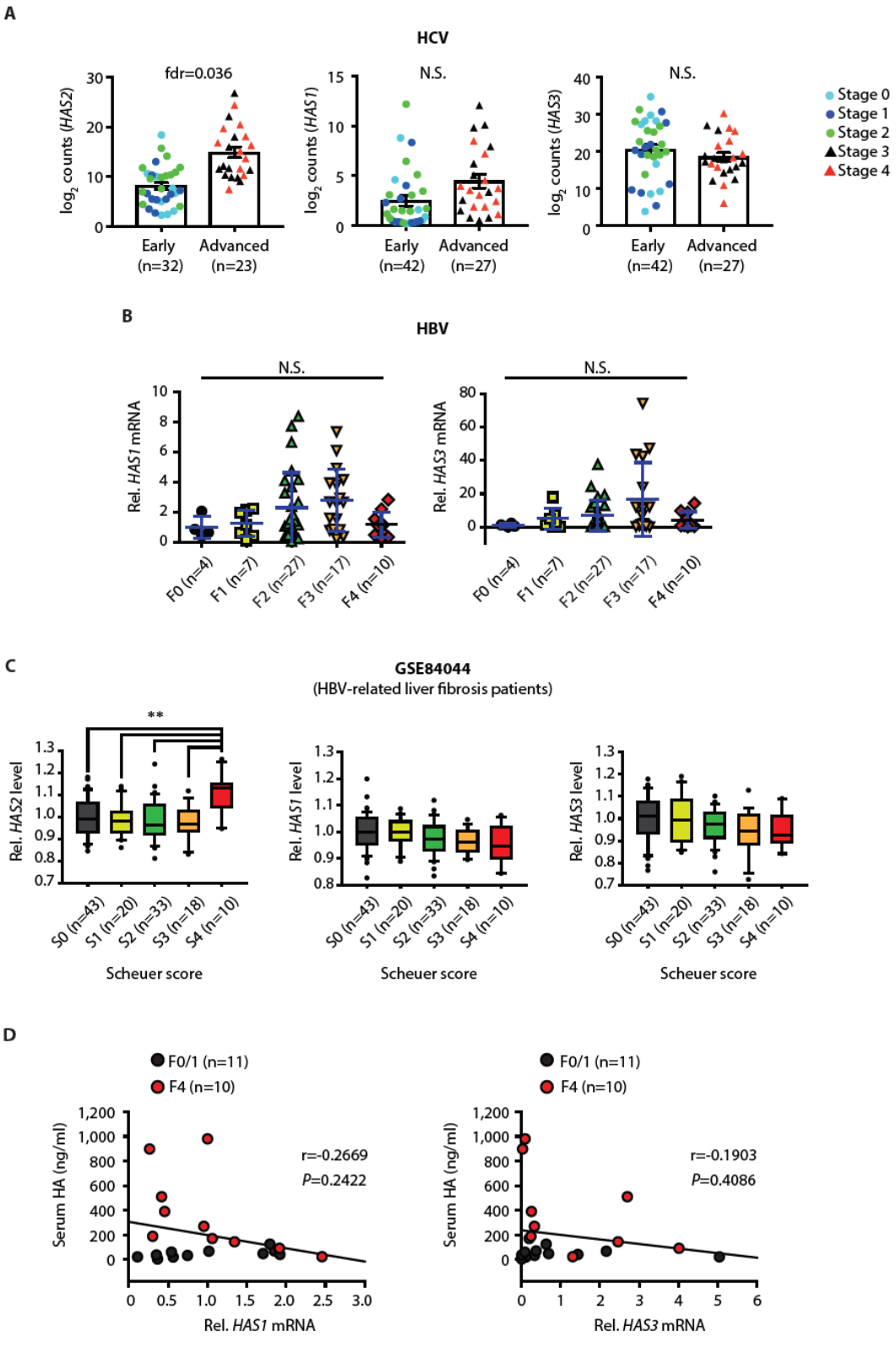
A
HCV
fdr=0.036
N.S.
N.S.
log2 counts (HAS2)
log2 counts (HAS1)
log2 counts (HAS3)
Early (n=32)
Advanced (n=23)
Early (n=42)
Advanced (n=27)
Early (n=42)
Advanced (n=27)
Stage 0
Stage 1
Stage 2
Stage 3
Stage 4
B
HBV
N.S.
N.S.
Rel. HAS1 mRNA
Rel. HAS3 mRNA
F0 (n=4)
F1 (n=7)
F2 (n=27)
F3 (n=17)
F4 (n=10)
C
GSE84044
(HBV-related liver fibrosis patients)
**
Rel. HAS2 level
Rel. HAS1 level
Rel. HAS3 level
S0 (n=43)
S1 (n=20)
S2 (n=33)
S3 (n=18)
S4 (n=10)
Scheuer score
D
F0/1 (n=11)
F4 (n=10)
Serum HA (ng/ml)
r=-0.2669
P=0.2422
r=-0.1903
P=0.4086
Rel. HAS1 mRNA
Rel. HAS3 mRNA

Fig. S1. Expression of *HAS* in patients with fibrosis. (**A**) *HAS1*, *HAS2*, and *HAS3* read counts in patients with HCV. HAS1, HAS2 and HAS3 corrected counts were plotted in early (stage 0-2) versus advanced (stage 3-4) stages of fibrosis in HCV-infected patients (n = 55 patients, each stage indicated in a different color). Data are mean ± s.e.m. N.S., not significant. FDR-values ≤ 0.05 were considered statistically significant. (**B**) Hepatic *HAS1* and *HAS3* mRNA expression in liver tissue of patients with fibrosis and chronic hepatitis B virus. Data are mean ± s.d. N.S., not significant. Statistical significance was calculated by one-way ANOVA with Tukey's *post hoc* analysis. (**C**) *HAS* mRNA expression in patients with HBV-related liver fibrosis. *HAS* mRNA expression was analyzed in patients with fibrosis (GSE84044). Liver fibrosis was classified into five stages, S0 to S4, according to Scheuer scoring systems. Data are shown as box and whisker plot. Box, interquartile range; whiskers, 5-95 percentiles; horizontal line within box, median. Statistical significance was calculated by one-way ANOVA with Tukey's *post hoc* analysis. (**D**) Correlation analysis of *HAS1* (or *HAS3*) mRNA expression and serum HA content. n = 21. Correlation was assessed by Pearson correlation coefficient analysis.

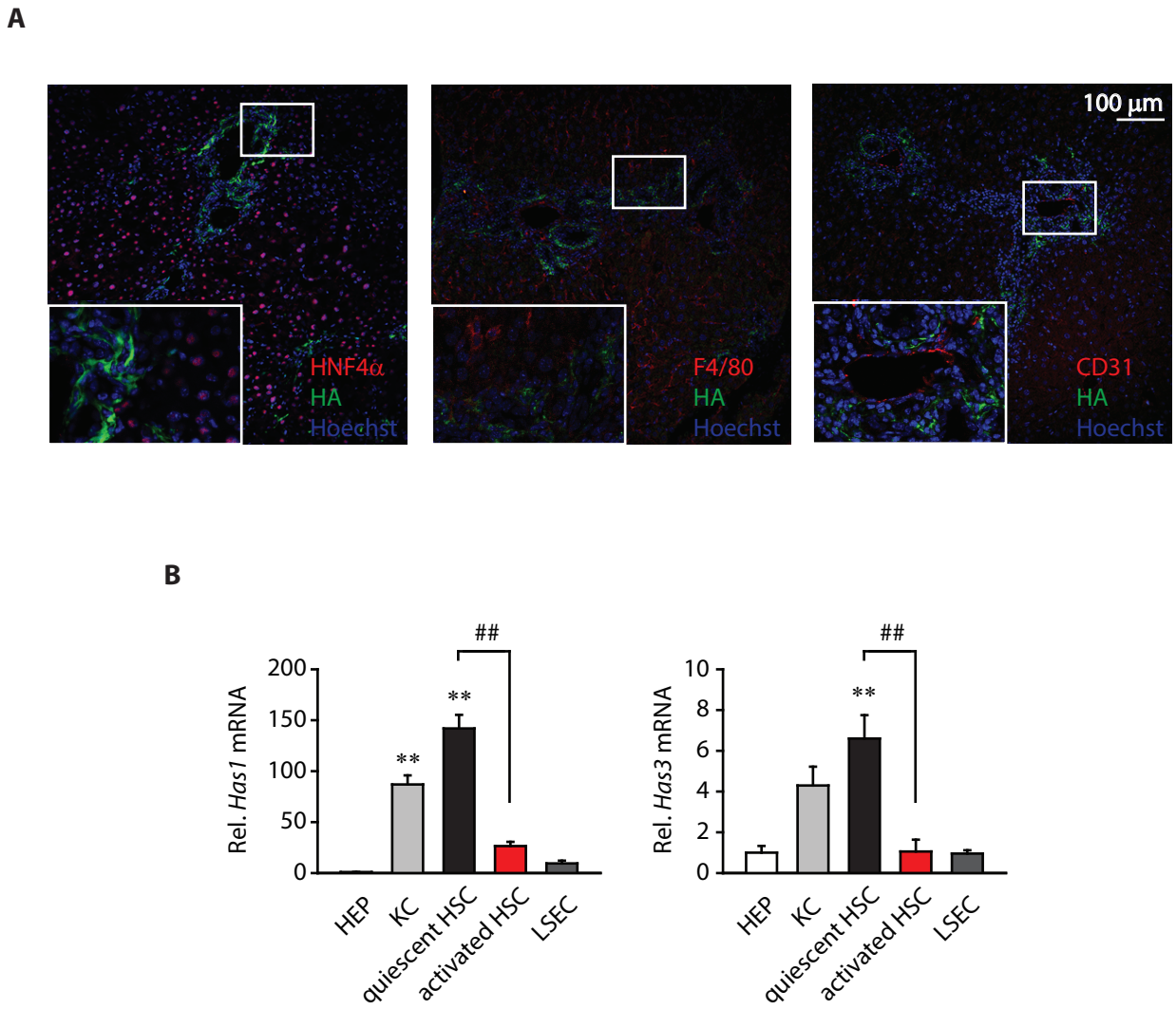

Fig. S2. Expression of HA and *Has* expression in mouse liver cells. (**A**) Localization of HA and HNF4α (a marker of hepatocytes), F4/80 (a marker of macrophages), and CD31 (a marker of endothelial cells) was determined by confocal microscopy in BDL-operated mouse liver. Boxed regions are shown at higher magnification. (**B**) qRT-PCR assays for *Has1* and *Has3* mRNA in mouse liver cells (n = 3/group). HEP, hepatocytes; KC, Kupffer cells; HSC, hepatic stellate cells; LSEC, liver sinusoidal endothelial cells. Data are mean ± s.e.m. $^{**}P<0.01$ versus HEP. Statistical significance was calculated by one-way ANOVA with Tukey's *post hoc* analysis.

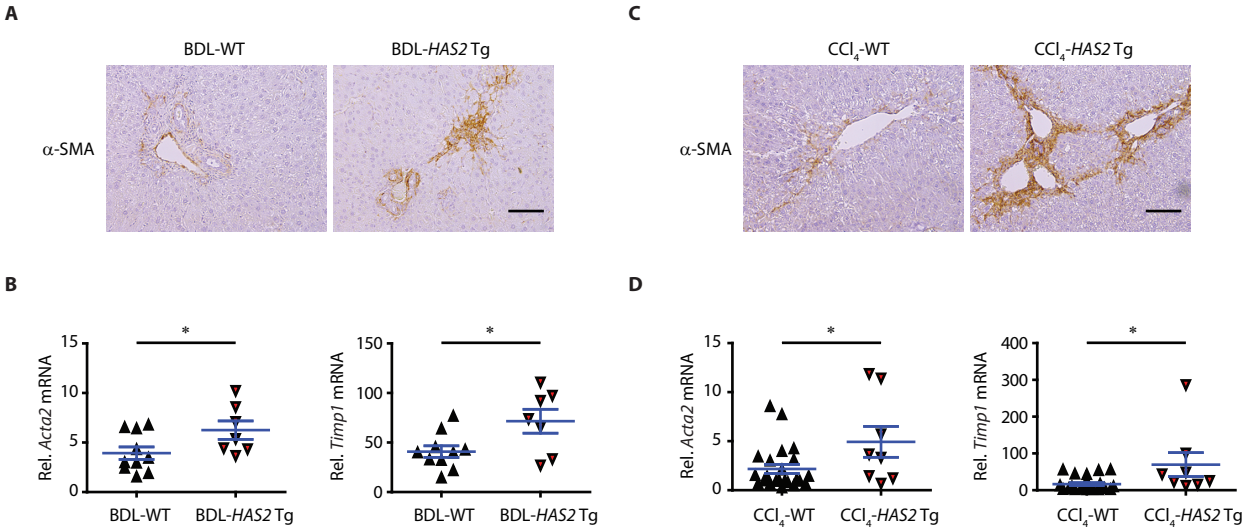

Fig. S3. Increased susceptibility to BDL- or CCl_4-induced liver fibrosis in *ASMA-HAS2* Tg mice. (**A**) Representative images of α-SMA staining in liver sections of wild-type (WT) or *ASMA-HAS2* Tg mice 2 weeks after BDL. Scale bars, 100 μm. (**B**) Hepatic *Acta2* or *Timp1* mRNA expression (BDL-WT, $n = 10$; BDL-*ASMA-HAS2* Tg, $n = 7$). Data are shown over Sham-WT value. Data are mean ± s.e.m. Statistical significance was calculated by two-tailed Student's *t*-test. (**C**) Representative images of α-SMA staining in liver sections of WT or *ASMA-HAS2* Tg mice 5 weeks after CCl_4 injection. Scale bars, 100 μm. (**D**) Hepatic *Acta2* or *Timp1* mRNA expression (CCl_4-WT, $n = 23$; CCl_4-*ASMA-HAS2* Tg, $n = 8$). Data are shown over Con-WT value. Data are mean ± s.e.m. Statistical significance was calculated by two-tailed Student's *t*-test.

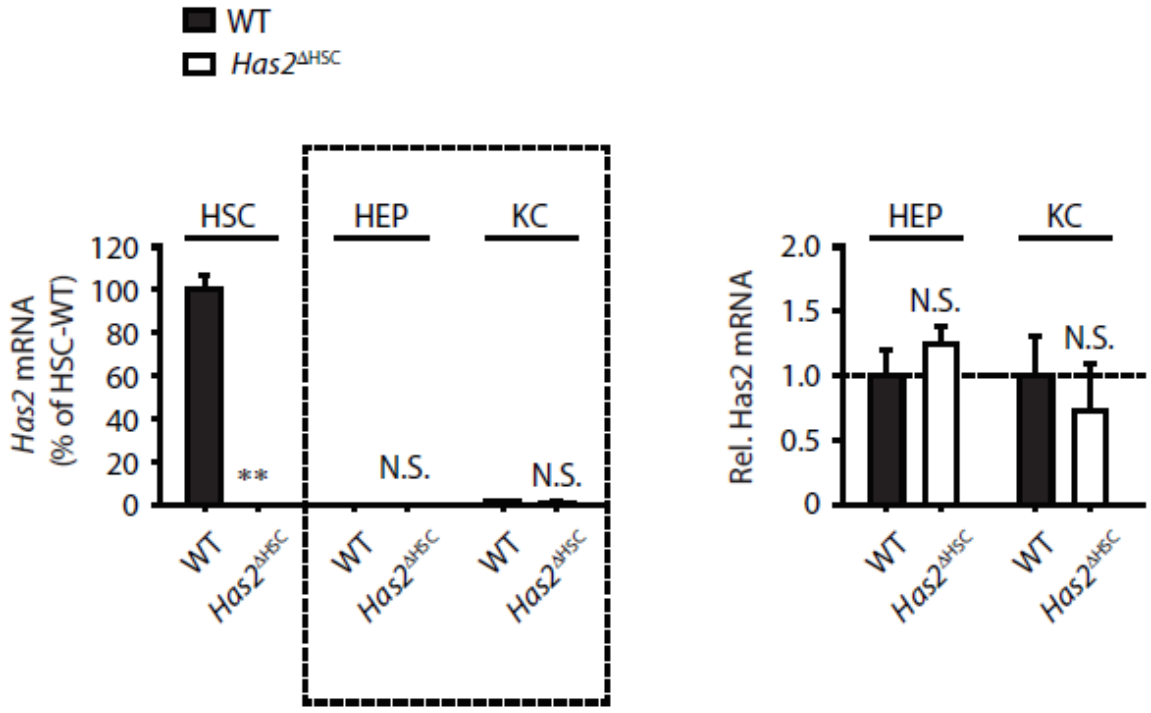

Fig. S4. Generation of HSC-specific *Has2* knockout mice. *Has2* mRNA expression in primary mouse hepatic stellate cells (HSC), hepatocytes (HEP), and Kupffer cells (KC) isolated from WT and $Has2^{\Delta HSC}$ mice (n = 3/group). Data are mean ± s.e.m. $^{**}P<0.01$ versus WT. Statistical significance was calculated by two-tailed Student's t-test. N.S., not significant.

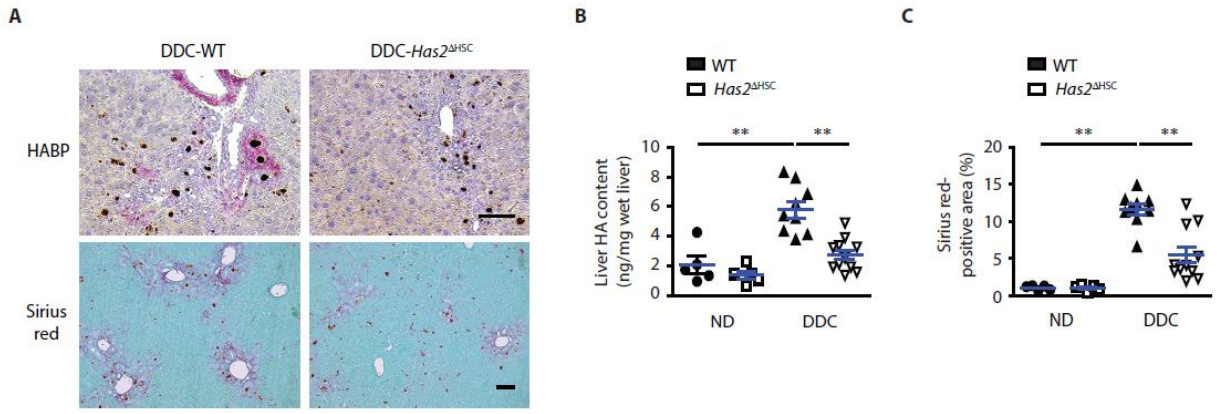

Fig. S5. Effect of HSC-specific *Has2* deficiency on DDC-induced liver fibrosis. (**A**) Representative images of HA (red) and Sirius red staining. WT and *Has2*$^{\Delta HSC}$ mice were fed with a normal diet (ND) or a 3,5-diethylcarbonyl-1,4-dihydrocollidine (DDC) diet for 4 weeks. HABP, hyaluronan binding protein. Scale bars, 100 μm. (**B**) Liver HA content was measured by ELISA (ND-WT, n = 5; ND- *Has2*$^{\Delta HSC}$, n = 5; DDC-WT, n = 9; DDC- *Has2*$^{\Delta HSC}$, n = 11). (**C**) Quantification of Sirius red-positive area by Image J. Data are mean ± s.e.m. $^{**}P<0.01$. Statistical significance was calculated by one-way ANOVA with Tukey's *post hoc* analysis.

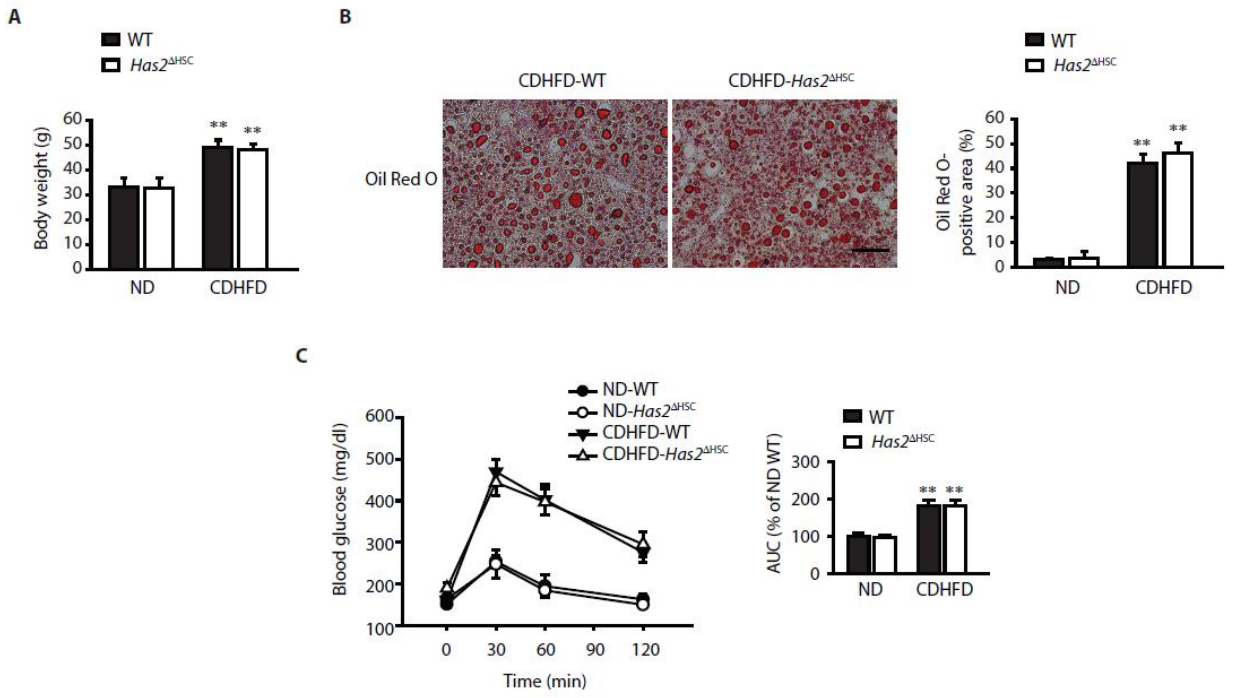

Fig. S6. No effect of HSC-specific *Has2* deletion on CDHFD-mediated fat accumulation or glucose intolerance. (**A**) Body weight. WT and *Has2*$^{\Delta HSC}$ mice were fed with a normal diet (ND) or a choline-deficient high fat diet (CDHFD) for 12 weeks (ND-WT, n = 5; ND-*Has2*$^{\Delta HSC}$, n = 5; CDHFD-WT, n = 10; CDHFD-*Has2*$^{\Delta HSC}$, n = 9). (**B**) Representative images of Oil Red O staining (left) and quantification by Image J (right). Scale bars, 100 μm. (**C**) Glucose tolerance test was conducted in WT and *Has2*$^{\Delta HSC}$ mice fed ND or CDHFD for 10 weeks (ND-WT, n = 5; ND-*Has2*$^{\Delta HSC}$, n = 5; CDHFD-WT, n = 13; CDHFD-*Has2*$^{\Delta HSC}$, n = 9). Data are mean ± s.e.m. **P<0.01 versus ND-WT. Statistical significance was calculated by one-way ANOVA with Tukey's *post hoc* analysis.

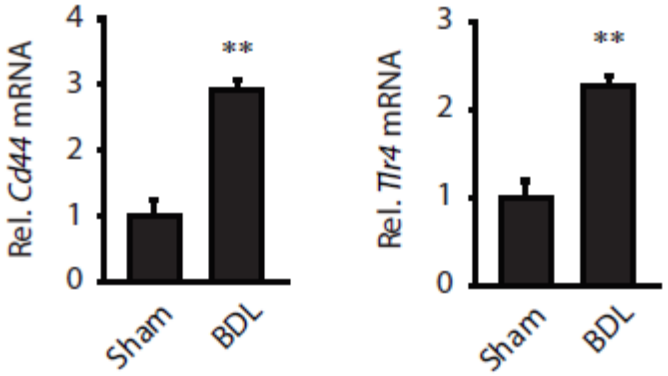

Fig. S7. *Cd44* and *Tlr4* mRNA expression in in vivo–activated HSCs. Primary hepatic stellate cells were isolated from sham- or BDL-operated mice 5 days after surgery (n = 3/group). $^{**}P<0.01$. Data are mean ± s.e.m. Statistical significance was calculated by two-tailed Student's t-test.

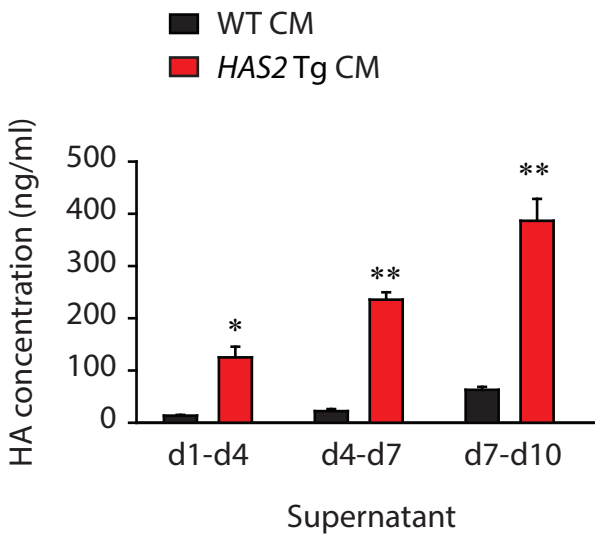

Fig. S8. HA concentration in the supernatant of WT HSCs or *HAS2* Tg HSCs. The supernatant (conditioned medium, CM) was collected at the indicated time points (n = 3/group). Data are mean ± s.e.m. *P<0.05, **P<0.01 versus WT CM. Statistical significance was calculated by one-way ANOVA with Tukey's *post hoc* analysis.

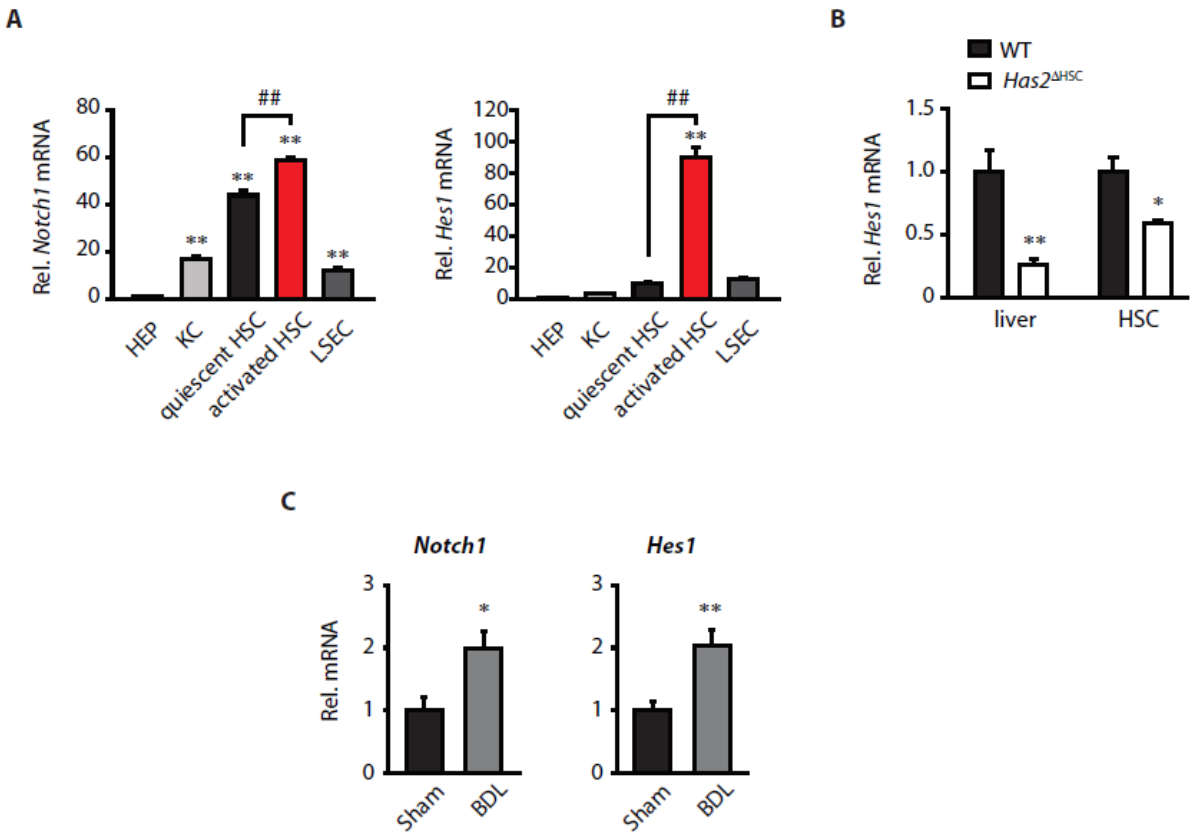

Fig. S9. *Notch1* and its target gene *Hes1* mRNA expression. (**A**) *Notch1* and *Hes1* mRNA expression in mouse liver cells (n = 3/group). $^{**}P<0.01$ versus hepatocytes; $^{\#\#}P<0.01$. HEP, hepatocytes; KC, Kupffer cells; HSC, hepatic stellate cells; LSEC, liver sinusoidal endothelial cells. (**B**) qRT-PCR for *Hes1* mRNA (liver, n = 8/group; HSCs, n = 3-4/group). $^{*}P<0.05$, $^{**}P<0.01$ versus WT. (**C**) qRT-PCR for *Notch1* and *Hes1* mRNA in WT mouse liver homogenates 3 weeks after BDL (n = 7-9/group). $^{*}P<0.05$, $^{**}P<0.01$ versus Sham. Data are mean ± s.e.m. Statistical significance was calculated by one-way ANOVA with Tukey's *post hoc* analysis (A) and two-tailed Student's *t*-test (B, C).

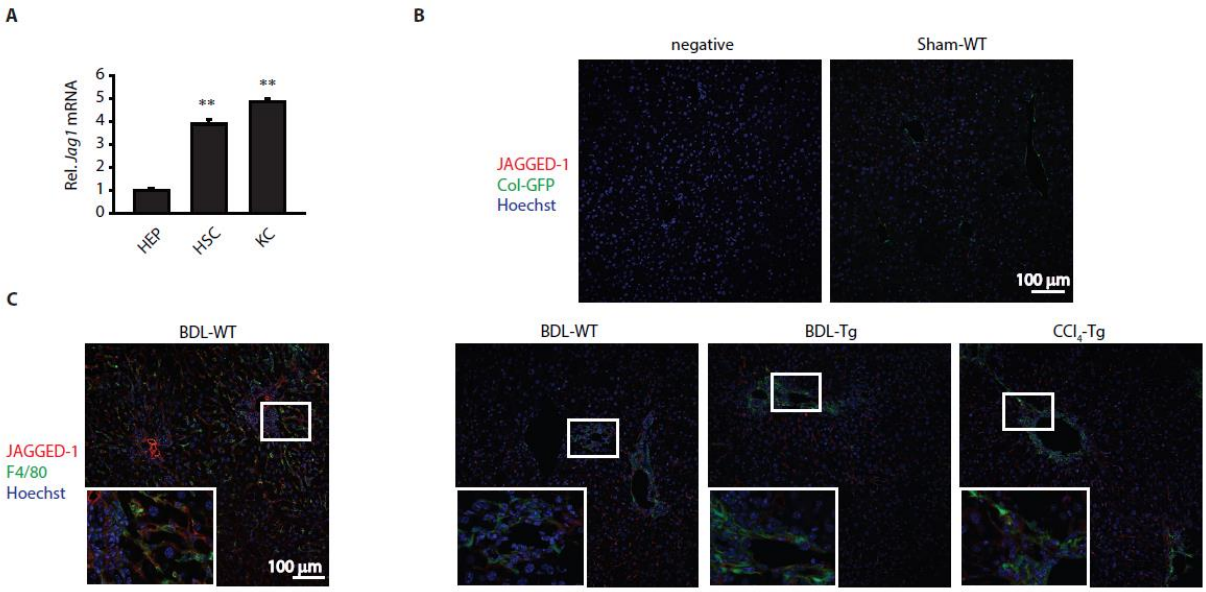

Fig. S10. Jagged-1 expression. (**A**) *Jag1* mRNA expression in mouse liver cells (n = 3/group). **P<0.01 versus hepatocytes. HEP, hepatocytes; HSC, hepatic stellate cells; KC, Kupffer cells. (**B**) Representative images of co-localization of HA and Col-GFP (collagen producing cells). Col-GFP/WT or Col-GFP/*ASMA-HAS2* Tg mouse liver sections were used. Mice underwent BDL for 2 weeks or were injected with CCl_4 intraperitoneally twice a week for 5 weeks. For negative control, the appropriate labeled secondary antibodies were applied to WT mouse liver sections without the primary antibody. (**C**) Co-localization of HA and F4/80 (a marker of macrophages) was determined by confocal microscopy in BDL-operated mouse liver. Boxed regions are shown at higher magnification.

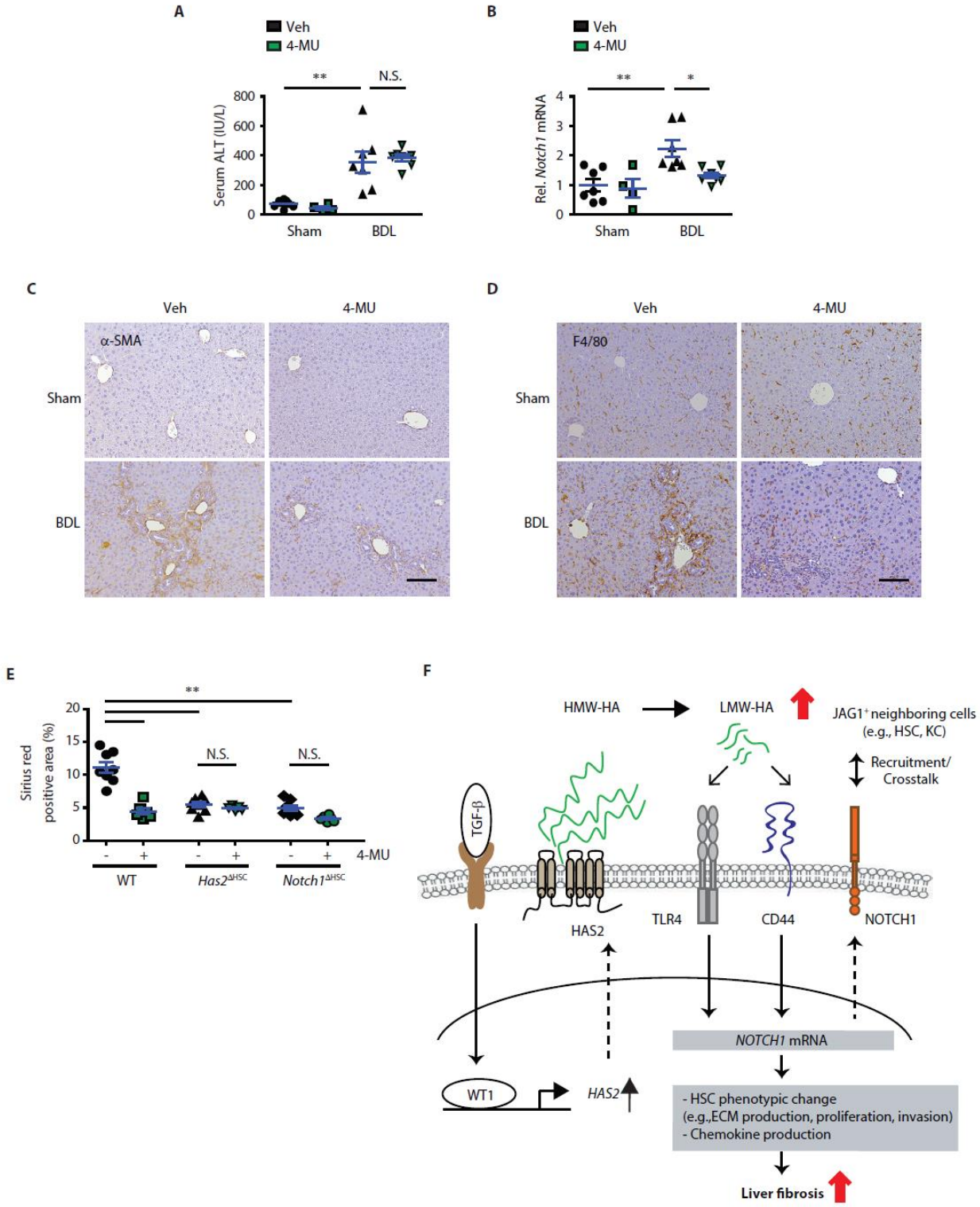
A
Veh
4-MU
**
N.S.
Serum ALT (IU/L)
800
600
400
200
0
Sham
BDL
B
Veh
4-MU
**
*
Rel. Notch1 mRNA
4
3
2
1
0
Sham
BDL
C
Veh
4-MU
α-SMA
Sham
BDL
D
Veh
4-MU
F4/80
Sham
BDL
E
**
Sirius red positive area (%)
20
15
10
5
0
N.S.
N.S.
- + - + - +
4-MU
WT
Has2ΔHSC
Notch1ΔHSC
F
HMW-HA
LMW-HA
JAG1+ neighboring cells (e.g., HSC, KC)
Recruitment/ Crosstalk
TGF-β
HAS2
TLR4
CD44
NOTCH1
NOTCH1 mRNA
WT1
HAS2
- HSC phenotypic change (e.g.,ECM production, proliferation, invasion)
- Chemokine production
Liver fibrosis

Fig. S11. Inhibition of BDL-induced HSC activation and macrophage infiltration by 4-MU treatment. (**A**) Serum ALT concentrations. Mice were orally gavaged once a day either with vehicle (Veh) or 4-methylumbelliferone (4-MU, 450 mg/kg) from day 7 to day 21 after bile duct ligation (BDL). (**B**) Hepatic *Notch1* mRNA expression. Sham-Veh, $n = 7$; Sham-4-MU, $n = 4$; BDL-Veh, $n = 7$; BDL-4-MU, $n = 7$. Data are mean ± s.e.m. $*P<0.05$, $**P<0.01$; N.S., not significant. Statistical significance was calculated by one-way ANOVA with Tukey's *post hoc* analysis. (**C**) Representative immunohistochemical images of α-SMA and (**D**) F4/80 staining. Scale bars, 100 μm. (**E**) Sirius red staining quantification (WT-Veh, $n = 8$; WT-4-MU, $n = 6$; $Has2^{\Delta HSC}$-Veh, $n = 8$; $Has2^{\Delta HSC}$-4-MU, $n = 5$; $Notch1^{\Delta HSC}$-Veh, $n = 7$; $Notch1^{\Delta HSC}$-4-MU, $n = 6$). Data are mean ± s.e.m. $**P<0.01$; N.S., not significant. Statistical significance was calculated by one-way ANOVA with Tukey's *post hoc* analysis. **(F)** Summary of the proposed model. Overexpressed HAS2 in hepatic stellate cells promotes liver fibrosis by producing HA. Low-molecular weight forms of HA, elevated in fibrotic livers and blood, induce hepatic stellate cell activation through the HA receptors CD44 and TLR4 and a newly identified effector Notch1. Dysregulated HAS2 expression is transcriptionally regulated by TGF-β through Wilms tumor 1.

Table S1. Sequence of primers used for qPCR.

Gene	Forward	Reverse
18S rRNA	5’-AGTCCCTGCCCTTTGTACACA-3’	5’-CGATCCGAGGGCCTCACTA-3’
hHAS1	5’-ACCCACTGCGATGAGACAG-3’	5’-GTCATGAGGCCCAGGATG-3’
hHAS2	5’-ACAGACAGGCTGAGGACGAC-3’	5’-AGCTGTGATTCCAAGGAGGA-3’
hHAS3	5’-AGATGCTTCGAGTCCTGGAG-3’	5’-CTTCCGTAGCTCTGACCTGG-3’
hNOTCH1	5’-GGTGAGACCTGCCTGAATG-3’	5’-GTTCTTGCAGGGGGTGC-3’
hWT1	5’-CAGCTTGAATGCATGACCTG-3’	5’-TATTCTGTATTGGGCTCCGC-3’
mHas1	5’-CTATGCTACCAAGTATACCTCG-3’	5’-TCTCGGAAGTAAGATTTGGAC-3’
mHas2	5’-TGAGCAGGAGCTGAACAAGA-3’	5’-GCCAACAATATAAGCAGCTGTG-3’
mHas3	5’-GATGTCCAAATCCTCAACAAG-3’	5’-CCCACTAATACATTGCACAC-3’
mItga1	5’-CCTTCCCTCGGATGTGAGTCA-3’	5’-AAGTTCTCCCCGTATGGTAAGA-3’
mFn1	5’-ATGTGGACCCCTCCTGATAGT-3’	5’-GCCCAGTGATTTCAGCAAAGG-3’
mCol4a1	5’-GTCTGGCTTCTGCTGCTCTT-3’	5’-CACATTTTCCACAGCCAGAG-3’
mCol1a1	5’-ACATGTTCAGCTTTGTGGACC-3’	5’-TAGGCCATTGTGTATGCAGC-3’
mActa2	5’-ACTGGGACGACATGGAAAAG-3’	5’-GTTCAGTGGTGCCTCTGTCA-3’
mTimp1	5’-GTAAGGCCTGTAGCTGTGCC-3’	5’-AGGTGGTCTCGTTGATTTCT-3’
mCcl2	5’-CCTGCTGTTCACAGTTGCC-3’	5’-ATTGGGATCATCTTGCTGGT-3’
mCxcl1	5’-TGCACCCAAACCGAAGTC-3’	5’-GTCAGAAGCCAGCGTTCACC-3’
mCd44	5’-GCACTGTGACTCATGGATCC-3’	5’-TTCTGGAATCTGAGGTCTCC-3’
mTlr4	5’-TGTTCTTCTCCTGCCTGACA-3’	5’-TGTCATCAGGGACTTTGCTG-3’
mNotch1	5’-GTGCCTGCCCTTTGAGTCTT-3’	5’-GCGATAGGAGCCGATCTCATTG-3’
mHes1	5’-ATAGCTCCCGGCATTCCAA-3’	5’-GCGCGGTATTTCCCCAACA-3’
mWt1	5’-AATGCGCCCTACCTGCCCA-3’	5’-CCGTCGAAAGTGACCGTGCTGTAT-3’
mJag1	5’-CAGTGCCTCTGTGAGACCAA-3’	5’-AGGGGTCAGAGAGACAAGCA-3’