Supplementary Materials and Methods

Hepatocyte toxicity assay. Freshly isolated hepatocytes were incubated for overnight with varying concentrations (0-125 μM) of sodium glycochenodeoxycholate (GCDC) or sodium glycocholate hydrate (GCA). Cell numbers were counted.

Bile acid assay. Bile acids were measured using Mouse Total Bile Acids Assay Kit (Crystal Chem) following the manufacturer's instructions.

In vivo localization of FITC-labeled proteins. CCN1 or JAG1 were fluorescein isothiocyanate (FITC)-labeled using a ProtOn Fluorescein labeling kit according to the manufacturer's instructions (Vector Laboratories). Proteins (CCN1, 1 mg/kg; Jag1, 1 mg/kg) were injected via retro-orbital and intraperitoneal delivery, and liver tissues were collected after 2 or 24 hours. Fluorescent signals were obtained using liver cryosections (7 μm).

Immunocytochemistry. For p16 staining, cholangiocytes were stained with anti-p16 antibody (Santa Cruz Biotech) and visualized with anti-rabbit IgG Alexa Fluor 488 (Invitrogen). TUNEL assay was performed using the ApopTaq Red detection kit (Millipore) following manufacturer's protocol.

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure S1. Bile acid accumulation and hepatocyte sensitivity to bile acid in *Ccn1*$^{wt/wt}$ and *Ccn1*$^{D125A/D125A}$ mice. (A) Primary hepatocytes were isolated from *Ccn1*$^{wt/wt}$ and *Ccn1*$^{D125A/D125A}$ mice and incubated with indicated concentration of GCDCA or GCA overnight, and cell numbers were counted. Average values from triplicate determination are expressed as mean ± s.d. (B) Liver bile acids and serum bile acids from *Ccn1*$^{wt/wt}$ and *Ccn1*$^{D125A/D125A}$ mice 7 days after BDL or sham operation were measured. Data expressed are mean ± s.d. n=6 per group. Bile acid production and hepatocyte sensitivity to bile acid in *Ccn1*$^{wt/wt}$ and *Ccn1*$^{D125A/D125A}$ mice were similar.

Supplemental Figure S2. Hepatocytes proliferation from *Ccn1*$^{wt/wt}$ and *Ccn1*$^{D125A/D125A}$ mice after BDL or DDC-diet were not different. Liver sections from *Ccn1*$^{wt/wt}$ and *Ccn1*$^{D125A/D125A}$ 7 days after BDL (upper panel) or DDC-diet for 6 weeks (lower panel) were immunostained for PCNA, and percentage of PCNA-positive hepatocytes were quantified and expressed as mean ± s.d. (n=6 per group). Bar=50 μm.

Supplemental Figure S3. Localization of FITC-labeled CCN1 or Jag1 in the liver. (A) FITC-labeled CCN1 protein or soluble Jag1 (1 mg/kg each) were delivered into WT mice via retro-orbital injection (A) or intraperitoneal injection (B), and liver tissues were collected after 2 or 24 hours. Fluorescent signals (494 nm excitation/518 nm emission) were obtained in liver cryosections (7 μm). Bar=100 μm.

Supplemental Figure S4. Knockdown of *Ccn1* did not induce cholangiocyte apoptosis or senescence. LMCCs were incubated with *siCcn1* or non-targeting siRNA control for 2 days,

and analyzed for TUNEL staining (left panel) and stained with antibody recognizing p16 (right panel). Bar = 50 μm.

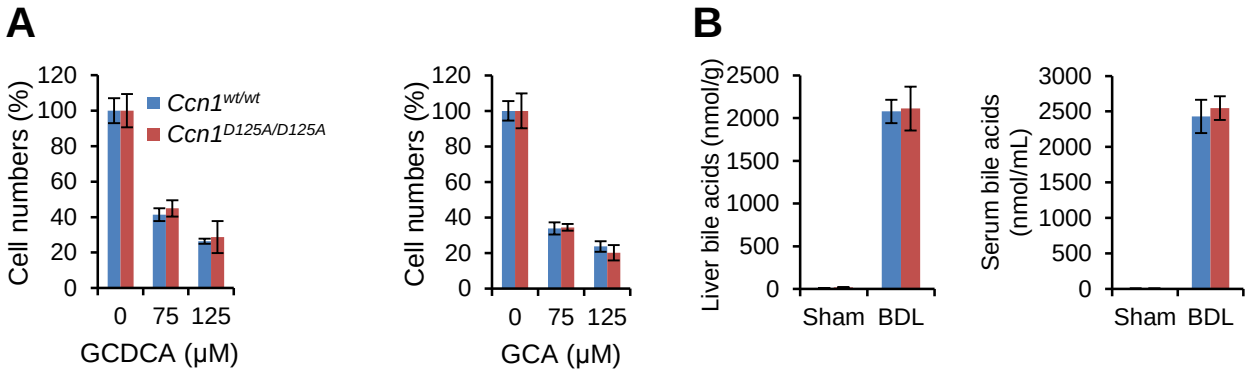
Supplemental Figure S5. Adenoviral overexpression of *Ccn1* enhanced cholangiocyte cell proliferation. LMCCs were transduced with adenovirus overexpressing *Ccn1* or *LacZ* as control. (A) Pictures showing cells 2 days after viral transduction. (B) Cells were incubated with BrdU for 25 min. and percentage of BrdU positive cells were counted. Data are expressed as mean ± s.d. of triplicate determinations. **p*<0.001, Student *t* test.

Supplemental Figure S6. Efficiency of siRNA knockdown. LMCCs were treated siRNA targeting indicated integrin subunits and non-targeting siRNA as control for 2 days. Total RNA was isolated and mRNA expression of genes indicated was evaluated by qRT-PCR. Data are expressed as mean ± s.d. of triplicate determinations. **p*<0.0001, Student *t* test.

Supplemental Figure S7. Expression of proliferation-related NFκB target genes. LMCCs were treated with *siCcn1*, *siJag1* or non-targeting siRNA as control for 2 day, or treated overnight with purified recombinant CCN1 or BSA as control (4 μg/ml each), BAY11-7082 (5 μM), control peptide (25 μM) or NBD (25 μM). Expression of cyclin D1, IL-6, and β-actin was measured by qRT-PCR of mRNA. Data are expressed as mean ± s.d. of triplicate determinations. **p*<0.005, Student *t* test.

Supplemental Figure S8. Gene expression in cholangiocytes treated with *siCcn1* or CCN1 protein. (A) LMCCs were treated with *siCcn1* or non-targeting siRNA as control for 2 days. Expression of indicated genes was measured by qRT-PCR of mRNA. (B) Cells were treated overnight with purified recombinant CCN1 or BSA as control (4 μg/ml each), and expression of

indicated genes was analyzed by qRT-PCR. Data are expressed as mean ± s.d. of triplicate determinations. *p<0.004, Student t test.



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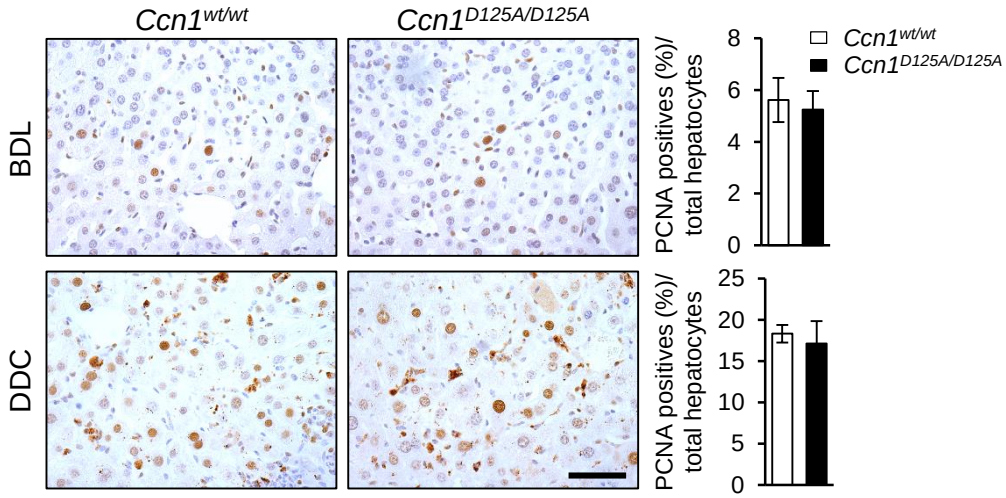

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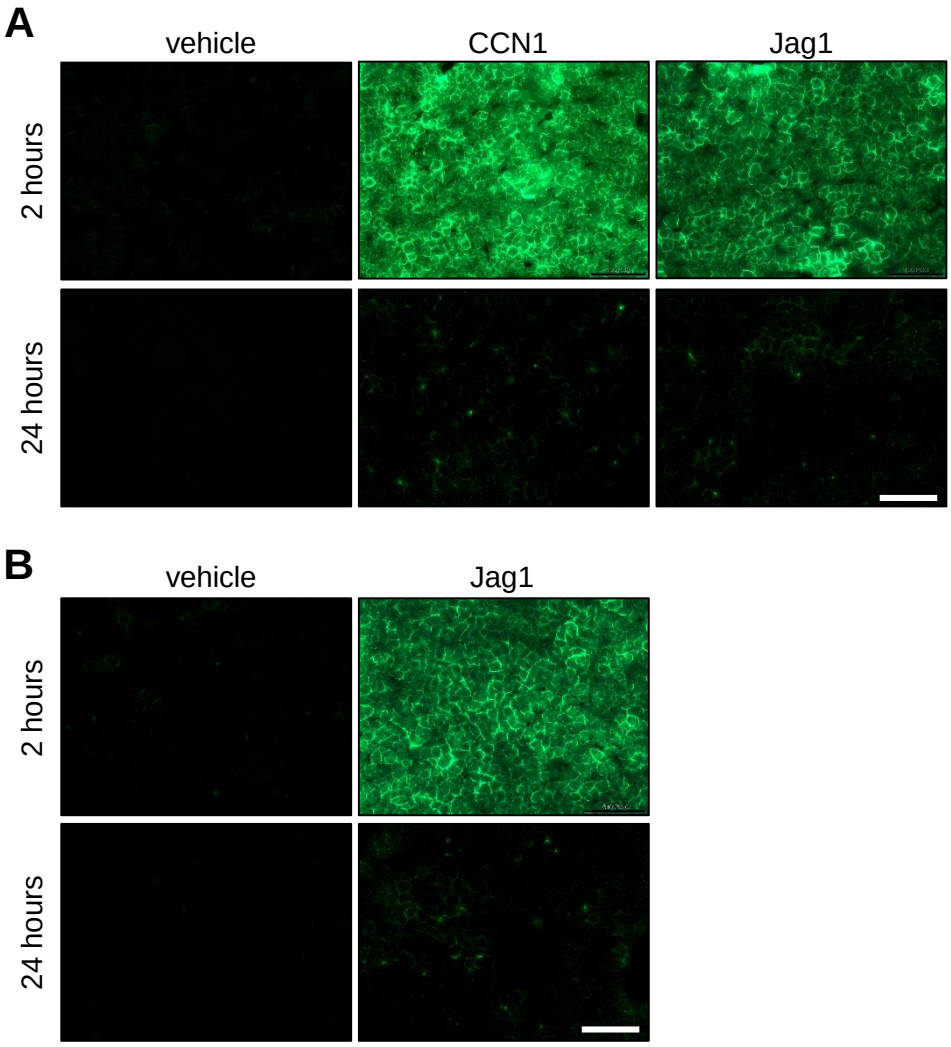

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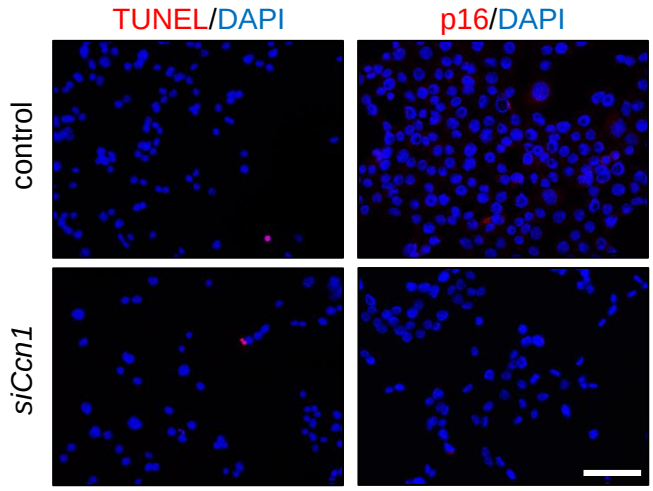

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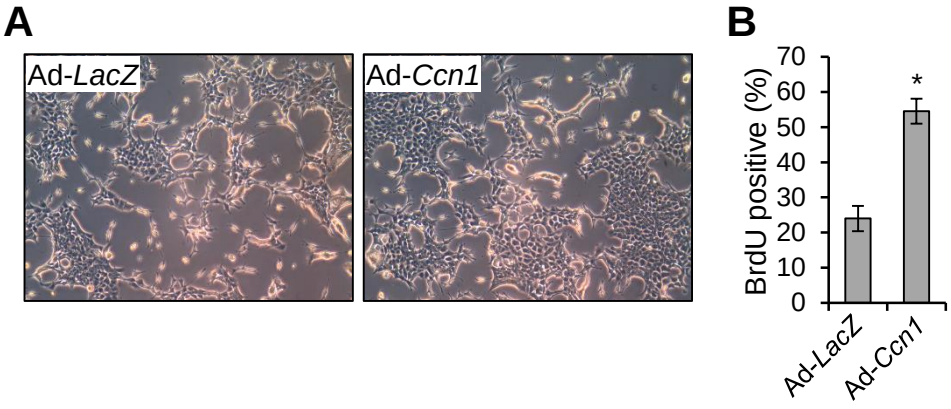

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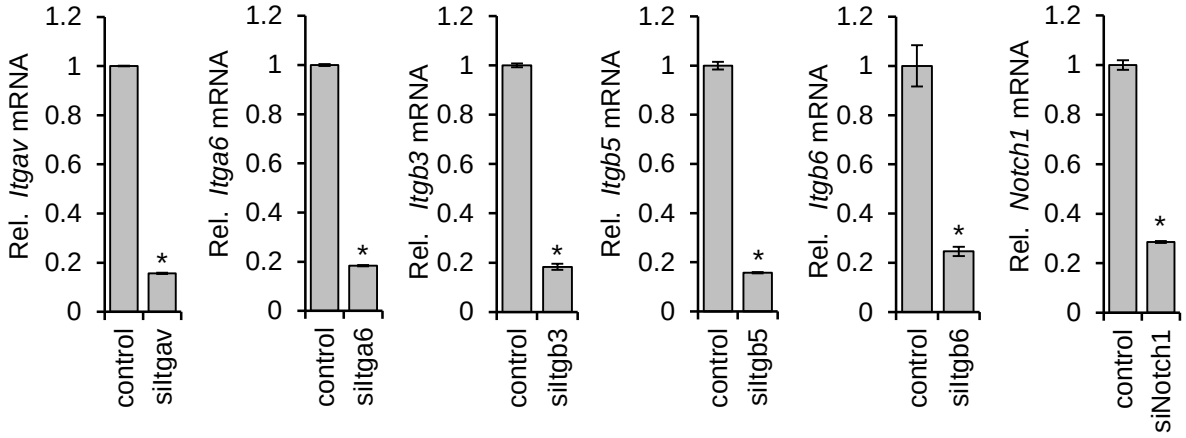

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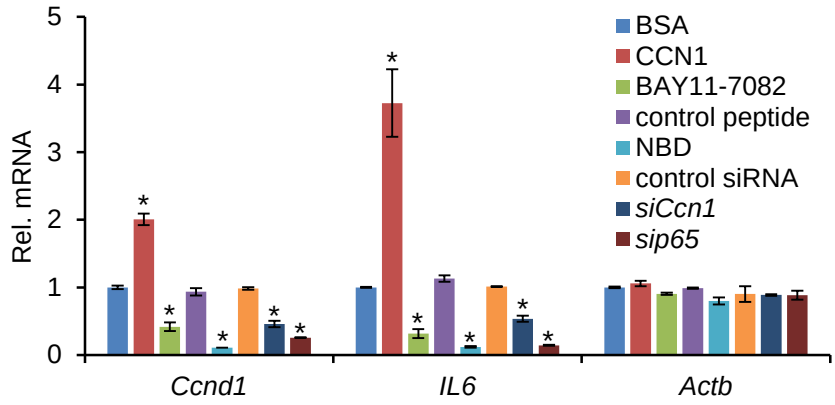

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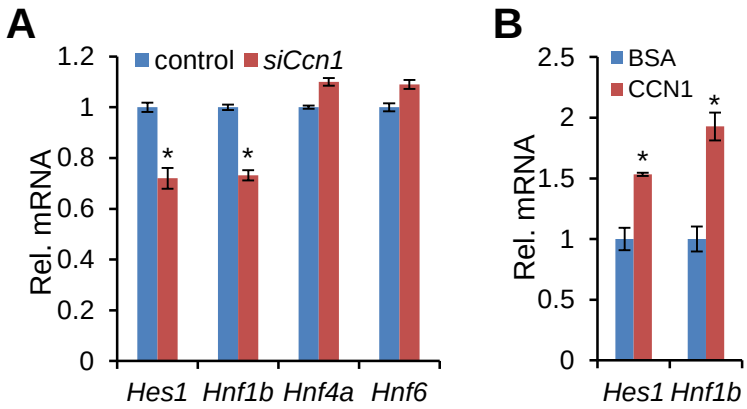

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Supplementary Table 1. Primer sequences used in this study.

Gene	Orientation	Sequence (5' to 3')
Actb	sense	ctaaggccaaccgtgaaag
	antisense	accagaggcatacagggaca
Ccn1	sense	aaaggcagctcactgaag
	antisense	gccggtatttcttgacac
Ccnd1	sense	taggccctcagcctcact
	antisense	ccacccctgggataaagcac
Cftr	sense	gctagtgctgatttggtgcg
	antisense	gtcagccactcccacgtaaa
Ck19	sense	acttgcgcgacaagattc
	antisense	aacttggttctgaagtcatctgc
CypE	sense	ttcacaaaccacaatggcacaggg
	antisense	tgccgtccagccaatctgtcttat
Dll1	sense	tacacatgttcctgccgacc
	antisense	aggtgcaagagaagctgtcc
Dll3	sense	gcaccttctccctcgtcatt
	antisense	gaagtgcaactcccatgtgc
Dll4	sense	ggtcgcctgtgcaatgaatg
	antisense	ttcttgcacggagagtggtg
Hes1	sense	agaggctgccaaggtttttg
	antisense	tcccactgttgctggtgtaga
Hnf1a	sense	ccacgccttatacagccaca
	antisense	atcaacatggtctgcgggag
Hnf1a	sense	ccacgccttatacagccaca
	antisense	atcaacatggtctgcgggag
Hnf1b	sense	catctgcaatggtggtcacag
	antisense	ggcttgcagtggcacctgttt
Hnf4a	sense	atgacacgtccccatctgaag
	antisense	ctcgaggctccgtagtgtttg
Hnf6	sense	caaatcaccatctcccagcag
	antisense	cagactcctcctcctggcatt
Il6	sense	accactcccaacagacc
	antisense	tccagaagaccagaggaa
Itgav	sense	cgtcctccaggatgtttctcc
	antisense	tccaaaccactggtgggact
Itga6	sense	tgccacctatcacaaggctg
	antisense	cggggaatgctgtcatcgta
Itgb3	sense	gctcattggccttgctactc
	antisense	cccggtaggtgatattggtg
Itgb5	sense	aatgtggaagtgcccccaat
	antisense	gtacaggggtttgaggctt

Gene	Oirentation	Sequence (5’ to 3’)
Itgb6	sense	tctgaggatggagtgctgtg
	antisense	ccatctgcagacaggtagca
Jag1	sense	cagtgcctctgtgagaccaa
	antisense	aggggtcagagagacaagca
Jag2	sense	gctacttgggcaagaactgc
	antisense	gttccatcctgacggacagt
Notch1	sense	cccactgtgaactgccctat
	antisense	ccccattcttgcagttgttt
Notch2	sense	aaaatctgccctccactgg
	antisense	ccgcttcataacttccctctc
Notch3	sense	tgaacaacgtggaggctacc
	antisense	gcagcctgtccaagtgatct
Notch4	sense	ctctgtcccccaggtttcac
	antisense	cccgggcttcacattcatct
Shh	sense	acccaactccgatgtgttccgtta
	antisense	tatataaccttgcctgccgctgct
Wnt3a	sense	tcactgcgaaagctactcca
	antisense	caccaccgtcagcaacag
Wnt7b	sense	tcccctgtctgtcatgtctctt
	antisense	ctgtttcaagcagaaggaggag

Supplementary Table 2. List of RNAi sequences

Protein	Target sequence (5' to 3')
Ccn1	taactcattgtttctcgttaactccac
Integrin alpha-v	gtcatatttagatatgatttctgccac
Integrin alpha-6	aaagggtaacatcaccttctattgcac
Integrin beta-3	attccttaactgcttgttctactactg
Integrin beta-5	ttccactagtgcatatgttgagccctg
Integrin beta-6	ataccataactaatacaatccttccat
Jagged1	agctatattacaggttgttccttccca
Notch1	atcttgtaaggaatattgaggctgcca
p65	gcgagttatagcttcagggtactccat