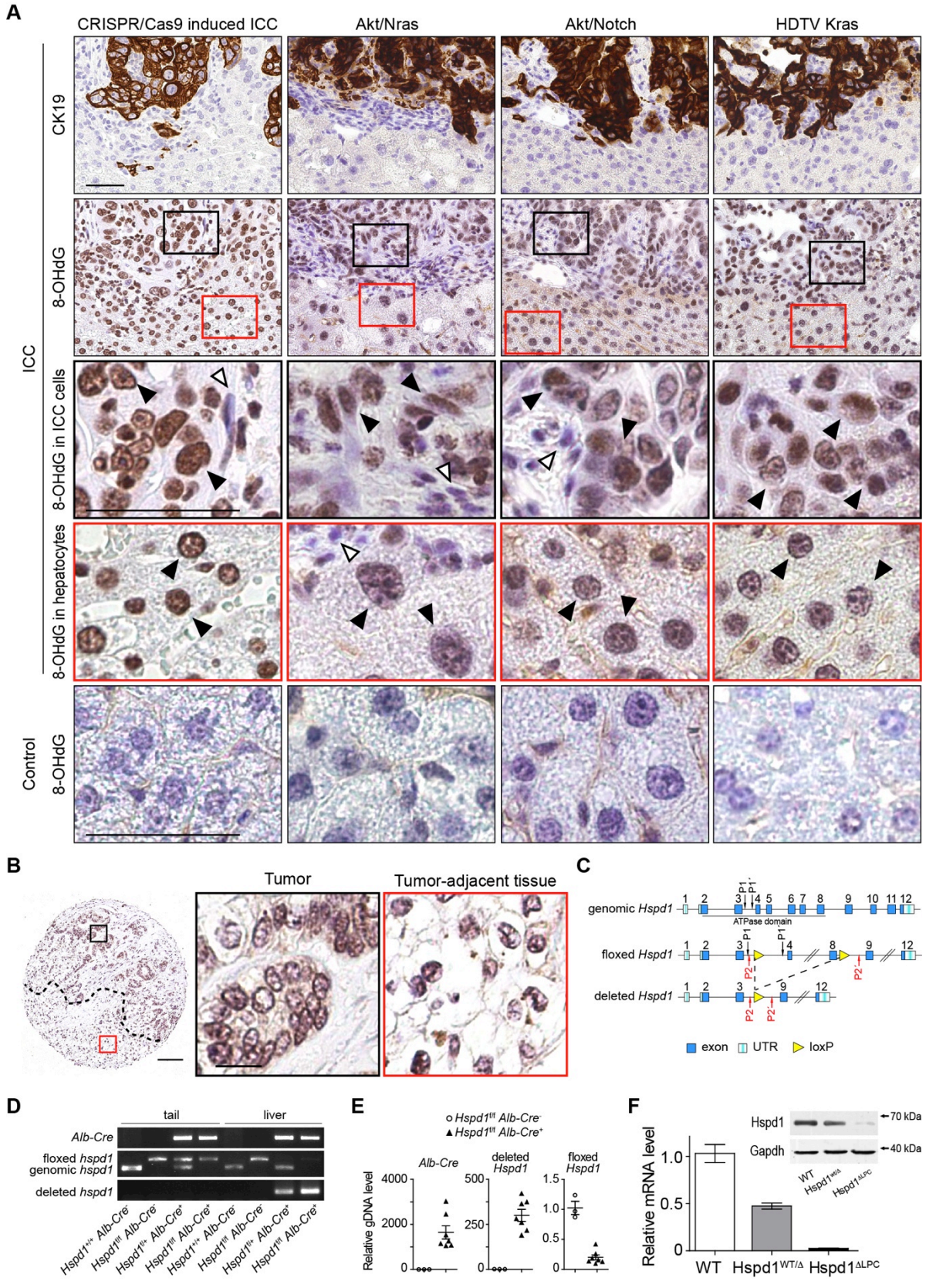
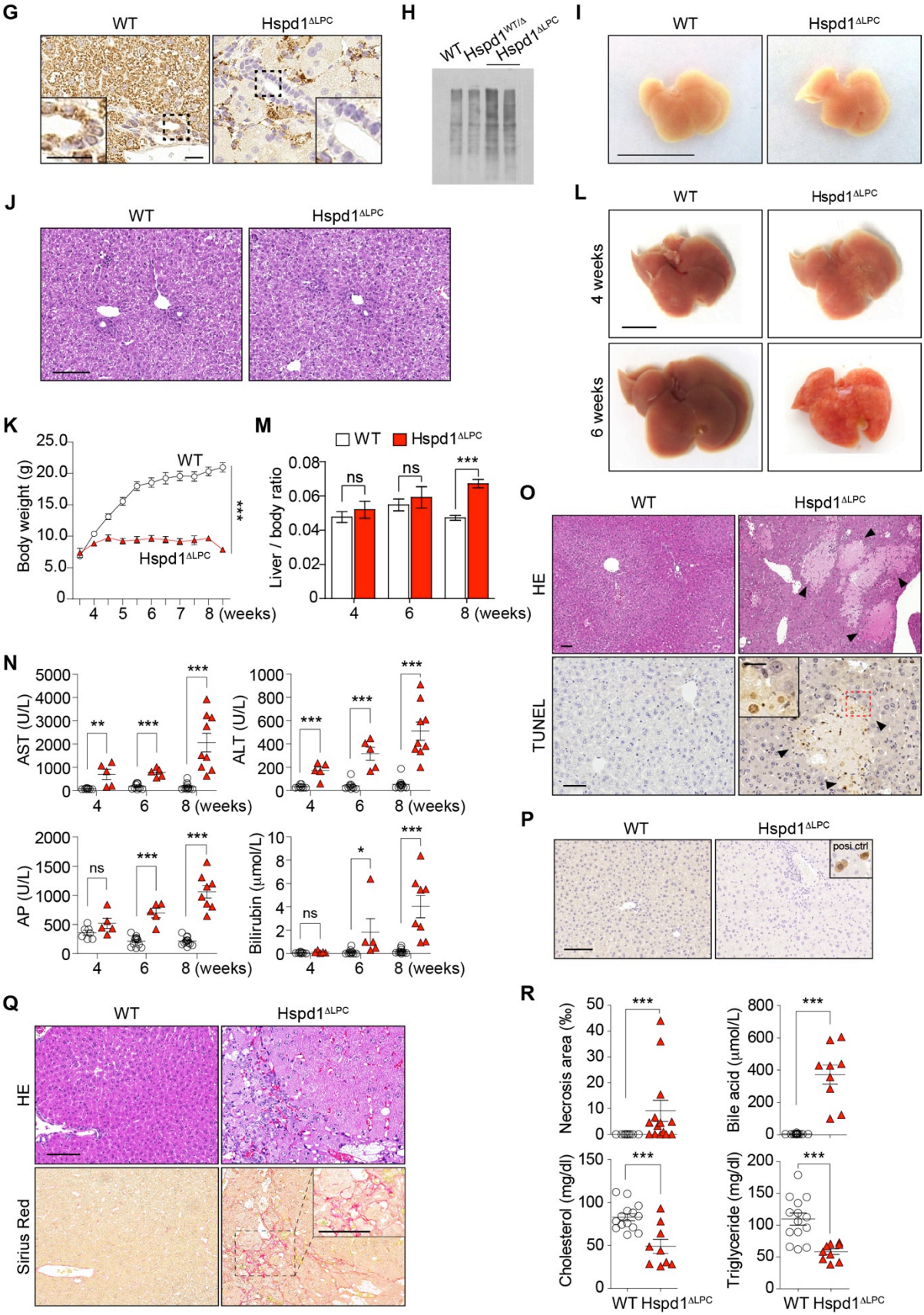
Supplemental Information


A
CRISPR/Cas9 induced ICC
Akt/Nras
Akt/Notch
HDTV Kras
ICC
CK19
8-OHdG
8-OHdG in ICC cells
8-OHdG in hepatocytes
Control
8-OHdG
B
Tumor
Tumor-adjacent tissue
C
genomic Hspd1
floxed Hspd1
deleted Hspd1
ATPase domain
exon
UTR
loxP
D
tail
liver
Alb-Cre
floxed hspd1
genomic hspd1
deleted hspd1
Hspd1+/+ Alb-Cre-
Hspd1f/f Alb-Cre-
Hspd1f/+ Alb-Cre-
Hspd1f/f Alb-Cre+
Hspd1+/+ Alb-Cre+
Hspd1f/f Alb-Cre+
Hspd1f/+ Alb-Cre+
Hspd1f/f Alb-Cre+
E
Hspd1f/f Alb-Cre-
Hspd1f/f Alb-Cre+
Alb-Cre
deleted Hspd1
floxed Hspd1
Relative gDNA level
F
Hspd1
Gapdh
70 kDa
40 kDa
WT
Hspd1WT/Δ
Hspd1ΔLPC
Relative mRNA level


G
WT
Hspd1ΔLPC
H
WT
Hspd1WT/Δ
Hspd1ΔLPC
I
WT
Hspd1ΔLPC
J
WT
Hspd1ΔLPC
L
WT
Hspd1ΔLPC
4 weeks
6 weeks
K
Body weight (g)
WT
Hspd1ΔLPC
***
4 5 6 7 8 (weeks)
M
WT
Hspd1ΔLPC
Liver / body ratio
ns
ns
***
4 6 8 (weeks)
O
WT
Hspd1ΔLPC
HE
TUNEL
N
AST (U/L)
ALT (U/L)
AP (U/L)
Bilirubin (μmol/L)
ns
4 6 8 (weeks)
P
WT
Hspd1ΔLPC
posi.ctrl
Q
WT
Hspd1ΔLPC
HE
Sirius Red
R
Necrosis area (‰)
Bile acid (μmol/L)
Cholesterol (mg/dl)
Triglyceride (mg/dl)
WT Hspd1ΔLPC

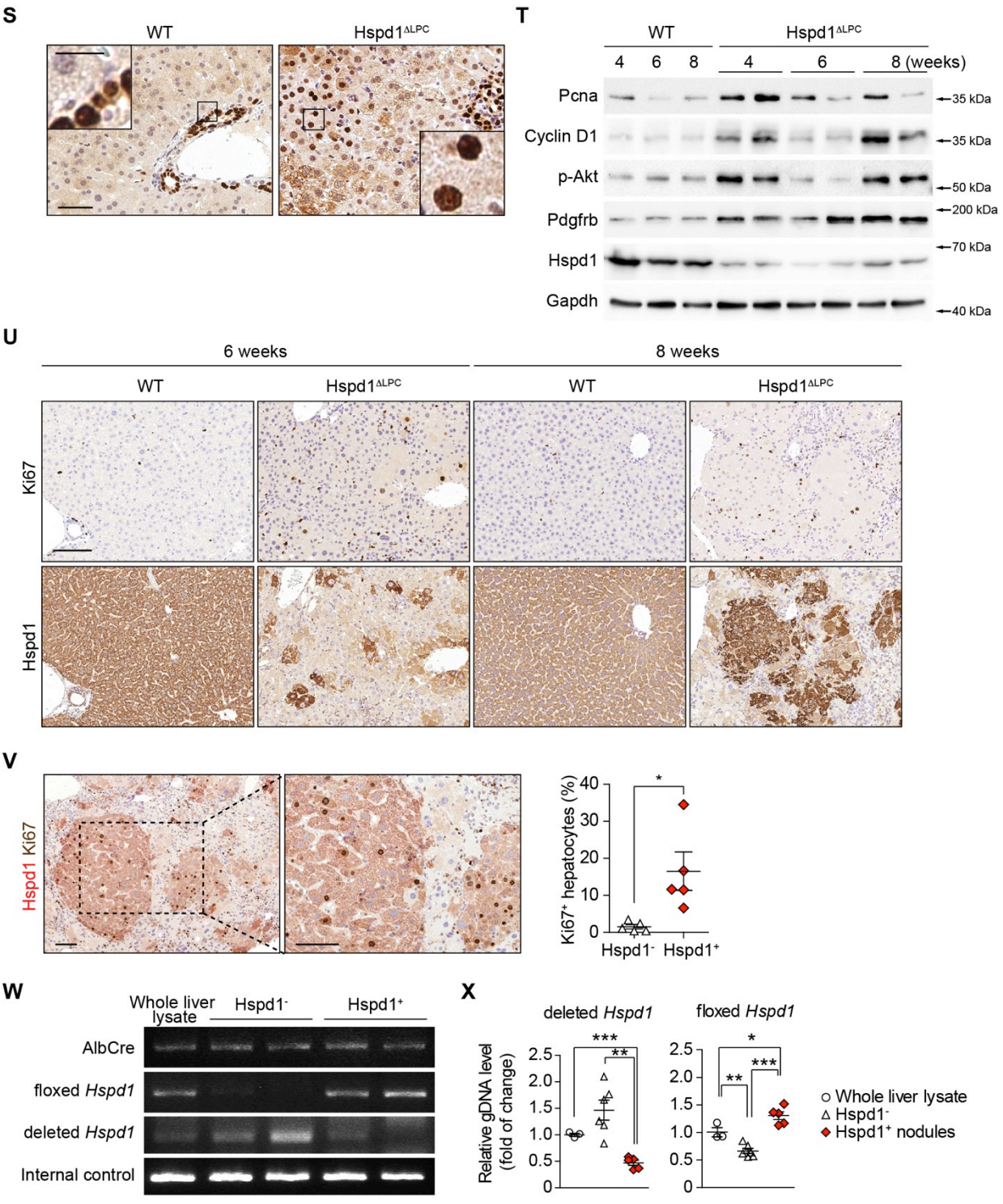
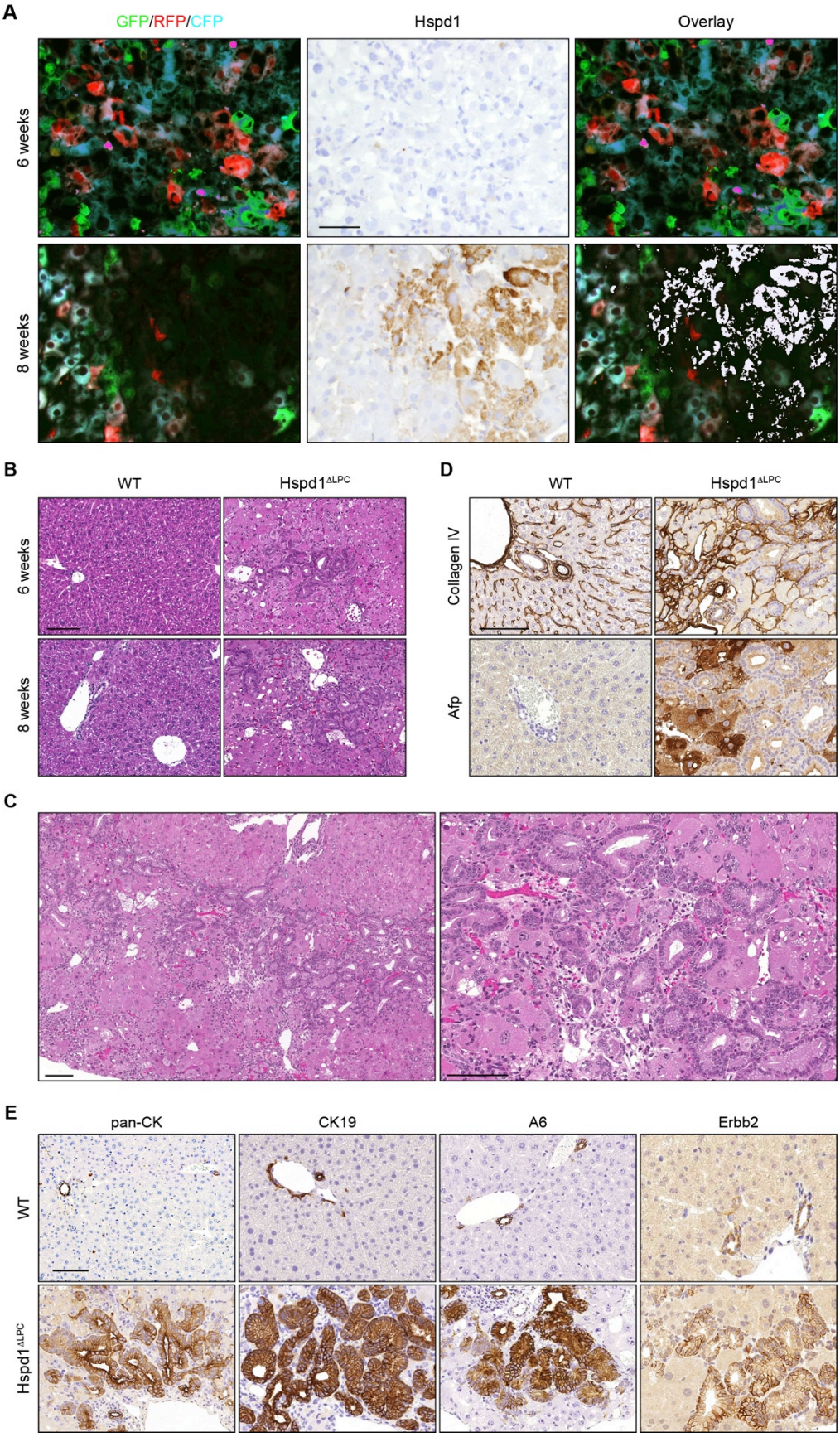
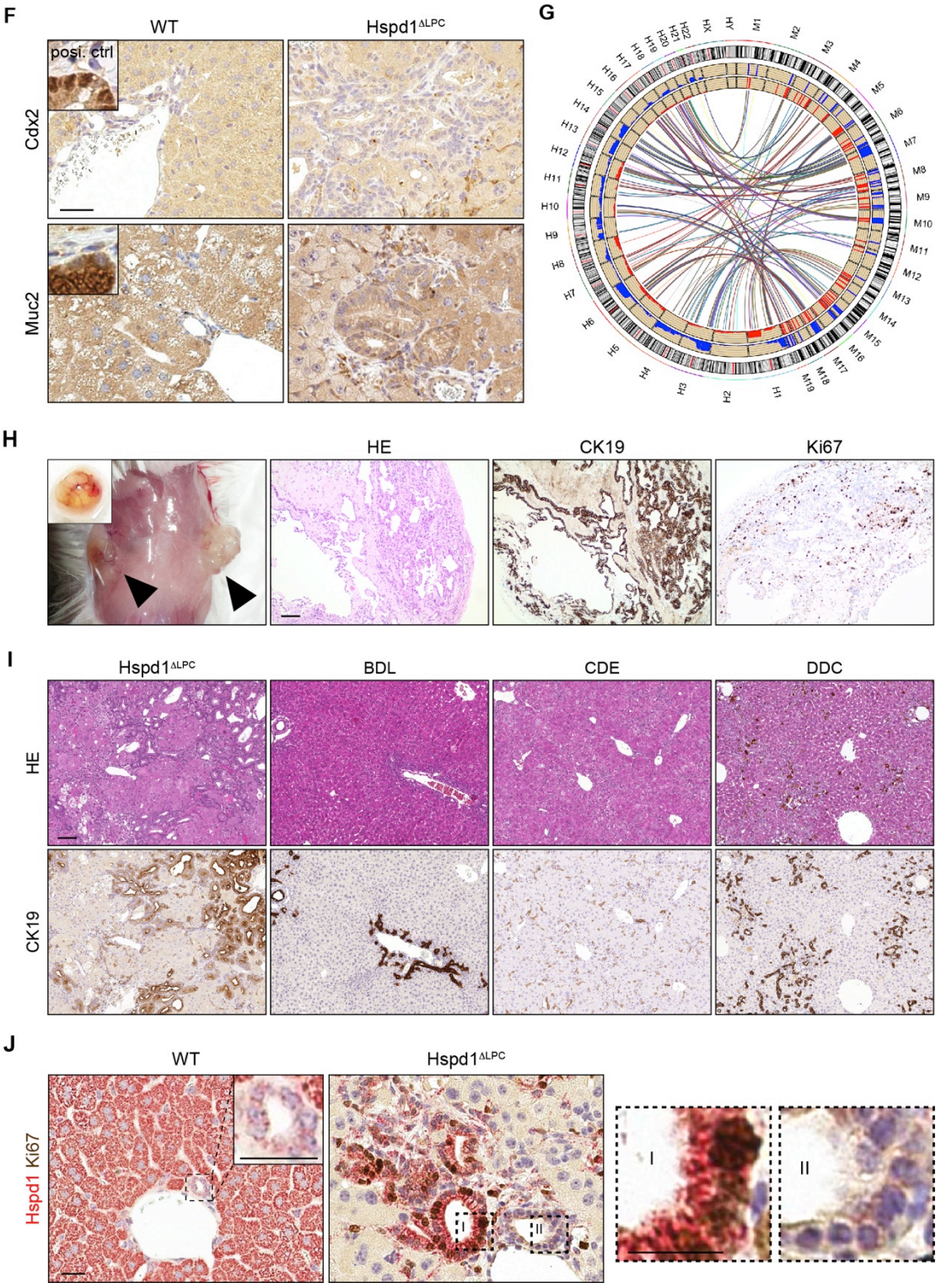


Figure S1. Hspd1$^{\Delta LPC}$ mice mimics ROS accumulation in livers of human ICC patients. Related to Figure 1. (A) Immunohistochemistry staining of CK19 and 8-OHdG of livers from four mouse ICC models as indicated. Black arrowheads indicate the positive staining of 8-OHdG in liver parenchymal cells, while white arrowheads indicate the negative staining in non-parenchymal cells. Scale bar: 50

μm. **(B)** Representative 8-OHdG staining of human ICC samples. Scale bar on the left: 100 μm. Scale bar on the right: 20 μm. **(C)** Schematic representation of genomic *Hspd1* (top), floxed *Hspd1* and deleted *Hspd1* (bottom) alleles. Exons are represented by blue boxes. The primer pairs detecting the floxed alleles (P1 and P1') and the deleted alleles (P2 and P2') are shown. **(D)** Genotypes were determined by PCR using tail genomic DNA and liver genomic DNA from the mice as indicated. **(E)** Relative DNA levels of *cre*, floxed *Hspd1* alleles and deleted *Hspd1* alleles were determined by qRT-PCR with liver genomic DNA from $Hspd1^{f/f}$ $AlbCre^{-}$ mice and $Hspd1^{f/f}$ $AlbCre^{+}$ mice. **(F)** Western blot and qRT-PCR of whole liver lysates from 6-week-old $Hspd1^{\Delta LPC}$ mice, $Hspd1^{\Delta LPC/+}$ mice and control littermates (WT). **(G)** Representative immunohistochemistry staining of Hspd1 of liver sections from 8-week-old $Hspd1^{\Delta LPC}$ mice and WT littermates. Scale bar: 20 μm. **(H)** OxyBlot of whole liver extracts from mice indicated. **(I)** Macroscopic appearance of livers from postnatal day 7 (P7) $Hspd1^{\Delta LPC}$ mouse and control littermate (WT). Scale bar: 1 cm. **(J)** HE staining of liver sections from postnatal day 7 (P7) $Hspd1^{\Delta LPC}$ mouse and control littermate (WT). Scale bar: 100 μm. **(K)** Body weights of $Hspd1^{\Delta LPC}$ mice and control littermates were measured. **(L)** Macroscopic appearance of livers from postnatal 4- and 6-week-old $Hspd1^{\Delta LPC}$ mice and control littermates (WT). Scale bar: 5 mm. **(M)** Liver weight to body weight ratios of 4-, 6- and 8-week-old $Hspd1^{\Delta LPC}$ mice and WT littermates. **(N)** Serum levels of AST, ALT, AP and Bilirubin in of 4-, 6- and 8-week-old $Hspd1^{\Delta LPC}$ mice and control littermates. **(O)** H&E and TUNEL staining of liver sections from 8-week-old $Hspd1^{\Delta LPC}$ mice and WT littermates. Scale bar: 100 μm. **(P)** Cleaved-Caspase3 (cl-Caspase3) staining of liver sections from 8-week-old $Hspd1^{\Delta LPC}$ mice and WT littermates. Scale bar: 100 μm. **(Q)** Sirius red and reticulin stainings of liver sections from 8-week-old $Hspd1^{\Delta LPC}$ mice and WT littermates. Scale bar: 100 μm. **(R)** Upper left: Quantification of necrotic areas in $Hspd1^{\Delta LPC}$ mice and WT littermates. Upper right and lower: Serum levels of bile acids, cholesterol and triglyceride in $Hspd1^{\Delta LPC}$ mice and WT littermates. **(S)** Sox9 staining of liver sections from 8-week-old $Hspd1^{\Delta LPC}$ mice and WT littermates. Scale bar: 50 μm. **(T)** Western blot analysis of cell-cycle related proteins Pcna, Cyclin D1, p-Akt, Pdgfrb, Hspd1 and Gapdh of whole liver protein extracts from 4-, 6- and 8-week-old $Hspd1^{\Delta LPC}$ mice and WT littermates. **(U)** Representative Ki67 and Hspd1 staining of consecutive liver sections from 6- and 8-week-old $Hspd1^{\Delta LPC}$ mice and WT littermate. Scale bar: 100 μm. **(V)** Double staining of Hspd1 (red) and Ki67 (brown) of liver sections from 8-week-old $Hspd1^{\Delta LPC}$ mice and WT littermates. Quantification of $Ki67^{+}$ hepatocytes is shown in the right panel. Scale bar: 50 μm. **(W)** Genotypes were determined by PCR using genomic DNA from $Hspd1^{+}$ areas and $Hspd1^{-}$ areas of the 8-week-old $Hspd1^{\Delta LPC}$ livers. **(X)** Relative DNA levels of floxed *hspd1* alleles and deleted *hspd1* alleles were determined by qRT-PCR with genomic DNA as indicated.

Data are represented as the mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. ns, no significance.


A
GFP/RFP/CFP
Hspd1
Overlay
6 weeks
8 weeks
B
WT
Hspd1ΔLPC
6 weeks
8 weeks
D
WT
Hspd1ΔLPC
Collagen IV
Afp
C
E
pan-CK
CK19
A6
Erbb2
WT
Hspd1ΔLPC


F
WT
Hspd1ΔLPC
posi. ctrl
Cdx2
Muc2
G
H
HE
CK19
Ki67
I
Hspd1ΔLPC
BDL
CDE
DDC
HE
CK19
J
WT
Hspd1ΔLPC
Hspd1 Ki67
I
II

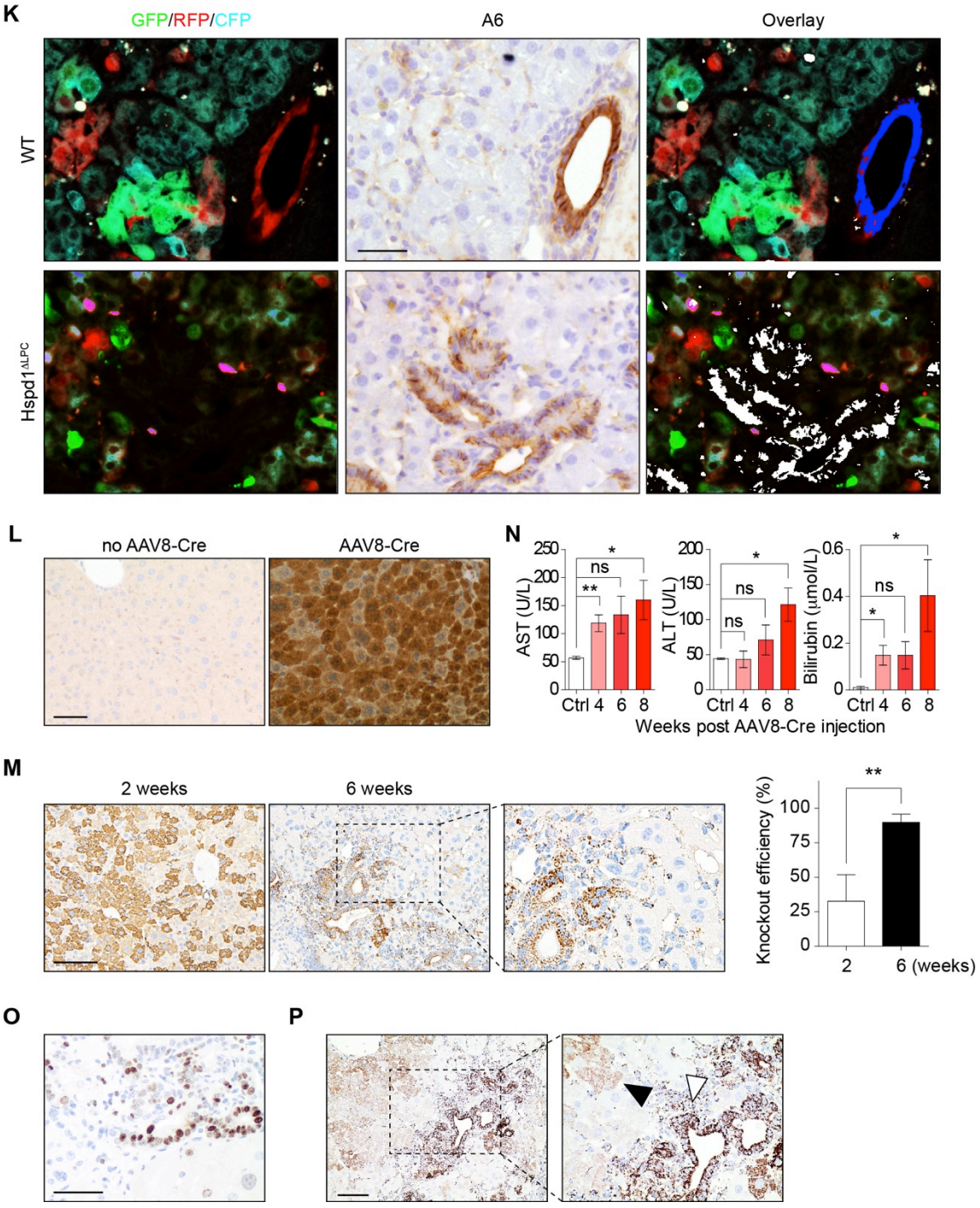


Figure S2. Cholangiocellular hyperproliferation in Hspd1$^{\Delta LPC}$ mice. Related to Figure 1. (A) Representative microscopy of liver sections from 6- and 8-week-old Hspd1$^{\Delta LPC}$-confetti mice. Unlabelled hepatocytes in 8-week-old Hspd1$^{\Delta LPC}$-confetti liver stained positive with Hspd1 antibody. Scale bar: 20 μm. **(B)** H&E staining of liver sections from 6- and 8-week-old Hspd1$^{\Delta LPC}$ mice and WT

littermates. Scale bar: 100 μm. **(C)** Representative H&E staining of liver sections showing widespread cholangiolar overgrowth from Hspd1$^{\Delta LPC}$ mice. Scale bar: 100 μm. **(D)** Collagen IV and Afstainings of liver sections from 8-week-old Hspd1$^{\Delta LPC}$ mice and WT littermates. Scale bar: 100 μm. **(E)** Immunohistochemistry analysis of biliary lineage markers cytokeratin, CK19, A6 and Erbb2 in liver sections from 8-week-old WT and Hspd1$^{\Delta LPC}$ mice. Scale bar: 50 μm. **(F)** Representative IHC staining of Cdx2 and Muc2 of liver sections from 8-week-old Hspd1$^{\Delta LPC}$ mice and WT littermates. Scale bar: 20 μm. **(G**) Synteny analysis for chromosomal aberrations of CK19^{+} cells in Hspd1$^{\Delta LPC}$ mice (M) and human ICC (H). Amplifications are indicated in red in the inner circle, deletions in blue in the outer circle of each circular plot. **(H)** H&E and CK19 staining of liver sections from different mouse liver injury models as indicated. Scale bar: 100 μm. **(I)** Representative photograph of SCID Beige mouse transplanted with liver pieces from 8-week-old Hspd1$^{\Delta LPC}$ mouse, and H&E, CK19, and Ki67 IHC of the tumor graft. Arrowheads indicate the tumor grafts. Scale bar: 100 μm. **(J)** Double staining of Hspd1 (red) and Ki67 (brown) of liver sections from 8-week-old Hspd1$^{\Delta LPC}$ mice and WT littermates. Scale bar: 20 μm. **(K)** Fluorescence microscopy and A6 staining of liver sections from 8-week-old WT and confetti-Hspd1$^{\Delta LPC}$ mice. Unlabelled cholangiocytes stained positive with A6 antibody. Scale bar: 20 μm. **(L)** EYFP staining of reporter mice receiving AAV8 injection or not. Scale bar: 50 μm. **(M)** Knockout efficiency determined by Hspd1 staining in liver sections from AAV8-Cre Hspd1$^{loxP/loxP}$ mice at 2 and 6 weeks post AAV8 injection. Scale bar: 50 μm. **(N)** Serum levels of AST, ALT and Bilirubin in AAV8-Cre Hspd1$^{loxP/loxP}$ and control mice. **(O-P)** Representative Ki67 (O，Scale bar: 50 μm) and Hspd1 (P, Scale bar: 100 μm) staining of liver sections from AAV8-Cre Hspd1$^{loxP/loxP}$ mice. White arrowhead indicates the Hspd1^{+} cholangiocytes, while black arrowhead indicates the Hspd1^{+} hepatocytes.

Data are represented as the mean ± SEM. *$p < 0.05$, **$p < 0.01$. ns, no significance.

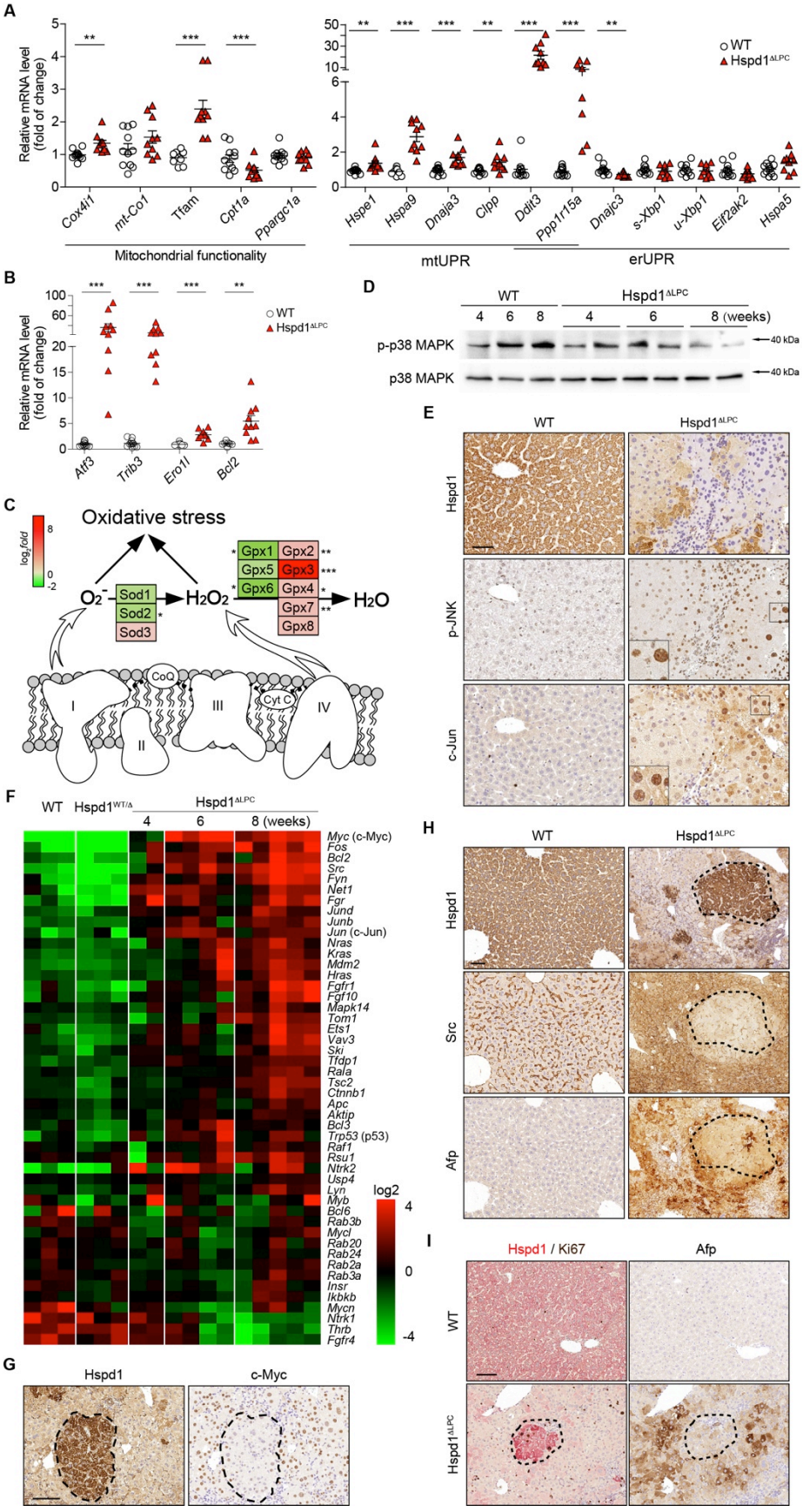
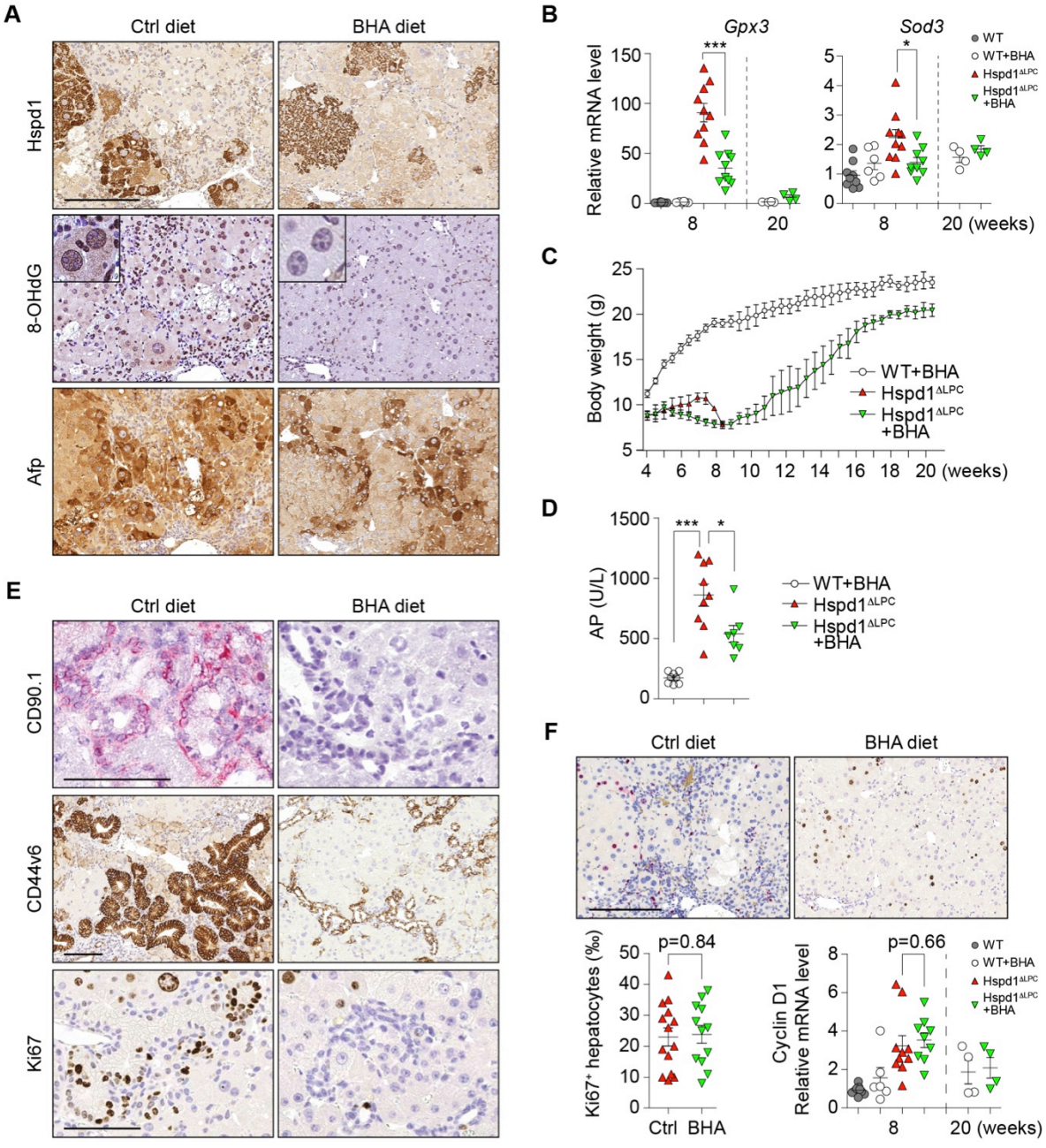

A
Relative mRNA level (fold of change)
WT
Hspd1ΔLPC
Cox4i1
mt-Co1
Tfam
Cpt1a
Ppargc1a
Mitochondrial functionality
Hspe1
Hspa9
Dnaja3
Clpp
Ddit3
Ppp1r15a
Dnajc3
s-Xbp1
u-Xbp1
Eif2ak2
Hspa5
mtUPR
erUPR
B
Atf3
Trib3
Ero1l
Bcl2
C
Oxidative stress
log2fold
O2-
Sod1
Sod2
Sod3
H2O2
Gpx1
Gpx2
Gpx5
Gpx3
Gpx6
Gpx4
Gpx7
Gpx8
H2O
CoQ
Cyt C
I
II
III
IV
D
WT
Hspd1ΔLPC
4
6
8
8 (weeks)
p-p38 MAPK
p38 MAPK
40 kDa
E
Hspd1
p-JNK
c-Jun
F
WT
Hspd1WT/Δ
Hspd1ΔLPC
Myc (c-Myc)
Fos
Bcl2
Src
Fyn
Net1
Fgr
Jund
Junb
Jun (c-Jun)
Nras
Kras
Mdm2
Hras
Fgfr1
Fgf10
Mapk14
Tom1
Ets1
Vav3
Ski
Tfdp1
Rala
Tsc2
Ctnnb1
Apc
Aktip
Bcl3
Trp53 (p53)
Raf1
Rsu1
Ntrk2
Usp4
Lyn
Myb
Bcl6
Rab3b
Mycl
Rab20
Rab24
Rab2a
Rab3a
Insr
Ikbkb
Mycn
Ntrk1
Thrb
Fgfr4
log2
G
Hspd1
c-Myc
H
Src
Afp
I
Hspd1 / Ki67
Afp
WT
Hspd1ΔLPC

Figure S3. Oxidative microenvironment and oncogenic signals in Hspd1$^{\Delta LPC}$ mice. Related to Figure 2. (A, B) qRT-PCR of whole liver lysates from 8-week-old Hspd1$^{\Delta LPC}$ mice and control littermates for the indicated genes. **(C)** Schematic representation of the expression of genes encoding antioxidant enzymes (Sod and Gpx families) in Hspd1 KO livers relative to WT livers. Gene expression levels by qRT-PCR were present in a color code: red=upregulated, green=downregulated. **(D)** Western blot analysis of p-p38 MAPK and total p38 MAPK in whole liver protein extracts from 4-, 6- and 8-week-old Hspd1$^{\Delta LPC}$ mice and WT littermates. Gapdh was used as internal control. **(E)** Immunohistochemistry analysis of Hspd1, p-JNK and c-Jun in consecutive liver sections from 8-week-old WT and Hspd1$^{\Delta LPC}$ mice. Scale bar: 50 μm. **(F)** Heat map of oncogenes in Hspd1$^{\Delta LPC}$ livers in comparison with WT livers over time. Gene expression levels by qRT-PCR were present in a color code: red=high, green=low. **(G)** Lower magnification of the main figure 2G. Immunohistochemistry analysis of Hspd1 and c-Myc in consecutive liver sections from 8-week-old WT and Hspd1$^{\Delta LPC}$ mice. Scale bar: 50 μm. **(H)** Immunohistochemistry analysis of Hspd1, Src, Afp in consecutive liver sections from 8-week-old WT and Hspd1$^{\Delta LPC}$ mice. Scale bar: 50 μm. **(I)** Left: double staining of Hspd1 (red) and Ki67 (brown) of liver sections from 8-week-old Hspd1$^{\Delta LPC}$ mice and WT littermates; Right: immunohistochemistry staining of Afp in the corresponding liver sections as shown in the left panel. Scale bar: 50 μm.

Data are represented as the mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.


A
Ctrl diet
BHA diet
Hspd1
8-OHdG
Afp
B
Gpx3
Sod3
Relative mRNA level
***
*
WT
WT+BHA
Hspd1ΔLPC
Hspd1ΔLPC +BHA
8
20
20 (weeks)
C
Body weight (g)
WT+BHA
Hspd1ΔLPC
Hspd1ΔLPC +BHA
4 6 8 10 12 14 16 18 20 (weeks)
D
AP (U/L)
***
*
WT+BHA
Hspd1ΔLPC
Hspd1ΔLPC +BHA
E
Ctrl diet
BHA diet
CD90.1
CD44v6
Ki67
F
Ctrl diet
BHA diet
Ki67+ hepatocytes (‰)
p=0.84
Ctrl BHA
Cyclin D1 Relative mRNA level
p=0.66
WT
WT+BHA
Hspd1ΔLPC
Hspd1ΔLPC +BHA
8
20 (weeks)

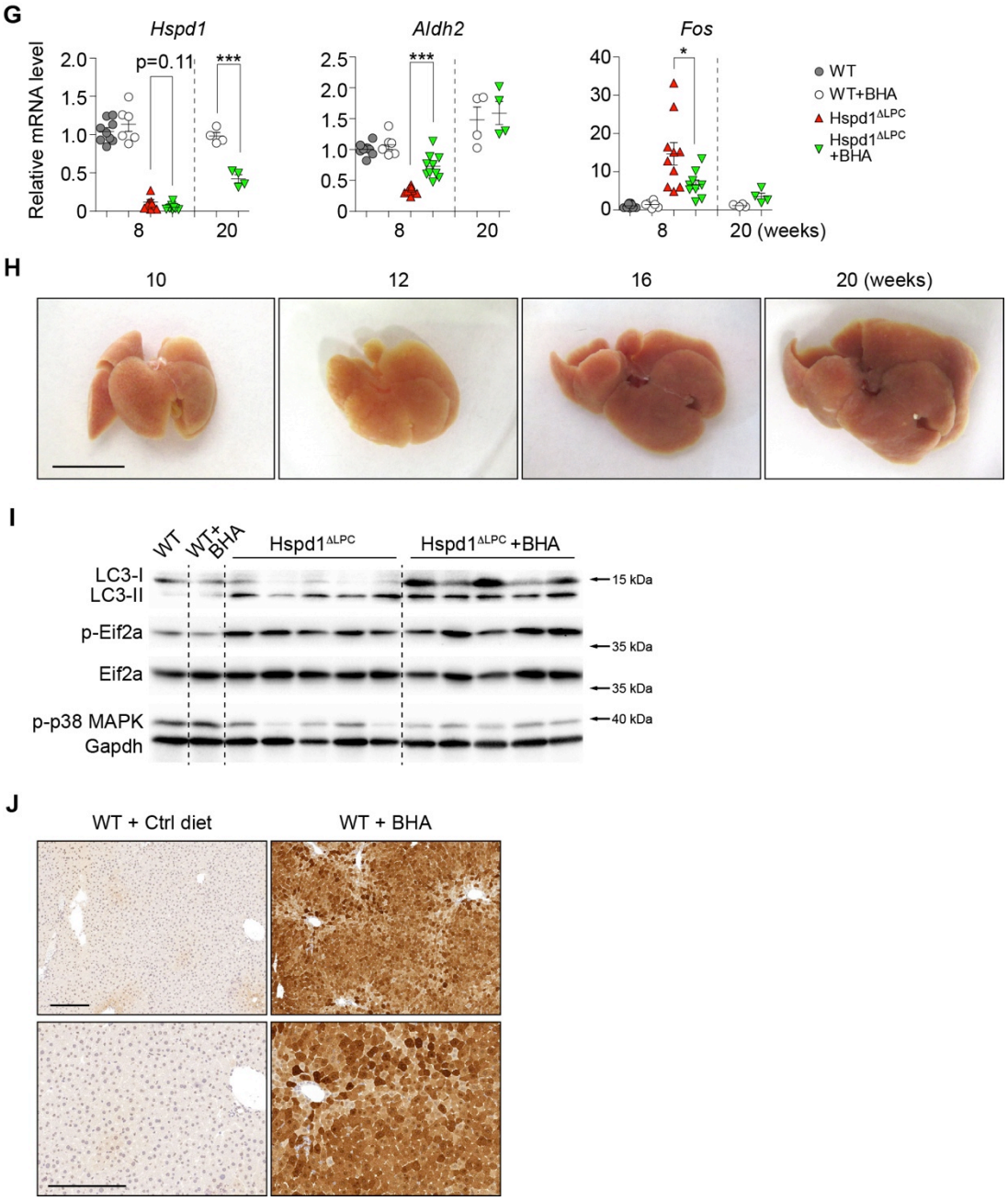
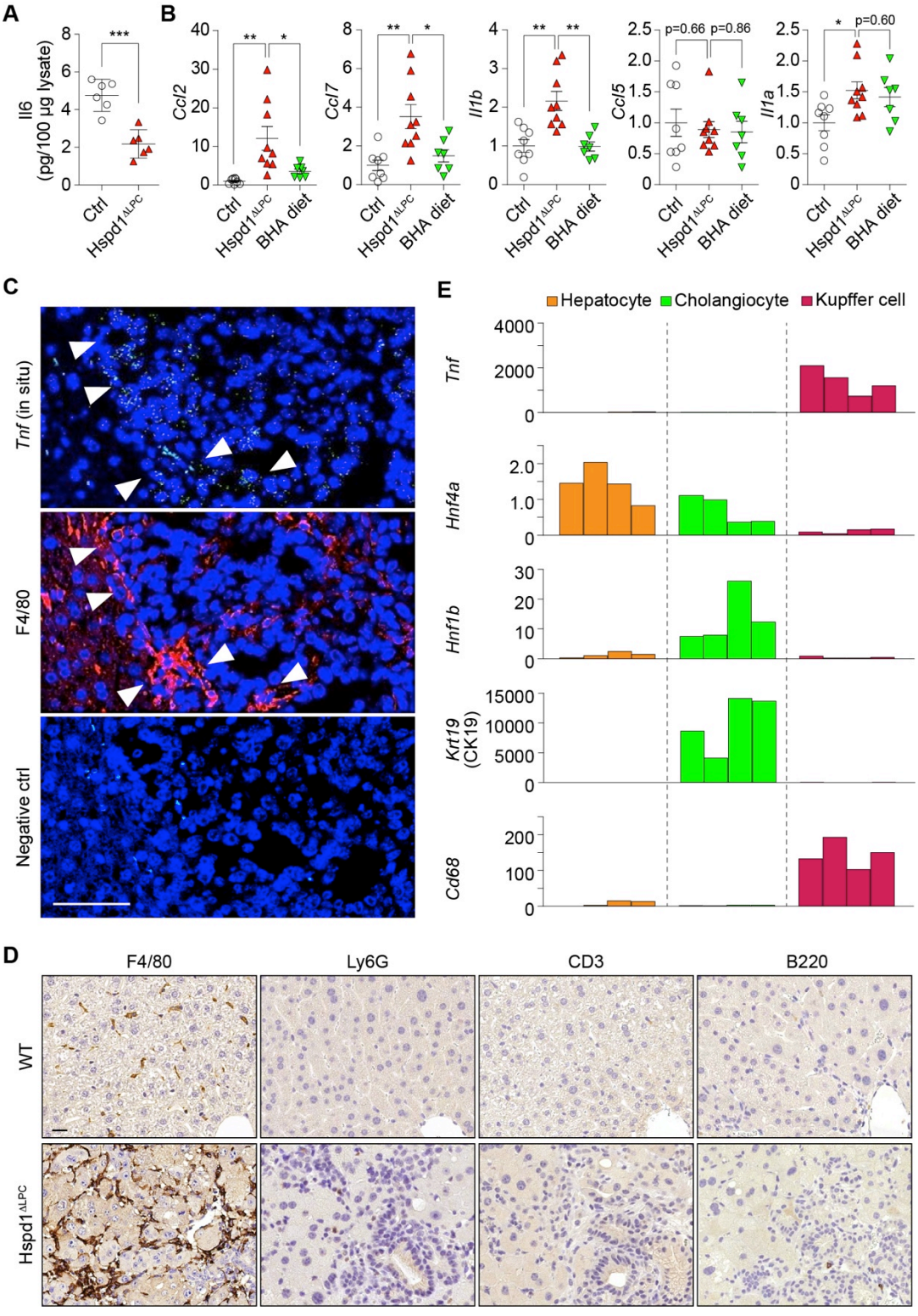
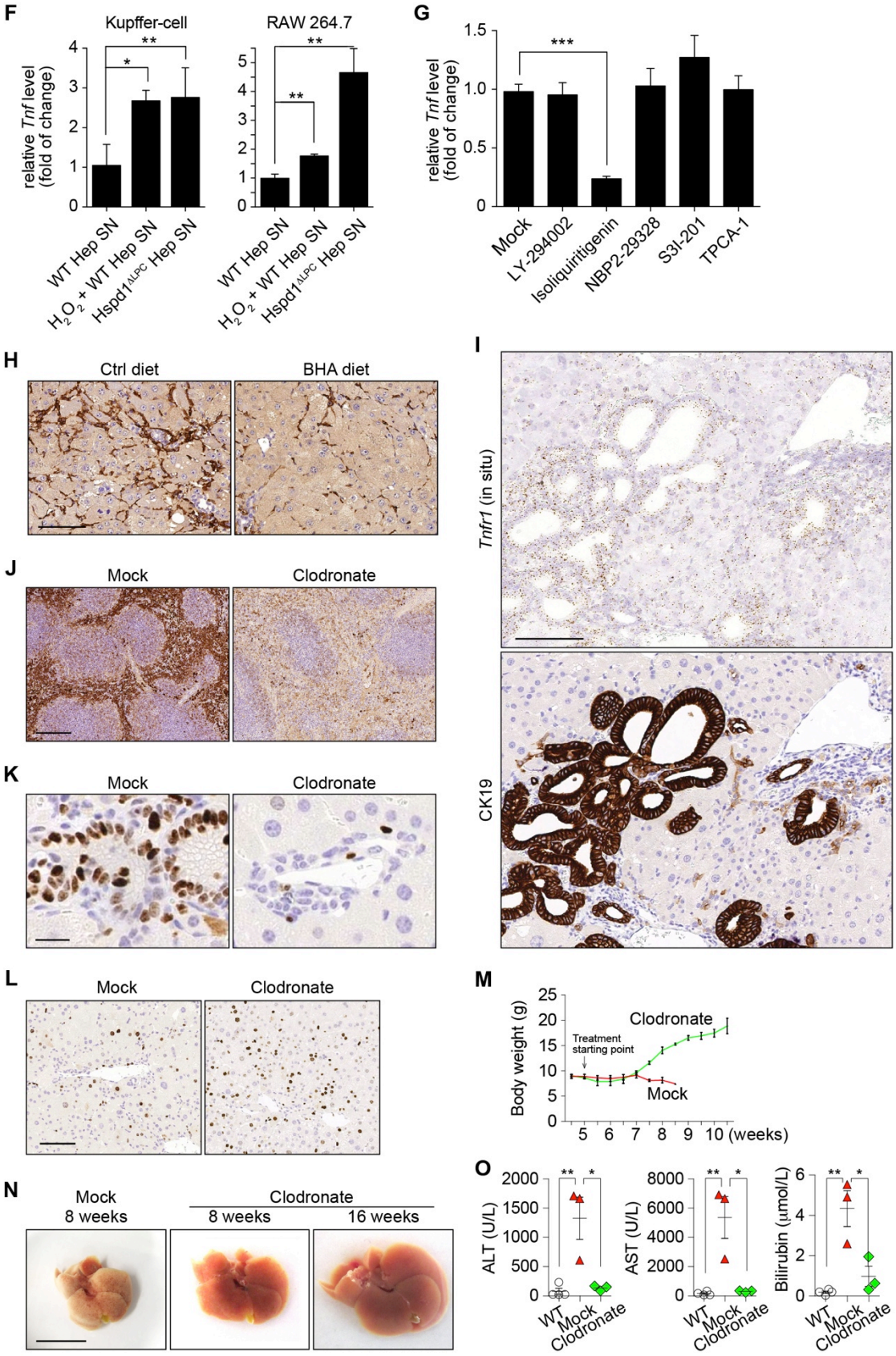


Figure S4. BHA-containing diet rescued Hspd1$^{\Delta LPC}$ mice by decreasing ROS driven oncogenic signaling. Related to Figure 3. (A) Immunohistochemistry staining of Hspd1, 8-OHdG and Afp in consecutive liver sections from 8-week-old Hspd1$^{\Delta LPC}$ mice fed with normal diet or BHA-containing diet. Scale bar: 100 μm. **(B)** qRT-PCR of whole liver lysates from 8- and 20-week-old Hspd1$^{\Delta LPC}$ mice fed with normal diet or BHA-containing diet for *Gpx3* and *Sod3*. **(C)** Body weight curve of Hspd1$^{\Delta LPC}$ mice fed with normal diet or BHA-containing diet. WT mice fed with BHA-containing diet were shown as reference. **(D)** Serum levels of AP in 8-week-old Hspd1$^{\Delta LPC}$ mice fed with normal diet or BHA-

containing diet. **(E)** Immunohistochemistry staining of CD90.1, CD44v6 and Ki67 in liver sections from 8-week-old Hspd1$^{\Delta LPC}$ mice fed with normal diet or BHA-containing diet. Scale bar: 100 μm. **(F)** Ki67 staining of liver sections from 8-week-old Hspd1$^{\Delta LPC}$ mice fed with normal diet or BHA-containing diet. Quantification of Ki67^{+} hepatocytes and qRT-PCR for Cyclin D1 were shown in lower panels. Scale bar: 100 μm. **(G)** qRT-PCR of whole liver lysates from 8- and 20-week-old Hspd1$^{\Delta LPC}$ mice fed with normal diet or BHA-containing diet for indicated genes. **(H)** Macroscopic appearance of livers from Hspd1$^{\Delta LPC}$ mice fed with BHA-containing diet at indicated ages. Scale bar: 1 cm. **(I)** Western blot analysis of p-Eif2α, total Eif2α, p-p38 MAPK, total p38 MAPK and LC3 in whole liver protein extracts from main figure 3G. **(J)** Immunohistochemistry staining of Chop in liver sections from 8-week-old WT mice fed with normal diet or BHA-containing diet. Scale bar: 100 μm.
Data are represented as the mean ± SEM. *$p < 0.05$, ***$p < 0.001$.


A
Il6 (pg/100 µg lysate)
***
Ctrl
Hspd1ΔLPC
B
Ccl2
Ccl7
Il1b
Ccl5
Il1a
**
*
p=0.66
p=0.86
p=0.60
Ctrl
Hspd1ΔLPC
BHA diet
C
Tnf (in situ)
F4/80
Negative ctrl
D
F4/80
Ly6G
CD3
B220
WT
Hspd1ΔLPC
E
Hepatocyte
Cholangiocyte
Kupffer cell
Tnf
Hnf4a
Hnf1b
Krt19 (CK19)
Cd68


F
Kupffer-cell
RAW 264.7
relative Tnf level (fold of change)
WT Hep SN
H2O2 + WT Hep SN
Hspd1ΔLPC Hep SN
G
relative Tnf level (fold of change)
Mock
LY-294002
Isoliquiritigenin
NBP2-29328
S3I-201
TPCA-1
H
Ctrl diet
BHA diet
I
Tnfr1 (in situ)
CK19
J
Mock
Clodronate
K
Mock
Clodronate
L
Mock
Clodronate
M
Body weight (g)
Clodronate
Treatment starting point
Mock
(weeks)
N
Mock
8 weeks
Clodronate
8 weeks
16 weeks
O
ALT (U/L)
AST (U/L)
Bilirubin (μmol/L)
WT
Mock
Clodronate

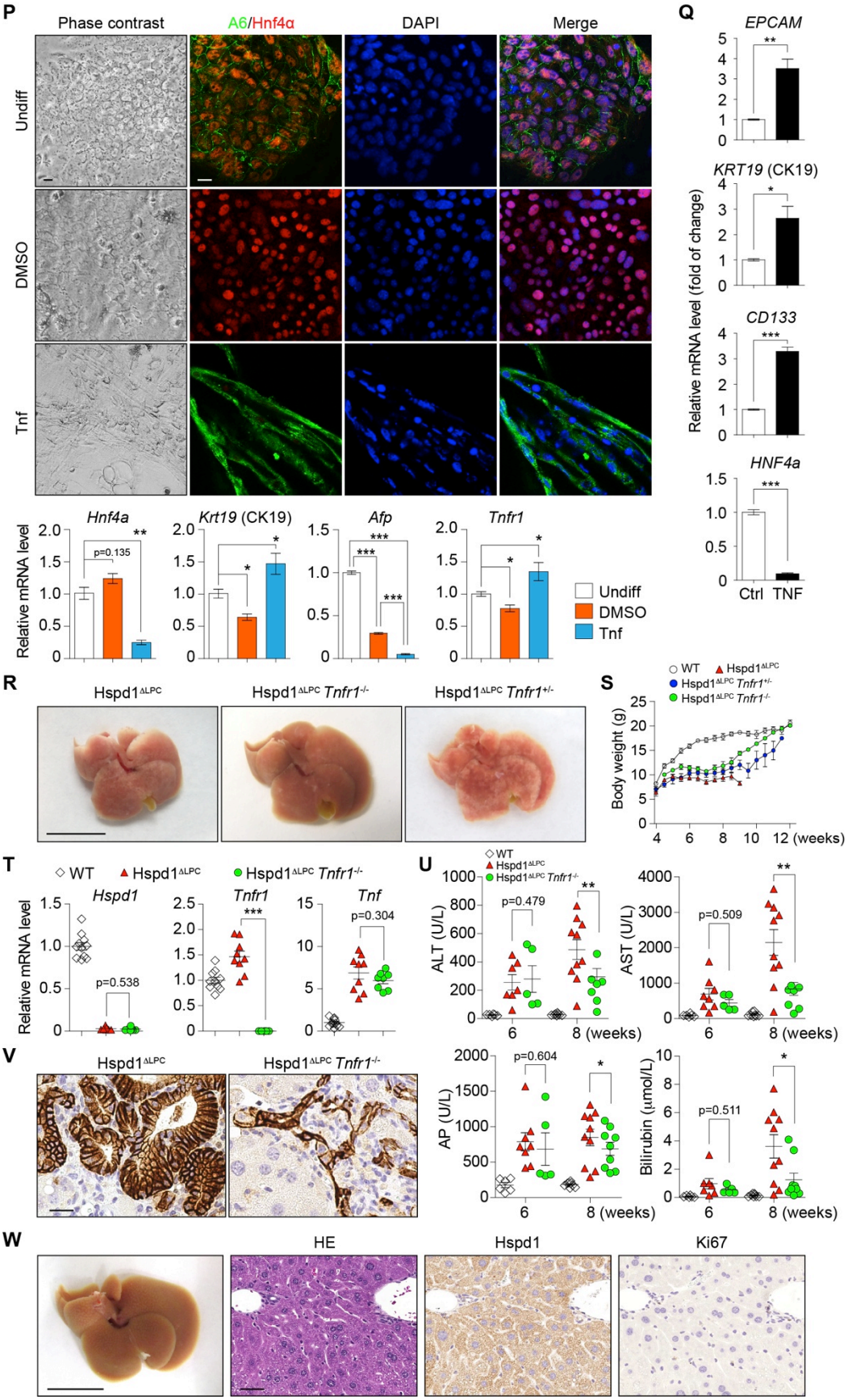
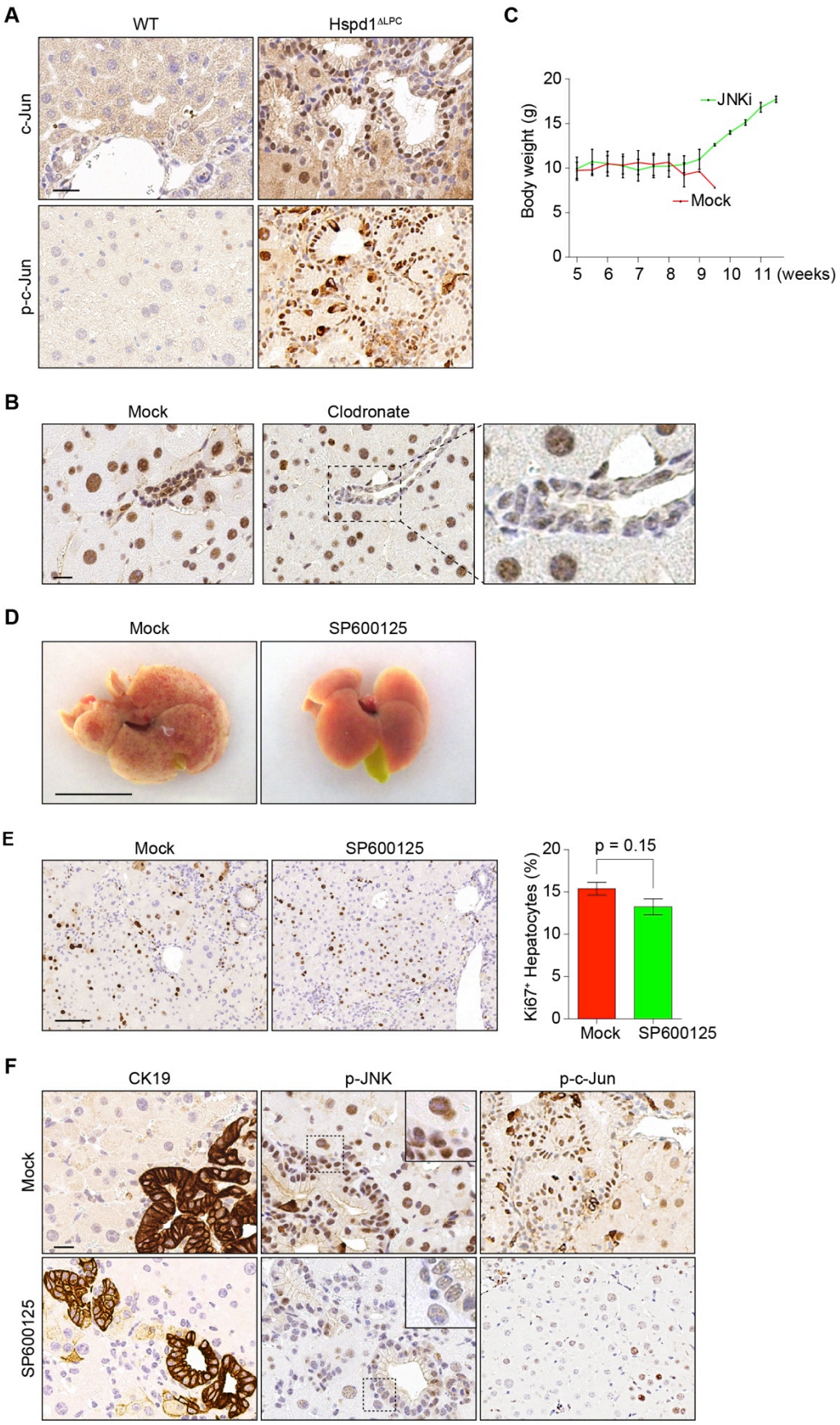

P
Phase contrast
A6/Hnf4α
DAPI
Merge
Undiff
DMSO
Tnf
Hnf4a
Krt19 (CK19)
Afp
Tnfr1
Relative mRNA level
p=0.135
Undiff
DMSO
Tnf
Q
EPCAM
KRT19 (CK19)
CD133
HNF4a
Relative mRNA level (fold of change)
Ctrl
TNF
R
Hspd1ΔLPC
Hspd1ΔLPC Tnfr1-/-
Hspd1ΔLPC Tnfr1+/-
S
WT
Hspd1ΔLPC
Hspd1ΔLPC Tnfr1+/-
Hspd1ΔLPC Tnfr1-/-
Body weight (g)
(weeks)
T
WT
Hspd1ΔLPC
Hspd1ΔLPC Tnfr1-/-
Hspd1
Tnfr1
Tnf
Relative mRNA level
p=0.538
p=0.304
U
WT
Hspd1ΔLPC
Hspd1ΔLPC Tnfr1-/-
ALT (U/L)
AST (U/L)
AP (U/L)
Bilirubin (μmol/L)
p=0.479
p=0.509
p=0.604
p=0.511
6
8 (weeks)
V
Hspd1ΔLPC
Hspd1ΔLPC Tnfr1-/-
W
HE
Hspd1
Ki67

Figure S5. Kupffer cell-produced Tnf is critical for cholangiolar overgrowth. Related to Figure 4 and 5. (A) Il6 ELISA of WT and 8-week-old Hspd1$^{\Delta LPC}$ livers. **(B)** qRT-PCR of whole liver lysates from 8-week-old Hspd1$^{\Delta LPC}$ mice fed with normal diet or BHA-containing diet for indicated genes. **(C)** *Tnf* in situ hybridization and F4/80 IF staining in consecutive liver sections from 8-week-old Hspd1$^{\Delta LPC}$ mice. Scale bar: 50 μm. **(D)** Immunohistochemistry staining of F4/80, Ly6G, CD3 and B220 in liver sections from 8-week-old Hspd1$^{\Delta LPC}$ and WT mice. Scale bar: 20 μm. **(E)** qRT-PCR of indicated genes in FACS sorted liver cells. **(F)** qRT-PCR of *Tnf* in primary Kupffer-cells (left) and RAW 264.7 cells (right) incubated with supernatant of WT primary hepatocytes, H_2O_2 treated WT primary hepatocytes or primary hepatocytes from Hspd1$^{\Delta LPC}$ mice. **(G)** qRT-PCR of *Tnf* in Raw 246.7 cells incubated with supernatant of WT primary hepatocytes treated with H_2O_2 together with the indicated inhibitors. **(H)** F4/80 staining in liver sections from 8-week-old Hspd1$^{\Delta LPC}$ mice on normal or BHA diet. Scale bar: 50 μm. **(I)** Lower magnification of the main figure 4F. Immunohistochemistry staining of CK19 and in situ hybridization of *Tnfr1* in consecutive liver sections from 8-week-old Hspd1$^{\Delta LPC}$ mice. Scale bar: 50 μm. **(J)** Immunohistochemistry staining of F4/80 in spleen sections from 8-week-old Hspd1$^{\Delta LPC}$ treated with mock or liposomal clodronate. Scale bar: 100 μm. **(K)** Ki67 staining in liver sections with cholangiocytes from 8-week-old Hspd1$^{\Delta LPC}$ mice treated with mock or liposomal clodronate. Scale bar: 20 μm. **(L)** Ki67 staining in liver sections with hepatocytes from 8-week-old Hspd1$^{\Delta LPC}$ mice treated with mock or liposomal clodronate. Scale bar: 100 μm. **(M)** Body weight curve of Hspd1$^{\Delta LPC}$ mice treated with mock or liposomal clodronate. **(N)** Macroscopic appearance of livers from 8- and 16-week-old Hspd1$^{\Delta LPC}$ mice treated with liposomal clodronate. Scale bar: 1 cm. **(O)** Serum levels of AST, ALT and Bilirubin in 8-week-old Hspd1$^{\Delta LPC}$ mice treated with mock or liposomal clodronate. **(P)** Images of hepatoblasts kept in undifferentiated medium (basal medium), hepatocytic differentiation medium (basal medium + DMSO) or cholangiocytic differentiation medium (basal medium + Tnf) for 7 days. Lower magnification of immunofluorescence images in Figure 5B with was shown. qRT-PCR of indicated genes was shown in the lower panel. Scale bar: 20 μm. **(Q)** qRT-PCR of the HepRG cells, a human bi-potential liver cancer cell line, cultured with or without Tnf for 3 days. **(R)** Macroscopic appearance of livers from 6-week-old Hspd1$^{\Delta LPC}$, Hspd1$^{\Delta LPC}$ *Tnfr1*$^{+/-}$ and Hspd1$^{\Delta LPC}$ *Tnfr1*$^{-/-}$ mice. Scale bar: 1 cm. **(S)** Body weight curves for WT, Hspd1$^{\Delta LPC}$, Hspd1$^{\Delta LPC}$ *Tnfr1*$^{+/-}$ and Hspd1$^{\Delta LPC}$ *Tnfr1*$^{-/-}$ mice. **(T)** qRT-PCR of whole liver extracts from 6- and 8-week-old WT, Hspd1$^{\Delta LPC}$ and Hspd1$^{\Delta LPC}$ *Tnfr1*$^{-/-}$ mice for indicated genes. **(U)** Serum levels of AST, ALT, AP and Bilirubin in 6- and 8-week-old WT, Hspd1$^{\Delta LPC}$, and Hspd1$^{\Delta LPC}$ *Tnfr1*$^{-/-}$ mice. **(V)** Immunohistochemistry staining of CD44v6 of liver sections from 8-week-old Hspd1$^{\Delta LPC}$ and Hspd1$^{\Delta LPC}$ *Tnfr1*$^{-/-}$ mice. Scale bar: 20 μm. **(W)** Macroscopic appearance of liver from

1-year-old Hspd1$^{\Delta LPC}$ *Tnfr1*$^{-/-}$ mouse. Scale bar: 1 cm. H&E staining and immunohistochemistry staining for Hspd1 and Ki67 were shown. Scale bar: 20 μm.

Data are represented as the mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001.


A
WT
Hspd1ΔLPC
c-Jun
p-c-Jun
C
Body weight (g)
JNKi
Mock
5 6 7 8 9 10 11 (weeks)
B
Mock
Clodronate
D
Mock
SP600125
E
Mock
SP600125
p = 0.15
Ki67+ Hepatocytes (%)
Mock SP600125
F
CK19
p-JNK
p-c-Jun
Mock
SP600125

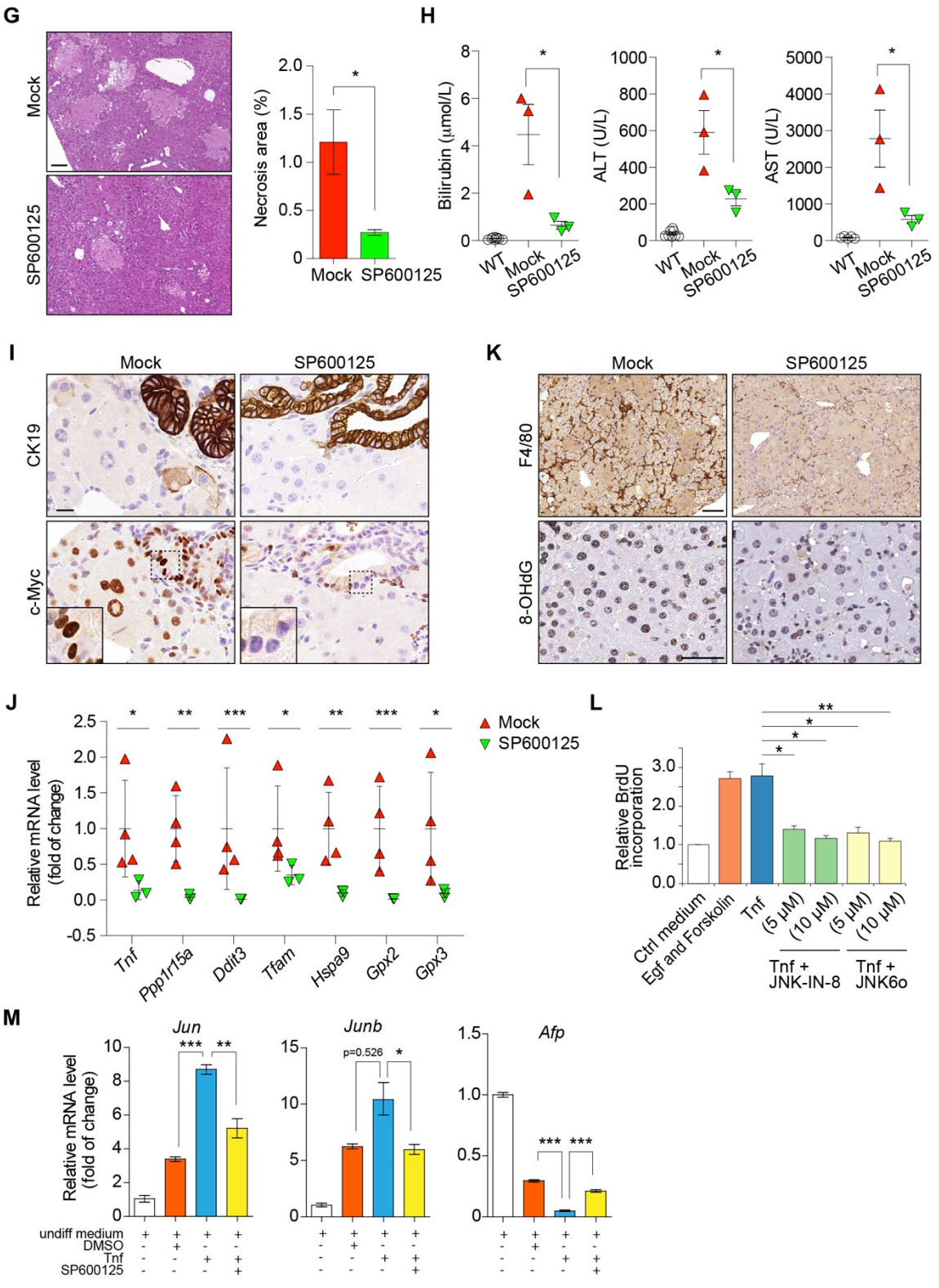
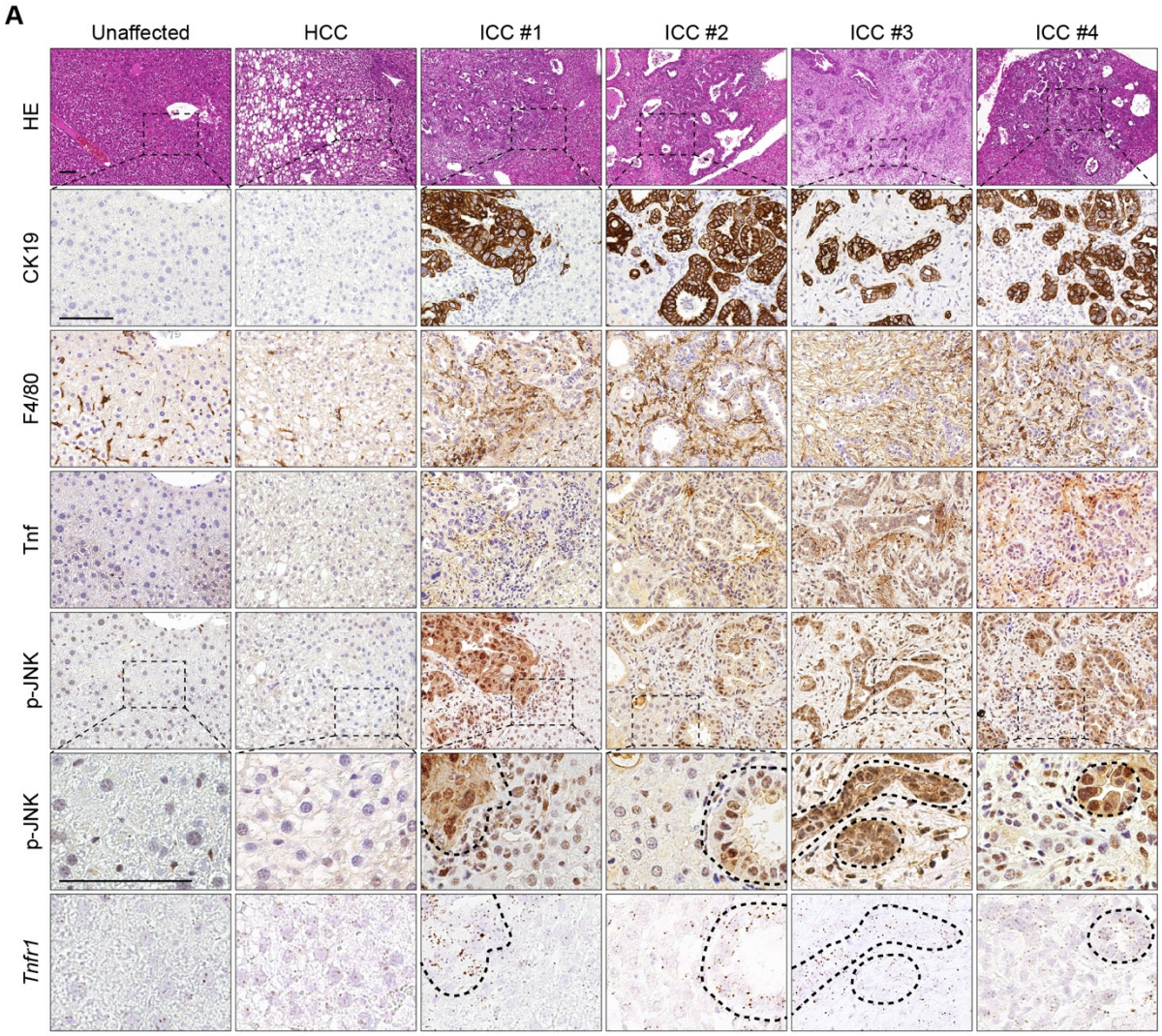
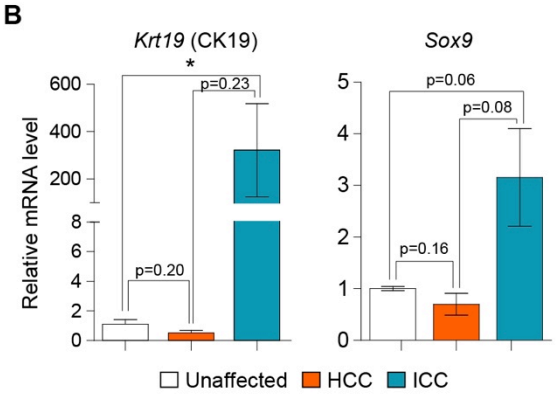
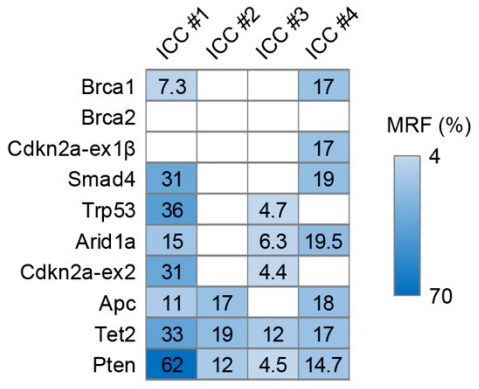

G
Mock
SP600125
Necrosis area (%)
Mock SP600125
H
Bilirubin (μmol/L)
ALT (U/L)
AST (U/L)
WT
Mock
SP600125
I
Mock
SP600125
CK19
c-Myc
K
F4/80
8-OHdG
J
Relative mRNA level (fold of change)
Tnf
Ppp1r15a
Ddit3
Tfam
Hspa9
Gpx2
Gpx3
L
Relative BrdU incorporation
Ctrl medium
Egf and Forskolin
Tnf
(5 μM)
(10 μM)
Tnf + JNK-IN-8
Tnf + JNK6o
M
Jun
Junb
Afp
p=0.526
undiff medium
DMSO
Tnf
SP600125

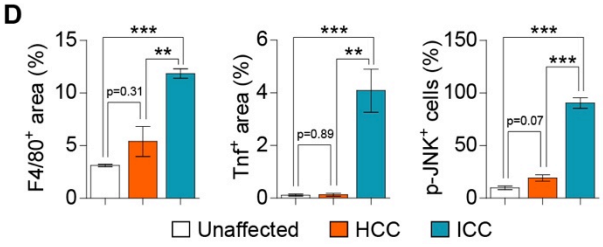
Figure S6. Pharmacological blockade of JNK activation rescued Hspd1$^{\Delta LPC}$ mice. Related to Figure 6. (A) Immunohistochemistry staining for c-Jun and p-c-Jun of liver sections from 8-week-old WT and Hspd1$^{\Delta LPC}$ mice. Scale bar: 20 μm. **(B)** Immunohistochemistry staining of p-JNK in liver sections from 8-week-old Hspd1$^{\Delta LPC}$ mice treated with mock or liposomal clodronate. Scale bar: 20 μm. **(C)** Body weight curves for Hspd1$^{\Delta LPC}$ mice treated with mock (red line) or SP600125 (green line). **(D)** Macroscopic appearance of liver from 8-week-old Hspd1$^{\Delta LPC}$ mice treated with mock or SP600125. Scale bar: 1 cm. **(E)** Ki67 staining in liver sections from 8-week-old Hspd1$^{\Delta LPC}$ mice treated with mock or SP600125. Quantification of Ki67^{+} hepatocytes was shown in the right panel. Scale bar: 100 μm. **(F)** Immunohistochemistry staining of CK19, p-JNK and p-c-Jun in liver sections from 8-week-old Hspd1$^{\Delta LPC}$ mice treated with mock or SP600125. Scale bar: 20 μm. **(G)** H&E staining in liver sections from 8-week-old Hspd1$^{\Delta LPC}$ mice treated with mock or SP600125. Quantification of necrotic areas was shown in the right panel. Scale bar: 100 μm. **(H)** Serum levels of AST, ALT, AP and Bilirubin in 8-week-old WT mice and Hspd1$^{\Delta LPC}$ mice treated with mock or SP600125. **(I-K)** Immunohistochemistry staining of CK19, c-Myc (I, Scale bar: 20 μm), F4/80 and 8-OHdG (K, Scale bar: 50 μm) in liver sections from 8-week-old Hspd1$^{\Delta LPC}$ mice treated with mock or SP600125. **(J)** qRT-PCR of indicated genes in 8-week-old WT mice and Hspd1$^{\Delta LPC}$ mice treated with mock or SP600125. **(L)** BrdU incorporation assay of primary cholangiocytes incubated with DMSO-containing starvation medium (Ctrl medium) or medium supplemented with growth factors Egf and Forskolin or Tnf (5 ng/ml) with or without JNK-IN-8 or JNK6o. **(M)** qRT-PCR of the cell extracts from main figure 6F for indicated genes.

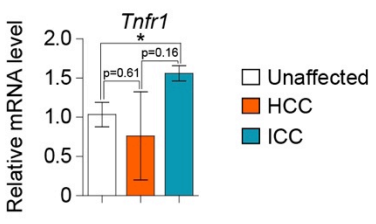
Data are represented as the mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001.


A
Unaffected
HCC
ICC #1
ICC #2
ICC #3
ICC #4
HE
CK19
F4/80
Tnf
p-JNK
p-JNK
Tnfr1


B
Krt19 (CK19)
Sox9
Relative mRNA level
p=0.23
p=0.20
p=0.06
p=0.08
p=0.16
Unaffected
HCC
ICC


C
ICC #1
ICC #2
ICC #3
ICC #4
Brca1 7.3 17
Brca2
Cdkn2a-ex1β 17
Smad4 31 19
Trp53 36 4.7
Arid1a 15 6.3 19.5
Cdkn2a-ex2 31 4.4
Apc 11 17 18
Tet2 33 19 12 17
Pten 62 12 4.5 14.7
MRF (%)
4
70


D
F4/80+ area (%)
Tnf+ area (%)
p-JNK+ cells (%)
p=0.31
p=0.89
p=0.07
Unaffected
HCC
ICC


E
Tnfr1
Relative mRNA level
p=0.61
p=0.16
Unaffected
HCC
ICC

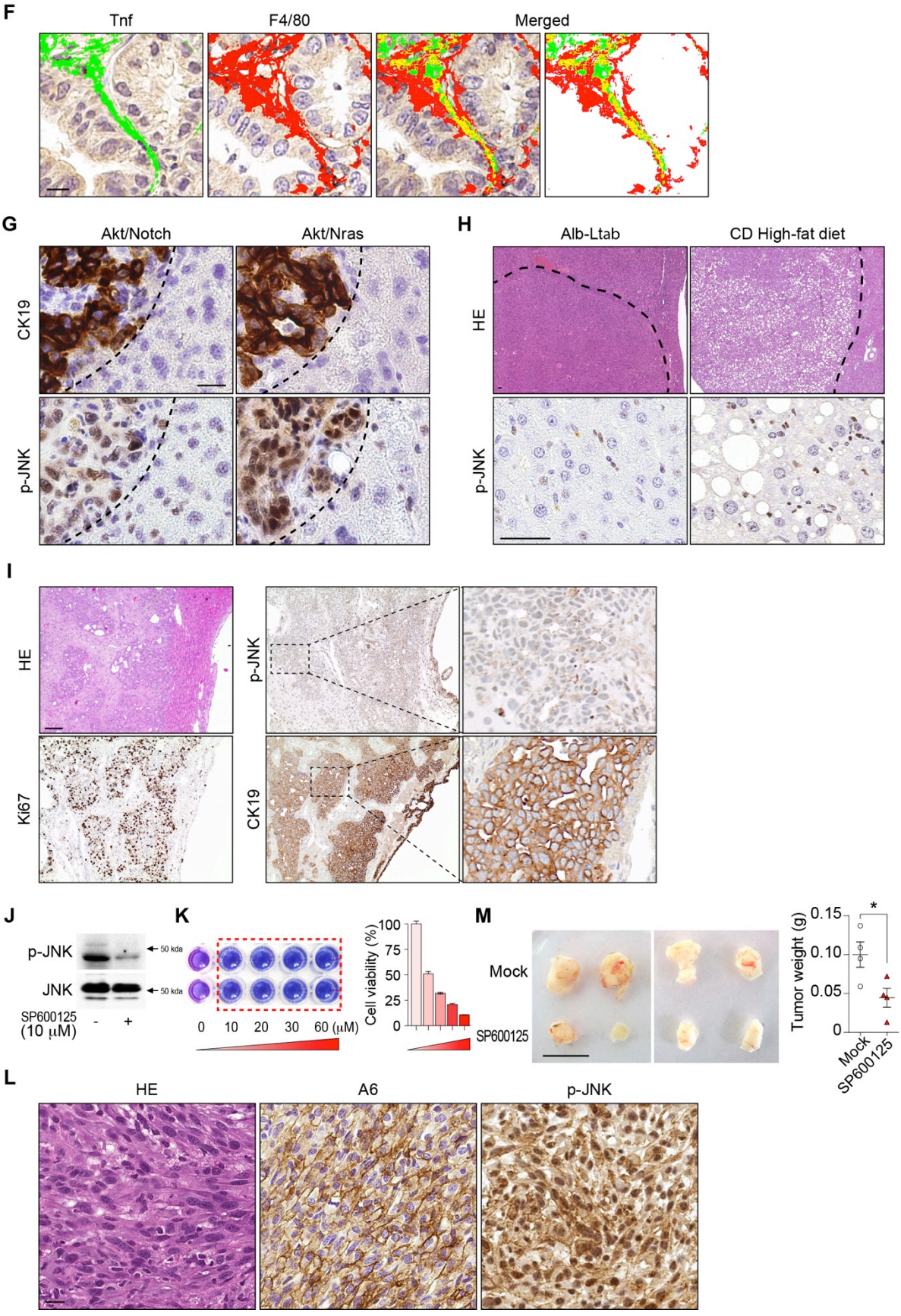

F
Tnf
F4/80
Merged
G
Akt/Notch
Akt/Nras
CK19
p-JNK
H
Alb-Ltab
CD High-fat diet
HE
p-JNK
I
HE
Ki67
p-JNK
CK19
J
p-JNK
JNK
50 kda
50 kda
SP600125
(10 μM)
-
+
K
0
10
20
30
60 (μM)
Cell viability (%)
100
75
50
25
0
M
Mock
SP600125
Tumor weight (g)
0.15
0.10
0.05
0
*
Mock
SP600125
L
HE
A6
p-JNK

Figure S7. JNK activation in four other ICC models and ICC cell lines. Related to Figure 7. (A) H&E, CK19, F4/80, Tnf and p-JNK IHC, and Tnfr1 in situ hybridization of consecutive sections from CRISPR/cas9 mediated multiplex mutagenesis livers. Scale bar: 100 μm. **(B)** qRT-PCR of the liver extracts from (A) for indicated genes. **(C)** Quantitative analysis of sgRNA distribution using qRT-PCR with guide-specific forward primers and a generic reverse primer. The results of the first three ICC samples were adopted from published paper (Weber et al., 2015). **(D)** Quantifications of $F4/80^+$ areas, Tnf^+ areas and p-JNK^+ cells in (A). **(E)** qRT-PCR of the liver extracts from (A) for indicated genes. **(F)** Immunohistochemistry staining of main figure 7B with highlighted colors. Scale bar: 20 μm. **(G)** p-JNK and CK19 IHC in consecutive sections from Akt/Notch- and Akt/Nras-induced ICC livers. Scale bar: 20 μm. **(H)** H&E staining and immunohistochemistry staining of p-JNK in liver sections from two HCC models as indicated. Scale bar: 50 μm. **(I)** H&E staining and immunohistochemistry staining of Ki67, p-JNK and CK19 in liver sections from $JNK1/2^{-LPC}$ mice in HDTV-Akt/Notch induced ICC model. Scale bar: 100 μm. **(J)** Western blot of p-JNK and total JNK in the mouse ICC cell line treated with mock control or SP600125 (20 μM) for 48 hours. **(K)** Cell viability was measured using CellTiter-Blue assay in presence or absence of SP600125 for 3 days with gradients of concentrations as indicated. Data represent percentage of viable cells relative to untreated controls. Representative images of cell plates after CellTiter-Blue incubation were shown on the left, with the red dashed boxes highlighting the responsive wells. **(L)** Representative H&E staining and immunohistochemistry staining of A6 and p-JNK in subcutaneous tumor formed by mICC cells. Scale bar: 20 μm. **(M)** Mouse ICC cells were subcutaneously injected to WT mice. Mice were treated with vehicle or SP600125 (50 mg/kg). Mice were sacrificed 2 weeks after the implantation and tumor weights were measured (n=4). Scale bar: 1 cm.

Data are represented as the mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

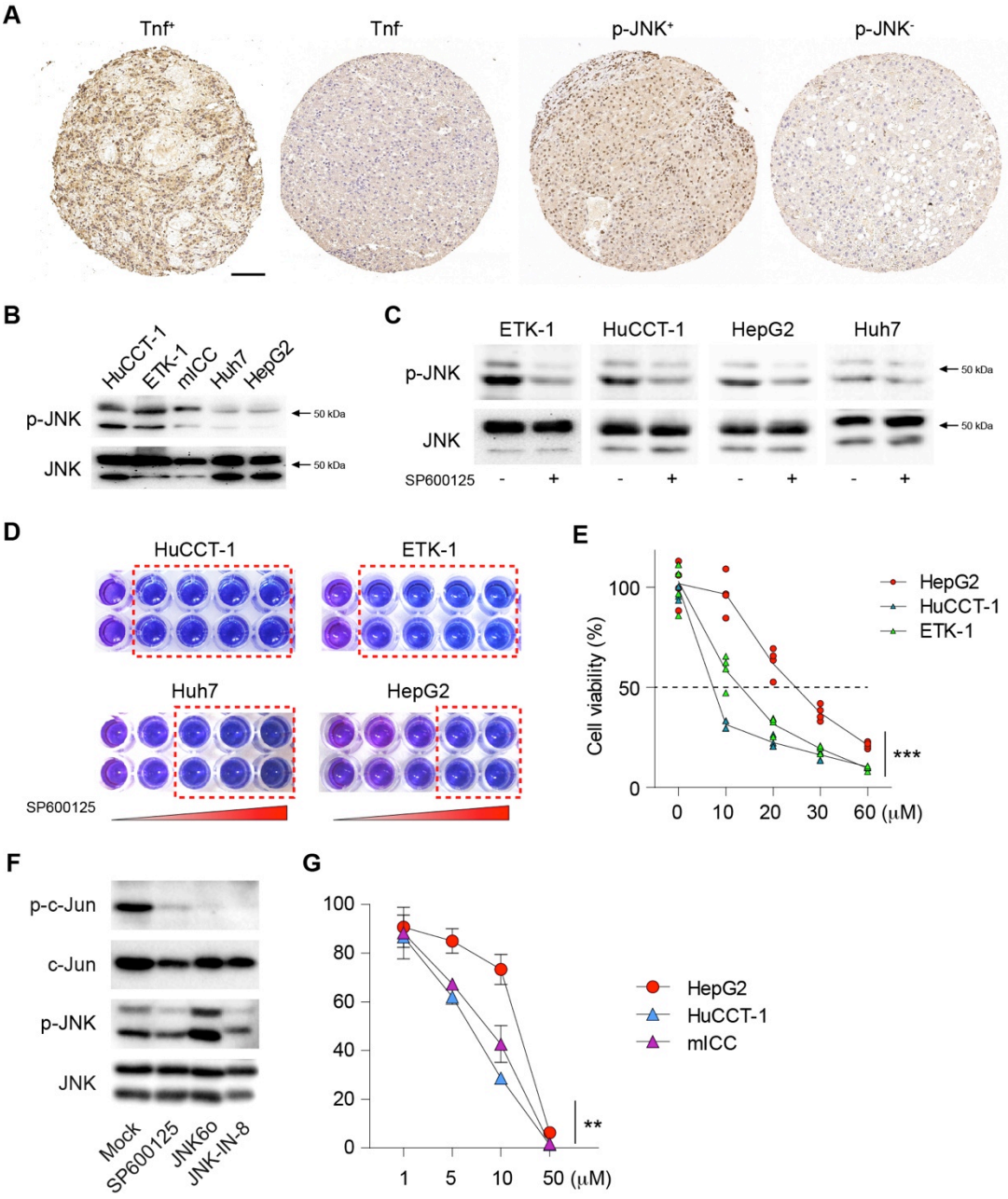


Figure S8: Tnf and p-JNK are specifically upregulated in ICC patient specimens and cell lines. Related to Figure 8. (A) Representative positive and negative staining of Tnf and p-JNK in human patient samples. Scale bar: 100 μm. **(B)** Western blot of p-JNK and total JNK in the indicated cell lines. **(C)** Western blot of p-JNK and total JNK in the cell lines treated with mock control or SP600125 (20 μM) for 48 hours. **(D, E)** Cell viability was measured using CellTiter-Blue assay in presence or absence of SP600125 for 3 days with gradients of concentrations as indicated in the x-axis. Data represent percentage of viable cells relative to untreated controls. Representative images

of cell plates after CellTiter-Blue incubation were shown in (D), with the red dashed boxes highlighting the responsive wells. **(F)** Western blot of indicated proteins in the mICC cells treated with mock control, SP600125, JNK6o or JNK-IN-8 (10 μM) for 2 hours. **(G)** Cell viability was measured using CellTiter-Blue assay in presence or absence of JNK-IN-8 for 3 days with gradients of concentrations as indicated in the x-axis. Data represent percentage of viable cells relative to untreated controls.

Data are represented as the mean ± SEM. $^{**}p < 0.01$, $^{***}p < 0.001$.