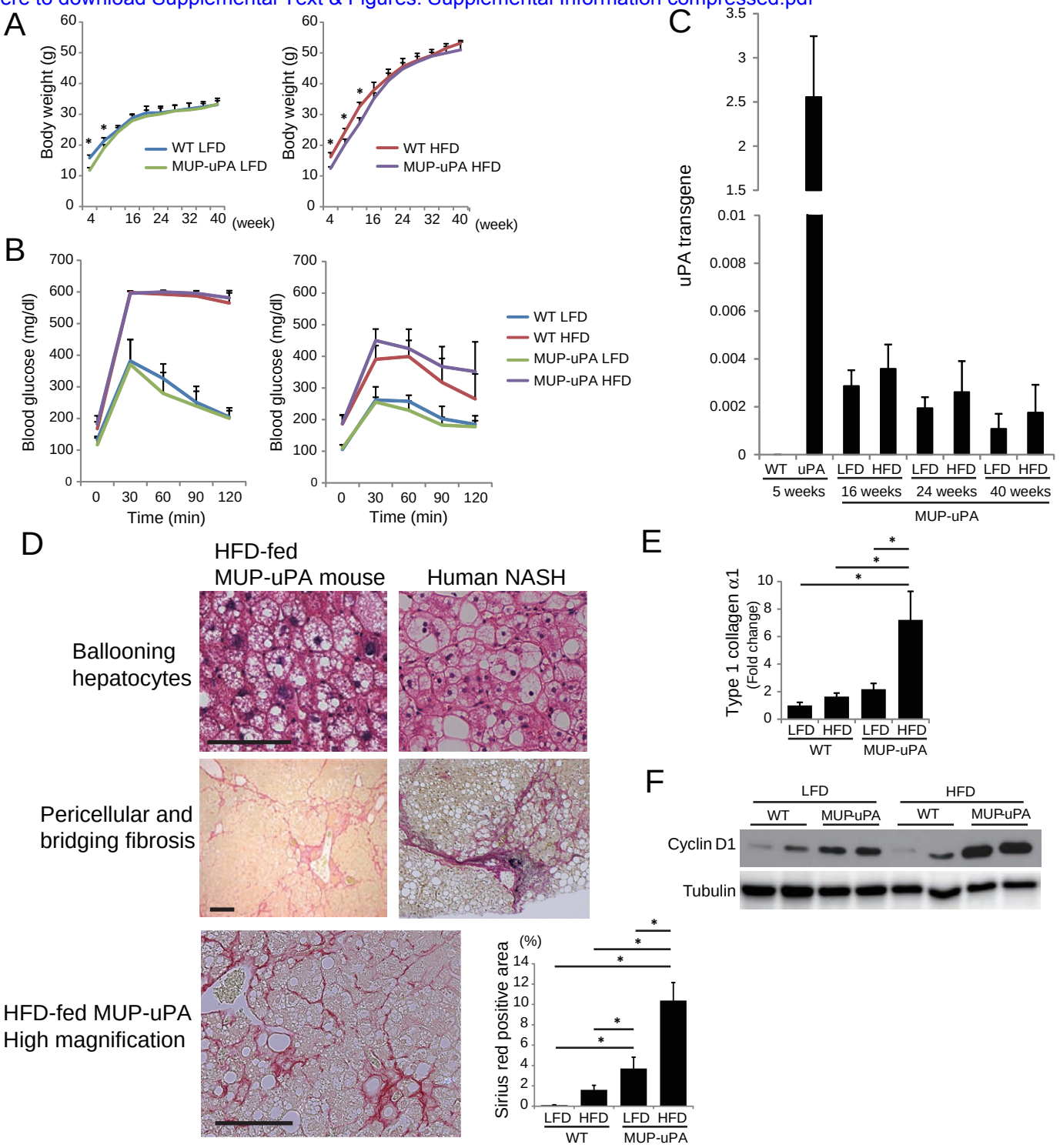
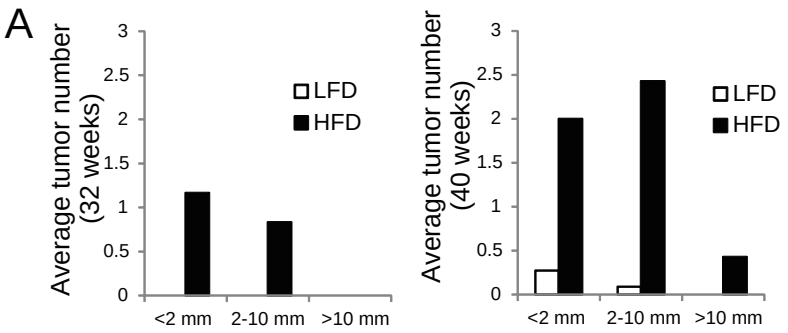


Figure S1 (Related to Figure 1). Effects of HFD on *MUP-uPA* mice. (A) Body weights of LFD- or HFD-fed *WT* and *MUP-uPA* mice. HFD was started at 6 weeks of age. Data are means ± S.D. (n = 6-8 per group). **p* < 0.05. (B) Glucose tolerance tests of 24 weeks old *WT* and *MUP-uPA* mice that were kept on LFD or HFD. Blood glucose was measured at the indicated time points after i.p. injection of 0.8 g/kg (right graph) or 2.0 g/kg (left graph) glucose. (n = 4-5 per group). (C) Expression of the *uPA* transgene was examined by real-time PCR. Results are presented as means ± S.D. (n = 3 per group). (D) Comparison of liver histology between HFD-fed *MUP-uPA* mouse (24 weeks old) and human NASH by H&E and Sirius red staining (scale bar = 100 μm). Bottom panel shows high magnification image of Sirius red staining of HFD-fed *MUP-uPA* mouse liver (scale bar = 100 μm). Sirius red positive areas in livers from 24 weeks old *WT* and *MUP-uPA* mice that were kept on LFD or HFD were quantified with Image J software and shown as bar graphs. Data are means ± S.D. (n = 4 per group). **p* < 0.05. (E) Relative mRNA amounts of type 1 collagen α1 were examined by real-time Q-PCR. Data are presented as means ± S.D. (LFD-fed *WT*, n = 3; others, n =5 per group). **p* < 0.05. (F) Immunoblot evaluation of cyclin D1 in livers of 24 weeks old mice kept on LFD or HFD.



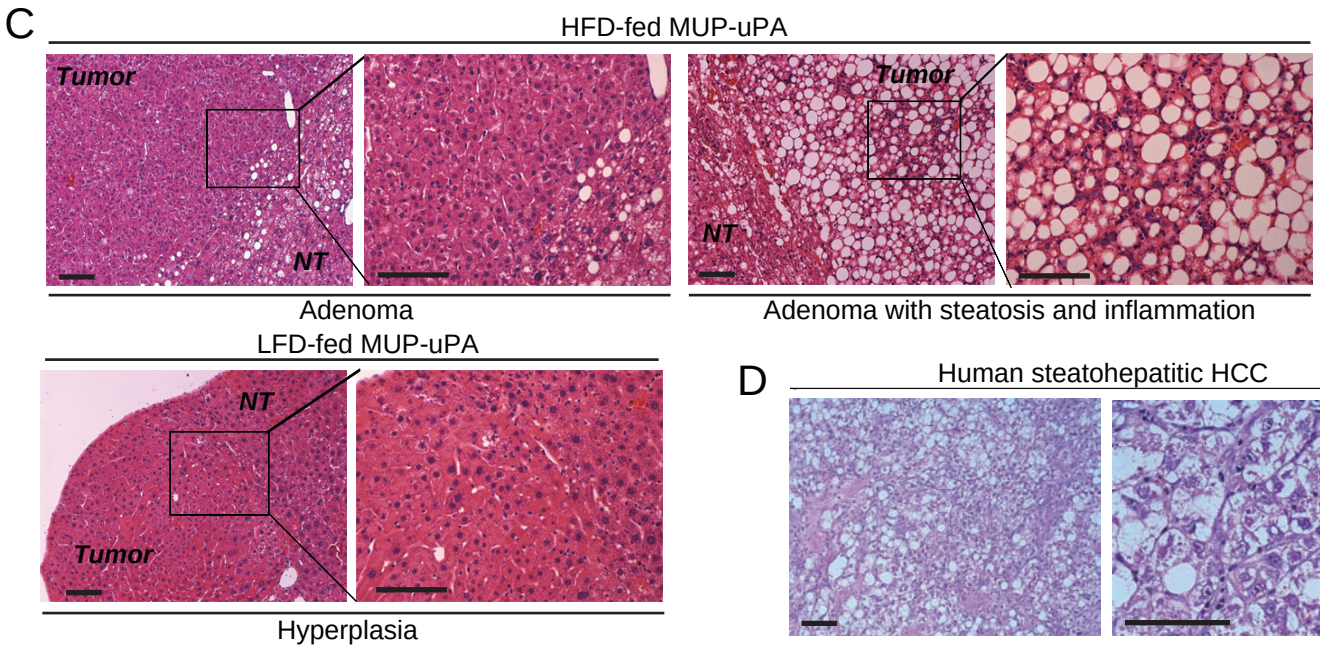
B

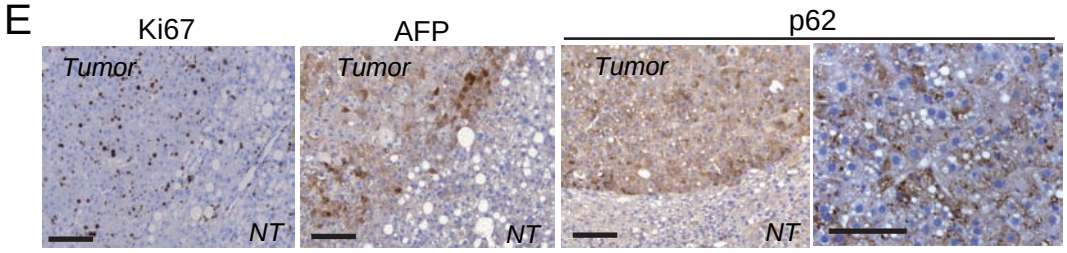
32 weeks

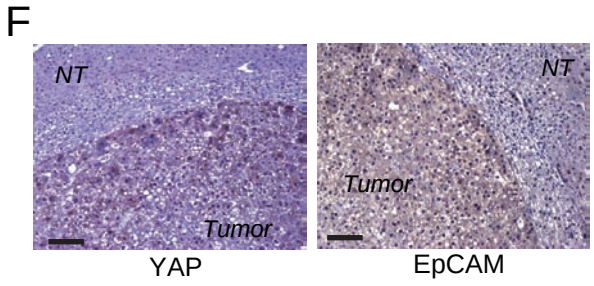
Histology	Adenoma	HCC
LFD	0/6 (0%)	0/6 (0%)
HFD	3/6 (50%)	1/6 (16.6%)
p	0.045	0.29

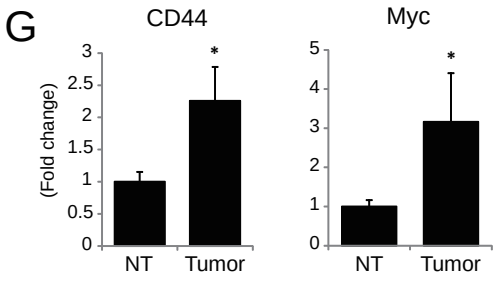
40 weeks

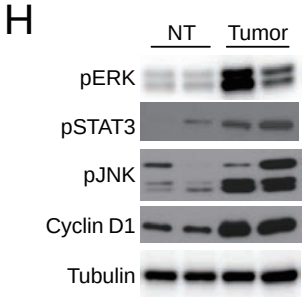
Histology	Adenoma	HCC
LFD	0/11 (0%)	0/11 (0%)
HFD	10/14 (71.4%)	7/14 (50%)
p	0.0057	0.0003

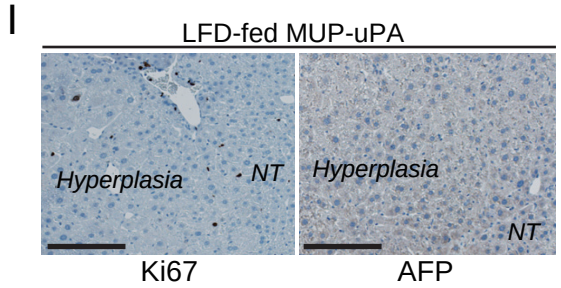












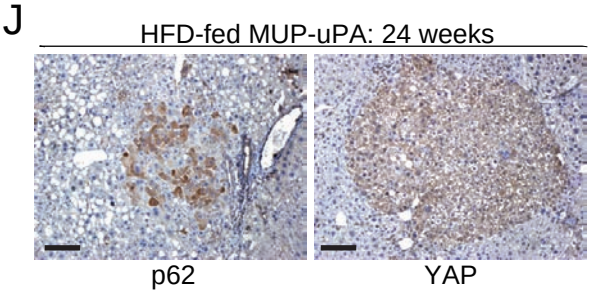


Figure S2 (Related to Figure 2). Characteristics of liver tumors in HFD-fed *MUP-uPA* mice. (A) The average numbers of liver tumors in LFD- or HFD-fed *MUP-uPA* mice at 32 and 40 weeks of age. (B) Frequencies of liver adenoma and HCC in LFD- or HFD-fed *MUP-uPA* mice at 32 and 40 weeks of age. (C) Representative H&E staining of tumor sections from 40 weeks old LFD- or HFD-fed *MUP-uPA* mice. Upper four panels show adenomas from HFD-fed *MUP-uPA* mice and lower two panels show hyperplastic nodule from LFD-fed *MUP-uPA* mice (scale bar = 100 μm). (D) Representative images of H&E stained human steatohepatitic HCC (scale bar = 100 μm). (E) IHC analysis of the indicated antigens in non-tumor (NT) and tumor areas of 40 weeks old HFD-fed *MUP-uPA* livers (scale bar = 100 μm). (F) IHC of YAP and EpCAM in tumor and non-tumor (NT) areas of HFD-fed *MUP-uPA* mouse livers (scale bar = 100 μm). (G) Relative CD44 and Myc mRNAs in tumor and NT areas of HFD-fed *MUP-uPA* mouse livers. Data are means ± S.D. (NT, n = 3; Tumor, n = 5). *$p < 0.05$. (H) IB analysis of the indicated proteins in liver tumors and non-tumor liver tissue (NT) from 40 weeks old HFD-fed *MUP-uPA* mice. (I) IHC of Ki67 and AFP in hyperplastic lesion from 40 weeks old LFD-fed *MUP-uPA* livers (scale bar = 100 μm). (J) IHC of p62 and YAP in liver premalignant foci of 24 weeks old HFD-fed *MUP-uPA* mice (scale bar = 100 μm).

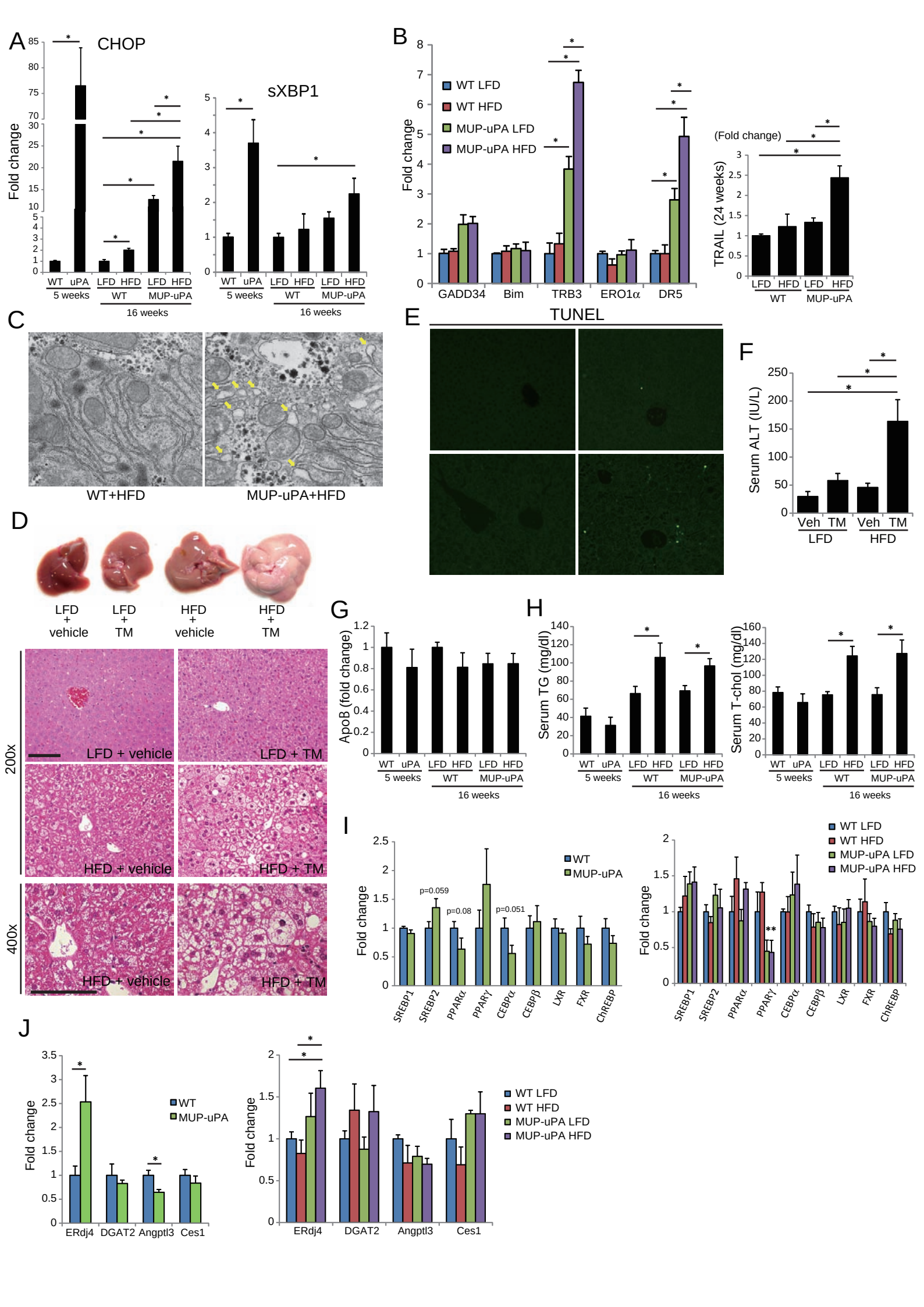

A
CHOP
sXBP1
Fold change
WT uPA LFD HFD LFD HFD
5 weeks
WT
MUP-uPA
16 weeks
B
WT LFD
WT HFD
MUP-uPA LFD
MUP-uPA HFD
Fold change
GADD34
Bim
TRB3
ERO1α
DR5
(Fold change)
TRAIL (24 weeks)
C
WT+HFD
MUP-uPA+HFD
D
LFD + vehicle
LFD + TM
HFD + vehicle
HFD + TM
200x
400x
E
TUNEL
F
Serum ALT (IU/L)
Veh TM Veh TM
LFD
HFD
G
ApoB (fold change)
H
Serum TG (mg/dl)
Serum T-chol (mg/dl)
I
WT
MUP-uPA
p=0.059
p=0.08
p=0.051
SREBP1
SREBP2
PPARα
PPARγ
CEBPα
CEBPβ
LXR
FXR
ChREBP
J
ERdj4
DGAT2
Angptl3
Ces1

Figure S3 (Related to Figure 3). ER stress is sustained by HFD in *MUP-uPA* mice. (A) Relative mRNA amounts of ER stress markers in livers of 5 weeks old *WT* and *MUP-uPA* mice and 16 weeks old *WT* and *MUP-uPA* mice that were kept on LFD or HFD. (B) Relative mRNA amounts of downstream targets for ER stress signaling in livers of 16 weeks old *WT* and *MUP-uPA* mice kept on LFD or HFD and TRAIL mRNA at 24 weeks. (C) Electron micrographs showing the ER in hepatocytes of HFD-fed *WT* and *MUP-uPA* mice. Arrows indicate dilated ER (scale bar = 1 μm). (D-F) LFD or HFD-fed 20 weeks old *WT* mice were intraperitoneally injected with 1.25 mg/kg tunicamycin (TM) or 150 mM dextrose (vehicle). Representative images of livers and H&E (D) and TUNEL (E) staining of liver sections prepared 36 hrs later (scale bar = 100 μm). (F) Serum ALT in LFD- or HFD-fed 20 weeks old *WT* mice at 36 hrs after injection of tunicamycin or vehicle. Data are means ± S.D. (n = 3-4 per group). (G) Relative expression of apoB mRNA in mouse livers described in **A**. (H) Serum TG and total cholesterol concentrations in 5 weeks and 16 weeks old *WT* and *MUP-uPA* mice kept on LFD or HFD. (I) Relative expression of lipogenic regulators in mouse livers described in **A** (left graph, 5 weeks old; right graph, 16 weeks old). (J) Relative expression of genes regulated by the IRE1α-XBP1 pathway in mouse livers described in **A** (left graph, 5 weeks old; right graph, 16 weeks old). All bar graphs represent means ± S.D. (n = 3 per group). *$p < 0.05$.

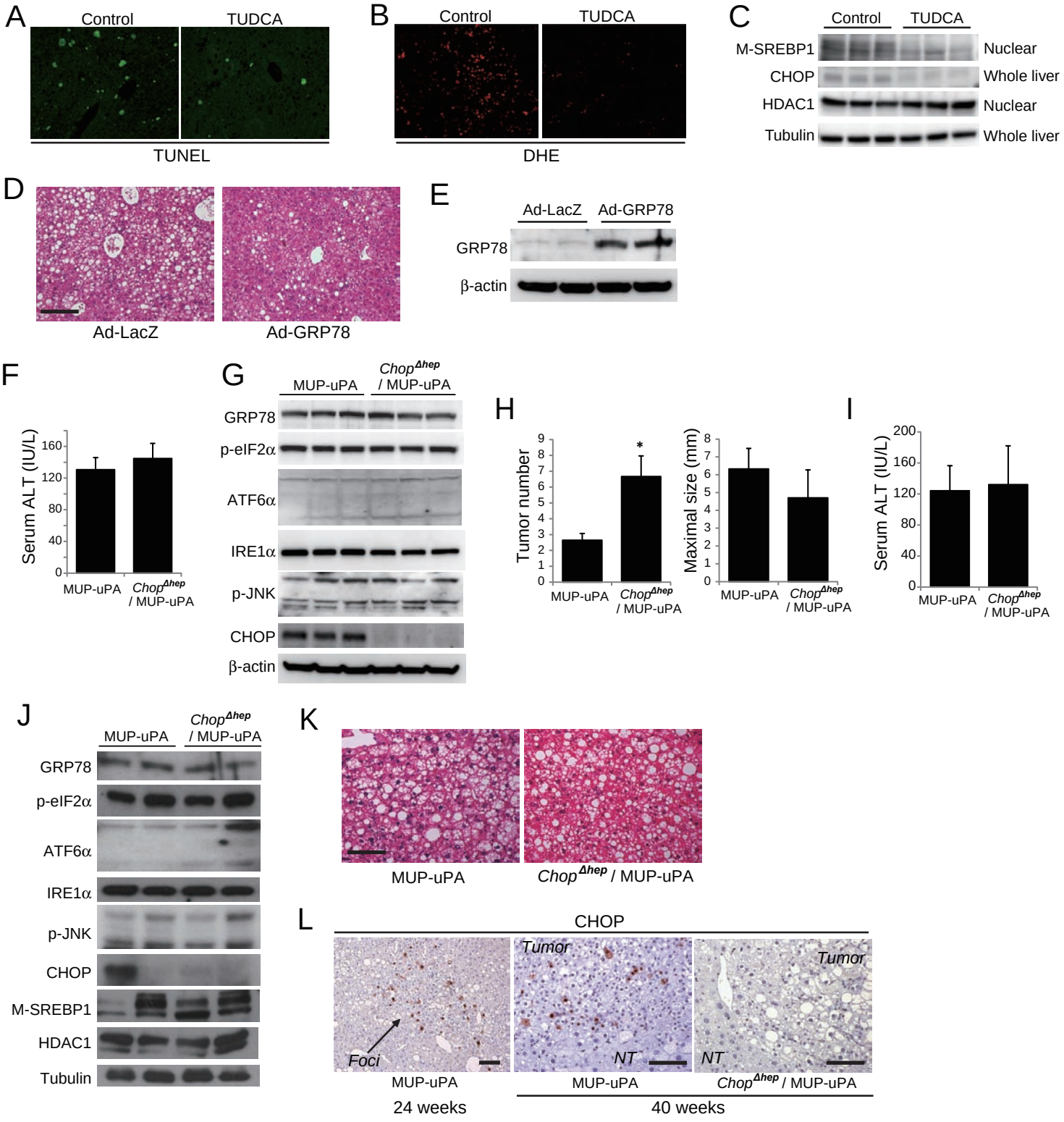

A
Control
TUDCA
TUNEL
B
Control
TUDCA
DHE
C
Control
TUDCA
M-SREBP1
Nuclear
CHOP
Whole liver
HDAC1
Nuclear
Tubulin
Whole liver
D
Ad-LacZ
Ad-GRP78
E
Ad-LacZ
Ad-GRP78
GRP78
β-actin
F
Serum ALT (IU/L)
160
120
80
40
0
MUP-uPA
Chop Δhep / MUP-uPA
G
MUP-uPA
Chop Δhep / MUP-uPA
GRP78
p-eIF2α
ATF6α
IRE1α
p-JNK
CHOP
β-actin
H
Tumor number
9
8
7
6
5
4
3
2
1
0
*
MUP-uPA
Chop Δhep / MUP-uPA
Maximal size (mm)
8
7
6
5
4
3
2
1
0
MUP-uPA
Chop Δhep / MUP-uPA
I
Serum ALT (IU/L)
200
160
120
80
40
0
MUP-uPA
Chop Δhep / MUP-uPA
J
MUP-uPA
Chop Δhep / MUP-uPA
GRP78
p-eIF2α
ATF6α
IRE1α
p-JNK
CHOP
M-SREBP1
HDAC1
Tubulin
K
MUP-uPA
Chop Δhep / MUP-uPA
L
CHOP
Tumor
Tumor
Foci
NT
NT
MUP-uPA
MUP-uPA
Chop Δhep / MUP-uPA
24 weeks
40 weeks
M
NT
Tumor
MUP-uPA
Chop Δhep / MUP-uPA
MUP-uPA
Chop Δhep / MUP-uPA
(number per area)
TUNEL positive cells
10
8
6
4
2
0
NT
p=0.075
1.2
1
0.8
0.6
0.4
0.2
0
Tumor
MUP-uPA
Chop Δhep / MUP-uPA

Figure S4 (Related to Figure 4). Effects of TUDCA and GRP78 overexpression and CHOP ablation on NASH and HCC development. (A,B) Cell death and ROS accumulation in livers of TUDCA- or vehicle-treated HFD-fed *MUP-uPA* mice were examined by TUNEL (A) and DHE staining (B), respectively (scale bar = 100 μm). (C) Effects of TUDCA treatment on ER stress and SREBP1 activation in HFD-fed *MUP-uPA* mouse livers. CHOP protein expression in whole liver and mature SREBP1 in the nuclear fraction were examined by IB analysis. Shown are three individual livers per condition. (D,E) Effects of GRP78 overexpression. HFD-fed *MUP-uPA* mice were intravenously injected with 1×10^9 pfu of Ad-LacZ or Ad-GRP78. After 6 days the mice (n=6 per group) were sacrificed and hepatic steatosis was analyzed by H&E staining (scale bar = 100 μm) (D) and GRP78 expression was determined by IB analysis (E). (F) Serum ALT in 5 weeks old $Chop^{\Delta hep}$*/MUP-uPA* and $Chop^{F/F}$*/MUP-uPA* mice. Data are means ± S.D. (n = 3 per group). (G) IB analysis of ER stress markers in livers of 5 weeks old $Chop^{\Delta hep}$*/MUP-uPA* and $Chop^{F/F}$*/MUP-uPA* mice. (H-K) Effect of hepatocyte CHOP ablation on tumor development and severity of NASH in HFD-fed *MUP-uPA* mice at 40 weeks of age. (H) Tumor numbers and maximal sizes are shown. Results are means ± S.E.M. ($Chop^{F/F}$*/MUP-uPA*, n = 17; $Chop^{\Delta hep}$*/MUP-uPA*, n = 11). *$p < 0.05$. Serum ALT (I), expression of indicated proteins in non-tumor tissue (J), and H&E staining of non-tumor areas (scale bar = 100 μm) (K) are shown. Data are presented as means ± S.D. (L) IHC analysis of CHOP expression in preneoplastic foci (24 weeks old) and liver tumors (40 weeks old) of HFD-fed $Chop^{F/F}$*/MUP-uPA* and $Chop^{\Delta hep}$*/MUP-uPA* mice (scale bar = 100 μm). (M) TUNEL staining of non-tumor (NT) and tumor areas of liver sections from 40 weeks old HFD-fed $Chop^{F/F}$*/MUP-uPA* and $Chop^{\Delta hep}$*/MUP-uPA* mice (scale bar = 100 μm). Bar graphs show numbers of TUNEL positive cells per 200× field. Data are means ± S.D. (n = 5-6 per group).

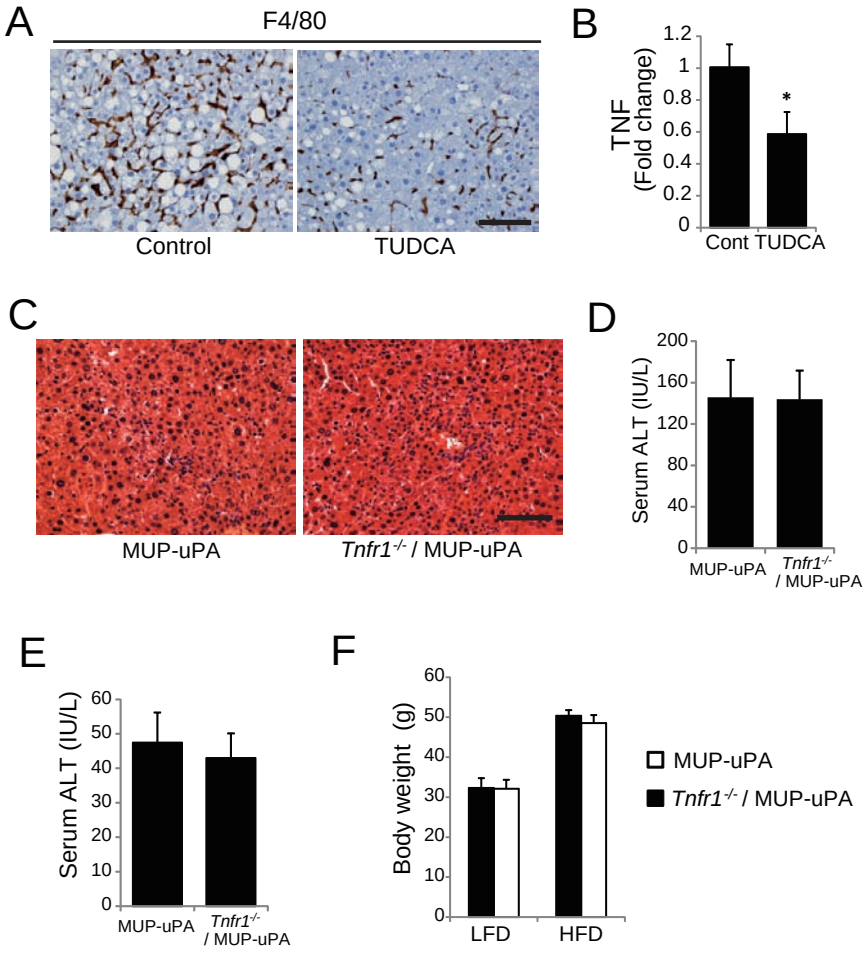


Figure S5 (Related to Figure 5). Characteristics of *Tnfr1*$^{-/-}$*/MUP-uPA* mice. (A, B) Effect of 4 weeks TUDCA treatment on HFD-fed *MUP-uPA* mouse liver. IHC of F4/80 (scale bar = 100 μm) (A) and TNF mRNA expression (B). Data are means ± S.D. (n = 5 per group). **p* < 0.05. (C) H&E staining of liver sections from 5 weeks old *MUP-uPA* and *Tnfr1*$^{-/-}$*/MUP-uPA* mice (scale bar = 100 μm). (D) Serum ALT in 5 weeks old *MUP-uPA* and *Tnfr1*$^{-/-}$*/MUP-uPA* mice. Data are presented as means ± S.D. (n = 3 per group). (E) Serum ALT in 40 weeks old LFD-fed *MUP-uPA* and *Tnfr1*$^{-/-}$*/MUP-uPA* mice. Data are presented as means ± S.D. (n = 5 per group). (F) Body weights of 40 weeks old LFD- or HFD-fed *MUP-uPA* and *Tnfr1*$^{-/-}$*/MUP-uPA* mice (LFD group, *MUP-uPA*, n = 11, *Tnfr1*$^{-/-}$*/MUP-uPA*, n = 10; HFD group, *MUP-uPA*, n = 14; *Tnfr1*$^{-/-}$*/MUP-uPA*, n = 11). Results are shown as means ± S.D.

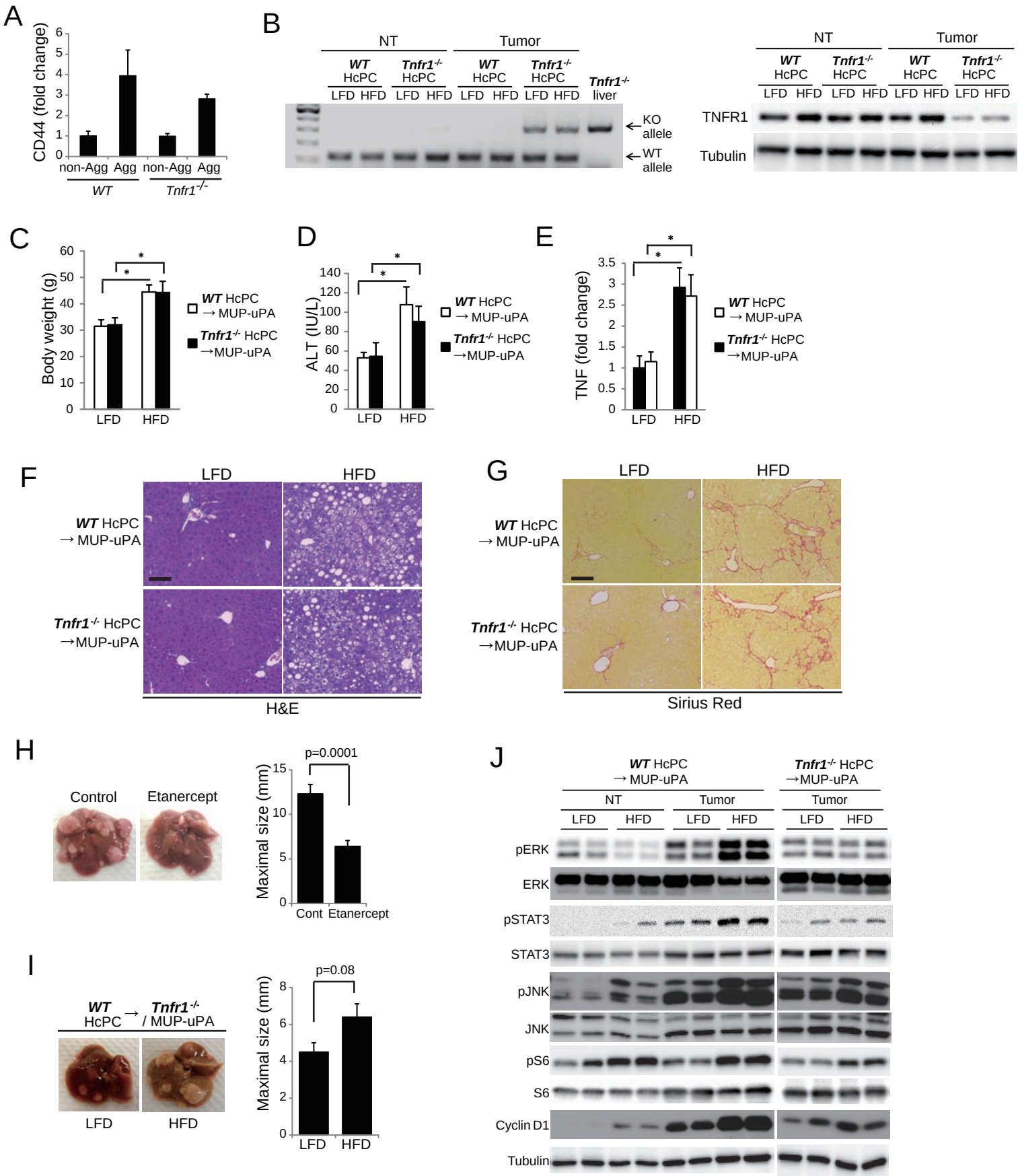

A
CD44 (fold change)
non-Agg
Agg
non-Agg
Agg
WT
Tnfr1-/-
B
NT
Tumor
WT HcPC
Tnfr1-/- HcPC
WT HcPC
Tnfr1-/- HcPC
Tnfr1-/- liver
LFD HFD LFD HFD LFD HFD LFD HFD
KO allele
WT allele
NT
Tumor
WT HcPC
Tnfr1-/- HcPC
WT HcPC
Tnfr1-/- HcPC
LFD HFD LFD HFD LFD HFD LFD HFD
TNFR1
Tubulin
C
Body weight (g)
WT HcPC → MUP-uPA
Tnfr1-/- HcPC →MUP-uPA
LFD
HFD
D
ALT (IU/L)
WT HcPC → MUP-uPA
Tnfr1-/- HcPC →MUP-uPA
LFD
HFD
E
TNF (fold change)
WT HcPC → MUP-uPA
Tnfr1-/- HcPC →MUP-uPA
LFD
HFD
F
LFD
HFD
WT HcPC → MUP-uPA
Tnfr1-/- HcPC →MUP-uPA
H&E
G
LFD
HFD
WT HcPC → MUP-uPA
Tnfr1-/- HcPC →MUP-uPA
Sirius Red
H
Control
Etanercept
p=0.0001
Maximal size (mm)
Cont
Etanercept
I
WT HcPC → Tnfr1-/- / MUP-uPA
LFD
HFD
p=0.08
Maximal size (mm)
LFD
HFD
J
WT HcPC → MUP-uPA
Tnfr1-/- HcPC →MUP-uPA
NT
Tumor
Tumor
LFD HFD LFD HFD LFD HFD
pERK
ERK
pSTAT3
STAT3
pJNK
JNK
pS6
S6
Cyclin D1
Tubulin

Figure S6 (Related to Figure 6). Analyses of HcPC transplanted *MUP-uPA* mice. (A) Relative mRNA amounts of CD44 in non-HcPC and HcPC isolated from DEN-treated *WT* and *Tnfr1*$^{-/-}$ mice. Data are means ± S.D. (n = 3 per group). (B) Confirmation of TNFR1 deletion in cancer cells but not in non-tumor liver tissues. Left panel shows that the deleted *Tnfr1* allele is detected only in tumor tissues of *Tnfr1*$^{-/-}$ HcPC-transplanted *MUP-uPA* mice by genomic DNA PCR. Right panel shows an immunoblot of TNFR1 protein expression. (C-F) No differences in the severity of NASH and TNF expression in the background liver of *MUP-uPA* mice transplanted with either *WT* and *Tnfr1*$^{-/-}$ HcPC. Body weight (C), serum ALT (D), expression of TNF mRNA in non-tumor tissues (E), liver steatosis (F) and fibrosis (G) in *MUP-uPA* mice transplanted with either *WT* or *Tnfr1*$^{-/-}$ HcPC and kept on LFD or HFD are shown (scale bar = 100 μm). Data are expressed as means ± S.D. (n = 10-11 per group). *$p < 0.05$. (H) Effect of the TNF antagonist etanercept on HFD-induced acceleration of tumor growth. *WT* HcPC-transplanted *MUP-uPA* mice were kept on HFD for 5 months as shown in Figure 6A. 5 mg/kg etanercept or vehicle control was i.p. injected three times a week during the last 7 weeks. Representative images of livers and maximal tumor sizes are shown. Data are expressed as means ± S.E.M. (control, n = 10; etanercept, n = 12). (I) HcPC were isolated from DEN- *WT* mice and transplanted into *Tnfr1*$^{-/-}$*/MUP-uPA* mice. HcPC-transplanted *Tnfr1*$^{-/-}$*/MUP-uPA* mice were divided into two groups that were fed with either LFD or HFD, and 5 months later tumorigenesis was assessed. Representative images of livers and maximal tumor sizes are shown. Data are expressed as means ± S.E.M. (n = 10 per group). (J) Non-tumor (NT) and tumor tissues from *MUP-uPA* mice transplanted with either *WT* or *Tnfr1*$^{-/-}$ HcPC and kept on LFD or HFD were IB analyzed for phosphorylation of ERK, STAT3, JNK, and S6, and expression of cyclin D1.

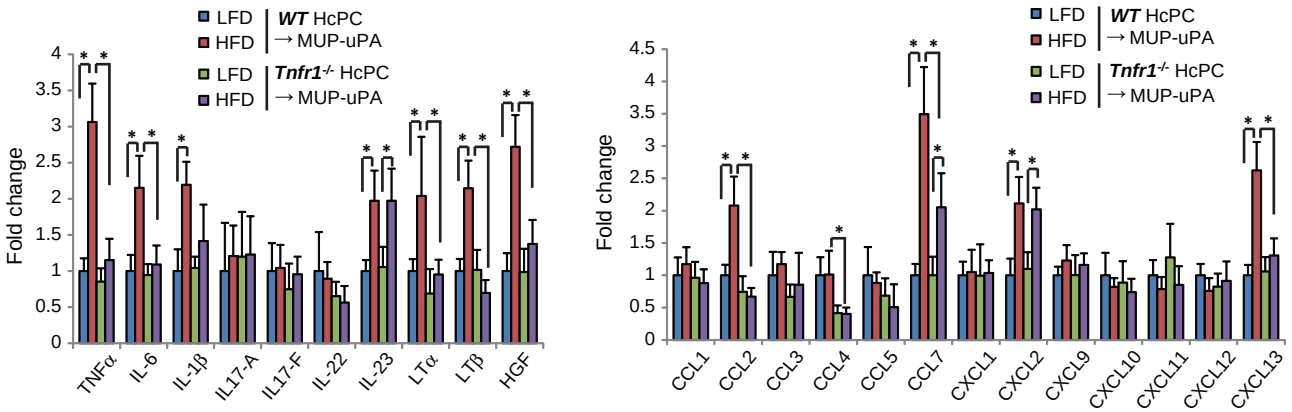


Figure S7 (Related to Figure 7). Expression of inflammatory cytokines and chemokines in tumor tissues. Relative mRNA amounts of inflammatory cytokines and chemokines in HCC tissues from *MUP-uPA* mice transplanted with either *WT* or *Tnfr1*$^{-/-}$ HcPC and kept on LFD or HFD were determined by real-time Q-PCR. (n = 5 per group). *$p < 0.05$. Results are shown as means ± S.D.