Supplementary Figure Legend:

Supplementary Figure 1. FKBP-Caspase 8 activation leads to apoptosis of target cells.
(**A**) The structure of the AFC8 transgene construct. Expression of the FKBP-Caspase 8 fusion gene is driven by the liver-specific albumin enhancer/promoter. M, myristoylation signal; FKBP, FK506 binding domain; Caspase 8, human active Caspase 8 (fragment Ser217-Asp479). Inducible activation of Caspase 8 through dimerization of FKBP-Caspase 8. The chemical dimerizer AP20187 leads to activation of Caspase 8 and apoptosis. (**B**) The FKBP-Caspase 8 gene driven by the CMV promoter (CFC8) was transfected into 293T cells. Activity of apoptosis effector caspase 3/7 was measured after treatment with increasing doses of AP20187, indicating induction of apoptosis. **(C-E)** Activation of FKBP-Caspase 8 leads to specific death of target cells with no bystander death in 293T or HepG2 cells. The FKBP-Caspase 8 gene driven by the CMV promoter (CFC8) or albumin promoter (AFC8) was co-transfected with a GFP-expressing plasmid into HepG2 cells. Transfected cells were cultured with indicated doses of AP20187 for 24h and analyzed by FACS for GFP expression and 7AAD uptake. **(C/D)** The CFC8 gene caused inducible, hepatocyte specific death in both 293T and HepG2 cells but the AFC8 gene caused inducible, hepatocyte specific death only in HepG2 cells. (**E**) Inducible, hepatocyte cell death was observed in GFP+ cells (**D**) but not in bystander GFP- cells (**E**). Data represent mean ± s.e.m.

Supplementary Figure 2. Generation of the AFC8 transgenic mouse and its specific expression in the liver of AFC8 transgenic mice.
Standard transgenic mouse procedure was used to inject the transgene construct into fertilized BalbC/Rag2-γC DKO embryos. (**A**) Transgenic founder mice were identified by PCR to detect the human FKBP-Caspase 8 region. The endogenous mouse p18 gene was used as a control. 300 fg of AFC8 plasmid DNA,100 ng of mouse genomic DNA, a mixture of 300 fg AFC8 plasmid + 100 ng mouse genomic DNA, water + PCR mixture, and DNA from a transgenic founder mouse are shown. (**B**) AFC8 founder mice were verified by genomic Southern blot. A probe that detects both the endogenous and transgenic albumin promoter region was labeled with ^{32}P and incubated with 2 and 5ug of genomic DNA from AFC8 and 5ug from control mice (Ctrl.). The bands were quantitated by a phosphoimager to determine that there are ~14 copies of the AFC8

transgene in the AFC8 founder mouse. (**C**) To demonstrate liver specific expression of FKBP-Caspase 8, RNA was isolated from the liver, spleen, heart, and kidneys of AFC8 mice. Real-time PCR demonstrated expression of human Caspase 8 mRNA only in the liver of AFC8 mice. Controls include samples without reverse transcriptase (-RT).

Supplementary Figure 3. Human hepatocyte gene expression in the liver of AFC8-hu mice. (**A**) Human gene-specific primers for hepatocyte gene expression analysis. Liver RNA was isolated from AFC8 mice with no transplant (mouse control) and HepG2 cells (human HCC cell line – human control). Quantitative real time PCR was performed using equal amount of mRNA with human-specific PCR primers. (**B**) Liver RNA was isolated from AFC8 mice with no transplant (control), AFC8-hu mice (Hep), and adult hepatocytes (Adult Hep.). Quantitative real time PCR was performed with human-specific PCR primers to detect expression of human albumin and human liver metabolism enzymes UGT2B7, Cyp2E9, Cyp2E1 in the liver of AFC8-hu HSC/Hep mice. Values shown are relative human gene expression normalized to human GAPDH. Data represent means ± s.e.m.

Supplementary Figure 4. Human immune reconstitution in the liver of AFC8-hu HSC/Hep mice.

We analyzed intrahepatic leukocytes in the liver of AFC8-hu HSC/Hep mice by FACS. To define each human leukocyte (hCD45+mCD45-) subset, human cells were stained for the following human markers: CD3, CD4, CD8, CD16, CD56, CD11c and CD123.

Supplementary Figure 5. HCV infection induced accumulation of multiple human leukocyte subsets in the liver of AFC8-hu mice.

We analyzed intrahepatic leukocytes in the liver of mock- or HCV-infected AFC8-hu mice by immuno-histochemistry and FACS. (**A**) Human CD3+ T cells were detected with anti-CD3 mAb in the same experimental animals as described in Fig. 2C. (**B**) Human leukocyte (hCD45+mCD45-) subsets were defined as follows: CD4+ T (CD3+CD4+), CD8+ T (CD3+CD8+), NK (CD3-CD56+), and pDC (CD3-CD4+CD11c-CD123+). Total cell number of each leukocyte subset from the liver is shown. Error bars indicate standard errors (SE, n=6 mice/group).

Figure S6. Specie-specific detection of hepatic fibrosis genes by Real-Time PCR analysis.

Liver RNA was isolated from AFC8 mice with no transplant (mouse control) and HepG2 cells (human HCC cell line – human control). Quantitative real time PCR was performed using equal amount of mRNA with human- (A and C) or mouse- (B and D) specific PCR primers for TIMP1 (A and B) and COL1A1 (C and D) genes.

Human or mouse specific quantitative real time PCR primers were designed using NCBI primer design program and Blast database, which include albumin, UGT2B7 (Human-FP TCAGCCCTGGCCCAGATCCC/RP ACAGCTGCTCCCCTGGCCTT), Cyp2C9 (Human-FP GCCTGCCCCATGCAGTGACC/RP CACAGCAGCCAGCCAGGCCAT), Cyp2E1 (Human-FP CCCCAGCGGCACCATGTCTG/RP TGGGCCAACCGGGTGAAGGAA), TIMP1 (Murine-FP GGCTCCTAGAGAGACACACCAGAGCA/RP CGTTCCTTAGGCGGCCCGTG, Human-FP CCCACAAACCGCAGCGAGGAG/RP GGCAGGCAAGGTGACGGGAC), COL1A1 (Murine-FP CTGCCCTCCTGACGCATGGC/RP AGCACTCGCCCTCCCGTCTT, Human-FP TCTGGCGCTCCCATGGCTCT/RP GCCCTGCGGCACAAGGGATT) and GAPDH (Murine-FP GGATGCAGGGATGATGTTC/RP TGCACCAACTGCTTAG, Human-FP CCACCTTTGACGCTGGG/RP CATACCAGGAAATGAGCTTGACA).

Figure S7. Detection of human leukocytes in the fibrotic area of liver from HCV-infected AFC8-hu HSC/Hep mice.

Serial adjacent liver sections of an AFC8-hu/HCV mouse (#109) were stained with Sirius Red/Fast Green, anti-human CD45 (hCD45, brown), anti- human CD68 (hCD68, brown), anti-human CD3 (hCD3, red), anti-human α-smooth muscle actin (hαSMA, brown) and anti-human albumin (hAlbumin, red). Shown are pictures taken from the same region of the liver from adjacent sections.

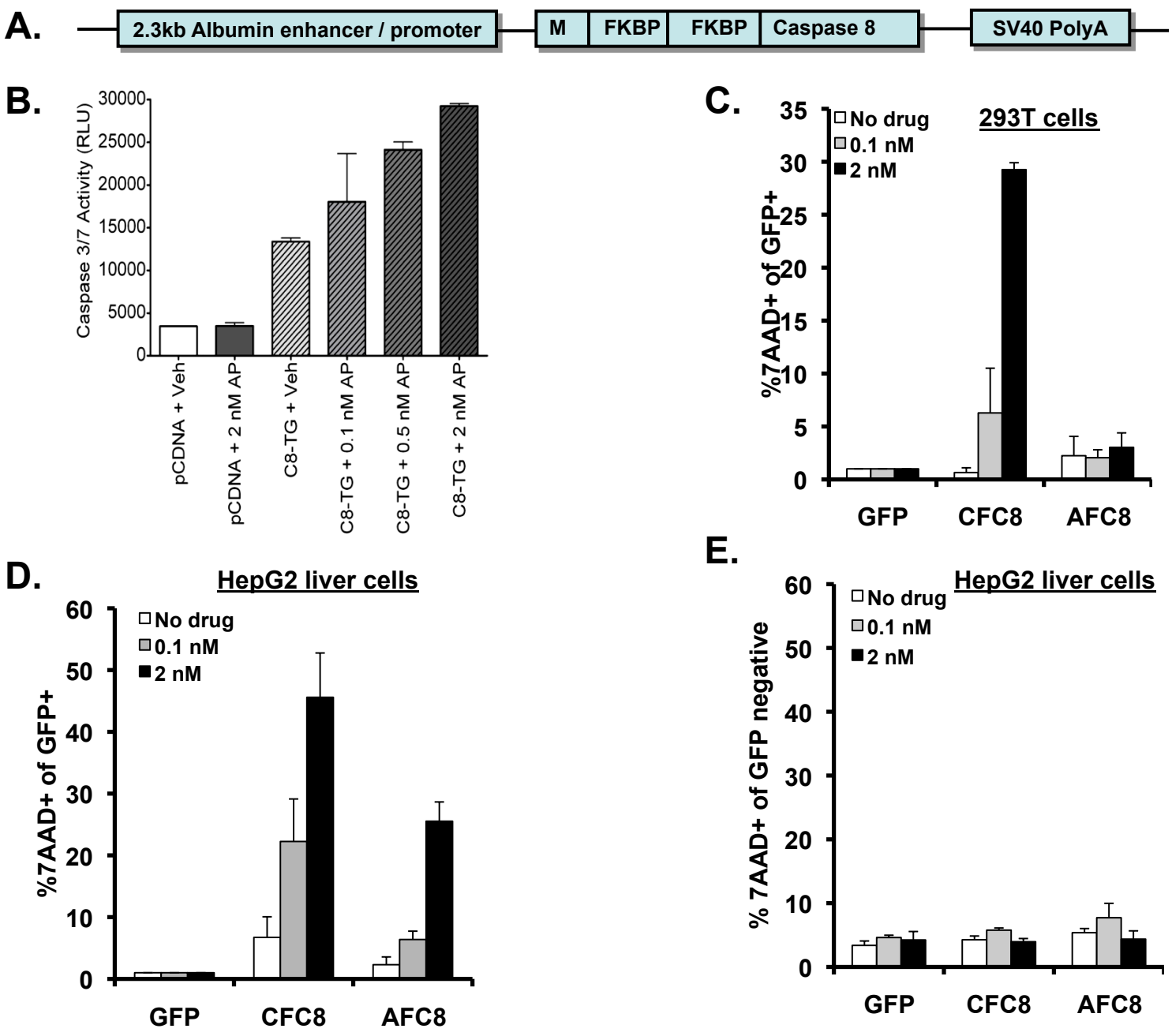
A.
2.3kb Albumin enhancer / promoter
M
FKBP
FKBP
Caspase 8
SV40 PolyA
B.
Caspase 3/7 Activity (RLU)
30000
25000
20000
15000
10000
5000
0
pCDNA + Veh
pCDNA + 2 nM AP
C8-TG + Veh
C8-TG + 0.1 nM AP
C8-TG + 0.5 nM AP
C8-TG + 2 nM AP
C.
293T cells
No drug
0.1 nM
2 nM
%7AAD+ of GFP+
35
30
25
20
15
10
5
0
GFP
CFC8
AFC8
D.
HepG2 liver cells
No drug
0.1 nM
2 nM
%7AAD+ of GFP+
60
50
40
30
20
10
0
GFP
CFC8
AFC8
E.
HepG2 liver cells
No drug
0.1 nM
2 nM
% 7AAD+ of GFP negative
60
50
40
30
20
10
0
GFP
CFC8
AFC8

Figure S1

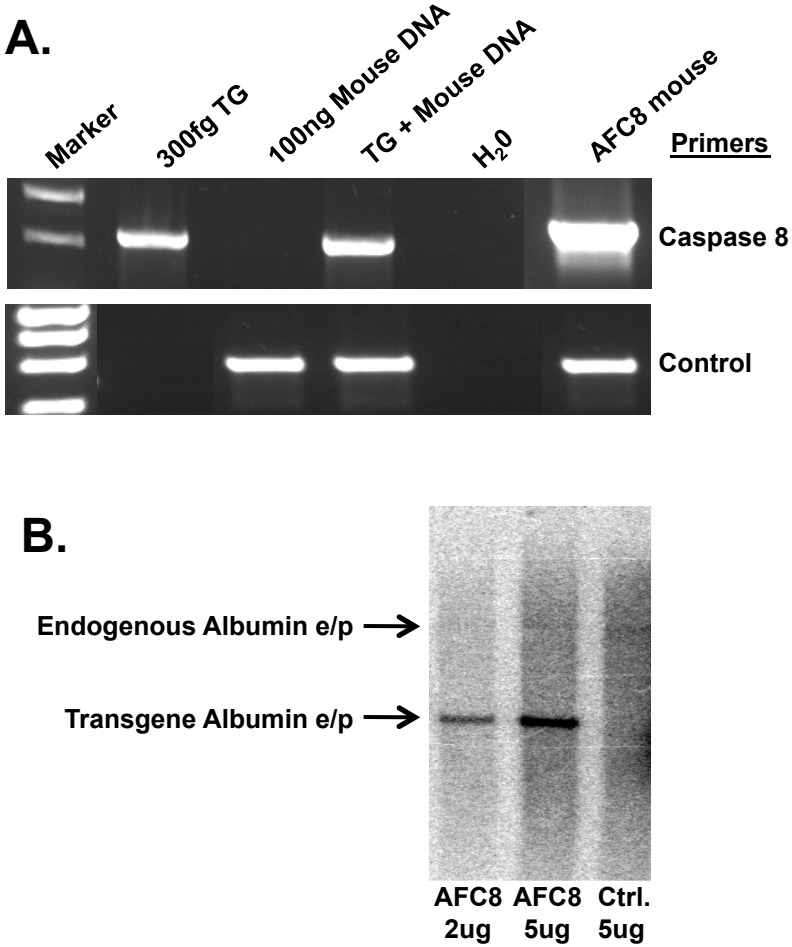
A.
Marker
300fg TG
100ng Mouse DNA
TG + Mouse DNA
H20
AFC8 mouse
Primers
Caspase 8
Control
B.
Endogenous Albumin e/p
Transgene Albumin e/p
AFC8 2ug
AFC8 5ug
Ctrl. 5ug
C.
Relative Caspase 8expression
0
1
2
3
4
5
6
*
Liver Control
Heart
Spleen
Kidney
Liver
Liver -RT
Water
AFC8

Figure S2

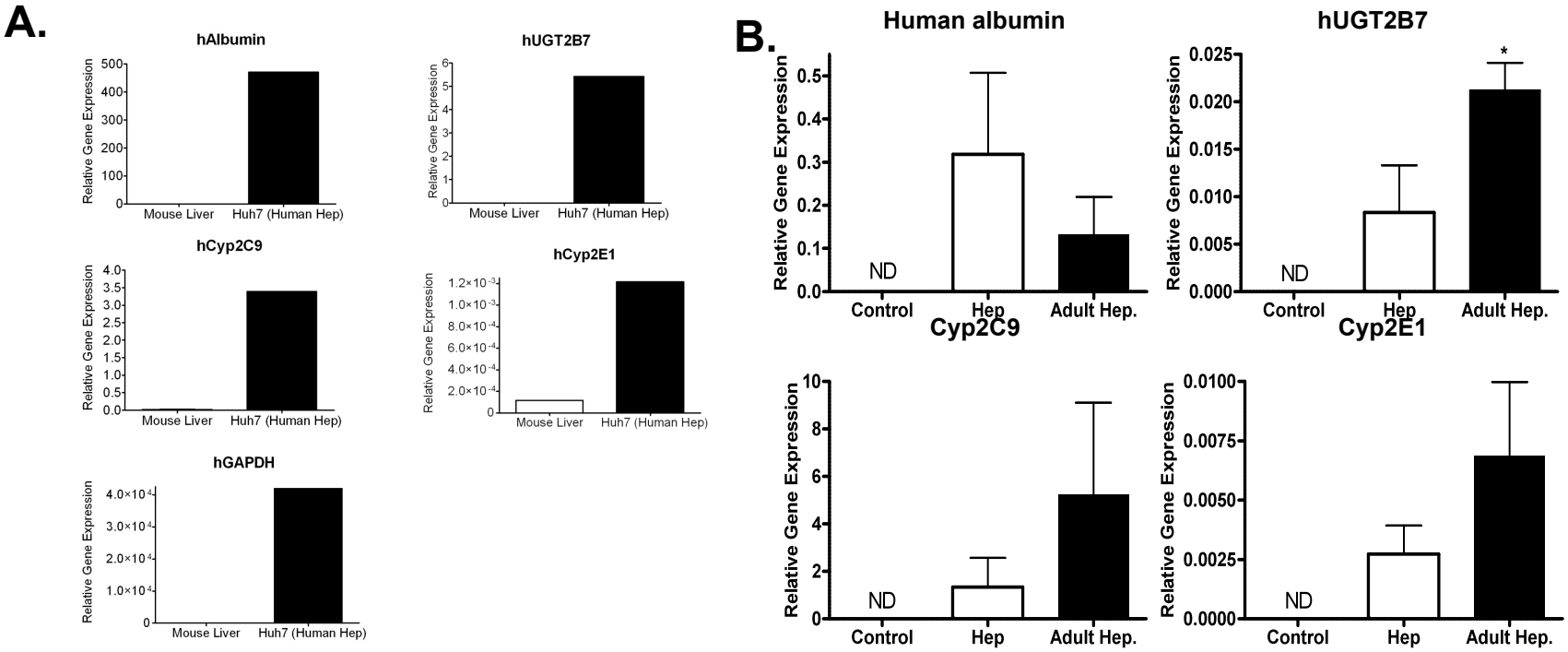
A.
hAlbumin
Relative Gene Expression
Mouse Liver
Huh7 (Human Hep)
hUGT2B7
hCyp2C9
hCyp2E1
hGAPDH
B.
Human albumin
hUGT2B7
ND
Control
Hep
Adult Hep.
Cyp2C9
Cyp2E1
*

Figure S3

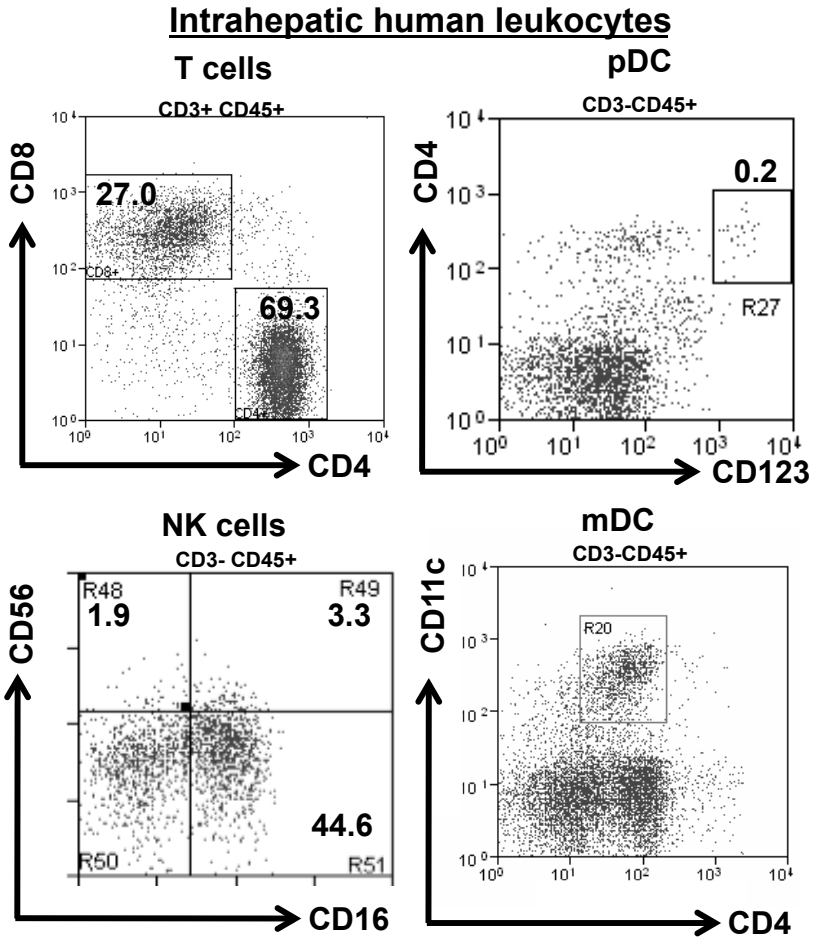
Intrahepatic human leukocytes
T cells
CD3+ CD45+
CD8
CD4
27.0
69.3
CD8+
CD4+
pDC
CD3-CD45+
CD4
CD123
0.2
R27
NK cells
CD3- CD45+
CD56
CD16
R48
1.9
R49
3.3
44.6
R50
R51
mDC
CD3-CD45+
CD11c
CD4
R20

Figure S4

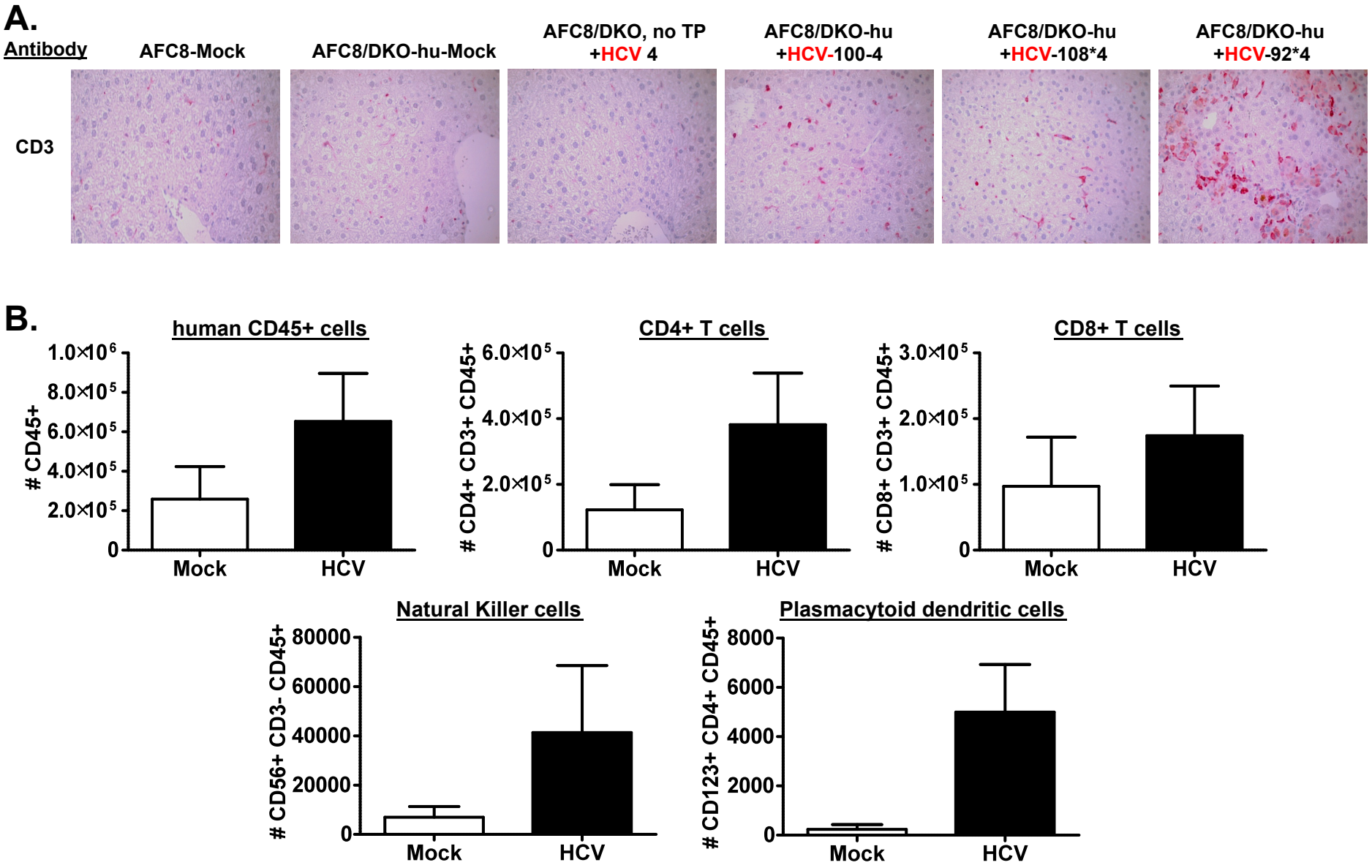
A.
Antibody
AFC8-Mock
AFC8/DKO-hu-Mock
AFC8/DKO, no TP
+HCV 4
AFC8/DKO-hu
+HCV-100-4
AFC8/DKO-hu
+HCV-108*4
AFC8/DKO-hu
+HCV-92*4
CD3
B.
human CD45+ cells
CD45+
1.0×10^6
8.0×10^5
6.0×10^5
4.0×10^5
2.0×10^5
0
Mock
HCV
CD4+ T cells
CD4+ CD3+ CD45+
6.0×10^5
4.0×10^5
2.0×10^5
0
Mock
HCV
CD8+ T cells
CD8+ CD3+ CD45+
3.0×10^5
2.0×10^5
1.0×10^5
0
Mock
HCV
Natural Killer cells
CD56+ CD3- CD45+
80000
60000
40000
20000
0
Mock
HCV
Plasmacytoid dendritic cells
CD123+ CD4+ CD45+
8000
6000
4000
2000
0
Mock
HCV

Figure S5

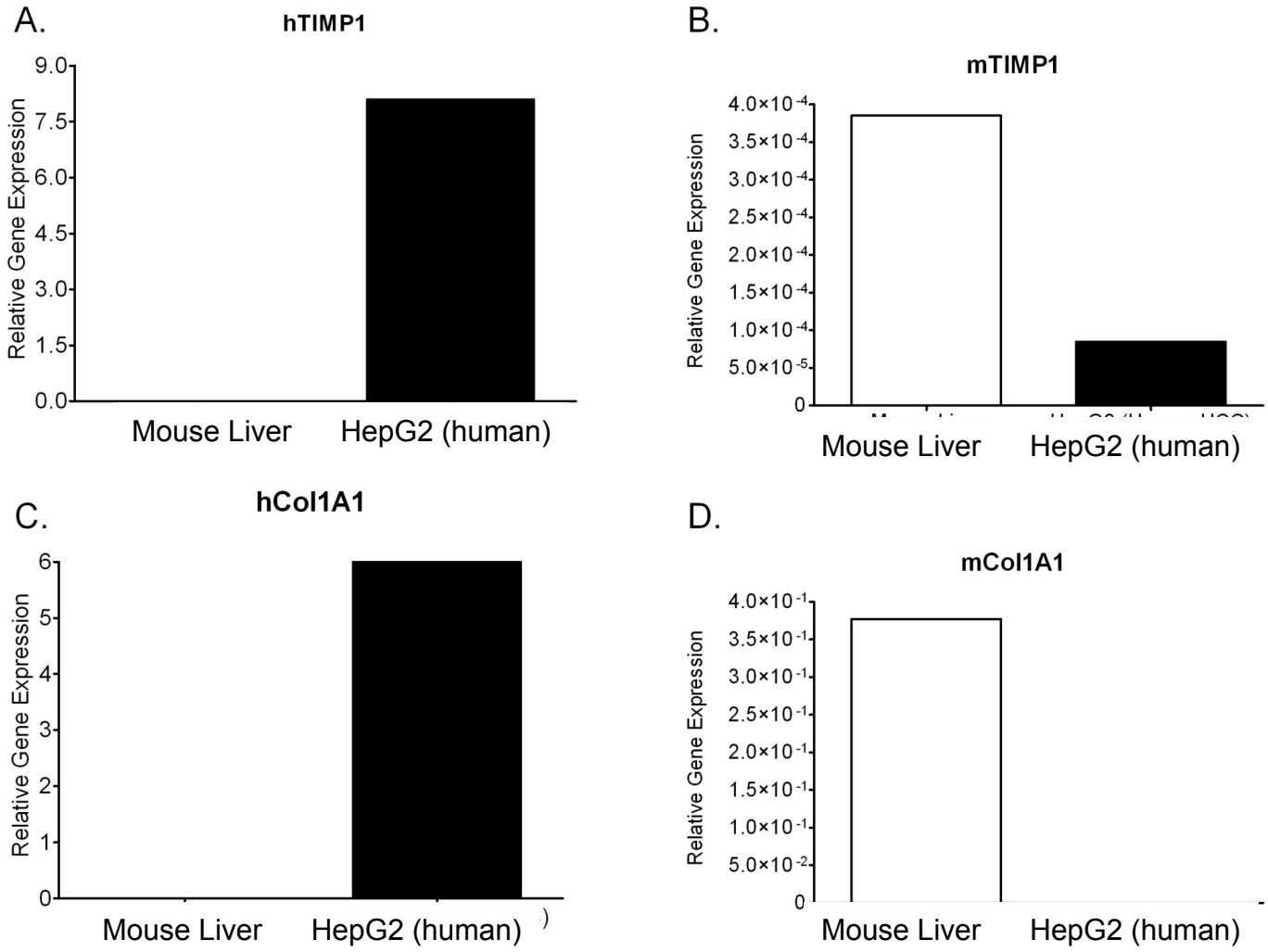
A.
hTIMP1
Relative Gene Expression
9.0
7.5
6.0
4.5
3.0
1.5
0.0
Mouse Liver
HepG2 (human)
B.
mTIMP1
Relative Gene Expression
4.0×10-4
3.5×10-4
3.0×10-4
2.5×10-4
2.0×10-4
1.5×10-4
1.0×10-4
5.0×10-5
0
Mouse Liver
HepG2 (human)
C.
hCol1A1
Relative Gene Expression
6
5
4
3
2
1
0
Mouse Liver
HepG2 (human)
D.
mCol1A1
Relative Gene Expression
4.0×10-1
3.5×10-1
3.0×10-1
2.5×10-1
2.0×10-1
1.5×10-1
1.0×10-1
5.0×10-2
0
Mouse Liver
HepG2 (human)

Figure S6

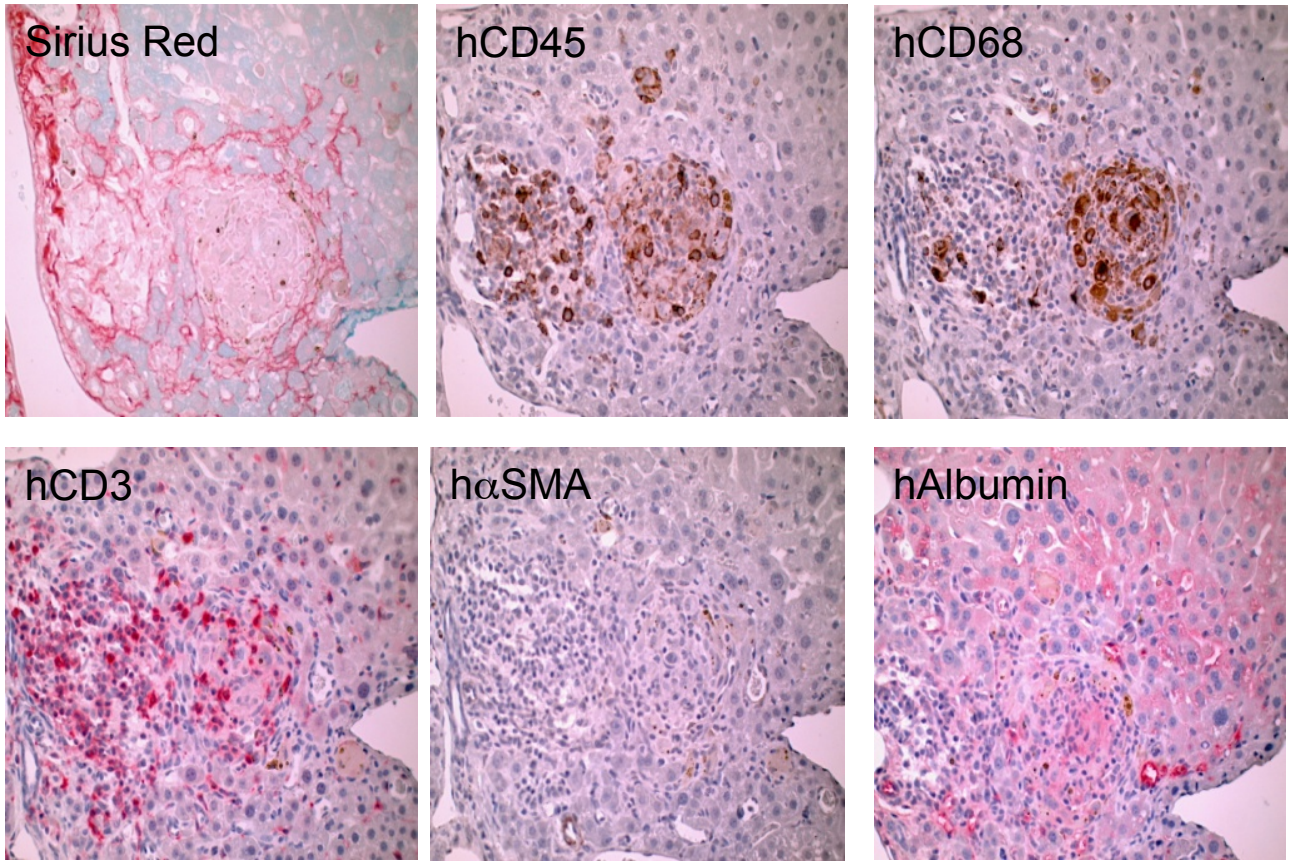

Figure S7