SUPPLEMENTARY MATERIALS

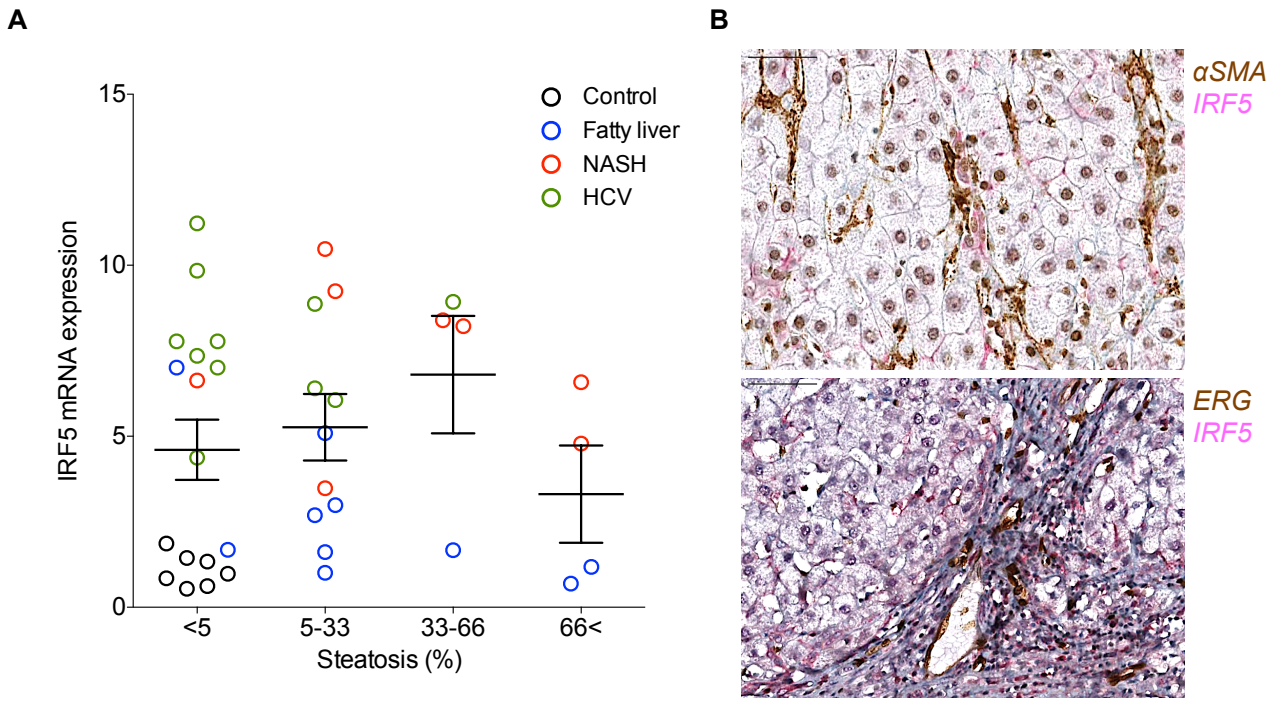

Figure S1. IRF5 mRNA expression is not expressed modulated by steatosis grade in human NAFLD and HVC and is not expressed in hepatic stellate cells of sinusoidal endothelial cells. A. Interferon regulatory factor (IRF) 5 mRNA expression in liver biopsies from control patients with normal liver (control; n=7), patients with fatty liver (n=10), non-alcoholic steatohepatitis (NASH; n=8) and with viral hepatitis C (HCV; n=11) stratified by their state of steatosis. **B**. Representative co-immunostaining images of IRF5 (pink stain) with erythroblast transformation-specific-related gene (ERG; brown stain) or α-smooth muscle actin (αSMA; brown stain) in liver sections from selected patients, scale bar=100μM. Differences between patient groups tested by one-way ANOVA.

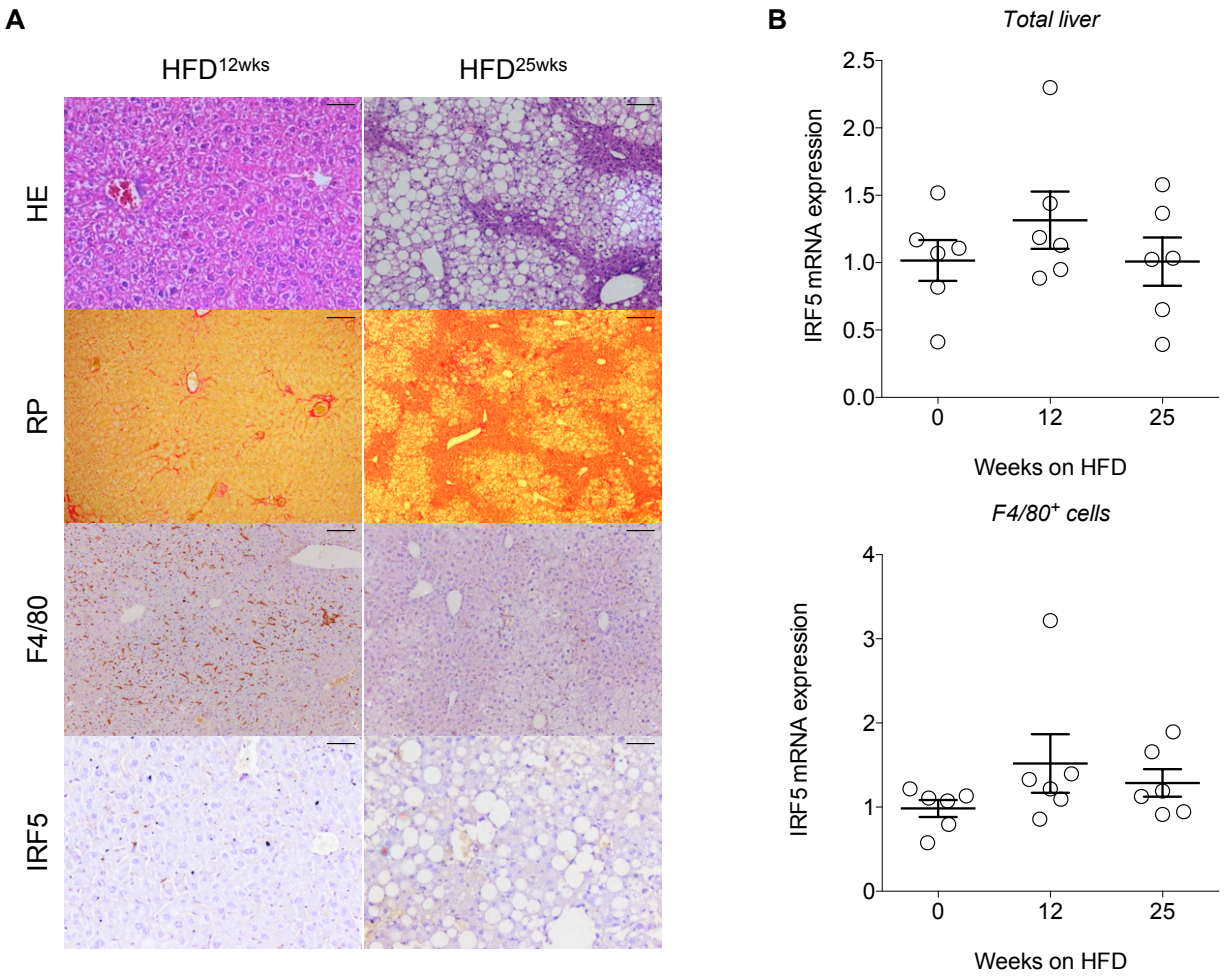

Figure S2. **IRF5 expression is not modulated under high-fat feeding in mice.** Representative images of histological analysis of liver sections from mice maintained on a high-fat diet (HFD) for 12 and 25 weeks. Liver sections stained with hematoxylin and eosin (HE) red picrosirius (RP) to visualize collagen fibres. Immunohistochemical analysis of F4/80 and interferon regulatory factor (IRF) 5. **B.** IRF5 mRNA expression in total liver lysate and liver F4/80^{+} cells. Differences between diets/treatments determined by one-way ANOVA. All values reported as mean ± SEM.

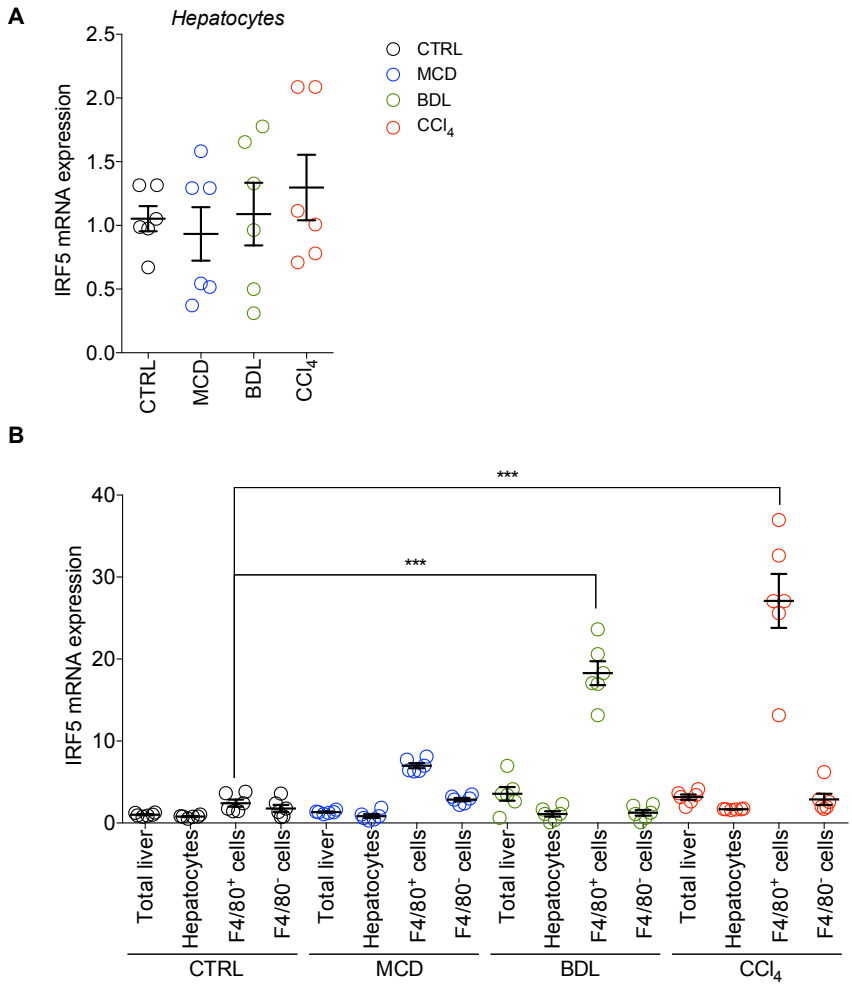

Figure S3. **IRF5 mRNA expression is not modulated in hepatocytes and is restricted to F4/80$^+$ cells. A.** Interferon regulatory factor (IRF) 5 mRNA expression in hepatocytes from mice upon normal chow (CTRL), methionine-and-choline deficient (MCD) feeding, bile duct ligation (BDL) or carbon tetrachloride (CCl_4) treatment. **B.** IRF5 mRNA expression from total liver lysate, isolated hepatocytes and immunoselected F4/80$^+$ and F4/80$^-$ cells of mice upon NC, MCD feeding, BDL or CCl_4. n=6 per group, differences between groups determined by two-way ANOVA. All values reported as mean ± SEM. *p<0,05 ** p<0,01 *** p<0,001.

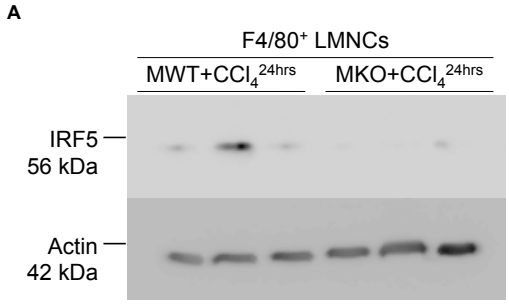

Figure S4. **Myeloid-specific deletion of IRF5 in liver F4/80$^+$ cells. A.** Immunoblot of interferon regulatory factor (IRF) 5 protein in immunoselected liver F4/80$^+$ cells from wild-type (MWT) mice and mice with a myeloid-specific deletion of IRF5 (MKO) upon carbon tetrachloride (CCl_4) treatment. n=3 per group, differences between groups determined by two-way ANOVA. All values reported as mean ± SEM. *p<0,05 ** p<0,01 *** p<0,001.

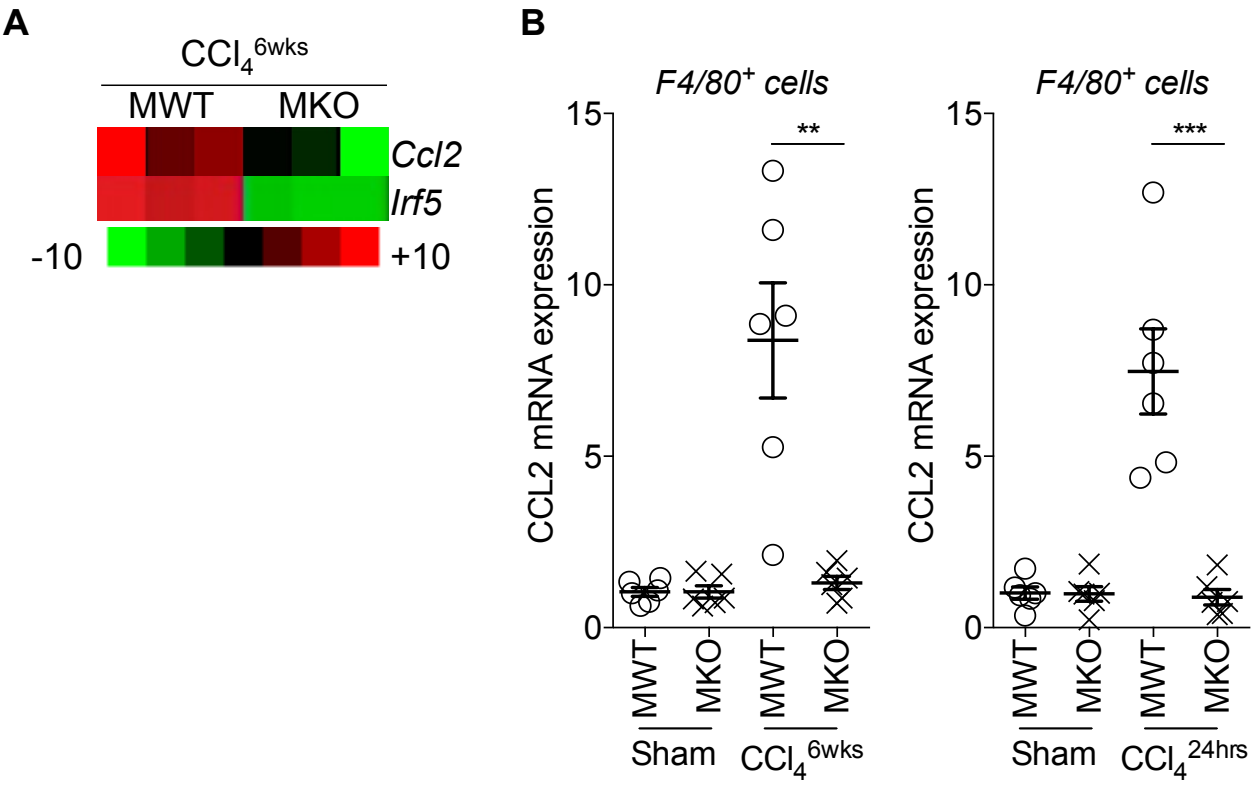

Figure S5. **Decreased CCL2 expression in IRF5-deficient macrophages. A.** heatmap from transcriptomic analysis of chemokine (C-C motif) ligand 2 (CCL2) and interferon regulatory factor (IRF) 5 mRNA expression in liver F4/80$^+$ cells from mice with myeloid specific deletion of IRF5 (MKO) and wild-type littermates (MWT) following experimental fibrosis (6wks) by carbon tetrachloride (CCl_4) administration. **B.** IRF5 mRNA expression in liver F4/80$^+$ cells following experimental fibrosis (6wks) and acute toxicity (24hrs) by CCl_4. N=6 per group. All values reported as mean ± SEM. *p<0,05 ** p<0,01 *** p<0,001.

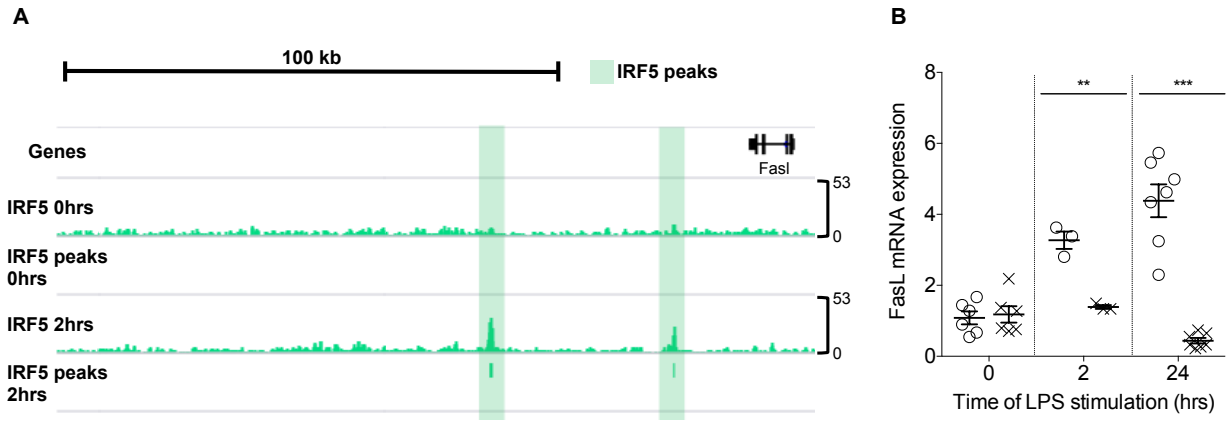

Figure S6. **IRF5 directly regulated FasL gene expression. A.** Representative USCS Genome Browser tracks in the Fas ligand (FasL) locus for interferon regulatory factor (IRF) 5 of unstimulated (0hrs) or LPS-stimulated (24hrs) bone marrow derived macrophages (BMDM). IRF5 expression and binding peaks highlighted by green bands. **B.** FasL and IRF5 mRNA expression in unstimulated (0hrs) and LPS-stimulated (2hrs and 24hrs) BMDM from wild-type mice (MWT) and mice with myeloid-deficiency of IRF5 (MKO). N=3. All values reported as mean ± SEM. *p<0,05 ** p<0,01 *** p<0,001.

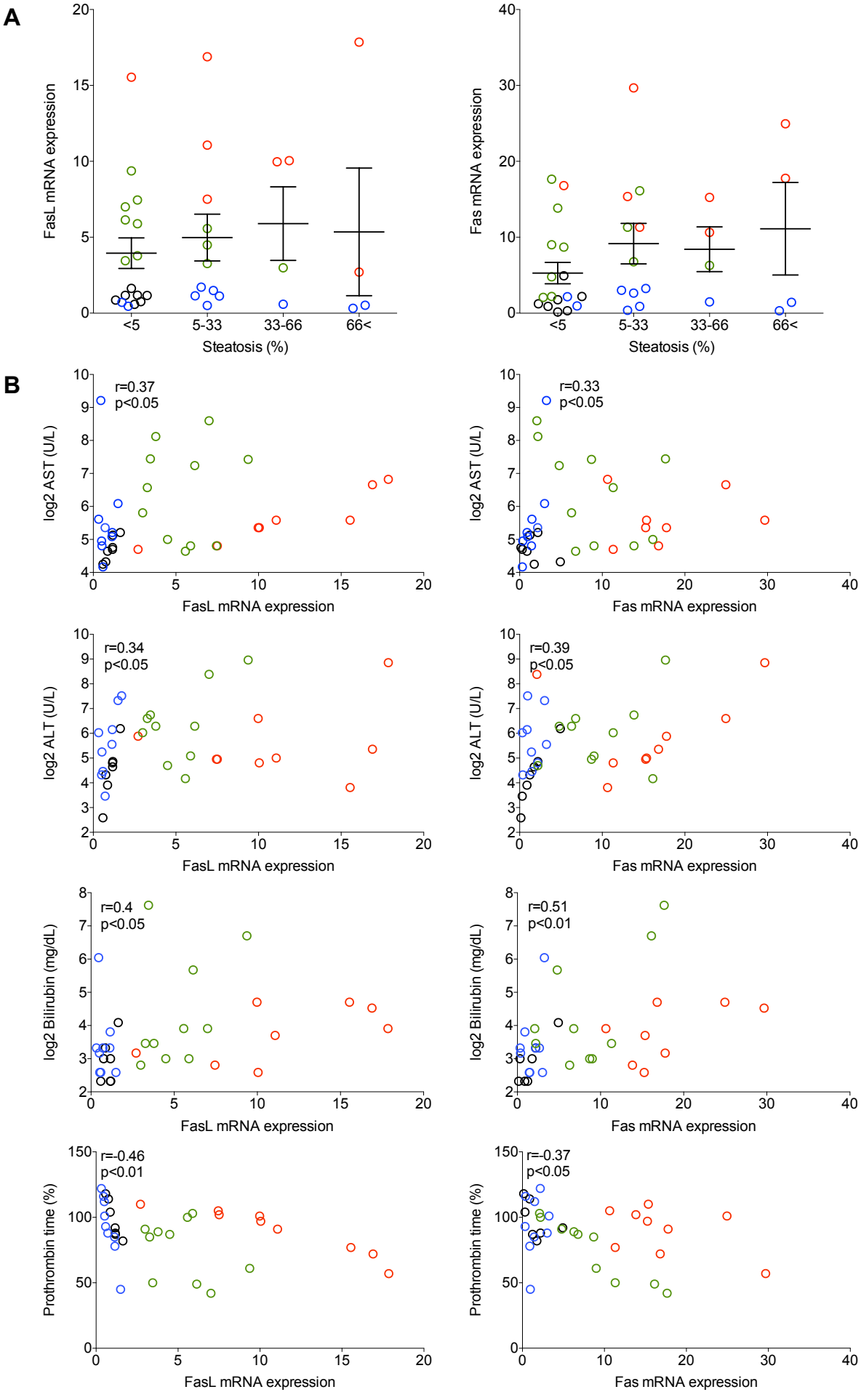
A
FasL mRNA expression
Fas mRNA expression
<5
5-33
33-66
66<
Steatosis (%)
B
log2 AST (U/L)
log2 ALT (U/L)
log2 Bilirubin (mg/dL)
Prothrombin time (%)
FasL mRNA expression
Fas mRNA expression
r=0.37
p<0.05
r=0.33
p<0.05
r=0.34
p<0.05
r=0.39
p<0.05
r=0.4
p<0.05
r=0.51
p<0.01
r=-0.46
p<0.01
r=-0.37
p<0.05

Figure S7. Fas and FasL expression are independent of steatosis in human liver but correlate to markers of liver damage. A. Fas ligand (FasL) and Fas mRNA expression in liver biopsies from control patients with normal liver (n=7), patients with fatty liver (n=10), non-alcoholic steatohepatitis (NASH; n=8) and with viral hepatitis C (HCV; n=11) stratified by steatosis grade. **B.** Correlative analyses between Fas and FasL mRNA expression with plasma aspartate and alanine transaminase (AST and ALT, respectively) levels, bilirubin levels and prothrombin time (PT) from the same cohort of patients (n=36). Differences between patient groups determined by one-way ANOVA. Correlative analyses were assessed by Spearman's test. All values reported as mean ± SEM. *p<0,05 ** p<0,01 *** p<0,001.

Table S1. Significantly enriched KEGG terms amongst up-regulated transcripts in IRF5 MKO mice versus MWT mice following experimental fibrosis by CCl_4

KEGG TERM	p-value
Glycine, serine and threonine metabolism	$3{,}8 \times 10^{-12}$
Tryptophan metabolism	$2{,}2 \times 10^{-8}$
Metabolism of xenobiotics by cytochrome P450	$5{,}1 \times 10^{-6}$
Fatty acid metabolism	$8{,}1 \times 10^{-6}$
PPAR signaling pathway	$1{,}6 \times 10^{-4}$

KEGG: Kyoto encyclopedia of genes and genomes; MKO: myeloid-specific knockout; MWT: wild-type; CCl_4: carbon tetrachloride.

Table S2. Significantly enriched GO terms amongst up-regulated transcripts in IRF5 MKO mice versus MWT mice following experimental fibrosis by CCl_4

GO TERM	p-value
Carboxylic acid metabolic process	$5{,}6 \times 10^{-43}$
Oxoacid metabolic process	$5{,}6 \times 10^{-43}$
Fatty acid metabolic process	$1{,}8 \times 10^{-11}$
Lipid metabolic process	$8{,}4 \times 10^{-11}$
Gluconeogenesis	$1{,}5 \times 10^{-7}$

GO: gene ontology; MKO: myeloid-specific knockout; MWT: wild-type; CCl_4: carbon tetrachloride.

Table S3. Primers applied for PCR genotyping of MWT and MKO mice

Primer	Sequence 5'-3'
IRF5 flox forward	CGT GTA GCA CTC CAT GCT CT
IRF5 flox reverse	AGG GCC TGT CCA GAA TTA GG
LyzM cre mutant	CCC AGA AAT GCC AGA TTA CG
LyzM cre wild-type	TTA CAG TCG GCC AGG CTG AC
LyzM cre common	CTT GGG CTG CCA GAA TTT CTC

Table S4. Primers applied for qRT-PCR analysis of gene expression in human and murine samples with use of SYBR green chemistry.

Gene	Forward sequence 5'-3'	Reverse sequence 5'-3'
IRF5 (mouse)	GATGGGGACAACACCATCTT	GGCTTTTGTTAAGGGCACAG
F4/80 (mouse)	CATCTGTGGCTGCCTCCCT	CCTTGGGAGCCTTCTGGATC
Col1α1 (mouse)	CACCCCAGCGAAGAACTCATA	GCCACCATTGATAGTCTCTCCTAAC
IRF5 (human)	TTATTCTGCATCCCCTGGAG	GCTCTTGTTAAGGGCACAGC
GAPDH (human)	AATCCCATCACCATCTTCCA	TGGACTCCACGACGTACTCA
18S (human)	TTCGAACGTCTGCCCTATCAA	ATGGTAGGCACGGCGACTA
TNF (human)	CAGCCTCTTCTCCTTCCTGA	GCCAGAGGGCTGATTAGAGA
IL1β (human)	ACAGATGAAGTGCTCCTTCCA	GTCGGAGATTCGTAGCTGGAT
αSMA (mouse)	GGCATCAATCACTTCAACAG	CCTATACGCTCTCAAATACC
IL1β (mouse)	CAACCAACAAGTGATATTCTCCATG	GATCCACACTCTCCAGCTGCA
MHCII (mouse)	GCTCTCGGAGACCTATGACG	ACAGGCAAACCTCTGGACAC
IL6 (mouse)	CCACGGCCTTCCCTACTTC	TCCACGATTTCCCAGAGAACA
TNF (mouse)	CCACCACGCTCTTCTGTCTA	CACTTGGTGGTTTGCTACGA
ARG1 (mouse)	CATTTGGGTGGATGCTCACA	TGGTACATCTGGGAACTTTCCTTT
TGFβ1 (mouse)	ACCCTGCCCCTATATTTGGA	TGGTTGTAGAGGGCAAGGAC
IL10 (mouse)	GGTTGCCAAGCCTTATCGGA	ACCTGCTCCACTGCCTTGCT
CD206 (mouse)	CCTCTGGTGAACGGAATGAT	CTTCCTTTGGTCAGCTTTGG
FasL (mouse)	CATCACAACCACTCCCACTG	GTTCTGCCAGTTCCTTCTGC
Fas (mouse)	TGTGAACATGGAACCCTTGA	TTCAGGGTCATCCTGTCTCC
BCL2 (mouse)	TGAGTACCTGAACCGGCATCT	GCATCCCAGCCTCCGTTAT
BCL-X_L (mouse)	GCTGGGACACTTTTGTGGAT	TGTCTGGTCACTTCCGACTG
Fas (human)	TCAGTACGGAGTTGGGGAAG	CAGGCCTTCCAAGTTCTGAG
FasL (human)	GCACTTTGGGATTCTTTCCA	CCTCCATTTGTCTGGCTCAT
GAPDH (mouse)	GTGGACCTCATGGCCTACAT	TGTGAGGGAGATGCTCAGTG
18S (mouse)	GGGAGCCTGAGAAACGGC	GGGTCGGGAGTGGGTAATTT

Table S5. Antibodies applied for immunohistochemical analysis of protein expression in human and murine samples.

Epitope	Working dilution	Company	Product number
F4/80 (mouse)	1:100	Abcam	ab6640
αSMA (mouse)	1:500	Abcam	ab5694
FasL (mouse)	1:100	Abcam	ab15285
IRF5 (mouse)	1:50	Abcam	ab33478
IRF5 (human)	1:50	Proteintech	10547-1-AP
CD68 (human)	1:500	Dako	M081401
ERG (human)	1:25	Abcam	ab92513
αSMA (human)	1:500	Dako	M0851

Table S6. Antibodies applied for flow cytometric analysis of murine leukocyte populations.

Marker	Flourochrome	Product number	Company	Working dilution	Epitope position
Viability	AmCyan	L34957	Life Tech	1:1000	surface
TCRβ	Brilliant Violet™ 711	563135	BD	1:100	surface
F4/80	PE-Cy7	25-480-82	eBioSciences	1:200	surface
CD8	Brilliant Violet™ 605	100743	BioLegend	1:200	surface
CD45	PE-eFlour® 610	61-0451-80	eBioSciences	1:100	surface
CD45	PerCP-Cy5.5	550994	BD	1:300	surface
CD4	Alexa Flour® 700	56-0041-80	eBioSciences	1:50	surface
CD25	APC	557192	BD	1:50	surface
CD206	FITC	141704	BioLegend	1:50	surface
CD19	Brilliant Violet™ 650	115541	BioLegend	1:300	surface
CD11c	APC	17-0114-81	eBioSciences	1:100	surface
CD11b	Brilliant Violet™ 785	101243	BioLegend	1:300	surface
TNF	eFlour® 450	48-7321-80	eBioSciences	1:200	intracellular
IL4	PE	12-7041-81	eBioSciences	1:100	intracellular
IL17	Brilliant Violet™ 786	564171	BD	1:100	intracellular
IL13	PECy7	25-7133-82	eBioSciences	1:100	intracellular
Il10	APC	17-7101-82	eBioSciences	1:100	intracellular
IFNγ	FITC	554411	BD	1:100	intracellular
FoxP3	PE	12-5773-80	eBioSciences	1:100	intracellular
FASL	PerCP-eFlour® 710	46-5911-80	eBioSciences	1:300	intracellular