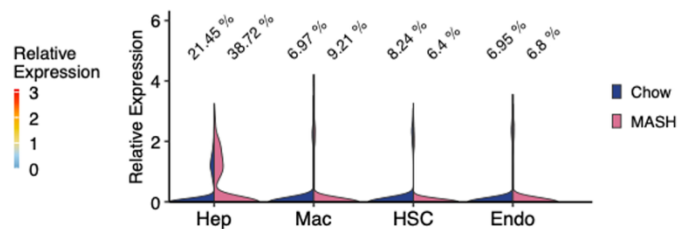
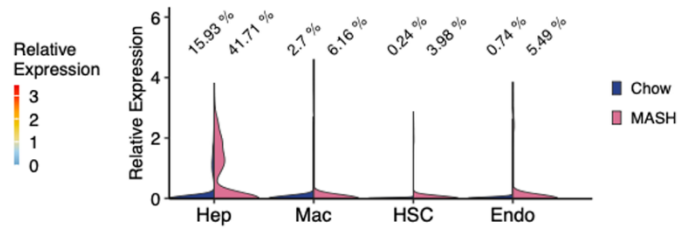
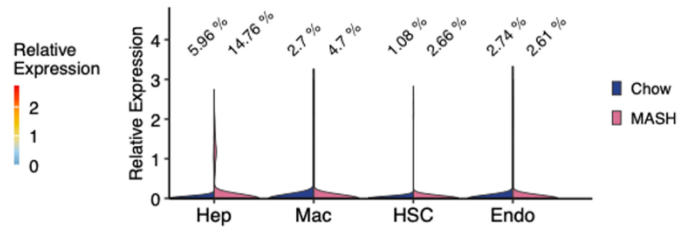
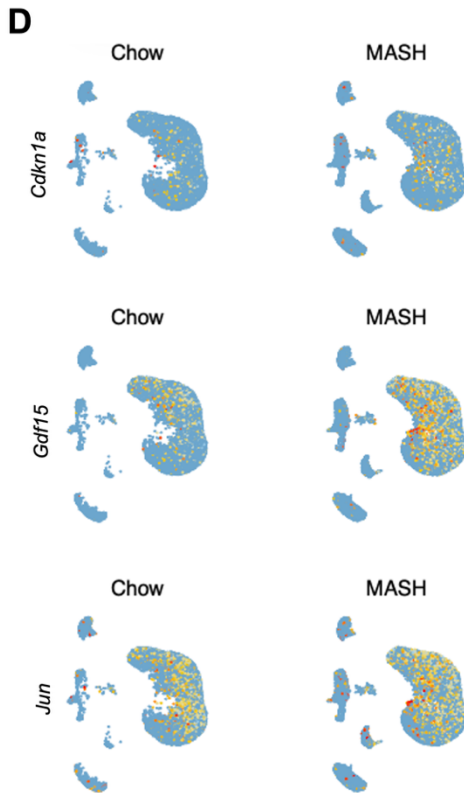
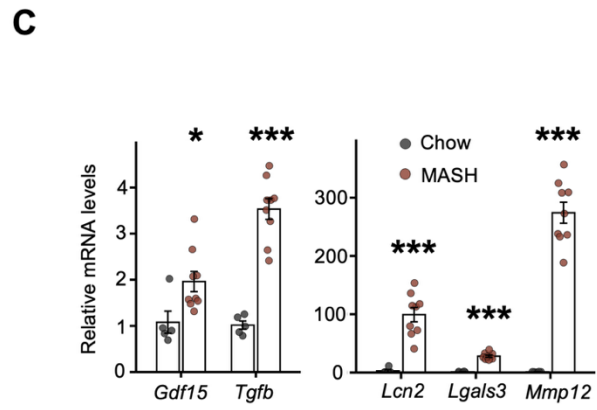
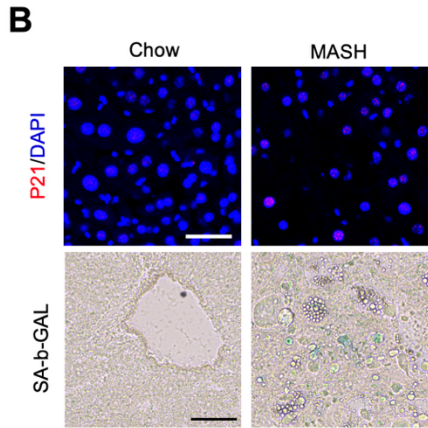
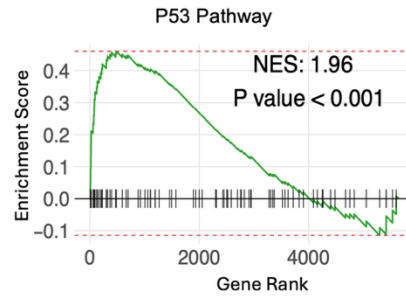
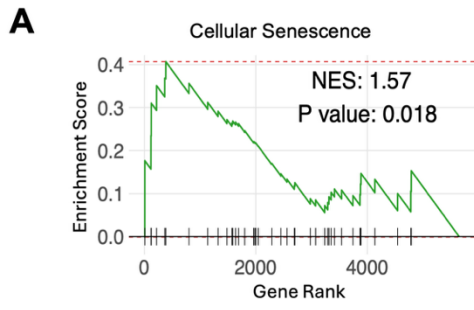


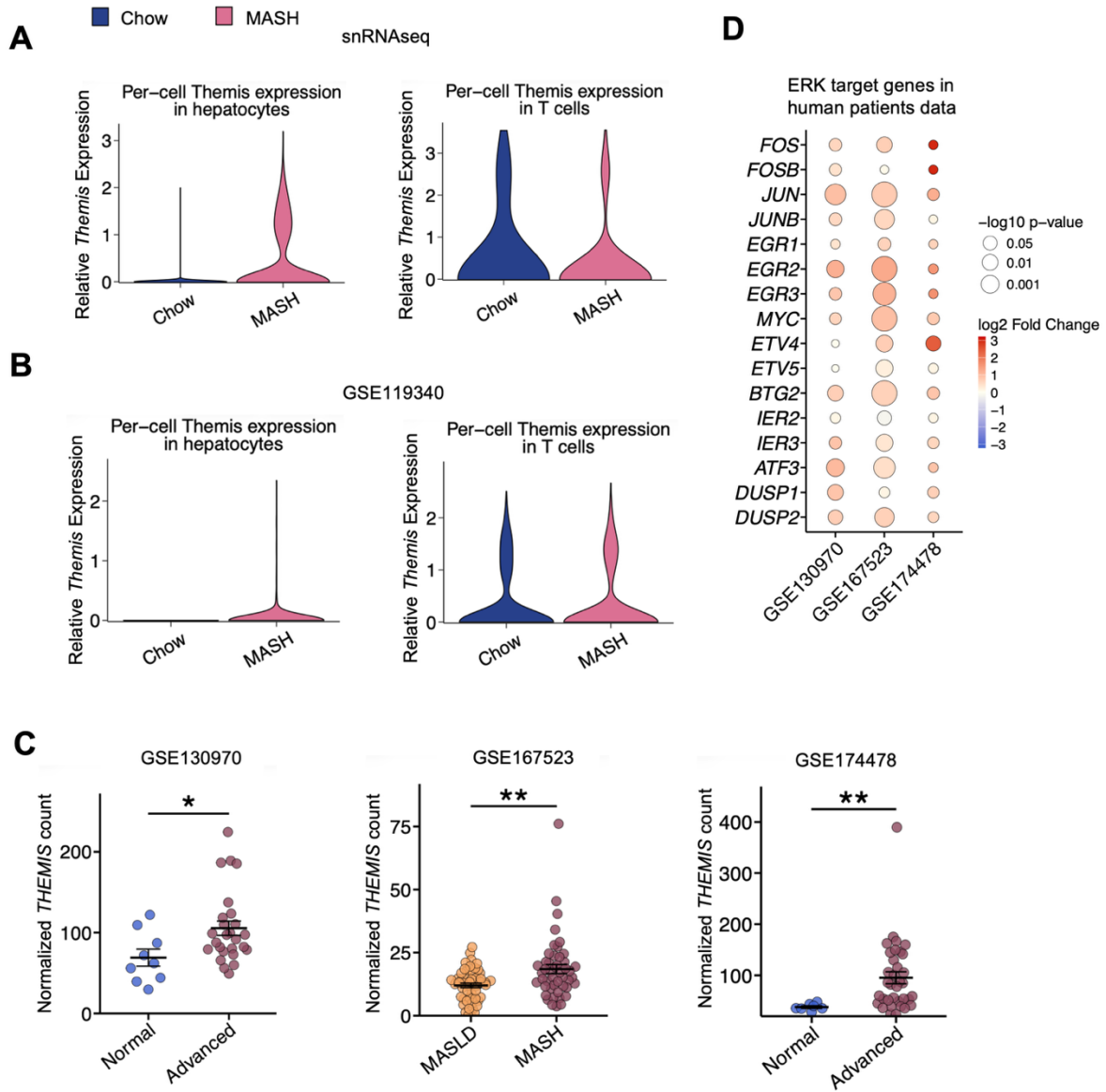
Supplementary Figure 1. Healthy and diet-induced MASH livers. (A) Stacked violin plot showing normalized expression of cell type-specific marker gene for each cluster. (B) UMAP and bar plots visualizing the proportion of nuclei from Chow and MASH samples. (C-D) Feature plots showing gene expression patterns in all hepatocytes. (E) Immunofluorescence staining for ANXA2 and CK8 of liver sections. (F) Heatmaps of genes associated with essential hepatic pathways grouped by non-daHep and daHep in MASH livers. Scale bars in (E) represent 100 μm .



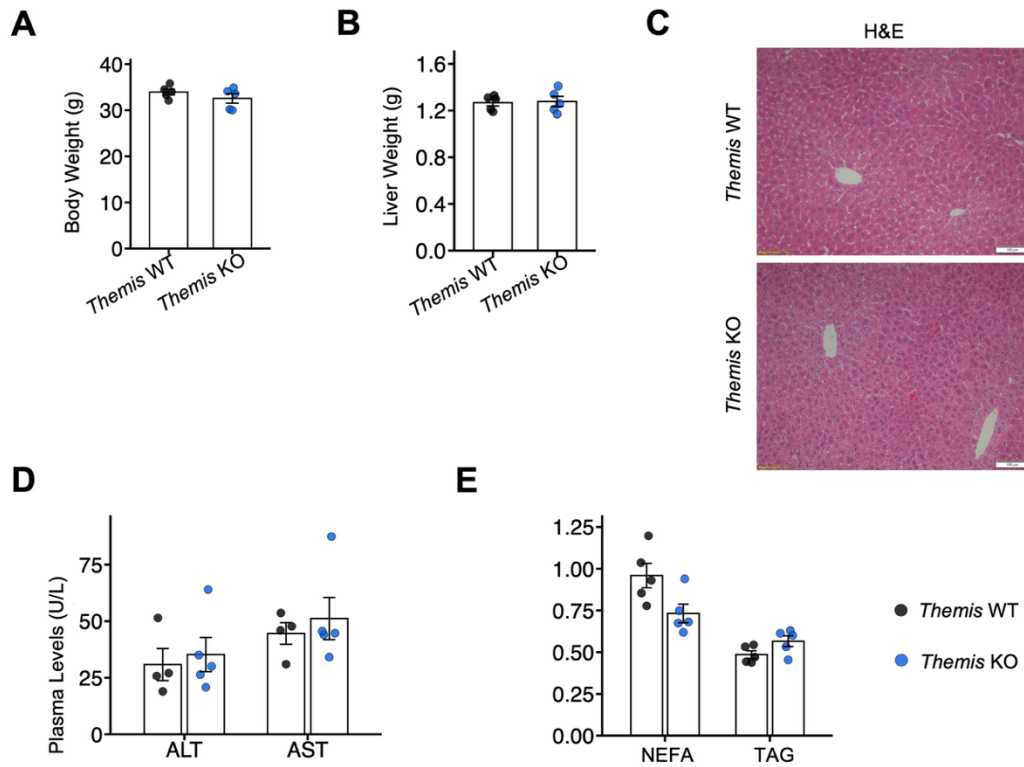
Supplementary Figure 2. Cellular senescence is induced during MASH progression. (A)

Gene sets enrichment analysis (GSEA) between daHep versus non-daHep cells in MASH highlighting Cellular Senescence pathway and P53 pathway are among the top up-regulated pathways. (B) Immunofluorescence staining for P21 and SA- β -GAL staining of Chow and MASH liver sections. (C) qPCR analysis of senescence-associated gene expression. (D) Feature plots and violin plots showing senescence-associated gene expression in hepatocytes and non-parenchymal cells.

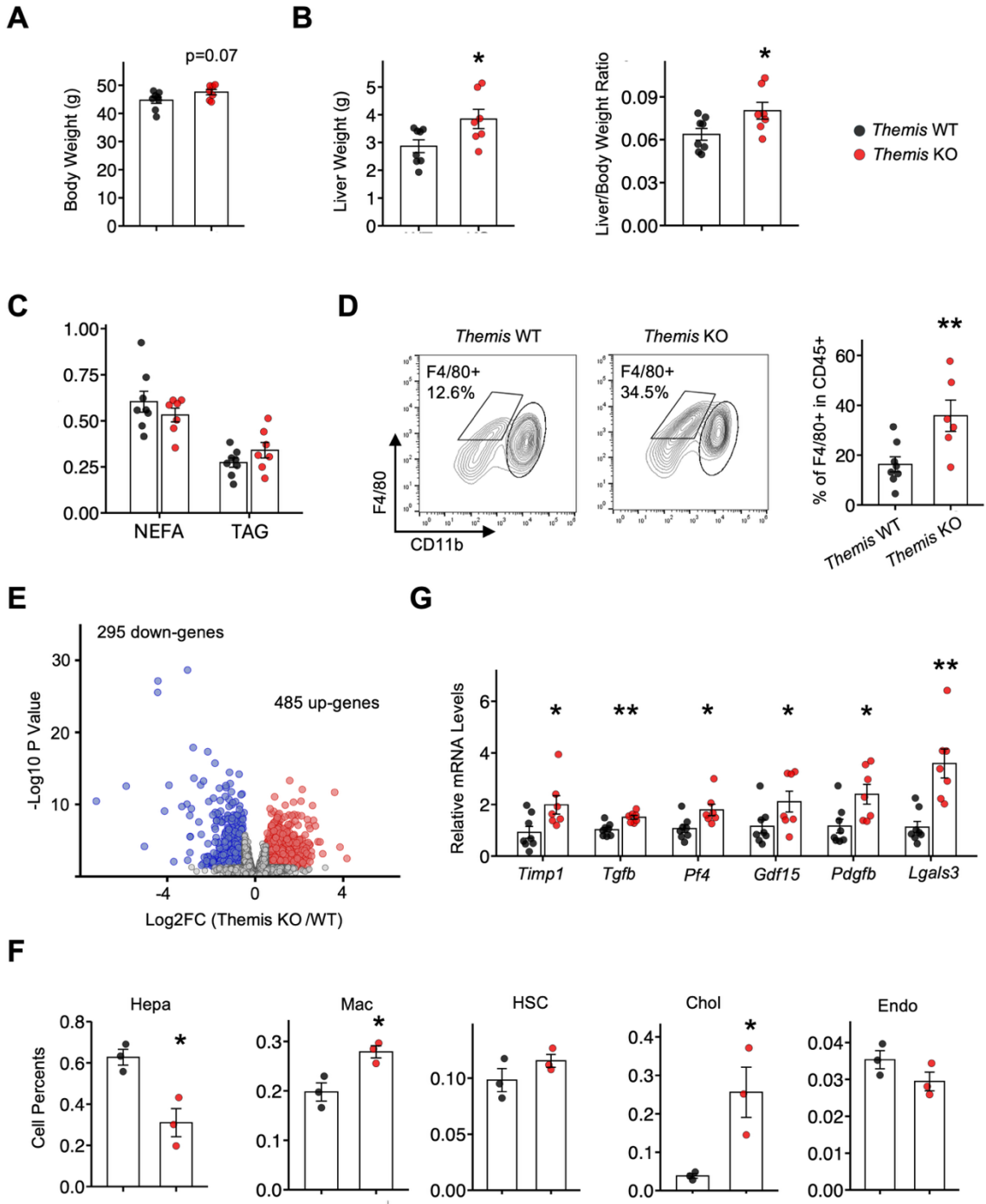
Data in (C) represent mean \pm SEM; two-tailed unpaired Student's *t* test. * $P < 0.05$ and *** $P < 0.001$. Scale bars in (B) represent 50 μ m.



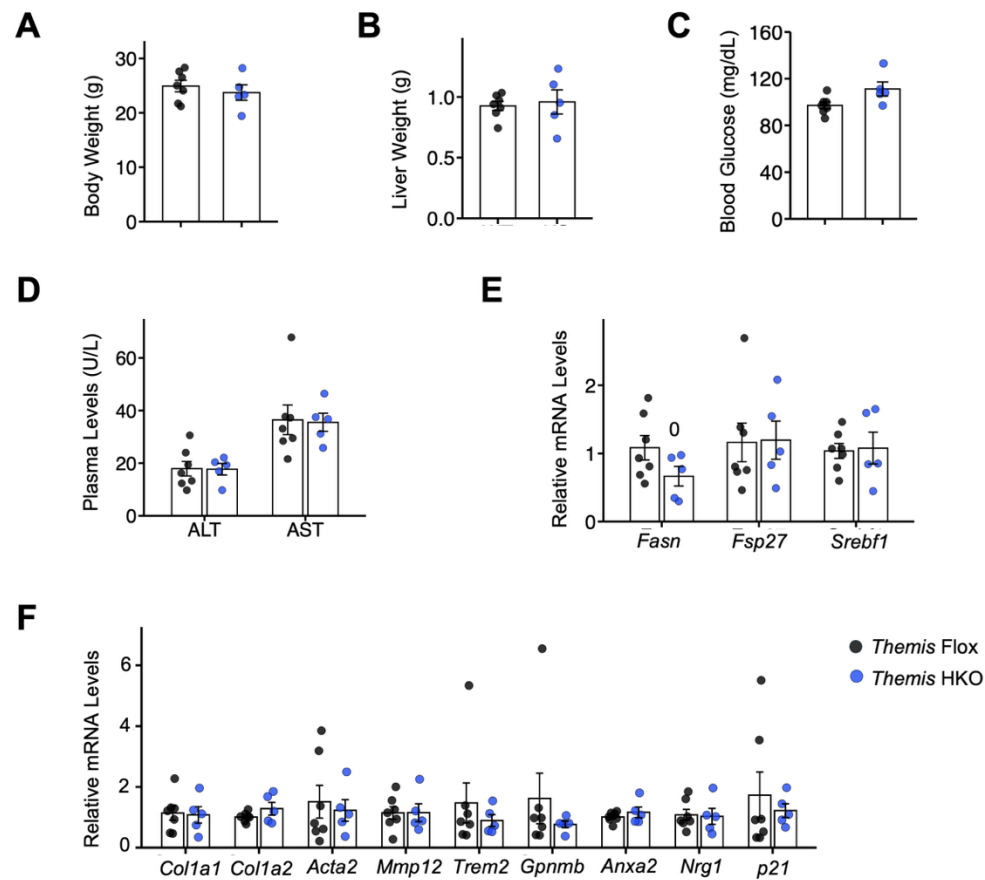
Supplementary Figure 3. Induction of Themis expression and ERK activation in MASH livers. (A-B) Violin plots showing *Themis* expression in hepatocytes and T cells using chow and MASH single-nucleus RNAseq dataset (A) and published single-cell RNAseq dataset (B). (C-D) Analysis of published bulk RNAseq datasets showing *Themis* expression (C) and ERK target gene expression (D) in human non-MASH, MASLD and MASH patients' livers. Data represent mean \pm SEM; two-tailed unpaired Student's *t* test. * $P < 0.05$ and ** $P < 0.01$.



Supplementary Figure 4. Physiological characterization of 9-month-old *Themis* WT and *Themis* KO mice fed on Chow diet. (A) Bar plot of body weight. (B) Bar plot of liver weight. (C) Histology of liver by H&E staining on liver sections. (D) Bar plot of plasma ALT and AST measurement. (E) Bar plot of plasma non-esterified fatty acids (NEFA; mmol/L) and plasma triacylglycerols (TAG; mg/mL) measurements. Values represent mean \pm SEM; two-tailed unpaired Student's *t* test.



Supplementary Figure 5. Whole-body Themis ablation. (A) Body weight of *Themis* WT and *Themis* KO mice fed on MASH diet for 3 months. (B) Liver weight and liver/body weight ratio. (C) Bar plot of plasma NEFA (mmol/L) and plasma TAG (mg/mL) measurements. (D) Flow cytometry analysis of liver macrophages in *Themis* WT and *Themis* KO mice fed on MASH diet, and quantitation of percentage of F4/80⁺ macrophage among all CD45⁺ cells. (E) Volcano plot of bulk RNA-seq on liver samples highlighting differentially expressed genes between *Themis* WT and *Themis* KO. (F) Estimated proportion for major liver cell types using deconvoluted bulk RNA-seq data. (G) Gene expression levels in liver measured by qPCR. Values represent mean \pm SEM; two-tailed unpaired Student's *t* test. * $P < 0.05$ and ** $P < 0.01$.



Supplementary Figure 6. Physiological characterization of 3-month-old *Themis Flox* and

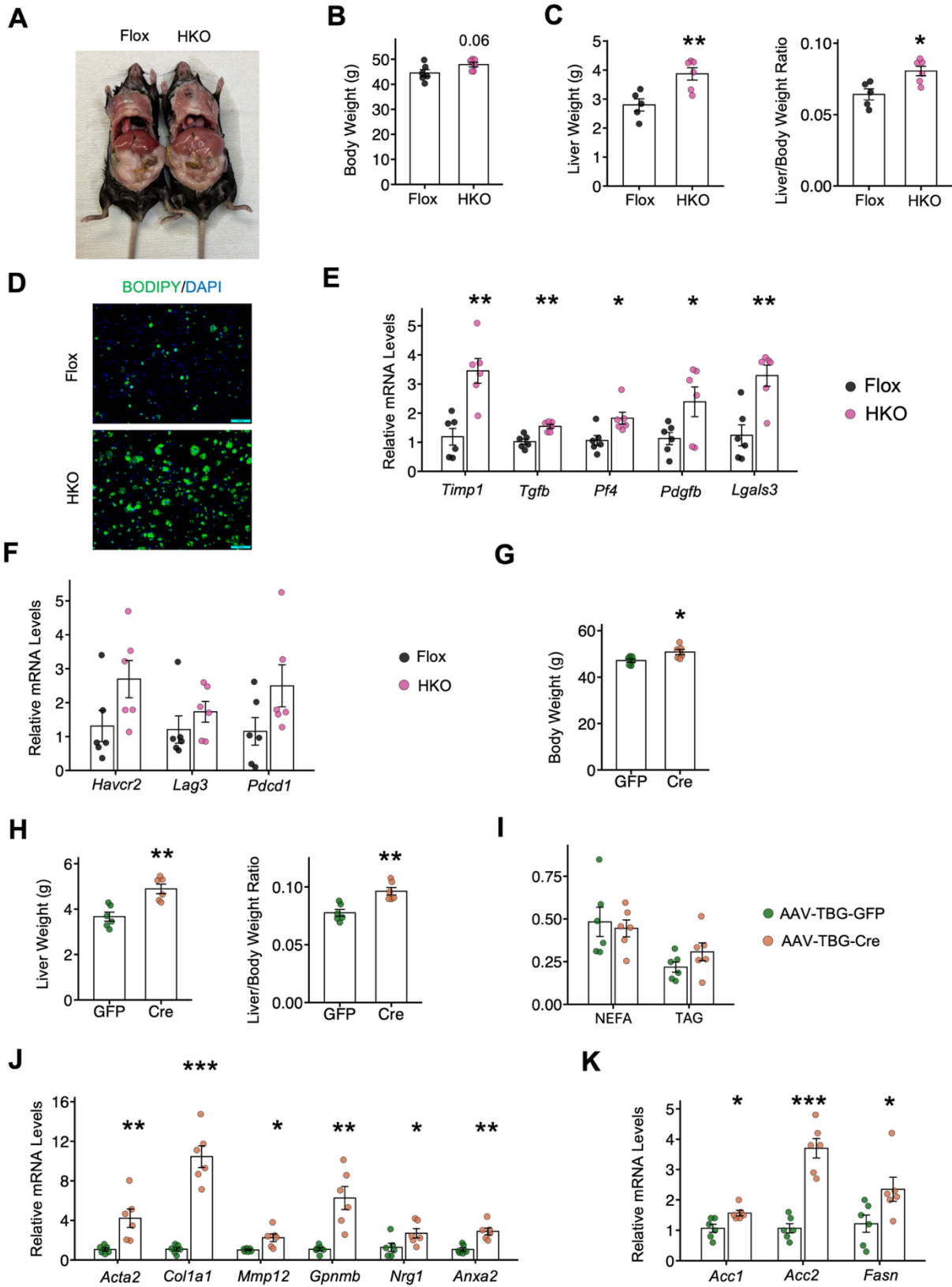
***Themis HKO* mice fed on Chow diet. (A) Bar plot of body weight. (B) Bar plots of liver**

weight. (C) Blood glucose after 24-hours fasting. (D) Bar plot of plasma ALT and AST

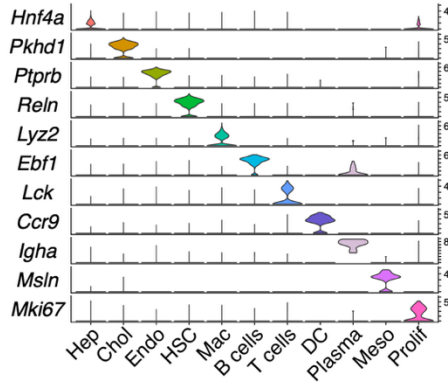
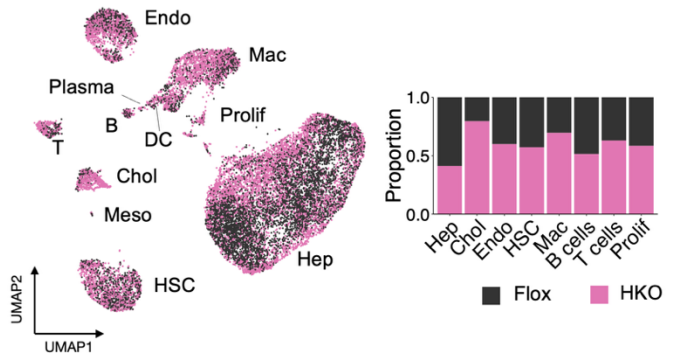
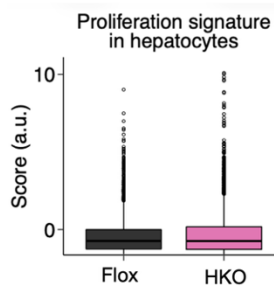
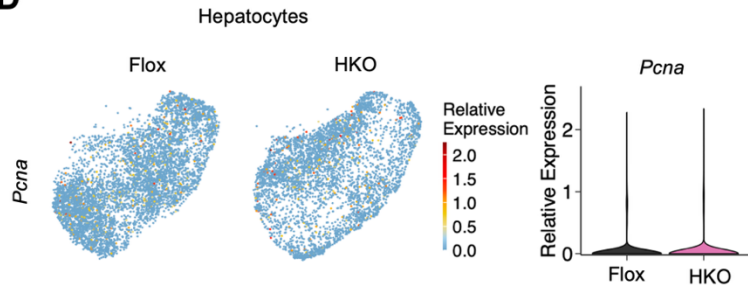
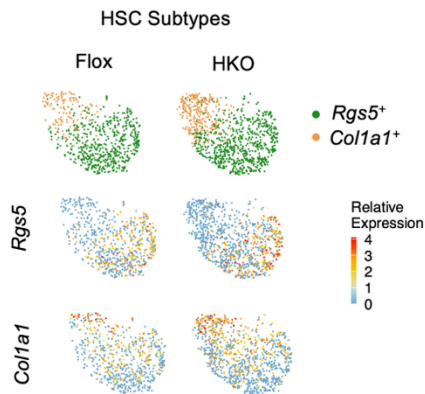
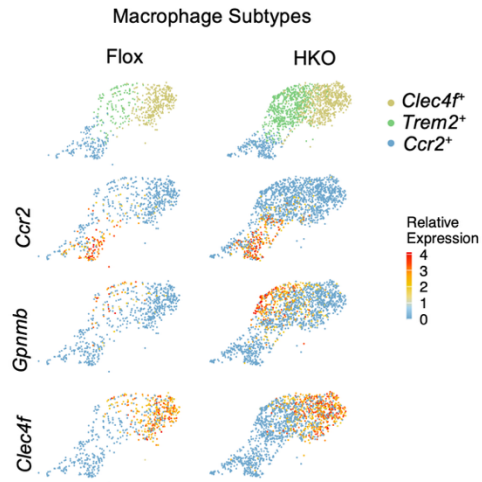
measurement. (E-F) Gene expression levels measured by qPCR. Values represent mean \pm SEM;

comparisons between genotypes were conducted with two-tailed unpaired Student's *t* test. * $P <$

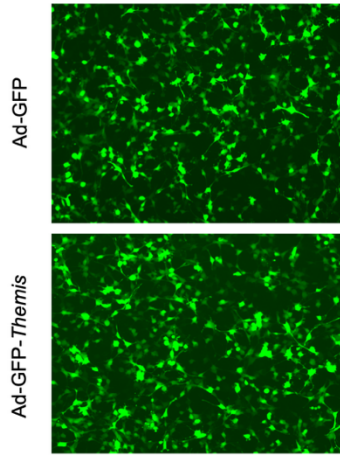
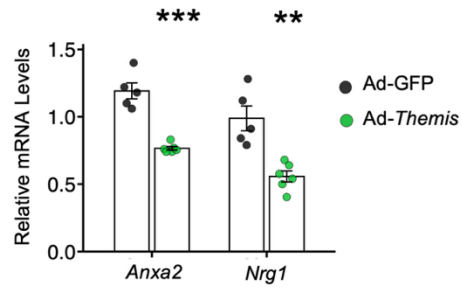
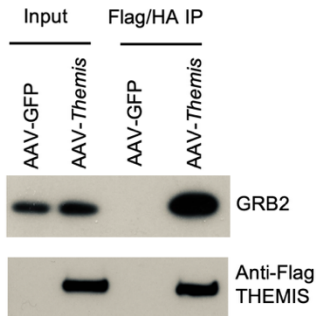
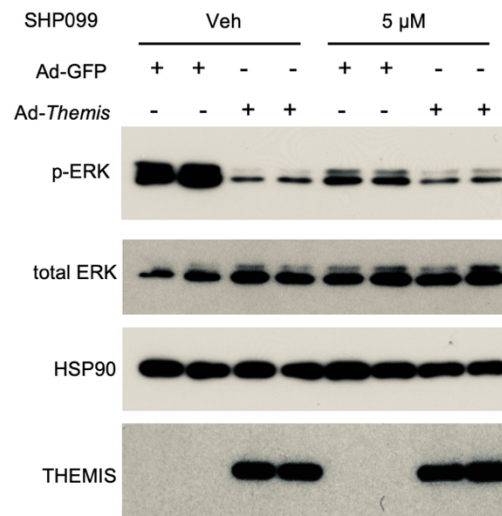
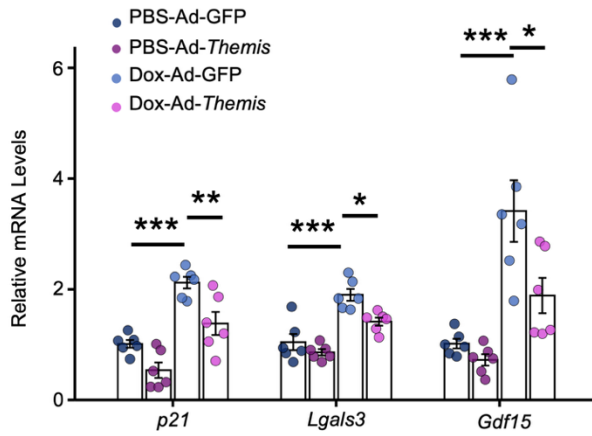
0.05.



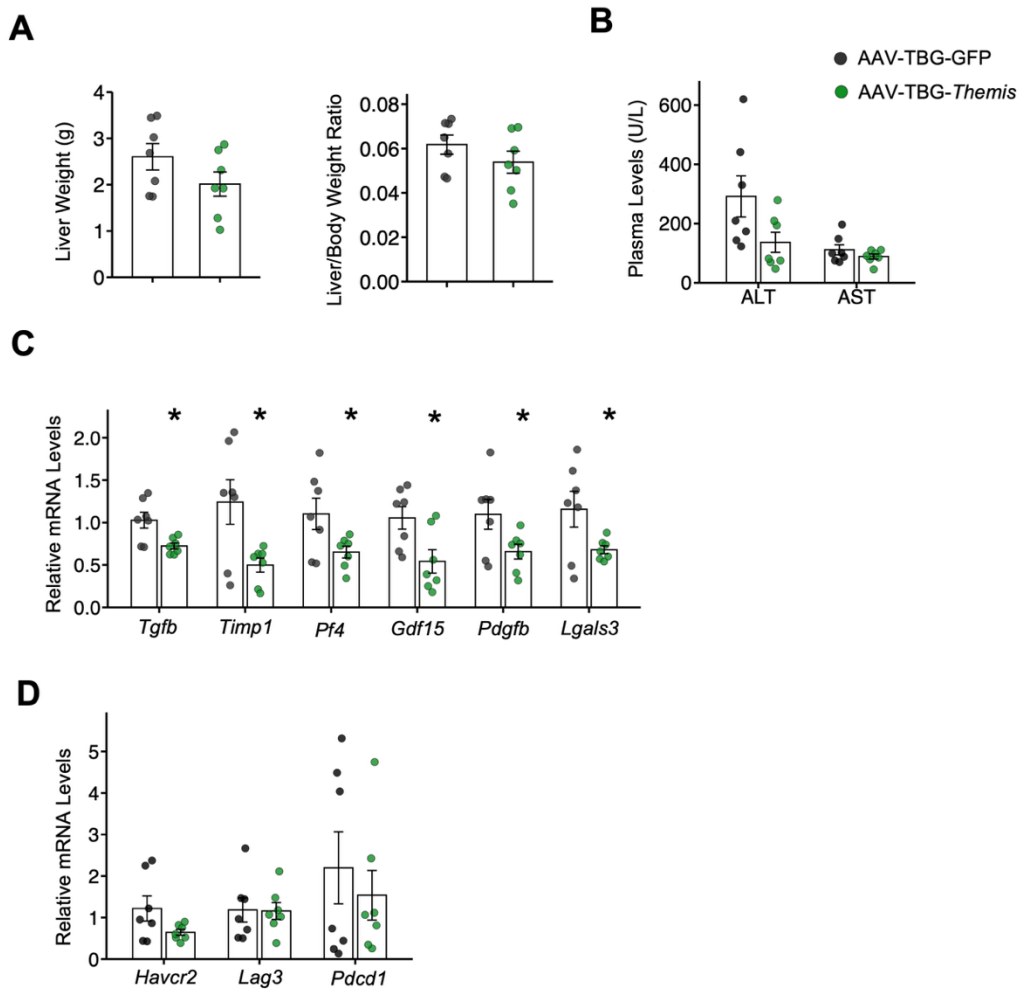
Supplementary Figure 7. Hepatocyte-specific THEMIS inactivation. (A-D) *Themis*^{flox/flox} (Flox) (n = 6) and *Themis*^{Alb-Cre} (HKO) (n = 6) male mice were fed on a MASH diet for 20 weeks. (A) Images of representative *Themis* Flox and HKO mice immediately after dissection. (B) Body weight. (C) Liver weight and liver/body weight ratio. (D) BODIPY staining of primary hepatocytes isolated from *Themis* Flox and HKO mice fed on a MASH diet. (E-F) qPCR analysis of gene expression in *Themis* Flox and *Themis* HKO livers. (G-K) *Themis* Flox mice were fed on a MASH diet for 12 weeks when those mice were tail-vein injected with either AAV-TBG-GFP (n = 6) or AAV-TBG-Cre (n = 6) and continued the MASH diet for another 8 weeks. (G) Body weight. (H) Liver weight and liver/body weight ratio. (I) Plasma NEFA (mmol/L) and plasma TAG (mg/mL) measurements (J-K) qPCR analysis of gene expression of mice injected with either AAV-TBG-GFP or AAV-TBG-Cre. Values represent mean \pm SEM; comparisons between genotypes were conducted with two-tailed unpaired Student's *t* test. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. Scale bars in (D) represent 100 μm .

A**B****C****D****E****F**

Supplementary Figure 8. snRNA-seq analysis of *Themis* Flox and HKO livers. (A) Stacked violin plot showing normalized expression of cell type-specific marker gene for each cluster. (B) Visualization of cell clusters grouped by genotype in UMAP coordinates, and a bar plot showing the proportion of nuclei from *Themis* Flox and HKO MASH livers grouped by cell type excluding rare cell types (< 0.5% total nuclei). (C) Analysis of proliferative gene signature scores of *Themis* Flox and HKO hepatocytes. (D) Feature plots and violin plots showing *Pcna* expression in *Themis* Flox and HKO hepatocytes. (E) Subclusters of HSC with marker genes. (F) Subclusters of macrophages with marker genes.

A**B****C****D****E**

Supplementary Figure 9. Adenoviral transduction in cultured hepatocytes. (A) Images of Hepa1 cells infected with either Ad-GFP or Ad-GFP-Themis under the GFP channel of a microscope. (B) qPCR analysis of gene expression from primary hepatocytes infected with either Ad-GFP or Ad-Themis. (C) Immunoprecipitation of Flag and HA double tagged THEMIS. (D) Immunoblotting of either Ad-GFP or Ad-GFP-Themis transduced human primary hepatocytes stimulated by SHP099 for 4 hours. (E) Hepa1 cells overexpressing either GFP or Themis were treated with 50nM doxorubicin (Dox) for 3 days. qPCR analysis of gene expression. Data in (B) represent mean \pm SEM; two-tailed unpaired Student's *t* test. ***p* < 0.01 and ****p* < 0.001. Data in (E) represent mean \pm SEM; One-way ANOVA with post-hoc Tukey's HSD test. * *P* < 0.05, ** *P* < 0.01 and *** *P* < 0.001.



Supplementary Figure 10. AAV-mediated THEMIS overexpression in the liver. Male

C57BL/6 mice were fed on a MASH diet for 14 weeks when mice were tail-vein injected with

either AAV-TBG-GFP (n = 7) or AAV-TBG-Themis (n = 7). Mice were continuously fed on a

MASH diet for another 6 weeks. (A) Tissue weight and liver/body weight ratio. (B) Plasma ALT

and AST levels. (C-D) qPCR analysis of gene expression. Values represent mean ± SEM; two-

tailed unpaired Student's *t* test. * *P* < 0.05.

Supplementary Table 1

Senescence	De novo lipogenesis	Fatty acid oxidation	Proliferation
Cxcr2	Acly	Acadl	Mki67
Ptges	Acsl3	Crat	Pcna
Itpka	Hsd17b12	Etfdh	Top2a
Il1a	Acss2	Hadh	Mcm2
Il1b	Elovl6	Acadm	Mcm3
Il7	Acacb	Cpt2	Mcm4
Il2	Hacd3	Crot	Mcm5
Fgf2	Elovl5	Hadha	Mcm6
Csf1	Srebf1	Hadhb	Mcm7
Gem	Acaca	Acox3	Ccna2
Tnfrsf1b	Fads6	Acads	Ccnb2
Selplg	Fasn	Acad10	Ccnd1
Serpine1	Elovl2	Fabp1	Ccnd2
Cd9	Hacd2	Etfb	Ccnd3
Spx	Fads2	Echs1	Cdk2
Plat	Fads1	Gcdh	Cdk4
Gdf15	Scd1	Acat1	Cdk6
Mmp2		Etfb	Tyms
Mmp13		Acad11	Rrm1
Mmp12		Acaa1a	Rrm2
Icam1		Acadvl	Ung
Sema3f		Acox2	Fen1
Mif		Ehhadh	Prim1
Igfbp3		Eci1	Pole
Cxcl16		Hsd17b4	Polq
Ccl2		Acaa2	Cdc6
Ccl7			Cenpf
Ccl5			Cenpe
Ccl4			Cenpa
Timp2			Aurka
Bmp6			Kif11
Edn1			Kif23
Plau			Bub1b
Ptger2			Mad2l1
Mmp14			Ndc80
Angpt1			Spc25
Tnfrsf11b			Cenpn
Acvr1b			Cenpk
Ets2			Hist1h1b
Cdkn1a			Ube2s
Areg			Anapc1
			Anapc5