

Supplemental information

**Liver-fibrosis-activated transcriptional
networks govern hepatocyte reprogramming
and intra-hepatic communication**

**Anne Loft, Ana Jimena Alfaro, Søren Fisker Schmidt, Felix Boel Pedersen, Mike Krogh
Terkelsen, Michele Puglia, Kan Kau Chow, Annette Feuchtinger, Maria
Troullinaki, Adriano Maida, Gretchen Wolff, Minako Sakurai, Riccardo Berutti, Bilgen
Ekim Üstünel, Peter Nawroth, Kim Ravnskjaer, Mauricio Berriel Diaz, Blagoy
Blagoev, and Stephan Herzig**

Figure S1

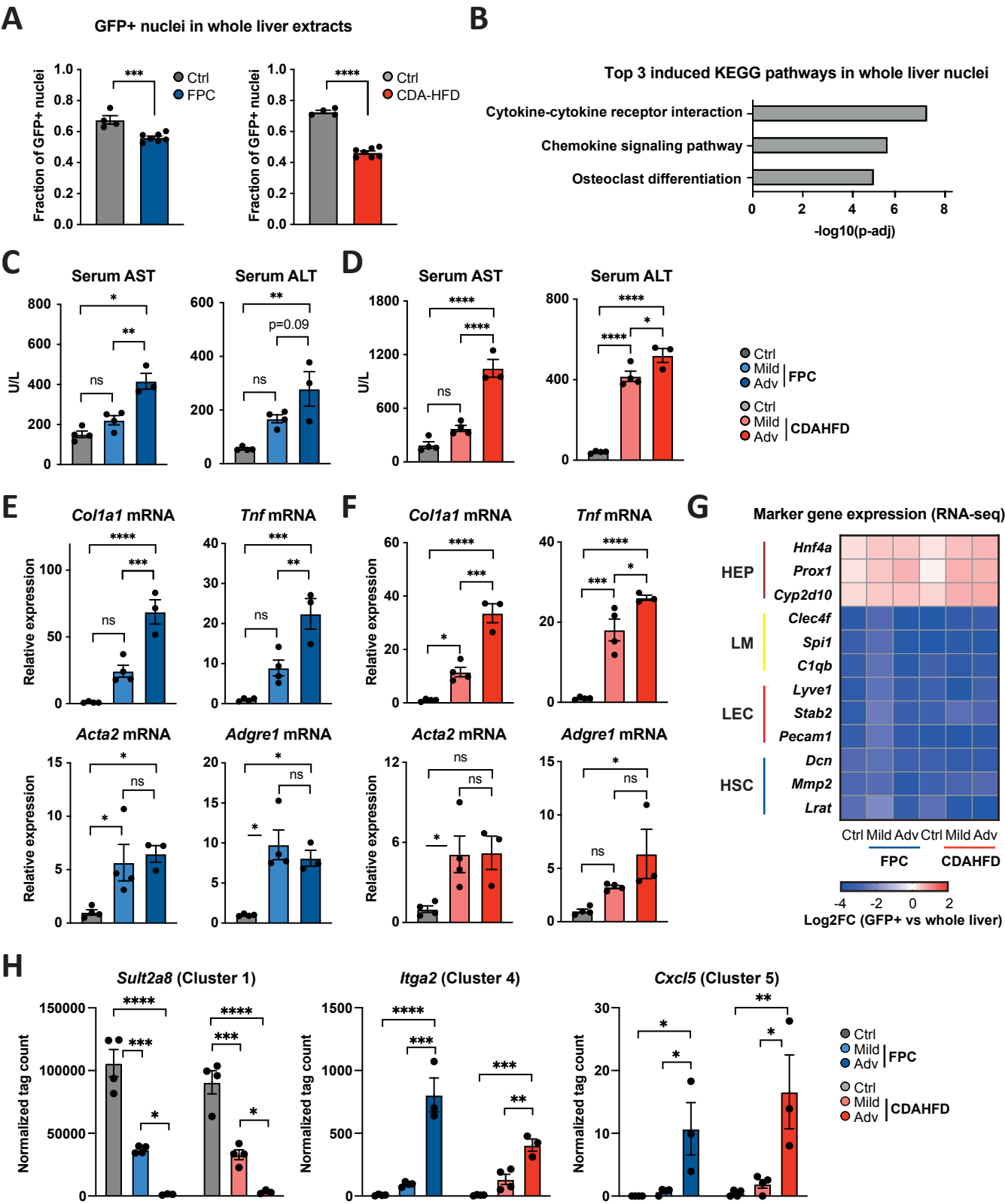


Figure S1. Characterization of mice with mild and advanced NASH. Related to Figure 1.

HEP-INTACT mice fed an FPC diet for 20 weeks, a CDAHFD for 7 weeks or the corresponding control diets.

(A) Fraction of GFP+ nuclei of whole liver nuclei counted using a fluorescence microscope in extracts obtained from HEP-INTACT mice (n = 4-7).

(B) Functional enrichment analyses using KEGG pathways for the common set of genes induced in NASH versus control in whole liver nuclei from both NASH models.

(C-D) Serum aspartate aminotransferase (AST) (left) or alanine aminotransferase (ALT) (right) in HEP-INTACT mice fed (C) an FPC diet or (D) a CDAHFD (n = 3-4).

(E-F) Expression (qPCR) of indicated genes in whole liver lysates from HEP INTACT fed (E) an FPC diet or (F) a CDAHFD (n = 3-4).

(G) Log2FC in expression of hepatocyte and NPC marker genes from the indicated cell types in GFP+ nuclei vs whole liver nuclei obtained from HEP INTACT mice. LM = liver macrophages; LEC = liver endothelial cells; HSC = hepatic stellate cells.

(H) Expression (RNA-seq) of *Sult2a8* (left), *Itga2* (middle), and *Cxcl5* (right) from the indicated RNA-seq clusters in GFP+ nuclei from HEP INTACT mice (n = 3-4).

(A, C-F, H) Every dot represents one individual mouse. Colored bars indicate mean \pm SEM. Significance was determined by (A) two-tailed, unpaired t-test, (C-F) one-way ANOVA with Tukey's multiple comparison test between the different conditions or (H) two-way ANOVA with Sidak's multiple comparison test between the different conditions for each study. *p < 0.05, ** p < 0.01, *** p < 0.001, ****p < 0.0001, ns = non-significant.

Figure S2

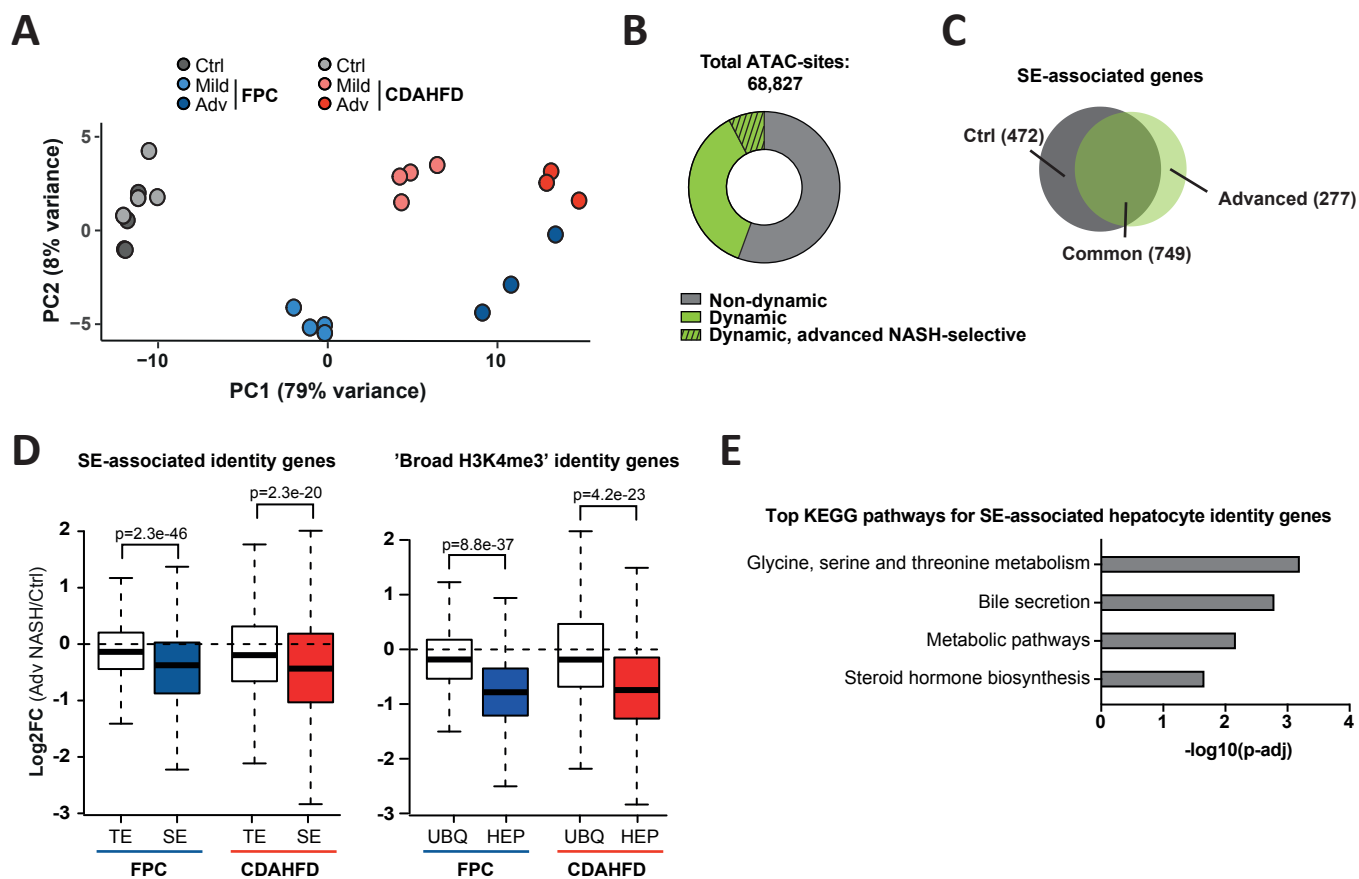


Figure S2. Chromatin remodeling and hepatocyte identity loss in advanced NASH. Related to Figure 2.

(A) Principal component analyses of the ATAC-seq data from GFP⁺ nuclei isolated from the HEP INTACT mice.

(B) Fraction of non-dynamic (grey) as well as dynamic (green) ATAC-seq sites changing accessibility in one or more condition during NASH progression. The shaded area marks ATAC-seq sites changing accessibility in advanced NASH specifically.

(C) Overlap between superenhancer (SE)-associated genes identified in the control condition and the advanced NASH condition.

(D) Log₂FC in gene expression (RNA-seq) in GFP⁺ nuclei from HEP-INTACT mice (advanced NASH vs control group) for genes associated with a typical enhancer (TE) or a SE identified in control mice (left) or genes associated with broad H3K4me₃ domains specific to the liver (HEP) or in ubiquitously (UBQ) broad H3K4me₃ domains (right).

(E) Functional enrichment analyses using KEGG pathways for SE-associated genes identified in the control condition.

(A) Every dot represents one individual mouse, (D) horizontal line indicates the median and whiskers indicate 1.5x IQR. (D) Significance was determined by two-sided Wilcoxon–Mann–Whitney test and indicated by exact p value.

Figure S3

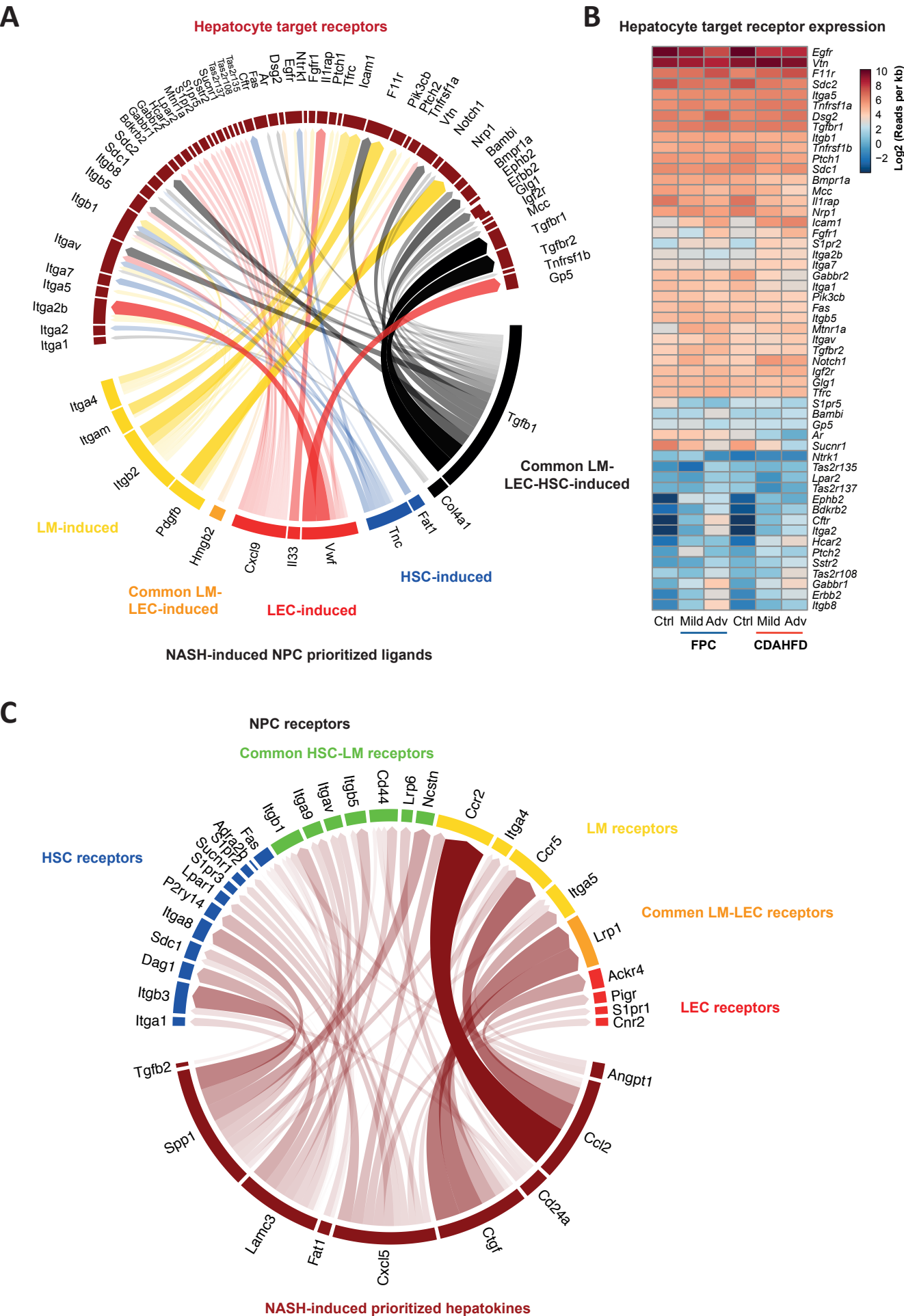


Figure S3. Interactions between NASH-activated ligands and target receptors. Related to Figure 3.

(A) Circle plot showing predicted interaction links between the NASH-induced NPC ligands identified in liver macrophages (LM) (yellow), liver endothelial cells (LEC) (red), hepatic stellate cells (HSC) (blue), both in LM and LEC (orange), or in all cell types (black) to their associated hepatocyte target receptors (bordeaux) related to the regulation of hepatocyte target genes as displayed in Figure 3C.

(B) Log2-normalized expression level (RNA-seq) of the hepatocyte target receptors shown in Figure S3A in GFP+ nuclei obtained from the indicated groups of HEP-INTACT mice.

(C) Circle plot showing the predicted interaction links between NASH-induced hepatokines (bordeaux) to their predicted target receptors in LM (yellow), LEC (red), HSC (blue), common in LM and LEC (orange) or common in HSC and LM (green) related to the regulation of NPC target genes as displayed in Figure 3F.

Figure S4

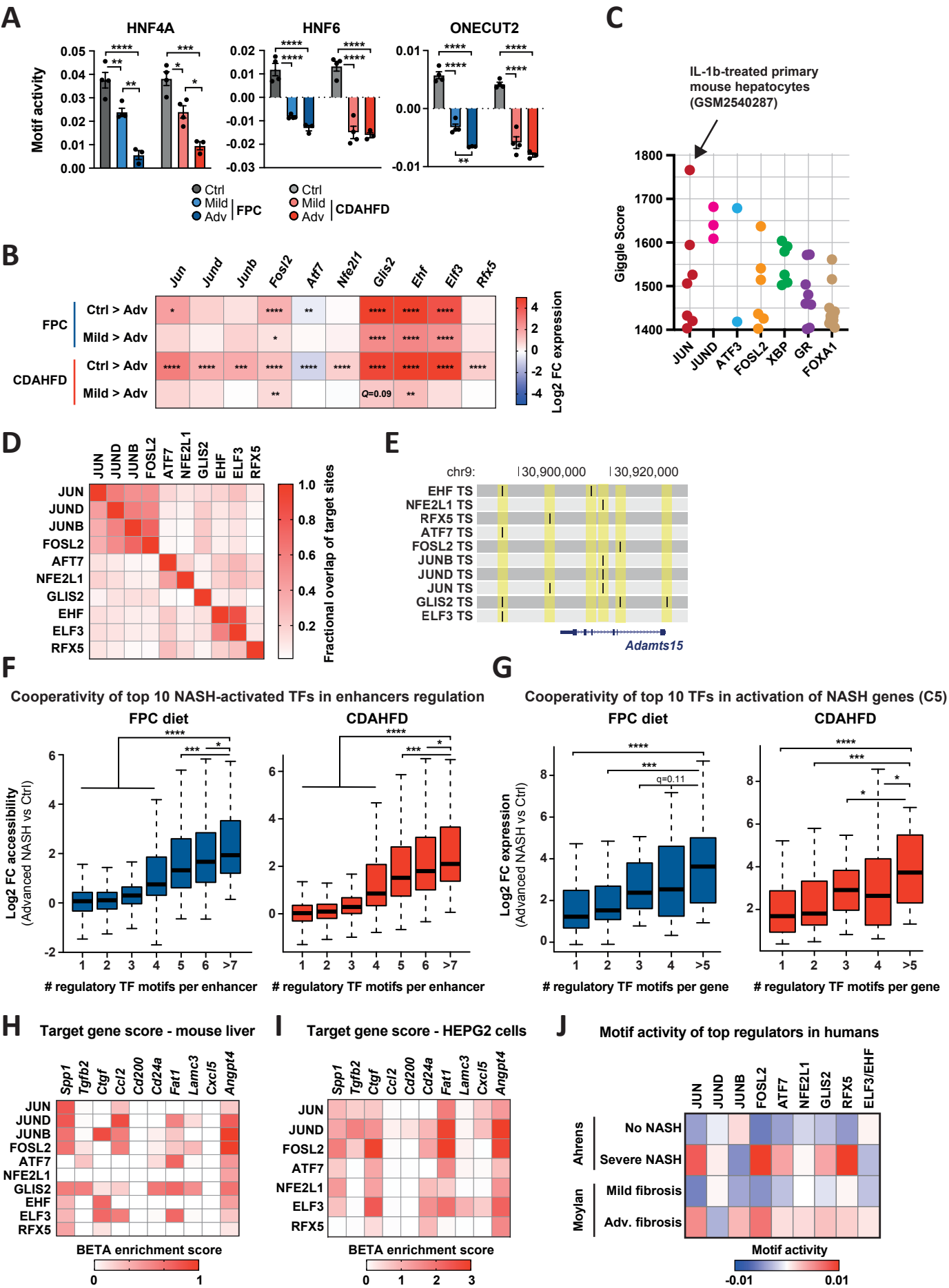


Figure S4. NASH-activated TFs cooperatively induce enhancers and genes. Related to Figure 4.

(A) Motif activity as calculated by IMAGE for HNF4A (left), HNF6/ONECUT1 (middle) and ONECUT2 (right) in GFP+ nuclei obtained from HEP-INTACT mice (n = 3-4).

(B) Heatmap showing log2FC in gene expression (RNA-seq) in GFP+ nuclei from HEP-INTACT mice (advanced NASH vs control group or advanced NASH vs mild NASH) for genes encoding the top predicted TFs with NASH-induced motif activity.

(C) Validation of IMAGE-predicted hepatocyte JUN target sites using the Gigggle score (i.e., a score for the similarity with public ChIP-seq profiles in CistromeDB).

(D) Similarity heatmap showing the fractional overlap between IMAGE-predicted target sites (TS) of the indicated NASH-activated TFs.

(E) IMAGE-predicted target sites of the indicated NASH-activated TFs in the *Adamts15* loci.

(F) Log2FC in accessibility (ATAC-seq) in GFP+ nuclei from HEP-INTACT mice (advanced NASH vs control group) receiving either an FPC diet (left) or a CDAHFD (right) for genomic regions associated with an increasing number of motifs for top NASH-activated TFs.

(G) Log2FC in expression (RNA-seq) in GFP+ nuclei from HEP-INTACT mice (advanced NASH vs control group) receiving either an FPC diet (left) or a CDAHFD (right) for hepatocyte genes in RNA-seq cluster 5 associated with an increasing number of motifs for top NASH-activated TFs.

(H-I) Beta enrichment score based on (H) IMAGE-predicted target sites in mouse control and NASH livers or (I) ChIP-seq binding sites in HEPG2 cells for the indicated TFs and their putative target genes.

(J) Heatmap showing the motif activity as calculated by ISMARA in human cohorts of NASH and liver fibrosis for top 10 TFs with NASH-induced motif activities in HEP-INTACT mice.

(A) Every dot represents one individual mouse and colored bars indicate mean \pm SEM, (F-G) horizontal line indicates the median and whiskers indicate 1.5x IQR. Significance was determined by (A) one-way ANOVA with Tukey's multiple comparison test between the different conditions, (B) FDR-corrected pairwise comparison between the indicated conditions or (F-G) Kruskal-Wallis one-way analysis with FDR-corrected multiple comparison test between (F) sites or (G) genes associated with (F) 7 or (G) 5 or more motifs and the other groups, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 or when corrected for FDR, *q < 0.05, ***q < 0.001, ****q < 0.0001, ns = non-significant.

Figure S5

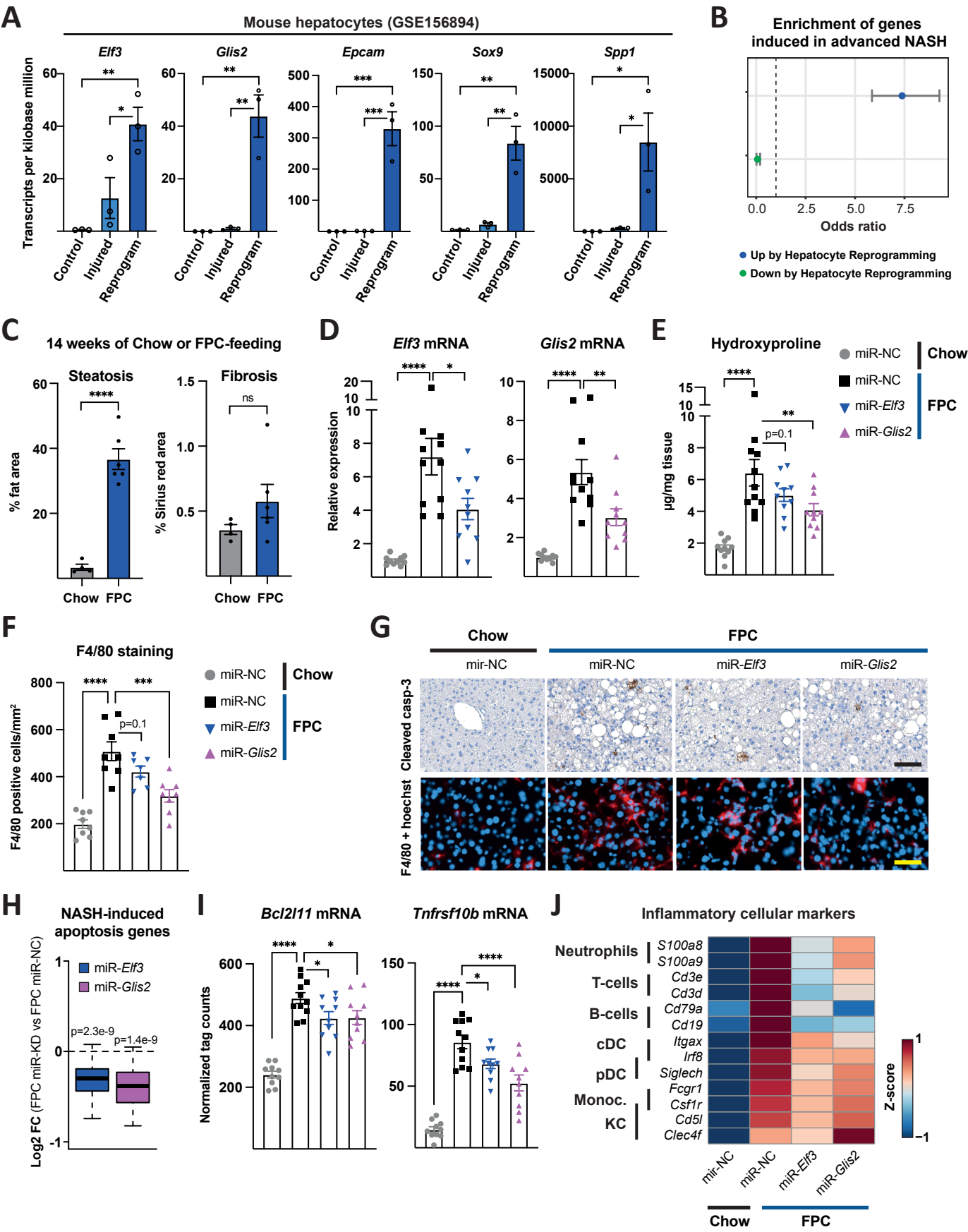


Figure S5. Loss of ELF3 and GLIS2 improves key NASH parameters. Related to Figure S5.

(A) Expression (RNA-seq) of the indicated genes in different types of mouse hepatocytes isolated following DDC-induced liver injury. Control mouse hepatocytes was collected from untreated mice. RNA-seq data was obtained from (Merrell et al., 2021).

(B) Enrichment (as indicated by odds ratio) of genes induced in advanced NASH for genes induced (blue) or repressed (green) in reprogrammed hepatocytes vs injured hepatocytes following DDC-induced liver injury (Merrell et al., 2021).

(C) Mice were fed an FPC diet or a control diet for 14 weeks. % fat area (left) and % Sirius red area (right) were measured in the liver (n = 4-6).

(D-J) Mice were fed a control or FPC diet for 14 weeks, injected with AAVs expressing non-coding miRNAs (miR-NC) or miRNA against *Elf3* (miR-*Elf3*) or *Glis2* (miR-*Glis2*) and kept on the diet for additional 10 weeks.

(D) Expression (RNA-seq) of *Elf3* (left) and *Glis2* (right) in whole liver (n = 10-11).

(E) Hydroxyproline content in liver sections (n = 10-11).

(F) F4/80 positive cells per mm² sectioned liver tissue (n = 7-8)

(G) Representative cleaved caspase-3 staining (upper), and F4/80 (red)/Hoechst (blue) staining of liver sections (lower). Black bar indicates 100 μ m and yellow bar indicates 50 μ m.

(H) Log2FC in expression (FPC miRNA-*Elf3* or FPC miRNA-*Glis2* vs FPC miR-NC) for NASH-induced ($P_{\text{adj}} < 0.01$, Log2FC > 1) genes annotated to the KEGG apoptosis pathway.

(I) Expression (RNA-seq) of selected apoptosis marker genes in whole liver (n = 10-11)

(J) Heatmap showing row-scaled expression values (RNA-seq) in whole livers of marker genes for a panel of inflammatory cells. pDC = plasmacytoid dendritic cells, cDC = conventional dendritic cells, Monoc. = monocytes, KC = kupffer cells.

(A, C-F, I) Every dot/square/triangle represents one individual mouse. Bars indicate mean \pm SEM.

(B) The circle indicates odds ratio with 95% confidence interval. Significance was determined by (A, D-F, I) one-way ANOVA with Dunnett's multiple comparison test between (A) different hepatocyte subtypes, (D-F, I) FPC-fed mice injected with miR-NC and the other conditions, (B) Fisher's exact test and indicated by the odds ratio, (C) two-tailed, unpaired t-test or (H) one-sample Wilcoxon signed rank test ($\mu = 0$). * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$ or exact p value, ns = non-significant.

Figure S6

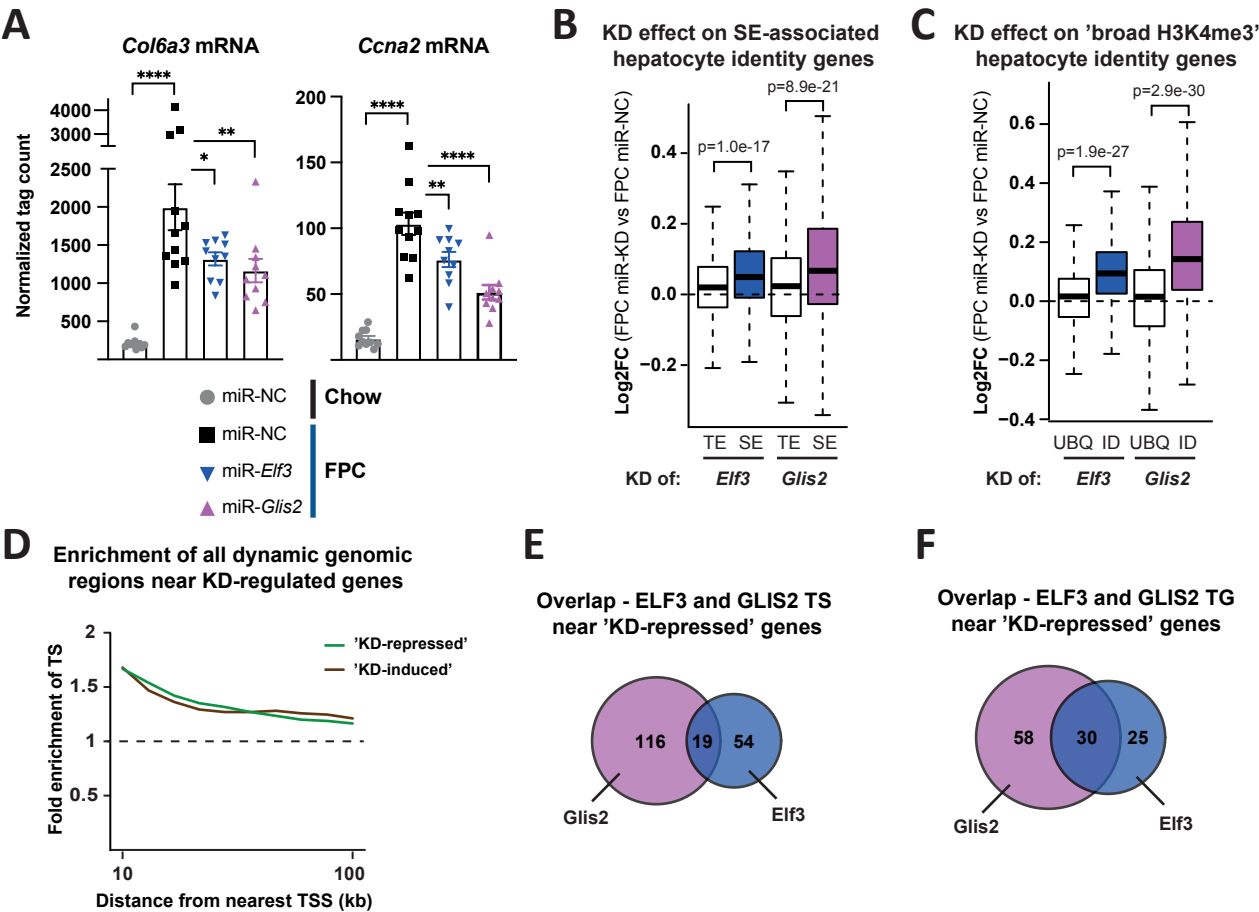


Figure S6. Regulation of NASH-activated hepatocyte gene programs by ELF3 and GLIS2. Related to Figure 6.

Mice were fed a control or an FPC diet for 14 weeks, injected with AAVs expressing non-coding miRNAs (miR-NC) or miRNA against *Elf3* (miR-*Elf3*) or *Glis2* (miR-*Glis2*) and kept on the diet for additional 10 weeks.

(A) Expression (RNA-seq) of *Col6a3* (left) and *Ccna2* (right) from the indicated RNA-seq clusters in whole liver (n = 10-11).

(B-C) Log2FC in expression (RNA-seq, FPC miRNA-*Elf3* or FPC miRNA-*Glis2* vs FPC miR_NC) for (B) genes associated with a typical enhancer or a superenhancer (SE) identified in control mice or (C) genes associated with broad H3K4me3 domains specific to the liver (HEP) or in ubiquitous (UBQ) broad H3K4me3 domains.

(D) Enrichment of all dynamic ATAC-sites in the vicinity of FPC-induced, ‘knock down (KD)-repressed’ hepatocyte genes (green) or FPC-repressed, ‘KD-induced’ hepatocyte genes (brown). Enrichment was determined as the number of target sites (TS) per gene within different distances from the transcription start site (TSS; 10–100 kb) of regulated genes relative to the number of binding sites per gene of all hepatocyte genes.

(E-F) Overlap between predicted regulatory ELF3 and GLIS2 (E) target sites and (F) target genes in the vicinity of FPC-induced, ‘KD-repressed’ hepatocyte genes.

(A) Every dot/square/triangle represents one individual mouse. Bars indicate mean \pm SEM. (B-C) Horizontal line indicates the median and whiskers indicate 1.5x IQR. Significance was determined by (A) one-way ANOVA with Dunnett’s multiple comparison test between FPC miR-NC mice and the other conditions or (B-C) two-sided Wilcoxon–Mann–Whitney test between indicated conditions.

*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 or exact p value.

Figure S7

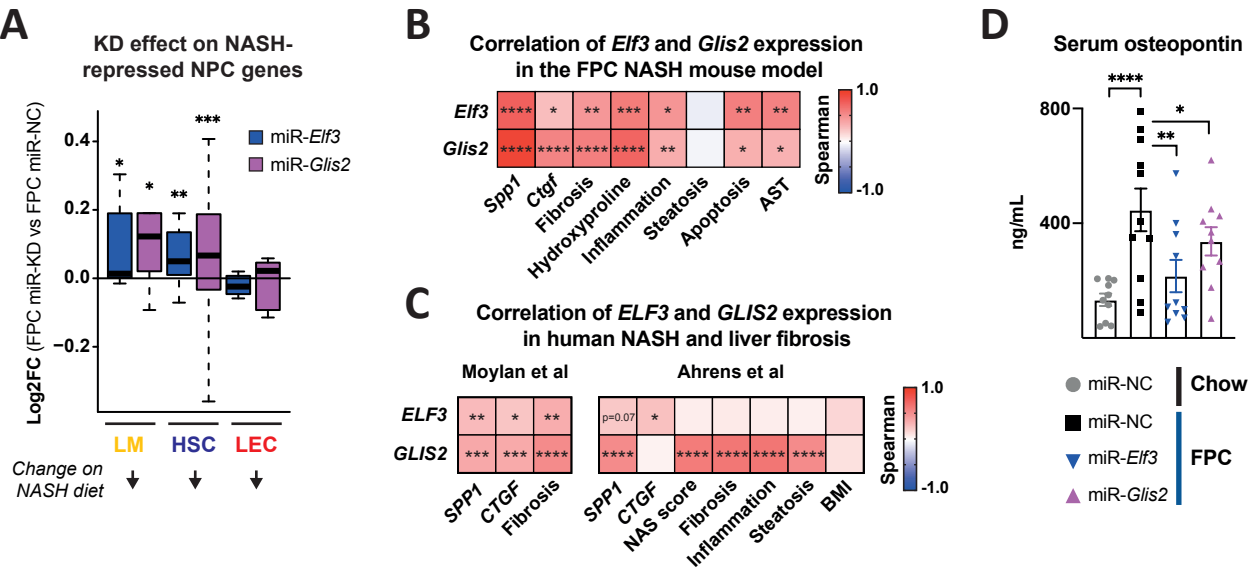


Figure S7. Modulation of NASH-activated HSC gene programs by ELF3 and GLIS2. Related to Figure 7.

(A) Log2FC in expression (RNA-seq, FPC miRNA-*Elf3* or FPC miRNA-*Glis2* vs FPC miR-NC) for NASH-repressed genes in liver macrophages (LM), hepatic stellate cells (HSC), and liver endothelial cells (LEC).

(B) Heatmap showing the Spearman correlation coefficient between *Elf3* or *Glis2* liver expression (RNA-seq) and the indicated parameters; *Spp1* and *Ctgf* liver expression (RNA-seq), fibrosis (Sirius red area in liver sections), hydroxyproline (hydroxyproline content in liver), inflammation (number of inflammatory foci/field in liver sections), steatosis (% fat area in liver sections), apoptosis (number of cleaved caspase-3 positive cells/field in liver sections), and AST (plasma activity of aspartate aminotransferase) in mice fed an FPC diet.

(C) Heatmap showing the Spearman correlation coefficient between *ELF3* or *GLIS2* expression (microarray) and the indicated parameters; *SPPI* and *CTGF* expression (microarray), NAS score, fibrosis score, inflammation score, steatosis (% liver fat area), BMI (body mass index, kg/m²) in two human cohorts of NASH and liver fibrosis.

(D) Serum osteopontin levels (n = 10-11).

(A) Horizontal line indicates the median and whiskers indicate 1.5x IQR. (D) Every dot represents one individual mouse. Colored bars indicate mean \pm SEM. Significance was determined by (A) one-sample Wilcoxon signed rank test ($\mu = 0$), (B-C) one-tailed *P* value or (D) one-way ANOVA with FDR-corrected multiple comparison test between FPC miR-NC mice and the other conditions. **p* < 0.05, ** *p* < 0.01, *** *p* < 0.001, **** *p* < 0.0001 or when FDR-corrected, **q* < 0.05, ** *q* < 0.01, **** *q* < 0.0001.

Table S1. Overview of anthropometric and blood/serum parameters in mice fed an FPC diet or a CDAHFD. Related to Figure 1.

	FPC-study				CDAHFD-study			
	Control	Mild	Adv	ANOVA	Control	Mild	Adv	ANOVA
Body weight [g]	37.13 ±1.97	36.33 ±1.51	33.47 ±0.95	0.4380	29.98 ±0.53	25.7 ±0.84 *	24.07 ±0.58	0.0026
Liver weight [g]	1.69 ± 0.04	3.30 ± 0.16 **	2.22 ± 0.36 #	0.0030	1.45 ± 0.03	1.85 ± 0.12 *	1.19 ± 0.05 ##	0.0054
Epididymal WAT weight [mg]	421.3 ± 133.5	815 ± 30.4	455.3 ± 114.5	0.0800	409.8 ± 23.4	330.0 ± 36.7	246.3 ± 20.1 *	0.0352
Inguinal WAT weight [mg]	349.5 ± 99.3	342.7 ± 47.2	249.3 ± 50.1	0.7249	345.3 ± 31.9	215.0 ± 17.9 *	179.7 ± 6.73 **	0.0063
Blood glucose [mg/dL]	155.0 ± 9.55	199.3 ± 15.0 p=0.11	175.7 ± 8.5	0.1239	180.8 ± 6.8	125.3 ± 4.35 **	116 ± 7.65 **	0.0007
Serum albumin [g/L]	31.2 ± 0.44	31.86 ± 0.80	25.55 ± 0.95 **/##	0.0032	30.11 ± 0.56	34.97 ± 0.61 **	28.36 ± 0.27 ##	0.0002
Serum LDL [mg/dL]	15.83 ± 0.89	51.6 ± 5.02 ***	41.33 ± 2.98 **	0.0006	28.7 ± 0.99	15.48 ± 1.16 **	22.3 ± 2.25	0.0023
Serum HDL [mg/dL]	69.03 ± 2.68	122.8 ± 5.14 ***	52.79 ± 5.54 ####	<0.0001	95.11 ± 2.30	48.66 ± 3.57 ****	24.75 ± 7.75 ****/###	<0.0001
Serum triglycerid [mg/dL]	167.25 ± 3.41	81.5 ± 9.5 ***	91.67 ± 7.9 ***	<0.0001	156 ± 16.33	107.5 ± 5.48 p=0.06	98.67 ± 5.54 *	<0.0001
Serum cholesterol [mg/dL]	103.75 ± 3.88	192.75 ± 11.88 ***	103.0 ± 3.17 ###	0.0002	140.5 ± 3.01	70 ± 4.39 ****	44.67 ± 2.36 ****/##	<0.0001

Data displayed are mean ± SEM (n = 3-4). Statistical significance was determined using a one-way ANOVA with post-hoc Tukey's multiple comparison test between conditions for each individual diet and indicated by *p < 0.01, **p < 0.01, ***p < 0.001, ****p < 0.0001 for mild (Mild) or advanced NASH (Adv) compared to control or #p < 0.01, ##p < 0.01, ###p < 0.001, ####p < 0.0001 for advanced NASH compared to mild NASH.

Table S4. Overview of anthropometric and blood/plasma parameters in mice fed an FPC diet with hepatocyte specific Glis2 or Elf3 knock down. Related to Figure 5.

	Chow	FPC			
	miR-NC	miR-NC	miR-Elf3	miR-Glis2	ANOVA
Body weight [g]	39.81 ± 1.23	41.35 ± 0.7	37.59 ± 1.01 *	39.56 ± 0.39	0.0435
Liver weight [g]	1.74 ± 0.06 ****	4.23 ± 0.29	4.05 ± 0.21	3.81 ± 0.13	<0.0001
Epididymal WAT weight [mg]	893.0 ± 64.6	1047.7 ± 35.3	864.15 ± 43.8 *	984.5 ± 41.44	0.0478
Inguinal WAT weight [mg]	670.3 ± 64.4	570.0 ± 23.7	451.9 ± 30.8	515.0 ± 29.7	0.0071
Blood glucose [mg/dL]	183.2 ± 10.7	171.36 ± 6.30	176 ± 9.78	176.8 ± 9.78	0.8449
Plasma AST [fold increase 23 vs 14 wks]	1.31 ± 0.02 **	2.04 ± 0.107	2.05 ± 0.061	1.66 ± 0.128 *	0.0005

Data displayed are mean ± SEM (n = 9-11). Statistical significance was determined using one-way ANOVA with post-hoc Dunnett's multiple comparison test between the mice fed an FPC diet and injected with control virus (miR-NC) and the other conditions and indicated by *p < 0.01, ****p < 0.0001.

Table S5. Overview of oligonucleotides. Related to STAR Methods.

qPCR primers

<i>Name</i>	<i>Sequence (5'→3')</i>
Coll1a1_F	CCTCAGGGTATTGCTGGACAAC
Coll1a1_R	CAGAAGGACCTTGTTTGCCAGG
Tnf_F	TCATCTTTCAAAATTCGAGTGACA
Tnf_R	GTTTGCTACGACGTGGGCTAC
Acta2_F	TGCTGACAGAGGCACCACTGAA
Acta2_R	CAGTTGTACGTCCAGAGGCATAG
Adgre1_F	TCATTCCAGCTCTGGCCCTTG
Adgre1_R	GCGCTAGTGCCCAAAACAAA
Elf3_F	TCCTCCGACTACCTTTGGCACT
Elf3_R	ACTCCAGAACCTGGGTCTTCGA
Glis2_F	GGAGAACCTGAAGATCCACAACC
Glis2_R	TGCTTGAAGCGGTCACCTGGAGT
Tbp_F	CTACCGTGAATCTTGGCTGTAAAC
Tbp_R	AATCAACGCAGTTGTCCGTGGC

miRNA targeting oligos

<i>Name</i>	<i>Sequence (5'→3')</i>
Elf3_F	TGCTGAATACCTCATGGCTCGGCTCAGTTTTGGCCACTGACTGACTGAGCCGACATGAGGTATT
Elf3_R	CCTGAATACCTCATGTCCGGCTCAGTCAGTCAGTGGCCAAAACCTGAGCCGAGCCATGAGGTATTC
Glis2_F	TGCTGATGCAAAGCATGATGCCGGACGTTTTGGCCACTGACTGACGTCCGGCAATGCTTTGCAT
Glis2_R	CCTGATGCAAAGCATTGCCGGACGTCAGTCAGTGGCCAAAACGTCCGGCATCATGCTTTGCATC