Fig. S1

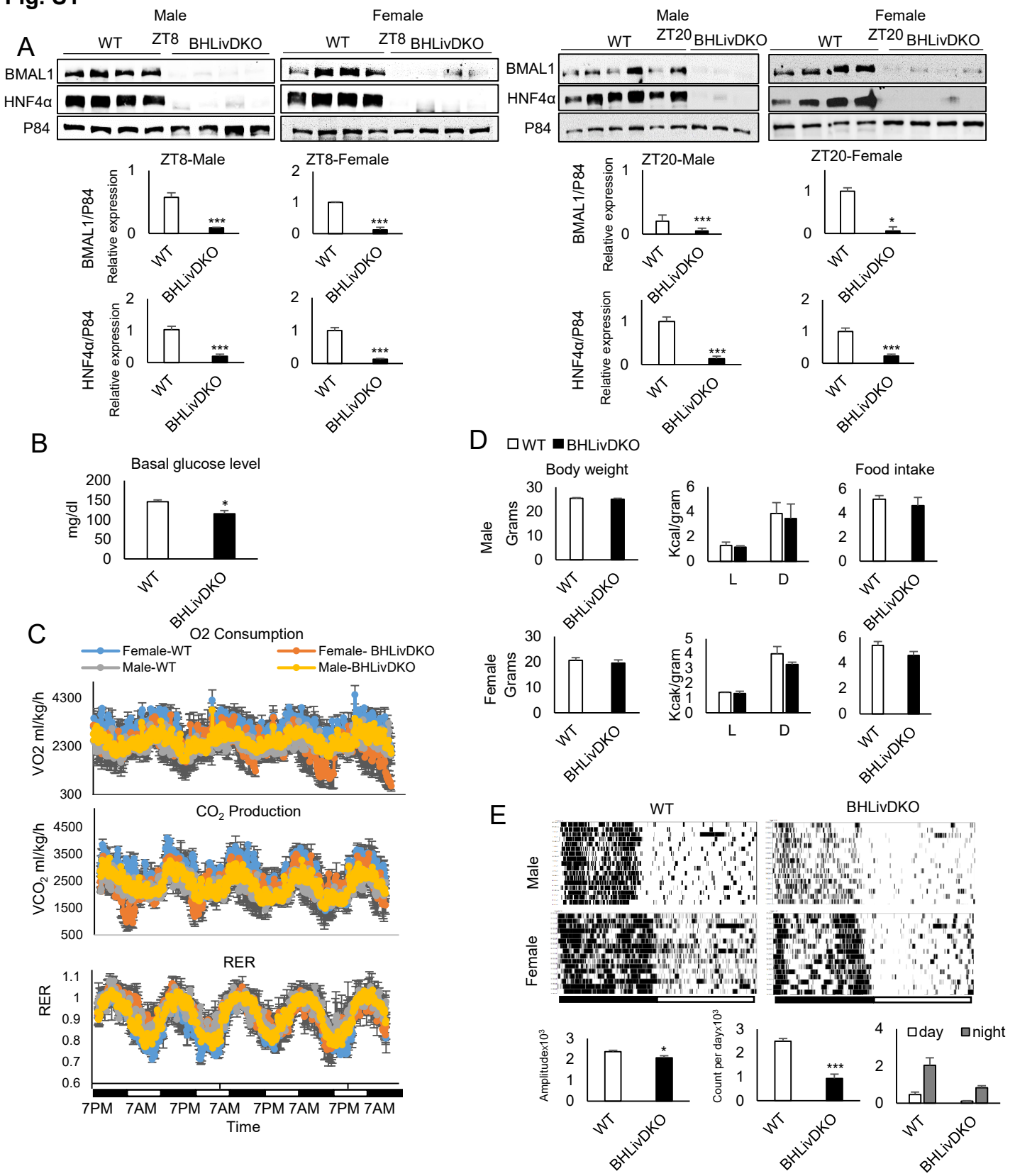

Fig S1. Loss of BMAL1 and HNF4α in the liver does not alter diurnal energy expenditure A. Western blot of whole-cell lysates from WT and BHLivDKO livers harvested at ZT8 (left panels) or ZT20 (right panels) reveals total HNF4α, BMAL1, and p84, 10 days following the first tamoxifen injection in male and female mice. Quantification of the immunoblots normalized to P84 (bottom panels). Student's *t*-test. $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$. **B.** Basal blood glucose levels in WT and BHLivDKO mice. **C.** Oxygen consumption, carbon dioxide emission, and Respiratory Exchange Ratio of male and female WT and BHLivDKO mice as measured by indirect calorimetry. **D.** Food intake patterns for all WT and BHLivDKO mice during the 24-hour cycle including the light/resting (L) and dark/active (D) phases. **E.** Actograms reveal the home cage activity of WT and BHLivDKO animals (top panel). Quantification (bottom panels). $^{*}P < 0.05$, $^{****}P < 0.0001$ was determined by Mann-Whitney U-test (N = 6-8).

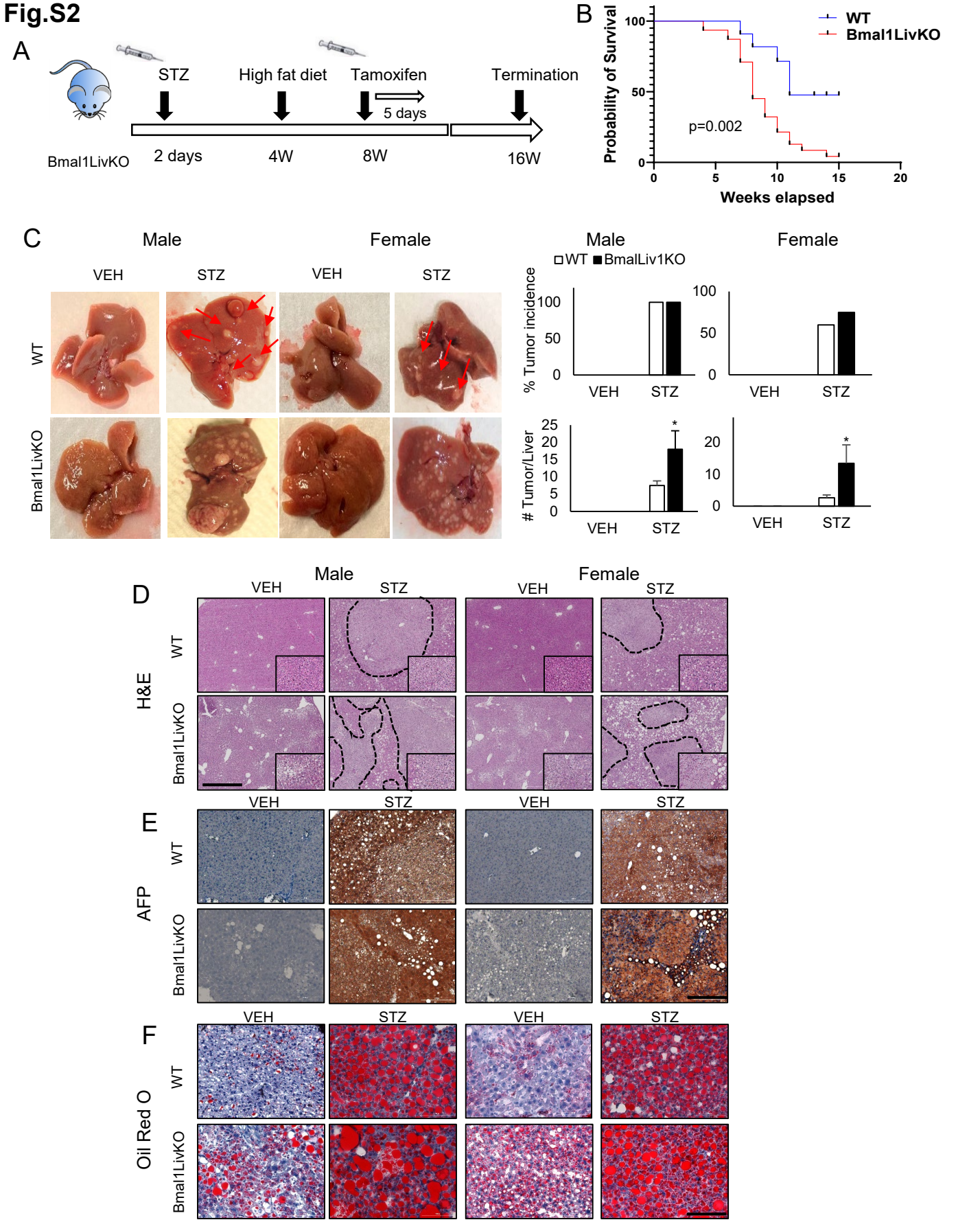

Fig S2. **Mice with hepatic BMAL1 deficiency are more likely to develop hepatocellular carcinoma with a poor survival rate. A.** Experimental timeline for STAM model of HCC in WT and Bmal1LivKO mice. **B.** Kaplan-Meier survival analysis for the WT and Bmal1LivKO mice treated with the streptozotocin (STZ). **C.** Whole livers were taken from mice treated with VEH or STZ followed by HFD, left panel. Percent tumor incidence and the number of tumors per liver per animal group (right panel). **D, E, F.** Staining of livers for H&E, AFP, and Oil Red O respectively (scale bar, 200 μm) (*N* = 3-6).

Fig. S3

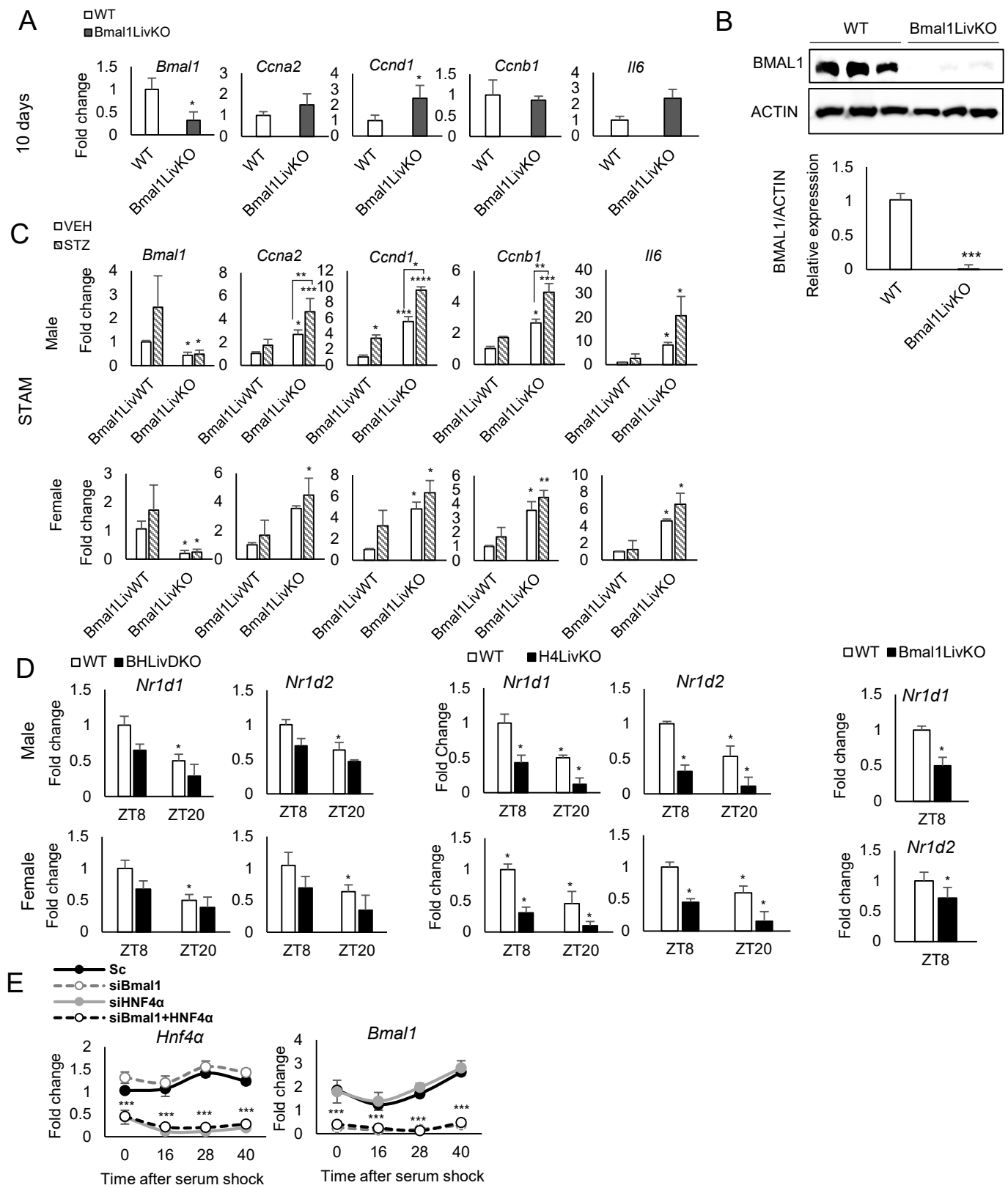

Fig S3. **Loss of hepatic BMAL1 in the STAM model of HCC significantly induce cyclin gene expression. A.** *Bmal1*, Cyclins, and *Il6* gene expression in Bmal1LivKO 10 days post tamoxifen treatment. **B.** Western blot of BMAL1 and ACTIN protein post tamoxifen treatment. **C.** Expression of *Bmal1*, Cyclins, and *Il6* at mRNA level in Bmal1LivKO liver post-VEH/STZ injection in STAM model. two-way ANOVA, Sidak's multiple comparisons test (N = 3-6): *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$. **D.** *Nr1d1* and *Nr1d1* gene expression in the liver of BHLivdKO, H4LivKO and Bmal1LivKO, and their WT. **E.** qPCR reveals expression of Hnf4α, and Bmal1 following siRNA or scrambled control.Two-way ANOVA, Sidak's multiple comparisons test (N = 6-10). ***, $P < 0.0005$.

Fig.S4

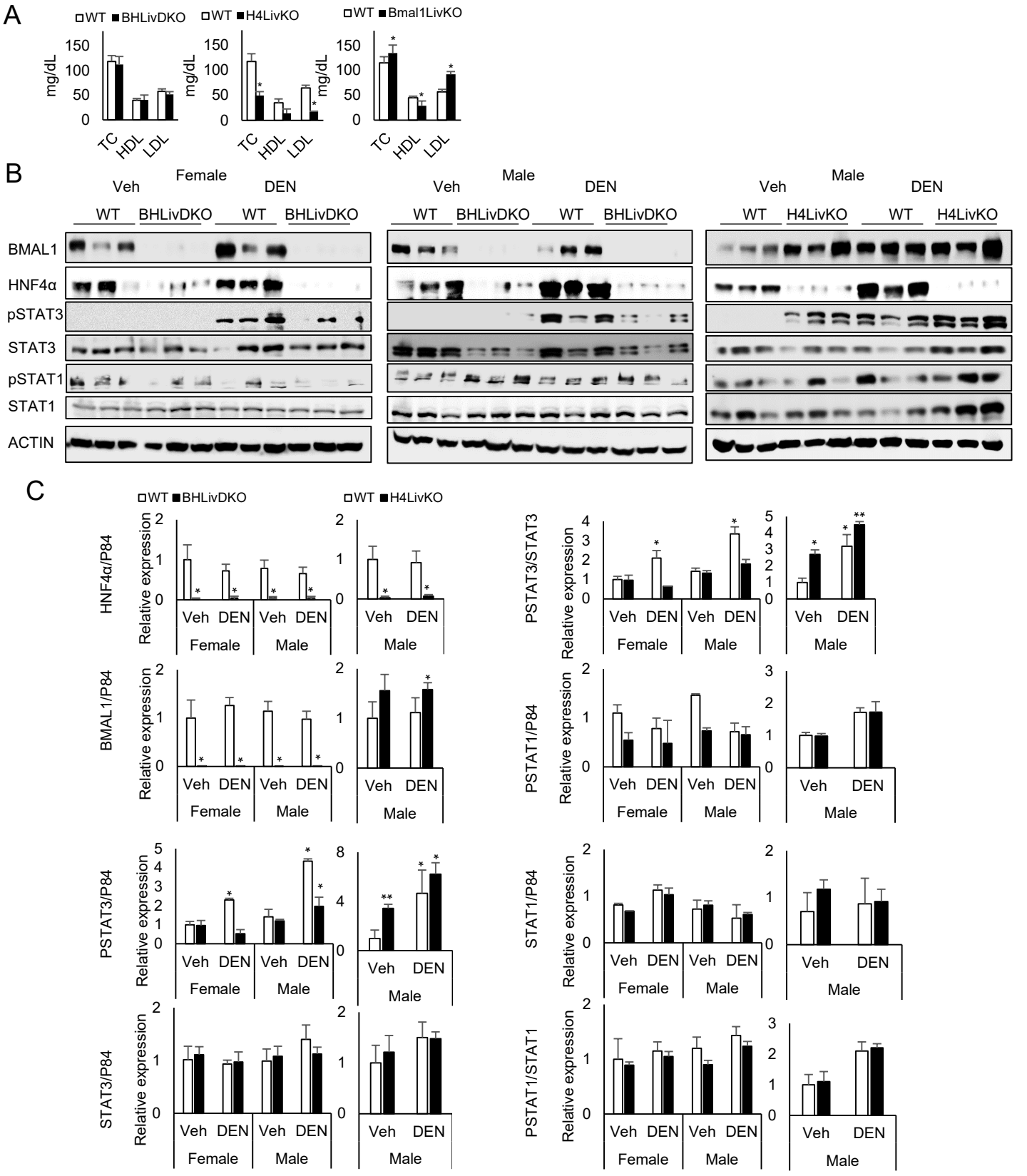

Fig S4. Loss of BMAL1 and HNF4α effects on IL-6 AND STAT3 signaling pathway. A. Serum total cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) levels in WT, BHLivDKO, H4LivKO, and Bmal1LivKO mice. Significance ($P < 0.05$) was determined by Mann-Whitney U-test (N = 4-5). **B**. Western blot of BMAL1, HNF4α, STAT1, STAT3, total STAT proteins, and ACTIN in the liver of BHLivDKo and H4LivKO mice post-DEN or VEH treatment following HFD (N = 3). **C.** Quantification of the HNF4α, BMAL1, STAT1, STAT3, and total STAT protein normalized to P84 or total STAT protein. Student's t-test. *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$. Quantification corresponds to the Western blot presented, technical replicates in Fig. 3E.

Fig.S5

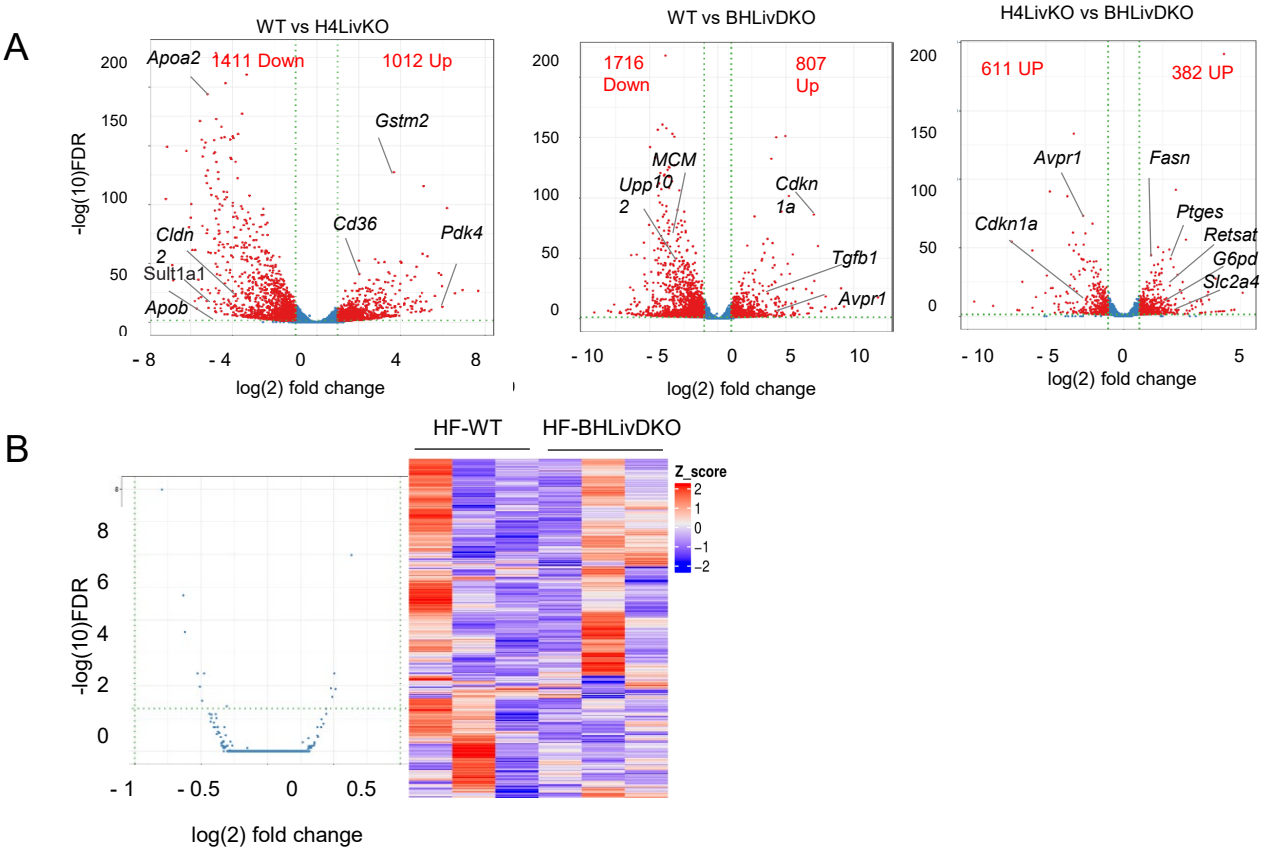

Fig S5. Distinct gene expression patterns in BHLivDKO livers compared to H4livKO livers. A. Volcano plots of all genes; the vertical dotted lines indicate the $|\log_2$ (fold change)| = 1; the horizontal dotted line denotes the $-\log_{10}$(FDR) = 1.3; red and blue dots represent the differentially expressed genes (DEGs) and the non-DEGs, respectively; key genes were highlighted in black; the number of upregulated and downregulated DEGs were shown in the top-left and top-right corners, respectively; WT vs. H4LivKO (top left panel), WT vs. BHLivDKO (top right panel) and H4LivKO vs. BHLivDKO (bottom panel) reveals differential gene expression across genotypes (*N = 3*). **B.** Volcano plot of all genes; the vertical dotted lines denote the $|\log_2$ (fold change) | = 1, the horizontal dotted line indicates the $-\log_{10}$(FDR) = 1.3; blue dots represent the non-DEGs (left panel), and heat map (right panel) of the relative expression of top 5 percent most variable genes in WT vs. BHLivDKO mice with fed HFD for 45 weeks.

Fig. S6

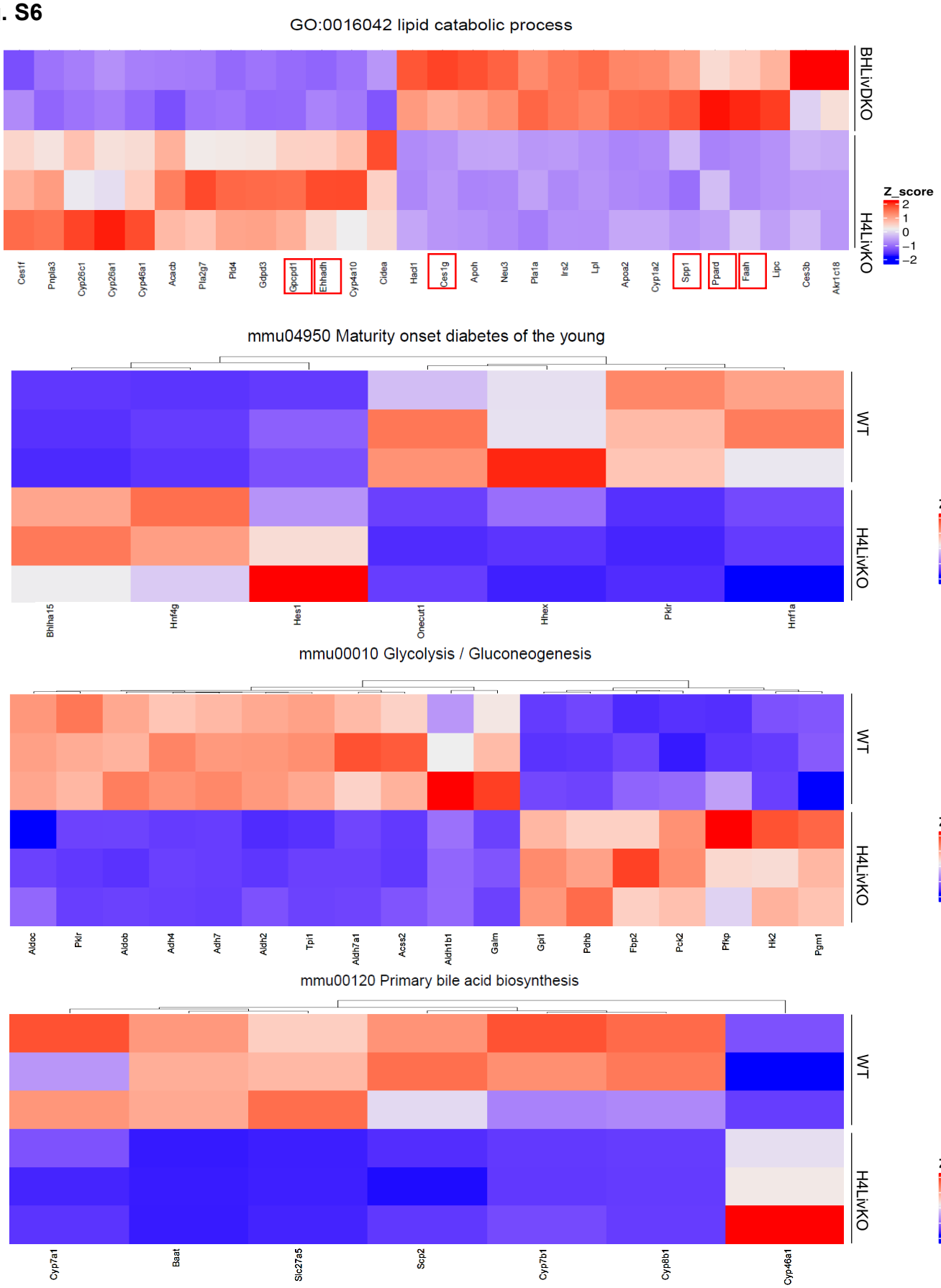

Fig S6. Heat maps showing differentially expressed genes in BHLivDKO vs. H4LivKO and in WT vs.H4LivKO

Fig. S7

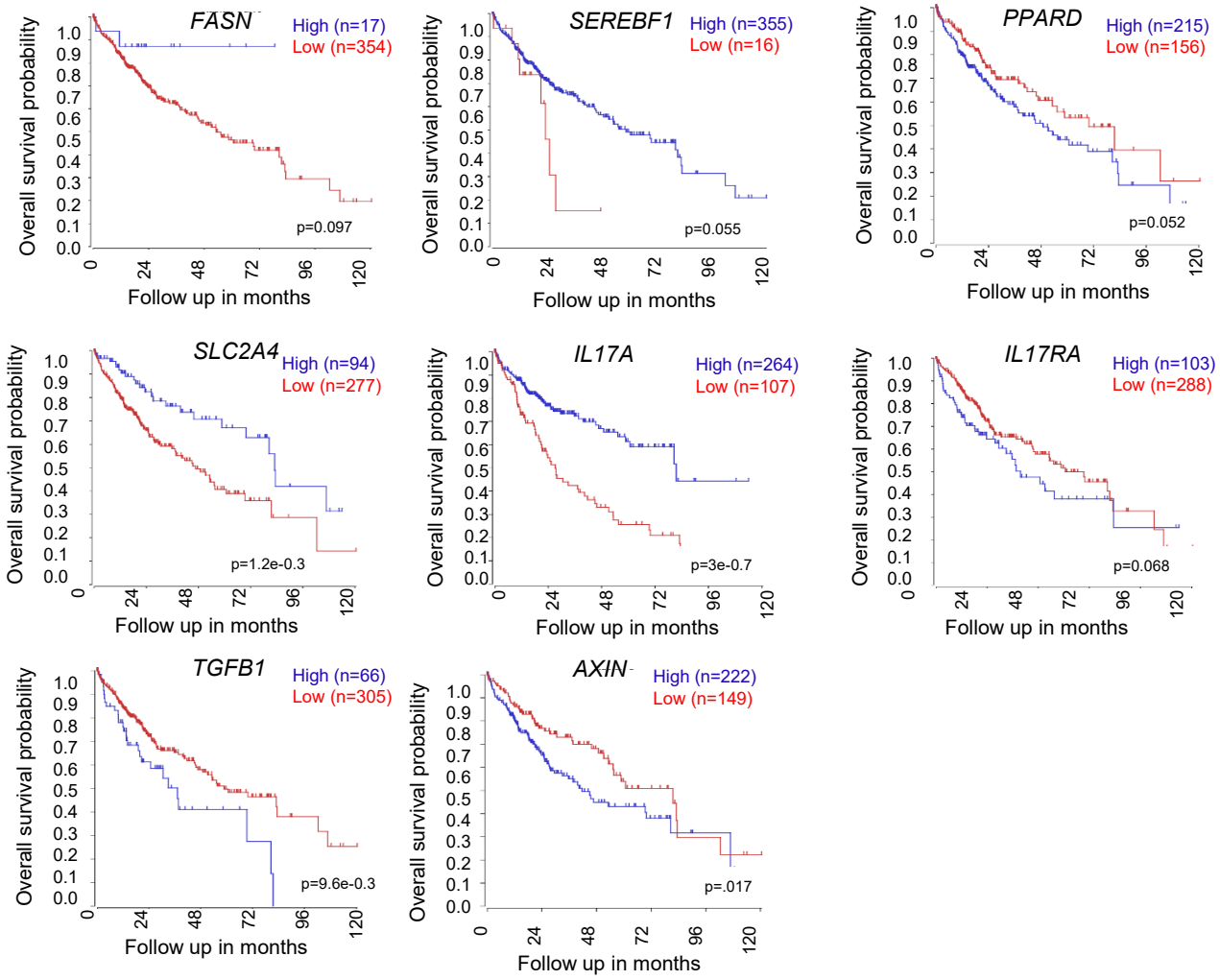

Fig S7. Kaplan-Meier survival curve for additional genes. Survival curves show *FASN*, *SEREBF1, PPARD, SLC2A4, IL17A, IL17RA, TGFB1, and AXIN* genes expression using the tumor liver hepatocellular carcinoma (TCGA) LIHC dataset using the R2 Genomics Analysis and Visualization Platform (http://r2.amc.nl). Survival time is measured from the time of initial diagnosis to the date of death or the date of the last follow-up. The survival distribution is estimated by the Kaplan-Meier method. $P < 0.05$ were considered to be statistically significant (N = 371, 250 Male and 121 Female).

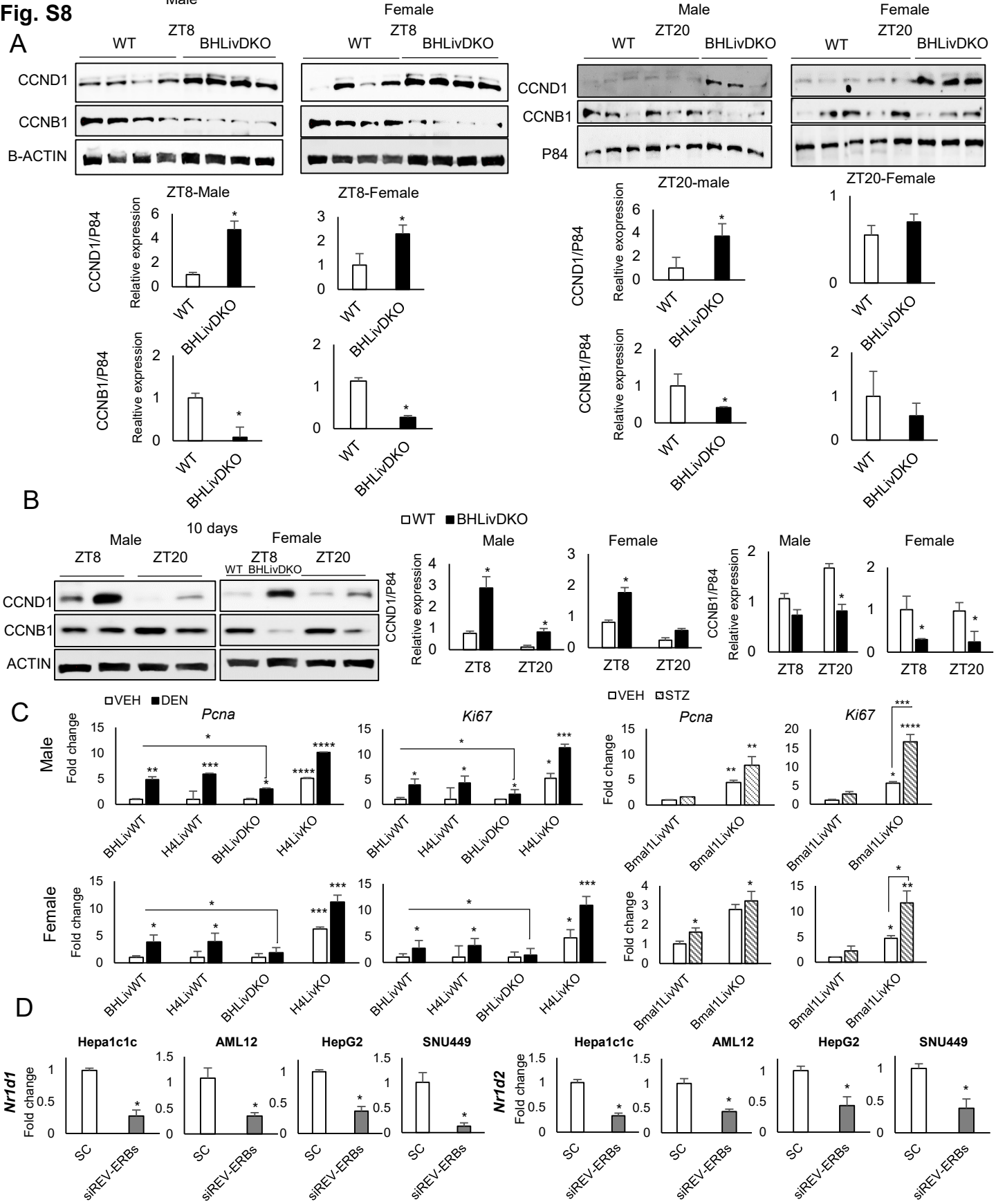

Fig S8. Alterations in cyclin D1 (CCND1) and cyclin B1 (CCNB1) in BHLivDKO and WT livers. A. Western blot of liver lysates from WT and BHLivDKO reveals total CCND1, CCNB1, P84, and ACTIN proteins 10 days following the first tamoxifen injection in the male and female individual animals at 2 *Zeitgeber* times (ZT8 and ZT20)(left panels). Quantification of the immunoblots normalized to P84 or ACTIN (right panels). Student's *t*-test. **P* ≤ 0.05. **B.** Expression of CCND1 and CCNB1 in pooled samples from each time point and sex (left panels). Quantification of the immunoblots normalized to ACTIN (right panels). Student's *t*-test. **P* ≤ 0.05. **C.** *Pcna* and *Ki67* gene expression in the liver of animals treated with DEN/VEH or STZ/VEH following HFD. **D.** REV-ERBα and β (*Nr1d1* and *Nr1d2*) gene expression to confirm siRNA knockdown in Hpa1c1c, AML12, HepG2, and SNU449 cell lines, Student's *t*-test, **P* ≤ 0.05.

Table S1: Liver evaluation

Sample ID	Treatment	fat score	ballooning	Mallory	lobular inflammation	Nodule size	HCC?
Male-WT	DEN	2	1	0	1	2.5 mm	yes
	DEN	1	1	0	0	6 mm	yes
	DEN	2	1	0	1	1.5mm	yes
	DEN	2	0	0	1	2 mm and 3 mm	yes
	DEN	2	1	0	1	2 mm	yes
	DEN	1	0	0	0	3 mm	yes
	DEN	1	0	0	1	1 mm x2	yes
Male-BH livDKO	DEN	1	0	0	0	0	0
	DEN	1	1	0	0	0	0
	DEN	0	0	0	0	0	0
	DEN	1	1	0	0	2 mm	yes
	DEN	0	0	0	0	1.5mm	yes
	DEN	1	1	0	1	0	0
	DEN	2	1	0	1	2 mm	yes
	DEN	1	2	yes	1	0	0
Male-WT	VEH	1	0	0	1	0	0
	VEH	0	0	0	0	0	0
	VEH	1	0	0	0	0	0
	VEH	1	0	0	1	0	0
	VEH	1	0	0	0	0	0
	VEH	1	0	0	1	0	0
Male-BHLiv DKO	VEH	1	0	0	0	0	0
	VEH	1	0	0	1	0	0
	VEH	1	0	0	0	0	0
	VEH	0	0	0	1	0	0
	VEH	1	0	0	1	0	0
Male-WT	DEN	1	1	0	0	2 mm	yes
	DEN	2	1	0	1	2.5 mm	yes
	DEN	1	0	0	0	3 mm	yes
Male-H4LivKO	DEN	3	2	yes	1	5 mm	yes
	DEN	2	1	0	2	5 mm	yes
	DEN	2	1	0	1	5 mm	yes
Female-WT	DEN	1	0	0	0	1 mm	yes
	DEN	1	1	0	1	3 mm	yes
	DEN	1	0	0	0	1 mm	yes
	DEN	0	0	0	1	0	0
	DEN	1	0	0	1	0	0
	DEN	0	0	0	0	0	0
	DEN	0	0	0	0	0	0
	DEN	0	0	0	0	0	0
	DEN	3	2	yes	1	5.5 mm and 1.5?	yes
Female-DKO	DEN	1	0	0	1	0	0
	DEN	1	0	0	1	0	0
	DEN	1	0	1	0	0	0
	DEN	1	1	0	1	0	?
	DEN	3	2	yes	2	1 mm	yes
	DEN	1	0	0	1	0	0
Female-WT	VEH	1	1	0	1	0	0
	VEH	1	1	0	1	0	0
	VEH	1	0	0	1	0	0
	VEH	0	0	0	0	0	0
	VEH	1	0	0	1	0	0
	VEH	1	0	0	0	0	0
	VEH	1	0	0	1	0	0
	VEH	1	0	0	1	0	0
	VEH	1	0	0	1	0	0
Female-DKO	VEH	1	0	0	1	0	0
	VEH	1	0	0	0	0	0
	VEH	0	0	0	0	0	0
	VEH	1	1	0	1	0	0
	VEH	1	1	0	0	0	0
	VEH	2	1	yes	1	0	0
	VEH	1	0	0	1	0	0
	VEH	1	0	0	1	0	0

Table S2: Antibodies list

Name	Catalog # and Company
P1/P2-HNF4α	#PP-H1415-00 R&D Systems
BMAL1	#93806 Abcam
CCND1	#EPR2241 Abcam
CCNB1	#SC-245 Santa Cruz biotechnology
ACTIN	# A5441 Sigma
P84	# GTX70220 GeneTex
STAT1	#9172S Cell signaling
PSTAT1	#9167S Cell signaling
STAT3	#9139S Cell signaling
PSTAT3	#9145S Cell signaling
AFP	#ab46799, Abcam
Rabbit specific HRP/DAB detection IHC kit	#ab64261, Abcam
Anti-Mouse-HRP	#1706516 BioRad
Anti-Rabbit-HRP	#1791019 BioRad

Table S3: Primers list

Name	Sequences(5`to 3`) Mouse
P1/P2 HNF4α F	ACCAAGAGGTCCATGGTGTTT
P1/P2 HNF4α R	GTGCCGAGGGACGATGTAG
P1HNF4α F	CATGGATATGGCCGACTACAG
P1HNF4α R	GCCCGAATGTCGCCATTGATCCCAGAGA
P2HNF4A F	GGTACCCTTGGTCATGGTCAGT
P2HNF4α R	TGGATGAATTGAGGTTGGCAC
Bmal1 F	GCAGTGCCACTGACTACCAAGA
Bmal1 R	TCCTGGACATTGCATTGCAT
DBP F	AATGACCTTTGAACCTGATCCCGCT
DBP R	GCTCCAGTACTTCTCATCCTTCTGT
Per2 F	CGCCTAGAATCCCTCCTGAGA
Per2 R	CCACCGGCCTGTAGGATCT
Pdk4 F	GAGCTGGTATATCCAGAGCCTGAT
Pdk4 R	CGAACTTTGACCAGCGTGTCT
CyclinD1 F	TGCTACCGCACAACGCA
CyclinD1 R	TCAATCTGTTCCTGGCAGGC
Cyclin B1 F	GGAAATTCTTGACAACGGTG
Cyclin B1 R	TGCCTTTGTCACGGCCTTAG
Cdh1 F	CAAAGTGACGCTGAAGTCCA
Cdh1 R	TGATGACACGGCATGAGAAT
Snail1 F	CACCCTCATCTGGGACTCTC
Snail1 R	CTTCACATCCGAGTGGGTTT
Ctnnb1 F	ACAAACTGTTTTGAAAATCCA
Ctnnb1 R	CGAGTCATTGCATACTGTCC
IL17ra F	TAGTGTTTCCTCTACCCAGCACG
IL17ra R	AGCCGCTCATTGGTGTTCAG
IL17a F	CAGACTACCTCAACCGTTCCAC
IL17a R	TCCAGCTTTCCCTCCGCATTGA
Tgfb1F	AGCCCGAAGCGGACTACTAT
Tgfb1R	TTCCACATGTTGCTCCACAC
PEG10-F	CCCCGGGCGCTGGTGTTG
PEG10-R	AGCGGGGCCGGGGAGTTTC
GPC3 F	AGGTAGCTGCGAGGAAAC
GPC3 F	AGGTCACGTCTTGCTCCTC
R-Spondin2 F	ATCTGGGGCTCGGTGTCCATAATAC
R-Spondin2 R	GCGGGTGTCGGCAAACTTTTTC
Avpr1a F	CATCCTCTGCTGGACACCTTTC
Avpr1a R	TCAAGGAAGCCAGTAACGCCGT
Fasn F	CACAGTGCTCAAAGGACATGCC
Fasn R	CACCAGGTGTAGTGCCTTCCTC
Ppard F	GGACCAGAACACACGCTTCCTT
Ppard R	CCGACATTCCATGTTGAGGCTG
Cdkn1a F	TGTCCAATCCTGGTGATGT
Cdkn1a R	CAACTGCTCACTGTCCAC
Ptges F	GGAAAGACTGGGAGGATGACTC
Ptges R	TCATCTGCTCCATCTACTTCTGG
Srebp1 F	CGACTACATCCGCTTCTTGCAG
Srebp1 R	CCTCCATAGACACATCTGTGCC
Slc2a4 F	GGTGTGGTCAATACGGTCTTCAC
Slc2a4 R	AGCAGAGCCACGGTCATCAAGA
G6pd	CCGCATCATAGTGGAGAAACCC
G6pd	TGTCCAGGTAGTGGTCAATGCG
Axin2 F	CTCCTTGGAGGCAAGAGC
Axin2 R	GGCCACGCAGCACCGCTG
PTEN F	AATTCCCAGTCAGAGGCGCTATGT
PTEN R	GATTGCAAGTTCCGCCACTGAA CA
Ki67 F	GCCTTGTGAAGCAGAAGAGAAAAC
Ki67 R	CTGGACCTCAAACACCTAACATCAG
PCNA F	CTGGACCTCAAACACCTAACATCAG
PCNA R	GCAAACGTTAGGTGAACAGGCTC
18s F	CGCCGCTAGAGGTGAAATTC
18S R	CGAACCTCCGACTTTCGTTCT