SUPPLEMENTAL MATERIAL

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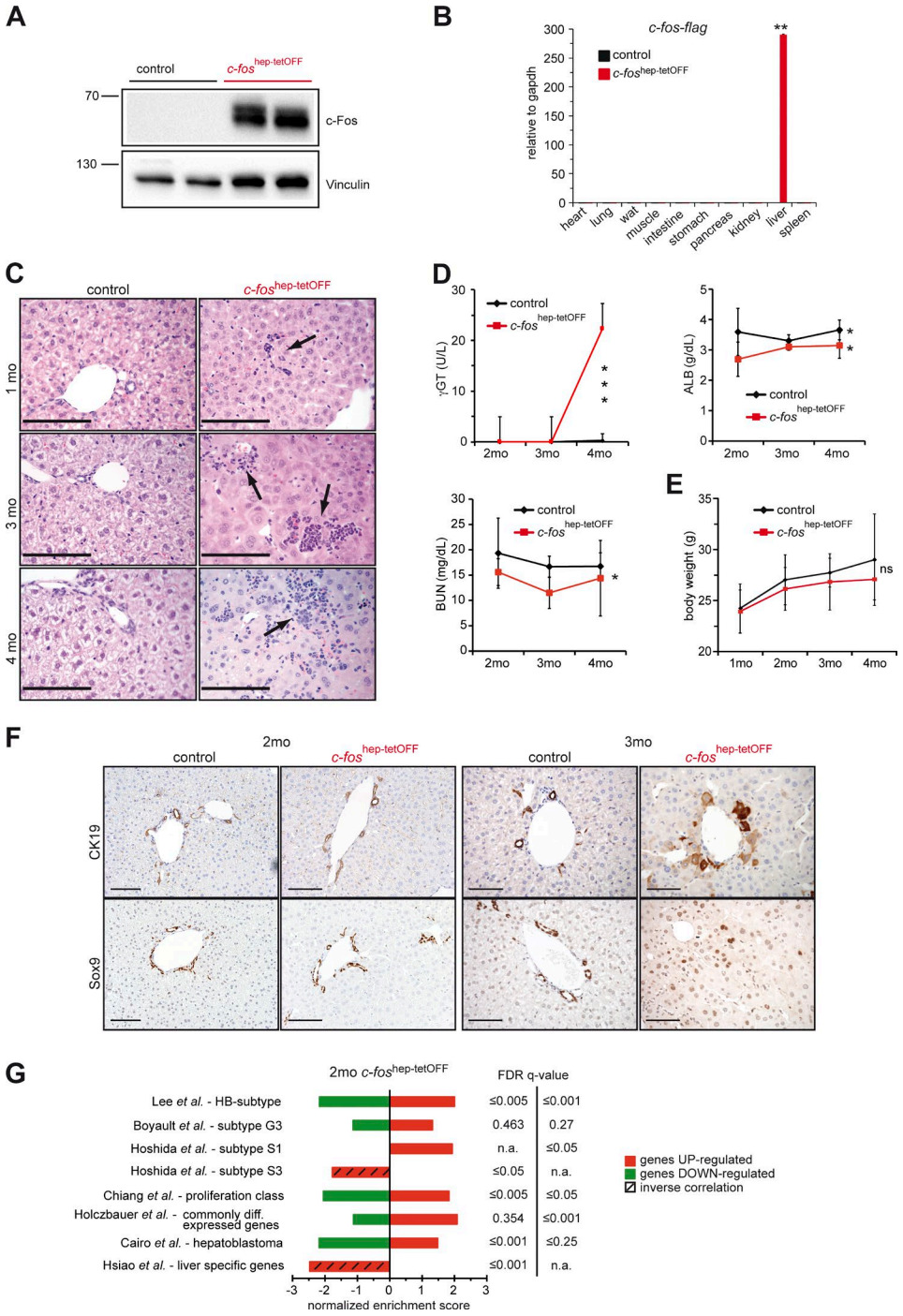

Figure S1. **Phenotypic consequences of hepatocyte-specific c-Fos expression.** (A) Representative c-Fos immunoblot in livers from *c-fos*$^{hep\text{-}tetOFF}$ and control mice 2 mo after doxycycline removal. Vinculin served as loading control. Molecular mass is indicated in kilodaltons. (B) qRT-PCR analyses of *c-fos-flag* in different tissues. Bar graphs represent mean ± SD relative to gapdh; $n = 2/3$; **, $P \leq 0.01$ by Student's *t* test. (C) Representative H&E in *c-fos*$^{hep\text{-}tetOFF}$ and control mice at the indicated time points after doxycycline removal. Bars, 100 µm. Arrows indicate necrotic foci. (D) Serum γ-glutamyl transferase (γGT), albumin (ALB), and blood urea nitrogen (BUN) at the indicated time points of doxycycline removal/c-Fos expression ($n = 13; 3; 9/11; 3; 6$). (E) Body weight ($n = 12; 14; 6; 15/10; 14; 7; 11$) at the indicated time points of doxycycline removal/c-Fos expression. Plots represent mean ± SD; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; ns, not significant by two-way ANOVA. (F) Representative IHC for CK19 and Sox9 at 2 and 3 mo of c-Fos expression. Bars, 100 µm. (G) Normalized enrichment scores at 2 mo of c-Fos expression relative to controls (RNA-seq, $n = 2$/cohort) compared with human HCC molecular classes and relevant gene signatures by GSEA. False discovery rate (FDR) *q*-values are indicated on the right side. n.a., not applicable, as the published gene signature was unidirectional (with only enriched genes). Hatched bars highlight inverse correlations computed with up-regulated (red) or down-regulated (green) gene sets.

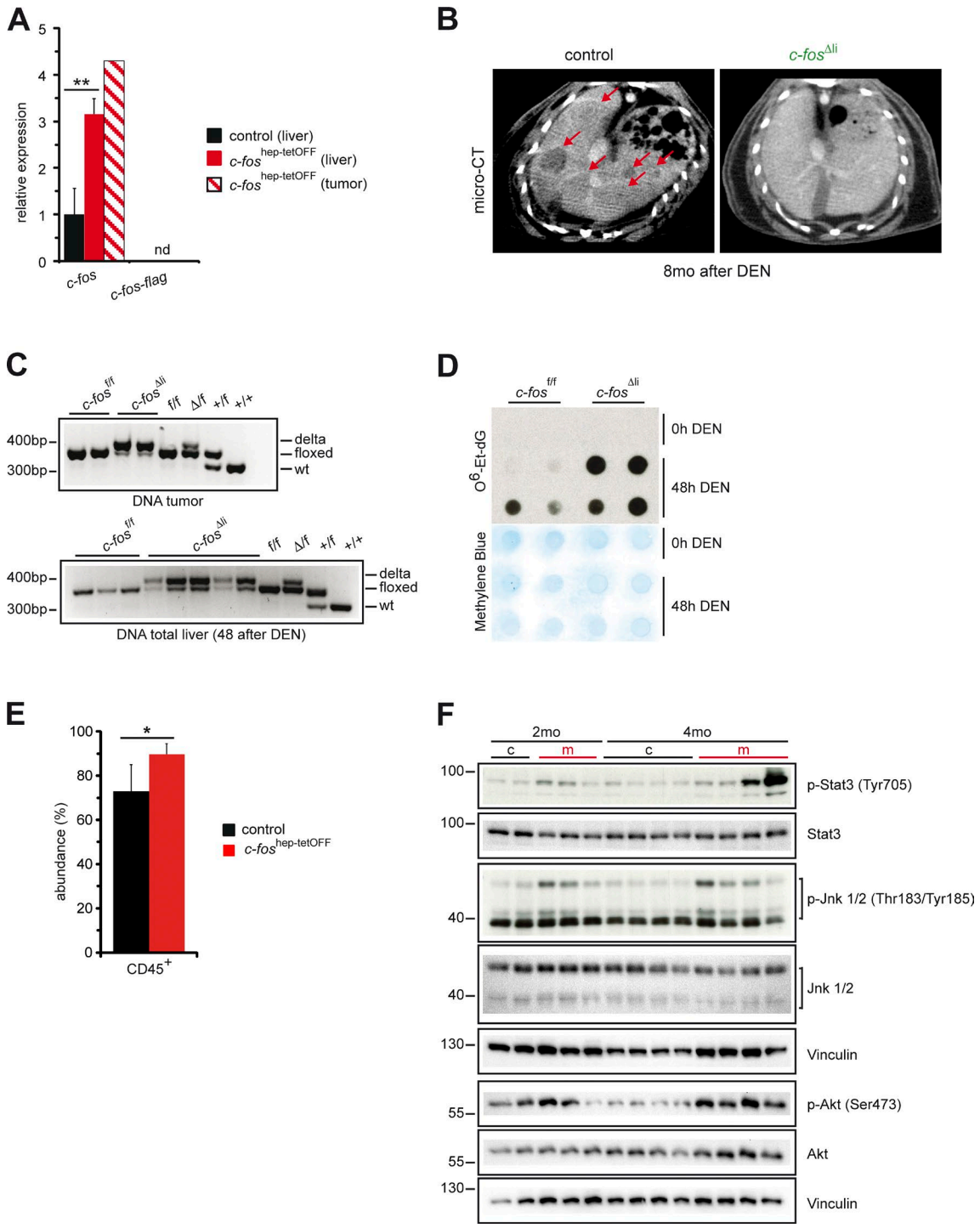

Figure S2. **c-Fos expression is essential for HCC development.** (A) qRT-PCR analyses (total *c-fos* and *c-fos-flag*) 7 mo after intraperitoneal DEN injection in mice with ectopic c-Fos expression between 3 and 9 wk and in control littermates. Bar graphs represent mean ± SD; $n = 4/3/1$; mean expression in controls set to 1; **, $P \leq 0.01$ by Student's *t* test. nd, not detected. (B) Representative micro-CT of one *c-fos*$^{\Delta li}$ mouse and its control littermate 8 mo after DEN injection. Arrows indicate liver tumors. (C) PCR analysis of genomic DNA from DEN-induced liver tumors (top) and total liver (bottom, 48 h after DEN injection) of *c-fos*$^{\Delta li}$ and *c-fos*$^{f/f}$ mice. The wild-type (wt), floxed (f), and deleted (Δ) alleles of *c-fos* are indicated. (D) Dot blot analysis of DEN-induced DNA adducts in liver DNA from *c-fos*$^{\Delta li}$ and control mice (without and 48 h after DEN) using an antibody against O^6-ethyl-2-deoxyguanosine (O6-Et-dG). Methylene blue staining served as loading control. (E) Flow cytometry analysis of isolated nonparenchymal liver cell fraction for CD45 positivity at 2 mo of ectopic c-Fos expression. Bar graphs represent mean ± SD; $n = 6$/cohort; *, $P \leq 0.05$ by Student's *t* test. (F) Western blot of total liver lysate of *c-fos*$^{hep\text{-}tetOFF}$ and control mice 2 and 4 mo after doxycycline removal. Vinculin served as loading control. Molecular mass is indicated in kilodaltons.

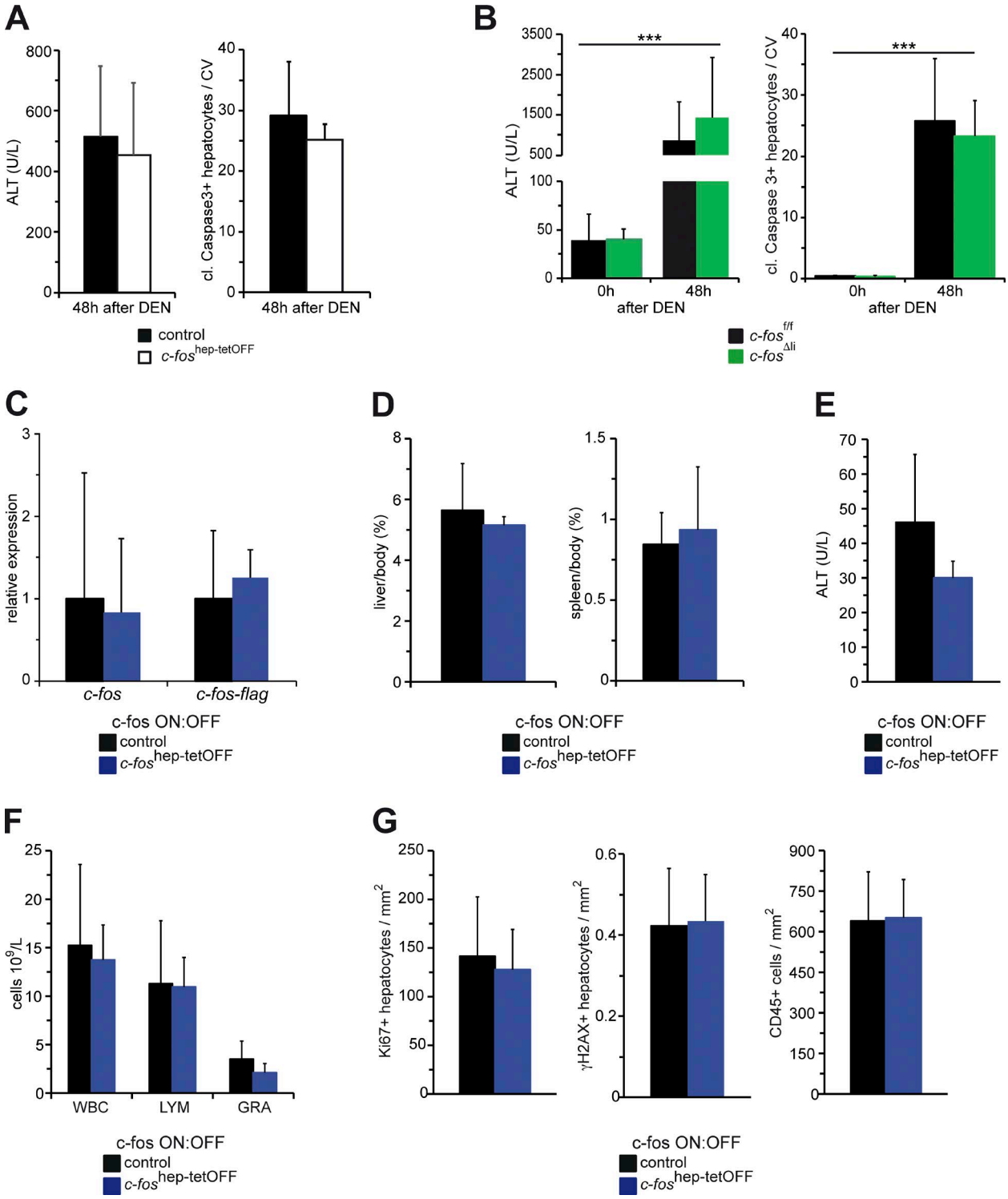

Figure S3. **DEN-induced events and c-Fos phenotype reversibility.** (A) Serum ALT (n = 5/5) and quantification of cleaved caspase 3–positive cells around the central vein (CV; n = 5/5) in liver sections from 8-wk-old *c-fos*$^{hep\text{-}tetOFF}$ and control mice 48 h after DEN. Plots represent mean ± SD. (B) Serum ALT (n = 4; 3/7; 6) and quantification of cleaved caspase 3–positive cells around the CV (n = 3; 3/22; 8) in liver sections from 8-wk-old *c-fos*$^{\Delta li}$ and control mice untreated (0) and 48 h after DEN. Plots represent mean ± SD; ***, P ≤ 0.001 by Student's t test. (C–G) qRT-PCR analyses (C; total *c-fos* and *c-fos-flag*), liver/body and spleen/body ratio (D), serum ALT (E), blood cell count of white blood cells (WBC), lymphocytes (LYM), and granulocytes (GRA; F), and quantification of Ki67- and γH2AX-positive hepatocytes and CD45-positive cells in liver sections (G) in *c-fos*$^{hep\text{-}tetOFF}$ and control mice 2 mo after the switch from Fos on to off state (4 mo Fos on/2 mo Fos off). Bar graphs represent mean ± SD; n = 3/5.

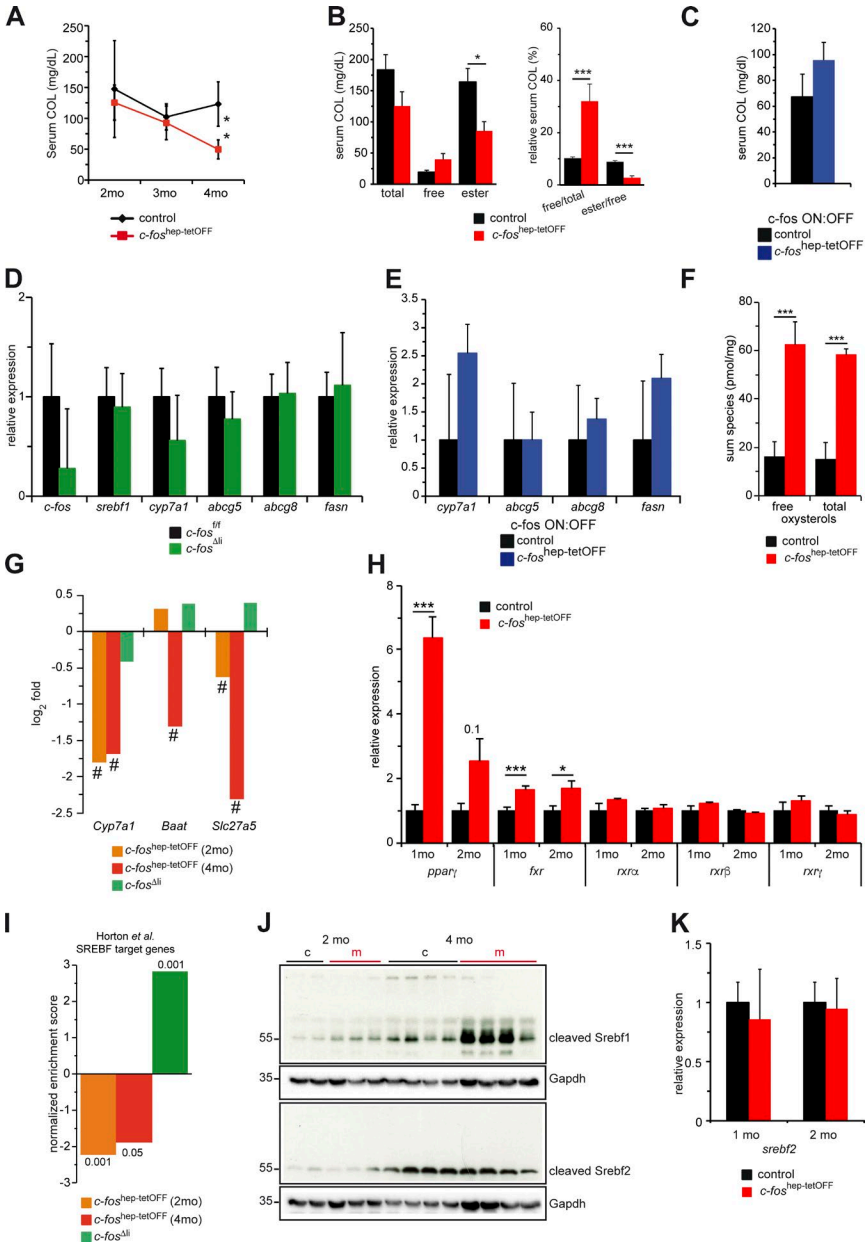

Figure S4. **Liver metabolic pathways affected by c-Fos.** (A) Serum total cholesterol (COL) at the indicated time points of c-Fos expression (n = 12; 5; 15/11; 7; 9). Plot represents mean ± SD; **, P ≤ 0.01 by two-way ANOVA. (B) Serum cholesterol species at 4 mo of c-Fos expression (n = 7/9). Bar graphs represent mean ± SD; *, P ≤ 0.05; ***, P ≤ 0.001 by Student's t test. (C) Serum total cholesterol (COL) in *c-fos*$^{hep\text{-}tetOFF}$ and control mice 2 mo after the switch from Fos on to off state (4 mo Fos on/2 mo Fos off). Bar graph represents mean ± SD; n = 3/5. (D and E) qRT-PCR for LXRα target genes in *c-fos*$^{\Delta li}$ and control mice (n = 6/cohort) at 48 h after DEN injection (D) and in *c-fos*$^{hep\text{-}tetOFF}$ and control mice 2 mo after the switch from Fos on to off state (E). Bar graphs represent mean ± SD; n = 3/5; mean expression in controls set to 1. (F) Free and total oxysterol in liver extracts from *c-fos*$^{hep\text{-}tetOFF}$ at 4 mo of c-Fos expression. Bar graphs represent mean ± SD of the sum of all oxysterols species resolved by mass spectrometry (n = 5/cohort). ***, P ≤ 0.0005 by Student's t test. (G) Relative expression of Cyp7a1 and BA amidation enzymes by RNA-seq in *c-fos*$^{hep\text{-}tetOFF}$ at 2 and 4 mo of ectopic c-Fos expression (n = 2; 3/cohort) and *c-fos*$^{\Delta li}$ 48h after DEN (n = 3/cohort) mice. Bar graphs represent mean fold changes (log2); # indicates significance after multiple testing corrections. (H) qRT-PCR analyses of the indicated nuclear receptors, including FXR encoded by Nr1h4, in total liver tissue of *c-fos*$^{hep\text{-}tetOFF}$ and control mice at 1 and 2 mo of c-Fos expression. Bar graphs represent mean ± SD; mean expression in each control group set to 1; n = 5; 5/5; 5. *, P ≤ 0.05; ***, P ≤ 0.001. (I) Normalized enrichment scores for each indicated genotype and condition derived from GSEA of the published Horton et al. (2003) SREBF signature (RNA-seq, n = 2; 3; 3/cohort). False discovery rate (FDR) q-values are indicated on each bar. (J) Srebf1 and Srebf2 immunoblot in liver lysates from *c-fos*$^{hep\text{-}tetOFF}$ and control mice 2 and 4 mo after doxycycline removal. Gapdh served as loading control. Molecular mass is indicated in kilodaltons. (K) qRT-PCR analyses of *srebf2* in total liver tissue of *c-fos*$^{hep\text{-}tetOFF}$ and control mice at 1 and 2 mo of c-Fos expression. Bar graphs represent mean ± SD; n = 5/cohort; mean expression in each control group set to 1.

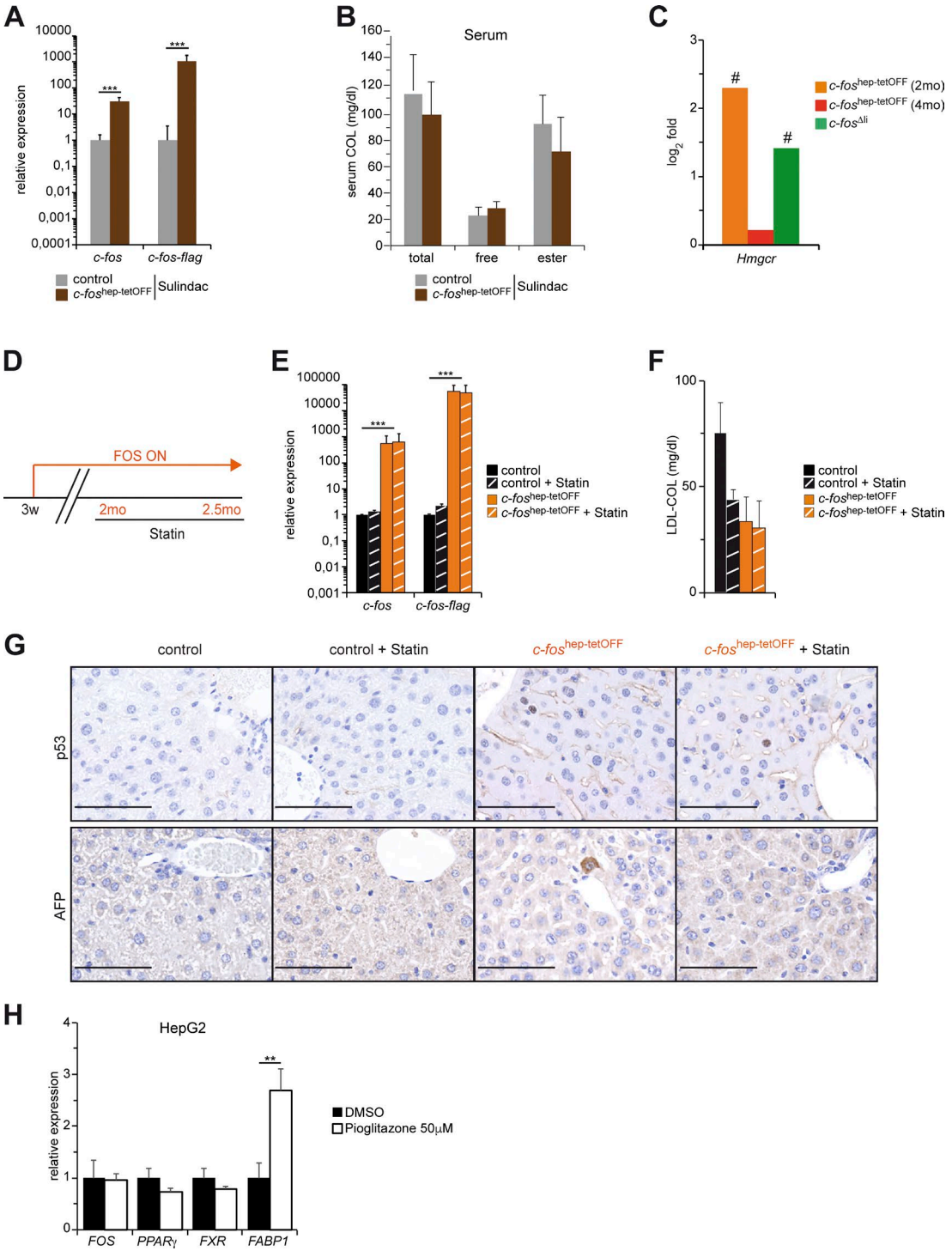

Figure S5. **Regulation of LXRα expression by c-Fos.** qRT-PCR analyses (A; total *c-fos* and *c-fos-flag*) and serum cholesterol species (B) in *c-fos*$^{hep\text{-}tetOFF}$ and control mice 2 mo after sulindac treatment. Bar graphs represent mean ± SD; $n = 6/6$; ***, $P \leq 0.001$ by Student's *t* test. (C) Relative expression of Hmgcr by RNA-seq in *c-fos*$^{hep\text{-}tetOFF}$ at 2 and 4 mo of ectopic c-Fos expression ($n = 2$; 3/cohort) and *c-fos*$^{\Delta li}$ 48 h after DEN ($n = 3$/cohort) mice. Bar graph represents mean fold changes (log2); # indicates significance after multiple testing corrections. (D) Ectopic expression of c-Fos was allowed during 2.5 mo and combined with daily statin treatment during the last 2 wk. (E and F) qRT-PCR analyses (E; total *c-fos* and *c-fos-flag*) and serum LDL cholesterol (F) in untreated and statin-treated *c-fos*$^{hep\text{-}tetOFF}$ and control mice. Bar graphs represent mean ± SD; $n = 2$; 2/4; 6. ***, $P \leq 0.001$. (G) AFP and p53 IHC in untreated and statin-treated *c-fos*$^{hep\text{-}tetOFF}$ and control mice. Bars, 100 µm. (H) Relative mRNA expression of LXRα and its target genes in HepG2 cells 24 h after treatment with the PPARγ agonist pioglitazone or vehicle (DMSO). Bar graph represents mean ± SD; $n = 3$; DMSO-treated cells set to 1. **, $P \leq 0.01$ by Student's *t* test.

Table S1. **Full and abbreviated name of BA and oxysterol species determined by MS**

Abbreviation	Full name
Free oxysterols	
25-OHC	25-Hydroxycholesterol
24S-OHC	24S-Hydroxycholesterol
24/25-EC	24,25-Epoxycholesterol
7b-OHC	7β-Hydroxycholesterol
Desmos	Desmosterol
Total oxysterols	
24S-OHC	24S-Hydroxycholesterol
24/25-EC	24,25-Epoxycholesterol
Desmos	Desmosterol
7-DC	7-Dehydrocholesterol
BAs	
CA	Cholic acid
CDCA	Chenodeoxycholic acid
LCA	Lithocholic acid
MCA(a)	α-Muricholic acid
TDCA	Taurodeoxycholic acid
TUDCA	Tauroursodeoxycholic acid
UDCA	Ursodeoxycholic acid

Table S2. **Specific primers used in this study**

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)
Mouse		
abcg5	TTGCGATACACAGCGATGC	ATCTAGCATCATGACCTTGGGG
abcg8	GTCGTCTCCAAATGTCACTCG	ACGAGCAGGAAGTGTAGAAGG
acaca	CTGGCTTACAGGATGGTTTGG	GCATTTCACTGCTGCAATACC
acly	CTTAGCCTGTGAGCTGATGG	TTGTACAGGAGTTCTTTGCCG
cyp51	ACAGGATAACCCAGCATCAGG	GGATTACTGGGTTTTCTGGGG
cyp7a1	GACATGGAGAAGGCTAAGACG	TGATGCTATCTAGTACTGGCAGG
fasn	AGGATCTCTCCAAGTTCGACG	GTCTCGGGATCTCTGCTAAGG
c-fos	CCAGTCAAGAGCATCAGCAA	TAAGTAGTGCAGCCCGGAGT
c-fos-flag	CCAGTCAAGAGCATCAGCAA	TGTCGTCGTCGTCCTTGTAG
fxr	AGATGGGGATGTTGGCTGAA	GTTCCGTTTTCTCCCTGCAA
gpam	CTTGCAGCATCTATGCAGTCC	GCCATACTGGATGAACTTGCC
hmgcs1	TACAAGCCTGACATGCTCTCC	GTCATTCAGGAACATCCGAGC
idl1	ACCTTTCCAGGTTGTTTCACC	CCAGATACCATCAGATTGGGC
krt19	ATGATCGTCTCGCCTCCTAC	TGGTTGTAATCTCGGGAGGG
lxra	AGGGAGGAGTGTGTGCTGTC	GCAGGACTTGAGGAGGTGAG
mvd	ATACACATTTGATGCTGGCCC	GTGGCAATGATGTACTGGACC
mylip	TAAAGAGTCCCTCTTGGCAGG	GCTGACACAATCTGCAGAACC
ppara	CATCACAGACACCCTCTCTCC	CGATCACACTTGTCGTACACC
pparg2	GAAGTTGGTGGGCCAGAATG	TTGACCCAGAGCATGGTGC
rxr-a	CATTGTTGGGCGACTTTTGC	AGTTCACCTGGGTAGAGAAG
rxr-b	TGACCTACTCGTGTCGTGAT	ATCTCCATCCCCGTCTTTGT
rxr-g	CACCCTCAGGAGCACTGG	TGCTTCCCTGAGGATCTGTCC
sqle	TGTCAGAAACCAACCAAGTGC	ACTTGGAGAAGAGTCCATCCG
srebf1	TTGTGGAGCTCAAAGACCTGG	TAAGCGTCTCCACCACTTCG
srebf2	GGTGAGACCTACCATGCATCC	CAGGTGTCTACCTCTCCATGC
thrsp	AACGACGCTGCTGAAACG	CAGTCTTCTCTCGTGTAAAGCG
Human		
ABCG5	CCTCAGAAACATACAAGAAATGCC	TCTAGATGTTGCACCTGGGC
ABCG8	AGACGAAGGATCTTGACGAGG	GGTCATTGACATCAGACAGGC
c-FOS	CGTTGTGAAGACCATGACAGG	CCTTCTCCTTCAGCAGGTTGG
CYP7A1	GTGCCAATCCTCTTGAGTTCC	GTGGTATTTCCATCCATCGGG
FABP2	CACCCCCTTGATATCCTTCC	TTCTCCGGCAAGTACCAACT
FASN	GCTGGAAGTCACCTATGAAGC	AGTCGAAGAAGAAGGAGAGCC
FXR	TGGGAATGTTGGCTGAATGT	TCACTTGTCGCAAGTCACGA
LXRa	GTTATAACCGGGAAGACTTTGCCA	GCCTCTCTACCTGGAGCTGGT
PPARG	CTCCTATTGACCCAGAAAGCGA	TGCCATGAGGGAGTTGGAAG

REFERENCE

Horton, J.D., N.A. Shah, J.A. Warrington, N.N. Anderson, S.W. Park, M.S. Brown, and J.L. Goldstein. 2003. Combined analysis of oligonucleotide microarray data from transgenic and knockout mice identifies direct SREBP target genes. *Proc. Natl. Acad. Sci. USA*. 100:12027–12032. http://dx.doi.org/10.1073/pnas.1534923100