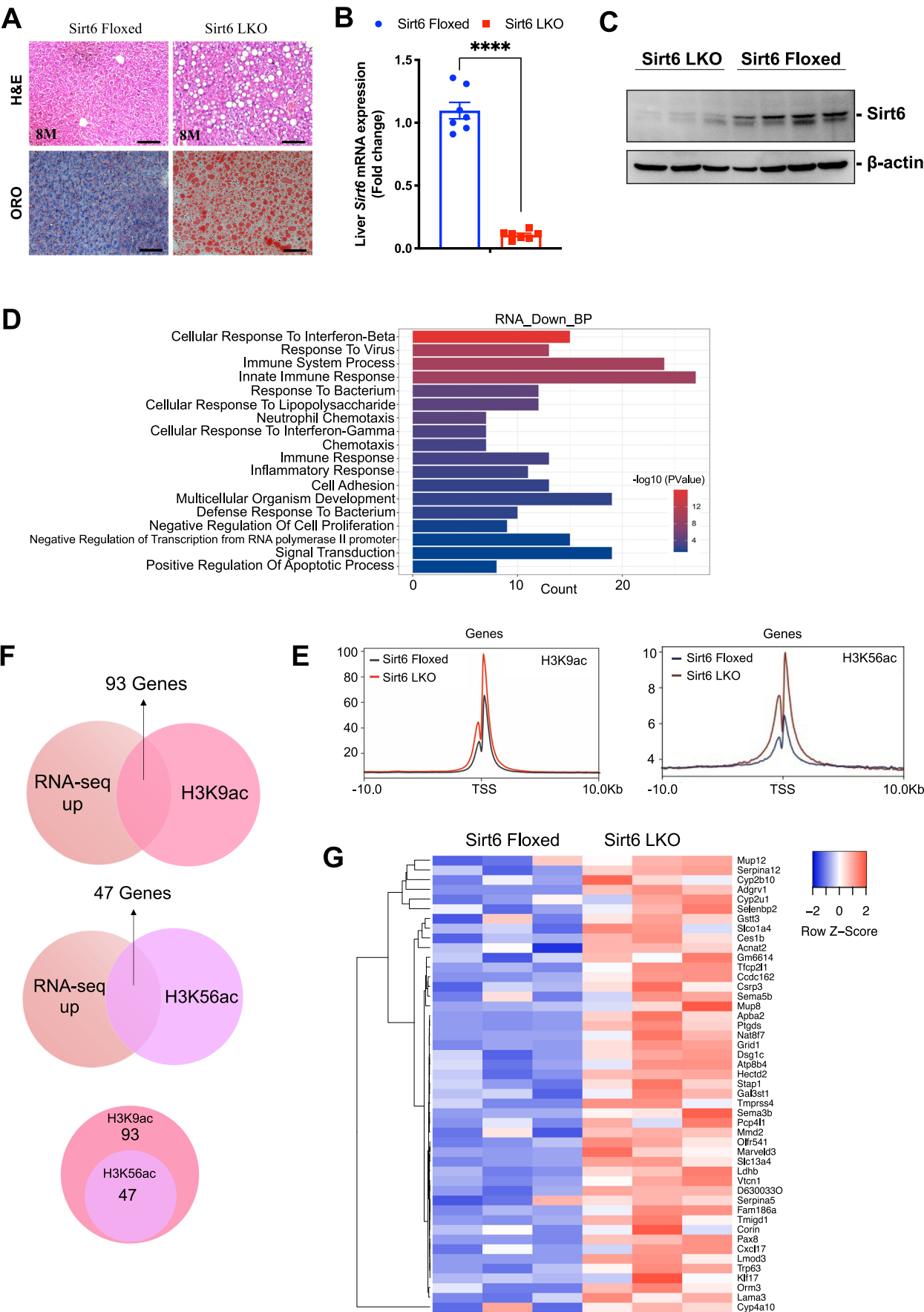


Expanded View Figures

Figure EV1. Sirt6 ablation induces formation of fatty liver accompanied by alterations of a broad lipid-related gene expression and increased H3K9 and H3K56 acetylation.

(A) H&E staining and Oil red O staining of Sirt6 Floxed and Sirt6 LKO mice liver sections of 8 month old mice. Scale bars:100µm. (B) Expression levels of Sirt6 revealed by qPCR in Sirt6 Floxed or Sirt6 LKO mice. $n = 7$ BR; error bars = SEM. t -test. **** $p < 0.001$. (C) Expression levels of Sirt6 revealed by Western blot in Sirt6 Floxed or Sirt6 LKO mice. (D) RNA-seq showing downregulated different express genes enriched based on the biological process. $n = 3$ BR, t -test, p value < 0.05 , $\text{Log}_2 \text{FC} < -1$. (E) Metagene analysis showing the bind pattern of H3K9ac or H3K56ac in Sirt6 Floxed or Sirt6 LKO mice liver. (F) Venn diagram showing that RNA-sequence upregulated genes and ChIP-H3K9Ac/H3K56Ac peaks. (G) Heatmap showed the 47 candidate genes' ranking list.



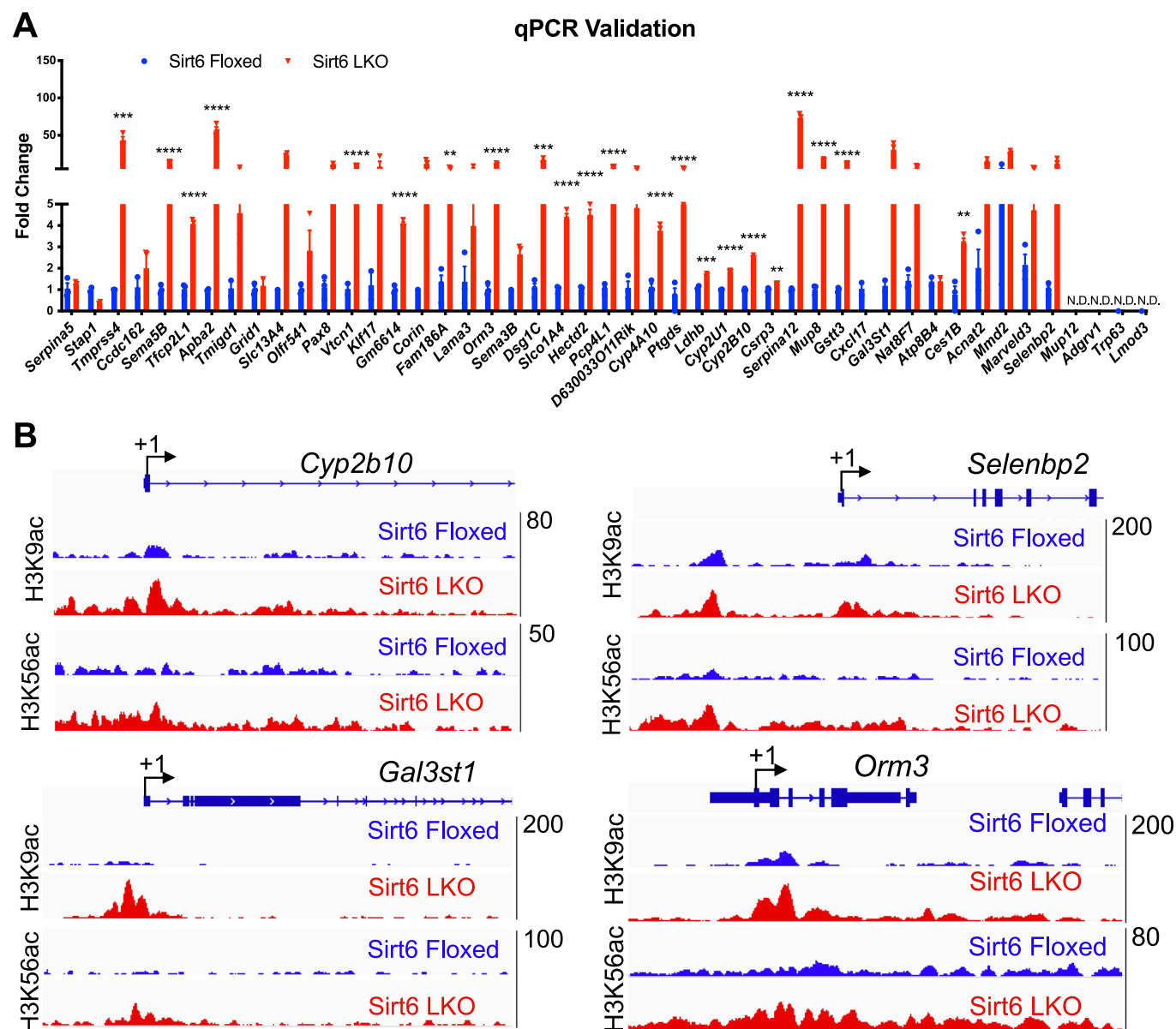


Figure EV2. Validation of candidates' expression and the levels of H3K9Ac and H3K56Ac around their promoters.

(A) qPCR validation of 47 candidate genes. Expression levels of Sirt6 revealed by qPCR in Sirt6 Floxed or Sirt6 LKO mice. $n = 3$ BR; error bars = SEM. Multiple t-test. $**p < 0.01$, $***p < 0.005$, $****p < 0.001$. (B) IGV browser of images of read coverage across the 4 candidate genes binding peaks in H3K9Ac and H3K56Ac ChIP-seq from Sirt6 Floxed versus Sirt6 LKO mice liver.

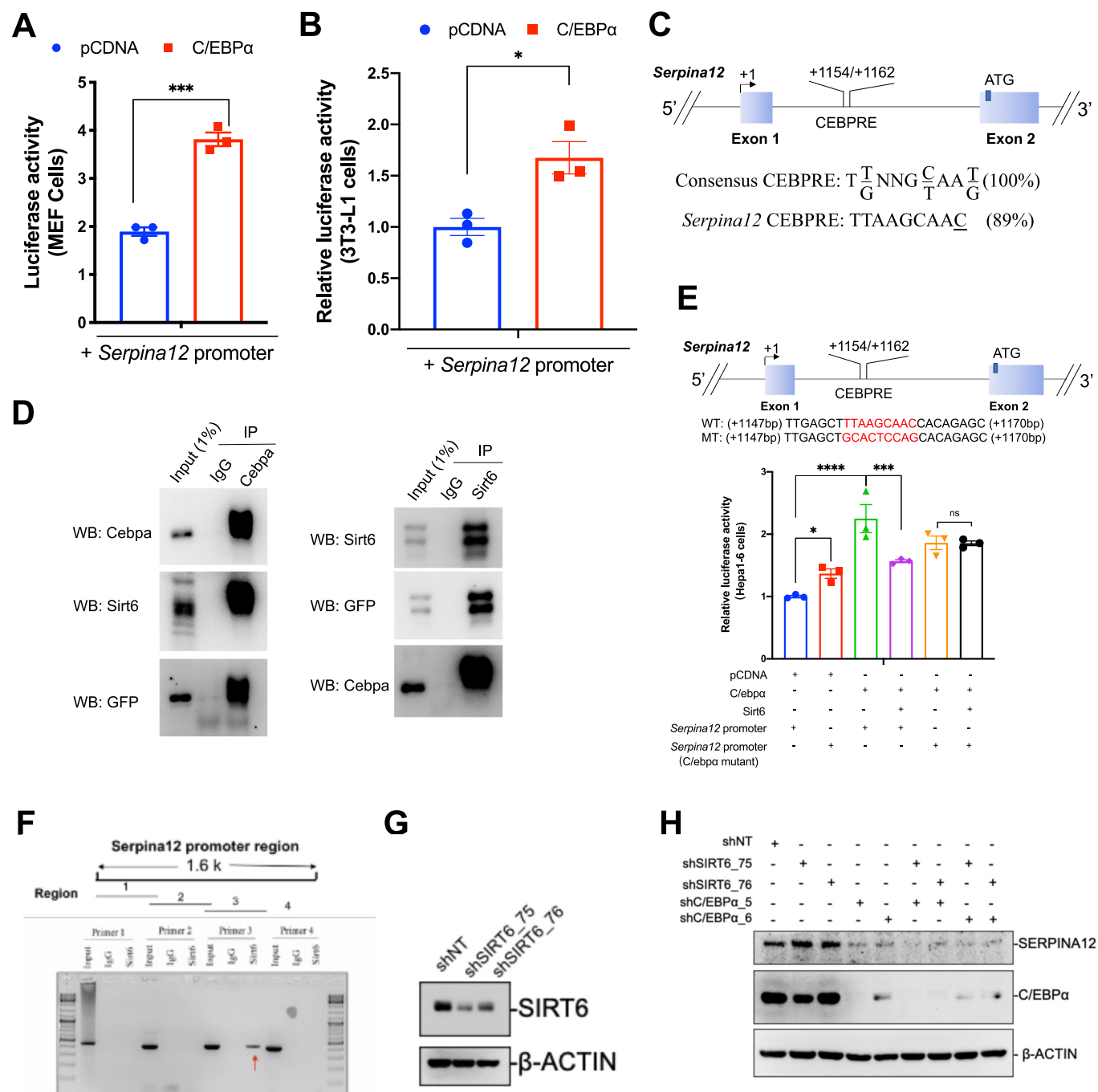


Figure EV3. *Serpina12* is upregulated in the liver after sirt6 ablation through transcription factor CEBPα.

(A) Luciferase activity after transfected promoter *m-serpina12-0.75k* and CEBPα in MEF cells. $n = 3$ BR. (B) Luciferase activity after transfected promoter *m-serpina12-0.75k* and CEBPα in 3T3-L1 cells. $n = 3$ BR. (C) Consensus CEBPRE binding region on *Serpina12* gene region. (D) IP experiments on lysates from 293T/17 cells detected by antibodies of Sirt6 and C/ebpα. Sirt6-GFP and C/ebpα expression was induced 48 h prior to IP. (E) Ectopic expression of C/ebpα increased *Serpina12* promoter activity, whereas mutation of C/ebpα-binding sites reduced the induction. Ectopic expression of Sirt6 represses the *Serpina12* promoter activity but not the mutant C/ebpα-binding sites promoter activity. $n = 3$ BR. (F) SIRT6 ChIP to analysis SERPINA12 promoter binding region in HepG2 cells. (G) Western blot analysis shows the SIRT6 protein level in SIRT6 WT and knockdown groups in HepG2 cells. (H) Western blot analysis shows the SERPINA12 protein level with either knockdown SIRT6 or CEBPα in HepG2 cells. Data information: For (A),(B) and (E), error bars = SEM. t-test or two-way ANOVA. * $p < 0.05$, *** $p < 0.005$, **** $p < 0.001$, ns no significant difference.

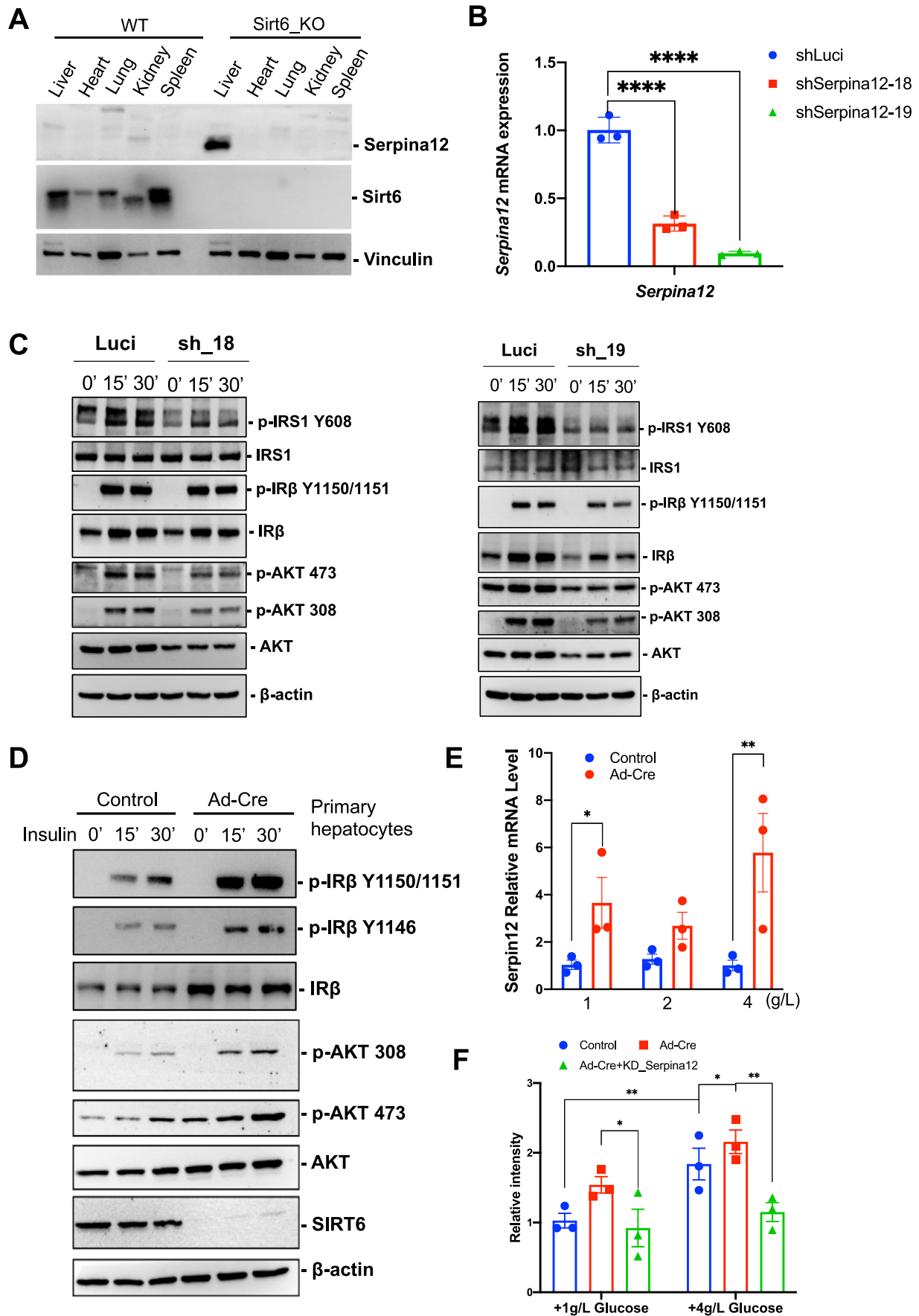
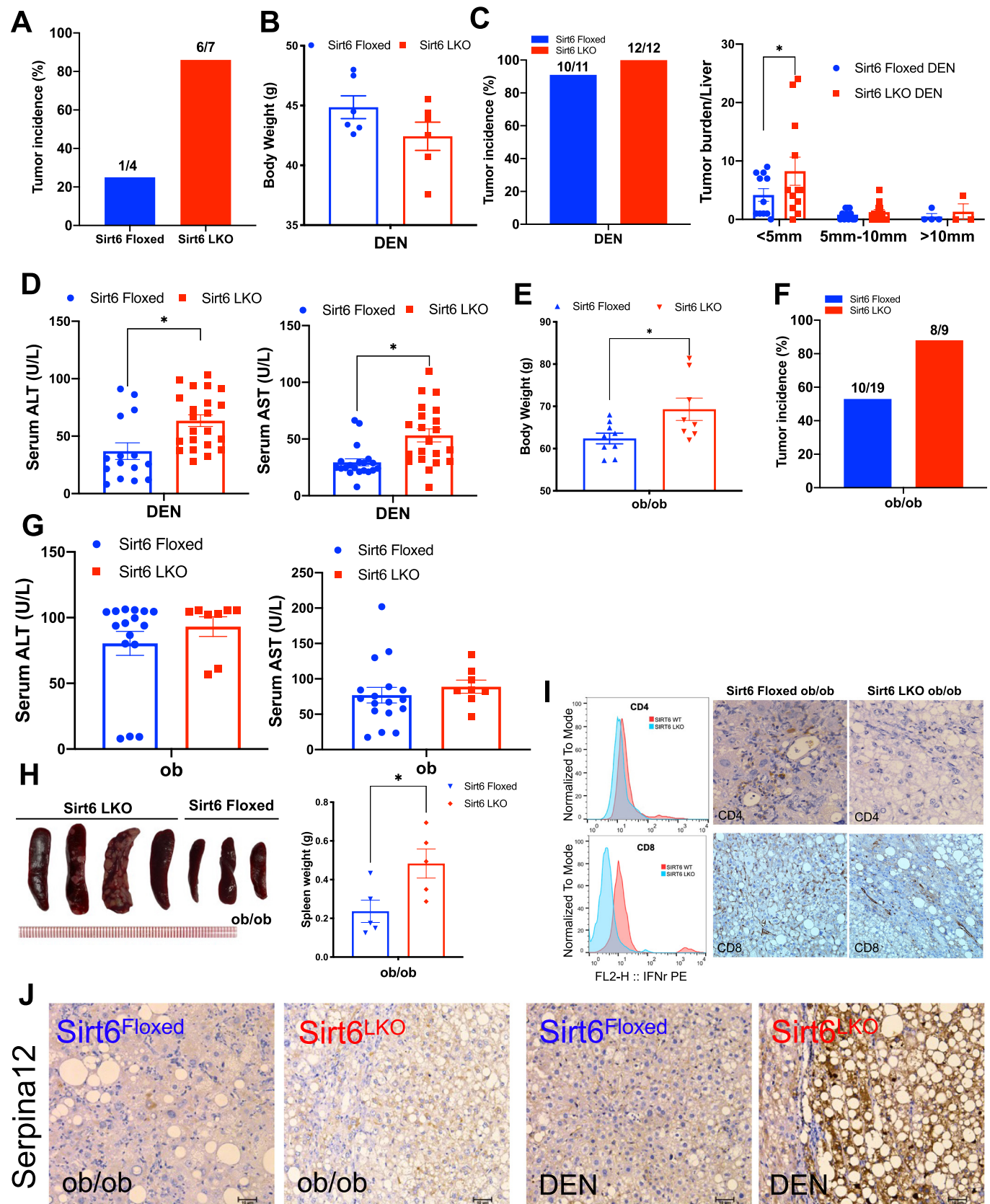


Figure EV4. SIRT6 affects insulin signaling by regulating the expression of Serpina12.

(A) Western blot shows the Serpina12 protein level in different tissue in SIRT6 WT or KO mice. (B) qPCR shows the knockdown efficiency of Serpina12 in primary hepatocytes. (C) Western blot analysis of insulin signaling following insulin treatment at 0 min, 15 min and 30 min by knockdown Luci control or two different Serpina12 shRNAs in primary hepatocytes. (D) Western blot analysis of insulin signaling following insulin treatment at 0 min, 15 min and 30 min in control or Ad-Cre in primary hepatocytes. (E) qPCR analysis of Serpina12 mRNA levels in different glucose concentration. (F) Relative intensity in control, knockout Sirt6 with or without knockdown Serpina12 primary hepatocytes. Data information: For (B),(E), and (F), $n = 3$ TR; error bars = SEM. Multiple t -test or two-way ANOVA. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.001$.



◀ **Figure EV5. Sirt6 deficiency causes lipid-rich environment and less CD8⁺ T cells infiltration in the liver thus accelerate tumor formation.**

(A) Tumor incident (%) of Sirt6 Floxed or Sirt6 LKO mice in 2-year-old. (B) Body weight of Sirt6 Floxed and Sirt6 LKO with DEN injection mice. $n = 6$ BR. (C) Tumor incident in Sirt6 Floxed and Sirt6 LKO with DEN injection mice; Tumor burden per liver of Sirt6 Floxed and Sirt6 LKO with DEN injection mice. $n = 11$ or 12 BR. (D) Serum ALT level and AST level of Sirt6 Floxed and Sirt6 LKO with DEN injection mice. For ALT, $n = 15$ or 22 BR; For AST, $n = 20$ or 21 BR; (E) Body weight of Sirt6 Floxed and Sirt6 LKO with ob/ob mice. $n = 8$ or 9 BR. (F) Tumor incident in Sirt6 Floxed and Sirt6 LKO with ob/ob mice (6–9-month-old). (G) Serum ALT level and AST level of Sirt6 Floxed and Sirt6 LKO with ob/ob mice. For ALT, $n = 16$ or 8 BR; For AST, $n = 17$ or 8 BR; (H) Spleen morphology of Sirt6 Floxed and Sirt6 LKO with ob/ob mice at 7-month-old; Spleen weight of Sirt6 Floxed and Sirt6 LKO with ob/ob mice at 7-month-old. $n = 5$ BR. (I) The CD4⁺ and CD8⁺ T cells amount in the spleen of Sirt6 Floxed and Sirt6 LKO with ob/ob mice; IHC staining of CD4⁺ and CD8⁺ in Sirt6 Floxed and Sirt6 LKO with ob/ob mice. (J) IHC staining of Seprina12 in Sirt6 Floxed and Sirt6 LKO with ob/ob or DEN injection mice. Data information: For (B–E),(G), and (H), error bars = SEM. t -test. $*p < 0.05$.