

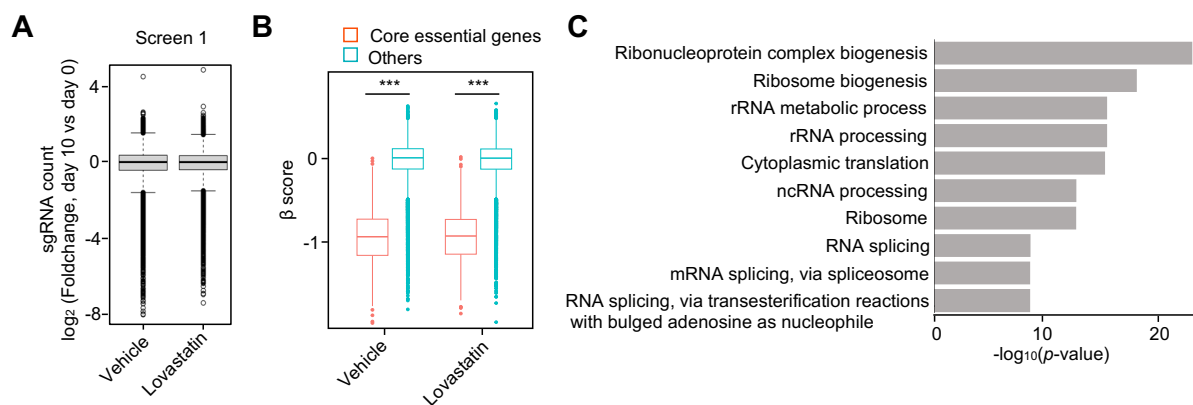


Fig. S1 Additional analysis of CRISPR screens (Screen 1) in HepG2 cells.

(A) The sgRNA count distribution during the CRISPR screening for each condition (Screen 1). (B) The core essential genes underwent significantly negative selection compared to other genes during the CRISPR screens as measured by β score (Screen 1). (C) The top ten enriched functional terms for synthetic lethal hits of the CRISPR screening (Screen 1) as determined by the GO and KEGG analysis.

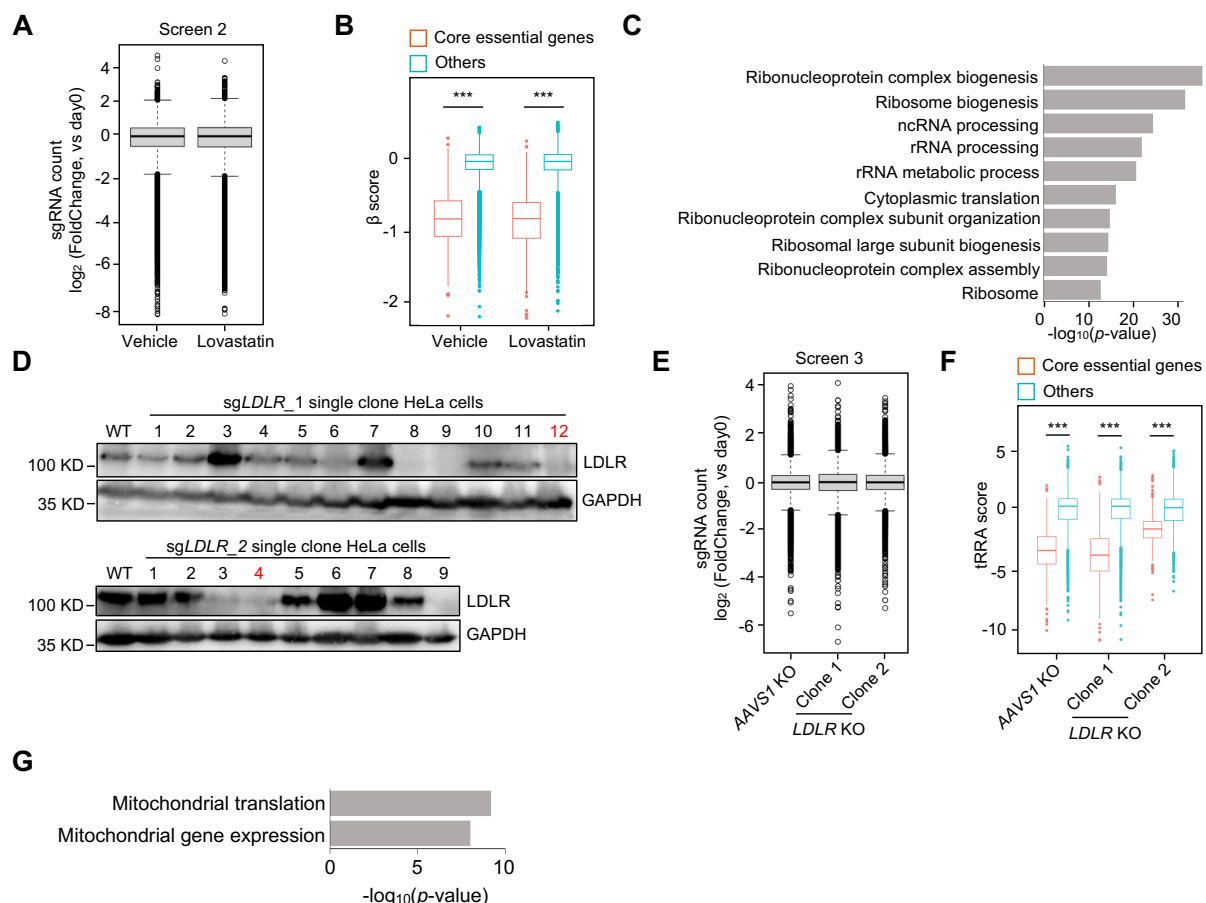


Fig. S2 Additional analysis of CRISPR screens (Screen 2 and 3) in HeLa cells.

(A) The sgRNA count distribution during the CRISPR screening for each condition (Screen 2). (B) The core essential genes underwent significantly negative selection compared to other genes during the CRISPR screens as measured by β score (Screen 2). (C) The top ten enriched functional terms for synthetic lethal hits of the CRISPR screening (Screen 2) as determined by the GO and KEGG analysis. (D) Immunoblot analysis of LDLR in HeLa cells validating the knockout status of *LDLR* in different single clonal cells. The #12 single clone of sgLDLR_1 group and #4 clone of sgLDLR_2 group are employed and designated as clone 1 and clone 2 in the following study. (E) The sgRNA count distribution during the CRISPR screening for each condition (Screen 3). (F) The core essential genes underwent significantly negative selection compared to other genes during the CRISPR screens as measured by tRRA score (Screen 3). (G) The significantly enriched functional terms for overlapped synthetic lethal hits of the CRISPR screening (Screen 3) as determined by the GO and KEGG analysis.

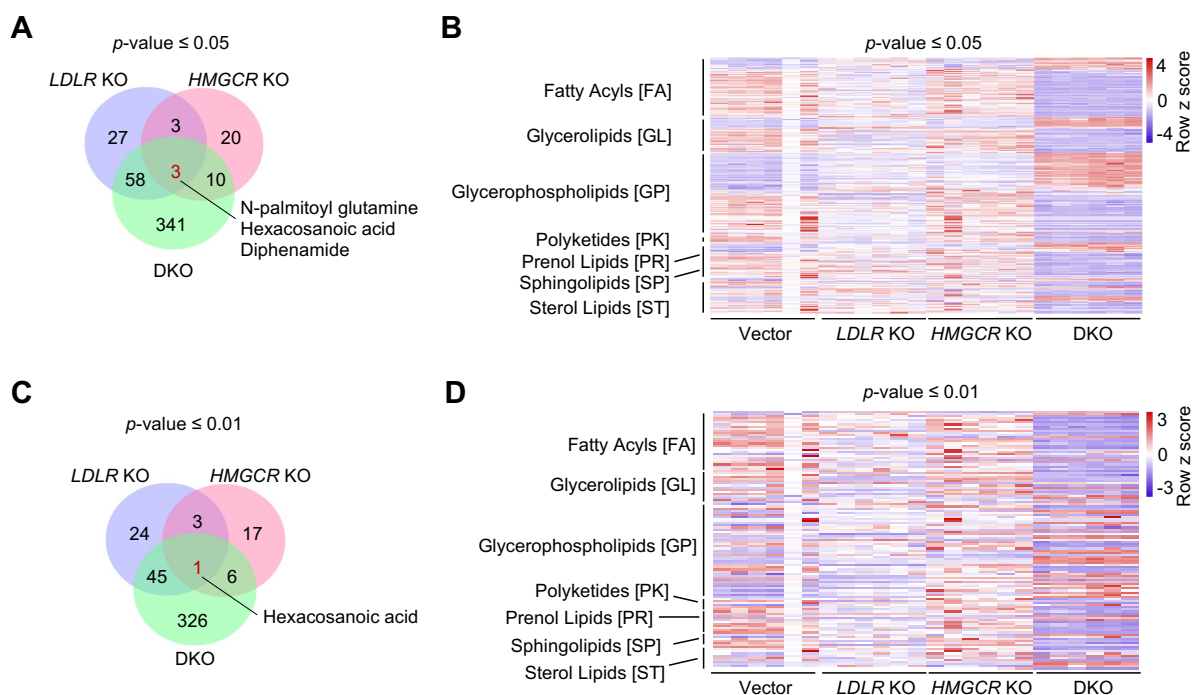


Fig. S3 Metabolomic analysis of genetically perturbed HepG2 cells.

(A) Venn diagram showing the overlap of differential metabolites in HepG2 cells upon *LDLR*, *HMGCR* KO or *LDLR/HMGCR* DKO identified by untargeted metabolomic profiling ($p\text{-value} \leq 0.05$). The common hits are highlighted. (B) Heatmap showing the clustering and magnitude of differential lipid molecules detected by metabolomic profiling of indicated cells ($p\text{-value} \leq 0.05$). (C) Venn diagram representing the overlap of differential metabolites in indicated cells by untargeted metabolomic profiling using a more stringent cutoff ($p\text{-value} \leq 0.01$). (D) Heatmap representing the clustering and magnitude of differential lipid molecules detected in indicated cells using a more stringent cutoff ($p\text{-value} \leq 0.01$). The detailed cutoff information can be found in Methods.

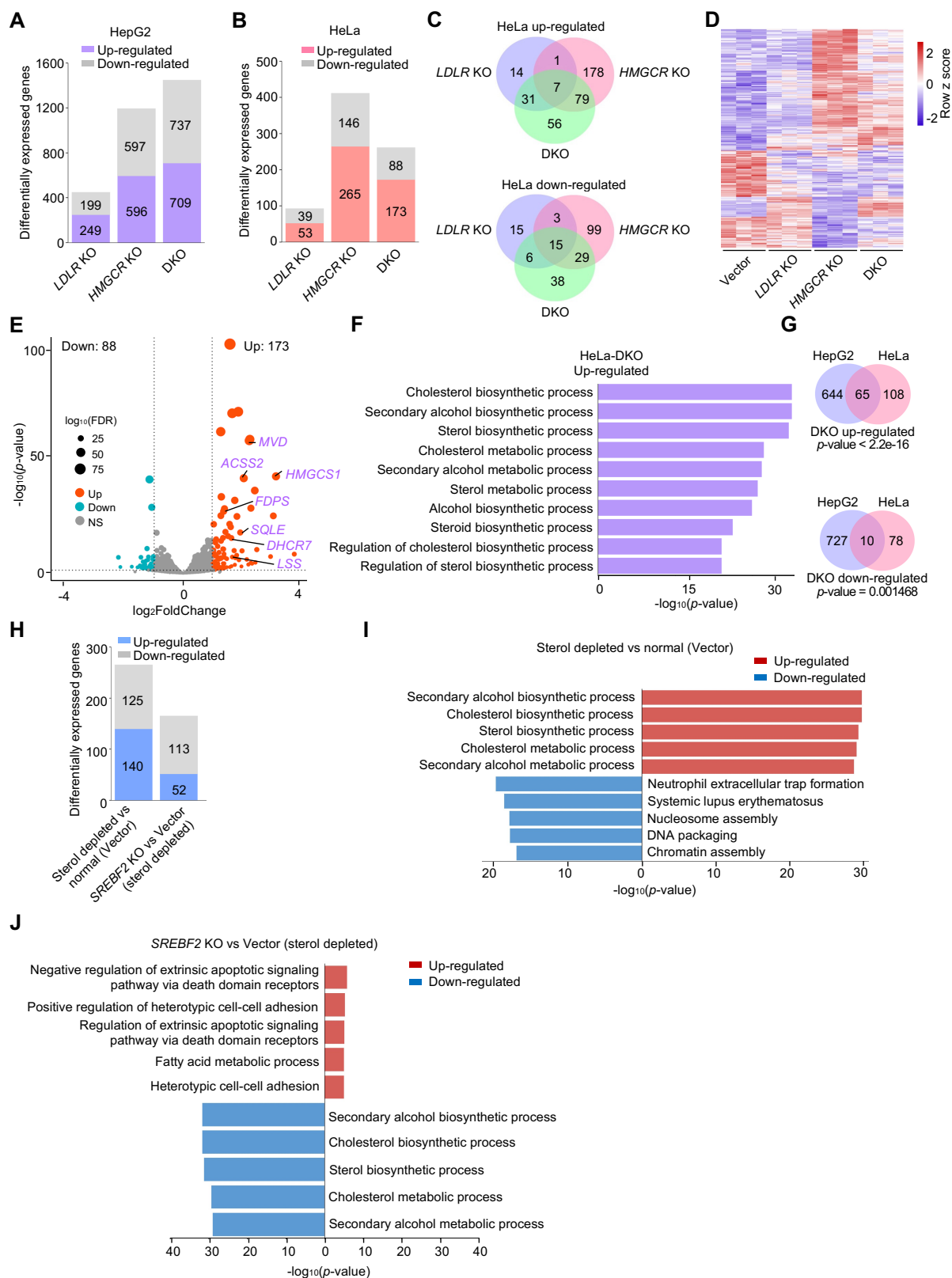


Fig. S4 Transcriptomic analysis for potential cholesterol regulators.

(A) The number of either up-regulated or down-regulated DEGs for indicated samples of HepG2 cells. (B) The number of either up-regulated or down-regulated DEGs for

indicated samples of HeLa cells. (C) Venn diagram showing the overlap of up- or down-regulated DEGs for indicated HeLa cells. (D) Heatmap showing the DEGs across different samples of HeLa cells. (E) Volcano plot showing the DEGs in *LDLR/HMGCR* DKO cells compared to control HeLa cells with several typical genes highlighted. (F) The top ten enriched functional terms for up-regulated DEGs in *LDLR/HMGCR* DKO cells compared to control HeLa cells by GO and KEGG analysis. (G) Venn diagram showing the overlap of up- or down-regulated DEGs upon *LDLR/HMGCR* DKO between HepG2 cells and HeLa cells. (H) The number of either up-regulated or down-regulated DEGs between sterol deprivation condition (sterol depleted vs. normal) and *SREBF2* KO under sterol deprivation (*SREBF2* KO vs. Vector control) in HepG2 cells. (I) The top five enriched functional terms for either up-regulated or down-regulated DEGs in sterol deprivation condition (sterol depleted vs. normal) of HepG2 cells. (J) The top five enriched functional terms for either up-regulated or down-regulated DEGs in *SREBF2* KO cells compared to Vector control HepG2 cells in sterol deprivation condition.

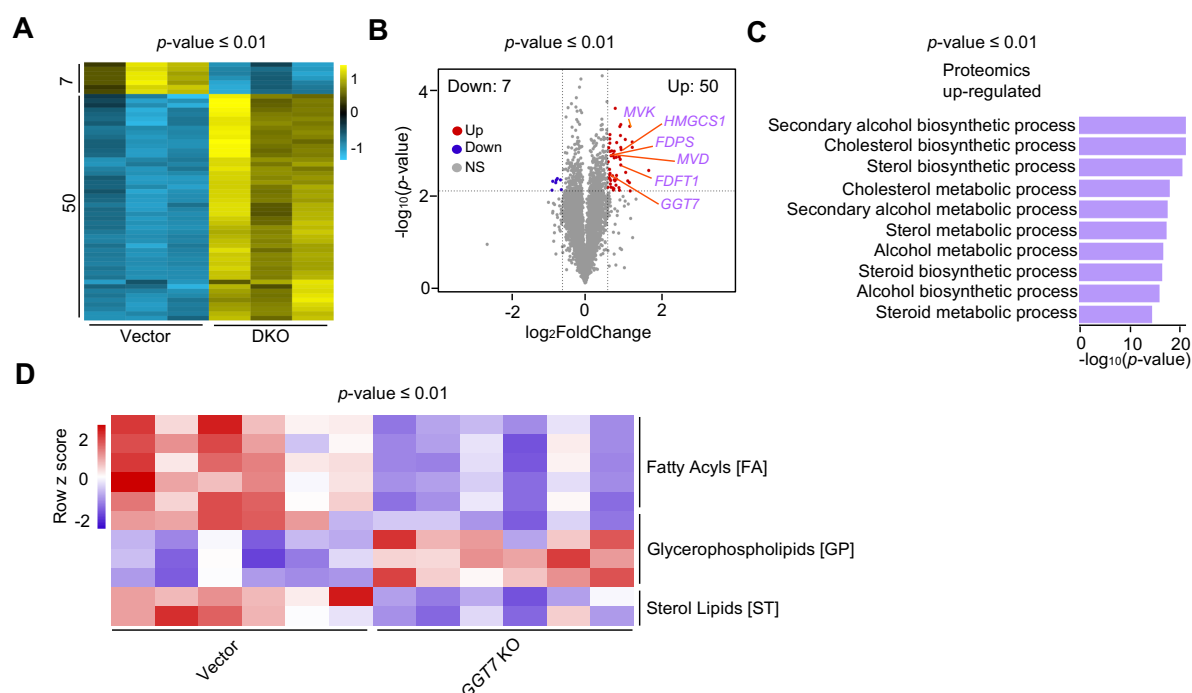


Fig. S5 Proteomics analysis of genetically perturbed HepG2 cells and metabolomic analysis of *GGT7* KO HepG2 cells using a more stringent cutoff.

(A) Heatmap showing the differentially expressed proteins in *LDLR/HMGCR* DKO cells compared to Vector control HepG2 cells determined by proteomics profiling when $p\text{-value}$ was ≤ 0.01 . (B) Volcano plot showing the differentially expressed proteins in *LDLR/HMGCR* DKO cells compared to Vector control HepG2 cells when $p\text{-value}$ was ≤ 0.01 . (C) The top ten enriched functional terms for up-regulated proteins in *LDLR/HMGCR* DKO cells compared to control HepG2 cells by GO and KEGG analysis when $p\text{-value}$ was ≤ 0.01 . (D) Decreased lipids upon *GGT7* KO in HepG2 cells determined by untargeted metabolomic profiling when $p\text{-value}$ was ≤ 0.01 . $n = 6$ biological replicates for each group.

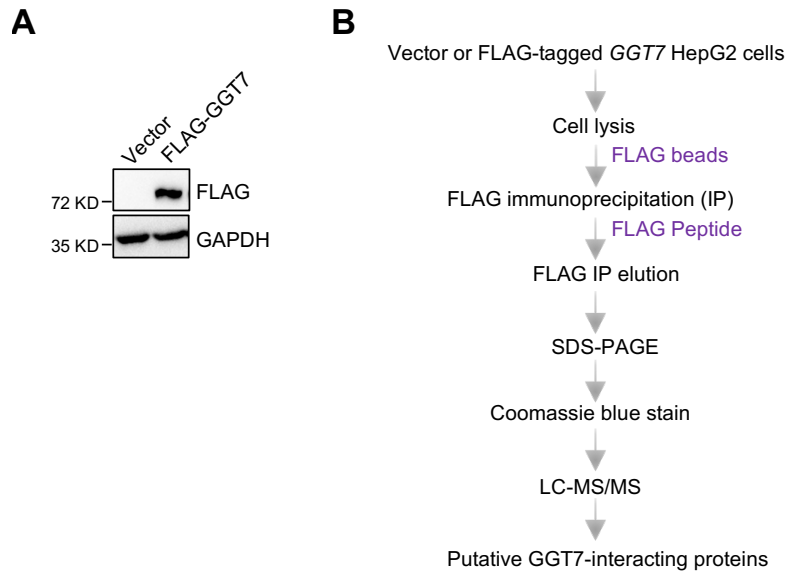


Fig. S6 Identification of GGT7-interacting proteins using immunoprecipitation coupled with mass spectrometry.

(A) Immunoblot analysis of GGT7 or GAPDH in HepG2 cells expressing vector control or FLAG-GGT7 via lentiviral infection. (B) The workflow of immunoprecipitation coupled with mass spectrometry to identify GGT7-interacting protein partners in HepG2 cells.

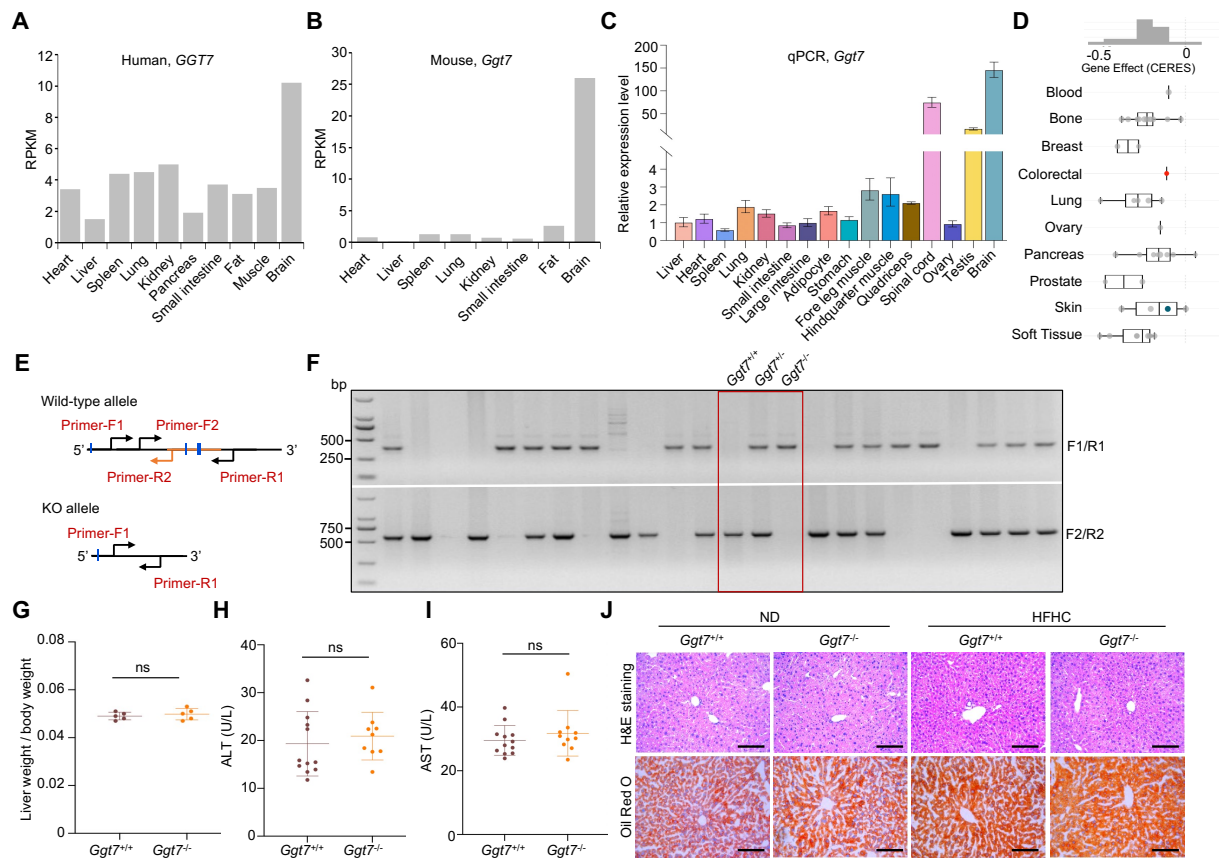


Fig. S7 Construction and analysis of the whole-body *Ggt7* knockout mice.

(A-B) The expression level of human *GGT7* (A) or mouse *Ggt7* (B) RNA as determined by RPKM (reads per kilobase per million reads) from RNA-seq data across multiple tissue types. The expression data were retrieved from NCBI gene database (BioProject: PRJEB4337 for human and BioProject: PRJNA66167 for mouse). (C) RT-qPCR validation of mouse *Ggt7* expression across multiple tissue types. (D) The CERES score of *GGT7* informing its essentiality across multiple tissue types. The data were extracted from DepMap portal (Avana public 19Q1 database). (E) The PCR primer positions and amplicons for genotyping *Ggt7* knockout mice. (F) The representative gel showing the genotyping PCR results for *Ggt7* knockout mice. (G-I) The liver index (measured by the values of liver weight / body weight) (G) (n = 5), liver alanine transaminase (ALT) levels (H) (n = 12 and 9, respectively), and liver aspartate aminotransferase (AST) levels (I) (n = 12 and 10, respectively) in *Ggt7*^{+/+} or *Ggt7*^{-/-} mice. Unpaired two-sided t test, ns means not significant. (J) Representative images of Hematoxylin and Eosin (H&E) staining and lipid droplet staining with Oil Red O of liver sections from *Ggt7*^{+/+} or *Ggt7*^{-/-} mice fed with ND or HFHC diet (n = 3).