

Figure S1. Validation of lipidotoxic cell models and related bioinformatics analysis. (A) AML12 cells were treated with PAOA for 48 h and lipid droplet changes were observed under a microscope (magnification, x20; with red representing lipid droplets and blue representing nuclei). (B) KEGG analysis indicated that inflammatory-related and lipid metabolism-related signaling pathways were affected in AML12 cells after 48 h of PAOA treatment. (C) Changes in lipid metabolism signaling pathways in AML12 cells after 48 h of PAOA treatment were measured. (D) Gene set enrichment analysis showed that inflammatory-related signaling pathways were affected in AML12 cells after 48 h of PAOA treatment. (E) mRNA levels of PLIN5 (fold) and (F) protein expression level of PLIN5 after PAOA treatment were detected. * $P < 0.05$, ** $P < 0.01$. (G) Protein-protein interaction analysis of PLIN5 and ferroptosis-associated proteins was conducted. PAOA, palmitic acid and oleic acid; BSA, bovine serum albumin; PLIN5, perilipin 5; KEGG Kyoto Encyclopedia of Genes and Genomes.

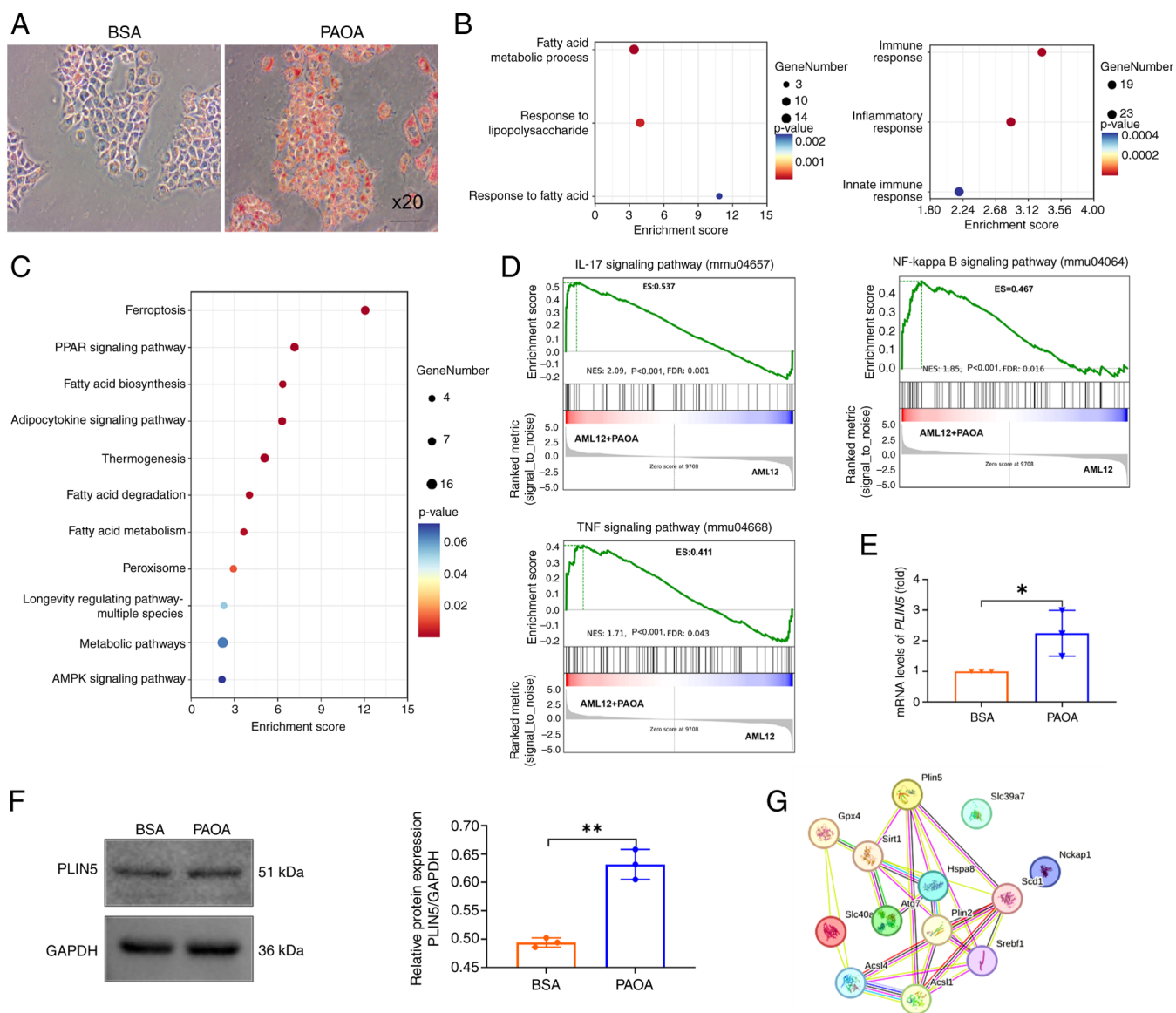


Figure S2. PLIN5 knockout alleviates hepatic steatosis and liver injury in metabolic-associated fatty liver disease. (A) mRNA levels of PLIN5 in mouse liver tissues were detected by quantitative polymerase chain reaction (n=5 mice/group). (B) Protein expression levels of PLIN5 in liver tissues were detected by western blotting using GAPDH as an internal control, with semi-quantitative analysis performed using ImageJ (n=3 mice/group). (C) Lipid droplet sizes in mouse liver tissues were observed under a microscope using H&E staining, with red representing the cytoplasm and blue representing nuclei (magnification, x10). (D) Liver tissue fibrosis was observed under a microscope using Masson's trichrome staining, with blue representing collagen fibers (magnification, x10). (E) TG, (F) TC and (G) ALT content in liver tissues from each group were detected (n=5 mice/group). All experiments were repeated three or more times (n=3). *P<0.05, **P<0.01. HFD, high-fat diet; ND, normal diet; PLIN5, perilipin 5; WT, wild-type. TG, triglyceride; TC, total cholesterol; ALT, alanine aminotransferase.

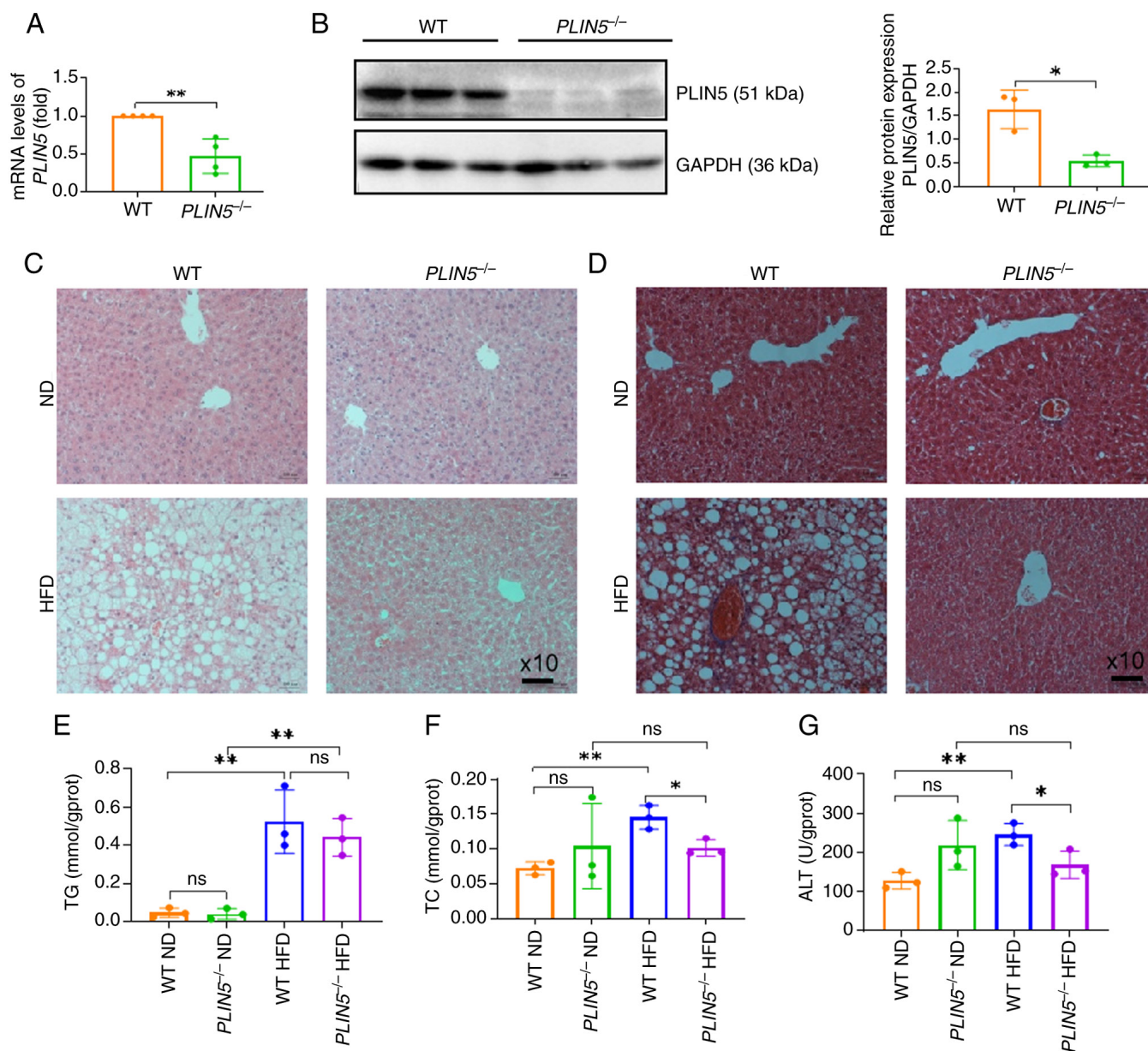


Figure S3. Single-cell transcriptome analysis of the expression of ACSL4 and SLC7A11. (A) ACSL4 and (B) SLC7A11 expression. * $P < 0.05$, n.s., not significant. HFD, high-fat diet; PLIN5, perilipin 5; FPKM, fragments per kilobase of transcript per million mapped fragments.

