

Supplementary Information

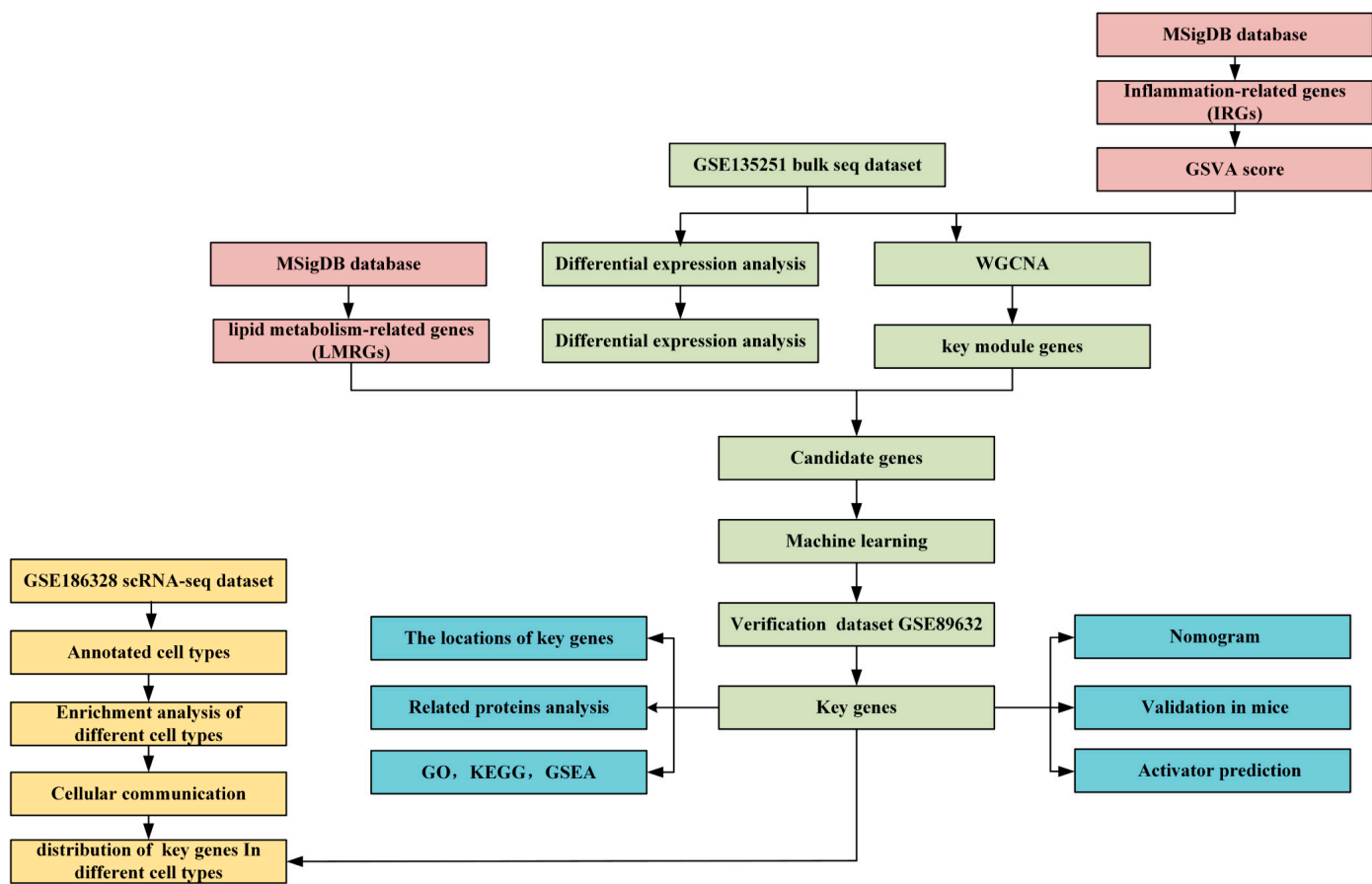


Figure S1. Flowchart of this study.

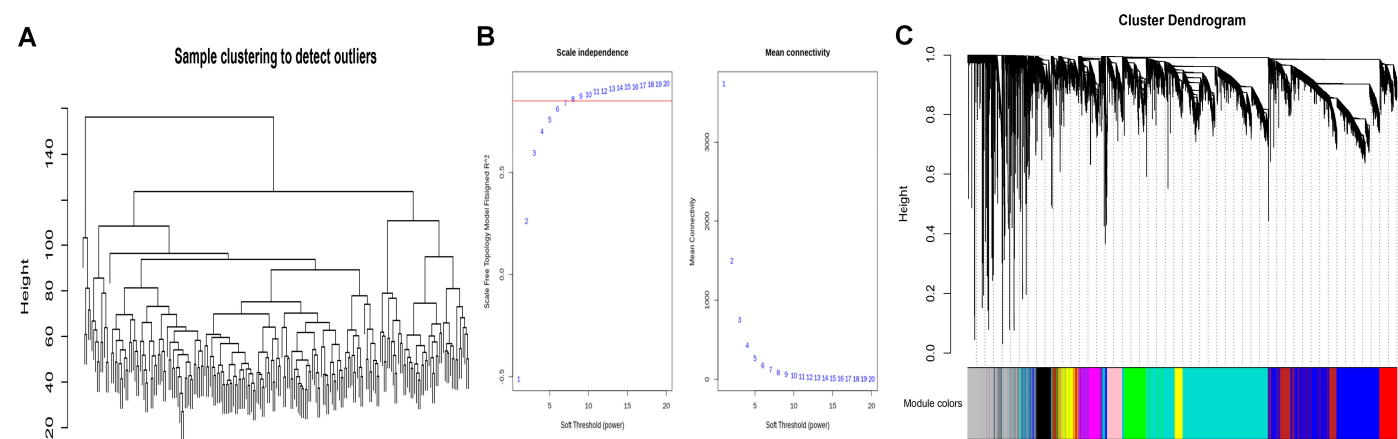


Figure S2. A WGCNA network was constructed to expand the IRG gene set (A) A WGCNA network was constructed using all GSE135251 samples, with no outliers detected. (B) Analysis of the scale-free index and the mean connectivity for various soft-threshold powers. (C) Displays the merged modules under the cluster tree. WGCNA: Weighted gene co-expression network analysis; IRG: inflammation-related genes

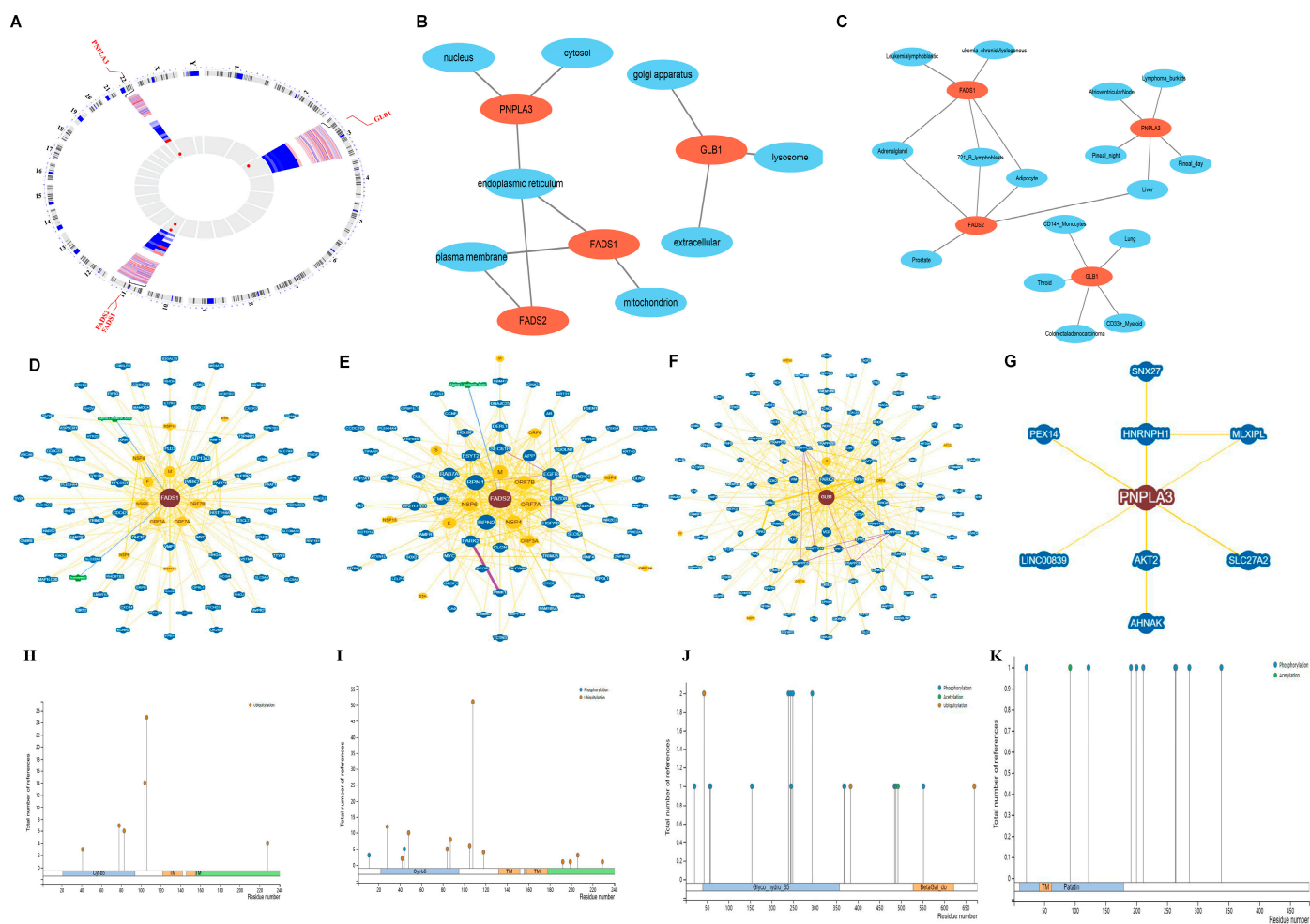


Figure S3. The localization, function, and post-translational modifications of the four key genes (A) The localization of key genes on chromosomes. (B) The subcellular distribution of the four key genes. (C) The distribution of the four key genes in organs and tissues. (D–G) The interaction between the proteins coded by the four key genes and other proteins. (H–K) The potential phosphorylation sites of the four key genes at the protein level.

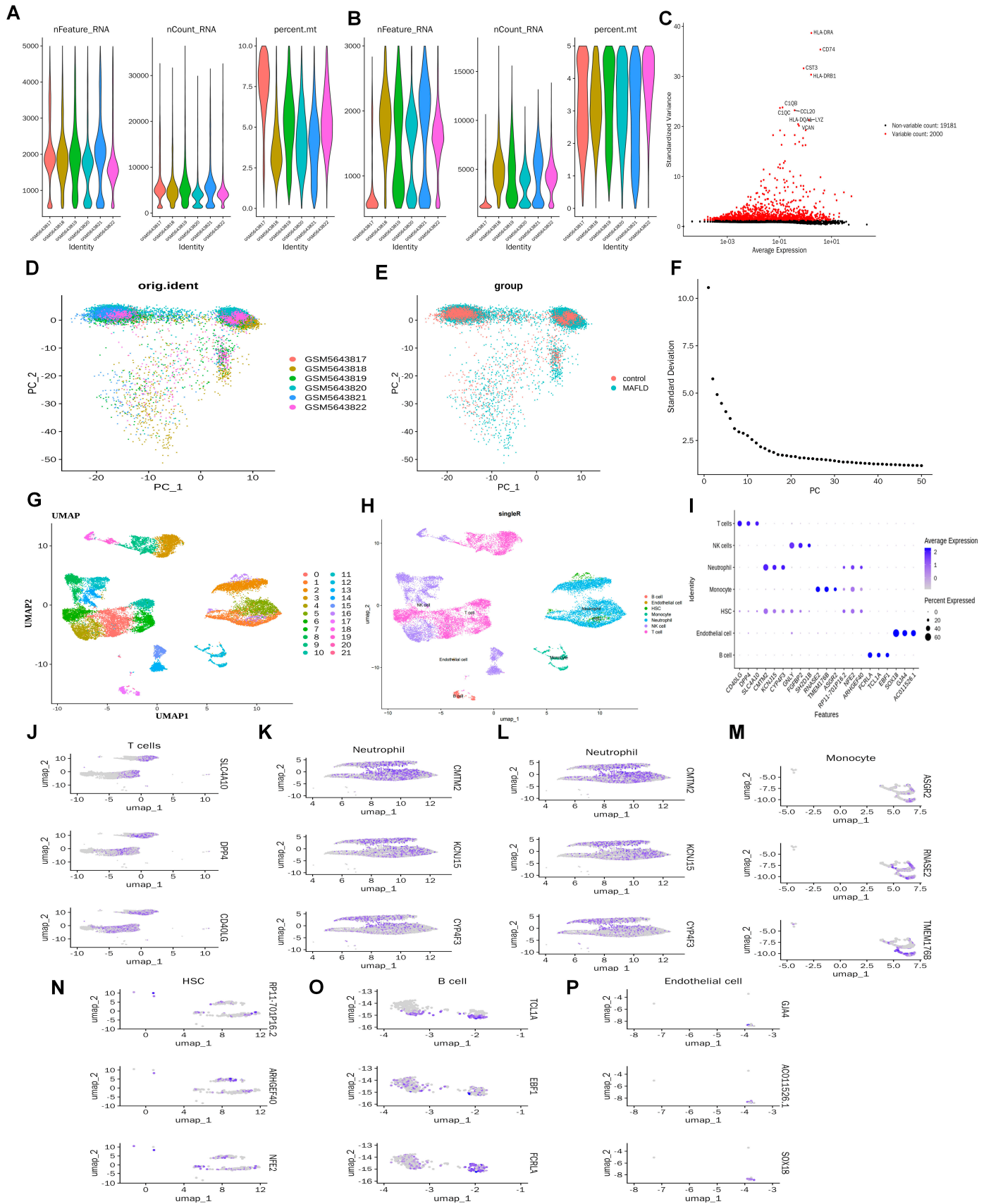


Figure S4. Acquisition of 7 annotated cell types in the scRNA-seq dataset GSE186328 (A) The distribution maps of nFeature_RNA, nCount_RNA, and percent.mt before quality control. (B) The distribution maps of nFeature_RNA, nCount_RNA, and percent.mt after quality control. (C) 2,000 highly variable genes were identified with top 10 hypervariable genes labeled. (D) DCA analysis showed the distribution of cells in each sample. (E) DCA analysis showed the distribution of cells between

MAFLD and control. **(F)** The top 50 PCs were visualized using the ElbowPlot function. **(G)** UMAP clustering delineated 22 distinct cell clusters. **(H)** Marker genes identified 7 cell types: B cells, endothelial cells, HSCs, monocytes, neutrophils, NK cells, and T cells. **(I)** Bubble plot of marker gene expression in different cell types. **(J–P)** The distribution of marker genes in different cells. PC: principal components; DCA: decision curve analysis; UMAP: uniform manifold approximation and projection; MAFLD: Metabolic Associated Fatty Liver Disease

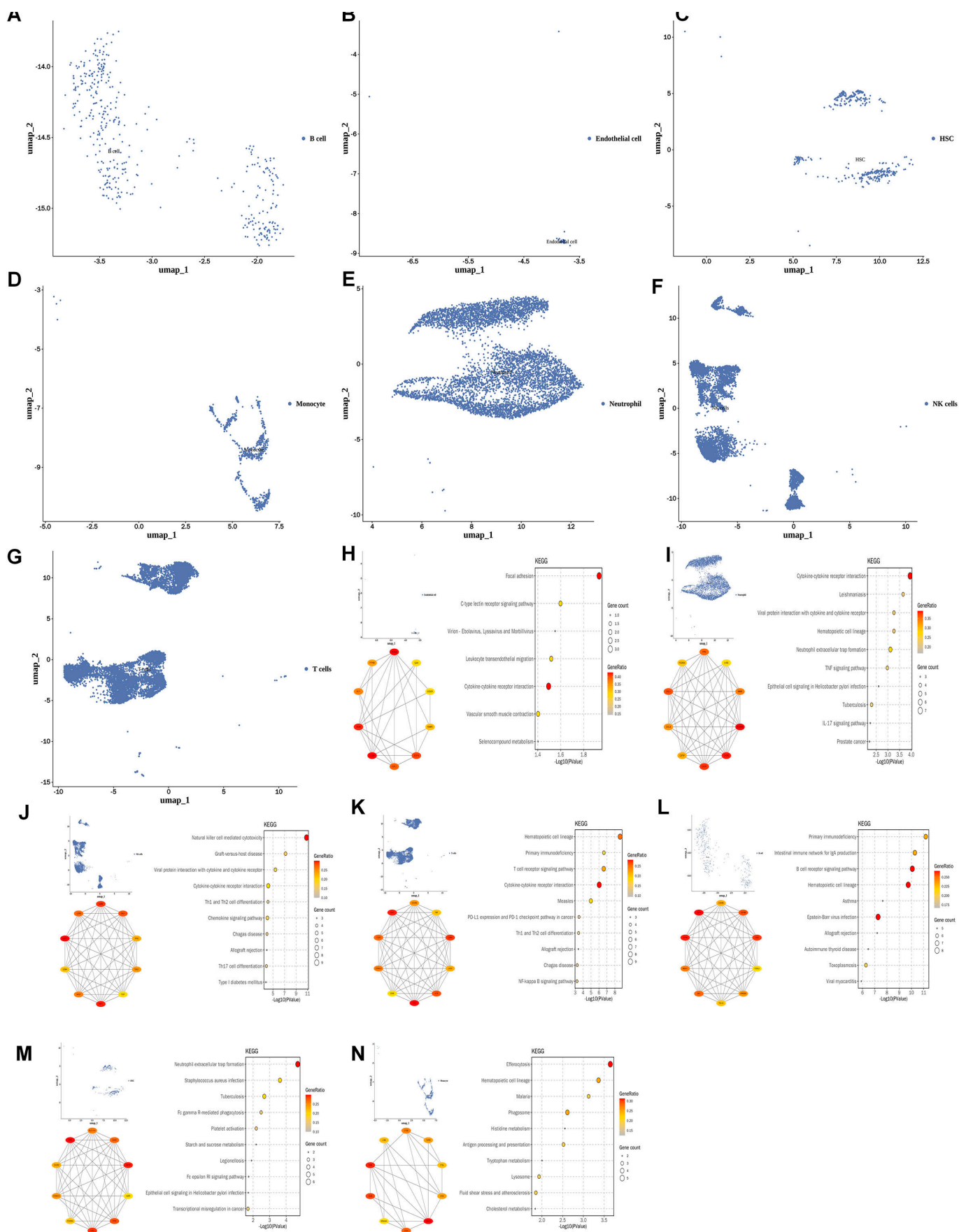


Figure S5. Enrichment analysis of marker genes of the 7 annotated cell types in GSE186328. (A–G) The 7 annotated cell types were presented with UMAP plots. (H–N) Enrichment analysis of the marker genes in the 7 annotated cell types. UMAP: uniform manifold approximation and projection.

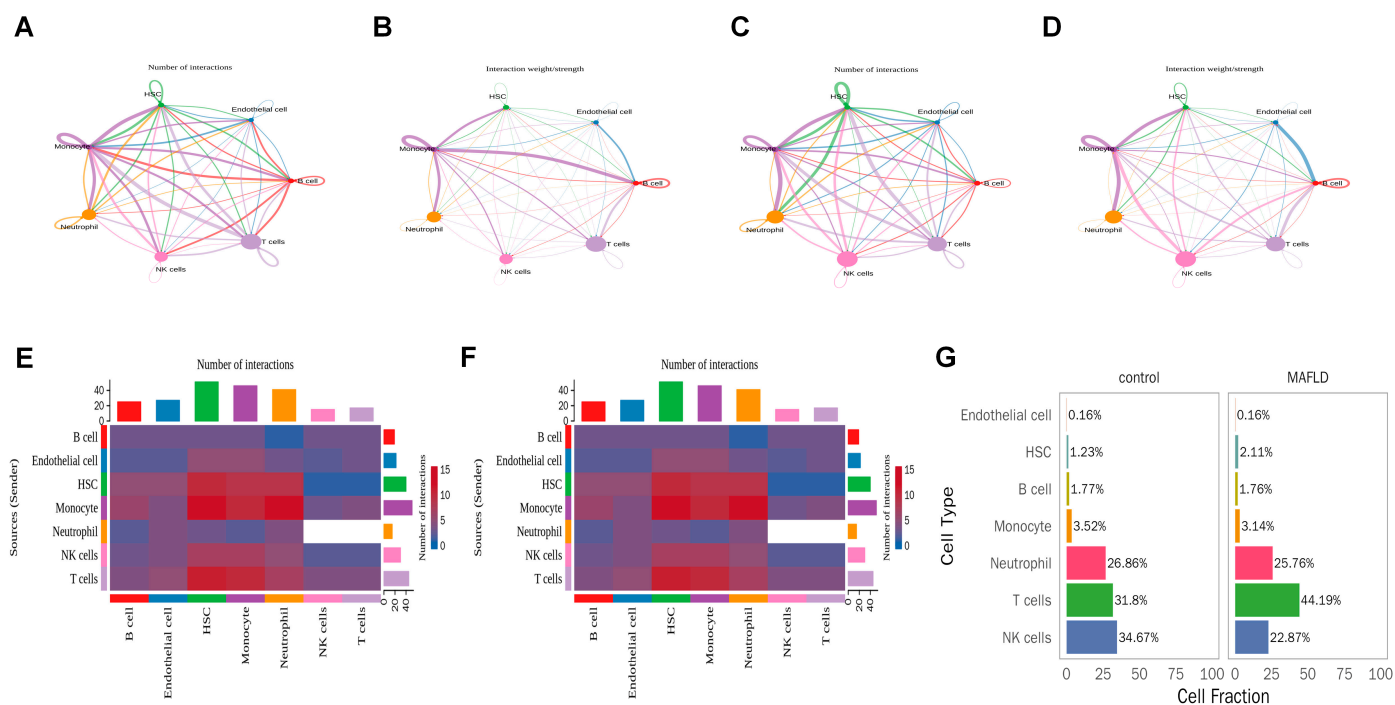


Figure S6. Cellular communication analysis in GSE186328. (A) The quantity of cell communication among different cell types in the MAFLD group. (B) The intensity of cell communication among different cell types in the MAFLD group. (C) The quantity of cell communication among different cell types in the control group. (D) The intensity of cell communication among different cell types in the control group. (E) The correlation of the quantity and intensity of intercellular interactions in the MAFLD group. (F) The correlation of the quantity and intensity of intercellular interactions in the MAFLD group. (G) The differences in the distribution of annotated cell types between the MAFLD and control groups. MAFLD: Metabolic Associated Fatty Liver Disease.

Table S1. The primer sequences of target genes.

Primer		Sequences
FADS1	F	CAACATCAGCGACTTCAGCC
FADS1	R	GCGCTTTATTCTTGTTGGGT
FADS2	F	CACAGCCTTTGTCCTCGCTA
FADS2	R	GGCTGAGGCACCCCTTTAAGT
GLB1	F	TCCGGATACCCCGCTTCTAC
GLB1	R	CACGGTCCCCAGAAAACCTCA
PNPLA3	F	CCCTCTCGGCCGTATAATGG
PNPLA3	R	CCCGTCTCTGATGCACTTGT
GAPDH	F	TGTGTCCGTCGTGGATCTGA
GAPDH	R	GAGTTGCTGTTGAAGTCGCA

Table S2. Protein classification information for FADS1, FADS2, GLB1, and PNPLA3.

ID	Gene name	Gene ID	PANTHER family/subfamily	PANTHER protein class
GLB1	Beta-galactosidase	HU- MAN HGNC=4298 U niProtKB=P16278	BETA-GALACTOSIDASE (PTHR23421:SF172)	galactosidase
FADS1	Acyl-CoA (8-3)-desaturase	HU- MAN HGNC=3574 U niProtKB=O60427	ACYL-COA (8-3)-DESATURASE (PTHR19353:SF57)	oxidoreductase
FADS2	Acyl-CoA 6-desaturase	HU- MAN HGNC=3575 U niProtKB=O95864	ACYL-COA 6-DESATURASE (PTHR19353:SF12)	oxidoreductase
PNPLA3	1-acylglycerol-3-phosphate O-acyltransferase PNPLA3	HU- MAN HGNC=18590 UniProtKB=Q9NST1	1-ACYLGLYCEROL-3-PHOSPHATE O-ACYLTRANSFERASE PNPLA3 (PTHR12406:SF22)	phospholipase

Table S3. Hydrogen bond interactions between estradiol and target proteins in molecular docking studies.

Gene	Drug	Residue(s)	Bond Distance(Å)	Binding Free Energy (kcal/mol)
FADS1	Estradiol	SER-185, LYS-78	2.1, 3.1	-7.7
FADS2	Estradiol	LEU-185	2.5	-8.6
GLB1	Estradiol	LEU-514, TRP-317	2.3, 3.2	-8.5
PNPLA3	Estradiol	SER-453	2.2	-9.6