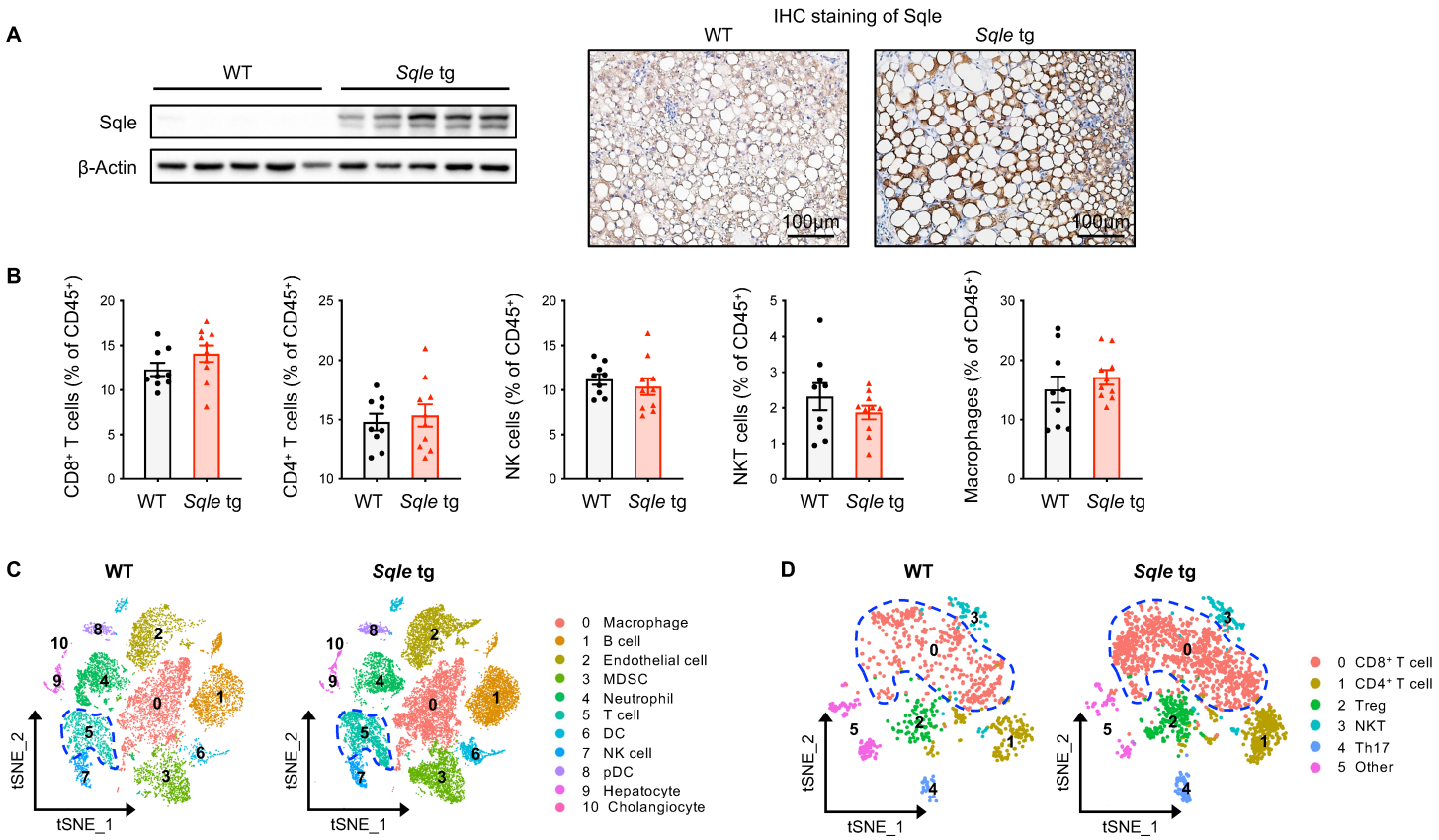
Fig. S1



Cluster	Cell type	Key differentially expressed transcripts
0	Macrophage	Itgam, Adgre1, C1qc, Mrc1, Cd5l
1	B cell	Cd79a, Cd79b, Ighm, Iglc2, Cd19
2	Endothelial cell	Sparc, Pecam1, Fabp4, Tek, Lyve1
3	MDSC	Itgam, Il1b, Fn1, Il1rn, Prdx1
4	Neutrophil	Itgam, S100a8, Retnlg, Hp, Msrb1
5	T cell	Cd3e, Cd3d, Trbc1, Thy1
6	DC	H2-Aa, Syngr2, Cd83, Cd74, Flt3, Batf3
7	NK cell	Ncr1, Klra7, Klre1
8	pDC	Siglech, Bst2, Ccr9
9	Hepatocyte	Alb, Serpina3k, Apoa1
10	Cholangiocyte	Epcam, Krt7, Krt19, Clu

Cluster	Cell type	Key differentially expressed transcripts
0	CD8⁺ T cell	Cd8a, Cd3e, Trbc1
1	CD4⁺ T cell	Cd4, Cd3e, Trbc1
2	Treg	Cd4, Foxp3, Ikzf2, Ccr8
3	NKT	Ncr1, Klra7, Klre1, Cd3e, Trbc1
4	Th17	Il17a, Il23r, Rorc, Furin
5	Other	Cd3e, Trbc1 (without high expression of neither Cd4 nor Cd8a)

Fig. S1. (**A**) Overexpression of Sqle in the livers of *Sqle tg* mice was confirmed by western blot (*Left*) and immunohistochemistry (IHC) staining (*Right*). (**B**) Percentages of intratumoral CD8$^+$ T cells, CD4$^+$ T cells, NK cells, NKT cells, and macrophages in WT and *Sqle* tg mice determined by flow cytometry. (**C**) tSNE plots of CD45$^+$ cells derived from tumors of WT or *Sqle* tg mice (*Upper*). Tumors isolated from three different mice of the same group were pooled per sample, and two samples per group were prepared for subsequent sequencing (n=2 per group). Two samples from the same group were presented in one tSNE plot. Each dot represents a single cell. Key differentially expressed transcripts that define each cell cluster are shown (*Down*). (**D**) tSNE plots of T lymphocytes (cluster #5 in fig. S1C) derived from tumors of WT or *Sqle* tg mice (*Upper*). Two samples from the same group were presented in one tSNE plot. Key differentially expressed transcripts that define each cell sub-cluster are shown (*Down*).

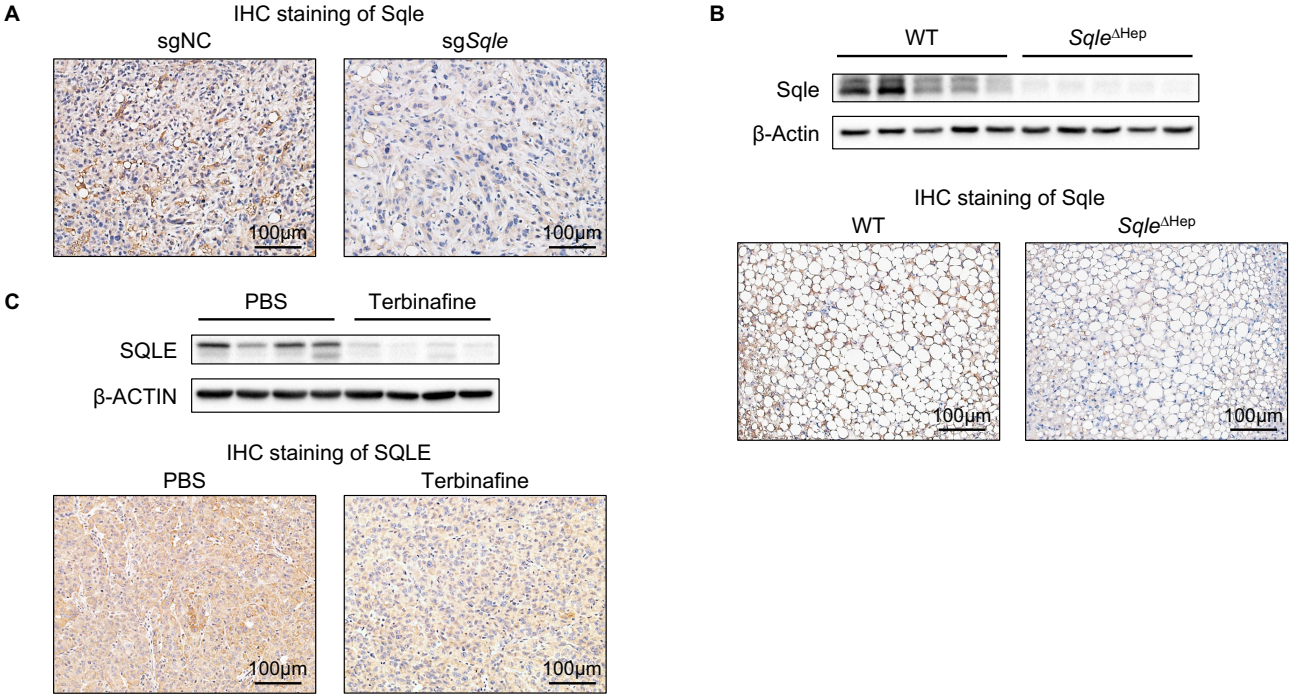


Fig. S2. (**A**) Knockout of Sqle in RIL-175 orthotopic tumors was confirmed by IHC staining. (**B**) Knockout of Sqle in the livers of *Sqle*$^{\Delta Hep}$ mice was confirmed by western blot (*Upper*) and IHC staining (*Down*). (**C**) Inhibition of terbinafine on SQLE of HKCI2 subcutaneous tumors in humanized mice was confirmed by western blot (*Upper*) and IHC staining (*Down*).

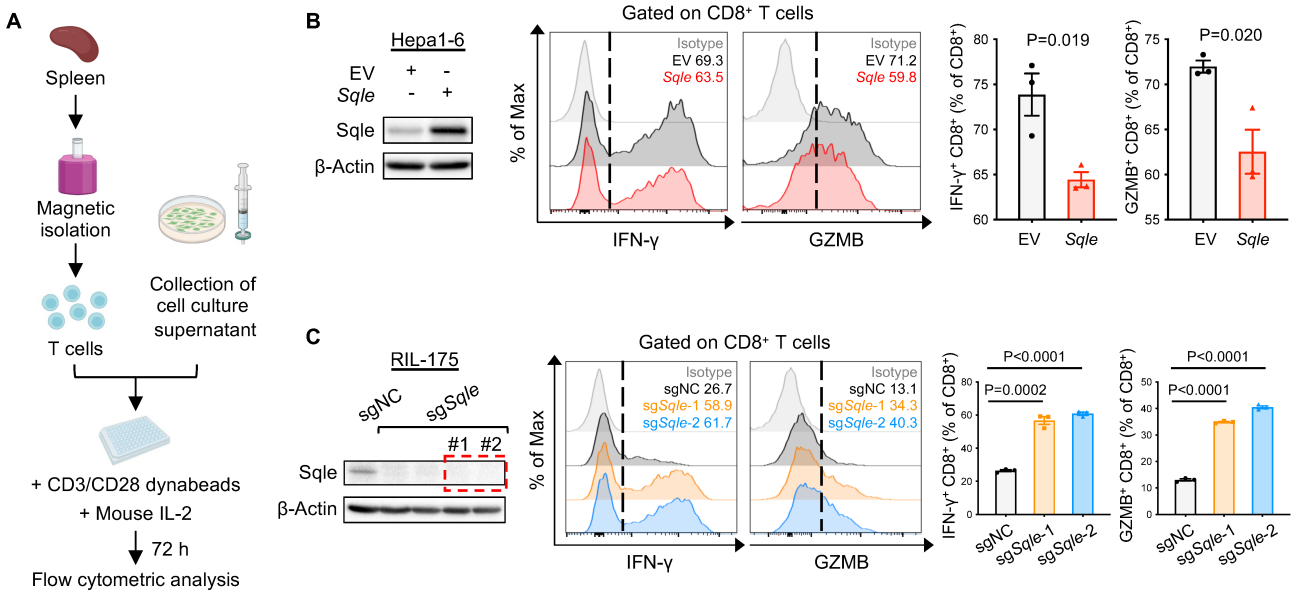


Fig. S3. (**A**) T cells harvested from tumor-free mouse spleens were treated with indicated conditioned medium for 72 h and were collected to be analyzed by flow cytometry. (**B**) Flow cytometry of IFN-γ$^+$ or Granzyme B$^+$ CD8$^+$ T cells treated with conditioned medium of Hepa1-6 cells with or without overexpression of SQLE. (**C**) Flow cytometry of IFN-γ$^+$ or Granzyme B$^+$ CD8$^+$ T cells treated with conditioned medium of RIL-175 cells with or without SQLE knockout.

Fig. S4

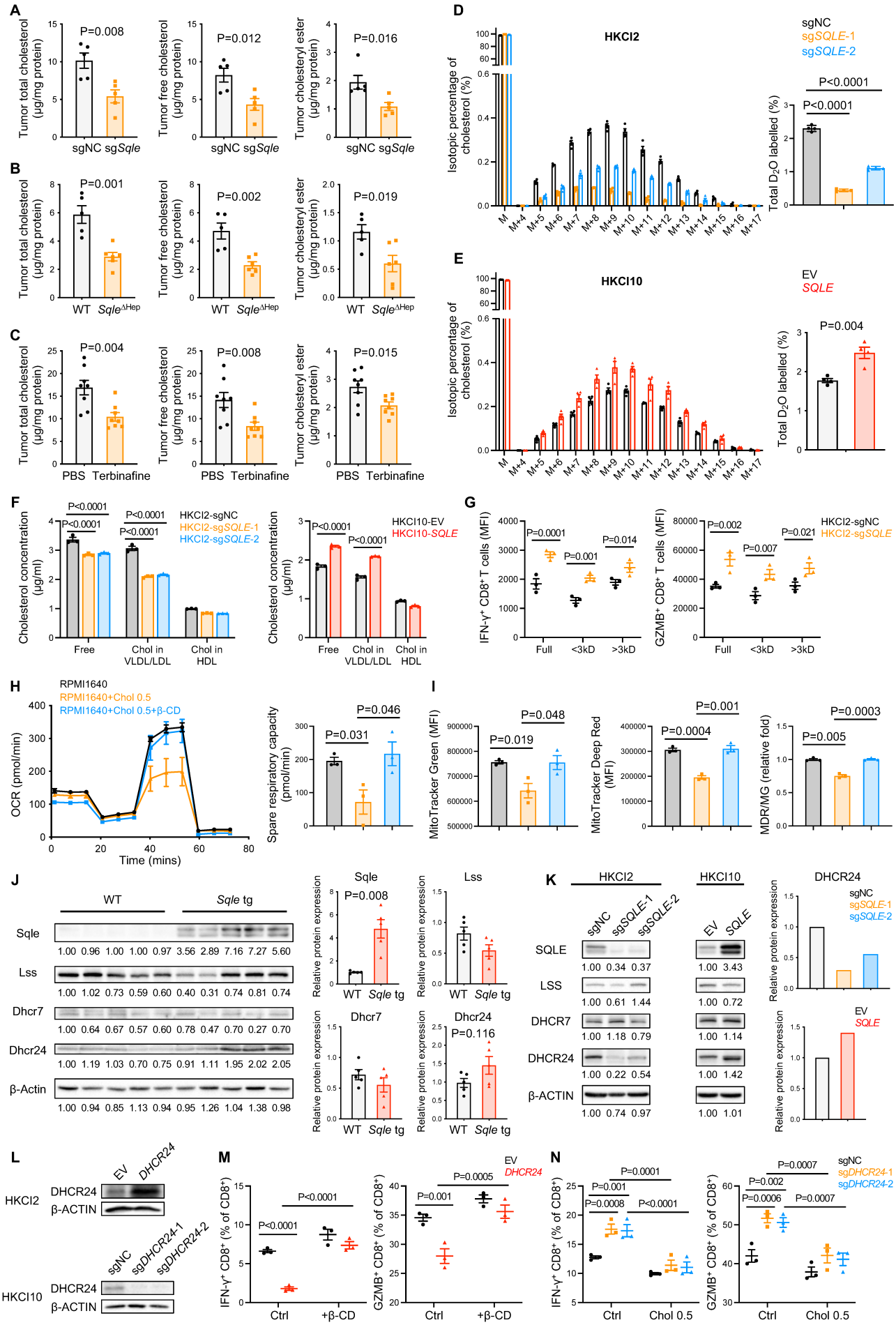

A
Tumor total cholesterol (μg/mg protein)
P=0.008
Tumor free cholesterol (μg/mg protein)
P=0.012
Tumor cholesteryl ester (μg/mg protein)
P=0.016
sgNC sgSqle
B
P=0.001
P=0.002
P=0.019
WT SqleΔHep
C
P=0.004
P=0.008
P=0.015
PBS Terbinafine
D
HKCI2
Isotopic percentage of cholesterol (%)
M M+4 M+5 M+6 M+7 M+8 M+9 M+10 M+11 M+12 M+13 M+14 M+15 M+16 M+17
sgNC
sgSQLE-1
sgSQLE-2
P<0.0001
P<0.0001
Total D2O labelled (%)
E
HKCI10
EV
SQLE
P=0.004
F
Cholesterol concentration (μg/ml)
HKCI2-sgNC
HKCI2-sgSQLE-1
HKCI2-sgSQLE-2
Free
Chol in VLDL/LDL
Chol in HDL
HKCI10-EV
HKCI10-SQLE
G
IFN-γ+ CD8+ T cells (MFI)
P=0.0001
P=0.001
P=0.014
Full
<3kD
>3kD
GZMB+ CD8+ T cells (MFI)
P=0.002
P=0.007
P=0.021
HKCI2-sgNC
HKCI2-sgSQLE
H
RPMI1640
RPMI1640+Chol 0.5
RPMI1640+Chol 0.5+β-CD
OCR (pmol/min)
Time (mins)
Spare respiratory capacity (pmol/min)
P=0.031
P=0.046
I
MitoTracker Green (MFI)
P=0.019
P=0.048
MitoTracker Deep Red (MFI)
P=0.0004
P=0.001
MDR/MG (relative fold)
P=0.005
P=0.0003
J
WT
Sqle tg
Sqle
1.00 0.96 1.00 1.00 0.97 3.56 2.89 7.16 7.27 5.60
Lss
1.00 1.02 0.73 0.59 0.60 0.40 0.31 0.74 0.81 0.74
Dhcr7
1.00 0.64 0.67 0.57 0.60 0.78 0.47 0.70 0.27 0.70
Dhcr24
1.00 1.19 1.03 0.70 0.75 0.91 1.11 1.95 2.02 2.05
β-Actin
1.00 0.94 0.85 1.13 0.94 0.95 1.26 1.04 1.38 0.98
Relative protein expression
P=0.008
P=0.116
K
HKCI2
HKCI10
sgNC
sgSQLE-1
sgSQLE-2
EV
SQLE
SQLE
1.00 0.34 0.37
1.00 3.43
LSS
1.00 0.61 1.44
1.00 0.72
DHCR7
1.00 1.18 0.79
1.00 1.14
DHCR24
1.00 0.22 0.54
1.00 1.42
β-ACTIN
1.00 0.74 0.97
1.00 1.01
DHCR24
sgNC
sgSQLE-1
sgSQLE-2
EV
SQLE
L
HKCI2
DHCR24
β-ACTIN
EV
DHCR24
HKCI10
sgNC
sgDHCR24-1
sgDHCR24-2
M
IFN-γ+ CD8+ (% of CD8+)
P<0.0001
P<0.0001
Ctrl
+β-CD
GZMB+ CD8+ (% of CD8+)
P=0.001
P=0.0005
EV
DHCR24
N
IFN-γ+ CD8+ (% of CD8+)
P=0.0001
P=0.001
P=0.0008
P<0.0001
Ctrl
Chol 0.5
GZMB+ CD8+ (% of CD8+)
P=0.0007
P=0.002
P=0.0006
P=0.0007
sgNC
sgDHCR24-1
sgDHCR24-2

Fig. S4

Fig. S4. (**A**) Total and free cholesterol concentrations in the tumors of orthotopic RIL-175 mouse model. (**B**) Total and free cholesterol concentrations in the tumors of DEN-injected CDHFD-fed MASH-HCC WT and hepatocyte-specific *Sqle* knockout mice. (**C**) Total and free cholesterol concentrations in HKCI2 subcutaneous tumors of humanized mouse model. (**D** and **E**) D_2O stable isotope labelling (48 h) of *de novo* synthesized cholesterol and LC-MS analysis of deuterium incorporated into cholesterol in HKCI2 (**D**) and HKCI10 (**E**) isogenic cells. (**F**) The conditioned medium of HKCI2 or HKCI10 cells was separated into low (<3kDa) and high (>3kDa) molecular weight fractions. Concentrations of free cholesterol and lipoprotein cholesterol were quantified separately. (**G**) The low (<3kDa) and high (>3kDa) molecular weight fractions of HKCI2-sgNC or -sg*SQLE* conditioned medium were used to culture T cells. $CD8^+$ T cell function was analyzed by flow cytometry. (**H**) Oxygen consumption rate (OCR) and spare respiratory capacity (SRC) were measured using seahorse analysis on $CD8^+$ T cells treated with RPMI1640 medium with or without the addition of cholesterol (0.5 μg/ml) and β-CD (0.5mM). (**I**) MFI of MitoTracker Green (MG) and MitoTracker Deep Red (MDR), and the ratio of MDR to MG of $CD8^+$ T cells treated with RPMI1640 medium with or without the addition of cholesterol (0.5 μg/ml) and β-CD (0.5mM). (**J** and **K**) Protein expression levels of SQLE downstream enzymes in cholesterol biosynthesis, namely LSS, DHCR7, and DHCR24, in *Sqle* tg mice (**J**), SQLE-knockout HKCI2 cells, and SQLE-overexpressing HKCI10 cells (**K**) were determined by western blot. (**L**) DHCR24 overexpression in HKCI2 cells and DHCR24 knockout in HKCI10 cells were validated by western blot. (**M**) Flow cytometric analysis of IFN-γ^+ or Granzyme B^+ $CD8^+$ T cells treated with the conditioned medium of HCKI2 cells with or without the overexpression of DHCR24, with or without β-CD (0.5mM). (**N**) Flow cytometric analysis of IFN-γ^+ or Granzyme B^+ $CD8^+$ T cells treated with the conditioned medium of HCKI10 cells with or without DHCR24 knockout, with or without the addition of cholesterol (0.5 μg/ml).

Fig. S5

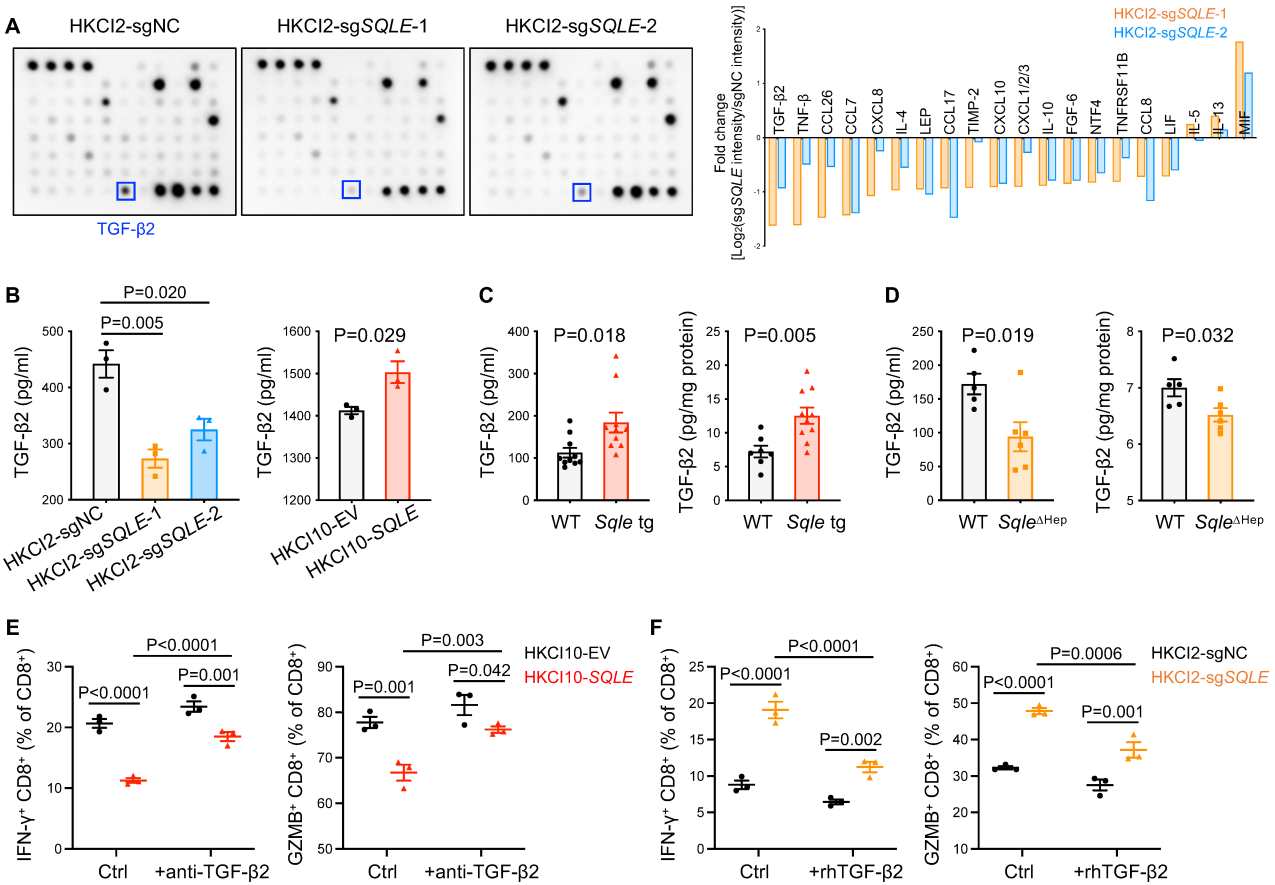


Fig. S5. (**A**) Cytokine antibody array was conducted on the conditioned medium of HKCI2 cells with or without SQLE knockout (*Left*). Densitometry showed TGF-β2 was the top down-regulated cytokine (*Right*). (**B**) ELISA measured TGF-β2 levels in the conditioned medium of SQLE-knockout and –overexpressing cells. (**C** and **D**) ELISA measured TGF-β2 levels in the serum and tumor lysates of hepatocyte-specific *Sqle* tg (**C**) and *Sqle* knockout (**D**) mice. (**E**) T cells were treated with the conditioned medium of HKCI10 cells with or without SQLE overexpression, with or without the addition of anti-TGF-β2 antibody (1 μg/ml), then measured by flow cytometry. (**F**) T cells were treated with the conditioned medium of HKCI2 cells with or without SQLE knockout, with or without the supplement of recombinant human TGF-β2 (10 ng/ml), then measured by flow cytometry.

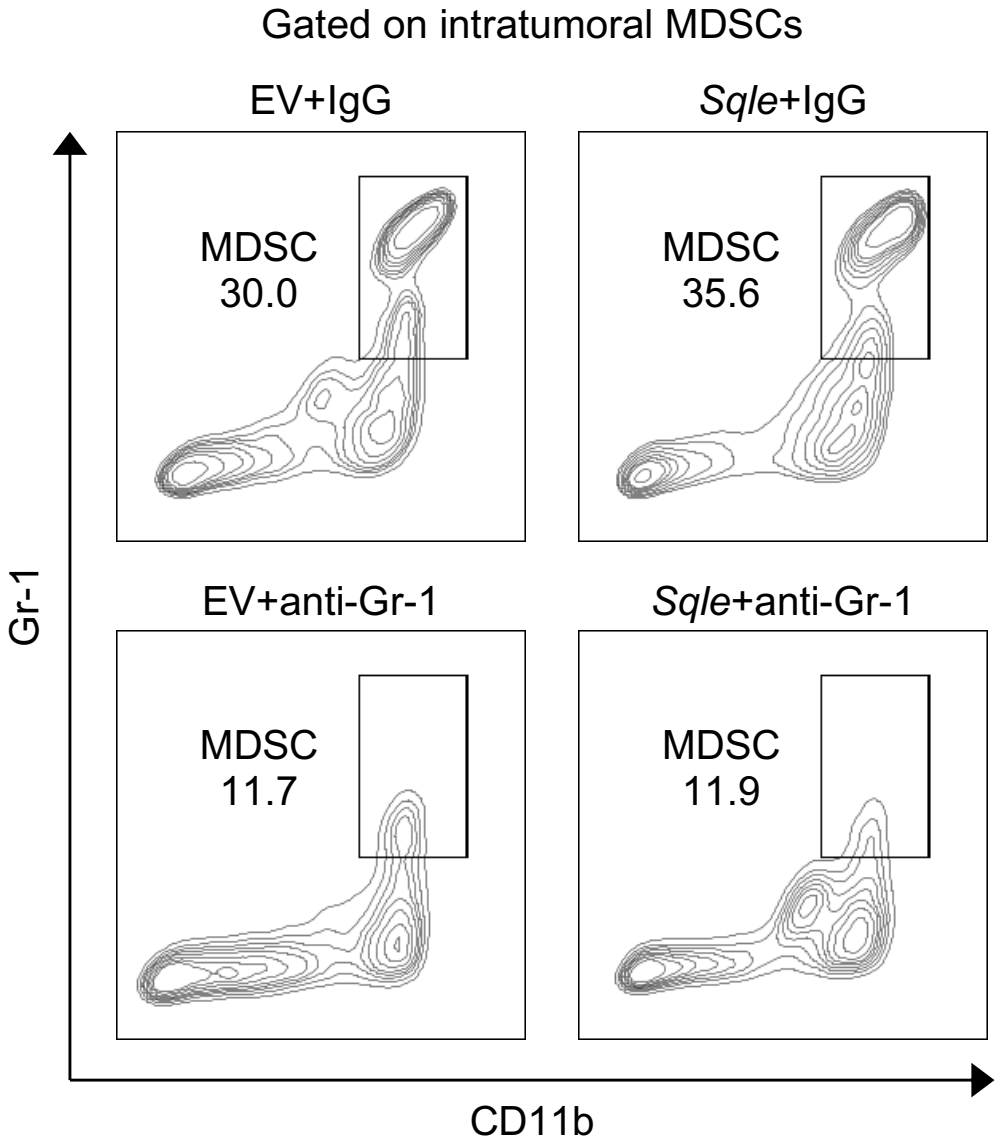


Fig. S6. Representative flow cytometric plots showed depletion efficiency of MDSCs in orthotopic tumors.

Fig. S7

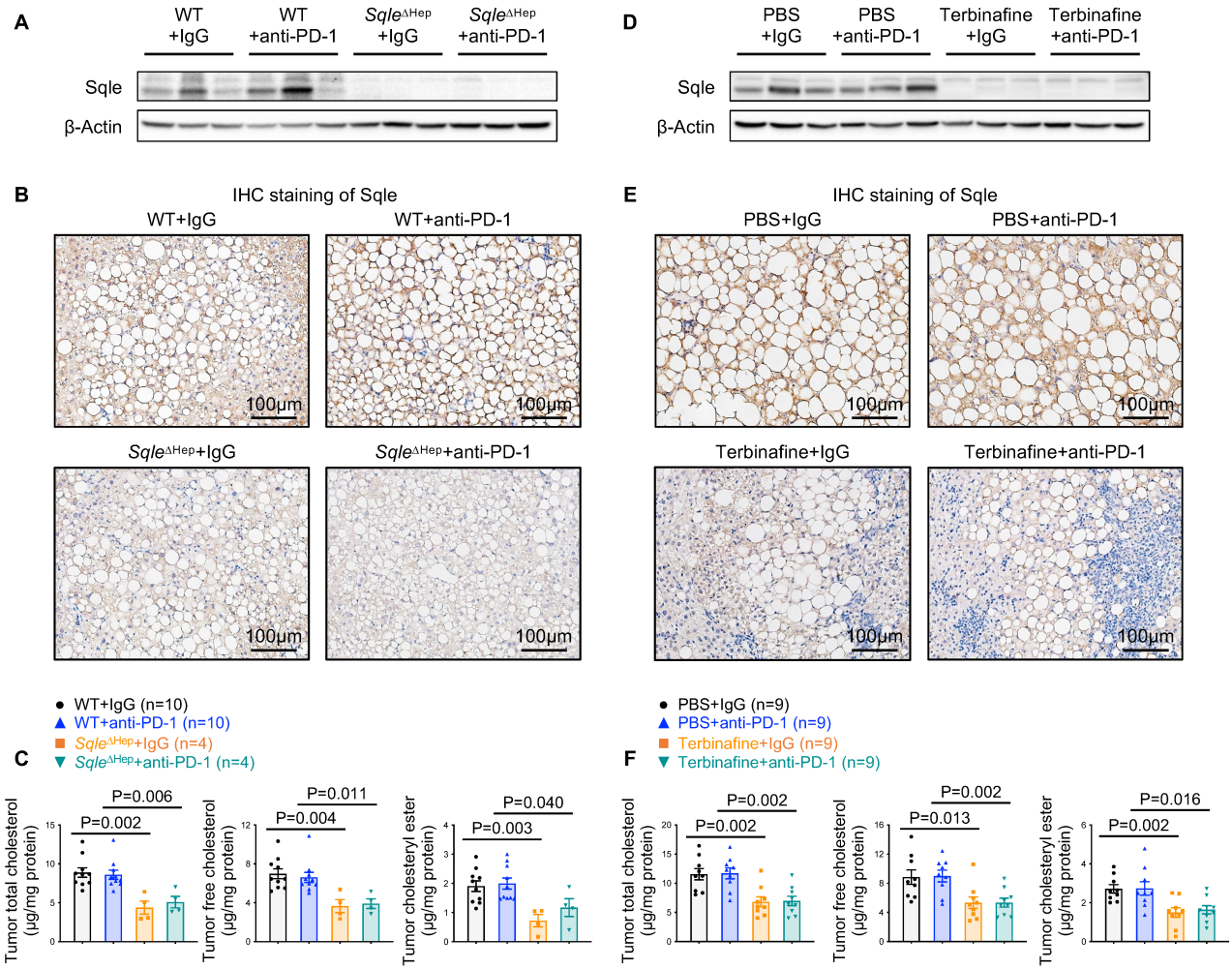


Fig. S7. (**A** and **B**) Knockout of Sqle in the livers of *Sqle*$^{\Delta Hep}$ mice in Fig. 7A was confirmed by western blot (**A**) and IHC staining (**B**). (**C**) Total cholesterol and free cholesterol concentrations in the tumors of mice in Fig. 7A were quantified. (**D** and **E**) Inhibition of terbinafine on Sqle in DEN-injected CDHFD-induced MASH-HCC mouse model in Fig. 7D was confirmed by western blot (**D**) and IHC staining (**E**). (**F**) Total cholesterol and free cholesterol concentrations in the tumors of mice in Fig. 7D were quantified.

Fig. S8

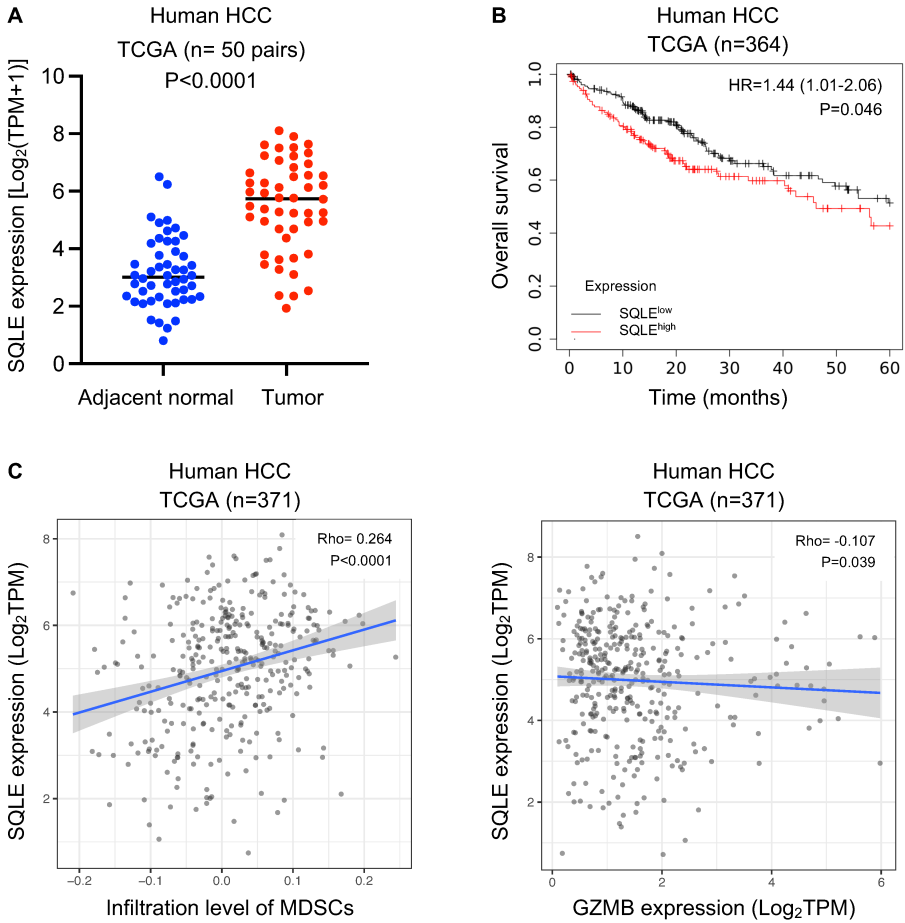


Fig. S8. (**A**) Expression of SQLE in human HCC and paired adjacent normal tissues was determined in TCGA cohort. (**B**) High SQLE expression was associated with poor survival in human HCC in TCGA cohort. (**C**) The association between SQLE expression and MDSC infiltration (*left*) or GZMB expression (*right*) was analyzed using TIMER2.0.

Supplementary Methods

Single-cell RNA sequencing

Fresh tumors isolated from WT or *Sqle* tg mouse of DEN-injected CDHFD-fed MASH-HCC were minced, washed with PBS, and digested twice in RPMI 1640 medium containing enzyme mixture of 0.35% collagenase IV, 120 U/ml DNase I, and 2 mg/ml papain for 15 mins at 37 °C with gentle rotation. Cell suspension was filtered through a 70 μm cell strainer, and tumor-infiltrating $CD45^+$ cells were enriched using CD45 (TIL) microbeads (Miltenyi Biotec). Tumors isolated from three different mice of the same group were pooled per sample, and two samples per group were prepared for library generation (10X Genomics Chromium Next GEM Single Cell 5' Kit) and sequenced on the Illumina NovaSeq 6000 system (double-end sequencing, 150bp).

scRNA-seq analyses

Libraries were sequenced at a mean sequencing depth ranging from 45,000 to 120,000 reads per cell. The scRNA-seq sequencing data were converted to FASTQ format using bcl2fastq software (v5.0.1). The sequencing data were compared to reference genome using Cell Ranger software (v7.0.0), and individual cellular 5' end transcripts were identified and counted. The expression profile matrix output by Cell Ranger was loaded into R (4.1.2) using Seurat (v4.1.0) for subsequent filtering of low-quality cells, normalization, dimensional reduction, and clustering. Single-cell transcripts were filtered according to the following criteria: (1) cells in which fewer than 500 genes were detected, or (2) cells with the proportion of mitochondrial transcripts > 25%, or (3) multiple cells in one GEM.

After filtration, the expression matrix contained 29,920 cells, with 11,011 cells from WT tumors and 18,909 cells from *Sqle* tg tumors. All downstream analyses were based on the

filtered high-quality cells. Dimensional reduction was accomplished by calculating gene expression values using the LogNormalize method, followed by performing principal component analysis (PCA) based on the normalized expression values using the top 20 principal components for clustering, and subsequently, visualized by t-Distributed Stochastic Neighbor Embedding (t-SNE). Major cell types were identified based on the expression of a set of indicative markers (figure S1C and S1D).

To assess differential enrichment of Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic pathways between $CD8^+$ T cells derived from *Sqle* tg and WT mouse tumors, gene set variation analysis (GSVA), a non-parametric unsupervised analysis method, was utilized. The result of GSVA was visualized using a bar plot.

Flow cytometric analysis

Tumors, liver tissues, and peripheral blood mononuclear cells (PBMCs) were harvested. After mechanical dissection, tumors and liver tissues were digested with Collagenase IV (Sigma-Aldrich) and DNase I (Sigma-Aldrich) at 37 °C for 30 mins with gentle rotation, followed by filtration through 70 μm strainers. Cells were collected, washed with PBS, and incubated with anti-CD16/32 antibody (Biolegend) to block Fc receptors for 10 mins at 4 °C, followed by immunostaining. For stimulation, cells were incubated at 37 °C under 5% CO_2 for 3 h, with a 1:500 cell stimulation cocktail (eBioscience). For intracellular staining, samples were fixed using Foxp3/transcription factor fixation/permeabilization set (eBioscience). Surface and intracellular staining were performed using antibodies listed in table S1. Samples were analyzed by FACSVerse or FACSCelesta (BD Biosciences), and the data were analyzed using FlowJo software (v10.8.1).

Assessment of human T cell effector function

PBMCs were freshly isolated from buffy coats of healthy donors using Ficoll-Paque Premium (Cytiva). Naïve T cells were isolated from PBMCs using human T cell isolation kit (STEMCELL) and cultured in cell culture supernatants in the presence of human T-activator CD3/CD28 dynabeads (Invitrogen) and recombinant human IL-2 (10 ng/ml, Biolegend). After 72 h co-culture, dynabeads were removed and cells were collected for further phenotypic and functional analysis using flow cytometry.

Mitochondrial function assays

Human peripheral blood $CD8^+$ T cells (STEMCELL) were cultured in the indicated conditioned medium for 48 h at 37 °C. Cells were then collected for MitoTracker Green and Mitotracker Deep Red (Invitrogen) staining to measure mitochondrial mass and membrane potential.

Seahorse culture plates were treated with 25 µl/well of 22.4 µg/ml of Cell-Tak (Corning) for 20 mins at RT. Cell-Tak solution was then removed and plates was washed twice with 200 µl sterile MilliQ water/well, air dried, and kept at 4 °C until the assay was conducted. Human peripheral blood $CD8^+$ T cells (STEMCELL) were cultured in the indicated conditioned medium for 48 h at 37 °C. Cells were then collected and seeded on Cell-Tak-coated plates (1×10^5 $CD8^+$ T cells per well) in Seahorse XF RPMI Medium supplemented with 1 mM pyruvate, 2 mM glutamine, and 10 mM glucose. Cells were centrifuged at 300 g for 2 mins, and incubated for 45 mins at 37 °C. Cells were monitored in basal respiration condition and stimulated with oligomycin (1.5 µM), FCCP (1 µM), and rotenone/antimycin A (0.5 µM) using Seahorse XFe96 Analyzer (Agilent). Spare respiratory capacity (SRC) was calculated as

the difference between the mean of initial oxygen consumption rate (OCR) values and the maximal OCR values achieved after FCCP added.

Evaluation of MDSC function and T cell suppression assay

Orthotopic tumors were freshly isolated from mice and immune cells were enriched by Percoll. Mouse spleen was minced, filtered through a 70 μm cell strainer, and then resuspended with 2 ml RBC lysis buffer on ice for 5 mins. The cell suspension derived from mouse tumor or spleen was purified for MDSCs using mouse MDSC ($CD11b^+Gr1^+$) isolation kit (STEMCELL). The isolated MDSCs from tumors were divided into three parts for RNA extraction, flow cytometry, and co-culture assays. Freshly isolated T cells from mouse spleen using mouse T cell isolation kit (STEMCELL) were labelled with or without carboxyfluorescein succinimidyl ester (CFSE; Invitrogen) and co-cultured with MDSCs (MDSC: T ratio=1:1 or 1:2) in the presence of mouse CD3/CD28 dynabeads (Invitrogen) and mouse IL-2 (10 ng/ml, Biolegend) for 72 h. After 72 h of co-culture, dynabeads were removed, and cells were collected for intracellular staining of IFN-γ and Granzyme B. CFSE signal intensity and functional marker intensity were acquired by flow cytometry. Human MDSCs were induced from PBMCs of healthy donors by adding recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF) (Biolegend) and IL-6 (Biolegend) (20 ng/ml) in RPMI1640 medium at 37 °C in a humidified incubator containing 5% CO_2 for 5 days. Yield and purity of MDSCs were confirmed by flow cytometry.

Cell lines and cell culture

Two human MASH-HCC cell lines HKCI2 and HKCI10 were generated previously from MASH-HCC patients by Prof. Nathalie Wong (Department of Surgery, CUHK)[1]. Murine HCC cell line RIL-175 was kindly provided by Dr. Greten and Prof. Zender[2]. The above cells were cultured in RPMI 1640 medium (Gibco, Thermo Fisher Scientific) supplemented with 10%

fetal bovine serum (Gibco) and maintained at 37 °C in a humidified incubator containing 5% CO_2.

Murine Hepa1-6 cells were purchased from purchased from the American Type Culture Collection (ATCC). The 293T cell line was purchased from Invitrogen (Thermo Fisher Scientific). The above two types of cell line were cultured in Dulbecco's Modified Eagle Medium (Gibco, Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (Gibco) and maintained at 37 °C in a humidified incubator containing 5% CO_2.

Construction of organoids derived from primary mouse liver tumors

Tumors derived from WT or *Sqle* tg mice were freshly collected and washed with PBS. Tumors were cut into small pieces, washed with PBS for three times to remove grease in the supernatant, minced into smaller pieces with the addition of digestion medium, and then incubated at 37 °C under 5% CO_2 for 2 h, with gentle shaking every 20 mins. The cell suspension was filtered through a 70 μm cell strainer, and then resuspended with 2 ml RBC lysis buffer (Biolegend) on ice for 5 mins. After centrifugation, the cell pellet was resuspended in appropriate volume of mouse liver organoid medium mixed with appropriate volume of Matrigel (Corning), and subsequently, seeded on the culture plate and maintained at 37 °C in a humidified incubator containing 5% CO_2.

Lentivirus packaging

Single guide RNAs (sgRNAs) targeting mouse Sqle (sgRNA1, CCGCTGTCGCCATCGACACGGGG; sgRNA2, CGTGCTGGTGTTCCTGTCGCTGG), human SQLE (sgRNA1, CGTGCTGGTGTTCCTCTCGCTGG; sgRNA2, CCGCTGTCGCCACCGAAACGGGG), or human DHCR24 (sgRNA1,

GTCATCAAGCTCAGGCAACA; sgRNA2, GCAAGACCTTCATGTGCACG) were cloned into pLenti-CRISPR v2 vector. The full-length open reading frame of human SQLE (NM_003129.4), mouse Sqle (NM_009270), or human DHCR24 (NM_014762.4) was cloned into pCDH-CMV-MCS-EF1-Puro vector. For lentivirus packaging, 6 μg of plasmid DNA, 4.5 μg of psPAX2 (Addgene), 1.5 μg of pMD2.G (Addgene), and 30 μl of lipofectamine 2000 (Invitrogen) were mixed in 1.5 ml Opti-MEM (Gibco) and added into the 293T cells in a 10 cm culture dish. The medium was changed at 6 h post-transfection, and the culture supernatant was collected at 48 h after transfection.

RNA extraction and real-time quantitative PCR

Total RNA was extracted using TRIzol reagent (Invitrogen). Subsequently, complementary DNA (cDNA) was synthesized from a total of 1 μg RNA using RT reagent kit (TaKaRa). Real-time PCR was conducted using aliquots of cDNA and SYBR Green PCR Master Mix (Applied Biosystems) on a LightCycler 480 real-time PCR system (Roche). All reactions were performed in triplicate. β-actin was used as an internal control. 2^(-ΔΔCt) method was applied to calculate the fold change of the target gene expression. Primers used are listed in table S2.

Western blot analysis

Total protein was resolved by 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene fluoride membrane. After blocking with 5% skim milk (Sigma-Aldrich) for 1 h at room temperature, the membrane was incubated with primary antibodies (table S1) overnight at 4 °C followed by the secondary antibody for 1 h at room temperature. The proteins of interest were detected using ECL Western Blotting Substrates (Bio-Rad).

Cholesterol/ cholesteryl ester concentration measurement

Free cholesterol and cholesteryl ester contents of cell culture supernatants and mouse tumor tissues were measured using the Cholesterol/ Cholesteryl Ester Quantification Assay kit (Abcam) according to the manufacturer's instructions. Lipoprotein cholesterol concentrations were measured using the Cholesterol Assay Kit-HDL and LDL/VLDL (Abcam) according to the manufacturer's instructions.

Cytokine antibody array and ELISA determination of TGF-β2

The conditioned medium of HKCI2 with or without SQLE knockout was collected after 48 h culture, filtered through a 0.22 μm strainer, and analyzed with Human Cytokine Antibody Array (Membrane, 80 Targets; Abcam) following the manufacturer's instructions. Signal intensity of each spot was measured with Image Lab software (Bio-Rad) and normalized by six positive control intensities on each membrane.

The concentrations of human TGF-β2 in cell culture conditioned medium were measured with Human TGF-beta2 Quantikine ELISA Kit (R&D Systems). Mouse TGF-β2 in the serum and tumor lysates was determined by Mouse/Rat/Canine/Porcine TGF-beta2 Quantikine ELISA Kit (R&D Systems). TGF-beta2 Antibody and Recombinant Human TGF-beta2 Protein (R&D Systems) were used in T cell co-culture assays *in vitro*.

Serum AFP measurement

Serum AFP levels of WT and *Sqle* tg or knockout mice were detected using the mouse AFP Quantikine ELISA kit (R&D Systems) according to the manufacturer's instructions.

Analysis of the Cancer Genome Atlas (TCGA)-Liver Hepatocellular Carcinoma dataset

The overall survival of HCC patients in relation to SQLE expression was assessed by the Kaplan-Meier survival curve and the log-rank test using KM Plotter[3]. The correlation between SQLE expression and MDSC infiltration or GZMB expression was evaluated with the Spearman's correlation coefficient Rho using TIMER 2.0[4].

References

1. Xu W, Zhang X, Wu JL, et al. O-GlcNAc transferase promotes fatty liver-associated liver cancer through inducing palmitic acid and activating endoplasmic reticulum stress. J Hepatol 2017;67:310-320.
2. Eggert T, Wolter K, Ji J, et al. Distinct Functions of Senescence-Associated Immune Responses in Liver Tumor Surveillance and Tumor Progression. Cancer Cell 2016;30:533-547.
3. Menyhárt O, Nagy Á, Győrffy B. Determining consistent prognostic biomarkers of overall survival and vascular invasion in hepatocellular carcinoma. R Soc Open Sci 2018;5:181006.
4. Li T, Fu J, Zeng Z, et al. TIMER2.0 for analysis of tumor-infiltrating immune cells. Nucleic Acids Res 2020;48:W509-w514.

Table S1. Antibodies used in this study

Antibodies	Source	Cat #
Brilliant Violet 605™ anti-mouse CD45 Antibody	Biolegend	103140
PE anti-mouse CD3 Antibody	Biolegend	100206
PE/Cyanine7 anti-mouse CD4 Antibody	Biolegend	100528
Brilliant Violet 711™ anti-mouse CD8a Antibody	Biolegend	100759
PE/Cyanine5 anti-mouse NK-1.1 Antibody	Biolegend	108716
Brilliant Violet 421™ anti-mouse CD279 (PD-1) Antibody	Biolegend	135221
FITC anti-mouse/human CD11b Antibody	Biolegend	101206
PE/Cyanine5 anti-mouse Ly-6G/Ly-6C (Gr-1) Antibody	Biolegend	108410
PE/Cyanine7 anti-mouse Ly-6G Antibody	Biolegend	127618
Brilliant Violet 421™ anti-mouse Ly-6C Antibody	Biolegend	128032
Arginase 1 Monoclonal Antibody (A1exF5), PE	eBioscience	12-3697-82
Arginase 1 Monoclonal Antibody (A1exF5), PE-Cyanine7	eBioscience	25-3697-82
PE/Cyanine5 anti-mouse F4/80 Antibody	Biolegend	123112
PE anti-mouse/human CD11b Antibody	Biolegend	101208
PE/Cyanine7 anti-mouse CD11c Antibody	Biolegend	117318
Brilliant Violet 421™ anti-mouse I-A/I-E Antibody	Biolegend	107632
Brilliant Violet 711™ anti-mouse CD206 (MMR) Antibody	Biolegend	141727
PE/Cyanine5 anti-mouse CD8a Antibody	Biolegend	100710
FITC anti-mouse TNF-α Antibody	Biolegend	506304
Brilliant Violet 421™ anti-mouse TNF-α Antibody	Biolegend	506328
Brilliant Violet 711™ anti-mouse IFN-γ Antibody	Biolegend	505836
Granzyme B Monoclonal Antibody (NGZB), PE-Cyanine7	eBioscience	25-8898-82
FITC anti-mouse CD3 Antibody	Biolegend	100203
PE anti-mouse CD186 (CXCR6) Antibody	Biolegend	151104

Purified anti-mouse CD16/32 Antibody	Biolegend	101302
APC/Cyanine7 anti-mouse CD45 Antibody	Biolegend	103116
FITC anti-mouse CD4 Antibody	Biolegend	100510
PerCP/Cyanine5.5 anti-mouse CD8a Antibody	Biolegend	100734
APC anti-mouse NK-1.1 Antibody	Biolegend	108709
Brilliant Violet 510™ anti-mouse CD19 Antibody	Biolegend	115546
Brilliant Violet 421™ anti-mouse Ly-6G/Ly-6C (Gr-1) Antibody	Biolegend	108445
FITC anti-mouse Ly-6G Antibody	Biolegend	127606
PerCP/Cyanine5.5 anti-mouse Ly-6C Antibody	Biolegend	128011
Arginase 1 Monoclonal Antibody (A1exF5), APC	eBioscience	17-3697-82
FITC anti-mouse F4/80 Antibody	Biolegend	123108
PerCP/Cyanine5.5 anti-mouse CD206 (MMR) Antibody	Biolegend	141715
APC anti-mouse CD11c Antibody	Biolegend	117309
Brilliant Violet 510™ anti-mouse TNF-α Antibody	Biolegend	506339
APC anti-mouse IFN-γ Antibody	Biolegend	505809
FITC anti-human/mouse Granzyme B Antibody	Biolegend	515403
SQLE Polyclonal antibody	Proteintech	12544-1-AP
β-Actin (13E5) Rabbit mAb	Cell Signaling	4970
Anti-DHCR7 antibody	Abcam	ab103296
DHCR24 Polyclonal antibody	Proteintech	10471-1-AP
LSS Polyclonal antibody	Proteintech	13715-1-AP
TGF-beta 2 Antibody	R&D Systems	AB-12-NA
InVivoPlus anti-mouse PD-1 (CD279)	BioXCell	BP0146
InVivoPlus rat IgG2a isotype control, anti-trinitrophenol	BioXCell	BP0089
InVivoMAb anti-mouse CD8α	BioXCell	BE0061
InVivoMAb rat IgG2b isotype control, anti-keyhole limpet hemocyanin	BioXCell	BE0090

InVivoMAb anti-mouse Ly6G/Ly6C (Gr-1)	BioXCell	BE0075

Table S2. Primer sequences

Primer name		Sequences
Arg1	Forward	CCTTTCTCAAAAGGACAGCCTC
	Reverse	CAGACCGTGGGTTCTTCACA
Nos2 (iNOS)	Forward	TCTAGTGAAGCAAAGCCCAACA
	Reverse	CTCTCCACTGCCCCAGTTTT
Ido1	Forward	GATGTTCGAAAGGTGCTGCC
	Reverse	AGAAGCTGCGATTTCCACCA
Tgfb1	Forward	GATACGCCTGAGTGGCTGTC
	Reverse	GGGGCTGATCCCGTTGATT
Cd274 (Pdl1)	Forward	CCTCGCCTGCAGATAGTTCC
	Reverse	CCCAGTACACCACTAACGCA
S100a9	Forward	GATGGAGCGCAGCATAACCA
	Reverse	GTTTGTGTCCAGGTCCTCCAT