Sub Class	Common Name	Synonyms	Molecular Formula	Yumm 5.2	B16F10	A375
Short-chain	Formic acid	C1:0	C1H2O1	1mM	1mM	1mM
	Acetic acid	C2:0	C2H4O2	1mM	1mM	1mM
	Propionic acid	C3:0	C3H6O2	1mM	1mM	1mM
	Butyric acid	C4:0	C4H8O2	1mM	1mM	1mM
	Isobutyric acid	C4:0	C4H8O2	1mM	1mM	1mM
	Valeric acid	C5:0	C5H10O2	1mM	1mM	1mM
Medium-chain	Caproic acid (Hexanoic acid)	C6:0	C6H12O2	100μM	100μM	100μM
	Caprylic acid (Octanoic acid)	C8:0	C8H16O2	100μM	100μM	100μM
	Capric acid (Decanoic acid)	C10:0	C10H20O2	100μM	100μM	100μM
	Lauric acid	C12:0	C12H24O2	40μM	100μM	100μM
Long-chain	Myristic acid	C14:0	C14H28O2	20μM	20μM	20μM
	Palmitic acid (PA)	C16:0	C16H32O2	20μM	20μM	20μM
	Stearic acid (SA)	C18:0	C18H36O2	20μM	20μM	20μM
	Arachidic acid	C20:0	C20H40O2	10μM	20μM	20μM
	Myristoleic acid	C14:1	C14H26O2	40μM	40μM	40μM
	Palmitoleic acid (POA)	C16:1	C16H30O2	40μM	40μM	40μM
	Oleic acid (OA)	C18:1	C18H34O2	40μM	40μM	40μM
	Linoleic acid (LA)	C18:2	C18H32O2	20μM	20μM	20μM
	Linoelaidic acid(γLA)	C18:2	C18H32O2	20μM	20μM	20μM
	α-Linolenic acid (αLA)	C18:3	C18H30O2	20μM	20μM	20μM
	Arachidonic acid (AA)	C20:4	C20H32O2	20μM	20μM	30μM
	Eicosapentaenoic acid (EPA)	C20:5	C20H30O2	20μM	20μM	20μM
Very long-chain	Behenic acid	22:0	C22H44O2	20μM	20μM	20μM
	Lignoceric acid	24:0	C24H48O2	10μM	10μM	10μM
	Erucic acid	22:1	C22H42O2	40μM	40μM	40μM
	Nervonic acid	24:1	C24H46O2	40μM	40μM	40μM
	Docosahexaenoic acid (DHA)	22:6	C22H32O2	20μM	20μM	

Table S1. All types of typical fatty acids that are used in this study, Related to Figure 1. The final concentrations of individual fatty acid applied to each cell lines are listed.

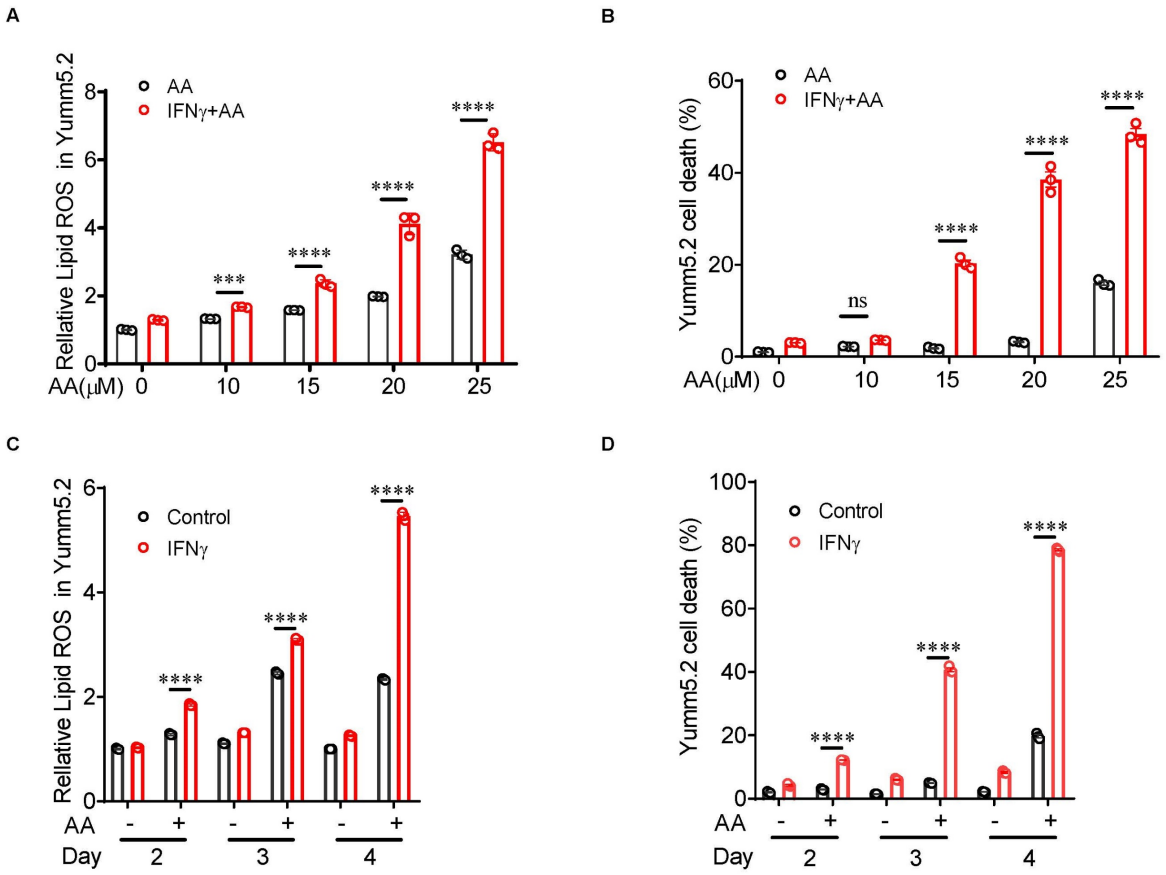


Figure S1. Effect of arachidonic acid and IFNγ on tumor cell death, Related to Figure 1.

(A and B) Percentage of relative lipid ROS (A) or dead cells (B) in Yumm5.2 cells treated with IFNγ plus different concentrations of arachidonic acid (AA) for 3 days. n = 3 biological replicates. ***P = 0.0003 and ****P < 0.0001 (A); ns, P = 0.64 and ****P < 0.0001 (B) (two-way ANOVA).

(C and D) Percentage of relative lipid ROS (C) or dead cells (D) in Yumm5.2 cells treated with IFNγ and AA for 2-4 days. n = 3 biological replicates. (two-way ANOVA). ****P < 0.0001 (C) and ****P < 0.0001 (D) (two-way ANOVA).

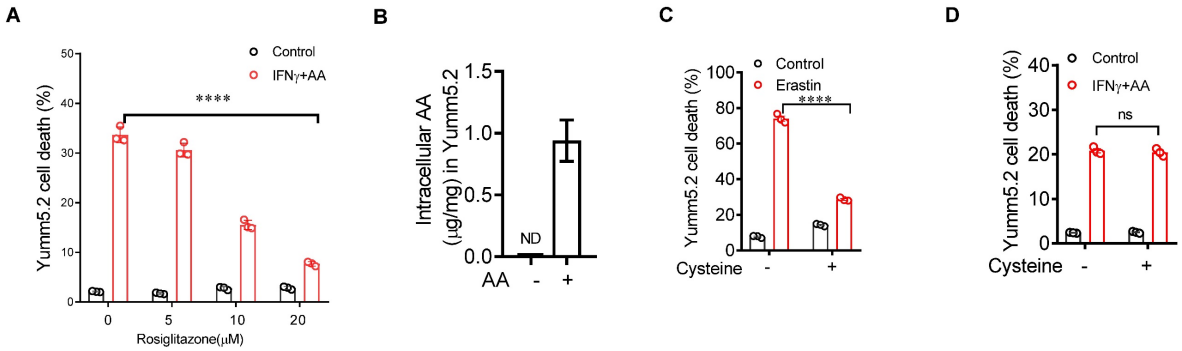


Figure S2. Effect of arachidonic acid and IFNγ on tumor ferroptosis, Related to Figure 2.

(A) Percentage of dead cells in Yumm5.2 cells treated with IFNγ plus AA for 3 days in the presence of different doses of Rosiglitazone. n = 3 biological replicates. ****$P < 0.0001$ (two-way ANOVA).

(B) AA levels in Acsl4$^{+/+}$ or Acsl4$^{-/-}$ Yumm5.2 cells treated AA (20 M) for 2 days. AA was detected by ELISA and normalized by tumor proteins. n = 2 biological replicates, ND, not detected.

(C) Percentage of dead Yumm5.2 cells treated with 2 M Erastin for 24 hours in the presence of 200 μM cysteine. n = 3 biological replicates. ****$P < 0.0001$ (two-way ANOVA).

(D) Percentage of dead Yumm5.2 cells treated with IFNγ and AA for 3 days in the presence of 200 μM cysteine. n = 3 biological replicates. ns, $P = 0.98$ (two-way ANOVA).

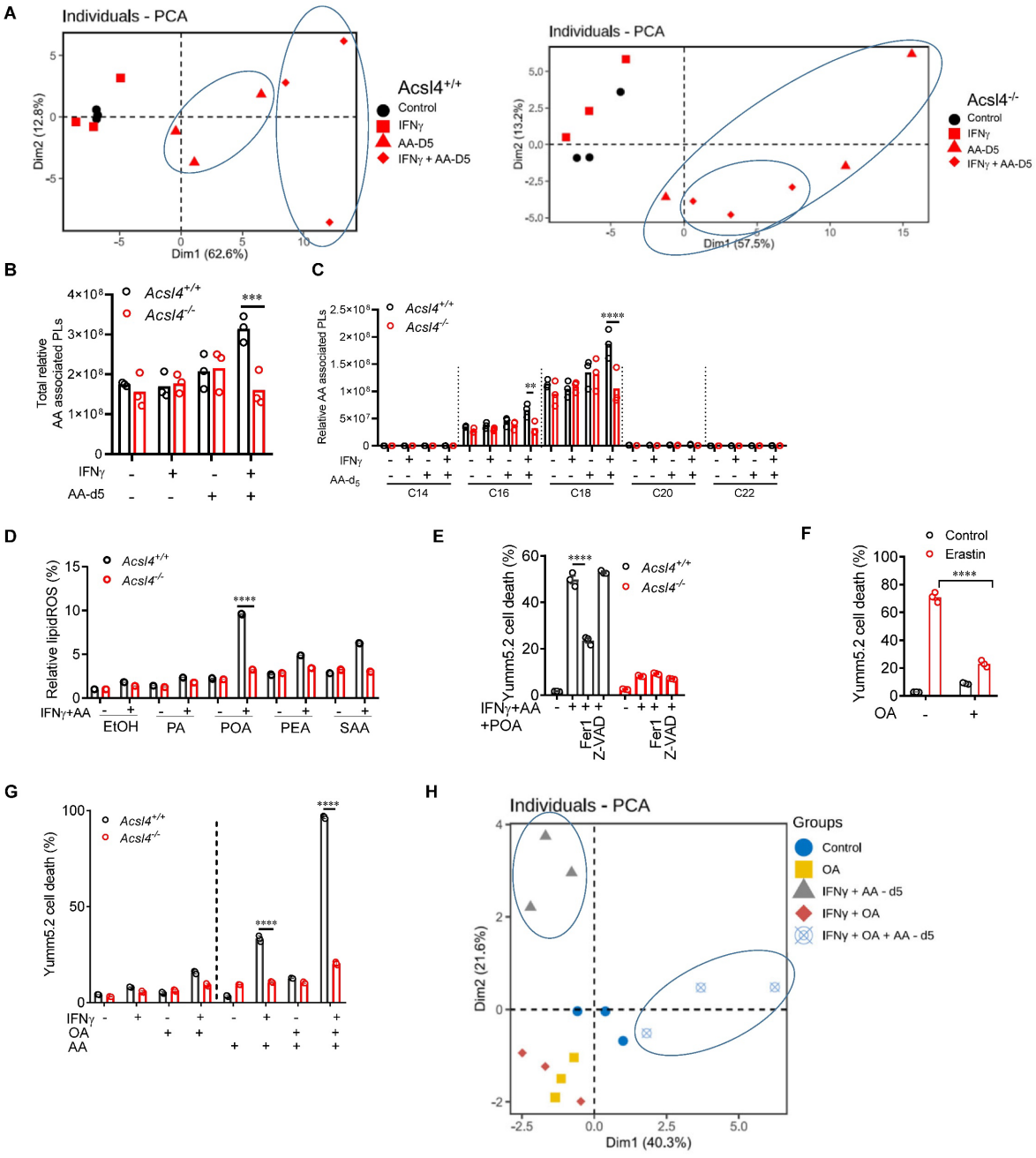

A
Individuals - PCA
Dim2 (12.8%)
Dim1 (62.6%)
Acsl4+/+
Control
IFNγ
AA-D5
IFNγ + AA-D5
Individuals - PCA
Dim2 (13.2%)
Dim1 (57.5%)
Acsl4-/-
Control
IFNγ
AA-D5
IFNγ + AA-D5
B
Total relative AA associated PLs
Acsl4+/+
Acsl4-/-
***
IFNγ
AA-d5
C
Relative AA associated PLs
Acsl4+/+
Acsl4-/-
**
****
IFNγ
AA-d5
C14
C16
C18
C20
C22
D
Relative lipidROS (%)
Acsl4+/+
Acsl4-/-
****
IFNγ+AA
EtOH
PA
POA
PEA
SAA
E
Yumm5.2 cell death (%)
Acsl4+/+
Acsl4-/-
****
IFNγ+AA +POA
Fer1
Z-VAD
Fer1
Z-VAD
F
Yumm5.2 cell death (%)
Control
Erastin
****
OA
G
Yumm5.2 cell death (%)
Acsl4+/+
Acsl4-/-
****
****
IFNγ
OA
AA
H
Individuals - PCA
Groups
Control
OA
IFNγ + AA - d5
IFNγ + OA
IFNγ + OA + AA - d5
Dim2 (21.6%)
Dim1 (40.3%)

Figure S3. Role of IFNγ in ACSL4 associated phospholipids, Related to Figure 4.

(A) Principal component analysis (PCA) of lipid distribution in *Acsl4*$^{+/+}$ and *Acsl4*$^{-/-}$ Yumm5.2 cells. Yumm5.2 cells were treated with IFNγ, AA-d_5, and their combination for 48 hours. Lipids were analyzed by ultra-performance liquid chromatography–tandem mass spectrometry.

(B) Total AA (non-deuterated AA-d_0 and deuterated AA-d_5) associated phospholipids (PLs) in *Acsl4*$^{+/+}$ or *Acsl4*$^{-/-}$ Yumm5.2 cells treated with IFNγ, AA-d_5, and their combination for 48 hours. n = 3 biological replicates, mean ± s.d. ***P = 0.0005 (two-way ANOVA).

(C) Distribution of different length fatty acids in AA associated PLs in *Acsl4*$^{+/+}$ or *Acsl4*$^{-/-}$ Yumm5.2 cells treated with IFNγ, AA-d_5, and their combination for 48 hours. n = 3 biological replicates, mean ± s.d. *P = 0.0407, and ****P < 0.0001 (two-way ANOVA).

(D) Relative lipid ROS in *Acsl4*$^{+/+}$ or *Acsl4*$^{-/-}$ Yumm5.2 cells treated with IFNγ and AA (10 μM) in the presence of PA (20 μM), POA (60 μM), PEA (60 μM), and SAA (60 μM) for 48 hours. n = 3 biological replicates, mean ± s.d. ****P < 0.0001 (two-way ANOVA).

(E) Percentage of dead cells in *Acsl4*$^{+/+}$ or *Acsl4*$^{-/-}$ Yumm5.2 cells treated with IFNγ, AA (10 μM), and POA (40 μM) in the presence of ferrostatin-1 (2 μM Fer1) or z-VAD-FMK (10 μM z-VAD) for 48 hours. n = 3 biological replicates, mean ± s.d. ****P < 0.0001 (two-way ANOVA).

(F) Percentage of dead Yumm5.2 cells treated with Erastin in the presence of OA (60 μM) for 24 hours. n = 3 biological replicates (mean ± s.d.). ****P < 0.0001 (two-way ANOVA).

(G) Percentage of dead cells in *Acsl4*$^{+/+}$ or *Acsl4*$^{-/-}$ Yumm5.2 cells treated with IFNγ, OA (60 μM), AA (20 μM), and their combination for 3 days. n = 3 biological replicates, mean ± s.d. ****P < 0.0001 (two-way ANOVA).

(H) Principal component analysis (PCA) of lipid distribution in Yumm5.2 cells treated with I IFNγ, OA and AA-d5. Cells were treated with IFNγ (10 ng/ml), OA (60 μM), AA-d5 (10 μM) or their combinations for 48 hours Whole cell phospholipids were analyzed by ultra-performance liquid chromatography–tandem mass spectrometry. n = 3 replicates.

Sub Class	Common Name	Abbreviation	Synonyms	Systematic Name	Molecular Formula	Exact Mass
Saturate	Palmitic Acid	PA	C16:0	hexadecanoic acid	C16H32O2	256.2
	Stearic Acid	SA	C18:0	octadecanoic acid	C18H36O2	284.3
Unsaturate	Palmitoleic acid	POA	C16:1n-7	9Z-hexadecenoic acid	C16H30O2	254.2
	Palmitelaidic acid	PEA	C16:1n-7	9E-hexadecenoic acid	C16H30O2	254.2
	Sapienic acid	SAA	C16:1n-10	6Z-hexadecenoic acid	C16H30O2	254.2
	Oleic acid	OA	C18:1n-9	9Z-octadecenoic acid	C18H34O2	282.3
	Elaidic acid	EA	C18:1n-9	9E-octadecenoic acid	C18H34O2	282.3
	trans-vaccenic acid	VA	C18:1n-7	11E-octadecenoic acid	C18H34O2	282.3

Table S2. Isomers of C16:1 and C18:1 fatty acids. Related to Figure 4.

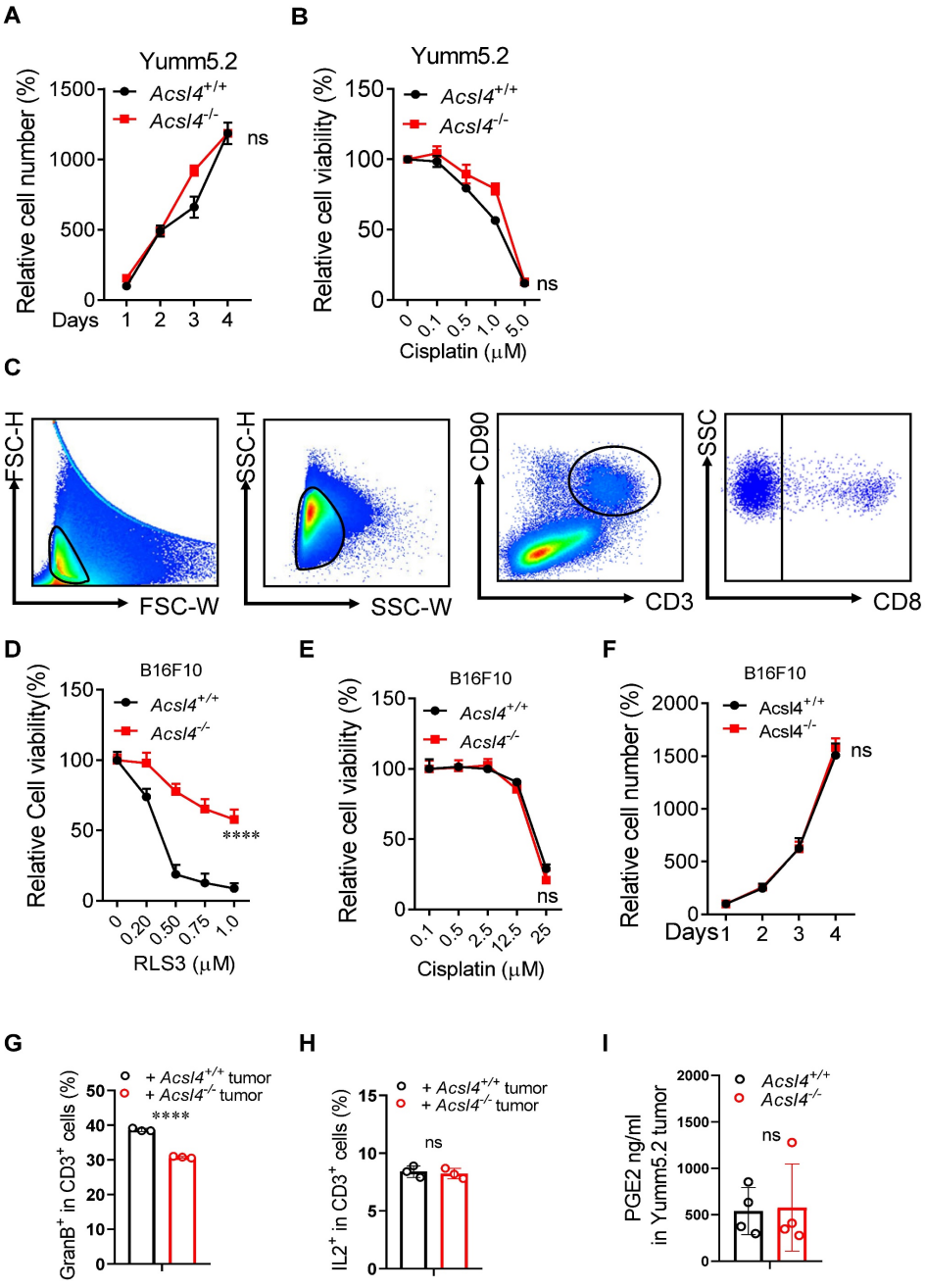


Figure S4. Role of tumor ACSL4 in tumor cells and T cells, Related to Figure 5.

(A) Relative cell numbers of *Acsl4*$^{+/+}$ or *Acsl4*$^{-/-}$ Yumm5.2 cells. Cell numbers were determined by alamarBlue assay on day 4. n = 4 biological replicates, mean ± s.d. ns, $P > 0.99$ (two-way ANOVA).

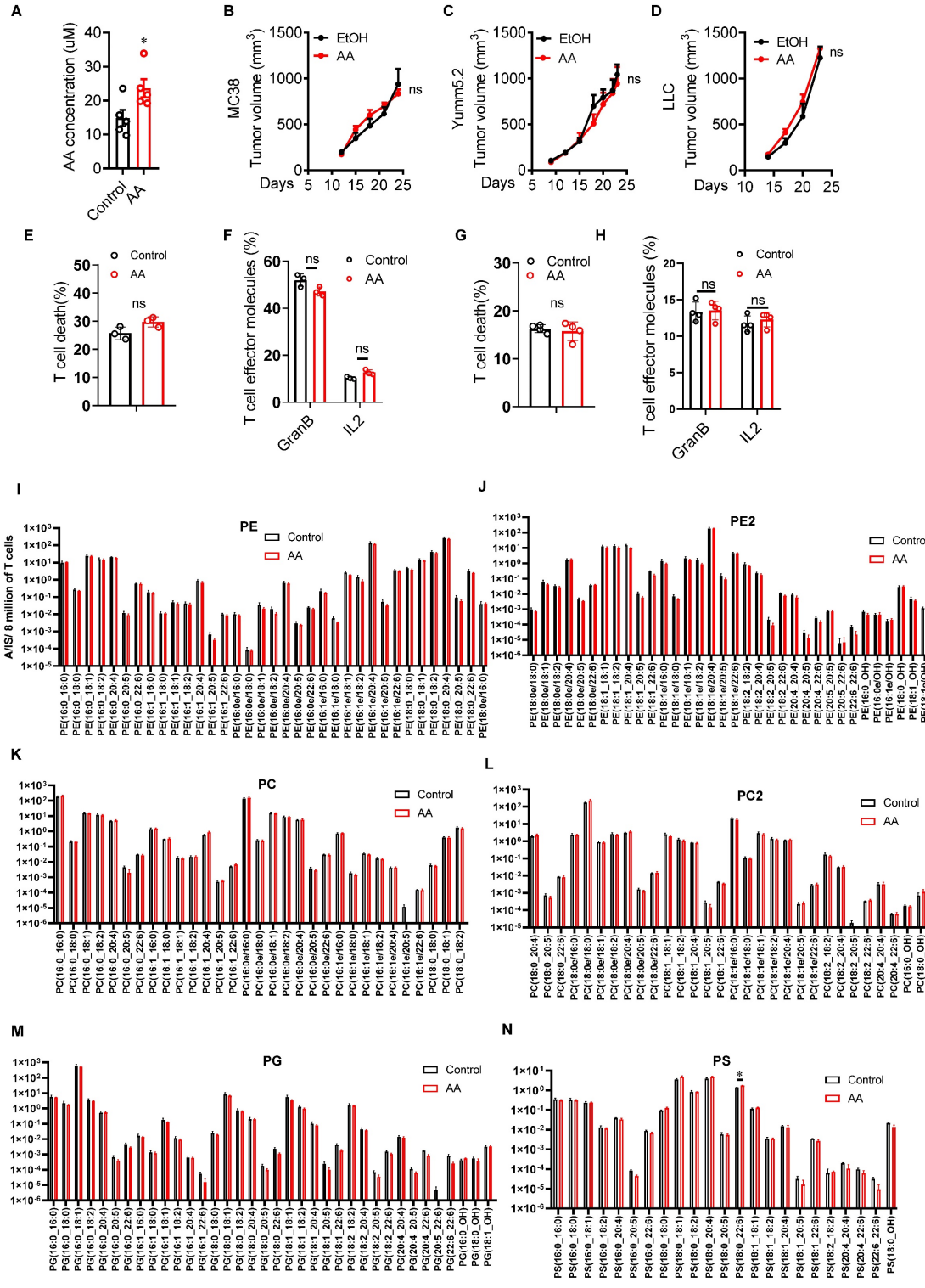
(B) *Acsl4*$^{+/+}$ or *Acsl4*$^{-/-}$ Yumm5.2 cell viability determined by alamarBlue assay. Tumor cells were treated with indicated concentrations of cisplatin for 24 hours. n = 4 biological replicates, mean ± s.d. ns, $P = 0.99$ (two-way ANOVA).

(C) Flow cytometry gating strategy showing T cell identification in the mouse tumor tissues.

(D - F) *Acsl4*$^{+/+}$ or *Acsl4*$^{-/-}$ B16F10 cells treated with different concentrations of RSL3 (D) or cisplatin (E) for 24 hours or cultured in complete medium for 1-4 days (F). Cell viability (D and E) and relative cell numbers (F) were determined by alamarBlue assay. n = 4 biological replicates. mean ± s.d. ****$P < 0.0001$ (D); ns, $P = 0.48$ (E) and ns, $P = 0.47$ (F) (two-way ANOVA).

(G and H) Percentages of granzyme B$^+$ (G) and IL-2$^+$ (H) in T cells co-cultured with *Acsl4*$^{+/+}$ or *Acsl4*$^{-/-}$ Yumm5.2 cells. ****$P < 0.0001$ (G); ns, $P = 0.69$ (H) (two-tailed t-test).

(I) PGE2 levels in *Acsl4*$^{+/+}$ or *Acsl4*$^{-/-}$ Yumm5.2 tumor tissues in tumor-bearing mice. PGE2 was measured by ELISA. n = 4 biological replicates. ns, $P = 0.89$ (two-tailed t-test).


A
AA concentration (uM)
Control
AA
B
MC38
Tumor volume (mm3)
EtOH
AA
Days
ns
C
Yumm5.2
D
LLC
E
T cell death(%)
F
T cell effector molecules (%)
GranB
IL2
G
H
I
PE
A/IS/ 8 million of T cells
J
PE2
K
PC
L
PC2
M
PG
N
PS

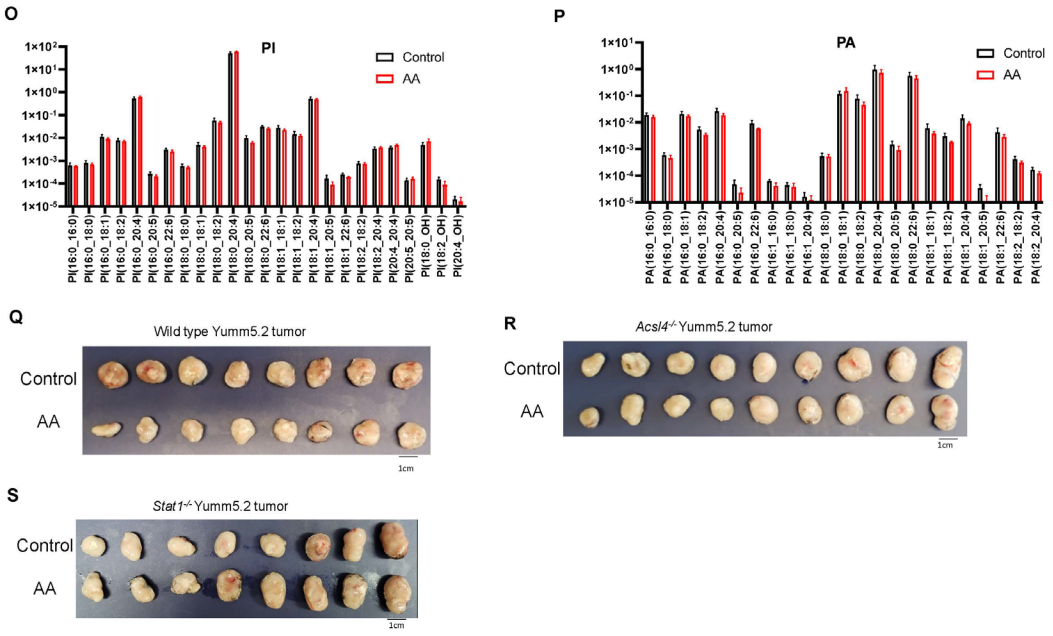


Figure S5. Impact of AA on T cells, Related to Figure 6.

(A) AA concentration in tumor tissue fluids. Yumm5.2 tumor-bearing mice were treated with PBS or AA. AA concentration was detected in tumor tissue fluids post one-day administration. **P* = 0.0407 (two-tailed t-test).

(B-D) Effect of AA administration on tumor growth in NSG mice. NSG mice bearing MC38 (B), Yumm5.2 (C), and LLC (D) tumors were treated with PBS and AA. Tumor volume is shown. n = 3- 5 mice /group, mean ± s.e.m. ns, *P* = 0.85 (B), ns, *P* = 0.99 (C) and ns, *P* = 0.88 (D) (two-way ANOVA).

(E) Percentage of dead T cells. T cells were treated with AA (20 μM) for 72 hours *in vitro*. n = 3 biological replicates (mean ± s.d.). ns, *P* = 0.07 (two-tailed t-test).

(F) Percentages of granzyme B^+ and IL-2^+ T cells. T cells were treated with AA (20 μM) for 72 hours *in vitro*. ns, *P* = 0.13 and ns, *P* = 0.06; (Multiple t tests).

(G) Percentage of dead T cells in the tumor microenvironment. Yumm5.2 tumor-bearing mice were treated with AA (2 mg/kg). n = 4 biological replicates (mean ± s.d.). ns, *P* = 0.63 (two-tailed t-test).

(H) Percentages of granzyme B^+ and IL-2^+ T cells in the tumor microenvironment. Yumm5.2 tumor-bearing mice were treated with AA (2 mg/kg). ns, *P* = 0.97 and ns, *P* = 0.70 (Multiple t tests).

(I-P) Lipidomics profiling in T cells. Yumm5.2 tumor-bearing mice were treated with AA (2 mg/kg) as indicated. T cells were isolated and sorted for lipidomic profiling. Data are the mean values of the area of analyte (A) over the internal standard (IS) in 8 x 10^6 T cells. n = 4 biological replicates. log10-transformation has been applied to visualize and compare the abundance of the different phospholipid species in the samples. *P = 0.013; multiple t-test with Sidak–Bonferroni correction for multiple comparisons. Phosphatidylethanolamine (PE) (I and J), phosphatidylcholine (PC) (K and L), phosphatidylglycerol (PG)(M), phosphatidylserine (PS)(N), phosphatidylinositol (PI)(O) and phosphatidic acid (PA)(P).

(Q-S) Effect of AA administration on tumor growth *in vivo*. Wild type (Q), *Acsl4*$^{-/-}$ (R), and *Stat1*$^{-/-}$ (S) Yumm5.2 tumor-bearing mice were treated with PBS and AA. Tumor mass images are shown. n = 8-9 tumors/group.

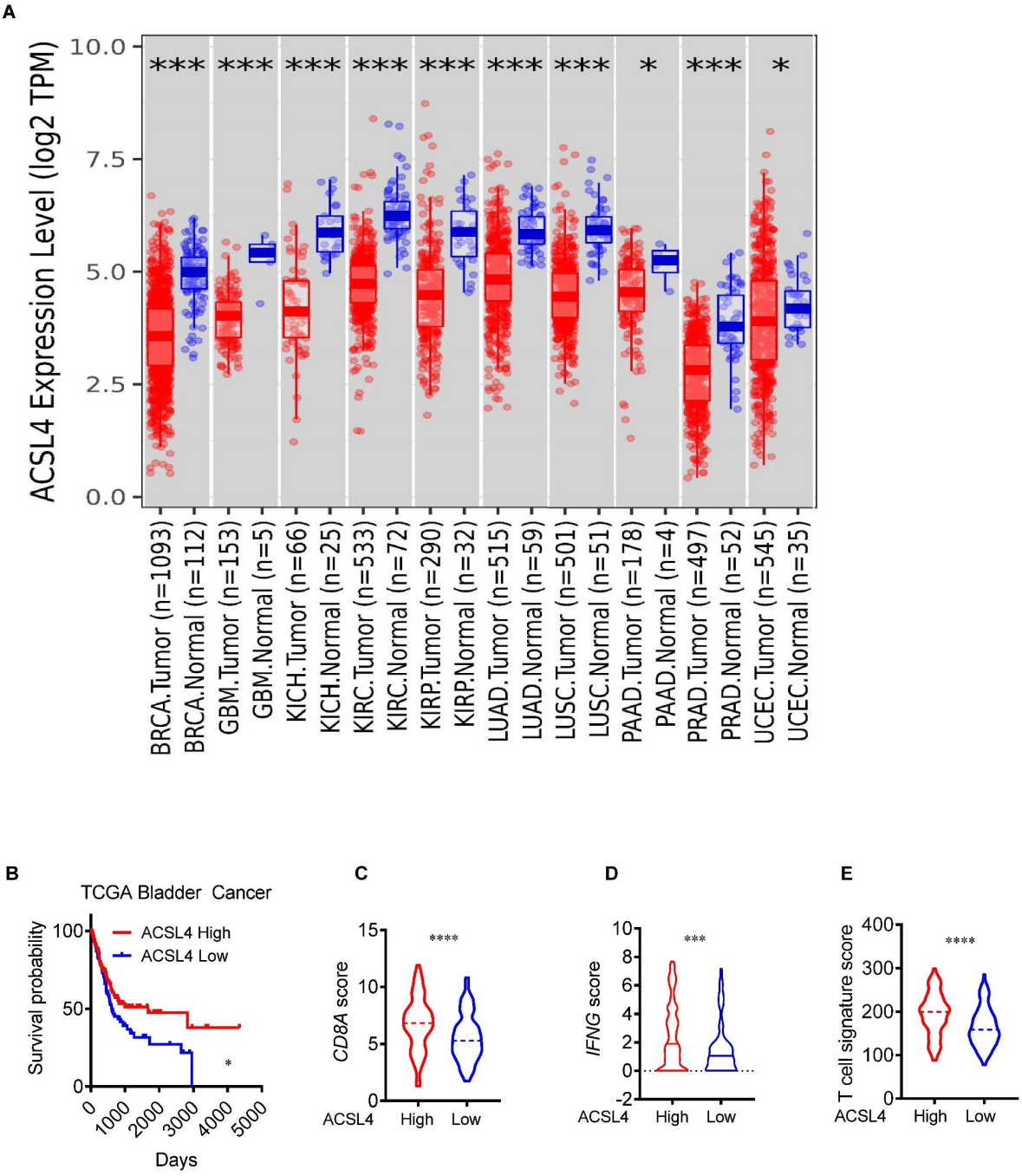


Figure S6. Clinical relevance of human tumor ACSL4, Related to Figure 6.

(A) ACSL4 expression in tumor and adjacent normal tissues in TCGA data sets. BRCA: Breast invasive carcinoma; GBM: Glioblastoma multiforme; KICH: Kidney Chromophobe; KIRC: Kidney renal clear cell carcinoma; KIRP: Kidney renal papillary cell carcinoma; LUAD:

Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; PAAD: Pancreatic adenocarcinoma; PRAD: Prostate adenocarcinoma; UCEC: Uterine Corpus Endometrial Carcinoma. The statistical significance computed by the Wilcoxon test is annotated by the number of stars (*P < 0.05; **P < 0.01; ***P < 0.001).

(B) Kaplan-Meier survival curves for bladder cancer patients with Low (bottom 25%) (n = 107) or High (top 25%) (n = 107) tumor ACSL4 transcripts in TCGA dataset. *P = 0.0489 (Log-rank test).

(C-E) Correlation between ACSL4 transcripts and immune genes - including CD8A of the minima, median, maxima and range for each blot are (1.28, 6.83, 11.92 and 10.64) (1.75, 5.29, 10.83 and 9.01) (C), IFNG of the minima, median, maxima and range for each blot are (0.0, 1.90, 7.68 and 7.68) (0.0, 1.05, 7.17 and 7.17) (D), and T cell signature score of the minima, median, maxima and range for each blot are (87.6, 199.7, 298.9 and 211.3) (77.5, 158.5, 285.6 and 208.1) (E) in TCGA dataset in patients with bladder cancer expressing High (n = 107) or Low (n = 107) levels of ACSL4. mean ± s.d. ****P < 0.0001 (C), ***P = 0.0009 (D) and ****P < 0.0001(E) (two-tailed t-test).

Binding Site (BS)	Primer Sequence
hACSL4-BS1	F:5'-CTCACTGCTGTTAGGCGCA-3'
	R:5'-CGATCCGCTTCTGTCAGTCTC-3'
hACSL4-BS2	F:5'-CAAAGCTGCGGTGACTTTTCC-3'
	R:5'-GTTAAGATCCCCGCTCACTCC-3'
hACSL4-BS3	F:5'-TGTAATCTCAGGTGGTAAGGCA-3'
	R:5'-TCCCTGATGCGTAATGGTGA-3'
hACSL4-BS4	F:5'-TCCGGGCGCGTCTTTTC-3'
	R:5'-AAGCTCGCAAAAAGGAACCG-3'
hACSL4-BS5	F:5'-CTCCGGGCGCGTCTTTTC-3'
	R:5'-AGCTCGCAAAAAGGAACCG-3'

Table S3. ChIP-qPCR primers for specific ACSL4 promotor region amplification, Related to STAR Methods.