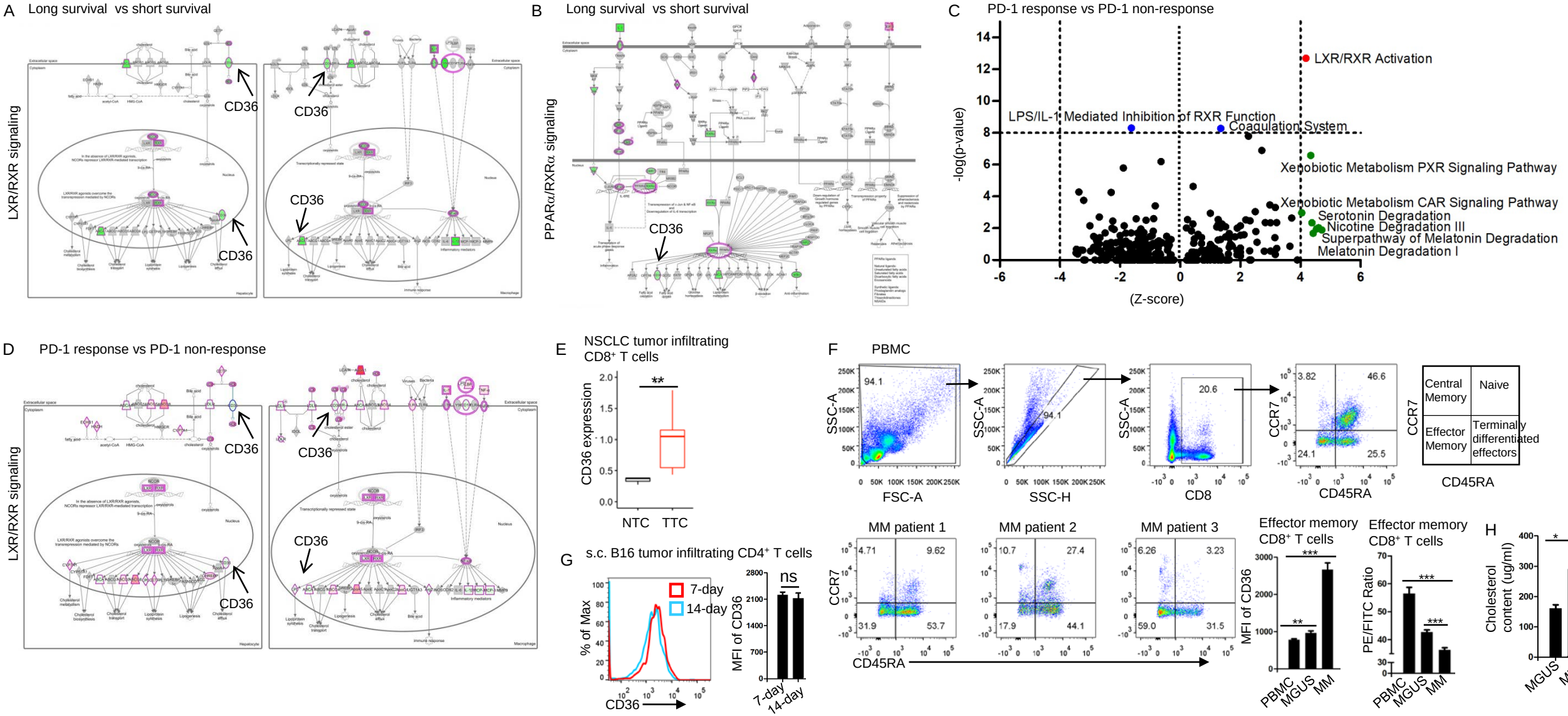
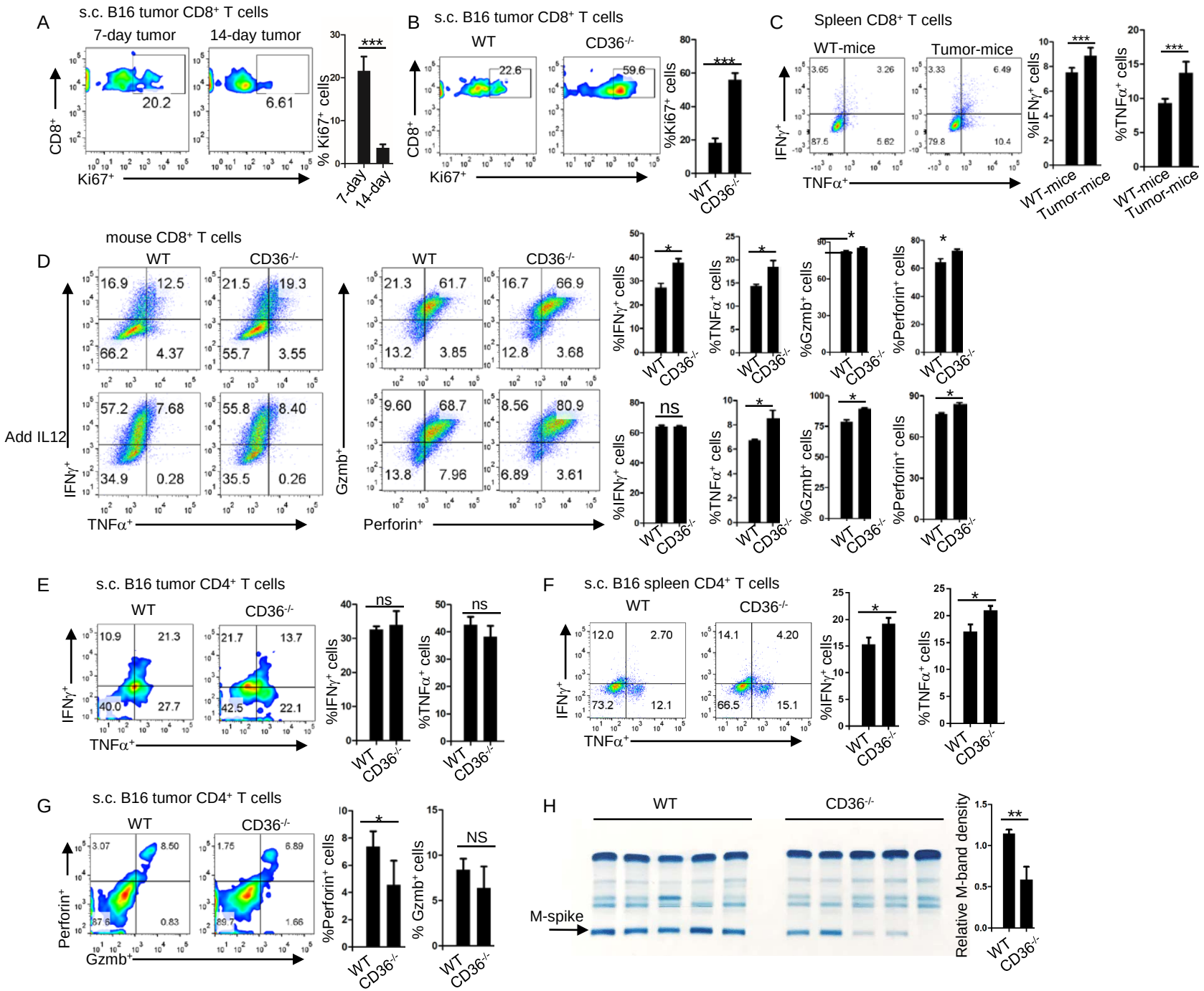

A
Long survival vs short survival
LXR/RXR signaling
CD36
CD36
CD36
CD36
B
Long survival vs short survival
PPARα/RXRα signaling
CD36
C
PD-1 response vs PD-1 non-response
-log(p-value)
(Z-score)
LXR/RXR Activation
LPS/IL-1 Mediated Inhibition of RXR Function
Coagulation System
Xenobiotic Metabolism PXR Signaling Pathway
Xenobiotic Metabolism CAR Signaling Pathway
Serotonin Degradation
Nicotine Degradation III
Superpathway of Melatonin Degradation
Melatonin Degradation I
D
PD-1 response vs PD-1 non-response
LXR/RXR signaling
CD36
CD36
CD36
CD36
E
NSCLC tumor infiltrating CD8+ T cells
CD36 expression
**
NTC
TTC
F
PBMC
SSC-A
FSC-A
SSC-H
CD8
CCR7
CD45RA
Central Memory
Naive
Effector Memory
Terminally differentiated effectors
MM patient 1
MM patient 2
MM patient 3
Effector memory CD8+ T cells
MFI of CD36
Effector memory CD8+ T cells
PE/FITC Ratio
PBMC
MGUS
MM
G
s.c. B16 tumor infiltrating CD4+ T cells
% of Max
7-day
14-day
CD36
MFI of CD36
ns
7-day
14-day
H
Cholesterol content (ug/ml)
MGUS
MM

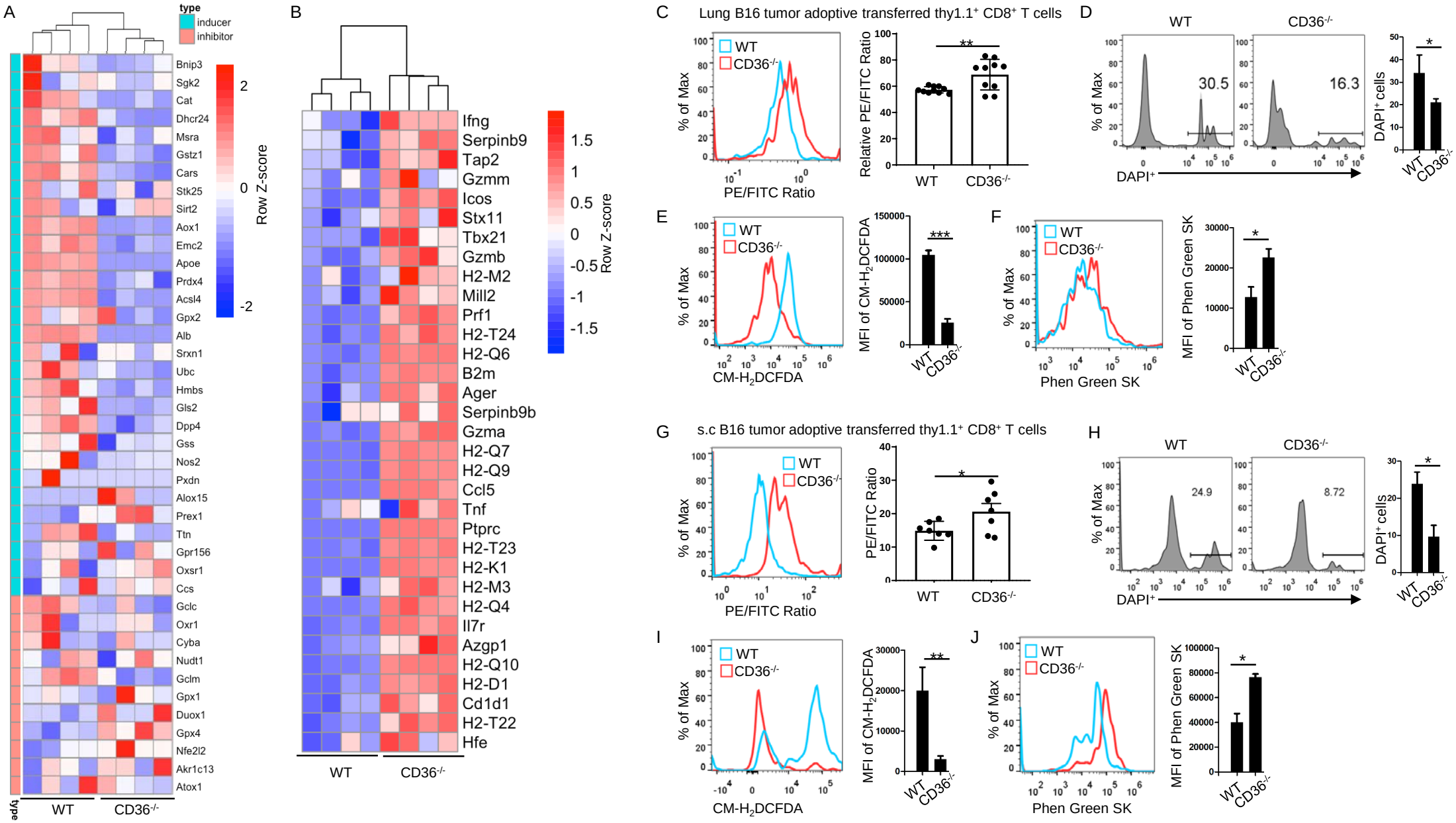
Supplementary Figure 1. Increased CD36 expression on tumor-infiltrating $CD8^+$ T cells is associated with tumor progression and poor survival in human and murine cancers, Related to Figure 1.

(A and B) IPA analysis of canonical pathway changes in tumor-infiltrating $CD8^+$ T cells between long- and short-survival melanoma patients. Shown are LXR/RXR signaling (A) and PPARα/RXRα signaling (B). Red: upregulated; Green: downregulated. **(C and D)** IPA analysis of canonical pathway changes between PD-1 treatment responder and non-responder melanoma patients (C). Shown is LXR/RXR signaling (D). Red: upregulated; Green: downregulated. **(E)** CD36 expression of $CD8^+$ T cells from non-small-cell lung cancer (NSCLC) tumor tissues and normal tissues. NTC: normal adjacent tissue cells; TTC: tumor tissue cells. Data are presented as mean ± SEM. $^{**}p < 0.01$.**(F)** $CD8^+$ T cells from MM or MGUS patient's bone marrow and blood were analyzed for the expression of CCR7, CD45RA, CD36 and lipid peroxidation. **(G)** B6 mice were injected s.c. with 1×10^6 B16 cells. Tumor-infiltrating $CD4^+$ T cells were analyzed for the expression of CD36 at days 7 and 14 after tumor injection. **(H)** Cholesterol content in bone marrow plasma of MGUS and MM patients. Data are presented as mean ± SEM. $^{**}p < 0.01$; $^{***}p < 0.001$.


A
s.c. B16 tumor CD8+ T cells
7-day tumor
14-day tumor
20.2
6.61
CD8+
Ki67+
% Ki67+ cells
7-day
14-day
***
B
s.c. B16 tumor CD8+ T cells
WT
CD36-/-
22.6
59.6
%Ki67+ cells
C
Spleen CD8+ T cells
WT-mice
Tumor-mice
3.65
3.26
87.5
5.62
3.33
6.49
79.8
10.4
IFNγ+
TNFα+
%IFNγ+ cells
%TNFα+ cells
D
mouse CD8+ T cells
Add IL12
16.9
12.5
66.2
4.37
21.5
19.3
55.7
3.55
57.2
7.68
34.9
0.28
55.8
8.40
35.5
0.26
Gzmb+
Perforin+
21.3
61.7
13.2
3.85
16.7
66.9
12.8
3.68
9.60
68.7
13.8
7.96
8.56
80.9
6.89
3.61
%Gzmb+ cells
%Perforin+ cells
ns
E
s.c. B16 tumor CD4+ T cells
10.9
21.3
40.0
27.7
21.7
13.7
42.5
22.1
F
s.c. B16 spleen CD4+ T cells
12.0
2.70
73.2
12.1
14.1
4.20
66.5
15.1
G
s.c. B16 tumor CD4+ T cells
3.07
8.50
87.6
0.83
1.75
6.89
89.7
1.66
%Perforin+ cells
% Gzmb+ cells
NS
H
M-spike
Relative M-band density
**

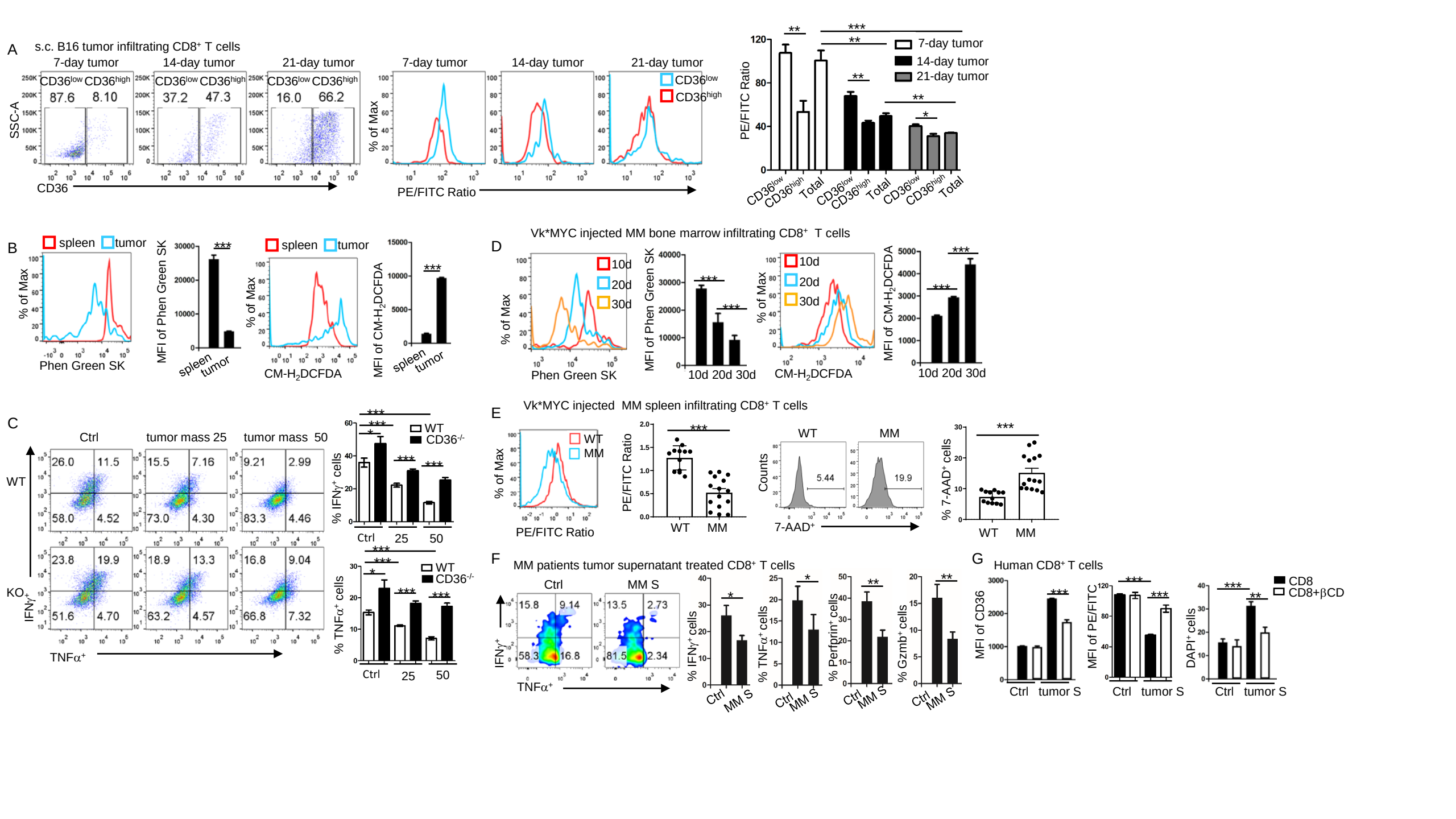
Supplementary Figure 2. Expression of CD36 reduces cytotoxic cytokine production in murine CD8$^+$ T cells but not CD4$^+$ T cells, Related to Figure 2.

(A) B6 mice were injected s.c. with 1 × 10^6 B16 cells. Tumor-infiltrating CD8$^+$ T cells were analyzed for the expression of Ki67 at day 7 and 14 after tumor injection. **(B)** WT and CD36$^{-/-}$ B6 mice were injected s.c. with 1 × 10^6 B16 cells. Tumor-infiltrating CD8$^+$ T cells were analyzed for the expression of Ki67 at day 14 after tumor injection. **(C)** B6 mice were injected s.c. with 1 × 10^6 B16 cells and maintained for 10 days. Spleen CD8$^+$ T cells from tumor free or tumor bearing mice were analyzed for the expression of IFNγ and TNFα. **(D)** Splenocytes from WT Pmel-1 and CD36$^{-/-}$ Pmel-1 mice were differentiated in vitro for 3 days in the presence of hgp$100_{25\text{-}33}$ peptide (1 μg/ml) and IL-2 (10 ng/ml). Some of the cells were added with IL-12 as indicated. T cells were examined for the production of IFNγ, TNFα, Gzmb and perforin on day 3. **(E-G)** WT and CD36$^{-/-}$ B6 mice were injected s.c. with 1 × 10^6 B16 cells. Tumor-infiltrating (E and G) and spleen (F) CD4$^+$ T cells were analyzed for the expression of IFNγ, TNFα, Gzmb or perforin at day 14 after tumor injection. **(H)** WT and CD36$^{-/-}$ B6 mice were injected i.v. with 1.5 × 10^6 Vk*MYC cells. Tumor burden is shown as bands of M-spike at day 28. Data are presented as mean ± SEM. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.


A
type
inducer
inhibitor
Bnip3
Sgk2
Cat
Dhcr24
Msra
Gstz1
Cars
Stk25
Sirt2
Aox1
Emc2
Apoe
Prdx4
Acsl4
Gpx2
Alb
Srxn1
Ubc
Hmbs
Gls2
Dpp4
Gss
Nos2
Pxdn
Alox15
Prex1
Ttn
Gpr156
Oxsr1
Ccs
Gclc
Oxr1
Cyba
Nudt1
Gclm
Gpx1
Duox1
Gpx4
Nfe2l2
Akr1c13
Atox1
2
0
-2
Row Z-score
type
WT
CD36-/-
B
Ifng
Serpinb9
Tap2
Gzmm
Icos
Stx11
Tbx21
Gzmb
H2-M2
Mill2
Prf1
H2-T24
H2-Q6
B2m
Ager
Serpinb9b
Gzma
H2-Q7
H2-Q9
Ccl5
Tnf
Ptprc
H2-T23
H2-K1
H2-M3
H2-Q4
Il7r
Azgp1
H2-Q10
H2-D1
Cd1d1
H2-T22
Hfe
1.5
1
0.5
0
-0.5
-1
-1.5
Row Z-score
WT
CD36-/-
C
Lung B16 tumor adoptive transferred thy1.1+ CD8+ T cells
WT
CD36-/-
% of Max
PE/FITC Ratio
Relative PE/FITC Ratio
**
WT
CD36-/-
D
WT
CD36-/-
% of Max
30.5
16.3
DAPI+
DAPI+ cells
*
WT
CD36-/-
E
WT
CD36-/-
% of Max
CM-H2DCFDA
MFI of CM-H2DCFDA
***
WT
CD36-/-
F
WT
CD36-/-
% of Max
Phen Green SK
MFI of Phen Green SK
*
WT
CD36-/-
G
s.c B16 tumor adoptive transferred thy1.1+ CD8+ T cells
WT
CD36-/-
% of Max
PE/FITC Ratio
PE/FITC Ratio
*
WT
CD36-/-
H
WT
CD36-/-
% of Max
24.9
8.72
DAPI+
DAPI+ cells
*
WT
CD36-/-
I
WT
CD36-/-
% of Max
CM-H2DCFDA
MFI of CM-H2DCFDA
**
WT
CD36-/-
J
WT
CD36-/-
% of Max
Phen Green SK
MFI of Phen Green SK
*
WT
CD36-/-

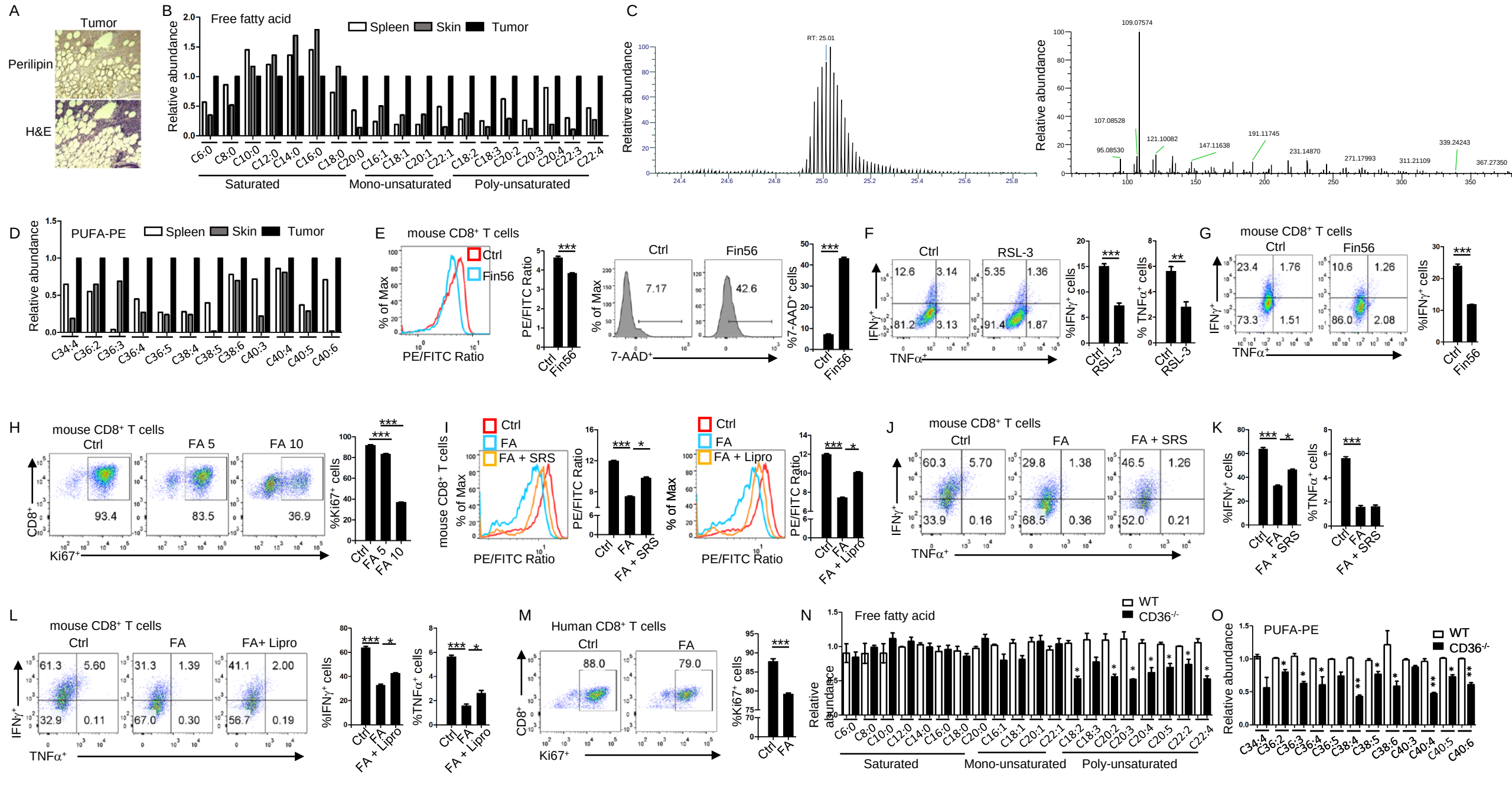
Supplementary Figure 3. CD36 regulates transcriptional and metabolic programs including lipid peroxidation in tumor-infiltrating murine CD8$^+$ T cells, Related to Figure 3.

WT and CD36$^{-/-}$ B6 mice were injected i.v. with 2 × 10^5 B16 cells, and 16 days later, spleen (A) and lung (B) of tumor-bearing mice were collected, sorted for CD8$^+$ T cells, and performed RNAseq on T cells. **(A)** Heatmap of lipid peroxidation- and ferroptosis-related genes. **(B)** Heatmap of T cell cytotoxicity-related genes. **(C-J)** Splenocytes from WT Pmel-1 and CD36$^{-/-}$ Pmel-1 mice were differentiated in vitro for 5 days in the presence of hgp100$_{25\text{-}33}$ peptide (1 μg/ml) and IL-2 (10 ng/ml) and then adoptively transferred into tumor-bearing mice. **(C-F)** B6 mice were injected i.v. with 2 × 10^5 B16 cells. At day 12 after tumor inoculation, 2 × 10^6 WT or CD36$^{-/-}$ CD8$^+$ Pmel-1 T cells were i.v. injected into tumor-bearing mice. At day 16, WT or CD36$^{-/-}$ Pmel-1 CD8$^+$ T cells in tumor were analyzed for lipid peroxidation, cell death, cytosolic ROS and iron. **(G-J)** B6 mice were injected s.c. with 1 × 10^6 B16 cells. Two weeks after tumor inoculation, 2 × 10^6 WT or CD36$^{-/-}$ Pmel-1 CD8$^+$ T cells were i.v. injected into tumor-bearing mice. Three days after T cell transfer, WT or CD36$^{-/-}$ Pmel-1 CD8$^+$ T cells in tumor were analyzed for lipid peroxidation, cell death, cytosolic ROS and iron. Data are presented as mean ± SEM. *p < 0.05; **p < 0.01; ***p < 0.001.


A
s.c. B16 tumor infiltrating CD8+ T cells
7-day tumor
14-day tumor
21-day tumor
CD36low CD36high
87.6 8.10
37.2 47.3
16.0 66.2
SSC-A
CD36
% of Max
PE/FITC Ratio
CD36low
CD36high
PE/FITC Ratio
7-day tumor
14-day tumor
21-day tumor
Total
B
spleen
tumor
% of Max
Phen Green SK
MFI of Phen Green SK
CM-H2DCFDA
MFI of CM-H2DCFDA
C
Ctrl
tumor mass 25
tumor mass 50
WT
KO
IFNγ+
TNFα+
26.0 11.5
58.0 4.52
15.5 7.16
73.0 4.30
9.21 2.99
83.3 4.46
23.8 19.9
51.6 4.70
18.9 13.3
63.2 4.57
16.8 9.04
66.8 7.32
CD36-/-
% IFNγ+ cells
% TNFα+ cells
Ctrl
25
50
D
Vk*MYC injected MM bone marrow infiltrating CD8+ T cells
10d
20d
30d
% of Max
Phen Green SK
MFI of Phen Green SK
CM-H2DCFDA
MFI of CM-H2DCFDA
E
Vk*MYC injected MM spleen infiltrating CD8+ T cells
WT
MM
% of Max
PE/FITC Ratio
Counts
5.44
19.9
7-AAD+
% 7-AAD+ cells
F
MM patients tumor supernatant treated CD8+ T cells
Ctrl
MM S
15.8 9.14
58.3 16.8
13.5 2.73
81.5 2.34
IFNγ+
TNFα+
% IFNγ+ cells
% TNFα+ cells
% Perforin+ cells
% Gzmb+ cells
G
Human CD8+ T cells
MFI of CD36
MFI of PE/FITC
DAPI+ cells
tumor S
CD8
CD8+βCD

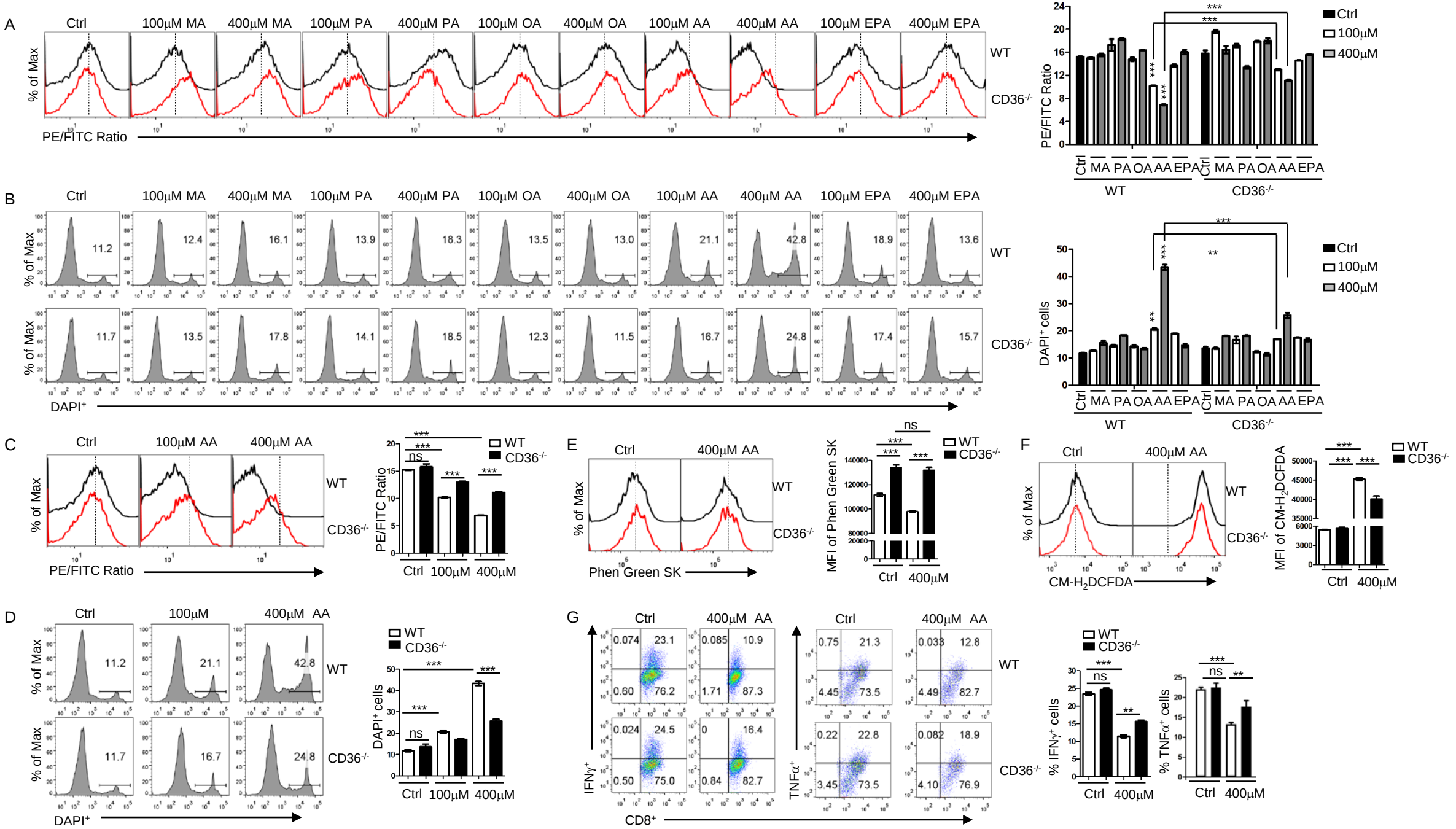
Supplementary Figure 4. CD36 mediates ferroptosis and reduces cytotoxic cytokine production in human and murine CD8$^+$ T cells, Related to Figure 4.

(A) B6 mice were injected s.c. with 1 × 10^6 B16 cells. At days 7, 14 and 21 after tumor inoculation, tumor-infiltrating CD8$^+$ T cells were analyzed for CD36 expression and lipid peroxidation. **(B)** B6 mice were injected s.c. with 1 × 10^6 B16 cells. Spleen and tumor-infiltrating CD8$^+$ T cells were analyzed for iron and ROS at day 14 after tumor injection. **(C)** WT Pmel-1 or CD36$^{-/-}$ Pmel-1 CD8$^+$ T cells were isolated and in vitro stimulated with CD3/CD28 antibodies in the presence of IL-2. Tumor mass (25 or 50 μl at 1 mg/ml) was added to the culture of T cells for 1 day as indicated. Cultured T cells were analyzed for IFNγ and TNFα expression. **(D)** B6 mice were injected i.v. with 1.5 × 10^6 Vk*MYC cells, and tumor-infiltrating CD8$^+$ T cells from bone marrow were analyzed for iron and cytosolic ROS at days 10, 20 and 30 after tumor injection. **(E)** B6 mice were injected i.v. with 1.5 × 10^6 Vk*MYC cells. Lipid peroxidation and 7-AAD level of spleen CD8$^+$ T cells from 4-week Vk*MYC MM-bearing mice or tumor-free mice were examined. **(F)** Human CD8$^+$ T cells were isolated from blood and in vitro stimulated with CD3/CD28 beads in the presence of IL-2. MM patient bone marrow plasma (100 μl) was added to the culture of T cells during T cell differentiation as indicated. Cultured human CD8$^+$ T cells were analyzed for Perforin, Gzmb, IFNγ, or TNFα production. **(G)** Human CD8$^+$ T cells were isolated from blood and in vitro stimulated with CD3/CD28 beads in the presence of IL-2. MM patient bone marrow plasma (100 μl) and β-CD (0.5 mM) were added to the culture of T cells during T cell differentiation as indicated. Cultured human CD8$^+$ T cells were analyzed for CD36, lipid peroxidation and cell death. Data are presented as mean ± SEM. S: supernatant. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.


A
Tumor
Perilipin
H&E
B
Free fatty acid
Spleen Skin Tumor
Relative abundance
C6:0 C8:0 C10:0 C12:0 C14:0 C16:0 C18:0 C20:0 C16:1 C18:1 C20:1 C22:1 C18:2 C18:3 C20:2 C20:3 C20:4 C22:3 C22:4
Saturated
Mono-unsaturated
Poly-unsaturated
C
RT: 25.01
Relative abundance
109.07574
107.08528
95.08530
121.10082
147.11638
191.11745
231.14870
271.17993
311.21109
339.24243
367.27350
D
PUFA-PE
Spleen Skin Tumor
Relative abundance
C34:4 C36:2 C36:3 C36:4 C36:5 C38:4 C38:5 C38:6 C40:3 C40:4 C40:5 C40:6
E
mouse CD8+ T cells
Ctrl
Fin56
% of Max
PE/FITC Ratio
7.17
42.6
7-AAD+
%7-AAD+ cells
F
Ctrl
RSL-3
12.6 3.14 81.2 3.13
5.35 1.36 91.4 1.87
IFNγ+
TNFα+
%IFNγ+ cells
% TNFα+ cells
G
mouse CD8+ T cells
Ctrl
Fin56
23.4 1.76 73.3 1.51
10.6 1.26 86.0 2.08
IFNγ+
TNFα+
%IFNγ+ cells
H
mouse CD8+ T cells
Ctrl
FA 5
FA 10
93.4
83.5
36.9
CD8+
Ki67+
%Ki67+ cells
I
mouse CD8+ T cells
Ctrl
FA
FA + SRS
FA + Lipro
% of Max
PE/FITC Ratio
J
mouse CD8+ T cells
Ctrl
FA
FA + SRS
60.3 5.70 33.9 0.16
29.8 1.38 68.5 0.36
46.5 1.26 52.0 0.21
IFNγ+
TNFα+
K
%IFNγ+ cells
%TNFα+ cells
L
mouse CD8+ T cells
Ctrl
FA
FA+ Lipro
61.3 5.60 32.9 0.11
31.3 1.39 67.0 0.30
41.1 2.00 56.7 0.19
IFNγ+
TNFα+
%IFNγ+ cells
%TNFα+ cells
M
Human CD8+ T cells
Ctrl
FA
88.0
79.0
CD8+
Ki67+
%Ki67+ cells
N
Free fatty acid
WT
CD36-/-
Relative abundance
C6:0 C8:0 C10:0 C12:0 C14:0 C16:0 C18:0 C20:0 C16:1 C18:1 C20:1 C22:1 C18:2 C18:3 C20:2 C20:3 C20:4 C20:5 C22:2 C22:4
Saturated
Mono-unsaturated
Poly-unsaturated
O
PUFA-PE
WT
CD36-/-
Relative abundance
C34:4 C36:2 C36:3 C36:4 C36:5 C38:4 C38:5 C38:6 C40:3 C40:4 C40:5 C40:6

Supplementary Figure 5. CD36 mediates ferroptosis and reduces cytotoxic cytokine production in CD8$^+$ T cells through uptake of fatty acid, Related to Figure 5.

(A) Immunohistochemistry showing the staining of perilipin (top) and H&E (bottom) on s.c. B16 tumors. **(B)** LC-MS analysis of indicated free fatty acid contents in tumor, adjacent skin, and spleen. **(C)** Representative normal-phase LS-MS chromatogram and mass spectra for arachidonic acid (AA). **(D)** LC-MS analysis of indicated poly unsaturated fatty acyl-phosphatidylethanolamines (PUFA-PE) contents in tumor, adjacent skin, and spleen. **(E-L)** Pmel-1 CD8$^+$ T cells were isolated and in vitro stimulated with CD3/CD28 antibodies in the presence of IL-2. **(E-G)** Fin56 (10 μM) or RSL-3 (10 μM) was added during T-cell differentiation. Lipid peroxidation, 7-AAD and cell viability (E), and IFNγ or TNFα expression (F and G) of the cells were measured. **(H)** Fatty acid (5 or 10 μl) was added during T-cell differentiation as indicated. Ki67 ratio of the cells was measured. **(I-L)** Fatty acid (5 μl), SRS (10 μM), or Lipro (2 μM) was added to the culture of T cells during T-cell differentiation as indicated. Cultured T cells were analyzed for lipid peroxidation (I) and IFNγ or TNFα expression (J-L). **(M)** Human CD8$^+$ T cells were isolated from blood and in vitro stimulated with CD3/CD28 beads in the presence of IL-2. Fatty acid (5 μl) was added during T-cell differentiation as indicated. Ki67 expression of the cells was measured. **(N and O)** LC-MS analysis of indicated free fatty acid contents (N) and PUFA-PE contents (O) in tumor-treated WT and CD36$^{-/-}$ CD8$^+$ T cells. Lipro: liproxstatin-1.SRS: SRS16-86. Data are presented as mean ± SEM. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.


A
Ctrl
100μM MA
400μM MA
100μM PA
400μM PA
100μM OA
400μM OA
100μM AA
400μM AA
100μM EPA
400μM EPA
% of Max
PE/FITC Ratio
WT
CD36-/-
PE/FITC Ratio
Ctrl
100μM
400μM
MA PA OA AA EPA
B
11.2
12.4
16.1
13.9
18.3
13.5
13.0
21.1
42.8
18.9
13.6
11.7
13.5
17.8
14.1
18.5
12.3
11.5
16.7
24.8
17.4
15.7
DAPI+
DAPI+ cells
C
PE/FITC Ratio
D
DAPI+
E
Phen Green SK
MFI of Phen Green SK
F
CM-H2DCFDA
MFI of CM-H2DCFDA
G
IFNγ+
TNFα+
CD8+
% IFNγ+ cells
% TNFα+ cells

Supplementary Figure 6. AA is an effective component in fatty acids that induces CD36-mediated ferroptosis and reduces cytotoxic cytokine production in $CD8^+$ T cells, Related to Figure 5.

WT Pmel-1 or $CD36^{-/-}$ Pmel-1 $CD8^+$ T cells were isolated and in vitro stimulated with CD3/CD28 antibodies in the presence of IL-2. Different free fatty acids were added during T cell differentiation as indicated. Lipid peroxidation **(A and C)**, cell death **(B and D)**, iron **(E)**, cytosolic ROS **(F)**, and IFNγ or TNFα expression **(G)** of the cells were measured. Data are presented as mean ± SEM. **p < 0.01; ***p < 0.001.