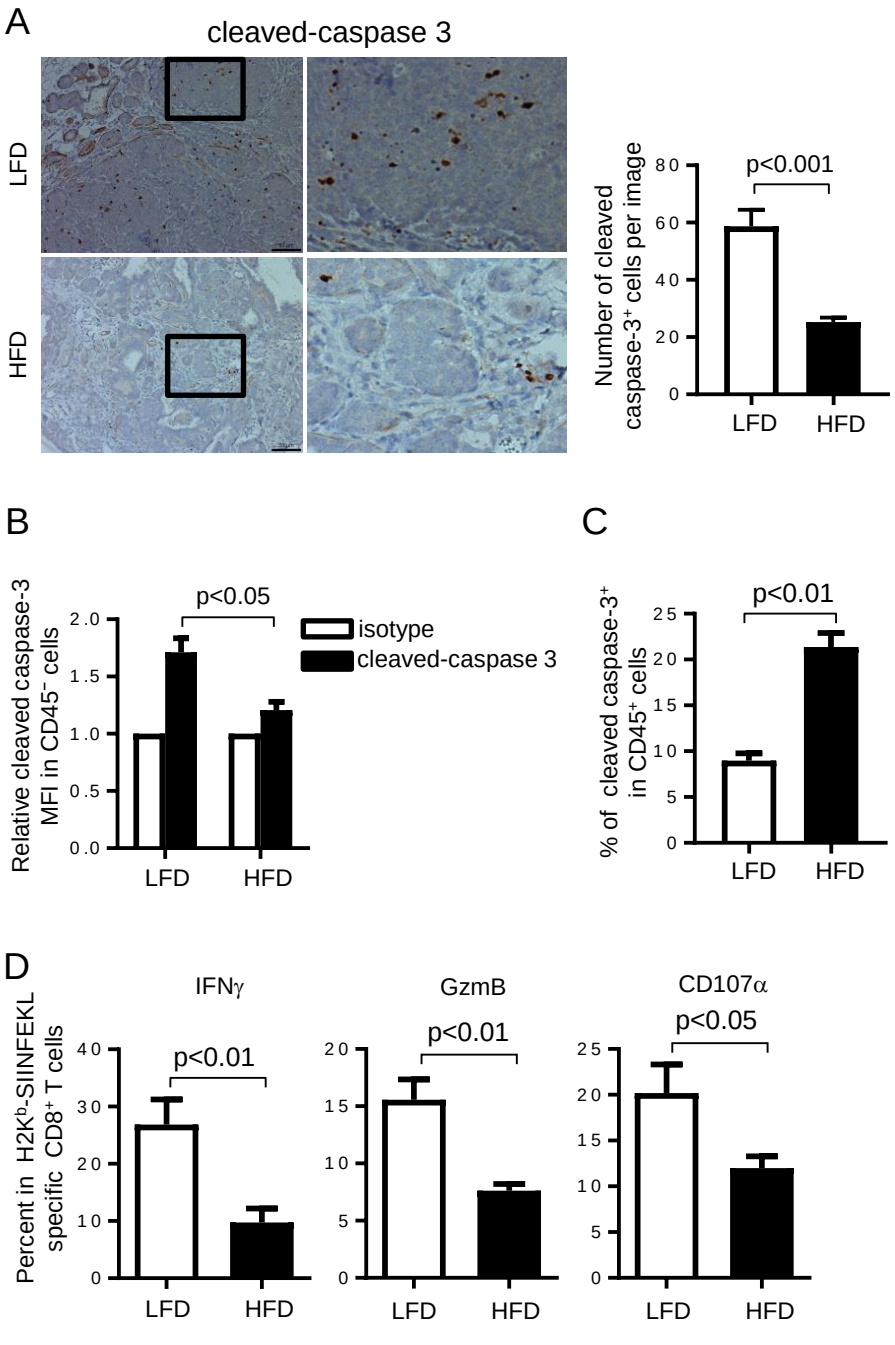
A
cleaved-caspase 3
LFD
HFD
Number of cleaved caspase-3+ cells per image
p<0.001
80
60
40
20
0
LFD
HFD
B
Relative cleaved caspase-3 MFI in CD45− cells
p<0.05
isotype
cleaved-caspase 3
2.0
1.5
1.0
0.5
0.0
LFD
HFD
C
% of cleaved caspase-3+ in CD45+ cells
p<0.01
25
20
15
10
5
0
LFD
HFD
D
Percent in H2Kb-SIINFEKL specific CD8+ T cells
IFNγ
p<0.01
40
30
20
10
0
LFD
HFD
GzmB
p<0.01
20
15
10
5
0
LFD
HFD
CD107α
p<0.05
25
20
15
10
5
0
LFD
HFD

Figure S1: Obesity correlates with reduced cleaved caspase-3 expression in $CD45^-$ cells (mainly tumor cells) and enhanced apoptosis of $CD45^+$ tumor-infiltrating immune cells, and this linked to loss of functional tumor antigen specific $CD8^+$ T_{EFF} cells, related to Figure 1.

PyMT mice were fed on a HFD or LFD from 8-10 weeks of age and tumors were collected. (A) Representative immunohistochemistry analysis of cleaved caspase-3 in tumor sections prepared from *PyMT* mice fed on a HFD or LFD, scale bar = 50 μm (left). Histograms showing cleaved caspase-3 expressing cells per view (right). Three views per slide from five tumors per group were analyzed. Results represent mean ± SEM, n = 5. (B-C) Single-cell suspensions prepared from the tumors were analyzed by flow cytometry for cleaved caspase-3 expression in $CD45^-$ cells (B) and percentage of cleaved $caspase\text{-}3^+$ cells in $CD45^+$ cells (C), respectively. Quantification of mean fluorescence intensity (MFI) is shown (normalized to isotype control; n = 4). (D) *PyMT ChOVA* mice were fed on a HFD or LFD from 8-10 weeks of age and tumors were collected. Single-cell suspensions were prepared and H-$2K^b$-SIINFEKL tetramer was utilized to detect $OVA_{SIINFEKL}$ specific $CD8^+$ T cells. Single-cell suspensions from the tumors were analyzed by flow cytometry for $IFN\gamma^+$, $CD107\alpha^+$ or $GzmB^+$ T_{EFF} cells among $OVA_{SIINFEKL}$ specific $CD8^+$ T cells. Data are shown with mean ± SEM (n = 5).

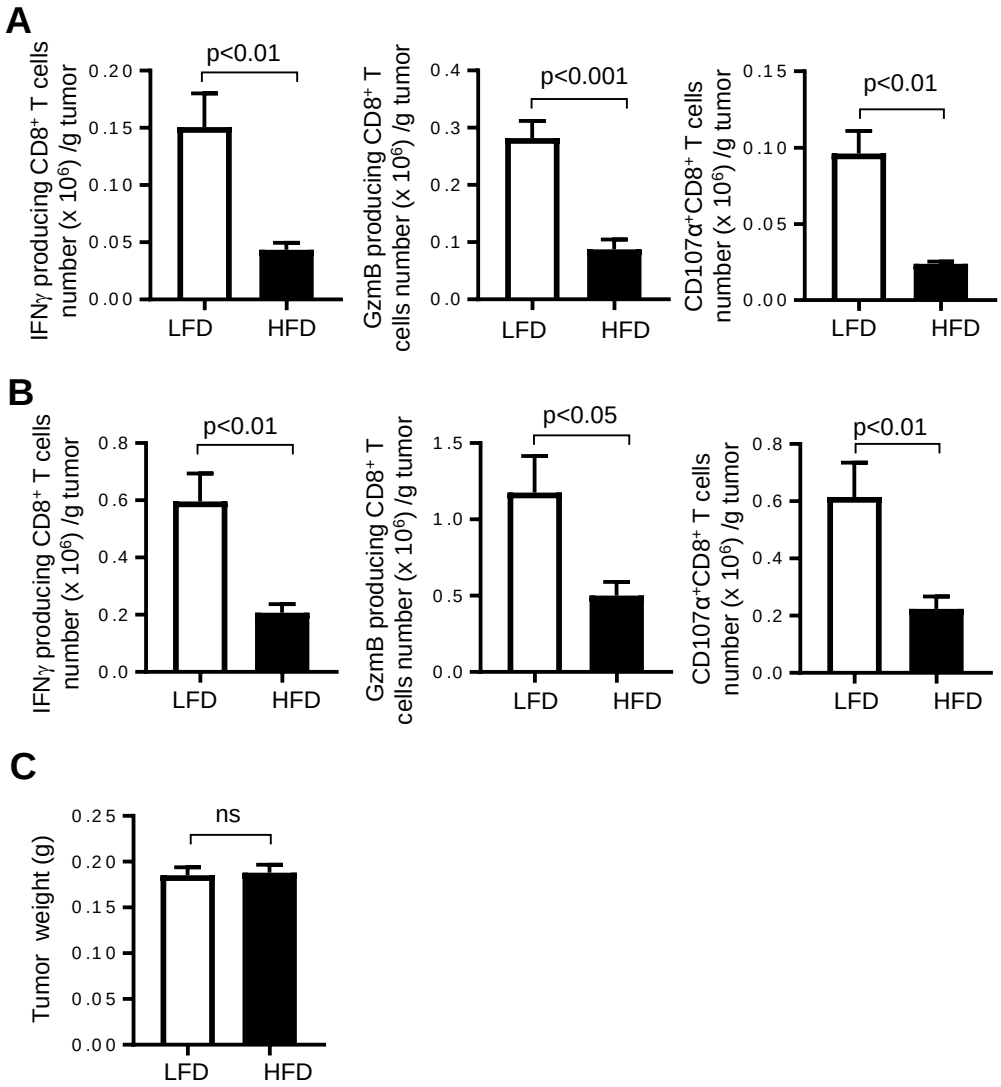

Figure S2: Obesity- promoted breast tumor development is associated with reduced tumor infiltrating functional CD8⁺ T cells, related to Figure 1.

(A) *PyMT* mice were fed on HFD or LFD from 8-10 weeks of age. Tumors were collected and single-cell suspensions prepared from the tumors were analyzed by flow cytometry to detect activated T_{EFF} (IFNγ^+, GzmB$^+$ n = 8 or CD107α^+ n = 4-5) (numbers of the activated T cells per gram tumor) with Precision Count Beads (Biolegend). (B-C) Wild type C57BL/6 mice were fed on HFD or LFD for 16 weeks, followed by tumor establishment with Py8119 tumor cells (n = 10). Single-cell suspension prepared from the tumors similar in weight were analyzed by flow cytometry to detect numbers of activated T_{EFF} (IFNγ^+, GzmB$^+$ or CD107α^+) per gram of tumor with Precision Count Beads (Biolegend) (n = 9-10) (B). The cell numbers were determined using the formula: Cell count (cells/µl) = (Cell count x Precision Count Beads volume) / (Precision Count beads Count x Cell Volume) x Count Beads Concentration. Tumors weight were measured on day 12 after tumor cell injection (n = 10) (C). All data are shown with mean ± SEM.

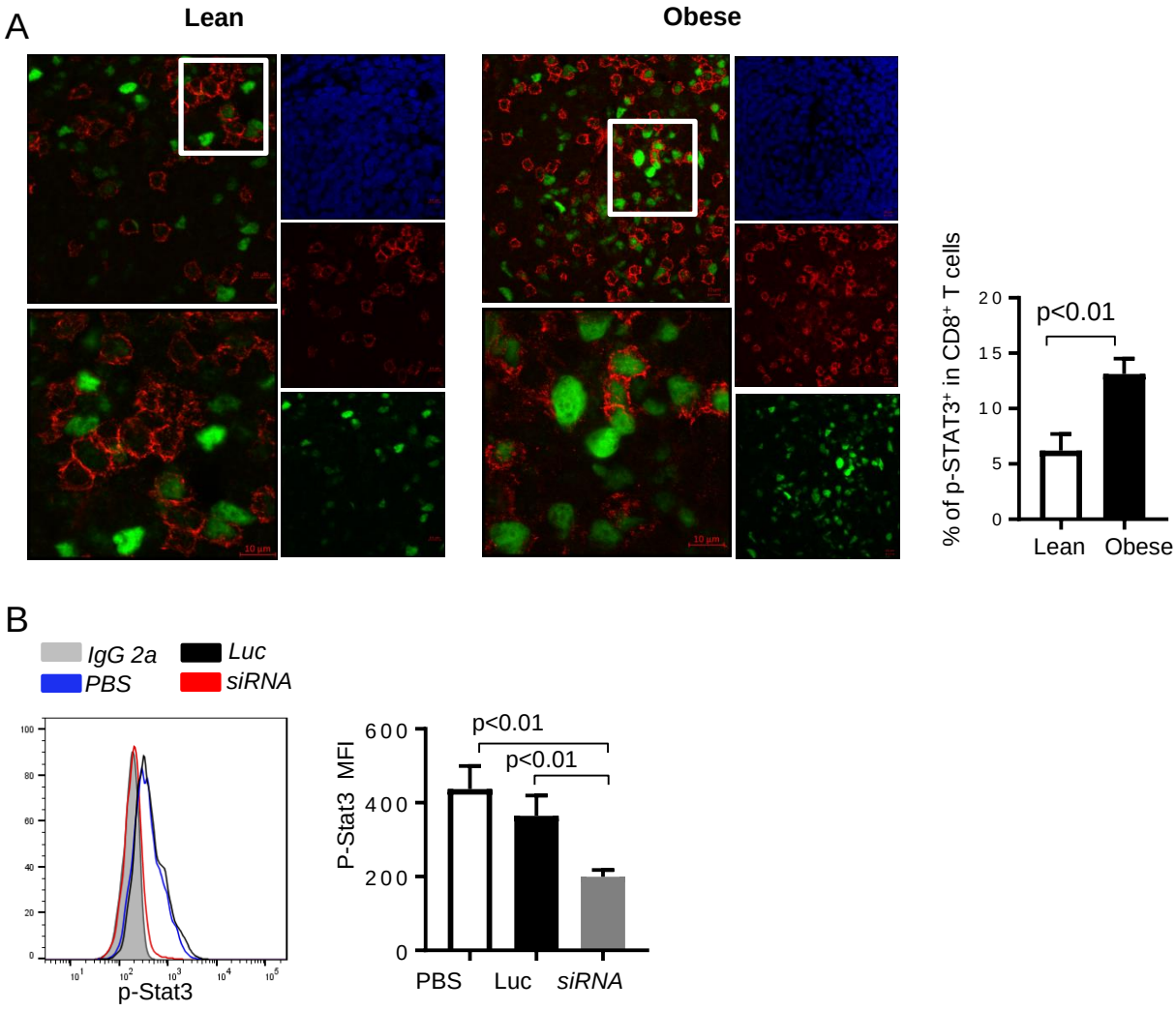

Figure S3: Obesity promotes STAT3 activation in tumor CD8⁺ T cells, related to Figure 2.

(A) Representative immunofluorescence images of CD8 (red), p-STAT3 (green) and Hoechst (blue) in tumor tissues from lean (BMI<25) or obese (BMI>32.5) breast cancer patients, scale bar = 10 μm (left). Histograms showing p-STAT3 expressing cell frequencies within CD8⁺ T cells (right). 5 views per slide from 5 tumors per group were analyzed. Results represent mean ± SEM, n = 5. (B) Testing the inhibiting effects of CTLA4 (apt)-Stat3 siRNA in tumor infiltrating CD8⁺ T cells. *PyMT* mice were treated with PBS (vehicle), Luc (CTLA4 (apt)-Luciferase siRNA) or siRNA (CTLA4 (apt)-Stat3 siRNA), once every two days for three weeks, (n = 6, mice). Tumors were then harvested for generating single-cell. Levels of p-Stat3 expression were measured by flow cytometry (left). Quantification of mean fluorescence intensity (MFI) is shown (n = 3, n is for number of samples, each of which was pooled from 2 mice) (right).

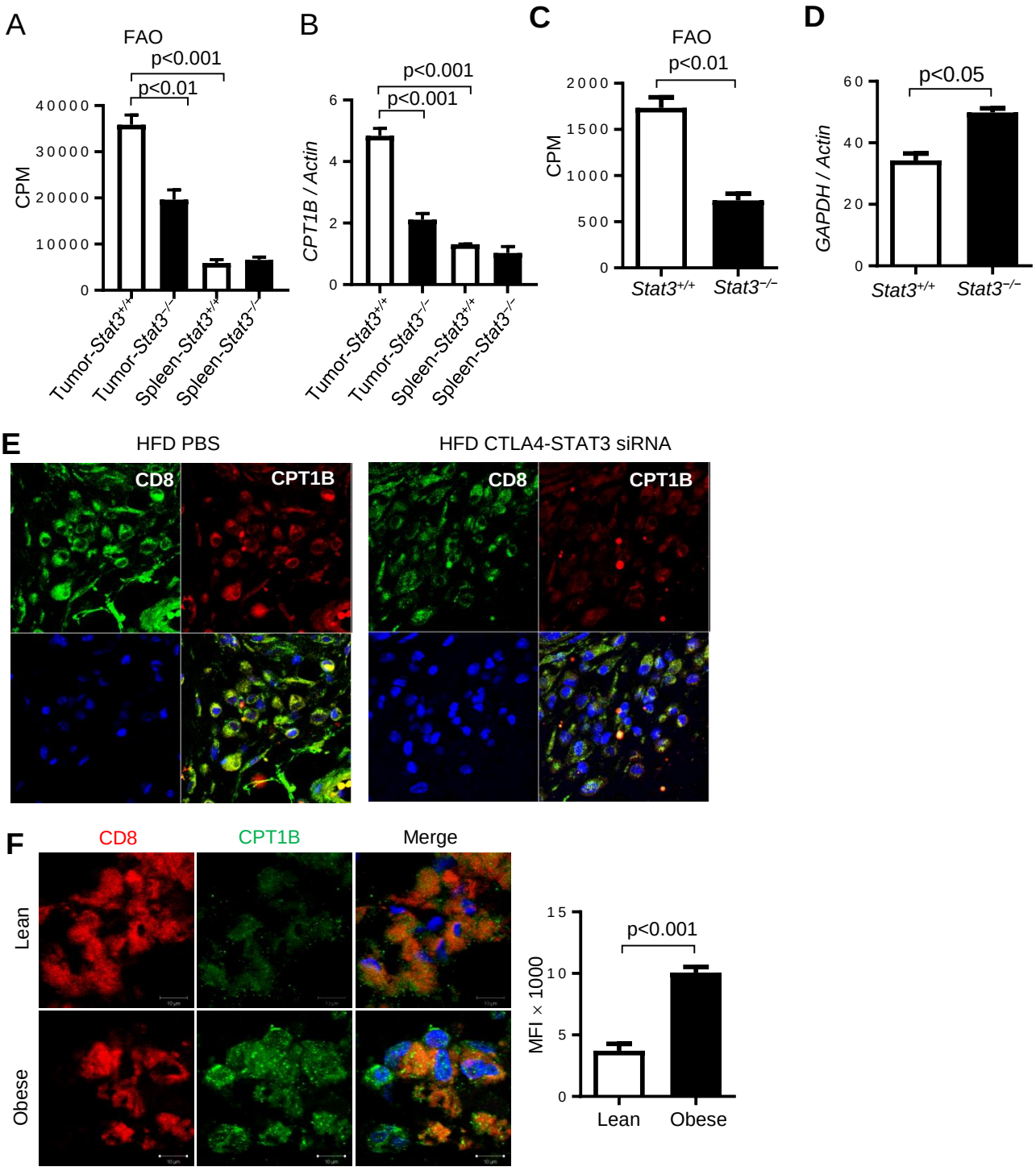
A
FAO
p<0.001
p<0.01
CPM
40000
30000
20000
10000
0
Tumor-Stat3+/+
Tumor-Stat3−/−
Spleen-Stat3+/+
Spleen-Stat3−/−
B
p<0.001
p<0.001
CPT1B / Actin
6
4
2
0
C
FAO
p<0.01
CPM
2000
1500
1000
500
0
Stat3+/+
Stat3−/−
D
p<0.05
GAPDH / Actin
60
40
20
0
E
HFD PBS
HFD CTLA4-STAT3 siRNA
CD8
CPT1B
F
CD8
CPT1B
Merge
Lean
Obese
MFI × 1000
15
10
5
0
p<0.001
Lean
Obese

Figure S4: Ablating *STAT3* in CD8$^+$ T cell enhanced CD8$^+$ T cell anti-tumor effect by reducing FAO while promoting glycolysis, related to Figure 3 and Figure 4.

(A-B) Tumors were harvested from HFD-fed *PyMT/Stat3*$^{+/+}$ and *PyMT/Stat3*$^{-/-}$ tumor bearing mice, and spleens were harvested from HFD-fed littermate *PyMT*$^-$ */Stat3*$^{+/+}$ and *PyMT*$^-$ */Stat3*$^{-/-}$ tumor-free mice. The splenic CD8$^+$ T cells and intra-tumor CD8$^+$ T cells were sorted for measurement of FAO (n = 3) (A). Sorted CD8$^+$ T cells were analyzed for expression of the *CPT1B* genes by q-PCR (n = 3) (B). (C-D) *PyMT* tumor-primed splenic CD3$^+$CD8$^+$ T cells were cultured *in vitro* with 10% TSN in the presence of CD3, CD28 antibodies and IL-2 for two weeks, followed by FAO measurement (n = 2) (C). Analysis of *GAPDH* mRNA expression from cells harvested in (n = 3) (D) (for C and D, n is for number of samples, each of which was pooled from 2-3) mice). (E) Representative immunofluorescence images of CD8 (green), CPT1B (red) and Hoechst (blue) on tumor sections derived from *PyMT* mice fed on HFD treated with PBS or CTLA4-*Stat3* siRNA. (F) Representative immunofluorescence images of CD8 (red), CPT1B (green) and Hoechst (blue) of tumor tissue sections from lean (BMI<25) or obese (BMI>32.5) breast cancer patients, and mean fluorescence index (MFI) was calculated from positive counts in five random views (CD8+areas) per patient specimen (n = 4). All data shown are shown with mean ± SEM.

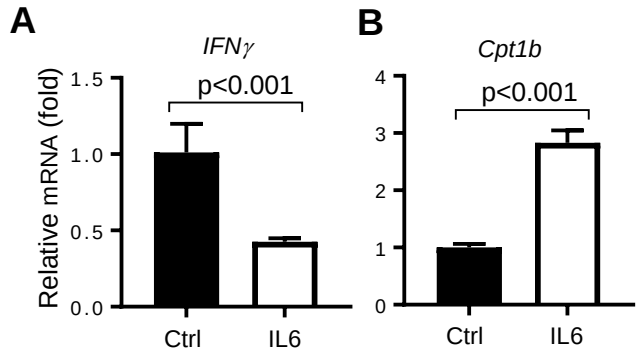

Figure S5: Activation Stat3 with IL6 suppressed *IFNg* and induced *Cpt1*b genes expression in CD8$^+$ T cells, related to Figure 3 and Figure 4.

(A-B) 2x10^6 of purified splenic mouse CD8$^+$ T cells were incubated with 20 ng/ml IL6 for 0.5h. Expression level of *IFNγ*, an activated T_{EFF} cell marker, in CD8$^+$ T cells was detected by qPCR (A). Expression of FAO key enzyme *Cpt1b* was detected by qPCR (B). All data shown are representative of three independent experiments, mean ±SEM.

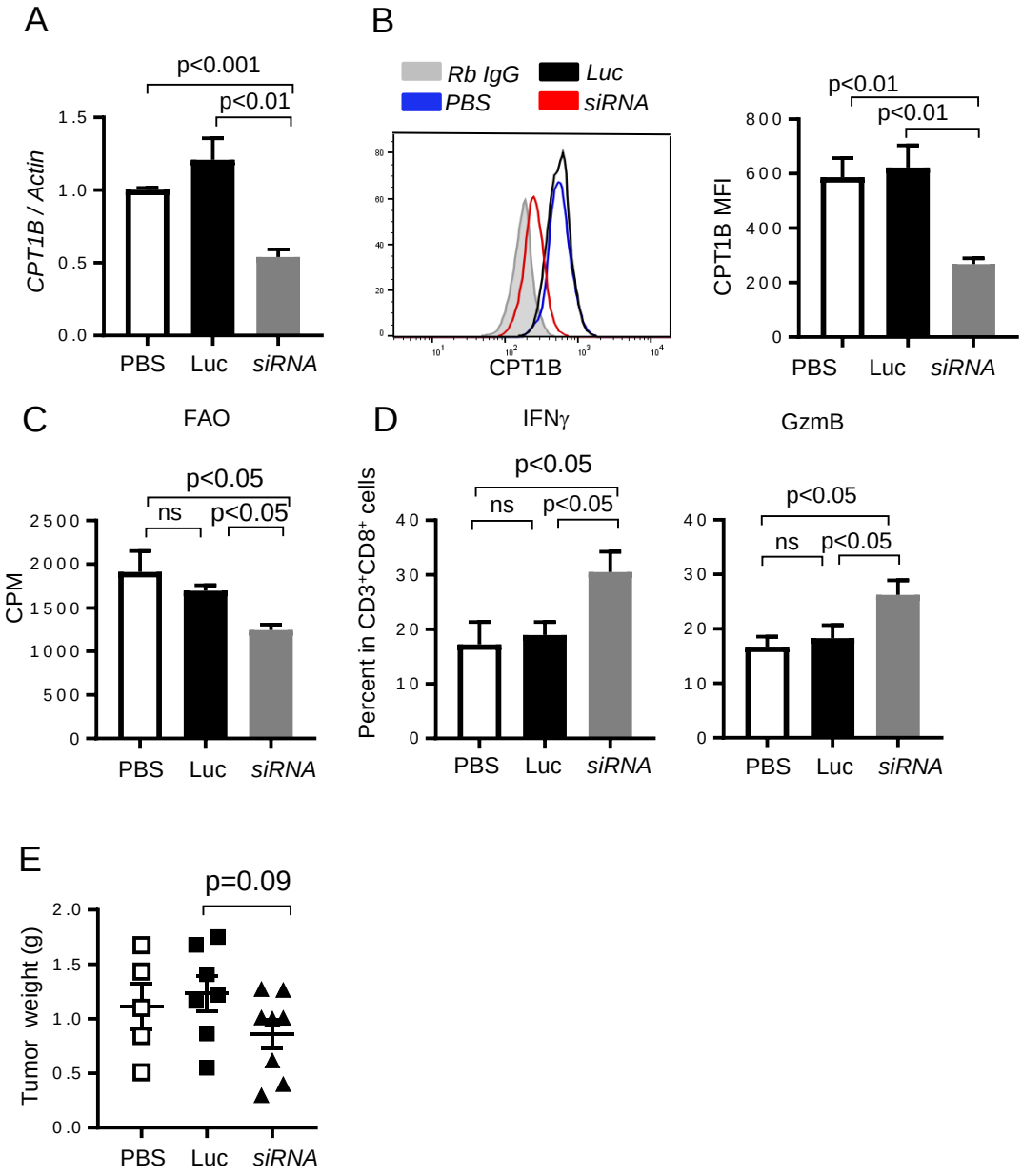

Figure S6: CPT1B specific inhibition improved tumor CD8⁺ T cell functions, related to Figure 5.

Py8119 tumor-bearing C57BL/6 mice were treated with PBS, CTLA4-aptamer-luc (luc)siRNA (luc) or CTLA4-aptamer-CPT1B siRNA (siRNA) every other day for 2 weeks (n = 5-8). (A-C) Sorted tumor-infiltrating CD8⁺ T cells from tumor-bearing mice were analyzed for *CPT1B* gene expression by q-PCR (A), protein expression by flow cytometry (n = 4-5) (B) and FAO rate (n = 4) (C). (D) Tumors were collected to prepare single-cell suspension for flow cytometry analysis of IFNγ and granzyme B producing CD8⁺ T cells (n = 5-8). (E) Tumors were collected and tumor weight were measured (n = 5-8). All data are shown with mean ± SEM.

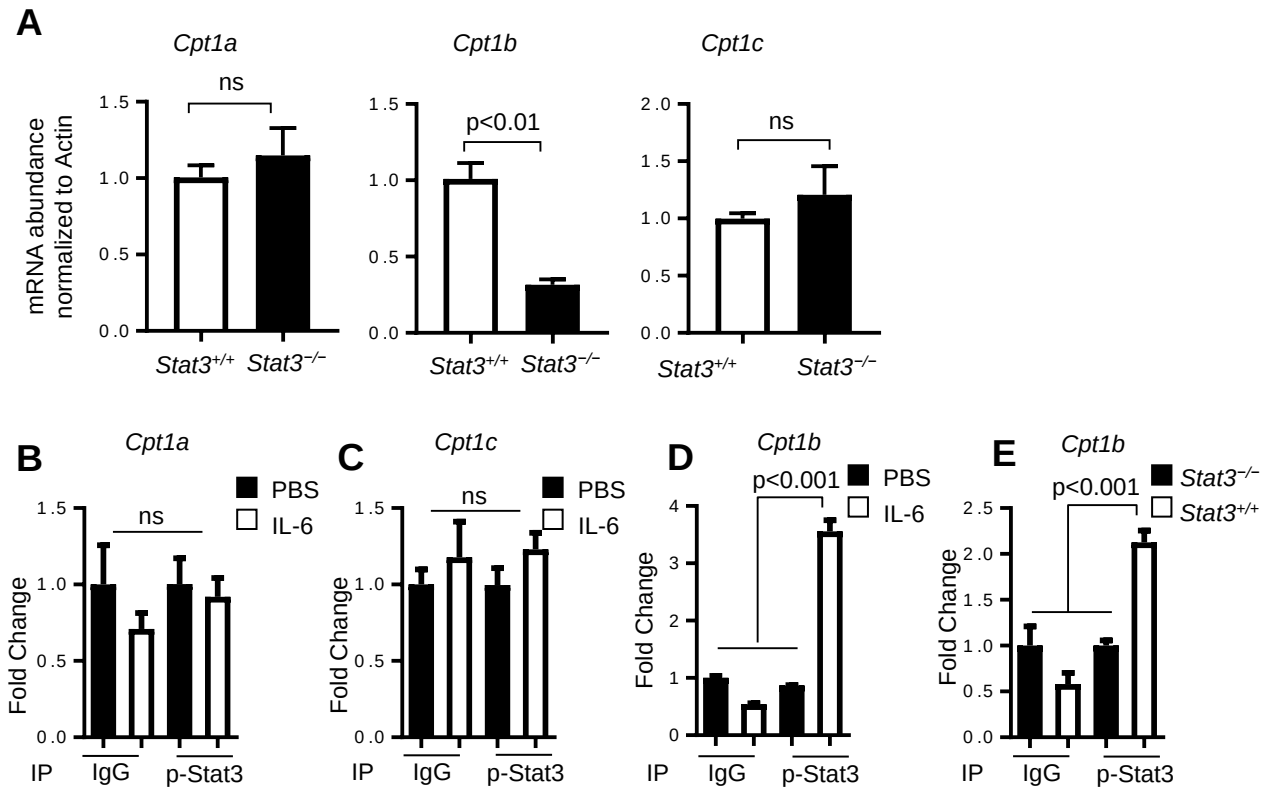

Figure S7: Stat3 enhances expression of *Cpt1b, but not Cpt1a, or Cpt1c,* by directly binding to the *Cpt1b* promoter in CD8 T cells in the tumor milieu, related to Figure 5.

(A) Tumors were prepared from *PyMT* mice with *Stat3*$^{+/+}$ and *Stat3*$^{-/-}$(Stat3 functional KO in which phospho-Stat3 (Tyr 705) is deleted) T cells. The intra-tumor CD3^{+}CD8^{+} T cells were sorted for measurement of mRNA expression of the indicated *Cpt1* genes using q-PCR (n = 3). (B-E) Splenic CD8^{+} T cells from C57BL/6 mice or *PyMT* tumor-bearing mice were used for chromatin immuno-precipitation (ChIP). Chromatins were prepared from IL-6 stimulated splenic CD8^{+} T cells from C57BL/6 mice (B-D) or splenic CD8^{+} T cells with/without functional *Stat3* from *PyMT* tumor-bearing mice (E). Quantitative real-time PCR showing the relative binding activities of p-STAT3 on the promoter regions of *CPT1a, CPT1b* or *CPT1c*. The results were normalized by PBS control (B-D) or Stat3$^{-/-}$ control (E) (fold change). Results are means ± SEM (n = 3).