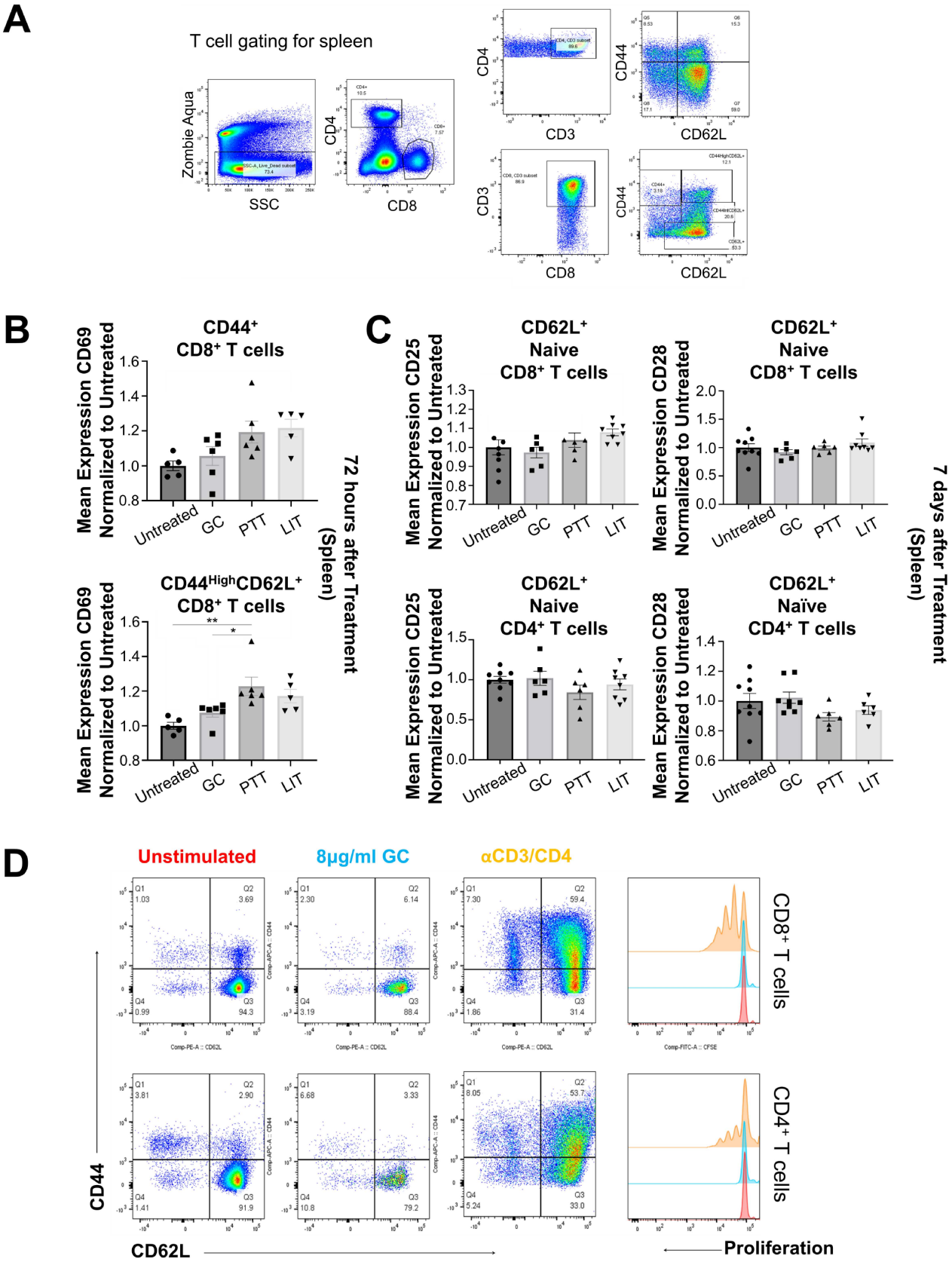

A
T cell gating for spleen
Zombie Aqua
SSC
CD4
CD8
CD3
CD44
CD62L
B
CD44+ CD8+ T cells
CD44HighCD62L+ CD8+ T cells
Mean Expression CD69 Normalized to Untreated
Untreated
GC
PTT
LIT
72 hours after Treatment (Spleen)
C
CD62L+ Naive CD8+ T cells
CD62L+ Naive CD4+ T cells
Mean Expression CD25 Normalized to Untreated
Mean Expression CD28 Normalized to Untreated
7 days after Treatment (Spleen)
D
Unstimulated
8µg/ml GC
αCD3/CD4
CD8+ T cells
CD4+ T cells
CD44
CD62L
Proliferation

Figure S2

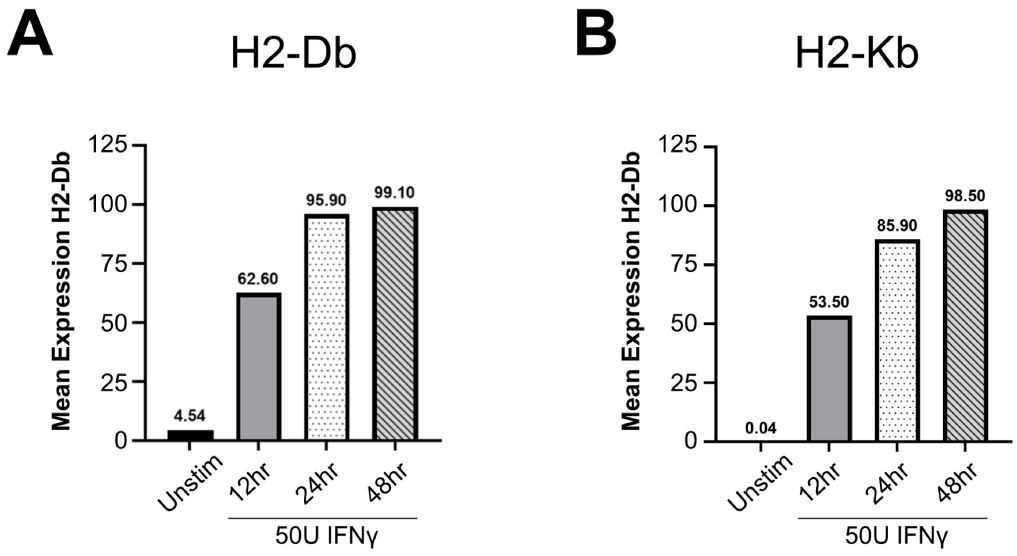

A
H2-Db
Mean Expression H2-Db
125
100
75
50
25
0
4.54
62.60
95.90
99.10
Unstim
12hr
24hr
48hr
50U IFNγ
B
H2-Kb
Mean Expression H2-Db
125
100
75
50
25
0
0.04
53.50
85.90
98.50
Unstim
12hr
24hr
48hr
50U IFNγ

Figure S3

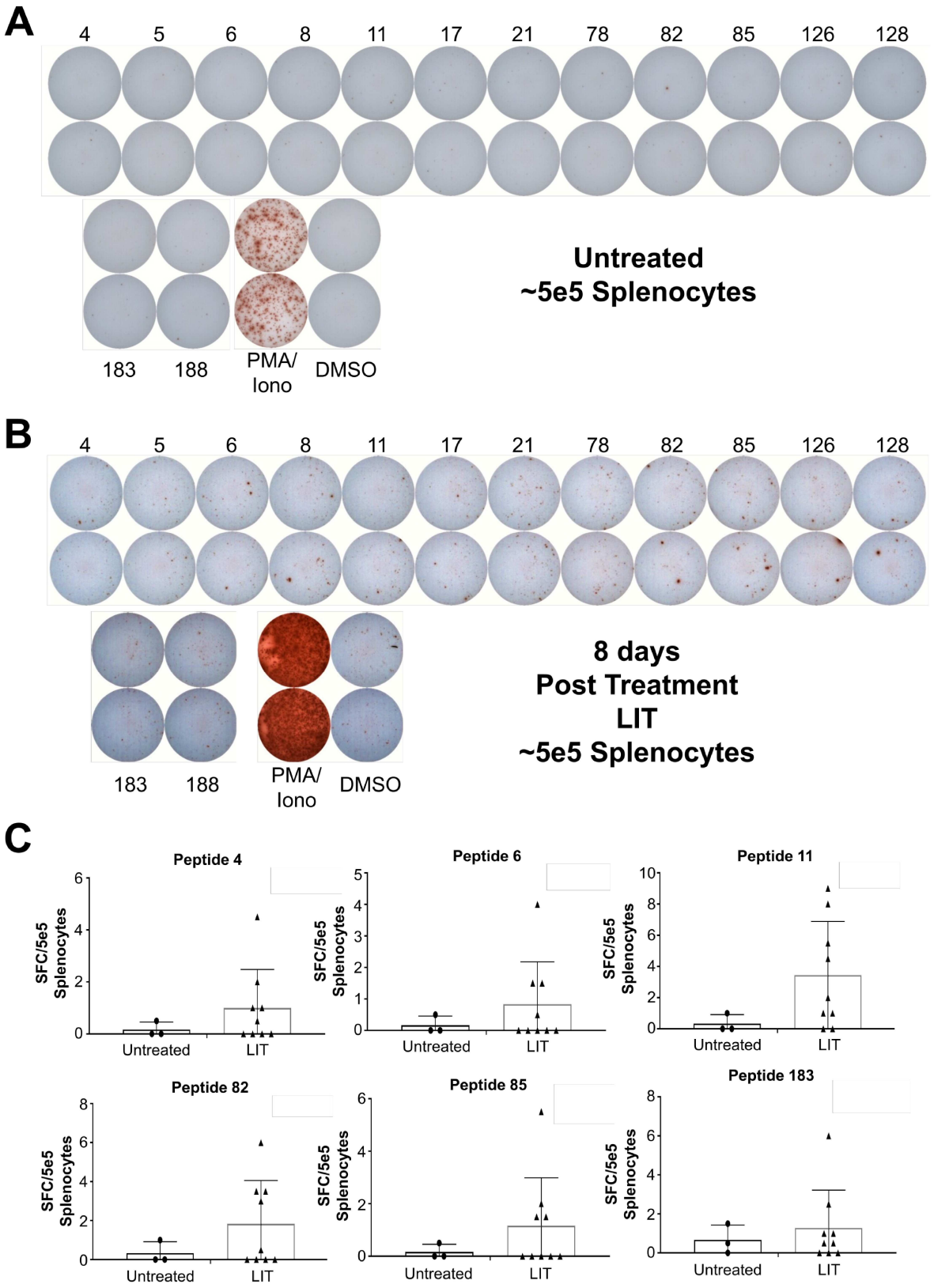
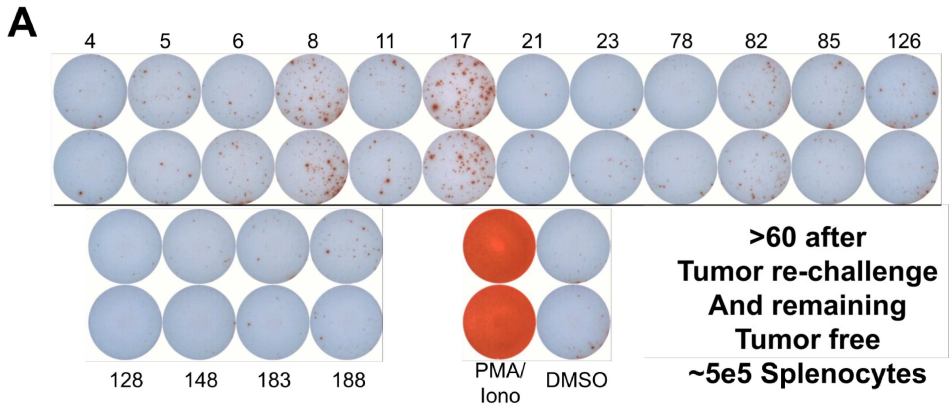
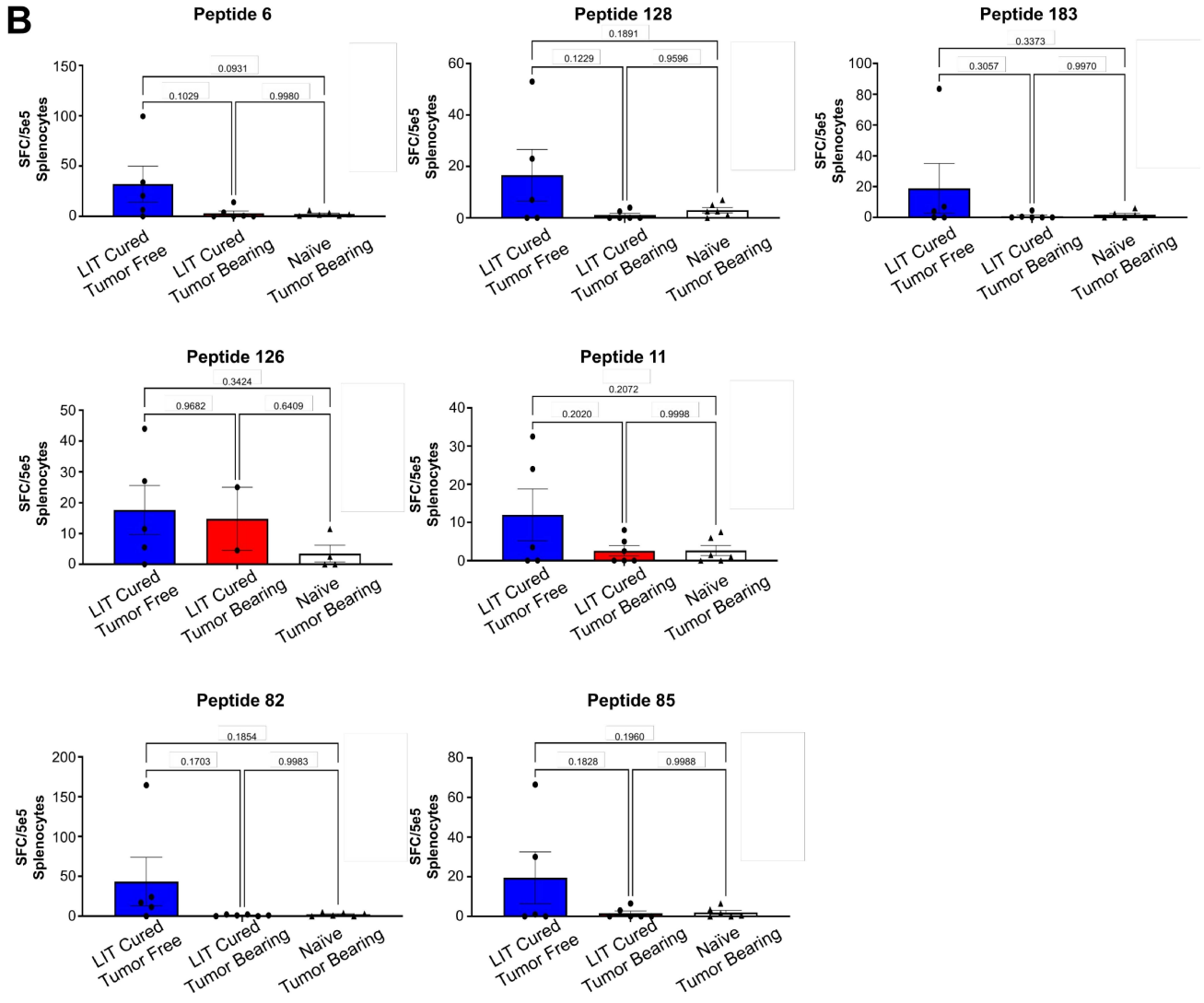

A
4 5 6 8 11 17 21 78 82 85 126 128
183 188 PMA/ Iono DMSO
Untreated
~5e5 Splenocytes
B
4 5 6 8 11 17 21 78 82 85 126 128
183 188 PMA/ Iono DMSO
8 days
Post Treatment
LIT
~5e5 Splenocytes
C
Peptide 4
Peptide 6
Peptide 11
Peptide 82
Peptide 85
Peptide 183
SFC/5e5 Splenocytes
Untreated
LIT

Figure S4


A
4
5
6
8
11
17
21
23
78
82
85
126
128
148
183
188
PMA/
Iono
DMSO
>60 after
Tumor re-challenge
And remaining
Tumor free
~5e5 Splenocytes


B
Peptide 6
Peptide 128
Peptide 183
Peptide 126
Peptide 11
Peptide 82
Peptide 85
SFC/5e5 Splenocytes
LIT Cured Tumor Free
LIT Cured Tumor Bearing
Naïve Tumor Bearing

Supplemental Figure 1

LIT activation of T cells within the spleen. (A) Flow cytometry gating used for analysis of the $CD4^+$ and $CD8^+$ T cells. (B) Flow cytometry analysis of the normalized mean expression of CD69 on the surface of $CD8^+$ effector and memory T cells from the spleens of animals in different groups 72 hours after treatment. (C) Flow cytometry analysis of the normalized mean expression of CD25 and CD25 on the surface of naïve CD4 and CD8 naïve T cells from the animal spleens seven days after treatment. One-way ANOVA was used for statistical analysis of the mean expression data. P values = *0.5, **0.05, **0.005, ****0.0005. (D) Flow cytometry analysis of CD44, CD62L, and CFSE expression of either purified $CD4^+$ or $CD8^+$ T cells from the spleens. T cells were incubated with either GC or anti-CD3/anti-CD28 for 72 hours prior to flow analysis.

Supplemental Figure 2

In vitro characterization of B16-F10 H2-Db and H2-Kb expression following 50 U/mL of IFNγ stimulation (A-B). Analysis of the expression of (A) H2Db and (B) H2Kb MHC-I molecules on B16-F10 cells grown in vitro. The mean expression of H2-Db or H2-Kb was examined after 12, 24, and 48 hours via flow cytometry. Expression of H2-Db or H2-Kb is represented as a bar graph.

Supplemental Figure 3

ELISpot screening of tumor specific peptides in untreated and LIT B16-F10 tumor-bearing animals. (A-C) C57BL/6 mice of 6-8-week-old were injected with 1e5 B16-F10 tumor cells. Once the tumors reached $0.5cm^3$, the tumors were treated with LIT or left untreated. Eight days after treatment splenocytes were isolated and used for ELISPOT. Approximately 5e5 total splenocytes were used for the ELISPOTs with ~1μM peptide. ELISPOTs were incubated for ~36 hours prior to developing the ELISPOT. Spots were counted using the default settings on the ELISPOT reader. Assays were performed in duplicate. The average number of spots in the negative control was then subtracted from the average number of spots in the peptide incubated wells to get the final spot count. (A) ELISPOT using splenocytes from untreated tumor-bearing animals. (B) ELISPOT using splenocytes from LIT treated animals 8 days after treatment. (C) Spot-forming cells (SFC) per well were calculated using the following formula for six selected peptides, Average spots peptide wells – Average spots DMSO wells = final spot count per well and graphed.

Supplemental Figure 4

Tumor specific peptide T cell reactivity in LIT-cured animals that resisted tumor rechallenge. (A-B) C57BL/6 mice of 6–8-week-old were injected with 1e5 B16-F10 tumor cells. Once the tumors reached $0.5cm^3$, the tumors were treated with LIT or left untreated. LIT-cured survivors were then rechallenged on the opposite flank after being tumor free for >60days. Once the animals were tumor free for >60days following tumor rechallenge, splenocytes were isolated and incubated with B16-F10 specific peptides for ~36 hours before being developed. Approximately 5e5 total splenocytes from LIT-cured tumor rechallenged non-tumor-bearing animals were used to ELISpots with ~1μM peptide. ELISpots were incubated for ~36 hours prior to developing the ELISpot. (A) ELISPOT using splenocytes from LIT-cured animals that resisted tumor rechallenge. (B) Spot-forming cells (SFC) were counted using the default settings on the ELISPOT reader for seven selected peptides. Assays were performed in duplicate. The average number of spots in the negative control was then subtracted from the average number of spots in the peptide incubated wells to get the final spot count. The number was graphed, and each dot represents an individual animal. One-Way ANOVA was used for statistical analysis. P values = *0.5, **0.05, **0.005, ****0.0005.