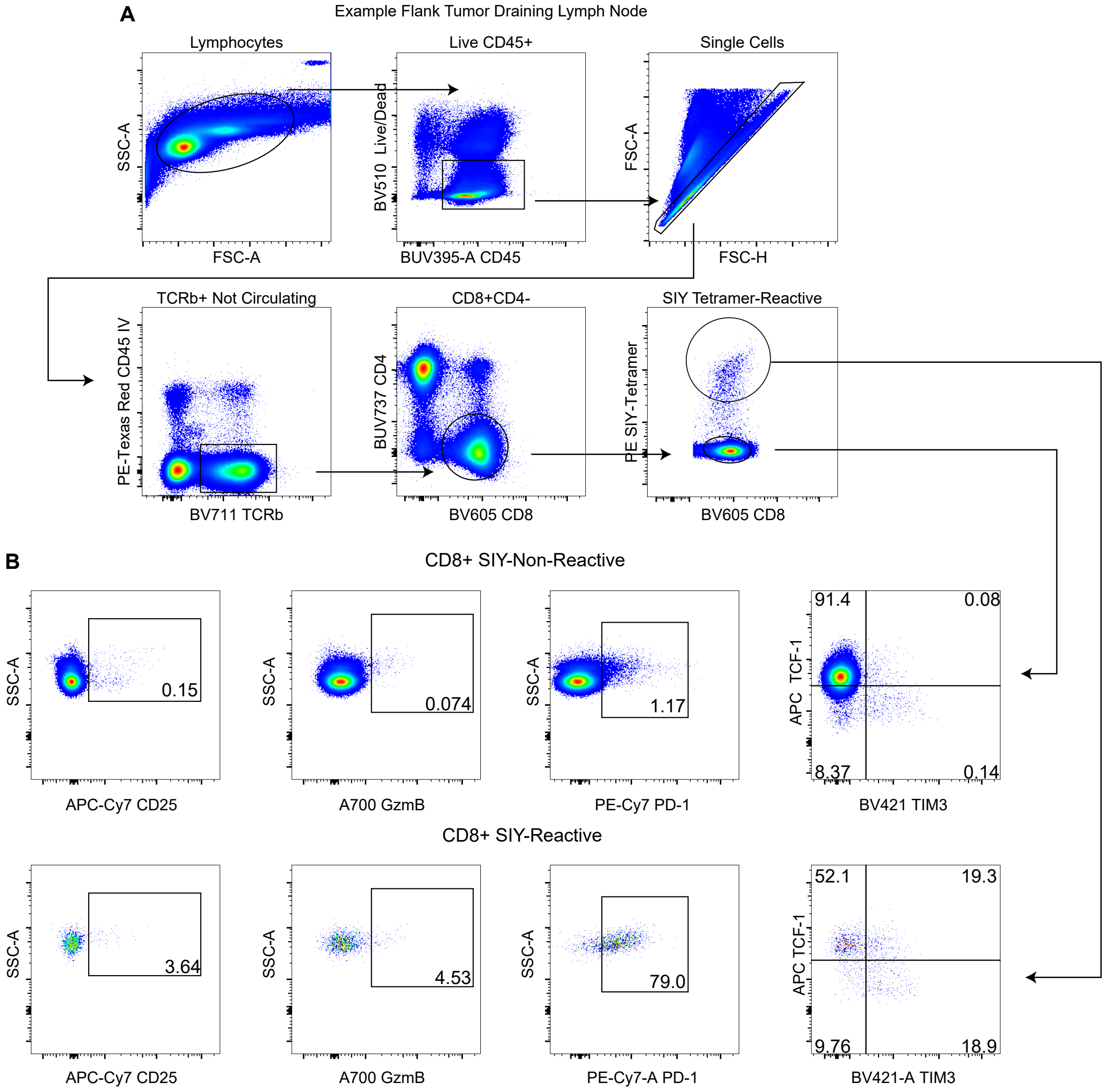
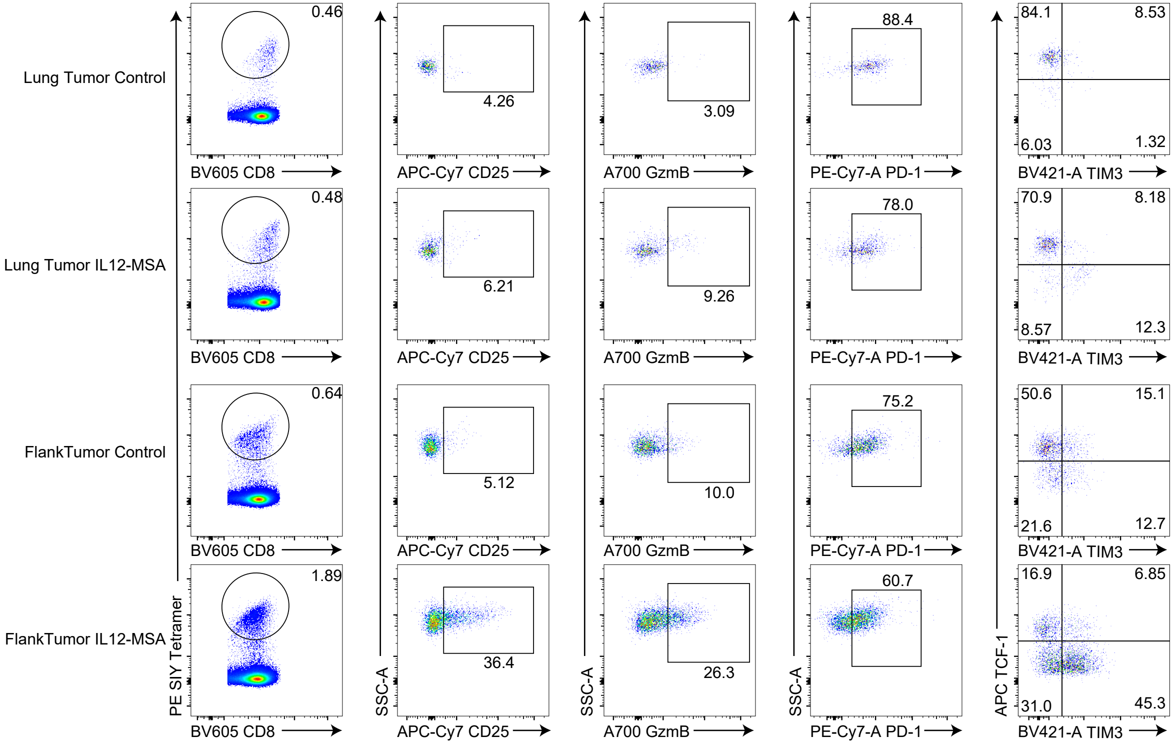

A
Example Flank Tumor Draining Lymph Node
Lymphocytes
SSC-A
FSC-A
Live CD45+
BV510 Live/Dead
BUV395-A CD45
Single Cells
FSC-A
FSC-H
TCRb+ Not Circulating
PE-Texas Red CD45 IV
BV711 TCRb
CD8+CD4-
BUV737 CD4
BV605 CD8
SIY Tetramer-Reactive
PE SIY-Tetramer
BV605 CD8
B
CD8+ SIY-Non-Reactive
SSC-A
0.15
APC-Cy7 CD25
SSC-A
0.074
A700 GzmB
SSC-A
1.17
PE-Cy7 PD-1
91.4
0.08
8.37
0.14
APC TCF-1
BV421 TIM3
CD8+ SIY-Reactive
SSC-A
3.64
APC-Cy7 CD25
SSC-A
4.53
A700 GzmB
SSC-A
79.0
PE-Cy7-A PD-1
52.1
19.3
9.76
18.9
APC TCF-1
BV421-A TIM3

**Supplemental Figure 1.**

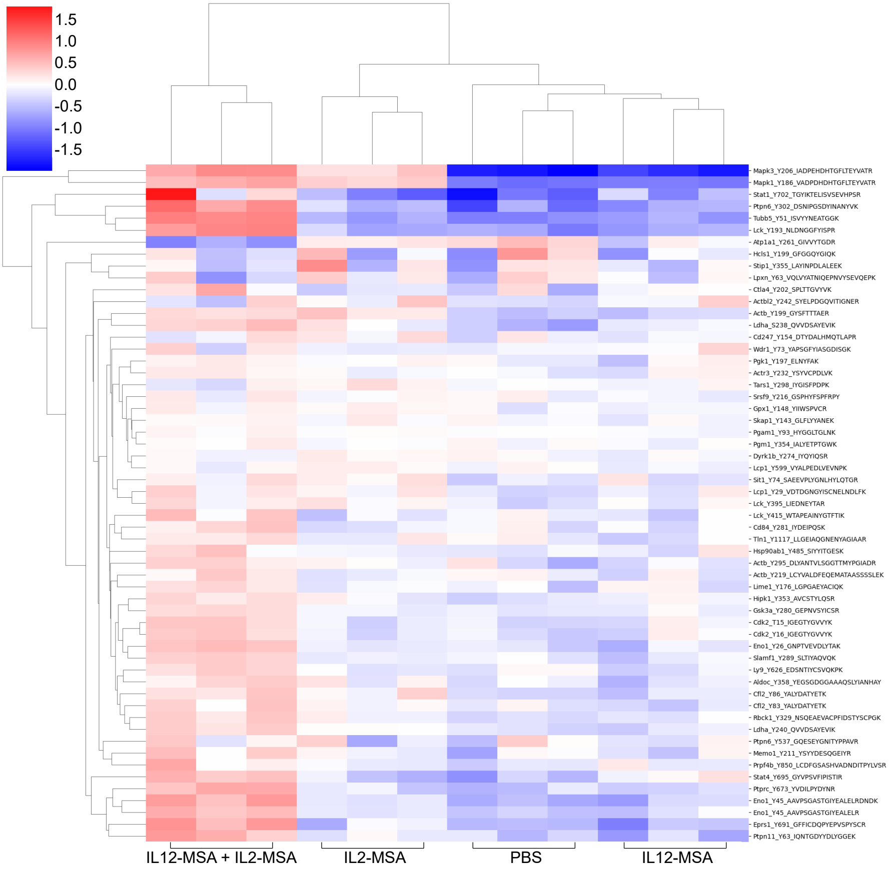
**(A)** Representative gating strategy to identify SIY tetramer-reactive CD8$^+$ T cells. **(B)** Representative gating of CD25, GzmB, PD-1, TCF-1, and TIM-3 on SIY-reactive and SIY non-reactive CD8$^+$ T cells.

Example Tumor-Draining Lymph Nodes



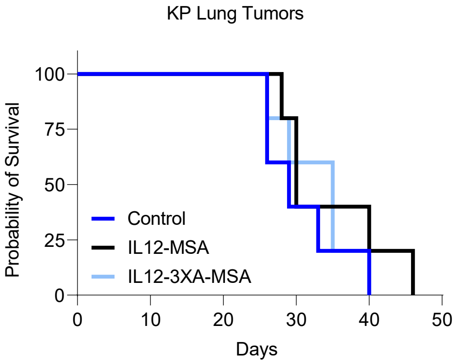
**Supplemental Figure 2.**

Representative gating of SIY tetramer-reactive CD8$^+$ T cells and their expression of CD25, GzmB, PD-1, TCF-1, and TIM-3 across different tumor sites and treatment conditions. Images depict tumor-draining lymph nodes. The same gating strategies were used for analyzing spleens and tumors.


1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
Mapk3_Y206_IADPEHDHTGFLTEYVATR
Mapk1_Y186_VADPDHDHTGFLTEYVATR
Stat1_Y702_TGYIKTELISVSEVHPSR
Ptpn6_Y302_DSNIPGSDYINANYVK
Tubb5_Y51_ISVYYNEATGGK
Lck_Y193_NLDNGGFYISPR
Atp1a1_Y261_GIVVYTGDR
Hcls1_Y199_GFGGQYGIQK
Stip1_Y355_LAYINPDLALEEK
Lpxn_Y63_VQLVYATNIQEPNVYSEVQEPK
Ctla4_Y202_SPLTTGVYVK
Actbl2_Y242_SYELPDGQVITIGNER
Actb_Y199_GYSFTTTAER
Ldha_S238_QVVDSAYEVIK
Cd247_Y154_DTYDALHMQTLAPR
Wdr1_Y73_YAPSGFYIASGDISGK
Pgk1_Y197_ELNYFAK
Actr3_Y232_YSYVCPDLVK
Tars1_Y298_IYGISFPDPK
Srsf9_Y216_GSPHYFSPFRPY
Gpx1_Y148_YIIWSPVCR
Skap1_Y143_GLFLYYANEK
Pgam1_Y93_HYGGLTGLNK
Pgm1_Y354_IALYETPTGWK
Dyrk1b_Y274_IYQYIQSR
Lcp1_Y599_VYALPEDLVEVNPK
Sit1_Y74_SAEEVPLYGNLHYLQTGR
Lcp1_Y29_VDTDGNGYISCNELNDLFK
Lck_Y395_LIEDNEYTAR
Lck_Y415_WTAPEAINYGTFTIK
Cd84_Y281_IYDEIPQSK
Tln1_Y1117_LLGEIAQGNENYAGIAAR
Hsp90ab1_Y485_SIYYITGESK
Actb_Y295_DLYANTVLSGGTTMYPGIADR
Actb_Y219_LCYVALDFEQEMATAASSSSLEK
Lime1_Y176_LGPGAEYACIQK
Hipk1_Y353_AVCSTYLQSR
Gsk3a_Y280_GEPNVSYICSR
Cdk2_T15_IGEGTYGVVYK
Cdk2_Y16_IGEGTYGVVYK
Eno1_Y26_GNPTVEVDLYTAK
Slamf1_Y289_SLTIYAQVQK
Ly9_Y626_EDSNTIYCSVQKPK
Aldoc_Y358_YEGSGDGGAAAQSLYIANHAY
Cfl2_Y86_YALYDATYETK
Cfl2_Y83_YALYDATYETK
Rbck1_Y329_NSQEAEVACPFIDSTYSCPGK
Ldha_Y240_QVVDSAYEVIK
Ptpn6_Y537_GQESEYGNITYPPAVR
Memo1_Y211_YSYYDESQGEIYR
Prpf4b_Y850_LCDFGSASHVADNDITPYLVSR
Stat4_Y695_GYVPSVFIPISTIR
Ptprc_Y673_YVDILPYDYNR
Eno1_Y45_AAVPSGASTGIYEALELRDNDK
Eno1_Y45_AAVPSGASTGIYEALELR
Eprs1_Y691_GFFICDQPYEPVSPYSCR
Ptpn11_Y63_IQNTGDYYDLYGGEK
IL12-MSA + IL2-MSA
IL2-MSA
PBS
IL12-MSA

**Supplemental Figure 3.**

Heatmap of selected phosphorylation sites. Unsupervised hierarchical clustering was performed on the basis of the Pearson correlation distance metric.


KP Lung Tumors
Probability of Survival
100
75
50
25
0
0
10
20
30
40
50
Days
Control
IL12-MSA
IL12-3XA-MSA

**Supplemental Figure 4.**

Kaplan-Meier plot of mice bearing KP lung tumors. Mice were treated with either IL12-MSA or IL12-3XA-MSA on days 7 and 14 post-tumor inoculation and monitored for survival. N = 5 for all conditions. Log-rank test (Mantel-Cox).

| Significantly Different Phospho Sites | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | P value | Mean of IL12+IL2 | Mean of PBS | Difference | SE of difference | t ratio | df | q value |
| Stat4_Y694 | 0.000581 | 1.654 | 0.5745 | 1.08 | 0.02603 | 41.49 | 2 | 0.015927 |
| Gsk3a_Y279 | 0.000631 | 1.333 | 0.8513 | 0.4818 | 0.01211 | 39.79 | 2 | 0.015927 |
| Tubb5_Y50 | 0.000758 | 2.594 | 0.3908 | 2.203 | 0.0607 | 36.29 | 2 | 0.015927 |
| Cdk2_Y15 | 0.002752 | 1.453 | 0.7831 | 0.6703 | 0.03523 | 19.02 | 2 | 0.037617 |
| Memo1_Y210 | 0.003528 | 1.382 | 0.8407 | 0.5417 | 0.03227 | 16.79 | 2 | 0.037617 |
| Eno1_Y25 | 0.003583 | 1.604 | 0.7866 | 0.8169 | 0.04903 | 16.66 | 2 | 0.037617 |
| Mapk1_Y185 | 0.005997 | 1.857 | 0.3432 | 1.514 | 0.1178 | 12.85 | 2 | 0.043623 |
| Actb_Y198 | 0.006111 | 1.323 | 0.6585 | 0.6643 | 0.05217 | 12.73 | 2 | 0.043623 |
| Mapk3_Y205 | 0.0065 | 2.263 | 0.159 | 2.104 | 0.1705 | 12.34 | 2 | 0.043623 |
| Eno1_Y44 | 0.006924 | 1.718 | 0.6569 | 1.061 | 0.08872 | 11.95 | 2 | 0.043623 |
| Lck_Y192 | 0.008918 | 2.432 | 0.4697 | 1.963 | 0.1866 | 10.52 | 2 | 0.048384 |
| Slamf1_Y288 | 0.009658 | 1.447 | 0.8968 | 0.5498 | 0.05442 | 10.1 | 2 | 0.048384 |
| Hipk1_Y352 | 0.009984 | 1.291 | 0.7696 | 0.5214 | 0.05249 | 9.933 | 2 | 0.048384 |
| Cdk2_T14 | 0.011685 | 1.466 | 0.781 | 0.6846 | 0.07466 | 9.169 | 2 | 0.049173 |
| Ptprc_Y672 | 0.01192 | 1.813 | 0.663 | 1.15 | 0.1267 | 9.077 | 2 | 0.049173 |
| Actr3_Y231 | 0.012488 | 1.148 | 0.9058 | 0.2422 | 0.02732 | 8.864 | 2 | 0.049173 |

**Supplemental Table 1.**

Phosphorylation sites that are statistically significantly different between control and IL2-MSA + IL12-MSA treated samples.