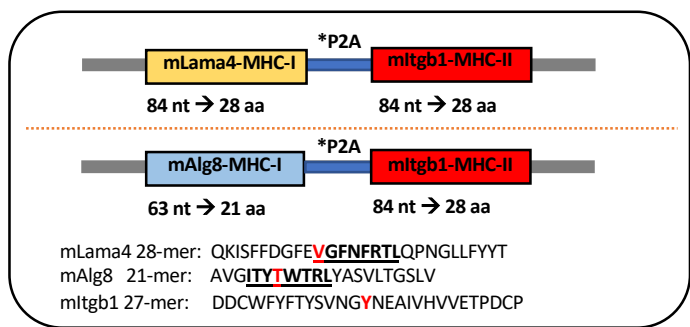
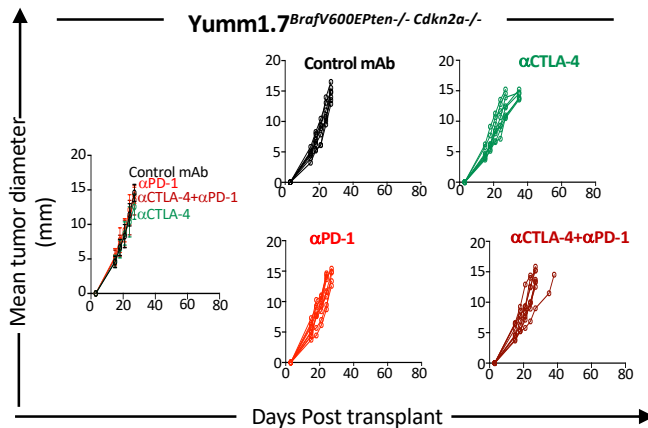


Figure S1

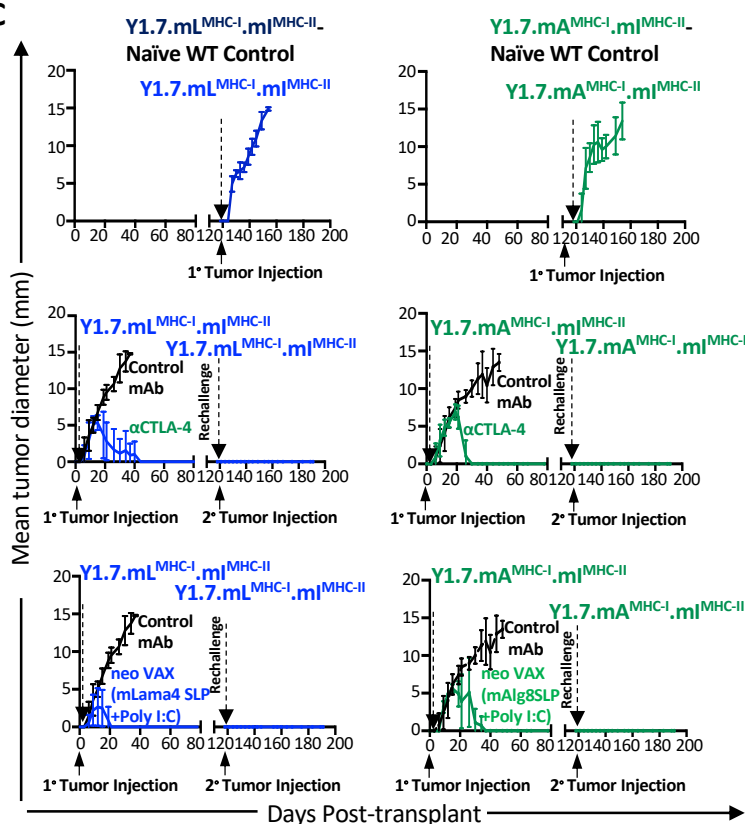
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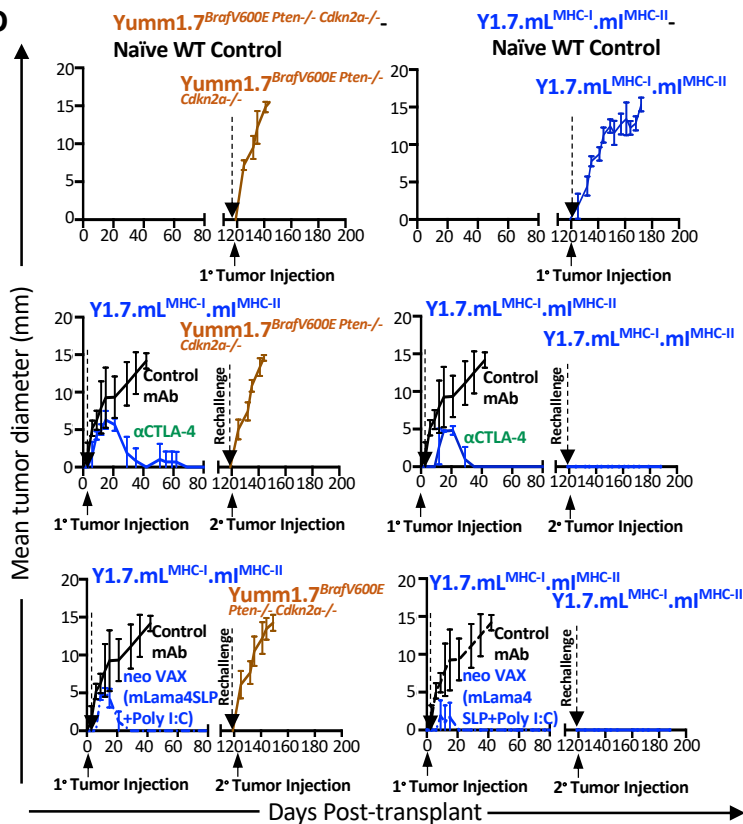
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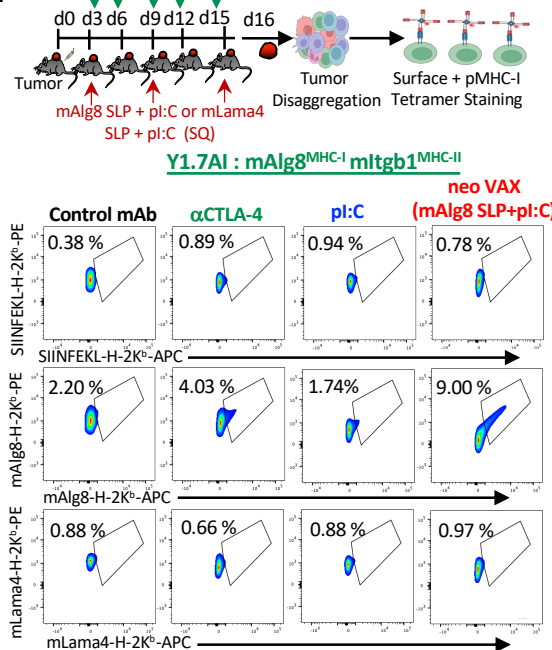
C



D



E



F

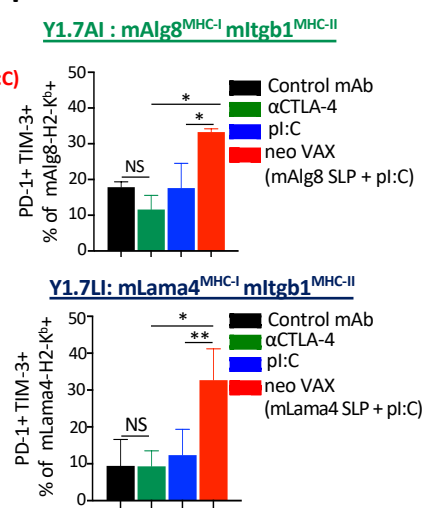
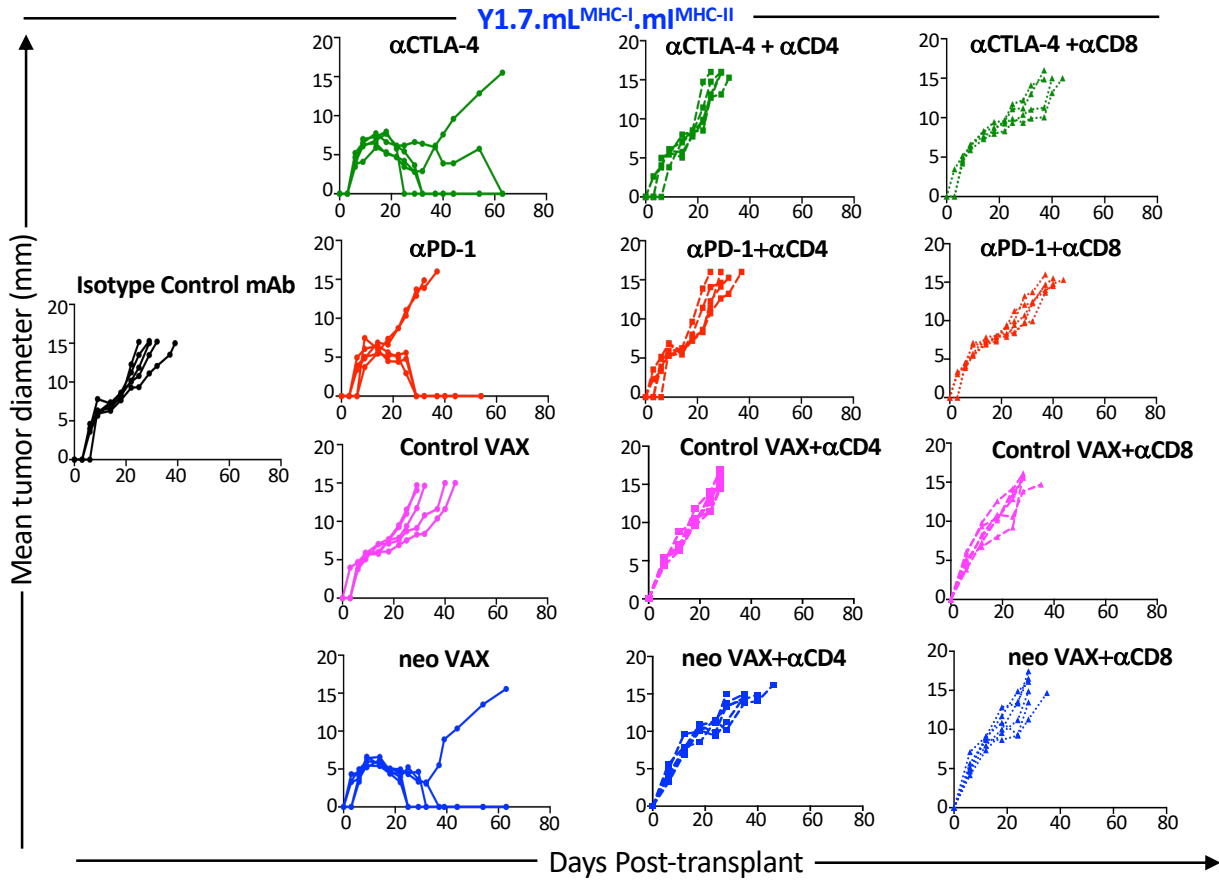
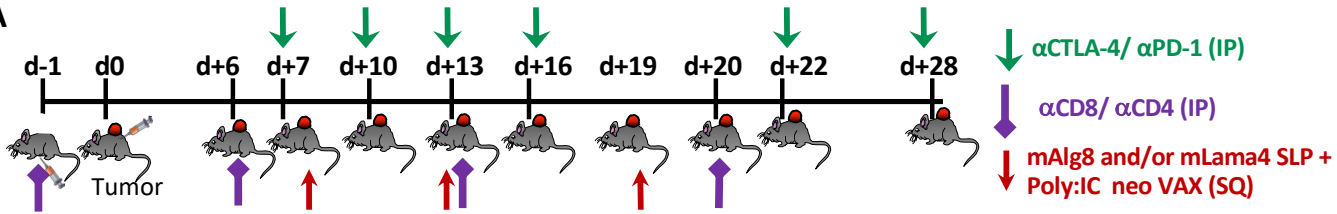


Figure S1. NeoAg vaccines and ICT induces long-term tumor protection in Y1.7AI and Y1.7LI melanoma models. Related to Figure 1.

(A) Depiction of NeoAg minigenes used to express NeoAgs in the parental *Braf*^{V600E} *Pten*^{-/-} *Cdkn2a*^{-/-} YUMM1.7 melanoma line, along with peptide sequences encoded by minigenes. mLama4 or mAlg8 and mltgb1 NeoAgs were separated by 2A peptides that induce ribosomal skipping during translation. (B) Tumor growth in WT C57BL/6J mice transplanted with parental *Braf*^{V600E} *Pten*^{-/-} *Cdkn2a*^{-/-} YUMM1.7 melanoma cells and treated with control mAb, anti-CTLA-4, anti-PD-1 or anti-CTLA4 + anti-PD-1 combination immune checkpoint therapy (ICT) on d. 3, 6, 9, 12, 18, 24 post tumor-transplant. (C) WT C57BL/6J mice were transplanted with Y1.7 mA^{MHC-I}.ml^{MHC-II} (Y1.7AI) and Y1.7 mL^{MHC-I}.ml^{MHC-II} (Y1.7LI) melanoma cells and treated with control mAb or anti-CTLA-4 on d. 3, 6, 9, 12, 18, 24 or mAlg8 NeoAg (relevant for Y1.7AI) synthetic long peptide (SLP) + poly I:C (pl:C) or mLama4 NeoAg (relevant for Y1.7LI) synthetic long peptide (SLP) + pl:C on d. 3, 9, 15. Mice were rechallenged with same tumor used for initial tumor challenge at least 60 days post-rejection of primary tumor. Naïve WT C57BL/6J mice transplanted with Y1.7AI or Y1.7LI tumor without any treatment were included as controls, indicating cell line preps used in rechallenge experiments were capable of tumor formation. (D) WT C57BL/6J mice were transplanted with Y1.7LI melanoma cells and treated with anti-CTLA-4 ICT on d. 3, 6, 9, 12, 18, 24 or mLama4 NeoAg SLP + pl:C on d. 3, 9, 15. Mice were rechallenged with either with same tumor used for initial tumor challenge (Y1.7LI) or parental *Braf*^{V600E} *Pten*^{-/-} *Cdkn2a*^{-/-} YUMM1.7 at least 60 days post-rejection of primary tumor. Naïve WT C57BL/6J mice transplanted with either Y1.7LI or parental YUMM1.7 without any treatment were included as controls. (E) Representative flow cytometry plots displaying mAlg8 or mLama4 tetramer-specific CD8 T cells in Y1.7AI and Y1.7LI tumors treated with control mAb, anti-CTLA-4, pl:C, mAlg8 SLP + pl:C NeoAg vaccine (for Y1.7AI) or mLama4 SLP + pl:C NeoAg vaccine (for Y1.7LI) and harvested on d. 16 post-tumor transplant. mAlg8-H2-K^b, mLama4-H2-K^b, or SIINFEKL-H2-K^b (irrelevant control) tetramers were labeled with PE and APC. Dot plots are gated on live CD45⁺ Thy1.2⁺ CD8 T cells. (F) Co-expression of PD-1 and TIM-3 on mAlg8- or mLama4-specific CD8 T cells in Y1.7AI and Y1.7LI tumors treated with control mAb, anti-CTLA-4, pl:C alone, mAlg8 SLP + pl:C NeoAg vaccine (for Y1.7AI), or mLama4 SLP + pl:C NeoAg vaccine (for Y1.7LI). Tumor growth data in (B), (C) and (D) are presented as individual mouse tumor growth as mean tumor diameter and are representative of three independent experiments.

Figure S2

A



B

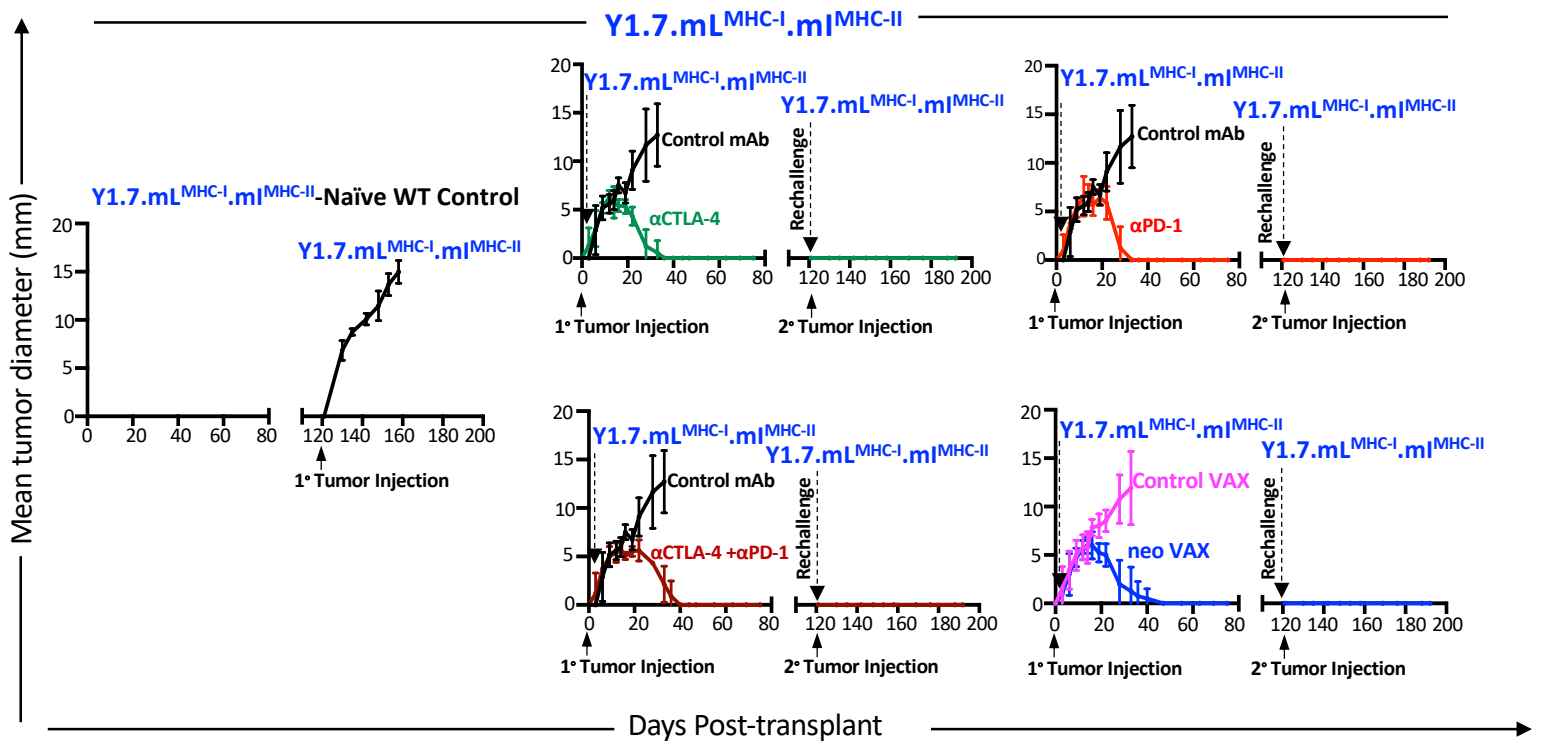
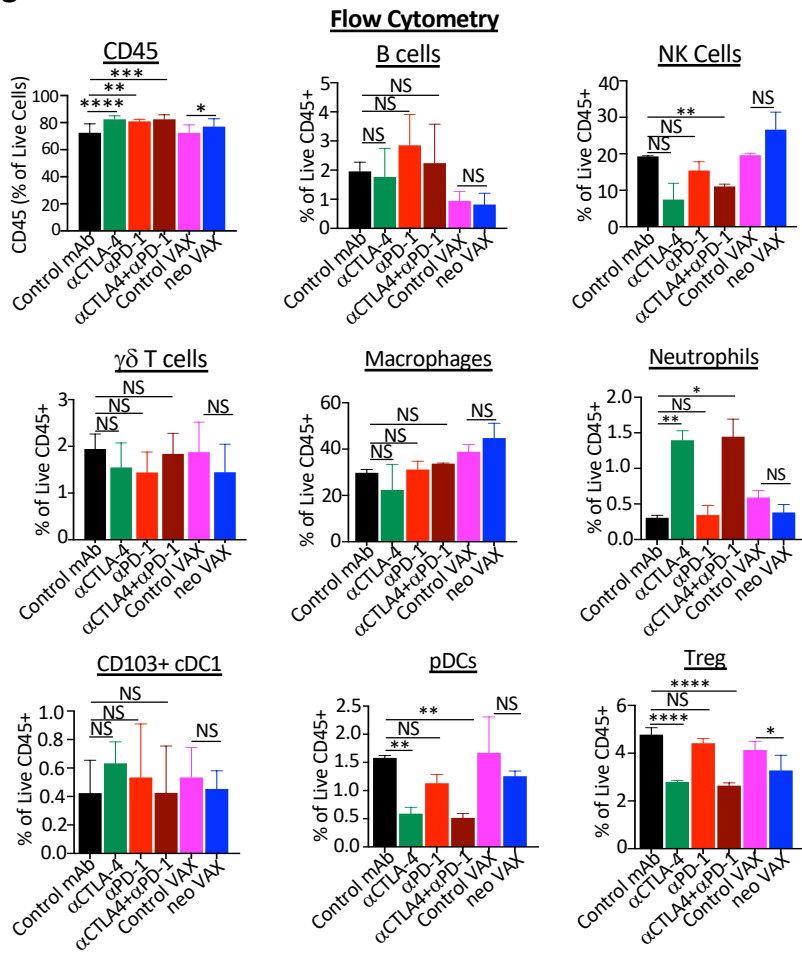


Figure S2. NeoAg vaccines and ICT induce long-term tumor protection in Y1.7LI melanoma models in a T cell-dependent manner. Related to Figure 1.

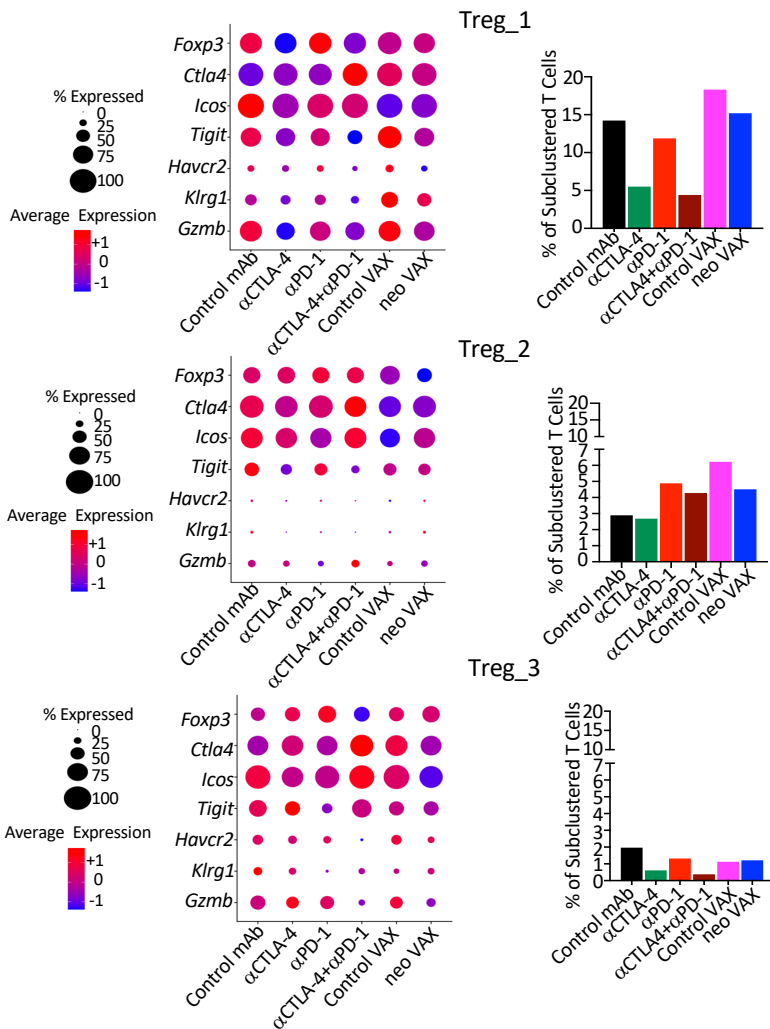
(A) Y1.7LI tumor growth in WT C57BL/6J mice treated with isotype control mAb, anti-CD4, or anti-CD8 α mAbs on d. -1, 6, 13, 20 and anti-CTLA-4 or anti-PD-1 on d. 7, 10, 13, 16, 22, 28 or irrelevant mAlg8 SLP + pl:C (Control VAX) or relevant mLama4 SLP + pl:C (neo VAX) on d. 7, 13, 19. (B) WT C57BL/6J mice transplanted with Y1.7LI melanoma cells were treated with control mAb, anti-CTLA-4, anti-PD-1, anti-CTLA-4 + anti-PD-1, irrelevant (for Y1.7LI) mAlg8 SLP + pl:C (Control VAX), or relevant mLama4 SLP + pl:C (neo VAX) starting on d. 7 post tumor-transplant, and subsequently on d. 10, 13, 16, 22, 28 for ICT and d. 13, 19 for NeoAg vaccines. Mice were rechallenged with the same tumor line used for initial tumor challenge (Y1.7LI) at least 60 days post-rejection of primary tumor. Naïve WT C57BL/6J mice transplanted with Y1.7LI tumor without any treatment was included as a control. Tumor growth data in (A) and (B) are presented as individual mouse tumor growth as mean tumor diameter and are representative of three independent experiments.

Figure S3

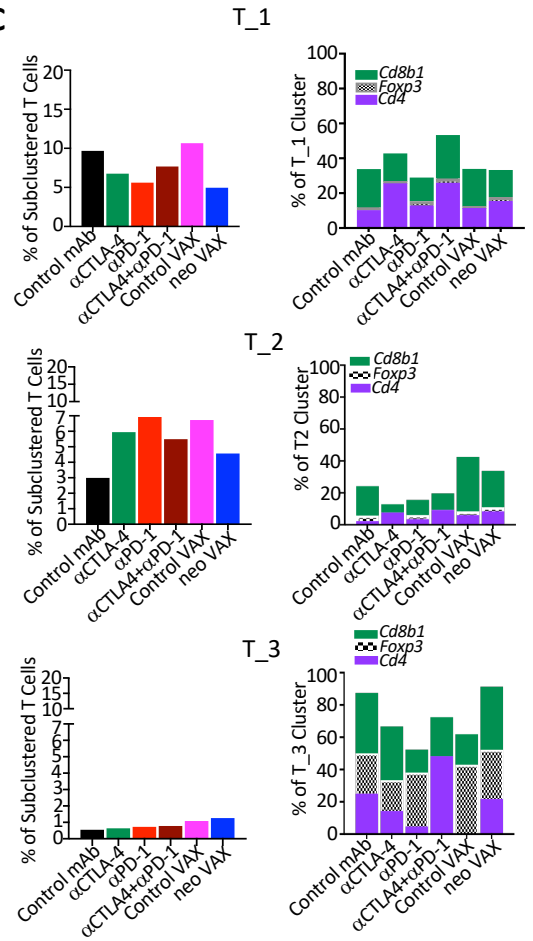
A



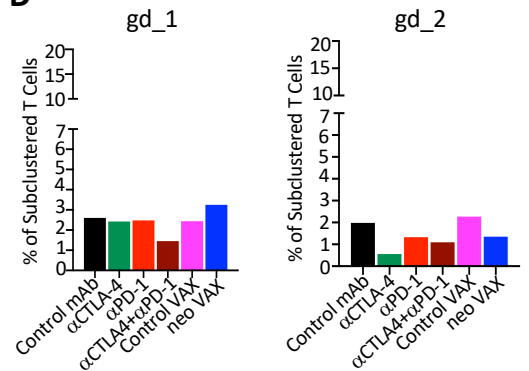
B



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D



E

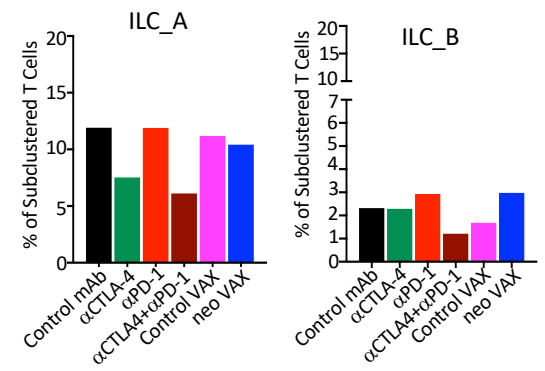


Figure S3. Flow cytometry and scRNAseq analysis of Y1.7LI intratumoral lymphoid and myeloid populations. Related to Figure 2.

(A) Graph of flow cytometry data displaying intratumoral lymphoid and myeloid cells as a percentage of intratumoral live or live CD45⁺ cells in Y1.7LI tumors treated with control mAb, anti-CTLA-4, anti-PD-1, anti-CTLA-4 + anti-PD-1, irrelevant (for Y1.7LI) mAlg8 SLP + pl:C (control VAX), or relevant mLama4 SLP + pl:C (neo VAX) beginning on d. 7 post-tumor transplant and harvested on d. 15. (B) Dot plot depicting expression level and percent of cells expressing *Foxp3*, *Ctla4*, *Icos*, *Tigit*, *Havcr2* (TIM-3), *Klrg1*, *Gzmb* and graph displaying frequency of regulatory T cell (Treg) scRNAseq clusters by treatment condition. (C) Graph displaying mixed T cell clusters represented as percentage of total subclustered T cells and percentage of Foxp3⁺ CD4 Tregs, conventional CD4 T cells, or CD8 T cells in clusters T_1, T_2, and T_3 by treatment condition. (D) Graph displaying $\gamma\delta$ T cell clusters represented as percentage of total subclustered T cells by treatment condition. (E) Graph displaying ILC clusters represented as percentage of total subclustered T cells by treatment condition. Bar graphs in (A) display mean \pm SEM and are representative of at least three independent experiments (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$, **** $P < 0.0001$, NS, not significant, unpaired t test).

**Figure S4. Heat map of the top 10 most DEG across T cell/ILC clusters (see also Figure 2D).
Related to Figure 2.**

Figure S5

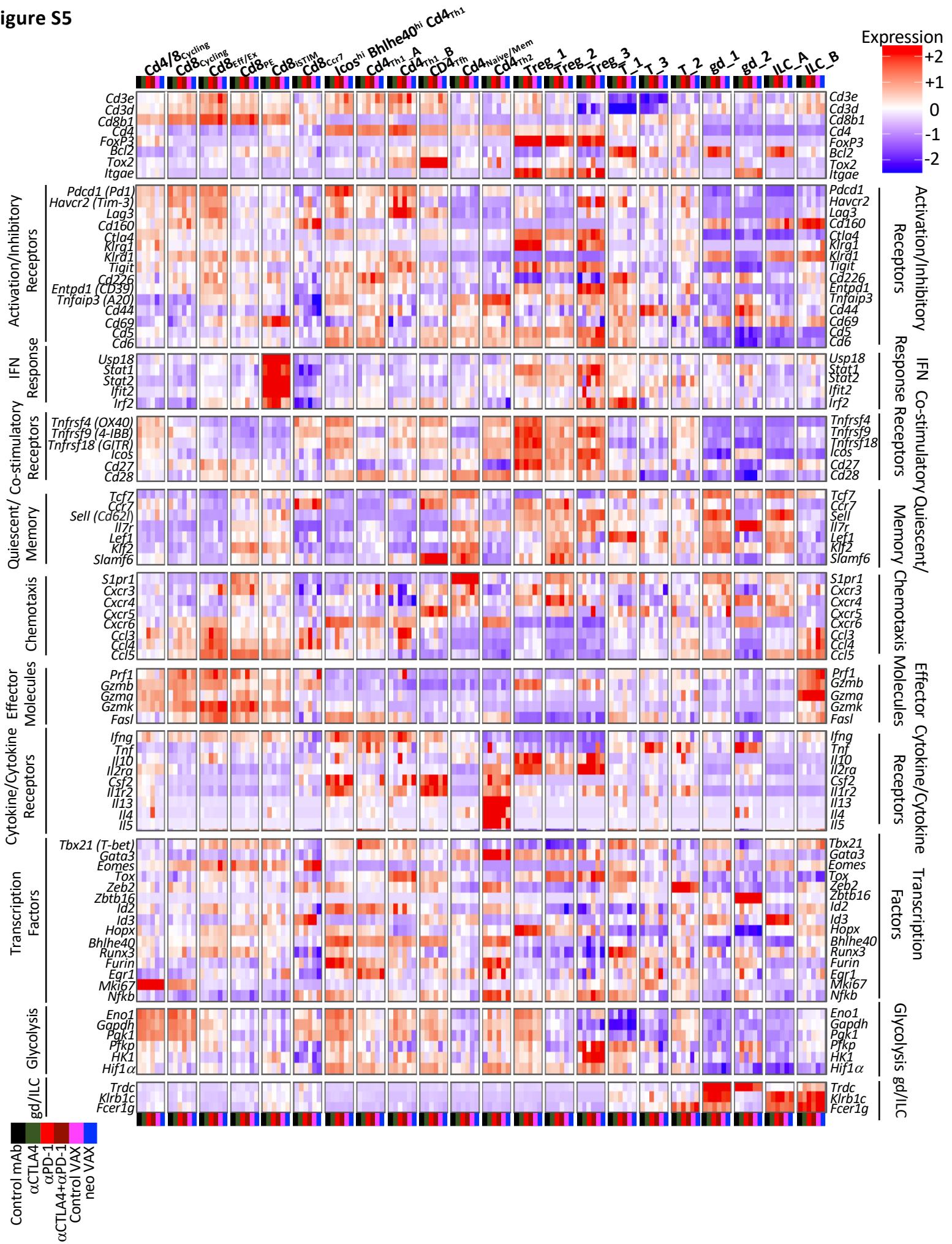
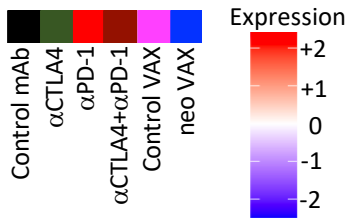
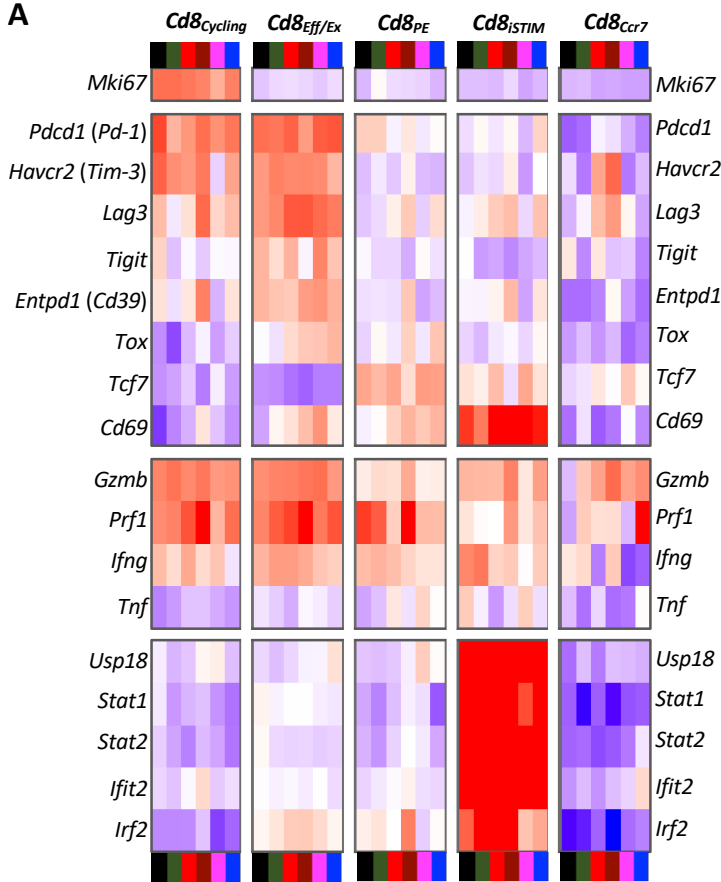


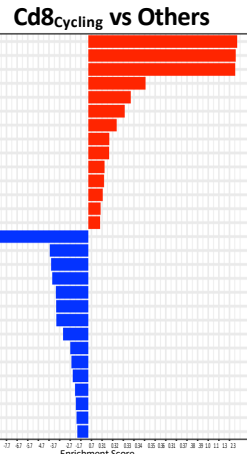
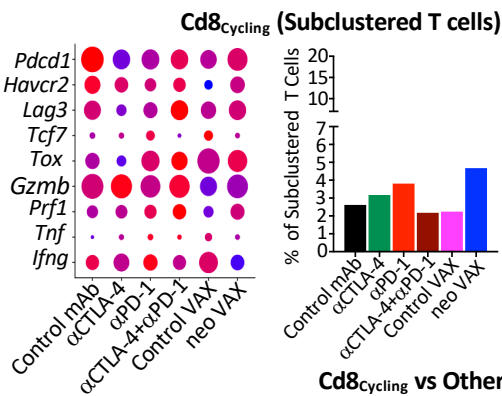
Figure S5. Heat map displaying normalized expression of select genes in each T cell/ILC cluster by treatment condition. Related to Figure 2.

Figure S6

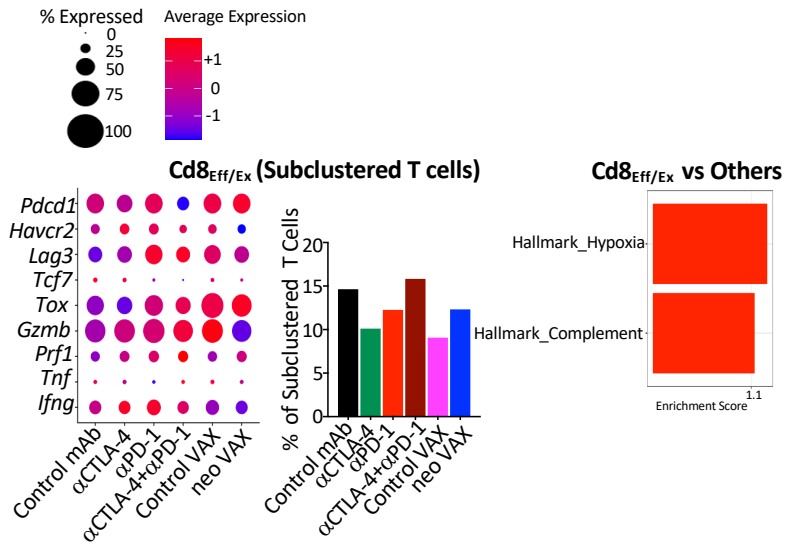
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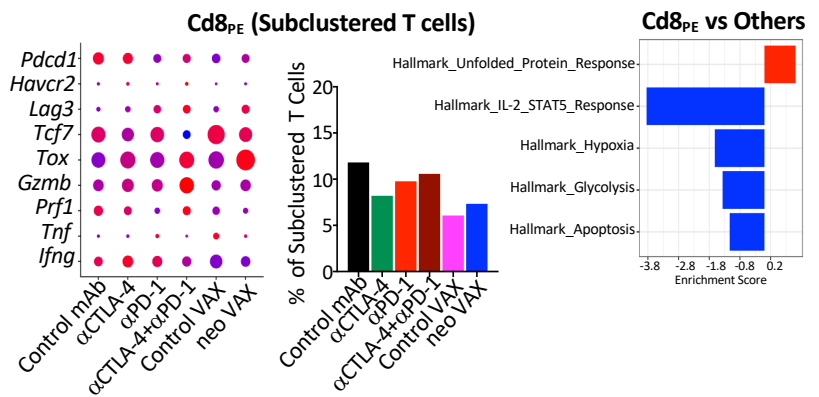
B



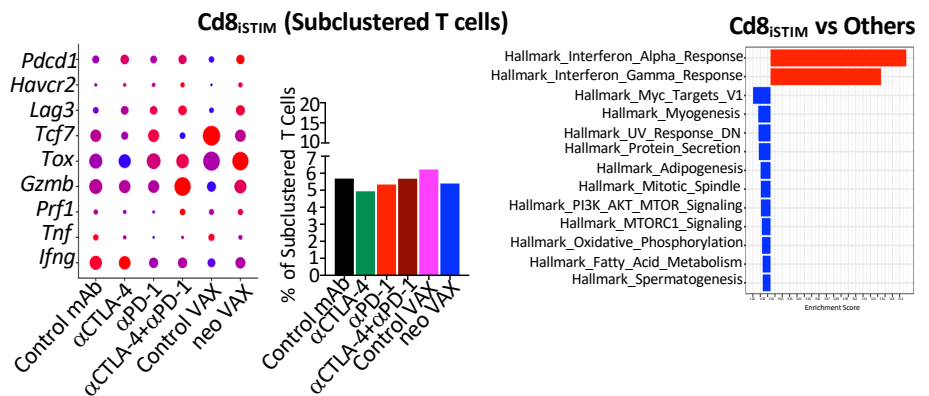
C



D



E



F

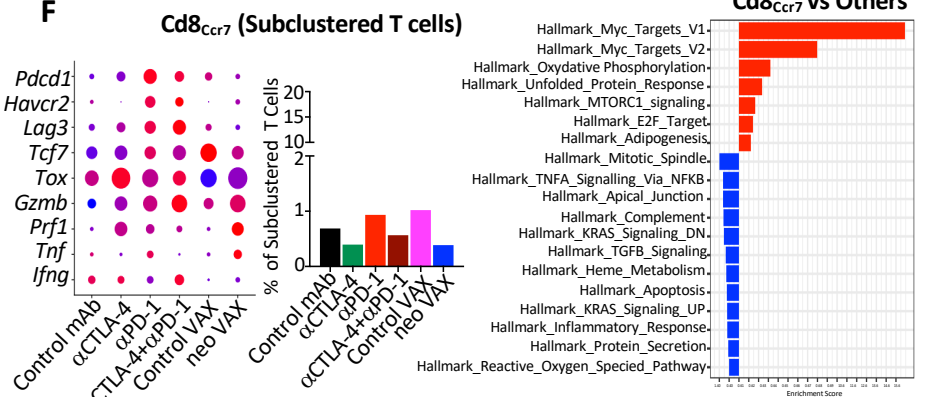
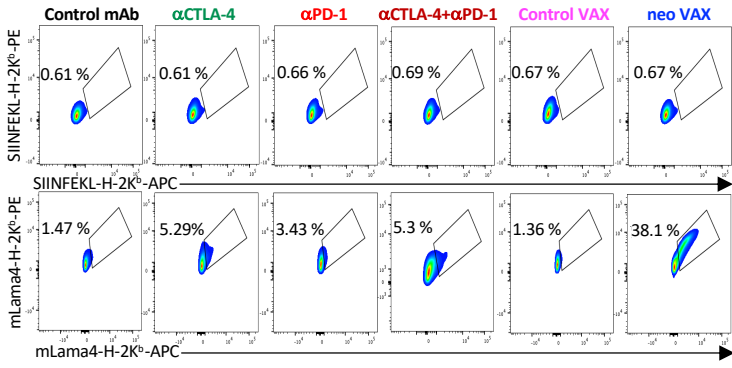


Figure S6. scRNAseq analysis of bulk CD8 T cells from Y1.7LI tumor bearing mice treated with NeoAg vaccines or ICT. Related to Figure 2.

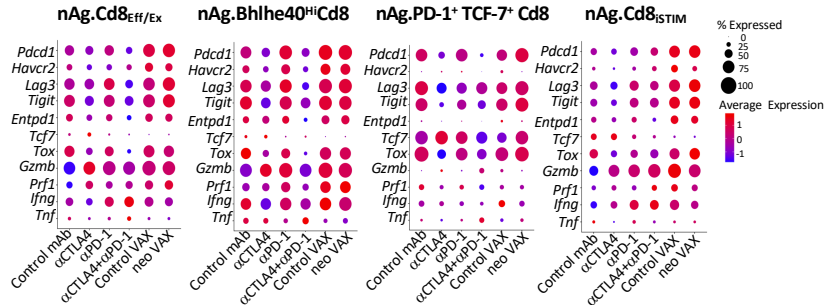
(A) Heat map displaying normalized expression of select genes in each bulk CD8 T cell clusters (see also Figures 2A and 2D). (B-F) scRNAseq dot plot depicting expression level/percent of cells expressing select transcripts, bar graphs depicting frequency of each CD8 T cell cluster by treatment condition, and GSEA displaying significantly enriched gene sets in each CD8 T cell cluster.

Figure S7

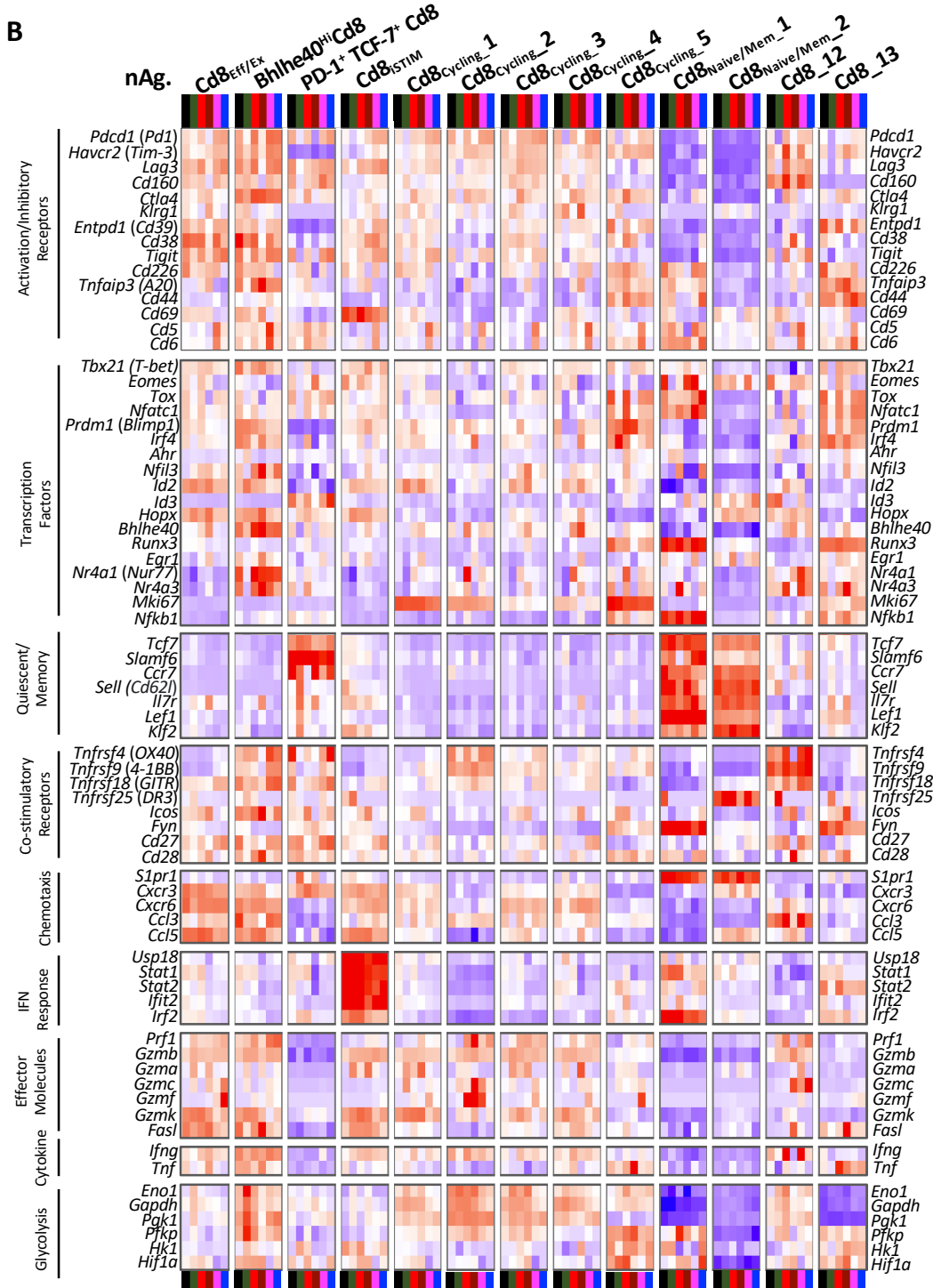
A



C



B



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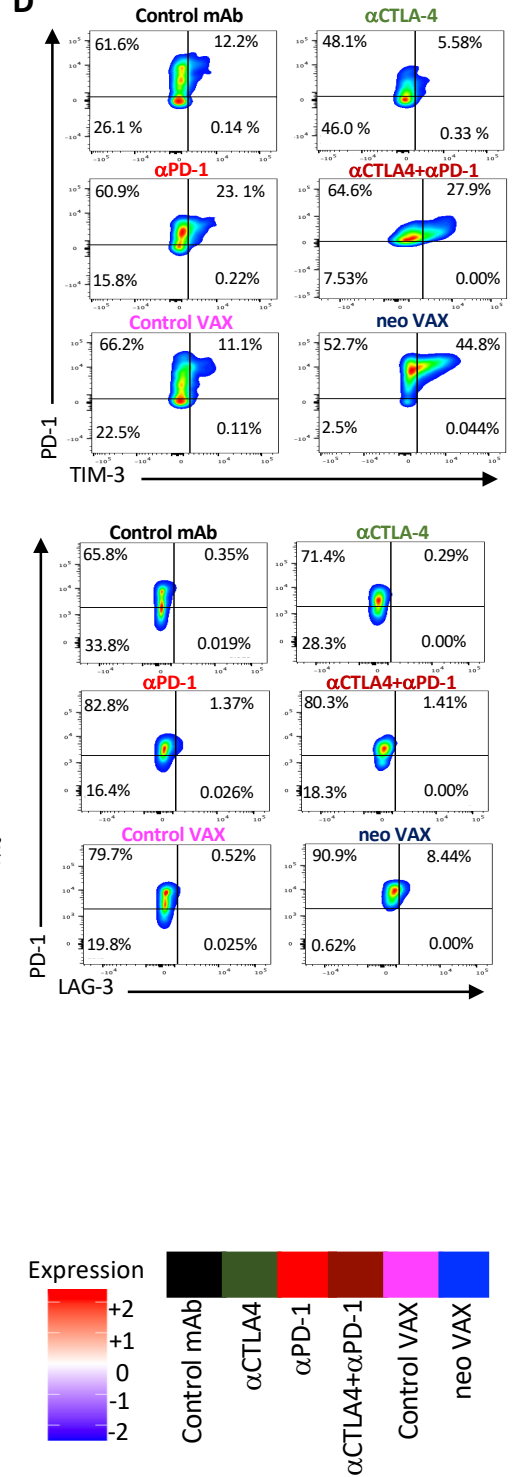


Figure S7. scRNAseq and flow cytometry profiling of mLama4 NeoAg-specific CD8 T cells from Y1.7LI tumor bearing mice treated with NeoAg vaccines or ICT. Related to Figure 3.

(A) Representative flow cytometry plots displaying mLama4 tetramer-specific CD8 T cells in Y1.7LI tumors treated with control mAb, anti-CTLA-4, anti-PD-1, anti-CTLA-4 + anti-PD-1, irrelevant (for Y1.7LI) mAlg8 SLP + pl:C (Control VAX), or relevant mLama4 SLP + pl:C (neo VAX) beginning on d. 7 and harvested on d. 15 post-tumor transplant. mLama4-H2-K^b or SIINFEKL-H2-K^b (irrelevant control) tetramers were labeled with PE and APC. Dot plots are gated on live CD45⁺ Thy1.2⁺ CD8 T cells (See also Figure S15). (B) Heat map displaying normalized expression of select genes in each mLama4 NeoAg-specific CD8 T cell clusters by treatment condition (see also Figure 3E). (C) scRNAseq dot plot depicting expression level/percent of cells expressing select transcripts within select mLama4 NeoAg-specific CD8 T cell clusters by treatment condition. (D) Representative flow cytometry plots displaying PD-1⁺ and/or TIM-3⁺/LAG-3⁺ after gating on mLama4 tetramer positive CD8 T cells (See also Figure S15).

Figure S8

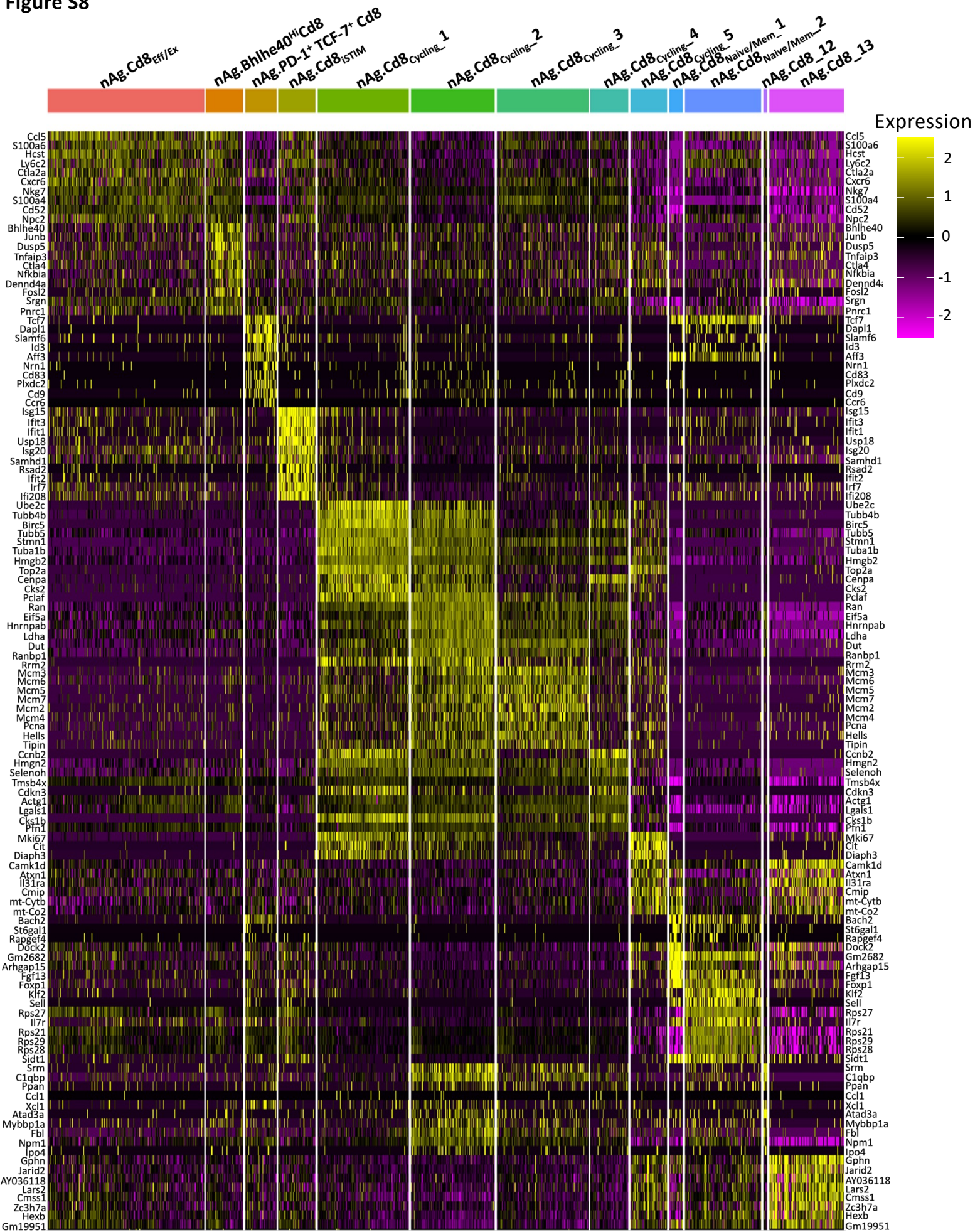


Figure S8. Heat map of the top 10 most DEG across NeoAg-specific CD8 T cells clusters. Related to Figure 3.

Figure S9

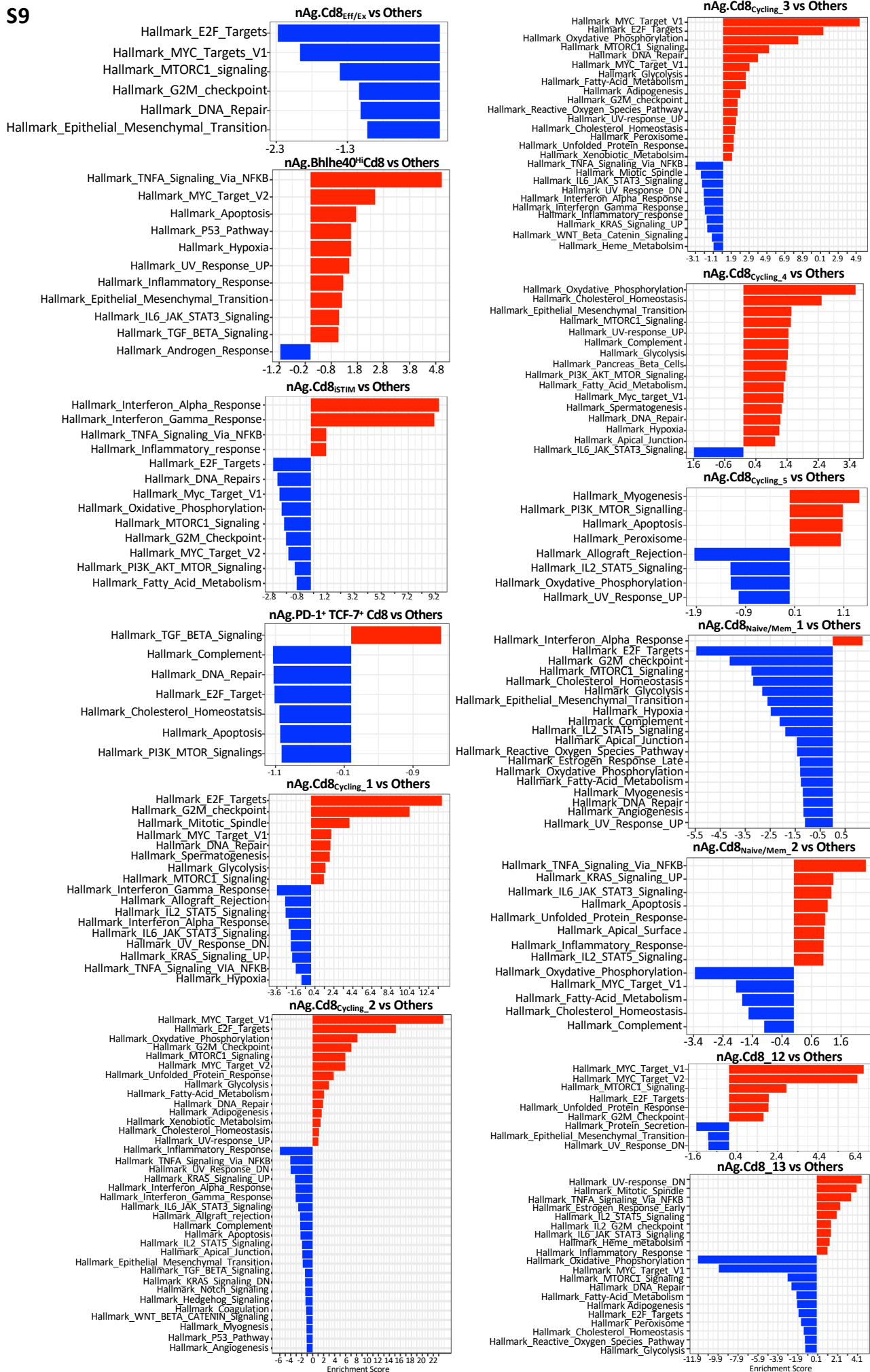
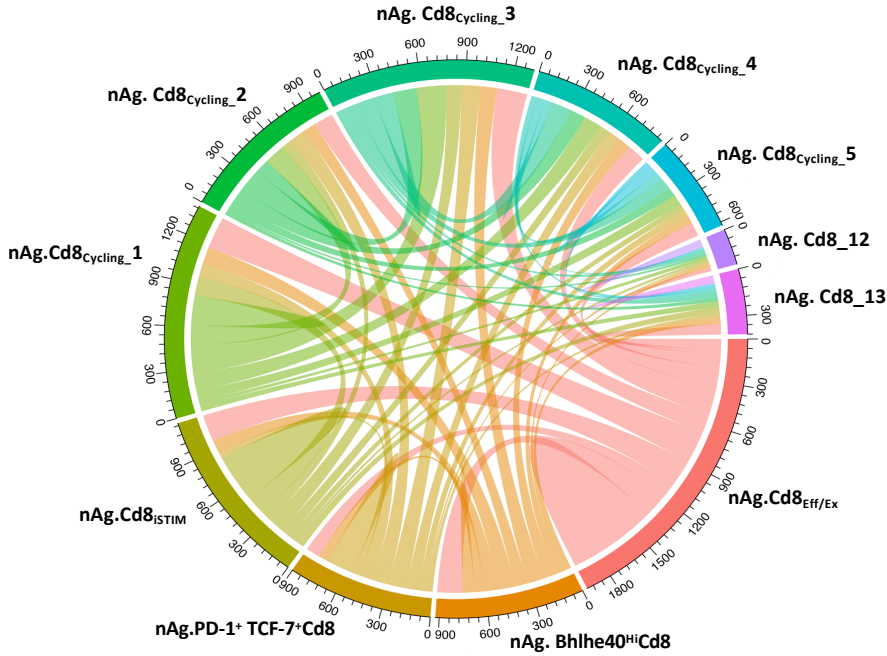


Figure S9. GSEA displaying significantly enriched gene sets for each NeoAg-specific CD8 T cells cluster. Related to Figure 3.

Figure S10

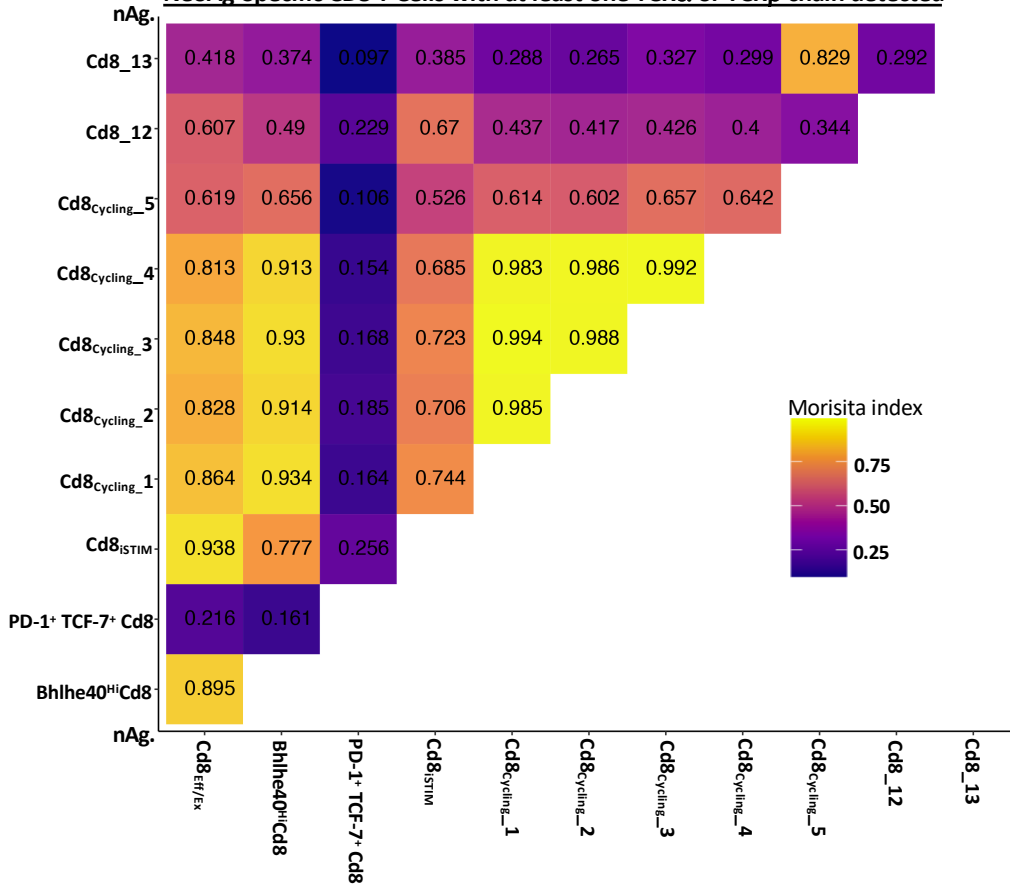
A

NeoAg-Specific CD8 T Cells with at least one TCR α or TCR β chain detected



B

NeoAg-Specific CD8 T Cells with at least one TCR α or TCR β chain detected



C

NeoAg-Specific CD8 T Cells with at least one TCR α or TCR β chain detected

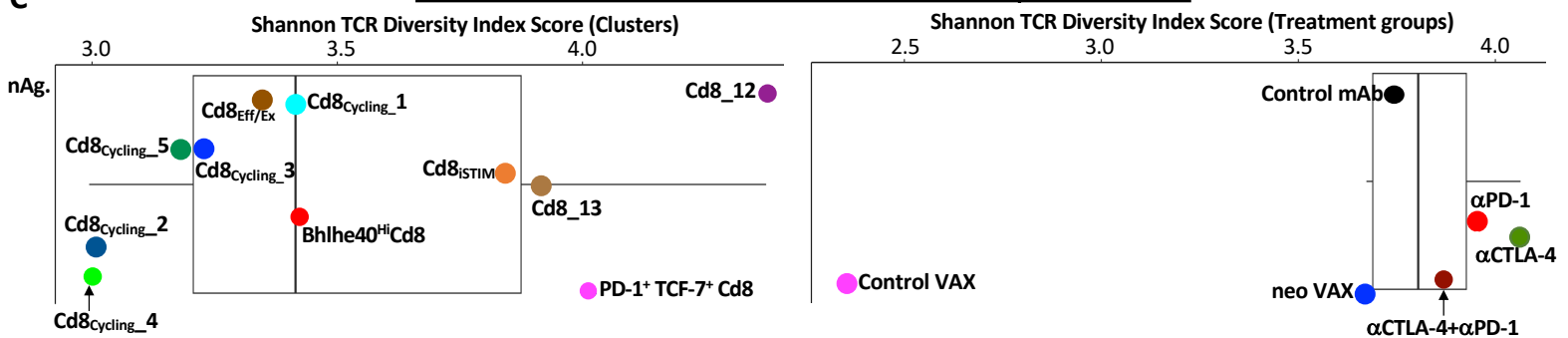
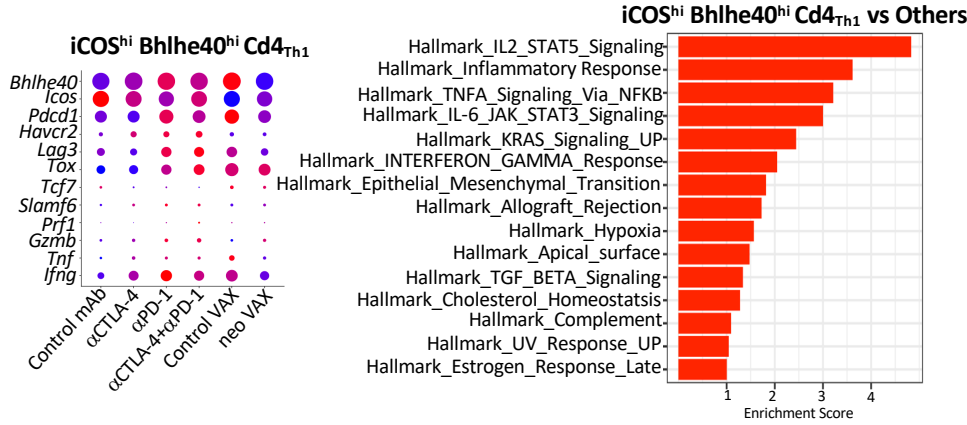


Figure S10. scTCRseq analysis of mLama4 NeoAg-specific CD8 T cells with at least one TCR alpha or beta or both chains. Related to Figure 4.

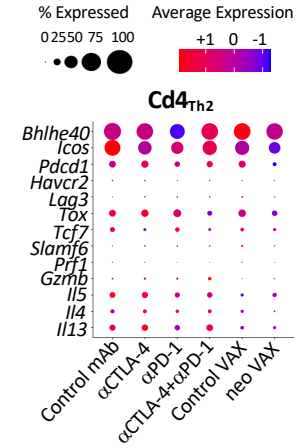
(A) Chord diagram displaying overlapping TCR clonotypes (TCR α /TCR β /TCR $\alpha\beta$) of mLama4 NeoAg-specific CD8 T cells by cluster. (B) Morisita index values depicting overlapping TCR clonotypes (TCR α /TCR β /TCR $\alpha\beta$) of mLama4 NeoAg-specific CD8 T cells by cluster. (C) Shannon TCR (TCR α /TCR β /TCR $\alpha\beta$) diversity index of by clusters and treatment groups.

Figure S11

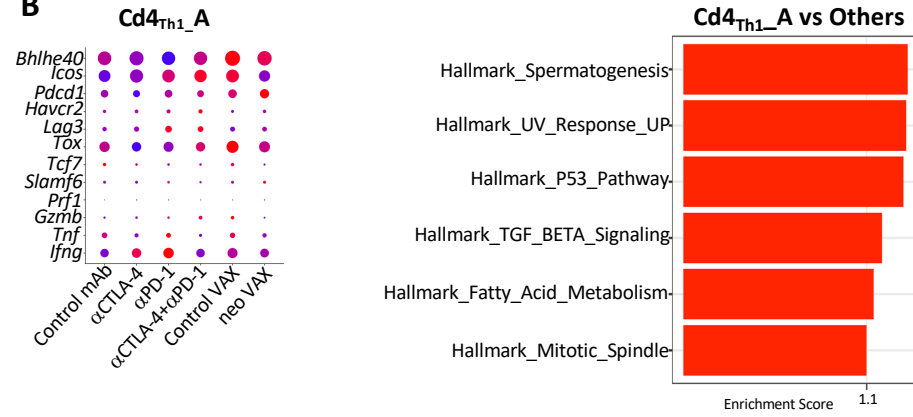
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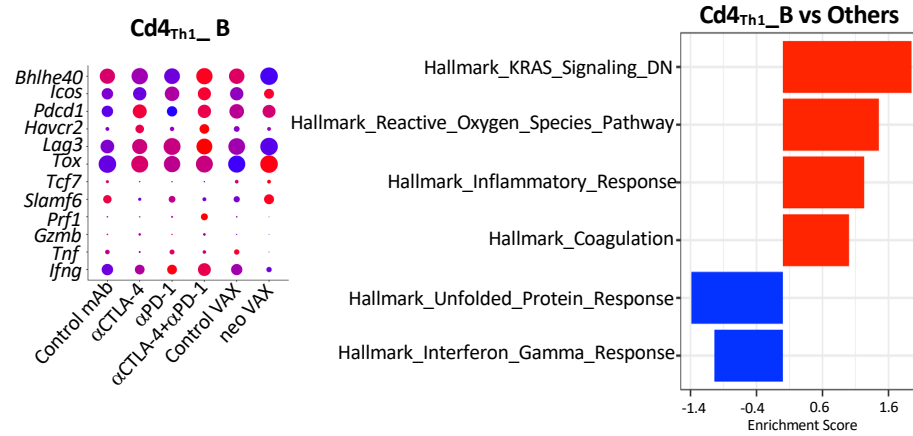
E



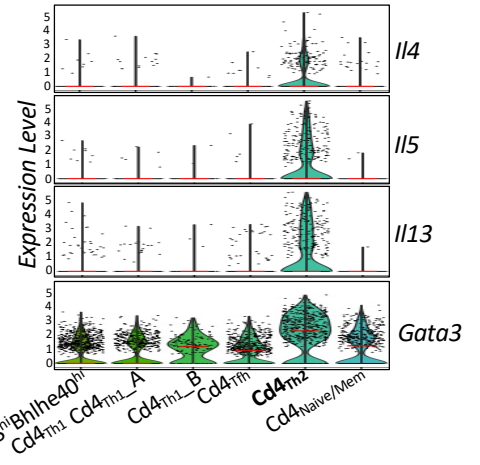
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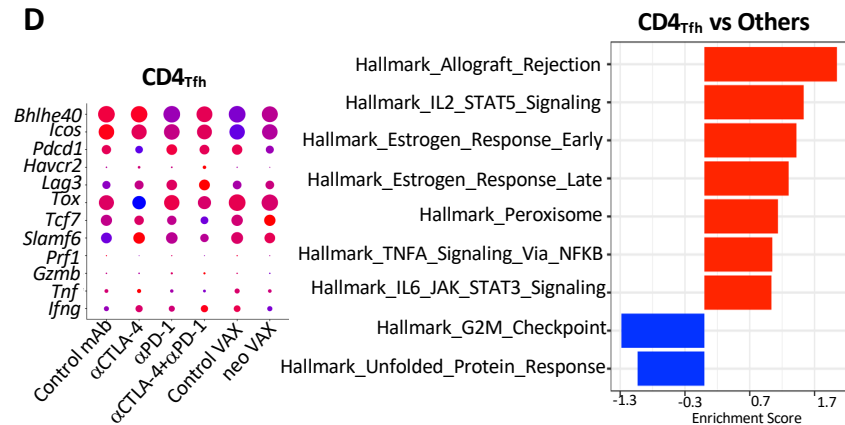
C



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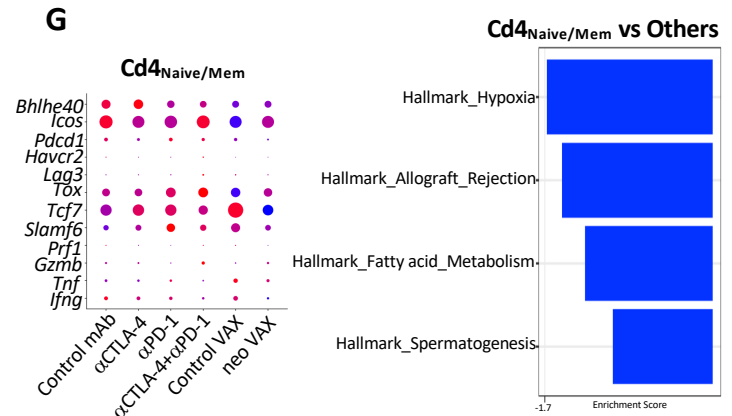


Figure S11. Select transcript alterations and cluster-specific enriched gene sets in conventional CD4 T cells (see also Figure 2A). Related to Figure 5.

(A-E and G) scRNAseq dot plots depicting expression level/percent of cells expressing select transcripts and GSEA displaying significantly enriched gene sets within each CD4 T cell cluster by treatment condition (see also Figure 2D). (F) Violin plots denoting expression level of select genes per CD4 T cell.

Figure S12

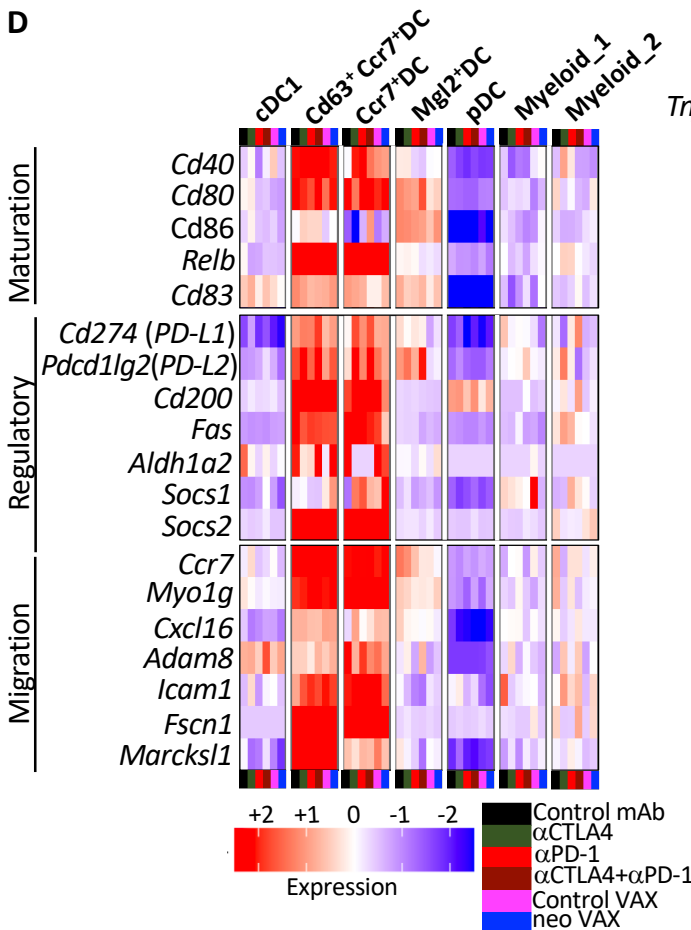
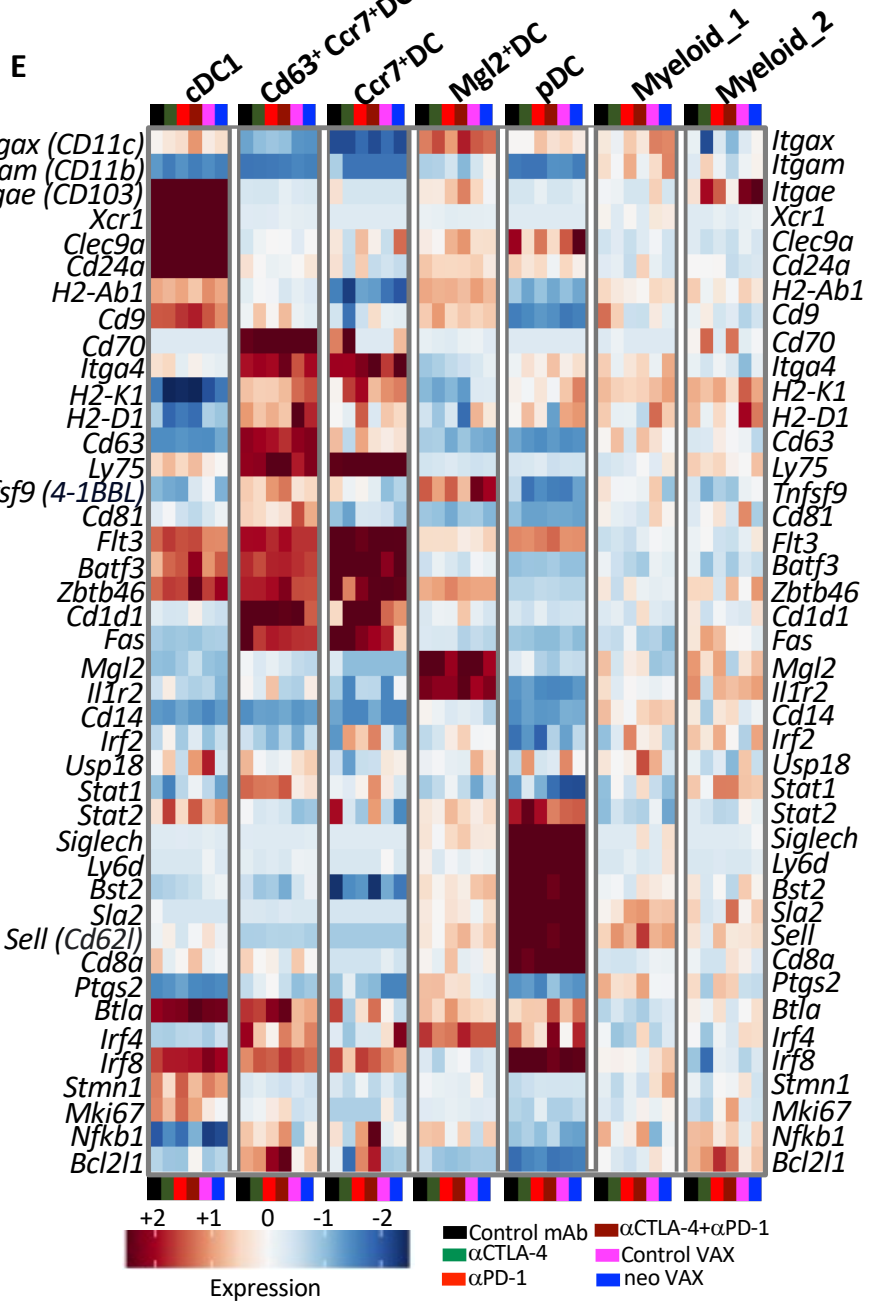
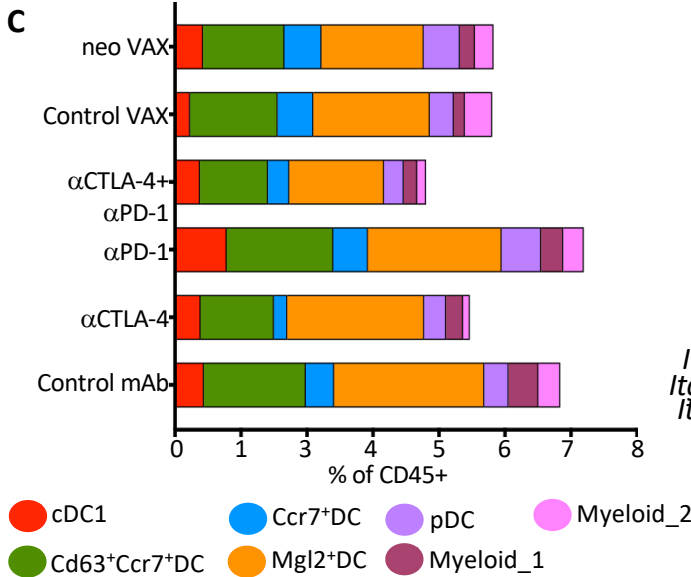
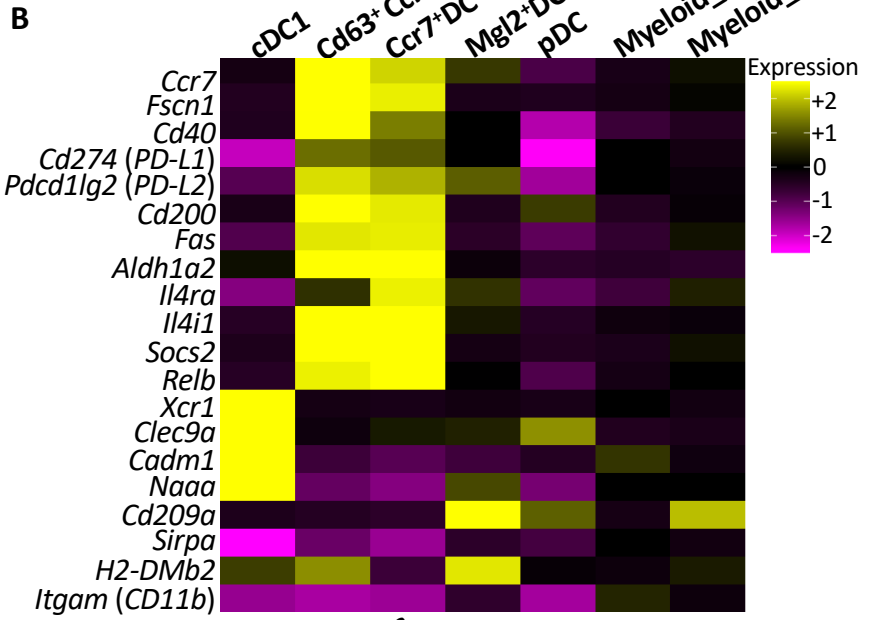
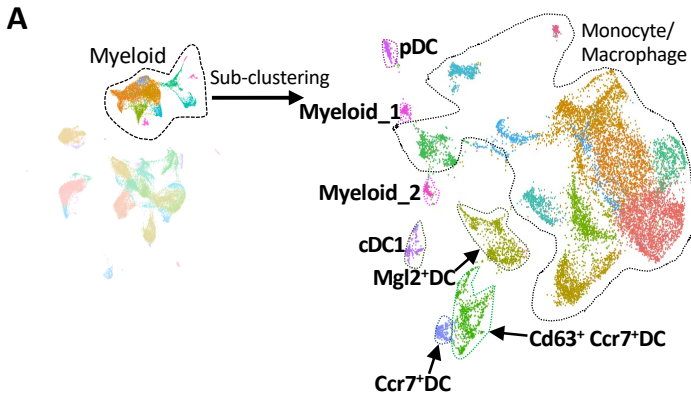


Figure S12. scRNAseq analysis of dendritic cell (DC) clusters from Y1.7LI tumor bearing mice treated with NeoAg vaccines or ICT.

(A) UMAP displaying myeloid cell sub-clustering and DC annotations (See also Figures 2A and 6A). (B) Heat map displaying normalized expression of select genes in each DC cluster. (C) Graphs depicting frequency of DCs in each cluster by condition and treatment represented as percent of live CD45⁺ cells. (D) Heat map displaying normalized expression of select genes⁶¹ in each DC cluster by treatment condition. (E) Heat map displaying normalized expression of select genes in each DC cluster by treatment condition.

Figure S13

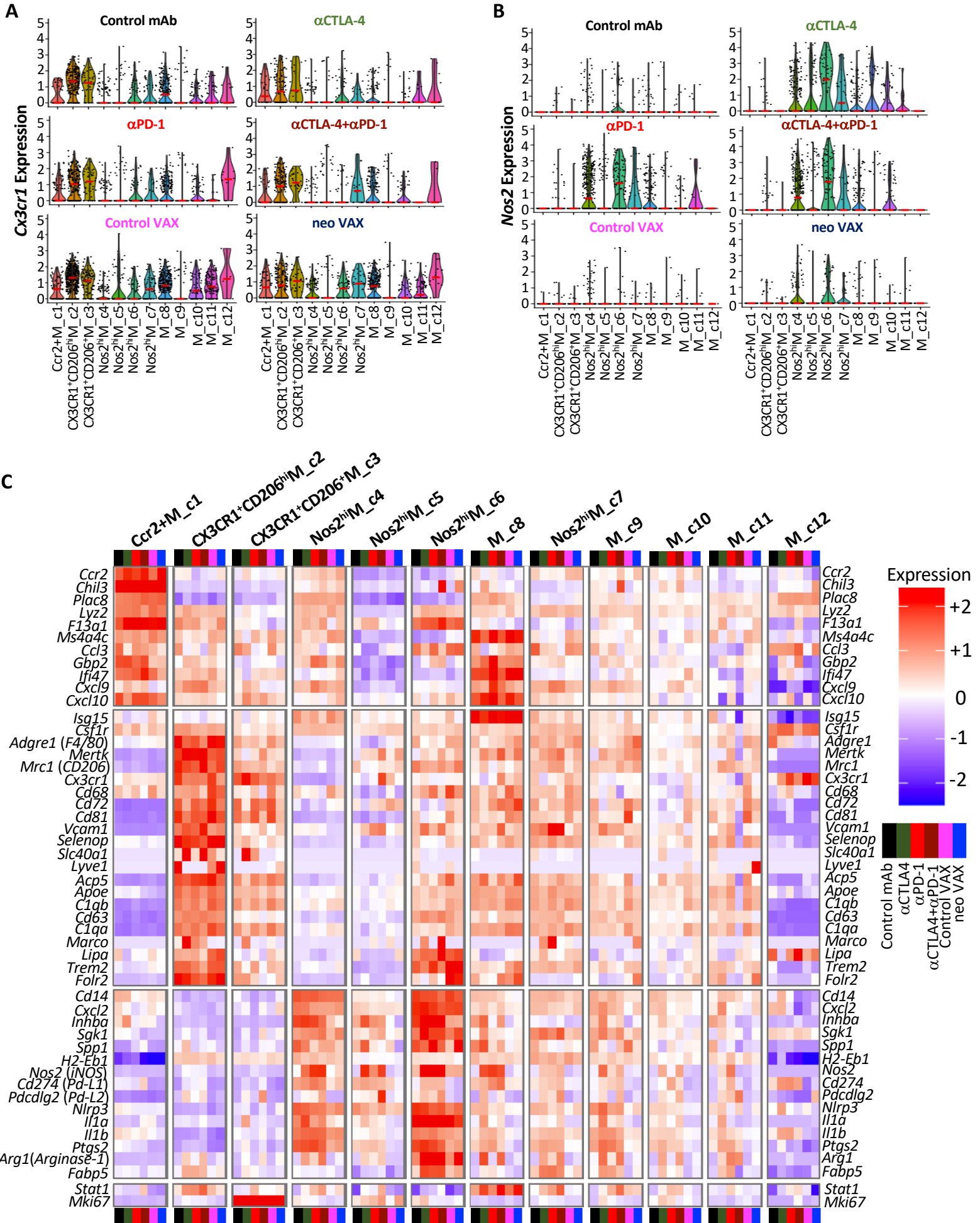


Figure S13. scRNAseq analysis of macrophage clusters from Y1.7LI tumor bearing mice treated with NeoAg vaccines or ICT. Related to Figure 6.

(A) Violin plots denoting expression level of *Cx3cr1* transcript per cell in each monocyte/macrophage cluster by treatment condition. (B) Violin plots denoting expression level of *Nos2* (iNOS) transcript per cell in each monocyte/macrophage cluster by treatment condition. (C) Heat map displaying normalized expression of select genes in each monocyte/macrophage cluster by treatment condition.

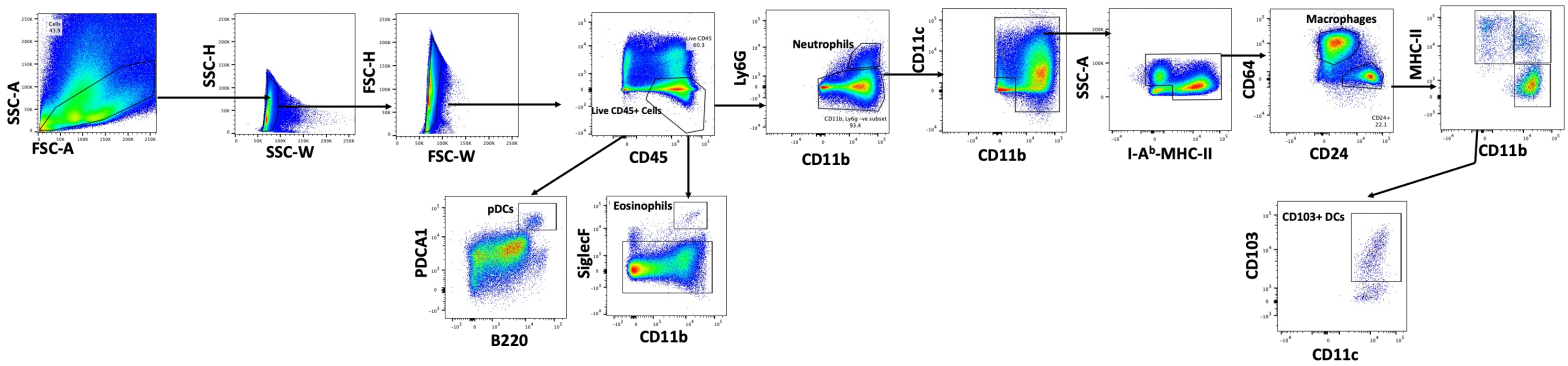
Figure S14



Figure S14. Principal component analysis of subclustered T Cells/ILCs. Each dot represents individual sample from different treatment conditions (see also Figures 2A and 2D). **Related to Figure 2.**

Figure S15

Myeloid Subsets



Lymphoid Subsets

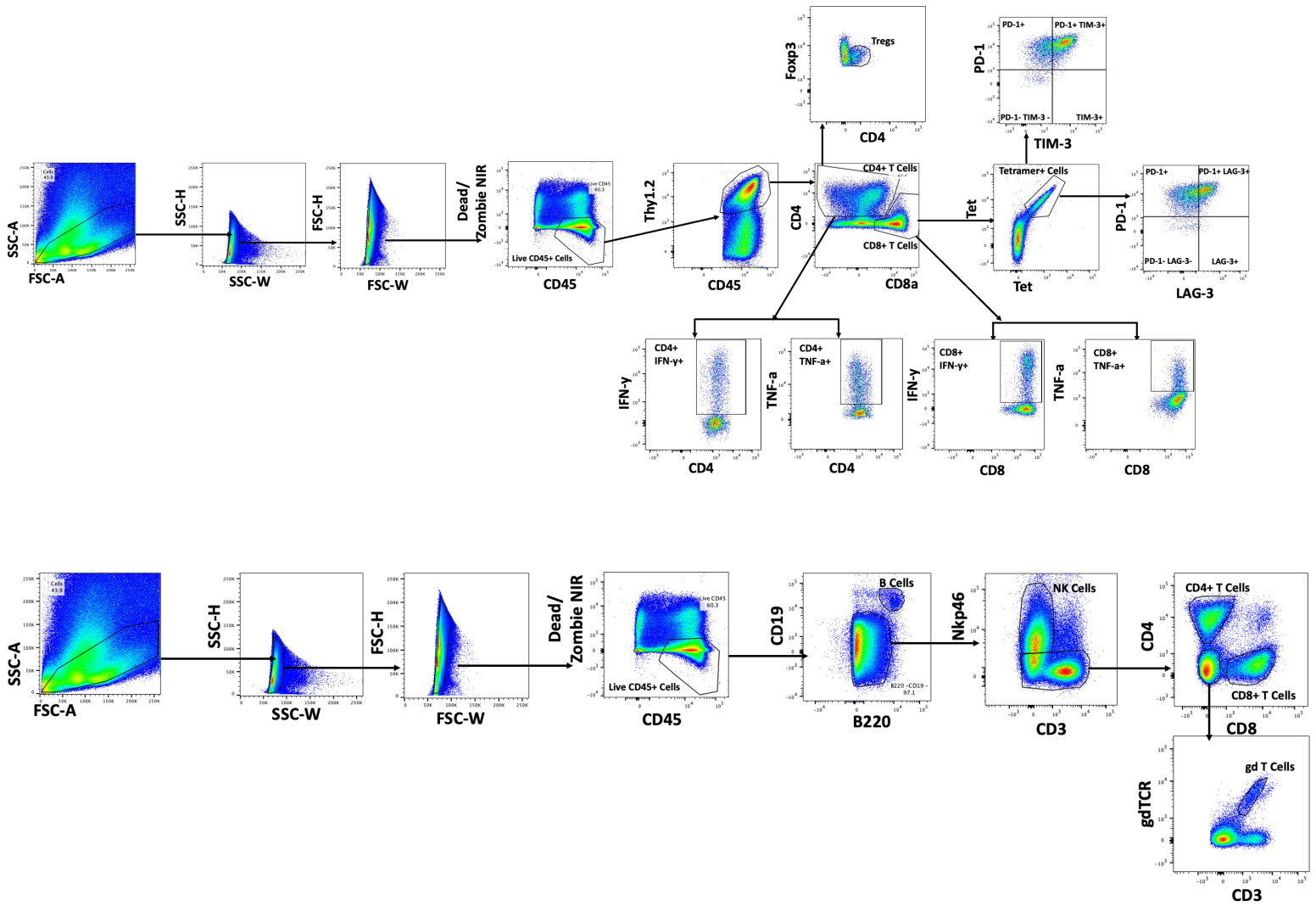


Figure S15. Gating strategy for identifying intratumoral immune cells. Flow cytometry dot plots and gating of intratumoral myeloid and lymphoid populations.