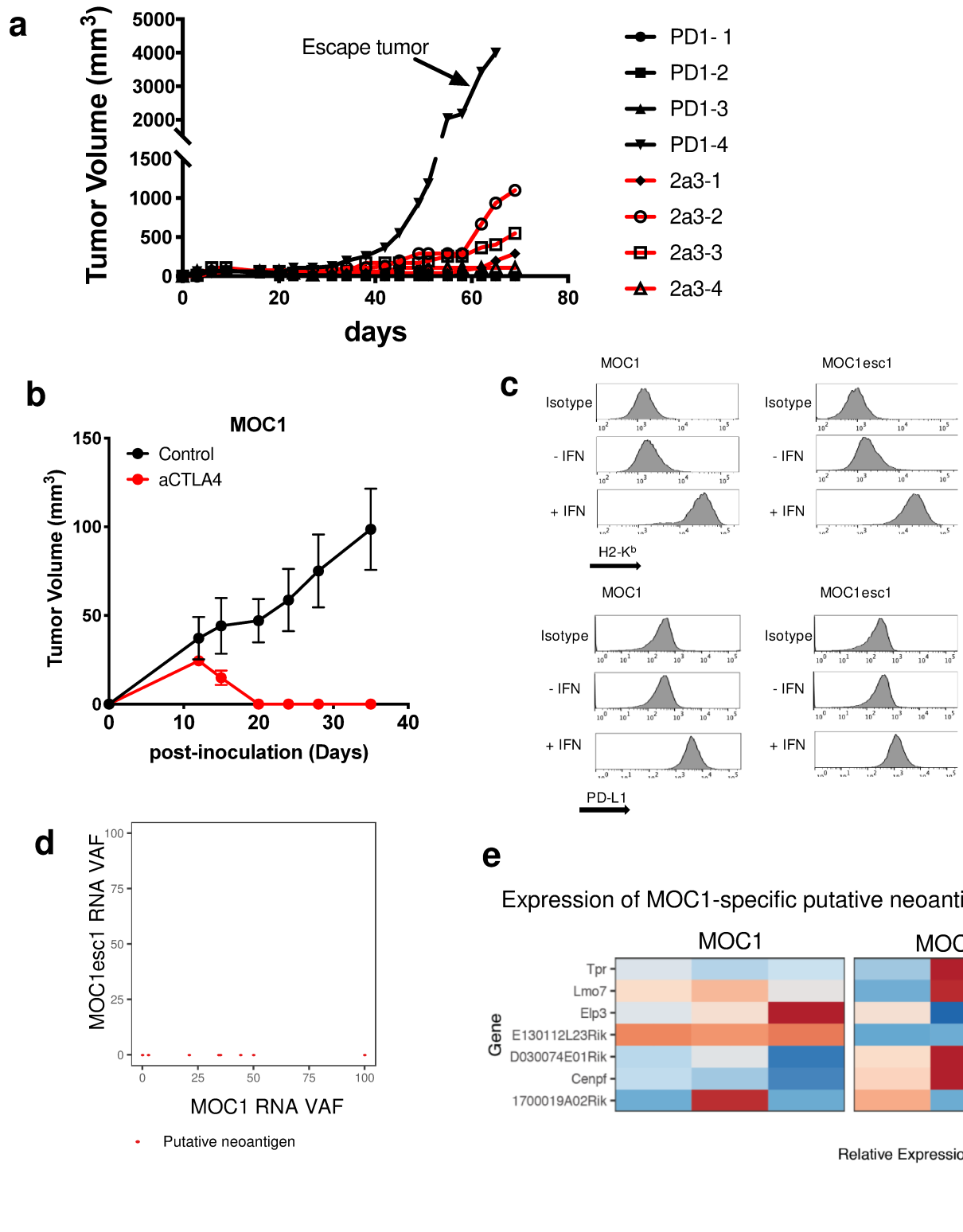
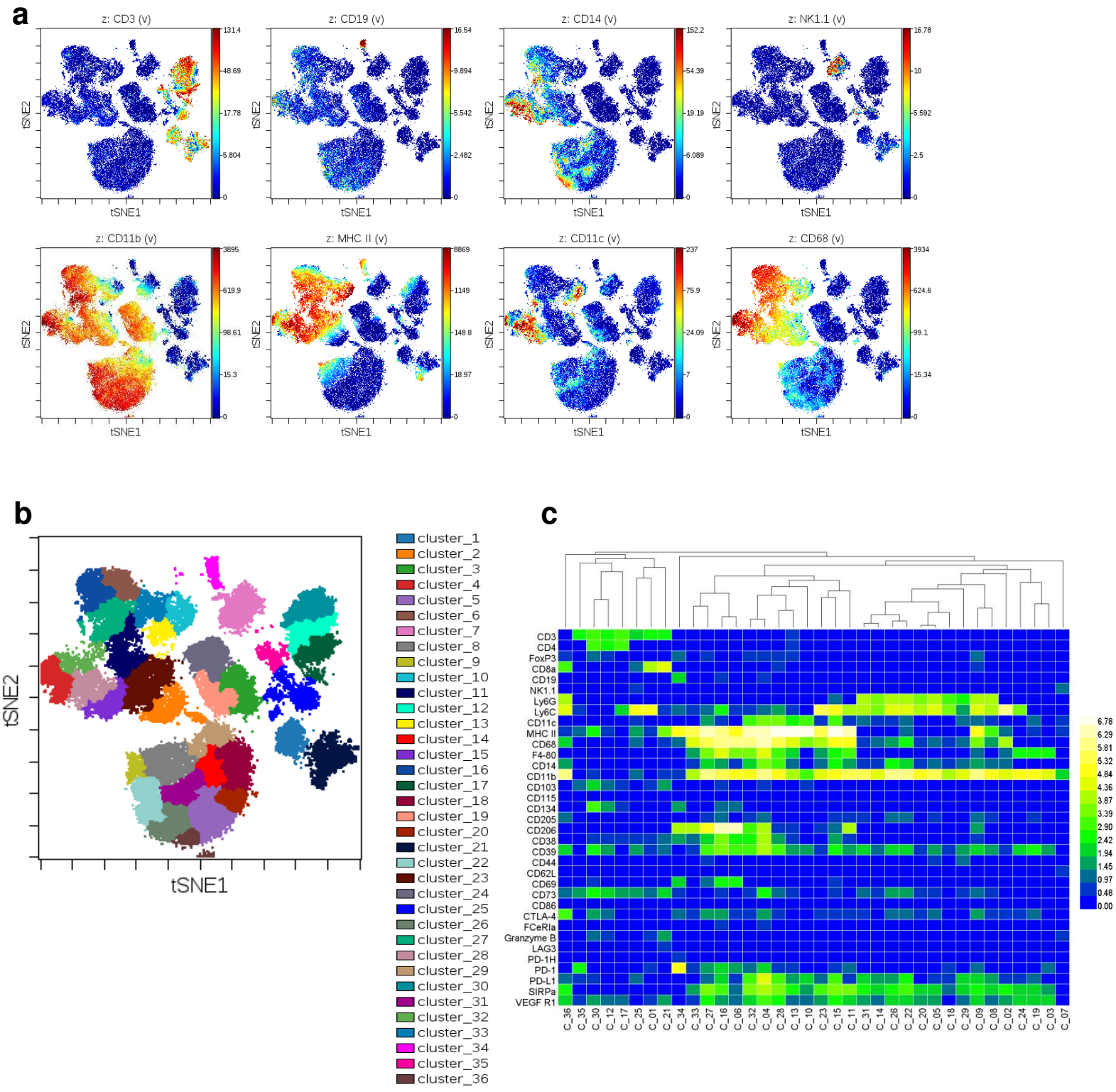
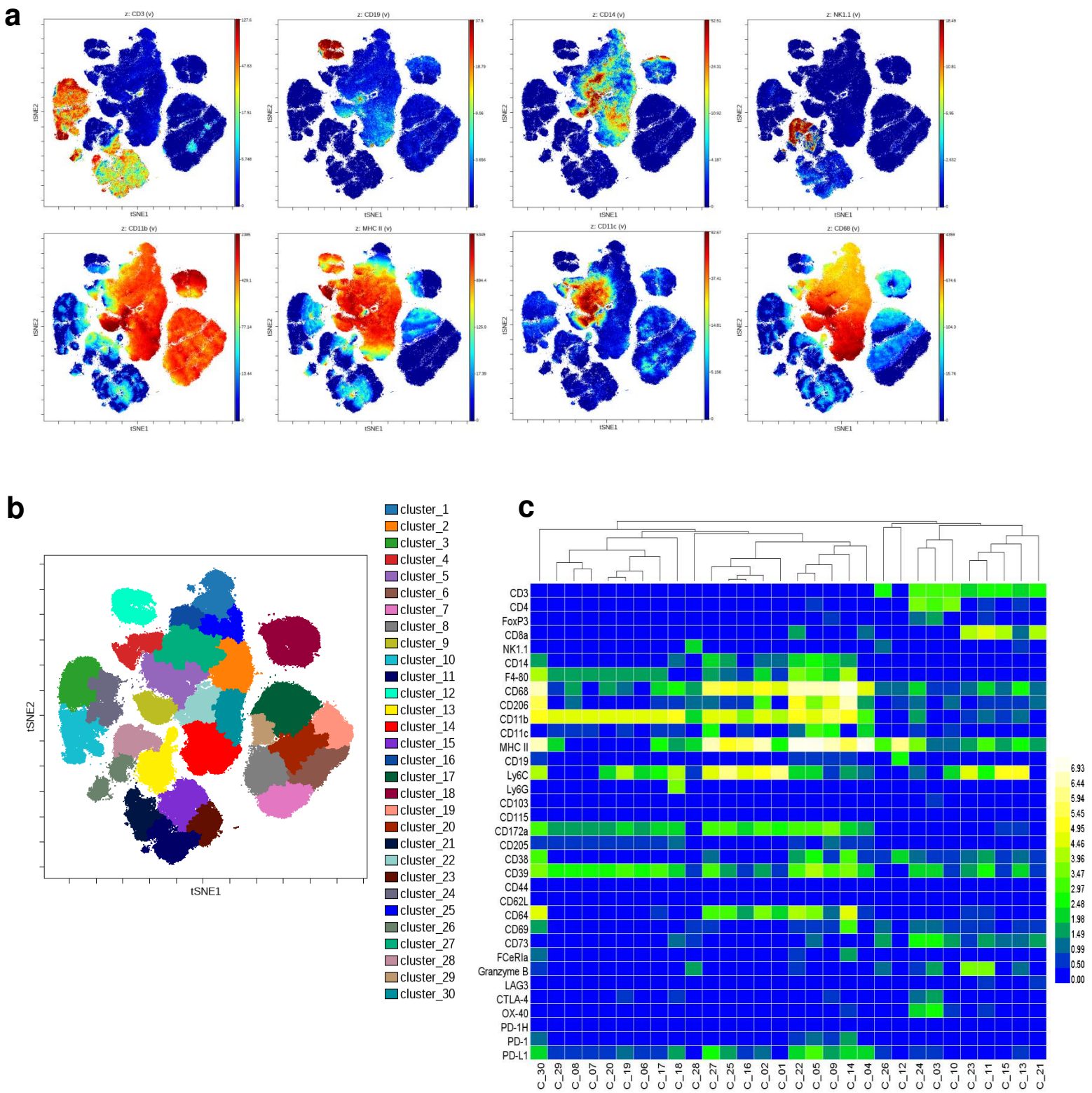


Supplementary Data 1. Isogenic MOC1/MOC1esc1 model characterization. a. Repeat experiment showing MOC1 anti-PD1 sensitivity and escape tumor growth. **b,** MOC1 tumors are sensitive to anti-CTLA4 treatment. 10^6 MOC1 cells were subcutaneously implanted in C57BL/6 mice. Tumor bearing mice were treated with anti-CTLA4 (clone 9D9) on Days 3, 6, 9 after tumor implantation (Data are shown as mean± SEM, N=5 per group. **c,** Representative histograms of cell surface protein levels of H2-K^b or PD-L1 and the appropriate isotype control antibody staining on MOC1 and MOC1esc1 cells under indicated IFNγ conditions. **d, e**. Whole exome sequencing and *in vitro* RNA-seq were performed on MOC1 and MOC1esc1 lines. Mutation-derived neoantigens were predicted. 20 of the 139 MOC1 specific DNA variants had predicted putative neoantigens and were colored (**d**), of which 7 were expressed. The expression levels of 7 potential neoantigens were visualized using a heatmap with replicates (**e**).

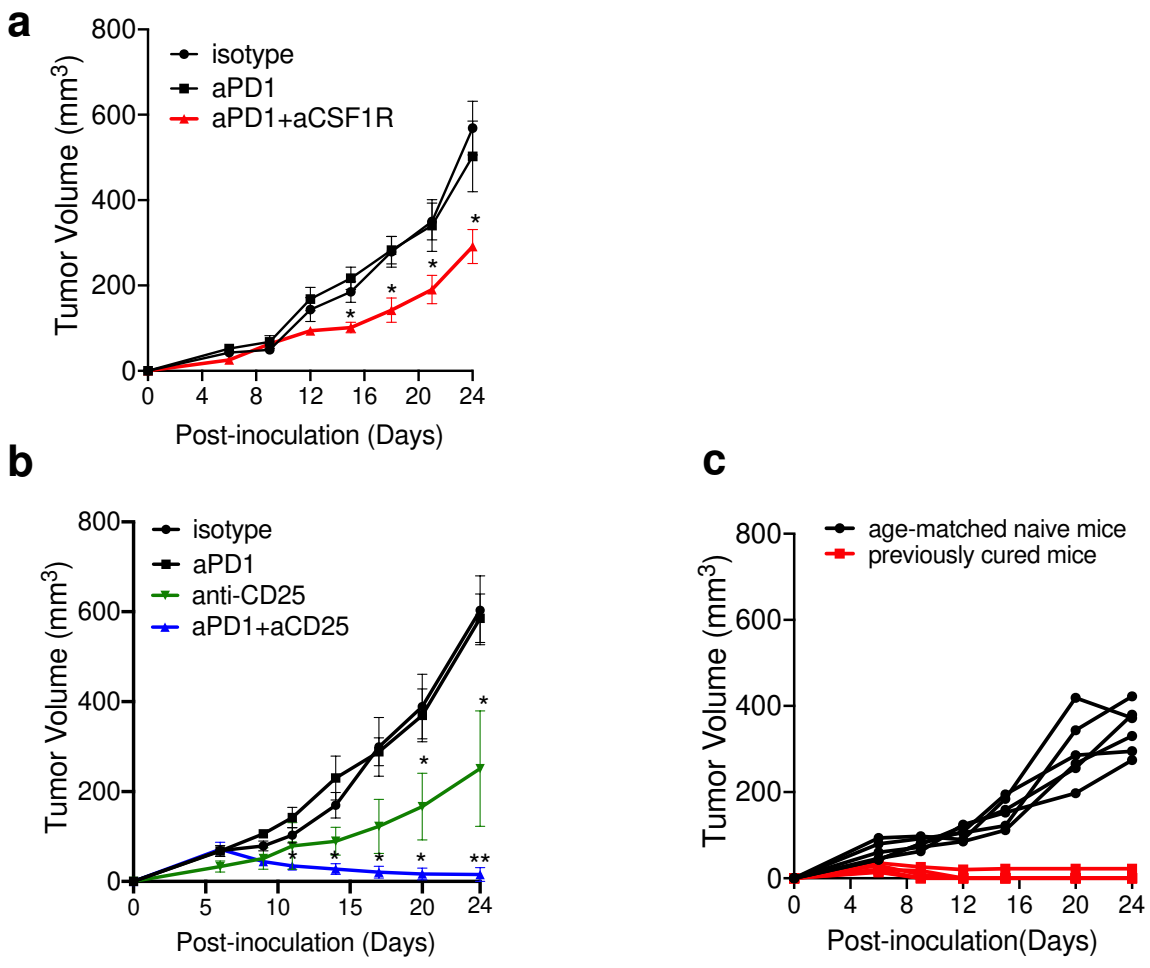


Supplementary Data 2. TME profiling in treatment naïve MOC1 and MOC1esc1 using mass cytometry.

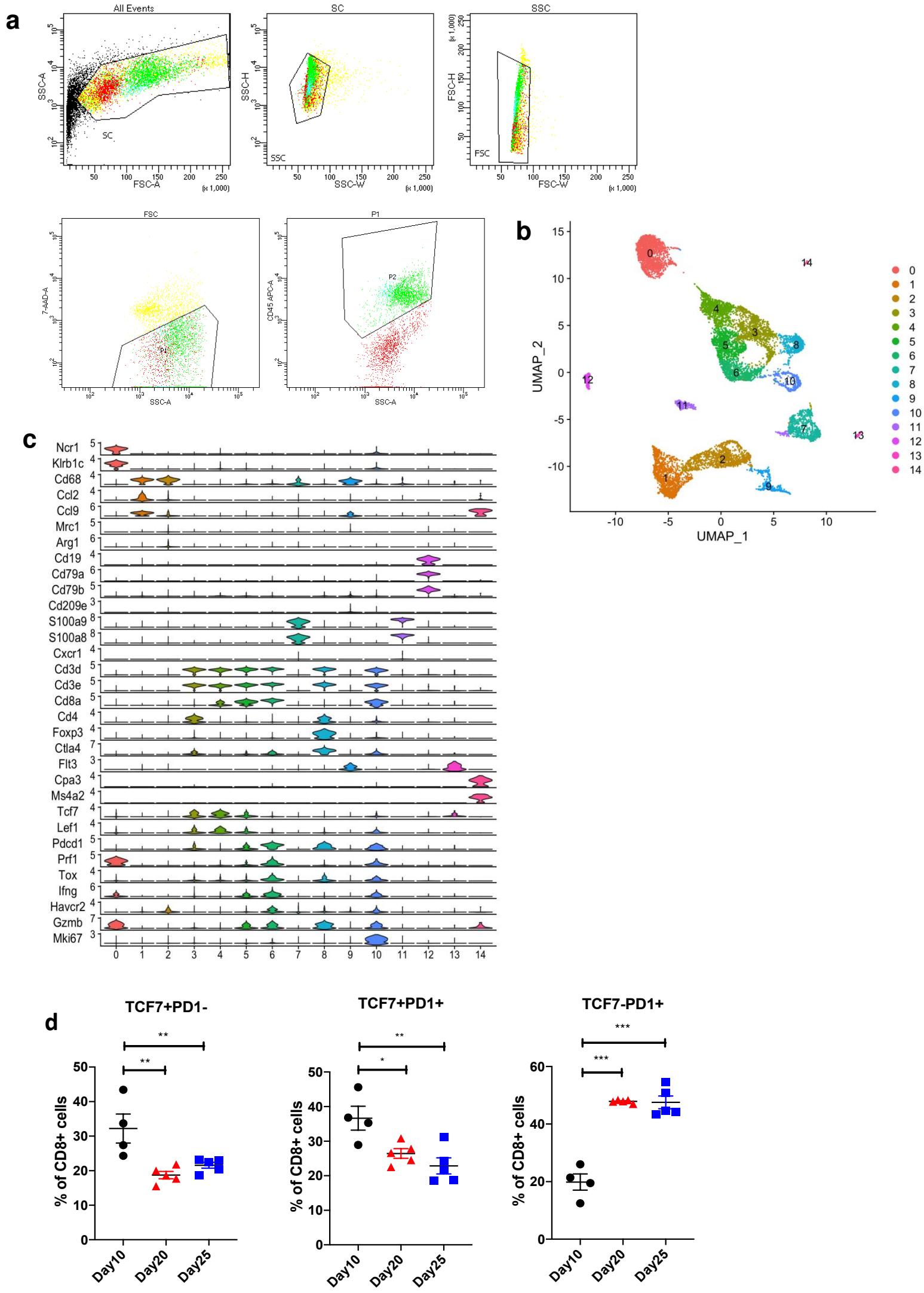
a, ViSNE (within Cytobank) plots of tumor infiltrating CD45+ cells overlaid with the expression of additional selected markers of major immune cell subpopulations. Cells were total CD45+ cells pooled from both groups of MOC1 and MOC1esc1 treatment naïve tumors. **b,** 36 clusters of phenotypically similar cells were identified using spanning-tree progression analysis of density-normalized events (SPADE) algorithm. **c,** Heatmap of the mean intensities of individual phenotypic markers across the 36 clusters after hierarchical clustering.



Supplementary Data 3. TME profiling of MOC1esc1 upon isotype control, anti-PD1, or anti-CTLA4 treatment using mass cytometry. a, ViSNE plots of tumor infiltrating CD45+ cells overlaid with the expression of additional selected markers of major immune cell subpopulations. Cells were total CD45+ cells pooled from MOC1esc1 tumors treated with isotype control, anti-PD1, or anti-CTLA4 antibodies **b,** 30 clusters of phenotypically similar cells were identified using SPADE algorithm. **c,** Heatmap of the mean intensities of individual phenotypic markers across the 30 clusters after hierarchical clustering.



Supplementary Data 4. ***In vivo*** **depletion experiments confirmed the contribution of Tregs and M2-like macrophages in MOC1esc1 anti-PD1 resistance. a,** Combination of anti-PD1 and anti-CSF1R significantly suppressed MOC1esc1 tumor growth. (**P*<0.05, ***P*<0.01, ****P*<0.001. Significance was calculated by one-way ANOVA. Data are shown as mean± SEM, N=4 per group.) **b,** Anti-CD25 monotherapy suppresses MOC1esc1 tumor progression (green line) and combination of anti-PD1 and anti-CD25 leads to complete rejection of tumors (blue line). (**P*<0.05, ***P*<0.01, ****P*<0.001. Significance was calculated by one-way ANOVA. Data are shown as mean± SEM, N=4 per group.) **c,** 6 weeks after tumor rejection, the previously cured of anti-CD25 and anti-PD1 treatment MOC1esc1 bearing mice were re-challenged with MOC1esc1. Age matched mice were injected with MOC1esc1 cells as control group. Tumor growth was monitored. (N=6 per group)



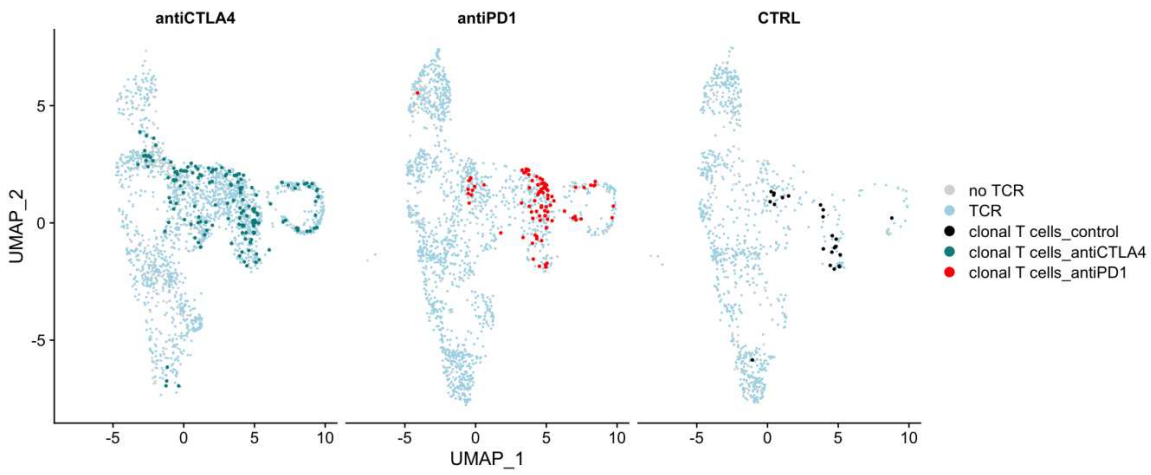
Supplementary Data 5.

Supplementary Data 5. ScRNAseq analysis of MOC1esc1 tumor infiltrating CD45+ cells. MOC1esc1 bearing mice were treated with isotype control, anti-PD1, or anti-CTLA4 on Days 3, 6, 9 post tumor implantations. Tumors were harvested on Day 12. 7AAD-CD45+ cells were flow sorted and subjected to 10x scRNA-seq and TCR-seq. **a,** Gating strategy for live CD45+ cells sorting of tumor samples. **b,** Unsupervised clustering identified 15 clusters of CD45+ cells pooled from 3 treatment groups. **c,** . Violin plots showing expression of selected immune cell marker genes across clusters. The y-axis represents the normalized gene expression levels. **d,** MOC1esc1 tumors harvested at indicated time points were analyzed by flow cytometry for T cell subset distribution. Percentages of CD8+ subsets in MOC1esc1 tumors at indicated time points post-inoculation. (*P<0.05, **P<0.01, ***P<0.001. Significance was calculated by one-way ANOVA. Data are shown as mean± SEM, N=4 or 5 mice per group.)

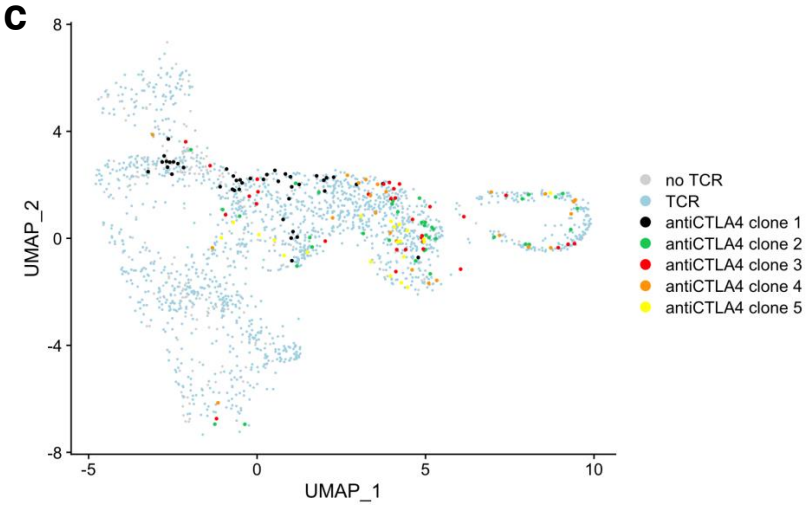
a

Groups	isotype	anti-PD1	anti-CTLA4
T cell #	994	1771	2133
T cell w/TCR #	738	1375	1554
% of T cells with productive TCR	74.2	77.6	72.9
Sequenced immune cell #	3405	4479	3066

b

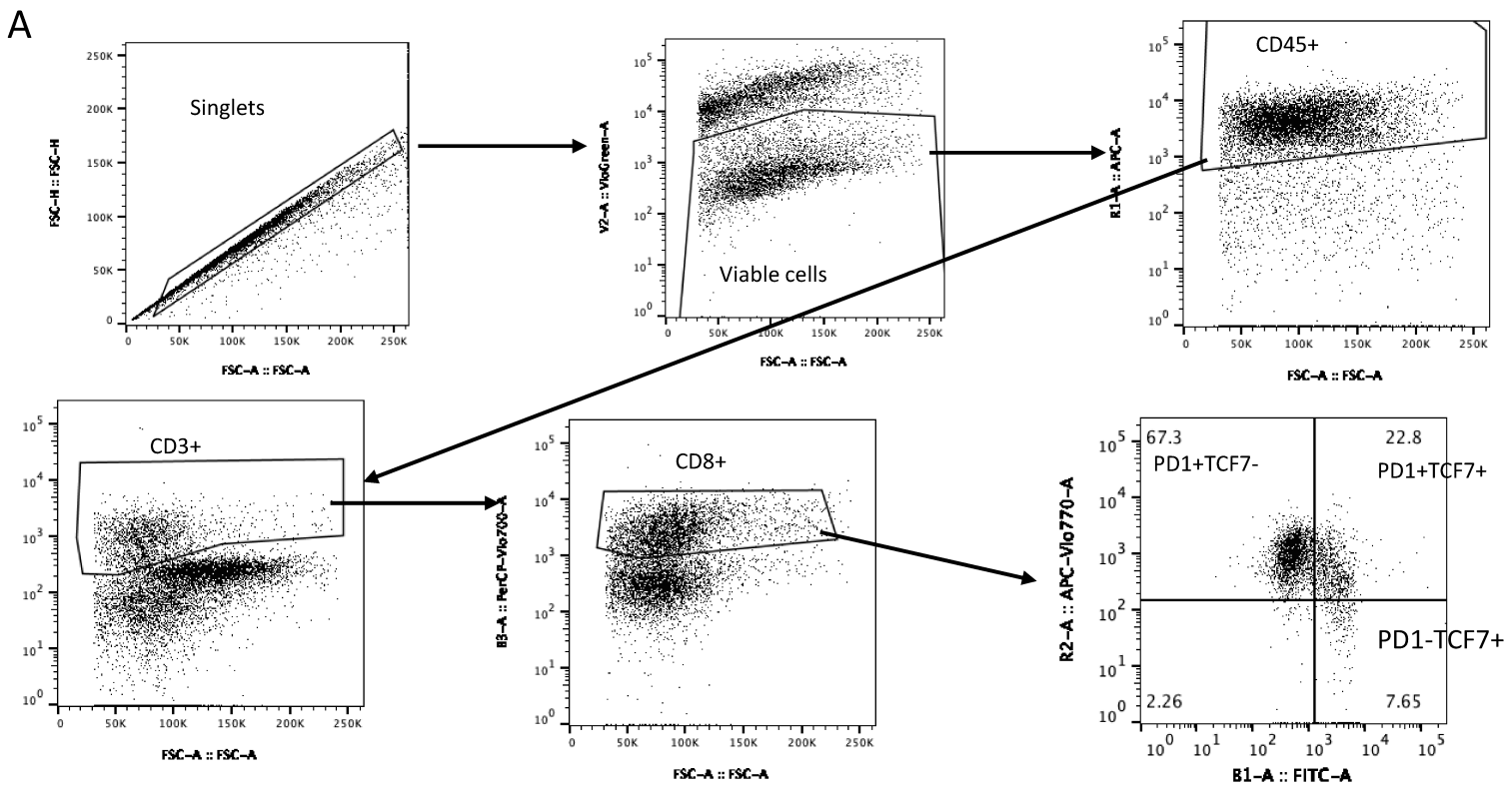


c



Supplementary Data 6. Extended data supporting the dynamics between major subsets of T cells in MOC1esc1 tumors. a, Summary of the number of T cells, the number of T cells with productive TCR, the percentage of T cells with productive TCR and the number of sequenced tumor infiltrating immune cells in each of the indicated MOC1esc1 treatment conditions. **b,** Clonal T cells were colored in indicated treatment conditions. Clonal T cells were defined as cells expressing TCR represented by two or more cells. **c,** UMAP of T cells in anti-CTLA4 treated condition with top 5 frequent clonotypes color coded.

Supplementary Data 6.



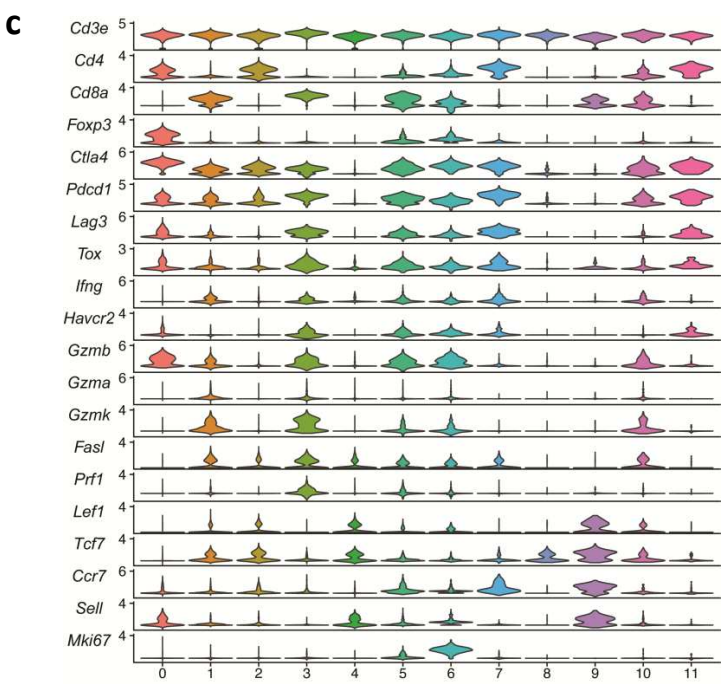
Supplementary Data 7.

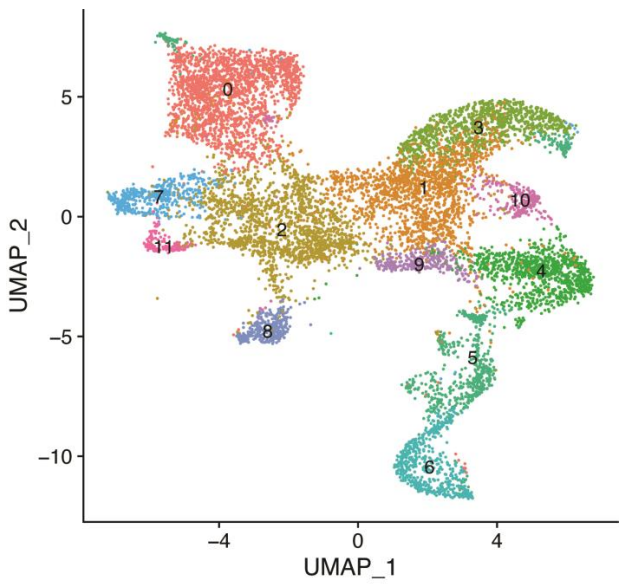
Supplementary Data 7. The effect of Tregs depletion on CD8+ T cell composition in MOC1esc1 tumors. MOC1esc1 tumors treated with isotype control, anti-PD1, anti-CD25, or anti-PD1 in combination with anti-CD25 were harvested at Day 10 post-inoculation and analyzed by flow cytometry for CD8+ T cell subset distribution. **a**, Gating strategy for CD8+ T cell subsets. **b**, Percentages of CD8+ T cell subsets in MOC1esc1 tumors at indicated treatment conditions are shown. **c**, Percentages of CD45+ cells in live events, CD3+ T cells in CD45+ cells, CD8+ T cells in total CD3+ T cells in MOC1esc1 tumors in indicated treatment conditions are shown. (*P<0.05, **P<0.01, ***P<0.001. Significance was calculated by one-way ANOVA. Data are shown as mean± SEM, N= 4-10 mice per group.)

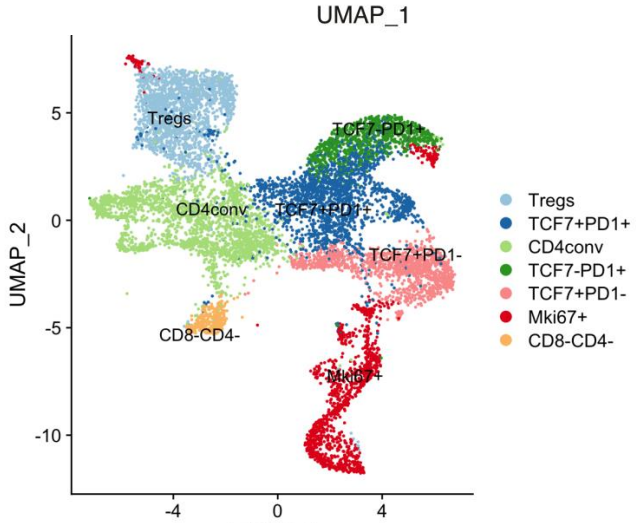
a

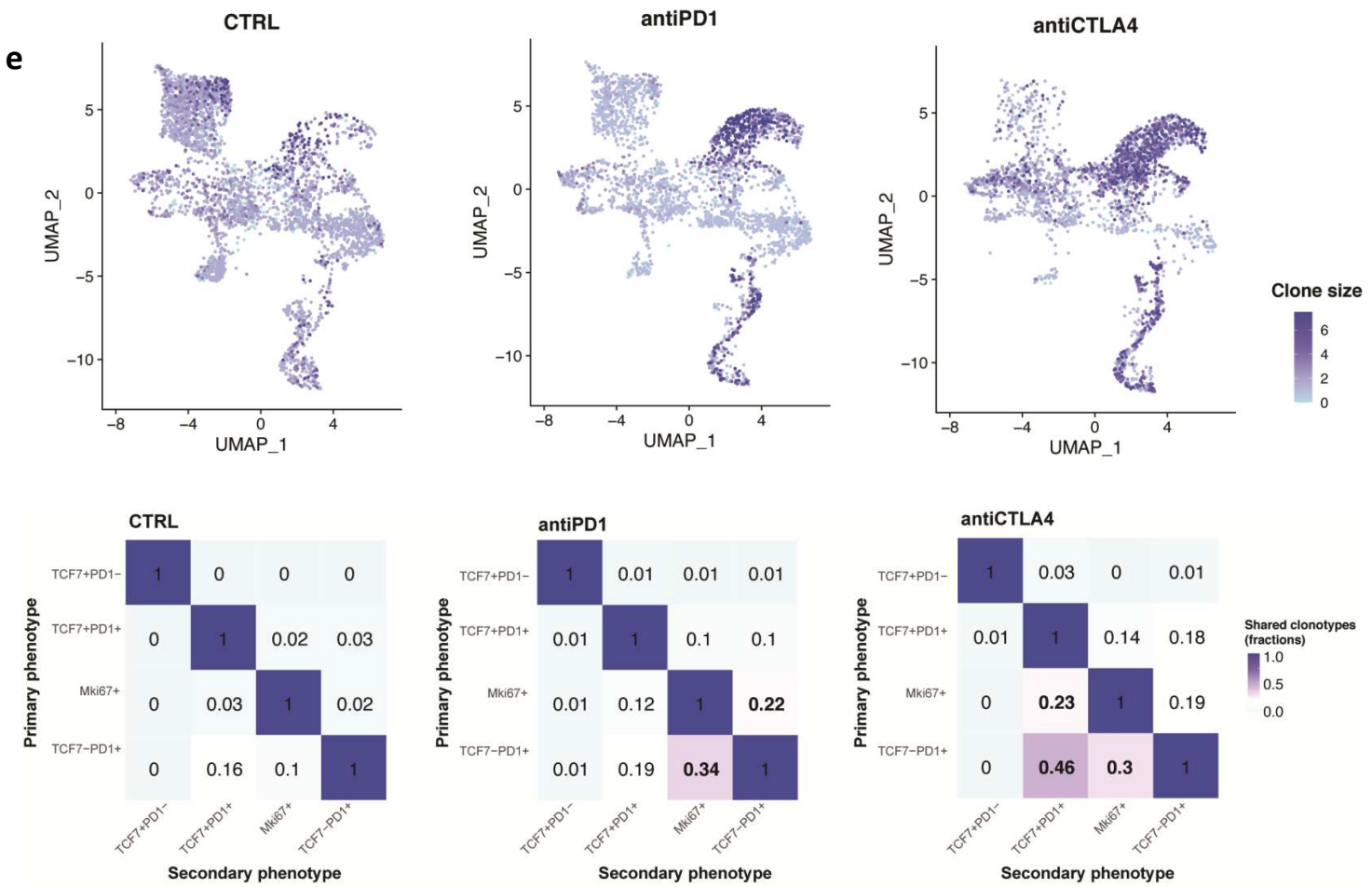
MOC1

Groups	isotype	anti-PD1	Anti-CTLA4
T cell #	3913	3090	2998
T cell w/TCR #	2083	2772	2803
% of T cells with productive TCR	53.2	89.7	93.4





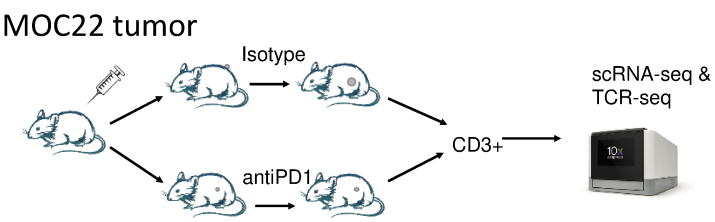




Supplementary Data 8.

Supplementary Data 8. Dynamics and lineage transitions in MOC1 CD8+ TILs. A total of 10,001 CD3+ T cells were sequenced in MOC1 tumors treated with isotype control, anti-PD1, or anti-CTLA4. **a,** Summary of the number of T cells, the number of T cells with productive TCR, the percentage of T cells with productive TCR in each of the indicated MOC1 treatment conditions. UMAP of T cells in MOC1 tumors colored by indicated major subsets from different treatment condition. **b**. Unsupervised clustering identified 12 clusters of T cells pooled from 3 treatment groups of MOC1 tumors. **c.** Violin plots showing expression of selected T cell function associated marker genes across clusters. The y-axis represents the normalized gene expression levels. **d**, UMAP of total T cells in MOC1 tumors colored by indicated major subsets. T cells from all 2 conditions were pooled for clustering analysis. **e**, Heatmap of TCR clone size. Cells were colored by TCR clone size in the UMAP of T cells in indicated conditions. **f**. Heatmap showing the shared fractions of TCR between primary and secondary phenotypes in indicated conditions.

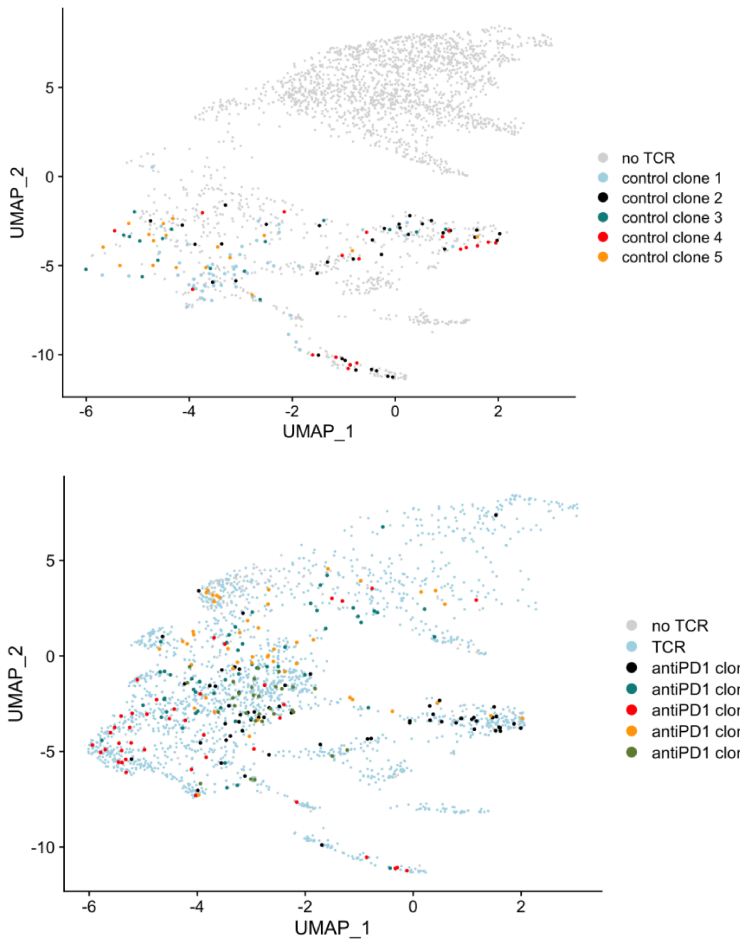
a



b

Groups	isotype	anti-PD1
T cell #	2811	2737
T cell w/TCR #	2223	2081
% of T cells with productive TCR	79.1	76

c



Supplementary Data 9. Clonal dynamics between major subsets of T cells in MOC22 tumors. a, Workflow of TIL characterization in MOC22 tumors. MOC22 bearing C57BL/6 mice were treated with isotype control or anti-PD1 on Days 3, 6, 9 post transplantation. Tumors were harvested on Day17. Live CD3+ T cells were flow sorted and subsequently subjected to 10x scRNA-seq and TCR-seq. **b,** Summary of the number of T cells, the number of T cells with productive TCR, the percentage of T cells with productive TCR in each indicated condition of MOC22 model. **c,** UMAP of T cells in each indicated condition with top 5 frequent clonotypes colored.

Supplementary Data 9.