Supplementary methods

Antibodies:

For *in vivo* study antibodies, rat IgG2a (2A3, Bioxcell), polyclonal hamster IgG (Bioxcell), rat IgG1 (TNP6A7, Bioxcell), anti-mouse PD-1 (RMP1-14, Bioxcell), anti-mouse CTLA-4 (9D9, Leinco Technologies), anti-mouse CD25 (PC-61.5.3, Bioxcell), and anti-mouse CSF1R (AFS98, Bioxcell) were purchased. For fluorescence conjugated antibodies, Rat IgG2b,κ (RTK4530, BioLegend), Mouse IgG2a,κ (MG2a-53, BioLegend), anti-mouse H2-K^b (AF6-88.5.5.3, Invitrogen), anti-mouse PD-L1(10F.9G2, BioLegend), anti-mouse CD45 (104, BioLegend), anti-mouse CD3(145-2C11, BioLegend), anti-mouse CD8a (53-6.7, Biolegend), anti-mouse PD1 (29F.1A12, Biolegend), anti-mouse TCF1/TCF7 (C63D9, Cell signaling), Rabbit IgG (DA1E, Cell signaling) were used. 7-AAD (BioLegend) and Zombie Aqua Fixable Viability Kit (BioLegend) were used for cellular viability staining.

Whole Exome sequencing, variant analysis, and neoantigen prediction

Genomic DNA was isolated using the DNeasy Blood and Tissue Kit (Qiagen). Library construction was performed using the Agilent Sure Select Whole Exome Capture and captured using the Agilent SureSelect XT Mouse All Exon probe sets according to the manufacturer protocol. Libraries were sequenced on the Illumina HiSeq 4000 platform. Reads were aligned to the mouse reference genome (mm9) using BWA-MEM version 0.7.10 and duplicates were marked using SAMBLASTER version 0.1.22 [66]. SNVs and small indels were detected using the Genome Modeling System [38]. Variants were annotated using Ensembl v67 and neoepitopes were predicted by NetMHC pan 4.0 [39] using epitopes of length 9 against alleles H2-K^b and H2-D^b.

Putative neoantigens were determined by filtering to those with a mutant epitope binding affinity of less than 500 nM and a fold-change difference (between mutant and wildtype epitopes) greater than 1.

Tumor re-challenge

Mice cured of MOC1esc1 (10^6 cells) were generated after anti-CTLA4 treatment coupled with surgical resection of any residual tumors (50% of mice). Mice were rested for 6 weeks and re-challenged with MOC1 (10^6) or MOC1esc1 (10^6) tumor lines in parallel with age-matched naïve mice. In re-challenge following anti-CD25 in combination with anti-PD1, all the initially established MOC1esc1 tumors rejected. After 6 weeks' rest, cured mice and age-matched controls were re-challenged with MOC1esc1 (10^6). Tumor growth was monitored 2-3 times per week.

Bulk RNA-seq analysis

Total RNA was extracted using RNeasy Plus Mini kit (QIAGEN) according to manufacturer's protocol. Standard mRNA library preparation kit (RS-122-2101, Illumina) was used for library preparation. Libraries were sequenced on Illumina NextSeq 500 platform. Paired read data was adapted and quality trimmed using Trimmomatic v0.36 [67]. Trimmed reads were quantified by pseudoalignment against mm10 using Kallisto v0.46.0 [68]. Differential expression analysis was performed using DESeq2 [69]. Hallmark gene signature enrichment [70] was calculated from signed -log10 padj values using GSEA Preranked [71]. The variance stabilizing transformed expression values of the core enrichment genes from each gene set with FDR < 0.001 were plotted after gene-wise z-score normalization.

Tumor dissociation and FACS analysis:

MOC1 and MOC1E1 tumors were harvested on day 12 after tumor inoculation. MOC22 tumors were harvested on day 17 after tumor inoculation. Fresh mouse tumors were minced and digested using mouse Tumor Dissociation kit (130-096-730) and gentleMACS Dissociator (130-093-235) from Miltenyi Biotec according to the manufacturer's instructions. After incubation for 45 min at 37 °C, cells were filtered and blocked using Rat Anti-Mouse CD16/CD32 (2.4G2, BD biosciences). Cells were first stained with Zombie Aqua in PBS to distinguish live/dead cells, then stained with surface marker antibodies for 20 mins at 4 C degrees. For TCF7 staining, after surface staining, the cells were fixed using Foxp3/ transcription factor staining buffer set (00-5523-00, eBioscience) and blocked with rat serum. The cells then were stained with intracellular antibody for mouse TCF7. All flow cytometry analyses were performed on a MACSQuant analyzer 10 (Miltenyi) and analyzed using FlowJo10 (Treestar).

ScRNA-seq data Quality Control, UMAP clustering, and cell cluster annotation

We used several QC metrics to identify low-quality cells based on their expression. The utilized criteria include library size, number of expressed genes, and proportion of reads mapped to mitochondria. These criteria were selected with the rationale that cells with small library sizes are of low quality because the RNA might have been lost during library preparation; cells with very few expressed genes are likely to be of poor quality as the diverse transcript population has not been successfully captured; cells with high proportion of mitochondrial-mapped genes are indicative low quality [67]. Ambient RNAs were ruled out with the restriction of cells having more than 200 sequenced genes. With the counts assessed and quantified, the generated raw gene count matrices were combined and subsequently converted to a Seurat object for read counts normalization, scaling, and clustering. A fraction of cells with high abundance (>5%)

mitochondrial RNA signature was filtered. We used adaptive threshold by identifying cells that are outliers for the various QC metrics based on the median absolute deviation (MAD) from the median value of each metric across all cells. a value is considered to be outlier if more than 2 MADs from the median. To remove doublets, we applied DoubletFinder (V2.0) to identify and remove doublets. To optimize the DoubletFinder performance, the parameter of pK value was pre-selected by optimizing the performance of Mean-variance normalized bimodality coefficient.

After quality control and unsupervised cell clustering, Uniform Manifold Approximation and Projection (UMAP) was performed for visualization, and each resulting cluster was conceptualized as a cell type. Cell type of each cluster was inferred by marker gene enrichment. For this purpose, a set of top-ranked canonical markers were derived by differential expression across cell clusters. With the derived marker genes, we used the LM22 profiled by CIBERSORT team to help cell type annotation. The cell types were annotated in a combination of automatic annotation and manual curations. To start, we performed automatic cell-type annotation in a supervised manner, which requires the pre-existing knowledge of marker genes for each cell type. Given the gene signatures of each cell type, for each cluster, we calculated the summed logFC (cells in one cluster versus all other cells, which could be both positive or negative) of marker genes divided by log2 total number of marker genes as the cell-type scores of the input gene signature; the cell type of gene signature with the highest score is annotated as the cell-type identity of that cluster. The minimum gene signature score is set to 0, and if the score of all input signatures is less than 0, the cluster will be annotated as “others.” The immune LM22 gene signature from CIBERSORT team [68] was used to annotate the cell types. After the automatic annotation, the annotated cell types were manually revised with known cell type specific marker genes including: CD3e (T cells), CD8a (CD8+ T

cells), CD4 (CD4+ T cells), Foxp3 (Tregs), Adgre1 (Macrophages), Arg1 (M2-like macrophages), Klrb1b (NK cells), S100a9 (Neutrophils), Siglech (plasmacytoid Dendritic cells), Ccl22 (Dendritic cells), Cd19, Cd79 (B cells), Cpa3 (Mast cells)."