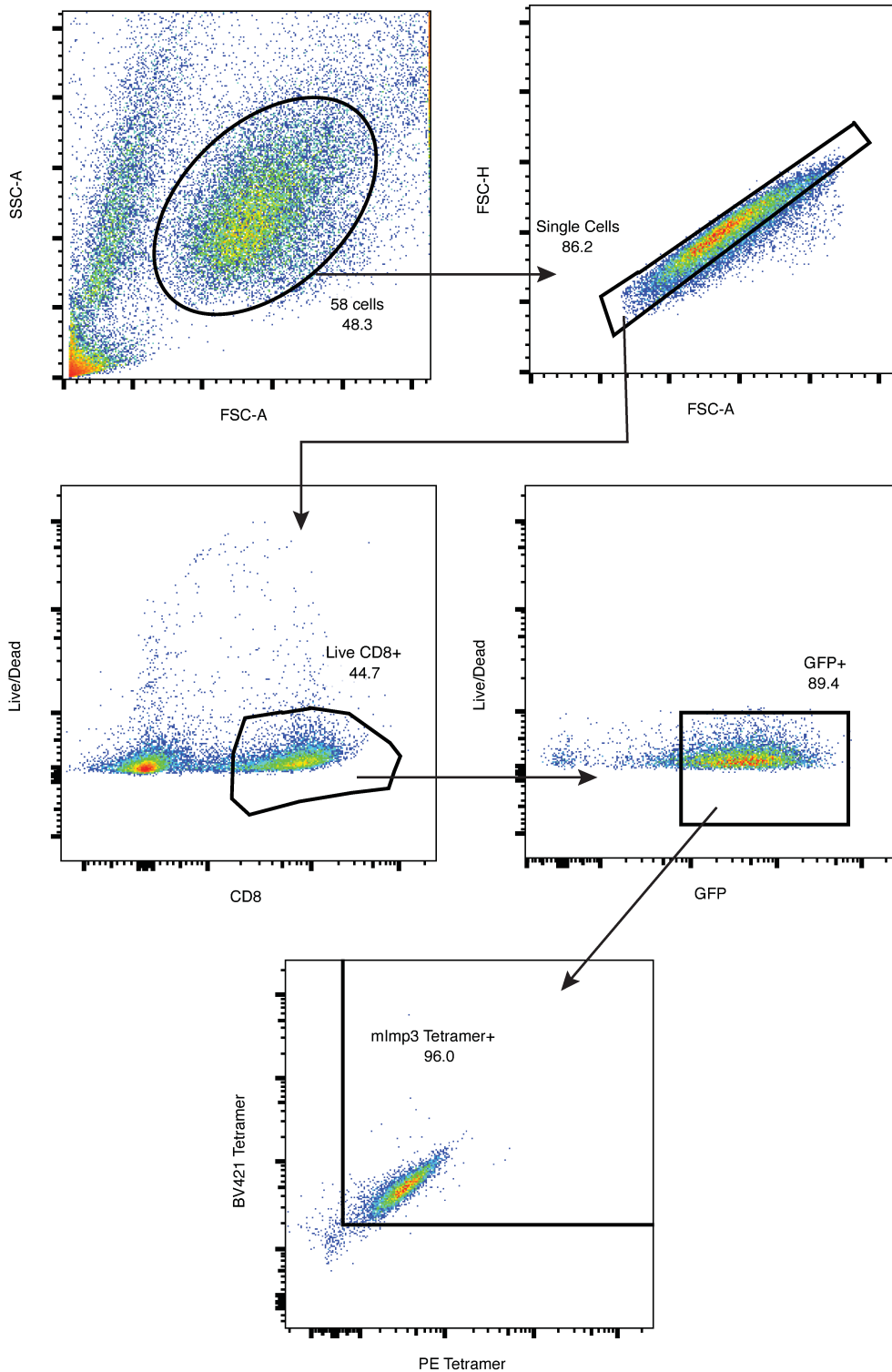


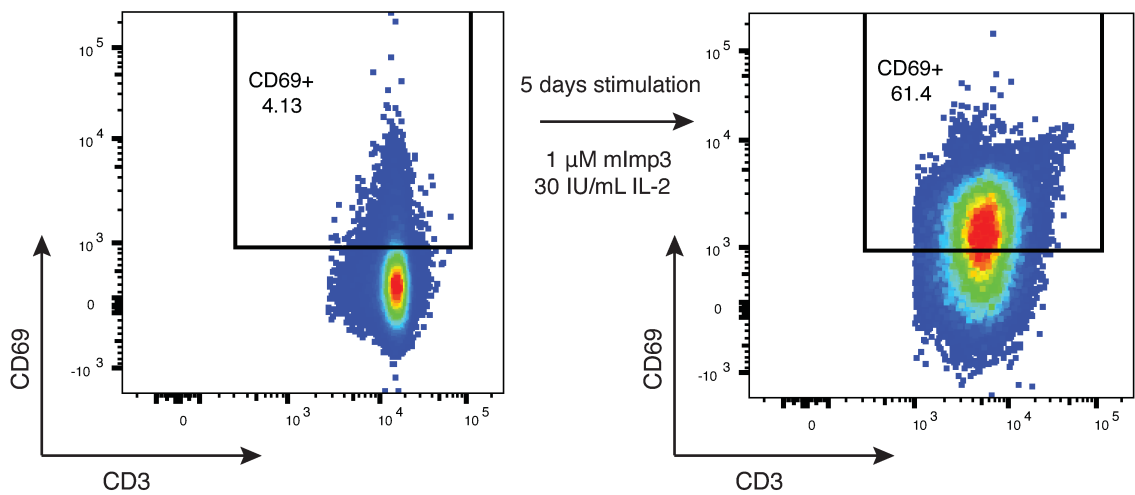
Supplemental Figure S1: Clonotype distributions of expanded populations. Pie charts depicting the frequency of each clonotype identified via single-cell PCR in either 1x stimulated splenocytes (top), 3x stimulated splenocytes (middle), or vaccine stimulated splenocytes (bottom). TCRs later screened for functional status are labeled with their specific name.

TCR	Alpha Chain	Alpha CDR3	Beta Chain	Beta CDR3
4.1B	TRAV6D/TRAJ47	CALGAEDYANKMIF	TRBV13/TRBJ2/TRBD1	CASGDWVGAETLYF
4.3D	TRAV13D/TRAJ26	CALEYAQGLTF	TRBV13/TRBJ2/TRBD2	CASGGLGDQDTQYF
V1.1D	TRAV6/TRAJ22	CVLGDSGSWQLIF	TRBV13/TRBJ2/TRBD2	CASGDAMGGAETLYF
V1.6C	TRAV6/TRAJ22	CVLAHASSGSWQLIF	TRBV29/TRBJ2/TRBD2	CASPTGGLAKTLYF
V1.3E	TRAV12D/TRAJ18	CALSDRGSALGRLHF	TRBV3/TRBJ2/TRBD1	CASSLEQGGGYNIAEQFF
3x1.1C	TRAV3D/TRAJ17	CAVGGSNSAGNKLTF	TRBV12/TRBJ1/TRBD1	CASSLEDREGSDYTF
3x1.4A	TRAV12D/TRAJ6	CALVPGGNYKPTF	TRBV26/TRBJ2/TRBD1	CASSPDSYEQYF
3x2.4D	TRAV12D/TRAJ6	CALIPGGNYKPTF	TRBV26/TRBJ2/TRBD1	CASSPDSYEQYF
3x2.5C	TRAV12D/TRAJ6	CALSEGNYKPTF	TRBV26/TRBJ2/TRBD1	CASSPDSYEQYF

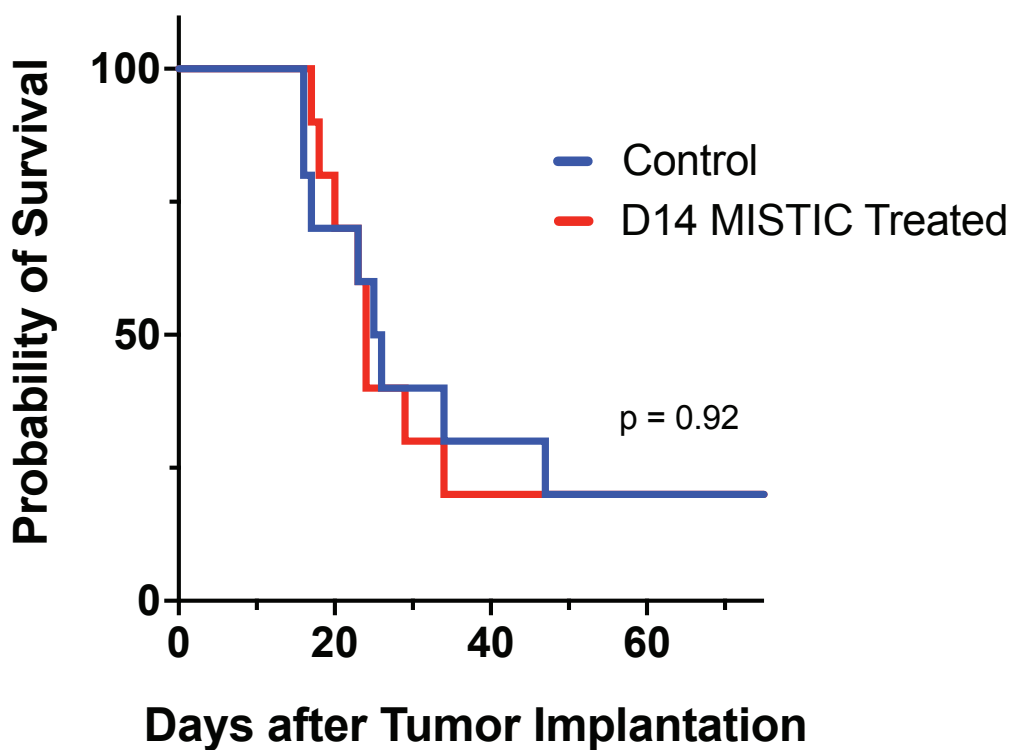
Supplemental Figure S2: Candidate neoantigen-specific TCRs. List of 9 candidate TCRs identified from single-cell PCR that were selected for further analysis with their indicated TCR characteristics.



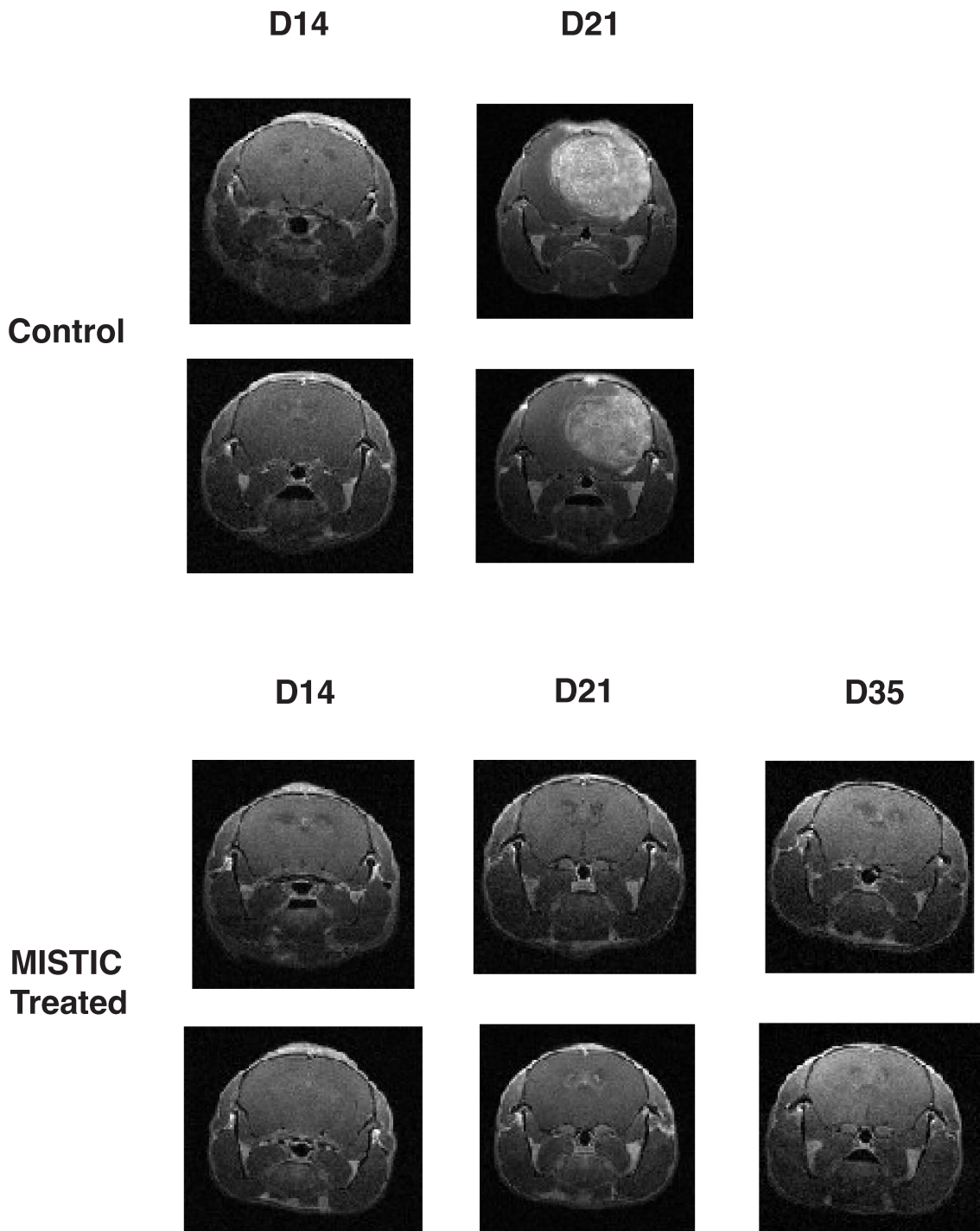
Supplemental Figure S3: Gating schema for 58 hybridoma cells. Example gating schema for 58 hybridoma cells transduced with candidate TCR (3x1.1C) and stained with cell-surface antibodies and fluorescently conjugated mImp3 tetramer.



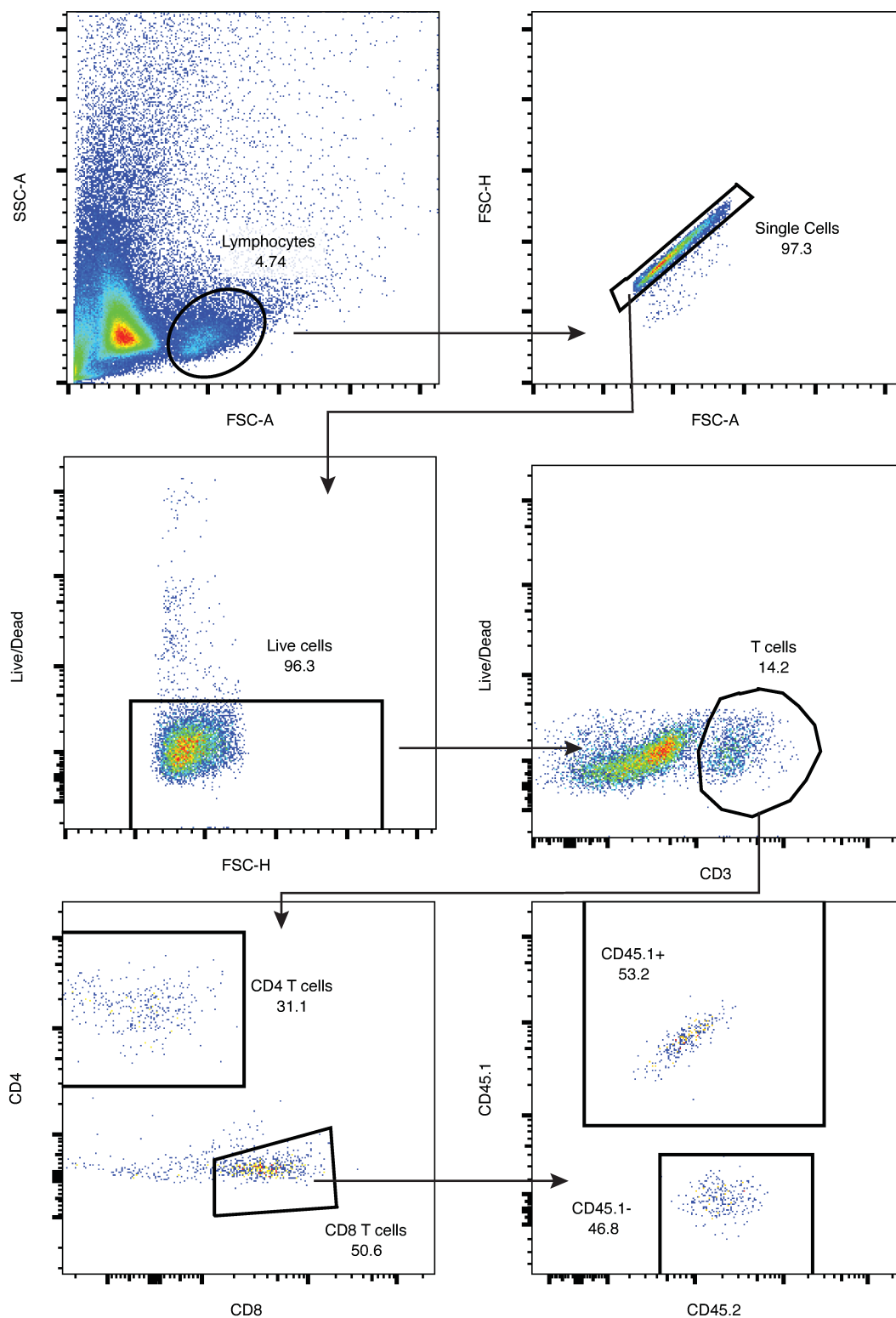
Supplemental Figure S4: MISTIC splenocyte peptide-specific activation. Representative flow cytometry plots of naive (left) or peptide-stimulated (right) CD8 T cells from the spleens of MISTIC transgenic mice after 5 days of in-vitro stimulation with mImp3 peptide and IL-2.



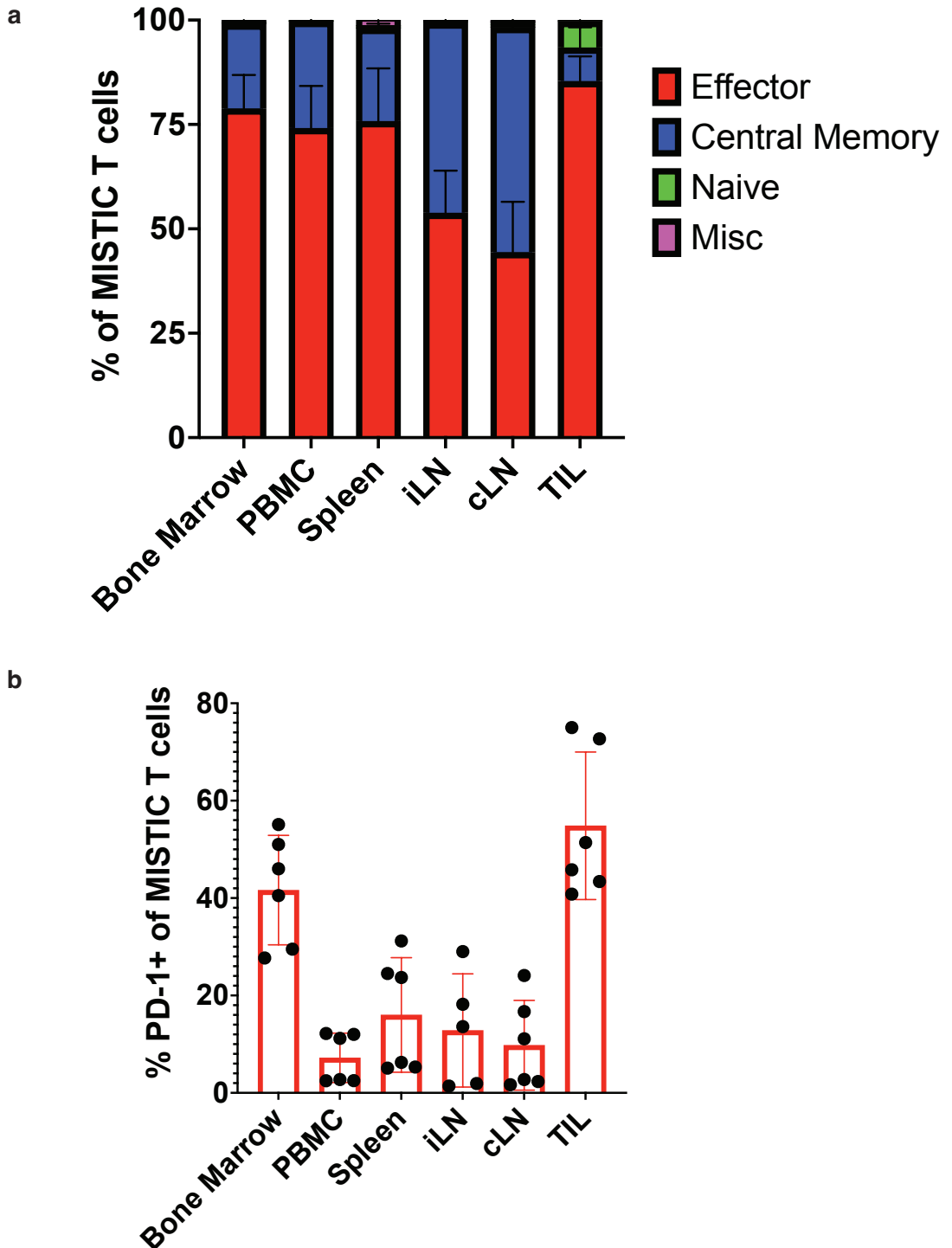
Supplemental Figure S5: Efficacy of delayed MISTIC adoptive cell therapy. Survival of GL261-bearing mice treated on day 14 with either irradiation and IL-2 (Control) or irradiation, IL-2, and MISTIC T cells (MISTIC Treated). N=10 for each group from 2 independent experiments. Significance assessed by log-rank test.



Supplemental Figure S6: Serial MRI of tumor-bearing mice. Representative T1-weighted MRI scans of either control or day 7 MISTIC treated mice 14, 21, or 35 days following tumor implantation.

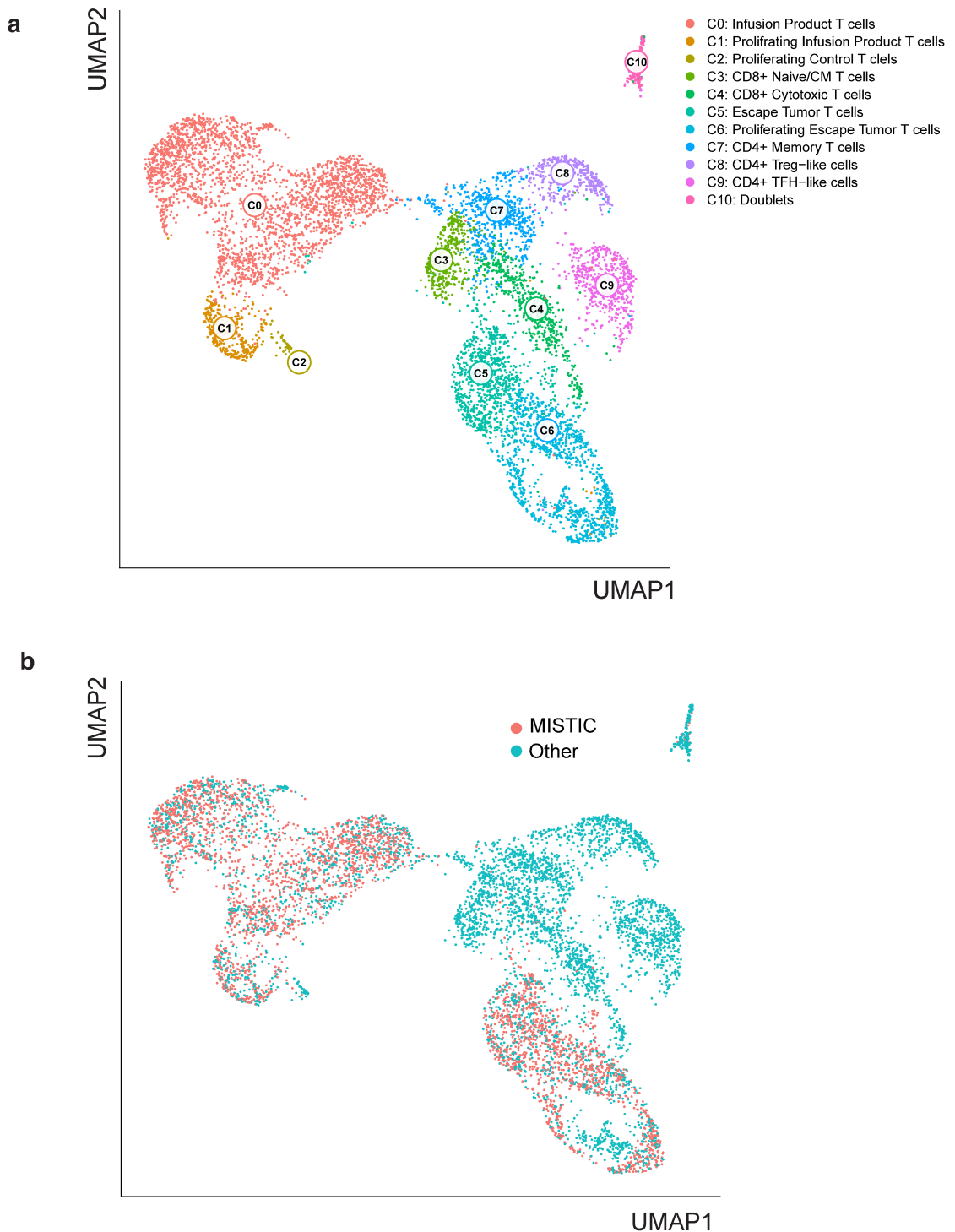


Supplemental Figure S7: Gating schema for adoptively transferred MISTIC T cells. Example gating schema for the identification of congenically labeled adoptively transferred MISTIC T cells. The provided example is from tumor tissue.



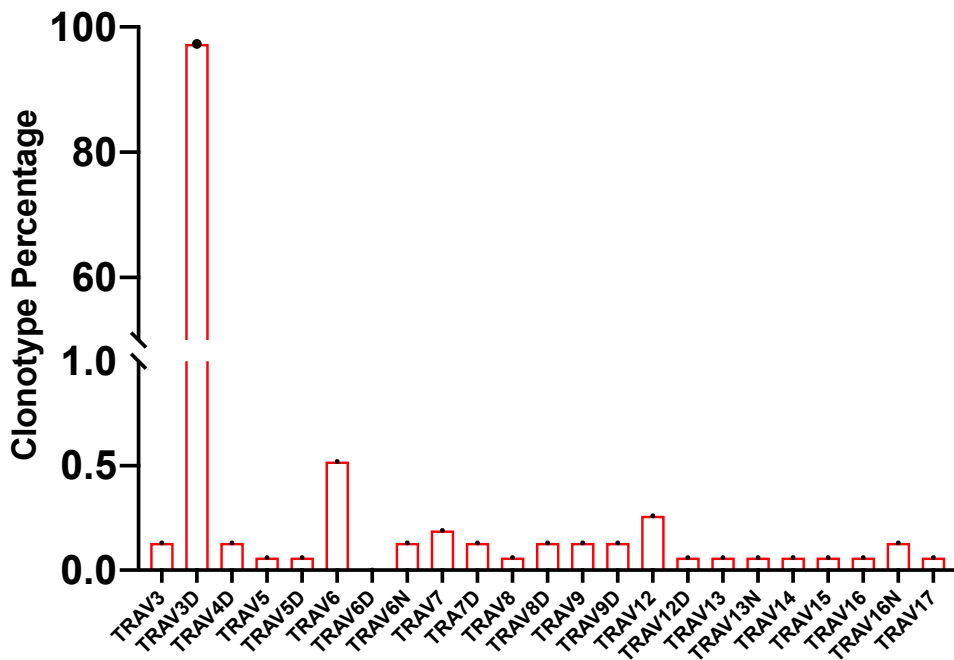
Supplemental Figure S8: Phenotypic characteristics of adoptively transferred MISTIC T cells.

a, Stacked bar plots displaying the proportion of MISTIC T cells of a given phenotypic state in each anatomical compartment on day 10 (3 days post-transfer). Phenotypic states defined as follows: effector (CD44+ CD62L⁻), central memory (CD44+ CD62L⁺), or naive (CD44⁻ CD62L⁺). N=6 from 2 independent experiments. **b**, Proportion of PD-1+ MISTIC T cells in each anatomical compartment on day 10 (3 days post-transfer). N=6 from 2 independent experiments.

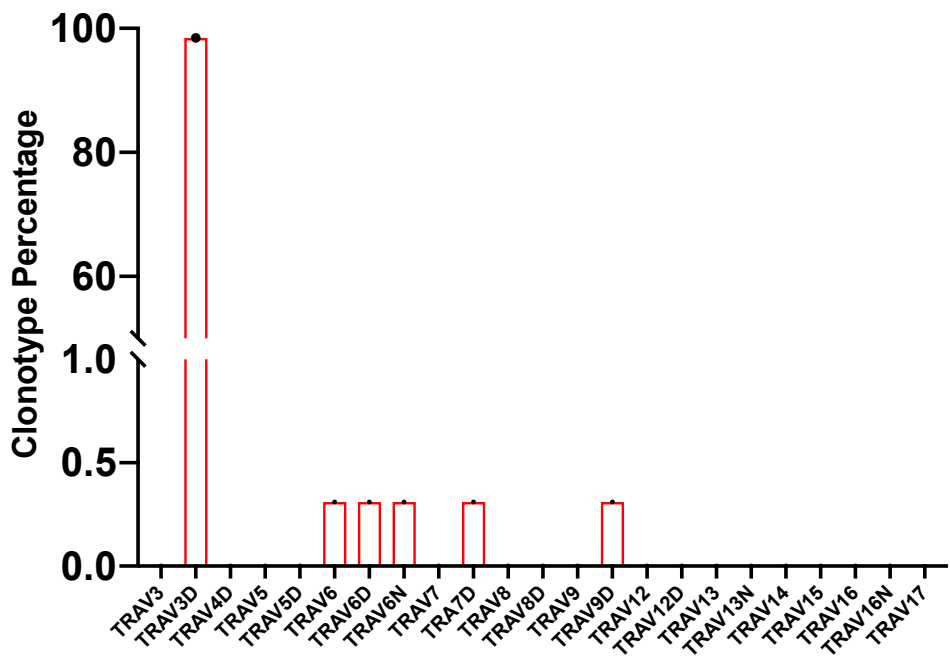


Supplemental Figure S9: Characterization of single-cell populations. a, UMAP representation dimensionality reduction of the scRNA-seq data with cell types labeled according to canonical marker genes. **b**, UMAP representation dimensionality reduction of the scRNA-seq data derived from the three populations with cells bearing the 3x1.1C TCR labeled as “MISTIC”.

CD8

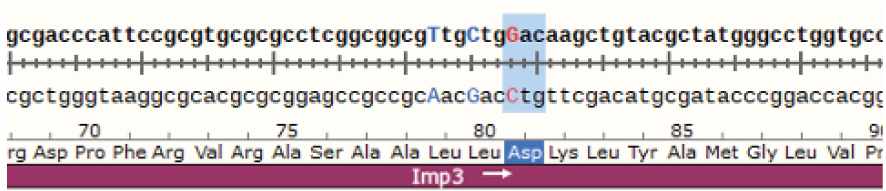


CD4

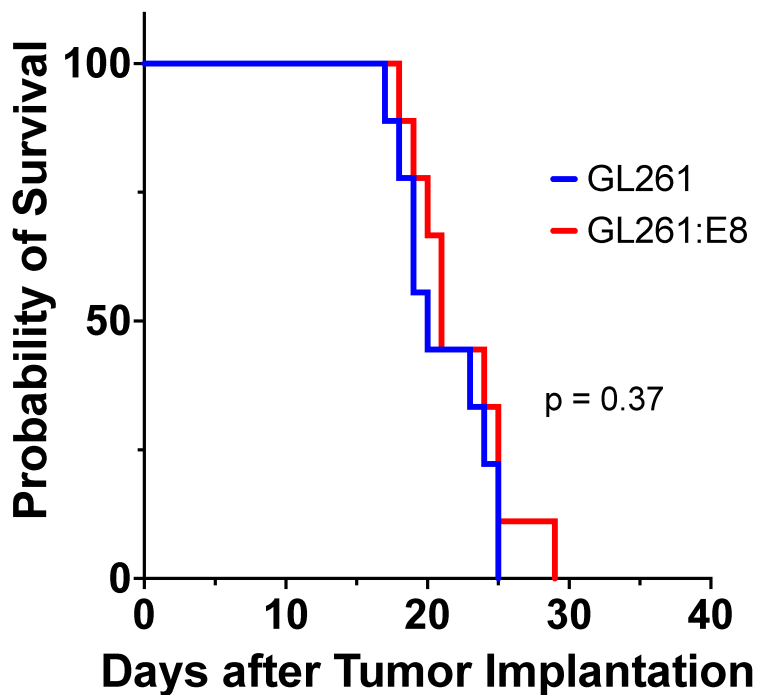


Supplemental Figure S10: Clonal architecture of MISTIC infusion product. TCR α chain usage from 1,543 CD8+ and 324 CD4+ T cells within the MISTIC infusion product as assessed via single-cell RNA-sequencing. TCR α chains not represented among either the CD8 or CD4 populations were omitted for clarity.

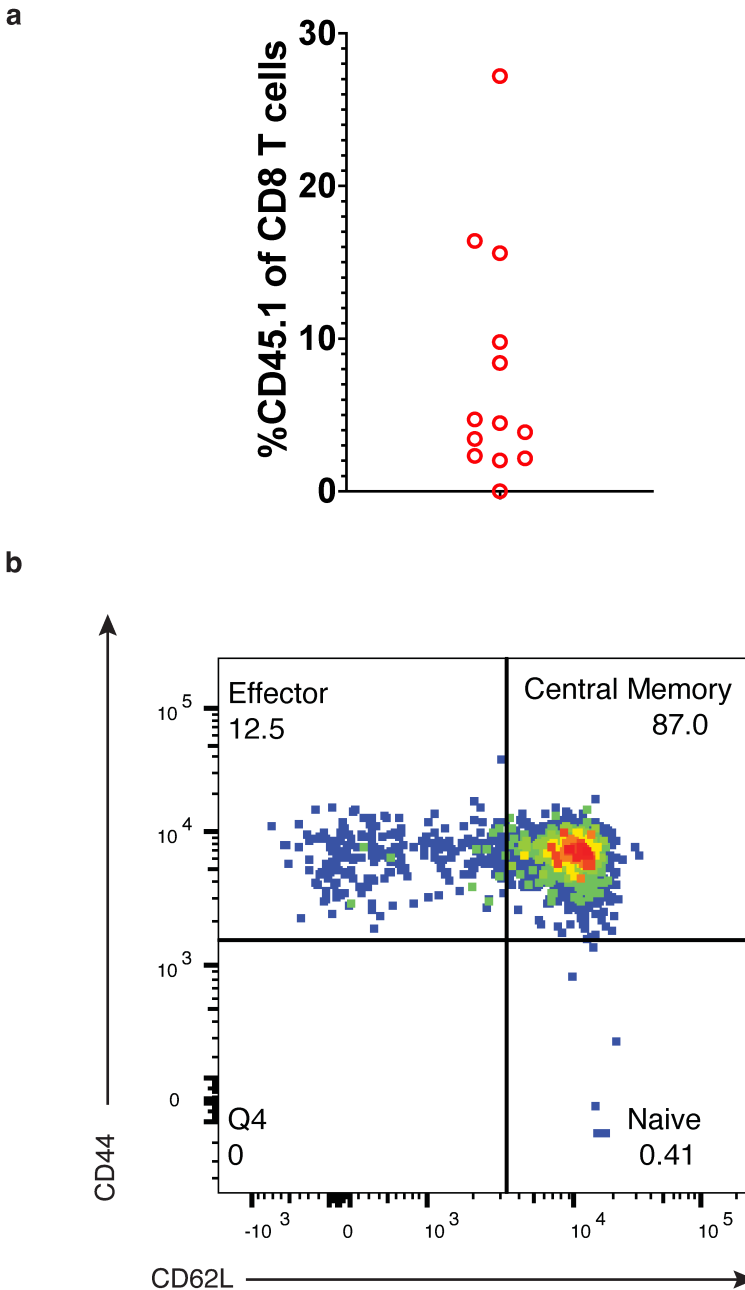
a



b



Supplemental Figure S11: Generation and characterization of GL261:E8 cell line. a, Sequencing results from the Imp3 locus in GL261:E8 confirming the N81D conversion to the wild-type Imp3 sequence. **b,** Survival of tumor-bearing mice following intracranial implantation with either GL261 or GL261:E8. N=10 mice in each group from 2 independent experiments. Significance by log-rank test.



Supplemental Figure S12: Persistence of MISTIC T cells following transfer. **a**, Frequency of MISTIC T cells among CD8 T cells in peripheral blood of treated mice on day 50 following tumor implantation. N=13 mice from 3 separate experimental replicates. **b**, Representative flow cytometry plot displaying expression of CD44 and CD62L in MISTIC T cells in the peripheral blood of treated mice on day 50 following tumor implantation. N=13 mice from 3 separate experimental replicates.