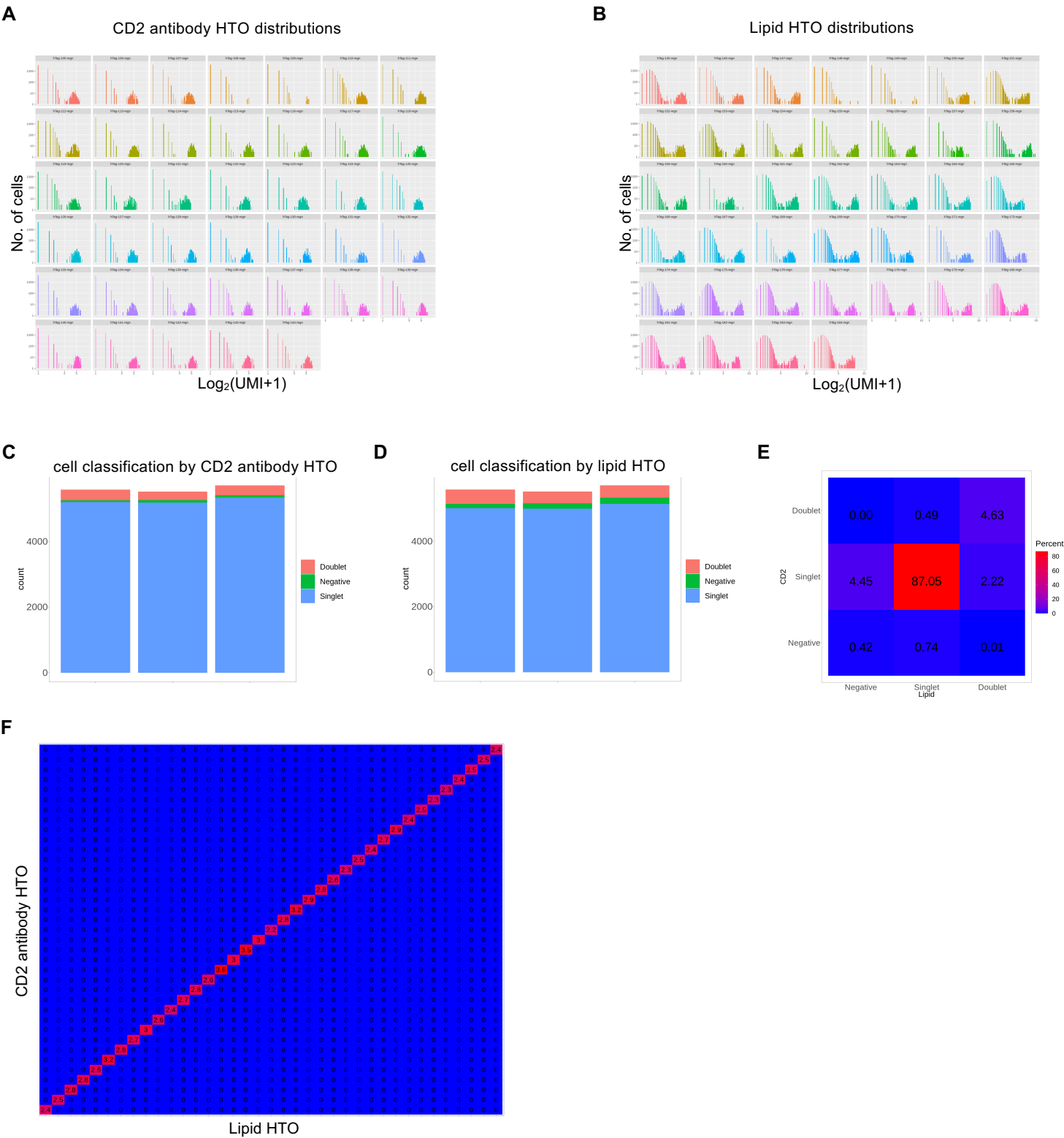
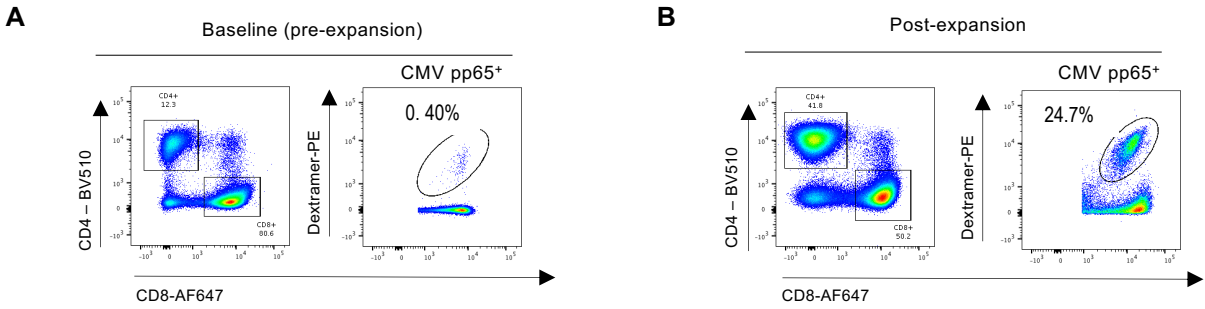


Supplementary Figure 1



Supplementary Figure 1: Both anti-CD2 antibody- and lipid-based hashing reagents enable efficient identification of T cells based on HTO classification. Each aliquot of T cells is labeled with two HTOs; a uniquely barcoded anti-CD2 antibody and a separate, uniquely barcoded lipid HTO. After staining, the aliquots are washed and pooled for capture. **(A and B)** Histograms of raw HTO UMI distributions for each anti-CD2 antibody **(A)** or lipid HTO **(B)**. **(C and D)** Number of cells identified as doublet, negative, or singlet based on independent classification using anti-CD2 antibody **(C)** or lipid HTO **(D)** demultiplexing. Triplicate captures are shown. **(E)** Comparison of cell classification between anti-CD2 antibody and lipid HTOs. Lipid HTO demultiplexing is largely concordant with anti-CD2 antibody HTO classification. **(F)** Matrix showing the percentage of total cells classified by all possible antibody:lipid HTO pairs. The input pairing for each T cell aliquot is on the diagonal and is recovered by HTO demultiplexing.

Supplementary Figure 2

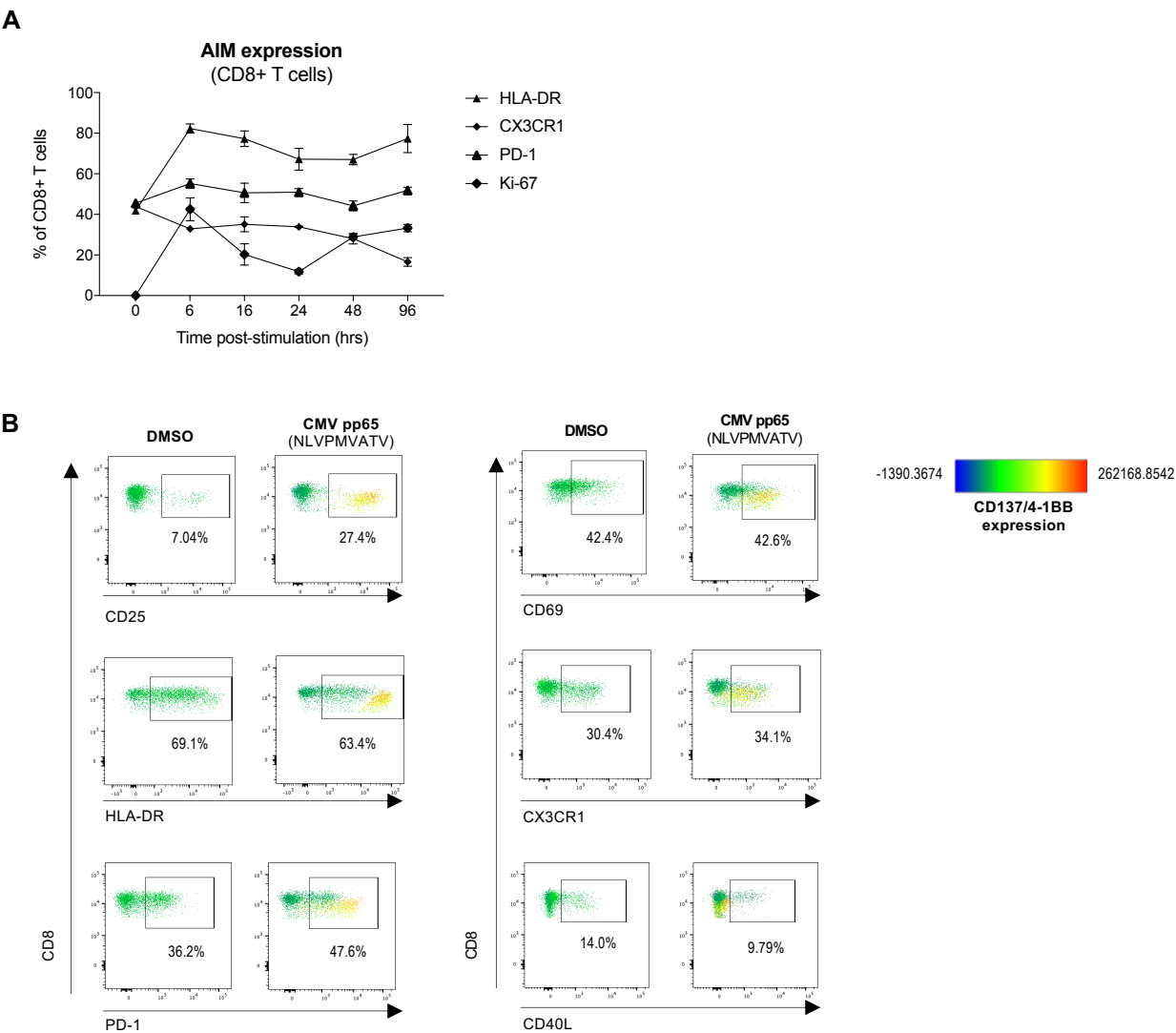


Supplementary Figure 2: CMV pp65⁺ T cells were expanded in culture to enable proof-of-concept experiments.

Related to Figure 2. (A) The baseline percentage of CMV pp65 dextramer⁺ CD8⁺ T cells from a CMV serum positive, HLA-A*02:01⁺ healthy donor (HD1) was assessed by flow cytometry.

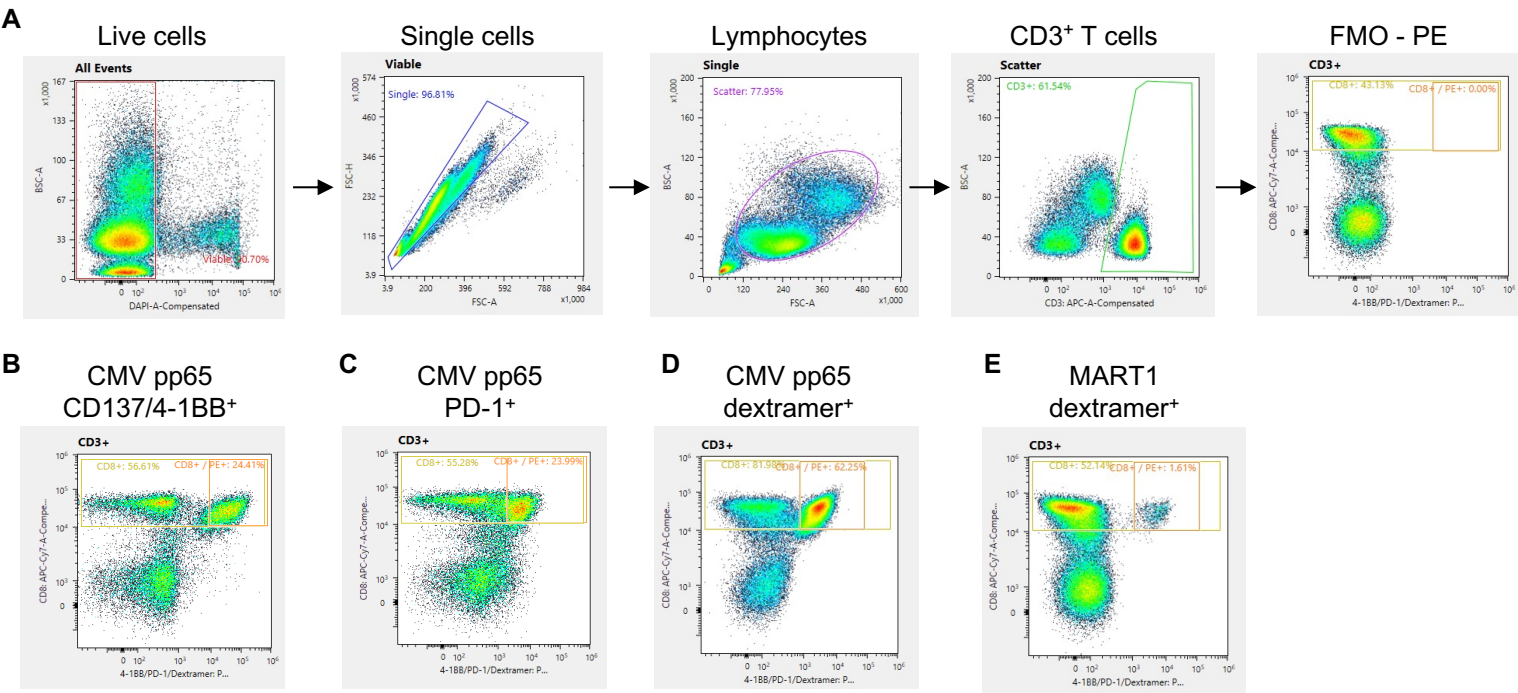
(B) HD1 T cells were expanded in culture for 10 days in the presence of CMV pp65 peptide (NLVPMVATV) and DC and T cell supporting cytokines. Post-expansion, the percentage of CMV pp65 dextramer⁺ CD8⁺ T cells was determined by flow cytometry.

Supplementary Figure 3



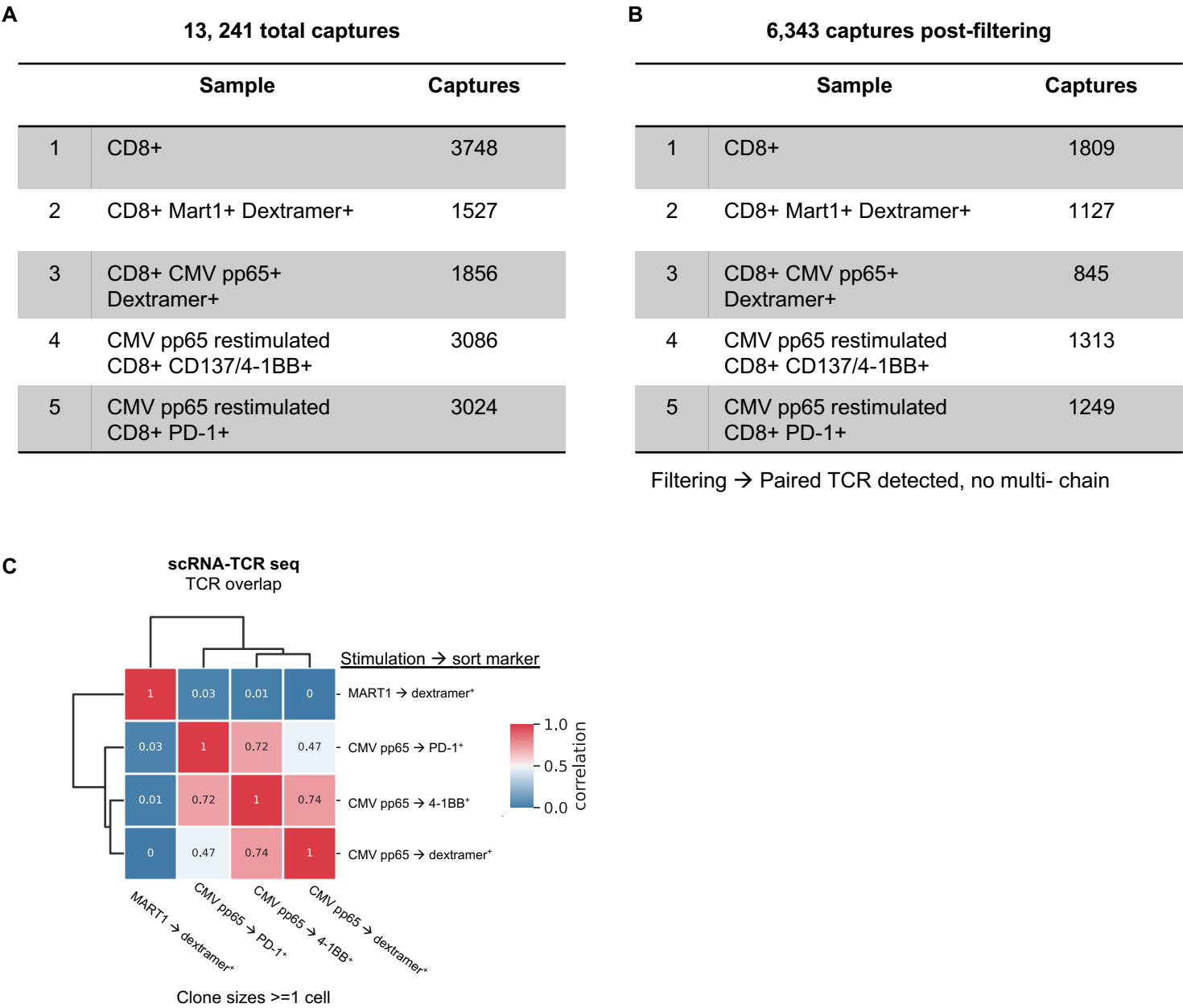
Supplementary Figure 3: Activation-induced markers are upregulated on antigen-specific T cells following antigen-specific activation. Related to Figure 2. T cells from a CMV serum positive, HLA-A*02:01⁺ healthy donor (HD1) were expanded for 10 days in the presence of CMV pp65 peptide (NLVPMVATV) to enable proof-of-concept tests. **(A)** Expanded T cells were restimulated with cognate CMV pp65 peptide in RAPTER assay culture conditions over a 96-hour time course. The percentages of AIM⁺ CD8⁺ T cells were assessed by flow cytometry. **(B)** Expanded T cells were restimulated with DMSO or CMV pp65 peptide (NLVPMVATV) for 24 hrs. The percentages of CD8⁺ T cells that stain positive for the indicated AIMs were assessed by flow cytometry. The CD137/4-1BB⁺ CD8⁺ T cells within each AIM⁺ gate are indicated by the yellow-red color scale.

Supplementary Figure 4



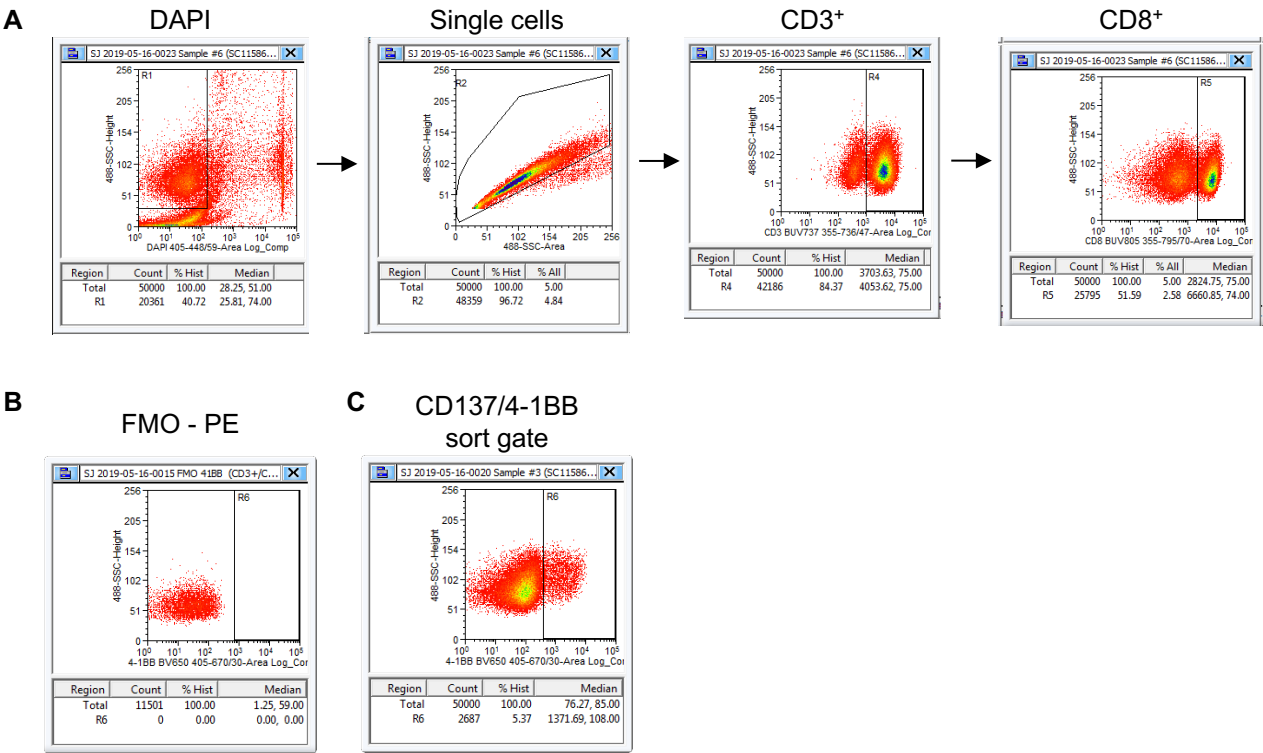
Supplementary Figure 4: Fluorescence activated cell sorting (FACS) gating strategy to isolate CD8⁺ T cell populations for scRNA- and TCR-seq analysis. Related to Figure 2. (A) FACS upper-level gating including the fluorescence minus one (FMO) gate for the PE channel. (B) CD137/4-1BB⁺ CD8⁺ T cells. (C) PD-1⁺ CD8⁺ T cells. (D) CMV pp65 dextramer⁺ CD8⁺ T cells. (E) MART1 dextramer⁺ CD8⁺ T cells.

Supplementary Figure 5



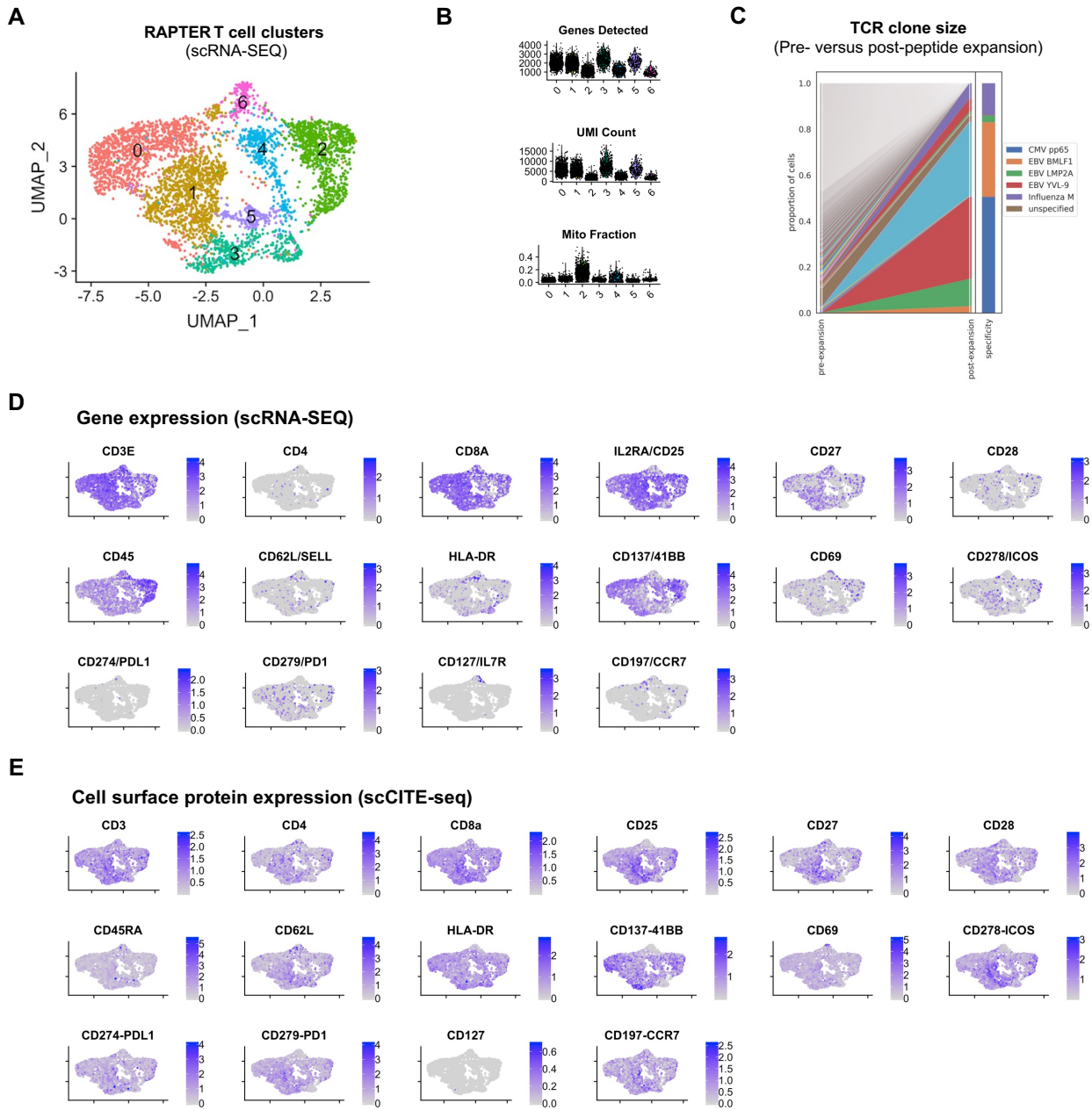
Supplementary Figure 5: CMV pp65 and MART1 dextramer⁺, and CMV pp65 CD137/4-1BB⁺, and PD-1⁺ CD8⁺ T cells were isolated by FACS then analyzed by scTCR-SEQ. Related to Figure 2. (A) Total scRNA-SEQ captures from all samples pre-filtering and (B) post-filtering to select TCR sequences with paired a and b chains. Any TCR captures with multiple chains were eliminated from analysis. (C) All dextramer⁺ TCRs were compared to CD137/4-1BB⁺, and PD-1⁺ TCRs with clone sizes >=1 cell.

Supplementary Figure 6



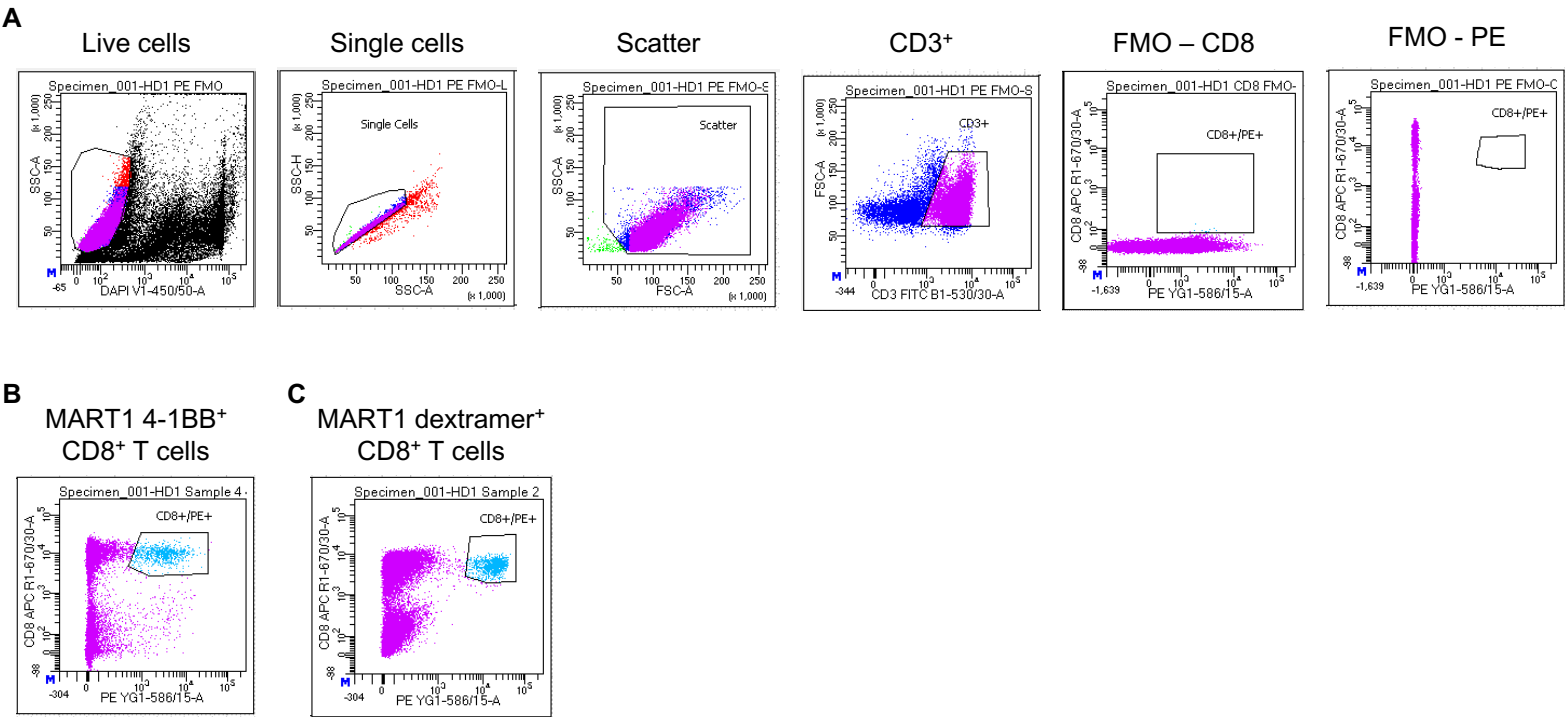
Supplementary Figure 6: FACS gating strategy to isolate CD8⁺ CD137/4-1BB⁺ T cell populations for scRNA- and TCR-seq. Related to Figure 3. PBMC from HD1 were cultured for 7 days with 5 viral peptides to expand the number of epitope-specific memory T cells. Expanded PBMC were then re-stimulated with DMSO or cognate peptides for 24 hours. **(A)** FACS upper-level gating. **(B)** FMO gate for the CD137/4-1BB (BV650) channel. **(C)** CD137/4-1BB⁺ CD8⁺ T cell sort gate.

Supplementary Figure 7



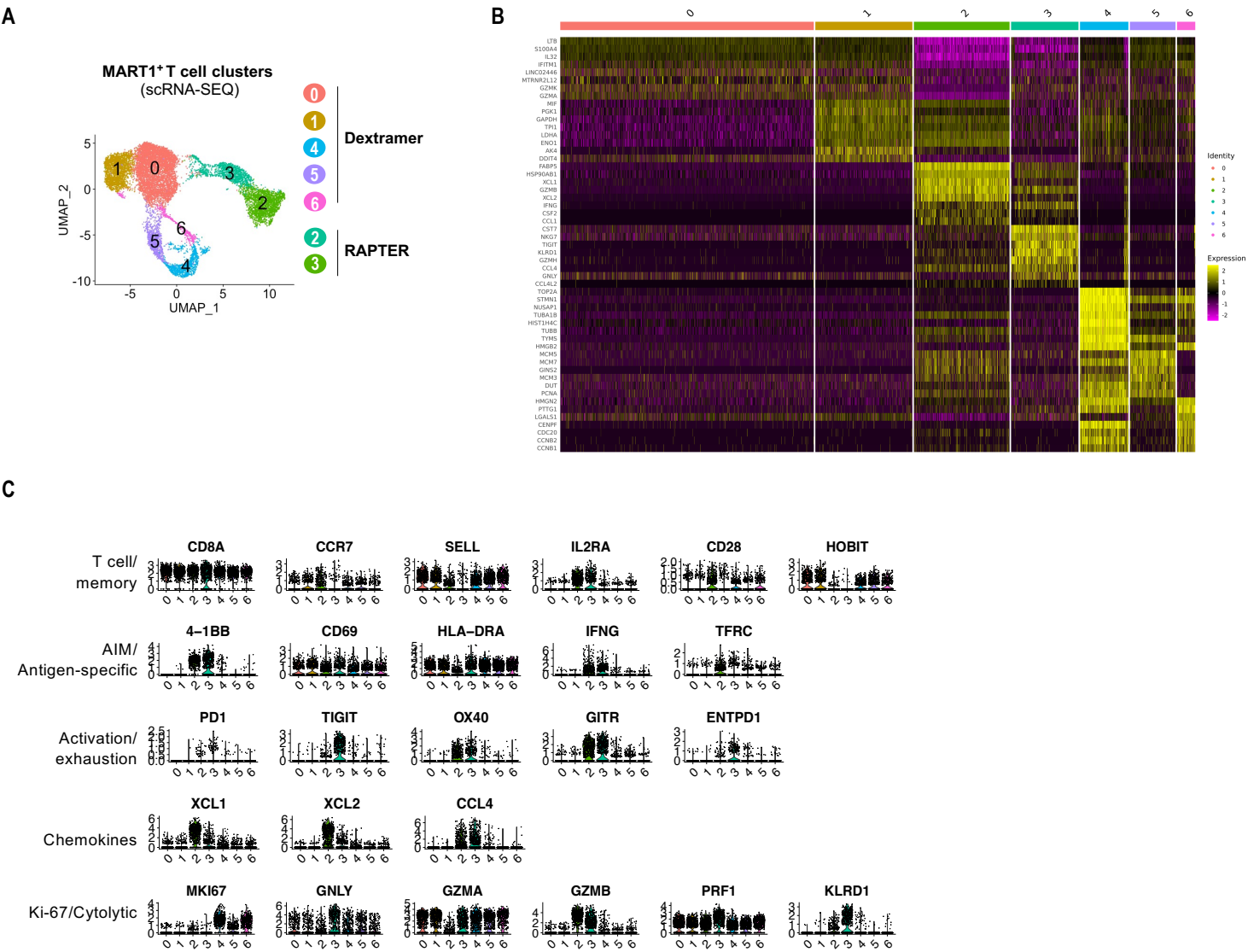
Supplementary Figure 7: scRNA-SEQ analysis of RAPTER CD8⁺ T cells. Related to Figure 3. PBMC from HD1 were cultured for 7 days with 5 viral peptides to expand the number of epitope-specific memory T cells. Expanded PBMC were then re-stimulated with cognate ligand for 24 hours and RAPTER was performed (Figure 3). Unexpanded CD8⁺ T cells were also analyzed by scRNA- and TCR-SEQ to enable pre- versus post-peptide expansion clone size analyses. **(A)** UMAP of total CD8⁺ and CD8⁺ CD137/4-1BB⁺ T cells enriched by FACS and analyzed by scRNA-SEQ. **(B)** Violin plots for number of genes expressed, unique molecular identifier (UMI) count, and fraction of mitochondrial RNA counts for each cluster. **(C)** Antigen-specific TCR clone size in pre- versus post-peptide expansion samples. **(D)** Feature maps showing selected expression of genes by scRNA-SEQ, and **(E)** scCITE-seq across clusters.

Supplementary Figure 8



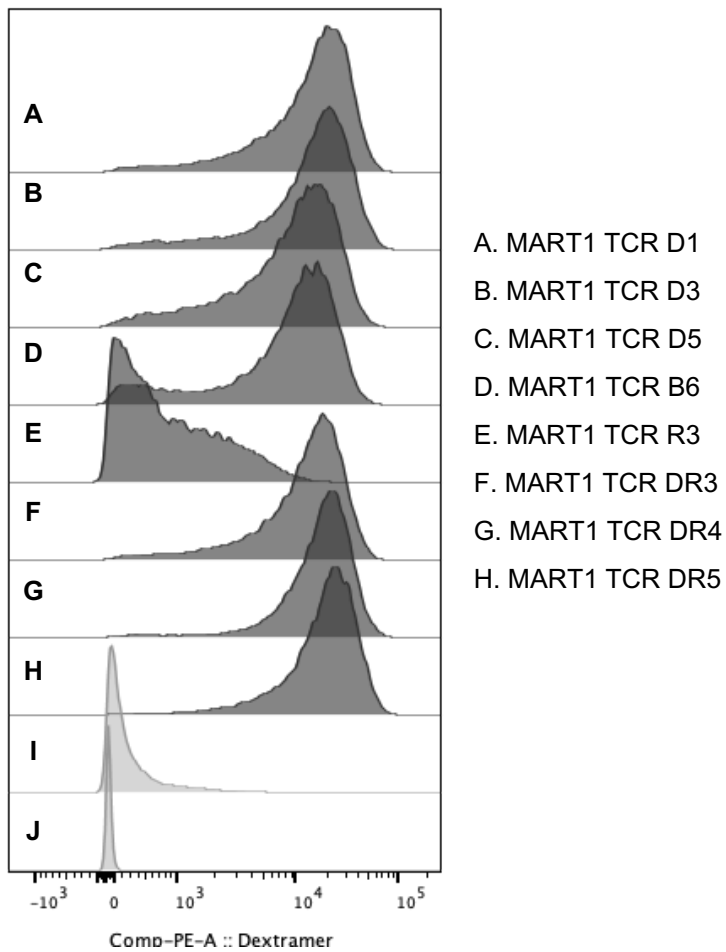
Supplementary Figure 8: Gating strategies to isolate antigen specific CD8⁺ T cells. Related to Figure 4. PBMC from an HLA-A*02:01⁺ healthy donor were cultured for 10 days with an HLA-A*02:01-restricted MART1 peptide (ELAGIGILTV) to expand epitope-specific T cells. MART1 peptide-expanded T cells were diluted with unexpanded autologous T cells in a 2-fold dilution series, then re-stimulated for 24 hr with cognate MART1 peptide. Each dilution point was uniquely hashed, then all samples were pooled (Figure 4B). (A) Upper-level FACS gating including the FMO gate for the PE channel. (B) CD137/4-1BB⁺ CD3⁺ T cells and (C) MART1 dextramer⁺ CD8⁺ T cells were isolated by FACS for scRNA/TCR-seq.

Supplementary Figure 9



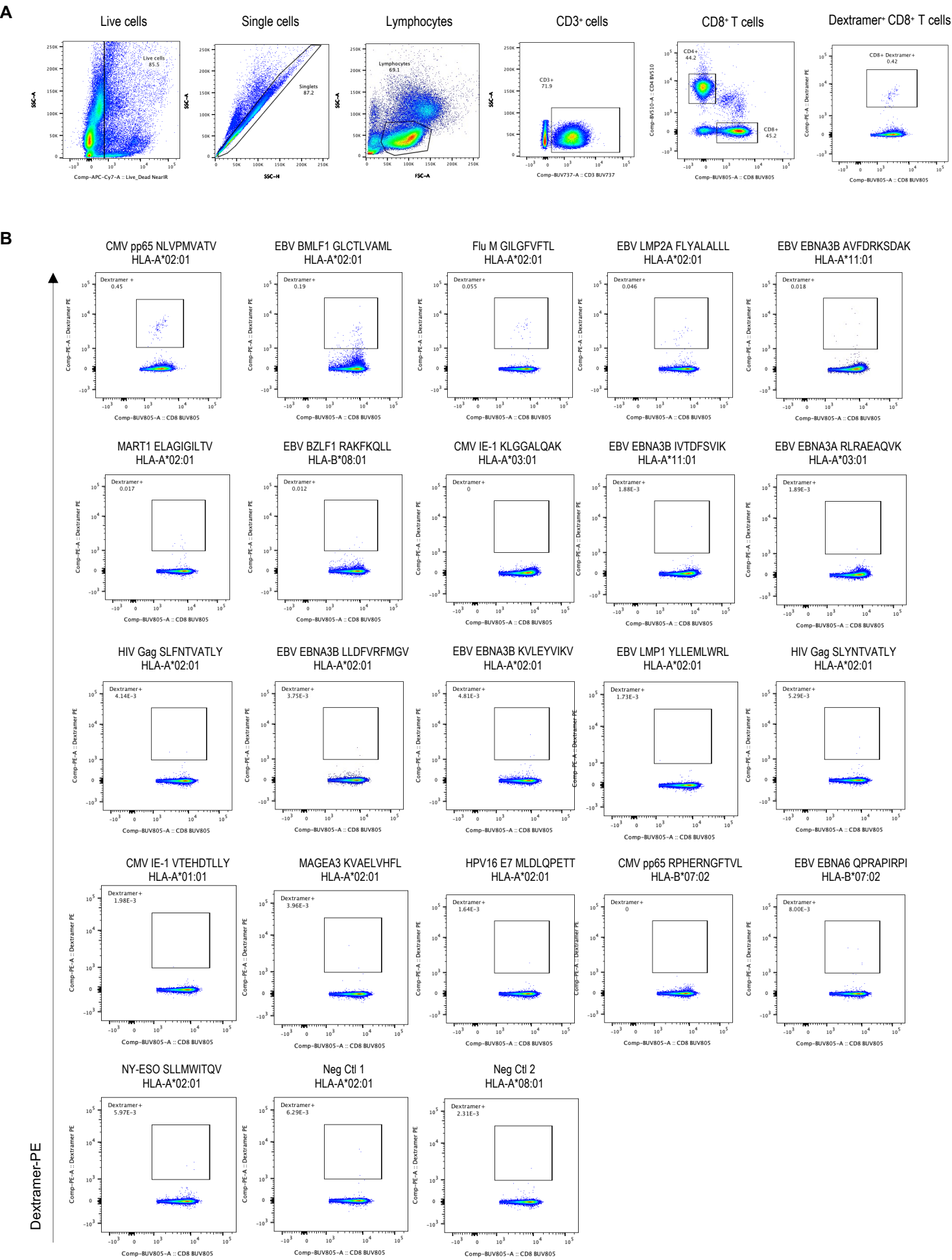
Supplementary Figure 9: MART1 RAPTER and dextramer sorted CD8⁺ T cell cluster maps and transcriptional phenotypes. Related to Figure 4. (A) Uniform Manifold Approximation and Projection (UMAP) plot of MART1⁺ dextramer-sorted and RAPTER-sorted CD8⁺ T cells. A total of 3,554 CD137/4-1BB⁺, MART1-reactive CD8⁺ T cells were captured and comprised 565 unique clones (red) and 9,930 of MART1 dextramer⁺ T cells were captured and comprised 587 unique clones (aqua). (B) Heat map showing the most differentially expressed genes in each cluster. (C) Violin plots of relevant phenotypic and functional T cell markers.

Supplementary Figure 10



Supplementary Figure 10. Flow cytometry plots of dextramer specificity of MART1 TCRs identified in the dextramer (A – D), RAPTER (E), or both assays (F – G). Related to Figure 4. TCR-engineered Jurkat cells expressing the MART1 ELAGIGILTV epitope reactive TCRs were stained with the MART1 ELAGIGILTV dextramer (A – H) or a negative control dextramer. Representative negative control dextramer stain (I) and FMO control (J) are shown.

Supplementary Figure 11



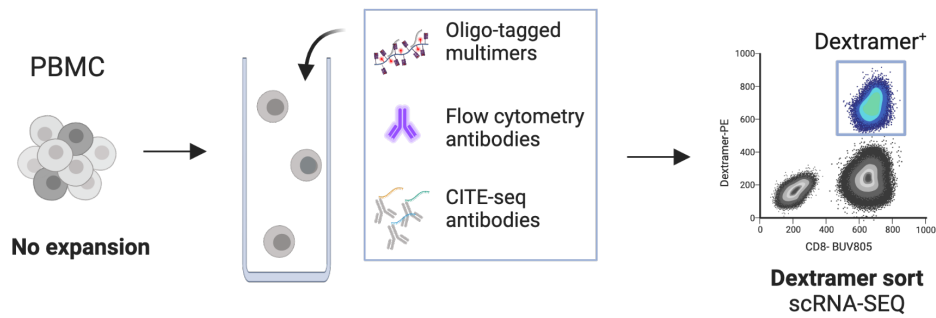
Supplementary Figure 11, con't

Supplementary Figure 11: Flow cytometry analyses of frequencies of antigen specific CD8⁺ T cells. Related to Figure 5. PBMCs from an HLA-A*02:01⁺ healthy donor were stained with individual PE-conjugated oligo-tagged dextramers (HLA haplotype matched and unmatched). Dextramer⁺ cells were gated to assess the abundance of antigen specific CD8⁺ T cells for each epitope. **(A)** Gating strategies for CD8⁺ dextramer⁺ T cells. **(B)**. Flow cytometry plots of CD8⁺ dextramer⁺ T cells associated with individual dextramer specificities. The percentage of CD8⁺ T cells that were stained positive for the indicated dextramer is shown in each plot.

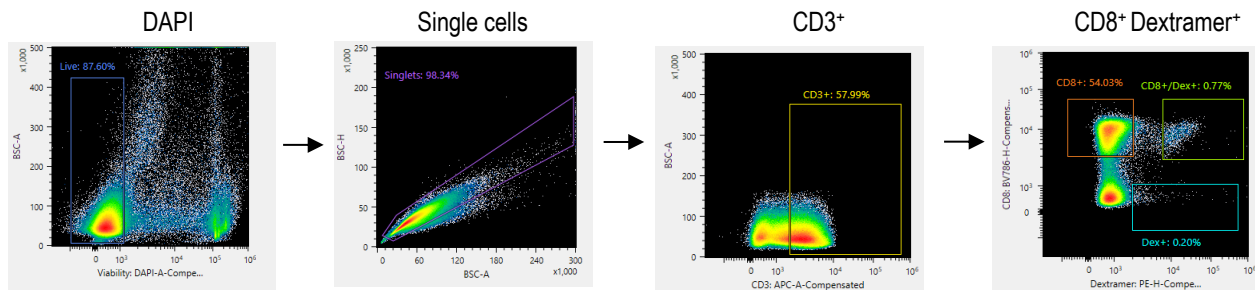
Supplementary Figure 12

A

Pooled dCODE dextramer workflow

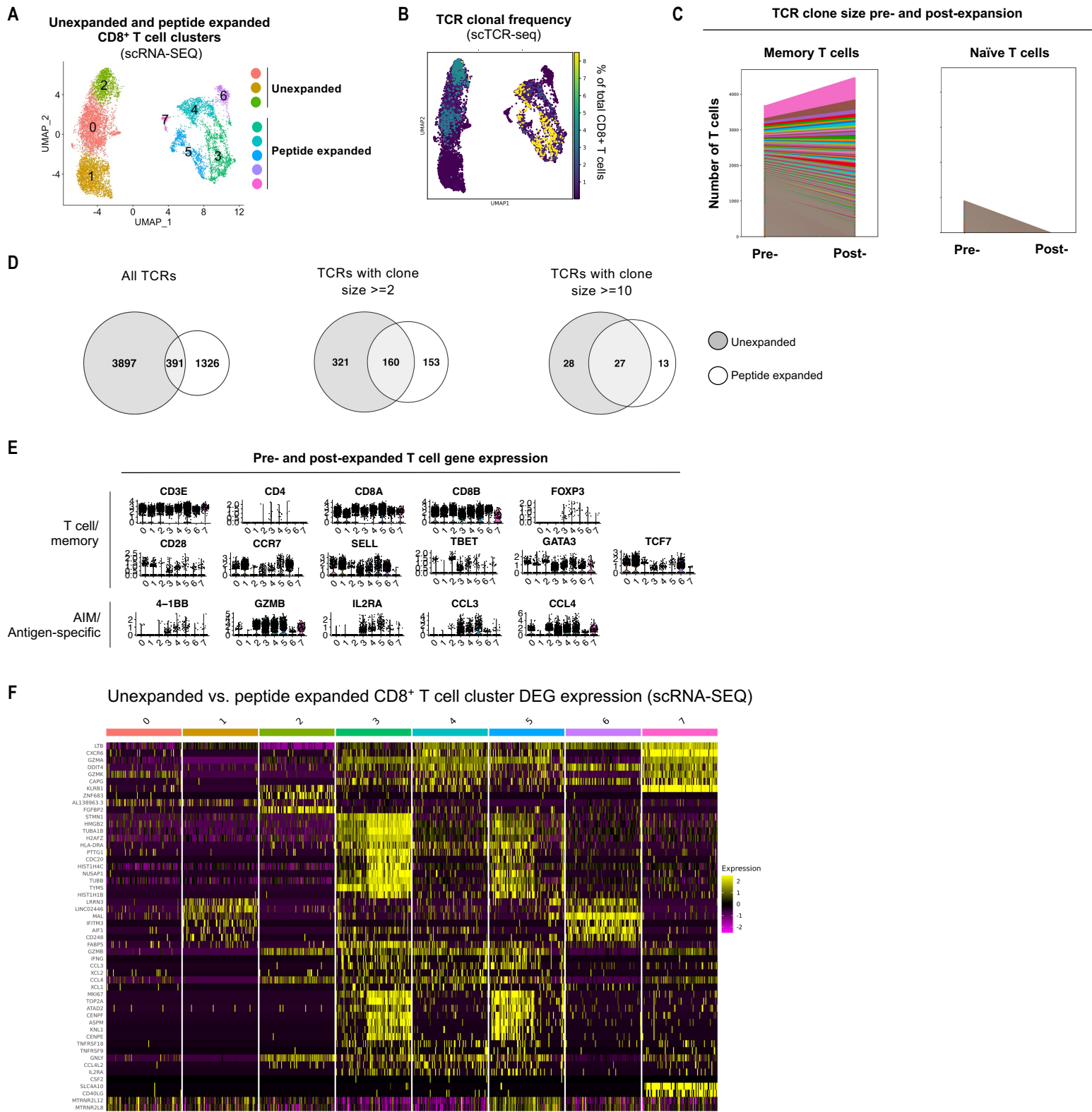


B



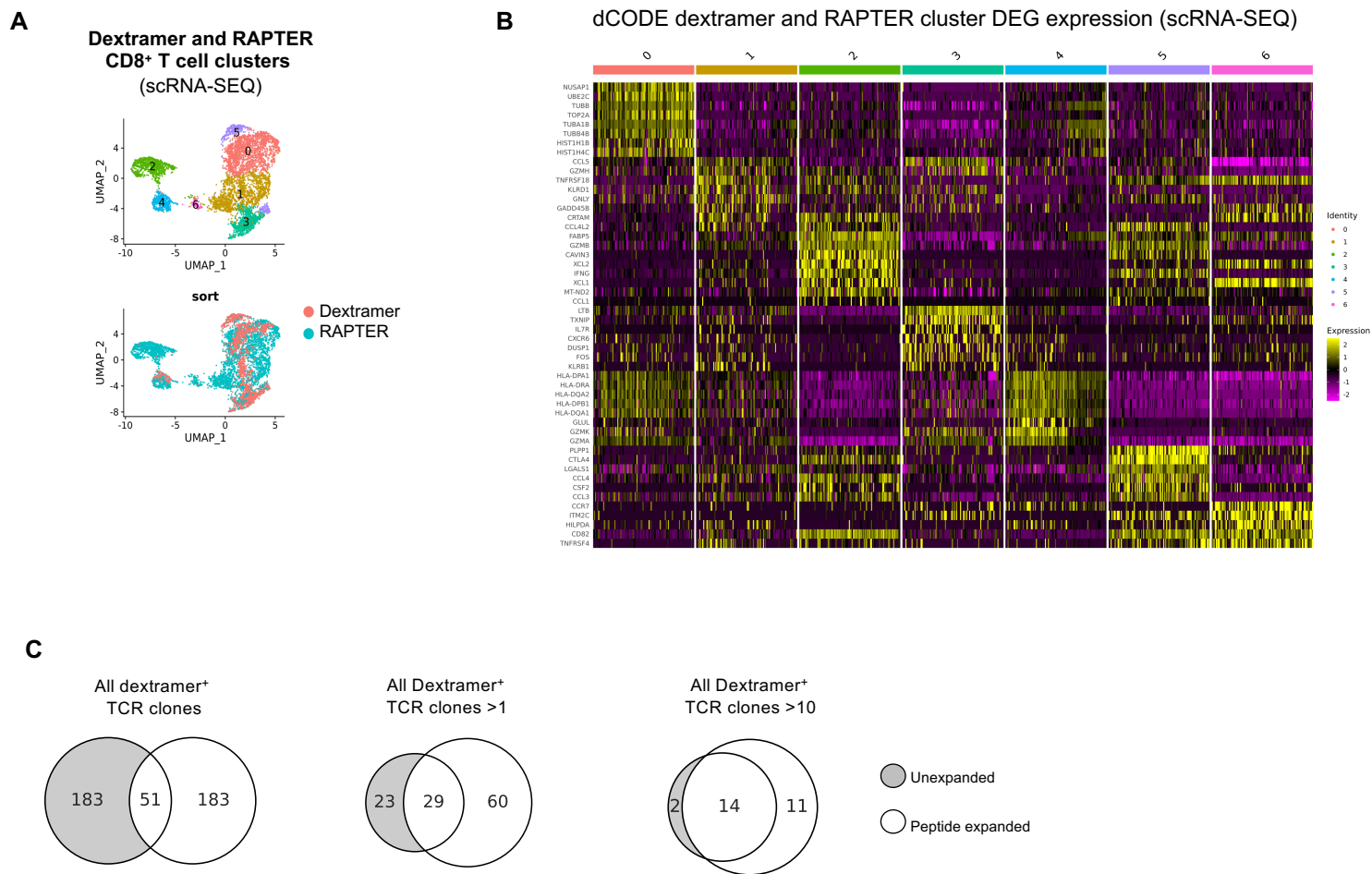
Supplementary Figure 12: Pooled oligo-tagged dCODE dextramer screen. Related to Figure 5. (A) Schematic summarizing the pooled oligo-tagged dextramer staining workflow. PBMC from a healthy donor were stained with a pool of 23 PE-conjugated oligo-tagged dCODE dextramers (Supplementary Table 1) to detect antigen-specific T cells. Additional flow cytometry and CITE-seq reagents to enable FACS and cell surface protein detection by CITE-seq were also applied. **(B)** Cells were sorted on a Sony MA900 FACS machine to isolate CD8⁺ dextramer⁺ T cells for scRNA-SEQ.

Supplementary Figure 13



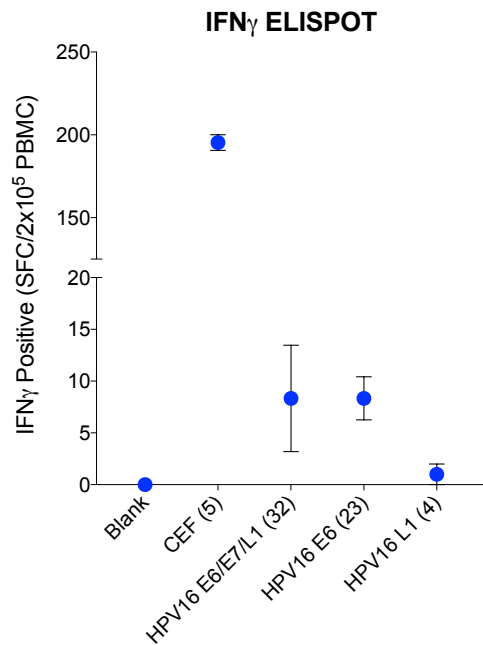
Supplementary Figure 13: Epitope-specific memory T cell expansion maintains clonal T cells. Related to Supplementary Methods. PBMC from an HLA-A*02:01⁺, A*29:02⁺, B*35:01⁺, and B*57:01⁺ healthy donor were expanded for 7 days with HLA haplotyped matched and unmatched peptides (Supplementary Table 2) to expand epitope-specific T cells. **(A)** UMAP plot of unexpanded (n=6592 cells) and peptide expanded (n=3453 cells) total CD8⁺ T cells resolved into 7 distinct UMAP clusters using scRNA-SEQ data. **(B)** Feature maps representing the clonal frequencies of total unexpanded and peptide expanded TCR sequences for paired TCR a/b chains. **(C)** Stacked plots representing individual paired TCR a/b chain sequences in the pre-expansion versus peptide expanded CD8⁺ T cells from the memory (left panel) and naïve (right panel) T cell compartments. T cell memory phenotype was determined using RNA- and CITE-seq data. **(D)** Venn diagrams representing paired TCR a/b chain sequence overlap between pre- (gray) and post-expansion (white) CD8⁺ T cells (All clones, clones >=2 cells, and clones >=10 cells). **(E)** Violin plots of relevant phenotypic and functional T cell markers for the unexpanded and expanded CD8⁺ T cells in (A). **(F)** Heat map showing the most differentially expressed genes in unexpanded versus peptide expanded T cell clusters.

Supplementary Figure 14



Supplementary Figure 14: Peptide expanded dCODE dexramer and RAPTER⁺ CD8⁺ T cell differential gene expression (DEG) analysis from scRNA-SEQ data. Related to Figure 5. Total CD8⁺ T cells from an HLA-A*02:01⁺ healthy donor were directly isolated by FACS and evaluated by scRNA-SEQ or expanded for 7 days with specific peptides and then isolated by FACS and evaluated by scRNA-SEQ. **(A)** UMAP displaying CD8⁺ T cell clusters from the dCODE dexramer and RAPTER assays. **(B)** Heat map showing the most differentially expressed genes in the dCODE dexramer and RAPTER⁺ T cell clusters. **(C)** Venn diagrams representing paired TCR a/b chain sequence overlap between dexramer⁺ CD8⁺ T cells pre- (gray) and post-expansion (white) for all TCR clones (left), clone sizes >1 (middle) and >10 (right).

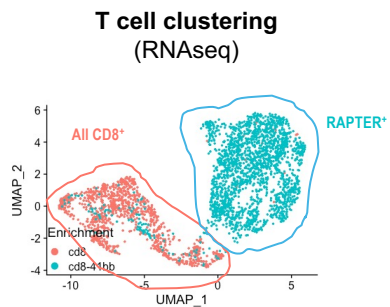
Supplementary Figure 15



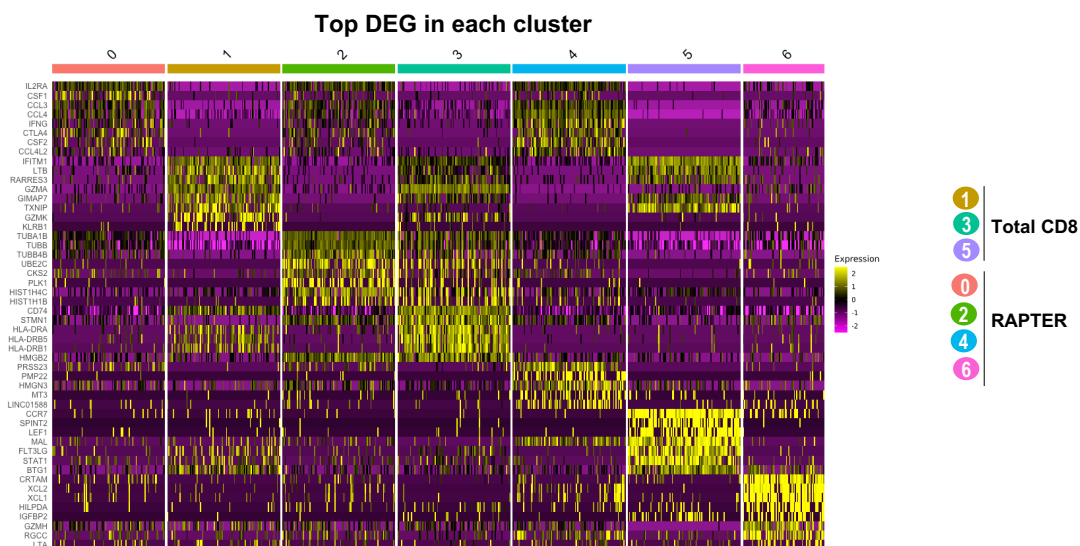
Supplementary Figure 15: HPV antigen-specific T cell reactivity in PBMC from an HPV⁺ cervical cancer patient. Related to Figure 6. Cryopreserved PBMC from an HLA-A*02:01⁺ cervical cancer patient were tested in an IFN γ ELISpot assay after resting overnight. The number of IFN γ ⁺ spots per 2x10⁵ PBMC indicated was obtained following an overnight stimulation with pools of 15-mer overlapping peptides derived from the HPV proteins E6, E7 and L1, or with short peptides of common viral antigens of CMV, EBV and influenza M (CEF).

Supplementary Figure 16

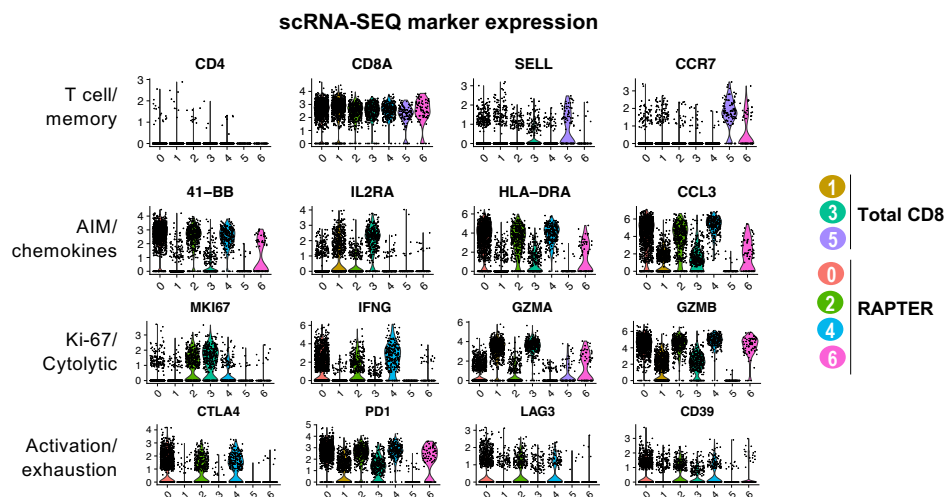
A



B



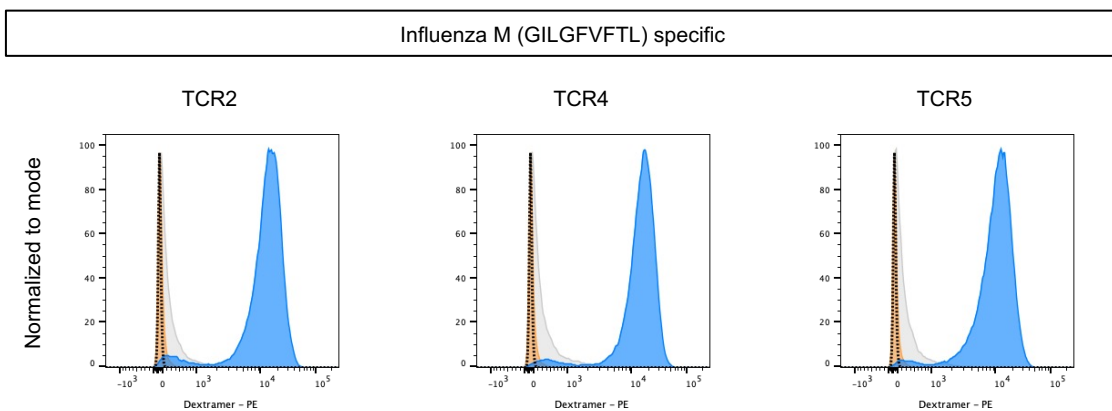
C



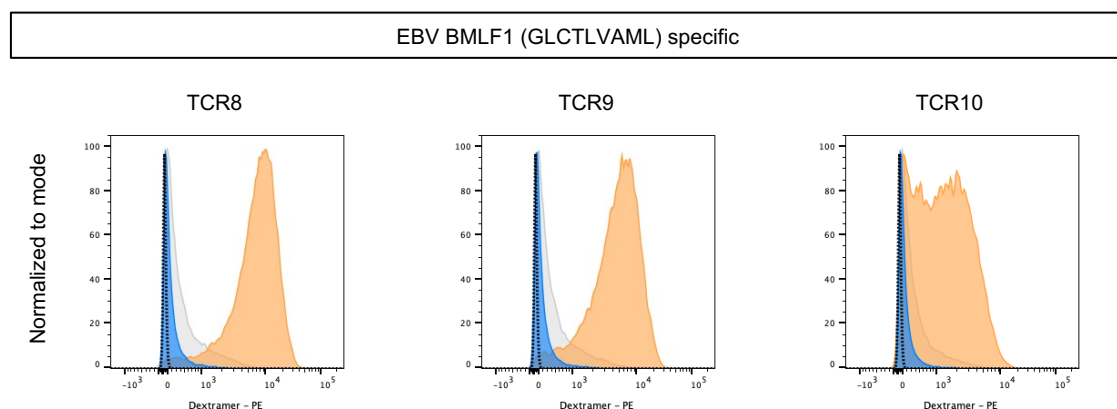
Supplementary Figure 16: Differential gene expression (DEG) of total CD8⁺ T cell and RAPTER⁺ clusters. Related to Figure 6. (A) UMAP of total sorted CD8⁺ T cells and CD137/4-1BB⁺ T cells from the RAPTER assay in Figure 6. (B) Heat map showing to 10 differentially expressed genes for each T cell cluster in (A). (C) Violin plots of relevant phenotypic and functional T cell markers.

Supplementary Figure 17

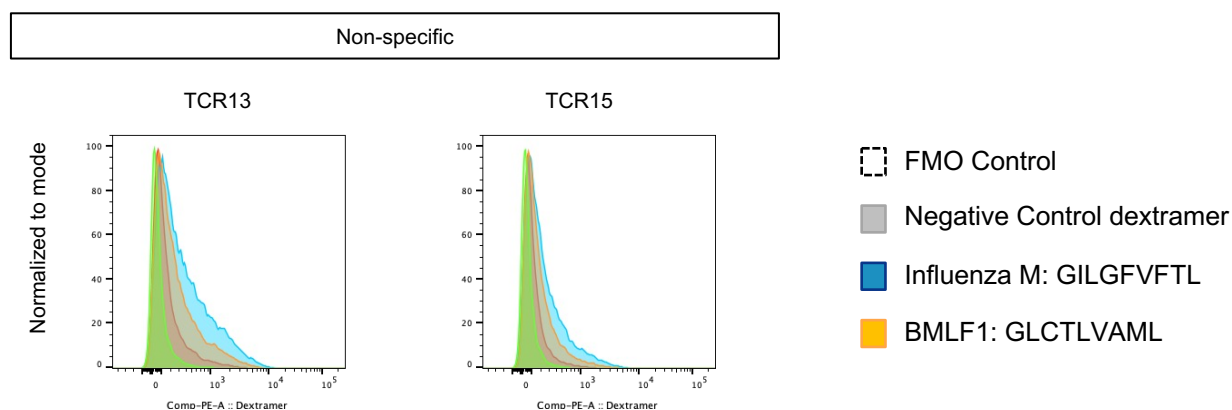
A



B



C



Supplementary Figure 17. Flow cytometry plots of dextramer specificity of TCRs identified in RAPTER assay. Related to Figure 7. TCR-engineered Jurkat cells expressing the (A) Influenza M (GILGFVFTL) epitope-reactive TCRs (TCR2, TCR4 and TCR6), (B) EBV BMLF1 (GLCTLVAML) epitope-reactive TCRs (TCR8, TCR9 and TCR10) or (C) two non-specific TCRs (TCR13 and TCR15) were stained with the indicated dextrans.