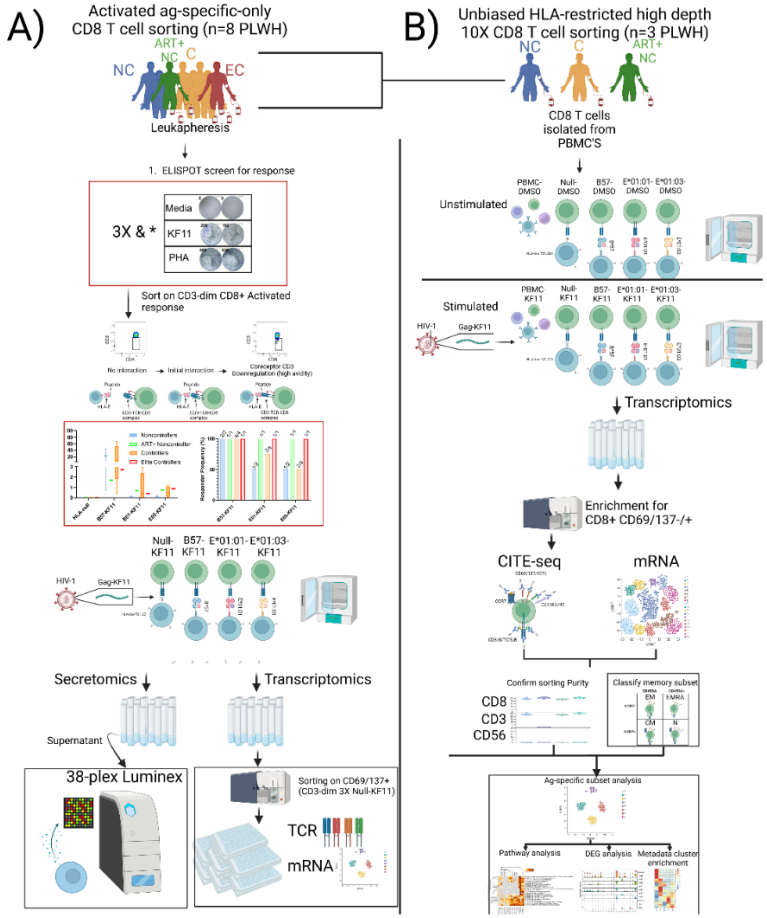
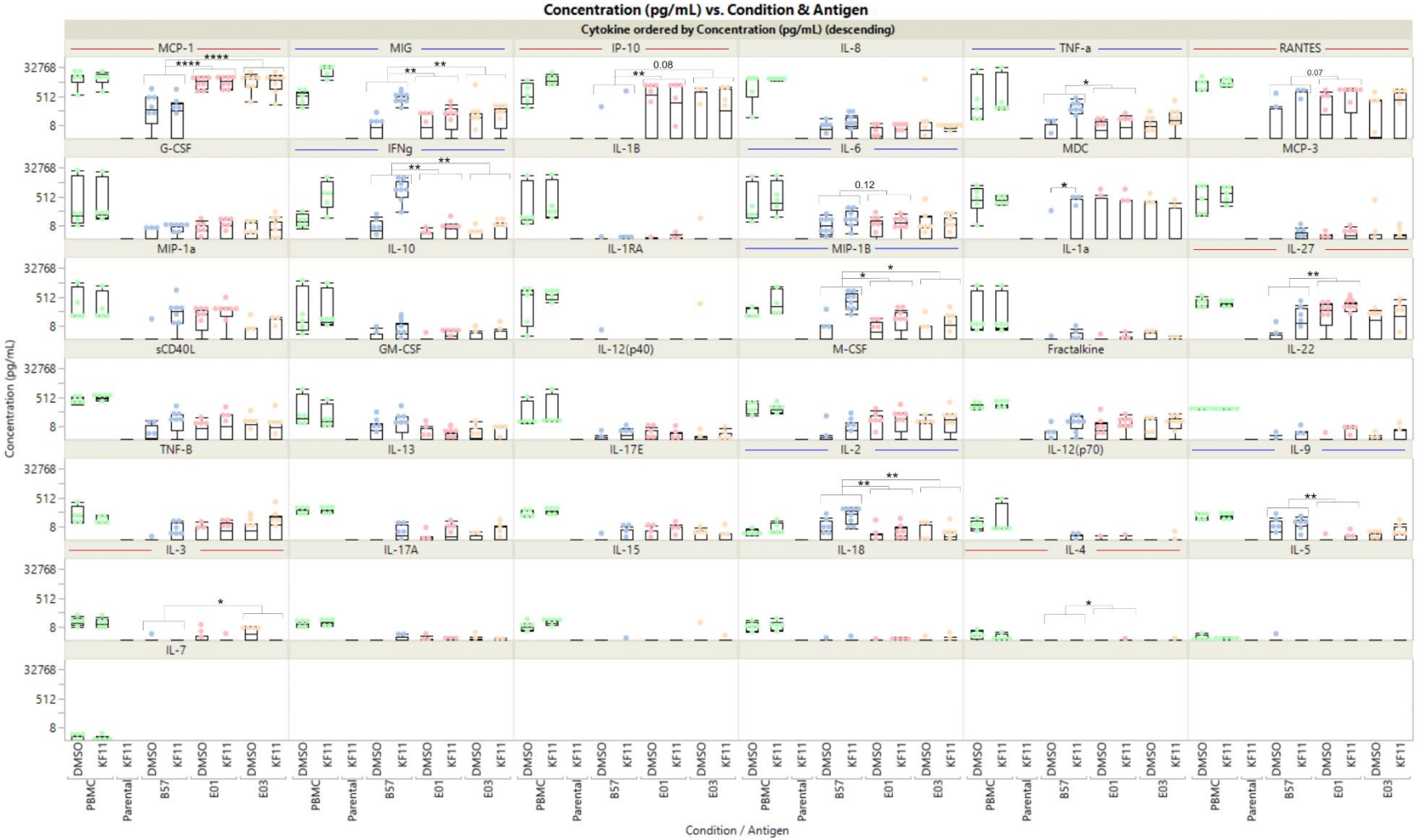
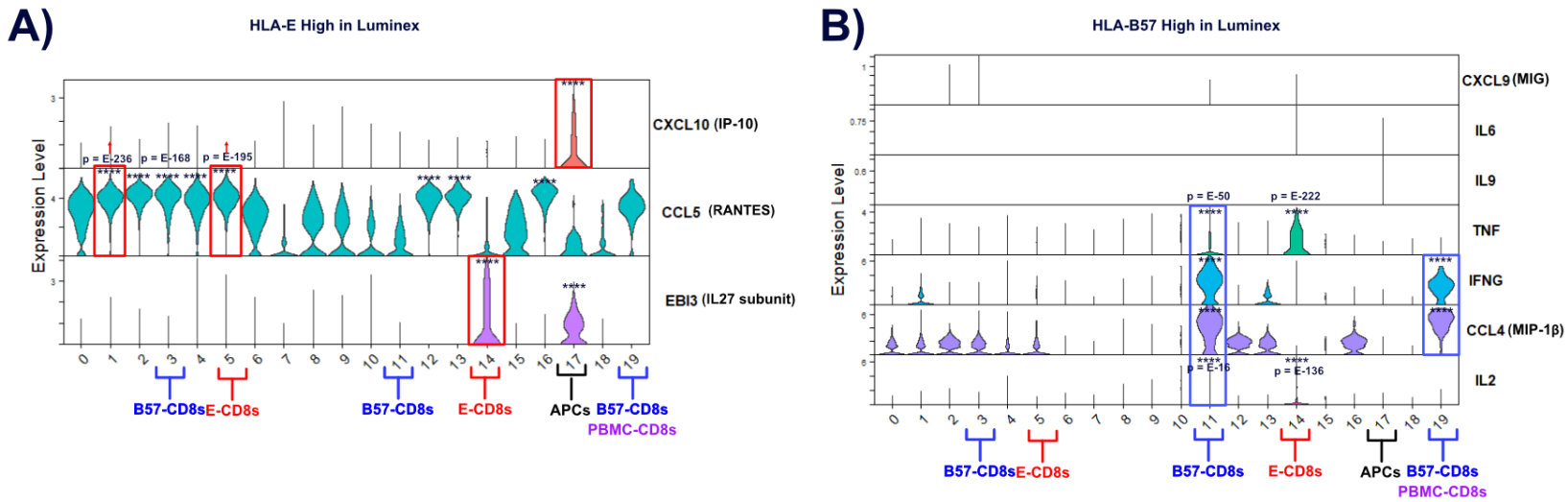


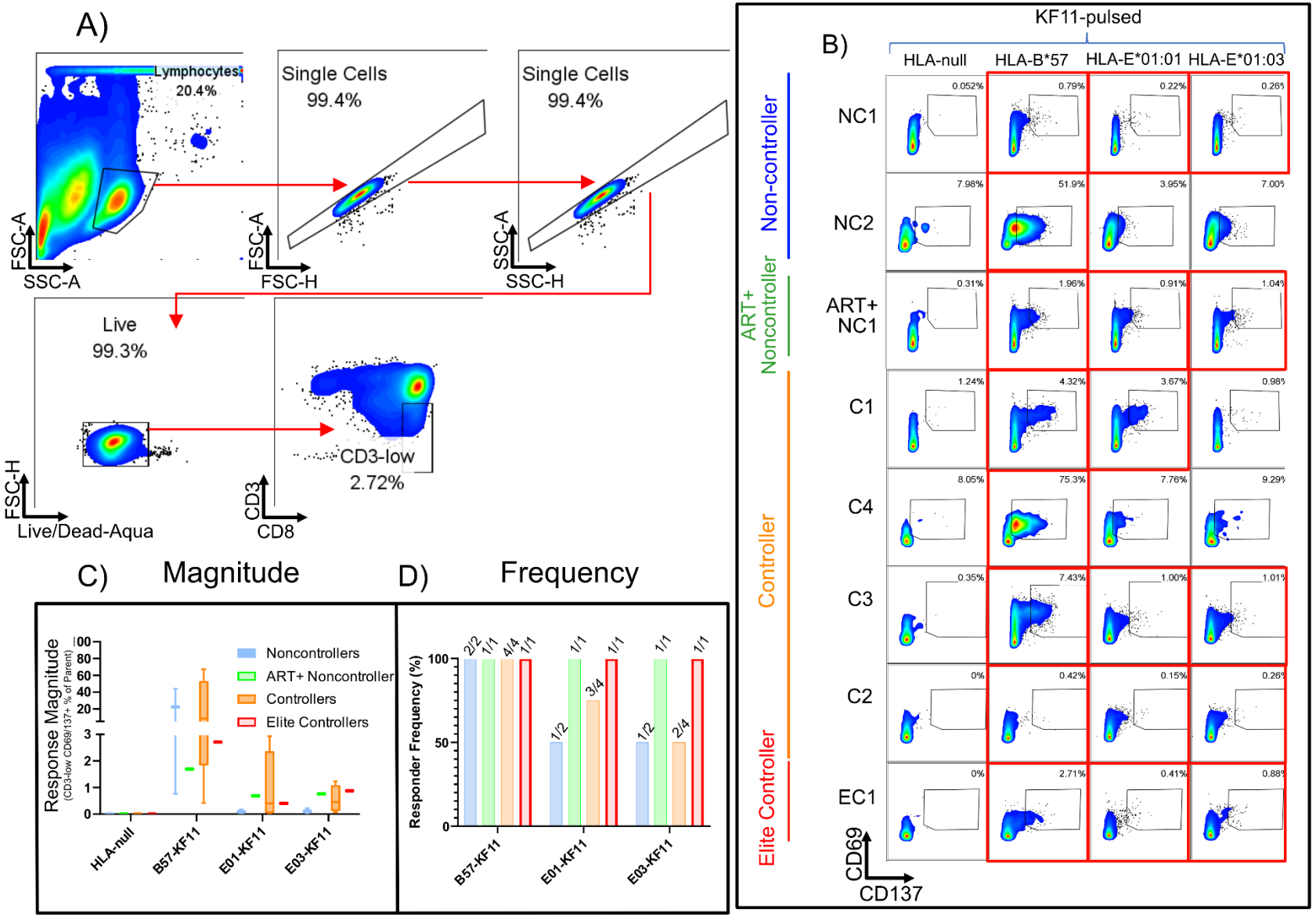
Experimental and analysis workflow graphical abstract: Graphical abstract generated through Biorender illustrating the 2 sets of experiments used to obtain the data in this study. A set of 8 PLW leukopheresis donor samples (2 NC, 1 ART+ NC, 4 C, and 1 EC) were used for all experiments. (Left side) Initial targeted antigen-specific scRNA/TCR-seq sorting and 38-plex Luminex strategy. (Right side) Follow-up higher-depth and broader/unbiased condition sorting strategy for 10X. Both unstimulated and stimulated (KF11 unpulsed or pulsed) conditions for all previous HLA's (null, B57, E01, E03) were utilized alongside PBMC's. Both enriched activated and unactivated CD8s were sorted to include resting memory repertoire and achieve sufficient cell number. These cells were multiplexed by patient and subjected to 10X multi-omic sequencing for scTCR, scRNA, and scCITE-seq expression data acquisition. The TCR data was analyzed through a novel Chi-squared approach to determine which TCR α/β paired families and clonotypes significantly increased in every combination of metadata from unstimulated to stimulated, and these significantly increasing pairs were then interrogated through metaclonotype clustering by dissimilarity distance using TCRDist3, clusters which were then compared to those obtained in the targeted ag-specific analysis through the previous strategy. First, cells were assayed for cell marker surface expression through CITE-seq to ensure purity of CD56- CD8+ T cell sorting. Cells were then subset by those whose TCR pairs matched the ones found to significantly increase in some condition/combination of conditions and classified into memory subsets based on their surface expression of CD45RA and internal expression of CCR7 and these clusters were interrogated for differential gene expression, gene ontology, and metadata-driven analysis.



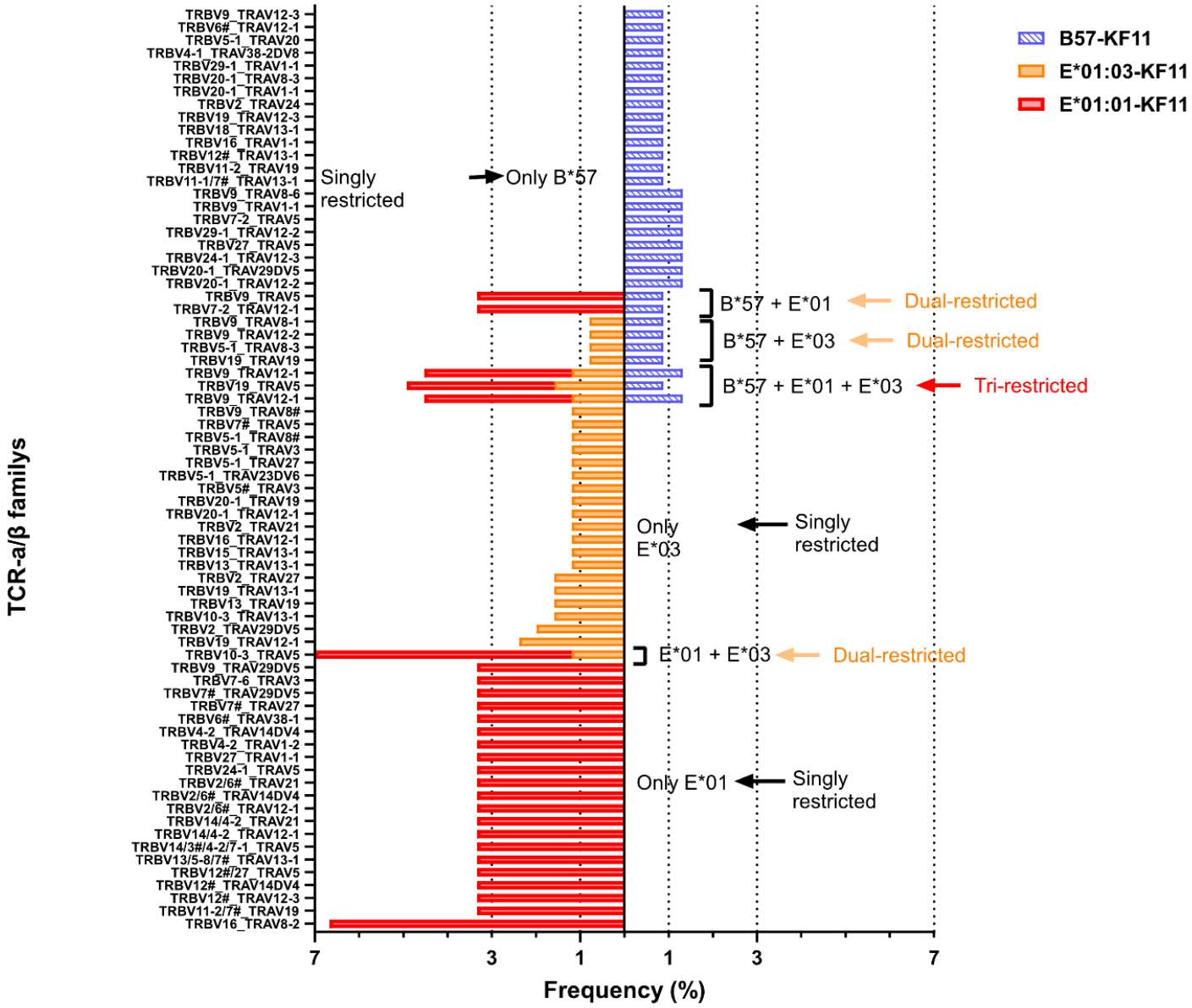
Supplemental Figure 2: Muliplex-ELISA Cytokine and chemokine Response Summary. Summary of all 8 HLA-class I restricted responses to either no peptide or KF11 across all responders, cytokines separated by boxes. All concentrations for each donor in each condition are normalized to Parental-cell line KF11 response (41A3.CD4 “null” cell line pulsed with KF11 and co-cultured with CD8s from all 8 donors) individually except for PBMC response. Higher HLA-B57-restricted or -E*01:01 response is represented by a blue or red line respectively.



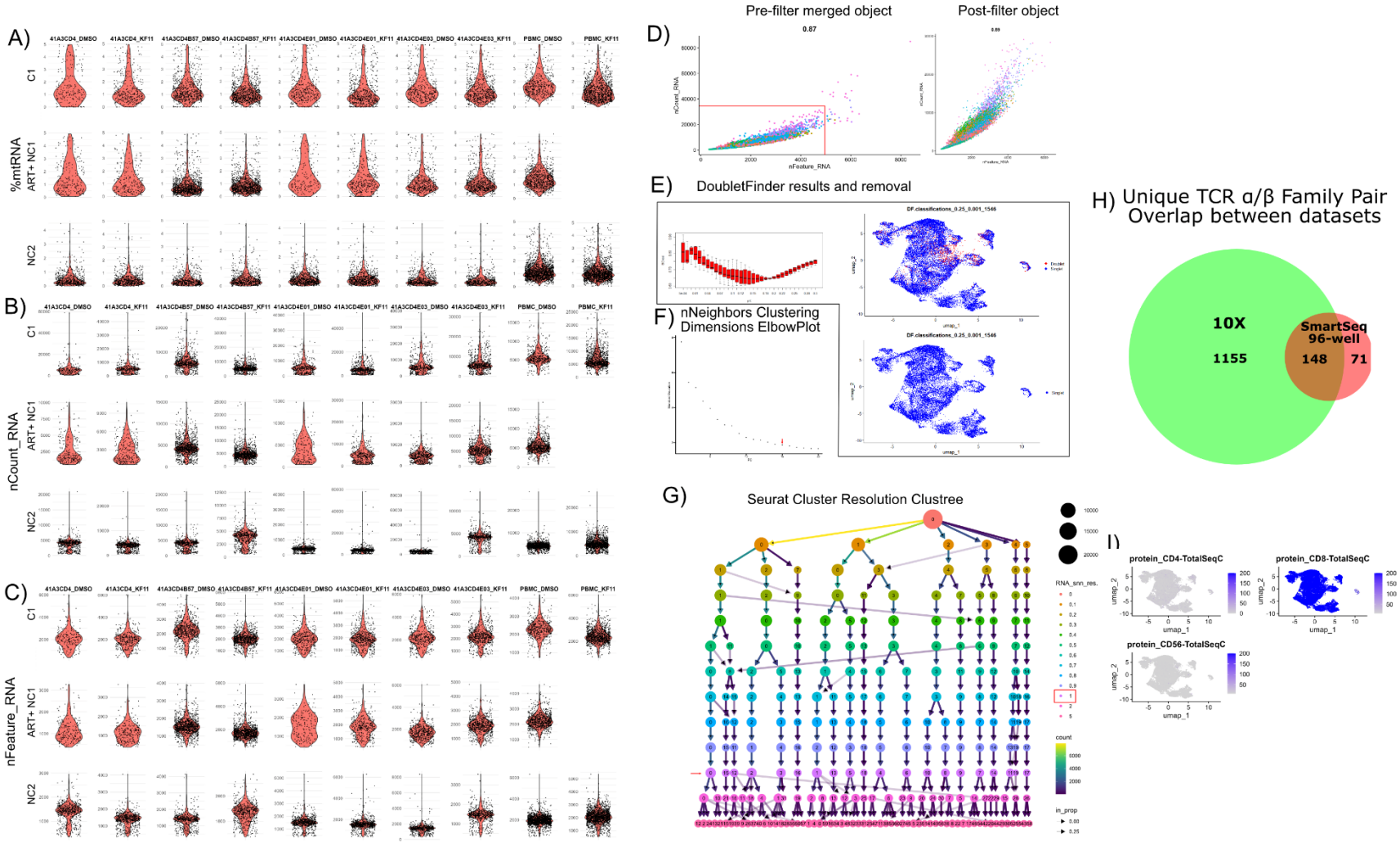
Supplemental Figure 3: Several secreted Cytokines and chemokines Confirmed to be Expressed within Appropriate HLA-restricted CD8s. Violin plot of RNA expression for those cytokines identified through high-parameter Luminex in HLA-restricted co-culture within 10X Seurat clusters. **A)** HLA-E- or **B)** -B57-restricted cytokines which are significantly increasing compared to all other clusters by MAST are boxed in red or blue respectively. Relevant clusters are marked as E-CD8s or B57-CD8s below depending on relative number of cells for HLA-restricted metadata in those clusters. Exact p values are given when given cytokine is positive in more than 1 cluster and in both an —CD8 and B57-CD8 cluster for comparison. All p values given are FDR-corrected (p = 0.05, 0.01, 0.001, 0.0001 represented as *, **, ***, **** respectively) .



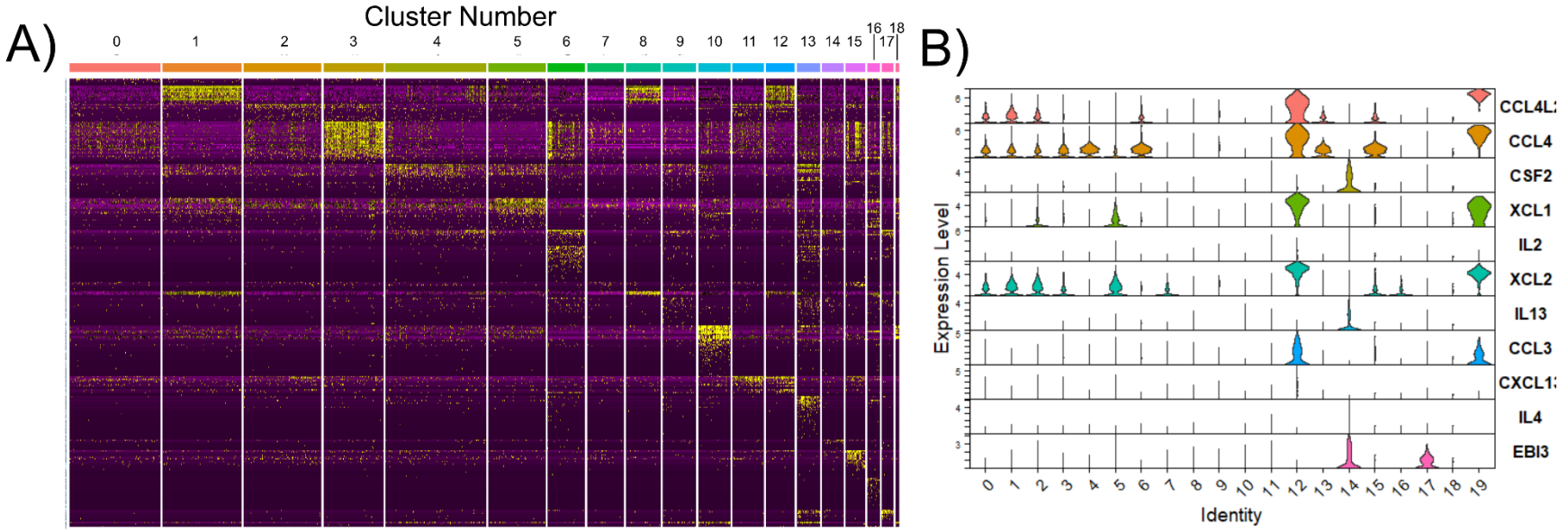
Supplemental Figure 4: Gating strategy and response magnitude for CD3-low HLA-class I restricted responses. A) Gating strategy for identifying CD3-low CD8+ T cells. **B)** Response magnitude of dually activated (CD69+ CD137+) CD3-low high avidity HLA-restricted CD8+ T cells to KF11 in each HLA group. **C)** Graph showing magnitude of CD69+ CD137+ response shown in (B). **D)** Bar graph showing response frequency among noncontrollers, ART-suppressed noncontroller, controllers, and elite controller in cohort relative to HLA-B*57:01-, E*01:01-, and E*01:03-restricted KF11 from (B).



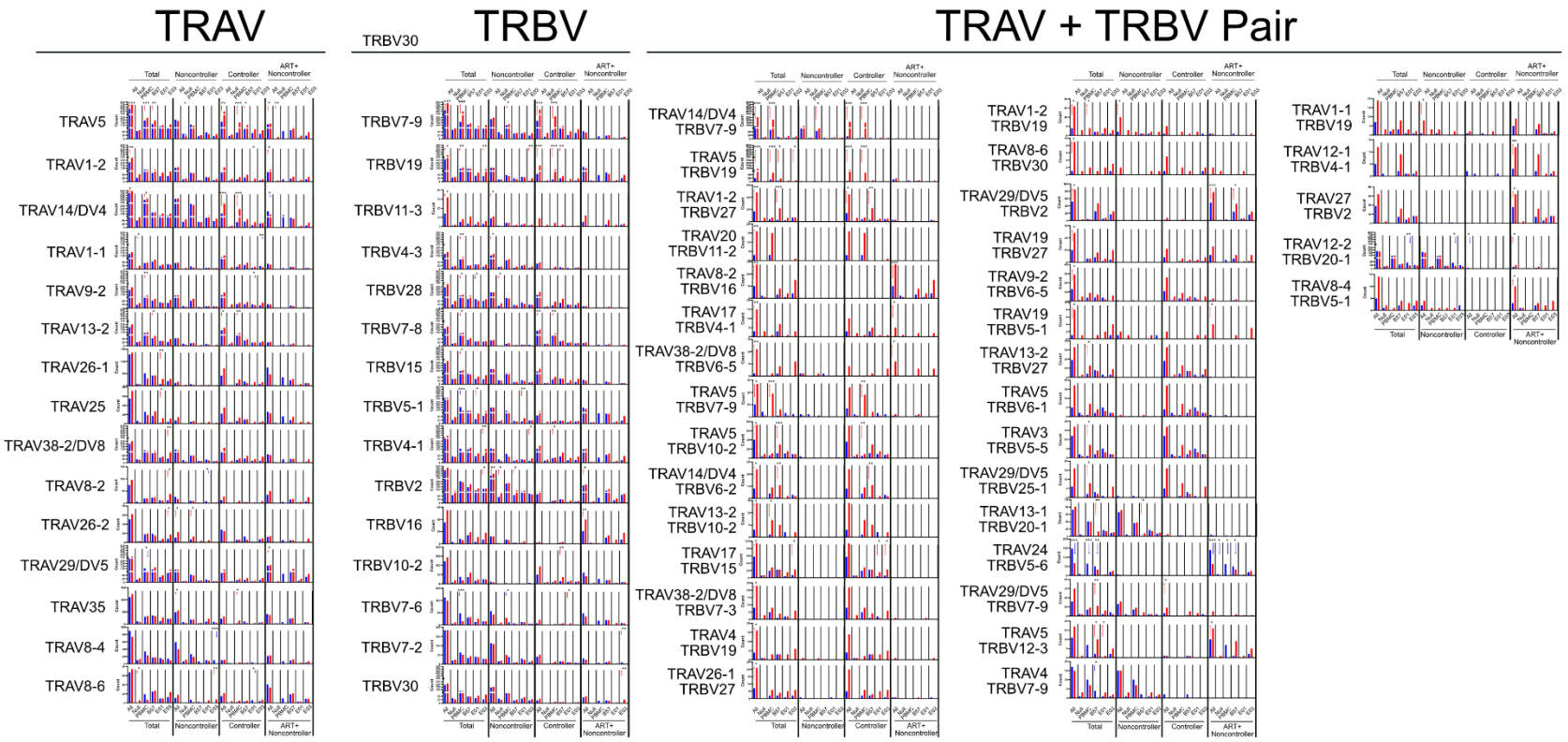
Supplemental Figure 5: Pyramid plot of TCR cross-restriction in one PWH. Pyramid plot in same style as Figure 2A, but for a single PWH to show cross-restriction at the donor level in activated CD8s.



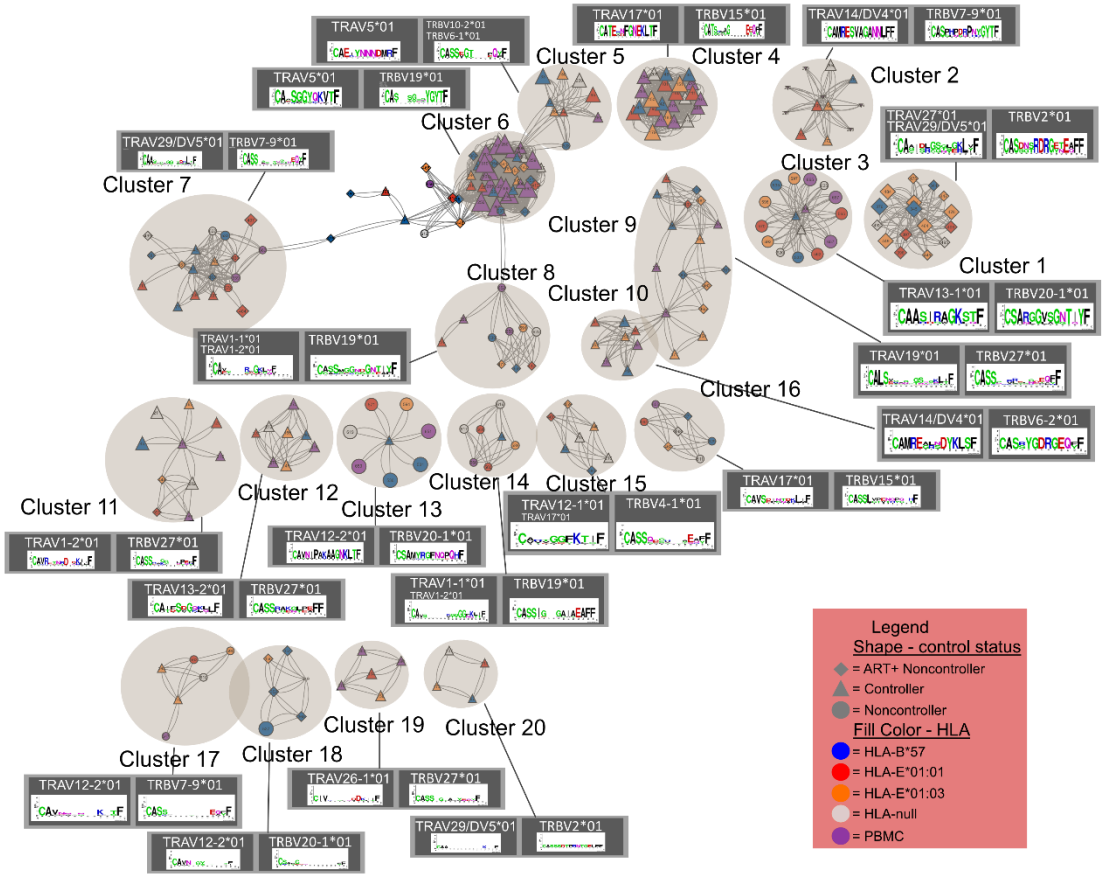
Supplemental Figure 6: 10X genomics QC metrics. Violin plots by donor (rows) and condition (columns) for post-filtered **A)** %miRNA, **B)** nCount_RNA, and **C)** nFeature_RNA values of merged dataset are shown. **D)** Scatterplot showing nCount_RNA to nFeature_RNA pre- and post-98th percentile filtering in an attempt to remove outliers from dataset. Additional **E)** DoubletFinder filtering used to more specifically identify and remove potential doublets. Left boxplot of BCReal vs pK simulation used to find optimal pK setting in package to identify potential doublets within dimplot shown at upper right. Lower-right dimplot represents post-filtered merged dataset. **F)** Elbowplot was used to determine optimal number of dimensions on which to plot the UMAP. **G)** Clustree used to determine optimal resolution which best identified distinct clusters of transcriptionally distinct CD8s that were not "overclustered", or do not have substantial cross-assignment to non-existent distinct clusters, as represented by non-opaque arrows. **H)** Overlap of unique TCR α/β family pairs observed in antigen-specific CD3-low high avidity scTCR-seq data and 10X scTCR-seq data.



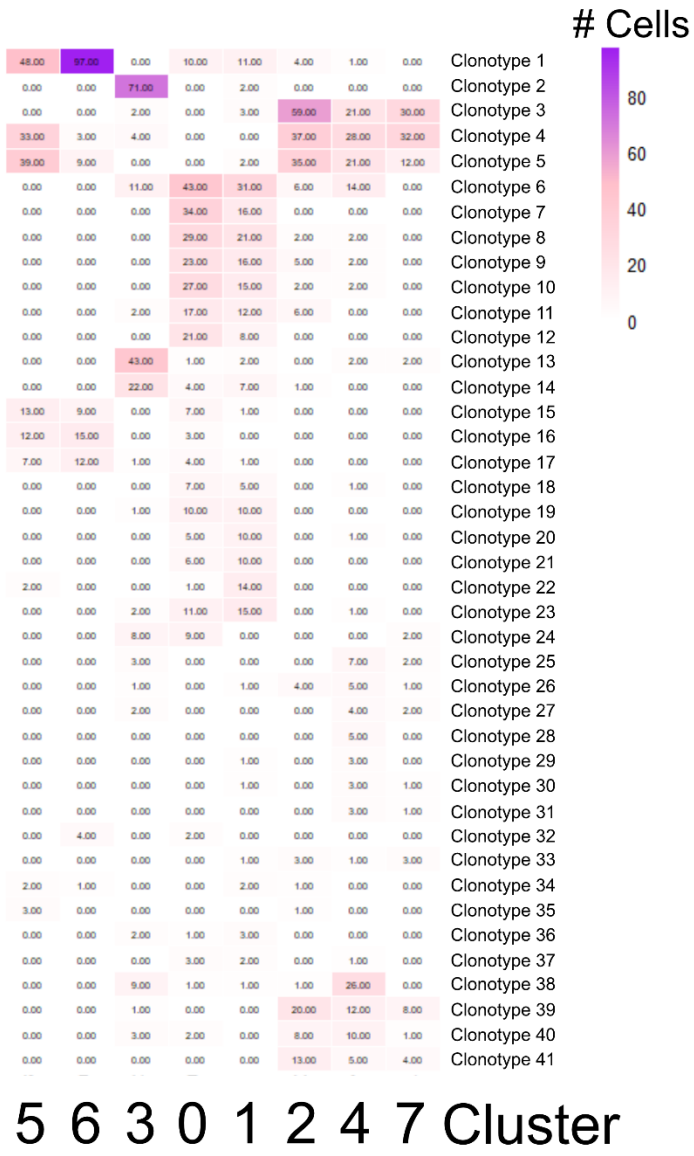
Supplemental Figure 7: Clusters have distinct transcriptional profiles. A) Dim Heatmap of top 50 markers identified per cluster by MAST, unlabeled. **B)** Violinplot of top variable cytokines across dataset separated by cluster.



Supplemental Figure 8: Stacked bar graph summary of significantly increasing α, β, or α/β paired families. Bar graphs of significantly increasing A) α, B) β, or C) α/β paired families showing count of each family or paired family as blue for unstimulated or red for stimulated, separated by first control status (Total, noncontroller, controller, ART-suppressed noncontroller) and sub-set by HLA status within those control statuses (All, Null, PBMC, B57, E01, and E03). Red up arrow and blue down arrow indicates increasing or decreasing family within the indicated metadata combination. Significance determined through novel Chi-squared comparison of count of cells between unstimulated and stimulated compared to counts of indicated family or family pair between unstimulated and stimulated metadata combination. ART-supprssed noncontroller PBMC data not available due to sorting error (1 out of 30 conditions), and so no significance applicable in that condition. Significance of increase or decrease indicated above (*, **, ***, **** mean $p < 0.05, 0.01, 0.001, 0.0001$ respectively).



Supplemental Figure 9: Antigen-specific clonotypes are multiply HLA-I restricted. TCRdist3 distance matrix-based clustering of KF11 stimulated significantly increasing α/β pair family clonotypes above proportion and TDU cutoffs. With increased cell number and therefore cluster number, clusters are labeled for reference in text. TCR families included in each cluster are also labeled by cluster alongside a Logo plot detailing the CDR3 sequences therein. PBMC, Null-, HLA-B*57:01-, E*01:01-, and E*01:03-restricted clonotypes are colored in purple, gray, blue, red, and orange respectively. Clonotypes identified in noncontrollers are represented as circles, while those in controllers and the ART-suppressed noncontroller are represented as triangles and diamonds respectively.



Supplemental Figure 10: Antigen-specific HLA-restricted clonotypes are transcriptionally distinct from one another. Heatmap of number cells with paired TCR clonotype sequence metadata (unique clonotype paired sequence 1, 2, 3, etc.) found within each Seurat cluster within the ag-specific subset of 10X data.

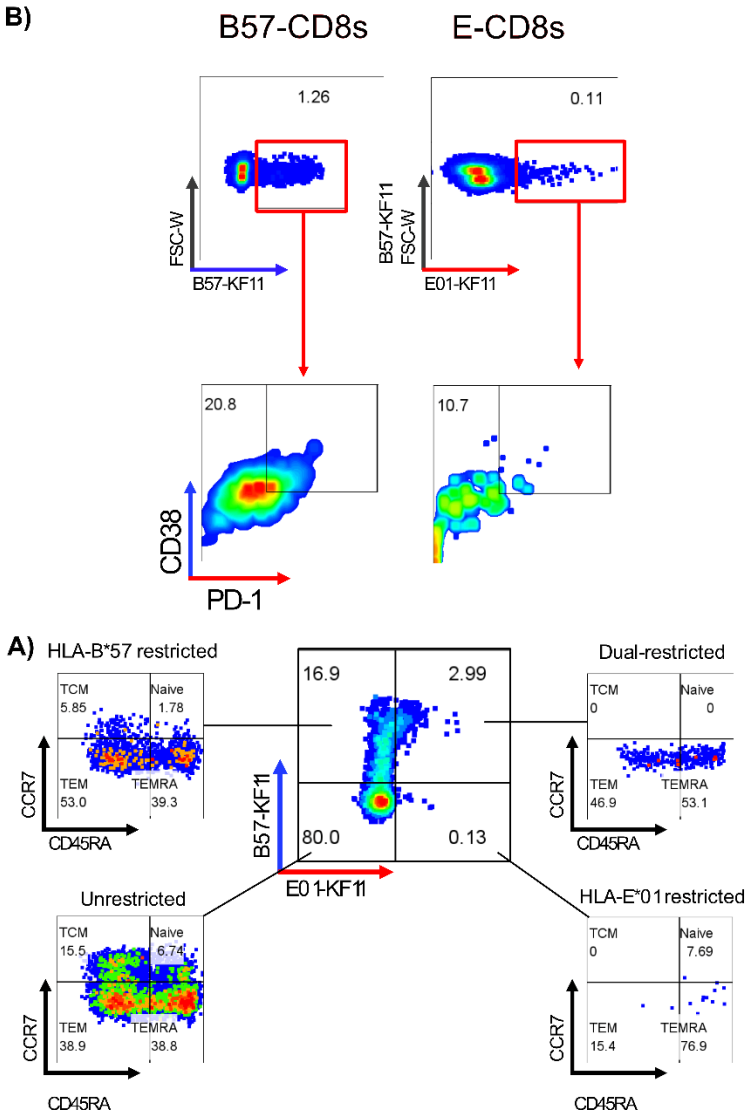


Figure S11: Gating for Dextramer staining based examination of CD8 Memory and Exhaustion. A) Representative gating for CD38+ PD1+ exhaustion within all B57-CD8s or E-CD8s within cohort. **B)** Memory state of all 4 quadrants of B57+/- and E01-KF11 +/- dextramer PWH EC2 αβ. Representative graph of memory state (CD45RA vs CCR7) within each dextramer-positive quadrant shown. Same gating used to determine memory state across rest of cohort.

Supplemental Table 1: Cohort demographics. Table for all donor samples used in study. Control status determined as stated in text for noncontrollers (NC), controllers (C), and elite controllers (EC) having a plasma viral load >2000 , <2000, or <200 cp/mL respectively. "KF11 ELISPOT IFNγ SFU/1e6 cells" shows ELISPOT IFNγ response magnitude of indicated donor PBMCs to KF11 peptide if tested based on sample availability.

Status	Alternate ID	Viral Load (cp/mL)	HLA-A1	HLA-A2	HLA-B1	HLA-B2	KF11 ELISPOT IFNγ SFU/1e6 cells
C 1	Ci10004	253	A*03:01	A*30:02	B*07:02	B*57	2400
C 2	Ci10060	722	A*01:01	A*33:01	B*42:01	B*57	415
C 3	Ci10067	215	A*30:02	A*33:01	B*13:02	B*57	610
C 4	Ci20018	1886	A*02:01	A*26:01	B*40:01	B*57	1520
EC 1	Ci10071	49	A*01:01	A*66:02	B*8:01	B*57	730
ART+ NC	Ci10074	49	A*01:01	A*26:01	B*8:01	B*57	770
NC 1	Ci10027	4827	A*01:01	A*02:01	B*8:01	B*57	714
NC 2	Ci10076	17693	A*02:01	A*30:02	B*35:01	B*57	N/A
EC 2	8219	47	A*02:01	A*30:02	B*07:05	B*57	2737
PWO 1		0	A*30:01	A*68:02	B*42:01	B*57	N/A

Supplemental Table 2: Antibody List - Sorting. Table for all antibodies describing the marker, fluorophore, clone, company source, and catalog number for the antibody "sorting mix" referenced in Methods section.

Marker	Fluorophore	Clone	Company	Catalog #
Live-dead	Aqua	N/A	Invitrogen	L34957
CD3	PacBlue	UCHT1	BD	558117
CD4	A780	RPA-T4	Invitrogen	47-0049-42
CD8	A700	RPA-T8	BD	561453
CD14	PerCP-Cy5.5	M5E2	BD	550787
CD16	PerCP-Cy5.5	3G8	BD	560717
CD19	PerCP-Cy5.5	SJ25C1	BD	340951
CD56	PerCP-Cy5.5	B159	BD	560842
CD69	APC	FN50	BD	555533
CD137	PE	4B4-1	BD	555956
CD94	PE-Cy7	DX22	Biolegend	305516
CD107a	FITC	H4A3	BD	555800

Supplemental Table 3: Antibody List – Multimer Testing. Table for all antibodies describing the marker, fluorophore, clone, company source, and catalog number for the antibody "multimer staining mix" referenced in Methods section.

Marker	Fluorophore	Clone	Company	Catalog #
Live-dead	Zombie Aqua	N/A	Invitrogen	L34957
CCR7	RealBlue 705	3D12	BD	756922
CD3	A780	UCHT1	Invitrogen	47-0038-42
CD4	BV711	SK3	Biolegend	344648
CD8	PE-Texas Red	3B5	Invitrogen	MHCD0817
CD14	BUV563	MϕP9	BD	741441
CD16	BUV563	B73.1	BD	741449
CD19	BUV563	SJ25C1	BD	612916
CD56	BUV805	B159	BD	742022
CD94	PE-Cy7	DX22	Biolegend	305516
CD38	StarBright Blue 615	AT13/5	BioRad	MCA1019SBB615
PD1	BV650	J105	Invitrogen	41-627-9942
CD45RA	StarBright Blue 765	F8-11-13	BioRad	MCA88SBB765
KLRC1/NKG2A	mFluor Violet 610	131411	Novus	FAB1059MFV610
KLRC2/NKG2C	A700	134522	R&D Systems	FAB1381N
TCR-a/B	BUV737	IP26	BD	749196
CD57	BV785	QA17A04	Biolegend	393330