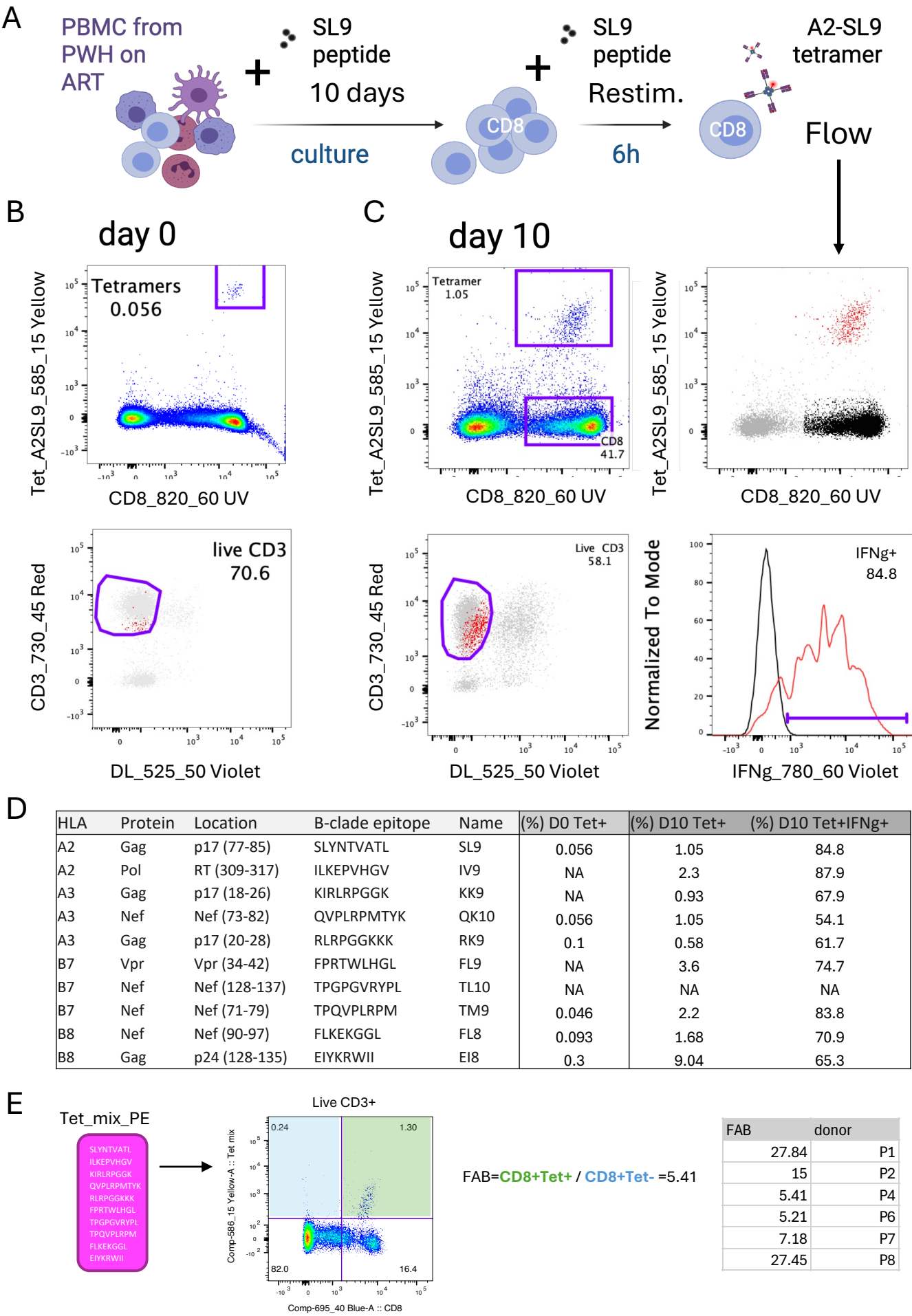


- 1 **In vivo effect of anti-PD-1 on HIV-specific CD8+ T-cells in men with HIV**
- 2 **Supplementary Figures and legends**
- 3

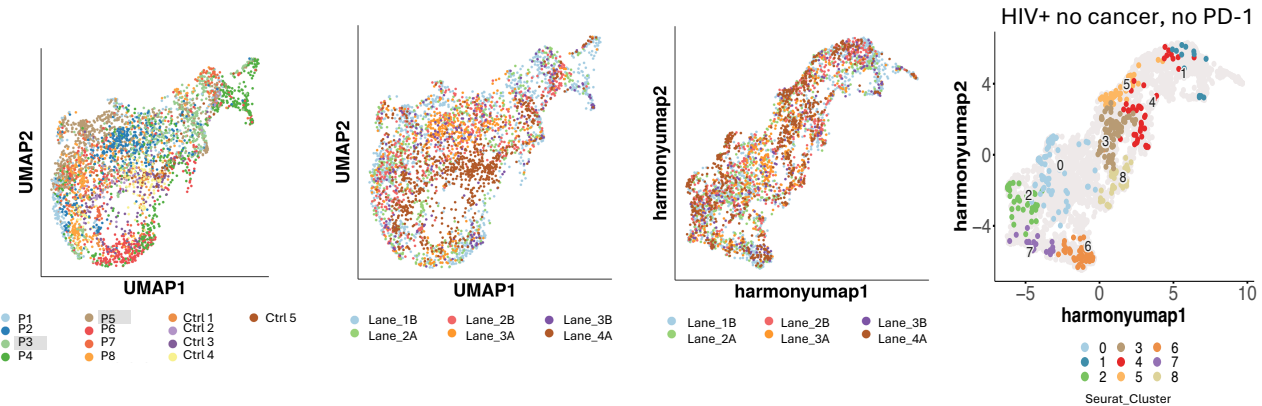
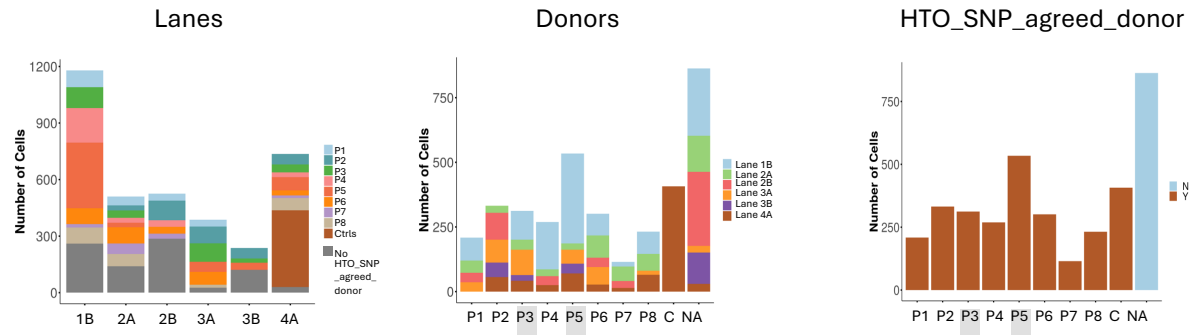
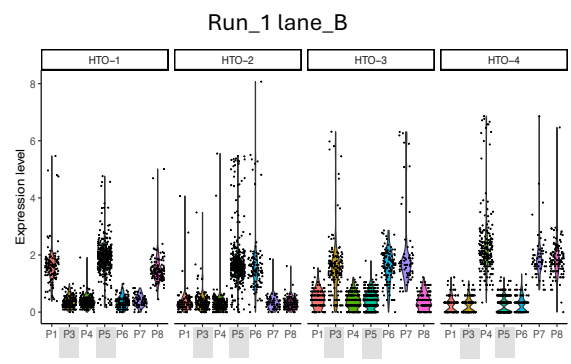
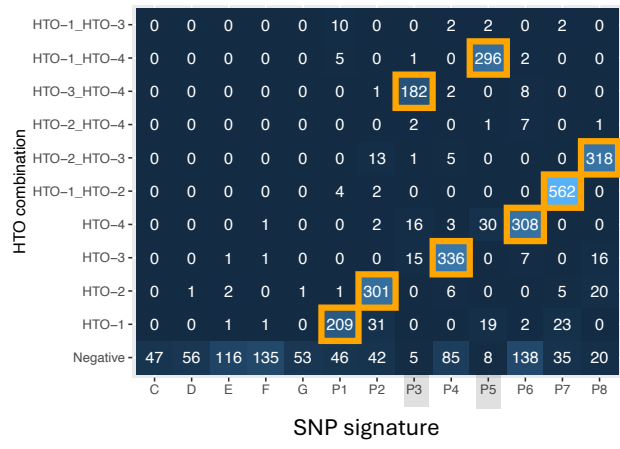
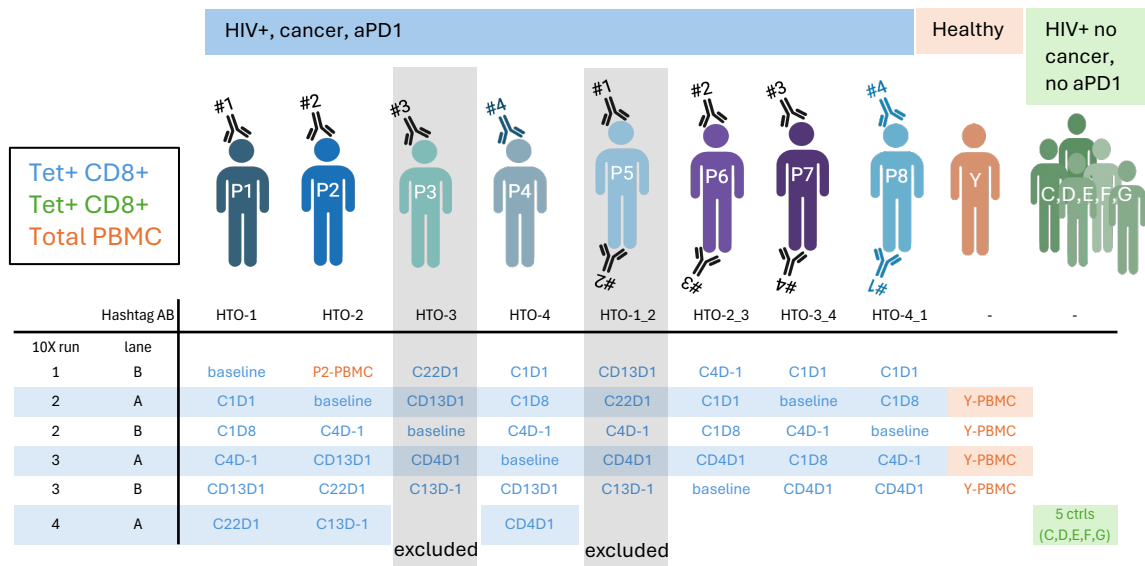
Supplementary Figure 1



Supplementary Figure 1

- A) Schematic of tetramer validation assay: PBMC were cultured with the same peptides as used for peptide:HLA tetramers, for 10 days in vitro. On day 10, restimulation with the relevant peptide in context of brefeldin A and monensin was performed for 6h and intracellular cytokine staining for interferon gamma (IFN γ) and tetramer surface staining was performed using flow cytometry. Illustrations were created by Gubser, C. w BioRender (2026). <https://BioRender.com/guinpdh>.
- B) Flow plot show CD8 $^{+}$ SL9-tetramer staining of PBMC from an A2 positive PWH on ART on day 0, ex vivo (top plot) and back gating of SL9+CD8 $^{+}$ T cells on live/dead CD3 $^{+}$ gate (bottom plot).
- C) Flow plot show CD8 $^{+}$ SL9-tetramer staining of PBMC from an A2 positive PWH on ART on day 10 after in vitro culture with SL9 peptide (upper plots) and back gating of SL9+CD8 $^{+}$ T cells on live/dead CD3 $^{+}$ gate (bottom left plot) and IFN γ histogram overlay (bottom right) of SL9+CD8 $^{+}$ T cells (red line) and SL9 $^{-}$ CD8 $^{+}$ T cells (black line).
- D) Table showing the ten tetramers used for live sorting of tetramer $_{\text{mix}}$ $^{+}$ CD8 $^{+}$ T cells as well as the frequency (%) of tetramer $^{+}$ CD8 $^{+}$ T-cells in HLA-matched donors ex vivo (D0) and the frequency (%) of tetramer $^{+}$ CD8 $^{+}$ T-cells producing IFN γ after 10-day in vitro culture with peptide as described in A and B.
- E) PBMC from study participants (P1, P2, P4, P6, P7, P8) were stained with a mix of all validated tetramers (tet $_{\text{mix}}$ $_{\text{PE}}$) and gated on live CD3 $^{+}$ cells (flow plot). A >2 fold change in frequency (%) of tet $_{\text{mix}}$ CD8 $^{+}$ T cells (green square) compared to tet $_{\text{mix}}$ CD8 $^{-}$ T cells (blue square), i.e. FAB (fold above background) was considered as specific for tet $_{\text{mix}}$ staining, i.e. HIV-specific CD8 $^{+}$ T cells.

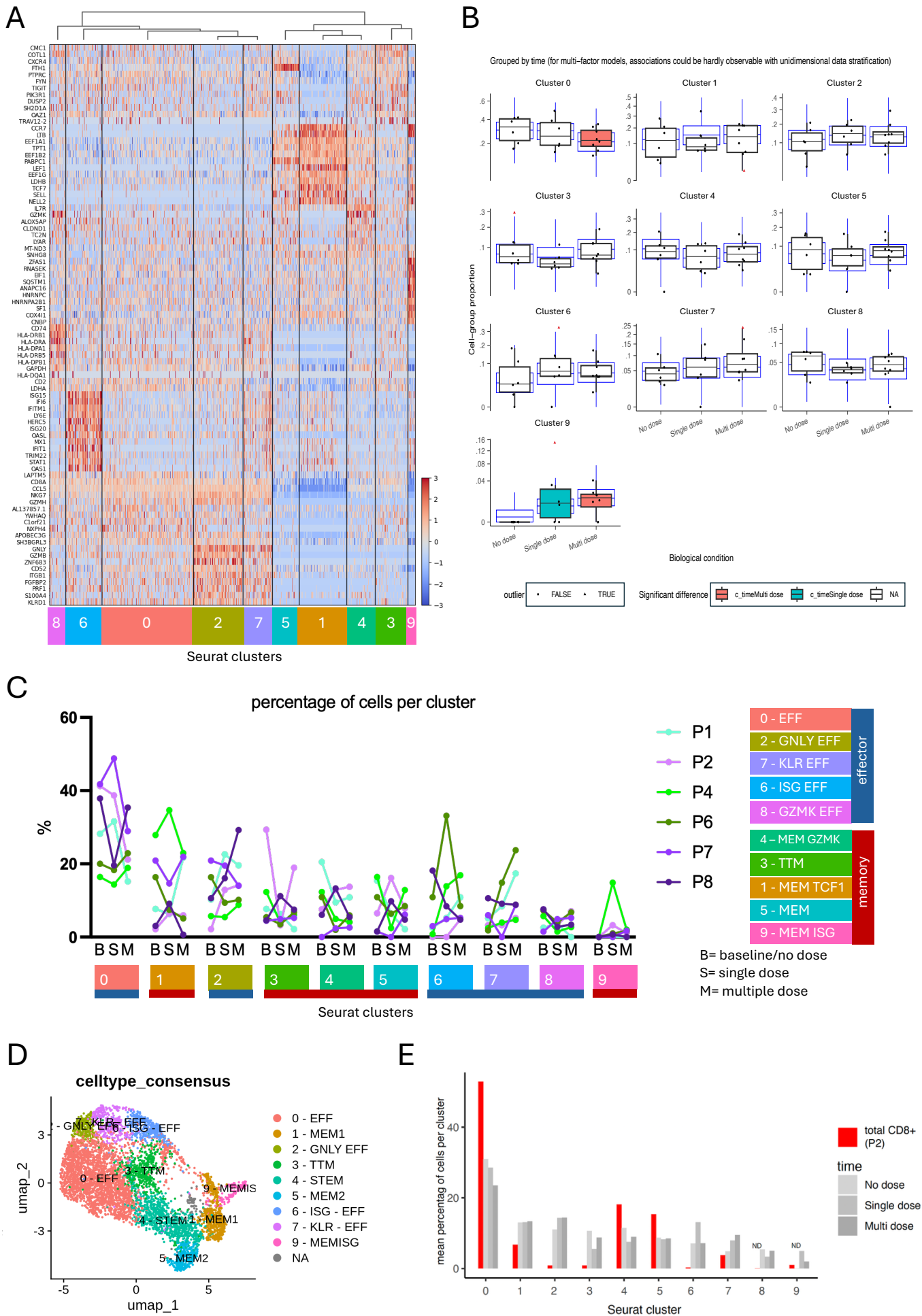
A



Supplementary Figure 2

- A) Schematic of sample matrix designed to perform four individual 10X runs containing two lanes (A, B) each. “Samples from donors were hash tagged (HTO) as indicated. Each lane contained one timepoint sample per donor to allow for demultiplexing via single nucleotide polymorphisms (SNP). Filler cells (orange) from an uninfected donor (Y) were added to lanes 2AB, 3AB to reduce loss of the cell populations of interest (sorted Tet⁺ CD8⁺ T-cells from P1, 2, 4, 6, 7, 8, blue). In one case (run 1 lane B, first row), the total PBMC from study participant P2 (P2-PBMC) were added instead of uninfected PBMC filler cells, as sample timepoint C1D8 from P2 did not yield enough live cells after sorting and hence this timepoint was lost. Run 4 lane A (green) contained tetramer sorted CD8⁺ T cells from five (C, D, E, F, G) PWH on ART without cancer and without anti-PD-1 treatment, referred to as ‘controls’. Illustrations were created by Gubser, C. w BioRender (2026). <https://BioRender.com/guinpdh>.
- B) Table shows cell numbers after demultiplexing based on SNP-signature and combination of hash tag (HTO) antibodies.
- C) Violin plot shows expression levels of HTO-1, -2, -3, and -4 on donor cells from run1 lane B.
- D) Stacked bar graphs show the total number of cells assigned to each donor across all runs/lanes (left graph) and total number of cells detected in each run/lanes from each donor and HTO-SNP consensus (middle and right graphs). NA bar contains cells that could not be assigned to a specific donor and did not have consensus from the HTO and SNP analyses.
- E) Unintegrated UMAPs with considerable inter-donor variability (first plot) but little inter-batch variability (second plot). Harmony integrated UMAP removed donor effects (second last plot). UMAP of tetramer⁺ CD8⁺T cells from 5 PWH on ART without cancer and no PD-1 (control cohort) distribution across Seurat clusters.

Supplementary Figure 3

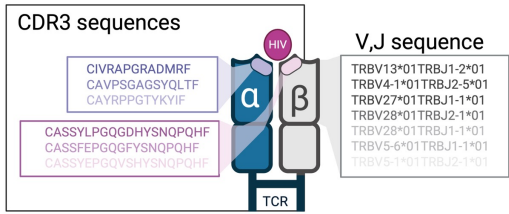


Supplementary Figure 3

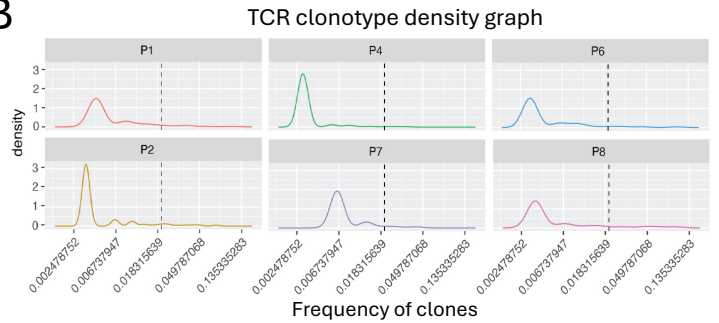
- A) Heatmap of top twelve differentially expressed genes (DEG) across cell clusters of total tetramer+ CD8+T cells.
- B) Black box plots show mean frequency of cells in Seurat Clusters 0-9 across time points of anti-PD-1 treatment (no dose, single dose and multiple dose). The blue boxplots represent the posterior predictive check. Turquoise and red colored boxes indicate statistically significant changes, $p < 0.05$. Source data are provided as a Source Data file, tab 3.
- C) Graph shows frequency (%) of tetramer+ CD8+T-cells in each cluster across anti-PD-1 treatment time (B, S, M) per donor (P1, P2, P4, P6, P7, P8).
- D) UMAP shows cell type composition of all tetramer+ CD8 T-cells merged with total CD8 T-cells from participant P2.
- E) Bar graph shows the proportion (%) of total CD8+ T-cells in participant P2 (red) and the mean frequency of all tetramer+ CD8+T-cells in each cluster across anti-PD-1 treatment time (B, S, M).

Supplementary Figure 4

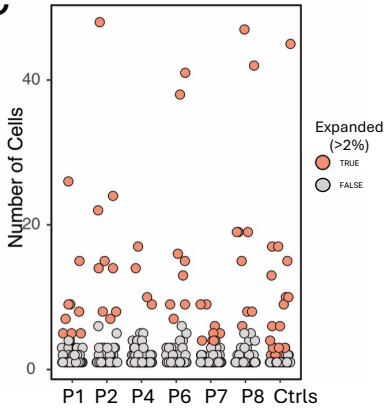
A



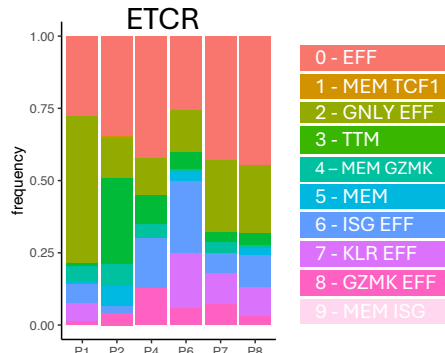
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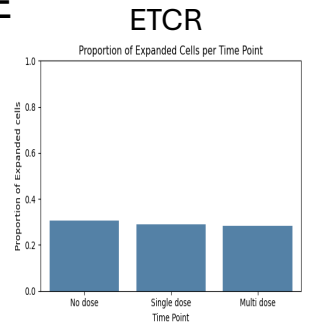
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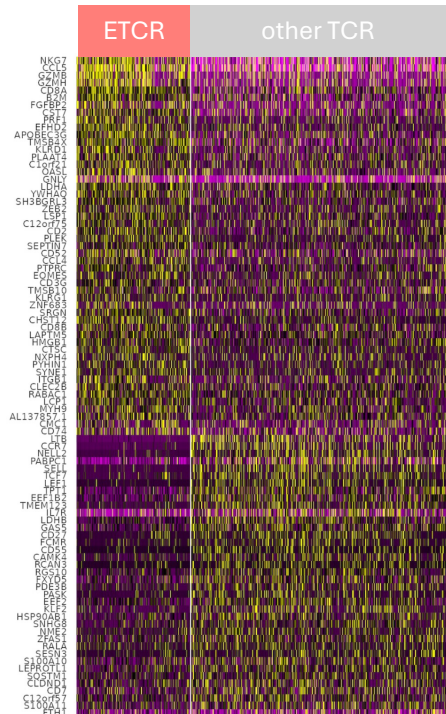
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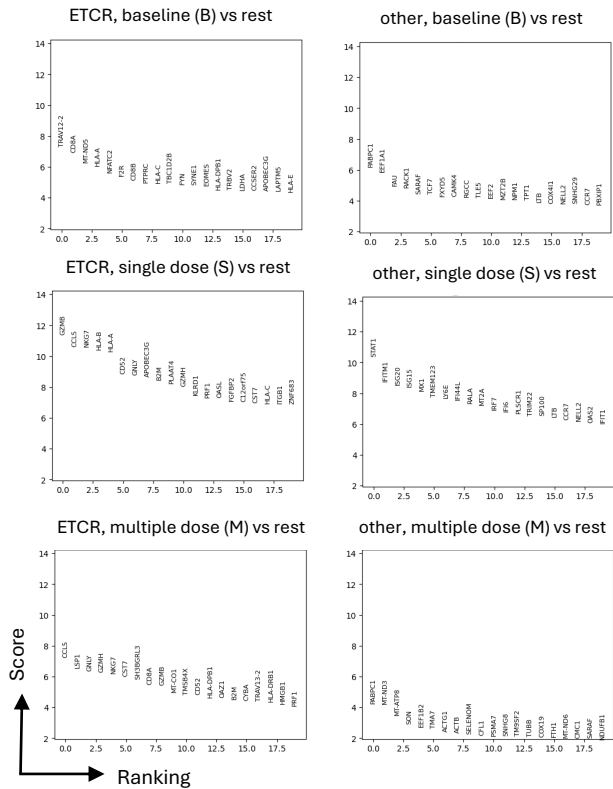
E



F



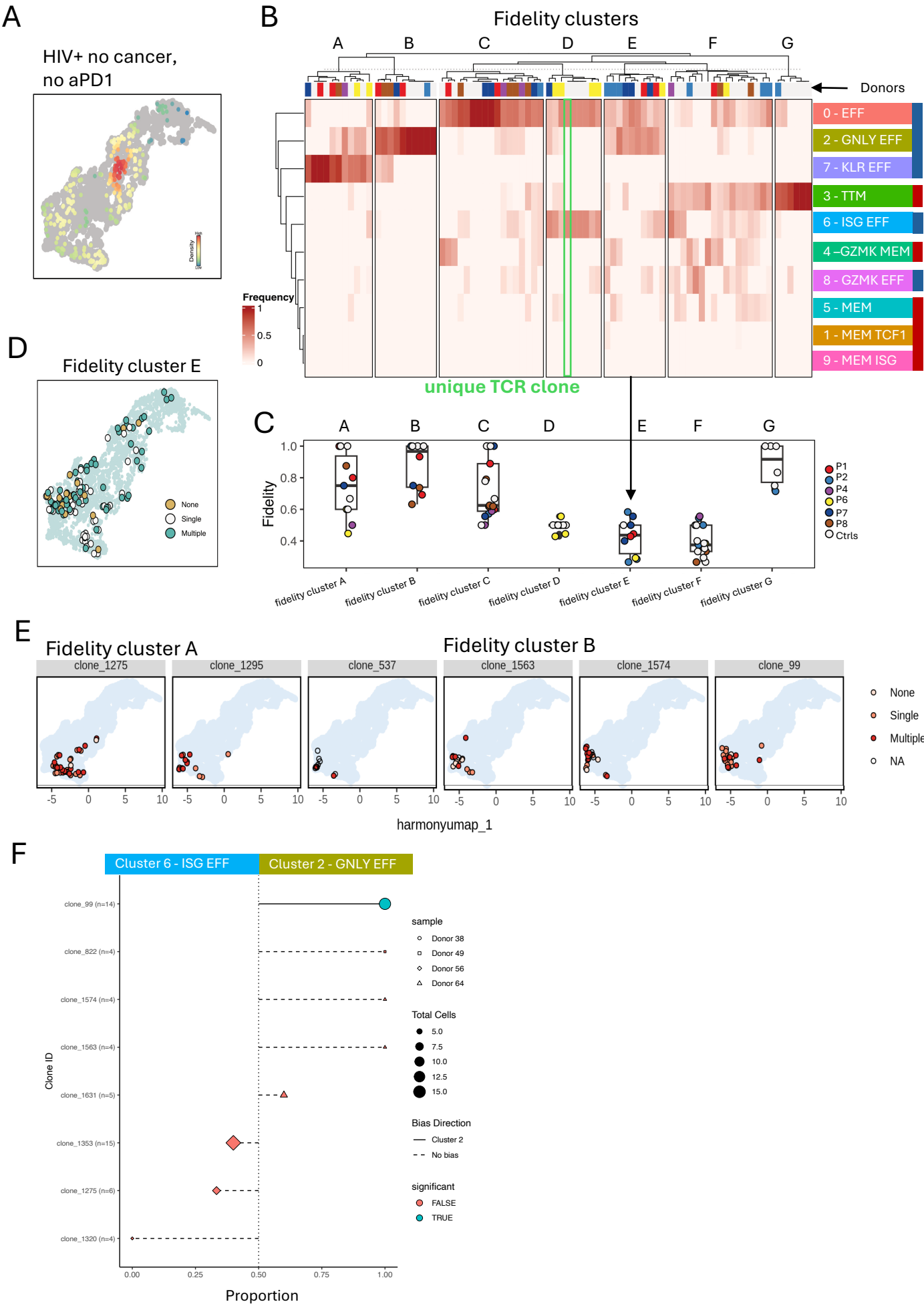
G



Supplementary Figure 4

- A) Schematic of T-cell receptor (TCR) and the CDR3alpha, beta sequences and V, J gene transcripts of the TCR beta chain. Illustrations were created by Gubser, C. w BioRender (2026). <https://BioRender.com/guinpdh>.
- B) Density graph shows frequency of TCR clones in each donor. Expanded TCR clones (ETCR) were defined as >2% of cells with an identical CDR3ab TCR sequences within a sample. Density of cells with a low-frequency TCR clone is high (left side of the density plot), whereas the density of cells with a high-frequency TCR is low (right part if the density plot)
- C) Plot depicts number of cells per donor with ETCR clones (red)
- D) Graph shows distribution of ETCR clones across annotated clusters in each donor.
- E) Bar graph shows the frequency of ETCR clones across anti-PD-1 treatment timepoints (B, S, M)
- F) Heatmap of top DEGs comparing ETCR clones (red) against the other TCRs (grey, non-expanded)
- G) Plots show score and ranking of differentially expressed genes between the 6 conditions: ETCR clones (baseline, single dose, multiple dose, red) and other TCRs (baseline, single dose, multiple dose, grey)

Supplementary Figure 5



Supplementary Figure 5

- A) UMAP shows density of ETCR clones from five control donors (Supp. Fig 2A), i.e. PWH on ART without cancer and without anti-PD-1 treatment
- B) Heatmap showing frequency of ETCR clones across cell types (Seurat clusters on the right). ETCR clones are hierarchically clustered into seven compositionally similar groups, we termed 'Fidelity clusters' A-G. Each colored rectangle on the top of the graph represents a donor to which each TCR clone belongs. The green rectangle indicates a unique TCR clone. Donors are color coded.
- C) Boxplot shows 'clonal fidelity', defined as the maximum frequency of cells for a given cell type per ETCR clone.
- D) UMAP shows cells belonging to low fidelity cluster E at different timepoints following anti-PD-1
- E) UMAPs show cells belonging to high fidelity clusters A and B at different timepoints following anti-PD-1
- F) Graph shows trajectories of expanded TCR clones that were trackable at both baseline and the single-dose time point. To evaluate cluster bias, a binomial exact test with FDR p-value correction was performed to determine if specific clones were biased toward GNLY-EFF (cluster 2, plotted to the right) or ISG-EFF (cluster 6, plotted to the left). The vertical line at 0.5 indicates the null hypothesis, assuming cells within a single clone are equally likely to reside in either cluster.