SUPPLEMENTARY FIGURES

Figure S1

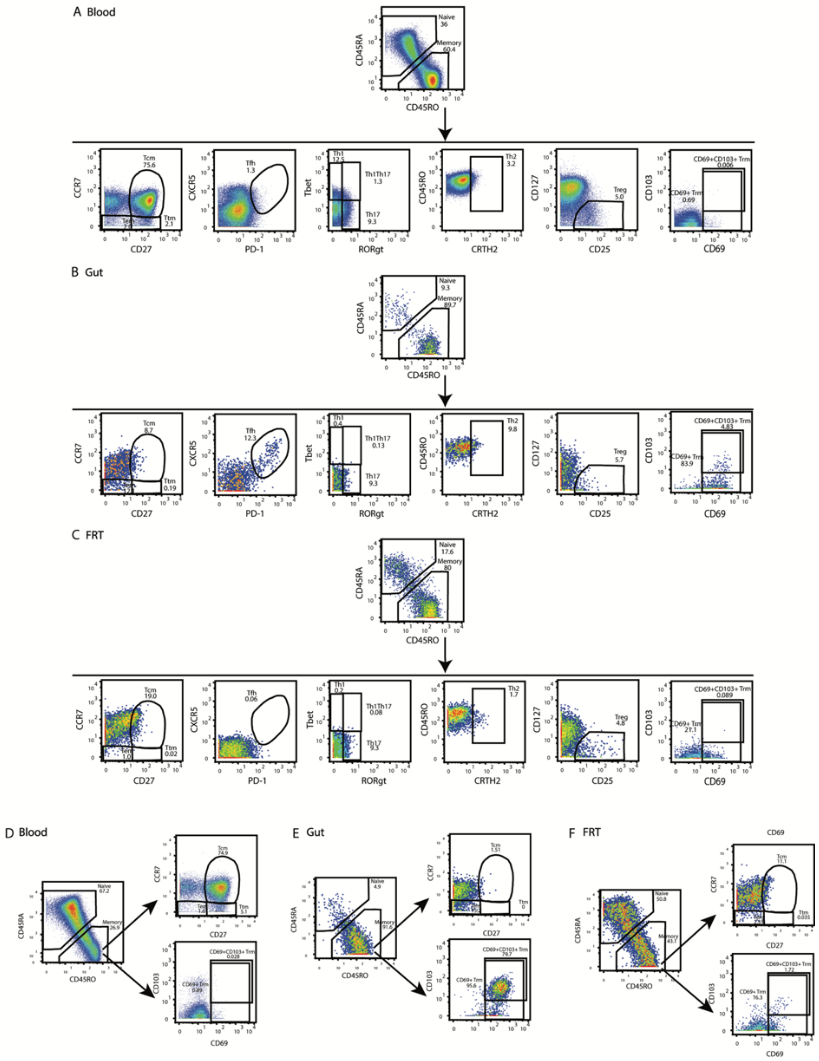

Figure S1. Gating strategies to identify canonical subsets of T cells. (A-C) CD4+ T cell gating. Live, singlet CD3+CD4+CD8- cells were gated for naïve vs. memory subsets based on CD45RA and CD45RO expression, followed by gating for the Tcm (CCR7+CD27+), Tem (CCR7-CD27-), Ttm (CCR7-CD27+), Tfh (PD1+CXCR5+), Th1 (Tbet+RORγt-), Th1Th17 (Tbet+RORγt+), Th17 (Tbet-RORγt+), Th2 (CRTH2+), Treg (CD25+CD127-), and CD69+ Trm subsets within the memory compartment as indicated. A subset of CD69+ Trm expressing CD103 (CD69+CD103+) was also analyzed. Shown are results from a representative donor from blood **(A)**, gut **(B),** and FRT **(C)**. **(D-F) CD8+ T cell gating.** Live, singlet CD3+CD4-CD8+ cells were gated for naïve vs. memory subsets based on CD45RA and CD45RO expression, followed by gating for the Tcm, Tem, Ttm, and Trm subsets within the memory compartment as indicated. Shown are results from a representative donor from blood **(D)**, gut **(E),** and FRT **(F)**.

Figure S2

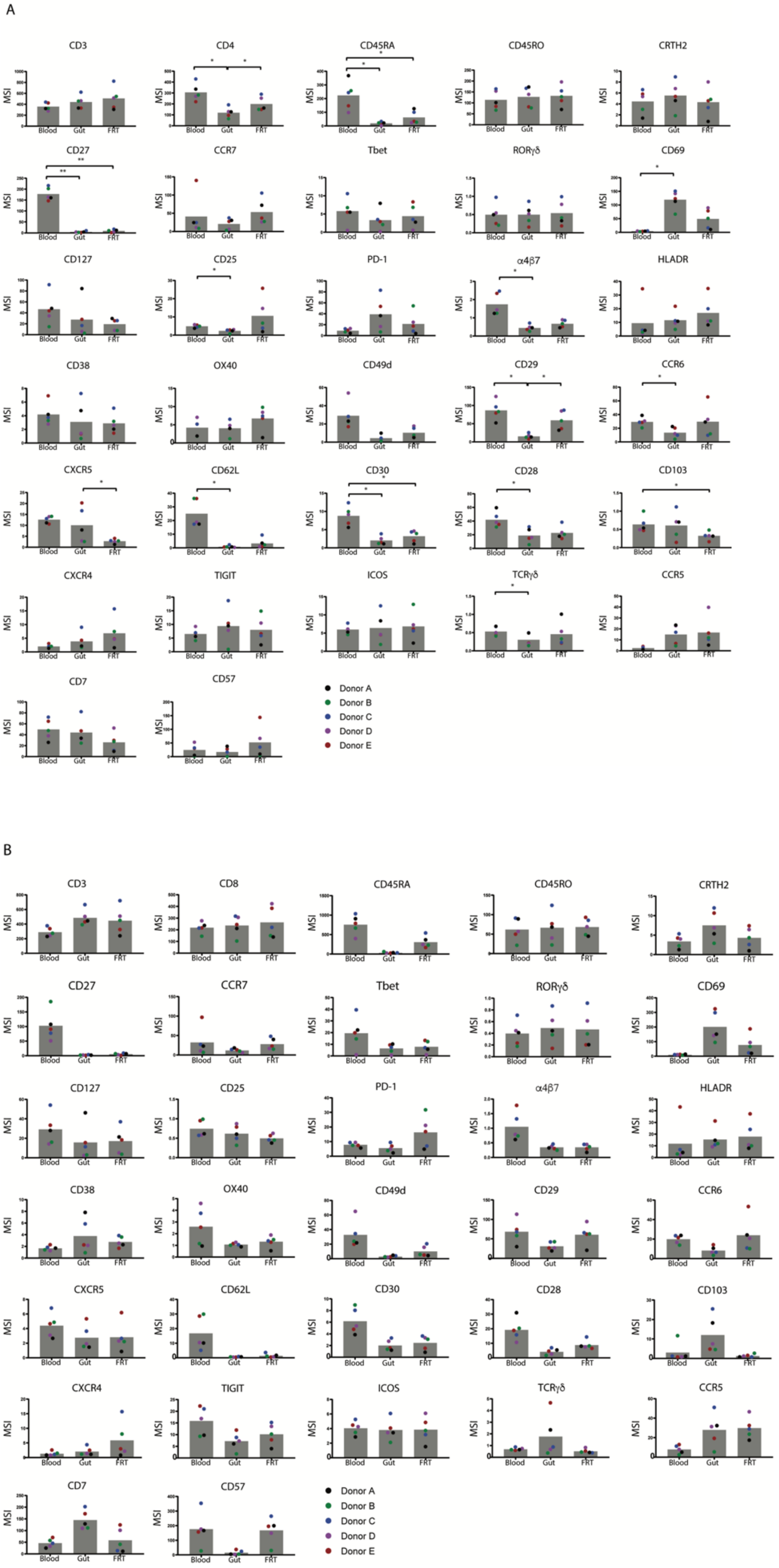

Figure S2. Antigen expression levels in T cells from participants. Shown are mean signal intensity (MSI) levels reflecting relative expression of the indicated antigens among CD4+ T cells **(A)** or CD8+ T cells **(B)** from blood, gut, and FRT. Datasets were pre-gated on live, singlet CD3+CD4+CD8- cells (A) or live, singlet CD3+CD4-CD8+ cells (B) prior to MSI calculation. *$p < 0.05$, as determined by a Student's paired t-test and adjusted for multiple testing using the Benjamini-Hochberg for FDR. Each colored dot corresponds to a different donor (n=5 donors).

Figure S3

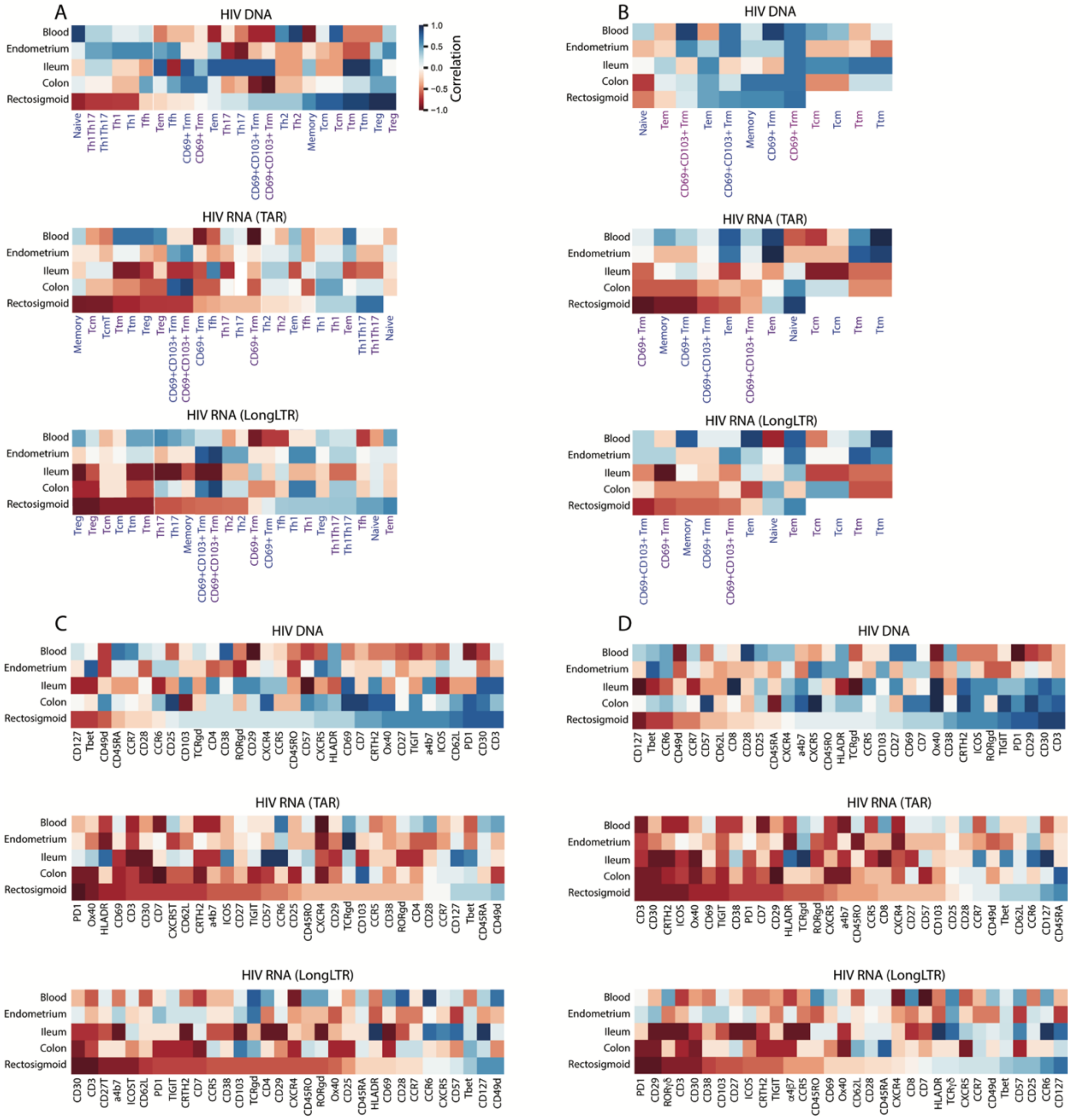

Figure S3. Associations of subset frequencies and antigen expression levels with HIV DNA and RNA levels in participant specimens. The relative frequencies of the canonical CD4+ **(A)** and CD8+ **(B)** T cell subsets analyzed from the indicated blood and tissue sites were assessed for association with levels of HIV DNA, HIV TAR RNA, and HIV Long LTR RNA from the same specimens of the 5 participants. Subset frequencies calculated as a fraction of total CD4+ or CD8+ T cells are colored in blue font, while those calculated as a fraction of memory CD4+ or CD8+ T cells are colored in purple font. Associations were also calculated between the HIV DNA and RNA levels and the relative expression levels of each of the measured antigens in total CD4+ **(C)** and CD8+ **(D)** T cells. Heatmaps represent Spearman correlations, and are ordered according to associations observed within the rectosigmoid specimens. Note that endocervix and ectocervix specimens were excluded from this analysis as not enough cells were obtained from some of these sites from some participants. HIV DNA and RNA levels were obtained from [10].

Figure S4

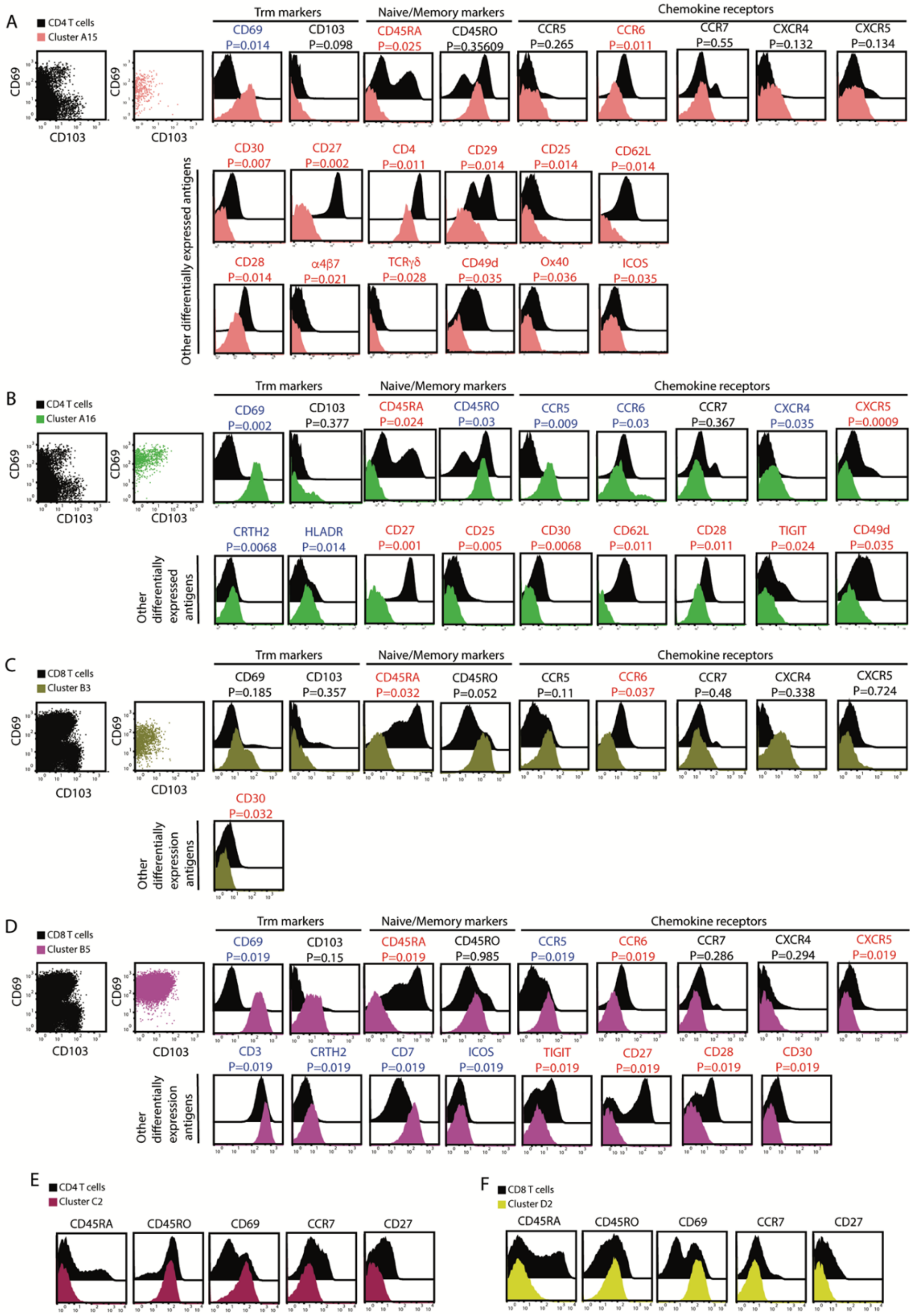

Figure S4. Antigens differentially expressed in select T cell clusters. (A-C) Antigens differentially expressed in T cell clusters over-represented in tissues. T cell clusters A15 **(A)**, A16 **(B)**, B3 **(C)**, and B5 **(D)**, all of which were over-represented in tissues are compared to blood, were assessed for differentially expressed antigens. The two sets of 2D dot plots on the left of each panel show the expression levels of the Trm markers CD69 and CD103 among total CD4+ or CD8+ T cells (black), vs. the cells within the clusters (color). While CD69 was preferentially expressed in cells from all four of these clusters, CD103 was preferentially expressed only in clusters A16 and B5. The histogram plots show antigens differentially expressed among total CD4+ or CD8+ T cells (black) as compared to cells within the clusters (color), and correspond to concatenated events from all donors. They are grouped by category (Trm markers, naïve/memory markers, chemokine receptors, and others). P-values compare expression levels between total CD4+ or CD8+ T cells and the corresponding cluster, and were determined by a Student's paired t-test and adjusted for multiple testing using the Benjamini-Hochberg for FDR. Markers significantly ($p < 0.05$) overexpressed in a particular tissue-associated cluster are in blue font, while markers significantly underexpressed are in red font. A total of 5 donors were analyzed. **(D, E)** Antigens differentially expressed in T cell clusters over-represented in gut as compared to FRT. CD4+ T cell cluster C2 **(D)** and CD8+ T cell cluster D2 **(E)**, both of which were over-represented in gut as compared to FRT, were assessed for differentially expressed antigens. The histogram plots show antigens differentially expressed among total CD4+ or CD8+ T cells (black) as compared to cells within the clusters (color), and correspond to concatenated events from all donors. A total of 5 donors were analyzed.