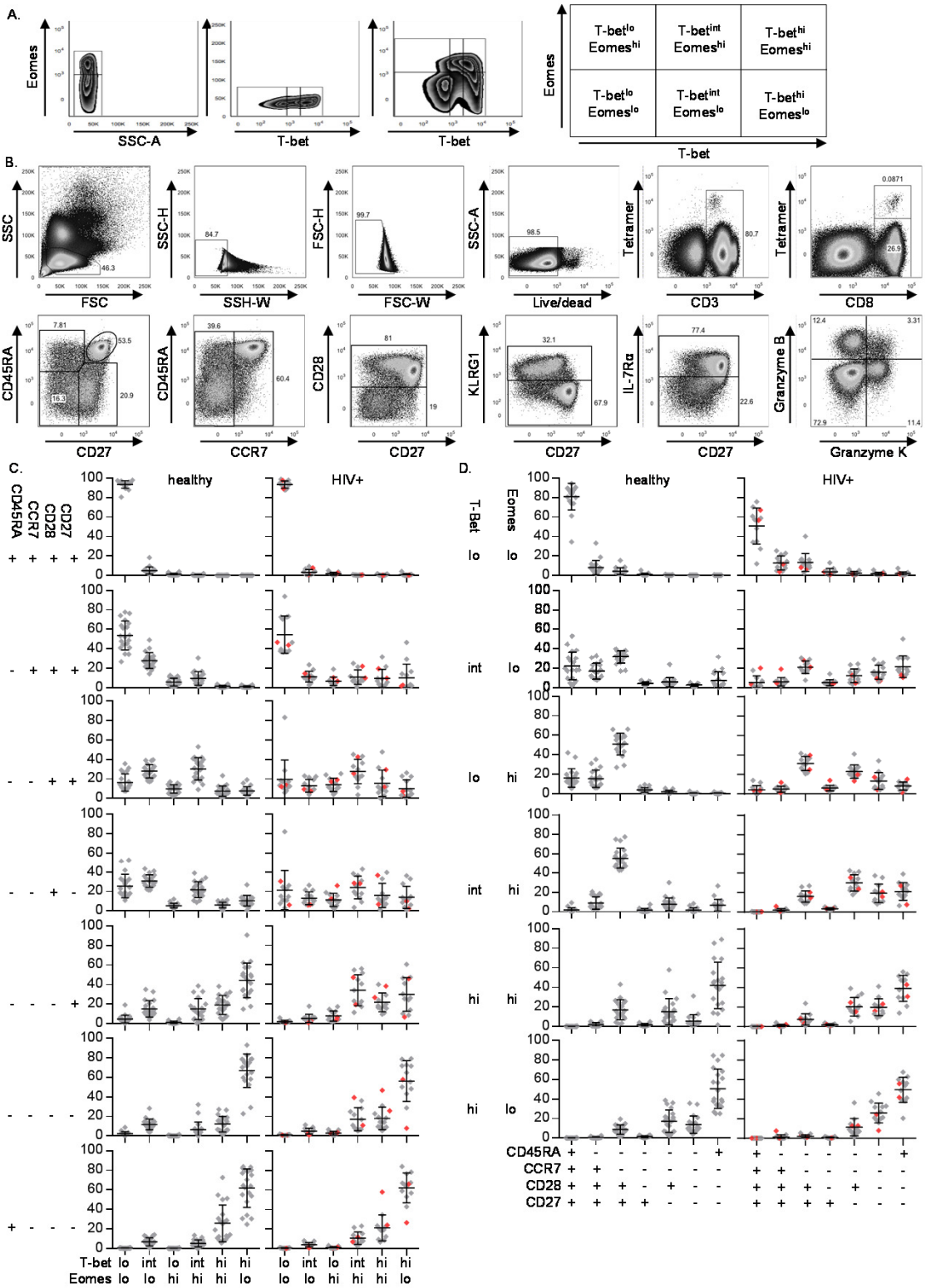

Figure S1. Shifting associations between the surface phenotype and the T-bet/Eomes expression levels (**a**) Representative dot plots showing the gating strategy used fpr defining the T-bet/Eomes gating populations, and (**b**) the gating strategy used for the other markers. Upper row from left to right: the lymphocyte gate (nb. after fixation and permeabilization), the sideward scatter width/height duplet exclusion, the forward scatter width/height duplet exclusion, the gating of alive cells, the gating of $CD3^+$ $tetramer^+$ events, the gating of $CD3^+CD8^+(tetramer^+)$ events. Lower row from left to right: the gating of the CD45RA/CD27 $CD8^+$ T-cell populations, the gating of the CCR7-positive and negative $CD8^+$ T-cell populations, the gating of the CD28-positive and negative $CD8^+$ T-cell populations, and the expression frequencies of KLRG1, IL-7Rα, granzyme K and granzyme B (lower row) (**c**) The distribution of the T-bet/Eomes expression states per CD45RA/CCR7/CD28/CD27-defined subset in 20 healthy- (left column) and 13 HIV-1-infected subjects (right column), mean/SD shown and (**c**), reciprocally, the distribution of the CD45RA/CCR7/CD28/CD27-defined subsets over the T-bet/Eomes populations in 20 healthy- (left column) and 13 HIV-1-infected subjects (right column), mean/SD shown. The two HIV-1-infected patients not co-infected with hCMV are shown in red. See Figure 1 for statistical analyses.

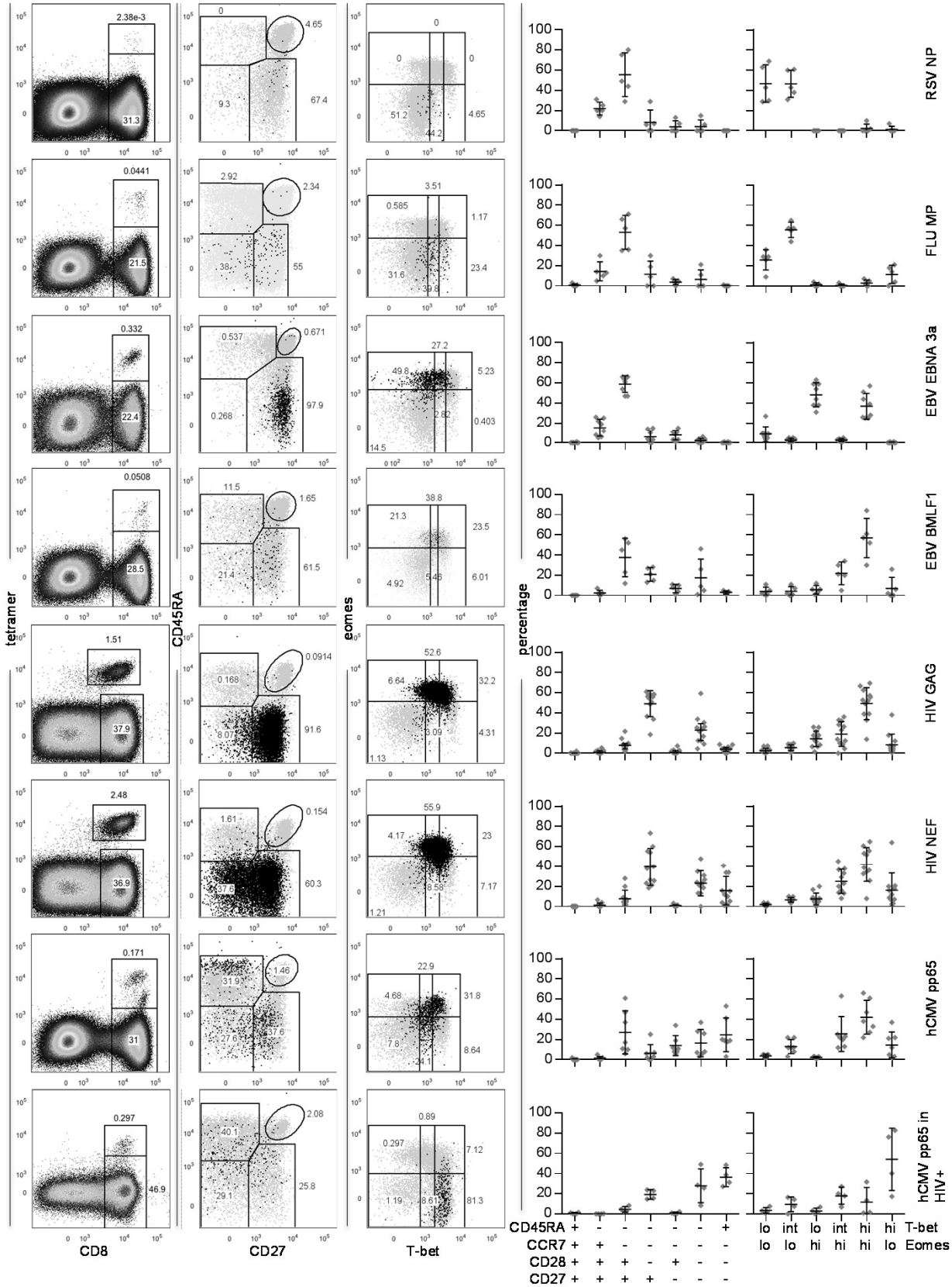

Figure S2. Virus-specific CD8⁺ T-cells display distinct CD45RA/CCR7/CD28/CD27 and T-bet/Eomes expression levels
Representative dot plots of the different virus-specific CD8⁺ T-cell populations as stained with tetramers (left column), where CD8 fluorescence intensity (FI) is plotted on the X-axis and tetramer FI on the Y-axis, as well as their CD45RA (Y-axis)/CD27 (X-axis) and T-bet (X-axis)/Eomes (Y-axis) phenotypes presented as an overlay of the respective tetramer⁺ population in black on the total CD8⁺ T-cell population in gray. (Second column) The range of CD45RA/CCR7/CD28/CD27 phenotypes found among RSV NP- (n=5), influenza (Flu) MP1- (n=5), EBV EBNA-3a- (n=8), EBV BMLF-1- (n=5), HIV-1 gag- (n=12), HIV-1 nef- (n=11) and hCMV pp65-specific CD8⁺ T-cells in healthy individuals (n=7) and in HIV-1-infected persons (n=4). (Middle column) The T-bet/Eomes expression states found among the same virus-specific populations, median and IQR shown. (Fourth and fifth columns) the surface marker- and T-bet/Eomes-defined subset distribution of hCMV pp65-specific cells circulating in healthy individuals (n=7) compared to the same populations in HIV-1-infected individuals (n=4), mean/SD shown. See Figure 2 for statistical analyses.

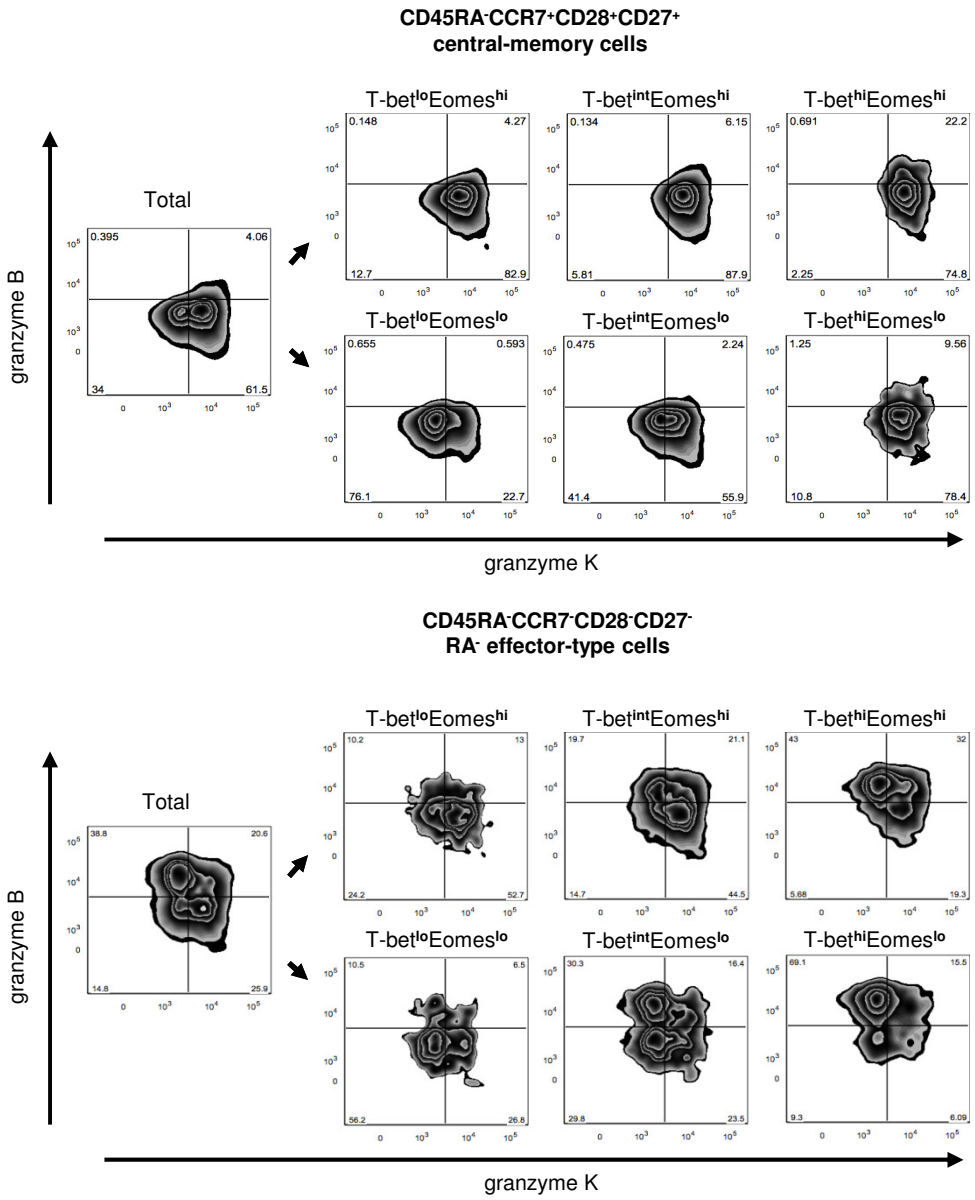

Figure S3. An individual CD45RA/CCR7/CD28/CD27-defined subset comprises cells in different T-bet/Eomes expression states that are linked to functional differences

Representative zebra plots of CD45RA$^-$ CCR7$^+$CD28$^+$CD27$^+$ central-memory cells (upper panel) and CD45RA$^-$ CCR7$^-$ CD28$^-$ CD27$^-$ effector-type cells (lower panel) plotting granzyme K fluorescence intensity on the X-axis and granzyme B fluorescence intensity on the Y-axis, and the shifts in the expression of these effector molecules per T-bet/Eomes expression state found in these CD45RA/CCR7/CD28/CD27-defined subsets.

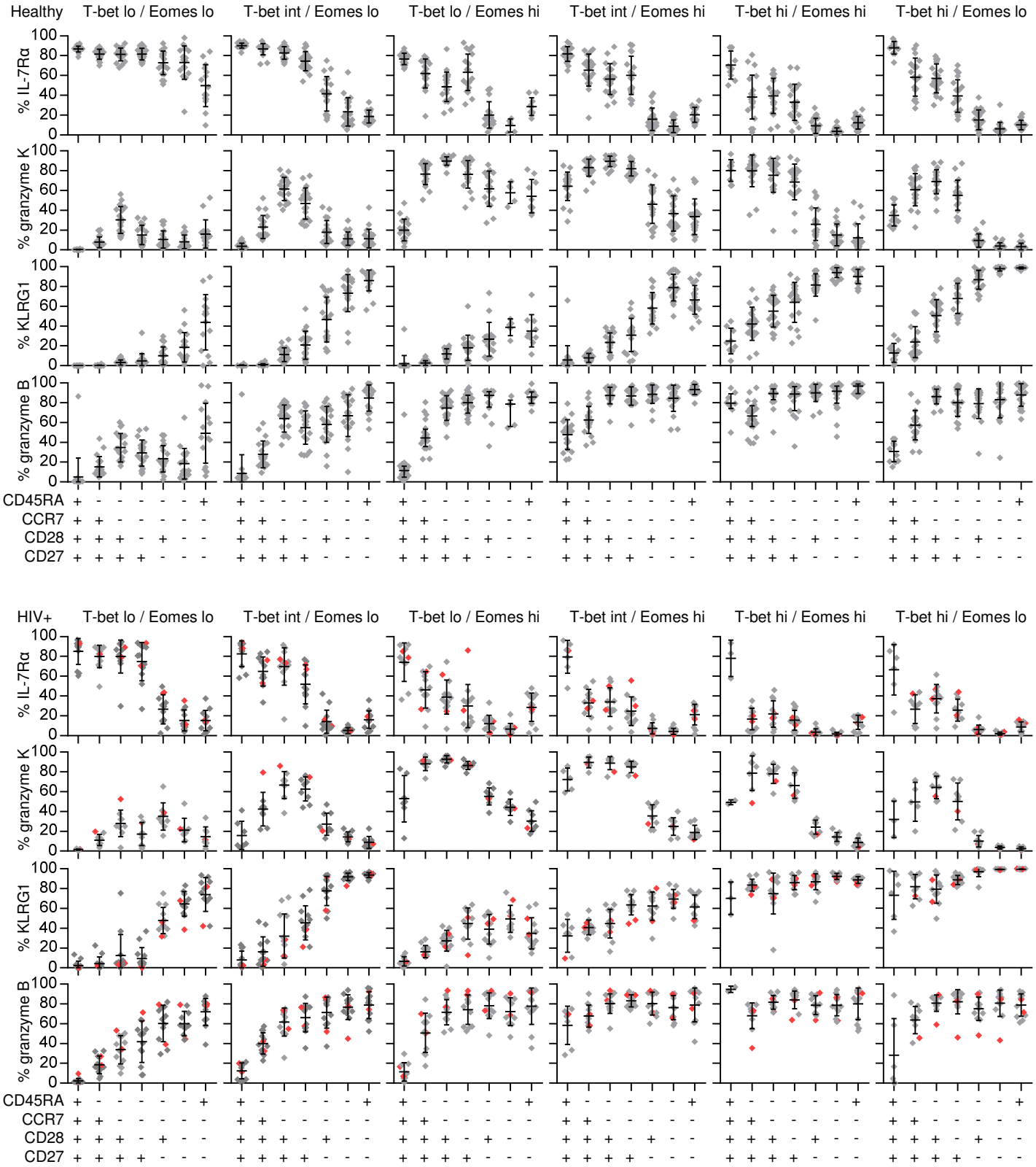

Figure S4. Combining the CD45RA/CCR7/CD28/CD27 dimension and the T-bet/Eomes dimension more accurately predicts CD8⁺ T-cell functional potential

Scatter plots displaying the expression of IL-7Rα (first row), granzyme K (second row), granzyme B (third row) and KLRG1 (last row) per T-bet^{lo}Eomes^{lo} (first column), T-bet^{int}Eomes^{lo} (second column), T-bet^{lo}Eomes^{hi} (third column), T-bet^{int}Eomes^{hi} (fourth column), T-bet^{hi}Eomes^{hi} (fifth column), T-bet^{hi}Eomes^{lo} (last column), and per CD45RA/CCR7/CD28/CD27-defined subset plotted on the X-axis in 20 healthy individuals (upper panel) and in 13 HIV-1-infected individuals (lower panel), mean/SD shown. The two HIV-1-infected patients not co-infected with hCMV are shown in red. See Figure 4 for statistical analyses.

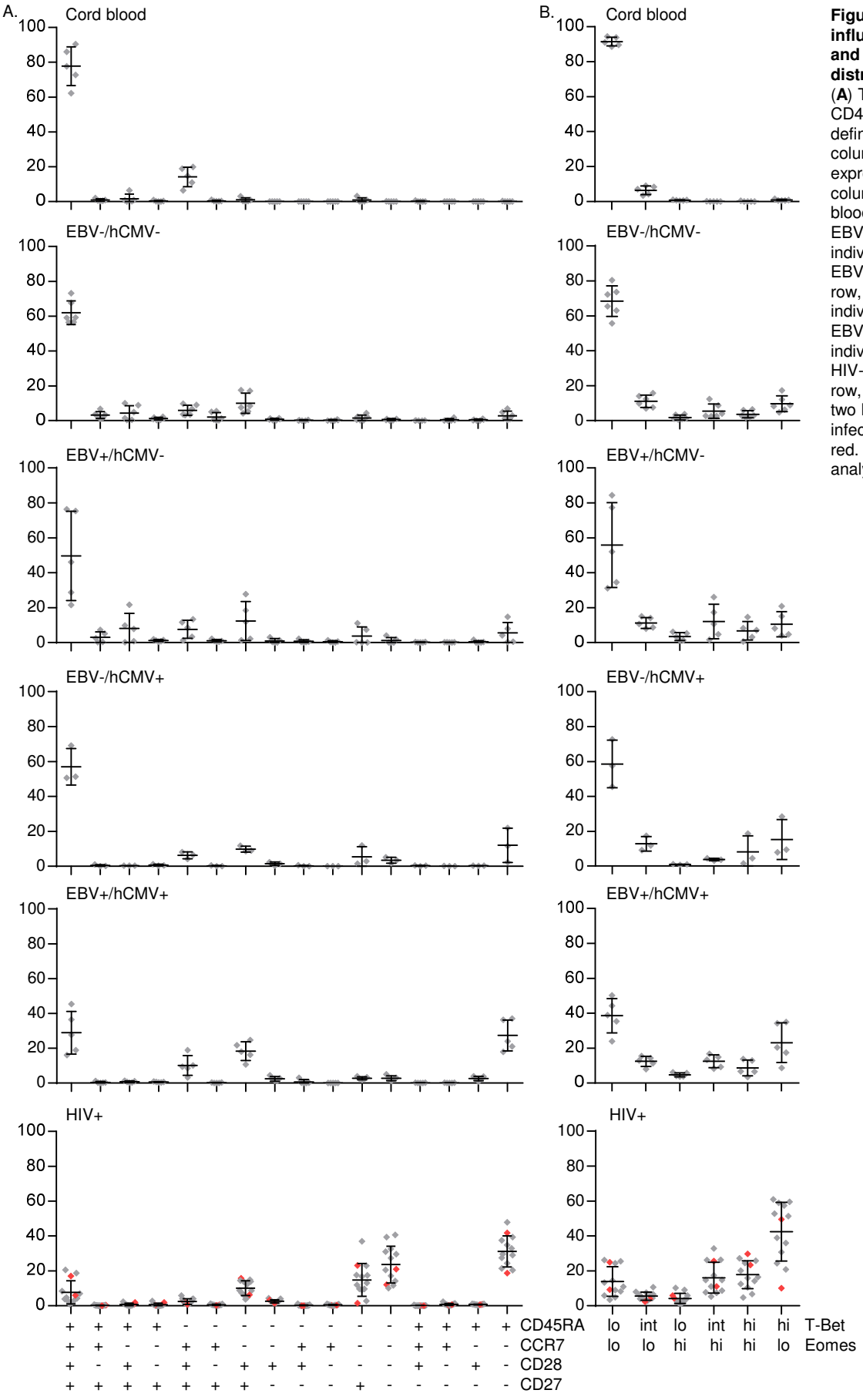

Figure S5. Infection history influences the surface marker- and T-bet/Eomes-defined subset distribution
(**A**) The CD45RA/CCR7/CD28/CD27-defined subset distribution (left column) and (**B**) the T-bet/Eomes expression state distribution (right column) over $CD8^+$ T-cells in cord blood samples (first row, n=5), EBV/hCMV seronegative individuals (second row, n=6), EBV seropositive individuals (third row, n=5), hCMV seropositive individuals (fourth row, n=3), EBV/hCMV double-infected individuals (fifth row, n=6) and HIV-1-infected individuals (last row, n=13), mean/SD shown. The two HIV-1-infected patients not co-infected with hCMV are shown in red. See Figure 5 for statistical analyses.

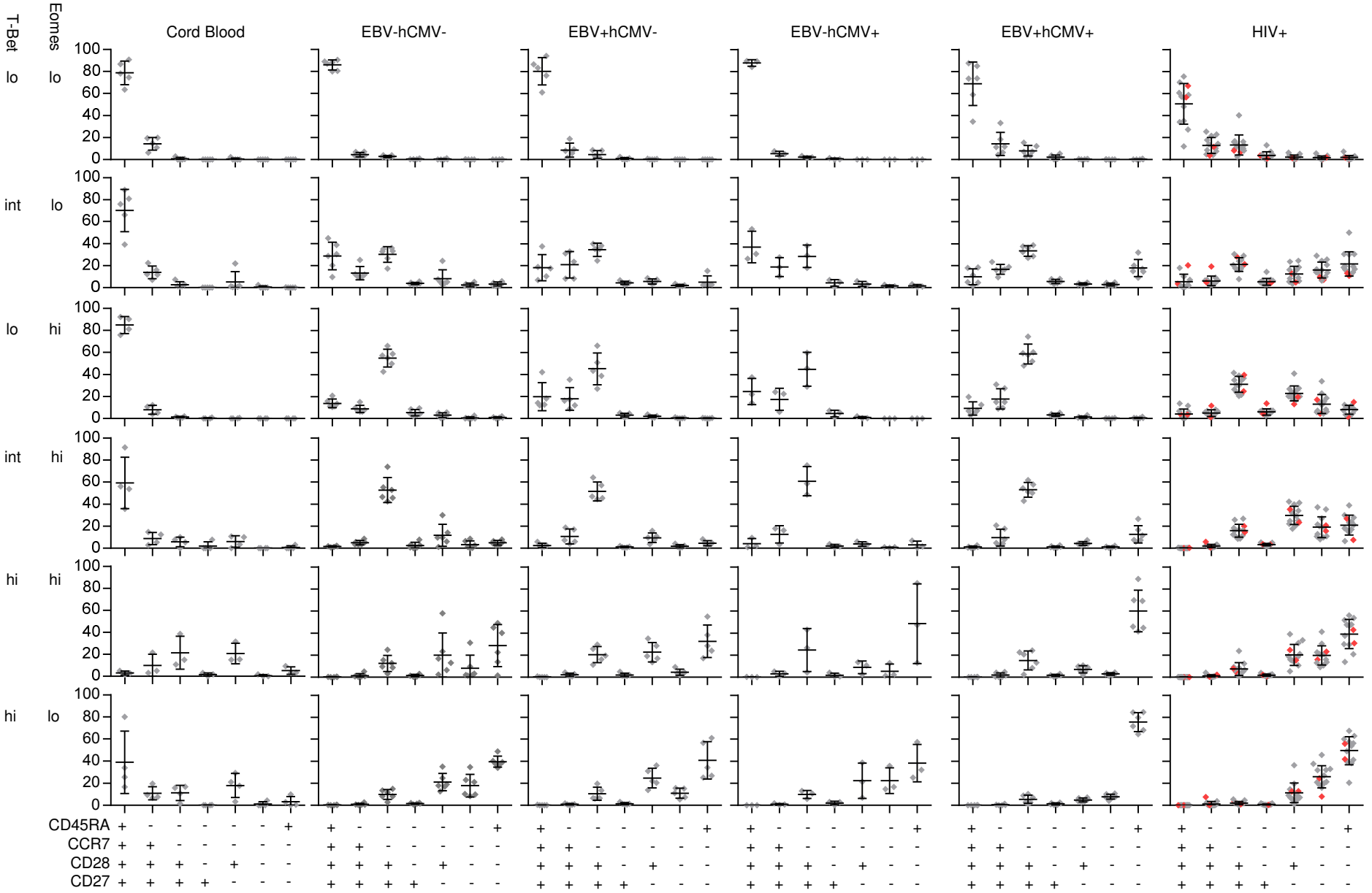

Figure S6. Infection history influences the associations between T-bet/Eomes expression levels and surface phenotype

Scatter plots displaying the relative distribution of the CD45RA/CCR7/CD28/CD27 phenotypes (plotted on the X-axis of each graph) over the different T-bet/Eomes expression states (rows) per study group: cord blood samples (first column, n=5), EBV/hCMV seronegative individuals (second column, n=6), EBV seropositive individuals (third column, n=5), hCMV seropositive individuals (fourth column, n=3), EBV/hCMV double-infected individuals (fifth column, n=6), and HIV-1-infected individuals (last column, n=13), mean/SD shown. The two HIV-1-infected patients not co-infected with hCMV are shown in red.

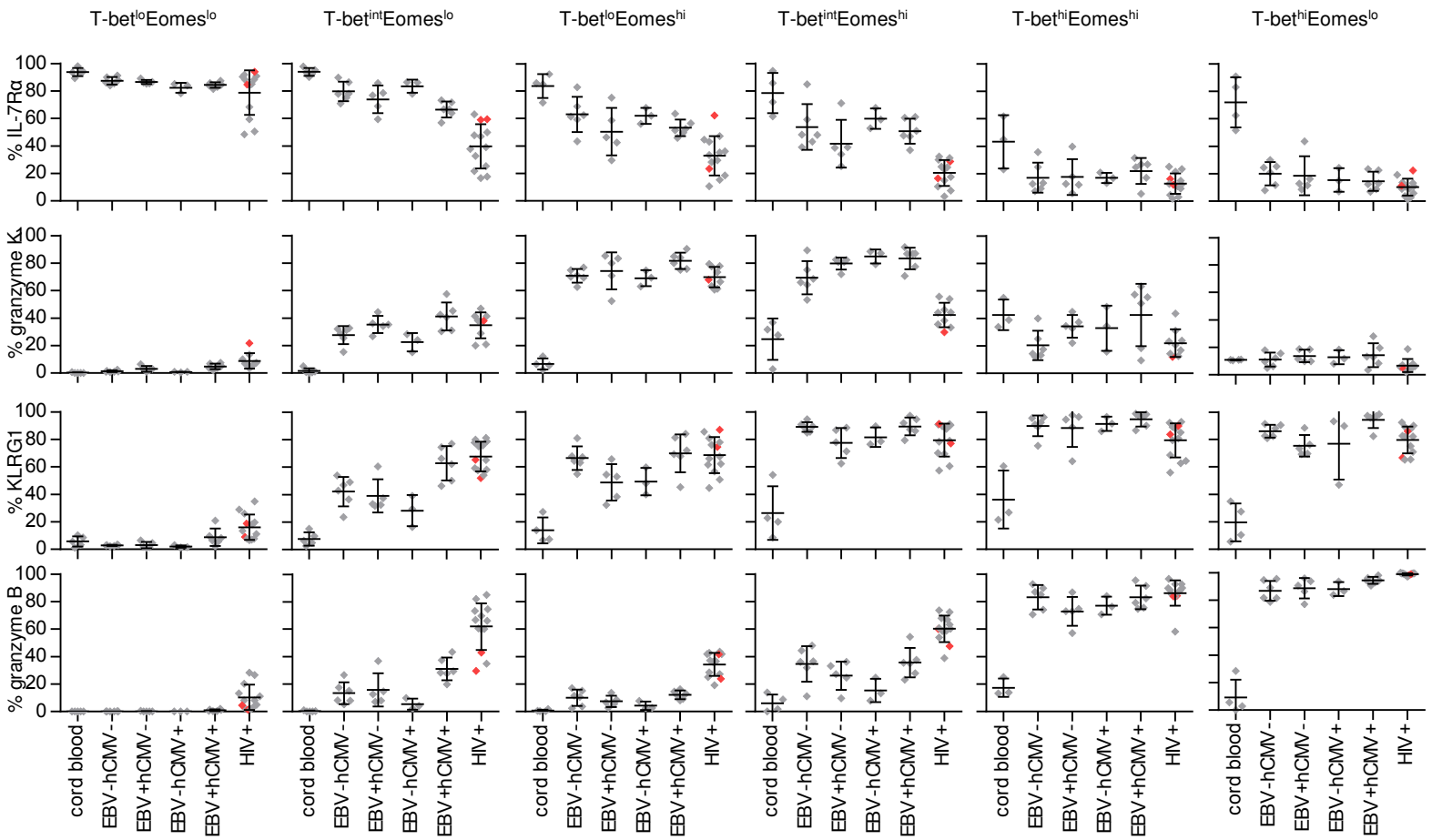

Figure S7. Viral infection history impacts on the associations between the T-bet/Eomes expression levels and the functional potential

Expression frequencies of IL-7Rα (first row), granzyme K (second row), KLRG1 (third row) and granzyme B (last row) per T-bet^{lo}Eomes^{lo} (first column), T-bet^{int}Eomes^{lo} (second column), T-bet^{lo}Eomes^{hi} (third column), T-bet^{int}Eomes^{hi} (fourth column), T-bet^{hi}Eomes^{hi} (fifth column) and T-bet^{hi}Eomes^{lo} cells (last column) and per study group: cord blood samples, n=5; EBV/hCMV seronegative individuals, n=6; EBV seropositive individuals, n=5, hCMV seropositive individuals, n=3, EBV/hCMV double-infected individuals, n=6, and HIV-1-infected infected individuals (n=13) plotted on the X-axis of each graph, median and IQR shown. Note that not all cord blood samples comprised all T-bet/Eomes expression states. The two HIV-1-infected patients not co-infected with hCMV are shown in red.

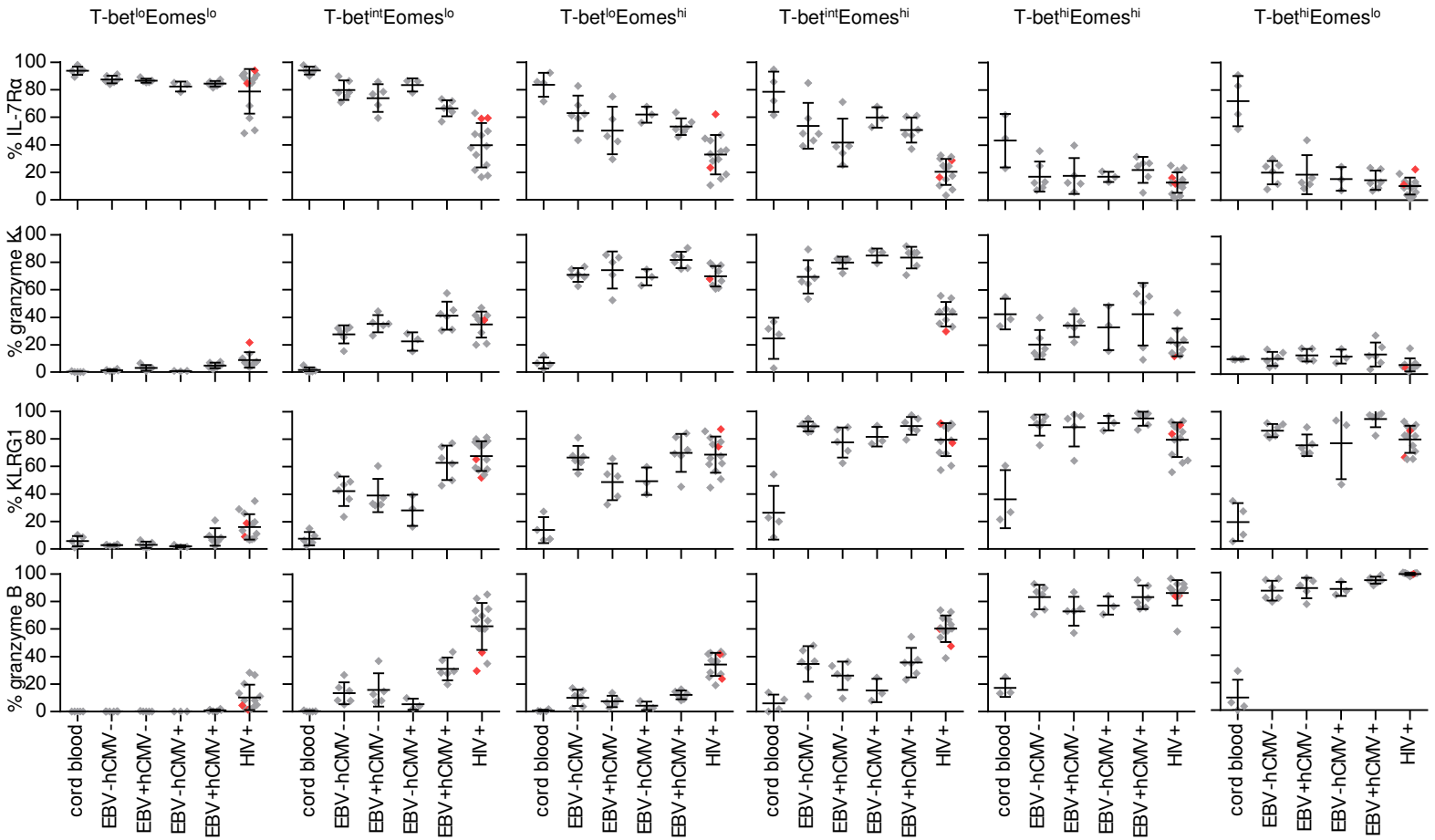

Figure S8. Viral infection history impacts on the associations between CD45RA/CCR7/CD28/CD27 phenotype and the functional potential
Expression frequencies of IL-7Rα (first row), granzyme K (second row), KLRG1 (third row) and granzyme B (last row) per CD45RA/CCR7/CD28/CD27-defined subset (columns) and per study group: cord blood samples, n=5; EBV/hCMV seronegative individuals, n=6; EBV seropositive individuals, n=5, hCMV seropositive individuals, n=3, EBV/hCMV double-infected individuals, n=6 and HIV-1-infected individuals (n=13) plotted on the X-axis of each graph, mean/SD shown. Note that early-like and RA^- effector-type populations were absent from cord blood, and that only two cord blood samples comprised sufficient RA^+ effector-type cells (>20 events) to be included in these analyses. The two HIV-1-infected patients not co-infected with hCMV are shown in red.

Figure S9. Infection history influences the associations between surface phenotype, T-bet/Eomes expression levels and the functional potential

Heat maps indicating the degree of expression of IL-7Rα (first column), granzyme K (second column), KLRG1 (third column) and granzyme B (last column) per combined surface marker- and T-bet/Eomes-defined CD8⁺ T-cell subset plotted on the X- and Y-axes, respectively, in cord blood samples (n=5, first row), EBV/hCMV seronegative individuals (n=6, second row), EBV mono-infected individuals (n=5, third row), hCMV mono-infected individuals (n=3, fourth row), EBV/hCMV double-infected individuals (n=6, fifth row) and HIV-1-infected individuals (n=13, last row). The degree of expression intensity is indicated by increments in color intensity and by the mean percentage of positive cells denoted in the boxes. Ns = not significant, * $p<0.05$, *** $P<0.001$.