Age-related changes in the TCR repertoire of human naïve and memory CD4$^+$ and CD8$^+$ T cells by longitudinal analysis

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Supplemental Figure S1: Changes of T cell counts in blood and memory T cell subsets with age.

Supplemental Figure S2: Variations of TCRα and TCRβ richness of naïve and memory CD4$^+$ and CD8$^+$ T cells isolated at the same time.

Supplemental Figure S3: CDR3 lengths of TCRα and TCRβ in total, naïve and memory CD4$^+$ and CD8$^+$ T cells in study subject.

Supplemental Figure S4: Development of equations to predict paired αβ TCR richness based on the separated TCRα and TCRβ sequences.

Supplemental Table S1: Demographics of study subjects and their blood T cell subset counts

Supplemental Table S2: Summary of αβ TCR repertoire of CD4$^+$ and CD8$^+$ T cells

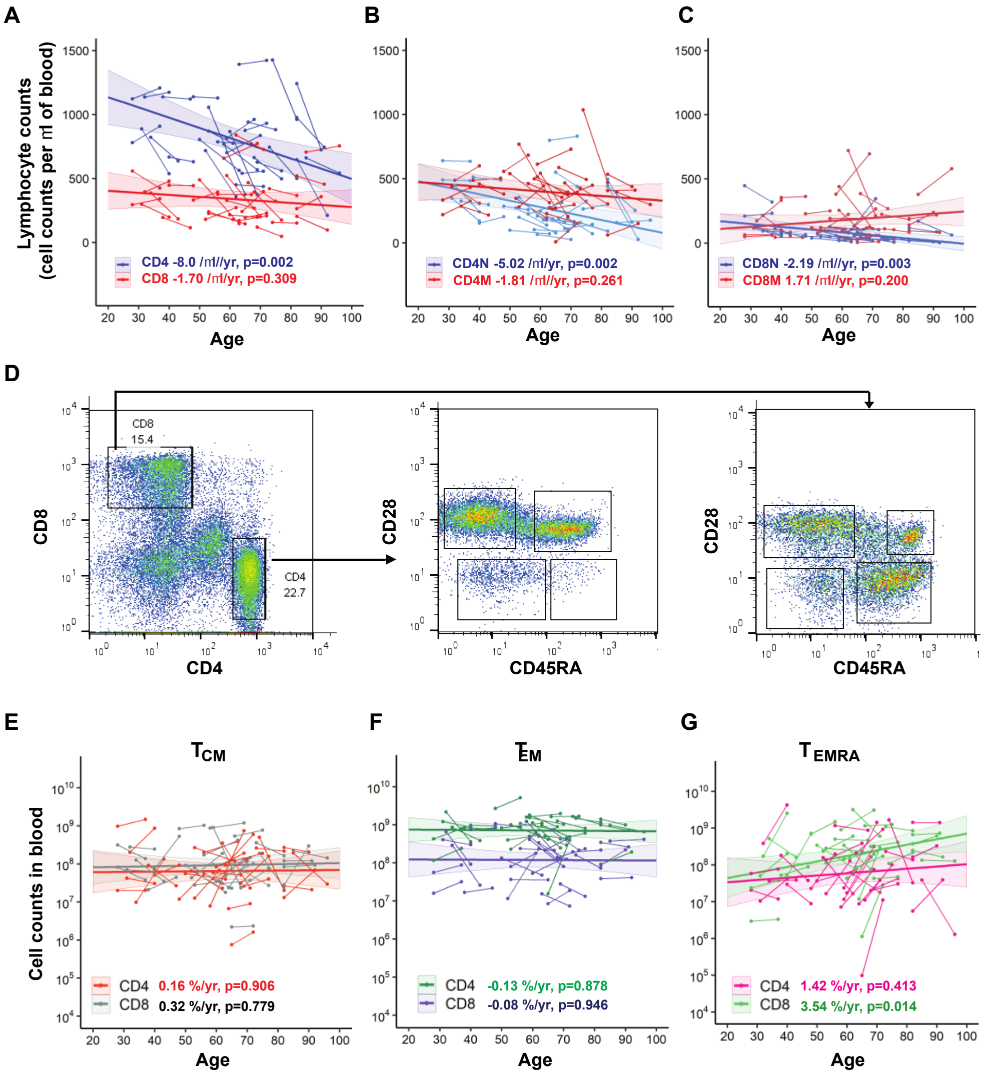
Supplemental Table S3: Summary of αβ TCR repertoire of naïve and memory CD4$^+$ and CD8$^+$ T cells

Supplemental Table S4: Overlap of TCR sequences between two visits

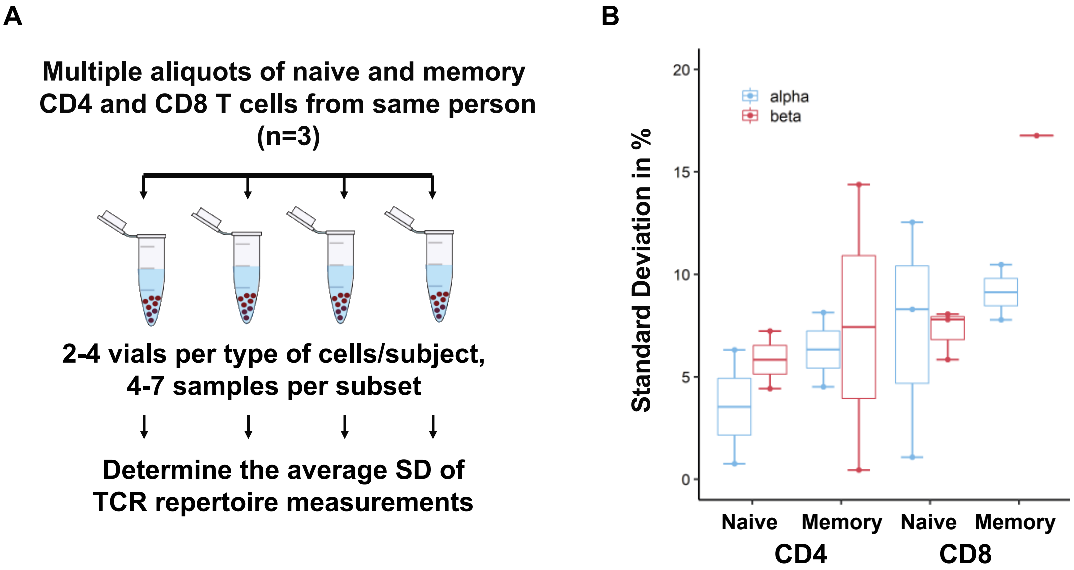
Supplemental Table S5: Overlap Percentages of TCR sequences between naïve and memory CD4$^+$ and CD8$^+$ T cells

Supplemental Table S6: Overlap of TCR sequences between CD4$^+$ and CD8$^+$ T cells

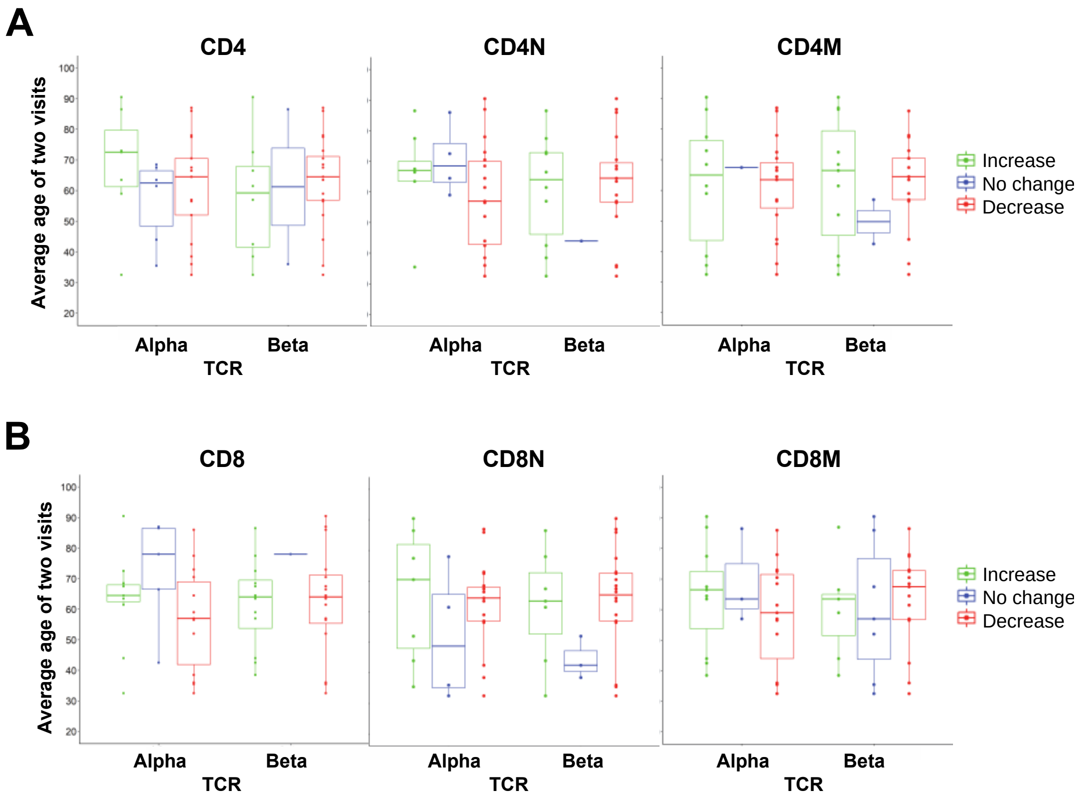
Supplemental Table S7: Oligos used in TCR library construction and sequencing



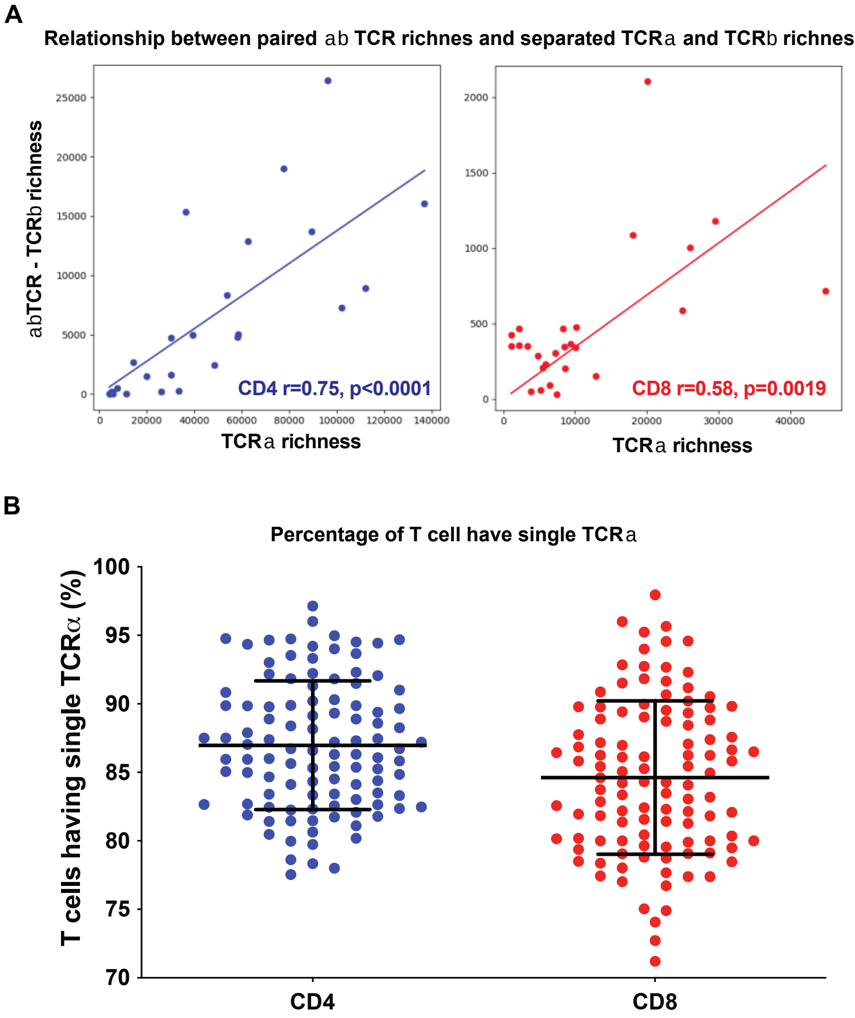
Supplemental Figure 1: Changes of T cell counts in blood and memory T cell subsets with age. A. Changes in CD4$^+$ and CD8$^+$ T cell counts in blood with age. Cell counts were calculated based on 1) lymphocyte counts per microliter of blood and 2) percentage of CD4$^+$ and CD8$^+$ T cells and naïve (CD45RA$^+$CD28$^+$) and memory cells in lymphocytes, calculated from flow cytometry. D. Gating of CD4$^+$ and CD8$^+$ T memory T cell subsets: T$_{CM}$(CD45RA$^-$CD28$^+$), T$_{EM}$(CD45RA$^-$CD28$^-$), and T$_{EMRA}$(CD45RA$^+$CD28$^-$). E-G, Numbers of CD4$^+$ and CD8$^+$ T$_{CM}$, T$_{EM}$, and T$_{EMRA}$ cells in blood at each visit and their change with age.



Supplemental Figure 2: Variations of TCRα and TCRβ richness of naïve and memory CD4$^+$ and CD8$^+$ T cells isolated at the same time. A. Experimental plan. Naïve and memory CD4$^+$ and CD8$^+$ T cells were isolated from blood (three healthy adults) by cell sort and equal number of sorted cells (0.5-1 x 10^6) were aliquot in 3-4 tubes. TCRα and TCRβ sequences in each tube were independently measured and then predicted to 10^7 by DivE method. B. Standard deviations of projected richness of TCRα and TCRβ are presented as percentages.



Supplemental Figure 3: Average age of three groups of TCRα and TCRβ richness changes in in total, naïve and memory CD4$^+$ and CD8$^+$ T cells. A. Box-Whisker plot of average age of three groups (decrease, no change, and increase) of richness changes with age in total, naïve (N), and memory (M) CD4$^+$ T cells.. B. Box-Whisker plot of average age of three groups of richness changes with age in total, naïve (N), and memory (M) CD8$^+$ T cells.



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Equations for calculating paired ab TCR richness based on separated TCRa and TCRb richness

CD4 **Paired ab TCR richness = 0.138 x [(0.870 x TCRa) + TCRb] + Max (0.870 x TCRa, TCRb)**

CD8 **Paired ab TCR richness = 0.035 x [(0.846 x TCRa) + TCRb] + Max (0.846 x TCRa, TCRb)**

Figure 4: Development of equations to predict paired αβ TCR richness based on the unpaired TCRα and TCRβ sequences. A. Linear relationship between 1) the paired αβ TCR richnesses minus the maximum unpaired richnesses and 2) the sum of the unpaired richnesses, TCRα and TCRβ, from αβ TCR data of single cell analysis. 745,182 and 158,305 αβ TCRs from CD4$^+$ and CD8$^+$ T cells, respectively, were used in this analysis. B. Percentage of T cells with a single TCRα. The values are derived via the analyses of 108 and 111 single T cell samples for CD4$^+$ and CD8$^+$, respectively, using published data with secondary TCRα information (see method for details). C. Equations for calculating paired αβ TCR richness based on unpaired TCRα and TCRβ richness of CD4$^+$ and CD8$^+$ T cells. TCRα richness is preceded by a factor indicating average contribution to TCRα richness by primary TCRα's, compensating contributions to TCRα richness by secondary TCRα's in bulk-sequencing.