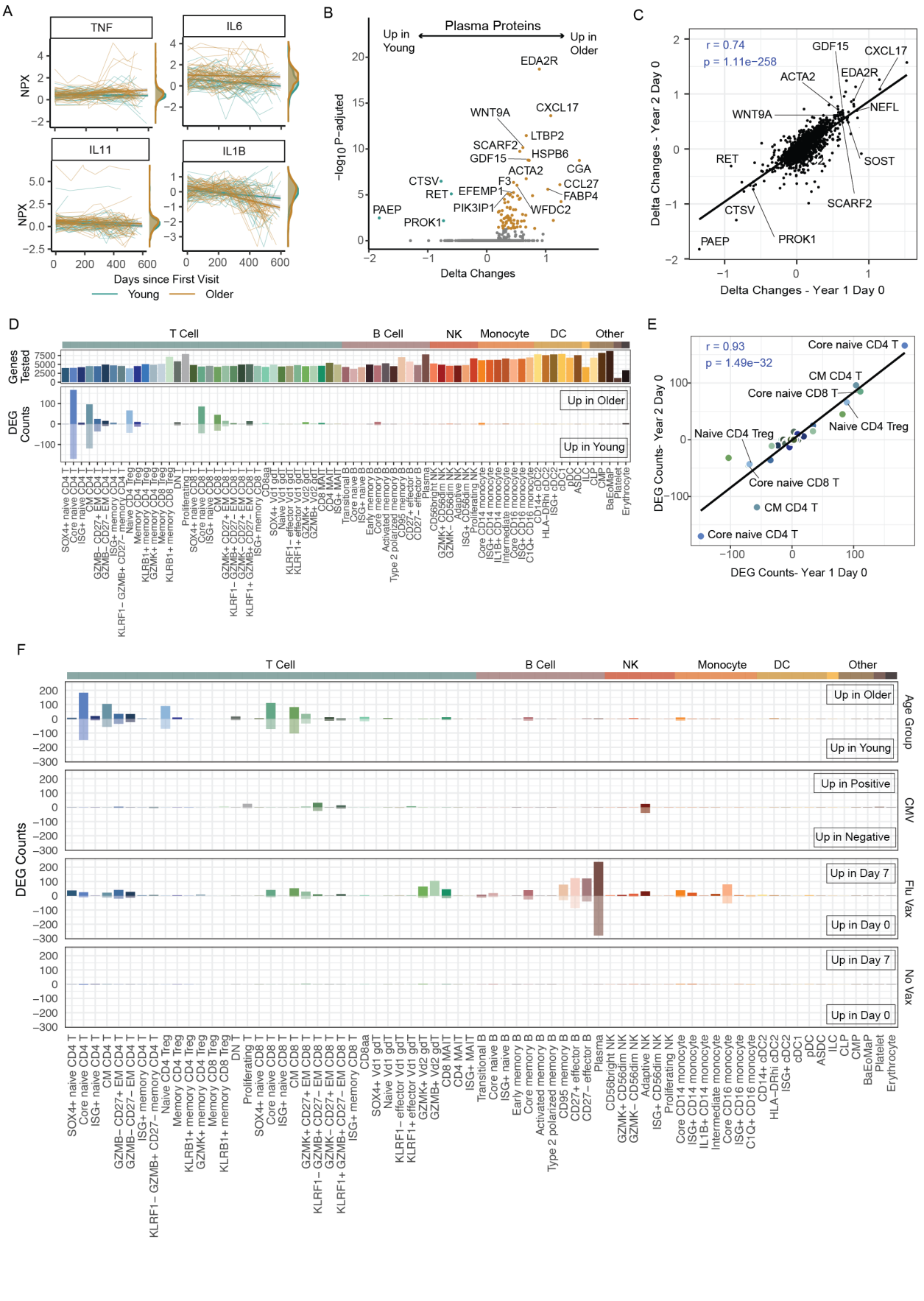


Figures 12

Supplemental Figure 1, in regard to Main Figure 2**. Impact of age, CMV infection and influenza vaccination on the healthy immune landscape. A.** Normalized protein expression (NPX) of inflammatory markers TNF, IL-6, IL-1b and IL-11 over time in young (teal) and older (bronze) adult plasma. **B**. Volcano plot of the age-related expression differences in circulating plasma proteome at baseline (Flu Vax Year 2 Day 0). **C**. Spearman correlation between age-related protein expression difference at Year 1 and Year 2. **D.** The number of down-regulated or up-regulated differential expressed genes (DEGs) from DEseq2 analysis of immune cell subsets from young (n=40) and older (n=44) adults at 'Flu Vax year 2 day 0' using DEseq2 analysis. **E**. Spearman correlation between the number of age-related DEGs in each immune cell subsets in 'Flu Vax year 1 day 0 and 'Flu Vax year 2 day 0'. **F.** The number of DEGs in immune cell subsets, comparing CMV (CMV-positive versus CMV-negative), Flu vaccination time series ("Flu Vax", Day 0 and Day 7 post-flu vaccination) and No Vax time series ("No Vax", Day 0 vs Day 7 after no vaccination) to that of age. DEGs were defined as log2fc >0.1 and padj<0.05 for all comparisons.

A

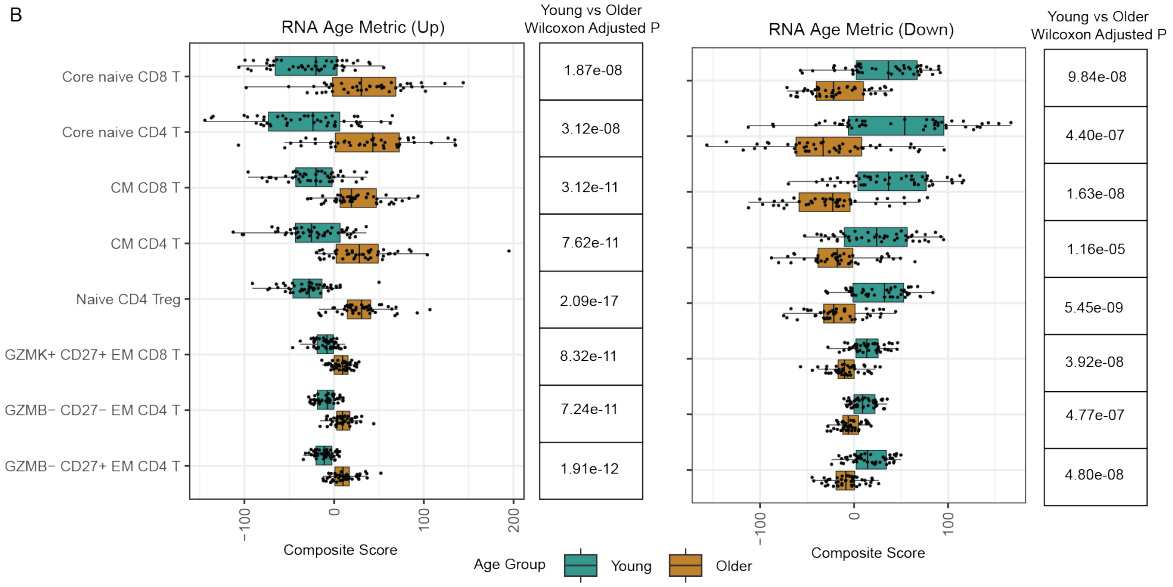
$$\text{Age Composite Metric}_i = \sum_{j=1}^{n} \frac{E_{ij} - \mu_j}{\sigma_j}$$

(Up or Down)

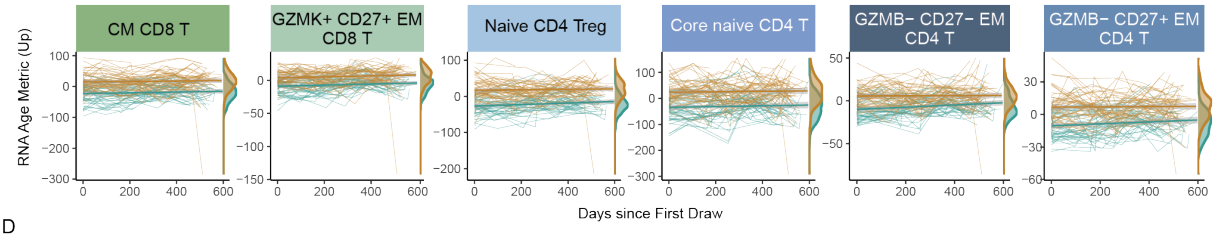
Where:

- Age Composite Metric$_i$ is the composite score for sample i.
- E_{ij} is the mean pseudo-bulked expression for gene j in sample i
- n is the total number of genes in the set, with n ≥ 20.
- μ_j is the mean expression of gene j across all samples.
- σ_j is the standard deviation of expression of gene j across all samples.
- The Age Composite Metric is calculated from genes that are differently regulated in older adults comparing with young adults. The 'Up' metric is based on up-regulated genes, and the 'Down' metric is based on down-regulated genes.

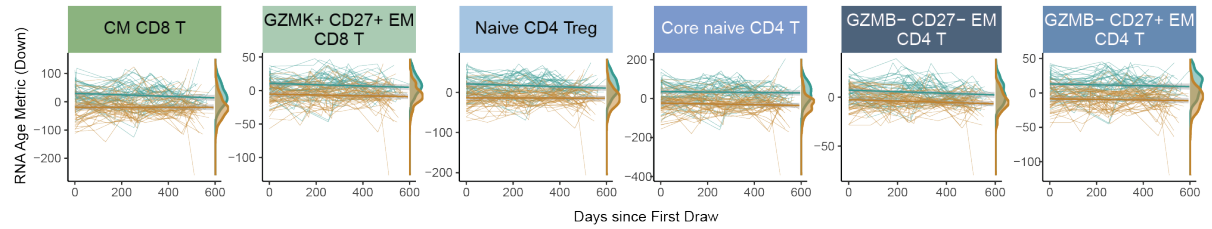
B



C

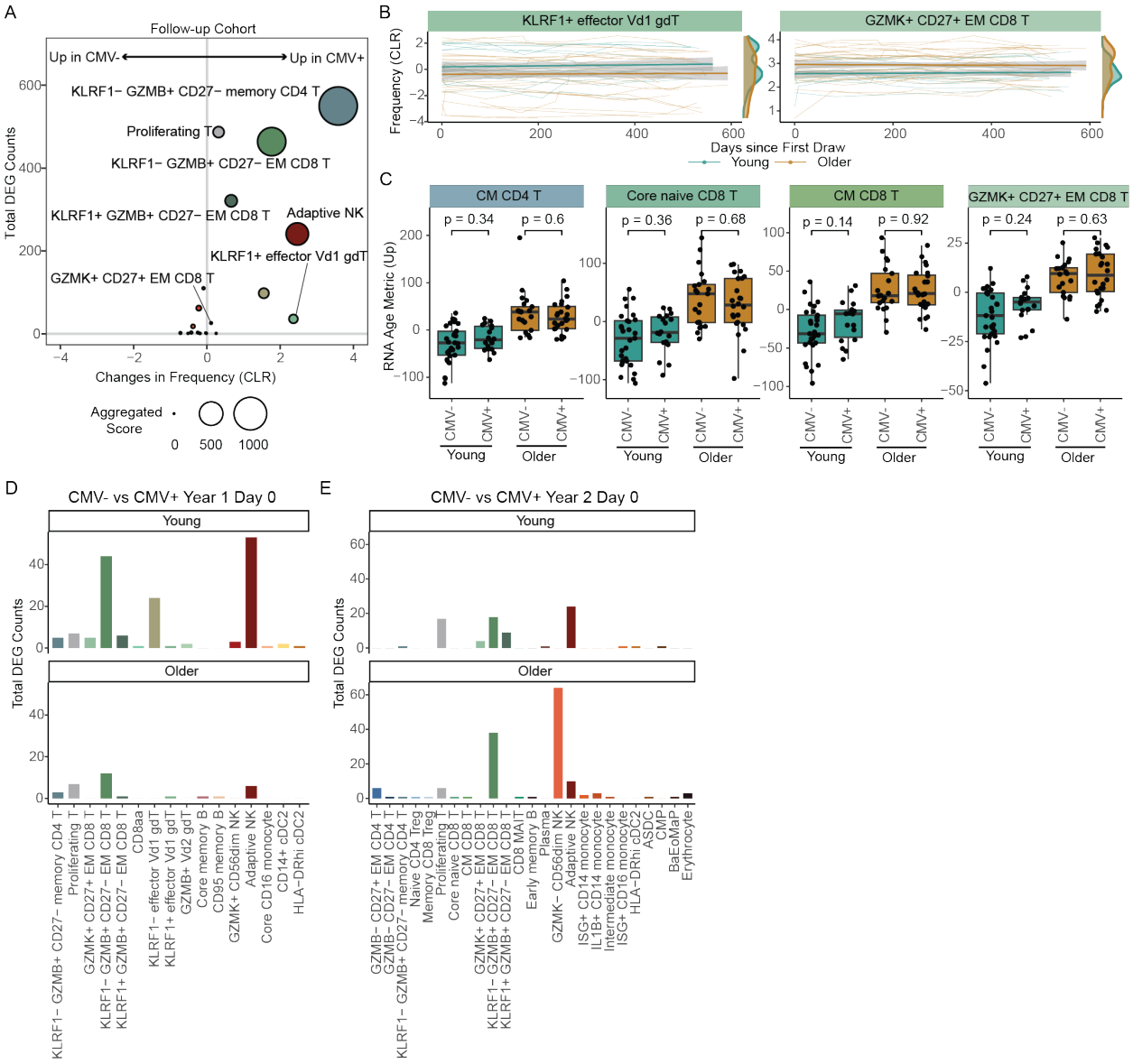


D

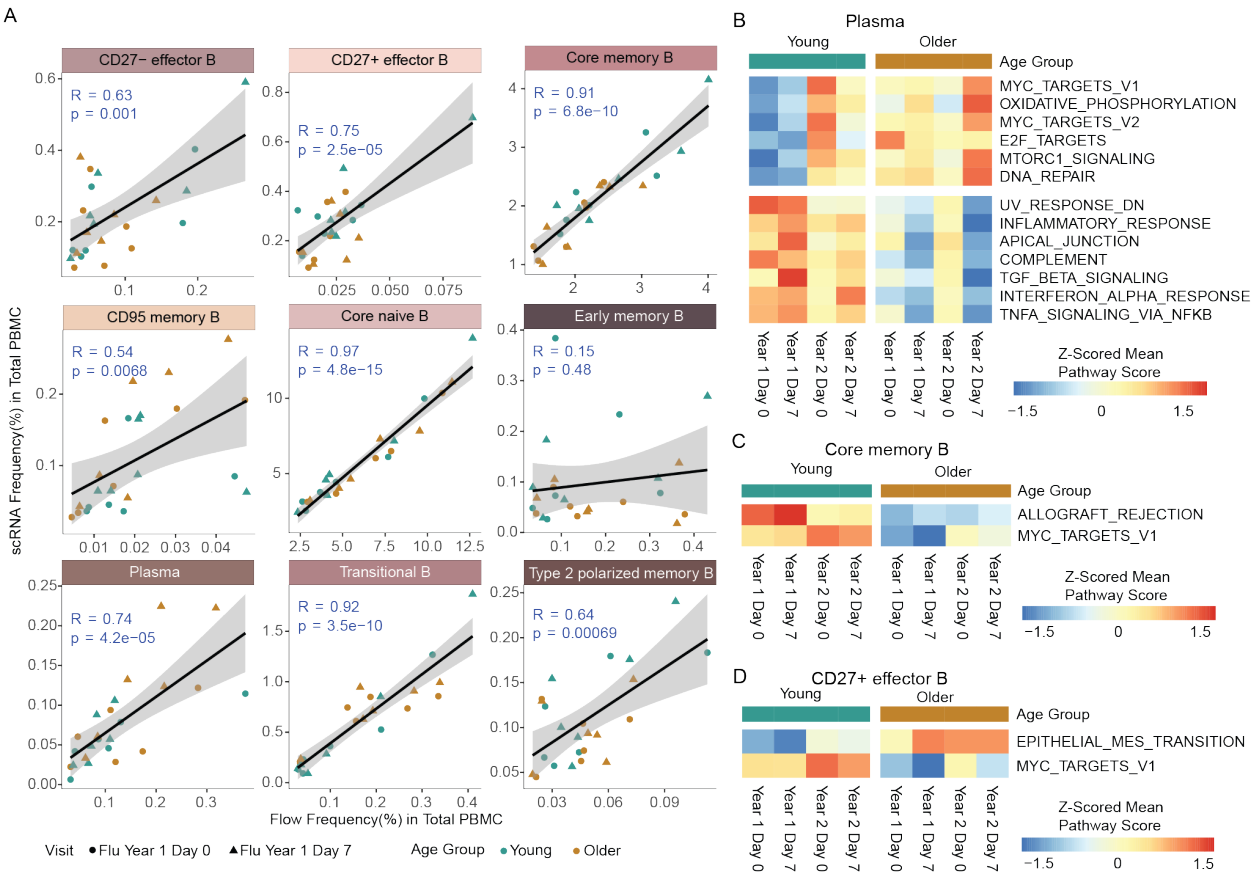


Supplemental Figure 2, in regard to Main Figure 2**. Maintained, age-related transcriptional signatures in healthy immune cell subsets. A.** Equation for calculating the composite age score in each immune cell subset with more than 20 DEGs between young and older adults. **B.** RNA age metric (upregulated and downregulated genes) in 8 subsets (all subsets with >20 DEGs) comparing young and older adults on year 1 day 0 samples. Each dot is from a single donor. P-value was determined using the Wilcoxon rank-sum test. **C**. RNA age metric (upregulated genes) in select subsets over time in young and older adults. Each donors' samples are connected with a thin line. **D**. RNA age metric (downregulated genes) in select

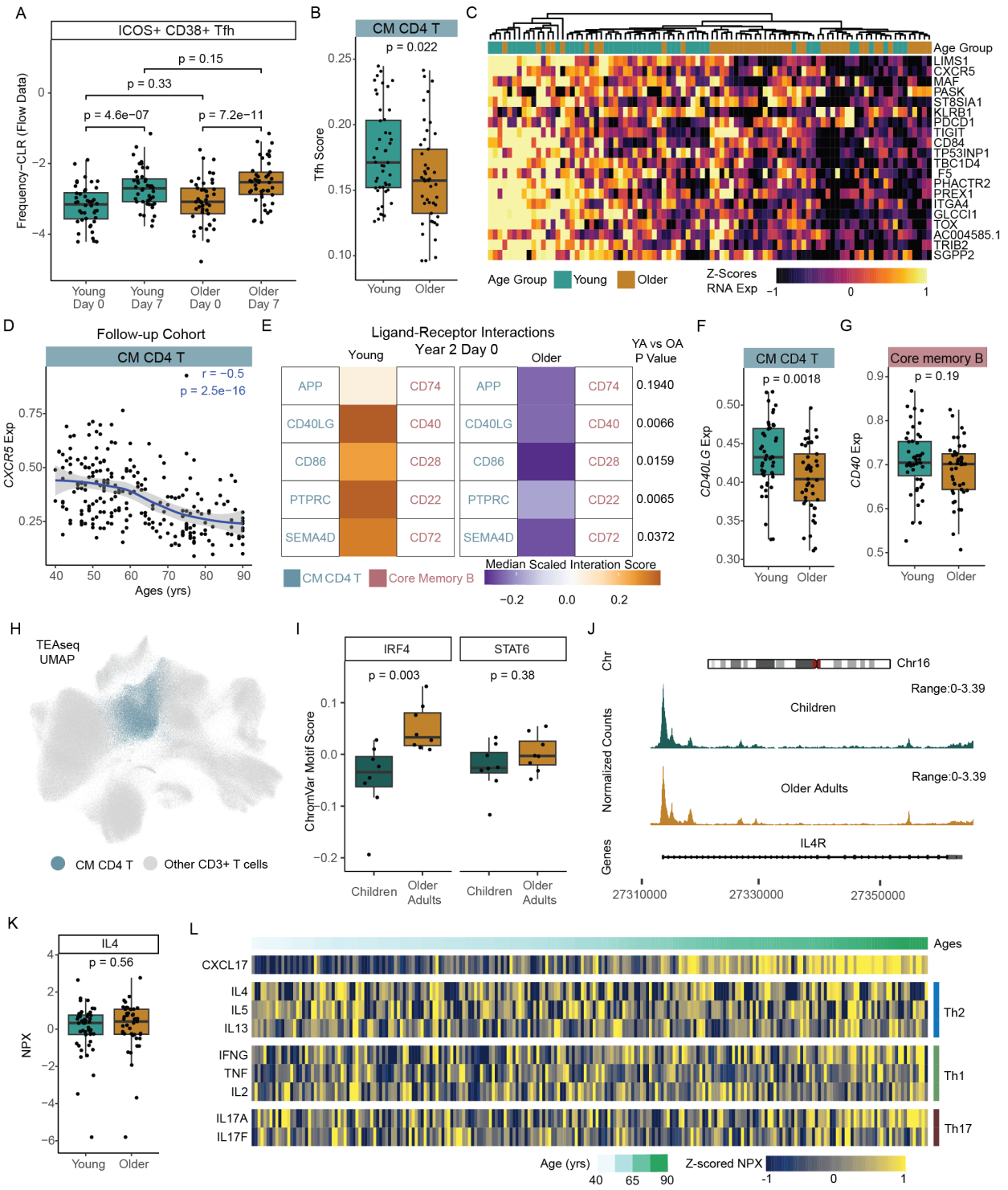
subsets over time in young and older adults. Each donors' samples are connected with a thin line.



Supplemental Figure 3, in regard to Main Figure 4**. Transcriptional landscape of healthy immune cell subsets altered by CMV and age. A.** Bubble plot comparison of the change in frequency (using centered log-ratio (CLR) transformation) and number of DEGs between CMV+ (n=136) and CMV- (n=97) adults in our follow-up cohort. Bubble size shows a combined metric of change defined as -log10(p.adj from CLR freq comparison) x DEG_Counts**. B.** Select subset frequencies in PBMCs shown over time. Teal dots are young adults. Bronze dots are older adults. Regression line shown. **C.** RNA age metric (upregulated genes) in select T cell subsets split by age and CMV infection status. P-values were calculated using Wilcoxon rank sum test. **D-E.** The number of DEGs (log2fc >0.1 and p.adj<0.05) in immune cell subsets comparing CMV+ and CMV- individuals, in young and older adults separately from our longitudinal cohort at **D.** 'Flu Vax year 1 day 0' and **E.** 'Flu Vax year 2 day 0'.



Supplemental Figure 4, in regard to Main Figure 5**. Age-associated responses of non-naive B cell subsets to flu vaccination. A**. Correlation plots of RNA-quantified (y-axis) and flow cytometry-quantified (x-axis) level 3 B cell population (individual plots) frequencies of total live PBMC. Data shown for 6 young and 6 older adult subjects in flu year 1 that were represented in both scRNA-seq and cytometry analyses. P-value and r values of the Pearson correlation are displayed. **B-D**. Sample level enrichment analysis (SLEA) scores for the top pathways in the Hallmark database at each timepoint for **B.** plasma cells, **C**. core memory B cells and **D**. CD27+ effector B cells in young and older adults.



Supplemental Figure 5, in regard to Main Figure 6**. Age-related transcriptional alterations in CM CD4 T cells independent from circulating cytokine signatures. A.** The CLR transformed frequency of ICOS+ CD38+ Tfh cells determined by spectral flow cytometry, at day 0 and day 7 post-influenza vaccination, comparing responses in young and older adults. P-values were calculated using Wilcoxon's signed-rank test (paired) for the comparison between Day 0 and Day 7, and using the Wilcoxon rank-sum test for all other comparisons. **B**. Tfh activity score was

determined by NMF projection in CM CD4 T cells in young and older adults. **C.** Expression of leading-edge genes in Tfh activity score in young and older adults. **D.** CXCR5 expression in CM CD4 T cells across age in our follow-up cohort. **E.** Receptor-ligand interaction prediction between CM CD4 T cells and core memory B cells in young (n=40) and older (n=44) adults from a single time point (Flu Year 2 Day 0). **F.** CD40LG expression in CM CD4 T cells in young and older adults. **G.** CD40 expression in core memory B cells in young and older adults. **H.** TEAseq UMAP of T cells based on RNA module, with CM CD4 T cells highlighted. **I**. IRF4 and STAT6 transcription factor (TF) activity based on Chromvar analysis of scATACseq data in CM CD4 T cells from children (n=8) and older adults (n=8). P-value was determined by Wilcoxon rank-sum or Wilcoxon signed-rank test, as appropriate, unless otherwise indicated in legend. **J.** Chromatin accessibility tracks of the *IL4R* gene region in CM CD4 T cell subsets from TEA-seq data, showing normalized read coverage. **K**. IL-4 normalized protein expression (NPX) in young (teal) and older (bronze) adult plasma. **L**. Normalized protein expression (NPX) of select Th1-, Th2- and Th17-related serum proteins in our follow-up cohort, with donors ordered by age.