Supplementary Figures

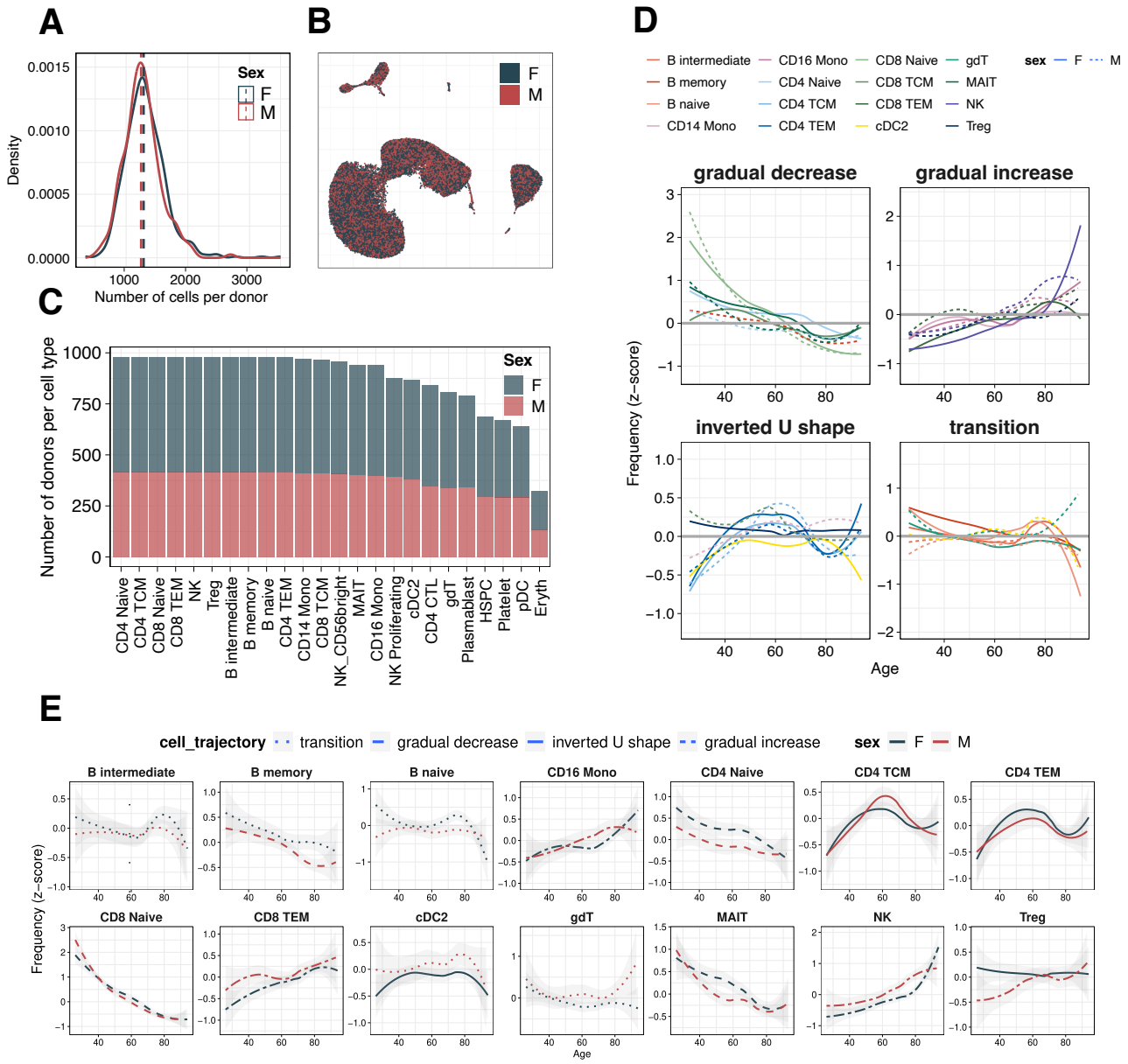

Figure S1. Data overview and immune cell trajectories across the lifespan. A. Distribution of the number of cells per donor, shown separately for males (red) and females (blue). **B.** UMAP plot of all immune cells, colored by sex. For visualization purposes we downsampled to 500,000 cells (representing ~50% of the total cells) **C.** Number of donors per cell type and sex. **D.** Immune cell trajectory groups over the lifespan. The y-axis represents normalized cell frequency (z-score), and the x-axis represents age. Color indicates cell type and line type indicates sex. **E.** Immune cell trajectory per cell type over the lifespan. The y-axis represents normalized cell frequency (z-score), and the x-axis represents age. Color indicates sex and line type indicates trajectory group.

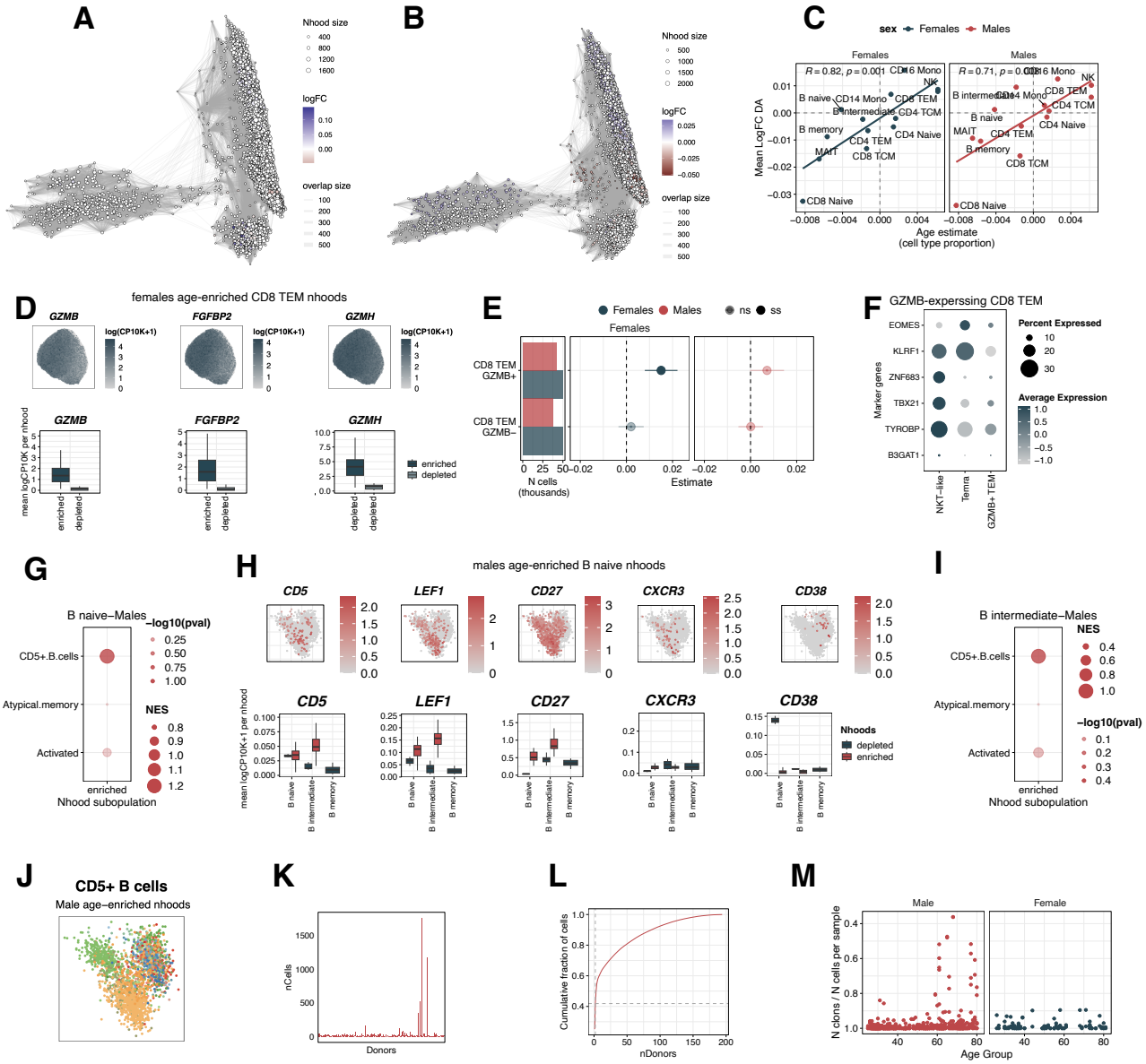

Figure S2. Sex-stratified differential abundance analysis. Nhood (neighborhood) graph of the results from differential abundance testing with age, separately by males **(A)** and females **(B).** Nodes represent Nhoods, colored when they are significant (Spatial-FDR < 0.05), and edges correspond to the number of cells shared between Nhoods. **C.** Correlation of the mean logFC per cell type across Nhoods (y-axis) versus the age-estimate of the cell type compositional analysis (x-axis). **D.** Top. UMAP plots of female-age-enriched CD8+ T effector memory (TEM) cells colored by the expression of the three top marker genes (*GZMB, FGFBP2*, and *GZMH*). Bottom. Average log-normalized, scaled expression per Nhood of the same genes. Only Nhoods that are significantly enriched or depleted with age are shown. **E.** Left. Total number of cells analyzed per CD8+ TEM subpopulation and sex. Center. Cell type composition analysis estimates with age from *GZMB*+/- CD8+ TEM subpopulations in females. Right. Cell type composition analysis estimates with age from CD8+ TEM subpopulations. **F.** Average expression of marker genes for *GZMB*-expressing cytotoxic subpopulations of CD8+ TEM. **G.** Gene set enrichment analysis (GSEA) of marker genes of age-enriched B naive Nhoods in males compared to transcriptional signatures of B naive subpopulations from Terekhova et al., 2023. Significant enrichments (FDR < 0.05) are highlighted. **H.** Top. UMAP plots of male-age-enriched B naive and intermediate cells, colored by the expression of the top marker genes. Bottom. Average log-normalized, scaled expression per Nhood in males of the same marker genes.Only Nhoods that are significantly enriched or depleted with age are shown. **I.** Same as panel **G** but for B intermediate cells. **J.** UMAP plot of male *CD5*+ B subpopulation colored by donor. **K.** Number of cells per male donor belonging to *CD5*+ subpopulation. Donors

are sorted by increasing age. **L.** Cumulative fraction of cells from *CD5*+ B subpopulation belonging to a particular donor. **M.** Ratio of number of clones by number of cells per sample in *CD5*+ B cells through the lifespan in males (left) and females (right)

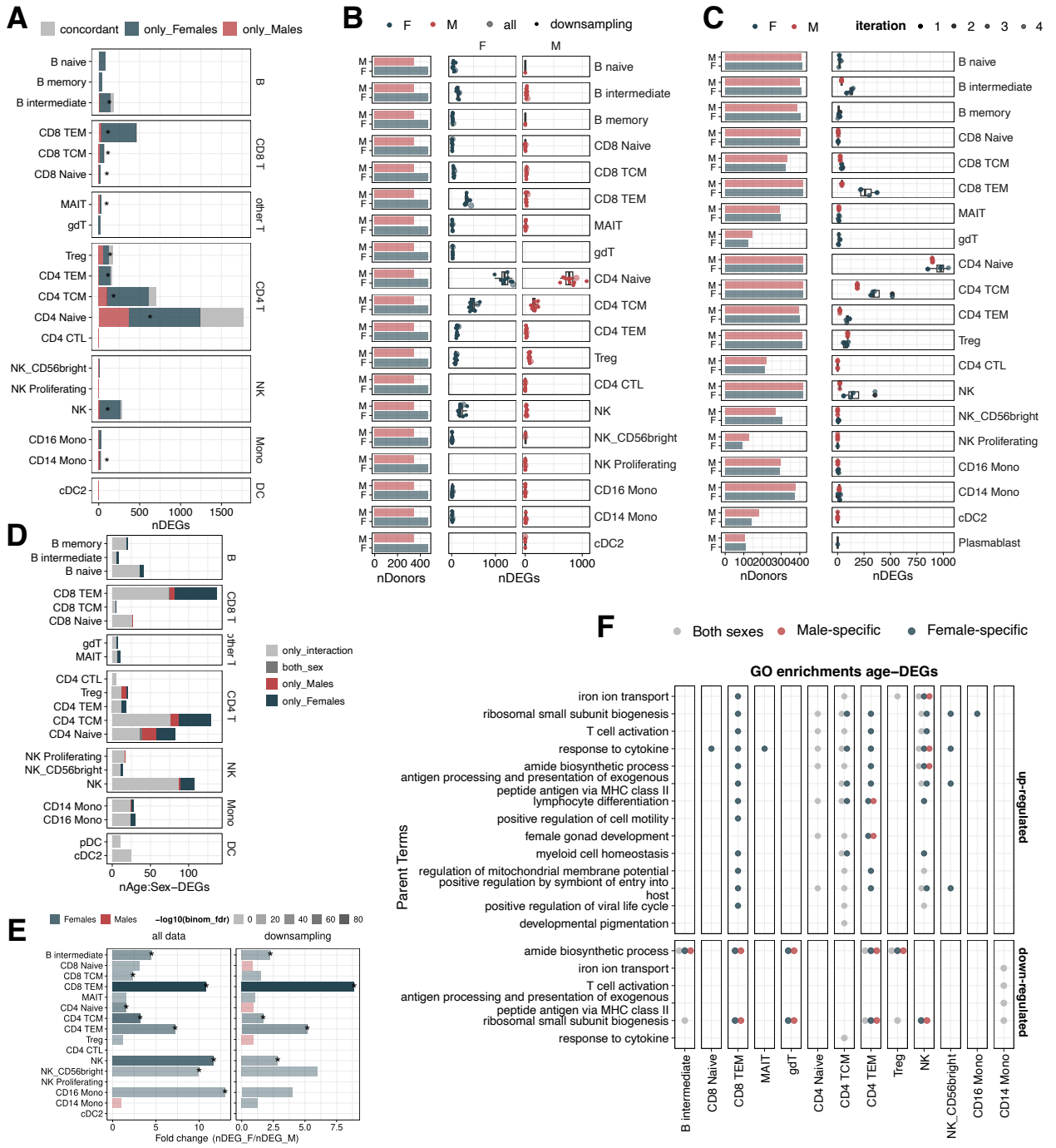

Figure S3. Sex-stratified linear age-differential expression analysis. A. Overlap of age-differentially expressed genes (age-DEGs) in each sex-stratified analysis. Asterisks indicate significant overlaps (FDR< 0.05). **B.** Left. Total number of donors analyzed per cell type. Right. Number of age-DEGs detected in males and females after downsampling donors to match the number of donors in the cell type with the lowest sample size that still yielded at least one age-associated DEG (i.e., 468 donors in female gdT cells and 347 donors in male CD4+ CTL cells). Light dots represent age-DEG counts from ten independent downsampling iterations, and the dark one correspond to the real number of age-DEGs. **C.** Same as panel **B** but downsampling to the same number of donors between sexes for each cell type. **D.** Number of DEGs (nominal p-value < 0.01) from a model including the Age:Sex interaction term, colored to indicate whether each gene was also detected in the sex-stratified age-DEA. **E.** Ratio of the number of age-DEGs in females versus males, colored by the sex with the higher number of age-DEGs. Asterisks indicate statistically significant sex bias (binomial test, FDR < 0.05). Intensity of the color corresponds to $-\log_{10}$(FDR) value of the binomial test. Results are shown for the full dataset (left) and after downsampling to equal numbers of donors between sexes (right). **D.** Non-redundant Gene Ontology (GO) terms from enrichment analysis of upregulated (top) and downregulated (bottom) age-

DEGs. Each dot indicates whether the corresponding GO term is enriched in sex-shared (grey), male-specific (red), or female-specific (blue) age-DEGs.

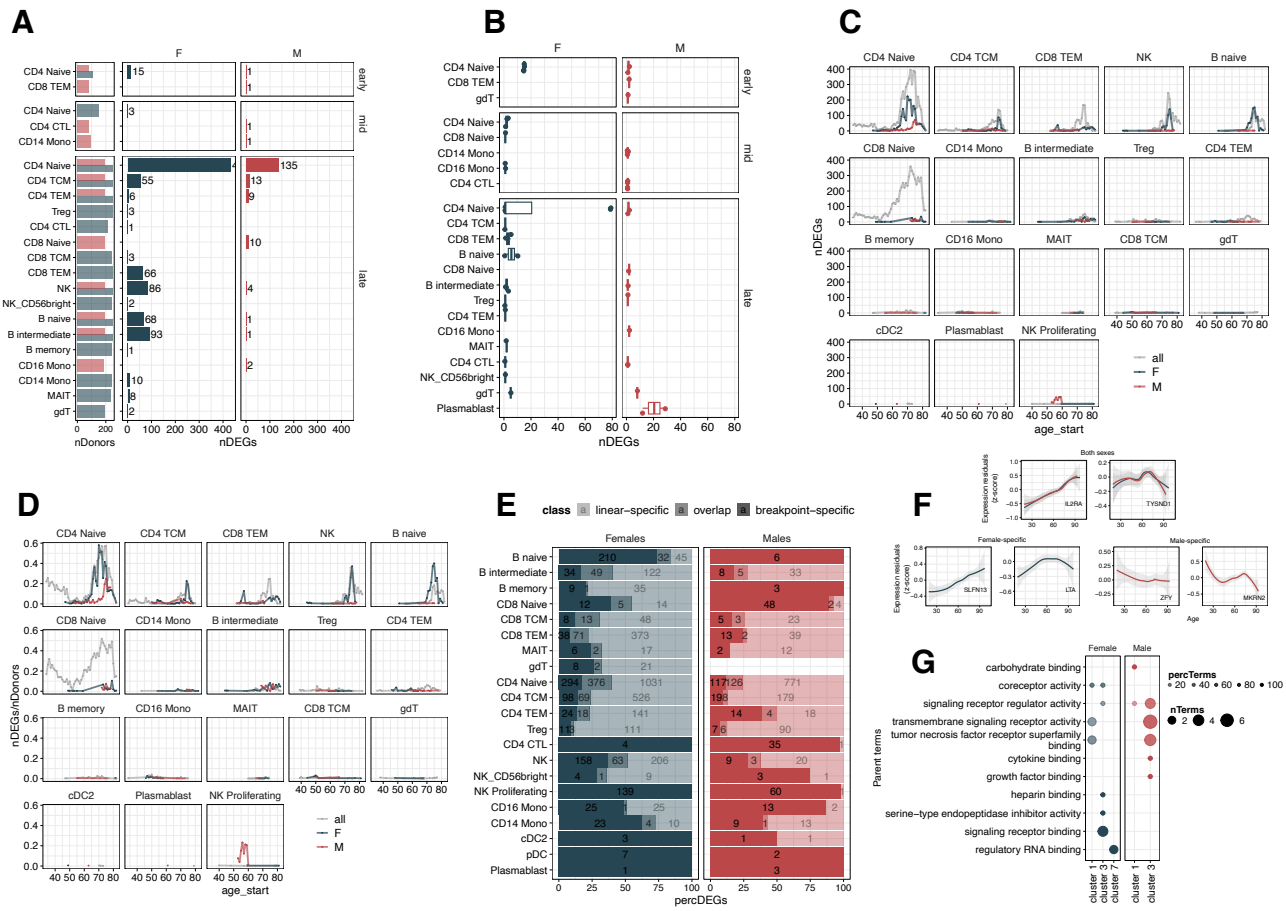

Figure S4. Sex-stratified non-linear age-differential expression analysis. A. Left. Number of donors per cell type. Right. Number of age-differentially expressed genes (age-DEGs) per cell type across age bins: early (<50 years old), mid (40–60 years old), and late (≥50 years old), separately in males and females. **B.** Number of age-DEGs detected in males and females after downsampling to match the number of donors in the smallest age group (early (<50 years old) in females —111 donors—, and mid (40–60 years old) in males —80 donors—). Each dot represents the result from one of the four independent downsampling iterations. **C.** Number of age-DEGs identified through breakpoint analysis in each cell type across the lifespan in females (blue), males (red) and the combined dataset (grey). **D.** Same as panel **C** but the y-axis shows the number of age-DEGs normalized by the number of donors in each comparison. **E.** Percentage of age-DEGs across cell types in each sex, identified trough: breakpoint analysis (breakpoint-specific), linear models (linear-specific) or both (overlap). **F.** Representative examples of gene expression trajectories illustrating linear (left) and non-linear (right) transcriptional changes in immune aging. Top: age-DEGs in both sexes. Bottom: Female-specific age-DEGs (left) and male-specific age-DEGs (right). **G.** Non-redundant Gene Ontology (GO)

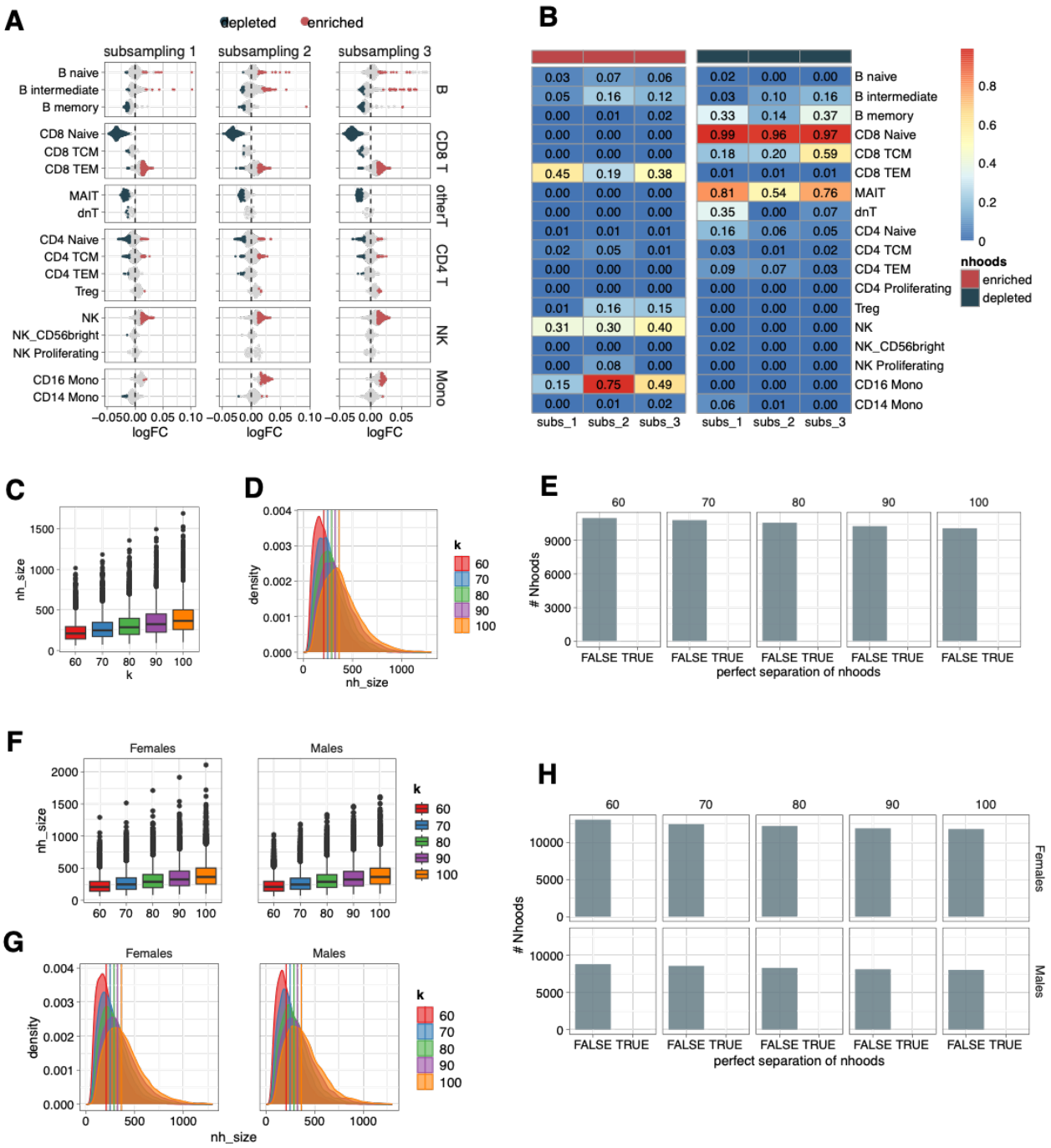

Figure S5. Differential abundance analysis: replication using data subsamplings and fine-tuning the *k* parameter for the KNN graph a. Beeswarm plot of the distribution of neighborhood (Nhood) logFC with age for each data subsampling (25% of the total cells; 316,939 cells out of a total of 1,272,489 cells). Each dot corresponds to a Nhood of cells assigned to a dominant cell type (>80% of cells within the neighborhood (see **Methods**)). Age-DA Nhoods (Spatial-FDR < 0.05) are colored. **b.** Proportion of age-DA enriched (left) and depleted (right) Nhoods in each data subsampling. **C.** and **D.** To identify the proper value of nearest neighbors to consider for the graph building (*k*), we set different *k* values (60, 70, 80, 90 and, 100), and compared the distribution of the number of Nhoods for each Nhood size **(C)** and the distribution of the Nhood size across different values of *k* **(D)**. Vertical lines in **C** correspond to the mean Nhood size. **E.** Number of Nhoods that have perfect data separation based on age categories (TRUE) versus the ones that do not (FALSE) at different values of *k*. Age categories have been defined as: young (<20 years), middle-aged (21–40 years), and old (>41 years) (see **Methods**)).