BIOINFORMATICS INSTITUTE

# Transcriptional signatures and age-related changes in $CD8^+HLA\text{-}DR^+$ regulatory T cells

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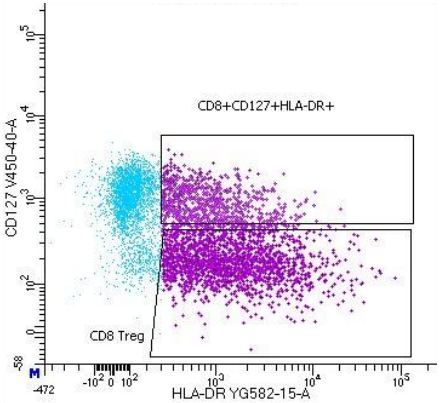
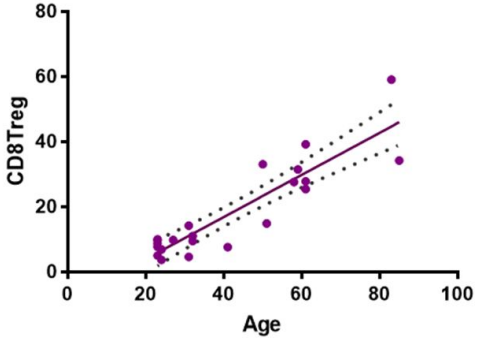
# About CD8⁺HLA-DR⁺ Treg

- First described in 2014, the CD8⁺HLA-DR⁺ regulatory T lymphocytes (CD8⁺Treg) subset is known for its role in suppressing effector T cells through checkpoint inhibitor molecules (such as CTLA-4 and PD-1), sharing certain features with conventional CD4⁺Treg cells.
- Despite this, the detailed nature and function of these cells are still not well understood. Understanding this subset is particularly significant in light of age-related alterations in the immune system and the heightened vulnerability of CD8⁺ T lymphocytes to such changes.
- Preliminary findings indicated that the CD8⁺HLA-DR⁺ population differentiates into two subpopulations based on CD127 (*IL7R*) surface expression. This led to defining the CD8⁺Treg phenotype as CD3⁺CD8⁺HLA-DR⁺CD127$^{low}$.

*Experimental data:*




*Flow cytometry data, gating from live CD3⁺CD8⁺ T cells*




*Correlation of the percentage of CD8⁺Treg (from CD8⁺ T cells) with the age of donors*

# Project aim and objectives

**Aim**:

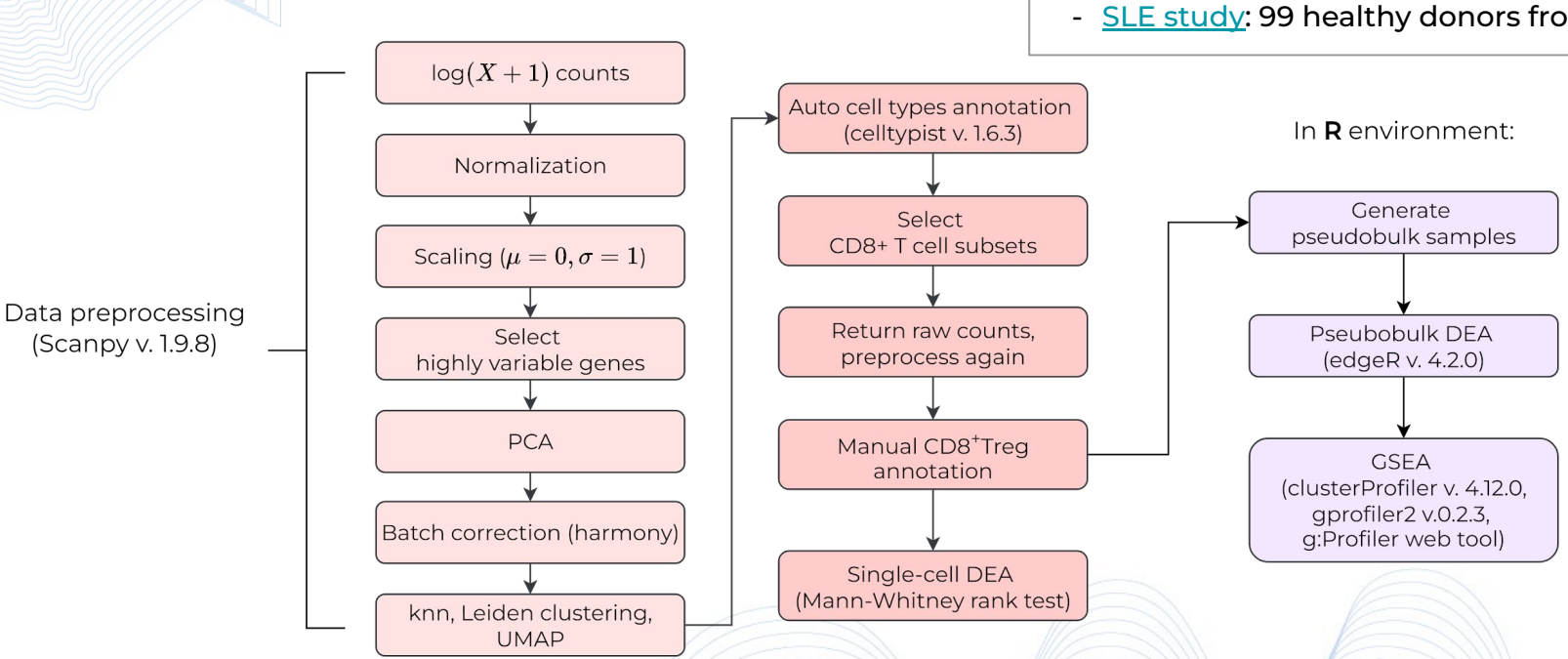
To determine transcriptional signatures and age-realed gene expression changes in the CD8+HLA-DR+ Treg (CD8+Treg) population using open single-cell RNA seq data

**Objectives:**

1. Develop a data processing pipeline, identify the $CD8^{+}$Treg cluster
2. Determine transcriptional signatures of $CD8^{+}$Treg
3. Evaluate age-related gene expression changes in the $CD8^{+}$Treg subset

# Methods

**Workflow scheme:**




**Data:**

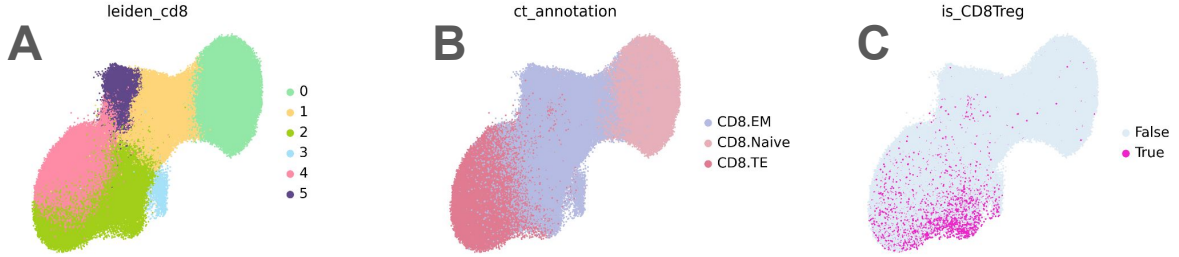
- OneK1K: 982 donors from 19 to 97 y.o.
- SLE study: 99 healthy donors from 22 to 75 y.o.

*The described analysis is available as Jupyter-notebooks on a GitHub page*

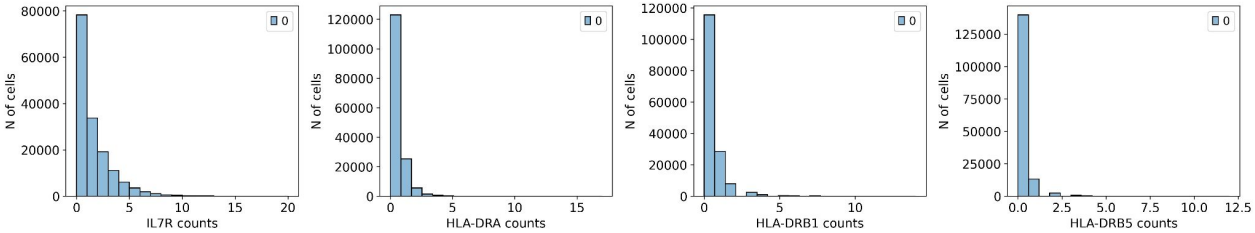
# CD8⁺Treg transcriptional signatures

To determine CD8⁺Treg signatures, scRNA-seq data from donors aged 19 to 60 years (n = 299) were analyzed. According to previous experimental results, T cells in this age range typically do not exhibit distinct age-related changes.

It should be noted that cells potentially corresponding to **CD8⁺Treg were not separated into a distinct cluster** using the Leiden algorithm but were distributed among CD8⁺ effector T cells. Therefore, the CD8⁺Treg population was defined by their phenotype as CD8⁺ effector T cells with **increased expression of *HLA-DRA*, *HLA-DRB1*, and *HLA-DRB5*** genes **and decreased expression of *IL7R* (CD127)**.




*CD8+ T lymphocytes annotation on UMAP representation.*
*A - Leiden clusters, B - automatic celltypist annotation, C - manual annotation of CD8⁺Treg cells based on HLA-DR and IL7R gene expression levels*

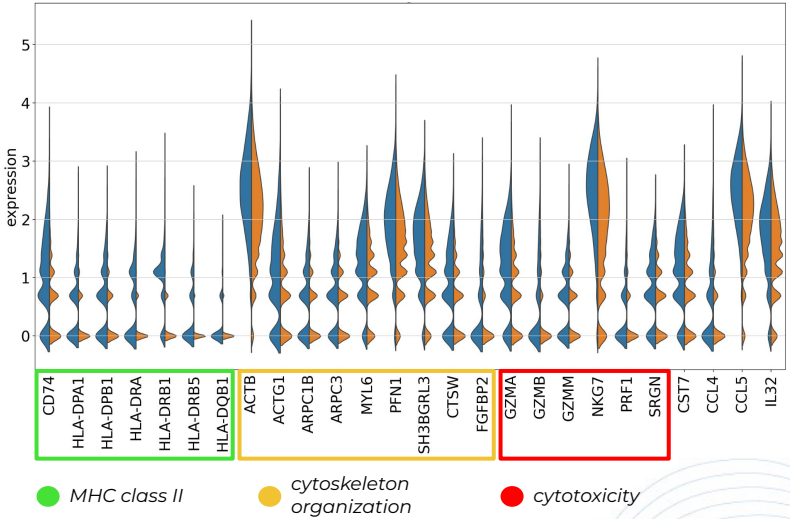



*Distribution of gene counts*

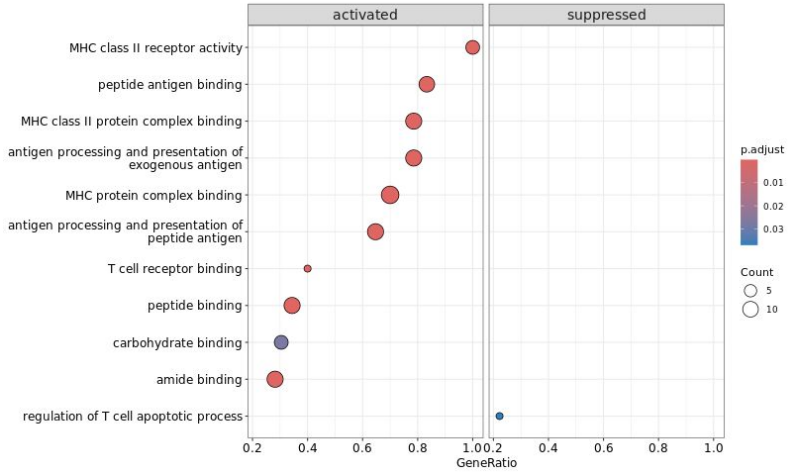
# CD8⁺Treg transcriptional signatures

DEA at the single-cell level revealed that the CD8⁺Treg, compared to the main population of CD8⁺ effector T cells, exhibited increased expression of **genes associated with MHC-II-mediated antigen presentation, cytotoxicity, and cytoskeletal organization**.

At the pseudobulk level, GSEA based on GO terms the CD8⁺Treg population also was characterized by increased expression of genes involved in antigen presentation, particularly those mediated by MHC class II molecules.




*DE genes of CD8⁺Treg (blue) vs. main CD8⁺ effector T cells population (orange) at the single-cell level*



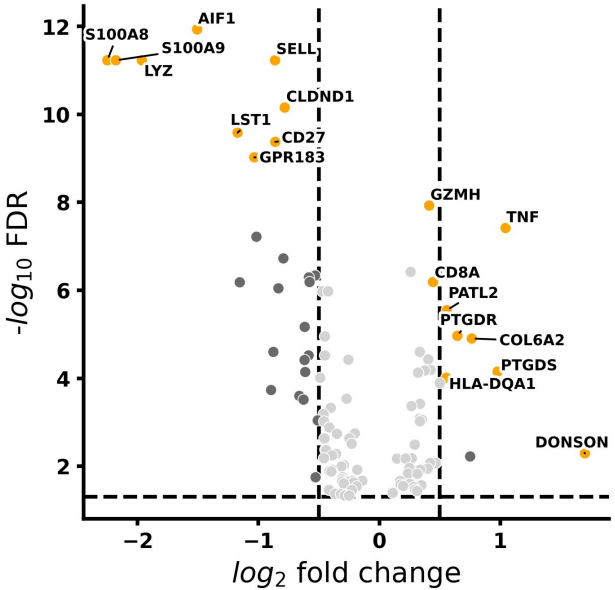

*GSEA results for CD8⁺Treg subset at the pseudobulk level*

# Age-related changes in CD8⁺Treg

To assess age-associated changes at the transcriptomic level, we analyzed CD8⁺Treg populations from young (20-35 years, n = 152) and old (70-97 years, n = 424) donors.

DEA at the pseudobulk level among older donors showed a shift in the CD8⁺Treg transcriptional profile **towards a terminally differentiated phenotype**. Specifically, a decrease in the expression of cytotoxic molecules such as *LYZ*, *GZMA*, and *GZMK* was observed, while the expression of *GZMH*, characteristic of terminally differentiated CD8⁺ T lymphocytes, increased.

Additionally, CD8⁺Treg were characterized by increased expression levels of MHC molecules, as confirmed by GSEA (next slide)

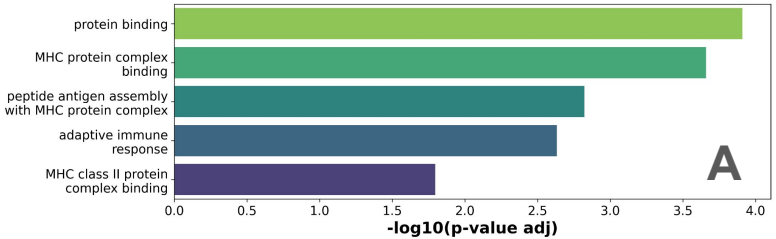


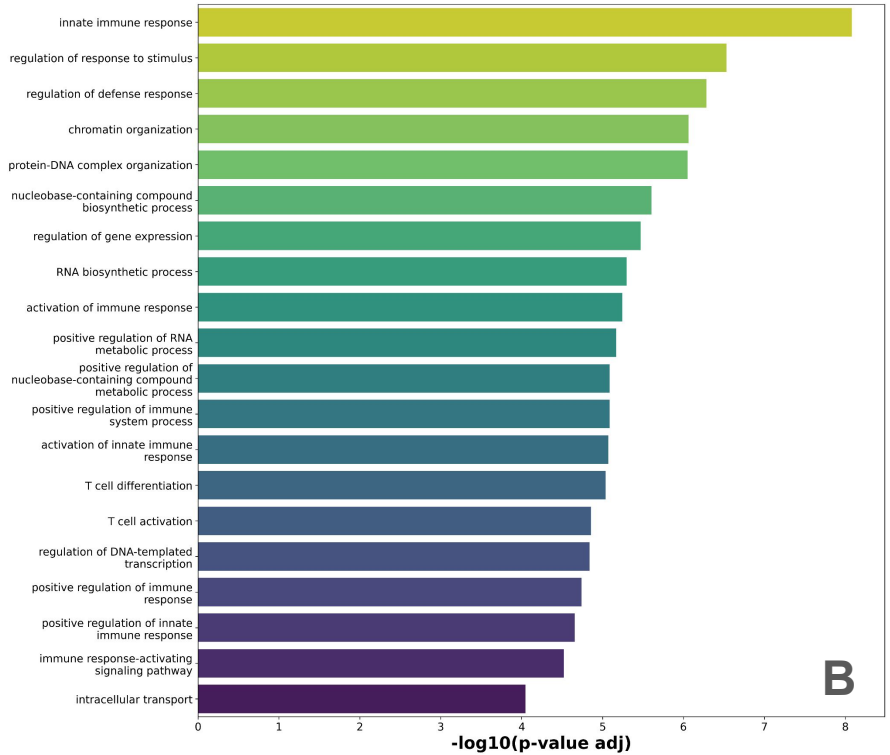

*DE genes of CD8⁺Treg subset from old donors (70-97 y.o.) at the pseudobulk level*

# Age-related changes in CD8⁺Treg

Furthermore, GSEA results indicated a **decrease** in the expression of genes **involved in RNA biosynthesis and metabolism, as well as T cell differentiation, activation**, and immune response with age. However, no increase in the expression of genes associated with exhaustion, such as PD-1, Tim3, Lag3, TIGIT, CD160, and CD244, was observed.

It can be inferred that with age, the CD8⁺Treg population transitions to a terminally differentiated phenotype and exhibits a decreased functional response capacity, but this population does not undergo cellular exhaustion.




*GSEA of upregulated (A) and downregulated (B) genes of CD8⁺Treg subset from old donors (70-97 y.o.) at the pseudobulk level*

# Conclusion

- $CD8^+$Treg is a heterogeneous subpopulation of effector $CD8^+$ T lymphocytes
- $CD8^+$Treg tends to increase expression of genes associated with cytotoxicity, cytoskeletal rearrangement, and MHC-II-mediated antigen presentation. This would suggest that $CD8^+$Treg-mediated suppression likely involves cell-contact dependent cytolysis of target cells
- In the old age group (70-97 y.o.), the $CD8^+$Treg population shows decreased expression of activation genes and a change in marker gene expression to a terminal differentiated cell phenotype. Despite this shift, there is no evidence of increased expression of exhaustion markers
- However, there is a decrease in the expression of genes regulating key processes of T cell activation and function in old age, suggesting that the suppressor function of $CD8^+$Treg decreases with age

GitHub: