Characterization of germinal center and circulating T follicular helper cells using both intracellular and surface CITE-seq

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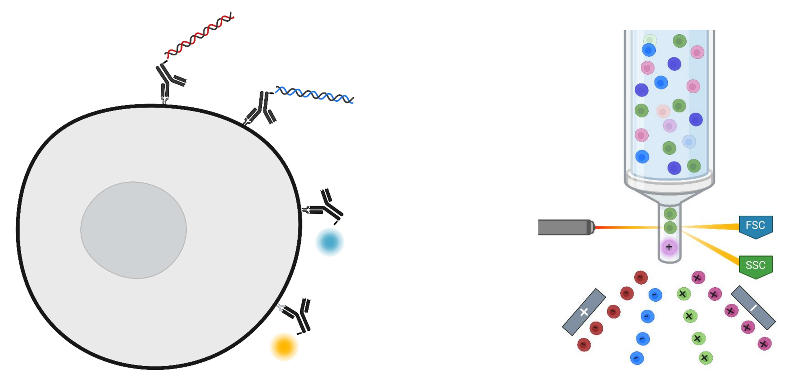
Abstract

T follicular helper (T_{FH}) cells are a CD4⁺ T cell subset that play a critical role in the formation of germinal centers (GC). T_{FH} cells are essential to produce protective antibody responses through the regulation of clonal selection and differentiation into activated antibody-secreting and memory B cells. T_{FH} cell differentiation is a multistep process that begins with the presentation of antigen by dendritic cells to naïve CD4⁺ T cells, where the CD4⁺ T cells commit to the T_{FH} lineage by expressing the lineage defining transcription factor B cell lymphoma 6 (Bcl-6). T_{FH} cells are phenotypically characterized by the high expression of C-X-C chemokine receptor 5 (CXCR5) and programmed cell death-1 (PD-1) on the cell surface. CXCR5⁺ memory CD4⁺ T cells present in peripheral blood represent a circulating T_{FH} (cT_{FH}) cell subset. In contrast to T_{FH} cells, cT_{FH} cells may express low levels of Bcl-6 with numerous reports of subsets that differ both phenotypically and functionally.

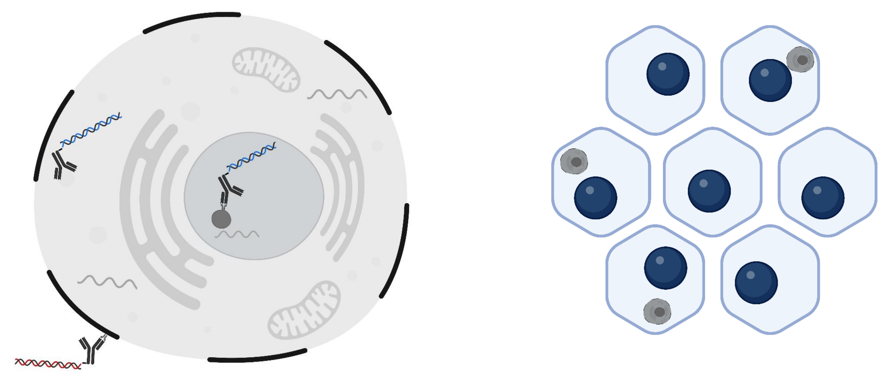
Here we characterize the heterogeneous subsets of T_{FH} and cT_{FH} cells using a combination of intracellular CITE-seq, surface CITE-seq and whole transcriptome single-cell RNA sequencing (scRNA-seq) analysis. Germinal center and circulating T_{FH} cells were enriched by sorting memory CD4⁺, CXCR5⁺, PD-1⁺ from a tonsil (n=1), and memory CD4⁺ CXCR5⁺ cells from a matched blood sample and a healthy blood donor (n=2). We examined the expression of Bcl-6 and other transcription factors, using protein and RNA expression data, across pre-T_{FH} (an early development stage in the germinal center), T_{FH} and cT_{FH} cells. Germinal center and circulating T_{FH} cells profiles were also compared.

Together, these data demonstrate how a combination of intracellular CITE-seq, surface CITE-seq and scRNA-seq may be used to deepen our understanding of the differentiation states and subsets of cells present at low abundance, such as T_{FH} cells, by enabling detection of intracellular and surface protein along with high-quality mRNA.

BD Rhapsody™ System Workflow



Single-cell suspensions from tonsil and blood were stained with a panel of antibodies including CD4, CD45-RA, PD-1 and CXCR5 to identify T_{FH} cells. Cells were also labeled with a 23-plex surface BD® AbSeq Panel. Cells were sorted using a 4-way sort on the BD FACSMelody™ Cell Sorter.



T_{FH} enriched immune cell suspensions were fixed using BD® Sample Preservation Buffer followed by permeabilization with BD Phosflow™ Perm Buffer III and stained with intracellular, oligo-conjugated BD® AbSeq Antibodies. Cells were counted with the BD Rhapsody™ System and loaded onto the cell capture cartridge. Libraries were prepared and indexed for Illumina sequencing followed by processing using the BD Rhapsody™ Whole Transcriptome Analysis Pipeline on Seven Bridges. Read count matrices were analyzed and visualized using Seurat and scCustomize in RStudio.[1,2] Briefly, standard QC metrics were applied followed by dataset integration, normalization, data scaling and visualization.

T_{FH} Enrichment

Figure 1. The BD FACSMelody™ Cell Sorter sorting strategy

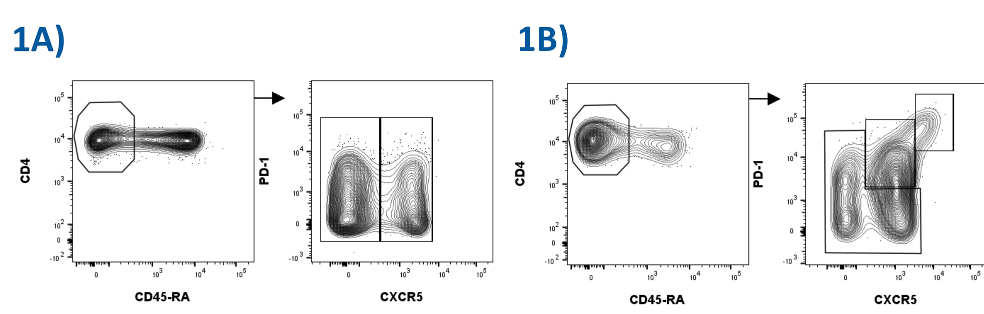


Figure 1. The BD FACSMelody™ Cell Sorter sorting strategy for **1A)** cT_{FH} in blood and **1B)** T_{FH} in tonsil prior to BD Rhapsody™ whole transcriptome workflow.

Identification and characterization of T_{FH} and cT_{FH} using intracellular and surface BD® AbSeq Antibodies

Figure 2. Tonsillar T_{FH} Cells

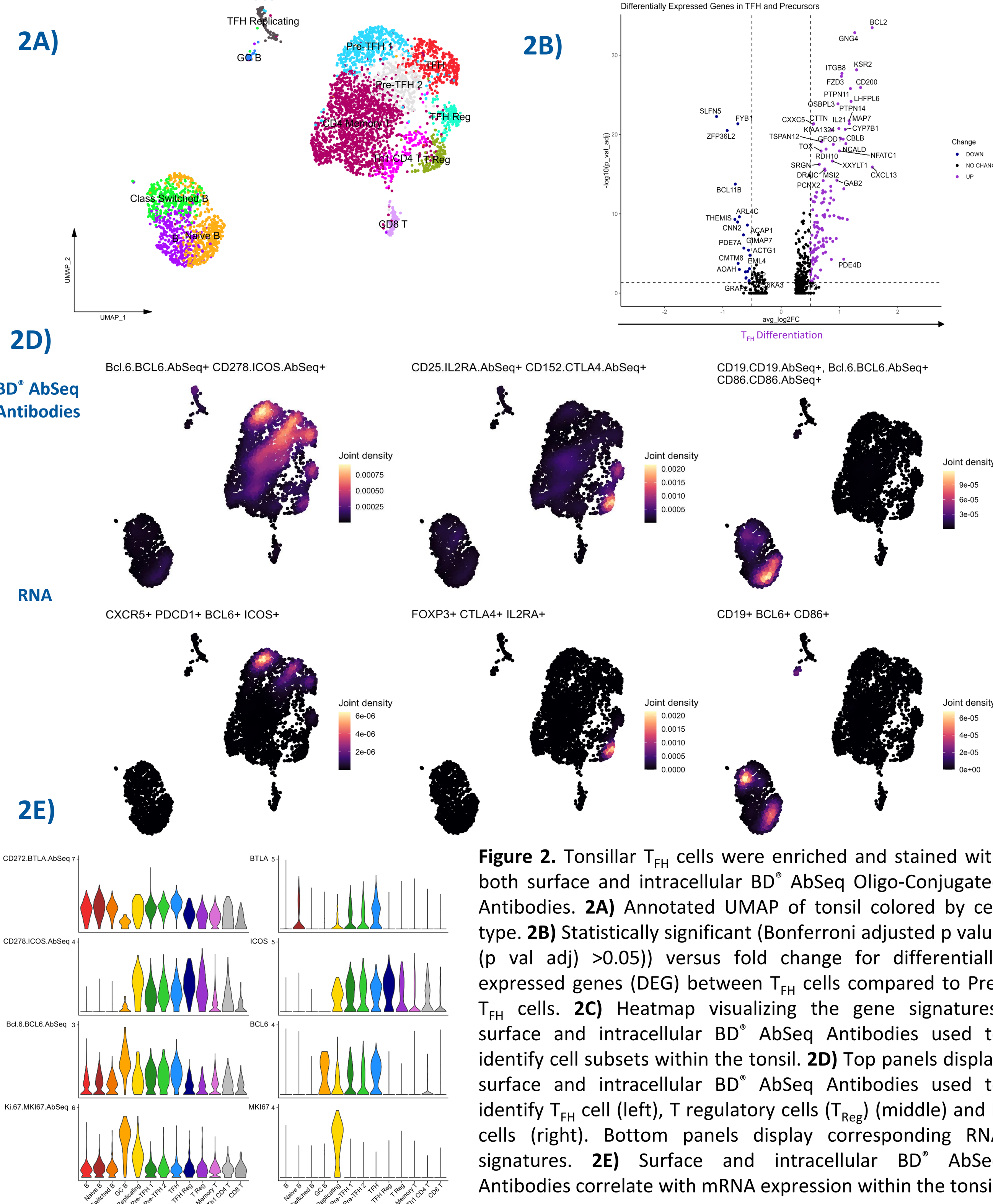


Figure 2. Tonsillar T_{FH} cells were enriched and stained with both surface and intracellular BD® AbSeq Oligo-Conjugated Antibodies. **2A)** Annotated UMAP of tonsil colored by cell type. **2B)** Statistically significant (Bonferroni adjusted p value (p val adj) >0.05)) versus fold change for differentially expressed genes (DEG) between T_{FH} cells compared to Pre-T_{FH} cells. **2C)** Heatmap visualizing the gene signatures, surface and intracellular BD® AbSeq Antibodies used to identify cell subsets within the tonsil. **2D)** Top panels display surface and intracellular BD® AbSeq Antibodies used to identify T_{FH} cell (left), T regulatory cells (T_{Reg}) (middle) and B cells (right). Bottom panels display corresponding RNA signatures. **2E)** Surface and intracellular BD® AbSeq Antibodies correlate with mRNA expression within the tonsil.

Figure 3. cT_{FH} Cells in Blood

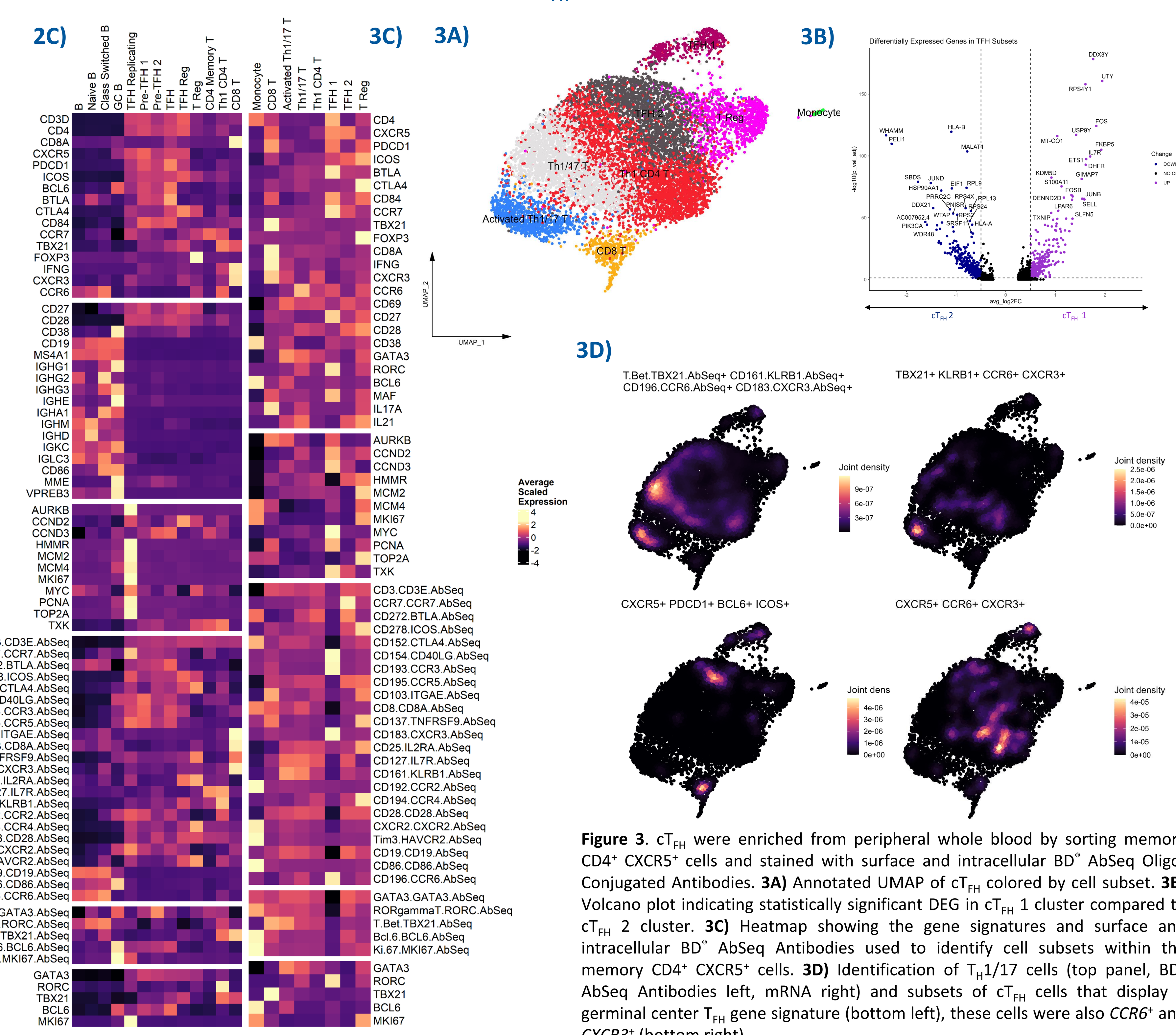


Figure 3. cT_{FH} were enriched from peripheral whole blood by sorting memory CD4⁺ CXCR5⁺ cells and stained with surface and intracellular BD® AbSeq Oligo-Conjugated Antibodies. **3A)** Annotated UMAP of cT_{FH} colored by cell subset. **3B)** Volcano plot indicating statistically significant DEG in cT_{FH} 1 cluster compared to cT_{FH} 2 cluster. **3C)** Heatmap showing the gene signatures and surface and intracellular BD® AbSeq Antibodies used to identify cell subsets within the memory CD4⁺ CXCR5⁺ cells. **3D)** Identification of $T_H1/17$ cells (top panel, BD® AbSeq Antibodies left, mRNA right) and subsets of cT_{FH} cells that display a germinal center T_{FH} gene signature (bottom left), these cells were also *CCR6*⁺ and *CXCR3*⁺ (bottom right)

Analysis of cell-cell communication

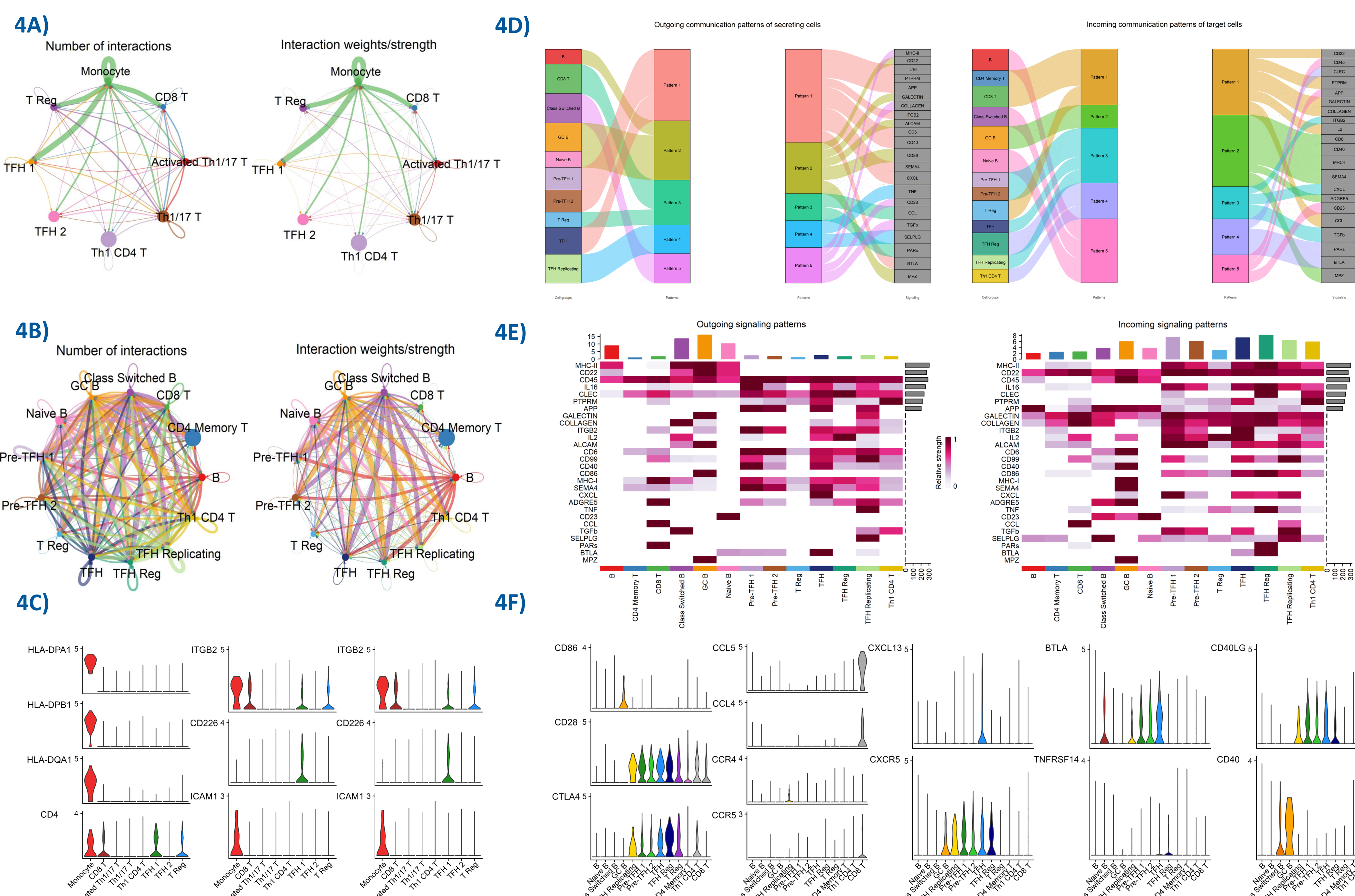


Figure 4. The probability of cell-cell communication was computed for tonsillar T_{FH} and cT_{FH} cells using CellChat.[3] **4A)** Aggregated cell-cell communication network with number of interaction and interaction weights for peripheral blood resident cells. **4B)** Aggregated cell-cell communication network with number of interaction and interaction weights for tonsil resident cells. **4C)** Gene expression of selected enriched signaling pathways in peripheral blood. **4D)** The top outgoing and incoming signaling patterns of secreting cells and target cells visualized by river plot, which indicates the coordination of cell types to global tonsil function. **4E)** Signals that contribute most to outgoing and incoming signals of cell groups in tonsil visualized by heatmap. **4F)** Gene expression of selected enriched signaling pathways in tonsil.

Comparison of T_{FH} and cT_{FH}

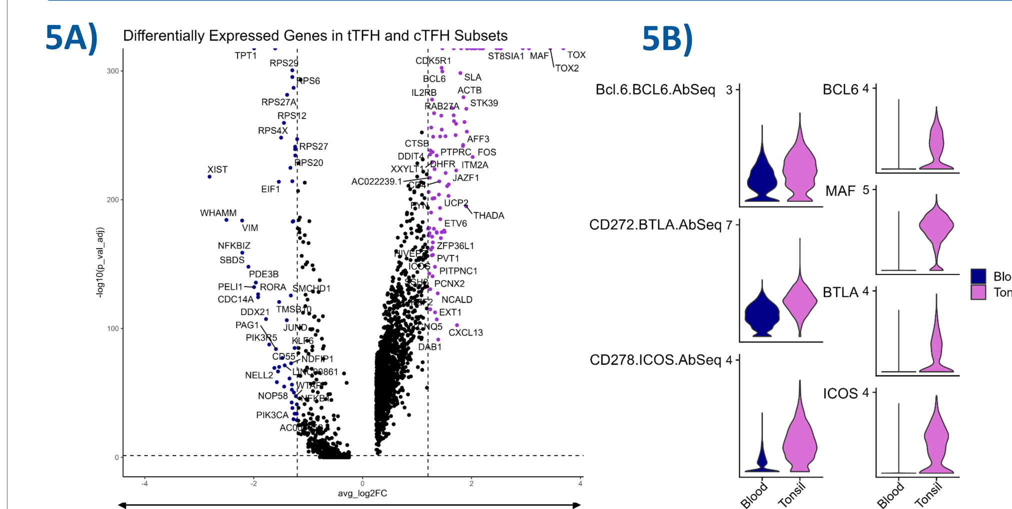


Figure 5. DEG were computed for tonsillar T_{FH} cells and cT_{FH}. **5A)** Volcano plot indicating statistically significant DEG in tonsillar T_{FH} cells and cT_{FH}. **5A)** Key gene and corresponding BD® AbSeq Antibody expression visualized with violin plot.

Conclusions

- We have demonstrated a workflow that enables the simultaneous detection of intracellular BD® AbSeq and surface BD® AbSeq Antibodies along with high-quality mRNA.
- Transcription factors detected with intracellular BD® AbSeq Antibodies showed a high concordance with mRNA expression.
- Our data demonstrate how a combination of intracellular BD® AbSeq Antibodies, surface BD® AbSeq Antibodies and scRNA-seq may be used to identify subsets of cells present at low abundance, such as T_{FH}, pre-T_{FH}, and cT_{FH} cells.

Refences:

1) Hao Y, Hao S, Andersen-Nissen E, et al. *Cell*. 2021;184(13):3573-3587.e29. doi: 10.1016/j.cell.2021.04.048.
2) Marsh SE. 2021. doi: 10.5281/zenodo.5706430.
3) Jin S, Guerrero-Juarez CF, Zhang L, et al. *Nat Commun*. 2021;12(1):1088. doi: 10.1038/s41467-021-21246-9.
4) Some images were created with BioRender.com

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