Simultaneous single cell analysis of multiple analytes resolves T cell populations at high resolution

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10x GENOMICS

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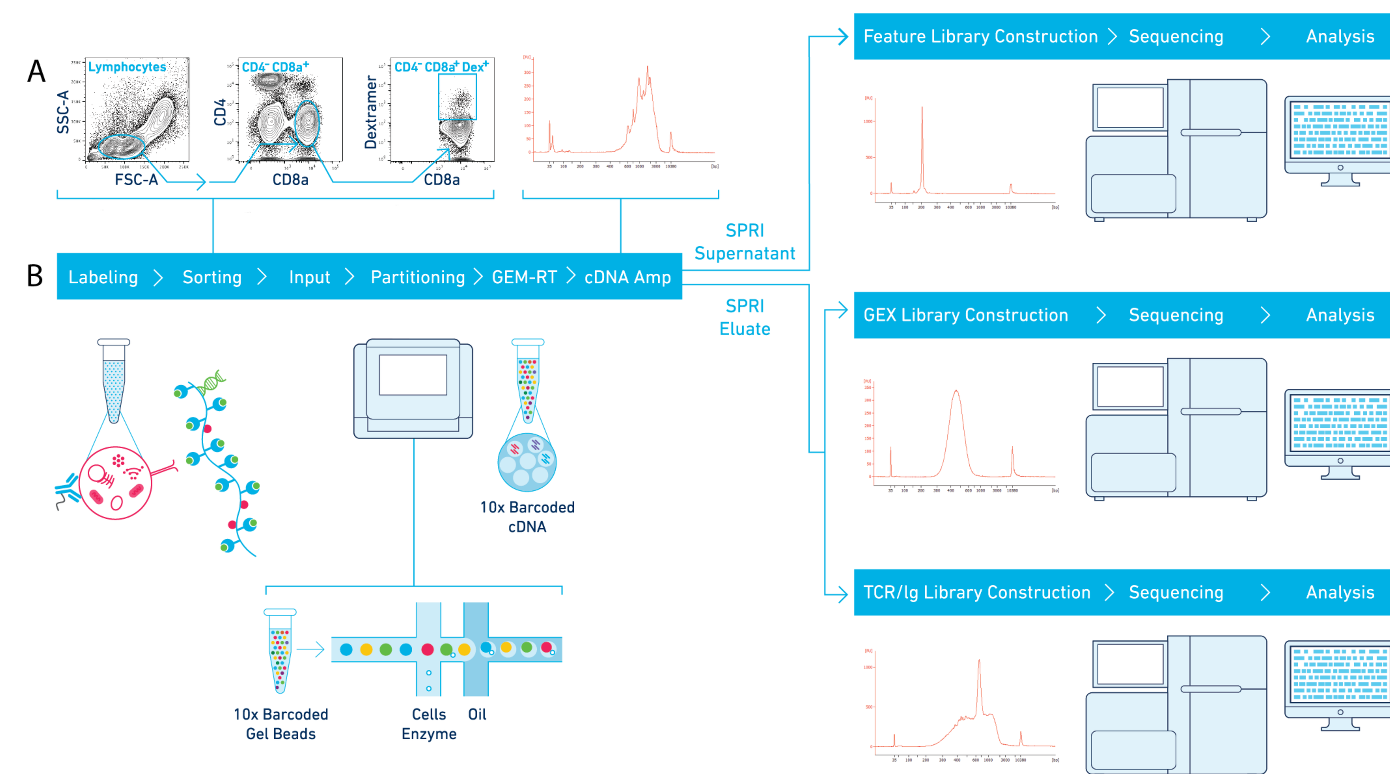
Introduction

- Characterization of lymphocyte types and understanding their antigen binding specificities is key to the development of effective therapeutics
- 10x Genomics have enabled the integration of ***transcriptome, cell-surface protein, immune repertoire and TCR antigen-specificity measurements from the same single cells***
- Combined with developments from Immudex and BioLegend we are able to provide an end-to-end solution for analysis of the tumor microenvironment

1. Feature Barcoding technology workflow

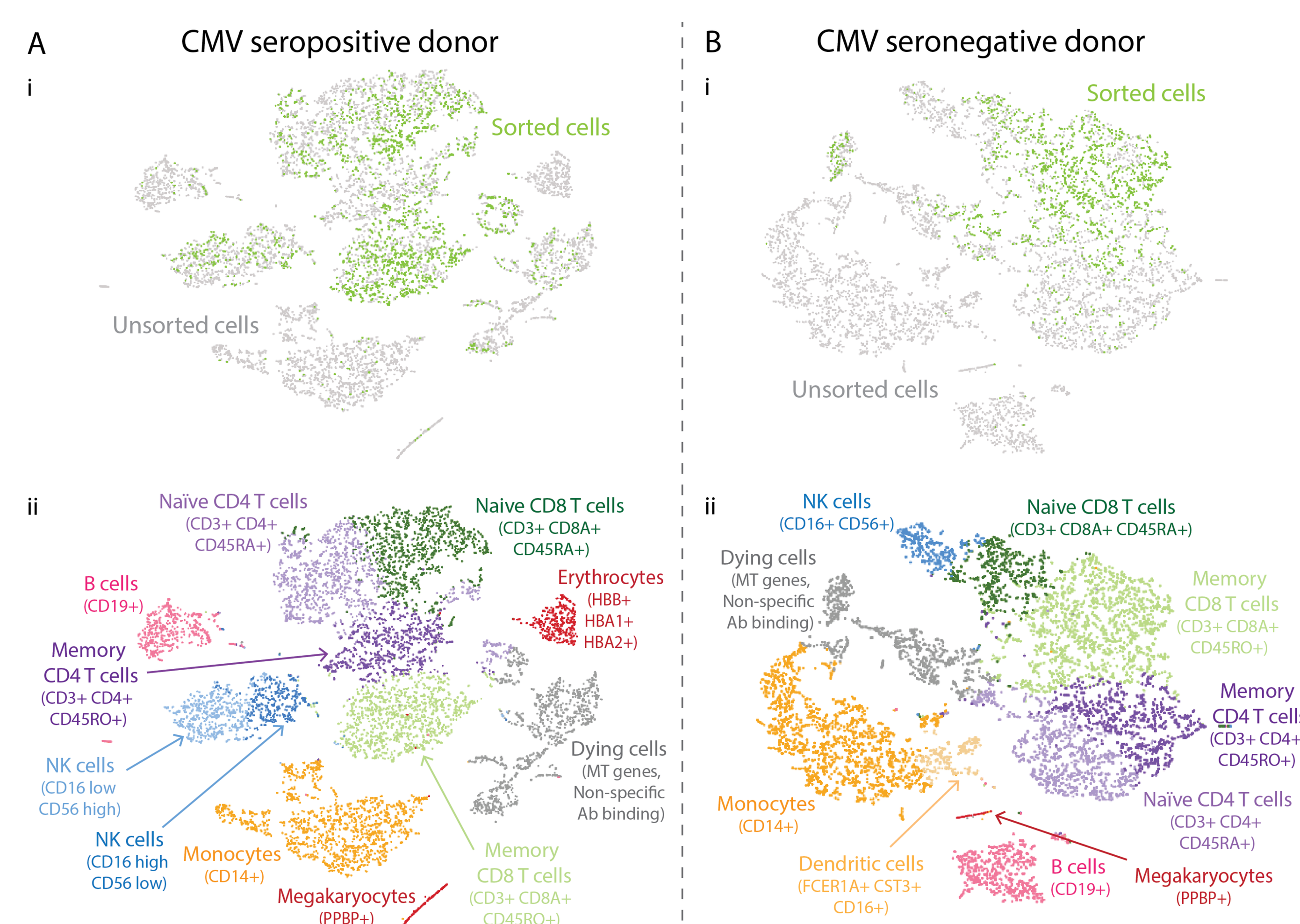


Samples were obtained from a CMV seropositive and seronegative donor. Cells were stained with panels of oligonucleotide conjugated antibodies (BioLegend) and Dextramers (Immudex). Each sample was split into two, one half was sorted for CD4-/CD8a+/Dextramer+ cells, and the other half left unsorted. The four samples (CMV seropositive and seronegative, sorted and unsorted) were taken through the workflow as outlined in Figure 1.

Feature Barcoding technology workflow for the multi-omic characterization of single cells.

A. Gating strategy used in flow cytometry to isolate CD4-/CD8a+/Dextramer+ cells.
B. Single Cell Immune Profiling with Feature Barcoding technology workflow where gene expression and immune repertoire libraries are generated alongside libraries from DNA barcodes conjugated to TotalSeq™-C antibodies or dCODE™ Dextramer® reagents, allowing quantification of cell surface proteins and identification of TCR specificities. Representative Bioanalyzer traces showing average size distribution for amplified cDNA and for each of the generated libraries are shown in the figure.

2. Cell type classification of PBMCs using gene and cell surface protein expression



The combination of gene expression and cell surface protein expression using labelled barcoded antibodies provides increased resolution of cell type characterization.

Unsorted PBMCs and CD4-/CD8a+/Dextramer+ sorted cells were aggregated, tSNE projections generated by Cell Ranger and visualized in Loupe Cell Browser. Cells were clustered on gene expression data with graph-based clustering. Each dot is a single cell.

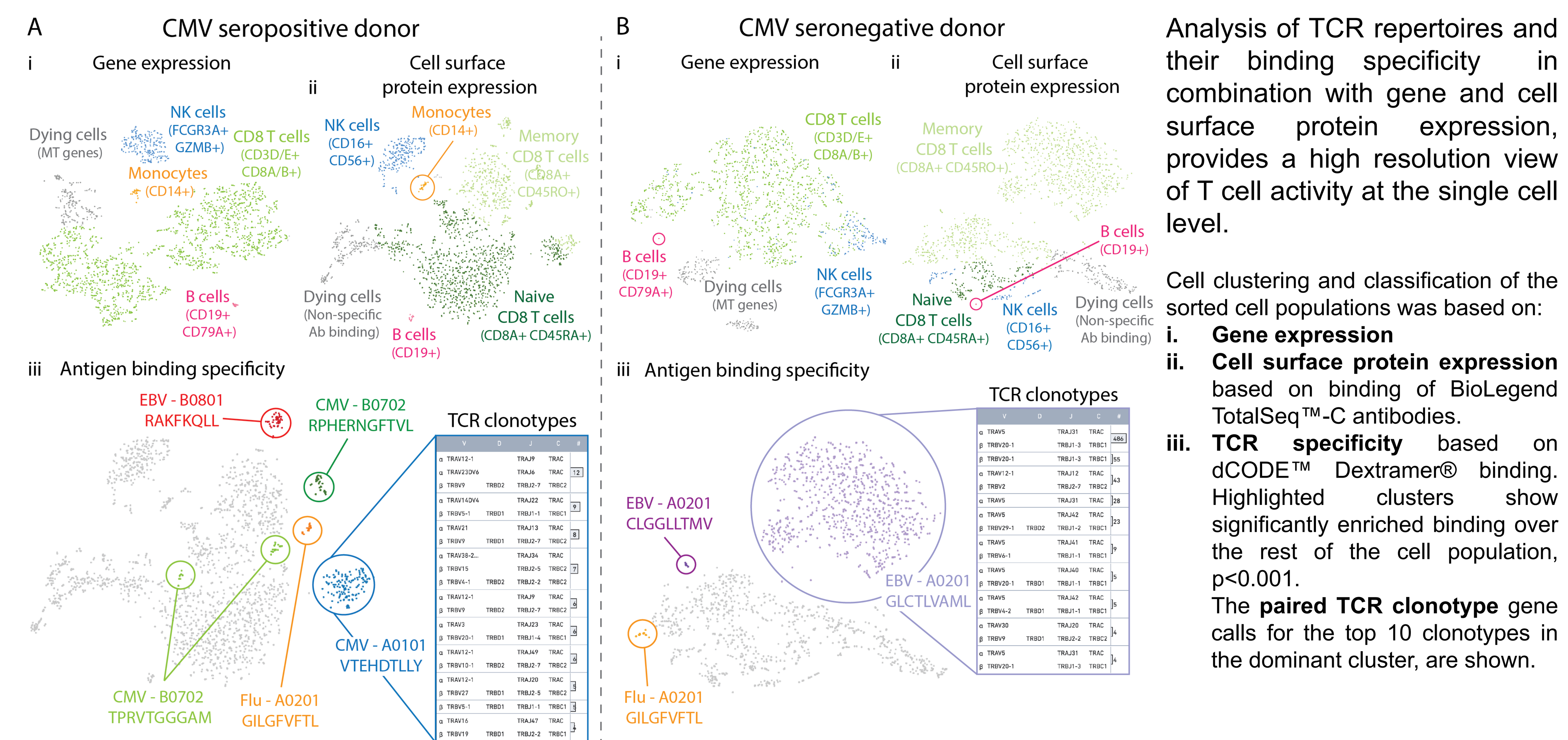
A. i. CMV seropositive donor colored based on sample. Unsorted PBMCs = grey (6770 cells), sorted cells = green (2038 cells).
B. i. CMV seronegative donor colored based on sample. Unsorted PBMCs = grey (5998 cells), sorted cells = green (1566 cells).

In ii. Cell type classification was performed using both gene expression data and surface protein expression profiles.

For a pdf version of this poster, visit the 10x Genomics website: https://www.10xgenomics.com/resources/posters/

For further information on dCODE™ Dextramers® see Immudex poster P56.

3. Multi-omic single cell characterization of T cell populations



Analysis of TCR repertoires and their binding specificity in combination with gene and cell surface protein expression, provides a high resolution view of T cell activity at the single cell level.

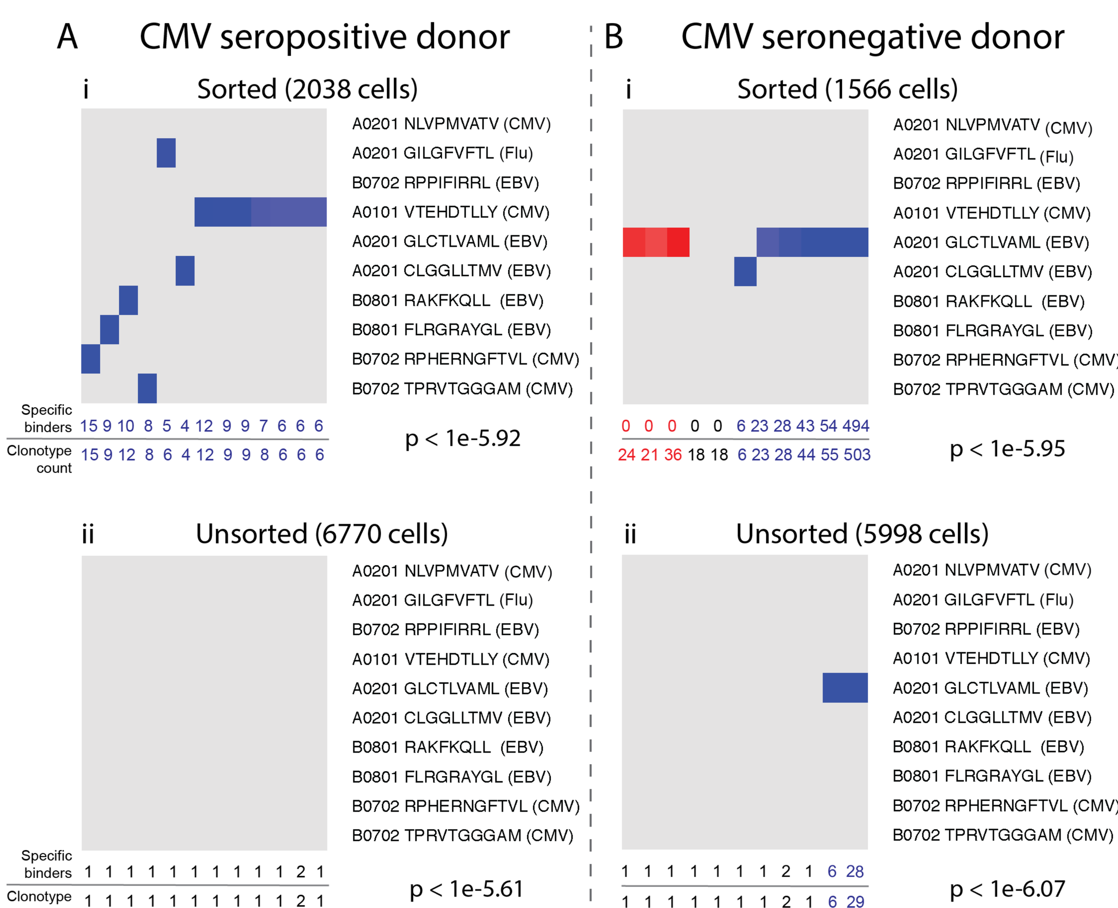
Cell clustering and classification of the sorted cell populations was based on:

i. **Gene expression**
ii. **Cell surface protein expression** based on binding of BioLegend TotalSeq™-C antibodies.
iii. **TCR specificity** based on dCODE™ Dextramer® binding. Highlighted clusters show significantly enriched binding over the rest of the cell population, p<0.001.

The **paired TCR clonotype** gene calls for the top 10 clonotypes in the dominant cluster, are shown.

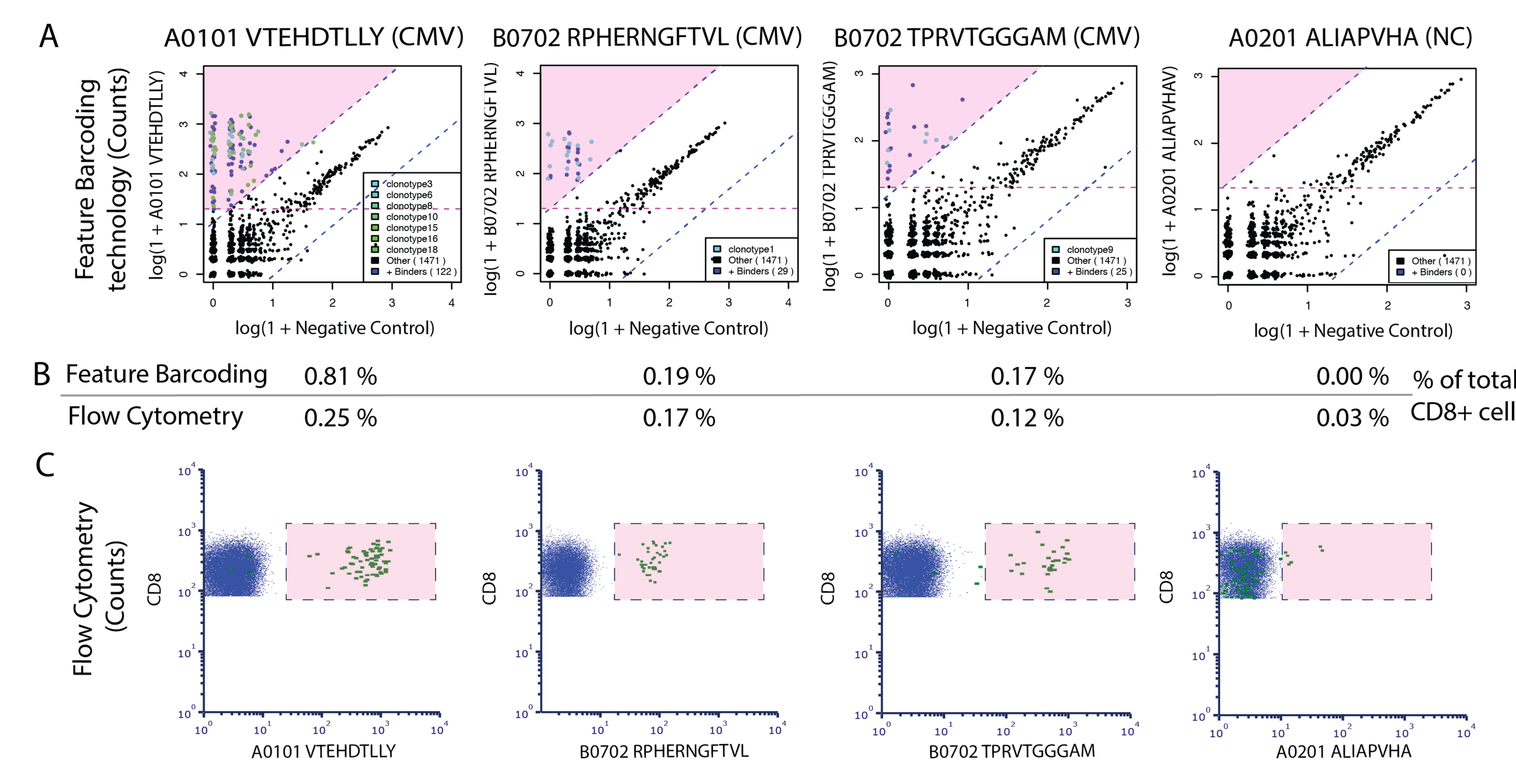
4. Analysis of flow sorted populations allows identification of more antigen specific cells

Many statistically significant specific TCR: dCODE™ Dextramer® binding events were identified in the sorted cells (i) whereas very few are identified in the unsorted PBMCs (ii).



Heatmaps show the significantly enriched (blue) or significantly under-represented (red) TCR clonotypes. Each column represents a TCR clonotype. The number of T cells with specificity for a particular Dextramer® (Specific binders) and the frequency of the T cell clonotype in the whole population (Clonotype count) are outlined below each heatmap. For all samples, the top cells ranked by magnitude of their log10 p-values are shown, and at least ten cells were included for each heatmap.

5. Feature Barcoding technology and flow cytometry analysis yield comparable data

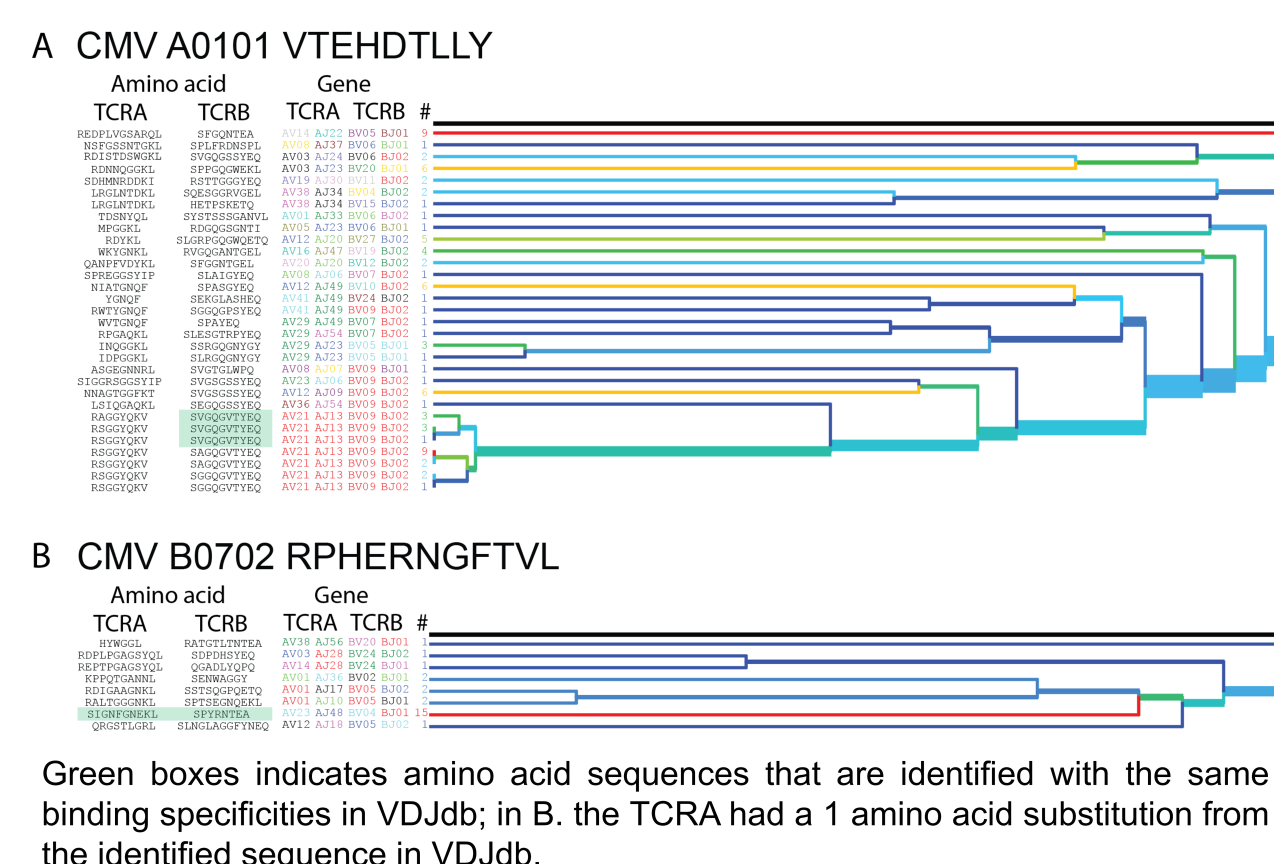


Feature Barcoding technology and flow cytometry identify similar frequencies of Dextramer® positive cells.

A. Feature Barcoding technology analysis with a CMV-specific dCODE™ Dextramer® panel. Positive cells are above the dashed lines with the background colored in pink.
B. Quantification of the fraction of positive binding cells as a percentage of the total number of CD8+ cells identified by Feature Barcoding technology and by flow cytometry.
C. Flow cytometric analysis of the same CMV seropositive sample with the same dCODE™ Dextramer® panel. Positive cells are inside the dashed box with pink background.

Based on analysis of the CMV seropositive donor CD4-/CD8a+/Dextramer+ sorted cells.

6. Clustering analysis of CMV specific TCR clonotypes



Green boxes indicates amino acid sequences that are identified with the same binding specificities in VDJdb; in B. the TCRA had a 1 amino acid substitution from the identified sequence in VDJdb.

Clustering analysis of TCR clonotypes for CMV specific dCODE™ Dextramers® reveals both novel and known CDR3 amino acid sequences.

Paired TCR sequences were clustered using TCRdist (Dash et al. 2017) to generate an average-linkage hierarchical tree, colored according to the number of cells that share each paired TCR sequence (at the nucleotide level). # is the frequency at which the specific clone was detected.

A. dCODE™ Dextramer® A*0101 VTEHDTLLY. 31 different TCR pairs identified.
B. dCODE™ Dextramer® B*0702 RPHERNGFTVL. 8 different TCR pairs identified.

Conclusions

This technology allows:

- Cell type characterization and identification of full length, paired TCR sequences with specificity for known antigens
- Characterization of the adaptive immune response at unprecedented resolution
- A truer understanding the complexity of the tumor microenvironment
- The potential for the discovery of novel TCR: antigen binding relationships

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