



Single-Cell Polyfunctional Proteomic Profiling Reveals Temporal and Niche Differences in CD4 and CD8 T cells in Acute Myeloid Leukemia Following PD-1 Blockade Therapy

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Background

Acute myeloid leukemia (AML) is a hematological malignancy characterized by rapid proliferation of abnormal myeloid cells in the bone marrow. Prognosis for patients is poor with a five-year overall survival rate of 25-30%.

Recent advancements in immune checkpoint blockade (ICB) therapies have revolutionized treatment in the solid tumor landscape, but ICB therapies in AML have not yielded similarly promising results.

Several studies explore therapies targeting T cell functionalities in AML, but a deeper understanding within the context of ICB is critical to determine underlying reasons for its limited success and to improve the therapeutic potential of immune checkpoint inhibition in leukemia.

Methods

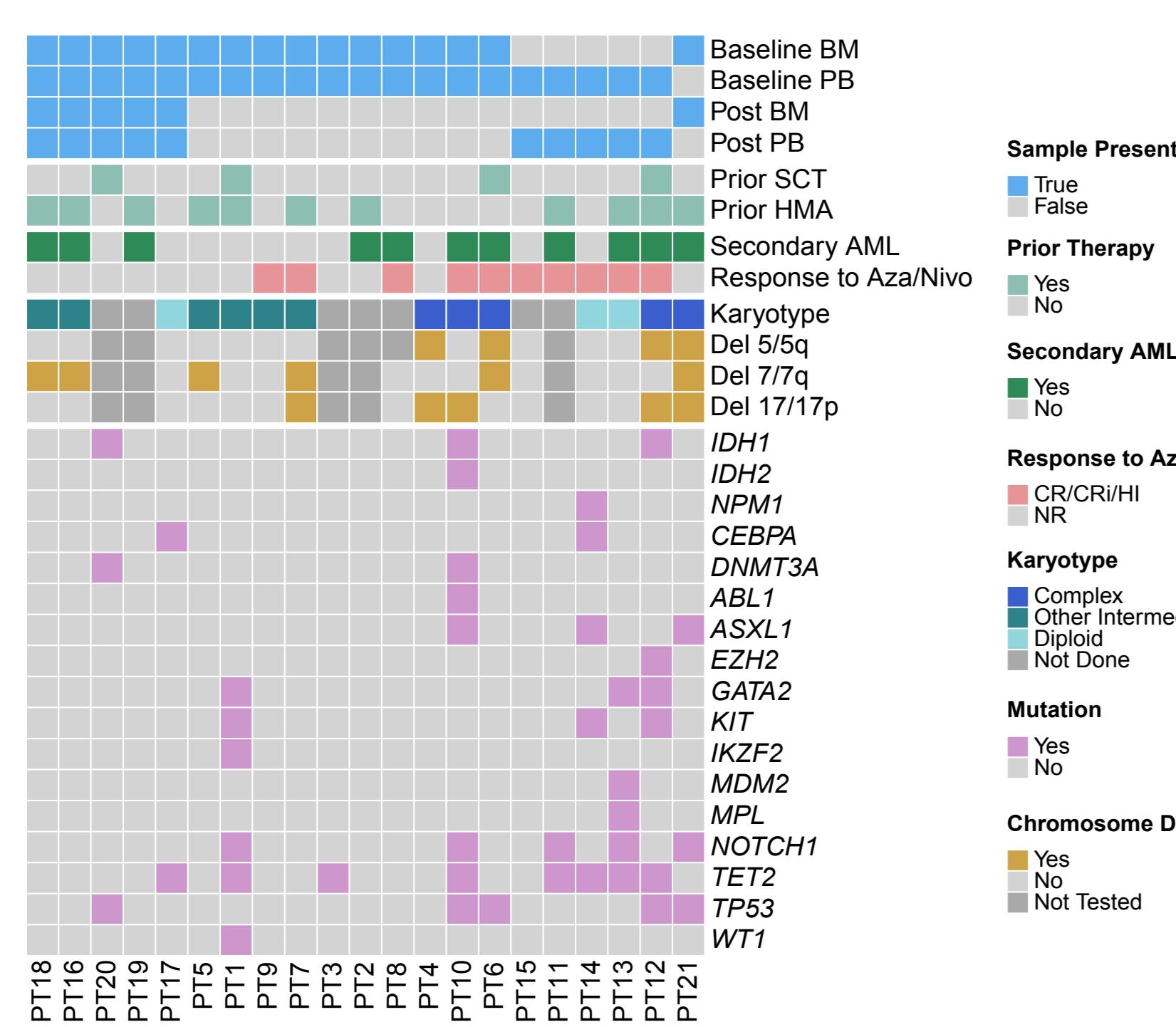


Fig 1. Clinical characteristics of Relapse/Refractory (RelRef) AML cohort for 21 patients involved in phase 2 clinical trial.

- Isolated CD4 and CD8 T cells by magnetic bead sorting from the peripheral blood (PB) and bone marrows (BMs) of 21 AML patients before (baseline) and after ICB (post-IO).

- Proteomic profiling via IsoPlexis polyfunctional multiplex assay isolating and measuring functionality of CD4 and CD8 T cells by the polyfunctional strength index (PSI). PSI is defined as the percentage of polyfunctional cells, multiplied by the sum of the mean fluorescence intensity (MFI) of the proteins secreted by those cells.

- PSI data were processed using an adapted single-cell RNA sequencing (scRNA seq) pipeline.

- 111,420 single cells; selected 101,120 after filtering out cells with > 3000 MFI.

- Seurat with Harmony-based batch correction and UMAP dimensional reduction.

Results

Establishing baseline functionality for T cells in AML.

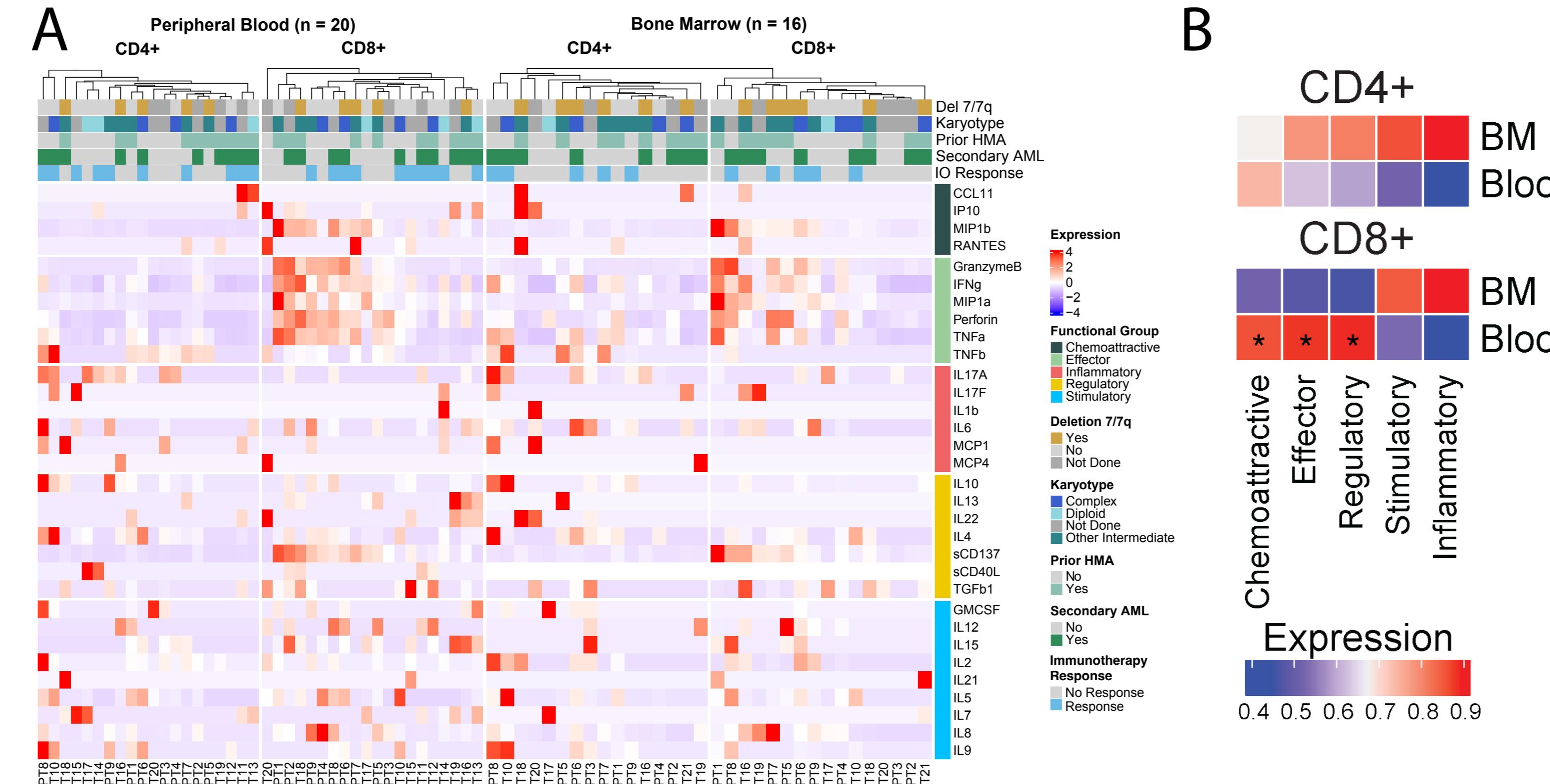


Fig 2. Baseline pseudobulk analysis revealed (A) similar patterns of CD8 cytokine expression in both the BM and PB at baseline (B) with significant differences in tumor microenvironments' (TMEs) expression defined by cytokine functional groups.

CD8 polyfunctionality differs in PB and BM TMEs.

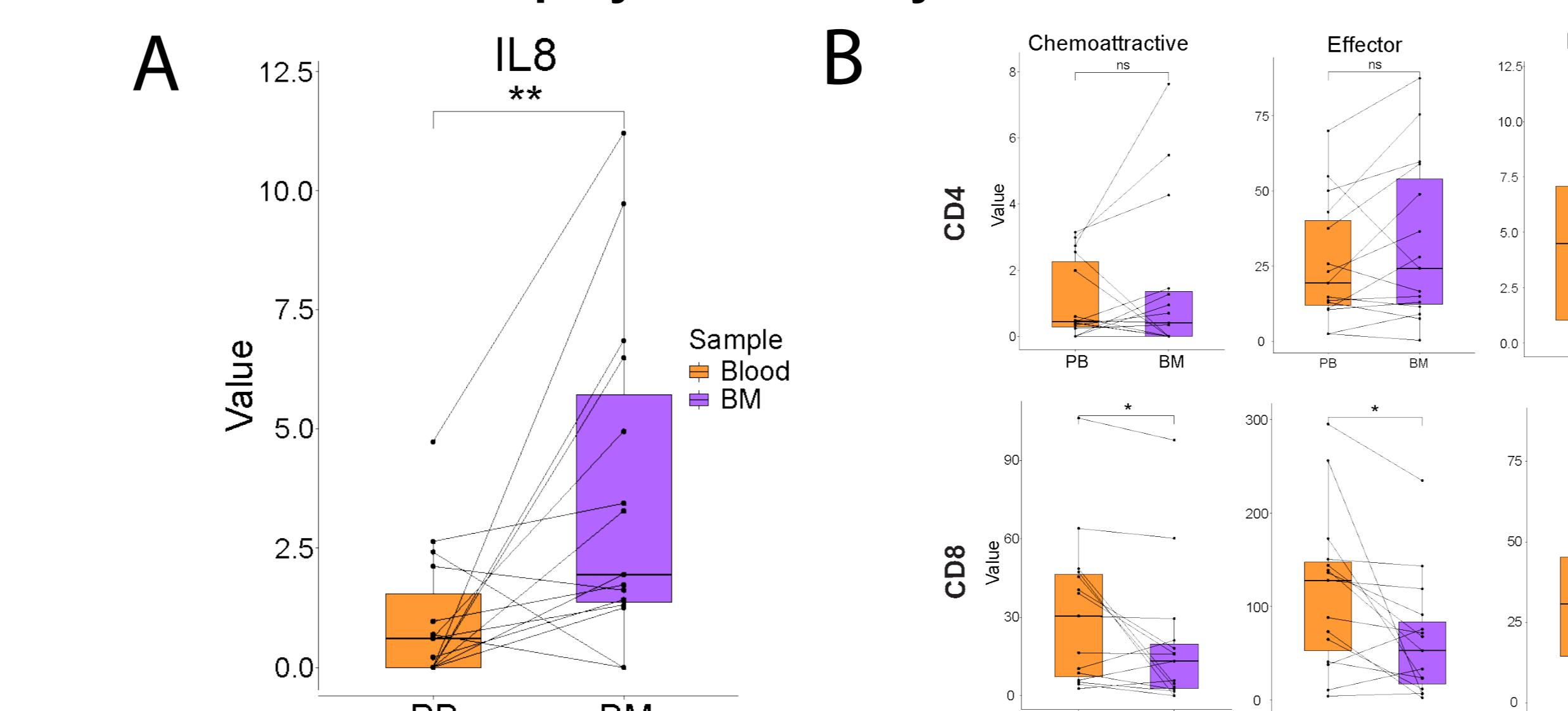


Fig 3. T cell expression within the TMEs show (A) IL-8 in CD4 T cells was the only individual cytokine with significant differences between TMEs and (B) CD8 T cell functionality differs in the local (BM) and systemic (PB) TMEs.

Differential effector cytokine activity between responders (CR) and nonresponders (NR) in CD4 cells.

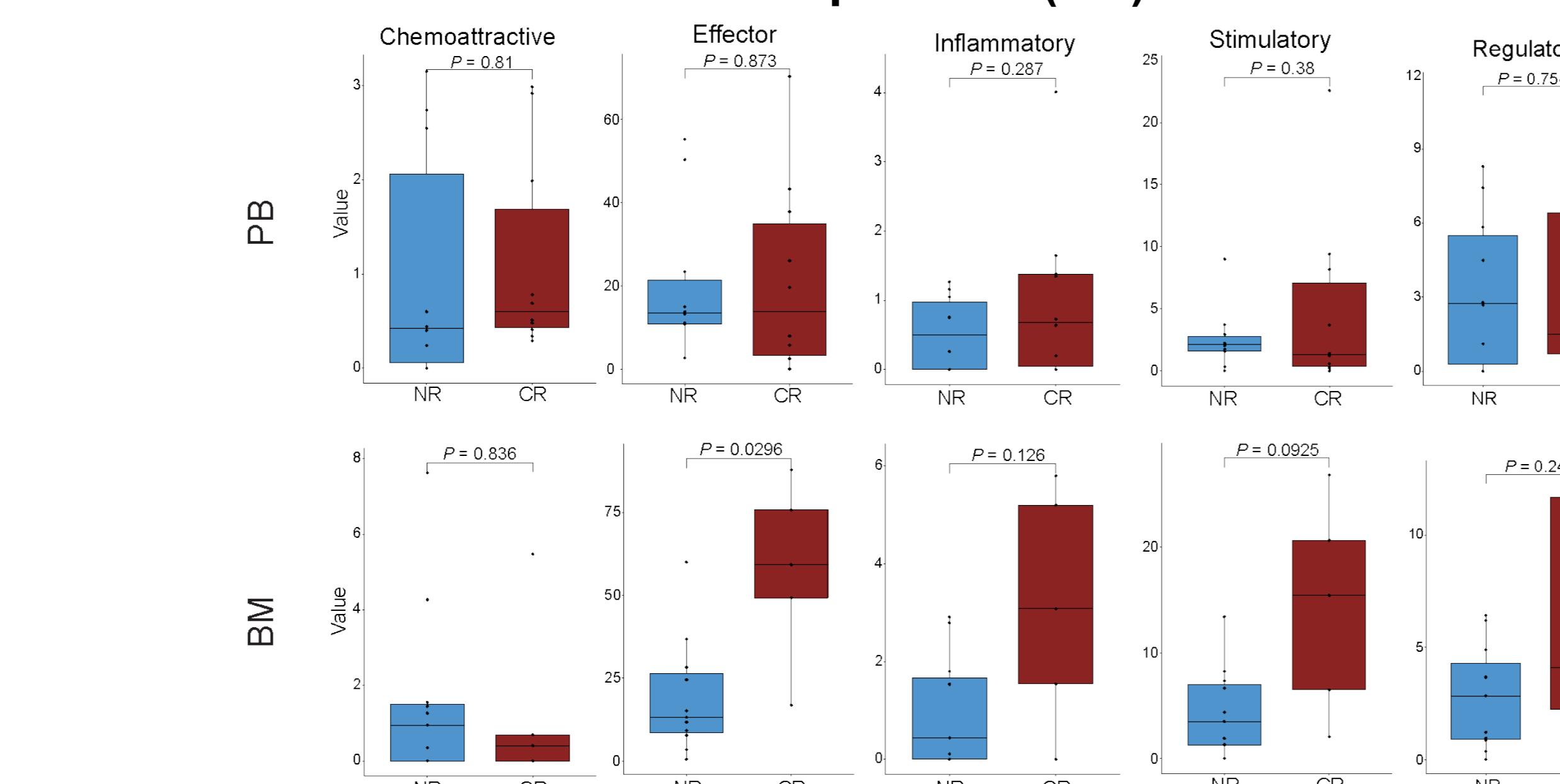


Fig 4. Comparisons between CR and NR at baseline revealed a significant increase in CD4 BM effector cytokine functional activity in responders.

Post-IO compartment is not a distinct functional state.

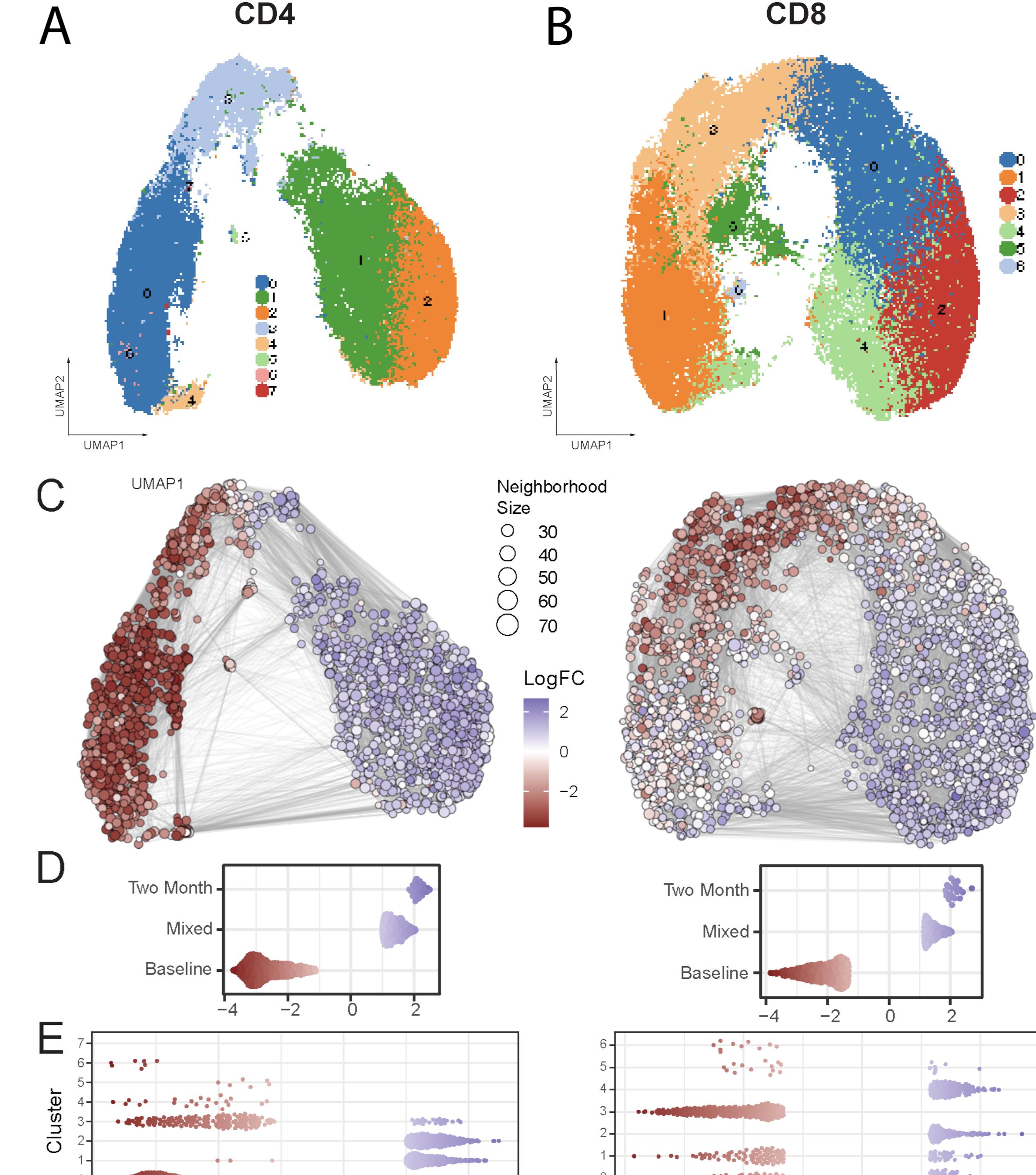


Fig 5. UMAP visualization for (A) CD4 cells and (B) CD8 cells. (C) Neighborhood graph of differential abundance testing results from miloR. Colors represent the log fold-change between baseline (red) and post-IO (blue) cells. White neighborhoods are non-differential (false discovery rate 10%). The edges depict links between cells shared by neighborhoods. Beeswarm plot of the neighborhood distribution by (D) time point and (E) UMAP-based cluster.

Conclusions

We conducted a novel analysis comparing PB and BM compartments in RelRef AML patients using single-cell and pseudobulk approaches.

Our findings indicate that it is critical to sample the BM tumor microenvironment given that T cell polyfunctionality differs between systemic and local microenvironments.

Post-IO T cells are not unique at the single cell level.

Unbiased, unsupervised clustering revealed novel functional groups that are potential biomarkers of response.

Differences between responders and non-responders at the single-cell level

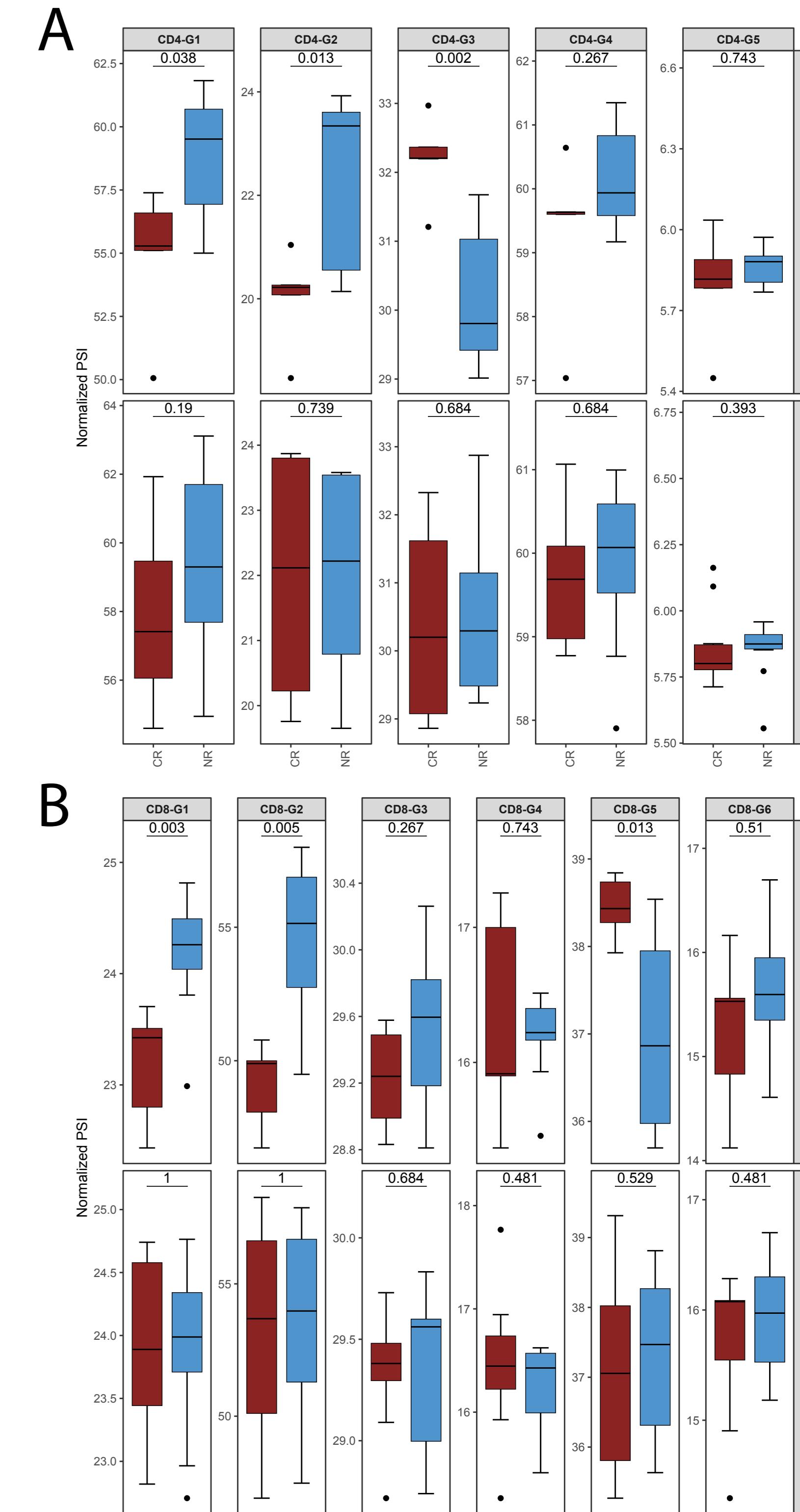


Fig 6. Single cell cytokine and polyfunctional group analysis depicting comparison of CR (brown) and NR (blue) at baseline by sample location in newly defined polyfunctional groups for (A) CD4 T cells, and (B) CD8 T cells.

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