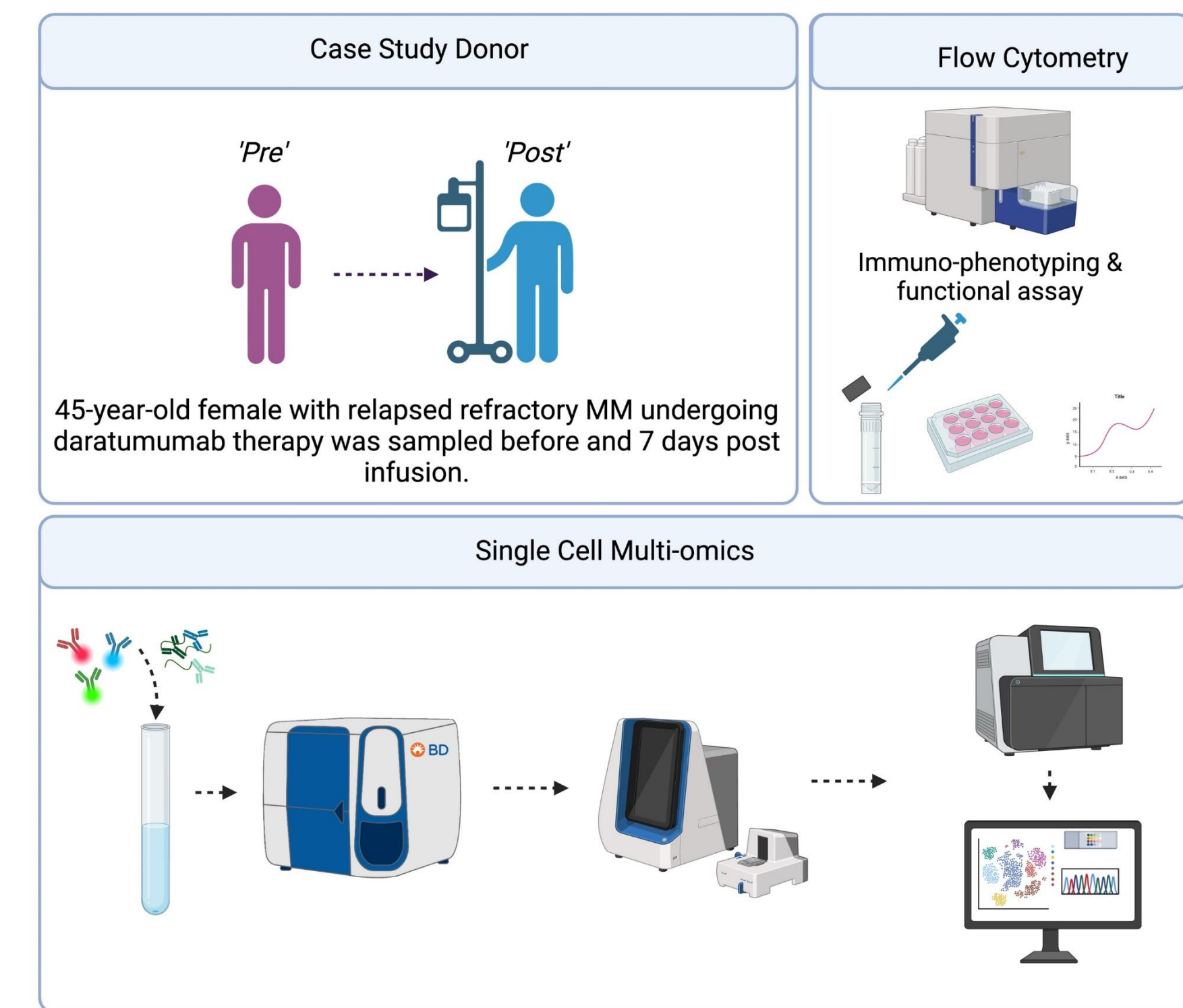


## Introduction

Multiple Myeloma (MM), a hematological malignancy, is characterized by the clonal proliferation of plasma cells in the bone marrow (BM). Despite recent advancements in MM treatment, many patients experience disease progression after multiple therapies. Daratumumab, a human IgG1 monoclonal antibody targeting CD38, has emerged as a breakthrough in the treatment of MM, including in cases of relapsed and refractory MM. However, MM remains incurable and therefore, understanding the impact of daratumumab on the immune microenvironment may enable the development of better-targeted therapies. This research study represents a case study of a 45-year-old donor with relapsed refractory MM undergoing daratumumab therapy, where we focused on the immediate impact of daratumumab on the immune microenvironment.

## Methods



- Samples were stained with a custom 18-plex BD® AbSeq panel and fluorescent antibodies, and viable lymphocytes were sorted on a BD FACSMelody™ Cell Sorter
- Single cells were partitioned on the BD Rhapsody™ Cartridge, and mRNA and BD® AbSeq were captured with BD Rhapsody™ Cell Capture Beads.
- Single-cell RNA sequencing (scRNA-seq) libraries were prepared and indexed for sequencing, followed by processing using the BD Rhapsody™ SevenBridges pipeline. Expression matrices were then analysed in R Studio using Seurat v4, SCpubr, scCustomize or custom base R workflows. Data was integrated using canonical correlation analysis.
- Additionally, flow cytometry was performed to characterize cell phenotypes and to assess effector function. For this, values from the donor used in our research study are displayed, along with the minimum, maximum, and mean values obtained from healthy cohorts (n=18) for each cell population.

## Conclusions

- This case study underscores daratumumab's rapid impact on the immune cell landscape within the BM immune microenvironment on this donor
- By combining flow cytometry with single-cell whole transcriptome sequencing and BD® AbSeq, this research study provided a comprehensive view of the effect of daratumumab therapy on the MM tumor immune microenvironment.
- Through BD® AbSeq, we identified the dynamic changes in the BM immune cell landscape and captured transcript expression patterns specific to targeted cell populations.
- This approach allowed us to link surface marker expression with gene expression profiles, enabling a comprehensive analysis of the immune microenvironment.

CD8<sup>+</sup> T cell clusters from the post-treatment sample were enriched in TNF and IFIT3

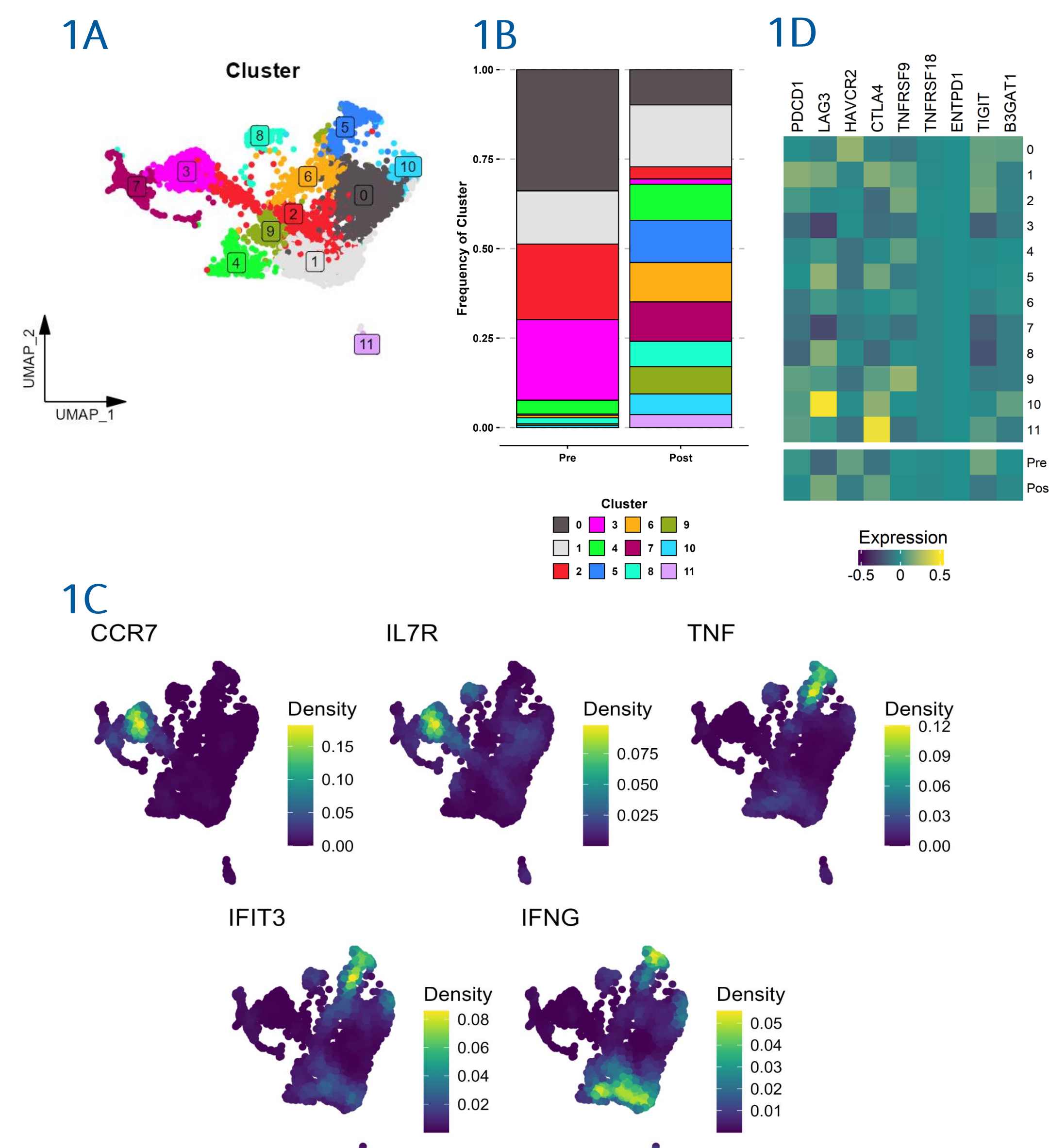


Figure 1. Analysis of the CD8<sup>+</sup> T cell subset identified clusters isolated to the post-treatment sample that were enriched in TNF and IFIT3. 1A) UMAP (uniform manifold approximation and projection) of re-clustered CD8<sup>+</sup> T cells. 1B) Cluster distribution before and after treatment. 1C) Expression of key cluster-defining genes in density plots. 1D) Heatmap of exhaustion marker transcript expression by cluster and sample.

Expanded memory T cells and increased granzyme post daratumumab therapy

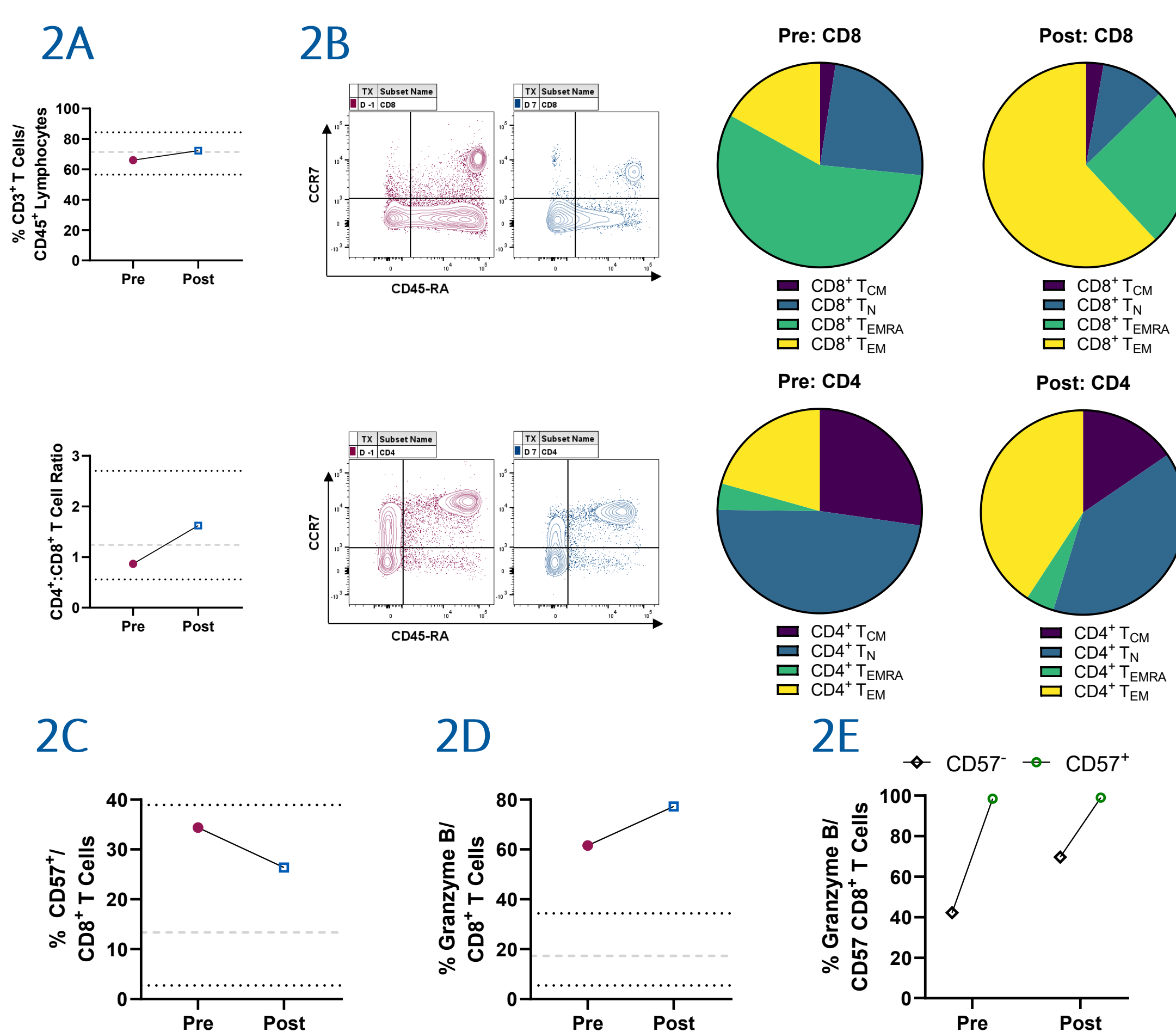


Figure 2. T cells show memory shifts post daratumumab therapy. 2A) The percentage of (lymphocytes, live, CD45<sup>+</sup>, CD14<sup>+</sup>, CD3<sup>+</sup>) T cells and the CD4:CD8 ratio pre- and post-daratumumab treatment. 2B) Contour plots demonstrating memory subset distribution in this patient and pie charts of the memory subsets as a percentage of the CD8 and CD4 T cell subsets. 2C) The percentage of CD57<sup>+</sup> CD8<sup>+</sup> T cells, 2D) the percentage of granzyme B CD8<sup>+</sup> T cells, and 2E) the percentage of granzyme B expressing cells in the CD57<sup>+</sup> and CD57<sup>-</sup> cell populations in CD8 T cells.

NK cells post-treatment displayed increased expression of interferon related genes

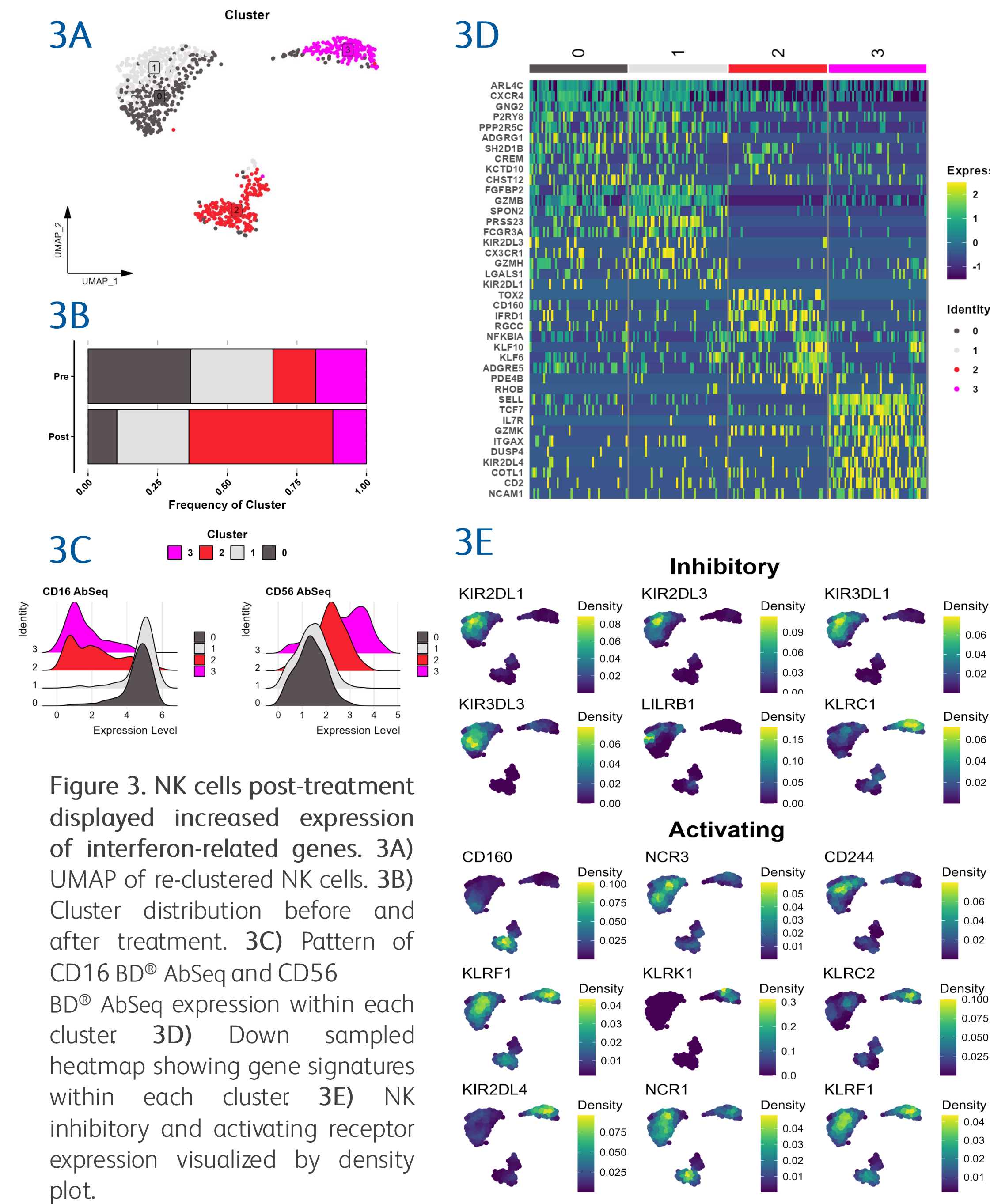


Figure 3. NK cells post-treatment displayed increased expression of interferon-related genes. 3A) UMAP of re-clustered NK cells. 3B) Cluster distribution before and after treatment. 3C) Pattern of CD16 BD® AbSeq and CD56 BD® AbSeq expression within each cluster. 3D) Down sampled heatmap showing gene signatures within each cluster. 3E) NK inhibitory and activating receptor expression visualized by density plot.

Increased CD56<sup>bright</sup>CD16<sup>-</sup> NK cell frequencies post daratumumab treatment

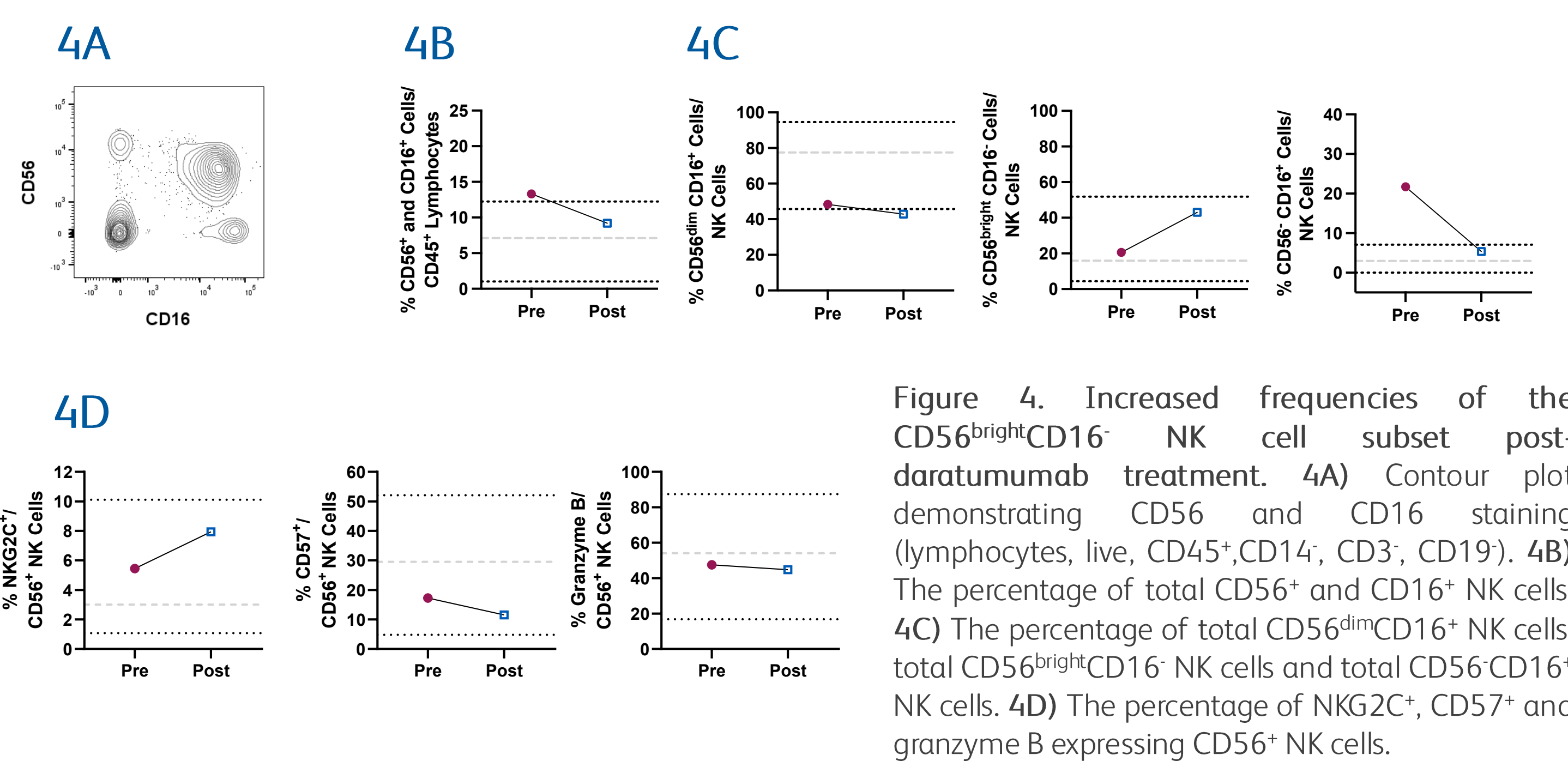


Figure 4. Increased frequencies of the CD56<sup>bright</sup>CD16<sup>-</sup> NK cell subset post-daratumumab treatment. 4A) Contour plot demonstrating CD56 and CD16 staining (lymphocytes, live, CD45<sup>+</sup>, CD14<sup>+</sup>, CD3<sup>+</sup>, CD19<sup>-</sup>). 4B) The percentage of total CD56<sup>+</sup> and CD16<sup>-</sup> NK cells. 4C) The percentage of total CD56<sup>dim</sup>CD16<sup>+</sup> NK cells. 4D) The percentage of CD56<sup>bright</sup>CD16<sup>-</sup> NK cells and total CD56<sup>bright</sup>CD16<sup>+</sup> NK cells.

CD8 NK-T-like cells increase post daratumumab treatment

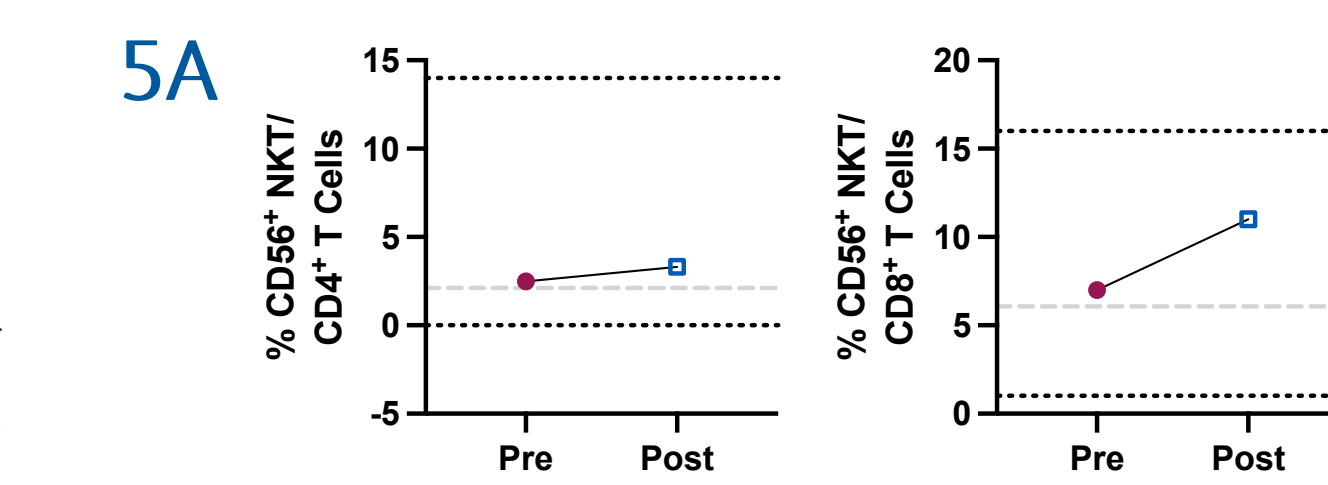


Figure 5. NK-T-like cells increase post-daratumumab therapy. 5A) The percentage of CD4<sup>+</sup>CD56<sup>+</sup> and CD8<sup>+</sup>CD56<sup>-</sup> NK-T-like (lymphocytes, live, CD45<sup>+</sup>, CD19<sup>-</sup>, CD14<sup>+</sup>, CD3<sup>+</sup>) cells pre- and post-daratumumab treatment.

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scRNA-seq characterization of CD4 demonstrates a change in the transcriptomic landscape

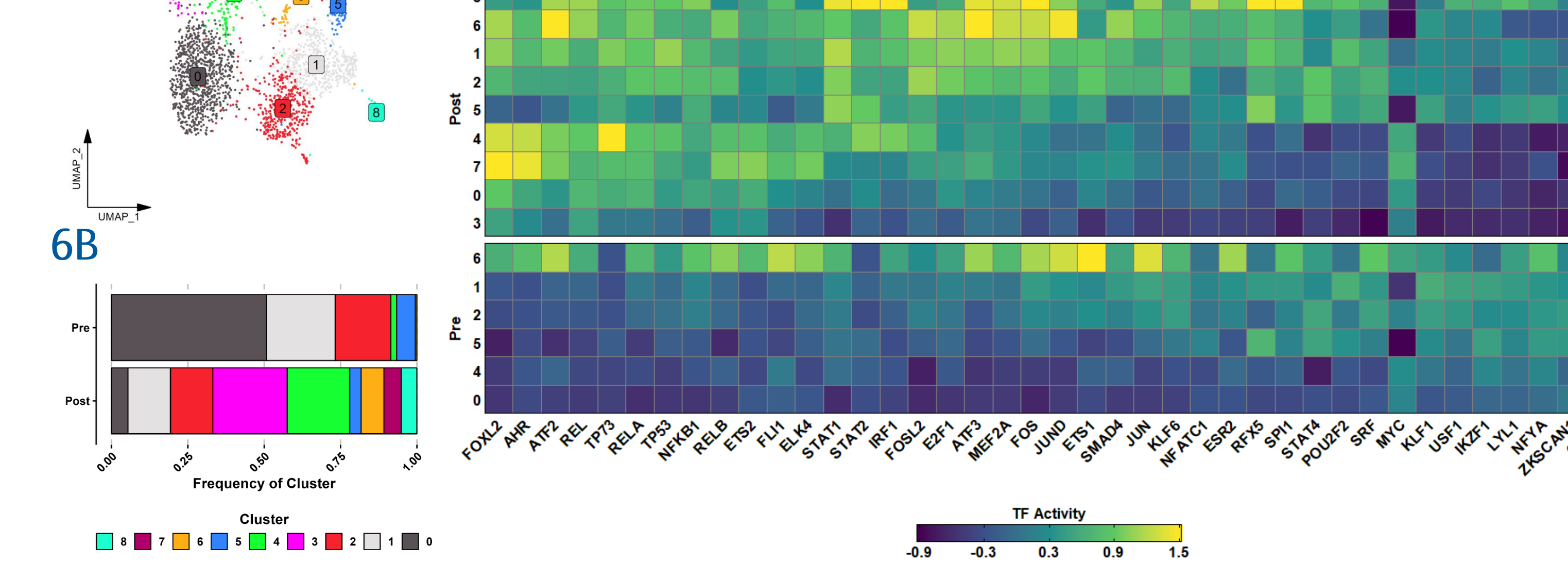


Figure 6. scRNA-seq characterization of CD4 pre- and post-daratumumab treatment demonstrates a more activated transcriptomic landscape. 6A) UMAP of re-clustered CD4<sup>+</sup> T cells 6B) Cluster distribution before and after treatment. 6C) Heatmap of transcription factor activity across CD4 T cell clusters pre- and post-treatment.

Increased degranulation in CD8 T cells post-daratumumab therapy

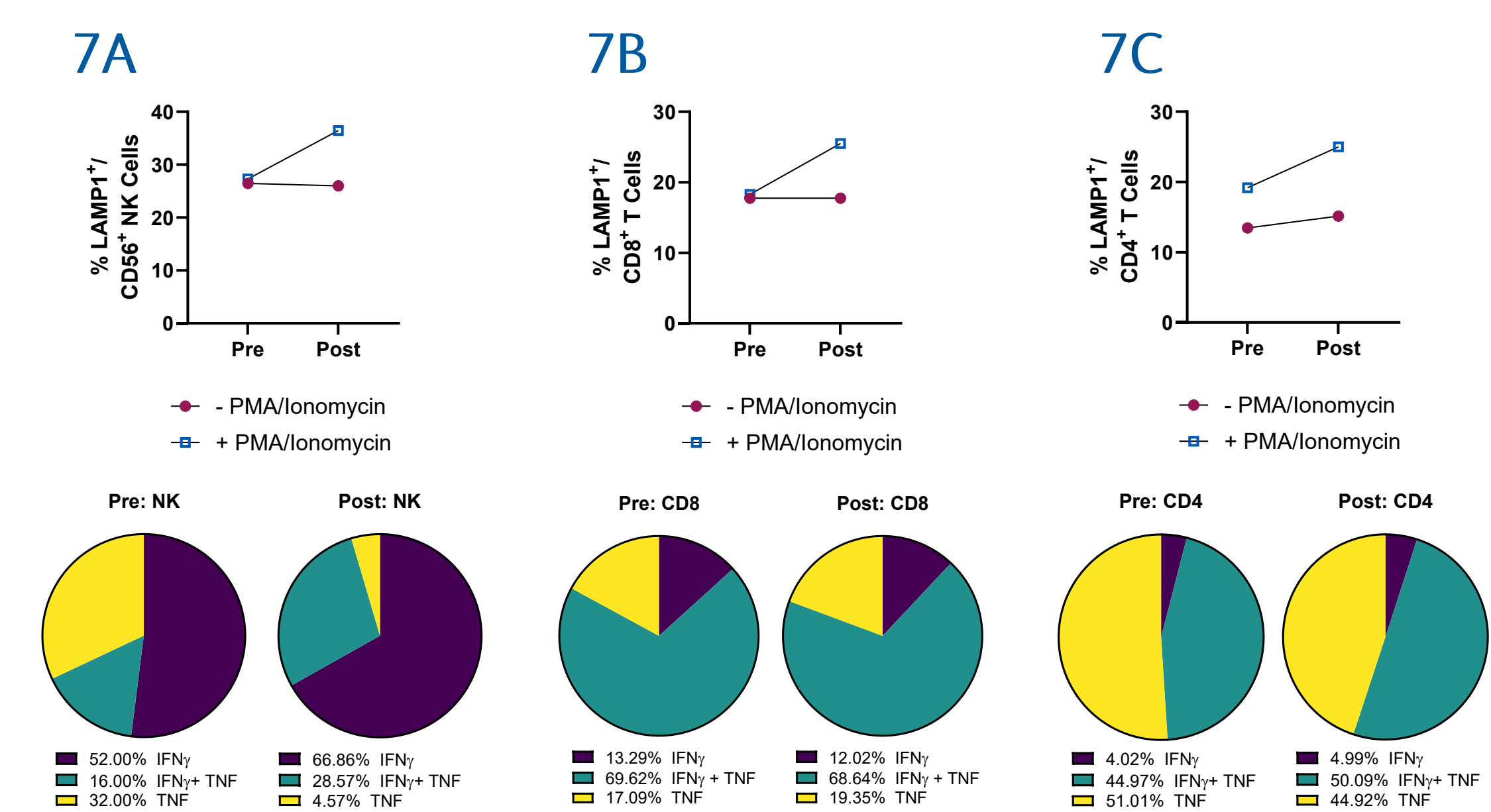


Figure 7. Increased degranulation post-daratumumab therapy. 7A) (lymphocytes, live, CD45<sup>+</sup>) CD56<sup>+</sup> NK cells, 7B) CD8<sup>+</sup> T cells, 7C) CD4<sup>+</sup> T cells. The top graph displays the percentage of LAMP1<sup>+</sup> cells. Pie charts display the percentage of single and dual positive cells as a percentage of the total IFN- $\gamma$  and TNF cytokine-producing cells.

Minor decrease in the B cell frequency post daratumumab treatment

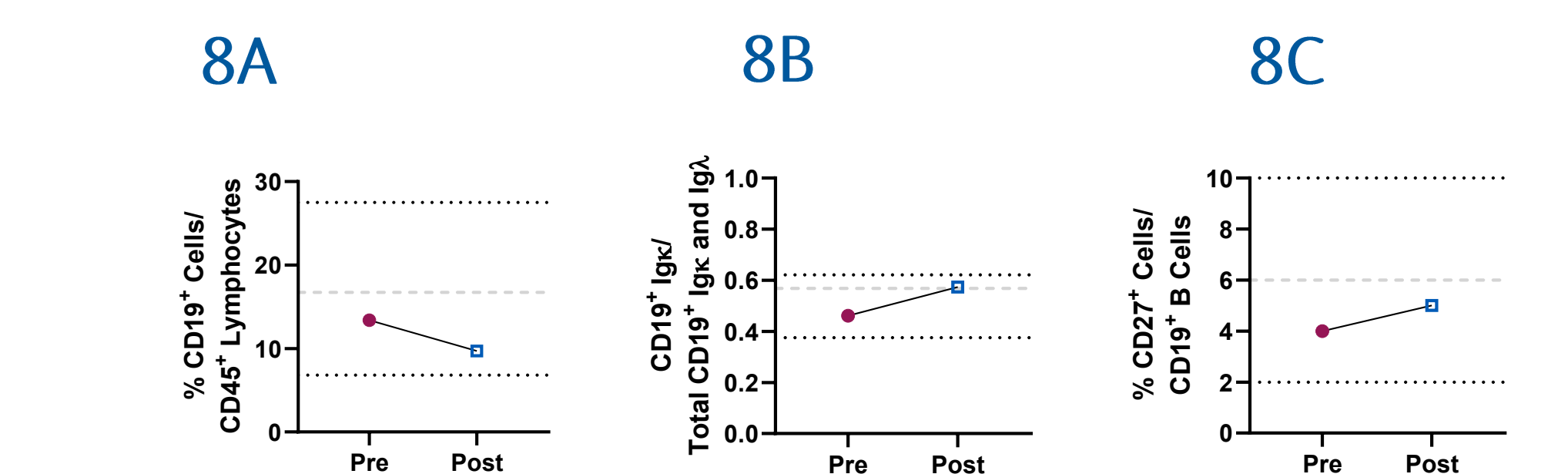


Figure 8. A minor decrease in B cell frequency was observed post-treatment. 8A) The percentage of total CD19<sup>+</sup> B cells (lymphocytes, live, CD45<sup>+</sup>, CD14<sup>+</sup>, CD3<sup>+</sup>) pre- and post-treatment. 8B) The ratio of Igk:IgI in CD19<sup>+</sup> B cells, 8C) and the frequency of CD27<sup>+</sup> cells within the CD19<sup>+</sup> B cell subset (lymphocytes, live, CD45<sup>+</sup>, CD19<sup>+</sup>, CD138<sup>-</sup>) pre- and post-treatment.

Monocytes increased in frequency post daratumumab therapy

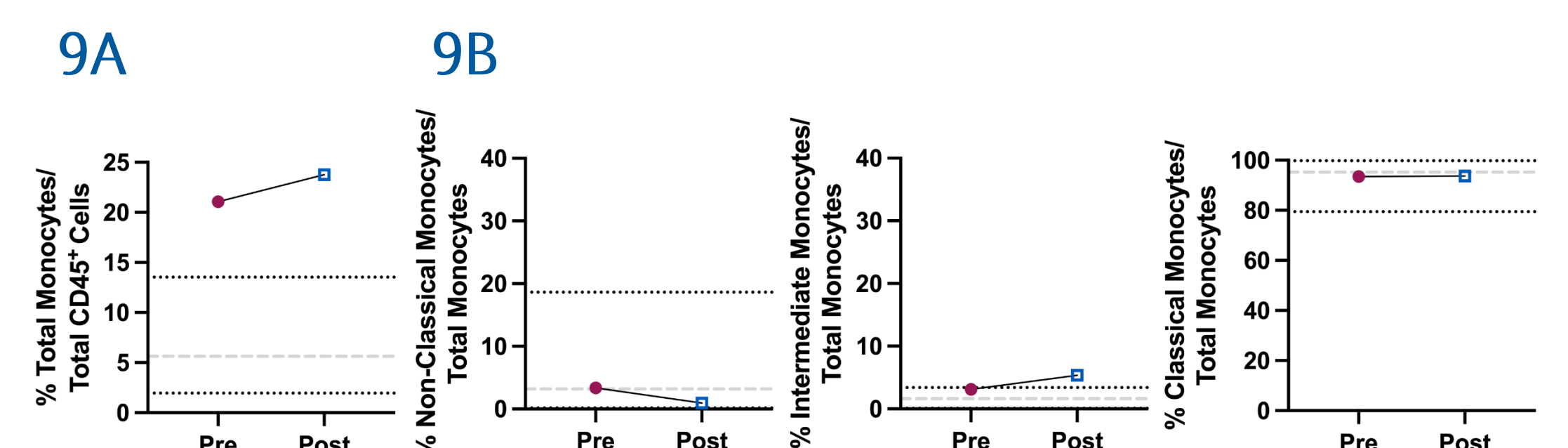


Figure 9. Monocytes increased in frequency post daratumumab therapy. 9A) The percentage of total (lymphocytes, live, CD45<sup>+</sup>, CD3<sup>+</sup>, CD4<sup>dim</sup>, CD19<sup>-</sup>, CD56<sup>-</sup>) CD16<sup>+</sup> and CD14<sup>+</sup> monocytes pre- and post-treatment. 9B) The percentage of monocyte subsets: non-classical (CD16<sup>+</sup>CD14<sup>-</sup>), intermediate (CD16<sup>+</sup>CD14<sup>+</sup>), and classical (CD16<sup>-</sup>CD14<sup>+</sup>) pre- and post-treatment.