Using Single Cell Melanoma Drug Treatment Data with Bulk RNA-Seq to Extract Clinical Insights

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INTRODUCTION

Melanoma affects over 100,000 Americans every year. We can combine more granular insights from single cell biology with clinical information from bulk RNA sequencing data. Using drug-labelled single cell data alongside melanoma clinical data from The Cancer Genome Atlas (TCGA) we extract promising general and drug response specific gene markers and examine their relationship with patient survival. We found gene signatures related to cell type, treated with drugs, and survival that will help design treatments to kill cancer cells rather than normal cells. We also generated a pipeline to do this analysis.

RESEARCH METHODOLOGIES

1. Download single cell RNA-seq melanoma data (Jerby-Arnon et. al, 2019) and bulk TCGA data with survival data.
2. Process Data Using R, Scanpy, and Seurat - normalize data and correct data for inconsistencies
3. Analyze Data
   - Use MSigDB to find gene sets that overlap with differentially expressed genes between treated and non-treated cells
   - Use STREAM to find cell state progression

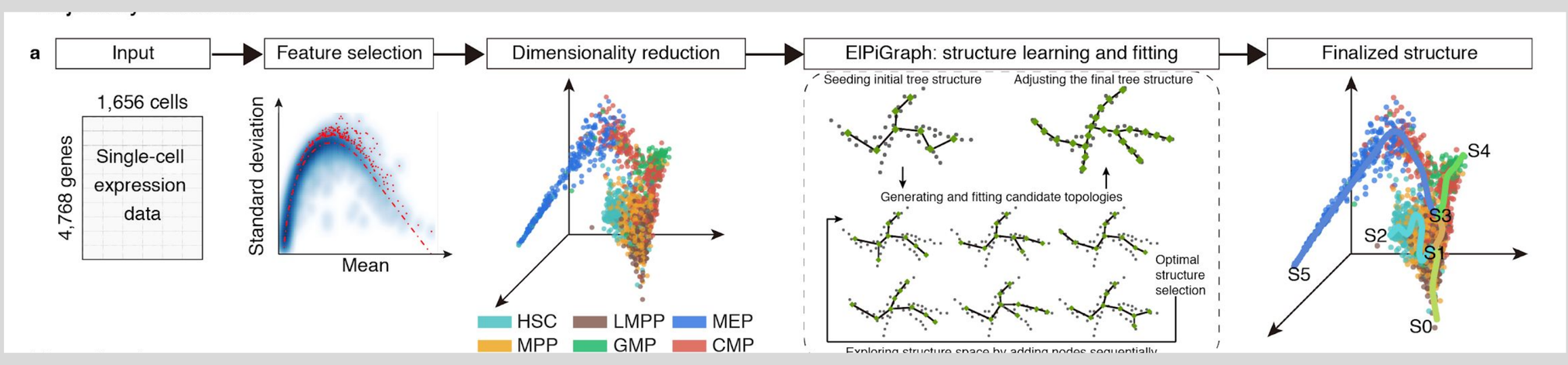

Figure 1. STREAM pipeline

   - Use Multi-subject Single Cell deconvolution (MuSiC) to deconvolve data from TCGA, connecting single cell drug treated dataset to bulk RNA-seq data.

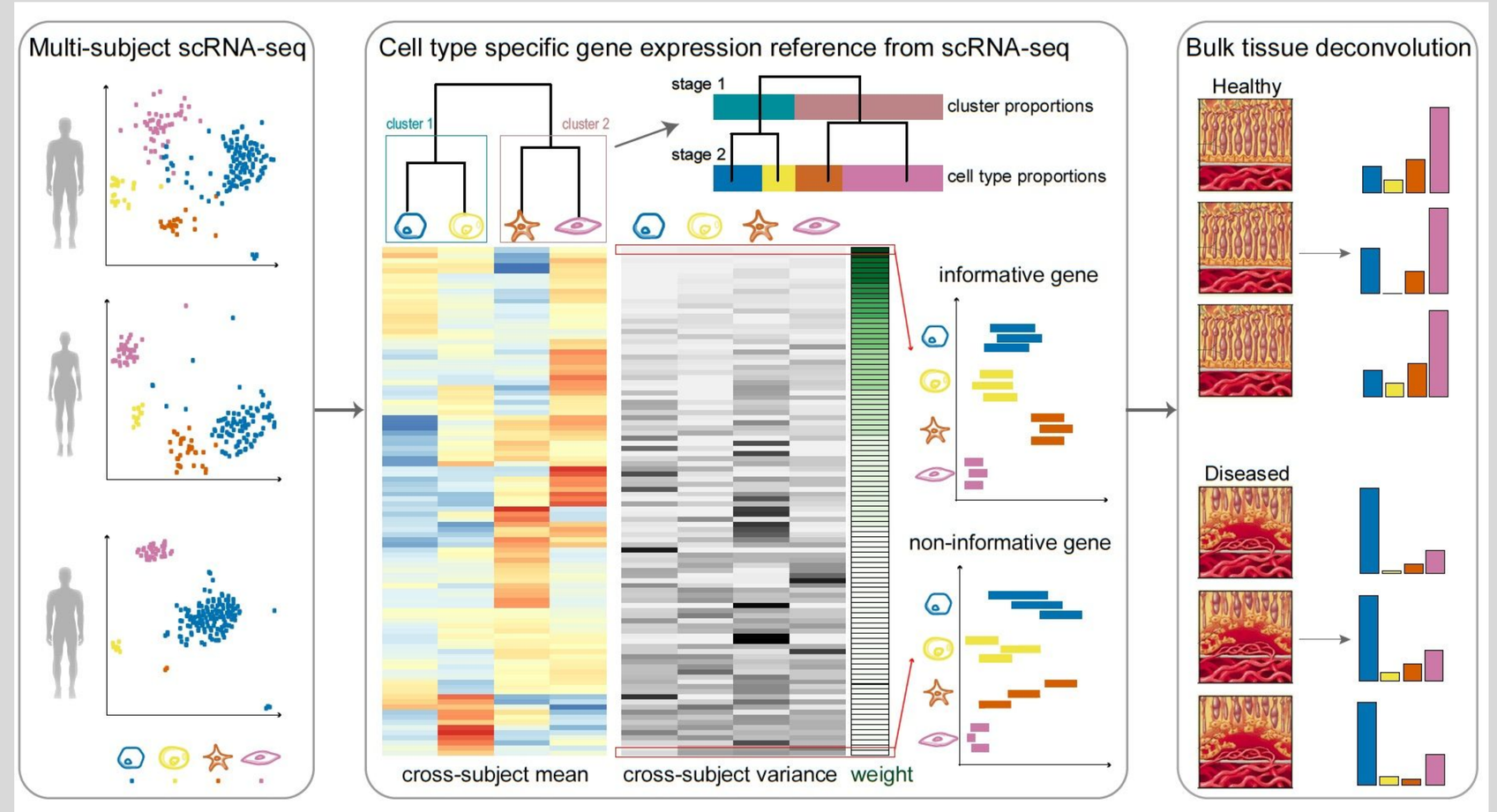

Figure 2. MuSiC pipeline to find fractions of different cell types within the bulk data

   - Use Kaplan-Meier plot to generate relationship between expression of malignant cell gene markers and survival
   - Plot heatmap of differentially expressed genes as well as marker genes found from CellMarker

IMPLICATIONS

These results are important because researchers can target differentially expressed genes and manipulate drugs to alter the pathways associated with those genes. We can extend this analysis to other cell types.

DATA AND FINDINGS

STEP 1: Curate Gene Markers from sc-RNA-seq Melanoma data

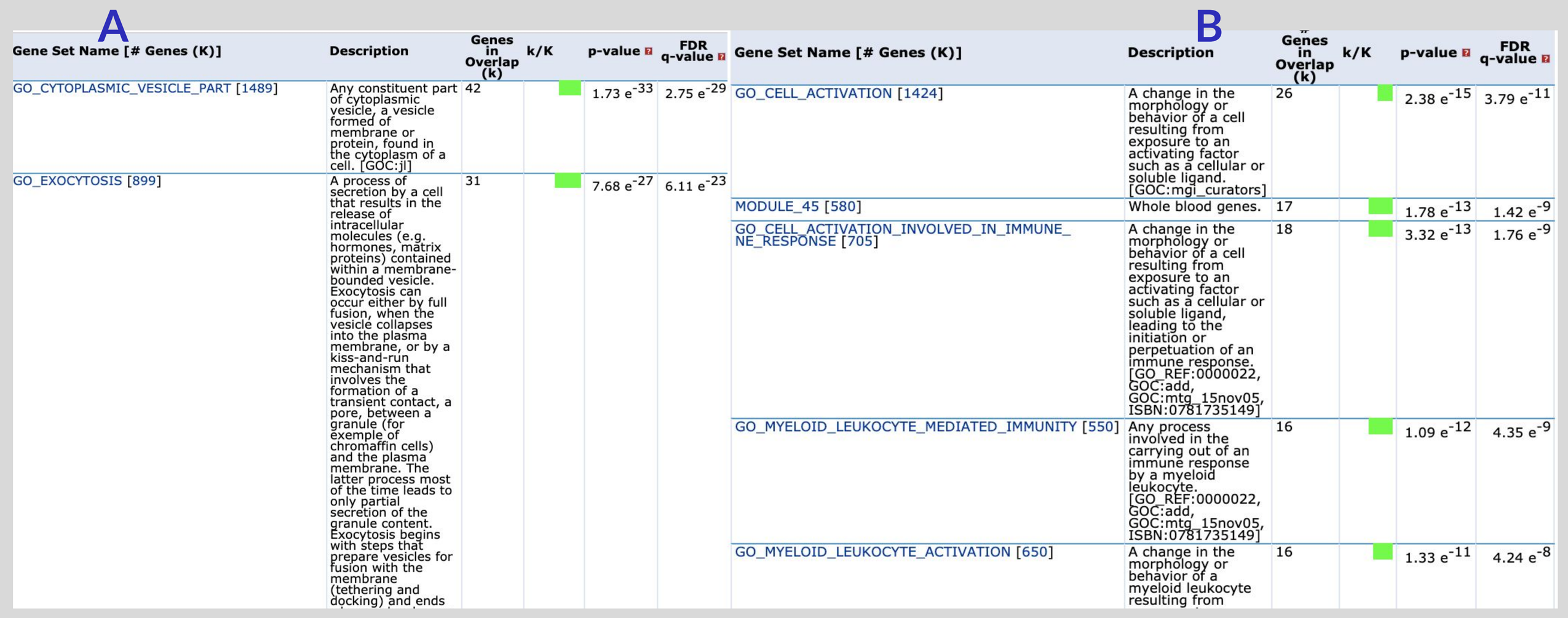

Figure 3. Top 5 MSigDB gene sets that overlap with 100 most differentially expressed genes for treated and untreated A) malignant cells and B) B cells

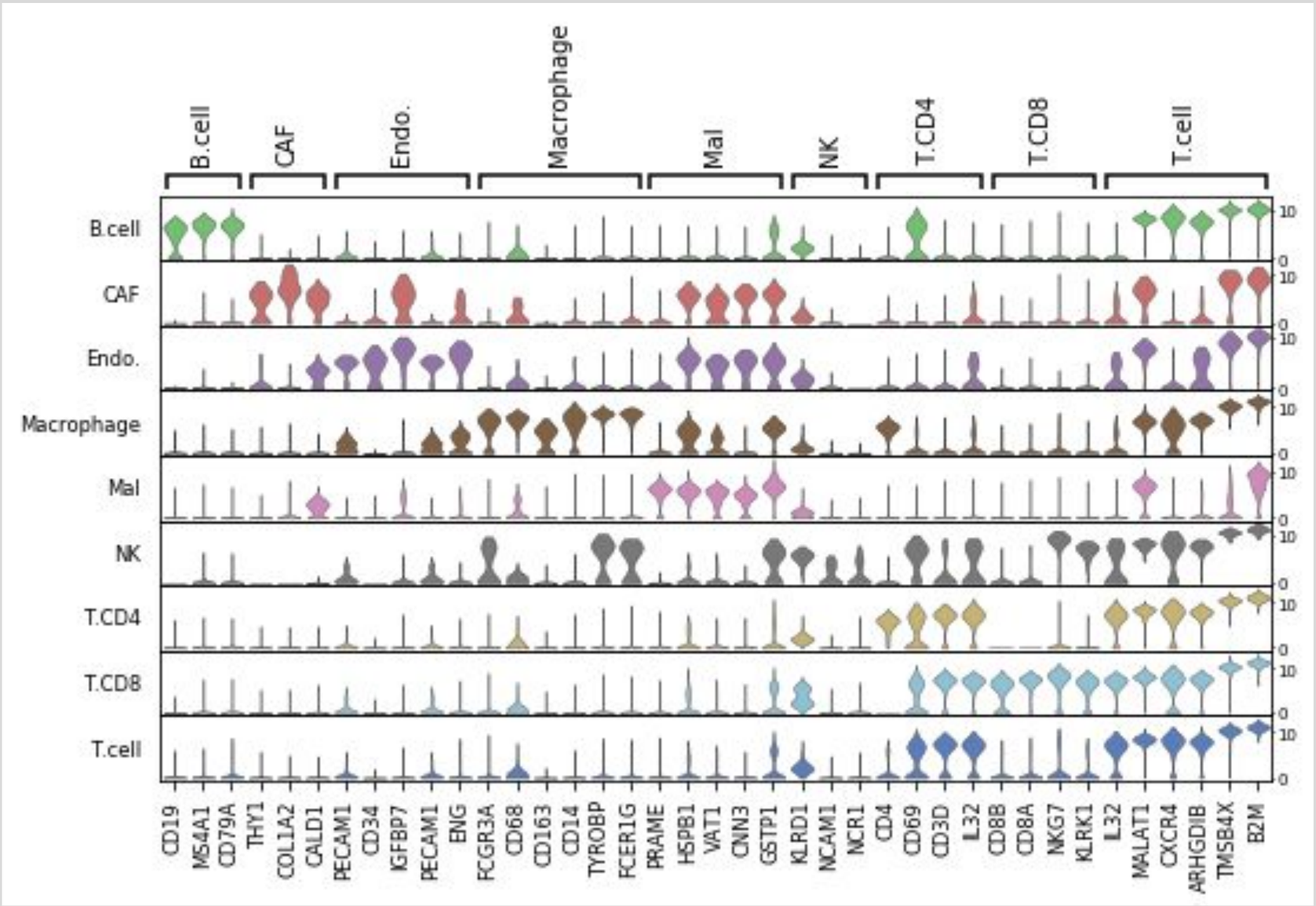

Figure 4. Violin plot that shows the distribution of the gene expression values of marker genes across cell types. Top 3 differentially expressed between the cell type and all other cell types

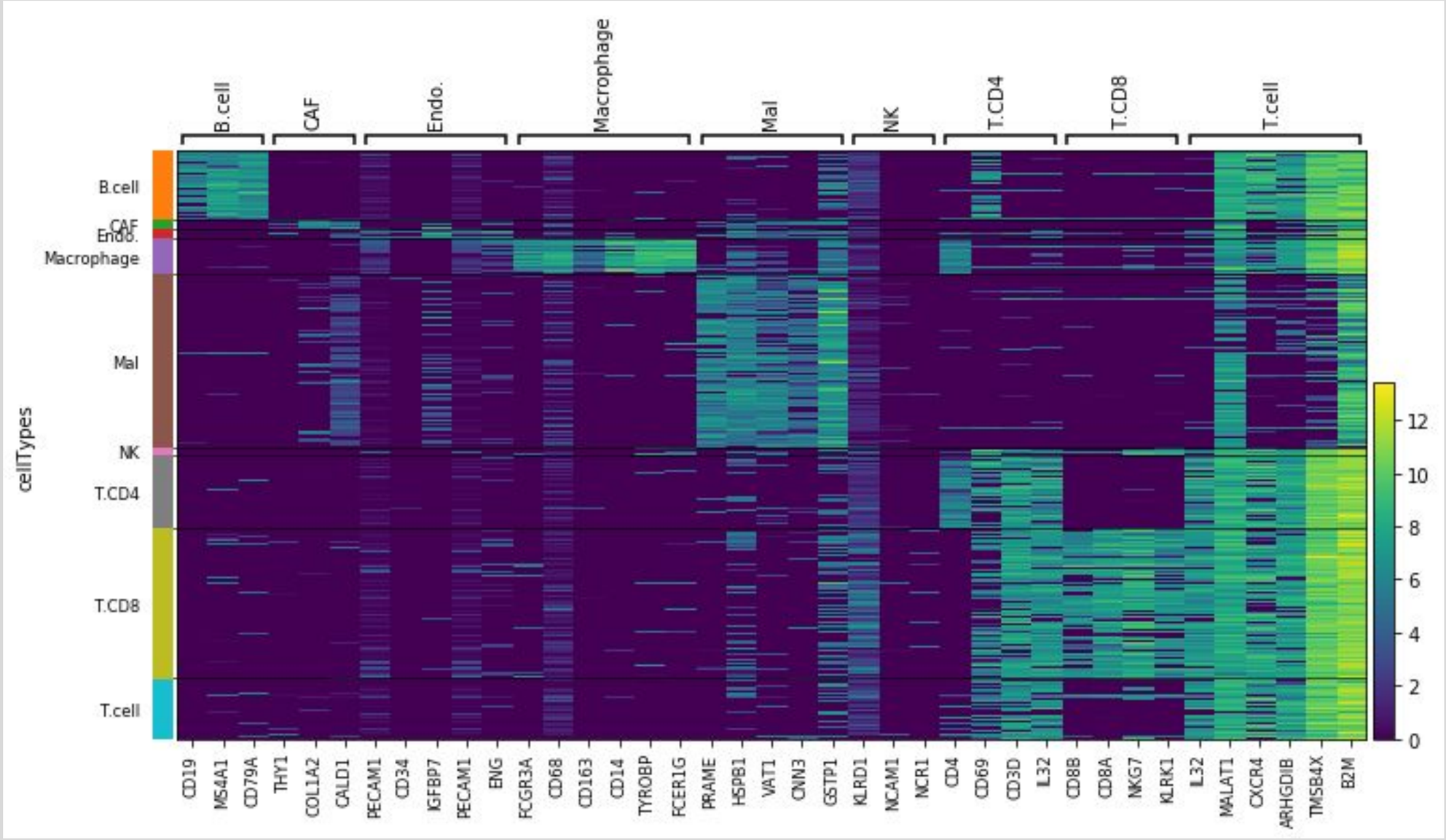

Figure 5. Heatmap that shows the expression of marker genes which consist of the top 3 differentially expressed between the cell type and all the other cell types as well as cell specific markers.

STEP 2: Analyze single cell data

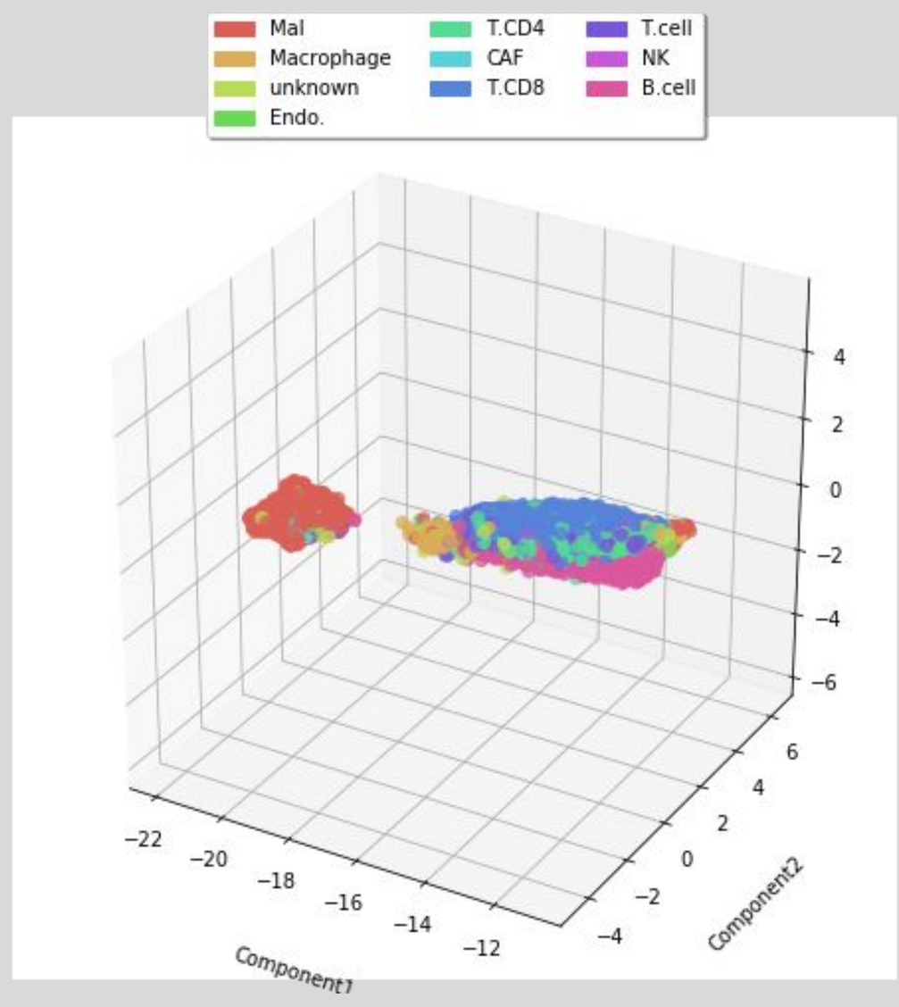

Figure 6.. STREAM plot showing time trajectories for CD8 and B cells.

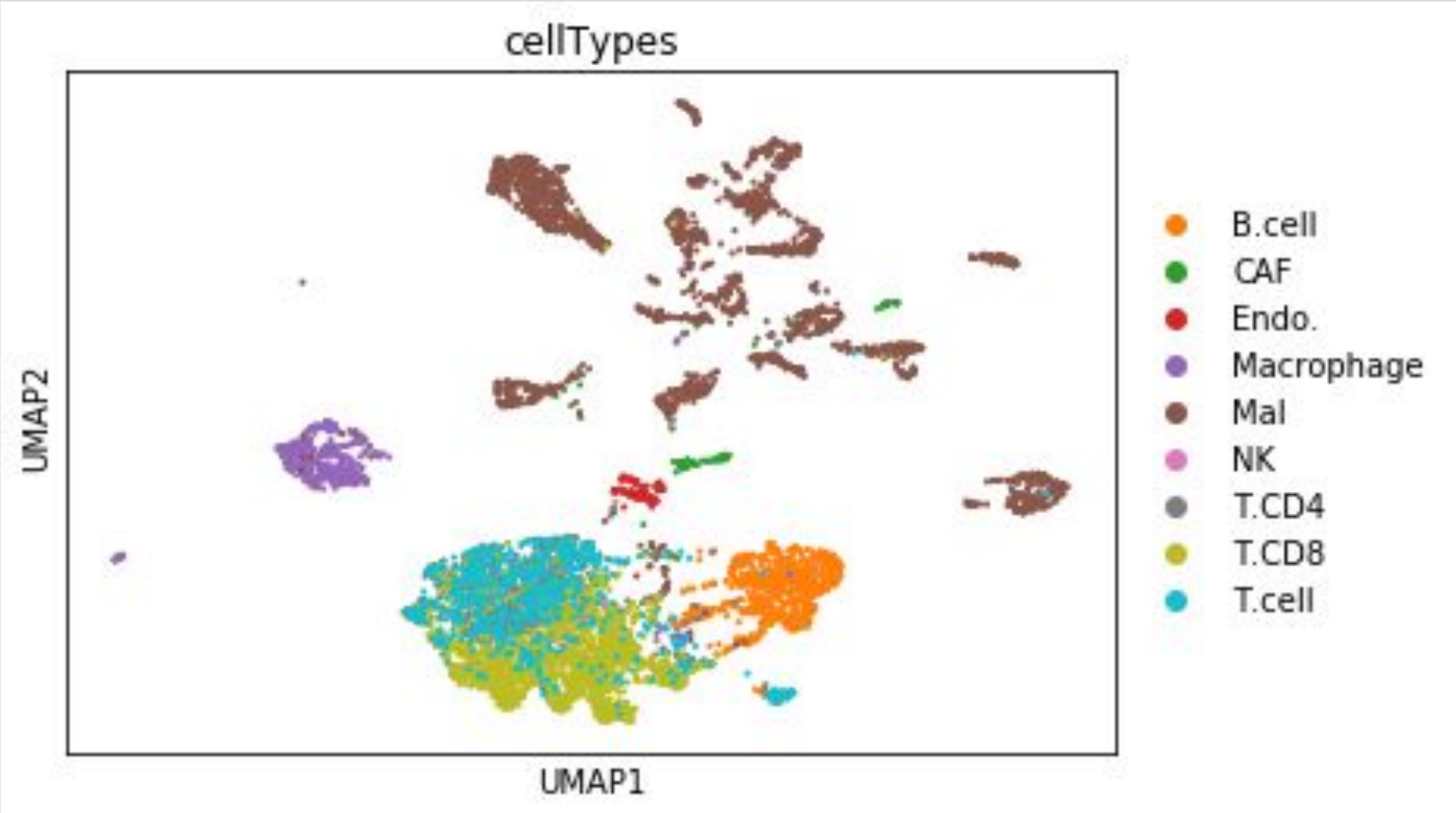

Figure 7. Clusters of cells generated through UMAP, showing distinctions between cell type

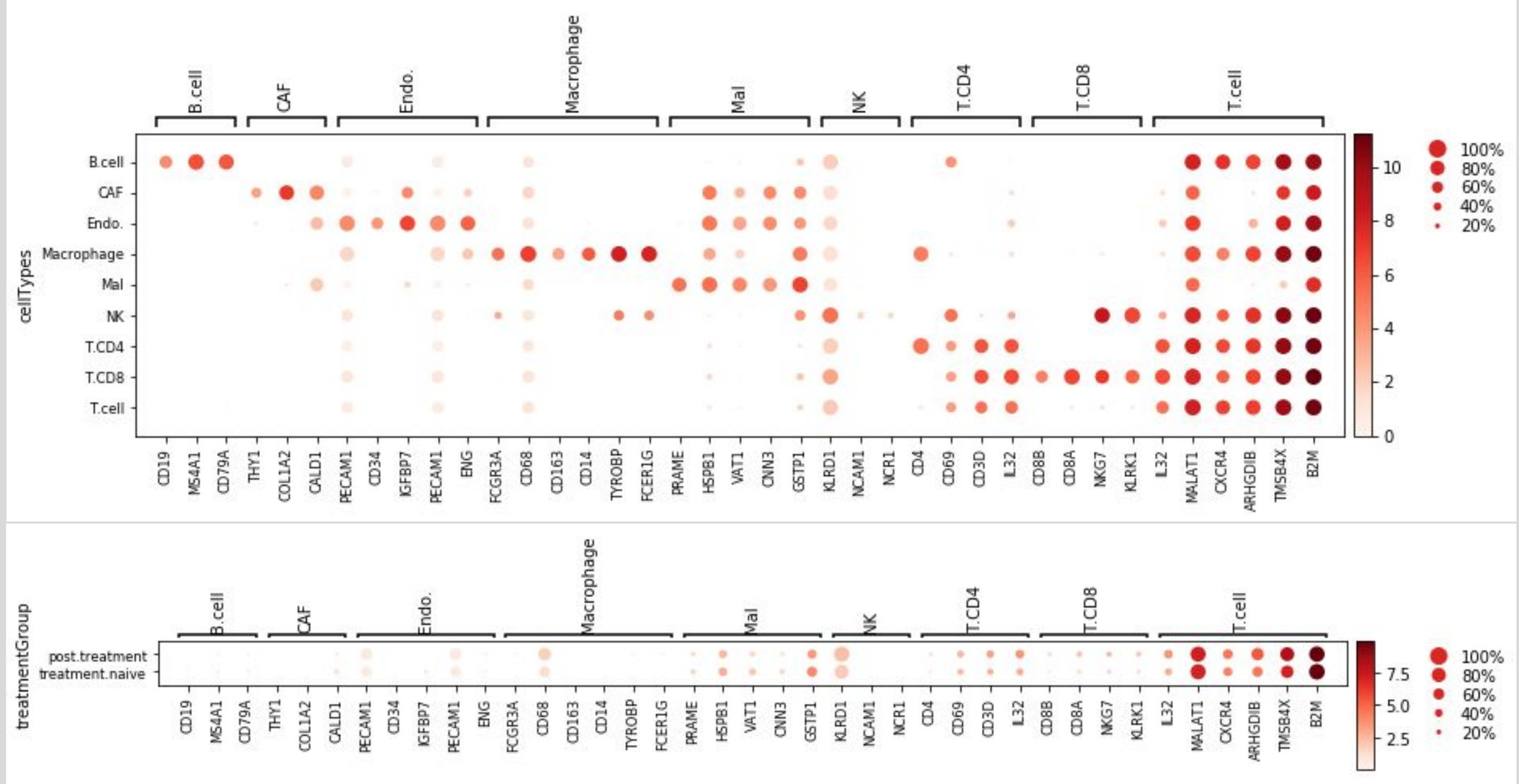

Figure 8. Dotplot that shows the fraction of cells with expression of each marker gene. The top graph shows a comparison between expression between cell types, while the bottom graph shows the expression of genes based on whether the cells were treated.

STEP 3: Combine single cell data with bulk data to get clinical insights

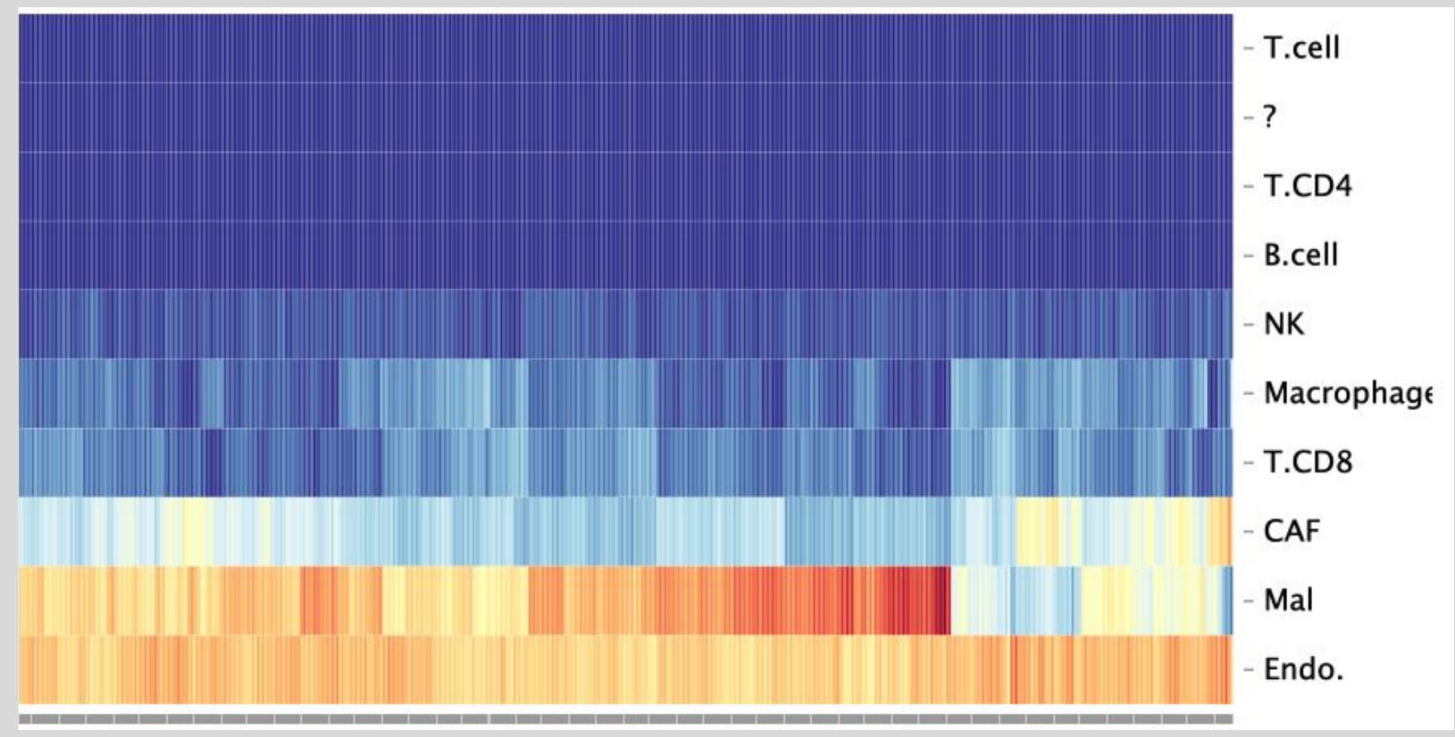

Figure 9. Deconvoluted TCGA data with two apparent malignant cell groups with differing expression

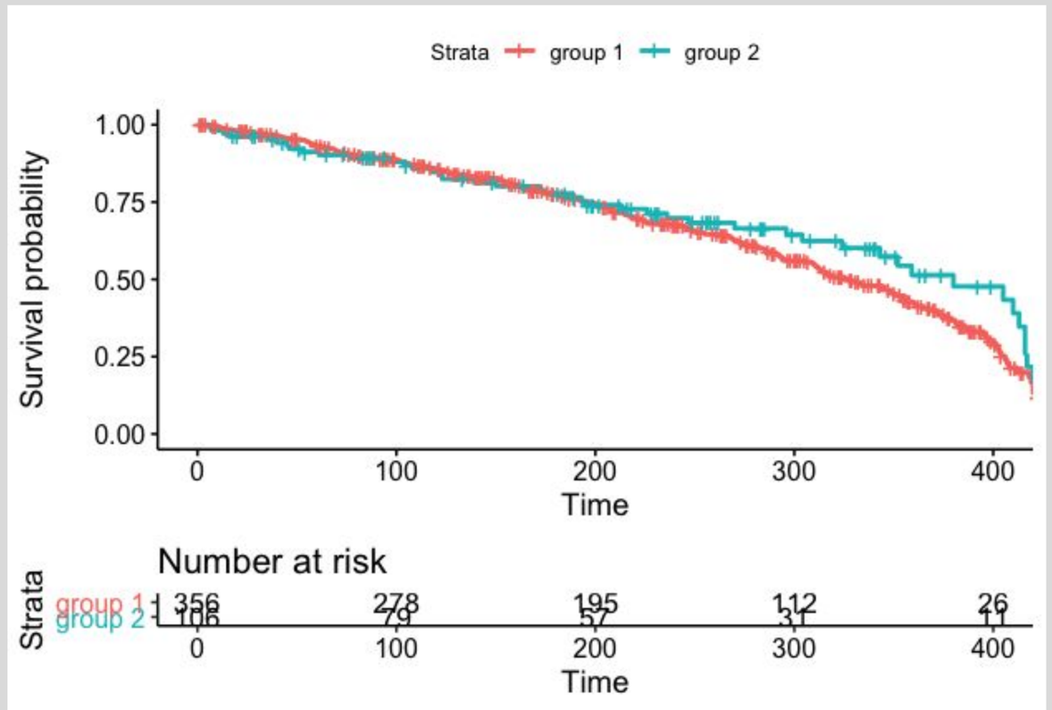

Figure 10. Kaplan-Meier Plot showing differences in survival between two malignant groups of cells

CONCLUSION

The genes that are most differentially expressed play a role in exocytosis, which suggests that there is a link between anti-PDL-1 therapy and altering the mechanism of exocytosis in malignant cells. The 34 clusters created suggest that there is some link to cancer associated fibroblasts as well as the differentially expressed genes between clusters. The Kaplan Meier Plot shows survival probability differences between two malignant groups of cells.

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