

Relative abundance

Summary proportion of clonotypes with specific frequencies

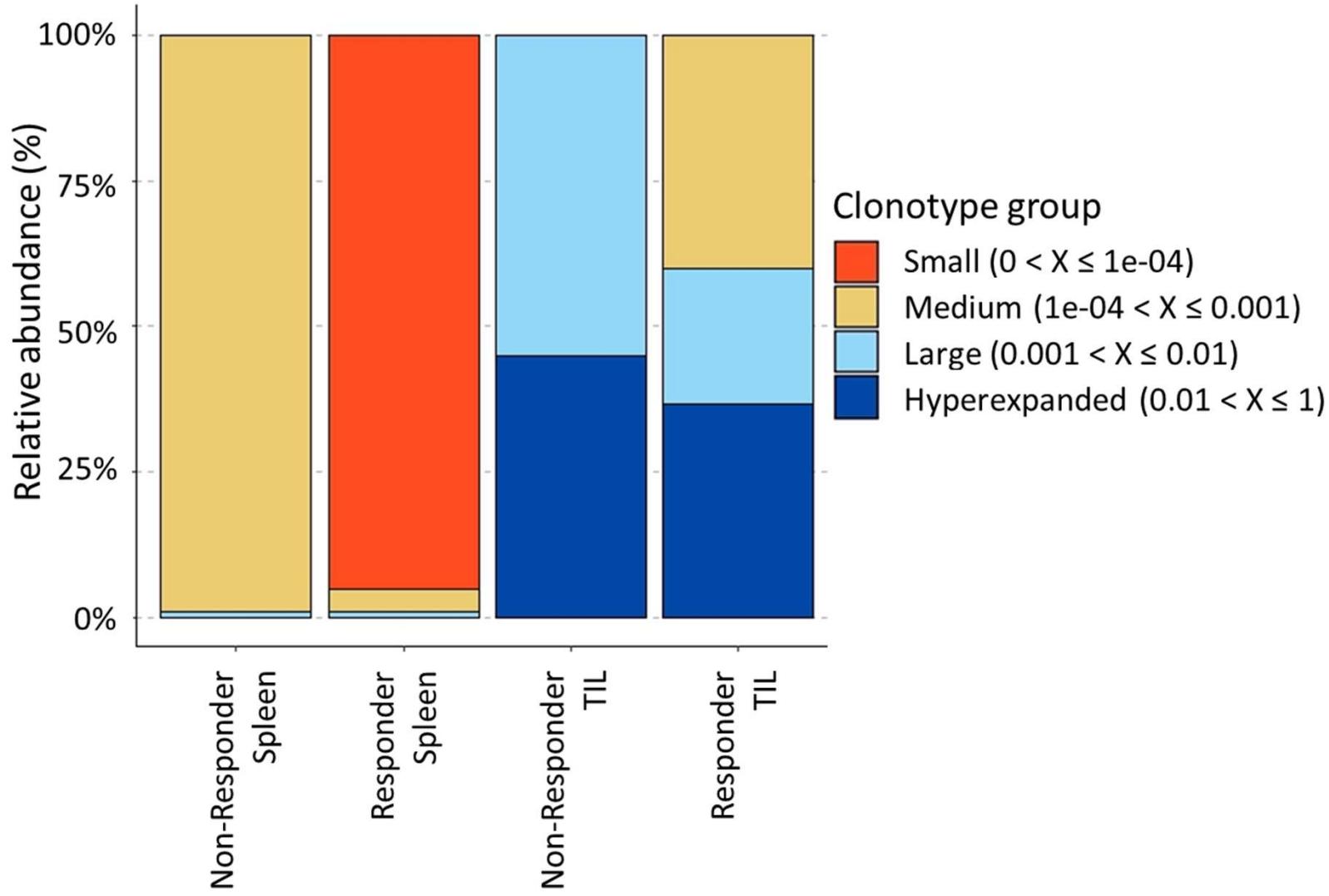


Figure 1. Relative abundance of TCR clonotypes in different samples. Relative abundance of TCR clonotypes in 4 sequenced samples including Non-Responder splenic CD8, Non-Responder CD8 TIL, Responder splenic CD8, and Responder CD8 TIL. In each sample, for each clonotype its relative abundance was calculated. These clonotypes were grouped based on their relative abundance as small ($0 < x \leq 1e-04$), medium ($1e-04 < x \leq 0.001$), large ($0.001 < x \leq 0.01$), and hyperexpanded ($0.01 < x \leq 1$).

Repertoire overlap

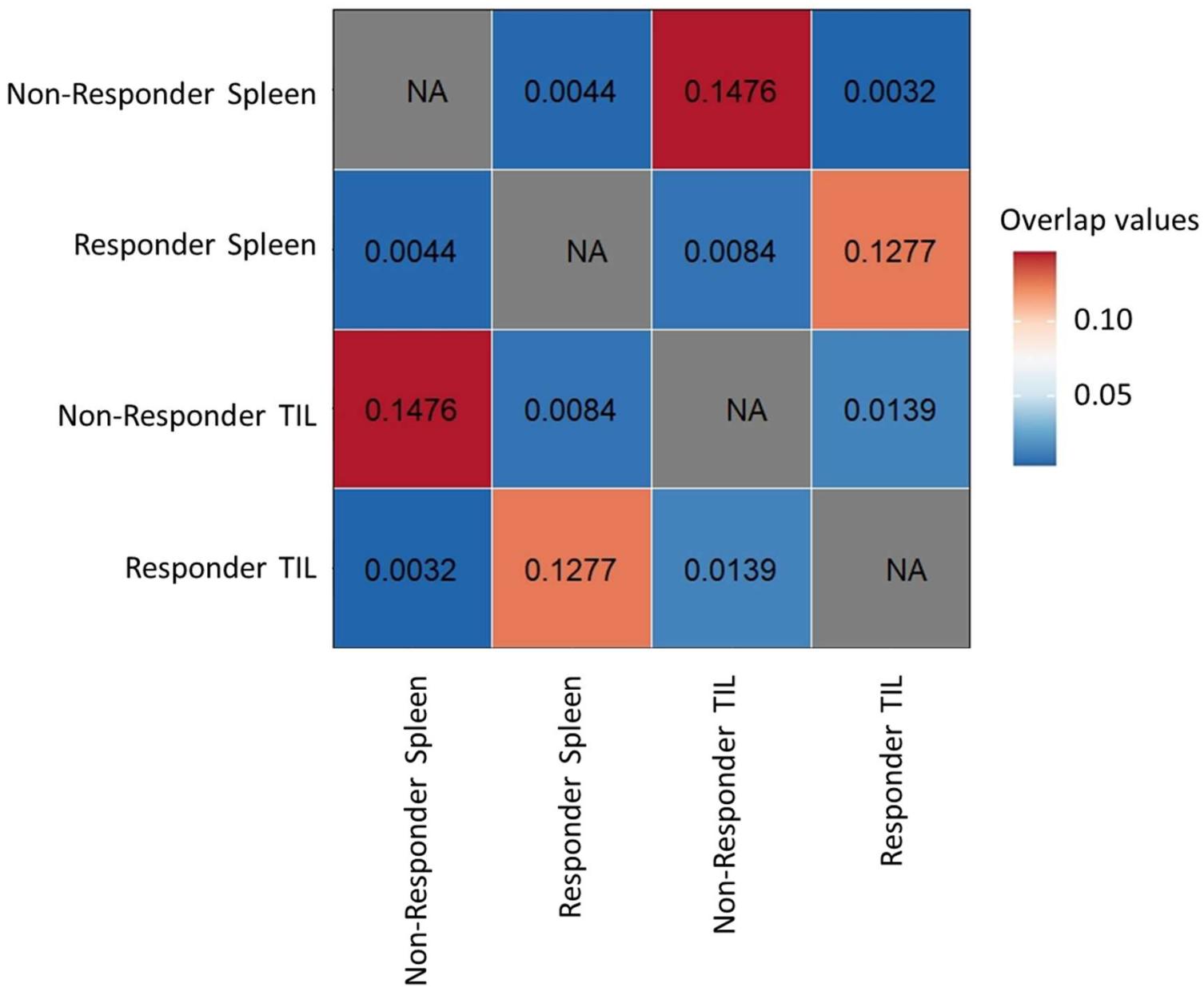


Figure 2. Repertoire overlap of TCR clonotypes between different samples. The overlap coefficient was calculated in a pairwise manner between each sample. The resulting matrix is plotted, showing the highest overlap between samples from the same individual (Non-Responder Spleen-Non-Responder TIL or Responder Spleen-Responder TIL).

Responder TIL TRAV-J Gene Usage

NonResponder TIL TRAV-J Gene Usage

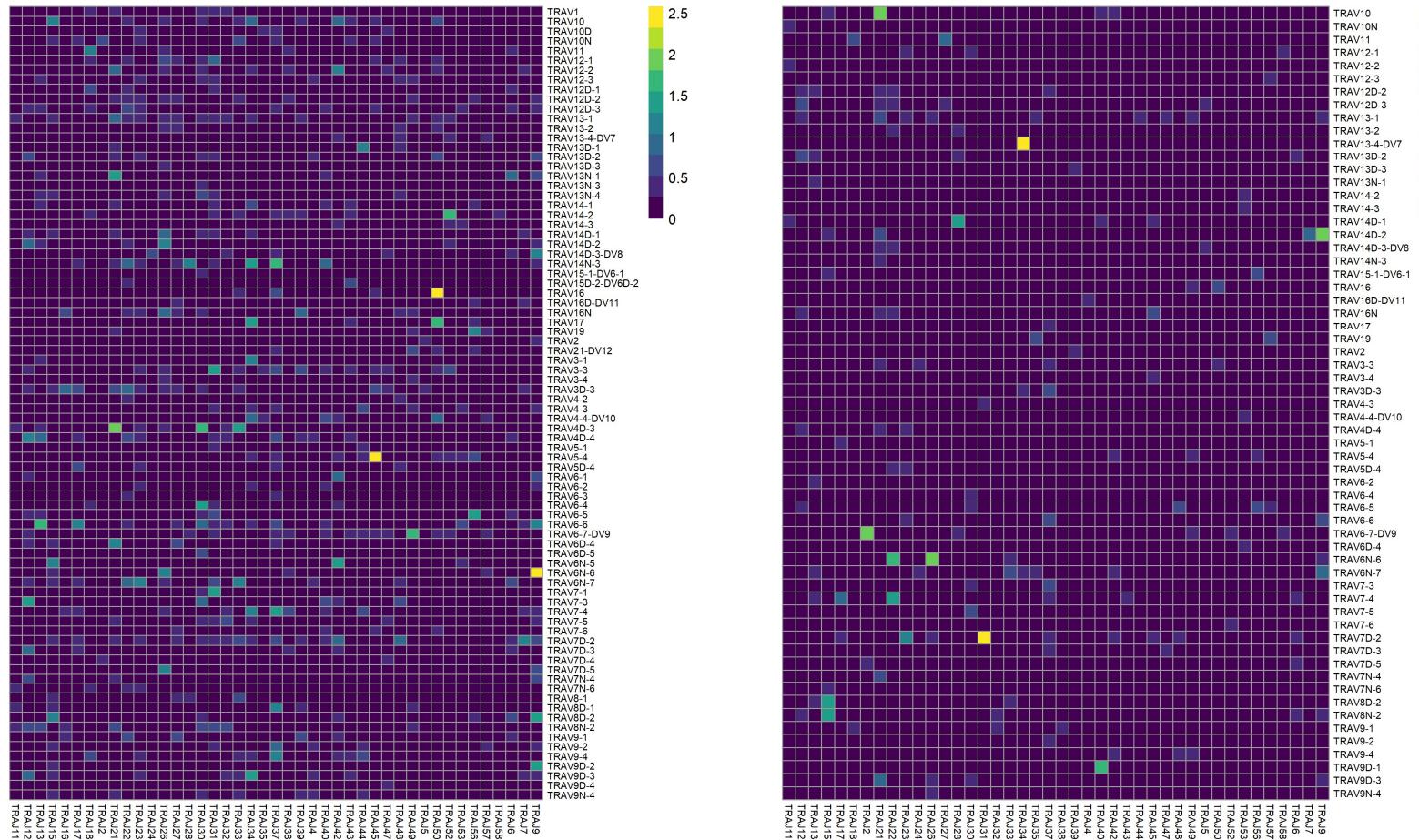


Figure 3. TCR α V-J gene usage in samples sequenced for TCR from Responder and Non-Responder TIL. TCR α V-J gene usage combinations in all T cells from each sample, colored by the log(# cells)

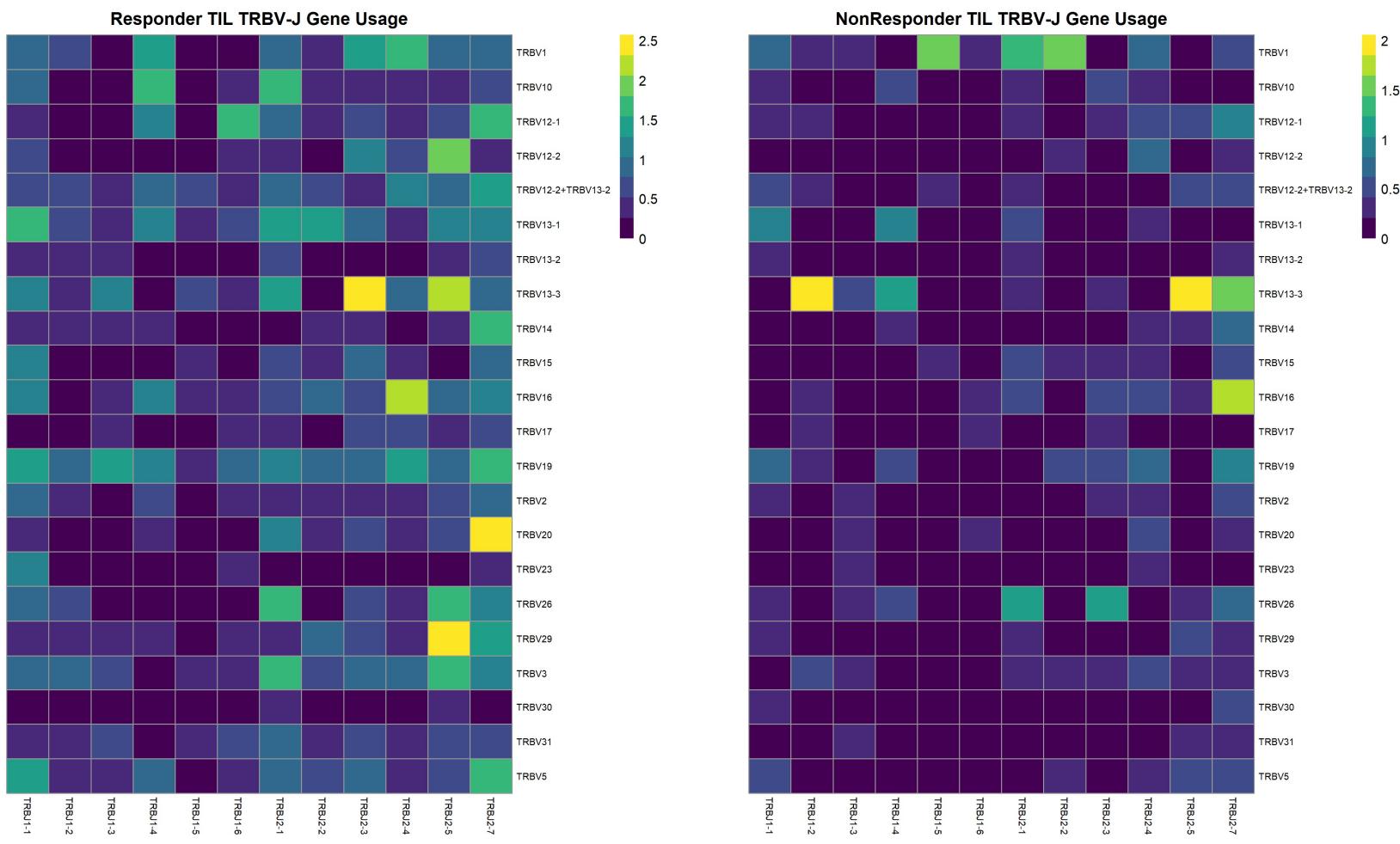


Figure 4. TCR β V-J gene usage in samples sequenced for TCR from Responder and Non-Responder TIL. TCR β V-J gene usage combinations in all T cells from each sample, colored by the log(# cells)

Log10-Transformed Values: Percents in GLIPH Groups

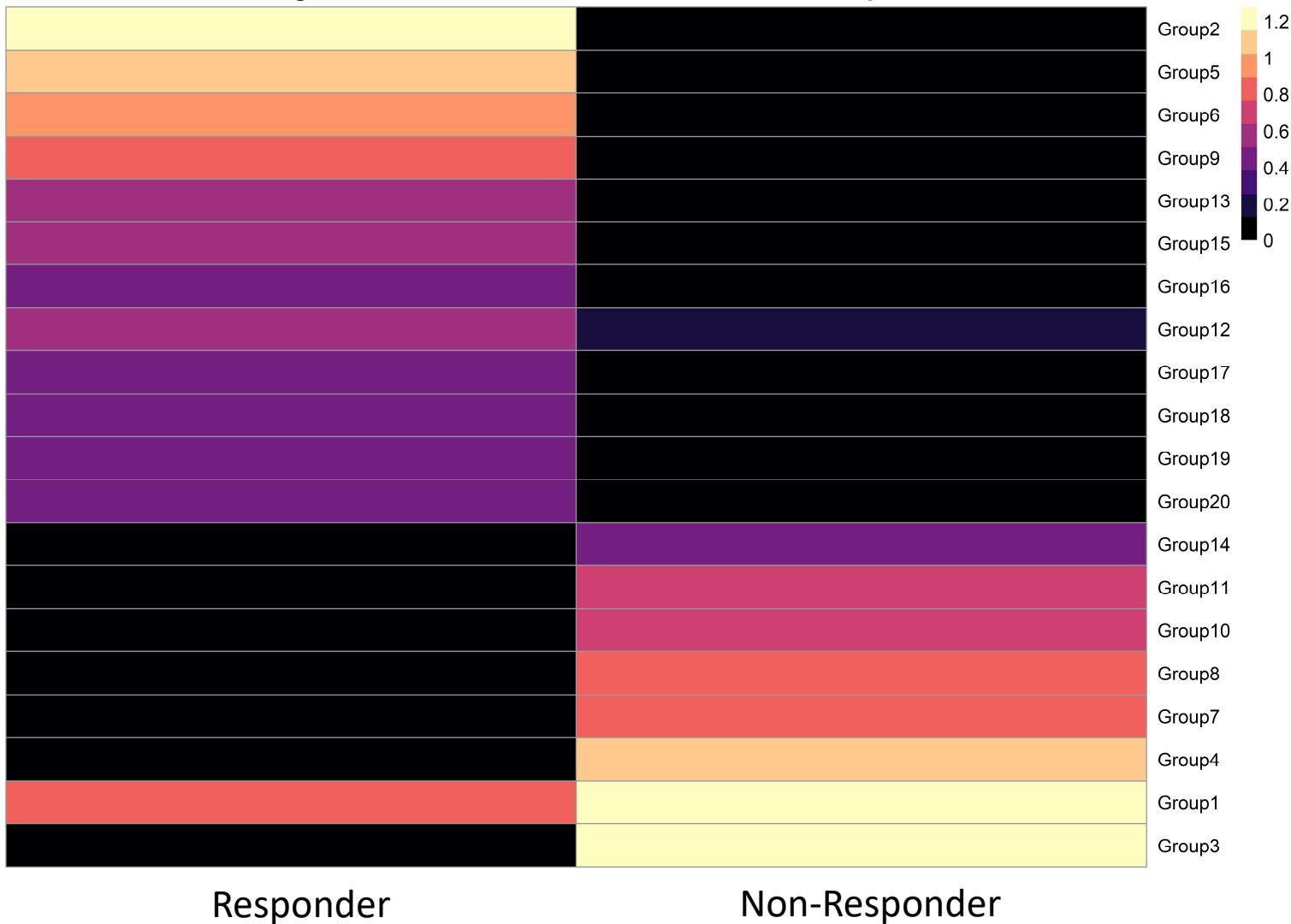


Figure 5. Percent of top 20 GLIPH groups in each Responder or Non-Responder TIL sample. The GLIPH algorithm was used to determine groups of clonotypes with high probability of having similar antigen recognition. The percentages of the top 20 groups in each sample is plotted, colored by the log10(percent).

Log Transformed Top 20 Responder and Non Responder Clonotypes

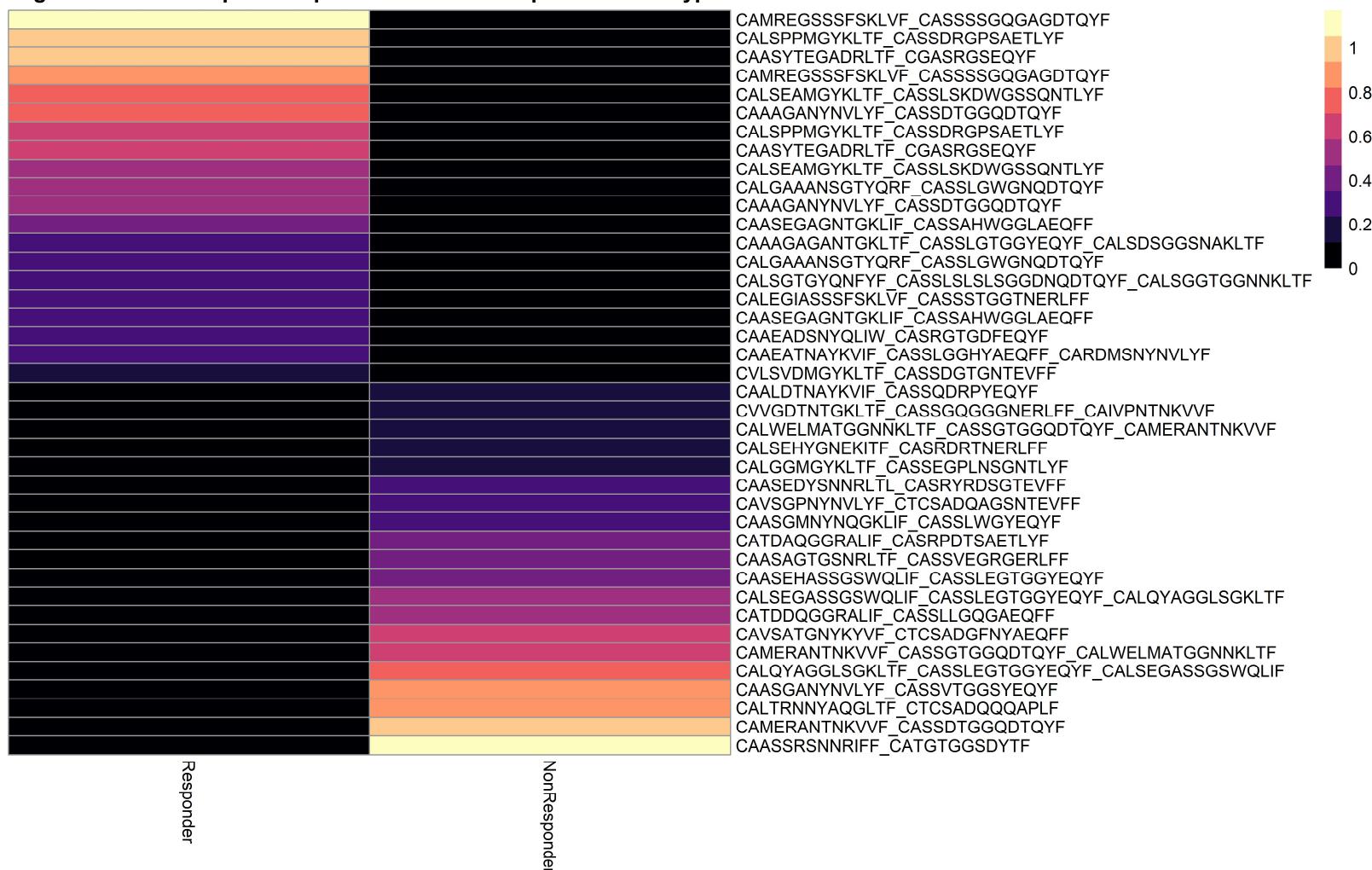


Figure 6. TCR clonotypes are mutually exclusive between responder and non-responder. Cells were grouped into clonotypes based on the paired amino acid sequences of their CDR3 α and CDR3 β regions. Top clonotypes are shown in a heatmap sorted abundance in the responder vs abundance in the non-responder.

Myeloid cells

T cells

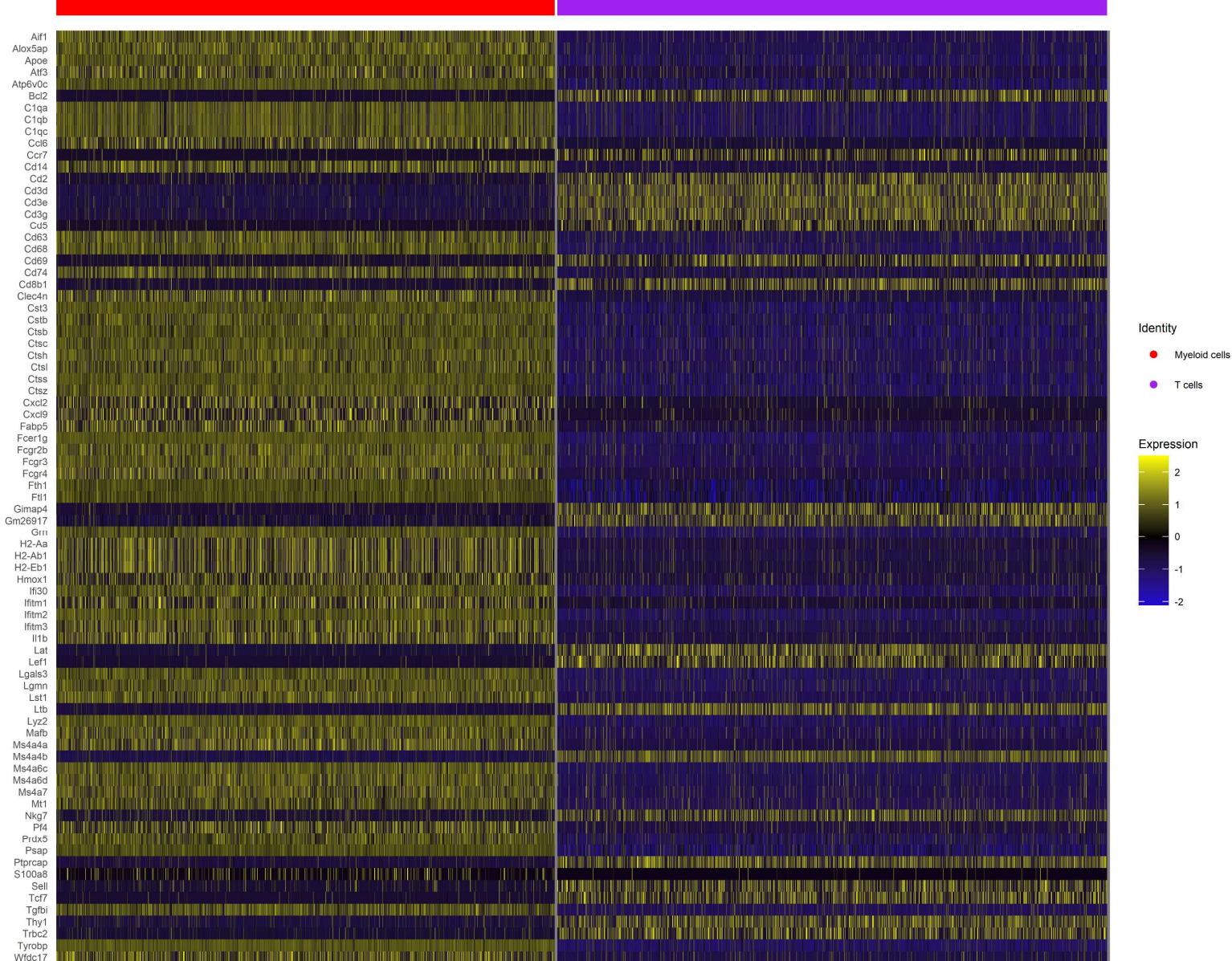


Figure 7. Myeloid and T cells have significantly different gene expression. The cells from all 4 samples were combined in Seurat. The T cells were separated from the myeloid cells by using the markers for each cluster determined with the FindMarkers function. Each population of cells was then randomly downsampled and plotted as a heatmap. The genes with the largest log fold change were plotted alphabetically.

Non-Responders

Responders

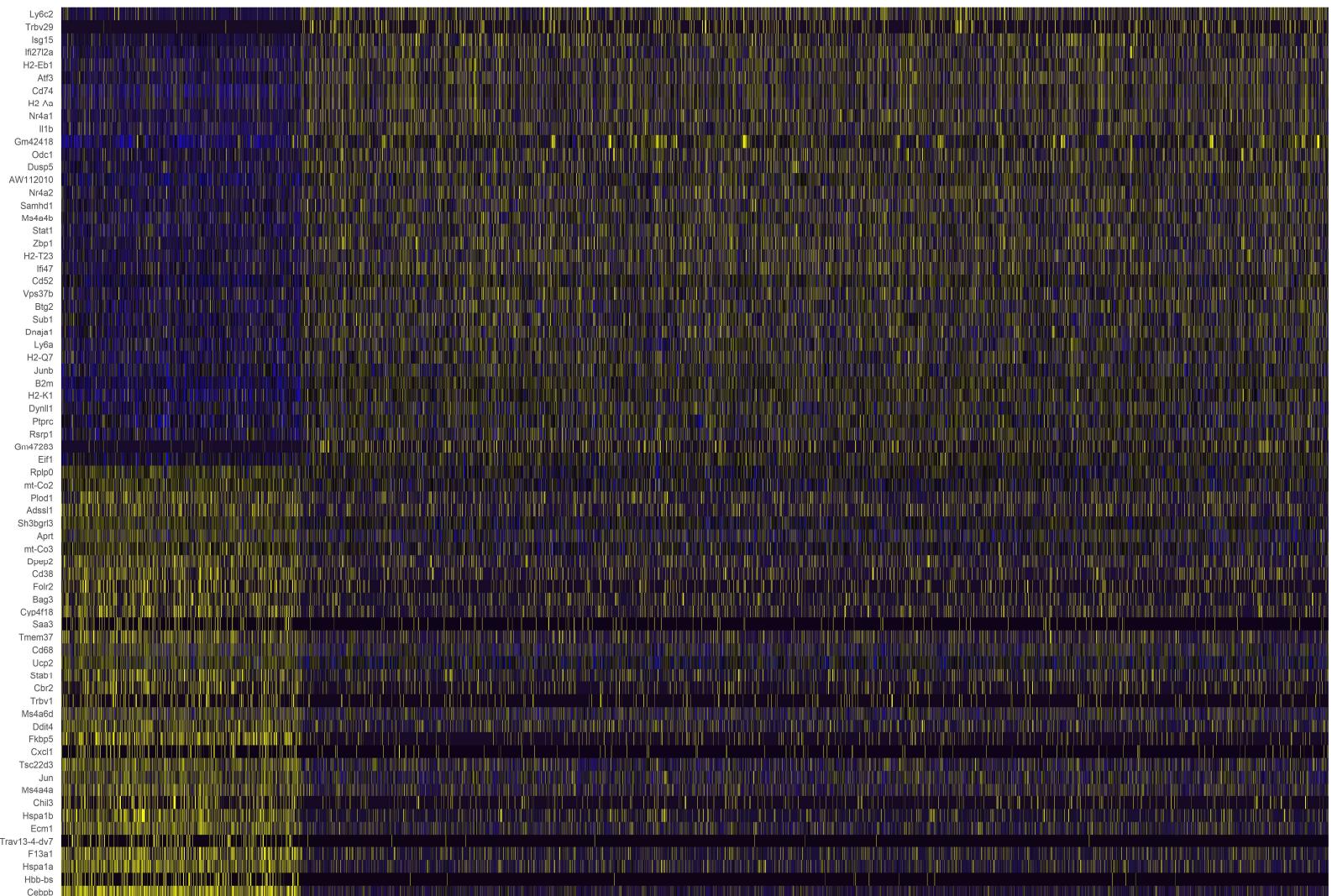


Figure 8. TIL T cells from Responder and Non-Responder mouse have different gene regulation. The T cells were extracted from the samples. The Responder and Non-Responder samples were compared using the FindMarkers function in Seurat. The genes with the largest fold change of Responder vs Non-Responder scaled expression were plotted as a heatmap.

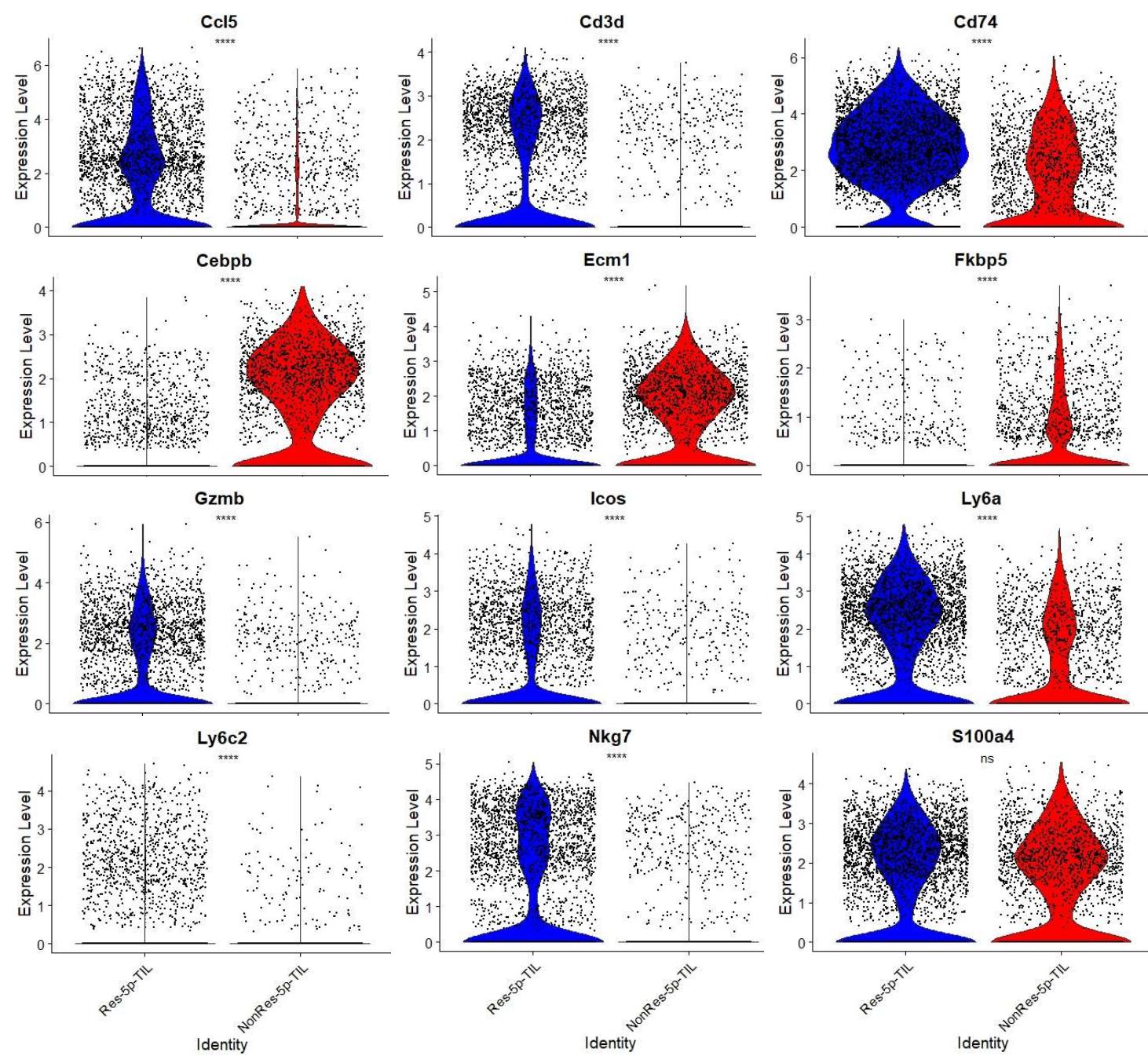


Figure 9. Violin plots of select differentially expressed genes between Responder and Non-Responder TIL T cells.
The normalized gene expression for each cell is shown as black dots. The groups were compared using Wilcoxon Test and the resulting p-value is shown. (**** $p \leq 0.0001$, ns $p > 0.01$)

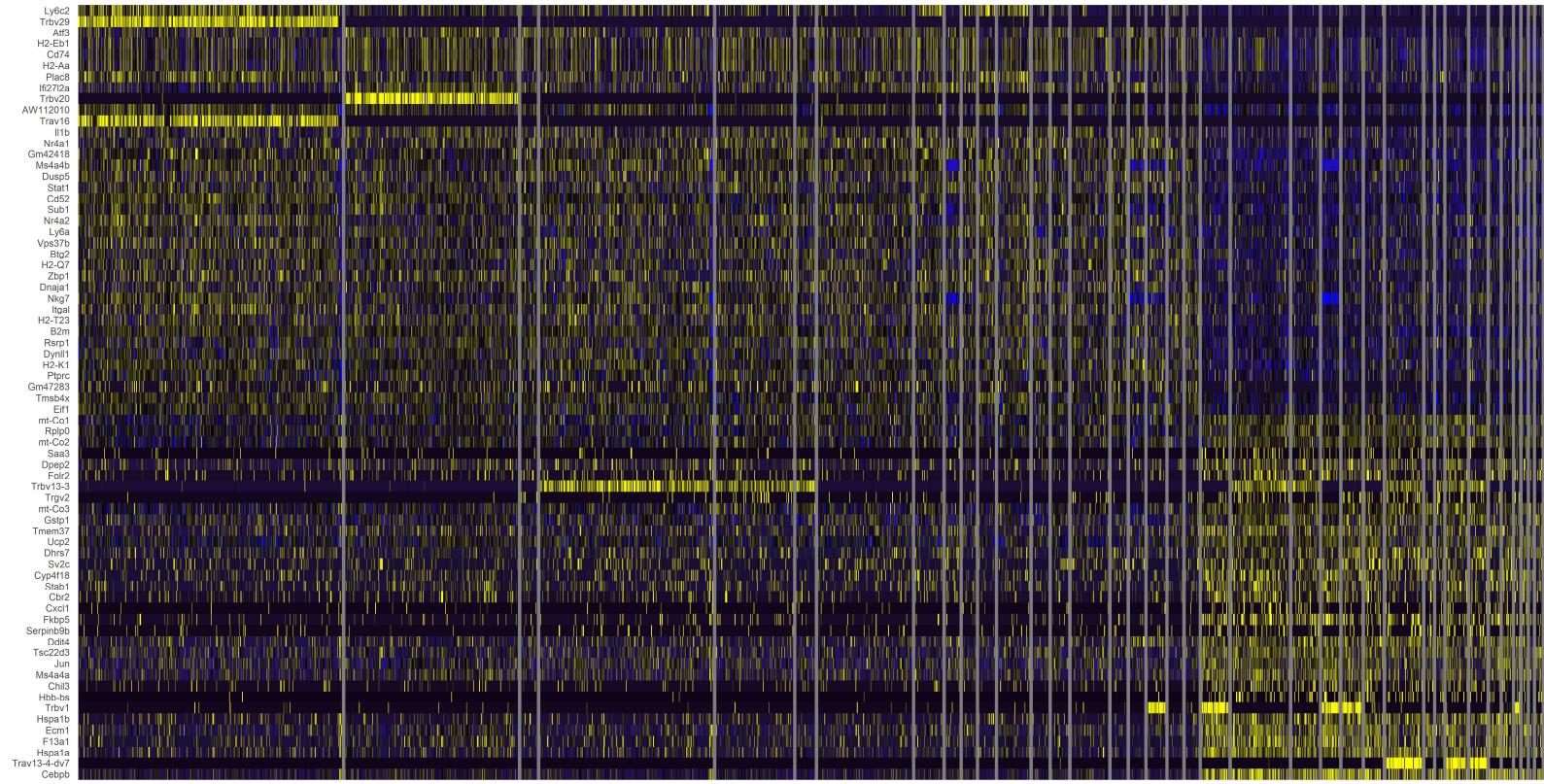


Figure 10. Top TCR clonotypes of Responder TIL versus Non-Responder TIL show differently activated genes. The expression for all cells with an identified clonotype was scaled. This was then used to compare the Responder sample from the Non-Responder sample using the FindMarkers function in Seurat. The clonotypes with the highest number of cells identified in the scRNA-seq data were determined. The gene expression is shown by heatmap where the values are the scaled gene expression, and the genes with the highest fold change are plotted.

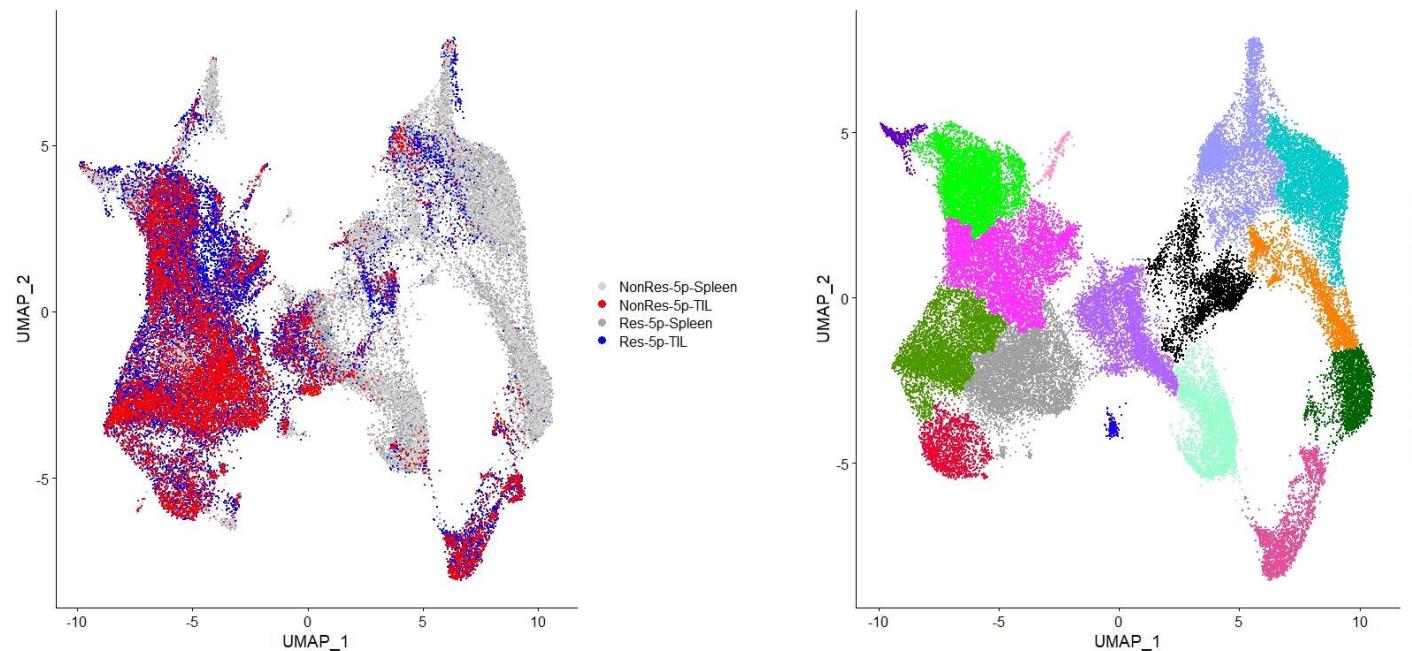


Figure 11. UMAP plots of combined scRNA-seq data colored by sample and cluster. The samples were integrated in Seurat and the FindNeighbors function was used with UMAP reduction. The FindClusters function was then used and resulting clusters plotted with 0.1 resolution. The TIL samples clustered together, and the spleen samples clustered together. The FindMarkers function was used to find gene markers of each cluster and used to identify cell types. There were many myeloid cells, a cluster of tumor cells, as well as many T cell clusters.

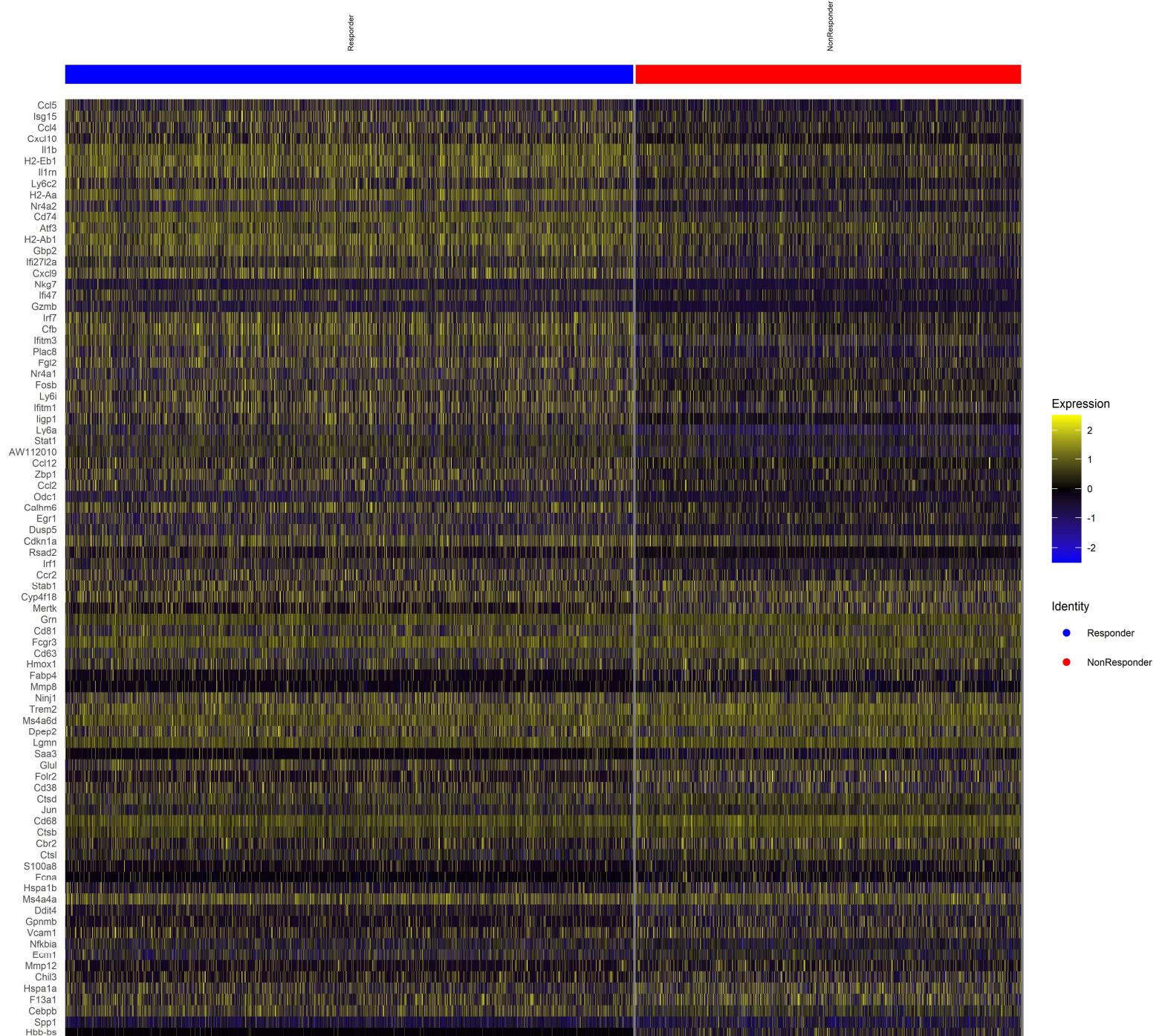


Figure 12. Myeloid cells from Responder and Non-Responder mouse do not have distinct gene regulation patterns. The myeloid cells were extracted from the samples. The Responder and Non-Responder samples were compared using the FindMarkers function in Seurat. The genes with the largest fold change of Responder vs Non-Responder scaled expression were plotted as a heatmap. There are significantly differently regulated genes but not in distinct patterns between the responders and non-responders. There are some upregulated genes related to antigen presentation.