

# **Case Study Presentation**

**Single Nucleus RNA Sequencing (snRNA-seq) Analysis  
of Tumor-Associated Macrophages**

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**Date: 3/31/2024**

## **Workflow for Analysis of snRNA-seq Data**

**Import and read the snRNA-seq data (Seurat)**



**Data normalization and scaling (Seurat)**



**Dimensionality reduction (PCA, UMAP, tSNE using Seurat)**



**Use macrophage gene markers to identify the macrophage clusters**

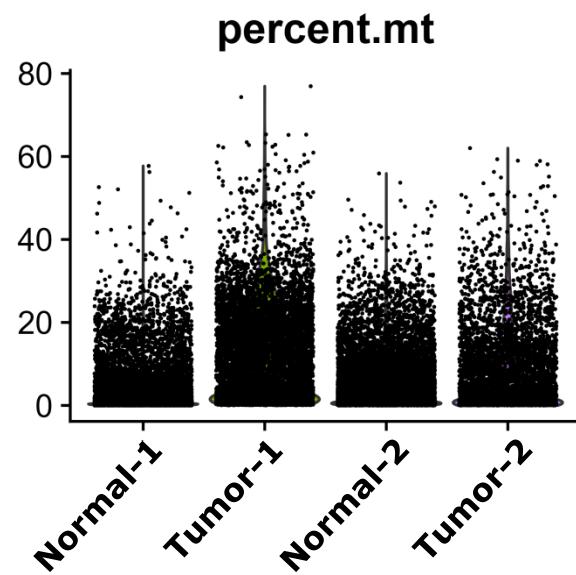
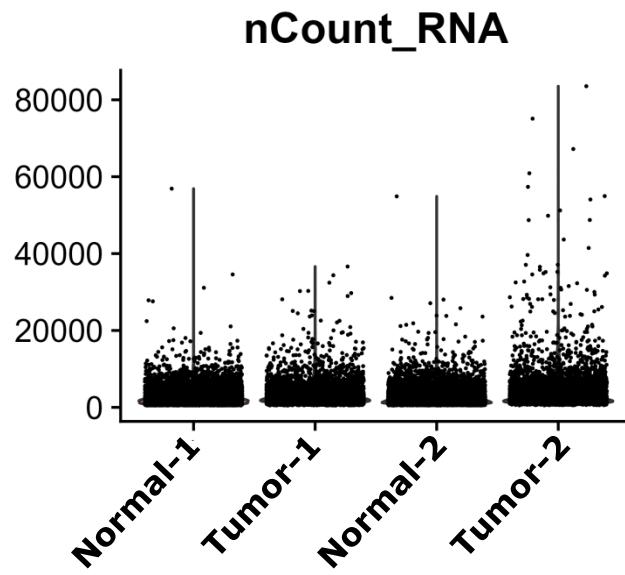
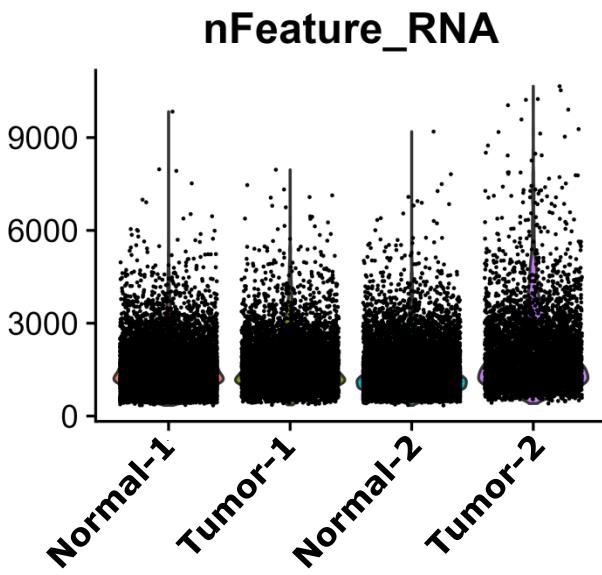


**Perform analysis of differentially expressed genes in TAMs**

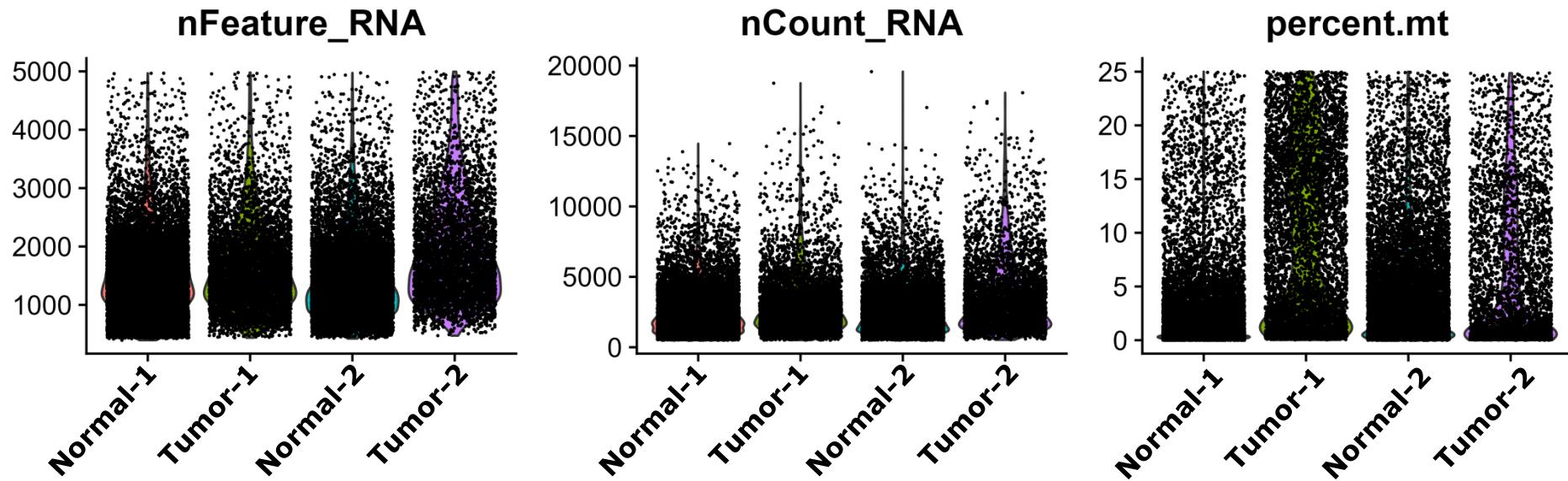


**Gene set enrichment analysis**

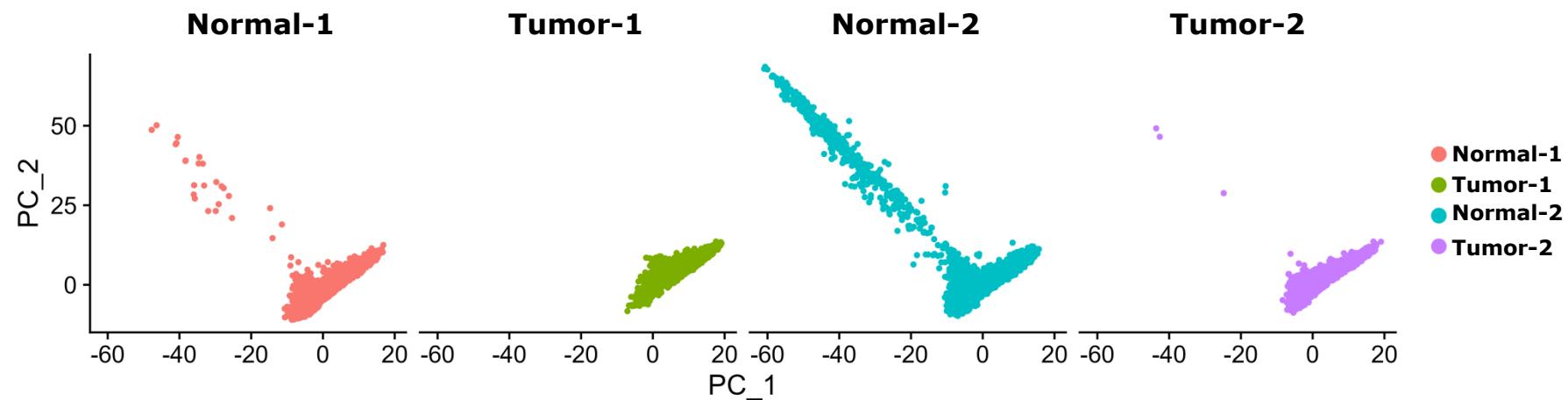
# QC Check of the snRNA-seq Data



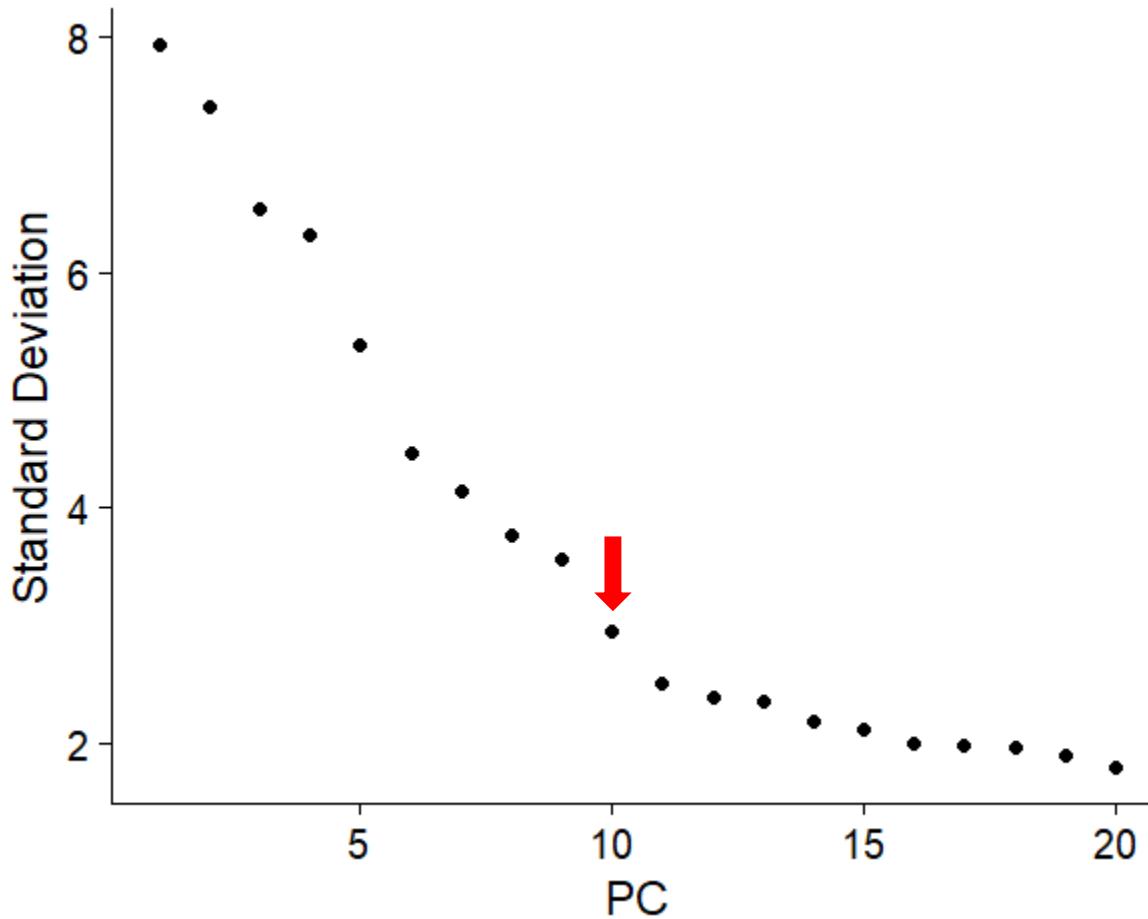
# QC Check of Processed snRNA-seq Data



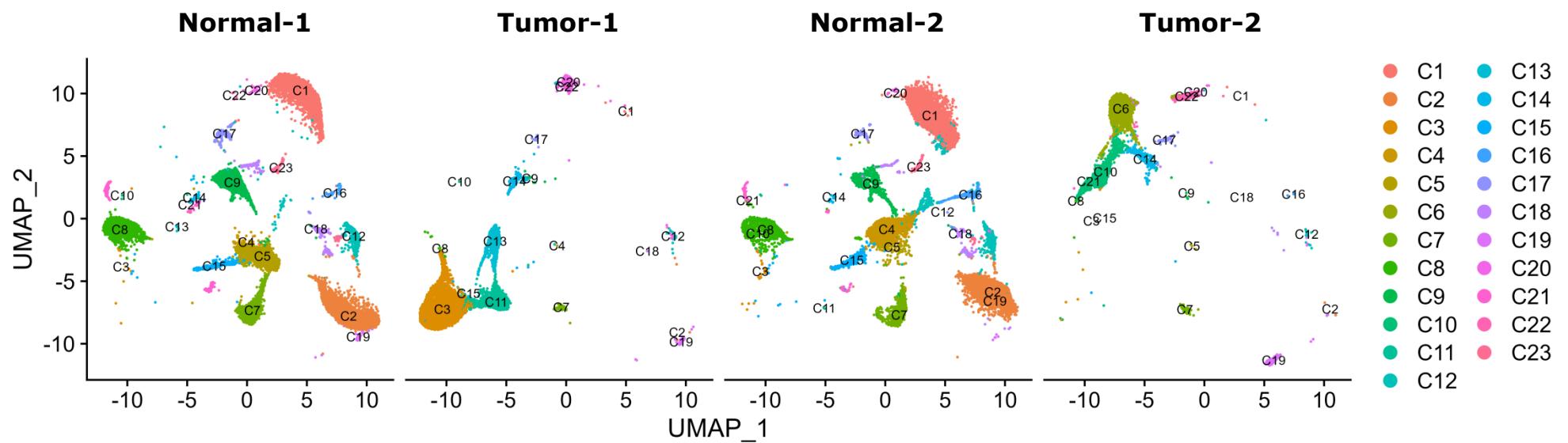
# PCA Plot Analysis of snRNA-seq Data After Dimensionality Reduction



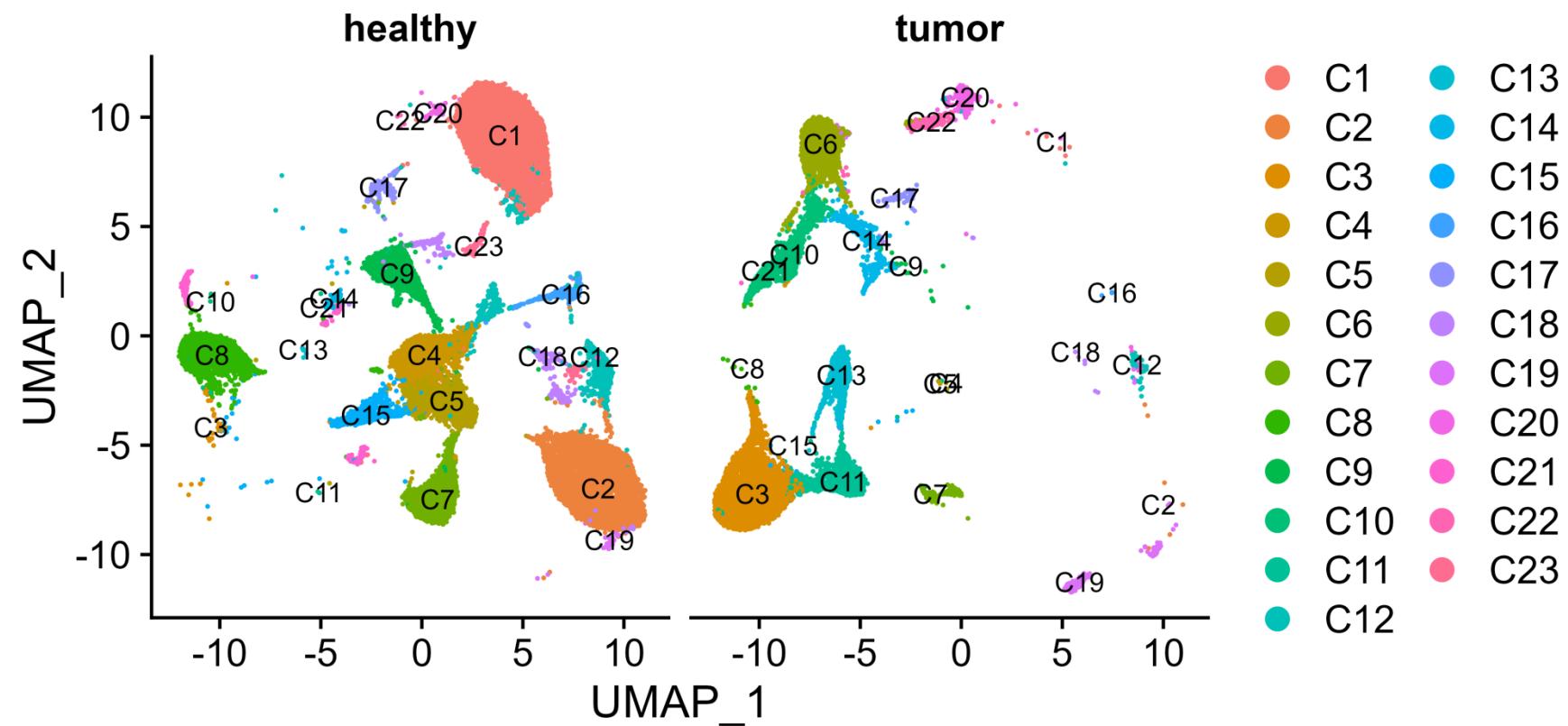
# ElbowPlot Analysis of Principal Components After Dimensionality Reduction



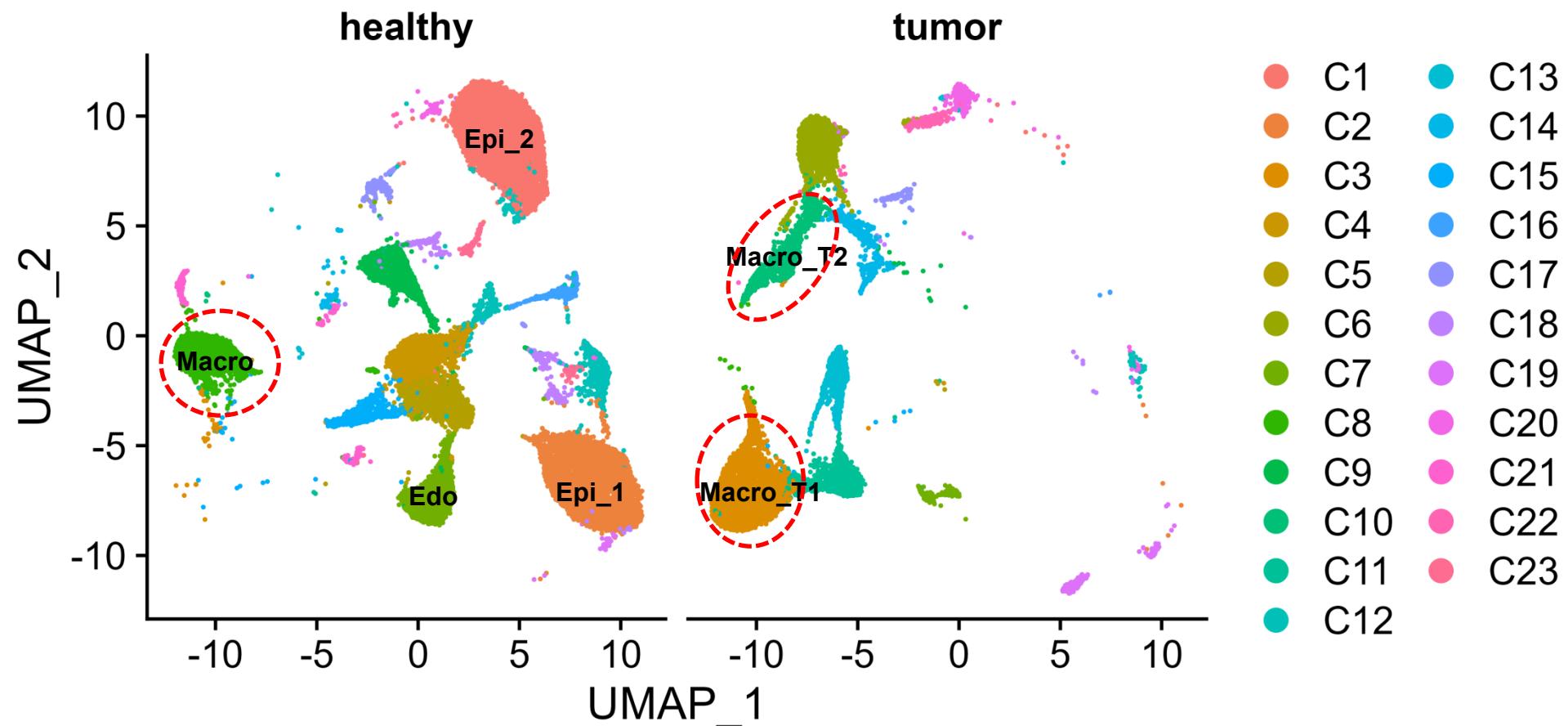
# Analysis of Cell Clusters Using the UMAP Plot (split by sample ID)



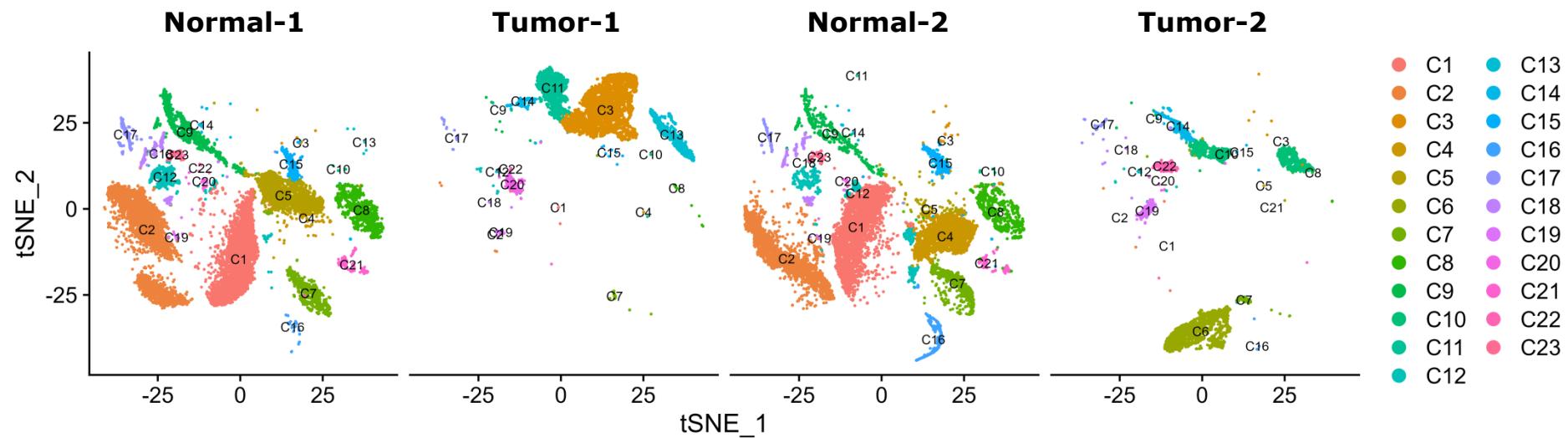
# Analysis of Cell Clusters Using the UMAP Plot (split by sample type)



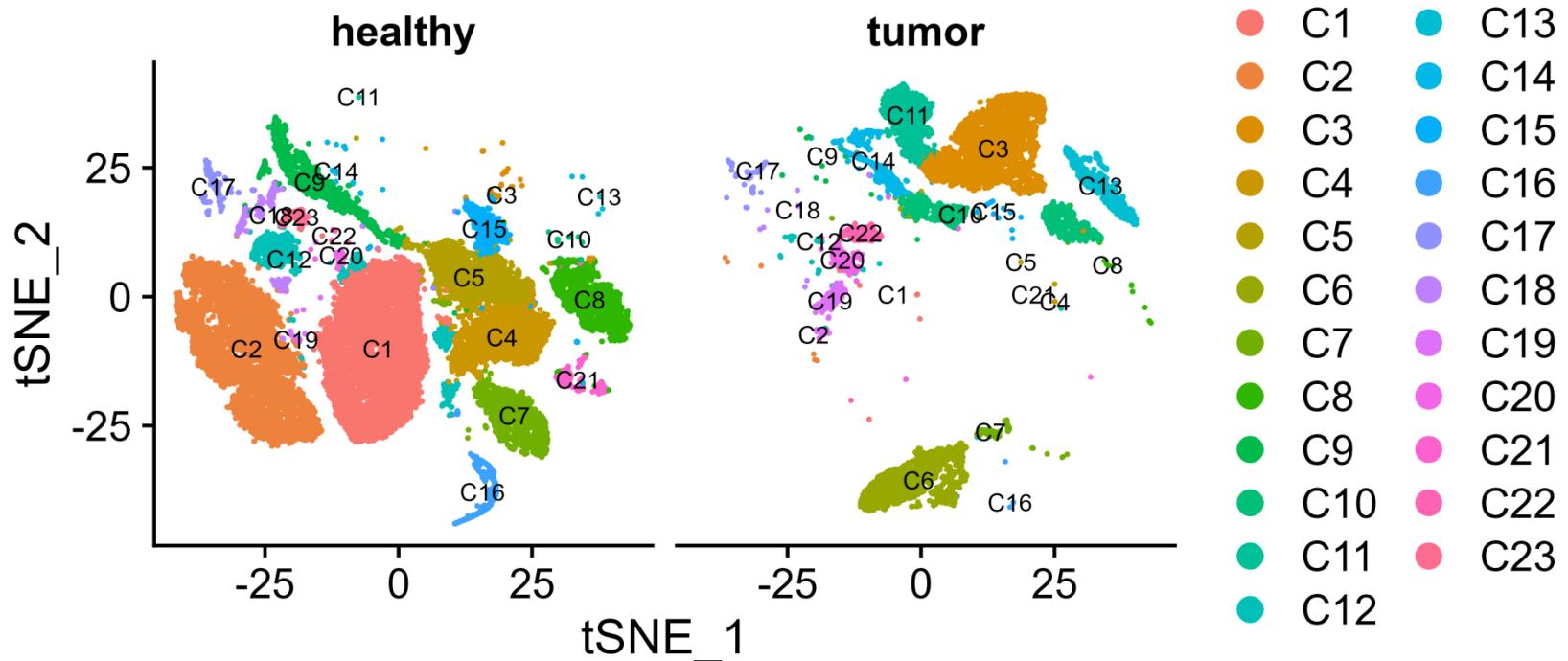
# Analysis of Cell Clusters Using the UMAP Plot (split by sample type)



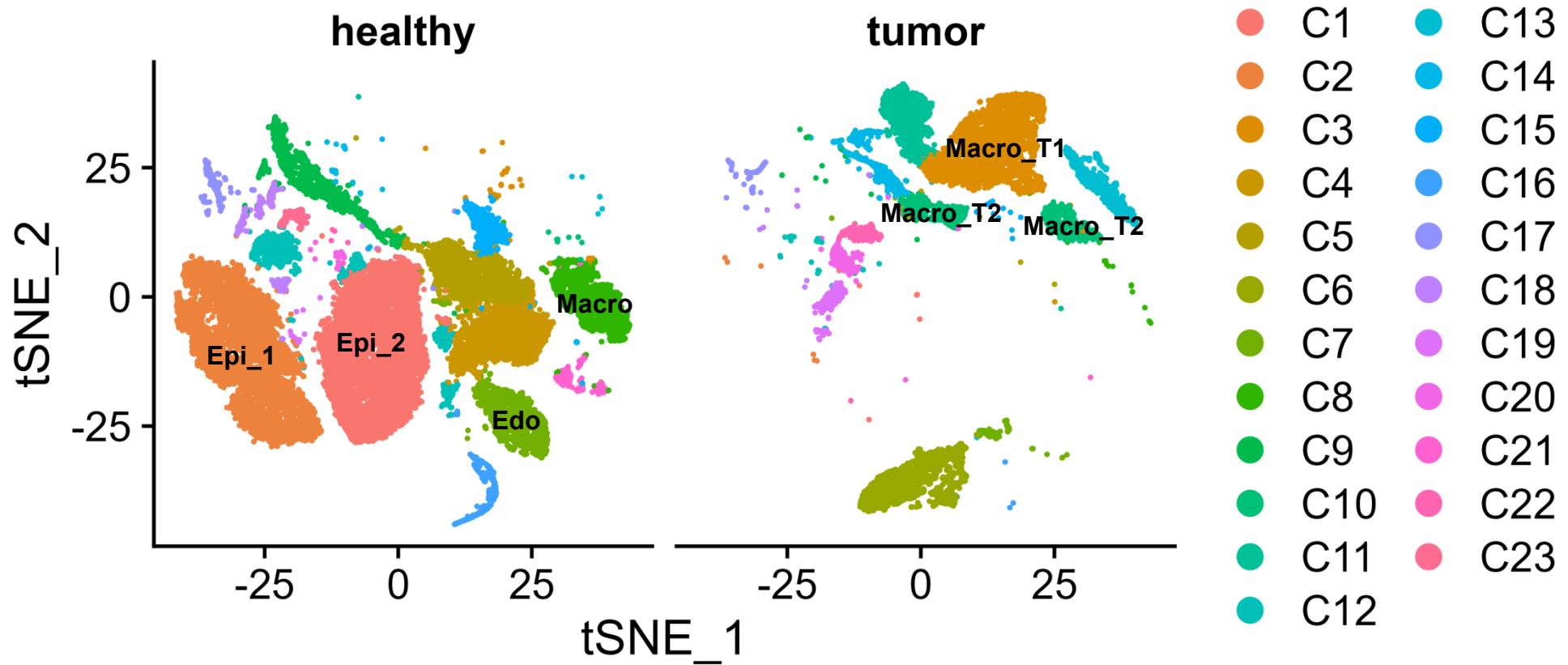
# Analysis of Cell Clusters Using the tSNE Plot (split by sample ID)



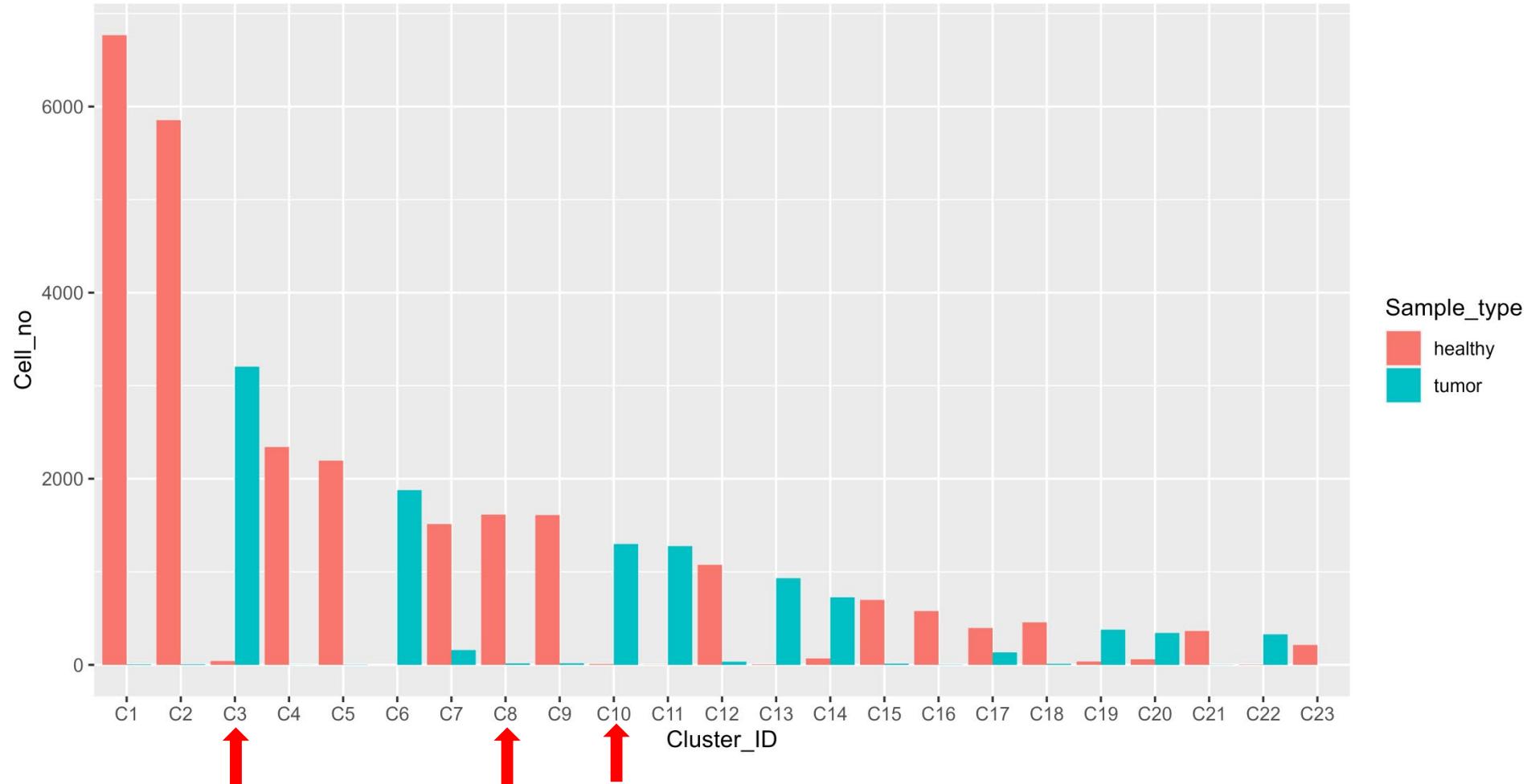
# Analysis of Cell Clusters Using the tSNE Plot (split by sample type)



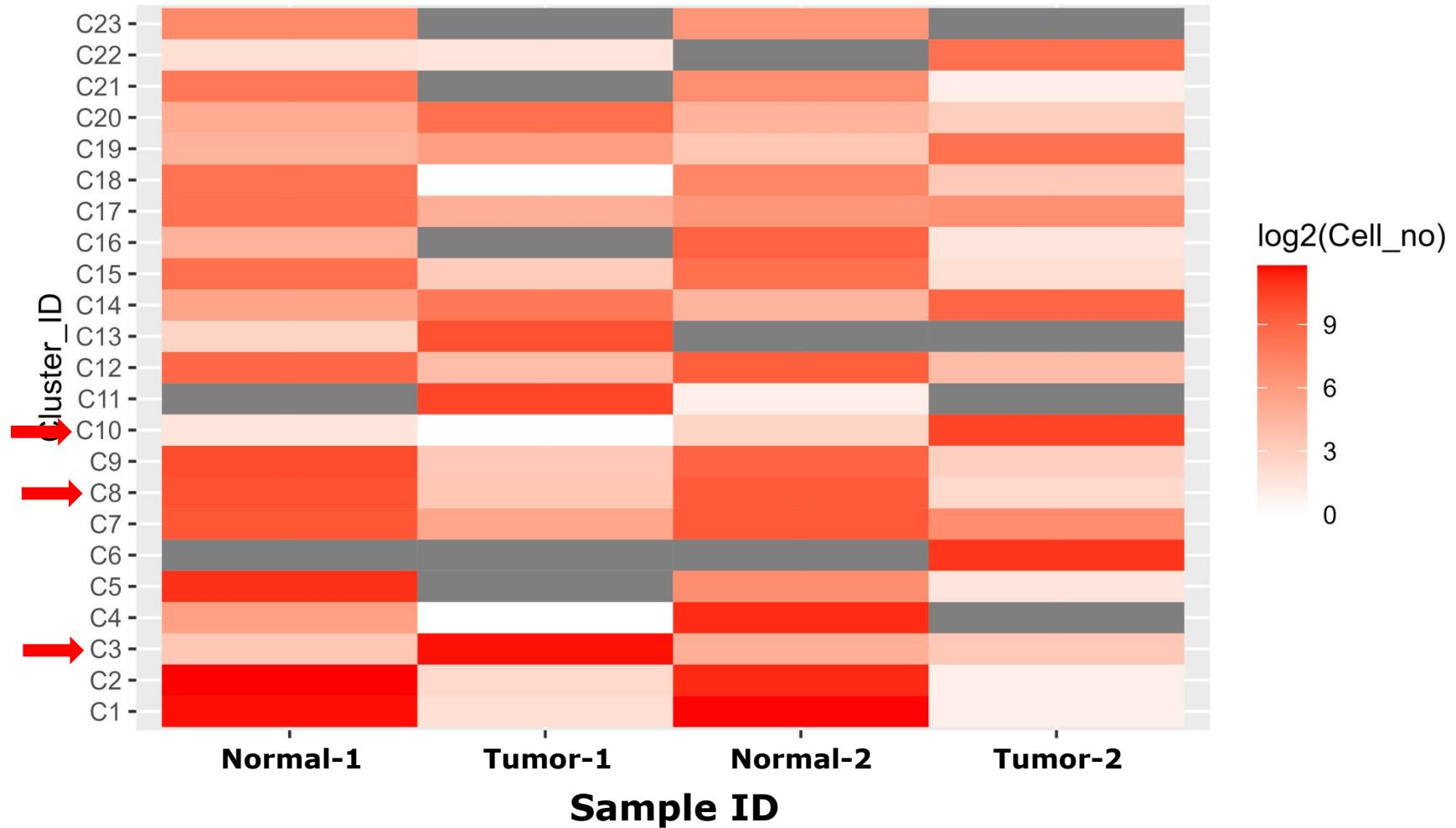
# Analysis of Cell Clusters Using the tSNE Plot (split by sample type)



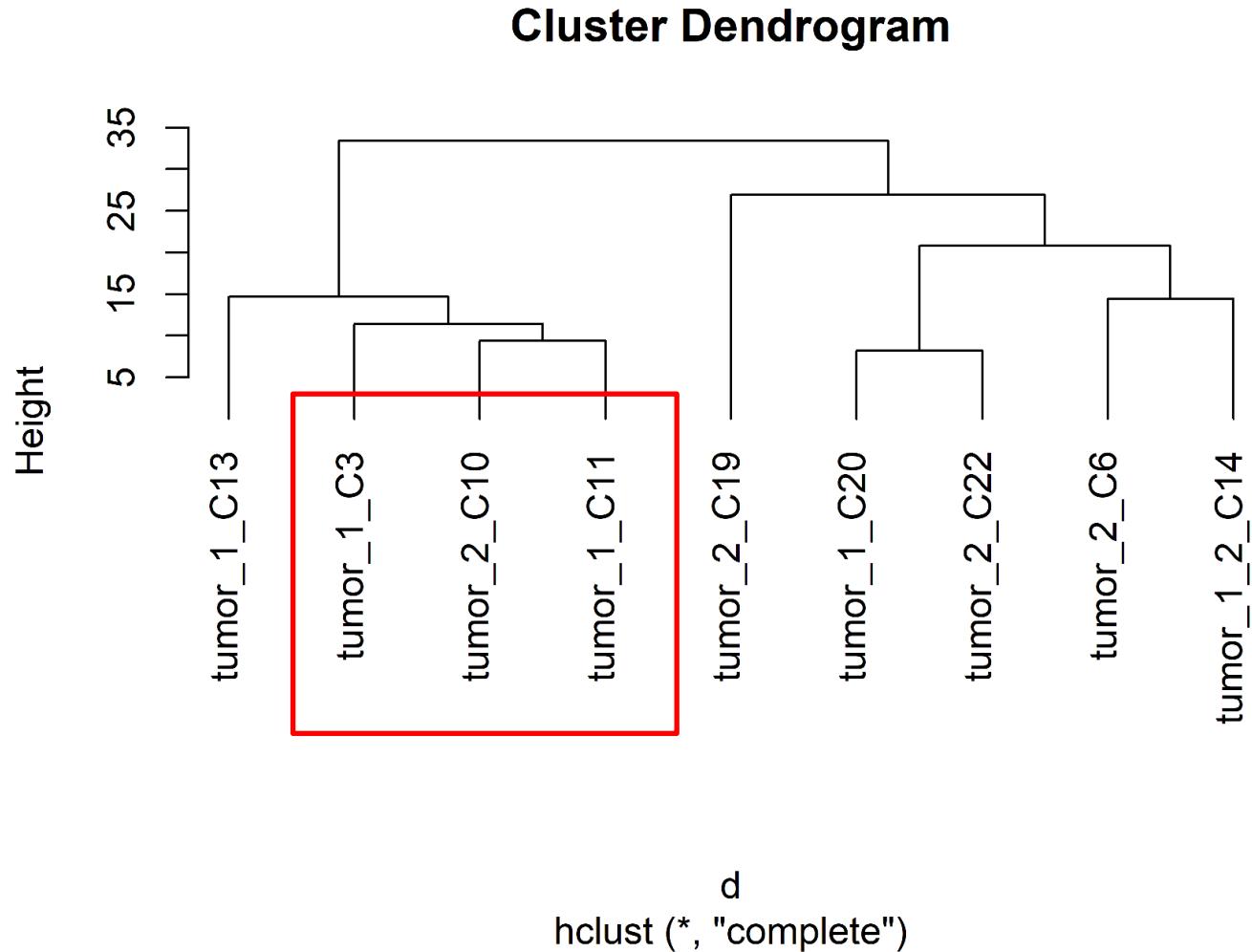
# Bar Plot Analysis of Cell Numbers of Cell Clusters (split by sample type)



## Heatmap Analysis of Cell Numbers of Cell Clusters (split by sample ID)



## Cluster Dendrogram Analysis of Tumor Clusters Identified From snRNA-seq



# Gene Marker for Macrophages

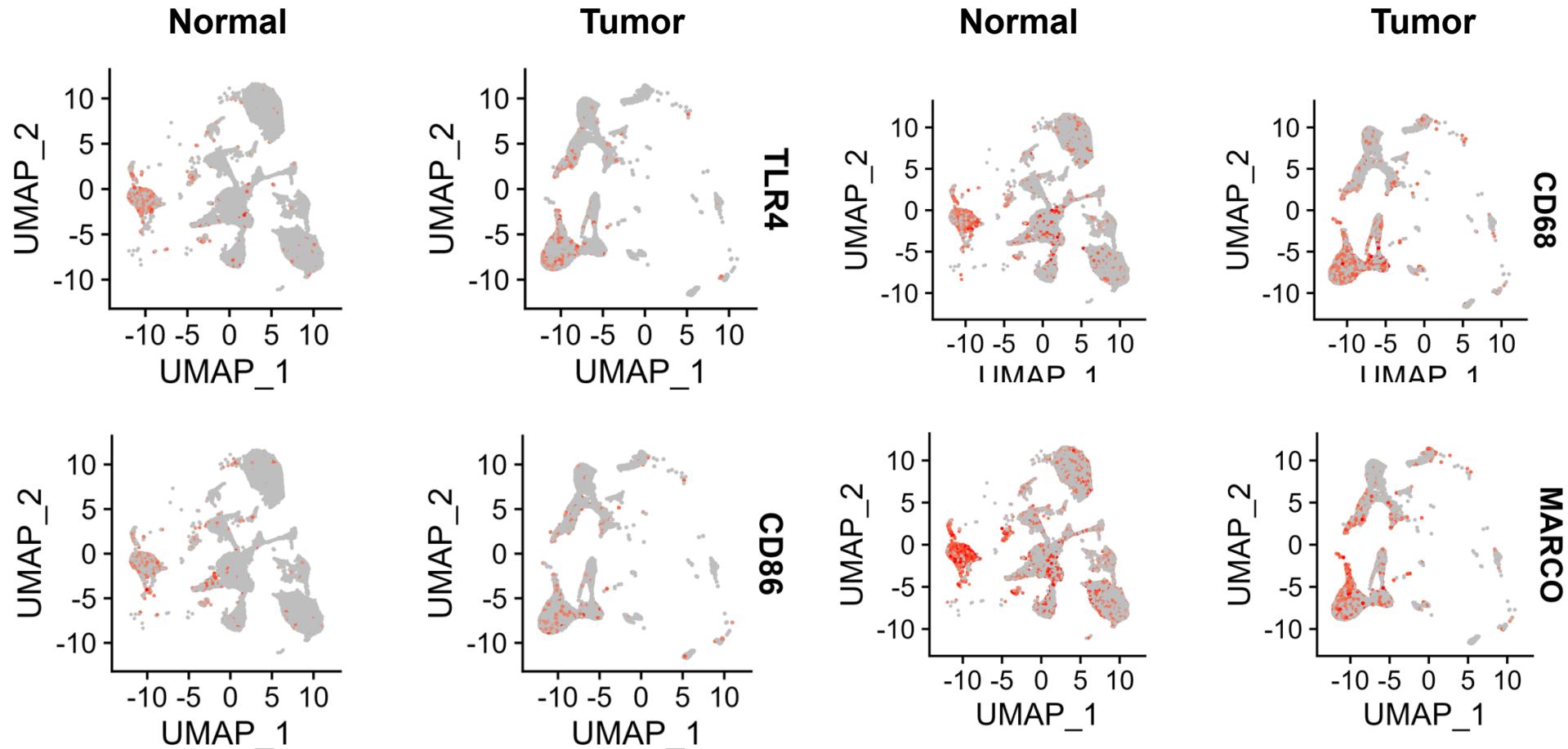
**M1 markers**: IL1A, IL1B, IL6, NOS2, TLR2, TLR4, CD80, CD86, SOCS3, CD68, MARCO, CSF2, TNF, CXCL2, IFNG, IL1R1

**M2 markers**: CSF1R, MRC1, PPARG, ARG1, CD163, CLEC10A, CLEC7A, PDCD1LG2, CCL22, FCGR1A, IL4, IRF4, PDGFB, STAT6

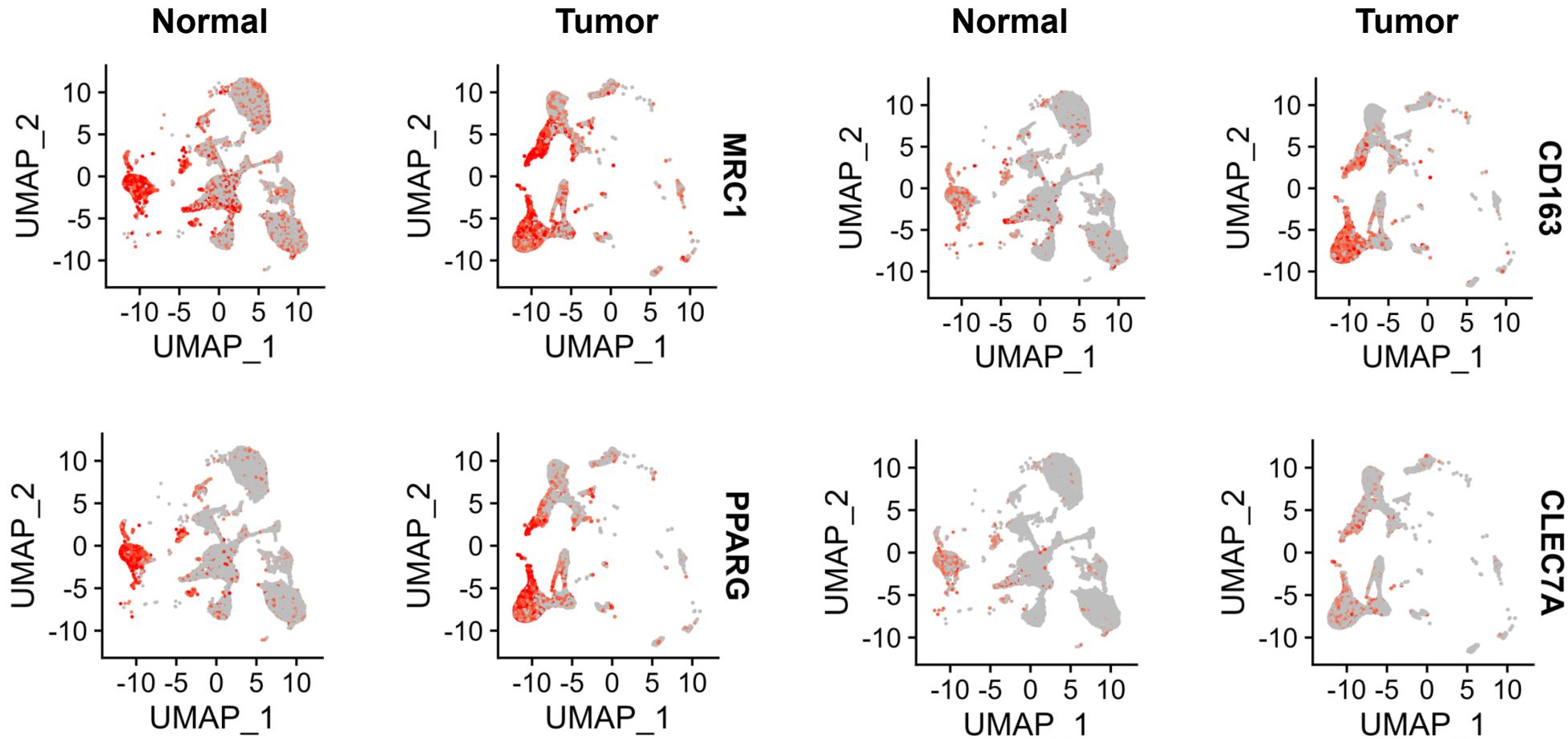
**TAM markers**: CCR2, CSF1R, MARCO, PDCD1LG2, CD40, CCL2, CSF1, FCGR3A, PDGFB, TLR7

**Pan markers**: ADGRE1, CD14, CD68, CSF1R, CX3CR1, FCGR1A, ITGAM, MERTK, CCR5, MSR1

## Analysis of Macrophage Type 1 Gene Markers on the UMAP Plot

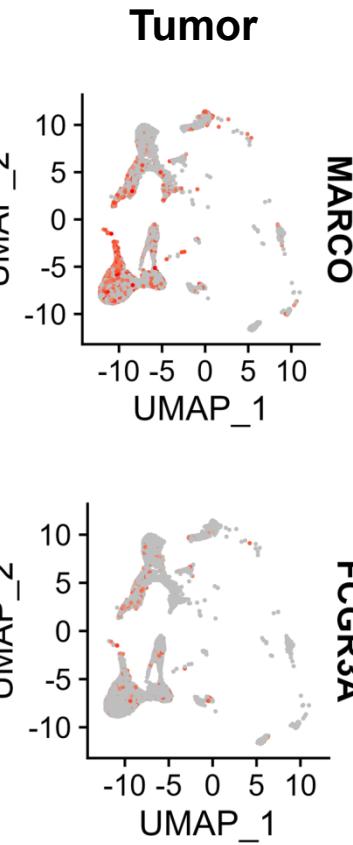
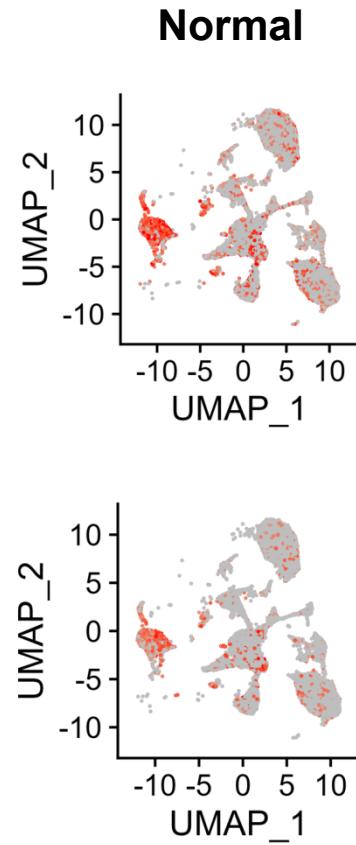


## Analysis of Macrophage Type 2 Gene Markers on the UMAP Plot



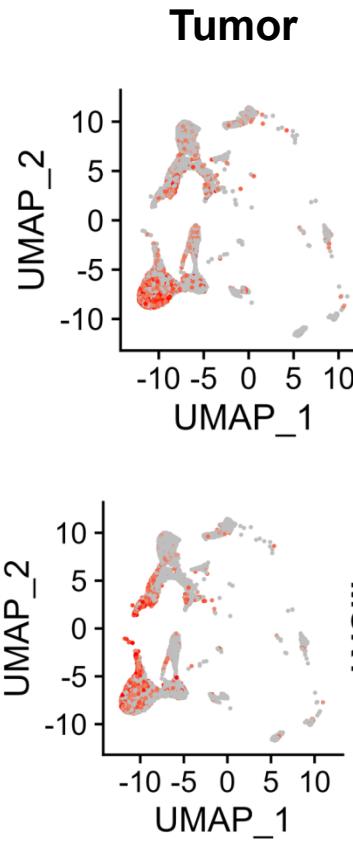
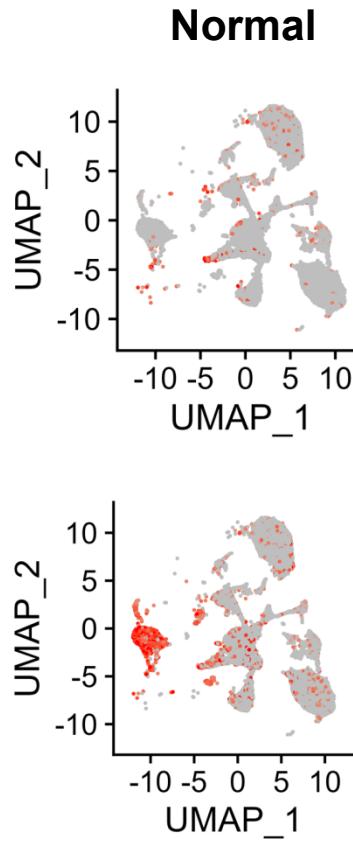
# Analysis of Other Macrophage Gene Markers on the UMAP Plot

## TAM Markers



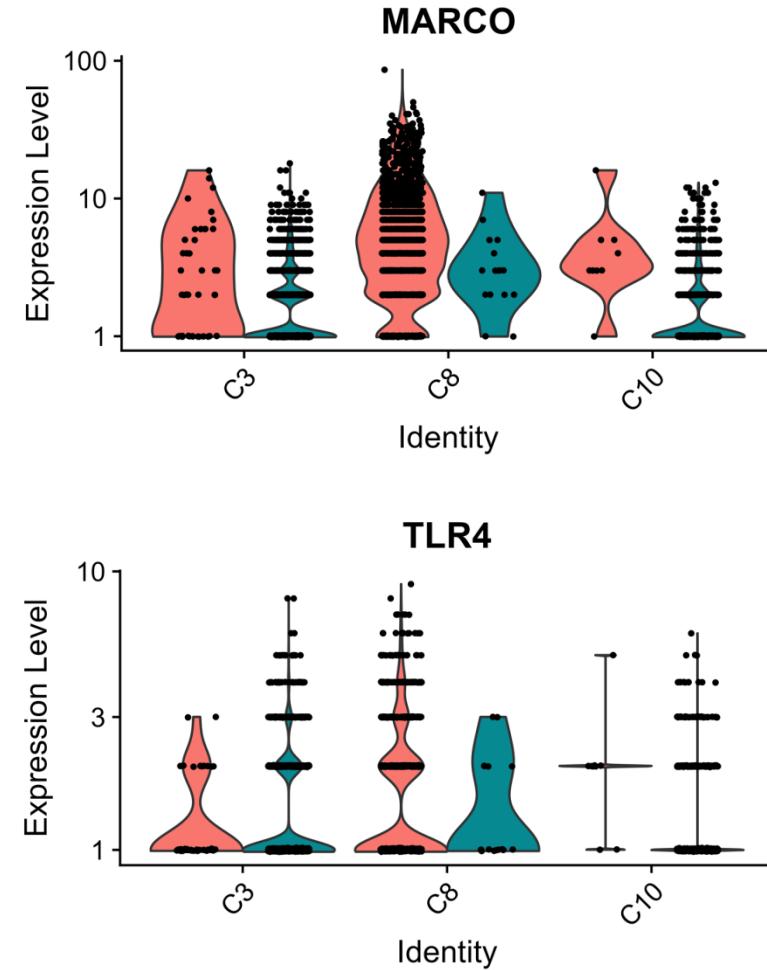
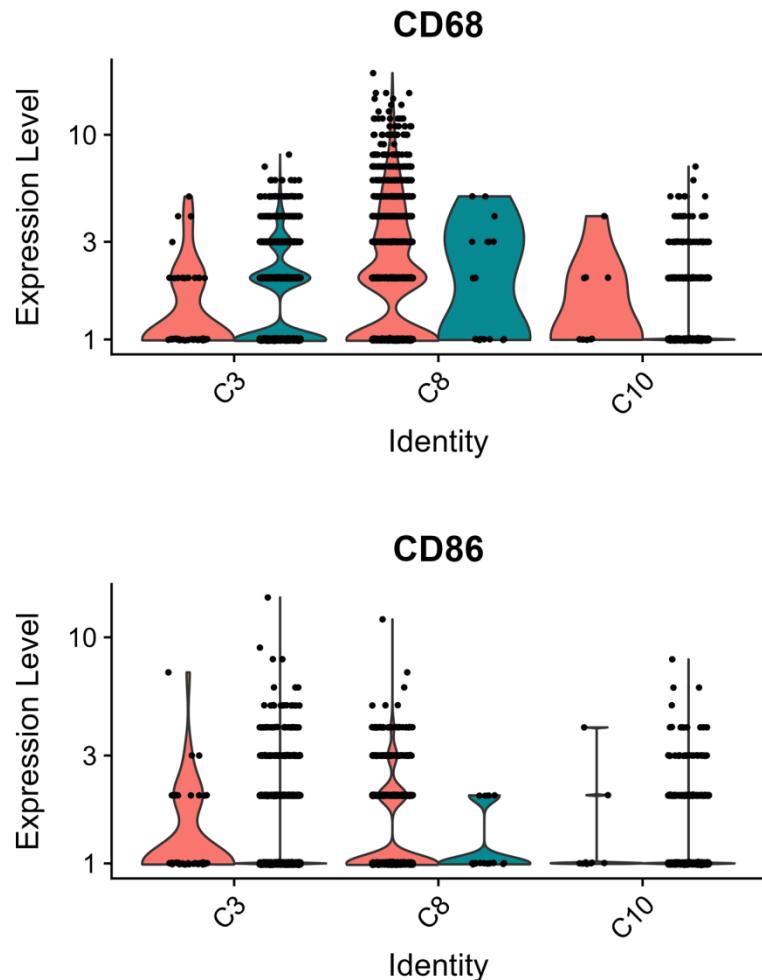
FCGR3A

## Pan Markers

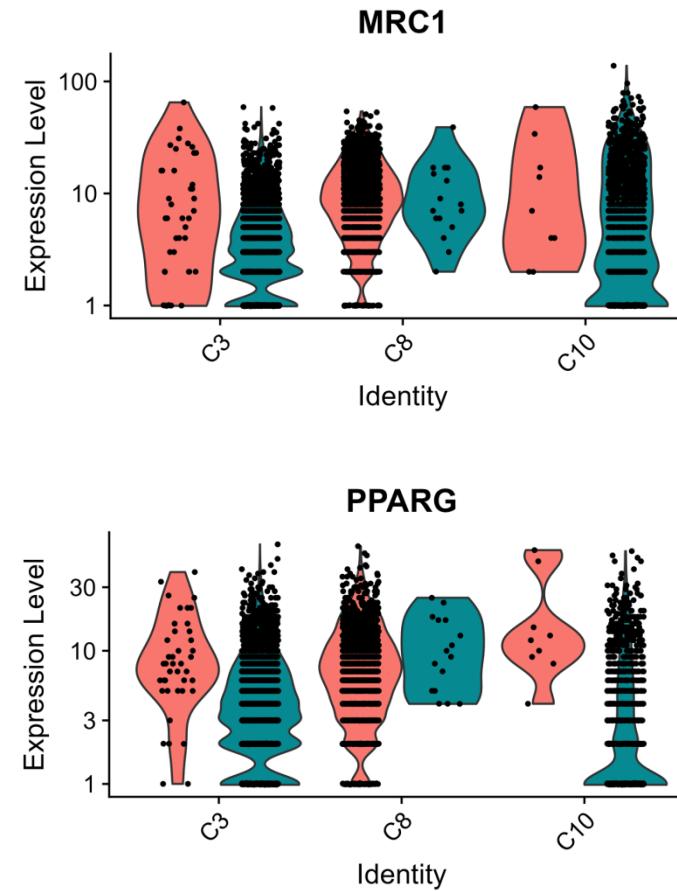
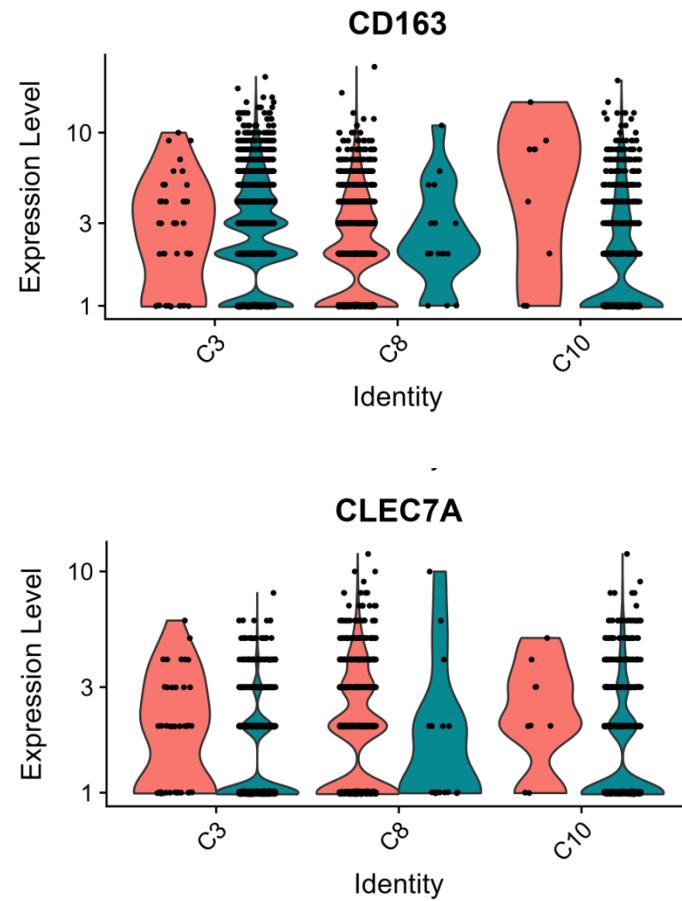


MSR1

# Analysis of Macrophage Type 1 Gene Markers on the VInPlot

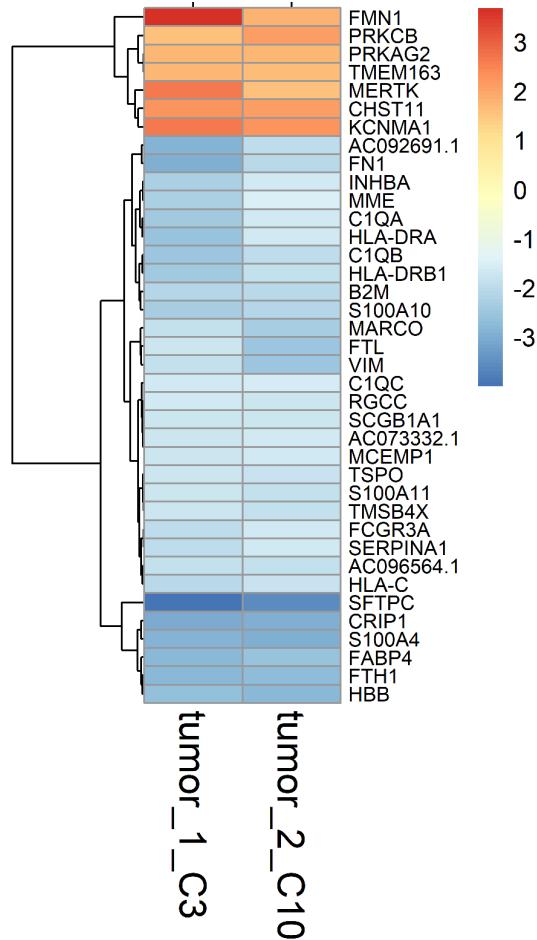


# Analysis of Macrophage Type 2 Gene Markers on the VlnPlot

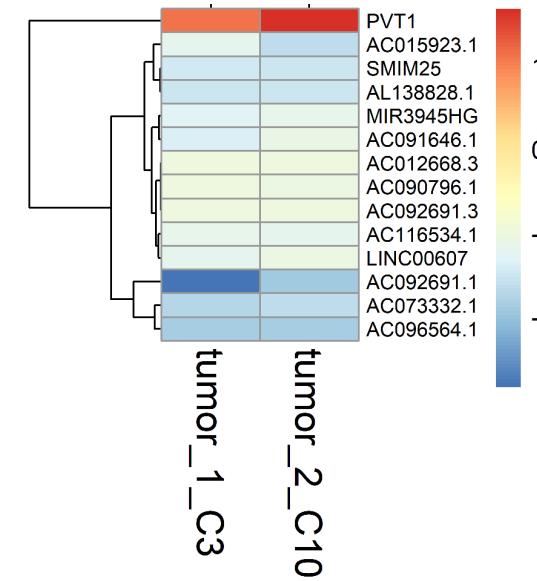


# Heatmap Analysis of Differentially Expressed Genes in TAMs

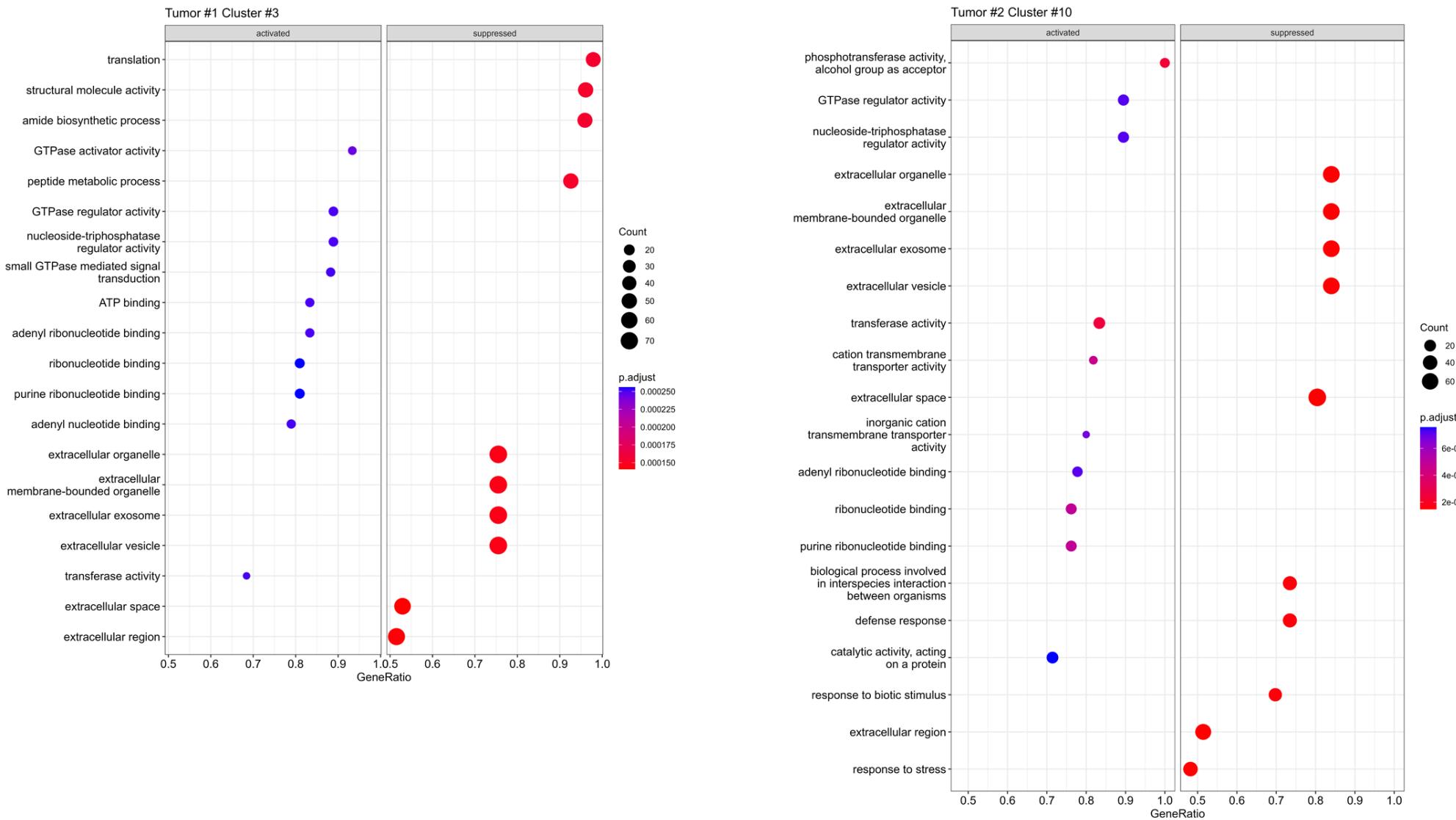
**>= 1.5 fold**



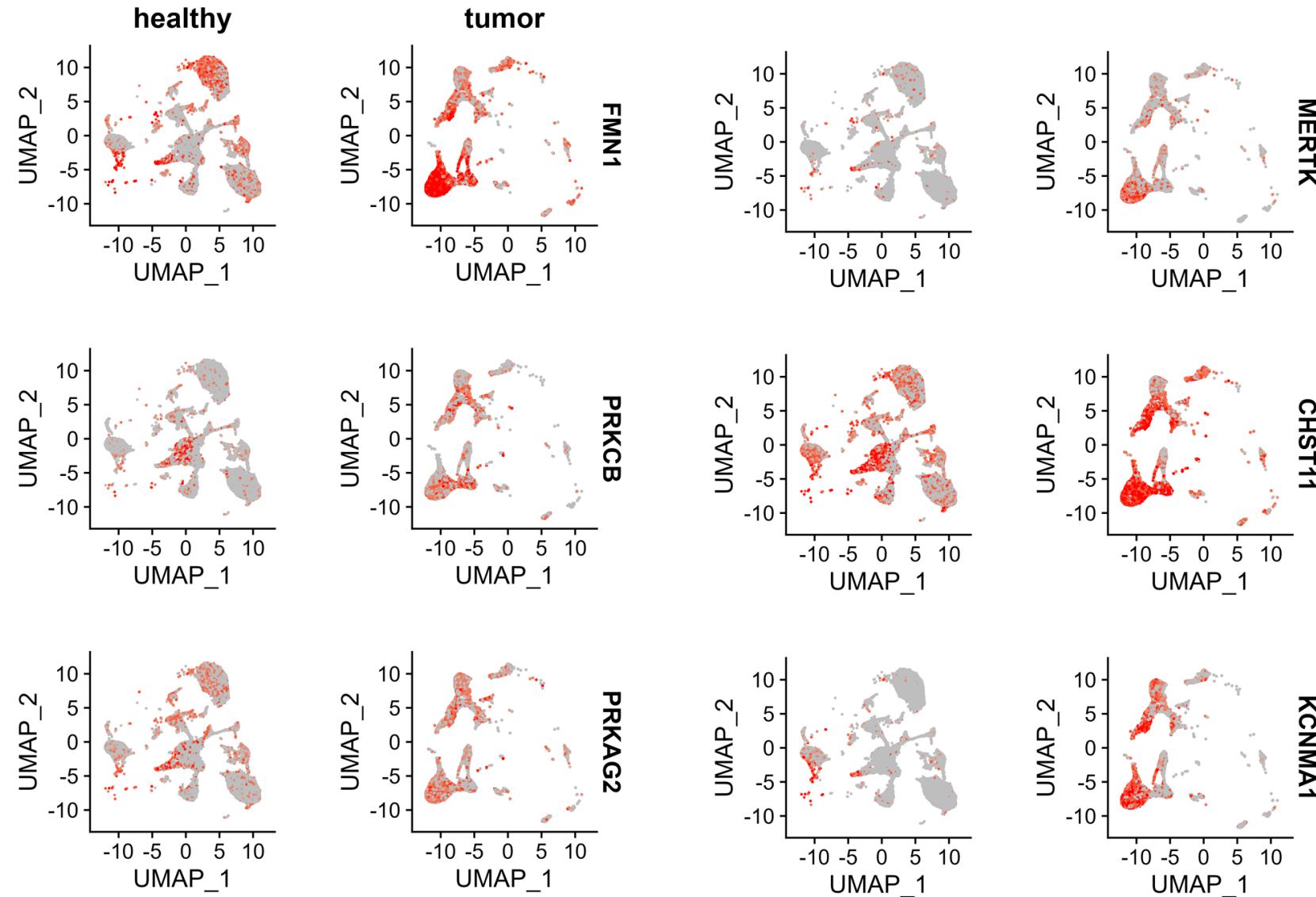
**lncRNA**



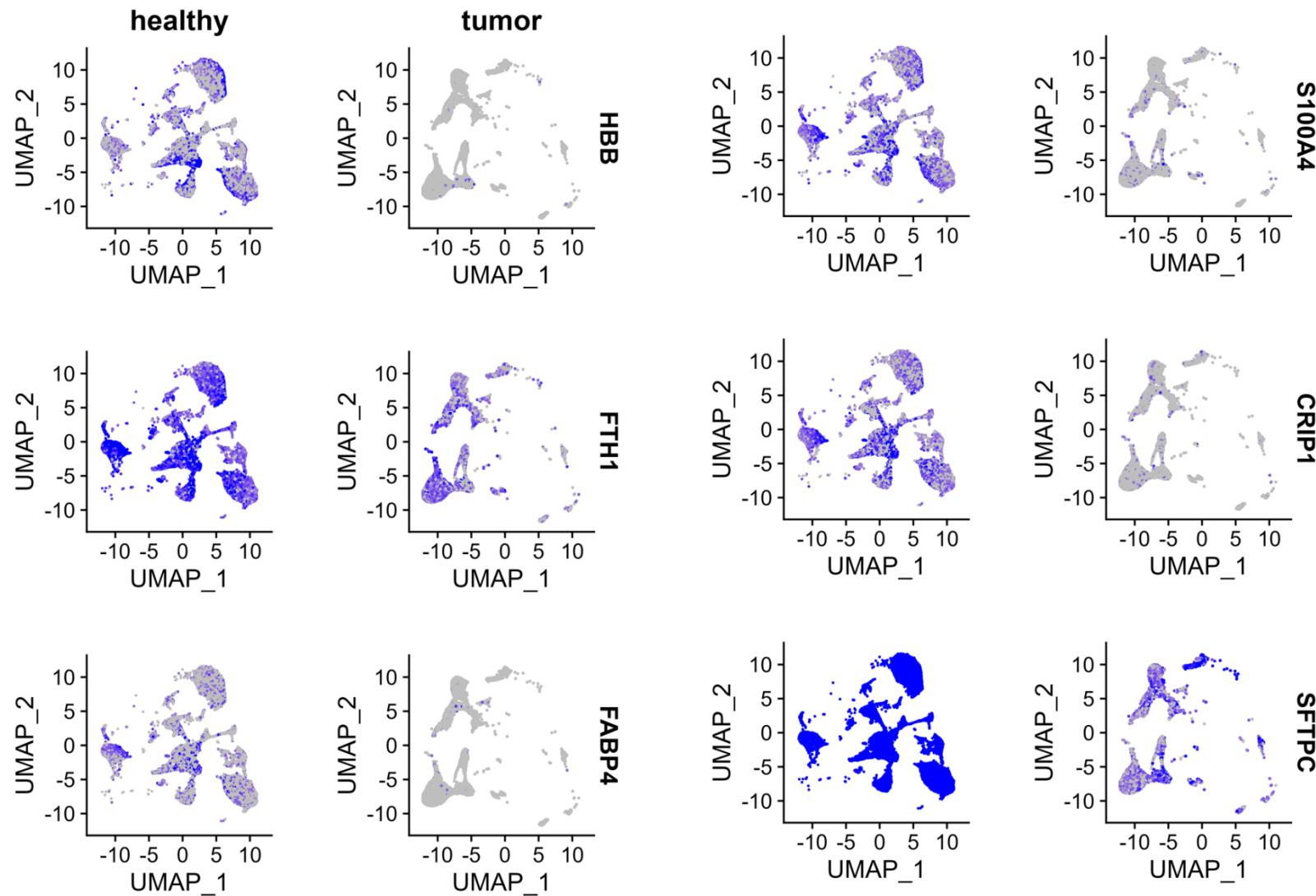
# Dotplot Analysis of Gene Set Enrichment in TAMs



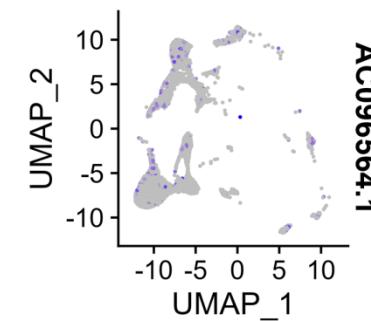
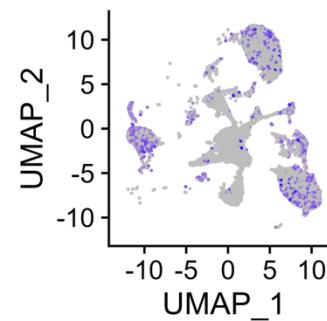
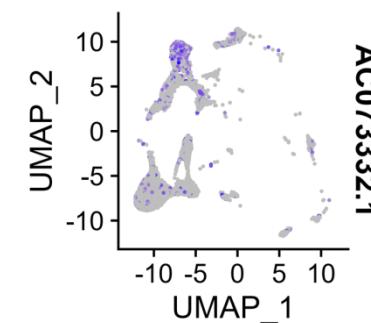
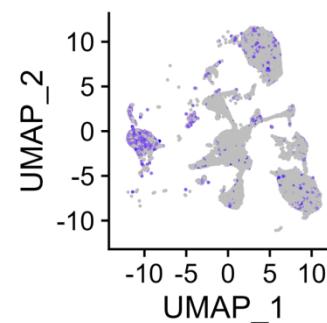
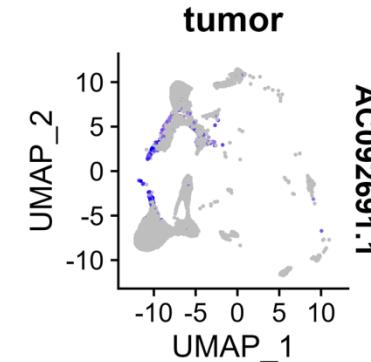
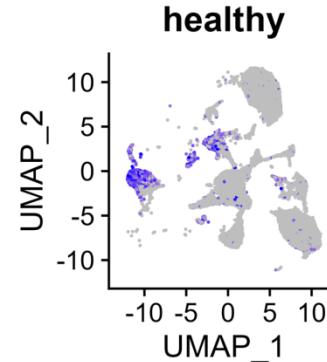
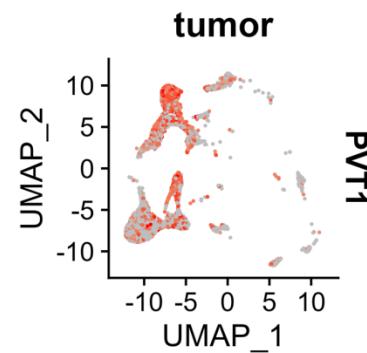
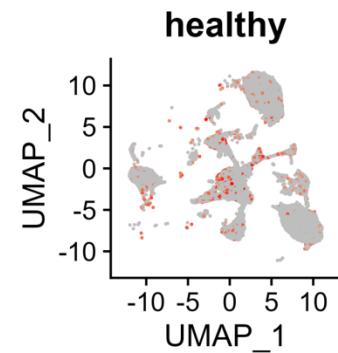
# Analysis of Upregulated Protein-Coding Genes in TAMs on the UMAP Plot



# Analysis of Downregulated Protein-Coding Genes in TAMs on the UMAP Plot



# Analysis of Differentially Expressed lncRNAs in TAMs on the UMAP Plot



## Mertk on tumor macrophages is a therapeutic target to prevent tumor recurrence following radiation therapy

Radiation therapy provides a means to kill large numbers of cancer cells in a controlled location resulting in the release of tumor-specific antigens and endogenous adjuvants. However, by activating pathways involved in apoptotic cell recognition and phagocytosis, irradiated cancer cells engender suppressive phenotypes in macrophages. We demonstrate that the macrophage-specific phagocytic receptor, Mertk is upregulated in macrophages in the tumor following radiation therapy. Ligation of Mertk on macrophages results in anti-inflammatory cytokine responses via NF- $\kappa$ B p50 upregulation, which in turn limits tumor control following radiation therapy. We demonstrate that in immunogenic tumors, loss of Mertk is sufficient to permit tumor cure following radiation therapy. However, in poorly immunogenic tumors, TGF $\beta$  inhibition is also required to result in tumor cure following radiation therapy. These data demonstrate that Mertk is a highly specific target whose absence permits tumor control in combination with radiation therapy.

## Comprehensive analysis of ferritin subunits expression and positive correlations with tumor-associated macrophages and T regulatory cells infiltration in most solid tumors

Ferritin is the most important iron storage form and is known to influence tumor immunity. We previously showed that expression of ferritin light chain (*FTL*) and ferritin heavy chain (*FTH1*) subunits is increased in head and neck squamous cell carcinoma (HNSC). Here, we analyzed solid tumor datasets from The Cancer Genome Atlas and Genotype-Tissue Expression databases to investigate correlations between *FTL* and *FTH1* expressions and (i) patient survival, using univariate, multivariate, Kaplan-Meier and Receiver Operator Characteristic analysis; and (ii) tumor-infiltrating immune cell subsets, using the bioinformatics tools Estimation of Stomal and Immune cells in Malignant Tumor tissues, Microenvironment Cell Population-counter, Tumor Immune Estimation Resource, and Tumor Immunology Miner. We found that *FTL* and *FTH1* are upregulated and downregulated, respectively, in most of the human cancers analyzed. Tumor *FTL* levels were associated with prognosis in patients with lower grade glioma (LGG), whereas *FTH1* levels were associated with prognosis in patients with liver hepatocellular carcinoma, HNSC, LGG, and kidney renal papillary cell carcinoma. In many cancers, *FTL* and *FTH1* levels was significantly positively correlated with tumor infiltration by tumor-associated macrophages and T regulatory cells. These results suggest an important role for *FTL* and *FTH1* in regulating tumor immunity to solid cancers.

# Summary

- The snRNA-seq profiling data showed that these two tumors significantly lost normal macrophages when compared to normal tissues.
- The snRNA-seq data analysis identified significant tumor-associated macrophages (TAMs) or TAM-like cells present in tumor tissues, which expressed pan macrophage, TAM and M2 gene markers such as CD163, CLEC7A, MRC1, PPARG, MARCO, MERTK, and MSR1.
- Analysis of differentially expressed genes in TAM-like cells in tumors indicates that macrophage-specific phagocytic receptor signaling, regulation of iron homeostasis, and long non-coding RNA (lncRNA)-mediated regulations (e.g., PVT1) may play roles in tumor development and progression.
- The snRNA-seq analysis of TAMs provides new insight into a mechanistic paradigm wherein macrophage-specific lncRNAs were downregulated in TAM-like cells, suggesting lncRNAs play important roles in regulation of immunological functions of macrophages.