

# Single Cell Analysis in Whole Bone Marrow

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# Intro

- Single cell analysis on mice
- Two different tissue types: whole bone marrow (WBM) and whole bone marrow enriched for megakaryocytes (enrBWM).
  - Not able to do FAC sorting because MKs are fragile so do a magnetic pull that enriches for them.
- Control mice and mice with a phenotype similar to primary myelofibrosis (PMF)
- Question: what is the contribution of megakaryocytes (MKs) in the development of bone marrow fibrosis in PMF?

# Analysis Steps

- Determine the origin of the cells (which state they belong too)
- Run Seurat with the Unknown cell and determine what to do with them.
- Re-run Seurat without the Unknown cells
- Label the clusters

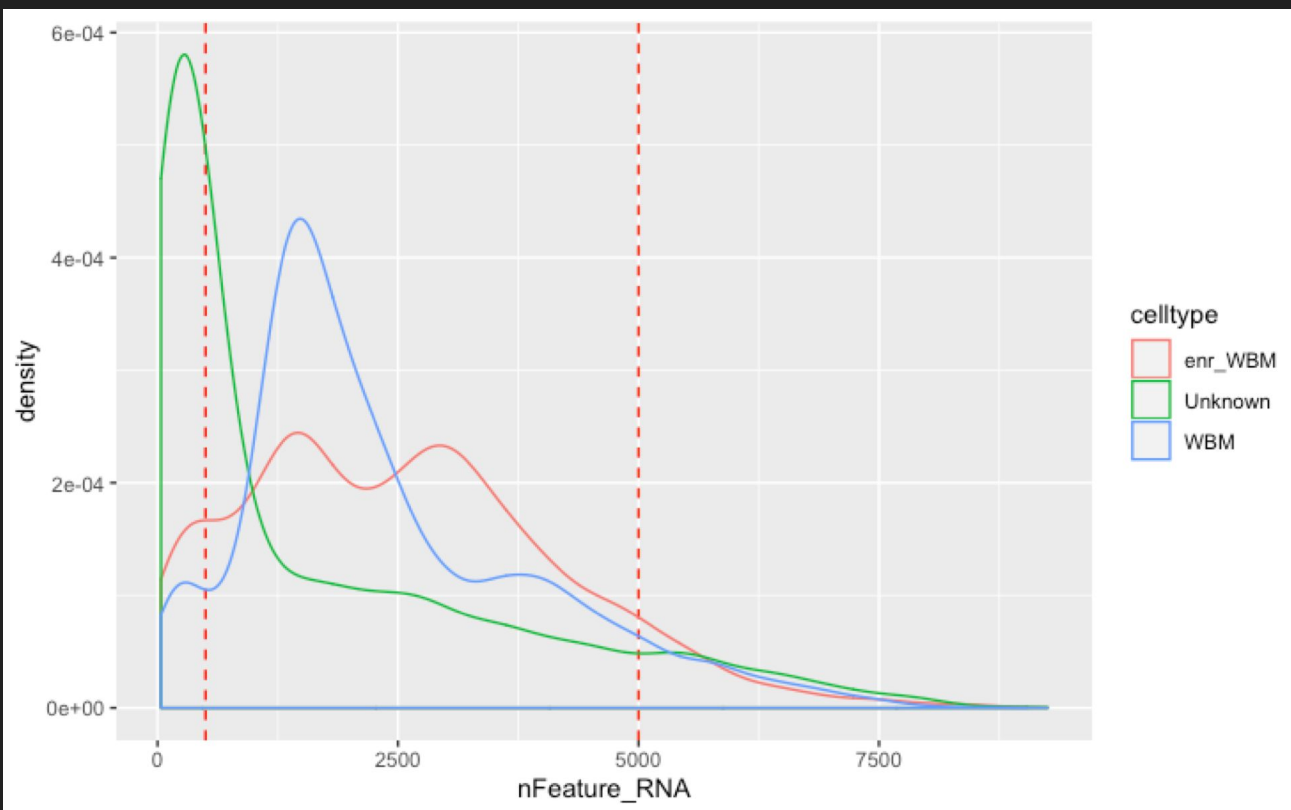
# HTO tagging

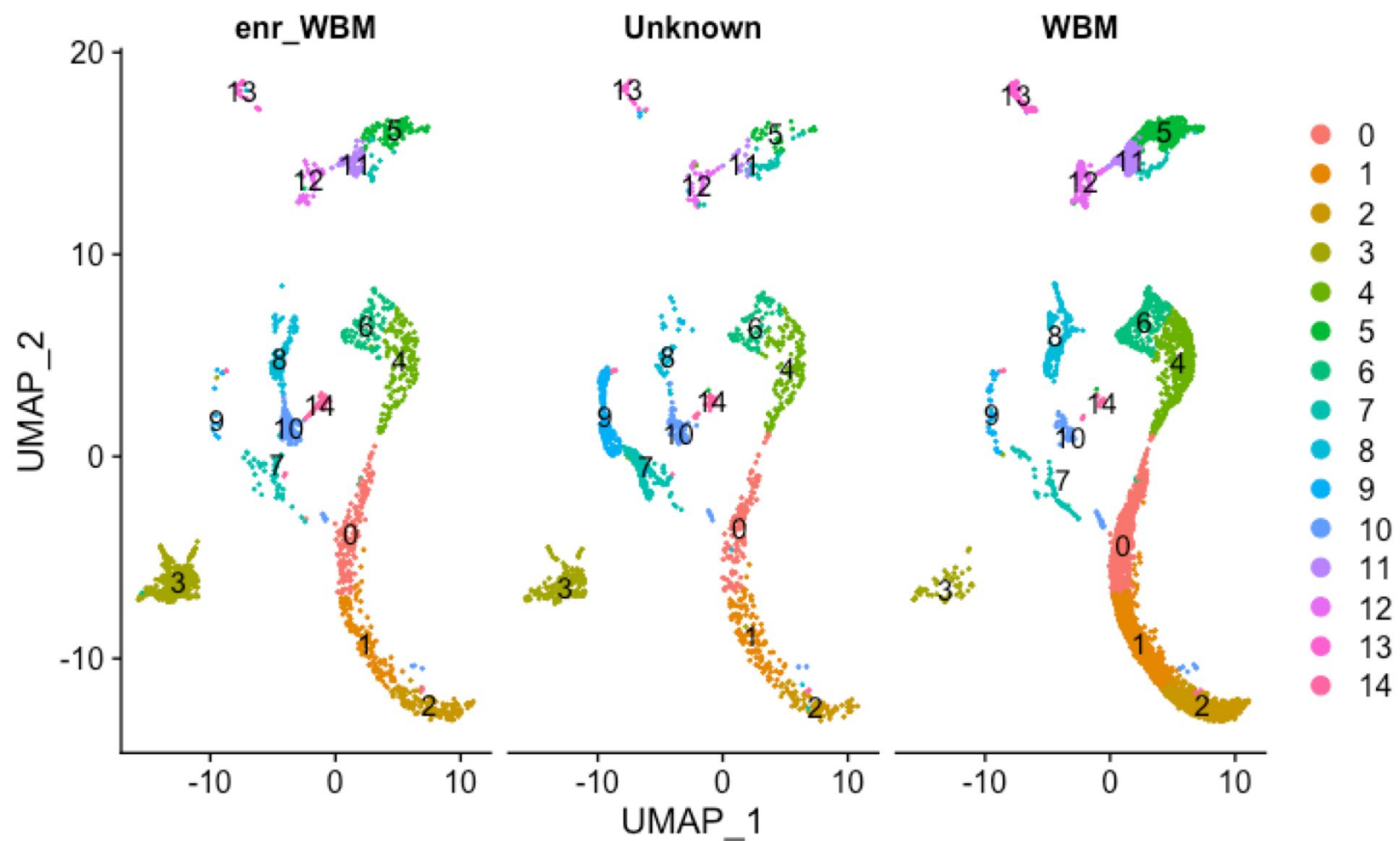
The cells were tagged and all ran at the same time.

Number of cells:

enrWBM control	enrWBM mutant	WBM control	WBM mutant	Unknown
797	1484	2425	2836	3736

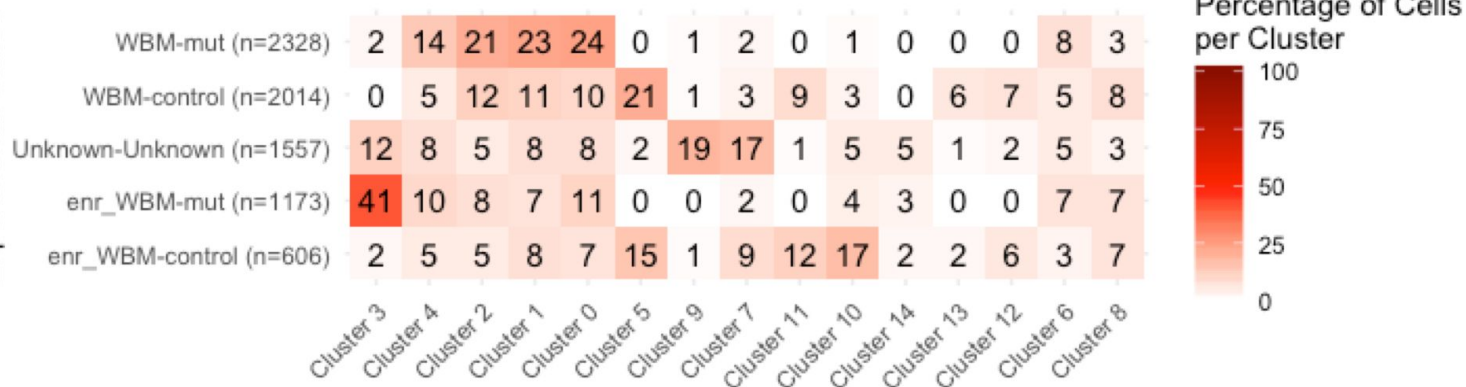
What to do with the Unknown cells?





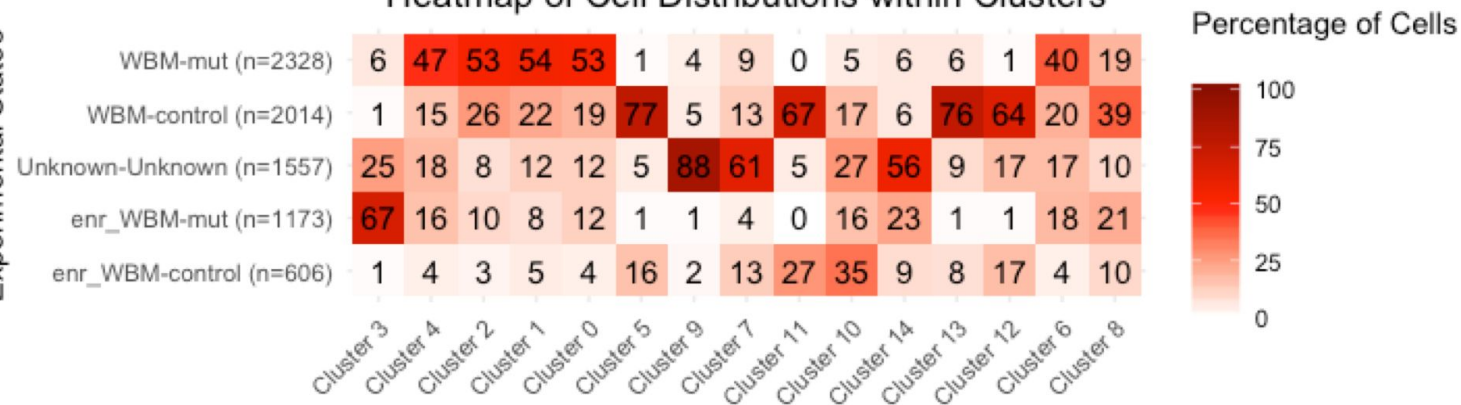
Heatmap of Cell Distributions within Experimental States

Experimental States

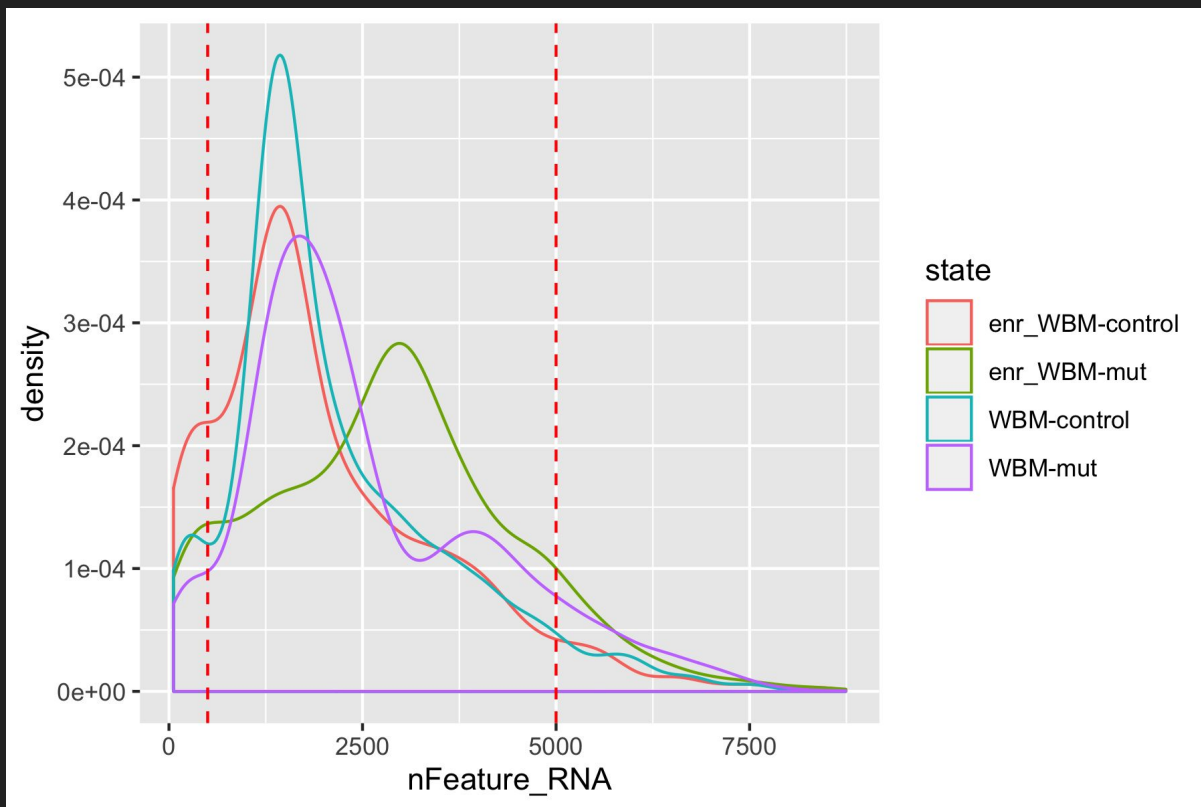


Heatmap of Cell Distributions within Clusters

Experimental States

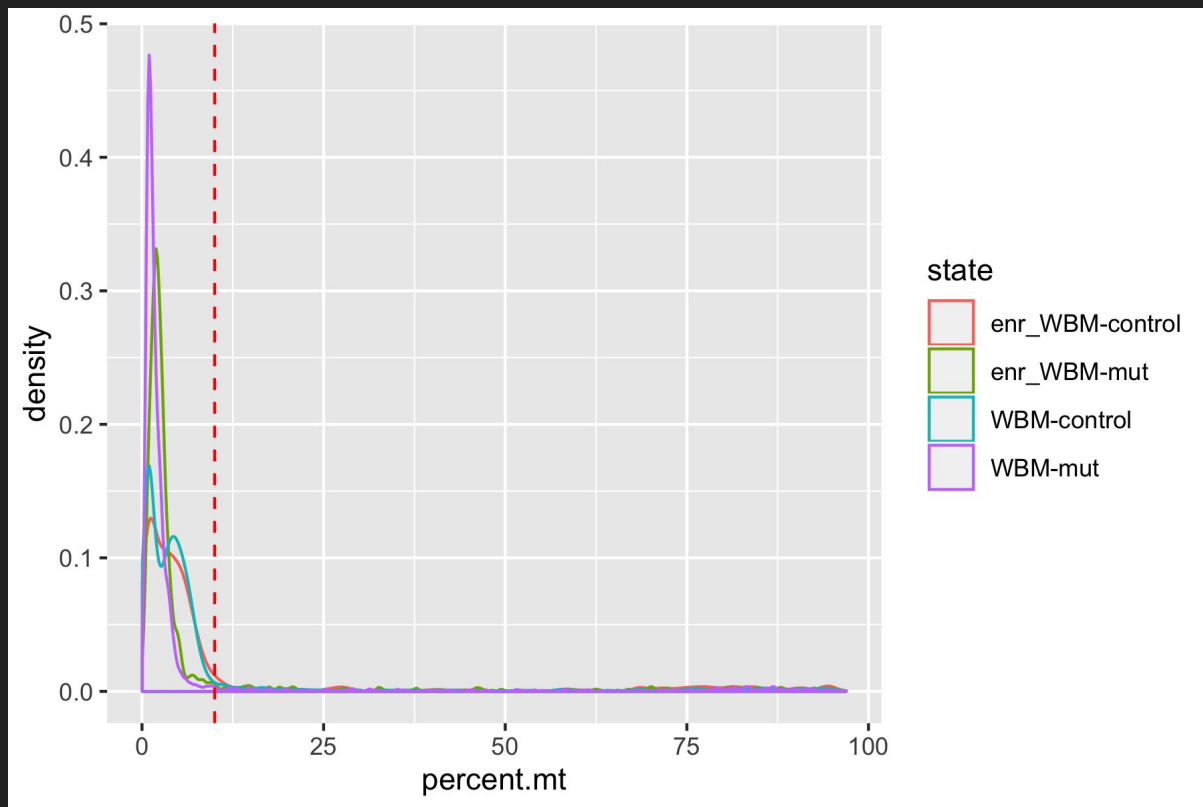


# SC data EDA

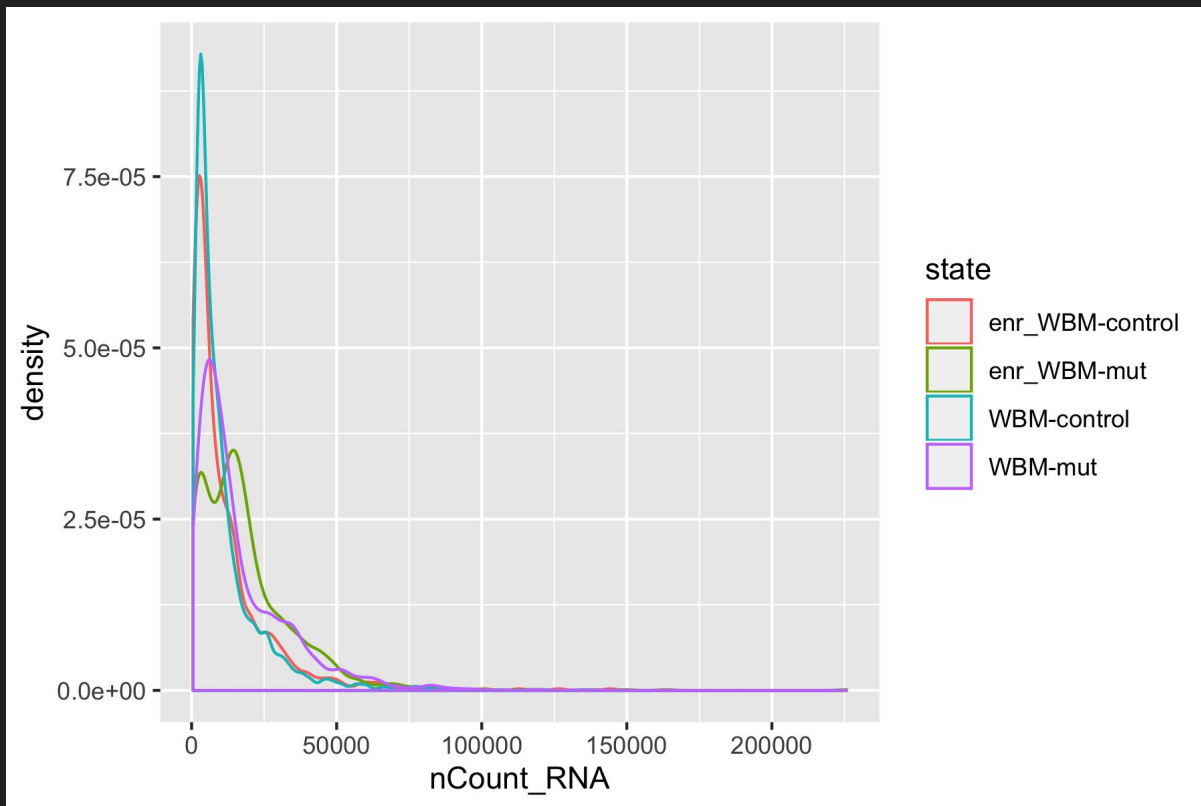




# SC data EDA

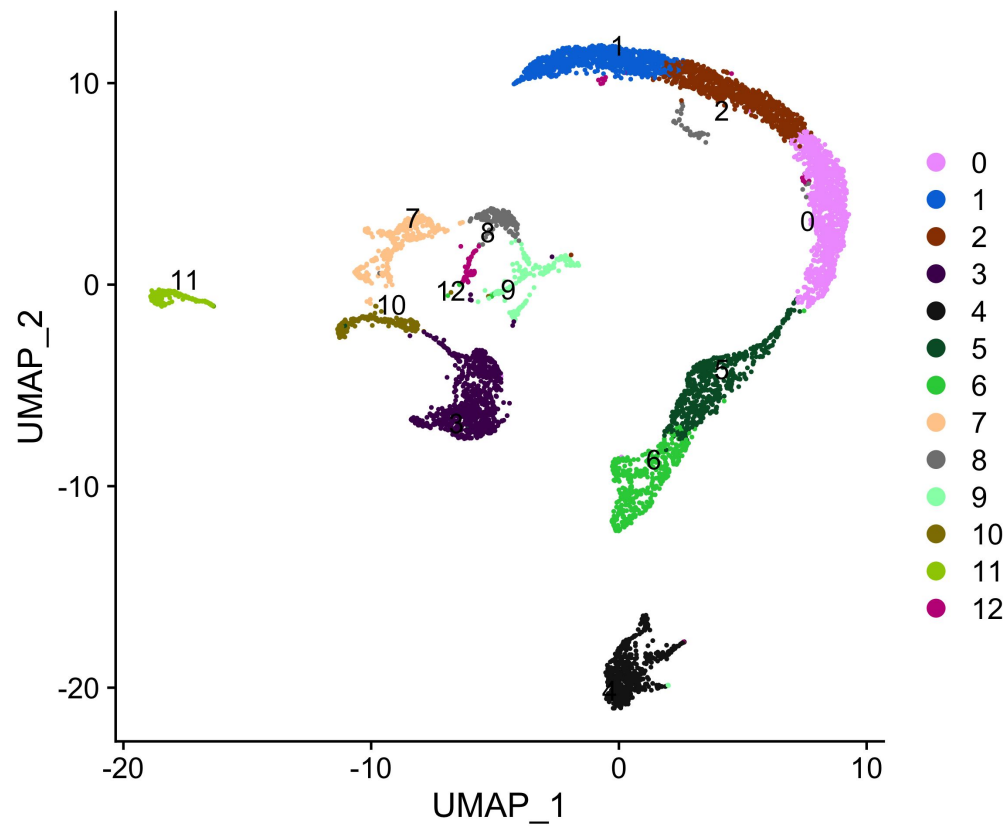


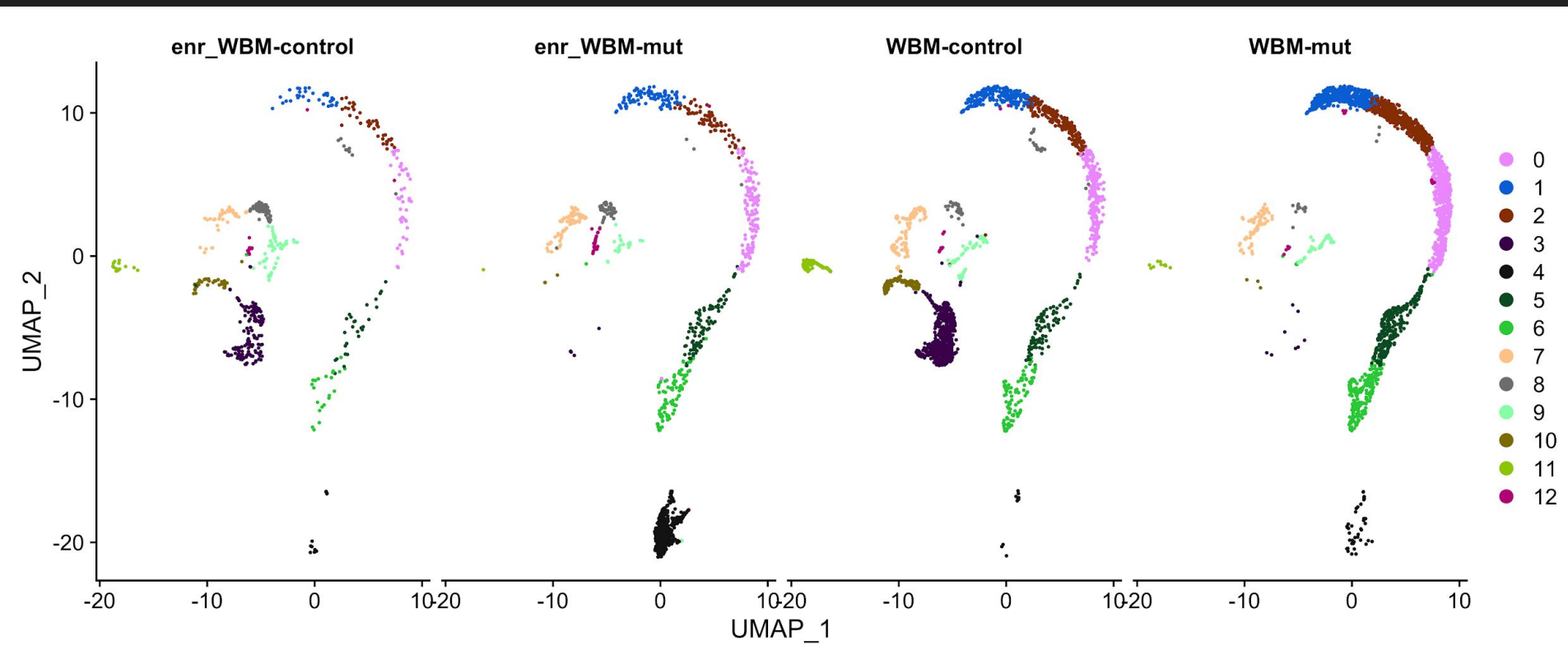
# SC data EDA



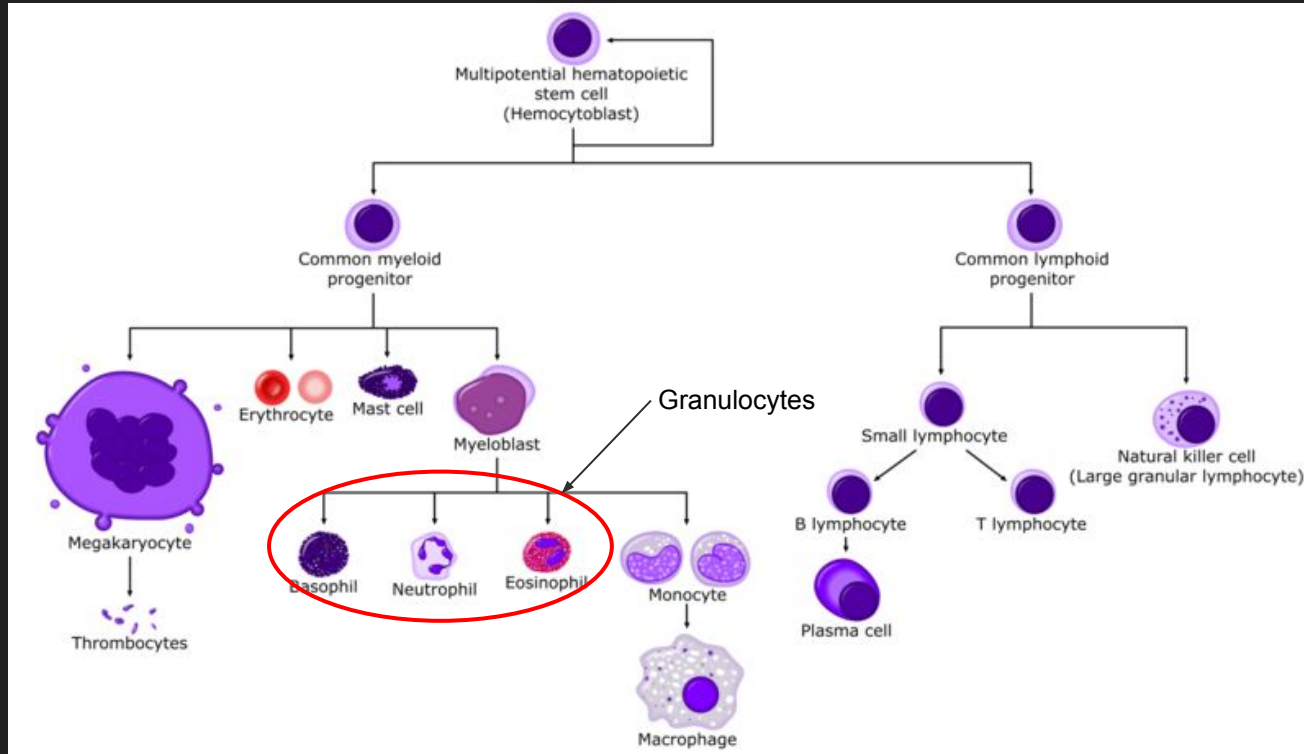
# Cell counts after subsetting

	enrWBM control	enrWBM mutant	WBM control	WBM mutant
Before subsetting	797	1484	2425	2836
After	606	1173	2014	2328
Percentage of Original	76%	79%	83%	82%





# Bone Marrow Differentiation



# Clustering, cont

- SingleR was useful for naming some of the cell types but others were not included in the databases (MKs, erythroids).
  - Did a literature search to find marker genes for these cell types
  - Also <https://panglaodb.se> was a great reference for marker genes.
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- I also looked at cluster markers and looked at where those genes were expressed.
    - The top markers for cluster 9 were erythroid-specific

# Naming clusters

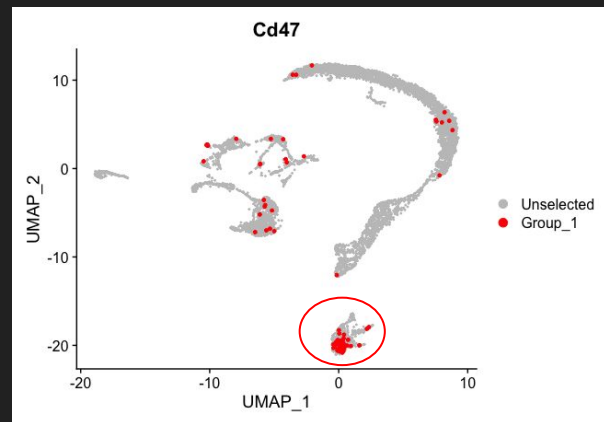
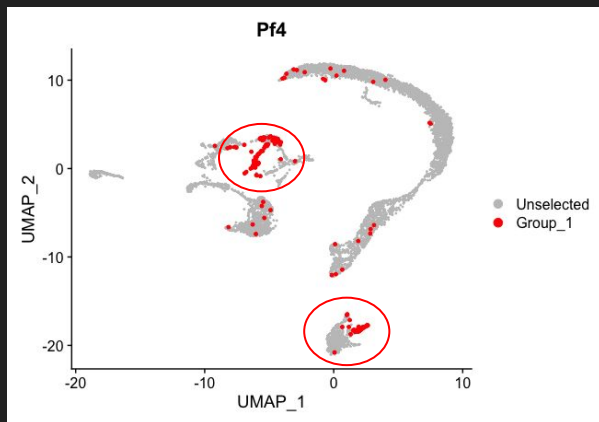
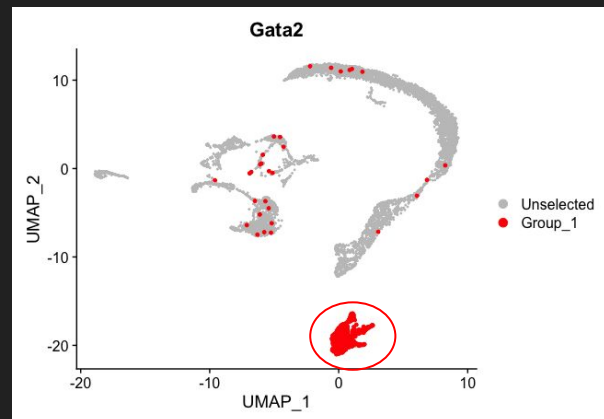
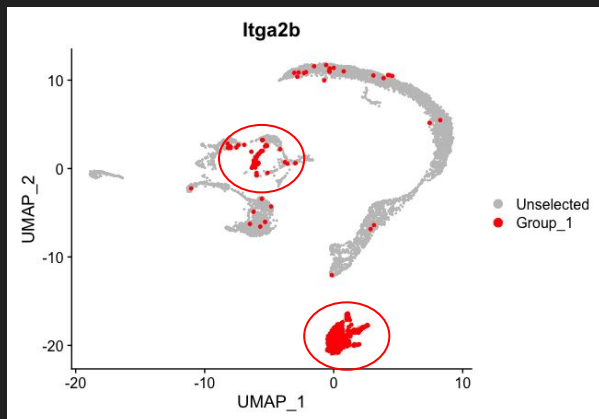
- Using SingleR with the help of Qianyi. Two datasets included

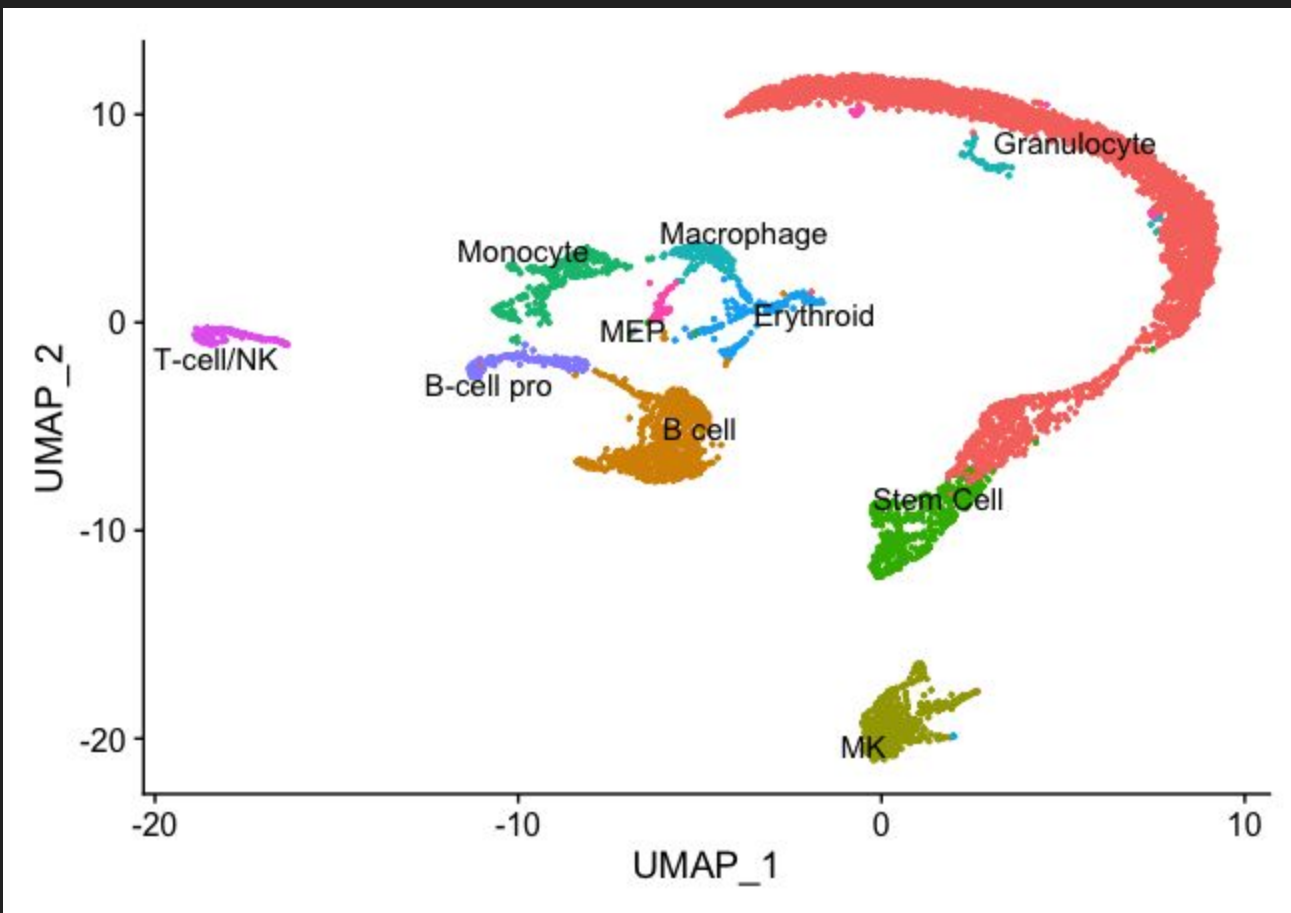
Dataset	0	1	2	3	4	5
1	Neutrophils	Neutrophils	Neutrophils	B cells	Basophils	Neutrophils
2	Granulocytes	Granulocytes	Granulocytes	B cells	Granulocytes	Granulocytes
Both	Granulocytes	Granulocytes	Granulocytes	B cells	Granulocytes	Granulocytes

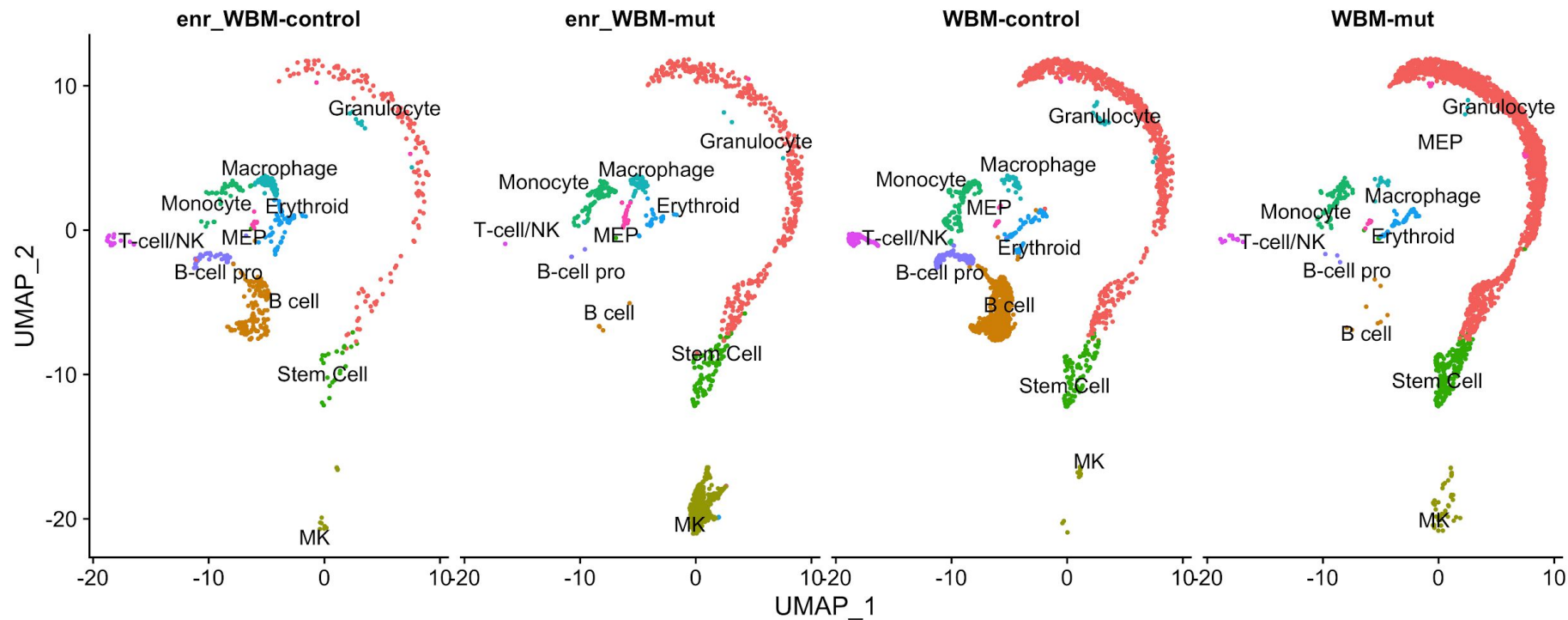
6	7	8	9	10	11	12
Stem cells	Monocytes	Macrophages	Neutrophils	B cells, pro	NKT	Neutrophils
Granulocytes	Monocytes	Granulocytes	Granulocytes	B cells	T cells	Granulocytes
Granulocytes	Monocytes	Granulocytes	Granulocytes	B cells	T cells	Granulocytes



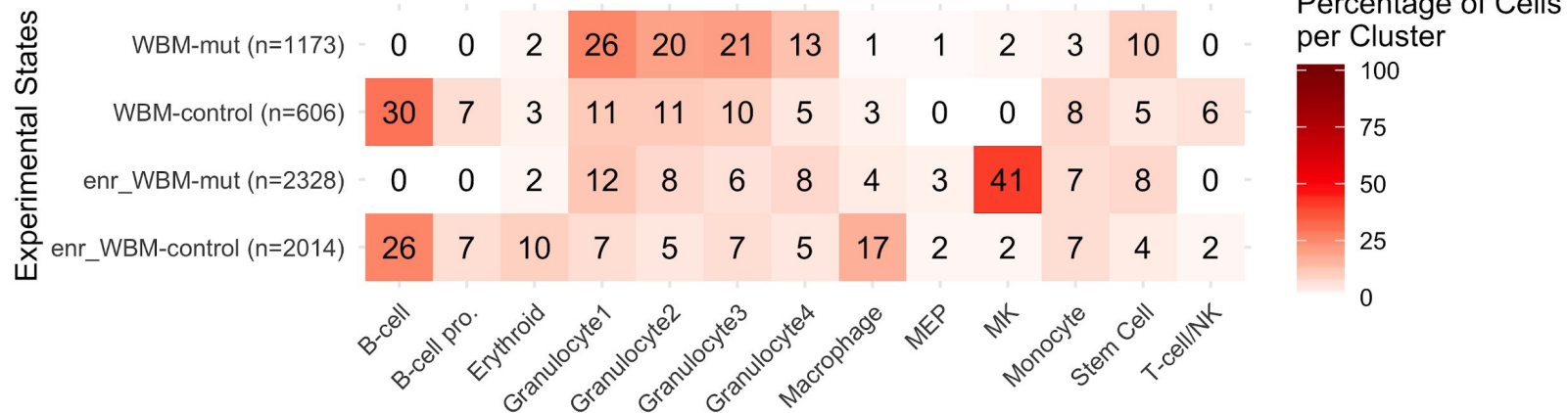
# Marker Gene Example for MKs







### Heatmap of Cell Distributions within Experimental States



### Heatmap of Cell Distributions within Clusters

