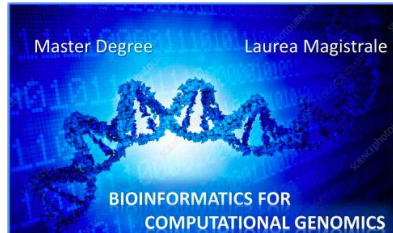


SINGLE-CELL RNA-SEQ ANALYSIS

Cominelli Marco, Sasso Elena



June 25th

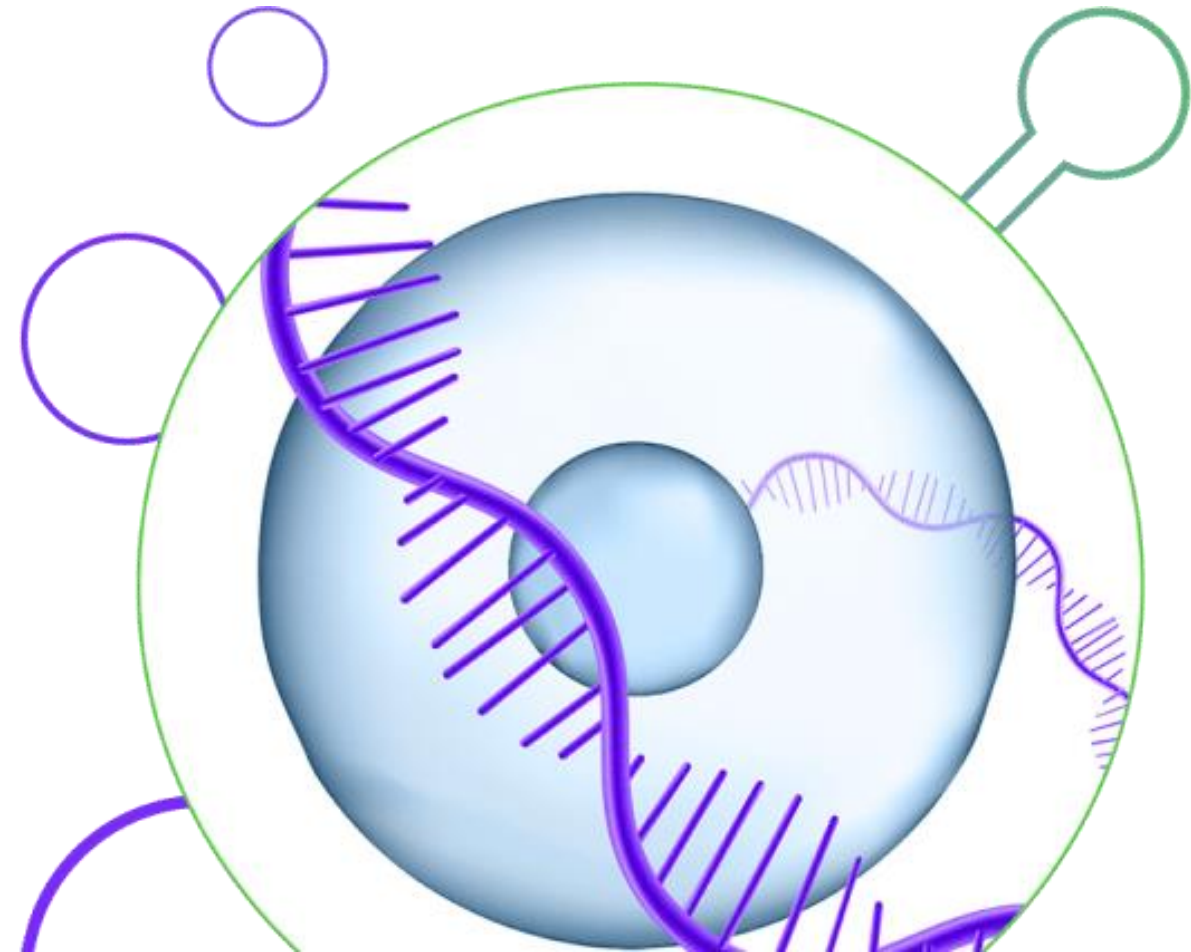


TABLE OF CONTENTS

1. General background

2. Cell quality control

3. Most variables genes

4. Elbow plot

5. Clustering

5.1 Possible biases

6. Clustering comparison

7. Marker genes

8. Final results

9. Comments

1. General background

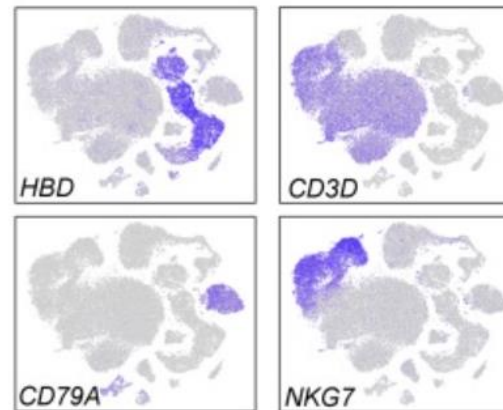
- Tissue: **bone marrow mononuclear cells (BMMCs)**

Cell types found by the authors of the article:

1. CD4+ naïve T cells
2. CD4+ memory T cells
3. CD14+ monocytes
4. CD8+ effector T cells (1)
5. CD20+ B cells
6. CD8+ effector T cells (2)
7. CD8+ naïve T cells
8. Late erythroid progenitors
9. Early erythrocytes
10. Natural killer cells
11. Late erythrocytes
12. Early erythroid progenitors
13. HSPCs
14. CD10+ B cells
15. CD16+ monocytes
16. Dendritic cells
17. Monocyte progenitors
18. Plasmacytoid dendritic cells
19. Plasma cells
20. Megakaryocytes



Putative marker genes reported by the authors:



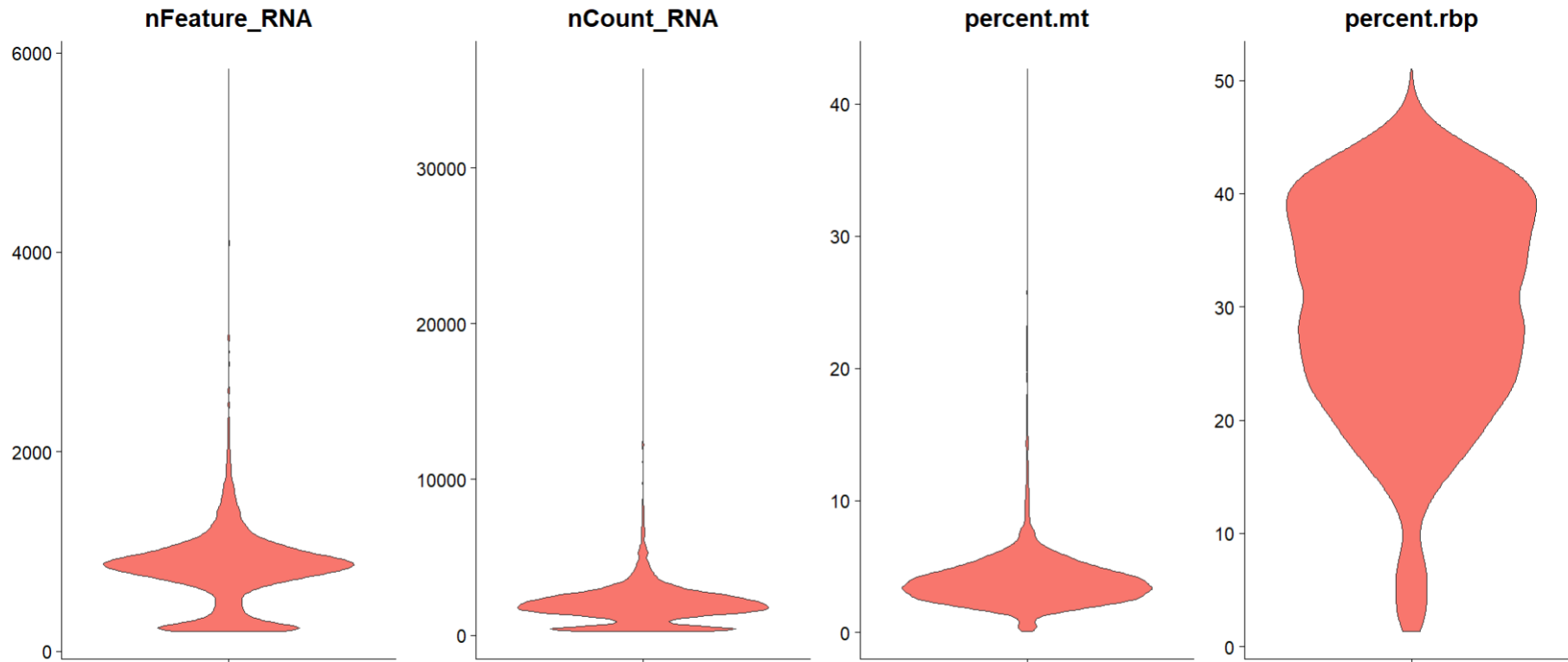
Cell types found by automated pipeline in PanglaoDB:

1. B cells (n=114)
2. B cells naïve (n=637)
3. Dendritic cells (n=88)
4. Erythroid-like and erythroid precursor cells (n=225)
5. Gamma delta T cells (n=582)
6. Langerhans cells (n=41)
7. Monocytes (n=590)
8. NK cells (n=697)
9. Plasma cells (n=29)
10. Plasmacytoid dendritic cells (n=36)
11. T cells (n=2355)
12. Unknown (n=54)

Oetjen KA, Lindblad KE, Goswami M, Gui G, Dagur PK, Lai C, Dillon LW, McCoy JP, Hourigan CS. Human bone marrow assessment by single-cell RNA sequencing, mass cytometry, and flow cytometry. JCI Insight. 2018 Dec 6;3(23):e124928. doi: 10.1172/jci.insight.124928. PMID: 30518681; PMCID: PMC6328018.v

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6328018/#sd>

2. Cell quality control

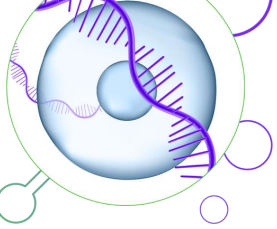


Thresholds used (by looking at the distributions):

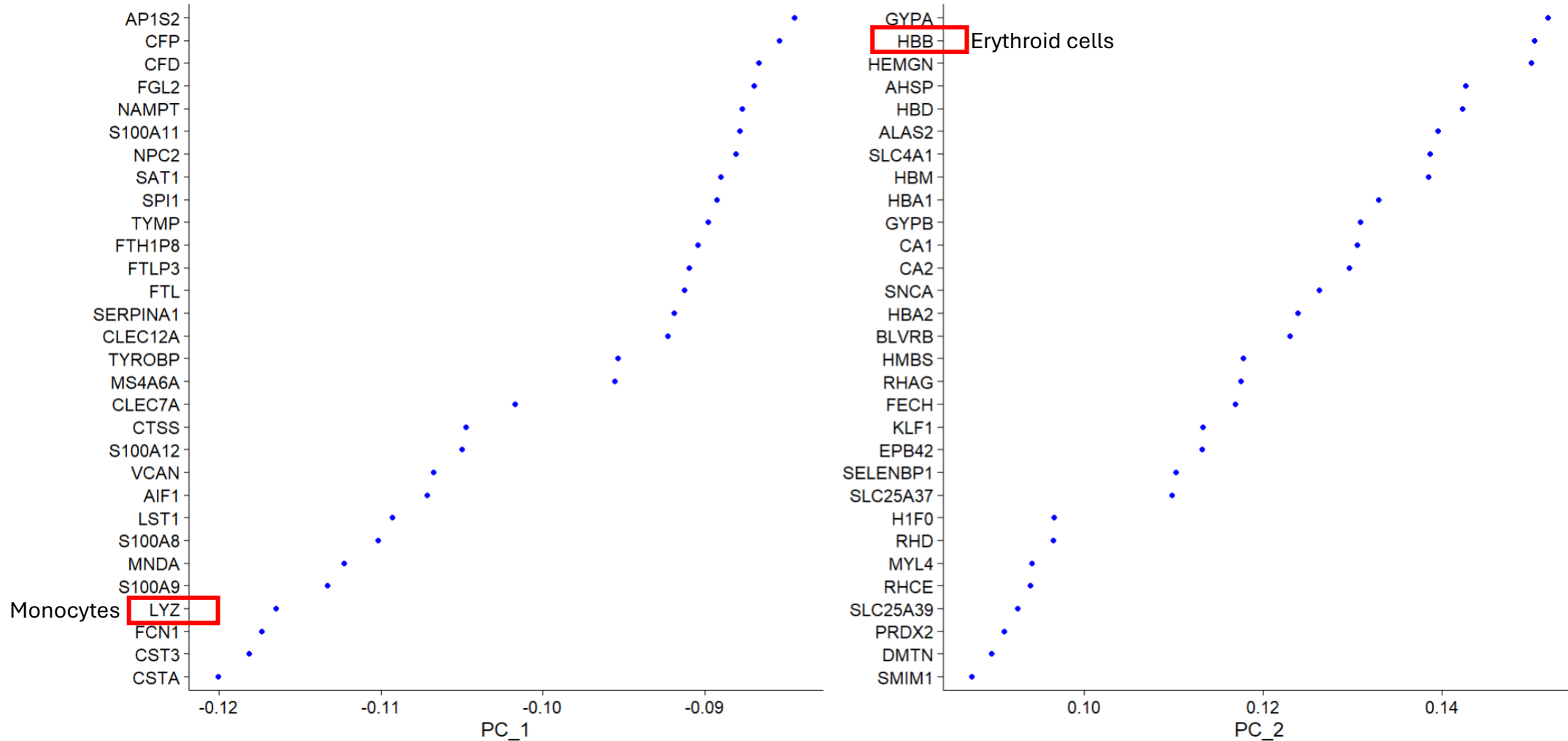
- $200 < \text{Number of genes detected} < 2500$
- $\% \text{ of reads mapping on mitochondrial genes} < 8\%$

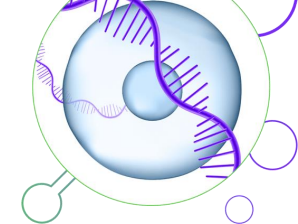
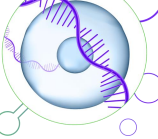


*From 6937 to 6662 → **275** cells removed*

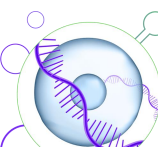
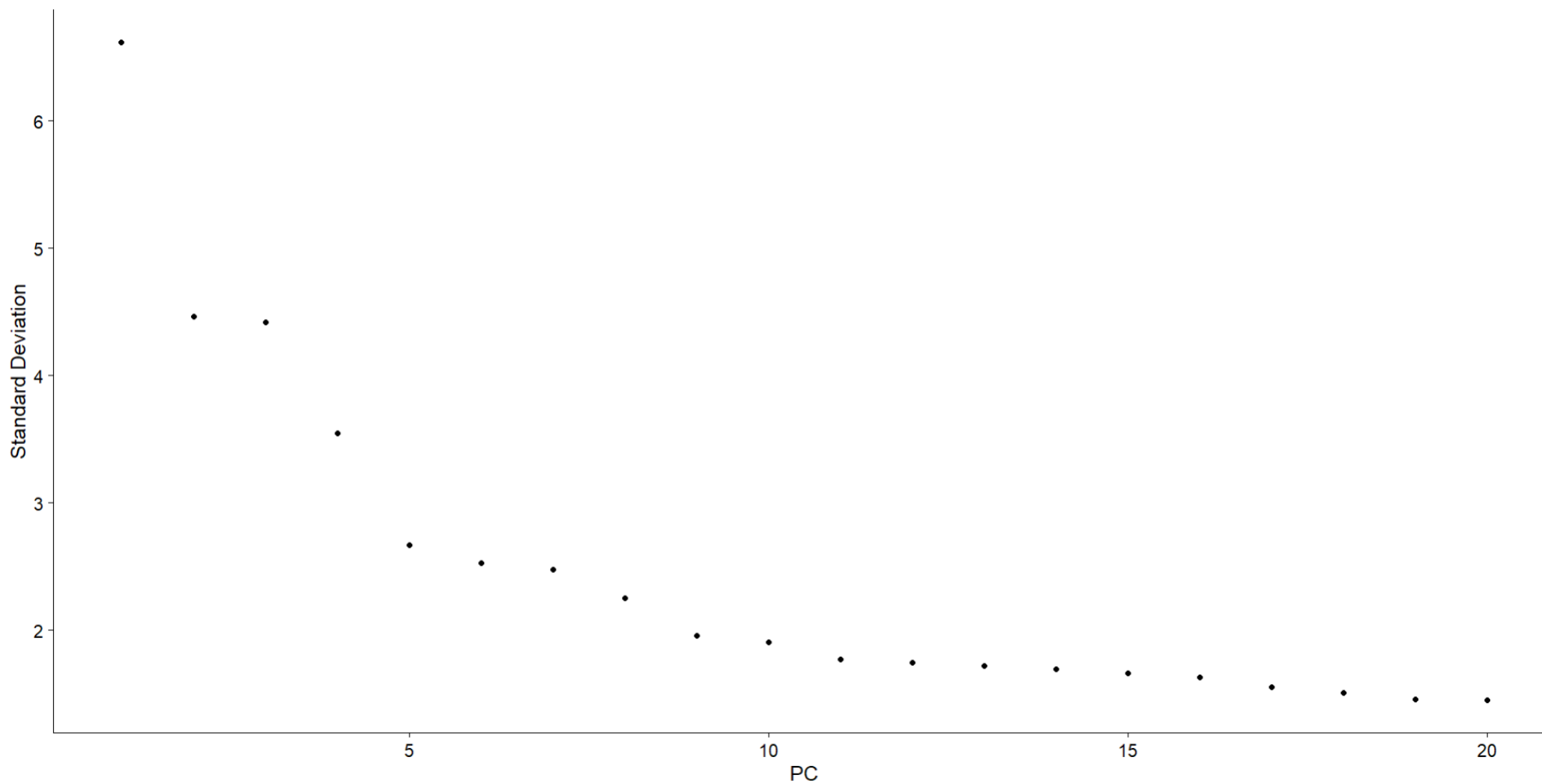


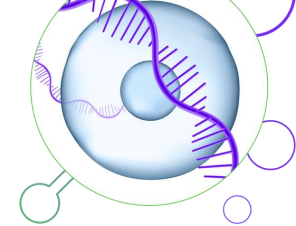
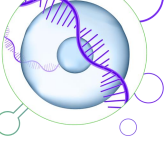
3. Most variable genes



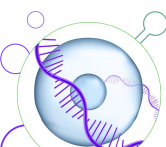
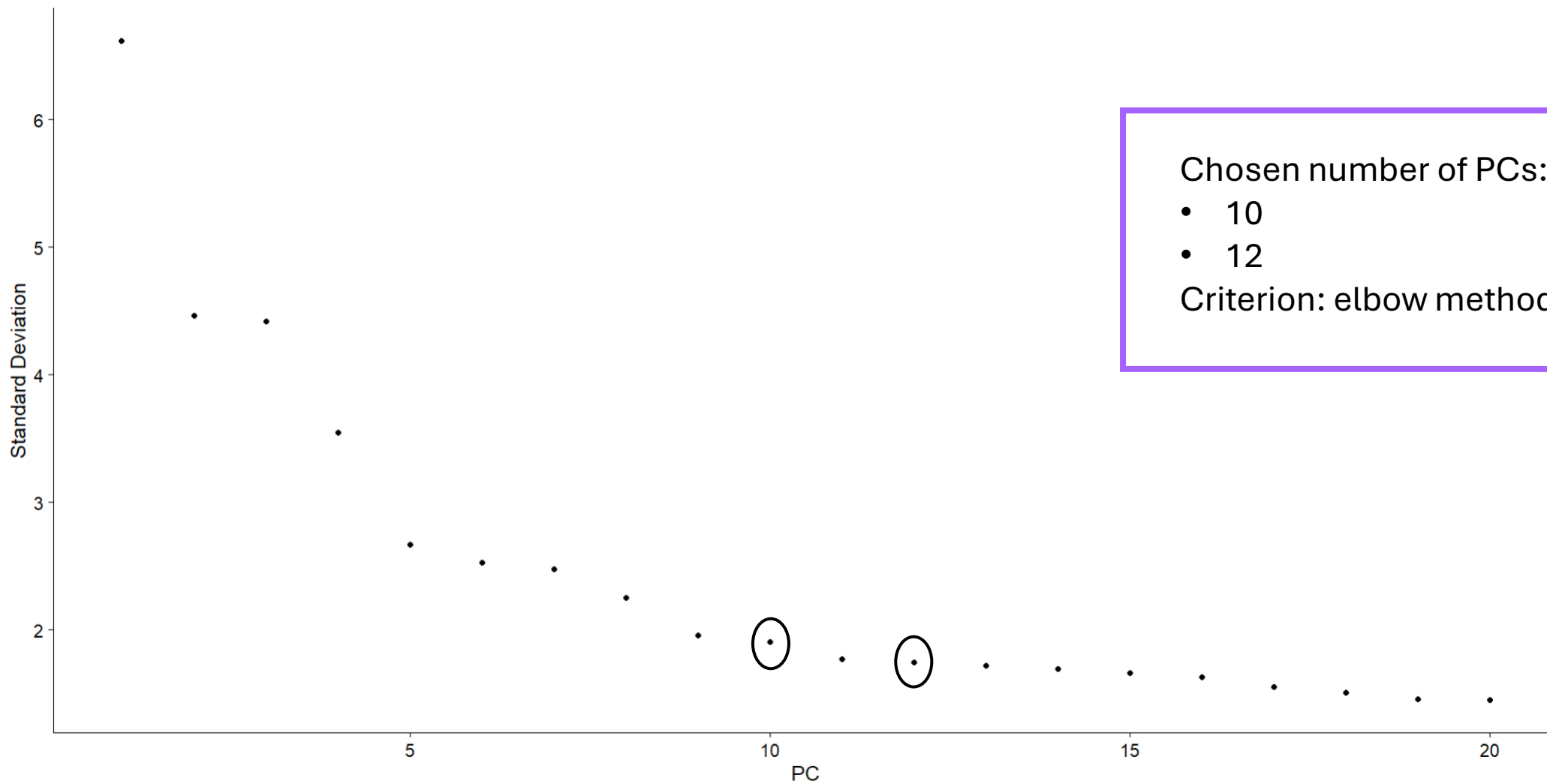


4. Elbow plot





4. Elbow plot

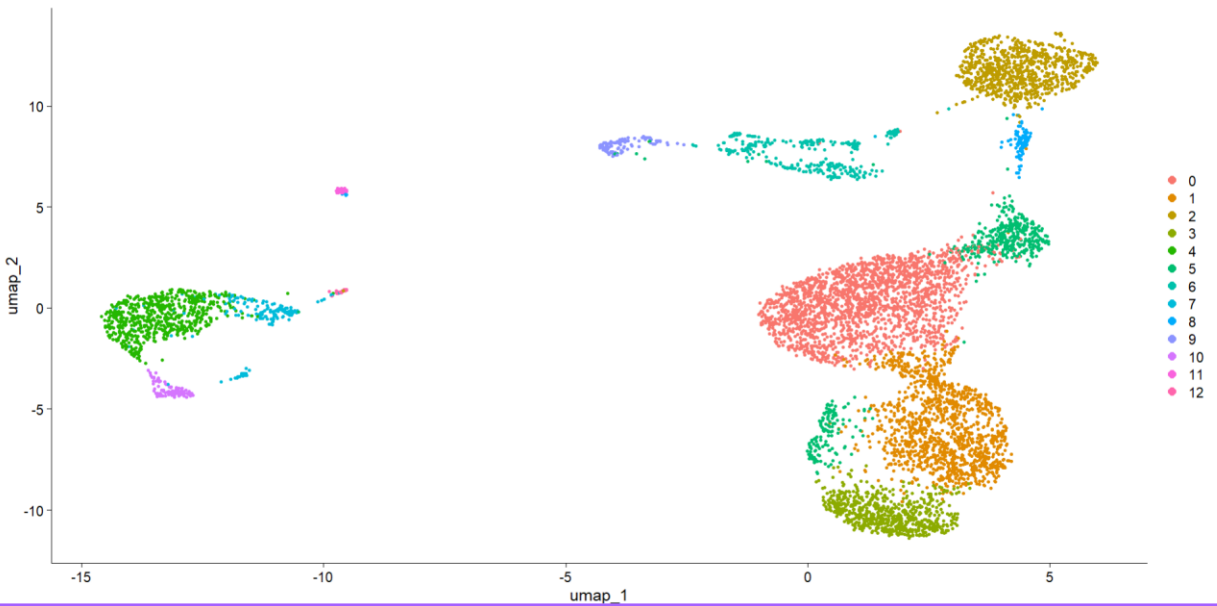


5. Clustering

- MDS technique: UMAP
- PCs: 10
- Resolution = 0.5

Number of cells for each cluster:

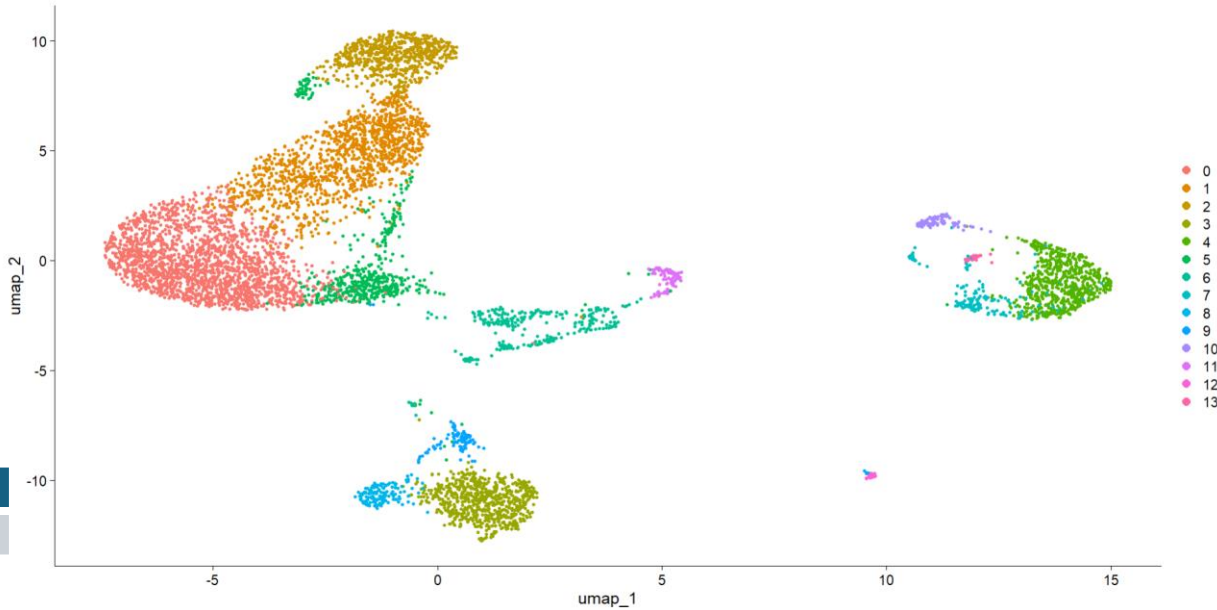
0	1	2	3	4	5	6	7	8	9	10	11	12
1919	1272	820	671	623	522	305	161	99	99	98	39	34



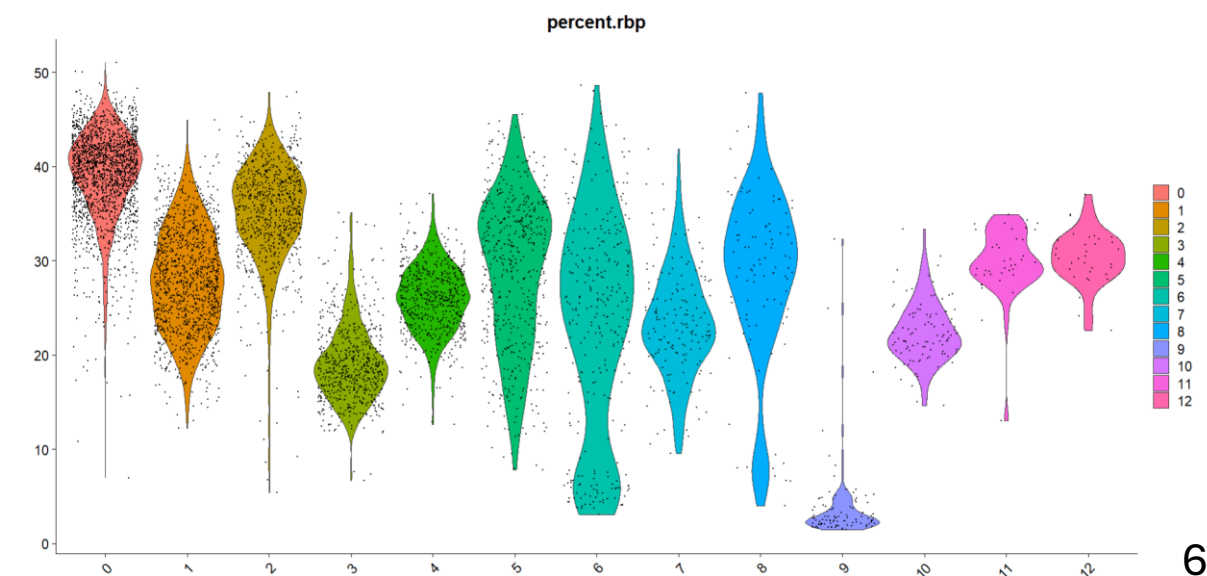
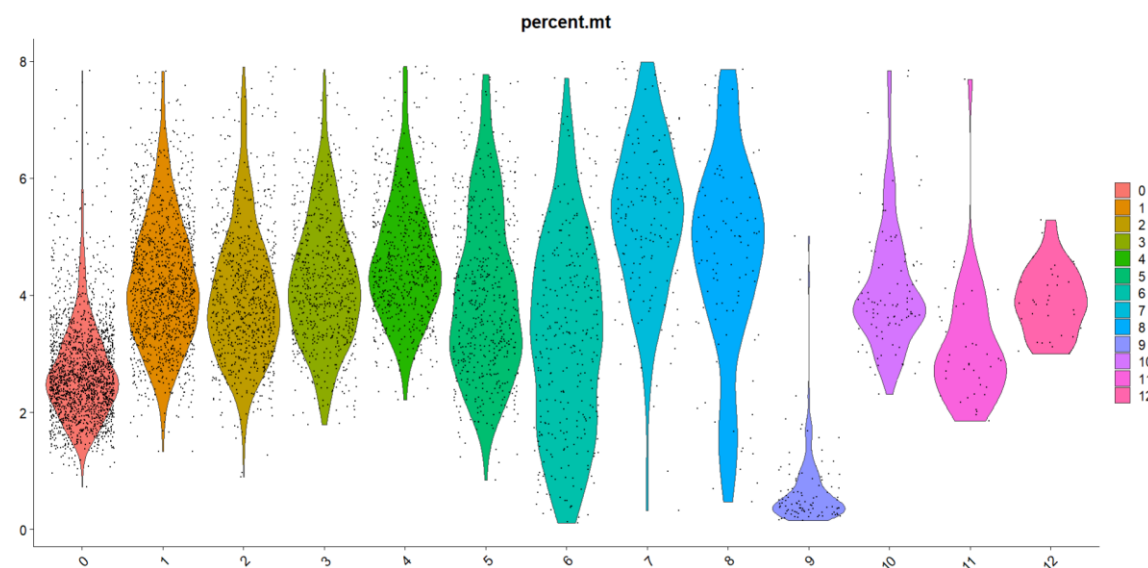
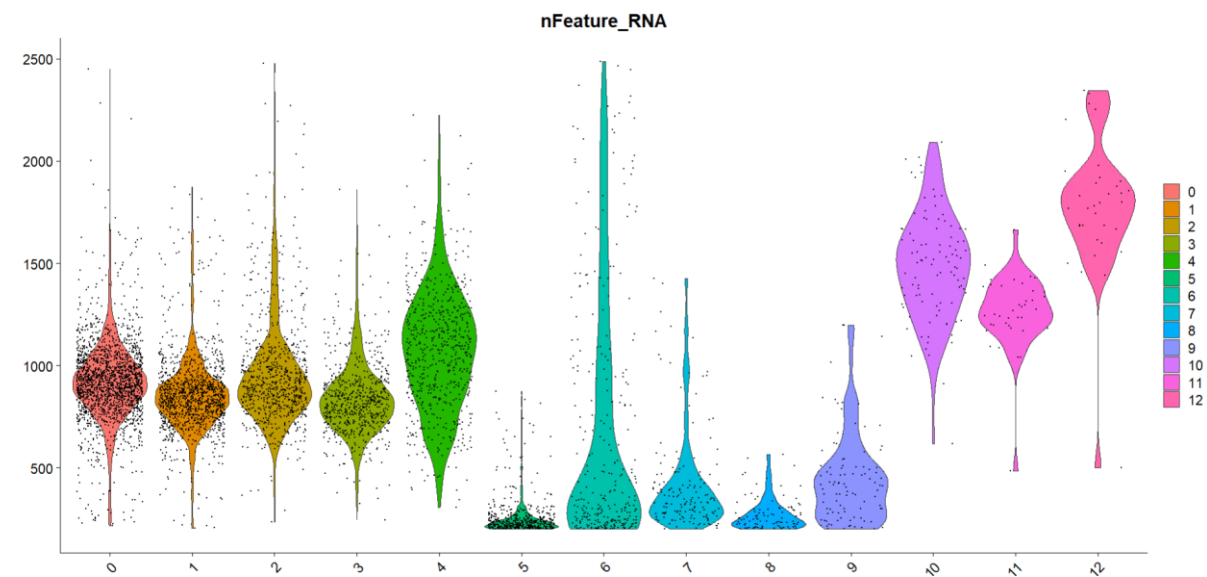
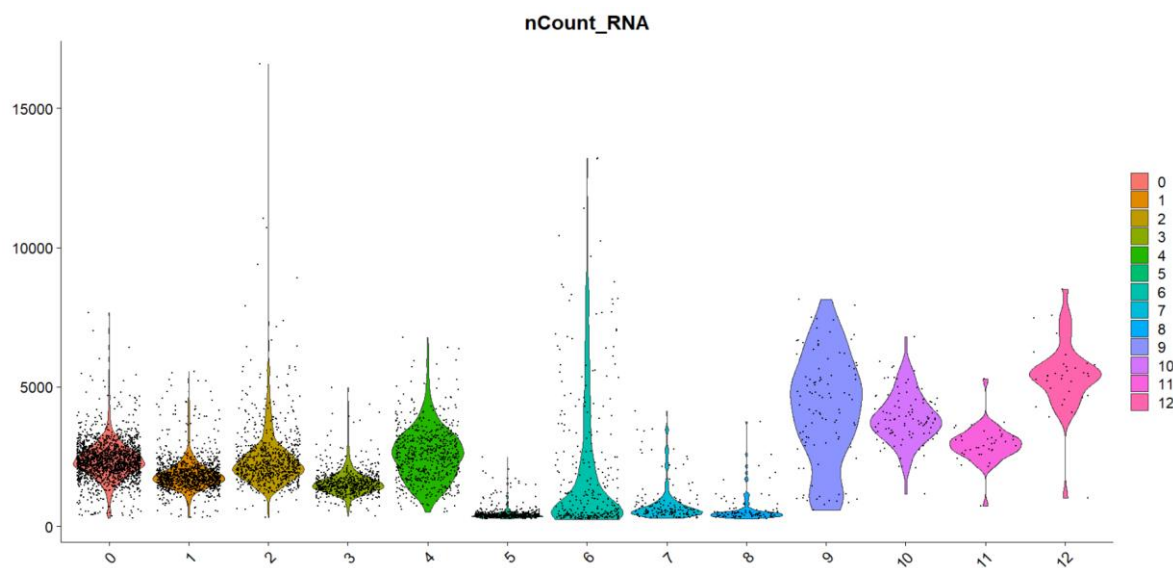
- MDS technique: UMAP
- PCs: 12
- Resolution = 0.5

Number of cells for each cluster:

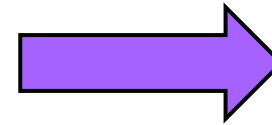
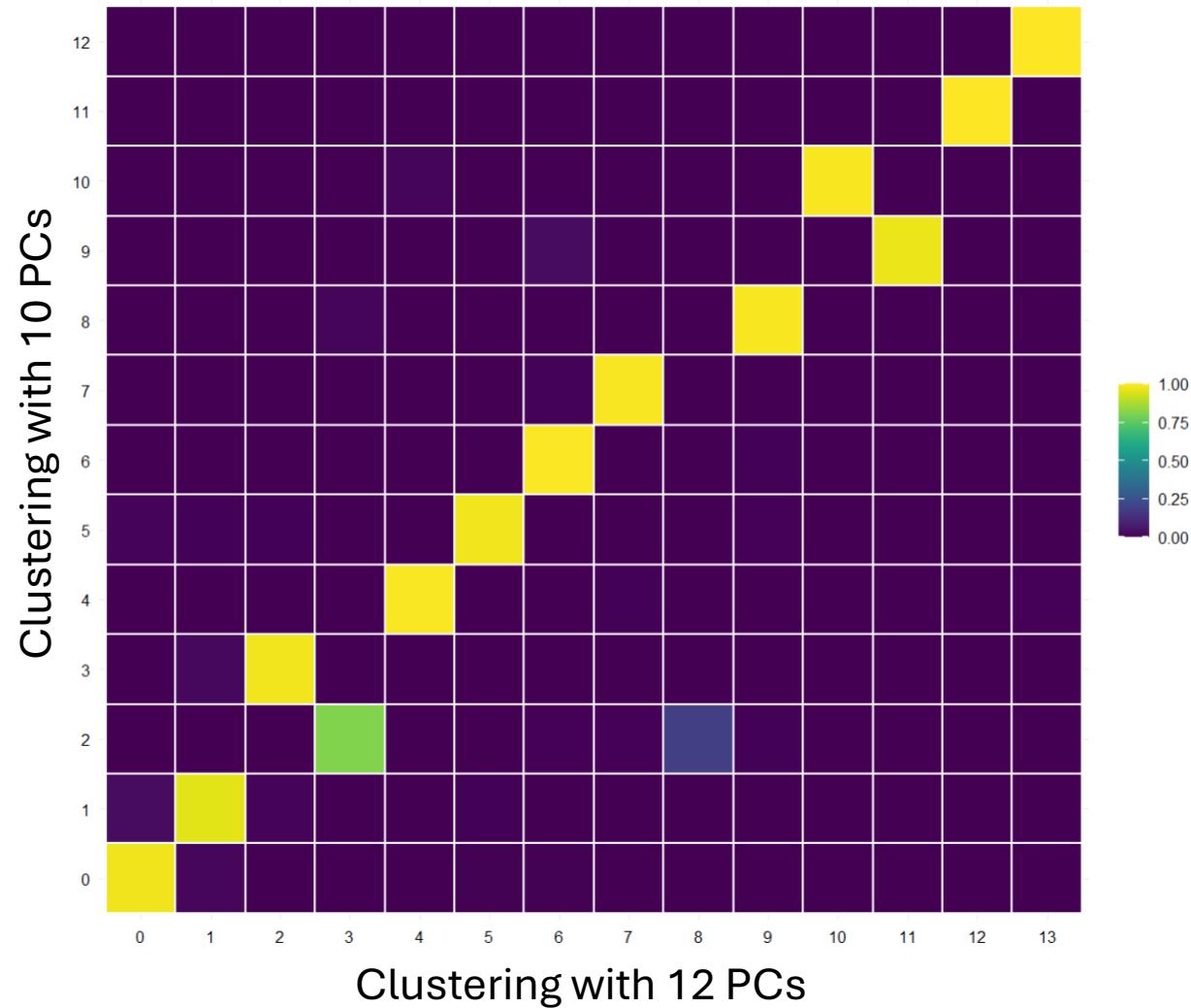
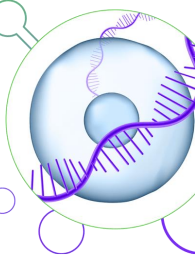
0	1	2	3	4	5	6	7	8	9	10	11	12	13
1921	1264	671	666	621	523	313	163	150	103	97	96	39	35



5.1. Possible biases

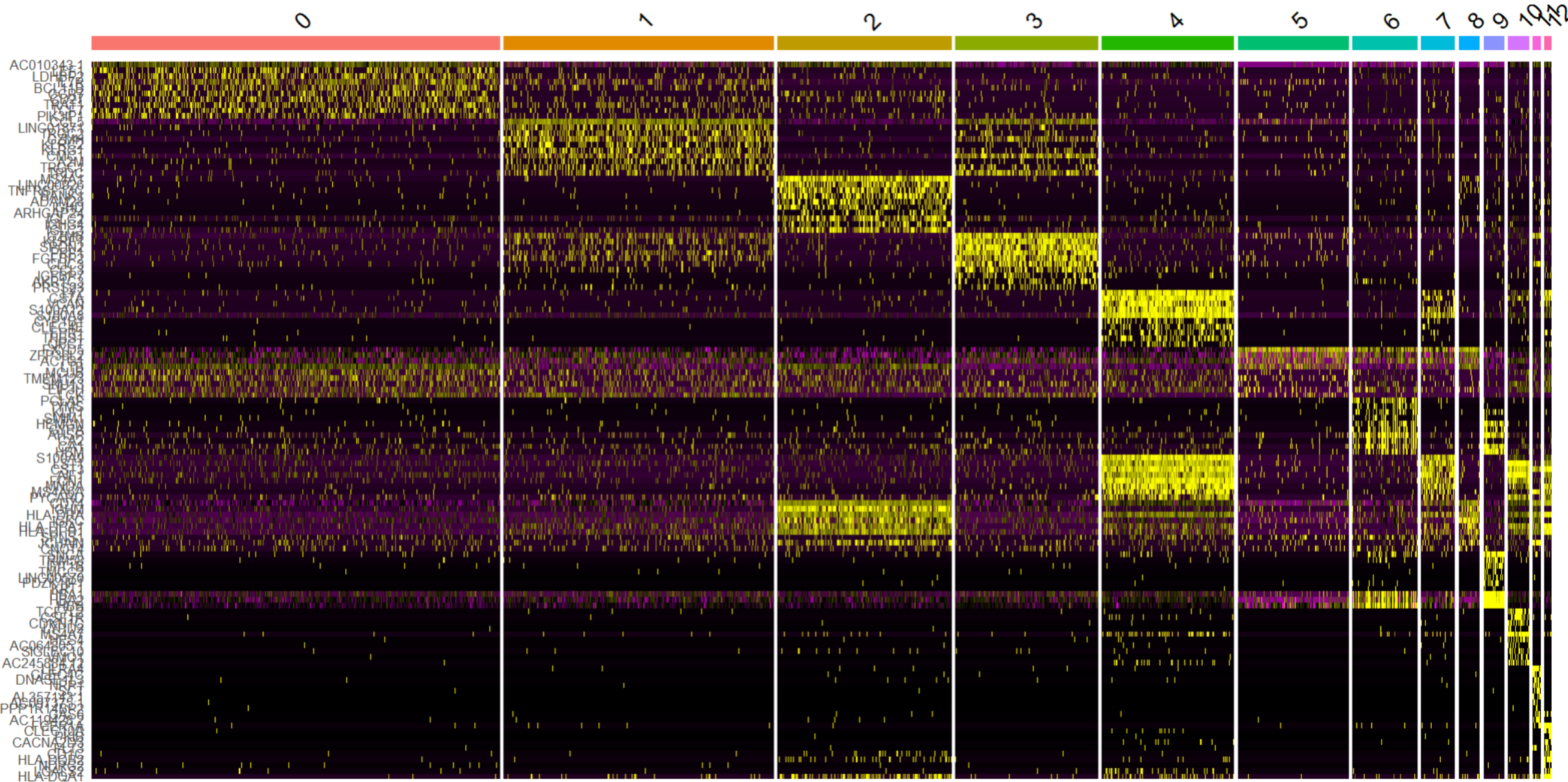


6. Clustering comparison

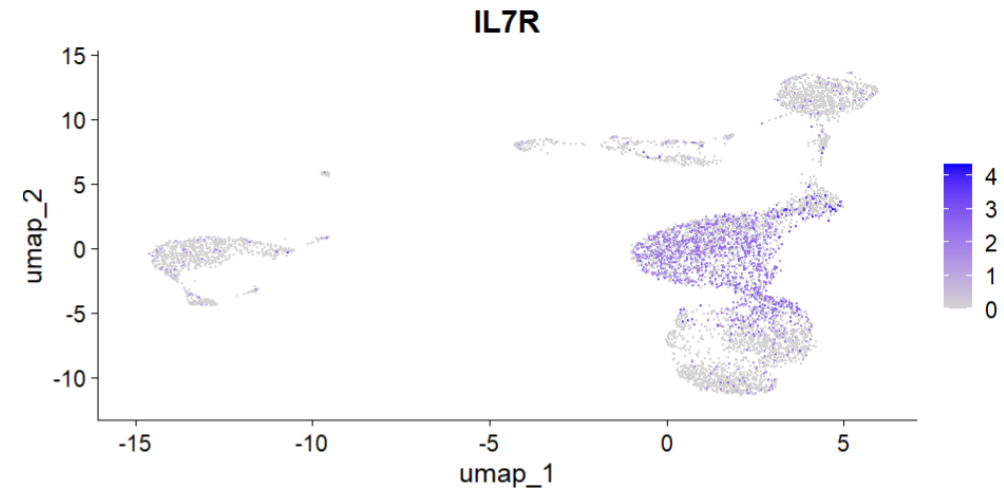
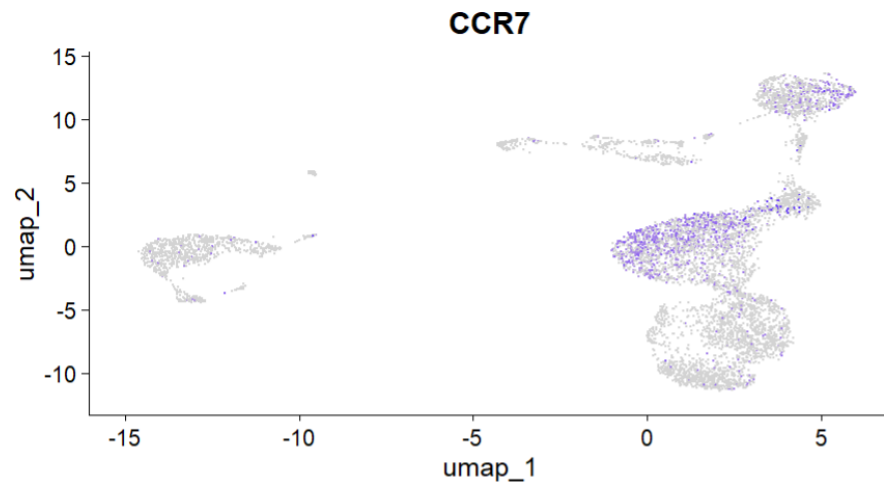


Cluster **2** computed by the clustering done choosing 10 PCs splitted into clusters **3** and **8** computed by the clustering done choosing 12 PCs.

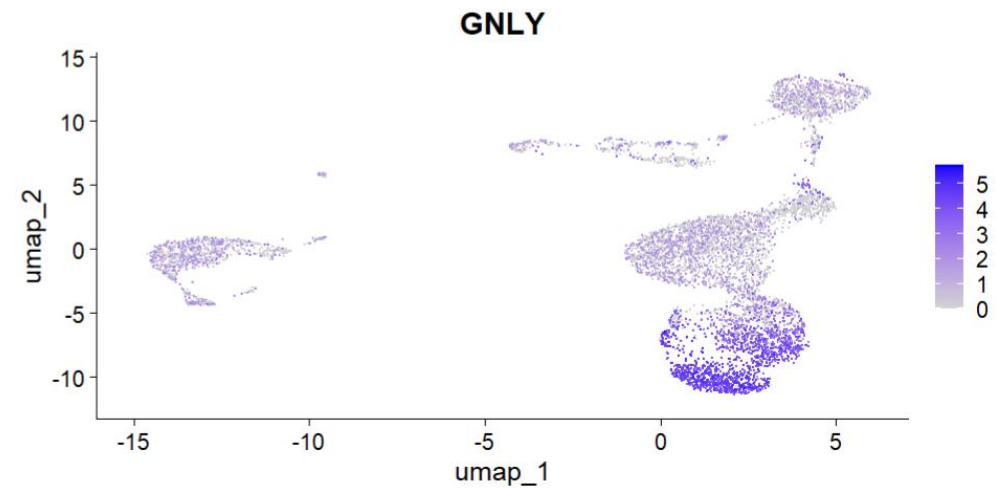
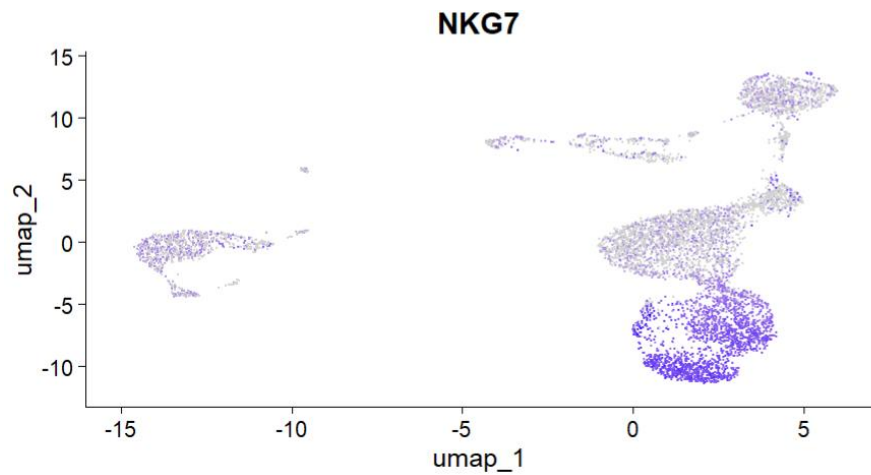
7. Marker Genes: One vs All comparison



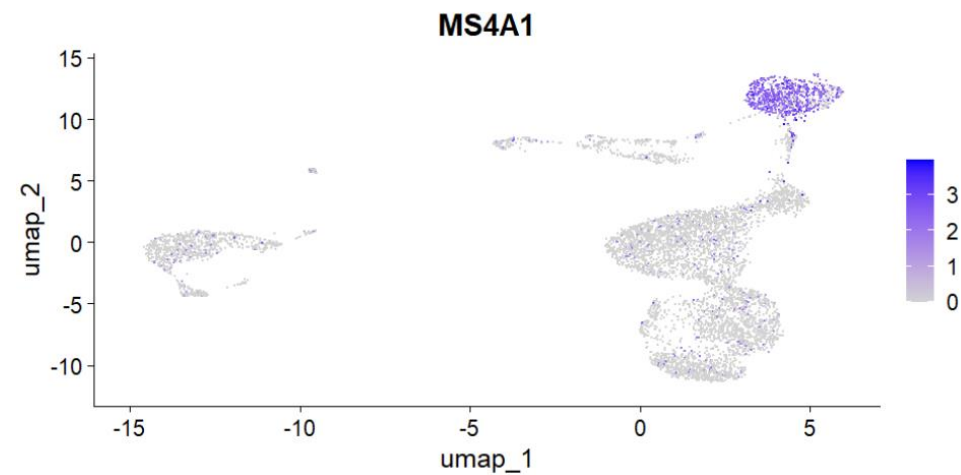
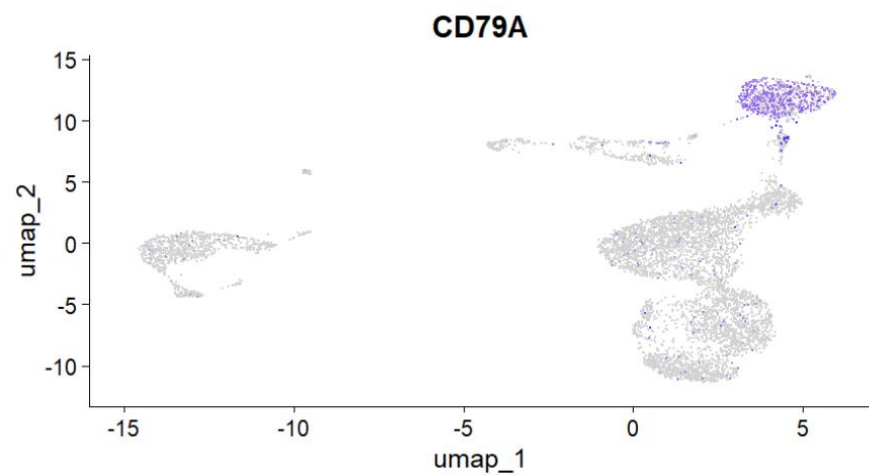
Cluster 0: Naive CD4+ T cells



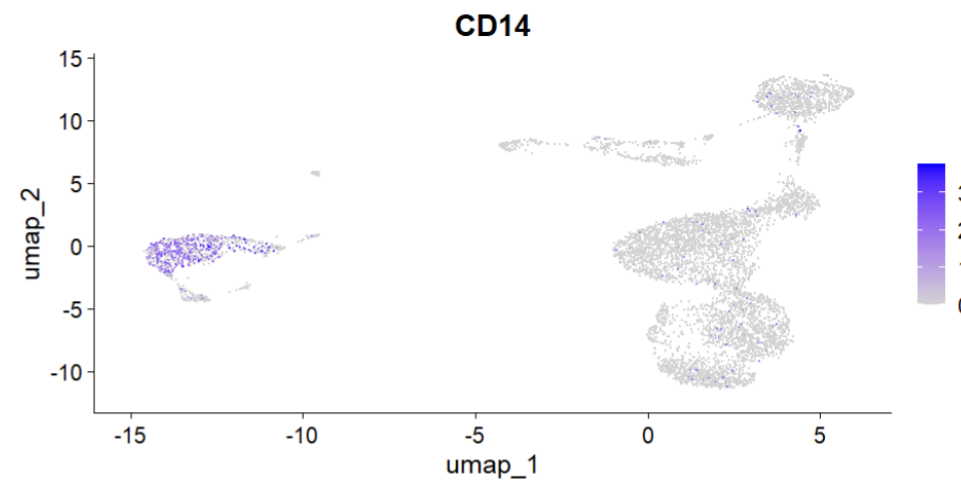
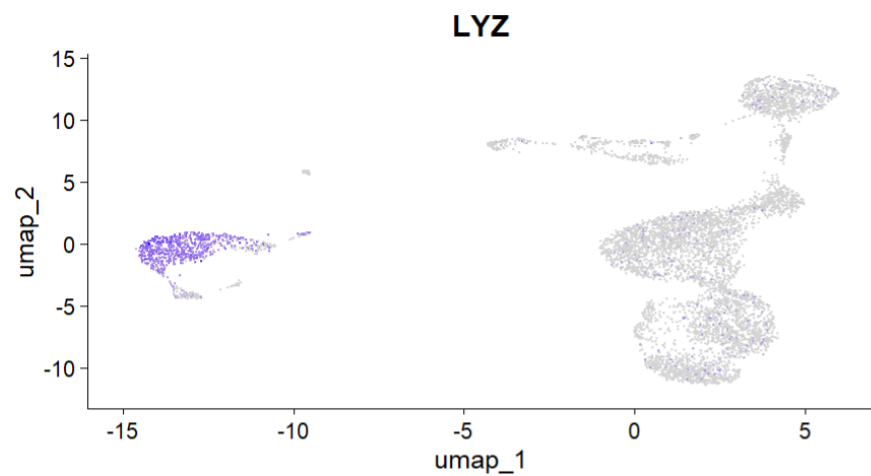
Cluster 1 and 3: Natural Killer (NK) cells



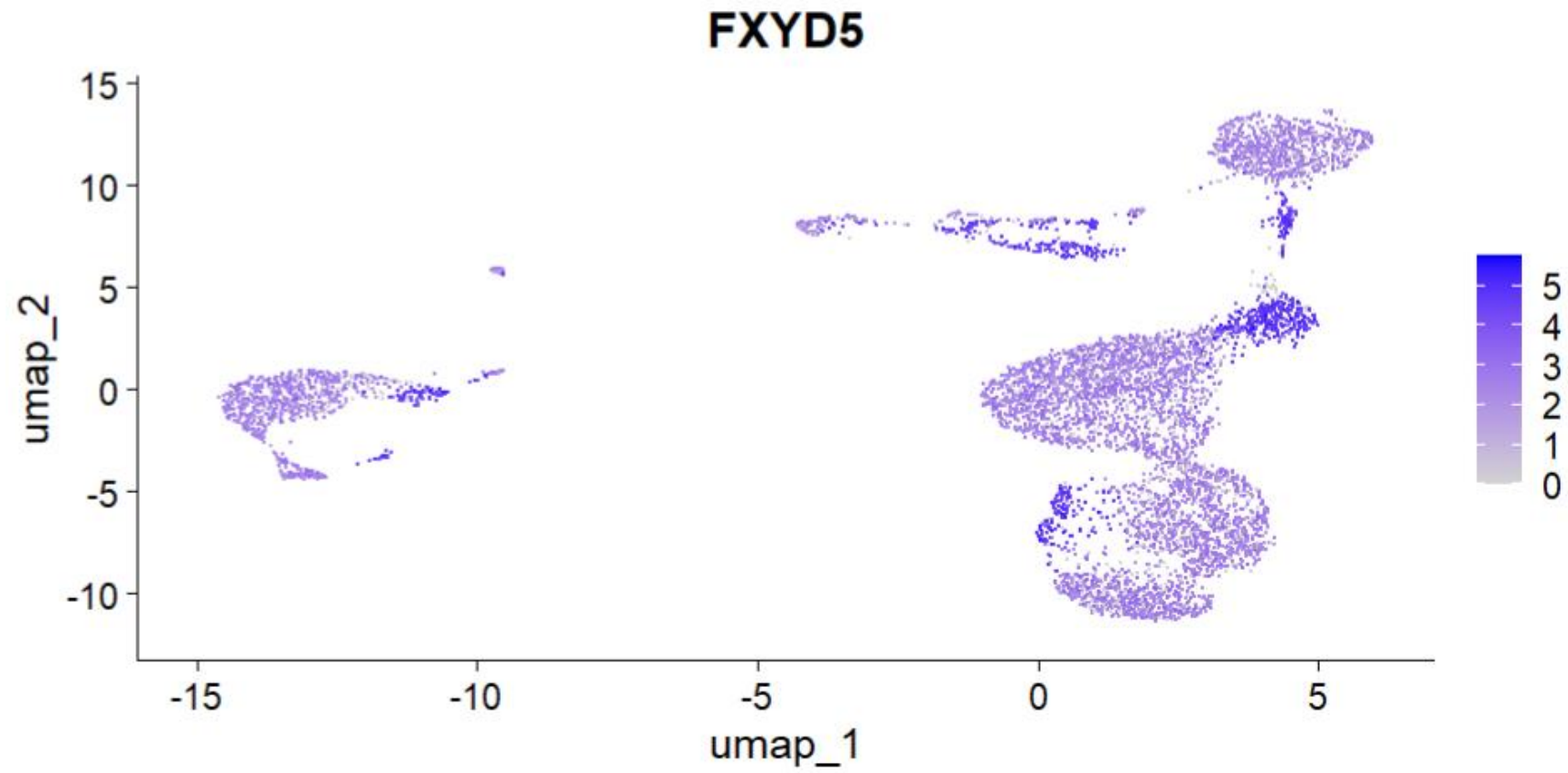
Cluster 2: Naive B cells



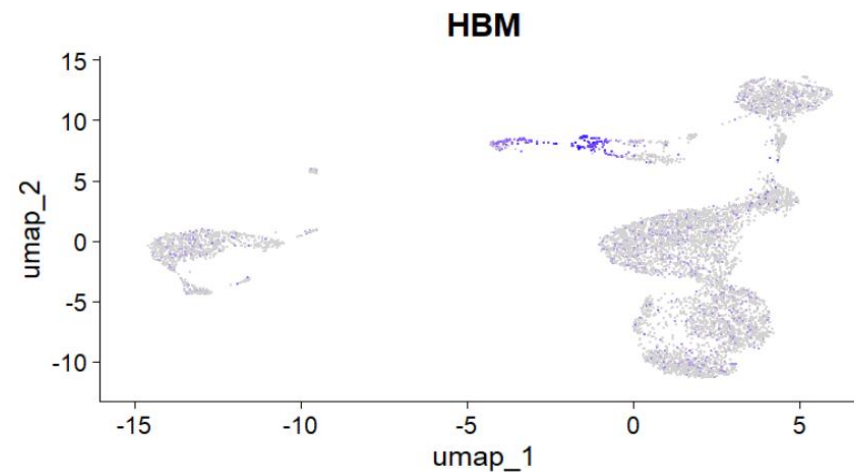
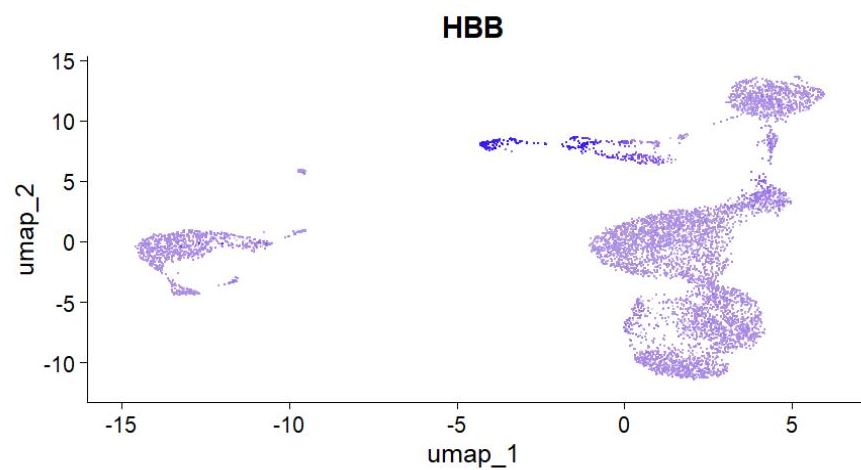
Cluster 4: CD14+ Monocytes



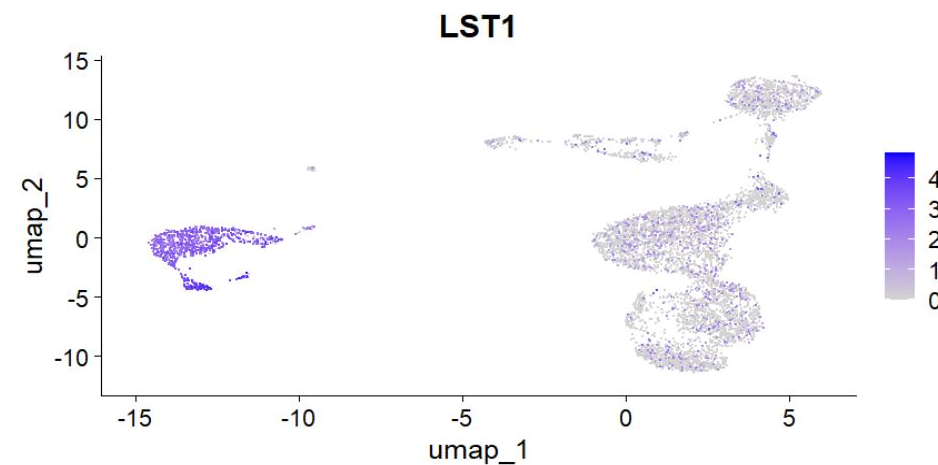
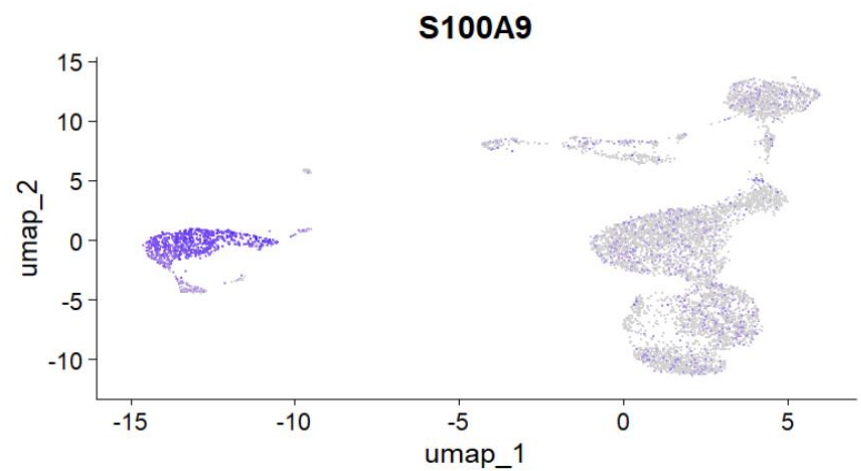
Cluster 5: T cells



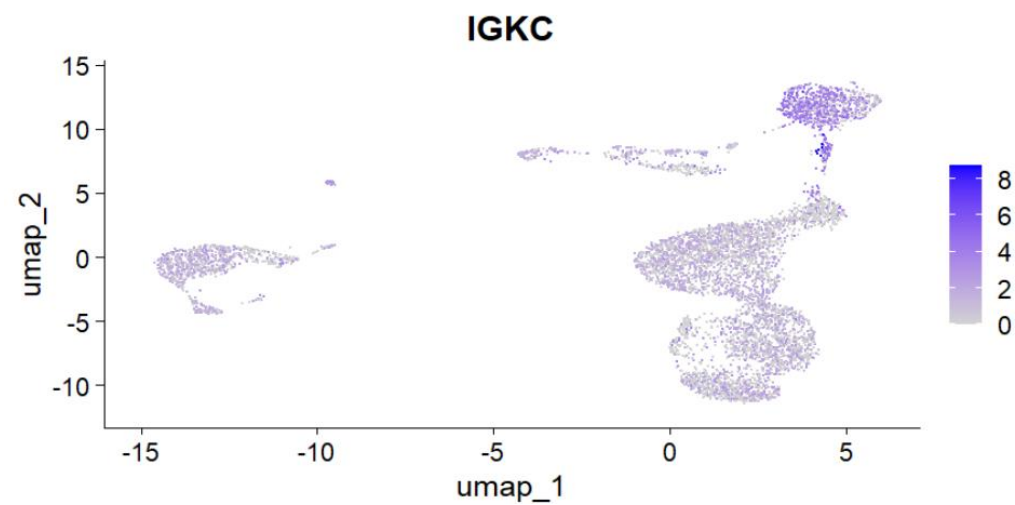
Cluster 6 and 9: Erythroid cells



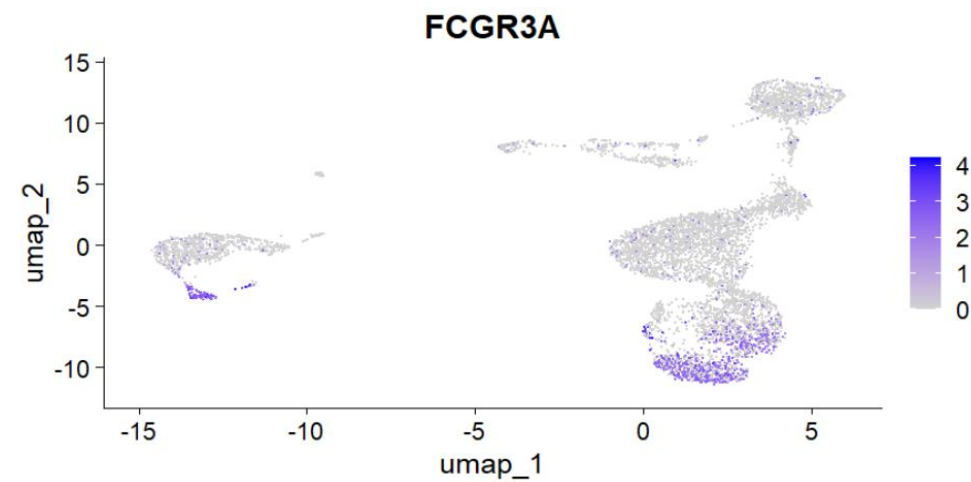
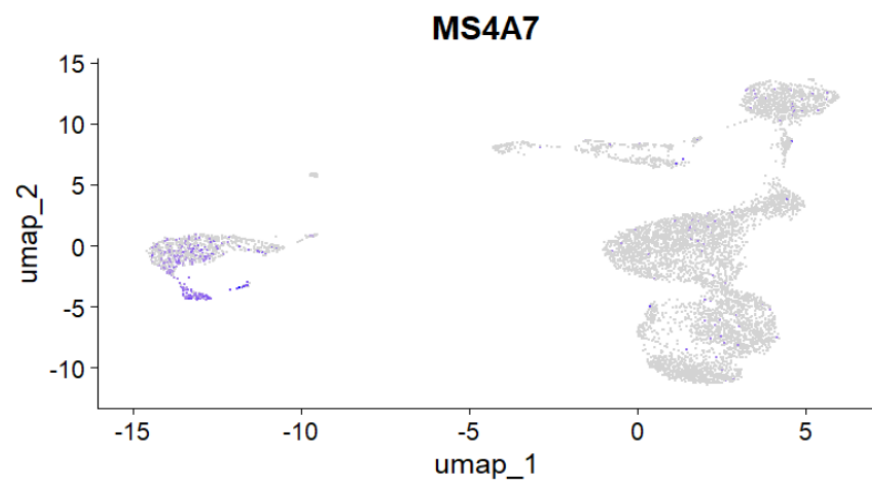
Cluster 7: Macrophages



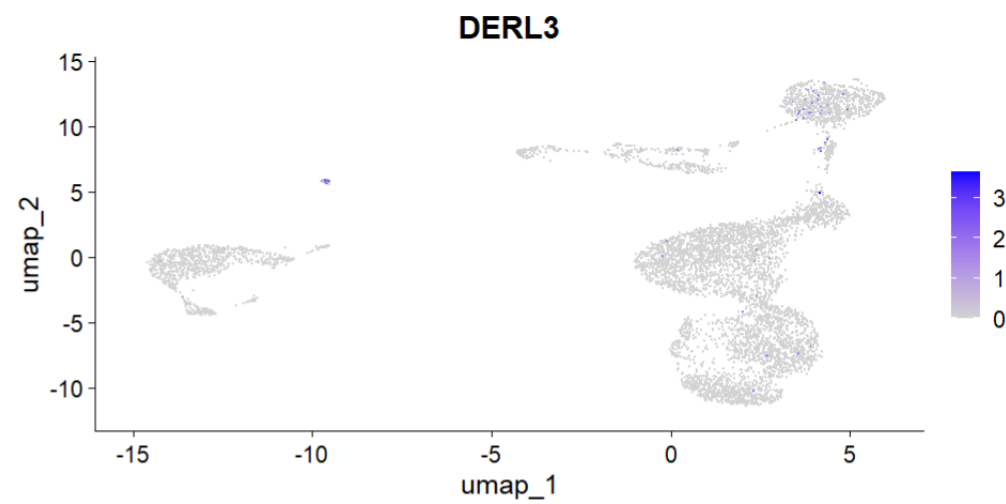
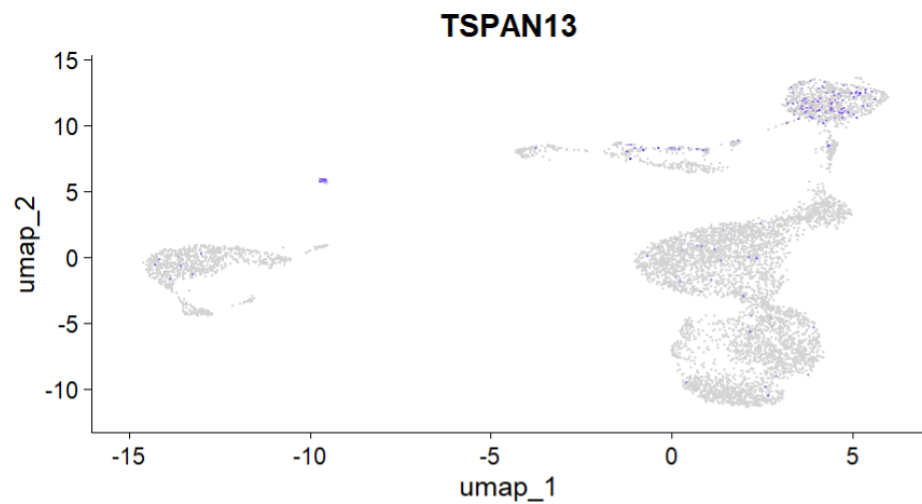
Cluster 8: Plasma cells



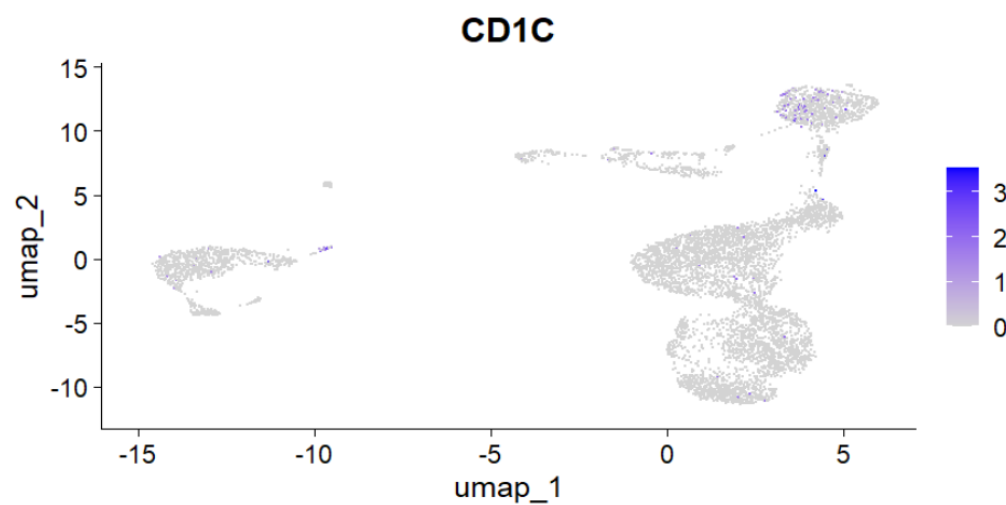
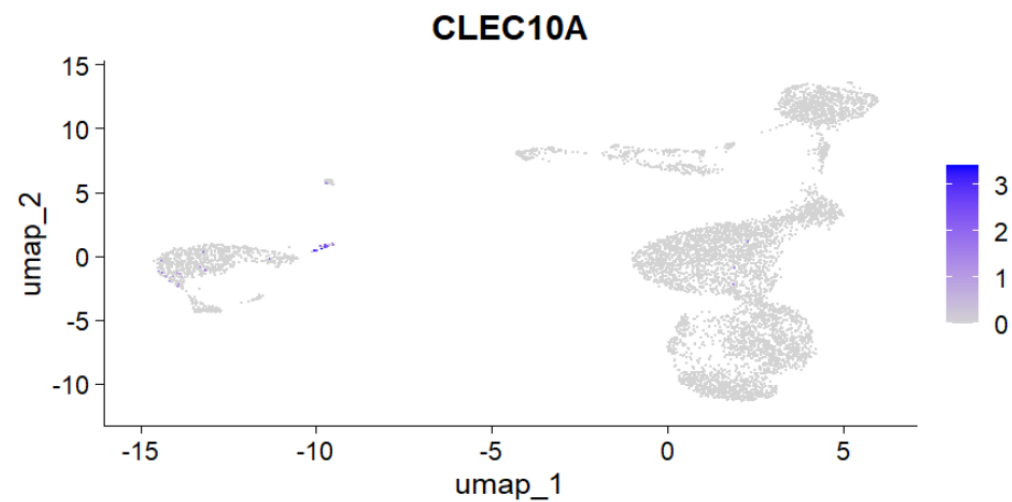
Cluster 10: FCGR3A+ Monocytes



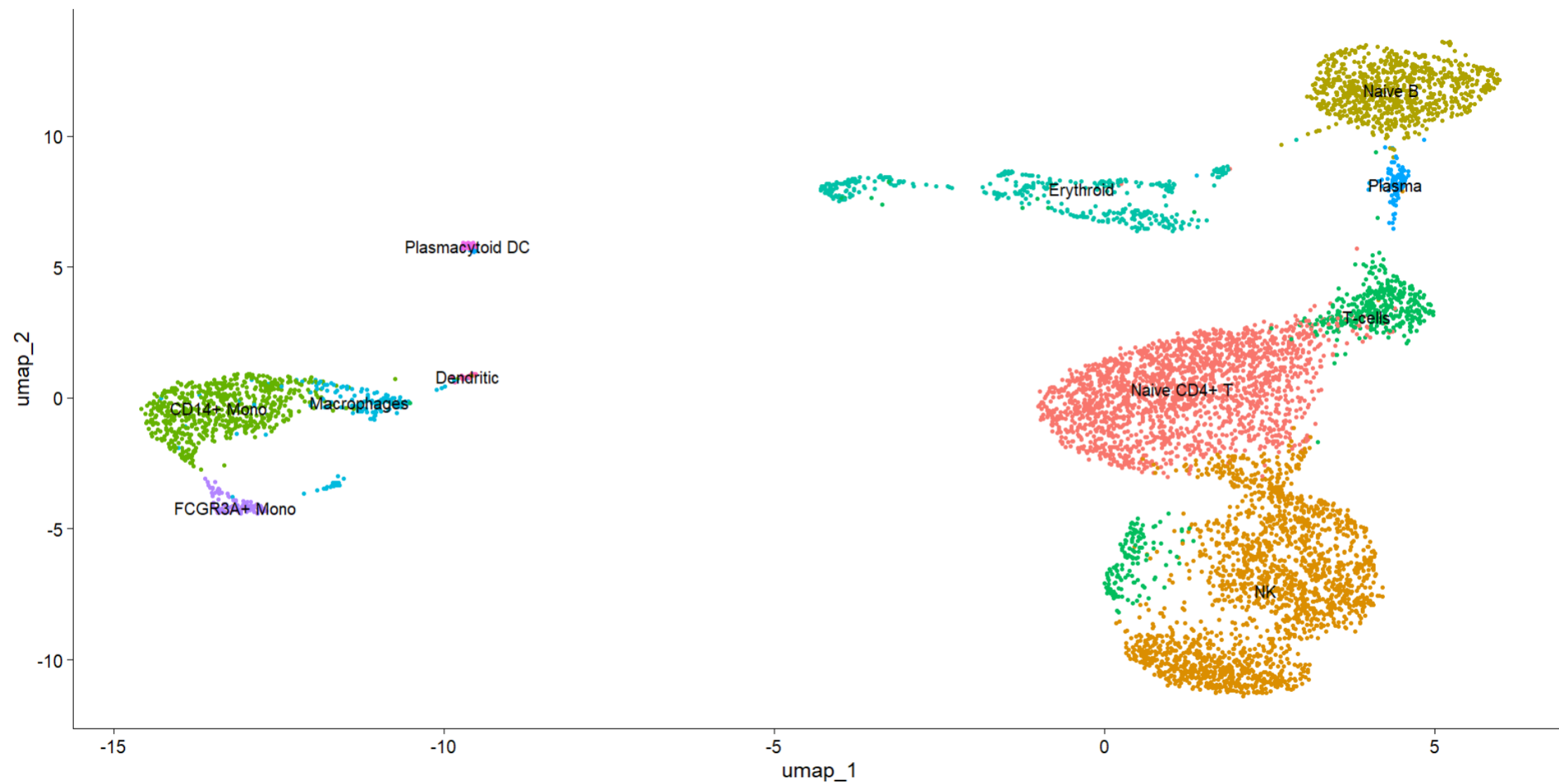
Cluster 11: Plasmacytoid dendritic cells



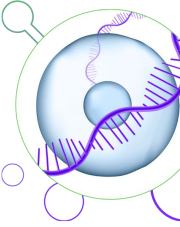
Cluster 12: Dendritic cells



8. Final result



9. Comments



Original paper:

1. CD4+ naïve T cells
2. CD4+ memory T cells
3. CD14+ monocytes
4. CD8+ effector T cells (1)
5. CD20+ B cells
6. CD8+ effector T cells (2)
7. CD8+ naïve T cells
8. Late erythroid progenitors
9. Early erythrocytes
10. Natural killer cells
11. Late erythrocytes
12. Early erythroid progenitors
13. HSPCs
14. CD10+ B cells
15. CD16+ monocytes
16. Dendritic cells
17. Monocyte progenitors
18. Plasmacytoid dendritic cells
19. Plasma cells
20. Megakaryocytes

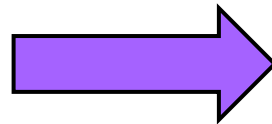
Us:

1. Naive CD4+ T cells
2. Natural Killer (NK) cells
3. Naive B cells
4. CD14+ Monocytes
5. T cells
6. Erythroid cells
7. Macrophages
8. Plasma cells
9. FCGR3A+ Monocytes
10. Dendritic cells
11. Plasmacytoid dendritic cells

PanglaoDB:

1. B cells (n=114)
2. B cells naïve (n=637)
3. Dendritic cells (n=88)
4. Erythroid-like and erythroid precursor cells (n=225)
5. Gamma delta T cells (n=582)
6. Langerhans cells (n=41)
7. Monocytes (n=590)
8. NK cells (n=697)
9. Plasma cells (n=29)
10. Plasmacytoid dendritic cells (n=36)
11. T cells (n=2355)
12. Unknown (n=54)

Our results differ from both the other two,
which differ also one from each other



Possible motivations:

- Subset of cells
- Number of PC component
- Modularity

THANKS FOR YOUR ATTENTION!