

2021_08_11 - Reanalysis

PAPER = "Single-cell transcriptomic analysis of Alzheimer's disease"

Data = 15 early AD, 9 late AD, 24 healthy control PFC brain nuclei

Downloaded = SYNAPSE

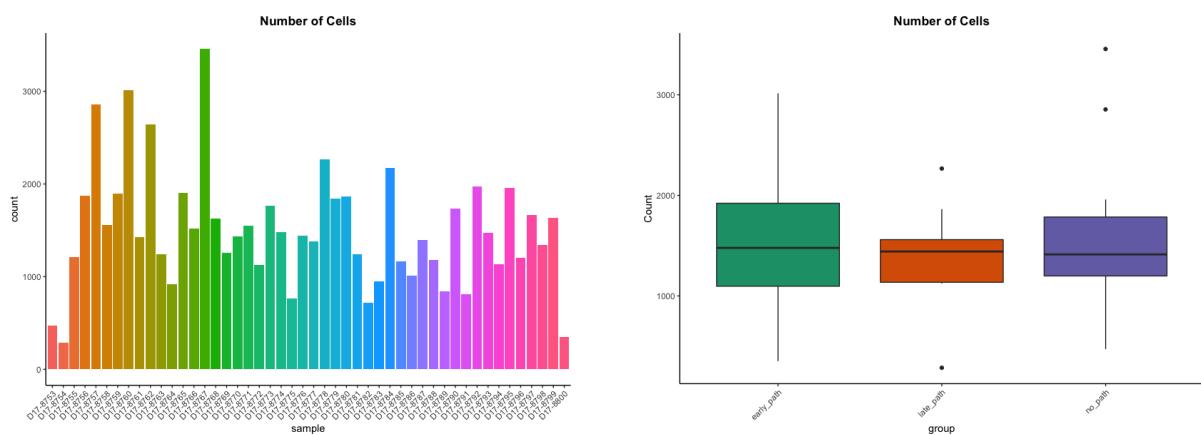
Cellranger-6.0.1

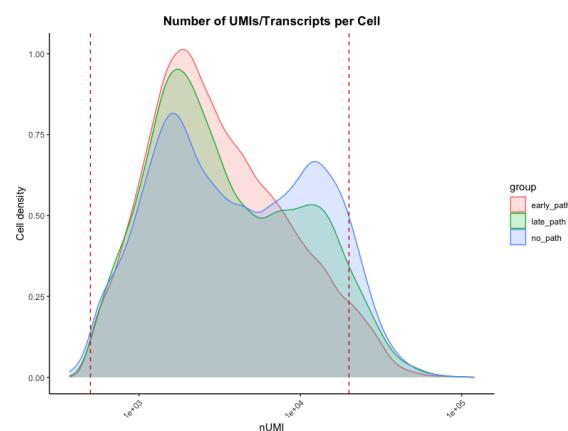
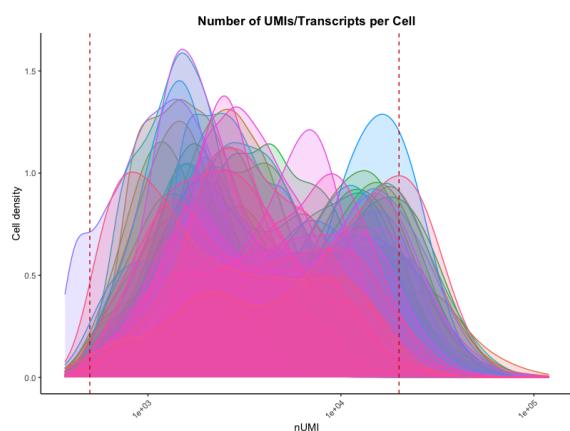
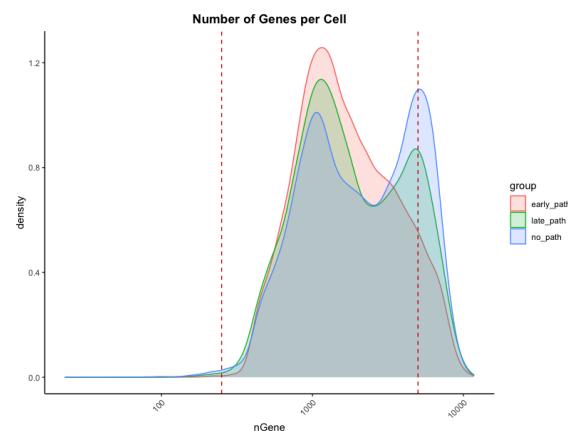
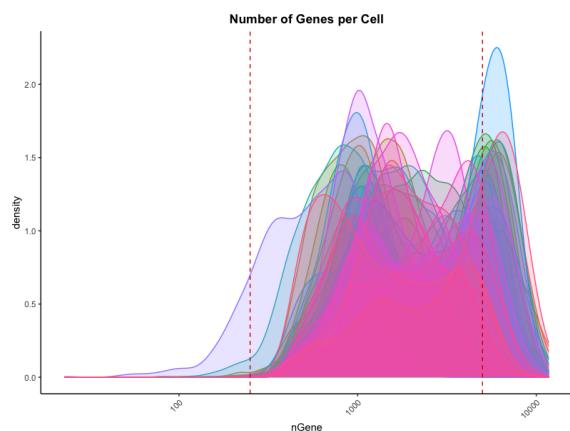
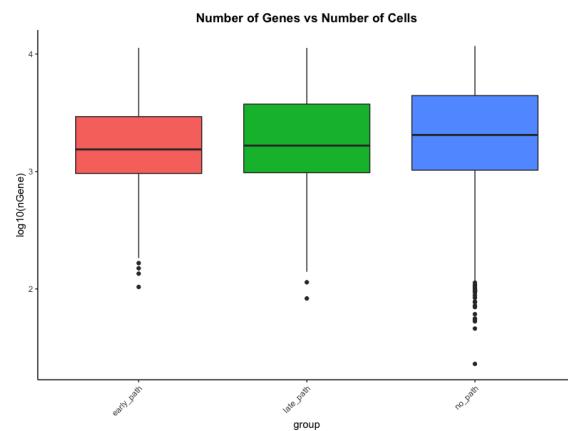
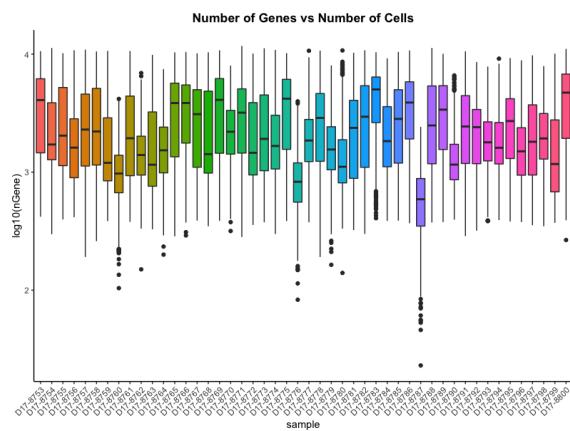
Re-aligned fastq data to both mRNA and mRNA+premRNA

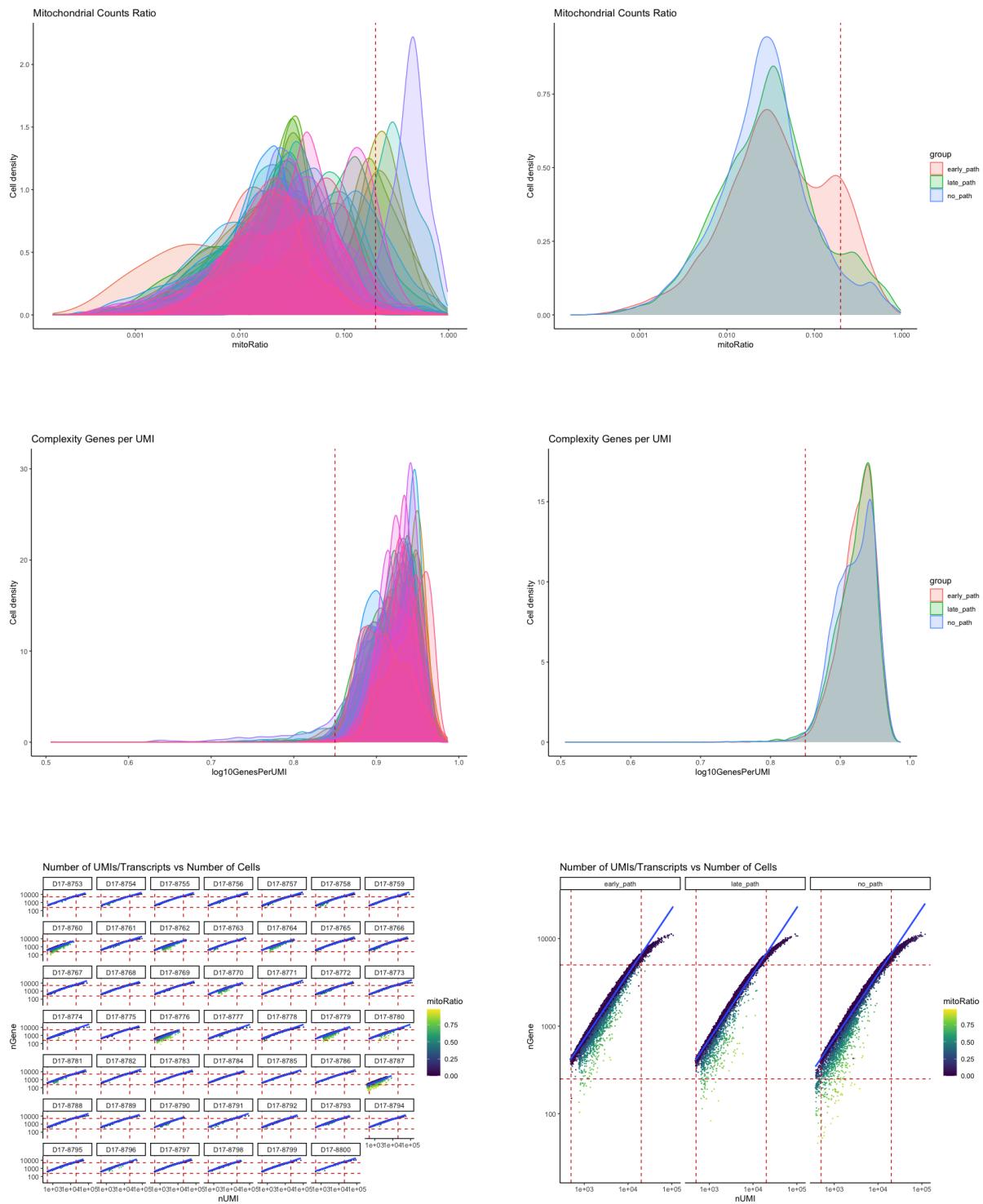
Because this is nuclei data, used mRNA+premRNA data for analysis

Seurat QC

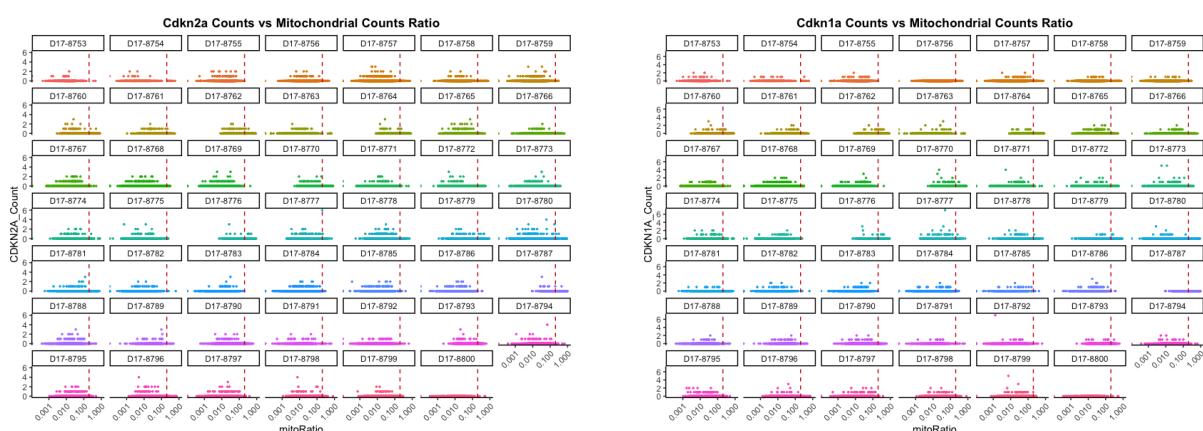
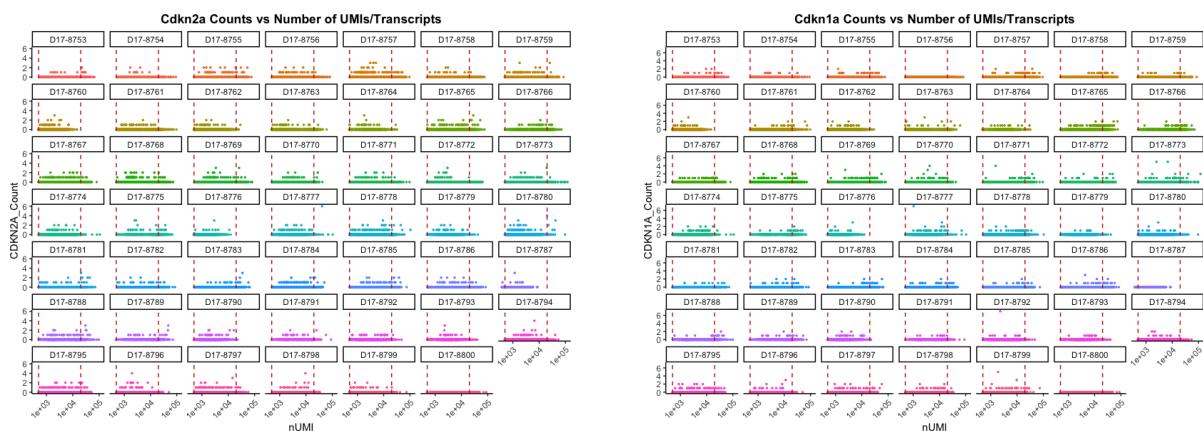
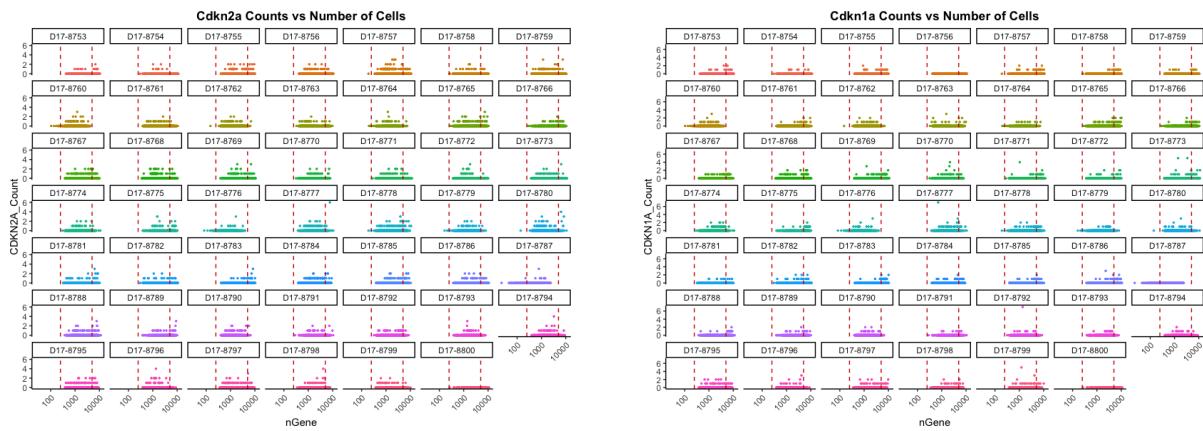
Quality control graphs used to identify filtering thresholds

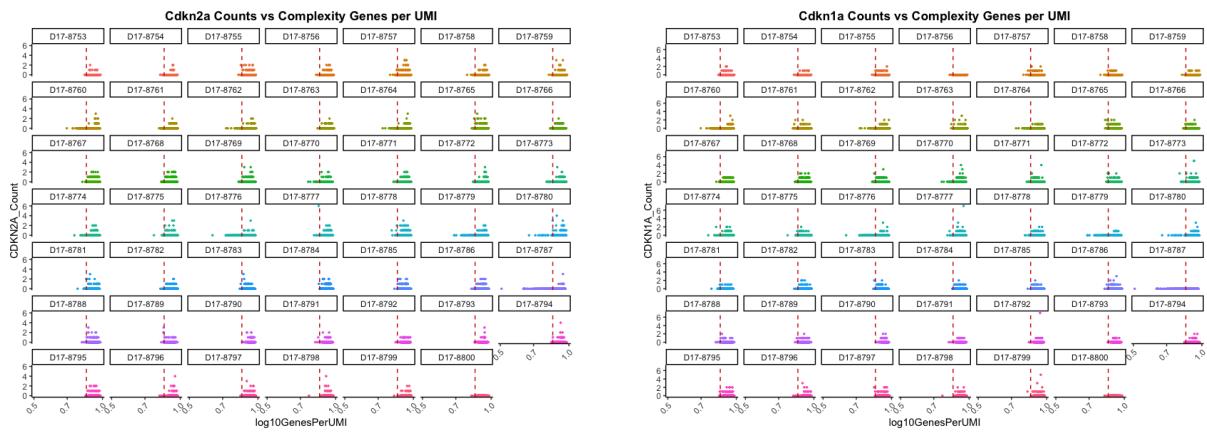






Senescence Cell not selectively removed

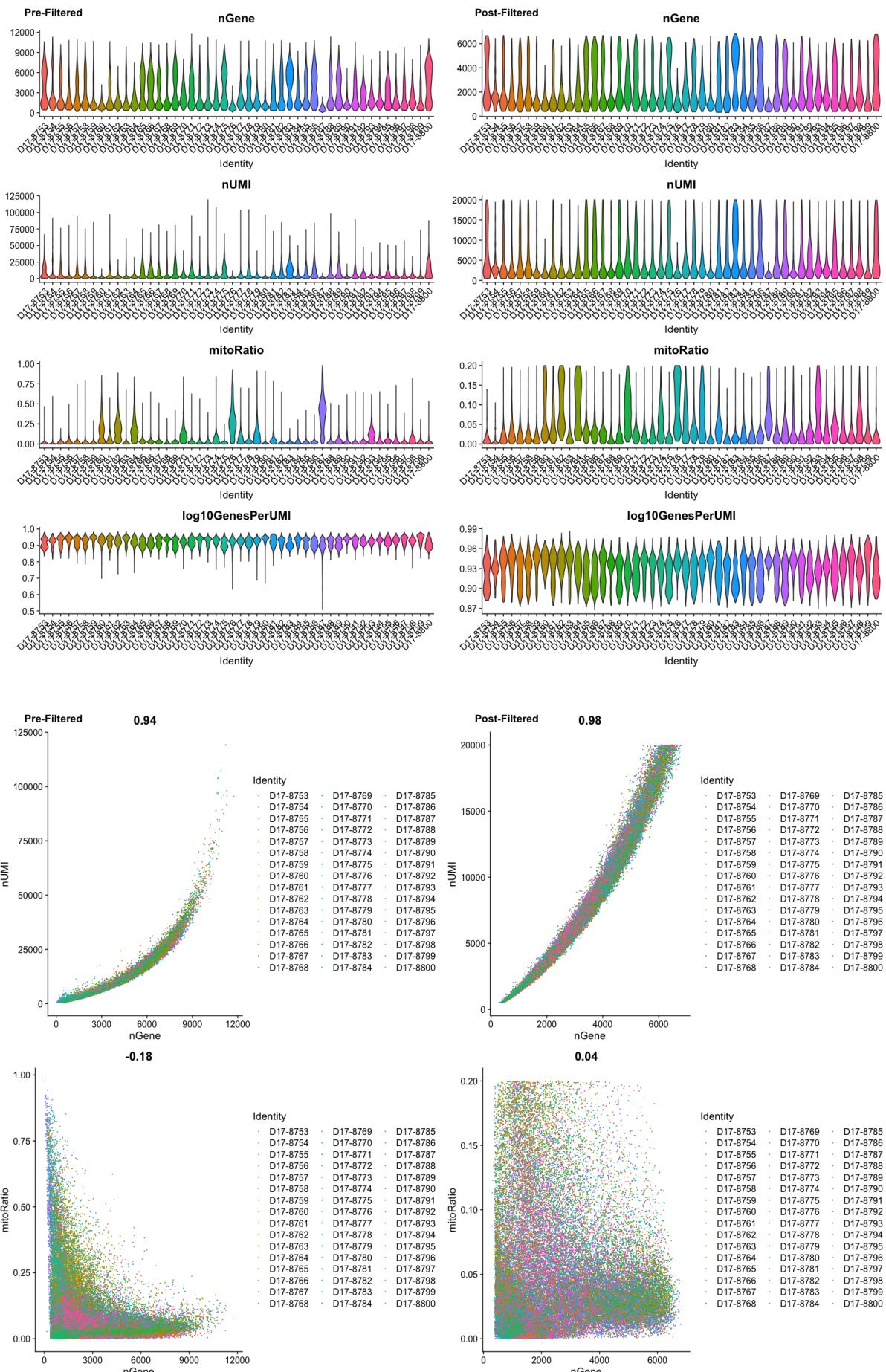




Seurat Filtering

Keep high quality cells without removing biologically relevant cells

- nUMI ≥ 500
- nUMI ≤ 20000
- nGene ≥ 250
- nGene ≤ 10000
- mitoRatio < 0.2
- $\log_{10}\text{GenesPerUMI} > 0.85$



Number of Filtered Cells		
sample	group	n
D17-8767	no_path	3,185
D17-8757	no_path	2,576
D17-8778	late_path	2,079
D17-8784	early_path	2,078
D17-8792	early_path	1,942
D17-8795	no_path	1,873
D17-8759	no_path	1,803
D17-8756	early_path	1,792
D17-8780	late_path	1,782
D17-8760	early_path	1,748
D17-8790	early_path	1,648
D17-8773	no_path	1,634
D17-8799	no_path	1,595
D17-8797	no_path	1,587
D17-8765	no_path	1,551
D17-8762	early_path	1,432
D17-8774	early_path	1,424
D17-8771	no_path	1,401
D17-8779	no_path	1,395
D17-8768	early_path	1,357
D17-8758	late_path	1,310
D17-8798	late_path	1,297
D17-8766	late_path	1,288
D17-8777	no_path	1,285
D17-8793	no_path	1,265
D17-8761	no_path	1,241
D17-8770	early_path	1,178
D17-8796	early_path	1,168
D17-8763	no_path	1,133
D17-8781	no_path	1,108
D17-8794	late_path	1,096
D17-8755	no_path	1,045
D17-8785	no_path	1,030
D17-8788	early_path	992
D17-8772	late_path	988
D17-8769	no_path	965
D17-8786	early_path	809
D17-8791	no_path	750
D17-8783	no_path	737
D17-8789	no_path	686
D17-8782	early_path	610
D17-8775	no_path	593
D17-8764	early_path	566
D17-8776	late_path	482
D17-8753	no_path	372
D17-8787	no_path	249
D17-8800	early_path	246
D17-8754	late_path	217

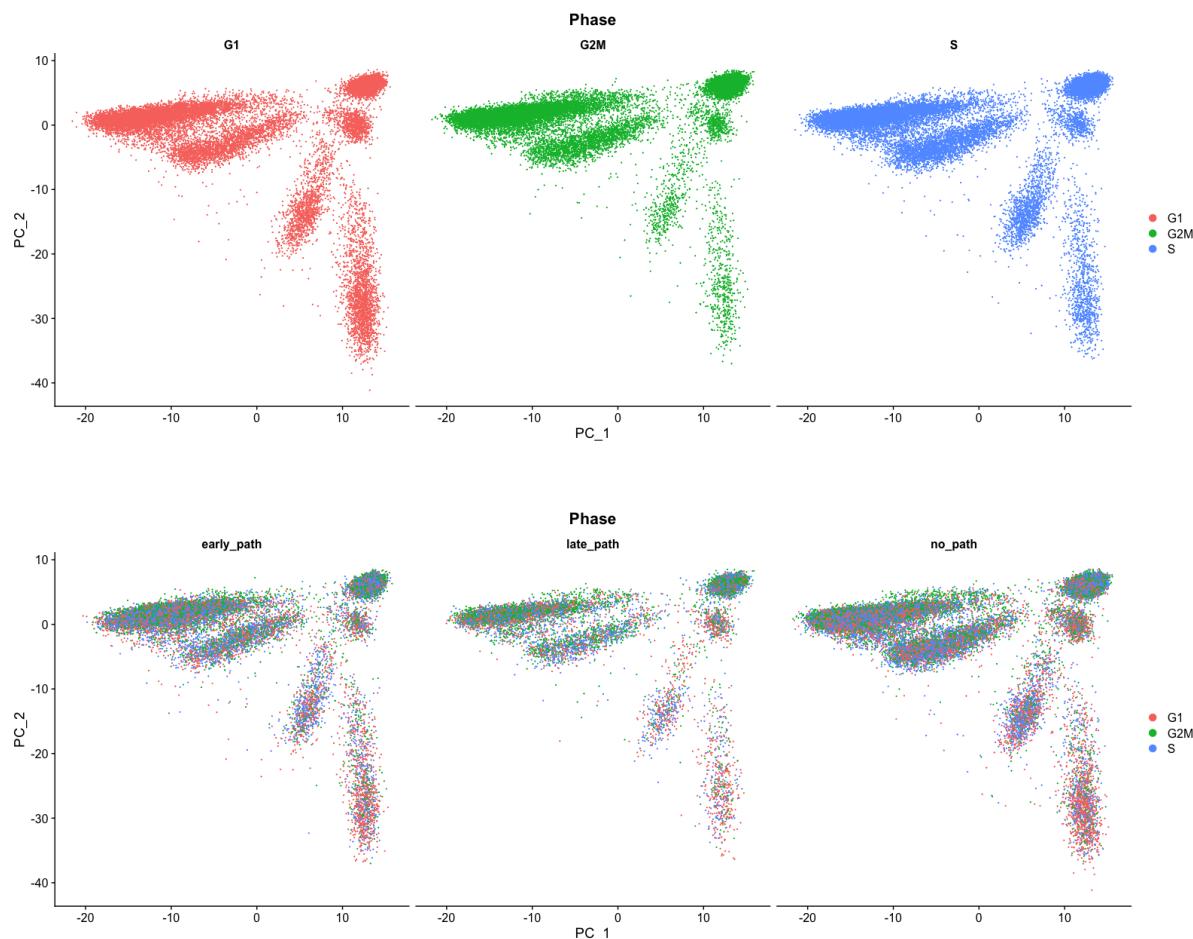
Number of Filtered Cells

group	n
no_path	31,059
early_path	18,990
late_path	10,539

Seurat Cell Cycle Scoring

Determine if cell cycle regression is required

No obvious difference in cell cycle clustering



Number of Cell Cycle Phase Cells

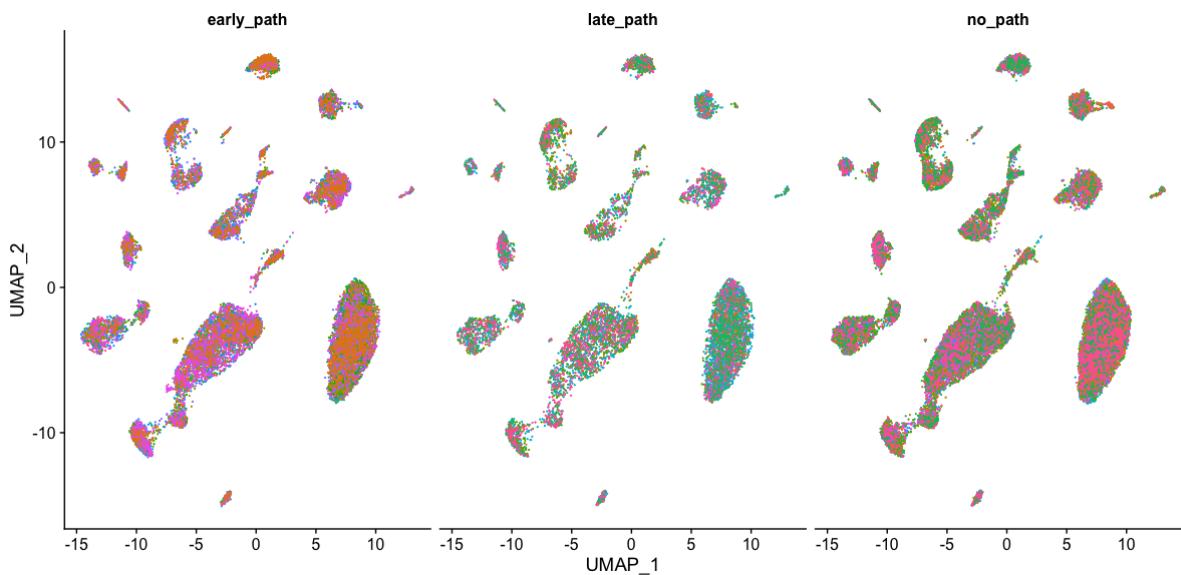
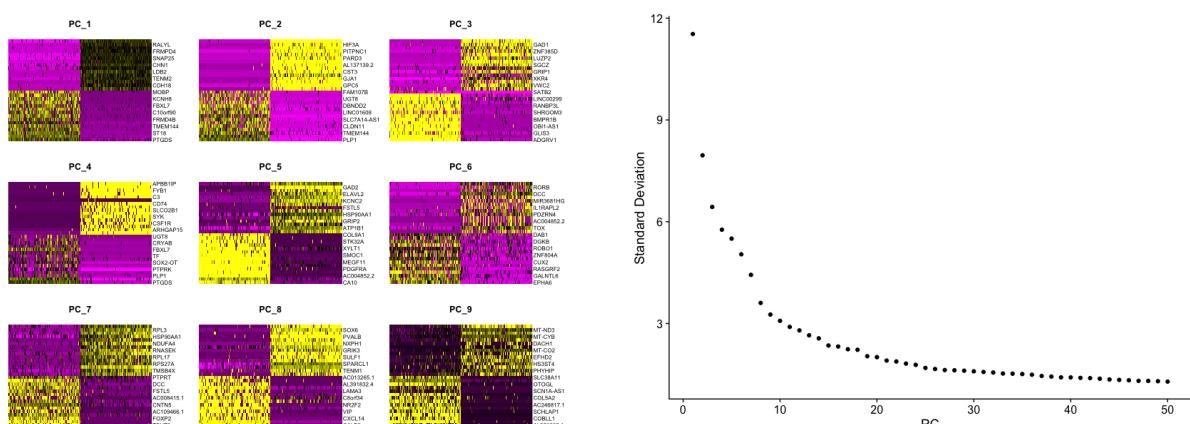
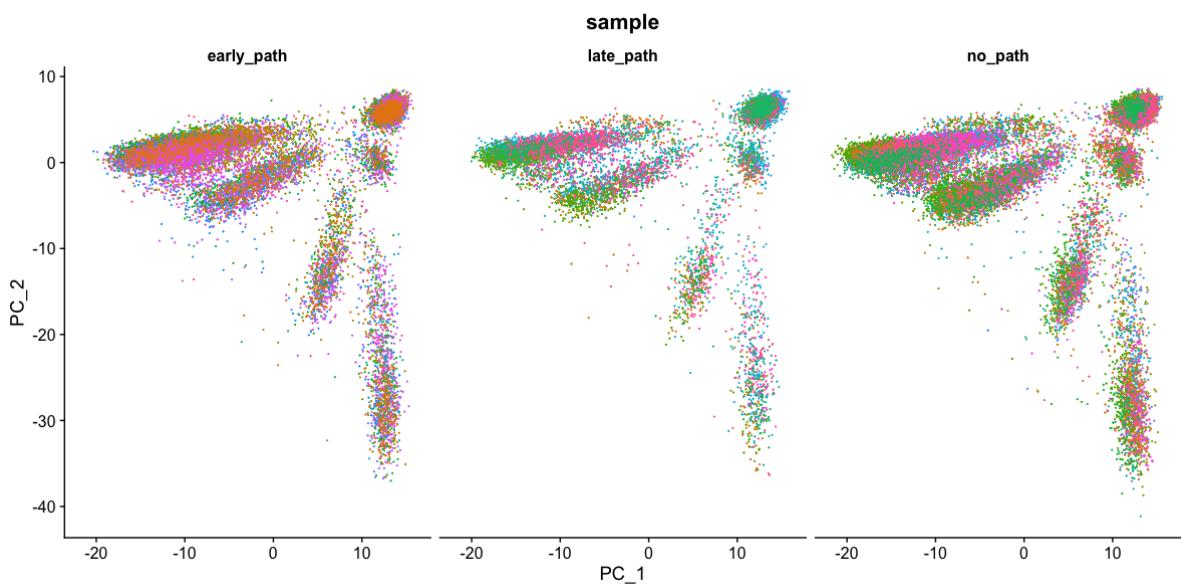
group	Phase	n
early_path	G1	5,747
early_path	G2M	6,277
early_path	S	6,966
late_path	G1	2,989
late_path	G2M	3,967
late_path	S	3,583
no_path	G1	9,129
no_path	G2M	10,215
no_path	S	11,715



Seurat Batch Correction

SCTransform batch correction ran out of memory

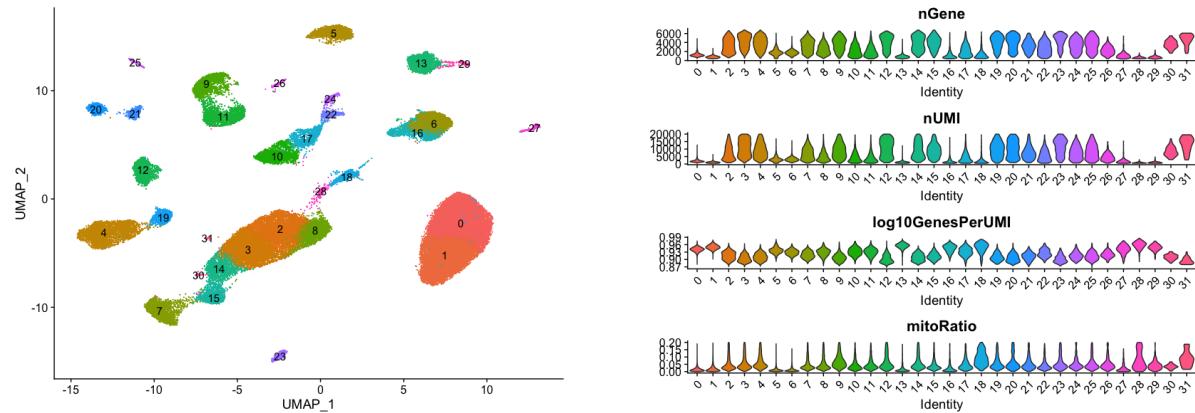
Harmony for batch integration was used



Seurat Clustering_Cells

Seurat uses a graph-based clustering approach, which embeds cells in a graph structure, using a K-nearest neighbor (KNN) graph

Resolution 1.2



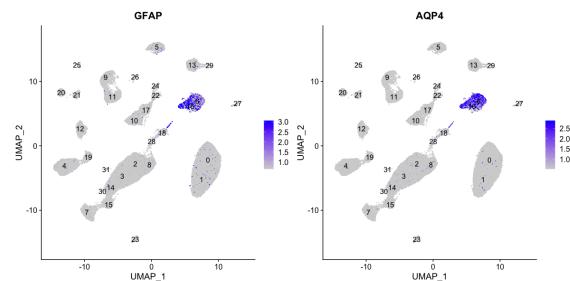
Seurat Marker_Identification

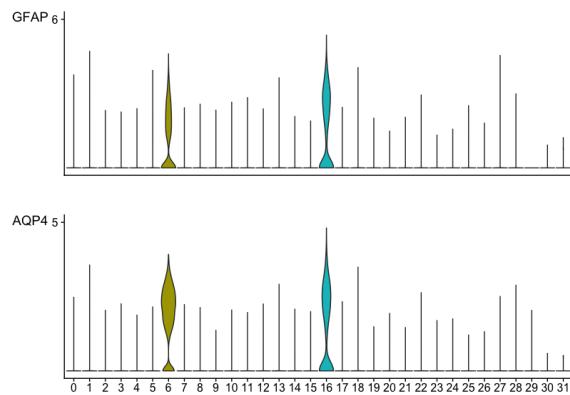
Used databases for cellmarkers aswell as original paper

- <http://bio-bigdata.hrbmu.edu.cn/CellMarker/index.jsp>
- <https://panglaodb.se/index.html>

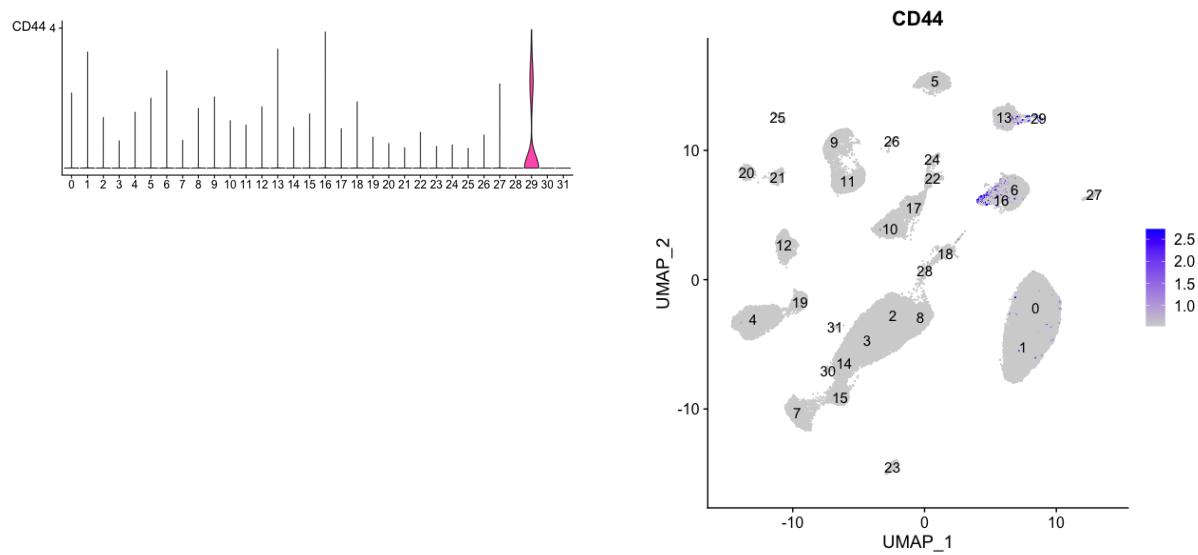
Could not find any immune cells, may be due to low cell numbers or nuclei isolation vs cell isolation

Astrocyte_Cells

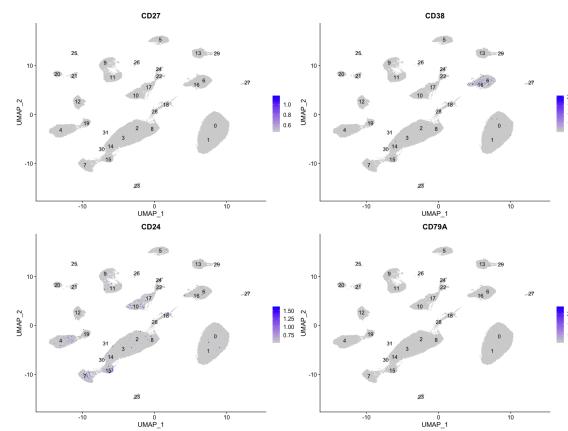
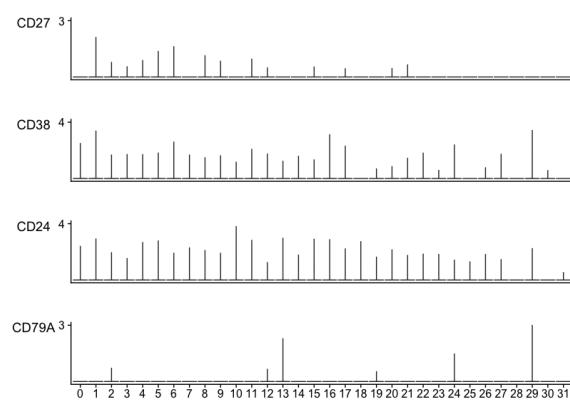




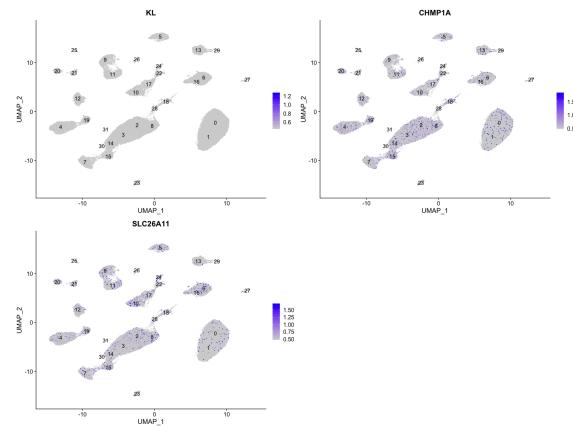
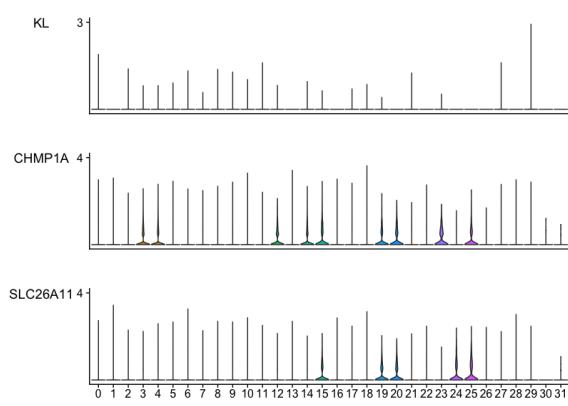
Astrocyte_Restricted_Precursors_Cells



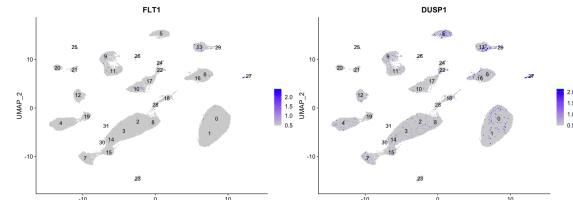
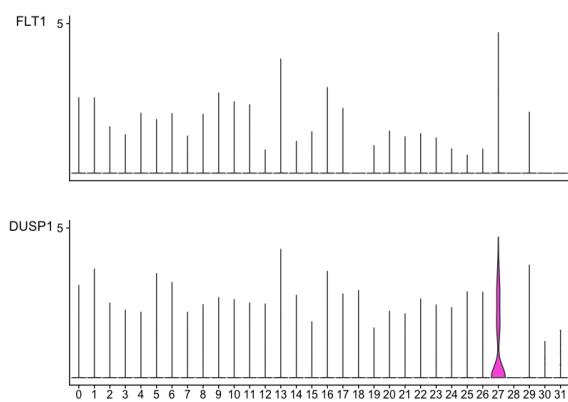
B_Cells



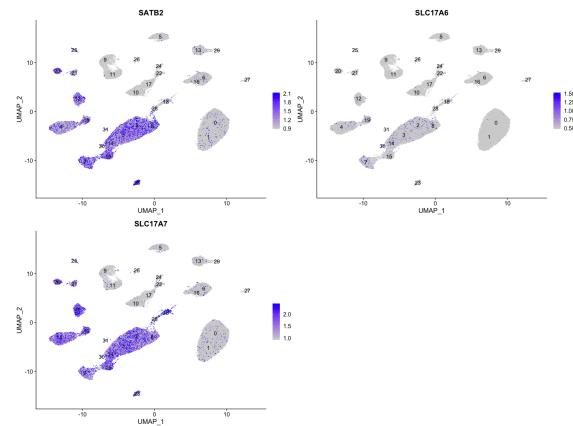
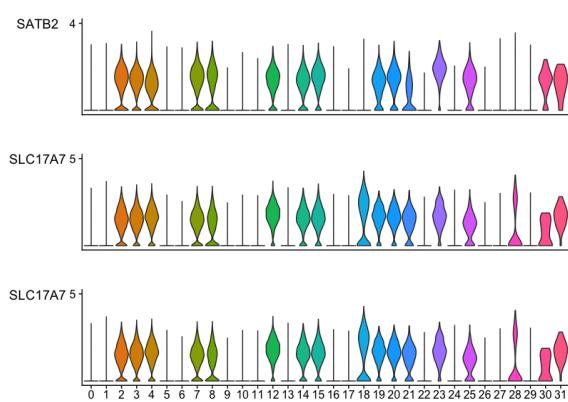
Choroid_Plexus_Cells



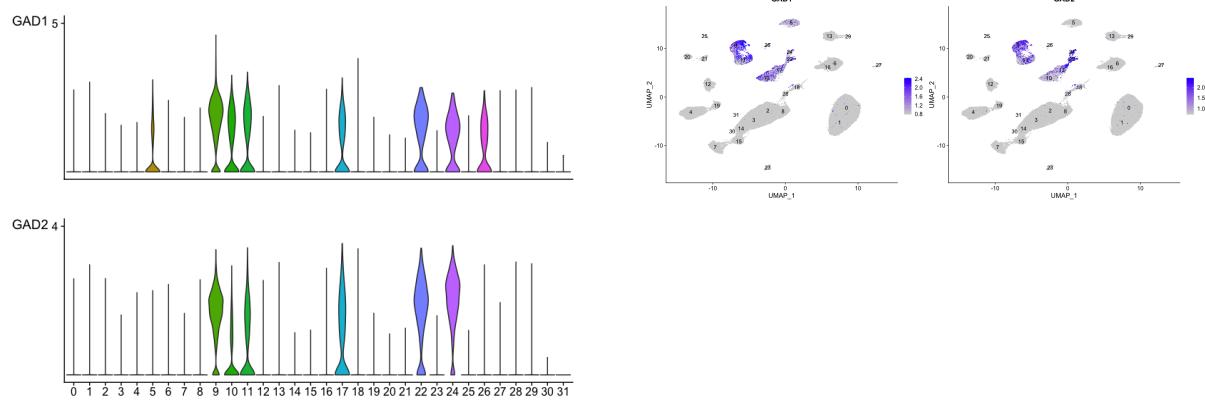
Endothelial_Cells



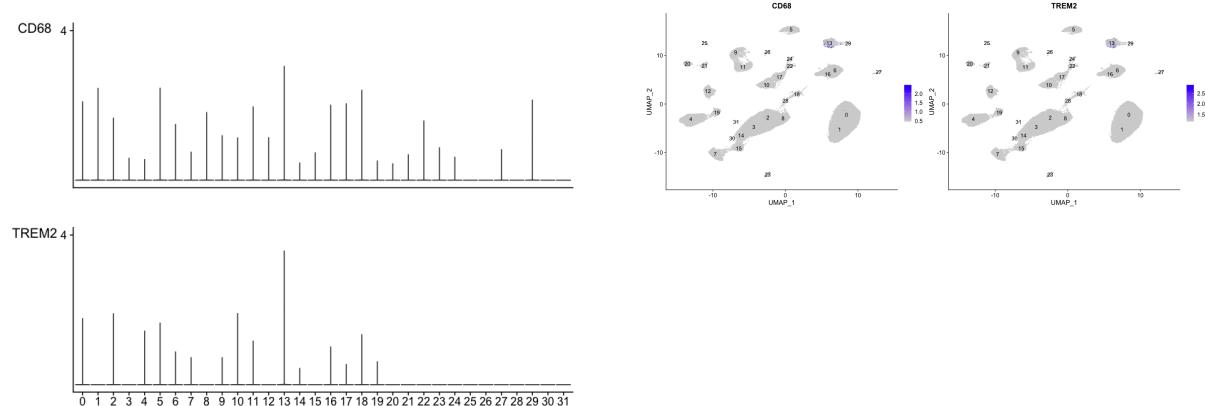
Excitatory_Neurons



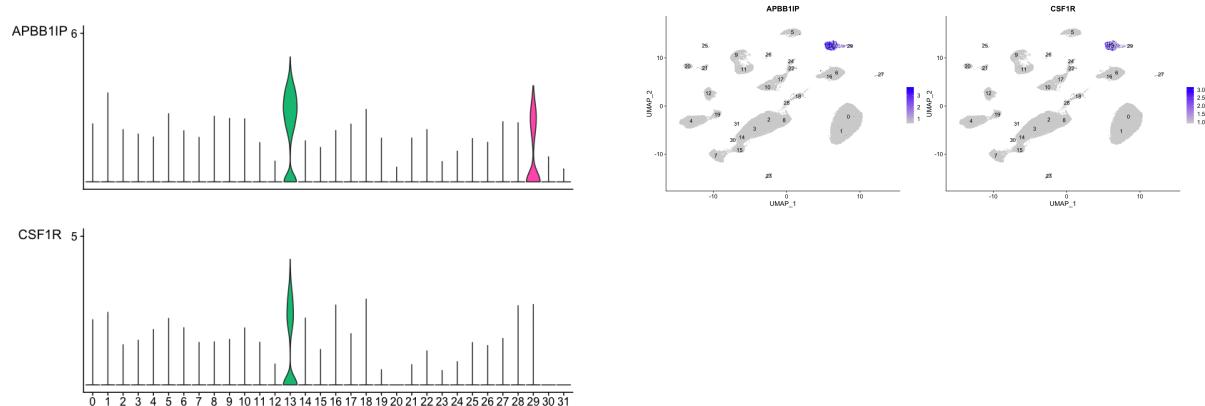
Inhibitory_Neurons



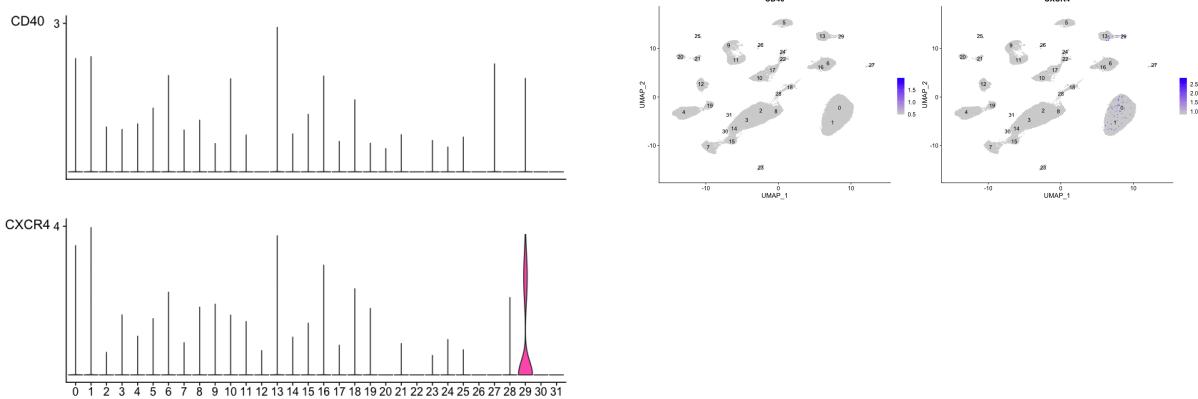
Macrophage_Cells



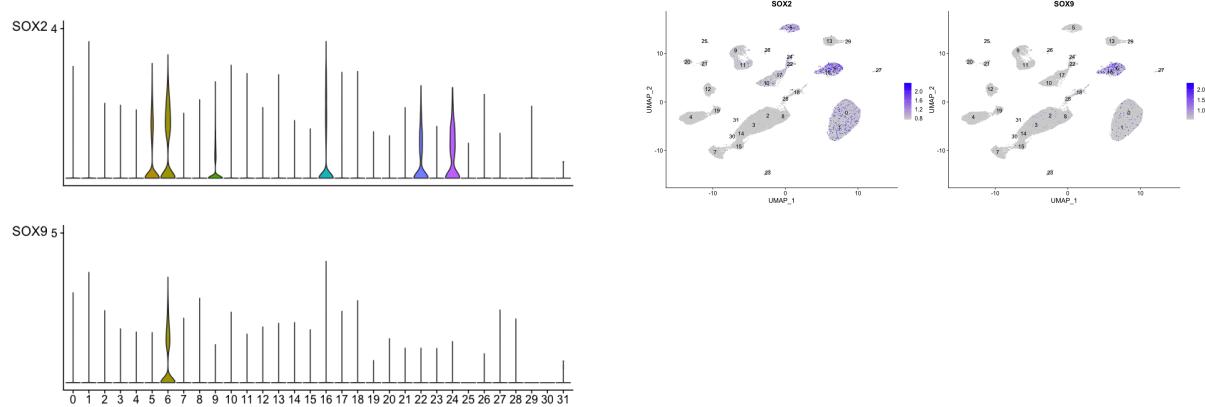
Microglial_Cells



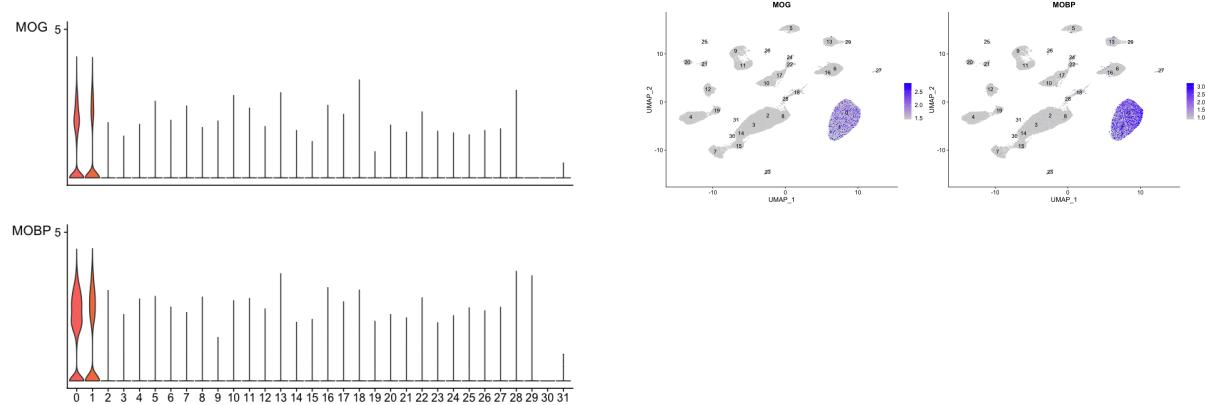
Monocyte_Cells



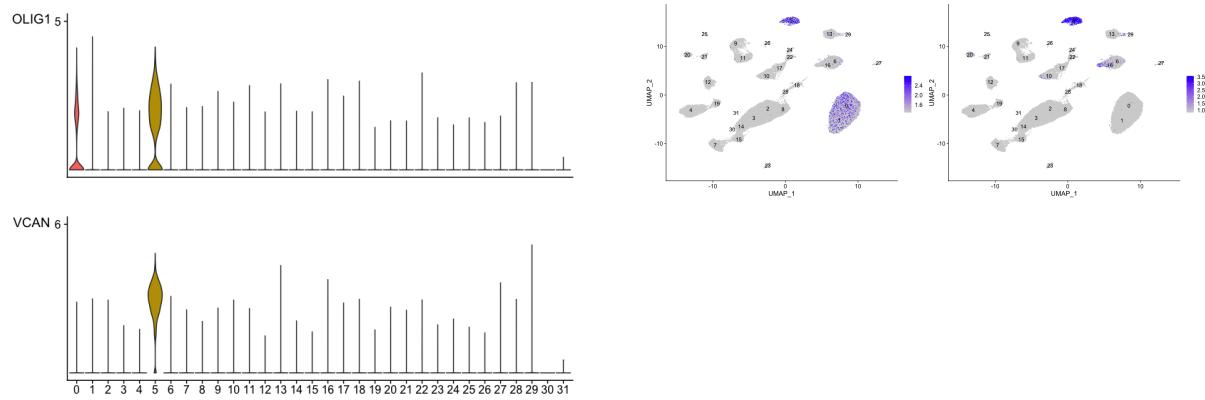
Neural_Stem_Cells



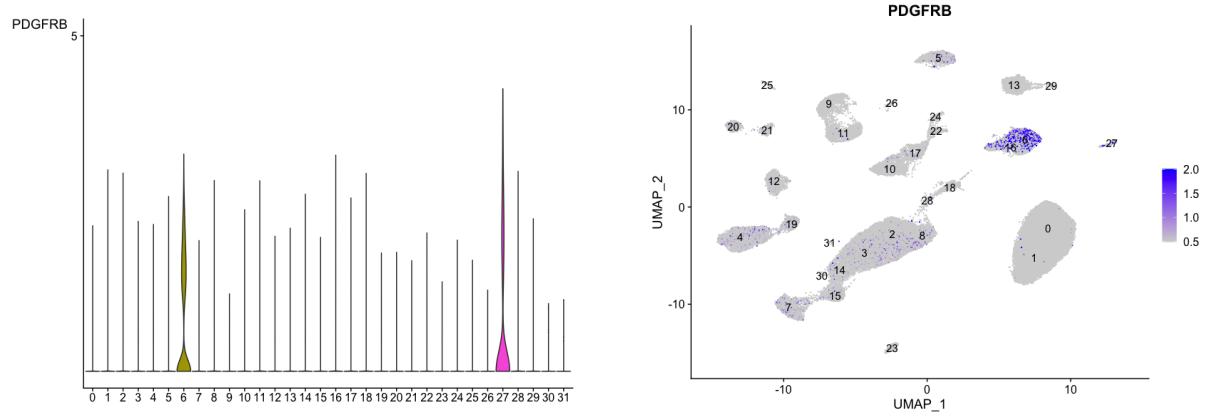
Oligodendrocyte_Cells



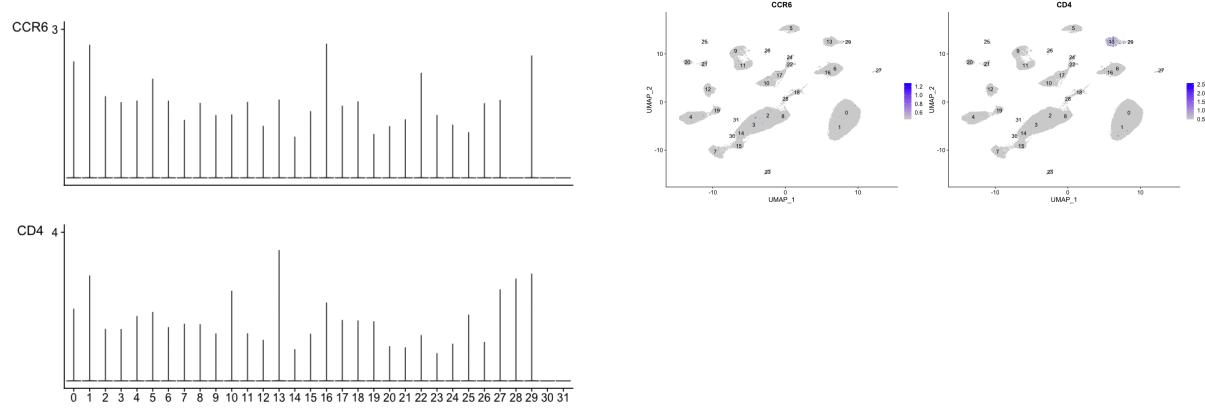
Oligodendrocyte_Precursor_Cells



Pericyte_Cells



T_Cells



Seurat Cluster_Annotation

Manually annotate cells

