

CCBR1045

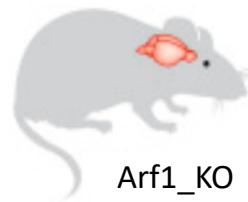
Identification of a unique microglia signature associated with *Arf1*-knockout neurodegenerative mice

2020.4.21

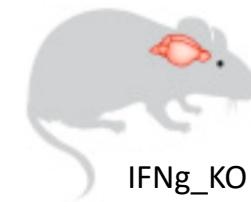
Da, Nathan, Guohao, Steve



WT



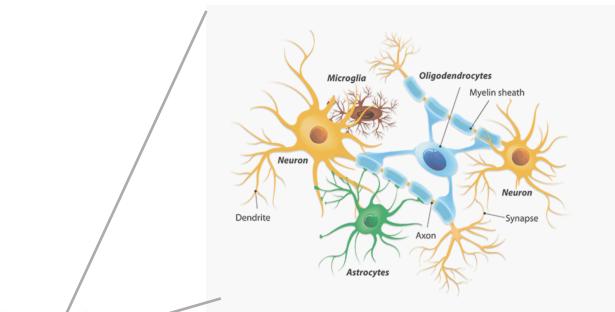
Arf1_KO

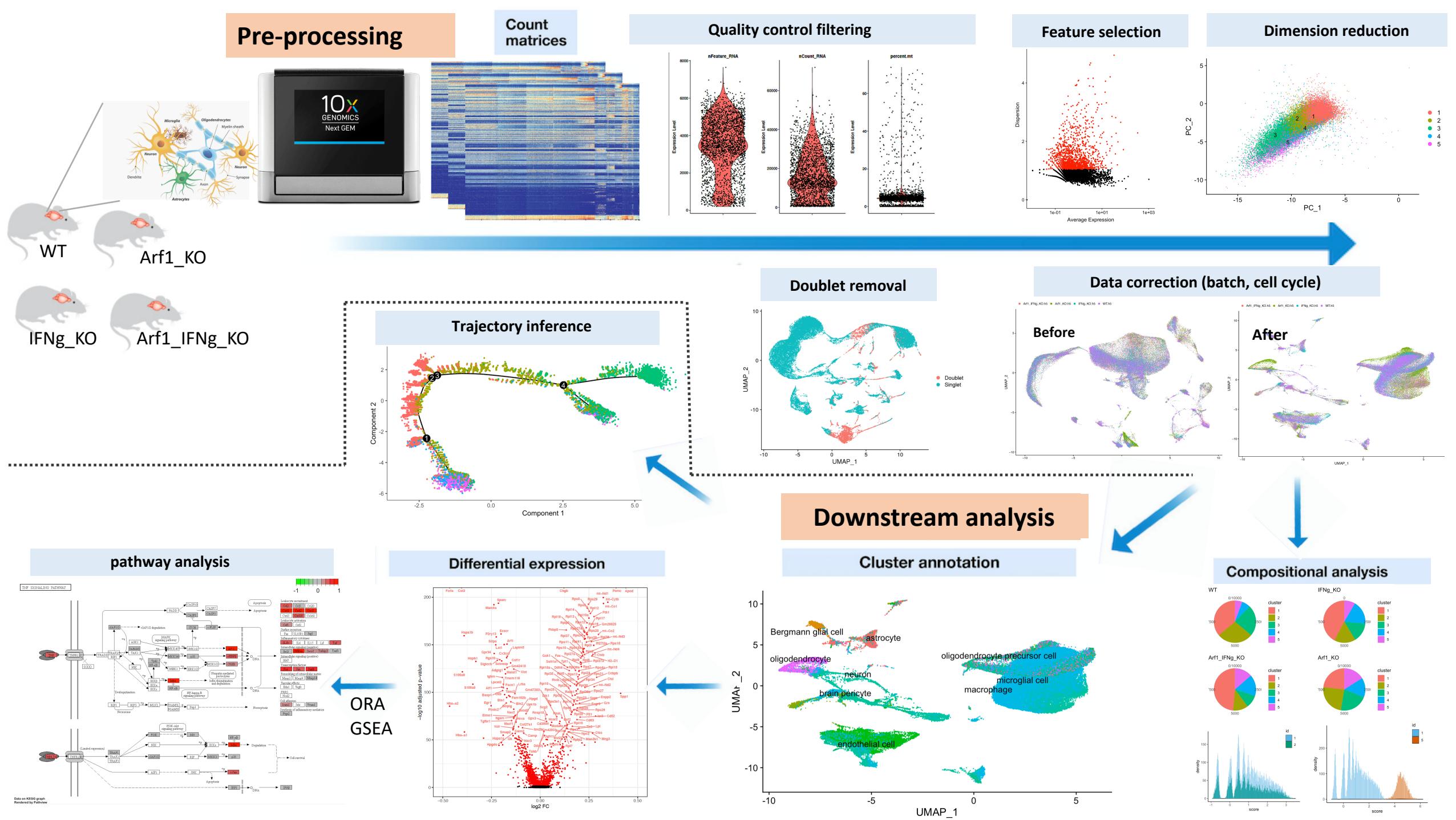


IFNg_KO

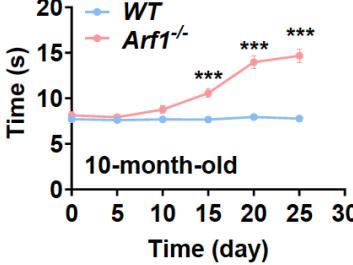
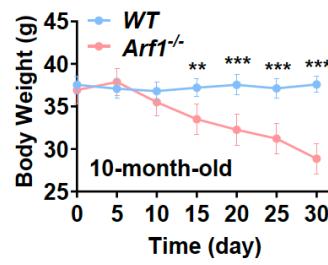
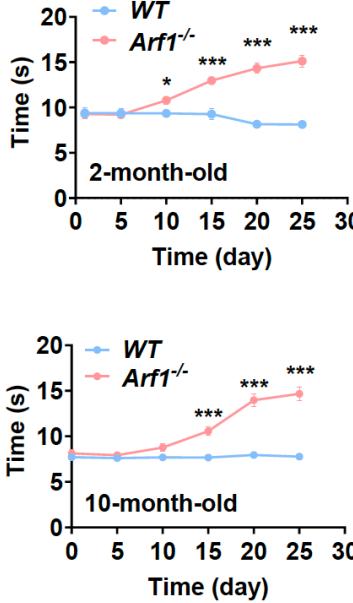
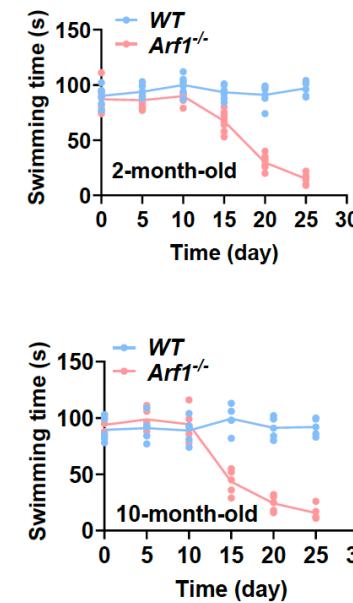
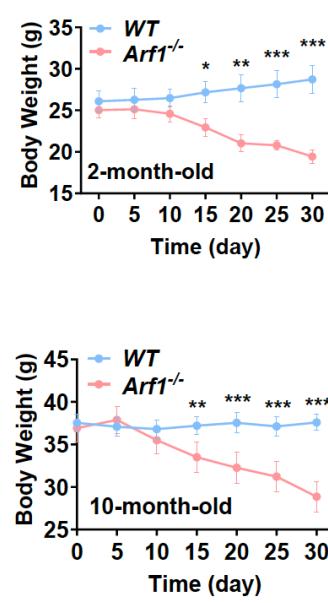
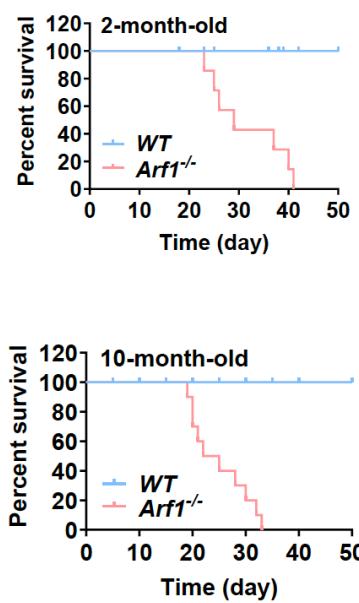
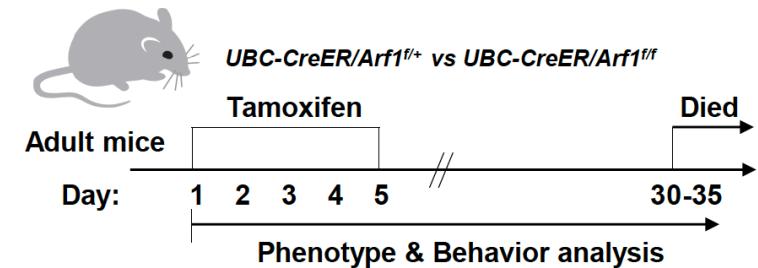


Arf1_IFNg_KO





Arf1 ablation induces neurodegenerative behaviors in adult mice



Arf1 knock-down, ubiquitous | disease



Arf1 knock-down, neuron | disease



Arf1 and IFNg knock-down, ubiquitous | normal



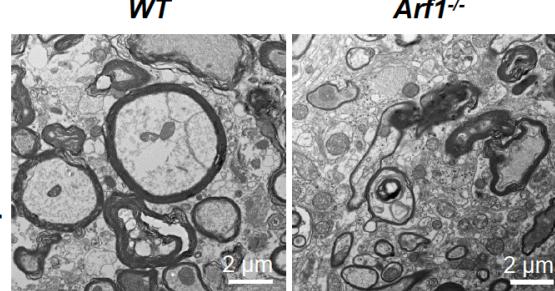
Dr. Guohao Wang (NIH)

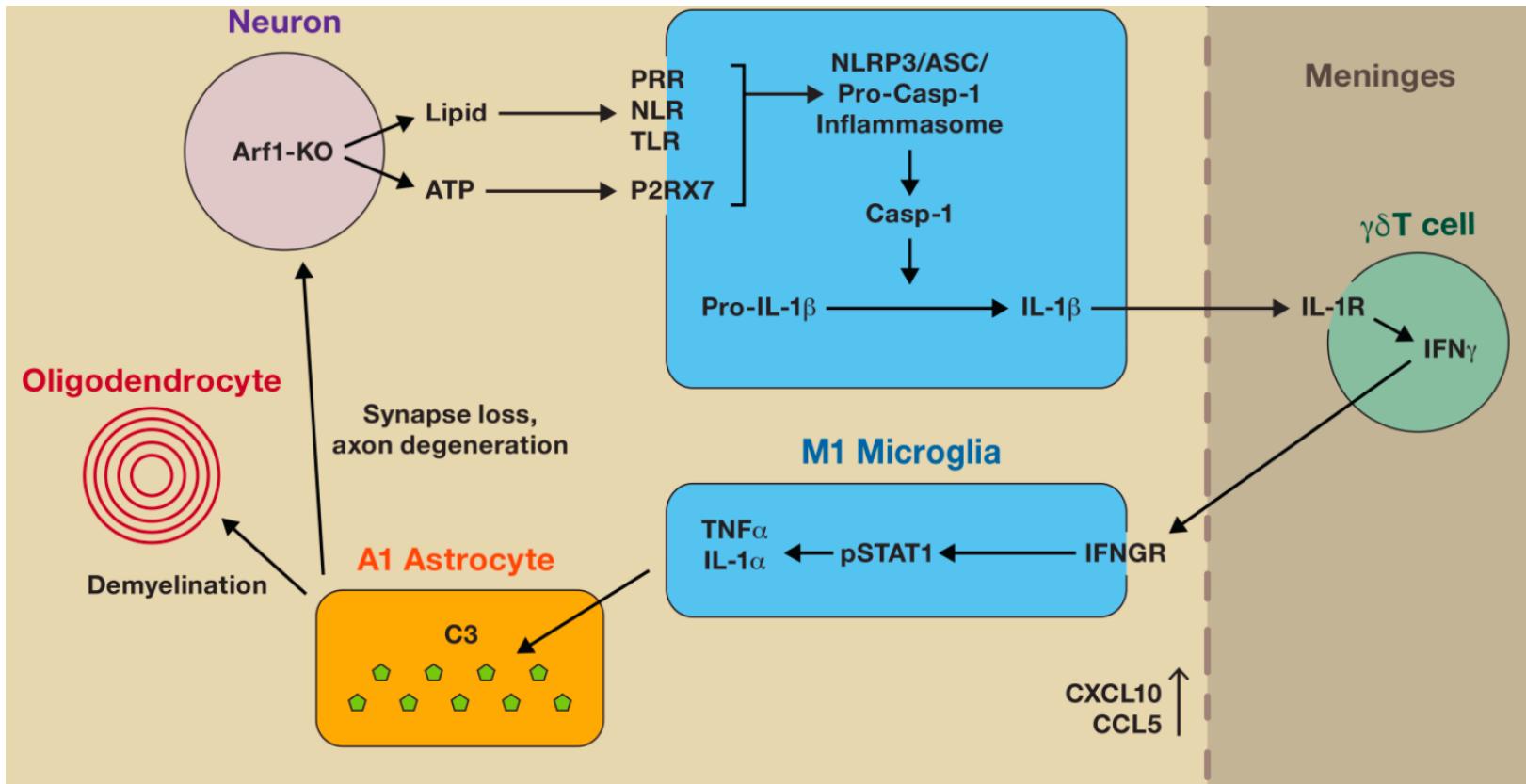
What do we know about Arf1

- *Arf1* is one of the most evolutionarily conserved genes between *Drosophila* and mouse, with an amino acid identity of 95.6% between the two species
- The ARF1 protein is localized to the Golgi apparatus and has a central role in intra-Golgi transport.
- Arf1-mediated lipid metabolism sustains cancer cells and its ablation induces anti-tumor immune responses in mice (Wang et al 2020)
- Arf1 regulates the initiation of myelination in the peripheral nervous system (Miyamoto et al 2018)

New findings (Guohao Wang)

- Arf1 ablation induces neurodegenerative behaviors in adult mice
- Arf1 deficiency promotes demyelination, axon degeneration, synapse loss
- The slow traveling in the balance beam tests, poor neurological score, synapse loss, axon demyelination, and axon degeneration associated with Arf1-ablated mice were almost completely suppressed in IFN γ -deficient mice

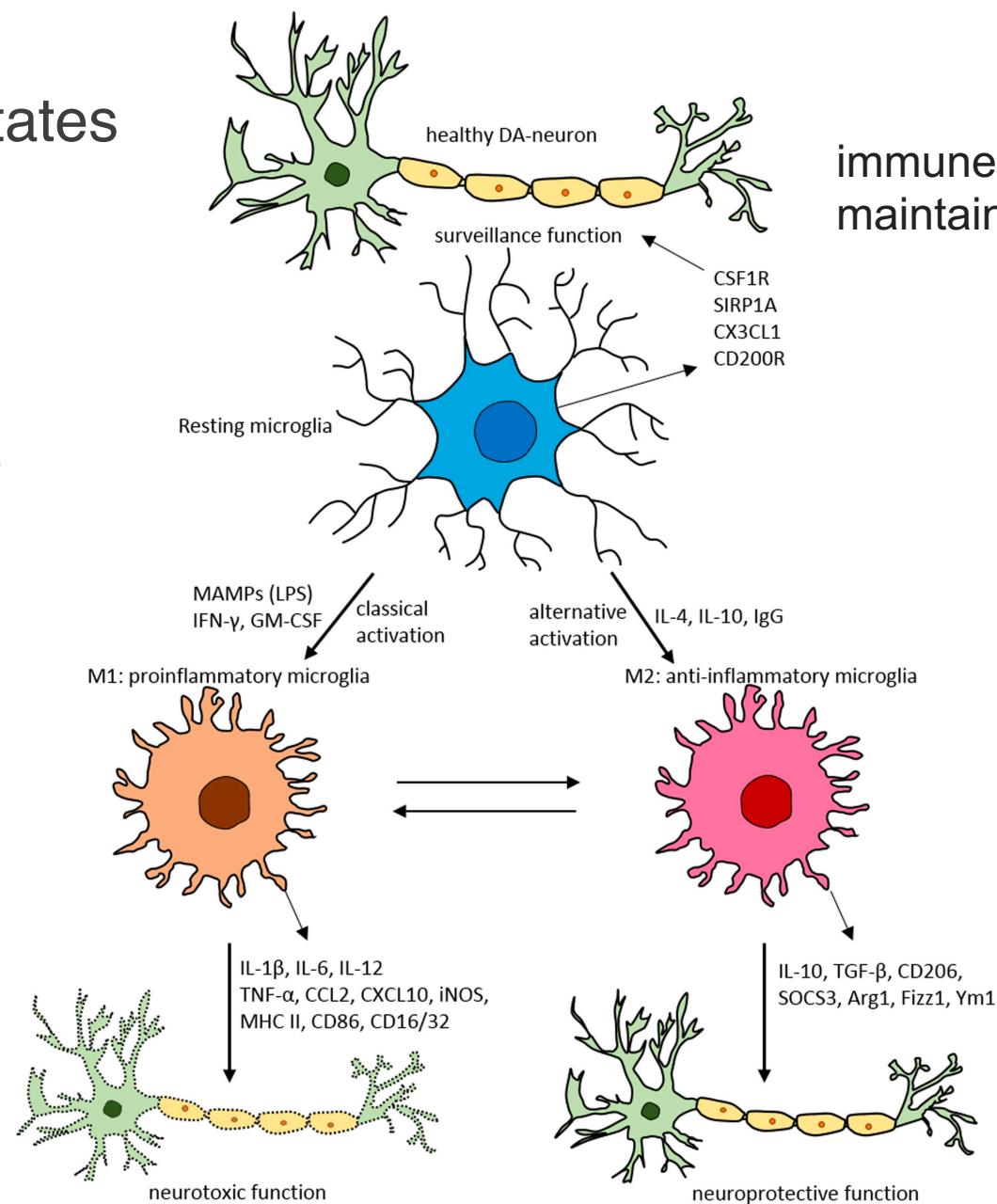




1. Knocking down Arf1 in neurons increases ROS and promotes the release of ATP, chemokines, and peroxidized lipids;
2. ATP and peroxidized lipids activate the inflammasome to produce IL-1 β in microglia;
3. Chemokines may help to recruit $\gamma\delta$ T cells to meninges; and the IL-1 β activates $\gamma\delta$ T cells to produce IFN γ , which enters parenchyma to activate a microglia–A1 astrocyte–C3 cascade;
4. Damage to neurons and oligodendrocytes, leading to neurodegeneration.

microglial polarization states

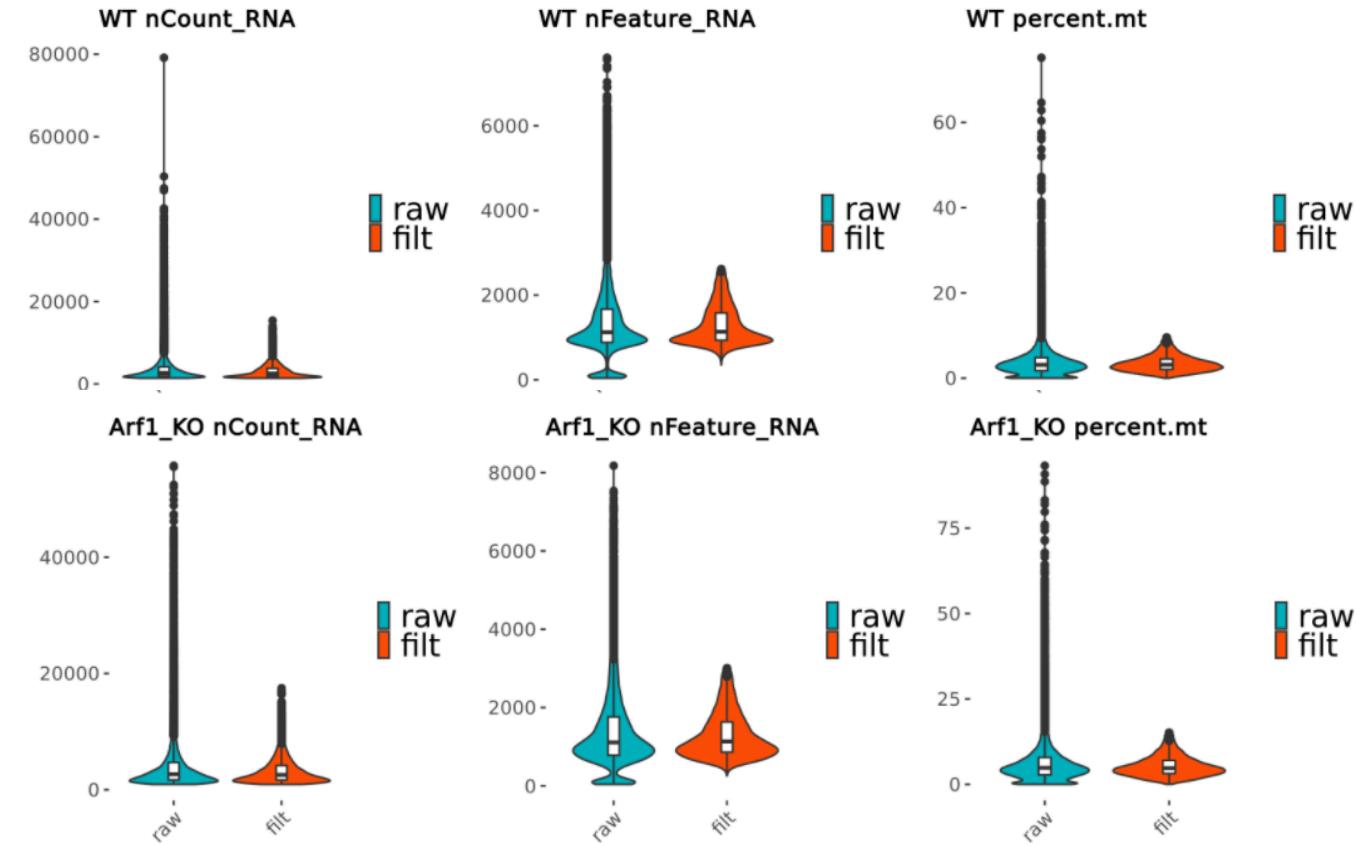
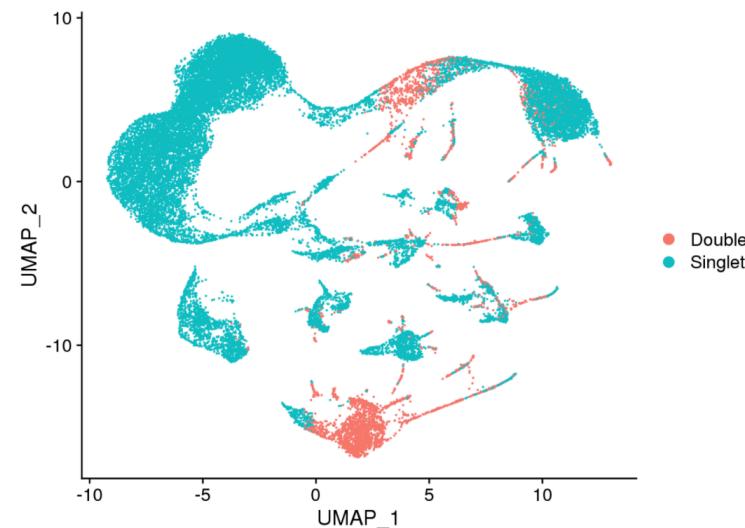
- Resting microglia state
- M1 pro-inflammatory state
- M2 anti-inflammatory state



immune response and maintaining homeostasis

pre-processing workflow

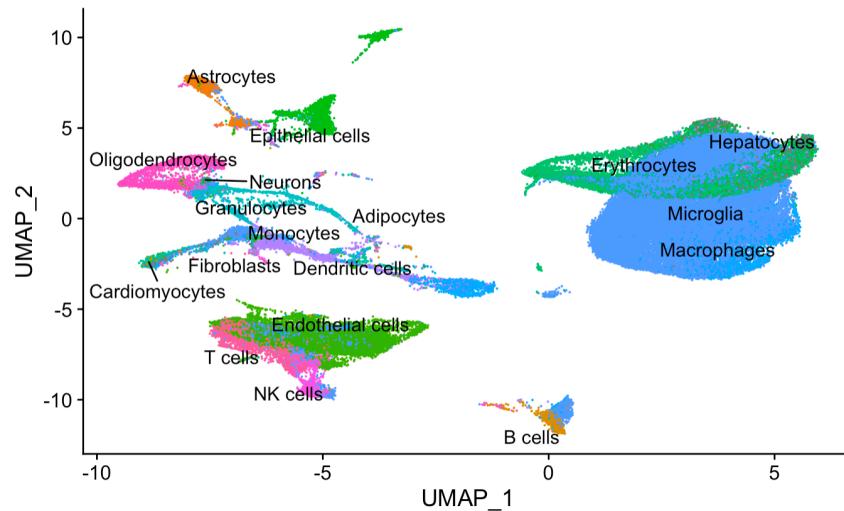
- Filtering out cells with nCount, nFeature, pct.mitochondria: mean absolute deviation (MAD) above 3
- Dimension reduction (PCA, URD), Clustering (Smart Local Moving) with resolution 0.2-1.2,
- Doublet removal(doubletFinder_v3), cell identity annotation (singleR)
- Integrate batches (four samples)



Sample	Estimated Number of Cells	Cells after filtering
WT	30,710	24,531
Arf1_KO	28,652	22,036
IFNg_KO	27,930	23,166
Arf1_IFNg_KO	28,695	23,484

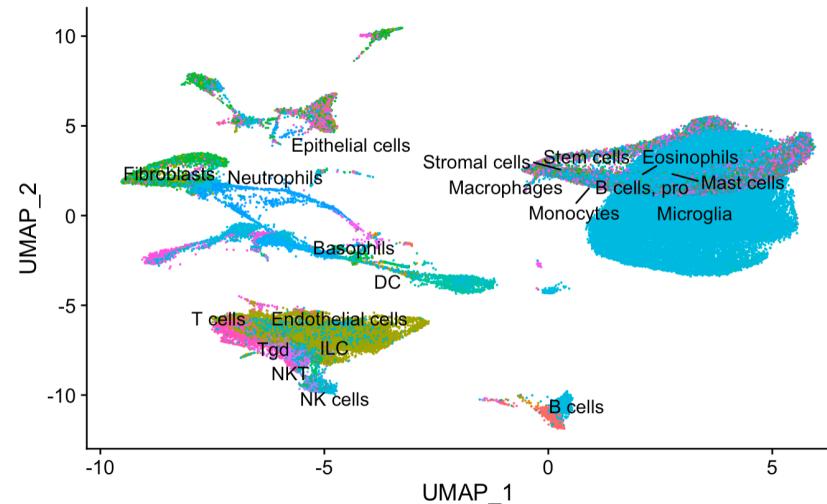
Comparison of cell identity annotation by three databases

mouseRNAseq_main



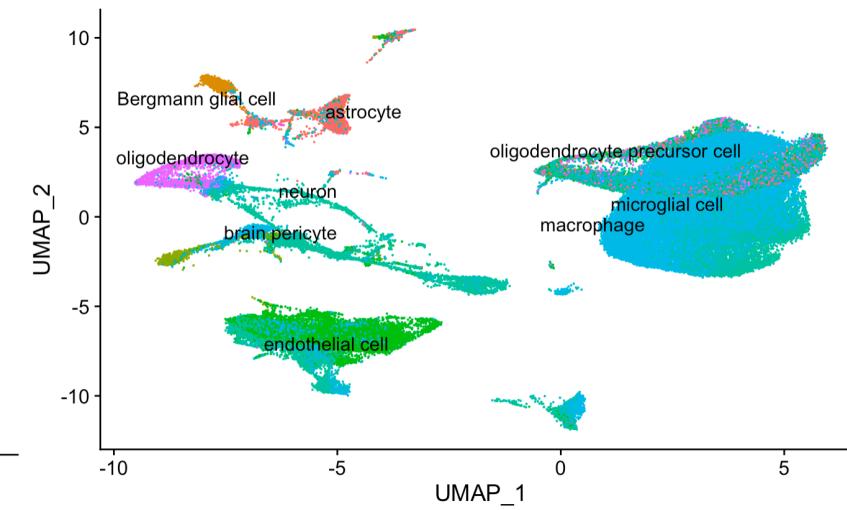
Microglia: 48,662

Immgen_main



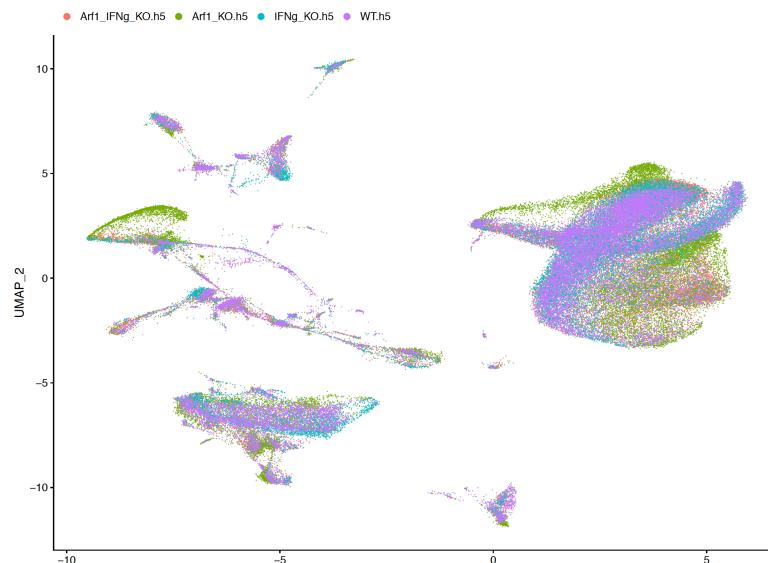
Microglia: 53,078

Tabulus Muris (mouse brain)

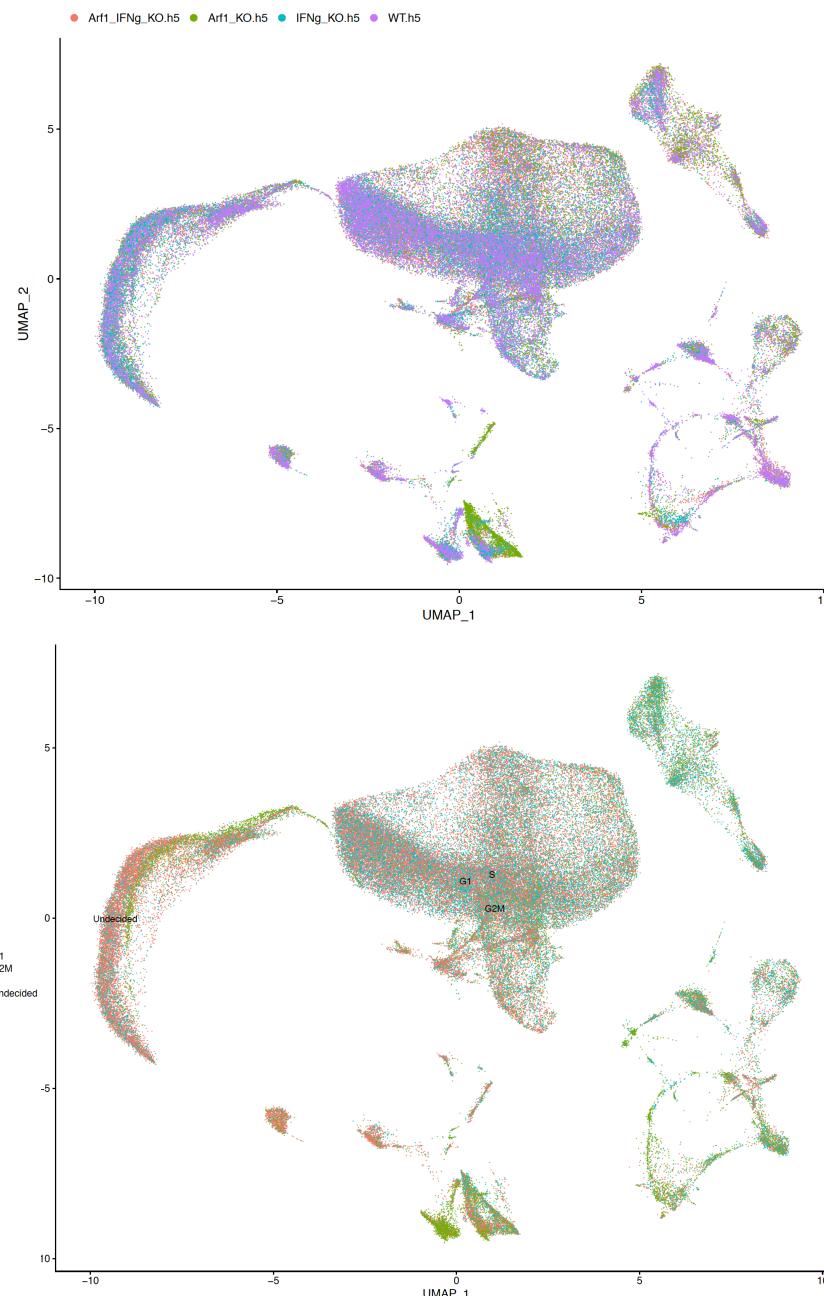


Microglial cell: 48,789

Before batch correction



Batch-corrected



Color by sample

Plotting all samples overlaid, colored by sample. Check to see if clustering behavior is tied to sample.

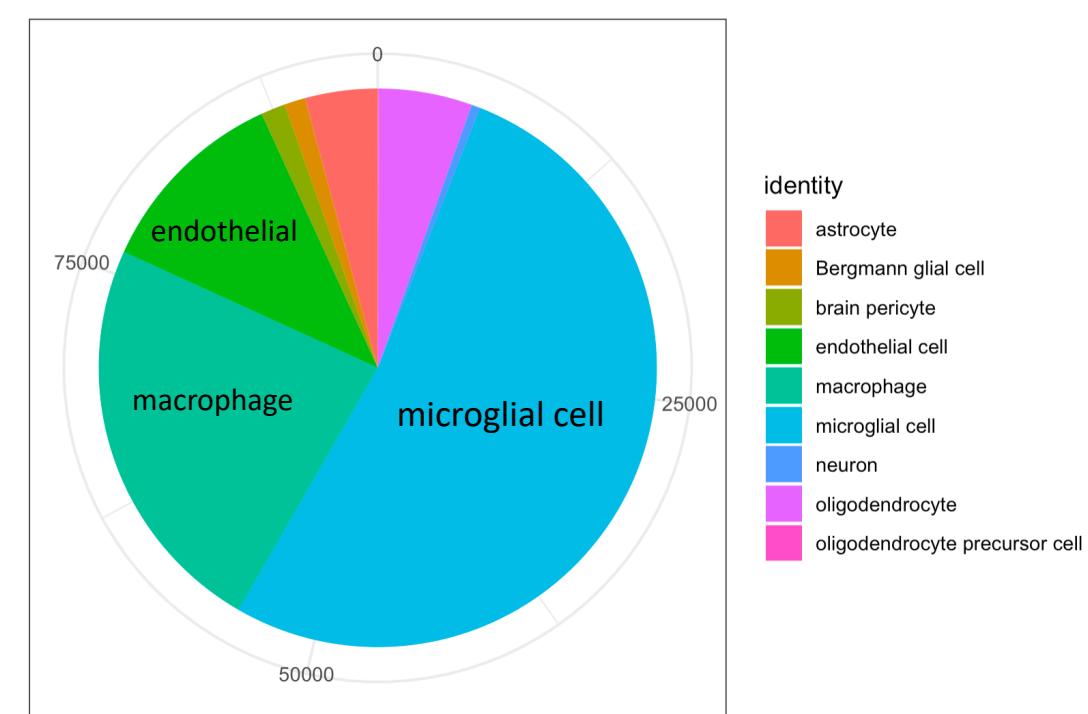
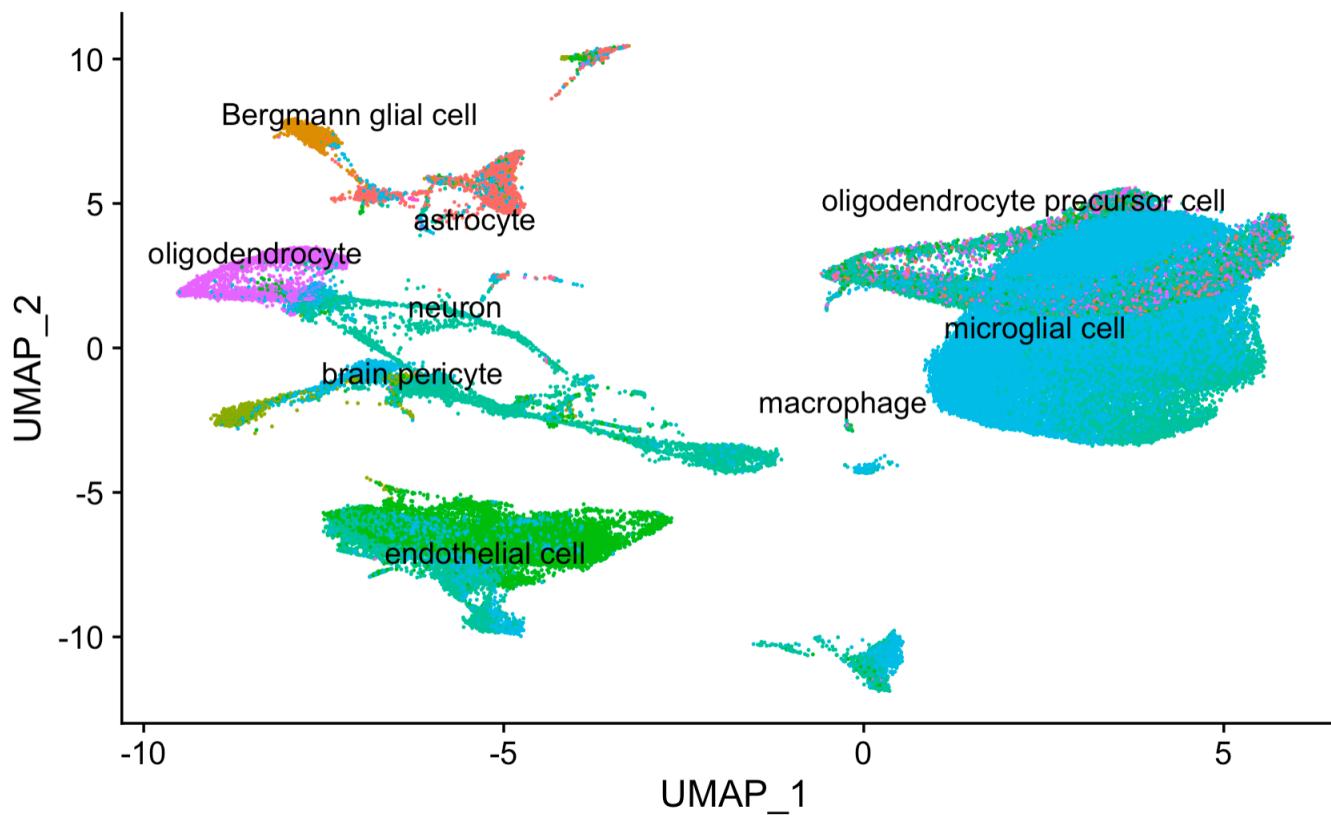
It looks like the four samples aren't separated before or after, which is expected.

Color by cell cycle

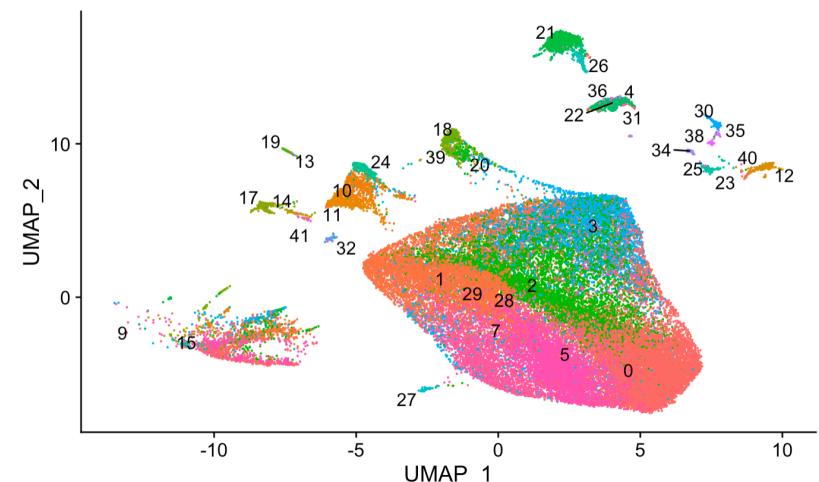
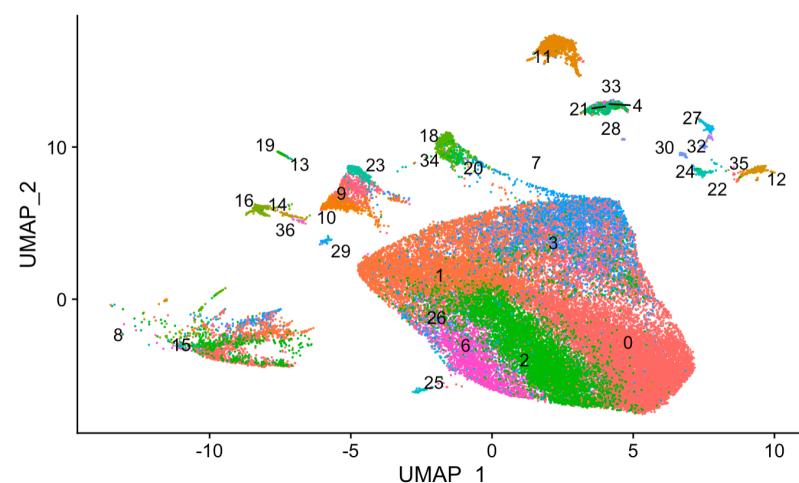
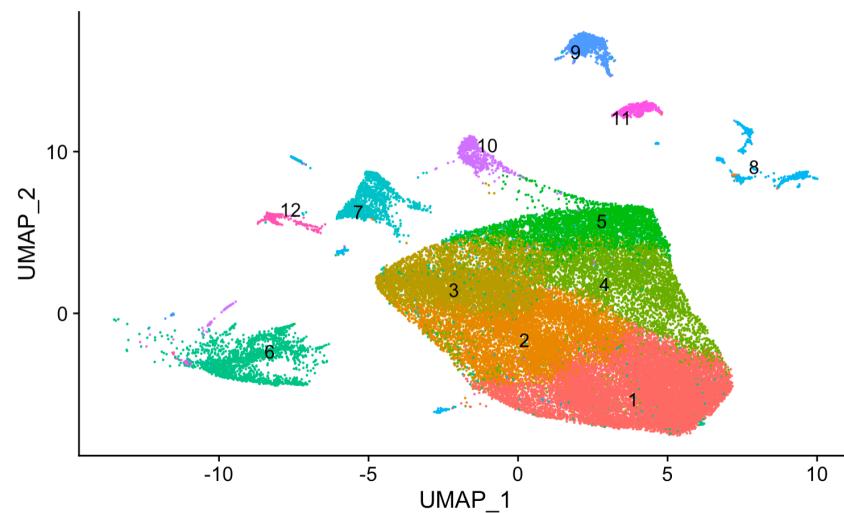
Plotting all samples overlaid, colored by phase. Check to see if clustering behavior is tied to cell cycle.

It looks like the microglial cluster(main cluster) isn't tied to cell cycle, good.

Cell identity annotation by Tabula Muris – over half of the cells are microglia

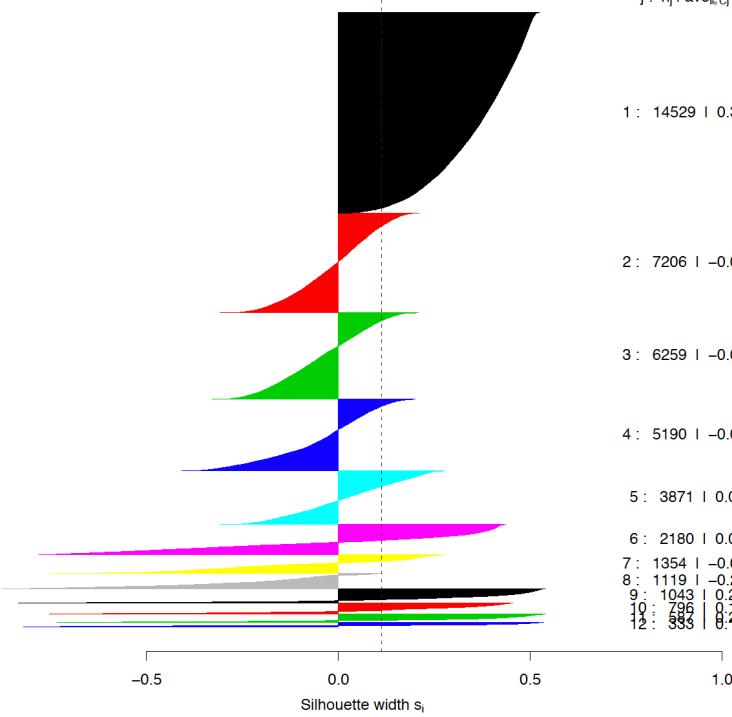


Only microglia cells – reshaped with 0.3, 0.6, 0.8 resolution into microglia subclusters

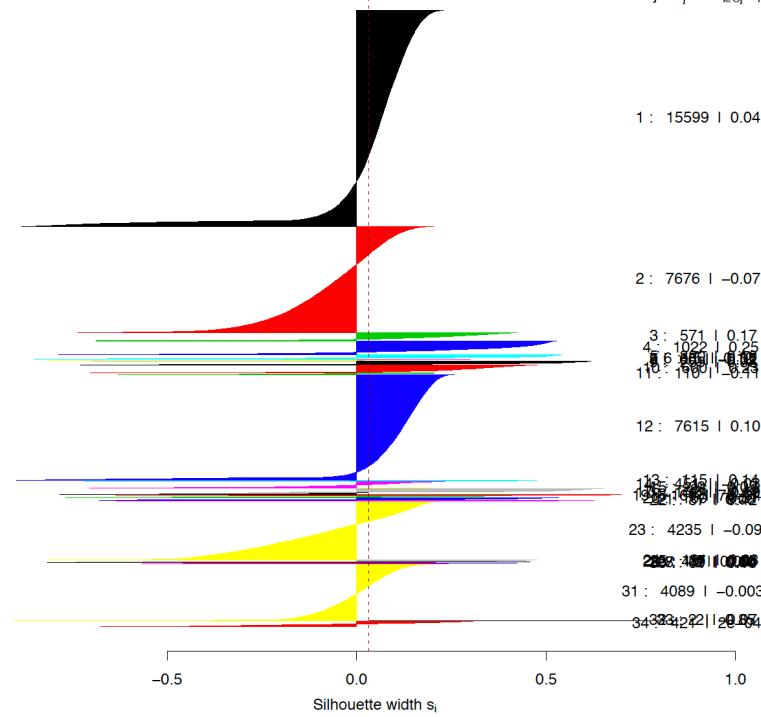


Assessing cluster separation with Silhouette Plot

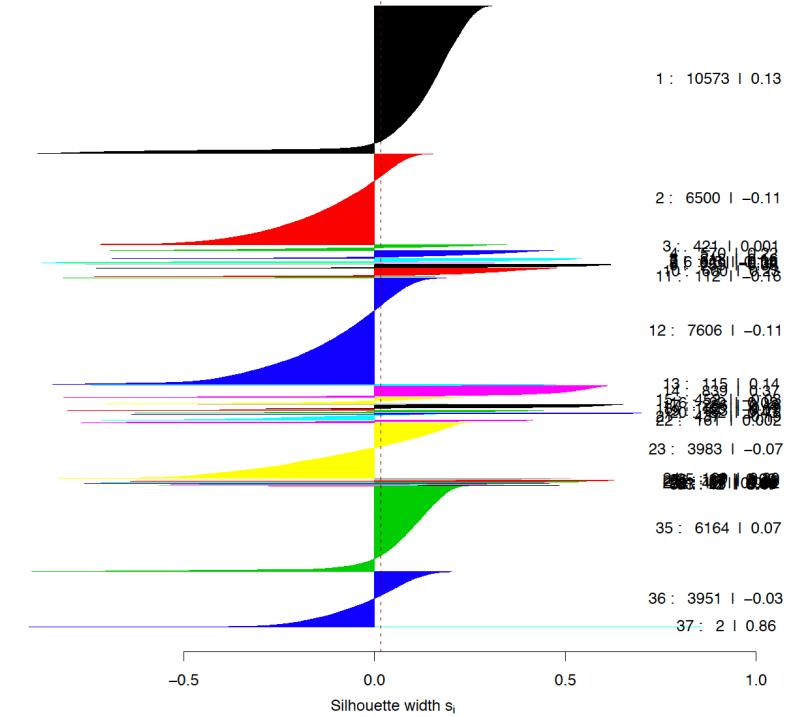
Silhouette plot of Seurat clustering – resolution 0.3
n = 44467



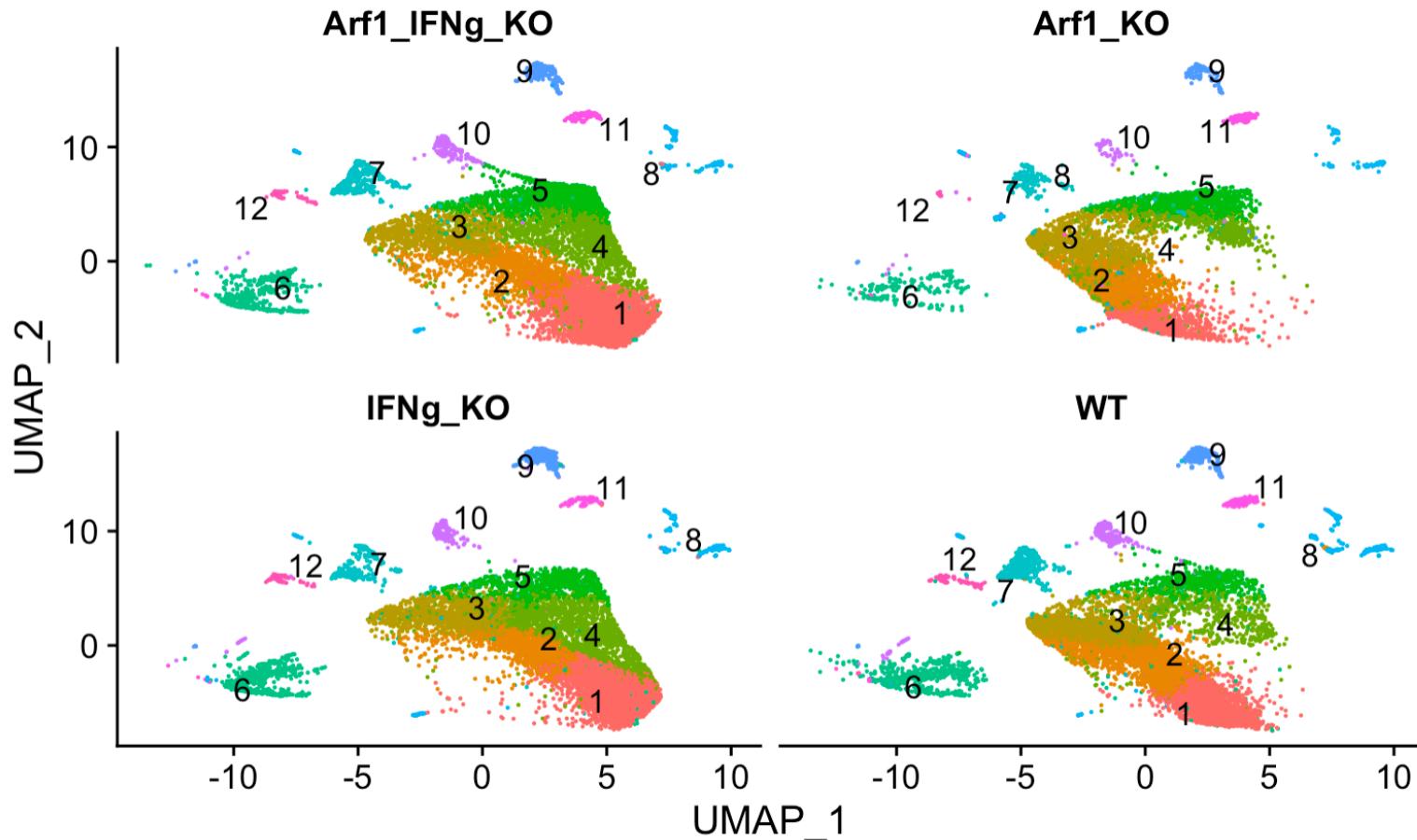
Silhouette plot of Seurat clustering – resolution 0.6
n = 44467



Silhouette plot of Seurat clustering – resolution 0.8
n = 44467



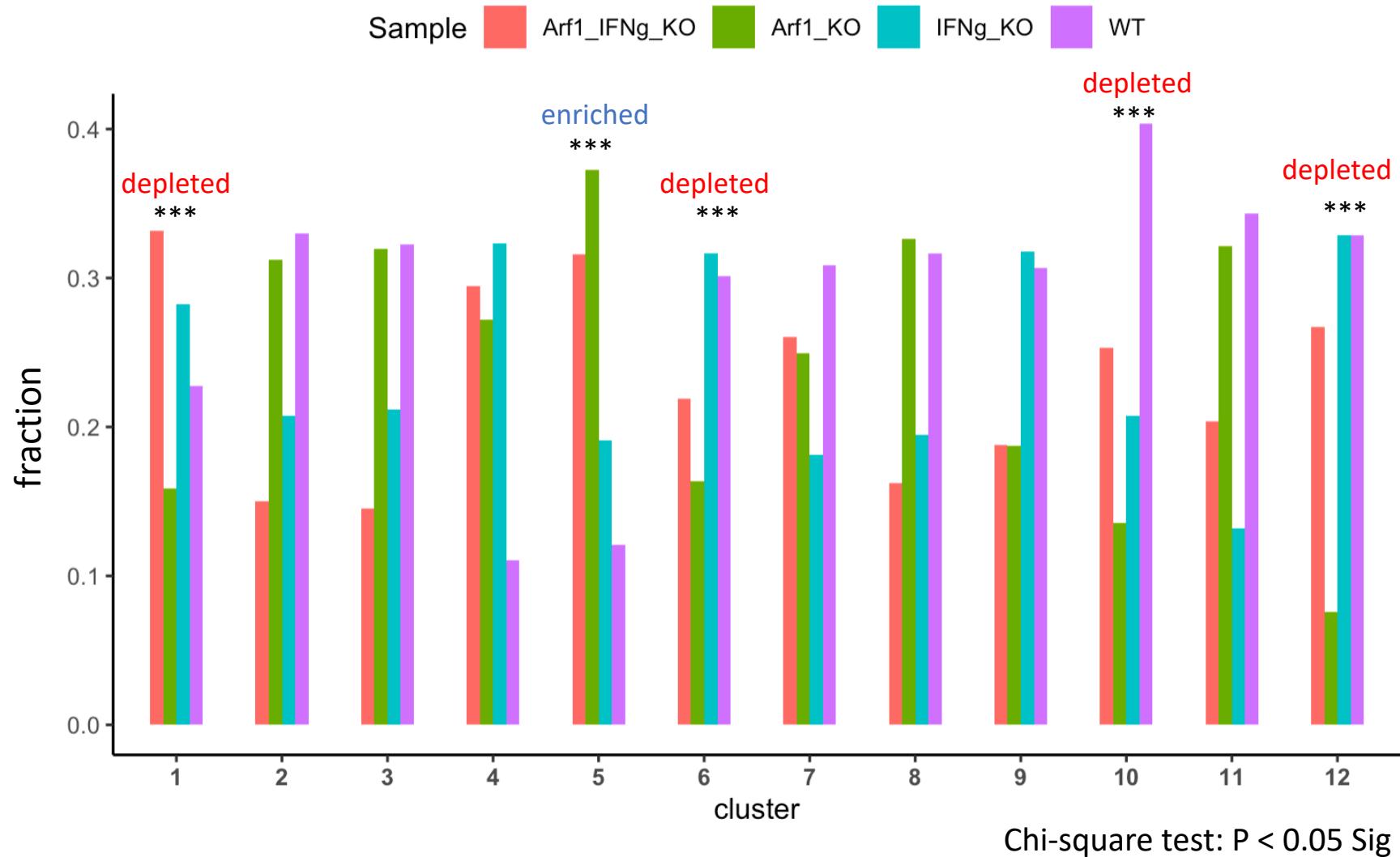
Only microglia cells – reshaped with 0.3 resolution into 12 microglia subclusters



Number of microglial cells for each cluster in each sample

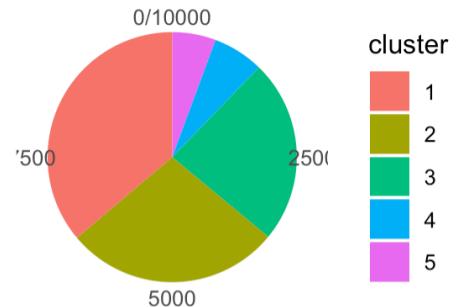
	Arf1_IFNg_KO	Arf1_KO	IFNg_KO	WT
1	4165	1990	3544	2855
2	1001	2080	1380	2198
3	844	1856	1230	1874
4	1392	1284	1527	522
5	1171	1380	707	447
6	412	308	596	567
7	317	304	221	376
8	169	340	203	330
9	171	170	289	279
10	172	92	141	274
11	111	175	72	187
12	74	21	91	91

Arf1_KO is enriched in Cluster 5

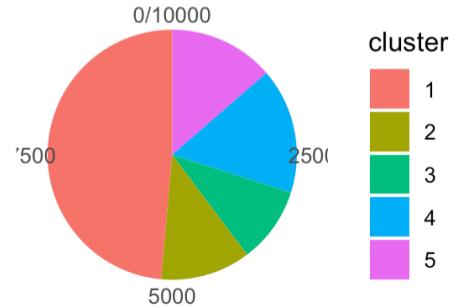


Proportion of cells for each cluster across samples

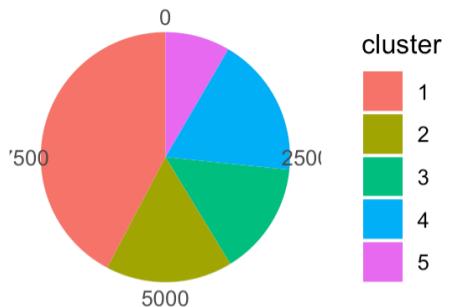
WT



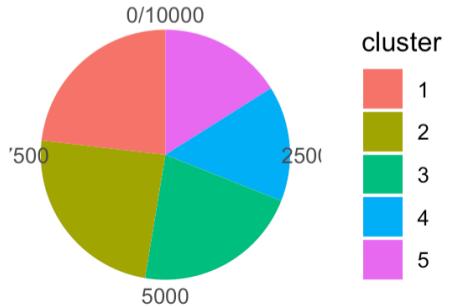
Arf1_IFNg_KO



IFNg_KO

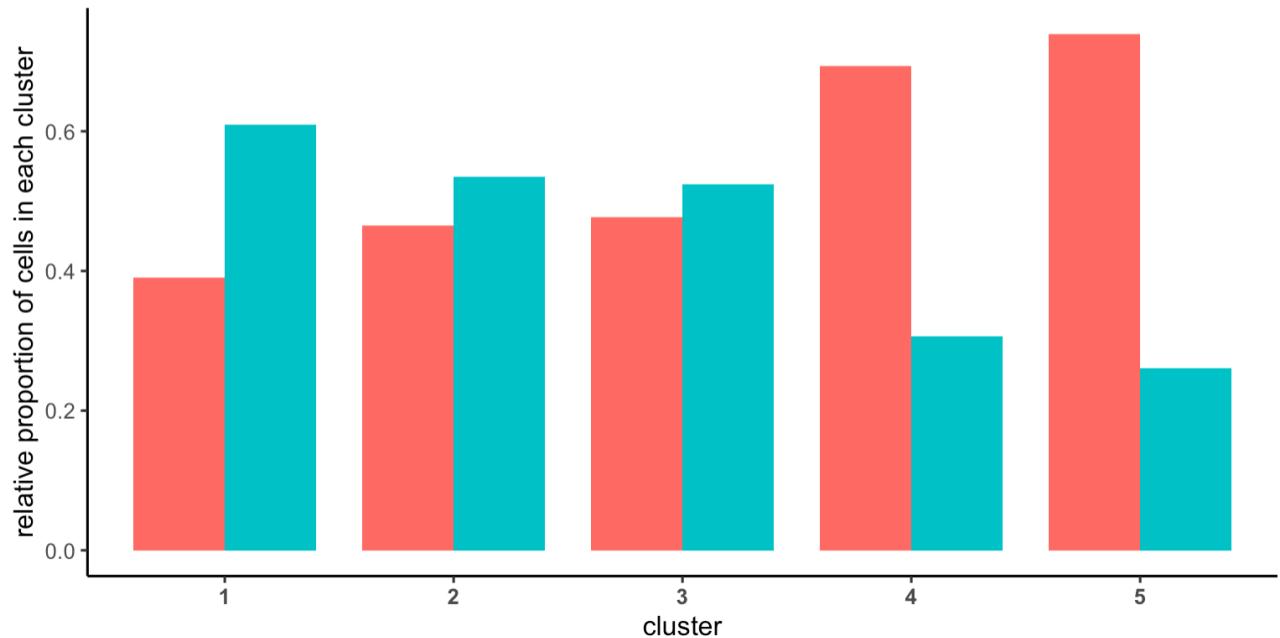


Arf1_KO

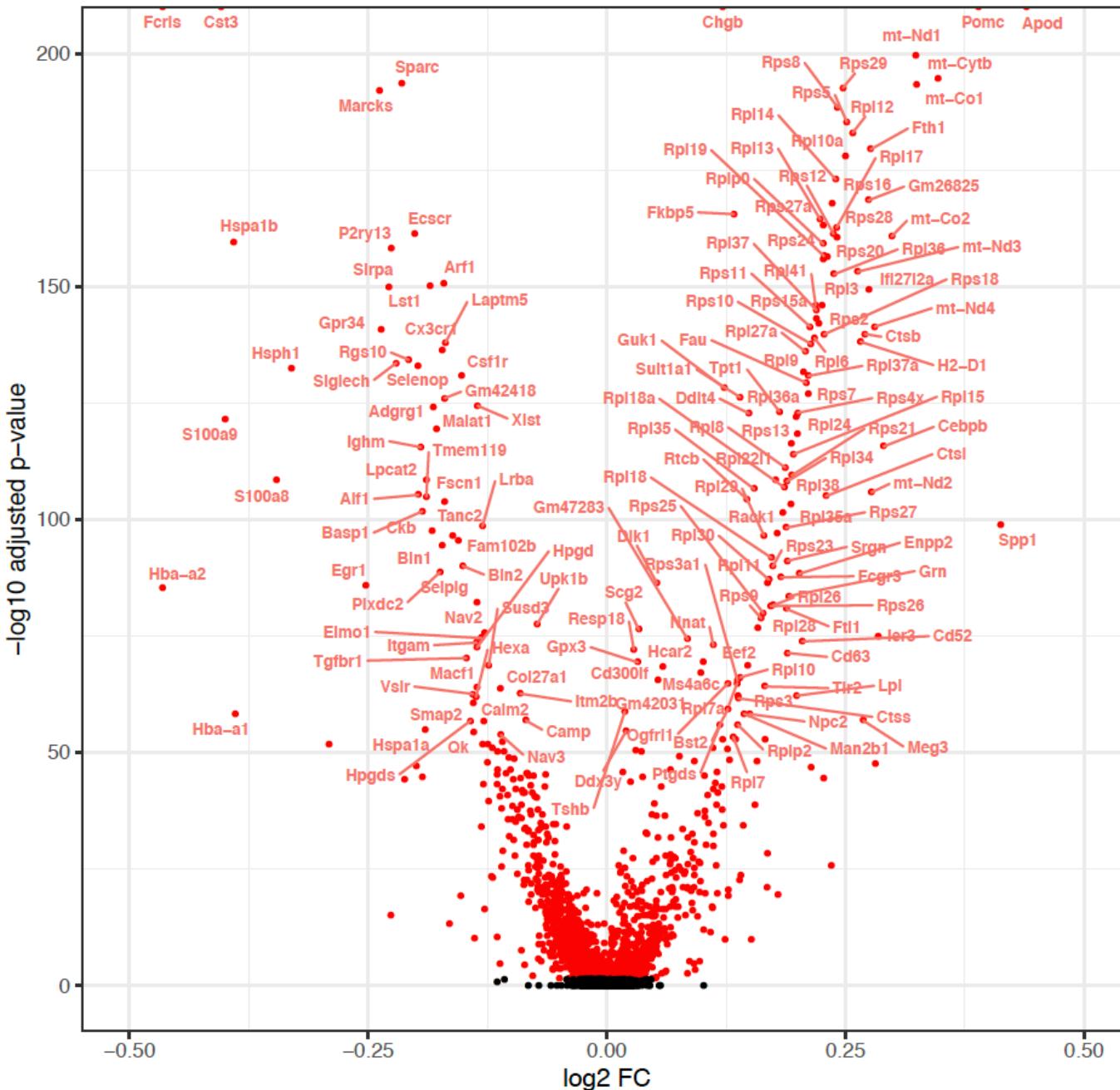


significantly higher proportion of cells in Arf1-KO cluster5

SampleName Arf1_KO WT

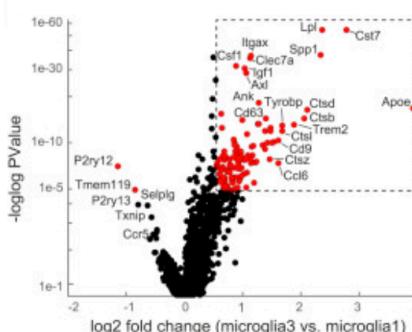


Arf1–WT

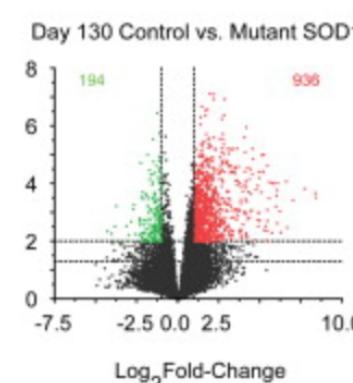


Curating published data to create a set of dysregulated genes in neurodegeneration

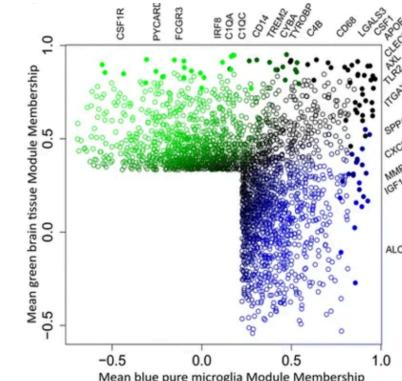
conditions	Model	Sequencing tech	source
multiple sclerosis	Mouse (EAE)	Nanostring	Kraseman et al., 2017
Amyotrophic lateral sclerosis (ALS)	Mouse (SODG93A)	Bulk RNA-seq	Chiu et al., 2013
Alzheimer's disease (AD)	Mouse	Single cell RNAseq	Keren-Shaul et al. 2017
aging	mouse	Bulk RNA-seq	Holtman et al., 2015



Alzheimer's disease (AD)

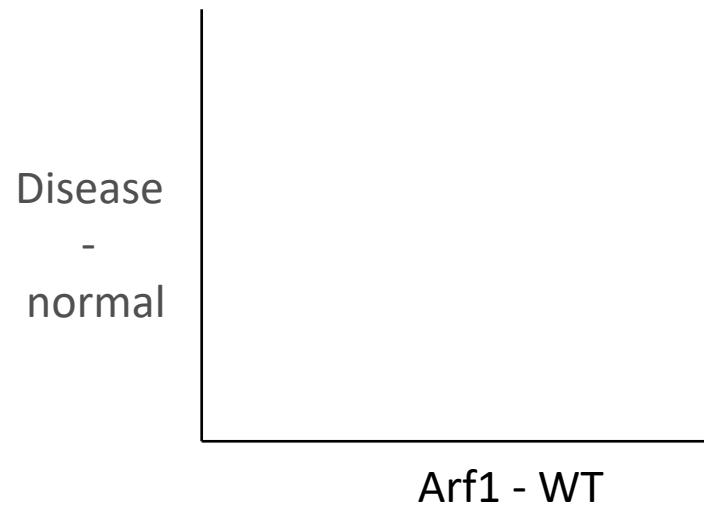


Amyotrophic lateral sclerosis (ALS)

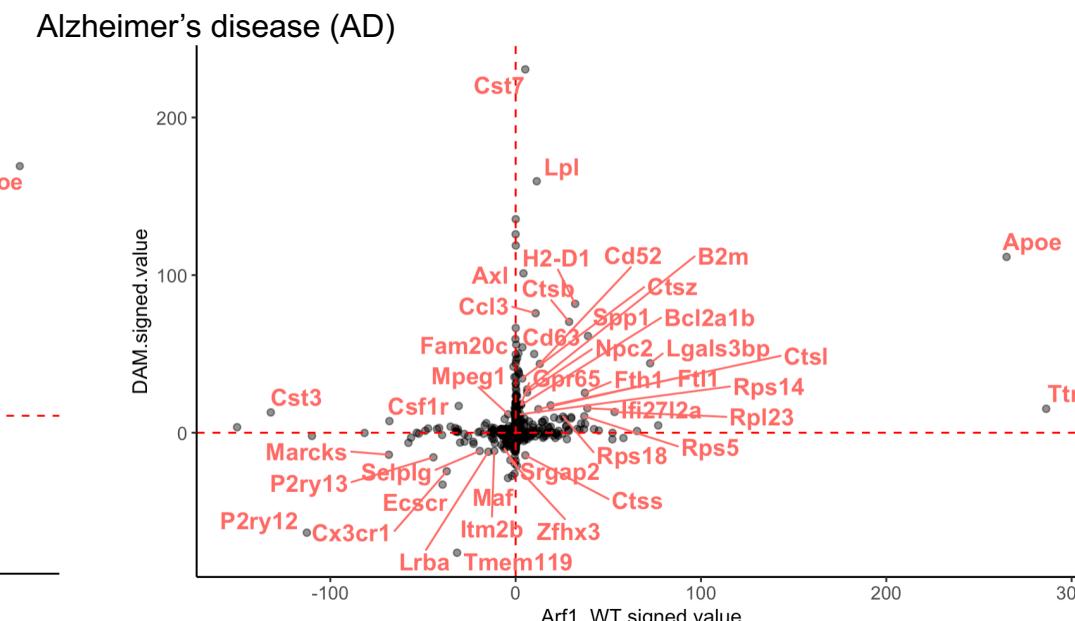
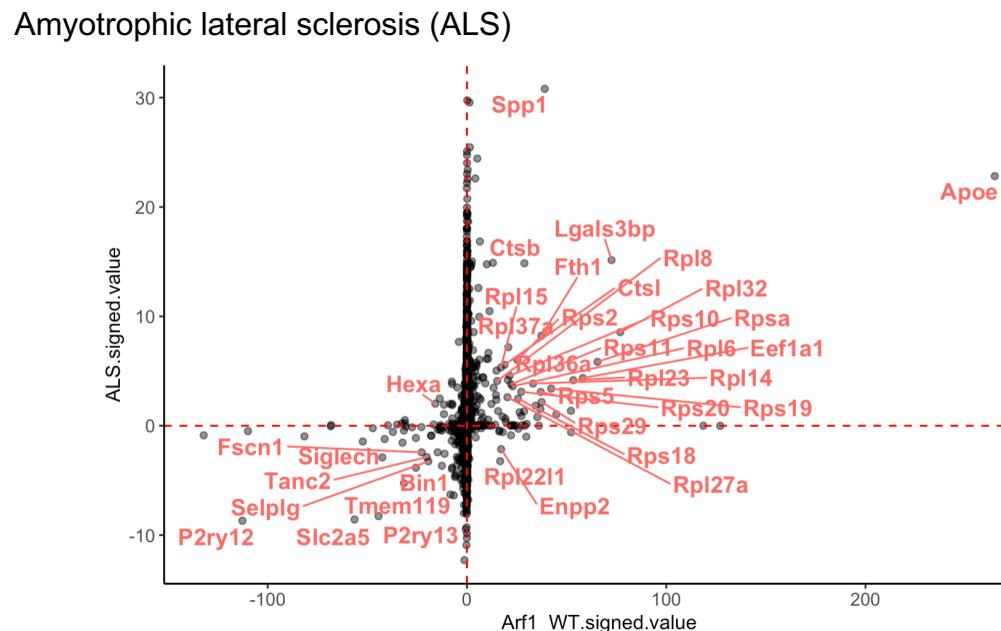
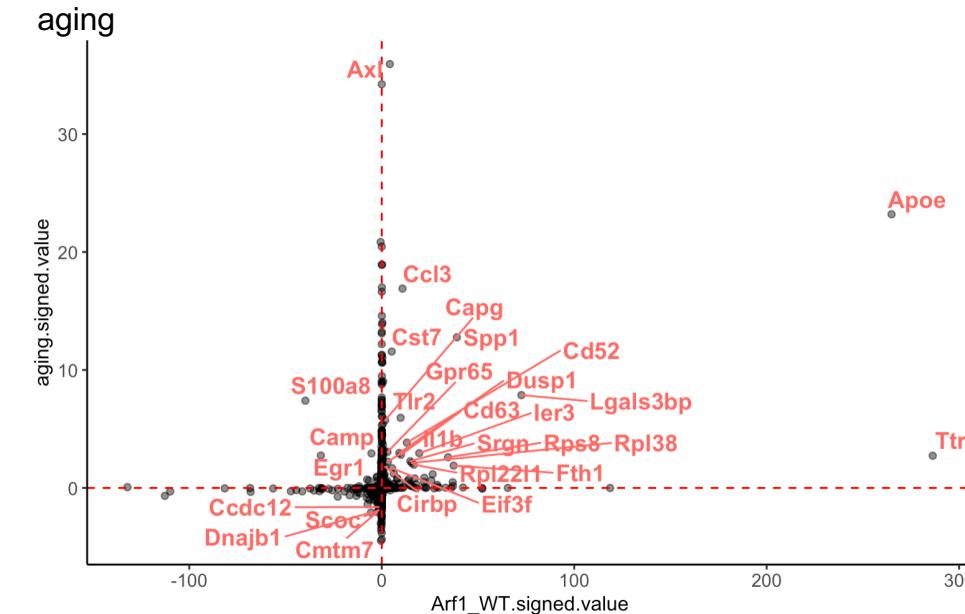
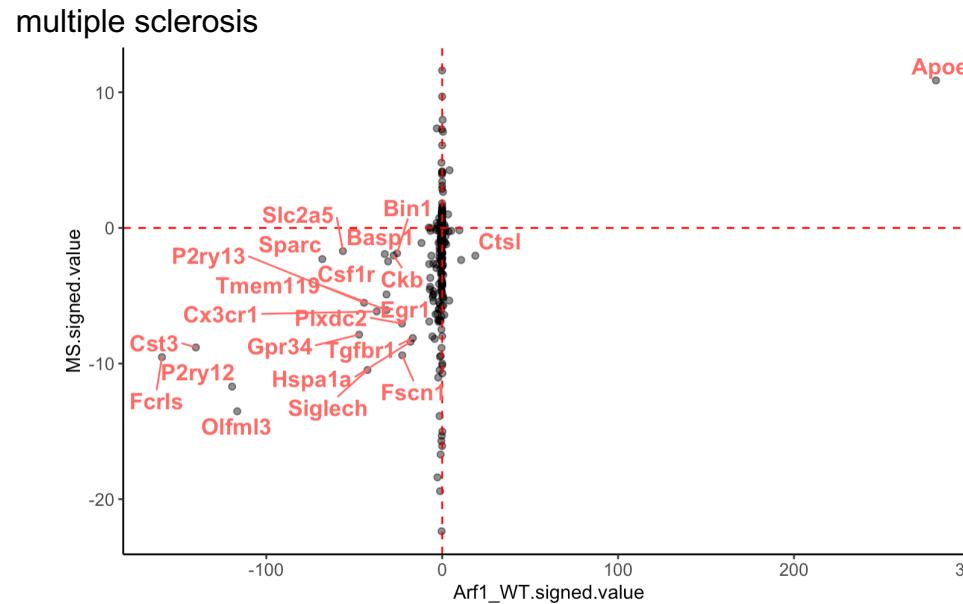


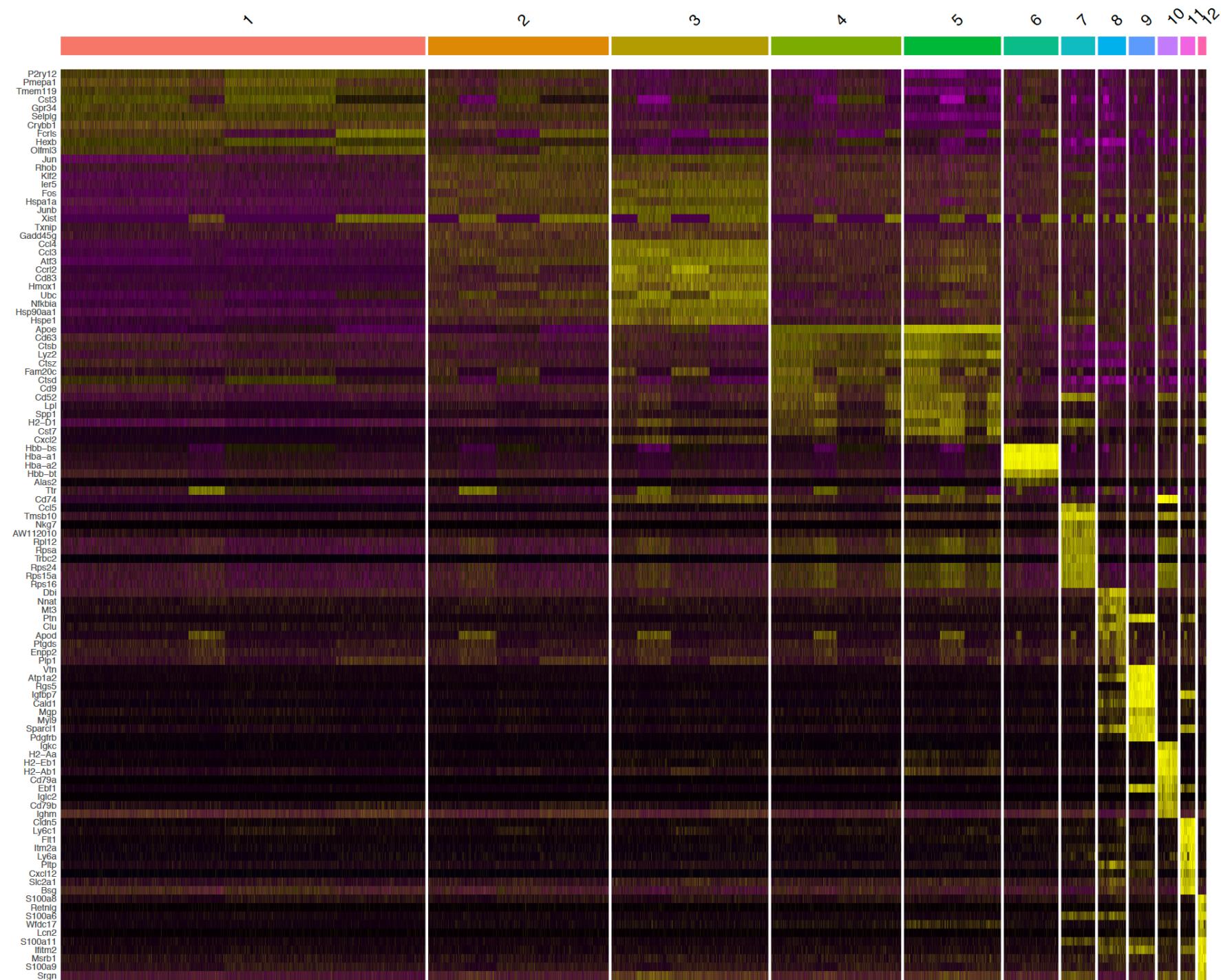
aging

Signed.value = sign(logFC) * log10(Pvalue) * abs(logFC) * -1



Top differentially expressed genes in Arf1-WT include some of the well-known neurodegenerative disease risk genes





Markers (top 10 by average fold change)
for each microglia
subcluster

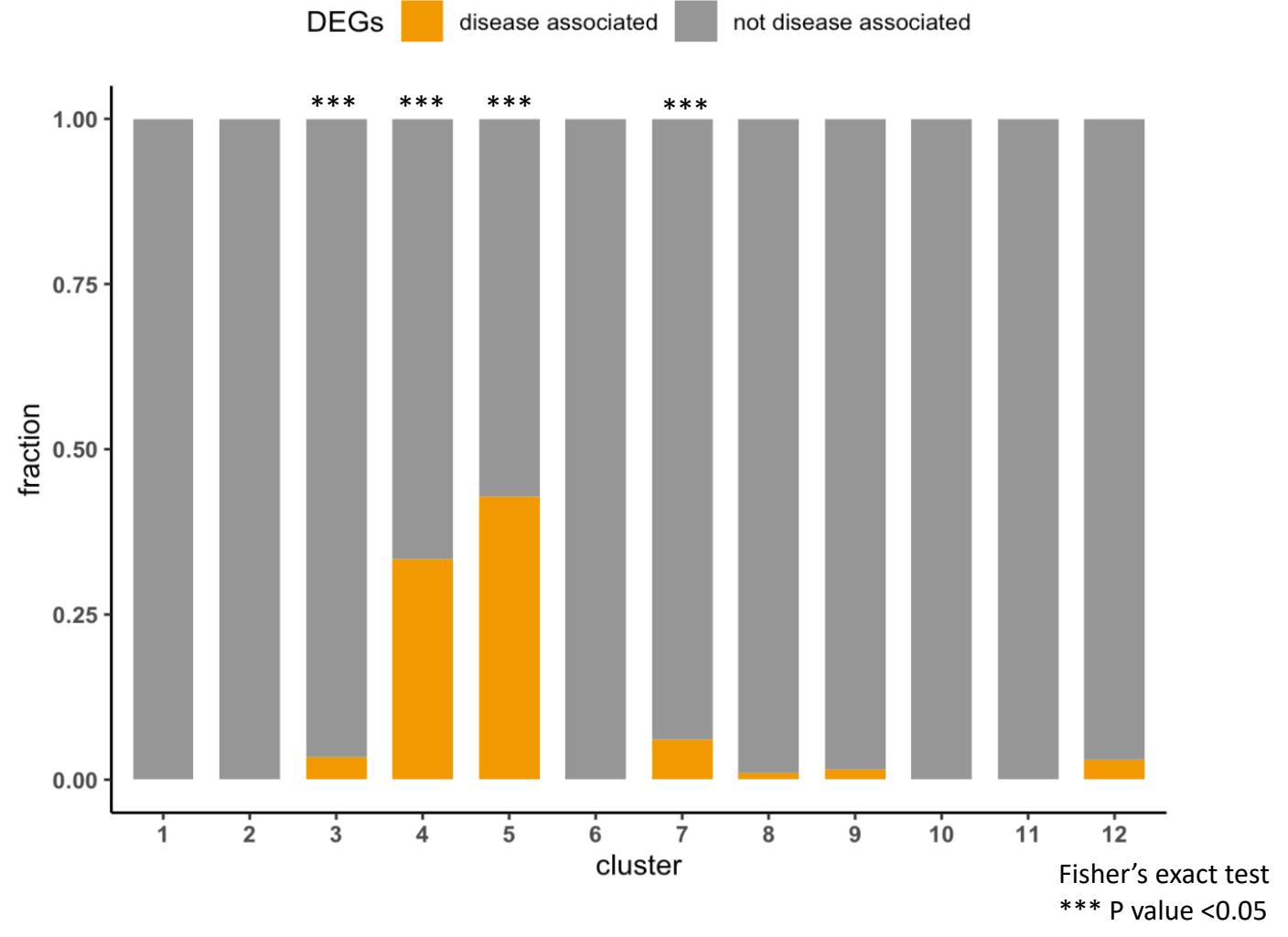
Does cluster5 DEGs have an over-representation of neurodegenerative disease-associated genes?

Commonly dysregulated genes in neurodegeneration:

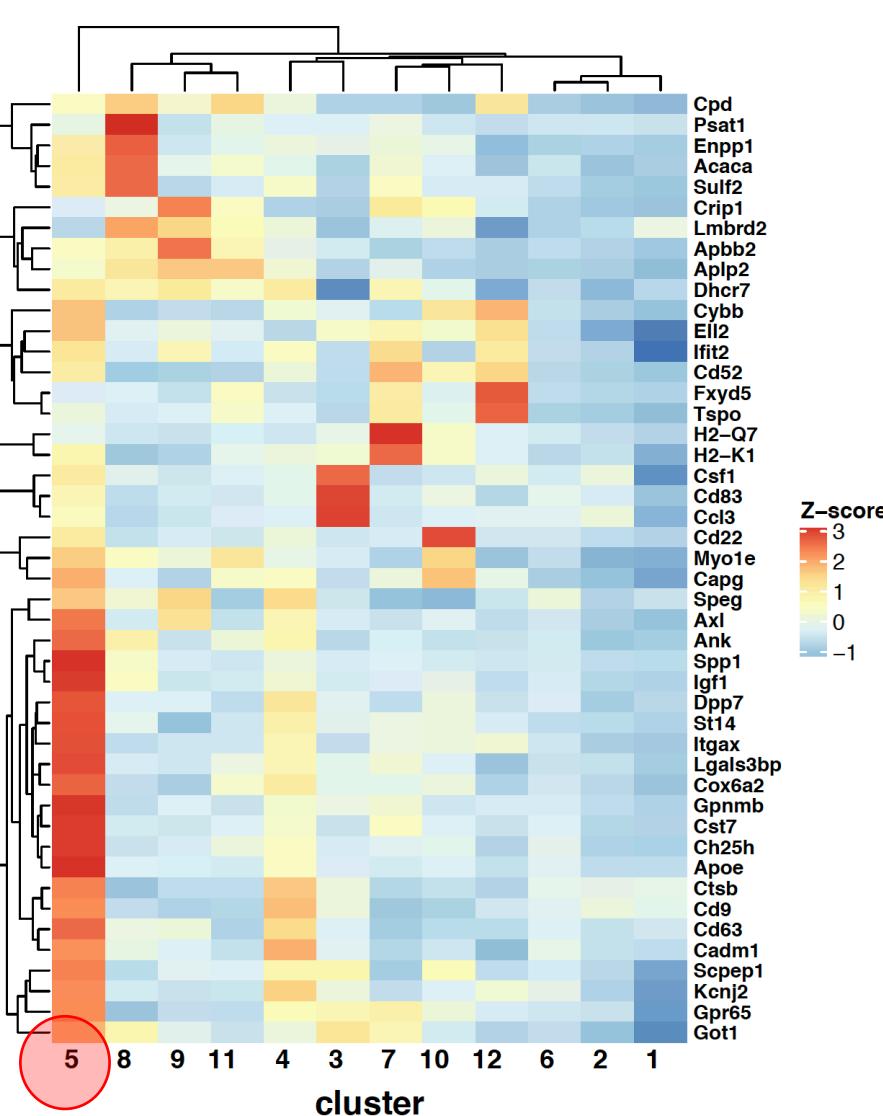
- Up-regulated
- Down-regulated

Cpd
Psat1
Enpp1
Acaca
Sulf2
Crip1
Lmbrd2
Appb2
Apol2
Dhcr7
Cybb
Elf2
Ifit2
Cd52
Fxyd5
Tspo
H2-Q7
H2-K1
Csf1
Cd83
Ccl3
Cd22
Myo1e
Capg
Speg
Axl
Ank
Spp1
Igf1
Dpp7
St14
Itgax
Lgals3bp
Cox6a2
Gpnmb
Cst7
Ch25h
Apoe
Ctsb
Cd9
Cd63
Cadm1
Sccep1
Kcnj2
Gpr65
Got1

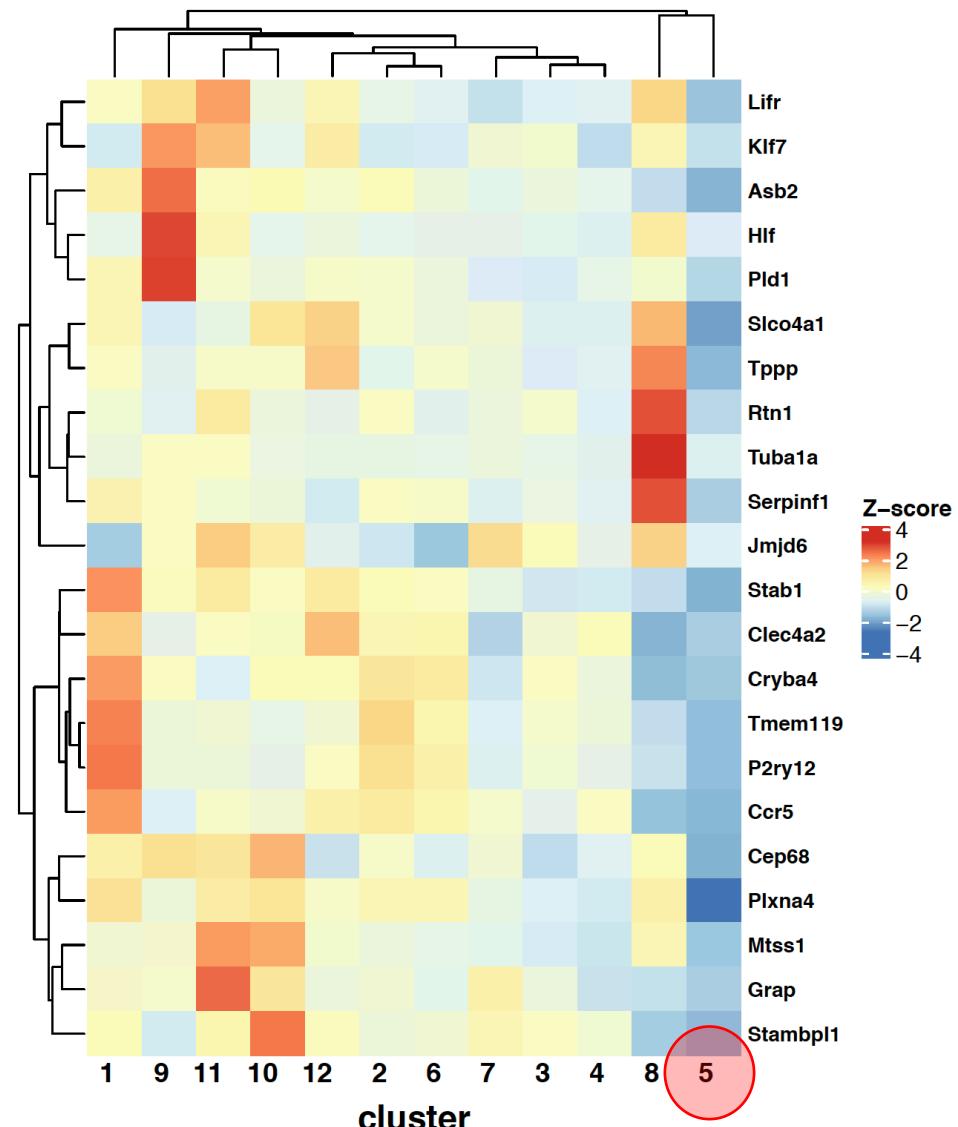
Lifr
Klf7
Asb2
Hif
Pld1
Slco4a1
Tpyp
Rtn1
Tuba1a
Serpinf1
Jmjdc6
Stab1
Clec4a2
Cryba4
Tmem119
P2ry12
Ccr5
Cep68
Plxna4
Mtss1
Grap
Stambpl1



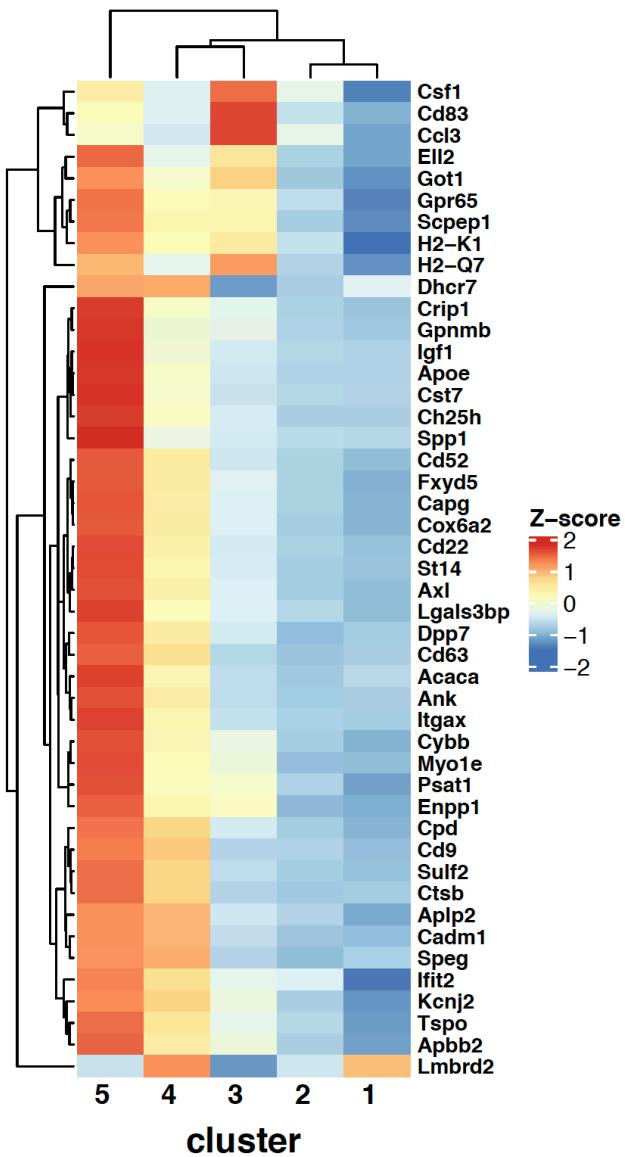
Up-regulated genes in neurodegeneration



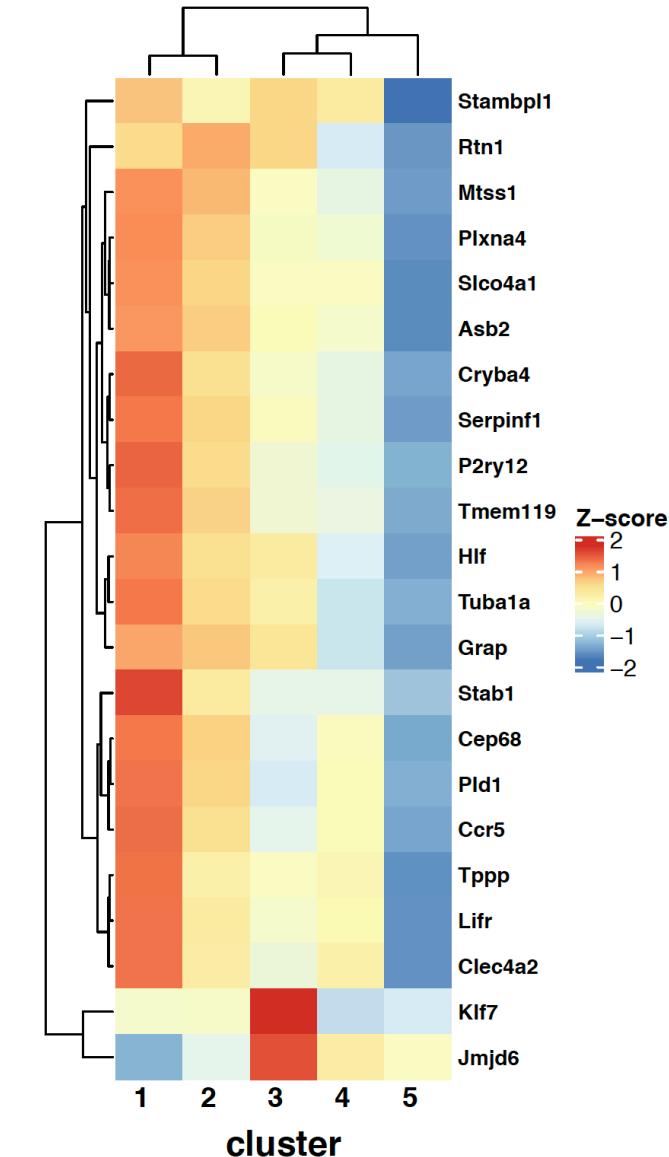
Down-regulated genes in neurodegeneration

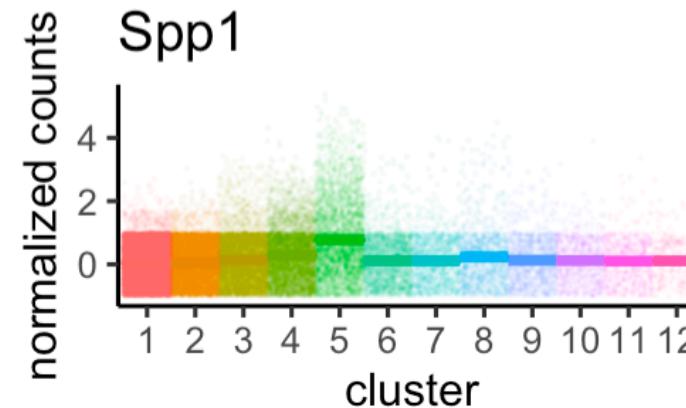
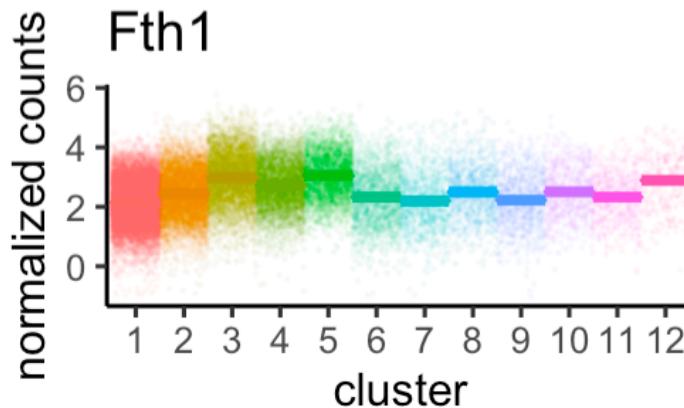
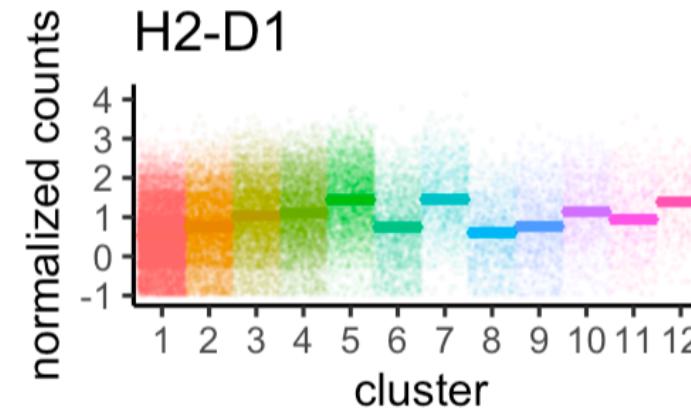
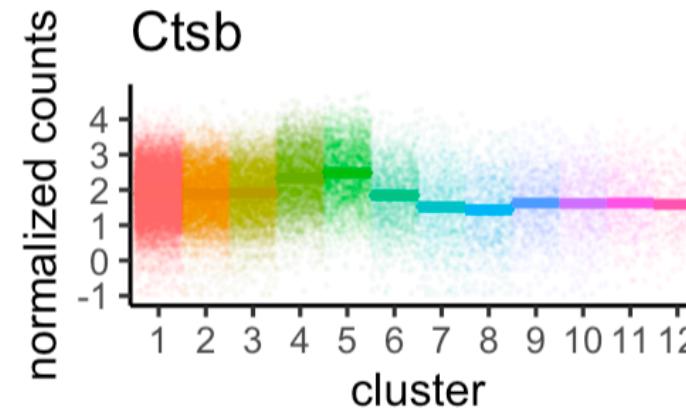
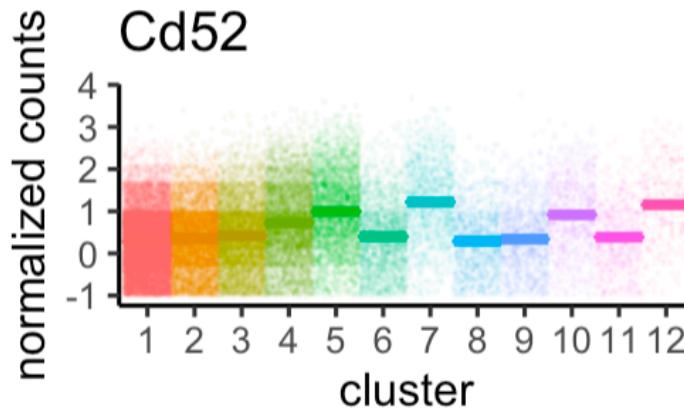
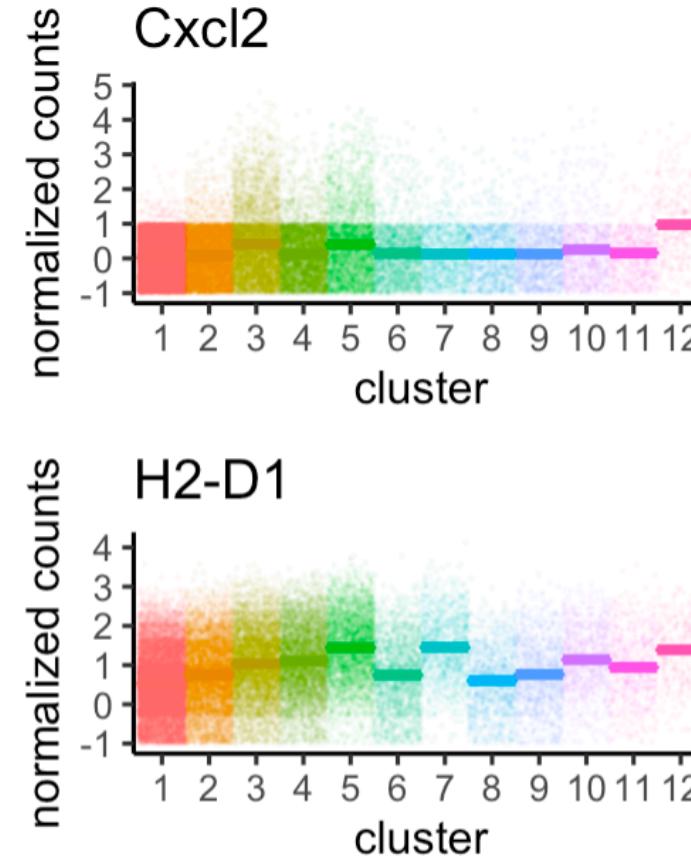
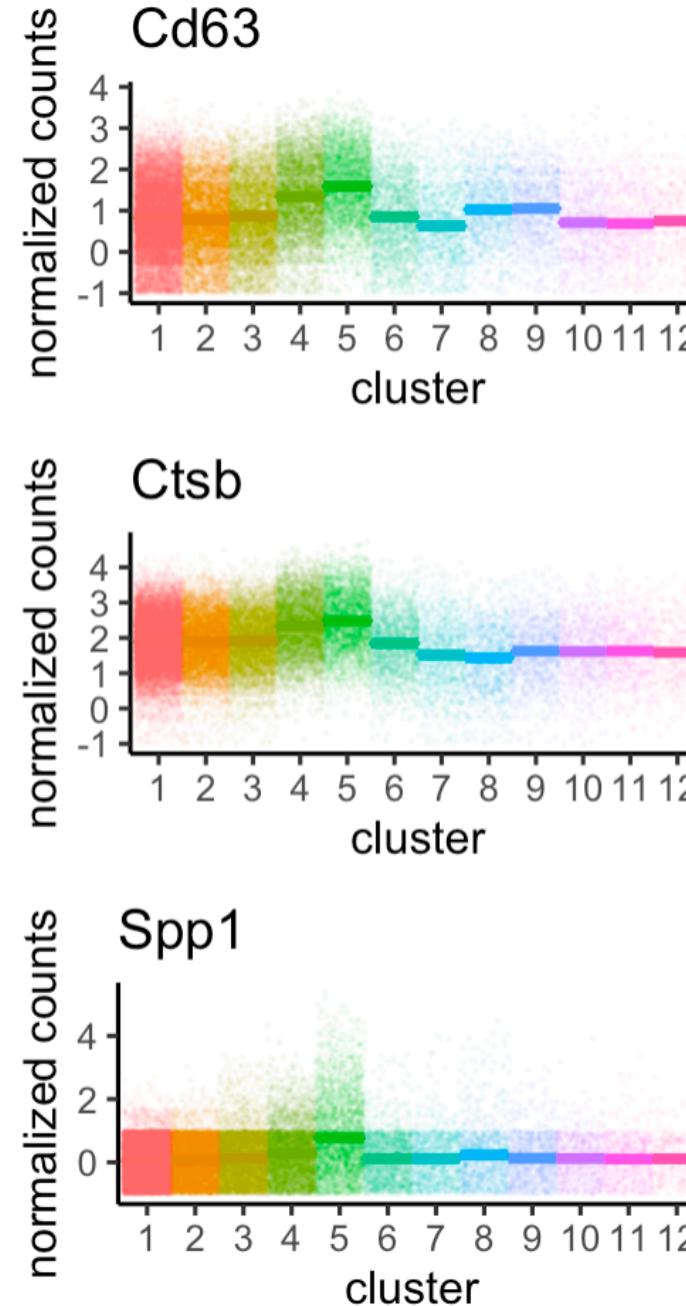
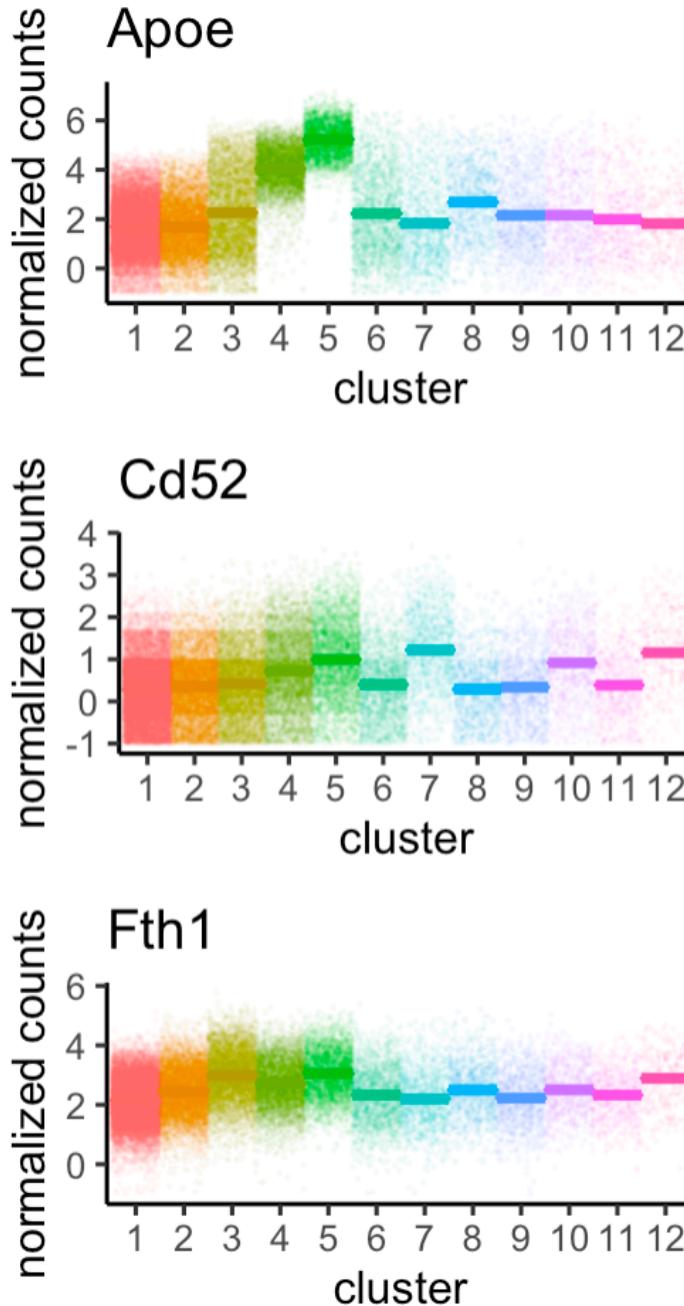


Up-regulated genes in neurodegeneration



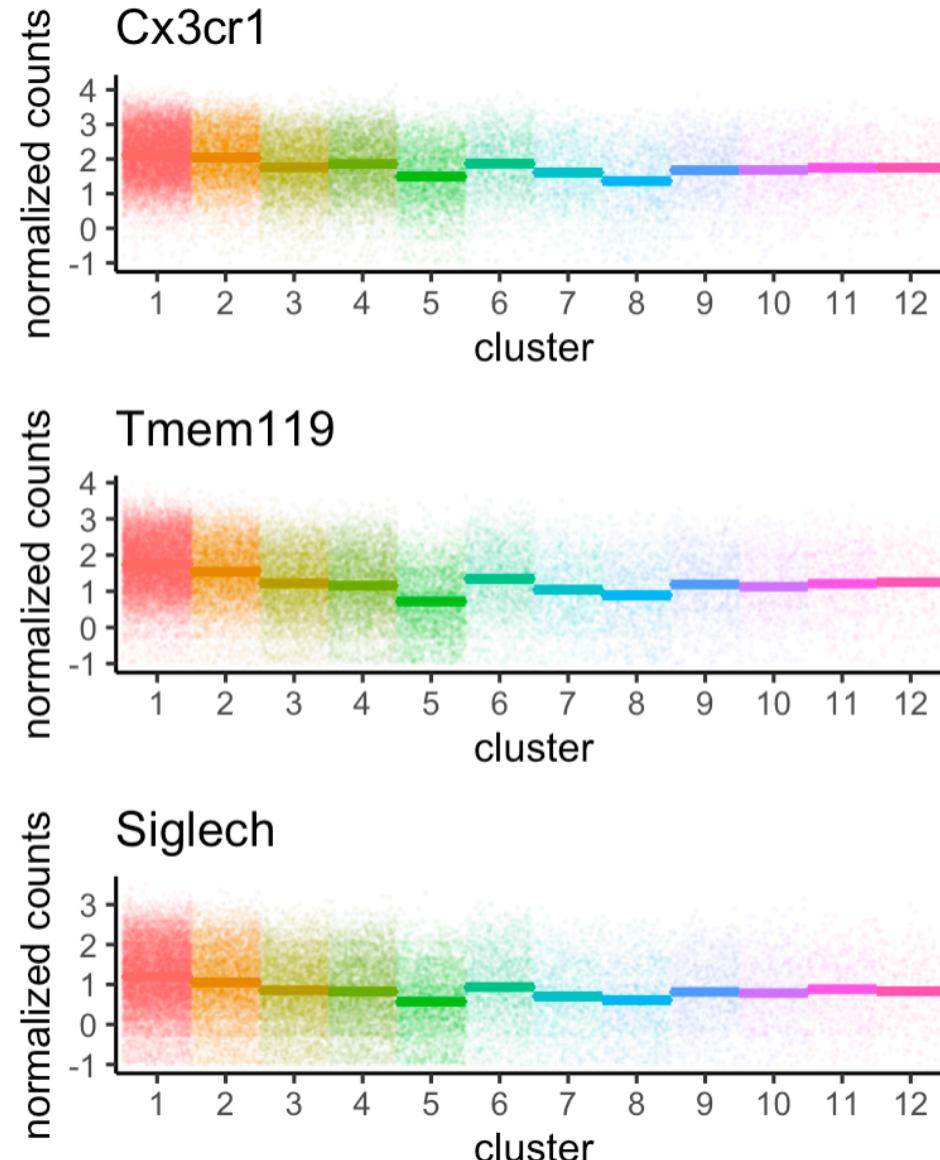
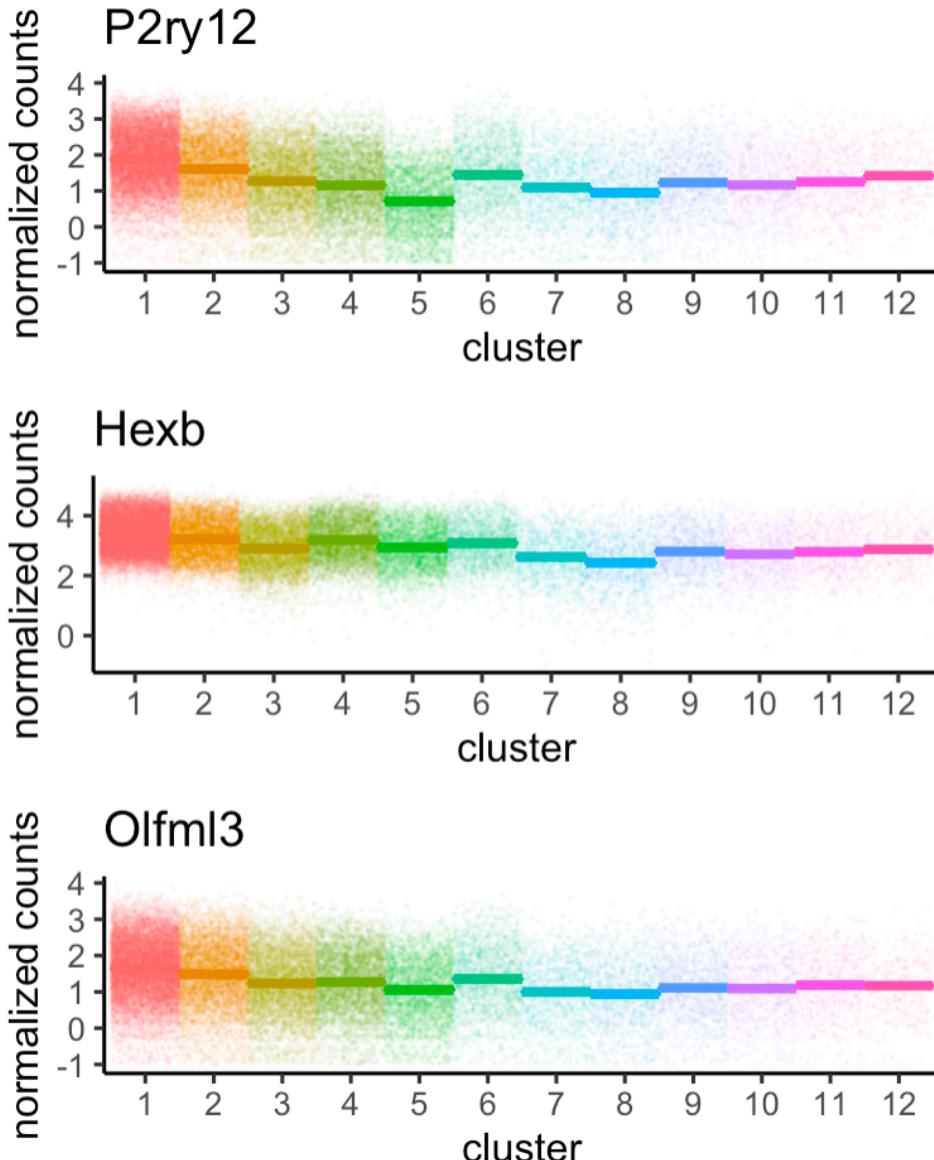
Down-regulated genes in neurodegeneration



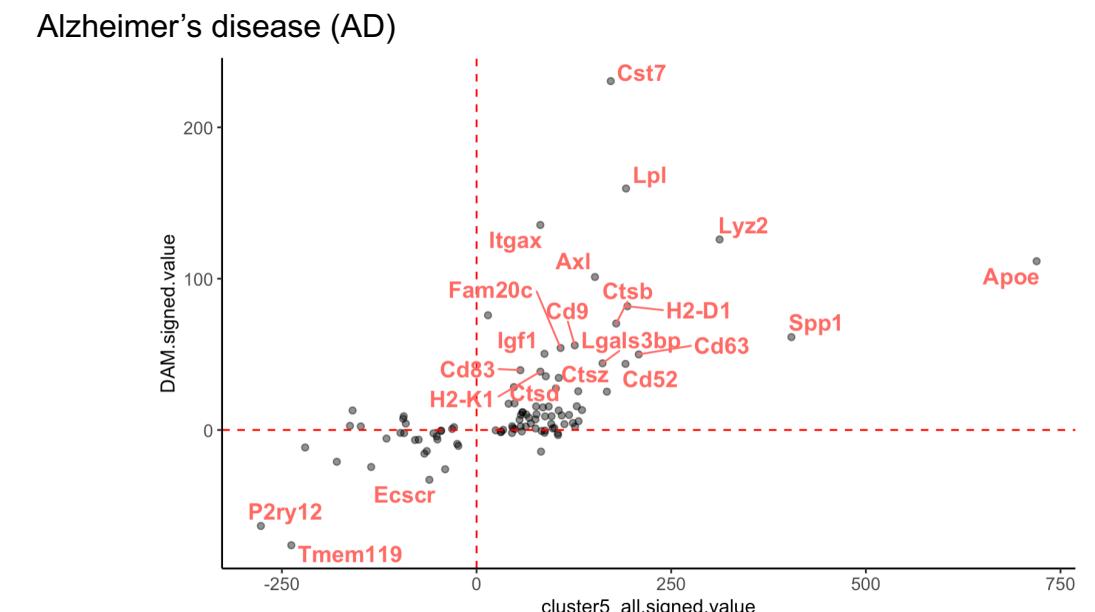
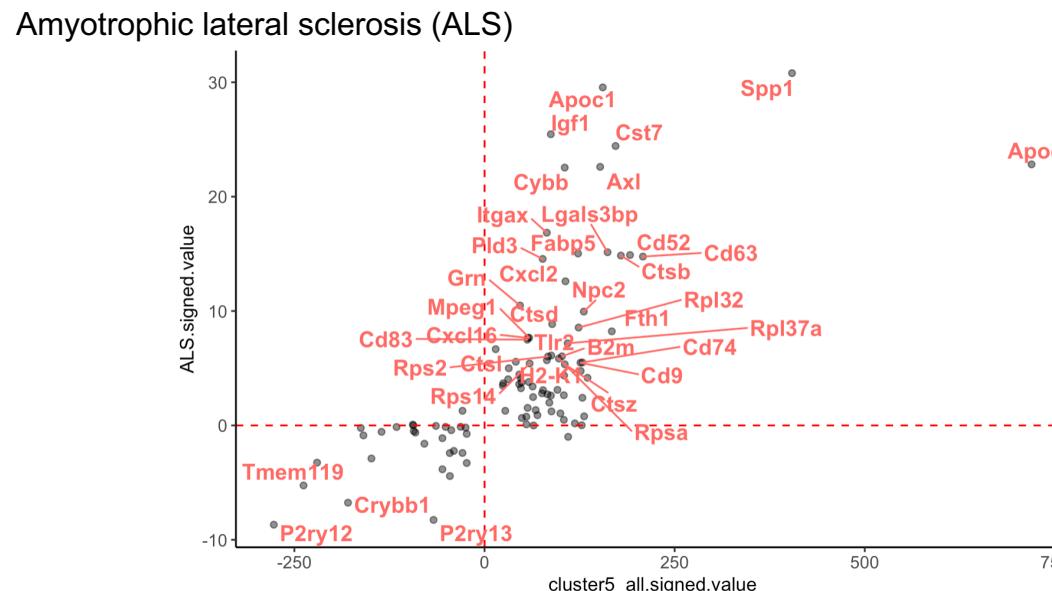
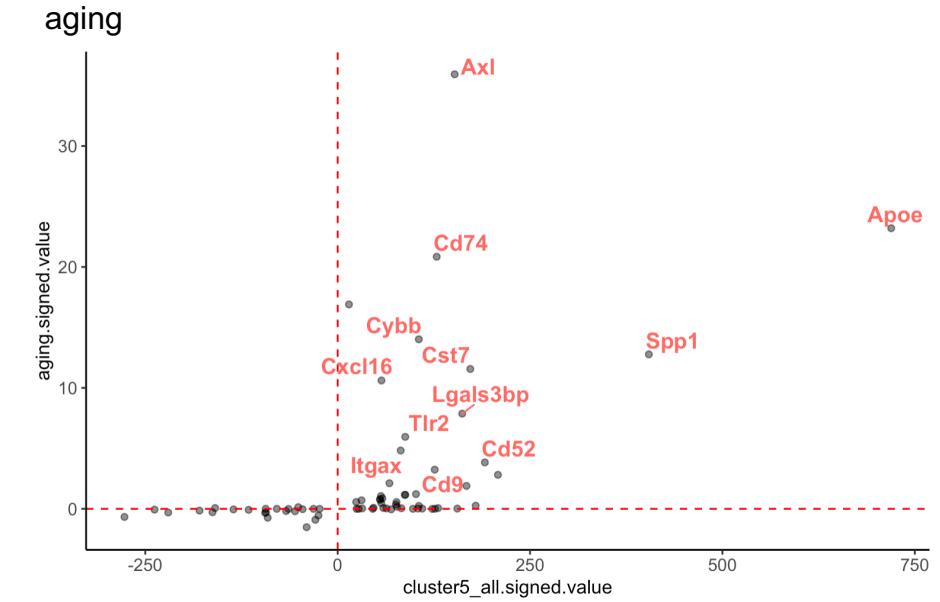
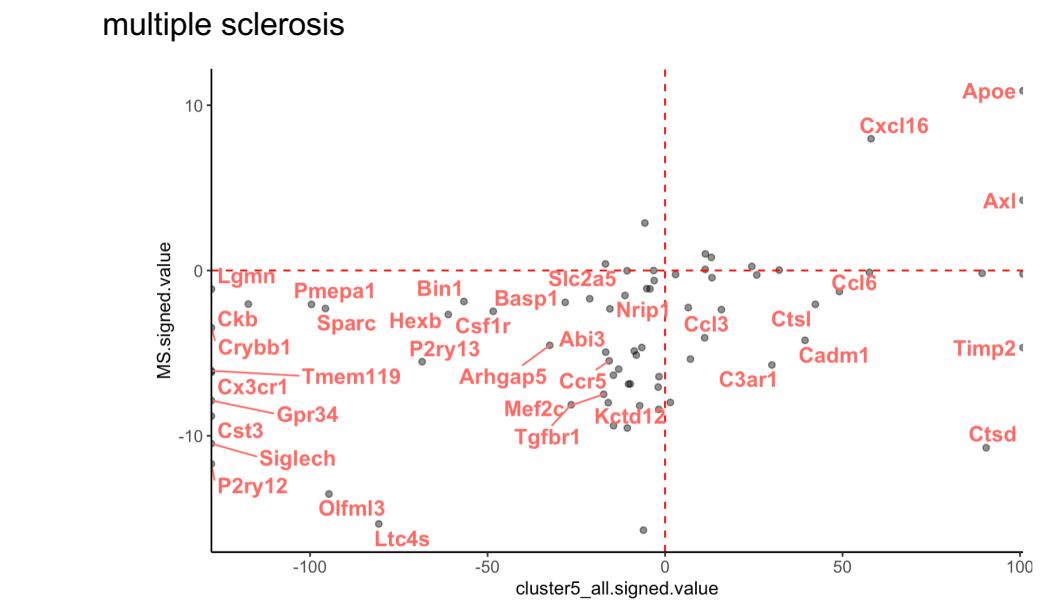


- Top markers for Cluster 5 include many known disease risk factors
- An increase in expression from Cluster 1 to Cluster 5

Top marker of Cluster 1 include mostly “microglial marker genes”

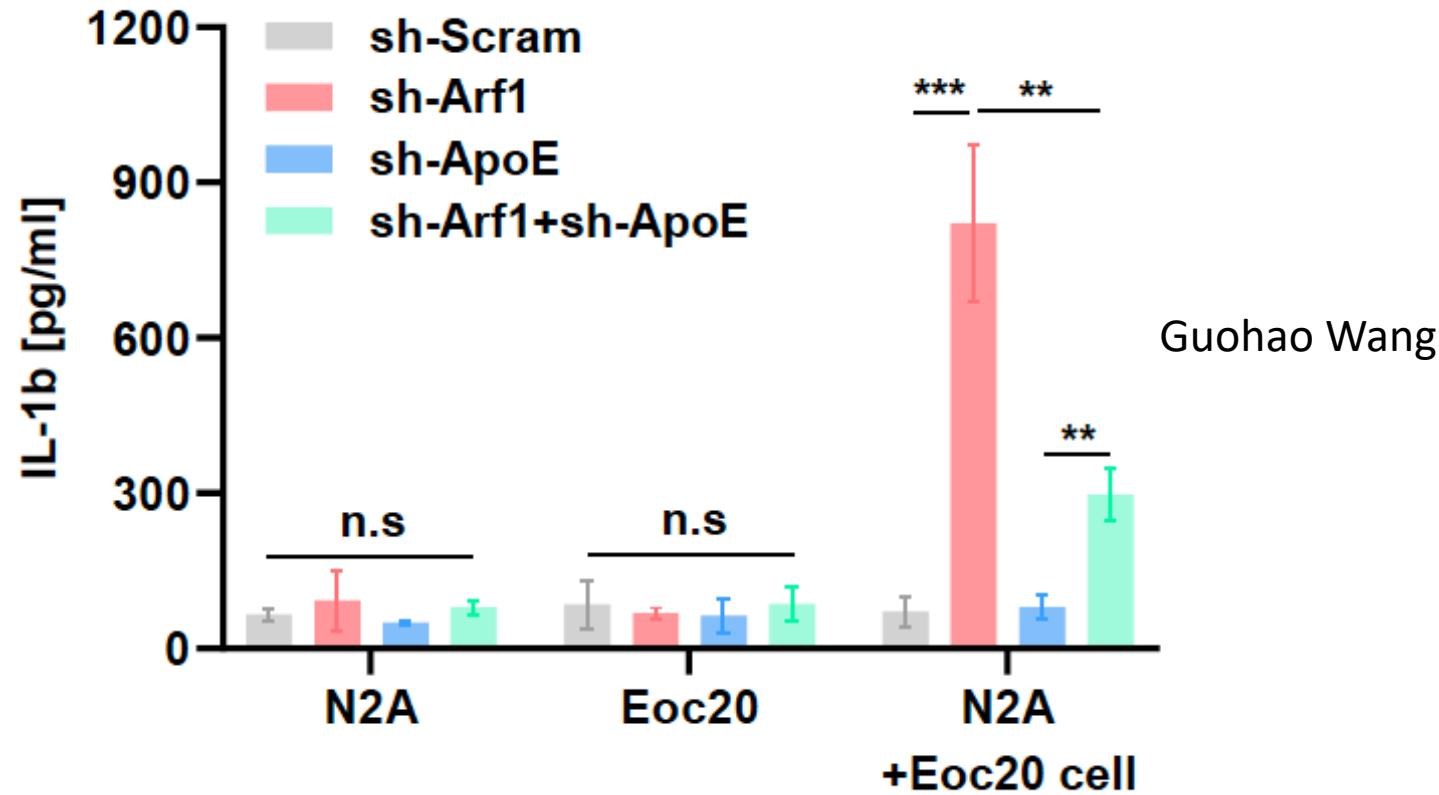


Top markers in both Cluster 5 include some of the well-known neurodegenerative disease risk genes

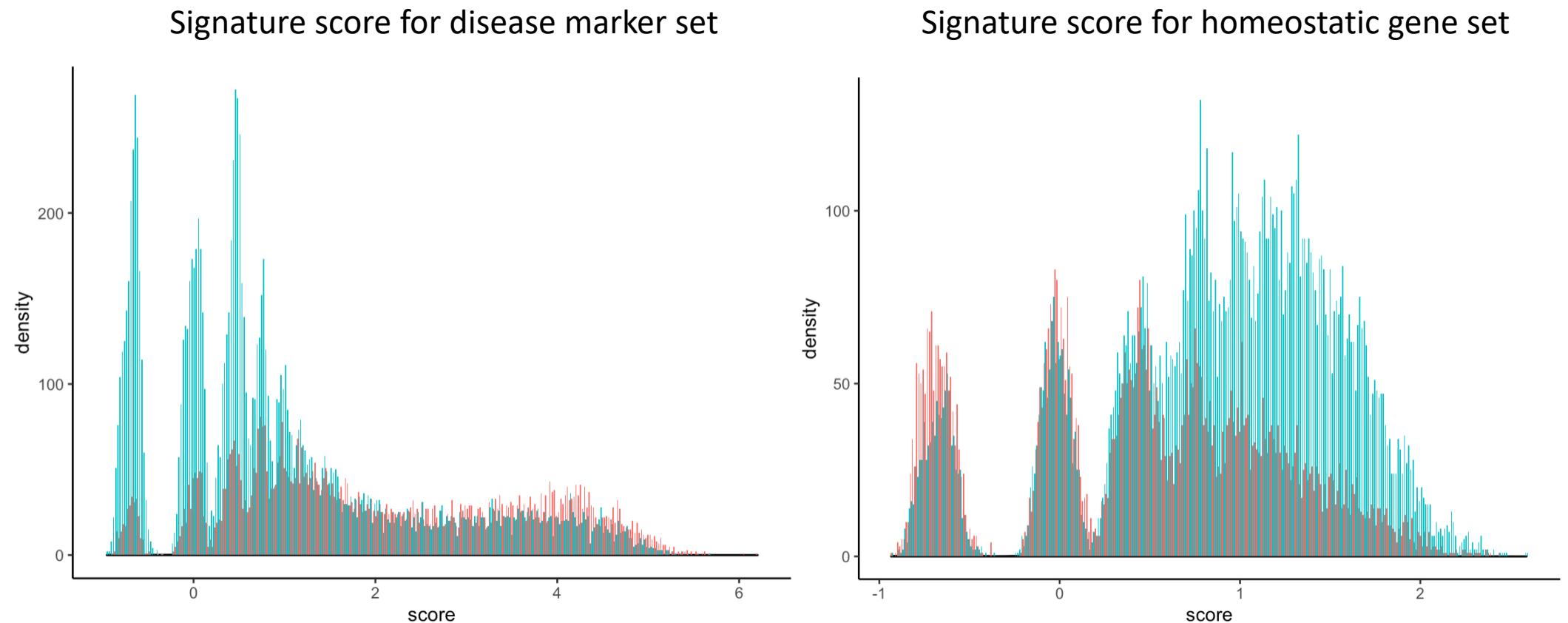


Increased IL-1 β in neuron (N2A) and microglia (Eoc20) co-culture can be significantly suppressed by knocking down *ApoE* (sh-ApoE).

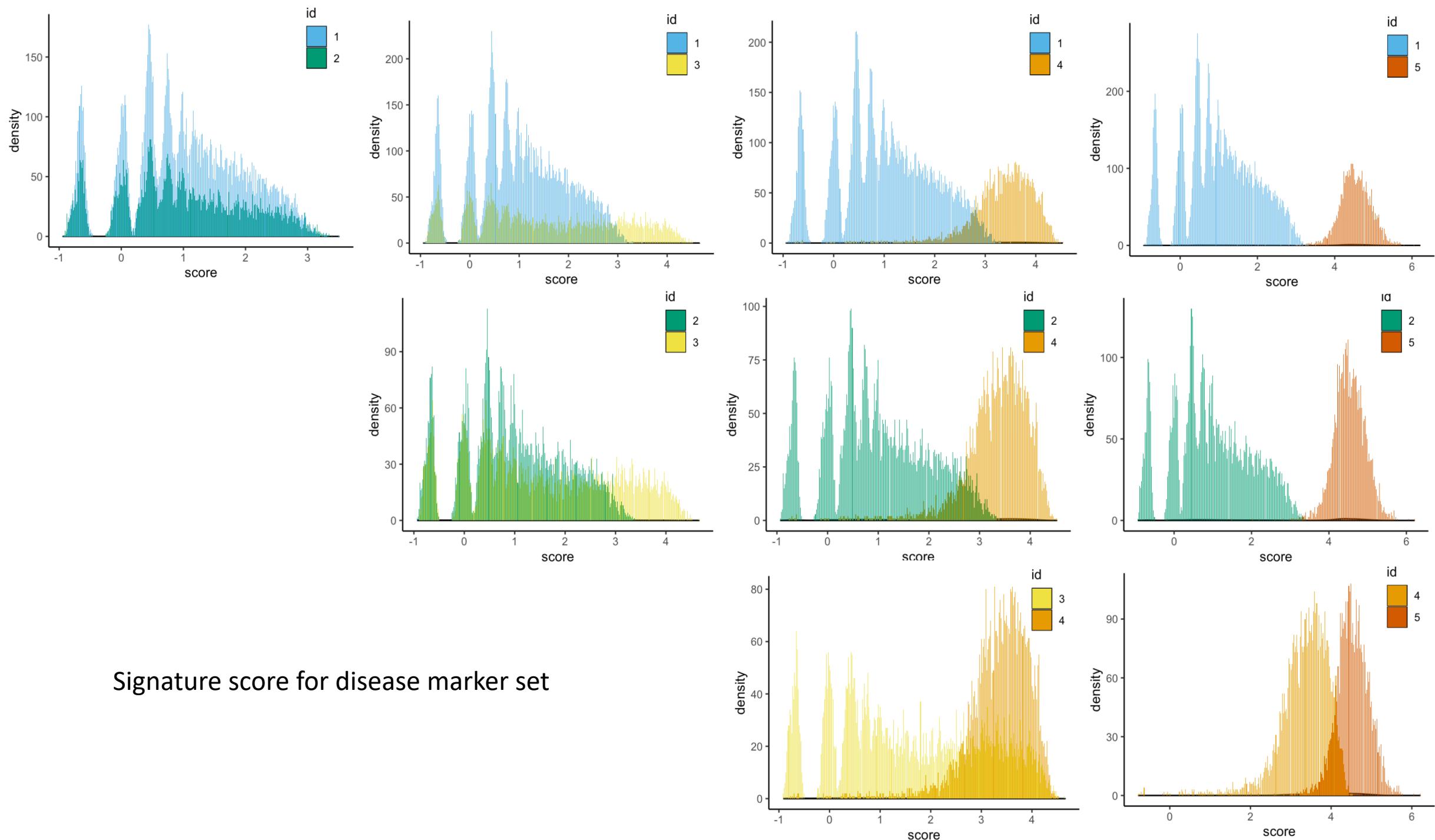
interleukin-1 β :
proinflammatory cytokines

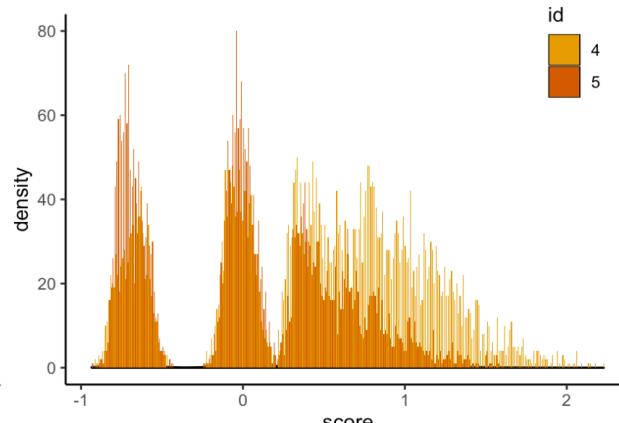
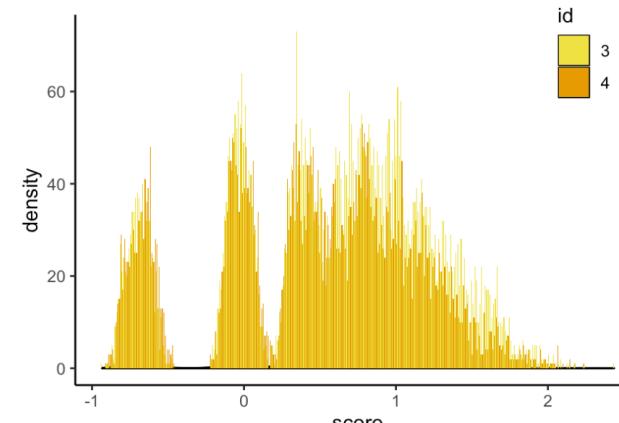
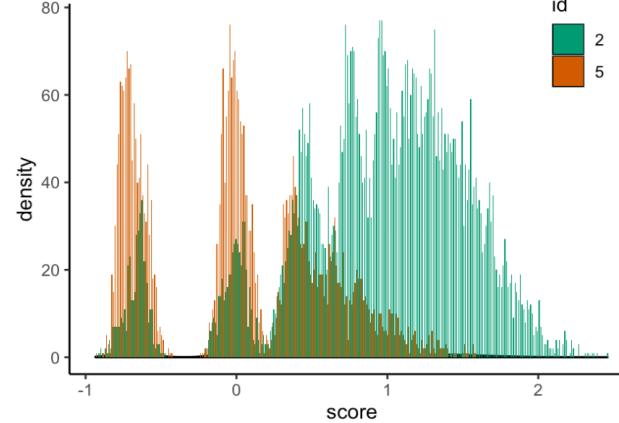
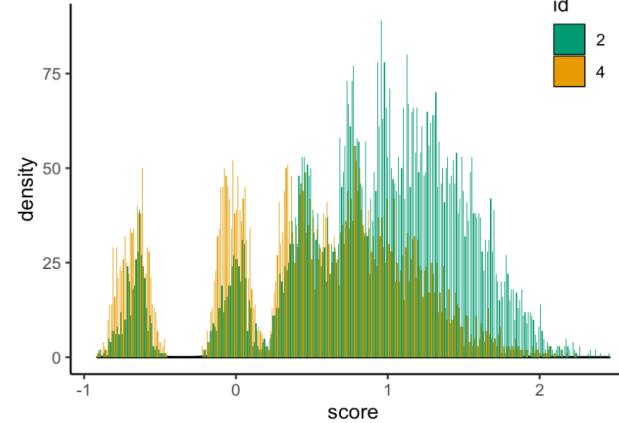
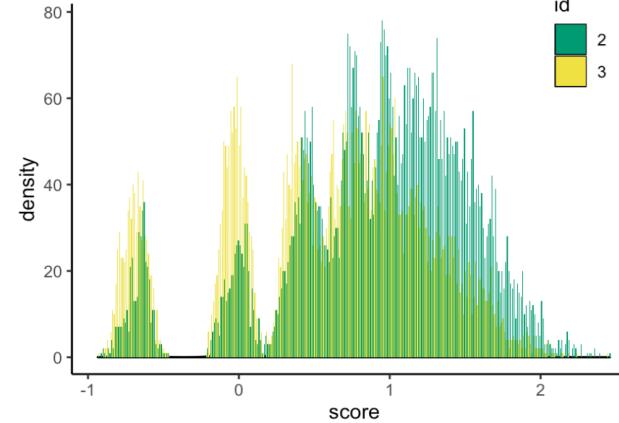
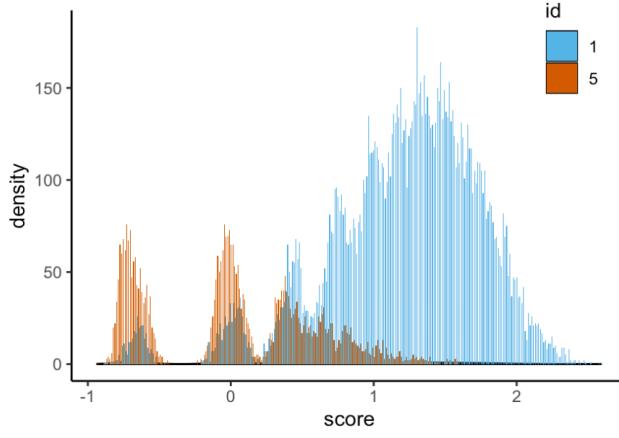
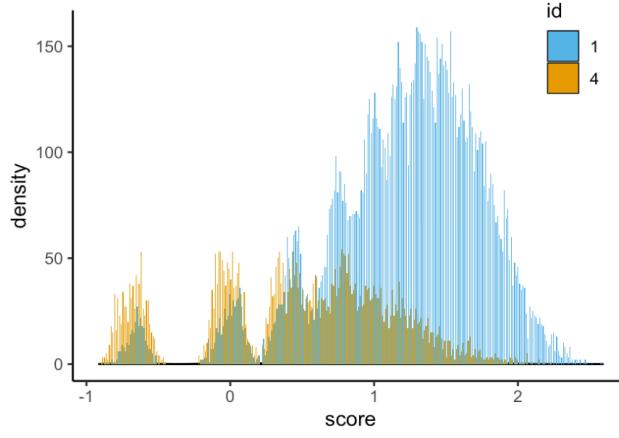
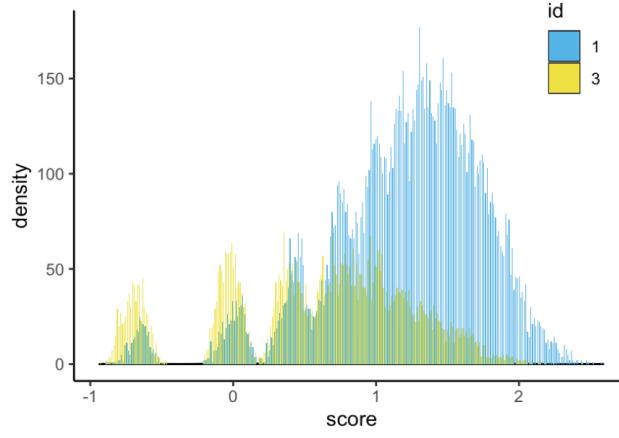
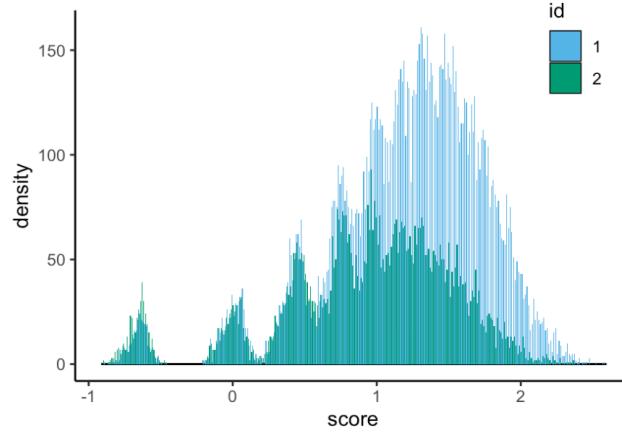


ApoE-mediated lipid transportation is important for IL-1 β induction in microglia



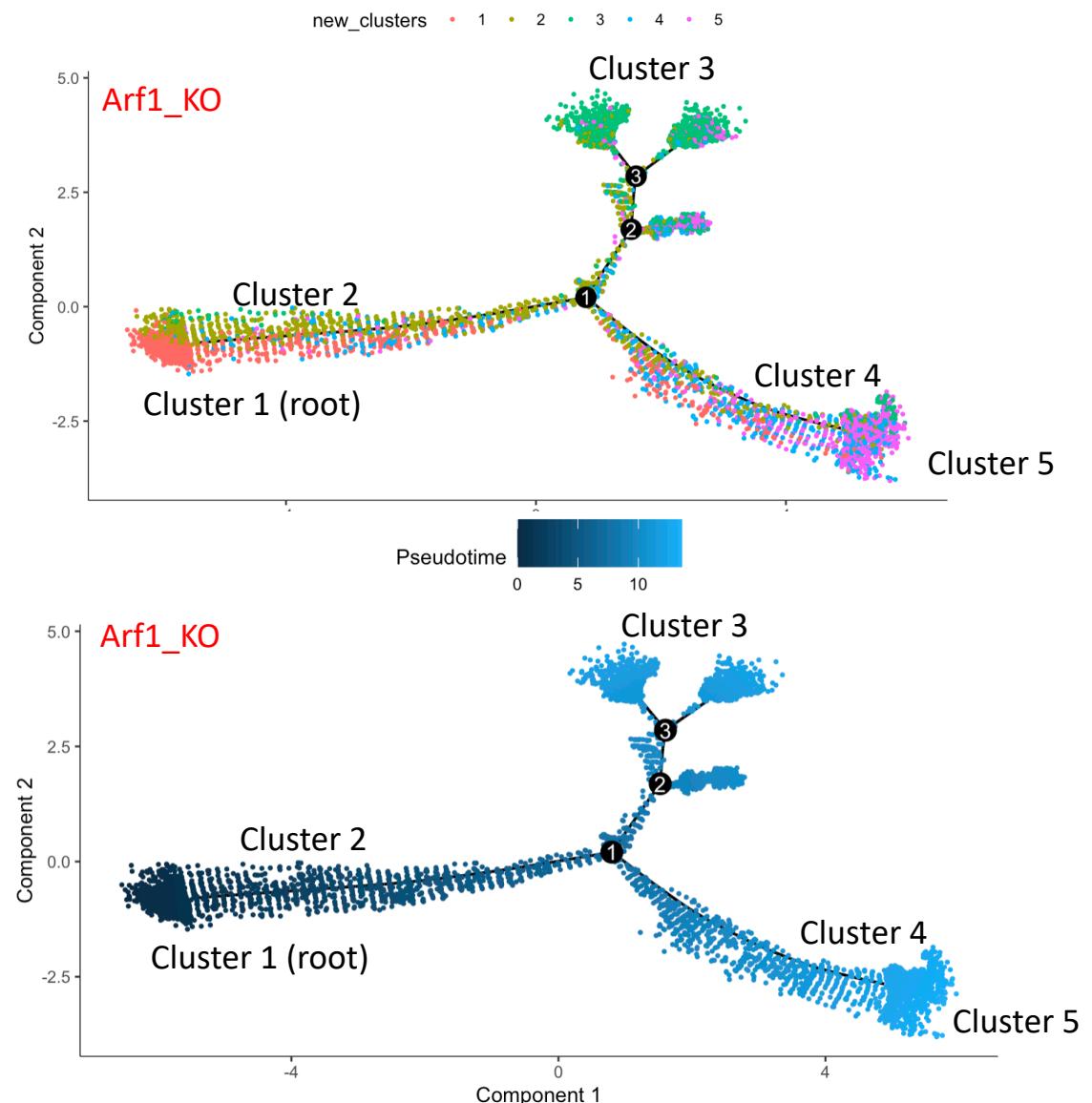
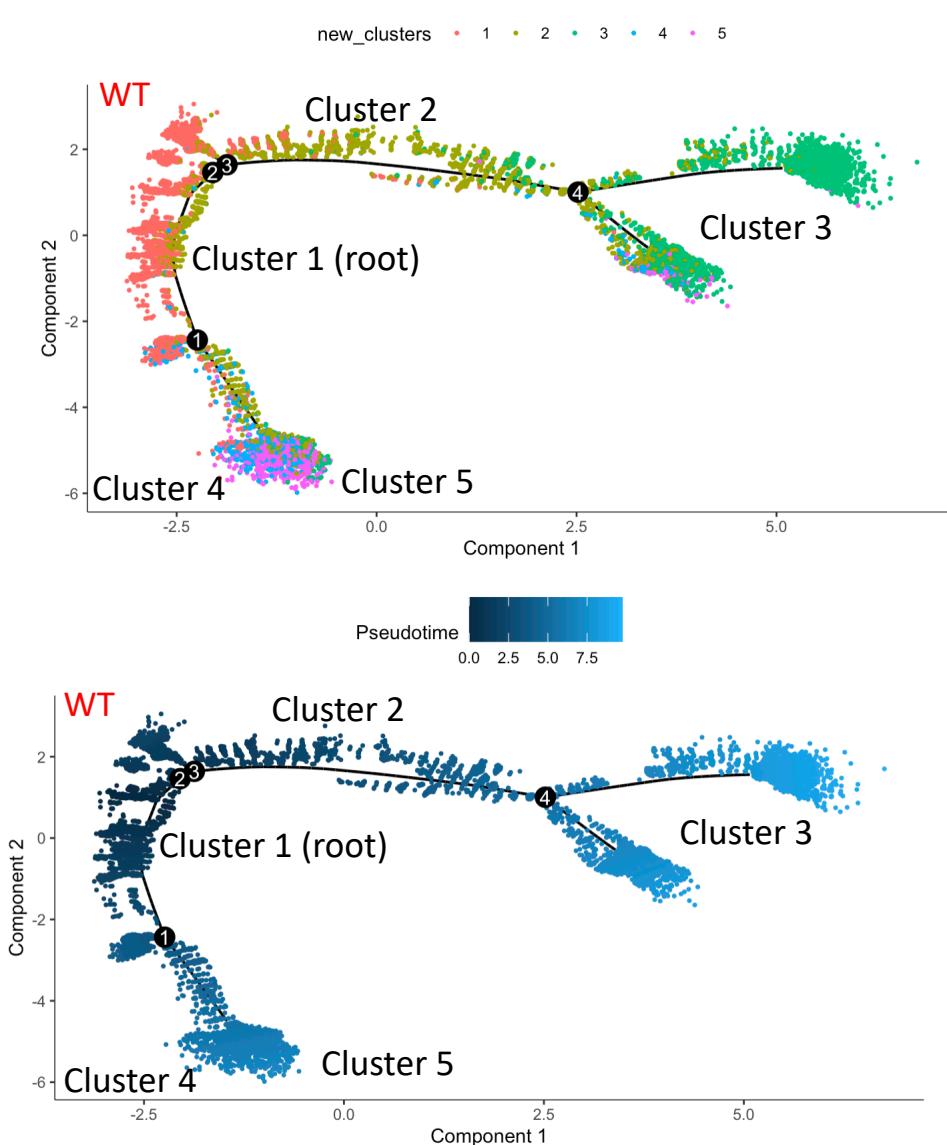
“Add Signature Score” aggregate individual marker score (AddModuleScore) from a gene list



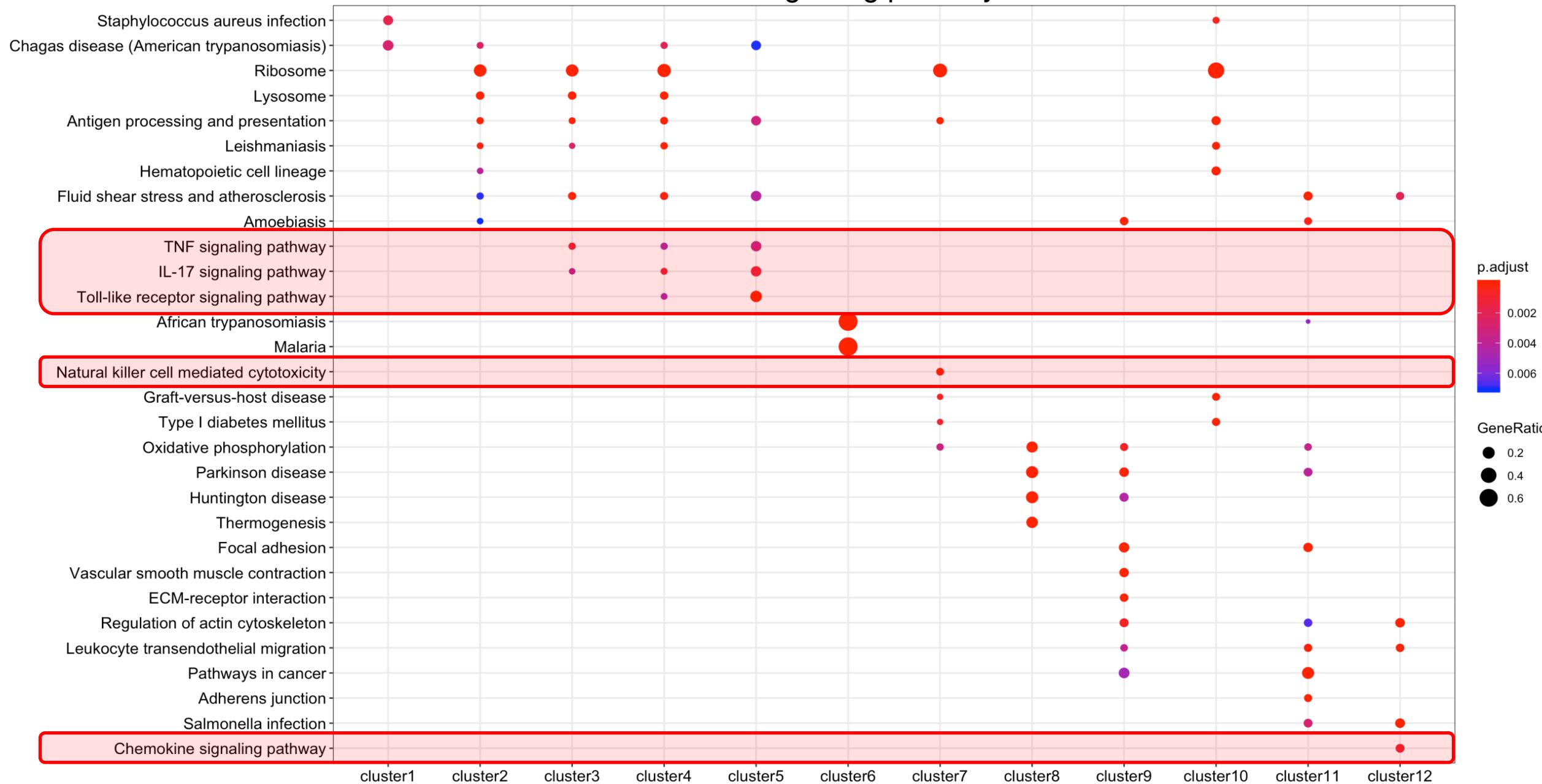


Signature score for homeostatic gene set

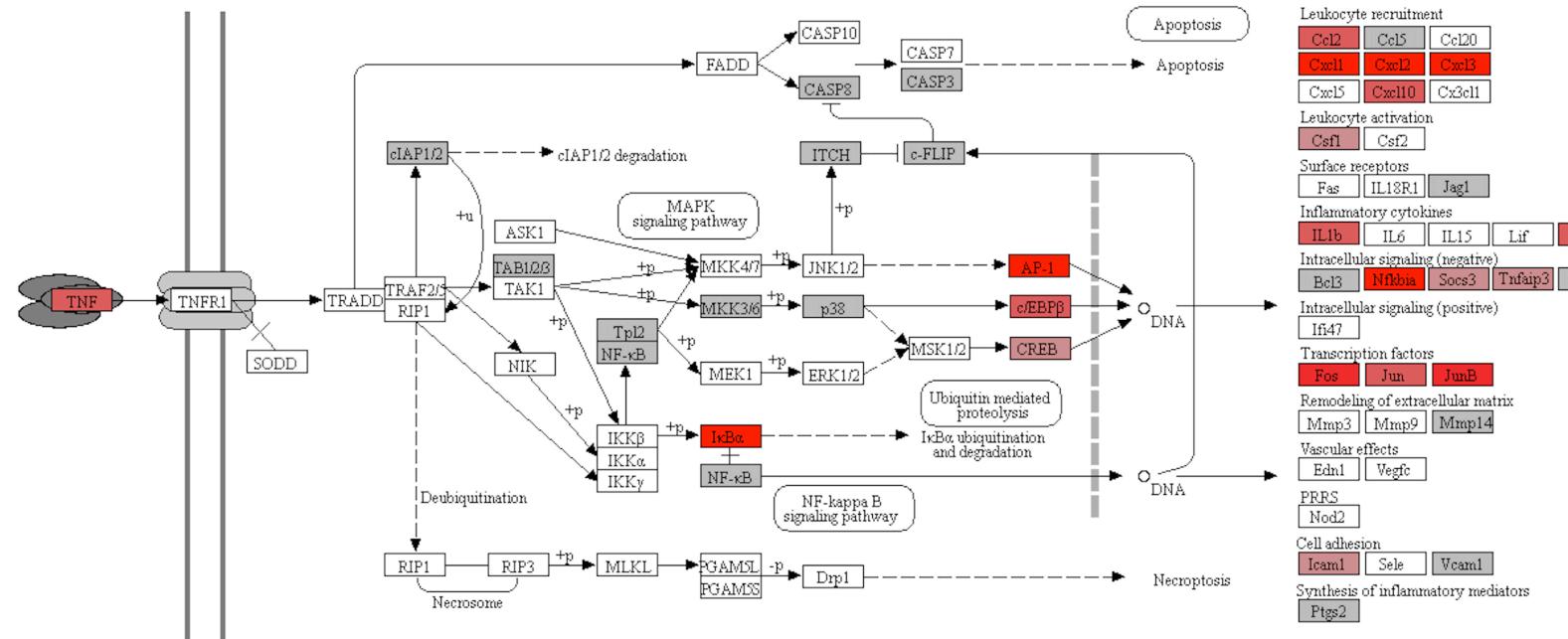
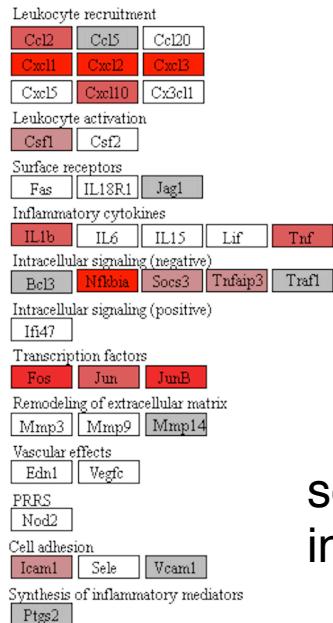
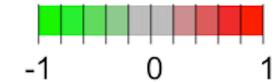
Trajectory analysis (pseudotime) shows Cluster3 has a different fate than Cluster4, 5 (1000 most variable genes across clusters)



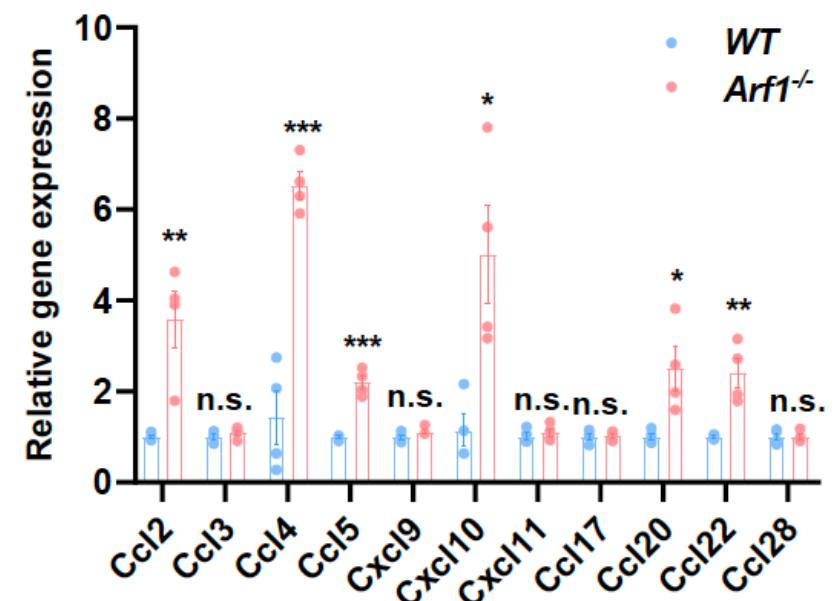
Inflammation signaling pathways

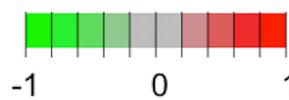


TNF SIGNALING PATHWAY

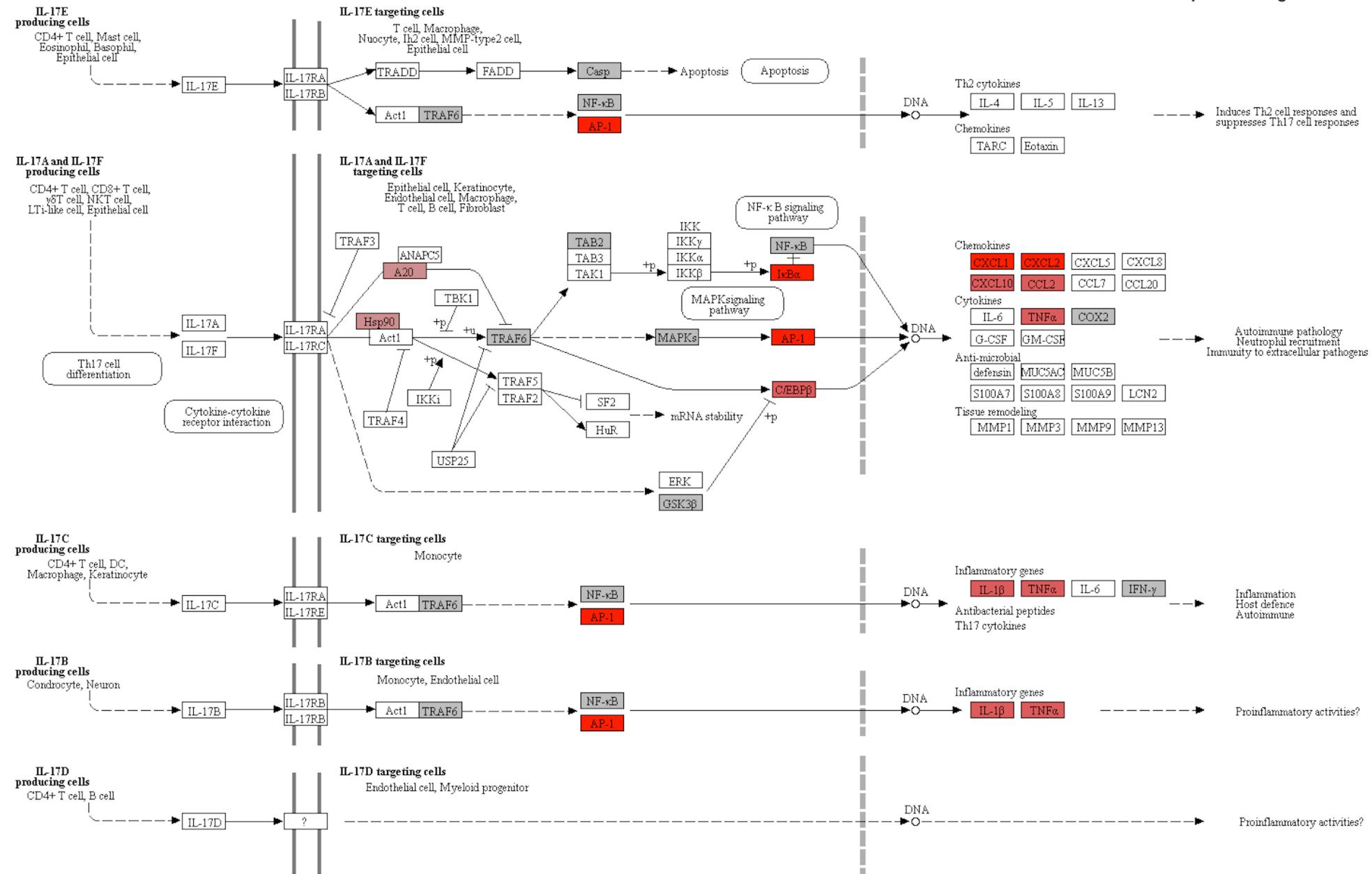


several chemokines were significantly increased in Arf1-ablated mice





IL-17 SIGNALING PATHWAY



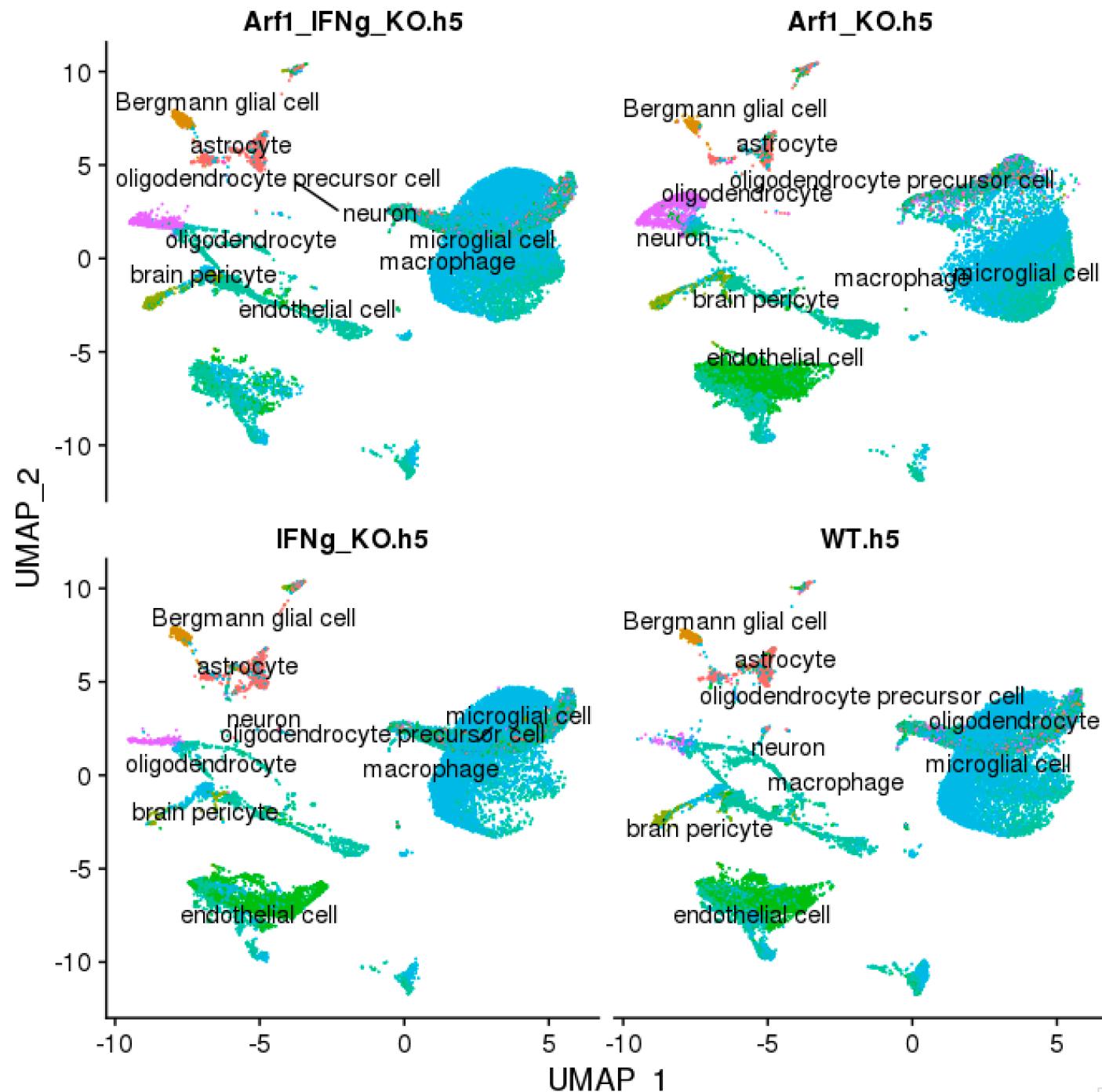
Summary

- Arf1 KO shows similar microglia gene dysregulation signature to AD, ALS, MS. Several chemokines were significantly increased in Arf1-ablated mice
- A microglia subtype - microglia Cluster 5 is identified and is strongly associated with Arf1-deficient neurodegeneration. Cluster 5 markers include some of the previously known neurodegenerative risk factors *Apoe*, *Cd63*, *Cxcl2*, *Cd52*, *Ctsb*, *H2-D1*, *Fth1*, and *Spp1*
- Cluster 5 is also characterized by down-regulation of several microglia homeostatic genes, including *P2ry12*, *P2ry13*, *Tmem119*, *Selplg*, *Cx3cr1*, *Hexb*, and *Siglech*
- Cluster1 has the opposite expression in “disease markers” and “homeostatic markers” compared to Cluster 5. Cluster 2 and Cluster 4 has “in-between” expression for these genes, and may represent transitional state from homeostatic to pro-inflammatory state.
- ORA indicates cluster 5 is enriched in inflammatory pathways including TNF signaling pathway and IL-17 signaling pathway.
- The pathogenic phenotype of Arf1 knock-out is associated with a shift in the distribution of number of cells from Cluster 1 to Cluster 5. Arf1-KO microglia cells are depleted in Cluster1 and enriched in Cluster 5 compared to three other samples. Percentage of cells in Cluster 5 for WT (5%), for Arf1_KO(15%).

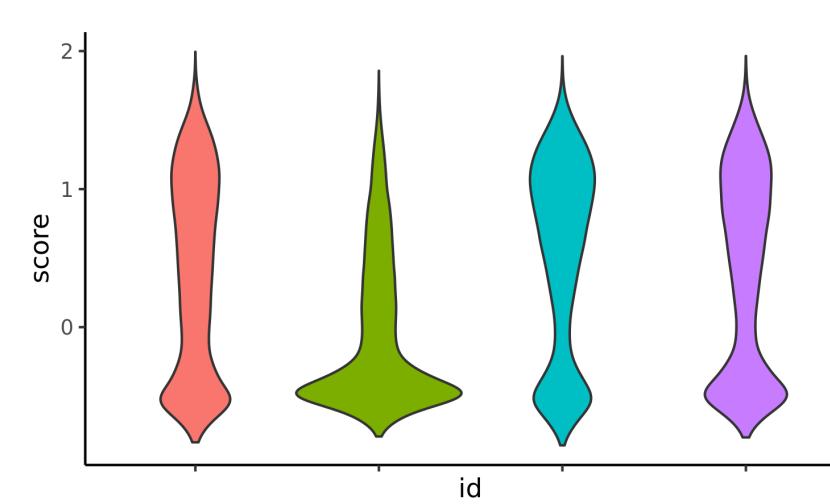
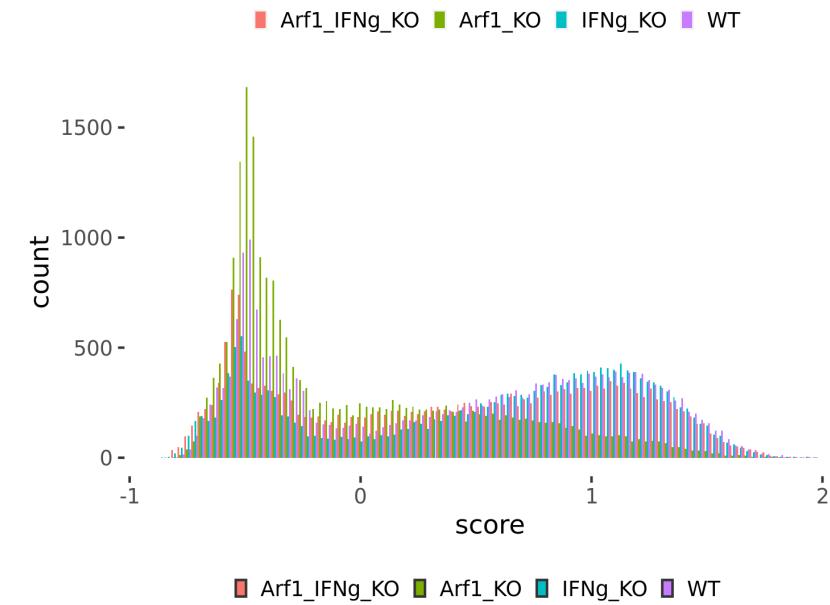
Supplementary

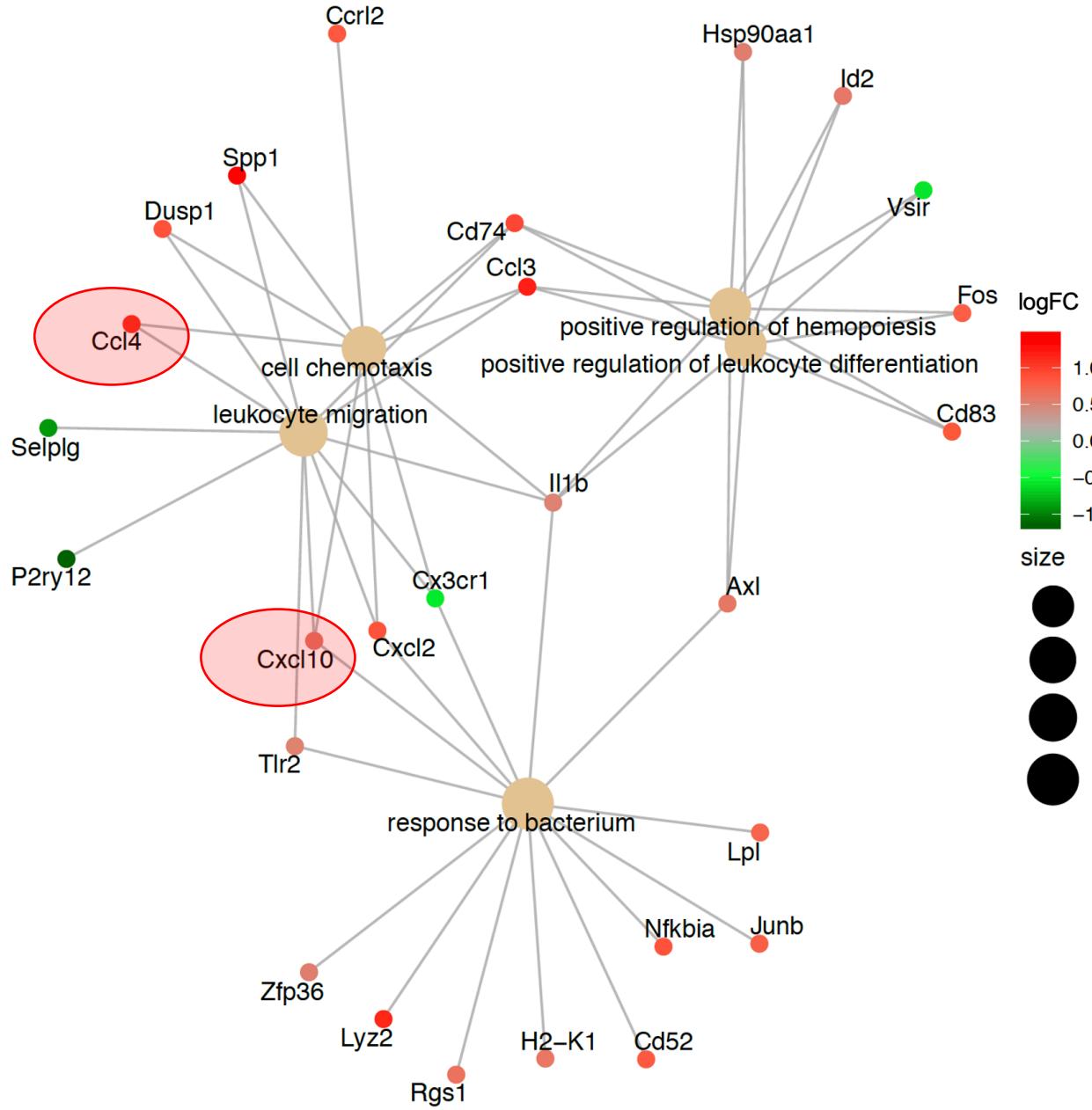
Cell identity and count by sample

cell identity	Arf1_IFNg_KO.h5	Arf1_KO.h5	IFNg_KO.h5	WT.h5
astrocyte	1136	554	1177	1049
Bergmann glial cell	380	230	230	297
brain pericyte	461	349	241	266
endothelial cell	788	3473	3053	3276
macrophage	6387	6463	3853	5193
microglial cell	13238	7683	14105	13763
neuron	56	282	58	81
oligodendrocyte	1023	2988	438	602
oligodendrocyte precursor cell	15	14	11	4
Total	23484	22036	23166	24531

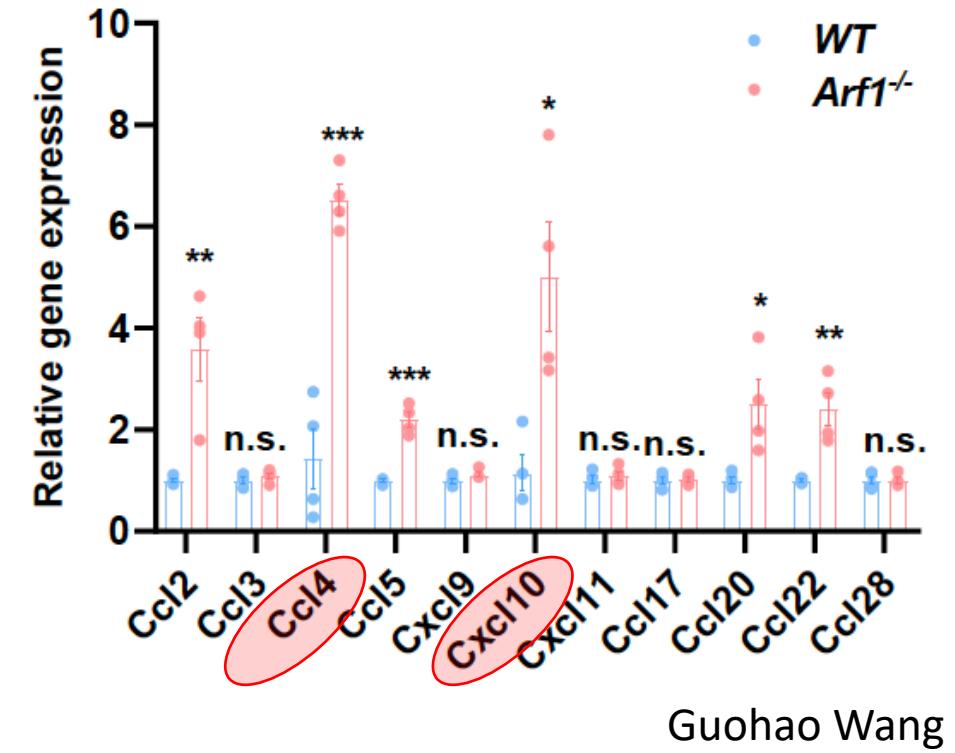


Down-regulation of microglia
markers in Arf1_KO compared to
three other samples



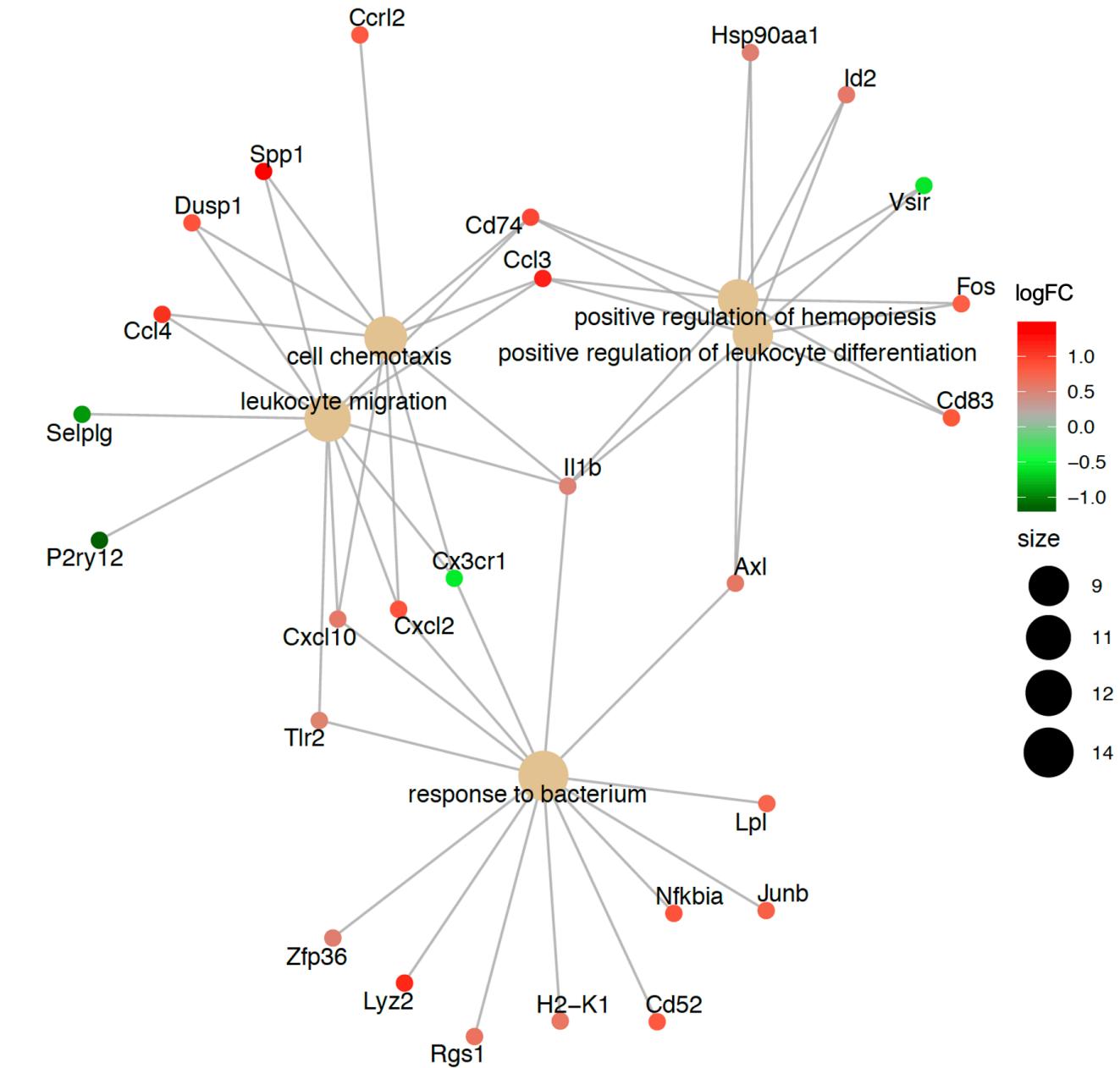
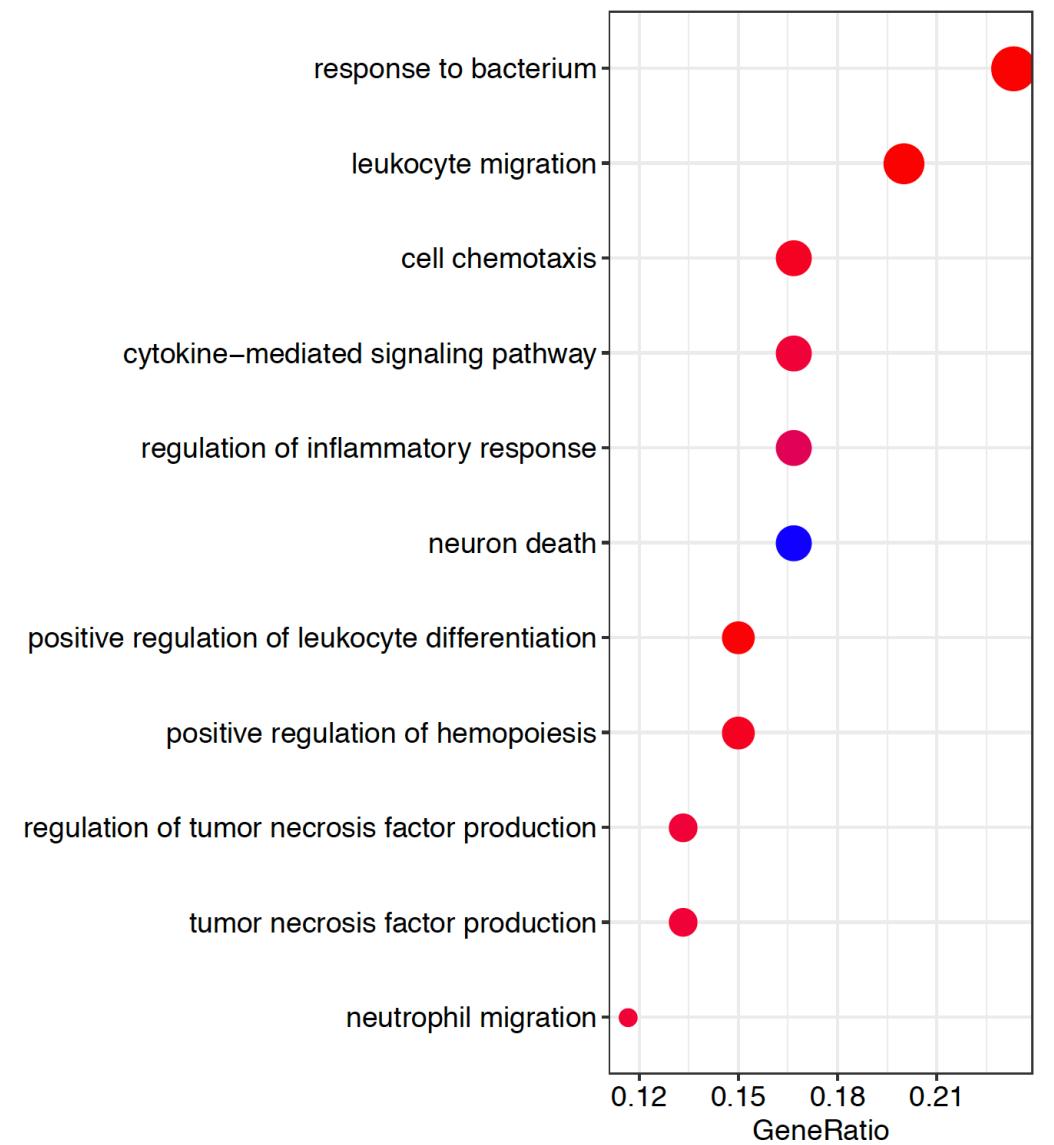


several chemokines were significantly increased in Arf1-ablated mice



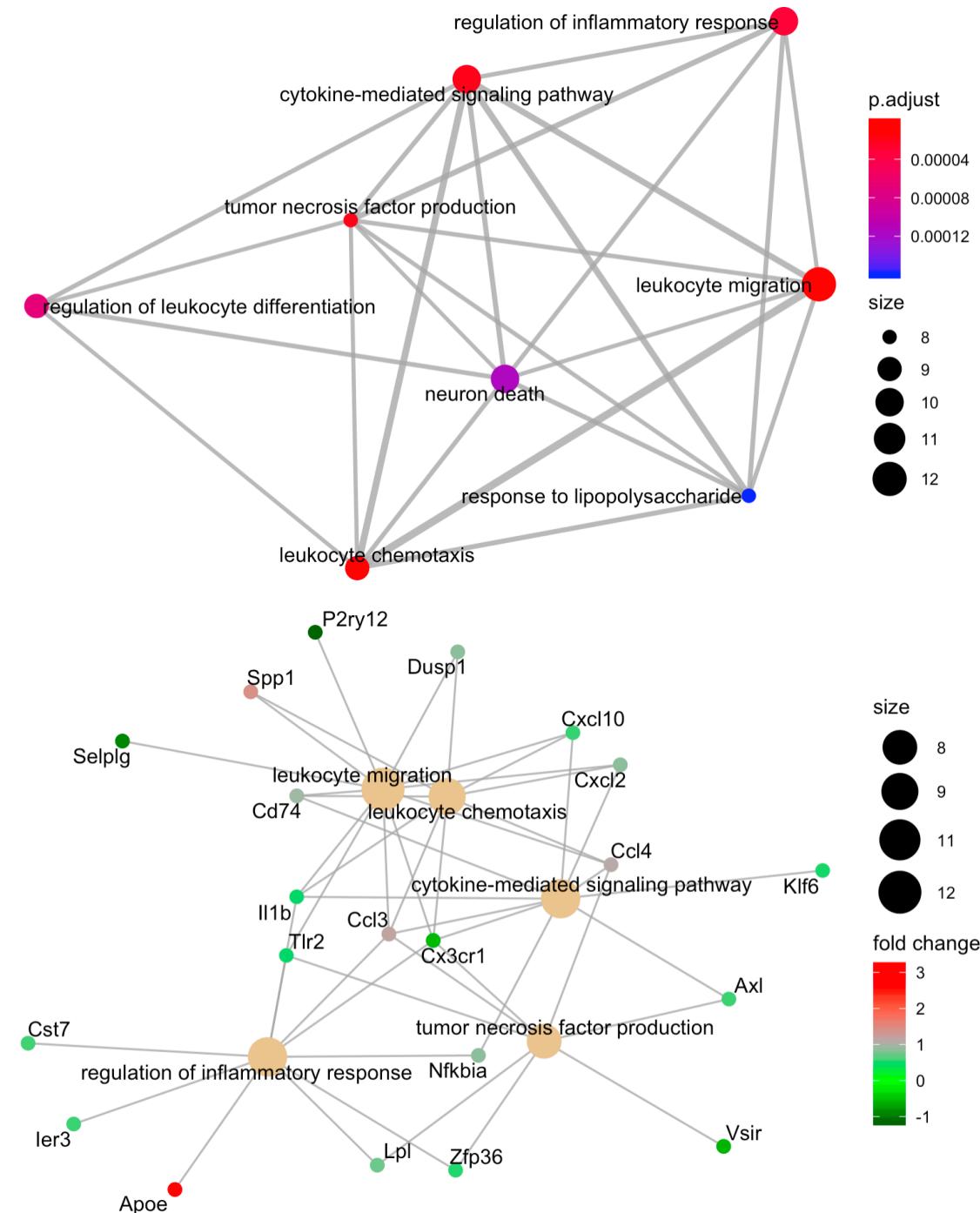
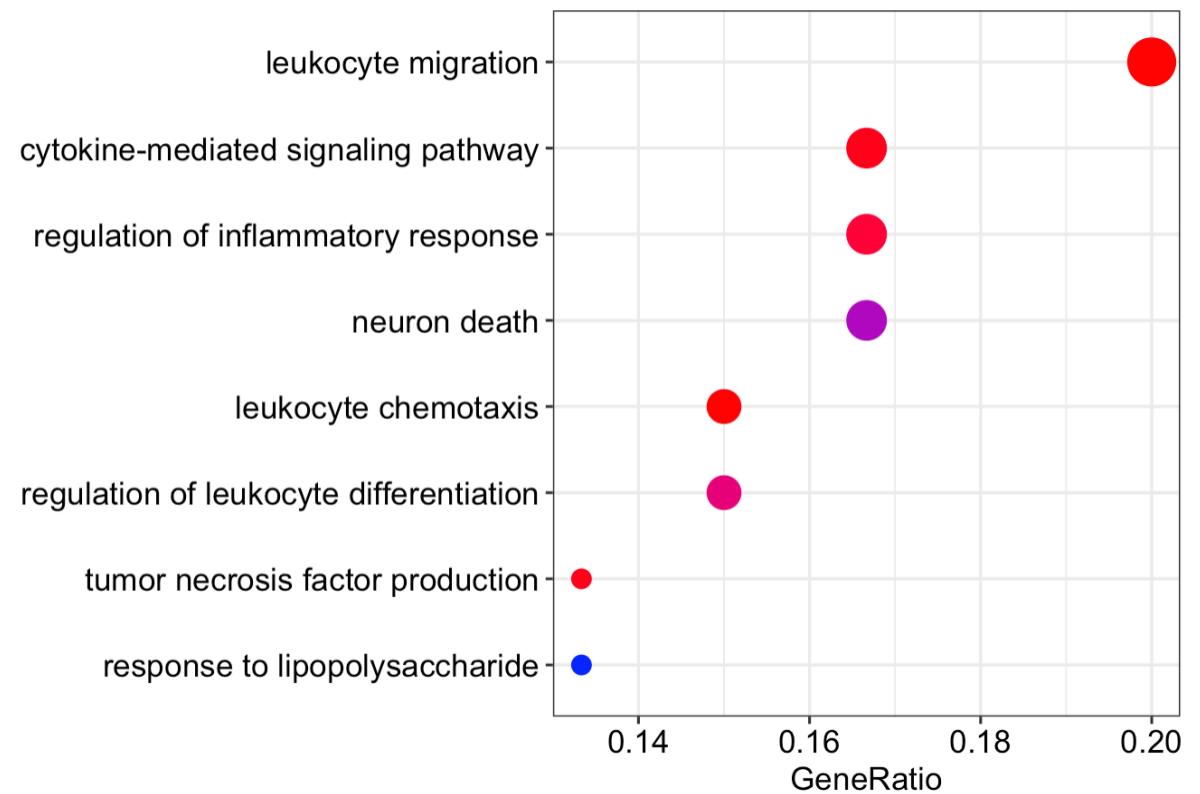
Guohao Wang

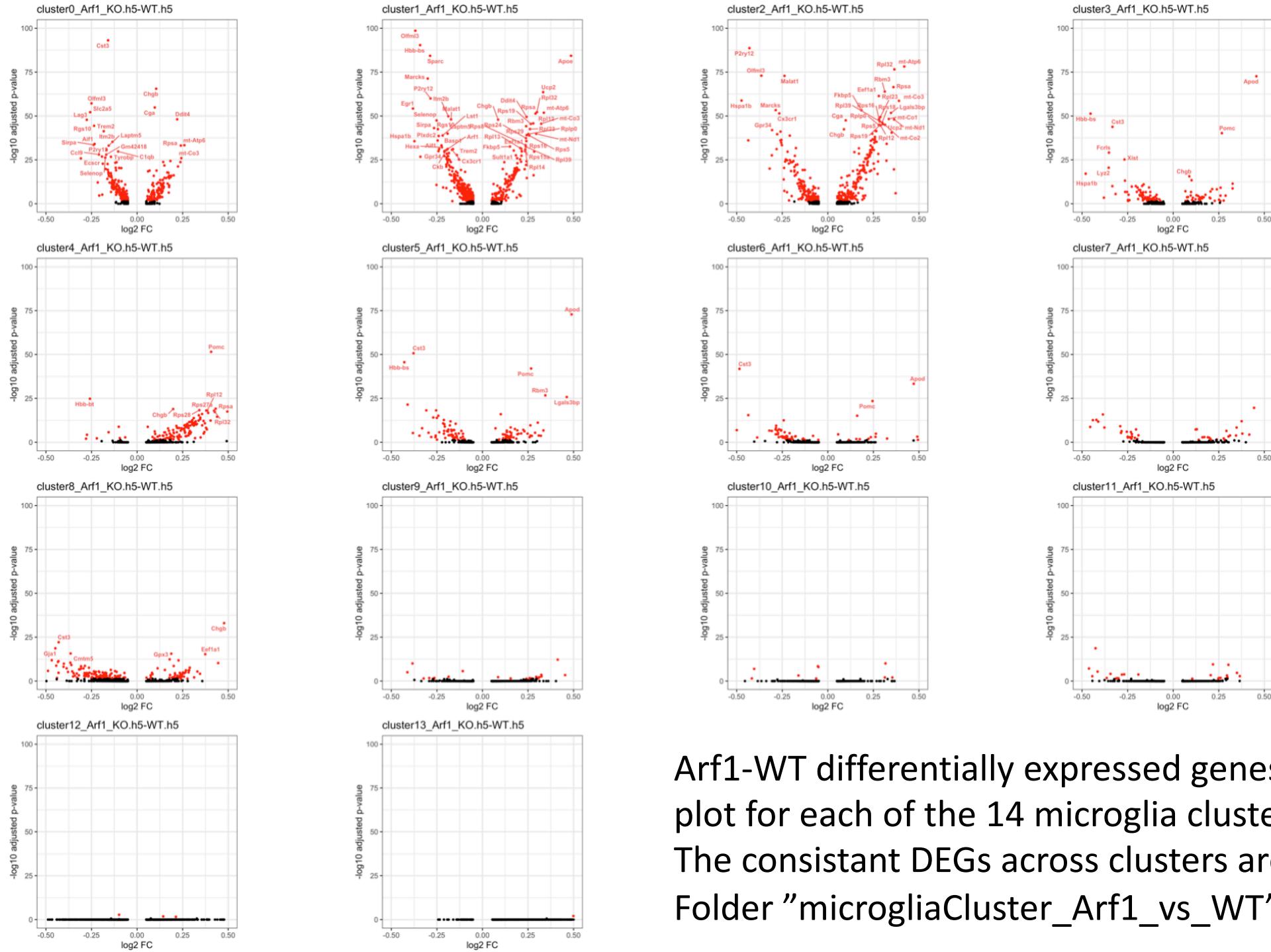
Cluster5 - enriched pathways



Cluster5 - enriched pathways

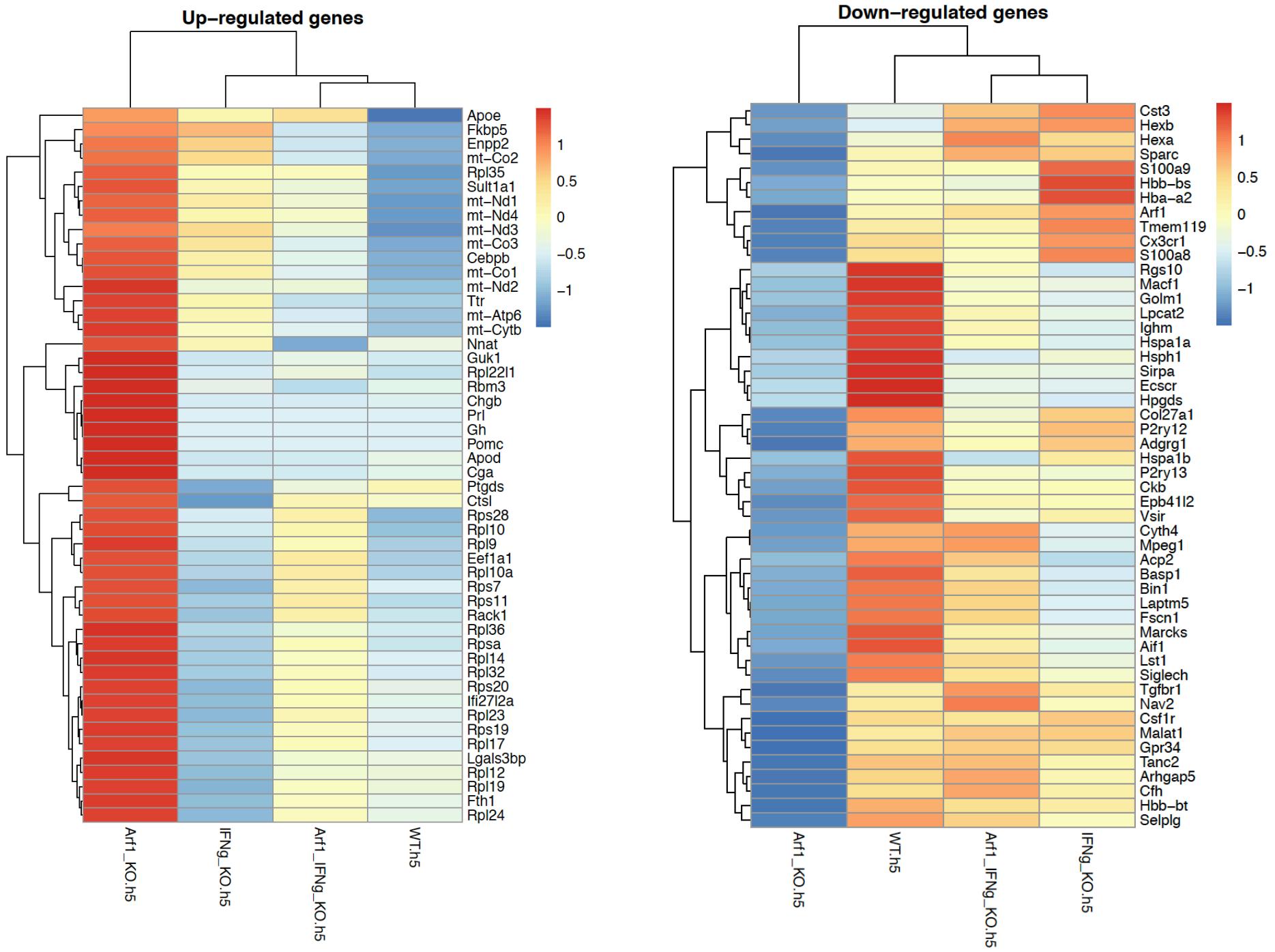
From file: myCluster5_cluster1_deg_ORA



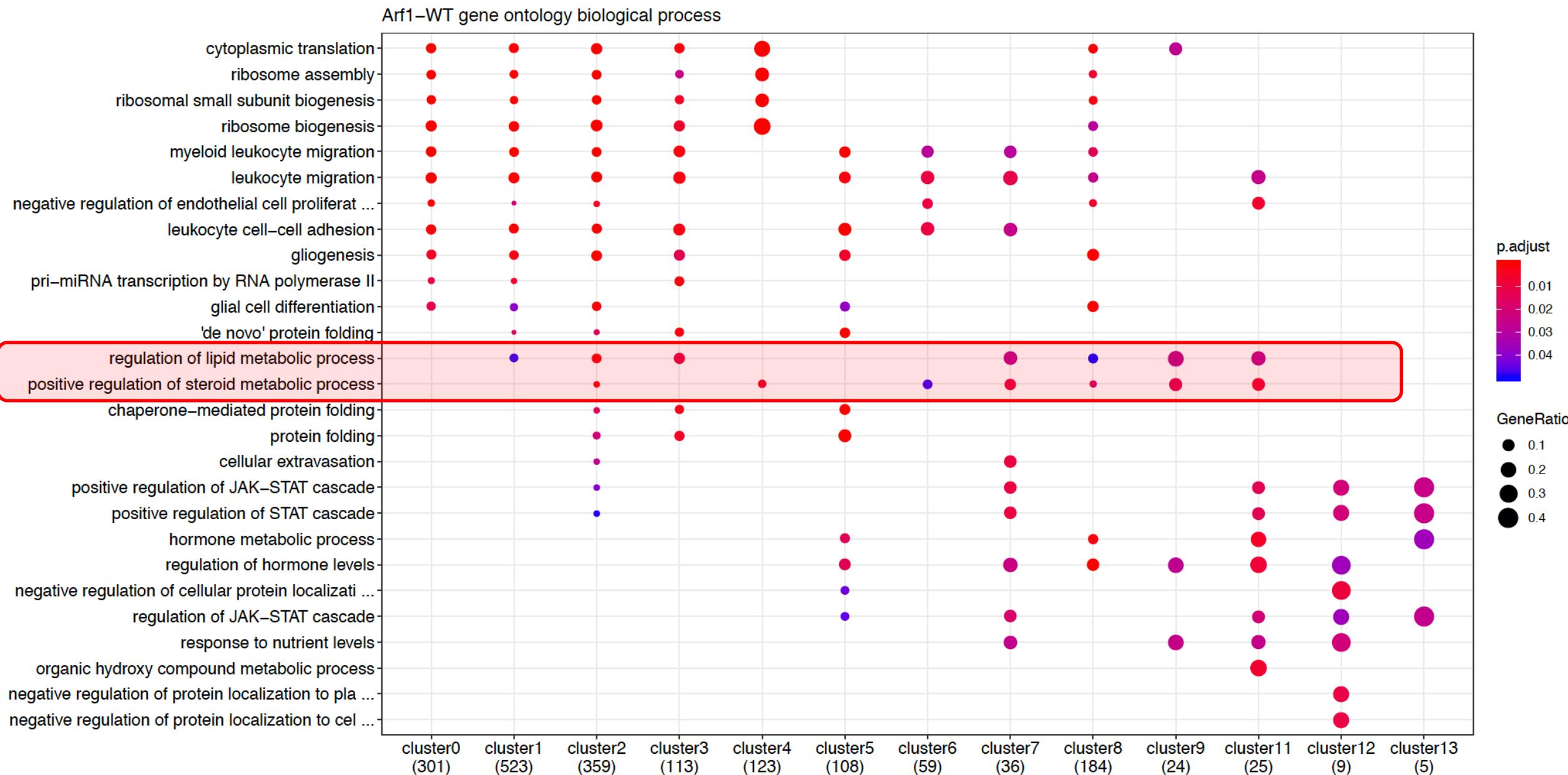


Arf1-WT differentially expressed genes volcano plot for each of the 14 microglia clusters
The consistant DEGs across clusters are in
Folder "microgliaCluster_Arf1_vs_WT"

Up and down-regulated genes in Arf1 vs. all

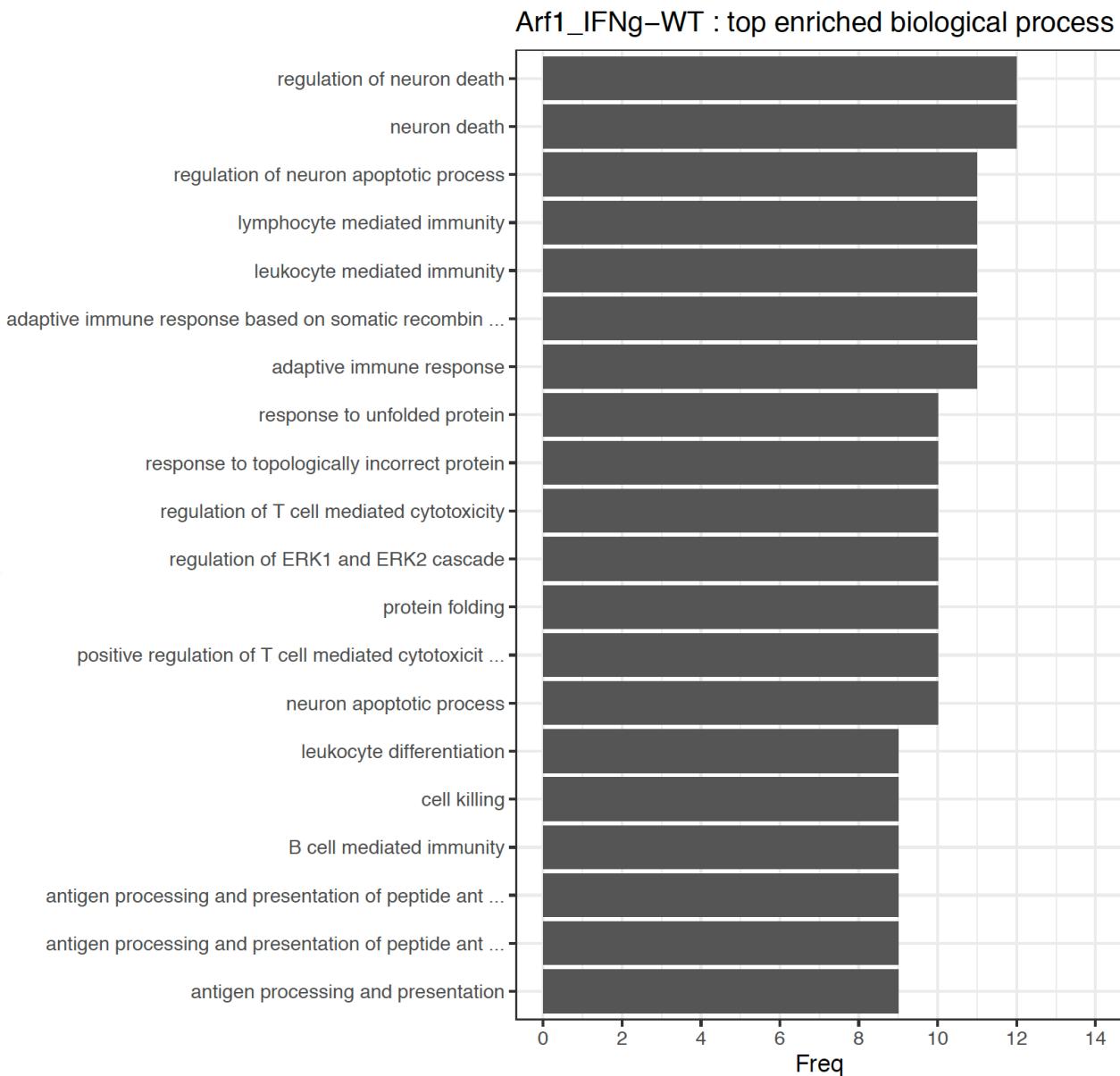
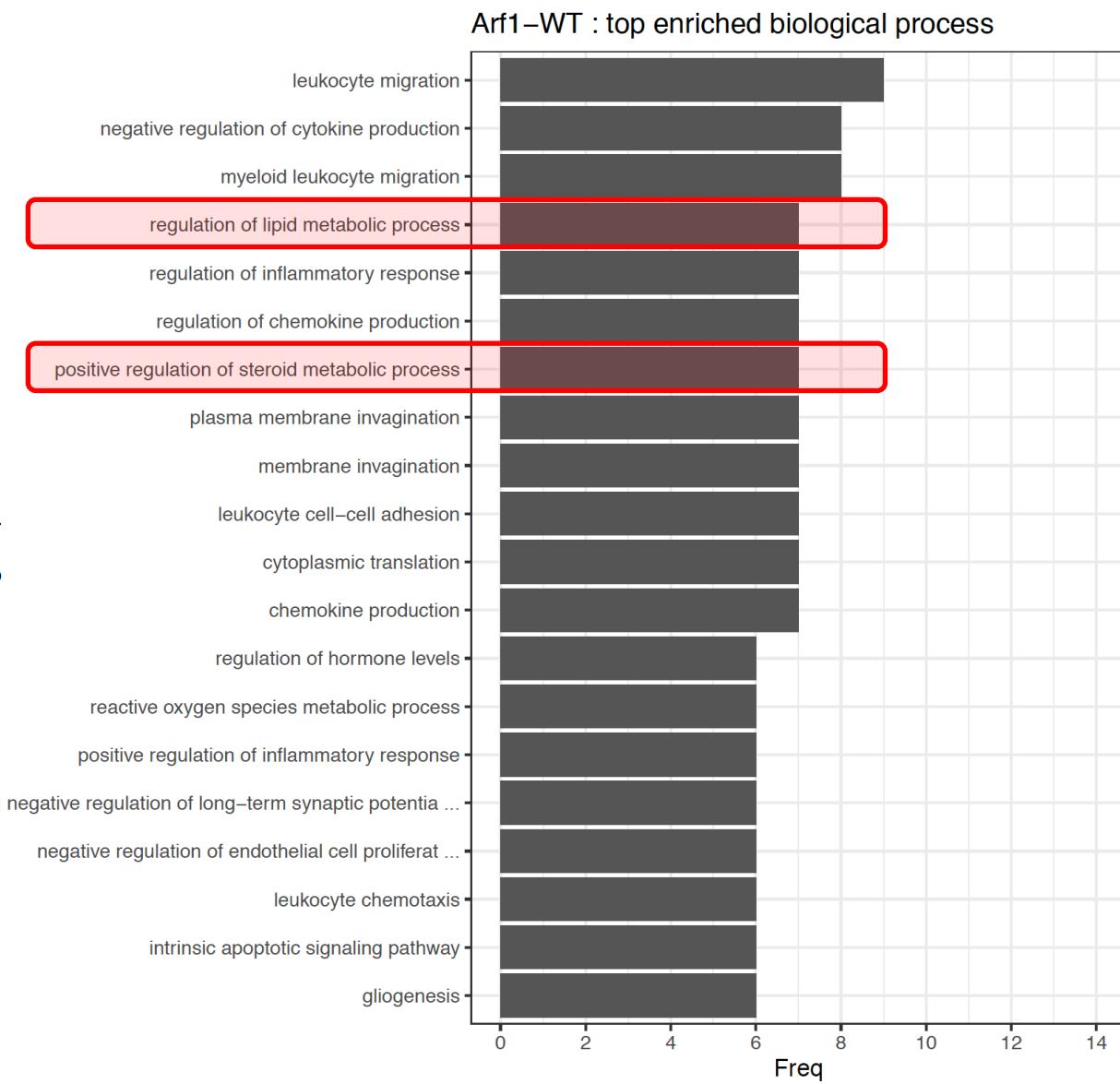


Overlap of significantly over-represented processes in Arf1-WT for each microglial cluster

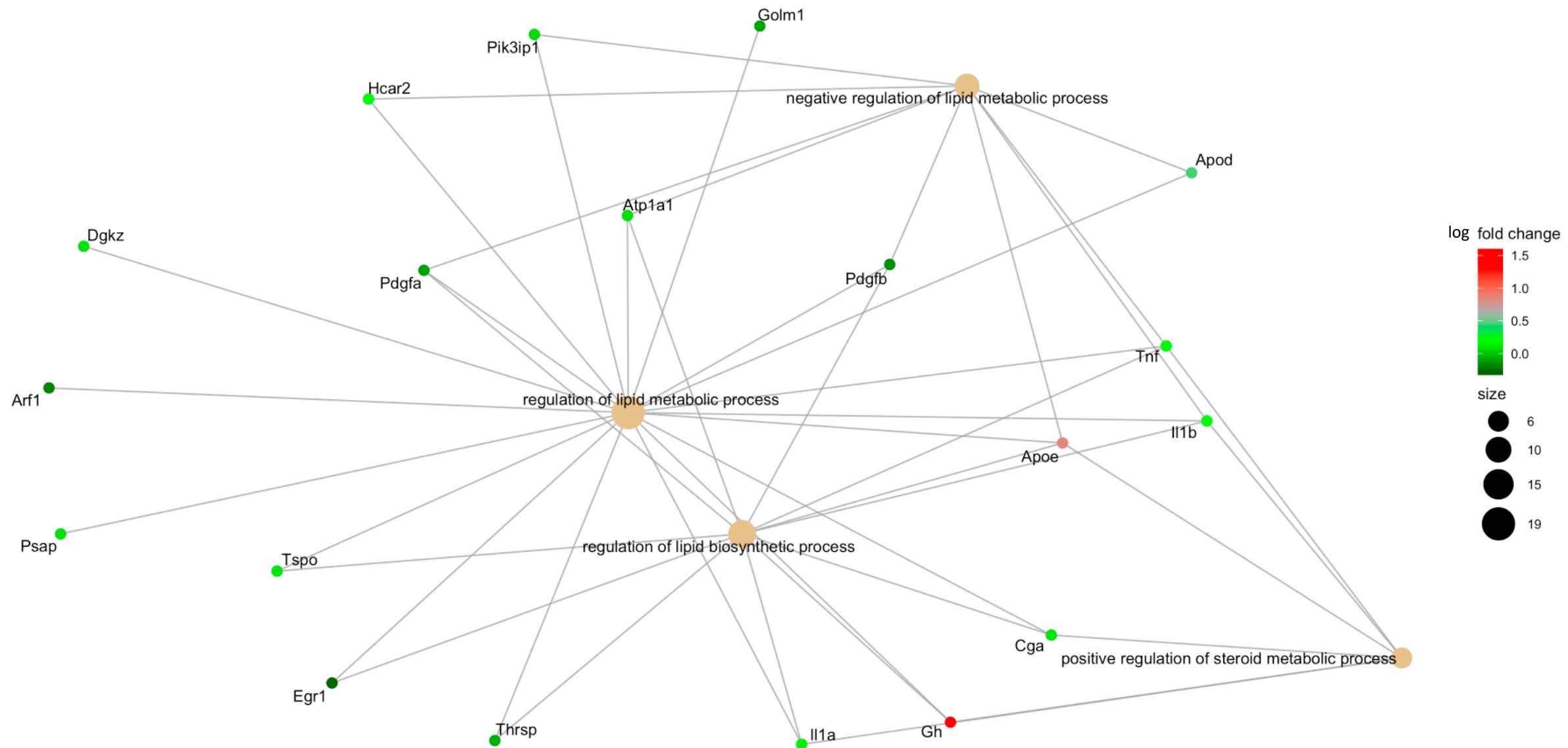


Most frequent biological processes in different sample comparisons

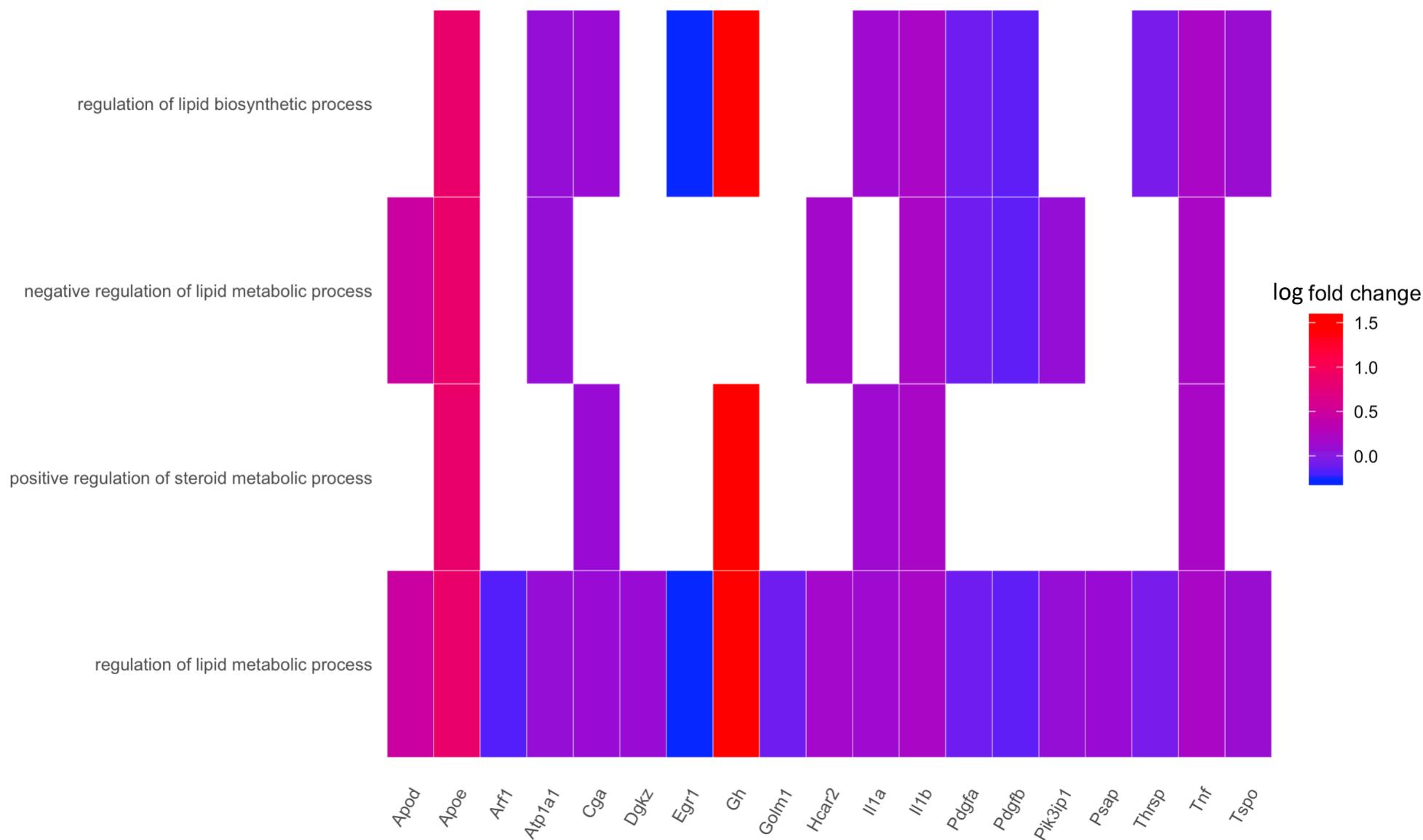
biological process



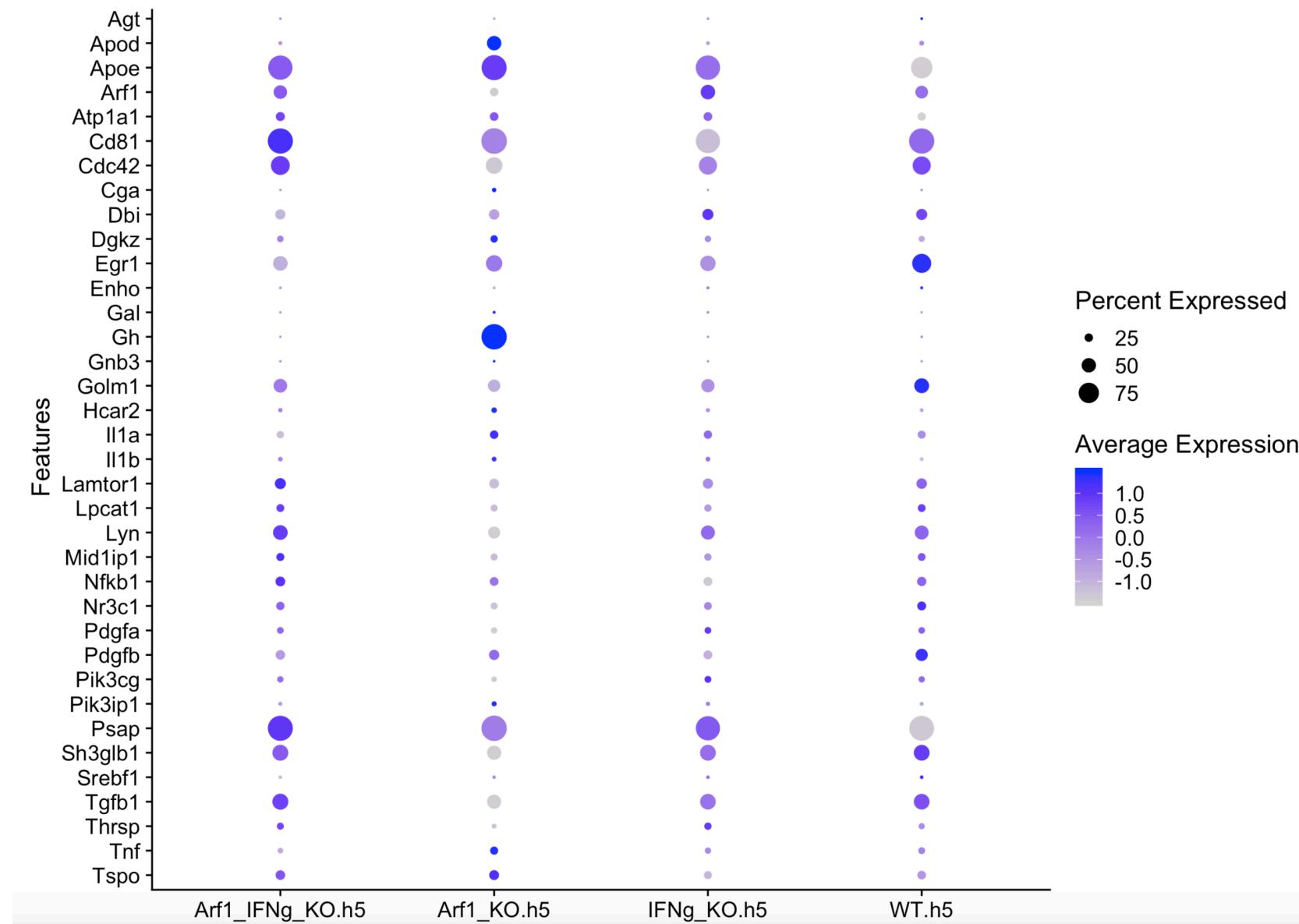
lipid metabolic process network



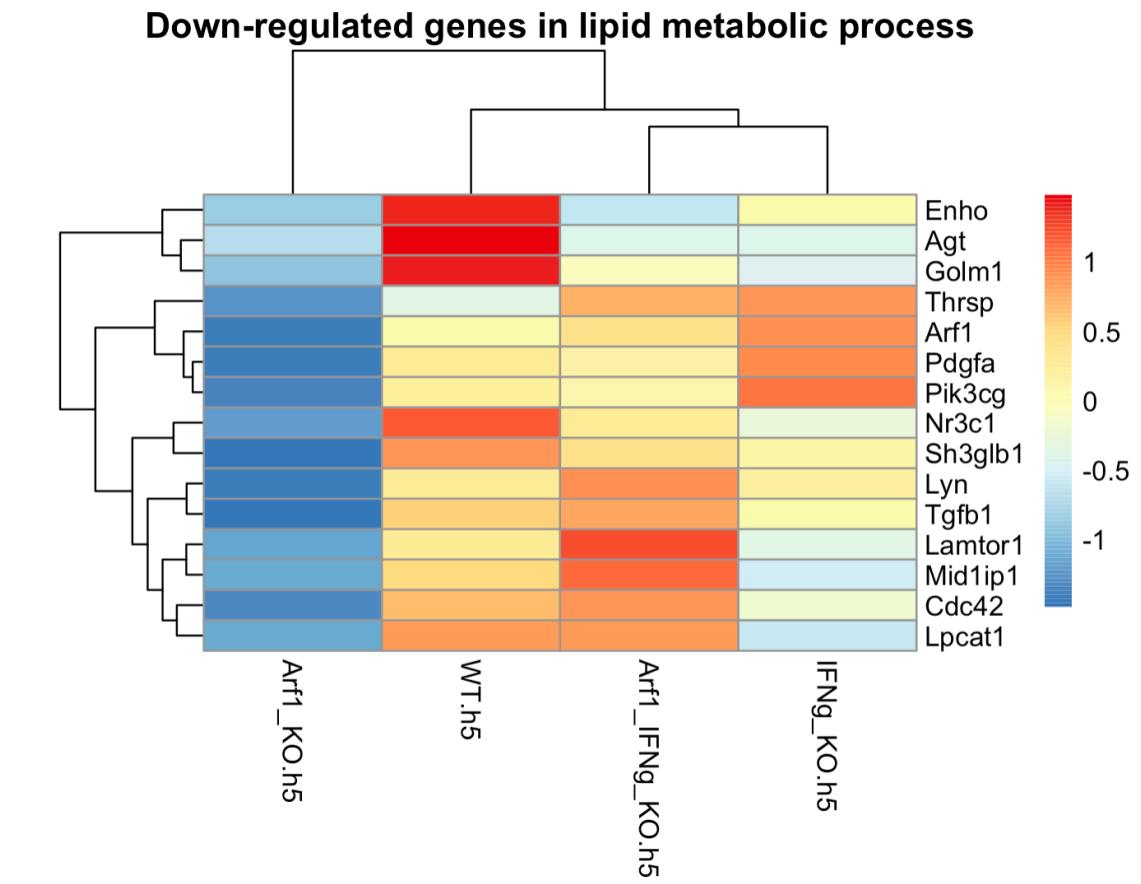
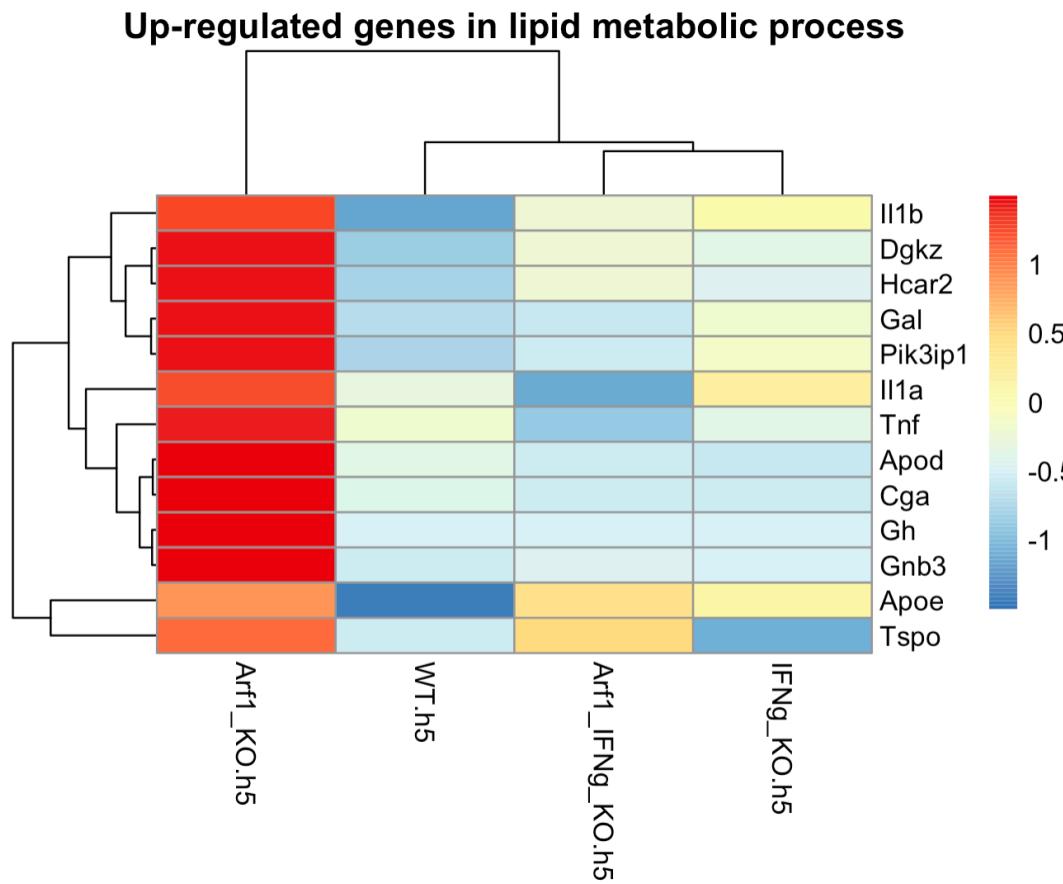
Differentially expressed genes in the lipid metabolic process



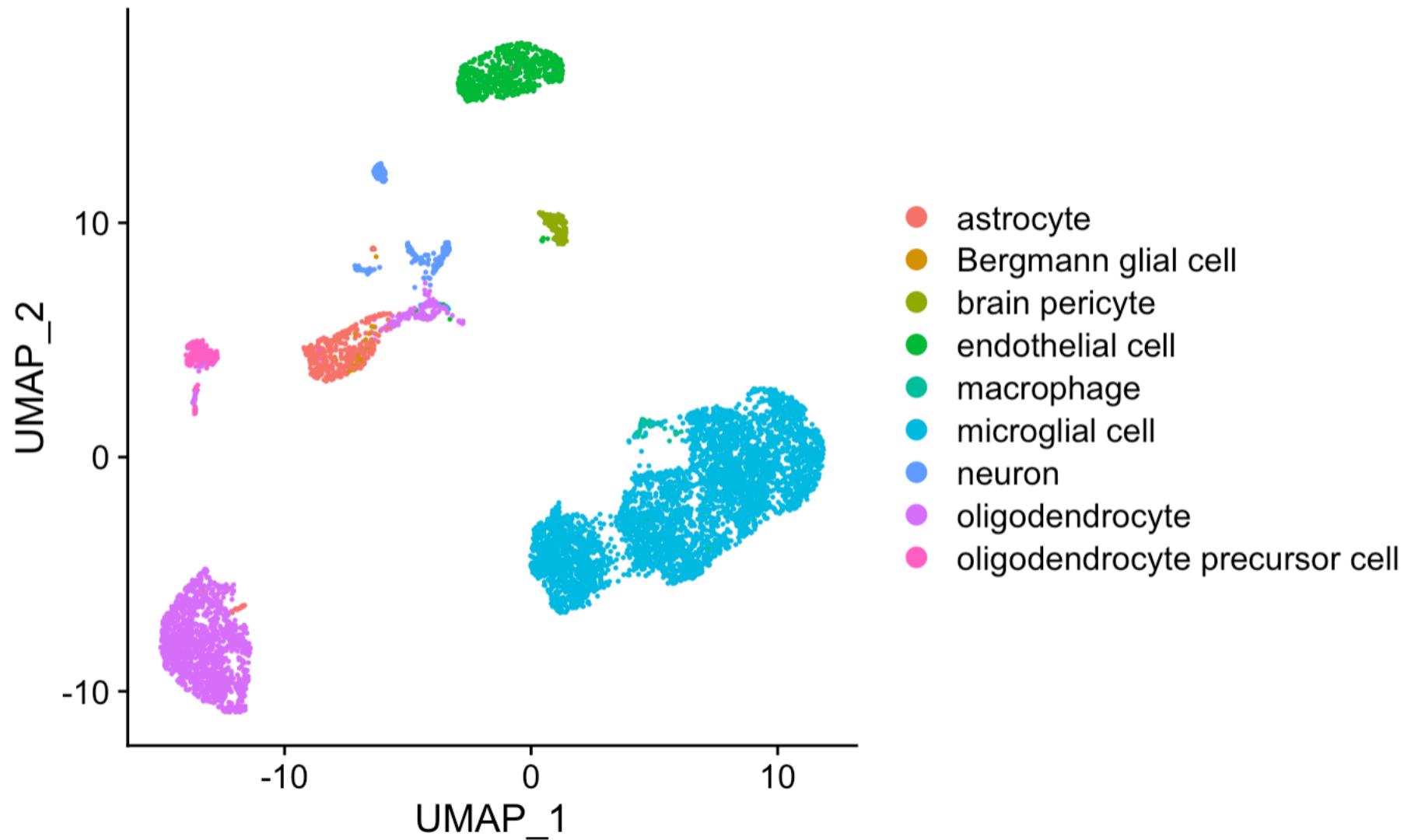
Differentially expressed genes in the lipid metabolic process – across samples



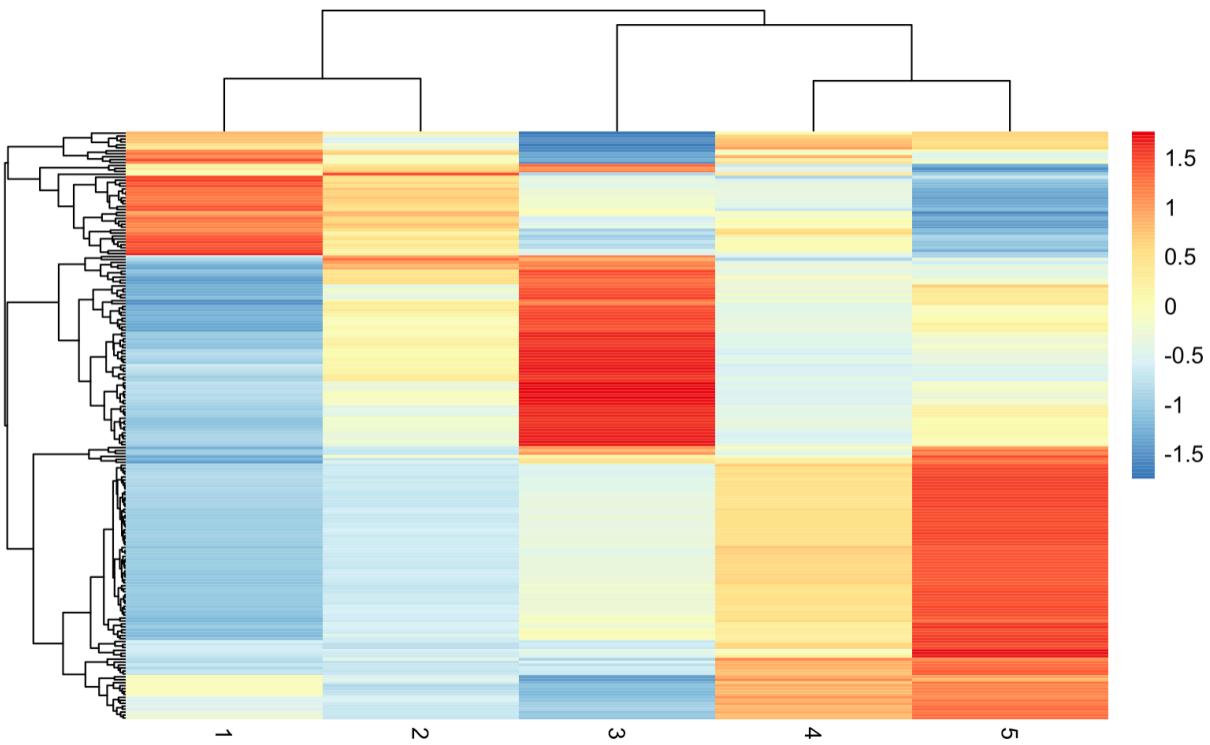
Differentially expressed genes in the lipid metabolic process – across samples



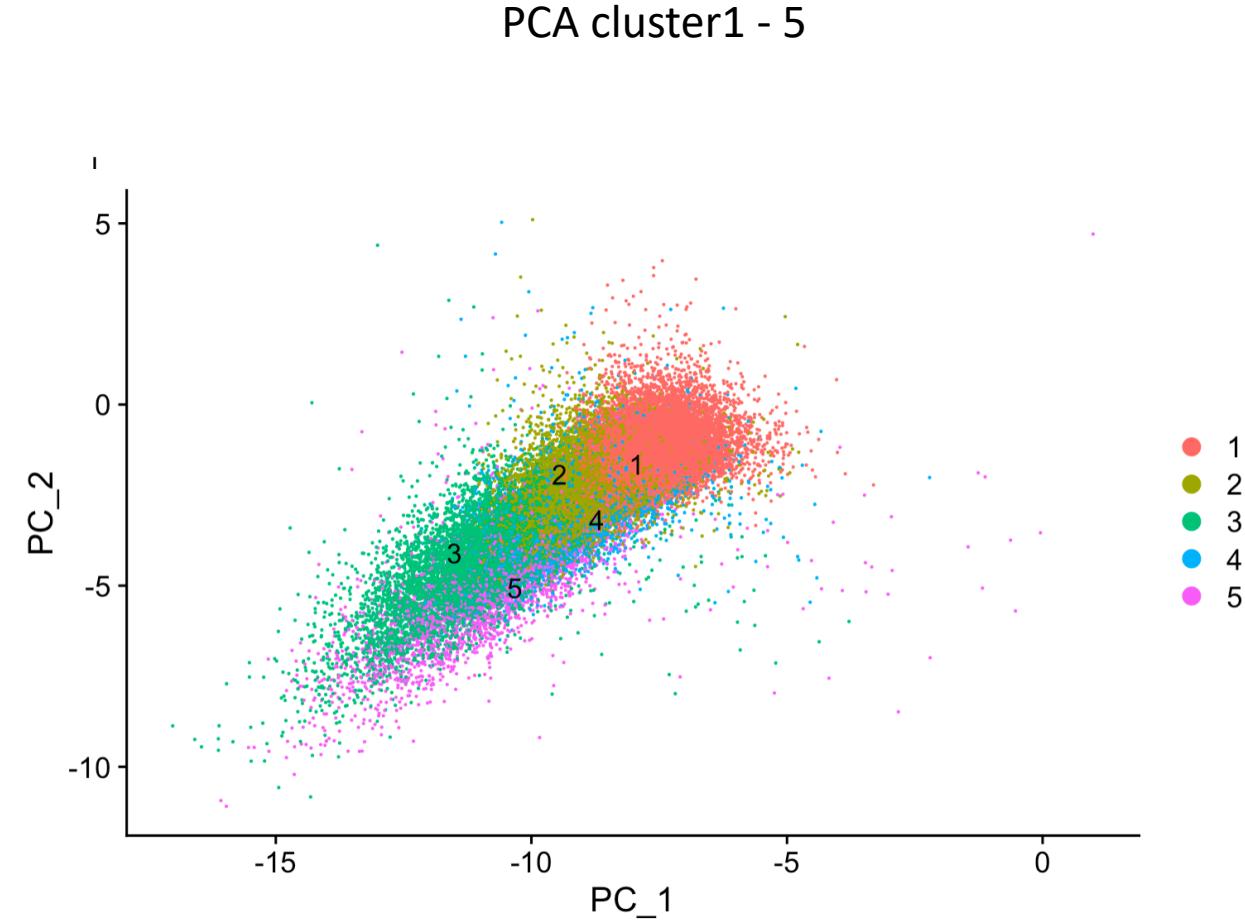
Tabula Muris mouse brain cell atlas



Top 500 most variable genes (mean expression) cluster1 - 5



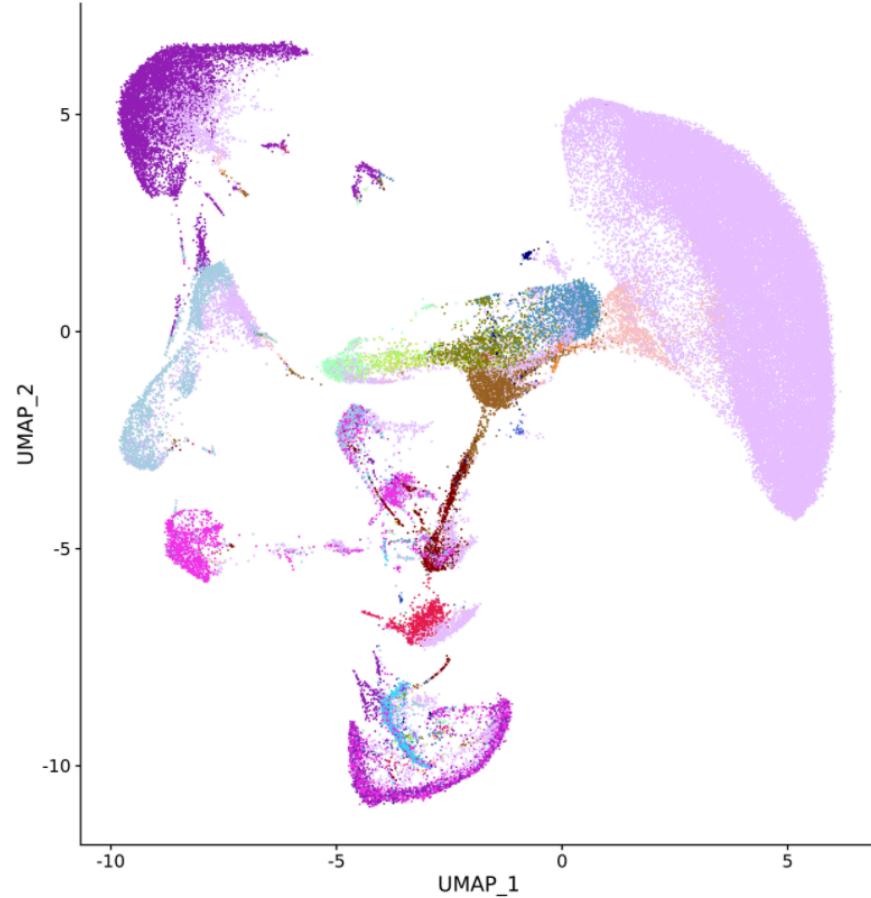
PCA cluster1 - 5



Mushrooms?!

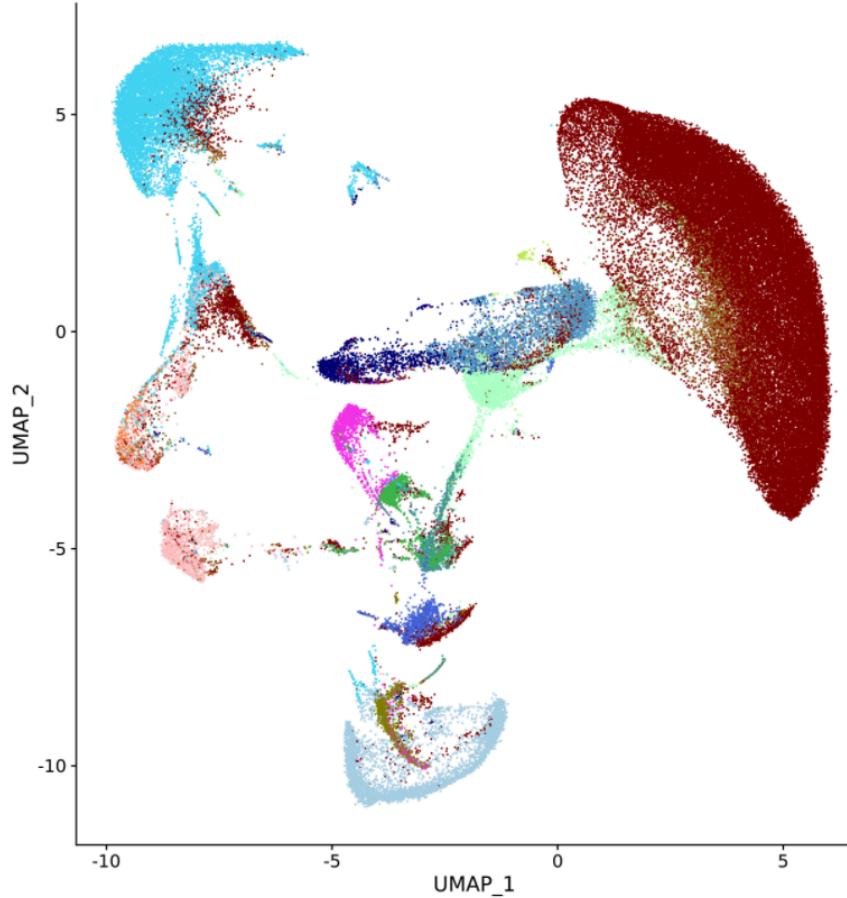
Immgen Main Cell Type Annotations

• B cells • Endothelial cells • Macrophages • Neutrophils • Stromal cells
• B cells, pro • Epithelial cells • Mast cells • NK cells • T cells
• Basophils • Fibroblasts • Microglia • NKT • Tgd
• DC • ILC • Monocytes • Stem cells



Mouse RNAseq Main Cell Type Annotations

• Adipocytes • Dendritic cells • Fibroblasts • Microglia • Oligodendrocyte
• Astrocytes • Endothelial cells • Granulocytes • Monocytes • T cells
• B cells • Epithelial cells • Hepatocytes • Neurons • NK cells
• Cardiomyocytes • Erythrocytes • Macrophages • Neurons • NK cells



<https://nidap.nih.gov/workspace/vector/view/ri.vector.main.workbook.498ab481-d107-4c9e-a7d3-3cca772543c2?branch=master>