

Re-analysis of Marin-Carli et al sMEC Data

Dave Bridges

January 4, 2021

Contents

1 Purpose	1
2 Data Input	1
2.1 Preprocessing	2
2.2 Highly Variable Features	3
2.3 Scaling	3
3 Dimensionality Reductions	3
4 Determination of Number of Clusters	6
5 Clustering Cell Types	8
5.1 Identification and Assignment of Clusters	8
5.2 Non-Linear Dimensionality Reduction	8
6 Feature Analysis	10
6.1 Annotation of clusters	10
6.2 Summary of Clusters	23
7 Session Information	33

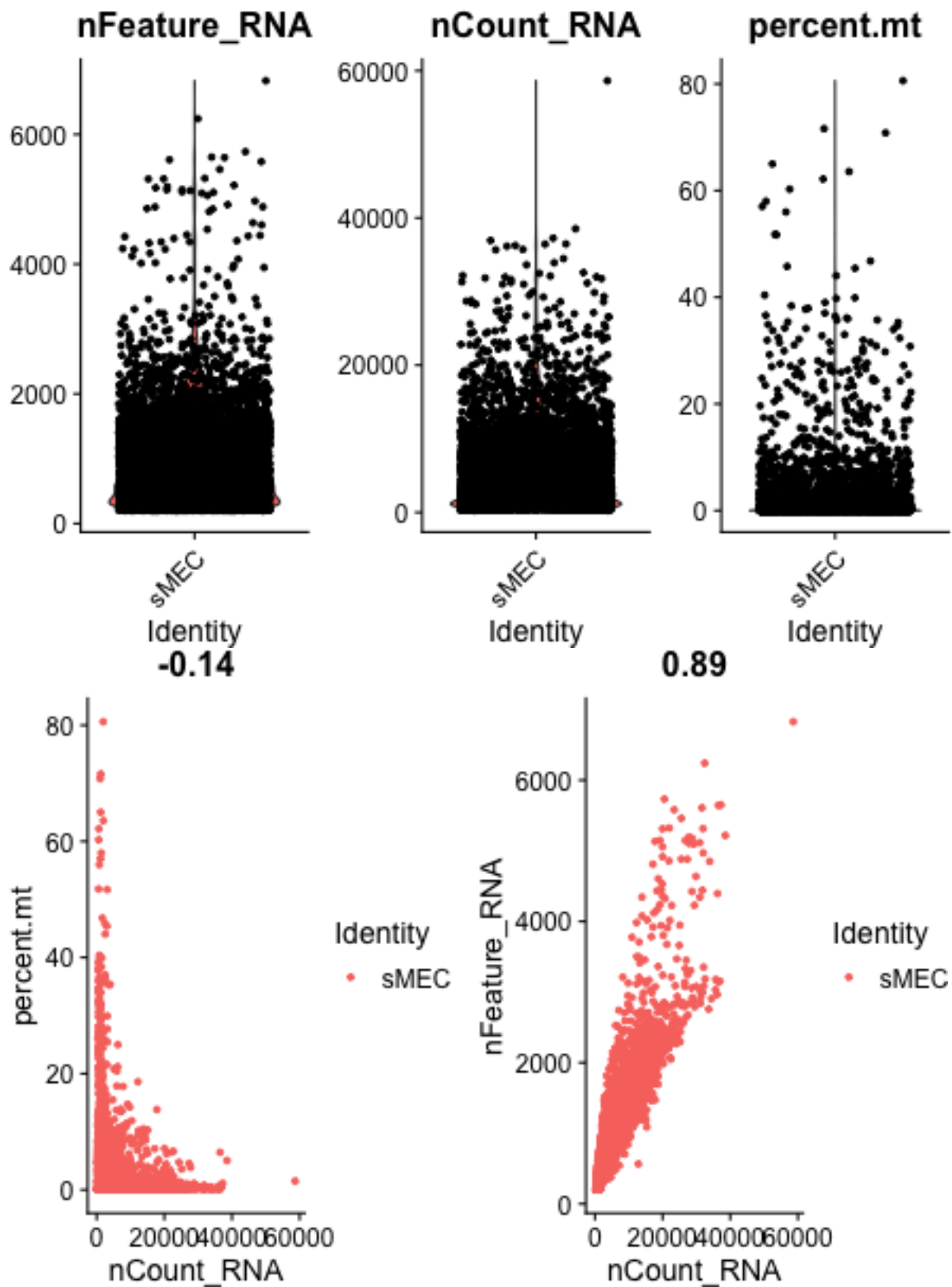
1 Purpose

To re-analyse cell populations from the Martin-Carli *et al*'s scRNAseq study of lactating mammary glands. This work is described in Martin Carli et al. (2020). This follows the analysis flow suggested for Seurat 3.2 seen at https://satijalab.org/seurat/v3.2/pbm3k_tutorial.html

2 Data Input

Downloaded the data from GSE15889 and removed prefixes from filenames.

2.1 Preprocessing



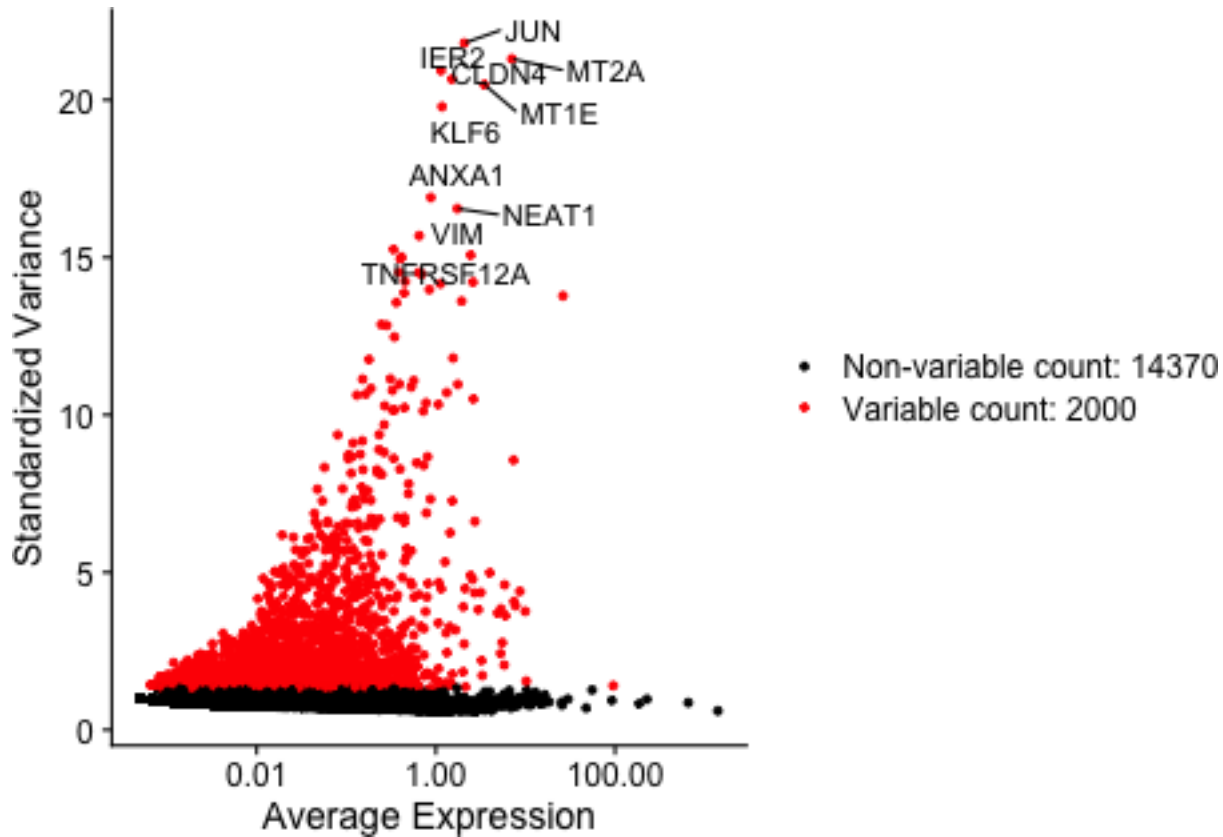
Normalizing Data

##

Normalizes the feature expression for each cell by the total expression x 1000, then log transforms

2.2 Highly Variable Features

Features with high cell to cell variability (highly expressed in some cells but not others)



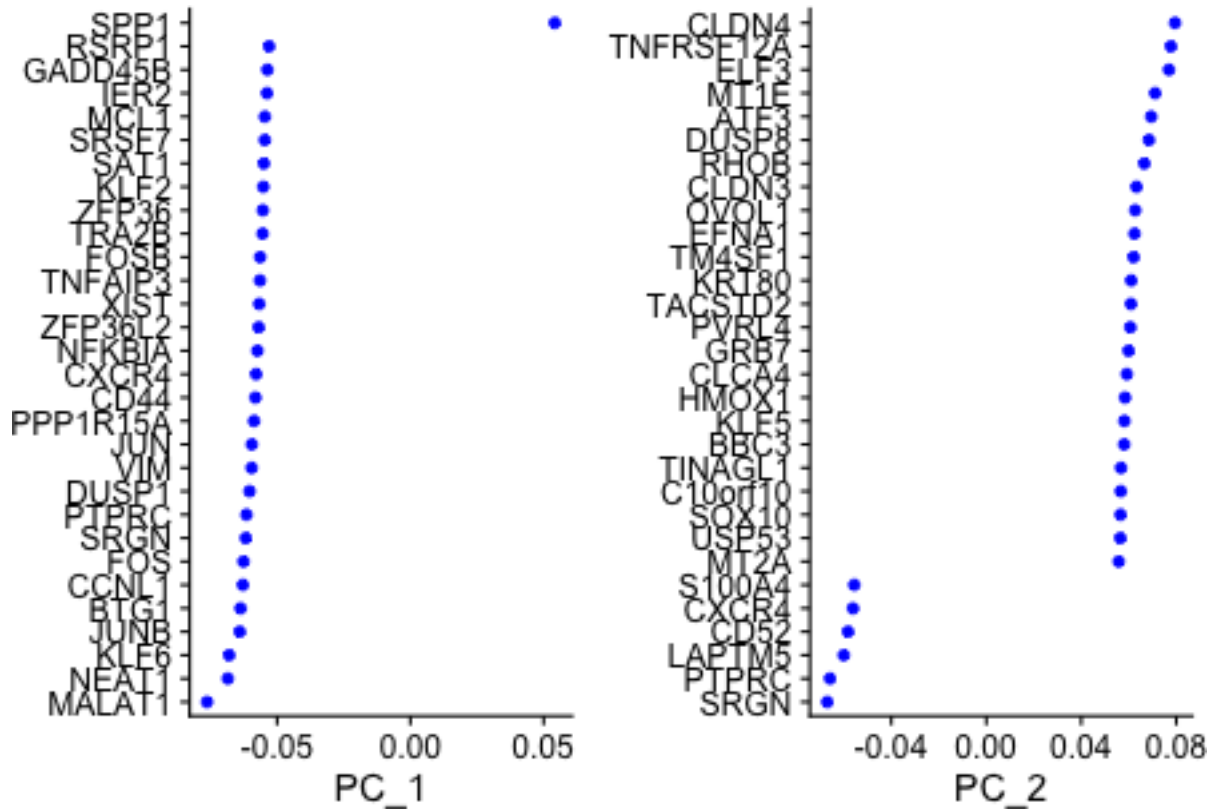
2.3 Scaling

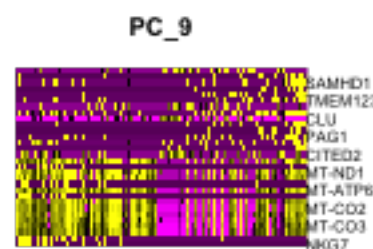
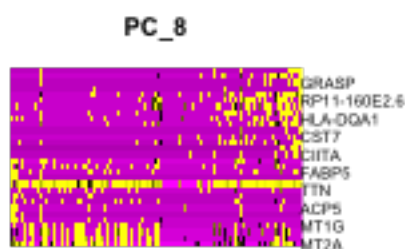
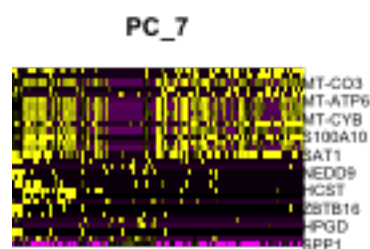
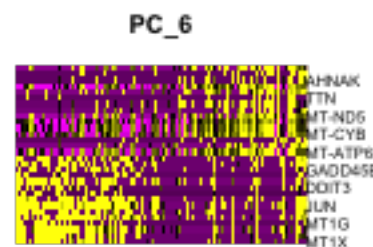
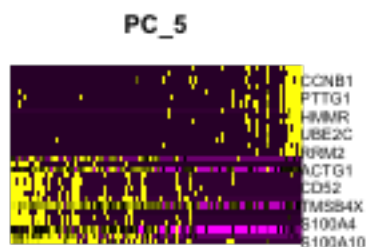
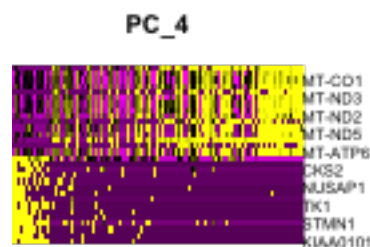
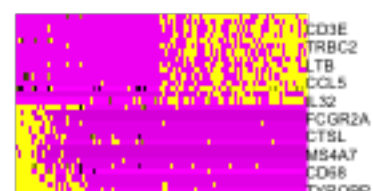
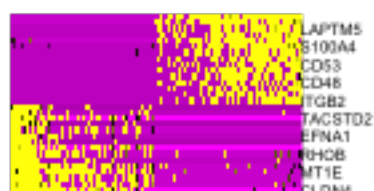
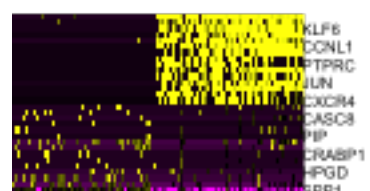
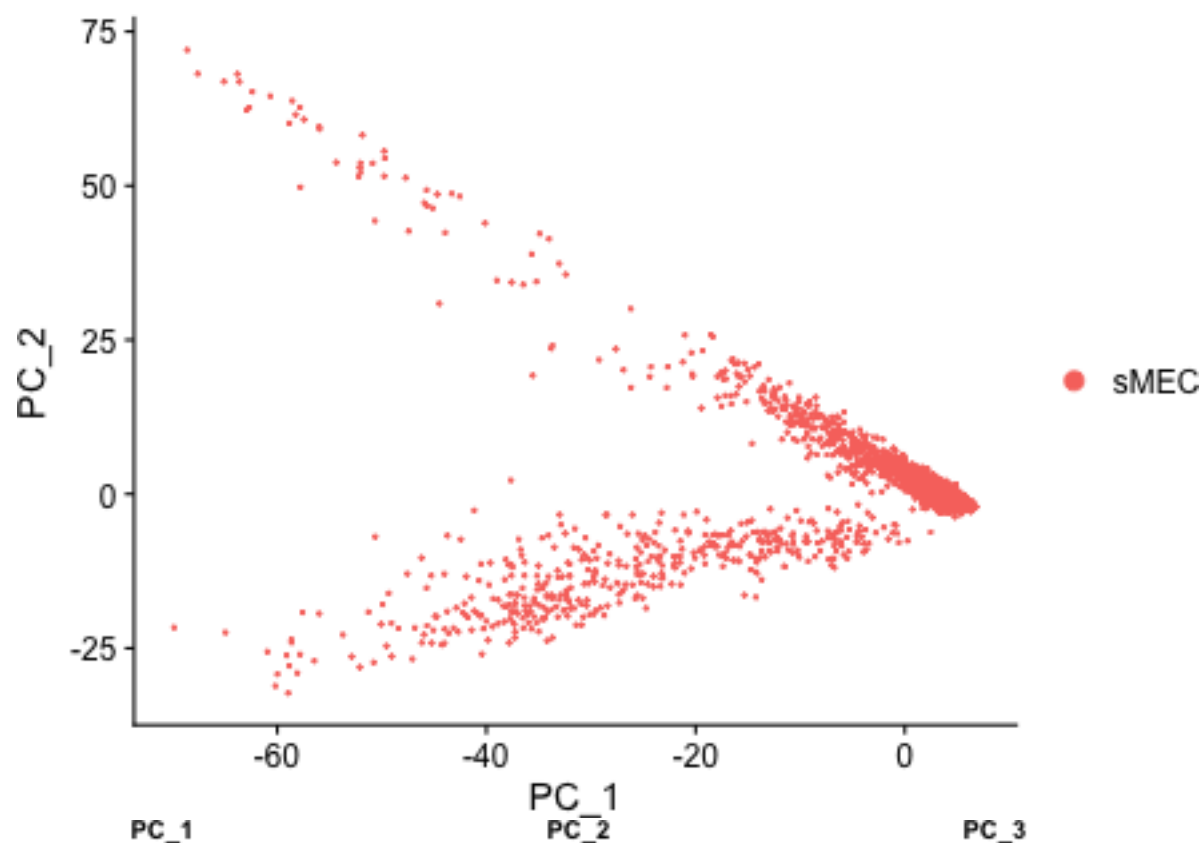
Shifts expression so that mean expression across cells is 0, and variance is 1. This reduces the impact of outliers on downstream analyses. We did not regress out specific sources of heterogeneity like mitochondrial contamination or cell cycle stage.

3 Dimensionality Reductions

```
## PC_ 1
## Positive: SPP1, CLU, SCGB3A1, HPGD, MYBPC1
## Negative: MALAT1, NEAT1, KLF6, JUNB, BTG1
## PC_ 2
## Positive: CLDN4, TNFRSF12A, ELF3, MT1E, ATF3
## Negative: SRGN, PTPRC, LAPTM5, CD52, CXCR4
## PC_ 3
## Positive: TYROBP, FCER1G, SPI1, CD68, FABP5
## Negative: ETS1, TRAC, CD3E, CD2, FYN
## PC_ 4
```

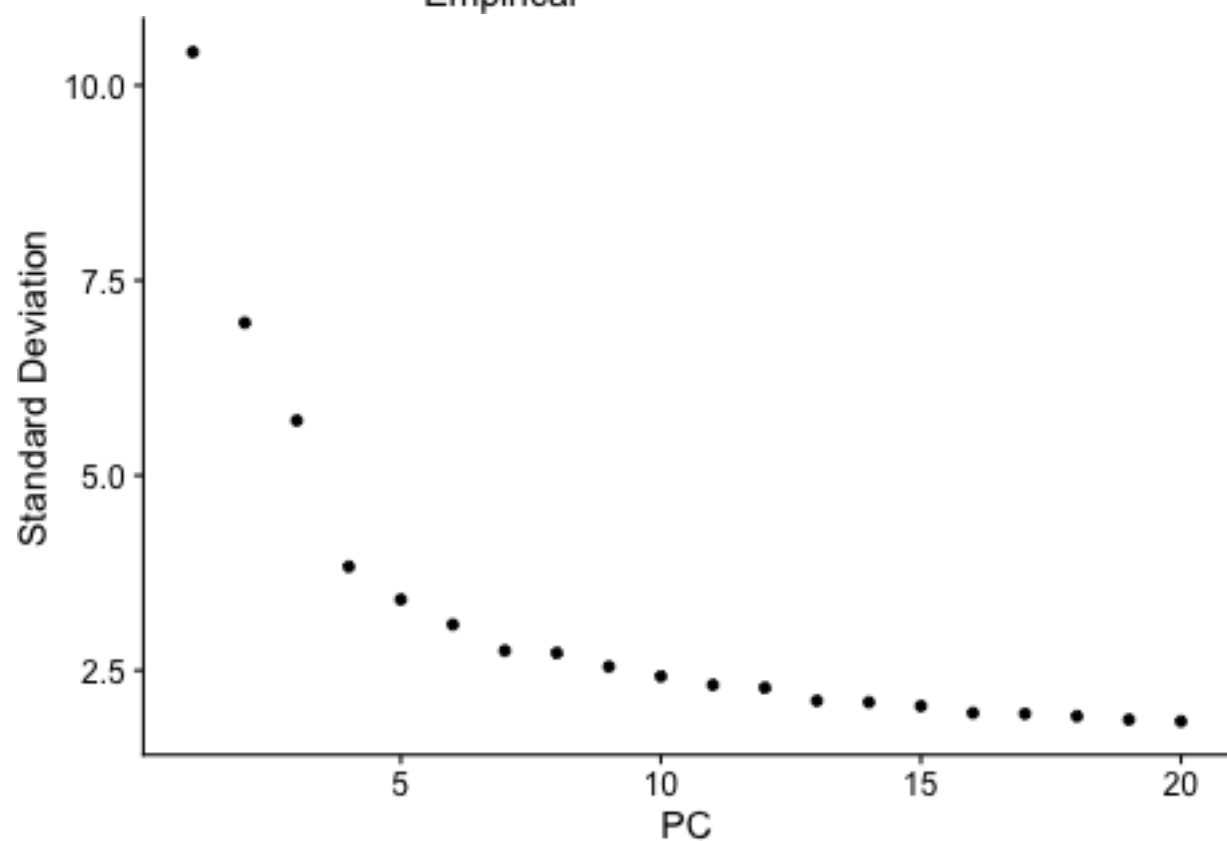
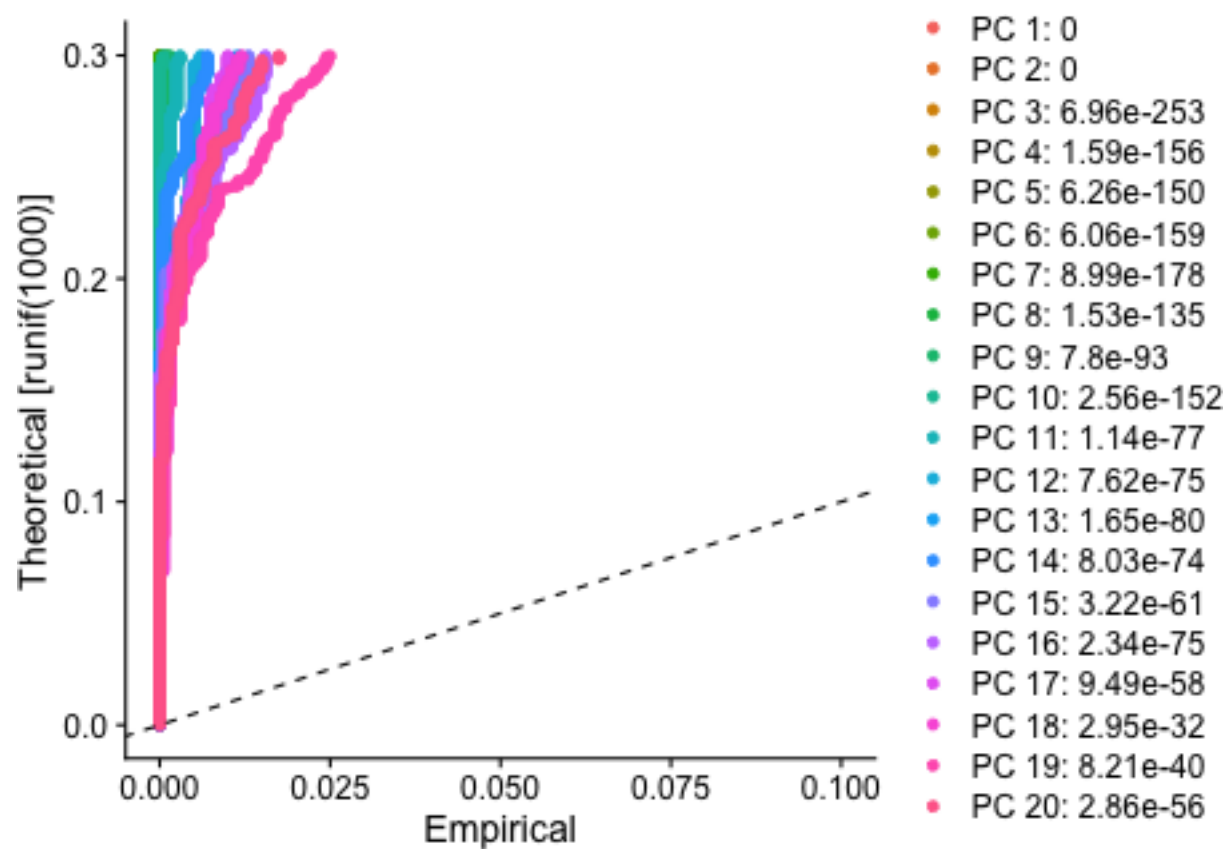
```
## Positive: KIAA0101, TYMS, PTTG1, STMN1, CCNB1
## Negative: NEAT1, MT-CO3, MT-CO1, MT-ND4, XIST
## PC_ 5
## Positive: S100A10, S100A6, FTL, S100A4, HCST
## Negative: KIAA0101, BIRC5, CCNB1, TYMS, TOP2A
```





4 Determination of Number of Clusters

Used the JackStraw procedure in Macosko et al. (2015), sampling 1% of the data re-running the PCA and constructing a null distribution of feature scores, then repeating. This identified ‘significant’ PCs. We also did an elbow plot.



Based on this we decided to use 7 PCs to cluster the cells.

5 Clustering Cell Types

Seurat 3.2 uses a K-nearest neighbor approach then tries to partition this into communities of cell types.

5.1 Identification and Assignment of Clusters

```
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
##
## Number of nodes: 5917
## Number of edges: 178451
##
## Running Louvain algorithm...
## Maximum modularity in 10 random starts: 0.7940
## Number of communities: 13
## Elapsed time: 0 seconds
```

5.2 Non-Linear Dimensionality Reduction

Did both UMAP and t-SNE plots using 7 clusters

5.2.1 t-SNE Plots

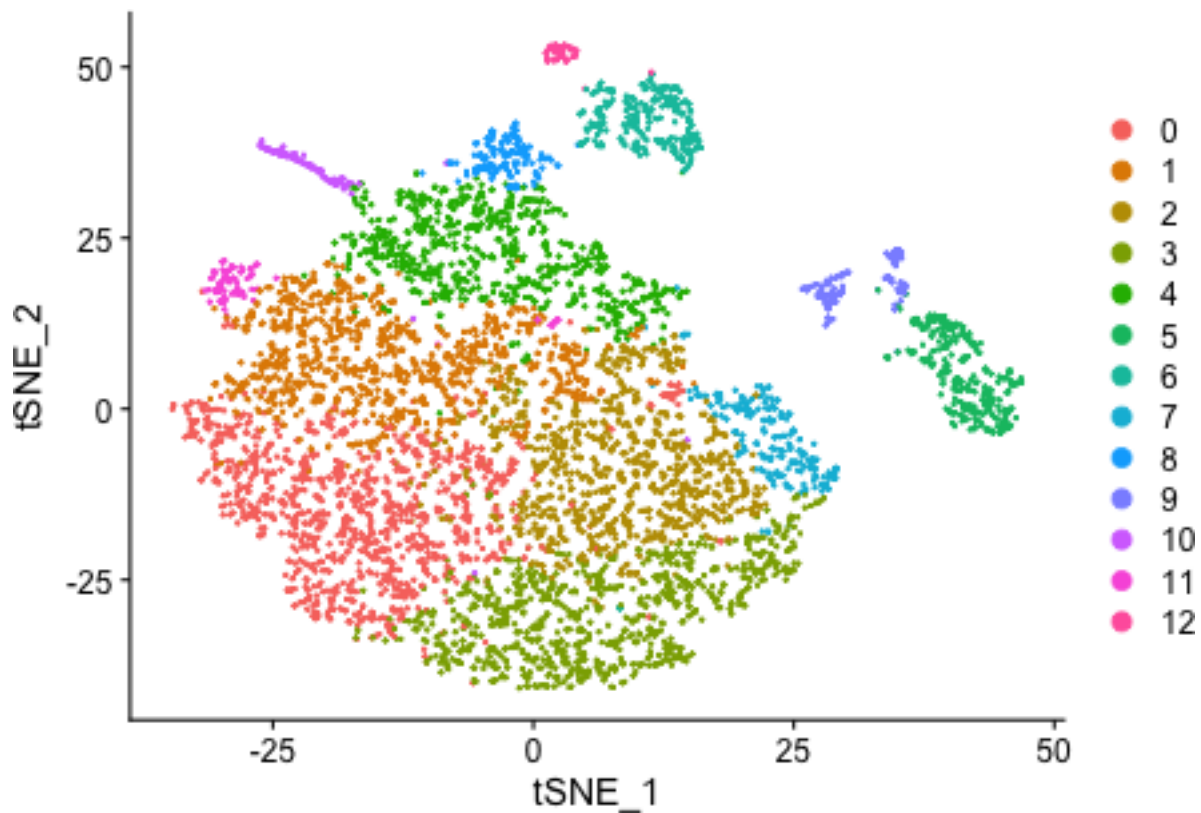


Figure 1: t-SNE plot of secreted MECs

5.2.2 UMAP

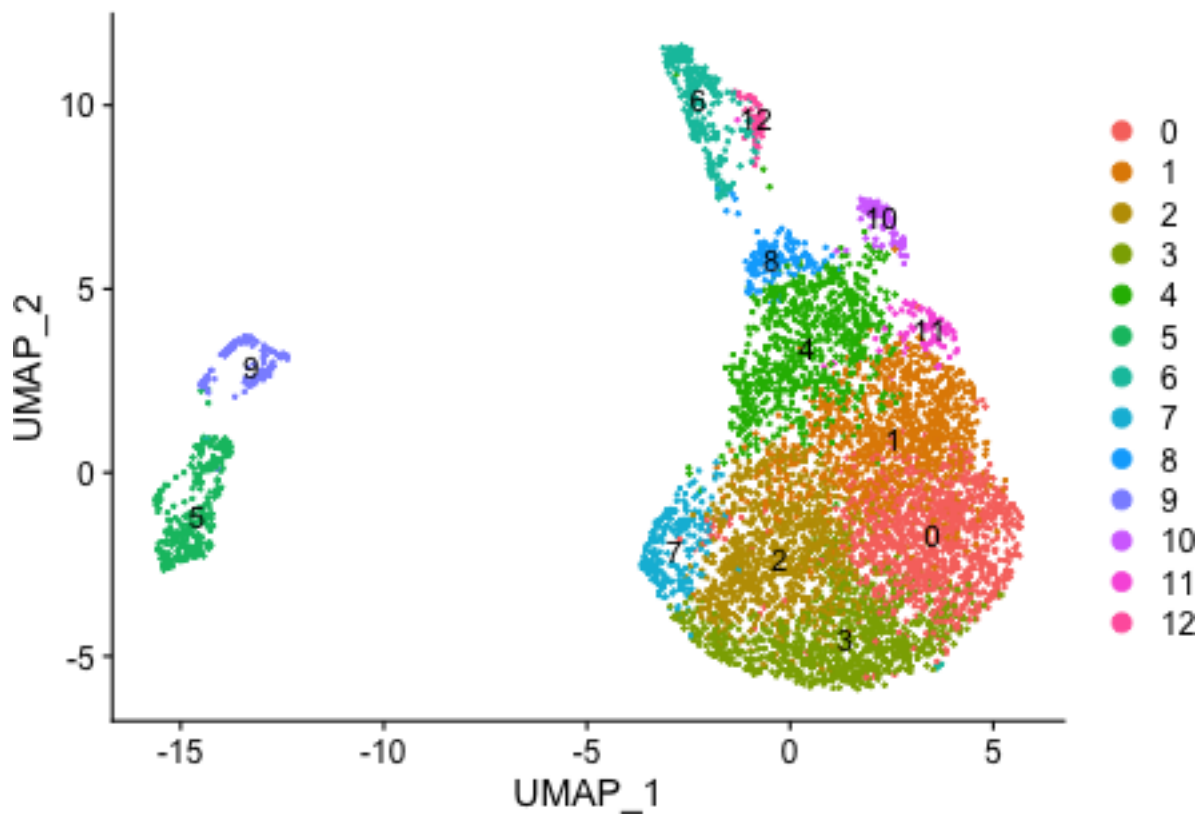


Figure 2: UMAP plot of secreted MECs

Table 1: Cell Specific Markers (All)

p_val	avg_logFC	pct.1	pct.2	p_val_adj	cluster	gene
0	0.916	0.611	0.176	0	4	LY6D
0	1.109	0.892	0.592	0	4	CLU
0	0.888	0.542	0.186	0	4	KRT15
0	3.261	0.997	0.137	0	5	MALAT1
0	2.638	0.772	0.012	0	5	PTPRC
0	2.774	0.994	0.545	0	5	MT-ATP6
0	2.964	0.846	0.085	0	6	JUN
0	3.724	0.643	0.050	0	6	MT1E
0	3.935	0.750	0.111	0	6	MT2A
0	1.590	1.000	0.664	0	7	MT-CO1
0	1.430	1.000	0.650	0	7	MT-CO3
0	1.373	0.860	0.358	0	7	MT-ND5
0	2.329	0.715	0.046	0	8	DEFB1
0	1.624	0.951	0.250	0	8	KRT7
0	2.003	0.812	0.215	0	8	KRT15
0	3.098	0.801	0.054	0	9	VIM
0	3.122	0.823	0.422	0	9	CD74
0	3.147	0.908	0.834	0	9	FTL
0	1.997	0.745	0.082	0	10	STMN1
0	1.974	0.816	0.319	0	10	H2AFZ

p_val	avg_logFC	pct.1	pct.2	p_val_adj	cluster	gene
0	1.914	0.857	0.474	0	10	TUBA1B
0	1.301	1.000	0.805	0	11	XDH
0	1.439	1.000	0.755	0	11	FASN
0	1.067	0.912	0.604	0	11	BTN1A1
0	3.137	0.882	0.070	0	12	MT1E
0	3.030	0.725	0.070	0	12	MT1X
0	3.073	0.922	0.133	0	12	MT2A

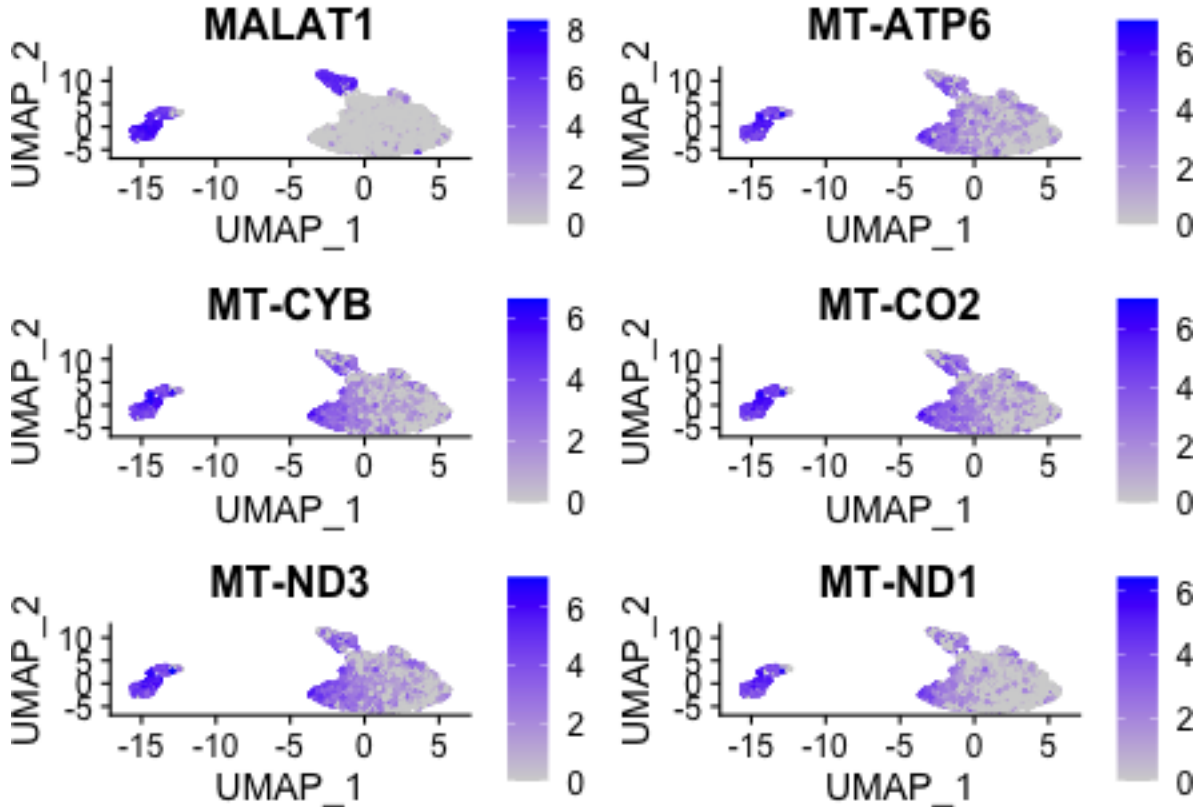
6 Feature Analysis

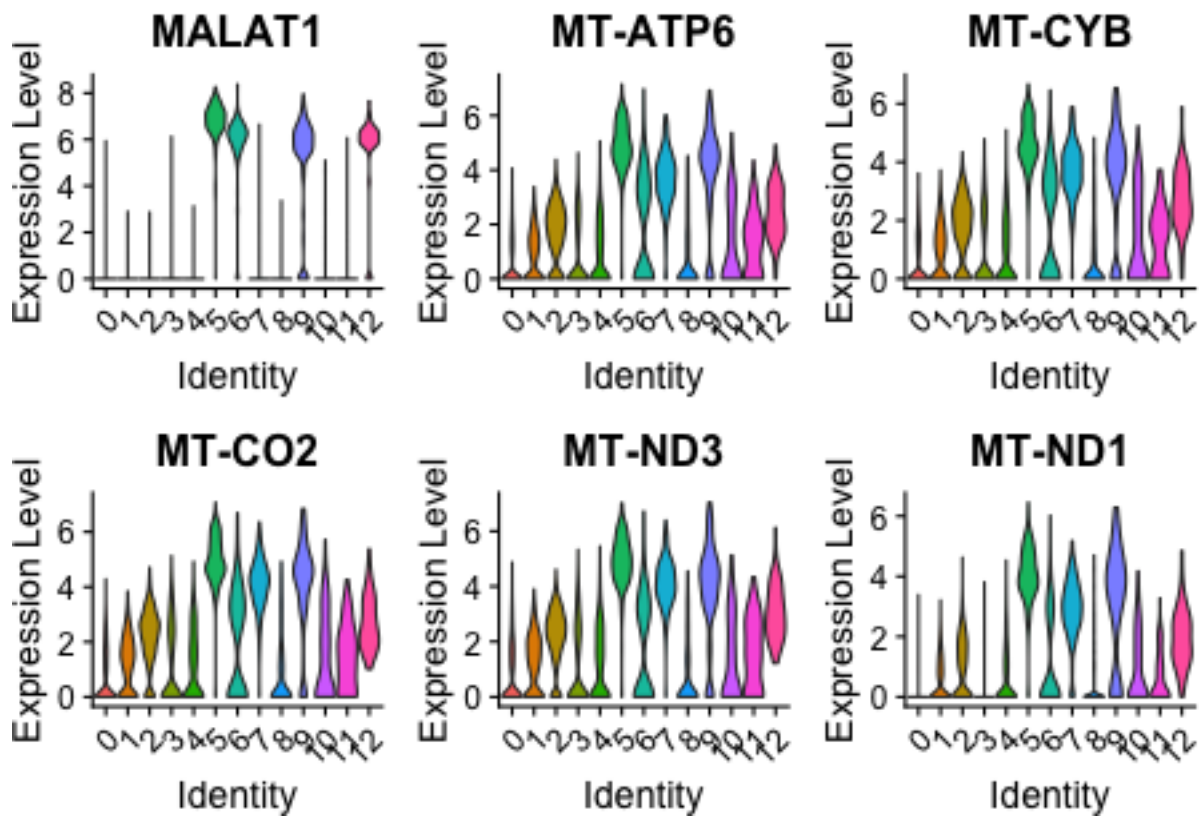
6.1 Annotation of clusters

Used for cell marker enrichment

Used CellMarker for enrichment analyses http://bio-bigdata.hrbmu.edu.cn/CellMarker/download/Human_cell_markers.txt. See Zhang et al. (2019) and <http://bio-bigdata.hrbmu.edu.cn/CellMarker> for details on this resource

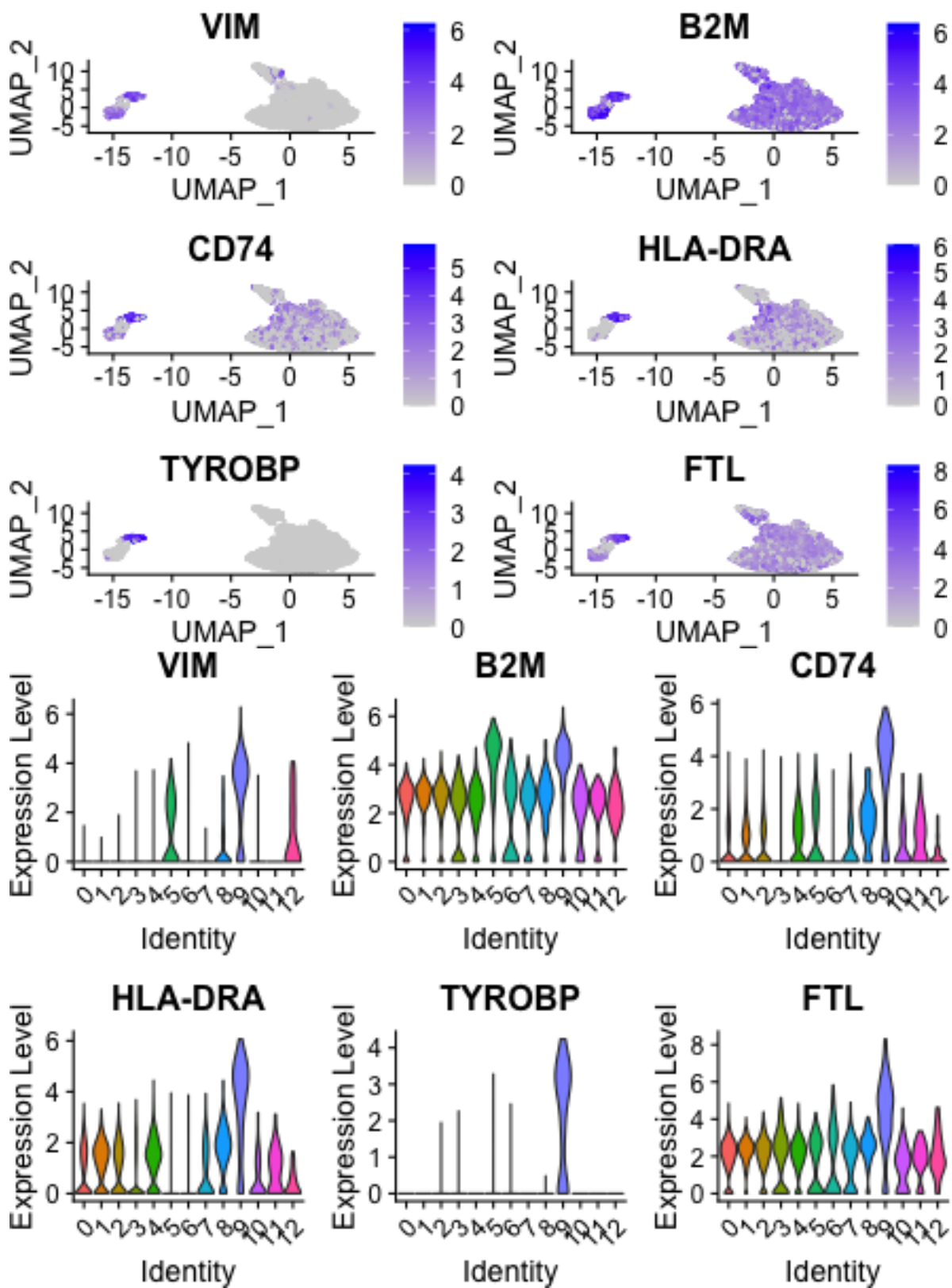
6.1.1 Cluster 5





ID	Description	Size	MFs	Pathways	Significance	Enrichment
Fetal	Fetal	399	-	0.001006436	tags	F99%
kid-kid-kid-	kid-kid-kid-	0.2219			list	B/C/C32/CXCR4/ZFP36L2/RNF213/SRGN/HLA-
ney,ney,ney,	ney,ney,ney,				signal	A/SDFK4/LTB/CD44/TNFAIP3/NKTR/ETS1/IL7R/EML4/HLA-
NorNorNor-	NorNorNor-					E/PRRC2C/CD52/FYN/S100A4/IRF1/B2M/ANKRD11/PIK3R1/ANKRD12/CCNL1/
malmalmal,	malmalmal,					C/FUS/SRSF11/CORO1A/MACF1/AHNAK/EVL/ATRAX/TXNIP/USP15/TNRC6B/I
NatNatNat-	NatNatNat-					F/RNF125/SP100/LCK/CD53/FMNL1/NCL/KMT2C/DUSP1/RNMT/PIM1/SMAP2/
u- u- u-	u- u- u-					
ral ral ral	ral ral ral					
killerkiller	killerkiller					
T T T	T T T					
(NK(NK(NKT)	(NK(NK(NKT)					
cell cell cell	cell cell cell					

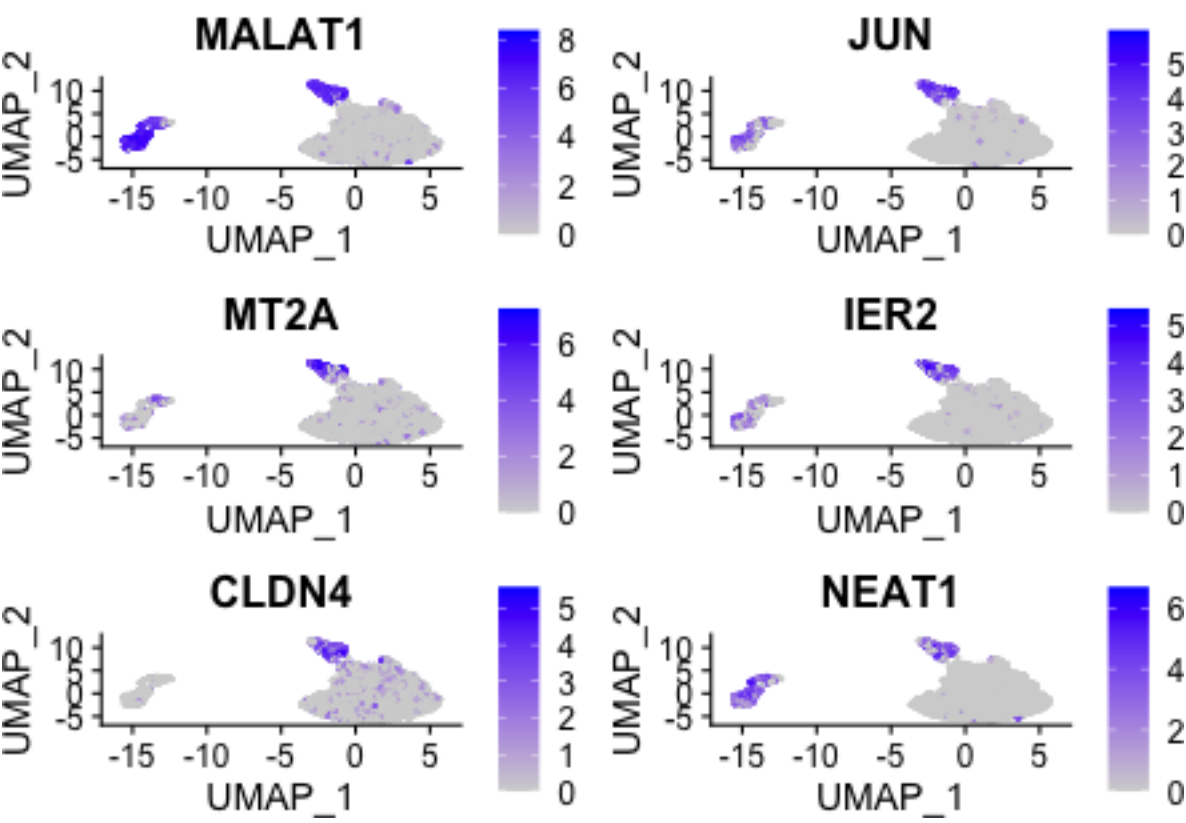
6.1.2 Cluster 9

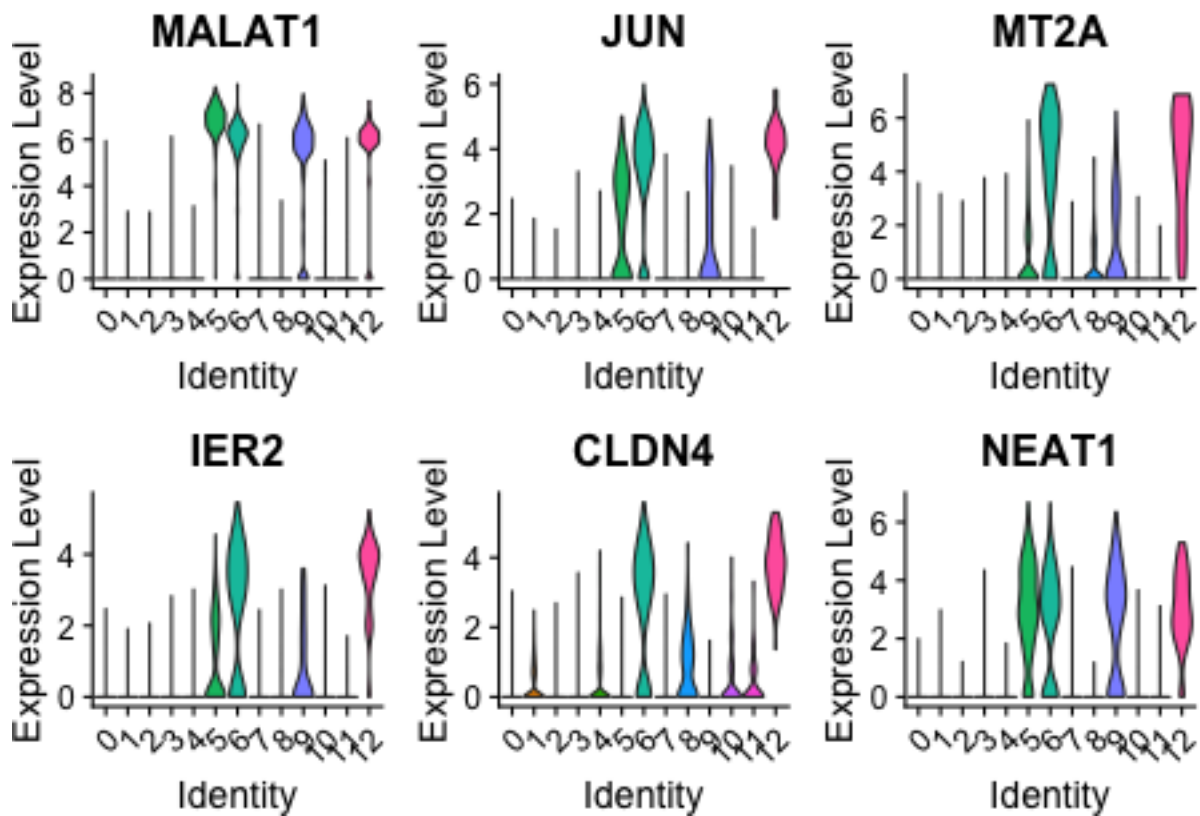


ID	Description	Size	Chrom	NFS	SNP	Struct	Shading	Enrichment
Lung, Normal, Sertoli cell	Lung, Normal, Sertoli cell	15.0	7.12	27.00	0.00	0.060	tags=67%, list=DRB1/HLA-DPA1/HLA-DQA1/HLA-DRA/HLA-DPB1/HLA-DQB1/HLA-DQB2/HLA-DQB3/CXCL8/HLA-A	
Embryonic pre-frontal cortex, Normal, Microglial cell	Embryonic pre-frontal cortex, Normal, Microglial cell	41.8	0.00	0.00	0.082	tags=70%, list=CD74/TYROBP/FABP5/CTSD/FCER1G/SRGN/SAT1/CTSB/APOC1/B/C/GPX1/CYBA/AIF1/NPC2/RGS1/CCL3/GPR183/CTSS/ITGB2/FOSB/GC/IGSF6/MS4A7/ARRB2/LST1/APLP2/UCP2/CD86/RGS10/BRI3/JUNB/E		
Large intestine, Normal, Paneth cell	Large intestine, Normal, Paneth cell	81.0	4.62	97.00	0.00	0.004	tags=59%, list=CD74/HLA-DPA1/HLA-DQA1/HLA-DRA/HLA-DPB1/HLA-DQB1/FCER1G/SRGN/HLA-DQB2/CTSL/CD68/LAPTM5/SOD2/CD83/AIF1/CTSZ/CXCL8/CCL3/ITGB2/DMB/CD48/LCP1/LSP1/CEBPB/CXCL16/ITGAX/METRNL/GMFG/CD53	
Fetal kidney, Normal, Monocyte	Fetal kidney, Normal, Monocyte	212.0	3.67	64.00	0.00	0.069	tags=76%, list=CD74/VIM/HLA-DRA/HLA-DPB1/HLA-DQB1/TYROBP/CTSD/LGALS1/FCER1G/SRGN/SAT1/TIMP1/CTSB/C11orf93/SOD2/CD83/TYMP/HLA-B/GPX1/CYBA/AIF1/CXCL8/EMP3/CTSS/LGALS3/BTG1/HLA-A/ITGB2/BASP1/S100A10/CD52/FOSB/PLAUR/CAPG/S100A6/HGST/ASPM/C/IGSF6/MS4A7/ARRB2/LST1/CST3/PKM/CTSH/APLP2/CD86/RGS10/IFI30/JAML/RAB31/CFLAR/FMNL1/NAMPT/SLC16A3/EMILIN2/GLUT1/DMA/CD63/RNASET2/QKI/VMP1/PFDN5/MTRNR2L8/RILPL2/RNH1/TNIP1	
Kidney, Renal Cell Carcinoma, B cell	Kidney, Renal Cell Carcinoma, B cell	23.0	5.72	7.00	0.00	0.063	tags=69%, list=CD74/HLA-DPA1/HLA-DQA1/HLA-DRA/HLA-DPB1/HLA-DQB1/HLA-DQB2/HLA-DQB3/CD83/GPR183/CD52	
Kidney, Normal, B cell	Kidney, Normal, B cell	38.0	4.77	7.80	0.00	0.066	tags=67%, list=CD74/HLA-DPA1/HLA-DQA1/HLA-DRA/HLA-DPB1/HLA-DQB1/HLA-DQB2/LAPTM5/HLA-DQB3/CD83/GPR183/BASP1/CD52/CAPG	

ID	Description	Size	MFSS	Score	Significance	Pathway	Enrichment
Kidney Re- nal Cell Car- ci- noma, Neutrophil	Kidney Re- nal Cell Car- ci- noma, Neutrophil	17	0.55	0.80	0.00846	0.0334	tags=71%, list=31%, signal=50%
							FIT1/TYROBP/SAT1/SOD2/FOS/CXCL8/RGS2/BCL2A1/S100A11/CEBPB

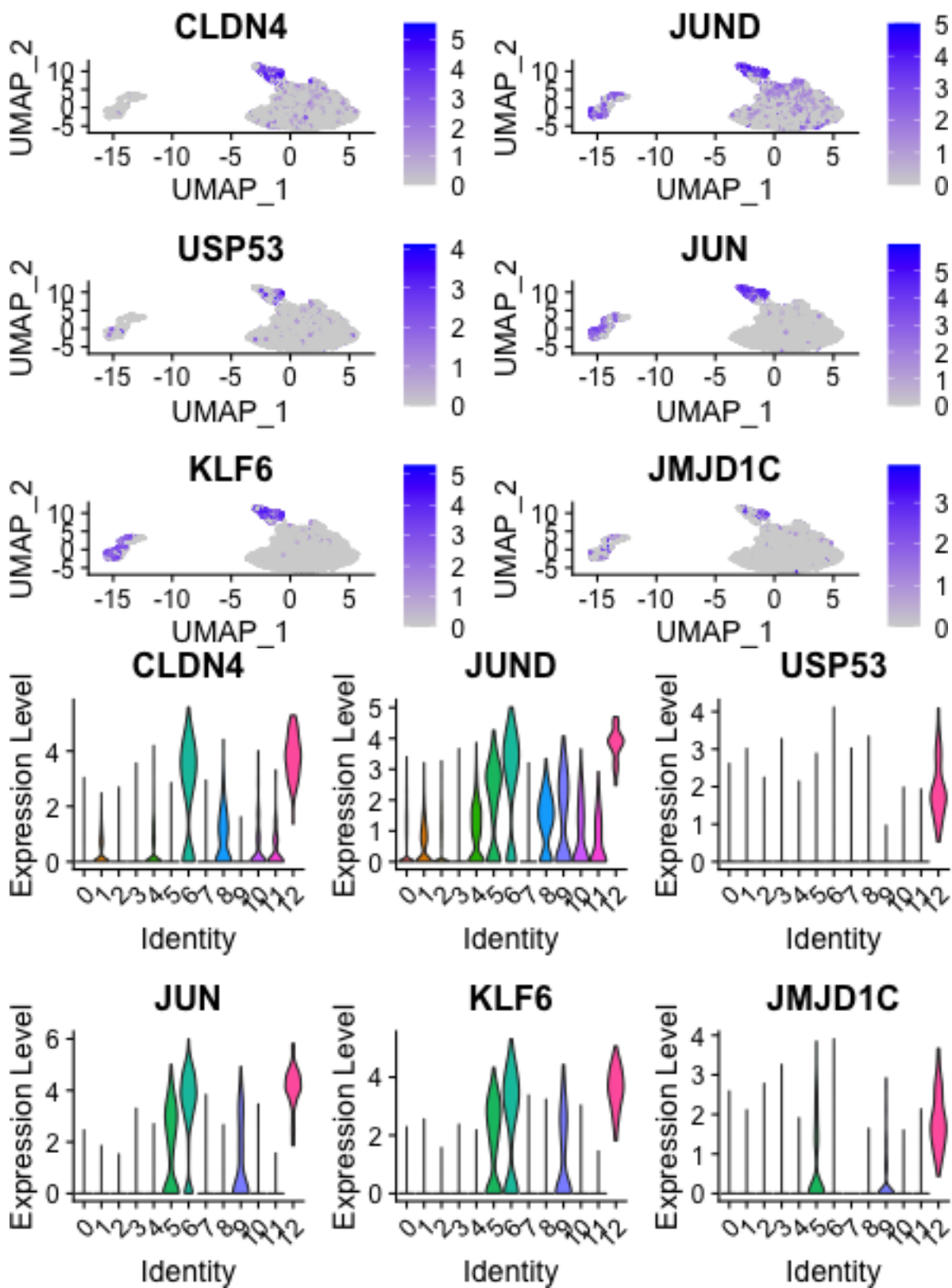
6.1.3 Cluster 6





	ID	Description	setSize	enrichment	NES	Score	adjusted	rank	leading_edge	edge_enrichment
Brain, Normal, Astrocyte	Brain, Normal, Astrocyte	Brain, Normal, Astrocyte	16	0.722	2.130	0.0000	0.00046		tags=94%, list=32%, signal=71%	MT2A/MT1X/JUN/IER2/ATF3/JUN
Embryonic prefrontal cortex, Normal, Microglial cell	Embryonic prefrontal cortex, Normal, Microglial cell	Embryonic prefrontal cortex, Normal, Microglial cell	20	0.637	1.950	0.0010	0.00146		tags=80%, list=32%, signal=63%	JUN/IER2/KLF6/DUSP1/IER3/FOS/

6.1.4 Cluster 12

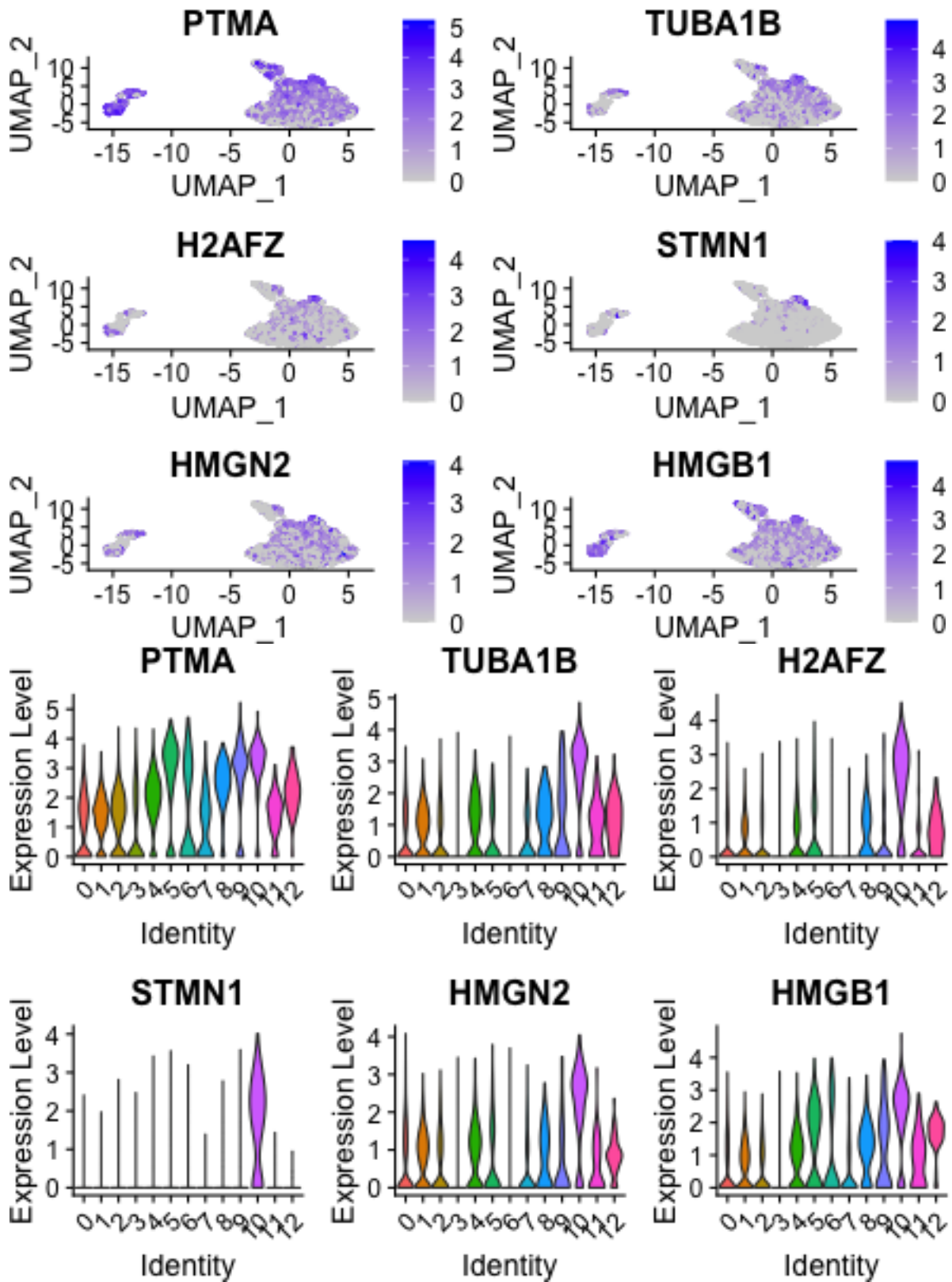


	ID	Description	setSize	enrichNES	negLog10P	adjustedP	leading edge	enrichment
Brain, Normal, Astrocyte	Brain, Normal, Astrocyte	Brain, Normal, Astrocyte	29	0.723	2.850	0.000	0.000	0.001 tags=59% list=9%, signal=55%
Embryonic pre-frontal cortex, Normal, Microglial cell	Embryonic pre-frontal cortex, Normal, Microglial cell	Embryonic pre-frontal cortex, Normal, Microglial cell	57	0.522	2.340	0.000	0.000	0.005 tags=44% list=15%, signal=40%
Fetal kidney, Normal, Monocyte	Fetal kidney, Normal, Monocyte	Fetal kidney, Normal, Monocyte	129	0.407	1.990	0.000	0.000	0.007 tags=45% list=27%, signal=39%
Liver, Normal, Liver bud hepatic cell	Liver, Normal, Liver bud hepatic cell	Liver, Normal, Liver bud hepatic cell	27	0.593	2.310	0.000	0.000	0.005 tags=48% list=15%, signal=42%
Liver, Hepatocellular Cancer, Regulatory T (Treg) cell	Liver, Hepatocellular Cancer, Regulatory T (Treg) cell	Liver, Hepatocellular Cancer, Regulatory T (Treg) cell	52	0.475	2.100	0.000	0.000	0.002 tags=50% list=22%, signal=41%
Blood, Normal, CD1C-CD141-	Blood, Normal, CD1C-CD141-	Blood, Normal, CD1C-CD141-	39	0.506	2.120	0.000	0.000	0.007 tags=61% list=27%, signal=46%
den-dritic cell	den-dritic cell	den-dritic cell						

	ID	Description	setSize	enrichNES	negLog10P	adjustedP	leading edge	enrichment
Kidney, Renal Cell Carcinoma, Neutrophil	Kidney, Renal Cell Carcinoma, Neutrophil	Kidney, Renal Cell Carcinoma, Neutrophil	10	0.722	2.130	0.000	0.001	50 tags=80% list=15%, signal=69%
Fetal gonadal, Normal, Granulosa cell	Fetal gonadal, Normal, Granulosa cell	Fetal gonadal, Normal, Granulosa cell	20	0.542	1.960	0.003	0.014	0.00897 tags=50% list=20%, signal=41%
Embryonic pre-frontal cortex, Normal, Astrocyte	Embryonic pre-frontal cortex, Normal, Astrocyte	Embryonic pre-frontal cortex, Normal, Astrocyte	37	0.446	1.850	0.003	0.014	0.00880 tags=38% list=18%, signal=32%
Embryo, Normal, Trophoblast, Fetal gonadal, Normal, Gonadal endothelial cell	Embryo, Normal, Trophoblast, Fetal gonadal, Normal, Gonadal endothelial cell	Embryo, Normal, Trophoblast, Fetal gonadal, Normal, Gonadal endothelial cell	25	0.497	1.900	0.004	0.017	0.01046 tags=41% list=15%, signal=39%
Fetal gonadal, Normal, Gonadal endothelial cell	Fetal gonadal, Normal, Gonadal endothelial cell	Fetal gonadal, Normal, Gonadal endothelial cell	63	0.375	1.720	0.006	0.017	0.01363 tags=57% list=36%, signal=39%
Umbilical cord blood, Normal, Multiphoid progenitor cell	Umbilical cord blood, Normal, Multiphoid progenitor cell	Umbilical cord blood, Normal, Multiphoid progenitor cell	52	0.398	1.760	0.006	0.017	0.01264 tags=46% list=26%, signal=36%

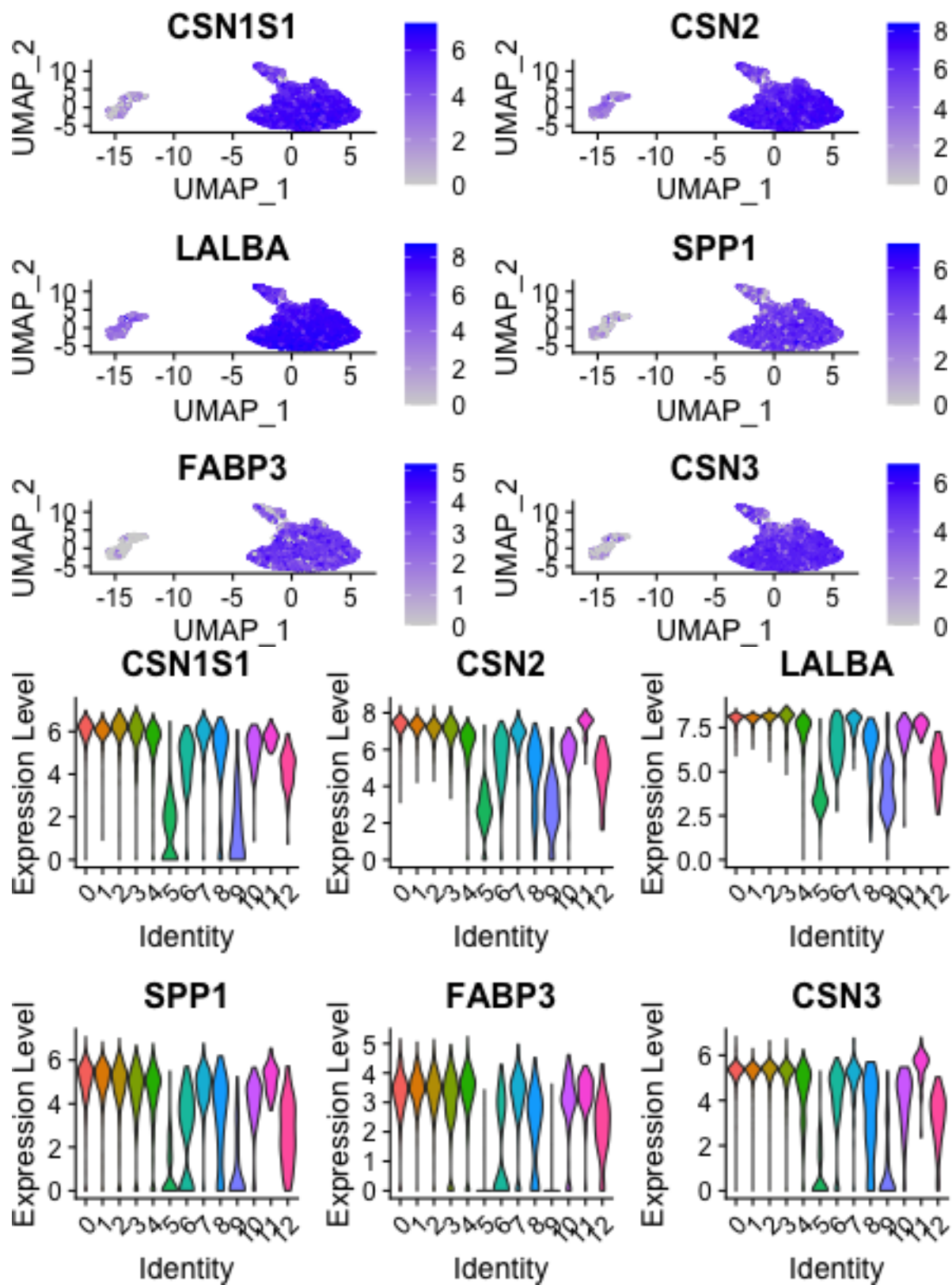
	ID	Description	set	Size	richNES	SetSize	Adjusted	Rank	leading	enrichment
Fetal gonad, Normal, Mitotic fetal germ cell	Fetal gonad, Normal, Mitotic fetal germ cell	Fetal gonad, Normal, Mitotic fetal germ cell	61	0.380	1.730	0.006	0.018	0.029	tags=41%, list=23%, signal=34%	ELF3/DDIT4/MCL1/PIM3/SPTSSA/SLC3A2/SOX4/GABA
Fetal gonad, Normal, Sertoli cell	Fetal gonad, Normal, Sertoli cell	Fetal gonad, Normal, Sertoli cell	62	0.371	1.690	0.006	0.018	0.036	tags=58%, list=37%, signal=39%	SYN/STD2/SLC3A2/GADD45G/TUBB4B/SDC4/S100A10/Z
Kidney, Normal, Mast cell	Kidney, Normal, Mast cell	Kidney, Normal, Mast cell	10	0.621	1.830	0.006	0.018	0.163	tags=50%, list=6%, signal=47%	MYF/EGR1/LMNA/TSC22D1/NFKBIA
Fetal gonad, Normal, Mitotic arrest phase fetal germ cell	Fetal gonad, Normal, Mitotic arrest phase fetal germ cell	Fetal gonad, Normal, Mitotic arrest phase fetal germ cell	110	0.325	1.580	0.009	0.024	0.169	tags=40%, list=27%, signal=33%	MYF/RHOB/PNRC1/JMJD1C/HSPB1/TXNIP/ANXA2/T
Large intestine, Normal, Paneth cell	Large intestine, Normal, Paneth cell	Large intestine, Normal, Paneth cell	19	0.488	1.740	0.019	0.046	0.072	tags=68%, list=33%, signal=47%	IL10/IL10X1/CXCL2/CEBPB/MAFB/SGK1/SLC25A37/PPIF/N

6.1.5 Cluster 10



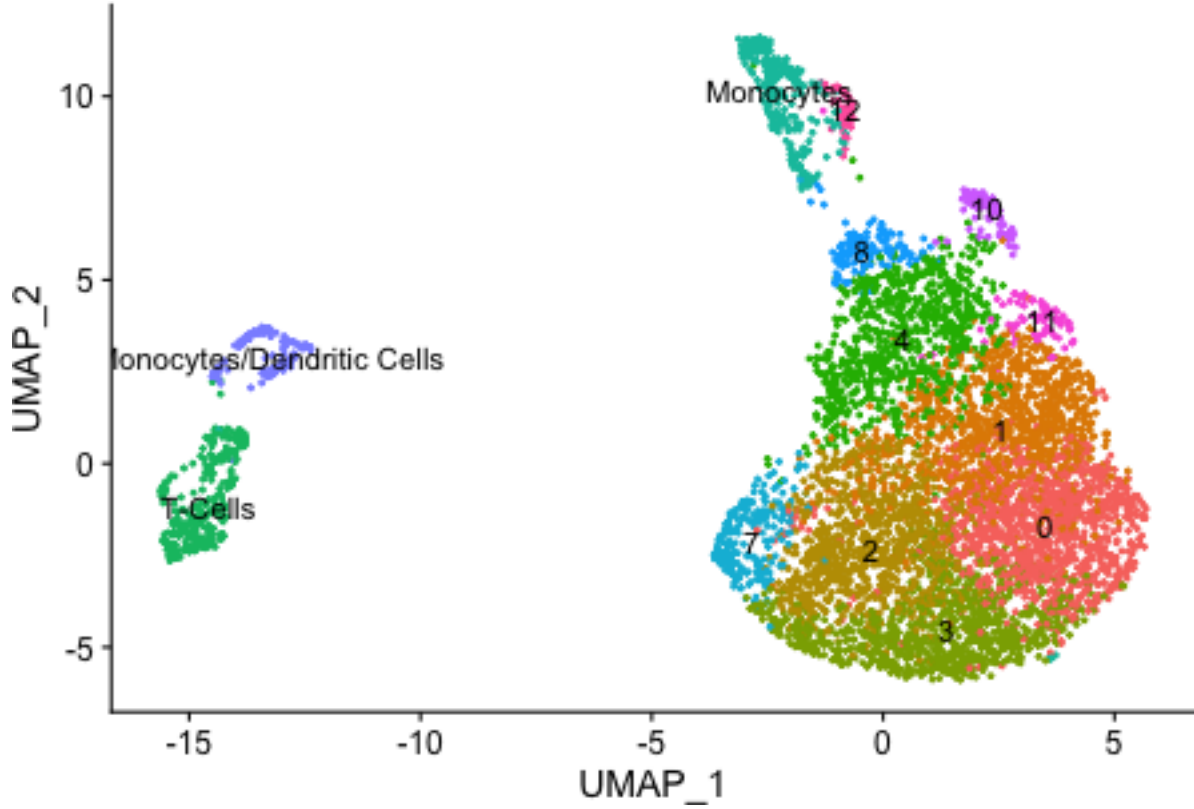
	ID	Description	set	Size	enrich	NES	Score	adjusted	task	leading_genes	enrichment
Embryonic pre-frontal cortex, Normal, Neural progenitor cell	Embryonic pre-frontal cortex, Normal, Neural progenitor cell	Embryonic pre-frontal cortex, Normal, Neural progenitor cell	49	0.6483	230.000	0.000	0.000	0.000	0.000	tags=69%, list=24%, signal=63%	H2AFZ/TUBA1B/CKS2/HMGN2/PTTG1/TOP2A
Large intestine, Normal, MKI67+ progenitor cell	Large intestine, Normal, MKI67+ progenitor cell	Large intestine, Normal, MKI67+ progenitor cell	20	0.7082	8.800	0.000	0.000	0.000	0.000	tags=75%, list=17%, signal=67%	PTTG1/TOP2A/HIST1H4C/TK1/CCNB1/CDC2
Fetal gonad, Normal, Endothelial cell	Fetal gonad, Normal, Endothelial cell	Fetal gonad, Normal, Endothelial cell	24	0.5582	3.340	0.000	0.000	0.000	0.000	tags=54%, list=19%, signal=48%	STMN1/H2AFZ/TUBB/HMGN2/PTTG1/HMGB
Lung, Normal, FOXN4+ cell	Lung, Normal, FOXN4+ cell	Lung, Normal, FOXN4+ cell	16	0.4961	1.800	0.010	0.040	0.020	0.023	tags=81%, list=40%, signal=51%	TYMS/PTMA/CDK1/PTGES3/RANBP1/UBE2

6.1.6 Other



ID	Description	setSize	enrichmentScore	NES	pvalue	p.adjust	qvalues
----	-------------	---------	-----------------	-----	--------	----------	---------

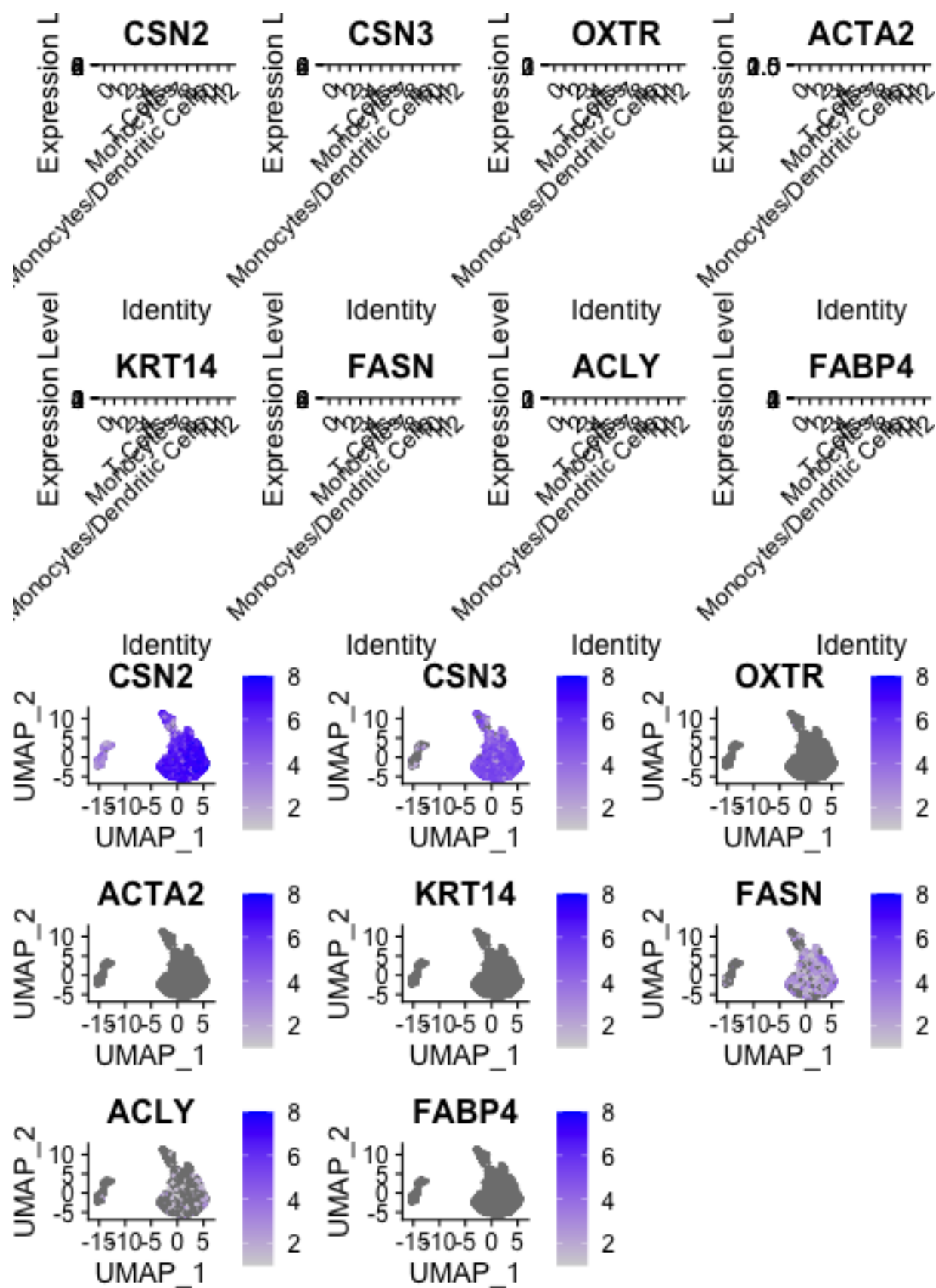
6.2 Summary of Clusters

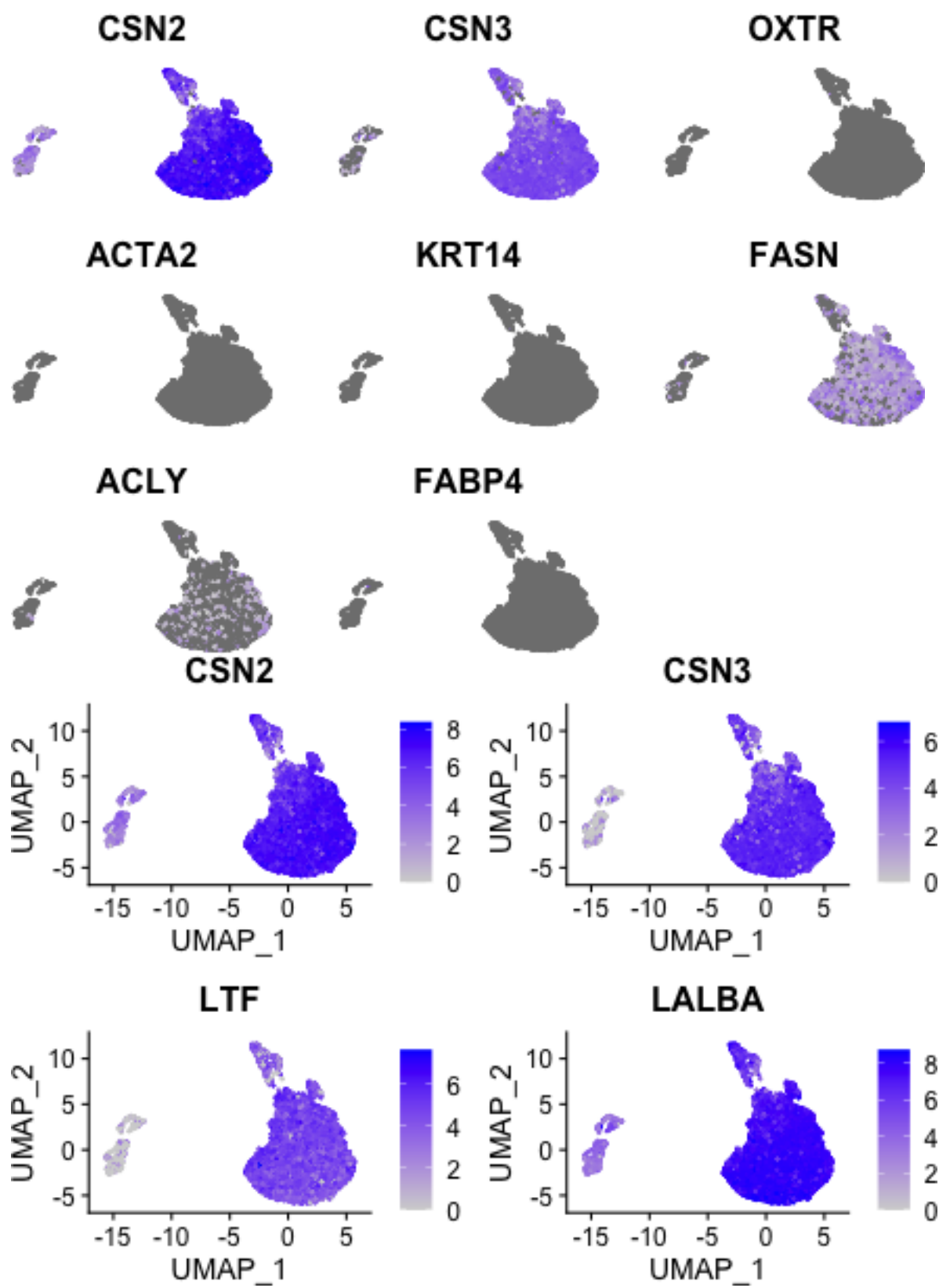


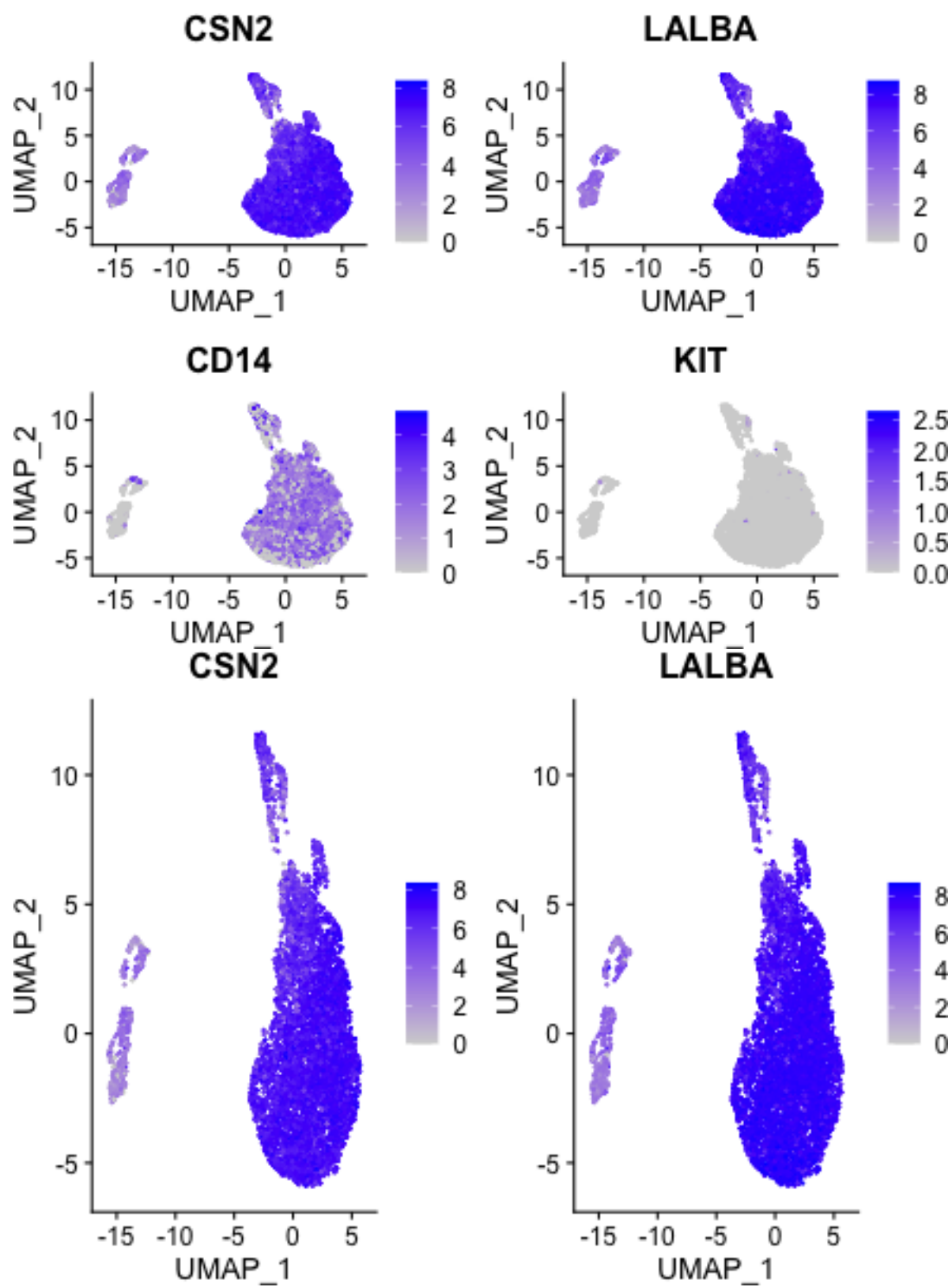
Analysis of clusters

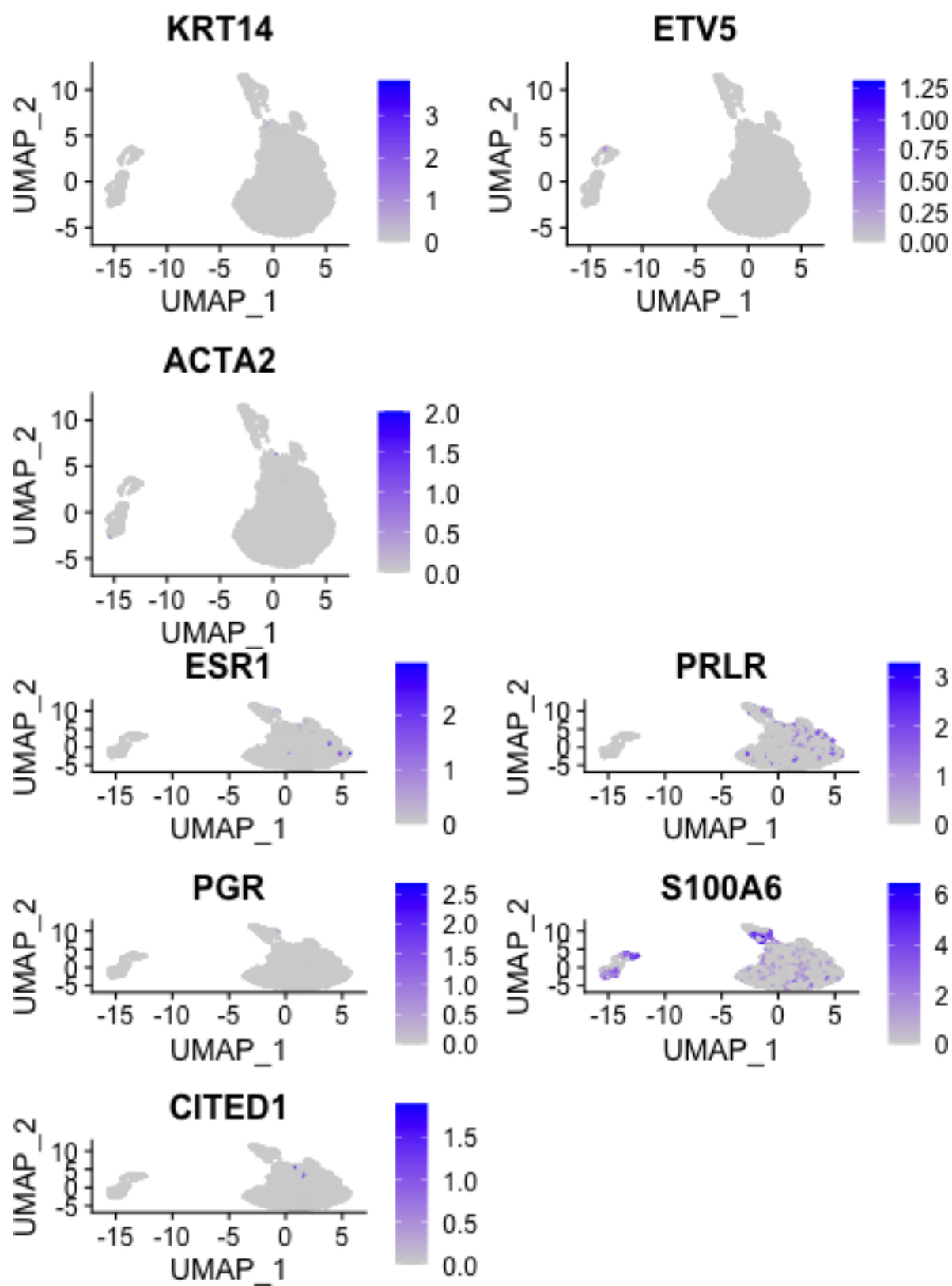
Table 8: Clusters of genes of interest

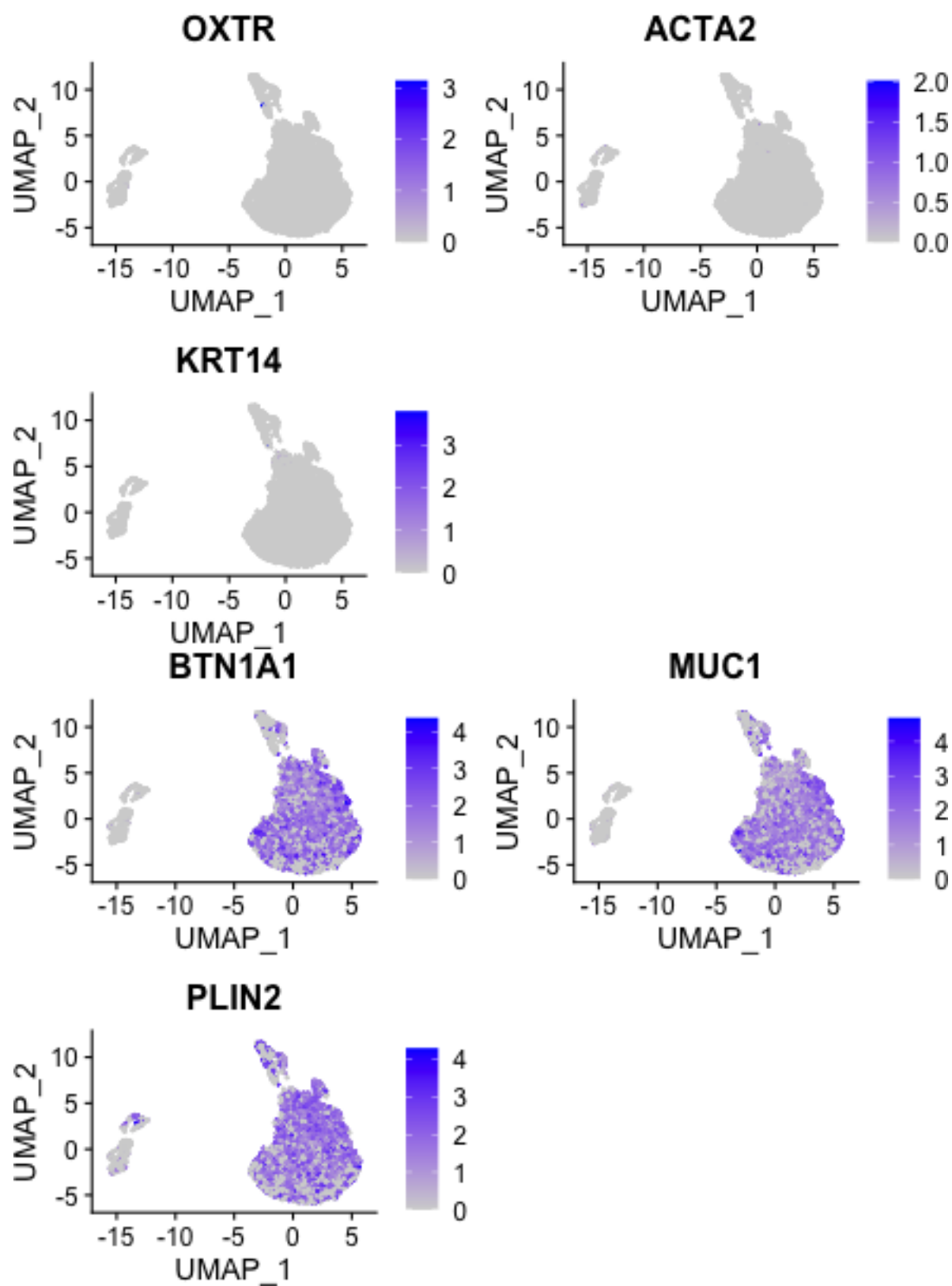
	p_val	avg_logFC	pct.1	pct.2	p_val_adj	cluster	gene
FASN	0	0.660	0.855	0.755	0	7	FASN
FASN1	0	1.439	1.000	0.755	0	11	FASN
ACLY	0	0.845	0.713	0.286	0	11	ACLY
CSN3	0	0.577	1.000	0.935	0	11	CSN3

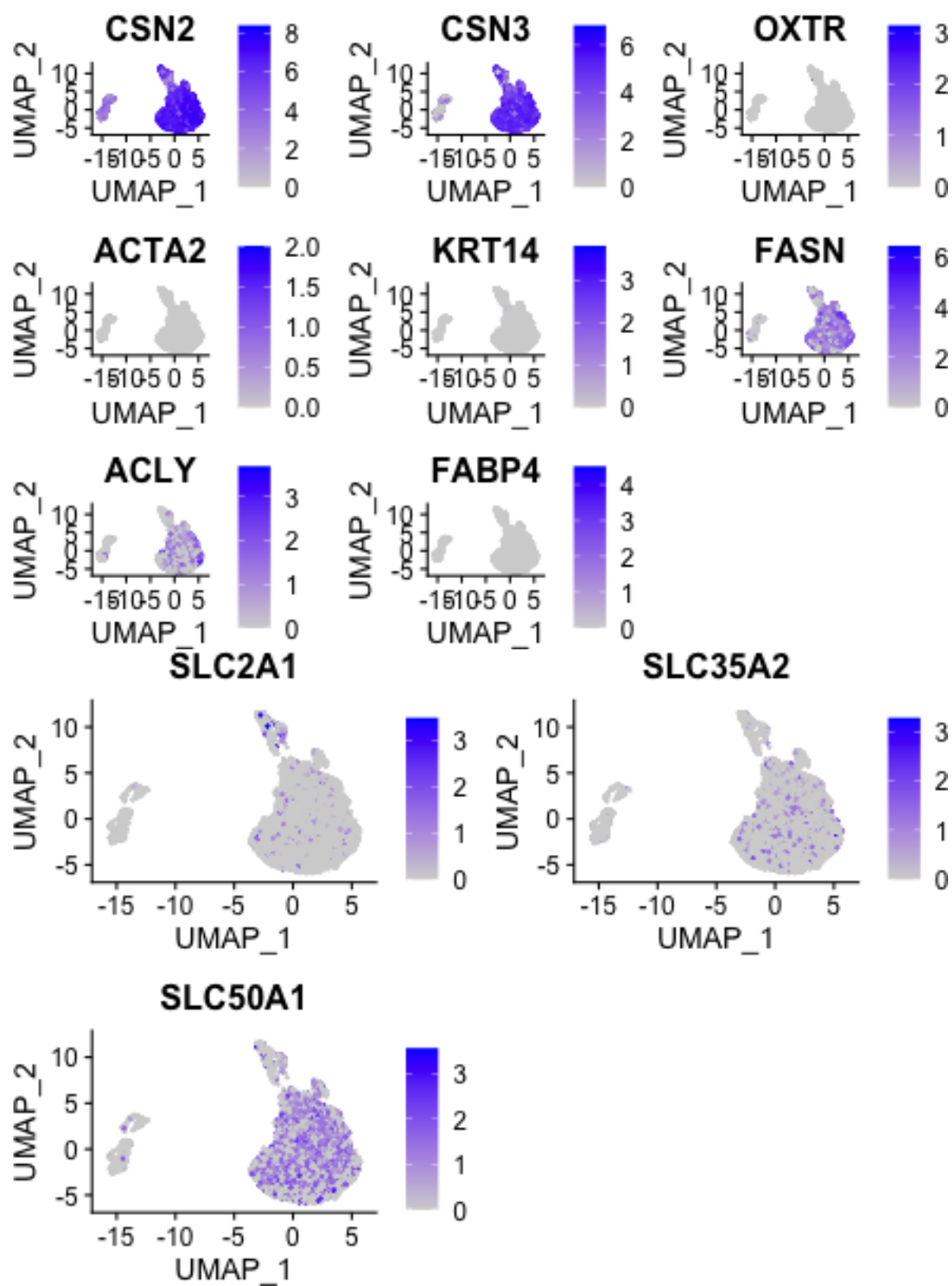


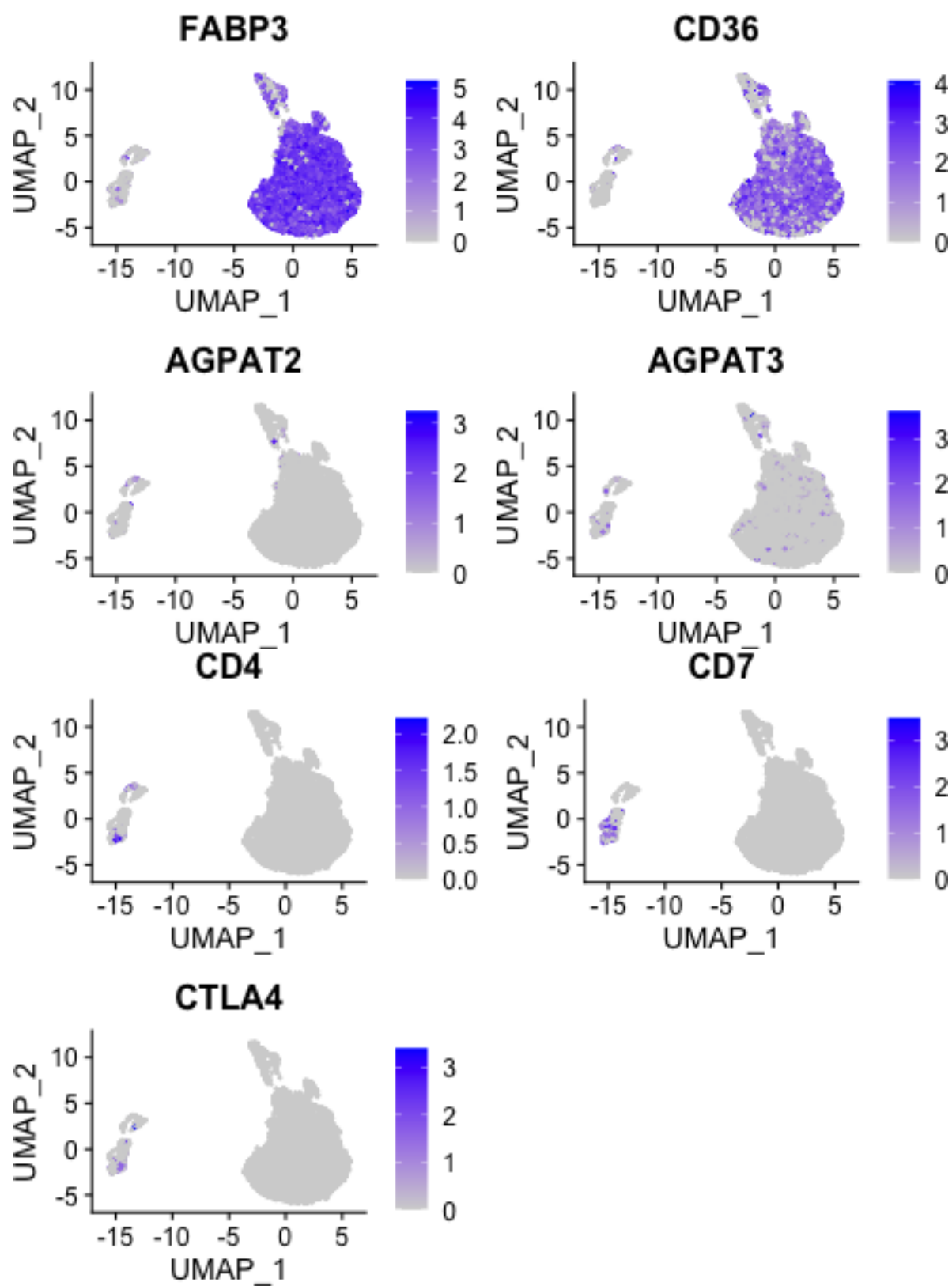


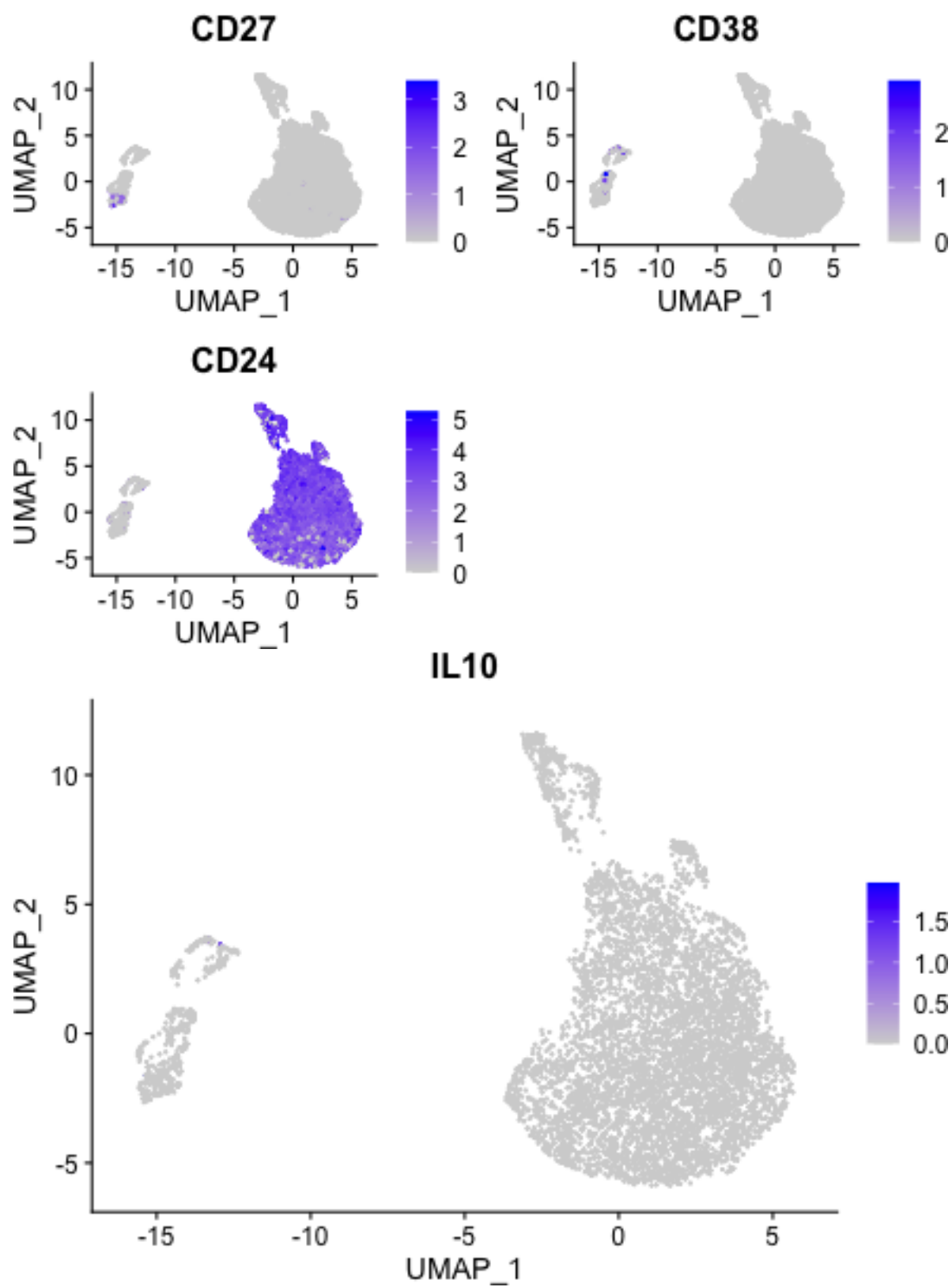












7 Session Information

```
sessionInfo()
```

```
## R version 4.0.2 (2020-06-22)
## Platform: x86_64-apple-darwin17.0 (64-bit)
## Running under: macOS 10.16
##
## Matrix products: default
## BLAS: /Library/Frameworks/R.framework/Versions/4.0/Resources/lib/libRblas.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.0/Resources/lib/libRlapack.dylib
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] stats      graphics  grDevices  utils      datasets  methods   base
##
## other attached packages:
## [1] ggplot2_3.3.3      clusterProfiler_3.16.1 Seurat_3.2.3
## [4] tibble_3.0.4       dplyr_1.0.2          tidyr_1.1.2
## [7] knitr_1.30
##
## loaded via a namespace (and not attached):
## [1] fastmatch_1.1-0      plyr_1.8.6           igraph_1.2.6
## [4] lazyeval_0.2.2       splines_4.0.2        BiocParallel_1.22.0
## [7] listenv_0.8.0        scattermore_0.7      urltools_1.7.3
## [10] digest_0.6.27        htmltools_0.5.0      GOsemSim_2.14.2
## [13] viridis_0.5.1        magick_2.5.2         GO.db_3.11.4
## [16] magrittr_2.0.1       memoise_1.1.0        tensor_1.5
## [19] cluster_2.1.0        ROCR_1.0-11          limma_3.44.3
## [22] globals_0.14.0       graphlayouts_0.7.1   matrixStats_0.57.0
## [25] vroom_1.3.2          prettyunits_1.1.1    enrichplot_1.8.1
## [28] colorspace_2.0-0     blob_1.2.1           ggrepel_0.9.0
## [31] xfun_0.19            crayon_1.3.4         jsonlite_1.7.2
## [34] scatterpie_0.1.5     spatstat_1.64-1      spatstat.data_1.7-0
## [37] survival_3.2-7       zoo_1.8-8            glue_1.4.2
## [40] polyclip_1.10-0      gtable_0.3.0         leiden_0.3.6
## [43] future.apply_1.6.0   BiocGenerics_0.34.0  abind_1.4-5
## [46] scales_1.1.1         DOSE_3.14.0          DBI_1.1.0
## [49] miniUI_0.1.1.1       Rcpp_1.0.5           progress_1.2.2
## [52] viridisLite_0.3.0    xtable_1.8-4         gridGraphics_0.5-1
## [55] reticulate_1.18      europepmc_0.4        bit_4.0.4
## [58] rsvd_1.0.3           stats4_4.0.2         htmlwidgets_1.5.3
## [61] httr_1.4.2           fgsea_1.14.0         RColorBrewer_1.1-2
## [64] ellipsis_0.3.1       ica_1.0-2            pkgconfig_2.0.3
## [67] farver_2.0.3         uwot_0.1.10          deldir_0.2-3
## [70] ggplotify_0.0.5      tidyselect_1.1.0     labeling_0.4.2
## [73] rlang_0.4.10         reshape2_1.4.4       later_1.1.0.1
## [76] AnnotationDbi_1.50.3 munsell_0.5.0         tools_4.0.2
## [79] downloader_0.4       generics_0.1.0       RSQLite_2.2.1
## [82] ggridges_0.5.2       evaluate_0.14        stringr_1.4.0
## [85] fastmap_1.0.1        yaml_2.2.1           goftest_1.2-2
```

## [88] bit64_4.0.5	fitdistrplus_1.1-3	tidygraph_1.2.0
## [91] purrr_0.3.4	RANN_2.6.1	ggraph_2.0.4
## [94] pbapply_1.4-3	future_1.21.0	nlme_3.1-151
## [97] mime_0.9	xml2_1.3.2	DO.db_2.9
## [100] compiler_4.0.2	plotly_4.9.2.2	curl_4.3
## [103] png_0.1-7	spatstat.utils_1.17-0	tweenr_1.0.1
## [106] stringi_1.5.3	highr_0.8	RSpectra_0.16-0
## [109] lattice_0.20-41	Matrix_1.3-0	vctrs_0.3.6
## [112] pillar_1.4.7	lifecycle_0.2.0	BiocManager_1.30.10
## [115] triebeard_0.3.0	lmtest_0.9-38	RcppAnnoy_0.0.18
## [118] data.table_1.13.6	cowplot_1.1.1	irlba_2.3.3
## [121] httpuv_1.5.4	patchwork_1.1.1	qvalue_2.20.0
## [124] R6_2.5.0	promises_1.1.1	KernSmooth_2.23-18
## [127] gridExtra_2.3	IRanges_2.22.2	parallelly_1.22.0
## [130] codetools_0.2-18	MASS_7.3-53	withr_2.3.0
## [133] sctransform_0.3.2	S4Vectors_0.26.1	hms_0.5.3
## [136] mgcv_1.8-33	parallel_4.0.2	grid_4.0.2
## [139] rpart_4.1-15	rvcheck_0.1.8	rmarkdown_2.6
## [142] Rtsne_0.15	ggforce_0.3.2	Biobase_2.48.0
## [145] shiny_1.5.0		

Macosko, Evan Z., Anindita Basu, Rahul Satija, James Nemesh, Karthik Shekhar, Melissa Goldman, Itay Tirosh, et al. 2015. “Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets.” *Cell* 161 (5). Elsevier: 1202–14. <https://doi.org/10.1016/j.cell.2015.05.002>.

Martin Carli, Jayne F., G. Devon Trahan, Kenneth L. Jones, Nicole Hirsch, Kristy P. Rolloff, Emily Z. Dunn, Jacob E. Friedman, et al. 2020. “Single Cell RNA Sequencing of Human Milk-Derived Cells Reveals Sub-Populations of Mammary Epithelial Cells with Molecular Signatures of Progenitor and Mature States: a Novel, Non-invasive Framework for Investigating Human Lactation Physiology.” *Journal of Mammary Gland Biology and Neoplasia*. Journal of Mammary Gland Biology; Neoplasia. <https://doi.org/10.1007/s10911-020-09466-z>.

Zhang, Xinxin, Yujia Lan, Jinyuan Xu, Fei Quan, Erjie Zhao, Chunyu Deng, Tao Luo, et al. 2019. “CellMarker: A manually curated resource of cell markers in human and mouse.” *Nucleic Acids Research* 47 (D1). Oxford University Press: D721–D728. <https://doi.org/10.1093/nar/gky900>.