

# **Effects of Nivolumab (PD-1 checkpoint blockade) on 2 specific people's T cell profiles**

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CMM 523

Updates as of November 1<sup>st</sup> 2024

[cell.com/cell-reports-medicine/pdf/S2666-3791\(22\)00432-3.pdf](https://cell.com/cell-reports-medicine/pdf/S2666-3791(22)00432-3.pdf)

# Cell Reports Medicine



## Article

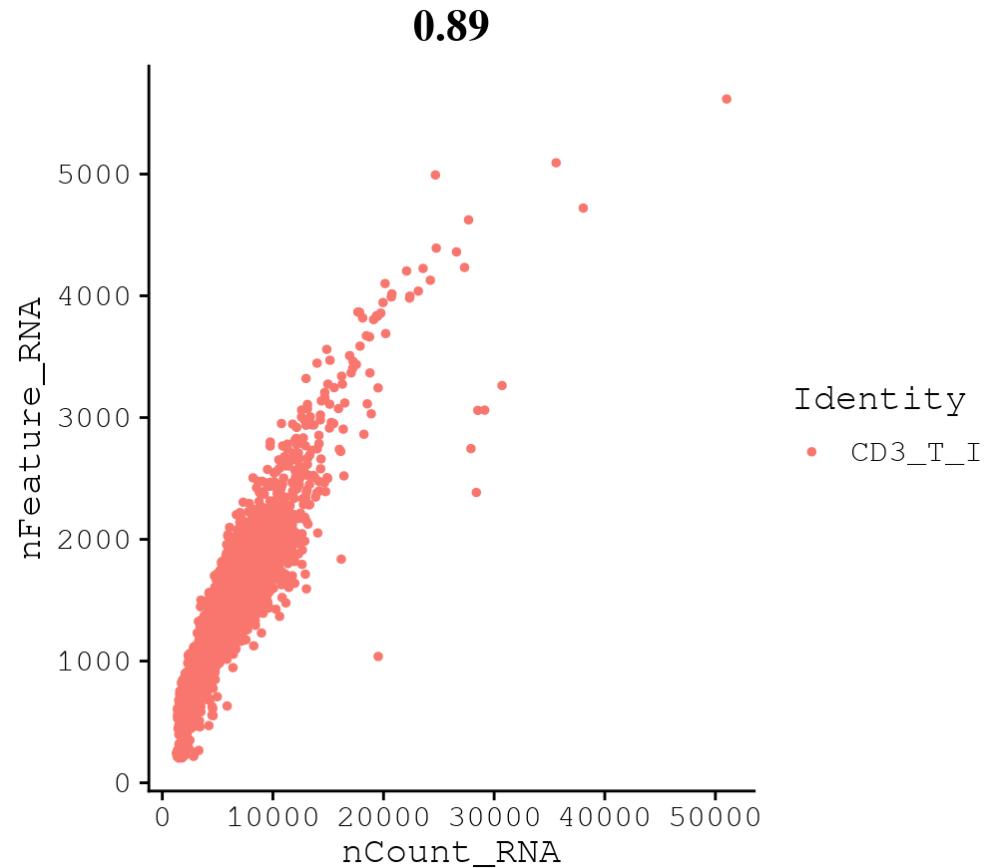
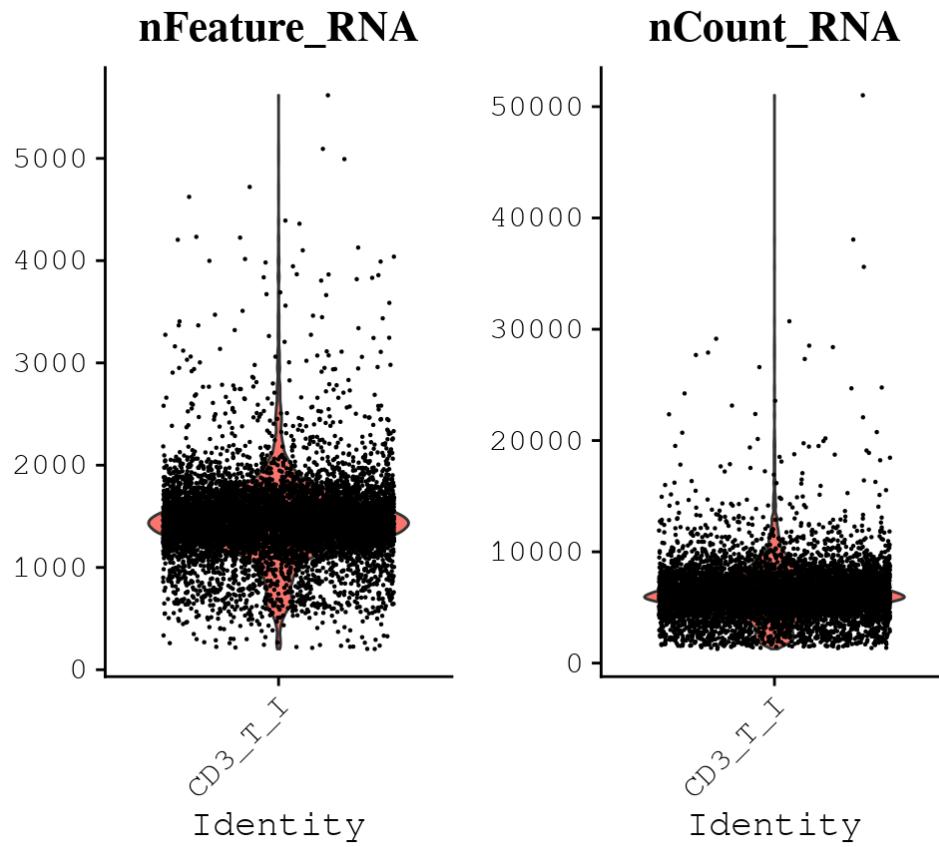
# Single-cell RNA sequencing reveals distinct T cell populations in immune-related adverse events of checkpoint inhibitors

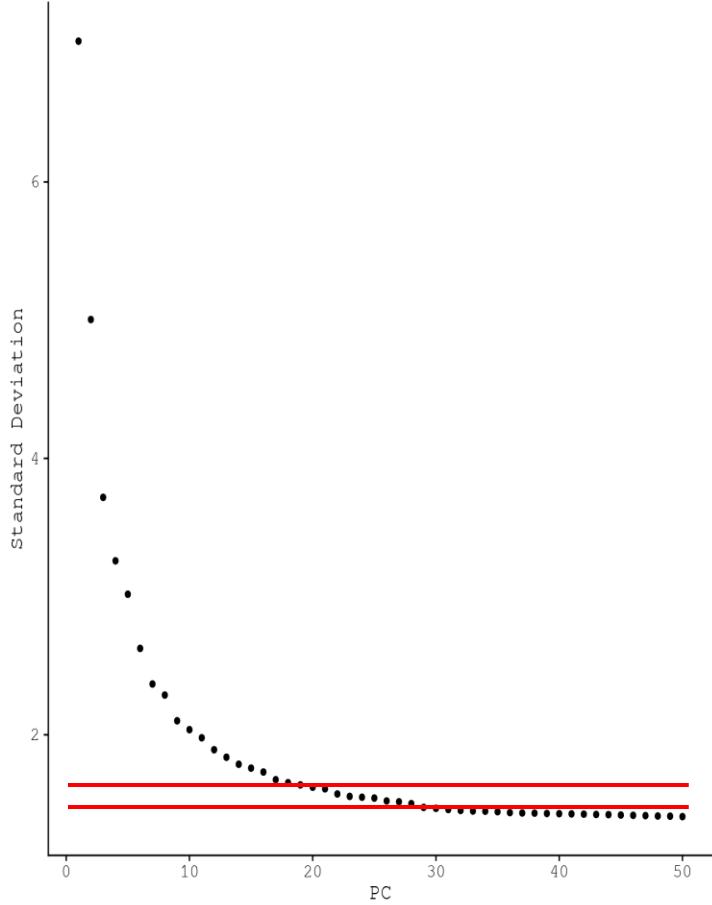
Shoib Bukhari,<sup>1</sup> Brian S. Henick,<sup>1,2</sup> Robert J. Winchester,<sup>1,3</sup> Shalom Lerrer,<sup>1</sup> Kieran Adam,<sup>1</sup> Yevgeniya Gartshteyn,<sup>3</sup> Rohan Maniar,<sup>2</sup> Ziyan Lin,<sup>4</sup> Alireza Khodadadi-Jamayran,<sup>4</sup> Aristotelis Tsirigos,<sup>4</sup> Mary M. Salvatore,<sup>5</sup> Galina G. Lagos,<sup>2</sup> Steven L. Reiner,<sup>6</sup> Matthew C. Dallos,<sup>2</sup> Matthen Mathew,<sup>2</sup> Naiyer A. Rizvi,<sup>2</sup> and Adam Mor<sup>1,2,3,7,\*</sup>

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- Their analysis focuses heavily on baseline T cell profiles
- Created my own subset of their data:
  - patients (mean age = 70) who had lung adenocarcinomas and received PD1 blockade
    - 2 patients experienced immune-related adverse events (pt #11, 16)
    - 2 patients did NOT experience side effects (pt #20, 22).
  - Whole blood was collected after irAE onset. CD3+ cells (T cells) were enriched and sequenced.

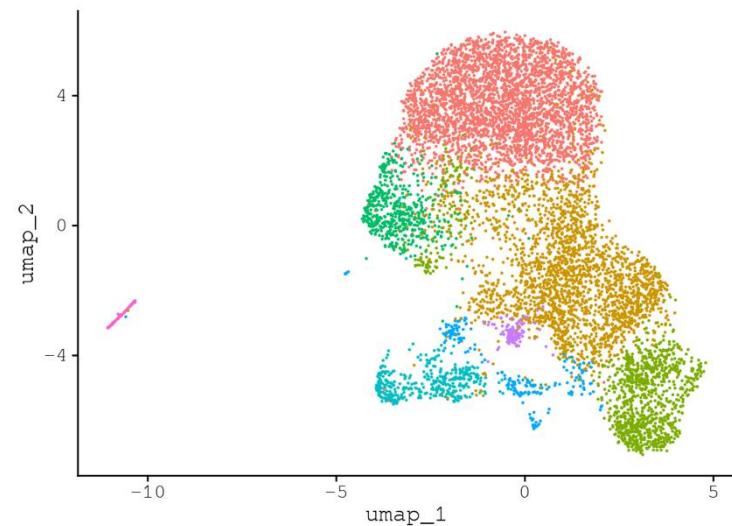
# Patient 11, post-treatment





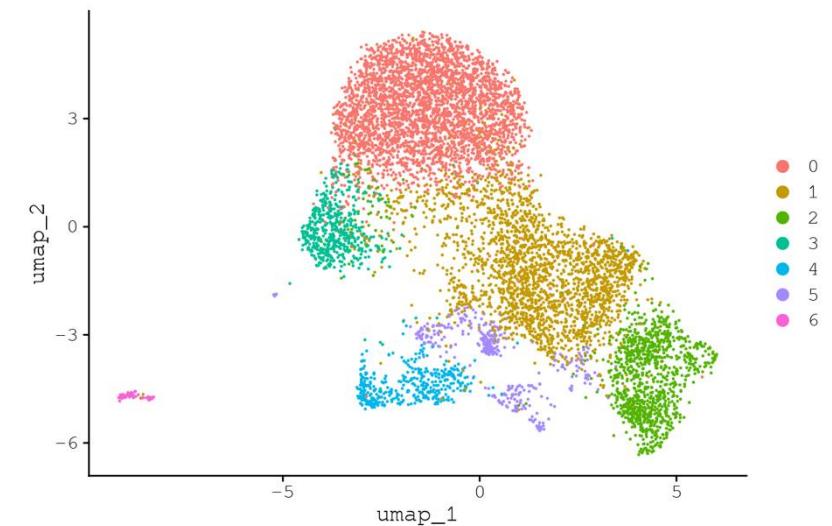
```
#cluster cells
P11T <- FindNeighbors(P11T, dims = 1:20)
P11T <- FindClusters(P11T, resolution = 0.5)

P11T <- RunUMAP(P11T, dims = 1:20)
DimPlot(P11T, reduction = "umap")
```



```
#cluster cells
P11T <- FindNeighbors(P11T, dims = 1:30)
P11T <- FindClusters(P11T, resolution = 0.5)

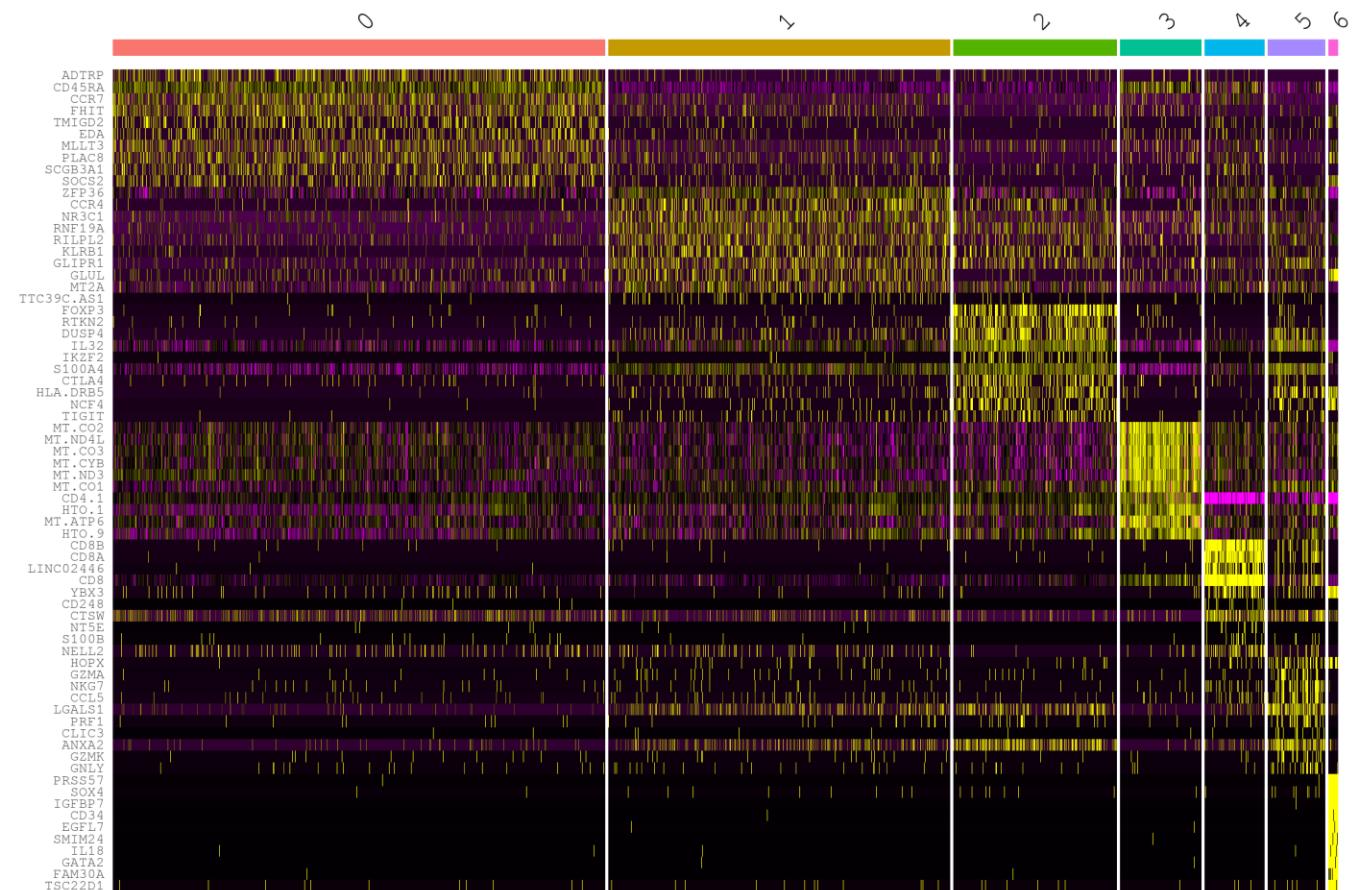
P11T <- RunUMAP(P11T, dims = 1:30)
DimPlot(P11T, reduction = "umap")
```

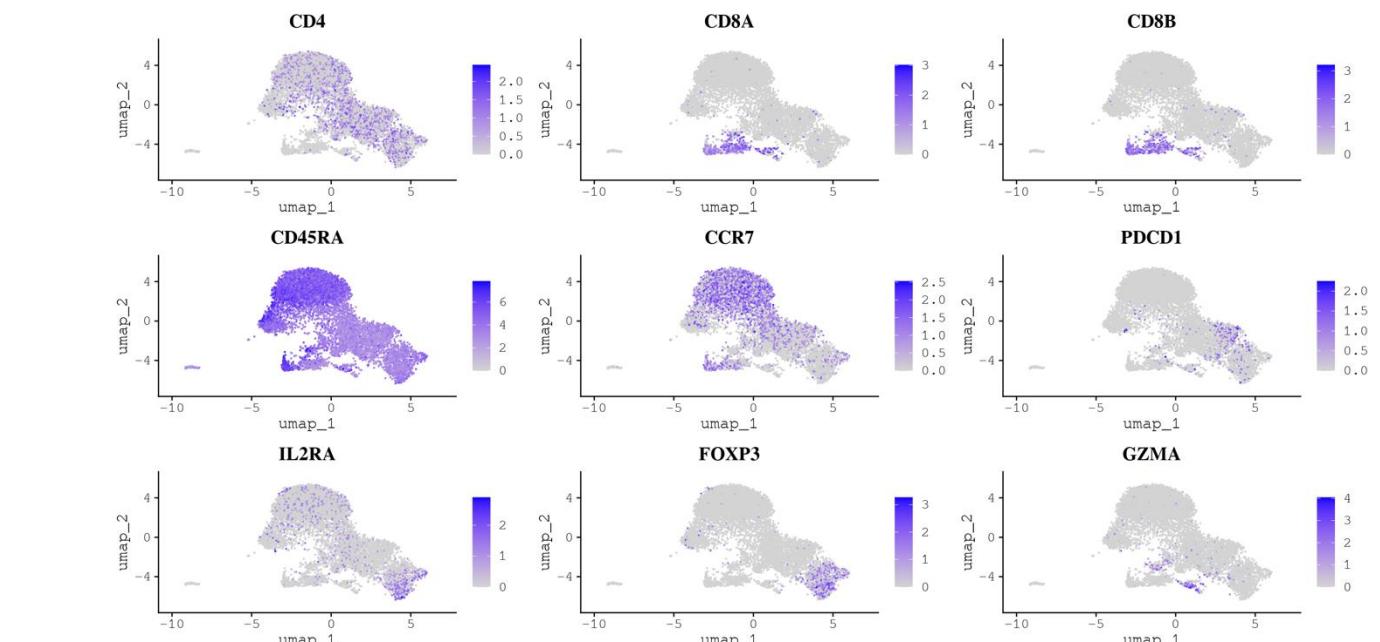
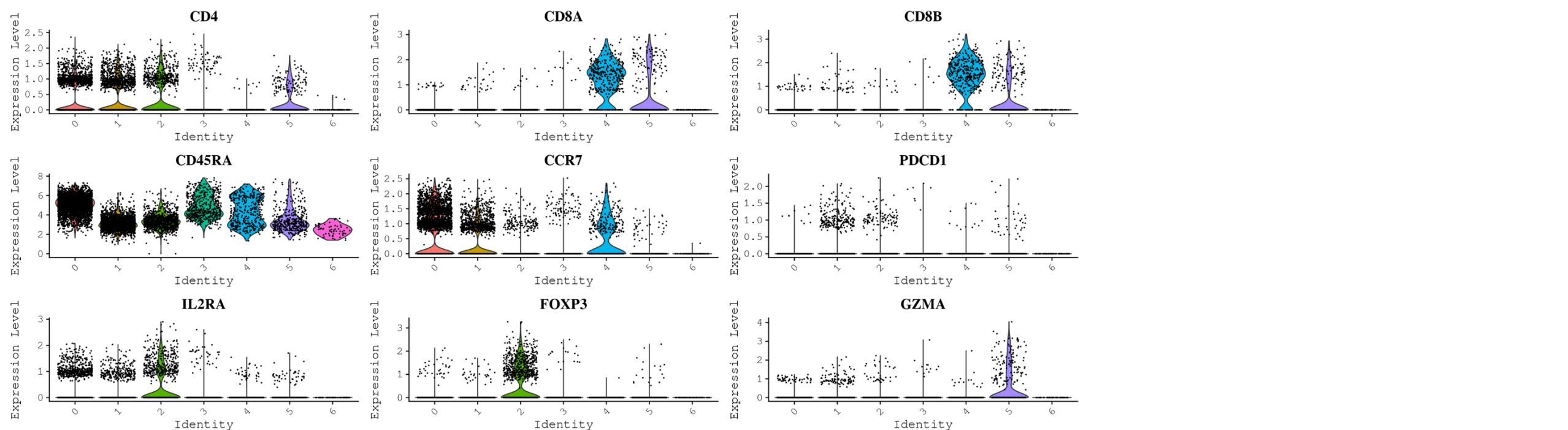


```
P11T.markers %>%
  group_by(cluster) %>%
  dplyr::filter(avg_log2FC > 1) %>%
  slice_min(p_val, n = 3) %>%
```

# Groups: cluster [7]

	p_val	avg_log2FC	pct.1	pct.2	p_val_adj	cluster	gene
	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<fct>	<chr>
1	0	2.48	0.484	0.106	0	0	ADTRP
2	0	1.48	1	1	0	0	CD45RA
3	4.54e-298	1.66	0.662	0.28	5.04e-294	0	CCR7
4	1.70e-203	1.06	0.975	0.875	1.89e-199	1	ZFP36
5	1.56e-179	1.87	0.383	0.105	1.74e-175	1	CCR4
6	1.82e-169	1.32	0.67	0.356	2.02e-165	1	NR3C1
7	0	5.28	0.509	0.016	0	2	FOXP3
8	0	3.59	0.444	0.056	0	2	RTKN2
9	1.93e-300	3.03	0.524	0.098	2.15e-296	2	DUSP4
10	1.05e-230	1.34	1	1	1.16e-226	3	MT.C02
11	5.49e-216	1.32	1	1	6.10e-212	3	MT.ND4L
12	1.64e-213	1.34	1	1	1.82e-209	3	MT.C03
13	0	5.51	0.901	0.027	0	4	CD8B
14	0	4.83	0.847	0.023	0	4	CD8A
15	0	6.11	0.55	0.011	0	4	LINC02446
16	3.25e-232	4.21	0.374	0.027	3.61e-228	5	HOPX
17	8.84e-202	5.30	0.345	0.027	9.82e-198	5	GZMA
18	1.74e-199	5.91	0.384	0.036	1.93e-195	5	NKG7
19	0	11.0	1	0.001	0	6	PRSS57
20	0	7.42	0.984	0.012	0	6	SOX4
21	0	9.09	0.937	0.002	0	6	IGFBP7





# Next! Annotation with SingleR

4

DatabaseImmuneCellExpressionData

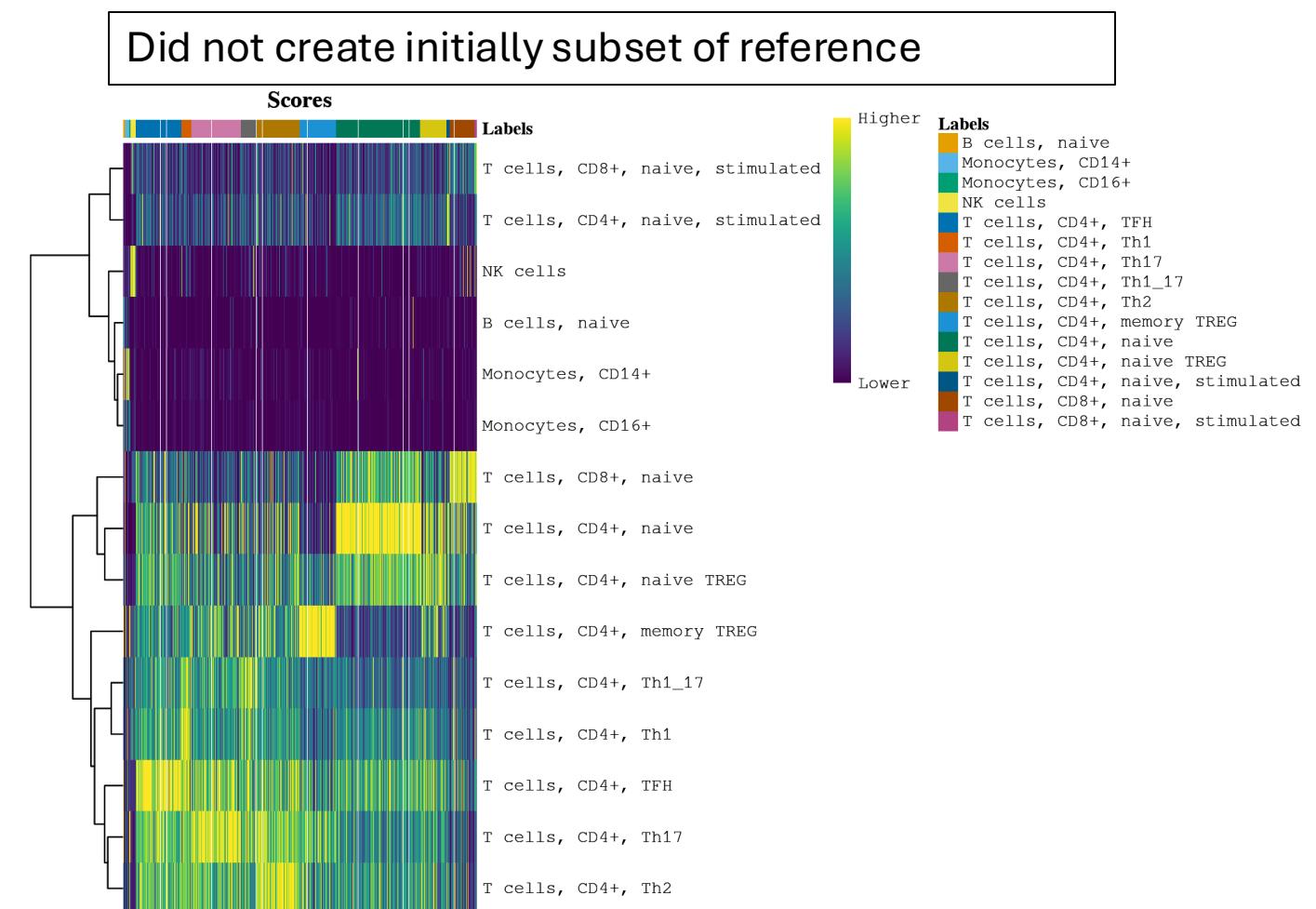
## Examples

```
ref.se <- BlueprintEncodeData(rm.NA = "rows")
```

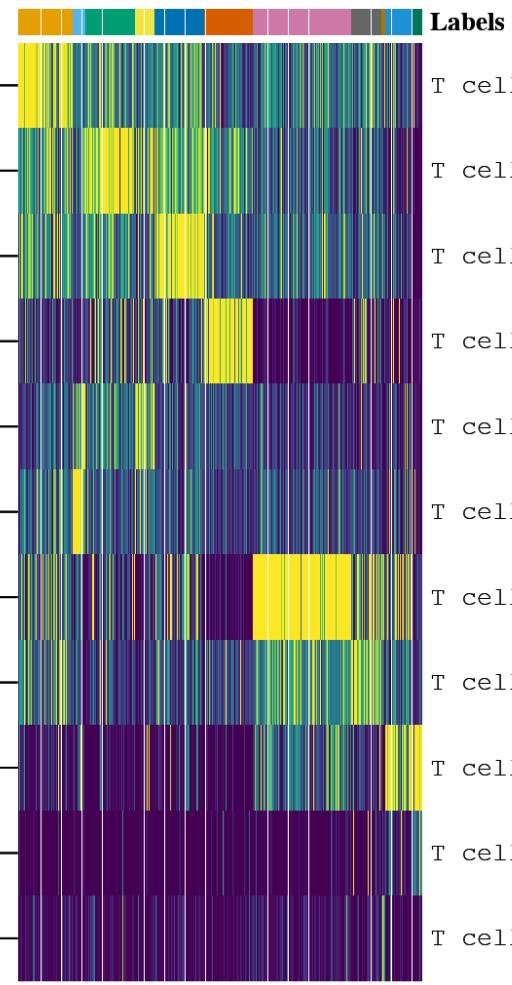
DatabaseImmuneCellExpressionData  
Obtain human bulk RNA-seq data from DICE

Samples were additionally annotated to 15 fine cell types ("label.fine"):

- B cells, naive
- Monocytes, CD14+
- Monocytes, CD16+
- NK cells
- T cells, memory TREG
- T cells, CD4+, naive
- T cells, CD4+, naive, stimulated
- T cells, CD4+, naive Treg
- T cells, CD4+, Th1
- T cells, CD4+, Th1\_17
- T cells, CD4+, Th2
- T cells, CD8+, naïve
- T cells, CD8+, naïve, stimulated
- T cells, CD4+, TFH
- T cells, CD4+, Th17

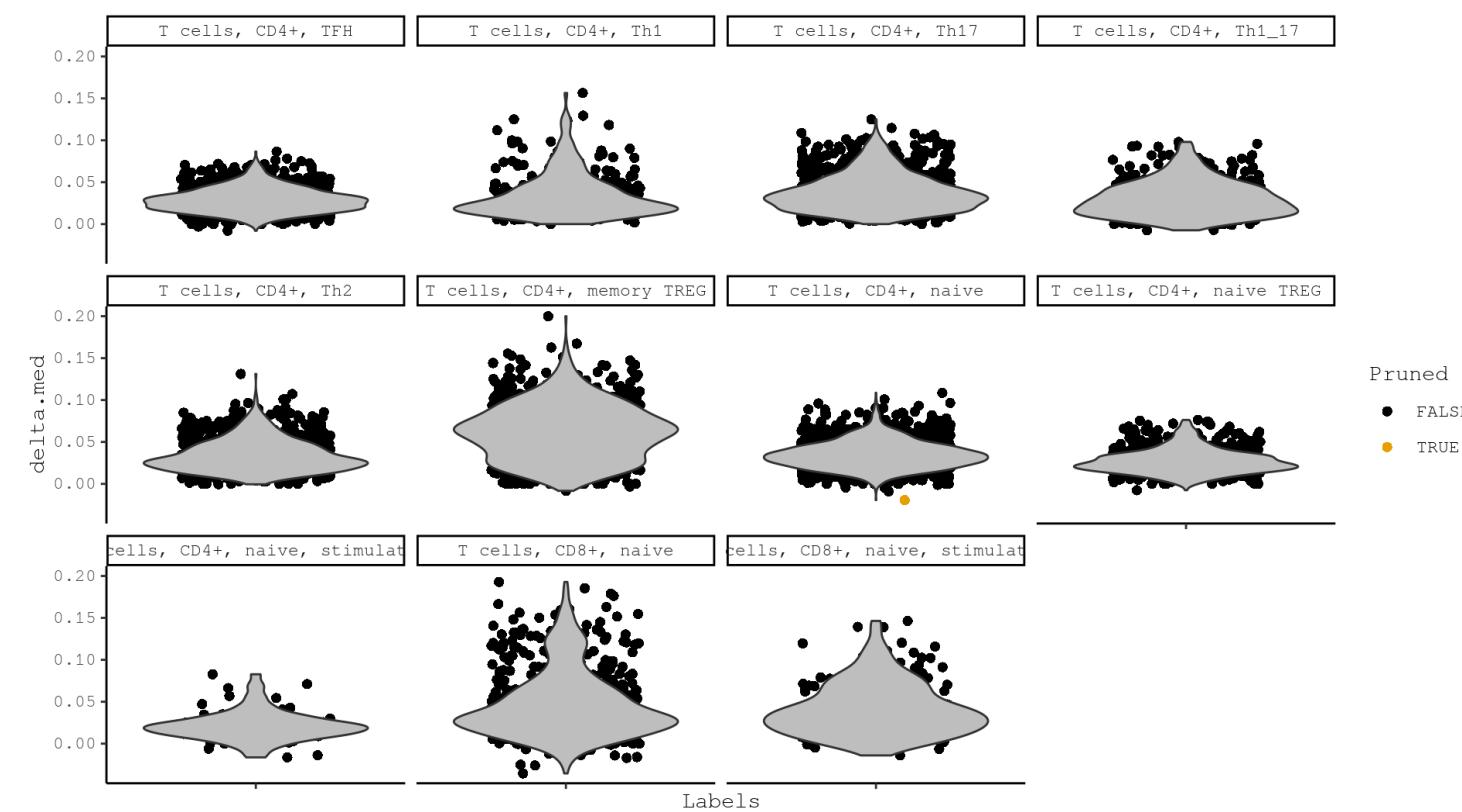


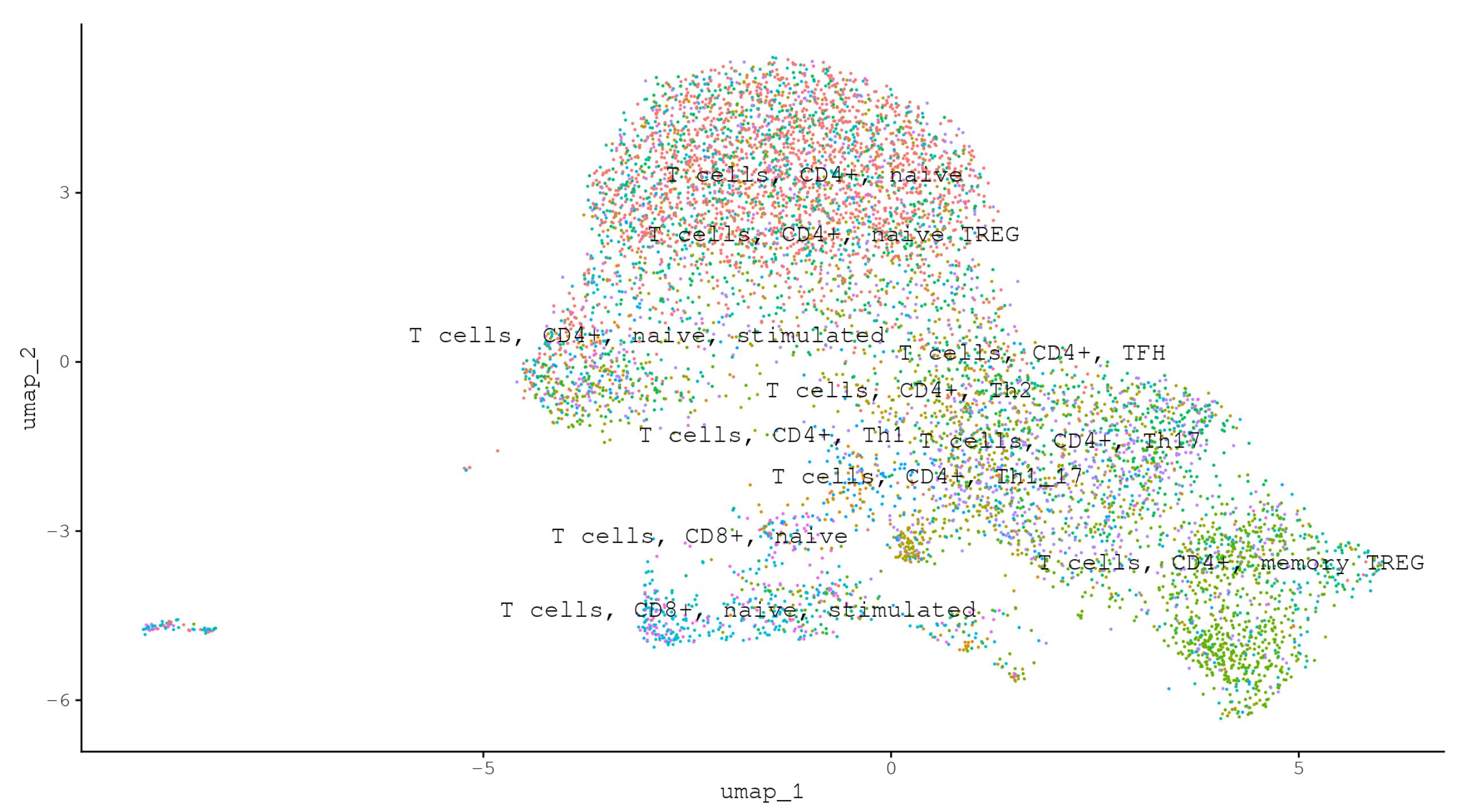
# Scores



# Labels

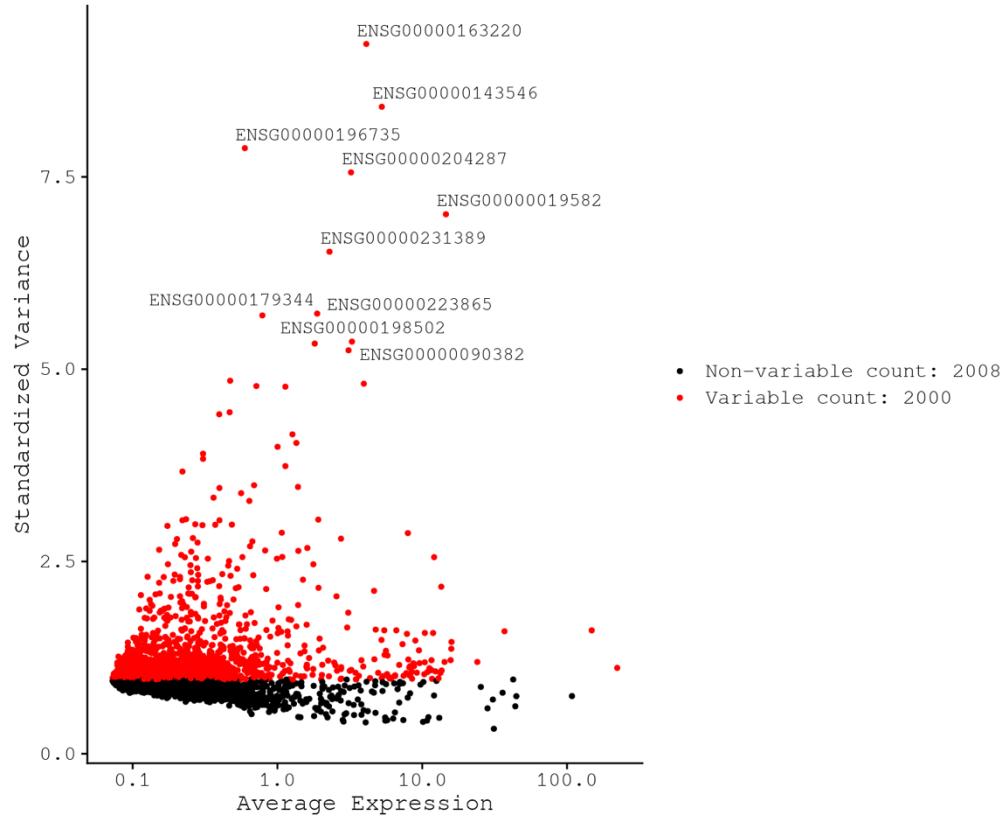
- T cells, CD4+, TFH
- T cells, CD4+, Th1
- T cells, CD4+, Th17
- T cells, CD4+, Th1\_17
- T cells, CD4+, Th2
- T cells, CD4+, memory TREG
- T cells, CD4+, naive
- T cells, CD4+, naive TREG
- T cells, CD4+, naive, stimulated
- T cells, CD8+, naive
- T cells, CD8+, naive, stimulated



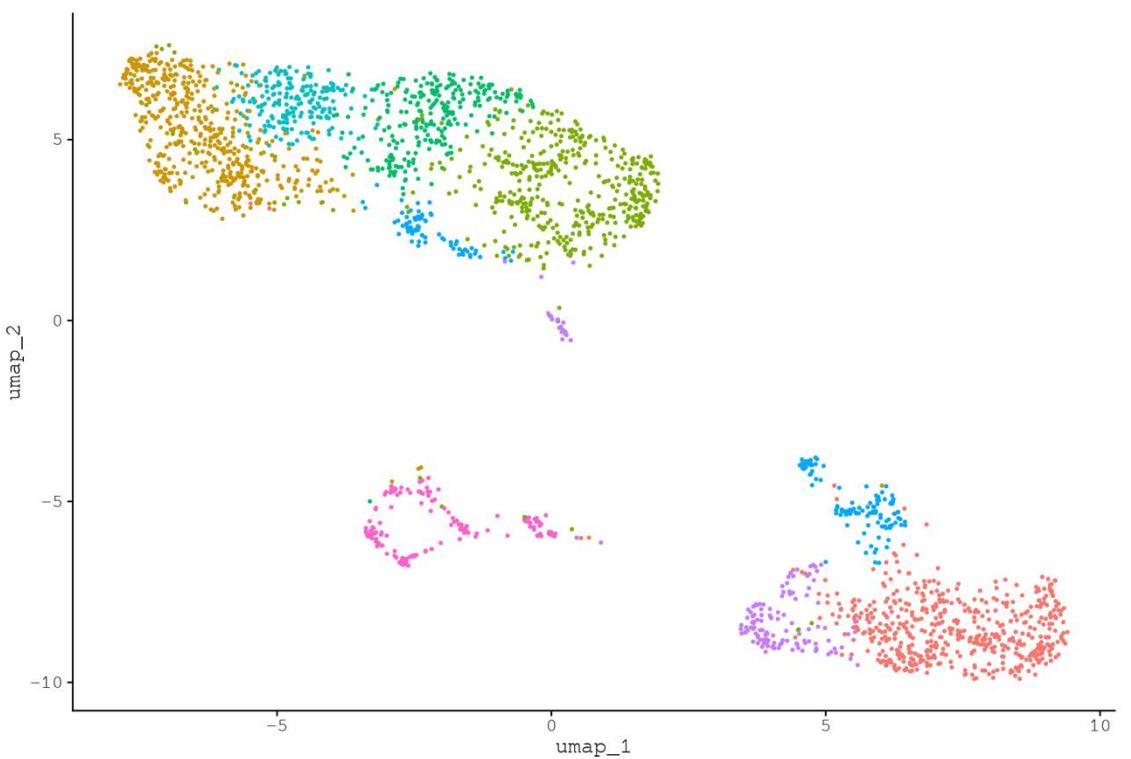
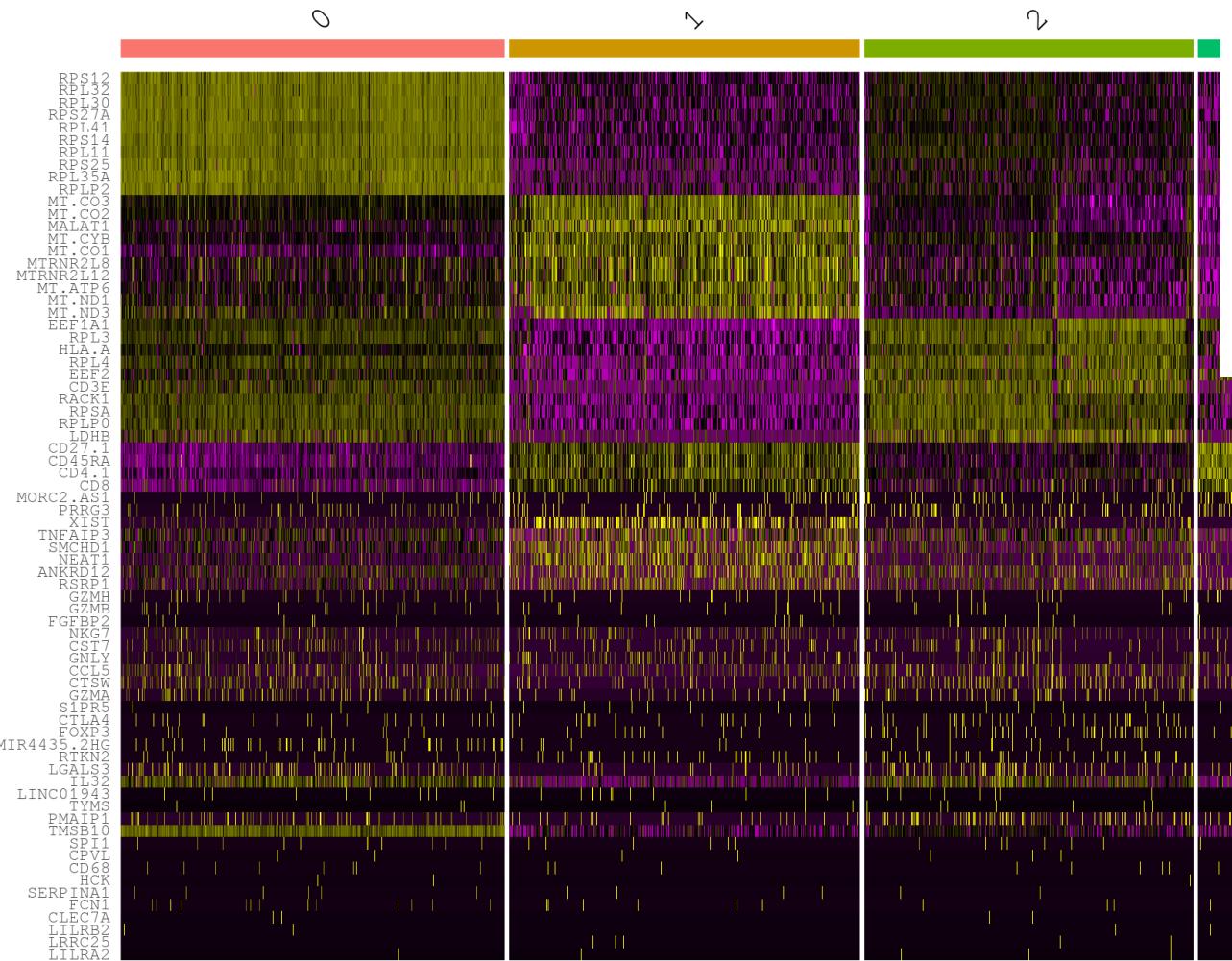


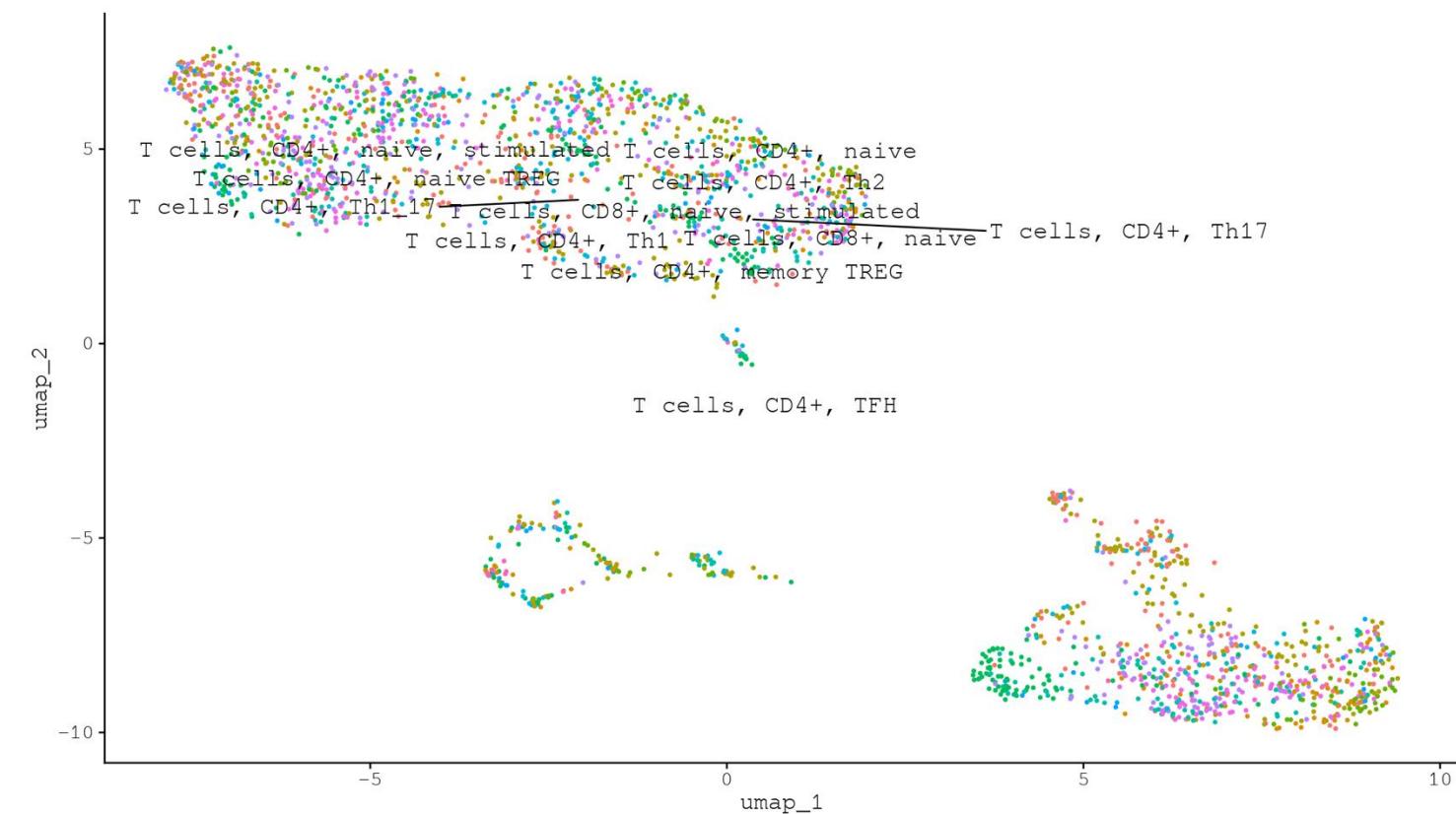
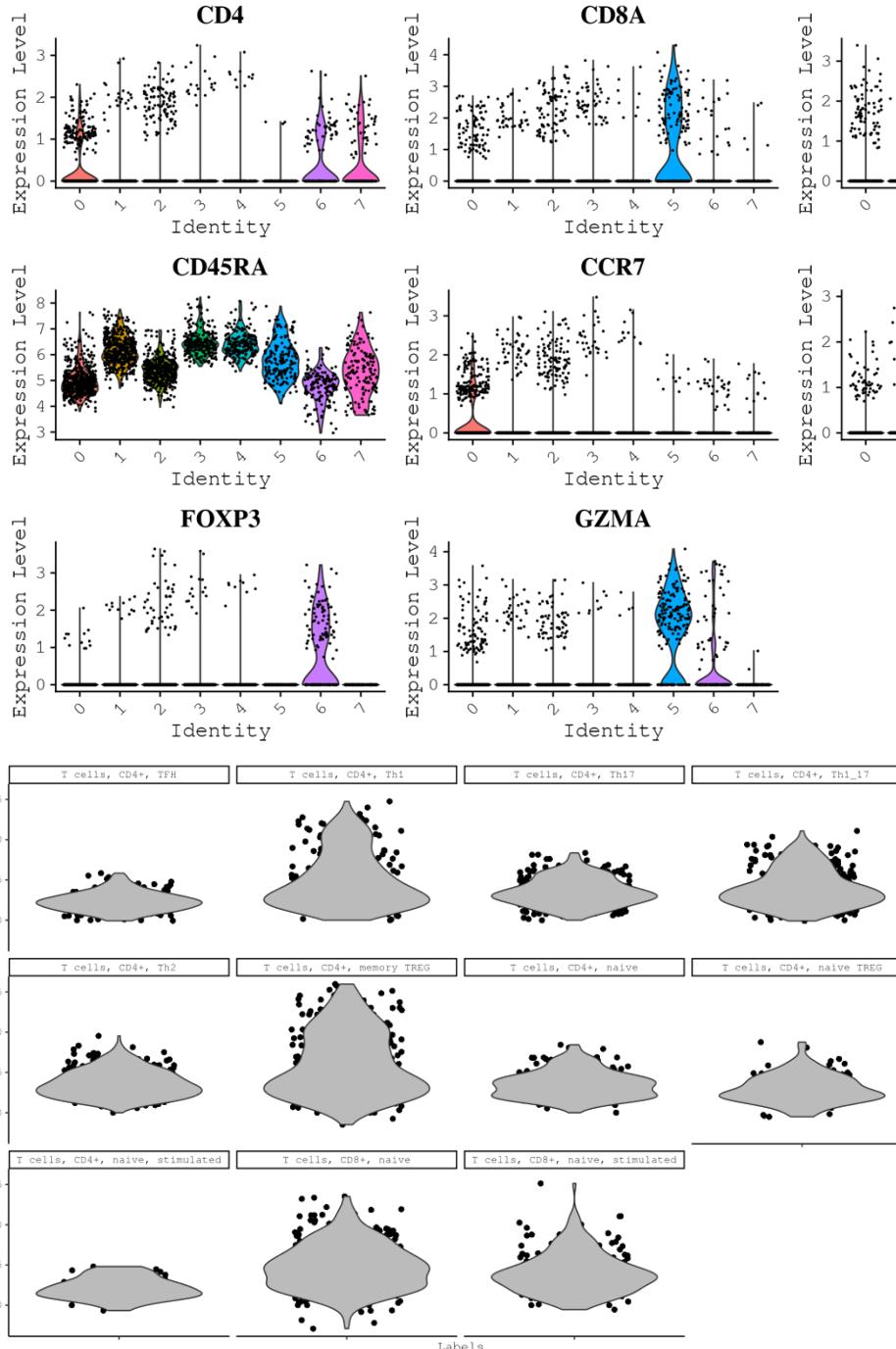
# Pause and repeat for the other patient

The other samples' genes were listed as Ensembl IDs?

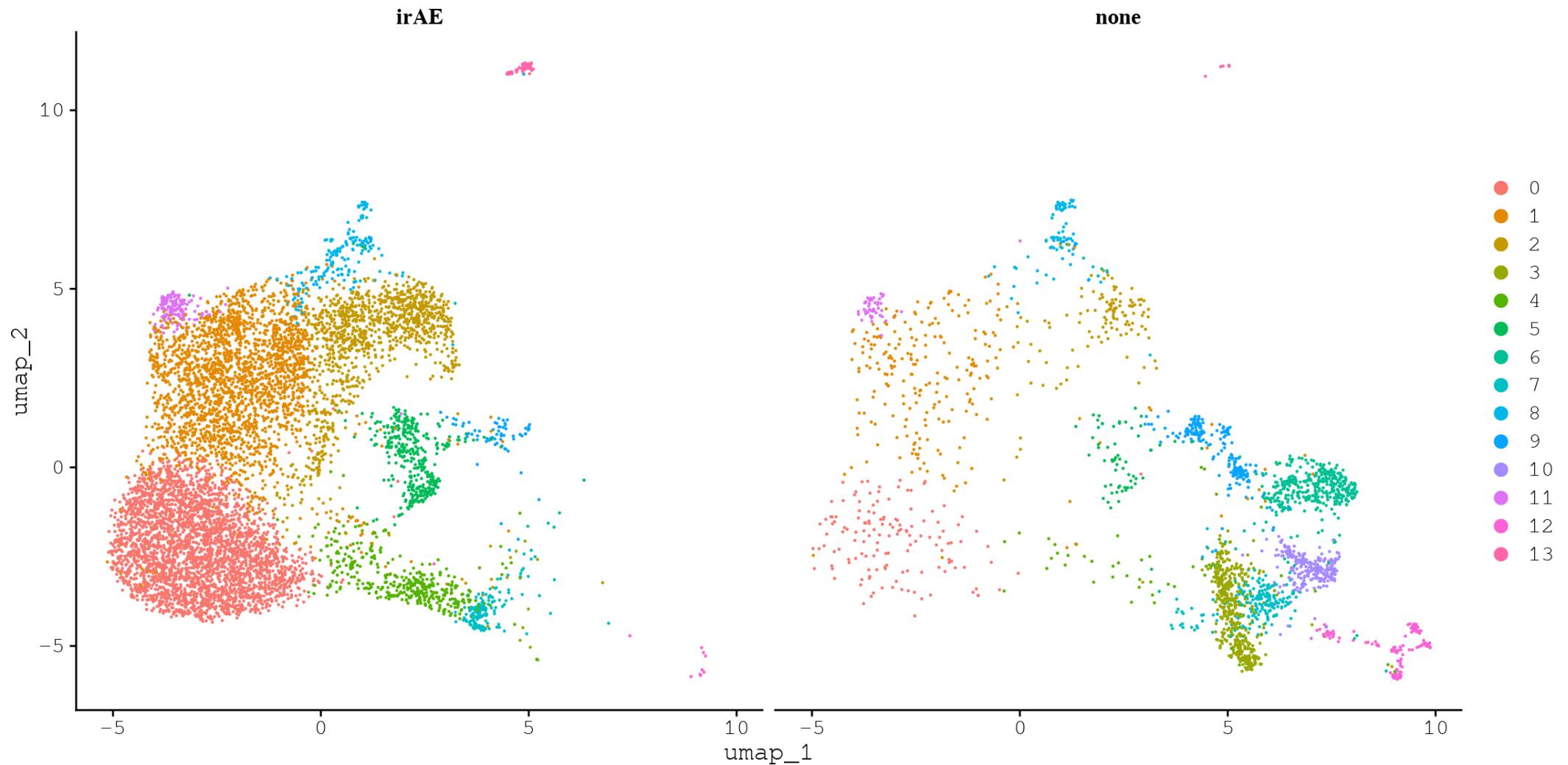


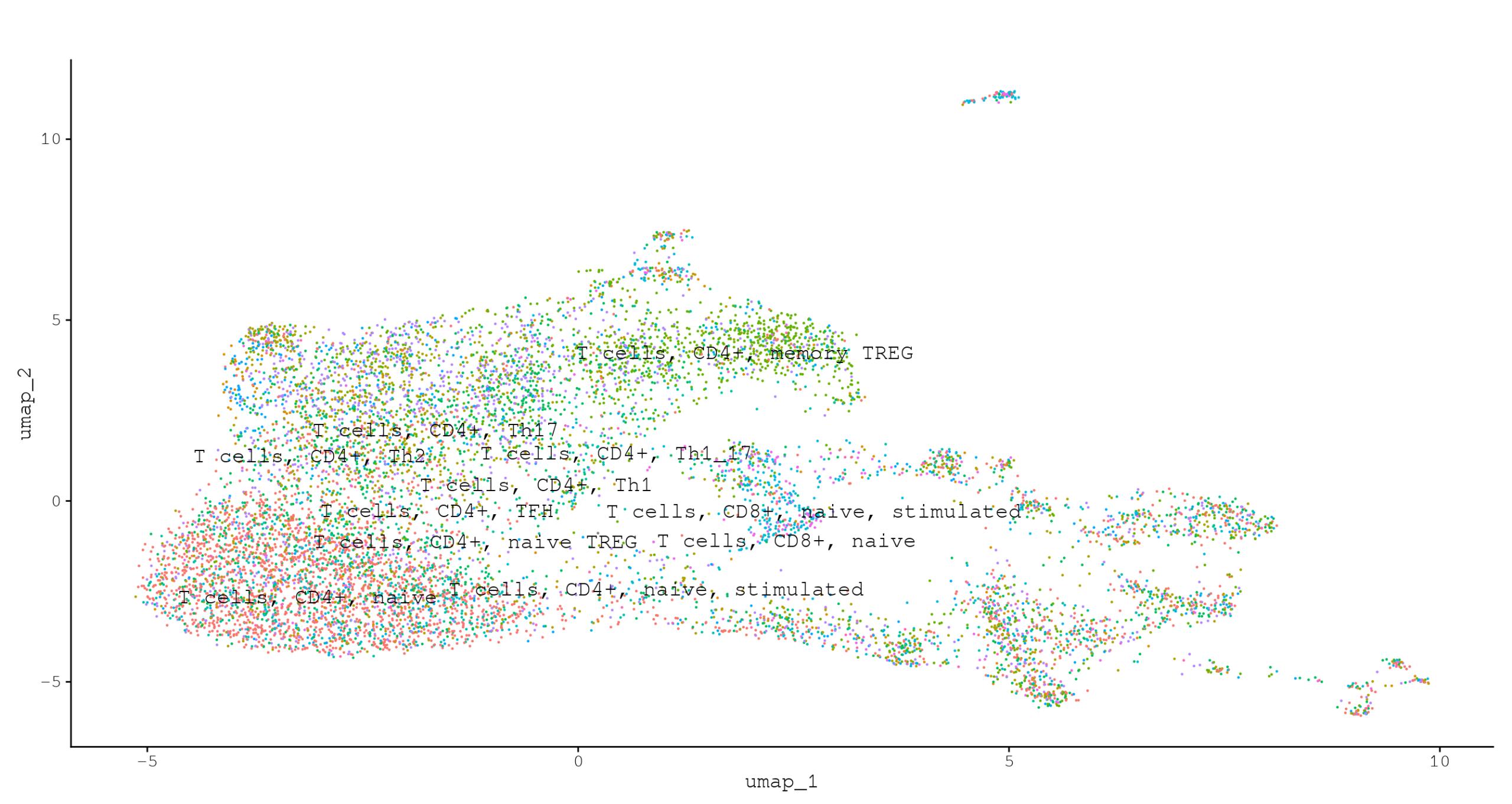
Spent 30 minutes trying to fix a problem that might not exist





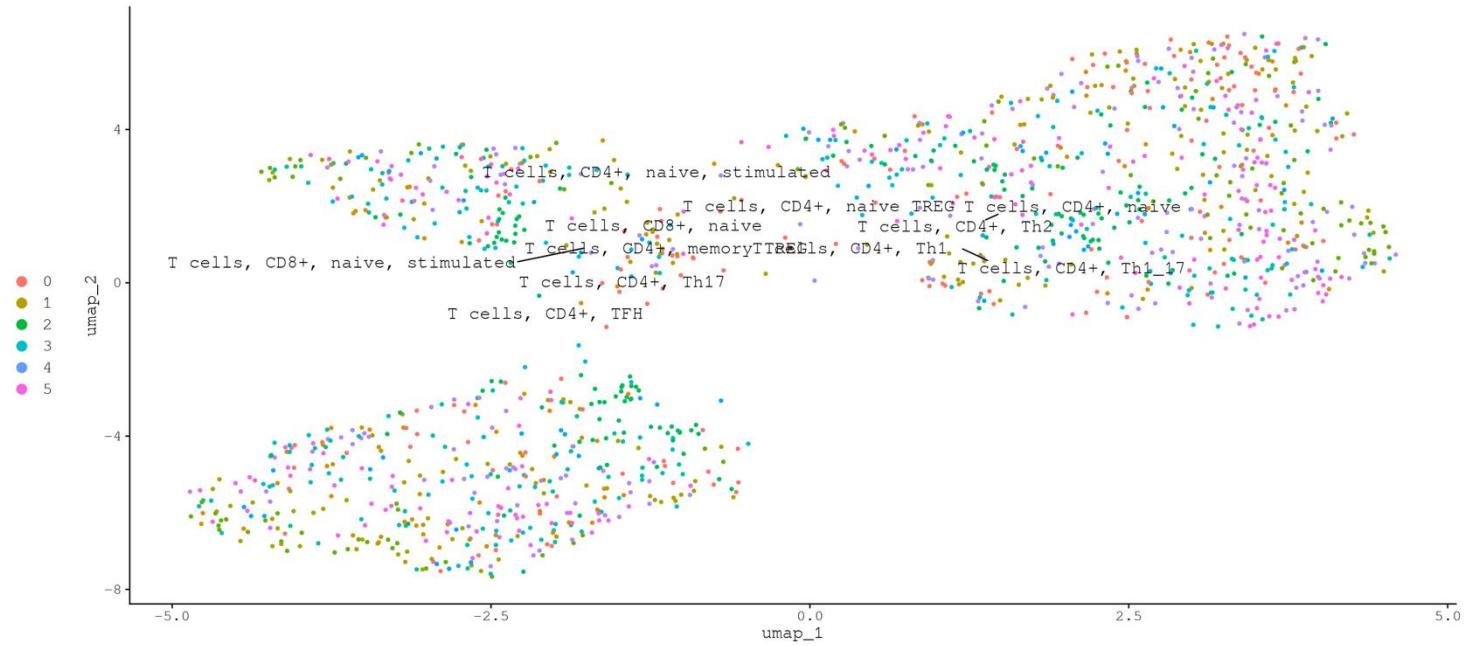
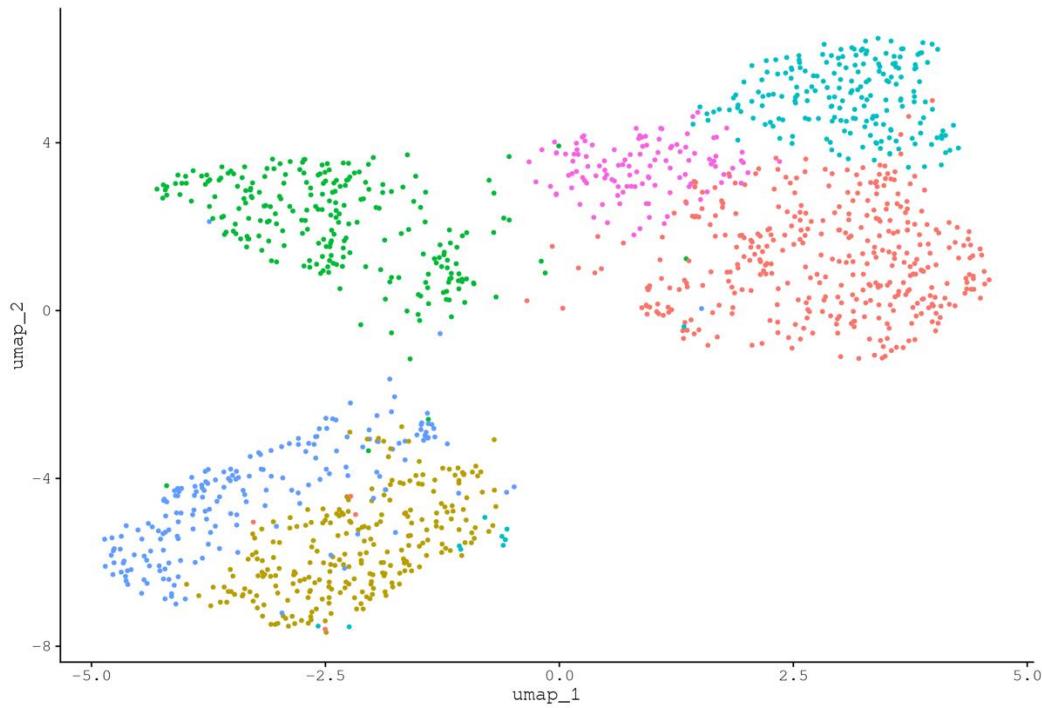
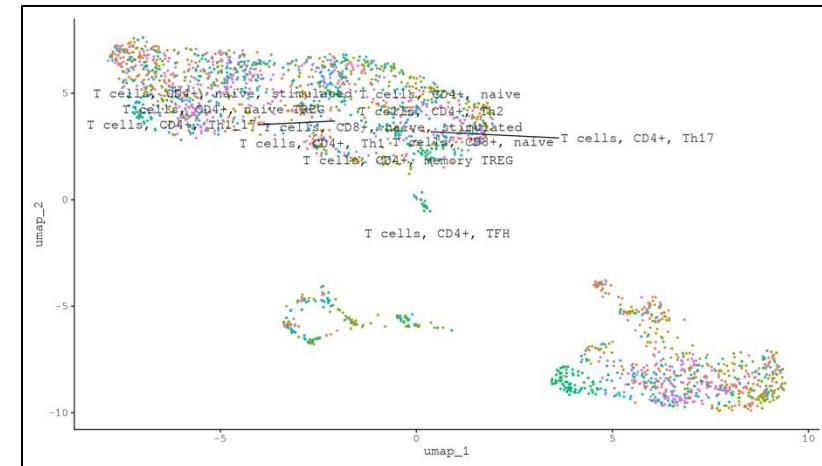
# Integration (?)

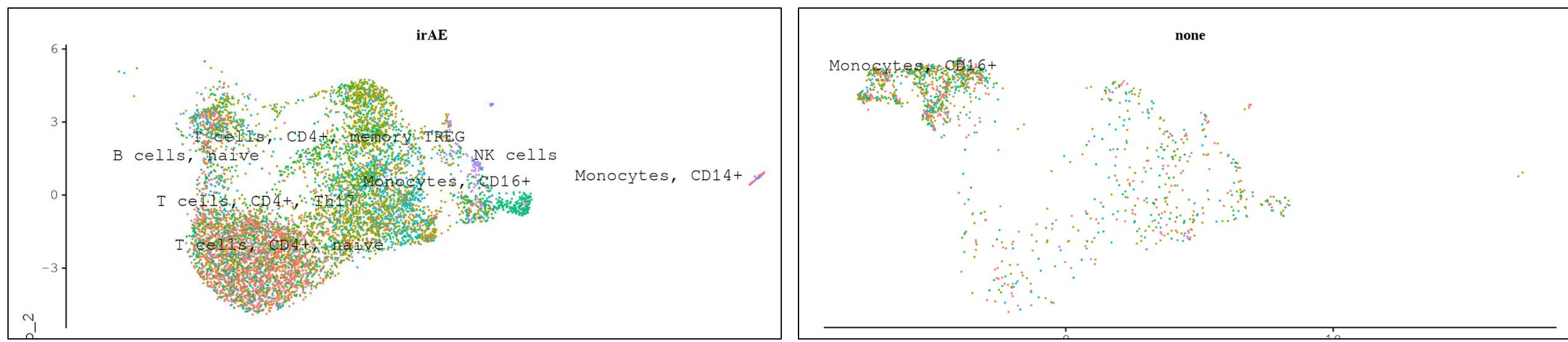
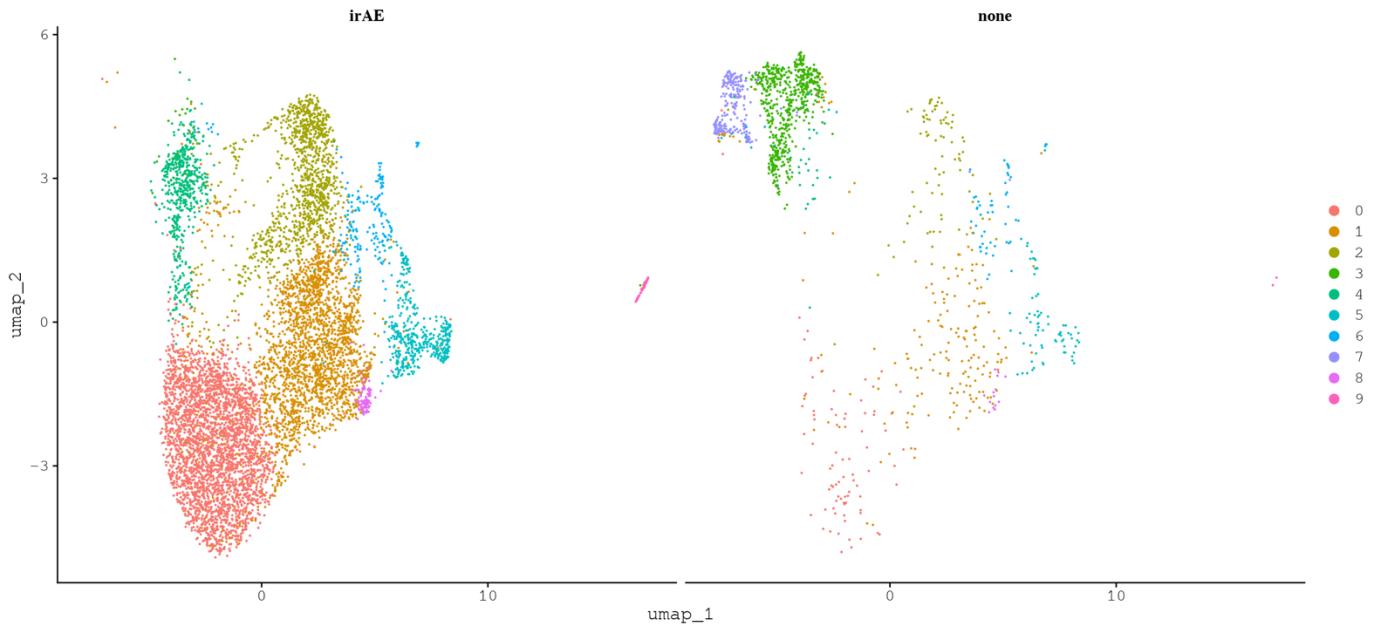




# Subset?

	p_val	avg_log2FC	pct.1	pct.2	p_val_adj	cluster	gene
	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<fctr>	<chr>
1	3.26e-136	2.04	1	0.985	2.58e-132	0	MALAT1
2	1.76e-70	1.86	0.788	0.324	1.39e-66	0	EML4
3	1.24e-62	1.72	0.759	0.319	9.78e-59	0	CCNL1
4	7.46e-118	1.92	1	0.941	5.89e-114	1	B2M
5	9.13e-117	2.29	1	0.67	7.21e-113	1	RPS4X
6	3.49e-115	2.18	1	0.662	2.75e-111	1	RPL7A
7	1.41e-33	1.03	0.914	0.684	1.11e-29	2	UBC
8	4.32e-11	1.26	0.537	0.41	3.41e-7	2	ITGB2
9	6.17e-8	4.01	0.053	0.007	4.87e-4	2	ADGRG1
10	4.54e-67	1.94	1	0.892	3.58e-63	3	MT.C02
11	4.48e-63	1.89	0.995	0.862	3.54e-59	3	MT.C03
12	1.21e-52	1.42	1	0.997	9.55e-49	3	MT.C01
13	1.47e-73	1.67	1	0.972	1.16e-69	4	EEF1A1
14	6.38e-70	1.39	1	0.927	5.04e-66	4	HLA.B
15	6.75e-60	1.35	0.995	0.837	5.33e-56	4	HLA.A
16	1.89e-5	1.95	0.212	0.099	1.50e-1	5	MORC2.AS1
17	2.10e-4	1.14	0.5	0.449	1	e+ 0	5
18	1.01e-3	1.06	0.458	0.394	1	e+ 0	5
							NEAT1





# Can draw conclusions?

- This is after aPD1 treatment, which is meant to undo T cell exhaustion.
- Patients with immune-related adverse events have significantly more T cells in circulation, compared to patients who do not have side effects
- Question: Does higher T cell count and immune-related adverse events also correspond to lower tumor burden? Or is this a double-negative situation?
- *Minuscule sample size*

# Future directions

**Goal: complete by December 1, 2024**

- Increase strength of conclusions by increasing sample size (up to 4 per group)
- As QC, calculate %MT genes per sample, and remove cells that show high values (are dying)
- Convert EnsemblIDs to HGNCs using BioMart (possible, although unlikely, increase in accuracy)
- Pseudobulk samples and calculate trajectories using Monocle3 to see how gene differentiation affects outcomes