Expanding Acne Dataset

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I'm working with a Seurat object for this analysis. Seurat objects are a representation of single-cell expression data for R, where we can analyze cell types and feature level (gene expression) of our sample conditions.

```
knitr::opts_chunk$set(echo = TRUE)

library(tidyverse) # load the tidyverse
```

```
## -- Attaching core tidyverse packages ------------------------ tidyverse 2.0.0 --
## v dplyr     1.1.3     v readr     2.1.4
## v forcats   1.0.0     v stringr   1.5.0
## v ggplot2   3.4.3     v tibble    3.2.1
## v lubridate 1.9.2     v tidyr     1.3.0
## v purrr     1.0.2
## -- Conflicts ------------------------------------------ tidyverse_conflicts() --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()    masks stats::lag()
## i Use the conflicted package (<http://conflicted.r-lib.org/>) to force all conflicts to become errors
```

```
library(Seurat) # we need this to work with Seurat objects
```

```
## The legacy packages maptools, rgdal, and rgeos, underpinning the sp package,
## which was just loaded, will retire in October 2023.
## Please refer to R-spatial evolution reports for details, especially
## https://r-spatial.org/r/2023/05/15/evolution4.html.
## It may be desirable to make the sf package available;
## package maintainers should consider adding sf to Suggests:.
## The sp package is now running under evolution status 2
##      (status 2 uses the sf package in place of rgdal)
## Attaching SeuratObject
```

```
# Check the working directory.
getwd()
```

```
## [1] "/Users/tamto/Desktop"
```

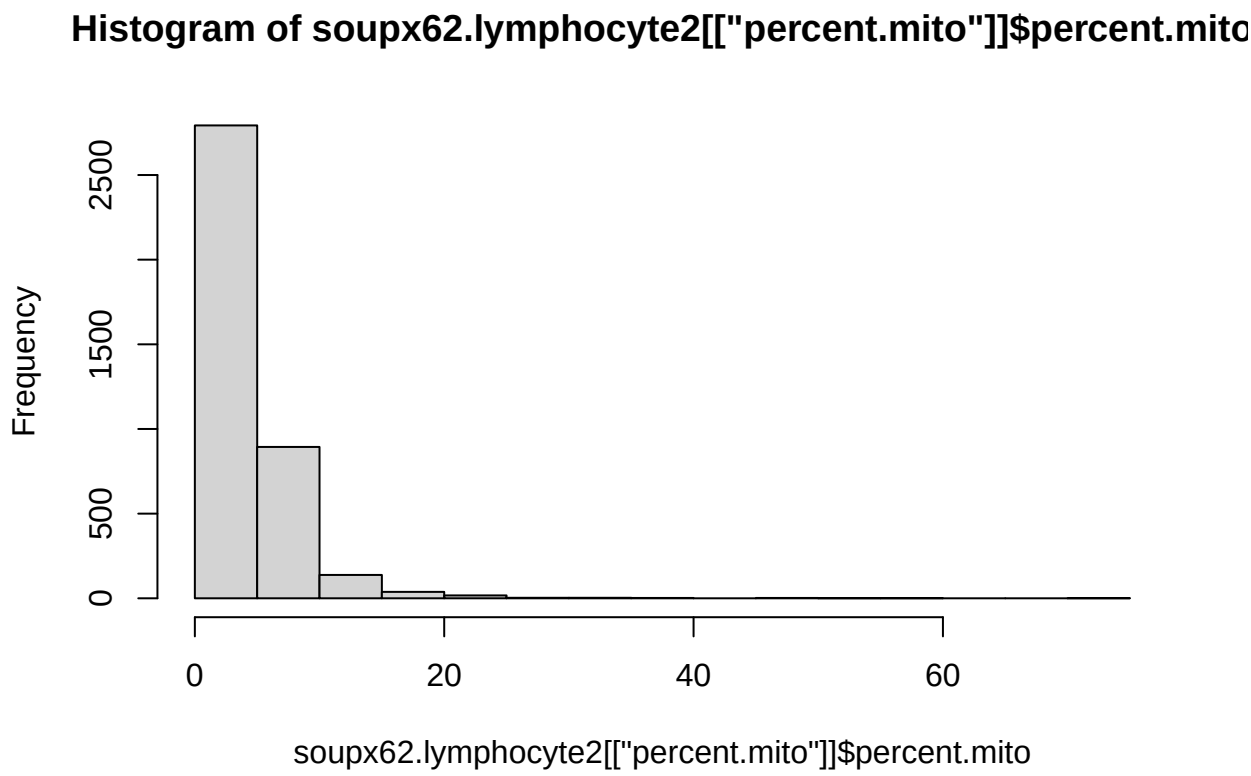
```
# Setting the wd to where the file is located (in this case it's saved on my desktop).
setwd("/Users/tamto/Desktop")

# Time to load the data in our global environment!
```

```r
load("/Users/tamto/Desktop/soupx62.lymphocyte2.Rdata")

# View the first 6 rows of the dataset.
head(soupx62.lymphocyte2, 6)
```

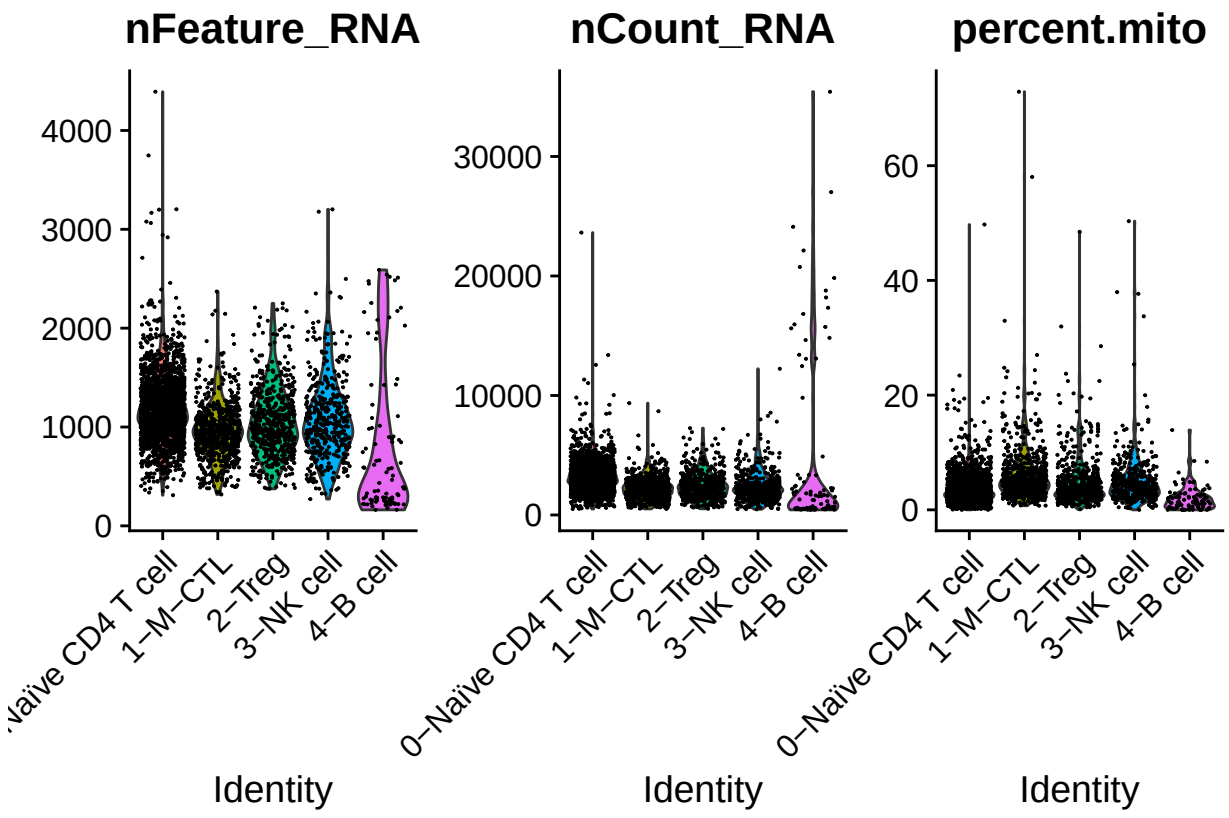
```
##                         orig.ident nCount_RNA nFeature_RNA percent.mito
## L_318A_AAACCCAGTCAGTCCG        318   5018.969         1571  0.016923572
## L_318A_AAAGAACGTGGATCAG        318   1174.791          752  0.001019867
## L_318A_AAAGAACTCTATGCCC        318   3070.307         1285  0.028881840
## L_318A_AAAGGTATCGCATGAT        318   2479.182          944  0.030006573
## L_318A_AAAGTGATCGTAGGGA        318   3225.081         1064  0.019521774
## L_318A_AACGGGAAGGTACATA        318   1931.879          845  0.028149236
##                         nCount_HTO nFeature_HTO      HTO_maxID
## L_318A_AAACCCAGTCAGTCCG        881            3    LesionalEpi
## L_318A_AAAGAACGTGGATCAG         84            4 LesionalDermis
## L_318A_AAAGAACTCTATGCCC         24            3 LesionalDermis
## L_318A_AAAGGTATCGCATGAT         19            4 LesionalDermis
## L_318A_AAAGTGATCGTAGGGA         29            2 LesionalDermis
## L_318A_AACGGGAAGGTACATA         15            2 LesionalDermis
##                              HTO_secondID HTO_margin         HTO_classification
## L_318A_AAACCCAGTCAGTCCG    LesionalDermis  1.2137600 LesionalDermis_LesionalEpi
## L_318A_AAAGAACGTGGATCAG NonlesionalDermis  1.9391469             LesionalDermis
## L_318A_AAAGAACTCTATGCCC       LesionalEpi  1.5604209             LesionalDermis
## L_318A_AAAGGTATCGCATGAT NonlesionalDermis  0.8417437             LesionalDermis
## L_318A_AAAGTGATCGTAGGGA    NonlesionalEpi  2.0494646             LesionalDermis
## L_318A_AACGGGAAGGTACATA    NonlesionalEpi  1.2800965             LesionalDermis
##                         HTO_classification.global        hash.ID      donor
## L_318A_AAACCCAGTCAGTCCG                   Doublet        Doublet Lesional 1
## L_318A_AAAGAACGTGGATCAG                   Singlet LesionalDermis Lesional 1
## L_318A_AAAGAACTCTATGCCC                   Singlet LesionalDermis Lesional 1
## L_318A_AAAGGTATCGCATGAT                   Singlet LesionalDermis Lesional 1
## L_318A_AAAGTGATCGTAGGGA                   Singlet LesionalDermis Lesional 1
## L_318A_AACGGGAAGGTACATA                   Singlet LesionalDermis Lesional 1
##                             stim RNA_snn_res.0.5 seurat_clusters
## L_318A_AAACCCAGTCAGTCCG lesional               2               0
## L_318A_AAAGAACGTGGATCAG lesional               2               2
## L_318A_AAAGAACTCTATGCCC lesional               2               0
## L_318A_AAAGGTATCGCATGAT lesional               2               1
## L_318A_AAAGTGATCGTAGGGA lesional               2               0
## L_318A_AACGGGAAGGTACATA lesional               2               0
##                                    celltype RNA_snn_res.0.9 RNA_snn_res.0.1
## L_318A_AAACCCAGTCAGTCCG 0-Naïve CD4 T cell               0               0
## L_318A_AAAGAACGTGGATCAG             2-Treg               4               2
## L_318A_AAAGAACTCTATGCCC 0-Naïve CD4 T cell               2               0
## L_318A_AAAGGTATCGCATGAT            1-M-CTL               3               1
## L_318A_AAAGTGATCGTAGGGA 0-Naïve CD4 T cell               0               0
## L_318A_AACGGGAAGGTACATA 0-Naïve CD4 T cell               2               0
```

```r
# I like to look at it using view() since the data is so big and it's easier to visualize in Rstudio.
view(soupx62.lymphocyte2)
```

```r
# We have to be cautious of mitochondrial RNA because the mitochondria has its own RNA that can get mix
soupx62.lymphocyte2[["percent.mito"]] <- PercentageFeatureSet(soupx62.lymphocyte2, pattern = "^MT-")
# We can view this as a histogram to see what percentage of mitochondrial RNA the cells have. This hist
hist(soupx62.lymphocyte2[["percent.mito"]]$percent.mito)
```




```r
# We can plot a violin plot to make sure we have a good reading on enough genes, there's a good reading
VlnPlot(soupx62.lymphocyte2, features = c("nFeature_RNA", "nCount_RNA", "percent.mito"), ncol = 3)
```




```
# These plots show that we have mostly around 1000 genes, less than 10,000 gene count, and less than 15

# This dataset has also already been normalized and scaled to make the values more comparable to each o

PCALymphocyte <- RunPCA(soupx62.lymphocyte2, features = VariableFeatures(object = soupx62.lymphocyte2))
```

```
## PC_ 1
## Positive:  LTB, CCR6, FTH1, RORA, VIM, S100A4, CD40LG, LGALS3, BATF, IL7R
##     TNFRSF4, S100A6, TMSB10, IL32, GAPDH, MAF, TSHZ2, PKM, NR3C1, ICOS
##     ZC3H12D, FRMD4B, TRBC2, AQP3, TYMP, JUNB, CTSH, CTLA4, CORO1B, SOD1
## Negative:  NKG7, CCL4, XCL2, CCL5, KLRD1, GZMB, TYROBP, CTSW, PRF1, GNLY
##     FCER1G, CST7, XCL1, GZMK, CCL4L2, GZMA, TRDC, KLRF1, CCL3, FCGR3A
##     SAMD3, GZMH, FGFBP2, MATK, CRTAM, HOPX, CCL3L1, PLEK, METRNL, IFNG
## PC_ 2
## Positive:  MZB1, IGKC, IGHG1, JCHAIN, DERL3, IGHG4, IGHG3, IGHG2, IGHGP, FCRL5
##     JSRP1, DNAAF1, IGHV3-23, CD79A, IGKV2-24, POU2AF1, IGKV4-1, IGHV3-33, IGF1, PRDX4
##     DPEP1, SCNN1B, IGLC2, LAMP5, SDC1, TNFRSF17, IGHV1-24, IGLV3-1, IGHA1, QPCT
## Negative:  MT-CO1, TMSB10, IL32, ANXA1, S100A4, MT-ATP6, JUNB, MT-ND3, FTH1, H3F3B
##     S100A6, ACTB, MT-ND4, MT-CYB, ZFP36L2, VIM, RGCC, MTRNR2L12, CD69, DUSP2
##     ZFP36, MT-ND2, ACTG1, LMNA, MT-ND1, MT-CO3, TRBC2, REL, PHLDA1, GAPDH
## PC_ 3
## Positive:  GZMK, KLF2, CCL5, GIMAP7, GIMAP4, CCL4L2, AP3M2, PASK, CCL4, PIK3R1
##     CCR7, CD8A, FCMR, SESN3, CD8B, ENC1, SELL, IFNG, MT-CO3, CCL3L1
##     GPR183, LYAR, KLRG1, MT-ND3, LINC02273, SH2D1A, DNAJB1, PDE3B, RILPL2, IGLC2
## Negative:  TNFRSF18, LGALS1, S100A4, S100A6, VIM, LMNA, CD63, CAPG, LGALS3, FCER1G
```

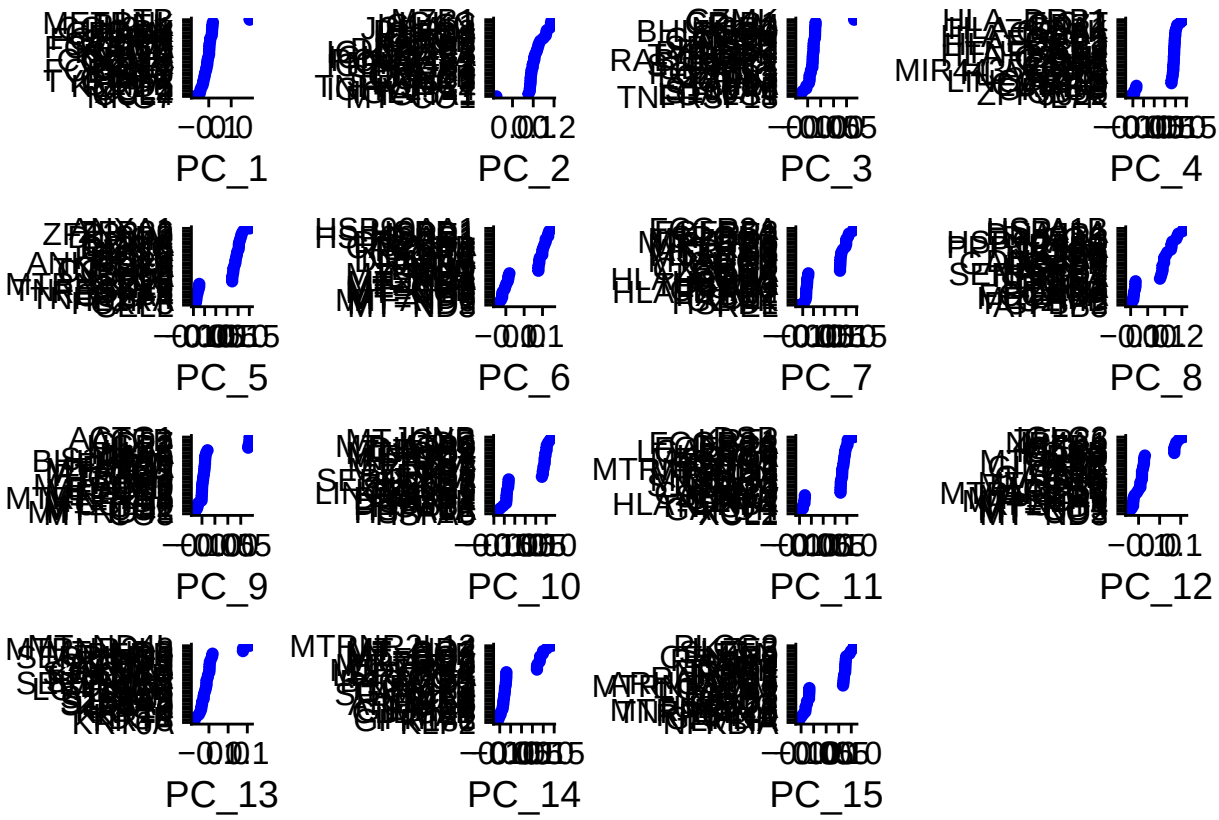
```
##     XCL1, SEPT11, S100A11, HOPX, RAB11FIP1, RBPJ, TYROBP, PHLDA1, KLRB1, SPINK2
##     CSF2, ACTB, GAPDH, CTSH, ENO1, BHLHE40, NCR3, PKM, CRIP1, GOLIM4
## PC_ 4
## Positive:  HLA-DRB1, TIGIT, CD74, HLA-DPA1, GZMK, CTLA4, HLA-DQB1, CCL5, FOXP3, HLA-DPB1
##     IL32, CCL4, TNFRSF9, HLA-DQA1, GZMH, GZMA, CD27, GBP5, CTSC, MIR4435-2HG
##     HLA-DRA, CST7, ACTB, LINC01943, CCL4L2, DUSP4, CYTOR, BATF, LAYN, PTTG1
## Negative:  IL7R, ZFP36L2, CD55, GRASP, AREG, FTH1, SPINK2, ANXA1, XCL1, FXYD7
##     KLRB1, FOS, CCR7, KIT, NFKB1, PLAC8, JUNB, FCER1G, SATB1, TYROBP
##     CD40LG, PLAUR, XBP1, BACH2, CD69, TRDC, AFF3, NFKBIA, ZFP36, CSF2
## PC_ 5
## Positive:  ANXA1, ZFP36, ZFP36L2, DUSP2, VIM, GZMA, LMNA, RGCC, GZMK, JUNB
##     CCL5, H3F3B, CCL4, ANKRD28, CD8A, TNFSF9, CCL4L2, CD69, CYBA, CLU
##     TNF, CD8B, DUSP1, DUSP5, GZMH, S100A4, TUBA4A, FOS, LINC01871, MT-CYB
## Negative:  SELL, FOXP3, IL2RA, TNFRSF4, CTLA4, RPS4Y1, TNFRSF18, GNLY, MTRNR2L8, GK
##     CD7, IKZF2, FCER1G, LAYN, SATB1, F5, TBC1D4, STAM, GBP5, PMAIP1
##     TYROBP, LAIR2, ENTPD1, HACD1, CARD16, IL18R1, MT-ND4L, TXK, PLAC8, BEX3
```

```
# I chose to look at 15 different dimensional variations since I'm trying to expand the dataset. We can

print(PCALymphocyte[['pca']], dims = 1:15, nfeatures = 5)
```

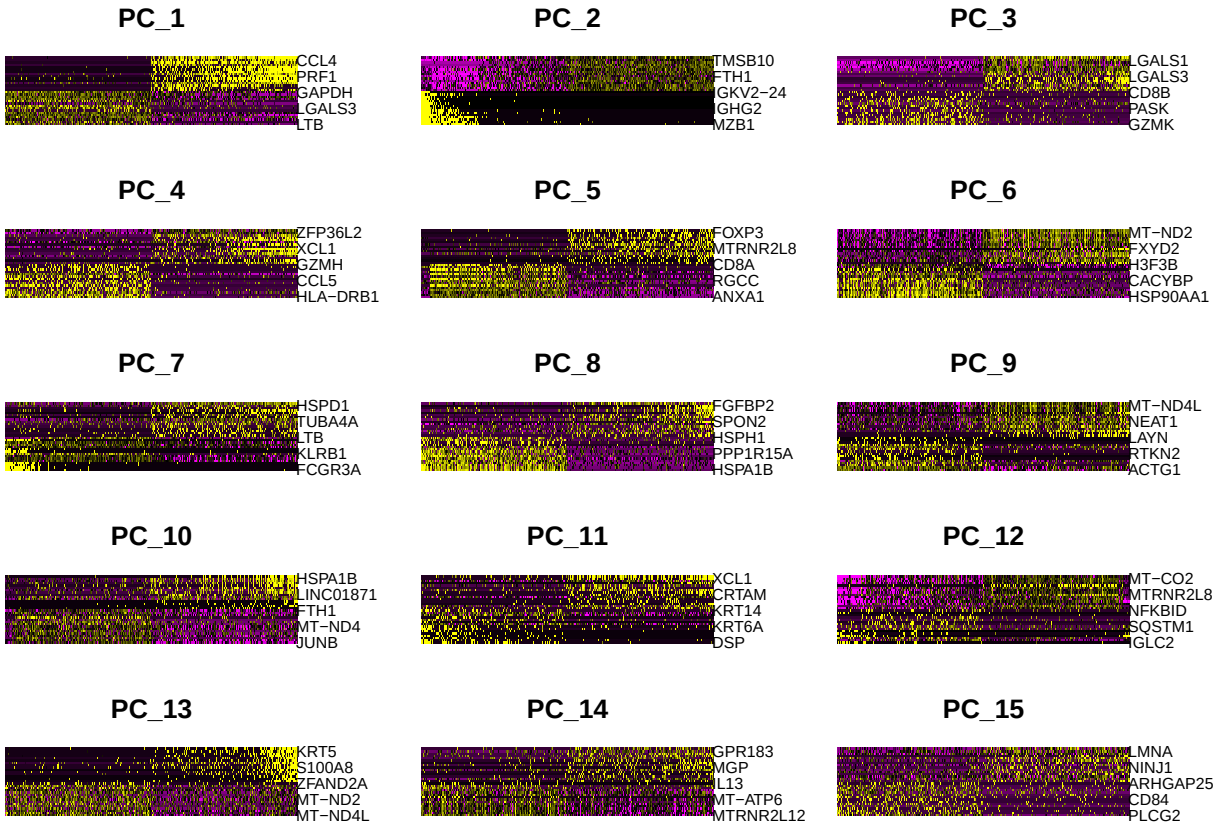
```
## PC_ 1
## Positive:  LTB, CCR6, FTH1, RORA, VIM
## Negative:  NKG7, CCL4, XCL2, CCL5, KLRD1
## PC_ 2
## Positive:  MZB1, IGKC, IGHG1, JCHAIN, DERL3
## Negative:  MT-CO1, TMSB10, IL32, ANXA1, S100A4
## PC_ 3
## Positive:  GZMK, KLF2, CCL5, GIMAP7, GIMAP4
## Negative:  TNFRSF18, LGALS1, S100A4, S100A6, VIM
## PC_ 4
## Positive:  HLA-DRB1, TIGIT, CD74, HLA-DPA1, GZMK
## Negative:  IL7R, ZFP36L2, CD55, GRASP, AREG
## PC_ 5
## Positive:  ANXA1, ZFP36, ZFP36L2, DUSP2, VIM
## Negative:  SELL, FOXP3, IL2RA, TNFRSF4, CTLA4
## PC_ 6
## Positive:  HSP90AA1, HSPD1, HSPE1, HSP90AB1, HSPH1
## Negative:  MT-ND3, MT-ND2, MT-ATP6, MT-ND1, MT-ND5
## PC_ 7
## Positive:  FCGR3A, FGFBP2, KLRF1, SPON2, MT-CO3
## Negative:  REL, HSPD1, GZMK, XCL1, FXYD2
## PC_ 8
## Positive:  HSPA1B, HSPA1A, DNAJB1, DUSP1, FOS
## Negative:  ATP1B3, FGFBP2, GZMB, METRNL, FCGR3A
## PC_ 9
## Positive:  ACTG1, ACTB, CD27, UGP2, IL32
## Negative:  MT-CO3, MT-ND4L, MT-CO1, MT-CO2, CCL20
## PC_ 10
## Positive:  JUNB, MT-CYB, ICOS, MT-ATP6, DUSP2
## Negative:  HSPA6, HSPA1B, HSPA1A, HSPB1, DNAJB4
## PC_ 11
## Positive:  DSP, KRT1, FCGR3A, DSC3, FGFBP2
## Negative:  XCL2, XCL1, GAPDH, TNFSF4, HLA-DRB1
```

```
## PC_ 12
## Positive:  IGLC2, ZFP36, NR4A1, MZB1, CCL3
## Negative:  MT-ND3, MT-CO2, MT-CO3, MT-ND4, RPS4Y1
## PC_ 13
## Positive:  MT-ND4L, MTRNR2L8, MT-ND3, TNFAIP3, SQSTM1
## Negative:  KRT6A, KRT5, DSP, KRT1, KRT14
## PC_ 14
## Positive:  MTRNR2L12, MT-ND4, MT-CO2, MT-CYB, MT-CO1
## Negative:  KLF2, GPR183, IFITM1, MAF, CLEC2B
## PC_ 15
## Positive:  PLCG2, IKZF3, SLFN5, TTN, CEMIP2
## Negative:  NFKBIA, LMNA, GAPDH, RPS4Y1, TNFRSF18
```

```
VizDimLoadings(PCALymphocyte, dims = 1:15, reduction = 'pca')
```

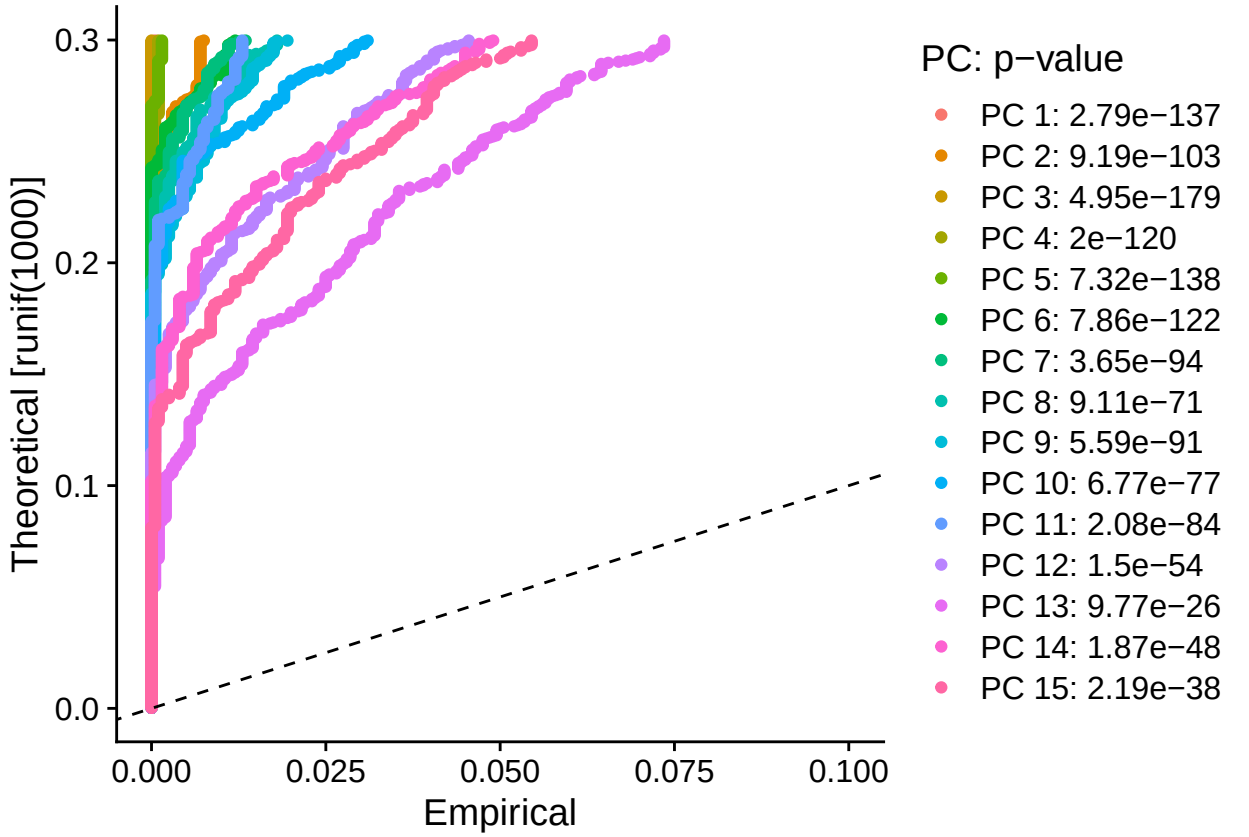



```
# DimHeatmap() function allows to explore heterogeneity in the dataset and allows us to decide which PC

DimHeatmap(PCALymphocyte, dims = 1:15, cells = 500, balanced = TRUE)
```



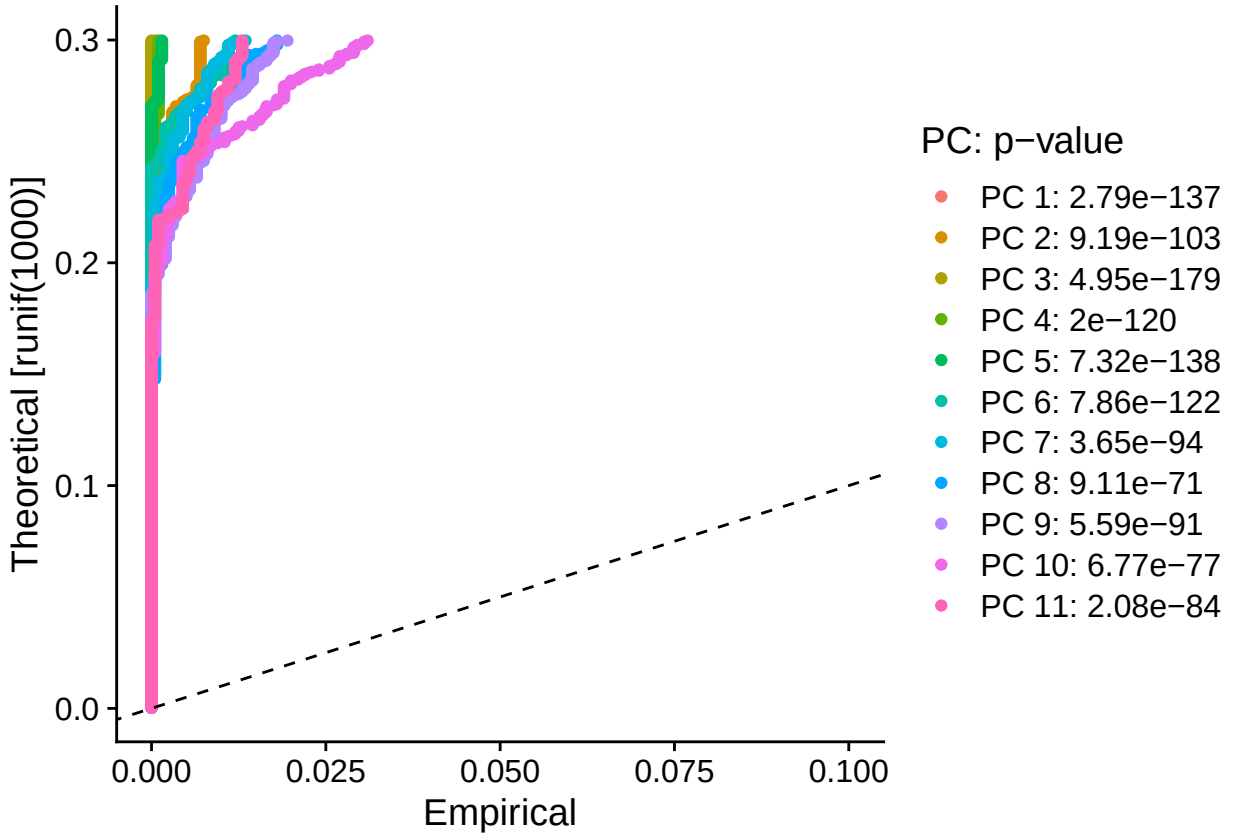

```
# We can create a JackStraw plot using the JackStrawPlot() function to verify which PCs are significant

PCALymphocyte <- JackStraw(PCALymphocyte, num.replicate = 100)
PCALymphocyte <- ScoreJackStraw(PCALymphocyte, dims = 1:15)
JackStrawPlot(PCALymphocyte, dims = 1:15)
```

```
## Warning: Removed 21000 rows containing missing values (‘geom_point()‘).
```

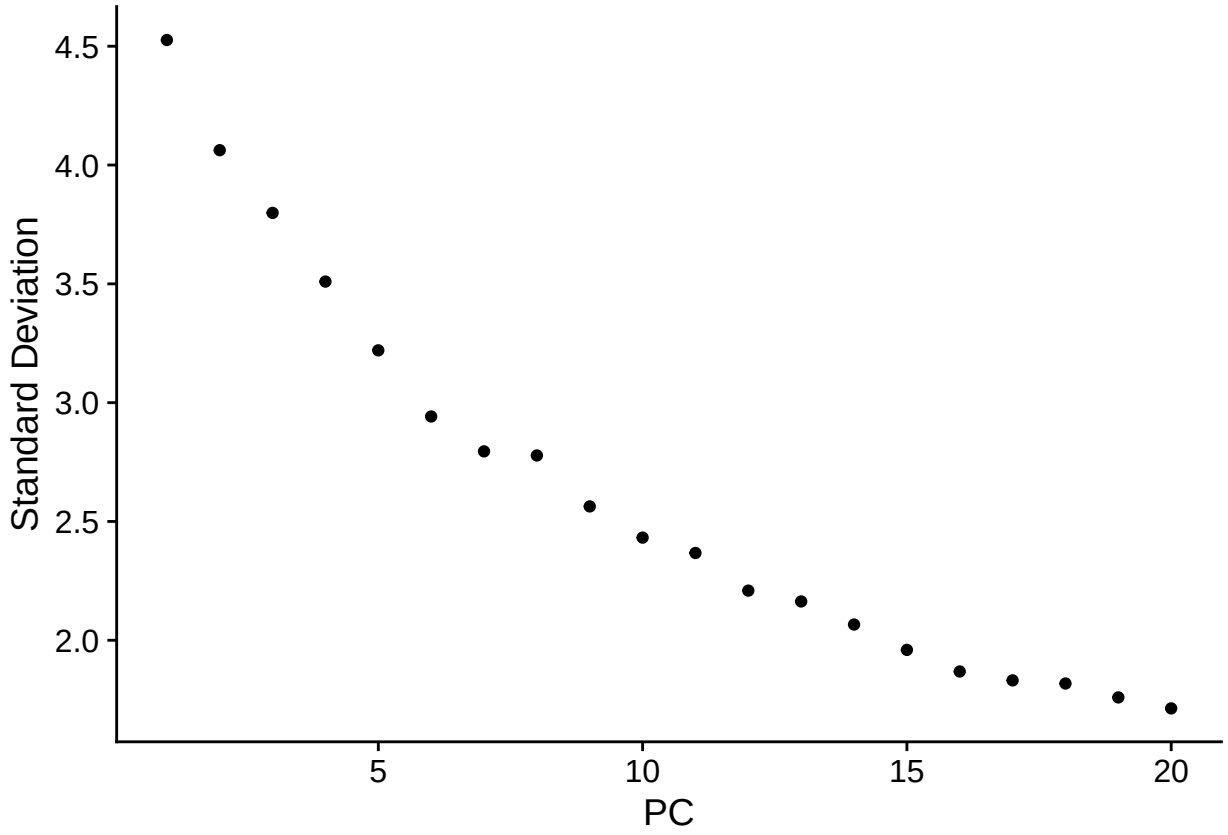



```
# The plot shows all 15 lines are above the dashed line, but PC 12-15 start to deviate a bit. I'm going

JackStrawPlot(PCALymphocyte, dims = 1:11)
```

```
## Warning: Removed 15400 rows containing missing values (`geom_point()`).
```




```
# We can verify this another way using an Elbow plot with the ElbowPlot() function that ranks PCs based

ElbowPlot(PCALymphocyte)
```




```
# We see there is also an elbow at PC 11 so it verifies the cut off I'm choosing here.

# This is a dimensionality reduction technique used for visualization. It captures the manifold (topolo

NewLymphocyteClusters <- FindNeighbors(PCALymphocyte, dims = 1:11)
```

```
## Computing nearest neighbor graph

## Computing SNN
```

```
NewLymphocyteClusters <- FindClusters(PCALymphocyte, resolution = 0.5)
```

```
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
##
## Number of nodes: 3893
## Number of edges: 125380
##
## Running Louvain algorithm...
## Maximum modularity in 10 random starts: 0.8607
## Number of communities: 11
## Elapsed time: 0 seconds

## Warning: Adding a command log without an assay associated with it
```

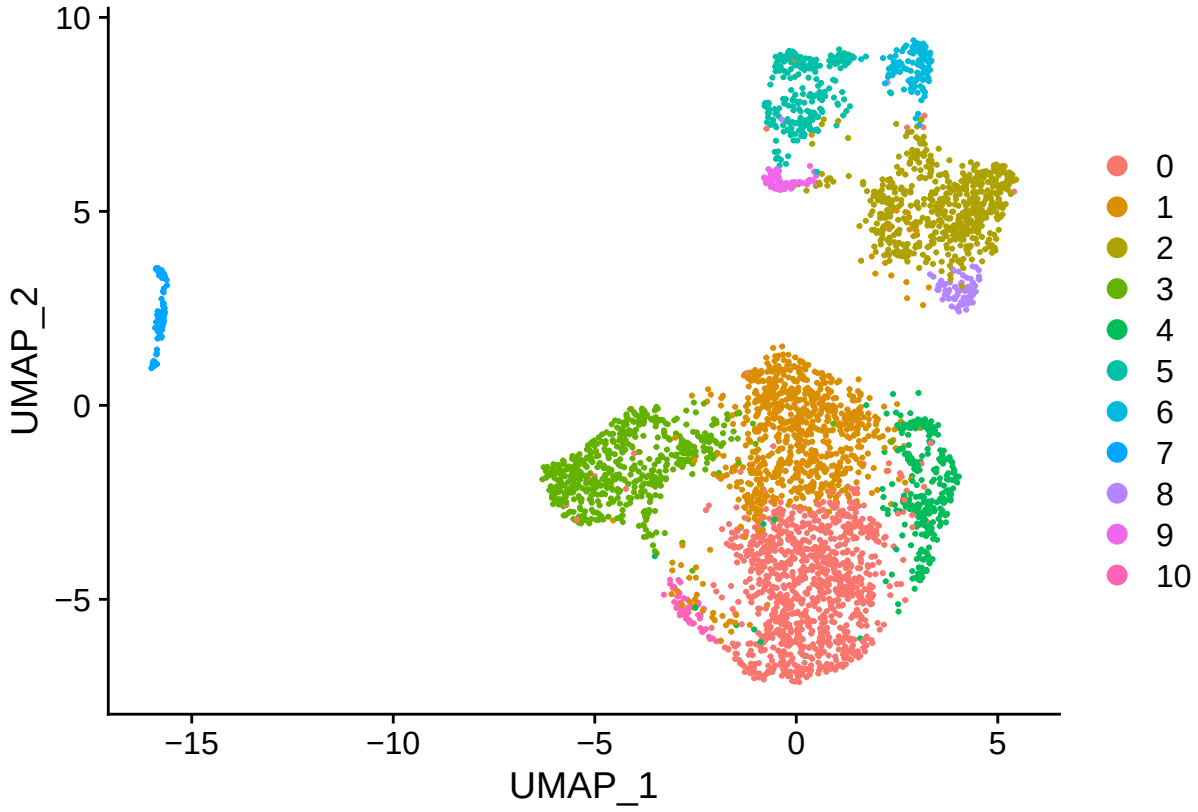
```
# Now we can verify what the cluster IDs of the first 5 cells are and that we have a total of 11 cluste
head(Idents(NewLymphocyteClusters), 5)
```

```
## L_318A_AAACCCAGTCAGTCCG L_318A_AAAGAACGTGGATCAG L_318A_AAAGAACTCTATGCCC
##                       4                       3                       1
## L_318A_AAAGGTATCGCATGAT L_318A_AAAGTGATCGTAGGGA
##                       2                       0
## Levels: 0 1 2 3 4 5 6 7 8 9 10
```

```
# Looks like we have 11 total levels (cell types) and they are named by number. We can create a UMAP to

NewLymphocyteClusters <- RunUMAP(NewLymphocyteClusters, reduction = "pca", dims = 1:11)
```

```
## Warning: The default method for RunUMAP has changed from calling Python UMAP via reticulate to the R-
## To use Python UMAP via reticulate, set umap.method to 'umap-learn' and metric to 'correlation'
## This message will be shown once per session

## 17:10:33 UMAP embedding parameters a = 0.9922 b = 1.112

## 17:10:33 Read 3893 rows and found 11 numeric columns

## 17:10:33 Using Annoy for neighbor search, n_neighbors = 30

## 17:10:33 Building Annoy index with metric = cosine, n_trees = 50

## 0%   10   20   30   40   50   60   70   80   90   100%

## [----|----|----|----|----|----|----|----|----|----|

## **************************************************|
## 17:10:33 Writing NN index file to temp file /var/folders/xk/m16nzsr50mj5_cn5n88svr_w0000gp/T//Rtmp3HI
## 17:10:33 Searching Annoy index using 1 thread, search_k = 3000
## 17:10:34 Annoy recall = 100%
## 17:10:34 Commencing smooth kNN distance calibration using 1 thread with target n_neighbors = 30
## 17:10:35 Initializing from normalized Laplacian + noise (using irlba)
## 17:10:35 Commencing optimization for 500 epochs, with 152338 positive edges
## 17:10:38 Optimization finished
```

```
DimPlot(NewLymphocyteClusters, reduction = "umap")
```




```
# Now we need to find out what each of these cells are. We can do this by finding out their top genes w

cluster_markers <- FindAllMarkers(NewLymphocyteClusters,
                                  only.pos = TRUE,
                                  min.pct = 0.25,
                                  logfc.threshold = 0.25)
```

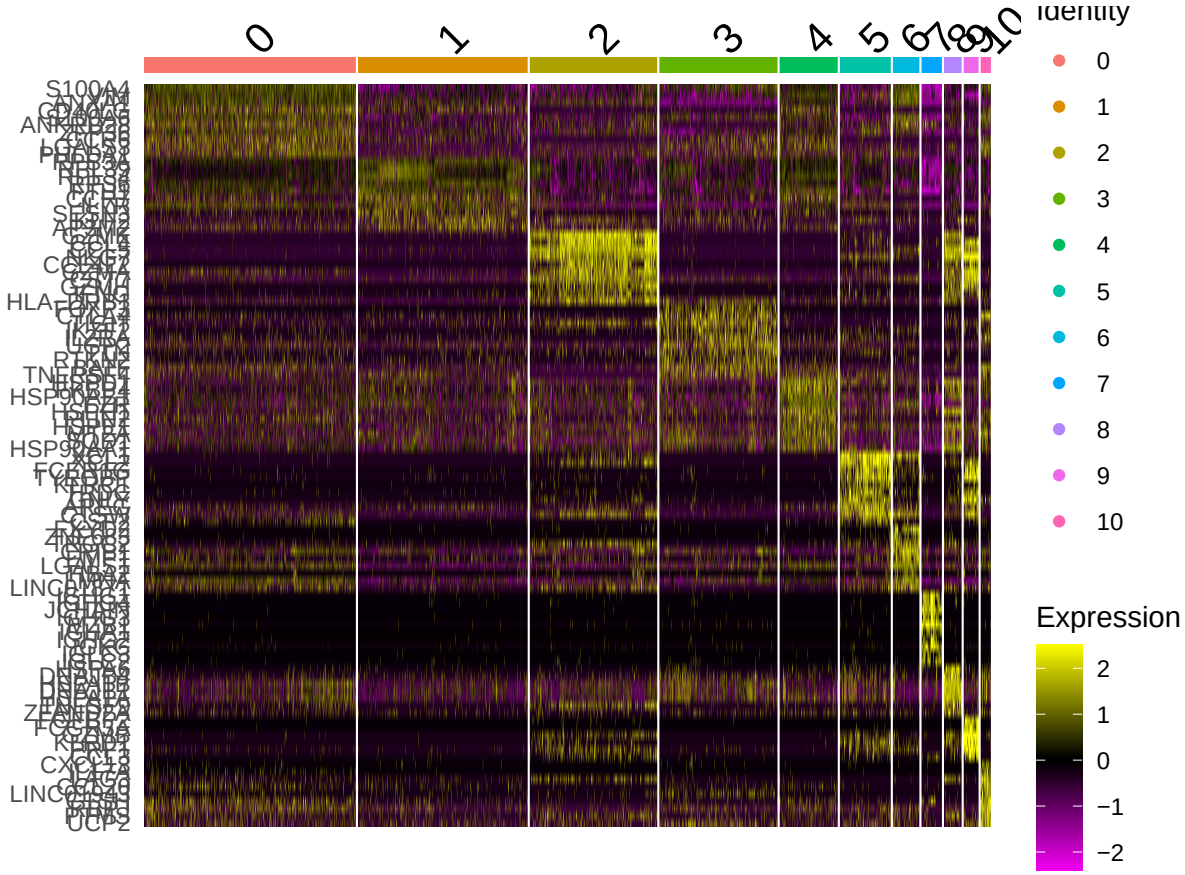
```
## Calculating cluster 0

## Calculating cluster 1

## Calculating cluster 2

## Calculating cluster 3

## Calculating cluster 4

## Calculating cluster 5

## Calculating cluster 6

## Calculating cluster 7

## Calculating cluster 8
```

```
## Calculating cluster 9

## Calculating cluster 10
```

```
# You can view the top genes for each cluster using slice_max().
cluster_markers %>%
  group_by(cluster) %>%
  slice_max(n = 1, order_by = avg_log2FC)
```

```
## # A tibble: 11 x 7
## # Groups:   cluster [11]
##        p_val avg_log2FC pct.1 pct.2 p_val_adj cluster gene
##        <dbl>      <dbl> <dbl> <dbl>     <dbl> <fct>   <chr>
##  1 5.42e-178       1.13 0.988 0.815 1.59e-173 0       S100A4
##  2 4.28e- 50       1.02 0.286 0.093 1.26e- 45 1       SESN3
##  3 1.80e-243       3.60 0.474 0.033 5.28e-239 2       CCL4L2
##  4 5.19e- 70       1.58 0.582 0.283 1.52e- 65 3       UGP2
##  5 9.01e- 65       1.79 0.898 0.52  2.65e- 60 4       HSPD1
##  6 0               4.32 0.838 0.062 0         5       XCL1
##  7 1.72e- 42       3.10 0.264 0.029 5.06e- 38 6       HBA2
##  8 4.80e-213      11.1  0.656 0.027 1.41e-208 7       IGKC
##  9 2.00e-119       4.01 0.602 0.04  5.88e-115 8       HSPA6
## 10 5.20e-188       4.34 0.986 0.065 1.53e-183 9       GZMB
## 11 4.70e- 92       4.78 0.396 0.012 1.38e- 87 10      CXCL13
```

```
# I want to make a heatmap of the top 10 genes since this gives us more information than just one top g
top10markers <- cluster_markers %>%
  group_by(cluster) %>%
  top_n(n = 10, wt = avg_log2FC)

DoHeatmap(NewLymphocyteClusters, features = top10markers$gene)
```




```
# Since the heatmap can get quite overwhelming with a lot of clusters, we can look at one cluster at a

cluster0.markers <- FindMarkers(NewLymphocyteClusters, ident.1 = 0, min.pct = 0.25)
head(cluster0.markers, n = 10)
```

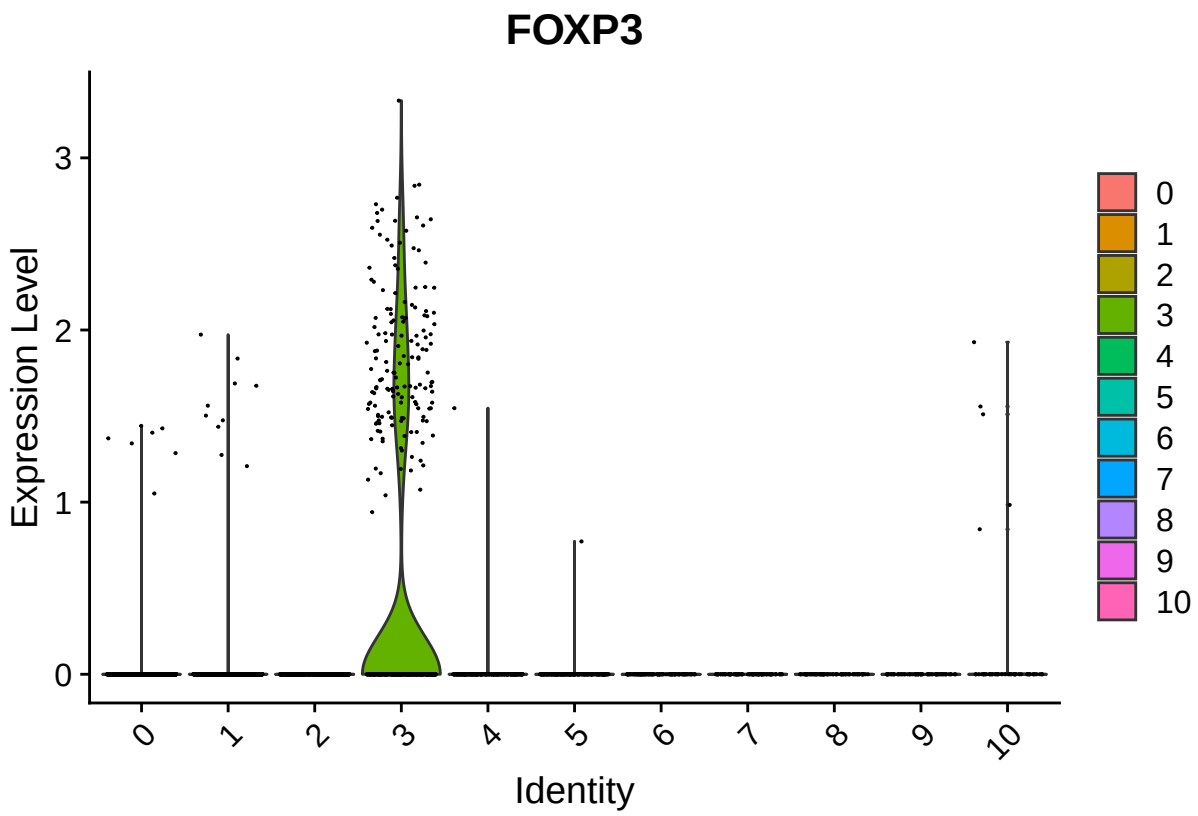
```
##                 p_val avg_log2FC pct.1 pct.2     p_val_adj
## S100A4  5.419047e-178  1.1316279 0.988 0.815 1.592658e-173
## VIM     1.700104e-119  0.8895228 0.993 0.860 4.996604e-115
## B2M     6.184956e-113  0.5123012 0.999 0.994 1.817758e-108
## ANXA1   6.488899e-103  0.8646855 0.965 0.675  1.907087e-98
## MYL12A  3.256195e-100  0.7691072 0.952 0.760  9.569957e-96
## CD40LG   3.662853e-97  0.9740685 0.484 0.150  1.076512e-92
## S100A6   5.912430e-92  0.8662376 0.966 0.757  1.737663e-87
## CAPG     1.201263e-89  0.8324390 0.482 0.157  3.530513e-85
## LMNA     8.818674e-84  0.8378498 0.908 0.634  2.591808e-79
## ANKRD28  1.632434e-83  0.8420013 0.533 0.200  4.797722e-79
```

```
# If it is difficult to distinguish a certain cluster from another cluster, we can use the following li

cluster8.markers <- FindMarkers(NewLymphocyteClusters, ident.1 = 8, ident.2 = 2, min.pct = 0.25)
head(cluster8.markers, n = 10)
```

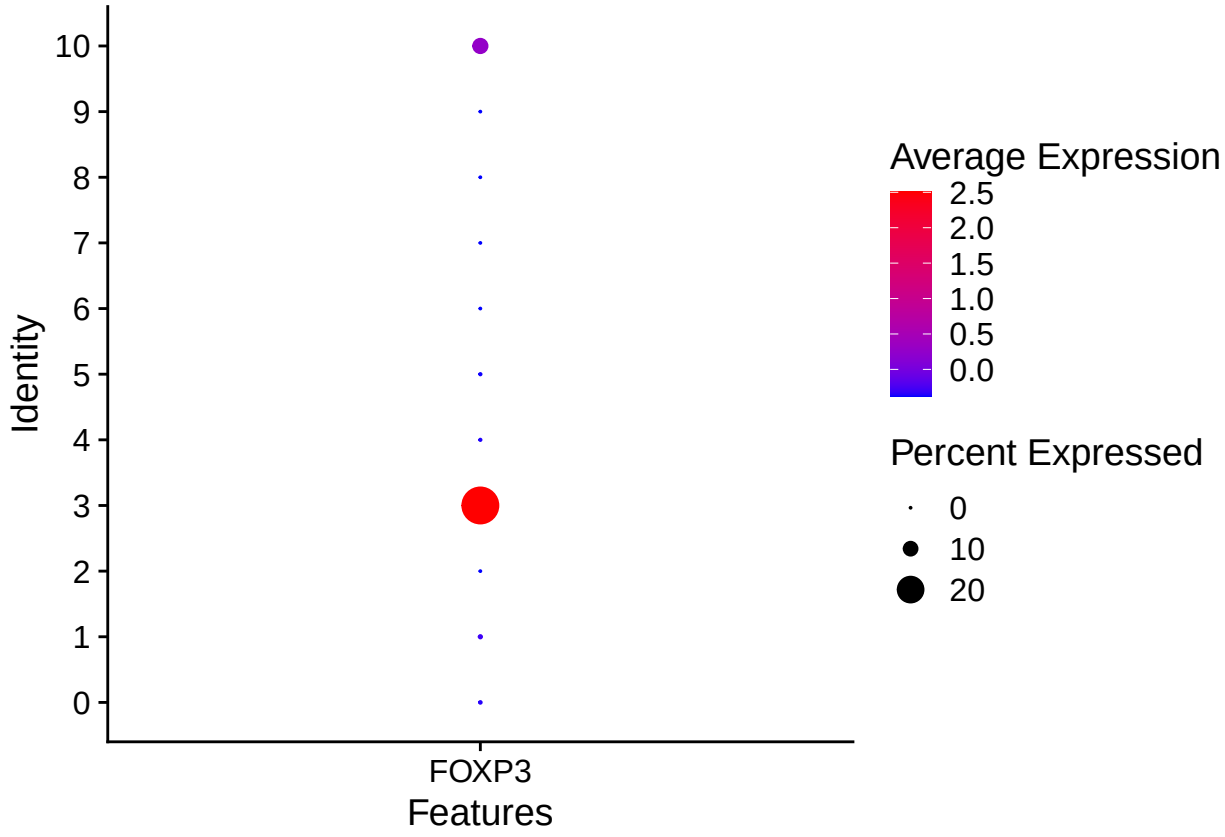
```
##                 p_val avg_log2FC pct.1 pct.2    p_val_adj
## HSPA6    4.064077e-70   4.596083 0.602 0.018 1.194432e-65
## HSPA1B   8.304835e-44   3.303275 0.988 0.453 2.440791e-39
```

```
## DNAJB4   2.479526e-42   2.539914 0.627 0.085 7.287327e-38
## HSPA1A   5.533323e-40   3.565678 0.964 0.547 1.626244e-35
## DNAJB1   1.305748e-36   2.955465 0.964 0.621 3.837593e-32
## DNAJA4   2.572017e-36   2.018598 0.434 0.033 7.559157e-32
## BAG3     1.880748e-34   1.872556 0.386 0.025 5.527518e-30
## HSPB1    2.743383e-34   2.259889 0.735 0.178 8.062804e-30
## HSP90AA1 3.260366e-31   2.237617 0.988 0.890 9.582217e-27
## HSPH1    9.261710e-31   2.407777 0.783 0.261 2.722017e-26
```

```
# I'm more of a visual analyzer, so I recommend making violin plots or dot plots of unique genes that a

VlnPlot(NewLymphocyteClusters, features = c("FOXP3"))
```



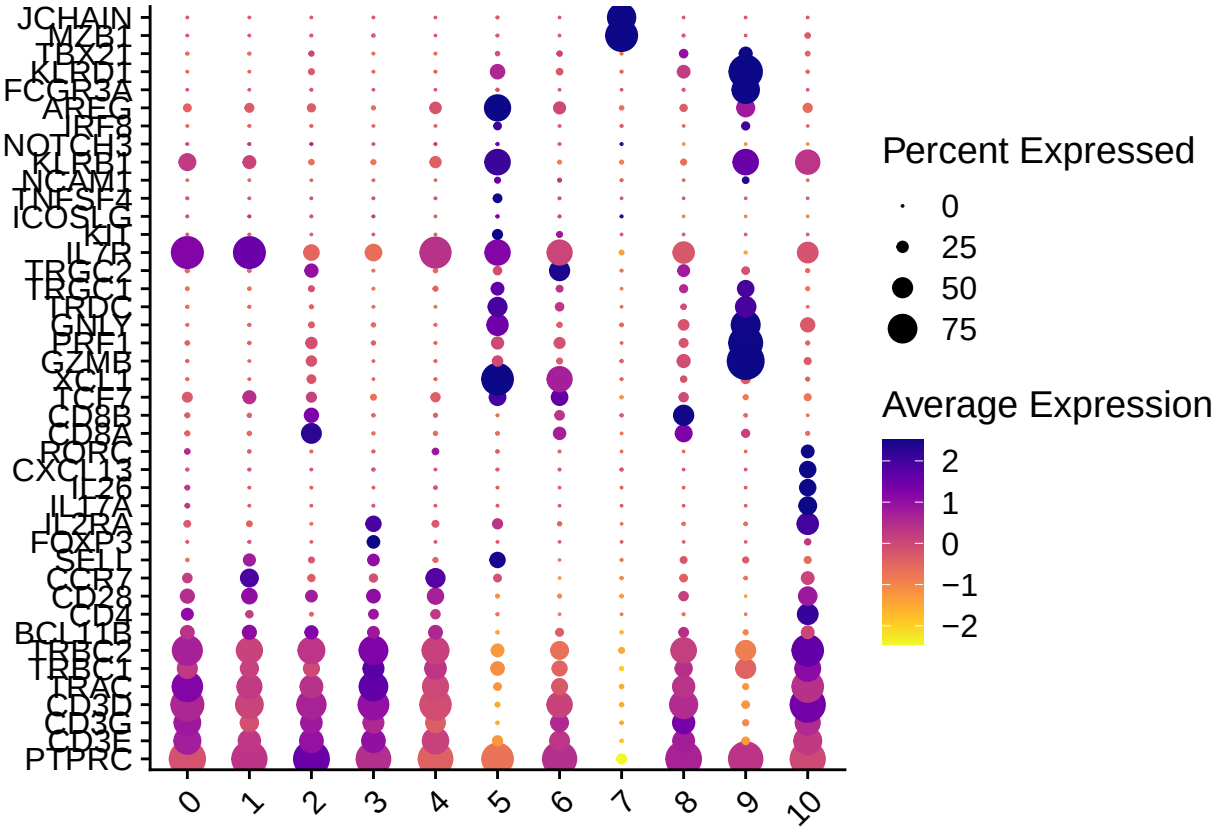

```
DotPlot(NewLymphocyteClusters, features = c("FOXP3"), cols = c("blue", "red"))
```



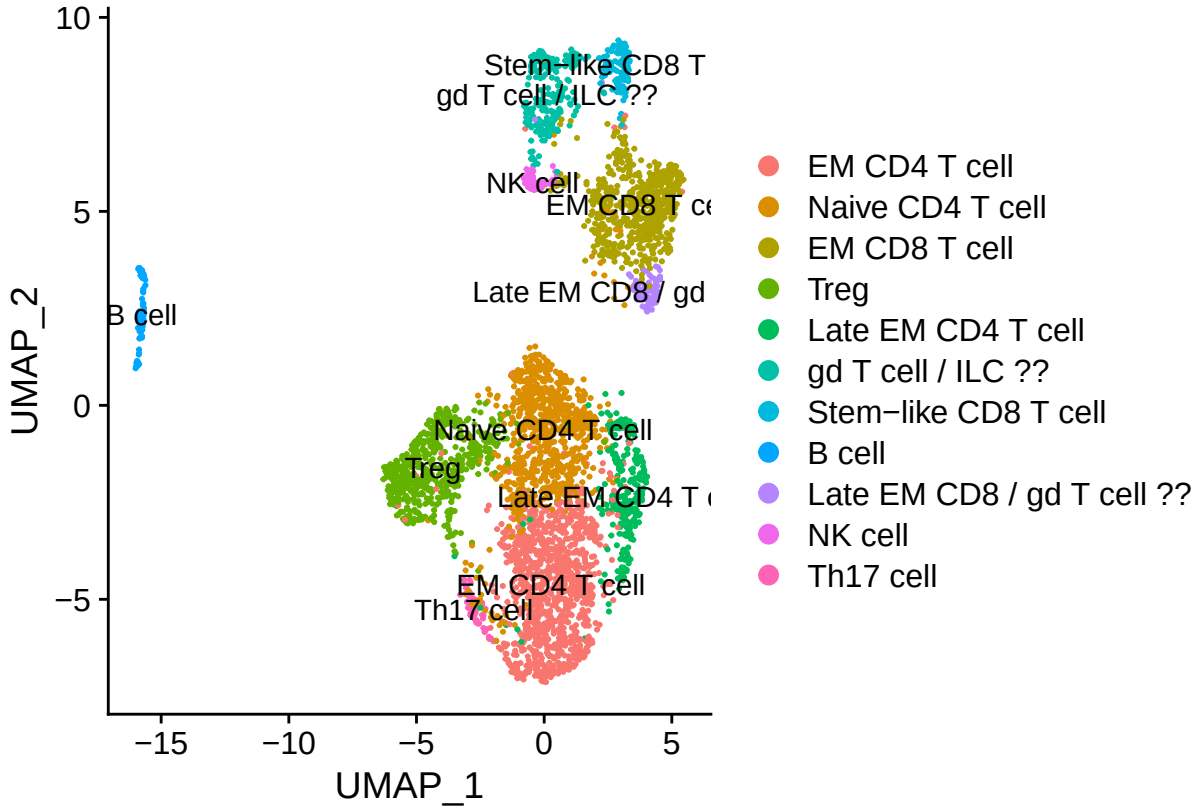

```
# Although we might be certain of a cluster based on specific genes, we also need to verify that these

# install.packages("scCustomize")
library(scCustomize)
```

```
## scCustomize v1.1.3
## If you find the scCustomize useful please cite.
## See 'samuel-marsh.github.io/scCustomize/articles/FAQ.html' for citation info.
```

```
genes <- c("PTPRC", "CD3E", "CD3G", "CD3D", "TRAC", "TRBC1", "TRBC2", "BCL11B", "CD4", "CD28", "CCR7",

DotPlot_scCustom(seurat_object = NewLymphocyteClusters, features = genes, flip_axes = T, x_lab_rotate =
```




```
# Now I've found out what each cluster may be based on their specific genes. It's time to rename the cl

new.cluster.ids <- c("EM CD4 T cell", "Naive CD4 T cell", "EM CD8 T cell", "Treg", "Late EM CD4 T cell"

names(new.cluster.ids) <- levels(NewLymphocyteClusters)

NewLymphocyteClusters <- RenameIdents(NewLymphocyteClusters, new.cluster.ids)

# View the new UMAP with the updated clusters!
DimPlot(NewLymphocyteClusters, reduction = 'umap', label = TRUE, pt.size = 0.4)
```

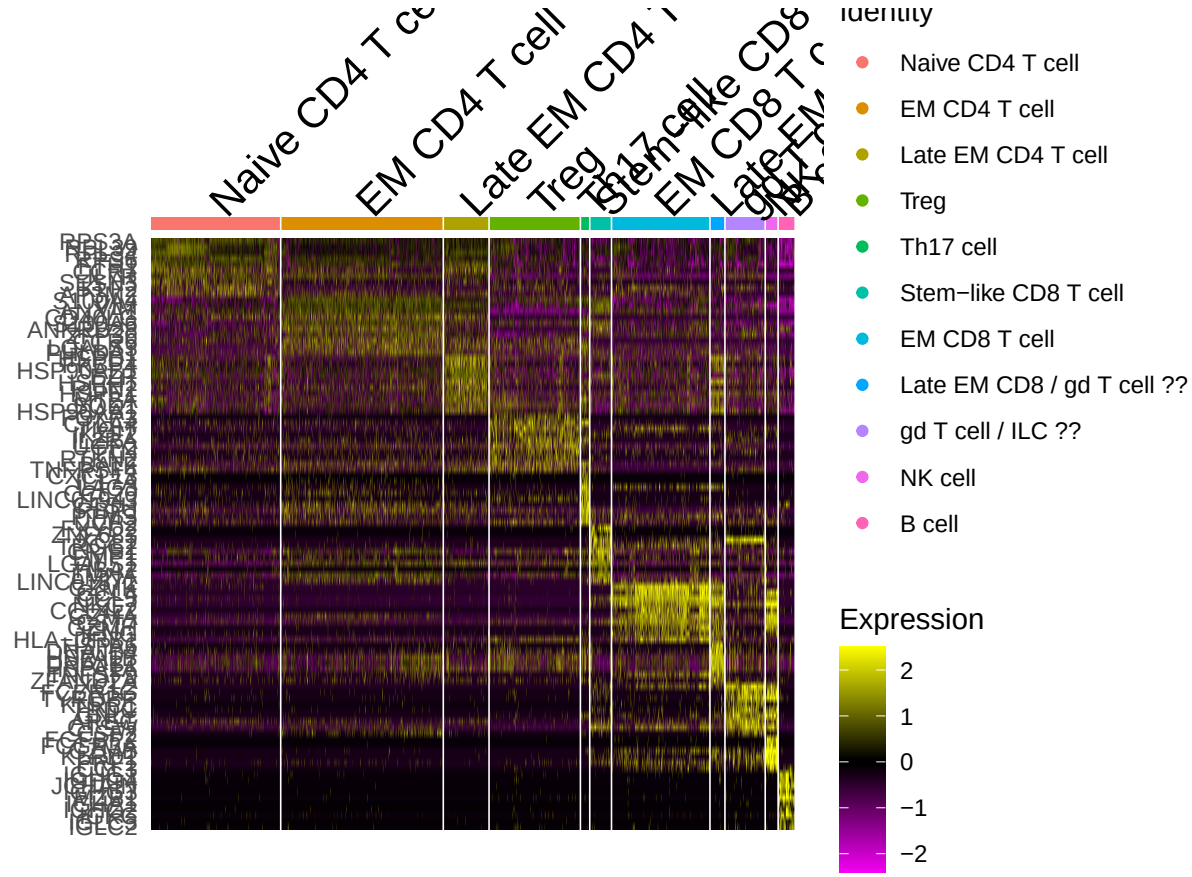



```
# I want to reorder the cell types so that it's easier to analyze when we make downstream analyses. Mak

NewLymphocyteClusters@active.ident <- factor(NewLymphocyteClusters@active.ident,
                                             levels = c("Naive CD4 T cell",
                                                        "EM CD4 T cell",
                                                        "Late EM CD4 T cell",
                                                        "Treg",
                                                        "Th17 cell",
                                                        "Stem-like CD8 T cell",
                                                        "EM CD8 T cell",
                                                        "Late EM CD8 / gd T cell ??",
                                                        "gd T cell / ILC ??",
                                                        "NK cell",
                                                        "B cell")
                                             )

NewLymphocyteClusters$celltype <- Idents(NewLymphocyteClusters)

# Verify each cluster and their top genes in an updated Heatmap that shows all the renamed clusters. We

cluster_markers <- FindAllMarkers(NewLymphocyteClusters, only.pos = TRUE, min.pct = 0.25, logfc.thresho
```

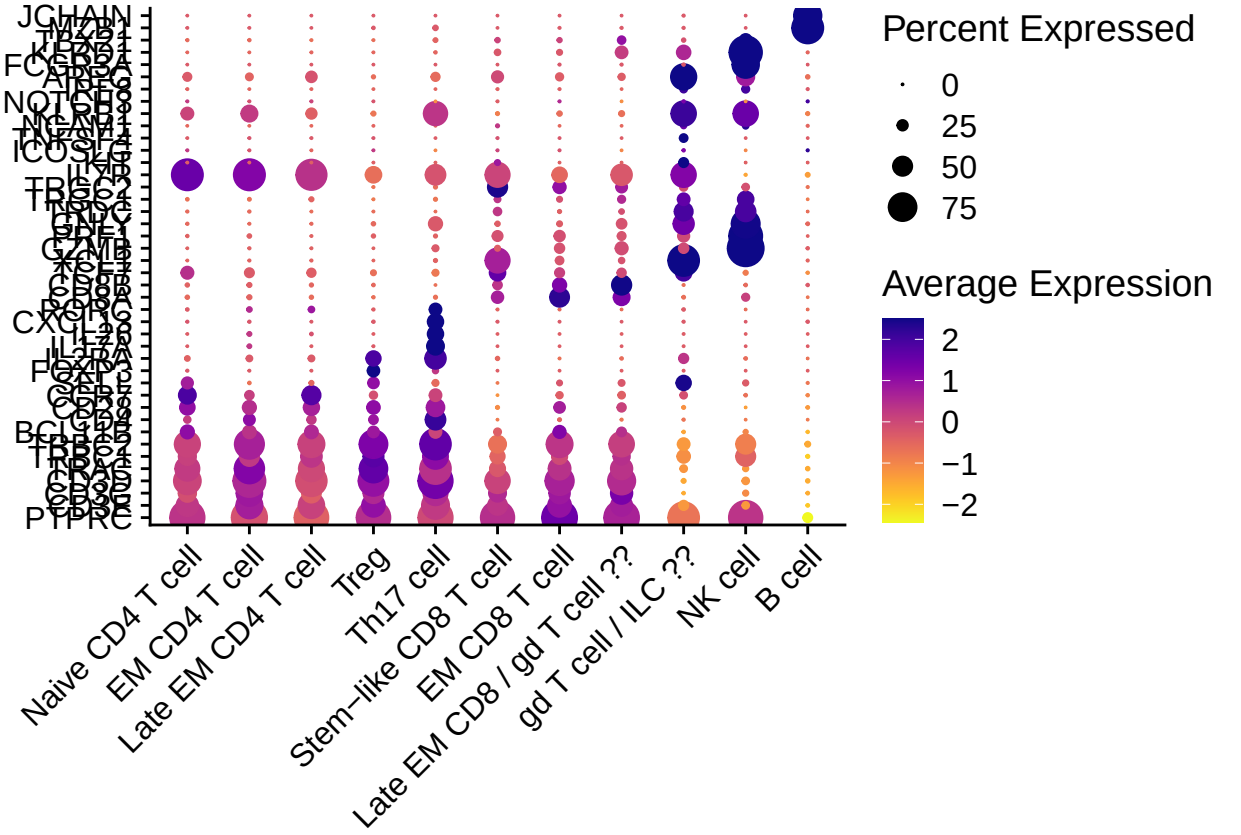
```
## Calculating cluster Naive CD4 T cell

## Calculating cluster EM CD4 T cell
```

```
## Calculating cluster Late EM CD4 T cell

## Calculating cluster Treg

## Calculating cluster Th17 cell

## Calculating cluster Stem-like CD8 T cell

## Calculating cluster EM CD8 T cell

## Calculating cluster Late EM CD8 / gd T cell ??

## Calculating cluster gd T cell / ILC ??

## Calculating cluster NK cell

## Calculating cluster B cell
```

```
top10markers <- cluster_markers %>%
  group_by(cluster) %>%
  top_n(n = 10, wt = avg_log2FC)

DoHeatmap(NewLymphocyteClusters, features = top10markers$gene)
```



```
DotPlot_scCustom(seurat_object = NewLymphocyteClusters, features = genes, flip_axes = T, x_lab_rotate =
```




```
# save(NewLymphocyteClusters, file = "NewLymphocyteClusters.Rdata")
```

CONCLUSION

Upon expanding this dataset, we were able to go from 5 clusters to 11 clusters ("Naive CD4 T cell", "EM CD4 T cell", "Late EM CD4 T cell", "Treg", "Th17 cell", "Stem-like CD8 T cell", "EM CD8 T cell", "Late EM CD8 / gd T cell ??", "gd T cell / ILC ??", "NK cell", "B cell"). There may be gd T cells and ILCs mixed within the same cluster because the ILC cluster seems to have cells expressing genes that represent gd T cells (TRDC, TRGC2, TRGC1) when they should not be positive for these genes. Moreover, gd T cells may be present in the Late EM CD8 T cell cluster as well due to similar functional gene signatures. In literature, these cell types have been shown to be quite similar, so we would need a dataset with lots more cells to make a more concrete verification that those are the correctly named clusters.

Overall, there are now 11 cell types distinguished in the lymphocyte dataset. Researchers can use this new information to generate hypotheses about genes of interest in more specific cell types.

Another method for annotating cell types may be to use the online source, Azimuth, at https://azimuth.hubmapconsortium.org/. It's super quick and easy to use because you just upload your data to their reference datasets that can then name the clusters, however, there are very limited reference datasets available. In this case, there is no human skin dataset on the site that we can refer to.

CELLxGENE (https://cellxgene.cziscience.com/) is another useful source where you can find single-cell data and explore gene expression across many tissues/cell types. It allows you to download and integrate data as well.

‘‘‘