

4-Functional characterization of clustered populations

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2021-10-07 12:08:04 +0200

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1 Description

Functional characterization of the cluster 1, 2, 3 and 4 by examining expression of deferentially expressing (DE) genes and functional markers with Seurat package[1].

To compare bulk and scRNA-seq data, AFlo and AFhi AM signatures were calculated by comparing AFlo AM samples with AFhi AM samples using DESeq2 package. A threshold of P adjusted < 0.05 and a biological FC > 2 was applied to obtain the signatures. For each cell, a score of signatures was calculated with VISION package [2] and the scores were presented using Seurat FeaturePlot function with the same embedding as in Figure 4A.

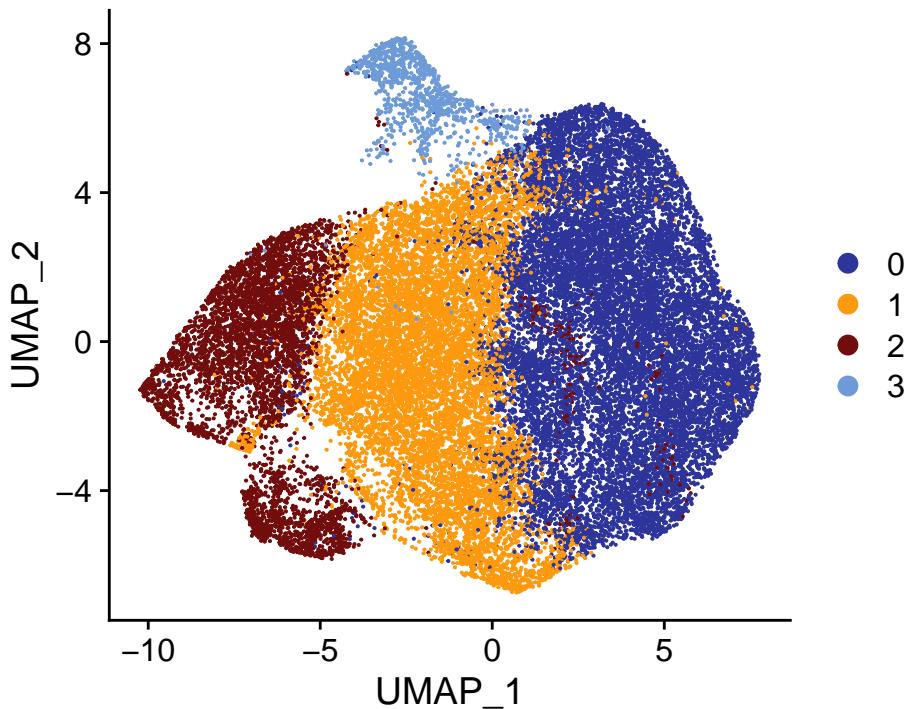
Notice: The cluster 1/2/3/4 in the manuscript referred to cluster 0/1/2/3 in the following codes.

2 Load data and packages

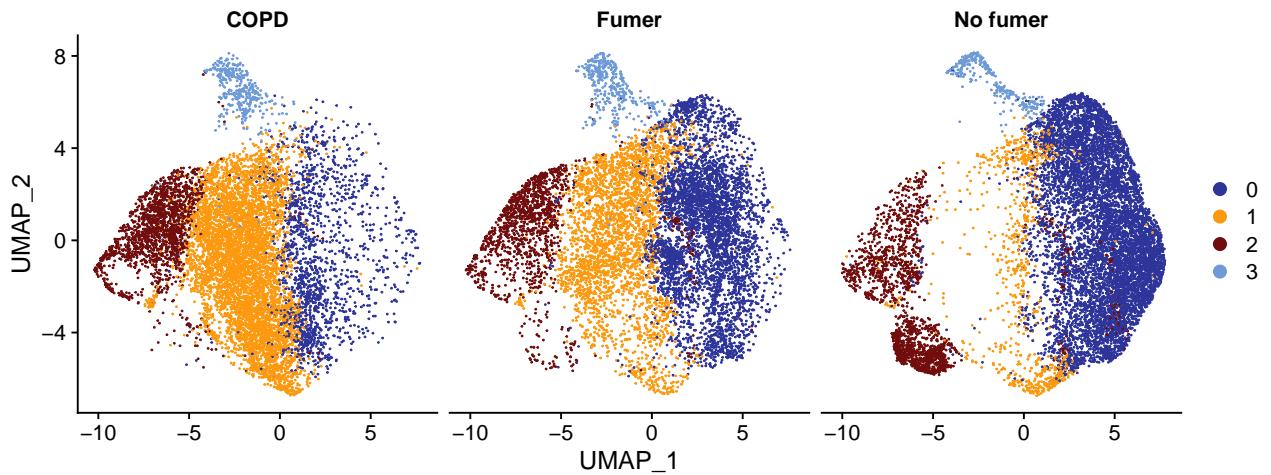
```
suppressMessages(library(Seurat))  
suppressMessages(library(dplyr))  
suppressMessages(library(ggplot2))  
results <- readRDS(file = "../3-Merge_and_cell_typing/so.merged_clusters.  
seuratObject.Rds")
```

3 Distribution of cells in clusters

```
pal_4c <- c("#2E359A", "#FC990E", "#720DOD", "#6E9BD8")  
p <- DimPlot(results, cols = pal_4c)  
p
```



UMAPplot split by group

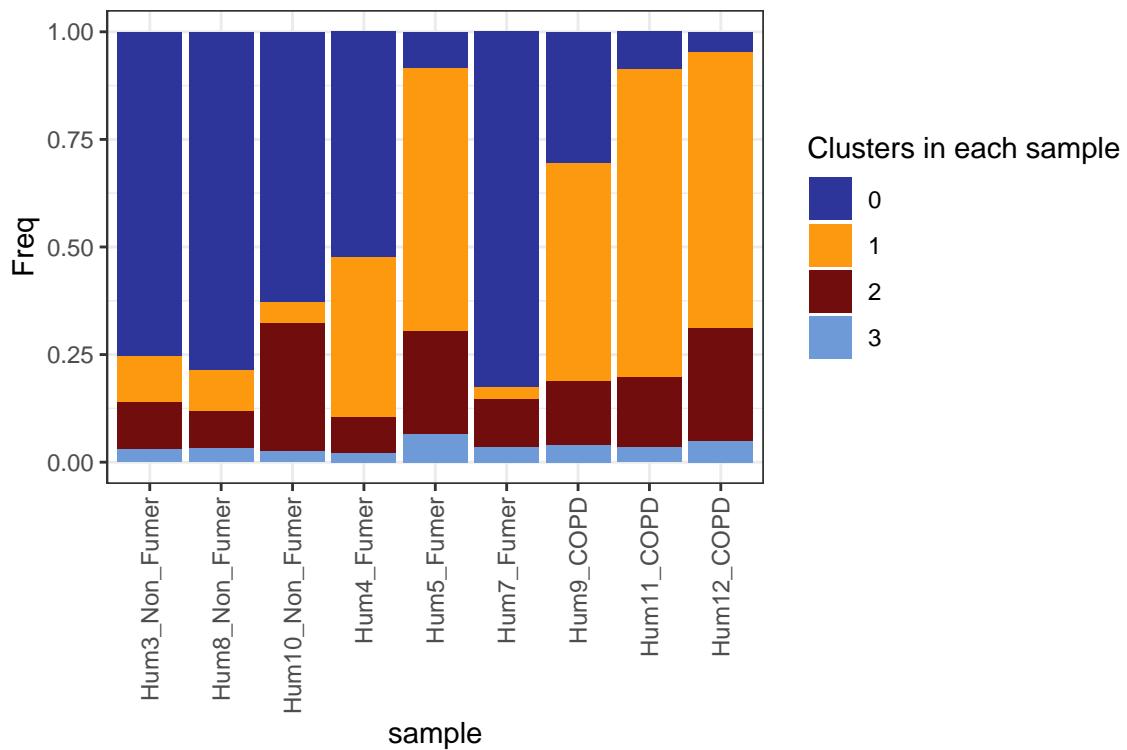


Barplot split by group

```

source("../R/barChart.R")
source("../R/SeuratFreqTable.R")
freq.celltype.list <- list(
  Hum3_Non_Fumer = Seurat2CellFreqTable(subset(results, subset = origin
    == "LBA_Hum3"), slotName = "seurat_clusters"),
  Hum8_Non_Fumer = Seurat2CellFreqTable(subset(results, subset = origin
    == "LBA_Hum8"), slotName = "seurat_clusters"),
  Hum10_Non_Fumer = Seurat2CellFreqTable(subset(results, subset = origin
    == "LBA_Hum10"), slotName = "seurat_clusters"),
  Hum4_Fumer = Seurat2CellFreqTable(subset(results, subset = origin == "
    LBA_Hum4"), slotName = "seurat_clusters"),
  Hum5_Fumer = Seurat2CellFreqTable(subset(results, subset = origin == "
    LBA_Hum5"), slotName = "seurat_clusters"),
  Hum7_Fumer = Seurat2CellFreqTable(subset(results, subset = origin == "
    LBA_Hum7"), slotName = "seurat_clusters"),
  Hum9_COPD = Seurat2CellFreqTable(subset(results, subset = origin == "
    LBA_Hum9"), slotName = "seurat_clusters"),
  Hum11_COPD = Seurat2CellFreqTable(subset(results, subset = origin == "
    LBA_Hum11"), slotName = "seurat_clusters"),
  Hum12_COPD = Seurat2CellFreqTable(subset(results, subset = origin == "
    LBA_Hum12"), slotName = "seurat_clusters")
)
p <- barChart(freq.celltype.list) + labs(fill = "Clusters in each sample"
  + scale_fill_manual(values = pal_4c) + theme(axis.text.x = element_
  text(angle = 90, vjust = 0.5, hjust=1))
p

```

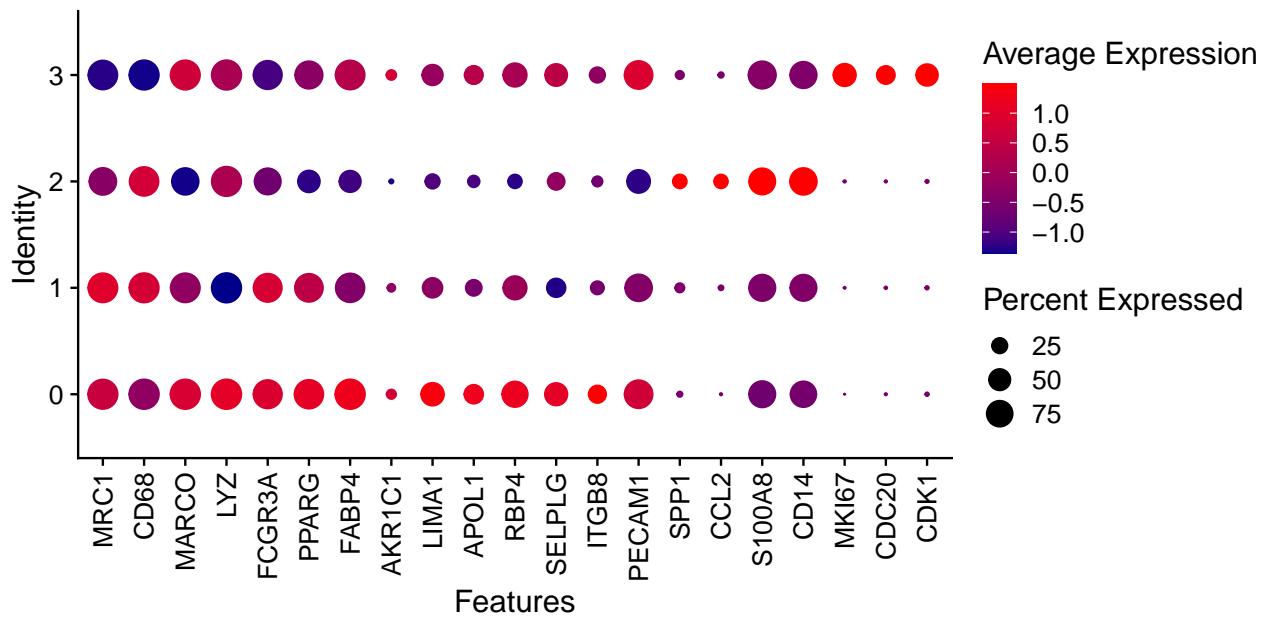


4 Expression of macrophage markers and Cluster 1/3/4 signatures

```

1 p <- DotPlot(results, features = c("MRC1", "CD68", "MARCO", "LYZ", "FCGR3A", # high expression of core macrophage genes
2   "PPARG", # AM-associated transcription factor
3   "FABP4", "AKR1C1", "LIMA1", "APOL1", "RBP4", # Cluster 1 upregulated
4   "SEPLIG", "ITGB8", "PECAM1", # Cluster 1 overexpressed genes coding for cell adhesion molecules
5   "SPP1", "CCL2", "S100A8", "CD14", # Cluster 3 overexpressed transcripts encoding monocyte lineage-associated molecules
6   "MKI67", "CDC20", "CDK1" # Cluster 4 upregulated cycling-related genes
7   ), assay = "RNA",
8   scale.by = "size",
9   cols = c("dark_blue", "red")) +
10  theme(axis.text.x = element_text(angle = 90,
11    vjust = 0.5,
12    hjust=1))
13

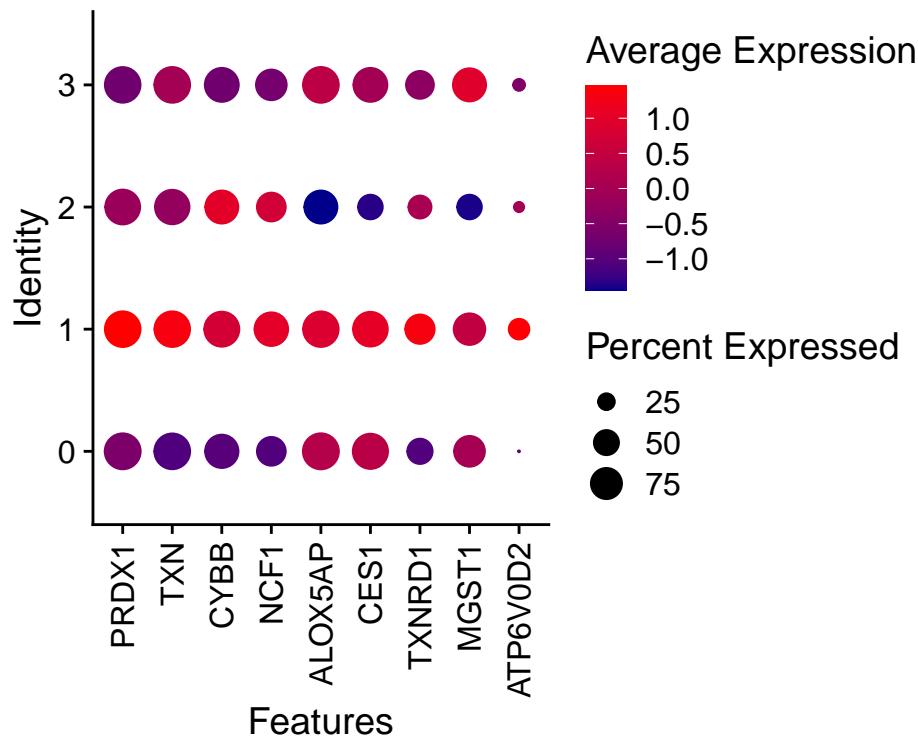
```



5 Expression of cluster 2 signature

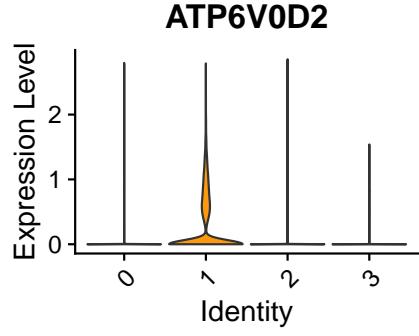
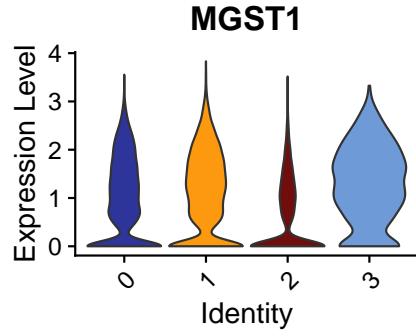
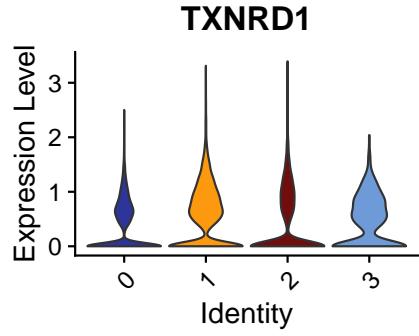
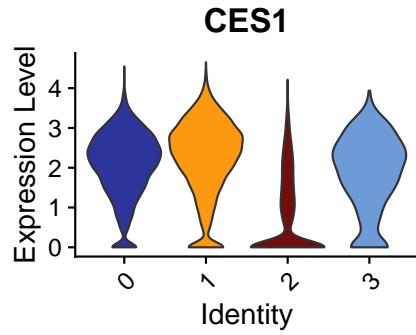
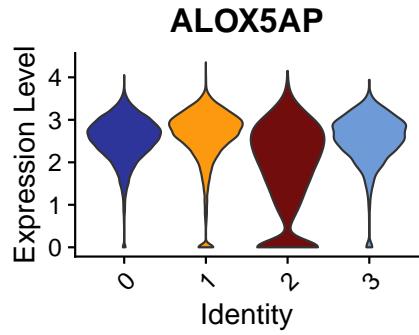
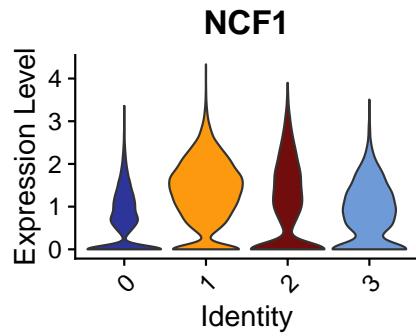
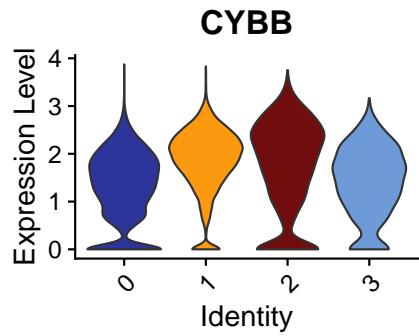
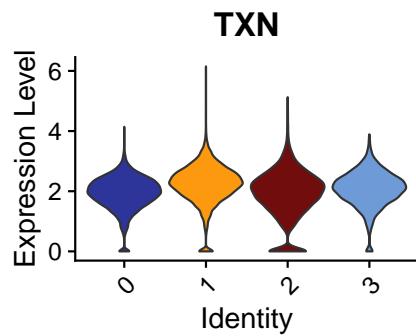
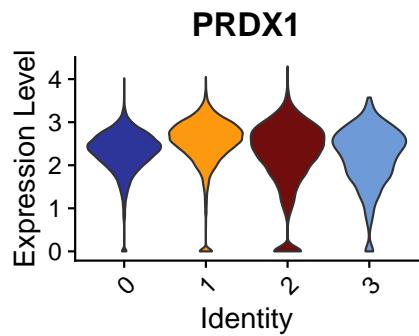
```

1 p <- DotPlot(results, features = c("PRDX1", "TXN", "CYBB", "NCF1", " "
2   ALOX5AP", "CES1", "TXNRD1", "MGST1", "ATP6V0D2"),
3     assay = "RNA",
4     scale.by = "size",
5     cols = c("darkblue", "red")) +
6     theme(axis.text.x = element_text(angle = 90,
7       vjust = 0.5,
8       hjust=1))
  
```



Signature of Cluster 2 in vlnplot:

```
p <- VlnPlot(results, features = c("PRDX1", "TXN", "CYBB", "NCF1", "ALOX5AP", "CES1", "TXNRD1", "MGST1", "ATP6V0D2"),
              pt.size = 0,
              cols = pal_4c,
              ncol = 2)
p
```



6 Focus on Cluster 3 and recluster

REMIND: The cluster 3 in the manuscript referred to cluster 2 in the following codes.

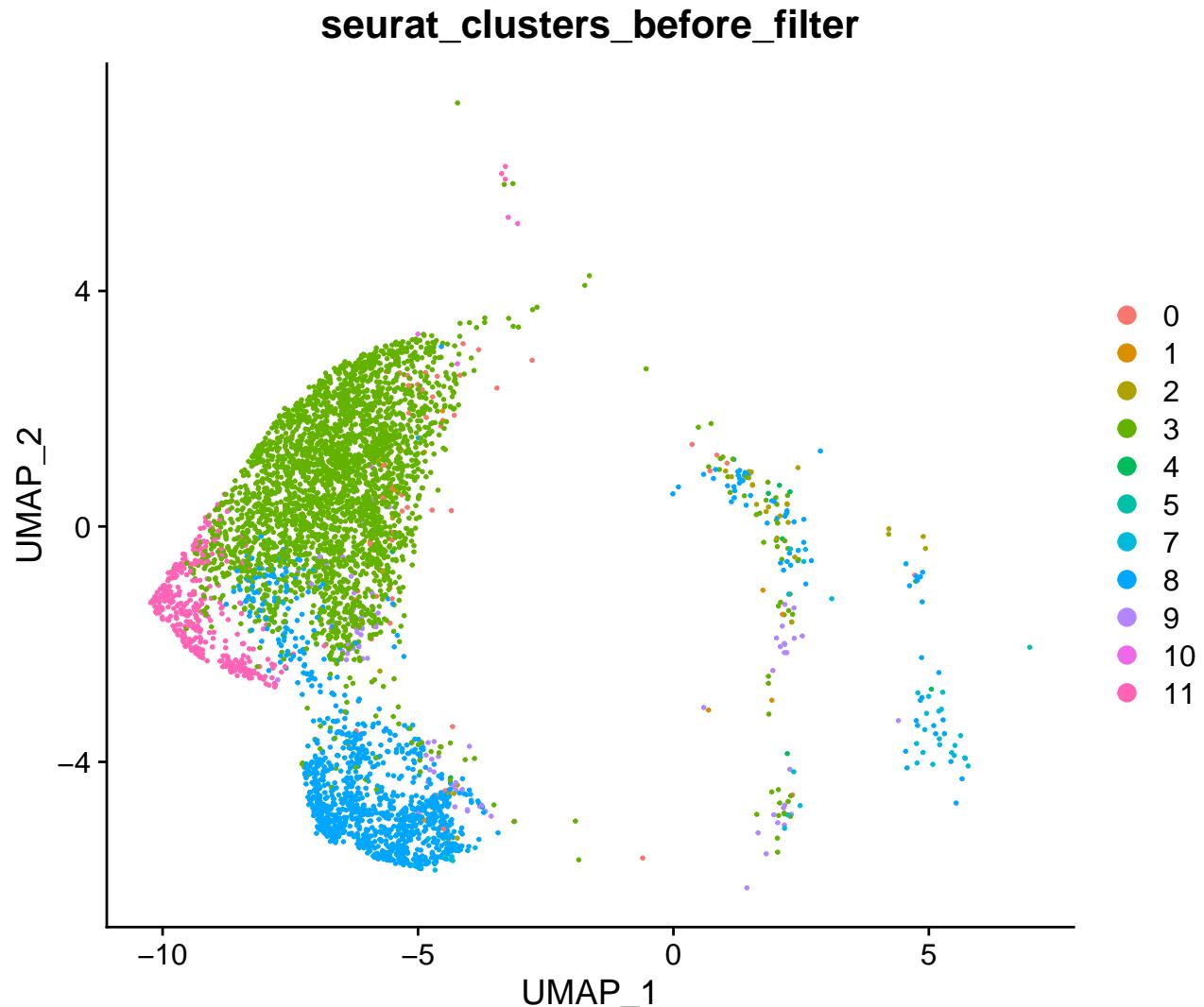
6.1 subsetdata

```
results.c2 <- subset(results, idents = 2) 1
results.c2 2

## An object of class Seurat
## 20050 features across 4897 samples within 1 assay 1
## Active assay: RNA (20050 features, 2000 variable features) 2
## 3 dimensional reductions calculated: pca, umap, tsne 3
## 4
```

As the DC-like population (cluster 11 in the pre-filter clustering) is mainly enriched in this cluster, let's first identify these cells.

```
DimPlot(results.c2, group.by = "seurat_clusters_before_filter") 1
```



6.2 Processing data (only cluster 3)

REMIND: The cluster 3 in the manuscript referred to cluster 2 in the following codes.

```

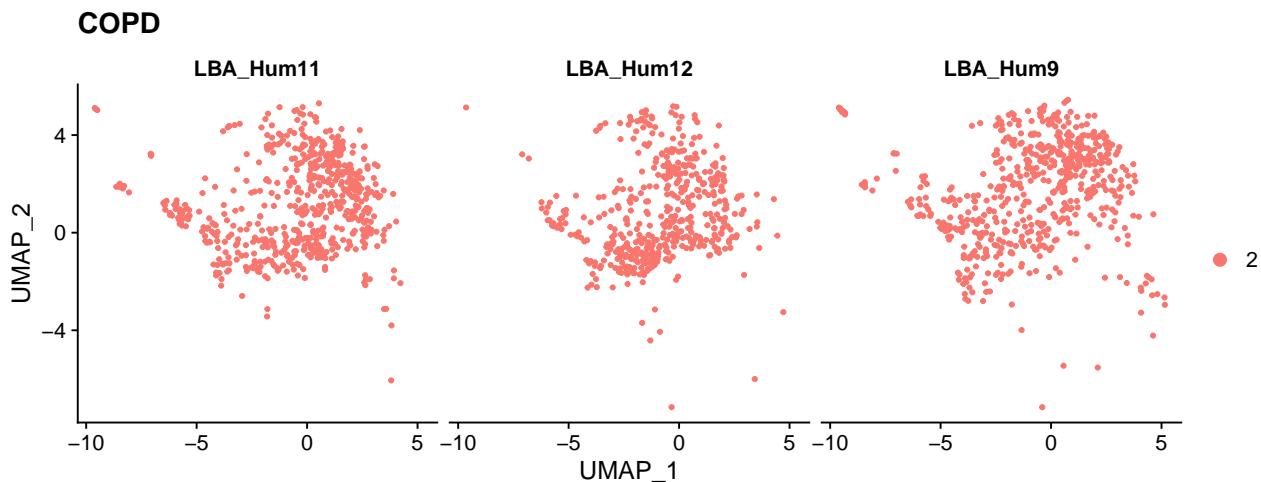
results.c2 <- NormalizeData(results.c2, verbose = FALSE)           1
results.c2 <- FindVariableFeatures(results.c2, selection.method = "vst", 2
  nfeatures = 2000, verbose = FALSE)
results.c2 <- ScaleData(results.c2, features = rownames(results.c2), 3
  verbose = FALSE)
results.c2 <- RunPCA(results.c2, features = VariableFeatures(results.c2), 4
  verbose = FALSE)
results.c2 <- RunUMAP(results.c2, dims = 1:10, verbose = FALSE)      5

```

```

DimPlot(subset(results.c2, subset = group == "COPD"), split.by = "origin") 1
+ ggtitle("COPD")

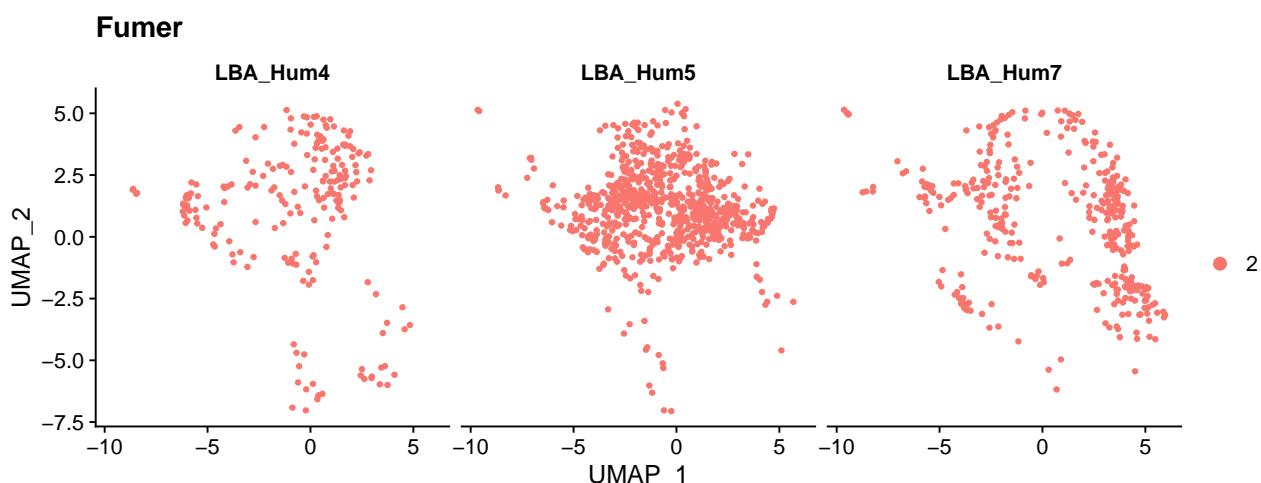
```



```

DimPlot(subset(results.c2, subset = group == "Fumer"), split.by = "origin" 1
) + ggtitle("Fumer")

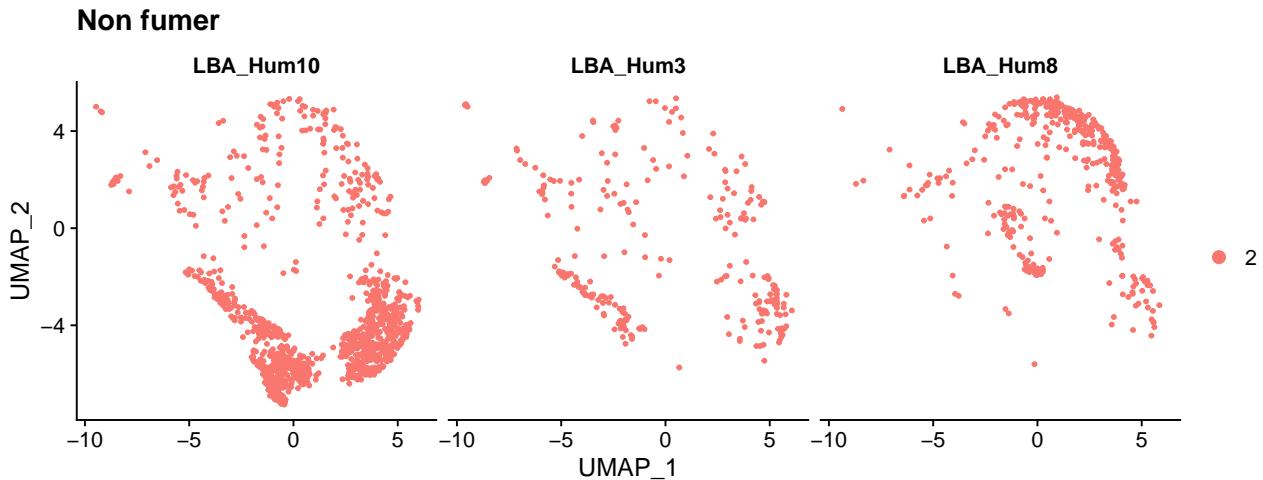
```



```

DimPlot(subset(results.c2, subset = group == "No_fumer"), split.by = " 1
origin") + ggtitle("Non_fumer")

```



Except the sample Hum10, other samples have relatively equal distribution of all subsets. the Hum10 represent two exceptional lower subsets.

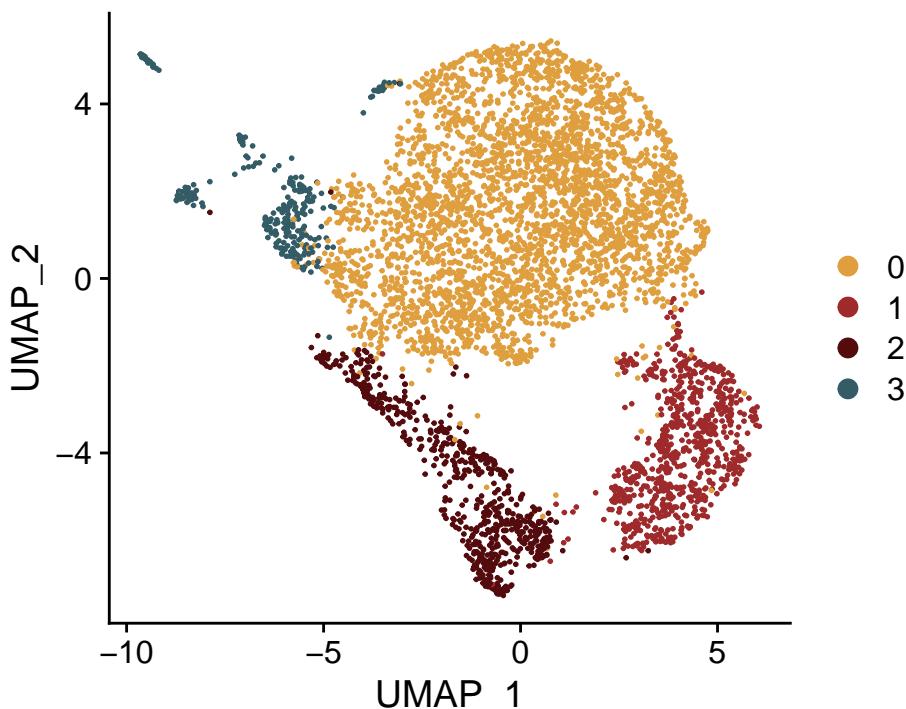
6.3 Re-cluster the Cluster 3

REMIND: The cluster 3 in the manuscript referred to cluster 2 in the following codes.

```

pal_4c.c2 <- c("#e09f3e", "#9e2a2b", "#540b0e", "#335c67")           1
results.c2 <- FindNeighbors(results.c2, reduction = "pca", dims = 1:10,      2
    verbose = FALSE)
results.c2 <- FindClusters(results.c2, resolution = 0.12, verbose = FALSE) 3
p <- DimPlot(results.c2, cols = pal_4c.c2)                                4
p

```



```

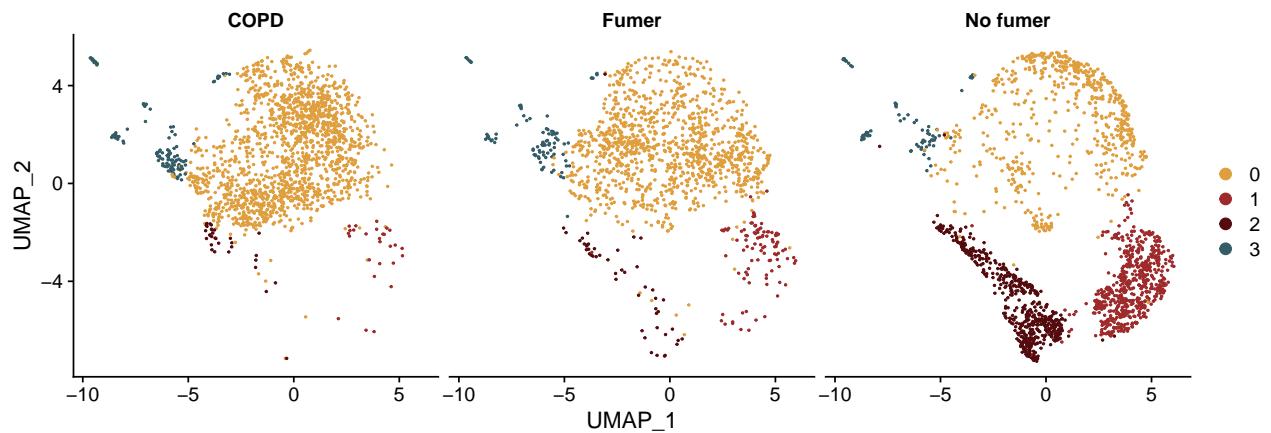
pal_4c.c2 <- c("#e09f3e", "#9e2a2b", "#540b0e", "#335c67")           1
results.c2 <- FindNeighbors(results.c2, reduction = "pca", dims = 1:10,      2
    verbose = FALSE)

```

```

results.c2 <- FindClusters(results.c2, resolution = 0.12, verbose = FALSE) 3
p <- DimPlot(results.c2, cols = pal_4c.c2, split.by = "group") 4
p 5

```

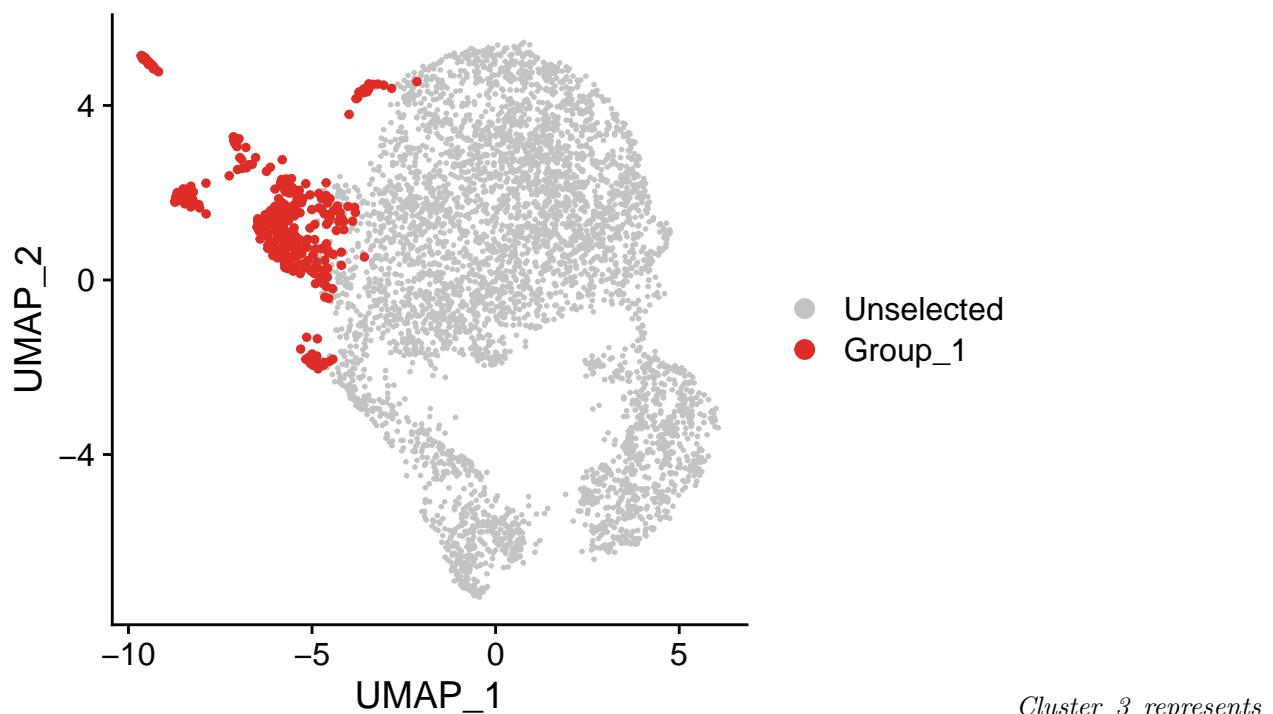


Where is the DC-like population located?

```

DimPlot(results.c2, cells.highlight = WhichCells(results.c2, expression =
  seurat_clusters_before_filter == "11")) 1

```



the old cluster 11 DC(-like).

Cluster 3 represents

```

saveRDS(results.c2, file = "./cluster2_clustered.seuratObject.Rds") 1

```

6.4 Statistic summary about the subpopulations in Cluster 3 (Mreg)

Cell number of each cluster for each samples

```

names.col <- unique(results.c2$origin)
names.row <- as.character(0:3)
df <- sapply(names.col, function(x) table(subset(results.c2, subset =
  origin == x)$seurat_clusters)[names.row])
df <- as.data.frame(df)
df

```

```

## # A tibble: 4 x 9
##   LBA_Hum3 LBA_Hum4 LBA_Hum5 LBA_Hum7 LBA_Hum8 LBA_Hum9 LBA_Hum10
##   <int>     <int>     <int>     <int>     <int>     <int>     <int>    <
## 1      85      151      715      255      351      527      166
##             539
## 2      77       17        7       77       41       13      497
##             11
## 3      70       12       13       22        5       13      464
##             8
## 4      35       28       34       29       16       50       28
##             49
## # ... with 1 more variable: LBA_Hum12 <int>

```

Distribution in %:

```

df <- apply(df, 2, function(x) round(x/sum(x)*100, 2) )
df <- as.data.frame(df)
df

```

```

## # A tibble: 4 x 9
##   LBA_Hum3 LBA_Hum4 LBA_Hum5 LBA_Hum7 LBA_Hum8 LBA_Hum9 LBA_Hum10
##   <dbl>     <dbl>     <dbl>     <dbl>     <dbl>     <dbl>     <dbl>    <
## 1      31.8      72.6      93.0      66.6      85.0      87.4      14.4
##             88.8
## 2      28.8      8.17      0.91      20.1      9.93      2.16      43.0
##             1.81
## 3      26.2      5.77      1.69      5.74      1.21      2.16      40.2
##             1.32
## 4      13.1      13.5      4.42      7.57      3.87      8.29      2.42
##             8.07
## # ... with 1 more variable: LBA_Hum12 <dbl>

```

```

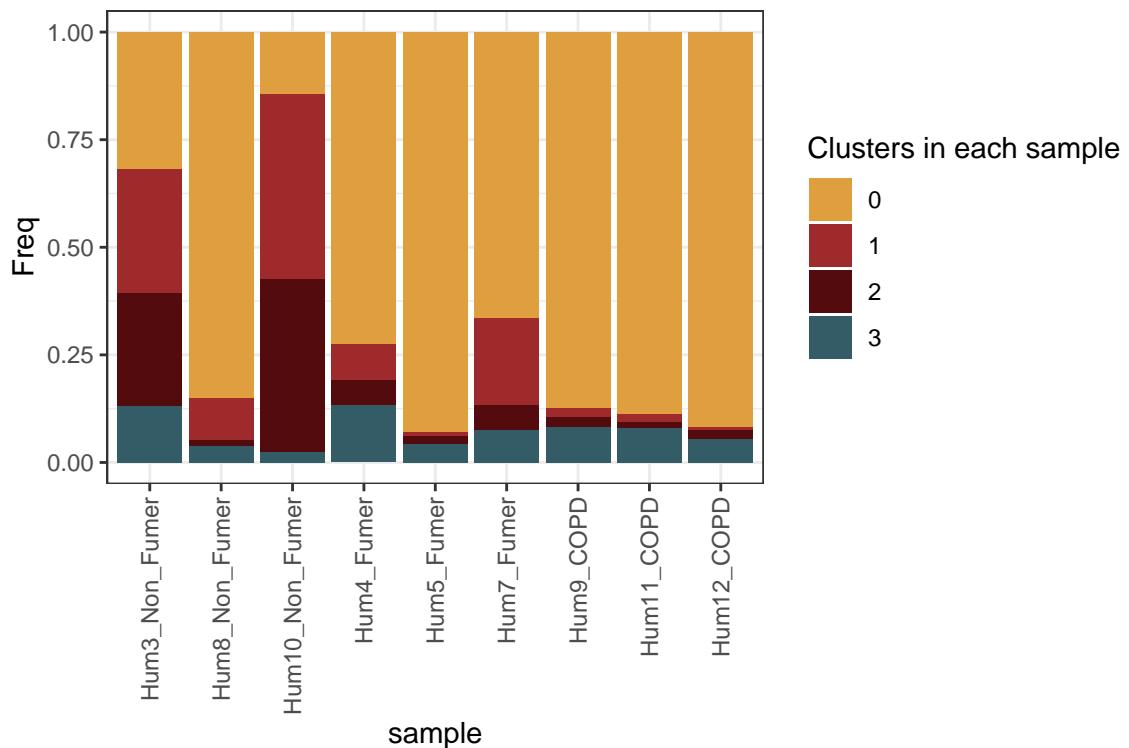
freq.celltype.list <- list(
  Hum3_Non_Fumer = Seurat2CellFreqTable(subset(results.c2, subset = origin
    == "LBA_Hum3"), slotName = "seurat_clusters"),
  Hum8_Non_Fumer = Seurat2CellFreqTable(subset(results.c2, subset = origin
    == "LBA_Hum8"), slotName = "seurat_clusters"),
  Hum10_Non_Fumer = Seurat2CellFreqTable(subset(results.c2, subset =
    origin == "LBA_Hum10"), slotName = "seurat_clusters"),
  Hum4_Fumer = Seurat2CellFreqTable(subset(results.c2, subset = origin ==
    "LBA_Hum4"), slotName = "seurat_clusters"),

```

```

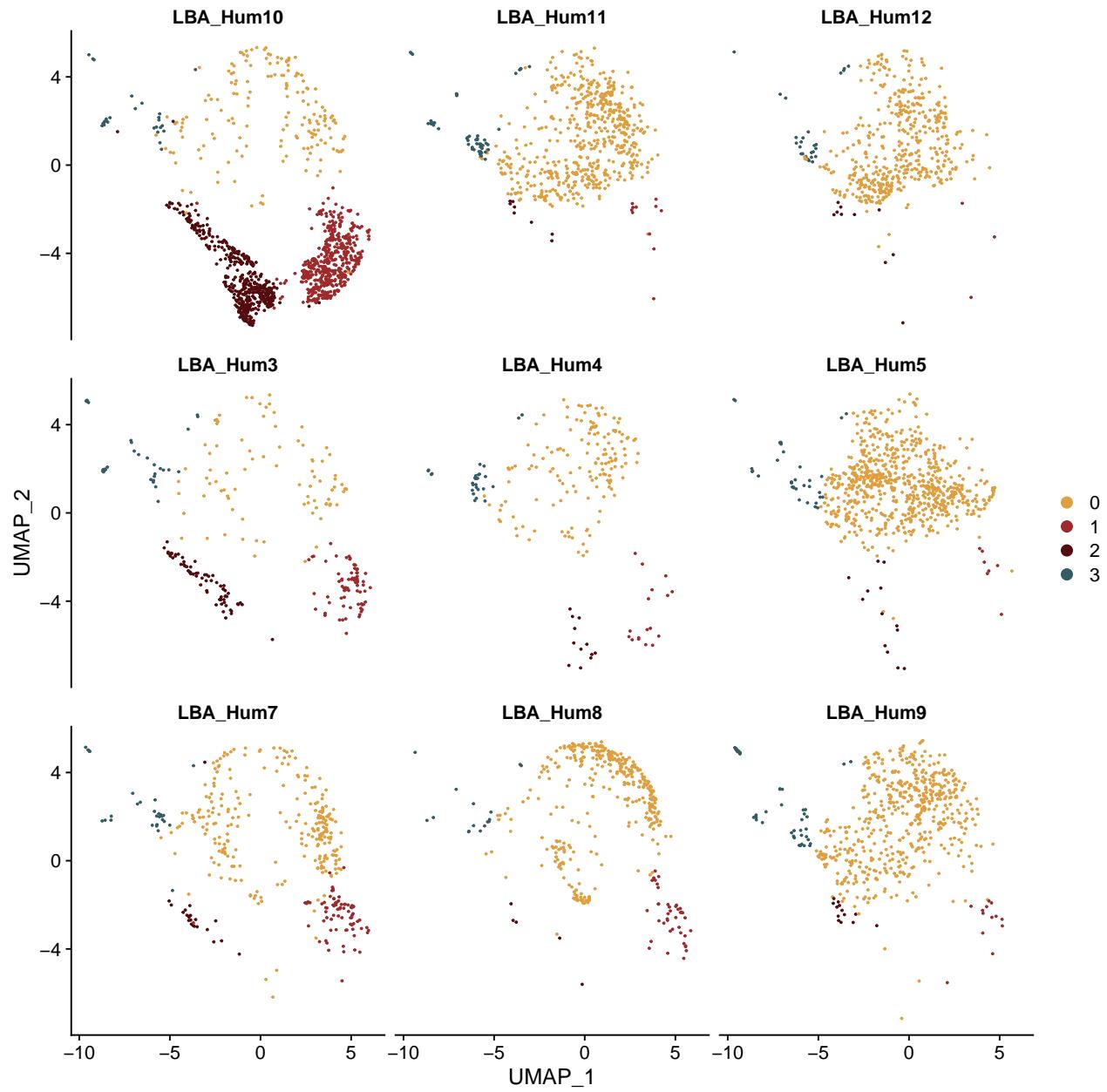
    Hum5_Fumer = Seurat2CellFreqTable(subset(results.c2, subset = origin == 6
      "LBA_Hum5"), slotName = "seurat_clusters"),
    Hum7_Fumer = Seurat2CellFreqTable(subset(results.c2, subset = origin == 7
      "LBA_Hum7"), slotName = "seurat_clusters"),
    Hum9_COPD = Seurat2CellFreqTable(subset(results.c2, subset = origin == 8
      "LBA_Hum9"), slotName = "seurat_clusters"),
    Hum11_COPD = Seurat2CellFreqTable(subset(results.c2, subset = origin == 9
      "LBA_Hum11"), slotName = "seurat_clusters"),
    Hum12_COPD = Seurat2CellFreqTable(subset(results.c2, subset = origin == 10
      "LBA_Hum12"), slotName = "seurat_clusters")
)
p <- barChart(freq.celltype.list) + labs(fill = "Clusters in each sample") 11
+ 12
  scale_fill_manual(values = pal_4c.c2) + 13
  theme(axis.text.x = element_text(angle = 90, vjust = 0.5, hjust=1)) 14
p 15

```



```
DimPlot(results.c2, split.by = "origin", ncol = 3, cols = pal_4c.c2)
```

1



6.5 Functional markers in subpopulations of Cluster 3 (Mreg)

```

1 p <- DotPlot(results.c2, features = c("FCER1A", "CD1A", "CD1C", "CD1E", "
2   CLEC10A",
3     "CCL18", "CCL4", "CCL3", "CXCL9", " "
4       CXCL10", "IL10",
5     "CALM1", "CALM2", "CCND2", "CCND3",
6     "FCGR2A", "FCGR2B",
7     "ADGRE5", "AGA", "ITGAM", "CLEC12A",
8     "ANXA11", "ARPC2", "ARPC5", "FCN1", " "
9       ICAM3"
),
assay = "RNA",
scale.by = "size",

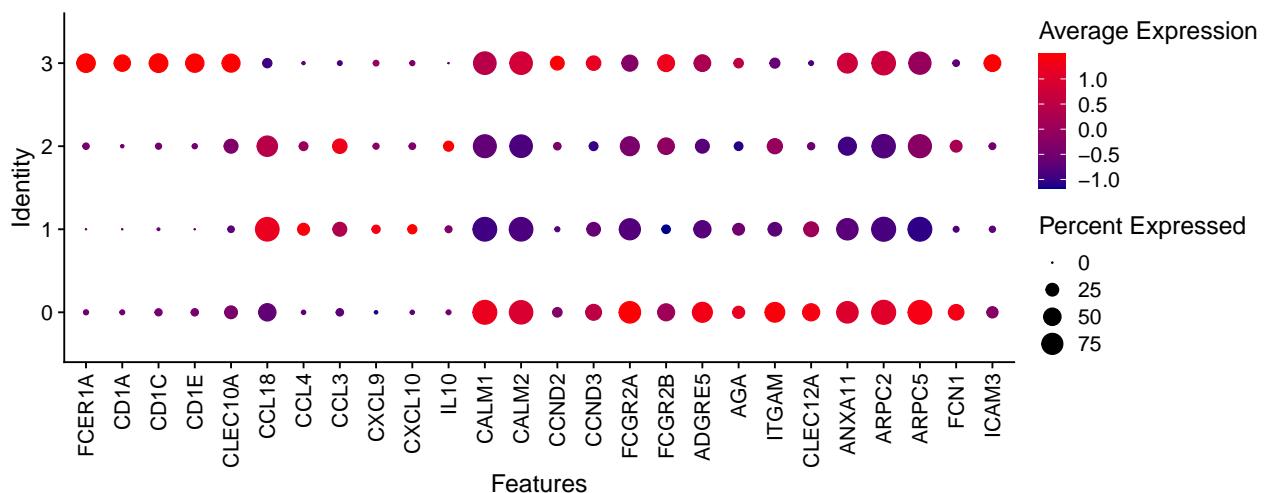
```

```

          cols = c("dark_blue", "red")) +
theme(axis.text.x = element_text(angle = 90,
vjust = 0.5,
hjust=1))
p

```

10
11
12
13
14



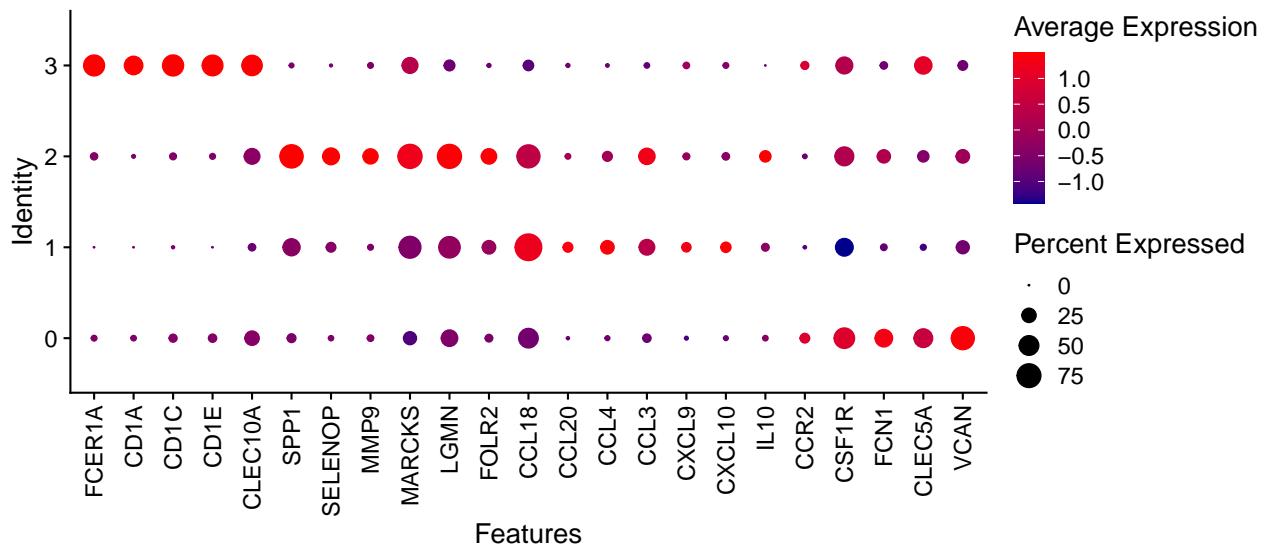
With another marker list

```

p <- DotPlot(results.c2, features = c("FCER1A", "CD1A", "CD1C", "CD1E", "CLEC10A",
                                         "SPP1", "SELENOP", "MMP9", "MARCKS",
                                         "LGMN", "FOLR2",
                                         "CCL18", "CCL20", "CCL4", "CCL3", "CXCL9",
                                         "CXCL10", "IL10",
                                         "CCR2", "CSF1R", "FCN1",
                                         "CLEC5A", "VCAN"
                                         ),
assay = "RNA",
scale.by = "size",
cols = c("dark_blue", "red")) +
theme(axis.text.x = element_text(angle = 90,
vjust = 0.5,
hjust=1))
p

```

1
2
3
4
5
6
7
8
9
10
11
12
13



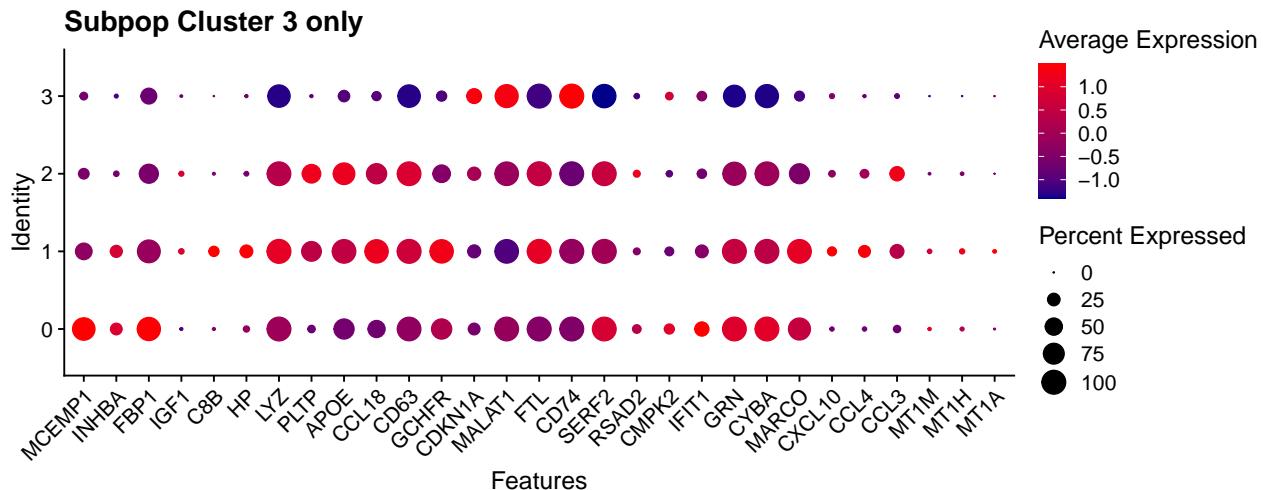
7 Compare to Mould et al. 2020

Let's focus on the Fig 2C of the report Mould et al. 2021[3]: Mould, K. J. et al. Airspace macrophages and monocytes exist in transcriptionally distinct subsets in healthy adults. Am. J. Respir. Crit. Care Med. (2021) doi:10.1164/RCCM.202005-1989OC.

```

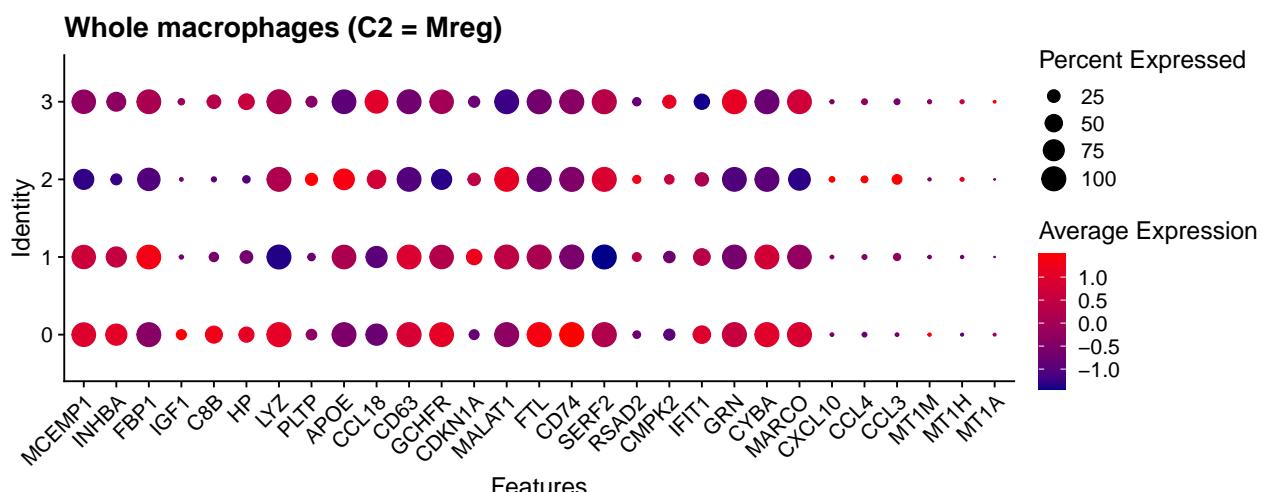
1 DotPlot(results.c2, features = c("MCEMP1", "INHBA", "FBP1",
2   "IGF1", "C8B", "HP",
3   "LYZ", "PLTP", "APOE",
4   "CCL18", "CD63", "GCHFR",
5   "CDKN1A", "MALAT1", "FTL",
6   "CD74", "SERF2", "RSAD2", "CMPK2",
7   "IFIT1", "GRN", "CYBA", "MARCO", "CXCL10"
8   ,
9   "CCL4", "CCL3",
10  "MT1M", "MT1H", "MT1A"),
11  scale.by = "size",
12  cols = c("darkblue", "red")) + theme(axis.text.x =
element_text(angle = 45, hjust=1))+
ggttitle("Subpop_Cluster_3_only")

```



How about in whole macrophages (not only cluster2)

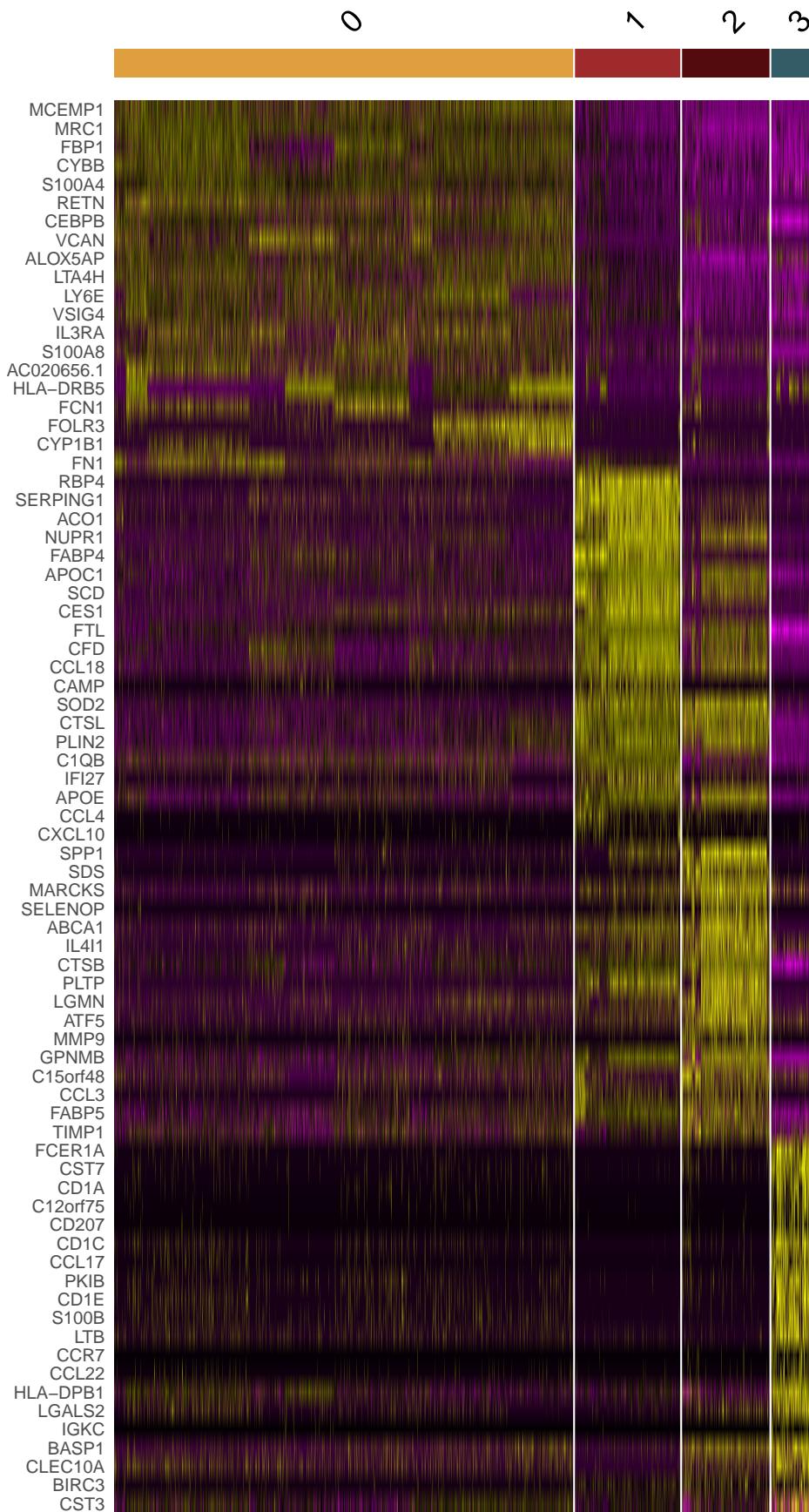
```
DotPlot(results, features = c("MCEMP1", "INHBA", "FBP1",
                               "IGF1", "C8B", "HP",
                               "LYZ", "PLTP", "APOE",
                               "CCL18", "CD63", "GCHFR",
                               "CDKN1A", "MALAT1", "FTL",
                               "CD74", "SERF2", "RSAD2", "CMPK2",
                               "IFIT1", "GRN", "CYBA", "MARCO", "CXCL10",
                               ,
                               "CCL4", "CCL3",
                               "MT1M", "MT1H", "MT1A"),
        scale.by = "size",
        cols = c("dark_blue", "red")) + theme(axis.text.x =
          element_text(angle = 45, hjust=1)) +
        ggtitle("Whole_macrophages_(C2_Mreg)")
```



Compare to Mould et al. Fig 2C, 1) the Mreg (C2 of whole macrophages) expresses higher cytokine, chemokines (CXCL10, CCL4, CCL3), thus may be the pro-inflammatory macrophage (m5) mentioned in their report; 2) Cluster 0 and cluster 1 are quite similar except IGF1 C8B HP, these 2 pops should represent continuous development of AM.

8 Find DE genes in subpopulations of the cluster 3 (Mreg)

```
all_cluster.markers <- FindAllMarkers(results.c2) 1  
  
require(dplyr)  
top20 <- all_cluster.markers %>% group_by(cluster) %>% top_n(n = 20, wt =  
    avg_log2FC) 2  
DoHeatmap(results.c2, features = top20$gene, group.colors = pal_4c.c2  
    ) + NoLegend() 3  
  4
```



9 Scoring of Mreg and AM signatures

```
library(Seurat)
library(VISION)
library(ggplot2)
```

9.1 Create signature from DE gene lists

The Mreg and AM signatures are the top 100 DE genes obtain from DESeq2 analysis with bulkRNAseq data (see bulkRNAseq_analysis for details).

```
sig <- read.table("./Mreg_MA_sig.csv", sep = "\t", header = T, as.is = T) 1
sig <- sig[-1, ] # remove description 2
sig <- as.data.frame(lapply(sig, unique), stringsAsFactors=FALSE) # remove 3
      doublon
sig <- as.data.frame(sapply(sig, as.character), stringsAsFactors=FALSE) 4
      # change to character
sig <- as.data.frame(sapply(sig, function(x) x <- x[! x == ""] ), 5
      stringsAsFactors=FALSE) # remove empty

sig.Mreg <- c(rep(1, length(sig$Mreg_sig))) 6
names(sig.Mreg) <- c(sig$Mreg_sig)

sig.AM <- c(rep(1, length(sig$MA_sig))) 7
names(sig.AM) <- c(sig$MA_sig)

sig.Mreg <- createGeneSignature(name = "Mreg", sigData = sig.Mreg) 8
sig.AM <- createGeneSignature(name = "AM", sigData = sig.AM) 9
sig.macro <- c(sig.Mreg, sig.AM) 10
```

9.2 Scoring for Mreg and AM signatures

Let's calculate scores for the Mreg and AM signatures in either total macrophages and cells in cluster 3 (referred to C2 in the codes).

```
vis <- Vision(results,
               signatures = sig.macro) 1
                  2
                  3
vis.c2 <- Vision(results.c2,
                  signatures = sig.macro) 4
                  5
```

Calculate score:

```
vis <- calcSignatureScores(vis) 1
vis.c2 <- calcSignatureScores(vis.c2) 2
```

9.3 Present signature score with existing embedding in Seurat object

Check cell names are the same in both analyses.

```
identical(colnames(results), rownames(vis@SigScores)) 1
## [1] TRUE 1
```

```
1 identical(colnames(results.c2), rownames(vis.c2@SigScores))
```

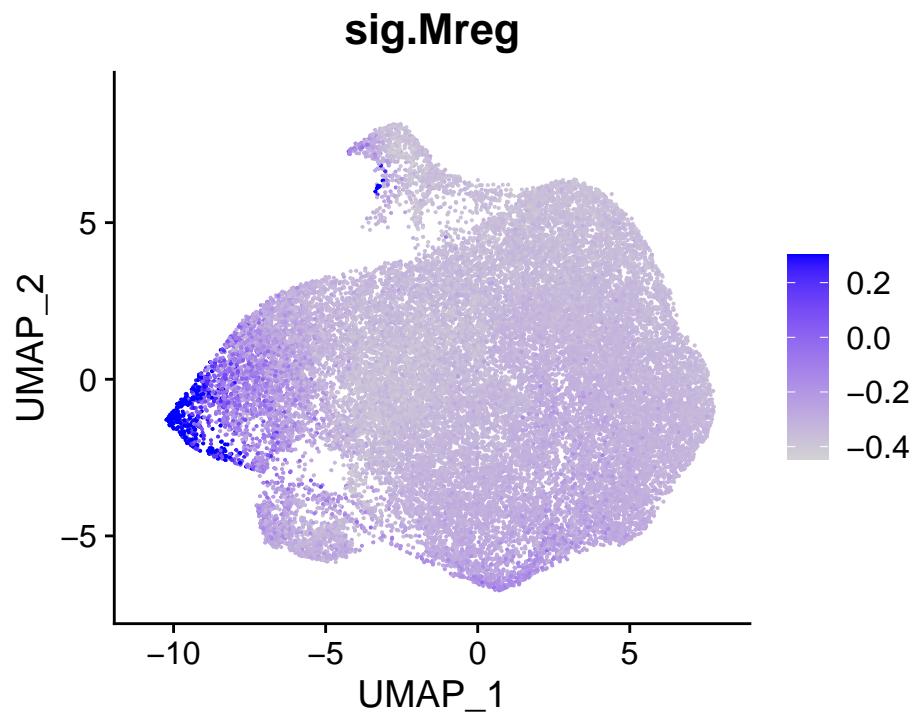
```
1 ## [1] TRUE
```

```
1 results$sig.Mreg <- vis@SigScores[, "Mreg"]  
2 results$sig.AM <- vis@SigScores[, "AM"]
```

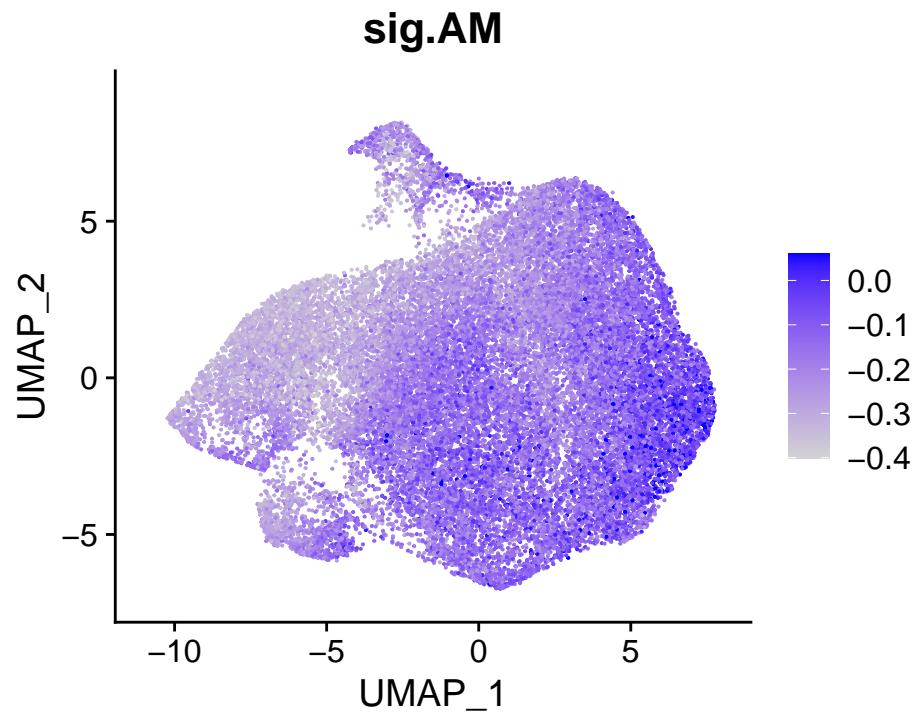
```
3 results.c2$sig.Mreg <- vis.c2@SigScores[, "Mreg"]  
4 results.c2$sig.AM <- vis.c2@SigScores[, "AM"]  
5
```

Plot signature scores with existing embedding in Seurat object:

```
1 FeaturePlot(results, features = "sig.Mreg"  
2 , min.cutoff = -0.45, max.cutoff = 0.3)
```



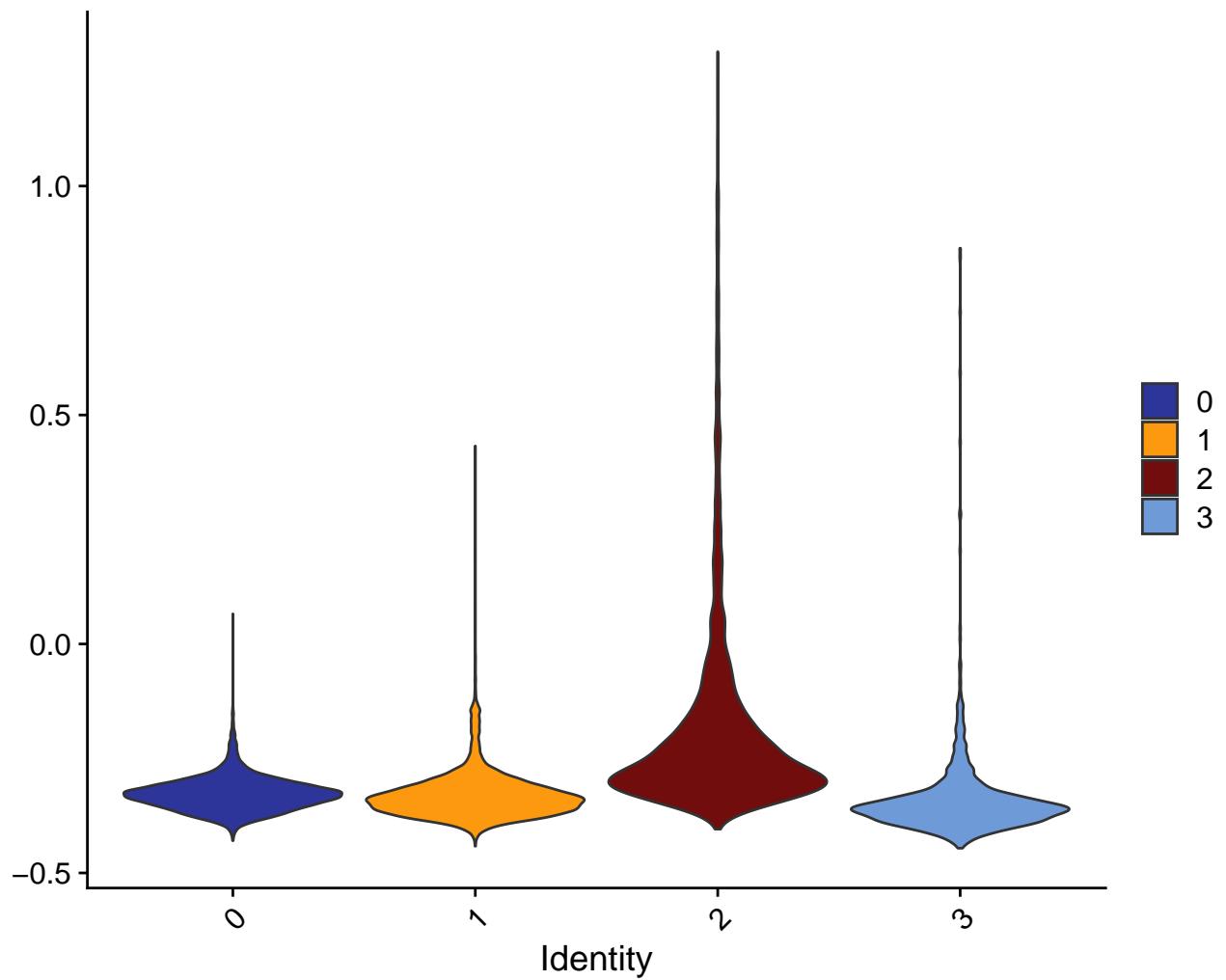
```
1 FeaturePlot(results, features = "sig.AM"  
2 , min.cutoff = -0.4, max.cutoff = 0.06)
```



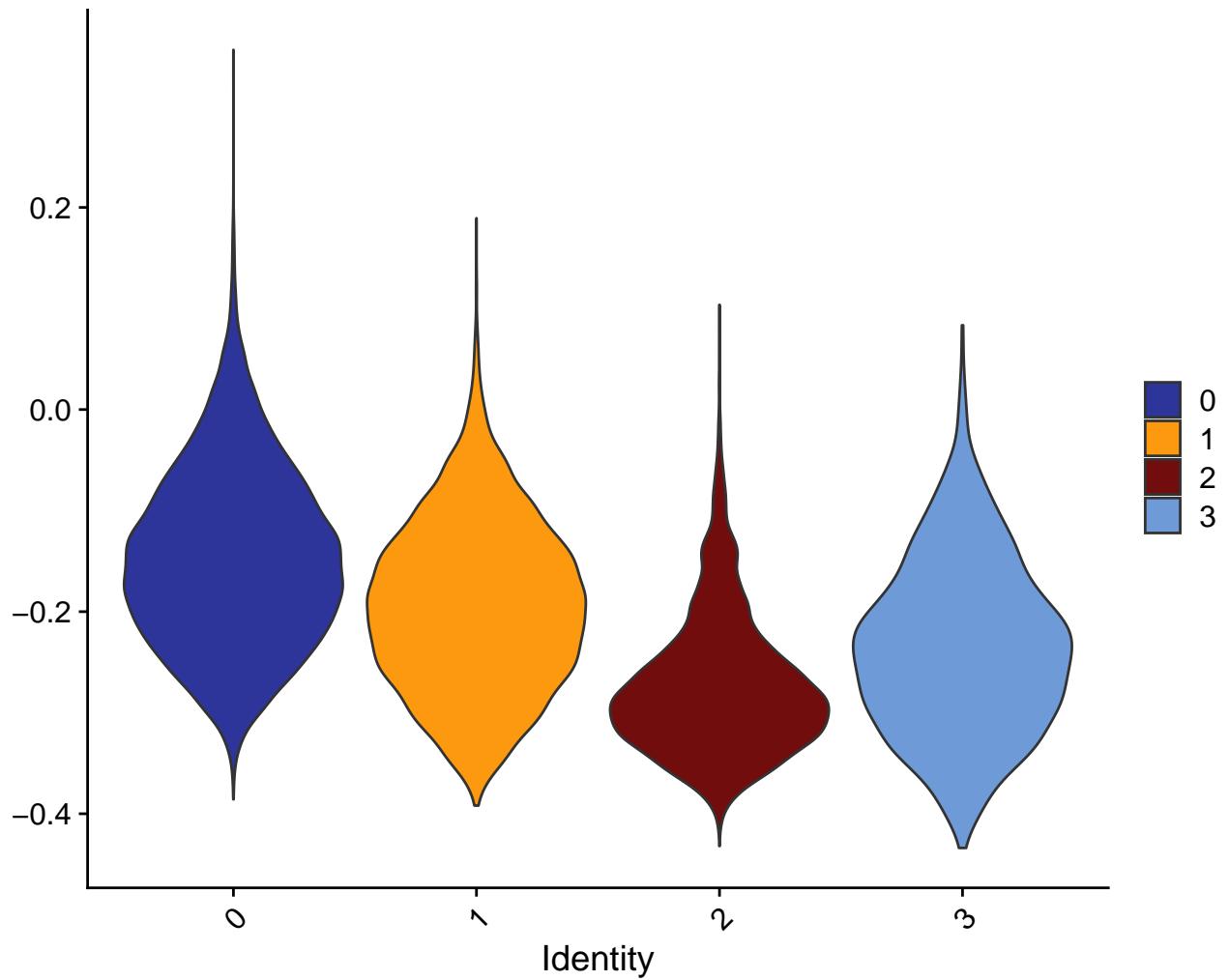
```
VlnPlot(results, features = "sig.Mreg", pt.size = 0, cols = pal_4c)
```

1

sig.Mreg

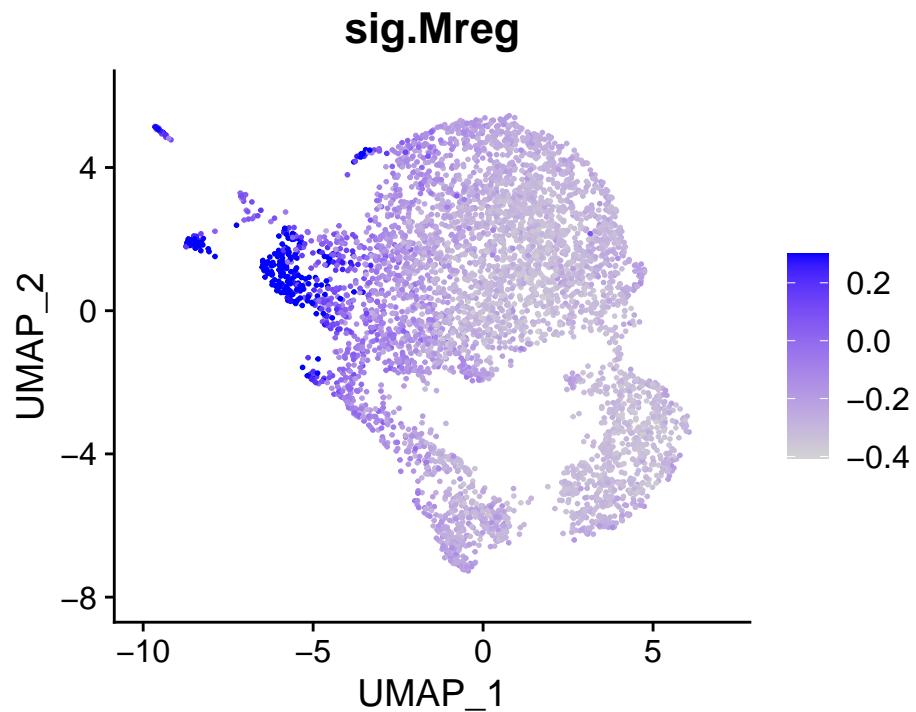


sig.AM

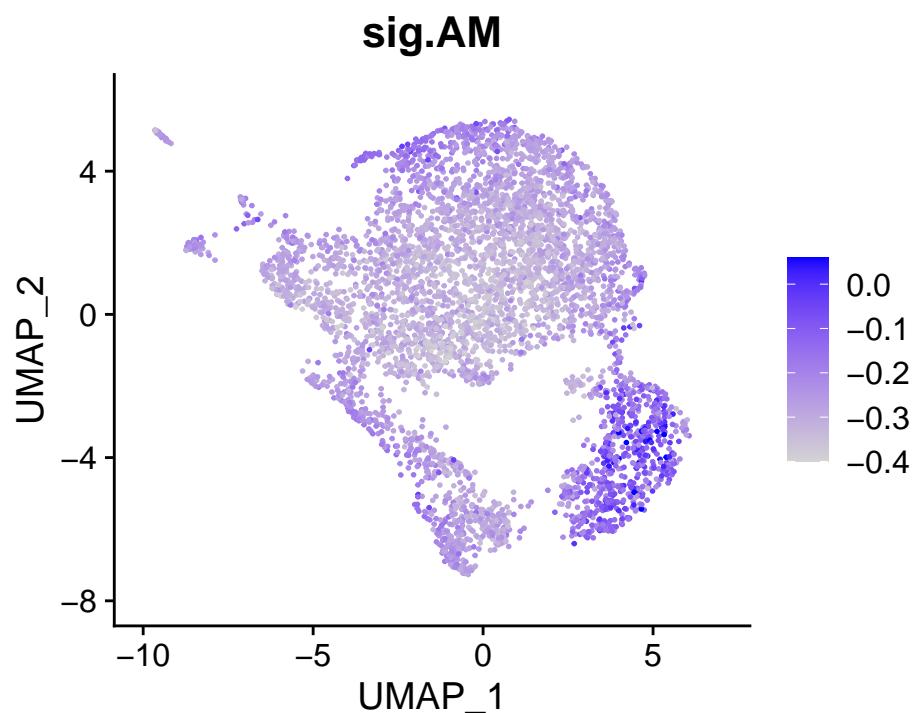


```
FeaturePlot(results.c2, features = "sig.Mreg"  
           , min.cutoff = -0.45, max.cutoff = 0.3)
```

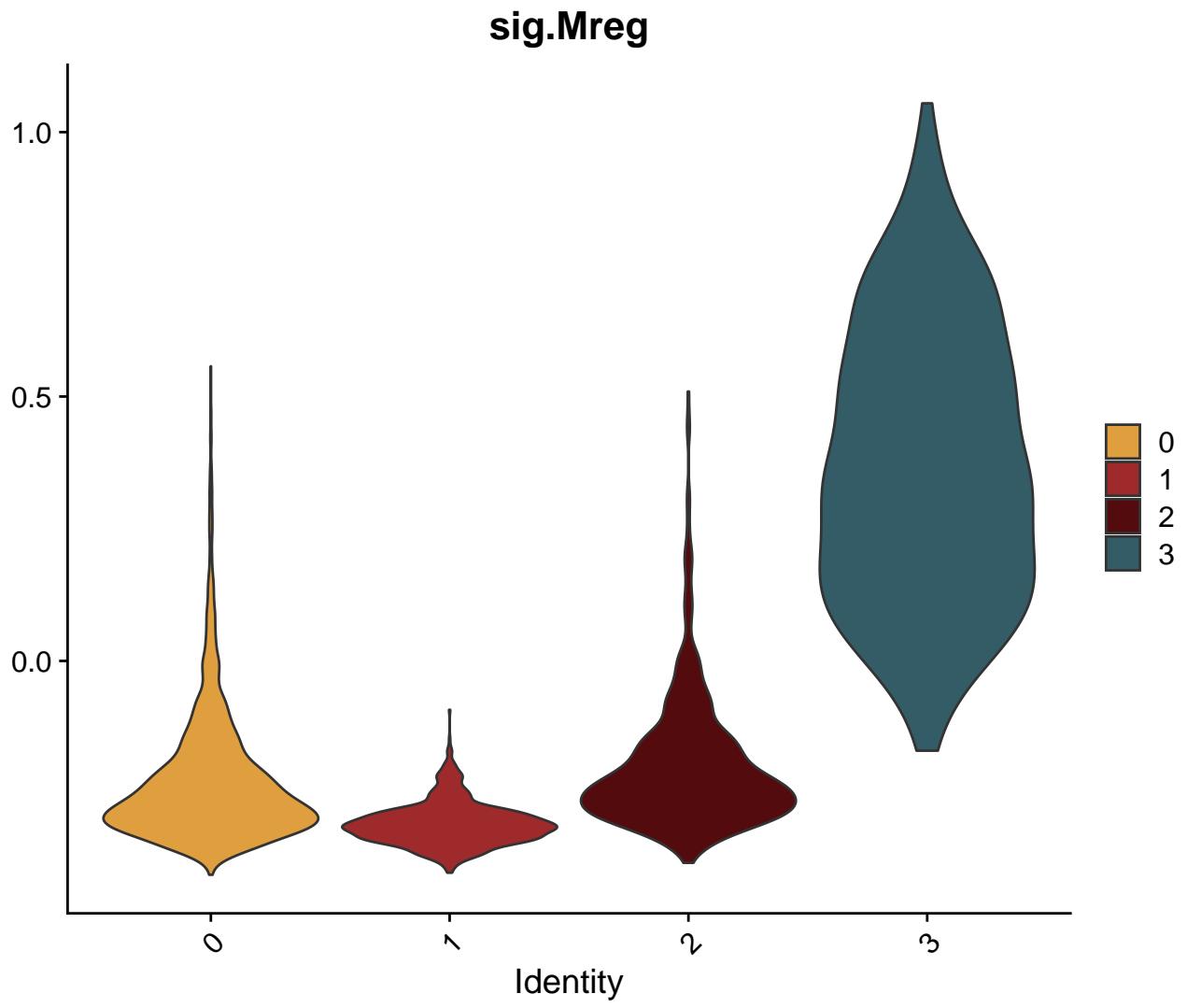
1
2

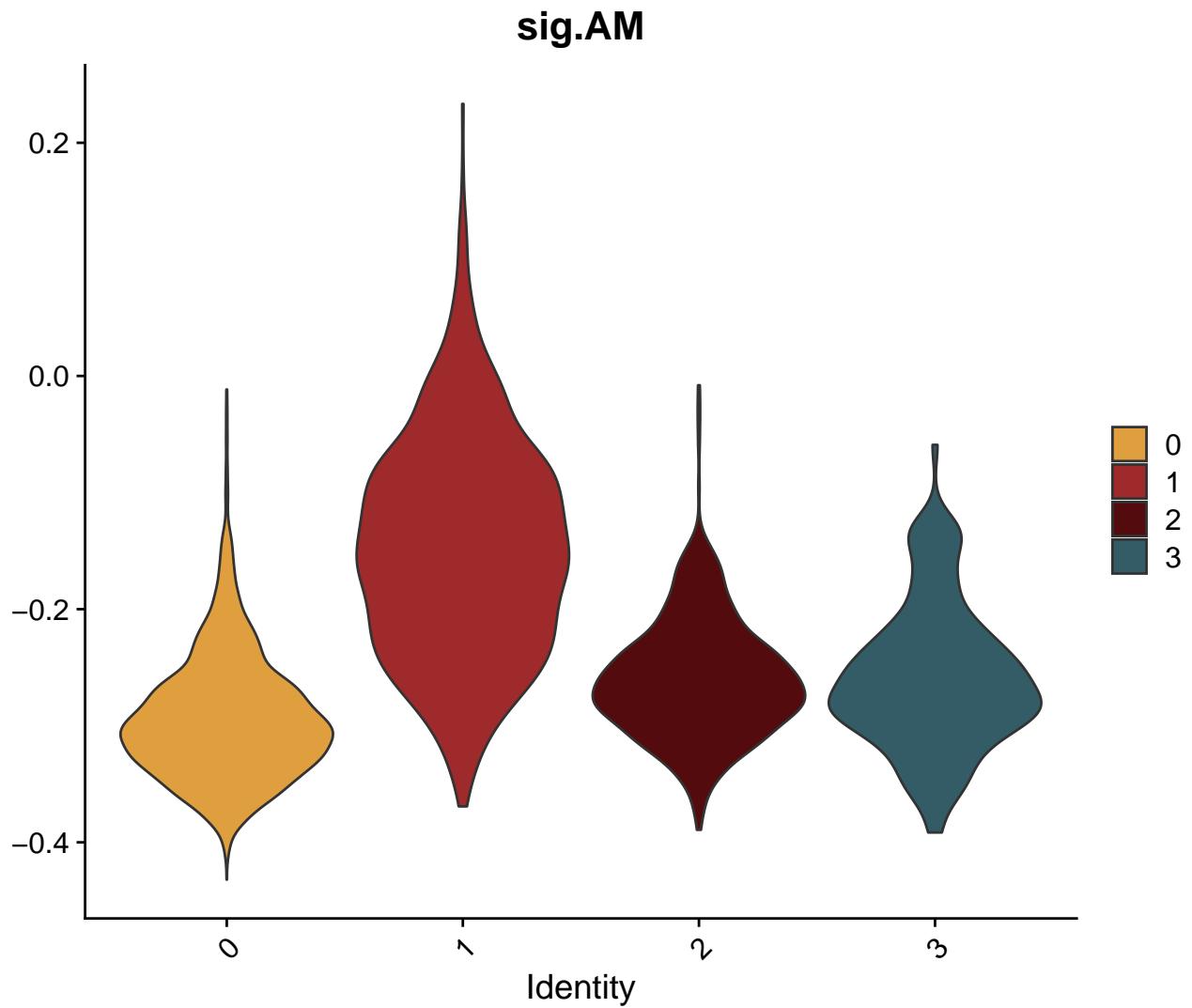


```
FeaturePlot(results.c2, features = "sig.Mreg"  
           , min.cutoff = -0.4, max.cutoff = 0.06)
```



```
VlnPlot(results.c2, features = "sig.AM", pt.size = 0, cols = pal_4c.c2)
```





10 Session information

```
sessionInfo()
## R version 4.0.3 (2020-10-10)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 20.04.3 LTS
##
## Matrix products: default
## BLAS:    /usr/lib/x86_64-linux-gnu/openblas-pthread/libblas.so.3
## LAPACK:  /usr/lib/x86_64-linux-gnu/openblas-pthread/liblapack.so.3
##
## locale:
## [1] LC_CTYPE=en_US.UTF-8          LC_NUMERIC=C
## [3] LC_TIME=en_GB.UTF-8          LC_COLLATE=en_US.UTF-8
## [5] LC_MONETARY=en_GB.UTF-8       LC_MESSAGES=en_US.UTF-8
## [7] LC_PAPER=en_GB.UTF-8          LC_NAME=C
## [9] LC_ADDRESS=C                  LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_GB.UTF-8   LC_IDENTIFICATION=C
```

```

##          16
## attached base packages:          17
## [1] stats      graphics   grDevices utils     datasets  methods   base    18
##          19
## other attached packages:         20
## [1] VISION_2.1.0       dplyr_1.0.7        RColorBrewer_1.1-2 ggplot2_3  21
##          .3.5
## [5] SeuratObject_4.0.2 Seurat_4.0.3
##          22
##          23
## loaded via a namespace (and not attached): 24
## [1] Rtsne_0.15           colorspace_2.0-2    deldir_0.2-10   25
## [4] ellipsis_0.3.2      ggridges_0.5.3     mclust_5.4.7    26
## [7] rstudioapi_0.13     spatstat.data_2.1-0 leiden_0.3.9    27
## [10] listenv_0.8.0       farver_2.1.0      ggrepel_0.9.1   28
## [13] RSpectra_0.16-0     fansi_0.5.0      logging_0.10-108 29
## [16] codetools_0.2-18    splines_4.0.3     knitr_1.33      30
## [19] polyclip_1.10-0    jsonlite_1.7.2    ica_1.0-2       31
## [22] cluster_2.1.0      png_0.1-7       uwot_0.1.10.9000 32
## [25] wordspace_0.2-6    shiny_1.6.0      sctransform_0.3.2 33
## [28] spatstat.sparse_2.0-0 plumber_1.1.0    compiler_4.0.3   34
## [31] httr_1.4.2          assertthat_0.2.1  Matrix_1.3-4    35
## [34] fastmap_1.1.0      lazyeval_0.2.2    limma_3.46.0    36
## [37] cli_3.0.1          later_1.2.0     htmltools_0.5.1.1 37
## [40] tools_4.0.3          rsvd_1.0.5      igraph_1.2.6    38
## [43] gtable_0.3.0        glue_1.4.2      RANN_2.6.1     39
## [46] reshape2_1.4.4      Rcpp_1.0.7      scattermore_0.7 40
## [49] vctrs_0.3.8          nlme_3.1-152   lmtest_0.9-38   41
## [52] xfun_0.24           stringr_1.4.0   webutils_1.1    42
## [55] globals_0.14.0      mime_0.11      miniUI_0.1.1.1 43
## [58] lifecycle_1.0.0      irlba_2.3.3    goftest_1.2-2   44
## [61] future_1.21.0       MASS_7.3-53    zoo_1.8-9      45
## [64] scales_1.1.1        loe_1.1       spatstat.core_2.3-0 46
## [67] ragg_1.1.3          promises_1.2.0.1 spatstat.utils_2.2-0 47
## [70] parallel_4.0.3      swagger_3.33.1  yaml_2.2.1     48
## [73] reticulate_1.20     pbapply_1.4-3   gridExtra_2.3   49
## [76] rpart_4.1-15        fastICA_1.2-2  stringi_1.7.3   50
## [79] highr_0.9           permute_0.9-5  rlang_0.4.11   51
## [82] pkgconfig_2.0.3     systemfonts_1.0.2 matrixStats_0.60.0 52
## [85] evaluate_0.14        lattice_0.20-41 ROCR_1.0-11   53
## [88] purrr_0.3.4          tensor_1.5     patchwork_1.1.1 54
## [91] htmlwidgets_1.5.3   labeling_0.4.2  cowplot_1.1.1   55
## [94] tidyselect_1.1.1    parallely_1.27.0 RcppAnnoy_0.0.19 56
## [97] plyr_1.8.6          magrittr_2.0.1  R6_2.5.0      57
## [100] generics_0.1.0     DBI_1.1.1     pillar_1.6.2   58
## [103] withr_2.4.2        mgcv_1.8-33   fitdistrplus_1.1-5 59
## [106] survival_3.2-7     abind_1.4-5   tibble_3.1.3   60
## [109] future.apply_1.7.0  crayon_1.4.1  KernSmooth_2.23-20 61
## [112] utf8_1.2.2          spatstat.geom_2.2-2 plotly_4.9.4.1 62
## [115] rmarkdown_2.9        grid_4.0.3     data.table_1.14.0 63
## [118] vegan_2.5-7          sparsesvd_0.2  digest_0.6.27   64
## [121] pbmcapply_1.5.0    xtable_1.8-4   tidyverse_1.1.3 65
## [124] httpuv_1.6.1        textshaping_0.3.5  munsell_0.5.0   66
## [127] viridisLite_0.4.0   iotools_0.3-2

```

References

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