

2-Sample QC and cell filtering

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Contents

| | |
|--|-----------|
| 1 Description | 2 |
| 2 Load data and package: | 2 |
| 3 QC for each sample | 2 |
| 3.1 Sample: NGS19-I415_Dim_LBA-Hum3 | 2 |
| 3.2 Sample: NGS19-I679_Dim_LBA-Hum4 | 4 |
| 3.3 Sample: NGS19-J028_Dim_LBA-Hum5 | 6 |
| 3.4 Sample: NGS19-J141_Dim_LBA-Hum7 | 8 |
| 3.5 Sample: NGS19-J142_Dim_LBA-Hum8 | 10 |
| 3.6 Sample: NGS19-J263_Dim_LBA-Hum9 | 12 |
| 3.7 Sample: NGS19-K359_Dim_LBA-Hum11 | 16 |
| 3.8 Sample: NGS19-K360_Dim_LBA-Hum12 | 18 |
| 4 Save data for other analyses | 20 |
| 5 Session information: | 21 |
| References | 22 |

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1 Description

Below the CellRanger count outputs were piped to Seurat package version 4.0.3 [1]. Briefly, filtered matrices containing cell IDs and feature names in each sample were used to build a Seurat object (`CreateSeuratObject` wrapped in `seurat.setup` function). We performed a quality control by filtering out the cells with less than 200 detected genes, the genes detected in less than 3 cells and the cells exhibiting more than 20% of mitochondrial genes. Gene counts in each sample was normalized separately by default method “LogNormalize” with scale factor 104 and log-transformation. Two thousands of highly variable features were identified with the “vst” method.

2 Load data and package:

```
# load package and data
library(Seurat)
library(ggpubr)
source("../R/seurat.setup.R")
dir.10x <- "Counts/scRNASeq" # 10X output directory
```

1
2
3
4
5

3 QC for each sample

3.1 Sample: NGS19-I415_Dim_LBA-Hum3

```
NGS19_I415_Dim_LBA_Hum3 <-
  seurat.setup(path.10x = file.path(dir.10x,
                                     "NGS19-I415_Dim_LBA-Hum3/outs/
                                     filtered_feature_bc_matrix/"),
               project = "Liegeois_humanAM",
               dimensionality = 1:20,
               mt.percentage = 20,
               human = TRUE)
```

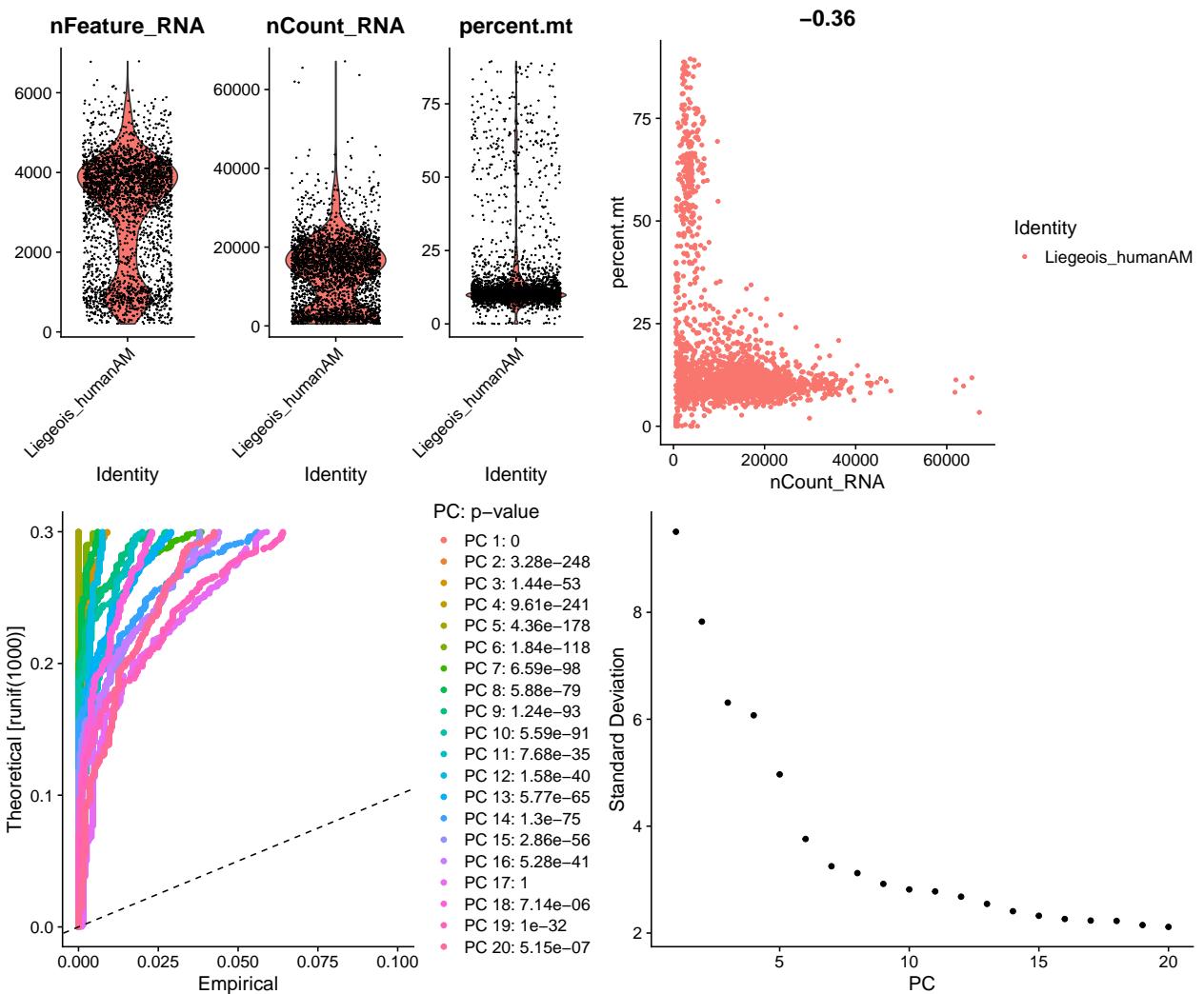
1
2
3
4
5
6
7

```
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
## Number of nodes: 2682
## Number of edges: 87787
##
## Running Louvain algorithm...
## Maximum modularity in 10 random starts: 0.7967
## Number of communities: 8
## Elapsed time: 0 seconds
```

1
2
3
4
5
6
7
8
9

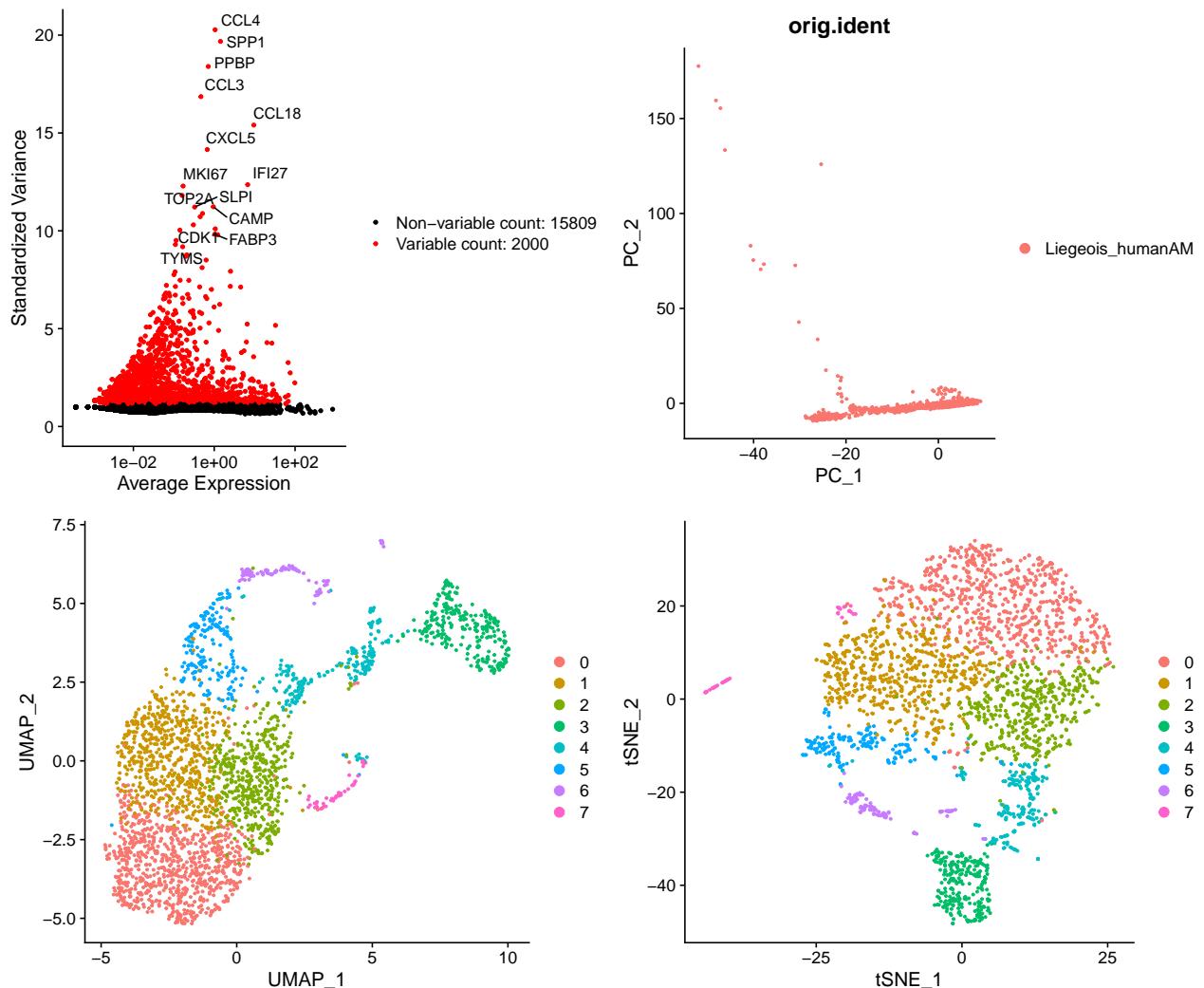
```
ggarrange(NGS19_I415_Dim_LBA_Hum3$plots$feature_vln,
          NGS19_I415_Dim_LBA_Hum3$plots$RNA_mt_pct_scatter,
          NGS19_I415_Dim_LBA_Hum3$plots$JackStrawPlot,
          NGS19_I415_Dim_LBA_Hum3$plots$ElbowPlot, ncol = 2, nrow = 2)
```

1
2
3
4



Sample: NGS19-I415_Dim_LBA-Hum3

```
1 ggarrange(NGS19_I415_Dim_LBA_Hum3$plots$variable_features ,
2             NGS19_I415_Dim_LBA_Hum3$plots$PCA_plot ,
3             NGS19_I415_Dim_LBA_Hum3$plots$UMAP_plot ,
4             NGS19_I415_Dim_LBA_Hum3$plots$TSNE_plot , ncol = 2, nrow = 2)
```



3.2 Sample: NGS19-I679_Dim_LBA-Hum4

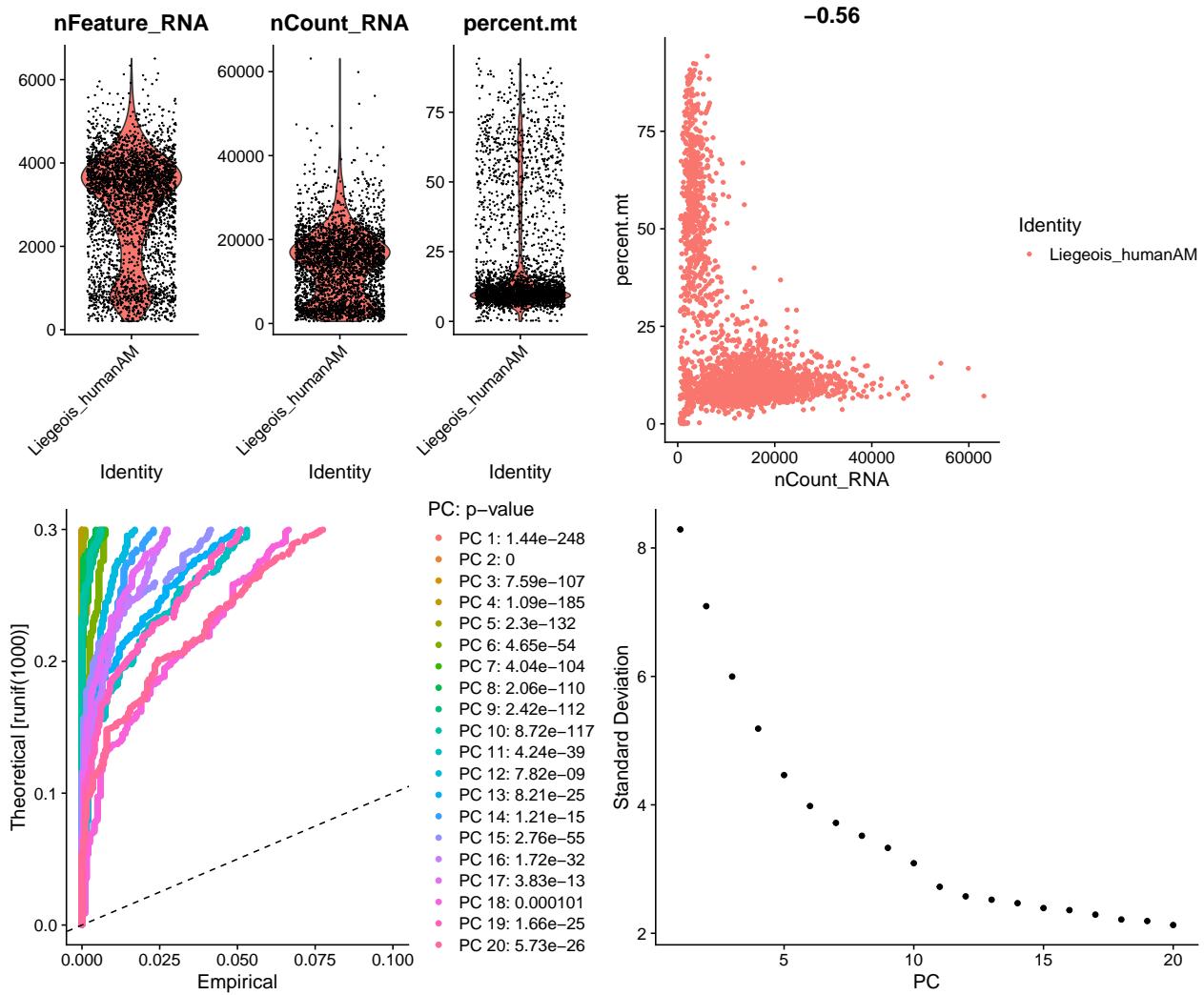
```
NGS19_I679_Dim_LBA_Hum4 <-
  seurat.setup(path.10x = file.path(dir.10x,
                                     "NGS19-I679_Dim_LBA-Hum4/outs/
                                     filtered_feature_bc_matrix/"),
  project = "Liegeois_humanAM",
  dimensionality = 1:20,
  mt.percentage = 20,
  human = TRUE)
```

```
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
## Number of nodes: 2598
## Number of edges: 86635
## Running Louvain algorithm...
## Maximum modularity in 10 random starts: 0.7716
## Number of communities: 8
## Elapsed time: 0 seconds
```

```

1 ggarrange(NGS19_I679_Dim_LBA_Hum4$plots$feature_vln,
2   NGS19_I679_Dim_LBA_Hum4$plots$RNA_mt_pct_scatter,
3   NGS19_I679_Dim_LBA_Hum4$plots$JackStrawPlot,
4   NGS19_I679_Dim_LBA_Hum4$plots$ElbowPlot, ncol = 2, nrow = 2)

```

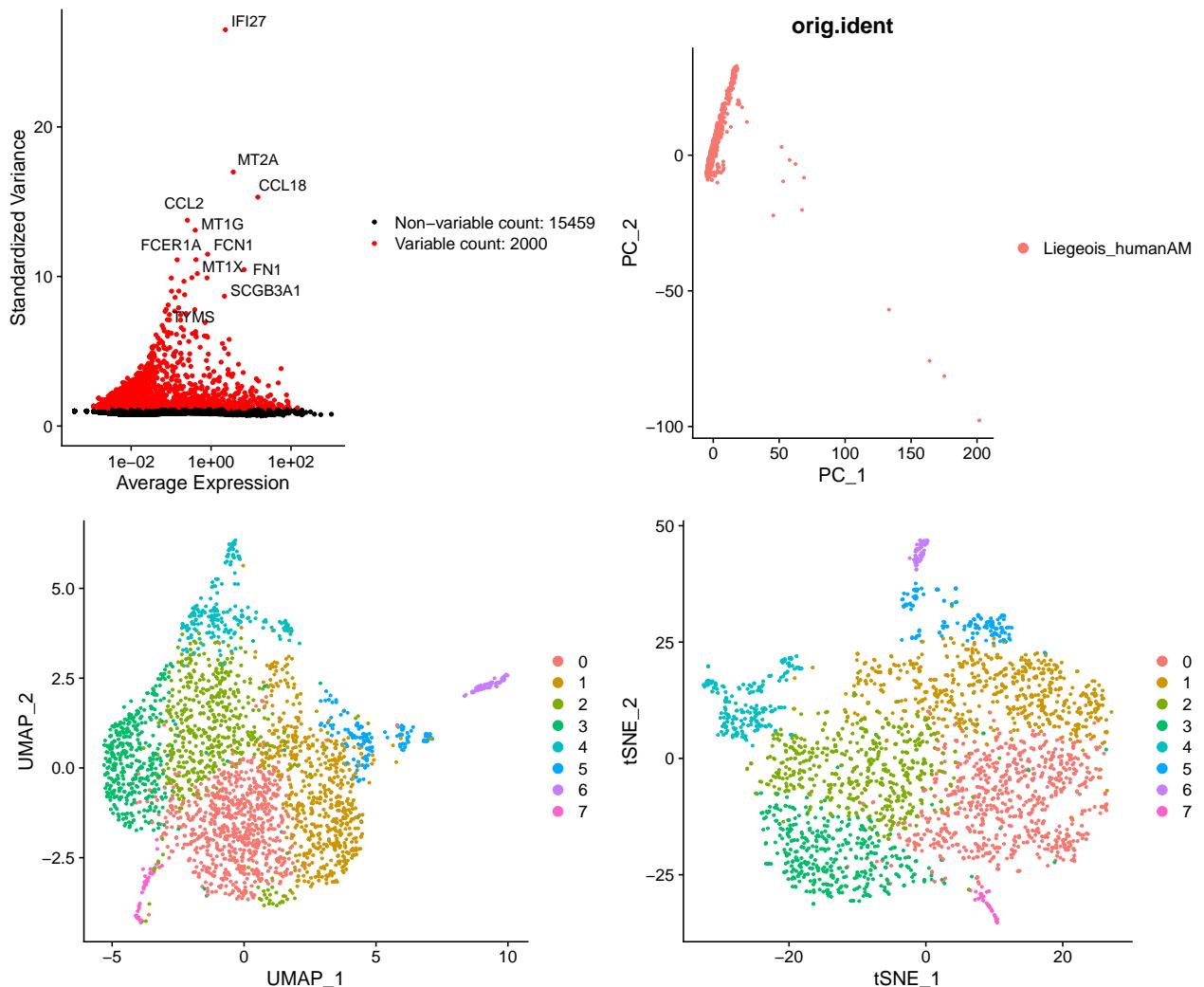


Sample: NGS19_I679_Dim_LBA-Hum4

```

1 ggarrange(NGS19_I679_Dim_LBA_Hum4$plots$variable_features,
2   NGS19_I679_Dim_LBA_Hum4$plots$PCA_plot,
3   NGS19_I679_Dim_LBA_Hum4$plots$UMAP_plot,
4   NGS19_I679_Dim_LBA_Hum4$plots$TSNE_plot, ncol = 2, nrow = 2)

```



3.3 Sample: NGS19-J028_Dim_LBA-Hum5

```

NGS19_J028_Dim_LBA_Hum5 <-
  seurat.setup(path.10x = file.path(dir.10x,
                                     "NGS19-J028_Dim_LBA-Hum5/outs/
                                     filtered_feature_bc_matrix/"),
  project = "Liegeois_humanAM",
  dimensionality = 1:20,
  mt.percentage = 20,
  human = TRUE)

```

```

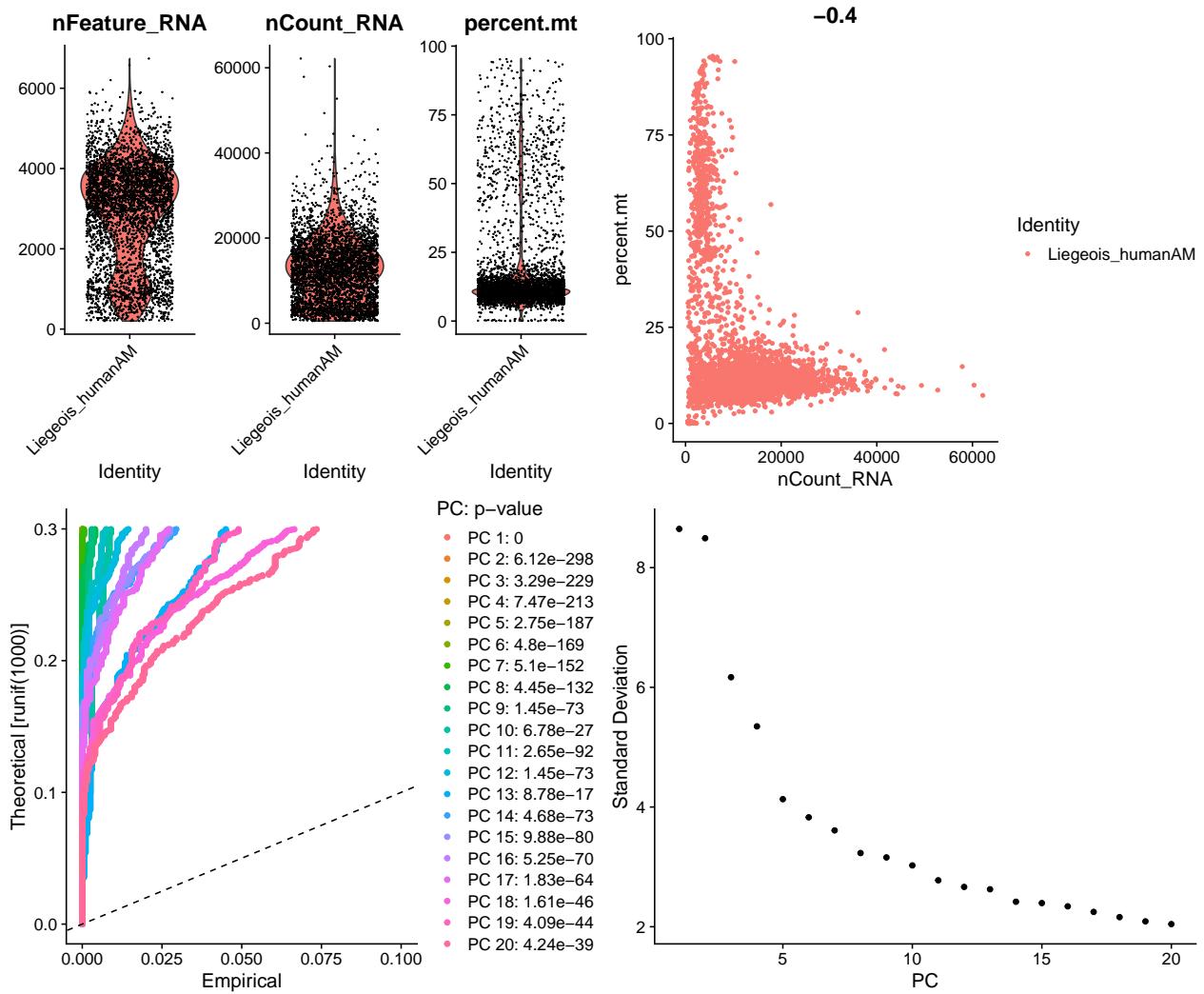
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
## 
## Number of nodes: 3347
## Number of edges: 113156
## 
## Running Louvain algorithm...
## Maximum modularity in 10 random starts: 0.8094
## Number of communities: 8
## Elapsed time: 0 seconds

```

```

1 ggarrange(NGS19_J028_Dim_LBA_Hum5$plots$feature_vln,
2           NGS19_J028_Dim_LBA_Hum5$plots$RNA_mt_pct_scatter,
3           NGS19_J028_Dim_LBA_Hum5$plots$JackStrawPlot,
4           NGS19_J028_Dim_LBA_Hum5$plots$ElbowPlot, ncol = 2, nrow = 2)

```

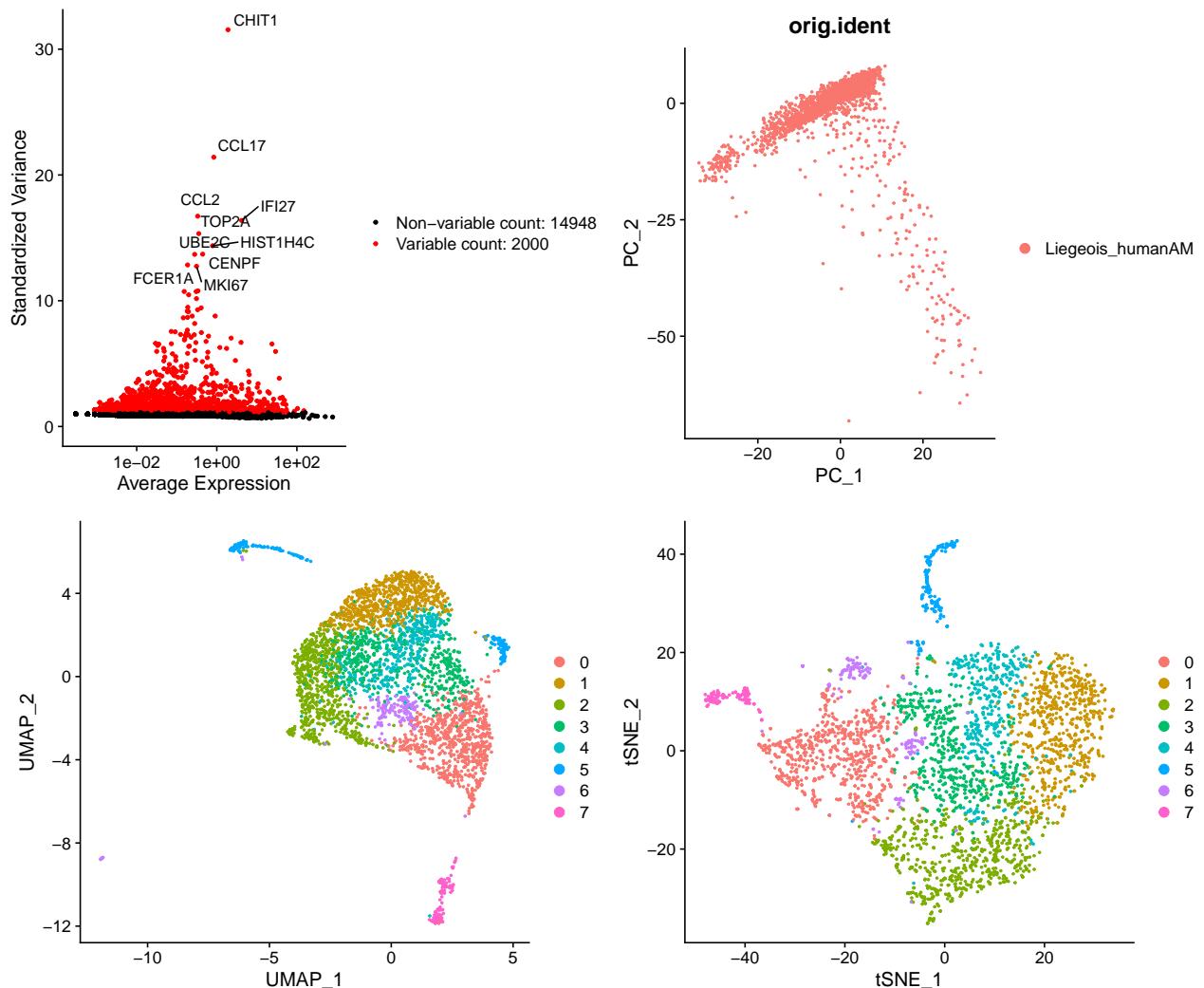


Sample: NGS19_J028_Dim_LBA-Hum5

```

1 ggarrange(NGS19_J028_Dim_LBA_Hum5$plots$variable_features,
2           NGS19_J028_Dim_LBA_Hum5$plots$PCA_plot,
3           NGS19_J028_Dim_LBA_Hum5$plots$UMAP_plot,
4           NGS19_J028_Dim_LBA_Hum5$plots$TSNE_plot, ncol = 2, nrow = 2)

```



3.4 Sample: NGS19-J141_Dim_LBA-Hum7

```

NGS19_J141_Dim_LBA_Hum7 <-
  seurat.setup(path.10x = file.path(dir.10x,
                                     "NGS19-J141_Dim_LBA-Hum7/outs/
                                     filtered_feature_bc_matrix/"),
  project = "Liegeois_humanAM",
  dimensionality = 1:20,
  mt.percentage = 20,
  human = TRUE)

```

```

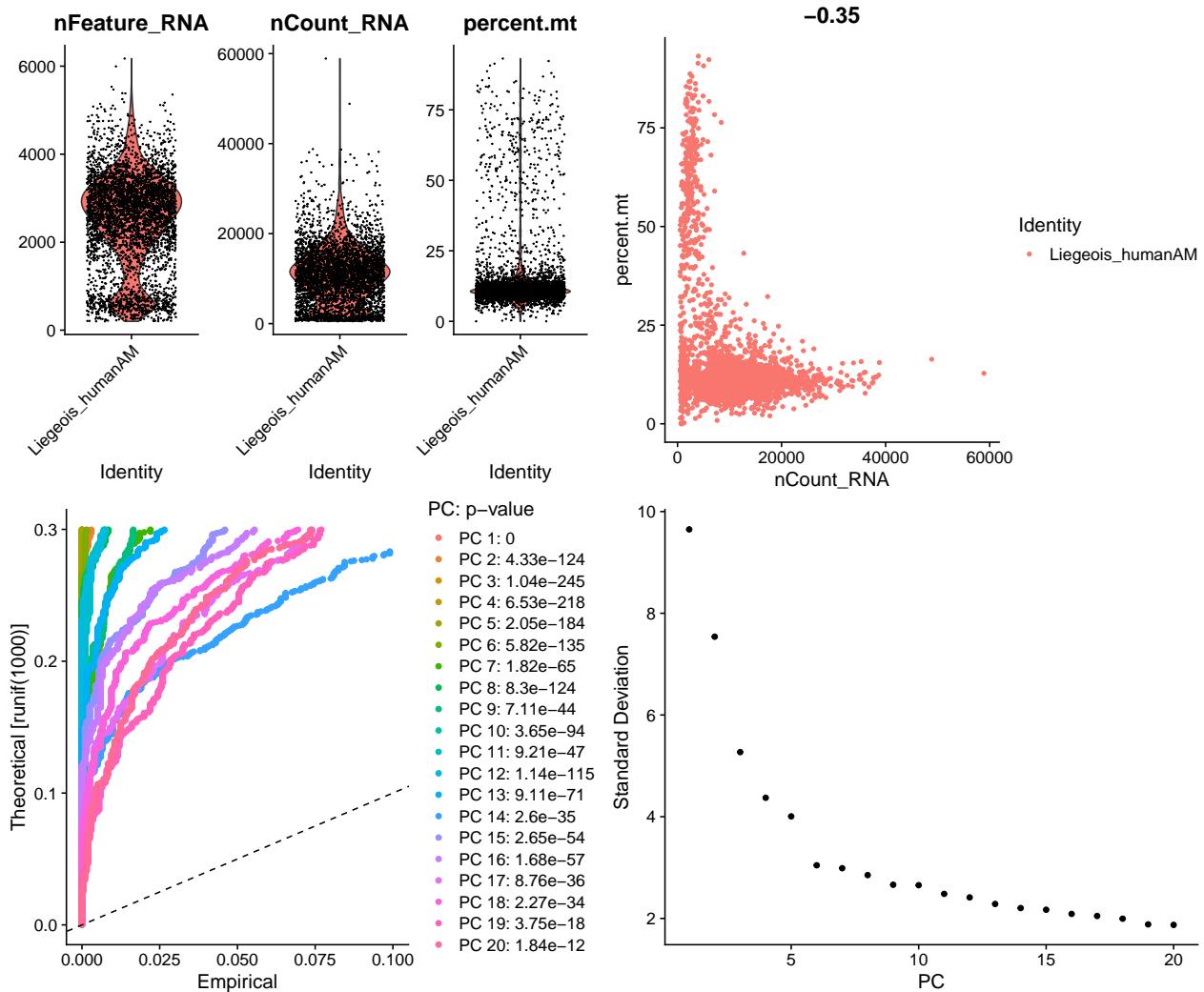
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
##
## Number of nodes: 3701
## Number of edges: 127998
##
## Running Louvain algorithm...
## Maximum modularity in 10 random starts: 0.8104
## Number of communities: 7
## Elapsed time: 0 seconds

```

```

1 ggarrange(NGS19_J141_Dim_LBA_Hum7$plots$feature_vln,
2           NGS19_J141_Dim_LBA_Hum7$plots$RNA_mt_pct_scatter,
3           NGS19_J141_Dim_LBA_Hum7$plots$JackStrawPlot,
4           NGS19_J141_Dim_LBA_Hum7$plots$ElbowPlot, ncol = 2, nrow = 2)

```

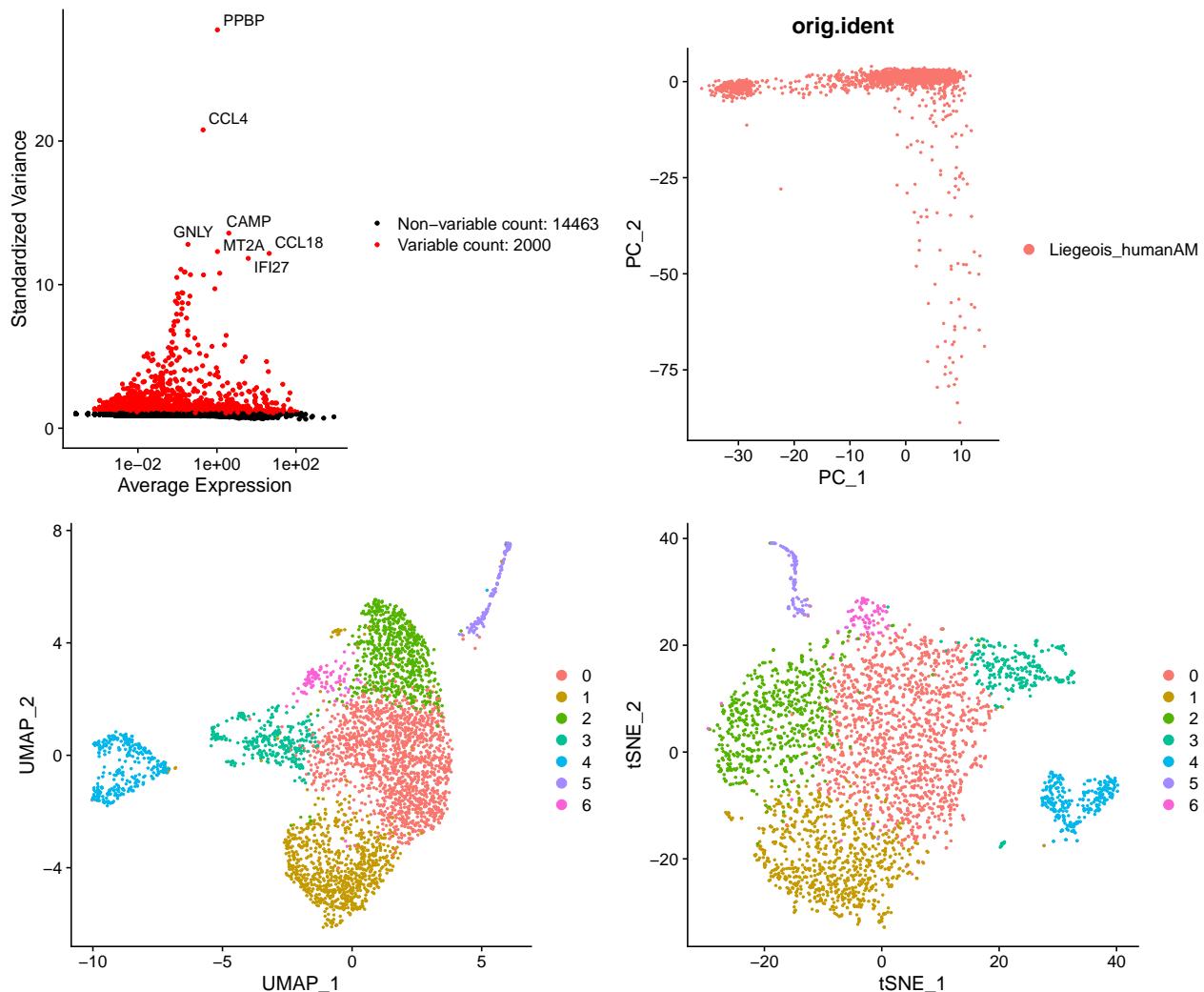


Sample: NGS19_J141_Dim_LBA-Hum7

```

1 ggarrange(NGS19_J141_Dim_LBA_Hum7$plots$variable_features,
2           NGS19_J141_Dim_LBA_Hum7$plots$PCA_plot,
3           NGS19_J141_Dim_LBA_Hum7$plots$UMAP_plot,
4           NGS19_J141_Dim_LBA_Hum7$plots$TSNE_plot, ncol = 2, nrow = 2)

```



3.5 Sample: NGS19-J142_Dim_LBA-Hum8

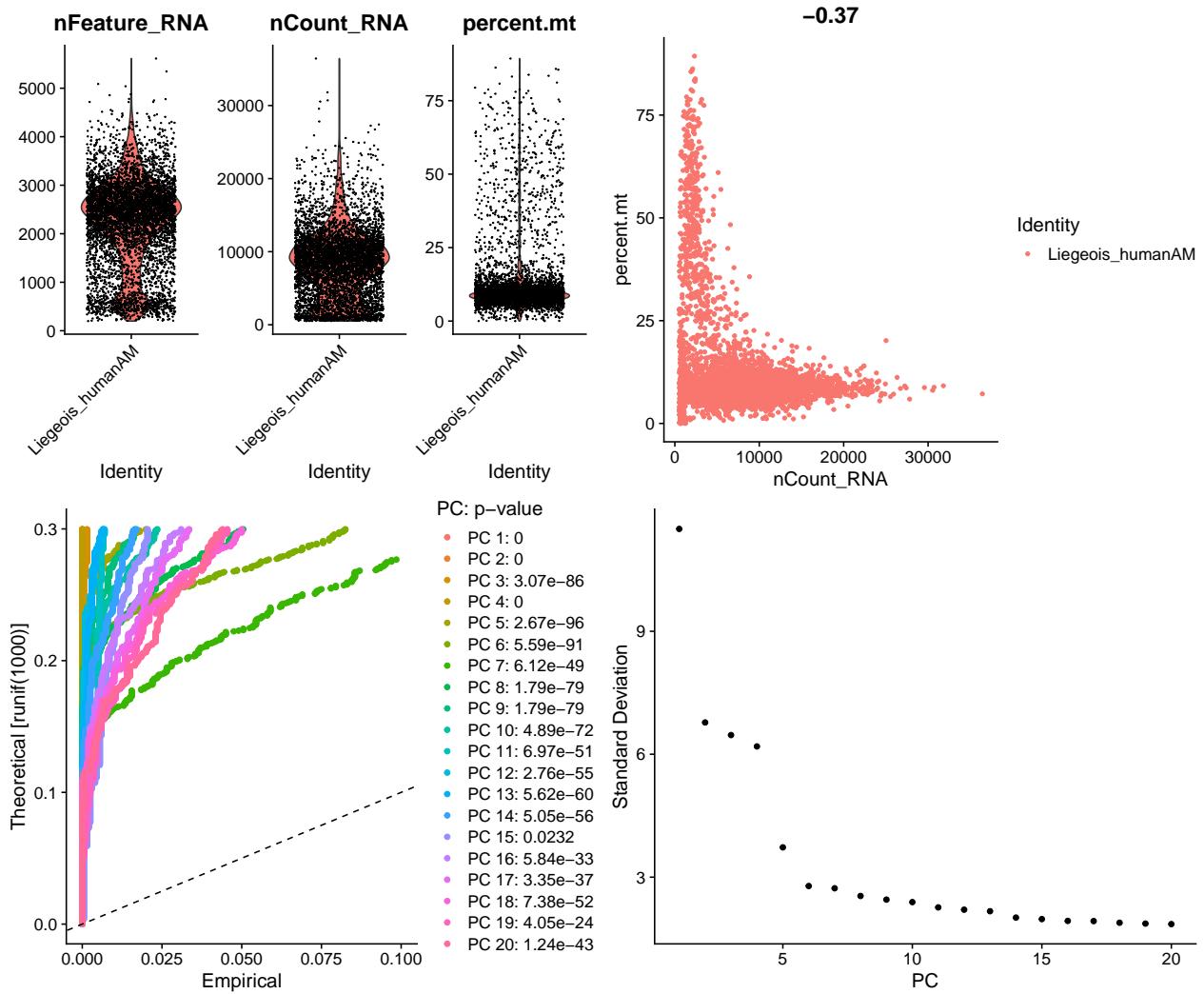
```
NGS19_J142_Dim_LBA_Hum8 <-
  seurat.setup(path.10x = file.path(dir.10x,
                                     "NGS19-J142_Dim_LBA-Hum8/outs/
                                     filtered_feature_bc_matrix/"),
  project = "Liegeois_humanAM",
  dimensionality = 1:20,
  mt.percentage = 20,
  human = TRUE)
```

```
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
## Number of nodes: 4993
## Number of edges: 163832
## Running Louvain algorithm...
## Maximum modularity in 10 random starts: 0.8019
## Number of communities: 10
## Elapsed time: 0 seconds
```

```

1 ggarrange(NGS19_J142_Dim_LBA_Hum8$plots$feature_vln,
2           NGS19_J142_Dim_LBA_Hum8$plots$RNA_mt_pct_scatter,
3           NGS19_J142_Dim_LBA_Hum8$plots$JackStrawPlot,
4           NGS19_J142_Dim_LBA_Hum8$plots$ElbowPlot, ncol = 2, nrow = 2)

```

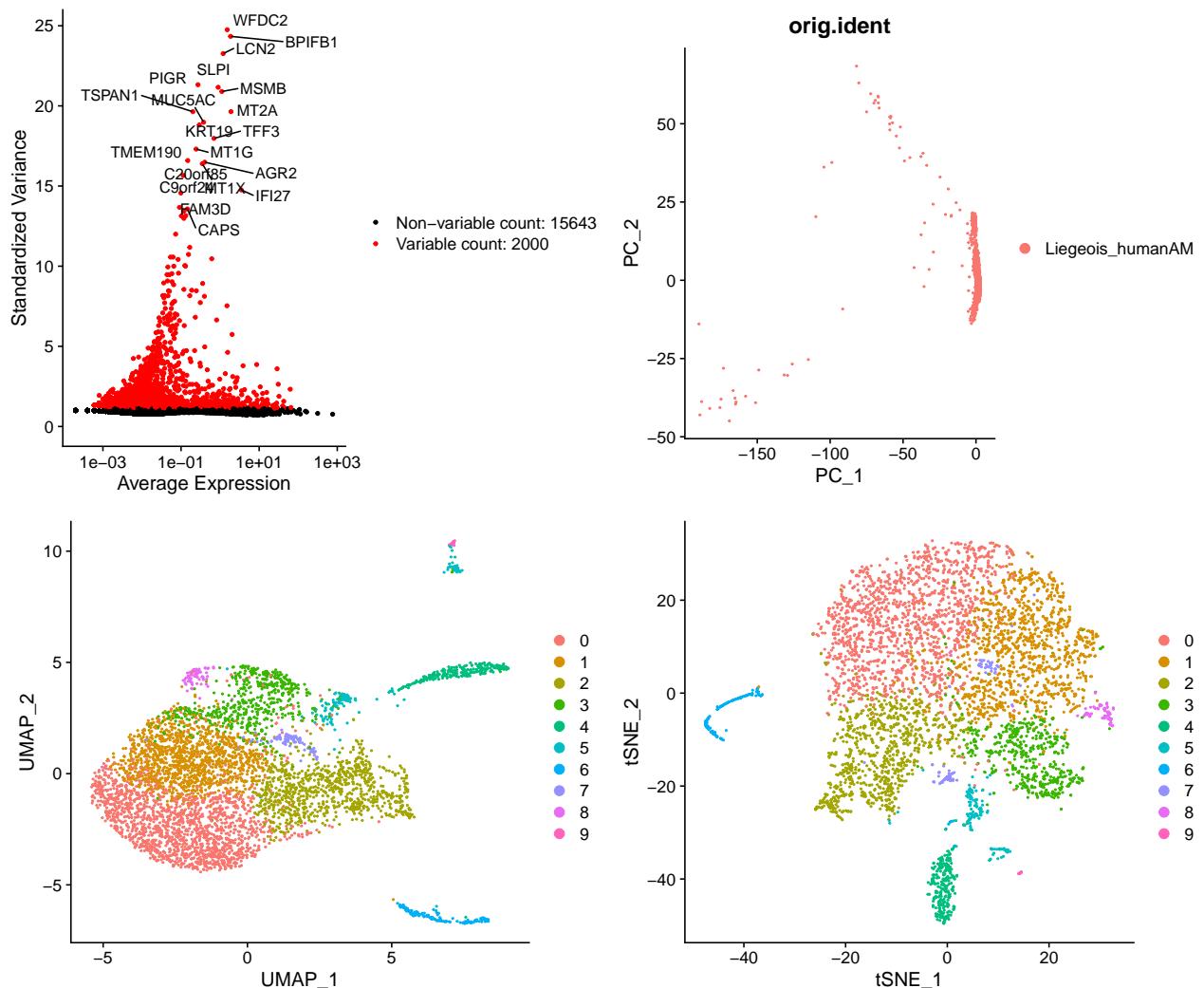


Sample: NGS19_J142_Dim_LBA-Hum8

```

1 ggarrange(NGS19_J142_Dim_LBA_Hum8$plots$variable_features,
2           NGS19_J142_Dim_LBA_Hum8$plots$PCA_plot,
3           NGS19_J142_Dim_LBA_Hum8$plots$UMAP_plot,
4           NGS19_J142_Dim_LBA_Hum8$plots$TSNE_plot, ncol = 2, nrow = 2)

```



3.6 Sample: NGS19-J263_Dim_LBA-Hum9

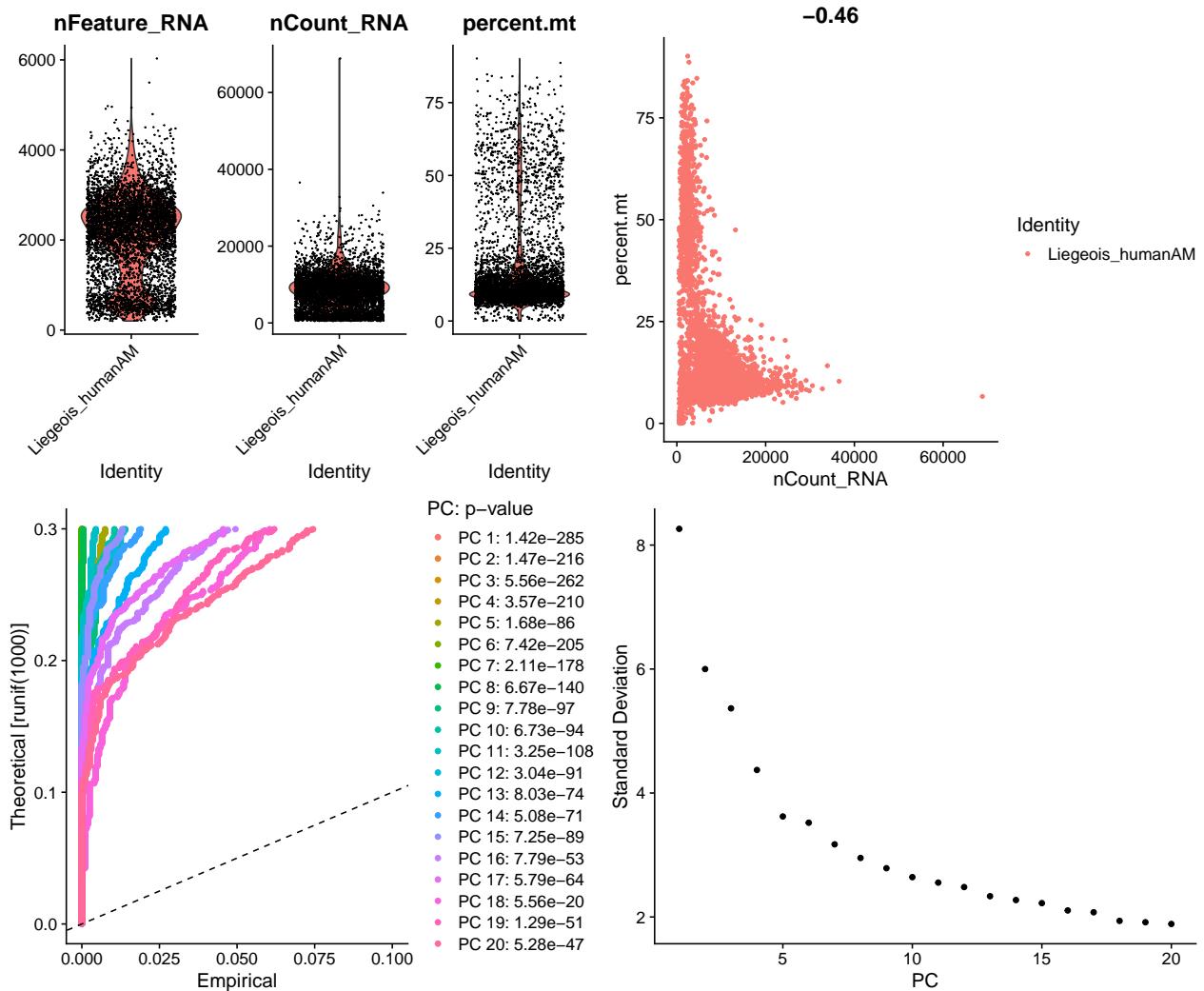
```
NGS19_J263_Dim_LBA_Hum9 <-
  seurat.setup(path.10x = file.path(dir.10x,
                                     "NGS19-J263_Dim_LBA-Hum9/outs/
                                     filtered_feature_bc_matrix/"),
  project = "Liegeois_humanAM",
  dimensionality = 1:20,
  mt.percentage = 20,
  human = TRUE)
```

```
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
## Number of nodes: 4320
## Number of edges: 147473
## Running Louvain algorithm...
## Maximum modularity in 10 random starts: 0.8098
## Number of communities: 9
## Elapsed time: 0 seconds
```

```

1 ggarrange(NGS19_J263_Dim_LBA_Hum9$plots$feature_vln,
2   NGS19_J263_Dim_LBA_Hum9$plots$RNA_mt_pct_scatter,
3   NGS19_J263_Dim_LBA_Hum9$plots$JackStrawPlot,
4   NGS19_J263_Dim_LBA_Hum9$plots$ElbowPlot, ncol = 2, nrow = 2)

```

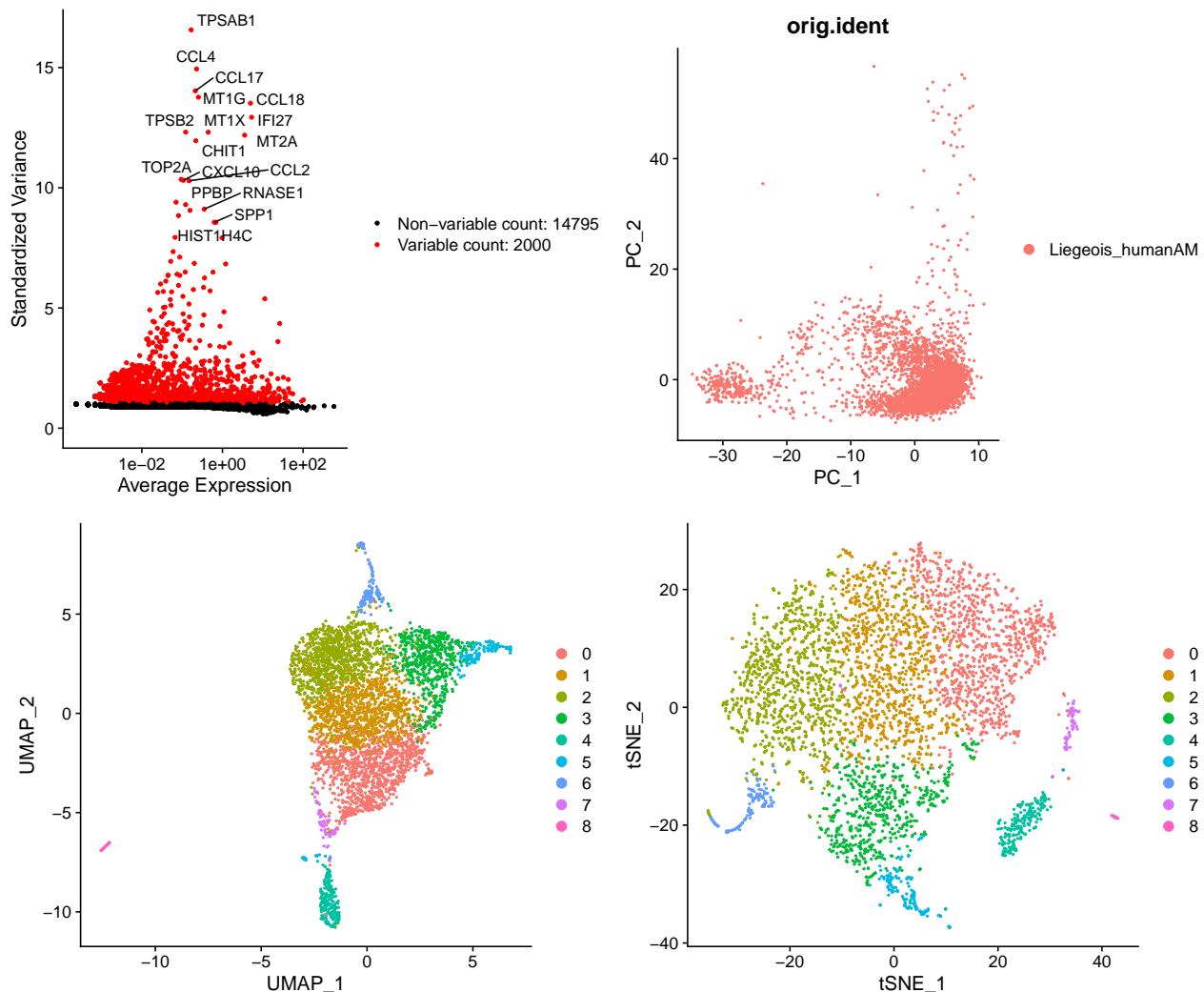


Sample: NGS19_J263_Dim_LBA-Hum9

```

1 ggarrange(NGS19_J263_Dim_LBA_Hum9$plots$variable_features,
2   NGS19_J263_Dim_LBA_Hum9$plots$PCA_plot,
3   NGS19_J263_Dim_LBA_Hum9$plots$UMAP_plot,
4   NGS19_J263_Dim_LBA_Hum9$plots$TSNE_plot, ncol = 2, nrow = 2)

```



Sample: NGS19-J264_Dim_LBA-Hum10

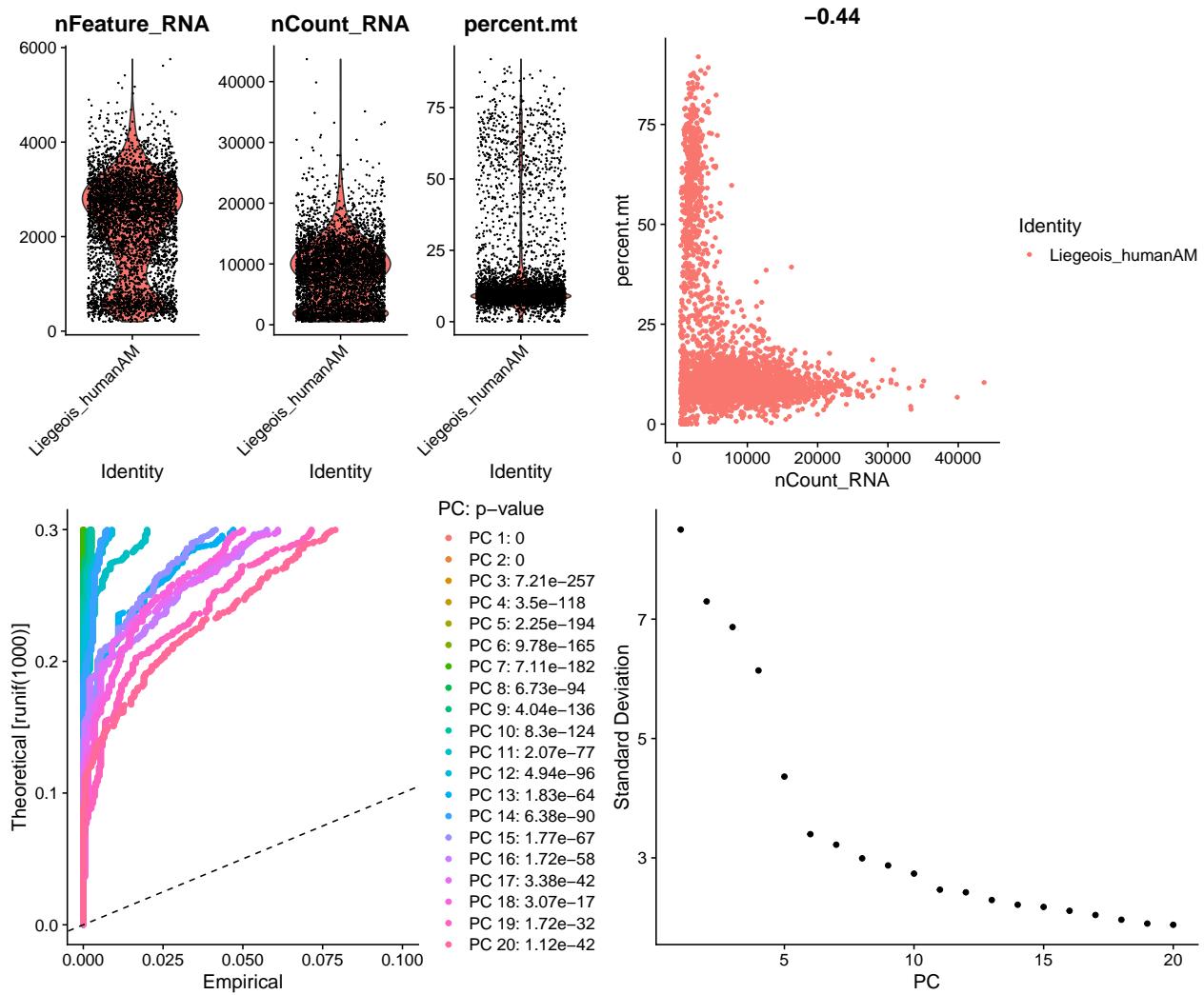
```
1 NGS19_J264_Dim_LBA_Hum10 <-
2   seurat.setup(path.10x = file.path(dir.10x,
3     "NGS19-J264_Dim_LBA-Hum10/outs/
4       filtered_feature_bc_matrix/"),
5     project = "Liegeois_humanAM",
6     dimensionality = 1:20,
7     mt.percentage = 20,
8     human = TRUE)
```

```
1 ## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
2 ##
3 ## Number of nodes: 4156
4 ## Number of edges: 145229
5 ##
6 ## Running Louvain algorithm...
7 ## Maximum modularity in 10 random starts: 0.8515
8 ## Number of communities: 9
9 ## Elapsed time: 0 seconds
```

```

1 ggarrange(NGS19_J264_Dim_LBA_Hum10$plots$feature_vln,
2   NGS19_J264_Dim_LBA_Hum10$plots$RNA_mt_pct_scatter,
3   NGS19_J264_Dim_LBA_Hum10$plots$JackStrawPlot,
4   NGS19_J264_Dim_LBA_Hum10$plots$ElbowPlot, ncol = 2, nrow = 2)

```

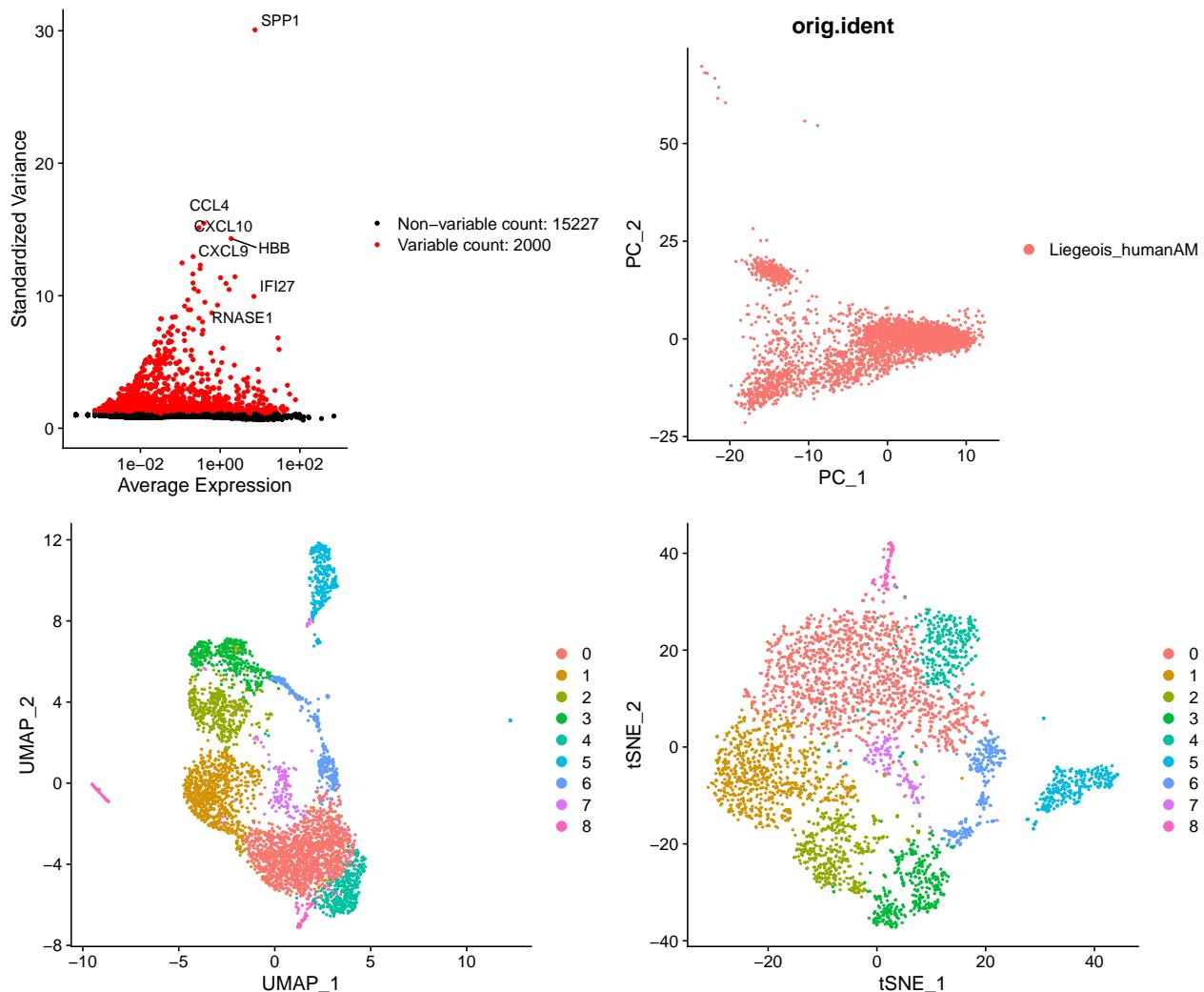


Sample: NGS19-J264_Dim_LBA-Hum10

```

1 ggarrange(NGS19_J264_Dim_LBA_Hum10$plots$variable_features,
2   NGS19_J264_Dim_LBA_Hum10$plots$PCA_plot,
3   NGS19_J264_Dim_LBA_Hum10$plots$UMAP_plot,
4   NGS19_J264_Dim_LBA_Hum10$plots$TSNE_plot, ncol = 2, nrow = 2)

```



3.7 Sample: NGS19-K359_Dim_LBA-Hum11

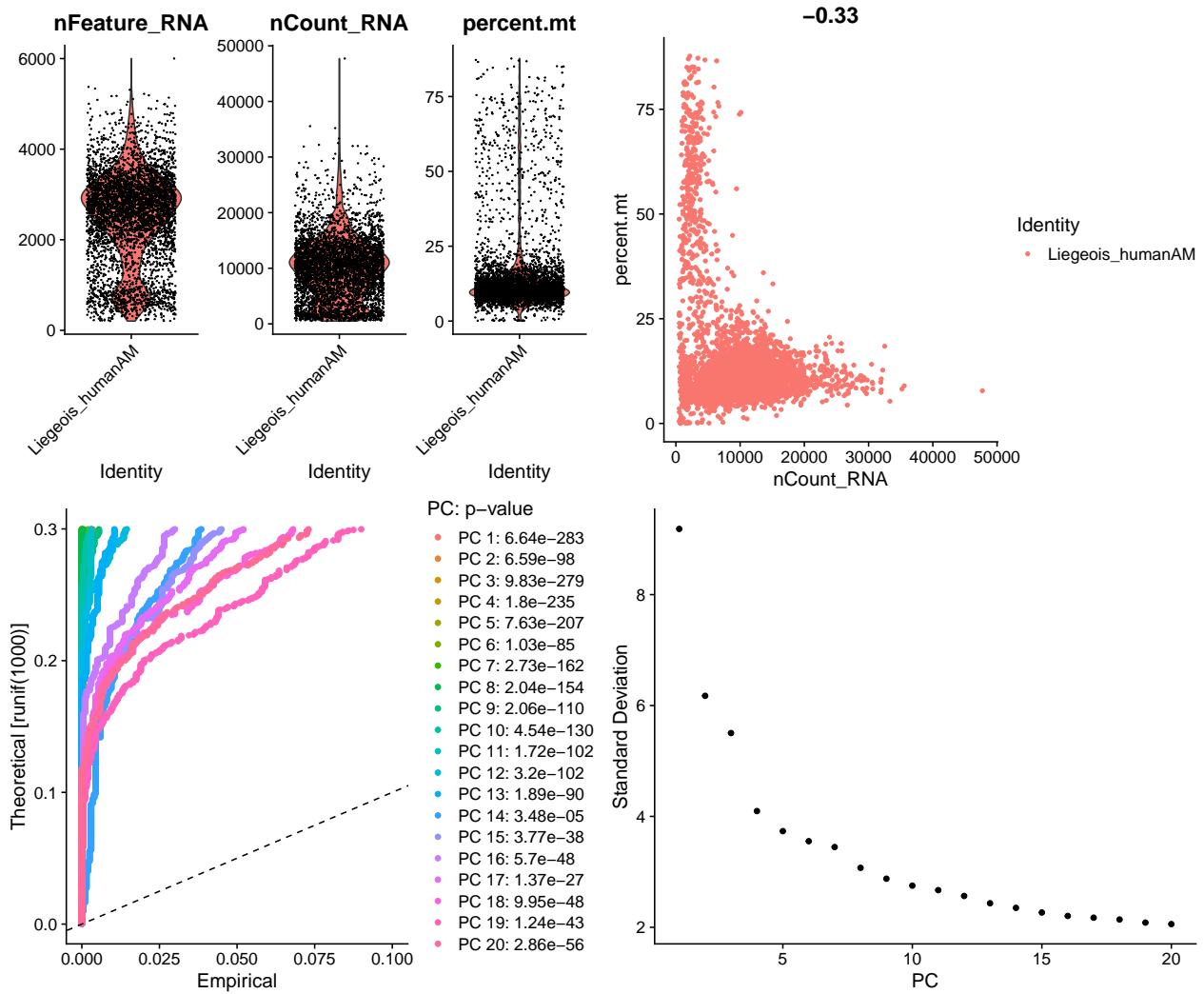
```
NGS19_K359_Dim_LBA_Hum11 <-
  seurat.setup(path.10x = file.path(dir.10x,
                                     "/NGS19-K359_Dim_LBA-Hum11/outs/
                                     filtered_feature_bc_matrix/"),
  project = "Liegeois_humanAM",
  dimensionality = 1:20,
  mt.percentage = 20,
  human = TRUE)
```

```
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
## Number of nodes: 3979
## Number of edges: 129686
## Running Louvain algorithm...
## Maximum modularity in 10 random starts: 0.7931
## Number of communities: 8
## Elapsed time: 0 seconds
```

```

1 ggarrange(NGS19_K359_Dim_LBA_Hum11$plots$feature_vln,
2   NGS19_K359_Dim_LBA_Hum11$plots$RNA_mt_pct_scatter,
3   NGS19_K359_Dim_LBA_Hum11$plots$JackStrawPlot,
4   NGS19_K359_Dim_LBA_Hum11$plots$ElbowPlot, ncol = 2, nrow = 2)

```

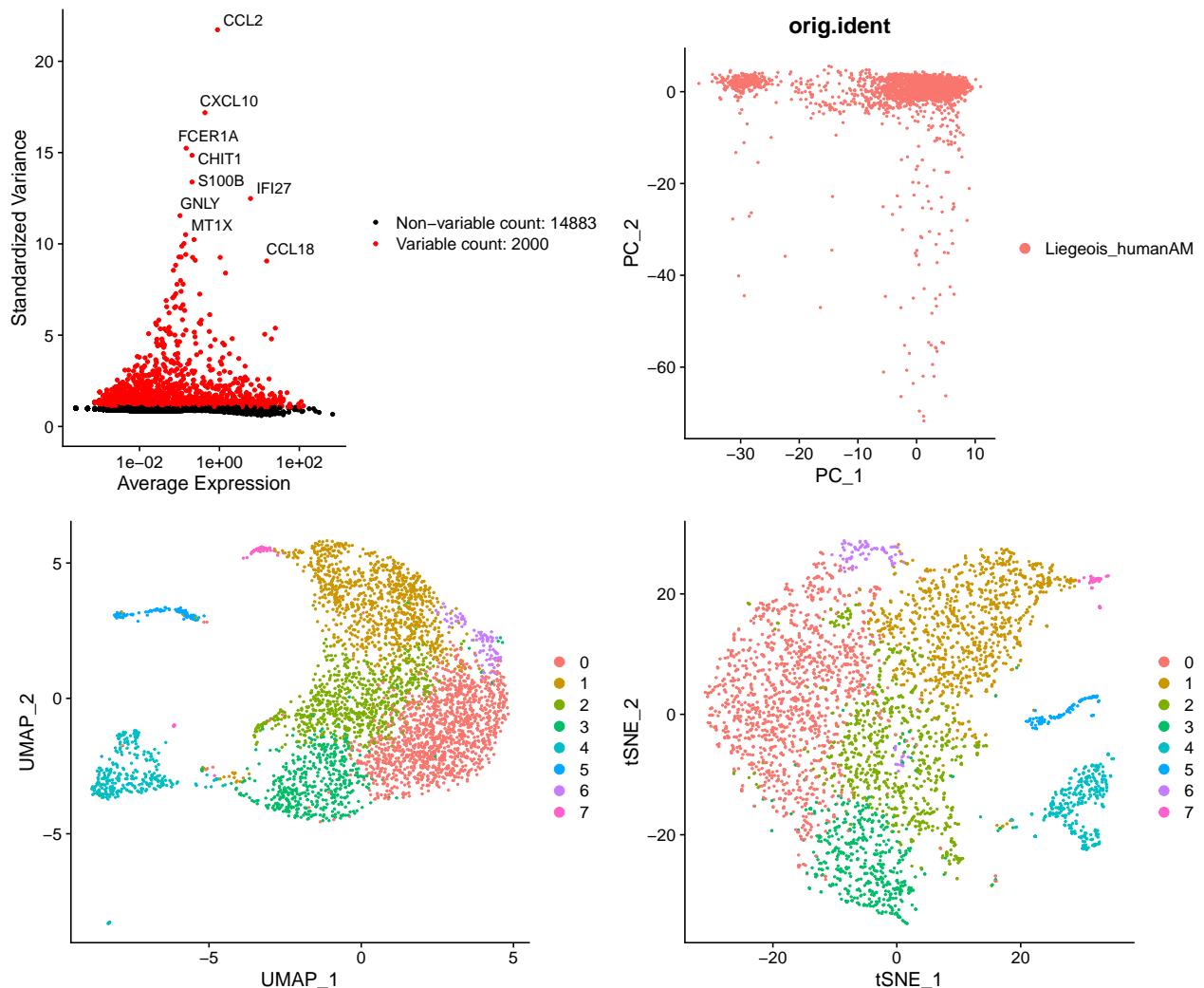


Sample: NGS19-K359_Dim_LBA-Hum11

```

1 ggarrange(NGS19_K359_Dim_LBA_Hum11$plots$variable_features,
2   NGS19_K359_Dim_LBA_Hum11$plots$PCA_plot,
3   NGS19_K359_Dim_LBA_Hum11$plots$UMAP_plot,
4   NGS19_K359_Dim_LBA_Hum11$plots$TSNE_plot, ncol = 2, nrow = 2)

```



3.8 Sample: NGS19-K360_Dim_LBA-Hum12

```

NGS19_K360_Dim_LBA_Hum12 <-
  seurat.setup(path.10x =  file.path(dir.10x,
                                         "NGS19-K360_Dim_LBA-Hum12/outs/
                                             filtered_feature_bc_matrix/"),
                                         project = "Liegeois_humanAM",
                                         dimensionality = 1:20,
                                         mt.percentage = 20,
                                         human = TRUE)

```

```

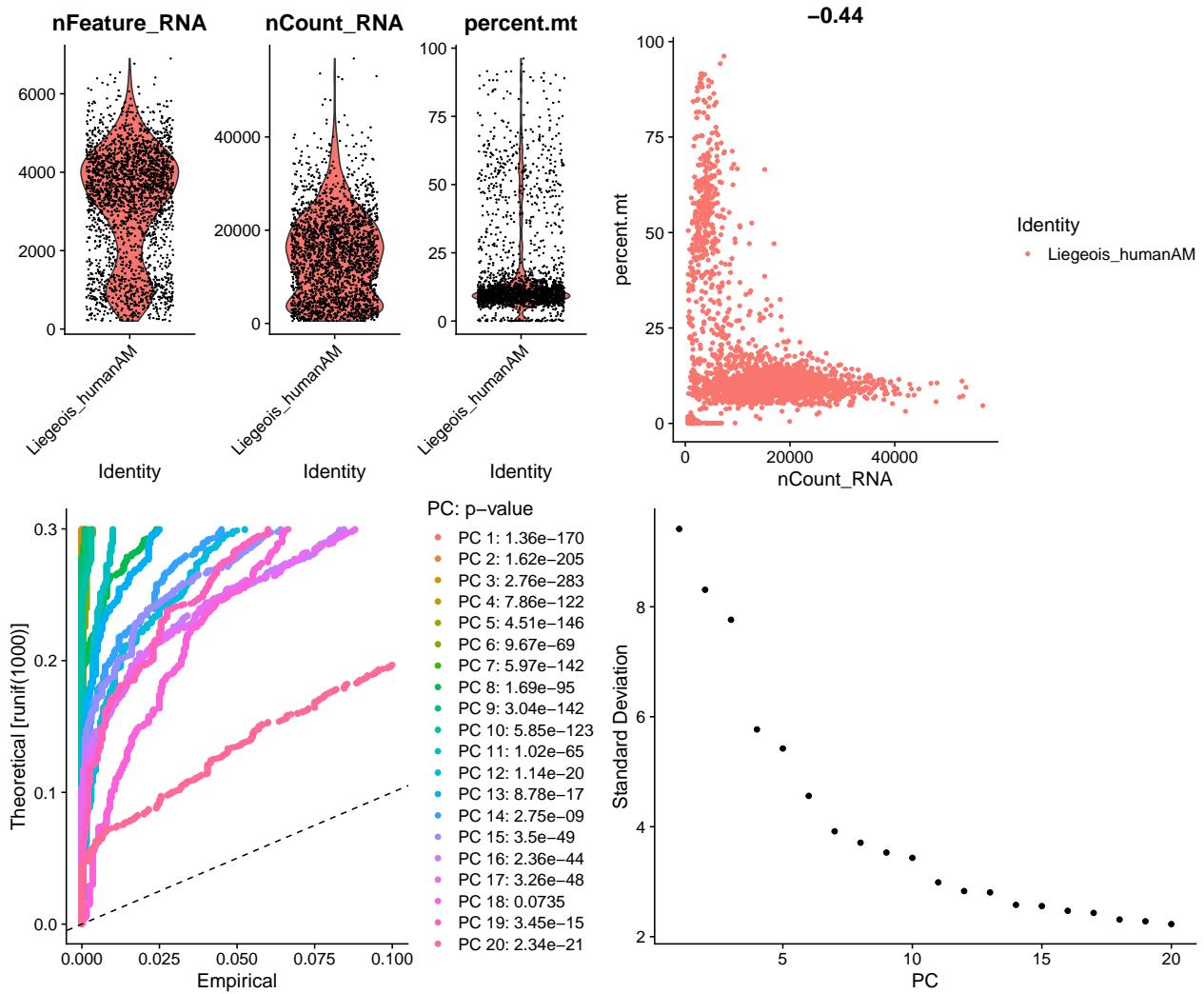
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
## Number of nodes: 1932
## Number of edges: 66552
## Running Louvain algorithm...
## Maximum modularity in 10 random starts: 0.7946
## Number of communities: 8
## Elapsed time: 0 seconds

```

```

1 ggarrange(NGS19_K360_Dim_LBA_Hum12$plots$feature_vln,
2   NGS19_K360_Dim_LBA_Hum12$plots$RNA_mt_pct_scatter,
3   NGS19_K360_Dim_LBA_Hum12$plots$JackStrawPlot,
4   NGS19_K360_Dim_LBA_Hum12$plots$ElbowPlot, ncol = 2, nrow = 2)

```

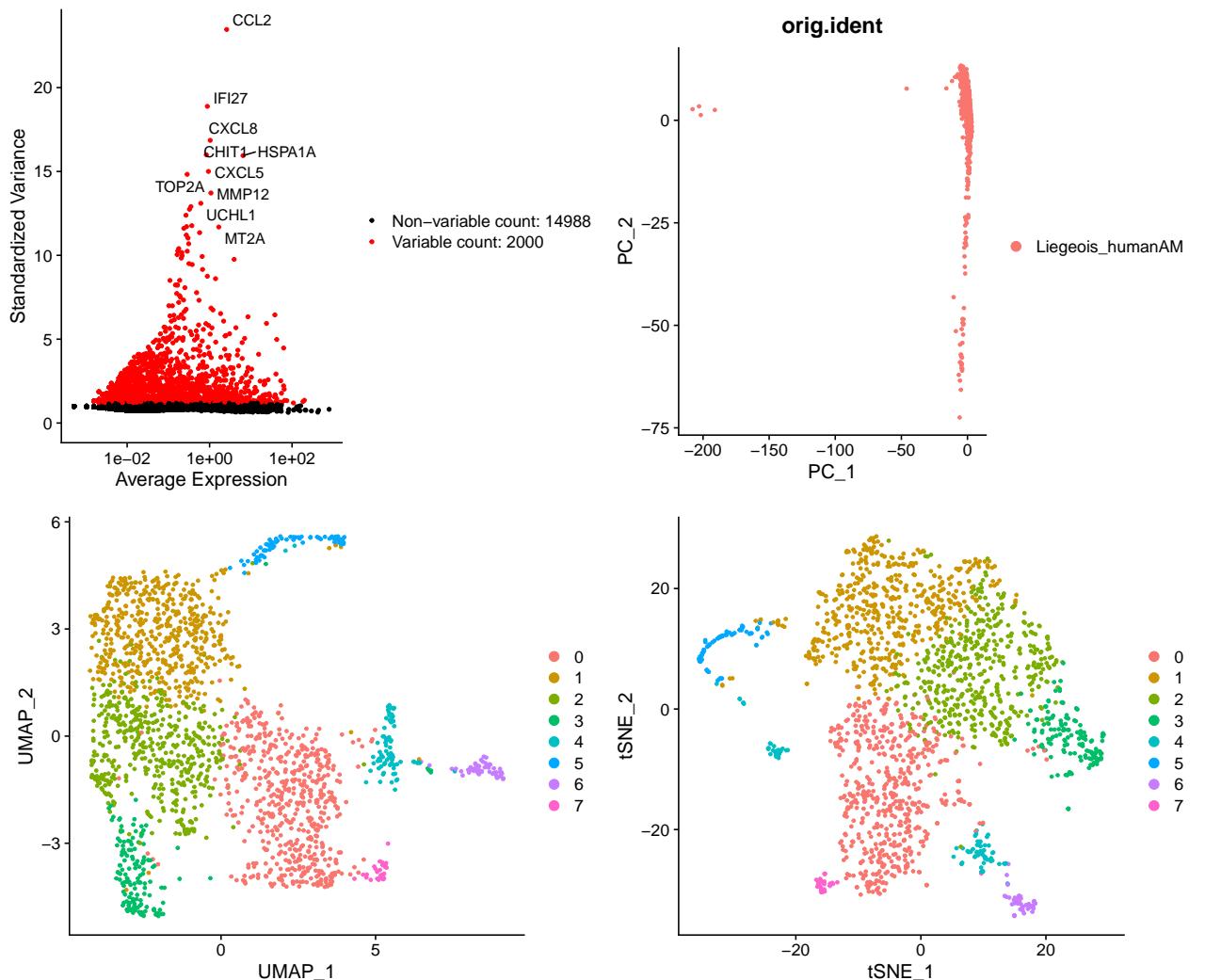


Sample: NGS19-K360_Dim_LBA-Hum12

```

1 ggarrange(NGS19_K360_Dim_LBA_Hum12$plots$variable_features,
2   NGS19_K360_Dim_LBA_Hum12$plots$PCA_plot,
3   NGS19_K360_Dim_LBA_Hum12$plots$UMAP_plot,
4   NGS19_K360_Dim_LBA_Hum12$plots$TSNE_plot, ncol = 2, nrow = 2)

```



4 Save data for other analyses

```

# save data for next use:
1
saveRDS(NGS18_G768_Dim_LBA_Hum1$seuratObject,
2      file = "./NGS18_G768_Dim_LBA_Hum1.seuratObject.rds")
3
saveRDS(NGS18_G904_Dim_LBA_Hum2$seuratObject,
4      file = "./NGS18_G904_Dim_LBA_Hum2.seuratObject.rds")
5
saveRDS(NGS19_I415_Dim_LBA_Hum3$seuratObject,
6      file = "./NGS19_I415_Dim_LBA_Hum3.seuratObject.rds")
7
saveRDS(NGS19_I679_Dim_LBA_Hum4$seuratObject,
8      file = "./NGS19_I679_Dim_LBA_Hum4.seuratObject.rds")
9
saveRDS(NGS19_J028_Dim_LBA_Hum5$seuratObject,
10     file = "./NGS19_J028_Dim_LBA_Hum5.seuratObject.rds")
11
saveRDS(NGS19_J140_Dim_LBA_Hum6$seuratObject,
12     file = "./NGS19_J140_Dim_LBA_Hum6.seuratObject.rds")
13
saveRDS(NGS19_J141_Dim_LBA_Hum7$seuratObject,
14     file = "./NGS19_J141_Dim_LBA_Hum7.seuratObject.rds")
15
saveRDS(NGS19_J142_Dim_LBA_Hum8$seuratObject,
16     file = "./NGS19_J142_Dim_LBA_Hum8.seuratObject.rds")
17
saveRDS(NGS19_J263_Dim_LBA_Hum9$seuratObject,
18

```

```

    file = "./NGS19_J263_Dim_LBA_Hum9.seuratObject.rds") 19
saveRDS(NGS19_J264_Dim_LBA_Hum10$seuratObject, 20
    file = "./NGS19_J264_Dim_LBA_Hum10.seuratObject.rds") 21
saveRDS(NGS19_K359_Dim_LBA_Hum11$seuratObject, 22
    file = "./NGS19_K359_Dim_LBA_Hum11.seuratObject.rds") 23
saveRDS(NGS19_K360_Dim_LBA_Hum12$seuratObject, 24
    file = "./NGS19_K360_Dim_LBA_Hum12.seuratObject.rds") 25

```

5 Session information:

| | |
|---------------|---|
| sessionInfo() | 1 |
|---------------|---|

```

## R version 4.0.3 (2020-10-10) 1
## Platform: x86_64-pc-linux-gnu (64-bit) 2
## Running under: Ubuntu 20.04.3 LTS 3
##
## Matrix products: default 4
## BLAS:    /usr/lib/x86_64-linux-gnu/openblas-pthread/libblas.so.3 5
## LAPACK:  /usr/lib/x86_64-linux-gnu/openblas-pthread/liblapack.so.3 6
##
## locale: 7
##   [1] LC_CTYPE=en_US.UTF-8          LC_NUMERIC=C 10
##   [3] LC_TIME=en_GB.UTF-8         LC_COLLATE=en_US.UTF-8 11
##   [5] LC_MONETARY=en_GB.UTF-8     LC_MESSAGES=en_US.UTF-8 12
##   [7] LC_PAPER=en_GB.UTF-8        LC_NAME=C 13
##   [9] LC_ADDRESS=C                 LC_TELEPHONE=C 14
##  [11] LC_MEASUREMENT=en_GB.UTF-8   LC_IDENTIFICATION=C 15
##
## attached base packages: 16
##   [1] stats      graphics   grDevices utils      datasets  methods   base 18
##
## other attached packages: 19
##   [1] dplyr_1.0.7       ggpubr_0.4.0       ggplot2_3.3.5 20
##   SeuratObject_4.0.2
##   [5] Seurat_4.0.3 21
##
## loaded via a namespace (and not attached): 22
##   [1] Rtsne_0.15        colorspace_2.0-2      ggsignif_0.6.2 23
##   [4] deldir_0.2-10     rio_0.5.27          ellipsis_0.3.2 24
##   [7] ggridges_0.5.3     spatstat.data_2.1-0   farver_2.1.0 25
##  [10] leiden_0.3.9      listenv_0.8.0        ggrepel_0.9.1 26
##  [13] RSpectra_0.16-0    fansi_0.5.0         codetools_0.2-18 27
##  [16] splines_4.0.3      knitr_1.33          polyclip_1.10-0 28
##  [19] jsonlite_1.7.2     broom_0.7.9         ica_1.0-2 29
##  [22] cluster_2.1.0     png_0.1-7          uwot_0.1.10.9000 30
##  [25] shiny_1.6.0        sctransform_0.3.2    spatstat.sparse_2.0-0 31
##  [28] compiler_4.0.3     httr_1.4.2          backports_1.2.1 32
##  [31] assertthat_0.2.1    Matrix_1.3-4        fastmap_1.1.0 33
##  [34] lazyeval_0.2.2      later_1.2.0         htmltools_0.5.1.1 34
##  [37] tools_4.0.3         igraph_1.2.6        gtable_0.3.0 35
##  [40] glue_1.4.2          RANN_2.6.1          reshape2_1.4.4 36
##  [43] Rcpp_1.0.7          carData_3.0-4       scattermore_0.7 37

```

| | | | | | |
|----|-------|----------------------|---------------------|--------------------|----|
| ## | [46] | cellranger_1.1.0 | vctrs_0.3.8 | nlme_3.1-152 | 40 |
| ## | [49] | lmtest_0.9-38 | xfun_0.24 | stringr_1.4.0 | 41 |
| ## | [52] | globals_0.14.0 | openxlsx_4.2.4 | mime_0.11 | 42 |
| ## | [55] | miniUI_0.1.1.1 | lifecycle_1.0.0 | irlba_2.3.3 | 43 |
| ## | [58] | rstatix_0.7.0 | goftest_1.2-2 | future_1.21.0 | 44 |
| ## | [61] | MASS_7.3-53 | zoo_1.8-9 | scales_1.1.1 | 45 |
| ## | [64] | spatstat.core_2.3-0 | hms_1.1.0 | promises_1.2.0.1 | 46 |
| ## | [67] | spatstat.utils_2.2-0 | parallel_4.0.3 | RColorBrewer_1.1-2 | 47 |
| ## | [70] | curl_4.3.2 | yaml_2.2.1 | reticulate_1.20 | 48 |
| ## | [73] | pbapply_1.4-3 | gridExtra_2.3 | rpart_4.1-15 | 49 |
| ## | [76] | stringi_1.7.3 | highr_0.9 | zip_2.2.0 | 50 |
| ## | [79] | rlang_0.4.11 | pkgconfig_2.0.3 | matrixStats_0.60.0 | 51 |
| ## | [82] | evaluate_0.14 | lattice_0.20-41 | ROCR_1.0-11 | 52 |
| ## | [85] | purrr_0.3.4 | tensor_1.5 | labeling_0.4.2 | 53 |
| ## | [88] | patchwork_1.1.1 | htmlwidgets_1.5.3 | cowplot_1.1.1 | 54 |
| ## | [91] | tidyselect_1.1.1 | parallelly_1.27.0 | RcppAnnoy_0.0.19 | 55 |
| ## | [94] | plyr_1.8.6 | magrittr_2.0.1 | R6_2.5.0 | 56 |
| ## | [97] | generics_0.1.0 | DBI_1.1.1 | haven_2.4.3 | 57 |
| ## | [100] | foreign_0.8-81 | pillar_1.6.2 | withr_2.4.2 | 58 |
| ## | [103] | mgcv_1.8-33 | fitdistrplus_1.1-5 | survival_3.2-7 | 59 |
| ## | [106] | abind_1.4-5 | tibble_3.1.3 | future.apply_1.7.0 | 60 |
| ## | [109] | crayon_1.4.1 | car_3.0-11 | 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 | readxl_1.3.1 | grid_4.0.3 | 63 |
| ## | [118] | data.table_1.14.0 | forcats_0.5.1 | digest_0.6.27 | 64 |
| ## | [121] | xtable_1.8-4 | tidyR_1.1.3 | httpuv_1.6.1 | 65 |
| ## | [124] | munsell_0.5.0 | viridisLite_0.4.0 | | 66 |

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