

```
In [1]:
library(devtools)
library(dplyr)
library(future)
library(Seurat)

Loading required package: usethis

Attaching package: 'dplyr'

The following objects are masked from 'package:stats':
  filter, lag

The following objects are masked from 'package:base':
  intersect, setdiff, setequal, union

Attaching SeuratObject
```

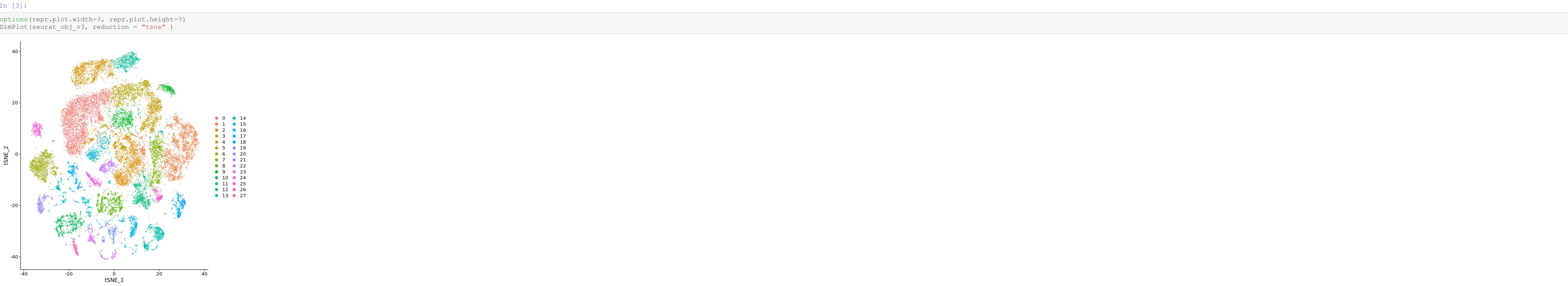
```
In [2]:
load("DSClusters.Robj")
seurat_obj_v3 ~ UpdateSeuratObject(seurat.obj)

seurat_obj_v3 ~ Seurat::NormalizeData(object=seurat_obj_v3) %>%
  FindVariableFeatures() %>%
  ScaleData() %>%
  RunPCA(verbose=F) %>%
  FindNeighbors(dims = 1:30) %>%
  FindClusters(resolution = 0.6) %>%
  RunTSNE(dims = 1:30,verbose=F)

Updating from v2.X to v3.X
Validating object structure
Updating object slots
Ensuring keys are in the proper structure
Ensuring feature names don't have underscores or pipes
Object representation is consistent with the most current Seurat version
Centering and scaling data matrix
Computing nearest neighbor graph
Computing SNN

Modularity Optimizer version 1.3.0 by Edo Maltman and Nees Jan van Eck
Number of nodes: 35235
Number of edges: 1415522

Running Louvain algorithm...
Maximum modularity in 10 random starts: 0.9282
Number of communities: 28
Elapsed time: 7 seconds
```

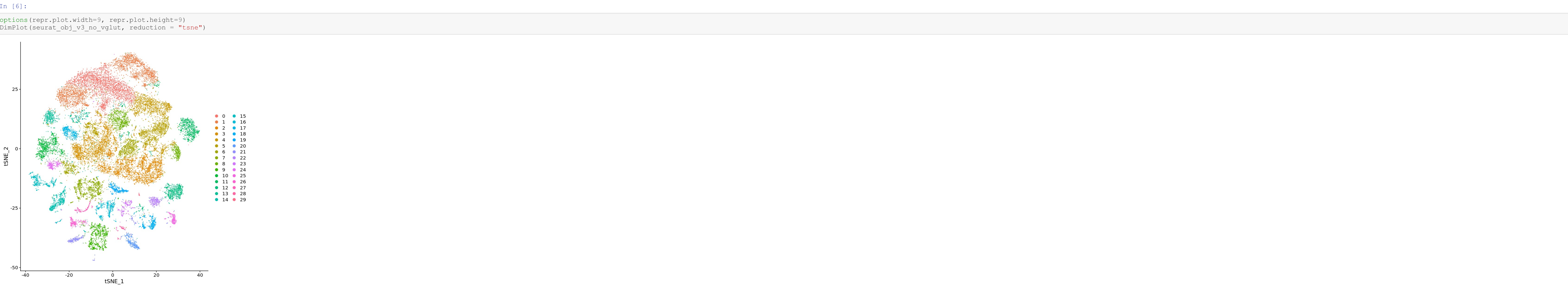


```
In [4]:
seurat_obj_v3_no_vglut ~ seurat_obj_v3[, as.numeric(seurat_obj_v3$RNA["Sic17a7", ]) == 0]
seurat_obj_v3_no_vglut ~ Seurat::NormalizeData(object=seurat_obj_v3_no_vglut) %>%
  FindVariableFeatures() %>%
  ScaleData() %>%
  RunPCA(verbose=F) %>%
  FindNeighbors(dims = 1:30) %>%
  FindClusters(resolution = 0.6) %>%
  RunTSNE(dims = 1:30,verbose=F)

Centering and scaling data matrix
Computing nearest neighbor graph
Computing SNN

Modularity Optimizer version 1.3.0 by Edo Maltman and Nees Jan van Eck
Number of nodes: 32449
Number of edges: 1310672

Running Louvain algorithm...
Maximum modularity in 10 random starts: 0.9240
Number of communities: 30
Elapsed time: 6 seconds
```



```
In [15]:
# clusterLabel is called 'RNA_snn_res.0.6'

# Number of cells?
print(paste0("Number of Cells Not Expressing Sic17a7: ",
  ncol(seurat_obj_v3_no_vglut)))

# How many clusters?
print(paste0("Number of Clusters For Cells Not Expressing Sic17a7: ",
  length(unique(seurat_obj_v3_no_vglut$RNA_snn_res.0.6))))

# Number of NeuN+ vs. NeuN-?
print(paste0("Number of NeuN+ Cells Not Expressing Sic17a7: ",
  sum(seurat_obj_v3_no_vglut$RNA["Mnfox3", ] > 0)))

print(paste0("Number of NeuN- Cells Not Expressing Sic17a7: ",
  sum(seurat_obj_v3_no_vglut$RNA["Mnfox3", ] == 0)))

# Number of cells that express glial markers
# AQP4 and GFAP are two common Astrocytic markers
# C1qa and Sall1 are Microglial markers
# Olig2 is an OPC marker
# MOG and Olig1 are Oligodendrocyte markers

print(paste0("Number of GFAP+ Cells Not Expressing Sic17a7: ",
  sum(seurat_obj_v3_no_vglut$RNA["Gfap", ] > 0)))

print(paste0("Number of AQP4+ Cells Not Expressing Sic17a7: ",
  sum(seurat_obj_v3_no_vglut$RNA["Aqp4", ] > 0)))

print(paste0("Number of C1qa+ Cells Not Expressing Sic17a7: ",
  sum(seurat_obj_v3_no_vglut$RNA["C1qa", ] > 0)))

print(paste0("Number of SALL1+ Cells Not Expressing Sic17a7: ",
  sum(seurat_obj_v3_no_vglut$RNA["Sall1", ] > 0)))

print(paste0("Number of OLIG2+ Cells Not Expressing Sic17a7: ",
  sum(seurat_obj_v3_no_vglut$RNA["Olig2", ] > 0)))

print(paste0("Number of OLIG1+ Cells Not Expressing Sic17a7: ",
  sum(seurat_obj_v3_no_vglut$RNA["Olig1", ] > 0)))

print(paste0("Number of MOG+ Cells Not Expressing Sic17a7: ",
  sum(seurat_obj_v3_no_vglut$RNA["Mog", ] > 0)))

# Number of cells that express our neuropeptides of interest (Sst, Pdyn, etc.)
print(paste0("Number of SST+ Cells Not Expressing Sic17a7: ",
  sum(seurat_obj_v3_no_vglut$RNA["Sst", ] > 0)))

print(paste0("Number of VIP+ Cells Not Expressing Sic17a7: ",
  sum(seurat_obj_v3_no_vglut$RNA["Vip", ] > 0)))

print(paste0("Number of PDYN+ Cells Not Expressing Sic17a7: ",
  sum(seurat_obj_v3_no_vglut$RNA["Pdyn", ] > 0)))

[[1] "Number of Cells Not Expressing Sic17a7: 32449"
[1] "Number of Clusters For Cells Not Expressing Sic17a7: 30"
[1] "Number of NeuN+ Cells Not Expressing Sic17a7: 1454"
[1] "Number of NeuN- Cells Not Expressing Sic17a7: 17904"
[1] "Number of GFAP+ Cells Not Expressing Sic17a7: 553"
[1] "Number of AQP4+ Cells Not Expressing Sic17a7: 669"
[1] "Number of C1QA+ Cells Not Expressing Sic17a7: 331"
[1] "Number of SALL1+ Cells Not Expressing Sic17a7: 431"
[1] "Number of OLIG2+ Cells Not Expressing Sic17a7: 626"
[1] "Number of OLIG1+ Cells Not Expressing Sic17a7: 1289"
[1] "Number of MOG+ Cells Not Expressing Sic17a7: 1503"
[1] "Number of SST+ Cells Not Expressing Sic17a7: 4419"
[1] "Number of VIP+ Cells Not Expressing Sic17a7: 161"
[1] "Number of PDYN+ Cells Not Expressing Sic17a7: 312"
```

```
In [17]:
# Top/unique genes to each cluster (violin plots for each cluster)

# Instead of finding 'unique' genes for each cluster (which could be due to noise),
# instead find significantly upregulated genes in each cluster

# save as a csv
plan(strategy = "multicore", workers = 12)

# Find all markers; adjust parameters as needed
# This can take a few minutes...
latsep_markers ~ FindAllMarkers(
  seurat_obj_v3_no_vglut,
  only.pos=TRUE)

# save latsep_markers as a csv
write.csv(latsep_markers, file="/mnt/accessory/seq_data/latsep/latsep_markers_no_vglut.csv")

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
Calculating cluster 11
Calculating cluster 12
```

Calculating cluster 13  
Calculating cluster 14  
Calculating cluster 15  
Calculating cluster 16  
Calculating cluster 17  
Calculating cluster 18  
Calculating cluster 19  
Calculating cluster 20  
Calculating cluster 21  
Calculating cluster 22  
Calculating cluster 23  
Calculating cluster 24  
Calculating cluster 25  
Calculating cluster 26  
Calculating cluster 27  
Calculating cluster 28  
Calculating cluster 29

```
In [10]:
sessionInfo()

R version 4.0.4 (2021-02-15)
Platform: x86_64-pc-linux-gnu (64-bit)
Running under: Debian GNU/Linux 11 (bullseye)

Matrix products: default
BLAS:   /usr/lib/x86_64-linux-gnu/blas/libblas.so.3.9.0
LAPACK: /usr/lib/x86_64-linux-gnu/lapack/liblapack.so.3.9.0

locale:
 [1] LC_CTYPE=C.UTF-8      LC_NUMERIC=C          LC_TIME=C.UTF-8
 [4] LC_COLLATE=C.UTF-8   LC_MONETARY=C.UTF-8  LC_MESSAGES=C.UTF-8
 [7] LC_PAPER=C.UTF-8     LC_NAME=C             LC_ADDRESS=C
[10] LC_TELEPHONE=C       LC_MEASUREMENT=C.UTF-8 LC_IDENTIFICATION=C

attached base packages:
[1] stats      graphics  grDevices  utils      datasets  methods   base

other attached packages:
[1] SeuratObject_4.1.4 Seurat_4.4.0      future_1.33.0     dplyr_1.1.4
[5] devtools_2.4.5    usethis_2.2.2

loaded via a namespace (and not attached):
 [1] Rtsne_0.17          colorspace_2.1-0    deldir_2.0-2
 [4] ellipsis_0.3.2      ggridge_0.5.4       IRdisplay_1.1
 [7] base64enc_0.1-3     fs_1.6.3            spatstat.data_3.0-3
[10] farver_2.1.1        leiden_0.4.3.1      listenv_0.9.0
[13] remotes_2.4.2.1     ggrepel_0.9.5       fansi_1.0.6
[16] codetools_0.2-19    splines_4.0.4       cachem_1.0.8
[19] plyr_1.8.4          pkgload_1.3.3       IRkernel_1.3.2.9000
[22] jsonlite_1.8.7      ica_1.0-3           cluster_2.1.6
[25] png_0.1-8           uwot_0.1-16         spatstat.sparse_3.0-3
[28] shiny_1.8.0         sctransform_0.4.1   compiler_4.0.4
[31] httr_1.4.7          Matrix_1.6-4        fastmap_1.1.1
[34] lazyeval_0.2.2      cli_3.6.2           later_1.3.2
[37] htmltools_0.5.7     tools_4.0.4         igraph_1.6.0
[40] grable_0.3.4        glue_1.7.0          RANN_2.6.1
[43] reshape2_1.4.4      Rcpp_1.0.12         scattermore_1.2
[46] vctrs_0.6.5         nlme_3.1-164        spatstat.explore_3.2-5
[49] progress_0.14.0     lme4_0.9-40         spatstat.random_3.2-2
[52] strings_1.5.1       lifecycle_1.0.4     mime_0.12
[55] miniUI_0.1.1.1      lifecycle_1.0.4     iriba_2.3.5.1
[58] gertstat_1.1-3      MASS_1.3-60         zoo_1.8-12
[61] scales_1.3.0        promises_1.2.1      spatstat.utils_3.0-4
[64] parallel_4.0.4      RColorBrewer_1.1-3  memoise_2.0.1
[67] rstatistx_1.34.0    pheatmap_1.7-2      gridExtra_2.3
[70] ggplot2_3.4.4        stringi_1.8.3       pkbuild_1.4.3
[73] repr_1.1.4          rlang_1.1.3         pkgconfig_2.0.3
[76] matrixStats_1.2.0   evaluate_0.23       lattice_0.22-5
[79] tensor_1.5          ROCR_1.0-11         purrr_1.0.2
[82] labeling_0.4.3      patchwork_1.2.0     htmlwidgets_1.6.4
[85] cowplot_1.1.2       tidyselect_1.2.0    parallelly_1.36.0
[88] RcppAnnoy_0.0.21    plyr_1.8.9          magrittr_2.0.3
[91] R6_2.5.1            generics_0.1.3      profvis_0.3.8
[94] pbdZMQ_0.3-10       withr_3.0.0         pillar_1.9.0
[97] fitdistrplus_1.1-11 ahind_1.4-5         survival_3.5-7
[100] sp_2.1-2            tidbits_3.2.1       future.apply_1.11.0
[103] crayon_1.5.2        uuid_1.1-1          KernSmooth_2.23-22
[106] utf8_1.2.4          spatstat.geom_3.2-7 plotly_4.10.3
[109] urlchecker_1.0.1    grid_4.0.4         data.table_1.14.10
[112] digest_0.6.33       xtable_1.8-4        tidyr_1.3.0
[115] httpuv_1.6.13       munsell_0.5.0       viridisLite_0.4.2
[118] sessioninfo_1.2.2
```