

```
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("nccClusters.Robj")
seurat_obj_v3 = UpdateSeuratObject(seurat_obj)
seurat_obj_v3 = Seurat::NormalizeData(object=seurat_obj_v3) %>%
  FindVariableFeatures() %>%
  ScaleData() %>%
  RunPCA(verbose=F) %>%
  FindNeighbors(dim = 1:30) %>%
  FindClusters(resolution = 0.6) %>%
  RunTSENE(dim = 1:30, verbose=F)
RunTSENE(dim = 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 Ludo Waltman and Nees Jan van Eck

Number of nodes: 35235  
Number of edges: 1415522

Running Louvain algorithm...

Maximum modularity in 10 random starts: 0.9282

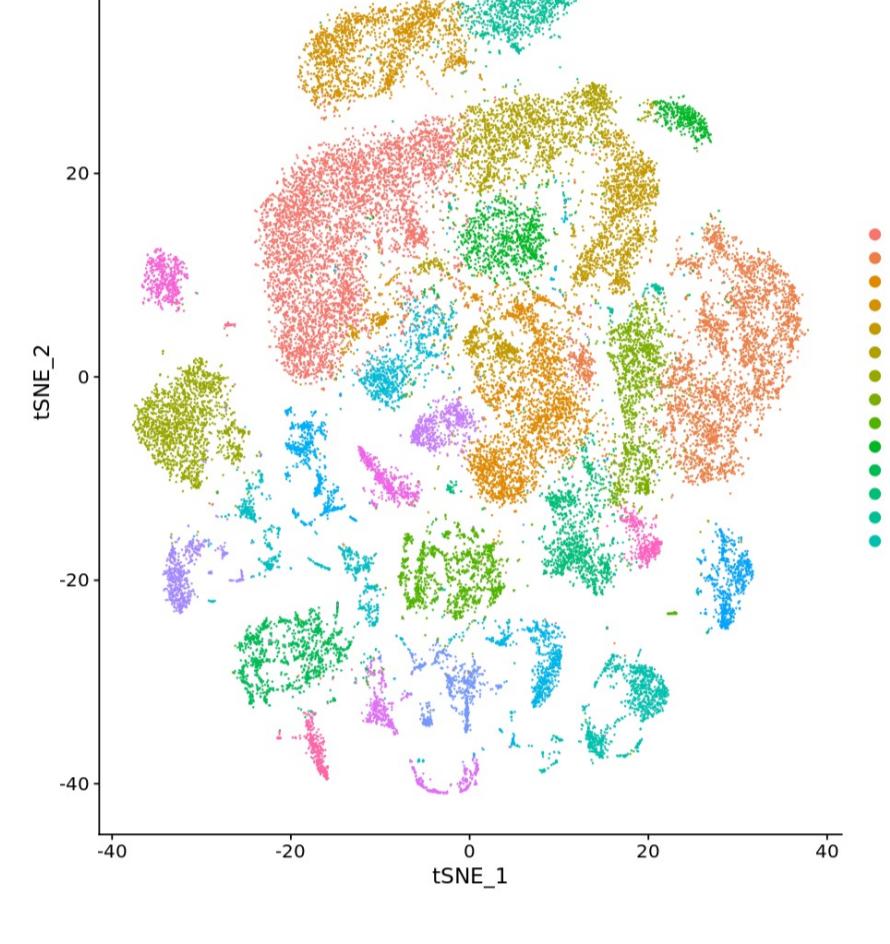
Number of communities: 28

Elapsed time: 7 seconds

In [3]:

options(repr.plot.width=9, repr.plot.height=9)

DimPlot(seurat\_obj\_v3, reduction = "tSNE")



In [4]:

seurat\_obj\_v3\_no\_vglut = seurat\_obj\_v3[, as.numeric(seurat\_obj\_v3\$RNA["Slc17a7", ]) == 0]

seurat\_obj\_v3\_no\_vglut = Seurat::NormalizeData(object=seurat\_obj\_v3\_no\_vglut) %>%

FindVariableFeatures() %>%
 ScaleData() %>%
 RunPCA(verbose=F) %>%

FindNeighbors(dim = 1:30) %>%
 FindClusters(resolution = 0.6) %>%

RunTSENE(dim = 1:30, verbose=F)

Centering and scaling data matrix

Computing nearest neighbor graph

Computing SNN

Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck

Number of nodes: 32449  
Number of edges: 1310672

Running Louvain algorithm...

Maximum modularity in 10 random starts: 0.9240

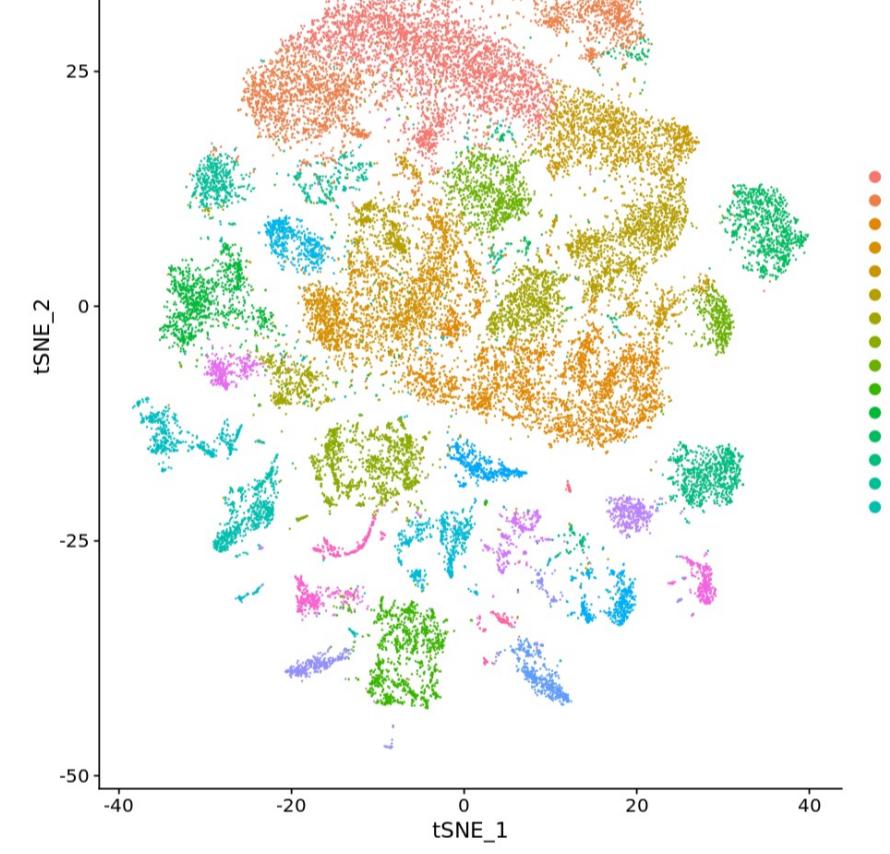
Number of communities: 30

Elapsed time: 6 seconds

In [6]:

options(repr.plot.width=9, repr.plot.height=9)

DimPlot(seurat\_obj\_v3\_no\_vglut, reduction = "tSNE")



In [15]:

# clusterlabel is called 'RNA\_snn\_res.0.6'

# Number of cells?

print(paste0("Number of Cells Not Expressing Slc17a7: ",

ncol(seurat\_obj\_v3\_no\_vglut)))

# How many clusters?

print(paste0("Number of Clusters For Cells Not Expressing Slc17a7: ",

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 Slc17a7: ",

sum(seurat\_obj\_v3\_no\_vglut\$RNA["Neurofox3", ] > 0)))

print(paste0("Number of NeuN- Cells Not Expressing Slc17a7: ",

sum(seurat\_obj\_v3\_no\_vglut\$RNA["Neurofox3", ] == 0)))

# Number of cells that express glial markers

# Gfap and Sall1 are two common Astrocytic markers

# Gfap and Sall1 are Microglial markers

# Olig2 is an OPC marker

# MOG and Olig1 are Oligodendrocyte markers

print(paste0("Number of Gfap+ Cells Not Expressing Slc17a7: ",

sum(seurat\_obj\_v3\_no\_vglut\$RNA["Gfap", ] > 0)))

print(paste0("Number of Afp+ Cells Not Expressing Slc17a7: ",

sum(seurat\_obj\_v3\_no\_vglut\$RNA["Afp", ] > 0)))

print(paste0("Number of C1q+ Cells Not Expressing Slc17a7: ",

sum(seurat\_obj\_v3\_no\_vglut\$RNA["C1q", ] > 0)))

print(paste0("Number of Sall1+ Cells Not Expressing Slc17a7: ",

sum(seurat\_obj\_v3\_no\_vglut\$RNA["Sall1", ] > 0)))

print(paste0("Number of Olig2+ Cells Not Expressing Slc17a7: ",

sum(seurat\_obj\_v3\_no\_vglut\$RNA["Olig2", ] > 0)))

print(paste0("Number of Olig1+ Cells Not Expressing Slc17a7: ",

sum(seurat\_obj\_v3\_no\_vglut\$RNA["Olig1", ] > 0)))

print(paste0("Number of MOG+ Cells Not Expressing Slc17a7: ",

sum(seurat\_obj\_v3\_no\_vglut\$RNA["MOG", ] > 0)))

# Number of cells that express our neuropoptides of interest (Sst, Pdyn, etc.)

print(paste0("Number of Sst+ Cells Not Expressing Slc17a7: ",

sum(seurat\_obj\_v3\_no\_vglut\$RNA["Sst", ] > 0)))

print(paste0("Number of Vip+ Cells Not Expressing Slc17a7: ",

sum(seurat\_obj\_v3\_no\_vglut\$RNA["Vip", ] > 0)))

print(paste0("Number of Pdyn+ Cells Not Expressing Slc17a7: ",

sum(seurat\_obj\_v3\_no\_vglut\$RNA["Pdyn", ] > 0)))

(1) "Number of Cells Not Expressing Slc17a7: 32449"

(1) "Number of Clusters For Cells Not Expressing Slc17a7: 30"

(1) "Number of NeuN+ Cells Not Expressing Slc17a7: 14545"

(1) "Number of NeuN- Cells Not Expressing Slc17a7: 17904"

(1) "Number of Gfap+ Cells Not Expressing Slc17a7: 669"

(1) "Number of C1q+ Cells Not Expressing Slc17a7: 331"

(1) "Number of Sall1+ Cells Not Expressing Slc17a7: 451"

(1) "Number of Olig2+ Cells Not Expressing Slc17a7: 1766"

(1) "Number of Olig1+ Cells Not Expressing Slc17a7: 1289"

(1) "Number of MOG+ Cells Not Expressing Slc17a7: 1503"

(1) "Number of Sst+ Cells Not Expressing Slc17a7: 104"

(1) "Number of Vip+ Cells Not Expressing Slc17a7: 161"

(1) "Number of Pdyn+ Cells Not Expressing Slc17a7: 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/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) Rtsniff_0.17       colorspace_2.1-0    deldir_2.0-2
```

```
(4) cli_2.5.0          grid_3.3.6         TBBdspkay_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) remote_2.4.2.1    grepel_0.9.5     fansi_1.0.4
```

```
(16) rlang_0.4.10       stringr_1.4.0     cachet_0.8
```

```
(19) polyclip_1.10-6   pkgload_1.3.3     IRkernel_1.3.2
```

```
(22) jsonlite_1.8-7    ica_1.0-5         cluster_2.1.6
```

```
(25) magrittr_2.0.1     usethis_1.16       spatstat.core_3.0-3
```

```
(28) shiny_1.8.0       strtransform_0.4.1  compiler_3.6.4
```

```
(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) pillar_1.5.7      tibble_3.1.6      later_1.3.0
```

```
(40) gttable_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_0.6.1       nime_3.1-16.1    spatstat.explore_3.2-5
```

```
(49) R6_2.5.1          mnorm_0.16.40    spatstat.random_3.2-2
```

```
(52) stringr_1.5.1     globals_0.16.2    mime_0.12
```

```
(55) minqa_0.1.1.1     lifecycle_1.0.4    irlib_2.3.5.1
```

```
(58) MASS_7.3-5.5      zoo_1.8-9         magrittr_2.0.3
```

```
(61) scales_1.1.0      promises_1.2.1    spatstat.util_3.0-4
```

```
(64) parallel_4.0.4    RColorBrewer_1.1-3  memoise_2.0.1
```

```
(67) reticulate_1.34.0  pbapply_1.7-2     gridExtra_2.1.0
```

```
(70) rlang_0.4.10       stringr_1.4.0      plogr_1.1.3
```

```
(73) repr_1.1.6        rlang_1.1.3       pkconfig_2.0.3
```

```
(76) matrixStats_1.2.0  evaluate_0.23    lattice_0.22-5
```

```
(79) tidyselect_1.1.0    RcppArmadillo_0.10.0  purrr_0.7.2
```

```
(82) labeling_0.4.3     patchwork_1.2.0    hms_1.1.1
```

```
(85) caret_6.0.8       tidyselect_1.2.0    parallelly_1.36.0
```

```
(88) RcppAnnoy_0.0.21  plyr_1.8.9        magrittr_2.0.3
```

```
(91) gridExtra_2.3.3   grid_3.3.6        profvis_0.3.8
```

```
(94) phdM2Q_0.1-10    withr_3.0.0       pillar_1.3.0
```

```
(97) fitdistrplus_1.1-11  abind_1.4-5       pillar_1.3.5-7
```

```
(100) sp_2.1-2          tibble_3.2.1       survival_3.5-7
```

```
(103) ggridges_0.5.2    withr_3.0.0       future.apply_1.11.0
```

```
(106) utf8_1.2.4        grid_4.0.4         KernSmooth_2.22-16
```

```
(109) uriehecker_1.0.1  spatstat.geom_3.2-7  plotly_4.10.3
```

```
(112) digest_0.6.25     Rtable_1.8-4       data.table_1.14.10
```

```
(115) httpuv_1.6.13    munspell_0.5.0     tidyverse_1.3.0
```

```
(118) sessioninfo_1.2.2 viridisLite_0.4.2
```