# GSE118020_Sample1_RAW

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## R Markdown

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When you click the **Knit** button a document will be generated that includes both content as well as the output of any embedded R code chunks within the document. You can embed an R code chunk like this:

```
##github. (2020). GitHub. Retrieved from https://github.com/MarcElosua/SPOTlight/tree/spotlight-0.1.7

#Load packages to CreateSeuratObject
library(SingleCellExperiment)
```

```
## Loading required package: SummarizedExperiment
```

```
## Loading required package: MatrixGenerics
```

```
## Loading required package: matrixStats
```

```
##
## Attaching package: 'MatrixGenerics'
```

```
## The following objects are masked from 'package:matrixStats':
##
##     colAlls, colAnyNAs, colAnys, colAvgsPerRowSet, colCollapse,
##     colCounts, colCummaxs, colCummins, colCumprods, colCumsums,
##     colDiffs, colIQRDiffs, colIQRs, colLogSumExps, colMadDiffs,
##     colMads, colMaxs, colMeans2, colMedians, colMins, colOrderStats,
##     colProds, colQuantiles, colRanges, colRanks, colSdDiffs, colSds,
##     colSums2, colTabulates, colVarDiffs, colVars, colWeightedMads,
##     colWeightedMeans, colWeightedMedians, colWeightedSds,
##     colWeightedVars, rowAlls, rowAnyNAs, rowAnys, rowAvgsPerColSet,
##     rowCollapse, rowCounts, rowCummaxs, rowCummins, rowCumprods,
##     rowCumsums, rowDiffs, rowIQRDiffs, rowIQRs, rowLogSumExps,
##     rowMadDiffs, rowMads, rowMaxs, rowMeans2, rowMedians, rowMins,
##     rowOrderStats, rowProds, rowQuantiles, rowRanges, rowRanks,
##     rowSdDiffs, rowSds, rowSums2, rowTabulates, rowVarDiffs, rowVars,
##     rowWeightedMads, rowWeightedMeans, rowWeightedMedians,
##     rowWeightedSds, rowWeightedVars
```

```
## Loading required package: GenomicRanges
```

```
## Loading required package: stats4
```

```
## Loading required package: BiocGenerics
```

```
##
## Attaching package: 'BiocGenerics'
```

```
## The following objects are masked from 'package:stats':
##
##     IQR, mad, sd, var, xtabs
```

```
## The following objects are masked from 'package:base':
##
##     anyDuplicated, append, as.data.frame, basename, cbind, colnames,
##     dirname, do.call, duplicated, eval, evalq, Filter, Find, get, grep,
##     grepl, intersect, is.unsorted, lapply, Map, mapply, match, mget,
##     order, paste, pmax, pmax.int, pmin, pmin.int, Position, rank,
##     rbind, Reduce, rownames, sapply, setdiff, sort, table, tapply,
##     union, unique, unsplit, which.max, which.min
```

```
## Loading required package: S4Vectors
```

```
##
## Attaching package: 'S4Vectors'
```

```
## The following objects are masked from 'package:base':
##
##     expand.grid, I, unname
```

```
## Loading required package: IRanges
```

```
##
## Attaching package: 'IRanges'
```

```
## The following object is masked from 'package:grDevices':
##
##     windows
```

```
## Loading required package: GenomeInfoDb
```

```
## Loading required package: Biobase
```

```
## Welcome to Bioconductor
##
##     Vignettes contain introductory material; view with
##     'browseVignettes()'. To cite Bioconductor, see
##     'citation("Biobase")', and for packages 'citation("pkgname")'.
```

```
##
## Attaching package: 'Biobase'
```

```
## The following object is masked from 'package:MatrixGenerics':
##
##     rowMedians
```

```
## The following objects are masked from 'package:matrixStats':
##
##     anyMissing, rowMedians
```

```
library(Seurat)
```

```
## Attaching SeuratObject
```

```
## Attaching sp
```

```
##
## Attaching package: 'Seurat'
```

```
## The following object is masked from 'package:SummarizedExperiment':
##
##     Assays
```

```
library(tidyverse)
```

```
## ── Attaching packages ─────────────────────────────────────── tidyverse 1.3.2 ──
```

```
## ✓ ggplot2 3.3.6     ✓ purrr   0.3.4
## ✓ tibble  3.1.8     ✓ dplyr   1.0.9
## ✓ tidyr   1.2.0     ✓ stringr 1.4.0
## ✓ readr   2.1.2     ✓ forcats 0.5.1
## ── Conflicts ────────────────────────────────────────── tidyverse_conflicts() ──
## ✖ dplyr::collapse()   masks IRanges::collapse()
## ✖ dplyr::combine()    masks Biobase::combine(), BiocGenerics::combine()
## ✖ dplyr::count()      masks matrixStats::count()
## ✖ dplyr::desc()       masks IRanges::desc()
## ✖ tidyr::expand()     masks S4Vectors::expand()
## ✖ dplyr::filter()     masks stats::filter()
## ✖ dplyr::first()      masks S4Vectors::first()
## ✖ dplyr::lag()        masks stats::lag()
## ✖ ggplot2::Position() masks BiocGenerics::Position(), base::Position()
## ✖ purrr::reduce()     masks GenomicRanges::reduce(), IRanges::reduce()
## ✖ dplyr::rename()     masks S4Vectors::rename()
## ✖ dplyr::slice()      masks IRanges::slice()
```

```
library(Matrix)
```

```
##
## Attaching package: 'Matrix'
##
## The following objects are masked from 'package:tidyr':
##
##     expand, pack, unpack
##
## The following object is masked from 'package:S4Vectors':
##
##     expand
```

```
library(scales)
```

```
##
## Attaching package: 'scales'
##
## The following object is masked from 'package:purrr':
##
##     discard
##
## The following object is masked from 'package:readr':
##
##     col_factor
```

```
library(cowplot)
library(RCurl)
```

```
##
## Attaching package: 'RCurl'
##
## The following object is masked from 'package:tidyr':
##
##     complete
```

```
#Download the file in GEO, and create the counts file for CreateSeuratObject
#set the path
getwd()
```

```
## [1] "D:/pankyung/intern_back_up/sub/GSE118020_RAW"
```

```
data_dir <- 'D:/pankyung/intern_back_up/sub/GSE118020_RAW'

#The list should show barcodes.tsv, features.tsv, and matrix.mtx
list.files(data_dir)
```

```
##  [1] "barcodes.tsv.gz"                  "cortex_sc_a.rds"
##  [3] "cortex_sc_a_o.rds"                "features.tsv.gz"
##  [5] "GSE118020_RAW.R"                  "GSE118020_RAW.Rmd"
##  [7] "GSE118020_RAW_nn.R"               "GSE118020_RAW_sam1_bar_feat_mat.R"
##  [9] "markers_sc_a.rds"                 "markers_sc_a_o.rds"
## [11] "matrix.mtx.gz"                    "posterior.R"
## [13] "spotlight_ls_a.rds"               "spotlight_ls_a_o.rds"
## [15] "spotlight_ls_p_o.rds"             "spotlight_ls_sss_o.rds"
## [17] "tissue_lowres_image(2).png"       "tissue_lowres_image.png"
```

```
#Use Read10X to create expression matrix
expression_matrix <- Read10X(data.dir = data_dir)

#Set the min.cells, min.features number for samples
cortex_sc_a = CreateSeuratObject(counts = expression_matrix, project='seurat', min.cells = 3,min.features = 200)

#This is the way that I can create contents in meta.data
cortex_sc_a[["percent.mt"]]<-PercentageFeatureSet(cortex_sc_a, pattern ="^MT-")
cortex_sc_a <- FindVariableFeatures(cortex_sc_a, selection.method = "vst", nfeatures = 2000)

#Bring the gene name
all.genes <- rownames(cortex_sc_a)

#Run RunPCA, RunUMAP to create object for SPotlight
set.seed(0812) #Set seed
cortex_sc_a <- Seurat::SCTransform(cortex_sc_a, verbose = FALSE) %>%
  Seurat::RunPCA(., verbose = FALSE) %>%
  Seurat::RunUMAP(., dims = 1:30, verbose = FALSE)
```

```
## Warning: Invalid name supplied, making object name syntactically valid. New
## object name is Seurat..SCTransform.RNA; see ?make.names for more details on
## syntax validity
```

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

```
#Run FindNeighbors, FindClusters to create object for SPotlight
cortex_sc_a <- FindNeighbors(cortex_sc_a, dims=1:10)
```

```
## Computing nearest neighbor graph
## Computing SNN
```

```
cortex_sc_a <- FindClusters(cortex_sc_a,resolution=0.5)
```

```
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
##
## Number of nodes: 6081
## Number of edges: 195802
##
## Running Louvain algorithm...
## Maximum modularity in 10 random starts: 0.9209
## Number of communities: 14
## Elapsed time: 0 seconds
```

```
#Use levels to find out how many clusters in Seurat object
d<-levels(cortex_sc_a)

#Use for loop to name each cluster easily
dl<-length(d)-1 #Cluseter starts at 0
nx<-c()
for (i in 0:dl){
  cc<-paste0('Ty',i)
  nx<-c(nx,cc)
}

#In this case, cell type will be just number, but if you can give cell name

#Name the levels with cell type
new.cluster.ids <- nx
names(new.cluster.ids) <- levels(cortex_sc_a) #Match the levels with cell type
cortex_sc_a <- RenameIdents(cortex_sc_a, new.cluster.ids) #Change the level of  each ident to cell type

#Add the subclass chapter in meta.data
cortex_sc_a[["subclass"]] <- as.character(Idents(object = cortex_sc_a))

#Save the cortex_sc_a in rds file
saveRDS(object = cortex_sc_a,file = "D:/pankyung/intern_back_up/sub/GSE118020_RAW/cortex_sc_a.rds")

#Find markers
cluster_markers_all_a <- Seurat::FindAllMarkers(object = cortex_sc_a,
                                                assay = "SCT",
                                                slot = "data",
                                                verbose = TRUE,
                                                only.pos = TRUE)
```

```
## Calculating cluster Ty0
## Calculating cluster Ty1
## Calculating cluster Ty2
## Calculating cluster Ty3
## Calculating cluster Ty4
## Calculating cluster Ty5
## Calculating cluster Ty6
## Calculating cluster Ty7
## Calculating cluster Ty8
## Calculating cluster Ty9
## Calculating cluster Ty10
## Calculating cluster Ty11
## Calculating cluster Ty12
## Calculating cluster Ty13
```

```
saveRDS(object = cluster_markers_all_a,
        file = here::here("D:/pankyung/intern_back_up/sub/GSE118020_RAW/markers_sc_a.rds"))

#=================================================
#Now apply the SeuratObject in the SPotlight vignette

#install.packages("devtools") #Install it if you need
#devtools::install_github("https://github.com/MarcElosua/SPOTlight/tree/spotlight-0.1.7") #Download the sample_data
#sample_data will saved in "D:WRWR-4.2.1WlibraryWSPOTlight" by default setting

#Bring the packages
library(Matrix)
library(data.table)
```

```
##
## Attaching package: 'data.table'
##
## The following objects are masked from 'package:dplyr':
##
##     between, first, last
##
## The following object is masked from 'package:purrr':
##
##     transpose
##
## The following object is masked from 'package:SummarizedExperiment':
##
##     shift
##
## The following object is masked from 'package:GenomicRanges':
##
##     shift
##
## The following object is masked from 'package:IRanges':
##
##     shift
##
## The following objects are masked from 'package:S4Vectors':
##
##     first, second
```

```
library(Seurat) #Single cell data package
library(SeuratData)
```

```
## ── Installed datasets ─────────────────────────────────────────────────────── SeuratData v0.2.2 ──
## ✓ stxBrain 0.1.1
##
## ──────────────────────────────────────────────────────── Key ─────────────────────────────────
──────────────────────
## ✓ Dataset loaded successfully
## › Dataset built with a newer version of Seurat than installed
## ❓ Unknown version of Seurat installed
```

```
library(dplyr) #data structure package
library(gt)
library(SPOTlight)
library(igraph)
```

```
##
## Attaching package: 'igraph'
##
## The following objects are masked from 'package:dplyr':
##
##     as_data_frame, groups, union
##
## The following objects are masked from 'package:purrr':
##
##     compose, simplify
##
## The following object is masked from 'package:tidyr':
##
##     crossing
##
## The following object is masked from 'package:tibble':
##
##     as_data_frame
##
## The following object is masked from 'package:GenomicRanges':
##
##     union
##
## The following object is masked from 'package:IRanges':
##
##     union
##
## The following object is masked from 'package:S4Vectors':
##
##     union
##
## The following objects are masked from 'package:BiocGenerics':
##
##     normalize, path, union
##
## The following objects are masked from 'package:stats':
##
##     decompose, spectrum
##
## The following object is masked from 'package:base':
##
##     union
```

```
library(RColorBrewer)
library(NMF)
```

```
## Loading required package: pkgmaker
## Loading required package: registry
##
## Attaching package: 'pkgmaker'
##
## The following object is masked from 'package:S4Vectors':
##
##     new2
##
## Loading required package: rngtools
## Loading required package: cluster
## NMF - BioConductor layer [OK] | Shared memory capabilities [NO: windows] | Cores 15/16
##
## Attaching package: 'NMF'
##
## The following objects are masked from 'package:igraph':
##
##     algorithm, compare
##
## The following object is masked from 'package:S4Vectors':
##
##     nrun
```

```
library(magrittr)
```

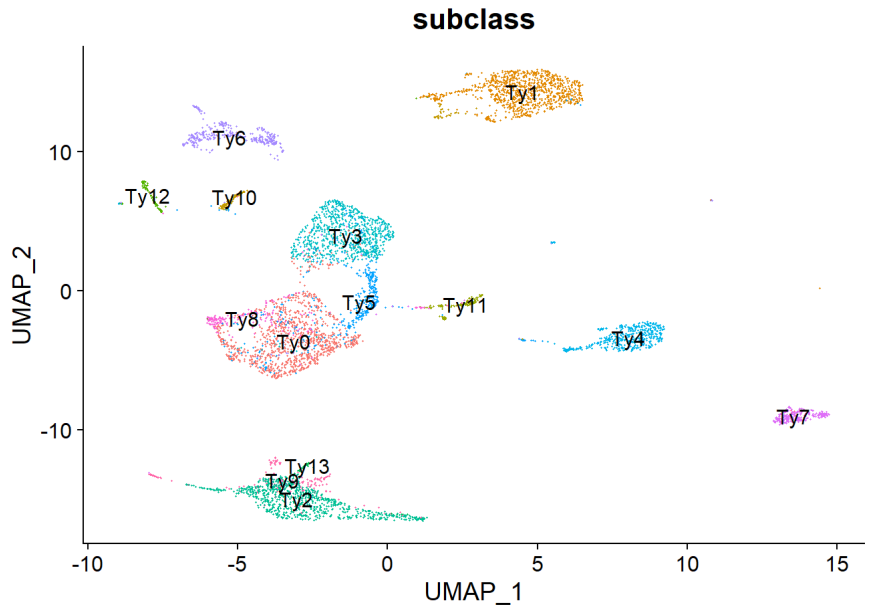
```
##
## Attaching package: 'magrittr'
##
## The following object is masked from 'package:purrr':
##
##     set_names
##
## The following object is masked from 'package:tidyr':
##
##     extract
##
## The following object is masked from 'package:GenomicRanges':
##
##     subtract
```

```
#Load data
path_to_data <- system.file(package = "SPOTlight")
cortex_sc_a <- readRDS(glue::glue("D:/pankyung/intern_back_up/sub/GSE118020_RAW/cortex_sc_a.rds"))

#Download the data from SeuratData
if (!("stxBrain.SeuratData" %in% rownames(InstalledData()))) {
  InstallData("stxBrain")
}

#Load SeuratData
anterior_a <- SeuratData::LoadData("stxBrain", type = "anterior1")

#Check clusters
Seurat::DimPlot(cortex_sc_a,
                group.by = "subclass",
                label = TRUE) + Seurat::NoLegend()
```




```
#Check the cell type
cortex_sc_a@meta.data %>%
  dplyr::count(subclass) %>%
  gt::gt(.[-1, ]) %>%
  gt::tab_header(
    title = "Cell types present in the reference dataset",
  ) %>%
  gt::cols_label(
    subclass = gt::html("Cell Type")
  )
```

```
## Warning in !is.null(rowname_col) && rowname_col %in% colnames(data_tbl):
## 'length(x) = 2 > 1' in coercion to 'logical(1)'
```

| Cell types present in the reference dataset | |
|---|---|
| Cell Type | n |
| Ty0 | 1050 |
| Ty1 | 1003 |

| Cell types present in the reference dataset | |
|---|---|
| Ty10 | 132 |
| Ty11 | 130 |
| Ty12 | 91 |
| Ty13 | 31 |
| Ty2 | 864 |
| Ty3 | 835 |
| Ty4 | 482 |
| Ty5 | 477 |
| Ty6 | 382 |
| Ty7 | 255 |
| Ty8 | 196 |
| Ty9 | 153 |

```
#I already found the markers at line 70
#Load markers
cluster_markers_all_a<- readRDS(file = "D:/pankyung/intern_back_up/sub/GSE118020_RAW/markers_sc_a.rds")

#SPOTlight Decomposition
set.seed(0812)
spotlight_ls_a <- spotlight_deconvolution(
  se_sc = cortex_sc_a,
  counts_spatial = anterior_a@assays$Spatial@counts,
  clust_vr = "subclass", # Variable in sc_seu containing the cell-type annotation
  cluster_markers = cluster_markers_all_a, # Dataframe with the marker genes
  cl_n = 100, # number of cells per cell type to use
  hvg = 3000, # Number of HVG to use
  ntop = NULL, # How many of the marker genes to use (by default all)
  transf = "uv", # Perform unit-variance scaling per cell and spot prior to factorzation and NLS
  method = "nsNMF", # Factorization method
  min_cont = 0 # Remove those cells contributing to a spot below a certain threshold
)
```

```
## [1] "Preparing Gene set"
## [1] "Normalizing count matrix"
## [1] "Seeding initial matrices"
## [1] "Training..."
## [1] "Time to train NMF model was 17.68mins"
## [1] "Deconvoluting spots"
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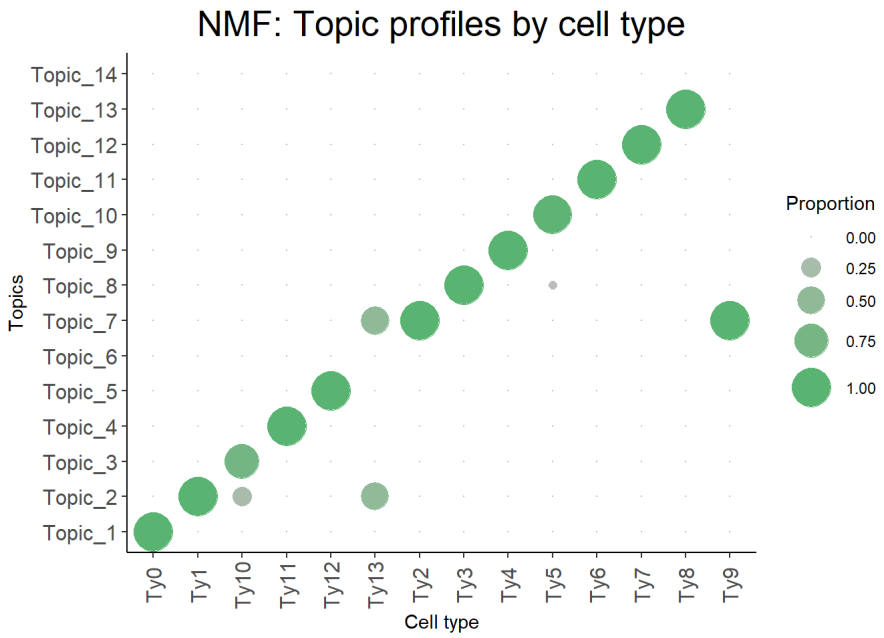
```
saveRDS(object = spotlight_ls_a, file = here::here("D:/pankyung/intern_back_up/sub/GSE118020_RAW/spotlight_ls_a.rds"))

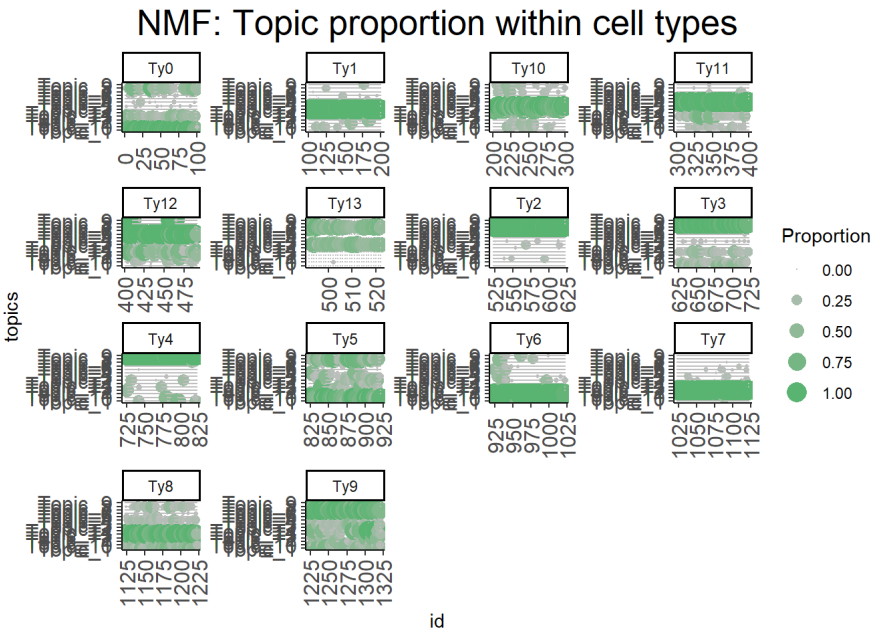
#Read spotlight object object
spotlight_ls_a <- readRDS(file = here::here("D:/pankyung/intern_back_up/sub/GSE118020_RAW/spotlight_ls_a.rds"))
nmf_mod <- spotlight_ls_a[[1]]
decon_mtrx <- spotlight_ls_a[[2]]

#Assess deconvolution
h <- NMF::coef(nmf_mod[[1]])
rownames(h) <- paste("Topic", 1:nrow(h), sep = "_")
topic_profile_plts_a <- SPOTlight::dot_plot_profiles_fun(
  h = h,
  train_cell_clust = nmf_mod[[2]])

topic_profile_plts_a[[2]] + ggplot2::theme(
  axis.text.x = ggplot2::element_text(angle = 90),
  axis.text = ggplot2::element_text(size = 12))
```




```
#Look at the individual topic profiles
topic_profile_plts_a[[1]] + theme(axis.text.x = element_text(angle = 90),
                                  axis.text = element_text(size = 12))
```




```
#Look at which genes are the most important
basis_spotlight_a <- data.frame(NMF::basis(nmf_mod[[1]]))

colnames(basis_spotlight_a) <- unique(stringr::str_wrap(nmf_mod[[2]], width = 30))

basis_spotlight_a %>%
  dplyr::arrange(desc(Ty0)) %>%
  round(., 5) %>%
  DT::datatable(., filter = "top")
```

Show 10 entries Search:

| | Ty0 | Ty1 | Ty10 | Ty11 | Ty12 | Ty13 | Ty2 | Ty3 | Ty4 | Ty5 |
|---|---|---|---|---|---|---|---|---|---|---|
| Tac1 | 0.00155 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00005 |
| Itgb1bp1 | 0.00148 | 0.00005 | 0.00002 | 0.00007 | 0 | 0 | 0.00008 | 0.00101 | 0 | 0.0002 |
| Fkbp1a | 0.00146 | 0 | 0.00015 | 0 | 0 | 0 | 0.00032 | 0.00145 | 0 | 0.00039 |
| Arpp21 | 0.00146 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00139 | 0 | 0.00006 |
| Chn1 | 0.00146 | 0 | 0 | 0.00004 | 0 | 0 | 0 | 0.00139 | 0 | 0.0002 |
| Pdyn | 0.00146 | 0 | 0 | 0.00004 | 0 | 0 | 0 | 0 | 0 | 0.0002 |
| Calm2 | 0.00145 | 0 | 0 | 0.00052 | 0.00002 | 0 | 0 | 0.00135 | 0 | 0.00018 |
| Ppp1r1b | 0.00141 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00123 | 0 | 0.0001 |
| Ramp1 | 0.00139 | 0.00035 | 0 | 0 | 0 | 0 | 0 | 0.00103 | 0 | 0 |
| Ap1s1 | 0.00137 | 0 | 0.00029 | 0.00007 | 0 | 0 | 0 | 0.00125 | 0 | 0.00031 |

Showing 1 to 10 of 4,587 entries Previous 1 2 3 4 5 … 459 Next

```
#Visualization
# This is the equivalent to setting min_cont to 0.04
decon_mtrx_sub <- decon_mtrx[, colnames(decon_mtrx) != "res_ss"] #Exclude res_ss column
decon_mtrx_sub[decon_mtrx_sub < 0.08] <- 0 #Convert the value that smaller that 0.08 to o
decon_mtrx <- cbind(decon_mtrx_sub, "res_ss" = decon_mtrx[, "res_ss"]) #Add res_ss column again
rownames(decon_mtrx) <- colnames(anterior_a) #Give the spot information

#I guess, with single cell data, we can classify the Ty9, but there is no such cell type in tissue which is used in reference
#I used different single cell data in this project, so probably it can happen

decon_df_a <- decon_mtrx %>%
  data.frame() %>%
  tibble::rownames_to_column("barcodes")

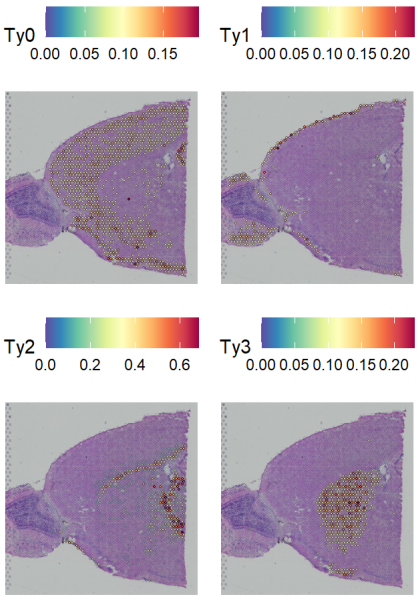
anterior_a@meta.data <- anterior_a@meta.data %>%
  tibble::rownames_to_column("barcodes") %>%
  dplyr::left_join(decon_df_a, by = "barcodes") %>%
  tibble::column_to_rownames("barcodes")

#See the Specific cell-types plot
#View the whole cell type
nx
```

```
##  [1] "Ty0"  "Ty1"  "Ty2"  "Ty3"  "Ty4"  "Ty5"  "Ty6"  "Ty7"  "Ty8"  "Ty9"
## [11] "Ty10" "Ty11" "Ty12" "Ty13"
```

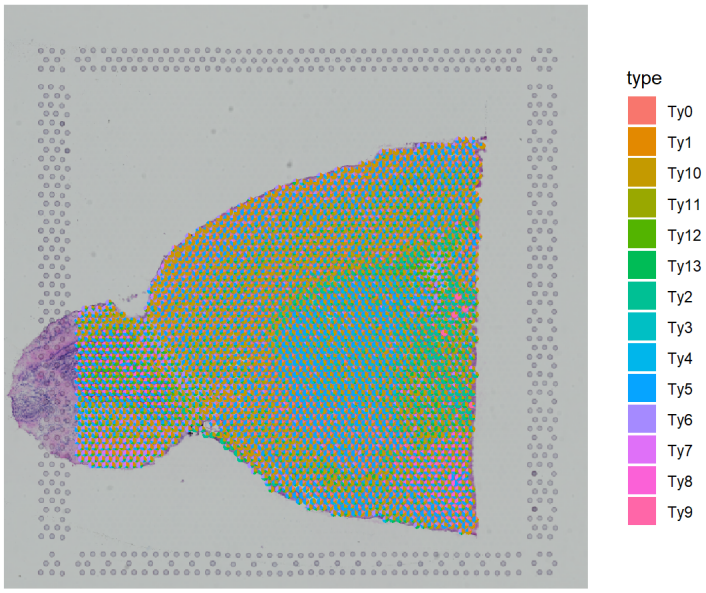
```
#Select cell types
nxs<-nx[1:4] #In this case, Ty1 to Ty4

#Draw the plot
Seurat::SpatialFeaturePlot(
  object = anterior_a,
  features = c(nxs),
  ncol = 2, #Set the display
  alpha = c(0.1, 1)) #Set the opacity
```




```
#Spatial_scatterpie plot will show spots containing at least one of cell types we select
#In this case, Spatial scatterpies for all cell types
cell_types_all_a <- colnames(decon_mtrx)[which(colnames(decon_mtrx) != "res_ss")]
SPOTlight::spatial_scatterpie(se_obj = anterior_a,
                              cell_types_all = cell_types_all_a,
                              img_path = "D:/pankyung/intern_back_up/sub/GSE118020_RAW/tissue_lowres_image.png",
                              pie_scale = 0.4)
```

```
## Warning in SPOTlight::spatial_scatterpie(se_obj = anterior_a, cell_types_all =
## cell_types_all_a, : Using slice anterior1
```




```
#Select cell types
nxs1<-nx[1:2] #In this case, Ty1 to Ty4

#Spatial scatterpies for specific cell type
SPOTlight::spatial_scatterpie(se_obj = anterior_a,
                              cell_types_all = cell_types_all_a,
                              img_path = "D:/pankyung/intern_back_up/sub/GSE118020_RAW/tissue_lowres_image.png",
                              cell_types_interest = nxs1, #Show spots which contain at least Ty0 or Ty1
                              pie_scale = 0.8)
```

```
## Warning in SPOTlight::spatial_scatterpie(se_obj = anterior_a, cell_types_all =
## cell_types_all_a, : Using slice anterior1
```