| university union |
| --- |
| itle: “R Notebook” |
| utput: html\_notebook |
| nit: |
| opts\_knit: |
| root.dir: “U:/USERS/CamaraH/Projects/Opn3\_scRNAseq\_analysis/Emont\_2022/mouse” |

# Load libraries  
library(SingleCellExperiment)

## Loading required package: SummarizedExperiment

## Loading required package: MatrixGenerics

## Loading required package: matrixStats

## Warning: package 'matrixStats' was built under R version 4.3.2

##   
## 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, aperm, 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

## Warning: package 'S4Vectors' was built under R version 4.3.2

##   
## Attaching package: 'S4Vectors'

## The following object is masked from 'package:utils':  
##   
## findMatches

## 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

## Warning: package 'GenomeInfoDb' was built under R version 4.3.3

## 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)

## Warning: package 'Seurat' was built under R version 4.3.3

## Loading required package: SeuratObject

## Warning: package 'SeuratObject' was built under R version 4.3.2

## Loading required package: sp

##   
## Attaching package: 'sp'

## The following object is masked from 'package:IRanges':  
##   
## %over%

## 'SeuratObject' was built with package 'Matrix' 1.6.3 but the current  
## version is 1.6.5; it is recomended that you reinstall 'SeuratObject' as  
## the ABI for 'Matrix' may have changed

##   
## Attaching package: 'SeuratObject'

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

## The following object is masked from 'package:GenomicRanges':  
##   
## intersect

## The following object is masked from 'package:GenomeInfoDb':  
##   
## intersect

## The following object is masked from 'package:IRanges':  
##   
## intersect

## The following object is masked from 'package:S4Vectors':  
##   
## intersect

## The following object is masked from 'package:BiocGenerics':  
##   
## intersect

## The following object is masked from 'package:base':  
##   
## intersect

##   
## Attaching package: 'Seurat'

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

library(tidyverse)

## Warning: package 'tidyverse' was built under R version 4.3.2

## Warning: package 'ggplot2' was built under R version 4.3.3

## Warning: package 'tidyr' was built under R version 4.3.3

## Warning: package 'readr' was built under R version 4.3.3

## Warning: package 'purrr' was built under R version 4.3.2

## Warning: package 'dplyr' was built under R version 4.3.2

## Warning: package 'stringr' was built under R version 4.3.2

## Warning: package 'lubridate' was built under R version 4.3.2

## ── Attaching core tidyverse packages ──────────────────────── tidyverse 2.0.0 ──  
## ✔ dplyr 1.1.4 ✔ readr 2.1.5  
## ✔ forcats 1.0.0 ✔ stringr 1.5.1  
## ✔ ggplot2 3.5.0 ✔ tibble 3.2.1  
## ✔ lubridate 1.9.3 ✔ tidyr 1.3.1  
## ✔ purrr 1.0.2

## ── Conflicts ────────────────────────────────────────── tidyverse\_conflicts() ──  
## ✖ lubridate::%within%() masks IRanges::%within%()  
## ✖ 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()  
## ✖ lubridate::second() masks S4Vectors::second()  
## ✖ lubridate::second<-() masks S4Vectors::second<-()  
## ✖ dplyr::slice() masks IRanges::slice()  
## ℹ Use the conflicted package (<http://conflicted.r-lib.org/>) to force all conflicts to become errors

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)

## Warning: package 'scales' was built under R version 4.3.2

##   
## Attaching package: 'scales'  
##   
## The following object is masked from 'package:purrr':  
##   
## discard  
##   
## The following object is masked from 'package:readr':  
##   
## col\_factor

library(cowplot)

## Warning: package 'cowplot' was built under R version 4.3.2

##   
## Attaching package: 'cowplot'  
##   
## The following object is masked from 'package:lubridate':  
##   
## stamp

library(RCurl)

## Warning: package 'RCurl' was built under R version 4.3.2

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

library(stringr)  
library(RColorBrewer)  
library(ggplot2)  
library(ggpubr)

##   
## Attaching package: 'ggpubr'  
##   
## The following object is masked from 'package:cowplot':  
##   
## get\_legend

###Create SeratObject

#The object was created from   
# Mm10X.data.barcodes.tsv.gz Cell barcodes for mouse adipose single-nucleus normalized expression data - https://singlecell.broadinstitute.org/single\_cell/data/public/SCP1376/a-single-cell-atlas-of-human-and-mouse-white-adipose-tissue?filename=Mm10X.data.barcodes.tsv.gz   
# Mm10X.data.features.tsv.gz Features for mouse adipose single-nucleus normalized expression data - https://singlecell.broadinstitute.org/single\_cell/data/public/SCP1376/a-single-cell-atlas-of-human-and-mouse-white-adipose-tissue?filename=Mm10X.data.features.tsv.gz   
# Mm10X.data.mtx.gz Mouse adipose single-nucleus normalized expression data -https://singlecell.broadinstitute.org/single\_cell/data/public/SCP1376/a-single-cell-atlas-of-human-and-mouse-white-adipose-tissue?filename=Mm10X.data.mtx.gz  
#Data was stored in a single folder named 10x\_norm\_data  
  
ctrl\_counts <- Read10X(data.dir = "10x\_norm\_data")  
# Turn count matrix into a Seurat object (output is a Seurat object)  
ctrl <- CreateSeuratObject(counts = ctrl\_counts)  
barcodes <- rownames(ctrl@meta.data)

###Checking the normal metadata  
all(barcodes %in% rownames(ctrl@meta.data))

## [1] TRUE

all(rownames(ctrl@meta.data) == barcodes)

## [1] TRUE

names(ctrl@meta.data)

## [1] "orig.ident" "nCount\_RNA" "nFeature\_RNA"

###Add metadata

#metadata - https://singlecell.broadinstitute.org/single\_cell/data/public/SCP1376/a-single-cell-atlas-of-human-and-mouse-white-adipose-tissue?filename=Mm.metadata.tsv  
#Read the metadata file----  
meta <- read.delim("metadata.tsv",sep="\t")

#Combine the full metadata with the single-cell object  
ctrl.meta = ctrl@meta.data  
ctrl.meta$cell\_id = rownames(ctrl.meta)  
mm\_meta = left\_join(ctrl.meta, meta, by = "cell\_id")  
ncol(ctrl) == nrow(mm\_meta) #Check if lenghts are equal

## [1] TRUE

dplyr::select(mm\_meta, -c("nCount\_RNA", "nFeature\_RNA", "orig.ident"))

## cell\_id biosample\_id biosample\_type  
## 1 Mm\_ING\_16-1\_TCAAGCACACAGCGCT Mm\_ING\_16-1 PrimaryBioSample\_Tissue  
## 2 Mm\_ING\_16-1\_CCTCCAAGTATGGAAT Mm\_ING\_16-1 PrimaryBioSample\_Tissue  
## 3 Mm\_ING\_16-1\_TTCTTCCAGCAAGCCA Mm\_ING\_16-1 PrimaryBioSample\_Tissue  
## 4 Mm\_ING\_16-1\_CACCGTTTCCGCTGTT Mm\_ING\_16-1 PrimaryBioSample\_Tissue  
rownames(mm\_meta) = mm\_meta$cell\_id  
ctrl@meta.data <- mm\_meta

### Saving the file

saveRDS(ctrl, "emont\_mouse\_seurat.rds")

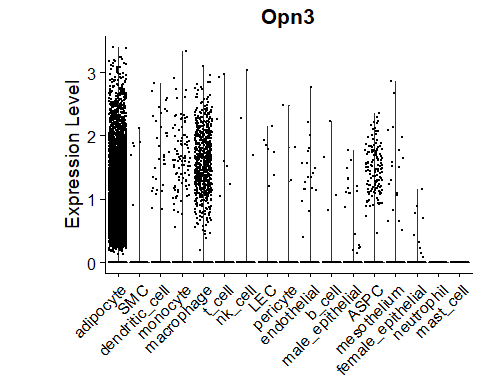
###Loading the object

ctrl = readRDS("emont\_mouse\_seurat.rds")

###PLOTTING

features <- c("Opn3")  
cluster\_sorted = sort(unique(ctrl$cell\_type\_\_custom))  
  
for(feat in features){  
Idents(ctrl) <- "cell\_type\_\_custom"  
unique(Idents(ctrl))  
plot <- Seurat::VlnPlot(ctrl,   
 features = feat,   
 raster = F) +  
 theme(axis.title.x = element\_blank(),  
 plot.margin = margin(l = 1.5, unit = "cm"))  
exp\_order = plot[[1]]$data %>%  
 group\_by(ident) %>%  
 summarise(avg\_exp = mean(!!sym(feat))) %>%  
 arrange(desc(avg\_exp)) %>%  
 pull(ident)  
  
plot = plot + scale\_x\_discrete(limits = exp\_order) + NoLegend()  
print(plot)  
  
ggsave(paste0("./figures/VlnPlot\_", feat, "\_all\_cells.png"), plot = plot, device = "png", width = 10, height = 8, dpi = 600)  
}

## Warning: Default search for "data" layer in "RNA" assay yielded no results;  
## utilizing "counts" layer instead.

 #DotPlot

###PLOTTING

library(ggplot2)  
library(ggpubr)  
  
features <- c("Opn1mw","Opn1sw","Opn3", "Opn4", "Opn5")  
cluster\_sorted = sort(unique(ctrl$cell\_type\_\_custom))  
  
#Plot all clusters by BMI   
Idents(ctrl) <- "cell\_type\_\_custom"  
unique(Idents(ctrl))

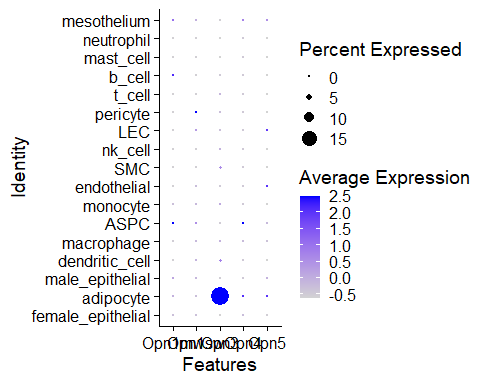
## [1] female\_epithelial adipocyte male\_epithelial dendritic\_cell   
## [5] macrophage ASPC monocyte endothelial   
## [9] SMC nk\_cell LEC pericyte   
## [13] t\_cell b\_cell mast\_cell neutrophil   
## [17] mesothelium   
## 17 Levels: female\_epithelial adipocyte male\_epithelial ... mesothelium

plot <- Seurat::DotPlot(ctrl,   
 features = features,   
 # split.by = "bmi\_\_group",   
 )

## Warning: No layers found matching search pattern provided

## Warning in FetchData.Assay5(object = object[[DefaultAssay(object = object)]], :  
## data layer is not found and counts layer is used

plot



dotplot\_data = plot$data  
dir.create("./data")

## Warning in dir.create("./data"): '.\data' already exists

#Write dot plot. This will also be used to generate the boxplot  
write.csv(dotplot\_data, paste0("./data/DotPlot\_Opn.csv"), row.names = F)

sessionInfo()

## R version 4.3.1 (2023-06-16 ucrt)  
## Platform: x86\_64-w64-mingw32/x64 (64-bit)  
## Running under: Windows 10 x64 (build 18363)  
##   
## Matrix products: default  
##   
##   
## locale:  
## [1] LC\_COLLATE=English\_United States.utf8   
## [2] LC\_CTYPE=English\_United States.utf8   
## [3] LC\_MONETARY=English\_United States.utf8  
## [4] LC\_NUMERIC=C   
## [5] LC\_TIME=English\_United States.utf8   
##   
## time zone: America/New\_York  
## tzcode source: internal  
##   
## attached base packages:  
## [1] stats4 stats graphics grDevices utils datasets methods   
## [8] base   
##   
## other attached packages:  
## [1] ggpubr\_0.6.0 RColorBrewer\_1.1-3   
## [3] RCurl\_1.98-1.14 cowplot\_1.1.3   
## [5] scales\_1.3.0 Matrix\_1.6-5   
## [7] lubridate\_1.9.3 forcats\_1.0.0   
## [9] stringr\_1.5.1 dplyr\_1.1.4   
## [11] purrr\_1.0.2 readr\_2.1.5   
## [13] tidyr\_1.3.1 tibble\_3.2.1   
## [15] ggplot2\_3.5.0 tidyverse\_2.0.0   
## [17] Seurat\_5.0.2 SeuratObject\_5.0.1   
## [19] sp\_2.1-3 SingleCellExperiment\_1.24.0  
## [21] SummarizedExperiment\_1.32.0 Biobase\_2.62.0   
## [23] GenomicRanges\_1.54.1 GenomeInfoDb\_1.38.8   
## [25] IRanges\_2.36.0 S4Vectors\_0.40.2   
## [27] BiocGenerics\_0.48.1 MatrixGenerics\_1.14.0   
## [29] matrixStats\_1.2.0   
##   
## loaded via a namespace (and not attached):  
## [1] RcppAnnoy\_0.0.22 splines\_4.3.1 later\_1.3.2   
## [4] bitops\_1.0-7 R.oo\_1.26.0 polyclip\_1.10-6   
## [7] fastDummies\_1.7.3 lifecycle\_1.0.4 rstatix\_0.7.2   
## [10] globals\_0.16.3 lattice\_0.22-5 MASS\_7.3-60.0.1   
## [13] backports\_1.4.1 magrittr\_2.0.3 plotly\_4.10.4   
## [16] rmarkdown\_2.26 yaml\_2.3.7 httpuv\_1.6.14   
## [19] sctransform\_0.4.1 spam\_2.10-0 spatstat.sparse\_3.0-3   
## [22] reticulate\_1.35.0 pbapply\_1.7-2 abind\_1.4-5   
## [25] zlibbioc\_1.48.2 Rtsne\_0.17 R.utils\_2.12.3   
## [28] GenomeInfoDbData\_1.2.11 ggrepel\_0.9.5 irlba\_2.3.5.1   
## [31] listenv\_0.9.1 spatstat.utils\_3.0-4 goftest\_1.2-3   
## [34] RSpectra\_0.16-1 spatstat.random\_3.2-3 fitdistrplus\_1.1-11   
## [37] parallelly\_1.37.1 leiden\_0.4.3.1 codetools\_0.2-19   
## [40] DelayedArray\_0.28.0 tidyselect\_1.2.1 farver\_2.1.1   
## [43] spatstat.explore\_3.2-6 jsonlite\_1.8.8 ellipsis\_0.3.2   
## [46] progressr\_0.14.0 ggridges\_0.5.6 survival\_3.5-8   
## [49] systemfonts\_1.0.6 tools\_4.3.1 ragg\_1.3.0   
## [52] ica\_1.0-3 Rcpp\_1.0.11 glue\_1.7.0   
## [55] gridExtra\_2.3 SparseArray\_1.2.4 xfun\_0.43   
## [58] withr\_3.0.0 fastmap\_1.1.1 fansi\_1.0.6   
## [61] digest\_0.6.33 timechange\_0.3.0 R6\_2.5.1   
## [64] mime\_0.12 textshaping\_0.3.7 colorspace\_2.1-0   
## [67] scattermore\_1.2 tensor\_1.5 spatstat.data\_3.0-4   
## [70] R.methodsS3\_1.8.2 utf8\_1.2.4 generics\_0.1.3   
## [73] data.table\_1.15.2 httr\_1.4.7 htmlwidgets\_1.6.4   
## [76] S4Arrays\_1.2.1 uwot\_0.1.16 pkgconfig\_2.0.3   
## [79] gtable\_0.3.5 lmtest\_0.9-40 XVector\_0.42.0   
## [82] htmltools\_0.5.7 carData\_3.0-5 dotCall64\_1.1-1   
## [85] png\_0.1-8 knitr\_1.46 rstudioapi\_0.15.0   
## [88] tzdb\_0.4.0 reshape2\_1.4.4 nlme\_3.1-164   
## [91] zoo\_1.8-12 KernSmooth\_2.23-22 parallel\_4.3.1   
## [94] miniUI\_0.1.1.1 vipor\_0.4.7 ggrastr\_1.0.2   
## [97] pillar\_1.9.0 grid\_4.3.1 vctrs\_0.6.5   
## [100] RANN\_2.6.1 promises\_1.2.1 car\_3.1-2   
## [103] xtable\_1.8-4 cluster\_2.1.6 beeswarm\_0.4.0   
## [106] evaluate\_0.23 cli\_3.6.1 compiler\_4.3.1   
## [109] rlang\_1.1.2 crayon\_1.5.2 future.apply\_1.11.2   
## [112] ggsignif\_0.6.4 labeling\_0.4.3 plyr\_1.8.9   
## [115] ggbeeswarm\_0.7.2 stringi\_1.8.3 viridisLite\_0.4.2   
## [118] deldir\_2.0-4 munsell\_0.5.1 lazyeval\_0.2.2   
## [121] spatstat.geom\_3.2-9 RcppHNSW\_0.6.0 hms\_1.1.3   
## [124] patchwork\_1.2.0 future\_1.33.2 shiny\_1.8.0   
## [127] highr\_0.10 ROCR\_1.0-11 igraph\_2.0.2   
## [130] broom\_1.0.5