**Spatial Transcriptomics: Streamlined Code for Analysis Using RStudio**

library(tidyverse)

library(Seurat)

library(RColorBrewer)

library(scales)

df <- readRDS("C:/Users/mergered8\_clustree.rds")

Idents(df) <- df$SCT\_snn\_res.0.9

mylist <- hue\_pal()(16)

**Figure 1A,D:**

SpatialDimPlot(df, ncol = 4, pt.size.factor = 1.2, alpha = c(0,0), stroke = 0.25, crop = FALSE, label = FALSE, label.size = 1, label.box = T) & NoLegend()

SpatialDimPlot(df, ncol = 4, pt.size.factor = 1.2, alpha = c(1,1), stroke = 0.25, crop = FALSE, label = FALSE, label.size = 1, label.box = T) & NoLegend()

SpatialDimPlot(df, ncol = 4, pt.size.factor = 1.2, alpha = c(1,1), stroke = 0.25, crop = FALSE, label = FALSE, label.size = 1, label.box = T)

**Figure 1B:**

levels(df) <- c("5","11","9","8","6","0","15","2","1","12","4","14","3","7","13","10")

df[["annotatation"]] <- Idents(df)

Idents(df) <- df$annotatation

DimPlot(df, label = T, cols = c(mylist[6],mylist[12],mylist[10],mylist[9],mylist[7],mylist[1],

mylist[16],mylist[3],mylist[2],mylist[13],mylist[5],mylist[15],

mylist[4],mylist[8],mylist[14],mylist[11])) + NoLegend()

**Figure 1C:**

Idents(df) <- df$SCT\_snn\_res.0.9

levels(df) <- c("5","11","9","8","6","0","15","2","1","12","4","14","3","7","13","10")

df <- RenameIdents(df, "5" = "NC-C","11" = "NC-P","9" = "CN","8" = "CN-M1", "6"= "CN-M2","0" = "M",

"15" = "OG","2" = "HEP", "1"="HEP", "12"= "HEP","4"= "HEP","14"= "HEP","3"= "HEP","7"= "HEP",

"13" = "EC","10" = "EC")

df[["zones"]] <- Idents(df)

df$library <- factor(df$library, c("1\_12\_hpi", "7\_24\_hpi", "9\_3\_dpi", "10\_5\_dpi", "11\_7\_dpi", "12\_10dpi", "13\_14dpi", "14\_21dpi"))

Timepoints <- c("0.5dpi", "1dpi", "3dpi", "5dpi", "7dpi", "10dpi", "14dpi", "21dpi")

levels(df$library) <- Timepoints

CellsbyTime\_table <- table(df$library, df$zones)

CellsbyTime\_table <- as.data.frame(CellsbyTime\_table)

colnames(CellsbyTime\_table)

colnames(CellsbyTime\_table)[1] <- "Timepoint"

colnames(CellsbyTime\_table)[2] <- "Zone"

ByDayPropTable <- CellsbyTime\_table %>% group\_by(Timepoint) %>% mutate(percent = prop.table(Freq))

Zone\_colors <- hue\_pal()(9)

dfMinusNonImmune <- subset(df, idents = c("NC-C","NC-P", "CN", "CN-M1", "CN-M2", "M", "OG"))

dfMinusNonImmune7dpi <- subset(dfMinusNonImmune, library != "7dpi")

dfMinusNonImmune7dpi$library <- droplevels(dfMinusNonImmune7dpi$library)

levels(dfMinusNonImmune7dpi$library)

dfMinusNonImmune7dpi$zones <- droplevels(dfMinusNonImmune7dpi$zones)

levels(dfMinusNonImmune7dpi$zones)

ggplot(data = ByDayPropTable, aes(x = Timepoint, y = Zone, size = percent)) +

geom\_point(color = "red") +

scale\_size(range = c(-.4,10), limits = c(0,1))

CellsbyTime\_table <- table(dfMinusNonImmune7dpi$library, dfMinusNonImmune7dpi$zones)

CellsbyTime\_table <- as.data.frame(CellsbyTime\_table)

colnames(CellsbyTime\_table)

colnames(CellsbyTime\_table)[1] <- "Timepoint"

colnames(CellsbyTime\_table)[2] <- "Zone"

ByDayPropTable <- CellsbyTime\_table %>% group\_by(Timepoint) %>% mutate(percent = prop.table(Freq))

Zone\_colors <- hue\_pal()(9)

ggplot(data = ByDayPropTable, aes(x = Timepoint, y = Zone, size = percent)) +

geom\_point(color = "red") +

scale\_size(range = c(-.4,10), limits = c(0,1))

**Figure 1E; Figure 2; Figure 3; Figure 1 – figure supplement 1C; Figure 4 – figure supplement 1 – figure supplement 6:**

Idents(df) <- df$zones

Idents(df) <- df$Timepoint

SpatialFeaturePlot(df, pt.size.factor = 1.2, alpha = c(1,1), stroke = 0.25, "Ccl2", crop = F, ncol = 4) &

scale\_fill\_gradientn(colours = rev(brewer.pal(n = 11, name = "RdYlBu")), limits = c(0,3), oob=squish)

**Figure 5A,B:**

Idents(df) <- df$SCT\_snn\_res.0.9

df[["annotatation"]] <- Idents(df)

df <- RenameIdents(df, "5" = "NC-C","11" = "NC-P","9" = "CN","8"= "CN-M1","6" = "CN-M2", "0" = "M",

"15" = "OG","2" = "HEP0", "1"="HEP1", "12"= "HEP2","4"= "HEP3","14"= "rep HEP","3"= "HEP4","7"= "HEP5",

"13" = "EC1","10" = "EC2")

FeaturePlot(df, c("Cxcl1"), label = T, max.cutoff = 1.5) + scale\_colour\_gradientn(colours = rev(brewer.pal(n = 7, name = "RdBu"))) + theme(text=element\_text(family="Arial"))

**Figure 5C,D:**

df <- RenameIdents(df, "5" = "NC-C","11" = "NC-P","9" = "CN","8"= "CN-M1","6" = "CN-M2", "0" = "M",

"15" = "OG","2" = "HEP0", "1"="HEP1", "12"= "HEP2","4"= "HEP3","14"= "rep HEP","3"= "HEP4","7"= "HEP5",

"13" = "EC1","10" = "EC2")

df[["zones"]] <- Idents(df)

VlnPlot(df, c("Cxcl1"), split.by = "zones", ncol = 1, cols = c(mylist[6],mylist[12],mylist[10],mylist[9],mylist[7],mylist[1], mylist[16],mylist[3],mylist[2],mylist[13],mylist[5],mylist[15],mylist[4],mylist[8],mylist[14],mylist[11]))

**Figure 5E,F:**

df$library <- factor(df$library, c("1\_12\_hpi", "7\_24\_hpi", "9\_3\_dpi", "10\_5\_dpi", "12\_10dpi", "13\_14dpi", "14\_21dpi"))

Timepoints <- c("0.5dpi", "1dpi", "3dpi", "5dpi", "10dpi", "14dpi", "21dpi")

levels(df$library) <- Timepoints

Idents(df) <- factor(df$library)

df[["Timepoint"]] <- Idents(df)

VlnPlot(df, c("Cxcl1"), split.by = "Timepoint", ncol = 1)

**Figure 1 – figure supplement 1A:**

SpatialFeaturePlot(df, pt.size.factor = 1.2, alpha = c(1,1), stroke = 0.25, features = "nCount\_Spatial", crop = F, ncol = 4) &

scale\_fill\_gradientn(colours = rev(brewer.pal(n = 11, name = "RdYlBu")), limits = c(0,60000), oob=squish)

**Figure 1 – figure supplement 1B:**

VlnPlot(df, features = "nCount\_Spatial", pt.size = 0.1, cols = c(mylist[6],mylist[12],mylist[10],mylist[9],mylist[7],mylist[1],

mylist[16],mylist[3],mylist[2],mylist[13],mylist[5],mylist[15],

mylist[4],mylist[8],mylist[14],mylist[11]))

**Table 4:**

PrepSCTFindMarkers(df)

markers <- FindAllMarkers(df, recorrect\_umi=FALSE, only.pos = T, min.pct = 0.25, assay = "SCT")

write.csv(markers, "C:/Users/13178/Desktop/myfile.csv")