

Figure-S2H.R

sokole

2024-07-23

```
# This Script Generates Figure S2H
# Script By: Eishani Kumar Sokolowski

# Empty the environment & suppress warnings
rm(list = ls())
options(warn=-1)

# Loading libraries
library(dplyr)
```

```
##
## 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
```

```
library(ggplot2)
library(cowplot)
library(ggpubr)
```

```
##
## Attaching package: 'ggpubr'
```

```
## The following object is masked from 'package:cowplot':
##
##   get_legend
```

```
library(tidyverse)
```

```
## — Attaching core tidyverse packages — tidyverse 2.0.0 —
## ✓ forcats    1.0.0    ✓ stringr    1.5.1
## ✓ lubridate  1.9.3    ✓ tibble     3.2.1
## ✓ purrr      1.0.2    ✓ tidyr      1.3.1
## ✓ readr      2.1.5
```

```
## — Conflicts ————— tidyverse_conflicts() —
## * dplyr::filter()   masks stats::filter()
## * dplyr::lag()      masks stats::lag()
## * lubridate::stamp() masks cowplot::stamp()
## i Use the conflicted package (<http://conflicted.r-lib.org/>) to force all conflicts
to become errors
```

```
library(plyr)
```

```
## -----
## You have loaded plyr after dplyr – this is likely to cause problems.
## If you need functions from both plyr and dplyr, please load plyr first, then dplyr:
## library(plyr); library(dplyr)
## -----
##
## Attaching package: 'plyr'
##
## The following object is masked from 'package:purrr':
##
##   compact
##
## The following object is masked from 'package:ggpubr':
##
##   mutate
##
## The following objects are masked from 'package:dplyr':
##
##   arrange, count, desc, failwith, id, mutate, rename, summarise,
##   summarize
```

```
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(reshape2)
```

```
##  
## Attaching package: 'reshape2'  
##  
## The following object is masked from 'package:tidyr':  
##  
##      smiths
```

```
library(Seurat)
```

```
## Loading required package: SeuratObject  
## Loading required package: sp  
## 'SeuratObject' was built under R 4.4.0 but the current version is  
## 4.4.1; it is recommended that you reinstall 'SeuratObject' as the ABI  
## for R may have changed  
##  
## Attaching package: 'SeuratObject'  
##  
## The following object is masked from 'package:base':  
##  
##      intersect
```

```
# Loading the seurat object  
islet <- readRDS("./Combined_Islet_150_Islet_162_Islet_168_Islet_67_Islet_116_Islet_117_  
Cluster_All_Cell_Type_Identities_Finalized.rds")  
  
# Subsetting seurat object by cell-type  
islet <- subset(x = islet, ids = "Acinar", invert=TRUE)  
islet <- subset(x = islet, ids = "Proliferating Alpha", invert=TRUE)  
islet <- subset(x = islet, ids = "Endothelial", invert=TRUE)  
islet <- subset(x = islet, ids = "Immune", invert=TRUE)  
islet <- subset(x = islet, ids = "Activated Stellate", invert=TRUE)  
islet <- subset(x = islet, ids = "Schwann", invert=TRUE)  
islet <- subset(x = islet, ids = "Gamma", invert=TRUE)  
islet <- subset(x = islet, ids = "Delta", invert=TRUE)  
islet <- subset(x = islet, ids = "Stellate", invert=TRUE)  
islet <- subset(x = islet, ids = "Ductal", invert=TRUE)  
  
# Making HTO label as ids and scaling it  
Ids(islet) <- "HTO_Label"  
islet <- ScaleData(object = islet, features = rownames(islet))
```

```
## Centering and scaling data matrix
```

```

# Loading the genes
genes.of.interest <- c("MAFB",
                      "SCG2",
                      "SHISAL2B")

# Create a new column
islet$Condition <- islet$HT0_Label
islet$Condition <- gsub("24 Hr DMSO","A_ERS",islet$Condition)
islet$Condition <- gsub("24 Hr Thapsigargin","A_ERS",islet$Condition)
islet$Condition <- gsub("24 Hr Untreated","B_CYT",islet$Condition)
islet$Condition <- gsub("24 Hr Cytokines","B_CYT",islet$Condition)

# Rename Treatment
islet$Treatment <- islet$HT0_Label
islet$Treatment <- gsub("24 Hr DMSO","A_24 Hr DMSO",islet$Treatment)
islet$Treatment <- gsub("24 Hr Thapsigargin","B_24 Hr Thapsigargin",islet$Treatment)
islet$Treatment <- gsub("24 Hr Untreated","C_24 Hr Untreated",islet$Treatment)
islet$Treatment <- gsub("24 Hr Cytokines","D_24 Hr Cytokines",islet$Treatment)

# Create a new column
islet$Cell_Type_Treatment <- paste0(islet$Cell_Type, " - ", islet$Treatment)

# Create a new column
islet$Type <- paste0(islet$Condition, " - ", islet$Cell_Type_Treatment)

# Plotting Violin Plots
VlnPlot(islet, genes.of.interest, split.by = "Treatment", group.by = "Type", stack = TRUE,
        sort = FALSE, flip = TRUE) +
  ylab("Normalized Gene Expression") +
  scale_fill_manual(values = c('grey50', 'forestgreen','grey','darkorange')) + theme(legend.position="bottom", axis.title.x = element_blank()) +
  theme(axis.text.x = element_text(size=5))

```

```

## The default behaviour of split.by has changed.
## Separate violin plots are now plotted side-by-side.
## To restore the old behaviour of a single split violin,
## set split.plot = TRUE.
##
## This message will be shown once per session.
## Scale for fill is already present.
## Adding another scale for fill, which will replace the existing scale.

```

