Figure-2D.R

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```
# This Script Generates Figure 2D
# 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)
```

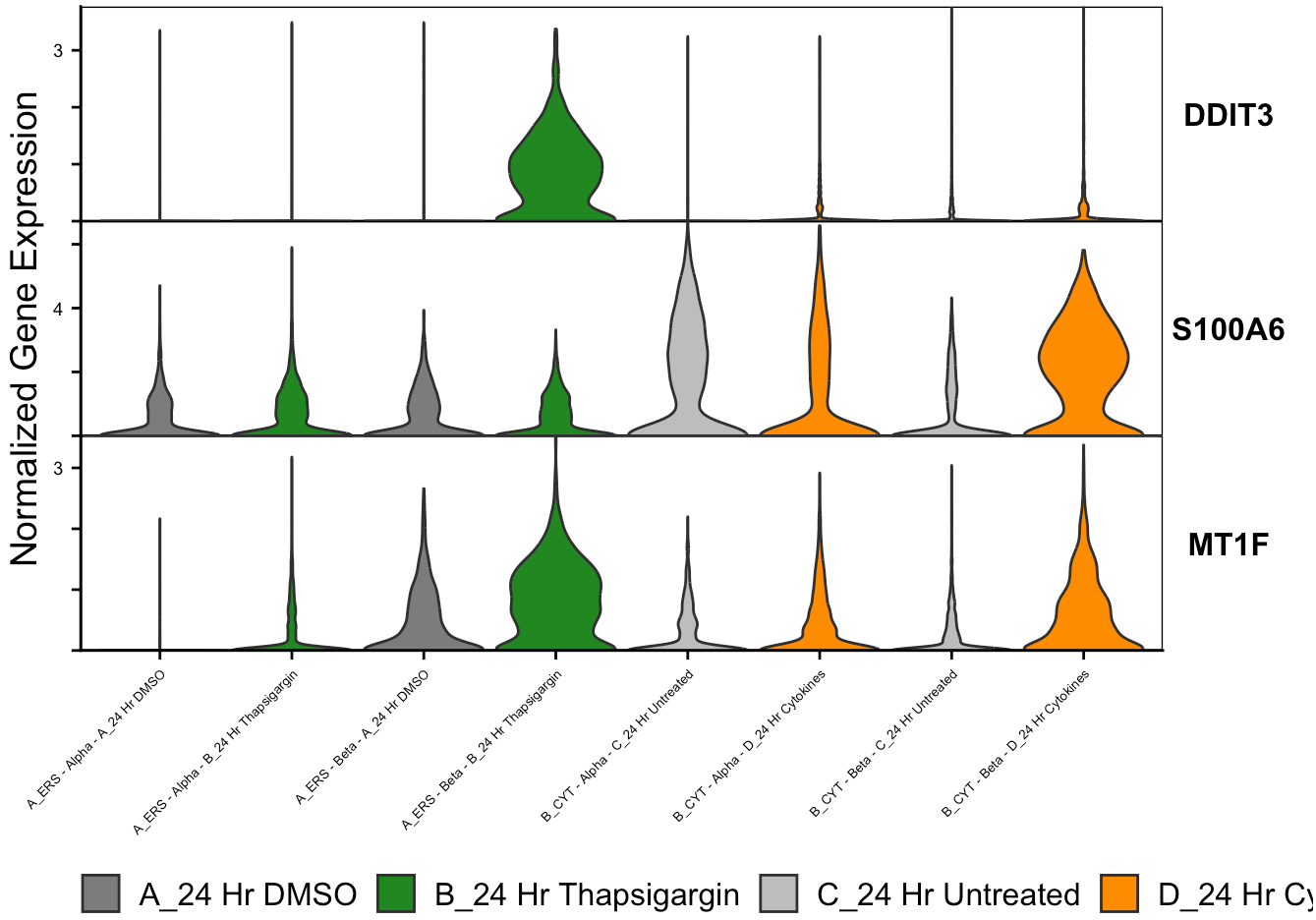
```
## Loading required package: SeuratObject
## Loading required package: sp
## 'SeuratObject' was built under R 4.4.0 but the current version is
## 4.4.1; it is recomended that you reinstall 'SeuratObject' as the ABI
## for R may have changed
##
## Attaching package: 'SeuratObject'
##
## The following 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, idents = "Acinar", invert=TRUE)
islet <- subset(x = islet, idents = "Proliferating Alpha", invert=TRUE)
islet <- subset(x = islet, idents = "Endothelial", invert=TRUE)
islet <- subset(x = islet, idents = "Immune", invert=TRUE)
islet <- subset(x = islet, idents = "Activated Stellate", invert=TRUE)
islet <- subset(x = islet, idents = "Schwann", invert=TRUE)
islet <- subset(x = islet, idents = "Gamma", invert=TRUE)
islet <- subset(x = islet, idents = "Delta", invert=TRUE)
islet <- subset(x = islet, idents = "Stellate", invert=TRUE)
islet <- subset(x = islet, idents = "Ductal", invert=TRUE)

# Making HTO label as idents and scaling it
Idents(islet) <- "HTO_Label"
islet <- ScaleData(object = islet, features = rownames(islet))
```

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

```
# Loading the genes
genes.of.interest <- c("DDIT3",
                       "S100A6",
                       "MT1F")

# Create a new column
islet$Condition <- islet$HTO_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$Treatment <- islet$HTO_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 = TRU
E, sort = FALSE, flip = TRUE) +
  ylab("Normalized Gene Expression") +
  scale_fill_manual(values = c('grey50', 'forestgreen','grey','darkorange')) + theme(leg
end.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.
```


Normalized Gene Expression
DDIT3
S100A6
MT1F
A_ERS - Alpha - A_24 Hr DMSO
A_ERS - Alpha - B_24 Hr Thapsigargin
A_ERS - Beta - A_24 Hr DMSO
A_ERS - Beta - B_24 Hr Thapsigargin
B_CYT - Alpha - C_24 Hr Untreated
B_CYT - Alpha - D_24 Hr Cytokines
B_CYT - Beta - C_24 Hr Untreated
B_CYT - Beta - D_24 Hr Cytokines
A_24 Hr DMSO
B_24 Hr Thapsigargin
C_24 Hr Untreated
D_24 Hr Cy