Supplemental code
The following code was used to generate the Supplemental-Fig-S3

#First load the libraries

```
library(dplyr)
library(Seurat)
library(patchwork)
library(ggplot2)
library(stringr)
library(SoupX)
library(reticulate)
library(glmpca)
library(SeuratWrappers)
library(scry)
library(reticulate)
library(monocle3)
library(FlexDotPlot)
library(cowplot)
library(tidyverse)
library(viridis)
library(scCustomize)
library(qs)
library(gridExtra)
library(plyr)
library(circlize)
library(ComplexHeatmap)
library(EnhancedVolcano)
library(data.table)
library(magrittr)
library(raster)
library(rgdal)
library(classInt)
library(RColorBrewer)
library(Seurat)
library(SeuratDisk)

rm(scrna.combined.seurat.hassan072022)
```

#load the object
```
scrna.combined.seurat.hassan072022 <- readRDS("/Volumes/Macintosh
HD/Users/hassanbukhari/Desktop/OneDrive - Mass General Brigham/Tau P251LKI figures
updated-02-27-223/New Seurat Object-2023/seurat_multi_rerun_annotations
2/scrna.combined.seurat.hassan_2022.rds")


p1 <- DimPlot(scrna.combined.seurat.hassan072022, reduction = "umap", label = TRUE, raster
= FALSE, repel = TRUE)
p1
```

```
#Rename the Seurat Object to scrna.combined

scrna.combined <-  scrna.combined.seurat.hassan072022
rm(scrna.combined.seurat.hassan072022)

#Now subset all the control. Save them and count their cells.


# Split the control1 object
Control1 <- subset(scrna.combined, subset= orig.ident=="control1")
p1 <- DimPlot(Control1, reduction = "umap", label = FALSE, repel = TRUE)
p1

saveRDS(Control1, paste0("/Volumes/Macintosh HD/Users/hassanbukhari/Desktop/OneDrive -
Mass General Brigham/Tau P251LKI figures updated-02-27-223/Figure 3/Figure 3
Supplementary files/", "Control1.rds"))

md1<- Control1@meta.data %>% as.data.table

a <-  md1[, .N, by = c("seurat_clusters")]

write.table (a, paste0("/Volumes/Macintosh HD/Users/hassanbukhari/Desktop/OneDrive - Mass
General Brigham/Tau P251LKI figures updated-02-27-223/Figure 3/Figure 3 Supplementary
files/", "Control1-Table.xls"),sep='\t',row.names=F,col.names=T, quote=F)

# Split the control3 object
Control3 <- subset(scrna.combined, subset= orig.ident=="CRN00224919")
p2 <- DimPlot(Control3, reduction = "umap", label = FALSE, repel = TRUE)
p2

saveRDS(Control3, paste0("/Volumes/Macintosh HD/Users/hassanbukhari/Desktop/OneDrive -
Mass General Brigham/Tau P251LKI figures updated-02-27-223/Figure 3/Figure 3
Supplementary files/", "Control3.rds"))

md1<- Control3@meta.data %>% as.data.table

a <-  md1[, .N, by = c("seurat_clusters")]

write.table (a, paste0("/Volumes/Macintosh HD/Users/hassanbukhari/Desktop/OneDrive - Mass
General Brigham/Tau P251LKI figures updated-02-27-223/Figure 3/Figure 3 Supplementary
files/", "Control3-Table.xls"),sep='\t',row.names=F,col.names=T, quote=F)


# Split the control2 object
Control2 <- subset(scrna.combined, subset= orig.ident=="CTRL2merged")
p2 <- DimPlot(Control2, reduction = "umap", label = FALSE, repel = TRUE)
p2

saveRDS(Control2, paste0("/Volumes/Macintosh HD/Users/hassanbukhari/Desktop/OneDrive -
Mass General Brigham/Tau P251LKI figures updated-02-27-223/Figure 3/Figure 3
Supplementary files/", "Control2.rds"))
```

```
md1<- Control2@meta.data %>% as.data.table

a <-  md1[, .N, by = c("seurat_clusters")]

write.table (a, paste0("/Volumes/Macintosh HD/Users/hassanbukhari/Desktop/OneDrive - Mass
General Brigham/Tau P251LKI figures updated-02-27-223/Figure 3/Figure 3 Supplementary
files/", "Control2-Table.xls"),sep='\t',row.names=F,col.names=T, quote=F)

# Split the P251L1 object
P251L1 <- subset(scrna.combined, subset= orig.ident=="P251L1merged")
p1 <- DimPlot(P251L1, reduction = "umap", label = FALSE, repel = TRUE)
p1

saveRDS(P251L1, paste0("/Volumes/Macintosh HD/Users/hassanbukhari/Desktop/OneDrive -
Mass General Brigham/Tau P251LKI figures updated-02-27-223/Figure 3/Figure 3
Supplementary files/", "P251L1.rds"))

md1<- P251L1@meta.data %>% as.data.table

a <-  md1[, .N, by = c("seurat_clusters")]

write.table (a, paste0("/Volumes/Macintosh HD/Users/hassanbukhari/Desktop/OneDrive - Mass
General Brigham/Tau P251LKI figures updated-02-27-223/Figure 3/Figure 3 Supplementary
files/", "P251L1.xls"),sep='\t',row.names=F,col.names=T, quote=F)

# Split the P251L2 object
P251L2 <- subset(scrna.combined, subset= orig.ident=="P251L2merged")
p1 <- DimPlot(P251L2, reduction = "umap", label = FALSE, repel = TRUE)
p1

saveRDS(P251L2, paste0("/Volumes/Macintosh HD/Users/hassanbukhari/Desktop/OneDrive -
Mass General Brigham/Tau P251LKI figures updated-02-27-223/Figure 3/Figure 3
Supplementary files/", "P251L2.rds"))

md1<- P251L2@meta.data %>% as.data.table

a <-  md1[, .N, by = c("seurat_clusters")]

write.table (a, paste0("/Volumes/Macintosh HD/Users/hassanbukhari/Desktop/OneDrive - Mass
General Brigham/Tau P251LKI figures updated-02-27-223/Figure 3/Figure 3 Supplementary
files/", "P251L2.xls"),sep='\t',row.names=F,col.names=T, quote=F)


# Split the P251L3 object
P251L3 <- subset(scrna.combined, subset= orig.ident=="LIB055588_CRN00233457")
p1 <- DimPlot(P251L3, reduction = "umap", label = FALSE, repel = TRUE)
p1
```

```
saveRDS(P251L3, paste0("/Volumes/Macintosh HD/Users/hassanbukhari/Desktop/OneDrive -
Mass General Brigham/Tau P251LKI figures updated-02-27-223/Figure 3/Figure 3
Supplementary files/", "P251L3.rds"))

md1<- P251L3@meta.data %>% as.data.table

a <-  md1[, .N, by = c("seurat_clusters")]

write.table (a, paste0("/Volumes/Macintosh HD/Users/hassanbukhari/Desktop/OneDrive - Mass
General Brigham/Tau P251LKI figures updated-02-27-223/Figure 3/Figure 3 Supplementary
files/", "P251L3.xls"),sep='\t',row.names=F,col.names=T, quote=F)


#Split the controls together
Controls <- subset(scrna.combined, subset= treatment =="control")

p1 <- DimPlot(Controls, reduction = "umap", label = FALSE, repel = FALSE)
p1

saveRDS(Controls, paste0("/Volumes/Macintosh HD/Users/hassanbukhari/Desktop/OneDrive -
Mass General Brigham/Tau P251LKI figures updated-02-27-223/Figure 3/Figure 3
Supplementary files/", "Controls.rds"))

md1<- Controls@meta.data %>% as.data.table

a <-  md1[, .N, by = c("seurat_clusters")]

write.table (a, paste0("/Volumes/Macintosh HD/Users/hassanbukhari/Desktop/OneDrive - Mass
General Brigham/Tau P251LKI figures updated-02-27-223/Figure 3/Figure 3 Supplementary
files//", "Controls.xls"),sep='\t',row.names=F,col.names=T, quote=F)

#Split the Knock ins together

rm(scrna.combined.seurat.hassan072022)

P251Ls <- subset(scrna.combined, subset= treatment =="P251L")


#load the knock in

#P251Ls  <- readRDS(paste0("/Volumes/shb27/SC-Hassan/Seurat Multi3/Samples
separated/P251Ls/P251Ls.rds"))

p1 <- DimPlot(P251Ls, reduction = "umap", label = FALSE, repel = TRUE)
p1

saveRDS(P251Ls, paste0("/Volumes/Macintosh HD/Users/hassanbukhari/Desktop/OneDrive -
Mass General Brigham/Tau P251LKI figures updated-02-27-223/Figure 3/Figure 3
Supplementary files/", "P251Ls.rds"))

md1<- P251Ls@meta.data %>% as.data.table
```

```
a <-  md1[, .N, by = c("seurat_clusters")]

write.table (a, paste0("/Volumes/Macintosh HD/Users/hassanbukhari/Desktop/OneDrive - Mass
General Brigham/Tau P251LKI figures updated-02-27-223/Figure 3/Figure 3 Supplementary
files/", "P251Ls.xls"),sep='\t',row.names=F,col.names=T, quote=F)


#to show representative Cholinergic

FeaturePlot(scrna.combined.seurat.hassan072022, "FBgn0270928", raster = FALSE) +
scale_colour_gradientn(colours = brewer.pal(n = 9, name = "PuBu"))

#to show representative GABAergic

FeaturePlot(scrna.combined.seurat.hassan072022, "FBgn0004516", raster = FALSE) +
scale_colour_gradientn(colours = brewer.pal(n = 9, name = "Greys"))

#to show representative Glutamatergic

FeaturePlot(scrna.combined.seurat.hassan072022, "FBgn0031424", raster = FALSE) +
scale_colour_gradientn(colours = brewer.pal(n = 9, name = "Greens"))

#to show representative Dopaminergic

FeaturePlot(scrna.combined.seurat.hassan072022, "FBgn0034136", raster = FALSE) +
scale_colour_gradientn(colours = brewer.pal(n = 9, name = "BuPu"))


#Now to determine the cells expressing > 2 of the gene within the all the datasets


#For Control 1

Control1
Control1 <- subset(scrna.combined, subset= orig.ident=="control1")

#cholinergic.neurons
cholinergic.neurons <- subset(x = Control1, subset = FBgn0270928 > 2)
cholinergic.neurons

#Glutamatergic.neurons
Glutamatergic.neurons <- subset(x = Control1, subset = FBgn0031424 > 2)
Glutamatergic.neurons

#GABAergic.neurons
GABAergic.neurons <- subset(x = Control1, subset = FBgn0004516 > 2)
GABAergic.neurons

#Dopaminergic.neurons
```

```r
Dopaminergic.neurons <- subset(x = Control1, subset = FBgn0034136 > 2)
Dopaminergic.neurons


#For Control2

Control2 <- subset(scrna.combined, subset= orig.ident=="CTRL2merged")
Control2
#cholinergic.neurons
cholinergic.neurons <- subset(x = Control2, subset = FBgn0270928 > 2)
cholinergic.neurons

#Glutamatergic.neurons
Glutamatergic.neurons <- subset(x = Control2, subset = FBgn0031424 > 2)
Glutamatergic.neurons

#GABAergic.neurons
GABAergic.neurons <- subset(x = Control2, subset = FBgn0004516 > 2)
GABAergic.neurons

#Dopaminergic.neurons
Dopaminergic.neurons <- subset(x = Control2, subset = FBgn0034136 > 2)
Dopaminergic.neurons

# For Control3
Control3 <- subset(scrna.combined, subset= orig.ident=="CRN00224919")
Control3
#cholinergic.neurons
cholinergic.neurons <- subset(x = Control3, subset = FBgn0270928 > 2)
cholinergic.neurons

#Glutamatergic.neurons
Glutamatergic.neurons <- subset(x = Control3, subset = FBgn0031424 > 2)
Glutamatergic.neurons

#GABAergic.neurons
GABAergic.neurons <- subset(x = Control3, subset = FBgn0004516 > 2)
GABAergic.neurons

#Dopaminergic.neurons
Dopaminergic.neurons <- subset(x = Control3, subset = FBgn0034136 > 2)
Dopaminergic.neurons

#For P251L1
P251L1 <- subset(scrna.combined, subset= orig.ident=="P251L1merged")
P251L1

#cholinergic.neurons
cholinergic.neurons <- subset(x = P251L1, subset = FBgn0270928 > 2)
cholinergic.neurons
```

```
#Glutamatergic.neurons
Glutamatergic.neurons <- subset(x = P251L1, subset = FBgn0031424 > 2)
Glutamatergic.neurons

#GABAergic.neurons
GABAergic.neurons <- subset(x = P251L1, subset = FBgn0004516 > 2)
GABAergic.neurons

#Dopaminergic.neurons
Dopaminergic.neurons <- subset(x = P251L1, subset = FBgn0034136 > 2)
Dopaminergic.neurons


#For P251L2
P251L2 <- subset(scrna.combined, subset= orig.ident=="P251L2merged")
P251L2

#cholinergic.neurons
cholinergic.neurons <- subset(x = P251L2, subset = FBgn0270928 > 2)
cholinergic.neurons

#Glutamatergic.neurons
Glutamatergic.neurons <- subset(x = P251L2, subset = FBgn0031424 > 2)
Glutamatergic.neurons

#GABAergic.neurons
GABAergic.neurons <- subset(x = P251L2, subset = FBgn0004516 > 2)
GABAergic.neurons

#Dopaminergic.neurons
Dopaminergic.neurons <- subset(x = P251L2, subset = FBgn0034136 > 2)
Dopaminergic.neurons


#For P251L3
P251L3 <- subset(scrna.combined, subset= orig.ident=="LIB055588_CRN00233457")
P251L3

#cholinergic.neurons
cholinergic.neurons <- subset(x = P251L3, subset = FBgn0270928 > 2)
cholinergic.neurons

#Glutamatergic.neurons
Glutamatergic.neurons <- subset(x = P251L3, subset = FBgn0031424 > 2)
Glutamatergic.neurons

#GABAergic.neurons
GABAergic.neurons <- subset(x = P251L3, subset = FBgn0004516 > 2)
GABAergic.neurons

#Dopaminergic.neurons
```

```
Dopaminergic.neurons <- subset(x = P251L3, subset = FBgn0034136 > 2)
Dopaminergic.neurons
```

#For the feature plots use the following code

```
#Kenyon cells #jdp #dac #crb
FeaturePlot(scrna.combined, features = c("FBgn0027654", "FBgn0005677", "FBgn0259685"),
raster = FALSE)


#MBON #Yp1 #Yp2 #Yp3
FeaturePlot(scrna.combined, features = c("FBgn0004045", "FBgn0005391", "FBgn0004047"),
raster = FALSE)

#Glia #MtnA #CG8369 #CG1552
FeaturePlot(scrna.combined, features = c("FBgn0002868", "FBgn0040532", "FBgn0030258"),
raster = FALSE)

#Medullary neurons #CG34355 #Gad1 #mamo
FeaturePlot(scrna.combined, features = c("FBgn0085384", "FBgn0004516", "FBgn0267033"),
raster = FALSE)

# T neurons #acj6 #Lim1 #Eaat1
FeaturePlot(scrna.combined, features = c("FBgn0000028", "FBgn0026411", "FBgn0026439"),
raster = FALSE)
```