Wagner20_Script_CortexMedulla

Packages used

```
library(Seurat)
library(ggplot2)
library(Matrix)
library(dplyr)
library(devtools)
library(umap)
library(clustree)
library(ggplot2)
library(gdata)
```

1. Load Seurat objects, Seurat v3

```
#### medulla: dataset from Fan et al. 2019, GEO
#### cortex: unsorted adult ovarian cortex dataset (run1) "Wagner2020_unsorted"

medulla <- readRDS("/san/lanner-lab/sharedJPLeni/Objects/medulla_fan2019.rds")
cortex <- readRDS("/san/lanner-lab/sharedJPLeni/Objects/cortex_run1.rds")
```

2. pre-processing and CCA integration

```
s.genes <- cc.genes$s.genes
g2m.genes <- cc.genes$g2m.genes

DefaultAssay(cortex) <- "RNA"

medulla<-NormalizeData(medulla, verbose = FALSE)
medulla<-FindVariableFeatures(medulla, selection.method = "vst", nfeatures = 2000,
verbose = FALSE)
cortex<-NormalizeData(cortex, verbose = FALSE)
cortex<-FindVariableFeatures(cortex, selection.method = "vst", nfeatures = 2000, v
erbose = FALSE)
cortex<-AddMetaData(cortex,metadata =cortex@active.ident, col.name="cluster")

anchors <- FindIntegrationAnchors(object.list = c(medulla,cortex), dims = 1:25)
integrated <- IntegrateData(anchorset = anchors, dims = 1:25)


integrated <- CellCycleScoring(integrated, s.features = s.genes, g2m.features = g2
m.genes, set.ident = TRUE)
```

3. ScaleData

```
DefaultAssay(integrated) <- "integrated"

integrated <- ScaleData(integrated, vars.to.regress = c("S.Score", "G2M.Score"), f
eatures = rownames(integrated))
integrated <- RunPCA(integrated, npcs = 21)
integrated <- RunUMAP(integrated, reduction = "pca", dims = 1:21)
```

4. Visualization, Renaming

```
integrated <- FindNeighbors(object = integrated, reduction = "pca", dims = 1:21)
integrated <- FindClusters(object = integrated,
                           dims = 1:21, resolution = 0.25, save.SNN = TRUE, fo
rce.recalc = TRUE, verbose = FALSE)
```

5. Figures

```
DefaultAssay(integrated) <- "RNA"

#Supplementary Fig. 2e
DimPlot(integrated, reduction = "umap", label = F, group.by = "cluster",order = c(
'oocytes','immune','granulosa','endothelial','perivascular','stroma',18:0), cols =
c("grey", "grey", "grey", "grey", "grey", "grey", "#FEE5D9", "#FCBBA1", "#FC9272",
"blue", "darkorchid3", "#FB6A4A", "#DE2D26",  "mediumspringgreen", "#BDD7E7", "#A1
D99B", "#6BAED6", "skyblue4", "violet", "mediumpurple1", "sienna1", "#08519C", "#3
1A354", "tan3", "#6A51A3")) + guides(colour = guide_legend(override.aes = list(siz
e=8)))

#Supplementary Fig. 2f
DimPlot(integrated, reduction = "umap", label = F, group.by = "cluster",order = c(
'1_oocytes','2_immune','3_gran','4_endo','5_pv','6_stroma',18:0), cols = c("lightg
rey", "lightgrey", "lightgrey", "lightgrey", "lightgrey", "lightgrey", "lightgrey"
,  "lightgrey", "lightgrey", "lightgrey", "lightgrey", "lightgrey", "lightgrey", "
lightgrey", "lightgrey", "lightgrey", "lightgrey", "lightgrey", "lightgrey", "gray
34", "#C49A00", "#53B400", "#00B6EB", "darkorchid", "#FB61D7")) + guides(colour =
guide_legend(override.aes = list(size=8)))
```