Wagner20_Script_Unsorted

Packages used

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

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1. Create Seurat objects, Seurat v3

```
#unsorted: filtered outs, Seurat objects created separately for TGP and C-Sec befo
re merging
#unsorted: TGP sample
ovar.data_062 <- Read10X(data.dir="/san/lanner-lab/sharedJPLeni/Objects/hg19_TGP")
ovar_062 <- CreateSeuratObject(counts = ovar.data_062, min.cells= 3, min.features
= 200, project = "10XProject-062")
ovar_062[["percent.mt"]] <-PercentageFeatureSet(object = ovar_062, pattern = "^MT-
")
ovar_062 <-subset(x = ovar_062, subset = nFeature_RNA>200&nFeature_RNA<7000&percen
t.mt<25)

#unsorted: C-Sec sample
ovar.data_063 <- Read10X(data.dir="/san/lanner-lab/sharedJPLeni/Objects/hg19_CSec"
)
ovar_063 <- CreateSeuratObject(counts = ovar.data_063, min.cells= 3, min.features
= 200, project = "10XProject-063")
ovar_063[["percent.mt"]] <-PercentageFeatureSet(object = ovar_063, pattern = "^MT-
")
ovar_063 <-subset(x = ovar_063, subset = nFeature_RNA>200&nFeature_RNA<7000&percen
t.mt<25)
```

2. pre-processing and CCA integration

```
ovar_unsorted_TGP <- NormalizeData(object = ovar_062, normalization.method = "LogN
ormalize",
                           scale.factor = 10000, verbose = FALSE)
ovar_unsorted_CSec <- NormalizeData(object = ovar_063, normalization.method = "Log
Normalize",
                           scale.factor = 10000, verbose = FALSE)

ovar_unsorted_TGP <- FindVariableFeatures(object = ovar_unsorted_TGP, selection.me
thod = "vst",
        nfeatures = 2000, verbose = FALSE)
ovar_unsorted_CSec <- FindVariableFeatures(object = ovar_unsorted_CSec, selection.
method = "vst",
        nfeatures = 2000, verbose = FALSE)

reference.list <- c(ovar_unsorted_TGP, ovar_unsorted_CSec)

Ovar_unsorted.anchors <- FindIntegrationAnchors(object.list = reference.list, dims
= 1:30)

Ovar_unsorted.integrated <- IntegrateData(anchorset = Ovar_unsorted.anchors, dims
= 1:30)
```

3. ScaleData

```
DefaultAssay(object = Ovar_unsorted.integrated) <- "integrated"

Ovar_unsorted.integrated <- ScaleData(object = Ovar_unsorted.integrated, verbose =
FALSE)
Ovar_unsorted.integrated <- RunPCA(object = Ovar_unsorted.integrated, npcs = 13, v
erbose = FALSE)
Ovar_unsorted.integrated <- RunUMAP(object = Ovar_unsorted.integrated, reduction =
"pca", dims = 1:13)
```

4. Visualization, Renaming

```
Ovar_unsorted.integrated <- FindNeighbors(object = Ovar_unsorted.integrated, reduc
tion = "pca", dims = 1:13)
Ovar_unsorted.integrated <- FindClusters(Ovar_unsorted.integrated, resolution = 0.
1)


Ovar_unsorted.integrated <- RenameIdents(object = Ovar_unsorted.integrated, '5' =
'1_oocytes', '4' = '2_immune', '3' = '3_gran', '2' = '4_endo', '1' = '5_pv', '0' =
'6_stroma')
levels(x = Ovar_unsorted.integrated)
```

5. Tables and Figures, no *ddx4

```
#Fig.2a
DimPlot(object = Ovar_unsorted.integrated, reduction = "umap", group.by = "orig.id
ent", cols = c("black", "grey"))
```

```
#Fig.2b
DimPlot(object = Ovar_unsorted.integrated, reduction = "umap", group.by = "ident",
cols = c("#FB61D7", "#A58AFF", "#00B6EB", "#53B400", "#C49A00", "grey"), label = F
, repel = TRUE)


#Fig. 2c,e Heatmap
###Assay RNA, slot "scale.data" after scaling rna data

DefaultAssay(object = Ovar_unsorted.integrated) <- "RNA"

###Downsample the clusters to a maximum of 300 cells each
Ovar_unsorted.integrated <- ScaleData(object = Ovar_unsorted.integrated, verbose =
FALSE)
Ovar_unsorted.integrated.small <- subset(Ovar_unsorted.integrated, downsample = 30
0, random.seed = 100)
heatmap300.markers <- FindAllMarkers(Ovar_unsorted.integrated.small, assay = "RNA"
, only.pos = TRUE)

DoHeatmap(Ovar_unsorted.integrated.small, features = unique(heatmap300.markers$gen
e), assay = "RNA", slot = 'scale.data', angle = 90, disp.max =3) + scale_fill_grad
ientn(colors = c("blue", "black", "yellow"))


#Fig.2d VlnPlots
###Stroma
stroma <- c("DCN", "PDGFRA", "APOE", "FHL2")
stroma_percent <- colSums(Ovar_unsorted.integrated@assays$RNA@counts[stroma, ])/co
lSums(Ovar_unsorted.integrated@assays$RNA@counts)
Ovar_unsorted.integrated <- AddMetaData(object = Ovar_unsorted.integrated, metadat
a = stroma_percent , col.name = "stroma_percent")
stroma <- VlnPlot(Ovar_unsorted.integrated, features = c("stroma_percent"), cols =
c("#FB61D7", "#A58AFF", "#00B6EB", "#53B400", "#C49A00", "grey"), pt.size=0.05) +
NoLegend() +
stat_summary(fun.y = median, geom='point', size = 20, colour = "black", shape = 95
)
###Perivascular
pv <- c("RGS5", "MCAM", "RERGL", "TAGLN", "MYH11")
pv_percent <- colSums(Ovar_unsorted.integrated@assays$RNA@counts[pv, ])/colSums(Ov
ar_unsorted.integrated@assays$RNA@counts)
Ovar_unsorted.integrated <- AddMetaData(object = Ovar_unsorted.integrated, metadat
a = pv_percent , col.name = "pv_percent")
pv <- VlnPlot(Ovar_unsorted.integrated, features = c("pv_percent"), cols = c("#FB6
1D7", "#A58AFF", "#00B6EB", "#53B400", "#C49A00", "grey"), pt.size=0.05) +  NoLege
nd() +
stat_summary(fun.y = median, geom='point', size = 20, colour = "black", shape = 95
)
###Endothelial
endo <- c("CD34", "VWF", "FLI1", "CDH5")
endo_percent <- colSums(Ovar_unsorted.integrated@assays$RNA@counts[endo, ])/colSum
s(Ovar_unsorted.integrated@assays$RNA@counts)
Ovar_unsorted.integrated <- AddMetaData(object = Ovar_unsorted.integrated, metadat
a = endo_percent , col.name = "endo_percent")
endo <- VlnPlot(Ovar_unsorted.integrated, features = c("endo_percent"), cols = c("
```

```
#FB61D7", "#A58AFF", "#00B6EB", "#53B400", "#C49A00", "grey"), pt.size=0.05) +  No
Legend() +
stat_summary(fun.y = median, geom='point', size = 20, colour = "black", shape = 95
)
###Granulosa
gran <- c("AMH", "FST", "FOXL2", "BEX1")
gran_percent <- colSums(Ovar_unsorted.integrated@assays$RNA@counts[gran, ])/colSum
s(Ovar_unsorted.integrated@assays$RNA@counts)
Ovar_unsorted.integrated <- AddMetaData(object = Ovar_unsorted.integrated, metadat
a = gran_percent , col.name = "gran_percent")
gran <- VlnPlot(Ovar_unsorted.integrated, features = c("gran_percent"), cols = c("
#FB61D7", "#A58AFF", "#00B6EB", "#53B400", "#C49A00", "grey"), pt.size=0.05) +  No
Legend() +
stat_summary(fun.y = median, geom='point', size = 20, colour = "black", shape = 95
)
###Immune
immune <- c("CD69", "ITGB2", "CXCR4", "CD14")
immune_percent <- colSums(Ovar_unsorted.integrated@assays$RNA@counts[immune, ])/co
lSums(Ovar_unsorted.integrated@assays$RNA@counts)
Ovar_unsorted.integrated <- AddMetaData(object = Ovar_unsorted.integrated, metadat
a = immune_percent , col.name = "immune_percent")
immune <- VlnPlot(Ovar_unsorted.integrated, features = c("immune_percent"), cols =
c("#FB61D7", "#A58AFF", "#00B6EB", "#53B400", "#C49A00", "grey"), pt.size=0.05) +
NoLegend() +
stat_summary(fun.y = median, geom='point', size = 20, colour = "black", shape = 95
)
###Oocyte
oo <- c("GDF9", "ZP3", "OOSP2", "FIGLA")
oo_percent <- colSums(Ovar_unsorted.integrated@assays$RNA@counts[oo, ])/colSums(Ov
ar_unsorted.integrated@assays$RNA@counts)
Ovar_unsorted.integrated <- AddMetaData(object = Ovar_unsorted.integrated, metadat
a = oo_percent , col.name = "oo_percent")
oo <- VlnPlot(Ovar_unsorted.integrated, features = c("oo_percent"), cols = c("#FB6
1D7", "#A58AFF", "#00B6EB", "#53B400", "#C49A00", "grey"), pt.size=0.05) + NoLegen
d() +
stat_summary(fun.y = median, geom='point', size = 20, colour = "black", shape = 95
)

CombinePlots(plots = list(oo, immune, gran, endo, pv, stroma))


#Fig.3a
DoHeatmap(Ovar_unsorted.integrated.small, features = c("DDX4", "DPPA3", "DAZL", "P
RDM1", "NANOS3", "TFAP2C", "POU5F1", "NANOG"), assay = "RNA", slot = 'scale.data',
angle = 90) + scale_fill_gradientn(colors = c("blue", "black", "yellow"))


#Fig.3b, left
plot <- FeaturePlot(Ovar_unsorted.integrated, features = "DDX4", cols = c("grey",
"red"), min.cutoff=0.0, pt.size = 1)
ddx4express <- colnames(Ovar_unsorted.integrated)[Ovar_unsorted.integrated@assays$
RNA@counts["DDX4",] > 0 ]
LabelPoints(plot = plot, points = ddx4express, labels = "*")
```

```
#Supplementary Fig.1a
###062=TGP, 063=CSec
table(Idents(object = Ovar_unsorted.integrated), Ovar_unsorted.integrated@meta.dat
a$orig.ident)

#Supplementary Fig.1c
VlnPlot(Ovar_unsorted.integrated, features = "IFITM3", cols = c("darkred", "#FB61D
7", "#A58AFF", "#00B6EB", "#53B400", "#C49A00", "grey"), pt.size=0.05) + NoLegend(
) +
stat_summary(fun.y = median, geom='point', size = 20, colour = "black", shape = 95
)

#Supplementary Data 1_Sheet 2
Ovar_unsorted.integrated_Markers <- FindAllMarkers(Ovar_unsorted.integrated, verbo
se= FALSE)
head(x = Ovar_unsorted.integrated_Markers)
write.csv(Ovar_unsorted.integrated_Markers, "SupplementaryData_1_Sheet2.csv")
```

6. Tables and Figures, Subcluster *ddx4 cells

```
###Determine 1_oocytes Cell-IDs prior to subclustering
WhichCells(object = Ovar_unsorted.integrated, idents = "1_oocytes")

## Cells expressing DDX4 in all clusters
ddx4express <- colnames(Ovar_unsorted.integrated)[Ovar_unsorted.integrated@assays$
RNA@counts["DDX4",] > 0 ]
Idents(object = Ovar_unsorted.integrated, cells = ddx4express) <- 'ddx4'
head(x = Idents(object = Ovar_unsorted.integrated))


## Re-establish 1_oocytes cluster
oocytes <- c("CGAGCCAGTAAGCACG_1", "CTGAAACAGAGCAATT_1", "GGCAATTGTCAGATAA_1", "TG
CCCTAGTGGAAAGA_1", "AAGGCAGTCGGCATCG_2",
        "AGATTGCTCTGTCTCG_2", "AGCAGCCTCGTACGGC_2", "CAGAGAGGTATCTGCA_2", "CCGGGAT
CACGAAGCA_2", "CGCCAAGCAGCTCGCA_2", "CTACCCAGTCAGAAGC_2",
        "CTGATCCTCAAAGACA_2", "GCGAGAATCCCGACTT_2", "GCTCCTAAGTACACCT_2", "TCATTAC
TCCGCATCT_2", "TGACAACCAAGTAATG_2", "TTAGGCAGTCTAAACC_2",
        "TTTCCTCTCTGGGCCA_2")

Idents(object = Ovar_unsorted.integrated, cells = oocytes) <- '1_oocytes'
head(x = Idents(object = Ovar_unsorted.integrated))
Ovar_unsorted.integrated <- RenameIdents(object = Ovar_unsorted.integrated, 'ddx4'
= '0_ddx4', '1_oocytes' = '1_oocytes', '2_immune' = '2_immune', '3_gran' = '3_gran
', '4_endo' = '4_endo', '5_pv' = '5_pv', '6_stroma' = '6_stroma')
table(Idents(object = Ovar_unsorted.integrated), Ovar_unsorted.integrated@meta.dat
a$orig.ident)

#Fig.3b, right
###DDX4
VlnPlot(Ovar_unsorted.integrated, features = "DDX4", cols = c("darkred", "#FB61D7"
, "#A58AFF", "#00B6EB", "#53B400", "#C49A00", "grey"), pt.size=0.05) + NoLegend()

#Fig.3c
```

```
###OOCYTE MARKERS
ddx4_oocyte <- c("GDF9", "ZP3", "OOSP2", "FIGLA")
ddx4_oocyte_percent <- colSums(Ovar_unsorted.integrated@assays$RNA@counts[ddx4_ooc
yte, ])/colSums(Ovar_unsorted.integrated@assays$RNA@counts)
Ovar_unsorted.integrated <- AddMetaData(object = Ovar_unsorted.integrated, metadat
a = ddx4_oocyte_percent , col.name = "ddx4_oocyte_percent")
ddx4_oocyte <- VlnPlot(Ovar_unsorted.integrated, features = c("ddx4_oocyte_percent
"), y.max = 0.012, cols = c("darkred", "#FB61D7", "#A58AFF", "#00B6EB", "#53B400",
"#C49A00", "grey"), pt.size=0.05) + NoLegend() +
stat_summary(fun.y = median, geom='point', size = 20, colour = "black", shape = 95
)

###OOGONIAL STEM CELL MARKERS
germline_ddx4 <- c("DPPA3", "DAZL", "PRDM1")
germline_ddx4_percent <- colSums(Ovar_unsorted.integrated@assays$RNA@counts[germli
ne_ddx4, ])/colSums(Ovar_unsorted.integrated@assays$RNA@counts)
Ovar_unsorted.integrated <- AddMetaData(object = Ovar_unsorted.integrated, metadat
a = germline_ddx4_percent , col.name = "germline_ddx4_percent")
germline_ddx4 <- VlnPlot(Ovar_unsorted.integrated, features = c("germline_ddx4_per
cent"), y.max = 0.002, cols = c("darkred", "#FB61D7", "#A58AFF", "#00B6EB", "#53B4
00", "#C49A00", "grey"), pt.size=0.05) + NoLegend() +
stat_summary(fun.y = median, geom='point', size = 20, colour = "black", shape = 95
)

###PLURIPOTENCY/GERMLINE MARKERS
pluri_ddx4 <- c("POU5F1", "NANOG", "TFAP2C")
pluri_ddx4_percent <- colSums(Ovar_unsorted.integrated@assays$RNA@counts[pluri_ddx
4, ])/colSums(Ovar_unsorted.integrated@assays$RNA@counts)
Ovar_unsorted.integrated <- AddMetaData(object = Ovar_unsorted.integrated, metadat
a = pluri_ddx4_percent , col.name = "pluri_ddx4_percent")
pluri_ddx4 <- VlnPlot(Ovar_unsorted.integrated, features = c("pluri_ddx4_percent")
, y.max = 0.002, cols = c("darkred", "#FB61D7", "#A58AFF", "#00B6EB", "#53B400", "
#C49A00", "grey"), pt.size=0.05) + NoLegend() +
stat_summary(fun.y = median, geom='point', size = 20, colour = "black", shape = 95
)

CombinePlots(plots = list(germline_ddx4, pluri_ddx4, ddx4_oocyte))

#Supplementary Data 1_Sheet 3
Ovar_unsorted.integrated_ddx4_Markers <- FindMarkers(Ovar_unsorted.integrated, ide
nt.1 = '0_ddx4', verbose= T)
write.csv(Ovar_unsorted.integrated_ddx4_Markers, "SupplementaryData_1_Sheet3.csv")
```

7. Figures, Superimposition of cultured DDX4 Ab+/- data (SmartSeq-2 data)

```
#Supplementary Fig.2i
###Top25 up in DDX4 Ab+
DDX4POS.DEG_SS2.new <- c("EREG", "CRISPLD2", "SCUBE3", "TIMP3", "LRRC32", "BEX1",
"FHL1", "RELN", "SCN3A", "SUSD2", "SRGN", "PDE1A", "TBX2", "HGF", "CYTIP", "S100A4
", "NTM", "MT1M", "AQP1", "CXCL12", "CDH6", "TOP2A", "ITPR3", "NTRK2", "MEOX2")
DDX4POS.DEG_SS2.new =intersect(DDX4POS.DEG_SS2.new, rownames(Ovar_unsorted.integra
ted@assays$RNA@counts))
DDX4POS.DEG_SS2_percent <- colSums(Ovar_unsorted.integrated@assays$RNA@counts[DDX4
POS.DEG_SS2.new, ])/colSums(Ovar_unsorted.integrated@assays$RNA@counts)
Ovar_unsorted.integrated <- AddMetaData(object = Ovar_unsorted.integrated, metadat
a = DDX4POS.DEG_SS2_percent , col.name = "DDX4POS.DEG_SS2_percent")
FeaturePlot(object = Ovar_unsorted.integrated, features = c("DDX4POS.DEG_SS2_perce
nt"),
            cols = c("grey", "red"), reduction = "umap", max.cutoff = 0.006, pt.si
ze=0.5)
###Top25 up in DDX4 Ab-
DDX4NEG.DEG_SS2.new <- c("DHRS3", "FGF7", "DSG2", "CD200", "CXCL1", "IL1B", "SPON2
", "GATA4", "PID1", "CXCL6", "NCAM1", "CXCL8", "SFRP4", "PTGFRN", "DIRAS3", "PDGFD
", "SOX4", "IL33", "TGFBI", "PDPN", "RARRES2", "ALDH1A3", "CPZ", "TSPAN13", "KCTD1
2")
DDX4NEG.DEG_SS2 =intersect(DDX4NEG.DEG_SS2.new, rownames(Ovar_unsorted.integrated@
assays$RNA@counts))
DDX4NEG.DEG_SS2_percent <- colSums(Ovar_unsorted.integrated@assays$RNA@counts[DDX4
NEG.DEG_SS2, ])/colSums(Ovar_unsorted.integrated@assays$RNA@counts)
Ovar_unsorted.integrated <- AddMetaData(object = Ovar_unsorted.integrated, metadat
a = DDX4NEG.DEG_SS2_percent , col.name = "DDX4NEG.DEG_SS2_percent")
FeaturePlot(object = Ovar_unsorted.integrated, features = "DDX4NEG.DEG_SS2_percent
",
            cols = c("grey", "blue"), reduction = "umap", max.cutoff = 0.002, pt.s
ize=0.5)
```