Wagner20_Script_Sorted

Packages used

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

1. Create Seurat objects, Seurat v3

```
#sorted: filtered outs, Seurat objects created separately for DDX4-POS and DDX4-NE
G, merged
#sorted: DDX4-POS
ovar.data_sorted_ddx4pos <- Read10X(data.dir="/san/lanner-lab/sharedJPLeni/Objects
/hg19_DDX4POS")
ovar_sorted_ddx4pos <- CreateSeuratObject(counts = ovar.data_sorted_ddx4pos, min.c
ells= 1, min.features = 200, project = "10XProject-sortedDDX4POS")
ovar_sorted_ddx4pos[["percent.mt"]] <-PercentageFeatureSet(object = ovar_sorted_dd
x4pos, pattern = "^MT-")
ovar_sorted_ddx4pos <-subset(x = ovar_sorted_ddx4pos, subset = nFeature_RNA>200&nF
eature_RNA<7000&percent.mt<25)

#sorted: DDX4-NEG
ovar.data_sorted_ddx4neg <- Read10X(data.dir="/san/lanner-lab/sharedJPLeni/Objects
/hg19_DDX4NEG")
ovar_sorted_ddx4neg <- CreateSeuratObject(counts = ovar.data_sorted_ddx4neg, min.c
ells= 1, min.features = 200, project = "10XProject-sortedDDX4NEG")
ovar_sorted_ddx4neg[["percent.mt"]] <-PercentageFeatureSet(object = ovar_sorted_dd
x4neg, pattern = "^MT-")
ovar_sorted_ddx4neg <-subset(x = ovar_sorted_ddx4neg, subset = nFeature_RNA>200&nF
eature_RNA<7000&percent.mt<25)
```

2. Merge, pre-processing, scaling

```
Ovar_sorted.combined <- merge(ovar_sorted_ddx4pos, y = ovar_sorted_ddx4neg, add.ce
ll.ids = c("DDX4POS", "DDX4NEG"), project = "sorted")

Ovar_sorted.combined <- NormalizeData(Ovar_sorted.combined, normalization.method =
"LogNormalize", scale.factor = 10000)

Ovar_sorted.combined <- FindVariableFeatures(Ovar_sorted.combined, selection.metho
d = "vst", nfeatures = 2000)

#Scale data
all.genes <- rownames(Ovar_sorted.combined)
Ovar_sorted.combined <- ScaleData(Ovar_sorted.combined, features = all.genes, vars
.to.regress = "nCount_RNA")
```

3. Visualization, Renaming

```
Ovar_sorted.combined <- readRDS("/san/lanner-lab/sharedJPLeni/200121_Ovar_sorted.c
ombined_scaled.rds")



Ovar_sorted.combined <- RunPCA(Ovar_sorted.combined, features = VariableFeatures(o
bject = Ovar_sorted.combined))

Ovar_sorted.combined <- FindNeighbors(object = Ovar_sorted.combined, reduction = "
pca", dims = 1:12)
Ovar_sorted.combined <- FindClusters(Ovar_sorted.combined, resolution = 0.1)


Ovar_sorted.combined <- RunPCA(object = Ovar_sorted.combined, npcs = 12, verbose =
FALSE)
Ovar_sorted.combined <- RunUMAP(object = Ovar_sorted.combined, reduction = "pca",
dims = 1:12)
```

4. Subcluster oocytes

```
###Subcluster oocytes
plot <- DimPlot(object = Ovar_sorted.combined, reduction = 'umap', pt.size = 0.05)
oocytes <- CellSelector(plot = plot)



Ovar_sorted.combined <- RenameIdents(object = Ovar_sorted.combined, 'oocytes' = '1
_oocytes', '5' = '2_mono', '4' = '3_t', '3' = '5_endo', '2' = '4_gran', '1' = '6_p
v', '0' = '7_stroma')
levels(x = Ovar_sorted.combined)
```

5. Figures and Tables, no *ddx4

```
#Fig.4a
DimPlot(object = Ovar_sorted.combined, reduction = "umap", group.by = "orig.ident"
, cols = c("darkblue", "red"))

#Fig.4b
DimPlot(object = Ovar_sorted.combined, reduction = "umap", group.by = "ident", col
```

```
s = c("grey", "#C49A00", "#00B6EB", "#53B400", "#A58AFF", "darkviolet", "#FB61D7")
)

#Fig.4c
DefaultAssay(object = Ovar_sorted.combined) <- "RNA"

VlnPlot(Ovar_sorted.combined, features = "DDX4", sort = "decreasing", group.by = "
orig.ident", cols = c("#FB61D7", "darkviolet", "#A58AFF", "#00B6EB", "#53B400", "#
C49A00", "grey"), pt.size=0.4) + NoLegend() +
stat_summary(fun.y = median, geom='point', size = 20, colour = "black", shape = 95
)

VlnPlot(Ovar_sorted.combined, features = "DDX4", cols = c("#FB61D7", "darkviolet",
"#A58AFF", "#00B6EB", "#53B400", "#C49A00", "grey"), pt.size=0.4) + NoLegend() +
stat_summary(fun.y = median, geom='point', size = 20, colour = "black", shape = 95
)

#Fig.5a
FeaturePlot(Ovar_sorted.combined, features = c("RGS5", "MCAM"), blend = T)

#Supplementary Fig.2c
table(Idents(object = Ovar_sorted.combined), Ovar_sorted.combined@meta.data$orig.i
dent)

#Supplementary Fig.2d
###Perivascular
pv <- c("RGS5", "MCAM", "RERGL", "TAGLN", "MYH11")
pv_percent <- colSums(Ovar_sorted.combined@assays$RNA@counts[pv, ])/colSums(Ovar_s
orted.combined@assays$RNA@counts)
Ovar_sorted.combined <- AddMetaData(object = Ovar_sorted.combined, metadata = pv_p
ercent , col.name = "pv_percent")
pv <- VlnPlot(Ovar_sorted.combined, features = c("pv_percent"), cols = c("#FB61D7"
, "darkviolet", "#A58AFF", "#00B6EB", "#53B400", "#C49A00", "grey"), pt.size=0.05)
+ NoLegend() +
stat_summary(fun.y = median, geom='point', size = 20, colour = "black", shape = 95
)
###Endothelial
endo <- c("CD34", "VWF", "FLI1", "CDH5")
endo_percent <- colSums(Ovar_sorted.combined@assays$RNA@counts[endo, ])/colSums(Ov
ar_sorted.combined@assays$RNA@counts)
Ovar_sorted.combined <- AddMetaData(object = Ovar_sorted.combined, metadata = endo
_percent , col.name = "endo_percent")
endo <- VlnPlot(Ovar_sorted.combined, features = c("endo_percent"), cols = c("#FB6
1D7", "darkviolet", "#A58AFF", "#00B6EB", "#53B400", "#C49A00", "grey"), pt.size=0
.05) + NoLegend() +
stat_summary(fun.y = median, geom='point', size = 20, colour = "black", shape = 95
)
###Granulosa
gran <- c("AMH", "FST", "FOXL2", "BEX1")
gran_percent <- colSums(Ovar_sorted.combined@assays$RNA@counts[gran, ])/colSums(Ov
ar_sorted.combined@assays$RNA@counts)
Ovar_sorted.combined <- AddMetaData(object = Ovar_sorted.combined, metadata = gran
_percent , col.name = "gran_percent")
gran <- VlnPlot(Ovar_sorted.combined, features = c("gran_percent"), cols = c("#FB6
```

```
1D7", "darkviolet", "#A58AFF", "#00B6EB", "#53B400", "#C49A00", "grey"), pt.size=0
.05) + NoLegend() +
stat_summary(fun.y = median, geom='point', size = 20, colour = "black", shape = 95
)
###T-cell
tcell <- c("CD69", "CCL5", "CXCR4", "CCL4")
tcell_percent <- colSums(Ovar_sorted.combined@assays$RNA@counts[tcell, ])/colSums(
Ovar_sorted.combined@assays$RNA@counts)
Ovar_sorted.combined <- AddMetaData(object = Ovar_sorted.combined, metadata = tcel
l_percent , col.name = "tcell_percent")
tcell <- FeaturePlot(object = Ovar_sorted.combined, features = c("tcell_percent"),
            cols = c("grey", "#A58AFF"), reduction = "umap")
tcell <- VlnPlot(Ovar_sorted.combined, features = c("tcell_percent"), cols = c("#F
B61D7", "darkviolet", "#A58AFF", "#00B6EB", "#53B400", "#C49A00", "grey"), pt.size
=0.05) + NoLegend() +
stat_summary(fun.y = median, geom='point', size = 20, colour = "black", shape = 95
)
###Monocytes
mono <- c("LYZ", "CD163", "HLA-DQA1", "CD14")
mono_percent <- colSums(Ovar_sorted.combined@assays$RNA@counts[mono, ])/colSums(Ov
ar_sorted.combined@assays$RNA@counts)
Ovar_sorted.combined <- AddMetaData(object = Ovar_sorted.combined, metadata = mono
_percent , col.name = "mono_percent")
mono <- VlnPlot(Ovar_sorted.combined, features = c("mono_percent"), cols = c("#FB6
1D7", "darkviolet", "#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_sorted.combined@assays$RNA@counts[oo, ])/colSums(Ovar_s
orted.combined@assays$RNA@counts)
Ovar_sorted.combined <- AddMetaData(object = Ovar_sorted.combined, metadata = oo_p
ercent , col.name = "oo_percent")
oo <- VlnPlot(Ovar_sorted.combined, features = c("oo_percent"), cols = c("#FB61D7"
, "darkviolet", "#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(oo, mono, tcell, gran, endo, pv))

#Supplementary Data 2_Sheet 2
Ovar_sorted.combined_Markers <- FindAllMarkers(Ovar_sorted.combined, verbose= T)
head(x = Ovar_sorted.combinted_Markers)
write.csv(Ovar_sorted.combined_Markers, "SupplementaryData2_Sheet2.csv")

#Supplementary Data 2_Sheet 3
Idents(Ovar_sorted.combined) <- Ovar_sorted.combined@meta.data$orig.ident
levels(x = Ovar_sorted.combined)
Ovar_sorted.combined_Markers_orig.ident <- FindAllMarkers(Ovar_sorted.combined, ve
rbose= T)
head(x = Ovar_sorted.combined_Markers_orig.ident)
write.csv(Ovar_sorted.combined_Markers_orig.ident, "SupplementaryData2_Sheet3.csv"
```

```
)
```

6. Figures and Tables, *ddx4

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

##Subcluster *ddx4 cells
ddx4express <- colnames(Ovar_sorted.combined)[Ovar_sorted.combined@assays$RNA@coun
ts["DDX4",] > 0 ]
Idents(object = Ovar_sorted.combined, cells = ddx4express) <- 'ddx4'
head(x = Idents(object = Ovar_sorted.combined))

##Re-establish 1_oocytes cluster and rename
oocytes <- c("DDX4POS_AGATGAACACCGGCTA", "DDX4POS_ATTACTCAGACGGATC", "DDX4POS_TCGA
CGGGTTCCTACC", "DDX4POS_TTAATCCAGCCATATC", "DDX4NEG_AAAGTGATCGTAGGGA", "DDX4NEG_AA
TAGAGAGGAATGTT", "DDX4NEG_ACAGGGATCTTCTTCC", "DDX4NEG_ACATCGACATTGCTGA", "DDX4NEG_
AGTAGTCAGTTTCTTC", "DDX4NEG_CATGGTACATCCTGTC", "DDX4NEG_CGAAGTTCAACTCGAT", "DDX4NE
G_CGATGGCCAATCCTTT", "DDX4NEG_CTCATTATCATCGCAA", "DDX4NEG_CTCCGATTCATCGTAG", "DDX4
NEG_GAATAGAGTATCCCAA", "DDX4NEG_GACACGCGTTAAACCC", "DDX4NEG_GAGGCAAAGGTGCAGT", "DD
X4NEG_GAGTCATAGATGAAGG", "DDX4NEG_GCCTGTTGTACGAGTG", "DDX4NEG_GGAAGTGCATGCCATA", "
DDX4NEG_GGCTTTCCAAGGTCAG", "DDX4NEG_GGGTGAATCATCCTAT", "DDX4NEG_GGTCACGAGTGGAAAG",
"DDX4NEG_TAACCAGAGACTACGG", "DDX4NEG_TAATTCCGTGGTCAAG", "DDX4NEG_TCCGGGATCGCGGACT"
, "DDX4NEG_TGTCCCACACAATTCG", "DDX4NEG_TTGCCTGAGCTAGAAT")
Idents(object = Ovar_sorted.combined, cells = oocytes) <- '1_oocytes'
head(x = Idents(object = Ovar_sorted.combined))
levels(Ovar_sorted.combined)

Ovar_sorted.combined <- RenameIdents(object = Ovar_sorted.combined, 'ddx4' = 'ddx4
', '1_oocytes' = 'oocytes', '6_immune' = 'monocytes', '5_immune' = 't cells', '4_e
ndo' = 'endothelial cells', '3_gran' = 'granulosa cells', '2_pv' = 'perivascular c
ells', '1_stroma' = 'stroma')
levels(x = Ovar_sorted.combined)

#Supplementary Fig.2e
VlnPlot(Ovar_sorted.combined, features= c("DDX4"), cols = c("darkred", "#FB61D7",
"darkviolet", "#A58AFF", "#00B6EB", "#53B400", "#C49A00", "grey"), pt.size=0.05) +
NoLegend()

#Supplementary Fig.2f
VlnPlot(Ovar_sorted.combined, features= c("IFITM3"), cols = c("darkred", "#FB61D7"
, "darkviolet", "#A58AFF", "#00B6EB", "#53B400", "#C49A00", "grey"), pt.size=0.05)
+ NoLegend()

#Supplementary Fig.2g
###OOCYTE MARKERS
ddx4_oocyte <- c("GDF9", "ZP3", "OOSP2", "FIGLA")
ddx4_oocyte_percent <- colSums(Ovar_sorted.combined@assays$RNA@counts[ddx4_oocyte,
])/colSums(Ovar_sorted.combined@assays$RNA@counts)
Ovar_sorted.combined <- AddMetaData(object = Ovar_sorted.combined, metadata = ddx4
_oocyte_percent , col.name = "ddx4_oocyte_percent")
ddx4_oocyte <- VlnPlot(Ovar_sorted.combined, features = c("ddx4_oocyte_percent"),
y.max = 0.012, cols = c("darkred", "#FB61D7", "darkviolet", "#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_sorted.combined@assays$RNA@counts[germline_d
dx4, ])/colSums(Ovar_sorted.combined@assays$RNA@counts)
Ovar_sorted.combined <- AddMetaData(object = Ovar_sorted.combined, metadata = germ
line_ddx4_percent , col.name = "germline_ddx4_percent")
germline_ddx4 <- VlnPlot(Ovar_sorted.combined, features = c("germline_ddx4_percent
"), y.max = 0.003, cols = c("darkred", "#FB61D7", "darkviolet", "#A58AFF", "#00B6E
B", "#53B400", "#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_sorted.combined@assays$RNA@counts[pluri_ddx4, ]
)/colSums(Ovar_sorted.combined@assays$RNA@counts)
Ovar_sorted.combined <- AddMetaData(object = Ovar_sorted.combined, metadata = plur
i_ddx4_percent , col.name = "pluri_ddx4_percent")
pluri_ddx4 <- VlnPlot(Ovar_sorted.combined, features = c("pluri_ddx4_percent"), y.
max = 0.003, cols = c("darkred", "#FB61D7", "darkviolet", "#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 2_Sheet 4
Ovar_sorted.combined.ddx4_Markers_ddx4 <- FindMarkers(Ovar_sorted.combined, verbos
e= T, ident.1 = 'ddx4')
head(x = Ovar_sorted.combined_Markers_ddx4)
write.csv(Ovar_sorted.combined_Markers_ddx4, "SupplementaryData2_Sheet4.csv")
```

7. Superimposition of Screen (FACS data Supplementary Table 1) and cultured DDX4 Ab+/- data (SmartSeq-2 data)

```
#Fig.4d
###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_sorted.combined@
assays$RNA@counts))
DDX4POS.DEG_SS2_percent <- colSums(Ovar_sorted.combined@assays$RNA@counts[DDX4POS.
DEG_SS2.new, ])/colSums(Ovar_sorted.combined@assays$RNA@counts)
Ovar_sorted.combined <- AddMetaData(object = Ovar_sorted.combined, metadata = DDX4
POS.DEG_SS2_percent , col.name = "DDX4POS.DEG_SS2_percent")
FeaturePlot(object = Ovar_sorted.combined, features = c("DDX4POS.DEG_SS2_percent")
,
            cols = c("grey", "red"), reduction = "umap", max.cutoff = 0.006, pt.si
ze=0.75)

###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.new =intersect(DDX4NEG.DEG_SS2.new, rownames(Ovar_sorted.combined@
assays$RNA@counts))
DDX4NEG.DEG_SS2_percent <- colSums(Ovar_sorted.combined@assays$RNA@counts[DDX4NEG.
DEG_SS2.new, ])/colSums(Ovar_sorted.combined@assays$RNA@counts)
Ovar_sorted.combined <- AddMetaData(object = Ovar_sorted.combined, metadata = DDX4
NEG.DEG_SS2_percent , col.name = "DDX4NEG.DEG_SS2_percent")
FeaturePlot(object = Ovar_sorted.combined, features = "DDX4NEG.DEG_SS2_percent",
            cols = c("grey", "darkblue"), reduction = "umap", max.cutoff = 0.001,
pt.size=0.75)


#Fig.6b
ddx4pos.pos.genes <- c("CD9", "ENTPD1", "CD44", "ITGA1", "ITGA3", "CDH5", "MCAM")
ddx4pos.neg.genes <- c("DPP4", "ITGA5", "ICAM1", "CD55", "SELE", "ENG", "CD200")

ddx4pos.pos.genes_percent <- colSums(Ovar_sorted.combined@assays$RNA@counts[ddx4po
s.pos.genes, ])/colSums(Ovar_sorted.combined@assays$RNA@counts)
Ovar_sorted.combined <- AddMetaData(object = Ovar_sorted.combined, metadata = ddx4
pos.pos.genes_percent , col.name = "ddx4pos.pos.genes_percent")
FeaturePlot(object = Ovar_sorted.combined, features = "ddx4pos.pos.genes_percent",
            cols = c("grey", "red"), reduction = "umap", max.cutoff = 0.002, pt.si
ze=0.75)

ddx4pos.neg.genes_percent <- colSums(Ovar_sorted.combined@assays$RNA@counts[ddx4po
s.neg.genes, ])/colSums(Ovar_sorted.combined@assays$RNA@counts)
Ovar_sorted.combined <- AddMetaData(object = Ovar_sorted.combined, metadata = ddx4
pos.neg.genes_percent , col.name = "ddx4pos.neg.genes_percent")
FeaturePlot(object = Ovar_sorted.combined, features = "ddx4pos.neg.genes_percent",
            cols = c("grey", "darkblue"), reduction = "umap", max.cutoff = 0.0015,
pt.size=0.75)
```