Wagner20_Script_Cultured

Packages used

```
library(data.table)
library(edgeR)
library(uwot)
```

DGE table/analysis

```
# Import gene counts
gene_counts<-data.table(read.delim("AdScript_Wagner20_Cultured_gene_counts_culture
d.txt", as.is=T))

# Make targets object
targets<-data.table(name=colnames(gene_counts)[4:43])
targets[, group:=gsub("[0-9]","",name)]

# Make design and contrasts
design<-model.matrix(~0+group, targets)
colnames(design)<-gsub("group","", colnames(design))
contrasts<-makeContrasts(CPCN=CP-CN, levels=design)

# Make DGE list objects
dge<-DGEList(gene_counts[, targets[, name], with=F], genes=gene_counts[,.(entrez,
symbol, length)], group=targets[, group])
dge<-calcNormFactors(dge)

# Remove low/non expressed genes and proceed with differential gene expression ana
lysis
dgecpm1_3<-dge[rowSums(cpm(dge)>=1)>=3,]
dgecpm1_3$samples$lib.size<-colSums(dgecpm1_3$counts)
dgecpm1_3<-calcNormFactors(dgecpm1_3)
dgecpm1_3<-estimateDisp(dgecpm1_3, design)
dgecpm1_3Fit<-glmFit(dgecpm1_3, design)
dgecpm1_3LRT<-glmLRT(dgecpm1_3Fit, contrast=contrasts)

# Data table with the results of the analysis
dgecpm1_3results<-data.table(topTags(dgecpm1_3LRT, n=Inf, sort.by="none")[[1]], DE
=c(decideTestsDGE(dgecpm1_3LRT)))
```

Figure

```
# Umap plot, Supplementary Fig.2h
set.seed(1)
dgecpm1_3_umap<-umap(scale(t(cpm(dgecpm1_3, log=T, prior.count=1)[, targets[, name
]])), scale=F, n_neighbors=7)
dgecpm1_3_umap_dt<-data.table(dgecpm1_3_umap, targets)
colnames(dgecpm1_3_umap_dt)[1:2]<-c("X", "Y")
ggplot(dgecpm1_3_umap_dt, aes(x=X, y=Y))+
stat_density2d(aes(fill=group, alpha=stat(level)), geom="polygon", bins=3, show.le
gend=F)+
stat_density2d(aes(color=group), contour =T, bins=3, show.legend=F)+
geom_point(aes(color=group), size=3)+geom_text_repel(aes(label=name), show.legend=
F)+
scale_color_manual(values=c("CP"="red", "CN"="darkblue"))+
scale_fill_manual(values=c("CP"="red", "CN"="darkblue"))+
scale_alpha_continuous(range=c(0,0.5))+
scale_x_continuous(limits=c(-3,4.5))+
scale_y_continuous(limits=c(-2,2.7))+
theme_bw(base_size=14)+
theme(axis.title=element_blank(), panel.grid=element_blank())#, limits=c(0.1,1)
```