###前期 ####

load("/home/yyyuxi/kidney/myproject/mouse\_kidney/fibrosis/IRI\_UIRI/ana/sce.rdata")

DimPlot(sce,group.by = "celltype",label = T,cols = mycolors1)

sce <- subset(sce,subset = celltype %in% c("Endo","GEC") & orig.ident %in% c("Our\_Con1","Our\_Con2","Our\_UIRI10D1",'Our\_UIRI10D2'))

sce$celltype <- plyr::mapvalues(sce$seurat\_clusters, from = c("5","19","24"),to = c("Capillary","Arterial","Glomerular"))

save(sce,file = "/home/yyyuxi/software/pyscenic/example/test2/sce.rdata")

#跑scenic流程同上

##1. SeuratExtend直接从loom文件load

load("/home/yyyuxi/software/pyscenic/example/test2/sce.rdata")

scenic\_output <- SeuratExtend::ImportPyscenicLoom("/home/yyyuxi/software/pyscenic/example/test2/sce\_SCENIC.loom")

#RegulonsAUC：量化每个 regulon 在单个细胞中的活性（AUC 值），用于分析细胞间的调控差异。

#Regulons：描述每个 regulon 的组成（转录因子及其靶基因），用于推断 regulon 的功能。

sce\_scenic\_output <- SeuratExtend::ImportPyscenicLoom("/home/yyyuxi/software/pyscenic/example/test2/sce\_SCENIC.loom", seu = sce )

sce\_scenic\_output@misc$SCENIC

##2. KS教程

library(SCopeLoomR)

library(AUCell)

library(SCENIC)

library(dplyr)

library(KernSmooth)

library(RColorBrewer)

library(plotly)

library(BiocParallel)

library(grid)

library(ComplexHeatmap)

library(data.table)

library(ggplot2)

library(pheatmap)

sce\_SCENIC <- SCopeLoomR::open\_loom("/home/yyyuxi/software/pyscenic/example/test2/sce\_SCENIC.loom")

#提取regulon

regulons\_incidMat <- get\_regulons(sce\_SCENIC, column.attr.name="Regulons")

regulons <- regulonsToGeneLists(regulons\_incidMat)

#提取regulonAUC

regulonAUC <- get\_regulons\_AUC(sce\_SCENIC, column.attr.name='RegulonsAUC')

regulonAucThresholds <- get\_regulon\_thresholds(sce\_SCENIC)

#sce对象

#cellinfo <- sce@meta.data[,c('celltype','group',"nFeature\_RNA","nCount\_RNA")]#细胞meta信息

#colnames(cellinfo)=c('celltype','group','nGene' ,'nUMI')

#把regulonAUC加入sce对象

next\_regulonAUC <- regulonAUC[,match(colnames(sce),colnames(regulonAUC))]

sce@meta.data = cbind(sce@meta.data ,t(SummarizedExperiment::assay(next\_regulonAUC[regulonAUC@NAMES,])))

####RSS 计算特异性TF

rss <- calcRSS(AUC = getAUC(regulonAUC), cellAnnotation= sce$celltype)

rss=na.omit(rss) #去除含有NA的行

rssPlot <-

plotRSS(

rss,

zThreshold = 3,

cluster\_columns = FALSE,

order\_rows = TRUE,

thr=0.1,

varName = "cellType",

col.low = '#330066',

col.mid = '#66CC66',

col.high = '#FFCC33')

rssPlot

FeaturePlot(sce,features = sub("\\(\\+)", "", unique(rssPlot$df$Topic)))

##### tips####

##1) 提取表达矩阵

# exprMat <- get\_dgem(sce\_SCENIC)#从sce\_SCENIC文件提取表达矩阵

# exprMat\_log <- log2(exprMat+1) # log处理

##2) 提取RSS数据进行可视化——热图与Rank图

#热图

{

rss\_data <- rssPlot$plot$data

library(ggheatmap)

library(reshape2)

rss\_data<-dcast(rss\_data,

Topic~rss\_data$cellType,

value.var = 'Z')

rownames(rss\_data) <- rss\_data[,1]

rss\_data <- rss\_data[,-1]

colnames(rss\_data)

col\_ann <- data.frame(group= c(rep("Neutrophil",1),

rep("Macrophage",1),

rep("mDC",1),

rep("T cell",1),

rep("Mast",1)))#列注释

rownames(col\_ann) <- colnames(rss\_data)

groupcol <- c("#D9534F", "#96CEB4", "#CBE86B", "#EDE574", "#0099CC")

names(groupcol) <- c("Neutrophil","Macrophage","mDC", "T cell","Mast")

col <- list(group=groupcol)

text\_columns <- sample(colnames(rss\_data),0)#不显示列名

p <- ggheatmap(rss\_data,color=colorRampPalette(c('#1A5592','white',"#B83D3D"))(100),

cluster\_rows = T,cluster\_cols = F,scale = "row",

annotation\_cols = col\_ann,

annotation\_color = col,

legendName="Relative value",

text\_show\_cols = text\_columns)

p

}

##rank图

{

B\_rss <- as.data.frame(rss)#rss特异性TF结果

#需要作图的细胞类型

celltype <- colnames(B\_rss)

rssRanklist <- list()

for(i in 1:length(celltype)) {

library(ggrepel)

data\_rank\_plot <- cbind(as.data.frame(rownames(B\_rss)),

as.data.frame(B\_rss[,celltype[i]]))#提取数据

colnames(data\_rank\_plot) <- c("TF", "celltype")

data\_rank\_plot=na.omit(data\_rank\_plot)#去除NA

data\_rank\_plot <- data\_rank\_plot[order(data\_rank\_plot$celltype,decreasing=T),]#降序排列

data\_rank\_plot$rank <- seq(1, nrow(data\_rank\_plot))#添加排序

p <- ggplot(data\_rank\_plot, aes(x=rank, y=celltype)) +

geom\_point(size=3, shape=16, color="#1F77B4",alpha =0.4)+

geom\_point(data = data\_rank\_plot[1:6,],

size=3, color='#DC050C')+ #选择前6个标记，自行按照需求选择

theme\_bw()+

theme(axis.title = element\_text(colour = 'black', size = 12),

axis.text = element\_text(colour = 'black', size = 10),

axis.text.x = element\_blank(),

axis.ticks.x = element\_blank())+

labs(x='Regulons Rank', y='Specificity Score',title =celltype[i])+

geom\_text\_repel(data= data\_rank\_plot[1:6,],

aes(label=TF), color="black", size=3, fontface="italic",

arrow = arrow(ends="first", length = unit(0.01, "npc")), box.padding = 0.2,

point.padding = 0.3, segment.color = 'black',

segment.size = 0.3, force = 1, max.iter = 3e3)

rssRanklist[[i]] <- p

}

}

##3）提取部分数据绘制RSS

cellTypes <- as.data.frame(subset(cellinfo,select = 'celltype'))

selectedResolution <- "celltype"

sub\_regulonAUC <- regulonAUC

rss <- calcRSS(AUC=getAUC(sub\_regulonAUC),#从aucellresults获取AUC矩阵

cellAnnotation=cellTypes[colnames(sub\_regulonAUC),

selectedResolution])