

Papalex CUL3 Analysis

2023-02-21

Goal

The aim of this report is to replicate the results of Papalex et al's pathway enrichment analysis.

```
# Load packages.
library(Seurat)

## Attaching SeuratObject

library(SeuratData)

## -- Installed datasets ----- SeuratData v0.2.2 --
## v thp1.eccite 3.1.5

## ----- Key -----

## v Dataset loaded successfully
## > Dataset built with a newer version of Seurat than installed
## (?) Unknown version of Seurat installed

library(ggplot2)
library(patchwork)
library(scales)
library(dplyr)

## 
## Attaching package: 'dplyr'

## The following objects are masked from 'package:stats':
## 
##     filter, lag

## The following objects are masked from 'package:base':
## 
##     intersect, setdiff, setequal, union

library(reshape2)
library(mixtools)

## mixtools package, version 2.0.0, Released 2022-12-04
## This package is based upon work supported by the National Science Foundation under Grant No. SES-051
```

```

library(stringr)
library(enrichR)

## Welcome to enrichR
## Checking connection ...

## Enrichr ... Connection is Live!
## FlyEnrichr ... Connection is available!
## WormEnrichr ... Connection is available!
## YeastEnrichr ... Connection is available!
## FishEnrichr ... Connection is available!
## OxEnrichr ... Connection is available!

library(kableExtra)

##
## Attaching package: 'kableExtra'
##
## The following object is masked from 'package:dplyr':
##
##     group_rows

```

Download Data

```

# Download dataset using SeuratData.
options(timeout = 1000)
InstallData(ds = "thp1.eccite")

## Warning: The following packages are already installed and will not be
## reinstalled: thp1.eccite

# Setup custom theme for plotting.
custom_theme <- theme(
  plot.title = element_text(size=16, hjust = 0.5),
  legend.key.size = unit(0.7, "cm"),
  legend.text = element_text(size = 14))

# Load object.
eccite <- LoadData(ds = "thp1.eccite")

```

Normalize and plot UMAP

```

# Normalize protein.
eccite <- NormalizeData(
  object = eccite,
  assay = "ADT",
  normalization.method = "CLR",
  margin = 2)

```

```

## Normalizing across cells

# Prepare RNA assay for dimensionality reduction:
# Normalize data, find variable features and scale data.
DefaultAssay(object = eccite) <- 'RNA'
eccite <- NormalizeData(object = eccite) %>% FindVariableFeatures() %>% ScaleData()

## Centering and scaling data matrix

# Run Principle Component Analysis (PCA) to reduce the dimensionality of the data.
eccite <- RunPCA(object = eccite)

## PC_ 1
## Positive: BIRC5, TOP2A, CDC20, MKI67, CENPF, TPX2, CDKN3, UBE2C, CKS1B, NUF2
##           CCNA2, NUSAP1, KIAAO101, CENPA, HMGB2, SGOL1, TYMS, STMN1, MYBL2, GTSE1
##           ASPM, H2AFZ, CDCA2, HMMR, CDCA8, KIF2C, CKAP2L, PTTG1, MND1, UBE2T
## Negative: FTH1, FCER1G, NEAT1, SOD2, FTL, MAFB, BTG1, NPC2, CTSL, CTSC
##            CTSB, SLC31A2, CHI3L1, FAM26F, TNFSF13B, GBP5, PLAUR, EVL, GK, ASAHI
##            HLA-DRB1, HLA-DRA, SPP1, SCPEP1, CD74, SAT1, GBP1, SLAMF7, WARS, SDS
## PC_ 2
## Positive: HYOU1, PDIA4, HSPA5, SDF2L1, MEI1, MANF, DNAJB9, NUCB2, TRIB3, WIPI1
##           CRELD2, HSP90B1, MSTO1, SLC39A14, HERPUD1, ALDH1L2, DERL3, VIMP, SEC11C, SERP1
##           PPAPDC1B, CDK2AP2, OSTC, DNAJB11, ERO1LB, SEC61G, SYVN1, TMED2, DNAJC3, PYCR1
## Negative: HSPA8, KIAAO101, TYMS, MKI67, FCER1G, CHI3L1, ACTG1, TOP2A, MYBL2, HSP90AA1
##            CCNA2, BIRC5, CLSPN, PKMYT1, NPC2, NUSAP1, HMGN2, ZWINT, CENPF, H2AFZ
##            TMEM106C, CENPW, TUBA1B, STMN1, CTSC, ASF1B, CDCA5, HMGA1, RRM2, GTSE1
## PC_ 3
## Positive: CDKN1A, ATF5, WARS, PLEK, CXCL10, IL1RN, SOD2, FAM26F, SLC31A2, GBP1
##           IDO1, SLAMF7, GK, HLA-DRA, ISG20, ICAM1, CD274, CCL2, ATF3, GBP5
##           CCL8, CD74, MTHFD2, IL8, FCER1G, GCH1, TNFSF13B, IL4I1, GLUL, RALA
## Negative: QPRT, S100A4, RPLPO, S100A6, ZFP36L2, ALOX5AP, SORL1, ANTXR1, C1orf162, VCAN
##            GLIPR1, CD1D, ID1, CAPN2, ID2, TGFBR1, RGS16, TKT, ITM2C, CDKN2C
##            HSPB1, ACTG1, CORO1A, SMYD3, ID3, RPSA, ALDH2, FOS, AZU1, THYN1
## PC_ 4
## Positive: RMDN3, GCHFR, GRN, DNASE2, WARS, SCCPDH, PSME2, LIPG, CTSD, HLA-DRB1
##           C19orf59, TSPO, HLA-DRB5, LTA4H, HLA-A, IFI30, AGT, GBP5, CEBPE, APOC1
##           GLUL, MARC1, CD74, CD1D, PPARG, ALOX5AP, CLDN23, CD68, S100A8, PLIN2
## Negative: CCL2, IGFBP3, PEA15, CCL3, NFKBIA, MMP9, CCL4, CCL5, POU2F2, IL1B
##            MARCKSL1, CXCL11, MX2, RGS1, CXCL9, USP18, PDPN, SPP1, CLEC5A, E2F1
##            TGFBR1, CKB, RUNX3, PTPN14, SMYD3, TGFB1, CCL8, TESC, GINS2, PNRC1
## PC_ 5
## Positive: FTL, FABP5, PLIN2, CSTB, CTSD, CD36, FTH1, HMGA1, SPOCD1, RMDN3
##           APOC1, DDT4L, AGPAT9, CDK4, MSR1, E2F1, SRM, GCHFR, GINS2, CLDN23
##           TOMM40, RND3, NCF2, FAM111B, DTL, APOE, STRA13, IL8, SLC11A1, CHCHD10
## Negative: TNFSF10, NCF1, PSMB9, CXCL11, CXCL10, IFI27, TMEM176B, RARRES3, TMEM176A, NFKBIA
##            IL32, SOCS1, IFITM1, GBP1, CCNB1, PLK1, HMMR, PNRC1, PSME2, RGS16
##            MY01G, CD74, ISG20, TNFSF13B, CXCL9, TMEM50B, CDC20, IFIT2, PTTG1, GLIPR1

# Run Uniform Manifold Approximation and Projection (UMAP) to visualize clustering in 2-D.
eccite <- RunUMAP(object = eccite, dims = 1:40)

## Warning: The default method for RunUMAP has changed from calling Python UMAP via reticulate to the R

```

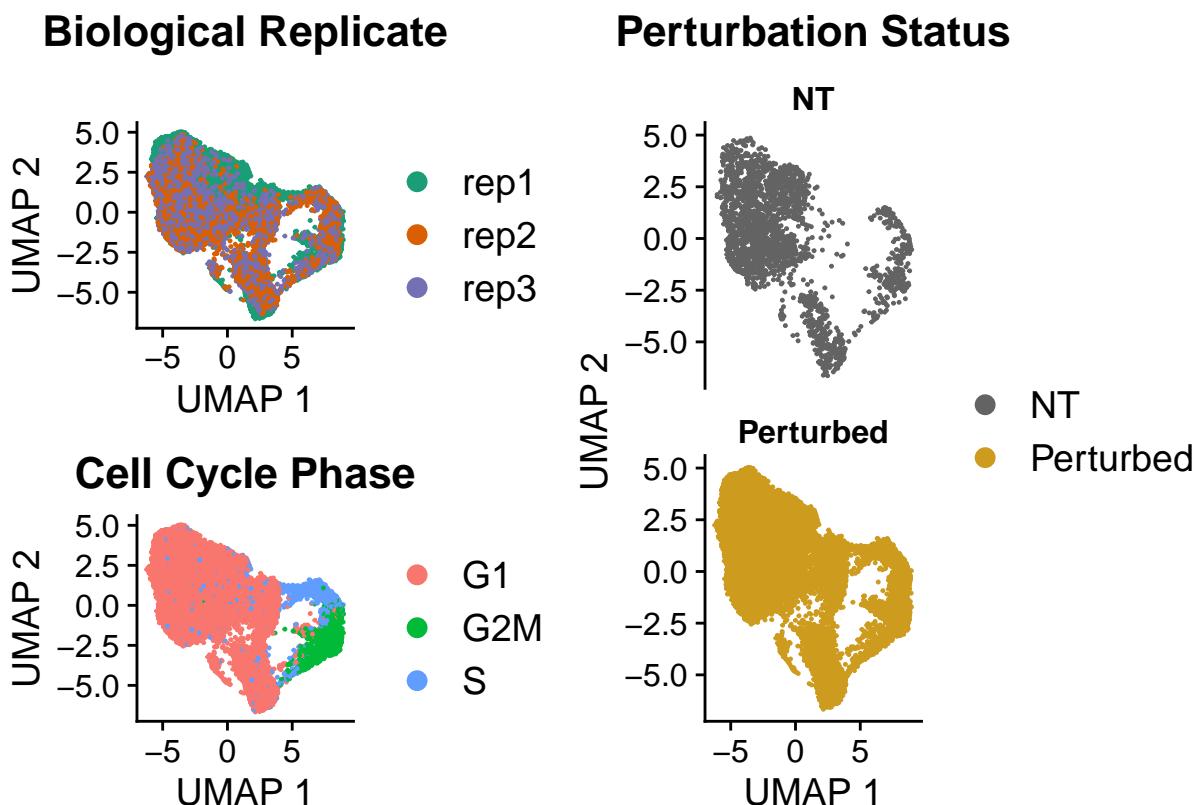
```
p2 <- DimPlot(
  object = eccite,
  group.by = 'Phase',
  label = F, pt.size = 0.2,
  reduction = "umap", repel = T) +
  ggtitle("Cell Cycle Phase") +
  ylab("UMAP 2") +
  xlab("UMAP 1") +
  custom_theme
```

```

p3 <- DimPlot(
  object = eccite,
  group.by = "crispr",
  pt.size = 0.2,
  reduction = "umap",
  split.by = "crispr",
  ncol = 1,
  cols = c("grey39","goldenrod3")) +
  ggtitle("Perturbation Status") +
  ylab("UMAP 2") +
  xlab("UMAP 1") +
  custom_theme

# Visualize plots.
((p1 / p2 + plot_layout(guides = 'auto')) | p3 )

```



Remove technical factors

```

# Calculate perturbation signature (PRTB).
eccite<- CalcPerturbSig(
  object = eccite,
  assay = "RNA",

```

```

slot = "data",
gd.class ="gene",
nt.cell.class = "NT",
reduction = "pca",
ndims = 40,
num.neighbors = 20,
split.by = "replicate",
new.assay.name = "PRTB")

```

```

## Processing rep1

## Processing rep3

## Processing rep2

# Prepare PRTB assay for dimensionality reduction:
# Normalize data, find variable features and center data.
DefaultAssay(object = eccite) <- 'PRTB'

# Use variable features from RNA assay.
VariableFeatures(object = eccite) <- VariableFeatures(object = eccite[["RNA"]])
eccite <- ScaleData(object = eccite, do.scale = F, do.center = T)

```

```

## Centering data matrix

# Run PCA to reduce the dimensionality of the data.
eccite <- RunPCA(object = eccite, reduction.key = 'prtbpca', reduction.name = 'prtbpca')

## Warning: Keys should be one or more alphanumeric characters followed by an
## underscore, setting key from prtbpca to prtbpca_

## Warning: All keys should be one or more alphanumeric characters followed by an
## underscore '_', setting key to prtbpca_

## prtbpca_1
## Positive: SPP1, S100A4, RPLPO, VCAN, ZFP36L1, TREM2, TGFB1, CAPN2, TGFBI, LGALS1
##           RPSA, SORL1, FSCN1, CSF1R, YWHAH, LMNA, RPS2, ADORA3, HSPB1, CORO1A
##           ID2, MMP9, VAT1, GLO1, COL6A1, AP1S2, NFKBIA, MGST3, APOE, IL8
## Negative: CD74, HLA-DRA, CXCL10, WARS, GBP5, GBP1, IFI27, HLA-DRB1, FAM26F, PSMB9
##           HLA-DRB5, IL18BP, PSME2, SOCS1, HLA-DPA1, HLA-DQB1, SOD2, IFITM1, NCF1, S100A8
##           HLA-A, GLUL, CTSL, CD70, FCGR1B, HLA-DMA, HLA-DPB1, FCER1G, LY6E, CHI3L1
## prtbpca_2
## Positive: CXCL10, CXCL11, CXCL9, GBP1, SOCS1, GBP5, SOD2, TNFSF13B, CCL2, IFIT3
##           IL32, MX1, GYPC, IL18BP, ISG20, WARS, TNFSF10, IDO1, LY6E, IFI27
##           RSAD2, BAZ1A, FAM26F, IFIT2, GCH1, CD274, USP18, FTH1, TMEM176A, H1F0
## Negative: S100A8, S100A9, ALOX5AP, S100A4, SPP1, CTSD, C19orf59, S100A6, GRN, APOC1
##           TREM2, CHI3L1, S100A10, ANXA2, GLO1, CALR, PPIB, TSPO, TIMP1, HLA-DRB5
##           IL8, SRGN, PLAUR, VIM, DNASE2, FABP5, LGALS1, HLA-DRB1, FN1, FCER1G
## prtbpca_3
## Positive: CXCL10, CCL2, CXCL11, S100A9, S100A8, ALOX5AP, ISG15, CXCL9, IFI6, MX1
##           LY6E, CCL8, CYP1B1, S100A10, IL32, MARCKSL1, NFKBIA, GLO1, AP1S2, IFITM1

```



```

## Warning: All keys should be one or more alphanumeric characters followed by an
## underscore '_', setting key to prtbumap_

# Generate plots to check if clustering is driven by biological replicate ID,
# cell cycle phase or target gene class.
q1 <- DimPlot(
  object = eccite,
  group.by = 'replicate',
  reduction = 'prtbumap',
  pt.size = 0.2, cols = "Dark2", label = F, repel = T) +
  scale_color_brewer(palette = "Dark2") +
  ggtitle("Biological Replicate") +
  ylab("UMAP 2") +
  xlab("UMAP 1") +
  custom_theme

## Scale for colour is already present.
## Adding another scale for colour, which will replace the existing scale.

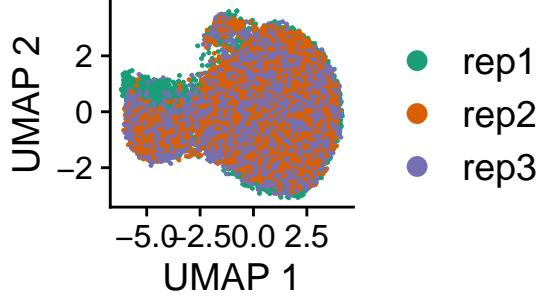
q2 <- DimPlot(
  object = eccite,
  group.by = 'Phase',
  reduction = 'prtbumap',
  pt.size = 0.2, label = F, repel = T) +
  ggtitle("Cell Cycle Phase") +
  ylab("UMAP 2") +
  xlab("UMAP 1") +
  custom_theme

q3 <- DimPlot(
  object = eccite,
  group.by = 'crispr',
  reduction = 'prtbumap',
  split.by = "crispr",
  ncol = 1,
  pt.size = 0.2,
  cols = c("grey39","goldenrod3")) +
  ggtitle("Perturbation Status") +
  ylab("UMAP 2") +
  xlab("UMAP 1") +
  custom_theme

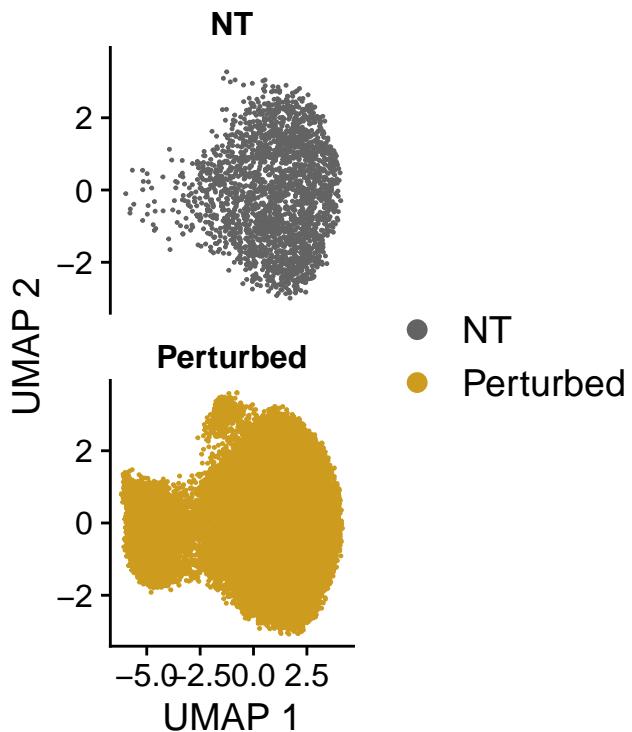
# Visualize plots.
(q1 / q2 + plot_layout(guides = 'auto') | q3)

```

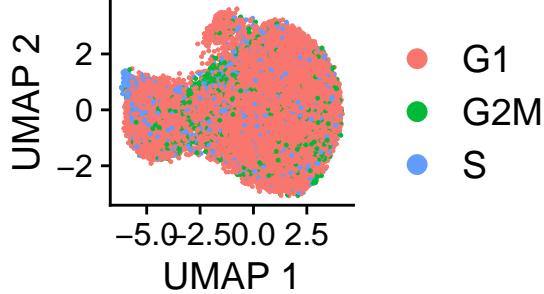
Biological Replicate



Perturbation Status



Cell Cycle Phase



Run mixscape

```
# Run mixscape.
eccite <- RunMixscape(
  object = eccite,
  assay = "PRTB",
  slot = "scale.data",
  labels = "gene",
  nt.class.name = "NT",
  min.de.genes = 5,
  iter.num = 10,
  de.assay = "RNA",
  verbose = F,
  prtb.type = "KO")

## Warning in FindMarkers.default(object = data.use, slot = data.slot, counts =
## counts, : No features pass logfc.threshold threshold; returning empty
## data.frame

## number of iterations= 95
## number of iterations= 187
## number of iterations= 172
```

```

## number of iterations= 18
## number of iterations= 6
## number of iterations= 18
## number of iterations= 11
## number of iterations= 11
## number of iterations= 59
## number of iterations= 43
## number of iterations= 42
## number of iterations= 19
## number of iterations= 12
## number of iterations= 12
## number of iterations= 23
## number of iterations= 19
## number of iterations= 19
## number of iterations= 51
## number of iterations= 51
## number of iterations= 51
## number of iterations= 36
## number of iterations= 26
## number of iterations= 25
## number of iterations= 20
## number of iterations= 12
## number of iterations= 12
## number of iterations= 17
## number of iterations= 15
## number of iterations= 14
## number of iterations= 13
## number of iterations= 73
## number of iterations= 46
## number of iterations= 41

# Calculate percentage of KO cells for all target gene classes.
df <- prop.table(table(eccite$mixscape_class.global, eccite$NT),2)

df2 <- reshape2::melt(df)
df2$Var2 <- as.character(df2$Var2)
test <- df2[which(df2$Var1 == "KO"),]
test <- test[order(test$value, decreasing = T),]
new.levels <- test$Var2
df2$Var2 <- factor(df2$Var2, levels = new.levels )
df2$Var1 <- factor(df2$Var1, levels = c("NT", "NP", "KO"))
df2$gene <- sapply(as.character(df2$Var2), function(x) strsplit(x, split = "g")[[1]][1])
df2$guide_number <- sapply(as.character(df2$Var2),
                           function(x) strsplit(x, split = "g")[[1]][2])
df3 <- df2[-c(which(df2$gene == "NT")),]

p1 <- ggplot(df3, aes(x = guide_number, y = value*100, fill= Var1)) +
  geom_bar(stat= "identity") +
  theme_classic()+
  scale_fill_manual(values = c("grey49", "grey79","coral1")) +
  ylab("% of cells") +
  xlab("sgRNA")

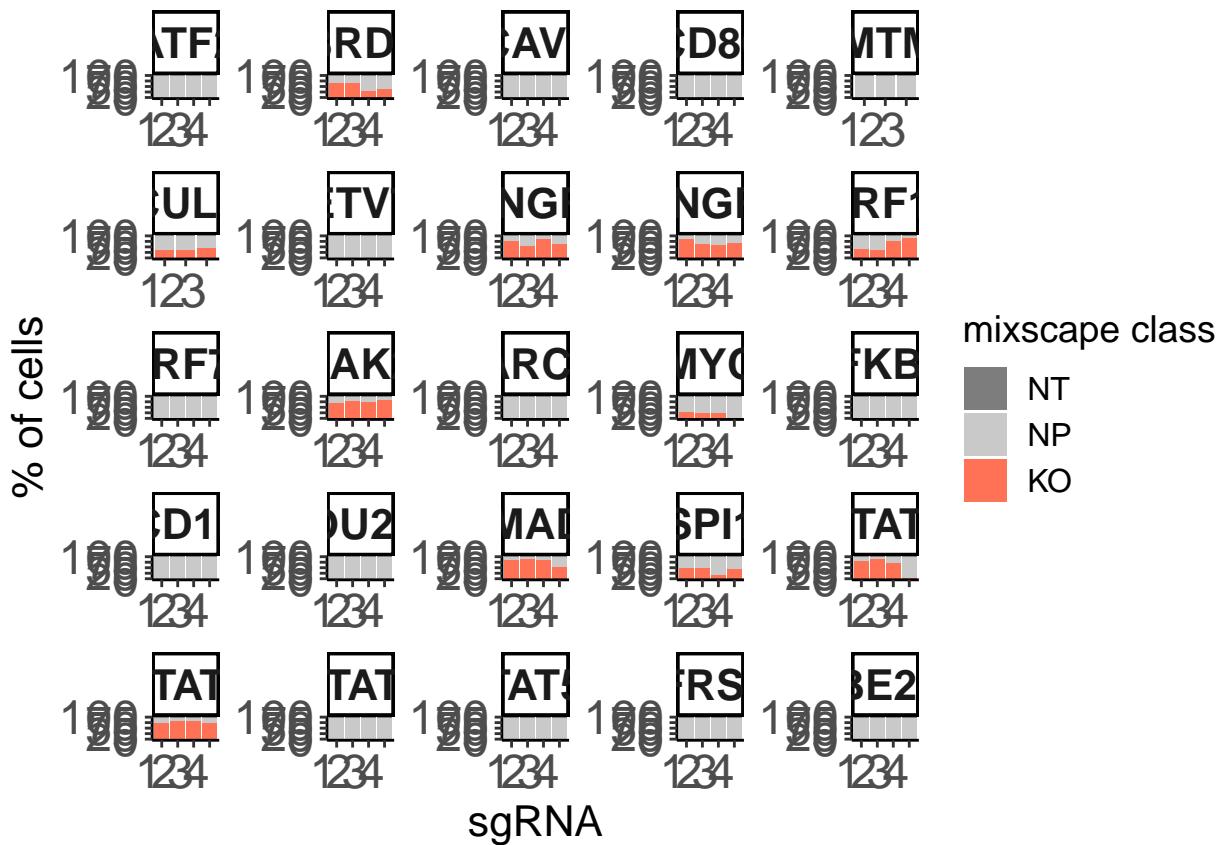
p1 + theme(axis.text.x = element_text(size = 18, hjust = 1),

```

```

axis.text.y = element_text(size = 18),
axis.title = element_text(size = 16),
strip.text = element_text(size=16, face = "bold")) +
facet_wrap(vars(gene), ncol = 5, scales = "free") +
labs(fill = "mixscape class") +theme(legend.title = element_text(size = 14),
legend.text = element_text(size = 12))

```



Perform Marker Gene Analysis/Pathway Enrichment Analysis

Papalex et reports 5 major pathways in CUL3 KO cells. Nuclear Receptors Meta-Pathway,NRF2 pathway,Phytochemical activity on NRF2 transcriptional activation,TYROBP causal network ,and complement system activation. We see pvalues that are approximately the same. However, we do see that the pathway “Phytochemical activity on NRF2 transcriptional activation” is ranked ahead of “TYROBP causal network” in this analysis whereas according to paplexi et al, the realtive pvalue rank should be flipped between these two pathways.

```

CUL3_marker = FindMarkers(eccite,ident.1 = 'CUL3 KO',ident.2 = 'NT',assay = 'RNA',only.pos = T)

## For a more efficient implementation of the Wilcoxon Rank Sum Test,
## (default method for FindMarkers) please install the limma package
## -----
## install.packages('BiocManager')
## BiocManager::install('limma')

```

```

## -----
## After installation of limma, Seurat will automatically use the more
## efficient implementation (no further action necessary).
## This message will be shown once per session

# get top 300 genes by log fold change
top = 300
CUL3_top = rownames(CUL3_marker)[order(CUL3_marker$avg_log2FC,decreasing = T)[1:top]]
# run pathway enrichment analysis
pathways = enrichr(CUL3_top,databases = 'WikiPathway_2021_Human')

## Uploading data to Enrichr... Done.
## Querying WikiPathway_2021_Human... Done.
## Parsing results... Done.

# get term and pvalue (using subset truncates pvalues for some reason)
pathways = cbind(pathways$WikiPathway_2021_Human$Term,
                  pathways$WikiPathway_2021_Human$Adjusted.P.value)[1:50,]
# make table
pathway_table = kable(pathways,booktabs = TRUE, linesep = "")
kable_styling(pathway_table,position = "center", latex_options = "scale_down")

```

Perform Marker Gene Analysis/Pathway Enrichment Analysis on Normalized Data

When using the normalized data, clearly the results are not at all the same.

```

CUL3_marker = FindMarkers(eccite,ident.1 = 'CUL3 KO',ident.2 = 'NT',
                           assay = 'PRTB',only.pos = T)

# get top 300 genes by log fold change
top = 300
CUL3_top = rownames(CUL3_marker)[order(CUL3_marker$avg_log2FC,decreasing = T)[1:top]]
# run pathway enrichment analysis
pathways = enrichr(CUL3_top,databases = 'WikiPathway_2021_Human')

## Uploading data to Enrichr... Done.
## Querying WikiPathway_2021_Human... Done.
## Parsing results... Done.

# get term and pvalue (using subset truncates pvalues for some reason)
pathways = cbind(pathways$WikiPathway_2021_Human$Term,
                  pathways$WikiPathway_2021_Human$Adjusted.P.value)[1:50,]
# make table
pathway_table = kable(pathways,booktabs = TRUE, linesep = "")
kable_styling(pathway_table,position = "center", latex_options = "scale_down")

```

Nuclear Receptors Meta-Pathway WP2882	1.19426453814473e-19
IL-18 signaling pathway WP4754	2.67460732896647e-15
NRF2 pathway WP2884	5.79777222817759e-14
Lung fibrosis WP3624	7.73036172305817e-10
Spinal Cord Injury WP2431	2.14847376311643e-08
Phytochemical activity on NRF2 transcriptional activation WP3	5.93505988471064e-08
SARS-CoV-2 innate immunity evasion and cell-specific immune response WP5039	2.04508944158617e-07
Ferroptosis WP4313	2.98431629435263e-07
Selenium Micronutrient Network WP15	3.77758943975651e-07
TYROBP causal network in microglia WP3945	8.24468762626036e-07
Regulation of toll-like receptor signaling pathway WP1449	8.24468762626036e-07
Chemokine signaling pathway WP3929	8.80226733902249e-07
NRF2-ARE regulation WP4357	9.41458352714735e-07
IL1 and megakaryocytes in obesity WP2865	1.12834924615539e-06
Photodynamic therapy-induced NFE2L2 (NRF2) survival signaling WP3612	1.12834924615539e-06
Toll-like Receptor Signaling Pathway WP75	1.12834924615539e-06
Photodynamic therapy-induced AP-1 survival signaling. WP3611	1.12834924615539e-06
p53 transcriptional gene network WP4963	1.18838612999873e-06
Senescence and Autophagy in Cancer WP615	1.18838612999873e-06
Apoptosis-related network due to altered Notch3 in ovarian cancer WP2864	1.63091836570387e-06
Allograft Rejection WP2328	1.70732311460629e-06
VEGFA-VEGFR2 Signaling Pathway WP3888	3.02979914894689e-06
Oxidative Stress WP408	8.15395718450203e-06
Vitamin D Receptor Pathway WP2877	1.08930924230432e-05
Vitamin B12 metabolism WP1533	1.10660025956448e-05
Photodynamic therapy-induced NF-kB survival signaling WP3617	1.10660025956448e-05
Pentose Phosphate Metabolism WP134	2.38995304927924e-05
Microglia Pathogen Phagocytosis Pathway WP3937	2.50226179206608e-05
Oxidative Damage WP3941	2.50226179206608e-05
COVID-19 adverse outcome pathway WP4891	2.50226179206608e-05
Complement and Coagulation Cascades WP558	2.898763346708e-05
Platelet-mediated interactions with vascular and circulating cells WP4462	4.7162128900405e-05
Aryl Hydrocarbon Receptor Netpath WP2586	6.02623334810642e-05
Glucocorticoid Receptor Pathway WP2880	0.00011140543473761
Complement system WP2806	0.000168882498235366
Kynurenone Pathway and links to Cellular Senescence WP5044	0.000211731198301808
Fibrin Complement Receptor 3 Signaling Pathway WP4136	0.000317322964328724
Vitamin D-sensitive calcium signaling in depression WP4698	0.000317322964328724
Cytokines and Inflammatory Response WP530	0.000368237795917242
Folate Metabolism WP176	0.000735492239449459
Tryptophan catabolism leading to NAD+ production WP4210	0.000735492239449459
RANKL/RANK signaling pathway WP2018	0.0015526187591333
miRNAs involvement in the immune response in sepsis WP4329	0.0018899088357796
Photodynamic therapy-induced HIF-1 survival signaling WP3614	0.0018899088357796
Complement Activation WP545	0.00250879992311433
Ebstein-Barr virus LMP1 signaling WP262	0.00287359608216466
Glutathione metabolism WP100	0.00287359608216466
Metabolic reprogramming in colon cancer WP4290	0.00318031259333159
NAD Metabolism in Oncogene-Induced Senescence and Mitochondrial Dysfunction-Associated Senescence WP5046	0.00320337734654777
Unfolded protein response WP4925	0.00320337734654777

Cytoplasmic Ribosomal Proteins WP477	2.7779458393605e-19
Type II interferon signaling (IFNG) WP619	2.88336633853203e-08
Immune response to tuberculosis WP4197	4.55179965834896e-08
SARS-CoV-2 innate immunity evasion and cell-specific immune response WP5039	2.61745740669961e-05
Ebola Virus Pathway on Host WP4217	0.00103683200835112
Allograft Rejection WP2328	0.00166570321050014
TYROBP causal network in microglia WP3945	0.00448002219138542
EPO Receptor Signaling WP581	0.00491768271537426
Non-genomic actions of 1,25 dihydroxyvitamin D3 WP4341	0.00626189707869799
Type I interferon induction and signaling during SARS-CoV-2 infection WP4868	0.00664639101752177
Prolactin Signaling Pathway WP2037	0.00664639101752177
Overview of interferons-mediated signaling pathway WP4558	0.00944175821459389
VEGFA-VEGFR2 Signaling Pathway WP3888	0.0117802347192378
Interleukin-11 Signaling Pathway WP2332	0.0134740709461464
IL-4 signaling pathway WP395	0.0213527634493522
TGF-beta Receptor Signaling WP560	0.0213527634493522
Pathways of nucleic acid metabolism and innate immune sensing WP4705	0.0231758327725216
TGF-beta receptor signaling in skeletal dysplasias WP4816	0.0231758327725216
Kit receptor signaling pathway WP304	0.0231758327725216
Leptin Insulin Overlap WP3935	0.0233506259852507
Endochondral Ossification with Skeletal Dysplasias WP4808	0.0252268949461796
Endochondral Ossification WP474	0.0252268949461796
AGE/RAGE pathway WP2324	0.0263258130335635
Regulation of toll-like receptor signaling pathway WP1449	0.0283176759290159
Cytosolic DNA-sensing pathway WP4655	0.0345180119754024
Unfolded protein response WP4925	0.0345180119754024
Hepatitis B infection WP4666	0.0345180119754024
Photodynamic therapy-induced unfolded protein response WP3613	0.0417671471037438
Interactions between immune cells and microRNAs in tumor microenvironment WP4559	0.0420190680618015
Acute viral myocarditis WP4298	0.0420190680618015
Selective expression of chemokine receptors during T-cell polarization WP4494	0.0420190680618015
PDGFR-beta pathway WP3972	0.0420190680618015
Host-pathogen interaction of human coronaviruses - interferon induction WP4880	0.0517524556048992
Complement system WP2806	0.0517524556048992
Toll-like Receptor Signaling Pathway WP75	0.0591140360480101
IL-5 signaling pathway WP127	0.0694241818682793
IL-6 signaling pathway WP364	0.0774997522713849
Thymic Stromal Lymphopoietin (TSLP) Signaling Pathway WP2203	0.0892600387947516
IL-3 signaling pathway WP286	0.0940504413214124
Adipogenesis WP236	0.0957026421727962
Interferon type I signaling pathways WP585	0.105308873961937
The Overlap Between Signal Transduction Pathways that Contribute to a Range of LMNA Laminopathies WP4879	0.105308873961937
Pathogenic Escherichia coli infection WP2272	0.105308873961937
MED and Pseudoachondroplasia genes WP4789	0.105308873961937
Brain-derived neurotrophic factor (BDNF) signaling pathway WP2380	0.110664250002887
Novel intracellular components of RIG-I-like receptor (RLR) pathway WP3865	0.116140718175124
Regulation of Actin Cytoskeleton WP51	0.117521829591053
Lung fibrosis WP3624	0.11999756090773
TFs regulate miRNAs related to cardiac hypertrophy WP1559	0.11999756090773
NAD Biosynthesis II (from tryptophan) WP2485	0.11999756090773