

Differential gene expression

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**** Introduction ****

In this code, we will use EDGER to analyze differentially expressed genes in RNA-Seq samples from the arcuate nucleus of the hypothalamus of exercised mice.

Groups:

CTL, 0h, 2h and 4h

1.0 Loadings

```
library(DESeq2)
library(ggplot2)
library(edgeR)
library(factoextra)
library(devtools)
library(tidyverse)
library(dplyr)
library(knitr)
library(stringr)
library(pheatmap)
library(RColorBrewer)
library(ggsci)
library(biomart)
library(dendextend)
library(tibble)
```

1.2 Loading Data

Loading:

1. eDat - Count matrix: quantification_raw
2. gDat - Group file

```
eDat <- read.table("Count.txt", header=TRUE, sep="\t", row.names = 1)
gDat <- read.table("Group.txt", header=TRUE, sep="\t")
```

exploring data

View data

head(eDat)

```
knitr::kable(head(eDat))
```

CTL	C1TL	C2TL	C3TL	C4TL	E5x0	E6x10	E7x20	E8x30	E9x40	E10x52	E11x62	E12x22	E13x32	E14x42	E15x54	E16x64	E17x24	E18x34	E19x44h	_5
0610005C13Rik	8	5	8	4	9	6	10	3	3	5	9	3	11	6	3	3	4	7		
0610006L08Rik	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	
0610009E02Rik	24	3	13	12	13	16	15	13	8	17	12	5	16	16	7	17	10	12		
0610009L18Rik	32	36	28	24	51	42	61	42	17	40	51	42	54	50	32	21	27	30		
0610009K12Rik	327	230	220	218	423	348	482	472	211	398	453	325	438	451	227	212	215	388		
0610022J13Rik	1	1	2	3	3	0	2	2	0	1	0	1	1	2	1	0	2	1		

```
# View data
```

head(gDat)

```
knitr::kable(head(gDat))
```

sample	treat
CTL_1	CTL
CTL_2	CTL
CTL_3	CTL
CTL_4	CTL
CTL_5	CTL
Exe0h_1	Exe0h

1.3 Exploring dataset

```
## How many transcripts are there in the entire library?
```

```
dim(eDat)
```

[1] 41079 20

```
## Count number of reads in each library
```

```
knitr:::kable(colSums(eDat))
```

	x
CTL_1	19659118
CTL_2	12709869
CTL_3	16504944
CTL_4	10506117

	x
CTL_5	12207032
Exe0h_1	10999794
Exe0h_2	23318452
Exe0h_3	17763551
Exe0h_4	22971441
Exe0h_5	22150547
Exe2h_1	9637053
Exe2h_2	18571904
Exe2h_3	22277769
Exe2h_4	18034142
Exe2h_5	21239057
Exe4h_1	19644975
Exe4h_2	11842915
Exe4h_3	12979164
Exe4h_4	13180388
Exe4h_5	15398202

```

## Load necessary libraries
library(ggdendro)
library(ggplot2)

# Function to plot dendrogram with legend
plot_dendrogram <- function(d, title, legend_labels, legend_colors) {
  plot(d, main = title)

  # Get plot coordinates
  coords <- par("usr")

  # Set legend coordinates
  legend_x <- coords[2] - 0.055 * diff(coords[1:2])
  legend_y <- coords[4] + 0.05 * diff(coords[3:4])

  # Add legend outside the plot
  legend(legend_x, legend_y, legend = legend_labels, fill = legend_colors, bty = "n", xpd = TRUE)
}

# Load data
eDat <- read.table("Count.txt", header=TRUE, sep="\t", row.names = 1)

# Calculate Spearman correlation
spear <- cor(eDat, method="spearman")

# Calculate distance matrix
d <- as.dist(1 - spear)

# Perform hierarchical clustering
upgma <- hclust(d, method="average")

# Function to set label color
labelCol <- function(x) {
  if (is.leaf(x)) {
    label <- attr(x, "label")
  }
}

```

```

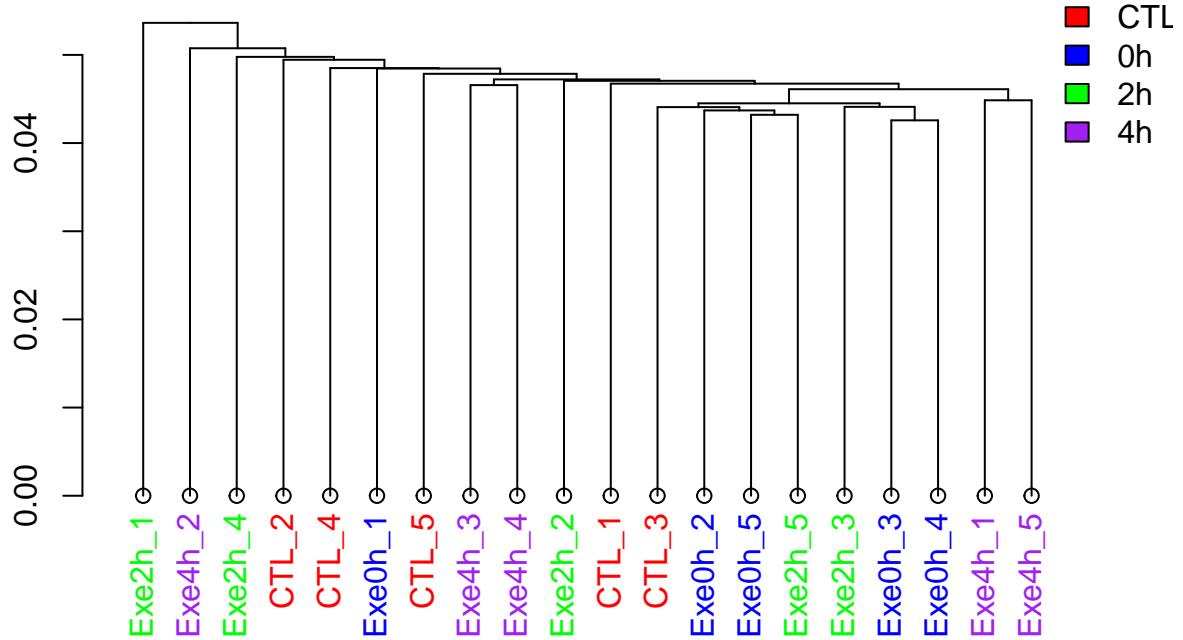
color <- ifelse(grepl("CTL", label), "red",
                ifelse(grepl("Exe0h", label), "blue",
                       ifelse(grepl("Exe2h", label), "green", "purple")))
attr(x, "nodePar") <- list(lab.col=color)
}
return(x)
}

# Apply labelCol on all nodes of the dendrogram
d <- dendrapply(as.dendrogram(upgma), labelCol)

## Plot dendrogram
plot_dendrogram(d, "Dendrogram before normalization", c("CTL", "0h", "2h", "4h"), c("red", "blue", "green"))

```

Dendrogram before normalization



```

# Save plot
png("plot1.png", width=7, height=7, units="in", res=300)
plot_dendrogram(d, "Dendrogram before normalization", c("CTL", "0h", "2h", "4h"), c("red", "blue", "green"))
dev.off()

## pdf
## 2

## Load necessary libraries
library(limma)

```

```

library(edgeR)

# Increase right margin
par(mar = c(5, 4, 4, 6) + 0.1)

# Define colors to the groups
colors <- c(rep("red", 5), rep("blue", 5), rep("green", 5), rep("purple", 5))

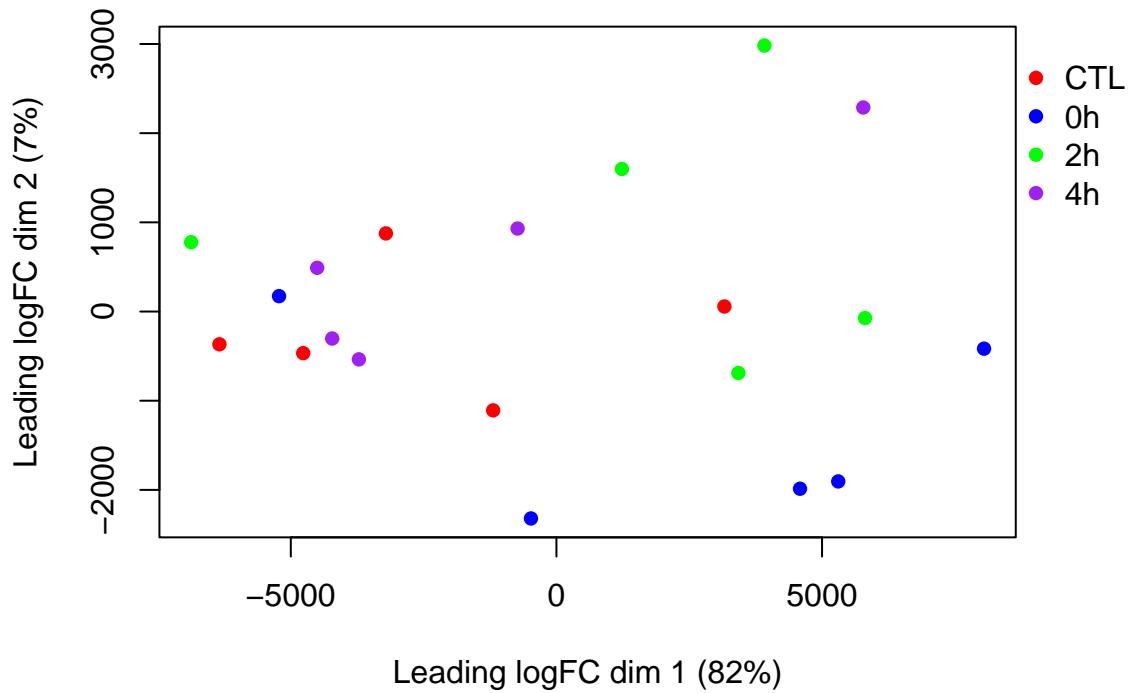
## Plot MDS plot
plotMDS(eDat, col = colors, pch = 16)

# Get the plot coordinates
plot_coord <- par("usr")

# Define coordinates for the legend
x_legend <- plot_coord[2] + 0.01 * diff(plot_coord[2:1]) # 1% to the left of the plot
y_legend <- plot_coord[4] - 0.05 * diff(plot_coord[4:3]) # 5% above the plot

# Add legend outside the plot
legend(x = x_legend, y = y_legend, legend = c("CTL", "0h", "2h", "4h"),
       col = c("red", "blue", "green", "purple"), pch = 16, title = "", bty = 'n',
       xpd = TRUE)

```



```

# Save plot
# Set up PNG device with desired resolution

```

```

png("plot2.png", width = 800, height = 600, units = "px", res = 300)

# Increase right margin
par(mar = c(5, 4, 4, 6) + 0.1)

# Define colors to the groups
colors <- c(rep("red", 5), rep("blue", 5), rep("green", 5), rep("purple", 5))

# Perform MDS plot
plotMDS(eDat, col = colors, pch = 16)

# Get the plot coordinates
plot_coord <- par("usr")

# Define coordinates for the legend
x_legend <- plot_coord[2] + 0.01 * diff(plot_coord[2:1]) # 1% to the left of the plot
y_legend <- plot_coord[4] - 0.05 * diff(plot_coord[4:3]) # 5% above the plot

# Add legend outside the plot
legend(x = x_legend, y = y_legend, legend = c("CTL", "0h", "2h", "4h"),
       col = c("red", "blue", "green", "purple"), pch = 16, title = "", bty = 'n',
       xpd = TRUE)

# Save the plot
dev.off()

```

```

## pdf
## 2

## Boxplot before normalization (outliers)

# Increase right margin
par(mar = c(5, 6, 4, 4) + 0.1)

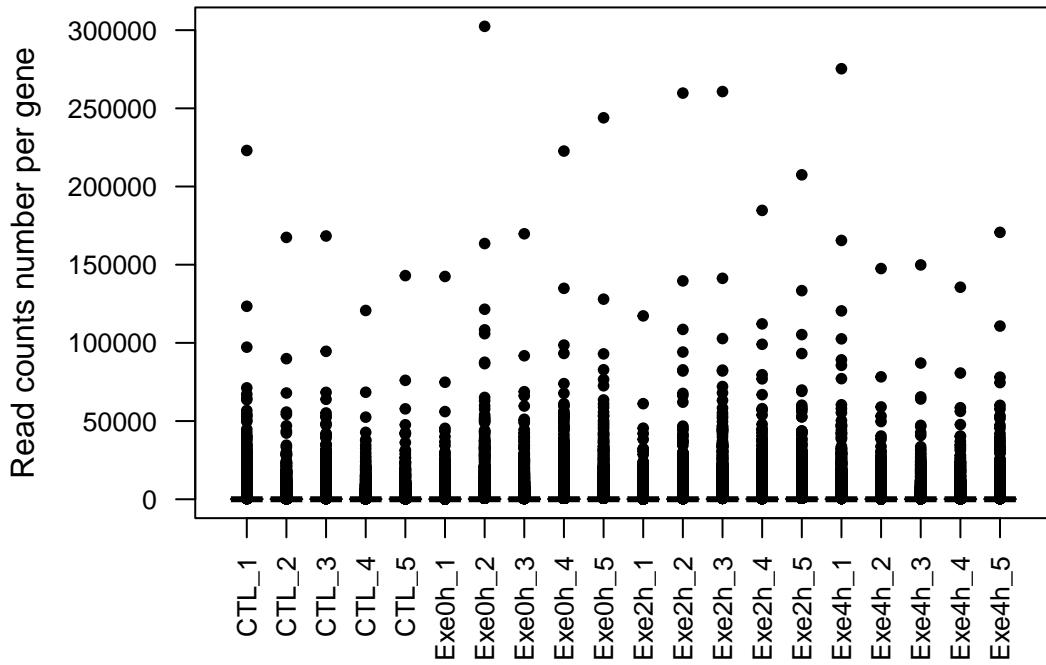
# Get the plot coordinates
plot_coord <- par("usr")

# plot
boxplot(eDat, col = colors, outline = T, pch = 20,
        main="Read counts number per gene for each sample\n(outliers)",
        xlab="",
        ylab="", las=2,cex.axis=0.8)

# Move y-axis label further to the left
mtext(side = 2, text = "Read counts number per gene", line = 4)

```

Read counts number per gene for each sample (outliers)



```

## Box plot without outliers
# plot
boxplot(eDat, col = colors, outline = F, pch = 20,
        main="Read counts number per gene for each sample\n (without outliers)",
        xlab="",
        ylab="", las=2,cex.axis=0.8)

# Move y-axis label further to the left
mtext(side = 2, text = "Read counts number per gene", line = 4)

# Get plot coordinates
coords <- par("usr")

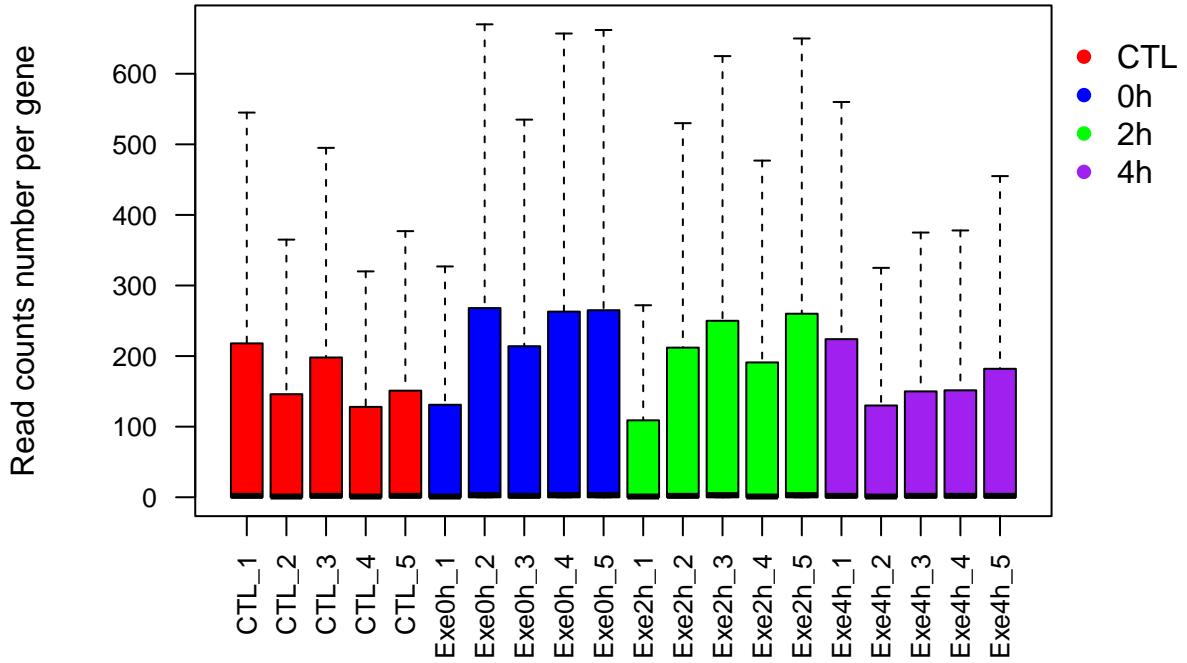
# Increase right margin
par(mar = c(5, 6, 4, 7) + 0.1)

# Set legend coordinates
legend_x <- coords[2] - 0.001 * diff(coords[1:2])
legend_y <- coords[4] + 0.05 * diff(coords[3:4])

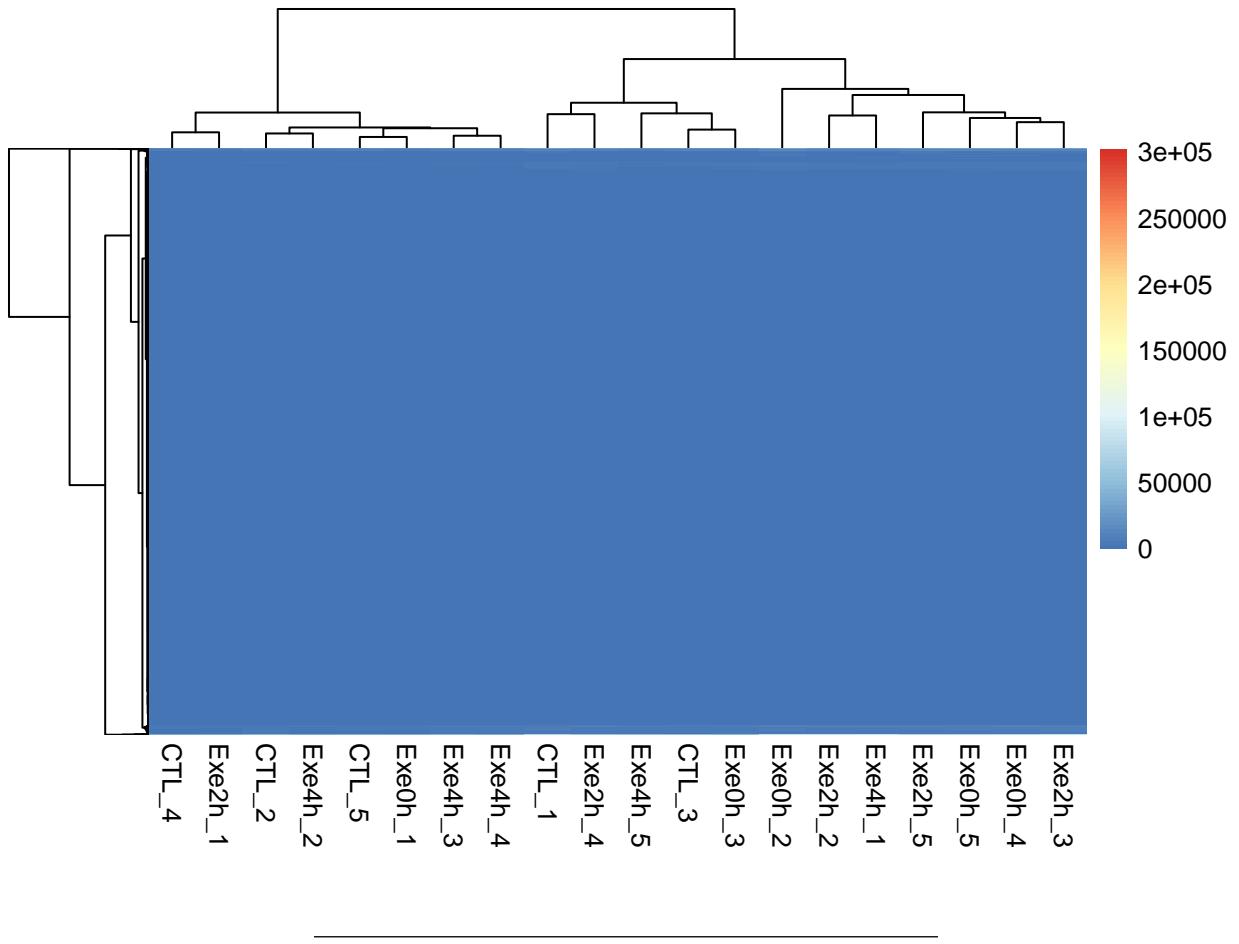
# Add legend outside the plot
legend(legend_x, legend_y, legend = c("CTL", "0h", "2h", "4h"),
       col = c("red", "blue", "green", "purple"), pch = 16, title = "", bty = 'n',
       xpd = TRUE)

```

Read counts number per gene for each sample (without outliers)



```
#Pheatmpa Distances
library(pheatmap)
d_matrix <- as.matrix(eDat)
pheatmap(d_matrix, show_rownames=F, show_colnames=T)
```



1.4 Groups (CTL vs 0 hours)

```

library(tibble)
library(ggrepel)

eDat <- read.table("Count.txt", header=TRUE, sep="\t", row.names = 1)
gDat <- read.table("Group.txt", header=TRUE, sep="\t")

## Counts
## filter only samples CTL and Exe0h
suppressMessages({library(dplyr)})

# Select only 'CTL' ou 'Exe0h'
selected_columns <- eDat[, grepl("^CTL|^Exe0h", names(eDat))]

# new table
new_table1 <- cbind(rownames = rownames(eDat), selected_columns)

## remove the first collumn
new_table1$rownames <- NULL

```

```

## Treat
new_group1 <- gDat[1:10, ]

## load EdgeR ##
library(edgeR)

# ATENTION!!
new_group1$treat

## [1] "CTL"    "CTL"    "CTL"    "CTL"    "CTL"    "ExeOh"  "ExeOh"  "ExeOh"  "ExeOh"
## [10] "ExeOh"

# Control treatment needs to appear as the first level for the Treat Vs. Control comparison
new_group1$treat <- factor(new_group1$treat, levels=c("CTL", "ExeOh"))

# Input data
y_adj <- DGEList(counts=new_table1, samples=new_group1, group=new_group1$treat)
design_adj <- model.matrix(~ treat - 1, data = new_group1)

# Filter lowly expressed genes
keep_adj <- rowSums(cpm(y_adj)>1) >= 2
sum(keep_adj)

## [1] 16131

y_adj <- y_adj[keep_adj, , keep.lib.sizes=FALSE]

## Explanation: ">= 2" is a condition to maintain the gene, which means that the gene needs to have at least two reads across all samples.

## Explanation: after filtering, 16131 genes remained that have sufficient expression in at least two replicates.

# Normalization for RNA composition
y_adj <- calcNormFactors(y_adj)
y_adj$samples

##      group lib.size norm.factors  sample treat
## CTL_1     CTL 19626485  0.9759532 CTL_1   CTL
## CTL_2     CTL 12687868  0.9810779 CTL_2   CTL
## CTL_3     CTL 16474303  1.0097562 CTL_3   CTL
## CTL_4     CTL 10484768  1.0226341 CTL_4   CTL
## CTL_5     CTL 12183173  1.0166898 CTL_5   CTL
## ExeOh_1   ExeOh 10979050  1.0019509 ExeOh_1 ExeOh
## ExeOh_2   ExeOh 23278095  0.9857161 ExeOh_2 ExeOh
## ExeOh_3   ExeOh 17729446  1.0168245 ExeOh_3 ExeOh
## ExeOh_4   ExeOh 22932476  0.9866500 ExeOh_4 ExeOh
## ExeOh_5   ExeOh 22110848  1.0040026 ExeOh_5 ExeOh

norm.expr_adj <- y_adj$samples

# norm counts

```

```

logcpm_adj <- cpm(y_adj, log=TRUE)

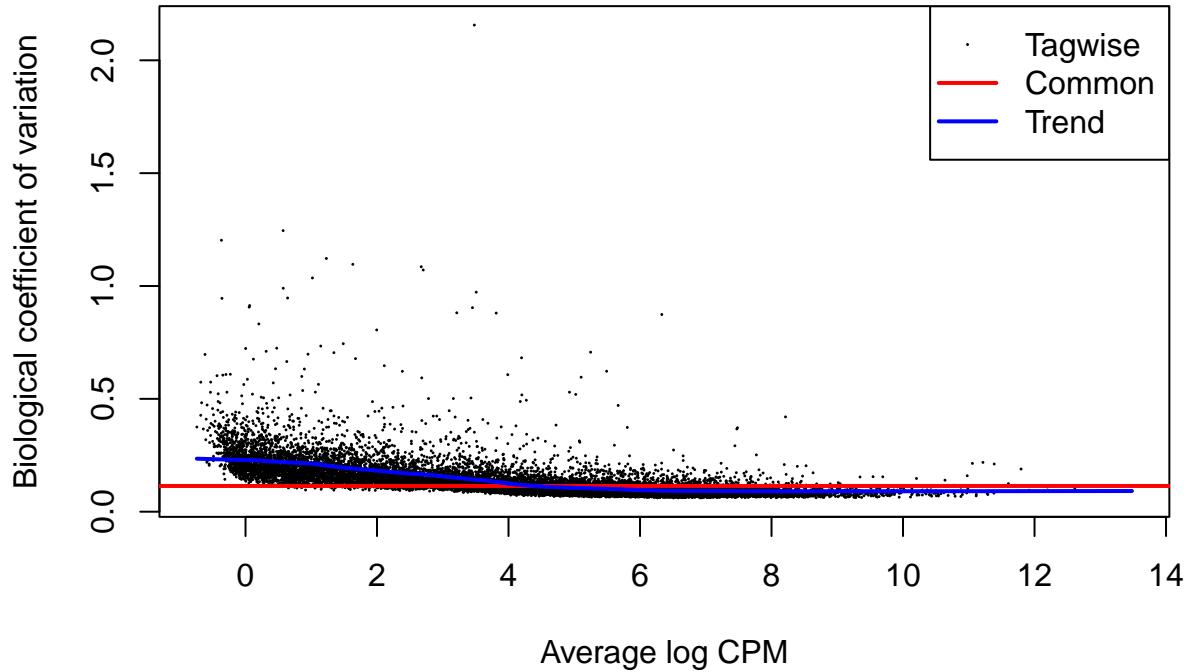
#####
##      Data analyses with edgeR      ##
#####

# Estimate dispersion with Cox-Reid profile-adjusted likelihood (CR) method
y_adj <- estimateDisp(y_adj, design_adj)
y_adj$common.dispersion

## [1] 0.01299319

## Explanation: A low dispersion value indicates that the expression levels of genes are relatively consis-
## plot
plotBCV(y_adj)

```



```

# Fit Generalized Linear Model (GLM)
fit <- glmFit(y_adj, design_adj)
colnames(fit)

## [1] "treatCTL"    "treatExe0h"

```

```

# Differential gene expression analysis
lrt <- glmLRT(fit, contrast=c(-1,1))

## Coefficients used in the contrast for the likelihood ratio test
topTags(lrt)

## Coefficient: -1*treatCTL 1*treatExe0h
##          logFC    logCPM      LR     PValue      FDR
## Arl4d    2.5283730 3.644635 218.91871 1.556835e-49 2.511330e-45
## Sgk1     1.7910453 6.203344 173.68018 1.162594e-39 9.376900e-36
## Tsc22d3 1.2179201 6.956926 167.50674 2.592640e-38 1.394063e-34
## Arrdc2   1.4305453 3.870079 128.18992 1.020005e-29 4.113426e-26
## Cebpd    1.7064096 2.835152 106.22413 6.584983e-25 2.124447e-21
## Klf15    0.7680026 5.568856 89.01382 3.920635e-21 1.054063e-17
## Slc2a1   0.7815728 6.858393 86.50986 1.390426e-20 3.204137e-17
## Plin4    2.2421116 3.404487 86.16906 1.651923e-20 3.330896e-17
## Gm19439  1.6073912 2.806024 84.70149 3.469883e-20 6.219186e-17
## Mfsd2a   0.9786308 5.408416 80.01067 3.723933e-19 6.007076e-16

## Explanation: It indicates that the comparison is between the coefficient for treatCTL (the reference

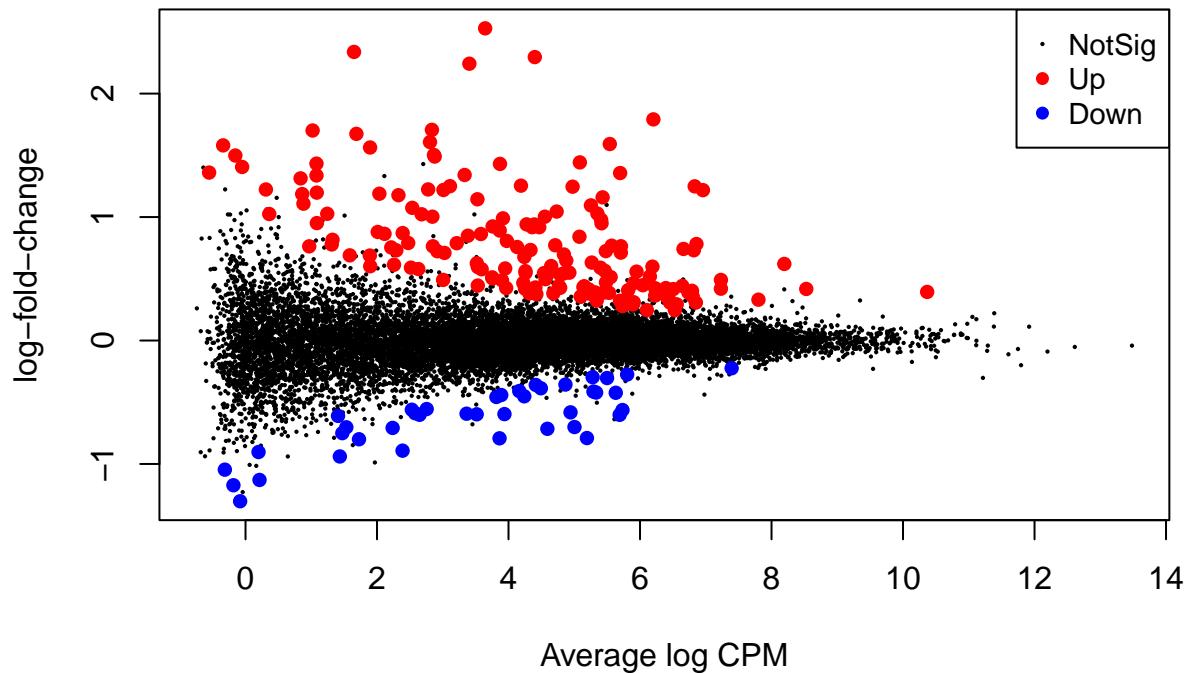
## Number of genes identified as differentially expressed (Disregarding parameters)
is.de <- decideTestsDGE(lrt)
summary(decideTestsDGE(lrt))

##          -1*treatCTL 1*treatExe0h
## Down                  40
## NotSig                15922
## Up                   169

## plot MD
plotMD(lrt, status=is.de)

```

-1*treatCTL 1*treatExe0h

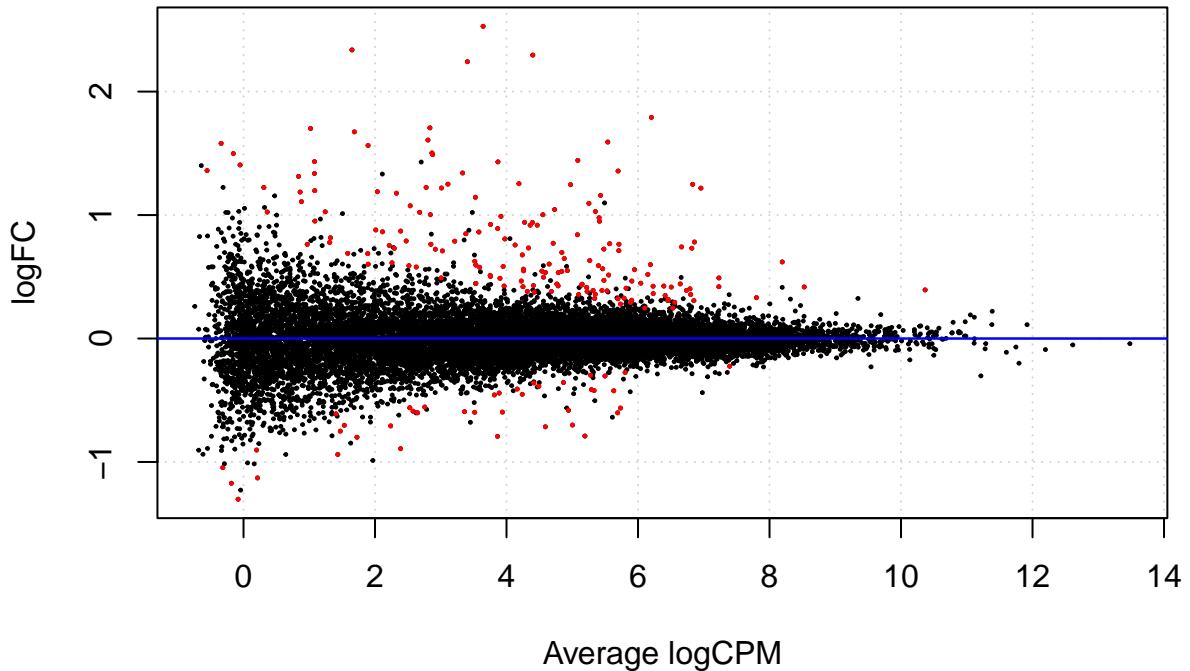


```
# Number of up and down-regulated. FDR < 5% and |log2 fold change| > 1
summary(de <- decideTestsDGE(lrt, lfc=0))
```

```
## -1*treatCTL 1*treatExe0h
## Down 40
## NotSig 15922
## Up 169
```

```
## Explanation: The absolute value of 1 on this scale indicates that there was a doubling (2-fold) change

# MA-plot
detags <- rownames(y_adj)[as.logical(de)]
plotSmear(lrt, de.tags=detags)
abline(h=c(-0, 0), col="blue")
```



```

# To export the results
result <- as.data.frame(topTags(lrt, n=1000000))
result <- add_column(result, symbol= rownames(result), .before = "logFC")

## save as table
write.table(result, "DEGs_CTL_vs_0h.txt", sep = "\t", row.names = FALSE)

## Check all the genes Downregulated and Upregulated

# Remove unnecessary columns
result$logCPM <- NULL
result$LR <- NULL

# Remove rows with FDR < 0.05 and logFC >= 0
result$expression <- ifelse(result$FDR < 0.05 & abs(result$logFC) >= 0,
                           ifelse(result$logFC >= 0, 'Up', 'Down'),
                           'Stable')

# Select only genes that are upregulated or downregulated
result_final <- subset(result, expression %in% c("Up", "Down"))

# Remove row names
rownames(result_final) <- NULL

## View the table

```

```
knitr::kable(result_final)
```

symbol	logFC	PValue	FDR	expression
Arl4d	2.5283730	0.0000000	0.0000000	Up
Sgk1	1.7910453	0.0000000	0.0000000	Up
Tsc22d3	1.2179201	0.0000000	0.0000000	Up
Arrdc2	1.4305453	0.0000000	0.0000000	Up
Cebpd	1.7064096	0.0000000	0.0000000	Up
Klf15	0.7680026	0.0000000	0.0000000	Up
Slc2a1	0.7815728	0.0000000	0.0000000	Up
Plin4	2.2421116	0.0000000	0.0000000	Up
Gm19439	1.6073912	0.0000000	0.0000000	Up
Mfsd2a	0.9786308	0.0000000	0.0000000	Up
Fos	1.5914162	0.0000000	0.0000000	Up
Fosb	2.2956568	0.0000000	0.0000000	Up
Usp2	1.0444512	0.0000000	0.0000000	Up
Nfkbia	0.9179199	0.0000000	0.0000000	Up
Per1	0.7312907	0.0000000	0.0000000	Up
Fosl2	1.3565789	0.0000000	0.0000000	Up
Plekhf1	1.5026662	0.0000000	0.0000000	Up
Atf3	2.3374572	0.0000000	0.0000000	Up
Mmp14	-0.7900059	0.0000000	0.0000000	Down
Ddit4	0.7118135	0.0000000	0.0000000	Up
Hif3a	1.3406345	0.0000000	0.0000000	Up
Errfi1	0.6312994	0.0000000	0.0000000	Up
Egr1	1.2487810	0.0000000	0.0000000	Up
Klf2	0.9240276	0.0000000	0.0000000	Up
Irs2	0.4906806	0.0000000	0.0000000	Up
Csrnp1	1.2501155	0.0000000	0.0000000	Up
Trib1	0.9407406	0.0000000	0.0000000	Up
Gadd45g	1.0027271	0.0000000	0.0000000	Up
Sik1	0.7241458	0.0000000	0.0000000	Up
Pdk4	0.8487158	0.0000000	0.0000000	Up
Rhou	0.5899716	0.0000000	0.0000000	Up
Kirrel2	1.5633416	0.0000000	0.0000000	Up
Arid5a	0.9896376	0.0000000	0.0000000	Up
Fam43a	0.7573944	0.0000000	0.0000000	Up
Nr4a3	1.2458588	0.0000000	0.0000000	Up
Nr4a1	1.1594934	0.0000000	0.0000000	Up
Fzd2	0.8066421	0.0000000	0.0000000	Up
Usp53	0.8406150	0.0000000	0.0000000	Up
Map3k6	1.0029438	0.0000000	0.0000000	Up
LOC118567915	1.2542226	0.0000000	0.0000000	Up
Hes5	-0.7921637	0.0000000	0.0000003	Down
Cldn5	-0.7149967	0.0000000	0.0000004	Down
Fbxw23	1.3133198	0.0000000	0.0000005	Up
Maff	1.1769639	0.0000000	0.0000005	Up
Cdkn1a	0.9177760	0.0000000	0.0000005	Up
Fzd1	0.5437749	0.0000000	0.0000007	Up
Apold1	1.4899841	0.0000000	0.0000007	Up
Btg2	0.9398285	0.0000000	0.0000014	Up
Fam20a	0.6967426	0.0000000	0.0000015	Up

symbol	logFC	PValue	FDR	expression
Rrad	1.0944230	0.0000000	0.0000036	Up
Dusp1	0.7333510	0.0000000	0.0000038	Up
Fam107a	0.6202189	0.0000000	0.0000043	Up
Klf4	0.7881507	0.0000000	0.0000086	Up
Ccn2	1.2236041	0.0000000	0.0000101	Up
Fn1	0.5138749	0.0000000	0.0000116	Up
Coq10b	0.5334168	0.0000000	0.0000116	Up
Txnip	0.9496076	0.0000001	0.0000152	Up
Per2	0.5649836	0.0000001	0.0000173	Up
Nedd9	0.5483248	0.0000001	0.0000173	Up
Rin2	0.4708987	0.0000001	0.0000173	Up
Emilin2	1.1889787	0.0000001	0.0000213	Up
Spsb1	0.4968295	0.0000001	0.0000215	Up
Cables1	0.4652885	0.0000001	0.0000215	Up
Gem	1.2192277	0.0000001	0.0000229	Up
Gm39080	0.8909155	0.0000001	0.0000229	Up
Gpt2	0.4479300	0.0000001	0.0000260	Up
F3	0.4073603	0.0000001	0.0000290	Up
Cx3cr1	-0.5819720	0.0000001	0.0000331	Down
Fzd4	0.5833852	0.0000002	0.0000484	Up
Klf13	0.4166384	0.0000002	0.0000514	Up
Junb	1.0283743	0.0000002	0.0000567	Up
Klf9	0.4025619	0.0000003	0.0000603	Up
Fkbp5	0.7634266	0.0000003	0.0000719	Up
Hr	0.5981013	0.0000004	0.0000899	Up
Fabp7	-0.7007317	0.0000004	0.0000899	Down
Rgcc	0.7097175	0.0000004	0.0000899	Up
Rrp8	0.5116345	0.0000005	0.0000993	Up
Zbtb16	1.4426385	0.0000006	0.0001341	Up
Arc	0.7714564	0.0000007	0.0001359	Up
Jun	0.5578433	0.0000007	0.0001384	Up
Serpine1	0.8791575	0.0000007	0.0001467	Up
Sdc4	0.4235128	0.0000008	0.0001615	Up
Rasd1	0.6772451	0.0000010	0.0001852	Up
Tcim	1.6737609	0.0000010	0.0001902	Up
Gucy2f	-0.9391819	0.0000010	0.0001990	Down
Etnppl	0.4248168	0.0000011	0.0002033	Up
Tiparp	0.5351864	0.0000013	0.0002325	Up
Il12rb1	1.3368817	0.0000014	0.0002540	Up
S100a16	-0.4232398	0.0000014	0.0002540	Down
Stk40	0.4327009	0.0000022	0.0003873	Up
Herpud1	0.3071754	0.0000028	0.0004876	Up
Ppp1r3g	1.7009525	0.0000029	0.0005057	Up
Mertk	0.6475418	0.0000030	0.0005274	Up
Mknk2	0.4396196	0.0000031	0.0005396	Up
P2ry13	-0.8921566	0.0000038	0.0006455	Down
Tmem254	-0.4212059	0.0000045	0.0007528	Down
Htra1	0.4421693	0.0000045	0.0007528	Up
Dnajb1	0.7417384	0.0000060	0.0009803	Up
Sult1a1	0.8626506	0.0000060	0.0009803	Up
Tmem252	1.1860994	0.0000061	0.0009850	Up
Mat2a	0.3307634	0.0000074	0.0011792	Up

symbol	logFC	PValue	FDR	expression
Sp140l1	-1.1293863	0.0000077	0.0012210	Down
Gm51447	1.4058459	0.0000078	0.0012210	Up
Pde4c	1.0264153	0.0000079	0.0012293	Up
Mchr1	0.4655748	0.0000085	0.0012991	Up
Gm53541	0.9511735	0.0000085	0.0012991	Up
Glul	0.3936292	0.0000090	0.0013538	Up
Gm33320	0.7630995	0.0000096	0.0014297	Up
Aff1	0.5455668	0.0000102	0.0015142	Up
Lgr6	-0.5960208	0.0000145	0.0021225	Down
Gjb6	0.3484156	0.0000153	0.0022256	Up
Plaur	1.4983028	0.0000166	0.0023856	Up
Hapln2	0.6031831	0.0000173	0.0024691	Up
Gpr6	0.6251288	0.0000180	0.0025438	Up
Aif1l	-0.5977313	0.0000185	0.0025987	Down
Gimap6	0.7538889	0.0000190	0.0026466	Up
Pim3	0.3886301	0.0000197	0.0027207	Up
Slc19a2	0.4412992	0.0000215	0.0029434	Up
Dzip1l	0.5495812	0.0000228	0.0030919	Up
Alkal2	1.4327290	0.0000235	0.0031629	Up
Lifr	0.3861406	0.0000253	0.0033747	Up
Dnajb5	0.5187529	0.0000256	0.0033803	Up
Lrrc8a	0.3897436	0.0000268	0.0035139	Up
Gadd45b	0.8700649	0.0000315	0.0040914	Up
Egr4	1.1439148	0.0000373	0.0048014	Up
Srxn1	0.3561115	0.0000375	0.0048014	Up
Gm11627	0.7781717	0.0000423	0.0053749	Up
Nostrin	0.6138901	0.0000438	0.0055192	Up
Ankrd13a	0.3101123	0.0000460	0.0057481	Up
Xdh	1.0222934	0.0000470	0.0058380	Up
Npas3	-0.3866928	0.0000475	0.0058462	Down
Gpr3	0.8643068	0.0000503	0.0061503	Up
Nfil3	0.4875412	0.0000509	0.0061794	Up
Ccn1	1.0748307	0.0000538	0.0064717	Up
Ciart	0.5028611	0.0000575	0.0068526	Up
Id2	-0.4137854	0.0000578	0.0068526	Down
Plk2	0.3221122	0.0000597	0.0070275	Up
Tekt4	0.7899856	0.0000621	0.0071742	Up
Slc25a33	0.3234592	0.0000622	0.0071742	Up
Daam2	0.2926685	0.0000623	0.0071742	Up
Gprc5c	-0.7028560	0.0000632	0.0072316	Down
Adipor2	0.3642144	0.0000650	0.0073865	Up
Prodh	0.4265908	0.0000774	0.0087281	Up
Pitpnm2	0.3071824	0.0000892	0.0099321	Up
LOC105243553	1.1978312	0.0000893	0.0099321	Up
5930403L14Rik	-0.4570878	0.0000911	0.0100657	Down
Ccdc60	-0.7078384	0.0000977	0.0107233	Down
Smarcd2	0.4200180	0.0001007	0.0109600	Up
Tma16	0.7306187	0.0001012	0.0109600	Up
Nckap5	-0.5550543	0.0001052	0.0113142	Down
Tmem44	-0.3598000	0.0001088	0.0115716	Down
Notch4	0.5772363	0.0001090	0.0115716	Up
Vgf	0.4175746	0.0001142	0.0120420	Up

symbol	logFC	PValue	FDR	expression
Otud7b	0.2918806	0.0001189	0.0123829	Up
Rtkn	0.3586093	0.0001190	0.0123829	Up
Pnpla7	0.3751529	0.0001256	0.0129893	Up
Gpd1	0.5616398	0.0001274	0.0130620	Up
Rsrp1	0.4197767	0.0001279	0.0130620	Up
Sap30	0.6017719	0.0001357	0.0137636	Up
P2ry12	-0.4092735	0.0001439	0.0145034	Down
Rapgef3	0.3264347	0.0001448	0.0145034	Up
Trac	-0.7996988	0.0001489	0.0148296	Down
Ada	0.6880614	0.0001535	0.0151878	Up
Gm51400	0.5902011	0.0001740	0.0171109	Up
Rasl11b	0.4250780	0.0001753	0.0171112	Up
Emp2	-0.3567410	0.0001761	0.0171112	Down
Slc6a8	0.2964498	0.0001971	0.0190376	Up
Fgfrl1	-0.4416060	0.0002108	0.0202427	Down
Ccr5	-0.7503239	0.0002152	0.0205378	Down
Tns2	0.3564482	0.0002246	0.0213101	Up
Sstr1	-0.2970437	0.0002331	0.0219866	Down
Zc3h6	-0.4518597	0.0002443	0.0229134	Down
Tob2	0.3805754	0.0002635	0.0245661	Up
Mc11	0.2462298	0.0002810	0.0260508	Up
Lcp2	-0.5606294	0.0002975	0.0274252	Down
Sall2	-0.2244158	0.0003052	0.0279726	Down
Gpr34	-0.6016994	0.0003114	0.0283826	Down
Plppb	0.2464454	0.0003155	0.0284566	Up
Ets1	-0.5927357	0.0003158	0.0284566	Down
C1ql4	1.2233344	0.0003251	0.0289106	Up
Gpr50	-0.6017445	0.0003253	0.0289106	Down
A730020M07Rik	1.1084004	0.0003262	0.0289106	Up
Vwf	0.4451426	0.0003286	0.0289691	Up
Dedd2	0.4141915	0.0003559	0.0311987	Up
Gm42084	1.5804071	0.0003787	0.0330174	Up
C330020E22Rik	1.3600788	0.0003874	0.0335992	Up
Cish	0.4909058	0.0003932	0.0339207	Up
Cebpb	0.7229472	0.0003995	0.0342768	Up
Phyhd1	0.3816493	0.0004254	0.0363056	Up
Iffo1	0.2790906	0.0004725	0.0399042	Up
Glce	-0.2753400	0.0004741	0.0399042	Down
Zfp276	0.3820236	0.0004750	0.0399042	Up
Syt15	-0.9032061	0.0004848	0.0405161	Down
Gm51879	-1.0459749	0.0004957	0.0411302	Down
Pnpla2	0.3895078	0.0004994	0.0411302	Up
Tnfrsf13b	-1.1721778	0.0004998	0.0411302	Down
Dmp1	0.7627408	0.0005157	0.0421984	Up
Kcnn2	0.4297634	0.0005180	0.0421984	Up
Pla1a	1.0245930	0.0005374	0.0435580	Up
Zfp469	0.5790404	0.0005495	0.0443209	Up
Gm10561	-0.5886712	0.0005621	0.0451101	Down
Mir6236	-0.5632525	0.0005752	0.0459338	Down
Irf8	-0.6104244	0.0005810	0.0461655	Down
Dusp4	0.5944496	0.0006185	0.0488515	Up
Tmem52	0.8159705	0.0006208	0.0488515	Up

symbol	logFC	PValue	FDR	expression
Arsi	-1.3017830	0.0006290	0.0491146	Down
Mxd4	0.3175590	0.0006303	0.0491146	Up
Tagap	0.6897825	0.0006361	0.0493284	Up
Trib2	-0.3036969	0.0006454	0.0498128	Down

```
#####
##### PLOTS #####
#####

## volcano plot

# Extract top tags from lrt
result_edgeR <- as.data.frame(topTags(lrt, n=1000000, adjust.method = "BH"))

# Extract log-transformed counts per million
count_CPM <- as.data.frame(logcpm_adj)

## Convert row names into first column
result_edgeR <- add_column(result_edgeR, gene = rownames(result_edgeR), .before = "logFC")

count_CPM <- add_column(count_CPM, gene = rownames(count_CPM), .before = "CTL_1")

## Order by gene
result_edgeR <- result_edgeR[order(result_edgeR$gene),]
count_CPM <- count_CPM[order(count_CPM$gene),]

## Combine result_edgeR columns with count_CPM
count_CPM <- add_column(count_CPM, logFC = result_edgeR$logFC, .before = "CTL_1")
count_CPM <- add_column(count_CPM, FDR = result_edgeR$FDR, .before = "CTL_1")
count_CPM <- add_column(count_CPM, gene2 = result_edgeR$gene, .before = "CTL_1")

# Check if gene and gene2 are the same and in the same order
if(all(count_CPM$gene == count_CPM$gene2)) {
  print("The values in 'gene' and 'gene2' are the same.")
} else {
  print("The values in 'gene' and 'gene2' are different.")
  different_indices <- which(count_CPM$gene != count_CPM$gene2)
  print(paste("The value in 'gene' is different at indices:", different_indices))
}

## [1] "The values in 'gene' and 'gene2' are the same.

## remove gene2
count_CPM$gene2 <- NULL

## Insert -log10 FDR
count_CPM <- add_column(count_CPM, logFDR = -log10(result_edgeR$FDR), .before = "CTL_1")

## Insert -log10 p-value
count_CPM <- add_column(count_CPM, logpvalue = -log10(result_edgeR$PValue), .before = "CTL_1")
```

```

## PLOT

# Assign count_CPM to expressao_genica
expressao_genica <- count_CPM

# Determine expression based on FDR and logFC thresholds
expressao_genica$expression <- ifelse(expressao_genica$FDR < 0.05 & abs(expressao_genica$logFC) >= 0,
                                         ifelse(expressao_genica$logFC >= 0, 'Up', 'Down'),
                                         'Stable')

# Count the number of genes categorized as Up and Down
sum(expressao_genica$expression == "Up")

## [1] 169

sum(expressao_genica$expression == "Down")

## [1] 40

# Create volcano plot

# Filter the data to include only "Down" and "Up" genes
expressao_genica_filtered <- expressao_genica[expressao_genica$expression %in% c("Down", "Up"), ]

# Order the filtered dataframe by logFC
expressao_genica_filtered <- expressao_genica_filtered[order(expressao_genica_filtered$logFC, decreasing = TRUE),]

# Select the top 5 upregulated genes and the top 5 downregulated genes
top_genes_up <- head(subset(expressao_genica_filtered, expression == "Up"), 5)
top_genes_down <- head(subset(expressao_genica_filtered, expression == "Down"), 5)

# Combine top_genes_up and top_genes_down into a single dataframe
top_genes <- rbind(top_genes_up, top_genes_down)

## see
print(top_genes)

##      gene    logFC      FDR    logFDR logpvalue     CTL_1     CTL_2
## Arl4d Arl4d 2.5283730 2.511330e-45 44.600096 48.807757 1.9552553 2.0481393
## Atf3   Atf3  2.3374572 5.008752e-11 10.300271 13.252659 0.1516988 -0.9416907
## Fosb   Fosb  2.2956568 2.138706e-15 14.669849 17.798329 2.0673245 2.3729756
## Plin4  Plin4 2.2421116 3.330896e-17 16.477439 19.782010 1.5803034 1.5902544
## Sgk1   Sgk1  1.7910453 9.376900e-36 35.027941 38.934572 4.6390729 4.9564345
## Sall2  Sall2 -0.2244158 2.797265e-02 1.553266 3.515415 7.4769055 7.5399012
## Glce   Glce -0.2753400 3.990423e-02 1.398981 3.324164 5.9268447 6.0442650
## Sstr1  Sstr1 -0.2970437 2.198656e-02 1.657843 3.632508 5.3847439 5.5825138
## Trib2   Trib2 -0.3036969 4.981276e-02 1.302659 3.190174 5.7755675 5.7873575
## Emp2   Emp2 -0.3567410 1.711119e-02 1.766720 3.754273 4.9746724 5.1286470
##           CTL_3     CTL_4     CTL_5  Exe0h_1  Exe0h_2  Exe0h_3  Exe0h_4
## Arl4d  1.776197 1.8358297 1.7434134 4.443234 4.237175 4.090626 4.807705
## Atf3   -0.251000 0.4129393 0.5766725 2.073710 1.850180 2.337934 2.973298

```

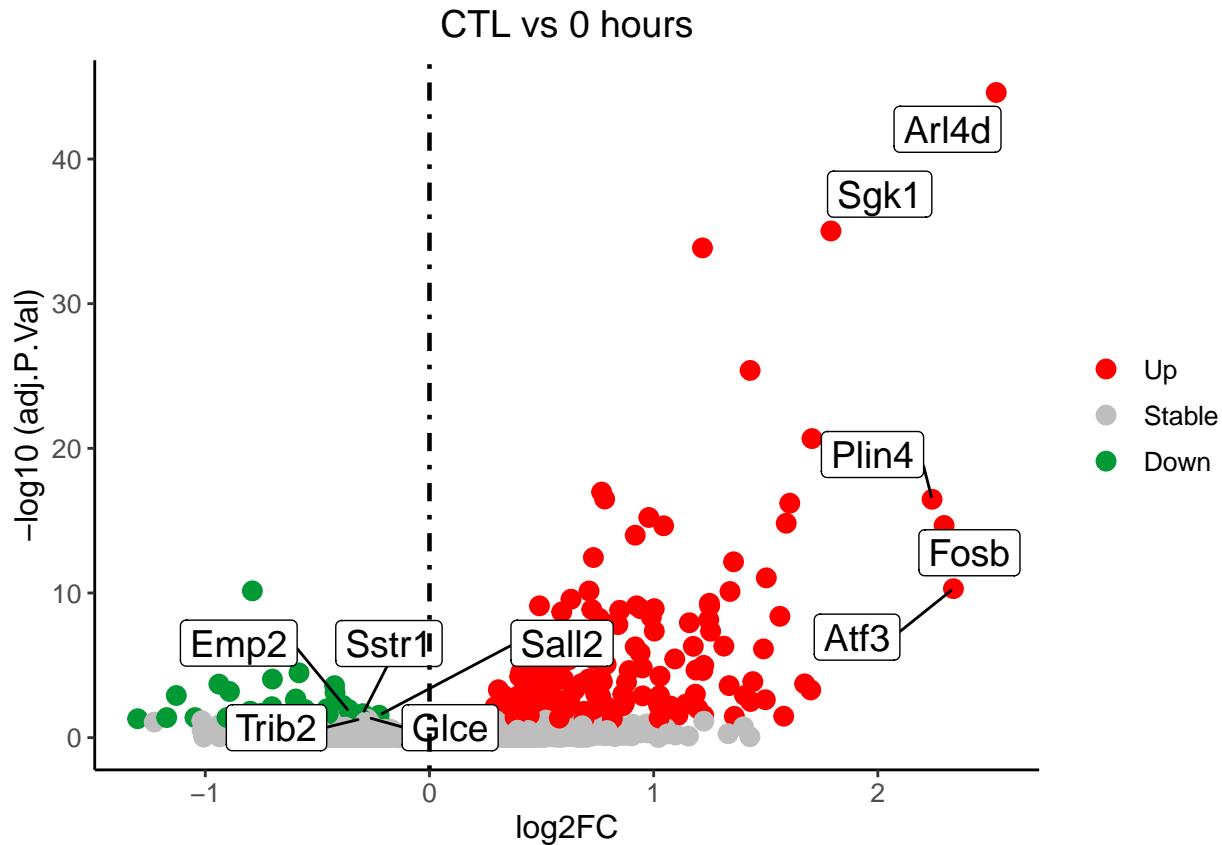
```

## Fosb  3.247433 3.0737466 3.1036501 5.716202 4.948945 4.661200 5.415487
## Plin4 2.133250 1.8358297 2.0550548 3.364618 3.831806 3.636137 4.470725
## Sgk1  5.411409 5.0298322 5.0679141 6.827420 7.119525 6.623199 6.924598
## Sall2 7.520794 7.4565149 7.5123494 7.391716 7.282261 7.254194 7.237609
## Glce   5.893643 5.8750342 5.9362567 5.728622 5.558978 5.721374 5.503856
## Sstr1  5.488770 5.3299520 5.2984816 5.224583 5.139367 5.122806 5.082571
## Trib2  5.602020 5.5584126 5.4606669 5.393218 5.483695 5.279362 5.203439
## Emp2   5.039188 4.9274327 5.0988322 4.765996 4.703203 4.707879 4.814519
##          ExeOh_5 expression
## Arl4d  4.144619      Up
## Atf3   1.806292      Up
## Fosb   4.483612      Up
## Plin4  4.589979      Up
## Sgk1   6.581915      Up
## Sall2  7.221756      Down
## Glce   5.784518      Down
## Sstr1  5.067954      Down
## Trib2  5.334330      Down
## Emp2   4.384417      Down

# volcano plot
plot1 <- ggplot(data = expressao_genica,
  aes(x = logFC,
      y = logFDR,
      colour = expression)) +
  geom_point(size = 3) +
  geom_label_repel(data = top_genes, aes(label = gene), size = 5, force = 5,
    fill = "white", color = "black", box.padding = unit(0.5, "lines")) + # Labeling top
  scale_color_manual(values = c("red", "grey", "#009933"),
    name = "",
    breaks = c("Up", "Stable", "Down")) +
  geom_vline(xintercept = c(-0, 0), lty = 4, col = "black", lwd = 0.8) +
  labs(x = "log2FC",
       y = "-log10 (adj.P.Val)") +
  theme_classic() +
  theme(plot.title = element_text(hjust = 0.5),
        legend.position = "right",
        legend.title = element_blank(),
        text = element_text(size = 12)) +
  ggtitle("CTL vs 0 hours")

# To display the volcano plot
plot1

```



```
## save as image
tiff("volcano_CTL_vs_0h.tiff", units="in", width=7, height=7, res=300)
plot1
dev.off()
```

```
## pdf
## 2
```

```
## heatmap
```

```
## Only genes Down and Upregulated
head(expressao_genica_filtered)
```

	gene	logFC	FDR	logFDR	logpvalue	CTL_1	CTL_2	
##	Arl4d	Arl4d	2.528373	2.511330e-45	44.60010	48.80776	1.9552553	2.0481393
##	Atf3	Atf3	2.337457	5.008752e-11	10.30027	13.25266	0.1516988	-0.9416907
##	Fosb	Fosb	2.295657	2.138706e-15	14.66985	17.79833	2.0673245	2.3729756
##	Plin4	Plin4	2.242112	3.330896e-17	16.47744	19.78201	1.5803034	1.5902544
##	Sgk1	Sgk1	1.791045	9.376900e-36	35.02794	38.93457	4.6390729	4.9564345
##	Cebpd	Cebpd	1.706410	2.124447e-21	20.67275	24.18145	1.4485270	1.6282417
##								
##		CTL_3	CTL_4	CTL_5	Exe0h_1	Exe0h_2	Exe0h_3	Exe0h_4
##	Arl4d	1.776197	1.8358297	1.7434134	4.443234	4.237175	4.090626	4.807705
##	Atf3	-0.251000	0.4129393	0.5766725	2.073710	1.850180	2.337934	2.973298
##	Fosb	3.247433	3.0737466	3.1036501	5.716202	4.948945	4.661200	5.415487
##	Plin4	2.133250	1.8358297	2.0550548	3.364618	3.831806	3.636137	4.470725

```

## Sgk1 5.411409 5.0298322 5.0679141 6.827420 7.119525 6.623199 6.924598
## Cebpd 2.152884 1.8358297 1.4342414 3.555179 3.265988 3.229964 3.559096
##     ExeOh_5 expression
## Arl4d 4.144619      Up
## Atf3  1.806292      Up
## Fosb  4.483612      Up
## Plin4 4.589979      Up
## Sgk1  6.581915      Up
## Cebpd 3.377056      Up

```

```

#remove the first five cols
expressao_genica_filtered <- expressao_genica_filtered[, -(1:5)]

## remove a col name expression
expressao_genica_filtered$expression <- NULL

# Set column names
colnames(expressao_genica_filtered) <- c(rep("CTL", 5), rep("Oh", 5))

# Convert dataframe to matrix
samp.with.rownames <- as.matrix(expressao_genica_filtered)

# Create column annotations for heatmap
suppressWarnings({
column_annotation <- c(rep("#74ADD1", 5), rep("#A6D96A", 5))
column_annotation <- as.matrix(column_annotation)
colnames(column_annotation) <- c("Variable X")
})

# plot
library("gplots")

```

```

##
## Attaching package: 'gplots'

## The following object is masked from 'package:stats':
##       lowess

heatmap.2(samp.with.rownames, scale="row", trace = "none", cexRow=1.5, cexCol=2, margins = c(5, 11), col
          = colorRampPalette(c("white", "#A6D96A", "#74ADD1"))(25))

## Warning in plot.window(...): "colSideColors" is not a graphical parameter

## Warning in plot.xy(xy, type, ...): "colSideColors" is not a graphical parameter

## Warning in title(...): "colSideColors" is not a graphical parameter

# Get plot coordinates
coords <- par("usr")

# Increase right margin

```

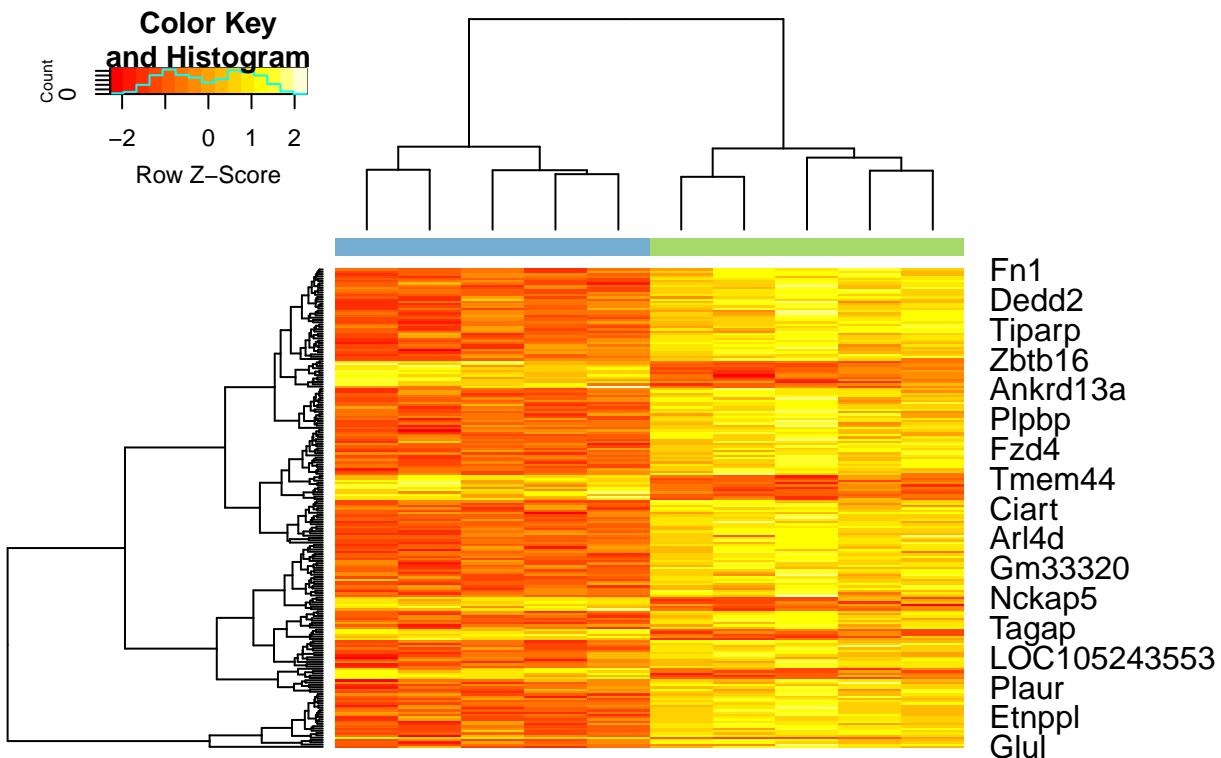
```

par(mar = c(5, 6, 4, 12) + 0.2)

# Set legend coordinates
legend_x <- coords[2] + 0.2 * diff(coords[1:2])
legend_y <- coords[4] + 0.05 * diff(coords[3:4])

# Add legend outside the plot
legend(legend_x, legend_y, legend = c("CTL", "Oh"),
       fill = c("#74ADD1", "#A6D96A"), border = FALSE, cex = 1.1,
       xpd = TRUE)

```



```

## save as image
suppressWarnings({
tiff("heatmap_CTL_vs_Oh.tiff", units="in", width=7, height=7, res=300)
heatmap.2(samp.with.rownames, scale="row", trace = "none", cexRow=1.5, cexCol=2, margins = c(5, 11), col
          = colorRampPalette(c("red", "yellow"))(100))

# Get plot coordinates
coords <- par("usr")

# Increase right margin
par(mar = c(5, 6, 4, 12) + 0.2)

# Set legend coordinates
legend_x <- coords[2] + 0.2 * diff(coords[1:2])
legend_y <- coords[4] + 0.05 * diff(coords[3:4])

```

```

# Add legend outside the plot
legend(legend_x, legend_y, legend = c("CTL", "0h"),
       fill = c("#74ADD1", "#A6D96A"), border = FALSE, cex = 1.1,
       xpd = TRUE)
dev.off()
})

## pdf
## 2

```

1.5 Groups (CTL vs 2 hours)

```

suppressWarnings({
  library(tibble)
  library(dplyr)
  library(edgeR)
  library(ggrepel)
})

## Import dataset
eDat <- read.table("Count.txt", header=TRUE, sep="\t", row.names = 1)
gDat <- read.table("Group.txt", header=TRUE, sep="\t")

## Counts
## filter only samples CTL and Exe2h

# Select only 'CTL' ou 'Exe2h'
selected_columns <- eDat[, grepl("CTL|Exe2h", names(eDat))]

# new table
new_table1 <- cbind(rownames = rownames(eDat), selected_columns)

## remove the first column
new_table1$rownames <- NULL

## Treat
new_group1 <- subset(gDat, treat == "CTL" | treat == "Exe2h")

# ATENTION!!
new_group1$treat

## [1] "CTL"    "CTL"    "CTL"    "CTL"    "CTL"    "Exe2h"  "Exe2h"  "Exe2h"  "Exe2h"
## [10] "Exe2h"

# Control treatment needs to appear as the first level for the Treat Vs. Control comparison
new_group1$treat <- factor(new_group1$treat, levels=c("CTL", "Exe2h"))

```

```

# Input data
y_adj <- DGEList(counts=new_table1, samples=new_group1, group=new_group1$treat)
design_adj <- model.matrix(~ treat - 1, data = new_group1)

# Filter lowly expressed genes
keep_adj <- rowSums(cpm(y_adj)>1) >= 2
sum(keep_adj)

## [1] 16010

y_adj <- y_adj[keep_adj, , keep.lib.sizes=FALSE]

## Explanation: ">= 2" is a condition to maintain the gene, which means that the gene needs to have at least two reads across all samples.

## Explanation: after filtering, 16010 genes remained that have sufficient expression in at least two replicates.

# Normalization for RNA composition
y_adj <- calcNormFactors(y_adj)
y_adj$samples

##          group lib.size norm.factors   sample treat
## CTL_1      CTL 19625276    0.9825876 CTL_1   CTL
## CTL_2      CTL 12686881    0.9952413 CTL_2   CTL
## CTL_3      CTL 16472724    1.0221810 CTL_3   CTL
## CTL_4      CTL 10483705    1.0202798 CTL_4   CTL
## CTL_5      CTL 12182156    1.0287885 CTL_5   CTL
## Exe2h_1    Exe2h 9621547    0.9901058 Exe2h_1 Exe2h
## Exe2h_2    Exe2h 18541201    0.9788466 Exe2h_2 Exe2h
## Exe2h_3    Exe2h 22239313    0.9865041 Exe2h_3 Exe2h
## Exe2h_4    Exe2h 18010377    0.9679394 Exe2h_4 Exe2h
## Exe2h_5    Exe2h 21196657    1.0298734 Exe2h_5 Exe2h

norm.expr_adj <- y_adj$samples

# norm counts
logcpm_adj <- cpm(y_adj, log=TRUE)

#####
## Data analyses with edgeR      ##
#####

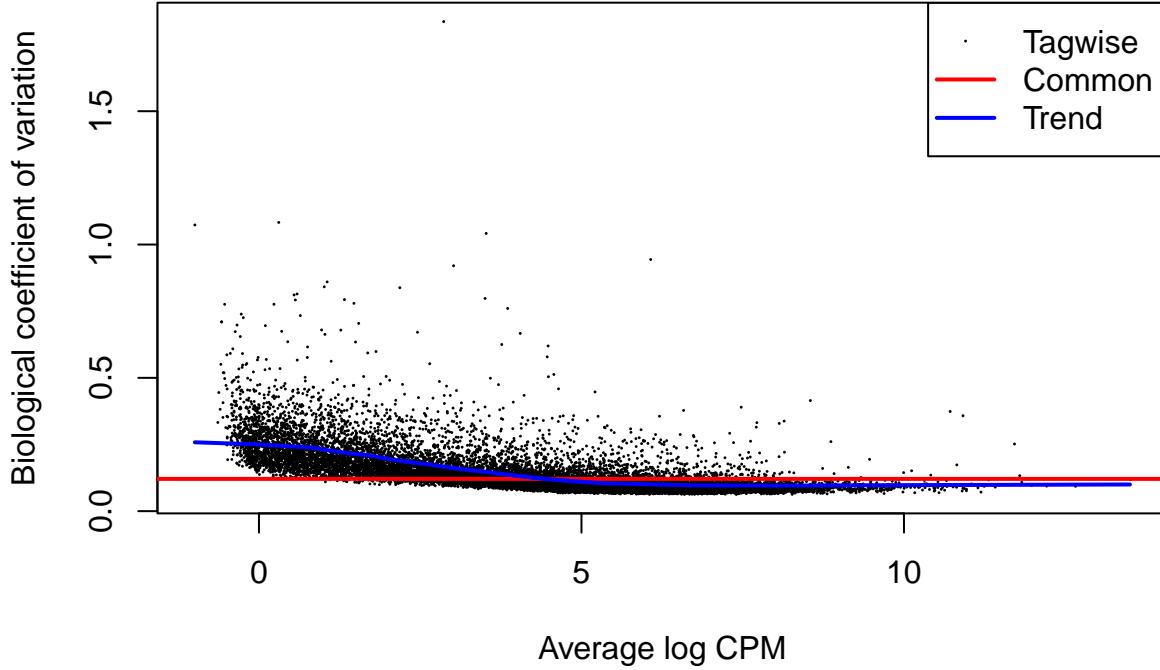
# Estimate dispersion with Cox-Reid profile-adjusted likelihood (CR) method
y_adj <- estimateDisp(y_adj, design_adj)
y_adj$common.dispersion

## [1] 0.01465838

## Explanation: A low dispersion value indicates that the expression levels of genes are relatively consistent across samples.

```

```
## plot
plotBCV(y_adj)
```



```
# Fit Generalized Linear Model (GLM)
fit <- glmFit(y_adj, design_adj)
colnames(fit)
```

```
## [1] "treatCTL"    "treatExe2h"
```

```
# Differential gene expression analysis
lrt <- glmLRT(fit, contrast=c(-1,1))

## Coefficients used in the contrast for the likelihood ratio test
topTags(lrt)
```

```
## Coefficient: -1*treatCTL 1*treatExe2h
##          logFC    logCPM       LR      PValue        FDR
## Fkbp5    1.1796386 5.968308 160.00988 1.125875e-36 1.802526e-32
## Plin4    1.9567219 3.139309 120.22510 5.647470e-28 4.520800e-24
## Tsc22d3  0.9247472 6.744314 106.62931 5.367342e-25 2.864372e-21
## Zbtb16   1.5649029 5.146535  94.80119 2.104995e-22 8.425243e-19
## Slc2a1   0.6882978 6.786838  85.54204 2.268280e-20 7.263032e-17
## P4ha2    0.9161265 5.076339  84.76129 3.366511e-20 8.982973e-17
```

```

## Mfsd2a  0.9055672 5.345205 79.11920 5.847328e-19 1.337367e-15
## Fam20a  1.0118445 5.030237 78.29276 8.884705e-19 1.778052e-15
## Fam107a 0.7732697 8.276181 77.06507 1.654175e-18 2.942594e-15
## Sdf2l1  0.8603870 4.172289 69.77987 6.630615e-17 1.061562e-13

```

```

## Explanation: It indicates that the comparison is between the coefficient for treatCTL (the reference
## Number of genes identified as differentially expressed (Disregarding parameters)
is.de <- decideTestsDGE(lrt)
summary(decideTestsDGE(lrt))

```

```

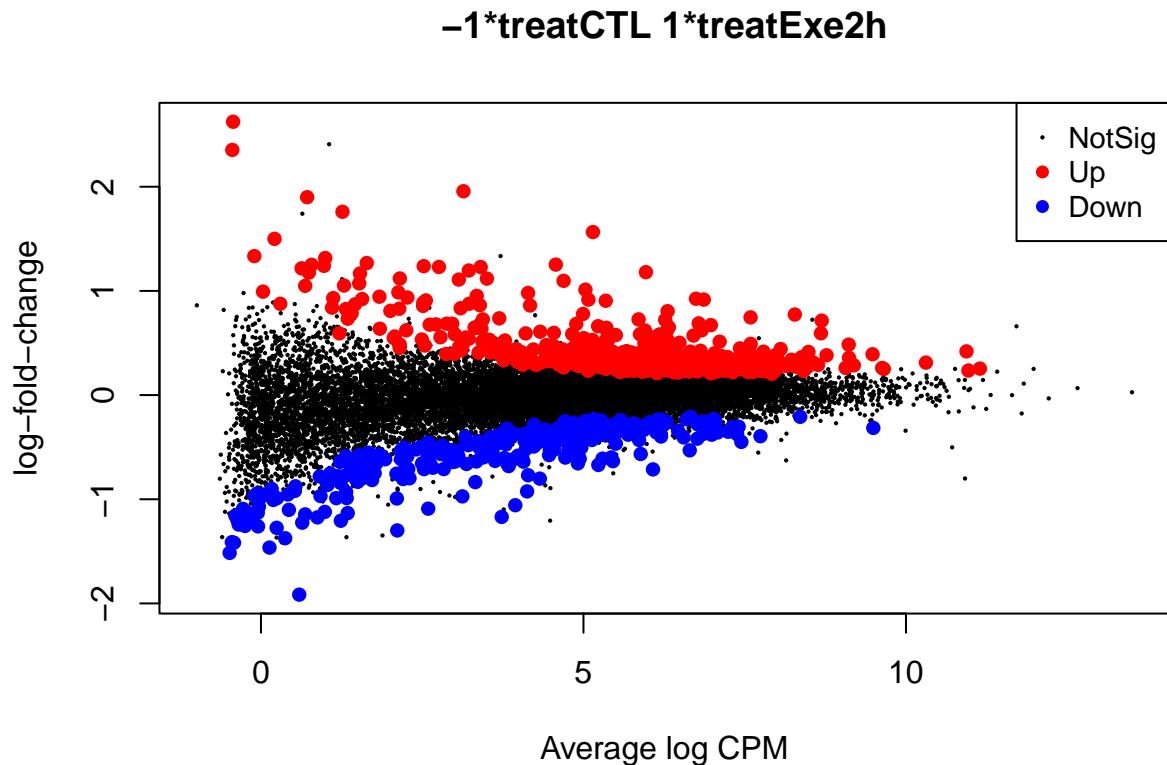
##          -1*treatCTL 1*treatExe2h
## Down                  297
## NotSig                15255
## Up                   458

```

```

## plot MD
plotMD(lrt, status=is.de)

```



```

# Number of up and down-regulated. FDR < 5% and |log2 fold change| > 0
summary(de <- decideTestsDGE(lrt, lfc=0))

```

```

##          -1*treatCTL 1*treatExe2h
## Down                  297

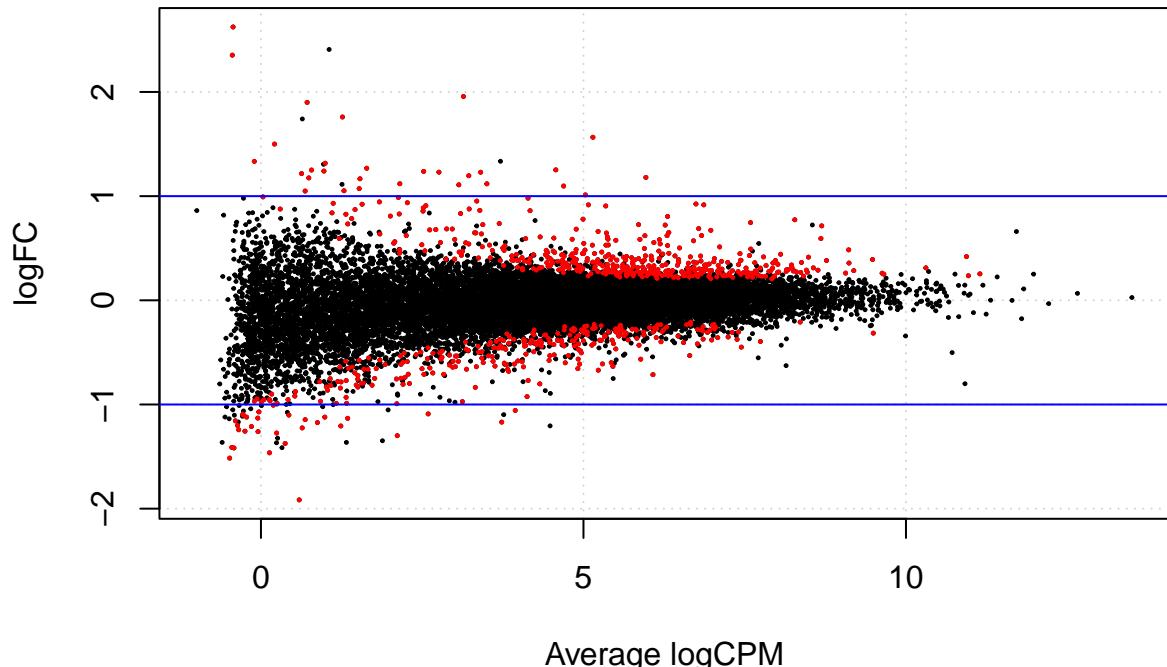
```

```

## NotSig          15255
## Up              458

# MA-plot
detags <- rownames(y_adj)[as.logical(de)]
plotSmear(lrt, de.tags=detags)
abline(h=c(-1, 1), col="blue")

```



```

# To export the results
result <- as.data.frame(topTags(lrt, n=1000000))
result <- add_column(result, symbol= rownames(result), .before = "logFC")

## save as table
write.table(result, "DEGs_CTL_vs_2h.txt", sep = "\t", row.names = FALSE)

## Check all the genes Downregulated and Upregulated

# Remove unnecessary columns
result$logCPM <- NULL
result$LR <- NULL

# Remove rows with FDR < 0.05 and logFC >= 0
result$expression <- ifelse(result$FDR < 0.05 & abs(result$logFC) >= 0,
                            ifelse(result$logFC >= 0, 'Up', 'Down'),
                            'Stable')

```

```

# Select only genes that are upregulated or downregulated
result_final <- subset(result, expression %in% c("Up", "Down"))

# Remove row names
rownames(result_final) <- NULL

## View the table
knitr::kable(result_final)

```

symbol	logFC	PValue	FDR	expression
Fkbp5	1.1796386	0.0000000	0.0000000	Up
Plin4	1.9567219	0.0000000	0.0000000	Up
Tsc22d3	0.9247472	0.0000000	0.0000000	Up
Zbtb16	1.5649029	0.0000000	0.0000000	Up
Slc2a1	0.6882978	0.0000000	0.0000000	Up
P4ha2	0.9161265	0.0000000	0.0000000	Up
Mfsd2a	0.9055672	0.0000000	0.0000000	Up
Fam20a	1.0118445	0.0000000	0.0000000	Up
Fam107a	0.7732697	0.0000000	0.0000000	Up
Sdf2l1	0.8603870	0.0000000	0.0000000	Up
Srxn1	0.6717424	0.0000000	0.0000000	Up
Cdkn1a	1.2523329	0.0000000	0.0000000	Up
Vgf	0.7147245	0.0000000	0.0000000	Up
Xbp1	0.5124732	0.0000000	0.0000000	Up
Hspa5	0.5932352	0.0000000	0.0000000	Up
Gpt2	0.5545965	0.0000000	0.0000000	Up
Manf	0.6211435	0.0000000	0.0000000	Up
Pdia4	0.6509220	0.0000000	0.0000000	Up
Tnfrsf12a	1.2296895	0.0000000	0.0000000	Up
Hr	0.8050511	0.0000000	0.0000000	Up
Mmp14	-0.6708801	0.0000000	0.0000000	Down
Creld2	0.7788021	0.0000000	0.0000000	Up
Mrps6	0.5464337	0.0000000	0.0000000	Up
Pdia6	0.4919749	0.0000000	0.0000000	Up
Lhfpl6	0.6610468	0.0000000	0.0000000	Up
Mapk4	0.5818033	0.0000000	0.0000000	Up
Kcna5	0.5759753	0.0000000	0.0000000	Up
Cish	0.8767606	0.0000000	0.0000000	Up
Zkscan16	-0.4495147	0.0000000	0.0000000	Down
Hmgcr	-0.5303673	0.0000000	0.0000001	Down
N4bp2l1	0.4974726	0.0000000	0.0000001	Up
Sytl2	-0.7694549	0.0000000	0.0000001	Down
Nr3c2	-0.6000270	0.0000000	0.0000001	Down
Sdc4	0.4436923	0.0000000	0.0000001	Up
Opalin	-1.0571251	0.0000000	0.0000001	Down
Insig1	-0.7133017	0.0000000	0.0000001	Down
Kirrel2	1.2676155	0.0000000	0.0000001	Up
Stip1	0.4141101	0.0000000	0.0000002	Up
Smim3	0.9524598	0.0000000	0.0000002	Up
Serpine1	1.1191032	0.0000000	0.0000003	Up
Hlcs	-0.6392316	0.0000000	0.0000004	Down
Baiap2	0.6214825	0.0000000	0.0000004	Up

symbol	logFC	PValue	FDR	expression
Chordc1	0.7221359	0.0000000	0.0000004	Up
Zdhhc14	0.4873288	0.0000000	0.0000006	Up
Etv5	0.5869395	0.0000000	0.0000007	Up
Alkal2	1.7596494	0.0000000	0.0000013	Up
Acss3	0.8629140	0.0000000	0.0000013	Up
Frmpd1	0.6827979	0.0000000	0.0000018	Up
Dynll1	0.4294586	0.0000000	0.0000020	Up
Ldlr	-0.5655004	0.0000000	0.0000020	Down
Htra1	0.5712579	0.0000000	0.0000022	Up
Gm11827	2.6237534	0.0000000	0.0000022	Up
P4ha1	0.5416503	0.0000000	0.0000022	Up
Camk1g	0.5260300	0.0000000	0.0000022	Up
Hdac4	0.5845020	0.0000000	0.0000023	Up
Pnpla2	0.5309186	0.0000000	0.0000023	Up
Fos	-0.9245320	0.0000000	0.0000033	Down
Elov17	-0.8355415	0.0000000	0.0000034	Down
Dnaja1	0.3824010	0.0000000	0.0000036	Up
Slc25a33	0.4380758	0.0000000	0.0000049	Up
Nmur2	0.9863616	0.0000000	0.0000054	Up
Gjb6	0.5025928	0.0000000	0.0000062	Up
Shtn1	-0.5644574	0.0000000	0.0000065	Down
Ftl1	0.4164790	0.0000000	0.0000081	Up
Rspo3	0.6845131	0.0000000	0.0000090	Up
Rhbdl3	0.4504631	0.0000000	0.0000103	Up
Hspf1	0.4844493	0.0000000	0.0000109	Up
Gm19439	1.2373401	0.0000000	0.0000117	Up
Hspa8	0.4192602	0.0000001	0.0000163	Up
Klf15	0.4172862	0.0000001	0.0000167	Up
Rab3ip	0.4288694	0.0000001	0.0000171	Up
Ezr	0.6203315	0.0000001	0.0000179	Up
Cc2d2a	-0.4861141	0.0000001	0.0000192	Down
Hif3a	1.1958431	0.0000001	0.0000209	Up
Herpud1	0.3660177	0.0000001	0.0000209	Up
Hspb1	1.2291340	0.0000001	0.0000219	Up
Calr	0.3819458	0.0000001	0.0000250	Up
Rxra	0.4262690	0.0000002	0.0000317	Up
Cited1	0.7265073	0.0000002	0.0000343	Up
Tns3	0.4006301	0.0000002	0.0000438	Up
Ucp2	0.3842927	0.0000002	0.0000438	Up
AgRP	1.0969935	0.0000003	0.0000514	Up
Fgfrl1	-0.6177103	0.0000003	0.0000514	Down
Emilin2	0.9442096	0.0000003	0.0000532	Up
Cacybp	0.3701425	0.0000003	0.0000599	Up
Hmggn1	0.4172969	0.0000004	0.0000666	Up
Btg1	0.4135062	0.0000004	0.0000751	Up
Nxpe4	-0.6320409	0.0000004	0.0000751	Down
Rgs2	0.3767711	0.0000004	0.0000754	Up
FosB	1.1183916	0.0000005	0.0000876	Up
Fkbp4	0.3586466	0.0000005	0.0000919	Up
Pnpla7	0.5975354	0.0000006	0.0000954	Up
Fbn1	-0.6625106	0.0000006	0.0000954	Down
Dcx	-0.5235255	0.0000006	0.0001020	Down

symbol	logFC	PValue	FDR	expression
Wipf3	0.5293587	0.0000007	0.0001107	Up
Itga6	-0.4391525	0.0000007	0.0001107	Down
Zfand4	-0.8061502	0.0000008	0.0001325	Down
Pierce1	0.7262492	0.0000008	0.0001373	Up
Dnajb11	0.3932245	0.0000009	0.0001398	Up
Gm27252	1.2163835	0.0000010	0.0001649	Up
Tnrc6c	-0.4063587	0.0000011	0.0001681	Down
Kcnf1	0.4025019	0.0000011	0.0001713	Up
Cited2	0.3871079	0.0000012	0.0001876	Up
Thr9	-1.2249799	0.0000012	0.0001882	Down
Atg101	0.4725416	0.0000012	0.0001882	Up
Xdh	0.9084248	0.0000013	0.0002011	Up
Ino80e	0.3897203	0.0000014	0.0002056	Up
Tekt4	0.8867928	0.0000015	0.0002282	Up
Gm36855	-1.3741311	0.0000016	0.0002307	Down
Junb	-0.5762193	0.0000016	0.0002365	Down
Cdkl1	-0.4377733	0.0000017	0.0002414	Down
Ahsa1	0.3740512	0.0000017	0.0002475	Up
Hnrnpc	0.3334773	0.0000018	0.0002508	Up
Tmem52	1.1666912	0.0000018	0.0002598	Up
Wee1	0.4597007	0.0000019	0.0002664	Up
Hnrnpm	0.3343662	0.0000020	0.0002733	Up
Zbtb40	0.5920373	0.0000021	0.0002814	Up
Tspan31	0.3740875	0.0000022	0.0002989	Up
Ptges3	0.3639532	0.0000025	0.0003319	Up
Khl12	0.3016195	0.0000025	0.0003332	Up
Atf3	1.1751594	0.0000026	0.0003417	Up
Rin2	0.3881096	0.0000027	0.0003479	Up
Mertk	0.5857355	0.0000028	0.0003709	Up
Itpk1	0.4247128	0.0000029	0.0003711	Up
Slco1a4	-0.4715205	0.0000036	0.0004592	Down
Cables1	0.4005090	0.0000037	0.0004716	Up
Cdk2ap2	0.4597639	0.0000038	0.0004738	Up
Hdx	-0.9314956	0.0000043	0.0005336	Down
Nkx6-2	-0.6119806	0.0000043	0.0005336	Down
Ptprd	-0.3730033	0.0000045	0.0005546	Down
Sst	0.4420368	0.0000047	0.0005782	Up
Rgcc	0.8331842	0.0000048	0.0005814	Up
Cdr2	0.4448605	0.0000050	0.0006010	Up
Gm32994	-1.1455232	0.0000051	0.0006038	Down
A730020M07Rik	1.3158706	0.0000051	0.0006060	Up
Gpr27	0.3427850	0.0000052	0.0006108	Up
Cebpd	0.9366364	0.0000053	0.0006108	Up
Ptges3-ps	0.7367851	0.0000053	0.0006108	Up
Sp140l1	-1.4639273	0.0000054	0.0006254	Down
Cnd3	0.4581467	0.0000057	0.0006489	Up
Rpl18	0.4252255	0.0000057	0.0006489	Up
F8a	0.4357927	0.0000058	0.0006594	Up
Rpn1	0.2771166	0.0000062	0.0006919	Up
Lrfn2	0.4823815	0.0000064	0.0007161	Up
Flt3	-0.7104512	0.0000068	0.0007463	Down
Brinp1	0.3479162	0.0000068	0.0007479	Up

symbol	logFC	PValue	FDR	expression
Ghsr	0.6081122	0.0000069	0.0007479	Up
Nav3	-0.4183559	0.0000069	0.0007479	Down
Phf14	-0.3342680	0.0000072	0.0007698	Down
Atic	0.3322275	0.0000075	0.0008022	Up
Zbtb25	-0.3880275	0.0000078	0.0008197	Down
Fcgr3	0.6479998	0.0000078	0.0008197	Up
Gm19500	-1.1739358	0.0000078	0.0008197	Down
Klf9	0.3590722	0.0000081	0.0008438	Up
Fbxw23	1.2505107	0.0000086	0.0008855	Up
Slc22a8	-0.6614678	0.0000088	0.0009005	Down
Gm31356	-0.4570680	0.0000090	0.0009208	Down
Tra2b	0.3170171	0.0000091	0.0009208	Up
Nudt11	0.3315525	0.0000093	0.0009383	Up
Noct	0.3318333	0.0000095	0.0009538	Up
D630039A03Rik	1.2405195	0.0000101	0.0010092	Up
Klhl29	0.3857467	0.0000108	0.0010708	Up
Ppargc1b	-0.5879650	0.0000111	0.0010942	Down
Fzd2	0.5238228	0.0000115	0.0011263	Up
Galnt18	0.3923071	0.0000124	0.0012066	Up
Lss	-0.4145642	0.0000126	0.0012145	Down
Pde10a	0.3649236	0.0000132	0.0012687	Up
Srsf3	0.2992163	0.0000134	0.0012767	Up
Cxcl12	-0.4310218	0.0000146	0.0013787	Down
Phldb1	-0.3585015	0.0000150	0.0014132	Down
Notch4	0.5303950	0.0000161	0.0015046	Up
Gpr17	0.3388269	0.0000162	0.0015046	Up
Hsp90b1	0.2852255	0.0000163	0.0015074	Up
Zfp687	-0.3049106	0.0000164	0.0015074	Down
Tnfrsf22	-0.9889281	0.0000165	0.0015101	Down
Micall2	-0.6422199	0.0000167	0.0015185	Down
Lifr	0.3860564	0.0000172	0.0015595	Up
Fancm	-0.4577664	0.0000176	0.0015839	Down
Plekh1	-0.5309223	0.0000179	0.0015943	Down
Exoc2	-0.3418023	0.0000179	0.0015943	Down
Pdgfrb	-0.3860391	0.0000184	0.0016301	Down
P2ry12	-0.5166999	0.0000187	0.0016457	Down
Frem1	-0.7551946	0.0000193	0.0016898	Down
9430065F17Rik	-1.5150132	0.0000197	0.0017104	Down
Cgrrf1	0.4128996	0.0000207	0.0017912	Up
Paqr8	0.4479062	0.0000214	0.0018407	Up
Mcam	-0.4934197	0.0000222	0.0018983	Down
Nxn	0.4631921	0.0000257	0.0021883	Up
Npy	0.9165497	0.0000276	0.0023404	Up
Tpt1	0.3115708	0.0000300	0.0025316	Up
Dhrs3	0.4409258	0.0000302	0.0025321	Up
Hsd17b7	-0.5858700	0.0000309	0.0025778	Down
Ramp2	0.5137190	0.0000317	0.0026329	Up
Atp13a5	-0.5556593	0.0000324	0.0026703	Down
Npas3	-0.4052222	0.0000327	0.0026814	Down
Hrh1	0.4349541	0.0000330	0.0026987	Up
Gm13889	0.4031370	0.0000338	0.0027481	Up
Fnbp1	-0.3033889	0.0000344	0.0027796	Down

symbol	logFC	PValue	FDR	expression
Pglyrp1	0.8286425	0.0000358	0.0028765	Up
Lrrc8a	0.3664958	0.0000360	0.0028833	Up
L3mbtl3	0.3445655	0.0000386	0.0030763	Up
Plce1	0.4214431	0.0000406	0.0032078	Up
Bcl2l1	0.2842614	0.0000408	0.0032078	Up
Prcc	0.3317262	0.0000409	0.0032078	Up
Tcerg1	-0.3040760	0.0000412	0.0032147	Down
Lrrcc1	-0.4252249	0.0000414	0.0032147	Down
Kcnn2	0.4793848	0.0000417	0.0032281	Up
Slc30a1	-0.3196461	0.0000459	0.0035339	Down
Slc16a1	-0.3840376	0.0000477	0.0036574	Down
Rplp2	0.3423227	0.0000496	0.0037849	Up
Igfbp7	0.4377681	0.0000506	0.0038359	Up
Fst14	0.3870948	0.0000513	0.0038745	Up
Fus	-0.3950814	0.0000540	0.0040562	Down
Wfdc1	0.6786006	0.0000557	0.0041634	Up
Apln	-0.5819615	0.0000622	0.0046354	Down
Trac	-0.8152523	0.0000633	0.0046916	Down
Sowaha	0.4376902	0.0000636	0.0046936	Up
Eml6	-0.3926909	0.0000643	0.0047253	Down
Tmem88b	-0.6344179	0.0000657	0.0048012	Down
Txlng	0.3817424	0.0000665	0.0048365	Up
Uvssa	-0.6816747	0.0000668	0.0048377	Down
Acox3	-0.3373688	0.0000690	0.0049557	Down
Map3k6	0.6760493	0.0000690	0.0049557	Up
Ociad2	0.3782798	0.0000697	0.0049795	Up
Pdia3	0.2895053	0.0000736	0.0052376	Up
Gprin2	-0.4192559	0.0000760	0.0053776	Down
Gm31513	-0.7943937	0.0000763	0.0053776	Down
Tpm4	0.3125529	0.0000766	0.0053776	Up
Mxd4	0.3369069	0.0000775	0.0054160	Up
Plppb	0.2900366	0.0000783	0.0054514	Up
Tinagl1	0.6853791	0.0000790	0.0054765	Up
Gm37885	0.5024653	0.0000796	0.0054911	Up
Akirin1	0.2682368	0.0000804	0.0055227	Up
Ralbp1	0.2959652	0.0000829	0.0056686	Up
Rev3l	-0.3577249	0.0000873	0.0059265	Down
Mdga2	-0.2875562	0.0000874	0.0059265	Down
Mc4r	0.5334931	0.0000881	0.0059480	Up
Atp6v0e	0.3722198	0.0000884	0.0059480	Up
Dnajc3	0.3560682	0.0000903	0.0060520	Up
Ets2	0.3606678	0.0000907	0.0060526	Up
9330117O12Rik	-1.1307634	0.0000914	0.0060686	Down
Usp36	-0.3380112	0.0000924	0.0061154	Down
Id1	0.5523052	0.0000928	0.0061172	Up
Tmem74b	-0.3840526	0.0000942	0.0061780	Down
Gm53598	-1.1215277	0.0000948	0.0061929	Down
Peg10	0.3918128	0.0000960	0.0062492	Up
St13	0.3099587	0.0001010	0.0065439	Up
Nptx2	0.4510795	0.0001022	0.0066005	Up
Kcnk1	0.3168042	0.0001034	0.0066478	Up
St7	0.3986395	0.0001050	0.0067211	Up

symbol	logFC	PValue	FDR	expression
Gm41442	-0.9689013	0.0001054	0.0067228	Down
Cet7	0.2786186	0.0001058	0.0067248	Up
Cep19	0.3152433	0.0001066	0.0067442	Up
Gfod2	0.3035208	0.0001072	0.0067550	Up
Gm28905	-0.7791850	0.0001090	0.0068422	Down
Galnt6	-0.5986017	0.0001097	0.0068589	Down
Ankrd44	-0.4092362	0.0001112	0.0069243	Down
Egln2	0.2635391	0.0001143	0.0070945	Up
Slc25a38	0.3764584	0.0001152	0.0071237	Up
Plod1	0.4004609	0.0001170	0.0072034	Up
Rps11	0.4725363	0.0001181	0.0072470	Up
Rpl36al	0.3694681	0.0001194	0.0072959	Up
Por	0.2862561	0.0001221	0.0074345	Up
Gm46065	-1.1018050	0.0001236	0.0074970	Down
D130040H23Rik	-0.4427307	0.0001254	0.0075786	Down
Eef1d	0.2676033	0.0001282	0.0077168	Up
Zfp428	0.3450023	0.0001300	0.0077947	Up
Hax1	0.3508339	0.0001308	0.0078160	Up
Cdr1os	-0.6553593	0.0001326	0.0078890	Down
Sat1	0.4495166	0.0001337	0.0079282	Up
Cdo1	0.3416937	0.0001347	0.0079592	Up
Fam43a	0.3863562	0.0001385	0.0081549	Up
Dnajb9	0.3204144	0.0001395	0.0081784	Up
Cdc37	0.2620805	0.0001502	0.0087641	Up
Ogfrl1	0.2989024	0.0001514	0.0087641	Up
Armcx5	-0.4010139	0.0001517	0.0087641	Down
Mrpl52	0.3338069	0.0001518	0.0087641	Up
Erbin	-0.3437350	0.0001522	0.0087641	Down
Rbmxl1	0.3910747	0.0001541	0.0088417	Up
Hes5	-0.6121214	0.0001547	0.0088454	Down
Itga7	-0.4345280	0.0001579	0.0089948	Down
Eif1b	0.3155314	0.0001589	0.0090232	Up
Nr4a1	-0.5311900	0.0001605	0.0090702	Down
Hspe1	0.3941804	0.0001609	0.0090702	Up
Gm10790	-1.2428235	0.0001618	0.0090885	Down
Th	0.4093228	0.0001654	0.0092602	Up
Nadk	0.2621932	0.0001663	0.0092744	Up
9330159F19Rik	0.2809722	0.0001693	0.0094109	Up
Nr2f6	0.2821691	0.0001701	0.0094252	Up
Atp10b	-0.8628921	0.0001716	0.0094722	Down
Snx3	0.2668053	0.0001738	0.0095626	Up
Nopchap1	0.2899316	0.0001761	0.0096538	Up
Rpl11	0.3087243	0.0001800	0.0097939	Up
Gm51447	1.3338589	0.0001802	0.0097939	Up
Dnajc21	0.3379632	0.0001805	0.0097939	Up
Mrpl43	0.3606256	0.0001830	0.0098971	Up
Plekha6	-0.3754768	0.0001848	0.0099437	Down
Shprh	-0.3444797	0.0001851	0.0099437	Down
Fau	0.3401152	0.0001865	0.0099851	Up
Cop1	0.2932988	0.0001899	0.0101319	Up
Pcdh15	-0.3891245	0.0001952	0.0103632	Down
Smndc1	0.3216627	0.0001955	0.0103632	Up

symbol	logFC	PValue	FDR	expression
Kctd16	-0.5983917	0.0001977	0.0104478	Down
Prdx6	0.3651202	0.0001997	0.0105176	Up
Garem2	-0.3667872	0.0002021	0.0106081	Down
Ccar1	0.3180370	0.0002036	0.0106225	Up
Mgme1	0.4700949	0.0002037	0.0106225	Up
Bcl7b	0.2968197	0.0002064	0.0107262	Up
Gm53211	-1.0068154	0.0002109	0.0109275	Down
Nox4	-1.4176626	0.0002127	0.0109858	Down
Erich3	-0.6030705	0.0002137	0.0109996	Down
Rplp0	0.3095686	0.0002169	0.0111280	Up
Rps9	0.2931234	0.0002199	0.0112466	Up
B3glct	-0.4026394	0.0002230	0.0113712	Down
Zmat1	-0.2906976	0.0002249	0.0113751	Down
F3	0.3882852	0.0002255	0.0113751	Up
Pop5	0.3660480	0.0002256	0.0113751	Up
Fmn1	-0.5862963	0.0002259	0.0113751	Down
Tubgcp2	-0.2733173	0.0002266	0.0113751	Down
Rpl6	0.3633828	0.0002292	0.0114668	Up
Zkscan2	-0.6105089	0.0002313	0.0114927	Down
Dapk1	0.3120810	0.0002316	0.0114927	Up
Lonrf3	0.2768471	0.0002319	0.0114927	Up
Tex15	-1.2743545	0.0002344	0.0115810	Down
Dhcr7	-0.2749063	0.0002357	0.0116087	Down
A930017M01Rik	-0.3025491	0.0002371	0.0116303	Down
Zeb1	-0.2350847	0.0002375	0.0116303	Down
Lars2	0.7456933	0.0002394	0.0116858	Up
Nherf2	0.3230145	0.0002438	0.0118641	Up
Dipk1c	-0.4569102	0.0002463	0.0119484	Down
Pebp1	0.3448421	0.0002510	0.0121403	Up
Osbpl3	-0.4979883	0.0002537	0.0122356	Down
Ube2g2	0.2684887	0.0002565	0.0123318	Up
Rab3b	0.2742632	0.0002594	0.0124364	Up
Snx1	0.2501472	0.0002618	0.0124600	Up
Tmcc3	0.2995074	0.0002632	0.0124600	Up
Rpph1	1.8995973	0.0002640	0.0124600	Up
Lactb2	-0.3751486	0.0002643	0.0124600	Down
Bcat2	0.4779584	0.0002644	0.0124600	Up
4931406C07Rik	-0.3320204	0.0002646	0.0124600	Down
Arhgef33	-0.7109430	0.0002723	0.0127806	Down
Mtarc2	0.3084814	0.0002741	0.0127806	Up
Trim66	-0.3645869	0.0002747	0.0127806	Down
Ubb	0.2600197	0.0002750	0.0127806	Up
Pde8a	-0.4276060	0.0002754	0.0127806	Down
Slc43a3	0.8258597	0.0002772	0.0128135	Up
Fads6	-0.3823693	0.0002777	0.0128135	Down
Rapgef6	-0.2779776	0.0002790	0.0128348	Down
Tet1	-0.4400846	0.0002808	0.0128568	Down
Rrp7a	0.2847014	0.0002811	0.0128568	Up
Rbm42	0.2794003	0.0002830	0.0129095	Up
Ubtf	0.2515382	0.0002851	0.0129676	Up
S100a10	0.3367730	0.0002886	0.0130345	Up
Serpinh1	0.6461781	0.0002888	0.0130345	Up

symbol	logFC	PValue	FDR	expression
Srsf10	0.3447392	0.0002894	0.0130345	Up
S100a16	-0.3169157	0.0002898	0.0130345	Down
Nudt10	0.2860799	0.0002964	0.0132912	Up
Gm33250	-0.4699125	0.0002983	0.0133392	Down
Gm50012	-0.7807115	0.0003011	0.0134272	Down
Rbbp7	0.2755187	0.0003020	0.0134324	Up
Adamts16	-0.7484109	0.0003044	0.0134929	Down
Gm30233	-1.2115205	0.0003051	0.0134929	Down
Atm	-0.3894590	0.0003095	0.0136354	Down
Pnrc2	0.2950390	0.0003100	0.0136354	Up
Ldlrad3	-0.4943213	0.0003267	0.0143117	Down
Derl2	0.2844698	0.0003272	0.0143117	Up
1500009L16Rik	0.4484481	0.0003317	0.0144698	Up
Chchd2	0.3836409	0.0003371	0.0146662	Up
Igdc4	-0.2624723	0.0003412	0.0148045	Down
Mvd	-0.4119849	0.0003443	0.0148985	Down
Rnasel	-0.6497992	0.0003466	0.0149551	Down
Metap1	0.2583734	0.0003504	0.0150628	Up
Castor1	0.4630543	0.0003509	0.0150628	Up
Gucy2e	-0.5217041	0.0003529	0.0151087	Down
Mxi1	0.3182783	0.0003548	0.0151460	Up
Jph1	-0.6559432	0.0003592	0.0152582	Down
Bltp3a	-0.3004992	0.0003593	0.0152582	Down
Ice1	-0.3806013	0.0003618	0.0153247	Down
Arrdc2	0.6468104	0.0003748	0.0158340	Up
Rnpep	0.3042817	0.0003819	0.0160921	Up
Gm12592	-0.7705076	0.0003838	0.0161043	Down
Gpx3	0.2890829	0.0003843	0.0161043	Up
Ppip5k2	-0.3315107	0.0003966	0.0165580	Down
Tmem200b	0.7331968	0.0003971	0.0165580	Up
Rufy4	1.4998305	0.0004004	0.0166369	Up
Amigo2	0.2625853	0.0004011	0.0166369	Up
Rps16	0.2921704	0.0004028	0.0166624	Up
Gm57798	-1.2063954	0.0004042	0.0166624	Down
Atp10a	0.4370301	0.0004049	0.0166624	Up
Bend3	-0.4009235	0.0004086	0.0167344	Down
Tmem114	0.5844553	0.0004087	0.0167344	Up
Epb41	-0.2603680	0.0004146	0.0169303	Down
Cracr2a	-0.7494430	0.0004156	0.0169303	Down
Dusp14	0.3754817	0.0004178	0.0169756	Up
Pllp	-0.4665864	0.0004286	0.0173734	Down
Pla2g4e	-0.6976322	0.0004332	0.0174931	Down
Fbxl4	-0.3144276	0.0004338	0.0174931	Down
Sprrb	0.2515068	0.0004393	0.0176722	Up
H2aj	0.3589879	0.0004430	0.0177766	Up
Epha8	-1.0903910	0.0004455	0.0178298	Down
Gm34256	-1.2605247	0.0004580	0.0182868	Down
Azi2	0.2626139	0.0004605	0.0183417	Up
Sertm1	0.3816969	0.0004710	0.0186754	Up
Rmrp	2.3532491	0.0004715	0.0186754	Up
Accs	-0.5354009	0.0004724	0.0186754	Down
Trim67	-0.4350454	0.0004754	0.0187051	Down

symbol	logFC	PValue	FDR	expression
Cspg4b	-0.4707467	0.0004755	0.0187051	Down
Egr1	-0.4314313	0.0004772	0.0187237	Down
B630019K06Rik	0.2535179	0.0004785	0.0187289	Up
Slc6a6	-0.2320426	0.0004802	0.0187503	Down
Tunar	0.2777197	0.0004823	0.0187687	Up
Adgrl4	0.3849404	0.0004830	0.0187687	Up
Vangl2	-0.2644610	0.0004865	0.0188593	Down
Fibin	0.5624601	0.0004916	0.0190110	Up
Ap1s2	0.2831065	0.0005009	0.0192175	Up
Scml4	-0.3743633	0.0005014	0.0192175	Down
Ints8	0.3100818	0.0005018	0.0192175	Up
Cog4	0.2485527	0.0005023	0.0192175	Up
Rfx7	-0.2905495	0.0005029	0.0192175	Down
Gsx1	0.5551146	0.0005089	0.0193806	Up
Gkap1	0.3087715	0.0005096	0.0193806	Up
Dennd4a	-0.2669510	0.0005116	0.0194102	Down
Pnoc	0.3400281	0.0005131	0.0194189	Up
Sik1	-0.3582685	0.0005153	0.0194561	Down
Lhfpl2	-0.4103969	0.0005206	0.0196056	Down
Cct3	0.2599236	0.0005229	0.0196056	Up
Gm26721	-0.8423454	0.0005229	0.0196056	Down
Rpsa	0.3576437	0.0005274	0.0197076	Up
Fbxl9	-0.8178539	0.0005281	0.0197076	Down
Gng5	0.3840924	0.0005304	0.0197486	Up
ND6	-0.3152207	0.0005522	0.0204650	Down
Cux2	-0.5042213	0.0005530	0.0204650	Down
Brd8	-0.2338885	0.0005535	0.0204650	Down
Ccdc187	-0.7391612	0.0005561	0.0205144	Down
Dda1	0.2456967	0.0005610	0.0205920	Up
Hexim1	0.2846320	0.0005626	0.0205920	Up
Klhl26	0.2516779	0.0005631	0.0205920	Up
Pcbp1	0.2365758	0.0005634	0.0205920	Up
Pip4p2	0.2699650	0.0005698	0.0207785	Up
Ppid	0.2347750	0.0005770	0.0209942	Up
Kcnj8	-0.5725844	0.0005797	0.0210452	Down
Kif21b	-0.3085428	0.0005832	0.0211251	Down
Brca2	0.3252161	0.0005850	0.0211291	Up
Cyyr1	-0.5943014	0.0005860	0.0211291	Down
Klrg2	0.9309579	0.0005934	0.0213259	Up
Adarb2	-0.4546183	0.0005941	0.0213259	Down
9330133O14Rik	-0.5051081	0.0005957	0.0213352	Down
Gm54170	-0.4974743	0.0006002	0.0214495	Down
Zfp558	-0.5087084	0.0006040	0.0215359	Down
Aspa	-0.6374602	0.0006072	0.0215590	Down
Rhoa	0.2479018	0.0006073	0.0215590	Up
Gpr6	0.4064967	0.0006215	0.0220134	Up
B230206H07Rik	-0.9196373	0.0006229	0.0220138	Down
Eif3b	0.2350820	0.0006253	0.0220523	Up
Rpl19	0.2812599	0.0006289	0.0221280	Up
Kcnma1	-0.2610025	0.0006332	0.0222328	Down
Ryr2	-0.4183967	0.0006420	0.0224724	Down
Hspb8	0.4231946	0.0006429	0.0224724	Up

symbol	logFC	PValue	FDR	expression
Rpl13	0.2932904	0.0006470	0.0225335	Up
Lncenc1	-0.8137076	0.0006474	0.0225335	Down
Hspd1	0.2691842	0.0006528	0.0226696	Up
Gramd1b	-0.2524155	0.0006547	0.0226869	Down
Mrfap1	0.2707883	0.0006605	0.0228381	Up
Fxr2	0.2223252	0.0006693	0.0230951	Up
Mipol1	-0.5608883	0.0006724	0.0231525	Down
Deup1	-0.6151781	0.0006751	0.0231945	Down
Ctnnbl1	0.2881272	0.0006903	0.0236227	Up
Parn	-0.2819512	0.0006905	0.0236227	Down
Mchr1	0.4031316	0.0006982	0.0238338	Up
Zc3h6	-0.4101859	0.0007091	0.0241533	Down
Galr2	0.8394091	0.0007115	0.0241866	Up
Vps13c	-0.2949009	0.0007158	0.0242437	Down
Gskip	0.2788402	0.0007173	0.0242437	Up
Dnajb4	0.2361958	0.0007178	0.0242437	Up
Golga7	0.2693816	0.0007224	0.0243488	Up
Kcnh7	-0.2807872	0.0007324	0.0245399	Down
Notch3	-0.3326877	0.0007332	0.0245399	Down
Sult1a1	0.6370207	0.0007344	0.0245399	Up
Prmt2	0.2051588	0.0007350	0.0245399	Up
Fmo1	0.3612377	0.0007374	0.0245399	Up
Umps	0.3562428	0.0007376	0.0245399	Up
Aim2	-0.6975023	0.0007391	0.0245399	Down
Morf4l2	0.2313777	0.0007418	0.0245399	Up
Pnrc1	0.2626552	0.0007419	0.0245399	Up
Ccn2	0.8563632	0.0007490	0.0247079	Up
Surf4	0.2432773	0.0007500	0.0247079	Up
Ppib	0.2736671	0.0007554	0.0248324	Up
Gm17244	-0.6625905	0.0007625	0.0250160	Down
Gm16170	-1.4123210	0.0007696	0.0251955	Down
Rps6	0.2700900	0.0007713	0.0252007	Up
Fam216a	0.2485020	0.0007775	0.0253251	Up
Ttc39b	-0.2995050	0.0007783	0.0253251	Down
Gm31159	-1.1330120	0.0007824	0.0254096	Down
Hsp90ab1	0.2356542	0.0007890	0.0255691	Up
Susd6	0.2875258	0.0007905	0.0255691	Up
Prdm4	-0.2681100	0.0007960	0.0256932	Down
Agtr1a	0.7845016	0.0008027	0.0258578	Up
Vim	0.4272717	0.0008058	0.0258954	Up
Cnih1	0.2369620	0.0008071	0.0258954	Up
Utp14b	-0.4809290	0.0008107	0.0259498	Down
Kirrel1	-0.4562917	0.0008129	0.0259498	Down
Qser1	-0.3177328	0.0008149	0.0259498	Down
Chst2	0.2862863	0.0008153	0.0259498	Up
Ppp1r3c	-0.3043891	0.0008195	0.0259878	Down
Mycl	0.3068606	0.0008212	0.0259878	Up
Eef1g	0.2697936	0.0008214	0.0259878	Up
Pex6	0.2802307	0.0008236	0.0259999	Up
Glul	0.3117428	0.0008263	0.0259999	Up
Ccdc116	-0.6562939	0.0008266	0.0259999	Down
Pabpc1l2a	0.4792475	0.0008285	0.0260097	Up

symbol	logFC	PValue	FDR	expression
Pdk4	0.4318281	0.0008325	0.0260831	Up
Pigz	-0.5665778	0.0008366	0.0261526	Down
Tle1	0.2804269	0.0008380	0.0261526	Up
Irf2bp1	0.2488360	0.0008414	0.0262063	Up
Dnajb2	0.2402186	0.0008477	0.0263316	Up
Scn8a	-0.2801614	0.0008487	0.0263316	Down
Onecut2	-0.9926489	0.0008571	0.0265260	Down
B230323A14Rik	-1.2994338	0.0008617	0.0265260	Down
Erf1	-1.9156933	0.0008623	0.0265260	Down
Palmd	0.2896199	0.0008629	0.0265260	Up
Paqr5	0.6369254	0.0008632	0.0265260	Up
U2surp	-0.2565323	0.0008674	0.0266051	Down
Rpl10	0.3248197	0.0008777	0.0268670	Up
Kcnh1	0.4153529	0.0008799	0.0268718	Up
Golph3	0.2286220	0.0008812	0.0268718	Up
Gm10524	-0.6206648	0.0008972	0.0272753	Down
Rad50	-0.3754488	0.0008978	0.0272753	Down
Gm16386	-0.7092974	0.0009045	0.0274277	Down
Ncoa5	0.2584024	0.0009169	0.0277495	Up
Ogfod2	0.3023721	0.0009315	0.0280850	Up
Zbtb34	-0.3860739	0.0009315	0.0280850	Down
Txnrd3	-0.4248052	0.0009354	0.0281346	Down
Arxes2	0.2255861	0.0009366	0.0281346	Up
Fam228b	-0.6372640	0.0009395	0.0281669	Down
Sacs	-0.2801911	0.0009462	0.0282642	Down
Gm36409	-0.5882846	0.0009463	0.0282642	Down
St8sia6	-0.6395681	0.0009585	0.0285755	Down
Ercc6l2	-0.2954769	0.0009619	0.0286012	Down
Wsb2	0.2592493	0.0009629	0.0286012	Up
Gm53294	-1.1212058	0.0009664	0.0286143	Down
Nav1	-0.3521017	0.0009669	0.0286143	Down
Gm33190	-0.7835364	0.0009697	0.0286449	Down
Crhr1	0.4076651	0.0009733	0.0286985	Up
Ostc	0.3006991	0.0009797	0.0288098	Up
Shc2	-0.2639367	0.0009807	0.0288098	Down
Cem2	0.2513582	0.0009838	0.0288156	Up
Pmm1	0.2452073	0.0009871	0.0288156	Up
Csnk1g2	0.2233730	0.0009878	0.0288156	Up
Axsl2	-0.2824613	0.0009881	0.0288156	Down
Cyth2	0.2772754	0.0009930	0.0289049	Up
Dock9	-0.2318978	0.0010006	0.0290726	Down
Sox12	-0.3829838	0.0010032	0.0290957	Down
Arsg	-0.3883237	0.0010058	0.0291187	Down
Gm51535	-1.1023855	0.0010155	0.0293476	Down
Kcna4	-0.2822641	0.0010220	0.0293802	Down
Rps4x	0.3020061	0.0010226	0.0293802	Up
Umodl1	-0.7571633	0.0010236	0.0293802	Down
Rgp1	-0.2427676	0.0010249	0.0293802	Down
Setd5	-0.2308902	0.0010258	0.0293802	Down
Selenok	0.2914268	0.0010411	0.0297649	Up
Crip2	0.2794062	0.0010486	0.0299062	Up
Ak3	0.2161181	0.0010498	0.0299062	Up

symbol	logFC	PValue	FDR	expression
Hmgm5	0.4101288	0.0010517	0.0299062	Up
Stx17	-0.2518529	0.0010556	0.0299213	Down
A930033H14Rik	-0.8306799	0.0010559	0.0299213	Down
Rpl7	0.2805197	0.0010588	0.0299504	Up
Slco3a1	0.2712736	0.0010616	0.0299763	Up
LOC118567337	-0.5352500	0.0010668	0.0300691	Down
Rn7sk	1.1086920	0.0010721	0.0301557	Up
Ppil4	0.2404199	0.0010749	0.0301557	Up
Rps8	0.2724532	0.0010771	0.0301557	Up
Rundc1	0.2324604	0.0010791	0.0301557	Up
Usp27x	0.2723899	0.0010810	0.0301557	Up
Rerg	0.3181346	0.0010812	0.0301557	Up
Csmd1	-0.3435970	0.0010832	0.0301603	Down
Myo5b	-1.1692111	0.0010883	0.0302500	Down
Cd24a	0.3247699	0.0010903	0.0302529	Up
Insc	-0.6601112	0.0010924	0.0302584	Down
Mettl23	0.3957499	0.0011031	0.0305023	Up
Pcdha2	-0.7520344	0.0011074	0.0305648	Down
Ly6c1	0.3287925	0.0011092	0.0305648	Up
Akirin2	0.2642628	0.0011293	0.0310657	Up
Fxr1	0.2270887	0.0011353	0.0311767	Up
Dlk1	0.2611708	0.0011388	0.0312195	Up
Rnf125	-0.6412352	0.0011460	0.0313332	Down
Gm46637	-1.2564386	0.0011469	0.0313332	Down
Usf1	0.2282942	0.0011491	0.0313404	Up
Gm16287	-1.1571693	0.0011556	0.0314636	Down
Thap4	0.3437515	0.0011673	0.0317300	Up
Pfn1	0.2787411	0.0011714	0.0317870	Up
Snrpa	0.2870286	0.0011788	0.0319323	Up
Rps17	0.2598969	0.0011842	0.0320266	Up
3300002A11Rik	-0.9525959	0.0011919	0.0321790	Down
Mob1b	-0.5265258	0.0012084	0.0325707	Down
Nudc	0.2536683	0.0012257	0.0329807	Up
Ppp4c	0.3085219	0.0012291	0.0330160	Up
Spats1	-0.4548718	0.0012385	0.0332124	Down
Zswim4	0.2652822	0.0012473	0.0333933	Up
Nkd2	0.3044099	0.0012513	0.0333933	Up
Htr1b	0.3667770	0.0012529	0.0333933	Up
Pabpc1l2b	0.4264533	0.0012536	0.0333933	Up
Ccdc74a	-0.3339271	0.0012564	0.0334132	Down
Ppp1r10	0.3527023	0.0012695	0.0337067	Up
Tek	-0.3948740	0.0012880	0.0341418	Down
Uhrf2	0.2821478	0.0012910	0.0341628	Up
Sec11a	0.3555276	0.0012973	0.0342552	Up
Rps2	0.3393056	0.0012987	0.0342552	Up
Rpl26	0.2822797	0.0013062	0.0343939	Up
Rpl8	0.2678023	0.0013131	0.0345207	Up
Fam237b	1.0515809	0.0013285	0.0348162	Up
Exog	0.2767927	0.0013287	0.0348162	Up
Hspa1a	0.9801778	0.0013328	0.0348665	Up
Ube2j2	0.2762319	0.0013398	0.0349933	Up
Samd14	0.2194611	0.0013619	0.0355116	Up

symbol	logFC	PValue	FDR	expression
Tmt1a	0.3300473	0.0013645	0.0355217	Up
Gpbp1	0.2078942	0.0013708	0.0356286	Up
Gm46358	-0.9529197	0.0013825	0.0358728	Down
Dad1	0.2350228	0.0013874	0.0358988	Up
Plekha7	-0.4155931	0.0013880	0.0358988	Down
Pdpr	-0.2713120	0.0013902	0.0358988	Down
Trib1	0.3839217	0.0014020	0.0361051	Up
Ankrd28	-0.2507414	0.0014098	0.0361051	Down
Mphosph9	-0.4048771	0.0014098	0.0361051	Down
5930430L01Rik	-0.5860018	0.0014109	0.0361051	Down
Pcdh11x	-0.3102968	0.0014115	0.0361051	Down
Otp	0.3907239	0.0014119	0.0361051	Up
F2rl2	0.6216482	0.0014162	0.0361051	Up
Asah2	-0.3811255	0.0014162	0.0361051	Down
Nek1	-0.3490392	0.0014372	0.0365820	Down
Npy1r	0.2973171	0.0014432	0.0366747	Up
Tmem125	-0.7986029	0.0014856	0.0376042	Down
P4hb	0.2219667	0.0014860	0.0376042	Up
Celrr	-0.8757792	0.0014868	0.0376042	Down
Gpr37	-0.3802613	0.0014929	0.0376754	Down
Ranbp9	0.2194164	0.0014943	0.0376754	Up
Scn1a	-0.3976766	0.0015158	0.0381118	Down
Ndn	0.2507984	0.0015190	0.0381118	Up
Tnik	-0.2859491	0.0015197	0.0381118	Down
Ngrn	0.2454252	0.0015226	0.0381118	Up
mt-Rnr1	0.2540581	0.0015235	0.0381118	Up
Sh2d3c	-0.2852408	0.0015281	0.0381674	Down
B3gnt9	-0.5756973	0.0015336	0.0382446	Down
Gm39260	-0.9888285	0.0015387	0.0383127	Down
Nalf2	0.2697947	0.0015479	0.0384817	Up
Rpl36	0.2546418	0.0015580	0.0386728	Up
Gm41361	-0.9893209	0.0015651	0.0387877	Down
Sf3b4	0.2699154	0.0015772	0.0390289	Up
Rps10	0.2824222	0.0015904	0.0392942	Up
Tcp1	0.2207880	0.0016116	0.0397571	Up
Tead2	0.4858789	0.0016151	0.0397808	Up
Serinc5	-0.2720965	0.0016315	0.0401226	Down
Rmdn3	0.2244068	0.0016454	0.0404032	Up
Gm40841	-0.8021549	0.0016501	0.0404261	Down
Prlh	0.9220536	0.0016514	0.0404261	Up
Lgi3	-0.2833879	0.0016592	0.0405549	Down
Aass	0.4754286	0.0016663	0.0406645	Up
Nrarp	-0.3317223	0.0016697	0.0406645	Down
Cdh11	-0.3827916	0.0016713	0.0406645	Down
Gnpda1	0.2629860	0.0016799	0.0408110	Up
Wifikkn2	0.8083203	0.0016854	0.0408846	Up
Fmo2	1.0719426	0.0016979	0.0411239	Up
Igfbp6	1.0487402	0.0017055	0.0411921	Up
Ccdc137	0.2906542	0.0017058	0.0411921	Up
Trub2	-0.2837507	0.0017433	0.0420325	Down
Anp32b	0.2834433	0.0017536	0.0421809	Up
Cebpzos	0.3157881	0.0017547	0.0421809	Up

symbol	logFC	PValue	FDR	expression
Gemin7	0.3001211	0.0017603	0.0422201	Up
Ltv1	0.2916888	0.0017633	0.0422201	Up
Rabif	0.2563519	0.0017642	0.0422201	Up
Gm34982	-0.7964407	0.0017731	0.0423690	Down
Phf24	-0.2758741	0.0017796	0.0424573	Down
Trpm2	-0.2468670	0.0017833	0.0424573	Down
Aldh1l2	-0.3214311	0.0017847	0.0424573	Down
Bag1	0.2473965	0.0017878	0.0424673	Up
Hspa2	0.3277009	0.0017930	0.0425273	Up
Cib2	0.2334081	0.0017971	0.0425622	Up
Sorbs1	0.2301305	0.0018068	0.0426597	Up
Kif26a	-0.5327492	0.0018095	0.0426597	Down
Sgk1	0.6086934	0.0018110	0.0426597	Up
Gm28913	-0.7211921	0.0018119	0.0426597	Down
D030056L22Rik	0.2914831	0.0018152	0.0426739	Up
Tmem44	-0.3313178	0.0018181	0.0426802	Down
1110046J04Rik	0.5922586	0.0018433	0.0431750	Up
Txnip	0.5294343	0.0018446	0.0431750	Up
Map1lc3b	0.2287399	0.0018619	0.0435162	Up
Stim2	0.2440324	0.0018719	0.0436326	Up
Trappc5	0.2340770	0.0018723	0.0436326	Up
Mctp2	-1.0709533	0.0018891	0.0439600	Down
Eif1	0.2846899	0.0018926	0.0439776	Up
Tbc1d24	-0.2349585	0.0019012	0.0439892	Down
Atoh8	0.8712152	0.0019014	0.0439892	Up
Cct4	0.2437138	0.0019035	0.0439892	Up
Fam174b	0.2925952	0.0019075	0.0439892	Up
Nkx2-2os	-0.9676797	0.0019078	0.0439892	Down
Rps23	0.2656852	0.0019096	0.0439892	Up
Gm42808	0.9928723	0.0019273	0.0443324	Up
Pih1d2	-0.6548325	0.0019379	0.0445139	Down
Gm35823	-0.6163093	0.0019428	0.0445615	Down
Fgf2	0.3815840	0.0019475	0.0446056	Up
Fcgr2b	0.5479508	0.0019558	0.0446551	Up
Npas2	0.3341000	0.0019575	0.0446551	Up
Ankrd12	-0.2759602	0.0019580	0.0446551	Down
Unc80	-0.2086690	0.0019650	0.0447510	Down
Lmntd1	-0.9643110	0.0019701	0.0448025	Down
Gm57732	-0.9759072	0.0019742	0.0448325	Down
Rnf38	-0.3033227	0.0019823	0.0448663	Down
Slc16a5	-0.9450832	0.0019836	0.0448663	Down
Gli1	-0.5409319	0.0019841	0.0448663	Down
Rnf216	0.2539982	0.0019907	0.0449514	Up
Spint2	0.2741767	0.0020101	0.0453255	Up
Yipf5	0.2226302	0.0020181	0.0454434	Up
Slx9	0.2518557	0.0020246	0.0455069	Up
Ifit3b	0.5405006	0.0020266	0.0455069	Up
Pmfbp1	-0.8325965	0.0020334	0.0455946	Down
Slc16a6	-0.5461547	0.0020403	0.0456866	Down
Ntn4	-0.4870053	0.0020498	0.0458341	Down
A830082N09Rik	-0.2311585	0.0020619	0.0459942	Down
Agpat4	0.2608891	0.0020627	0.0459942	Up

symbol	logFC	PValue	FDR	expression
Gpn3	0.3171790	0.0020944	0.0465378	Up
Gm38732	-0.9002773	0.0020954	0.0465378	Down
Agpat1	0.2357546	0.0020958	0.0465378	Up
C230012O17Rik	-0.9550670	0.0021193	0.0469940	Down
Muc3a	-0.9725101	0.0021408	0.0474046	Down
Apba3	0.3743285	0.0021498	0.0475395	Up
Stk4	0.2357954	0.0021623	0.0476696	Up
Bag2	0.3091288	0.0021644	0.0476696	Up
Plcl2	0.2677575	0.0021694	0.0476696	Up
H1f0	-0.3003297	0.0021713	0.0476696	Down
Dnaaf9	-0.2408053	0.0021744	0.0476696	Down
Stk11	0.2545812	0.0021778	0.0476696	Up
Cet5	0.2305921	0.0021790	0.0476696	Up
Hyou1	0.2340986	0.0021808	0.0476696	Up
Gm36845	-1.2010626	0.0021854	0.0476696	Down
Gm53991	-1.0925538	0.0021856	0.0476696	Down
Kbtbd11	0.2511402	0.0021900	0.0476696	Up
Blm	-0.5532860	0.0021914	0.0476696	Down
Pipox	-0.5047407	0.0021997	0.0477727	Down
Clstn2	-0.3074358	0.0022036	0.0477727	Down
H2-D1	0.2342106	0.0022051	0.0477727	Up
Foxred2	-0.2434675	0.0022214	0.0480460	Down
5930403L14Rik	-0.3513621	0.0022237	0.0480460	Down
Irs2	0.2244791	0.0022484	0.0485132	Up
Rap1b	0.2093458	0.0022521	0.0485270	Up
Gm51801	-0.5350077	0.0022659	0.0487119	Down
Rpp25l	0.3083030	0.0022667	0.0487119	Up
Kif13b	-0.2408904	0.0022713	0.0487403	Down
Zar1	0.4537431	0.0022769	0.0487403	Up
Creld1	0.2355694	0.0022772	0.0487403	Up
Ctdnep1	0.2663372	0.0022865	0.0488746	Up
Glipr2	0.3949789	0.0022988	0.0490727	Up
Gm53913	0.8763219	0.0023090	0.0491723	Up
AI849053	-0.4418484	0.0023097	0.0491723	Down
Bud23	0.3221908	0.0023168	0.0492181	Up
Akt3	-0.2094329	0.0023180	0.0492181	Down
H2-T24	-0.5854173	0.0023521	0.0498775	Down

```
#####
##### PLOTS #####
#####

## volcano plot

# Extract top tags from lrt
result_edgeR <- as.data.frame(topTags(lrt, n=1000000, adjust.method = "BH"))

# Extract log-transformed counts per million
count_CPM <- as.data.frame(logcpm_adj)

## Convert row names into first column
result_edgeR <- add_column(result_edgeR, gene = rownames(result_edgeR), .before = "logFC")
```

```

count_CPM <- add_column(count_CPM, gene = rownames(count_CPM), .before = "CTL_1")

## Order by gene
result_edgeR <- result_edgeR[order(result_edgeR$gene),]
count_CPM <- count_CPM[order(count_CPM$gene),]

## Combine result_edgeR columns with count_CPM
count_CPM <- add_column(count_CPM, logFC = result_edgeR$logFC, .before = "CTL_1")
count_CPM <- add_column(count_CPM, FDR = result_edgeR$FDR, .before = "CTL_1")
count_CPM <- add_column(count_CPM, gene2 = result_edgeR$gene, .before = "CTL_1")

# Check if gene and gene2 are the same and in the same order
if(all(count_CPM$gene == count_CPM$gene2)) {
  print("The values in 'gene' and 'gene2' are the same.")
} else {
  print("The values in 'gene' and 'gene2' are different.")
  different_indices <- which(count_CPM$gene != count_CPM$gene2)
  print(paste("The value in 'gene' is different at indices:", different_indices))
}

## [1] "The values in 'gene' and 'gene2' are the same."


## remove gene2
count_CPM$gene2 <- NULL

## Insert -log10 FDR
count_CPM <- add_column(count_CPM, logFDR = -log10(result_edgeR$FDR), .before = "CTL_1")

## Insert -log10 p-value
count_CPM <- add_column(count_CPM, logpvalue = -log10(result_edgeR$PValue), .before = "CTL_1")


## PLOT

# Assign count_CPM to expressao_genica
expressao_genica <- count_CPM

# Determine expression based on FDR and logFC thresholds
expressao_genica$expression <- ifelse(expressao_genica$FDR < 0.05 & abs(expressao_genica$logFC) >= 0,
                                         ifelse(expressao_genica$logFC >= 0, 'Up', 'Down'),
                                         'Stable')

# Count the number of genes categorized as Up and Down
sum(expressao_genica$expression == "Up")

## [1] 458

sum(expressao_genica$expression == "Down")

## [1] 297

```

```

# Create volcano plot

# Filter the data to include only "Down" and "Up" genes
expressao_genica_filtered <- expressao_genica[expressao_genica$expression %in% c("Down", "Up"), ]

# Order the filtered dataframe by logFC
expressao_genica_filtered <- expressao_genica_filtered[order(expressao_genica_filtered$logFC, decreasing = TRUE),]

# Select the top 5 upregulated genes and the top 5 downregulated genes
top_genes_up <- head(subset(expressao_genica_filtered, expression == "Up"), 5)
top_genes_down <- head(subset(expressao_genica_filtered, expression == "Down"), 5)

# Combine top_genes_up and top_genes_down into a single dataframe
top_genes <- rbind(top_genes_up, top_genes_down)

## see
print(top_genes)

```

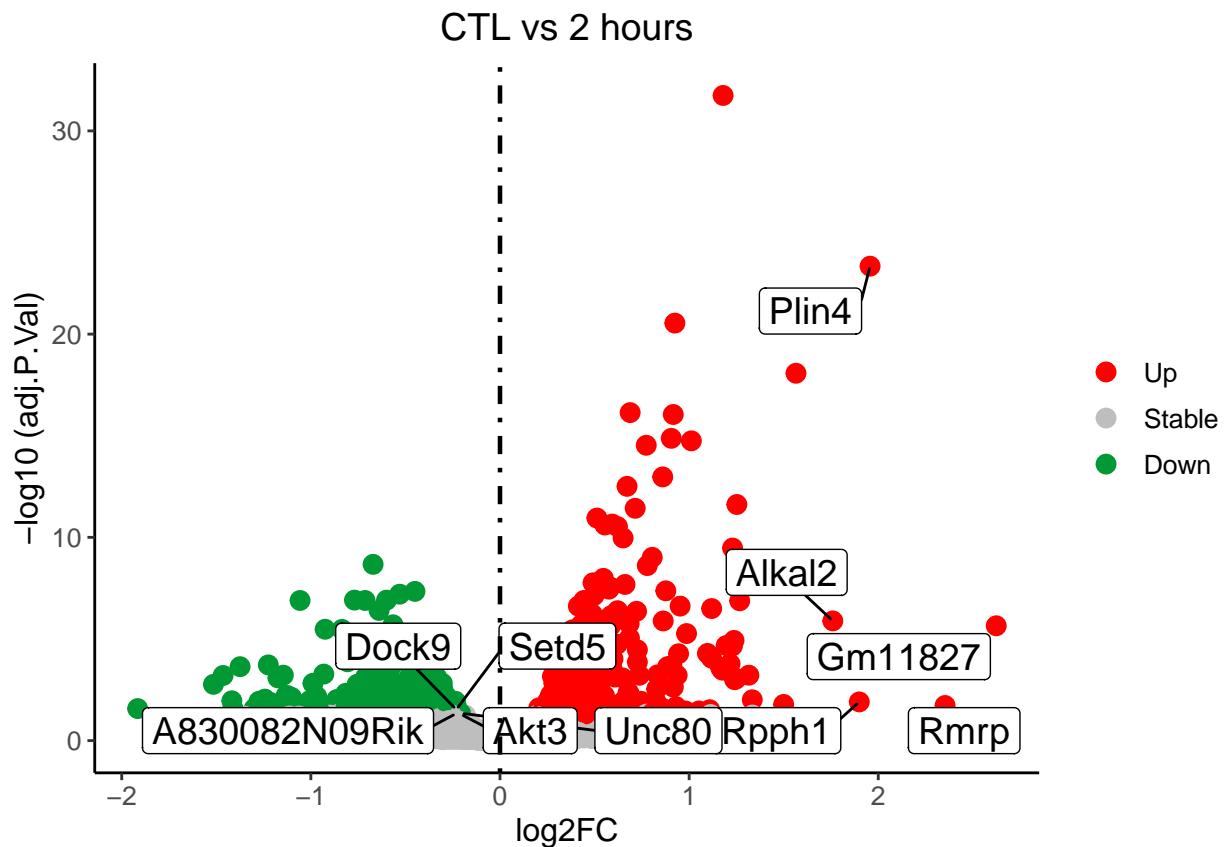
	gene	logFC	FDR	logFDR	logpvalue
## Gm11827	Gm11827	2.6237534	2.240810e-06	5.649595	8.132866
## Rmrp	Rmrp	2.3532491	1.867542e-02	1.728730	3.326473
## Plin4	Plin4	1.9567219	4.520800e-24	23.344785	27.248146
## Rpph1	Rpph1	1.8995973	1.246002e-02	1.904481	3.578465
## Alkal2	Alkal2	1.7596494	1.287091e-06	5.890391	8.432024
## Unc80	Unc80	-0.2086690	4.475099e-02	1.349197	2.706633
## Akt3	Akt3	-0.2094329	4.921812e-02	1.307875	2.634895
## Setd5	Setd5	-0.2308902	2.938023e-02	1.531945	2.988924
## A830082N09Rik	A830082N09Rik	-0.2311585	4.599416e-02	1.337297	2.685734
## Dock9	Dock9	-0.2318978	2.907260e-02	1.536516	2.999756
	CTL_1	CTL_2	CTL_3	CTL_4	CTL_5
## Gm11827	-1.836971552	-2.2966566	-2.0402953	-1.3046966	-1.816463
## Rmrp	-2.504767843	-3.0076864	-0.8888099	-0.7568513	-3.007686
## Plin4	1.573620022	1.5731223	2.1180145	1.8413577	2.040488
## Rpph1	0.008509575	-1.8224894	0.1037477	-0.5452415	-1.173005
## Alkal2	-0.637306189	-0.3998762	0.1813575	0.2050514	1.028075
## Unc80	8.461042851	8.3435764	8.5706698	8.3908588	8.524645
## Akt3	6.805108111	6.7882546	6.8075273	6.6886066	6.728001
## Setd5	7.034137539	7.0776541	7.2706896	7.0910595	7.191999
## A830082N09Rik	5.284133360	5.2945607	5.3037553	5.1968817	5.296506
## Dock9	6.646130094	6.5807011	6.7640435	6.7908134	6.766574
	Exe2h_1	Exe2h_2	Exe2h_3	Exe2h_4	Exe2h_5
## Gm11827	-0.4070428	0.4767689	0.62042978	-0.6425058	0.1194492
## Rmrp	-0.6232747	0.1585001	-1.50537514	1.1305289	0.1790382
## Plin4	3.3207304	3.7145543	4.12096293	3.7720876	3.7451169
## Rpph1	-0.4070428	2.0897678	-0.08194076	1.4050616	1.5899018
## Alkal2	1.6152050	2.2653739	1.84336411	1.6879193	1.5224889
## Unc80	8.2584371	8.1796480	8.28470739	8.2970484	8.2388622
## Akt3	6.6125478	6.4368030	6.55839297	6.6536476	6.5151800
## Setd5	6.9018618	6.8120097	6.91999711	6.9377389	6.9492896
## A830082N09Rik	5.1834734	4.9862438	5.02581254	5.0689605	4.9899655
## Dock9	6.4999289	6.3873319	6.53801586	6.4585542	6.5101133

```

# volcano plot
plot2 <- ggplot(data = expressao_genica,
  aes(x = logFC,
      y = logFDR,
      colour = expression)) +
  geom_point(size = 3) +
  geom_label_repel(data = top_genes, aes(label = gene), size = 5, force = 5,
    fill = "white", color = "black", box.padding = unit(0.5, "lines")) + # Labeling top
  scale_color_manual(values = c("red", "grey", "#009933"),
    name = "",
    breaks = c("Up", "Stable", "Down")) +
  geom_vline(xintercept = c(-0, 0), lty = 4, col = "black", lwd = 0.8) +
  labs(x = "log2FC",
    y = "-log10 (adj.P.Val)") +
  theme_classic() +
  theme(plot.title = element_text(hjust = 0.5),
    legend.position = "right",
    legend.title = element_blank(),
    text = element_text(size = 12)) +
  ggtitle("CTL vs 2 hours")

# To display the volcano plot
plot2

```



```

## save as image
tiff("volcano_CTL_vs_2h.tiff", units="in", width=7, height=7, res=300)
plot2
dev.off()

## pdf
## 2

## heatmap

## Only genes Down and Upregulated
head(expressao_genica_filtered)

##          gene    logFC        FDR    logFDR logpvalue      CTL_1
## Gm11827 Gm11827 2.623753 2.240810e-06 5.649595 8.132866 -1.836971552
## Rmrp     Rmrp    2.353249 1.867542e-02 1.728730 3.326473 -2.504767843
## Plin4    Plin4   1.956722 4.520800e-24 23.344785 27.248146 1.573620022
## Rpph1    Rpph1   1.899597 1.246002e-02 1.904481 3.578465 0.008509575
## Alkal2   Alkal2  1.759649 1.287091e-06 5.890391 8.432024 -0.637306189
## Zbtb16   Zbtb16  1.564903 8.425243e-19 18.074418 21.676749 3.820270968
##          CTL_2    CTL_3    CTL_4    CTL_5  Exe2h_1  Exe2h_2
## Gm11827 -2.2966566 -2.0402953 -1.3046966 -1.816463 -0.4070428 0.4767689
## Rmrp     -3.0076864 -0.8888099 -0.7568513 -3.007686 -0.6232747 0.1585001
## Plin4    1.5731223 2.1180145 1.8413577 2.040488 3.3207304 3.7145543
## Rpph1    -1.8224894 0.1037477 -0.5452415 -1.173005 -0.4070428 2.0897678
## Alkal2   -0.3998762 0.1813575 0.2050514 1.028075 1.6152050 2.2653739
## Zbtb16   3.7809891 3.9875432 4.5828163 4.458569 5.5624486 5.4388722
##          Exe2h_3  Exe2h_4  Exe2h_5 expression
## Gm11827 0.62042978 -0.6425058 0.1194492      Up
## Rmrp     -1.50537514 1.1305289 0.1790382      Up
## Plin4    4.12096293 3.7720876 3.7451169      Up
## Rpph1    -0.08194076 1.4050616 1.5899018      Up
## Alkal2   1.84336411 1.6879193 1.5224889      Up
## Zbtb16   5.78383610 5.9954130 5.7350487      Up

#remove the first five cols
expressao_genica_filtered <- expressao_genica_filtered[, -(1:5)]

## remove a col name expression
expressao_genica_filtered$expression <- NULL

# Set column names
colnames(expressao_genica_filtered) <- c(rep("CTL", 5), rep("2h", 5))

# Convert dataframe to matrix
samp.with.rownames <- as.matrix(expressao_genica_filtered)

# Create column annotations for heatmap
suppressWarnings({
column_annotation <- c(rep("#74ADD1", 5), rep("purple", 5))
column_annotation <- as.matrix(column_annotation)
colnames(column_annotation) <- c("Variable X")
})

```

```

})

# plot
library("gplots")

heatmap.2(samp.with.rownames, scale="row", trace = "none", cexRow=1.5, cexCol=2, margins = c(5, 11), co

## Warning in plot.window(...): "colSideColors" is not a graphical parameter

## Warning in plot.xy(xy, type, ...): "colSideColors" is not a graphical parameter

## Warning in title(...): "colSideColors" is not a graphical parameter

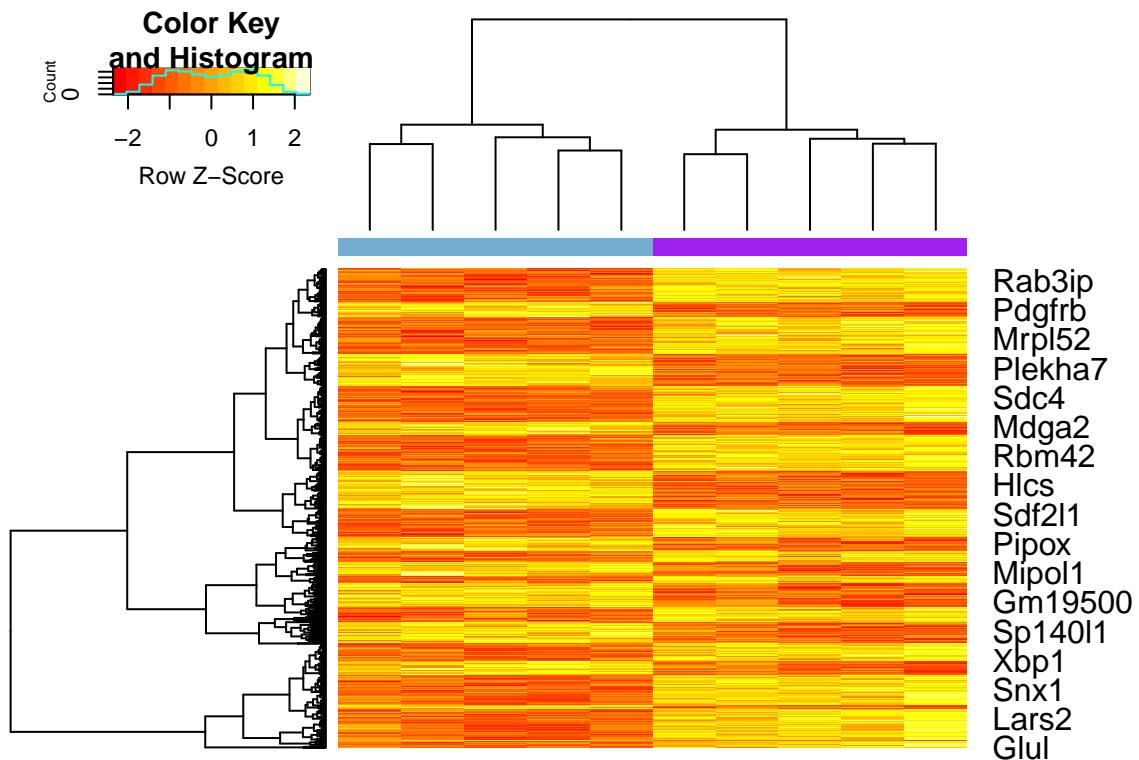
# Get plot coordinates
coords <- par("usr")

# Increase right margin
par(mar = c(5, 6, 4, 12) + 0.2)

# Set legend coordinates
legend_x <- coords[2] + 0.2 * diff(coords[1:2])
legend_y <- coords[4] + 0.05 * diff(coords[3:4])

# Add legend outside the plot
legend(legend_x, legend_y, legend = c("CTL", "2h"),
       fill = c("#74ADD1", "purple"), border = FALSE, cex = 1.1,
       xpd = TRUE)

```



```

## save as image
suppressWarnings({
tiff("heatmap_CTL_vs_2h.tiff", units="in", width=7, height=7, res=300)
heatmap.2(samp.with.rownames, scale="row", trace = "none", cexRow=1.5, cexCol=2, margins = c(5, 11), col=heatcols)

# Get plot coordinates
coords <- par("usr")

# Increase right margin
par(mar = c(5, 6, 4, 12) + 0.2)

# Set legend coordinates
legend_x <- coords[2] + 0.2 * diff(coords[1:2])
legend_y <- coords[4] + 0.05 * diff(coords[3:4])

# Add legend outside the plot
legend(legend_x, legend_y, legend = c("CTL", "2h"),
       fill = c("#74ADD1", "purple"), border = FALSE, cex = 1.1,
       xpd = TRUE)
})

```

1.6 Groups (CTL vs 4 hours)

```
library(tibble)
eDat <- read.table("Count.txt", header=TRUE, sep="\t", row.names = 1)
gDat <- read.table("Group.txt", header=TRUE, sep="\t")

## Counts
## filter only samples CTL and Exe4h
suppressMessages({library(dplyr)})

# Select only 'CTL' ou 'Exe4h'
selected_columns <- eDat[, grep("CTL|Exe4h", names(eDat))]

# new table
new_table1 <- cbind(rownames = rownames(eDat), selected_columns)

## remove the first column
new_table1$rownames <- NULL

## Treat
new_group1 <- subset(gDat, treat == "CTL" | treat == "Exe4h")

## load EdgeR ##
library(edgeR)

# ATENTION!!
new_group1$treat

## [1] "CTL"    "CTL"    "CTL"    "CTL"    "CTL"    "Exe4h"  "Exe4h"  "Exe4h"  "Exe4h"
## [10] "Exe4h"

# Control treatment needs to appear as the first level for the Treat Vs. Control comparison
new_group1$treat <- factor(new_group1$treat, levels=c("CTL", "Exe4h"))

# Input data
y_adj <- DGEList(counts=new_table1, samples=new_group1, group=new_group1$treat)
design_adj <- model.matrix(~ treat - 1, data = new_group1)

# Filter lowly expressed genes
keep_adj <- rowSums(cpm(y_adj)>1) >= 2
sum(keep_adj)

## [1] 16133

y_adj <- y_adj[keep_adj, , keep.lib.sizes=FALSE]

## Explanation: ">= 2" is a condition to maintain the gene, which means that the gene needs to have at least two reads across all samples.

## Explanation: after filtering, 16133 genes remained that have sufficient expression in at least two replicates.
```

```

# Normalization for RNA composition
y_adj <- calcNormFactors(y_adj)
y_adj$samples

##          group lib.size norm.factors   sample treat
## CTL_1      CTL 19626815    0.9857001 CTL_1   CTL
## CTL_2      CTL 12688047    0.9909214 CTL_2   CTL
## CTL_3      CTL 16474230    1.0176986 CTL_3   CTL
## CTL_4      CTL 10484733    1.0308495 CTL_4   CTL
## CTL_5      CTL 12183302    1.0229663 CTL_5   CTL
## Exe4h_1    Exe4h 19612309    0.9746790 Exe4h_1 Exe4h
## Exe4h_2    Exe4h 11823508    0.9790218 Exe4h_2 Exe4h
## Exe4h_3    Exe4h 12955300    0.9992526 Exe4h_3 Exe4h
## Exe4h_4    Exe4h 13156538    1.0004134 Exe4h_4 Exe4h
## Exe4h_5    Exe4h 15370027    1.0000724 Exe4h_5 Exe4h

norm.expr_adj <- y_adj$samples

# norm counts
logcpm_adj <- cpm(y_adj, log=TRUE)

#####
## Data analyses with edgeR      ##
#####

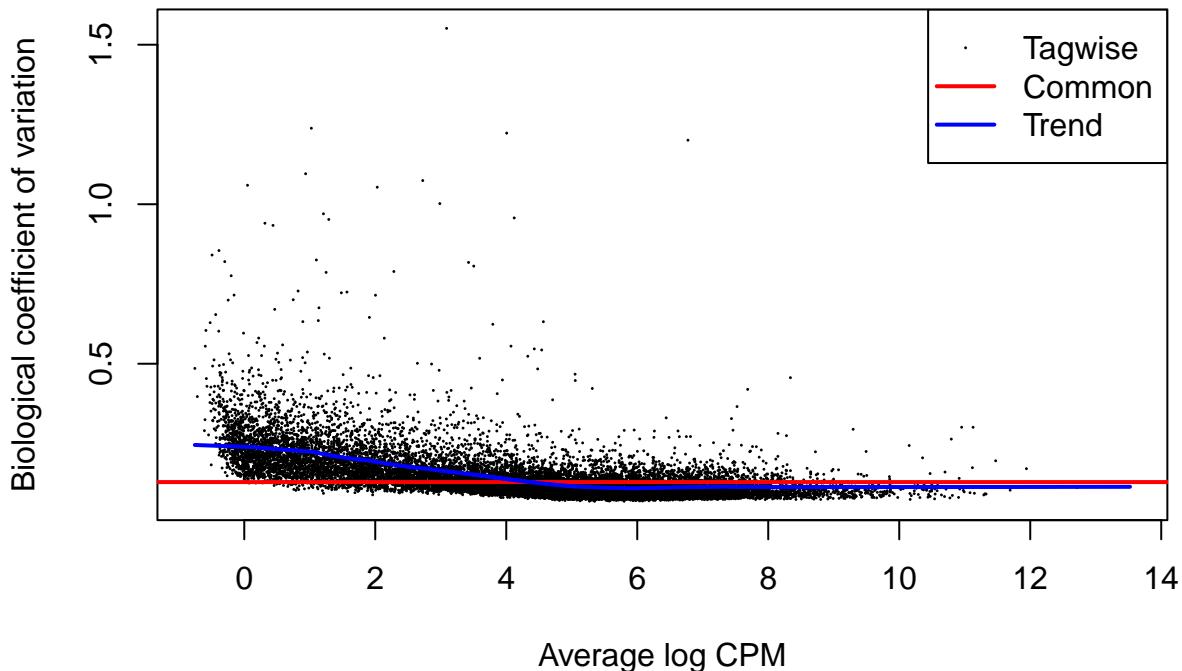
# Estimate dispersion with Cox-Reid profile-adjusted likelihood (CR) method
y_adj <- estimateDisp(y_adj, design_adj)
y_adj$common.dispersion

## [1] 0.01667379

## Explanation: A low dispersion value indicates that the expression levels of genes are relatively consis

## plot
plotBCV(y_adj)

```



```

# Fit Generalized Linear Model (GLM)
fit <- glmFit(y_adj, design_adj)
colnames(fit)

## [1] "treatCTL"      "treatExe4h"

# Differential gene expression analysis
lrt <- glmLRT(fit, contrast=c(-1,1))

## Coefficients used in the contrast for the likelihood ratio test
topTags(lrt)

## Coefficient: -1*treatCTL 1*treatExe4h
##          logFC    logCPM       LR      PValue        FDR
## Plin4     2.1134843 3.238554 105.23348 1.085599e-24 1.751397e-20
## Vgf      0.7907188 8.741153  87.99161 6.573272e-21 5.302330e-17
## Mfsd2a   0.8579491 5.311378  73.44214 1.036300e-17 5.572876e-14
## Mmp14   -0.7764059 5.191883  55.94235 7.462748e-14 2.442541e-10
## Fos     -1.0782993 4.083970  55.91429 7.570015e-14 2.442541e-10
## Pnpla7   0.6455701 4.567108  52.99245 3.348325e-13 9.003089e-10
## Fkbp5    0.6907586 5.646474  46.84586 7.679524e-12 1.769911e-08
## Srxn1    0.6435836 6.956585  43.60105 4.026232e-11 8.119400e-08
## Kirrel2   1.5298748 1.810158  43.31656 4.656252e-11 8.346590e-08
## Fam107a  0.6395114 8.193163  42.65976 6.513853e-11 1.050880e-07

```

```

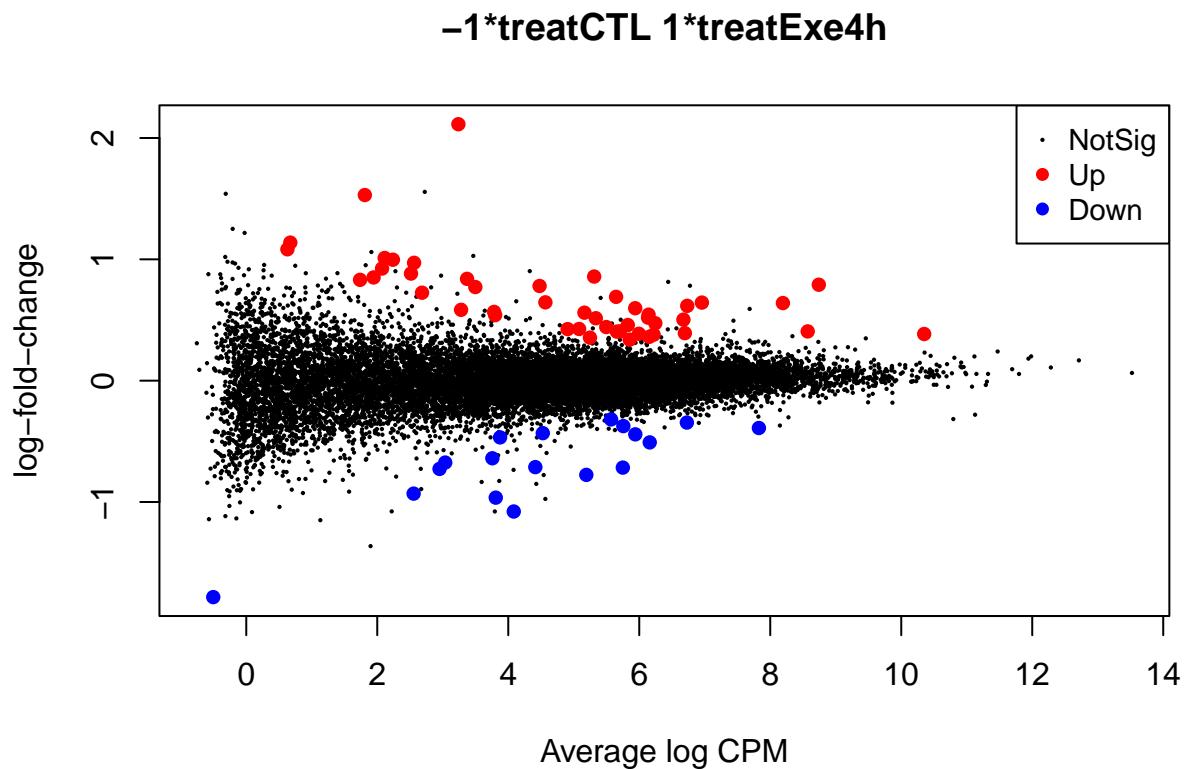
## Explanation: It indicates that the comparison is between the coefficient for treatCTL (the reference

## Number of genes identified as differentially expressed (Disregarding parameters)
is.de <- decideTestsDGE(lrt)
summary(decideTestsDGE(lrt))

##          -1*treatCTL 1*treatExe4h
## Down                  18
## NotSig                16069
## Up                   46

## plot MD
plotMD(lrt, status=is.de)

```



```

# Number of up and down-regulated. FDR < 5% and |log2 fold change| > 0
summary(de <- decideTestsDGE(lrt, lfc=0))

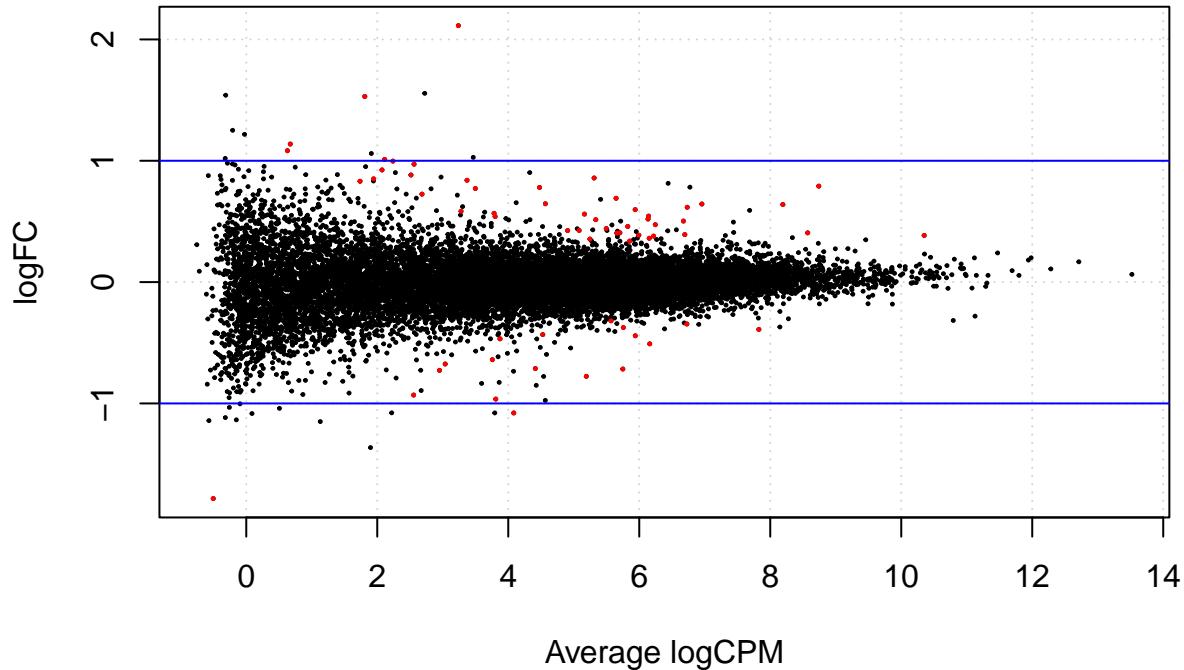
```

```

##          -1*treatCTL 1*treatExe4h
## Down                  18
## NotSig                16069
## Up                   46

```

```
# MA-plot
detags <- rownames(y_adj)[as.logical(de)]
plotSmear(lrt, de.tags=detags)
abline(h=c(-1, 1), col="blue")
```



```
# To export the results
result <- as.data.frame(topTags(lrt, n=1000000))
result <- add_column(result, symbol= rownames(result), .before = "logFC")

## save as table
write.table(result, "DEGs_CTL_vs_4h.txt", sep = "\t", row.names = FALSE)

## Check all the genes Downregulated and Upregulated

# Remove unnecessary columns
result$logCPM <- NULL
result$LR <- NULL

# Remove rows with FDR < 0.05 and logFC >= 0
result$expression <- ifelse(result$FDR < 0.05 & abs(result$logFC) >= 0,
                            ifelse(result$logFC >= 0, 'Up', 'Down'),
                            'Stable')

# Select only genes that are upregulated or downregulated
result_final <- subset(result, expression %in% c("Up", "Down"))
```

```

# Remove row names
rownames(result_final) <- NULL

## View the table
knitr::kable(result_final)

```

symbol	logFC	PValue	FDR	expression
Plin4	2.1134843	0.0000000	0.0000000	Up
Vgf	0.7907188	0.0000000	0.0000000	Up
Mfsd2a	0.8579491	0.0000000	0.0000000	Up
Mmp14	-0.7764059	0.0000000	0.0000000	Down
Fos	-1.0782993	0.0000000	0.0000000	Down
Pnpla7	0.6455701	0.0000000	0.0000000	Up
Fkbp5	0.6907586	0.0000000	0.0000000	Up
Srxn1	0.6435836	0.0000000	0.0000001	Up
Kirrel2	1.5298748	0.0000000	0.0000001	Up
Fam107a	0.6395114	0.0000000	0.0000001	Up
Hr	0.5440553	0.0000000	0.0000003	Up
Acss3	0.8390226	0.0000000	0.0000048	Up
Tekt4	0.9714225	0.0000000	0.0000093	Up
Htra1	0.6167338	0.0000000	0.0000175	Up
Etv5	0.5144178	0.0000000	0.0000399	Up
Egr1	-0.7164387	0.0000001	0.0000621	Down
Nmur2	0.9244320	0.0000001	0.0000786	Up
Ezr	0.5970915	0.0000002	0.0001733	Up
Nr4a3	-0.9641904	0.0000003	0.0002375	Down
Tnfrsf12a	0.8825754	0.0000009	0.0006983	Up
Slc2a1	0.5030374	0.0000010	0.0007548	Up
Ldlr	-0.4424429	0.0000012	0.0008571	Down
Stra6	0.8310502	0.0000014	0.0009832	Up
Gpt2	0.3864174	0.0000023	0.0015750	Up
Sdc4	0.4732810	0.0000027	0.0017142	Up
Mir12181	-1.7838005	0.0000038	0.0023207	Down
Gm37885	0.5669039	0.0000039	0.0023207	Up
Fbxw23	1.1378079	0.0000041	0.0023726	Up
Galnt18	0.4246700	0.0000044	0.0024437	Up
Aass	0.7248498	0.0000049	0.0026410	Up
Fn1	0.4417829	0.0000058	0.0029904	Up
Pglyrp1	0.9968174	0.0000059	0.0029904	Up
Mvd	-0.6396134	0.0000063	0.0031035	Down
Col19a1	-0.9308547	0.0000082	0.0038992	Down
2310022B05Rik	-0.3900274	0.0000092	0.0042480	Down
Hmcn1	-0.7272891	0.0000119	0.0053173	Down
Fmn1	-0.6748519	0.0000126	0.0054783	Down
Gm34159	1.0827956	0.0000131	0.0055497	Up
Ociad2	0.4259610	0.0000140	0.0057900	Up
Baiap2	0.3916015	0.0000154	0.0062287	Up
Pdia4	0.3612952	0.0000191	0.0073954	Up
Fstl4	0.5194462	0.0000193	0.0073954	Up
Ppp1r10	0.4587666	0.0000205	0.0077055	Up
Lhfpl6	0.5603434	0.0000221	0.0081050	Up
Nr4a1	-0.7124929	0.0000247	0.0088478	Down

symbol	logFC	PValue	FDR	expression
Wfikkn2	1.0110742	0.0000286	0.0100148	Up
Insig1	-0.5087029	0.0000429	0.0147282	Down
Hspa5	0.4060055	0.0000458	0.0154035	Up
N4bp2l1	0.3379418	0.0000565	0.0184991	Up
Camk1g	0.3789170	0.0000573	0.0184991	Up
Dhcr7	-0.3191487	0.0000756	0.0235005	Down
Slc24a4	0.3544053	0.0000757	0.0235005	Up
Glul	0.3846019	0.0000812	0.0247286	Up
Agrp	0.7802298	0.0000837	0.0250005	Up
Ctla2a	0.8508337	0.0000916	0.0268737	Up
Arrdc2	0.5838322	0.0000991	0.0285389	Up
Sult1a1	0.7715509	0.0001102	0.0311898	Up
Plcl1	0.4063886	0.0001122	0.0312208	Up
Stab1	0.5400489	0.0001258	0.0343921	Up
Plce1	0.4042226	0.0001281	0.0344308	Up
Hmgcr	-0.3452072	0.0001419	0.0375378	Down
Nol4l	-0.3744962	0.0001627	0.0423264	Down
Bcorl1	-0.4324290	0.0001910	0.0488987	Down
Fgfrl1	-0.4673777	0.0001940	0.0489077	Down

```
#####
##### PLOTS #####
#####

## volcano plot

# Extract top tags from lrt
result_edgeR <- as.data.frame(topTags(lrt, n=1000000, adjust.method = "BH"))

# Extract log-transformed counts per million
count_CPM <- as.data.frame(logcpm_adj)

## Convert row names into first column
result_edgeR <- add_column(result_edgeR, gene = rownames(result_edgeR), .before = "logFC")

count_CPM <- add_column(count_CPM, gene = rownames(count_CPM), .before = "CTL_1")

## Order by gene
result_edgeR <- result_edgeR[order(result_edgeR$gene),]
count_CPM <- count_CPM[order(count_CPM$gene),]

## Combine result_edgeR columns with count_CPM
count_CPM <- add_column(count_CPM, logFC = result_edgeR$logFC, .before = "CTL_1")
count_CPM <- add_column(count_CPM, FDR = result_edgeR$FDR, .before = "CTL_1")
count_CPM <- add_column(count_CPM, gene2 = result_edgeR$gene, .before = "CTL_1")

# Check if gene and gene2 are the same and in the same order
if(all(count_CPM$gene == count_CPM$gene2)) {
  print("The values in 'gene' and 'gene2' are the same.")
} else {
  print("The values in 'gene' and 'gene2' are different.")
  different_indices <- which(count_CPM$gene != count_CPM$gene2)
```

```

    print(paste("The value in 'gene' is different at indices:", different_indices))
}

## [1] "The values in 'gene' and 'gene2' are the same."

## remove gene2
count_CPM$gene2 <- NULL

## Insert -log10 FDR
count_CPM <- add_column(count_CPM, logFDR = -log10(result_edgeR$FDR), .before = "CTL_1")

## Insert -log10 p-value
count_CPM <- add_column(count_CPM, logpvalue = -log10(result_edgeR$PValue), .before = "CTL_1")

## PLOT

# Assign count_CPM to expressao_genica
expressao_genica <- count_CPM

# Determine expression based on FDR and logFC thresholds
expressao_genica$expression <- ifelse(expressao_genica$FDR < 0.05 & abs(expressao_genica$logFC) >= 0,
                                       ifelse(expressao_genica$logFC >= 0, 'Up', 'Down'),
                                       'Stable')

# Count the number of genes categorized as Up and Down
sum(expressao_genica$expression == "Up")

## [1] 46

sum(expressao_genica$expression == "Down")

## [1] 18

# Create volcano plot

# Filter the data to include only "Down" and "Up" genes
expressao_genica_filtered <- expressao_genica[expressao_genica$expression %in% c("Down", "Up"), ]

# Order the filtered dataframe by logFC
expressao_genica_filtered <- expressao_genica_filtered[order(expressao_genica_filtered$logFC, decreasing = TRUE),]

# Select the top 5 upregulated genes and the top 5 downregulated genes
top_genes_up <- head(subset(expressao_genica_filtered, expression == "Up"), 5)
top_genes_down <- head(subset(expressao_genica_filtered, expression == "Down"), 5)

# Combine top_genes_up and top_genes_down into a single dataframe
top_genes <- rbind(top_genes_up, top_genes_down)

# volcano plot
plot3 <- ggplot(data = expressao_genica,

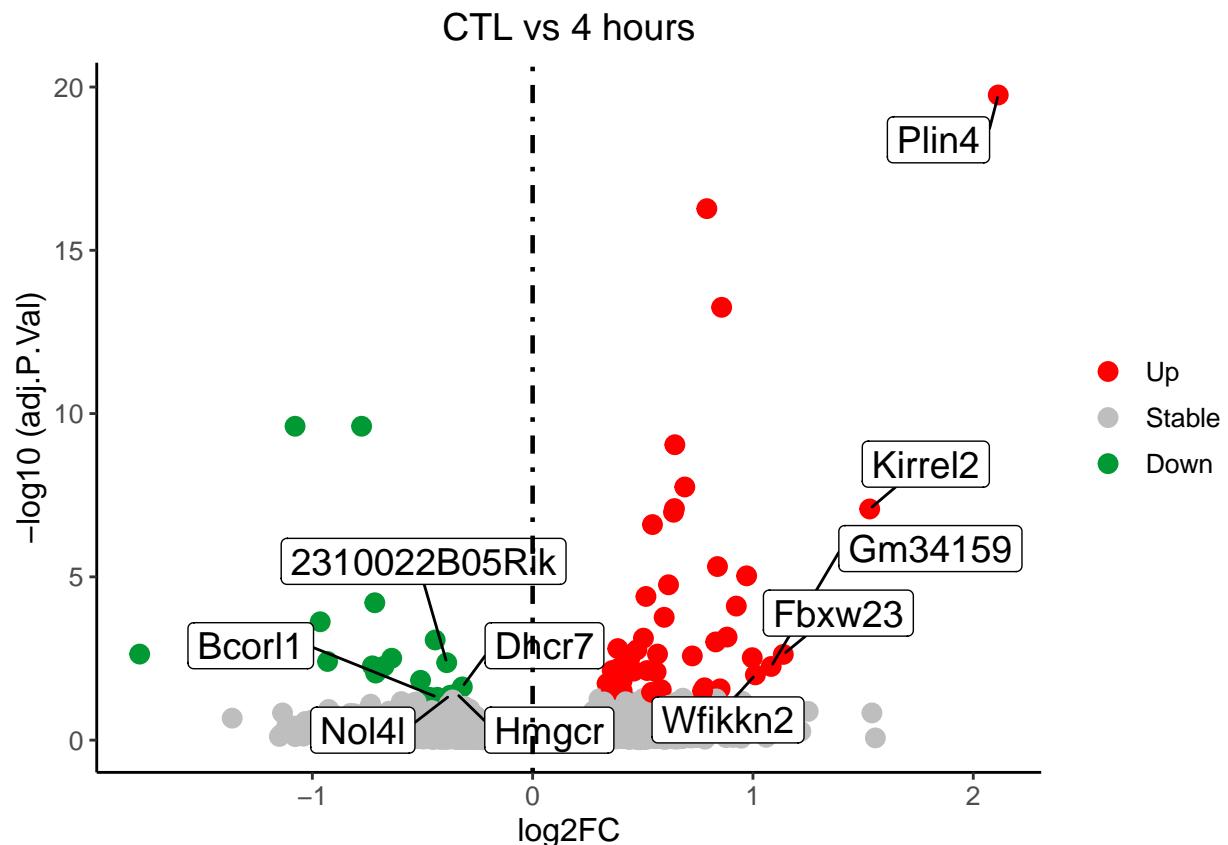
```

```

aes(x = logFC,
    y = logFDR,
    colour = expression)) +
geom_point(size = 3) +
geom_label_repel(data = top_genes, aes(label = gene), size = 5, force = 5,
                 fill = "white", color = "black", box.padding = unit(0.5, "lines")) + # Labeling top
scale_color_manual(values = c("red", "grey", "#009933"),
                    name = "",
                    breaks = c("Up", "Stable", "Down")) +
geom_vline(xintercept = c(-0, 0), lty = 4, col = "black", lwd = 0.8) +
labs(x = "log2FC",
     y = "-log10 (adj.P.Val)") +
theme_classic() +
theme(plot.title = element_text(hjust = 0.5),
      legend.position = "right",
      legend.title = element_blank(),
      text = element_text(size = 12)) +
ggtitle("CTL vs 4 hours")

# To display the volcano plot
plot3

```



```

## save as image
tiff("volcano_CTL_vs_4h.tiff", units="in", width=7, height=7, res=300)
plot3

```

```

dev.off()

## pdf
## 2

## heatmap

## Only genes Down and Upregulated
head(expressao_genica_filtered)

##          gene      logFC       FDR     logFDR logpvalue    CTL_1
## Plin4    Plin4 2.1134843 1.751397e-20 19.756615 23.964331 1.57613941
## Kirrel2 Kirrel2 1.5298748 8.346590e-08  7.078491 10.331964 0.96192850
## Fbxw23  Fbxw23 1.1378079 2.372606e-03  2.624774  5.385332 0.02502139
## Gm34159 Gm34159 1.0827956 5.549688e-03  2.255731  4.883663 0.09651100
## Wfikkn2 Wfikkn2 1.0110742 1.001477e-02  1.999359  4.544316 0.96192850
## Pglyrp1 Pglyrp1 0.9968174 2.990423e-03  2.524267  5.226833 1.80678075
##          CTL_2      CTL_3      CTL_4      CTL_5 Exe4h_1 Exe4h_2 Exe4h_3
## Plin4    1.5859613 2.12881375 1.83272761 2.0533222 3.921493 3.161968 4.011561
## Kirrel2  1.0889403 0.85505941 0.85133686 0.6626882 2.716610 1.946176 2.409870
## Fbxw23  -0.2266873 0.04746236 0.08948753 0.3358061 1.287200 1.145427 1.296401
## Gm34159 -0.3676228 0.27784334 -0.34661939 0.3358061 1.123649 1.087956 1.250299
## Wfikkn2  1.1418994 1.30154698 1.97533262 2.0809453 2.848201 2.339984 2.158516
## Pglyrp1  1.6236861 1.36972942 1.67446476 1.8109417 3.132644 2.435236 2.183264
##          Exe4h_4     Exe4h_5 expression
## Plin4    4.1448065 4.1618328      Up
## Kirrel2  2.6361908 1.8162842      Up
## Fbxw23   0.5910501 1.0182207      Up
## Gm34159  1.0272798 0.6507836      Up
## Wfikkn2  2.7050458 2.4000646      Up
## Pglyrp1  2.2318477 2.8669147      Up

#remove the fisrt five cols
expressao_genica_filtered <- expressao_genica_filtered[, -(1:5)]

## remove a col name expression
expressao_genica_filtered$expression <- NULL

# Set column names
colnames(expressao_genica_filtered) <- c(rep("CTL", 5), rep("4h", 5))

# Convert dataframe to matrix
smp.with.rownames <- as.matrix(expressao_genica_filtered)

# Create column annotations for heatmap
suppressWarnings({
  column_annotation <- c(rep("#74ADD1", 5), rep("#fd61bf", 5))
  column_annotation <- as.matrix(column_annotation)
  colnames(column_annotation) <- c("Variable X")
})

# plot

```

```

library("gplots")

heatmap.2(samp.with.rownames, scale="row", trace = "none", cexRow=1.5, cexCol=2, margins = c(5, 11), col
          = colorRampPalette(c("red", "white", "blue"))(100))

## Warning in plot.window(...): "colSideColors" is not a graphical parameter

## Warning in plot.xy(xy, type, ...): "colSideColors" is not a graphical parameter

## Warning in title(...): "colSideColors" is not a graphical parameter

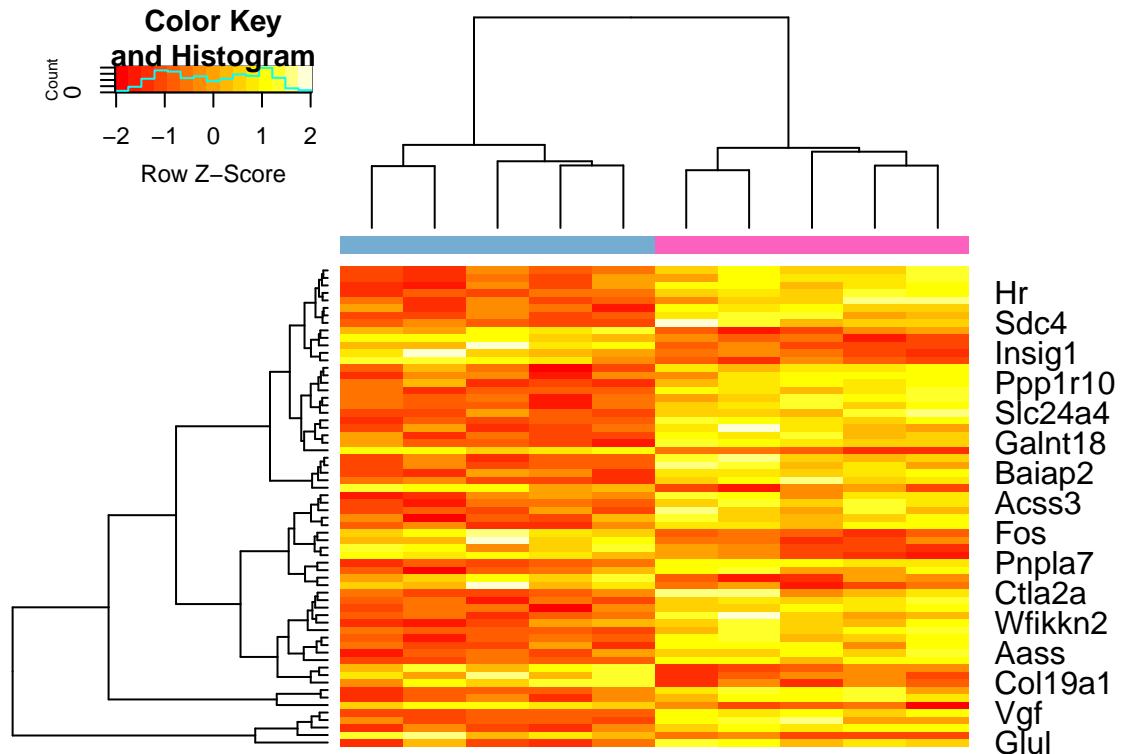
# Get plot coordinates
coords <- par("usr")

# Increase right margin
par(mar = c(5, 6, 4, 12) + 0.2)

# Set legend coordinates
legend_x <- coords[2] + 0.2 * diff(coords[1:2])
legend_y <- coords[4] + 0.05 * diff(coords[3:4])

# Add legend outside the plot
legend(legend_x, legend_y, legend = c("CTL", "4h"),
       fill = c("#74ADD1", "#fd61bf"), border = FALSE, cex = 1.1,
       xpd = TRUE)

```



```

## save as image
suppressWarnings({
tiff("heatmap_CTL_vs_4h.tiff", units="in", width=7, height=7, res=300)
heatmap.2(samp.with.rownames, scale="row", trace = "none", cexRow=1.5, cexCol=2, margins = c(5, 11), col=)

# Get plot coordinates
coords <- par("usr")

# Increase right margin
par(mar = c(5, 6, 4, 12) + 0.2)

# Set legend coordinates
legend_x <- coords[2] + 0.2 * diff(coords[1:2])
legend_y <- coords[4] + 0.05 * diff(coords[3:4])

# Add legend outside the plot
legend(legend_x, legend_y, legend = c("CTL", "4h"),
       fill = c("#74ADD1", "#fd61bf"), border = FALSE, cex = 1.1,
       xpd = TRUE)
})

```

1.7 KEGG pathway over-representation analysis using ‘clusterProfiler’

```

# Load the library
library("clusterProfiler")

## Registered S3 methods overwritten by 'treeio':
##   method           from
##   MRCA.phylo      tidytree
##   MRCA.treedata   tidytree
##   Nnode.treedata  tidytree
##   Ntip.treedata   tidytree
##   ancestor.phylo tidytree
##   ancestor.treedata tidytree
##   child.phylo    tidytree
##   child.treedata  tidytree
##   full_join.phylo tidytree
##   full_join.treedata tidytree
##   groupClade.phylo tidytree
##   groupClade.treedata tidytree
##   groupOTU.phylo  tidytree
##   groupOTU.treedata tidytree
##   is.rooted.treedata tidytree
##   nodeid.phylo    tidytree
##   nodeid.treedata  tidytree
##   nodelab.phylo   tidytree
##   nodelab.treedata tidytree

```

```

##  offspring.phylo      tidytree
##  offspring.treedata   tidytree
##  parent.phylo         tidytree
##  parent.treedata     tidytree
##  root.treedata       tidytree
##  rootnode.phylo      tidytree
##  sibling.phylo       tidytree

## clusterProfiler v4.6.2  For help: https://yulab-smu.top/biomedical-knowledge-mining-book/
##
## If you use clusterProfiler in published research, please cite:
## T Wu, E Hu, S Xu, M Chen, P Guo, Z Dai, T Feng, L Zhou, W Tang, L Zhan, X Fu, S Liu, X Bo, and G Yu.

##
## Attaching package: 'clusterProfiler'

## The following object is masked from 'package:stats':
##
##     filter

library("org.Mm.eg.db")

## Loading required package: AnnotationDbi

## Loading required package: stats4

## Loading required package: BiocGenerics

##
## Attaching package: 'BiocGenerics'

## The following objects are masked from 'package:dplyr':
##
##     combine, intersect, setdiff, union

## The following object is masked from 'package:limma':
##
##     plotMA

## The following objects are masked from 'package:stats':
##
##     IQR, mad, sd, var, xtabs

## The following objects are masked from 'package:base':
##
##     anyDuplicated, aperm, append, as.data.frame, basename, cbind,
##     colnames, dirname, do.call, duplicated, eval, evalq, Filter, Find,
##     get, grep, grepl, intersect, is.unsorted, lapply, Map, mapply,
##     match, mget, order, paste, pmax, pmax.int, pmin, pmin.int,
##     Position, rank, rbind, Reduce, rownames, sapply, setdiff, sort,
##     table, tapply, union, unique, unsplit, which.max, which.min

```

```

## Loading required package: Biobase

## Welcome to Bioconductor
##
##      Vignettes contain introductory material; view with
##      'browseVignettes()'. To cite Bioconductor, see
##      'citation("Biobase")', and for packages 'citation("pkgname")'.

## Loading required package: IRanges

## Loading required package: S4Vectors

##
## Attaching package: 'S4Vectors'

## The following object is masked from 'package:clusterProfiler':
##      rename

## The following object is masked from 'package:gplots':
##      space

## The following objects are masked from 'package:dplyr':
##      first, rename

## The following objects are masked from 'package:base':
##      expand.grid, I, unname

##
## Attaching package: 'IRanges'

## The following object is masked from 'package:clusterProfiler':
##      slice

## The following objects are masked from 'package:dplyr':
##      collapse, desc, slice

##
## Attaching package: 'AnnotationDbi'

## The following object is masked from 'package:clusterProfiler':
##      select

## The following object is masked from 'package:dplyr':
##      select

```

```

## library("pathview")

## #####
## Pathview is an open source software package distributed under GNU General
## Public License version 3 (GPLv3). Details of GPLv3 is available at
## http://www.gnu.org/licenses/gpl-3.0.html. Particullary, users are required to
## formally cite the original Pathview paper (not just mention it) in publications
## or products. For details, do citation("pathview") within R.
##
## The pathview downloads and uses KEGG data. Non-academic uses may require a KEGG
## license agreement (details at http://www.kegg.jp/kegg/legal.html).
## #####
library("ggplot2")
library("cowplot")

### Control vs 0 hours ###

## import DEGs
DEGs <- read.delim("~/VanessaPD/differential_expression/DEGs_CTL_vs_0h_NOFC.txt")

## select only genes with FDR <0.05
significant_genes <- DEGs[DEGs$FDR < 0.05, ]

# For KEGG pathway enrichment using the gseKEGG() function, we need to convert id types. We can use the
# In the bitr function, the param fromType should be the same as keyType from the gseGO function above
# toType: in the bitr function has to be one of the available options from keyTypes(org.Dm.eg.db) and m

# Convert gene IDs for gseKEGG function
# We will lose some genes here because not all IDs will be converted
ids = bitr(significant_genes$symbol, fromType = "SYMBOL", toType = "ENTREZID", OrgDb = org.Mm.eg.db)

## 'select()' returned 1:1 mapping between keys and columns

## Warning in bitr(significant_genes$symbol, fromType = "SYMBOL", toType =
## "ENTREZID", : 0.48% of input gene IDs are fail to map...

# Create a new dataframe df2 which has the respective entrez IDs for the gene symbols.
colnames(ids) = c("symbol", "EntrezID")
df2 = merge(significant_genes, ids, by = "symbol")

# we want the log2 fold change
original_gene_list = df2$logFC

# name the vector
names(original_gene_list) <- df2$EntrezID

```

```

# omit any NA values
gene_list<-na.omit(original_gene_list)

# sort the list in decreasing order (required for clusterProfiler)
gene_list = sort(gene_list, decreasing = TRUE)

## KEGG

kegg_ctl_vs_0h <- enrichKEGG(
  names(gene_list),
  organism = "mmu",
  keyType = "ncbi-geneid",
  pvalueCutoff = 0.05,
  pAdjustMethod = "BH",
  minGSSize = 10,
  maxGSSize = 500,
  qvalueCutoff = 0.2,
  use_internal_data = FALSE
)

```

```
## Reading KEGG annotation online: "https://rest.kegg.jp/link/mmu/pathway"...
```

```
## Reading KEGG annotation online: "https://rest.kegg.jp/list/pathway/mmu"...
```

```
## Reading KEGG annotation online: "https://rest.kegg.jp/conv/ncbi-geneid/mmu"...
```

```

# table
t_kegg_ctl_vs_0h <- as.data.frame(kegg_ctl_vs_0h)

## convert gene ID to Symbol
edox <- setReadable(kegg_ctl_vs_0h, 'org.Mm.eg.db', 'ENTREZID')

# table
t_kegg_ctl_vs_0h <- as.data.frame(edox)

```

```

# Split gene IDs in t_kegg_ctl_vs_0h and create a data frame with separate rows for each gene ID
geneIDs_split <- strsplit(t_kegg_ctl_vs_0h$geneID, "/")
t_kegg_ctl_vs_0h_expanded <- data.frame(geneID = unlist(geneIDs_split), t_kegg_ctl_vs_0h[rep(seq_len(nr
  # Merge the tables based on geneID
merged_data <- merge(t_kegg_ctl_vs_0h_expanded, df2, by.x = "geneID", by.y = "symbol", all.x = TRUE)

# Print the merged data
print(merged_data)

```

```

##      geneID      ID
## 1    Adipor2 mmu04920
## 2      Aff1 mmu05202
## 3      Arc mmu05031
## 4      Ccn2 mmu04390
## 5     Cdkn1a mmu04928

```

```
## 6      Cdkn1a mmu05205
## 7      Cdkn1a mmu05225
## 8      Cdkn1a mmu05210
## 9      Cdkn1a mmu05202
## 10     Cdkn1a mmu04068
## 11     Cdkn1a mmu05222
## 12     Cdkn1a mmu05226
## 13     Cdkn1a mmu05224
## 14     Cdkn1a mmu05217
## 15     Cdkn1a mmu04934
## 16     Cdkn1a mmu05216
## 17     Cdkn1a mmu05211
## 18     Cebpb mmu04148
## 19     Cebpb mmu04657
## 20     Cebpb mmu04668
## 21     Cebpb mmu05202
## 22     Cx3cr1 mmu04148
## 23     Dusp1 mmu04010
## 24     Dusp4 mmu04010
## 25     Dusp4 mmu04148
## 26     Egr1 mmu04928
## 27     Egr1 mmu04933
## 28     Ets1 mmu05211
## 29     F3 mmu04933
## 30     Fn1 mmu04933
## 31     Fn1 mmu05205
## 32     Fn1 mmu05222
## 33     Fos mmu05224
## 34     Fos mmu05210
## 35     Fos mmu04928
## 36     Fos mmu05031
## 37     Fos mmu04210
## 38     Fos mmu04668
## 39     Fos mmu04380
## 40     Fos mmu04010
## 41     Fos mmu04657
## 42     Fosb mmu04380
## 43     Fosb mmu05031
## 44     Fosb mmu04657
## 45     Fosl2 mmu04380
## 46     Fzd1 mmu05217
## 47     Fzd1 mmu04550
## 48     Fzd1 mmu04390
## 49     Fzd1 mmu05226
## 50     Fzd1 mmu05205
## 51     Fzd1 mmu05224
## 52     Fzd1 mmu05225
## 53     Fzd1 mmu04934
## 54     Fzd2 mmu05217
## 55     Fzd2 mmu04934
## 56     Fzd2 mmu05226
## 57     Fzd2 mmu05205
## 58     Fzd2 mmu04390
## 59     Fzd2 mmu05224
```

```

## 60      Fzd2 mmu04550
## 61      Fzd2 mmu05225
## 62      Fzd4 mmu04390
## 63      Fzd4 mmu05217
## 64      Fzd4 mmu05224
## 65      Fzd4 mmu05225
## 66      Fzd4 mmu05205
## 67      Fzd4 mmu04550
## 68      Fzd4 mmu04934
## 69      Fzd4 mmu05226
## 70      Gadd45b mmu04210
## 71      Gadd45b mmu04010
## 72      Gadd45b mmu05202
## 73      Gadd45b mmu05217
## 74      Gadd45b mmu05210
## 75      Gadd45b mmu05225
## 76      Gadd45b mmu05222
## 77      Gadd45b mmu05224
## 78      Gadd45b mmu05216
## 79      Gadd45b mmu05226
## 80      Gadd45b mmu04068
## 81      Gadd45g mmu04010
## 82      Gadd45g mmu05224
## 83      Gadd45g mmu05217
## 84      Gadd45g mmu05222
## 85      Gadd45g mmu04068
## 86      Gadd45g mmu05216
## 87      Gadd45g mmu05210
## 88      Gadd45g mmu05202
## 89      Gadd45g mmu04210
## 90      Gadd45g mmu05225
## 91      Gadd45g mmu05226
## 92      Hes5 mmu05224
## 93      Id2 mmu05202
## 94      Id2 mmu04390
## 95      Id2 mmu04550
## 96      Irs2 mmu04920
## 97      Irs2 mmu04068
## 98      Jun mmu04380
## 99      Jun mmu04933
## 100     Jun mmu05224
## 101     Jun mmu05211
## 102     Jun mmu04210
## 103     Jun mmu05031
## 104     Jun mmu04668
## 105     Jun mmu04657
## 106     Jun mmu05210
## 107     Jun mmu04010
## 108     Junb mmu04380
## 109     Junb mmu04668
## 110     Klf2 mmu04068
## 111     Klf4 mmu04550
## 112     Lcp2 mmu04380
## 113     Lifr mmu04550

```

```

## 114 Map3k6 mmu04010
## 115 Mcl1 mmu04210
## 116 Mertk mmu04148
## 117 Mknk2 mmu04010
## 118 Mmp14 mmu04668
## 119 Mmp14 mmu04928
## 120 Nfil3 mmu04710
## 121 Nfkbia mmu04380
## 122 Nfkbia mmu05222
## 123 Nfkbia mmu04668
## 124 Nfkbia mmu04657
## 125 Nfkbia mmu04210
## 126 Nfkbia mmu04920
## 127 Notch4 mmu05224
## 128 Nr4a1 mmu04934
## 129 Nr4a1 mmu04010
## 130 Nr4a3 mmu05202
## 131 P2ry12 mmu04148
## 132 Pde4c mmu04928
## 133 Per1 mmu04710
## 134 Per2 mmu05202
## 135 Per2 mmu04710
## 136 Plaur mmu05205
## 137 Plk2 mmu04068
## 138 Rasd1 mmu04934
## 139 Sdc4 mmu05205
## 140 Serpine1 mmu04390
## 141 Serpine1 mmu04933
## 142 Sgk1 mmu04068
## 143 Sgk1 mmu04148
## 144 Sgk1 mmu04928
## 145 Slc2a1 mmu05211
## 146 Slc2a1 mmu04920
## 147 Slc2a1 mmu04148
## 148 Smarcd2 mmu05225
## 149 Zbtb16 mmu05202
##                                         Description
## 1             Adipocytokine signaling pathway - Mus musculus (house mouse)
## 2             Transcriptional misregulation in cancer - Mus musculus (house mouse)
## 3             Amphetamine addiction - Mus musculus (house mouse)
## 4             Hippo signaling pathway - Mus musculus (house mouse)
## 5             Parathyroid hormone synthesis, secretion and action - Mus musculus (house mouse)
## 6             Proteoglycans in cancer - Mus musculus (house mouse)
## 7             Hepatocellular carcinoma - Mus musculus (house mouse)
## 8             Colorectal cancer - Mus musculus (house mouse)
## 9             Transcriptional misregulation in cancer - Mus musculus (house mouse)
## 10            FoxO signaling pathway - Mus musculus (house mouse)
## 11            Small cell lung cancer - Mus musculus (house mouse)
## 12            Gastric cancer - Mus musculus (house mouse)
## 13            Breast cancer - Mus musculus (house mouse)
## 14            Basal cell carcinoma - Mus musculus (house mouse)
## 15            Cushing syndrome - Mus musculus (house mouse)
## 16            Thyroid cancer - Mus musculus (house mouse)
## 17            Renal cell carcinoma - Mus musculus (house mouse)

```

```

## 18 Efferocytosis - Mus musculus (house mouse)
## 19 IL-17 signaling pathway - Mus musculus (house mouse)
## 20 TNF signaling pathway - Mus musculus (house mouse)
## 21 Transcriptional misregulation in cancer - Mus musculus (house mouse)
## 22 Efferocytosis - Mus musculus (house mouse)
## 23 MAPK signaling pathway - Mus musculus (house mouse)
## 24 MAPK signaling pathway - Mus musculus (house mouse)
## 25 Efferocytosis - Mus musculus (house mouse)
## 26 Parathyroid hormone synthesis, secretion and action - Mus musculus (house mouse)
## 27 AGE-RAGE signaling pathway in diabetic complications - Mus musculus (house mouse)
## 28 Renal cell carcinoma - Mus musculus (house mouse)
## 29 AGE-RAGE signaling pathway in diabetic complications - Mus musculus (house mouse)
## 30 AGE-RAGE signaling pathway in diabetic complications - Mus musculus (house mouse)
## 31 Proteoglycans in cancer - Mus musculus (house mouse)
## 32 Small cell lung cancer - Mus musculus (house mouse)
## 33 Breast cancer - Mus musculus (house mouse)
## 34 Colorectal cancer - Mus musculus (house mouse)
## 35 Parathyroid hormone synthesis, secretion and action - Mus musculus (house mouse)
## 36 Amphetamine addiction - Mus musculus (house mouse)
## 37 Apoptosis - Mus musculus (house mouse)
## 38 TNF signaling pathway - Mus musculus (house mouse)
## 39 Osteoclast differentiation - Mus musculus (house mouse)
## 40 MAPK signaling pathway - Mus musculus (house mouse)
## 41 IL-17 signaling pathway - Mus musculus (house mouse)
## 42 Osteoclast differentiation - Mus musculus (house mouse)
## 43 Amphetamine addiction - Mus musculus (house mouse)
## 44 IL-17 signaling pathway - Mus musculus (house mouse)
## 45 Osteoclast differentiation - Mus musculus (house mouse)
## 46 Basal cell carcinoma - Mus musculus (house mouse)
## 47 Signaling pathways regulating pluripotency of stem cells - Mus musculus (house mouse)
## 48 Hippo signaling pathway - Mus musculus (house mouse)
## 49 Gastric cancer - Mus musculus (house mouse)
## 50 Proteoglycans in cancer - Mus musculus (house mouse)
## 51 Breast cancer - Mus musculus (house mouse)
## 52 Hepatocellular carcinoma - Mus musculus (house mouse)
## 53 Cushing syndrome - Mus musculus (house mouse)
## 54 Basal cell carcinoma - Mus musculus (house mouse)
## 55 Cushing syndrome - Mus musculus (house mouse)
## 56 Gastric cancer - Mus musculus (house mouse)
## 57 Proteoglycans in cancer - Mus musculus (house mouse)
## 58 Hippo signaling pathway - Mus musculus (house mouse)
## 59 Breast cancer - Mus musculus (house mouse)
## 60 Signaling pathways regulating pluripotency of stem cells - Mus musculus (house mouse)
## 61 Hepatocellular carcinoma - Mus musculus (house mouse)
## 62 Hippo signaling pathway - Mus musculus (house mouse)
## 63 Basal cell carcinoma - Mus musculus (house mouse)
## 64 Breast cancer - Mus musculus (house mouse)
## 65 Hepatocellular carcinoma - Mus musculus (house mouse)
## 66 Proteoglycans in cancer - Mus musculus (house mouse)
## 67 Signaling pathways regulating pluripotency of stem cells - Mus musculus (house mouse)
## 68 Cushing syndrome - Mus musculus (house mouse)
## 69 Gastric cancer - Mus musculus (house mouse)
## 70 Apoptosis - Mus musculus (house mouse)
## 71 MAPK signaling pathway - Mus musculus (house mouse)

```

```

## 72 Transcriptional misregulation in cancer - Mus musculus (house mouse)
## 73 Basal cell carcinoma - Mus musculus (house mouse)
## 74 Colorectal cancer - Mus musculus (house mouse)
## 75 Hepatocellular carcinoma - Mus musculus (house mouse)
## 76 Small cell lung cancer - Mus musculus (house mouse)
## 77 Breast cancer - Mus musculus (house mouse)
## 78 Thyroid cancer - Mus musculus (house mouse)
## 79 Gastric cancer - Mus musculus (house mouse)
## 80 FoxO signaling pathway - Mus musculus (house mouse)
## 81 MAPK signaling pathway - Mus musculus (house mouse)
## 82 Breast cancer - Mus musculus (house mouse)
## 83 Basal cell carcinoma - Mus musculus (house mouse)
## 84 Small cell lung cancer - Mus musculus (house mouse)
## 85 FoxO signaling pathway - Mus musculus (house mouse)
## 86 Thyroid cancer - Mus musculus (house mouse)
## 87 Colorectal cancer - Mus musculus (house mouse)
## 88 Transcriptional misregulation in cancer - Mus musculus (house mouse)
## 89 Apoptosis - Mus musculus (house mouse)
## 90 Hepatocellular carcinoma - Mus musculus (house mouse)
## 91 Gastric cancer - Mus musculus (house mouse)
## 92 Breast cancer - Mus musculus (house mouse)
## 93 Transcriptional misregulation in cancer - Mus musculus (house mouse)
## 94 Hippo signaling pathway - Mus musculus (house mouse)
## 95 Signaling pathways regulating pluripotency of stem cells - Mus musculus (house mouse)
## 96 Adipocytokine signaling pathway - Mus musculus (house mouse)
## 97 FoxO signaling pathway - Mus musculus (house mouse)
## 98 Osteoclast differentiation - Mus musculus (house mouse)
## 99 AGE-RAGE signaling pathway in diabetic complications - Mus musculus (house mouse)
## 100 Breast cancer - Mus musculus (house mouse)
## 101 Renal cell carcinoma - Mus musculus (house mouse)
## 102 Apoptosis - Mus musculus (house mouse)
## 103 Amphetamine addiction - Mus musculus (house mouse)
## 104 TNF signaling pathway - Mus musculus (house mouse)
## 105 IL-17 signaling pathway - Mus musculus (house mouse)
## 106 Colorectal cancer - Mus musculus (house mouse)
## 107 MAPK signaling pathway - Mus musculus (house mouse)
## 108 Osteoclast differentiation - Mus musculus (house mouse)
## 109 TNF signaling pathway - Mus musculus (house mouse)
## 110 FoxO signaling pathway - Mus musculus (house mouse)
## 111 Signaling pathways regulating pluripotency of stem cells - Mus musculus (house mouse)
## 112 Osteoclast differentiation - Mus musculus (house mouse)
## 113 Signaling pathways regulating pluripotency of stem cells - Mus musculus (house mouse)
## 114 MAPK signaling pathway - Mus musculus (house mouse)
## 115 Apoptosis - Mus musculus (house mouse)
## 116 Efferocytosis - Mus musculus (house mouse)
## 117 MAPK signaling pathway - Mus musculus (house mouse)
## 118 TNF signaling pathway - Mus musculus (house mouse)
## 119 Parathyroid hormone synthesis, secretion and action - Mus musculus (house mouse)
## 120 Circadian rhythm - Mus musculus (house mouse)
## 121 Osteoclast differentiation - Mus musculus (house mouse)
## 122 Small cell lung cancer - Mus musculus (house mouse)
## 123 TNF signaling pathway - Mus musculus (house mouse)
## 124 IL-17 signaling pathway - Mus musculus (house mouse)
## 125 Apoptosis - Mus musculus (house mouse)

```

```

## 126 Adipocytokine signaling pathway - Mus musculus (house mouse)
## 127 Breast cancer - Mus musculus (house mouse)
## 128 Cushing syndrome - Mus musculus (house mouse)
## 129 MAPK signaling pathway - Mus musculus (house mouse)
## 130 Transcriptional misregulation in cancer - Mus musculus (house mouse)
## 131 Efferocytosis - Mus musculus (house mouse)
## 132 Parathyroid hormone synthesis, secretion and action - Mus musculus (house mouse)
## 133 Circadian rhythm - Mus musculus (house mouse)
## 134 Transcriptional misregulation in cancer - Mus musculus (house mouse)
## 135 Circadian rhythm - Mus musculus (house mouse)
## 136 Proteoglycans in cancer - Mus musculus (house mouse)
## 137 FoxO signaling pathway - Mus musculus (house mouse)
## 138 Cushing syndrome - Mus musculus (house mouse)
## 139 Proteoglycans in cancer - Mus musculus (house mouse)
## 140 Hippo signaling pathway - Mus musculus (house mouse)
## 141 AGE-RAGE signaling pathway in diabetic complications - Mus musculus (house mouse)
## 142 FoxO signaling pathway - Mus musculus (house mouse)
## 143 Efferocytosis - Mus musculus (house mouse)
## 144 Parathyroid hormone synthesis, secretion and action - Mus musculus (house mouse)
## 145 Renal cell carcinoma - Mus musculus (house mouse)
## 146 Adipocytokine signaling pathway - Mus musculus (house mouse)
## 147 Efferocytosis - Mus musculus (house mouse)
## 148 Hepatocellular carcinoma - Mus musculus (house mouse)
## 149 Transcriptional misregulation in cancer - Mus musculus (house mouse)

##   GeneRatio BgRatio      pvalue    p.adjust     qvalue
## 1       4/99  72/9710 6.158703e-03 0.0462325693 0.0334904860
## 2       9/99  226/9710 4.806604e-04 0.0178805655 0.0129525319
## 3       4/99  69/9710 5.296506e-03 0.0462325693 0.0334904860
## 4       6/99  158/9710 5.453036e-03 0.0462325693 0.0334904860
## 5       6/99  116/9710 1.160000e-03 0.0291830185 0.0211399342
## 6       7/99  204/9710 4.750804e-03 0.0462325693 0.0334904860
## 7       7/99  175/9710 2.026291e-03 0.0376890207 0.0273016109
## 8       5/99  88/9710 1.998769e-03 0.0376890207 0.0273016109
## 9       9/99  226/9710 4.806604e-04 0.0178805655 0.0129525319
## 10      7/99  132/9710 3.856274e-04 0.0178805655 0.0129525319
## 11      5/99  94/9710 2.669491e-03 0.0381942552 0.0276675985
## 12      6/99  150/9710 4.238239e-03 0.0462325693 0.0334904860
## 13      10/99 147/9710 2.294065e-06 0.0004266961 0.0003090951
## 14      6/99  63/9710 4.098185e-05 0.0038113125 0.0027608828
## 15      6/99  162/9710 6.148124e-03 0.0462325693 0.0334904860
## 16      3/99  37/9710 6.214055e-03 0.0462325693 0.0334904860
## 17      4/99  70/9710 5.574279e-03 0.0462325693 0.0334904860
## 18      7/99  161/9710 1.255184e-03 0.0291830185 0.0211399342
## 19      5/99  93/9710 2.547761e-03 0.0381942552 0.0276675985
## 20      6/99  114/9710 1.059758e-03 0.0291830185 0.0211399342
## 21      9/99  226/9710 4.806604e-04 0.0178805655 0.0129525319
## 22      7/99  161/9710 1.255184e-03 0.0291830185 0.0211399342
## 23      9/99  299/9710 3.373862e-03 0.0418358849 0.0303055646
## 24      9/99  299/9710 3.373862e-03 0.0418358849 0.0303055646
## 25      7/99  161/9710 1.255184e-03 0.0291830185 0.0211399342
## 26      6/99  116/9710 1.160000e-03 0.0291830185 0.0211399342
## 27      5/99  101/9710 3.642863e-03 0.0423482836 0.0306767420
## 28      4/99  70/9710 5.574279e-03 0.0462325693 0.0334904860
## 29      5/99  101/9710 3.642863e-03 0.0423482836 0.0306767420

```

```

## 30      5/99 101/9710 3.642863e-03 0.0423482836 0.0306767420
## 31      7/99 204/9710 4.750804e-03 0.0462325693 0.0334904860
## 32      5/99 94/9710 2.669491e-03 0.0381942552 0.0276675985
## 33      10/99 147/9710 2.294065e-06 0.0004266961 0.0003090951
## 34      5/99 88/9710 1.998769e-03 0.0376890207 0.0273016109
## 35      6/99 116/9710 1.160000e-03 0.0291830185 0.0211399342
## 36      4/99 69/9710 5.296506e-03 0.0462325693 0.0334904860
## 37      6/99 135/9710 2.515570e-03 0.0381942552 0.0276675985
## 38      6/99 114/9710 1.059758e-03 0.0291830185 0.0211399342
## 39      7/99 136/9710 4.620155e-04 0.0178805655 0.0129525319
## 40      9/99 299/9710 3.373862e-03 0.0418358849 0.0303055646
## 41      5/99 93/9710 2.547761e-03 0.0381942552 0.0276675985
## 42      7/99 136/9710 4.620155e-04 0.0178805655 0.0129525319
## 43      4/99 69/9710 5.296506e-03 0.0462325693 0.0334904860
## 44      5/99 93/9710 2.547761e-03 0.0381942552 0.0276675985
## 45      7/99 136/9710 4.620155e-04 0.0178805655 0.0129525319
## 46      6/99 63/9710 4.098185e-05 0.0038113125 0.0027608828
## 47      6/99 140/9710 3.016553e-03 0.0400770606 0.0290314870
## 48      6/99 158/9710 5.453036e-03 0.0462325693 0.0334904860
## 49      6/99 150/9710 4.238239e-03 0.0462325693 0.0334904860
## 50      7/99 204/9710 4.750804e-03 0.0462325693 0.0334904860
## 51      10/99 147/9710 2.294065e-06 0.0004266961 0.0003090951
## 52      7/99 175/9710 2.026291e-03 0.0376890207 0.0273016109
## 53      6/99 162/9710 6.148124e-03 0.0462325693 0.0334904860
## 54      6/99 63/9710 4.098185e-05 0.0038113125 0.0027608828
## 55      6/99 162/9710 6.148124e-03 0.0462325693 0.0334904860
## 56      6/99 150/9710 4.238239e-03 0.0462325693 0.0334904860
## 57      7/99 204/9710 4.750804e-03 0.0462325693 0.0334904860
## 58      6/99 158/9710 5.453036e-03 0.0462325693 0.0334904860
## 59      10/99 147/9710 2.294065e-06 0.0004266961 0.0003090951
## 60      6/99 140/9710 3.016553e-03 0.0400770606 0.0290314870
## 61      7/99 175/9710 2.026291e-03 0.0376890207 0.0273016109
## 62      6/99 158/9710 5.453036e-03 0.0462325693 0.0334904860
## 63      6/99 63/9710 4.098185e-05 0.0038113125 0.0027608828
## 64      10/99 147/9710 2.294065e-06 0.0004266961 0.0003090951
## 65      7/99 175/9710 2.026291e-03 0.0376890207 0.0273016109
## 66      7/99 204/9710 4.750804e-03 0.0462325693 0.0334904860
## 67      6/99 140/9710 3.016553e-03 0.0400770606 0.0290314870
## 68      6/99 162/9710 6.148124e-03 0.0462325693 0.0334904860
## 69      6/99 150/9710 4.238239e-03 0.0462325693 0.0334904860
## 70      6/99 135/9710 2.515570e-03 0.0381942552 0.0276675985
## 71      9/99 299/9710 3.373862e-03 0.0418358849 0.0303055646
## 72      9/99 226/9710 4.806604e-04 0.0178805655 0.0129525319
## 73      6/99 63/9710 4.098185e-05 0.0038113125 0.0027608828
## 74      5/99 88/9710 1.998769e-03 0.0376890207 0.0273016109
## 75      7/99 175/9710 2.026291e-03 0.0376890207 0.0273016109
## 76      5/99 94/9710 2.669491e-03 0.0381942552 0.0276675985
## 77      10/99 147/9710 2.294065e-06 0.0004266961 0.0003090951
## 78      3/99 37/9710 6.214055e-03 0.0462325693 0.0334904860
## 79      6/99 150/9710 4.238239e-03 0.0462325693 0.0334904860
## 80      7/99 132/9710 3.856274e-04 0.0178805655 0.0129525319
## 81      9/99 299/9710 3.373862e-03 0.0418358849 0.0303055646
## 82      10/99 147/9710 2.294065e-06 0.0004266961 0.0003090951
## 83      6/99 63/9710 4.098185e-05 0.0038113125 0.0027608828

```

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## 84      5/99  94/9710 2.669491e-03 0.0381942552 0.0276675985
## 85      7/99  132/9710 3.856274e-04 0.0178805655 0.0129525319
## 86      3/99  37/9710 6.214055e-03 0.0462325693 0.0334904860
## 87      5/99  88/9710 1.998769e-03 0.0376890207 0.0273016109
## 88      9/99  226/9710 4.806604e-04 0.0178805655 0.0129525319
## 89      6/99  135/9710 2.515570e-03 0.0381942552 0.0276675985
## 90      7/99  175/9710 2.026291e-03 0.0376890207 0.0273016109
## 91      6/99  150/9710 4.238239e-03 0.0462325693 0.0334904860
## 92      10/99 147/9710 2.294065e-06 0.0004266961 0.0003090951
## 93      9/99  226/9710 4.806604e-04 0.0178805655 0.0129525319
## 94      6/99  158/9710 5.453036e-03 0.0462325693 0.0334904860
## 95      6/99  140/9710 3.016553e-03 0.0400770606 0.0290314870
## 96      4/99  72/9710 6.158703e-03 0.0462325693 0.0334904860
## 97      7/99  132/9710 3.856274e-04 0.0178805655 0.0129525319
## 98      7/99  136/9710 4.620155e-04 0.0178805655 0.0129525319
## 99      5/99  101/9710 3.642863e-03 0.0423482836 0.0306767420
## 100     10/99 147/9710 2.294065e-06 0.0004266961 0.0003090951
## 101     4/99  70/9710 5.574279e-03 0.0462325693 0.0334904860
## 102     6/99  135/9710 2.515570e-03 0.0381942552 0.0276675985
## 103     4/99  69/9710 5.296506e-03 0.0462325693 0.0334904860
## 104     6/99  114/9710 1.059758e-03 0.0291830185 0.0211399342
## 105     5/99  93/9710 2.547761e-03 0.0381942552 0.0276675985
## 106     5/99  88/9710 1.998769e-03 0.0376890207 0.0273016109
## 107     9/99  299/9710 3.373862e-03 0.0418358849 0.0303055646
## 108     7/99  136/9710 4.620155e-04 0.0178805655 0.0129525319
## 109     6/99  114/9710 1.059758e-03 0.0291830185 0.0211399342
## 110     7/99  132/9710 3.856274e-04 0.0178805655 0.0129525319
## 111     6/99  140/9710 3.016553e-03 0.0400770606 0.0290314870
## 112     7/99  136/9710 4.620155e-04 0.0178805655 0.0129525319
## 113     6/99  140/9710 3.016553e-03 0.0400770606 0.0290314870
## 114     9/99  299/9710 3.373862e-03 0.0418358849 0.0303055646
## 115     6/99  135/9710 2.515570e-03 0.0381942552 0.0276675985
## 116     7/99  161/9710 1.255184e-03 0.0291830185 0.0211399342
## 117     9/99  299/9710 3.373862e-03 0.0418358849 0.0303055646
## 118     6/99  114/9710 1.059758e-03 0.0291830185 0.0211399342
## 119     6/99  116/9710 1.160000e-03 0.0291830185 0.0211399342
## 120     3/99  34/9710 4.892277e-03 0.0462325693 0.0334904860
## 121     7/99  136/9710 4.620155e-04 0.0178805655 0.0129525319
## 122     5/99  94/9710 2.669491e-03 0.0381942552 0.0276675985
## 123     6/99  114/9710 1.059758e-03 0.0291830185 0.0211399342
## 124     5/99  93/9710 2.547761e-03 0.0381942552 0.0276675985
## 125     6/99  135/9710 2.515570e-03 0.0381942552 0.0276675985
## 126     4/99  72/9710 6.158703e-03 0.0462325693 0.0334904860
## 127     10/99 147/9710 2.294065e-06 0.0004266961 0.0003090951
## 128     6/99  162/9710 6.148124e-03 0.0462325693 0.0334904860
## 129     9/99  299/9710 3.373862e-03 0.0418358849 0.0303055646
## 130     9/99  226/9710 4.806604e-04 0.0178805655 0.0129525319
## 131     7/99  161/9710 1.255184e-03 0.0291830185 0.0211399342
## 132     6/99  116/9710 1.160000e-03 0.0291830185 0.0211399342
## 133     3/99  34/9710 4.892277e-03 0.0462325693 0.0334904860
## 134     9/99  226/9710 4.806604e-04 0.0178805655 0.0129525319
## 135     3/99  34/9710 4.892277e-03 0.0462325693 0.0334904860
## 136     7/99  204/9710 4.750804e-03 0.0462325693 0.0334904860
## 137     7/99  132/9710 3.856274e-04 0.0178805655 0.0129525319

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	geneID.1	Count	logFC
## 138	6/99 162/9710 6.148124e-03 0.0462325693 0.0334904860		
## 139	7/99 204/9710 4.750804e-03 0.0462325693 0.0334904860		
## 140	6/99 158/9710 5.453036e-03 0.0462325693 0.0334904860		
## 141	5/99 101/9710 3.642863e-03 0.0423482836 0.0306767420		
## 142	7/99 132/9710 3.856274e-04 0.0178805655 0.0129525319		
## 143	7/99 161/9710 1.255184e-03 0.0291830185 0.0211399342		
## 144	6/99 116/9710 1.160000e-03 0.0291830185 0.0211399342		
## 145	4/99 70/9710 5.574279e-03 0.0462325693 0.0334904860		
## 146	4/99 72/9710 6.158703e-03 0.0462325693 0.0334904860		
## 147	7/99 161/9710 1.255184e-03 0.0291830185 0.0211399342		
## 148	7/99 175/9710 2.026291e-03 0.0376890207 0.0273016109		
## 149	9/99 226/9710 4.806604e-04 0.0178805655 0.0129525319		
##			
## 1	Nfkbia/Slc2a1/Irs2/Adipor2	4	0.3642144
## 2	Zbtb16/Nr4a3/Gadd45g/Cdkn1a/Gadd45b/Cebpb/Per2/Aff1/Id2	9	0.5455668
## 3	Fosb/Fos/Arc/Jun	4	0.7714564
## 4	Ccn2/Serpine1/Fzd2/Fzd4/Fzd1/Id2	6	1.2236041
## 5	Sgk1/Fos/Egr1/Pde4c/Cdkn1a/Mmp14	6	0.9177760
## 6	Plaur/Cdkn1a/Fzd2/Fzd4/Fzd1/Fn1/Sdc4	7	0.9177760
## 7	Gadd45g/Cdkn1a/Gadd45b/Fzd2/Fzd4/Fzd1/Smarcd2	7	0.9177760
## 8	Fos/Gadd45g/Cdkn1a/Gadd45b/Jun	5	0.9177760
## 9	Zbtb16/Nr4a3/Gadd45g/Cdkn1a/Gadd45b/Cebpb/Per2/Aff1/Id2	9	0.9177760
## 10	Sgk1/Gadd45g/Klf2/Cdkn1a/Gadd45b/Irs2/Plk2	7	0.9177760
## 11	Gadd45g/Nfkbia/Cdkn1a/Gadd45b/Fn1	5	0.9177760
## 12	Gadd45g/Cdkn1a/Gadd45b/Fzd2/Fzd4/Fzd1	6	0.9177760
## 13	Fos/Gadd45g/Cdkn1a/Gadd45b/Fzd2/Fzd4/Notch4/Jun/Fzd1/Hes5	10	0.9177760
## 14	Gadd45g/Cdkn1a/Gadd45b/Fzd2/Fzd4/Fzd1	6	0.9177760
## 15	Nr4a1/Cdkn1a/Fzd2/Rasd1/Fzd4/Fzd1	6	0.9177760
## 16	Gadd45g/Cdkn1a/Gadd45b	3	0.9177760
## 17	Cdkn1a/Slc2a1/Jun/Ets1	4	0.9177760
## 18	Sgk1/Slc2a1/Cebpb/Mertk/Dusp4/P2ry12/Cx3cr1	7	0.7229472
## 19	Fosb/Fos/Nfkbia/Cebpb/Jun	5	0.7229472
## 20	Fos/Junb/Nfkbia/Cebpb/Jun/Mmp14	6	0.7229472
## 21	Zbtb16/Nr4a3/Gadd45g/Cdkn1a/Gadd45b/Cebpb/Per2/Aff1/Id2	9	0.7229472
## 22	Sgk1/Slc2a1/Cebpb/Mertk/Dusp4/P2ry12/Cx3cr1	7	-0.5819720
## 23	Fos/Nr4a1/Map3k6/Gadd45g/Gadd45b/Dusp1/Dusp4/Jun/Mknk2	9	0.7333510
## 24	Fos/Nr4a1/Map3k6/Gadd45g/Gadd45b/Dusp1/Dusp4/Jun/Mknk2	9	0.5944496
## 25	Sgk1/Slc2a1/Cebpb/Mertk/Dusp4/P2ry12/Cx3cr1	7	0.5944496
## 26	Sgk1/Fos/Egr1/Pde4c/Cdkn1a/Mmp14	6	1.2487810
## 27	Egr1/Serpine1/Jun/Fn1/F3	5	1.2487810
## 28	Cdkn1a/Slc2a1/Jun/Ets1	4	-0.5927357
## 29	Egr1/Serpine1/Jun/Fn1/F3	5	0.4073603
## 30	Egr1/Serpine1/Jun/Fn1/F3	5	0.5138749
## 31	Plaur/Cdkn1a/Fzd2/Fzd4/Fzd1/Fn1/Sdc4	7	0.5138749
## 32	Gadd45g/Nfkbia/Cdkn1a/Gadd45b/Fn1	5	0.5138749
## 33	Fos/Gadd45g/Cdkn1a/Gadd45b/Fzd2/Fzd4/Notch4/Jun/Fzd1/Hes5	10	1.5914162
## 34	Fos/Gadd45g/Cdkn1a/Gadd45b/Jun	5	1.5914162
## 35	Sgk1/Fos/Egr1/Pde4c/Cdkn1a/Mmp14	6	1.5914162
## 36	Fosb/Fos/Arc/Jun	4	1.5914162
## 37	Fos/Gadd45g/Nfkbia/Gadd45b/Jun/Mcl1	6	1.5914162
## 38	Fos/Junb/Nfkbia/Cebpb/Jun/Mmp14	6	1.5914162
## 39	Fosb/Fos/Fosl2/Junb/Nfkbia/Jun/Lcp2	7	1.5914162
## 40	Fos/Nr4a1/Map3k6/Gadd45g/Gadd45b/Dusp1/Dusp4/Jun/Mknk2	9	1.5914162
## 41	Fosb/Fos/Nfkbia/Cebpb/Jun	5	1.5914162

## 42	Fosb/Fos/Fosl2/Junb/Nfkbia/Jun/Lcp2	7	2.2956568
## 43	Fosb/Fos/Arc/Jun	4	2.2956568
## 44	Fosb/Fos/Nfkbia/Cebpb/Jun	5	2.2956568
## 45	Fosb/Fos/Fosl2/Junb/Nfkbia/Jun/Lcp2	7	1.3565789
## 46	Gadd45g/Cdkn1a/Gadd45b/Fzd2/Fzd4/Fzd1	6	0.5437749
## 47	Fzd2/Klf4/Fzd4/Fzd1/Lifr/Id2	6	0.5437749
## 48	Ccn2/Serpine1/Fzd2/Fzd4/Fzd1/Id2	6	0.5437749
## 49	Gadd45g/Cdkn1a/Gadd45b/Fzd2/Fzd4/Fzd1	6	0.5437749
## 50	Plaur/Cdkn1a/Fzd2/Fzd4/Fzd1/Fn1/Sdc4	7	0.5437749
## 51	Fos/Gadd45g/Cdkn1a/Gadd45b/Fzd2/Fzd4/Notch4/Jun/Fzd1/Hes5	10	0.5437749
## 52	Gadd45g/Cdkn1a/Gadd45b/Fzd2/Fzd4/Fzd1/Smarcd2	7	0.5437749
## 53	Nr4a1/Cdkn1a/Fzd2/Rasd1/Fzd4/Fzd1	6	0.5437749
## 54	Gadd45g/Cdkn1a/Gadd45b/Fzd2/Fzd4/Fzd1	6	0.8066421
## 55	Nr4a1/Cdkn1a/Fzd2/Rasd1/Fzd4/Fzd1	6	0.8066421
## 56	Gadd45g/Cdkn1a/Gadd45b/Fzd2/Fzd4/Fzd1	6	0.8066421
## 57	Plaur/Cdkn1a/Fzd2/Fzd4/Fzd1/Fn1/Sdc4	7	0.8066421
## 58	Ccn2/Serpine1/Fzd2/Fzd4/Fzd1/Id2	6	0.8066421
## 59	Fos/Gadd45g/Cdkn1a/Gadd45b/Fzd2/Fzd4/Notch4/Jun/Fzd1/Hes5	10	0.8066421
## 60	Fzd2/Klf4/Fzd4/Fzd1/Lifr/Id2	6	0.8066421
## 61	Gadd45g/Cdkn1a/Gadd45b/Fzd2/Fzd4/Fzd1/Smarcd2	7	0.8066421
## 62	Ccn2/Serpine1/Fzd2/Fzd4/Fzd1/Id2	6	0.5833852
## 63	Gadd45g/Cdkn1a/Gadd45b/Fzd2/Fzd4/Fzd1	6	0.5833852
## 64	Fos/Gadd45g/Cdkn1a/Gadd45b/Fzd2/Fzd4/Notch4/Jun/Fzd1/Hes5	10	0.5833852
## 65	Gadd45g/Cdkn1a/Gadd45b/Fzd2/Fzd4/Fzd1/Smarcd2	7	0.5833852
## 66	Plaur/Cdkn1a/Fzd2/Fzd4/Fzd1/Fn1/Sdc4	7	0.5833852
## 67	Fzd2/Klf4/Fzd4/Fzd1/Lifr/Id2	6	0.5833852
## 68	Nr4a1/Cdkn1a/Fzd2/Rasd1/Fzd4/Fzd1	6	0.5833852
## 69	Gadd45g/Cdkn1a/Gadd45b/Fzd2/Fzd4/Fzd1	6	0.5833852
## 70	Fos/Gadd45g/Nfkbia/Gadd45b/Jun/Mcl1	6	0.8700649
## 71	Fos/Nr4a1/Map3k6/Gadd45g/Gadd45b/Dusp1/Dusp4/Jun/Mknk2	9	0.8700649
## 72	Zbtb16/Nr4a3/Gadd45g/Cdkn1a/Gadd45b/Cebpb/Per2/Aff1/Id2	9	0.8700649
## 73	Gadd45g/Cdkn1a/Gadd45b/Fzd2/Fzd4/Fzd1	6	0.8700649
## 74	Fos/Gadd45g/Cdkn1a/Gadd45b/Jun	5	0.8700649
## 75	Gadd45g/Cdkn1a/Gadd45b/Fzd2/Fzd4/Fzd1/Smarcd2	7	0.8700649
## 76	Gadd45g/Nfkbia/Cdkn1a/Gadd45b/Fn1	5	0.8700649
## 77	Fos/Gadd45g/Cdkn1a/Gadd45b/Fzd2/Fzd4/Notch4/Jun/Fzd1/Hes5	10	0.8700649
## 78	Gadd45g/Cdkn1a/Gadd45b	3	0.8700649
## 79	Gadd45g/Cdkn1a/Gadd45b/Fzd2/Fzd4/Fzd1	6	0.8700649
## 80	Sgk1/Gadd45g/Klf2/Cdkn1a/Gadd45b/Irs2/Plk2	7	0.8700649
## 81	Fos/Nr4a1/Map3k6/Gadd45g/Gadd45b/Dusp1/Dusp4/Jun/Mknk2	9	1.0027271
## 82	Fos/Gadd45g/Cdkn1a/Gadd45b/Fzd2/Fzd4/Notch4/Jun/Fzd1/Hes5	10	1.0027271
## 83	Gadd45g/Cdkn1a/Gadd45b/Fzd2/Fzd4/Fzd1	6	1.0027271
## 84	Gadd45g/Nfkbia/Cdkn1a/Gadd45b/Fn1	5	1.0027271
## 85	Sgk1/Gadd45g/Klf2/Cdkn1a/Gadd45b/Irs2/Plk2	7	1.0027271
## 86	Gadd45g/Cdkn1a/Gadd45b	3	1.0027271
## 87	Fos/Gadd45g/Cdkn1a/Gadd45b/Jun	5	1.0027271
## 88	Zbtb16/Nr4a3/Gadd45g/Cdkn1a/Gadd45b/Cebpb/Per2/Aff1/Id2	9	1.0027271
## 89	Fos/Gadd45g/Nfkbia/Gadd45b/Jun/Mcl1	6	1.0027271
## 90	Gadd45g/Cdkn1a/Gadd45b/Fzd2/Fzd4/Fzd1/Smarcd2	7	1.0027271
## 91	Gadd45g/Cdkn1a/Gadd45b/Fzd2/Fzd4/Fzd1	6	1.0027271
## 92	Fos/Gadd45g/Cdkn1a/Gadd45b/Fzd2/Fzd4/Notch4/Jun/Fzd1/Hes5	10	-0.7921637
## 93	Zbtb16/Nr4a3/Gadd45g/Cdkn1a/Gadd45b/Cebpb/Per2/Aff1/Id2	9	-0.4137854
## 94	Ccn2/Serpine1/Fzd2/Fzd4/Fzd1/Id2	6	-0.4137854
## 95	Fzd2/Klf4/Fzd4/Fzd1/Lifr/Id2	6	-0.4137854

## 96	Nfkbia/Slc2a1/Irs2/Adipor2	4	0.4906806
## 97	Sgk1/Gadd45g/Klf2/Cdkn1a/Gadd45b/Irs2/Plk2	7	0.4906806
## 98	Fosb/Fos/Fosl2/Junb/Nfkbia/Jun/Lcp2	7	0.5578433
## 99	Egr1/Serpine1/Jun/Fn1/F3	5	0.5578433
## 100	Fos/Gadd45g/Cdkn1a/Gadd45b/Fzd2/Fzd4/Notch4/Jun/Fzd1/Hes5	10	0.5578433
## 101	Cdkn1a/Slc2a1/Jun/Ets1	4	0.5578433
## 102	Fos/Gadd45g/Nfkbia/Gadd45b/Jun/Mcl1	6	0.5578433
## 103	Fosb/Fos/Arc/Jun	4	0.5578433
## 104	Fos/Junb/Nfkbia/Cebpb/Jun/Mmp14	6	0.5578433
## 105	Fosb/Fos/Nfkbia/Cebpb/Jun	5	0.5578433
## 106	Fos/Gadd45g/Cdkn1a/Gadd45b/Jun	5	0.5578433
## 107	Fos/Nr4a1/Map3k6/Gadd45g/Gadd45b/Dusp1/Dusp4/Jun/Mknk2	9	0.5578433
## 108	Fosb/Fos/Fosl2/Junb/Nfkbia/Jun/Lcp2	7	1.0283743
## 109	Fos/Junb/Nfkbia/Cebpb/Jun/Mmp14	6	1.0283743
## 110	Sgk1/Gadd45g/Klf2/Cdkn1a/Gadd45b/Irs2/Plk2	7	0.9240276
## 111	Fzd2/Klf4/Fzd4/Fzd1/Lifr/Id2	6	0.7881507
## 112	Fosb/Fos/Fosl2/Junb/Nfkbia/Jun/Lcp2	7	-0.5606294
## 113	Fzd2/Klf4/Fzd4/Fzd1/Lifr/Id2	6	0.3861406
## 114	Fos/Nr4a1/Map3k6/Gadd45g/Gadd45b/Dusp1/Dusp4/Jun/Mknk2	9	1.0029438
## 115	Fos/Gadd45g/Nfkbia/Gadd45b/Jun/Mcl1	6	0.2462298
## 116	Sgk1/Slc2a1/Cebpb/Mertk/Dusp4/P2ry12/Cx3cr1	7	0.6475418
## 117	Fos/Nr4a1/Map3k6/Gadd45g/Gadd45b/Dusp1/Dusp4/Jun/Mknk2	9	0.4396196
## 118	Fos/Junb/Nfkbia/Cebpb/Jun/Mmp14	6	-0.7900059
## 119	Sgk1/Fos/Egr1/Pde4c/Cdkn1a/Mmp14	6	-0.7900059
## 120	Per1/Per2/Nfil3	3	0.4875412
## 121	Fosb/Fos/Fosl2/Junb/Nfkbia/Jun/Lcp2	7	0.9179199
## 122	Gadd45g/Nfkbia/Cdkn1a/Gadd45b/Fn1	5	0.9179199
## 123	Fos/Junb/Nfkbia/Cebpb/Jun/Mmp14	6	0.9179199
## 124	Fosb/Fos/Nfkbia/Cebpb/Jun	5	0.9179199
## 125	Fos/Gadd45g/Nfkbia/Gadd45b/Jun/Mcl1	6	0.9179199
## 126	Nfkbia/Slc2a1/Irs2/Adipor2	4	0.9179199
## 127	Fos/Gadd45g/Cdkn1a/Gadd45b/Fzd2/Fzd4/Notch4/Jun/Fzd1/Hes5	10	0.5772363
## 128	Nr4a1/Cdkn1a/Fzd2/Rasd1/Fzd4/Fzd1	6	1.1594934
## 129	Fos/Nr4a1/Map3k6/Gadd45g/Gadd45b/Dusp1/Dusp4/Jun/Mknk2	9	1.1594934
## 130	Zbtb16/Nr4a3/Gadd45g/Cdkn1a/Gadd45b/Cebpb/Per2/Aff1/Id2	9	1.2458588
## 131	Sgk1/Slc2a1/Cebpb/Mertk/Dusp4/P2ry12/Cx3cr1	7	-0.4092735
## 132	Sgk1/Fos/Egr1/Pde4c/Cdkn1a/Mmp14	6	1.0264153
## 133	Per1/Per2/Nfil3	3	0.7312907
## 134	Zbtb16/Nr4a3/Gadd45g/Cdkn1a/Gadd45b/Cebpb/Per2/Aff1/Id2	9	0.5649836
## 135	Per1/Per2/Nfil3	3	0.5649836
## 136	Plaur/Cdkn1a/Fzd2/Fzd4/Fzd1/Fn1/Sdc4	7	1.4983028
## 137	Sgk1/Gadd45g/Klf2/Cdkn1a/Gadd45b/Irs2/Plk2	7	0.3221122
## 138	Nr4a1/Cdkn1a/Fzd2/Rasd1/Fzd4/Fzd1	6	0.6772451
## 139	Plaur/Cdkn1a/Fzd2/Fzd4/Fzd1/Fn1/Sdc4	7	0.4235128
## 140	Ccn2/Serpine1/Fzd2/Fzd4/Fzd1/Id2	6	0.8791575
## 141	Egr1/Serpine1/Jun/Fn1/F3	5	0.8791575
## 142	Sgk1/Gadd45g/Klf2/Cdkn1a/Gadd45b/Irs2/Plk2	7	1.7910453
## 143	Sgk1/Slc2a1/Cebpb/Mertk/Dusp4/P2ry12/Cx3cr1	7	1.7910453
## 144	Sgk1/Fos/Egr1/Pde4c/Cdkn1a/Mmp14	6	1.7910453
## 145	Cdkn1a/Slc2a1/Jun/Ets1	4	0.7815728
## 146	Nfkbia/Slc2a1/Irs2/Adipor2	4	0.7815728
## 147	Sgk1/Slc2a1/Cebpb/Mertk/Dusp4/P2ry12/Cx3cr1	7	0.7815728
## 148	Gadd45g/Cdkn1a/Gadd45b/Fzd2/Fzd4/Fzd1/Smarcd2	7	0.4200180
## 149	Zbtb16/Nr4a3/Gadd45g/Cdkn1a/Gadd45b/Cebpb/Per2/Aff1/Id2	9	1.4426385

##	logCPM	LR	PValue	FDR	expression	EntrezID
## 1	6.2310617	15.95043	6.502308e-05	7.386531e-03	Up	68465
## 2	4.5389664	19.46769	1.023160e-05	1.514182e-03	Up	17355
## 3	4.7102717	24.71224	6.656004e-07	1.359089e-04	Up	11838
## 4	2.7781207	30.48023	3.372871e-08	1.007552e-05	Up	14219
## 5	4.3602137	36.56108	1.479564e-09	5.303745e-07	Up	12575
## 6	4.3602137	36.56108	1.479564e-09	5.303745e-07	Up	12575
## 7	4.3602137	36.56108	1.479564e-09	5.303745e-07	Up	12575
## 8	4.3602137	36.56108	1.479564e-09	5.303745e-07	Up	12575
## 9	4.3602137	36.56108	1.479564e-09	5.303745e-07	Up	12575
## 10	4.3602137	36.56108	1.479564e-09	5.303745e-07	Up	12575
## 11	4.3602137	36.56108	1.479564e-09	5.303745e-07	Up	12575
## 12	4.3602137	36.56108	1.479564e-09	5.303745e-07	Up	12575
## 13	4.3602137	36.56108	1.479564e-09	5.303745e-07	Up	12575
## 14	4.3602137	36.56108	1.479564e-09	5.303745e-07	Up	12575
## 15	4.3602137	36.56108	1.479564e-09	5.303745e-07	Up	12575
## 16	4.3602137	36.56108	1.479564e-09	5.303745e-07	Up	12575
## 17	4.3602137	36.56108	1.479564e-09	5.303745e-07	Up	12575
## 18	2.9179557	12.53462	3.994811e-04	3.427676e-02	Up	12608
## 19	2.9179557	12.53462	3.994811e-04	3.427676e-02	Up	12608
## 20	2.9179557	12.53462	3.994811e-04	3.427676e-02	Up	12608
## 21	2.9179557	12.53462	3.994811e-04	3.427676e-02	Up	12608
## 22	4.9430808	27.72994	1.394874e-07	3.308929e-05	Down	13051
## 23	4.3341760	32.49193	1.196886e-08	3.785681e-06	Up	19252
## 24	3.5348699	11.71932	6.185456e-04	4.885146e-02	Up	319520
## 25	3.5348699	11.71932	6.185456e-04	4.885146e-02	Up	319520
## 26	6.8314238	51.45182	7.337586e-13	5.146200e-10	Up	13653
## 27	6.8314238	51.45182	7.337586e-13	5.146200e-10	Up	13653
## 28	3.3621949	12.97444	3.157730e-04	2.845661e-02	Down	23871
## 29	5.8194909	28.01172	1.205827e-07	2.903164e-05	Up	14066
## 30	5.5530617	30.16395	3.970221e-08	1.157060e-05	Up	14268
## 31	5.5530617	30.16395	3.970221e-08	1.157060e-05	Up	14268
## 32	5.5530617	30.16395	3.970221e-08	1.157060e-05	Up	14268
## 33	5.5402178	78.00764	1.026427e-18	1.505209e-15	Up	14281
## 34	5.5402178	78.00764	1.026427e-18	1.505209e-15	Up	14281
## 35	5.5402178	78.00764	1.026427e-18	1.505209e-15	Up	14281
## 36	5.5402178	78.00764	1.026427e-18	1.505209e-15	Up	14281
## 37	5.5402178	78.00764	1.026427e-18	1.505209e-15	Up	14281
## 38	5.5402178	78.00764	1.026427e-18	1.505209e-15	Up	14281
## 39	5.5402178	78.00764	1.026427e-18	1.505209e-15	Up	14281
## 40	5.5402178	78.00764	1.026427e-18	1.505209e-15	Up	14281
## 41	5.5402178	78.00764	1.026427e-18	1.505209e-15	Up	14281
## 42	4.3999833	77.14197	1.591003e-18	2.138706e-15	Up	14282
## 43	4.3999833	77.14197	1.591003e-18	2.138706e-15	Up	14282
## 44	4.3999833	77.14197	1.591003e-18	2.138706e-15	Up	14282
## 45	5.6977337	65.15288	6.930707e-16	6.987452e-13	Up	14284
## 46	4.8405033	35.92240	2.053348e-09	7.200556e-07	Up	14362
## 47	4.8405033	35.92240	2.053348e-09	7.200556e-07	Up	14362
## 48	4.8405033	35.92240	2.053348e-09	7.200556e-07	Up	14362
## 49	4.8405033	35.92240	2.053348e-09	7.200556e-07	Up	14362
## 50	4.8405033	35.92240	2.053348e-09	7.200556e-07	Up	14362
## 51	4.8405033	35.92240	2.053348e-09	7.200556e-07	Up	14362
## 52	4.8405033	35.92240	2.053348e-09	7.200556e-07	Up	14362
## 53	4.8405033	35.92240	2.053348e-09	7.200556e-07	Up	14362

## 54	3.9742444	43.81548	3.608402e-11	1.544054e-08	Up	57265
## 55	3.9742444	43.81548	3.608402e-11	1.544054e-08	Up	57265
## 56	3.9742444	43.81548	3.608402e-11	1.544054e-08	Up	57265
## 57	3.9742444	43.81548	3.608402e-11	1.544054e-08	Up	57265
## 58	3.9742444	43.81548	3.608402e-11	1.544054e-08	Up	57265
## 59	3.9742444	43.81548	3.608402e-11	1.544054e-08	Up	57265
## 60	3.9742444	43.81548	3.608402e-11	1.544054e-08	Up	57265
## 61	3.9742444	43.81548	3.608402e-11	1.544054e-08	Up	57265
## 62	3.9491574	26.96826	2.068239e-07	4.835182e-05	Up	14366
## 63	3.9491574	26.96826	2.068239e-07	4.835182e-05	Up	14366
## 64	3.9491574	26.96826	2.068239e-07	4.835182e-05	Up	14366
## 65	3.9491574	26.96826	2.068239e-07	4.835182e-05	Up	14366
## 66	3.9491574	26.96826	2.068239e-07	4.835182e-05	Up	14366
## 67	3.9491574	26.96826	2.068239e-07	4.835182e-05	Up	14366
## 68	3.9491574	26.96826	2.068239e-07	4.835182e-05	Up	14366
## 69	3.9491574	26.96826	2.068239e-07	4.835182e-05	Up	14366
## 70	2.3900346	17.32806	3.145080e-05	4.091394e-03	Up	17873
## 71	2.3900346	17.32806	3.145080e-05	4.091394e-03	Up	17873
## 72	2.3900346	17.32806	3.145080e-05	4.091394e-03	Up	17873
## 73	2.3900346	17.32806	3.145080e-05	4.091394e-03	Up	17873
## 74	2.3900346	17.32806	3.145080e-05	4.091394e-03	Up	17873
## 75	2.3900346	17.32806	3.145080e-05	4.091394e-03	Up	17873
## 76	2.3900346	17.32806	3.145080e-05	4.091394e-03	Up	17873
## 77	2.3900346	17.32806	3.145080e-05	4.091394e-03	Up	17873
## 78	2.3900346	17.32806	3.145080e-05	4.091394e-03	Up	17873
## 79	2.3900346	17.32806	3.145080e-05	4.091394e-03	Up	17873
## 80	2.3900346	17.32806	3.145080e-05	4.091394e-03	Up	17873
## 81	4.5532428	49.38466	2.103840e-12	1.212037e-09	Up	23882
## 82	4.5532428	49.38466	2.103840e-12	1.212037e-09	Up	23882
## 83	4.5532428	49.38466	2.103840e-12	1.212037e-09	Up	23882
## 84	4.5532428	49.38466	2.103840e-12	1.212037e-09	Up	23882
## 85	4.5532428	49.38466	2.103840e-12	1.212037e-09	Up	23882
## 86	4.5532428	49.38466	2.103840e-12	1.212037e-09	Up	23882
## 87	4.5532428	49.38466	2.103840e-12	1.212037e-09	Up	23882
## 88	4.5532428	49.38466	2.103840e-12	1.212037e-09	Up	23882
## 89	4.5532428	49.38466	2.103840e-12	1.212037e-09	Up	23882
## 90	4.5532428	49.38466	2.103840e-12	1.212037e-09	Up	23882
## 91	4.5532428	49.38466	2.103840e-12	1.212037e-09	Up	23882
## 92	3.8635126	37.64832	8.471962e-10	3.333200e-07	Down	15208
## 93	5.2922060	16.17424	5.777442e-05	6.852641e-03	Down	15902
## 94	5.2922060	16.17424	5.777442e-05	6.852641e-03	Down	15902
## 95	5.2922060	16.17424	5.777442e-05	6.852641e-03	Down	15902
## 96	7.2308034	50.49707	1.193430e-12	7.700486e-10	Up	384783
## 97	7.2308034	50.49707	1.193430e-12	7.700486e-10	Up	384783
## 98	5.9470345	24.65356	6.861806e-07	1.383597e-04	Up	16476
## 99	5.9470345	24.65356	6.861806e-07	1.383597e-04	Up	16476
## 100	5.9470345	24.65356	6.861806e-07	1.383597e-04	Up	16476
## 101	5.9470345	24.65356	6.861806e-07	1.383597e-04	Up	16476
## 102	5.9470345	24.65356	6.861806e-07	1.383597e-04	Up	16476
## 103	5.9470345	24.65356	6.861806e-07	1.383597e-04	Up	16476
## 104	5.9470345	24.65356	6.861806e-07	1.383597e-04	Up	16476
## 105	5.9470345	24.65356	6.861806e-07	1.383597e-04	Up	16476
## 106	5.9470345	24.65356	6.861806e-07	1.383597e-04	Up	16476
## 107	5.9470345	24.65356	6.861806e-07	1.383597e-04	Up	16476

## 108	5.3516594	26.60373	2.497616e-07	5.674513e-05	Up	16477
## 109	5.3516594	26.60373	2.497616e-07	5.674513e-05	Up	16477
## 110	3.7582093	50.50600	1.188009e-12	7.700486e-10	Up	16598
## 111	3.2125387	30.81748	2.834724e-08	8.627723e-06	Up	16600
## 112	2.5296424	13.08590	2.975270e-04	2.742519e-02	Down	16822
## 113	5.5299917	17.74080	2.531384e-05	3.374691e-03	Up	16880
## 114	2.8425243	41.73915	1.042992e-10	4.313976e-08	Up	53608
## 115	6.5302894	13.19297	2.810017e-04	2.605080e-02	Up	17210
## 116	4.8783604	21.79063	3.040820e-06	5.274352e-04	Up	17289
## 117	5.1505132	21.72637	3.144396e-06	5.395984e-04	Up	17347
## 118	5.1927613	55.71257	8.388001e-14	7.121413e-11	Down	17387
## 119	5.1927613	55.71257	8.388001e-14	7.121413e-11	Down	17387
## 120	3.9008820	16.41247	5.094882e-05	6.179363e-03	Up	18030
## 121	4.4675277	73.76902	8.781424e-18	1.011808e-14	Up	18035
## 122	4.4675277	73.76902	8.781424e-18	1.011808e-14	Up	18035
## 123	4.4675277	73.76902	8.781424e-18	1.011808e-14	Up	18035
## 124	4.4675277	73.76902	8.781424e-18	1.011808e-14	Up	18035
## 125	4.4675277	73.76902	8.781424e-18	1.011808e-14	Up	18035
## 126	4.4675277	73.76902	8.781424e-18	1.011808e-14	Up	18035
## 127	3.5913813	14.97340	1.090373e-04	1.157158e-02	Up	18132
## 128	5.4313163	44.49954	2.544168e-11	1.139999e-08	Up	15370
## 129	5.4313163	44.49954	2.544168e-11	1.139999e-08	Up	15370
## 130	4.9745727	45.47248	1.547986e-11	7.134444e-09	Up	18124
## 131	4.1631706	14.45088	1.438624e-04	1.450341e-02	Down	70839
## 132	1.2432407	19.95578	7.925420e-06	1.229278e-03	Up	110385
## 133	6.8181185	66.62242	3.288249e-16	3.536183e-13	Up	18626
## 134	5.4631373	29.27817	6.269798e-08	1.730599e-05	Up	18627
## 135	5.4631373	29.27817	6.269798e-08	1.730599e-05	Up	18627
## 136	-0.1548604	18.54853	1.656326e-05	2.385553e-03	Up	18793
## 137	5.6948621	16.11264	5.968440e-05	7.027512e-03	Up	20620
## 138	4.2402654	24.02146	9.526812e-07	1.851530e-04	Up	19416
## 139	6.2299717	24.30780	8.210608e-07	1.615187e-04	Up	20971
## 140	2.0111691	24.51656	7.367405e-07	1.467205e-04	Up	18787
## 141	2.0111691	24.51656	7.367405e-07	1.467205e-04	Up	18787
## 142	6.2033443	173.68018	1.162594e-39	9.376900e-36	Up	20393
## 143	6.2033443	173.68018	1.162594e-39	9.376900e-36	Up	20393
## 144	6.2033443	173.68018	1.162594e-39	9.376900e-36	Up	20393
## 145	6.8583930	86.50986	1.390426e-20	3.204137e-17	Up	20525
## 146	6.8583930	86.50986	1.390426e-20	3.204137e-17	Up	20525
## 147	6.8583930	86.50986	1.390426e-20	3.204137e-17	Up	20525
## 148	4.2509885	15.12379	1.006863e-04	1.095998e-02	Up	83796
## 149	5.0848748	24.76292	6.483287e-07	1.340794e-04	Up	235320

```

## remove (mus musculos name)
edox@result$Description <- gsub(pattern = " - Mus musculus (house mouse)", replacement = "", edox@result)

## table
t_kegg_ctl_vs_0h <- as.data.frame(edox)

## plot
cnetplot1 <- cnetplot(edox, color.params = list(foldChange = gene_list), circular = F, colorEdge = TRUE,
showCategory = c("Breast cancer", "Basal cell carcinoma", "Transcriptional misregulation in cancer",
"Colorectal cancer", "Hepatocellular carcinoma", "Small cell lung cancer",

```

```

    "AGE-RAGE signaling pathway in diabetic complications", "Gastric cancer",
    "Proteoglycans in cancer", "Renal cell carcinoma", "Amphetamine addiction"))
scale_colour_gradient2(name = "fold change", low = "darkblue", mid = "white", high = "red") +
theme(
  plot.title = element_text(size = 12, face = "bold", hjust = 0.5),
  plot.subtitle = element_text(size = 10, hjust = 0.5)) +
labs(
  title = "KEGG: Kyoto Encyclopedia of Genes and Genomes\nCategory: Human Diseases",
  subtitle = NULL
)

## Warning in cnetplot.enrichResult(x, ...): Use 'color.params = list(edge = your_value)' instead of 'colorEdge'.
##   The colorEdge parameter will be removed in the next version.

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

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

cnetplot2 <- cnetplot(edox, color.params = list(foldChange = gene_list), circular = F, colorEdge = TRUE
                      showCategory = c("FoxO signaling pathway",
                                      "TNF signaling pathway",
                                      "MAPK signaling pathway",
                                      "Hippo signaling pathway")) +
scale_colour_gradient2(name = "fold change", low = "darkblue", mid = "white", high = "red") +
theme(
  plot.title = element_text(size = 12, face = "bold", hjust = 0.5),
  plot.subtitle = element_text(size = 10, hjust = 0.5)) +
labs(
  title = "KEGG: Kyoto Encyclopedia of Genes and Genomes\nCategory: Environmental Information Processing",
  subtitle = NULL
)

## Warning in cnetplot.enrichResult(x, ...): Use 'color.params = list(edge = your_value)' instead of 'colorEdge'.
##   The colorEdge parameter will be removed in the next version.

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

cnetplot3 <- cnetplot(edox, color.params = list(foldChange = gene_list), circular = F, colorEdge = TRUE
                      showCategory = c("Efferocytosis",
                                      "Apoptosis",
                                      "Signaling pathways regulating pluripotency of stem cells")) +
scale_colour_gradient2(name = "fold change", low = "darkblue", mid = "white", high = "red") +
theme(
  plot.title = element_text(size = 12, face = "bold", hjust = 0.5),
  plot.subtitle = element_text(size = 10, hjust = 0.5)) +
labs(

```

```

    title = "KEGG: Kyoto Encyclopedia of Genes and Genomes\nCategory: Cellular Processes",
    subtitle = NULL
)

## Warning in cnetplot.enrichResult(x, ...): Use 'color.params = list(edge = your_value)' instead of 'co
##   The colorEdge parameter will be removed in the next version.

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

cnetplot4 <- cnetplot(edox, color.params = list(foldChange = gene_list), circular = F, colorEdge = TRUE
                      showCategory = c("Circadian rhythm",
                                      "Osteoclast differentiation",
                                      "Parathyroid hormone synthesis, secretion and action",
                                      "IL-17 signaling pathway",
                                      "Adipocytokine signaling pathway")) +
  scale_colour_gradient2(name = "fold change", low = "darkblue", mid = "white", high = "red") +
  theme(
    plot.title = element_text(size = 12, face = "bold", hjust = 0.5),
    plot.subtitle = element_text(size = 10, hjust = 0.5)) +
  labs(
    title = "KEGG: Kyoto Encyclopedia of Genes and Genomes\nCategory: Organismal Systems",
    subtitle = NULL
)

## Warning in cnetplot.enrichResult(x, ...): Use 'color.params = list(edge = your_value)' instead of 'co
##   The colorEdge parameter will be removed in the next version.

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

## put all of the together
KEGG_CTL_vs_0h_graph<- plot_grid(cnetplot1, cnetplot2, cnetplot3, cnetplot4, nrow = 2, ncol = 2, labels

## save image
tiff('KEGG_CTL_vs_0h_graph.tiff', units="in", width=17, height=17, res=600)
KEGG_CTL_vs_0h_graph
dev.off()

## pdf
## 2

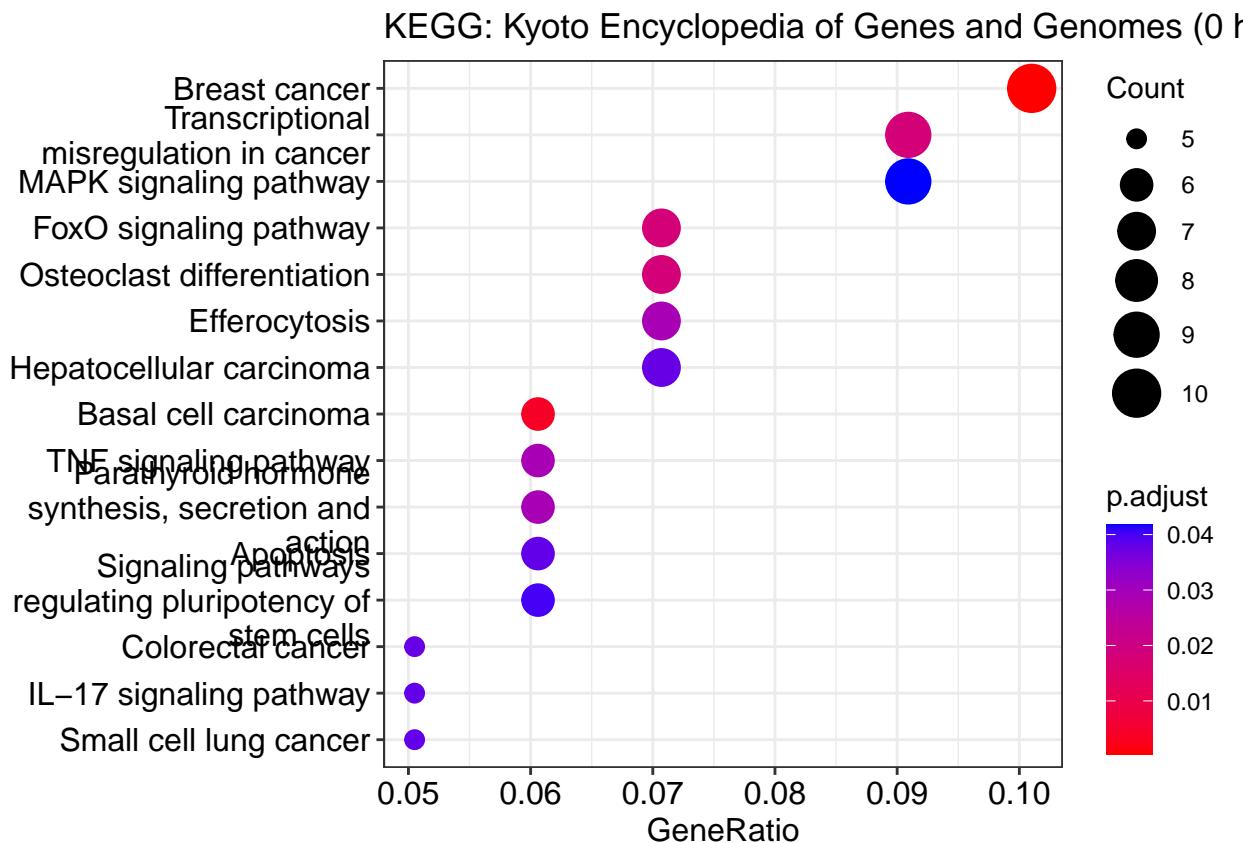
## save table
write.table(t_kegg_ctl_vs_0h, file = "t_kegg_ctl_vs_0h.txt", sep = "\t", row.names = FALSE)

## dotplot

```

```
options(enrichplot.colours = c("red","blue"))

dotplot(edox, size = "Count", color = "p.adjust", showCategory = 15, title = "KEGG: Kyoto Encyclopedia of Genes and Genomes (0 hour)"
```



```
# pathview
library("pathview")
pathways <- pathview(gene.data = gene_list,
                      pathway.id = c("mmu04657", "mmu04710"),
                      species = "mmu",
                      limit = list(gene=max(abs(gene_list)), cpd=1))

## 'select()' returned 1:1 mapping between keys and columns
## Info: Working in directory /home/vgsouza/VanessaPD/differential_expression
## Info: Writing image file mmu04657.pathview.png
## 'select()' returned 1:1 mapping between keys and columns
## Info: Working in directory /home/vgsouza/VanessaPD/differential_expression
## Info: Writing image file mmu04710.pathview.png

## Bar graph
barplot1 <- barplot(edox, showCategory=30) +
  ggtitle("KEGG: Kyoto Encyclopedia of Genes and Genomes (0 hour)") +
  theme(plot.title = element_text(hjust = 0.5, size = 15, face = "bold"))
```

```

### Control vs 2 hours ###

## import DEGs
DEGs <- read.delim("~/VanessaPD/differential_expression/DEGs_CTL_vs_2h_NOFC.txt")

## select only genes with FDR <0.05
significant_genes <- DEGs[DEGs$FDR < 0.05, ]

# For KEGG pathway enrichment using the gseKEGG() function, we need to convert id types. We can use the
# In the bitr function, the param fromType should be the same as keyType from the gseGO function above
# toType: in the bitr function has to be one of the available options from keyTypes(org.Dm.eg.db) and m

# Convert gene IDs for gseKEGG function
# We will lose some genes here because not all IDs will be converted
ids = bitr(significant_genes$symbol, fromType = "SYMBOL", toType = "ENTREZID", OrgDb = org.Mm.eg.db)

## 'select()' returned 1:1 mapping between keys and columns

## Warning in bitr(significant_genes$symbol, fromType = "SYMBOL", toType =
## "ENTREZID", : 1.32% of input gene IDs are fail to map...

# Create a new dataframe df2 which has the respective entrez IDs for the gene symbols.
colnames(ids) = c("symbol", "EntrezID")
df2 = merge(significant_genes, ids, by = "symbol")

# we want the log2 fold change
original_gene_list = df2$logFC

# name the vector
names(original_gene_list) <- df2$EntrezID

# omit any NA values
gene_list<-na.omit(original_gene_list)

# sort the list in decreasing order (required for clusterProfiler)
gene_list = sort(gene_list, decreasing = TRUE)

## KEGG

kegg_ctl_vs_2h <- enrichKEGG(
  names(gene_list),
  organism = "mmu",
  keyType = "ncbi-geneid",
  pvalueCutoff = 0.05,
  pAdjustMethod = "BH",
  minGSSize = 10,
  maxGSSize = 500,
  qvalueCutoff = 0.2,
  use_internal_data = FALSE
)

# table
t_kegg_ctl_vs_2h <- as.data.frame(kegg_ctl_vs_2h)

```

```

## convert gene ID to Symbol
edox <- setReadable(kegg_ctl_vs_2h, 'org.Mm.eg.db', 'ENTREZID')

# table
t_kegg_ctl_vs_2h <- as.data.frame(edox)

# Split gene IDs in t_kegg_ctl_vs_2h and create a data frame with separate rows for each gene ID
geneIDs_split <- strsplit(t_kegg_ctl_vs_2h$geneID, "/")
t_kegg_ctl_vs_2h_expanded <- data.frame(geneID = unlist(geneIDs_split), t_kegg_ctl_vs_2h[rep(seq_len(nr
# Merge the tables based on geneID
merged_data <- merge(t_kegg_ctl_vs_2h_expanded, df2, by.x = "geneID", by.y = "symbol", all.x = TRUE)

# Print the merged data
print(merged_data)

```

```

##      geneID      ID
## 1     Agtr1a mmu05171
## 2      Bag1 mmu04141
## 3      Bag2 mmu04141
## 4      Calr mmu04141
## 5      Dad1 mmu04141
## 6      Derl2 mmu04141
## 7     Dnaja1 mmu04141
## 8    Dnajb11 mmu04141
## 9    Dnajb2 mmu04141
## 10   Dnajc3 mmu04141
## 11      Fau mmu05171
## 12      Fau mmu03010
## 13     Fcgr3 mmu05171
## 14      Fos mmu05171
## 15   Herpud1 mmu04141
## 16  Hsp90ab1 mmu04141
## 17  Hsp90b1 mmu04141
## 18   Hspa1a mmu04141
## 19   Hspa5 mmu04141
## 20   Hspa8 mmu04141
## 21   Hspf1 mmu04141
## 22   Hyou1 mmu04141
## 23   Mrps6 mmu03010
## 24      Ostc mmu04141
## 25     P4hb mmu04141
## 26     Pdia3 mmu04141
## 27     Pdia4 mmu04141
## 28     Pdia6 mmu04141
## 29     Rpl10 mmu05171
## 30     Rpl10 mmu03010
## 31     Rpl11 mmu05171
## 32     Rpl11 mmu03010
## 33     Rpl13 mmu05171
## 34     Rpl13 mmu03010
## 35     Rpl18 mmu05171

```

```

## 36      Rpl18 mmu03010
## 37      Rpl19 mmu05171
## 38      Rpl19 mmu03010
## 39      Rpl26 mmu05171
## 40      Rpl26 mmu03010
## 41      Rpl36 mmu05171
## 42      Rpl36 mmu03010
## 43      Rpl36al mmu05171
## 44      Rpl36al mmu03010
## 45      Rpl6 mmu05171
## 46      Rpl6 mmu03010
## 47      Rpl7 mmu05171
## 48      Rpl7 mmu03010
## 49      Rpl8 mmu05171
## 50      Rpl8 mmu03010
## 51      Rplp0 mmu05171
## 52      Rplp0 mmu03010
## 53      Rplp2 mmu05171
## 54      Rplp2 mmu03010
## 55      Rpn1 mmu04141
## 56      Rps10 mmu05171
## 57      Rps10 mmu03010
## 58      Rps11 mmu03010
## 59      Rps11 mmu05171
## 60      Rps16 mmu05171
## 61      Rps16 mmu03010
## 62      Rps17 mmu05171
## 63      Rps17 mmu03010
## 64      Rps2 mmu05171
## 65      Rps2 mmu03010
## 66      Rps23 mmu05171
## 67      Rps23 mmu03010
## 68      Rps4x mmu05171
## 69      Rps4x mmu03010
## 70      Rps6 mmu05171
## 71      Rps6 mmu03010
## 72      Rps8 mmu05171
## 73      Rps8 mmu03010
## 74      Rps9 mmu03010
## 75      Rps9 mmu05171
## 76      Rpsa mmu05171
## 77      Rpsa mmu03010
## 78      Ube2g2 mmu04141
## 79      Ube2j2 mmu04141
## 80      Xbp1 mmu04141
##                                         Description
## 1      Coronavirus disease - COVID-19 - Mus musculus (house mouse)
## 2      Protein processing in endoplasmic reticulum - Mus musculus (house mouse)
## 3      Protein processing in endoplasmic reticulum - Mus musculus (house mouse)
## 4      Protein processing in endoplasmic reticulum - Mus musculus (house mouse)
## 5      Protein processing in endoplasmic reticulum - Mus musculus (house mouse)
## 6      Protein processing in endoplasmic reticulum - Mus musculus (house mouse)
## 7      Protein processing in endoplasmic reticulum - Mus musculus (house mouse)
## 8      Protein processing in endoplasmic reticulum - Mus musculus (house mouse)

```



```

## 63                   Ribosome - Mus musculus (house mouse)
## 64   Coronavirus disease - COVID-19 - Mus musculus (house mouse)
## 65                   Ribosome - Mus musculus (house mouse)
## 66   Coronavirus disease - COVID-19 - Mus musculus (house mouse)
## 67                   Ribosome - Mus musculus (house mouse)
## 68   Coronavirus disease - COVID-19 - Mus musculus (house mouse)
## 69                   Ribosome - Mus musculus (house mouse)
## 70   Coronavirus disease - COVID-19 - Mus musculus (house mouse)
## 71                   Ribosome - Mus musculus (house mouse)
## 72   Coronavirus disease - COVID-19 - Mus musculus (house mouse)
## 73                   Ribosome - Mus musculus (house mouse)
## 74                   Ribosome - Mus musculus (house mouse)
## 75   Coronavirus disease - COVID-19 - Mus musculus (house mouse)
## 76   Coronavirus disease - COVID-19 - Mus musculus (house mouse)
## 77                   Ribosome - Mus musculus (house mouse)
## 78 Protein processing in endoplasmic reticulum - Mus musculus (house mouse)
## 79 Protein processing in endoplasmic reticulum - Mus musculus (house mouse)
## 80 Protein processing in endoplasmic reticulum - Mus musculus (house mouse)
##   GeneRatio    BgRatio      pvalue     p.adjust      qvalue
## 1    28/321 334/9710 5.386959e-06 5.225350e-04 5.027828e-04
## 2    26/321 175/9710 9.871261e-11 2.872537e-08 2.763953e-08
## 3    26/321 175/9710 9.871261e-11 2.872537e-08 2.763953e-08
## 4    26/321 175/9710 9.871261e-11 2.872537e-08 2.763953e-08
## 5    26/321 175/9710 9.871261e-11 2.872537e-08 2.763953e-08
## 6    26/321 175/9710 9.871261e-11 2.872537e-08 2.763953e-08
## 7    26/321 175/9710 9.871261e-11 2.872537e-08 2.763953e-08
## 8    26/321 175/9710 9.871261e-11 2.872537e-08 2.763953e-08
## 9    26/321 175/9710 9.871261e-11 2.872537e-08 2.763953e-08
## 10   26/321 175/9710 9.871261e-11 2.872537e-08 2.763953e-08
## 11   28/321 334/9710 5.386959e-06 5.225350e-04 5.027828e-04
## 12   26/321 265/9710 6.362687e-07 9.257710e-05 8.907762e-05
## 13   28/321 334/9710 5.386959e-06 5.225350e-04 5.027828e-04
## 14   28/321 334/9710 5.386959e-06 5.225350e-04 5.027828e-04
## 15   26/321 175/9710 9.871261e-11 2.872537e-08 2.763953e-08
## 16   26/321 175/9710 9.871261e-11 2.872537e-08 2.763953e-08
## 17   26/321 175/9710 9.871261e-11 2.872537e-08 2.763953e-08
## 18   26/321 175/9710 9.871261e-11 2.872537e-08 2.763953e-08
## 19   26/321 175/9710 9.871261e-11 2.872537e-08 2.763953e-08
## 20   26/321 175/9710 9.871261e-11 2.872537e-08 2.763953e-08
## 21   26/321 175/9710 9.871261e-11 2.872537e-08 2.763953e-08
## 22   26/321 175/9710 9.871261e-11 2.872537e-08 2.763953e-08
## 23   26/321 265/9710 6.362687e-07 9.257710e-05 8.907762e-05
## 24   26/321 175/9710 9.871261e-11 2.872537e-08 2.763953e-08
## 25   26/321 175/9710 9.871261e-11 2.872537e-08 2.763953e-08
## 26   26/321 175/9710 9.871261e-11 2.872537e-08 2.763953e-08
## 27   26/321 175/9710 9.871261e-11 2.872537e-08 2.763953e-08
## 28   26/321 175/9710 9.871261e-11 2.872537e-08 2.763953e-08
## 29   28/321 334/9710 5.386959e-06 5.225350e-04 5.027828e-04
## 30   26/321 265/9710 6.362687e-07 9.257710e-05 8.907762e-05
## 31   28/321 334/9710 5.386959e-06 5.225350e-04 5.027828e-04
## 32   26/321 265/9710 6.362687e-07 9.257710e-05 8.907762e-05
## 33   28/321 334/9710 5.386959e-06 5.225350e-04 5.027828e-04
## 34   26/321 265/9710 6.362687e-07 9.257710e-05 8.907762e-05
## 35   28/321 334/9710 5.386959e-06 5.225350e-04 5.027828e-04

```

```

## 36 26/321 265/9710 6.362687e-07 9.257710e-05 8.907762e-05
## 37 28/321 334/9710 5.386959e-06 5.225350e-04 5.027828e-04
## 38 26/321 265/9710 6.362687e-07 9.257710e-05 8.907762e-05
## 39 28/321 334/9710 5.386959e-06 5.225350e-04 5.027828e-04
## 40 26/321 265/9710 6.362687e-07 9.257710e-05 8.907762e-05
## 41 28/321 334/9710 5.386959e-06 5.225350e-04 5.027828e-04
## 42 26/321 265/9710 6.362687e-07 9.257710e-05 8.907762e-05
## 43 28/321 334/9710 5.386959e-06 5.225350e-04 5.027828e-04
## 44 26/321 265/9710 6.362687e-07 9.257710e-05 8.907762e-05
## 45 28/321 334/9710 5.386959e-06 5.225350e-04 5.027828e-04
## 46 26/321 265/9710 6.362687e-07 9.257710e-05 8.907762e-05
## 47 28/321 334/9710 5.386959e-06 5.225350e-04 5.027828e-04
## 48 26/321 265/9710 6.362687e-07 9.257710e-05 8.907762e-05
## 49 28/321 334/9710 5.386959e-06 5.225350e-04 5.027828e-04
## 50 26/321 265/9710 6.362687e-07 9.257710e-05 8.907762e-05
## 51 28/321 334/9710 5.386959e-06 5.225350e-04 5.027828e-04
## 52 26/321 265/9710 6.362687e-07 9.257710e-05 8.907762e-05
## 53 28/321 334/9710 5.386959e-06 5.225350e-04 5.027828e-04
## 54 26/321 265/9710 6.362687e-07 9.257710e-05 8.907762e-05
## 55 26/321 175/9710 9.871261e-11 2.872537e-08 2.763953e-08
## 56 28/321 334/9710 5.386959e-06 5.225350e-04 5.027828e-04
## 57 26/321 265/9710 6.362687e-07 9.257710e-05 8.907762e-05
## 58 26/321 265/9710 6.362687e-07 9.257710e-05 8.907762e-05
## 59 28/321 334/9710 5.386959e-06 5.225350e-04 5.027828e-04
## 60 28/321 334/9710 5.386959e-06 5.225350e-04 5.027828e-04
## 61 26/321 265/9710 6.362687e-07 9.257710e-05 8.907762e-05
## 62 28/321 334/9710 5.386959e-06 5.225350e-04 5.027828e-04
## 63 26/321 265/9710 6.362687e-07 9.257710e-05 8.907762e-05
## 64 28/321 334/9710 5.386959e-06 5.225350e-04 5.027828e-04
## 65 26/321 265/9710 6.362687e-07 9.257710e-05 8.907762e-05
## 66 28/321 334/9710 5.386959e-06 5.225350e-04 5.027828e-04
## 67 26/321 265/9710 6.362687e-07 9.257710e-05 8.907762e-05
## 68 28/321 334/9710 5.386959e-06 5.225350e-04 5.027828e-04
## 69 26/321 265/9710 6.362687e-07 9.257710e-05 8.907762e-05
## 70 28/321 334/9710 5.386959e-06 5.225350e-04 5.027828e-04
## 71 26/321 265/9710 6.362687e-07 9.257710e-05 8.907762e-05
## 72 28/321 334/9710 5.386959e-06 5.225350e-04 5.027828e-04
## 73 26/321 265/9710 6.362687e-07 9.257710e-05 8.907762e-05
## 74 26/321 265/9710 6.362687e-07 9.257710e-05 8.907762e-05
## 75 28/321 334/9710 5.386959e-06 5.225350e-04 5.027828e-04
## 76 28/321 334/9710 5.386959e-06 5.225350e-04 5.027828e-04
## 77 26/321 265/9710 6.362687e-07 9.257710e-05 8.907762e-05
## 78 26/321 175/9710 9.871261e-11 2.872537e-08 2.763953e-08
## 79 26/321 175/9710 9.871261e-11 2.872537e-08 2.763953e-08
## 80 26/321 175/9710 9.871261e-11 2.872537e-08 2.763953e-08
##
## 1 Agtr1a/Fcgr3/Rps11/Rpl18/Rpl36al/Rpl6/Rpsa/Rplp2/Fau/Rps2/Rpl10/Rplp0/Rpl11/Rps4x/Rpl13/Rps9/1
## 2 Hspa1a/Pdia4/Hspa5/Xbp1/Pdia6/Hspf1/Hspa8/Dnajb11/Dnaja1/Calr/Herpud1/Dnajc3/Bag2/Ostc/Pdia3/Hsp90
## 3 Hspa1a/Pdia4/Hspa5/Xbp1/Pdia6/Hspf1/Hspa8/Dnajb11/Dnaja1/Calr/Herpud1/Dnajc3/Bag2/Ostc/Pdia3/Hsp90
## 4 Hspa1a/Pdia4/Hspa5/Xbp1/Pdia6/Hspf1/Hspa8/Dnajb11/Dnaja1/Calr/Herpud1/Dnajc3/Bag2/Ostc/Pdia3/Hsp90
## 5 Hspa1a/Pdia4/Hspa5/Xbp1/Pdia6/Hspf1/Hspa8/Dnajb11/Dnaja1/Calr/Herpud1/Dnajc3/Bag2/Ostc/Pdia3/Hsp90
## 6 Hspa1a/Pdia4/Hspa5/Xbp1/Pdia6/Hspf1/Hspa8/Dnajb11/Dnaja1/Calr/Herpud1/Dnajc3/Bag2/Ostc/Pdia3/Hsp90
## 7 Hspa1a/Pdia4/Hspa5/Xbp1/Pdia6/Hspf1/Hspa8/Dnajb11/Dnaja1/Calr/Herpud1/Dnajc3/Bag2/Ostc/Pdia3/Hsp90
## 8 Hspa1a/Pdia4/Hspa5/Xbp1/Pdia6/Hspf1/Hspa8/Dnajb11/Dnaja1/Calr/Herpud1/Dnajc3/Bag2/Ostc/Pdia3/Hsp90

```



```

## 63 Mrps6/Rps11/Rpl18/Rpl36al/Rpl6/Rpsa/Rplp2/Fau/Rps2/Rpl10/Rplp0/Rpl11/Rps4x/Rpl13/Rp
## 64 Agtr1a/Fcgr3/Rps11/Rpl18/Rpl36al/Rpl6/Rpsa/Rplp2/Fau/Rps2/Rpl10/Rplp0/Rpl11/Rps4x/Rpl13/Rp
## 65 Mrps6/Rps11/Rpl18/Rpl36al/Rpl6/Rpsa/Rplp2/Fau/Rps2/Rpl10/Rplp0/Rpl11/Rps4x/Rpl13/Rp
## 66 Agtr1a/Fcgr3/Rps11/Rpl18/Rpl36al/Rpl6/Rpsa/Rplp2/Fau/Rps2/Rpl10/Rplp0/Rpl11/Rps4x/Rpl13/Rp
## 67 Mrps6/Rps11/Rpl18/Rpl36al/Rpl6/Rpsa/Rplp2/Fau/Rps2/Rpl10/Rplp0/Rpl11/Rps4x/Rpl13/Rp
## 68 Agtr1a/Fcgr3/Rps11/Rpl18/Rpl36al/Rpl6/Rpsa/Rplp2/Fau/Rps2/Rpl10/Rplp0/Rpl11/Rps4x/Rpl13/Rp
## 69 Mrps6/Rps11/Rpl18/Rpl36al/Rpl6/Rpsa/Rplp2/Fau/Rps2/Rpl10/Rplp0/Rpl11/Rps4x/Rpl13/Rp
## 70 Agtr1a/Fcgr3/Rps11/Rpl18/Rpl36al/Rpl6/Rpsa/Rplp2/Fau/Rps2/Rpl10/Rplp0/Rpl11/Rps4x/Rpl13/Rp
## 71 Mrps6/Rps11/Rpl18/Rpl36al/Rpl6/Rpsa/Rplp2/Fau/Rps2/Rpl10/Rplp0/Rpl11/Rps4x/Rpl13/Rp
## 72 Agtr1a/Fcgr3/Rps11/Rpl18/Rpl36al/Rpl6/Rpsa/Rplp2/Fau/Rps2/Rpl10/Rplp0/Rpl11/Rps4x/Rpl13/Rp
## 73 Mrps6/Rps11/Rpl18/Rpl36al/Rpl6/Rpsa/Rplp2/Fau/Rps2/Rpl10/Rplp0/Rpl11/Rps4x/Rpl13/Rp
## 74 Mrps6/Rps11/Rpl18/Rpl36al/Rpl6/Rpsa/Rplp2/Fau/Rps2/Rpl10/Rplp0/Rpl11/Rps4x/Rpl13/Rp
## 75 Agtr1a/Fcgr3/Rps11/Rpl18/Rpl36al/Rpl6/Rpsa/Rplp2/Fau/Rps2/Rpl10/Rplp0/Rpl11/Rps4x/Rpl13/Rp
## 76 Agtr1a/Fcgr3/Rps11/Rpl18/Rpl36al/Rpl6/Rpsa/Rplp2/Fau/Rps2/Rpl10/Rplp0/Rpl11/Rps4x/Rpl13/Rp
## 77 Mrps6/Rps11/Rpl18/Rpl36al/Rpl6/Rpsa/Rplp2/Fau/Rps2/Rpl10/Rplp0/Rpl11/Rps4x/Rpl13/Rp
## 78 Hspa1a/Pdia4/Hspa5/Xbp1/Pdia6/Hsph1/Hspa8/Dnajb11/Dnaja1/Calr/Herpud1/Dnajc3/Bag2/Ostc/Pdia3/Hsp9
## 79 Hspa1a/Pdia4/Hspa5/Xbp1/Pdia6/Hsph1/Hspa8/Dnajb11/Dnaja1/Calr/Herpud1/Dnajc3/Bag2/Ostc/Pdia3/Hsp9
## 80 Hspa1a/Pdia4/Hspa5/Xbp1/Pdia6/Hsph1/Hspa8/Dnajb11/Dnaja1/Calr/Herpud1/Dnajc3/Bag2/Ostc/Pdia3/Hsp9
## Count logFC PValue FDR expression EntrezID
## 1 28 0.7845016 8.027053e-04 2.585777e-02 Up 11607
## 2 26 0.2473965 1.787819e-03 4.246733e-02 Up 12017
## 3 26 0.3091288 2.164371e-03 4.766957e-02 Up 213539
## 4 26 0.3819458 1.201620e-07 2.498434e-05 Up 12317
## 5 26 0.2350228 1.387358e-03 3.589882e-02 Up 13135
## 6 26 0.2844698 3.271745e-04 1.431165e-02 Up 116891
## 7 26 0.3824010 1.310611e-08 3.556420e-06 Up 15502
## 8 26 0.3932245 8.643920e-07 1.397870e-04 Up 67838
## 9 26 0.2402186 8.477005e-04 2.633165e-02 Up 56812
## 10 26 0.3560682 9.034597e-05 6.052046e-03 Up 100037258
## 11 28 0.3401152 1.864800e-04 9.985101e-03 Up 14109
## 12 26 0.3401152 1.864800e-04 9.985101e-03 Up 14109
## 13 28 0.6479998 7.821987e-06 8.196950e-04 Up 14131
## 14 28 -0.9245320 1.183020e-08 3.322833e-06 Down 14281
## 15 26 0.3660177 9.794209e-08 2.090737e-05 Up 64209
## 16 26 0.2356542 7.890200e-04 2.556906e-02 Up 15516
## 17 26 0.2852255 1.633665e-05 1.507427e-03 Up 22027
## 18 26 0.9801778 1.332812e-03 3.486653e-02 Up 193740
## 19 26 0.5932352 2.072199e-14 2.211727e-11 Up 14828
## 20 26 0.4192602 7.033597e-08 1.631998e-05 Up 15481
## 21 26 0.4844493 4.570364e-08 1.092112e-05 Up 15505
## 22 26 0.2340986 2.180818e-03 4.766957e-02 Up 12282
## 23 26 0.5464337 1.494227e-11 1.040112e-08 Up 121022
## 24 26 0.3006991 9.797144e-04 2.880983e-02 Up 66357
## 25 26 0.2219667 1.486046e-03 3.760419e-02 Up 18453
## 26 26 0.2895053 7.360801e-05 5.237619e-03 Up 14827
## 27 26 0.6509220 1.203844e-13 1.070752e-10 Up 12304
## 28 26 0.4919749 2.578986e-11 1.720399e-08 Up 71853
## 29 28 0.3248197 8.776656e-04 2.686697e-02 Up 110954
## 30 26 0.3248197 8.776656e-04 2.686697e-02 Up 110954
## 31 28 0.3087243 1.800450e-04 9.793881e-03 Up 67025
## 32 26 0.3087243 1.800450e-04 9.793881e-03 Up 67025
## 33 28 0.2932904 6.469674e-04 2.253346e-02 Up 270106
## 34 26 0.2932904 6.469674e-04 2.253346e-02 Up 270106
## 35 28 0.4252255 5.714709e-06 6.488829e-04 Up 19899

```

```

## 36   26  0.4252255 5.714709e-06 6.488829e-04      Up   19899
## 37   28  0.2812599 6.288711e-04 2.212797e-02      Up   19921
## 38   26  0.2812599 6.288711e-04 2.212797e-02      Up   19921
## 39   28  0.2822797 1.306150e-03 3.439385e-02      Up   19941
## 40   26  0.2822797 1.306150e-03 3.439385e-02      Up   19941
## 41   28  0.2546418 1.558022e-03 3.867276e-02      Up   54217
## 42   26  0.2546418 1.558022e-03 3.867276e-02      Up   54217
## 43   28  0.3694681 1.193964e-04 7.295941e-03      Up   66483
## 44   26  0.3694681 1.193964e-04 7.295941e-03      Up   66483
## 45   28  0.3633828 2.291934e-04 1.146683e-02      Up   19988
## 46   26  0.3633828 2.291934e-04 1.146683e-02      Up   19988
## 47   28  0.2805197 1.058833e-03 2.995037e-02      Up   19989
## 48   26  0.2805197 1.058833e-03 2.995037e-02      Up   19989
## 49   28  0.2678023 1.313123e-03 3.452070e-02      Up   26961
## 50   26  0.2678023 1.313123e-03 3.452070e-02      Up   26961
## 51   28  0.3095686 2.168614e-04 1.112805e-02      Up   11837
## 52   26  0.3095686 2.168614e-04 1.112805e-02      Up   11837
## 53   28  0.3423227 4.964596e-05 3.784913e-03      Up   67186
## 54   26  0.3423227 4.964596e-05 3.784913e-03      Up   67186
## 55   26  0.2771166 6.180168e-06 6.919195e-04      Up   103963
## 56   28  0.2824222 1.590421e-03 3.929420e-02      Up   67097
## 57   26  0.2824222 1.590421e-03 3.929420e-02      Up   67097
## 58   26  0.4725363 1.181431e-04 7.247015e-03      Up   27207
## 59   28  0.4725363 1.181431e-04 7.247015e-03      Up   27207
## 60   28  0.2921704 4.027714e-04 1.666236e-02      Up   20055
## 61   26  0.2921704 4.027714e-04 1.666236e-02      Up   20055
## 62   28  0.2598969 1.184244e-03 3.202661e-02      Up   20068
## 63   26  0.2598969 1.184244e-03 3.202661e-02      Up   20068
## 64   28  0.3393056 1.298746e-03 3.425522e-02      Up   16898
## 65   26  0.3393056 1.298746e-03 3.425522e-02      Up   16898
## 66   28  0.2656852 1.909585e-03 4.398915e-02      Up   66475
## 67   26  0.2656852 1.909585e-03 4.398915e-02      Up   66475
## 68   28  0.3020061 1.022554e-03 2.938023e-02      Up   20102
## 69   26  0.3020061 1.022554e-03 2.938023e-02      Up   20102
## 70   28  0.2700900 7.712908e-04 2.520075e-02      Up   20104
## 71   26  0.2700900 7.712908e-04 2.520075e-02      Up   20104
## 72   28  0.2724532 1.077145e-03 3.015565e-02      Up   20116
## 73   26  0.2724532 1.077145e-03 3.015565e-02      Up   20116
## 74   26  0.2931234 2.198740e-04 1.124659e-02      Up   76846
## 75   28  0.2931234 2.198740e-04 1.124659e-02      Up   76846
## 76   28  0.3576437 5.274482e-04 1.970762e-02      Up   16785
## 77   26  0.3576437 5.274482e-04 1.970762e-02      Up   16785
## 78   26  0.2684887 2.564943e-04 1.233175e-02      Up   22213
## 79   26  0.2762319 1.339842e-03 3.499327e-02      Up   140499
## 80   26  0.5124732 9.776675e-15 1.118033e-11      Up   22433

```

```

## remove (mus musculos name)
edox@result$Description <- gsub(pattern = " - Mus musculus (house mouse)", replacement = "", edox@resul-
```

```

## table
t_kegg_ctl_vs_2h <- as.data.frame(edox)
```

```

## plot
```

```

cnetplot1 <- cnetplot(edox, color.params = list(foldChange = gene_list), circular = F, colorEdge = TRUE
                      showCategory = c("Protein processing in endoplasmic reticulum",
                                      "Ribosome")) +
  scale_colour_gradient2(name = "fold change", low = "darkblue", mid = "white", high = "red") +
  theme(
    plot.title = element_text(size = 12, face = "bold", hjust = 0.5),
    plot.subtitle = element_text(size = 10, hjust = 0.5)) +
  labs(
    title = "KEGG: Kyoto Encyclopedia of Genes and Genomes\nCategory: Genetic Information Processing",
    subtitle = NULL
  )

## Warning in cnetplot.enrichResult(x, ...): Use 'color.params = list(edge = your_value)' instead of 'co
##   The colorEdge parameter will be removed in the next version.

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

cnetplot2 <- cnetplot(edox, color.params = list(foldChange = gene_list), circular = F, colorEdge = TRUE
                      showCategory = c("Coronavirus disease - COVID-19")) +
  scale_colour_gradient2(name = "fold change", low = "darkblue", mid = "white", high = "red") +
  theme(
    plot.title = element_text(size = 12, face = "bold", hjust = 0.5),
    plot.subtitle = element_text(size = 10, hjust = 0.5)) +
  labs(
    title = "KEGG: Kyoto Encyclopedia of Genes and Genomes\nCategory: Human Diseases",
    subtitle = NULL
  )

## Warning in cnetplot.enrichResult(x, ...): Use 'color.params = list(edge = your_value)' instead of 'co
##   The colorEdge parameter will be removed in the next version.

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

## put all of the together
KEGG_CTL_vs_2h_graph<- plot_grid(cnetplot1, cnetplot2, ncol = 2, labels = c("A", "B"))

## save image
tiff('KEGG_CTL_vs_2h_graph.tiff', units="in", width=15, height=12, res=600)
KEGG_CTL_vs_2h_graph
dev.off()

## pdf
## 2

```

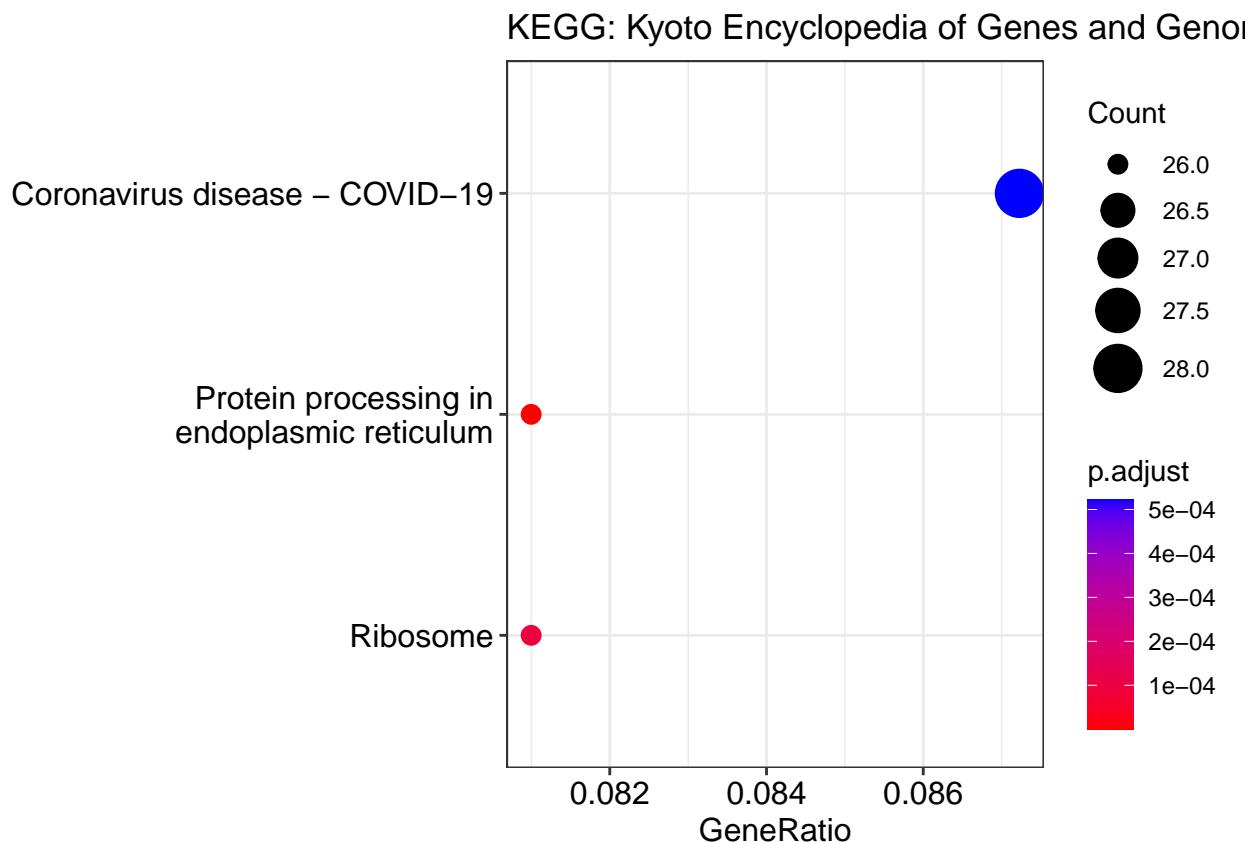
```

## save table
write.table(t_kegg_ctl_vs_2h, file = "t_kegg_ctl_vs_2h.txt", sep = "\t", row.names = FALSE)

## dotplot
options(enrichplot.colours = c("red","blue"))

dotplot(edox, size = "Count", color = "p.adjust", showCategory = 15, title = "KEGG: Kyoto Encyclopedia of Genes and Genomes")

```



```

# pathview
library("pathview")
pathways <- pathview(gene.data = gene_list,
                      pathway.id = c("mmu04657", "mmu04710"),
                      species = "mmu",
                      limit = list(gene=max(abs(gene_list)), cpd=1))

```

```

## 'select()' returned 1:1 mapping between keys and columns
## Info: Working in directory /home/vgsouza/VanessaPD/differential_expression
## Info: Writing image file mmu04657.pathview.png
## 'select()' returned 1:1 mapping between keys and columns
## Info: Working in directory /home/vgsouza/VanessaPD/differential_expression
## Info: Writing image file mmu04710.pathview.png

```

```
### Control vs 4 hours ###
```

```

## import DEGs
DEGs <- read.delim("~/VanessaPD/differential_expression/DEGs_CTL_vs_4h_NOFC.txt")

## select only genes with FDR < 0.05
significant_genes <- DEGs[DEGs$FDR < 0.05, ]

# For KEGG pathway enrichment using the gseKEGG() function, we need to convert id types. We can use the
# In the bitr function, the param fromType should be the same as keyType from the gseGO function above
# toType: in the bitr function has to be one of the available options from keyTypes(org.Dm.eg.db) and m

# Convert gene IDs for gseKEGG function
# We will lose some genes here because not all IDs will be converted
ids = bitr(significant_genes$symbol, fromType = "SYMBOL", toType = "ENTREZID", OrgDb = org.Mm.eg.db)

## 'select()' returned 1:1 mapping between keys and columns

## Warning in bitr(significant_genes$symbol, fromType = "SYMBOL", toType =
## "ENTREZID", : 1.56% of input gene IDs are fail to map...

# Create a new dataframe df2 which has the respective entrez IDs for the gene symbols.
colnames(ids) = c("symbol", "EntrezID")
df2 = merge(significant_genes, ids, by = "symbol")

# we want the log2 fold change
original_gene_list = df2$logFC

# name the vector
names(original_gene_list) <- df2$EntrezID

# omit any NA values
gene_list<-na.omit(original_gene_list)

# sort the list in decreasing order (required for clusterProfiler)
gene_list = sort(gene_list, decreasing = TRUE)

## KEGG

kegg_ctl_vs_4h <- enrichKEGG(
  names(gene_list),
  organism = "mmu",
  keyType = "ncbi-geneid",
  pvalueCutoff = 1,
  pAdjustMethod = "BH",
  minGSSize = 10,
  maxGSSize = 500,
  qvalueCutoff = 0.2,
  use_internal_data = FALSE
)

# table
t_kegg_ctl_vs_4h <- as.data.frame(kegg_ctl_vs_4h)

## convert gene ID to Symbol

```

```

edox <- setReadable(kegg_ctl_vs_4h, 'org.Mm.eg.db', 'ENTREZID')

# table
t_kegg_ctl_vs_4h <- as.data.frame(edox)

# Split gene IDs in t_kegg_ctl_vs_0h and create a data frame with separate rows for each gene ID
geneIDs_split <- strsplit(t_kegg_ctl_vs_4h$geneID, "/")
t_kegg_ctl_vs_4h_expanded <- data.frame(geneID = unlist(geneIDs_split), t_kegg_ctl_vs_4h[rep(seq_len(nr
    # Merge the tables based on geneID
merged_data <- merge(t_kegg_ctl_vs_4h_expanded, df2, by.x = "geneID", by.y = "symbol", all.x = TRUE)

# Print the merged data
print(merged_data)

##      geneID          ID
## 1  Baiap2 mmu05135
## 2  Camk1g mmu04925
## 3    Egr1 mmu04928
## 4    Egr1 mmu04933
## 5    Ezr mmu05205
## 6    Fn1 mmu05205
## 7    Fn1 mmu04933
## 8    Fn1 mmu05135
## 9    Fos mmu04928
## 10   Fos mmu05135
## 11   Glul mmu00250
## 12   Glul mmu00220
## 13   Gpt2 mmu00220
## 14   Gpt2 mmu00250
## 15   Hmgcr mmu04976
## 16   Hmgcr mmu00900
## 17   Ldlr mmu04976
## 18   Ldlr mmu04925
## 19   Mmp14 mmu04928
## 20     Mvd mmu00900
## 21   Nr4a1 mmu04925
## 22   Plce1 mmu04933
## 23   Plce1 mmu05205
## 24   Sdc4 mmu05205
## 25  Slc2a1 mmu04976
##                                         Description
## 1                               Yersinia infection - Mus musculus (house mouse)
## 2           Aldosterone synthesis and secretion - Mus musculus (house mouse)
## 3 Parathyroid hormone synthesis, secretion and action - Mus musculus (house mouse)
## 4 AGE-RAGE signaling pathway in diabetic complications - Mus musculus (house mouse)
## 5             Proteoglycans in cancer - Mus musculus (house mouse)
## 6             Proteoglycans in cancer - Mus musculus (house mouse)
## 7 AGE-RAGE signaling pathway in diabetic complications - Mus musculus (house mouse)
## 8                               Yersinia infection - Mus musculus (house mouse)
## 9 Parathyroid hormone synthesis, secretion and action - Mus musculus (house mouse)
## 10                          Yersinia infection - Mus musculus (house mouse)

```

```

## 11 Alanine, aspartate and glutamate metabolism - Mus musculus (house mouse)
## 12 Arginine biosynthesis - Mus musculus (house mouse)
## 13 Arginine biosynthesis - Mus musculus (house mouse)
## 14 Alanine, aspartate and glutamate metabolism - Mus musculus (house mouse)
## 15 Bile secretion - Mus musculus (house mouse)
## 16 Terpenoid backbone biosynthesis - Mus musculus (house mouse)
## 17 Bile secretion - Mus musculus (house mouse)
## 18 Aldosterone synthesis and secretion - Mus musculus (house mouse)
## 19 Parathyroid hormone synthesis, secretion and action - Mus musculus (house mouse)
## 20 Terpenoid backbone biosynthesis - Mus musculus (house mouse)
## 21 Aldosterone synthesis and secretion - Mus musculus (house mouse)
## 22 AGE-RAGE signaling pathway in diabetic complications - Mus musculus (house mouse)
## 23 Proteoglycans in cancer - Mus musculus (house mouse)
## 24 Proteoglycans in cancer - Mus musculus (house mouse)
## 25 Bile secretion - Mus musculus (house mouse)

##   GeneRatio BgRatio      pvalue    p.adjust     qvalue      geneID.1 Count
## 1    3/37 136/9710 0.014755253 0.1983762 0.1794791 Fn1/Baiap2/Fos 3
## 2    3/37 103/9710 0.006933882 0.1453060 0.1314643 Camk1g/Ldlr/Nr4a1 3
## 3    3/37 116/9710 0.009607005 0.1453060 0.1314643 Egr1/Mmp14/Fos 3
## 4    3/37 101/9710 0.006568046 0.1453060 0.1314643 Fn1/Plce1/Egr1 3
## 5    4/37 204/9710 0.007263582 0.1453060 0.1314643 Ezr/Sdc4/Fn1/Plce1 4
## 6    4/37 204/9710 0.007263582 0.1453060 0.1314643 Ezr/Sdc4/Fn1/Plce1 4
## 7    3/37 101/9710 0.006568046 0.1453060 0.1314643 Fn1/Plce1/Egr1 3
## 8    3/37 136/9710 0.014755253 0.1983762 0.1794791 Fn1/Baiap2/Fos 3
## 9    3/37 116/9710 0.009607005 0.1453060 0.1314643 Egr1/Mmp14/Fos 3
## 10   3/37 136/9710 0.014755253 0.1983762 0.1794791 Fn1/Baiap2/Fos 3
## 11   2/37 39/9710 0.009581168 0.1453060 0.1314643 Gpt2/Glul 2
## 12   2/37 20/9710 0.002570920 0.1453060 0.1314643 Gpt2/Glul 2
## 13   2/37 20/9710 0.002570920 0.1453060 0.1314643 Gpt2/Glul 2
## 14   2/37 39/9710 0.009581168 0.1453060 0.1314643 Gpt2/Glul 2
## 15   3/37 101/9710 0.006568046 0.1453060 0.1314643 Slc2a1/Hmgcr/Ldlr 3
## 16   2/37 23/9710 0.003398863 0.1453060 0.1314643 Hmgcr/Mvd 2
## 17   3/37 101/9710 0.006568046 0.1453060 0.1314643 Slc2a1/Hmgcr/Ldlr 3
## 18   3/37 103/9710 0.006933882 0.1453060 0.1314643 Camk1g/Ldlr/Nr4a1 3
## 19   3/37 116/9710 0.009607005 0.1453060 0.1314643 Egr1/Mmp14/Fos 3
## 20   2/37 23/9710 0.003398863 0.1453060 0.1314643 Hmgcr/Mvd 2
## 21   3/37 103/9710 0.006933882 0.1453060 0.1314643 Camk1g/Ldlr/Nr4a1 3
## 22   3/37 101/9710 0.006568046 0.1453060 0.1314643 Fn1/Plce1/Egr1 3
## 23   4/37 204/9710 0.007263582 0.1453060 0.1314643 Ezr/Sdc4/Fn1/Plce1 4
## 24   4/37 204/9710 0.007263582 0.1453060 0.1314643 Ezr/Sdc4/Fn1/Plce1 4
## 25   3/37 101/9710 0.006568046 0.1453060 0.1314643 Slc2a1/Hmgcr/Ldlr 3

##   logFC      PValue      FDR expression EntrezID
## 1  0.3916015 1.544338e-05 6.228700e-03 Up 108100
## 2  0.3789170 5.733301e-05 1.849907e-02 Up 215303
## 3 -0.7164387 6.162919e-08 6.214149e-05 Down 13653
## 4 -0.7164387 6.162919e-08 6.214149e-05 Down 13653
## 5  0.5970915 1.933896e-07 1.733308e-04 Up 22350
## 6  0.4417829 5.767331e-06 2.990423e-03 Up 14268
## 7  0.4417829 5.767331e-06 2.990423e-03 Up 14268
## 8  0.4417829 5.767331e-06 2.990423e-03 Up 14268
## 9 -1.0782993 7.570015e-14 2.442541e-10 Down 14281
## 10 -1.0782993 7.570015e-14 2.442541e-10 Down 14281
## 11  0.3846019 8.123816e-05 2.472859e-02 Up 14645
## 12  0.3846019 8.123816e-05 2.472859e-02 Up 14645

```

```

## 13 0.3864174 2.342966e-06 1.574961e-03 Up 108682
## 14 0.3864174 2.342966e-06 1.574961e-03 Up 108682
## 15 -0.3452072 1.419332e-04 3.753784e-02 Down 15357
## 16 -0.3452072 1.419332e-04 3.753784e-02 Down 15357
## 17 -0.4424429 1.168819e-06 8.571164e-04 Down 16835
## 18 -0.4424429 1.168819e-06 8.571164e-04 Down 16835
## 19 -0.7764059 7.462748e-14 2.442541e-10 Down 17387
## 20 -0.6396134 6.348202e-06 3.103501e-03 Down 192156
## 21 -0.7124929 2.467928e-05 8.847795e-03 Down 15370
## 22 0.4042226 1.280509e-04 3.443076e-02 Up 74055
## 23 0.4042226 1.280509e-04 3.443076e-02 Up 74055
## 24 0.4732810 2.656390e-06 1.714221e-03 Up 20971
## 25 0.5030374 9.825681e-07 7.548462e-04 Up 20525

## remove (mus musculos name)
edox$result$Description <- gsub(pattern = " - Mus musculus (house mouse)", replacement = "", edox$result)

## table
t_kegg_ctl_vs_4h <- as.data.frame(edox)

## plot
cnetplot1 <- cnetplot(edox, color.params = list(foldChange = gene_list), circular = F, colorEdge = TRUE,
                      showCategory = c("AGE-RAGE signaling pathway in diabetic complications",
                                      "Proteoglycans in cancer",
                                      "Yersinia infection")) +
  scale_colour_gradient2(name = "fold change", low = "darkblue", mid = "white", high = "red") +
  theme(
    plot.title = element_text(size = 12, face = "bold", hjust = 0.5),
    plot.subtitle = element_text(size = 10, hjust = 0.5)) +
  labs(
    title = "KEGG: Kyoto Encyclopedia of Genes and Genomes\nCategory: Human Diseases",
    subtitle = NULL
  )

## Warning in cnetplot.enrichResult(x, ...): Use 'color.params = list(edge = your_value)' instead of 'co
## The colorEdge parameter will be removed in the next version.

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

cnetplot2 <- cnetplot(edox, color.params = list(foldChange = gene_list), circular = F, colorEdge = TRUE,
                      showCategory = c("Arginine biosynthesis",
                                      "Terpenoid backbone biosynthesis",
                                      "Alanine, aspartate and glutamate metabolism")) +
  scale_colour_gradient2(name = "fold change", low = "darkblue", mid = "white", high = "red") +
  theme(
    plot.title = element_text(size = 12, face = "bold", hjust = 0.5),
    plot.subtitle = element_text(size = 10, hjust = 0.5)) +
  labs(
    title = "KEGG: Kyoto Encyclopedia of Genes and Genomes\nCategory: Metabolism",

```

```

    subtitle = NULL
)

## Warning in cnetplot.enrichResult(x, ...): Use 'color.params = list(edge = your_value)' instead of 'co
##   The colorEdge parameter will be removed in the next version.

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

cnetplot3 <- cnetplot(edox, color.params = list(foldChange = gene_list), circular = F, colorEdge = TRUE
                      showCategory = c("Bile secretion",
                                      "Aldosterone synthesis and secretion",
                                      "Parathyroid hormone synthesis, secretion and action")) +
  scale_colour_gradient2(name = "fold change", low = "darkblue", mid = "white", high = "red") +
  theme(
    plot.title = element_text(size = 12, face = "bold", hjust = 0.5),
    plot.subtitle = element_text(size = 10, hjust = 0.5)) +
  labs(
    title = "KEGG: Kyoto Encyclopedia of Genes and Genomes\nCategory: Organismal Systems",
    subtitle = NULL
  )

## Warning in cnetplot.enrichResult(x, ...): Use 'color.params = list(edge = your_value)' instead of 'co
##   The colorEdge parameter will be removed in the next version.

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

## put all of the together
KEGG_CTL_vs_4h_graph<- plot_grid(cnetplot1, cnetplot2, cnetplot3, ncol = 2, nrow = 2, labels = c("A", "B"))

## save image
tiff('KEGG_CTL_vs_4h_graph.tiff', units="in", width=17, height=17, res=600)
KEGG_CTL_vs_4h_graph
dev.off()

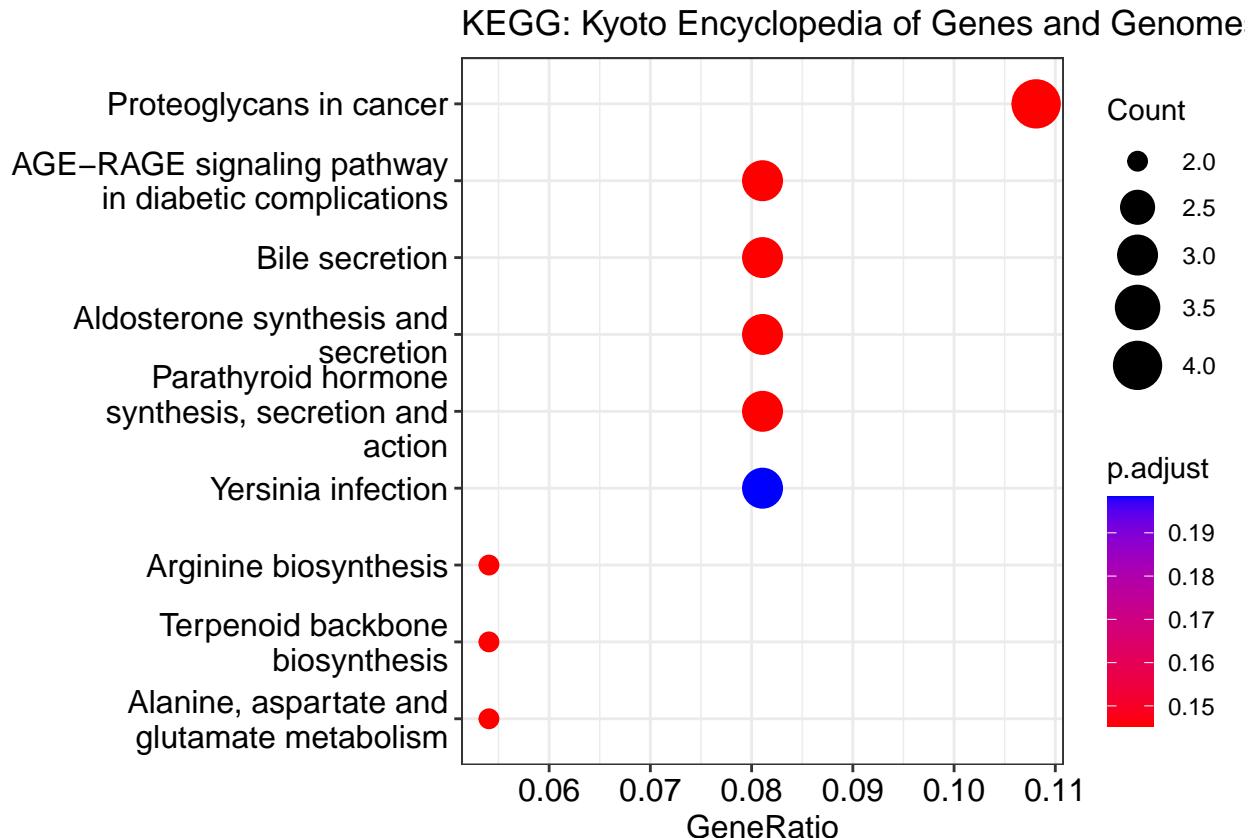
## pdf
## 2

## save table
write.table(t_kegg_ctl_vs_4h, file = "t_kegg_ctl_vs_4h.txt", sep = "\t", row.names = FALSE)

## dotplot
options(enrichplot.colours = c("red","blue"))

dotplot(edox, size = "Count", color = "p.adjust", showCategory = 15, title = "KEGG: Kyoto Encyclopedia o

```



```
# pathview
library("pathview")
pathways <- pathview(gene.data = gene_list,
                      pathway.id = c("mmu04657", "mmu04710"),
                      species = "mmu",
                      limit = list(gene=max(abs(gene_list)), cpd=1))

## 'select()' returned 1:1 mapping between keys and columns
## Info: Working in directory /home/vgsouza/VanessaPD/differential_expression
## Info: Writing image file mmu04657.pathview.png
## Warning: None of the genes or compounds mapped to the pathway!
## Argument gene.idtype or cpd.idtype may be wrong.
## 'select()' returned 1:1 mapping between keys and columns
## Info: Working in directory /home/vgsouza/VanessaPD/differential_expression
## Info: Writing image file mmu04710.pathview.png
```