

# DEseq2 Analysis

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## Install required packages

```
if (!requireNamespace("BiocManager", quietly = TRUE))
  install.packages("BiocManager")
BiocManager::install("DESeq2")
install.packages("pheatmap")
install.packages("RColorBrewer")
install.packages("dplyr")
install.packages("ggplot2")
BiocManager::install("genefilter")
BiocManager::install("ashr")
BiocManager::install("VennDetail")
install.packages("tidyverse")
install.packages("kableExtra")
```

## Load required packages

```
library(DESeq2)
library(RColorBrewer)
library(dplyr)
library(ggplot2)
library(genefilter)
library(ashr)
library(VennDetail)
library(tidyverse)
library(kableExtra)
library(pheatmap)
library(ComplexHeatmap)
library(circlize)
```

## Count Matrix Construction

```

pheno.data <- read.csv("pheno_data.csv")
gene.count.matrix <- read.table(file = "gene_count_matrix.csv", header = T,
                                sep = ",", row.names = NULL)
head(gene.count.matrix)

##          gene_id sample_01 sample_02 sample_03 sample_04 sample_05
## 1      Cre14.g619950.v5.5      115      127      93      162      167
## 2      Cre01.g048900.v5.5       0       0      12      10      42
## 3 Cre12.g543400.v5.5|FDH1     131      110      114      118      175
## 4      Cre16.g685837.v5.5      63       56      53      29      109
## 5      Cre02.g119526.v5.5       0       0       0       0       0
## 6      Cre06.g278159.v5.5     2707     2417     2316     2354     3916
##   sample_06 sample_07 sample_08 sample_09 sample_10 sample_11 sample_12
## 1      127      128      188      134       66      81      88
## 2      36       28       9       33       17      28      22
## 3     138      180      170       62      92      70      76
## 4     108      89      115       55      68      51      45
## 5       0       0       0       0       0       0       0
## 6    3663     3556     3537     1918     1959     1914     1826
##   sample_13 sample_14 sample_15 sample_16 sample_17 sample_18 sample_19
## 1      21       9      24       22       67      73      59
## 2       0      11      10       0      60      47      56
## 3      44      12      47       34      197     169     175
## 4      27      35      20       19      94     117     104
## 5       0       0       0       0       0       0       0
## 6    737      807      649      688     3347     3055     2937
##   sample_20 sample_21 sample_22 sample_23 sample_24 sample_25 sample_26
## 1      69      76      73       52      51     143      99
## 2      39      22      15       24       0      57      50
## 3     167     175     233     223     211      52      66
## 4      89      45      56       50      36      83      65
## 5       0       0       0       0       0       0       0
## 6   3173     2702     2480     2466     2429     2193     2079
##   sample_27 sample_28 sample_29 sample_30 sample_31 sample_32 sample_33
## 1     136      93     143     122     122     158     166
## 2      54      43       0       0       0       0      28
## 3      77      58      96     131      98     121     170
## 4      56      47     107      78     110      78     107
## 5       0       0       0       0       0       0       0
## 6   2104     1966     4211     4262     4106     3964     3586
##   sample_34 sample_35 sample_36 sample_37 sample_38 sample_39 sample_40
## 1     175     145     105      48      46      44      49
## 2      17      11      21       0       7       0       0
## 3     146     186      81      87      61      63      71
## 4     97     114     103      30      65      59      31
## 5       0       0       0       0       0       0       0
## 6   3233     3417     3375     1573     1519     1486     1466
##   sample_41 sample_42 sample_43 sample_44 sample_45 sample_46 sample_47
## 1     111      81      61      63       0       0       0
## 2      72      63      31      77       0       0       0
## 3     205     167     158     151      91     106      82
## 4     109      61      61      88      62      61      63
## 5       0       0       0       0       0       0       0

```

```

## 6      4477     4189     4127     4133     2854     2824     2724
##   sample_48
## 1      5
## 2      0
## 3    101
## 4    58
## 5      0
## 6   2738

gene.ids <- sapply(X = strsplit(x = gene.count.matrix$gene_id, split = "\\|"),
                     FUN = function(x){return(x[1])})
gene.count.matrix <- gene.count.matrix[,-1]

rownames(gene.count.matrix) = make.names(gene.ids, unique=TRUE)
head(gene.count.matrix)

##          sample_01 sample_02 sample_03 sample_04 sample_05 sample_06
## Cre14.g619950.v5.5     115     127      93     162     167     127
## Cre01.g048900.v5.5      0       0      12      10      42      36
## Cre12.g543400.v5.5    131     110     114     118     175     138
## Cre16.g685837.v5.5     63      56      53      29     109     108
## Cre02.g119526.v5.5      0       0       0       0       0       0
## Cre06.g278159.v5.5    2707    2417    2316    2354    3916    3663
##          sample_07 sample_08 sample_09 sample_10 sample_11 sample_12
## Cre14.g619950.v5.5    128     188     134      66      81      88
## Cre01.g048900.v5.5     28       9      33      17      28      22
## Cre12.g543400.v5.5    180     170      62      92      70      76
## Cre16.g685837.v5.5     89     115      55      68      51      45
## Cre02.g119526.v5.5      0       0       0       0       0       0
## Cre06.g278159.v5.5    3556    3537    1918    1959    1914    1826
##          sample_13 sample_14 sample_15 sample_16 sample_17 sample_18
## Cre14.g619950.v5.5     21       9      24      22      67      73
## Cre01.g048900.v5.5      0      11      10       0      60      47
## Cre12.g543400.v5.5     44      12      47      34     197     169
## Cre16.g685837.v5.5     27      35      20      19      94     117
## Cre02.g119526.v5.5      0       0       0       0       0       0
## Cre06.g278159.v5.5    737     807     649     688     3347    3055
##          sample_19 sample_20 sample_21 sample_22 sample_23 sample_24
## Cre14.g619950.v5.5     59      69      76      73      52      51
## Cre01.g048900.v5.5     56      39      22      15      24       0
## Cre12.g543400.v5.5    175     167     175     233     223     211
## Cre16.g685837.v5.5    104     89      45      56      50      36
## Cre02.g119526.v5.5      0       0       0       0       0       0
## Cre06.g278159.v5.5   2937    3173    2702    2480    2466    2429
##          sample_25 sample_26 sample_27 sample_28 sample_29 sample_30
## Cre14.g619950.v5.5    143     99     136      93     143     122
## Cre01.g048900.v5.5     57      50      54      43       0       0
## Cre12.g543400.v5.5     52      66      77      58      96     131
## Cre16.g685837.v5.5     83      65      56      47     107      78
## Cre02.g119526.v5.5      0       0       0       0       0       0
## Cre06.g278159.v5.5   2193    2079    2104    1966    4211    4262
##          sample_31 sample_32 sample_33 sample_34 sample_35 sample_36
## Cre14.g619950.v5.5    122     158     166     175     145     105
## Cre01.g048900.v5.5      0       0      28      17      11      21

```

```

## Cre12.g543400.v5.5      98      121      170      146      186      81
## Cre16.g685837.v5.5     110      78       107      97       114      103
## Cre02.g119526.v5.5      0       0        0        0        0        0
## Cre06.g278159.v5.5    4106     3964     3586     3233     3417     3375
##           sample_37 sample_38 sample_39 sample_40 sample_41 sample_42
## Cre14.g619950.v5.5      48       46       44       49      111       81
## Cre01.g048900.v5.5      0        7        0        0       72       63
## Cre12.g543400.v5.5     87       61       63       71      205      167
## Cre16.g685837.v5.5     30       65       59       31      109       61
## Cre02.g119526.v5.5      0       0        0        0        0        0
## Cre06.g278159.v5.5    1573     1519     1486     1466     4477     4189
##           sample_43 sample_44 sample_45 sample_46 sample_47 sample_48
## Cre14.g619950.v5.5      61       63       0        0        0        5
## Cre01.g048900.v5.5     31       77       0        0        0        0
## Cre12.g543400.v5.5    158      151      91      106      82      101
## Cre16.g685837.v5.5     61       88       62       61      63      58
## Cre02.g119526.v5.5      0       0        0        0        0        0
## Cre06.g278159.v5.5    4127     4133     2854     2824     2724     2738

```

## Creating DEseq data set object

```

dds <- DESeqDataSetFromMatrix(countData=gene.count.matrix, colData=pheno.data,
                               design = ~genotype+ condition +
                               genotype:condition)

```

```

## Warning in DESeqDataSet(se, design = design, ignoreRank): some variables in
## design formula are characters, converting to factors

```

```
dds$genotype
```

```

## [1] wt   atg8 atg8 atg8
## [16] atg8 wt   wt   wt   wt   wt
## [31] wt   wt   wt   wt   wt   atg8 atg8 atg8 atg8 atg8 atg8 atg8 atg8 atg8 atg8
## [46] atg8 atg8 atg8
## Levels: atg8 wt

```

```
dds$condition
```

```

## [1] 0h 4h
## [26] 4h 4h
## Levels: 0h 4h

```

```
dds
```

```

## class: DESeqDataSet
## dim: 17749 48
## metadata(1): version
## assays(1): counts
## rownames(17749): Cre14.g619950.v5.5 Cre01.g048900.v5.5 ...

```

```

##  Cre21.g753197.v5.5 Cre10.g441750.v5.5
##  rowData names(0):
##  colnames(48): sample_01 sample_02 ... sample_47 sample_48
##  colData names(3): sample genotype condition

nrow(dds)

## [1] 17749

# Our experiment consists of 12 samples each with 3 replicates:

dds$sample <- factor(paste0("sample_", rep(c(1,2,3,4,5,6,7,8,9,10,11,12),
                             each=4)),
                      levels = c("sample_1", "sample_2", "sample_3", "sample_4",
                                "sample_5", "sample_6",
                                "sample_7", "sample_8", "sample_9", "sample_10",
                                "sample_11", "sample_12"))

dds$sample

##  [1] sample_1  sample_1  sample_1  sample_1  sample_1  sample_2  sample_2  sample_2
##  [8] sample_2  sample_3  sample_3  sample_3  sample_3  sample_4  sample_4  sample_4
## [15] sample_4  sample_4  sample_5  sample_5  sample_5  sample_5  sample_5  sample_6
## [22] sample_6  sample_6  sample_6  sample_7  sample_7  sample_7  sample_7  sample_7
## [29] sample_8  sample_8  sample_8  sample_8  sample_9  sample_9  sample_9  sample_9
## [36] sample_9  sample_10 sample_10 sample_10 sample_10 sample_11 sample_11 sample_11
## [43] sample_11 sample_11 sample_12 sample_12 sample_12 sample_12 sample_12
## 12 Levels: sample_1 sample_2 sample_3 sample_4 sample_5 sample_6 ... sample_12

dds <- collapseReplicates(dds, dds$sample)

# Only keep counts without 0:

keep <- rowSums(counts(dds) > 1) >=3
dds <- dds[keep,]
nrow(dds)

## [1] 13977

gene.ids <- rownames(dds)

```

Set WT and condition 0h as the reference level

```

dds$genotype <- relevel(dds$genotype, ref = "wt")
dds$condition <- relevel(dds$condition, ref = "0h")

```

## Executing DEseq2

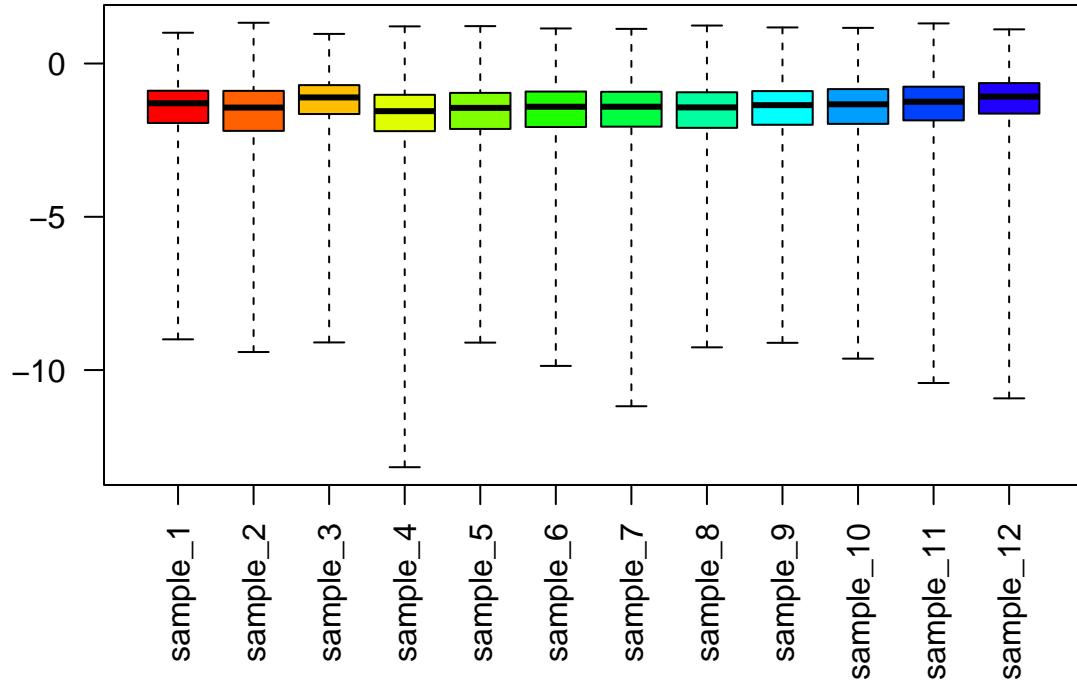
```

dds <- DESeq(dds, minReplicatesForReplace = Inf)
resultsNames(dds)

## [1] "Intercept"           "genotype_atg8_vs_wt"
## [3] "condition_4h_vs_0h"  "genotypeatg8.condition4h"

# Data visualization according to Cooks coefficient
par(mar=c(8,5,2,2))
boxplot(log10(assays(dds)[["cooks"]]), range=0, las=2, col=rainbow(16))

```



## Data previsualization

### Data transformations

Normal logarithm, variance stabilizing and regularized transformations were performed in order to visualize data; finally, the variance stabilizing transformation was selected.

```

ntd <- normTransform(dds)
vsd <- vst(dds, blind=FALSE)

## -- note: fitType='parametric', but the dispersion trend was not well captured by the

```

```

##      function: y = a/x + b, and a local regression fit was automatically substituted.
##      specify fitType='local' or 'mean' to avoid this message next time.

rld <- rlog(dds, blind = FALSE)

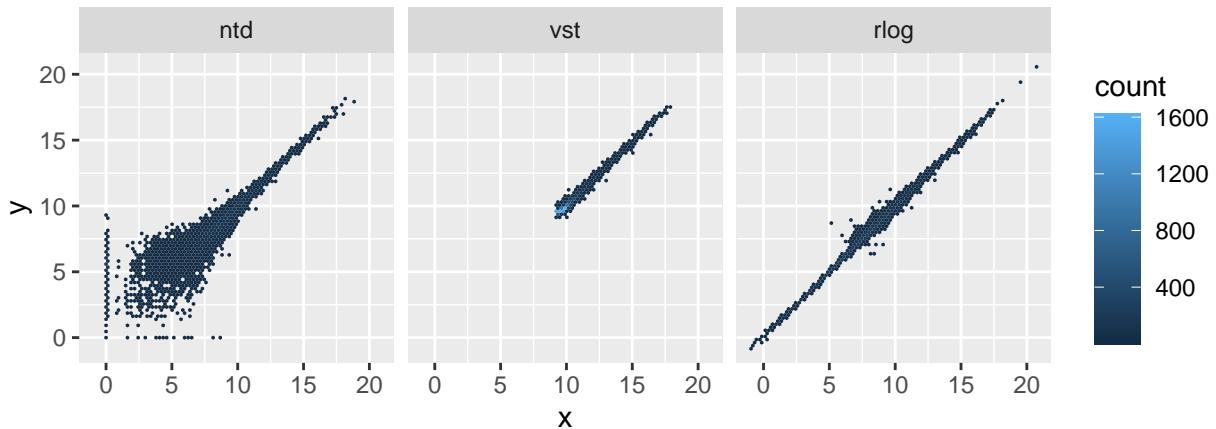
df <- bind_rows(as_data_frame(assay(ntd)[, 1:2]) %>% mutate(transformation = "ntd"),
                 as_data_frame(assay(vsd)[, 1:2]) %>% mutate(transformation = "vst"),
                 as_data_frame(assay(rld)[, 1:2]) %>% mutate(transformation = "rlog"))

## Warning: 'as_data_frame()' was deprecated in tibble 2.0.0.
## Please use 'as_tibble()' instead.
## The signature and semantics have changed, see '?as_tibble'.
## This warning is displayed once every 8 hours.
## Call 'lifecycle::last_lifecycle_warnings()' to see where this warning was generated.

# Plot exploring every transformation

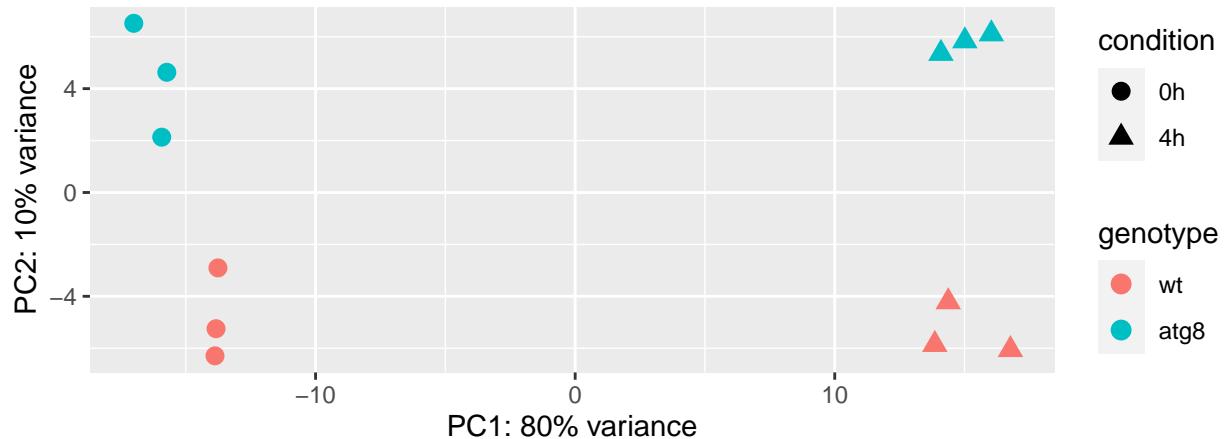
colnames(df)[1:2] <- c("x", "y")
lvls <- c("ntd", "vst", "rlog")
df$transformation <- factor(df$transformation, levels=lvls)
ggplot(df, aes(x = x, y = y)) + geom_hex(bins = 80) +
  coord_fixed() + facet_grid( . ~ transformation)

```



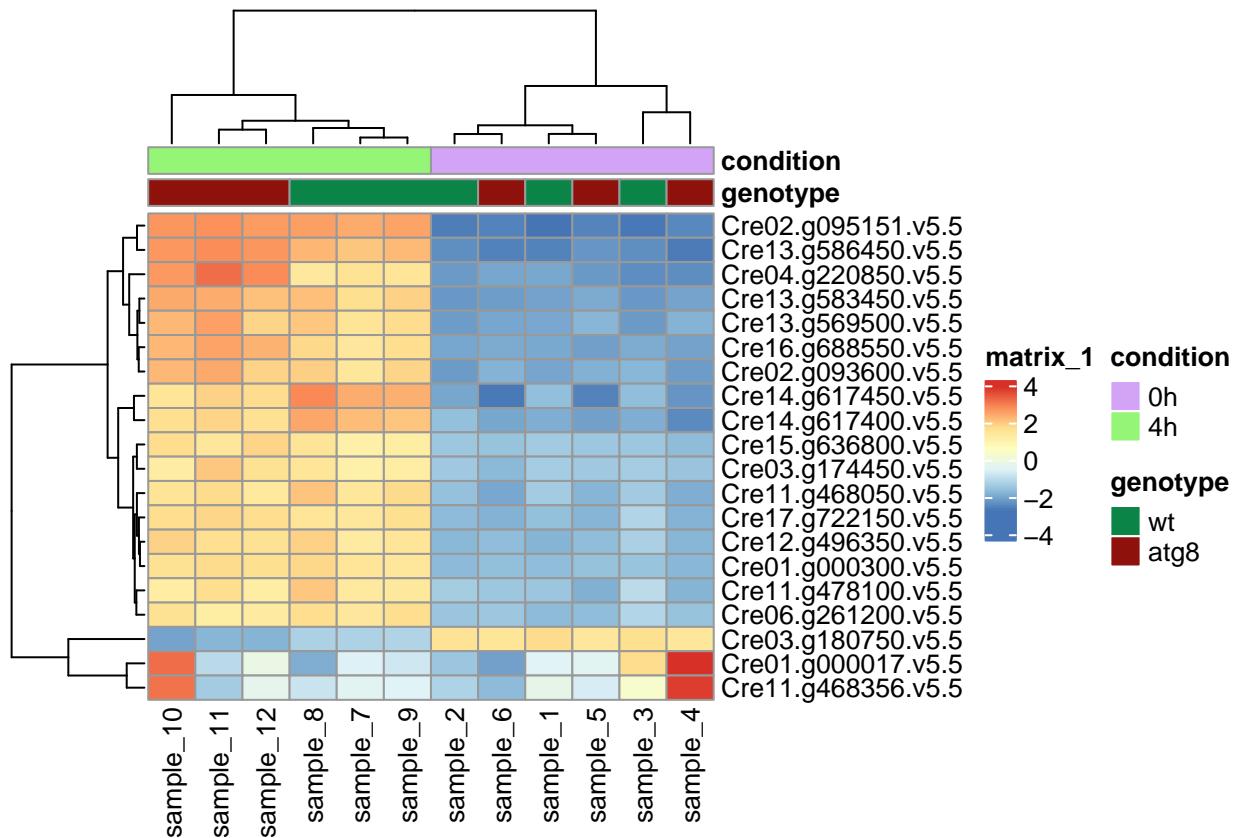
## Principal Component Analysis

```
pcaData <- plotPCA(vsd, intgroup=c("genotype", "condition"), returnData=TRUE)
percentVar <- round(100 * attr(pcaData, "percentVar"))
ggplot(pcaData, aes(PC1, PC2, color=genotype, shape=condition)) +
  geom_point(size=3) +
  xlab(paste0("PC1: ", percentVar[1], "% variance")) +
  ylab(paste0("PC2: ", percentVar[2], "% variance")) +
  coord_fixed()
```



## Gene clustering visualization

```
topVarGenes <- head(order(rowVars(assay(vsd))), decreasing = TRUE), 20
mat <- assay(vsd)[ topVarGenes, ]
mat <- mat - rowMeans(mat)
anno <- as.data.frame(colData(vsd)[, c("genotype", "condition")])
pheatmap(mat, annotation_col = anno)
```



## Gene Differential Expression Analysis

Five different contrasts were performed in order to understand the effects of cerulenin in *C. reinhardtii* cells. Those were:

- Effect of cerulenin in wild-type (0h vs 4h)
- Effect of cerulenin in mutant (0h vs 4h)
- Difference between wild-type and mutant 0h
- Difference between wild-type and mutant 4h
- Different response for both genotypes (the interaction term)

## Load some useful markers

```
ATG_markers <- read.table(file = "ATG marker genes .txt", header = T,
                           sep = "\t", row.names = NULL)

Chloro_stress_markers <- read.table(file = "Chloroplast chaperones and stress marker genes.txt", header =
                                      sep = "\t", row.names = NULL)

Chloro_redox_markers <- read.table(file = "Chloroplast redox marker genes.txt", header = T,
                                     sep = "\t", row.names = NULL)
```

## Effect of cerulenin in wild-type (0h vs 4h)

### Gene obtention

```
resWT = results(dds, contrast=c("condition", "4h", "0h"), cooksCutoff = FALSE,
                 independentFiltering = FALSE)
resWT

## log2 fold change (MLE): condition 4h vs 0h
## Wald test p-value: condition 4h vs 0h
## DataFrame with 13977 rows and 6 columns
##           baseMean log2FoldChange      lfcSE      stat     pvalue
##             <numeric>       <numeric> <numeric> <numeric> <numeric>
## Cre14.g619950.v5.5    307.8280   0.0281565  0.717068  0.0392662  0.968678
## Cre01.g048900.v5.5     76.4131   0.3799222  1.828657  0.2077602  0.835416
## Cre12.g543400.v5.5    428.9410  -0.2585408  0.320513 -0.8066479  0.419869
## Cre16.g685837.v5.5    255.7288   0.2418724  0.453962  0.5328032  0.594170
## Cre06.g278159.v5.5    9770.0557   0.1504420  0.124149  1.2117907  0.225592
## ...
##           padj
##             <numeric>
## Cre14.g619950.v5.5    0.997405
## Cre01.g048900.v5.5    0.990087
## Cre12.g543400.v5.5    0.862068
## Cre16.g685837.v5.5    0.942215
## Cre06.g278159.v5.5    0.652547
## ...
##           padj
##             <numeric>
## Cre06.g278104.v5.5    0.550696
## Cre17.g717150.v5.5    0.996460
## Cre10.g443600.v5.5    0.324773
## Cre14.g621400.v5.5    0.997405
## Cre10.g441750.v5.5    0.870623

summary(resWT)

##
## out of 13977 with nonzero total read count
## adjusted p-value < 0.1
## LFC > 0 (up)      : 909, 6.5%
## LFC < 0 (down)    : 980, 7%
## outliers [1]       : 0, 0%
## low counts [2]     : 0, 0%
## (mean count < 0)
## [1] see 'cooksCutoff' argument of ?results
## [2] see 'independentFiltering' argument of ?results
```

```

log.fold.change_WT <- resWT$log2FoldChange
q.value_WT <- resWT$padj
names(log.fold.change_WT) <- gene.ids
names(q.value_WT) <- gene.ids
base.meanWT <- resWT$baseMean
names(base.meanWT) <- gene.ids

# Take a look at upregulated and downregulated genes:

top_20_overexpressed_genes_WT <- resWT[order(resWT$log2FoldChange, decreasing = T),]
kable(top_20_overexpressed_genes_WT[1:20,-(3:4)])

```

	baseMean	log2FoldChange	pvalue	padj
Cre10.g465763.v5.5	17.445204	20.612283	0.0000012	0.0000297
Cre07.g331350.v5.5	19.178658	20.226262	0.0000004	0.0000097
Cre10.g466200.v5.5	15.235117	18.706363	0.0000109	0.0002224
Cre02.g143107.v5.5	7.114070	17.784866	0.0000295	0.0005527
Cre06.g250650.v5.5	13.336058	15.503286	0.0002575	0.0037533
Cre02.g102300.v5.5	33.165537	8.911089	0.0019320	0.0205662
Cre17.g735876.v5.5	22.382807	8.046166	0.0126038	0.0945220
Cre02.g116900.v5.5	12.062879	7.629730	0.0605932	0.2956062
Cre08.g364850.v5.5	8.610772	7.489975	0.0320338	0.1896386
Cre16.g680454.v5.5	350.851311	7.410076	0.0001349	0.0021043
Cre02.g091226.v5.5	16.082502	7.296935	0.0859688	0.3740927
Cre12.g511952.v5.5	11.269179	7.184714	0.0909203	0.3867668
Cre02.g095151.v5.5	13189.468430	7.063199	0.0000000	0.0000000
Cre07.g341750.v5.5	36.286173	6.880834	0.0077292	0.0644578
Cre08.g369200.v5.5	5.101397	6.578353	0.1158762	0.4496394
Cre12.g540351.v5.5	46.825918	6.539815	0.0033205	0.0323643
Cre03.g167690.v5.5	3.495144	6.501141	0.0738629	0.3369917
Cre03.g144444.v5.5	8.399966	6.444530	0.1295947	0.4817355
Cre04.g229422.v5.5	3.341127	6.298153	0.0952044	0.3978966
Cre12.g554929.v5.5	123.196764	6.295387	0.0000011	0.0000260

```

top_20_overrepressed_genes_WT <- resWT[order(resWT$log2FoldChange, decreasing = F),]
kable(top_20_overrepressed_genes_WT[1:20,-(3:4)])

```

	baseMean	log2FoldChange	pvalue	padj
Cre01.g043250.v5.5	102.22785	-22.99906	0e+00	0.0e+00
Cre16.g658300.v5.5	46.21710	-22.89138	0e+00	0.0e+00
Cre02.g115400.v5.5	20.74418	-22.35411	0e+00	9.0e-07
Cre12.g500250.v5.5	28.14109	-22.03587	0e+00	0.0e+00
Cre03.g179400.v5.5	39.29917	-22.00429	0e+00	0.0e+00
Cre06.g292249.v5.5	18.96145	-21.81929	1e-07	2.8e-06
Cre06.g283000.v5.5	20.54063	-21.80617	0e+00	1.0e-07
Cre17.g735750.v5.5	27.85918	-21.60836	0e+00	0.0e+00
Cre01.g034380.v5.5	38.57187	-21.42391	0e+00	0.0e+00
Cre13.g588250.v5.5	49.57133	-21.38082	0e+00	0.0e+00
Cre16.g684715.v5.5	14.85207	-21.37278	4e-07	9.9e-06
Cre03.g143827.v5.5	66.50156	-21.31874	0e+00	0.0e+00
Cre14.g629840.v5.5	37.01919	-21.31223	0e+00	0.0e+00
Cre14.g632350.v5.5	93.48560	-21.17466	0e+00	0.0e+00
Cre12.g554100.v5.5	20.57133	-21.16066	0e+00	2.0e-07
Cre09.g402050.v5.5	54.88133	-21.12392	0e+00	0.0e+00
Cre02.g083050.v5.5	18.89988	-21.07576	1e-07	4.0e-06
Cre09.g397050.v5.5	47.73692	-20.98995	0e+00	0.0e+00
Cre16.g658400.v5.5	59.75973	-20.89439	0e+00	0.0e+00
Cre04.g226550.v5.5	12.19448	-20.86036	9e-07	2.3e-05

```
# Differentially activated and repressed genes with a log fold change and p-adj threshold of 1.5 and 0.05
```

```
activated.genes.deseq2_WT <- gene.ids[log.fold.change_WT > 1 & q.value_WT < 0.05]
activated.genes.deseq2_WT <- activated.genes.deseq2_WT[!is.na(activated.genes.deseq2_WT)]
activated.genes.deseq2_WT_good <- substr(activated.genes.deseq2_WT,1,nchar(activated.genes.deseq2_WT)-5)
write.table(activated.genes.deseq2_WT_good,file="activated.genes.deseq2_WT.txt",sep="\t",quote=F,row.names=F)

repressed.genes.deseq2_WT <- gene.ids[log.fold.change_WT < - 1 & q.value_WT < 0.05]
repressed.genes.deseq2_WT <- represed.genes.deseq2_WT[!is.na(repressed.genes.deseq2_WT)]
represed.genes.deseq2_WT_good <- substr(represed.genes.deseq2_WT,1,nchar(represaed.genes.deseq2_WT)-5)
write.table(represaed.genes.deseq2_WT_good,file="represaed.genes.deseq2_WT.txt",sep="\t",quote=F,row.names=F)
```

```
length(activated.genes.deseq2_WT_good)
```

```
## [1] 458
```

```
length(repressed.genes.deseq2_WT_good)
```

```
## [1] 350
```

MA plot

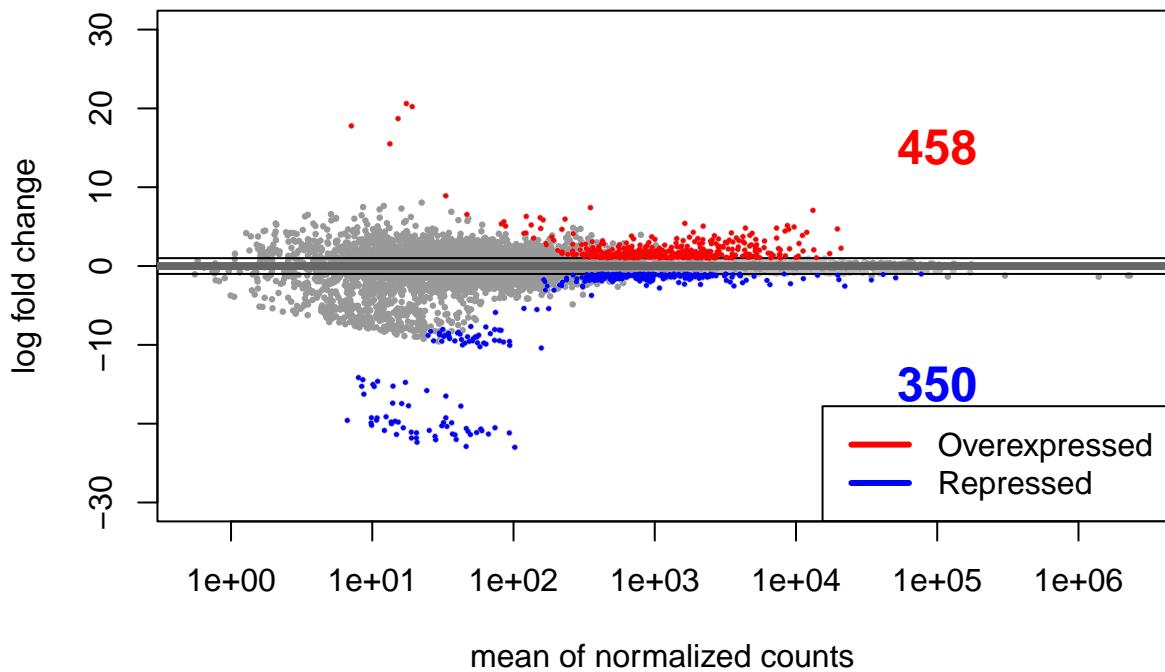
```
plotMA(resWT,alpha=0.05, ylim=c(-30,30), colSig="grey")
abline(h=c(-1,1), col="black", lwd=1)
points(x = base.meanWT[activated.genes.deseq2_WT],
       y = log.fold.change_WT[activated.genes.deseq2_WT], col="red", cex=0.2, pch=19)
points(x = base.meanWT[represed.genes.deseq2_WT],
       y = log.fold.change_WT[represed.genes.deseq2_WT], col="blue", cex=0.2, pch=19)
```

```

text(x =100000, y = 15, label = length(activated.genes.deseq2_WT_good),
     col = "red",      # Color del texto
     font = 2,          # Estilo
     cex = 1.5)        # Tamaño
text(x =100000, y = -15, label = length(repressed.genes.deseq2_WT_good),
     col = "blue",     # Color del texto
     font = 2,          # Estilo
     cex = 1.5)        # Tamaño

legend("bottomright", legend = c("Overexpressed", "Repressed"),
       lwd = 3, col = c("red", "blue"))

```



### Effect of cerulenin in mutant (0h vs 4h)

#### Gene obtention

```

res_atg8 <- results(dds, list(c("genotypeatg8.condition4h","condition_4h_vs_0h")),
                      cooksCutoff = FALSE, independentFiltering = FALSE)
res_atg8

```

```

## log2 fold change (MLE): genotypeatg8.condition4h+condition_4h_vs_0h effect
## Wald test p-value: genotypeatg8.condition4h+condition_4h_vs_0h effect
## DataFrame with 13977 rows and 6 columns

```

```

##          baseMean log2FoldChange      lfcSE       stat      pvalue
## Cre14.g619950.v5.5    307.8280 -0.0177850  0.721654 -0.0246447 9.80338e-01
## Cre01.g048900.v5.5     76.4131 -0.3532859  1.831528 -0.1928914 8.47044e-01
## Cre12.g543400.v5.5    428.9410 -0.0527444  0.321006 -0.1643096 8.69487e-01
## Cre16.g685837.v5.5    255.7288  0.3030096  0.456488  0.6637846 5.06828e-01
## Cre06.g278159.v5.5    9770.0557  0.5915680  0.124397  4.7554709 1.97984e-06
## ...
##          ...
##          padj
##          <numeric>
## Cre14.g619950.v5.5 9.98339e-01
## Cre01.g048900.v5.5 9.70421e-01
## Cre12.g543400.v5.5 9.75746e-01
## Cre16.g685837.v5.5 8.13498e-01
## Cre06.g278159.v5.5 3.98446e-05
## ...
##          ...
## Cre06.g278104.v5.5   0.517192
## Cre17.g717150.v5.5   0.490686
## Cre10.g443600.v5.5   0.972125
## Cre14.g621400.v5.5   0.847746
## Cre10.g441750.v5.5   0.747101

log.fold.change_atg8 <- res_atg8$log2FoldChange
q.value_atg8 <- res_atg8$padj
names(log.fold.change_atg8) <- gene.ids
names(q.value_atg8) <- gene.ids
base.mean_atg8 <- res_atg8$baseMean
names(base.mean_atg8) <- gene.ids

# Take a look at upregulated and downregulated genes:

top_20_overexpressed_genes_atg8 <- res_atg8[order(res_atg8$log2FoldChange, decreasing = T),]
kable(top_20_overexpressed_genes_atg8[1:20,-(3:4)])

```

	baseMean	log2FoldChange	pvalue	padj
Cre01.g031700.v5.5	71.40996	22.31599	0e+00	0.00e+00
Cre03.g169150.v5.5	84.33879	22.21561	0e+00	0.00e+00
Cre03.g206900.v5.5	39.26690	22.20814	0e+00	0.00e+00
Cre05.g242750.v5.5	31.80983	22.17803	0e+00	0.00e+00
Cre03.g183150.v5.5	52.34164	22.08262	0e+00	0.00e+00
Cre16.g650000.v5.5	31.64993	21.81398	0e+00	2.00e-07
Cre01.g055457.v5.5	18.41569	21.70069	3e-07	8.00e-06
Cre06.g282826.v5.5	42.54245	21.65897	0e+00	0.00e+00
Cre16.g688900.v5.5	26.82071	21.59666	0e+00	3.00e-07
Cre24.g755097.v5.5	25.65882	21.46373	0e+00	4.00e-07
Cre06.g305302.v5.5	18.84289	21.35537	5e-07	1.18e-05
Cre07.g346900.v5.5	87.77094	21.16046	0e+00	0.00e+00
Cre01.g009650.v5.5	19.95885	21.02765	0e+00	2.00e-07
Cre13.g581700.v5.5	50.68467	20.99081	0e+00	0.00e+00
Cre13.g569000.v5.5	31.53099	20.90911	0e+00	0.00e+00
Cre15.g637315.v5.5	10.27450	20.87907	9e-07	1.98e-05
Cre12.g544113.v5.5	21.59394	20.87294	0e+00	2.00e-07
Cre17.g724873.v5.5	12.39615	20.76123	1e-06	2.25e-05
Cre04.g216700.v5.5	20.06815	20.75520	0e+00	3.00e-07
Cre02.g109900.v5.5	70.00800	20.72860	0e+00	0.00e+00

```
top_20_overrepressed_genes_atg8 <- res_atg8[order(res_atg8$log2FoldChange, decreasing = F),]
kable(top_20_overrepressed_genes_atg8[1:20,-(3:4)])
```

	baseMean	log2FoldChange	pvalue	padj
Cre06.g283000.v5.5	20.54063	-29.73628	0e+00	0.0e+00
Cre17.g735750.v5.5	27.85918	-29.05778	0e+00	0.0e+00
Cre06.g292249.v5.5	18.96145	-26.98877	0e+00	0.0e+00
Cre12.g500250.v5.5	28.14109	-26.72339	0e+00	0.0e+00
Cre02.g115400.v5.5	20.74418	-25.68656	0e+00	0.0e+00
Cre16.g684715.v5.5	14.85207	-25.34339	0e+00	1.0e-07
Cre06.g294250.v5.5	123.06077	-24.04041	0e+00	0.0e+00
Cre08.g376740.v5.5	77.07946	-23.39426	0e+00	0.0e+00
Cre03.g205800.v5.5	69.35132	-23.21297	0e+00	1.3e-06
Cre13.g604550.v5.5	56.05740	-23.16705	0e+00	0.0e+00
Cre07.g315050.v5.5	88.92973	-23.13839	0e+00	0.0e+00
Cre09.g412450.v5.5	61.27340	-23.03721	0e+00	0.0e+00
Cre10.g428000.v5.5	52.12164	-23.01926	0e+00	0.0e+00
Cre13.g592350.v5.5	49.26531	-22.72924	0e+00	0.0e+00
Cre07.g325713.v5.5	47.76235	-22.60765	0e+00	0.0e+00
Cre06.g278168.v5.5	57.62603	-22.41235	0e+00	0.0e+00
Cre12.g547727.v5.5	29.91381	-22.32585	0e+00	0.0e+00
Cre05.g246250.v5.5	43.36180	-22.26541	0e+00	0.0e+00
Cre01.g010832.v5.5	37.26769	-22.11360	0e+00	0.0e+00
Cre16.g695200.v5.5	28.69367	-22.10441	1e-07	2.5e-06

```
activated.genes.deseq2_atg8 <- gene.ids$log.fold.change_atg8 > 1 & q.value_atg8 < 0.05]
activated.genes.deseq2_atg8 <- activated.genes.deseq2_atg8[!is.na(activated.genes.deseq2_atg8)]
activated.genes.deseq2_atg8_good <- substr(activated.genes.deseq2_atg8,1,nchar(activated.genes.deseq2_atg8))
write.table(activated.genes.deseq2_atg8_good,file="activated.genes.deseq2_atg8.txt",sep="\t",quote=F,rownames=FALSE)

repressed.genes.deseq2_atg8 <- gene.ids$log.fold.change_atg8 < -1 & q.value_atg8 < 0.05]
```

```

repressed.genes.deseq2_atg8 <- repressed.genes.deseq2_atg8[!is.na(repressed.genes.deseq2_atg8)]
repressed.genes.deseq2_atg8_good <- substr(repressed.genes.deseq2_atg8,1,nchar(repressed.genes.deseq2_atg8))
write.table(repressed.genes.deseq2_atg8_good,file="repressed.genes.deseq2_atg8.txt",sep="\t",quote=F,rows=1)

length(activated.genes.deseq2_atg8_good)

## [1] 618

length(repressed.genes.deseq2_atg8_good)

## [1] 441

```

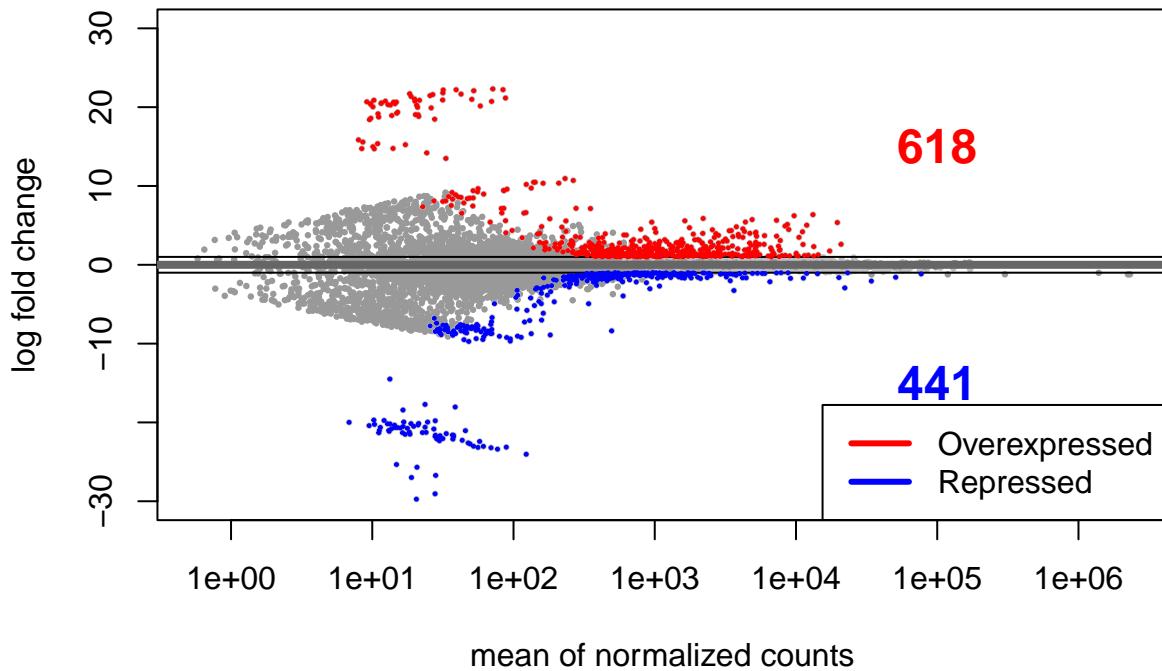
## MA plot

```

plotMA(res_atg8,alpha=0.05, ylim=c(-30,30),colSig="grey")
abline(h=c(-1,1), col="black", lwd=1)
points(x = base.meanWT[activated.genes.deseq2_atg8],
       y = log.fold.change_atg8[activated.genes.deseq2_atg8],col="red",cex=0.2,pch=19)
points(x = base.mean_atg8[repressed.genes.deseq2_atg8],
       y = log.fold.change_atg8[repressed.genes.deseq2_atg8],col="blue",cex=0.2,pch=19)
text(x =100000, y = 15, label = length(activated.genes.deseq2_atg8_good),
      col = "red",    # Color del texto
      font = 2,        # Estilo
      cex = 1.5)       # Tamaño
text(x =100000, y = -15, label = length(repressed.genes.deseq2_atg8_good),
      col = "blue",   # Color del texto
      font = 2,        # Estilo
      cex = 1.5)       # Tamaño

legend("bottomright", legend = c("Overexpressed", "Repressed"),
       lwd = 3, col = c("red", "blue"))

```



Plotting log2 fold change with target genes for wt and atg8 treated with cerulenin

```
# Test if lists of target genes are in the filtered gene.expression matrix
intersect(ATG_markers$PhyCode, gene.ids) # Check
```

```
## [1] "Cre09.g391245.v5.5" "Cre01.g045600.v5.5" "Cre02.g102350.v5.5"
## [4] "Cre12.g510100.v5.5" "Cre14.g630907.v5.5" "Cre03.g165215.v5.5"
## [7] "Cre16.g689650.v5.5" "Cre09.g391500.v5.5" "Cre12.g557000.v5.5"
## [10] "Cre16.g659000.v5.5" "Cre08.g377600.v5.5" "Cre10.g457550.v5.5"
## [13] "Cre07.g334700.v5.5" "Cre06.g290500.v5.5" "Cre01.g035500.v5.5"
```

```
intersect(Chloro_stress_markers$PhyCode, gene.ids) # Check
```

```
## [1] "Cre13.g583550.v5.5" "Cre11.g468050.v5.5" "Cre06.g250100.v5.5"
## [4] "Cre03.g145787.v5.5" "Cre14.g617450.v5.5" "Cre14.g617400.v5.5"
## [7] "Cre02.g090850.v5.5" "Cre17.g696400.v5.5" "Cre12.g559150.v5.5"
## [10] "Cre07.g341600.v5.5" "Cre12.g507650.v5.5" "Cre01.g009900.v5.5"
## [13] "Cre10.g430400.v5.5" "Cre12.g520600.v5.5"
```

```
intersect(Chloro_redox_markers$PhyCode, gene.ids) # Check
```

```

## [1] "Cre02.g087700.v5.5" "Cre06.g285150.v5.5" "Cre10.g458450.v5.5"
## [4] "Cre07.g325743.v5.5" "Cre16.g688550.v5.5" "Cre01.g054150.v5.5"
## [7] "Cre10.g422300.v5.5"

```

## ATG Markers

```

bar.names_ATG <- ATG_markers$Name
bar.phycodes_ATG <- ATG_markers$PhyCode

wt_treatment_ATG <- as.data.frame(resWT) %>% rownames_to_column("GeneID")
wt_treatment_ATG <- wt_treatment_ATG[wt_treatment_ATG$GeneID %in% bar.phycodes_ATG,]
wt_treatment_ATG$GeneID <- ATG_markers$Name[match(wt_treatment_ATG$GeneID, ATG_markers$PhyCode)]
row.names(wt_treatment_ATG) <- wt_treatment_ATG$GeneID
wt_treatment_ATG_fold.change <- wt_treatment_ATG[-c(1:2,4:7)]
colnames(wt_treatment_ATG_fold.change) <- c("WT cerulenin")
wt_treatment_ATG_fold.change.matrix <- as.matrix(wt_treatment_ATG_fold.change)
wt_treatment_ATG$GeneID[wt_treatment_ATG$log2FoldChange >1 & wt_treatment_ATG$padj < 0.05]

## [1] "ATG7"    "ATG3"    "ATG14"   "ATG2"    "ATG101"   "ATG8"

atg8_treatment_ATG <- as.data.frame(res_atg8) %>% rownames_to_column("GeneID")
atg8_treatment_ATG <- atg8_treatment_ATG[atg8_treatment_ATG$GeneID %in% bar.phycodes_ATG,]
atg8_treatment_ATG$GeneID <- ATG_markers$Name[match(atg8_treatment_ATG$GeneID, ATG_markers$PhyCode)]
row.names(atg8_treatment_ATG) <- atg8_treatment_ATG$GeneID
atg8_treatment_ATG_fold.change <- atg8_treatment_ATG[-c(1:2,4:7)]
colnames(atg8_treatment_ATG_fold.change) <- c("atg8 cerulenin")
atg8_treatment_ATG$GeneID[atg8_treatment_ATG$log2FoldChange >1 & atg8_treatment_ATG$padj < 0.05]

## [1] "VPS34"   "ATG7"    "ATG3"    "ATG14"   "ATG2"    "ATG101"   "ATG8"    "ATG13"

atg8_treatment_ATG_fold.change.matrix <- as.matrix(atg8_treatment_ATG_fold.change)

wt_atg8_treatment_ATG_fold.change.matrix <- cbind(wt_treatment_ATG_fold.change.matrix,
                                                   atg8_treatment_ATG_fold.change.matrix)
# Transpose matrix:

wt_atg8_treatment_ATG_fold.change.matrix <- t(wt_atg8_treatment_ATG_fold.change.matrix)

# Remove columns where for both genotypes target ATG genes have log2 fold change < 1
# and padj > 0.05

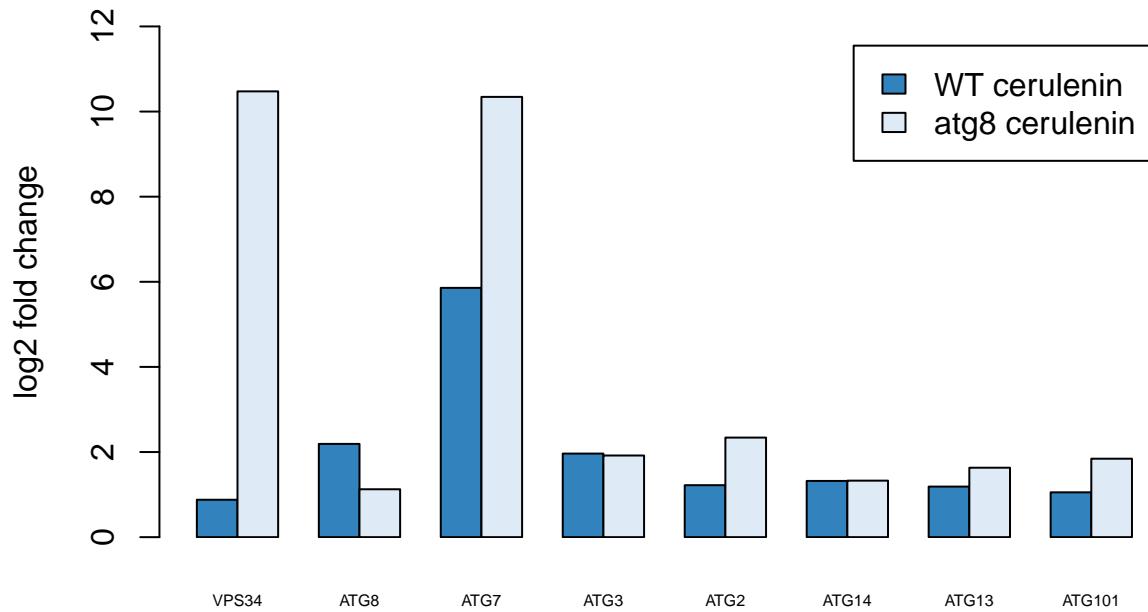
# Cols to remove: VPS15, ATG5, ATG12, ATG9, ATG5, ATG6, ATG1, ATG18, ATG4

wt_atg8_treatment_ATG_fold.change.matrix <- wt_atg8_treatment_ATG_fold.change.matrix[, -c(1,5,7:8,13:16)]
wt_atg8_treatment_ATG_fold.change.matrix<-wt_atg8_treatment_ATG_fold.change.matrix[,order(colnames(wt_atg8_treatment_ATG_fold.change.matrix))]

barplot(wt_atg8_treatment_ATG_fold.change.matrix, beside = TRUE,
        legend.text = TRUE, ylab = "log2 fold change", ylim = c(0,12),
        xpd = FALSE, cex.names = 0.5, col=colorRampPalette(rev(brewer.pal(2,"Blues")))(2))

## Warning in brewer.pal(2, "Blues"): minimal value for n is 3, returning requested palette with 3 diff

```



## Chloroplast chaperones and stress markers

```

bar.names_ChlStress <- Chloro_stress_markers>Name
bar.phycodes_ChlStress <- Chloro_stress_markers$PhyCode

wt_treatment_ChlStress <- as.data.frame(resWT) %>% rownames_to_column("GeneID")
wt_treatment_ChlStress <- wt_treatment_ChlStress[wt_treatment_ChlStress$GeneID %in% bar.phycodes_ChlStress]
wt_treatment_ChlStress$GeneID <- Chloro_stress_markers>Name[match(wt_treatment_ChlStress$GeneID,
Chloro_stress_markers$PhyCode)]
row.names(wt_treatment_ChlStress) <- wt_treatment_ChlStress$GeneID
wt_treatment_ChlStress_fold.change <- wt_treatment_ChlStress[-c(1:2,4:7)]
colnames(wt_treatment_ChlStress_fold.change) <- c("WT cerulenin")
wt_treatment_ChlStress_fold.change.matrix <- as.matrix(wt_treatment_ChlStress_fold.change)
wt_treatment_ChlStress$GeneID[wt_treatment_ChlStress$log2FoldChange >1 & wt_treatment_ChlStress$padj < 0.05] <- "Significant"

## [1] "VIPIP2"   "HSP22E"   "VIPIP1"   "HSP22F"   "CLPB3"

atg8_treatment_ChlStress <- as.data.frame(res_atg8) %>% rownames_to_column("GeneID")
atg8_treatment_ChlStress <- atg8_treatment_ChlStress[atg8_treatment_ChlStress$GeneID %in% bar.phycodes_ChlStress]
atg8_treatment_ChlStress$GeneID <- Chloro_stress_markers>Name[match(atg8_treatment_ChlStress$GeneID,
Chloro_stress_markers$PhyCode)]
row.names(atg8_treatment_ChlStress) <- atg8_treatment_ChlStress$GeneID
atg8_treatment_ChlStress_fold.change <- atg8_treatment_ChlStress[-c(1:2,4:7)]

```

```

colnames(atg8_treatment_Ch1Stress_fold.change) <- c("atg8_cerulenin")
atg8_treatment_Ch1Stress$GeneID[atg8_treatment_Ch1Stress$log2FoldChange >1 & atg8_treatment_Ch1Stress$p

## [1] "VIPP2"   "HSP22E"  "VIPP1"   "HSP22F"  "CLPB3"

atg8_treatment_Ch1Stress_fold.change.matrix <- as.matrix(atg8_treatment_Ch1Stress_fold.change)

wt_atg8_treatment_Ch1Stress_fold.change.matrix <- cbind(wt_treatment_Ch1Stress_fold.change.matrix,
                                         atg8_treatment_Ch1Stress_fold.change.matrix)
# Transpose matrix:

wt_atg8_treatment_Ch1Stress_fold.change.matrix <- t(wt_atg8_treatment_Ch1Stress_fold.change.matrix)

# Remove columns where for both genotypes target ATG genes have log2 fold change < 1
# and padj > 0.05

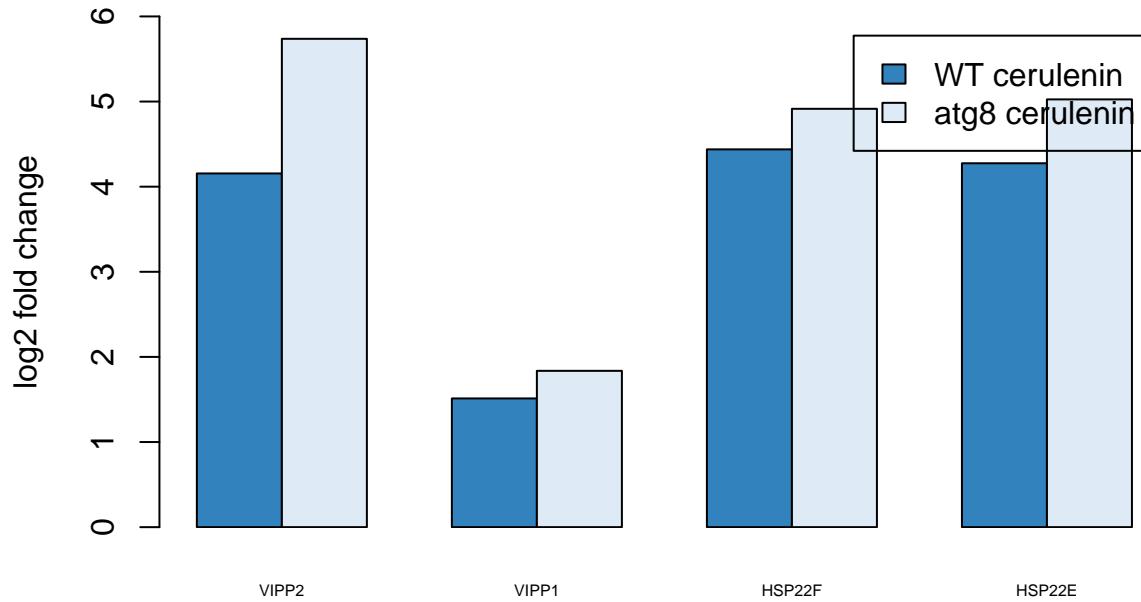
# Cols to remove: HSP22C, RPL37, CDJ2, CDJ1, CGE1, HSP70B, CDJ3, CLPS1, PRPS6, CLPS2

wt_atg8_treatment_Ch1Stress_fold.change.matrix <- wt_atg8_treatment_Ch1Stress_fold.change.matrix[, -c(2)
wt_atg8_treatment_Ch1Stress_fold.change.matrix<-wt_atg8_treatment_Ch1Stress_fold.change.matrix[,order(c

barplot(wt_atg8_treatment_Ch1Stress_fold.change.matrix, beside = TRUE,
        legend.text = TRUE, ylab = "log2 fold change", ylim = c(0,6),
        xpd = FALSE, cex.names = 0.5, col=colorRampPalette(rev(brewer.pal(2,"Blues")))(2))

## Warning in brewer.pal(2, "Blues"): minimal value for n is 3, returning requested palette with 3 different colors

```



### Chloroplast redox markers

```

bar.names_ChlRed <- Chloro_redox_markers$Name
bar.phycodes_ChlRed <- Chloro_redox_markers$PhyCode

wt_treatment_ChlRed <- as.data.frame(resWT) %>% rownames_to_column("GeneID")
wt_treatment_ChlRed <- wt_treatment_ChlRed[wt_treatment_ChlRed$GeneID %in% bar.phycodes_ChlRed,]
wt_treatment_ChlRed$GeneID <- Chloro_redox_markers$Name[match(wt_treatment_ChlRed$GeneID,
                                                               Chloro_redox_markers$PhyCode)]
row.names(wt_treatment_ChlRed) <- wt_treatment_ChlRed$GeneID
wt_treatment_ChlRed_fold.change <- wt_treatment_ChlRed[-c(1:2,4:7)]
colnames(wt_treatment_ChlRed_fold.change) <- c("WT cerulenin")
wt_treatment_ChlRed_fold.change.matrix <- as.matrix(wt_treatment_ChlRed_fold.change)
wt_treatment_ChlRed$GeneID[wt_treatment_ChlRed$log2FoldChange >1 & wt_treatment_ChlRed$padj < 0.05]

## [1] "GSTS1" "APX1"   "GPX5"

atg8_treatment_ChlRed <- as.data.frame(res_atg8) %>% rownames_to_column("GeneID")
atg8_treatment_ChlRed <- atg8_treatment_ChlRed[atg8_treatment_ChlRed$GeneID %in% bar.phycodes_ChlRed,]
atg8_treatment_ChlRed$GeneID <- Chloro_redox_markers$Name[match(atg8_treatment_ChlRed$GeneID,
                                                               Chloro_redox_markers$PhyCode)]
row.names(atg8_treatment_ChlRed) <- atg8_treatment_ChlRed$GeneID
atg8_treatment_ChlRed_fold.change <- atg8_treatment_ChlRed[-c(1:2,4:7)]

```

```

colnames(atg8_treatment_Ch1Red_fold.change) <- c("atg8 cerulenin")
atg8_treatment_Ch1Red$GeneID[atg8_treatment_Ch1Red$log2FoldChange >1 & atg8_treatment_Ch1Red$padj < 0.05]

## [1] "GSTS1" "APX1"  "GPX5"

atg8_treatment_Ch1Red_fold.change.matrix <- as.matrix(atg8_treatment_Ch1Red_fold.change)

wt_atg8_treatment_Ch1Red_fold.change.matrix <- cbind(wt_treatment_Ch1Red_fold.change.matrix,
                                                       atg8_treatment_Ch1Red_fold.change.matrix)
# Transpose matrix:

wt_atg8_treatment_Ch1Red_fold.change.matrix <- t(wt_atg8_treatment_Ch1Red_fold.change.matrix)

# Remove columns where for both genotypes target ATG genes have log2 fold change < 1
# and padj > 0.05

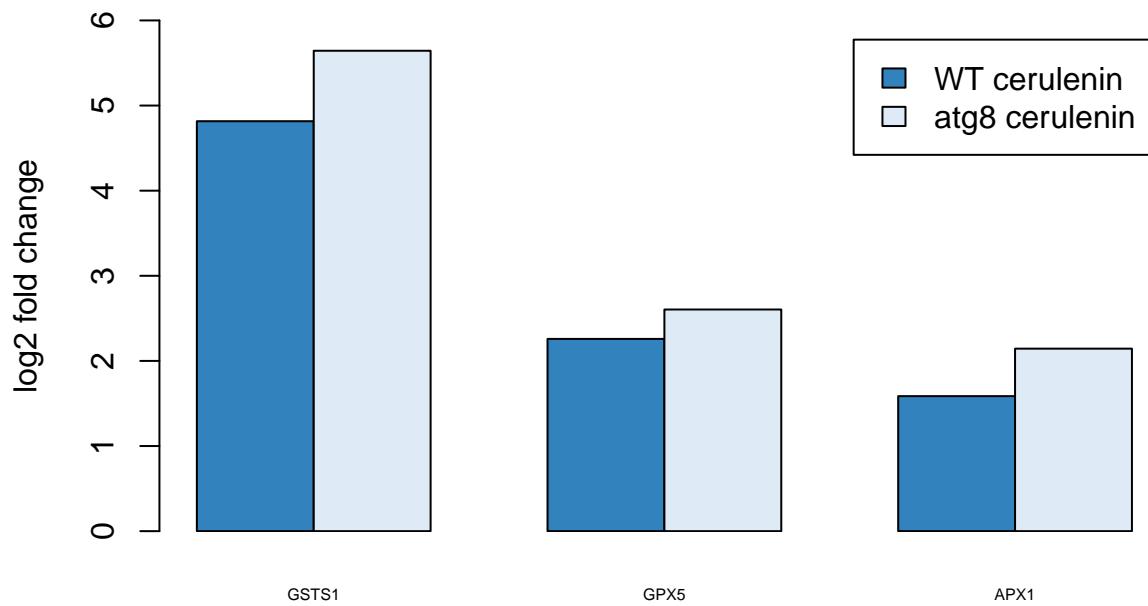
# Cols to remove: APX2, GRX3, PRX6, NTRC1

wt_atg8_treatment_Ch1Red_fold.change.matrix <- wt_atg8_treatment_Ch1Red_fold.change.matrix[, -c(3:6)]
wt_atg8_treatment_Ch1Red_fold.change.matrix<-wt_atg8_treatment_Ch1Red_fold.change.matrix[,order(colnames(wt_atg8_treatment_Ch1Red_fold.change.matrix))]

barplot(wt_atg8_treatment_Ch1Red_fold.change.matrix, beside = TRUE,
        legend.text = TRUE, ylab = "log2 fold change", ylim = c(0,6),
        xpd = FALSE, cex.names = 0.5, col=colorRampPalette(rev(brewer.pal(2,"Blues")))(2))

## Warning in brewer.pal(2, "Blues"): minimal value for n is 3, returning requested palette with 3 different colors

```



## Difference between wild-type and mutant 0h

## Gene obtention

```
res_WT_atg8_0h = results(dds, contrast=c("genotype","atg8","wt"),
                         cooksCutoff = FALSE, independentFiltering = FALSE)
res_WT_atg8_0h
```

```

## log2 fold change (MLE): genotype atg8 vs wt
## Wald test p-value: genotype atg8 vs wt
## DataFrame with 13977 rows and 6 columns
##           baseMean log2FoldChange      lfcSE      stat     pvalue
## <numeric>    <numeric>    <numeric>    <numeric>    <numeric>
## Cre14.g619950.v5.5 307.8280 -1.0486329 0.719601 -1.457241 0.145050
## Cre01.g048900.v5.5 76.4131  0.2567535 1.830265  0.140282 0.888437
## Cre12.g543400.v5.5 428.9410  0.4253013 0.320785  1.325814 0.184901
## Cre16.g685837.v5.5 255.7288  0.0698002 0.456223  0.152996 0.878402
## Cre06.g278159.v5.5 9770.0557 -0.0796437 0.124401 -0.640217 0.522031
## ...
##           ...       ...       ...       ...
## Cre06.g278104.v5.5 889.54623 -0.604567 0.310855 -1.944851 0.0517930
## Cre17.g717150.v5.5 146.81853  0.994081 1.170782  0.849074 0.3958403
## Cre10.g443600.v5.5 170.10265  0.127679 1.079078  0.118322 0.9058126
## Cre14.g621400.v5.5 5.13639   6.786913 4.117852  1.648168 0.0993182

```

```

## Cre10.g441750.v5.5 1599.45474      0.269795  0.181866  1.483486 0.1379453
##                               padj
##                               <numeric>
## Cre14.g619950.v5.5  0.728842
## Cre01.g048900.v5.5  0.999313
## Cre12.g543400.v5.5  0.799370
## Cre16.g685837.v5.5  0.999313
## Cre06.g278159.v5.5  0.999313
## ...
## Cre06.g278104.v5.5  0.445209
## Cre17.g717150.v5.5  0.981999
## Cre10.g443600.v5.5  0.999313
## Cre14.g621400.v5.5  0.619202
## Cre10.g441750.v5.5  0.711462

log.fold.change_WT_atg8_0h <- res_WT_atg8_0h$log2FoldChange
q.value_WT_atg8_0h <- res_WT_atg8_0h$padj
names(log.fold.change_WT_atg8_0h) <- gene.ids
names(q.value_WT_atg8_0h) <- gene.ids
base.mean_WT_atg8_0h <- res_WT_atg8_0h$baseMean
names(base.mean_WT_atg8_0h) <- gene.ids

# Take a look at upregulated and downregulated genes:

top_20_overexpressed_genes_WT_atg8_0h <- res_WT_atg8_0h[order(res_WT_atg8_0h$log2FoldChange, decreasing = TRUE), 1:20]
kable(top_20_overexpressed_genes_WT_atg8_0h[1:20,-(3:4)])

```

	baseMean	log2FoldChange	pvalue	padj
Cre10.g466200.v5.5	15.235117	20.585945	0.0000013	0.0001480
Cre07.g331350.v5.5	19.178658	19.940809	0.0000005	0.0000721
Cre02.g143107.v5.5	7.114070	19.404147	0.0000051	0.0004478
Cre10.g465763.v5.5	17.445204	18.935435	0.0000086	0.0006709
Cre06.g250650.v5.5	13.336058	14.980359	0.0004177	0.0159081
Cre13.g567450.v5.5	47.816726	9.974608	0.0028980	0.0675091
Cre06.g310900.v5.5	12.697053	8.158493	0.0541851	0.4562322
Cre03.g167622.v5.5	13.099835	8.019118	0.0284711	0.3099224
Cre08.g358565.v5.5	7.390831	7.384139	0.0824190	0.5624718
Cre02.g120200.v5.5	7.613366	7.380495	0.0815050	0.5587032
Cre15.g635100.v5.5	16.735402	7.208932	0.0296900	0.3160524
Cre17.g713500.v5.5	5.698800	7.072735	0.0671212	0.5118263
Cre13.g569900.v5.5	12.543505	6.987735	0.1003772	0.6221609
Cre09.g404552.v5.5	7.466950	6.930540	0.1032099	0.6306568
Cre14.g621400.v5.5	5.136393	6.786913	0.0993182	0.6192016
Cre11.g480502.v5.5	4.849025	6.781913	0.0473071	0.4194994
Cre09.g395925.v5.5	10.571970	6.551579	0.1236302	0.6819178
Cre03.g144444.v5.5	8.399966	6.412211	0.1318948	0.6990198
Cre06.g265200.v5.5	3.559183	6.353395	0.1041611	0.6328170
Cre02.g091226.v5.5	16.082502	6.198403	0.1454064	0.7288417

```

top_20_overrepressed_genes_WT_atg8_0h <- res_WT_atg8_0h[order(res_WT_atg8_0h$log2FoldChange, decreasing = TRUE), 1:20]
kable(top_20_overrepressed_genes_WT_atg8_0h[1:20,-(3:4)])

```

	baseMean	log2FoldChange	pvalue	padj
Cre07.g346900.v5.5	87.770943	-23.11461	0.0e+00	0.0000000
Cre08.g364650.v5.5	58.216483	-22.48245	0.0e+00	0.0000000
Cre02.g109900.v5.5	70.008000	-22.42246	0.0e+00	0.0000000
Cre03.g183150.v5.5	52.341644	-21.67106	0.0e+00	0.0000000
Cre10.g433650.v5.5	19.955260	-21.51465	1.0e-07	0.0000204
Cre02.g077951.v5.5	22.406577	-21.47873	1.0e-07	0.0000134
Cre03.g169150.v5.5	84.338786	-21.16178	0.0e+00	0.0000000
Cre02.g095141.v5.5	19.617552	-21.06563	3.0e-07	0.0000407
Cre13.g581700.v5.5	50.684675	-21.00907	0.0e+00	0.0000000
Cre10.g452500.v5.5	27.671211	-20.91627	5.0e-07	0.0000661
Cre06.g282826.v5.5	42.542451	-20.82930	0.0e+00	0.0000000
Cre12.g494600.v5.5	20.255885	-20.54698	3.0e-07	0.0000407
Cre07.g350050.v5.5	12.690641	-20.50715	1.4e-06	0.0001607
Cre01.g031700.v5.5	71.409961	-20.47323	0.0e+00	0.0000000
Cre03.g206900.v5.5	39.266900	-20.37765	0.0e+00	0.0000001
Cre09.g395732.v5.5	26.133934	-20.36151	1.0e-07	0.0000217
Cre06.g297049.v5.5	9.569606	-20.31073	1.2e-06	0.0001435
Cre17.g698950.v5.5	9.775073	-20.25779	1.9e-06	0.0002031
Cre03.g191650.v5.5	21.334812	-20.15767	0.0e+00	0.0000030
Cre16.g669350.v5.5	14.906514	-20.03924	9.0e-07	0.0001214

```

activated.genes.deseq2_WT_atg8_0h <- gene.ids[log.fold.change_WT_atg8_0h > 1 & q.value_WT_atg8_0h < 0.05]
activated.genes.deseq2_WT_atg8_0h <- activated.genes.deseq2_WT_atg8_0h[!is.na(activated.genes.deseq2_WT_atg8_0h)]
activated.genes.deseq2_WT_atg8_0h_good <- substr(activated.genes.deseq2_WT_atg8_0h,1,nchar(activated.genes.deseq2_WT_atg8_0h))
write.table(activated.genes.deseq2_WT_atg8_0h_good,file="activated.genes.deseq2_WT_atg8_0h.txt",sep="\t")

repressed.genes.deseq2_WT_atg8_0h <- gene.ids[log.fold.change_WT_atg8_0h < -1 & q.value_WT_atg8_0h < 0.05]
repressed.genes.deseq2_WT_atg8_0h <- represed.genes.deseq2_WT_atg8_0h[!is.na(repressed.genes.deseq2_WT_atg8_0h)]
repressed.genes.deseq2_WT_atg8_0h_good <- substr(repressed.genes.deseq2_WT_atg8_0h,1,nchar(repressed.genes.deseq2_WT_atg8_0h))
write.table(repressed.genes.deseq2_WT_atg8_0h_good,file="represed.genes.deseq2_WT_atg8_0h.txt",sep="\t")

length(activated.genes.deseq2_WT_atg8_0h_good)

```

```
## [1] 83
```

```
length(repressed.genes.deseq2_WT_atg8_0h_good)
```

```
## [1] 117
```

## MA plot

```

plotMA(res_WT_atg8_0h,alpha=0.05, ylim=c(-30,30),colSig="grey")
abline(h=c(-1,1), col="black", lwd=1)
points(x = base.mean_WT_atg8_0h[activated.genes.deseq2_WT_atg8_0h],
       y = log.fold.change_WT_atg8_0h[activated.genes.deseq2_WT_atg8_0h],col="red",cex=0.2,pch=19)
points(x = base.mean_WT_atg8_0h[represed.genes.deseq2_WT_atg8_0h],
       y = log.fold.change_WT_atg8_0h[represed.genes.deseq2_WT_atg8_0h],col="blue",cex=0.2,pch=19)

text(x =100000, y = 15, label = length(activated.genes.deseq2_WT_atg8_0h_good),

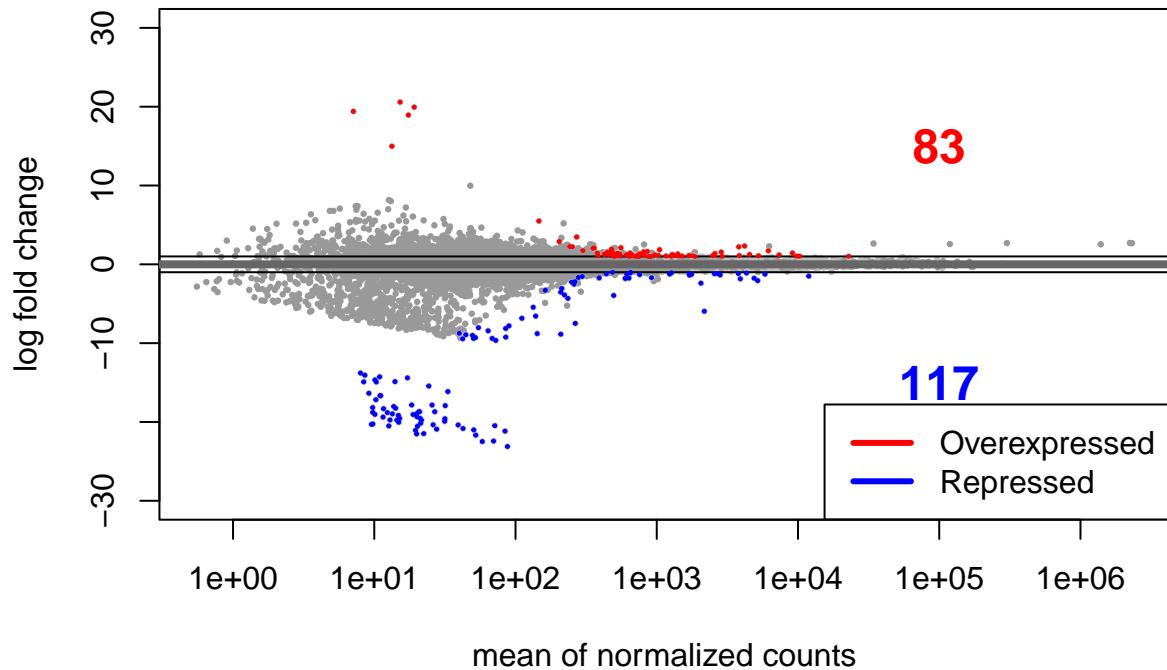
```

```

col = "red",    # Color del texto
font = 2,       # Estilo
cex = 1.5)      # Tamaño
text(x =100000, y = -15, label = length(repressed.genes.deseq2_WT_atg8_0h_good),
col = "blue",   # Color del texto
font = 2,       # Estilo
cex = 1.5)      # Tamaño

legend("bottomright", legend = c("Overexpressed", "Repressed"),
lwd = 3, col = c("red", "blue"))

```



## Difference between wild-type and mutant 4h

### Gene obtention

```

res_WT_atg8_4h = results(dds, list( c("genotype_atg8_vs_wt","genotypeatg8.condition4h")),
cooksCutoff = FALSE, independentFiltering = FALSE )
res_WT_atg8_4h

```

```

## log2 fold change (MLE): genotype_atg8_vs_wt+genotypeatg8.condition4h effect
## Wald test p-value: genotype_atg8_vs_wt+genotypeatg8.condition4h effect
## DataFrame with 13977 rows and 6 columns
##           baseMean log2FoldChange      lfcSE      stat     pvalue

```

```

## <numeric> <numeric> <numeric> <numeric>
## Cre14.g619950.v5.5 307.8280 -1.094574 0.719128 -1.522086 0.12798748
## Cre01.g048900.v5.5 76.4131 -0.476455 1.829920 -0.260369 0.79457914
## Cre12.g543400.v5.5 428.9410 0.631098 0.320734 1.967667 0.04910641
## Cre16.g685837.v5.5 255.7288 0.130937 0.454228 0.288264 0.77314498
## Cre06.g278159.v5.5 9770.0557 0.361482 0.124145 2.911778 0.00359378
## ...
## ...
## Cre06.g278104.v5.5 889.54623 0.228314 0.310320 0.735738 0.4618902
## Cre17.g717150.v5.5 146.81853 -0.644081 1.173279 -0.548958 0.5830341
## Cre10.g443600.v5.5 170.10265 1.887998 1.082569 1.743998 0.0811594
## Cre14.g621400.v5.5 5.13639 4.605473 4.141349 1.112071 0.2661077
## Cre10.g441750.v5.5 1599.45474 0.277861 0.181312 1.532499 0.1253994
## padj
## <numeric>
## Cre14.g619950.v5.5 0.5198724
## Cre01.g048900.v5.5 0.9823882
## Cre12.g543400.v5.5 0.3269940
## Cre16.g685837.v5.5 0.9793068
## Cre06.g278159.v5.5 0.0577359
## ...
## Cre06.g278104.v5.5 0.879075
## Cre17.g717150.v5.5 0.930899
## Cre10.g443600.v5.5 0.420310
## Cre14.g621400.v5.5 0.733029
## Cre10.g441750.v5.5 0.515712

```

```

log.fold.change_WT_atg8_4h <- res_WT_atg8_4h$log2FoldChange
q.value_WT_atg8_4h <- res_WT_atg8_4h$padj
names(log.fold.change_WT_atg8_4h) <- gene.ids
names(q.value_WT_atg8_4h) <- gene.ids
base.mean_WT_atg8_4h <- res_WT_atg8_4h$baseMean
names(base.mean_WT_atg8_4h) <- gene.ids

```

*# Take a look at upregulated and downregulated genes:*

```

top_20_overexpressed_genes_WT_atg8_4h <- res_WT_atg8_4h[order(res_WT_atg8_4h$log2FoldChange, decreasing = TRUE), 1:20]
kable(top_20_overexpressed_genes_WT_atg8_4h[1:20,-(3:4)])

```

	baseMean	log2FoldChange	pvalue	padj
Cre17.g712700.v5.5	42.409362	23.20189	0e+00	0.00e+00
Cre16.g658400.v5.5	59.759725	23.01070	0e+00	0.00e+00
Cre12.g524450.v5.5	32.024953	21.89046	0e+00	4.00e-07
Cre13.g588250.v5.5	49.571334	21.80803	0e+00	0.00e+00
Cre16.g687966.v5.5	33.784426	21.79817	0e+00	0.00e+00
Cre03.g143827.v5.5	66.501562	21.66314	0e+00	0.00e+00
Cre15.g636600.v5.5	58.721594	21.62000	0e+00	0.00e+00
Cre07.g336500.v5.5	36.496724	21.41570	0e+00	4.00e-07
Cre01.g043250.v5.5	102.227854	21.36041	0e+00	0.00e+00
Cre03.g195410.v5.5	16.175576	21.30459	0e+00	5.00e-07
Cre12.g524350.v5.5	18.072167	21.27414	6e-07	3.66e-05
Cre03.g179400.v5.5	39.299172	21.24146	0e+00	0.00e+00
Cre11.g467532.v5.5	33.222102	21.23809	0e+00	0.00e+00
Cre05.g240100.v5.5	25.335507	21.21101	0e+00	3.60e-06
Cre05.g239000.v5.5	46.353687	21.15574	0e+00	0.00e+00
Cre06.g292600.v5.5	15.273678	21.14644	7e-07	4.17e-05
Cre01.g034380.v5.5	38.571871	21.10793	0e+00	0.00e+00
Cre10.g422350.v5.5	8.728187	21.10641	7e-07	4.32e-05
Cre09.g402050.v5.5	54.881325	21.10392	0e+00	0.00e+00
Cre14.g629840.v5.5	37.019186	21.03051	0e+00	0.00e+00

```
top_20_overrepressed_genes_WT_atg8_4h <- res_WT_atg8_4h[order(res_WT_atg8_4h$log2FoldChange, decreasing = TRUE), 1:20, -(3:4)]
```

	baseMean	log2FoldChange	pvalue	padj
Cre02.g077951.v5.5	22.40658	-27.39922	0e+00	0.00e+00
Cre10.g433650.v5.5	19.95526	-26.86531	0e+00	0.00e+00
Cre07.g350050.v5.5	12.69064	-26.22043	0e+00	1.00e-07
Cre02.g095141.v5.5	19.61755	-26.18287	0e+00	0.00e+00
Cre07.g315050.v5.5	88.92973	-23.62099	0e+00	0.00e+00
Cre16.g684861.v5.5	36.98465	-22.61599	0e+00	0.00e+00
Cre07.g327079.v5.5	23.64919	-22.52874	1e-07	6.40e-06
Cre13.g592350.v5.5	49.26531	-22.51609	0e+00	0.00e+00
Cre14.g623300.v5.5	27.93290	-22.39287	0e+00	0.00e+00
Cre13.g604550.v5.5	56.05740	-22.25126	0e+00	0.00e+00
Cre02.g118450.v5.5	30.20969	-22.24960	0e+00	4.50e-06
Cre03.g205800.v5.5	69.35132	-22.19388	2e-07	1.33e-05
Cre12.g509600.v5.5	38.66322	-22.15181	0e+00	0.00e+00
Cre06.g278168.v5.5	57.62603	-22.07722	0e+00	0.00e+00
Cre03.g153250.v5.5	28.01849	-22.07050	1e-07	9.40e-06
Cre07.g314700.v5.5	37.60714	-21.95988	0e+00	0.00e+00
Cre16.g678997.v5.5	30.44633	-21.93106	0e+00	0.00e+00
Cre06.g294250.v5.5	123.06077	-21.90895	0e+00	0.00e+00
Cre15.g636250.v5.5	24.67298	-21.89186	0e+00	1.80e-06
Cre01.g010832.v5.5	37.26769	-21.87643	0e+00	0.00e+00

```
activated.genes.deseq2_WT_atg8_4h <- gene.ids$log.fold.change_WT_atg8_4h > 1 & q.value_WT_atg8_4h < 0.05
activated.genes.deseq2_WT_atg8_4h <- activated.genes.deseq2_WT_atg8_4h[!is.na(activated.genes.deseq2_WT_atg8_4h)]
activated.genes.deseq2_WT_atg8_4h_good <- substr(activated.genes.deseq2_WT_atg8_4h, 1, nchar(activated.genes.deseq2_WT_atg8_4h))
write.table(activated.genes.deseq2_WT_atg8_4h_good, file = "activated.genes.deseq2_WT_atg8_4h.txt", sep = "\t")
```

```

repressed.genes.deseq2_WT_atg8_4h <- gene.ids[log.fold.change_WT_atg8_4h < -1 & q.value_WT_atg8_4h < 0.05]
repressed.genes.deseq2_WT_atg8_4h <- represed.genes.deseq2_WT_atg8_4h[!is.na(repressed.genes.deseq2_WT_atg8_4h)]
repressed.genes.deseq2_WT_atg8_4h_good <- substr(repressed.genes.deseq2_WT_atg8_4h,1,nchar(repressed.genes.deseq2_WT_atg8_4h))
write.table(repressed.genes.deseq2_WT_atg8_4h_good,file="represed.genes.deseq2_WT_atg8_4h.txt",sep="\t")

length(activated.genes.deseq2_WT_atg8_4h)

## [1] 193

length(repressed.genes.deseq2_WT_atg8_4h)

## [1] 187

```

## MA plot

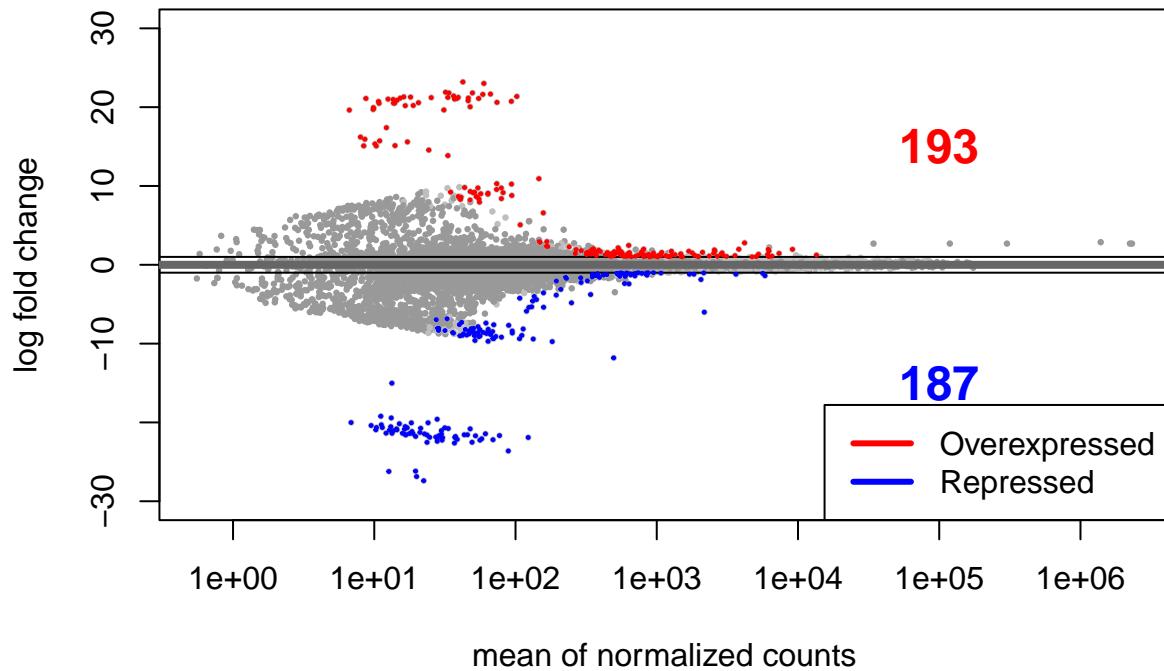
```

plotMA(res_WT_atg8_4h, ylim=c(-30,30),colSig="grey")
abline(h=c(-1,1), col="black", lwd=1)
points(x = base.mean_WT_atg8_4h[activated.genes.deseq2_WT_atg8_4h],
       y = log.fold.change_WT_atg8_4h[activated.genes.deseq2_WT_atg8_4h],col="red",cex=0.2,pch=19)
points(x = base.mean_WT_atg8_4h[repressed.genes.deseq2_WT_atg8_4h],
       y = log.fold.change_WT_atg8_4h[repressed.genes.deseq2_WT_atg8_4h],col="blue",cex=0.2,pch=19)

text(x =100000, y = 15, label = length(activated.genes.deseq2_WT_atg8_4h_good),
      col = "red",    # Color del texto
      font = 2,        # Estilo
      cex = 1.5)      # Tamaño
text(x =100000, y = -15, label = length(repressed.genes.deseq2_WT_atg8_4h_good),
      col = "blue",   # Color del texto
      font = 2,        # Estilo
      cex = 1.5)      # Tamaño

legend("bottomright", legend = c("Overexpressed", "Repressed"),
      lwd = 3, col = c("red", "blue"))

```



Different response for both genotypes (the interaction term)

Gene obtention

```
res_interaction = results(dds, name="genotypeatg8.condition4h",
                         cooksCutoff = FALSE, independentFiltering = FALSE)
res_interaction
```

```
## log2 fold change (MLE): genotypeatg8.condition4h
## Wald test p-value: genotypeatg8.condition4h
## DataFrame with 13977 rows and 6 columns
##           baseMean log2FoldChange      lfcSE       stat     pvalue
##           <numeric>      <numeric> <numeric>      <numeric> <numeric>
## Cre14.g619950.v5.5    307.8280   -0.0459415  1.017335 -0.0451587 0.9639808
## Cre01.g048900.v5.5     76.4131   -0.7332081  2.588131 -0.2832963 0.7769497
## Cre12.g543400.v5.5    428.9410    0.2057964  0.453622  0.4536733 0.6500640
## Cre16.g685837.v5.5    255.7288    0.0611372  0.643788  0.0949648 0.9243428
## Cre06.g278159.v5.5   9770.0557    0.4411260  0.175749  2.5099837 0.0120737
## ...
##           ...        ...       ...       ...     ...
## Cre06.g278104.v5.5   889.54623   0.83288039  0.439237  1.896199 0.0579338
## Cre17.g717150.v5.5   146.81853   -1.63816151  1.657499 -0.988333 0.3229896
## Cre10.g443600.v5.5   170.10265   1.76031972  1.528515  1.151653 0.2494635
## Cre14.g621400.v5.5    5.13639   -2.18143969  5.840032 -0.373532 0.7087524
```

```

## Cre10.g441750.v5.5 1599.45474      0.00806577  0.256806   0.031408  0.9749441
##                               padj
##                               <numeric>
## Cre14.g619950.v5.5  0.999419
## Cre01.g048900.v5.5  0.999419
## Cre12.g543400.v5.5  0.999419
## Cre16.g685837.v5.5  0.999419
## Cre06.g278159.v5.5  0.366382
## ...
## Cre06.g278104.v5.5  0.772653
## Cre17.g717150.v5.5  0.999419
## Cre10.g443600.v5.5  0.999419
## Cre14.g621400.v5.5  0.999419
## Cre10.g441750.v5.5  0.999419

log.fold.change_interaction <- res_interaction$log2FoldChange
q.value_interaction <- res_interaction$padj
names(log.fold.change_interaction) <- gene.ids
names(q.value_interaction) <- gene.ids
base.mean_interaction <- res_interaction$baseMean
names(base.mean_interaction) <- gene.ids

# Take a look at upregulated and downregulated genes:

top_20_overexpressed_genes_interaction <- res_interaction[order(res_interaction$log2FoldChange, decreasing = TRUE), 1:20]
kable(top_20_overexpressed_genes_interaction[1:20,-(3:4)])

```

	baseMean	log2FoldChange	pvalue	padj
Cre04.g214769.v5.5	8.578910	30.00000	0.0000006	0.0001753
Cre07.g331114.v5.5	14.030683	30.00000	0.0000006	0.0001753
Cre03.g196000.v5.5	10.329530	30.00000	0.0000006	0.0001753
Cre04.g213950.v5.5	7.990901	30.00000	0.0000006	0.0001753
Cre06.g292450.v5.5	8.428540	30.00000	0.0000006	0.0001753
Cre11.g481104.v5.5	17.175085	30.00000	0.0000003	0.0001134
Cre04.g219750.v5.5	10.123388	30.00000	0.0000006	0.0001753
Cre02.g087500.v5.5	10.921086	30.00000	0.0000006	0.0001753
Cre14.g633900.v5.5	24.339891	30.00000	0.0000001	0.0000342
Cre02.g099100.v5.5	33.227640	30.00000	0.0000000	0.0000036
Cre16.g658300.v5.5	46.217102	23.52922	0.0000000	0.0000006
Cre03.g183150.v5.5	52.341644	22.83219	0.0000000	0.0000245
Cre03.g206900.v5.5	39.266900	22.28228	0.0000005	0.0001753
Cre07.g319600.v5.5	9.767238	22.09547	0.0001150	0.0143253
Cre02.g098550.v5.5	10.725460	22.02765	0.0001957	0.0199689
Cre17.g699550.v5.5	11.544327	22.01875	0.0001931	0.0199689
Cre07.g346900.v5.5	87.770943	21.99156	0.0000000	0.0000000
Cre07.g330150.v5.5	6.660717	21.94332	0.0002175	0.0218705
Cre03.g179400.v5.5	39.299172	21.70125	0.0000006	0.0001753
Cre16.g658400.v5.5	59.759725	21.68779	0.0000001	0.0000285

```

top_20_overrepressed_genes_interaction <- res_interaction[order(res_interaction$log2FoldChange, decreasing = TRUE), 1:20]
kable(top_20_overrepressed_genes_interaction[1:20,-(3:4)])

```

	baseMean	log2FoldChange	pvalue	padj
Cre06.g250650.v5.5	13.336058	-30.00000	0.0000006	0.0001753
Cre16.g691351.v5.5	18.498013	-24.96833	0.0000052	0.0012079
Cre07.g315050.v5.5	88.929727	-24.78043	0.0000000	0.0000000
Cre02.g118450.v5.5	30.209685	-24.24031	0.0000181	0.0034299
Cre13.g592350.v5.5	49.265306	-24.11483	0.0000000	0.0000053
Cre13.g604550.v5.5	56.057399	-23.90740	0.0000000	0.0000001
Cre16.g678997.v5.5	30.446334	-23.88634	0.0000003	0.0001089
Cre16.g695200.v5.5	28.693675	-23.54711	0.0000408	0.0066344
Cre12.g544327.v5.5	9.507146	-23.34612	0.0000802	0.0112272
Cre09.g413000.v5.5	10.374182	-23.30969	0.0000816	0.0112272
Cre03.g153250.v5.5	28.018485	-23.29247	0.0000548	0.0086987
Cre16.g684861.v5.5	36.984651	-23.08733	0.0000000	0.0000077
Cre12.g486450.v5.5	11.227031	-22.92555	0.0001045	0.0133968
Cre08.g382825.v5.5	12.828425	-22.85710	0.0001092	0.0138783
Cre14.g609150.v5.5	11.132919	-22.63246	0.0001300	0.0152511
Cre03.g151300.v5.5	13.711523	-22.62959	0.0001261	0.0150679
Cre03.g205800.v5.5	69.351320	-22.56410	0.0001282	0.0151822
Cre10.g466200.v5.5	15.235117	-22.51100	0.0001425	0.0161548
Cre17.g736350.v5.5	15.843663	-22.49394	0.0001378	0.0159119
Cre04.g215001.v5.5	11.408657	-22.48296	0.0001406	0.0161085

```

activated.genes.deseq2_interaction <- gene.ids[log.fold.change_interaction > 1 & q.value_interaction <
activated.genes.deseq2_interaction <- activated.genes.deseq2_interaction[!is.na(activated.genes.deseq2_
activated.genes.deseq2_interaction_good <- substr(activated.genes.deseq2_interaction,1,nchar(activated.
write.table(activated.genes.deseq2_interaction_good,file="activated.genes.deseq2_interaction.txt",sep="

repressed.genes.deseq2_interaction <- gene.ids[log.fold.change_interaction < -1 & q.value_interaction <
repressed.genes.deseq2_interaction <- repressed.genes.deseq2_interaction[!is.na(repressed.genes.deseq2_
repressed.genes.deseq2_interaction_good <- substr(repressed.genes.deseq2_interaction,1,nchar(repressed.
write.table(repressed.genes.deseq2_interaction_good,file="repressed.genes.deseq2_interaction.txt",sep="

length(activated.genes.deseq2_interaction)

## [1] 103

length(repressed.genes.deseq2_interaction)

## [1] 87

```

### MA plot

```

plotMA(res_interaction, ylim=c(-30,30),colSig="grey")
abline(h=c(-1,1), col="black", lwd=1)
points(x = base.mean_interaction[activated.genes.deseq2_interaction],
       y = log.fold.change_interaction[activated.genes.deseq2_interaction],col="red",cex=0.2,pch=19)
points(x = base.mean_interaction[repressed.genes.deseq2_interaction],
       y = log.fold.change_interaction[repressed.genes.deseq2_interaction],col="blue",cex=0.2,pch=19)

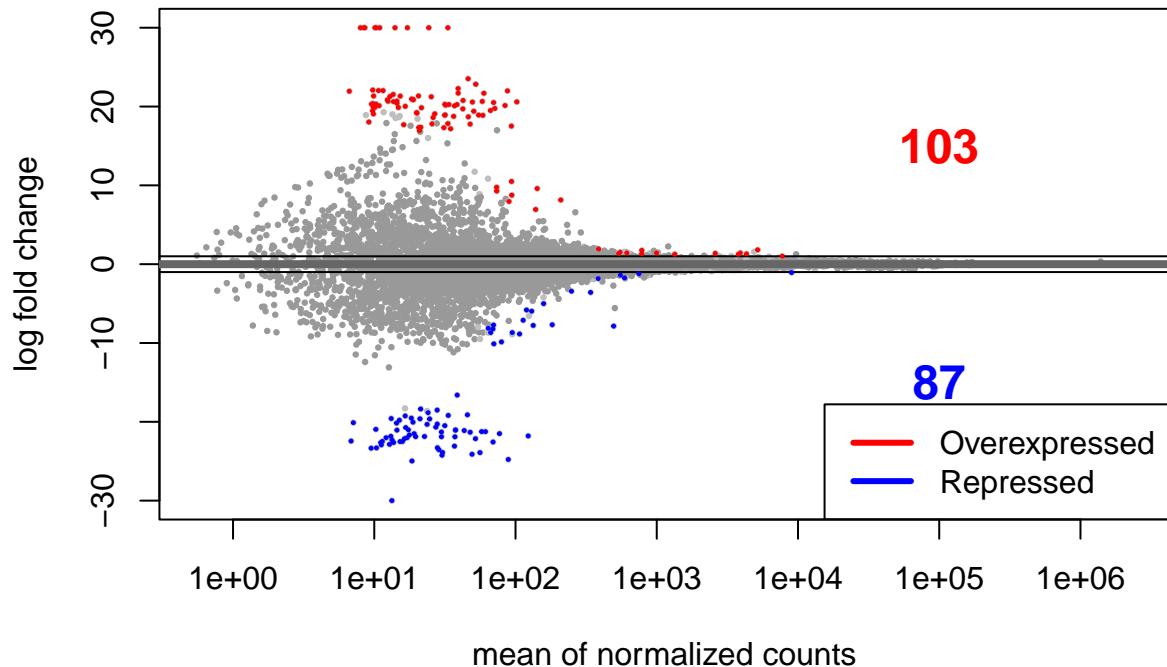
```

```

text(x = 100000, y = 15, label = length(activated.genes.deseq2_interaction_good),
      col = "red",    # Color del texto
      font = 2,        # Estilo
      cex = 1.5)       # Tamaño
text(x = 100000, y = -15, label = length(repressed.genes.deseq2_interaction_good),
      col = "blue",   # Color del texto
      font = 2,        # Estilo
      cex = 1.5)       # Tamaño

legend("bottomright", legend = c("Overexpressed", "Repressed"),
      lwd = 3, col = c("red", "blue"))

```



## Heatmap

```

interaction_sigs <- res_interaction[res_interaction$padj < 0.05,]
interaction_df <- as.data.frame(interaction_sigs)
interaction_dftop <- interaction_df[interaction_df$baseMean > 50 &
                                         abs(interaction_df$log2FoldChange) > 1,]
interaction_dftop <- interaction_dftop[order(interaction_dftop$log2FoldChange, decreasing=TRUE),]
mat_int <- assay(vsd)[rownames(interaction_dftop), rownames(colData(vsd))]
colnames(mat_int) <- rownames(colData(vsd))
base_mean_int <- rowMeans(mat_int)
mat_int_scaled <- t(apply(mat_int, 1, scale))

```

```

colnames(mat_int_scaled) <- colnames(mat_int) # Original code, changed sample names in final figure
num_keep <- 25
rows_keep <- c(seq(1:num_keep), seq((nrow(mat_int_scaled)-num_keep),nrow(mat_int_scaled)))
l2_val <- as.matrix(interaction_dftop[rows_keep,]$log2FoldChange)
colnames(l2_val) <- "logFC"
mean <- as.matrix(interaction_dftop[rows_keep,]$baseMean)
colnames(mean) <- "AveExp"

# maps values between b/w/r for min and max l2 values
col_logFC <- colorRamp2(c(min(l2_val),0, max(l2_val)),c("blue", "white","red"))

# maps between 0% quantile, and 75% quantile of mean values
col_AveExpr <- colorRamp2(c(quantile(mean)[1],quantile(mean)[4]),c("white", "red"))

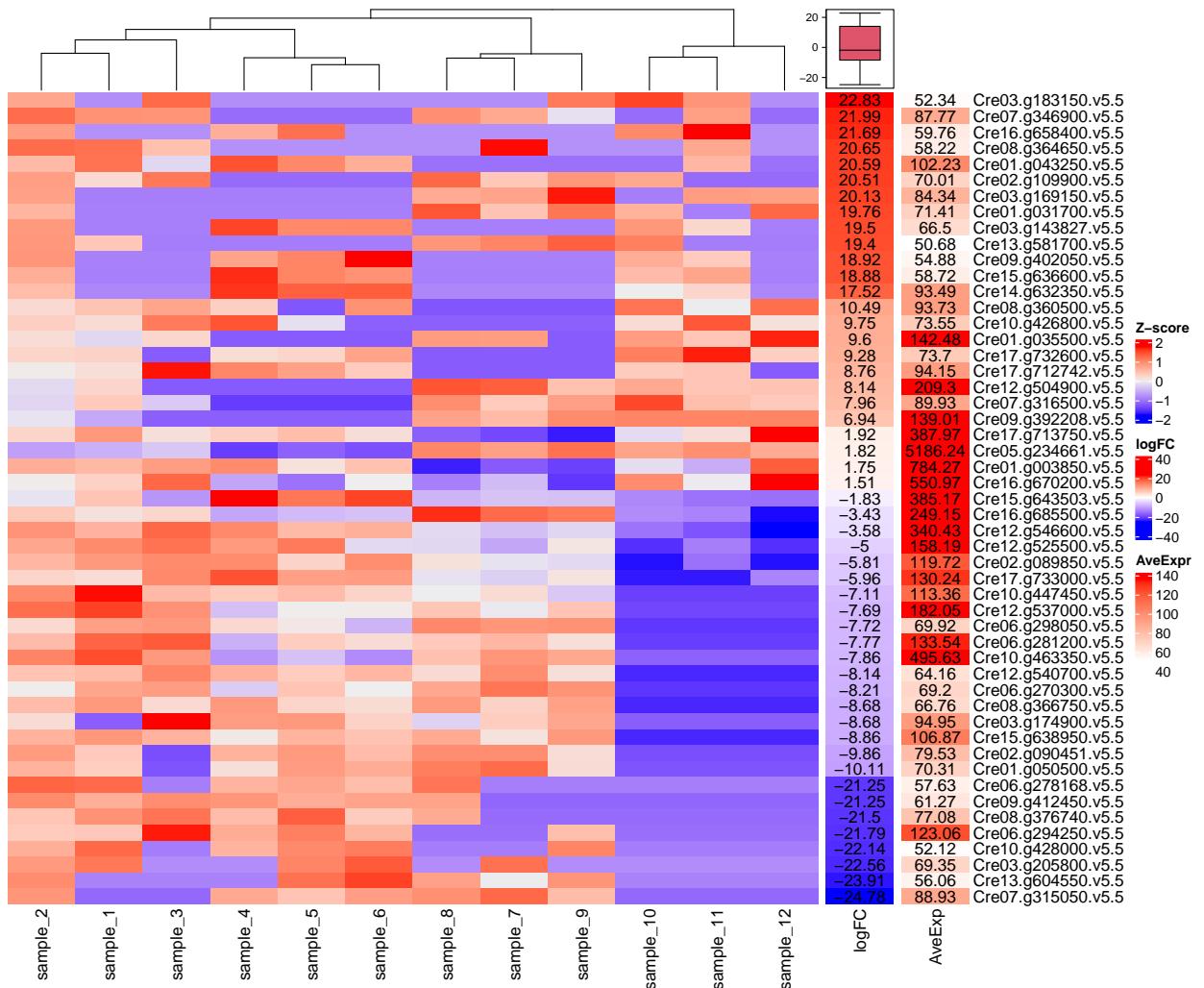
ha <- HeatmapAnnotation(summary = anno_summary(gp=gpar(fill=2), height =unit(2, "cm")))

h1 <- Heatmap(mat_int_scaled[rows_keep,],cluster_rows = F, column_labels = colnames(mat_int_scaled), na_color="white")
h2 <- Heatmap(l2_val, row_labels = rownames(interaction_dftop[rows_keep,]), cluster_rows = F, name= "logFC")
grid.text(round(l2_val[i,j],2),x,y)
})

h3 <- Heatmap(mean, row_labels = rownames(interaction_dftop[rows_keep,]), cluster_rows = F, name= "AveExp")
grid.text(round(mean[i,j],2),x,y)
}

h <- h1+h2+h3
h

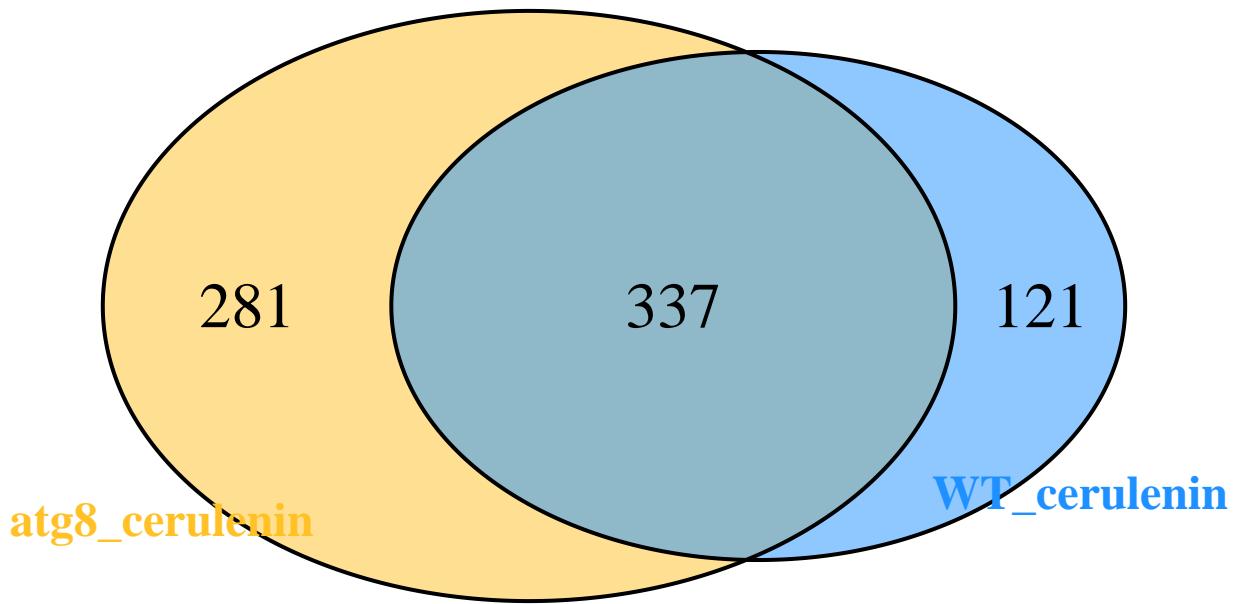
```



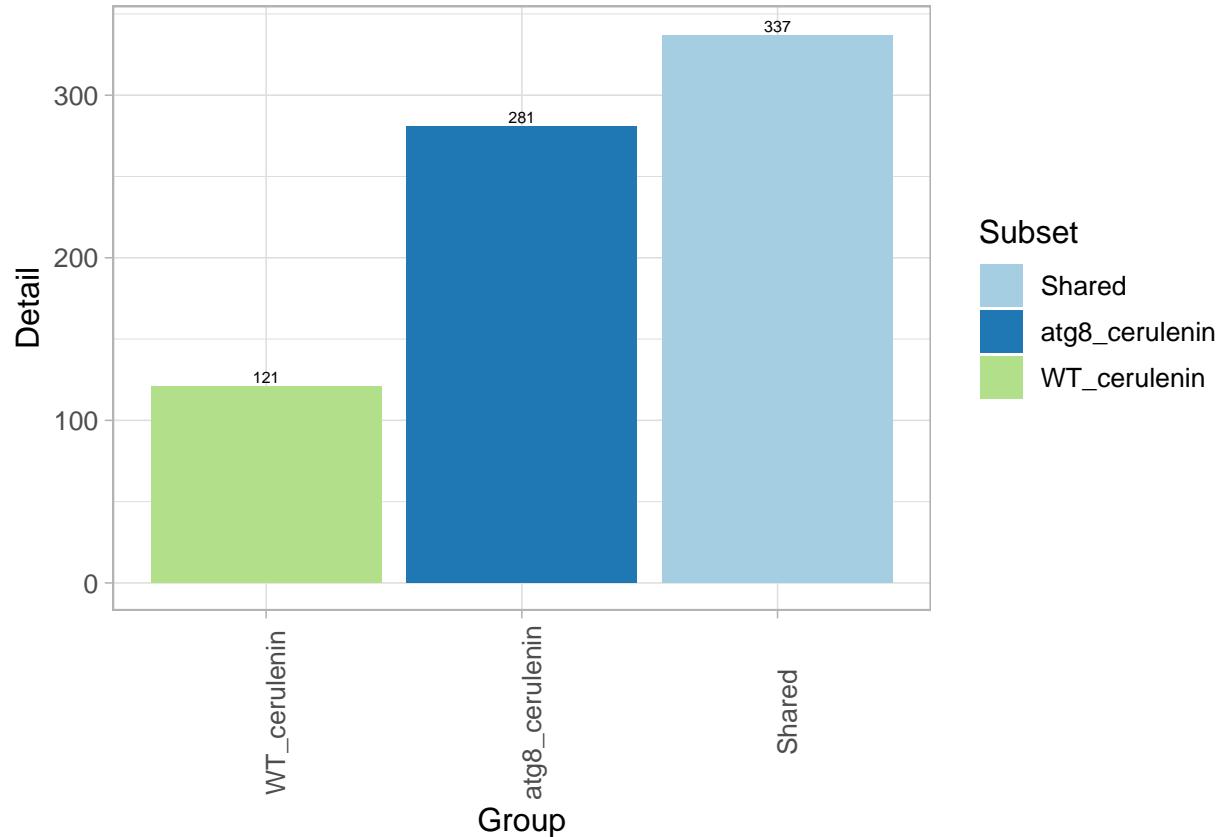
Are there any common genes between WT and atg8 the activated and/or repressed genes with treatment?

### Venn Diagrams

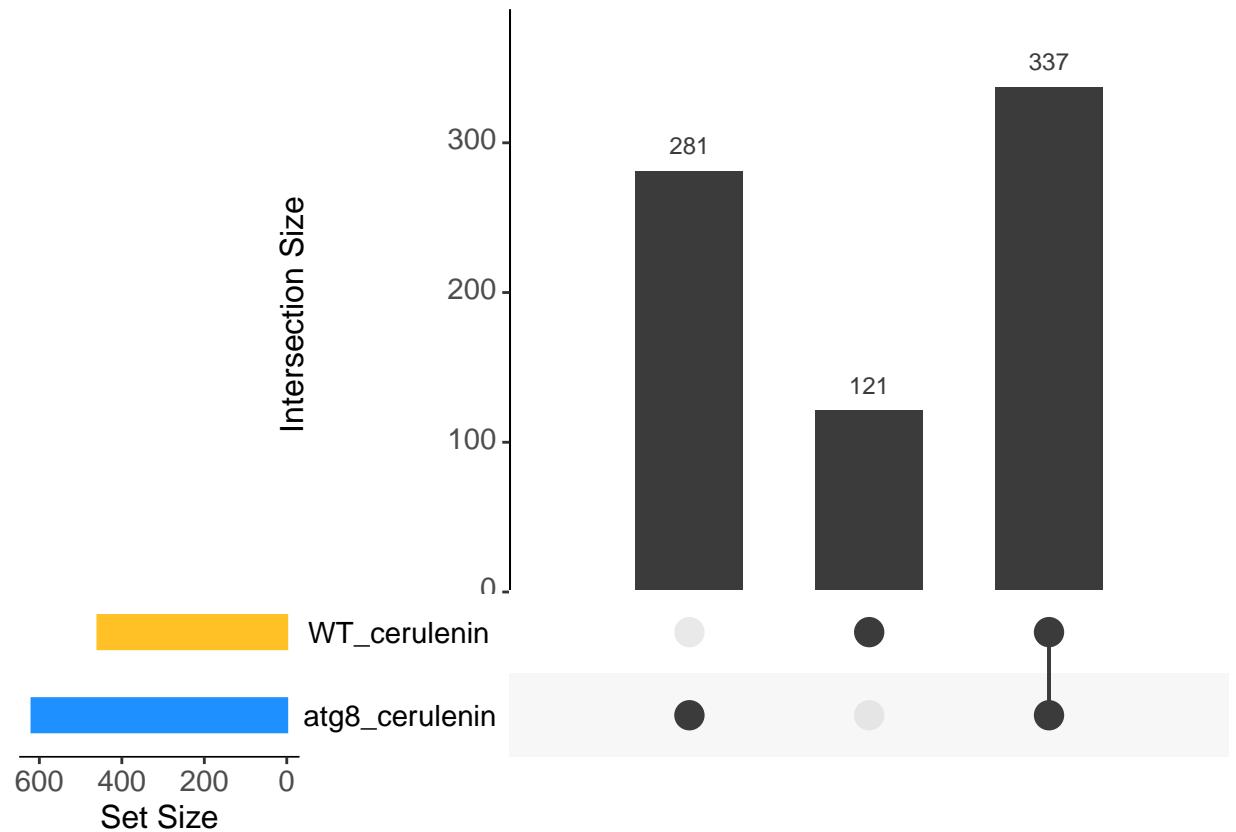
```
ven <- venndetail(list(WT_cerulenin= activated.genes.deseq2_WT_good, atg8_cerulenin=activated.genes.des
plot(ven, order=FALSE)
```



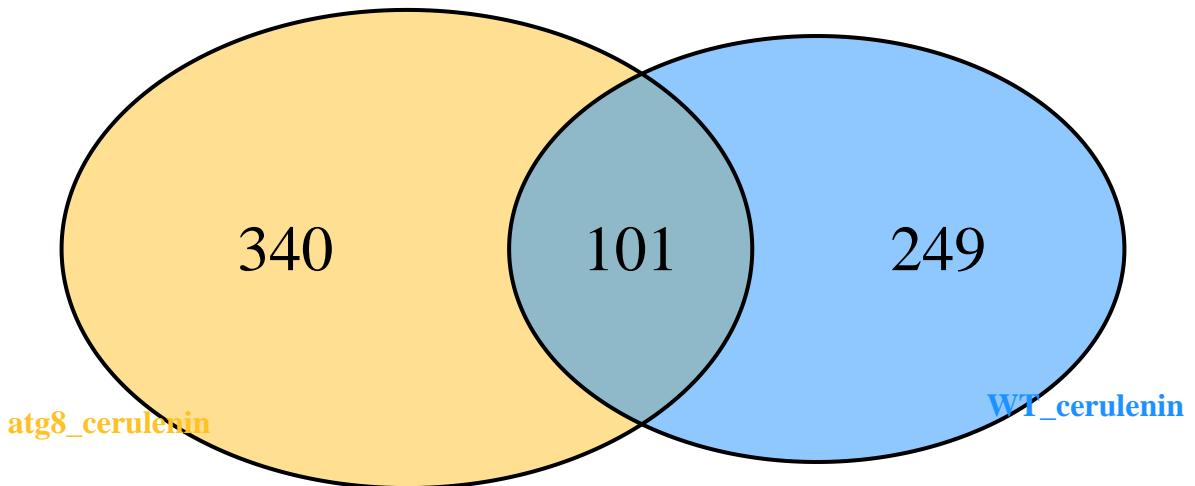
```
dplot(ven, order = TRUE, textsize = 2)
```



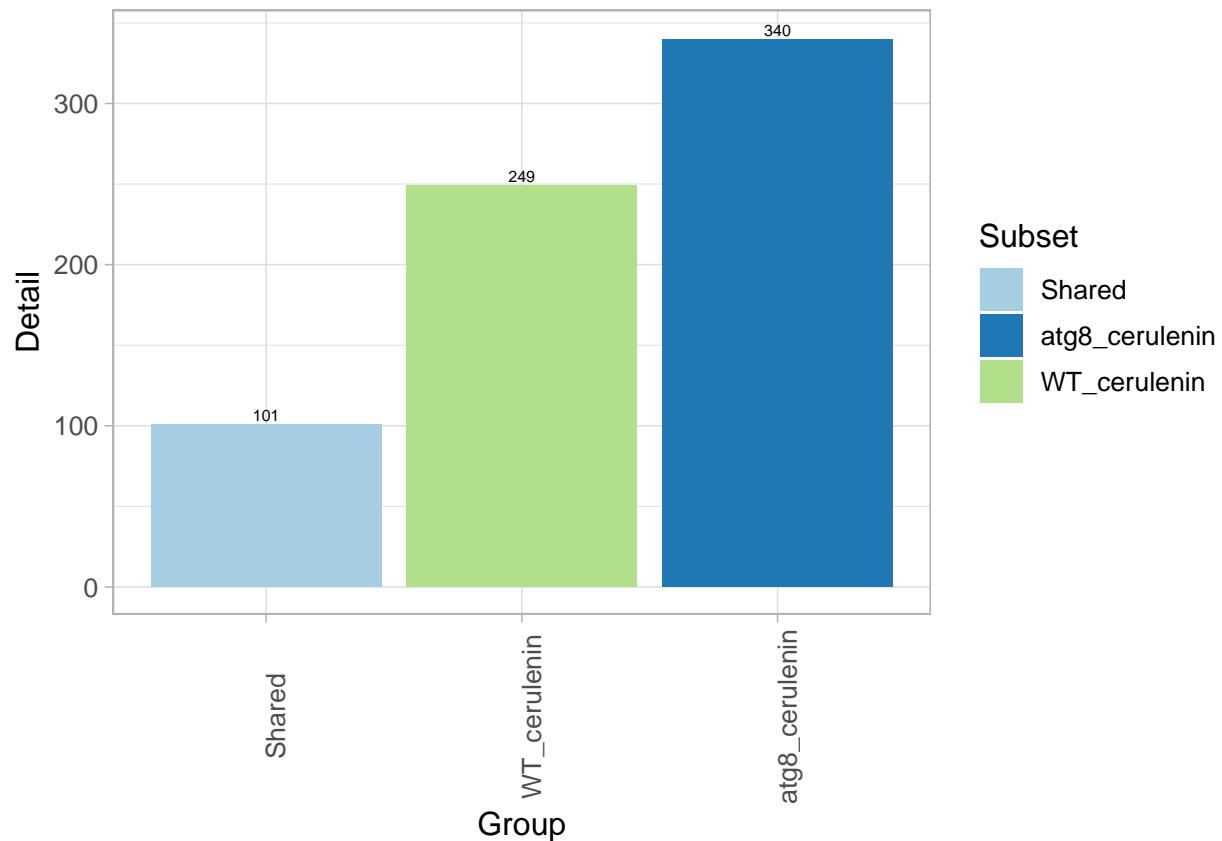
```
plot(ven, type = "upset")
```



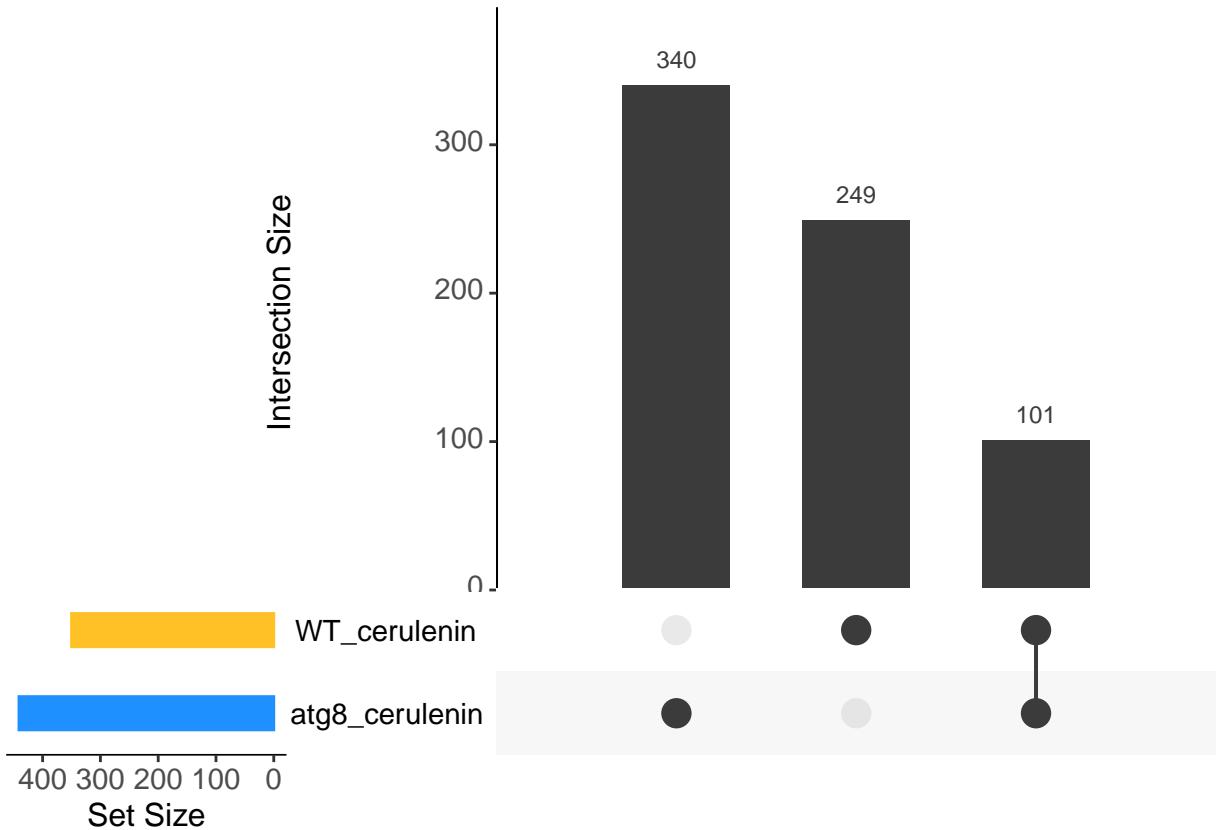
```
ven2 <- venndetail(list(WT_cerulenin= repressed.genes.deseq2_WT_good, atg8_cerulenin= repressed.genes.deseq2_atg8_good))
plot(ven2, cat.cex=1, order=FALSE)
```



```
dplot(ven2, order = TRUE, textsize = 2)
```



```
plot(ven2, type = "upset")
```



### Lists of shared genes

```

shared.activated <- result(ven)
shared.activated_cerulenin <- shared.activated %>% filter(shared.activated$Subset=="Shared")
shared.repressed <- result(ven2)
shared.repressed_cerulenin <- shared.repressed %>% filter(shared.repressed$Subset=="Shared")

```

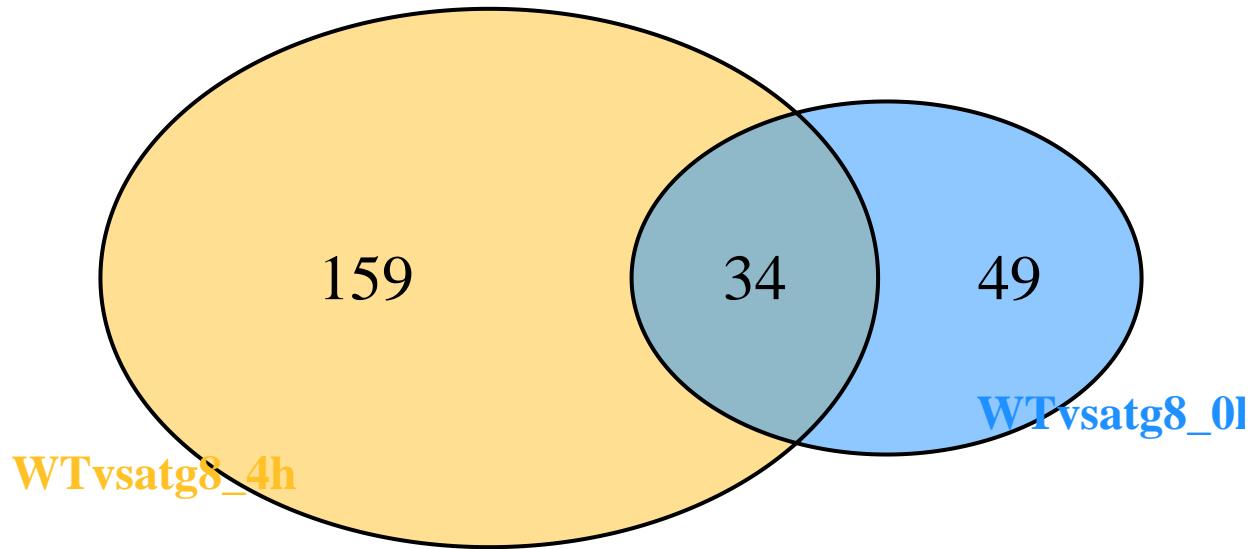
Are there any common genes between WT and atg8 activated and/or repressed genes with and without treatment?

### Venn Diagrams

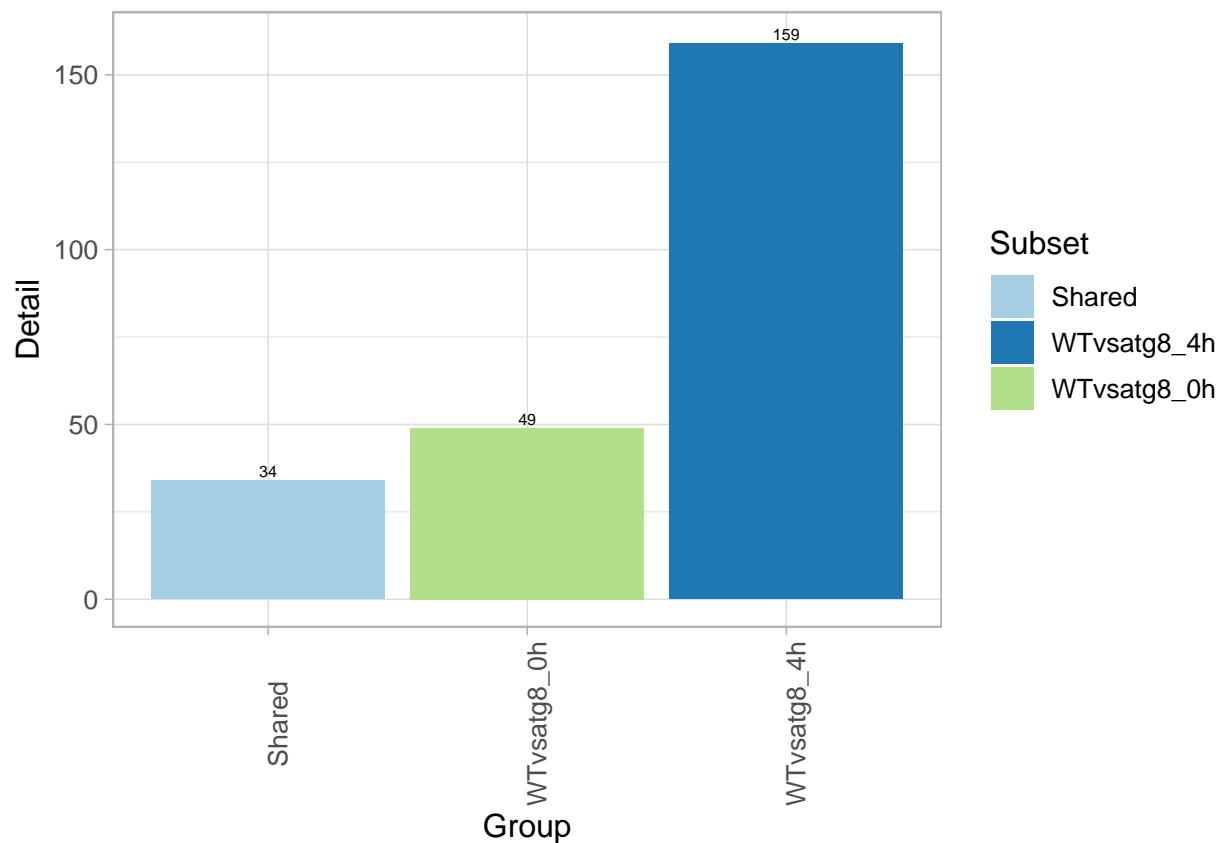
```

ven3 <- venndetail(list(WTvsatg8_0h= activated.genes.deseq2_WT_atg8_0h_good, WTvsatg8_4h=activated.genes.deseq2_WT_atg8_4h_good))
plot(ven3)

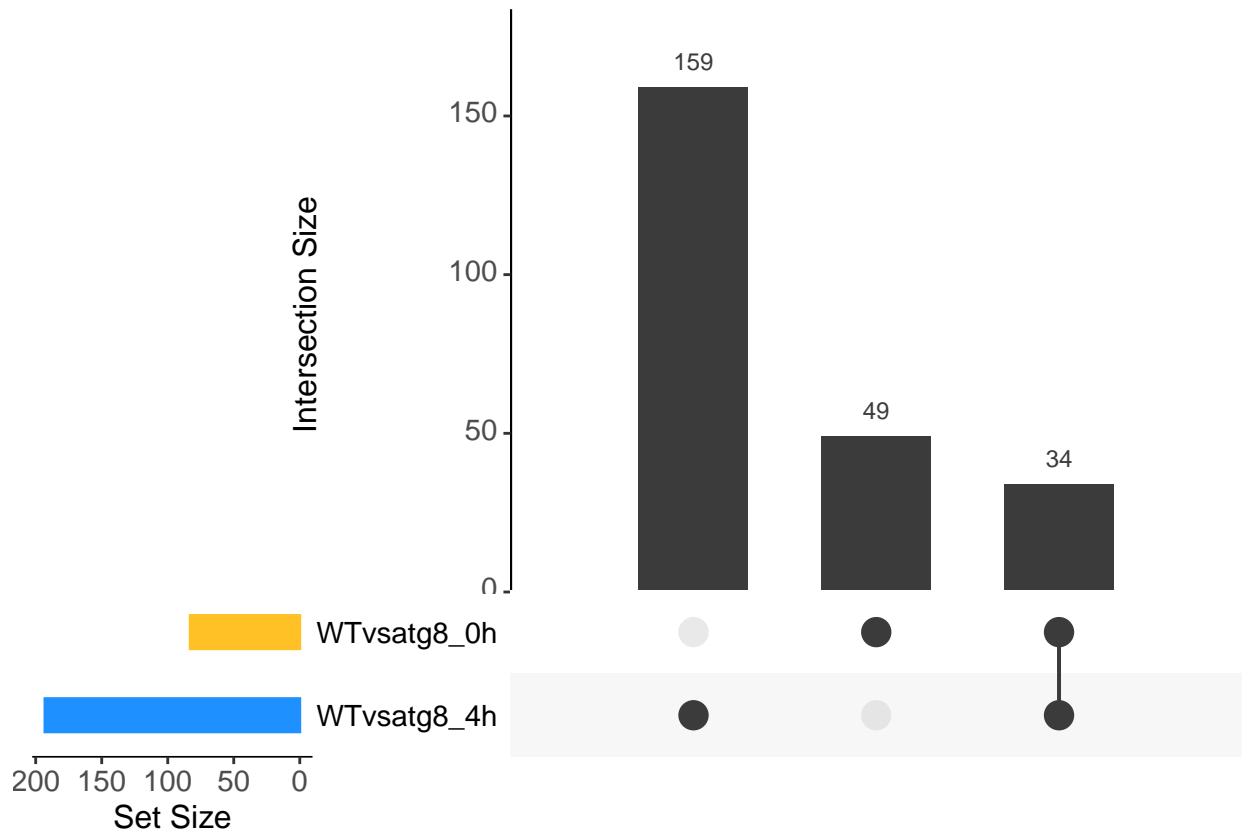
```



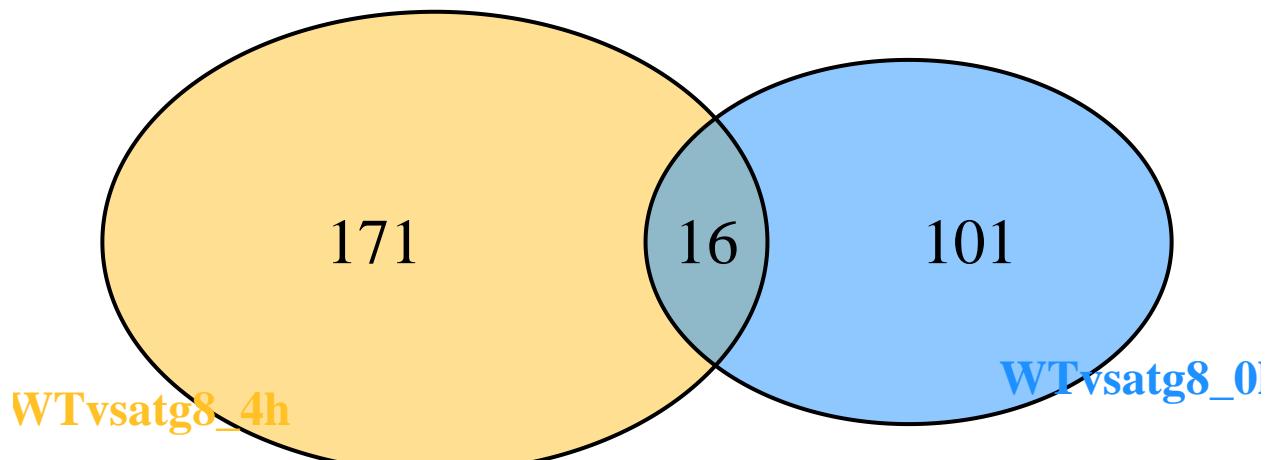
```
dplot(ven3, order = TRUE, textsize = 2)
```



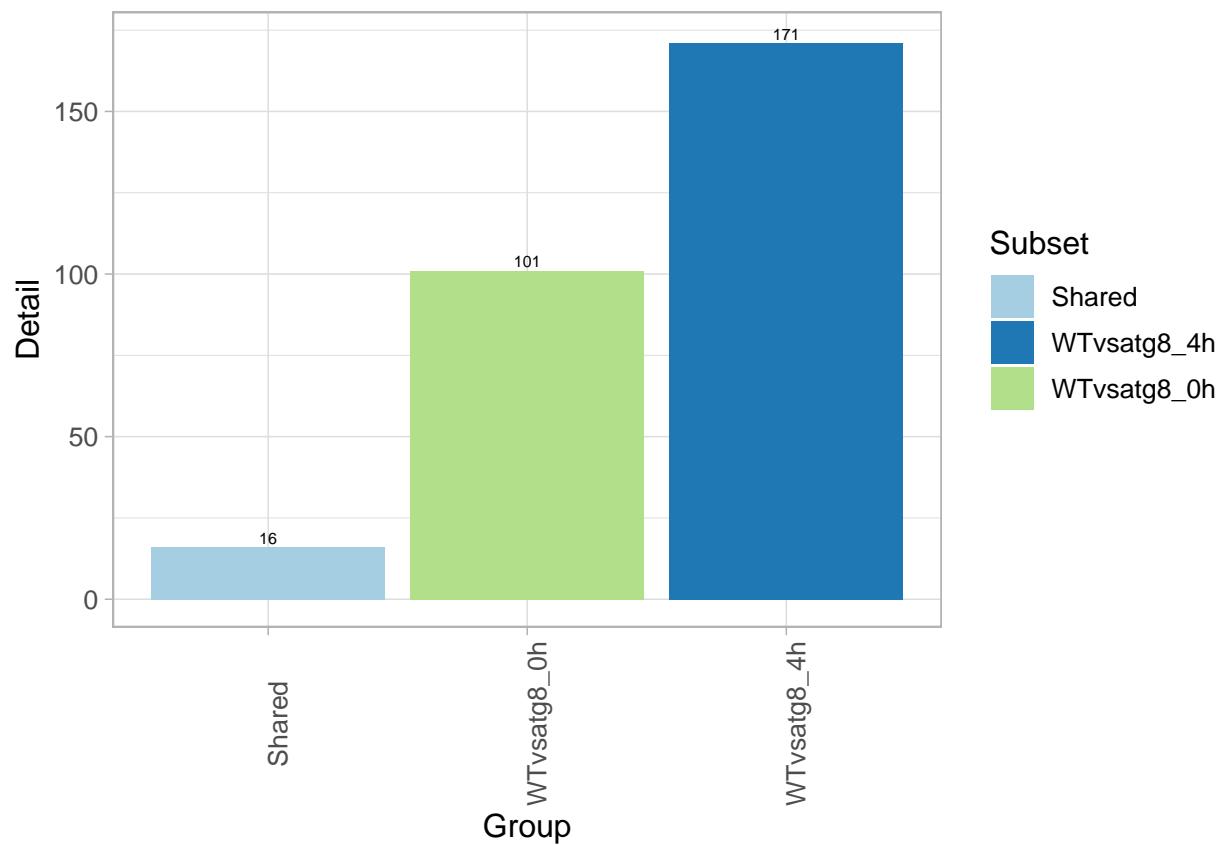
```
plot(ven3, type = "upset")
```



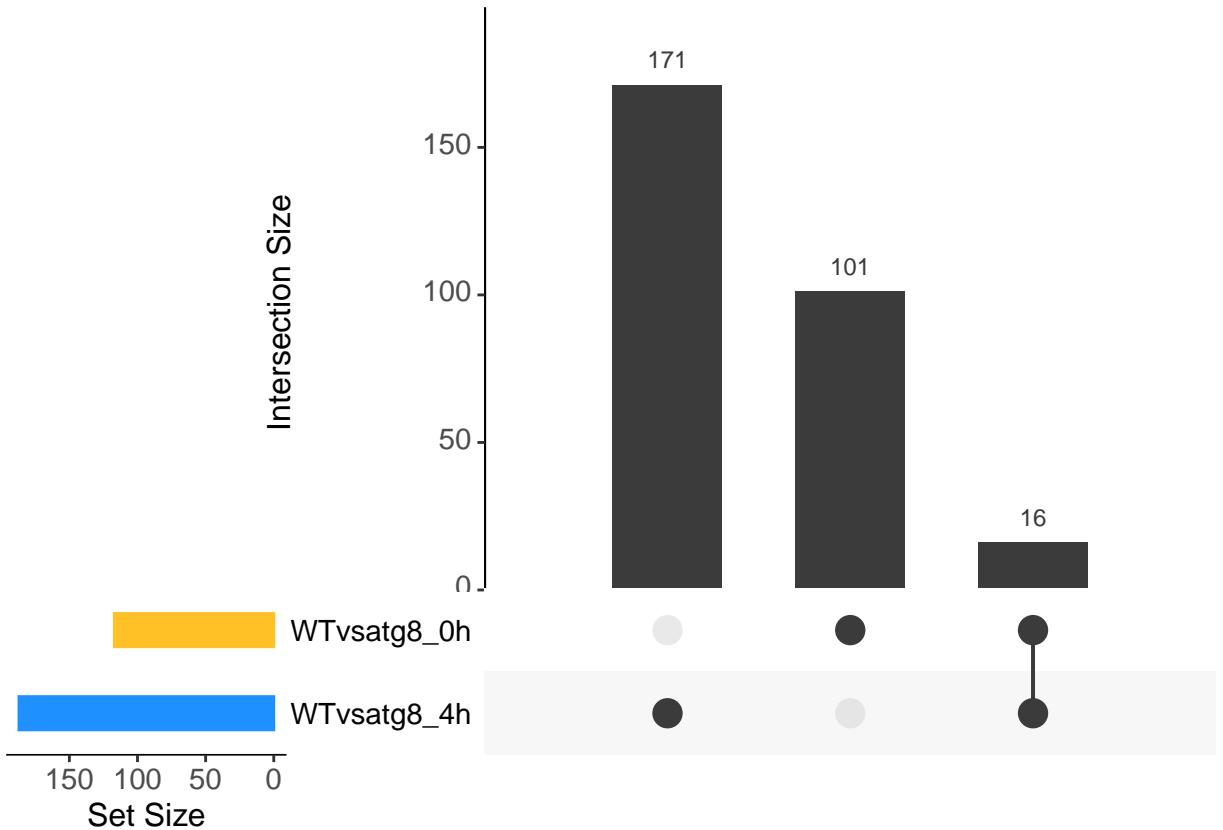
```
ven4 <- venndetail(list(WTvsatg8_0h= repressed.genes.deseq2_WT_atg8_0h_good, WTvsatg8_4h= repressed.genes.deseq2_WT_atg8_4h_good))  
plot(ven4)
```



```
dplot(ven4, order = TRUE, textsize = 2)
```



```
plot(ven4, type = "upset")
```



#### Lists of shared genes

```

shared.activated2 <- result(ven3)
shared.activated_0h <- shared.activated2 %>% filter(shared.activated2$Subset=="Shared")
shared.repressed2 <- result(ven4)
shared.repressed_4h <- shared.repressed2 %>% filter(shared.repressed2$Subset=="Shared")

```