DE analysis - Day8

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Overview

- Principal Investigator: Beth Overmoyer
- Experiment: RNAseq_analysis_of_inflammatory_breast_cancer_hbc04141
- study 6 was excluded because if low read depth in 3373-3
- https://www.bioconductor.org/packages/release/bioc/vignettes/DEGreport/inst/doc/DEGreport.html
- AnnotationHub. We use ensembl version matching bcbio pipeline - v94.
- HBC materials
- HBC materials - functional analysis
- http://bioconductor.org/packages/release/bioc/vignettes/DESeq2/inst/doc/DESeq2.html
- this is DE for Day8 samples

```
## Setup
### Bioconductor and CRAN libraries used

library(DESeq2)
```

```
## Loading required package: S4Vectors
## Loading required package: stats4
## Loading required package: BiocGenerics
## Loading required package: parallel
##
## Attaching package: 'BiocGenerics'
## The following objects are masked from 'package:parallel':
##
##     clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,
##     clusterExport, clusterMap, parApply, parCapply, parLapply,
##     parLapplyLB, parRapply, parSapply, parSapplyLB
## The following objects are masked from 'package:stats':
##
##     IQR, mad, sd, var, xtabs
## The following objects are masked from 'package:base':
##
##     anyDuplicated, 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
##
## Attaching package: 'S4Vectors'
```

```
## The following object is masked from 'package:base':
##
##     expand.grid
## Loading required package: IRanges
## Loading required package: GenomicRanges
## Loading required package: GenomeInfoDb
## Loading required package: SummarizedExperiment
## Loading required package: MatrixGenerics
## Loading required package: matrixStats
##
## Attaching package: 'MatrixGenerics'
## The following objects are masked from 'package:matrixStats':
##
##     colAlls, colAnyNAs, colAnys, colAvgsPerRowSet, colCollapse,
##     colCounts, colCummaxs, colCummins, colCumprods, colCumsums,
##     colDiffs, colIQRDiffs, colIQRs, colLogSumExps, colMadDiffs,
##     colMads, colMaxs, colMeans2, colMedians, colMins, colOrderStats,
##     colProds, colQuantiles, colRanges, colRanks, colSdDiffs, colSds,
##     colSums2, colTabulates, colVarDiffs, colVars, colWeightedMads,
##     colWeightedMeans, colWeightedMedians, colWeightedSds,
##     colWeightedVars, rowAlls, rowAnyNAs, rowAnys, rowAvgsPerColSet,
##     rowCollapse, rowCounts, rowCummaxs, rowCummins, rowCumprods,
##     rowCumsums, rowDiffs, rowIQRDiffs, rowIQRs, rowLogSumExps,
##     rowMadDiffs, rowMads, rowMaxs, rowMeans2, rowMedians, rowMins,
##     rowOrderStats, rowProds, rowQuantiles, rowRanges, rowRanks,
##     rowSdDiffs, rowSds, rowSums2, rowTabulates, rowVarDiffs, rowVars,
##     rowWeightedMads, rowWeightedMeans, rowWeightedMedians,
##     rowWeightedSds, rowWeightedVars
## 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")'.
##
## Attaching package: 'Biobase'
## The following object is masked from 'package:MatrixGenerics':
##
##     rowMedians
## The following objects are masked from 'package:matrixStats':
##
##     anyMissing, rowMedians
library(tidyverse)
## -- Attaching packages --------------------------------------- tidyverse 1.3.0 --
## v ggplot2 3.3.3     v purrr   0.3.4
## v tibble  3.1.0     v dplyr   1.0.5
```

```
## v tidyr   1.1.3     v stringr 1.4.0
## v readr   1.4.0     v forcats 0.5.1
```

```
## -- Conflicts ---------------------------------------- tidyverse_conflicts() --
## x dplyr::collapse()   masks IRanges::collapse()
## x dplyr::combine()    masks Biobase::combine(), BiocGenerics::combine()
## x dplyr::count()      masks matrixStats::count()
## x dplyr::desc()       masks IRanges::desc()
## x tidyr::expand()     masks S4Vectors::expand()
## x dplyr::filter()     masks stats::filter()
## x dplyr::first()      masks S4Vectors::first()
## x dplyr::lag()        masks stats::lag()
## x ggplot2::Position() masks BiocGenerics::Position(), base::Position()
## x purrr::reduce()     masks GenomicRanges::reduce(), IRanges::reduce()
## x dplyr::rename()     masks S4Vectors::rename()
## x dplyr::slice()      masks IRanges::slice()
```

```
library(RColorBrewer)
library(pheatmap)
library(DEGreport)
library(tximport)
library(ggplot2)
library(ggrepel)
library(knitr)
library(AnnotationHub)
```

```
## Loading required package: BiocFileCache
```

```
## Loading required package: dbplyr
```

```
##
## Attaching package: 'dbplyr'
```

```
## The following objects are masked from 'package:dplyr':
##
##     ident, sql
```

```
##
## Attaching package: 'AnnotationHub'
```

```
## The following object is masked from 'package:Biobase':
##
##     cache
```

```
library(ensembldb)
```

```
## Loading required package: GenomicFeatures
```

```
## Loading required package: AnnotationDbi
```

```
##
## Attaching package: 'AnnotationDbi'
```

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

```
## Loading required package: AnnotationFilter
```

```
##
## Attaching package: 'ensembldb'
```

```
## The following object is masked from 'package:dplyr':
##
##     filter

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

```
library(org.Hs.eg.db)
```

```
##
```

```
library(clusterProfiler)
```

```
##

## clusterProfiler v3.18.1  For help: https://guangchuangyu.github.io/software/clusterProfiler
##
## If you use clusterProfiler in published research, please cite:
## Guangchuang Yu, Li-Gen Wang, Yanyan Han, Qing-Yu He. clusterProfiler: an R package for comparing biol

##
## Attaching package: 'clusterProfiler'

## The following objects are masked from 'package:ensembldb':
##
##     filter, select

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

## The following object is masked from 'package:purrr':
##
##     simplify

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

## The following object is masked from 'package:S4Vectors':
##
##     rename

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

```
ggplot2::theme_set(theme_light(base_size = 14))

opts_chunk[["set"]](
    cache = FALSE,
    dev = c("png", "pdf"),
    error = TRUE,
    highlight = TRUE,
    message = FALSE,
    prompt = FALSE,
    tidy = FALSE,
    warning = FALSE)
```

```r
# Have a folder called 'data', and copy your Salmon folders here from the cluster.
## List all directories containing data
### change the pattern to something specific to your Salmon folders
samples <- list.files(path = "./data/final",
                      full.names = T,
                      pattern = "^S")

## Obtain a vector of all filenames including the path
files <- file.path(samples, "salmon", "quant.sf")
files
```

```
##  [1] "./data/final/S3154-2/salmon/quant.sf"
##  [2] "./data/final/S3169-2/salmon/quant.sf"
##  [3] "./data/final/S3188-2/salmon/quant.sf"
##  [4] "./data/final/S3190-4/salmon/quant.sf"
##  [5] "./data/final/S3193-1/salmon/quant.sf"
##  [6] "./data/final/S3194-3/salmon/quant.sf"
##  [7] "./data/final/S3220-1/salmon/quant.sf"
##  [8] "./data/final/S3234-1/salmon/quant.sf"
##  [9] "./data/final/S3291-3/salmon/quant.sf"
## [10] "./data/final/S3292-3/salmon/quant.sf"
## [11] "./data/final/S3372-1/salmon/quant.sf"
## [12] "./data/final/S3374-2/salmon/quant.sf"
## [13] "./data/final/S3404-1/salmon/quant.sf"
## [14] "./data/final/S3424-1/salmon/quant.sf"
## [15] "./data/final/S3474-3/salmon/quant.sf"
## [16] "./data/final/S3477-1/salmon/quant.sf"
## [17] "./data/final/S3563-3/salmon/quant.sf"
## [18] "./data/final/S3582-4/salmon/quant.sf"
## [19] "./data/final/S3644-1/salmon/quant.sf"
## [20] "./data/final/S3652-1/salmon/quant.sf"
## [21] "./data/final/S3688-2/salmon/quant.sf"
## [22] "./data/final/S3697-3/salmon/quant.sf"
## [23] "./data/final/S3713-1/salmon/quant.sf"
## [24] "./data/final/S3715-2/salmon/quant.sf"
## [25] "./data/final/S3723-1/salmon/quant.sf"
## [26] "./data/final/S3728-3/salmon/quant.sf"
## [27] "./data/final/S3732-1/salmon/quant.sf"
## [28] "./data/final/S3741-3/salmon/quant.sf"
## [29] "./data/final/S3816-1/salmon/quant.sf"
## [30] "./data/final/S3822-1/salmon/quant.sf"
## [31] "./data/final/S3825-1/salmon/quant.sf"
## [32] "./data/final/S3837-2/salmon/quant.sf"
## [33] "./data/final/S4047-1/salmon/quant.sf"
## [34] "./data/final/S4056-1/salmon/quant.sf"
## [35] "./data/final/S4089-1/salmon/quant.sf"
## [36] "./data/final/S4101-3/salmon/quant.sf"
## [37] "./data/final/S4136-1/salmon/quant.sf"
## [38] "./data/final/S4144-2/salmon/quant.sf"
## [39] "./data/final/S4172-1/salmon/quant.sf"
## [40] "./data/final/S4176-3/salmon/quant.sf"
## [41] "./data/final/S4237-1/salmon/quant.sf"
## [42] "./data/final/S4249-1/salmon/quant.sf"
## [43] "./data/final/S4261-1/salmon/quant.sf"
```

```
## [44] "./data/final/S4295-5/salmon/quant.sf"
```

```
## Since all quant files have the same name it is useful to have names for each element
### change the string in str_replace so the pattern matches your filenames
names(files) <- str_replace(samples, "./data/final/", "")
```

```
# Load the data and metadata
meta <- read_csv("tables/metadata_corrected.csv") %>%
  column_to_rownames(var = "samplename") %>%
  dplyr::filter(treatment == "post") %>%
  drop_na(response)
protein_coding_genes <- read_csv("tables/ensembl_w_description.protein_coding.csv")
```

```
# Connect to AnnotationHub
ah <- AnnotationHub()

# Query AnnotationHub
hs_ens <- query(ah, c("Homo sapiens", "EnsDb"))

# Get Ensembl94 - used in bcbio
hs_ens <- hs_ens[["AH64923"]]

# Extract gene-level information
txdb <- transcripts(hs_ens,
                    return.type = "data.frame") %>%
  dplyr::select(tx_id, gene_id)

genedb <- genes(hs_ens,
                return.type = "data.frame") %>%
  dplyr::select(gene_id, gene_name, symbol)

gene_symbol <- genedb %>% dplyr::select(gene_id, symbol)

hsdb <- inner_join(txdb, genedb)
write.table(hsdb,
            file = "data/ensembl94_hg38_annotations.txt",
            sep = "\t",
            row.names = F,
            quote = F)
```

```
# Read in  a tx2gene file with transcript identifiers in the first column and gene identifiers in the s
#wormdb <- read.table("ensembl94_WBcel235_annotations.txt", sep="\t", header=T)
tx2gene <- hsdb[, c("tx_id", "gene_id")]


# Run tximport
files <- files[rownames(meta)]
txi_file <- "data/txi.day8.RDS"
if (file.exists(txi_file)){
    txi <- readRDS(txi_file)
}else{
    txi <- tximport(files,
                type = "salmon",
                tx2gene = tx2gene,
                countsFromAbundance = "lengthScaledTPM",
```

```
                ignoreTxVersion = FALSE)
    saveRDS(txi, txi_file)
}

# Look at the counts
class(txi)
```

```
## [1] "list"
```

```
attributes(txi)
```

```
## $names
## [1] "abundance"           "counts"              "length"
## [4] "countsFromAbundance"
```

```
txi$counts %>% View()
```

Checking to see that the transcript to gene mapping is correct

When you have annotations that are from a different source from your reference you can run into problems (i.e lose genes). Some checks you can do before proceeding:

1. Look at the dimensions of your count matrix. Do you have ~20k genes present? `dim(txi$counts)`
2. When running `tximport()` you will get a message in your console. If you see something like `transcripts missing from tx2gene` start troubleshooting.

```
dim(txi$counts)
```

```
## [1] 58735    20
```

Sanity check that metadata matches your expression

It is always a good idea to check if:

1. Do you have expression data for all samples listed in your metadata?
2. Are the samples in your expression data in the same order as your metadata?

```
### Check that sample names match in both files
all(colnames(txi$counts) %in% rownames(meta))
```

```
## [1] TRUE
```

```
# Not the same? Make them the same
### This will change depending on what names you have listed!
#paste0(meta$samplename, "_", meta$library)
#rownames(meta) <- paste0(meta$samplename, "_", meta$library)
#meta$genotype <- relevel(meta$genotype, ref="Wildtype")

### Check that sample names match in both files
all(colnames(txi$counts) %in% rownames(meta))
```

```
## [1] TRUE
```

```
### Check that all samples are in the same order
meta <- meta[colnames(txi$counts),]
all(colnames(txi$counts) == rownames(meta))
```

```
## [1] TRUE
```

Run DESeq2

```
estimating size factors
estimating dispersions
gene-wise dispersion estimates
mean-dispersion relationship
final dispersion estimates
fitting model and testing
```

- Estimating size factors and count normalization
- Gene-wise dispersions
- Mean-dispersion(variance) relationship and the Negative Binomial Model
- Model fitting and hypothesis testing

Wald test

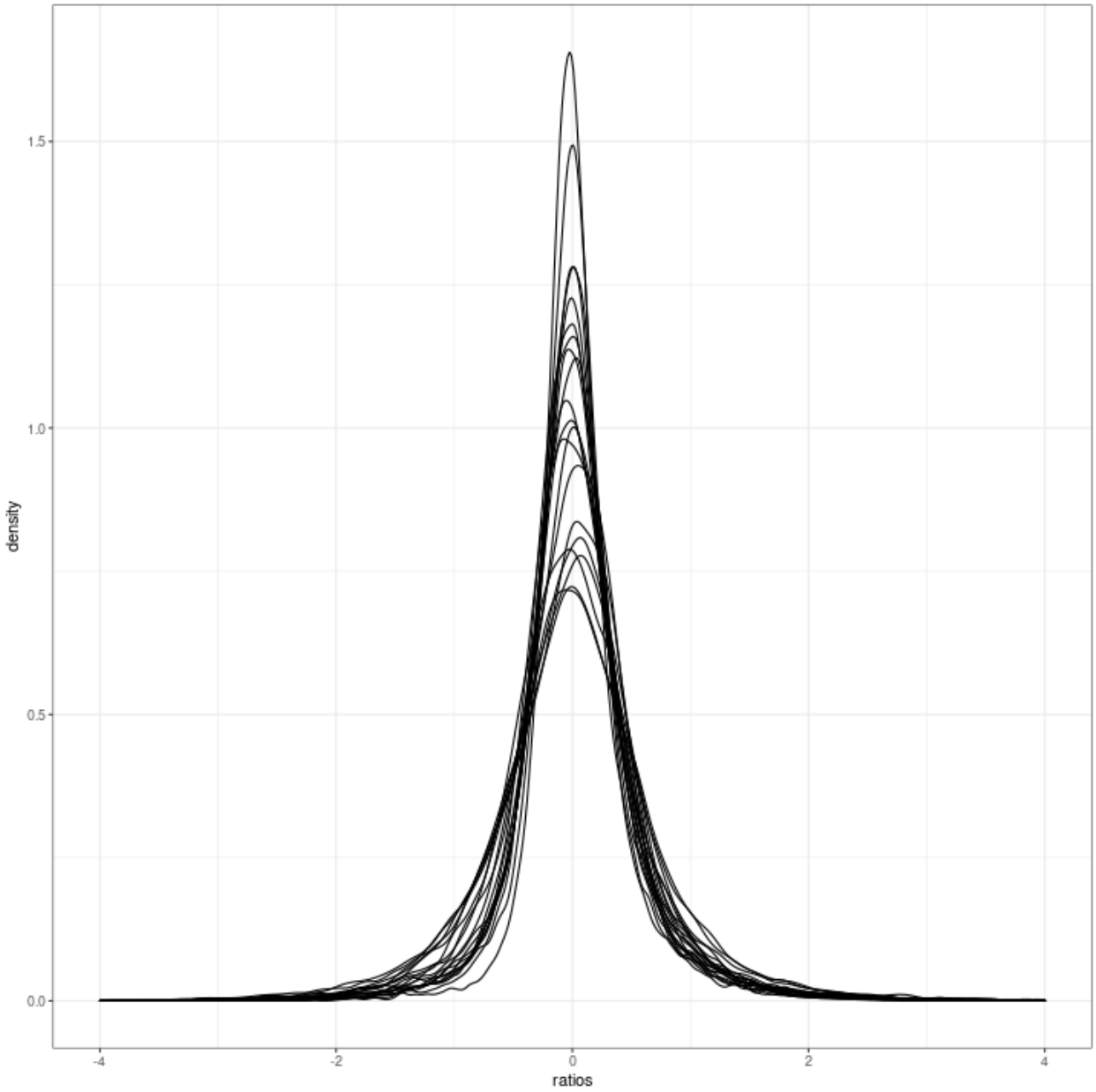
Here we subset protein coding genes.

```
## Create DESeq2Dataset object
dds_file <- "data/dds.day8.RDS"
meta$treatment <- as.factor(meta$treatment)
meta$response <- as.factor(meta$response)
meta$er <- as.factor(meta$er)
meta$date_of <- as.factor(meta$date_of)
meta$tumor_percentage <- as.factor(meta$tumor_percentage)
meta$tumor_percentage_high <- as.factor(meta$tumor_percentage_high)

non_responders <- meta %>% dplyr::filter(study_id %in% c(2, 19)) %>% row.names()

if (file.exists(dds_file)){
    dds <- readRDS(dds_file)
}else{
    dds <- DESeqDataSetFromTximport(txi,
                                    colData = meta,
                                    design = ~response)

    #dds <- dds[,!colnames(dds) %in% non_responders]
    design(dds) <- formula(~response + er + tumor_percentage_high + date_of)

    # subset protein-coding genes
    pc_genes <- intersect(protein_coding_genes$ensembl_gene_id, row.names(dds))
    dds <- dds[pc_genes,]
    # 100 reads / 20 samples
    keep <- rowSums(counts(dds)) >= 100
    dds <- dds[keep,]

    # Run DESeq2
    dds <- DESeq(dds)
    saveRDS(dds, dds_file)
}
```

DEGreport QC

Size factor QC - samples 1-20

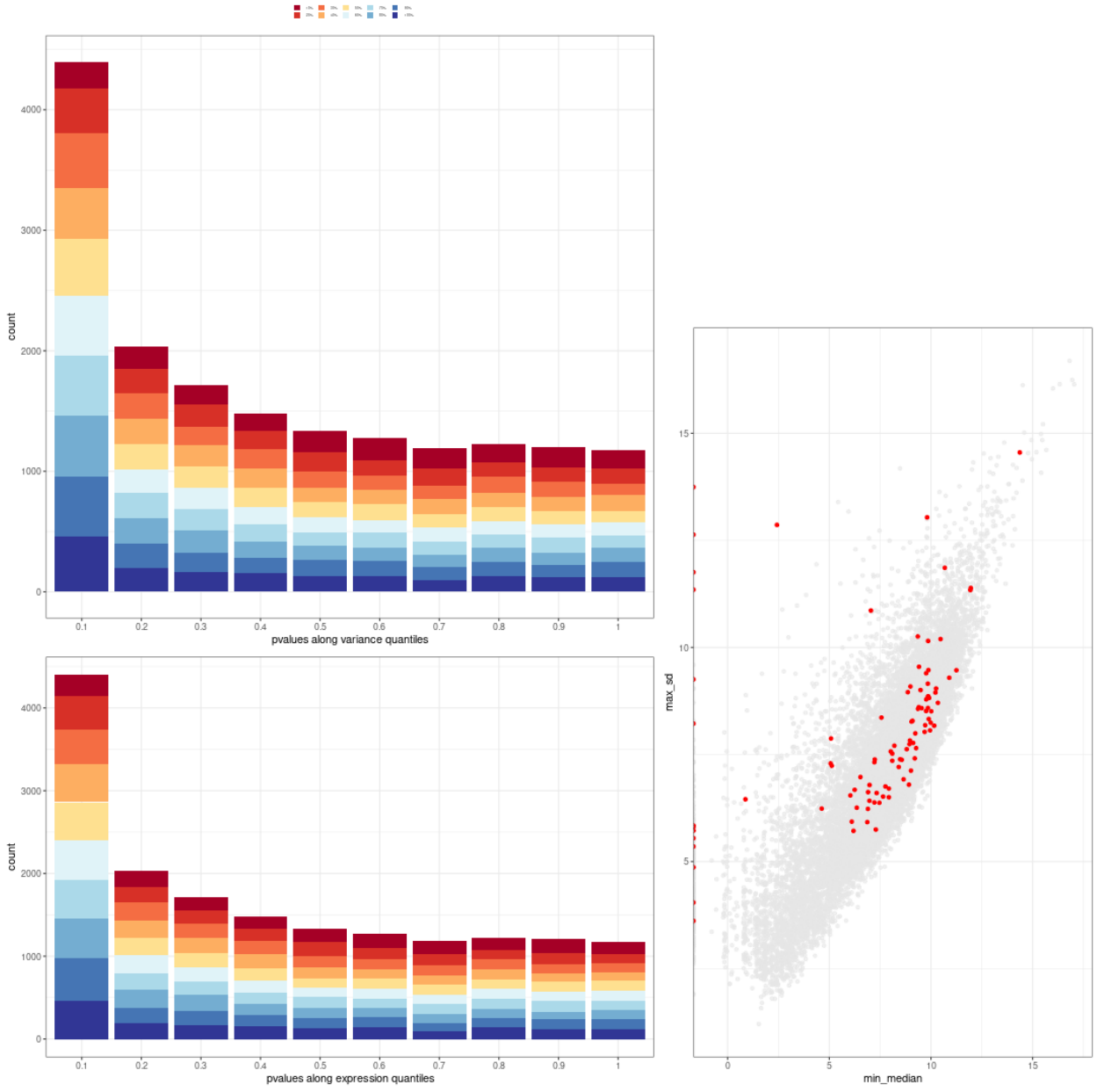
```
counts <- counts(dds, normalized = TRUE)
design <- as.data.frame(colData(dds))
degCheckFactors(counts[, 1:20])
```



Mean-Variance QC plots

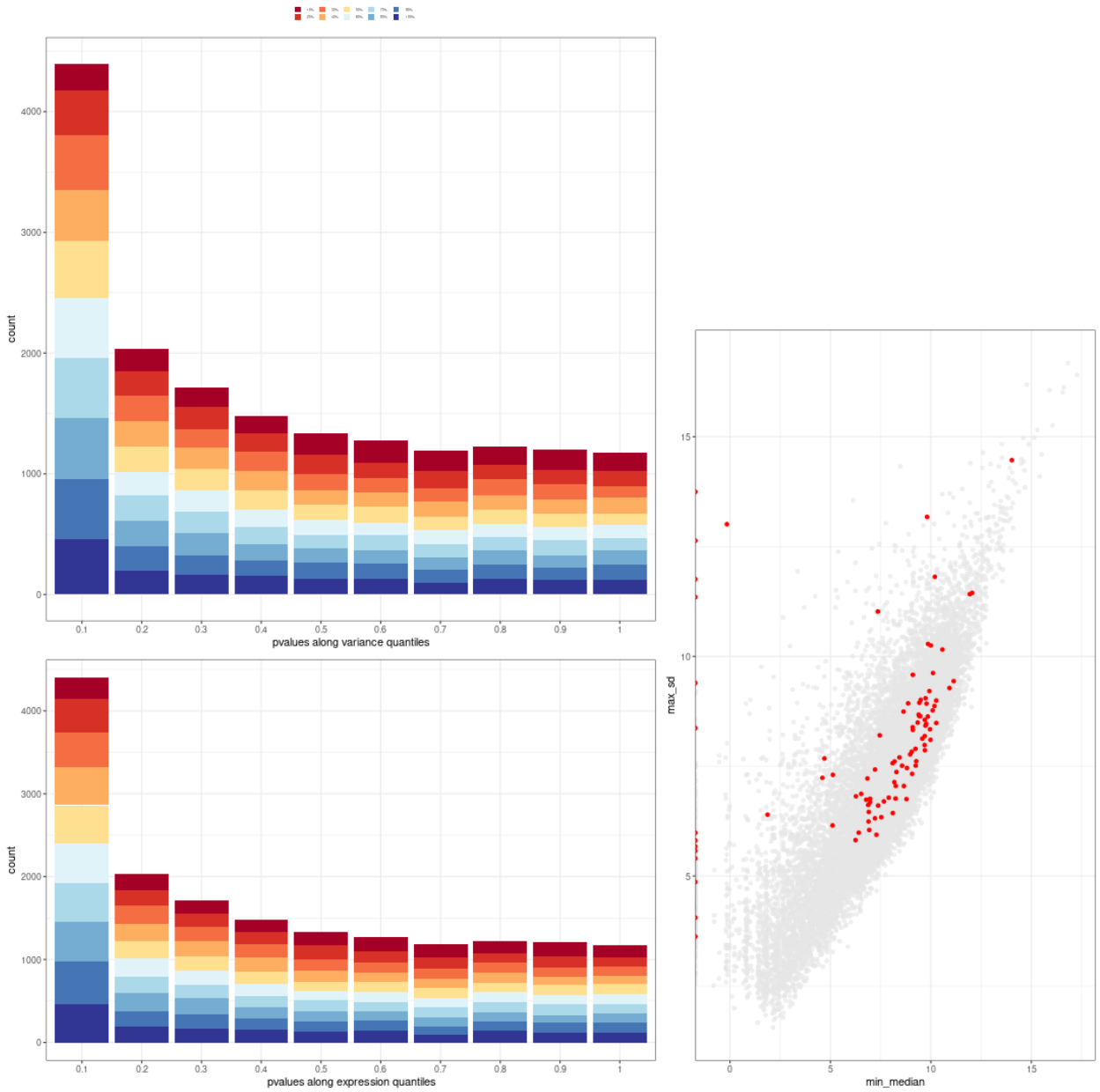
response

```
res <- results(dds)
degQC(counts, design[["response"]], pvalue = res[["pvalue"]])
```



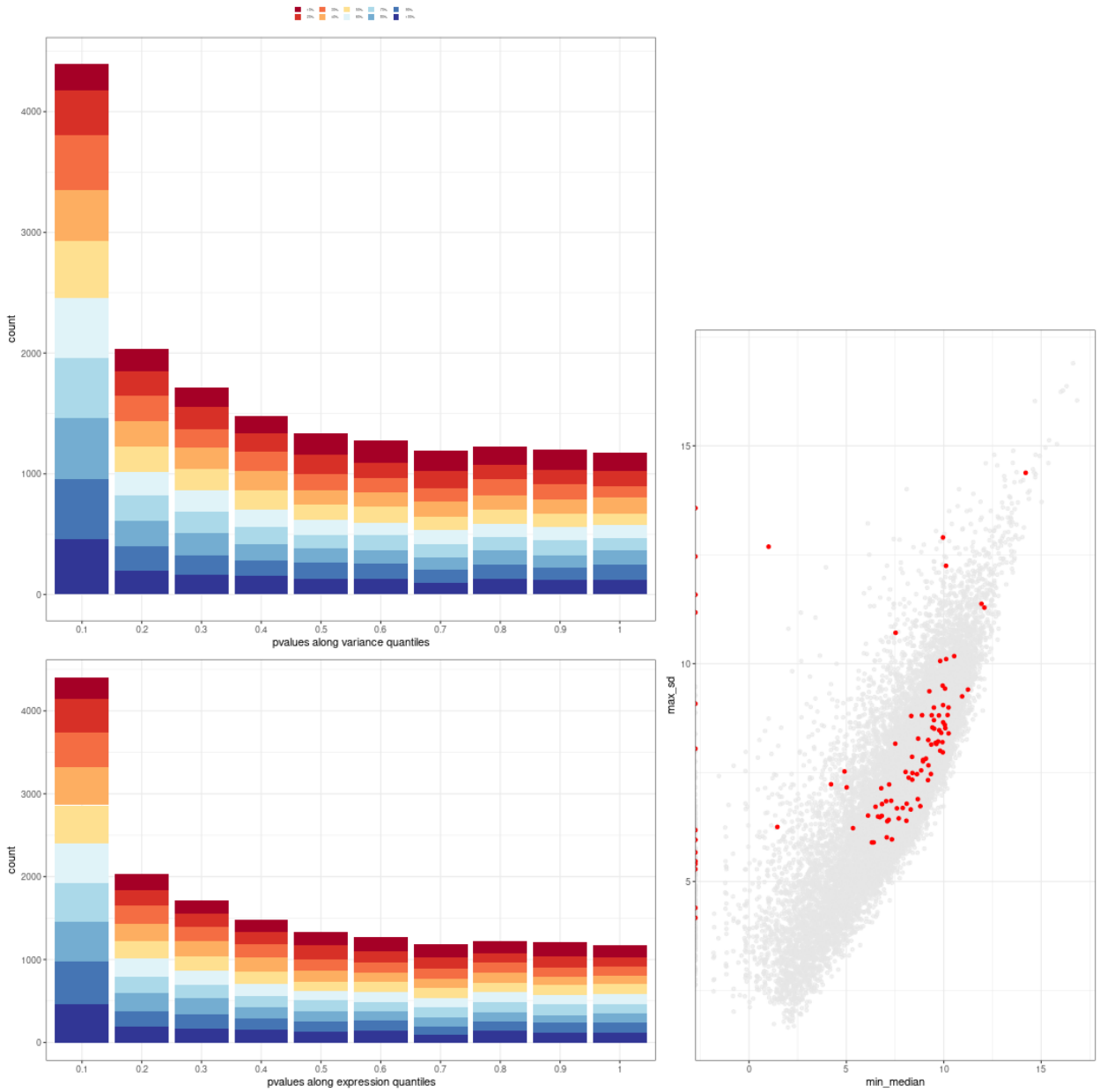
ER

```
degQC(counts, design[["er"]], pvalue = res[["pvalue"]])
```



tumor_percentage_high

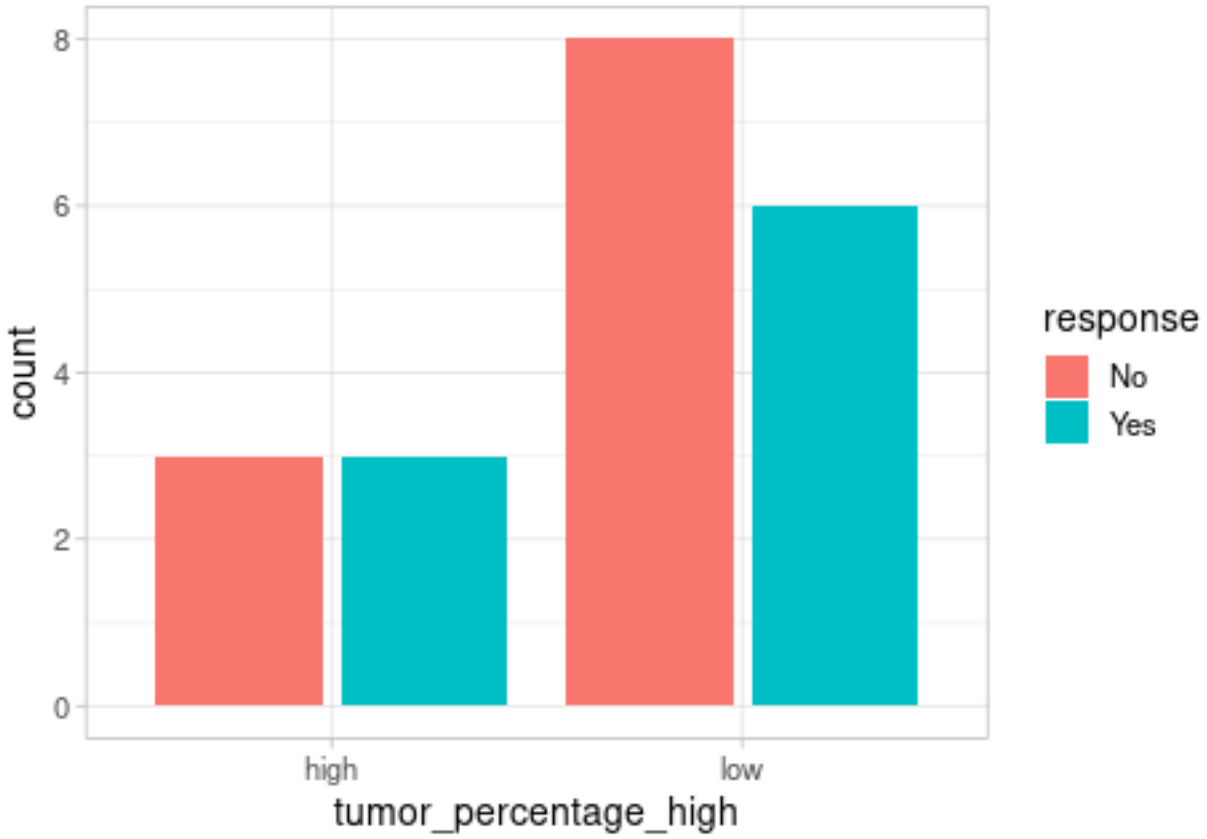
```
degQC(counts, design[["tumor_percentage_high"]], pvalue = res[["pvalue"]])
```



Covariates effect on count data

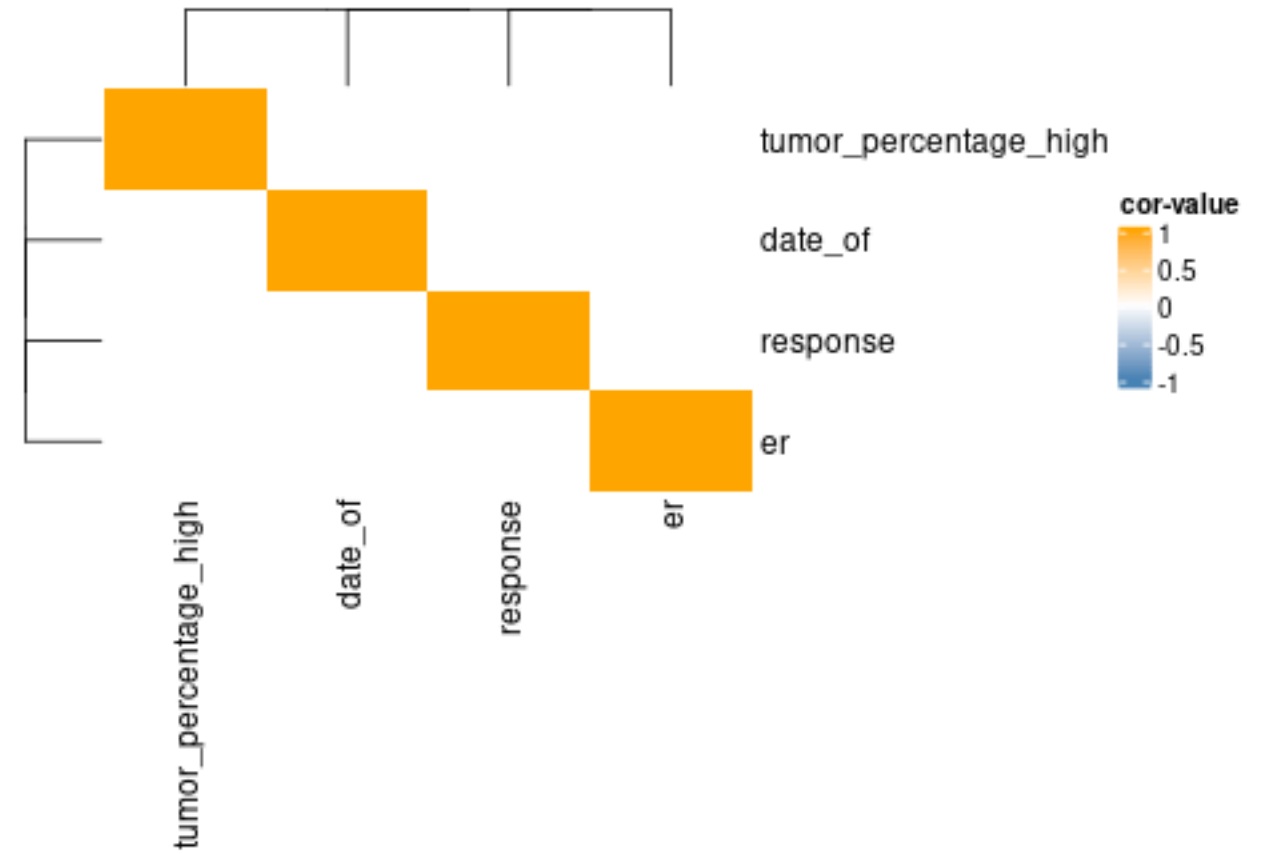
```
mdata <- colData(dds) %>%  as.data.frame()  %>%
  dplyr::select(response, er, date_of, tumor_percentage_high)

#resCov <- degCovariates(log2(counts(dds)+0.5), mdata)
```

```
mdata %>% ggplot(aes(tumor_percentage_high, fill = response)) + geom_bar(position = "dodge2")
```

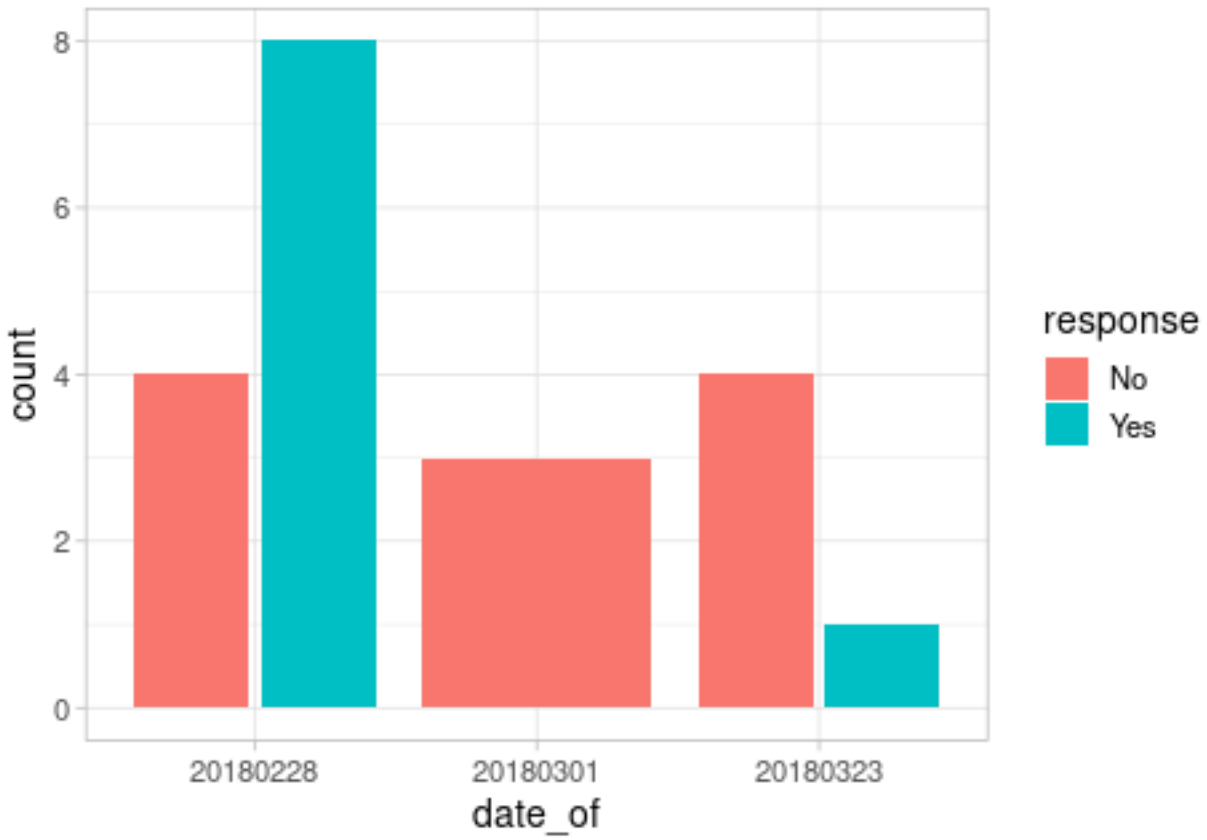


Covariates correlation with metrics

```
cor <- degCorCov(mdata)
```




```
mdata %>% ggplot(aes(date_of, fill = response)) + geom_bar(position = "dodge2")
```



Sample-level QC analysis

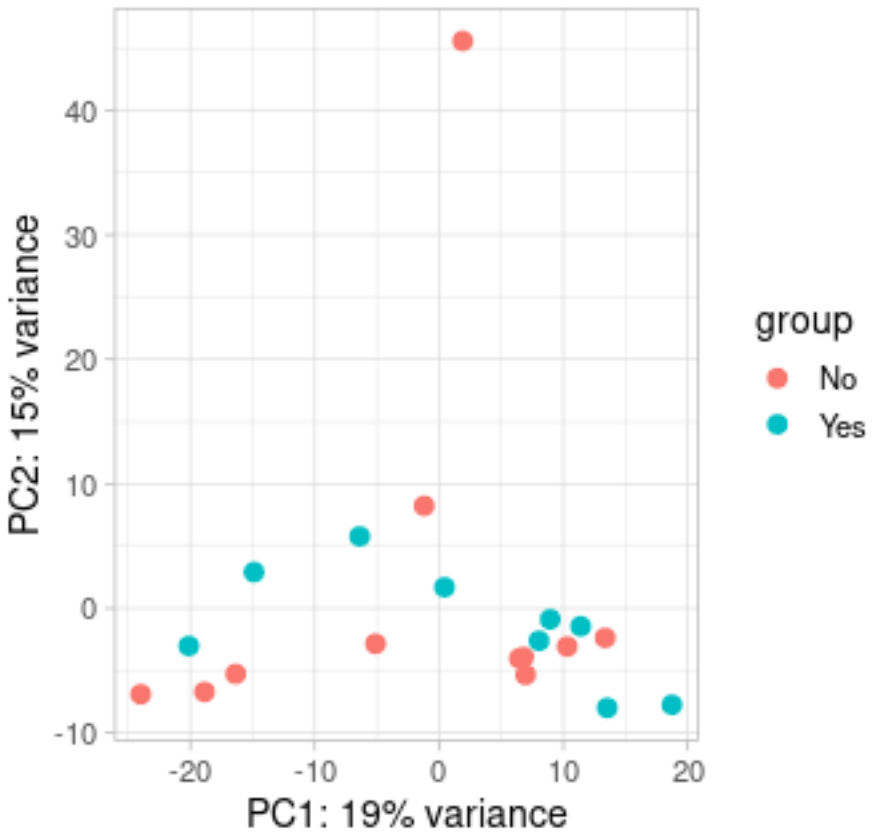
```
### Transform counts for data visualization (unsupervised analysis)
rld_file <- "data/rld.day8.RDS"
if (file.exists(rld_file)){
    rld <- readRDS(rld_file)
}else{
    rld <- rlog(dds, blind = TRUE)
    saveRDS(rld, rld_file)
}
class(rld) # what type of object is this
```

```
## [1] "DESeqTransform"
## attr(,"package")
## [1] "DESeq2"
```

```
# we also need just a matrix of transformed counts
rld_mat <- assay(rld)
```

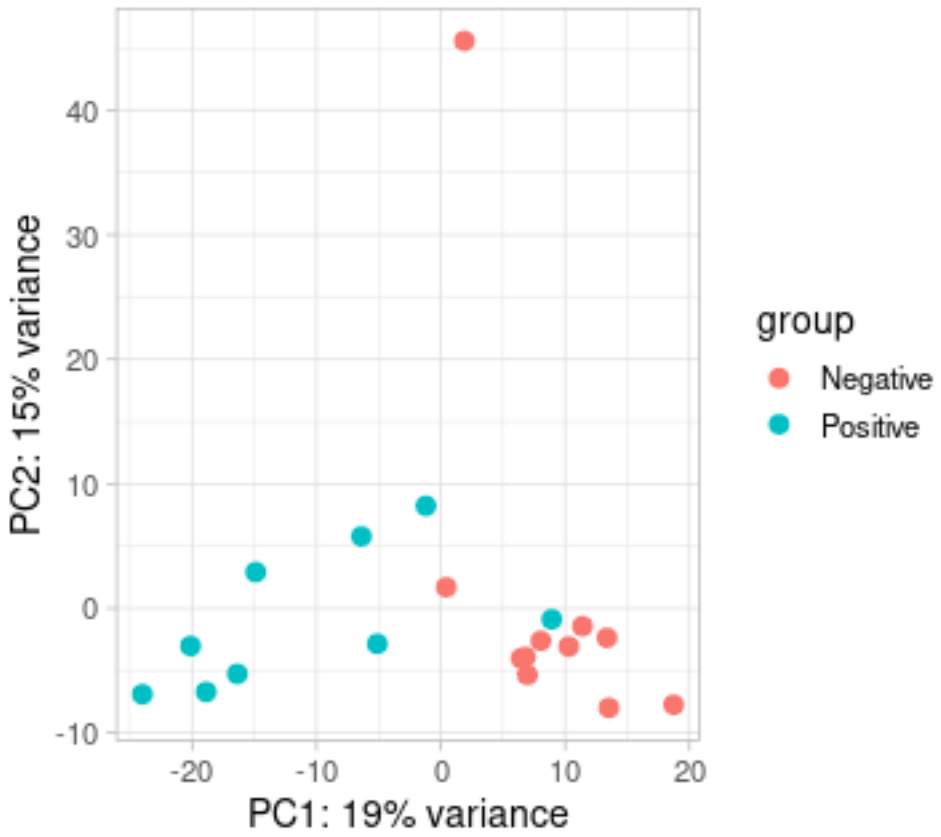
PCA - response

```
# Use the DESeq2 function
plotPCA(rld, intgroup = c("response"))
```



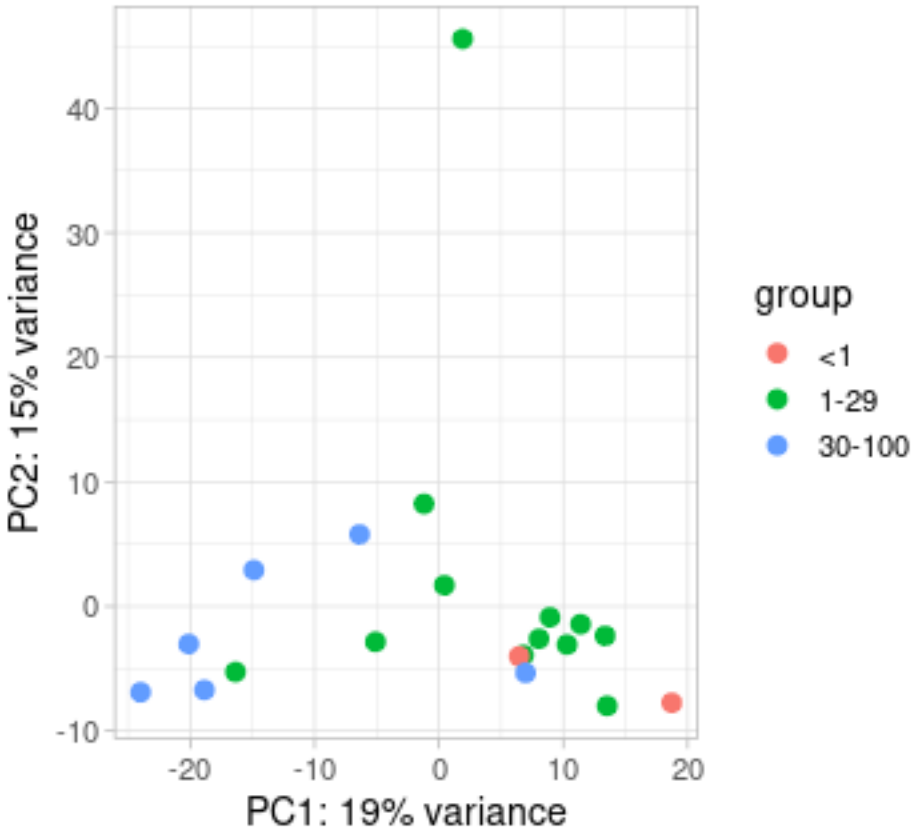
PCA - ER

```
# Use the DESeq2 function
plotPCA(rld, intgroup = c("er"))
```



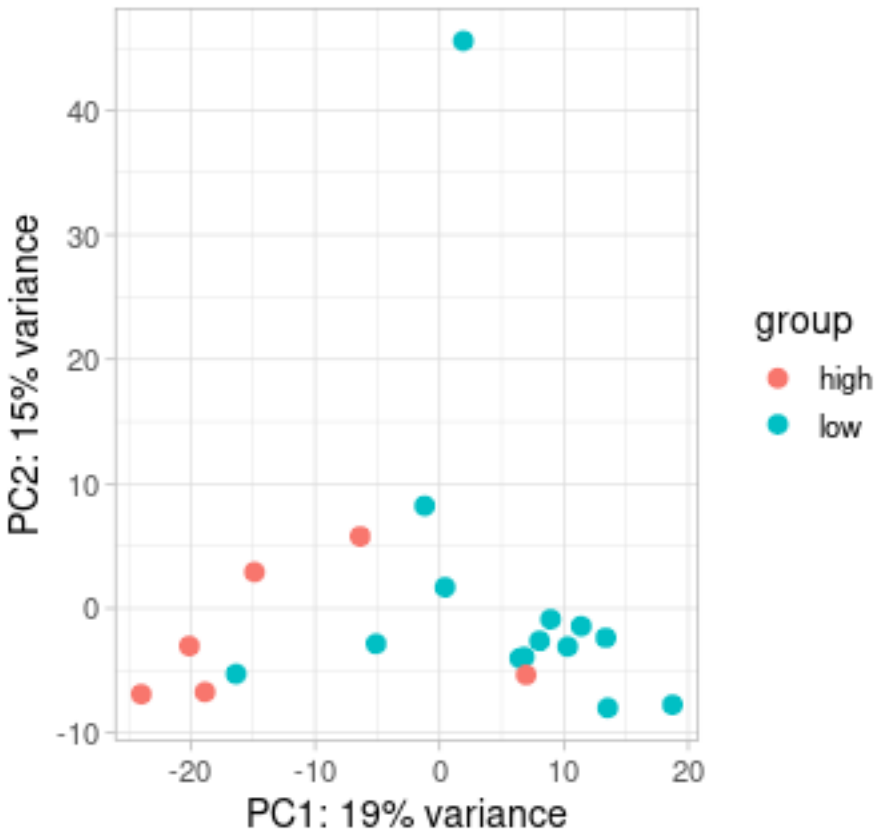
PCA - tumor_percentage

```
# Use the DESeq2 function
plotPCA(rld, intgroup = c("tumor_percentage"))
```



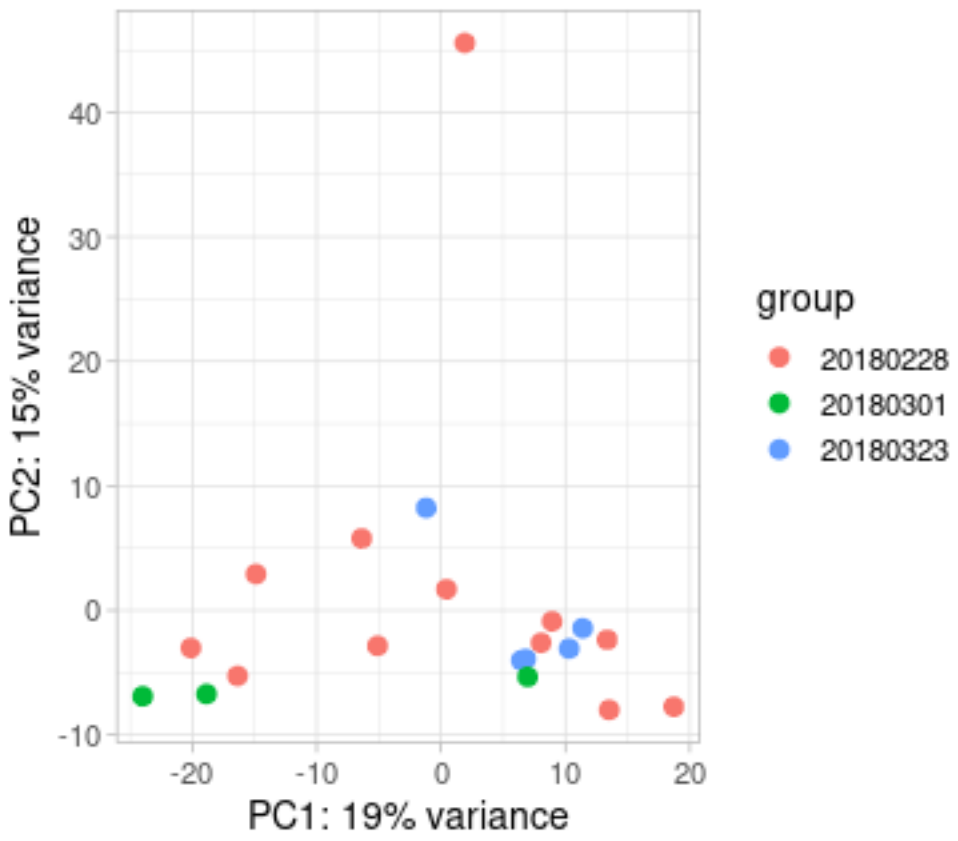
PCA - tumor_percentage_high

```
# Use the DESeq2 function
plotPCA(rld, intgroup = c("tumor_percentage_high"))
```



PCA - date_of

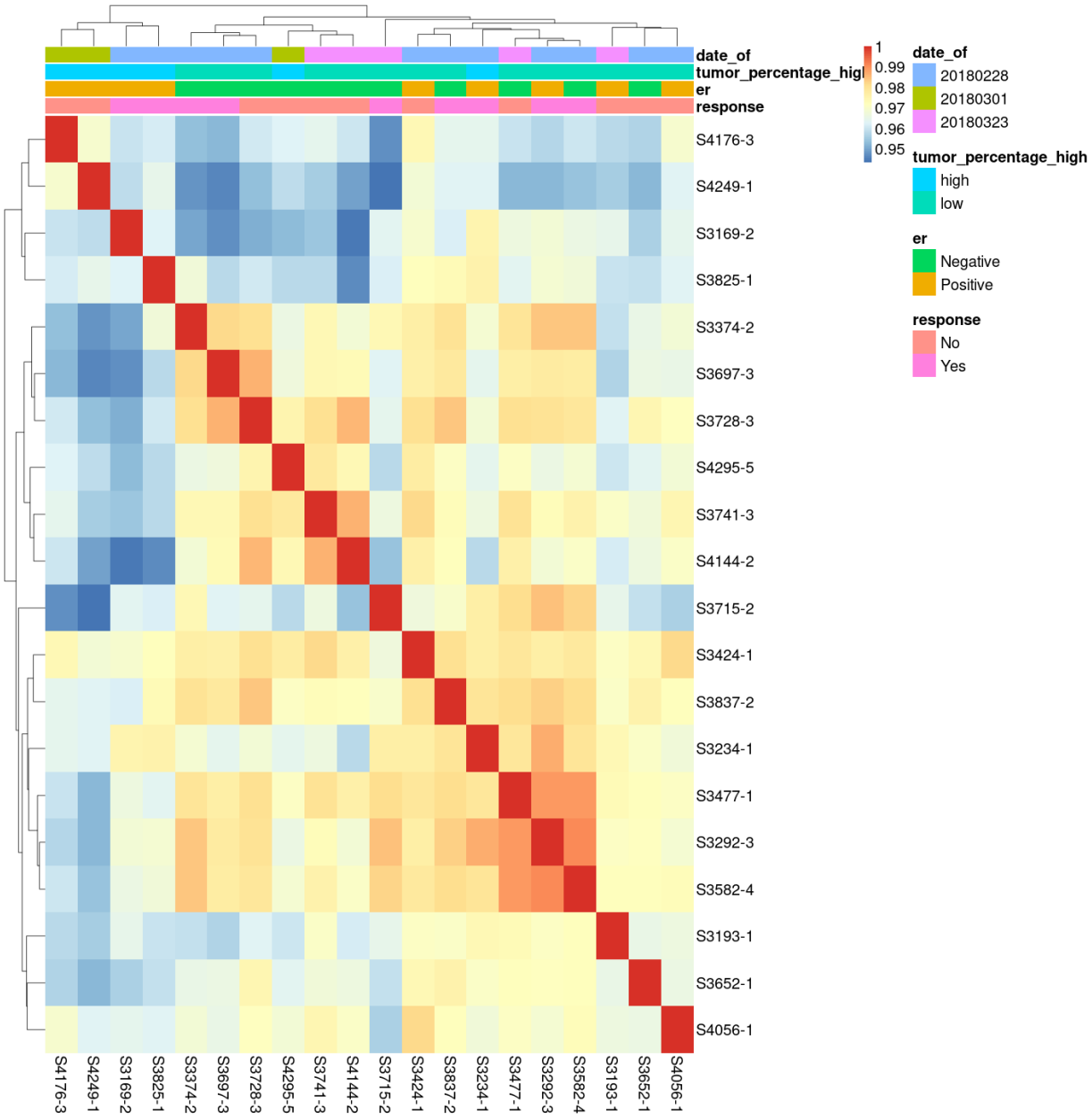
```
# Use the DESeq2 function
plotPCA(rld, intgroup = c("date_of"))
```



Inter-correlation analysis

Without study_id

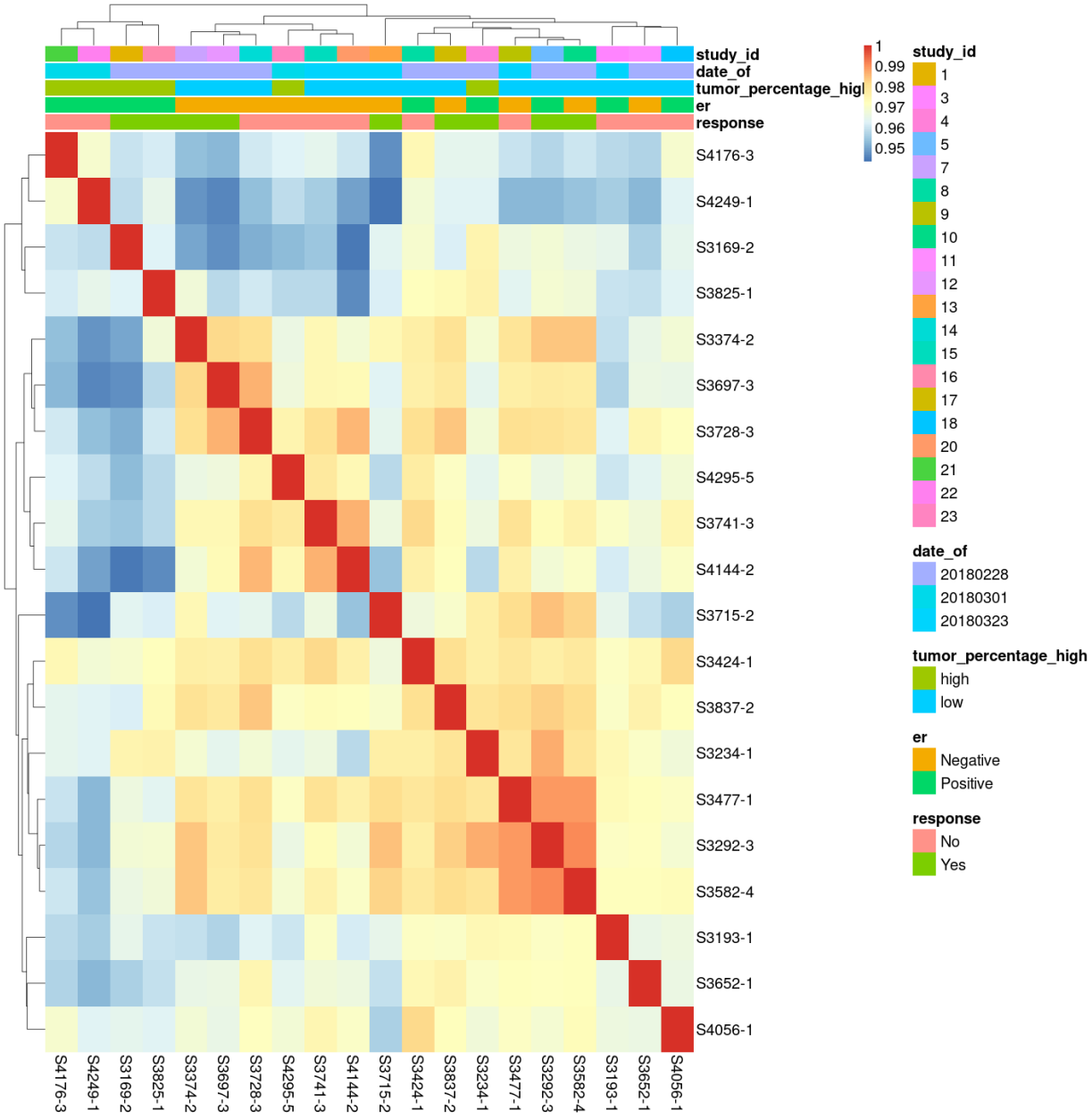
```
# Correlation matrix
rld_cor <- cor(rld_mat)

meta$study_id <- as.factor(meta$study_id)
# Create annotation file for samples
annotation <- meta[, c("response", "er", "tumor_percentage_high", "date_of")]

# Change colors
heat.colors <- brewer.pal(6, "Blues")

# Plot heatmap
pheatmap(rld_cor,
         annotation = annotation,
         border = NA,
         fontsize = 20)
```



With study_id

```
# Correlation matrix
rld_cor <- cor(rld_mat)

meta$study_id <- as.factor(meta$study_id)
# Create annotation file for samples
annotation <- meta[, c("response", "er", "tumor_percentage_high", "date_of", "study_id")]

# Change colors
heat.colors <- brewer.pal(6, "Blues")

# Plot heatmap
```

```
pheatmap(rld_cor,
         annotation = annotation,
         border = NA,
         fontsize = 20)
```



Response Yes vs No for Day 8 - see Table13

```
# Get results for rescue vs wt
contrast <- c("response", "Yes", "No")
resResponse <- results(dds, contrast = contrast, alpha = 0.05)
length(which(resResponse$padj < 0.05))
```

```
## [1] 818
```

```r
# Add annotations
resResponse_tb <- resResponse %>%
  data.frame() %>%
  rownames_to_column(var = "gene") %>%
  as_tibble() %>%
  left_join(gene_symbol, by = c("gene" = "gene_id"))

resResponse_tb_significant <- dplyr::filter(resResponse_tb, padj < 0.05)

samples_no <- meta %>% dplyr::filter(response == "No") %>% row.names()

counts_no <- txi$abundance %>%
                as.data.frame() %>%
                dplyr::select(any_of(samples_no)) %>%
                rowSums() %>%
                as.data.frame() %>%
                rownames_to_column(var = "ensembl_gene_id")
colnames(counts_no) <- c("ensembl_gene_id", "no_expression_mean_tpm")

samples_yes <- meta %>% dplyr::filter(response == "Yes") %>% row.names()

counts_yes <- txi$abundance %>%
                as.data.frame() %>%
                dplyr::select(any_of(samples_yes)) %>%
                rowSums() %>%
                as.data.frame() %>%
                rownames_to_column(var = "ensembl_gene_id")

colnames(counts_yes) <- c("ensembl_gene_id", "yes_expression_mean_tpm")

counts_yes <-counts_yes %>%
            left_join(counts_no,
                      by = c("ensembl_gene_id" = "ensembl_gene_id"))

resResponse_tb_significant <- resResponse_tb_significant %>%
          left_join(counts_yes, by = c("gene" = "ensembl_gene_id"))


write_csv(resResponse_tb_significant,
          "tables/T13.DE_response_day8.csv")

# Separate into up and doum-regulated gene sets
sigResponse_up <- rownames(resResponse)[which(resResponse$padj < 0.01 & resResponse$log2FoldChange > 0)]
sigResponse_down <- rownames(resResponse)[which(resResponse$padj < 0.01 & resResponse$log2FoldChange < 0
```

ER : Positive vs Negative for Day8 - Table 14

```r
contrast <- c("er", "Positive", "Negative")
resER <- results(dds, contrast = contrast, alpha = 0.05)
length(which(resER$padj < 0.05))
```

```
## [1] 182
```

```r
# Add annotations
resER_tb <- resER %>%
  data.frame() %>%
  rownames_to_column(var = "gene") %>%
  as_tibble() %>%
  left_join(gene_symbol, by = c("gene" = "gene_id"))

resER_tb_significant <- dplyr::filter(resER_tb, padj < 0.05)

samples_pos <- meta %>% dplyr::filter(er == "Positive") %>% row.names()

counts_pos <- txi$abundance %>%
                as.data.frame() %>%
                dplyr::select(any_of(samples_pos)) %>%
                rowMeans() %>%
                as.data.frame() %>%
                rownames_to_column(var = "ensembl_gene_id")
colnames(counts_pos) <- c("ensembl_gene_id", "Positive_expression_mean_tpm")

samples_neg <- meta %>% dplyr::filter(er == "Negative") %>% row.names()

counts_neg <- txi$abundance %>%
                as.data.frame() %>%
                dplyr::select(any_of(samples_neg)) %>%
                rowMeans() %>%
                as.data.frame() %>%
                rownames_to_column(var = "ensembl_gene_id")

colnames(counts_neg) <- c("ensembl_gene_id", "Negative_expression_mean_tpm")

counts_pos <-counts_pos %>%
            left_join(counts_neg,
                      by = c("ensembl_gene_id" = "ensembl_gene_id"))

resER_tb_significant <- resER_tb_significant %>%
          left_join(counts_pos, by = c("gene" = "ensembl_gene_id"))


write_csv(resER_tb_significant,
          "tables/T14.DE_ER.day8.csv")

# Separate into up and down-regulated gene sets
sigER_up <- rownames(resER)[which(resER$padj < 0.01 & resER$log2FoldChange > 0)]
sigER_down <- rownames(resER)[which(resER$padj < 0.01 & resER$log2FoldChange < 0)]
```

tumor_percentage_high : High vs Low for Day8- Table 15

```r
contrast <- c("tumor_percentage_high", "high", "low")
resTP <- results(dds, contrast = contrast, alpha = 0.05)
length(which(resTP$padj < 0.05))
```

```
## [1] 128
```

```r
# Add annotations
resTP_tb <- resTP %>%
  data.frame() %>%
  rownames_to_column(var = "gene") %>%
  as_tibble() %>%
  left_join(gene_symbol, by = c("gene" = "gene_id"))

resTP_tb_significant <- dplyr::filter(resTP_tb, padj < 0.05)

samples_high <- meta %>% dplyr::filter(tumor_percentage_high == "high") %>% row.names()

counts_high <- txi$abundance %>%
                as.data.frame() %>%
                dplyr::select(any_of(samples_high)) %>%
                rowMeans() %>%
                as.data.frame() %>%
                rownames_to_column(var = "ensembl_gene_id")
colnames(counts_high) <- c("ensembl_gene_id", "High_expression_mean_tpm")

samples_low <- meta %>% dplyr::filter(tumor_percentage_high == "low") %>% row.names()

counts_low <- txi$abundance %>%
                as.data.frame() %>%
                dplyr::select(any_of(samples_low)) %>%
                rowMeans() %>%
                as.data.frame() %>%
                rownames_to_column(var = "ensembl_gene_id")

colnames(counts_low) <- c("ensembl_gene_id", "Low_expression_mean_tpm")

counts_high <-counts_high %>%
            left_join(counts_low,
                      by = c("ensembl_gene_id" = "ensembl_gene_id"))

resTP_tb_significant <- resTP_tb_significant %>%
          left_join(counts_high, by = c("gene" = "ensembl_gene_id"))


write_csv(resTP_tb_significant,
          "tables/T15.DE_tumor_percentage_high.day8.csv")

# Separate into up and down-regulated gene sets
sigTP_up <- rownames(resTP)[which(resTP$padj < 0.01 & resTP$log2FoldChange > 0)]
sigTP_down <- rownames(resTP)[which(resTP$padj < 0.01 & resTP$log2FoldChange < 0)]
```

date_of: 20180323 vs 20180228 - for Day8: Table 16

```r
contrast <- c("date_of", "20180323", "20180228")
resDO <- results(dds, contrast = contrast, alpha = 0.05)
length(which(resDO$padj < 0.05))
```

```
## [1] 408
```

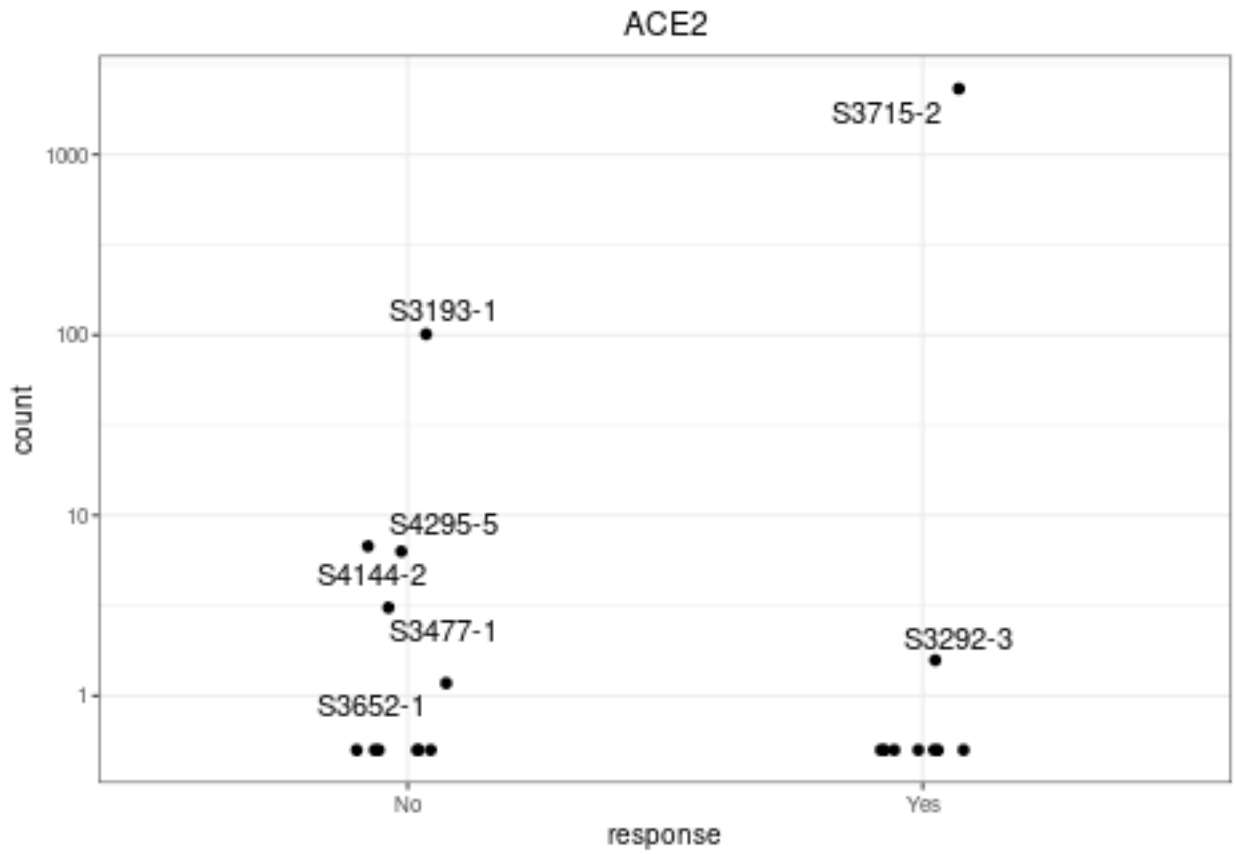
```r
# Add annotations
resDO_tb <- resDO %>%
  data.frame() %>%
  rownames_to_column(var = "gene") %>%
  as_tibble() %>%
  left_join(gene_symbol, by = c("gene" = "gene_id"))

resDO_tb_significant <- dplyr::filter(resDO_tb, padj < 0.05)

samples_23 <- meta %>% dplyr::filter(date_of == "20180323") %>% row.names()

counts_23 <- txi$abundance %>%
                as.data.frame() %>%
                dplyr::select(any_of(samples_23)) %>%
                rowMeans() %>%
                as.data.frame() %>%
                rownames_to_column(var = "ensembl_gene_id")
colnames(counts_23) <- c("ensembl_gene_id", "20180323_expression_mean_tpm")

samples_28 <- meta %>% dplyr::filter(date_of == "20180228") %>% row.names()

counts_28 <- txi$abundance %>%
                as.data.frame() %>%
                dplyr::select(any_of(samples_28)) %>%
                rowMeans() %>%
                as.data.frame() %>%
                rownames_to_column(var = "ensembl_gene_id")

colnames(counts_28) <- c("ensembl_gene_id", "20180228_expression_mean_tpm")

counts_23 <-counts_23 %>%
            left_join(counts_28,
                      by = c("ensembl_gene_id" = "ensembl_gene_id"))

resDO_tb_significant <- resDO_tb_significant %>%
          left_join(counts_23, by = c("gene" = "ensembl_gene_id"))


write_csv(resDO_tb_significant,
          "tables/T16.DE_date_of.day8.csv")

# Separate into up and doum-regulated gene sets
sigDO_up <- rownames(resDO)[which(resDO$padj < 0.01 & resDO$log2FoldChange > 0)]
sigDO_down <- rownames(resDO)[which(resDO$padj < 0.01 & resDO$log2FoldChange < 0)]
```

Visualization

Gene example

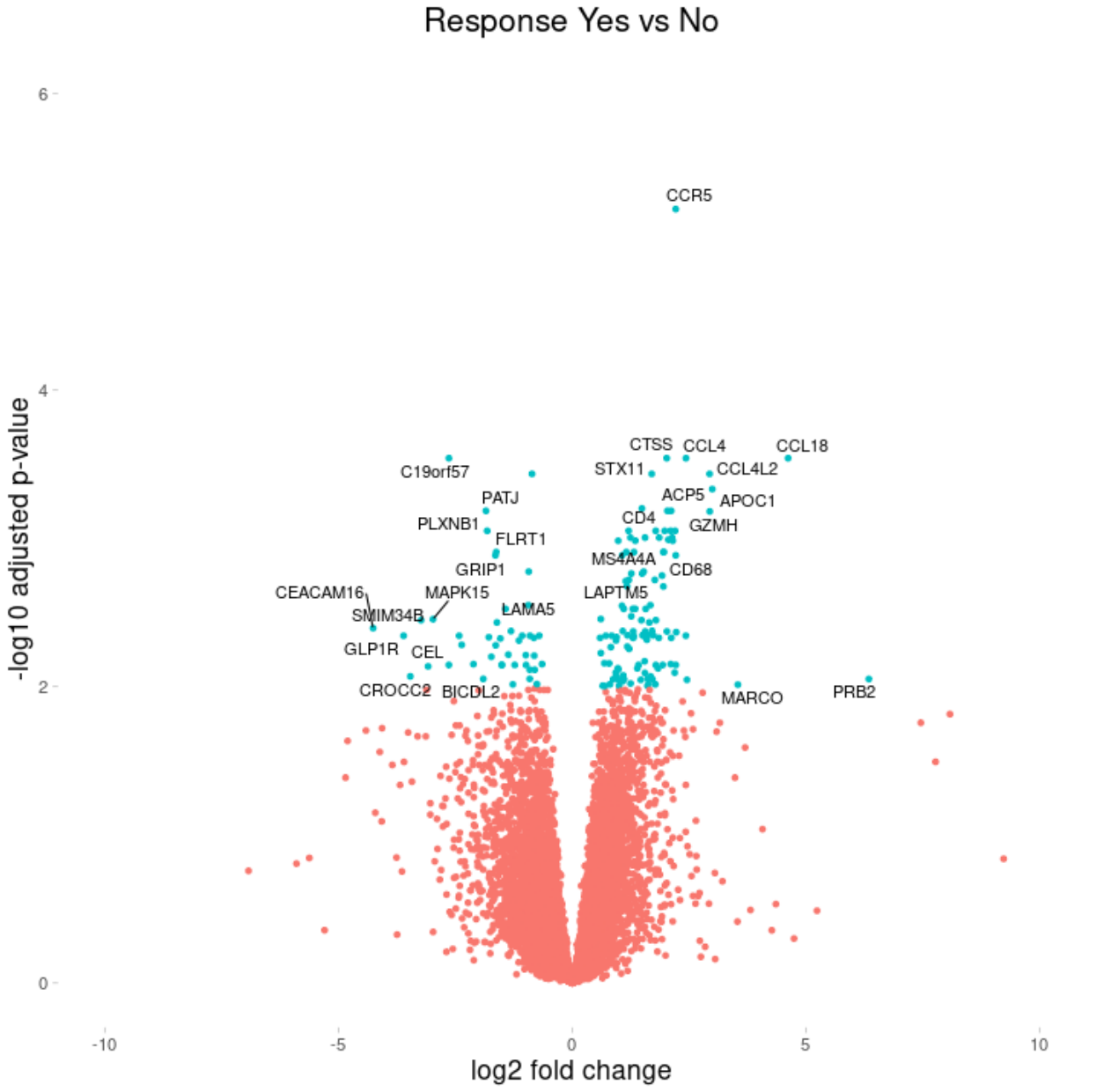
```r
d <- plotCounts(dds,
                gene = "ENSG00000130234",
                intgroup = "response",
                returnData = TRUE)
```

```
ggplot(d, aes(x = response, y = count)) +
     geom_point(position = position_jitter(w = 0.1, h = 0)) +
     geom_text_repel(aes(label = rownames(d))) +
     theme_bw(base_size = 10) +
     ggtitle("ACE2") +
     theme(plot.title = element_text(hjust = 0.5)) +
     scale_y_log10()
```



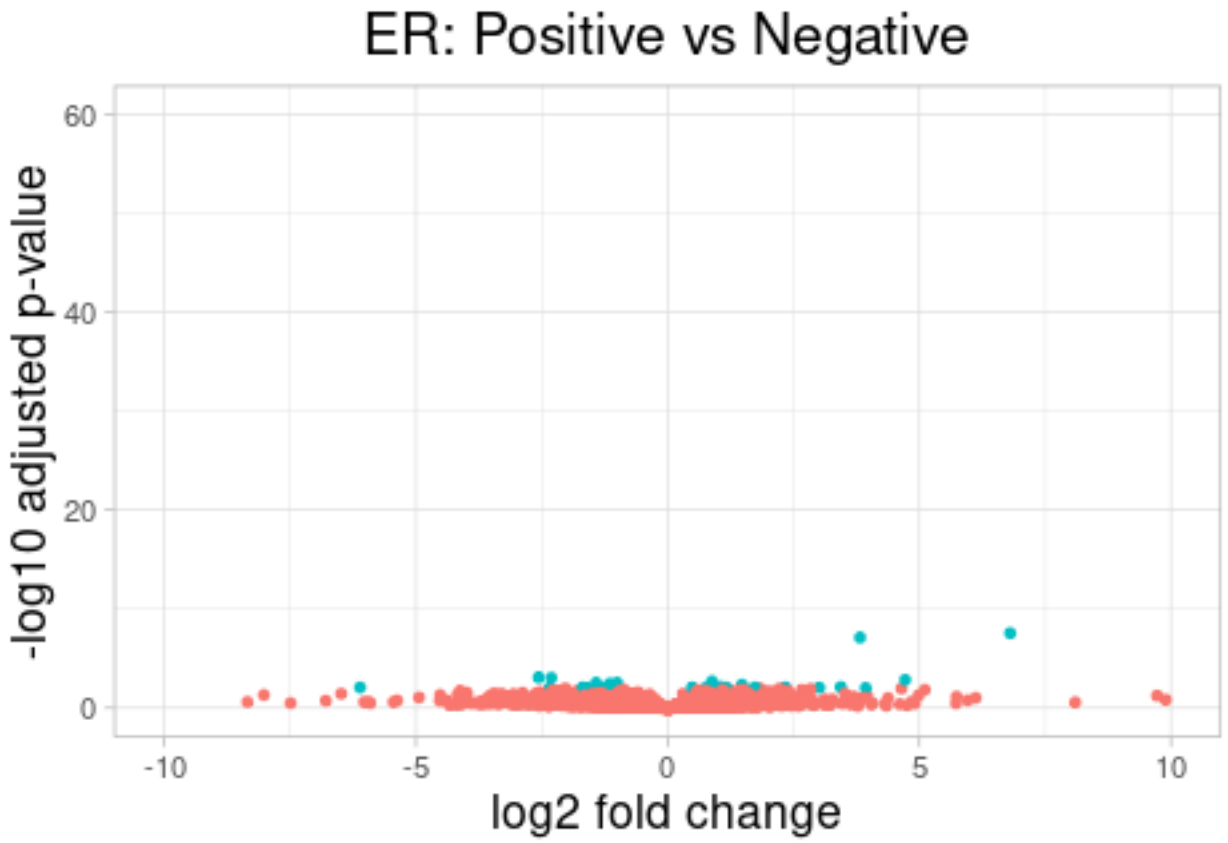

```
# Add a column for significant genes
resResponse_tb_vis <- resResponse_tb %>% mutate(threshold = padj < 0.01)

resResponse_tb_vis$symbol <- ifelse((abs(resResponse_tb_vis$log2FoldChange) > 1.5),
                                resResponse_tb_vis$symbol, NA)

resResponse_tb_vis$symbol <- ifelse(resResponse_tb_vis$threshold,
                                    resResponse_tb_vis$symbol, NA)

ggplot(resResponse_tb_vis,
       aes(log2FoldChange, -log10(padj), label = symbol)) +
  geom_point(aes(colour = threshold)) +
  ggtitle("Response Yes vs No") +
  xlab("log2 fold change") +
  ylab("-log10 adjusted p-value") +
  scale_x_continuous(limits = c(-10,10)) +
  scale_y_continuous(limits = c(0, 6))+
  theme(legend.position = "none",
```

```
        plot.title = element_text(size = rel(1.5), hjust = 0.5),
        axis.title = element_text(size = rel(1.25)),
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        panel.border = element_blank(),
        panel.background = element_blank()) +
  geom_text_repel(aes(label = symbol))
```

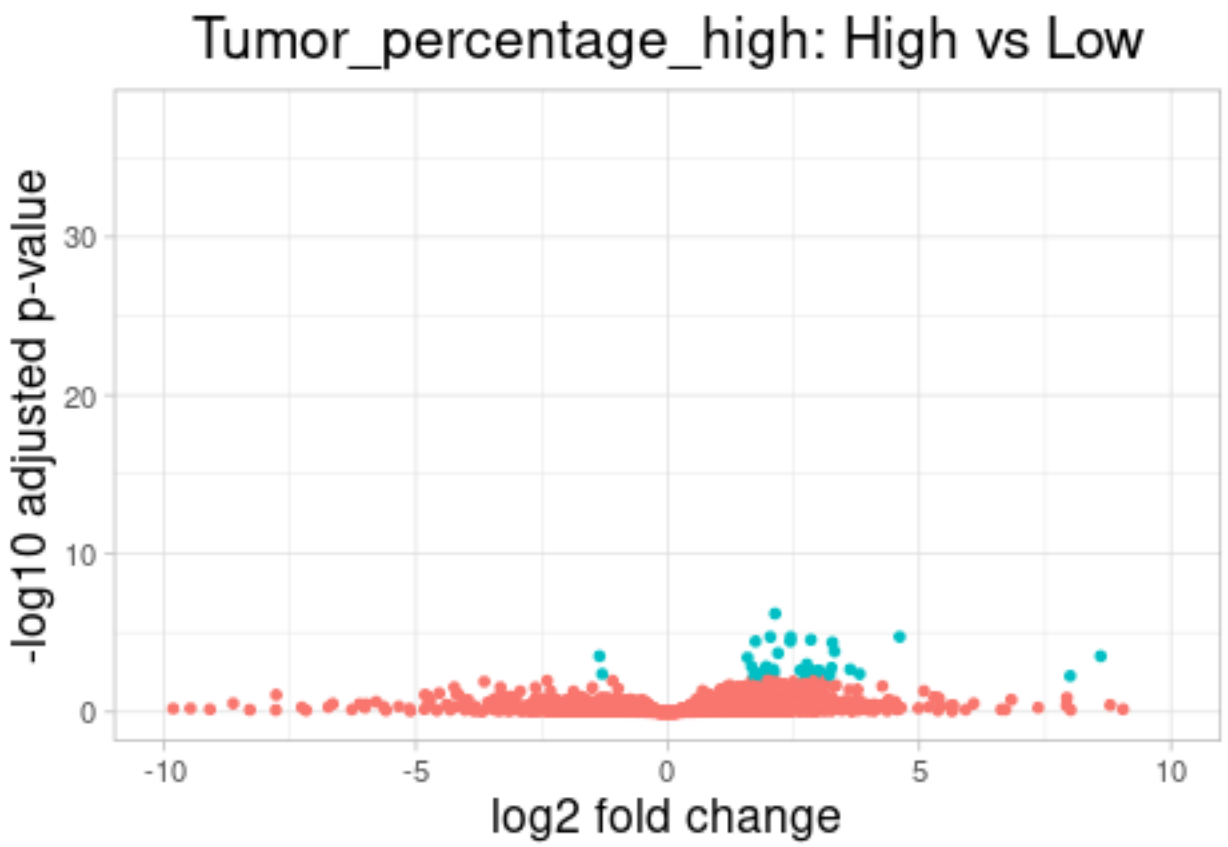



```
# Add a column for significant genes
resER_tb <- resER_tb %>% mutate(threshold = padj < 0.01)

ggplot(resER_tb) +
  geom_point(aes(x = log2FoldChange, y = -log10(padj), colour = threshold)) +
  ggtitle("ER: Positive vs Negative") +
```

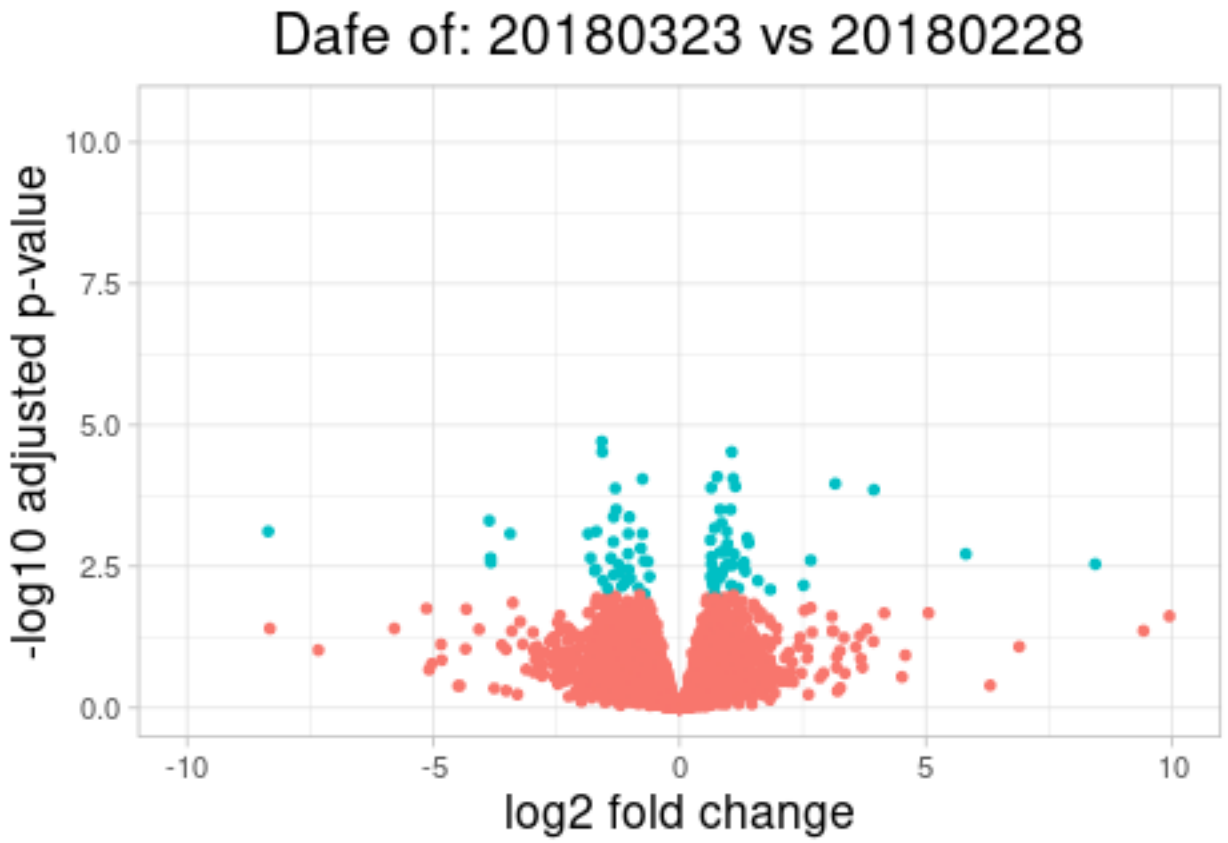
```
  xlab("log2 fold change") +
  ylab("-log10 adjusted p-value") +
  scale_x_continuous(limits = c(-10,10)) +
  theme(legend.position = "none",
        plot.title = element_text(size = rel(1.5), hjust = 0.5),
        axis.title = element_text(size = rel(1.25)))
```




```
# Add a column for significant genes
resTP_tb <- resTP_tb %>% mutate(threshold = padj < 0.01)

ggplot(resTP_tb) +
  geom_point(aes(x = log2FoldChange, y = -log10(padj), colour = threshold)) +
  ggtitle("Tumor_percentage_high: High vs Low") +
  xlab("log2 fold change") +
  ylab("-log10 adjusted p-value") +
  scale_x_continuous(limits = c(-10,10)) +
  theme(legend.position = "none",
        plot.title = element_text(size = rel(1.5), hjust = 0.5),
        axis.title = element_text(size = rel(1.25)))
```



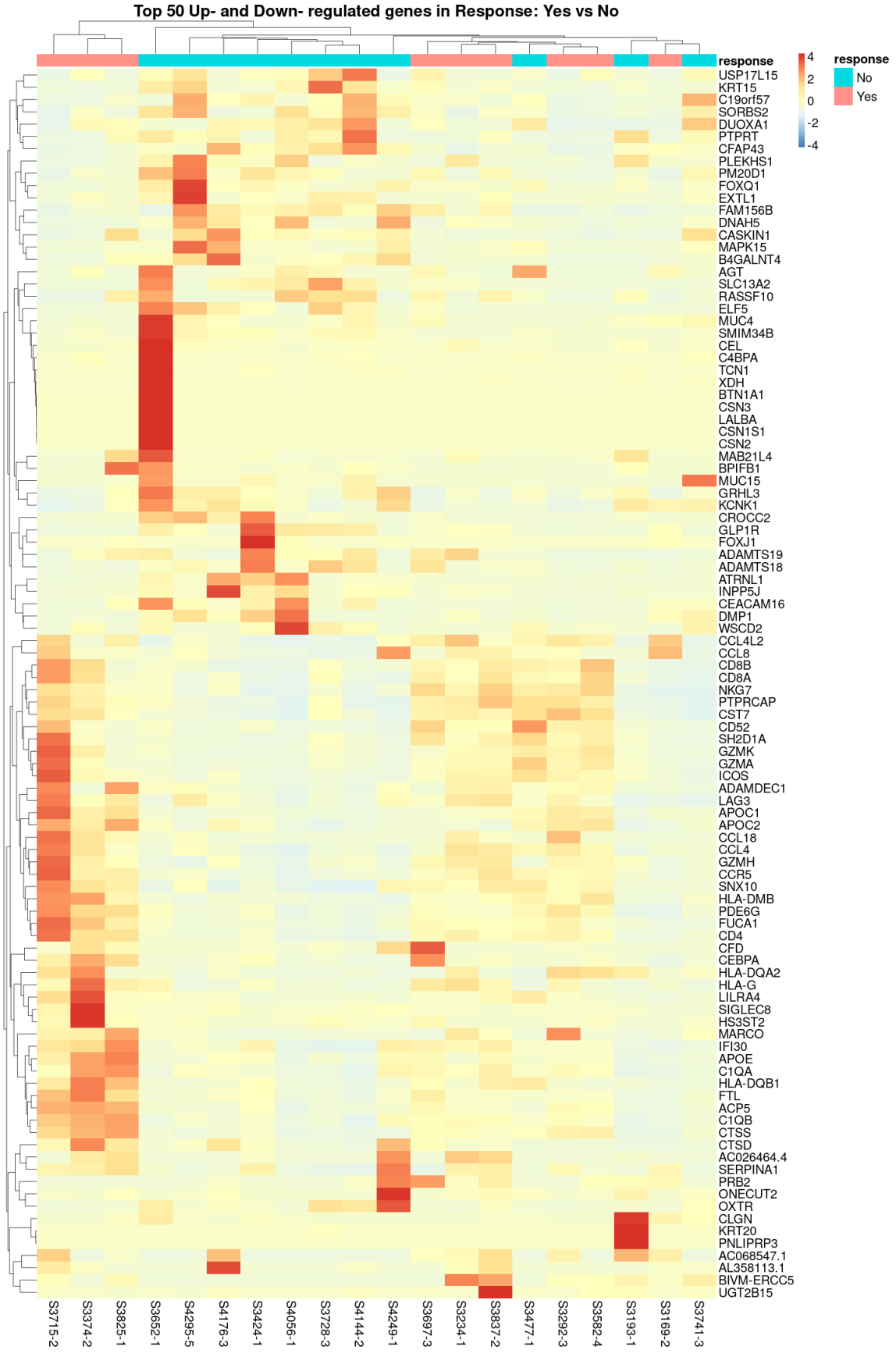

```
# Add a column for significant genes
resDO_tb <- resDO_tb %>% mutate(threshold = padj < 0.01)

ggplot(resDO_tb) +
  geom_point(aes(x = log2FoldChange, y = -log10(padj), colour = threshold)) +
  ggtitle("Dafe of: 20180323 vs 20180228") +
  xlab("log2 fold change") +
  ylab("-log10 adjusted p-value") +
  scale_x_continuous(limits = c(-10,10)) +
  theme(legend.position = "none",
        plot.title = element_text(size = rel(1.5), hjust = 0.5),
        axis.title = element_text(size = rel(1.25)))
```



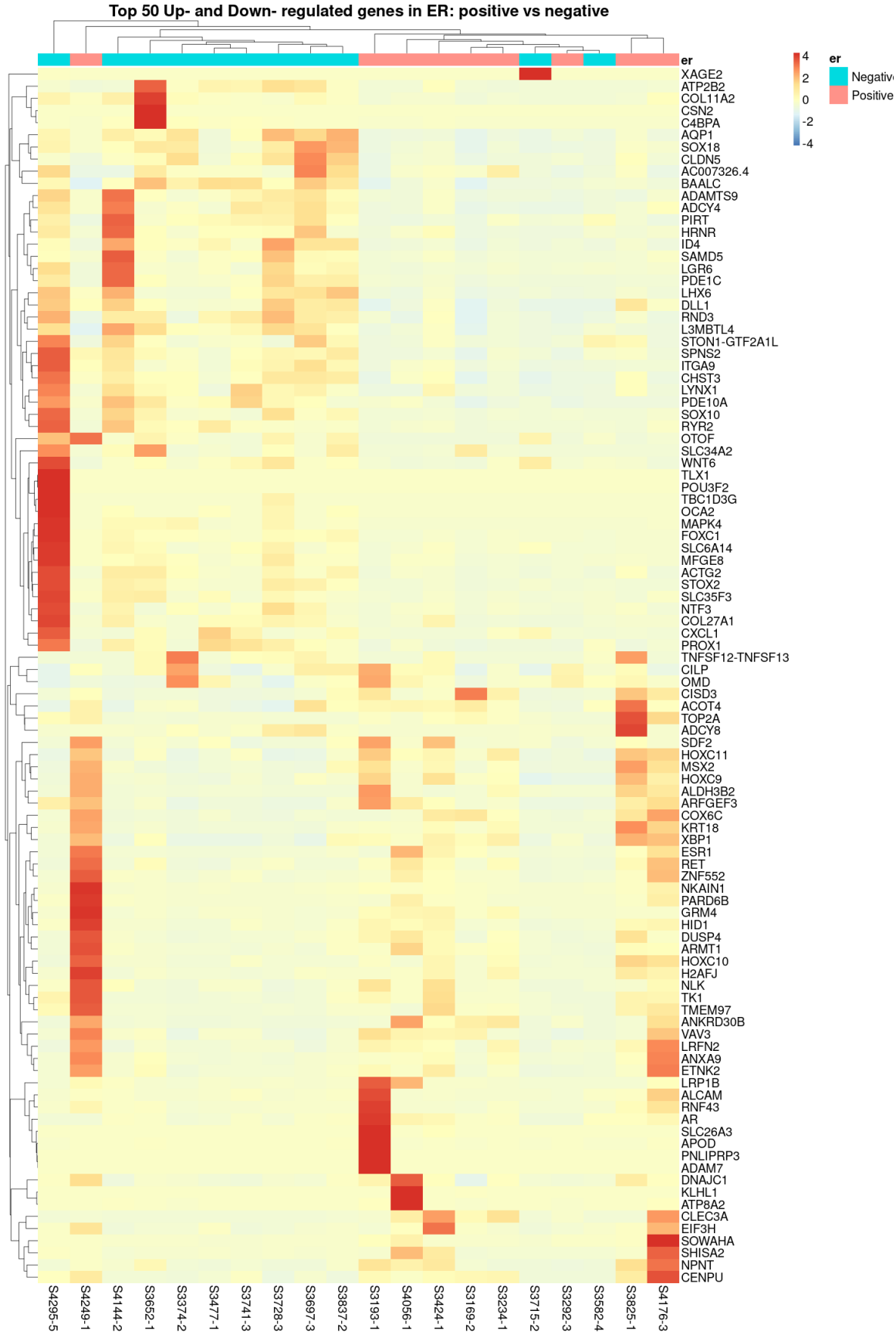
Heatmaps

```
# Create a matrix of normalized expression
sig_up <- resResponse_tb_significant %>% arrange(-log2FoldChange) %>% head(50) %>% pull(gene)
sig_down <- resResponse_tb_significant %>% arrange(log2FoldChange) %>% head(50) %>% pull(gene)
sig <- c(sig_up, sig_down)

row_annotation <- gene_symbol %>%
                    as_tibble() %>%
                    dplyr::filter(gene_id %in% sig)

plotmat <- txi$abundance[c(sig_up, sig_down),] %>% as.data.frame() %>%
          rownames_to_column(var = "ensembl_gene_id") %>%
          left_join(gene_symbol, by = c("ensembl_gene_id" = "gene_id")) %>%
          drop_na(symbol)

plotmat$ensembl_gene_id <- NULL

plotmat <- plotmat %>% column_to_rownames(var = "symbol") %>% as.matrix()

# Color palette
heat.colors <- brewer.pal(6, "YlOrRd")

# Plot heatmap
pheatmap(plotmat,
         scale = "row",
         show_rownames = TRUE,
```

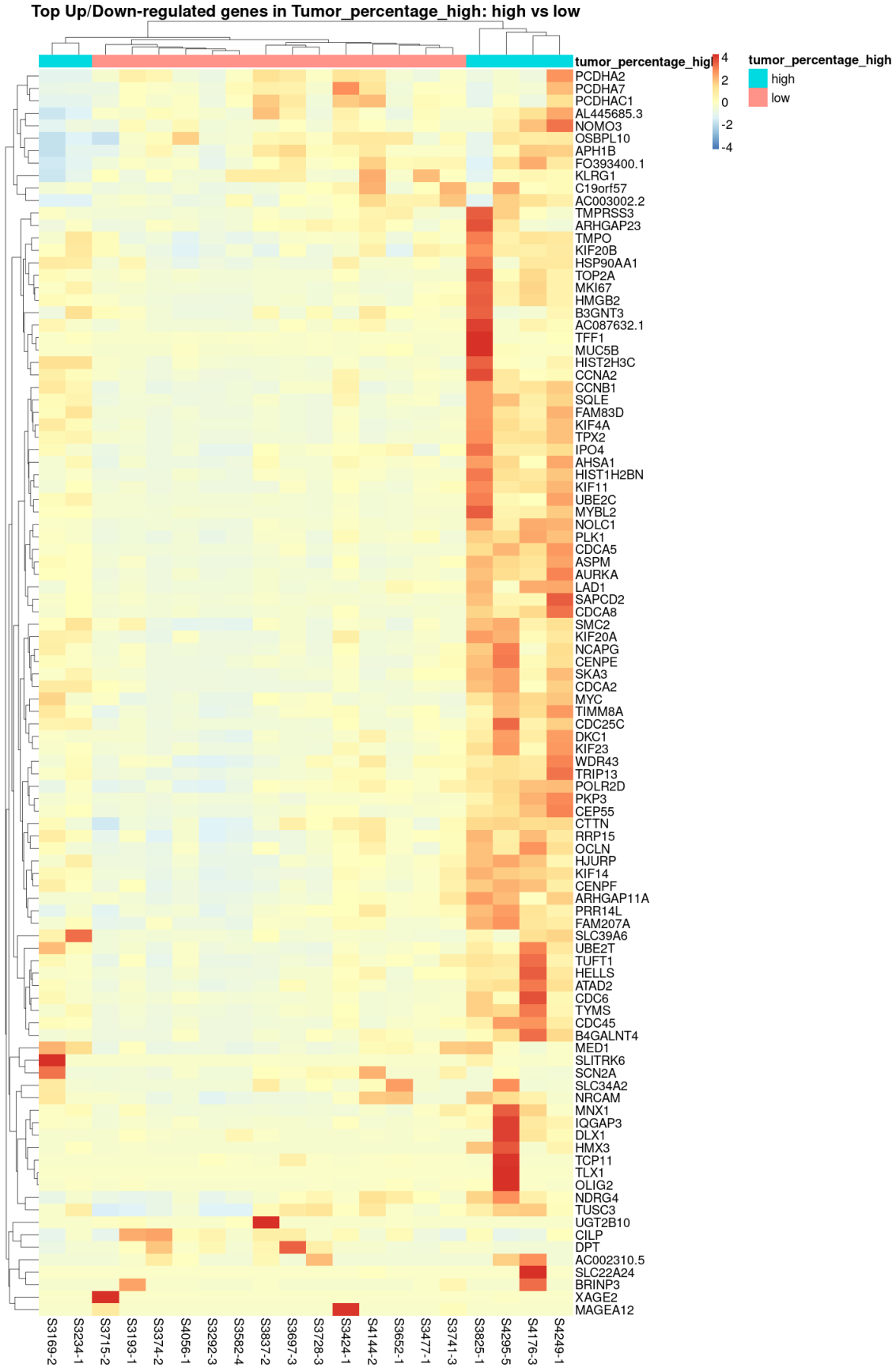
```
         border = FALSE,
         annotation = meta[, c("response"), drop = FALSE],
         main = "Top 50 Up- and Down- regulated genes in Response: Yes vs No",
         fontsize = 20)
```


Top 50 Up- and Down- regulated genes in Response: Yes vs No
response
No
Yes
4
2
0
-2
-4
USP17L15
KRT15
C19orf57
SORBS2
DUOXA1
PTPRT
CFAP43
PLEKHS1
PM20D1
FOXQ1
EXTL1
FAM156B
DNAH5
CASKIN1
MAPK15
B4GALNT4
AGT
SLC13A2
RASSF10
ELF5
MUC4
SMIM34B
CEL
C4BPA
TCN1
XDH
BTN1A1
CSN3
LALBA
CSN1S1
CSN2
MAB21L4
BPIFB1
MUC15
GRHL3
KCNK1
CROCC2
GLP1R
FOXJ1
ADAMTS19
ADAMTS18
ATRNL1
INPP5J
CEACAM16
DMP1
WSCD2
CCL4L2
CCL8
CD8B
CD8A
NKG7
PTPRCAP
CST7
CD52
SH2D1A
GZMK
GZMA
ICOS
ADAMDEC1
LAG3
APOC1
APOC2
CCL18
CCL4
GZMH
CCR5
SNX10
HLA-DMB
PDE6G
FUCA1
CD4
CFD
CEBPA
HLA-DQA2
HLA-G
LILRA4
SIGLEC8
HS3ST2
MARCO
IFI30
APOE
C1QA
HLA-DQB1
FTL
ACP5
C1QB
CTSS
CTSD
AC026464.4
SERPINA1
PRB2
ONECUT2
OXTR
CLGN
KRT20
PNLIPRP3
AC068547.1
AL358113.1
BIVM-ERCC5
UGT2B15
S3715-2
S3374-2
S3825-1
S3652-1
S4295-5
S4176-3
S3424-1
S4056-1
S3728-3
S4144-2
S4249-1
S3697-3
S3234-1
S3837-2
S3477-1
S3292-3
S3582-4
S3193-1
S3169-2
S3741-3

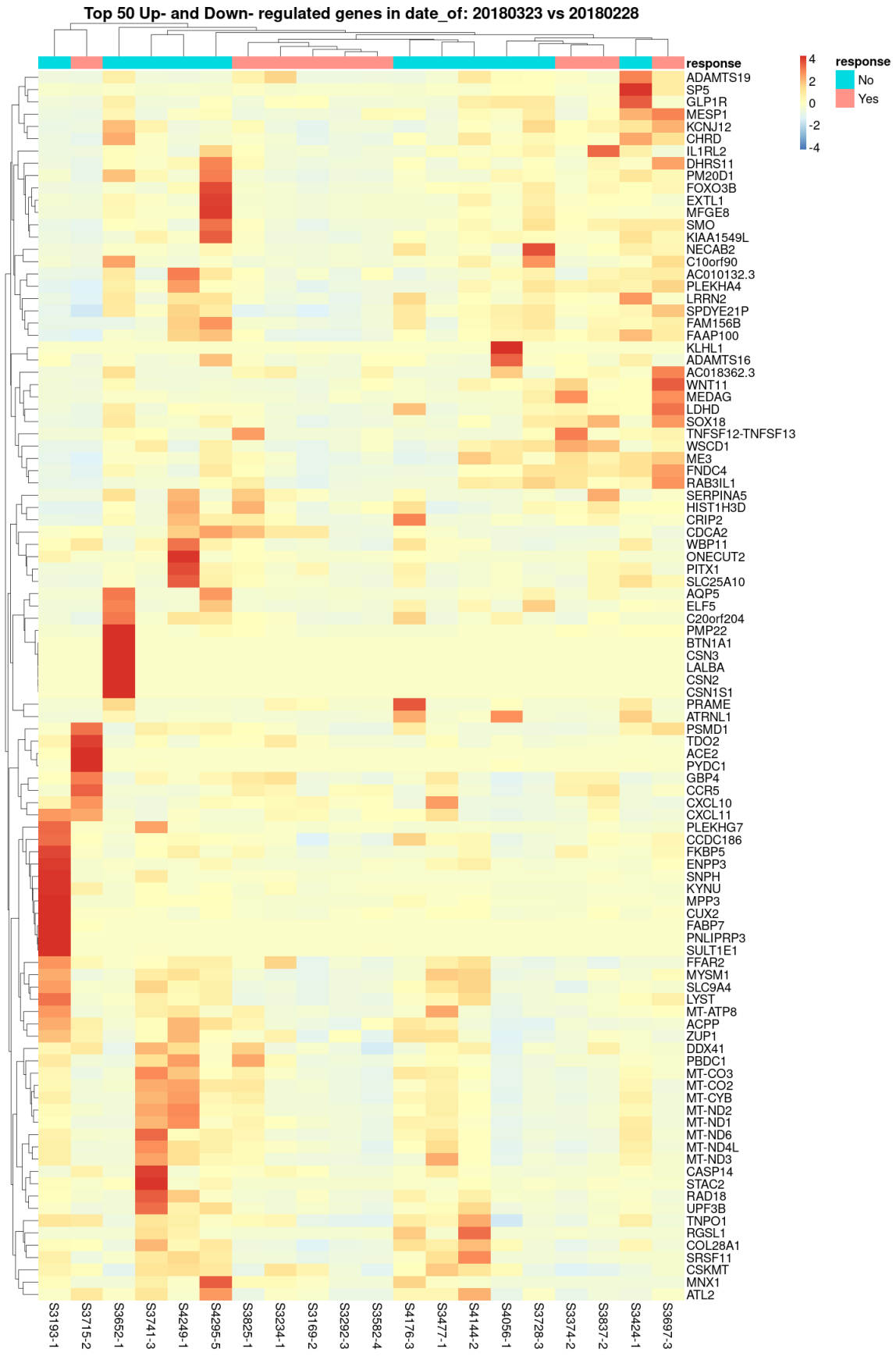
```r
# Create a matrix of normalized expression
sig_up <- resER_tb_significant %>% arrange(-log2FoldChange) %>% head(50) %>% pull(gene)
sig_down <- resER_tb_significant %>% arrange(log2FoldChange) %>% head(50) %>% pull(gene)
sig <- c(sig_up, sig_down)

row_annotation <- gene_symbol %>%
                    as_tibble() %>%
                    dplyr::filter(gene_id %in% sig)

plotmat <- txi$abundance[c(sig_up, sig_down),] %>% as.data.frame() %>%
          rownames_to_column(var = "ensembl_gene_id") %>%
          left_join(gene_symbol, by = c("ensembl_gene_id" = "gene_id")) %>%
          drop_na(symbol)

plotmat$ensembl_gene_id <- NULL

plotmat <- plotmat %>% column_to_rownames(var = "symbol") %>% as.matrix()

# Color palette
heat.colors <- brewer.pal(6, "YlOrRd")

# Plot heatmap
pheatmap(plotmat,
         scale = "row",
         show_rownames = TRUE,
         border = FALSE,
         annotation = meta[, c("er"), drop = FALSE],
         main = "Top 50 Up- and Down- regulated genes in ER: positive vs negative",
         fontsize = 20)
```


Top 50 Up- and Down- regulated genes in ER: positive vs negative
er
Negativ
Positive
4
2
0
-2
-4
XAGE2
ATP2B2
COL11A2
CSN2
C4BPA
AQP1
SOX18
CLDN5
AC007326.4
BAALC
ADAMTS9
ADCY4
PIRT
HRNR
ID4
SAMD5
LGR6
PDE1C
LHX6
DLL1
RND3
L3MBTL4
STON1-GTF2A1L
SPNS2
ITGA9
CHST3
LYNX1
PDE10A
SOX10
RYR2
OTOF
SLC34A2
WNT6
TLX1
POU3F2
TBC1D3G
OCA2
MAPK4
FOXC1
SLC6A14
MFGE8
ACTG2
STOX2
SLC35F3
NTF3
COL27A1
CXCL1
PROX1
TNFSF12-TNFSF13
CILP
OMD
CISD3
ACOT4
TOP2A
ADCY8
SDF2
HOXC11
MSX2
HOXC9
ALDH3B2
ARFGEF3
COX6C
KRT18
XBP1
ESR1
RET
ZNF552
NKAIN1
PARD6B
GRM4
HID1
DUSP4
ARMT1
HOXC10
H2AFJ
NLK
TK1
TMEM97
ANKRD30B
VAV3
LRFN2
ANXA9
ETNK2
LRP1B
ALCAM
RNF43
AR
SLC26A3
APOD
PNLIPRP3
ADAM7
DNAJC1
KLHL1
ATP8A2
CLEC3A
EIF3H
SOWAHA
SHISA2
NPNT
CENPU
S4295-5
S4249-1
S4144-2
S3652-1
S3374-2
S3477-1
S3741-3
S3728-3
S3697-3
S3837-2
S3193-1
S4056-1
S3424-1
S3169-2
S3234-1
S3715-2
S3292-3
S3582-4
S3825-1
S4176-3

```r
# Create a matrix of normalized expression
sig_up <- resTP_tb_significant %>% arrange(-log2FoldChange) %>% head(50) %>% pull(gene)
sig_down <- resTP_tb_significant %>% arrange(log2FoldChange) %>% head(50) %>% pull(gene)
sig <- c(sig_up, sig_down)

row_annotation <- gene_symbol %>%
                    as_tibble() %>%
                    dplyr::filter(gene_id %in% sig)

plotmat <- txi$abundance[c(sig_up, sig_down),] %>% as.data.frame() %>%
          rownames_to_column(var = "ensembl_gene_id") %>%
          left_join(gene_symbol, by = c("ensembl_gene_id" = "gene_id")) %>%
          drop_na(symbol)

plotmat$ensembl_gene_id <- NULL

plotmat <- plotmat %>% column_to_rownames(var = "symbol") %>% as.matrix()

# Color palette
heat.colors <- brewer.pal(6, "YlOrRd")

# Plot heatmap
pheatmap(plotmat,
         scale = "row",
         show_rownames = TRUE,
         border = FALSE,
         annotation = meta[, c("tumor_percentage_high"), drop = FALSE],
         main = "Top Up/Down-regulated genes in Tumor_percentage_high: high vs low",
         fontsize = 20)
```


Top Up/Down-regulated genes in Tumor_percentage_high: high vs low
tumor_percentage_high
4
2
0
-2
-4
tumor_percentage_high
high
low
PCDHA2
PCDHA7
PCDHAC1
AL445685.3
NOMO3
OSBPL10
APH1B
FO393400.1
KLRG1
C19orf57
AC003002.2
TMPRSS3
ARHGAP23
TMPO
KIF20B
HSP90AA1
TOP2A
MKI67
HMGB2
B3GNT3
AC087632.1
TFF1
MUC5B
HIST2H3C
CCNA2
CCNB1
SQLE
FAM83D
KIF4A
TPX2
IPO4
AHSA1
HIST1H2BN
KIF11
UBE2C
MYBL2
NOLC1
PLK1
CDCA5
ASPM
AURKA
LAD1
SAPCD2
CDCA8
SMC2
KIF20A
NCAPG
CENPE
SKA3
CDCA2
MYC
TIMM8A
CDC25C
DKC1
KIF23
WDR43
TRIP13
POLR2D
PKP3
CEP55
CTTN
RRP15
OCLN
HJURP
KIF14
CENPF
ARHGAP11A
PRR14L
FAM207A
SLC39A6
UBE2T
TUFT1
HELLS
ATAD2
CDC6
TYMS
CDC45
B4GALNT4
MED1
SLITRK6
SCN2A
SLC34A2
NRCAM
MNX1
IQGAP3
DLX1
HMX3
TCP11
TLX1
OLIG2
NDRG4
TUSC3
UGT2B10
CILP
DPT
AC002310.5
SLC22A24
BRINP3
XAGE2
MAGEA12
S3169-2
S3234-1
S3715-2
S3193-1
S3374-2
S4056-1
S3292-3
S3582-4
S3837-2
S3697-3
S3728-3
S3424-1
S4144-2
S3652-1
S3477-1
S3741-3
S3825-1
S4295-5
S4176-3
S4249-1

```r
# Create a matrix of normalized expression
sig_up <- resDO_tb_significant %>% arrange(-log2FoldChange) %>% head(50) %>% pull(gene)
sig_down <- resDO_tb_significant %>% arrange(log2FoldChange) %>% head(50) %>% pull(gene)
sig <- c(sig_up, sig_down)

row_annotation <- gene_symbol %>%
                    as_tibble() %>%
                    dplyr::filter(gene_id %in% sig)

plotmat <- txi$abundance[c(sig_up, sig_down),] %>% as.data.frame() %>%
          rownames_to_column(var = "ensembl_gene_id") %>%
          left_join(gene_symbol, by = c("ensembl_gene_id" = "gene_id")) %>%
          drop_na(symbol)

plotmat$ensembl_gene_id <- NULL

plotmat <- plotmat %>% column_to_rownames(var = "symbol") %>% as.matrix()

# Color palette
heat.colors <- brewer.pal(6, "YlOrRd")

# Plot heatmap
pheatmap(plotmat,
         scale = "row",
         show_rownames = TRUE,
         border = FALSE,
         annotation = meta[, c("response"), drop = FALSE],
         main = "Top 50 Up- and Down- regulated genes in date_of: 20180323 vs 20180228",
         fontsize = 20)
```


Top 50 Up- and Down- regulated genes in date_of: 20180323 vs 20180228
response
No
Yes
4
2
0
-2
-4
ADAMTS19
SP5
GLP1R
MESP1
KCNJ12
CHRD
IL1RL2
DHRS11
PM20D1
FOXO3B
EXTL1
MFGE8
SMO
KIAA1549L
NECAB2
C10orf90
AC010132.3
PLEKHA4
LRRN2
SPDYE21P
FAM156B
FAAP100
KLHL1
ADAMTS16
AC018362.3
WNT11
MEDAG
LDHD
SOX18
TNFSF12-TNFSF13
WSCD1
ME3
FNDC4
RAB3IL1
SERPINA5
HIST1H3D
CRIP2
CDCA2
WBP11
ONECUT2
PITX1
SLC25A10
AQP5
ELF5
C20orf204
PMP22
BTN1A1
CSN3
LALBA
CSN2
CSN1S1
PRAME
ATRNL1
PSMD1
TDO2
ACE2
PYDC1
GBP4
CCR5
CXCL10
CXCL11
PLEKHG7
CCDC186
FKBP5
ENPP3
SNPH
KYNU
MPP3
CUX2
FABP7
PNLIPRP3
SULT1E1
FFAR2
MYSM1
SLC9A4
LYST
MT-ATP8
ACPP
ZUP1
DDX41
PBDC1
MT-CO3
MT-CO2
MT-CYB
MT-ND2
MT-ND1
MT-ND6
MT-ND4L
MT-ND3
CASP14
STAC2
RAD18
UPF3B
TNPO1
RGSL1
COL28A1
SRSF11
CSKMT
MNX1
ATL2
S3193-1
S3715-2
S3652-1
S3741-3
S4249-1
S4295-5
S3825-1
S3234-1
S3169-2
S3292-3
S3582-4
S4176-3
S3477-1
S4144-2
S4056-1
S3728-3
S3374-2
S3837-2
S3424-1
S3697-3

```
#  prepares an expression profile for GSEA
#  http://software.broadinstitute.org/cancer/software/gsea/wiki/index.php/Data_formats#Expression_Data_
#  for GSEA it is important to report all genes - genome wide
#  hopefully cpms are better than logcpms
counts <- counts[rowSums(counts)>0,]
result_file <-  paste0("tables/8day.4gsea.txt")

counts_gsea <- counts %>% as.data.frame() %>%
    rownames_to_column(var = "ensembl_gene_id") %>%
    left_join(gene_symbol, by = c("ensembl_gene_id" = "gene_id")) %>%
    dplyr::relocate(symbol)
#%>%
#    dplyr::relocate(ensembl_gene_id)

colnames(counts_gsea)[1:2] <- c("NAME", "DESCRIPTION")

d <-duplicated(counts_gsea$NAME)
o <-  order(rowSums(counts_gsea[,rownames(meta)]),decreasing = T)
counts_gsea <- counts_gsea[o, ]
counts_gsea <- counts_gsea[!d, ]


samples_yes <- meta %>% dplyr::filter(response == "Yes") %>% row.names()
samples_no <- meta %>% dplyr::filter(response == "No") %>% row.names()

counts_gsea <- counts_gsea[,c("NAME", "DESCRIPTION", samples_yes, samples_no)]
# gsea now supports ENSEMBL_IDs
write_tsv(counts_gsea, result_file)
```

Functional analysis

Biological Process (BP)

```
bg_genes <- rownames(resResponse)

## Run GO enrichment analysis
compGO <- enrichGO(gene = sigResponse_up,
                   universe = bg_genes,
                   keyType = "ENSEMBL",
                   OrgDb = "org.Hs.eg.db",
                   ont = "BP",
                   qvalueCutoff  = 0.05,
                   pAdjustMethod = "BH",
                   readable = TRUE)

#dotplot(compGO,
#        showCategory = 20,
#        title = "GO (Biological Process) Enrichment \n Analysis for UP in Responders)",
#        label_format = 20,
#        font.size = 10)
# image pdf 12 x 12
```

```
## Output results from GO analysis to a table
print("UP")
```

```
## [1] "UP"
```

```
results_up <- data.frame(compGO@result) %>% dplyr::filter(p.adjust < 0.05)
nrow(results_up)
```

```
## [1] 252
```

```
write_csv(results_up, "tables/T20.day8.GO_BP_UP.csv")

compGO <- enrichGO(gene = sigResponse_down,
                   universe = bg_genes,
                   keyType = "ENSEMBL",
                   OrgDb = "org.Hs.eg.db",
                   ont = "BP",
                   qvalueCutoff  = 0.05,
                   pAdjustMethod = "BH",
                   readable = TRUE)


results_down <- data.frame(compGO@result) %>% dplyr::filter(p.adjust < 0.05)
print("Down")
```

```
## [1] "Down"
```

```
nrow(results_down)
```

```
## [1] 0
```

Molecular Function (MF)

```
bg_genes <- rownames(resResponse)

## Run GO enrichment analysis
compGO <- enrichGO(gene = sigResponse_up,
                   universe = bg_genes,
                   keyType = "ENSEMBL",
                   OrgDb = "org.Hs.eg.db",
                   ont = "MF",
                   qvalueCutoff  = 0.05,
                   pAdjustMethod = "BH",
                   readable = TRUE)

#dotplot(compGO,
#        showCategory = 20,
#        title = "GO (Biological Process) Enrichment \n Analysis for UP in Responders)",
#        label_format = 20,
#        font.size = 10)
# image pdf 12 x 12

## Output results from GO analysis to a table
print("UP")
```

```
## [1] "UP"
```

```r
results_up <- data.frame(compGO@result) %>% dplyr::filter(p.adjust < 0.05)
nrow(results_up)
```

```
## [1] 20
```

```r
write_csv(results_up, "tables/T21.day8.GO_MF_UP.csv")

compGO <- enrichGO(gene = sigResponse_down,
                   universe = bg_genes,
                   keyType = "ENSEMBL",
                   OrgDb = "org.Hs.eg.db",
                   ont = "BP",
                   qvalueCutoff  = 0.05,
                   pAdjustMethod = "BH",
                   readable = TRUE)


results_down <- data.frame(compGO@result) %>% dplyr::filter(p.adjust < 0.05)
print("Down")
```

```
## [1] "Down"
```

```r
nrow(results_down)
```

```
## [1] 0
```

Cellular Compartment (CC)

```r
bg_genes <- rownames(resResponse)

## Run GO enrichment analysis
compGO <- enrichGO(gene = sigResponse_up,
                   universe = bg_genes,
                   keyType = "ENSEMBL",
                   OrgDb = "org.Hs.eg.db",
                   ont = "CC",
                   qvalueCutoff  = 0.05,
                   pAdjustMethod = "BH",
                   readable = TRUE)

#dotplot(compGO,
#        showCategory = 20,
#        title = "GO (Biological Process) Enrichment \n Analysis for UP in Responders)",
#        label_format = 20,
#        font.size = 10)
# image pdf 12 x 12

## Output results from GO analysis to a table
print("UP")
```

```
## [1] "UP"
```

```r
results_up <- data.frame(compGO@result) %>% dplyr::filter(p.adjust < 0.05)
nrow(results_up)
```

```
## [1] 60
```

```
write_csv(results_up, "tables/T22.day8.GO_CC_UP.csv")

compGO <- enrichGO(gene = sigResponse_down,
                   universe = bg_genes,
                   keyType = "ENSEMBL",
                   OrgDb = "org.Hs.eg.db",
                   ont = "BP",
                   qvalueCutoff  = 0.05,
                   pAdjustMethod = "BH",
                   readable = TRUE)


results_down <- data.frame(compGO@result) %>% dplyr::filter(p.adjust < 0.05)
print("Down")
```

```
## [1] "Down"
```

```
nrow(results_down)
```

```
## [1] 0
```

R session

```
sessionInfo()
```

```
## R version 4.0.3 (2020-10-10)
## Platform: x86_64-redhat-linux-gnu (64-bit)
## Running under: Fedora 32 (Workstation Edition)
##
## Matrix products: default
## BLAS/LAPACK: /usr/lib64/libopenblas-r0.3.12.so
##
## locale:
##  [1] LC_CTYPE=en_CA.UTF-8       LC_NUMERIC=C
##  [3] LC_TIME=en_CA.UTF-8        LC_COLLATE=en_CA.UTF-8
##  [5] LC_MONETARY=en_CA.UTF-8    LC_MESSAGES=en_CA.UTF-8
##  [7] LC_PAPER=en_CA.UTF-8       LC_NAME=C
##  [9] LC_ADDRESS=C               LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_CA.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] parallel  stats4    stats     graphics  grDevices utils     datasets
## [8] methods   base
##
## other attached packages:
##  [1] clusterProfiler_3.18.1      org.Hs.eg.db_3.12.0
##  [3] ensembldb_2.14.0            AnnotationFilter_1.14.0
##  [5] GenomicFeatures_1.42.1      AnnotationDbi_1.52.0
##  [7] AnnotationHub_2.22.0        BiocFileCache_1.14.0
##  [9] dbplyr_2.1.0                knitr_1.30
## [11] ggrepel_0.9.1               tximport_1.18.0
## [13] DEGreport_1.26.0            pheatmap_1.0.12
## [15] RColorBrewer_1.1-2          forcats_0.5.1
## [17] stringr_1.4.0               dplyr_1.0.5
```

```
## [19] purrr_0.3.4                readr_1.4.0
## [21] tidyr_1.1.3                tibble_3.1.0
## [23] ggplot2_3.3.3              tidyverse_1.3.0
## [25] DESeq2_1.30.1              SummarizedExperiment_1.20.0
## [27] Biobase_2.50.0             MatrixGenerics_1.2.1
## [29] matrixStats_0.58.0         GenomicRanges_1.42.0
## [31] GenomeInfoDb_1.26.2        IRanges_2.24.1
## [33] S4Vectors_0.28.1           BiocGenerics_0.36.0
##
## loaded via a namespace (and not attached):
##   [1] utf8_1.1.4                   tidyselect_1.1.0
##   [3] RSQLite_2.2.3                grid_4.0.3
##   [5] BiocParallel_1.24.1          scatterpie_0.1.5
##   [7] munsell_0.5.0                withr_2.4.1
##   [9] colorspace_2.0-0             GOSemSim_2.16.1
##  [11] rstudioapi_0.13              DOSE_3.16.0
##  [13] labeling_0.4.2               lasso2_1.2-21.1
##  [15] GenomeInfoDbData_1.2.4       polyclip_1.10-0
##  [17] mnormt_2.0.2                 farver_2.1.0
##  [19] bit64_4.0.5                  downloader_0.4
##  [21] vctrs_0.3.6                  generics_0.1.0
##  [23] xfun_0.19                    R6_2.5.0
##  [25] graphlayouts_0.7.1           clue_0.3-58
##  [27] locfit_1.5-9.4               bitops_1.0-6
##  [29] cachem_1.0.4                 reshape_0.8.8
##  [31] fgsea_1.16.0                 DelayedArray_0.16.2
##  [33] assertthat_0.2.1             promises_1.2.0.1
##  [35] scales_1.1.1                 ggraph_2.0.5
##  [37] enrichplot_1.10.2            gtable_0.3.0
##  [39] Cairo_1.5-12.2               tidygraph_1.2.0
##  [41] rlang_0.4.10                 genefilter_1.72.1
##  [43] GlobalOptions_0.1.2          splines_4.0.3
##  [45] rtracklayer_1.50.0           lazyeval_0.2.2
##  [47] broom_0.7.5                  BiocManager_1.30.10
##  [49] yaml_2.2.1                   reshape2_1.4.4
##  [51] modelr_0.1.8                 backports_1.2.1
##  [53] httpuv_1.5.5                 qvalue_2.22.0
##  [55] tools_4.0.3                  psych_2.0.12
##  [57] logging_0.10-108             ellipsis_0.3.1
##  [59] ggdendro_0.1.22              Rcpp_1.0.6
##  [61] plyr_1.8.6                   progress_1.2.2
##  [63] zlibbioc_1.36.0              RCurl_1.98-1.2
##  [65] prettyunits_1.1.1            openssl_1.4.3
##  [67] viridis_0.5.1                GetoptLong_1.0.5
##  [69] cowplot_1.1.1                haven_2.3.1
##  [71] cluster_2.1.0                fs_1.5.0
##  [73] magrittr_2.0.1               data.table_1.14.0
##  [75] DO.db_2.9                    circlize_0.4.12
##  [77] reprex_1.0.0                 tmvnsim_1.0-2
##  [79] ProtGenerics_1.22.0          hms_1.0.0
##  [81] mime_0.9                     evaluate_0.14
##  [83] xtable_1.8-4                 XML_3.99-0.5
##  [85] readxl_1.3.1                 gridExtra_2.3
##  [87] shape_1.4.5                  compiler_4.0.3
```

```
##  [89] biomaRt_2.46.3              shadowtext_0.0.7
##  [91] crayon_1.4.1                htmltools_0.5.1.1
##  [93] later_1.1.0.1               geneplotter_1.68.0
##  [95] lubridate_1.7.10            DBI_1.1.1
##  [97] tweenr_1.0.1                ComplexHeatmap_2.6.2
##  [99] MASS_7.3-53                 rappdirs_0.3.3
## [101] Matrix_1.2-18               cli_2.3.1
## [103] igraph_1.2.6                pkgconfig_2.0.3
## [105] rvcheck_0.1.8               GenomicAlignments_1.26.0
## [107] xml2_1.3.2                  annotate_1.68.0
## [109] XVector_0.30.0              rvest_1.0.0
## [111] digest_0.6.27               ConsensusClusterPlus_1.54.0
## [113] Biostrings_2.58.0           rmarkdown_2.5
## [115] cellranger_1.1.0            fastmatch_1.1-0
## [117] edgeR_3.32.1                curl_4.3
## [119] shiny_1.6.0                 Rsamtools_2.6.0
## [121] rjson_0.2.20                lifecycle_1.0.0
## [123] nlme_3.1-149                jsonlite_1.7.1
## [125] viridisLite_0.3.0           askpass_1.1
## [127] limma_3.46.0                fansi_0.4.2
## [129] pillar_1.5.1                lattice_0.20-41
## [131] Nozzle.R1_1.1-1             fastmap_1.1.0
## [133] httr_1.4.2                  survival_3.2-7
## [135] GO.db_3.12.1                interactiveDisplayBase_1.28.0
## [137] glue_1.4.2                  png_0.1-7
## [139] BiocVersion_3.12.0          bit_4.0.4
## [141] ggforce_0.3.3               stringi_1.5.3
## [143] blob_1.2.1                  memoise_2.0.0
```