DAAD RNA-seq course - lessons 3-4

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Contents

Overview

- Petrenko2024 RNA-seq experiment for reanalysis

Load annotations

```
annotation_file <- "../../99_technical/ensembl112_mm10_annotations.txt"
gene_symbol_file <- "../../99_technical/gene_symbol.txt"
if (file.exists(annotation_file)){
    mmdb <- read_tsv(annotation_file)
    gene_symbol <- read_tsv(gene_symbol_file)
}else{
    # Connect to AnnotationHub
    ah <- AnnotationHub()
    # Query AnnotationHub
    mm_ens <- query(ah, c("Mus musculus", "EnsDb"))

    # Get Ensembl 112
    # AH116909
    mm_ens <- mm_ens[["AH116909"]]

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

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

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

    mmdb <- inner_join(txdb, genedb)
    write.table(mmdb,
            file = annotation_file ,
            sep = "\t",
            row.names = F,
            quote = F)
}
tx2gene <- mmdb[, c("tx_id", "gene_id")]
```

Load Counts

```
# raw counts downloaded from
# https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-13804

# setwd("02_classes/03_rnaseq_intro_part1/")

protein_coding_genes <- read_csv("../../99_technical/ensembl_w_description.mouse.protein_coding.csv")

counts_csv <- "../../01_data/counts.csv"
counts_tpm_csv <- "../../01_data/count_matrix_tpm.csv"
if (file.exists(counts_csv)){
    counts_prepared <- read_csv(counts_csv)
    counts_tpm <- read_csv(counts_tpm_csv)
}else{
    counts_raw_csv <- "../../01_data/count_matrix_raw.csv"
    counts_raw <- read_csv(counts_raw_csv)
    colnames(counts_raw)[1] <- "gene_name"
    # counts_tpm <- read_csv(counts_tpm_csv)
    # use Ensembl_Gene_id
    # remove genes with NA
    # filter protein coding genes

    counts_prepared <- counts_raw %>% left_join(gene_symbol, by = c("gene_name" = "symbol")) %>%
       dplyr::select(-gene_name) %>% drop_na(gene_id) %>%
       semi_join(protein_coding_genes, by = c("gene_id" = "ensembl_gene_id")) %>%
       relocate(gene_id) %>% rename(ensembl_gene_id = gene_id)

    gene_length <- read_tsv("../../99_technical/GC_lengths.tsv")
    counts <- counts_prepared %>% arrange(ensembl_gene_id)
    gene_ids <- intersect(counts$ensembl_gene_id, gene_length$ensembl_gene_id)

    v_len <- gene_length %>% dplyr::filter(ensembl_gene_id %in% gene_ids)
    counts_prepared <- counts %>% dplyr::filter(ensembl_gene_id %in% gene_ids)

    write_csv(counts_prepared, counts_csv)
    counts <- counts %>% column_to_rownames("ensembl_gene_id")

    x <- counts / v_len$Length
    counts_tpm <- t(t(x) * 1e6 / colSums(x)) %>% as.data.frame() %>% round(2) %>%
      rownames_to_column("ensembl_gene_id") %>% left_join(gene_symbol,
                                                          by = c("ensembl_gene_id" = "gene_id")) %>%
      write_csv(counts_tpm_csv)

}
counts <- counts_prepared %>% column_to_rownames(var = "ensembl_gene_id")
```

Cleanup and load metadata

```
# Load the data and metadata
# remove duplicate rows
metadata_csv <- "../../01_data/metadata.csv"
if (file.exists(metadata_csv)){
     metadata <- read_csv(metadata_csv)
}else{
    metadata_raw <- read_tsv("../../01_data/E-MTAB-13804.sdrf.txt") %>%
                      dplyr::select(-any_of(c("Scan Name", "Comment[SUBMITTED_FILE_NAME]",
                            "Comment[ENA_RUN]", "Comment[FASTQ_URI]"))) %>% distinct() %>%
                            dplyr::rename(sample_id = `Source Name`)
    colnames(metadata_raw)[7] <- "age"
    colnames(metadata_raw)[44] <- "stimulus1"
    colnames(metadata_raw)[46] <- "factor_value"


    metadata_raw$stimulus <- str_replace_all(metadata_raw$stimulus1, "control \\(olive oil\\)", "olive_
    metadata_raw$stimulus <- str_replace_all(metadata_raw$stimulus, "control \\(saline\\)", "saline")
    metadata_raw$stimulus <- str_replace_all(metadata_raw$stimulus, "carbon tetrachloride", "carbon_tet

    metadata <- metadata_raw %>% separate(factor_value, sep = "_",
                                          into = c("experiment", "treatment")) %>%
         dplyr::select(sample_id, experiment, treatment, stimulus)

    write_csv(metadata, metadata_csv)
}

metadata <- metadata %>% column_to_rownames(var = "sample_id")
```

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

```
## Create DESeq2Dataset object
dds_file <- "../../data/dds.RDS"

if (file.exists(dds_file)){
    dds <- readRDS(dds_file)
}else{
    dds <- DESeqDataSetFromMatrix(countData = counts,
                                  colData = metadata,
                                  design = ~treatment)


    # subset protein-coding genes
    pc_genes <- intersect(protein_coding_genes$ensembl_gene_id, row.names(dds))
    dds <- dds[pc_genes,]
    # 9509 genes left
    keep <- rowMeans(counts(dds)) >= 100
    dds <- dds[keep, ]

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

Convenience functions

```r
# return mean counts for a group of sample in a column
get_counts_for_samples <- function(ctpm, samples, column_name){
    tpm_counts <- ctpm %>%
        column_to_rownames("ensembl_gene_id") %>%
        dplyr::select(any_of(samples)) %>%
        rowMeans() %>%
        as.data.frame() %>%
        round(2) %>%
        rownames_to_column("ensembl_gene_id")

    colnames(tpm_counts) <- c("ensembl_gene_id", "tpm")

    tpm_counts <- tpm_counts %>%
        dplyr::mutate("{column_name}" := round(tpm, 2)) %>%
        dplyr::select(-tpm)

    return(tpm_counts)
}

# get rid of excess precision
comb_de_result_table <- function(results){
    results <- results %>%
        mutate(baseMean = round(baseMean, 2),
               log2FoldChange = round(log2FoldChange, 2),
               lfcSE = round(lfcSE, 2),
               stat = round(stat, 2),
               pvalue = format(pvalue, scientific = TRUE, digits = 2),
               padj = format(padj, scientific = TRUE, digits = 2))
    return(results)
}
```

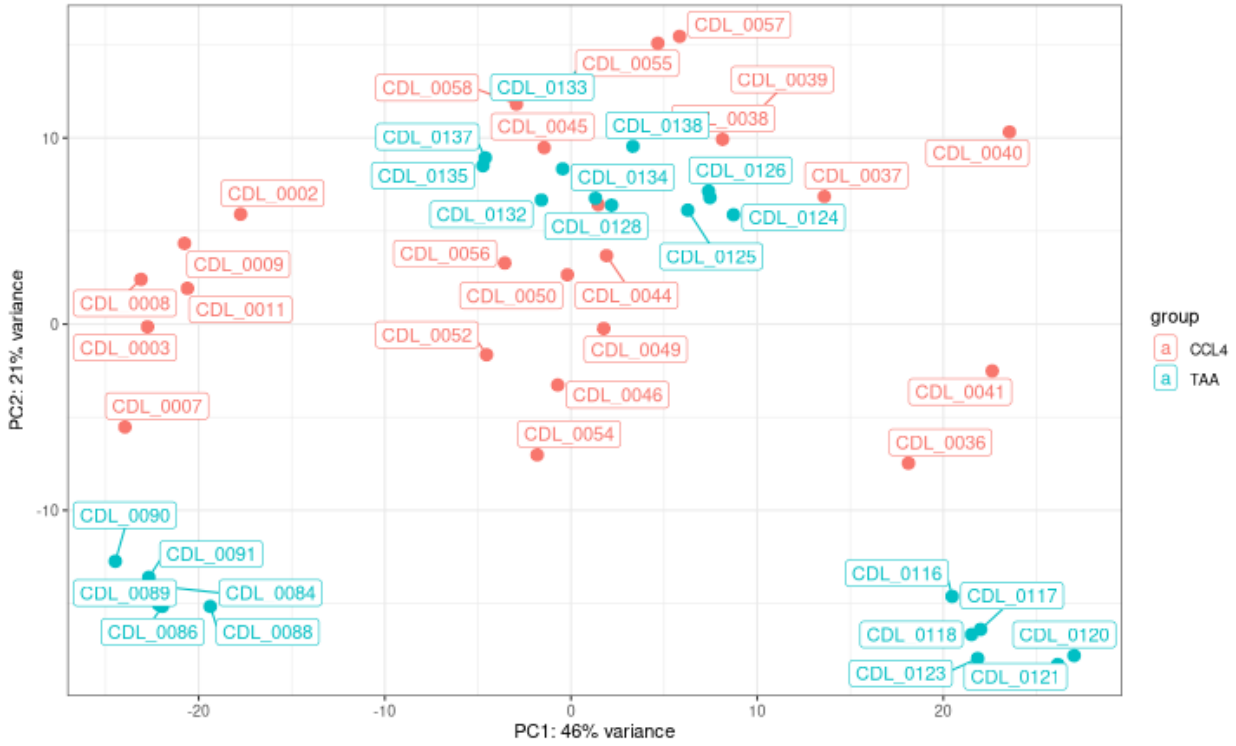
Sample-level QC analysis

PCA - treatment

```
plotPCA(rld, intgroup = c("treatment")) +
  geom_label_repel(aes(label = name)) +
  theme_bw()
```

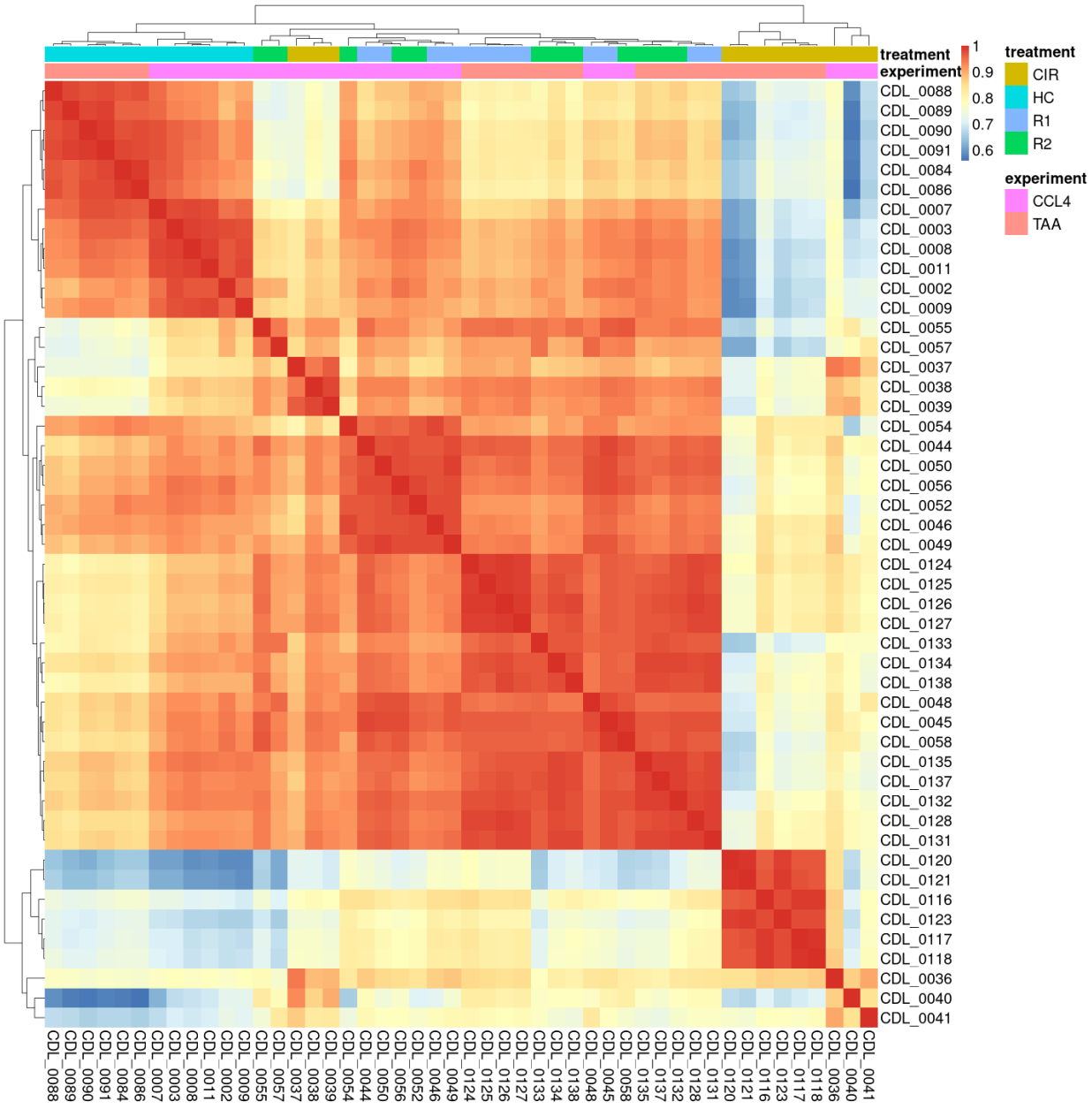
PCA - experiment

```
plotPCA(rld, intgroup = c("experiment")) + geom_label_repel(aes(label = name)) + theme_bw()
```



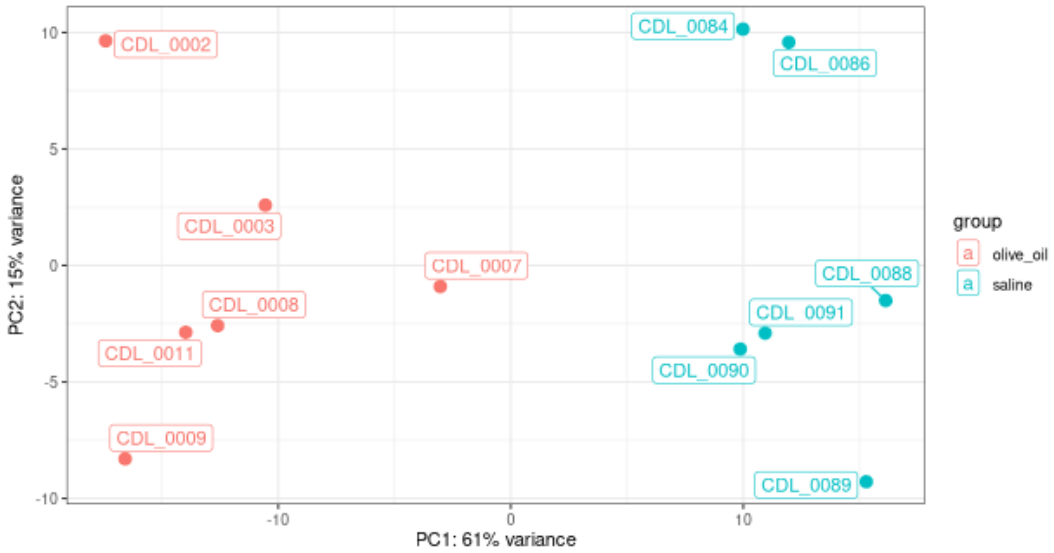
Clustering using top 1000 variable genes

```
rv <- rowVars(rld_mat)
rv <- order(rv, decreasing = TRUE) %>% head(1000)
rld_mat_1000 <- rld_mat[rv,]
annotation <- metadata[, c("experiment", "treatment")]

# Change colors
heat.colors <- brewer.pal(6, "Blues")
rld_cor <- cor(rld_mat_1000)
# Plot heatmap
pheatmap(rld_cor,
         annotation = annotation,
         border = NA,
         fontsize = 20)
```


treatment
experiment
CDL_0088
CDL_0089
CDL_0090
CDL_0091
CDL_0084
CDL_0086
CDL_0007
CDL_0003
CDL_0008
CDL_0011
CDL_0002
CDL_0009
CDL_0055
CDL_0057
CDL_0037
CDL_0038
CDL_0039
CDL_0054
CDL_0044
CDL_0050
CDL_0056
CDL_0052
CDL_0046
CDL_0049
CDL_0124
CDL_0125
CDL_0126
CDL_0127
CDL_0133
CDL_0134
CDL_0138
CDL_0048
CDL_0045
CDL_0058
CDL_0135
CDL_0137
CDL_0132
CDL_0128
CDL_0131
CDL_0120
CDL_0121
CDL_0116
CDL_0123
CDL_0117
CDL_0118
CDL_0036
CDL_0040
CDL_0041
1
0.9
0.8
0.7
0.6
treatment
CIR
HC
R1
R2
experiment
CCL4
TAA

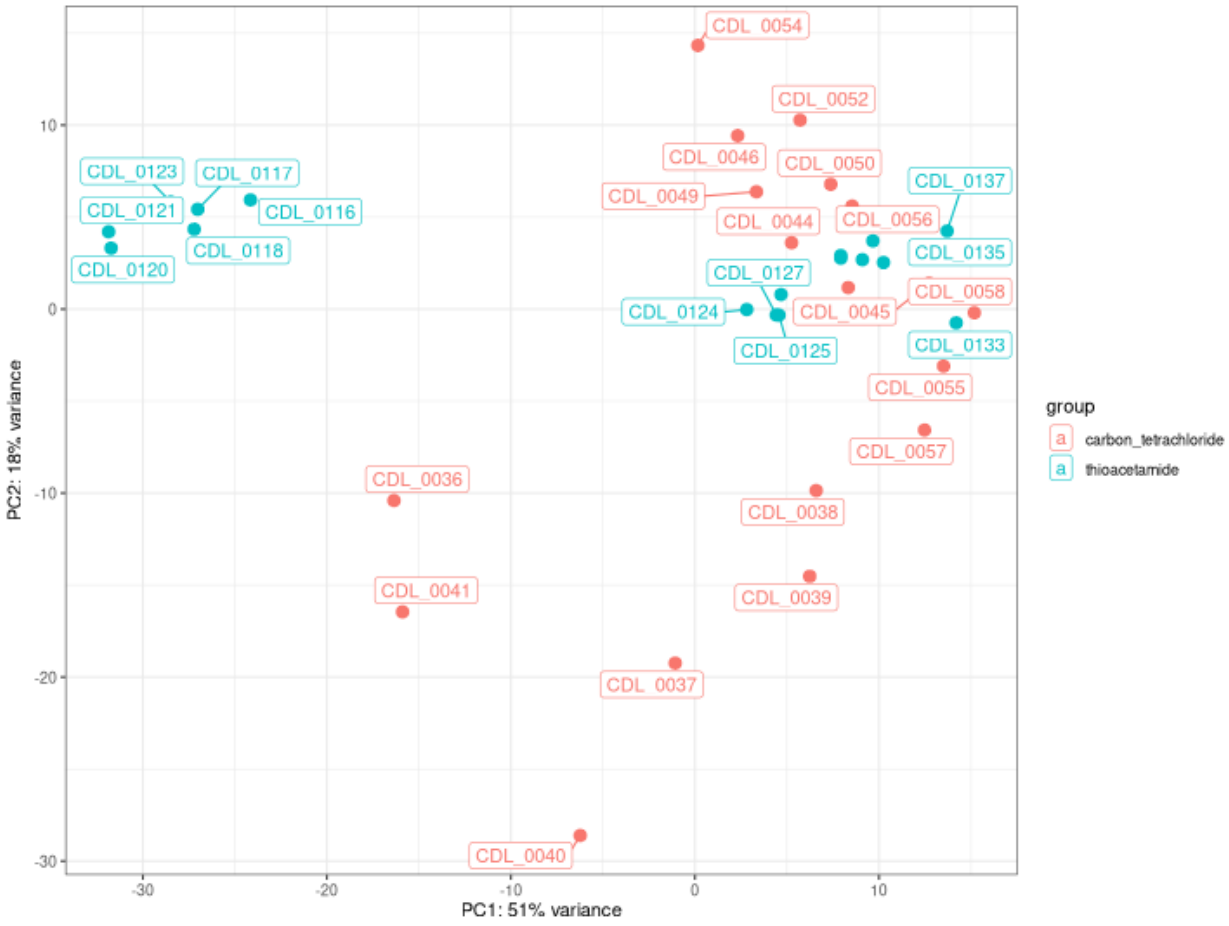
PCA: Controls

```
rld.sub <- rld[ , rld$stimulus %in% c("saline", "olive_oil") ]
plotPCA(rld.sub, intgroup = c("stimulus")) +
  geom_label_repel(aes(label = name)) +
  theme_bw()
```



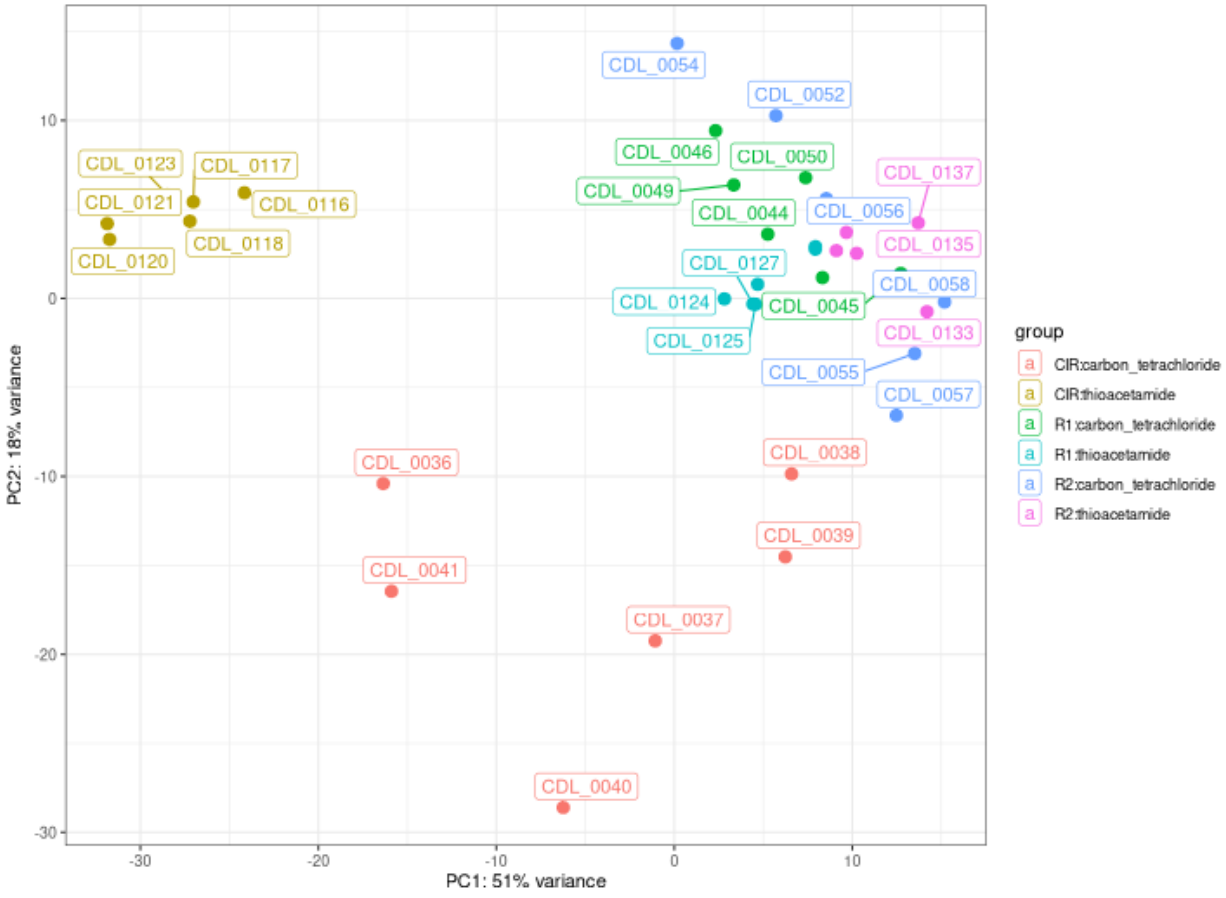
PCA: TAA and CCL4

```
rld.sub <- rld[ , rld$stimulus %in% c("carbon_tetrachloride", "thioacetamide")]
plotPCA(rld.sub, intgroup = c("stimulus")) +
  geom_label_repel(aes(label = name)) +
  theme_bw()
```



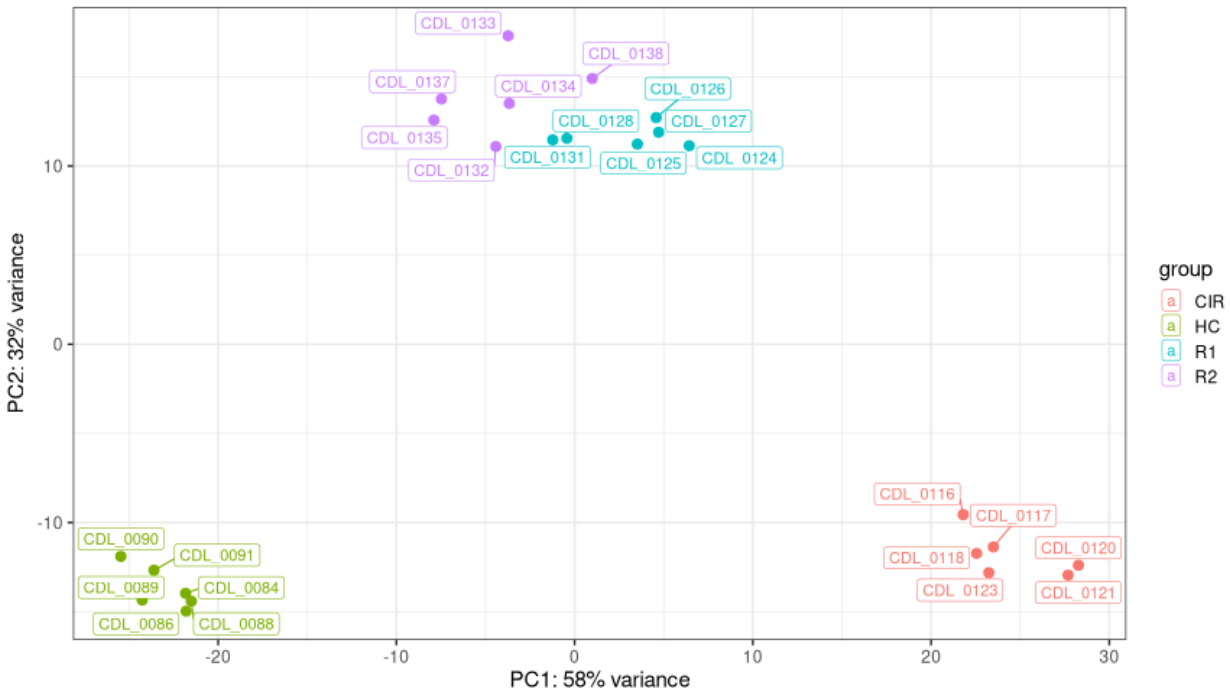
PCA: TAA and CCL4 - treatment

```
rld.sub <- rld[ , rld$stimulus %in% c("carbon_tetrachloride", "thioacetamide")]
plotPCA(rld.sub, intgroup = c("treatment", "stimulus")) +
  geom_label_repel(aes(label = name)) +
  theme_bw()
```



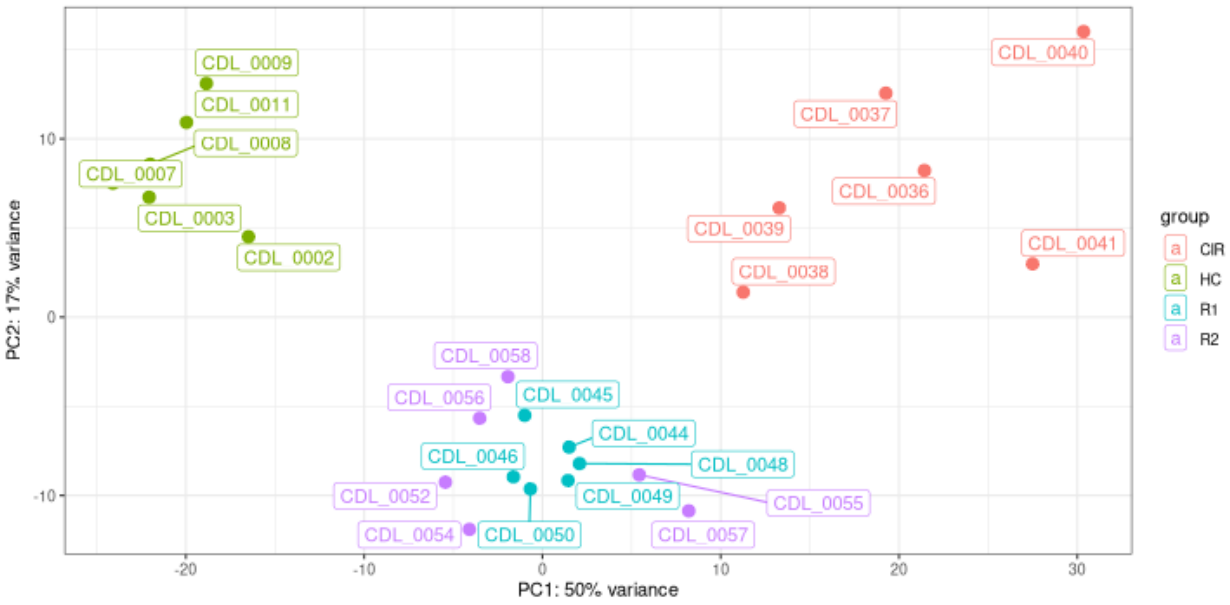
PCA: TAA experiment

```
rld.sub <- rld[ , rld$experiment %in% c("TAA")]
plotPCA(rld.sub, intgroup = c("treatment")) +
  geom_label_repel(aes(label = name)) + theme_bw(base_size = 15)
```



PCA: CCL4 experiment

```
rld.sub <- rld[ , rld$experiment %in% c("CCL4")]
plotPCA(rld.sub, intgroup = c("treatment")) +
  geom_label_repel(aes(label = name)) +
  theme_bw()
```



DE in TAA

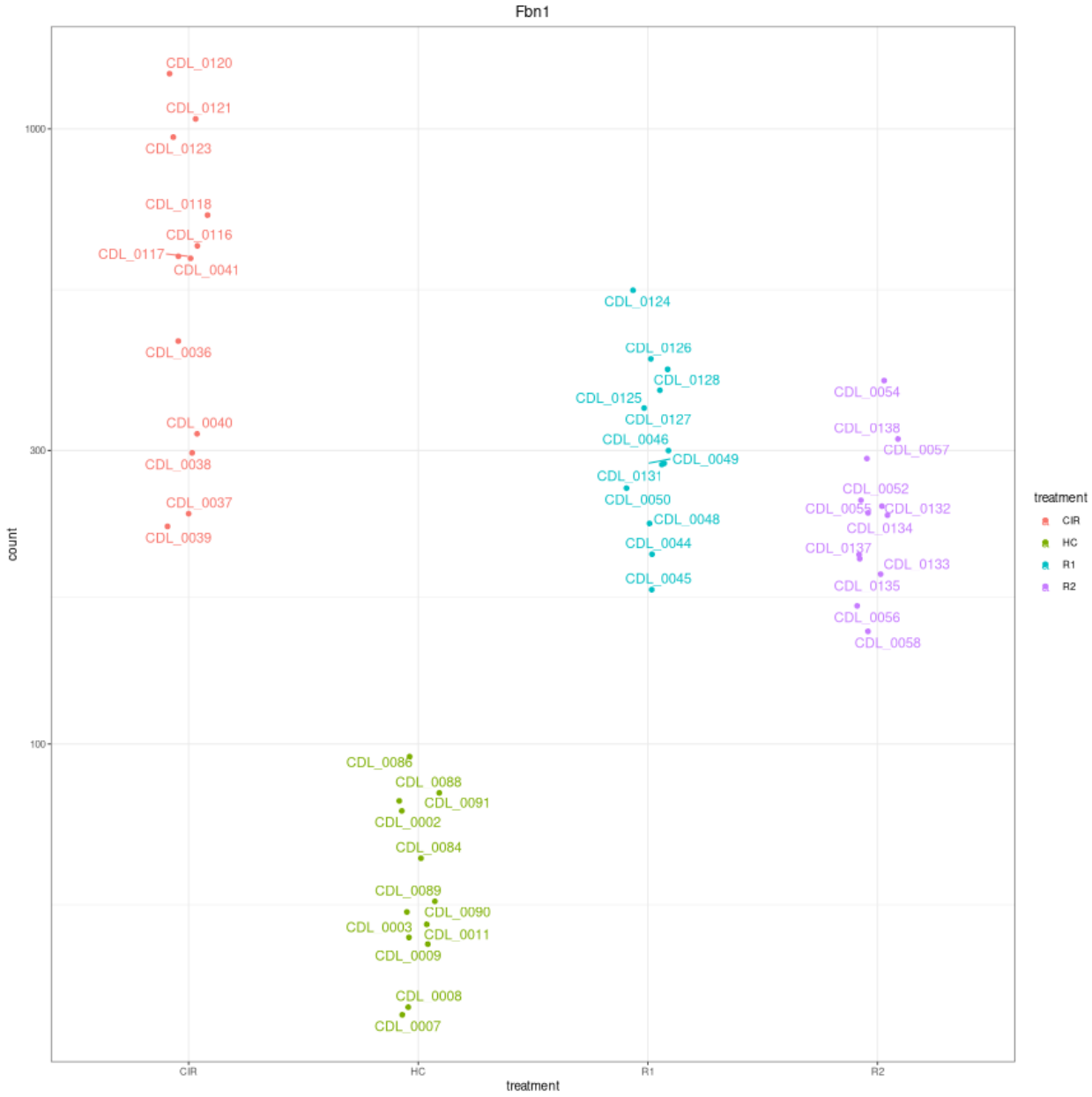
```
ddsTAA <- subset(dds, select = colData(dds)$experiment == "TAA")
ddsTAA <- subset(ddsTAA, select = colData(ddsTAA)$treatment == "HC" | colData(ddsTAA)$treatment == "CIR
ddsTAA$treatment <- droplevels(ddsTAA$treatment)
ddsTAA$treatment <- relevel(ddsTAA$treatment, ref = "HC")

contrast <- c("treatment", "CIR", "HC")
ddsTAA <- DESeq(ddsTAA)

resTreatment <- results(dds, contrast = contrast, alpha = 0.05)
length(which(resTreatment$padj < 0.05))
```

```
## [1] 5447
```

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

resTreatment_tb_significant <- dplyr::filter(resTreatment_tb, padj < 0.05) %>%
                        dplyr::filter(abs(log2FoldChange) > 1) %>%
                        comb_de_result_table()

write_csv(resTreatment_tb_significant, "../../03_outputs/T4.TAA_results.csv")

samples_control <- metadata %>% rownames_to_column("sample") %>%
         dplyr::filter(experiment == "TAA" & treatment == "HC")  %>% pull(sample)

counts_tpm$symbol <- NULL
tpm_control <- get_counts_for_samples(counts_tpm, samples_control, "HC_mean_tpm")

samples_effect <- metadata %>% dplyr::filter(experiment == "TAA" & treatment == "CIR") %>% row.names()
tpm_effect <- get_counts_for_samples(counts_tpm, samples_effect, "CIR_tpm")

tpm_counts <- tpm_effect %>%
              left_join(tpm_control,
                        by = c("ensembl_gene_id" = "ensembl_gene_id"))

resTreatment_tb_significant <- resTreatment_tb_significant %>%
          left_join(tpm_counts, by = c("gene" = "ensembl_gene_id")) %>%
          arrange(log2FoldChange)

write_xlsx(list(T4.TAA_results = resTreatment_tb_significant),
          "../../03_outputs/T4.DE_TAA.xlsx")

# Separate into up and doum-regulated gene sets
sigTreatment_up <- rownames(resTreatment)[which(resTreatment$padj < 0.01 & resTreatment$log2FoldChange
sigTreatment_down <- rownames(resTreatment)[which(resTreatment$padj < 0.01 & resTreatment$log2FoldChang
```

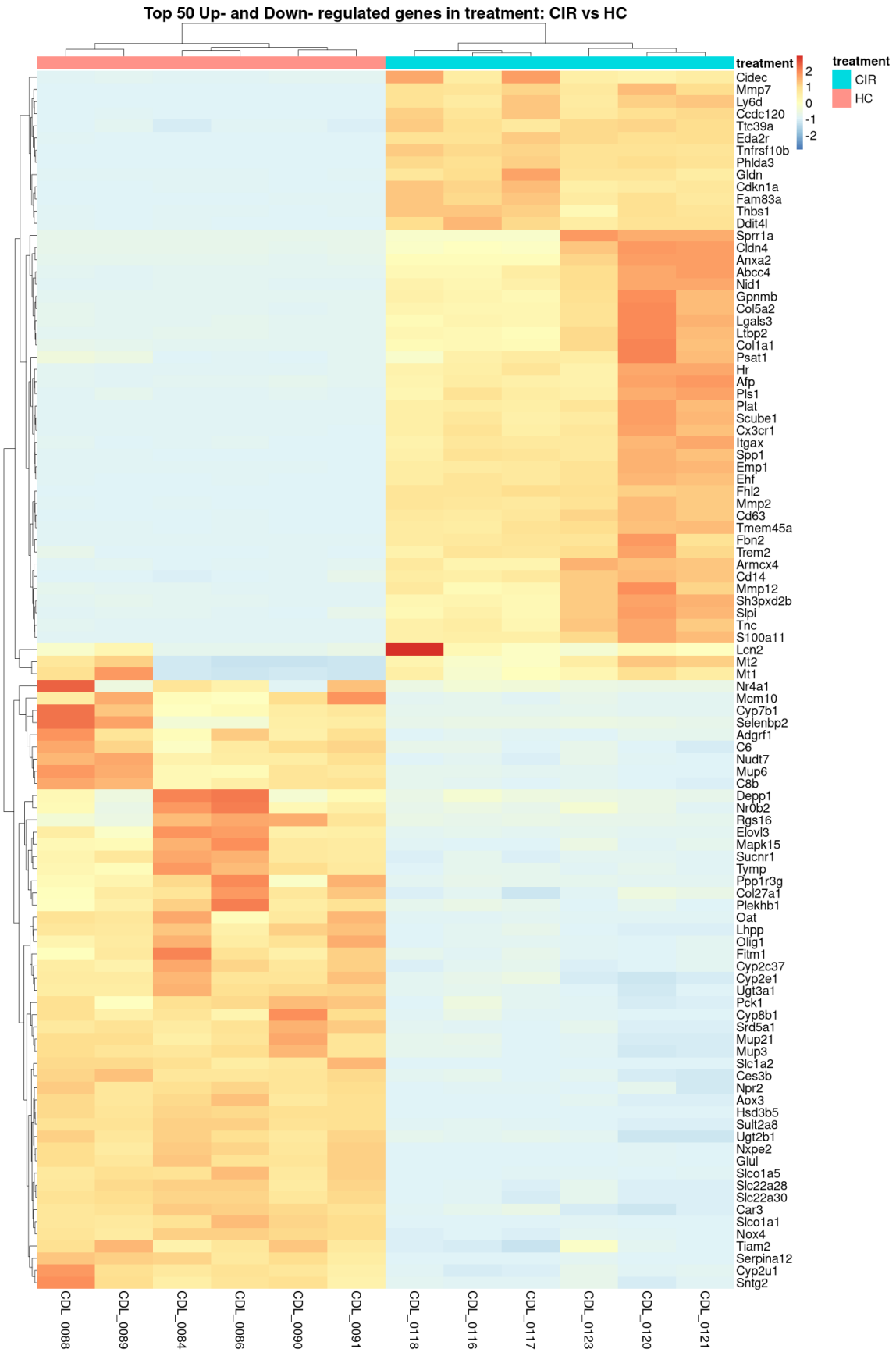
Visualization - Gene example

```
d <- plotCounts(dds,
                gene = "ENSMUSG00000027204",
                intgroup = "treatment",
                returnData = TRUE)

ggplot(d, aes(x = treatment, y = count, color = treatment)) +
     geom_point(position = position_jitter(w = 0.1, h = 0)) +
     geom_text_repel(aes(label = rownames(d))) +
     theme_bw(base_size = 10) +
     ggtitle("Fbn1") +
     theme(plot.title = element_text(hjust = 0.5)) +
     scale_y_log10()
```



Heatmaps

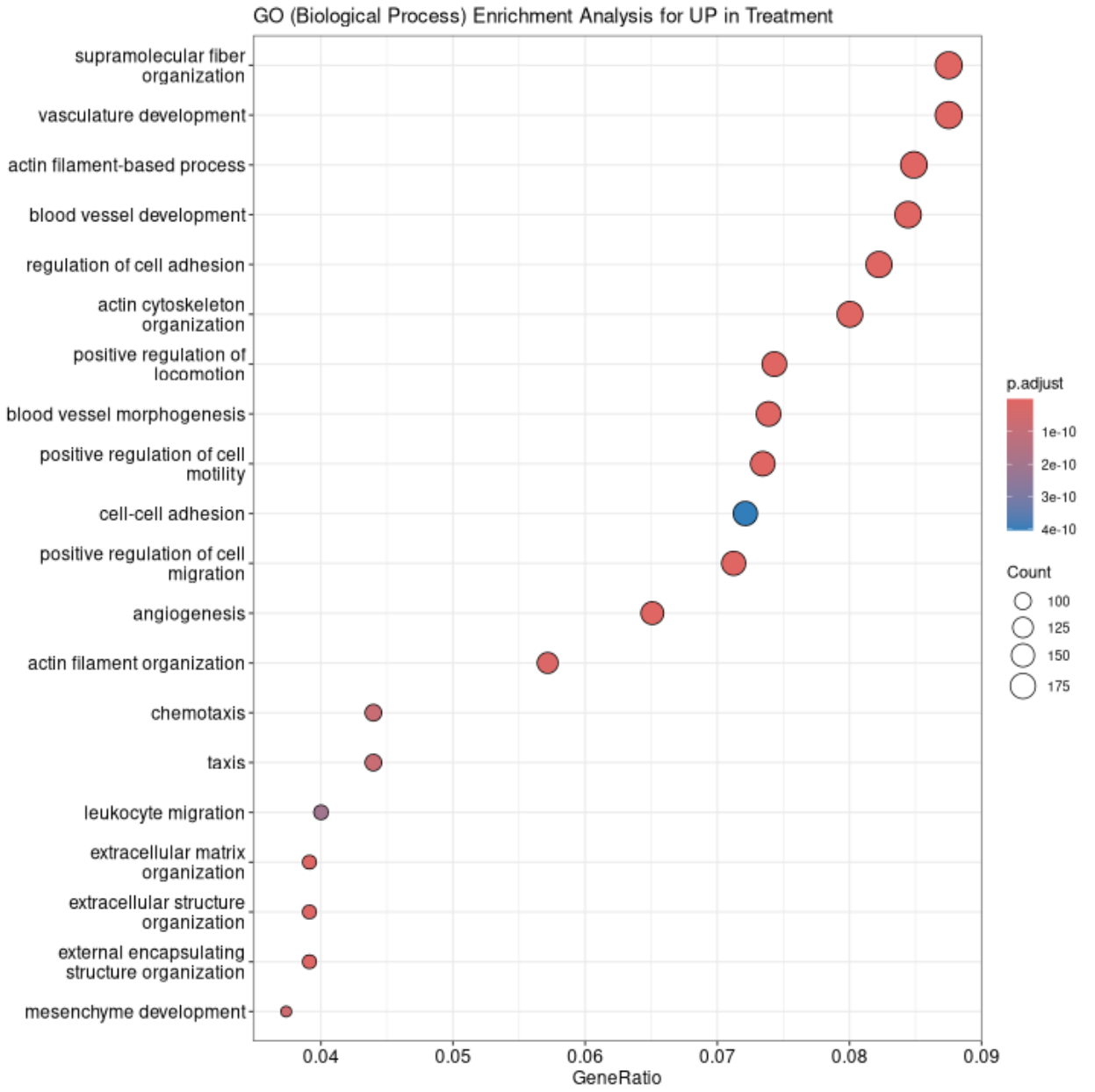
```
# Create a matrix of normalized expression
sig_up <- resTreatment_tb_significant %>% arrange(-log2FoldChange) %>% head(50) %>% pull(gene)
sig_down <- resTreatment_tb_significant %>% arrange(log2FoldChange) %>% head(50) %>% pull(gene)
sig <- c(sig_up, sig_down)

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

plotmat <- counts_tpm %>% column_to_rownames("ensembl_gene_id") %>%
     dplyr::select(any_of(c(samples_control, samples_effect)))

plotmat <- plotmat[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
# color = heat.colors,
pheatmap(plotmat,
         scale = "row",
         show_rownames = TRUE,
         border = FALSE,
         annotation = metadata[, c("treatment"), drop = FALSE],
         main = "Top 50 Up- and Down- regulated genes in treatment: CIR vs HC",
         fontsize = 20)
```


Top 50 Up- and Down- regulated genes in treatment: CIR vs HC
treatment
CIR
HC
2
1
0
-1
-2
Cidec
Mmp7
Ly6d
Ccdc120
Ttc39a
Eda2r
Tnfrsf10b
Phlda3
Gldn
Cdkn1a
Fam83a
Thbs1
Ddit4l
Sprr1a
Cldn4
Anxa2
Abcc4
Nid1
Gpnmb
Col5a2
Lgals3
Ltbp2
Col1a1
Psat1
Hr
Afp
Pls1
Plat
Scube1
Cx3cr1
Itgax
Spp1
Emp1
Ehf
Fhl2
Mmp2
Cd63
Tmem45a
Fbn2
Trem2
Armcx4
Cd14
Mmp12
Sh3pxd2b
Slpi
Tnc
S100a11
Lcn2
Mt2
Mt1
Nr4a1
Mcm10
Cyp7b1
Selenbp2
Adgrf1
C6
Nudt7
Mup6
C8b
Depp1
Nr0b2
Rgs16
Elovl3
Mapk15
Sucnr1
Tymp
Ppp1r3g
Col27a1
Plekhb1
Oat
Lhpp
Olig1
Fitm1
Cyp2c37
Cyp2e1
Ugt3a1
Pck1
Cyp8b1
Srd5a1
Mup21
Mup3
Slc1a2
Ces3b
Npr2
Aox3
Hsd3b5
Sult2a8
Ugt2b1
Nxpe2
Glul
Slco1a5
Slc22a28
Slc22a30
Car3
Slco1a1
Nox4
Tiam2
Serpina12
Cyp2u1
Sntg2
CDL_0088
CDL_0089
CDL_0084
CDL_0086
CDL_0090
CDL_0091
CDL_0118
CDL_0116
CDL_0117
CDL_0123
CDL_0120
CDL_0121

Functional analysis

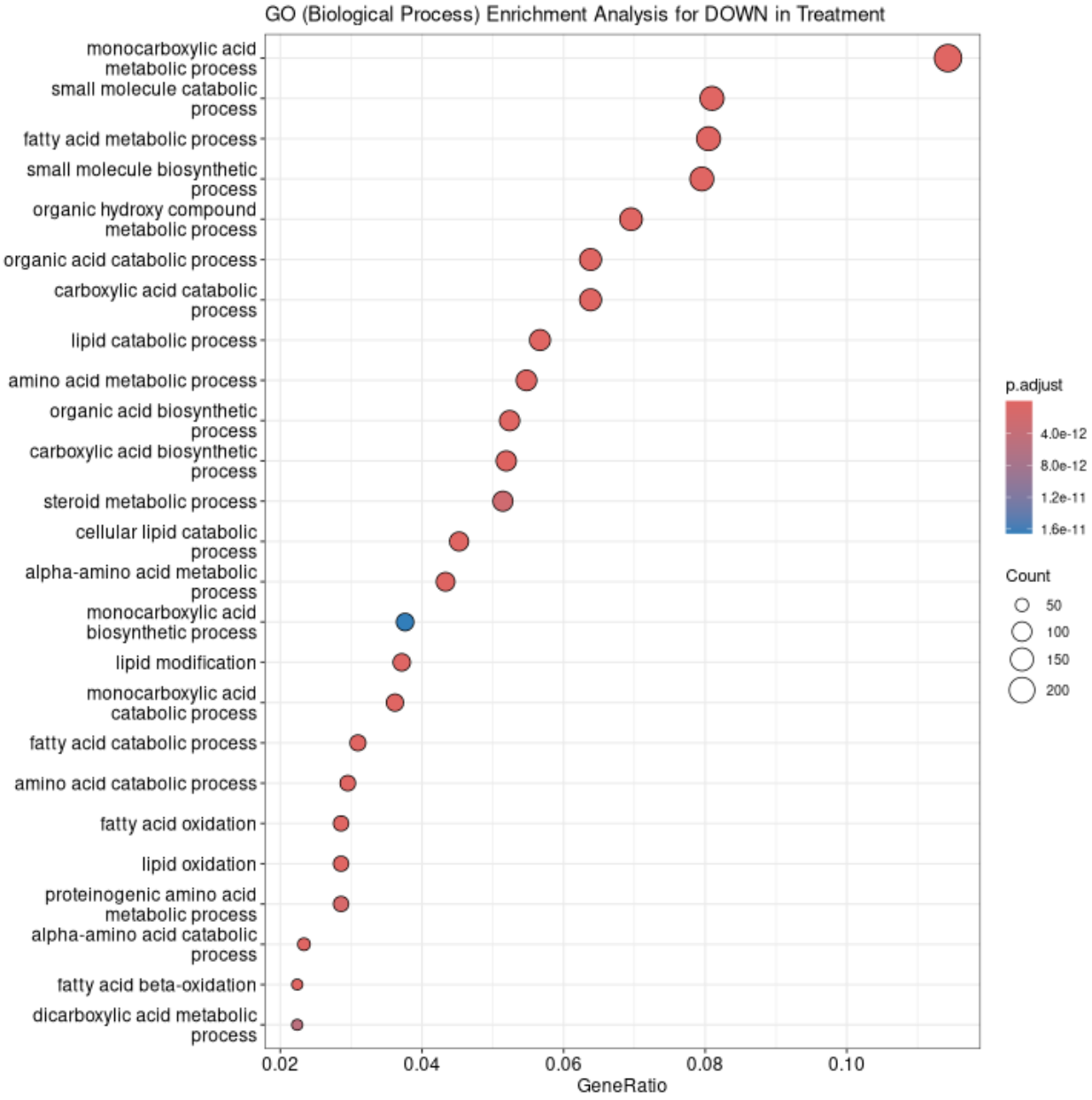
Create background dataset for hypergeometric testing using all genes tested for significance in the results

```
bg_genes <- resTreatment_tb$gene

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

dotplot(compGO,
        showCategory = 20,
        title = "GO (Biological Process) Enrichment Analysis for UP in Treatment")
```




```
# image pdf 12 x 12
```

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

dotplot(compGO, showCategory = 25, title = "GO (Biological Process) Enrichment Analysis for DOWN in Trea
```


GO (Biological Process) Enrichment Analysis for DOWN in Treatment
monocarboxylic acid metabolic process
small molecule catabolic process
fatty acid metabolic process
small molecule biosynthetic process
organic hydroxy compound metabolic process
organic acid catabolic process
carboxylic acid catabolic process
lipid catabolic process
amino acid metabolic process
organic acid biosynthetic process
carboxylic acid biosynthetic process
steroid metabolic process
cellular lipid catabolic process
alpha-amino acid metabolic process
monocarboxylic acid biosynthetic process
lipid modification
monocarboxylic acid catabolic process
fatty acid catabolic process
amino acid catabolic process
fatty acid oxidation
lipid oxidation
proteinogenic amino acid metabolic process
alpha-amino acid catabolic process
fatty acid beta-oxidation
dicarboxylic acid metabolic process
0.02
0.04
0.06
0.08
0.10
GeneRatio
p.adjust
4.0e-12
8.0e-12
1.2e-11
1.6e-11
Count
50
100
150
200

R session

```
sessionInfo()
```

```
## R version 4.4.1 (2024-06-14)
## Platform: x86_64-redhat-linux-gnu
## Running under: Fedora Linux 40 (Workstation Edition)
##
## Matrix products: default
## BLAS/LAPACK: FlexiBLAS OPENBLAS-OPENMP;  LAPACK version 3.11.0
##
## locale:
##  [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C
##  [3] LC_TIME=en_US.UTF-8        LC_COLLATE=en_US.UTF-8
##  [5] LC_MONETARY=en_US.UTF-8    LC_MESSAGES=en_US.UTF-8
##  [7] LC_PAPER=en_US.UTF-8       LC_NAME=C
##  [9] LC_ADDRESS=C               LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C
##
## time zone: America/Toronto
## tzcode source: system (glibc)
##
## attached base packages:
## [1] stats4    stats     graphics  grDevices utils     datasets  methods
## [8] base
##
## other attached packages:
##  [1] org.Mm.eg.db_3.19.1         clusterProfiler_4.12.1
##  [3] writexl_1.5.0               ggplotify_0.1.2
##  [5] knitr_1.48                  ggrepel_0.9.5
##  [7] tximport_1.32.0             DEGreport_1.40.1
##  [9] pheatmap_1.0.12             DESeq2_1.44.0
## [11] SummarizedExperiment_1.34.0 MatrixGenerics_1.16.0
## [13] matrixStats_1.3.0           RColorBrewer_1.1-3
## [15] ensembldb_2.28.0            AnnotationFilter_1.28.0
## [17] GenomicFeatures_1.56.0      AnnotationDbi_1.66.0
## [19] Biobase_2.64.0              GenomicRanges_1.56.1
## [21] GenomeInfoDb_1.40.1         IRanges_2.38.1
## [23] S4Vectors_0.42.1            AnnotationHub_3.12.0
## [25] BiocFileCache_2.12.0        dbplyr_2.5.0
## [27] BiocGenerics_0.50.0         lubridate_1.9.3
## [29] forcats_1.0.0               stringr_1.5.1
## [31] dplyr_1.1.4                 purrr_1.0.2
## [33] readr_2.1.5                 tidyr_1.3.1
## [35] tibble_3.2.1                ggplot2_3.5.1
## [37] tidyverse_2.0.0
##
## loaded via a namespace (and not attached):
##   [1] splines_4.4.1               BiocIO_1.14.0
##   [3] bitops_1.0-7                filelock_1.0.3
##   [5] polyclip_1.10-7             XML_3.99-0.17
##   [7] lifecycle_1.0.4             edgeR_4.2.1
##   [9] doParallel_1.0.17           vroom_1.6.5
##  [11] lattice_0.22-6              MASS_7.3-60.2
```

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##  [13] backports_1.5.0            magrittr_2.0.3
##  [15] limma_3.60.4               rmarkdown_2.27
##  [17] yaml_2.3.9                 cowplot_1.1.3
##  [19] DBI_1.2.3                  ConsensusClusterPlus_1.68.0
##  [21] abind_1.4-5                zlibbioc_1.50.0
##  [23] ggraph_2.2.1               RCurl_1.98-1.16
##  [25] yulab.utils_0.1.5          tweenr_2.0.3
##  [27] rappdirs_0.3.3             circlize_0.4.16
##  [29] GenomeInfoDbData_1.2.12    enrichplot_1.24.2
##  [31] tidytree_0.4.6             codetools_0.2-20
##  [33] DelayedArray_0.30.1        DOSE_3.30.2
##  [35] ggforce_0.4.2              tidyselect_1.2.1
##  [37] shape_1.4.6.1              aplot_0.2.3
##  [39] UCSC.utils_1.0.0           farver_2.1.2
##  [41] viridis_0.6.5              GenomicAlignments_1.40.0
##  [43] jsonlite_1.8.8             GetoptLong_1.0.5
##  [45] tidygraph_1.3.1            iterators_1.0.14
##  [47] foreach_1.5.2              tools_4.4.1
##  [49] treeio_1.28.0              Rcpp_1.0.13
##  [51] glue_1.7.0                 gridExtra_2.3
##  [53] mnormt_2.1.1               SparseArray_1.4.8
##  [55] xfun_0.45                  qvalue_2.36.0
##  [57] withr_3.0.0                BiocManager_1.30.23
##  [59] fastmap_1.2.0              fansi_1.0.6
##  [61] digest_0.6.36              timechange_0.3.0
##  [63] R6_2.5.1                   gridGraphics_0.5-1
##  [65] colorspace_2.1-0           GO.db_3.19.1
##  [67] RSQLite_2.3.7              utf8_1.2.4
##  [69] generics_0.1.3             data.table_1.15.4
##  [71] rtracklayer_1.64.0         graphlayouts_1.1.1
##  [73] httr_1.4.7                 S4Arrays_1.4.1
##  [75] scatterpie_0.2.3           pkgconfig_2.0.3
##  [77] gtable_0.3.5               blob_1.2.4
##  [79] ComplexHeatmap_2.20.0      XVector_0.44.0
##  [81] shadowtext_0.1.4           htmltools_0.5.8.1
##  [83] fgsea_1.30.0               ProtGenerics_1.36.0
##  [85] clue_0.3-65                scales_1.3.0
##  [87] logging_0.10-108           png_0.1-8
##  [89] ggfun_0.1.5                ggdendro_0.2.0
##  [91] rstudioapi_0.16.0          tzdb_0.4.0
##  [93] reshape2_1.4.4             rjson_0.2.21
##  [95] nlme_3.1-164               curl_5.2.1
##  [97] cachem_1.1.0               GlobalOptions_0.1.2
##  [99] BiocVersion_3.19.1         parallel_4.4.1
## [101] HDO.db_0.99.1              restfulr_0.0.15
## [103] pillar_1.9.0               grid_4.4.1
## [105] reshape_0.8.9              vctrs_0.6.5
## [107] cluster_2.1.6              evaluate_0.24.0
## [109] cli_3.6.3                  locfit_1.5-9.10
## [111] compiler_4.4.1             Rsamtools_2.20.0
## [113] rlang_1.1.4                crayon_1.5.3
## [115] labeling_0.4.3             plyr_1.8.9
## [117] fs_1.6.4                   stringi_1.8.4
## [119] psych_2.4.6.26             viridisLite_0.4.2
```

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## [121] BiocParallel_1.38.0         munsell_0.5.1
## [123] Biostrings_2.72.1           lazyeval_0.2.2
## [125] GOSemSim_2.30.0             Matrix_1.7-0
## [127] patchwork_1.2.0             hms_1.1.3
## [129] bit64_4.0.5                 KEGGREST_1.44.1
## [131] statmod_1.5.0               highr_0.11
## [133] igraph_2.0.3                broom_1.0.6
## [135] memoise_2.0.1               ggtree_3.12.0
## [137] fastmatch_1.1-4             bit_4.0.5
## [139] gson_0.1.0                  ape_5.8
```