Differential Expression Analysis for bulk RNA-seq data

CTRL Condition: Vehicle vs BPN

Ximing Ran

2025-03-25

Contents


```
library(tibble)
library(tidyr)
library(dplyr)
library(rtracklayer)
```

```
# load function from local files
source(here::here("source", "DEG_functions.R"))
```

1. Read the count data

In this section, we will read the clean count data from the synaptosomes_bulkRNA folder. We will read the data and merge them into a single table. The final table will be stored in `../dataresults/bulkRNA_counts_clean.csv`.

```
input_count <- read.csv(here::here("data", "bulkRNA",
                                   "bulkRNA_counts_cleaned.csv"))
counts <-  as.data.frame(input_count) %>%
  column_to_rownames(var = "gene")
colnames(counts) <- gsub("_", "-", colnames(counts))

# if the colname is start with X, remove it
colnames(counts) <- gsub("^X", "", colnames(counts))


# raw sample list
sample_list_raw <- read.csv(here::here("data", "bulkRNA",
                                   "sample_info_FXS.csv")) %>%
                    mutate(condition = Label,
                           sample = gsub("_", "-", Sample_ID))


# Ensure the column names of counts exist in Sample.name
new_colnames <- sample_list_raw$sample[match(colnames(counts), sample_list_raw$sample )]

# Assign new column names
colnames(counts) <- new_colnames


# sort the columns by the colname
condition_list <- data.frame(
  group =sample_list_raw$condition
)

row.names(condition_list) <- sample_list_raw$sample

counts<- counts[, rownames(condition_list)]

gene_name_mapping<- readRDS(here::here("data","ref" ,"gene_name_mapping.rds"))
```

2. Differential expression analysis

In this section, we will perform differential expression analysis using DESeq2.

```
# Init the result folder structure for the result
result_folder_all = './results/DEG-CTRL_Veh_vs_BPN'
result_folder = result_folder_all
Result_folder_structure(result_folder)

# load the comparison group information
reference_group <- "CTRL_VEH"
compare_group <- "CTRL_BPN"
reference_group_short <- reference_group
compare_group_short <- compare_group
```

```r
filter_sample_info <- condition_list %>%
  filter(group %in% c(reference_group, compare_group))
filter_counts <- counts[, rownames(filter_sample_info)]

# Run the DESeq2 analysis
dds_obj <- DEAnalysis(counts =filter_counts,
                      reference_group = reference_group,
                      compare_group = compare_group,
                      condition_list = filter_sample_info,
                      target_gene = target_gene,
                      result_folder = result_folder)


res <- results(dds_obj)
resOrdered <- res[order(res$padj), ]

# omit the NA values
resOrdered <- resOrdered[!is.na(resOrdered$padj),]
dds_obj <- dds_obj[rownames(resOrdered),]
write.csv(resOrdered, file.path(result_folder,"02-DEG", "01_all_gene_results.csv"))

# DEG with log2fc > 1 and padj < 0.05
deg_1 <- resOrdered %>% as.data.frame() %>% rownames_to_column(var = "gene") %>%
  filter(padj < 0.05 & abs(log2FoldChange) > 1) %>% arrange(padj)
deg_1 <- deg_1[!is.na(deg_1$padj),]
write.csv(deg_1, file.path(result_folder,"02-DEG","02_DEG_log2fc_1.csv"), row.names = FALSE)

# DEG with log2fc > 1.5 and padj < 0.05
deg_1.5 <- resOrdered %>% as.data.frame() %>% rownames_to_column(var = "gene") %>%
  filter(padj < 0.05 & abs(log2FoldChange) > 1.5) %>% arrange(padj)
deg_1.5  <- deg_1.5 [!is.na(deg_1.5 $padj),]
write.csv(deg_1.5 , file.path(result_folder,"02-DEG","03_DEG_log2fc_1_5.csv"), row.names = FALSE)
print("DEG analysis is done")
```

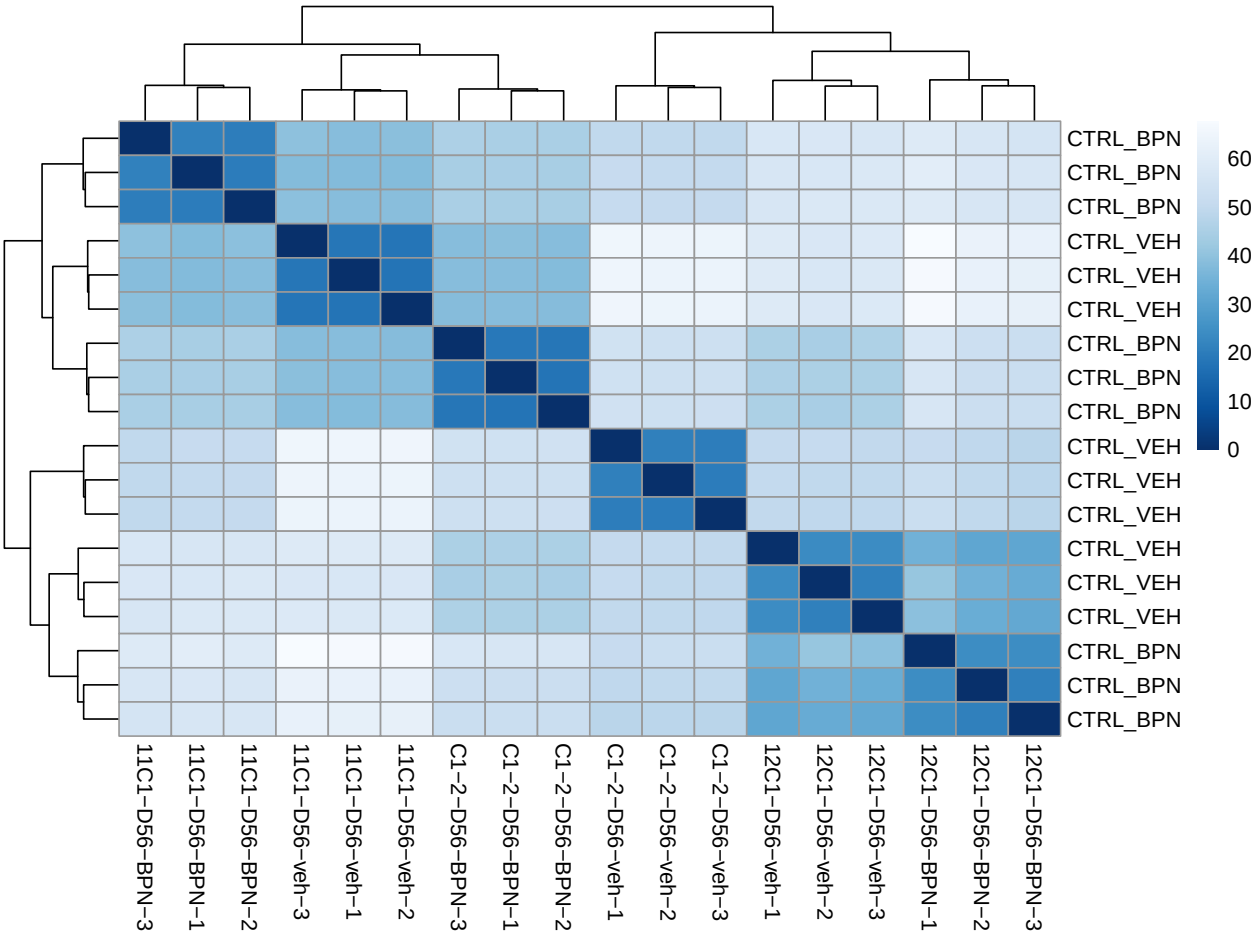
```
## [1] "DEG analysis is done"
```

```r
# Save the normalized counts
normalized_counts <- counts(dds_obj, normalized = TRUE)
write.csv(normalized_counts, file.path(result_folder,"02-DEG", "DESeq2_normalized_counts.csv"))
```

3. Visualization for reuslt

(1) Sample information

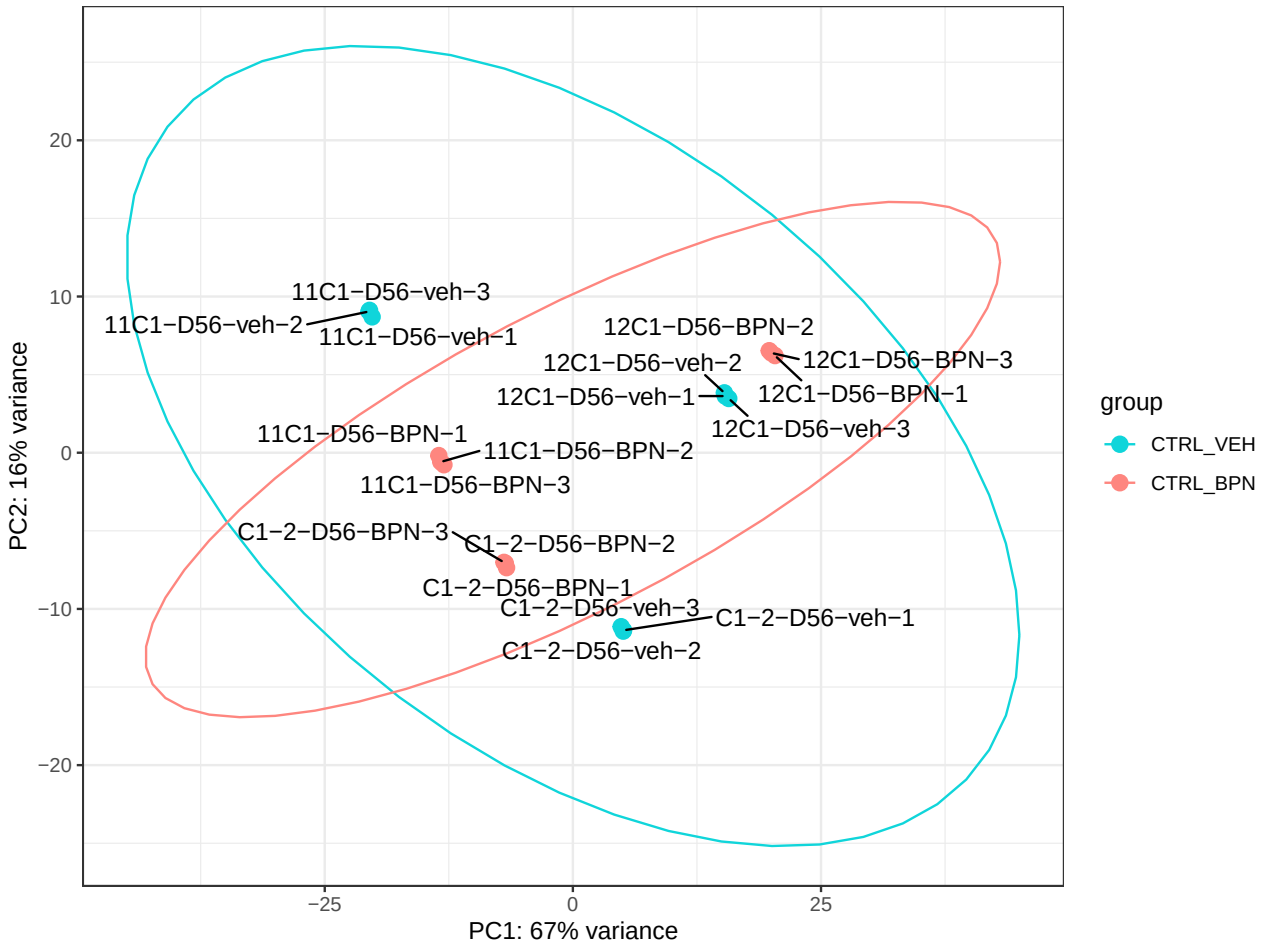
```
# Plot the sample distance
plot_sample_heatmap(dds_obj, figure_folder =
                    file.path(result_folder,"01-Sample_info"),
                    file_name = "01_sample_distance_heatmap")
```




```
## [1] "Sample distance heatmap is done"
```

```
# Plot the PCA plot for the sample
plot_sample_PCA_plot(dds_obj,figure_folder = file.path(result_folder,"01-Sample_info"),
                    file_name = "02_sample_PCA_plot",
                    reference_group, compare_group)
```

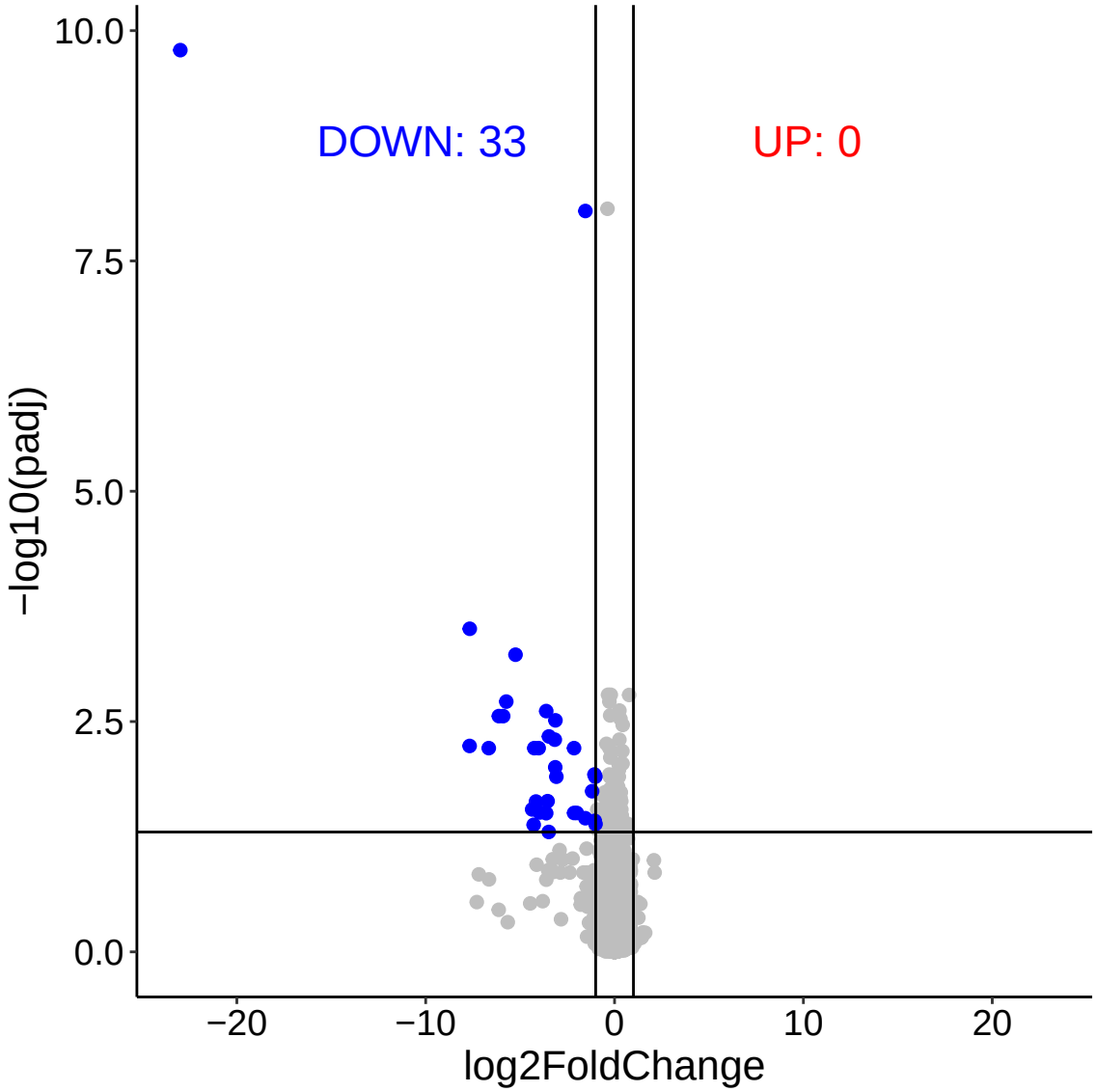
```
## [1] "PCA plot is done"
```


20
10
0
−10
−20
PC2: 16% variance
−25
0
25
PC1: 67% variance
11C1−D56−veh−3
11C1−D56−veh−2
11C1−D56−veh−1
12C1−D56−BPN−2
12C1−D56−veh−2
12C1−D56−BPN−3
12C1−D56−veh−1
12C1−D56−BPN−1
12C1−D56−veh−3
11C1−D56−BPN−1
11C1−D56−BPN−2
11C1−D56−BPN−3
C1−2−D56−BPN−3
C1−2−D56−BPN−2
C1−2−D56−BPN−1
C1−2−D56−veh−3
C1−2−D56−veh−1
C1−2−D56−veh−2
group
CTRL_VEH
CTRL_BPN

(2) DEG visualization - Volcano plot and Heatmap

```
result_df <- results(dds_obj) %>%
  as.data.frame() %>%
  rownames_to_column(var = "GeneName") %>%
  dplyr::select(GeneName, everything()) %>%
  filter(!is.na(padj)) %>%  # Correct way to filter non-NA values
  arrange(padj)

# Plot the volcano plot for the DEG
plot_volcano_plot(result_df=result_df,
                  figure_folder = file.path(result_folder,"02-DEG"),
                  file_name = "02_volcano_plot_log2fc_1",
                  thread = 1 , dot_size =2, label_gene = NULL)
```

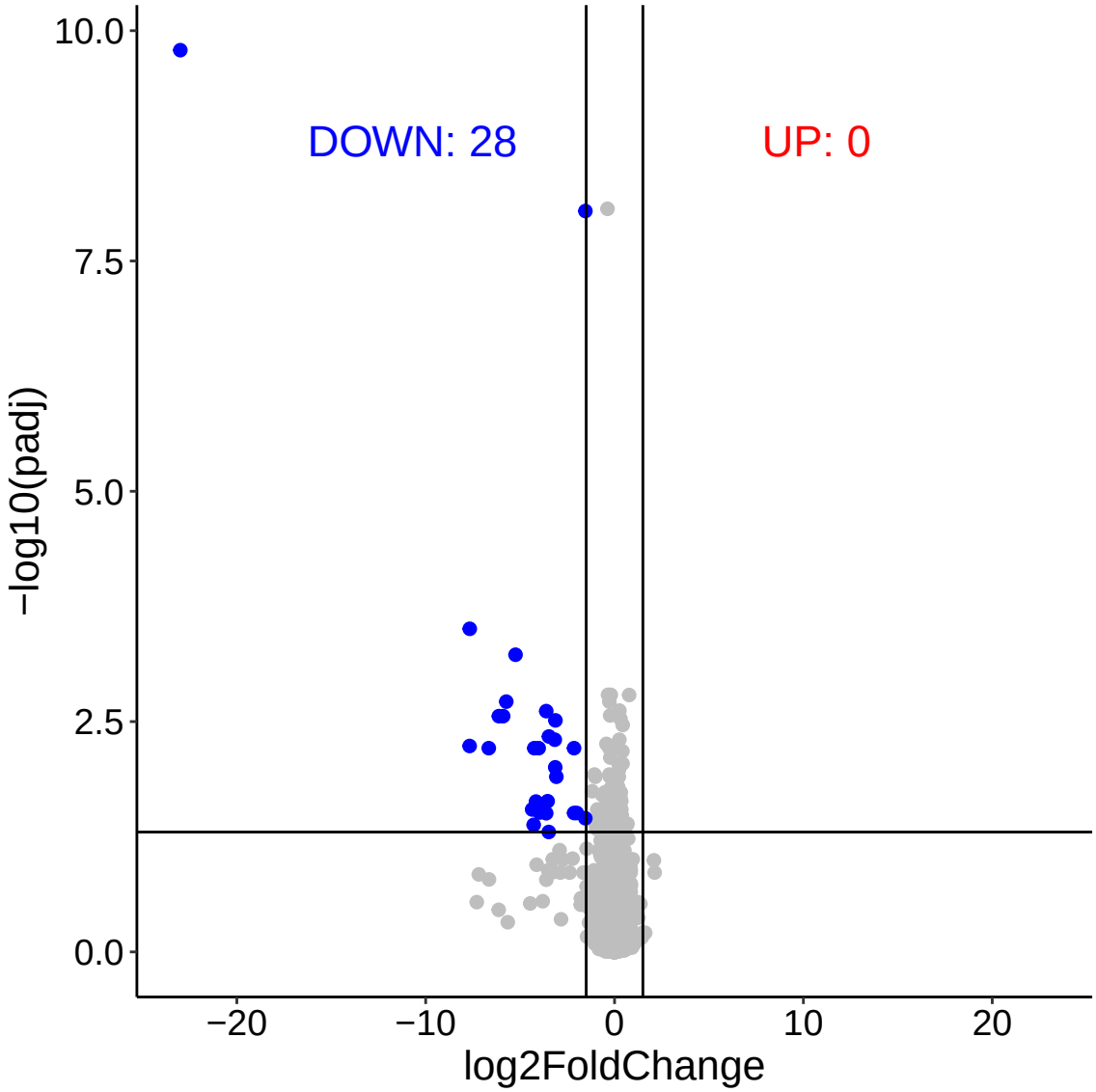
```
## [1] "Volcano plot for 02_volcano_plot_log2fc_1"
```




```
plot_volcano_plot(result_df=result_df,
```

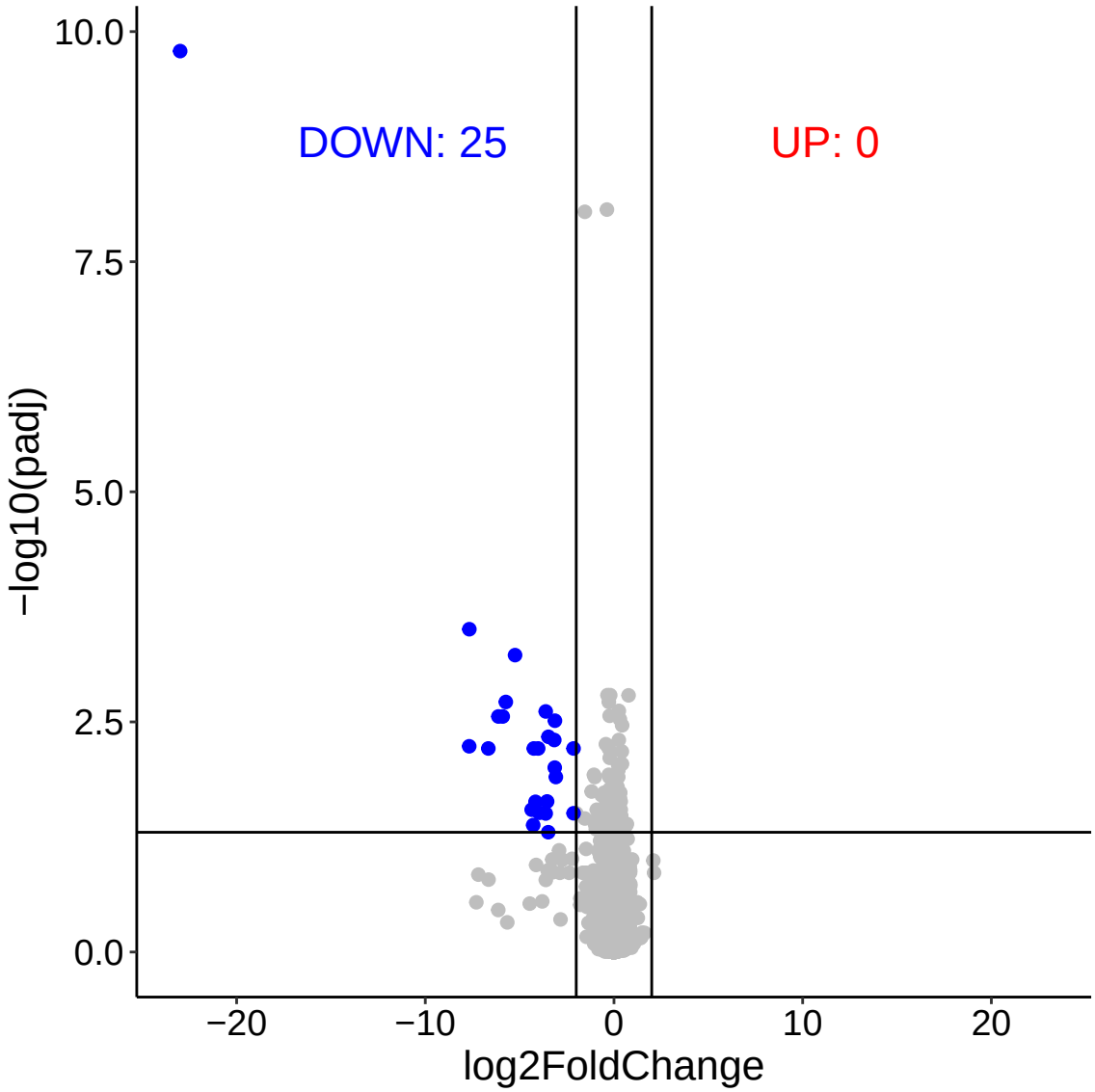
```
                figure_folder = file.path(result_folder,"02-DEG"),
                file_name = "03_volcano_plot_log2fc_1.5",
                thread = 1.5 ,  dot_size =2,label_gene = NULL)
```

```
## [1] "Volcano plot for 03_volcano_plot_log2fc_1.5"
```



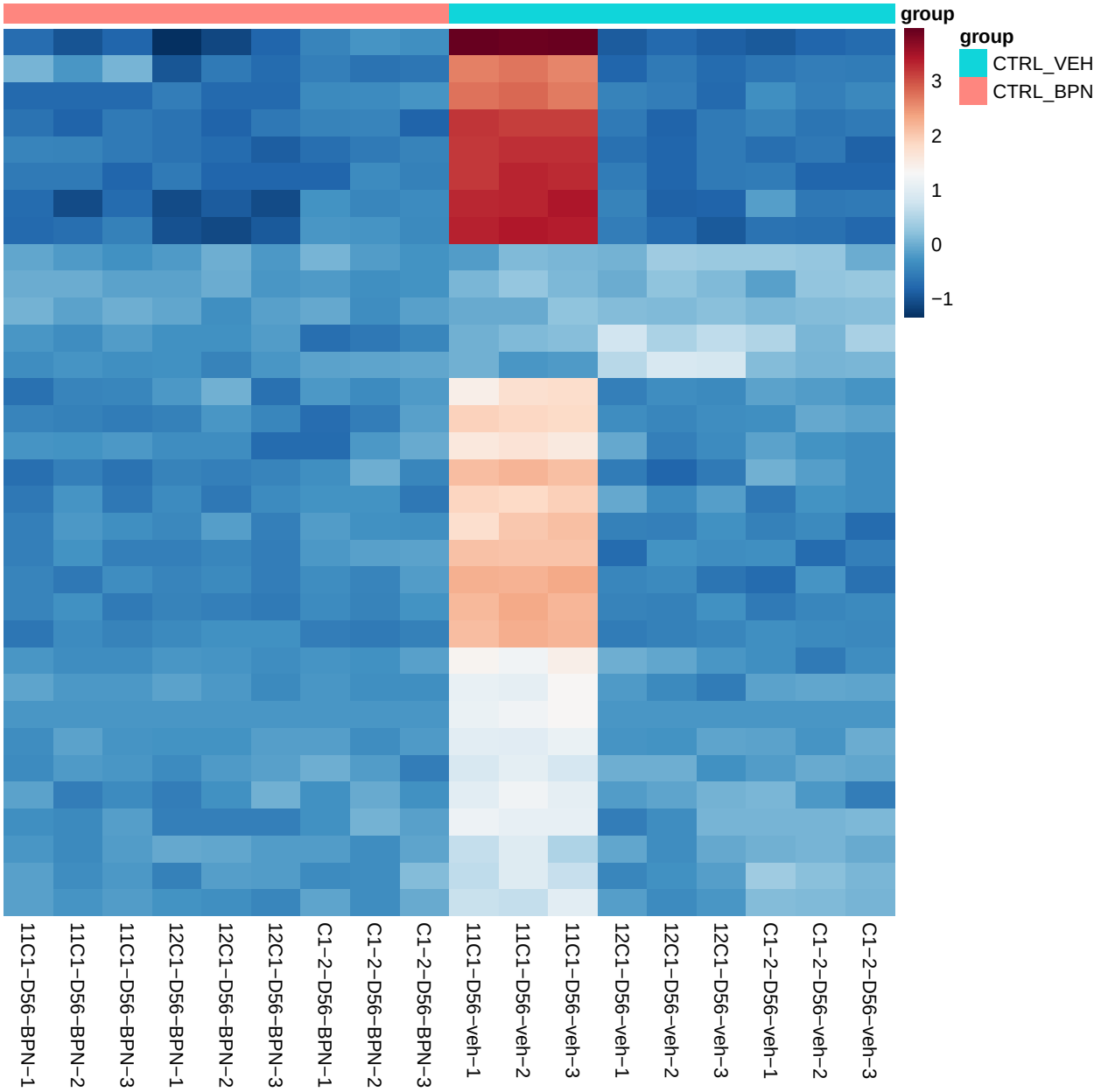

```
plot_volcano_plot(result_df=result_df,
                figure_folder = file.path(result_folder,"02-DEG"),
                file_name = "03_volcano_plot_log2fc_2",
                thread = 2 ,  dot_size =2,label_gene = NULL)
```

```
## [1] "Volcano plot for 03_volcano_plot_log2fc_2"
```



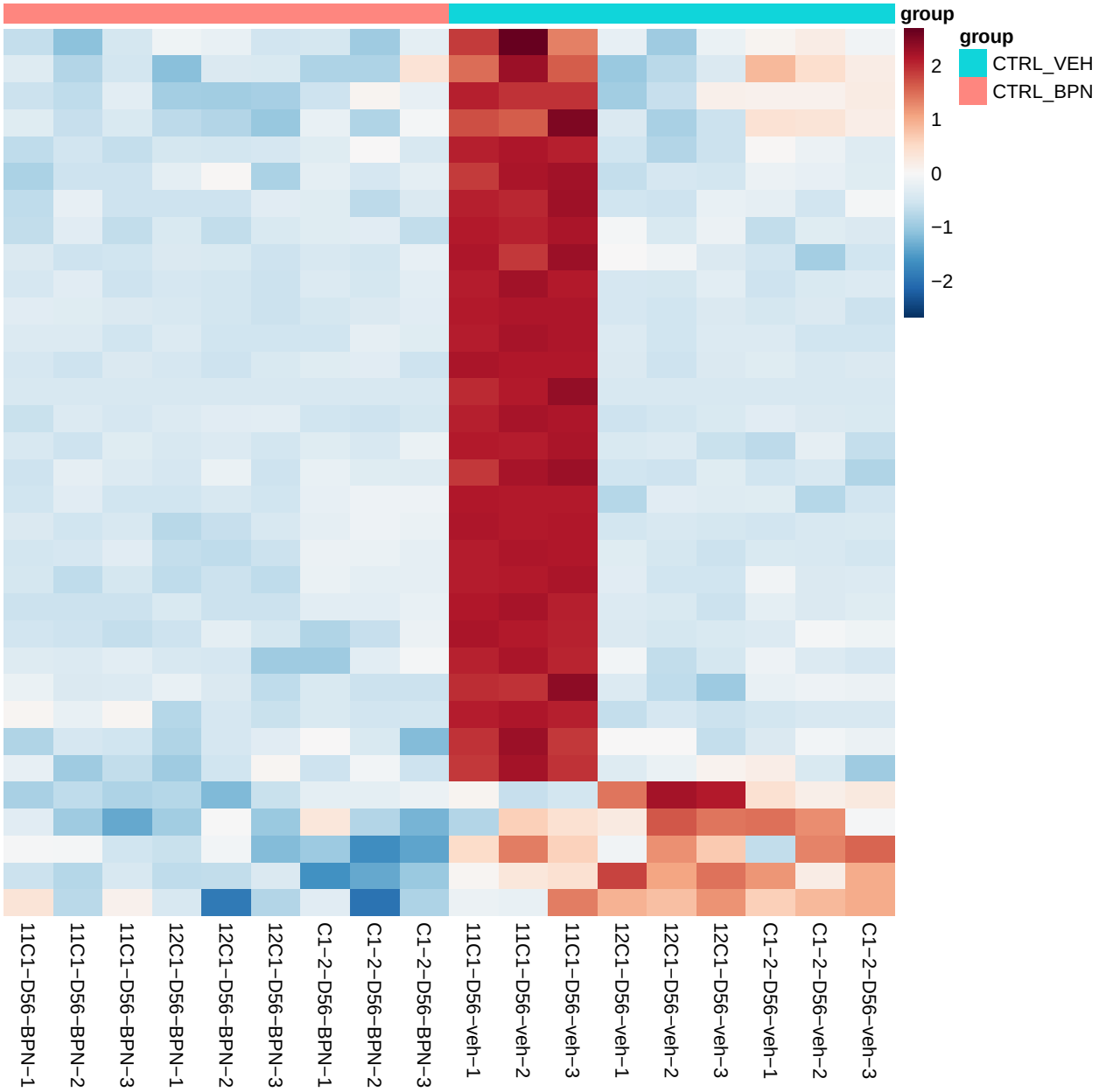

```
# Plot the heatmap for the DEG
vsd_obj <- varianceStabilizingTransformation(dds_obj, blind = TRUE)

DEG_gene_1 <- result_df %>% filter(abs(log2FoldChange) > 1 & padj <0.05) %>%  pull(GeneName)
DEG_gene_1.5 <- result_df %>% filter(abs(log2FoldChange) > 1.5 & padj <0.05) %>% pull(GeneName)
DEG_gene_2 <- result_df %>% filter(abs(log2FoldChange) > 2 & padj <0.05) %>% pull(GeneName)

plot_gene_heatmap(vsd_obj, gene_list = DEG_gene_1,
                  figure_folder = file.path(result_folder,"02-DEG"),
                  file_name = "02_heatmap_log2fc_1",
                  reference_group, compare_group,
                  cluster_rows = TRUE, cluster_cols = FALSE,
                  scale = "none")
```

```
## [1] "Heatmap for 02_heatmap_log2fc_1 "
```



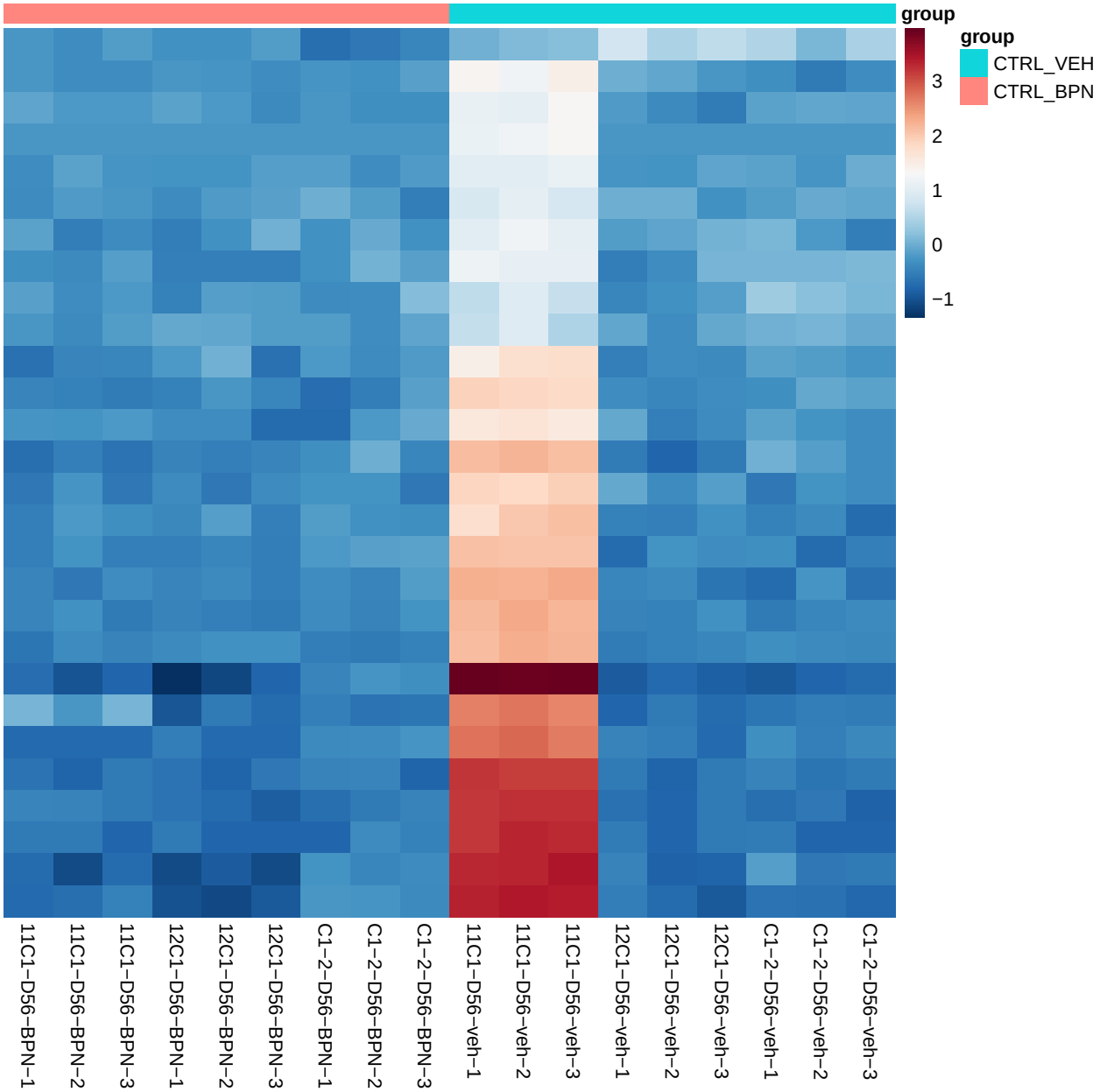

```
plot_gene_heatmap(vsd_obj, gene_list = DEG_gene_1,
                  figure_folder = file.path(result_folder,"02-DEG"),
                  file_name = "02_heatmap_log2fc_1_row",
                  reference_group, compare_group,
                  cluster_rows = TRUE, cluster_cols = FALSE,
                  scale = "row")
```

```
## [1] "Heatmap for 02_heatmap_log2fc_1_row "
```



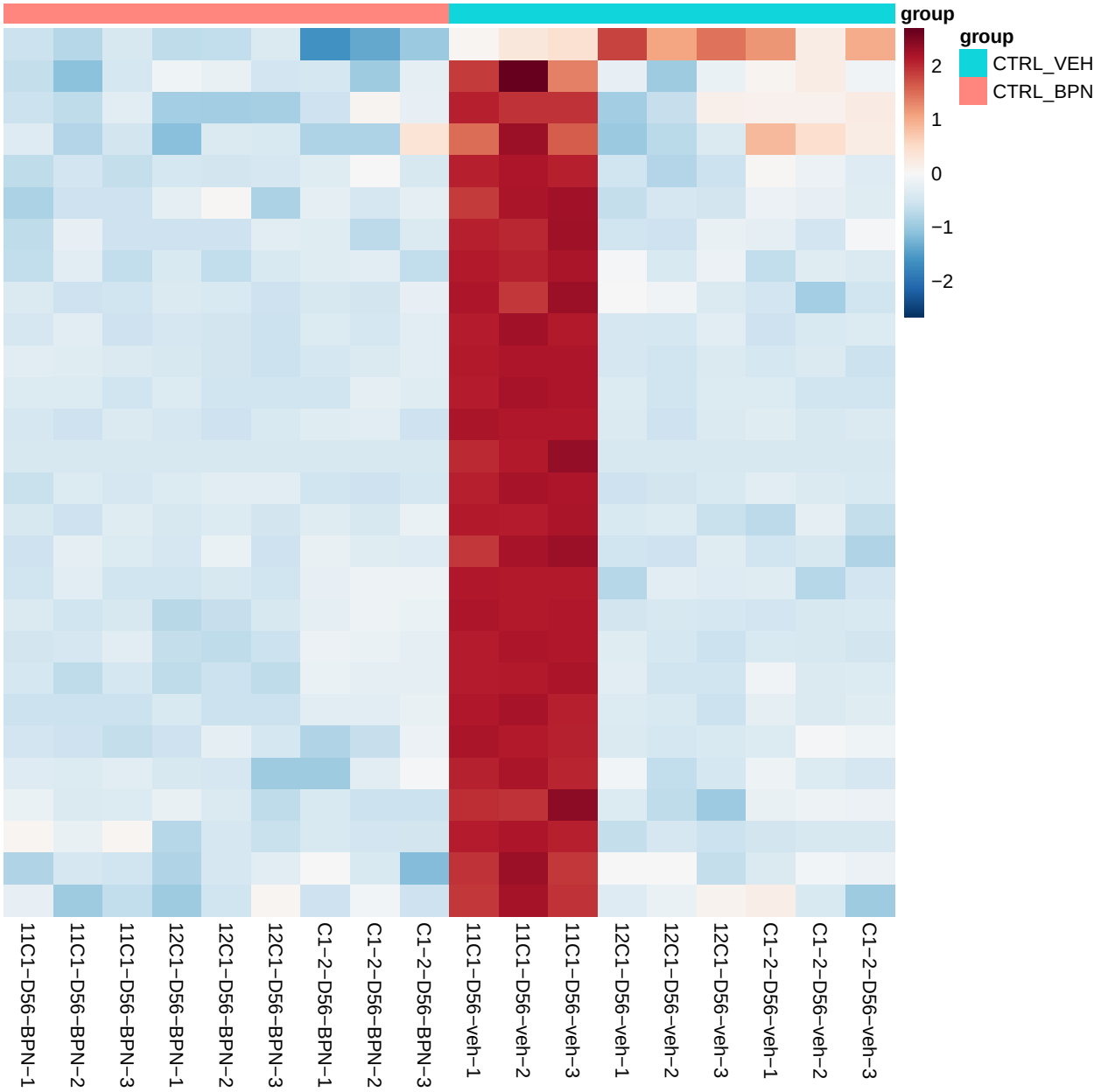

```
plot_gene_heatmap(vsd_obj, gene_list = DEG_gene_1.5,
                  figure_folder = file.path(result_folder,"02-DEG"),
                  file_name = "03_heatmap_log2fc_1.5",
                  reference_group, compare_group,
                  cluster_rows = TRUE, cluster_cols = FALSE,
                  scale = "none")
```

```
## [1] "Heatmap for 03_heatmap_log2fc_1.5 "
```



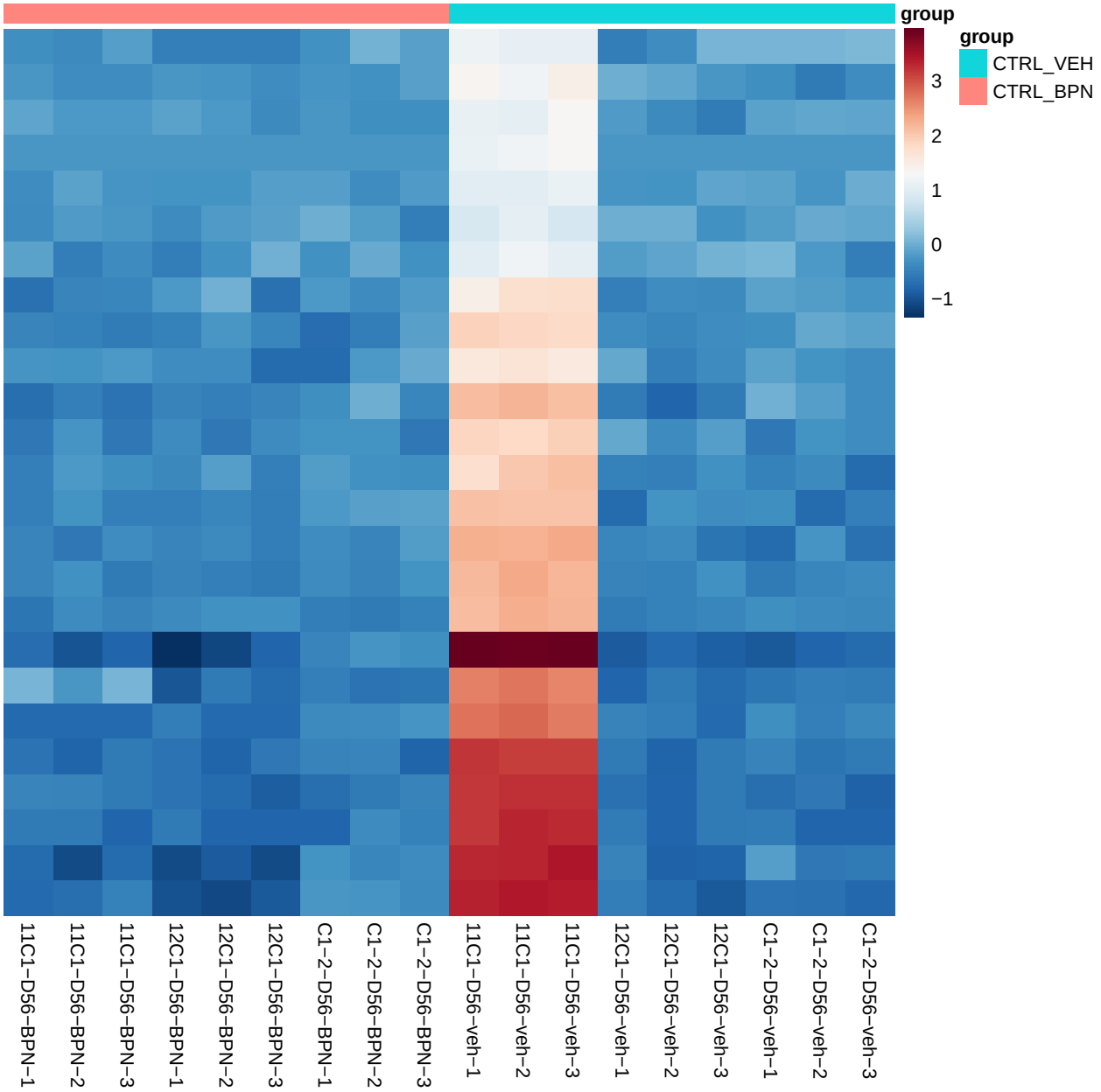

```
plot_gene_heatmap(vsd_obj, gene_list = DEG_gene_1.5,
                  figure_folder = file.path(result_folder,"02-DEG"),
                  file_name = "03_heatmap_log2fc_1.5_row",
                  reference_group, compare_group,
                  cluster_rows = TRUE, cluster_cols = FALSE,
                  scale = "row")
```

```
## [1] "Heatmap for 03_heatmap_log2fc_1.5_row "
```



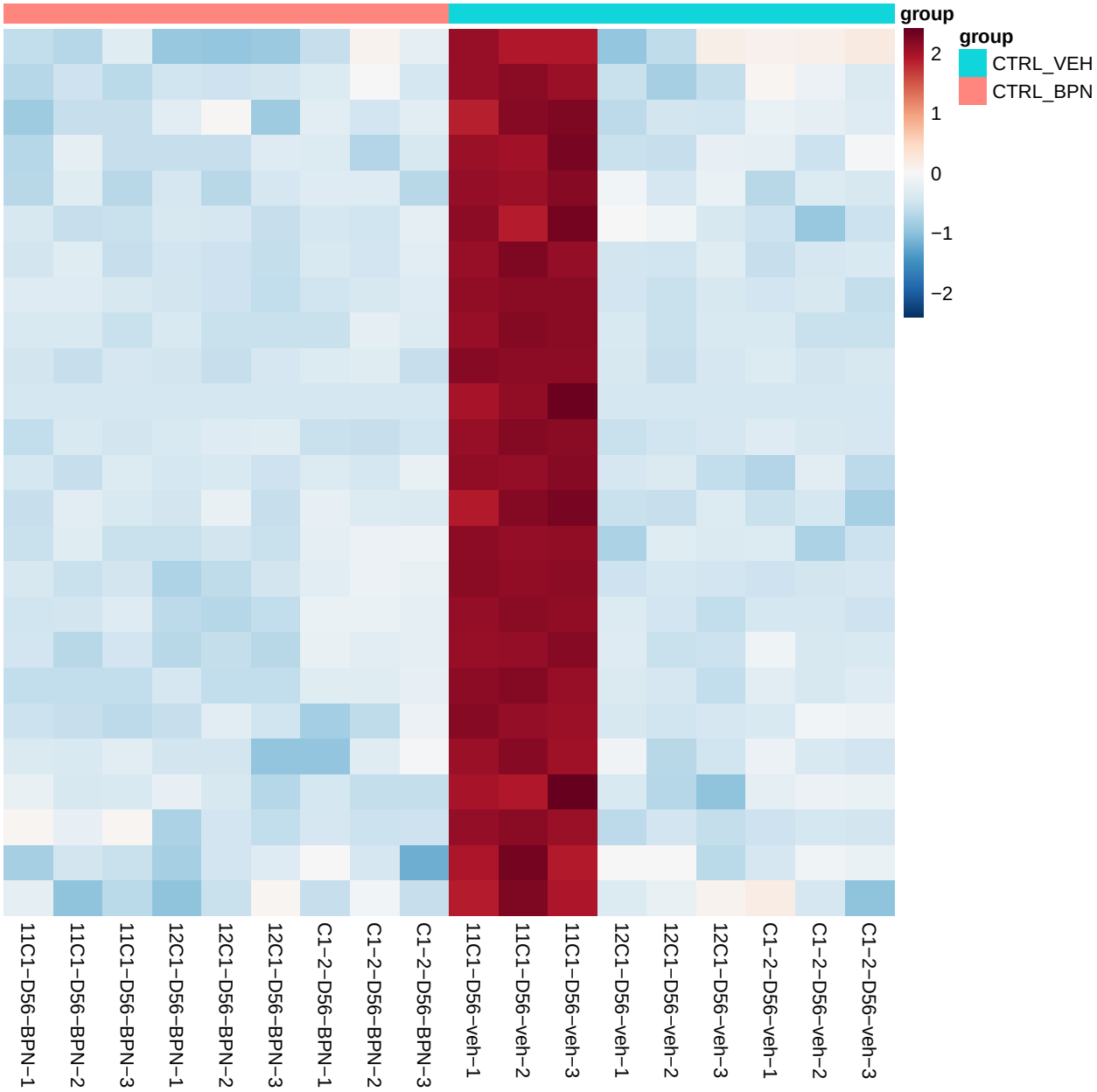

```
plot_gene_heatmap(vsd_obj, gene_list = DEG_gene_2,
                  figure_folder = file.path(result_folder,"02-DEG"),
                  file_name = "03_heatmap_log2fc_2",
                  reference_group, compare_group,
                  cluster_rows = TRUE, cluster_cols = FALSE,
                  scale = "none")
```

```
## [1] "Heatmap for 03_heatmap_log2fc_2 "
```




```
plot_gene_heatmap(vsd_obj, gene_list = DEG_gene_2,
                  figure_folder = file.path(result_folder,"02-DEG"),
                  file_name = "03_heatmap_log2fc_2_row",
                  reference_group, compare_group,
                  cluster_rows = TRUE, cluster_cols = FALSE,
                  scale = "row")
```

```
## [1] "Heatmap for 03_heatmap_log2fc_2_row "
```


group
group
CTRL_VEH
CTRL_BPN
2
1
0
−1
−2
11C1−D56−BPN−1
11C1−D56−BPN−2
11C1−D56−BPN−3
12C1−D56−BPN−1
12C1−D56−BPN−2
12C1−D56−BPN−3
C1−2−D56−BPN−1
C1−2−D56−BPN−2
C1−2−D56−BPN−3
11C1−D56−veh−1
11C1−D56−veh−2
11C1−D56−veh−3
12C1−D56−veh−1
12C1−D56−veh−2
12C1−D56−veh−3
C1−2−D56−veh−1
C1−2−D56−veh−2
C1−2−D56−veh−3

4. GSVA analysis

```
# The following code is used to generate the GSVA matrix , only need to run once
gmxFile <- here::here("data", "ref", "c5.go.v2023.1.Hs.symbols.gmt")
go_list <- getGmt(gmxFile)

geneset <- go_list
dat <- as.matrix(counts)

gsvapar <- gsvaParam(dat, geneset, maxDiff=TRUE)
gsva_es <- gsva(gsvapar)
```

```
## No annotation package name available in the input data object.
## Attempting to directly match identifiers in data to gene sets.
```

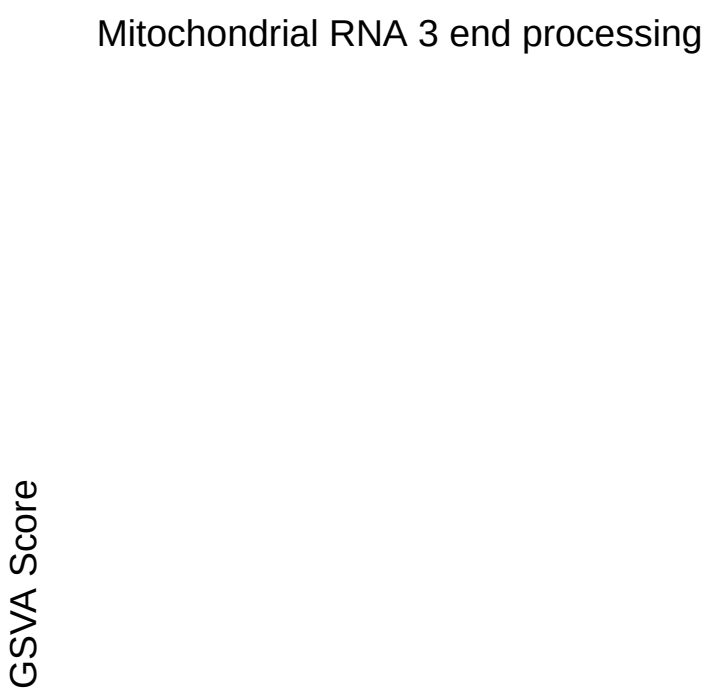
```
## Estimating GSVA scores for 10532 gene sets.
## Estimating ECDFs with Gaussian kernels
##   |                                                                      |
```

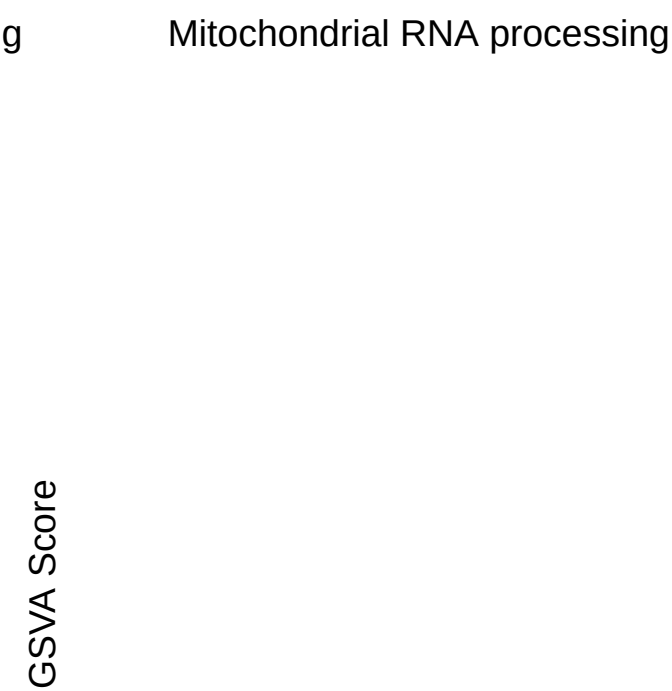
```
gsva_matrix <- as.data.frame(gsva_es)

# save the result
write.csv(gsva_matrix, file.path(result_folder,"04-GSVA", "01_GSVA_matrix.csv"))



gsva_matrix <- read.csv(file.path(result_folder,"04-GSVA", "01_GSVA_matrix.csv"),
                        row.names = 1)

colnames(gsva_matrix) <- sub("^X", "", colnames(gsva_matrix))
condition_list_label <- condition_list %>%
  filter(group %in% c(reference_group, compare_group)) %>%
  mutate(group = case_when(
    group == reference_group ~ reference_group_short,
    group == compare_group ~ compare_group_short,
    TRUE ~ group  # fallback just in case
  ))


# plot the heatmap for the GSVA result
pathway_list <- read.csv(here::here("data", "ref", "focus-pathway_2024_10_03.csv"))


# # plot for all pathway
# for (i in 1:nrow(pathway_list)){
#   if (i %% 10 == 0) print(i)
#   pathway_name <- pathway_list$pathway[i]
#   plot_gsva_boxplot(gsva_matrix,
#                     condition_list_label =condition_list_label,
#                     pathway_name =  pathway_name,
#                     figure_folder = file.path(result_folder,"04-GSVA","Boxplot"),
#                     file_name = paste0("GSVA_", pathway_name),
#                     fig.height = 6, fig.width = 4,
#                     reference_group =reference_group_short ,
#                     compare_group = compare_group_short)
```

```
#
# }

box_plot_folder<- file.path(result_folder,"04-GSVA","Boxplot")
# create the folder
dir.create(box_plot_folder, showWarnings = FALSE)


# plot for the focus pathway
for (i in 1:2){
  pathway_name <- pathway_list$pathway[i]
  print(pathway_name)
  p<-plot_gsva_boxplot(gsva_matrix,
                       condition_list_label =condition_list_label,
                       pathway_name =  pathway_name,
                       figure_folder = file.path(result_folder,"04-GSVA","Boxplot"),
                       file_name = paste0("GSVA_", pathway_name),
                       fig.height = 6, fig.width = 4,
                       reference_group =reference_group_short ,
                       compare_group = compare_group_short)
  print(p)
}
```

```
## [1] "GOBP_MITOCHONDRIAL_RNA_3_END_PROCESSING"
```

```
## [1] "GOBP_MITOCHONDRIAL_RNA_PROCESSING"
```





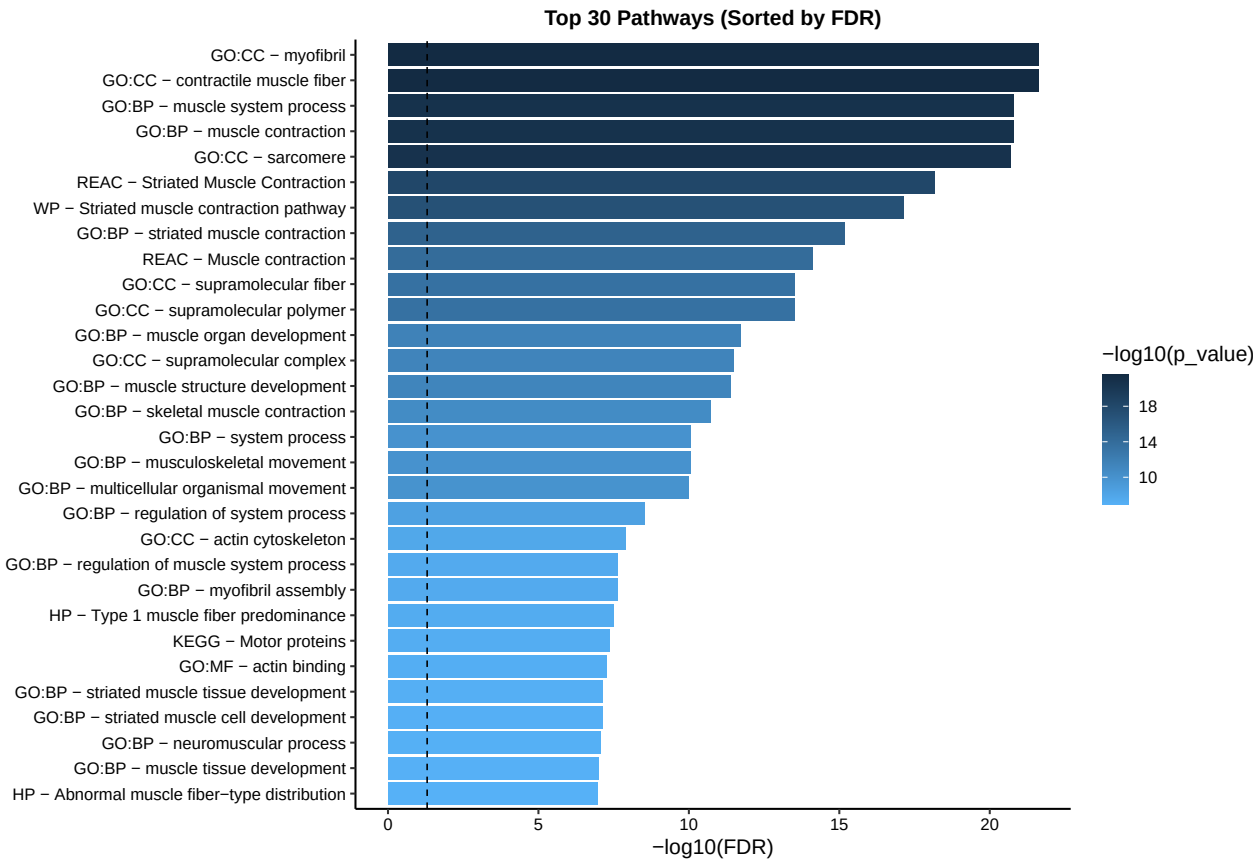
5. Pathway Enrichment Analysis

```
deg1 <- result_df %>% filter(padj < 0.05 & abs(log2FoldChange) > 1)
up_gene_1 <- deg1 %>% filter(log2FoldChange > 0) %>% pull(GeneName)
down_gene_1 <- deg1 %>% filter(log2FoldChange < 0) %>% pull(GeneName)


# test for the function
if (length(up_gene_1) == 0) {
  print("The up_gene_1 is empty, skip the analysis")
} else {
  Enrichment_analysis(gene_list = up_gene_1,
                      result_folder = file.path(result_folder,"03-Enrichment"),
                      file_name = "02-DEG_1_up", gene_name_mapping, flag = "Up")
}
```

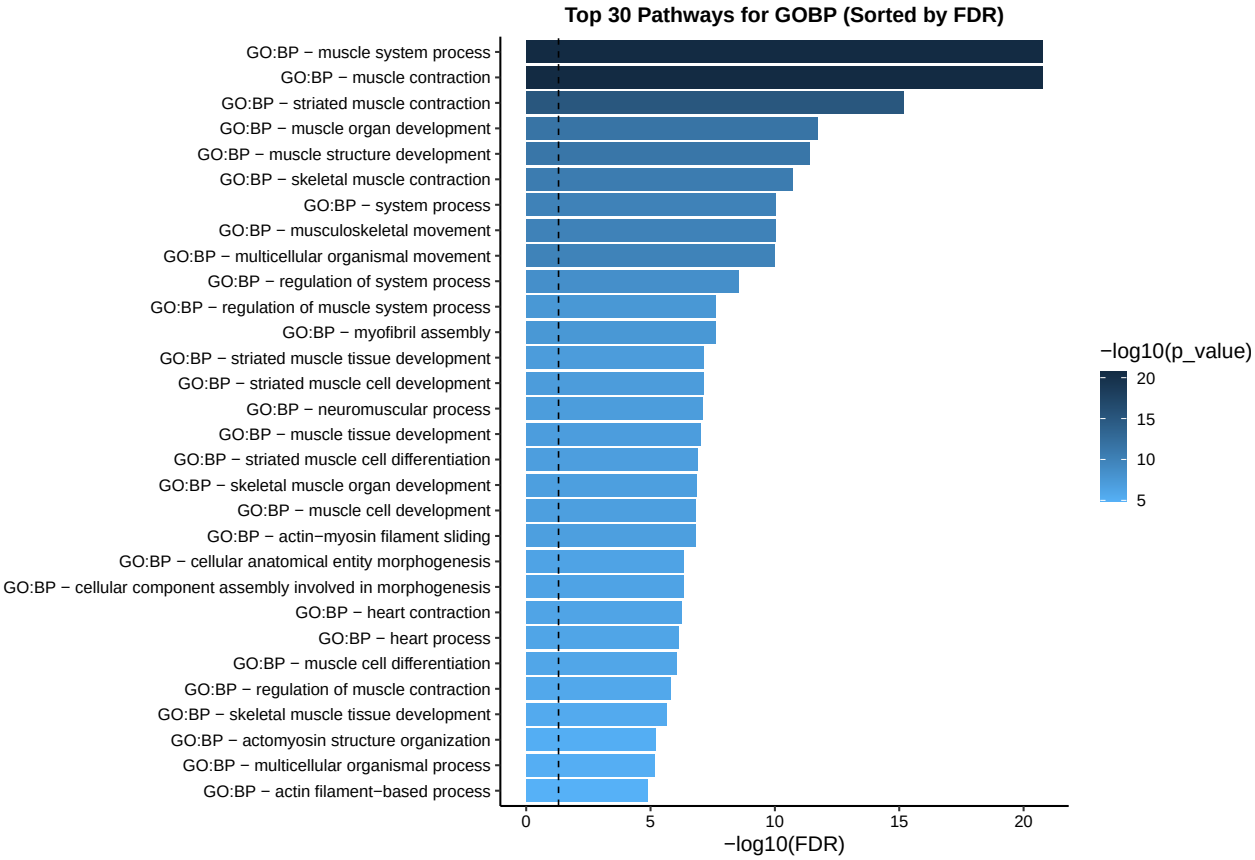
```
## [1] "The up_gene_1 is empty, skip the analysis"
```

```
if (length(down_gene_1) == 0) {
  print("The down_gene_1 is empty, skip the analysis")
} else {
  Enrichment_analysis(gene_list = down_gene_1,
                      result_folder = file.path(result_folder,"03-Enrichment"),
                      file_name = "02-DEG_1_down", gene_name_mapping, flag = "Down")
}
```

```
## [1] "Enrichment analysis for 02-DEG_1_down "
```



```
## [1] "Enrichment analysis for GOBP 02-DEG_1_down "
```

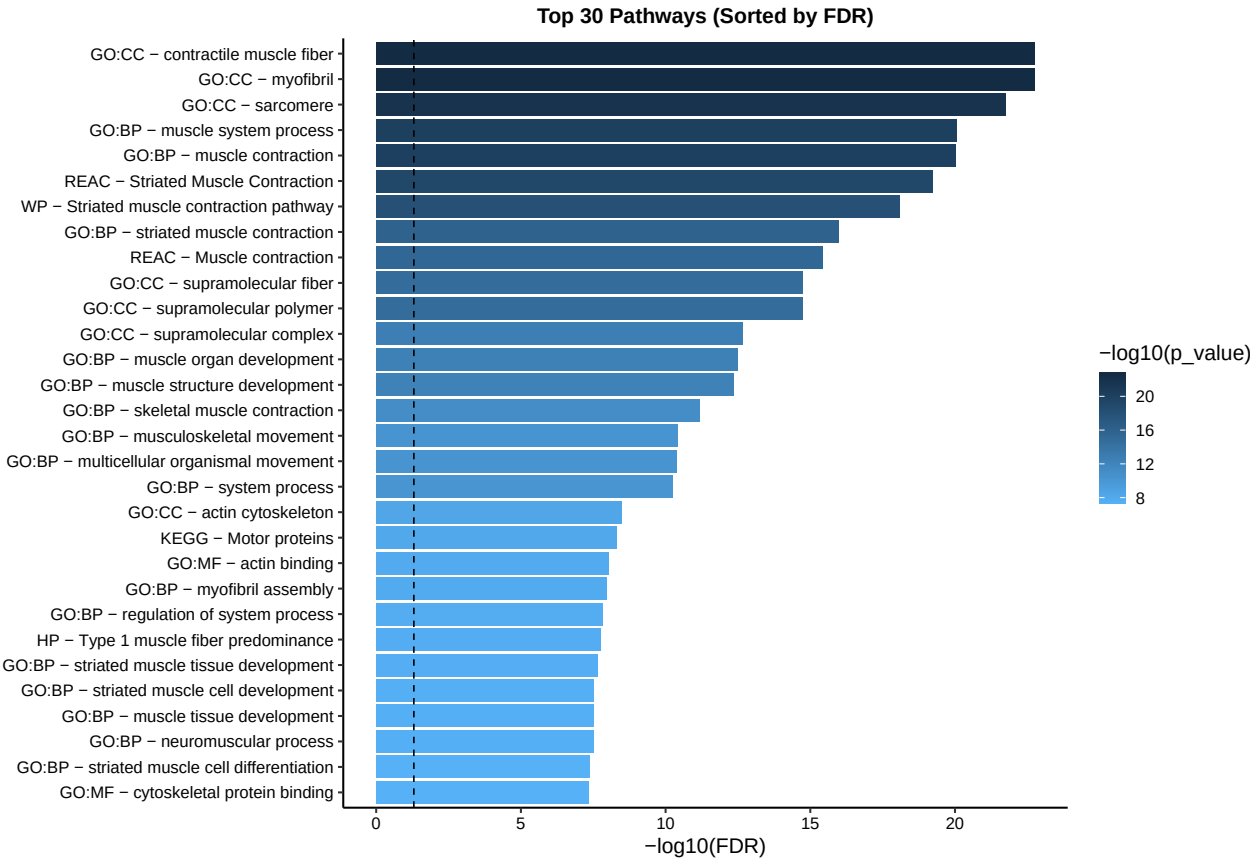


```
deg1.5 <- result_df %>% filter(padj < 0.05 & abs(log2FoldChange) > 1.5)
up_gene_1.5 <- deg1.5 %>% filter(log2FoldChange > 0) %>% pull(GeneName)
down_gene_1.5 <- deg1.5 %>% filter(log2FoldChange < 0) %>% pull(GeneName)

# test for the function
# if the up_gene_1.5 is empty, we will skip the analysis
if (length(up_gene_1.5) == 0) {
  print("The up_gene_1.5 is empty, skip the analysis")
} else {
  Enrichment_analysis(gene_list = up_gene_1.5,
                      result_folder = file.path(result_folder,"03-Enrichment"),
                      file_name = "02-DEG_1.5_up", gene_name_mapping, flag = "Up")
}
```

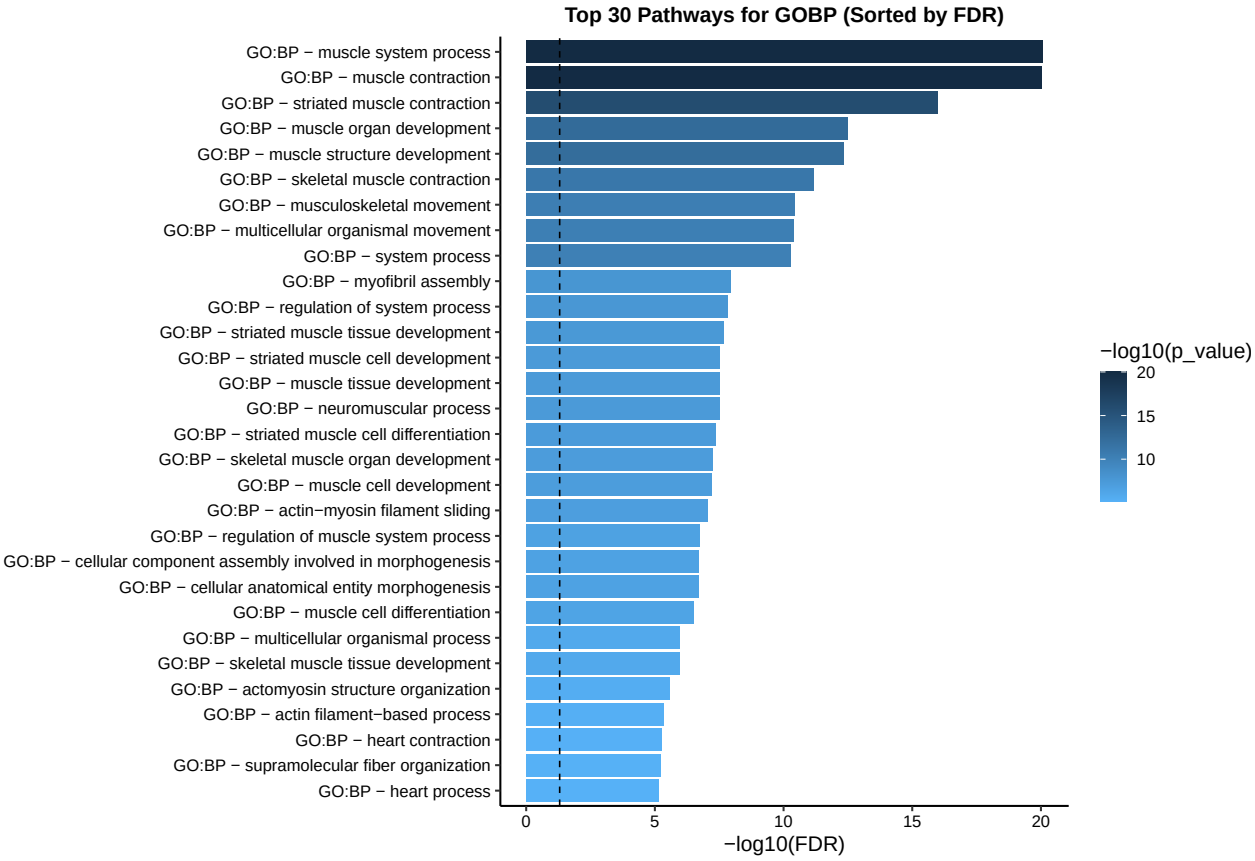
```
## [1] "The up_gene_1.5 is empty, skip the analysis"
```

```
if (length(down_gene_1.5) == 0) {
  print("The down_gene_1.5 is empty, skip the analysis")
} else {
  Enrichment_analysis(gene_list = down_gene_1.5,
                      result_folder = file.path(result_folder,"03-Enrichment"),
                      file_name = "02-DEG_1.5_down", gene_name_mapping, flag = "Down")
}
```

```
## [1] "Enrichment analysis for 02-DEG_1.5_down "
```




```
## [1] "Enrichment analysis for GOBP 02-DEG_1.5_down "
```

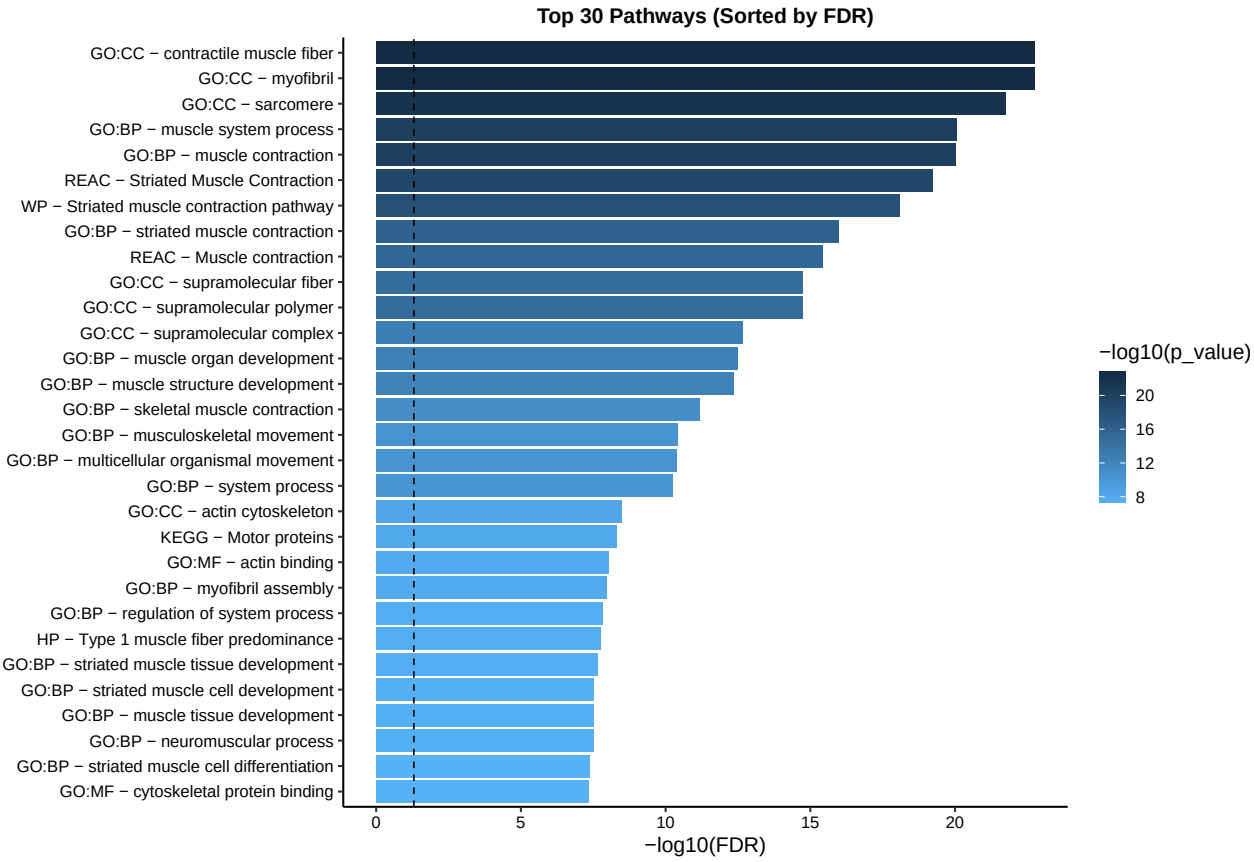

Top 30 Pathways for GOBP (Sorted by FDR)
GO:BP − muscle system process
GO:BP − muscle contraction
GO:BP − striated muscle contraction
GO:BP − muscle organ development
GO:BP − muscle structure development
GO:BP − skeletal muscle contraction
GO:BP − musculoskeletal movement
GO:BP − multicellular organismal movement
GO:BP − system process
GO:BP − myofibril assembly
GO:BP − regulation of system process
GO:BP − striated muscle tissue development
GO:BP − striated muscle cell development
GO:BP − muscle tissue development
GO:BP − neuromuscular process
GO:BP − striated muscle cell differentiation
GO:BP − skeletal muscle organ development
GO:BP − muscle cell development
GO:BP − actin−myosin filament sliding
GO:BP − regulation of muscle system process
GO:BP − cellular component assembly involved in morphogenesis
GO:BP − cellular anatomical entity morphogenesis
GO:BP − muscle cell differentiation
GO:BP − multicellular organismal process
GO:BP − skeletal muscle tissue development
GO:BP − actomyosin structure organization
GO:BP − actin filament−based process
GO:BP − heart contraction
GO:BP − supramolecular fiber organization
GO:BP − heart process
0
5
10
15
20
−log10(FDR)
−log10(p_value)
20
15
10

```r
deg2 <- result_df %>% filter(padj < 0.05 & abs(log2FoldChange) > 1.5)
up_gene_2 <- deg2 %>% filter(log2FoldChange > 0) %>% pull(GeneName)
down_gene_2 <- deg2 %>% filter(log2FoldChange < 0) %>% pull(GeneName)
result_folder = result_folder_all
# test for the function
if (length(up_gene_2) == 0) {
  print("The up_gene_2 is empty, skip the analysis")
} else {
  Enrichment_analysis(gene_list = up_gene_2,
                      result_folder = file.path(result_folder,"03-Enrichment"),
                      file_name = "02-DEG_2_up", gene_name_mapping, flag = "Up")
}
```

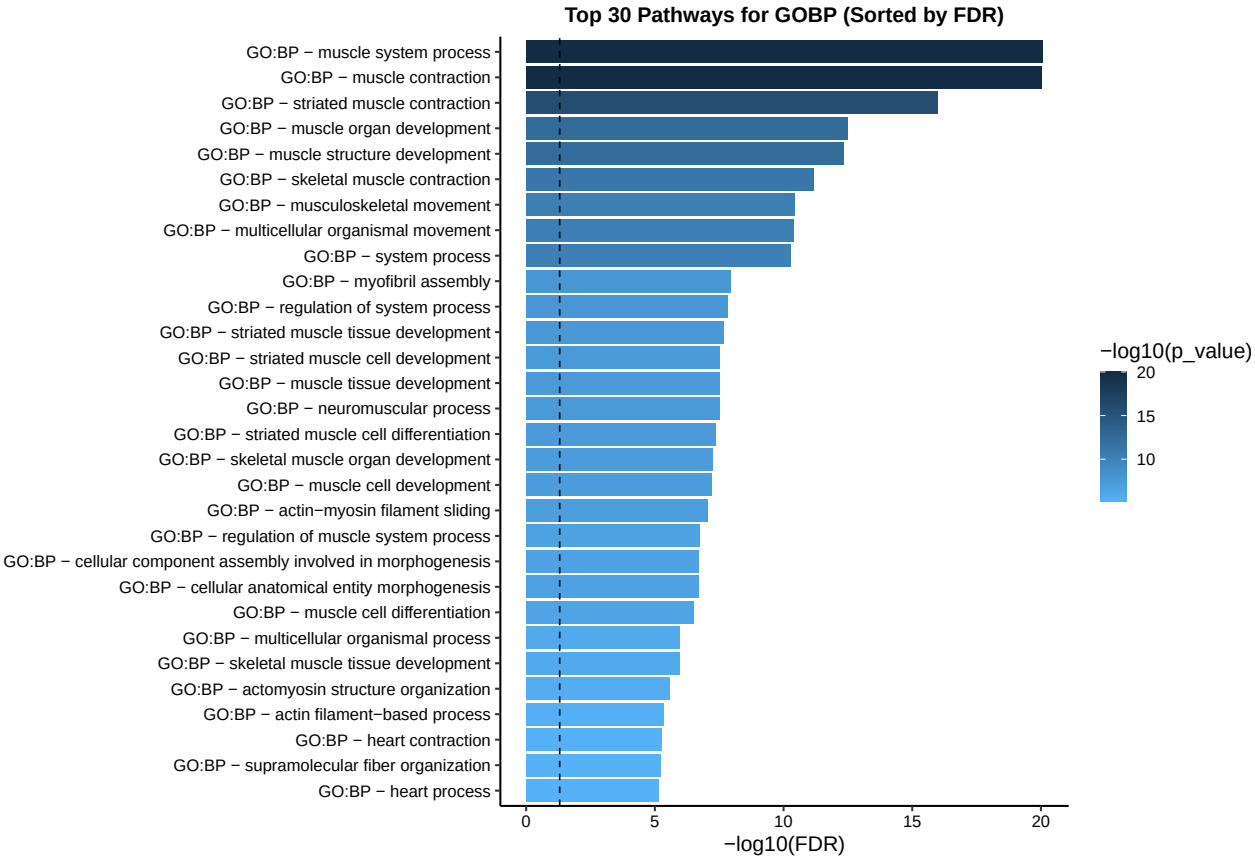
```
## [1] "The up_gene_2 is empty, skip the analysis"
```

```r
if (length(down_gene_2) == 0) {
  print("The down_gene_2 is empty, skip the analysis")
} else {
  Enrichment_analysis(gene_list = down_gene_2,
                      result_folder = file.path(result_folder,"03-Enrichment"),
                      file_name = "02-DEG_2_down", gene_name_mapping, flag = "Down")
}
```

```
## [1] "Enrichment analysis for 02-DEG_2_down "
```




```
## [1] "Enrichment analysis for GOBP 02-DEG_2_down "
```


Top 30 Pathways for GOBP (Sorted by FDR)
GO:BP − muscle system process
GO:BP − muscle contraction
GO:BP − striated muscle contraction
GO:BP − muscle organ development
GO:BP − muscle structure development
GO:BP − skeletal muscle contraction
GO:BP − musculoskeletal movement
GO:BP − multicellular organismal movement
GO:BP − system process
GO:BP − myofibril assembly
GO:BP − regulation of system process
GO:BP − striated muscle tissue development
GO:BP − striated muscle cell development
GO:BP − muscle tissue development
GO:BP − neuromuscular process
GO:BP − striated muscle cell differentiation
GO:BP − skeletal muscle organ development
GO:BP − muscle cell development
GO:BP − actin−myosin filament sliding
GO:BP − regulation of muscle system process
GO:BP − cellular component assembly involved in morphogenesis
GO:BP − cellular anatomical entity morphogenesis
GO:BP − muscle cell differentiation
GO:BP − multicellular organismal process
GO:BP − skeletal muscle tissue development
GO:BP − actomyosin structure organization
GO:BP − actin filament−based process
GO:BP − heart contraction
GO:BP − supramolecular fiber organization
GO:BP − heart process
0
5
10
15
20
−log10(FDR)
−log10(p_value)
20
15
10

Session information

```
sessionInfo()
```

```
## R version 4.4.0 (2024-04-24)
## Platform: aarch64-apple-darwin20
## Running under: macOS Sonoma 14.3.1
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/4.4-arm64/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.4-arm64/Resources/lib/libRlapack.dylib;  LAPACK v
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## time zone: America/New_York
## tzcode source: internal
##
## attached base packages:
## [1] parallel  stats4    stats     graphics  grDevices utils     datasets
## [8] methods   base
##
## other attached packages:
##  [1] GSEABase_1.66.0             graph_1.82.0
##  [3] annotate_1.82.0             XML_3.99-0.18
##  [5] extrafont_0.19              ggsignif_0.6.4
##  [7] patchwork_1.3.0             decoupleR_2.10.0
##  [9] GSVA_1.52.3                 BiocParallel_1.38.0
## [11] edgeR_4.2.2                 limma_3.60.6
## [13] GenomicFeatures_1.56.0      biomaRt_2.60.1
## [15] gprofiler2_0.2.3            RColorBrewer_1.1-3
## [17] data.table_1.16.4           org.Hs.eg.db_3.19.1
## [19] AnnotationDbi_1.66.0        clusterProfiler_4.12.6
## [21] ggfortify_0.4.17            pheatmap_1.0.12
## [23] EnhancedVolcano_1.22.0      ggrepel_0.9.6
## [25] apeglm_1.26.1               DESeq2_1.44.0
## [27] SummarizedExperiment_1.34.0 Biobase_2.64.0
## [29] MatrixGenerics_1.16.0       matrixStats_1.5.0
## [31] reshape2_1.4.4              Matrix_1.7-2
## [33] Signac_1.14.0               Seurat_5.2.1
## [35] SeuratObject_5.0.2          sp_2.2-0
## [37] rtracklayer_1.64.0          GenomicRanges_1.56.2
## [39] GenomeInfoDb_1.40.1         IRanges_2.38.1
## [41] S4Vectors_0.42.1            BiocGenerics_0.50.0
## [43] knitr_1.49                  lubridate_1.9.4
## [45] forcats_1.0.0               stringr_1.5.1
## [47] dplyr_1.1.4                 purrr_1.0.4
## [49] readr_2.1.5                 tidyr_1.3.1
## [51] tibble_3.2.1                ggplot2_3.5.1
## [53] tidyverse_2.0.0
##
## loaded via a namespace (and not attached):
##   [1] SpatialExperiment_1.14.0    R.methodsS3_1.8.2
##   [3] progress_1.2.3              goftest_1.2-3
```

```
##   [5] HDF5Array_1.32.1           Biostrings_2.72.1
##   [7] vctrs_0.6.5                spatstat.random_3.3-2
##   [9] digest_0.6.37              png_0.1-8
##  [11] deldir_2.0-4               parallelly_1.42.0
##  [13] magick_2.8.5               MASS_7.3-64
##  [15] httpuv_1.6.15              qvalue_2.36.0
##  [17] withr_3.0.2                xfun_0.51
##  [19] ggfun_0.1.8                survival_3.8-3
##  [21] memoise_2.0.1              gson_0.1.0
##  [23] systemfonts_1.2.1          ragg_1.3.3
##  [25] tidytree_0.4.6             zoo_1.8-12
##  [27] pbapply_1.7-2              R.oo_1.27.0
##  [29] prettyunits_1.2.0          KEGGREST_1.44.1
##  [31] promises_1.3.2             httr_1.4.7
##  [33] restfulr_0.0.15            rhdf5filters_1.16.0
##  [35] globals_0.16.3             fitdistrplus_1.2-2
##  [37] rhdf5_2.48.0               rstudioapi_0.17.1
##  [39] UCSC.utils_1.0.0           miniUI_0.1.1.1
##  [41] generics_0.1.3             DOSE_3.30.5
##  [43] curl_6.2.1                 zlibbioc_1.50.0
##  [45] ScaledMatrix_1.12.0        ggraph_2.2.1
##  [47] polyclip_1.10-7            GenomeInfoDbData_1.2.12
##  [49] SparseArray_1.4.8          xtable_1.8-4
##  [51] evaluate_1.0.3             S4Arrays_1.4.1
##  [53] BiocFileCache_2.12.0       hms_1.1.3
##  [55] irlba_2.3.5.1              colorspace_2.1-1
##  [57] filelock_1.0.3             ROCR_1.0-11
##  [59] reticulate_1.40.0          spatstat.data_3.1-4
##  [61] magrittr_2.0.3             lmtest_0.9-40
##  [63] later_1.4.1                viridis_0.6.5
##  [65] ggtree_3.12.0              lattice_0.22-6
##  [67] spatstat.geom_3.3-5        future.apply_1.11.3
##  [69] scattermore_1.2            shadowtext_0.1.4
##  [71] cowplot_1.1.3              RcppAnnoy_0.0.22
##  [73] pillar_1.10.1              nlme_3.1-167
##  [75] compiler_4.4.0             beachmat_2.20.0
##  [77] RSpectra_0.16-2            stringi_1.8.4
##  [79] tensor_1.5                 GenomicAlignments_1.40.0
##  [81] plyr_1.8.9                 crayon_1.5.3
##  [83] abind_1.4-8                BiocIO_1.14.0
##  [85] gridGraphics_0.5-1         emdbook_1.3.13
##  [87] locfit_1.5-9.11            graphlayouts_1.2.2
##  [89] bit_4.5.0.1                fastmatch_1.1-6
##  [91] textshaping_1.0.0          codetools_0.2-20
##  [93] BiocSingular_1.20.0        plotly_4.10.4
##  [95] mime_0.12                  splines_4.4.0
##  [97] Rcpp_1.0.14                fastDummies_1.7.5
##  [99] sparseMatrixStats_1.16.0   dbplyr_2.5.0
## [101] Rttf2pt1_1.3.12            blob_1.2.4
## [103] here_1.0.1                 fs_1.6.5
## [105] listenv_0.9.1              ggplotify_0.1.2
## [107] statmod_1.5.0              tzdb_0.4.0
## [109] tweenr_2.0.3               pkgconfig_2.0.3
## [111] tools_4.4.0                cachem_1.1.0
```

```
## [113] RSQLite_2.3.9              viridisLite_0.4.2
## [115] DBI_1.2.3                  numDeriv_2016.8-1.1
## [117] fastmap_1.2.0              rmarkdown_2.29
## [119] scales_1.3.0               grid_4.4.0
## [121] ica_1.0-3                  Rsamtools_2.20.0
## [123] coda_0.19-4.1              dotCall64_1.2
## [125] RANN_2.6.2                 farver_2.1.2
## [127] tidygraph_1.3.1            scatterpie_0.2.4
## [129] yaml_2.3.10                cli_3.6.4
## [131] lifecycle_1.0.4            uwot_0.2.2
## [133] mvtnorm_1.3-3              timechange_0.3.0
## [135] gtable_0.3.6               rjson_0.2.23
## [137] ggridges_0.5.6             progressr_0.15.1
## [139] ape_5.8-1                  jsonlite_1.9.0
## [141] RcppHNSW_0.6.0             bitops_1.0-9
## [143] bit64_4.6.0-1              Rtsne_0.17
## [145] yulab.utils_0.2.0          spatstat.utils_3.1-2
## [147] bdsmatrix_1.3-7            GOSemSim_2.30.2
## [149] spatstat.univar_3.1-1      R.utils_2.12.3
## [151] lazyeval_0.2.2             shiny_1.10.0
## [153] htmltools_0.5.8.1          enrichplot_1.24.4
## [155] GO.db_3.19.1               sctransform_0.4.1
## [157] rappdirs_0.3.3             tinytex_0.55
## [159] glue_1.8.0                 spam_2.11-1
## [161] httr2_1.1.0                XVector_0.44.0
## [163] RCurl_1.98-1.16            rprojroot_2.0.4
## [165] treeio_1.28.0              gridExtra_2.3
## [167] extrafontdb_1.0            igraph_2.1.4
## [169] R6_2.6.1                   SingleCellExperiment_1.26.0
## [171] labeling_0.4.3             RcppRoll_0.3.1
## [173] cluster_2.1.8              bbmle_1.0.25.1
## [175] Rhdf5lib_1.26.0            aplot_0.2.4
## [177] DelayedArray_0.30.1        tidyselect_1.2.1
## [179] ggforce_0.4.2              xml2_1.3.6
## [181] future_1.34.0              rsvd_1.0.5
## [183] munsell_0.5.1              KernSmooth_2.23-26
## [185] htmlwidgets_1.6.4          fgsea_1.30.0
## [187] rlang_1.1.5                spatstat.sparse_3.1-0
## [189] spatstat.explore_3.3-4
```