Differential Expression Analysis for bulk RNA-seq data

CTRL Condition: Vehicle 100 vs 10

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2025-03-24

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))


# raw sample list
sample_list_raw <- read.csv(here::here("data", "bulkRNA",
                                   "sample_info_AD.csv")) %>%
                    mutate(condition = paste0(Diagosis, "_", Treatment),
                           sample = gsub("_", "-", Sample.name))


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

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. We will compare the 22q vs Control in the vehicle condition. The results will be stored in `results/02-DEG-V_10/DESeq2_results.csv`.

```
# Init the result folder structure for the result
result_folder_all = './results/02-DEG-AD_10_vs_100'
result_folder = result_folder_all
Result_folder_structure(result_folder)

# load the comparison group information
reference_group <- "AD_Veh_10"
compare_group <- "AD_Veh_100"
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")
```

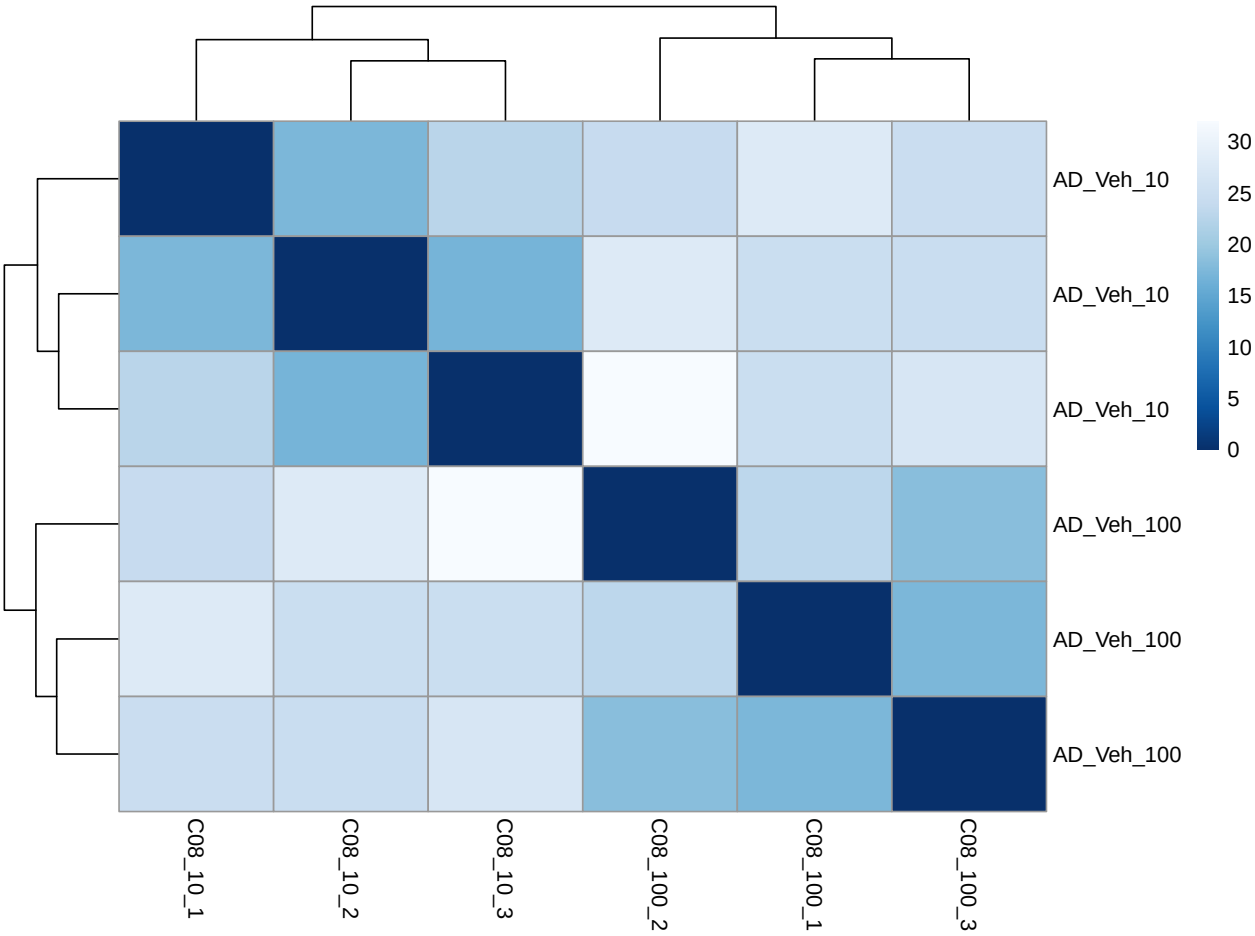
```
## [1] "DEG analysis is done"
```

```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

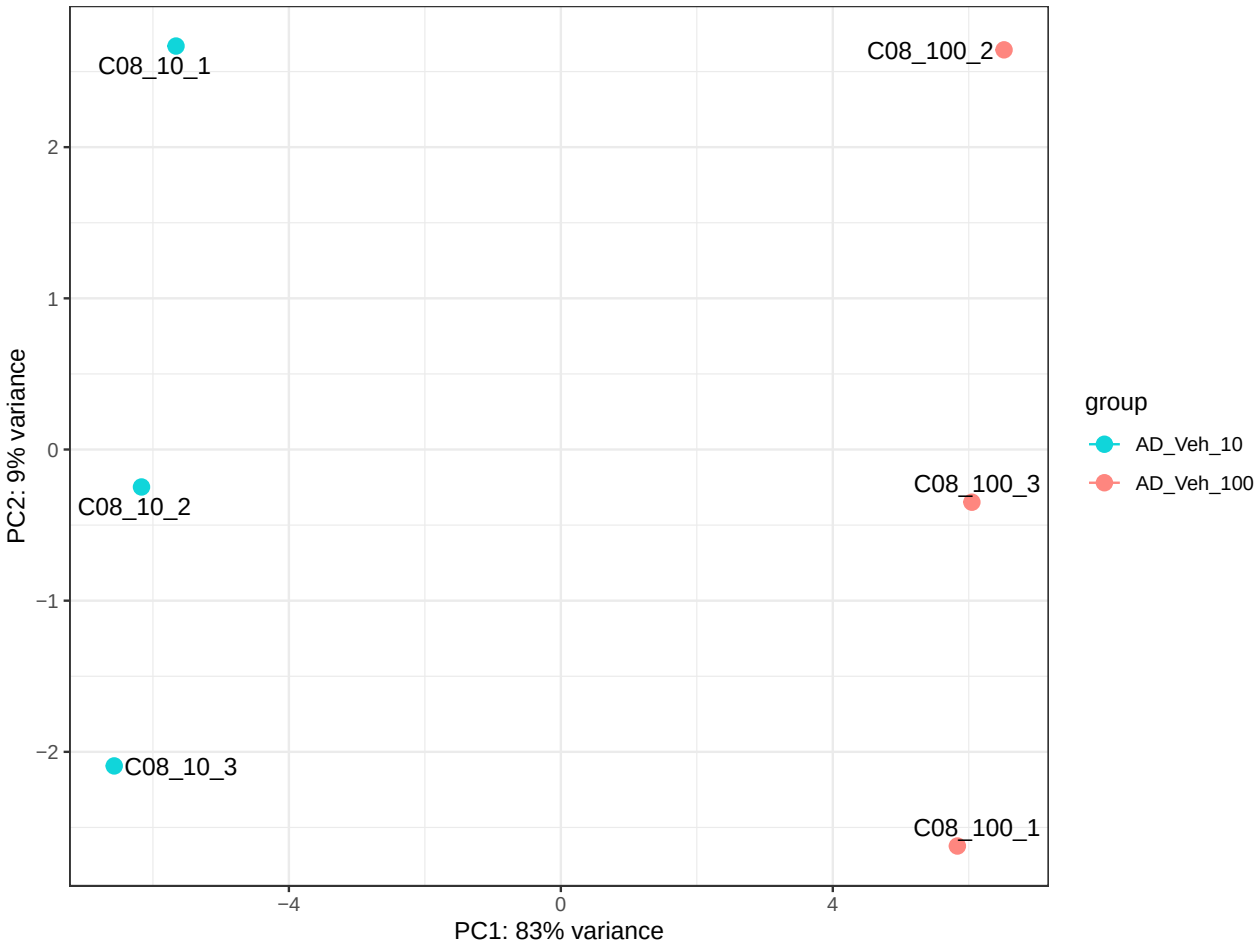
```
# Plot the sample distance
plot_sample_heatmap(dds_obj, figure_folder =
                    file.path(result_folder,"01-Sample_info"),
                    file_name = "01_sample_distance_heatmap")
```




```
## [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)
```

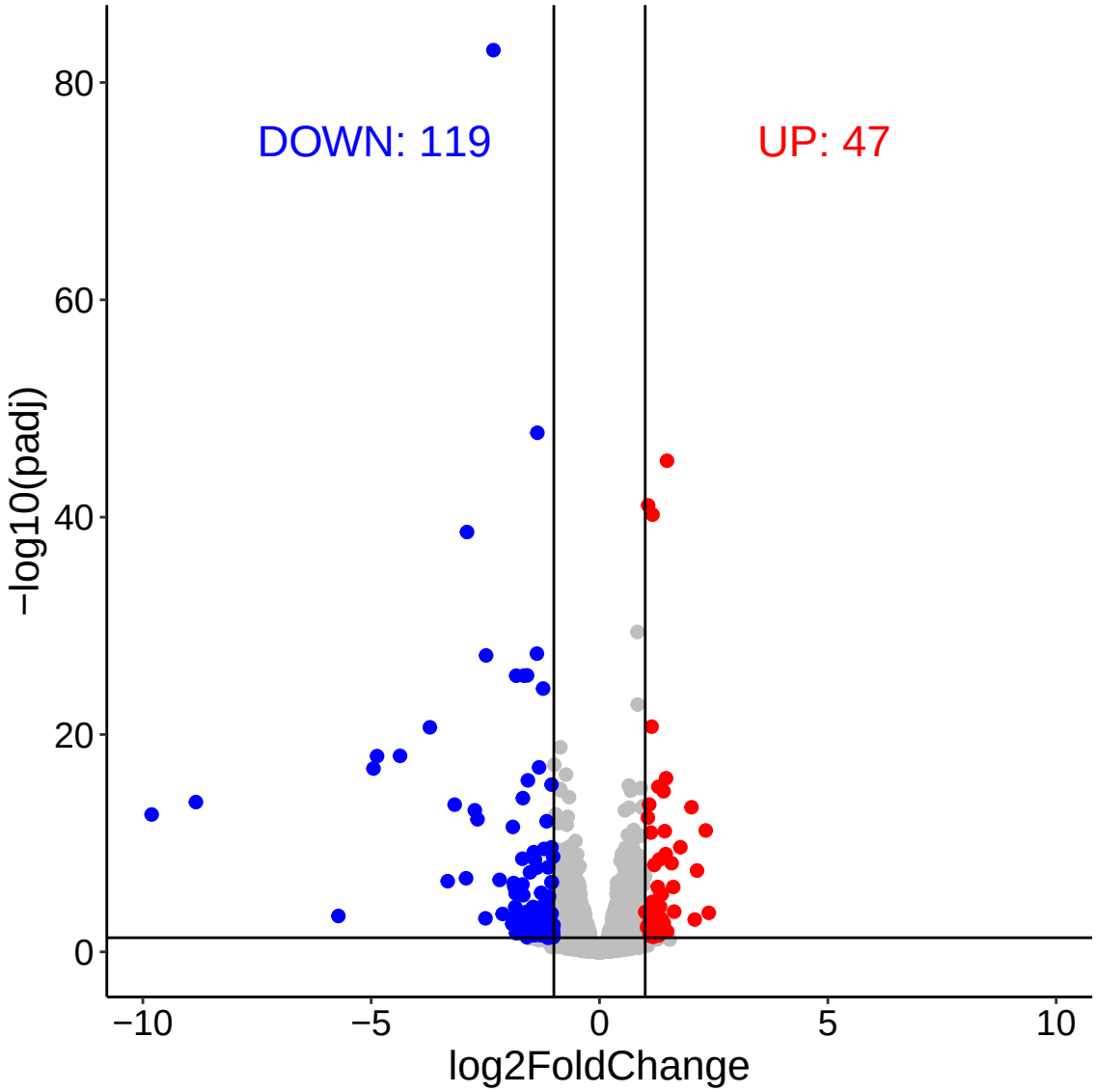
```
## [1] "PCA plot is done"
```


C08_10_1
C08_100_2
C08_10_2
C08_100_3
C08_10_3
C08_100_1
2
1
0
−1
−2
−4
0
4
PC2: 9% variance
PC1: 83% variance
group
AD_Veh_10
AD_Veh_100

(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)
```

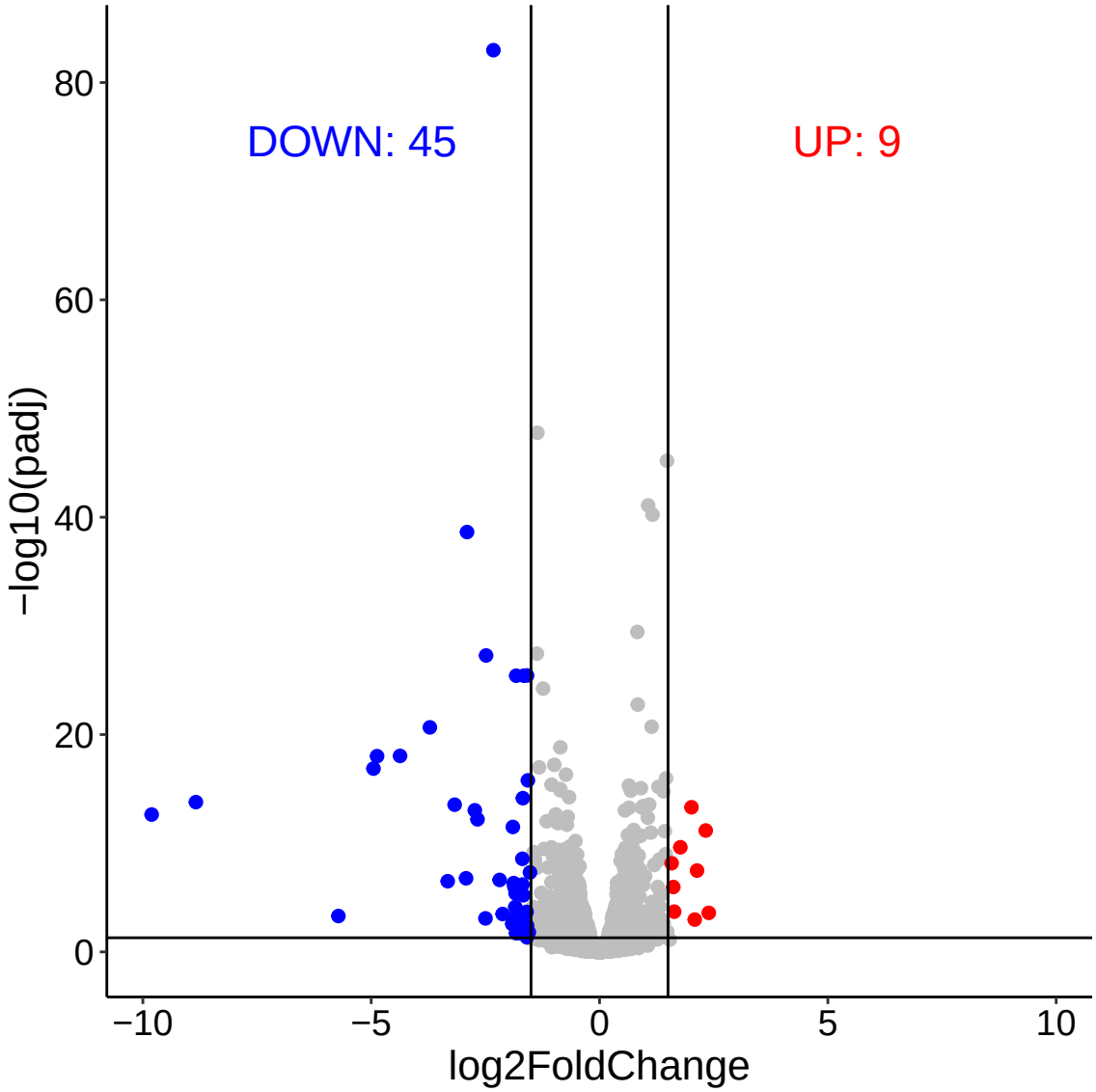
```
## [1] "Volcano plot for 02_volcano_plot_log2fc_1"
```




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

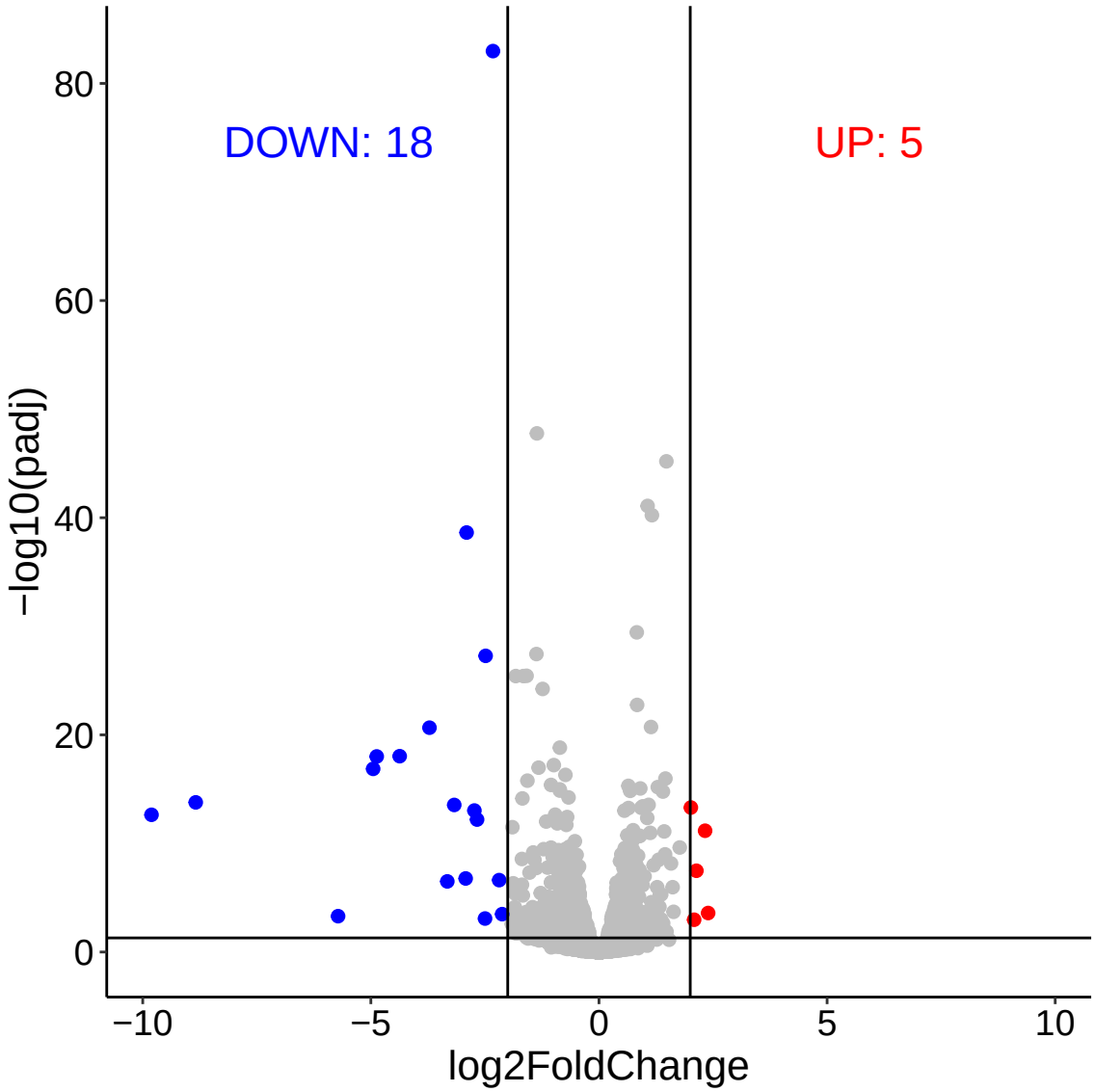
```
                  figure_folder = file.path(result_folder,"02-DEG"),
                  file_name = "03_volcano_plot_log2fc_1.5",
                  thread = 1.5 ,  dot_size =2,label_gene = NULL)
```

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



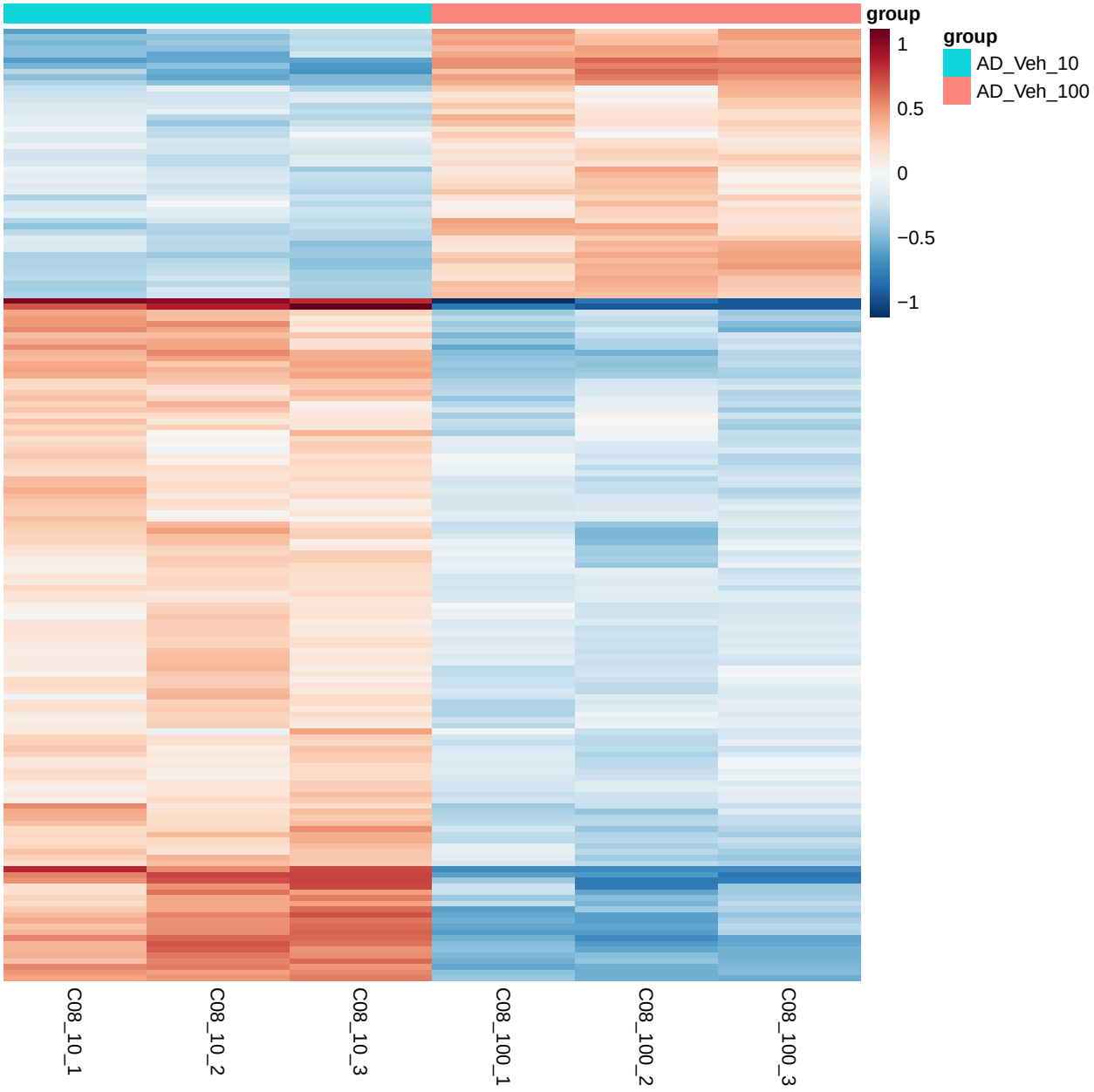

```
plot_volcano_plot(result_df=result_df,
                  figure_folder = file.path(result_folder,"02-DEG"),
                  file_name = "03_volcano_plot_log2fc_2",
                  thread = 2 ,  dot_size =2,label_gene = NULL)
```

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



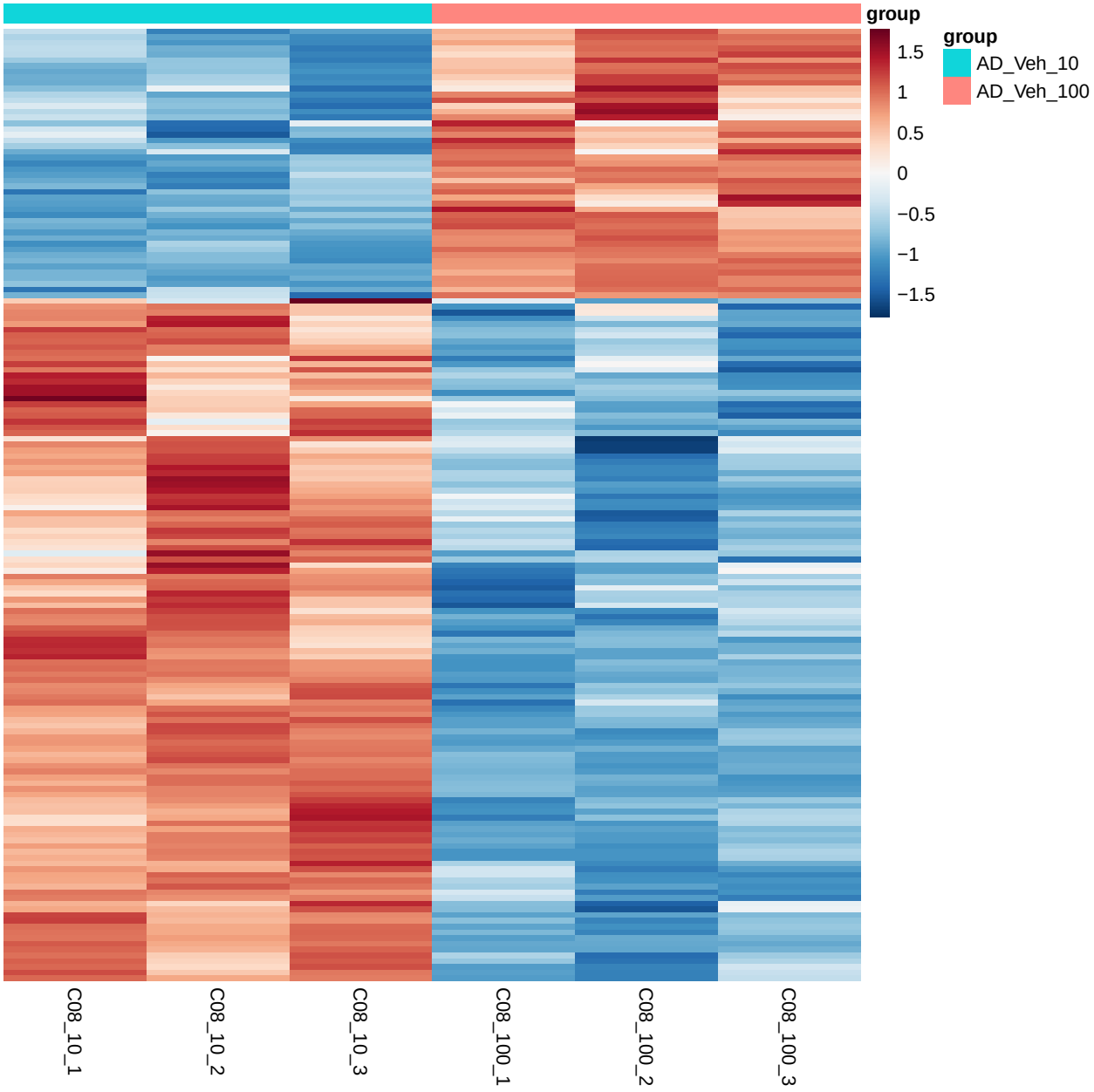

```
# Plot the heatmap for the DEG
vsd_obj <- varianceStabilizingTransformation(dds_obj, blind = TRUE)

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 "
```



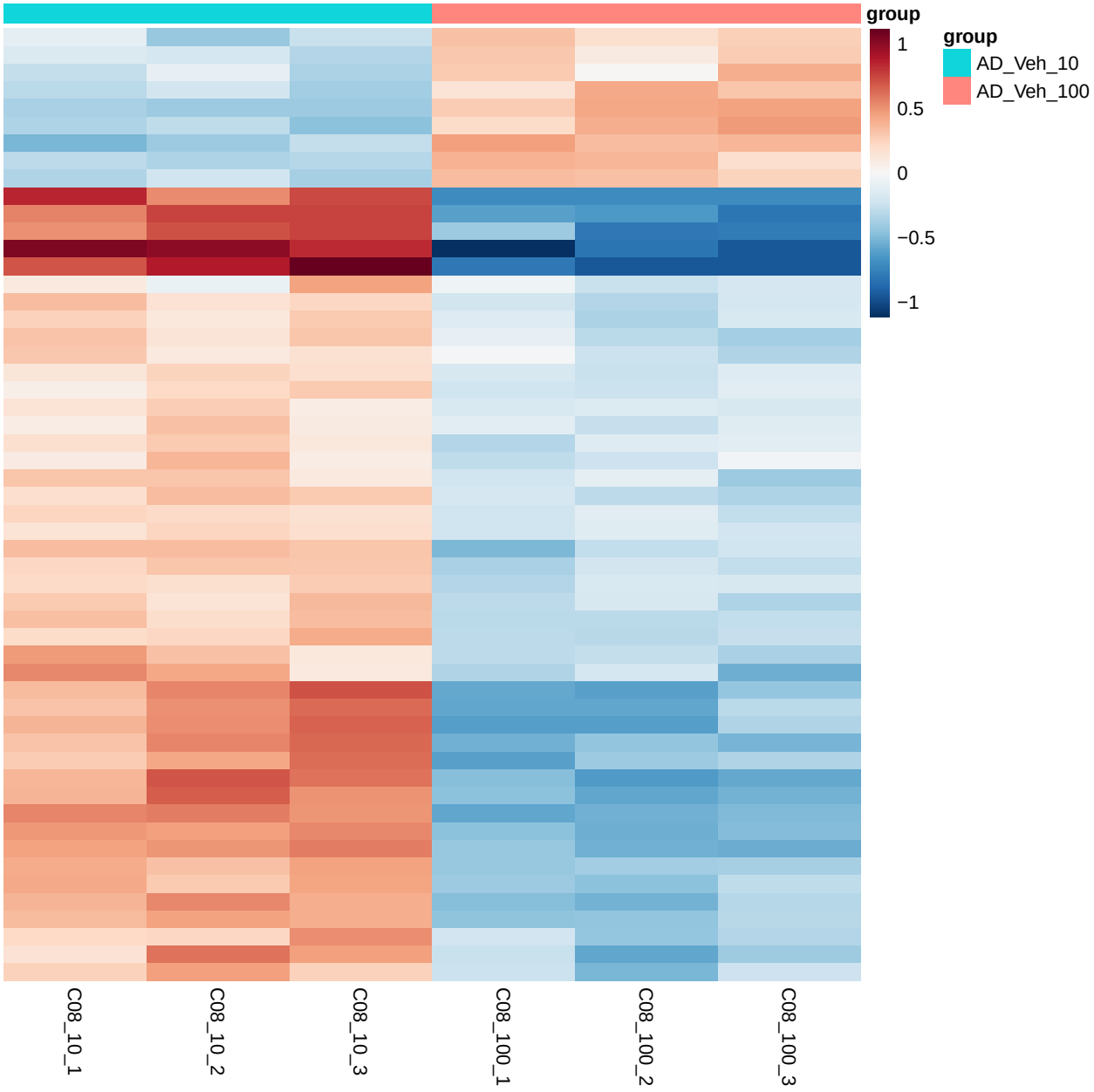

```
plot_gene_heatmap(vsd_obj, gene_list = DEG_gene_1,
                  figure_folder = file.path(result_folder,"02-DEG"),
                  file_name = "02_heatmap_log2fc_1_row",
                  reference_group, compare_group,
                  cluster_rows = TRUE, cluster_cols = FALSE,
        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

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



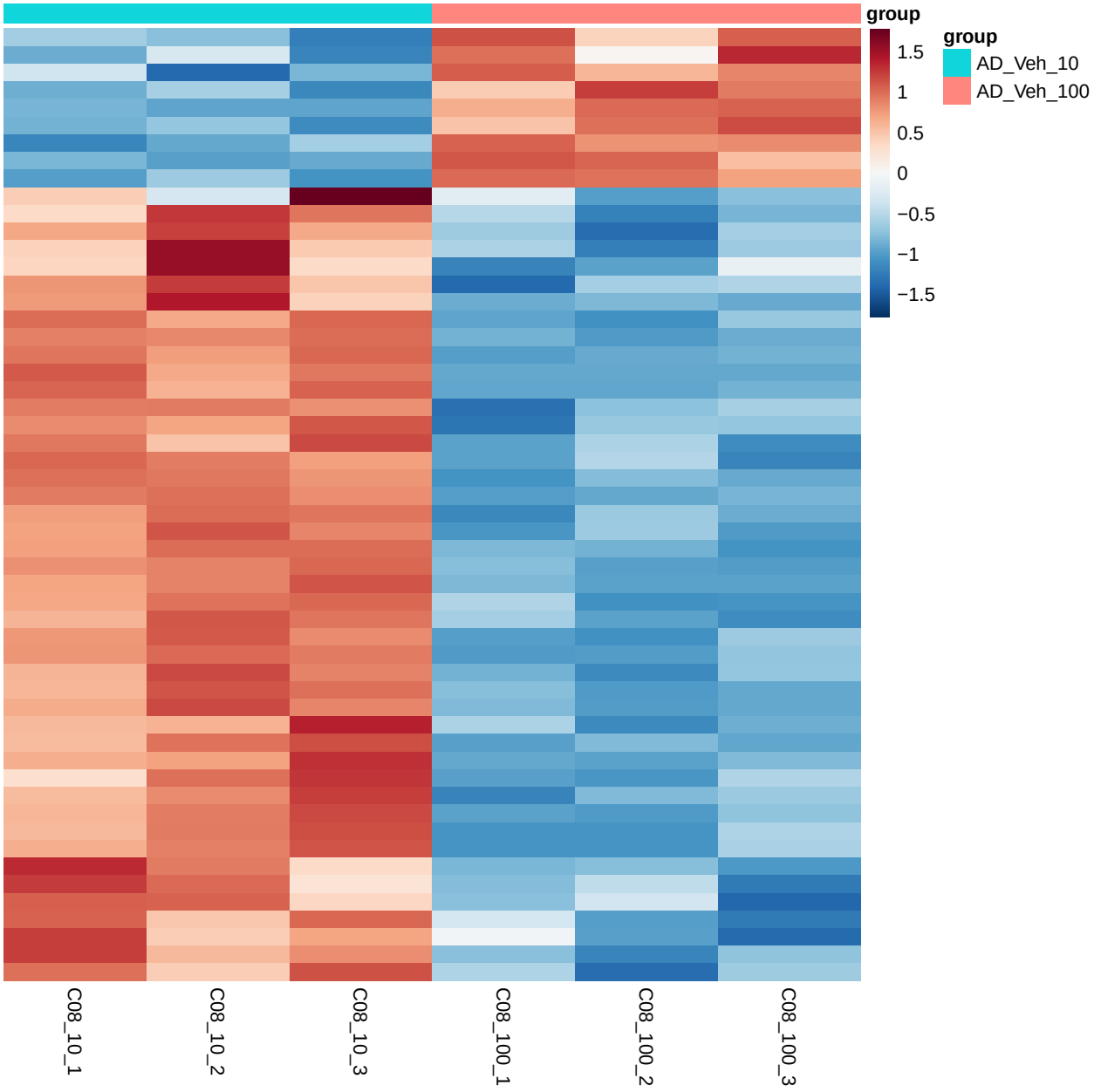

```
plot_gene_heatmap(vsd_obj, gene_list = DEG_gene_1.5,
                  figure_folder = file.path(result_folder,"02-DEG"),
                  file_name = "03_heatmap_log2fc_1.5",
                  reference_group, compare_group,
                  cluster_rows = TRUE, cluster_cols = FALSE,
        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

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



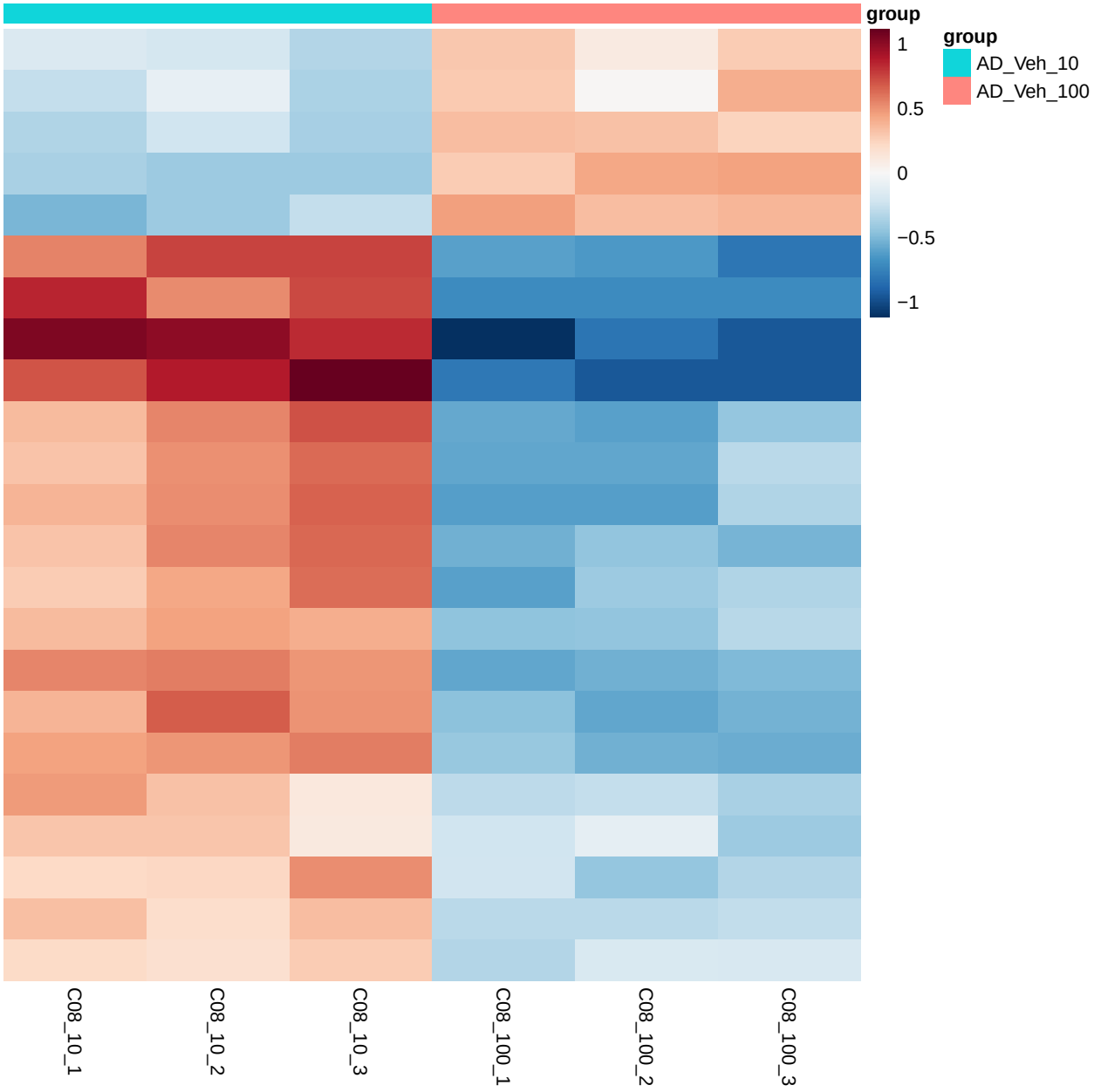

```
plot_gene_heatmap(vsd_obj, gene_list = DEG_gene_1.5,
                  figure_folder = file.path(result_folder,"02-DEG"),
                  file_name = "03_heatmap_log2fc_1.5_row",
                  reference_group, compare_group,
                  cluster_rows = TRUE, cluster_cols = FALSE,
    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

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



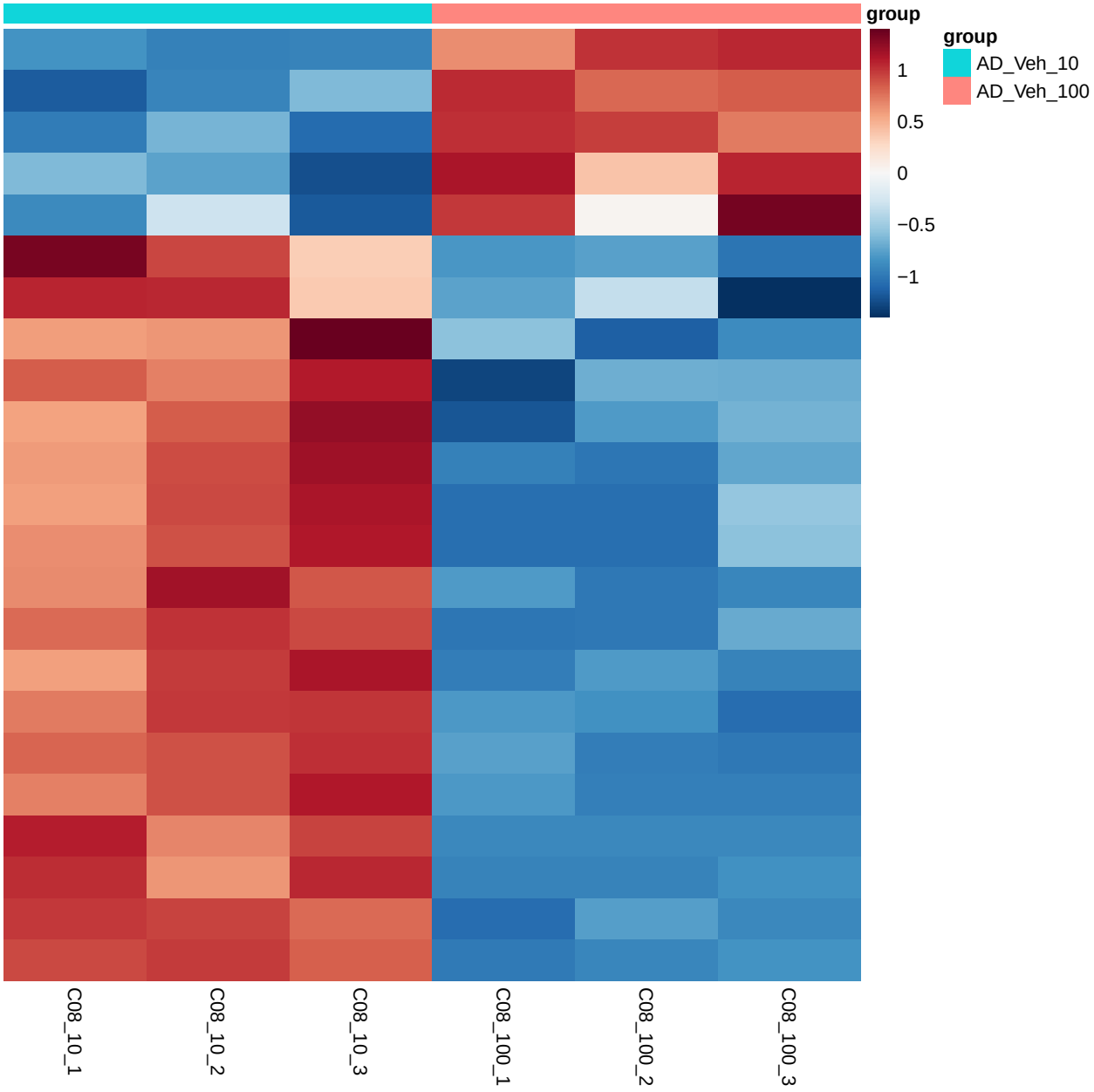

```
plot_gene_heatmap(vsd_obj, gene_list = DEG_gene_2,
                  figure_folder = file.path(result_folder,"02-DEG"),
                  file_name = "03_heatmap_log2fc_2",
                  reference_group, compare_group,
                  cluster_rows = TRUE, cluster_cols = FALSE,
                  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
AD_Veh_10
AD_Veh_100
1
0.5
0
−0.5
−1
C08_10_1
C08_10_2
C08_10_3
C08_100_1
C08_100_2
C08_100_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.
```

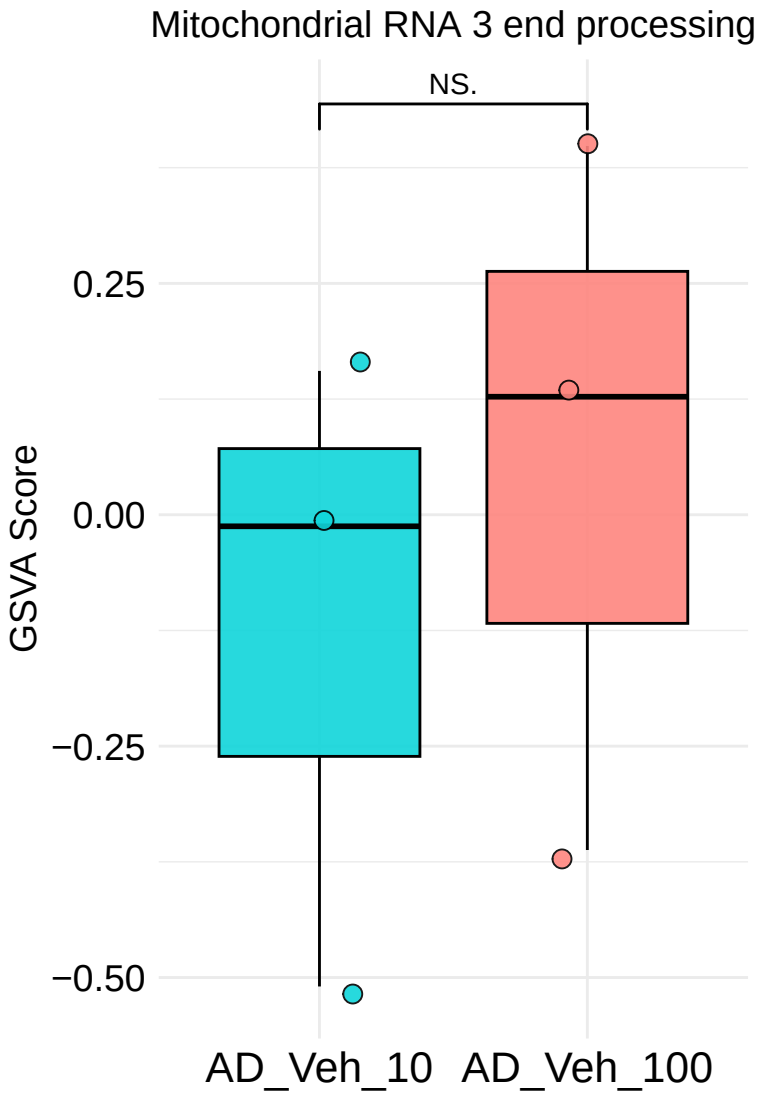
```
## Estimating GSVA scores for 10531 gene sets.
## Estimating ECDFs with Gaussian kernels
##   |                                                                      |
```

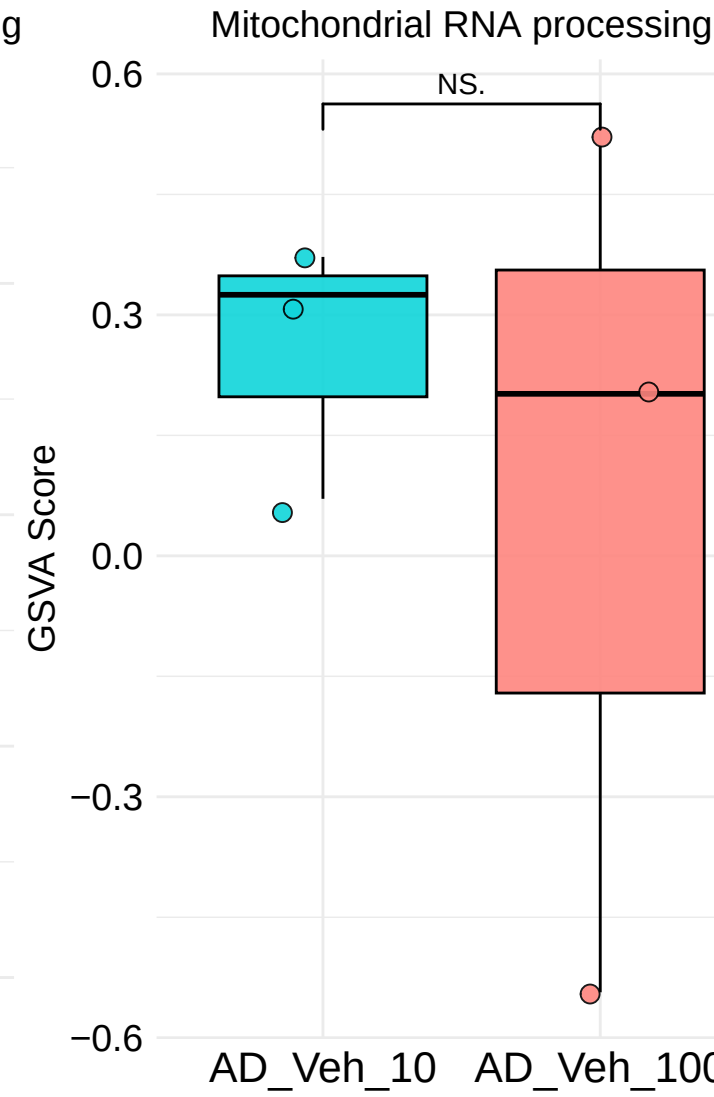
```
gsva_matrix <- as.data.frame(gsva_es)

# 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)
```

```r
#
# }

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"
```

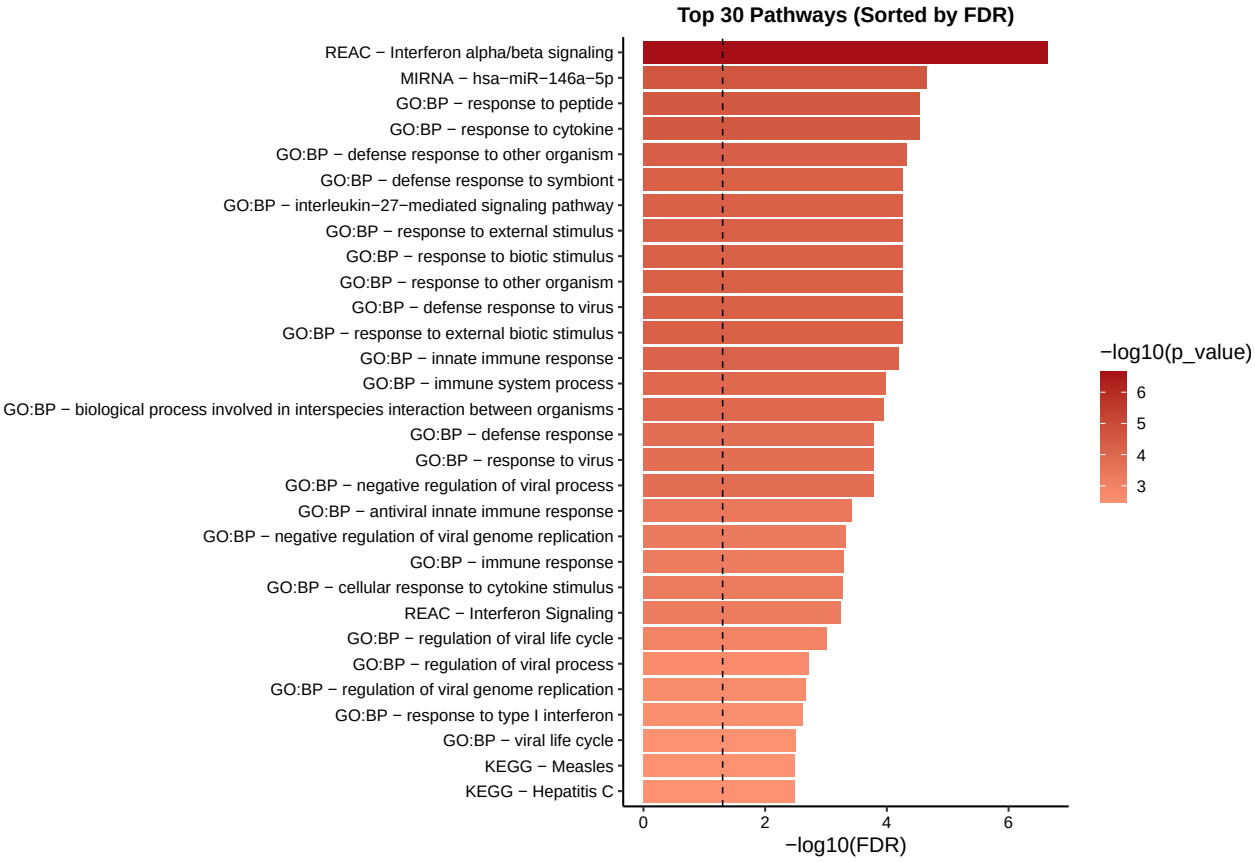

Mitochondrial RNA 3 end processing
NS.
0.25
0.00
−0.25
−0.50
GSVA Score
AD_Veh_10
AD_Veh_100


Mitochondrial RNA processing
NS.
0.6
0.3
0.0
−0.3
−0.6
GSVA Score
AD_Veh_10
AD_Veh_100

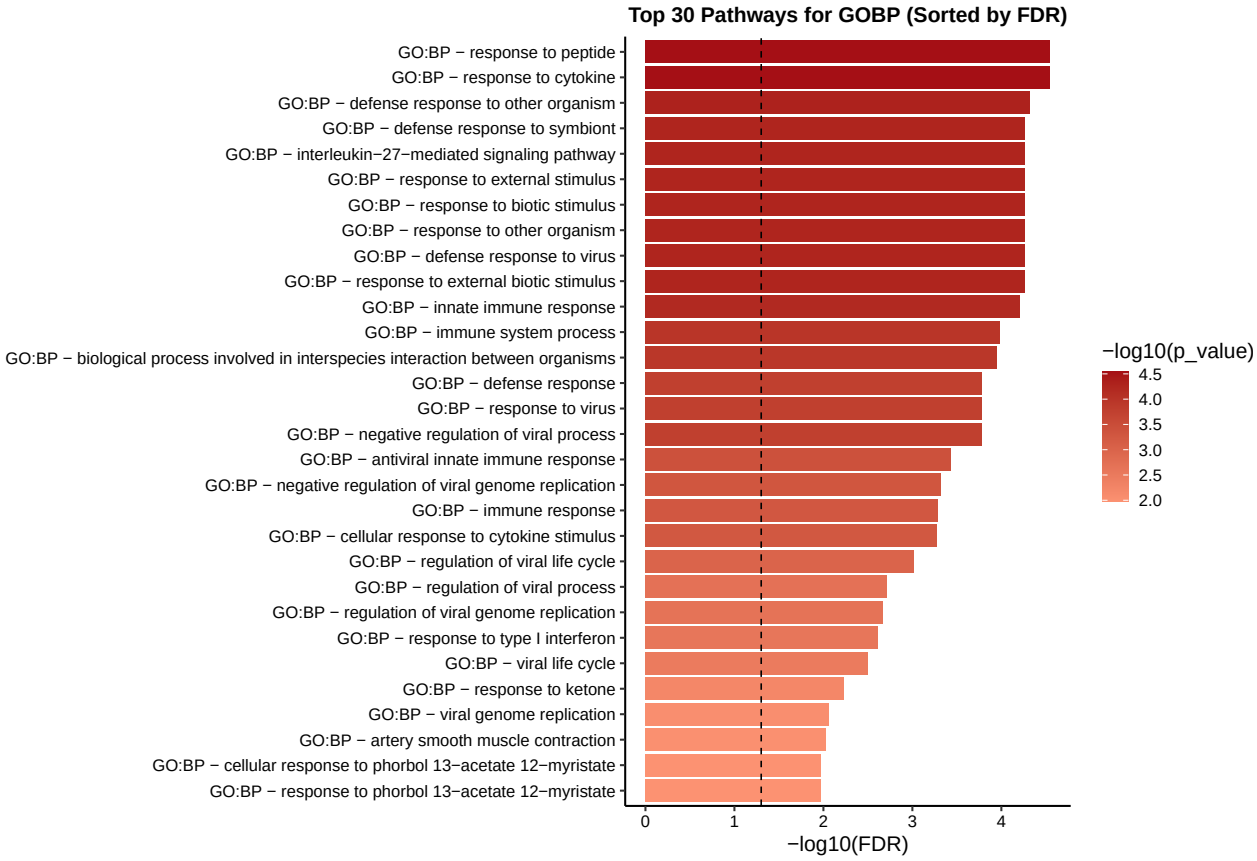
5. Pathway Enrichment Analysis

```
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
Enrichment_analysis(gene_list = up_gene_1,
                    result_folder = file.path(result_folder,"03-Enrichment"),
                    file_name = "01-DEG_1.0_up", gene_name_mapping, flag = "Up")
```

```
## [1] "Enrichment analysis for 01-DEG_1.0_up "
```

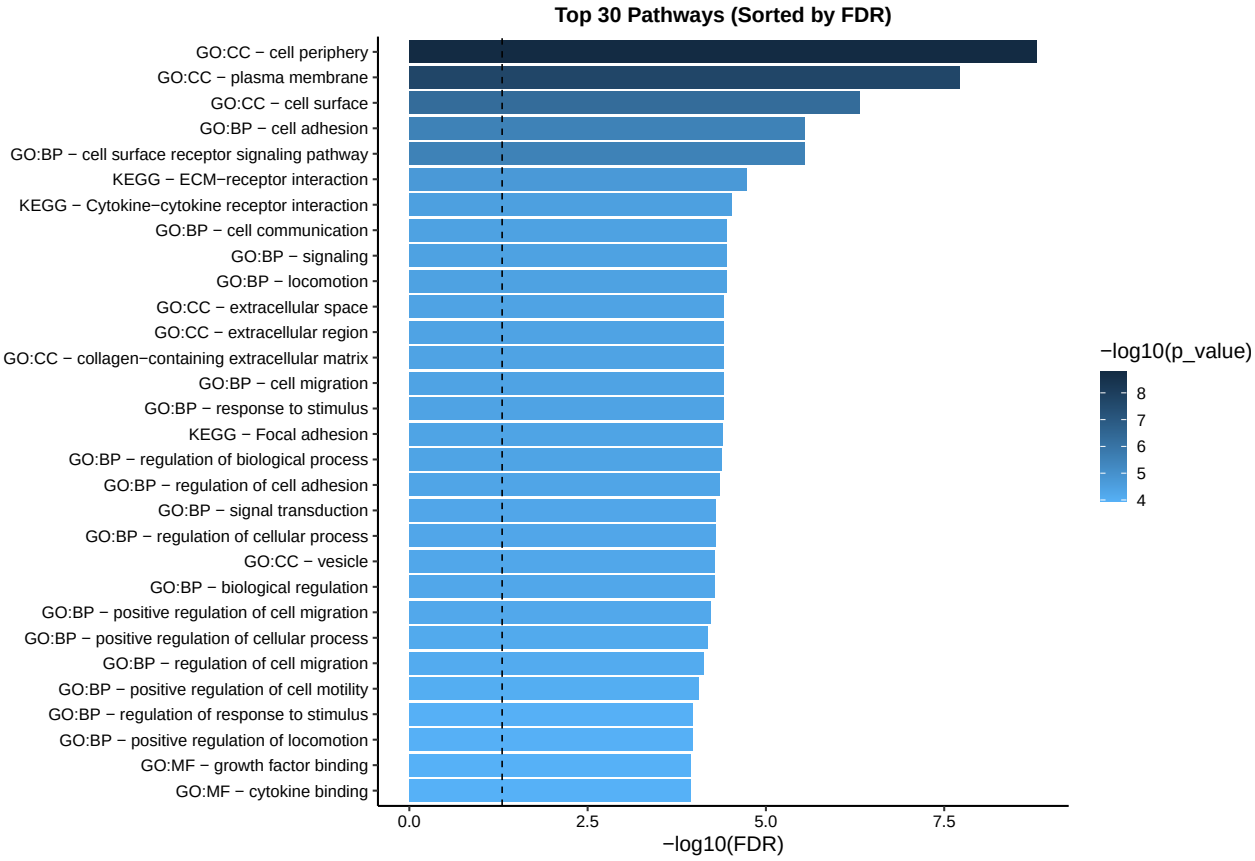



```
## [1] "Enrichment analysis for GOBP 01-DEG_1.0_up "
```

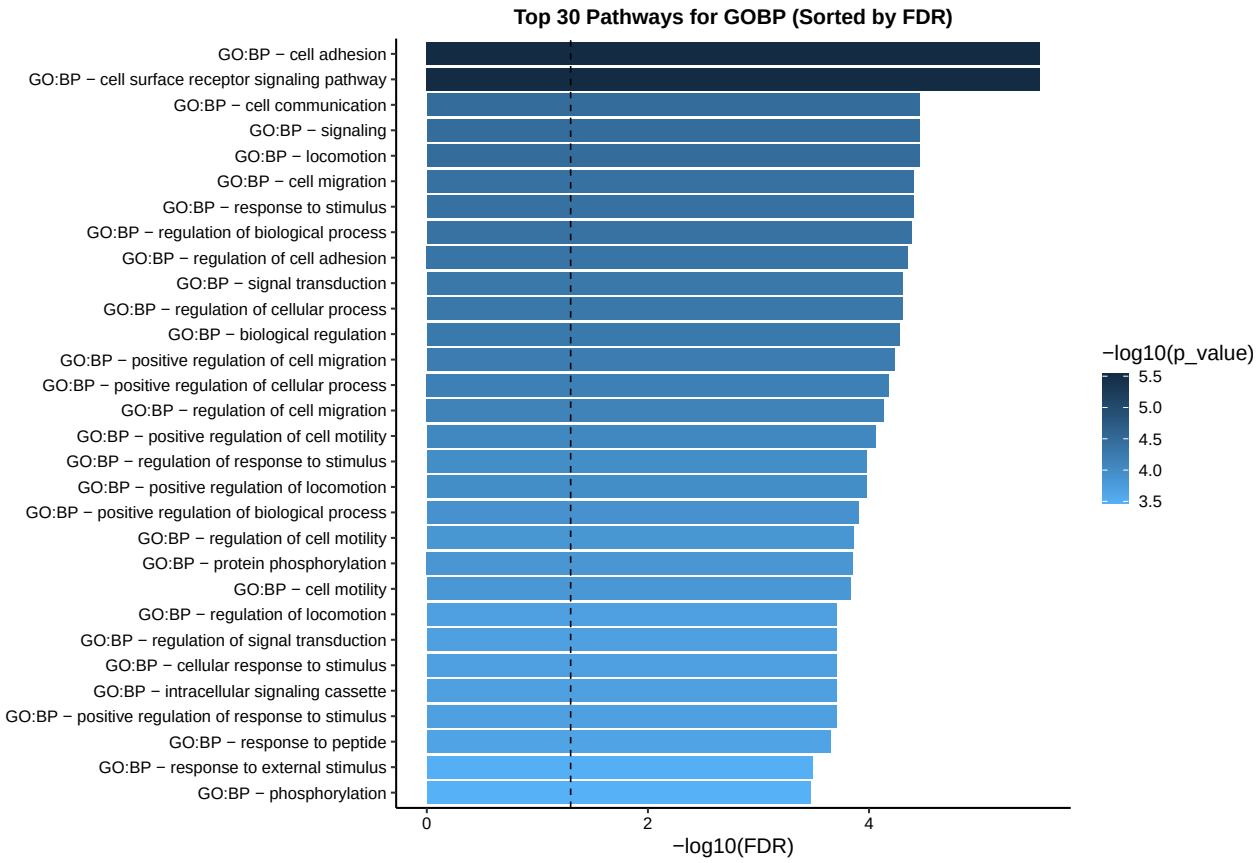



```
Enrichment_analysis(gene_list = down_gene_1,
                    result_folder = file.path(result_folder,"03-Enrichment"),
                    file_name = "01-DEG_1.0_down", gene_name_mapping, flag = "Down")
```

```
## [1] "Enrichment analysis for 01-DEG_1.0_down "
```

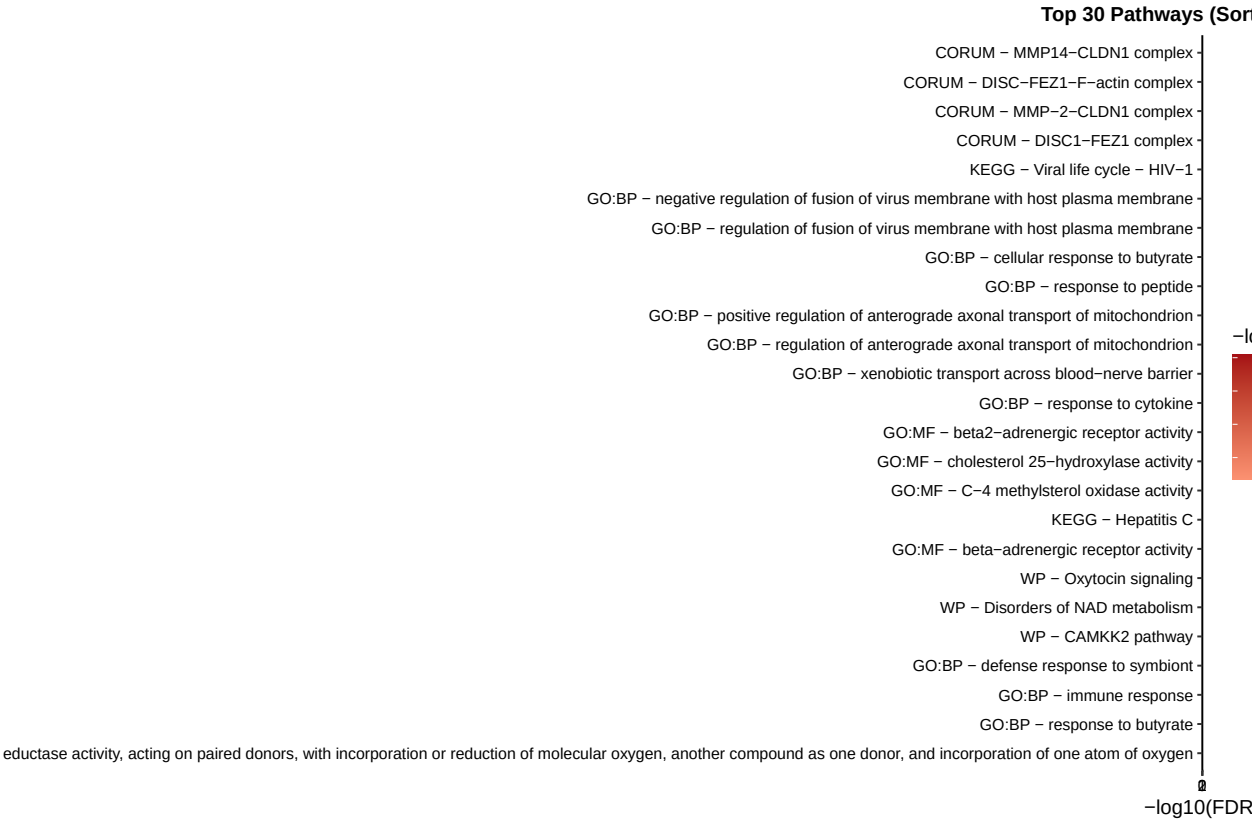



```
## [1] "Enrichment analysis for GOBP 01-DEG_1.0_down "
```

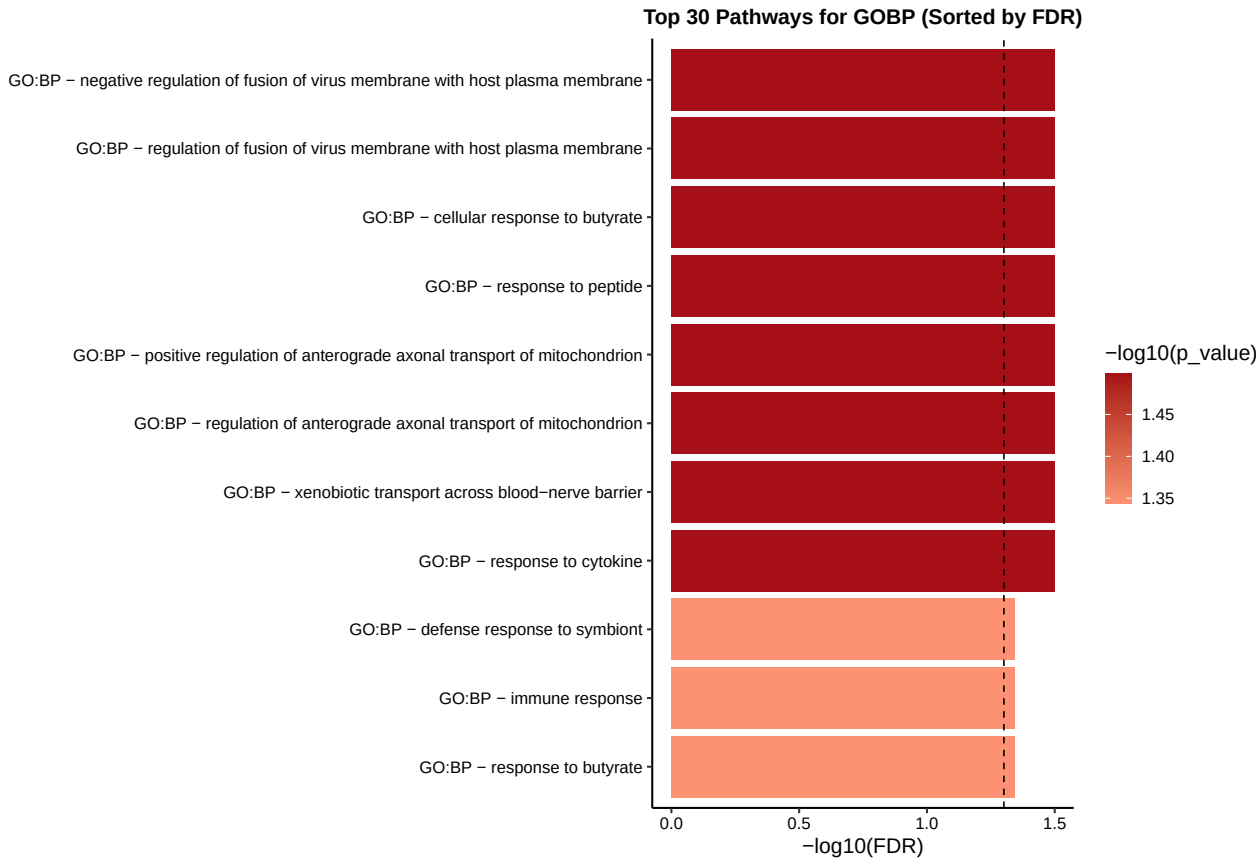



```r
deg1.5 <- result_df %>% filter(padj < 0.05 & abs(log2FoldChange) > 1.5)
up_gene_1.5 <- deg1.5 %>% filter(log2FoldChange > 0) %>% pull(GeneName)
down_gene_1.5 <- deg1.5 %>% filter(log2FoldChange < 0) %>% pull(GeneName)

# test for the function
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] "Enrichment analysis for 02-DEG_1.5_up "
```

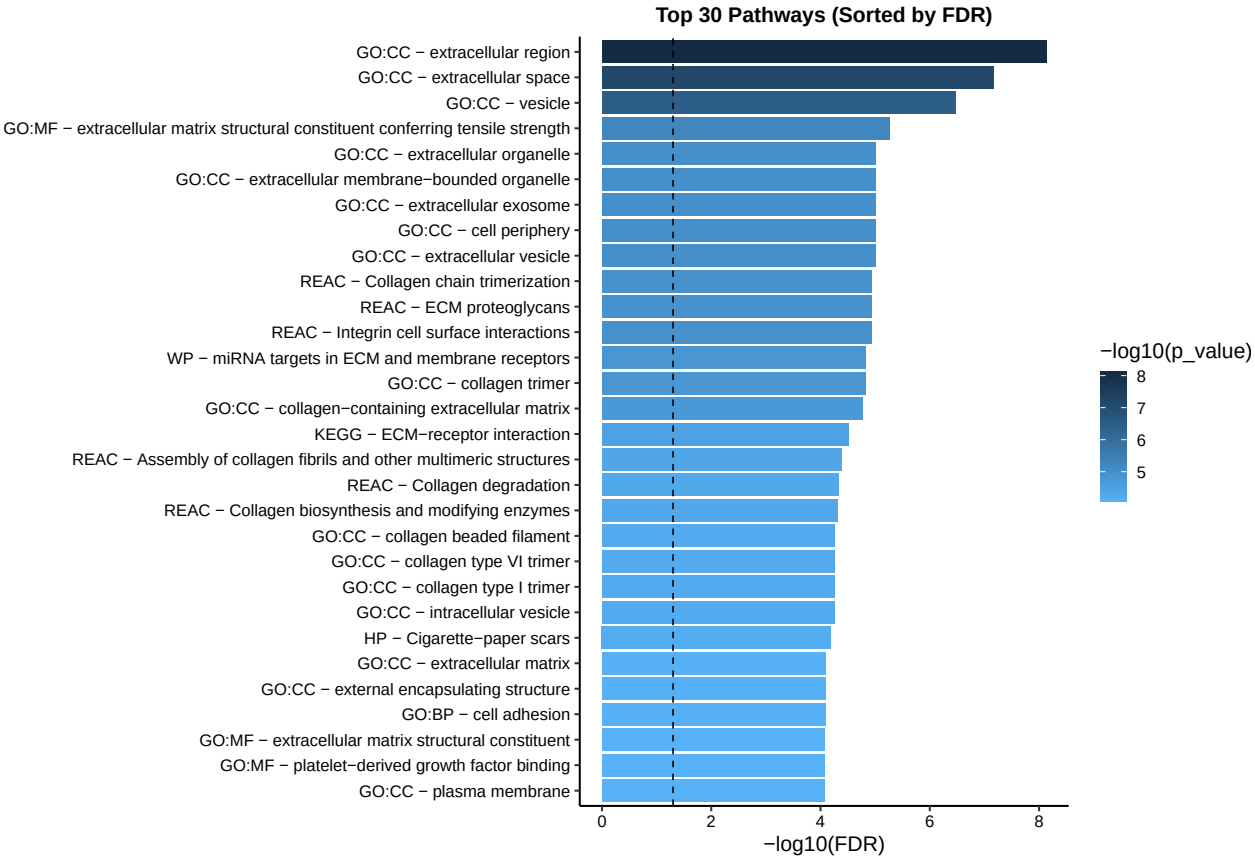



```
## [1] "Enrichment analysis for GOBP 02-DEG_1.5_up "
```

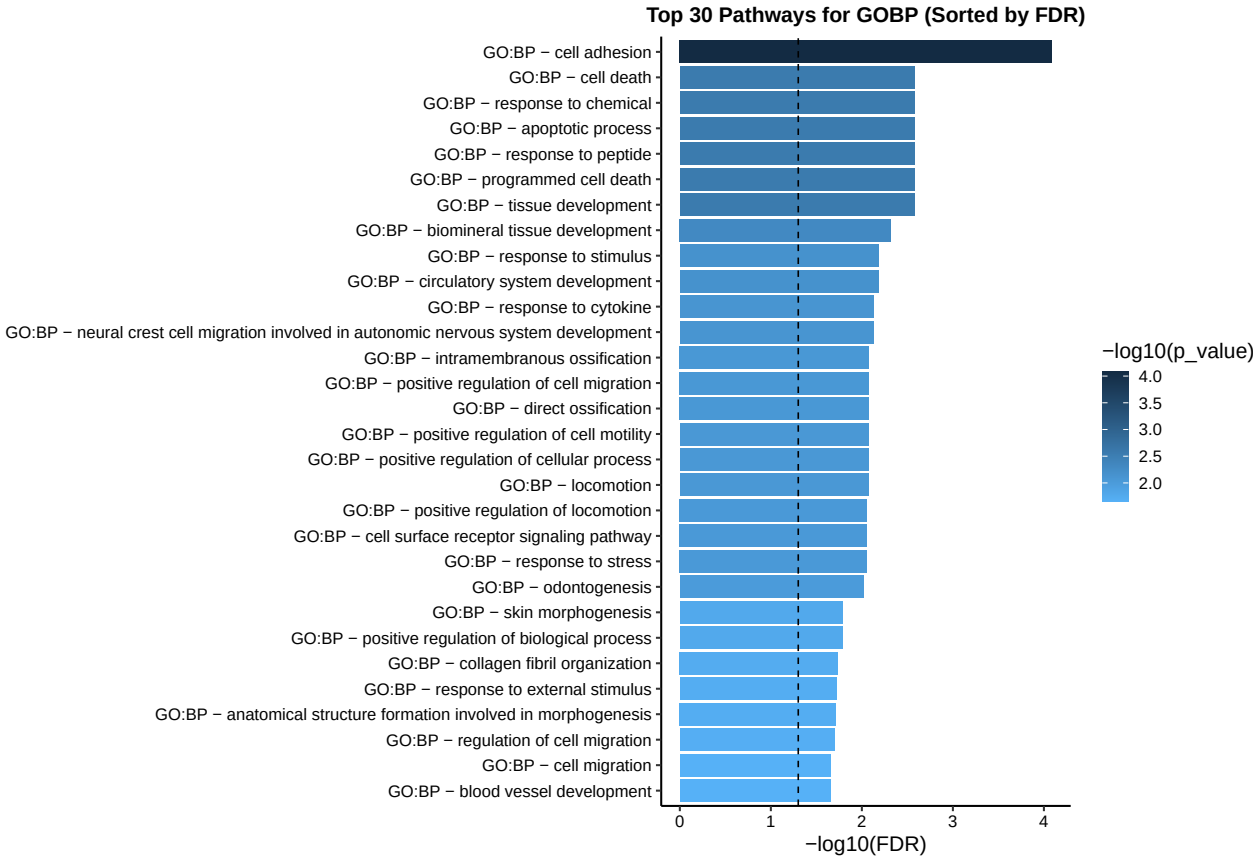



```
Enrichment_analysis(gene_list = down_gene_1.5,
                    result_folder = file.path(result_folder,"03-Enrichment"),
                    file_name = "02-DEG_1.5_down", gene_name_mapping, flag = "Down")
```

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

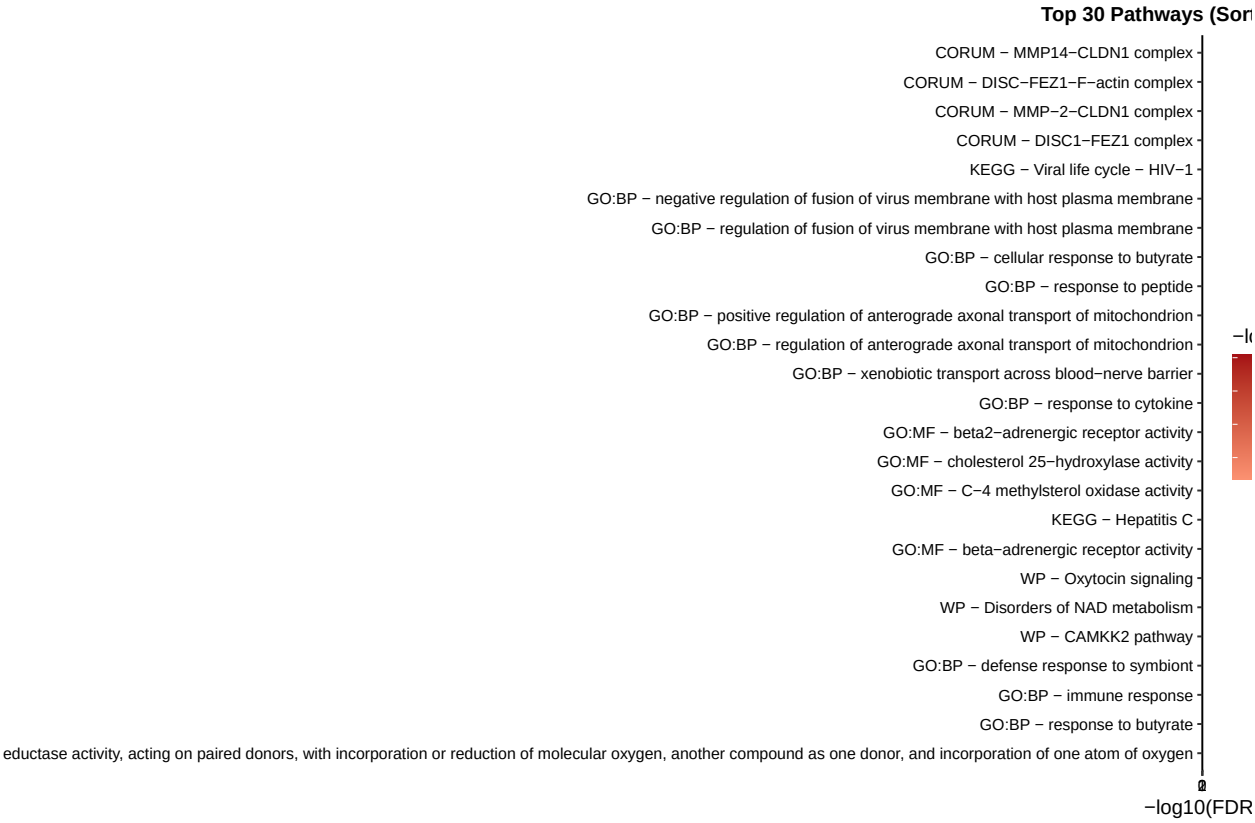



```
## [1] "Enrichment analysis for GOBP 02-DEG_1.5_down "
```

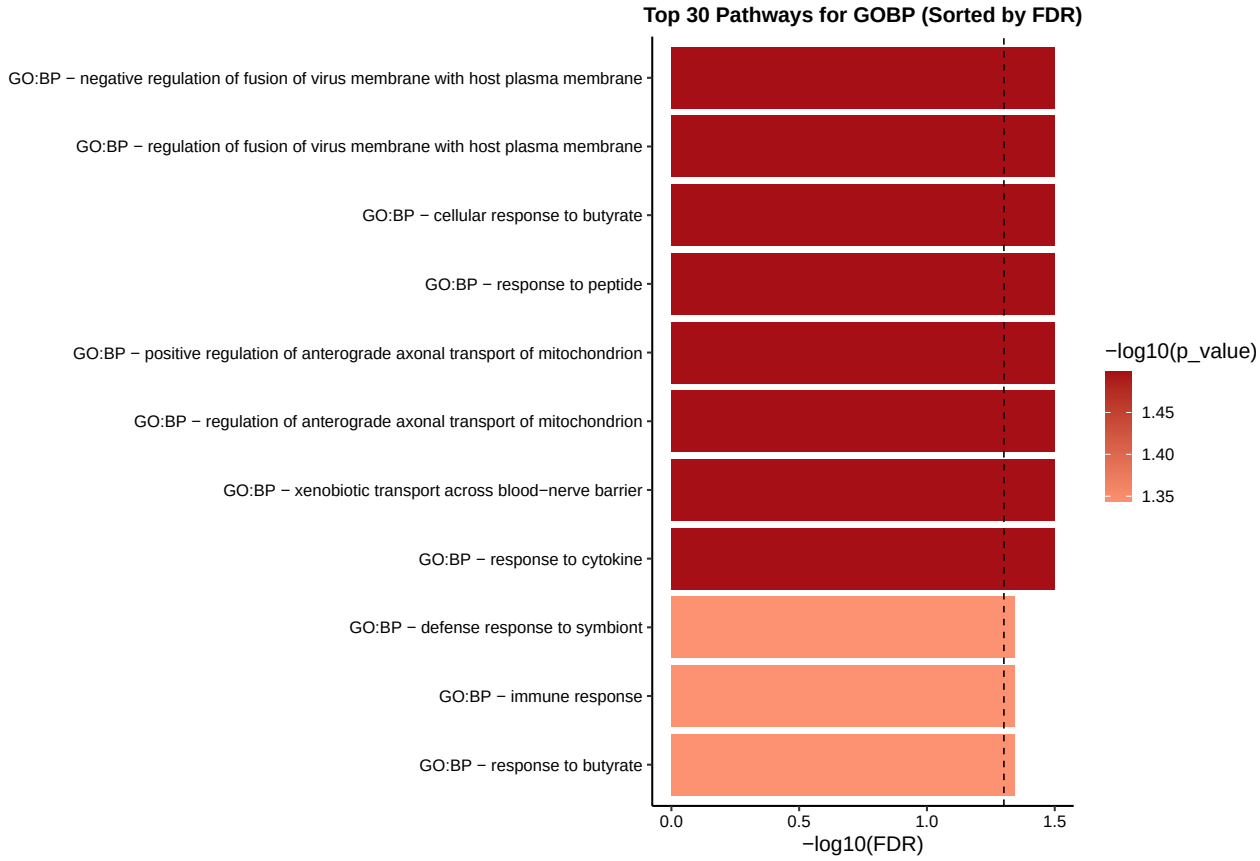



```
deg2 <- result_df %>% filter(padj < 0.05 & abs(log2FoldChange) > 1.5)
up_gene_2 <- deg2 %>% filter(log2FoldChange > 0) %>% pull(GeneName)
down_gene_2 <- deg2 %>% filter(log2FoldChange < 0) %>% pull(GeneName)
result_folder = result_folder_all
# test for the function
Enrichment_analysis(gene_list = up_gene_2,
                    result_folder = file.path(result_folder,"03-Enrichment"),
                    file_name = "03-DEG_2_up", gene_name_mapping, flag = "Up")
```

```
## [1] "Enrichment analysis for 03-DEG_2_up "
```

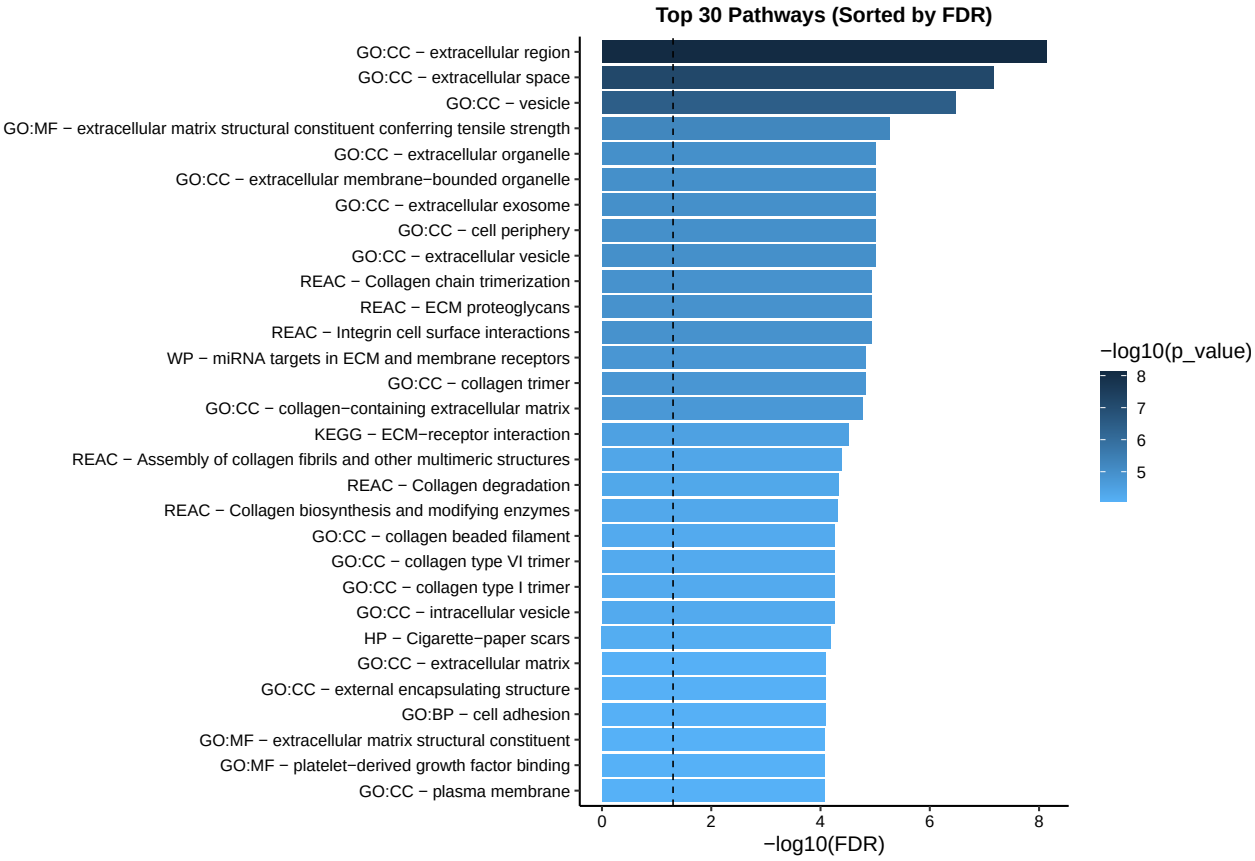



```
## [1] "Enrichment analysis for GOBP 03-DEG_2_up "
```

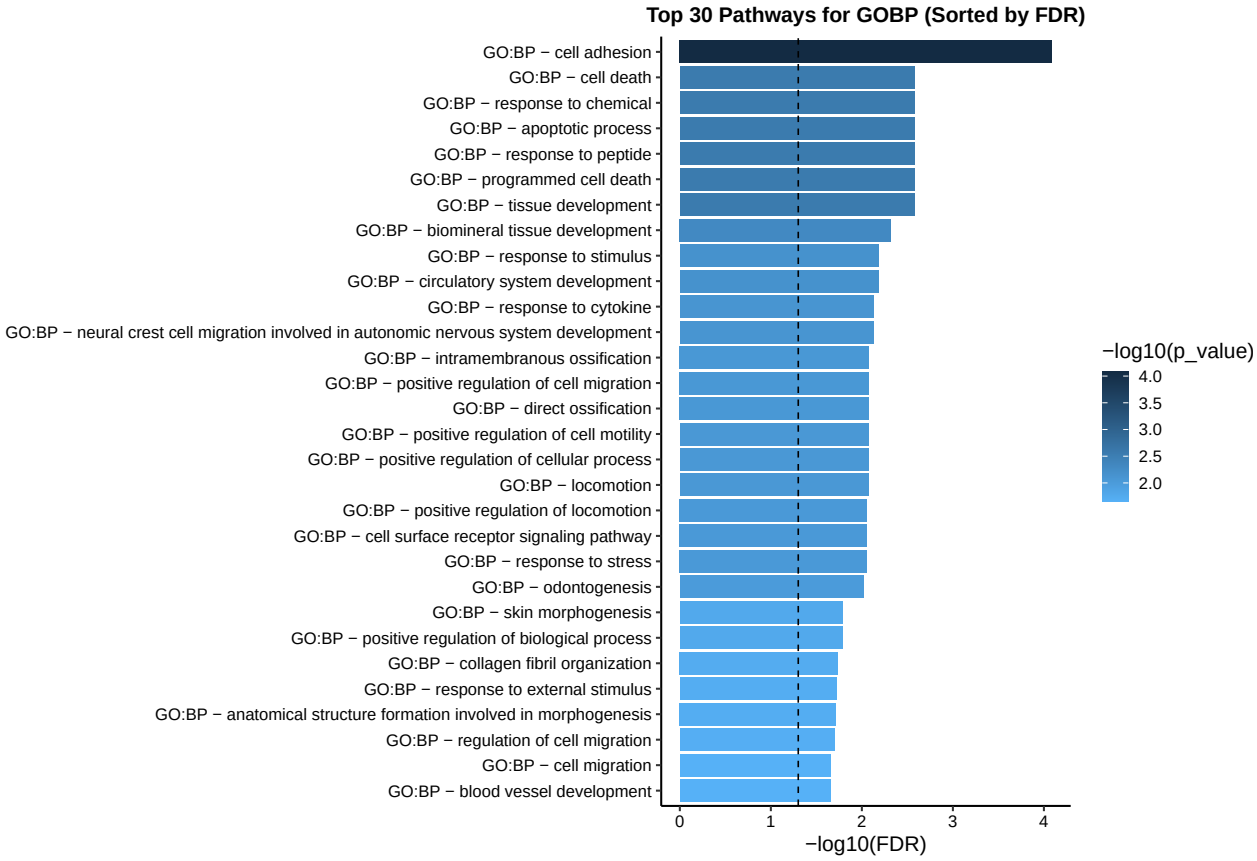



```
Enrichment_analysis(gene_list = down_gene_2,
                    result_folder = file.path(result_folder,"03-Enrichment"),
                    file_name = "03-DEG_2_down", gene_name_mapping, flag = "Down")
```

```
## [1] "Enrichment analysis for 03-DEG_2_down "
```




```
## [1] "Enrichment analysis for GOBP 03-DEG_2_down "
```


Top 30 Pathways for GOBP (Sorted by FDR)
GO:BP − cell adhesion
GO:BP − cell death
GO:BP − response to chemical
GO:BP − apoptotic process
GO:BP − response to peptide
GO:BP − programmed cell death
GO:BP − tissue development
GO:BP − biomineral tissue development
GO:BP − response to stimulus
GO:BP − circulatory system development
GO:BP − response to cytokine
GO:BP − neural crest cell migration involved in autonomic nervous system development
GO:BP − intramembranous ossification
GO:BP − positive regulation of cell migration
GO:BP − direct ossification
GO:BP − positive regulation of cell motility
GO:BP − positive regulation of cellular process
GO:BP − locomotion
GO:BP − positive regulation of locomotion
GO:BP − cell surface receptor signaling pathway
GO:BP − response to stress
GO:BP − odontogenesis
GO:BP − skin morphogenesis
GO:BP − positive regulation of biological process
GO:BP − collagen fibril organization
GO:BP − response to external stimulus
GO:BP − anatomical structure formation involved in morphogenesis
GO:BP − regulation of cell migration
GO:BP − cell migration
GO:BP − blood vessel development
0
1
2
3
4
−log10(FDR)
−log10(p_value)
4.0
3.5
3.0
2.5
2.0

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
```