Differential Expression Analysis for bulk RNA-seq data

Vehicle contition: 22q vs Control

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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 `results/02-DEG-Vehicle/synaptosomes_bulkRNA_counts`

```
input_count <- read.csv(here::here("data", "synaptosomes_bulkRNA",
                                   "synaptosomes_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", "synaptosomes_bulkRNA",
                                   "sample_info_22q.csv")) %>%
                    mutate(condition = paste0(Diagosis, "_", Treatment))


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

# 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)]

# load the 22q gene_list
target_22q_gene <- read.csv(here::here("data","ref" ,"22q_gene_2024_10_17.csv"))
target_gene <- target_22q_gene$gene
target_gene <- target_gene[1:(length(target_gene) - 4)]

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-Vehicle/DESeq2_results.csv`.

```
# Init the result folder structure for the result
result_folder = file.path("results", "02-DEG-Vehicle")
Result_folder_structure(result_folder)

# load the comparison group information
reference_group <- "CTRL_Vehicle"
compare_group <- "22q_Vehicle"
```

```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")
```

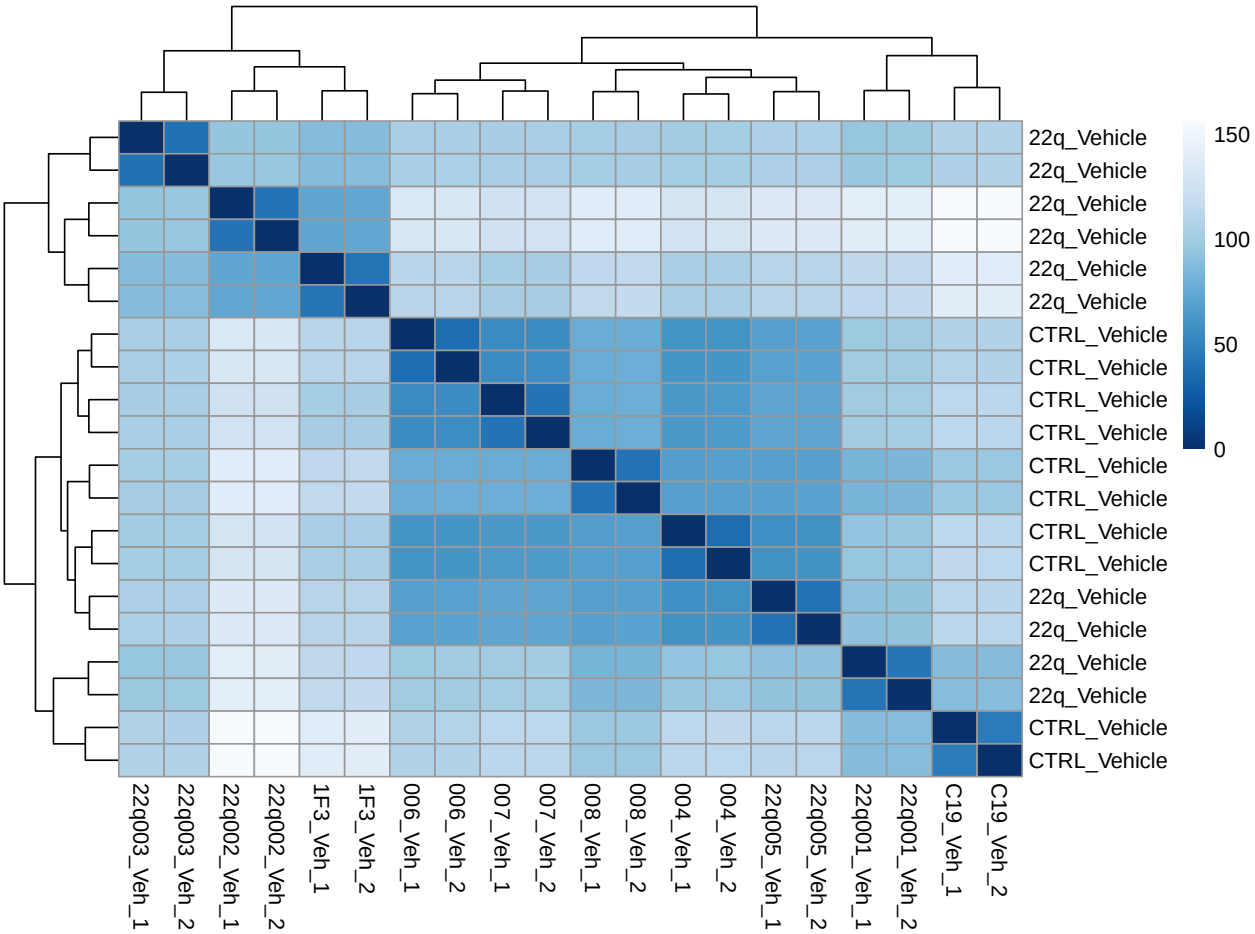
```
## [1] "DEG analysis is done"
```

```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

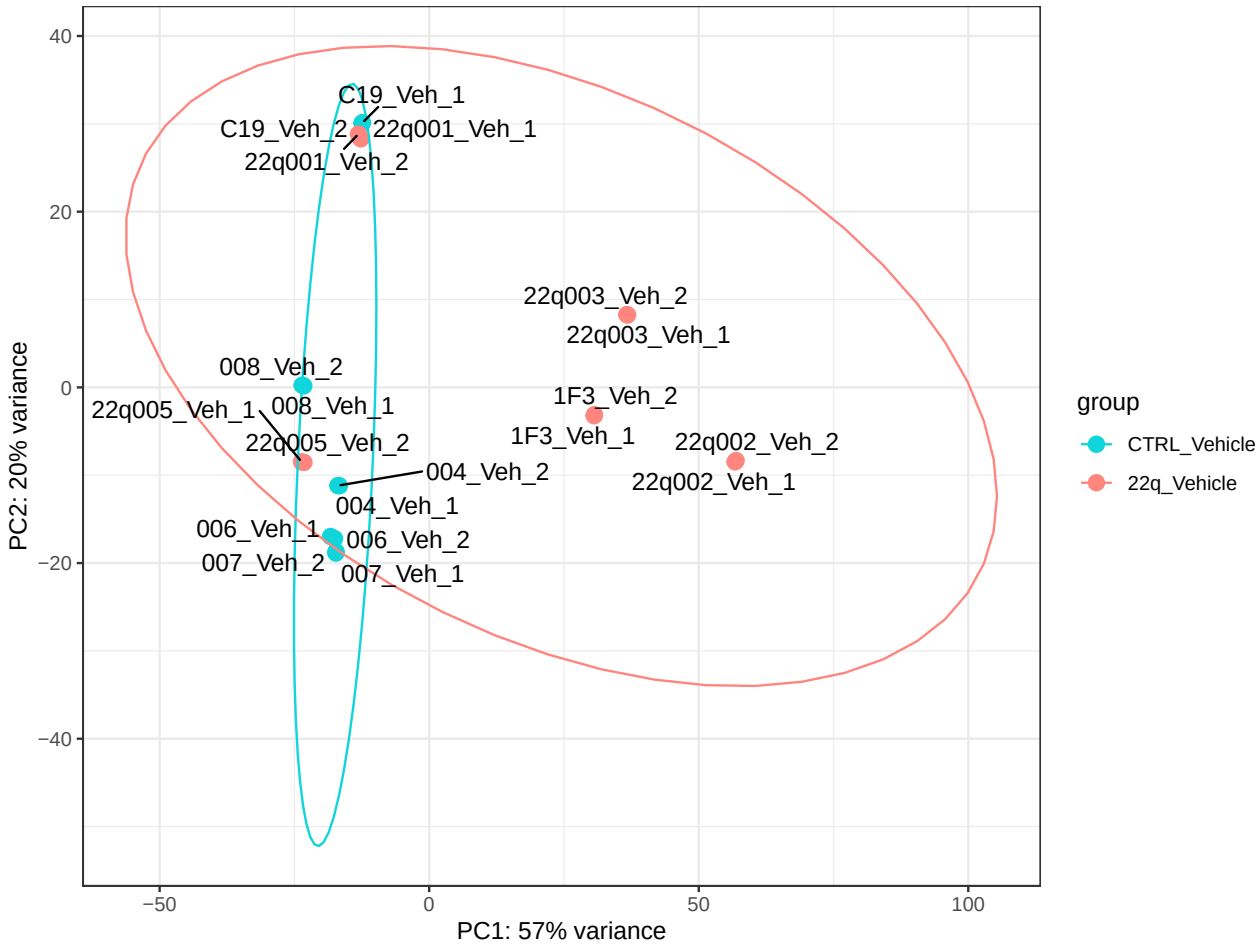
```
# Plot the sample distance
plot_sample_heatmap(dds_obj, figure_folder =
                    file.path(result_folder,"01-Sample_info"),
                    file_name = "01_sample_distance_heatmap")
```




```
## [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)
```

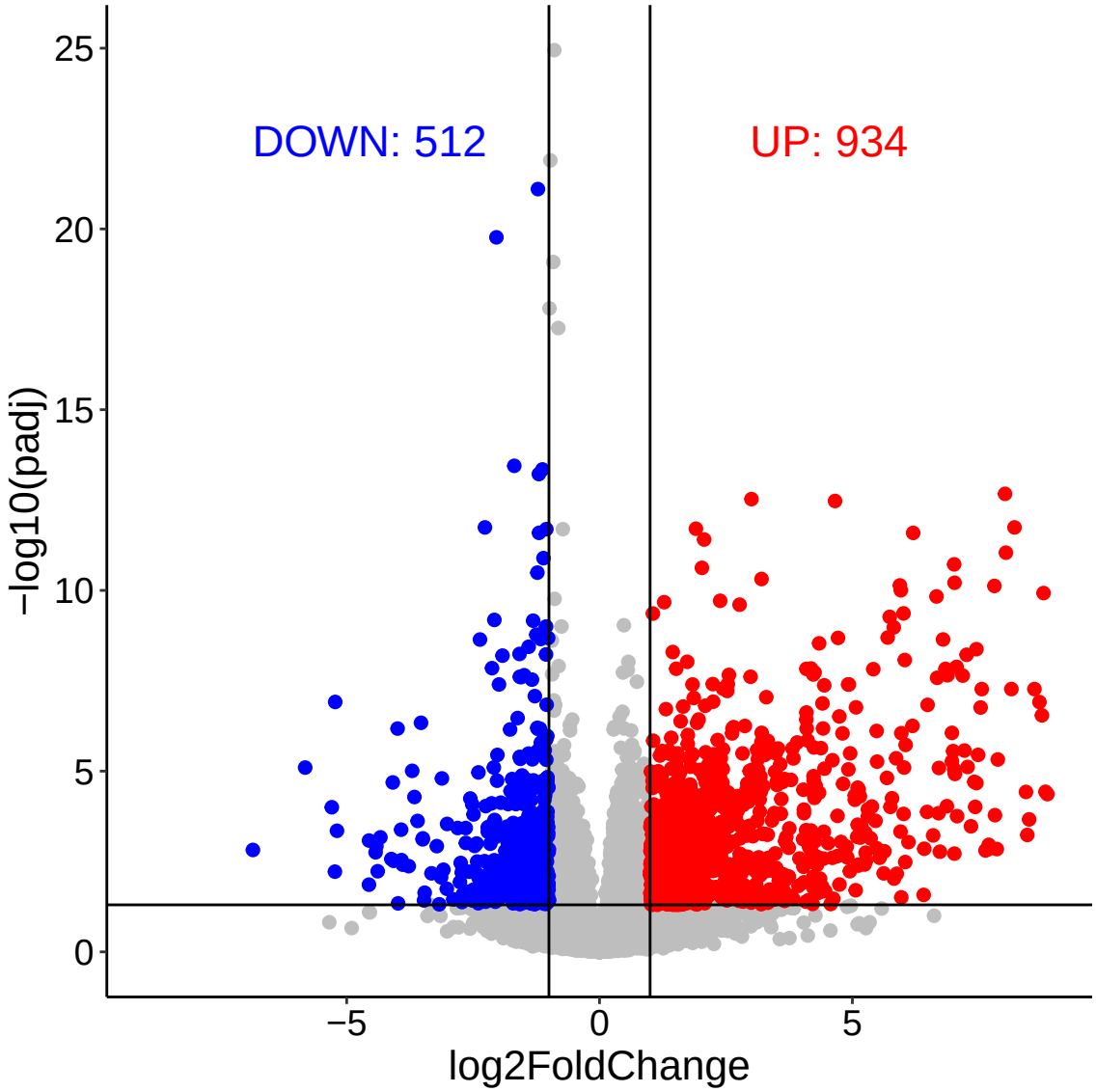
```
## [1] "PCA plot is done"
```


C19_Veh_1
C19_Veh_2
22q001_Veh_1
22q001_Veh_2
22q003_Veh_2
22q003_Veh_1
008_Veh_2
008_Veh_1
22q005_Veh_1
22q005_Veh_2
1F3_Veh_2
1F3_Veh_1
22q002_Veh_2
22q002_Veh_1
004_Veh_2
004_Veh_1
006_Veh_1
006_Veh_2
007_Veh_2
007_Veh_1
PC2: 20% variance
PC1: 57% variance
40
20
0
−20
−40
−50
0
50
100
group
CTRL_Vehicle
22q_Vehicle

(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,
                  figure_folder = file.path(result_folder,"02-DEG"),
                  file_name = "02_volcano_plot_log2fc_1",
                  thread = 1 , dot_size =2, label_gene = NULL)
```

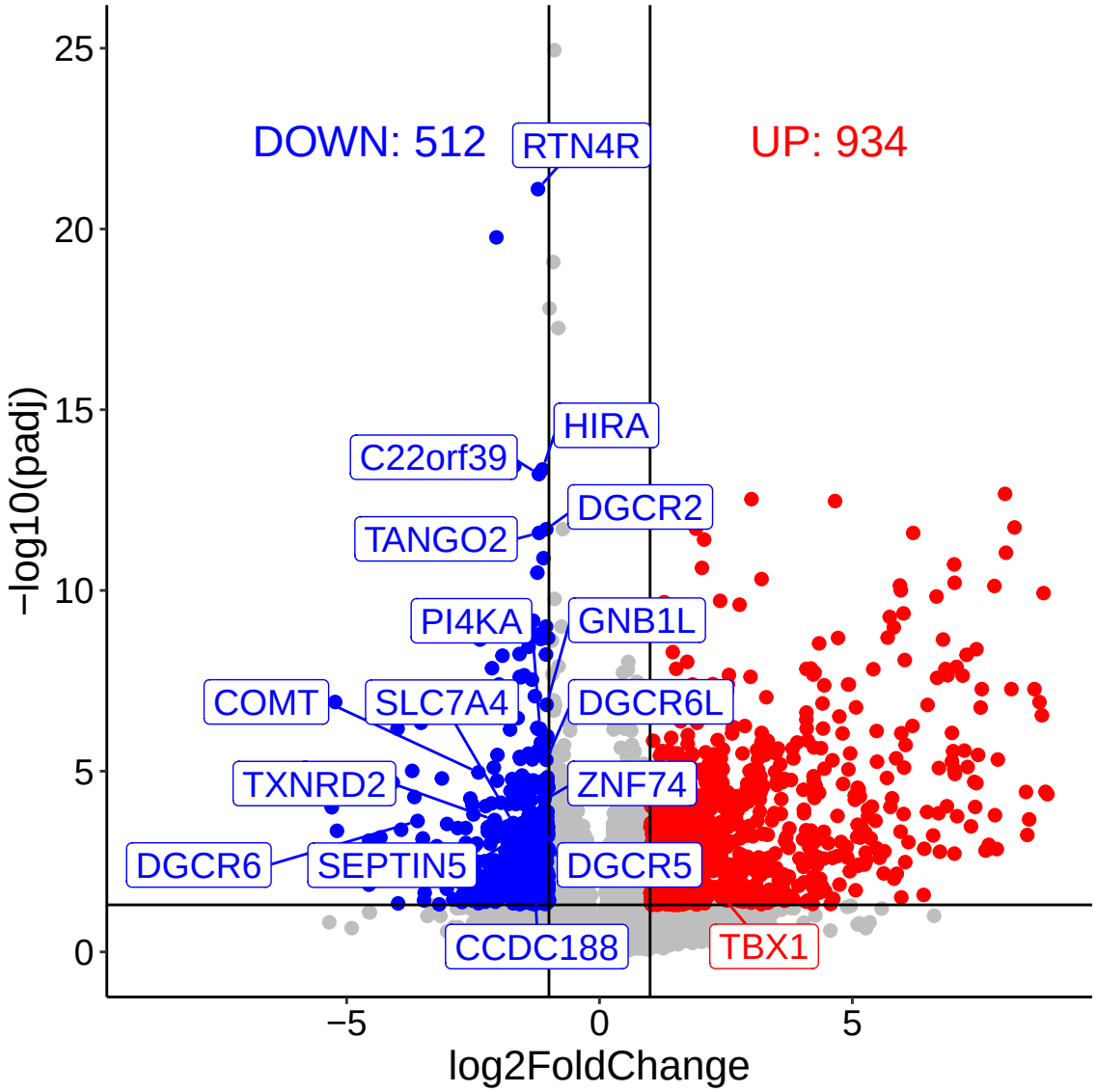
```
## [1] "Volcano plot for 02_volcano_plot_log2fc_1"
```




```
plot_volcano_plot(result_df,
```

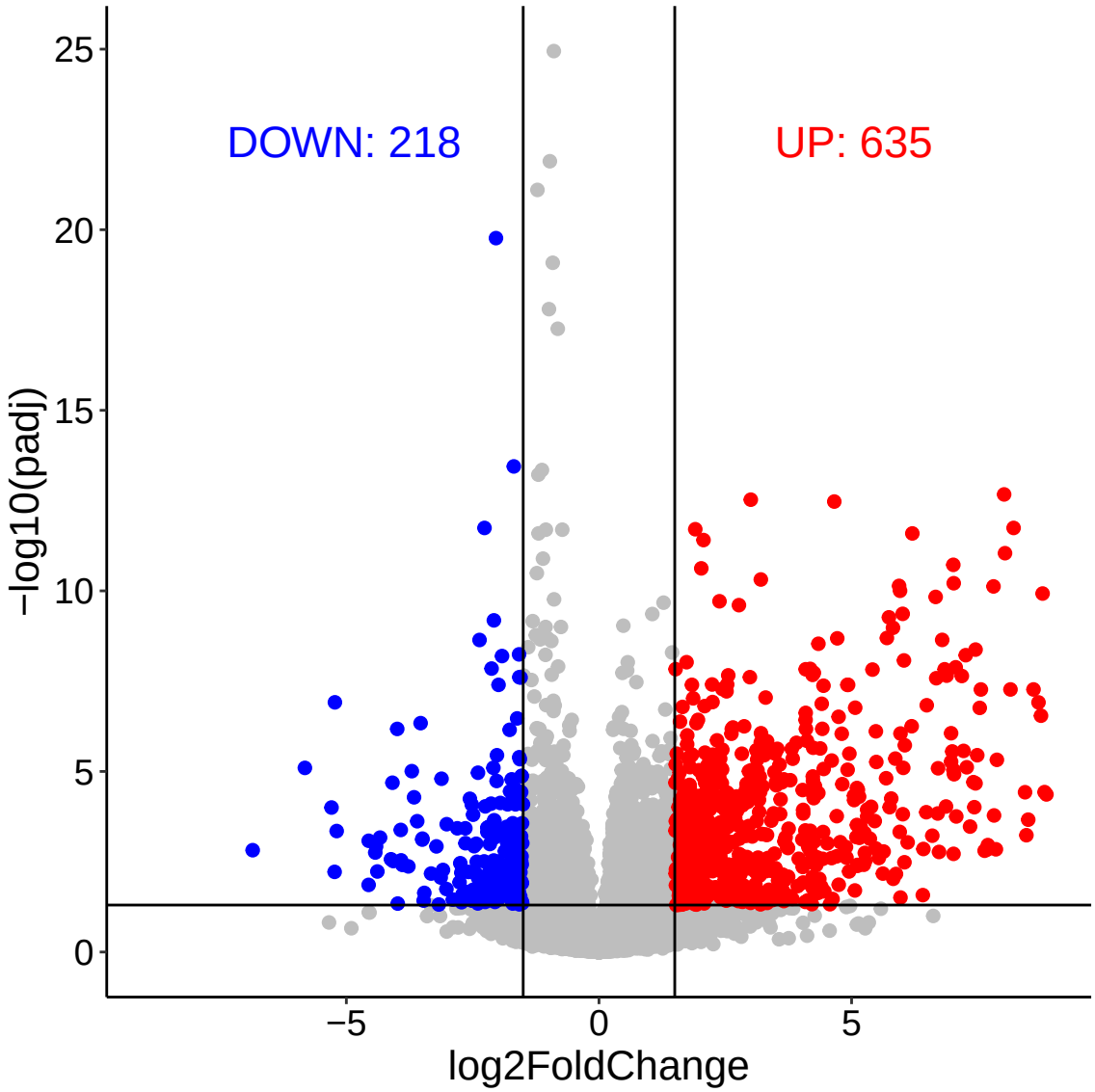
```
                  figure_folder = file.path(result_folder,"02-DEG"),
                  file_name = "02_volcano_plot_log2fc_1_with_22q_gene",
                  thread = 1 , dot_size =2, label_gene = target_gene)
```

```
## [1] "Volcano plot for 02_volcano_plot_log2fc_1_with_22q_gene"
```



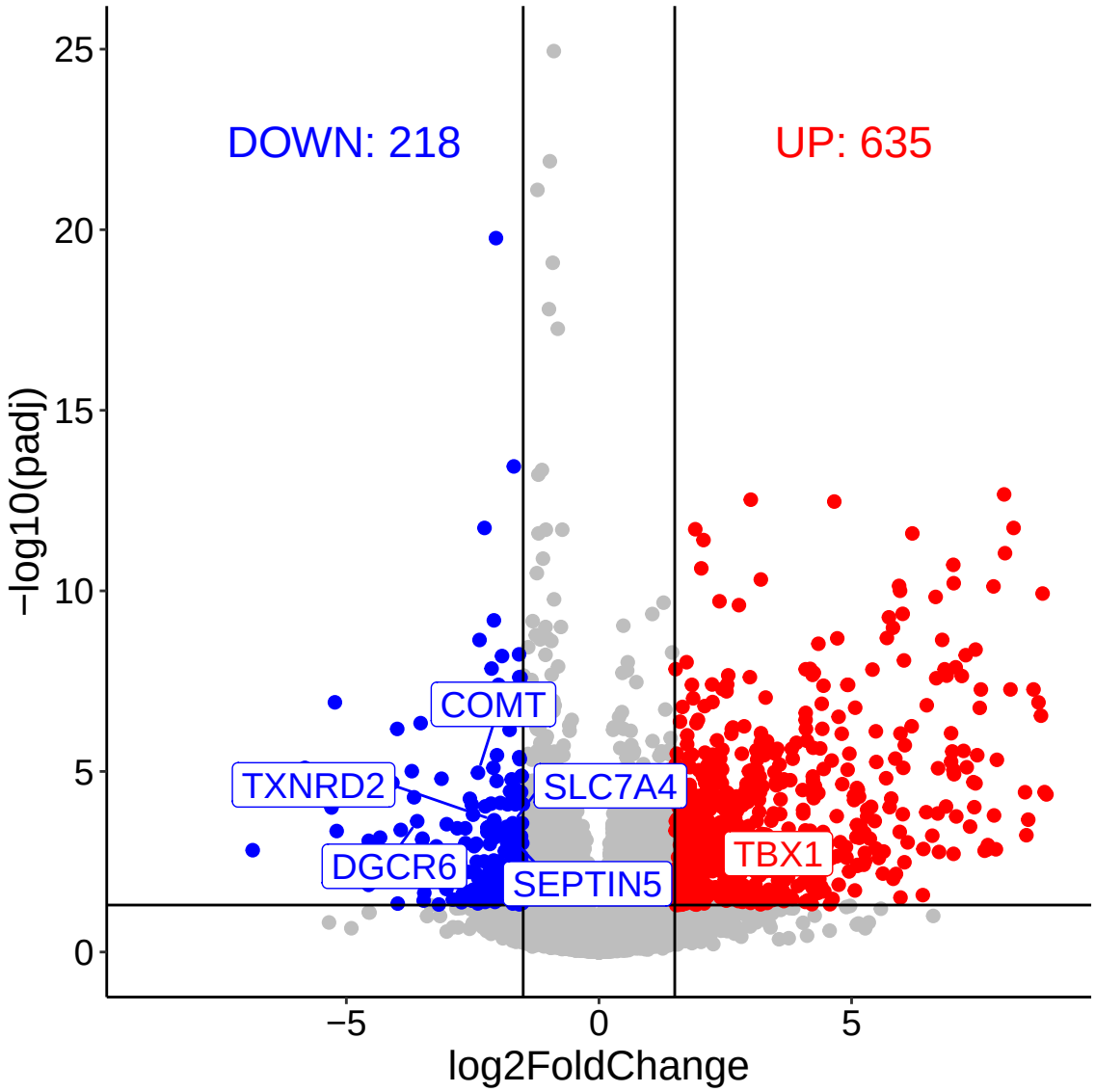

```
plot_volcano_plot(result_df,
                  figure_folder = file.path(result_folder,"02-DEG"),
                  file_name = "03_volcano_plot_log2fc_1.5",
                  thread = 1.5 ,  dot_size =2,label_gene = NULL)
```

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



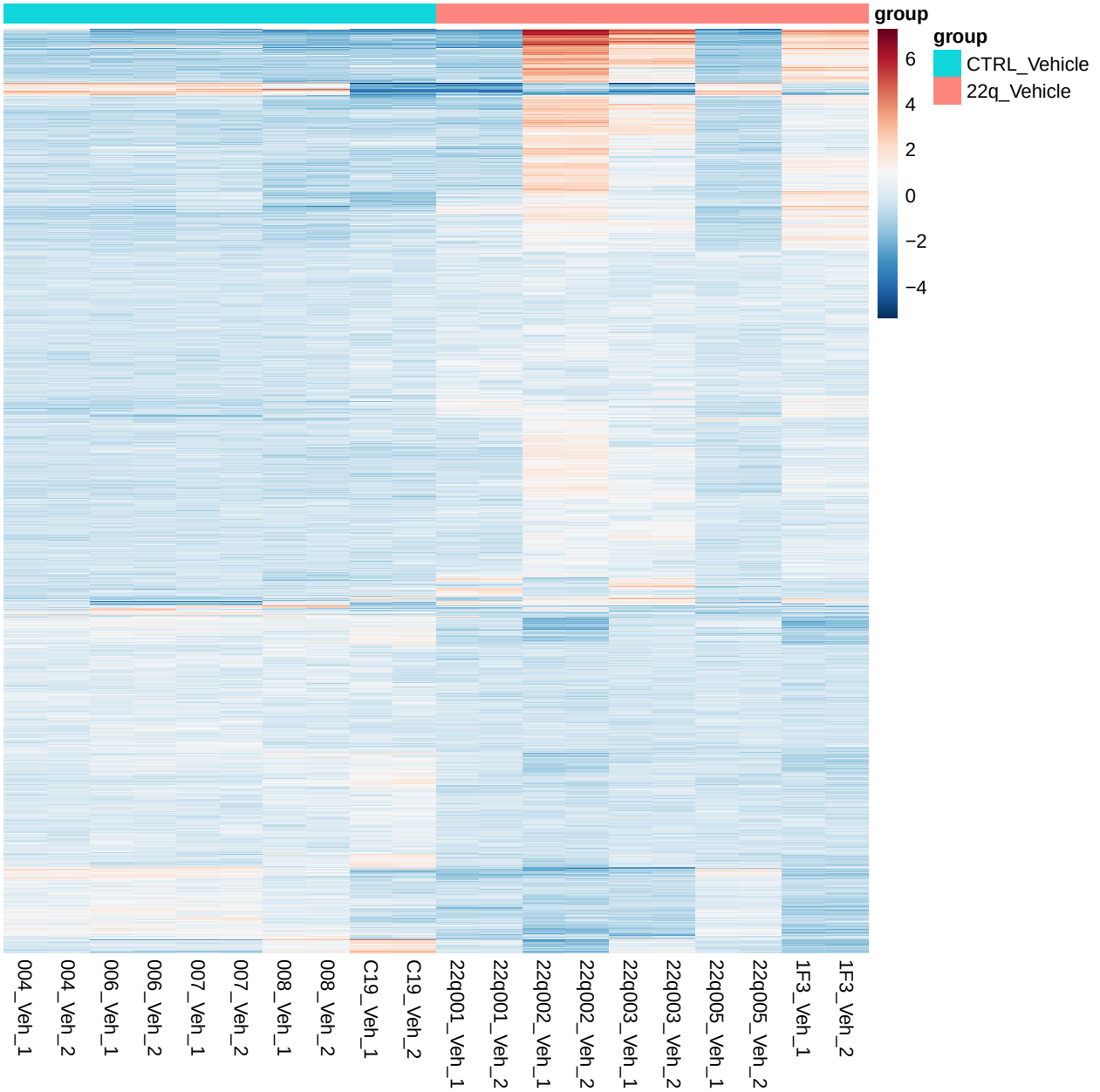

```
plot_volcano_plot(result_df,
                  figure_folder = file.path(result_folder,"02-DEG"),
                  file_name = "03_volcano_plot_log2fc_1.5_with_22q_gene",
                  thread = 1.5 , dot_size =2, label_gene = target_gene)
```

```
## [1] "Volcano plot for 03_volcano_plot_log2fc_1.5_with_22q_gene"
```



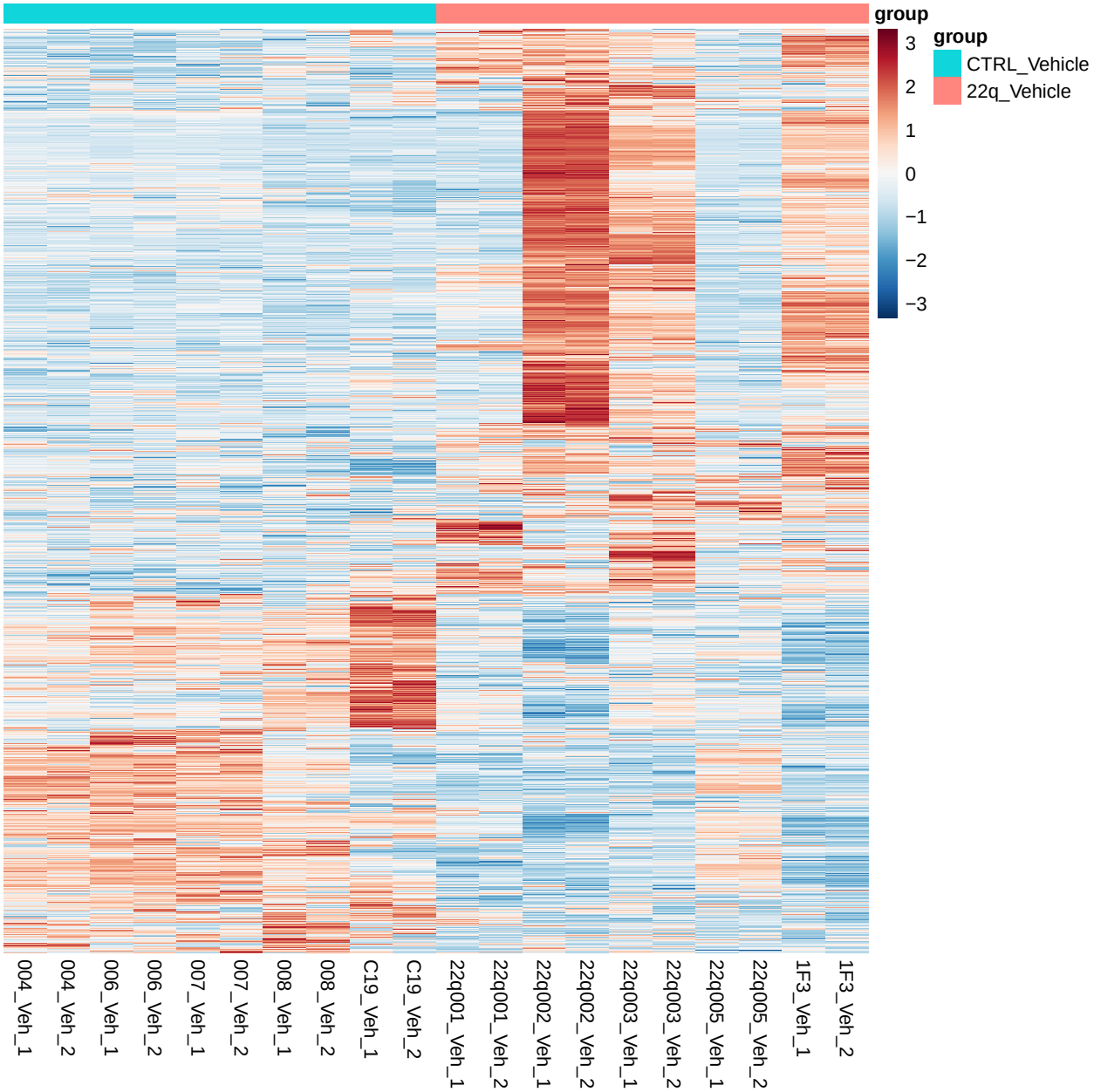

```
# Plot the heatmap for the DEG
vsd_obj <- varianceStabilizingTransformation(dds_obj, blind = TRUE)

DEG_gene_1 <- result_df %>% filter(abs(log2FoldChange) > 1) %>%  pull(GeneName)
DEG_gene_1.5 <- result_df %>% filter(abs(log2FoldChange) > 1.5) %>% 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 "
```



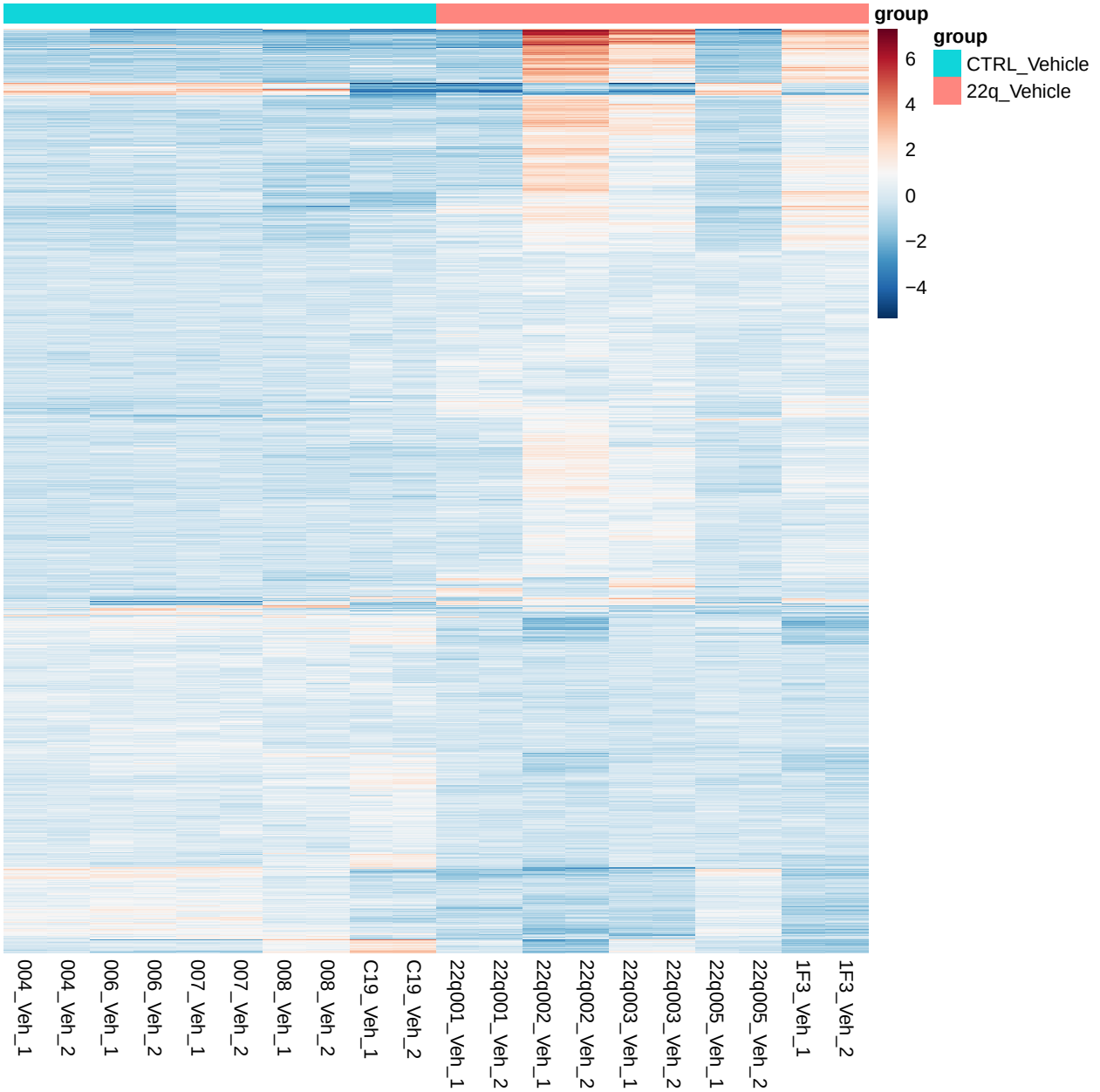

```
plot_gene_heatmap(vsd_obj, gene_list = DEG_gene_1,
                  figure_folder = file.path(result_folder,"02-DEG"),
                  file_name = "02_heatmap_log2fc_1_row",
                  reference_group, compare_group,
                  cluster_rows = TRUE, cluster_cols = FALSE,
        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

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



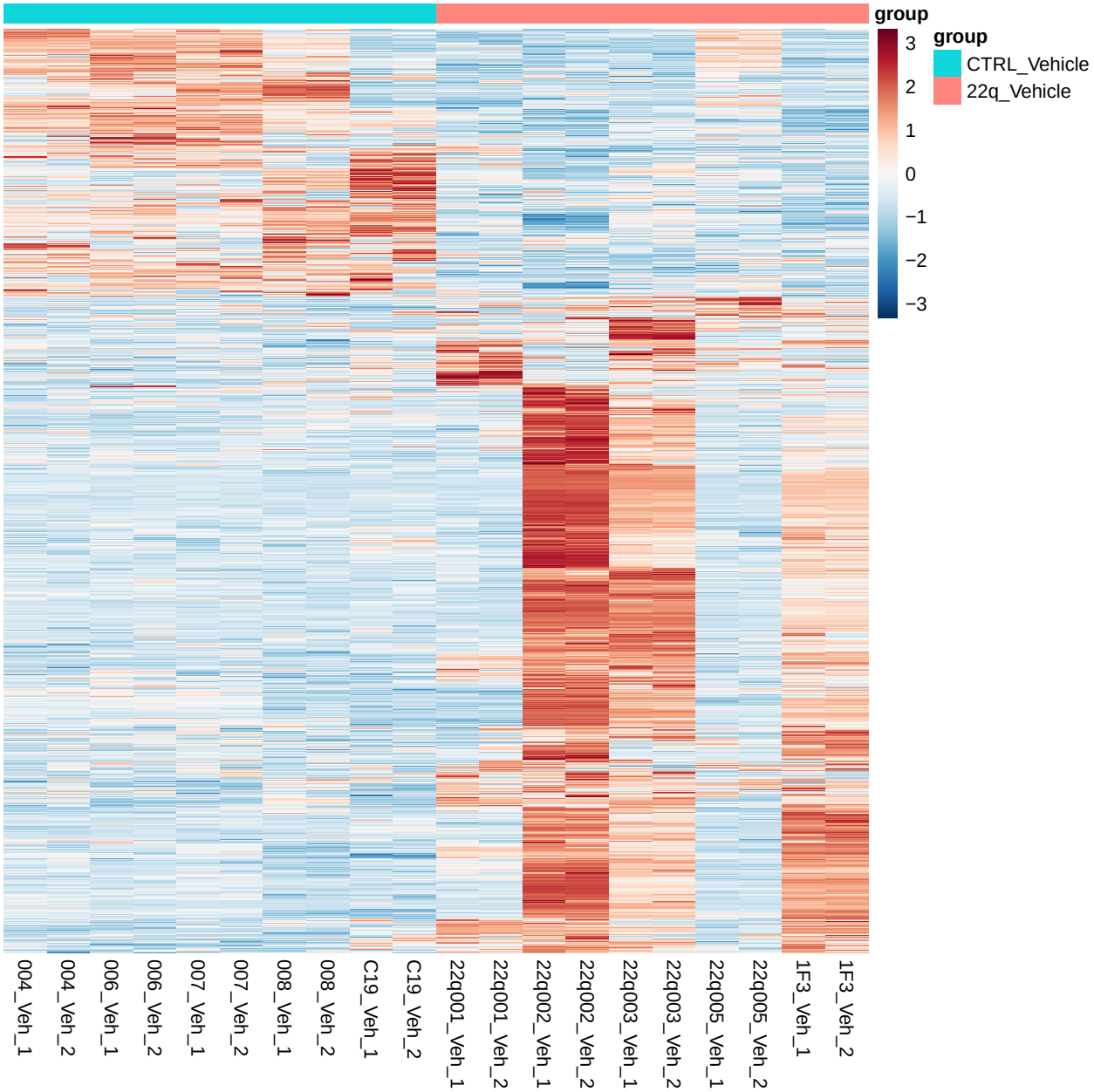

```
plot_gene_heatmap(vsd_obj, gene_list = DEG_gene_1,
                  figure_folder = file.path(result_folder,"02-DEG"),
                  file_name = "02_heatmap_log2fc_1",
                  reference_group, compare_group,
                  cluster_rows = TRUE, cluster_cols = FALSE,
            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

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



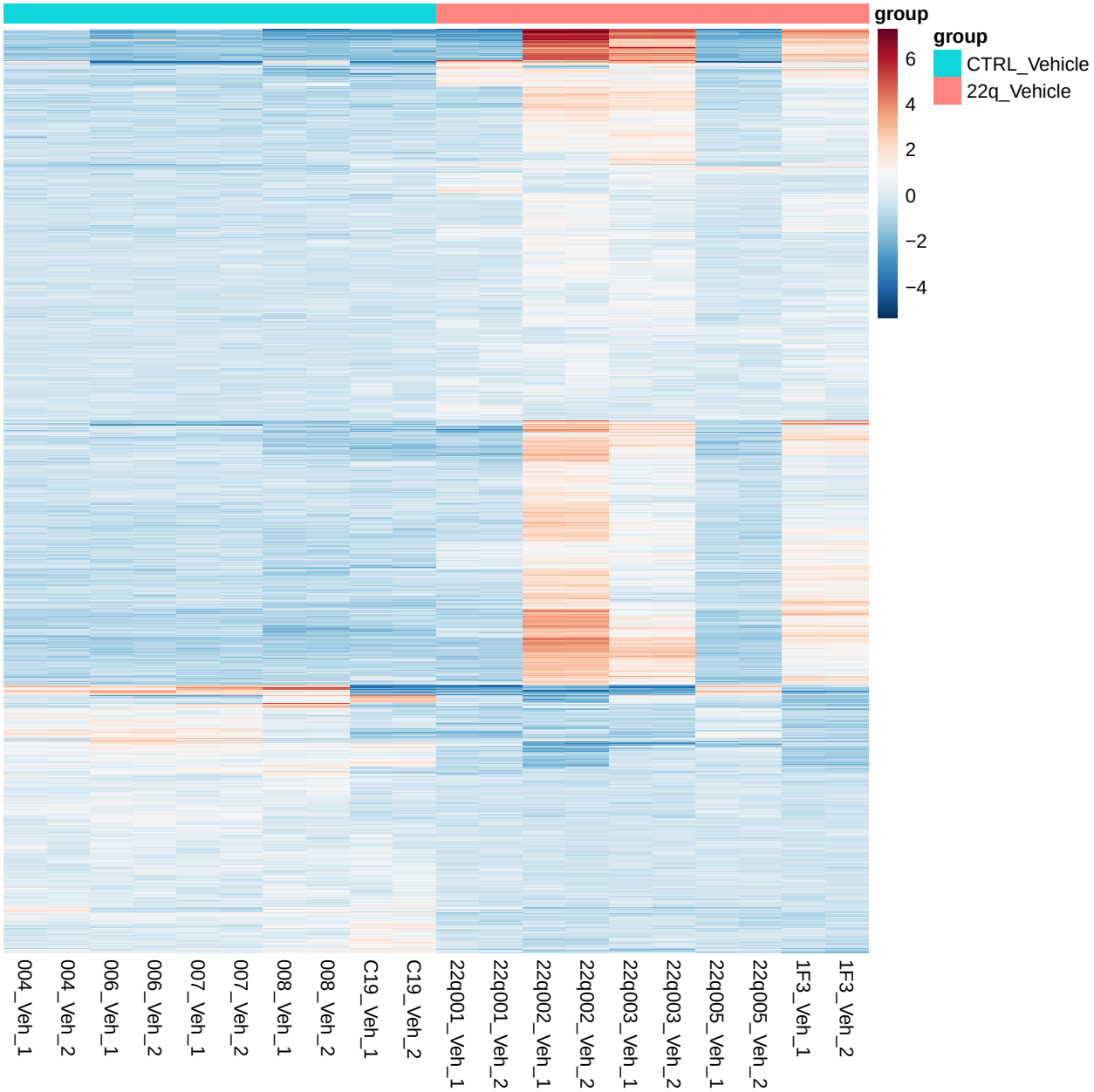

```
plot_gene_heatmap(vsd_obj, gene_list = DEG_gene_1.5,
                  figure_folder = file.path(result_folder,"02-DEG"),
                  file_name = "03_heatmap_log2fc_1.5_row",
                  reference_group, compare_group,
                  cluster_rows = TRUE, cluster_cols = FALSE,
    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

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




```
plot_gene_heatmap(vsd_obj, gene_list = DEG_gene_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")
```

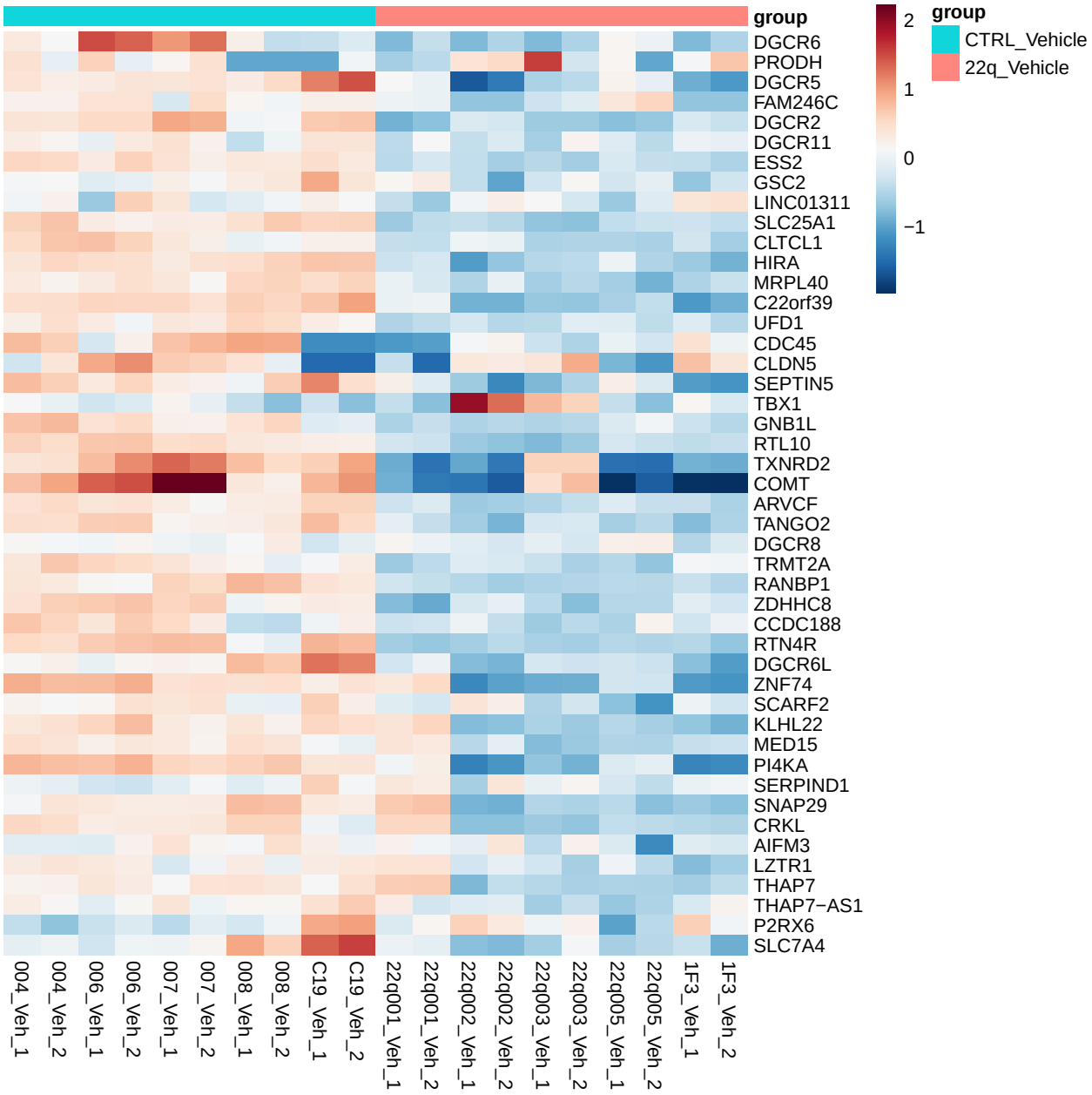
```
## [1] "Heatmap for 03_heatmap_log2fc_1.5 "
```


group
group
CTRL_Vehicle
22q_Vehicle
6
4
2
0
−2
−4
004_Veh_1
004_Veh_2
006_Veh_1
006_Veh_2
007_Veh_1
007_Veh_2
008_Veh_1
008_Veh_2
C19_Veh_1
C19_Veh_2
22q001_Veh_1
22q001_Veh_2
22q002_Veh_1
22q002_Veh_2
22q003_Veh_1
22q003_Veh_2
22q005_Veh_1
22q005_Veh_2
1F3_Veh_1
1F3_Veh_2

(3) 22q gene visualization - Heatmap

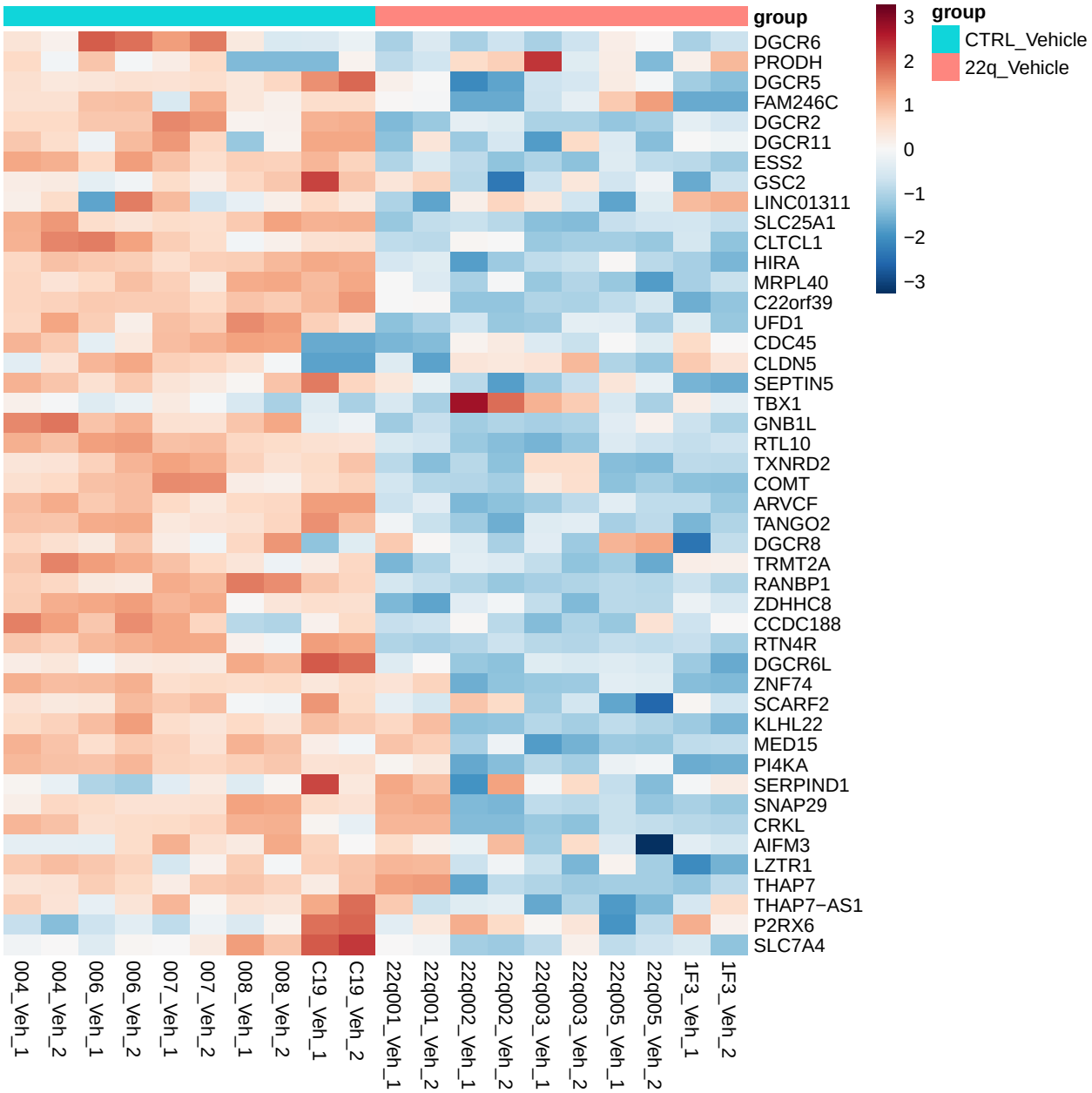
```
# Plot the heatmap for the 22q gene

target_gene <- intersect(target_gene, rownames(vsd_obj))

plot_gene_heatmap(vsd_obj, gene_list = target_gene ,
                  figure_folder = file.path(result_folder,"05-22q_Gene"),
                  file_name = "01_heatmap_22q_gene",
                  reference_group, compare_group,
                  cluster_rows = FALSE, cluster_cols = FALSE,
                  scale = "none", show_rownames =TRUE)
```

```
## [1] "Heatmap for 01_heatmap_22q_gene "
```



```
plot_gene_heatmap(vsd_obj, gene_list = target_gene ,
                  figure_folder = file.path(result_folder,"05-22q_Gene"),
                  file_name = "01_heatmap_22q_gene_row",
                  reference_group, compare_group,
                  cluster_rows = FALSE, cluster_cols = FALSE,
                  scale = "row", show_rownames =TRUE)
```

```
## [1] "Heatmap for 01_heatmap_22q_gene_row "
```



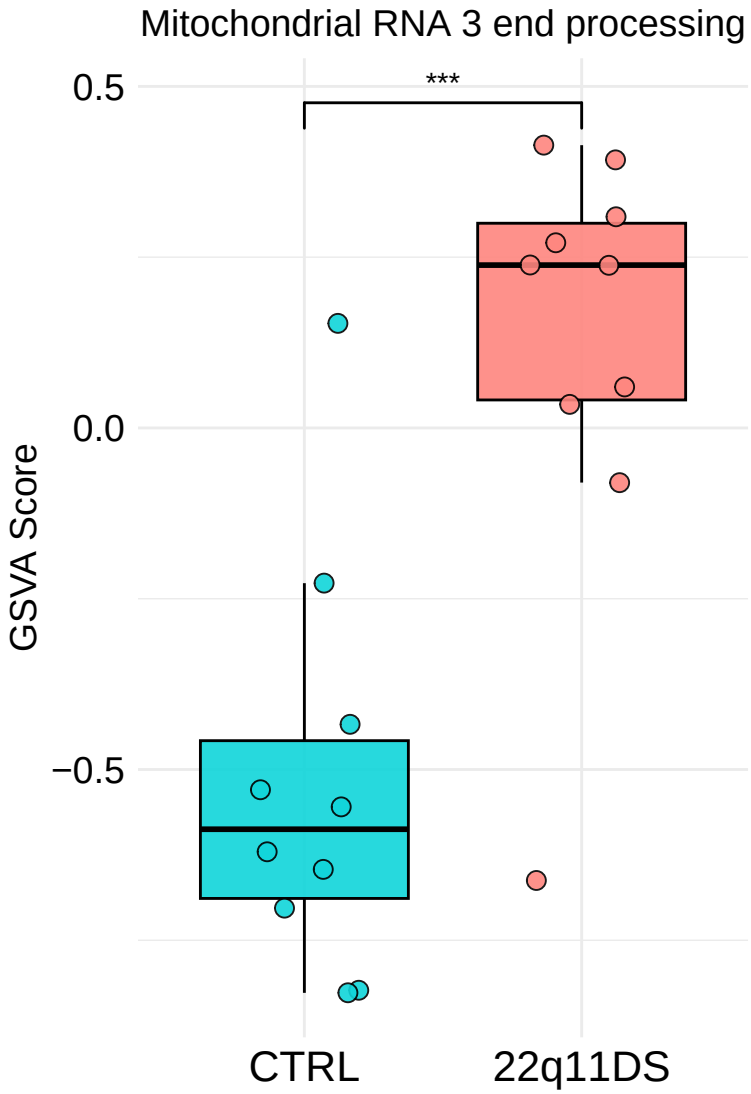
4. GSVA analysis

```
#
# 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)
# 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) <- gsub("X", "", colnames(gsva_matrix))
condition_list_label <- condition_list  %>%
                        filter(group %in% c("CTRL_Vehicle","22q_Vehicle")) %>%
  mutate(group = recode(group,
                        "CTRL_Vehicle" = "CTRL",
                        "22q_Vehicle" = "22q11DS"))

reference_group = "CTRL"
compare_group = "22q11DS"

# 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, compare_group)
#
# }


# plot for the focus pathway
for (i in 1:3){
  pathway_name <- pathway_list$pathway[i]
```

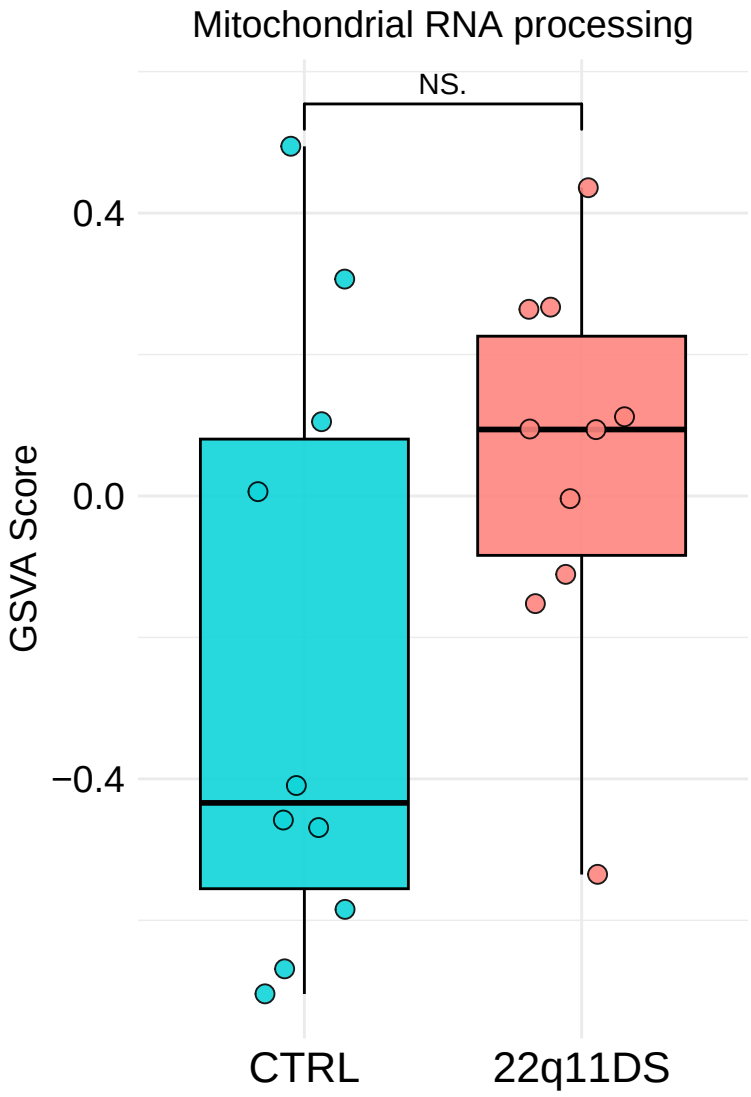
```
  print(pathway_name)
  p<-plot_gsva_boxplot(gsva_matrix,
                       condition_list_label =condition_list_label,
                       pathway_name =  pathway_name,
                       figure_folder = file.path(result_folder,"04-GSVA","Boxplot"),
                       file_name = paste0("GSVA_", pathway_name),
                       fig.height = 6, fig.width = 4,
                       reference_group, compare_group)
  print(p)
}
```

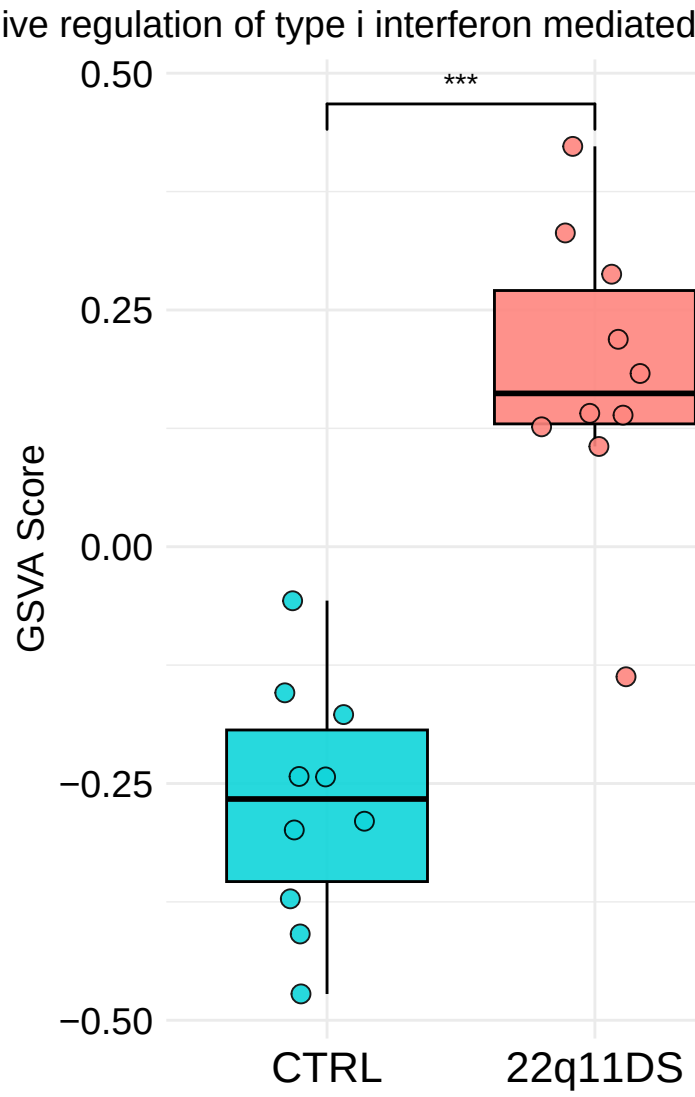
```
## [1] "GOBP_MITOCHONDRIAL_RNA_3_END_PROCESSING"
```

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




```
## [1] "GOBP_POSITIVE_REGULATION_OF_TYPE_I_INTERFERON_MEDIATED_SIGNALING_PATHWAY"
```

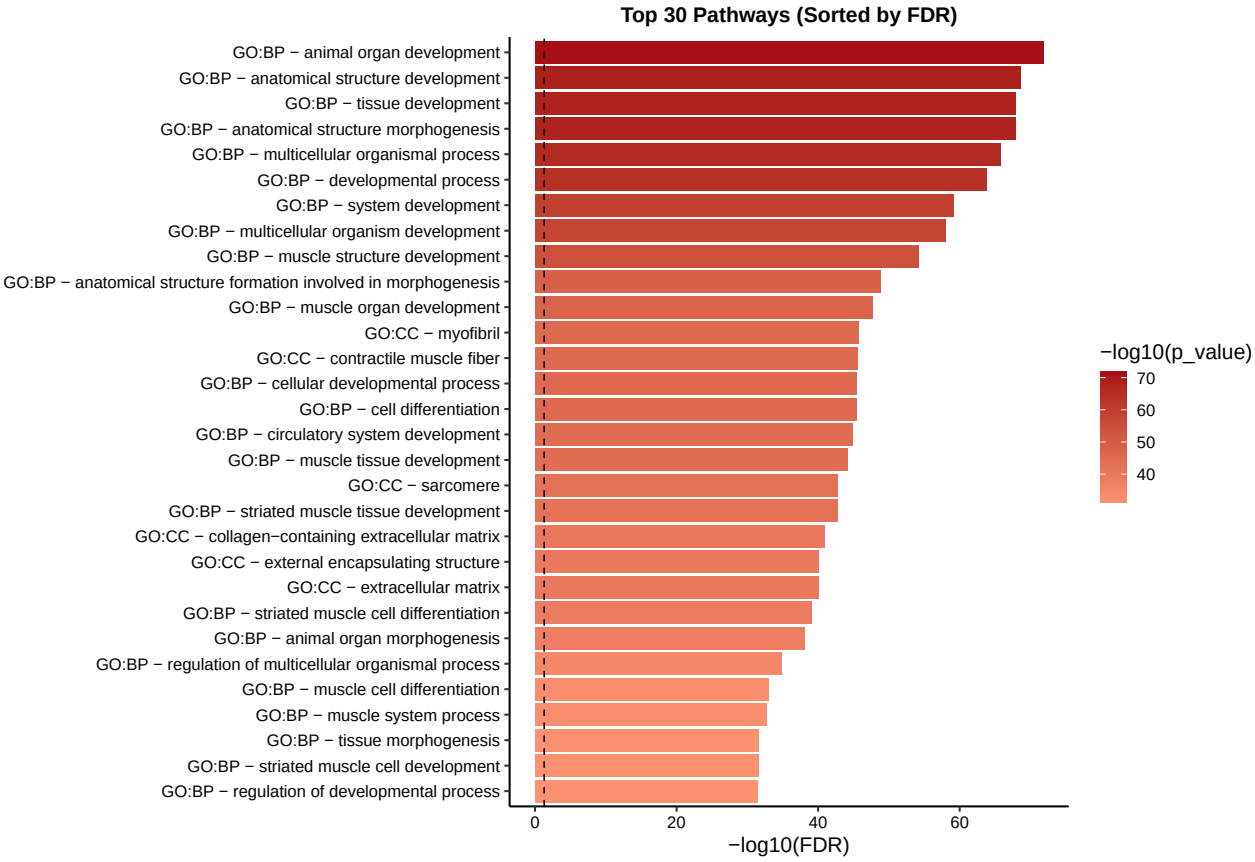

Mitochondrial RNA processing
NS.
0.4
0.0
−0.4
GSVA Score
CTRL
22q11DS


ive regulation of type i interferon mediated
***
0.50
0.25
0.00
−0.25
−0.50
GSVA Score
CTRL
22q11DS

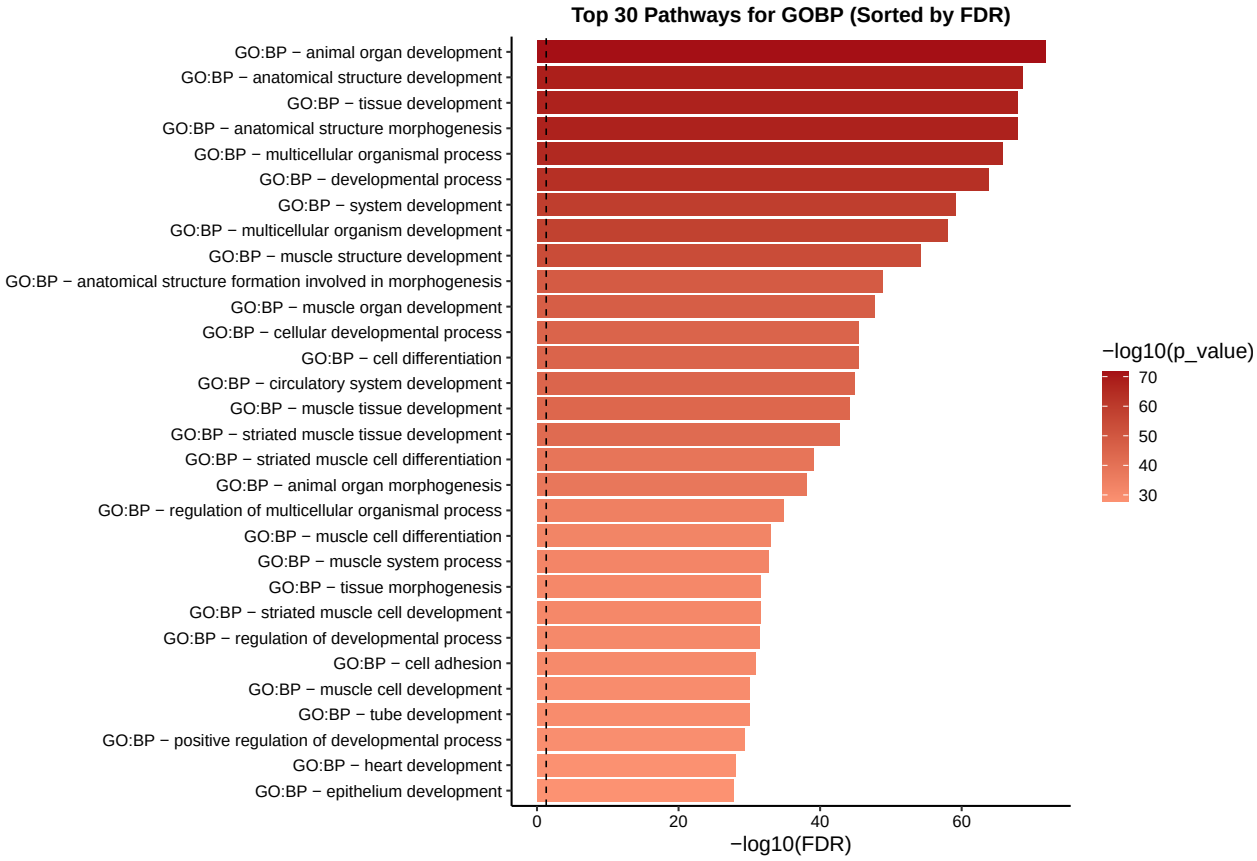
5. Pathway Enrichment Analysis

```
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 "
```

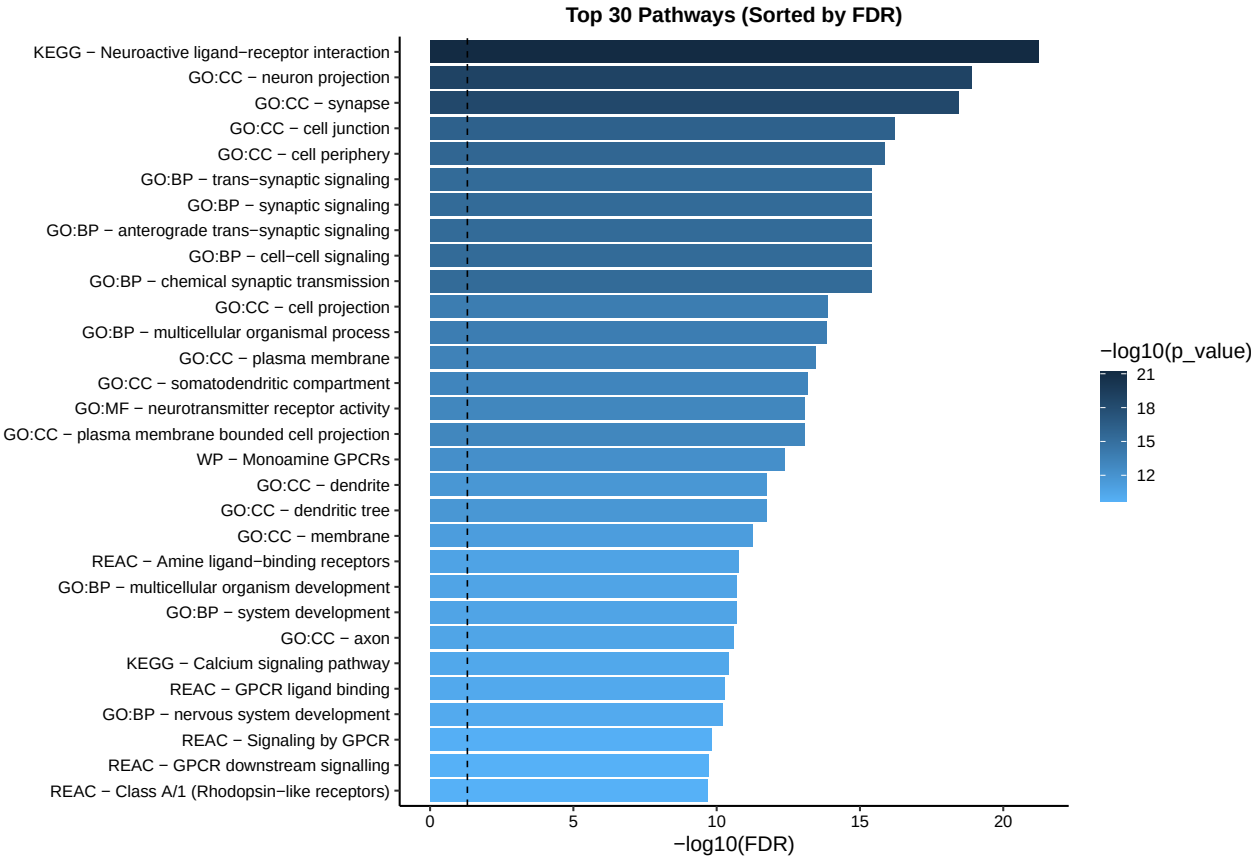



```
## [1] "Enrichment analysis for GOBP 01-DEG_1.0_up "
```

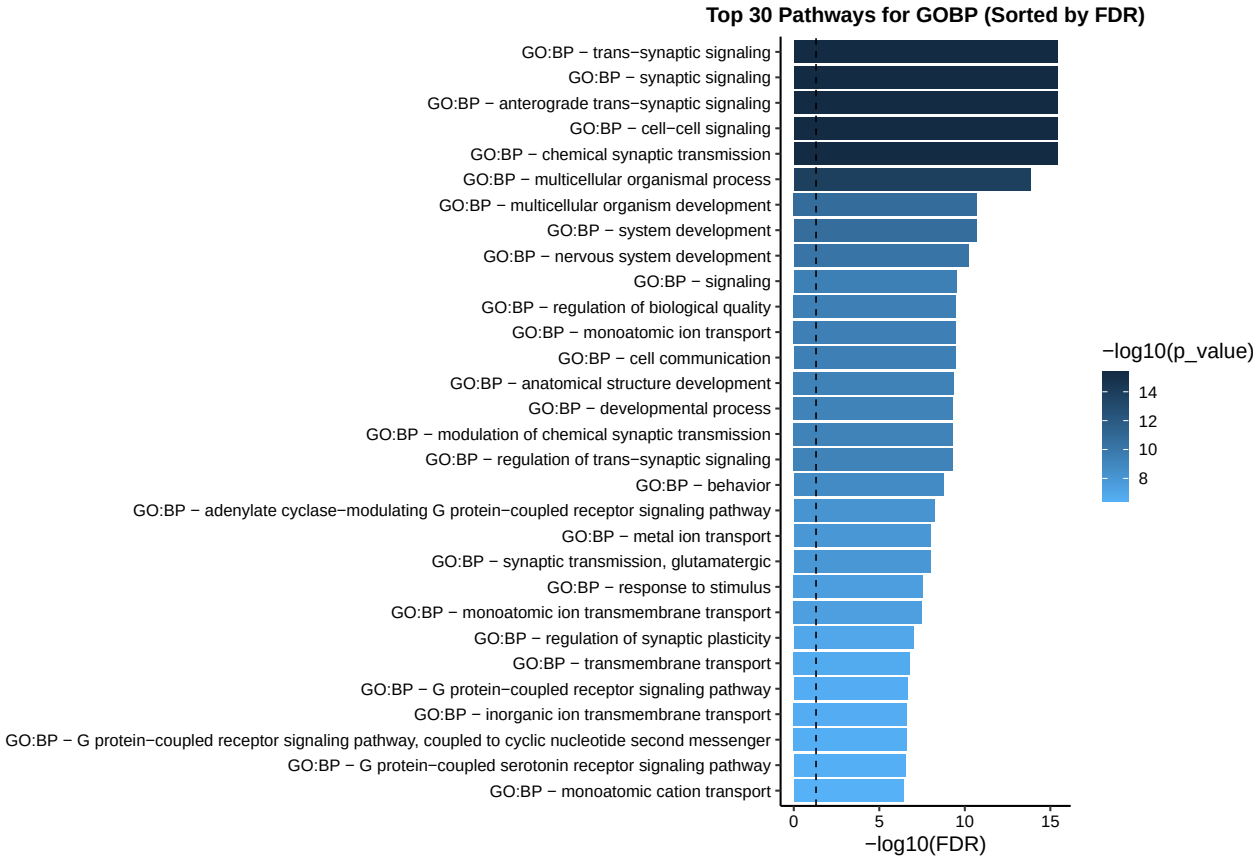



```
Enrichment_analysis(gene_list = down_gene_1,
                    result_folder = file.path(result_folder,"03-Enrichment"),
                    file_name = "01-DEG_1.0_down", gene_name_mapping, flag = "Down")
```

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

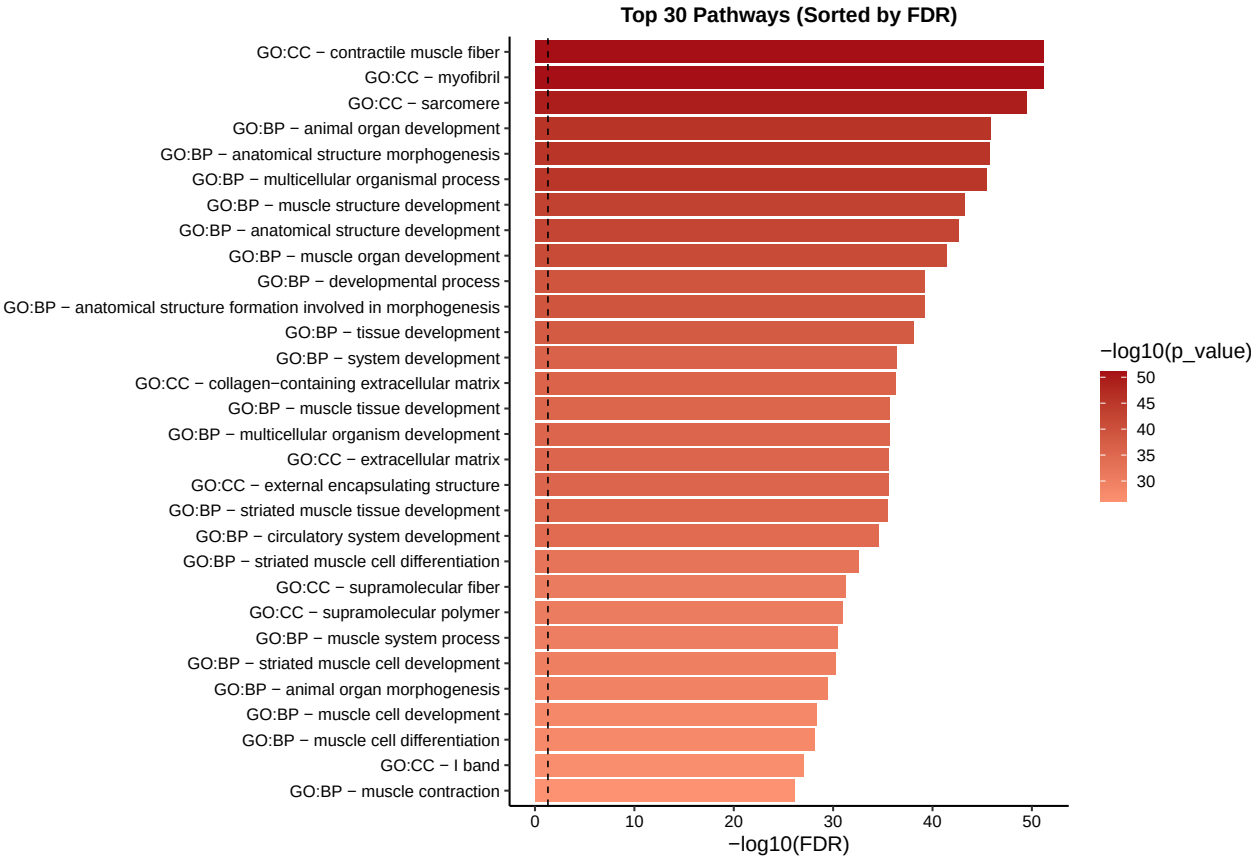



```
## [1] "Enrichment analysis for GOBP 01-DEG_1.0_down "
```

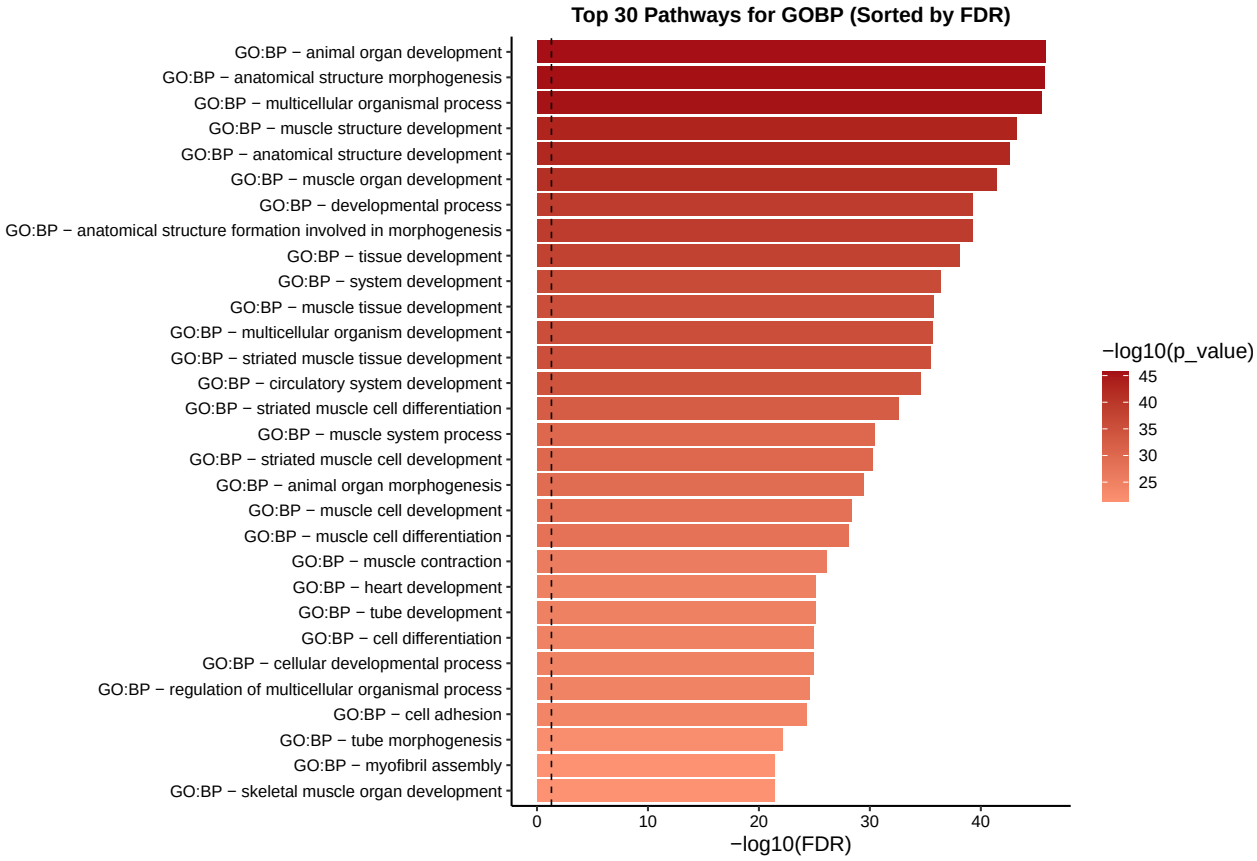



```
deg1.5 <- result_df %>% filter(padj < 0.05 & abs(log2FoldChange) > 1.5)
up_gene_1.5 <- deg1.5 %>% filter(log2FoldChange > 0) %>% pull(GeneName)
down_gene_1.5 <- deg1.5 %>% filter(log2FoldChange < 0) %>% pull(GeneName)

# 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 "
```

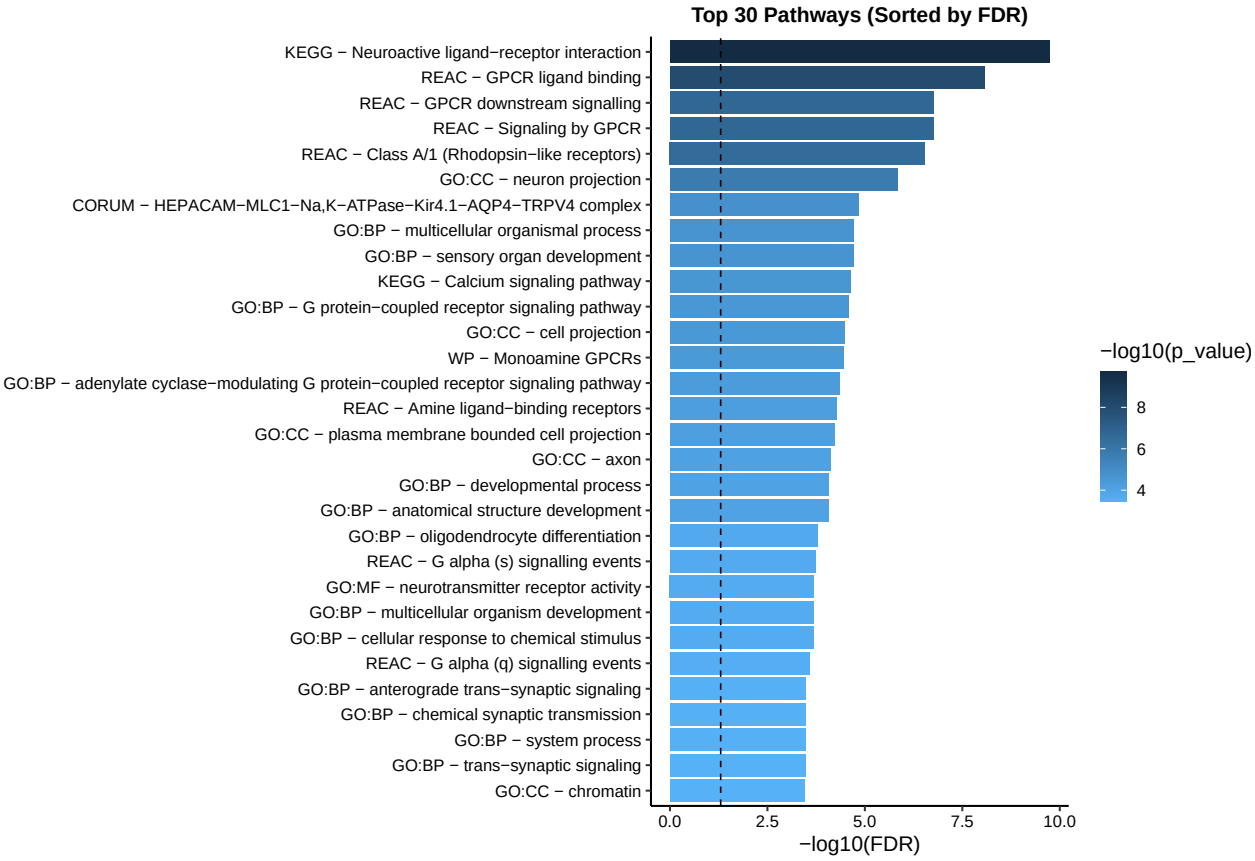



```
## [1] "Enrichment analysis for GOBP 02-DEG_1.5_up "
```

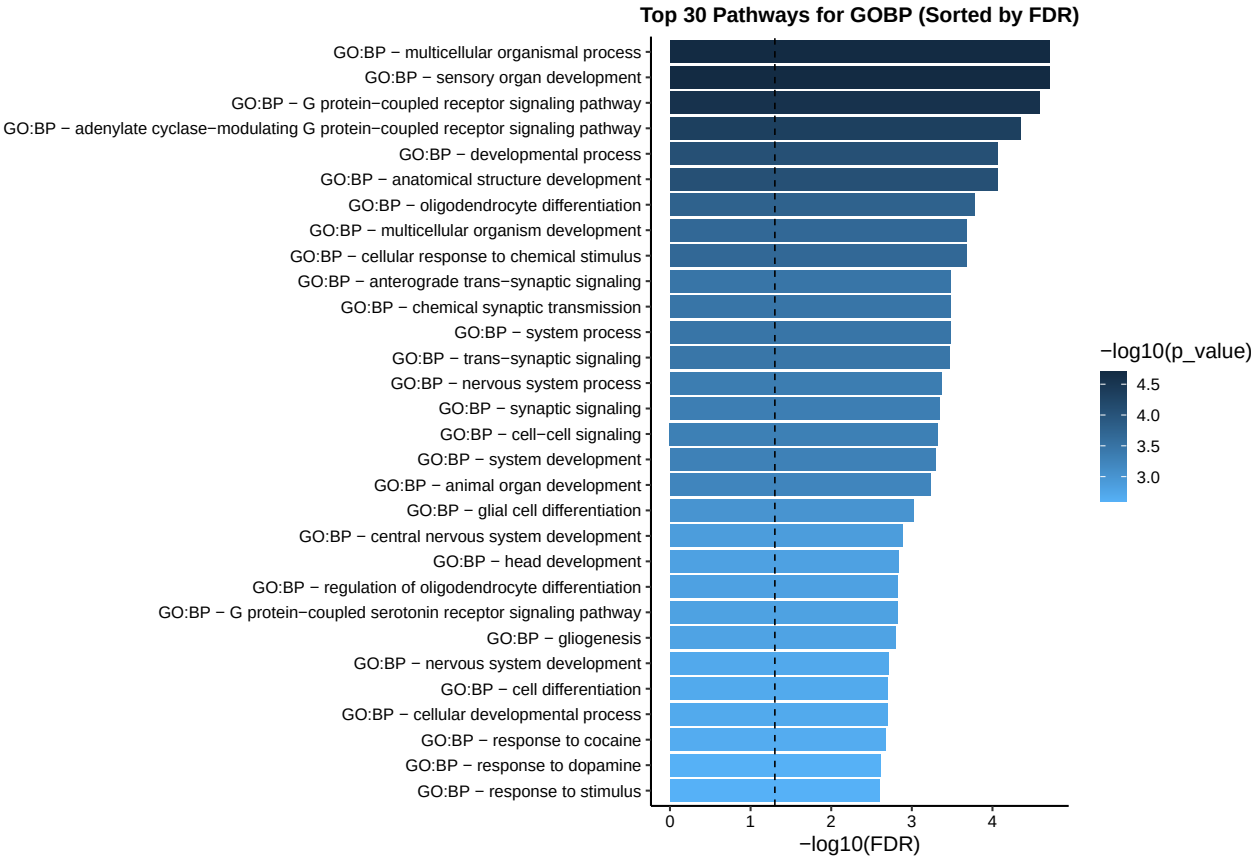



```
Enrichment_analysis(gene_list = down_gene_1.5,
                    result_folder = file.path(result_folder,"03-Enrichment"),
                    file_name = "02-DEG_1.5_down", gene_name_mapping, flag = "Down")
```

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




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


Top 30 Pathways for GOBP (Sorted by FDR)
GO:BP − multicellular organismal process
GO:BP − sensory organ development
GO:BP − G protein−coupled receptor signaling pathway
GO:BP − adenylate cyclase−modulating G protein−coupled receptor signaling pathway
GO:BP − developmental process
GO:BP − anatomical structure development
GO:BP − oligodendrocyte differentiation
GO:BP − multicellular organism development
GO:BP − cellular response to chemical stimulus
GO:BP − anterograde trans−synaptic signaling
GO:BP − chemical synaptic transmission
GO:BP − system process
GO:BP − trans−synaptic signaling
GO:BP − nervous system process
GO:BP − synaptic signaling
GO:BP − cell−cell signaling
GO:BP − system development
GO:BP − animal organ development
GO:BP − glial cell differentiation
GO:BP − central nervous system development
GO:BP − head development
GO:BP − regulation of oligodendrocyte differentiation
GO:BP − G protein−coupled serotonin receptor signaling pathway
GO:BP − gliogenesis
GO:BP − nervous system development
GO:BP − cell differentiation
GO:BP − cellular developmental process
GO:BP − response to cocaine
GO:BP − response to dopamine
GO:BP − response to stimulus
0
1
2
3
4
−log10(FDR)
−log10(p_value)
4.5
4.0
3.5
3.0

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
```