

# Differential Expression Analysis for bulk RNA-seq data

TTX contition: 22q vs Control

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```
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_cleaned.csv`.

```
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(Diagnosis, "_", 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-TTX")
Result_folder_structure(result_folder)

# load the comparison group information
reference_group <- "CTRL_TTX"
compare_group <- "22q_TTX"
```

```

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)

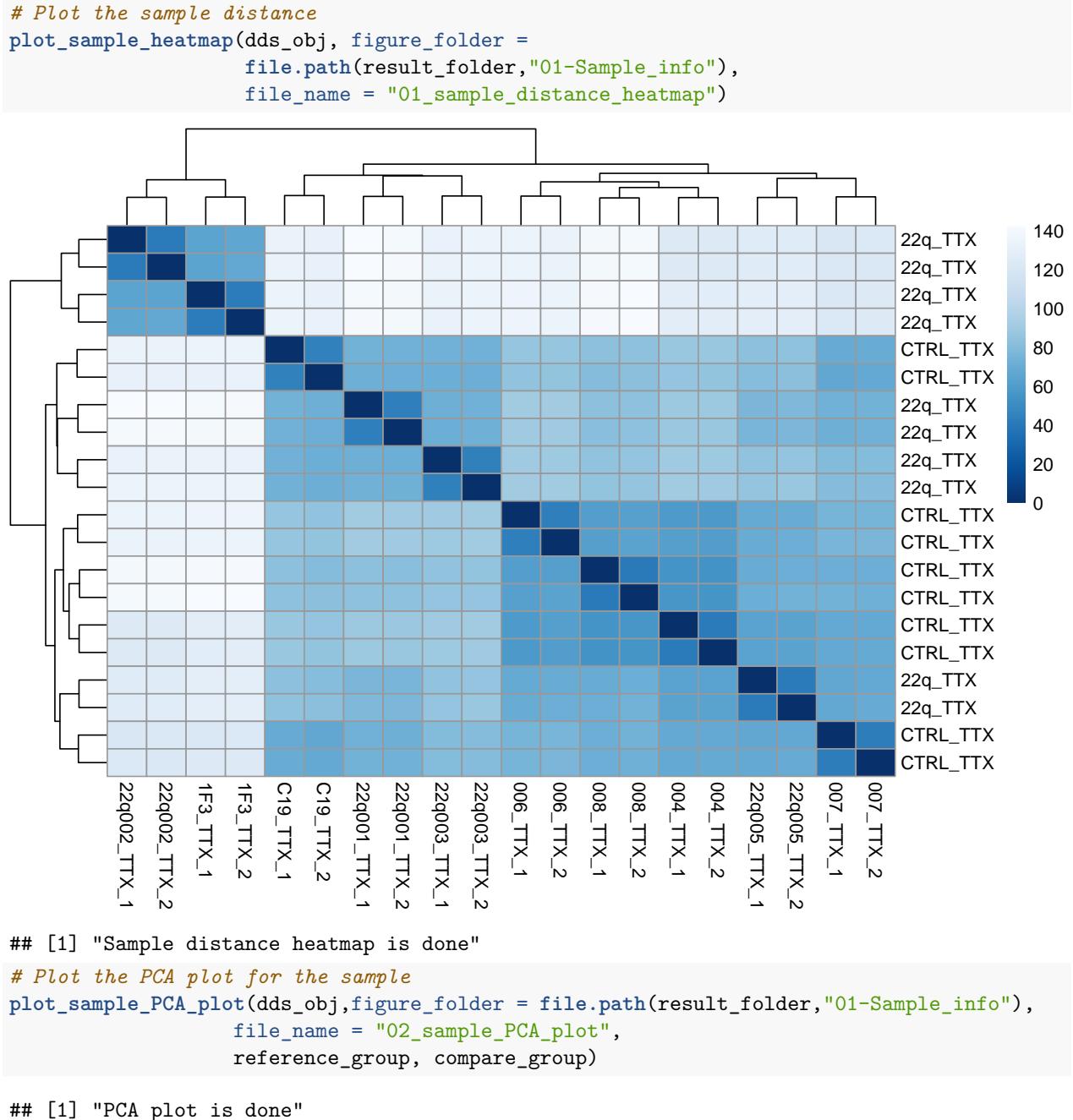
# DEG with log2fc > 1.5 and padj < 0.05
deg_1.5 <- resOrdered %>% as.data.frame() %>% rownames_to_column(var = "gene") %>%
  filter(padj < 0.05 & abs(log2FoldChange) > 1.5) %>% arrange(padj)
deg_1.5 <- deg_1.5 [!is.na(deg_1.5 $padj),]
write.csv(deg_1.5 , file.path(result_folder,"02-DEG","03_DEG_log2fc_1_5.csv"),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

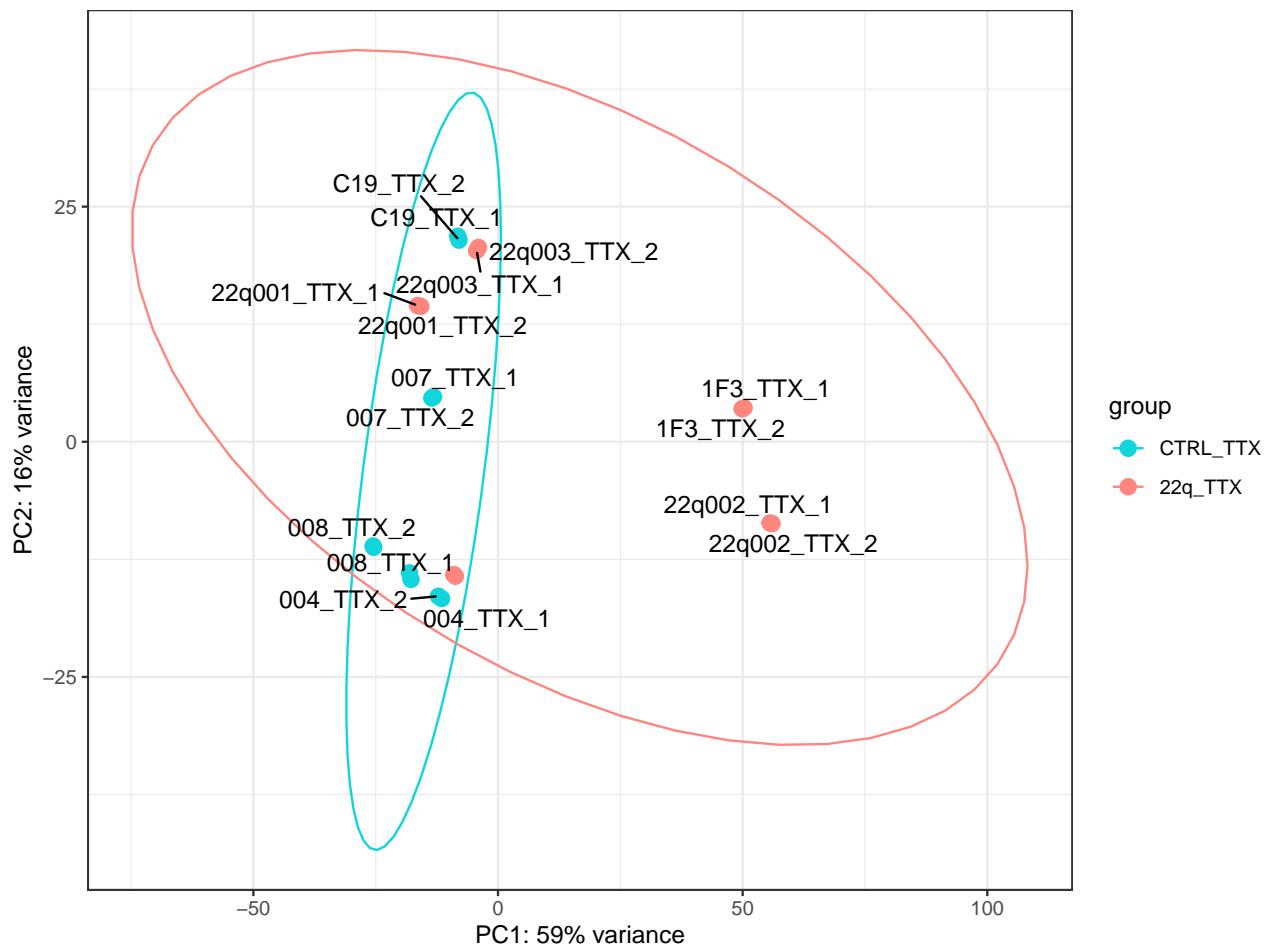
## [1] "DEG analysis is done"
# Save the normalized counts
normalized_counts <- counts(dds_obj, normalized = TRUE)
write.csv(normalized_counts, file.path(result_folder,"02-DEG", "DESeq2_normalized_counts.csv"))

```

### 3. Visualization for result

#### (1) Sample information



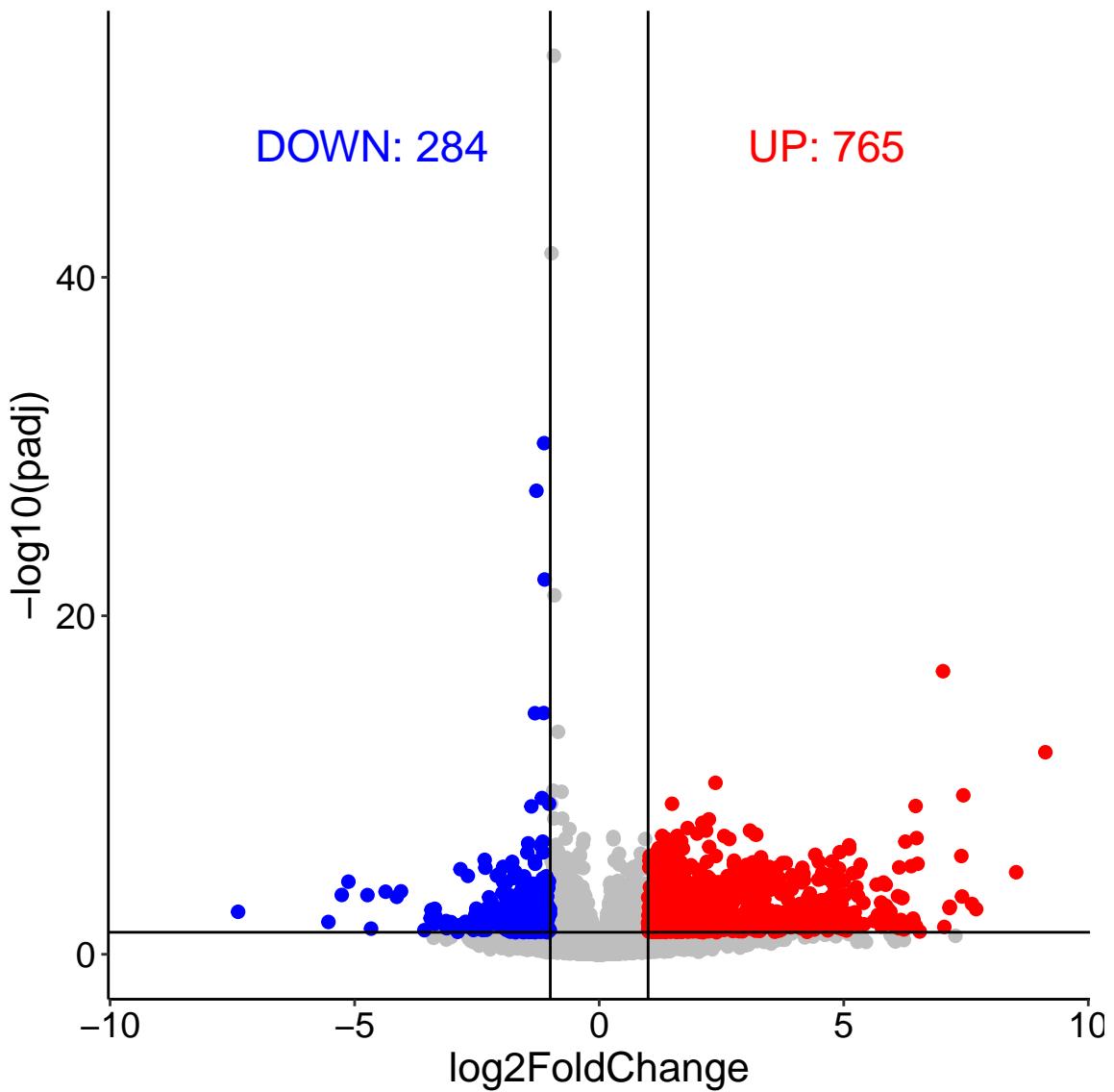


## (2) DEG visualization - Volcano plot and Heatmap

```
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"
```



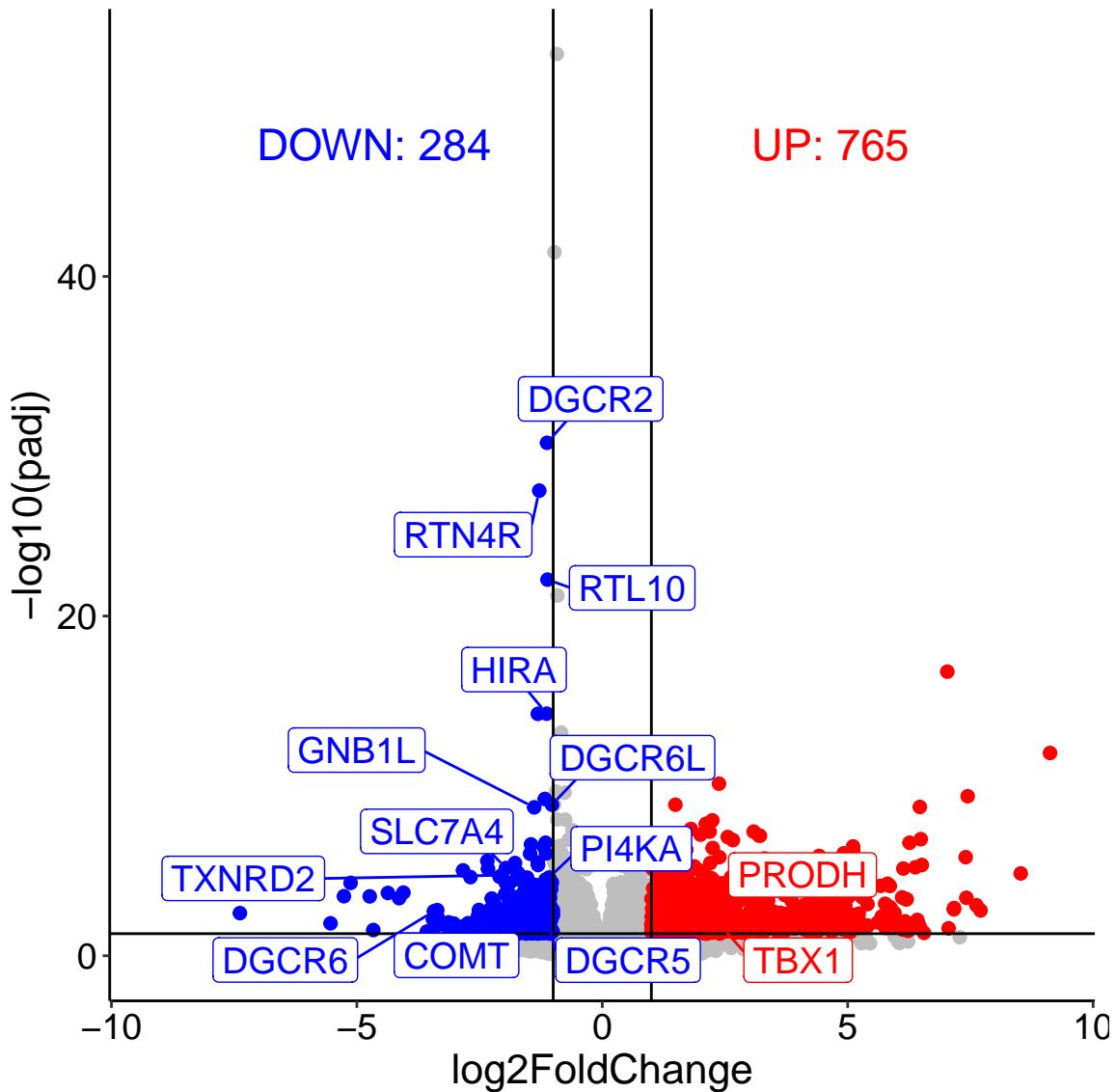
```
plot_volcano_plot(result_df,
```

```

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"
```

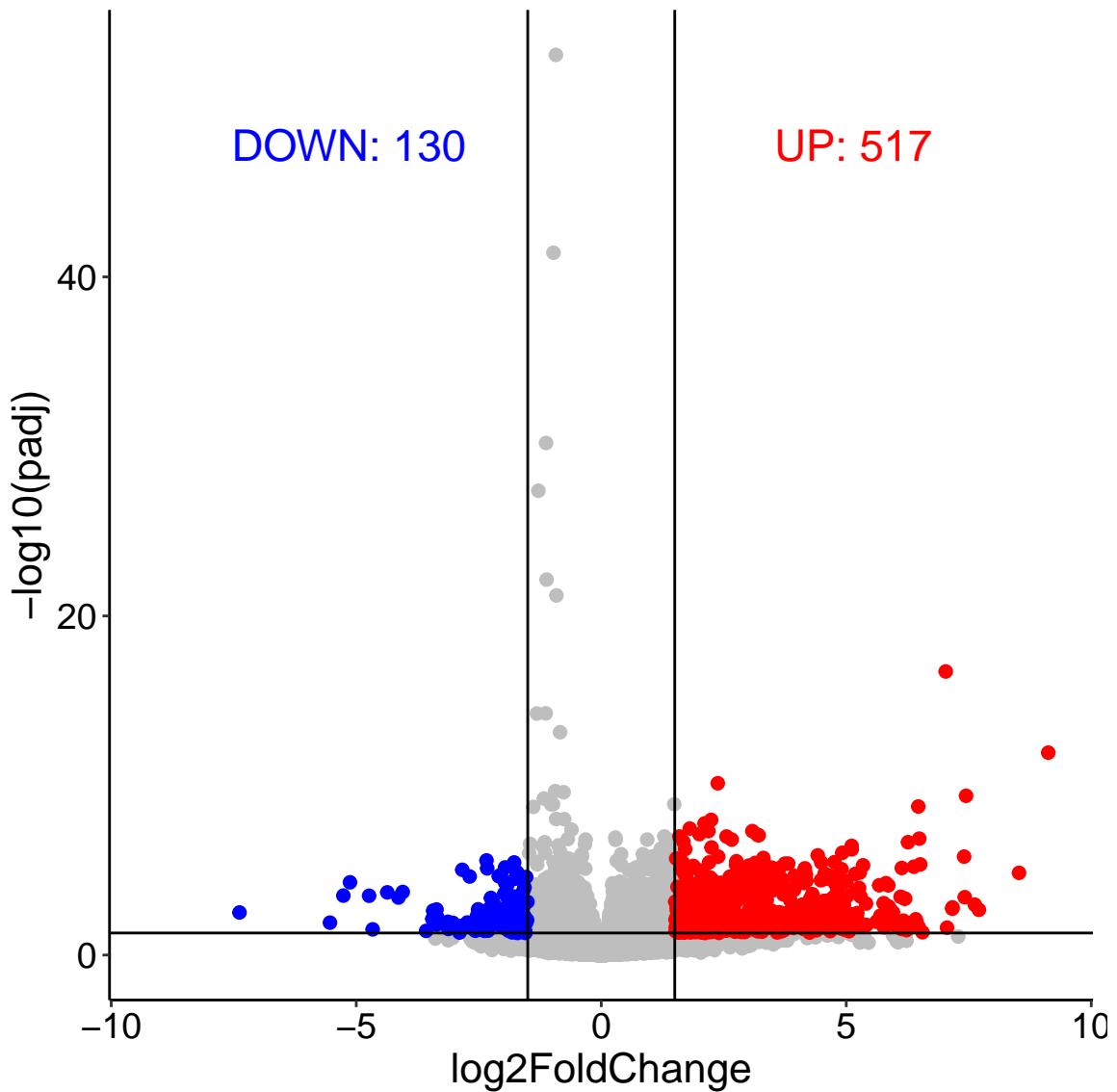


```

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)

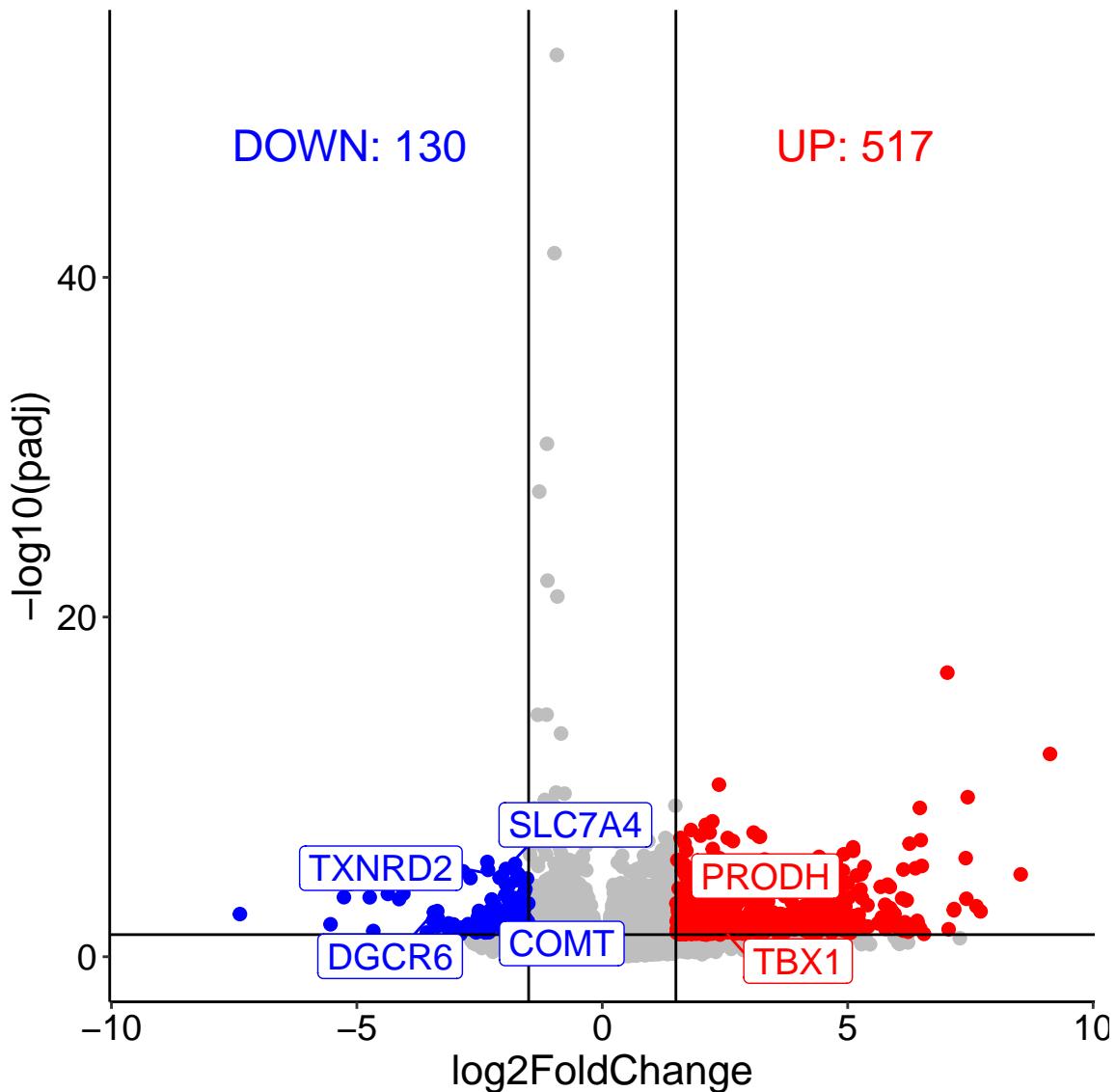
```

```
## [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"
```



```

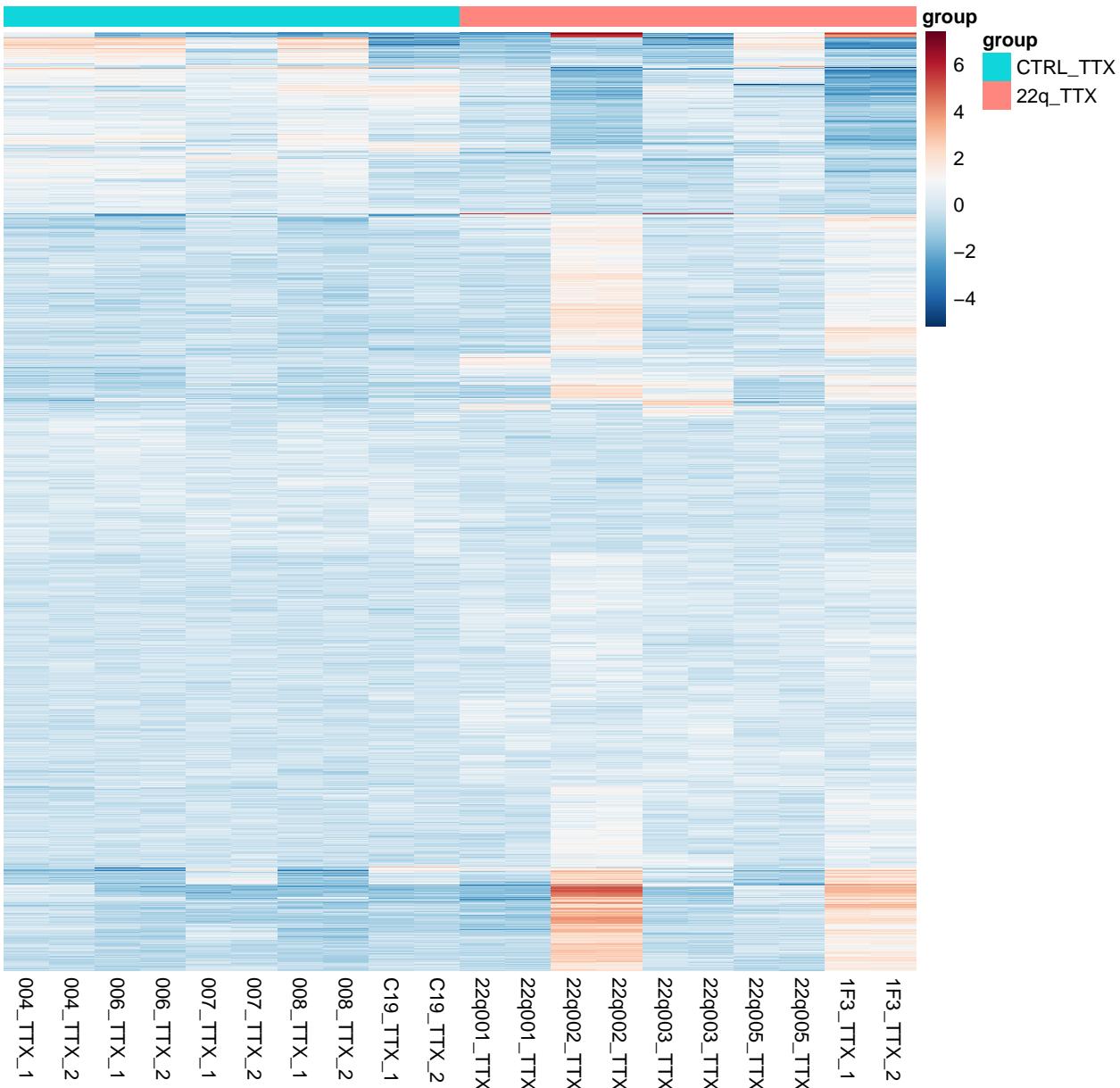
# Plot the heatmap for the DEG
vsd_obj <- varianceStabilizingTransformation(dds_obj, blind = TRUE)

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")

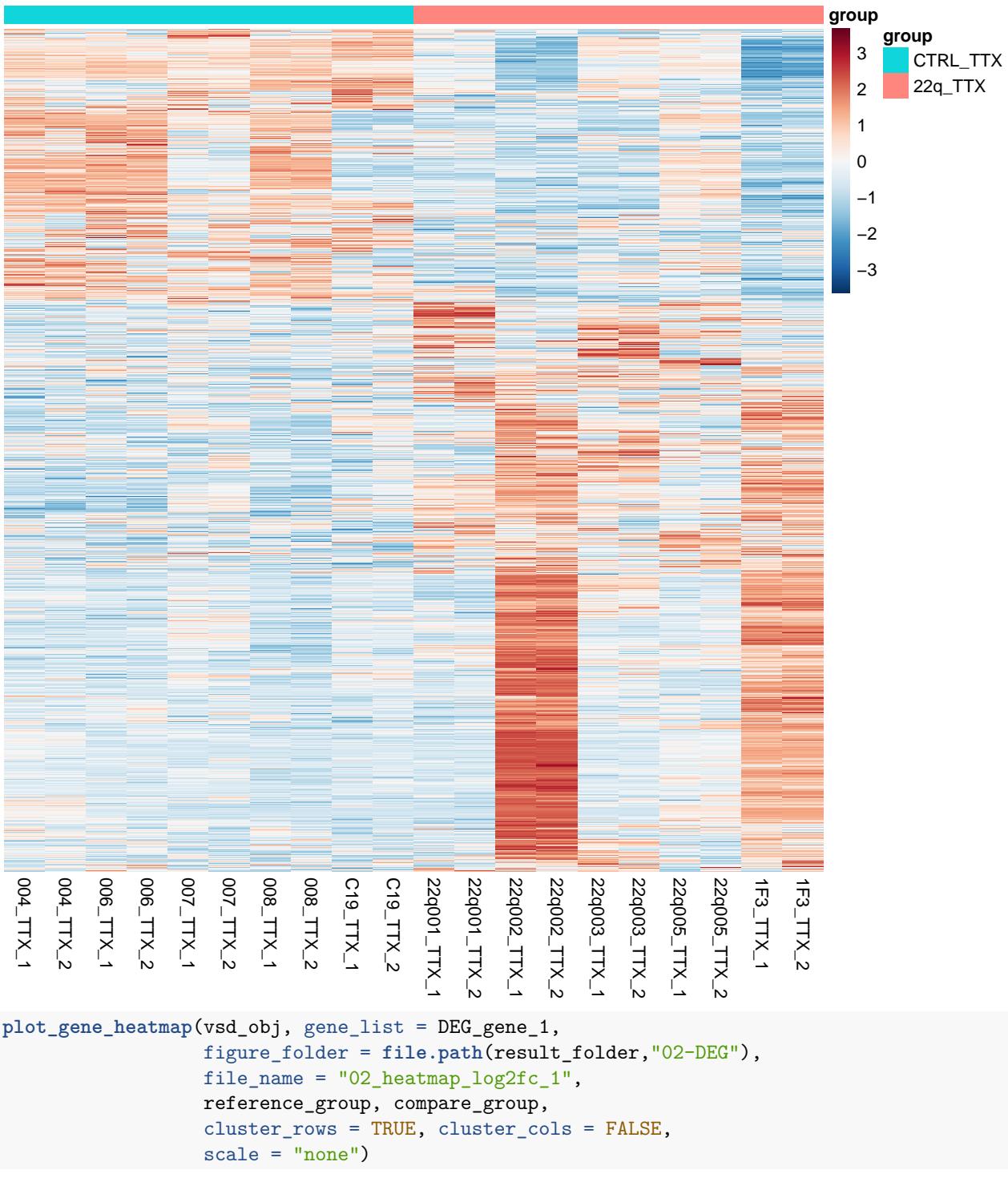
## [1] "Heatmap for 02_heatmap_log2fc_1 "

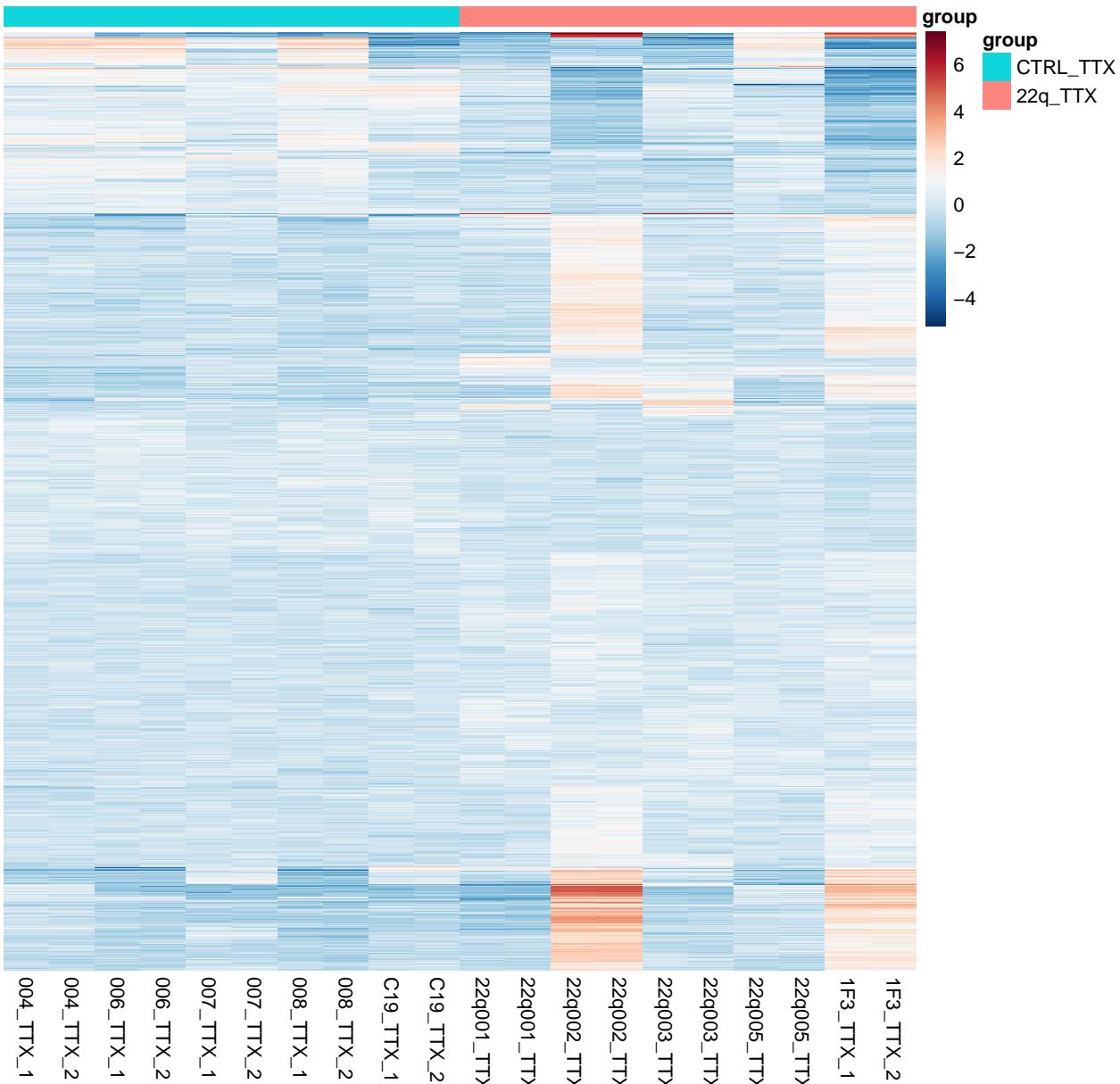
```



```
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")
```

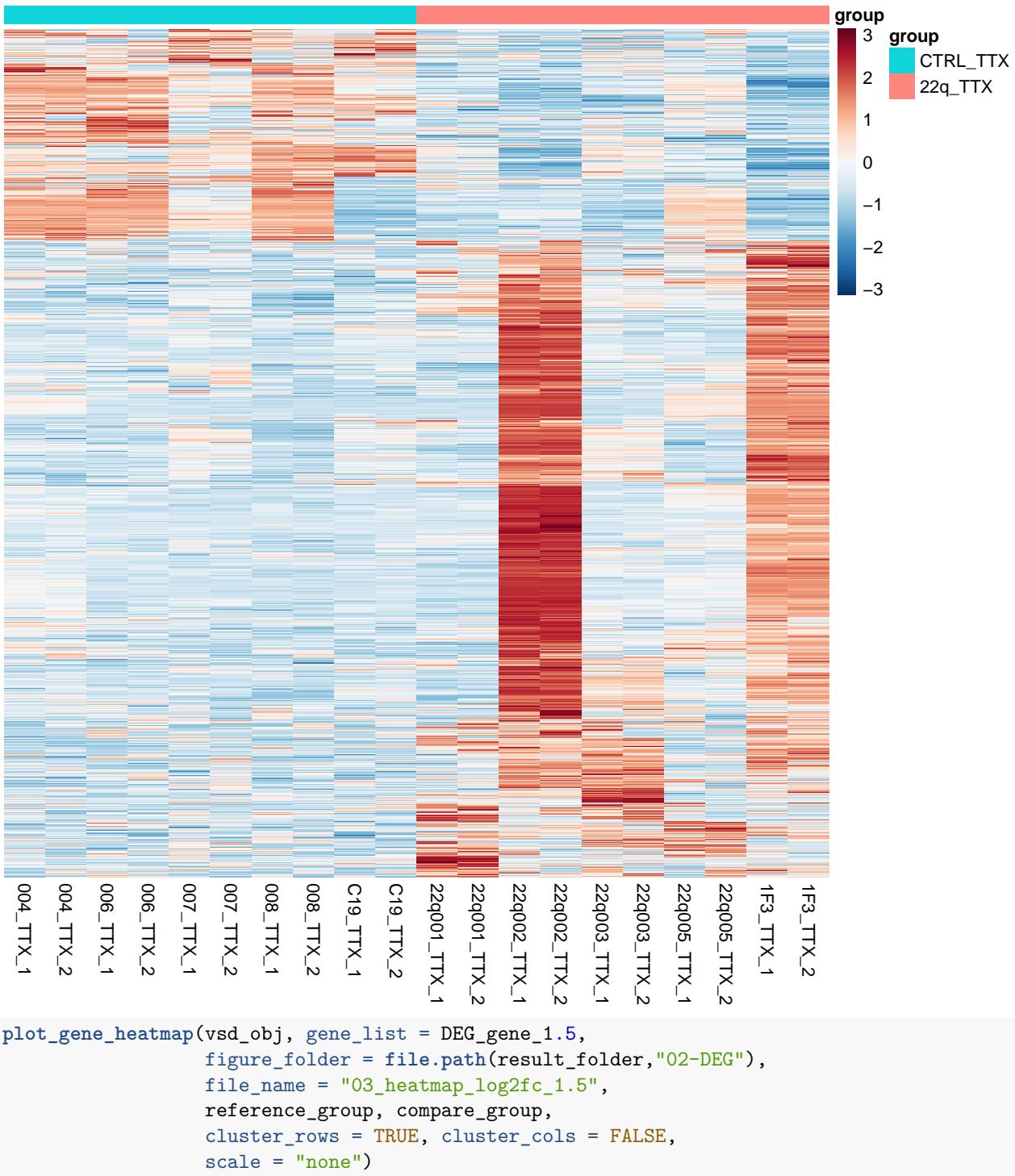
```
## [1] "Heatmap for 02_heatmap_log2fc_1_row "
```

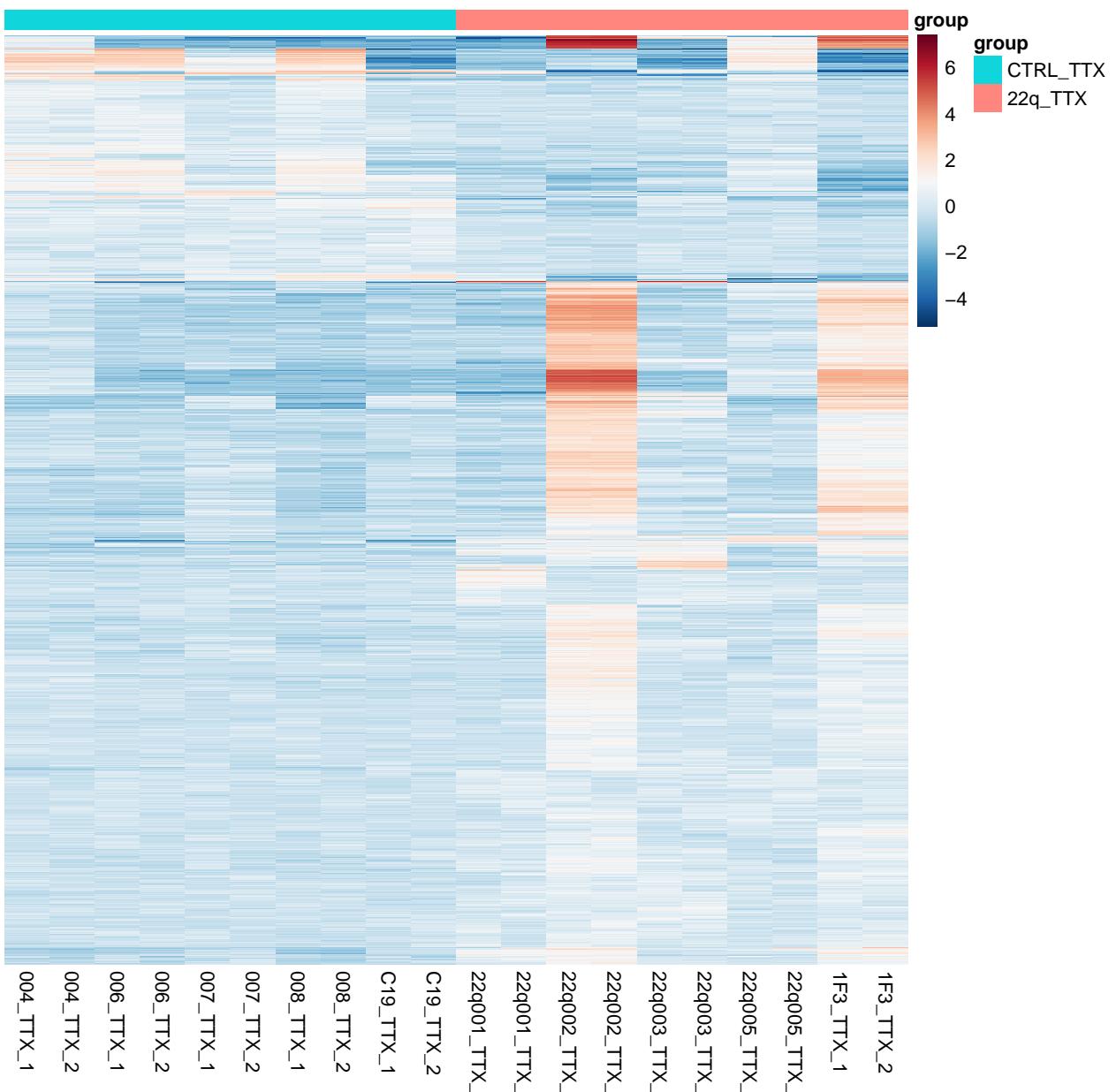




```
plot_gene_heatmap(vsd_obj, gene_list = DEG_gene_1.5,
                  figure_folder = file.path(result_folder, "02-DEG"),
                  file_name = "03_heatmap_log2fc_1.5_row",
                  reference_group, compare_group,
                  cluster_rows = TRUE, cluster_cols = FALSE,
   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

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





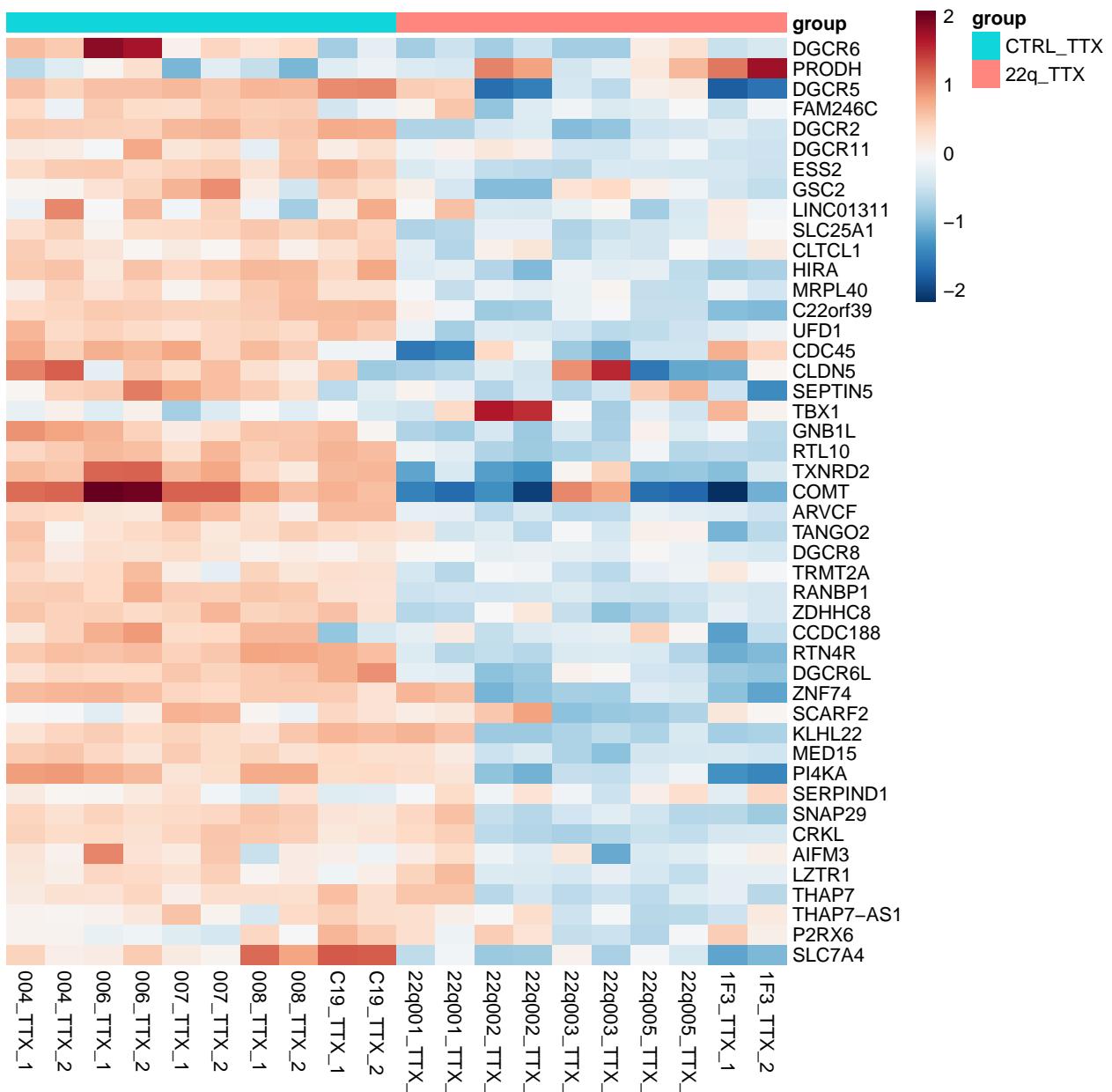
### (3) 22q gene visualization - Heatmap

```
# 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 "
```

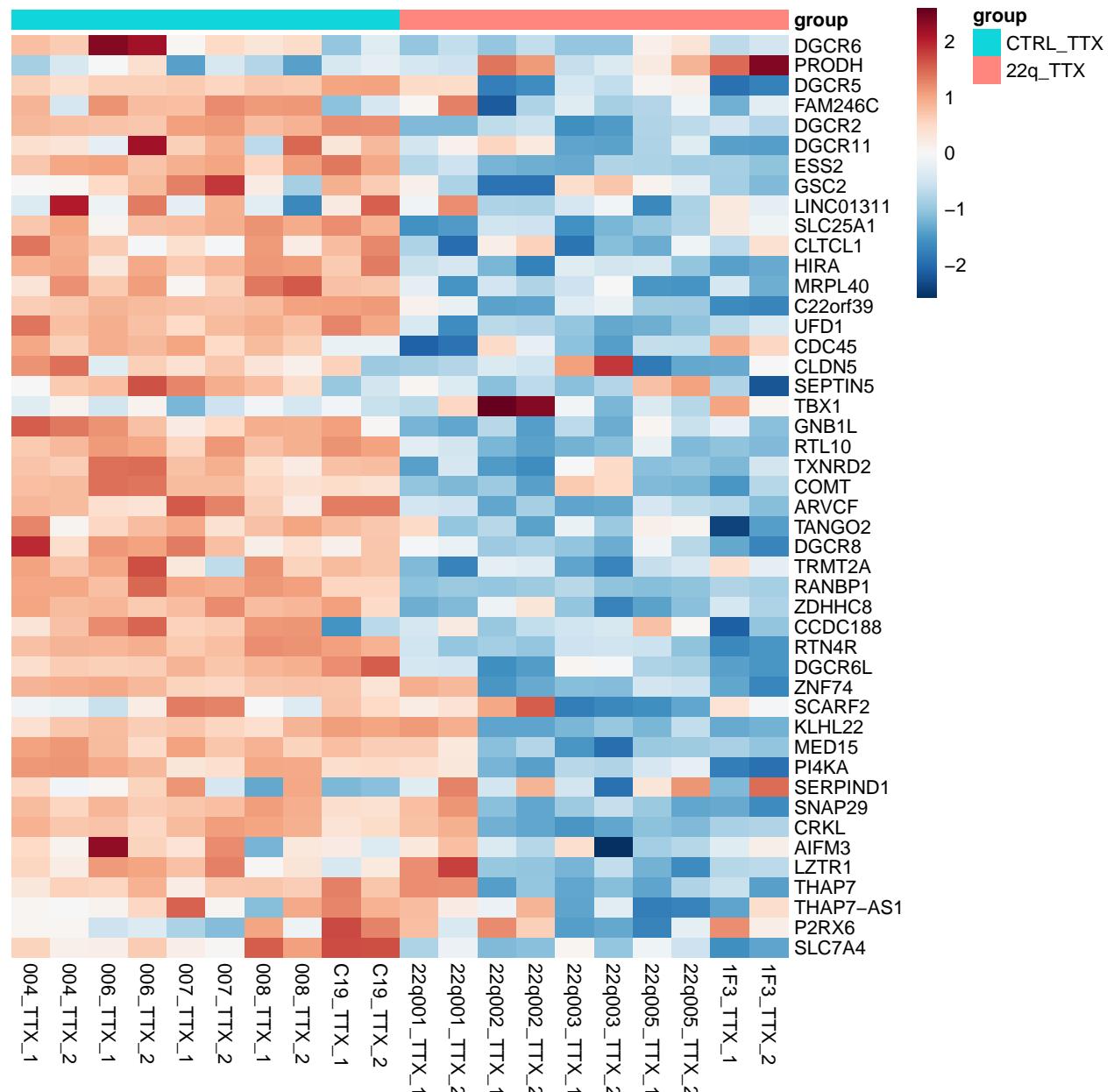


```

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

```
# # 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)
# 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 = recode(group,
                          "CTRL_TTX" = "CTRL",
                          "22q_TTX" = "22q11DS"))

reference_group_short = "CTRL"
compare_group_short = "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_gsва_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)
#
# }

# plot for the focus pathway
for (i in 1:3){
```

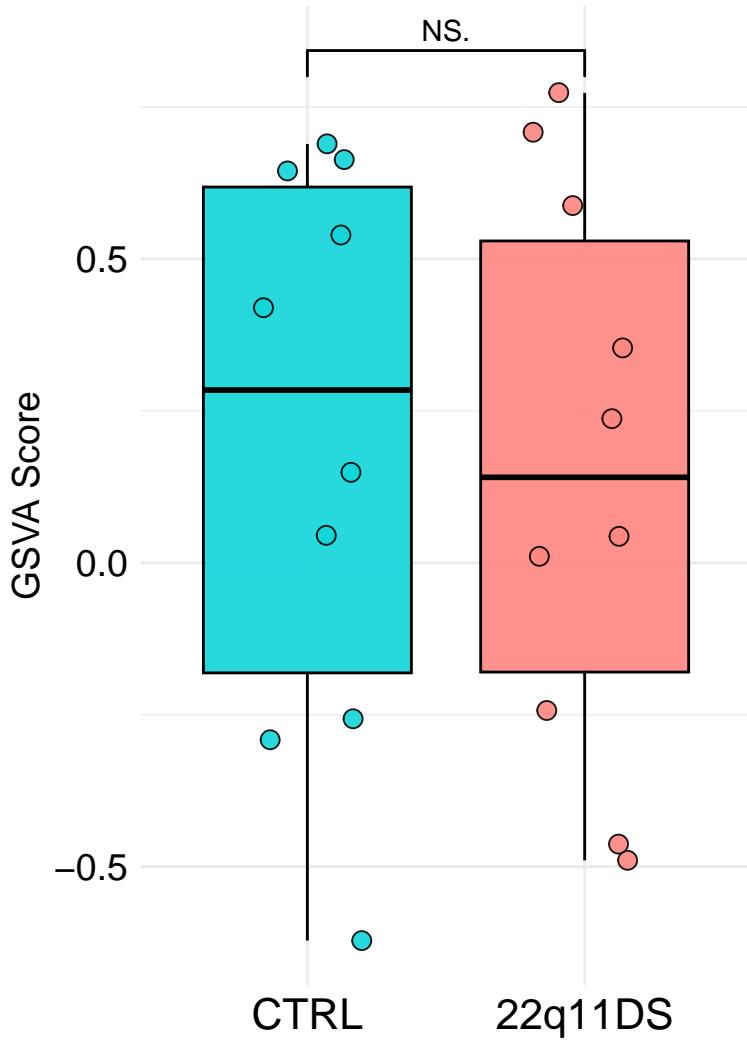
```

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 =reference_group_short ,
                       compare_group = compare_group_short)
print(p)
}

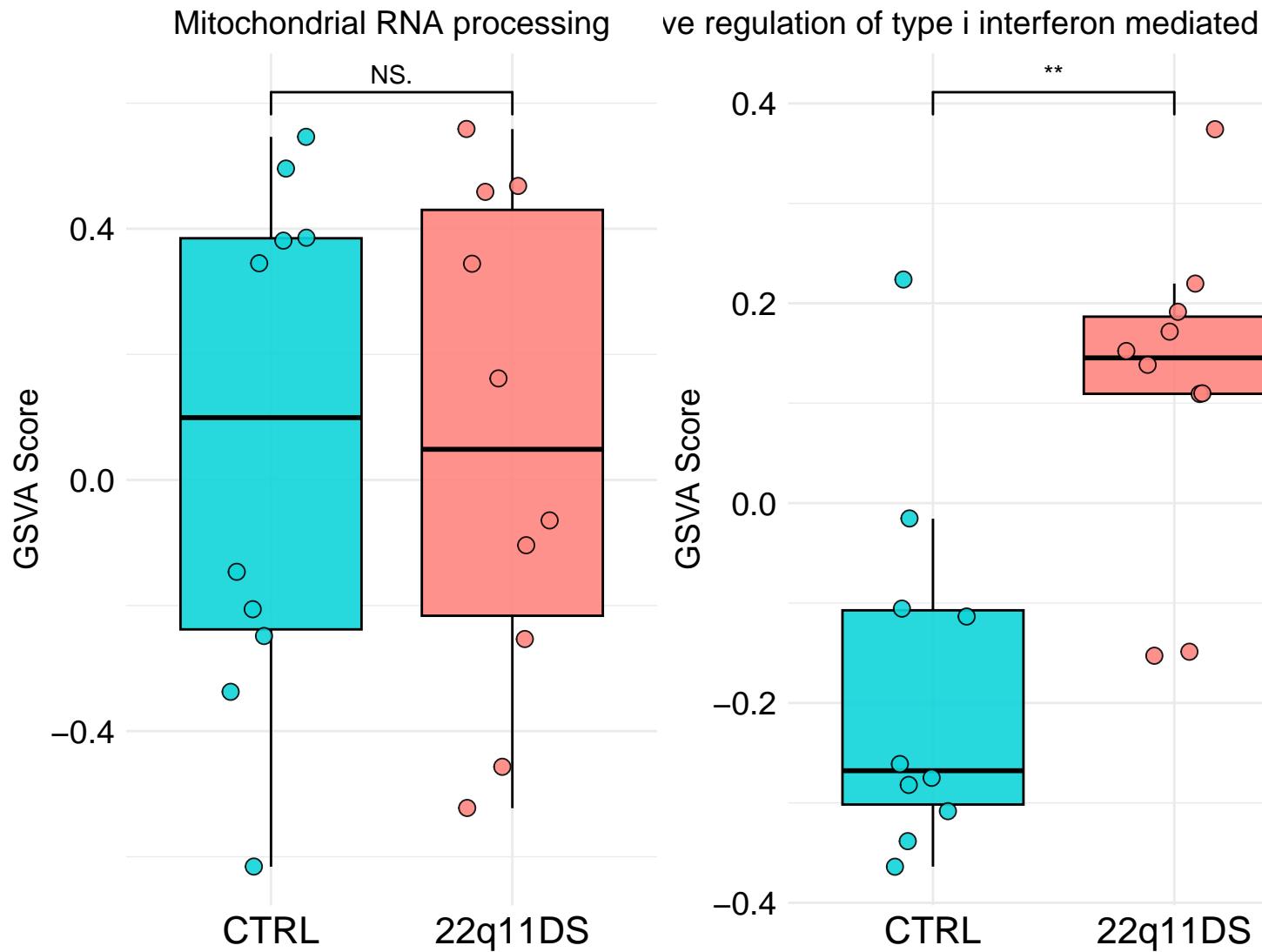
## [1] "GOBP_MITOCHONDRIAL_RNA_3_END_PROCESSING"
## [1] "GOBP_MITOCHONDRIAL_RNA_PROCESSING"

```

### Mitochondrial RNA 3 end processing



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



## 5. Pathway Enrichment Analysis

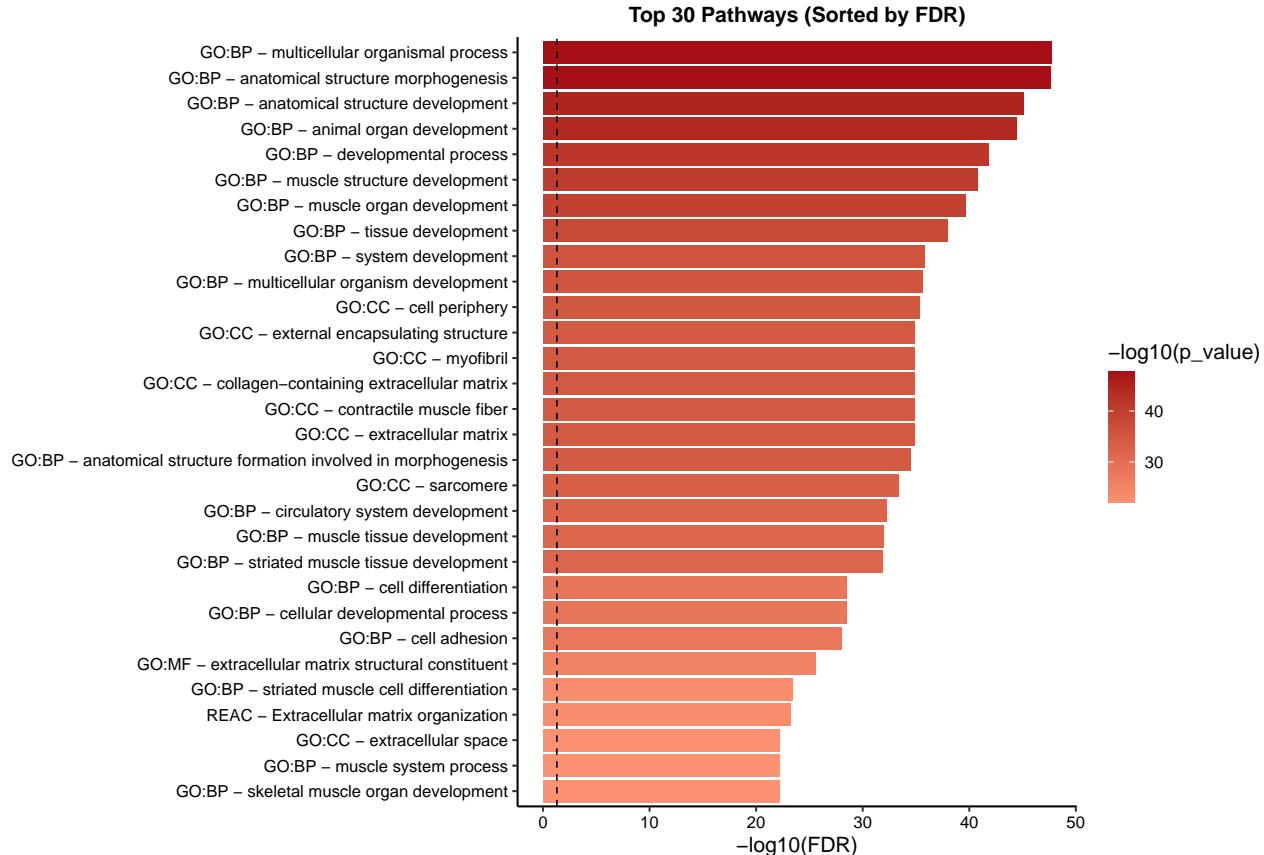
```

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)

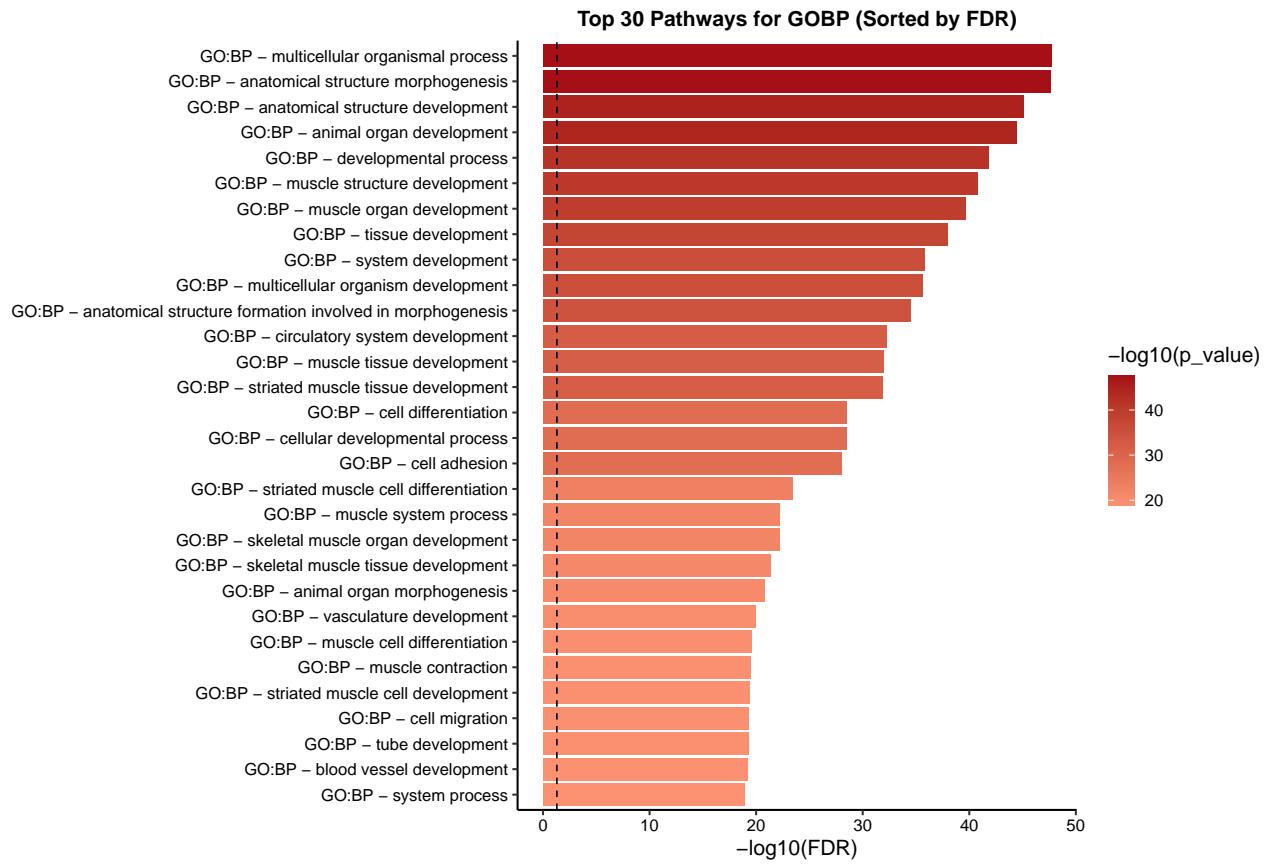
# test for the function
Enrichment_analysis(gene_list = up_gene_1,
                     result_folder = file.path(result_folder, "03-Enrichment"),
                     file_name = "01-DEG_1.0_up", gene_name_mapping, flag = "Up")

```

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

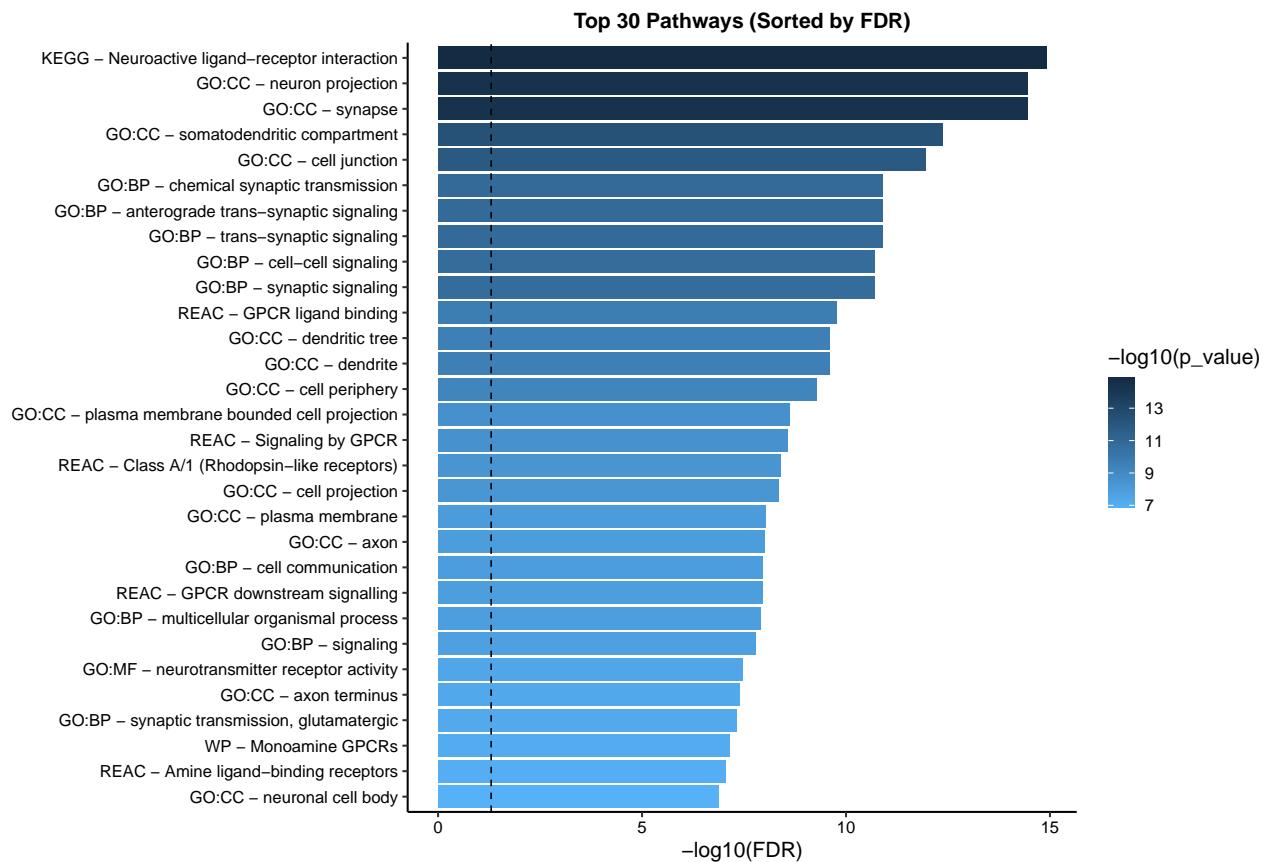


```
## [1] "Enrichment analysis for GOBP 01-DEG_1.0_up "
```

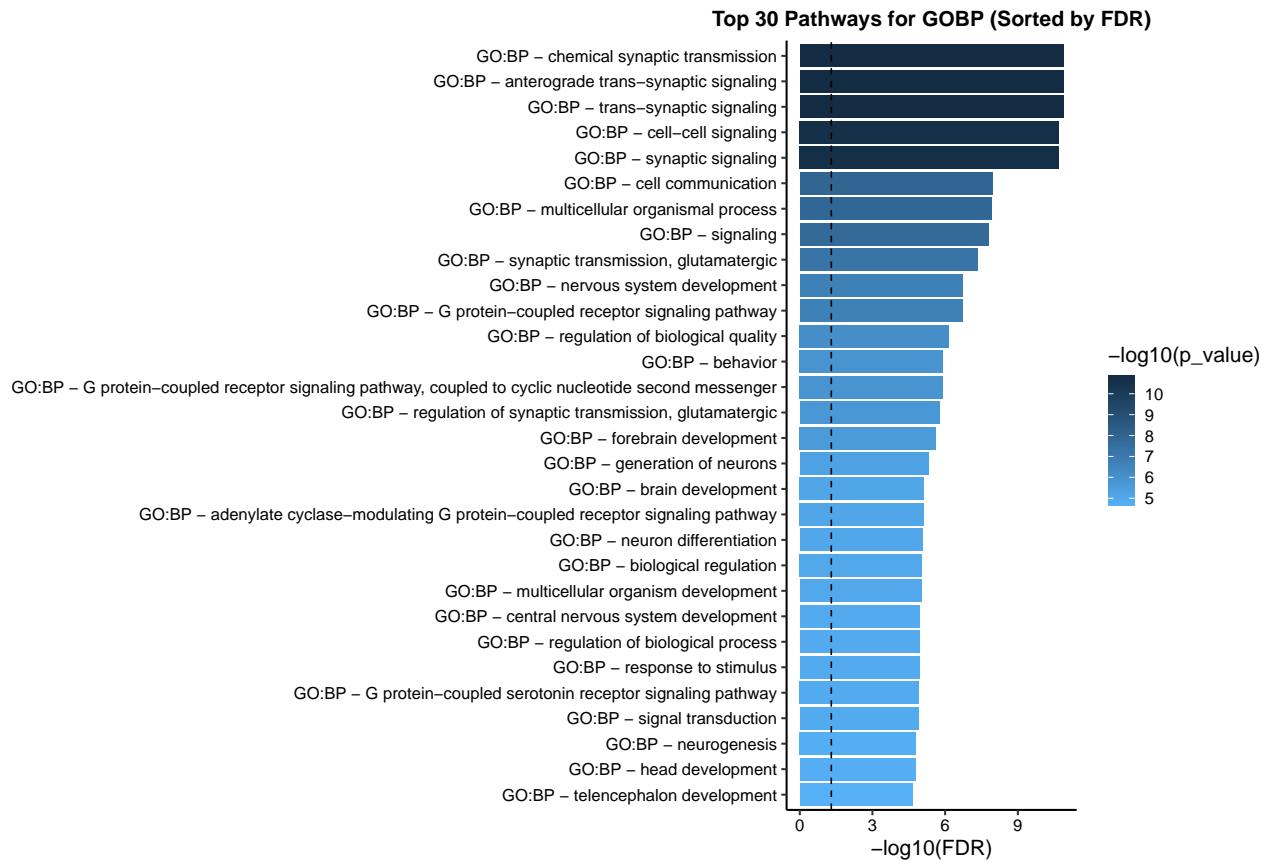


```
Enrichment_analysis(gene_list = down_gene_1,
                    result_folder = file.path(result_folder,"03-Enrichment"),
                    file_name = "01-DEG_1.0_down", gene_name_mapping, flag = "Down")

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



```
## [1] "Enrichment analysis for GOBP_01-DEG_1.0_down "
```



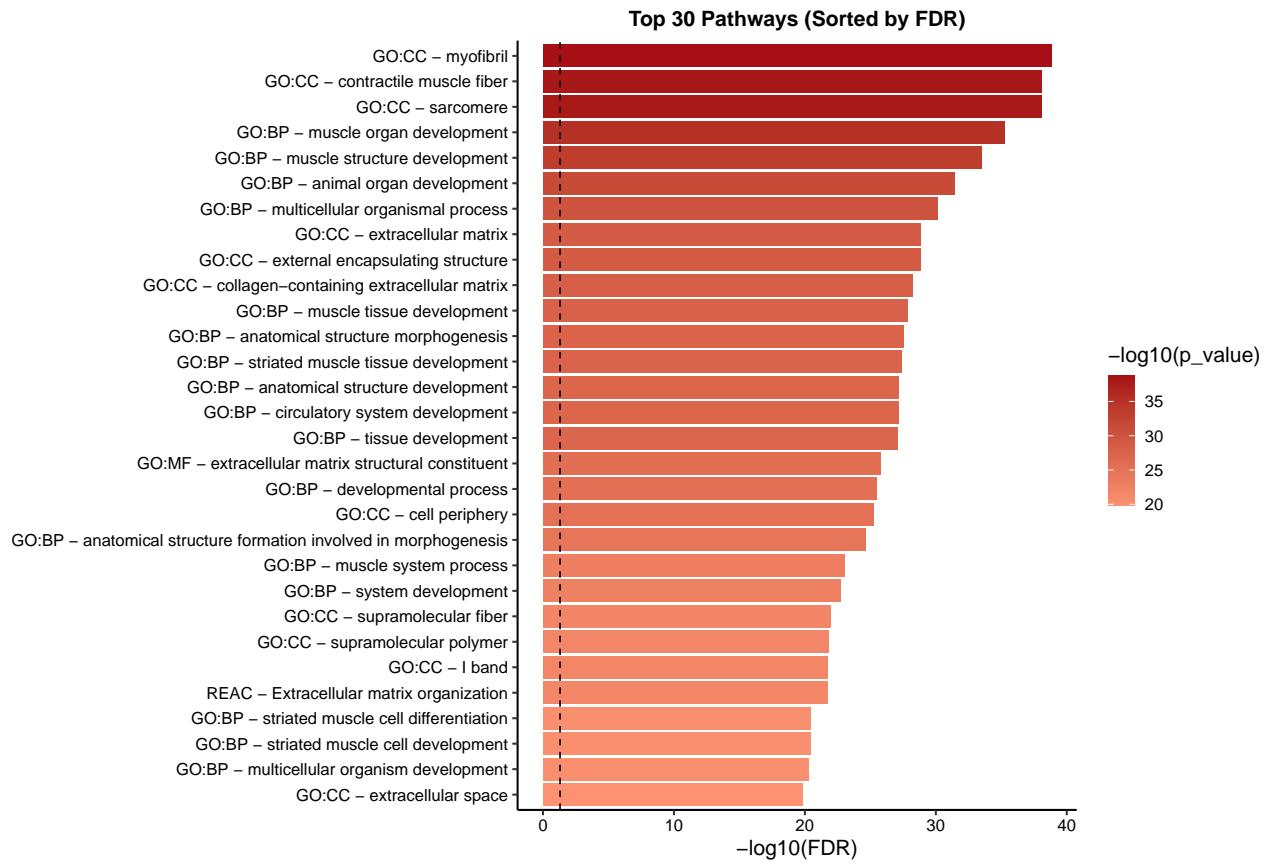
```

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)

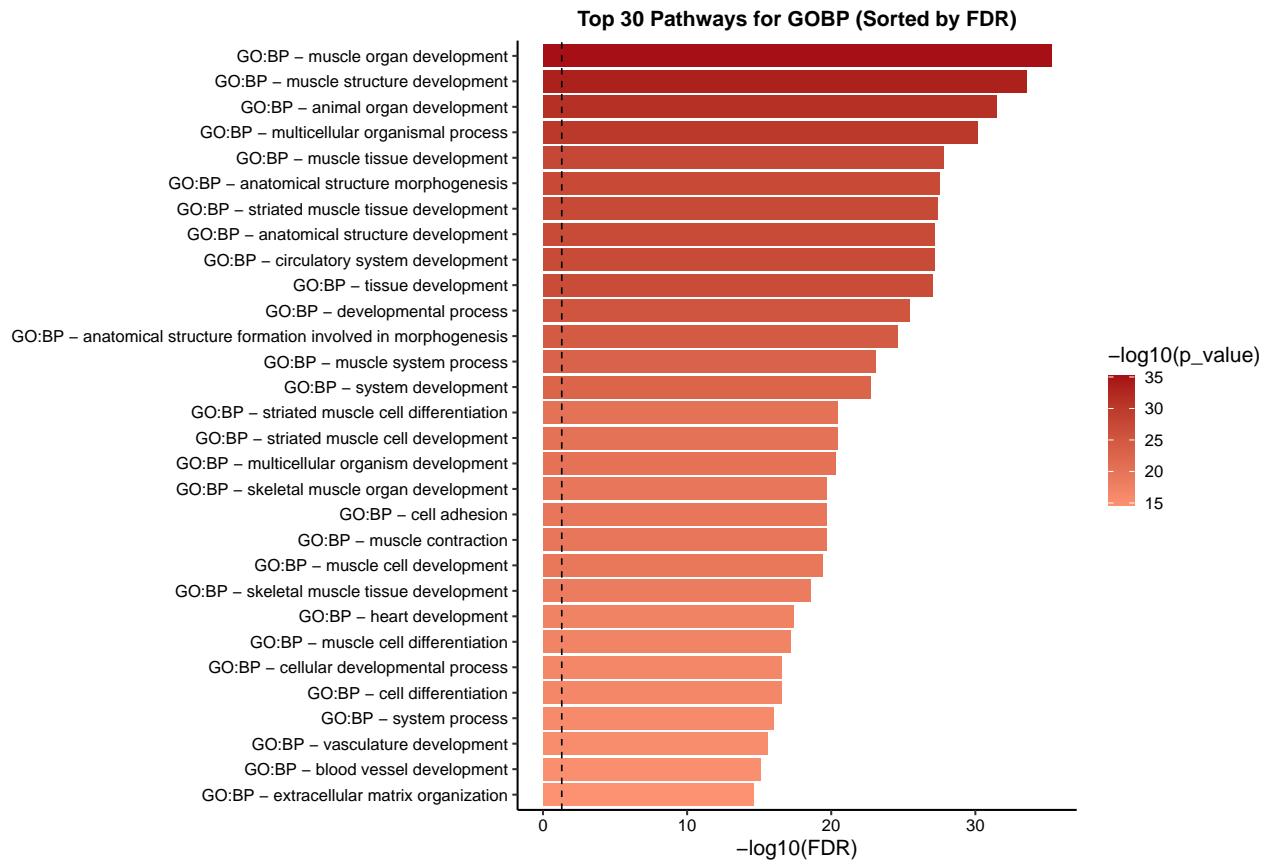
# test for the function
Enrichment_analysis(gene_list = up_gene_1.5,
                     result_folder = file.path(result_folder, "03-Enrichment"),
                     file_name = "02-DEG_1.5_up", gene_name_mapping, flag = "Up")

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

```

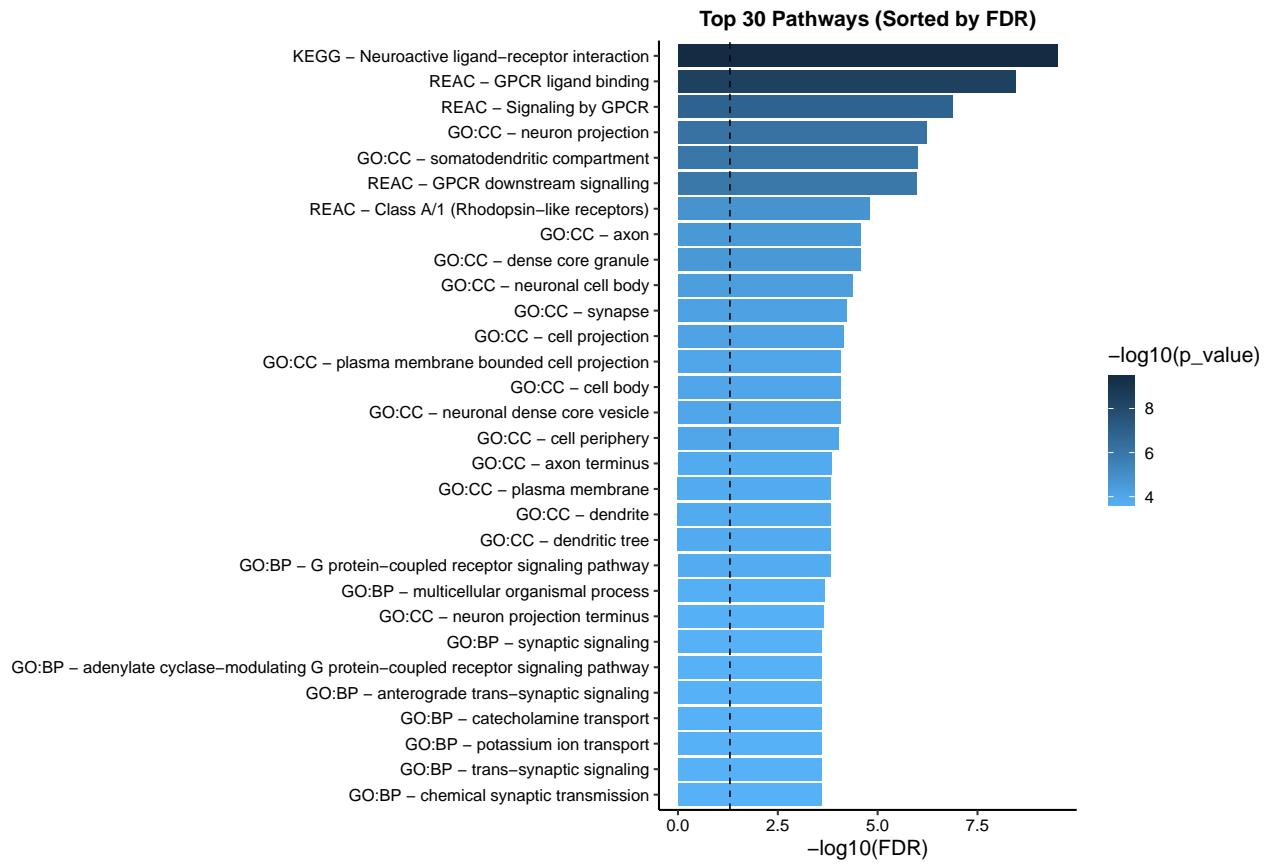


```
## [1] "Enrichment analysis for GOBP 02-DEG_1.5_up "
```

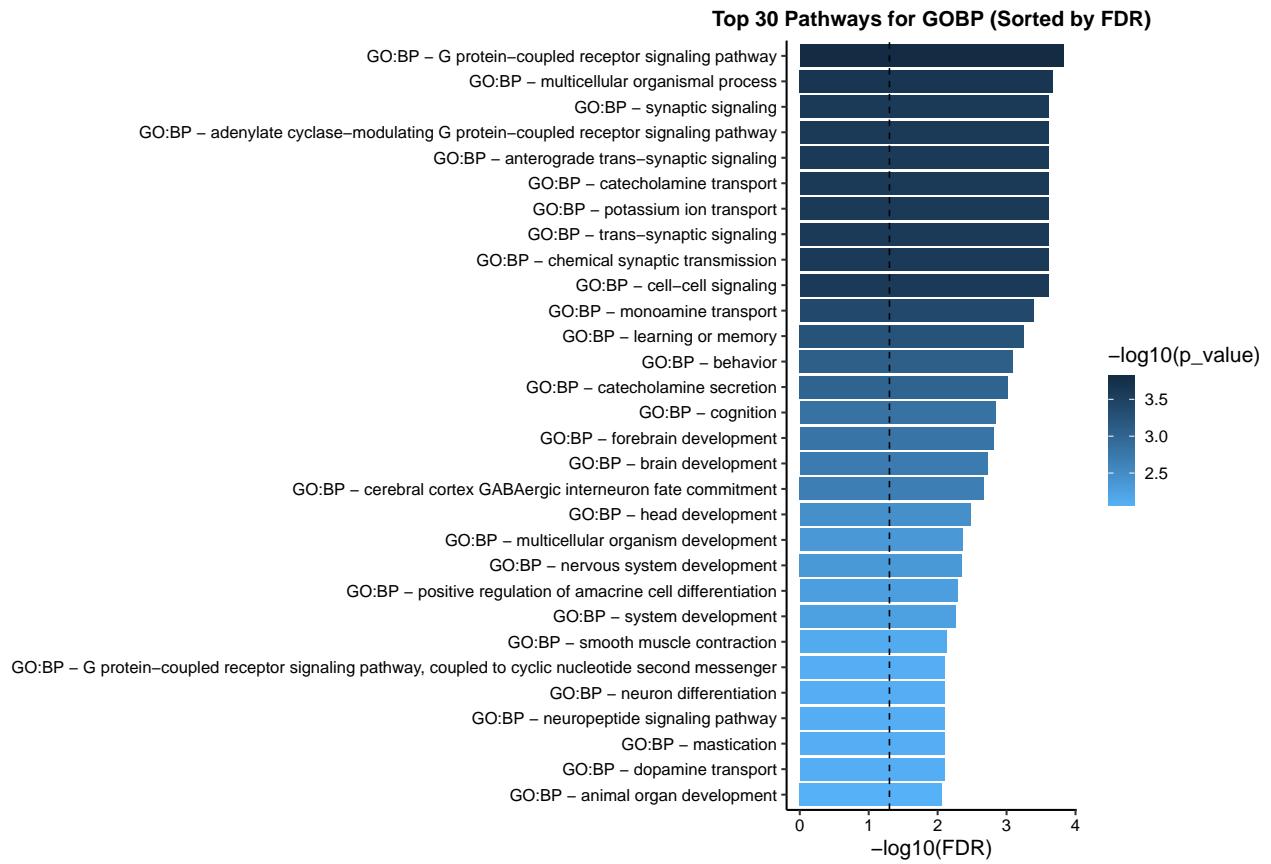


```
Enrichment_analysis(gene_list = down_gene_1.5,
                    result_folder = file.path(result_folder,"03-Enrichment"),
                    file_name = "02-DEG_1.5_down", gene_name_mapping, flag = "Down")

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



```
## [1] "Enrichment analysis for GOBP_02-DEG_1.5_down "
```



## 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                purrrr_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] ggrep_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
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## [107] statmod_1.5.0              tzdb_0.4.0
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## [111] tools_4.4.0                cachem_1.1.0

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## [121] ica_1.0-3              Rsamtools_2.20.0
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## [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
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## [133] mvtnorm_1.3-3          timechange_0.3.0
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## [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
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## [147] bdsmatrix_1.3-7         GOSemSim_2.30.2
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## [151] lazyeval_0.2.2           shiny_1.10.0
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## [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
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## [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
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## [189] spatstat.explore_3.3-4

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