Differential Expression Analysis for bulk RNA-seq data AD Condition: Vehicle 100 vs 10

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2025-03-24

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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.

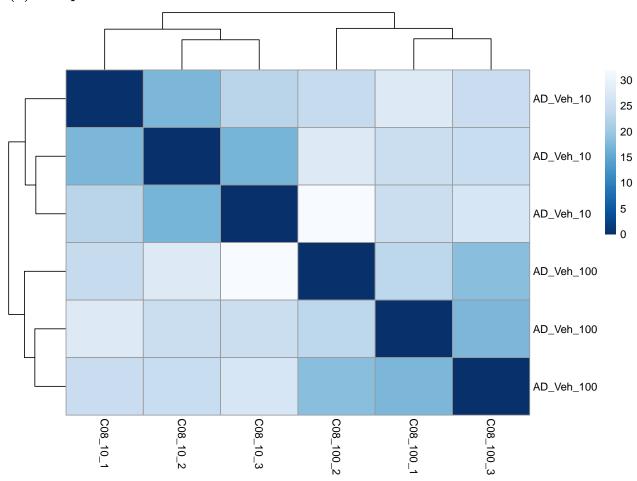
2. Differential expression analysis

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.

[1] "DEG analysis is done"

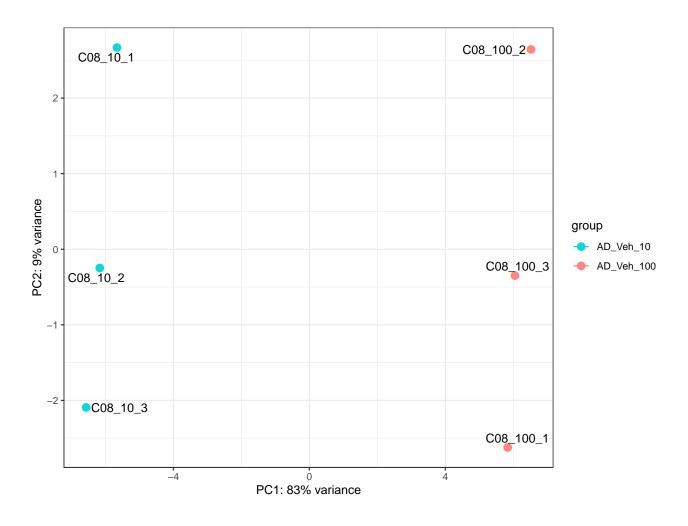
3. Visualization for reuslt

(1) Sample information



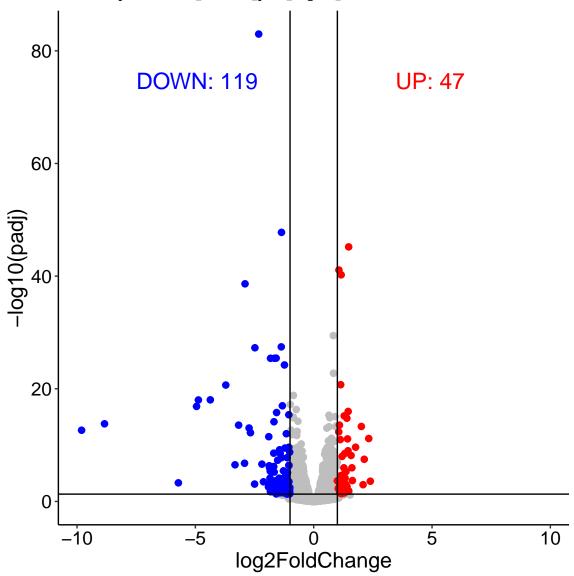
[1] "Sample distance heatmap is done"

[1] "PCA plot is done"

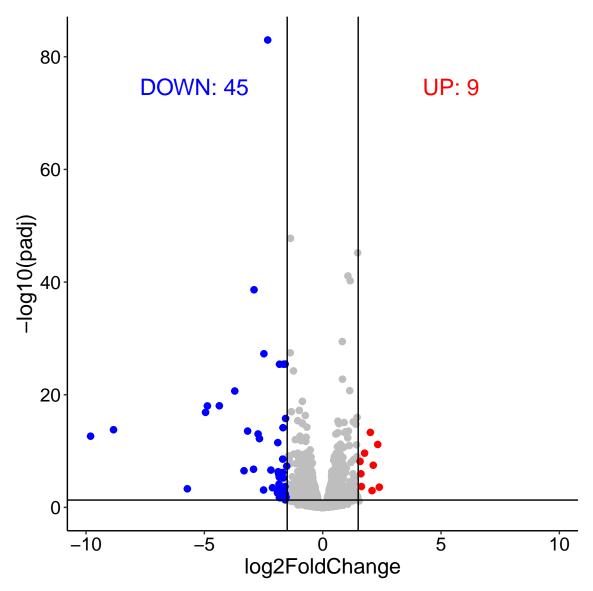


(2) DEG visualization - Volcano plot and Heatmap

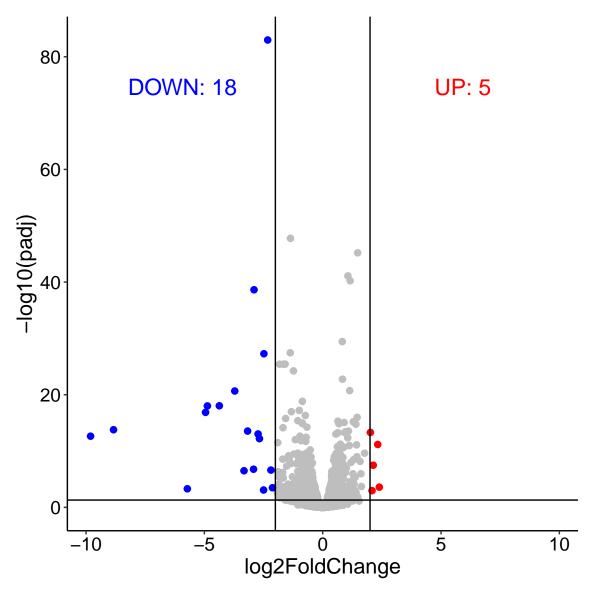
[1] "Volcano plot for 02_volcano_plot_log2fc_1"



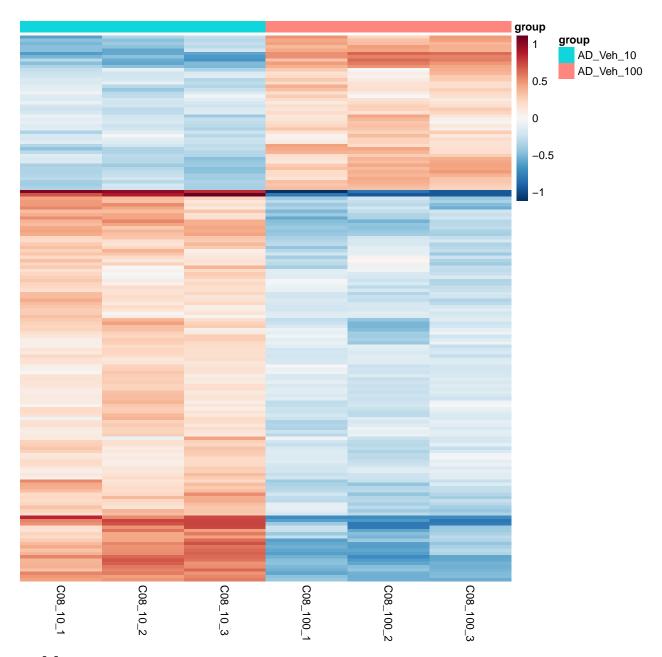
[1] "Volcano plot for 03_volcano_plot_log2fc_1.5"



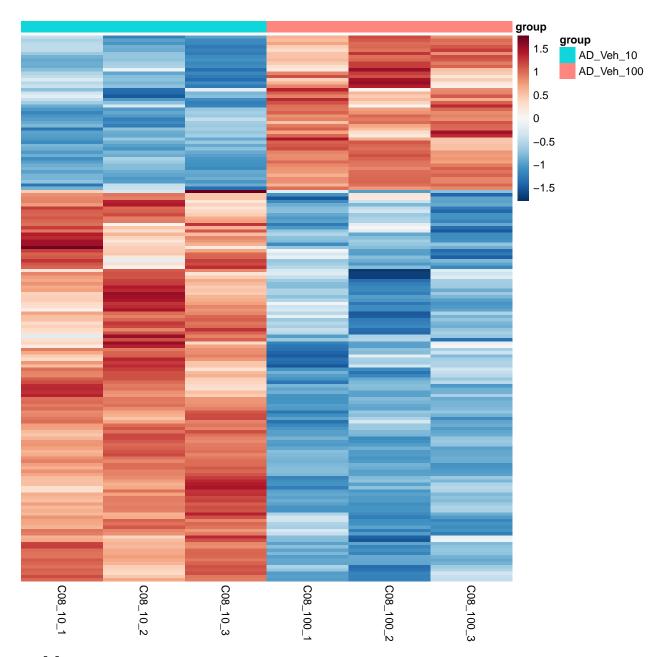
[1] "Volcano plot for 03_volcano_plot_log2fc_2"



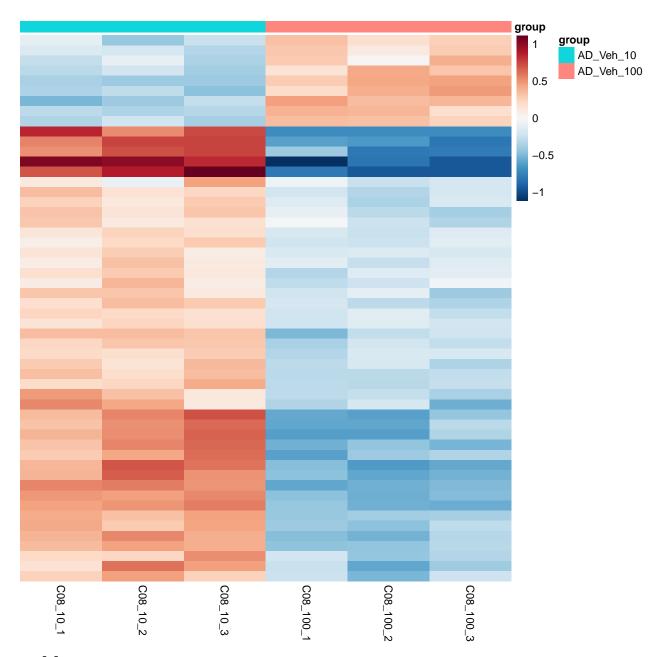
[1] "Heatmap for 02_heatmap_log2fc_1 "



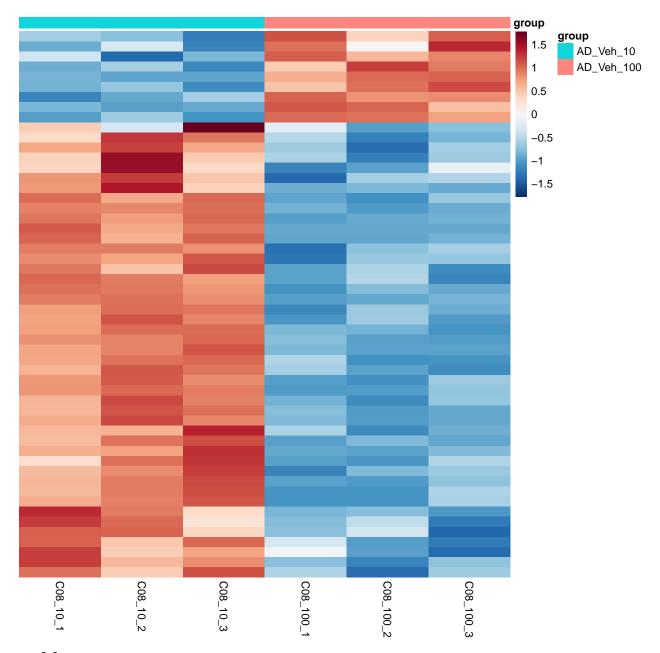
[1] "Heatmap for 02_heatmap_log2fc_1_row "



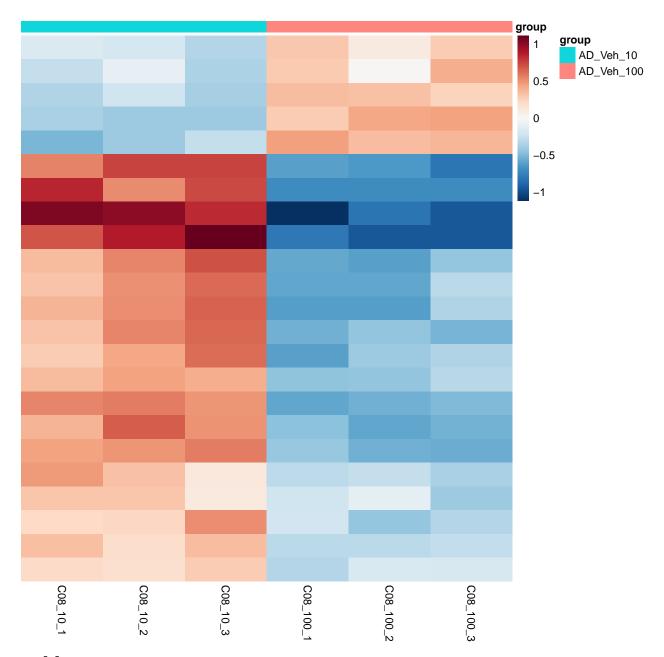
[1] "Heatmap for 03_heatmap_log2fc_1.5 " $\,$



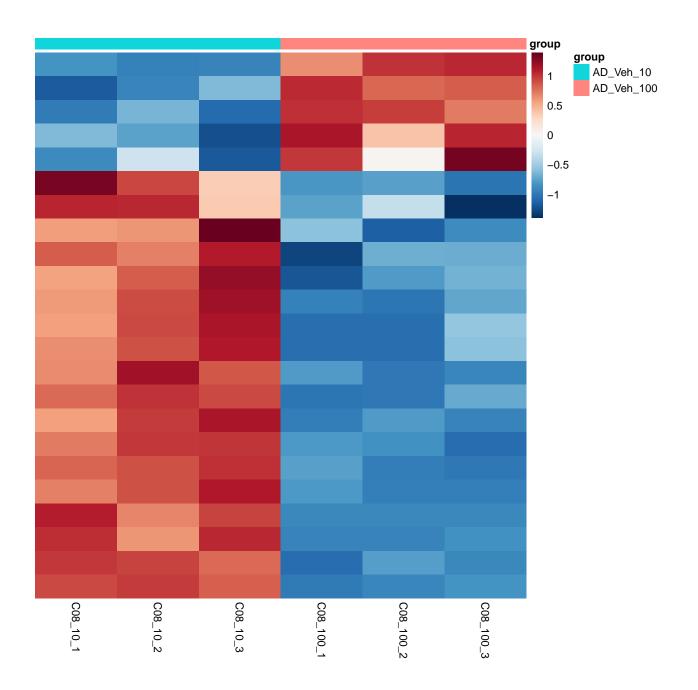
[1] "Heatmap for 03_heatmap_log2fc_1.5_row " $\,$



[1] "Heatmap for 03_heatmap_log2fc_2 " $\,$

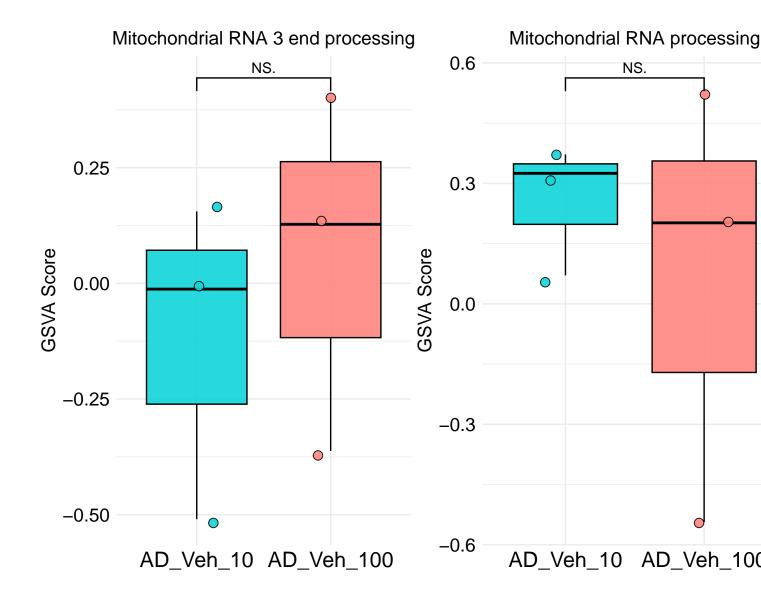


[1] "Heatmap for 03_heatmap_log2fc_2_row " $\,$



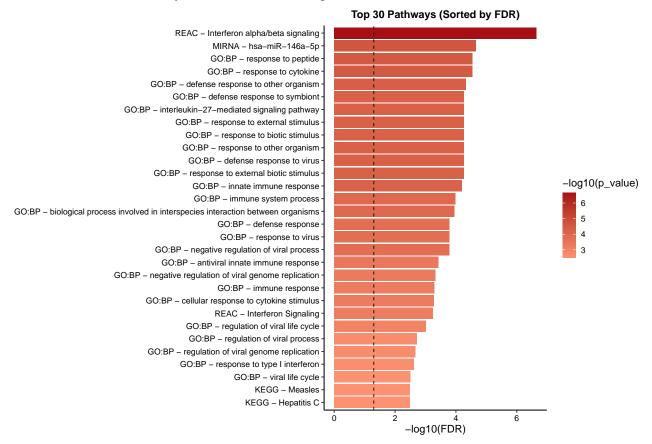
4. GSVA analysis

```
## 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
## |
```

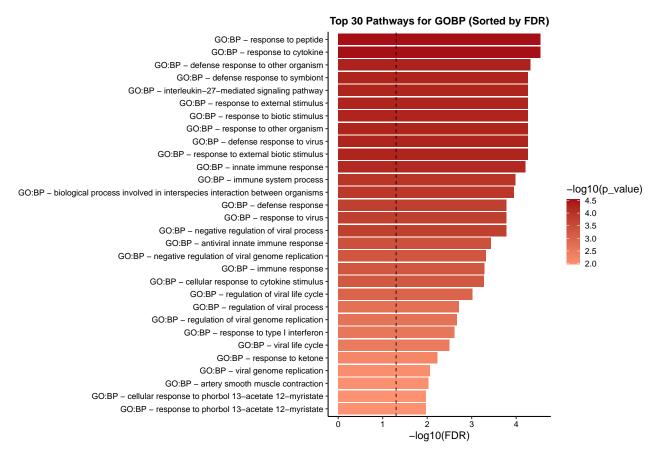


5. Pathway Enrichment Analysis

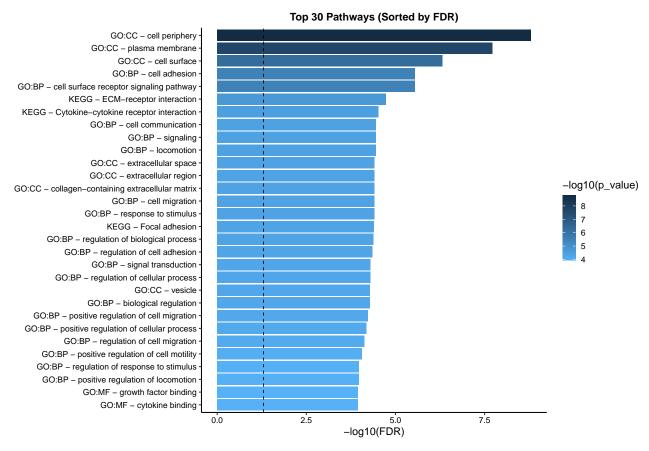
[1] "Enrichment analysis for 01-DEG_1.0_up "



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

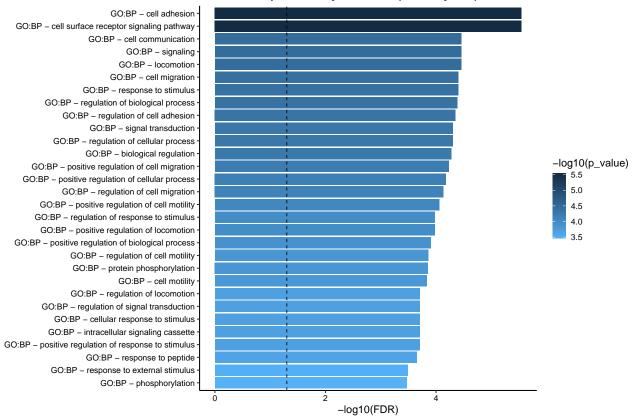


[1] "Enrichment analysis for 01-DEG_1.0_down "



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

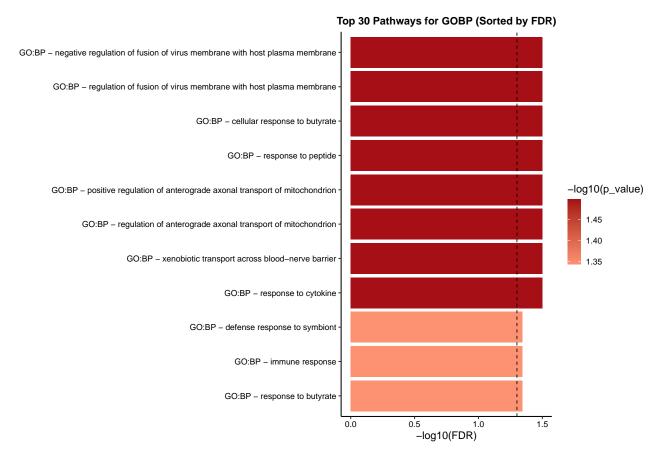




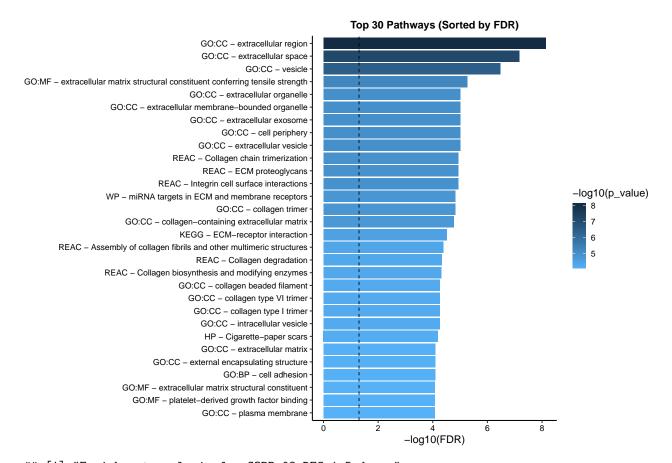
Top 30 Pathways (Sort

```
CORUM - MMP14-CLDN1 complex
                                                                                                                     CORUM - DISC-FEZ1-F-actin complex
                                                                                                                         CORUM - MMP-2-CLDN1 complex
                                                                                                                            CORUM - DISC1-FEZ1 complex
                                                                                                                              KEGG - Viral life cycle - HIV-1
                                                                            GO:BP - negative regulation of fusion of virus membrane with host plasma membrane
                                                                                    GO:BP - regulation of fusion of virus membrane with host plasma membrane
                                                                                                                        GO:BP - cellular response to butyrate
                                                                                                                                GO:BP - response to peptide
                                                                                    GO:BP - positive regulation of anterograde axonal transport of mitochondrion
                                                                                           GO:BP - regulation of anterograde axonal transport of mitochondrion
                                                                                                       GO:BP - xenobiotic transport across blood-nerve barrier
                                                                                                                               GO:BP - response to cytokine
                                                                                                                  GO:MF - beta2-adrenergic receptor activity
                                                                                                                 GO:MF - cholesterol 25-hydroxylase activity
                                                                                                                    GO:MF - C-4 methylsterol oxidase activity
                                                                                                                                        KEGG - Hepatitis C
                                                                                                                    GO:MF - beta-adrenergic receptor activity
                                                                                                                                    WP - Oxytocin signaling
                                                                                                                          WP - Disorders of NAD metabolism
                                                                                                                                    WP - CAMKK2 pathway
                                                                                                                      GO:BP - defense response to symbiont
                                                                                                                                 GO:BP - immune response
                                                                                                                               GO:BP - response to butyrate
eductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, another compound as one donor, and incorporation of one atom of oxygen
                                                                                                                                                     -log10(FDR
```

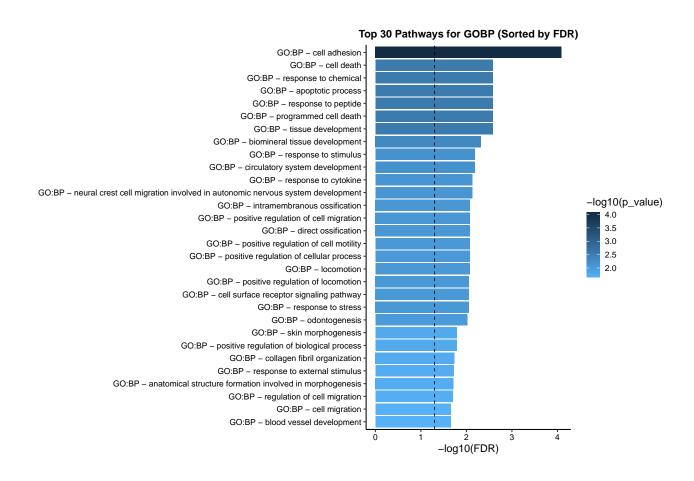
[1] "Enrichment analysis for GOBP 02-DEG_1.5_up "



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



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

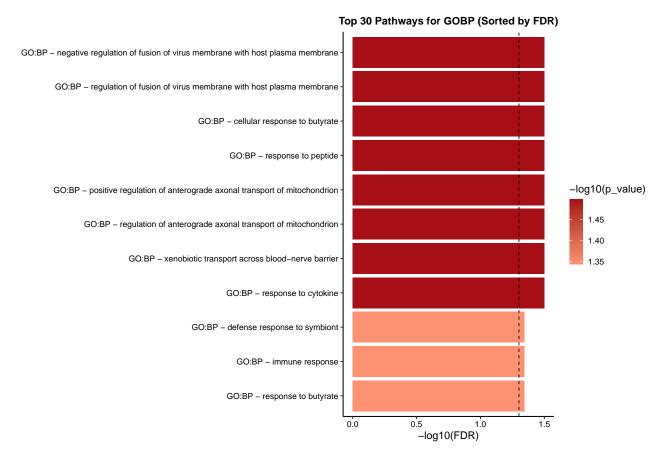


Top 30 Pathways (Sor

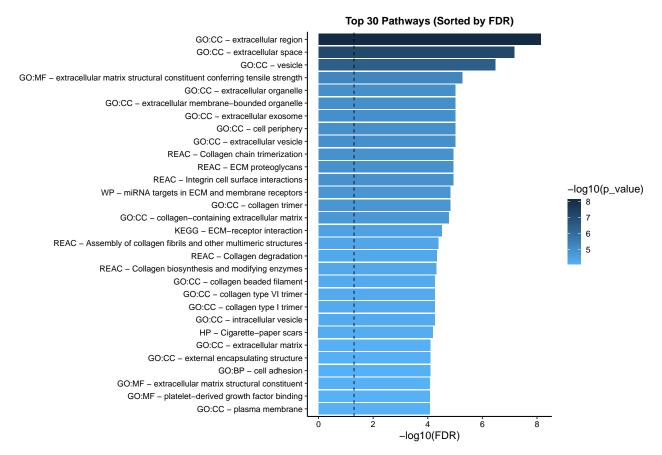
-log10(FDR

```
CORUM - MMP14-CLDN1 complex
                                                                                                                      CORUM - DISC-FEZ1-F-actin complex
                                                                                                                         CORUM - MMP-2-CLDN1 complex
                                                                                                                            CORUM - DISC1-FEZ1 complex
                                                                                                                              KEGG - Viral life cycle - HIV-1
                                                                            GO:BP - negative regulation of fusion of virus membrane with host plasma membrane
                                                                                    GO:BP - regulation of fusion of virus membrane with host plasma membrane
                                                                                                                        GO:BP - cellular response to butyrate
                                                                                                                                GO:BP - response to peptide
                                                                                    GO:BP - positive regulation of anterograde axonal transport of mitochondrion
                                                                                            GO:BP - regulation of anterograde axonal transport of mitochondrion
                                                                                                       GO:BP - xenobiotic transport across blood-nerve barrier
                                                                                                                               GO:BP - response to cytokine
                                                                                                                   GO:MF - beta2-adrenergic receptor activity
                                                                                                                  GO:MF - cholesterol 25-hydroxylase activity
                                                                                                                    GO:MF - C-4 methylsterol oxidase activity
                                                                                                                                         KEGG - Hepatitis C
                                                                                                                    GO:MF - beta-adrenergic receptor activity
                                                                                                                                     WP - Oxytocin signaling
                                                                                                                          WP - Disorders of NAD metabolism
                                                                                                                                     WP - CAMKK2 pathway
                                                                                                                       GO:BP - defense response to symbiont
                                                                                                                                  GO:BP - immune response
                                                                                                                               GO:BP - response to butyrate
eductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, another compound as one donor, and incorporation of one atom of oxygen
```

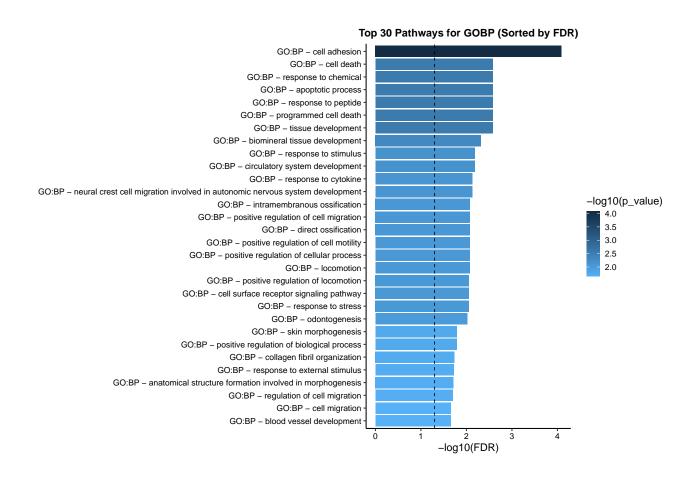
[1] "Enrichment analysis for GOBP 03-DEG_2_up "



[1] "Enrichment analysis for 03-DEG_2_down "



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



Session information

```
## R version 4.4.0 (2024-04-24)
## Platform: aarch64-apple-darwin20
## Running under: macOS Sonoma 14.3.1
## Matrix products: default
          /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
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## [11] edgeR 4.2.2
                                    limma 3.60.6
## [13] GenomicFeatures_1.56.0
                                    biomaRt_2.60.1
## [15] gprofiler2_0.2.3
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## [17] data.table_1.16.4
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## [19] AnnotationDbi_1.66.0
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## [25] apeglm_1.26.1
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## [27] SummarizedExperiment_1.34.0 Biobase_2.64.0
## [29] MatrixGenerics_1.16.0
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## [41] S4Vectors_0.42.1
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## [43] knitr_1.49
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## [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
```

```
png_0.1-8
     [9] digest_0.6.37
##
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                                     parallelly_1.42.0
                                     MASS 7.3-64
   [13] magick_2.8.5
                                     qvalue_2.36.0
  [15] httpuv_1.6.15
##
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                                     xfun_0.51
##
  [19] ggfun_0.1.8
                                     survival 3.8-3
  [21] memoise_2.0.1
                                     gson 0.1.0
##
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                                     ragg_1.3.3
##
   [25] tidytree_0.4.6
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##
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   [29] prettyunits_1.2.0
                                     KEGGREST_1.44.1
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##
   [31] promises_1.3.2
##
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##
  [65] ggtree_3.12.0
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##
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  [73] pillar_1.10.1
                                     nlme_3.1-167
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                                     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
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##
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## [113] RSQLite_2.3.9
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## [115] DBI_1.2.3
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```

```
## [117] fastmap_1.2.0
                                     rmarkdown 2.29
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## [121] ica 1.0-3
                                     Rsamtools 2.20.0
## [123] coda_0.19-4.1
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## [125] RANN_2.6.2
                                     farver_2.1.2
## [127] tidygraph 1.3.1
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                                     cli 3.6.4
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                                     timechange_0.3.0
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## [135] gtable_0.3.6
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## [139] ape_5.8-1
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## [141] RcppHNSW_0.6.0
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## [175] Rhdf5lib_1.26.0
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## [183] munsell_0.5.1
                                     KernSmooth_2.23-26
## [185] htmlwidgets 1.6.4
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## [187] rlang_1.1.5
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## [189] spatstat.explore 3.3-4
```