

# Differential Expression Analysis for bulk RNA-seq data

Vehicle Condition: CTRL vs FXS

Ximing Ran

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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`.

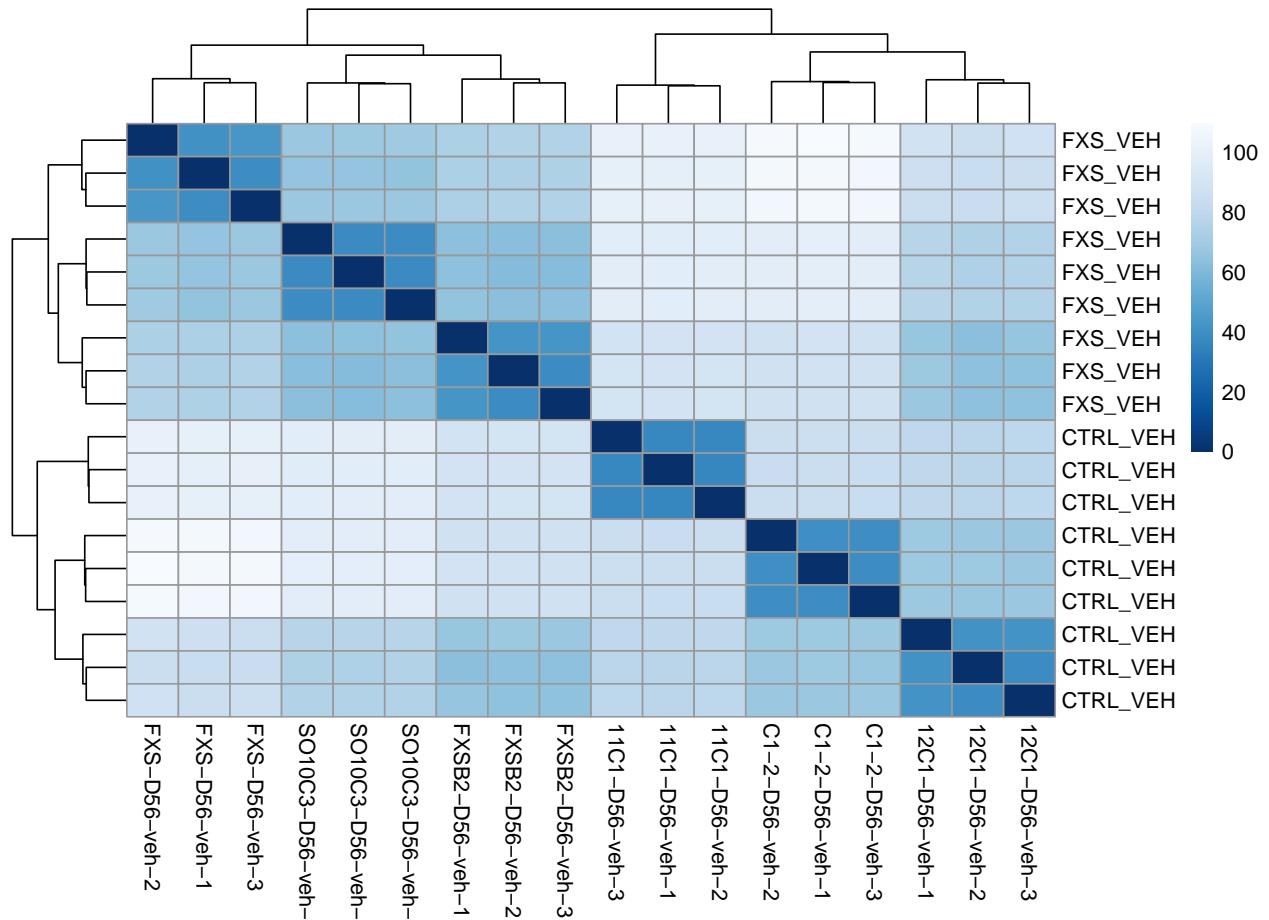
## **2. Differential expression analysis**

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

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

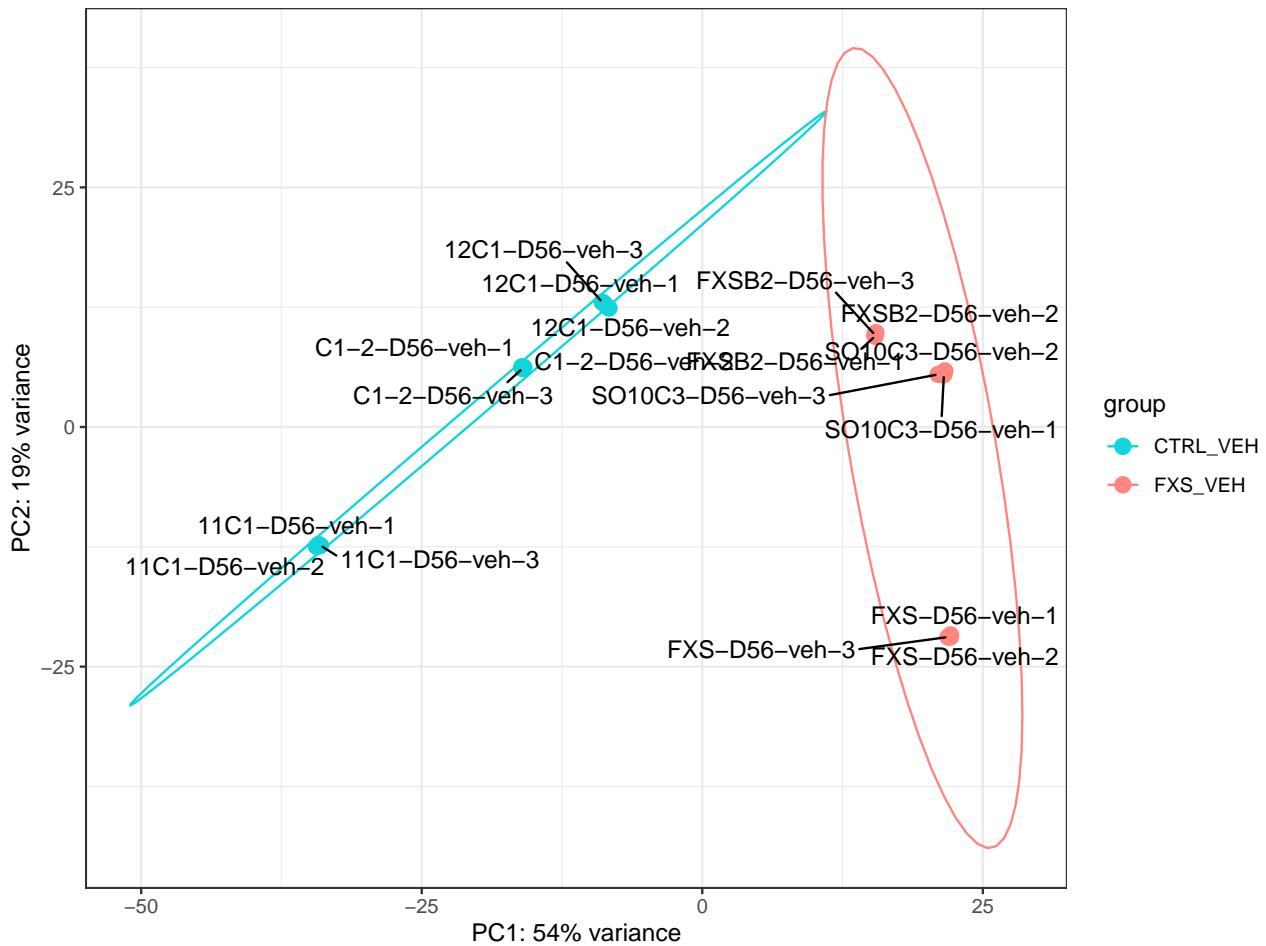
### 3. Visualization for result

#### (1) Sample information



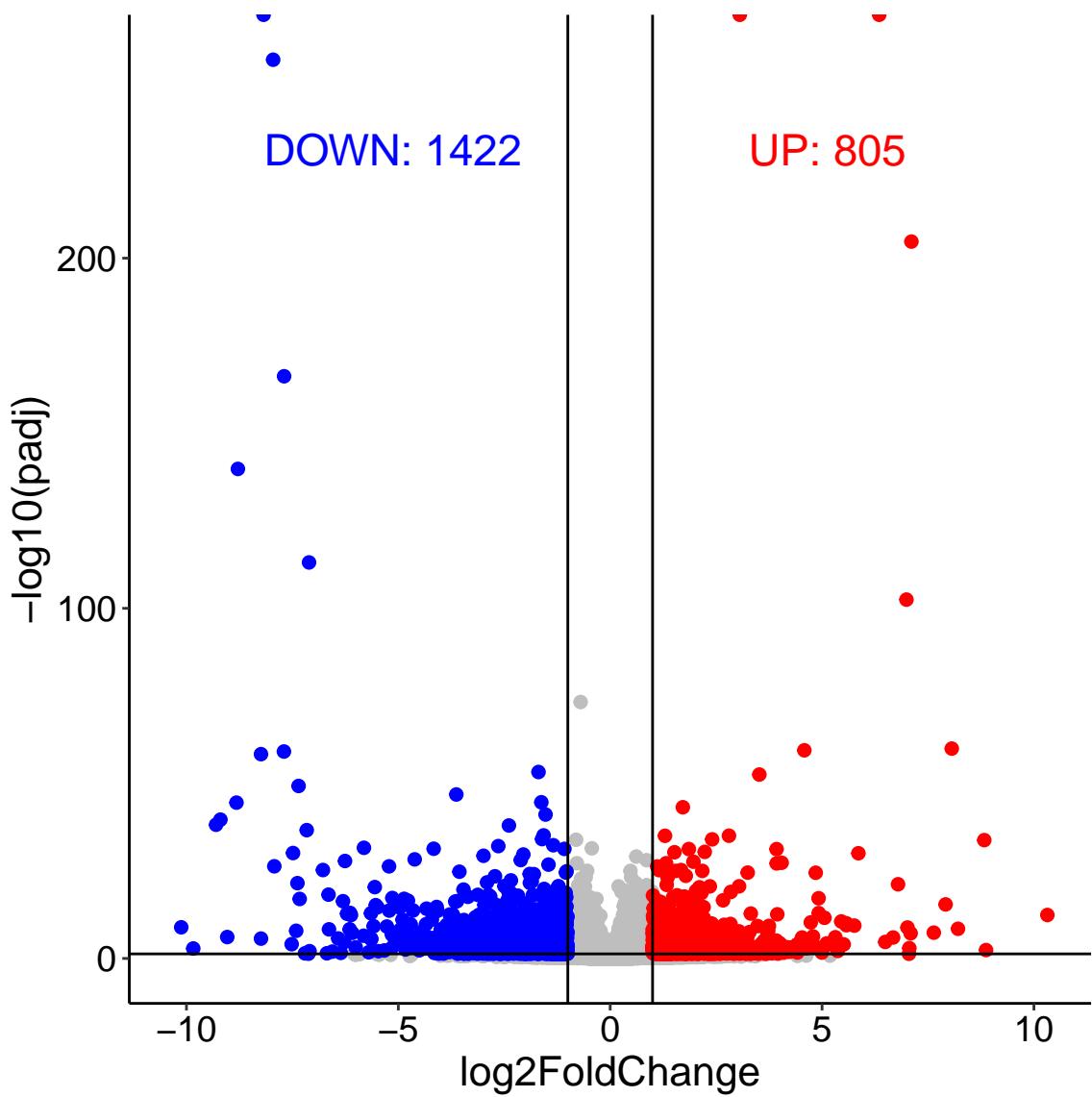
```
## [1] "Sample distance heatmap is done"
```

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

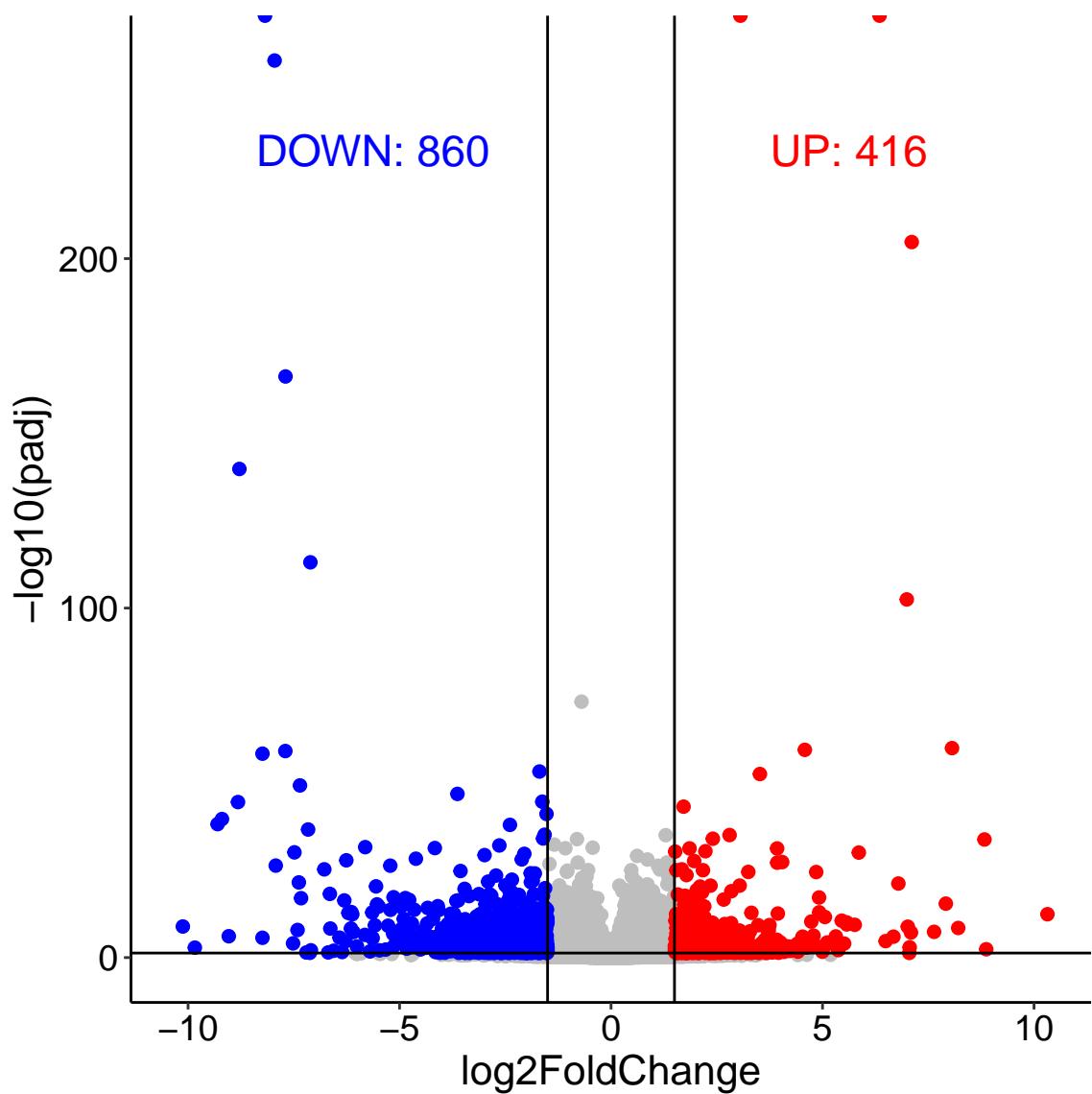


## (2) DEG visualization - Volcano plot and Heatmap

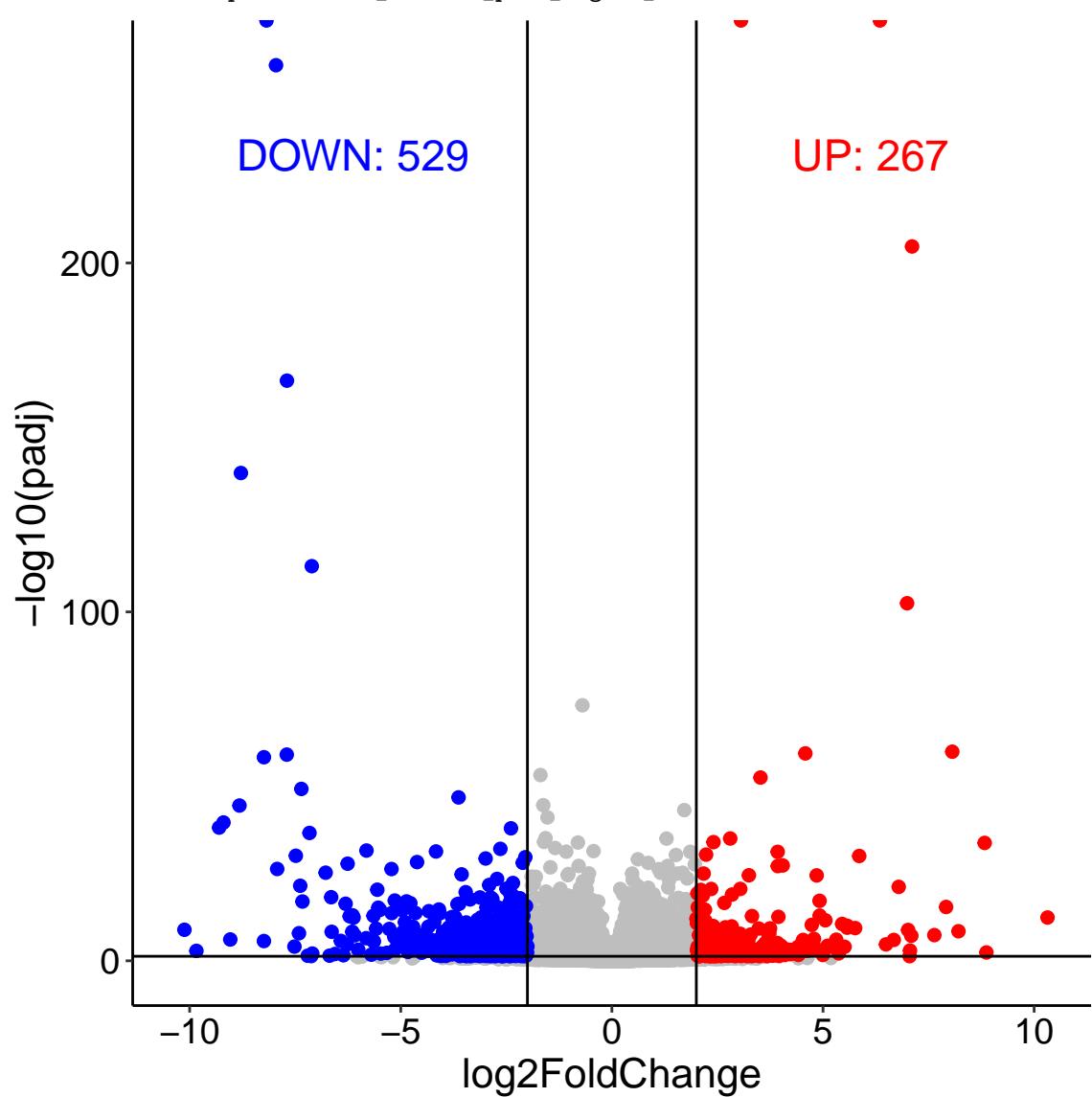
```
## [1] "Volcano plot for 02_volcano_plot_log2fc_1"
```



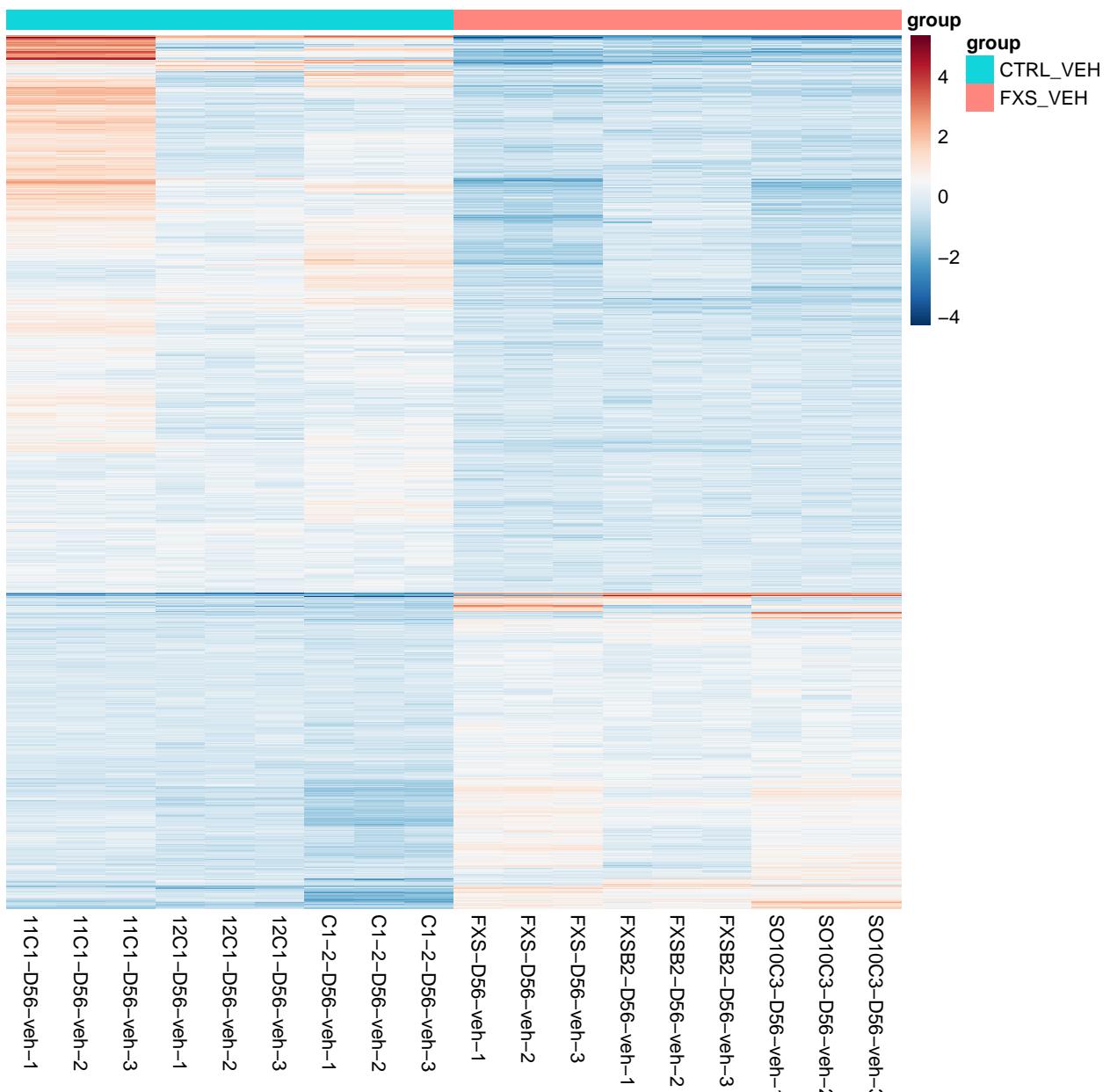
```
## [1] "Volcano plot for 02_volcano_plot_log2fc_1.5"
```



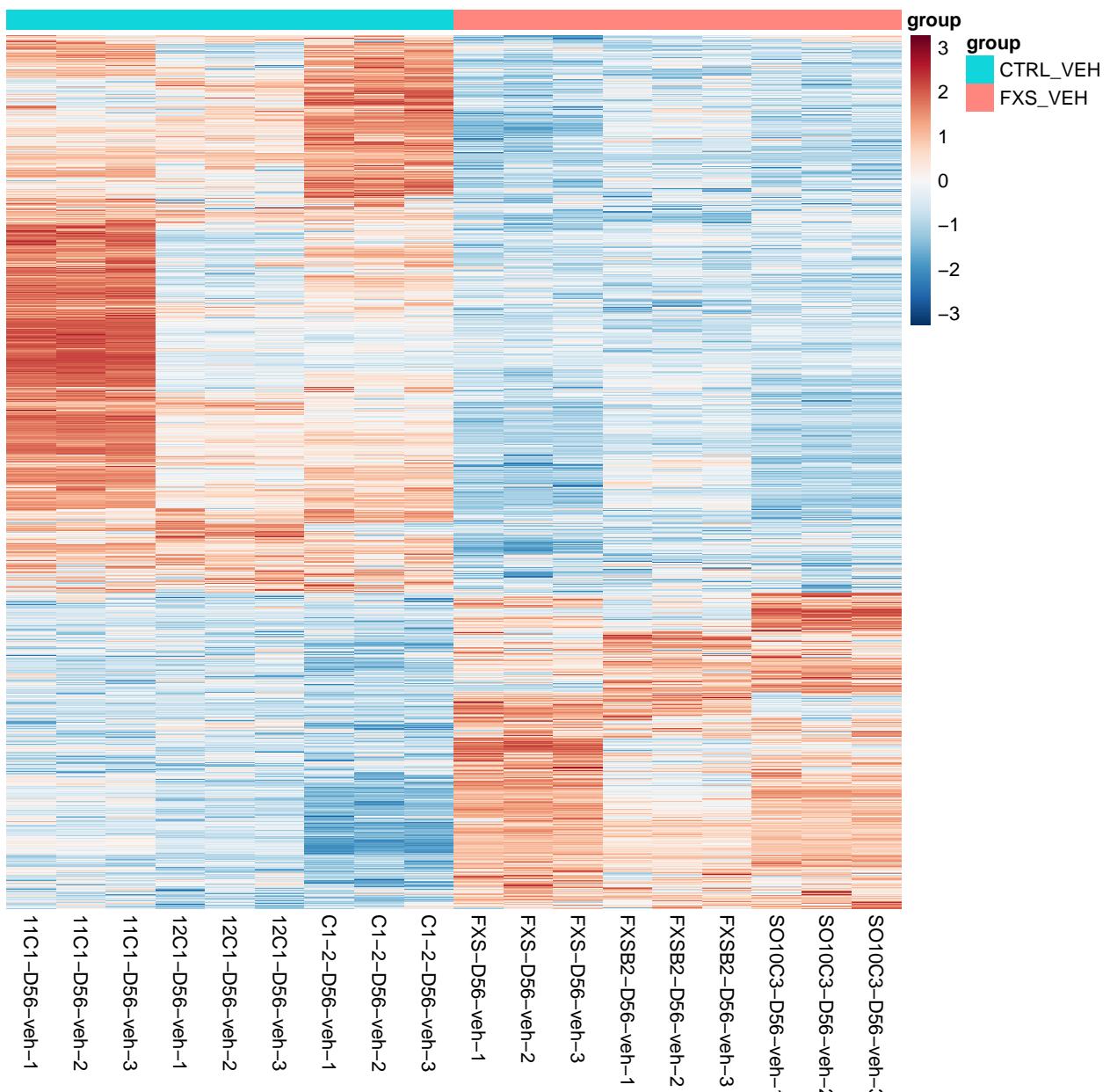
```
## [1] "Volcano plot for 02_volcano_plot_log2fc_2"
```



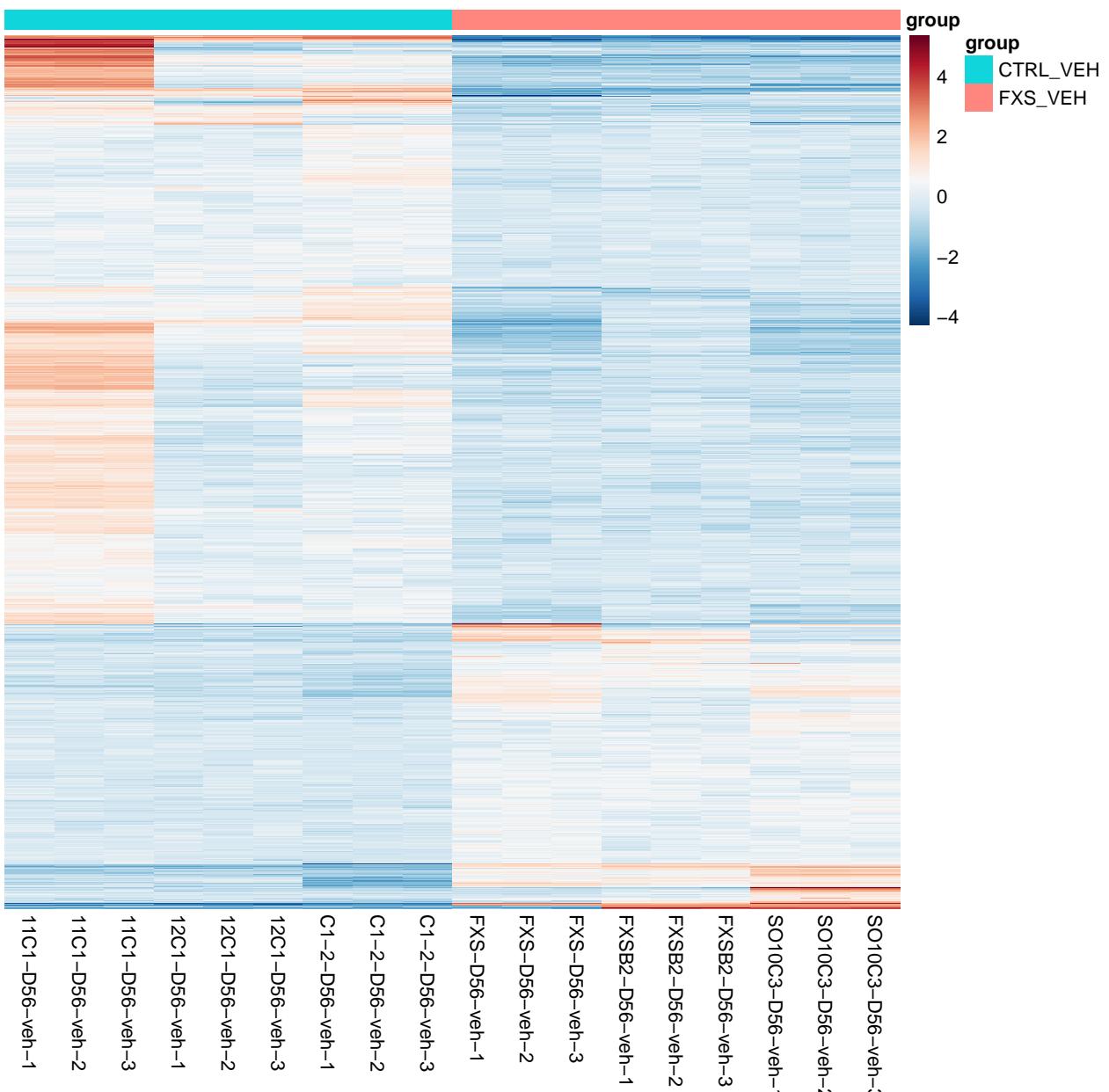
```
## [1] "Heatmap for 03_heatmap_log2fc_1 "
```



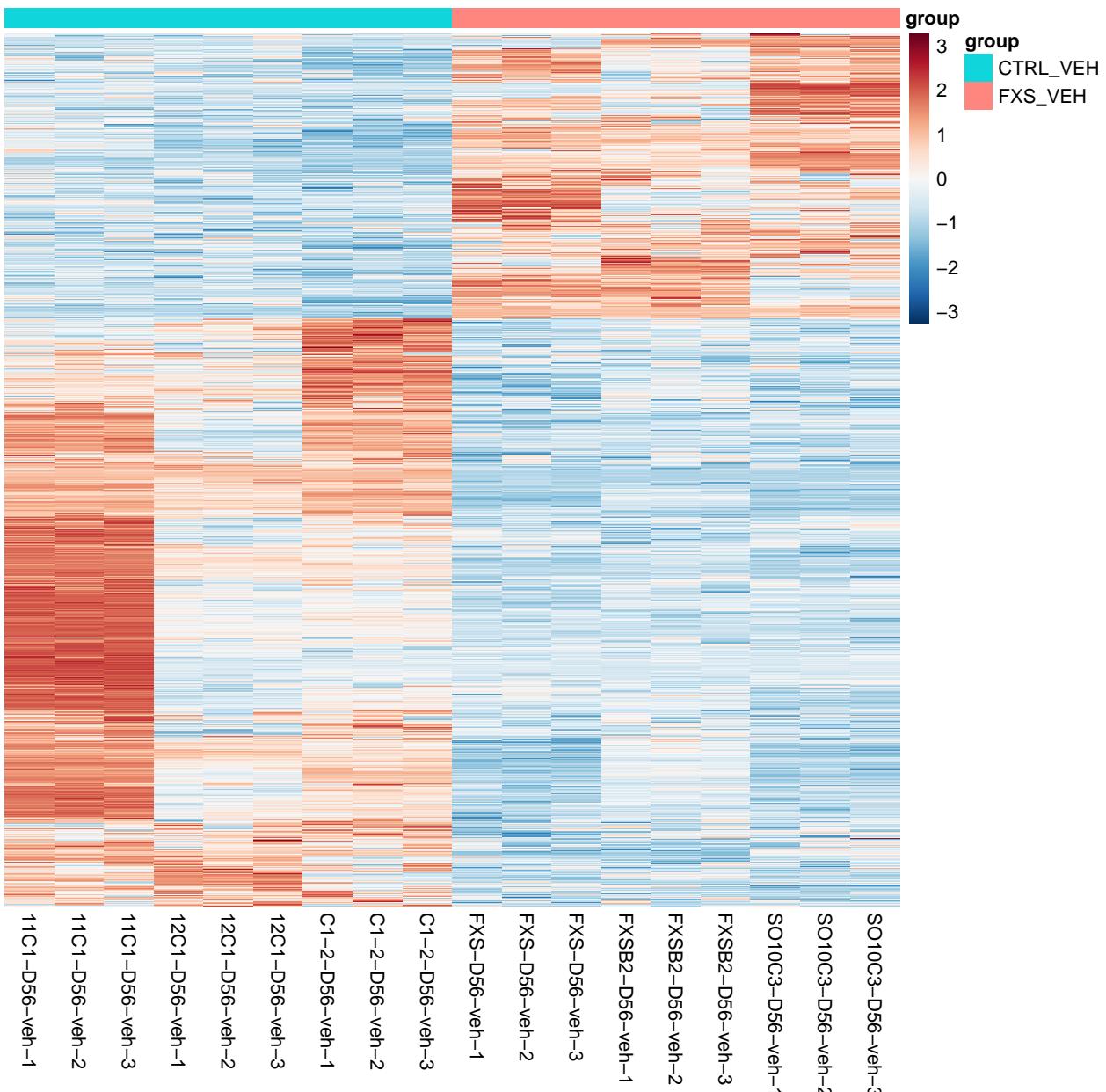
```
## [1] "Heatmap for 03_heatmap_log2fc_1_row "
```



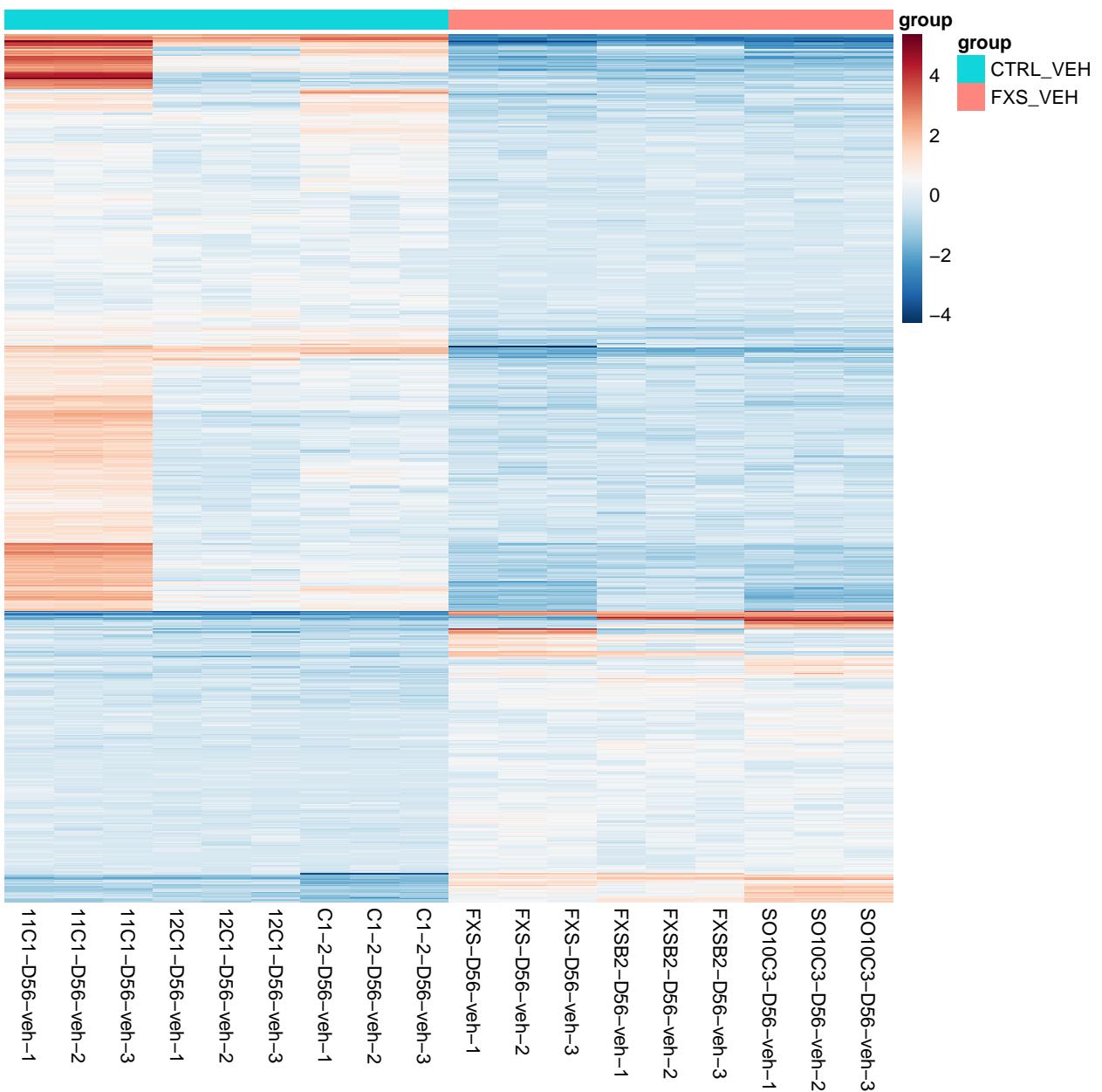
```
## [1] "Heatmap for 03_heatmap_log2fc_1.5 "
```



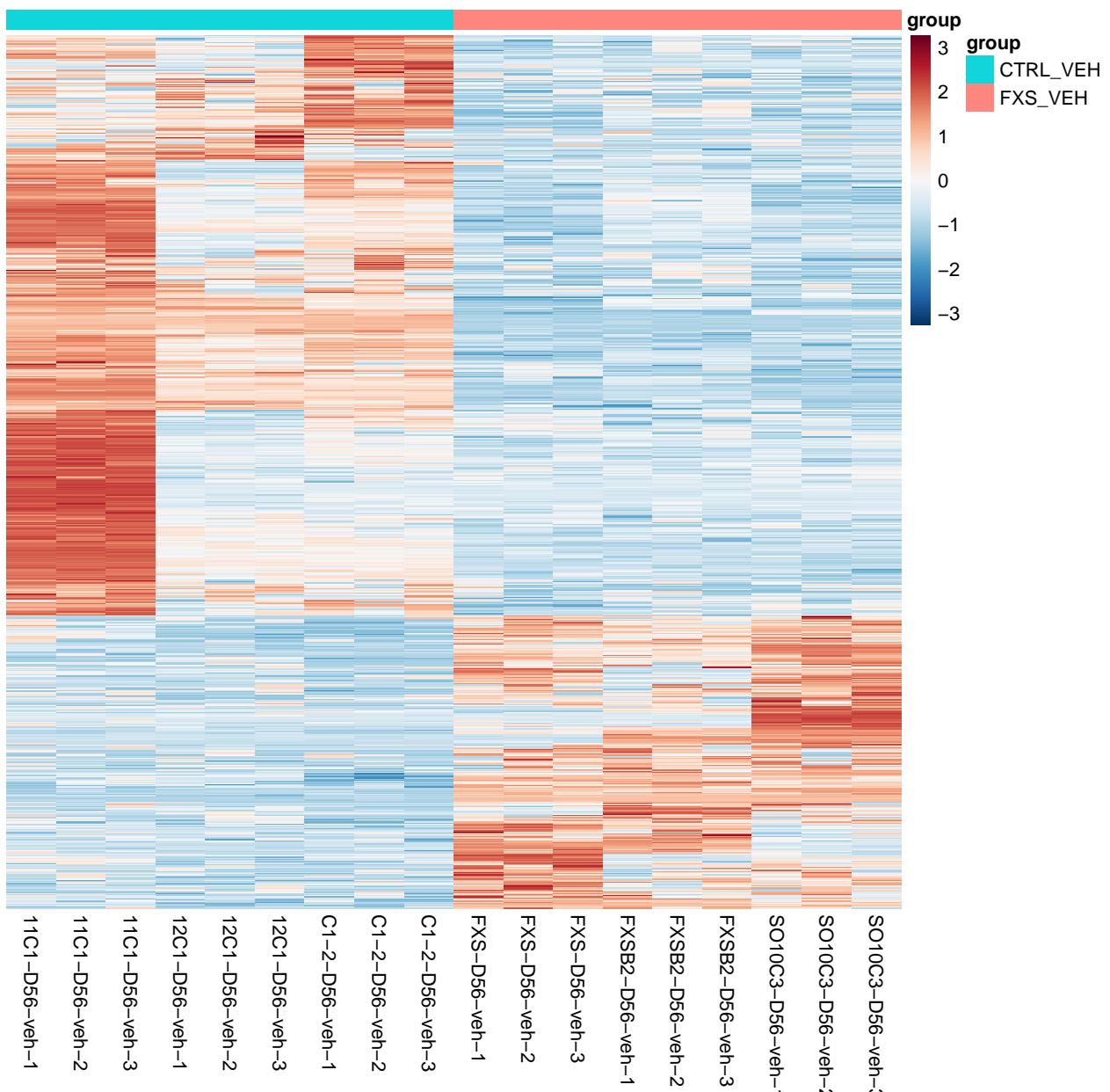
```
## [1] "Heatmap for 03_heatmap_log2fc_1.5_row "
```



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

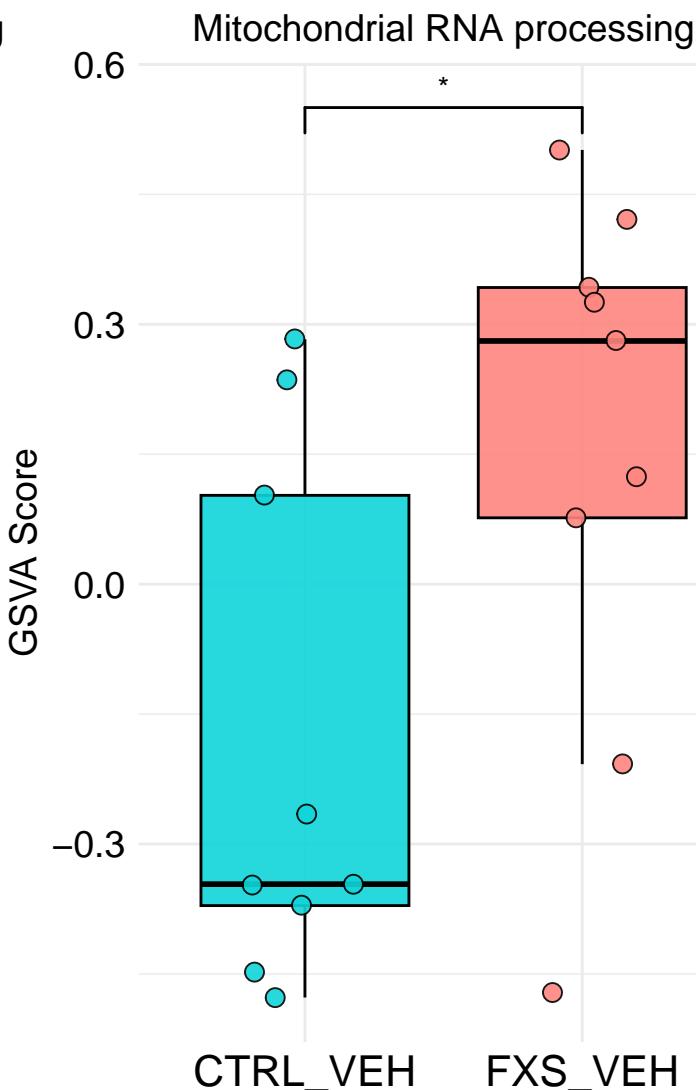
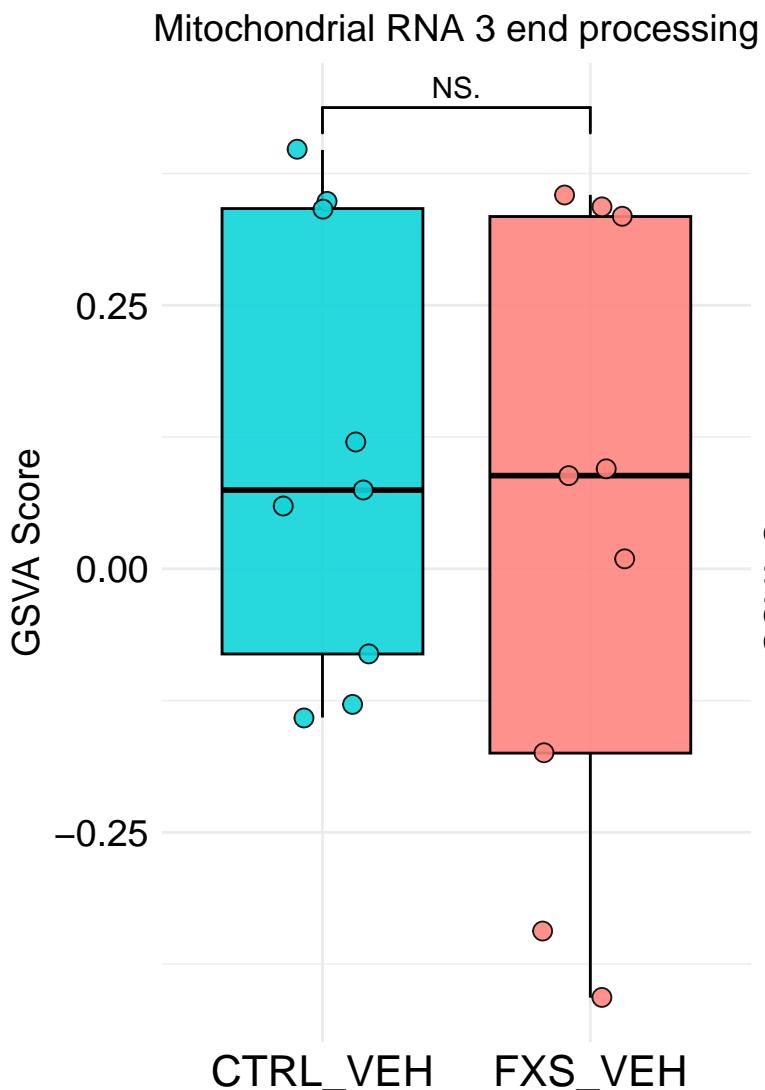


```
## [1] "Heatmap for 03_heatmap_log2fc_2_row "
```



#### 4. GSVA analysis

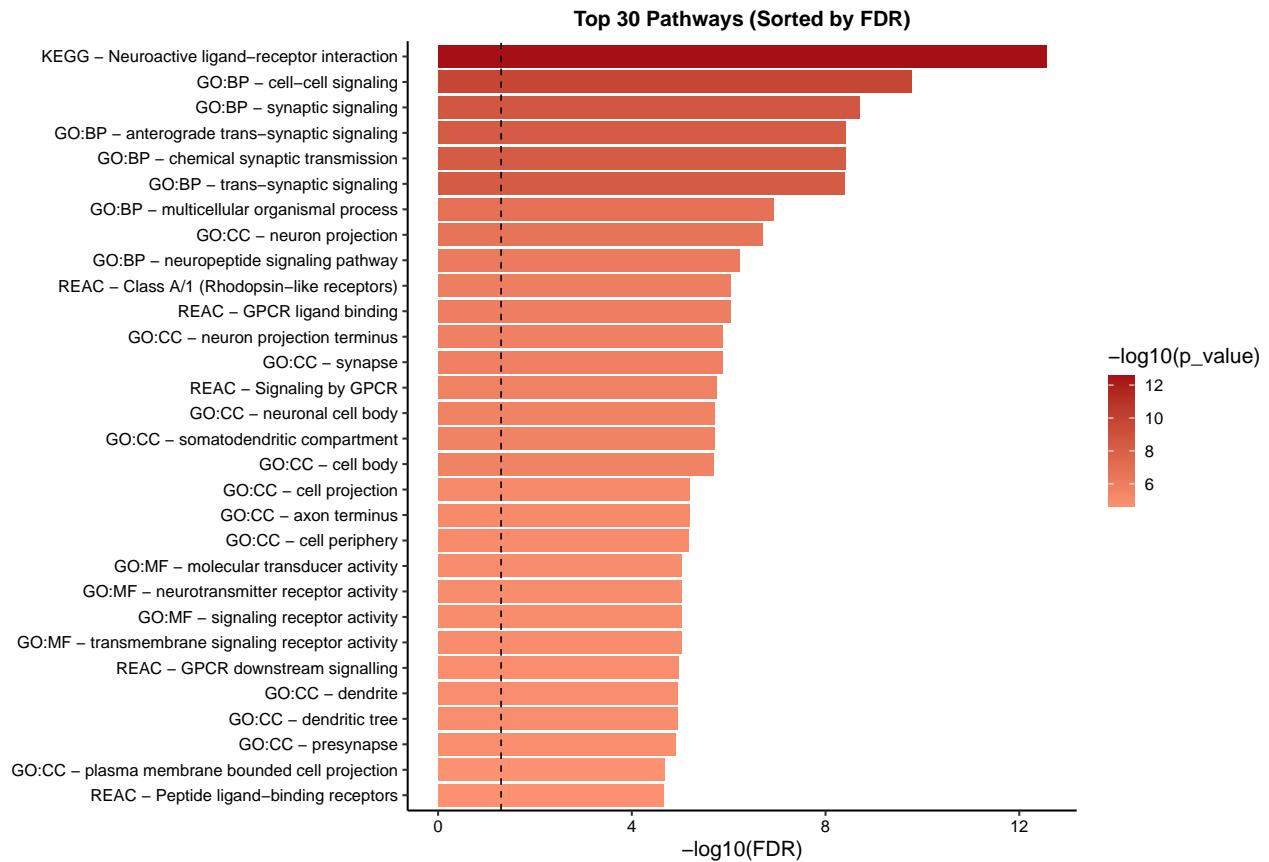
```
## [1] "GOBP_MITOCHONDRIAL_RNA_3_END_PROCESSING"  
## [1] "GOBP_MITOCHONDRIAL_RNA_PROCESSING"
```



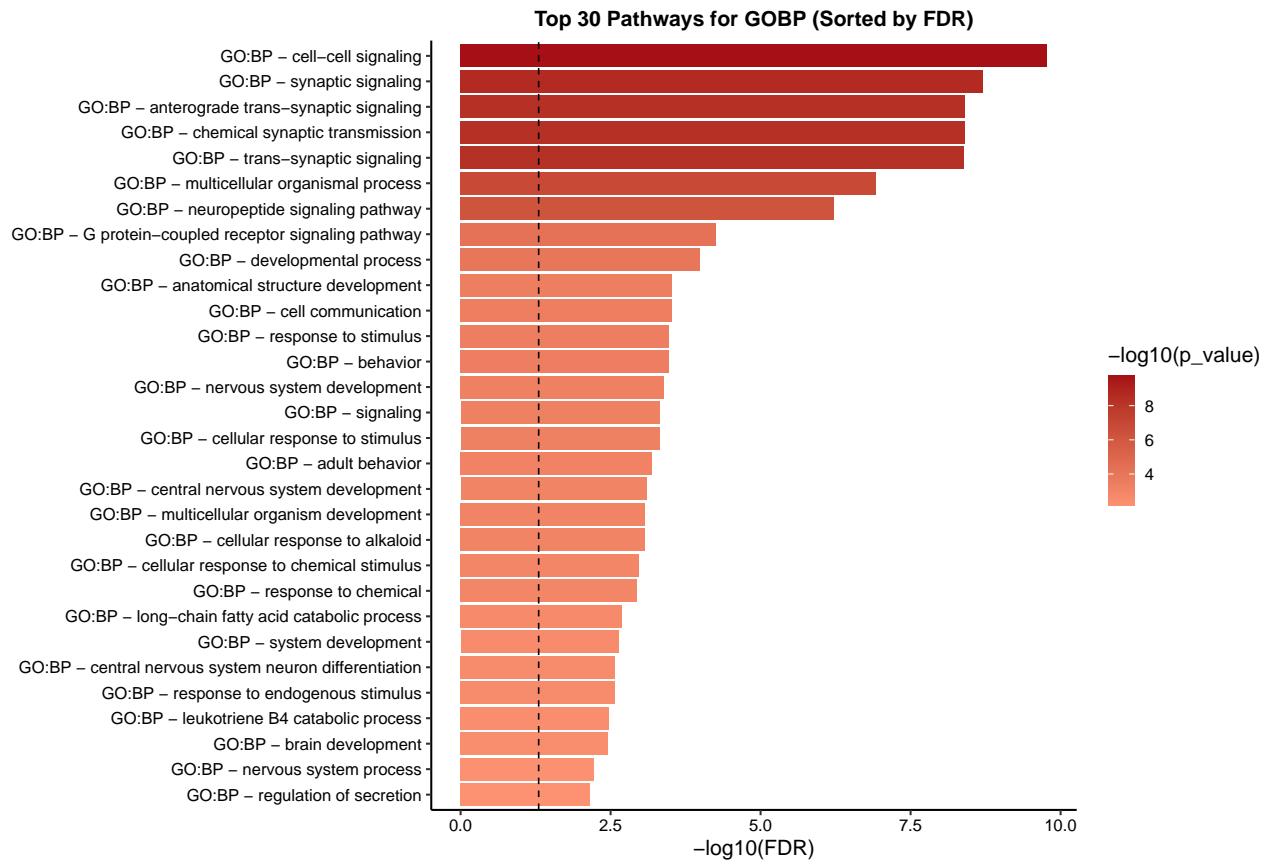
## 5. Pathway Enrichment Analysis

### (1) DEG threshold: $\log_{2}fc > 1$

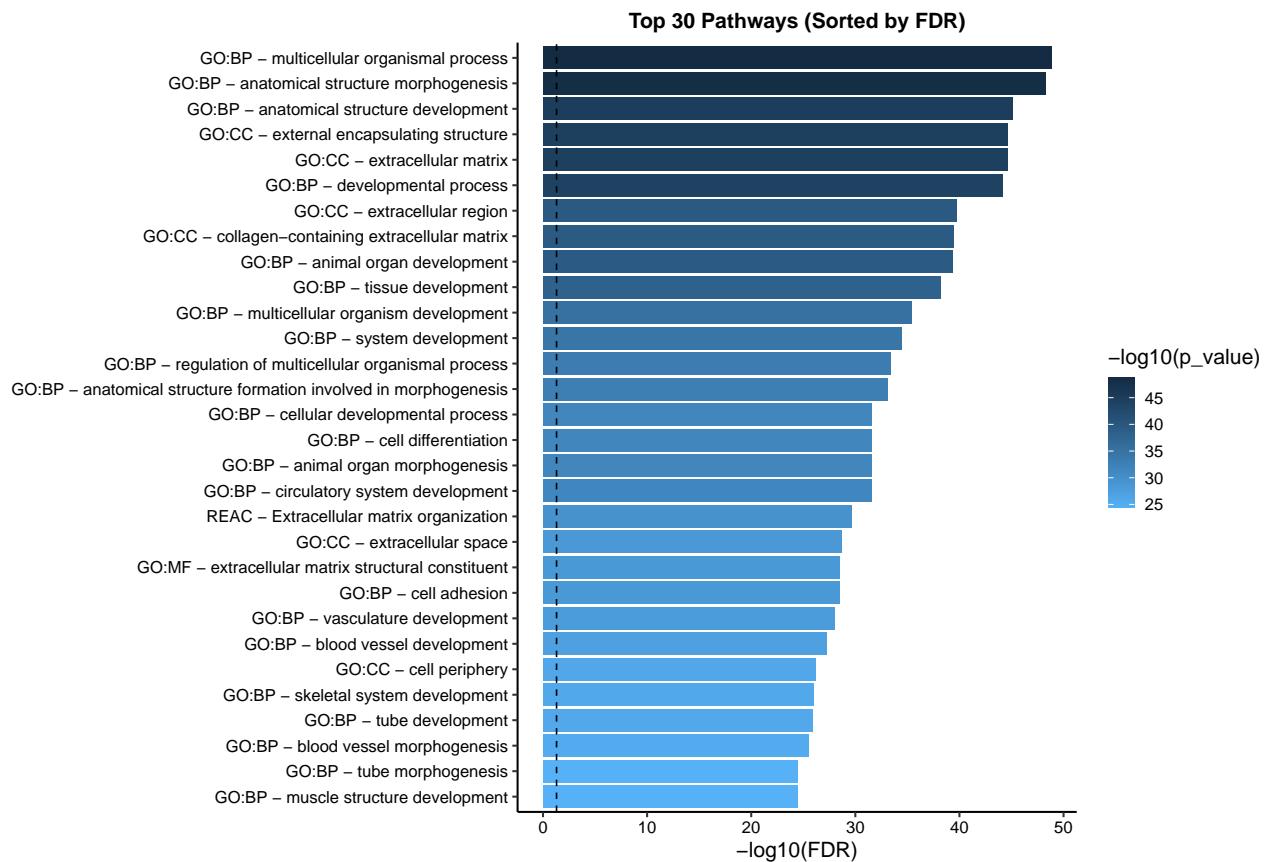
```
## [1] "Enrichment analysis for 02-DEG_1_up "
```



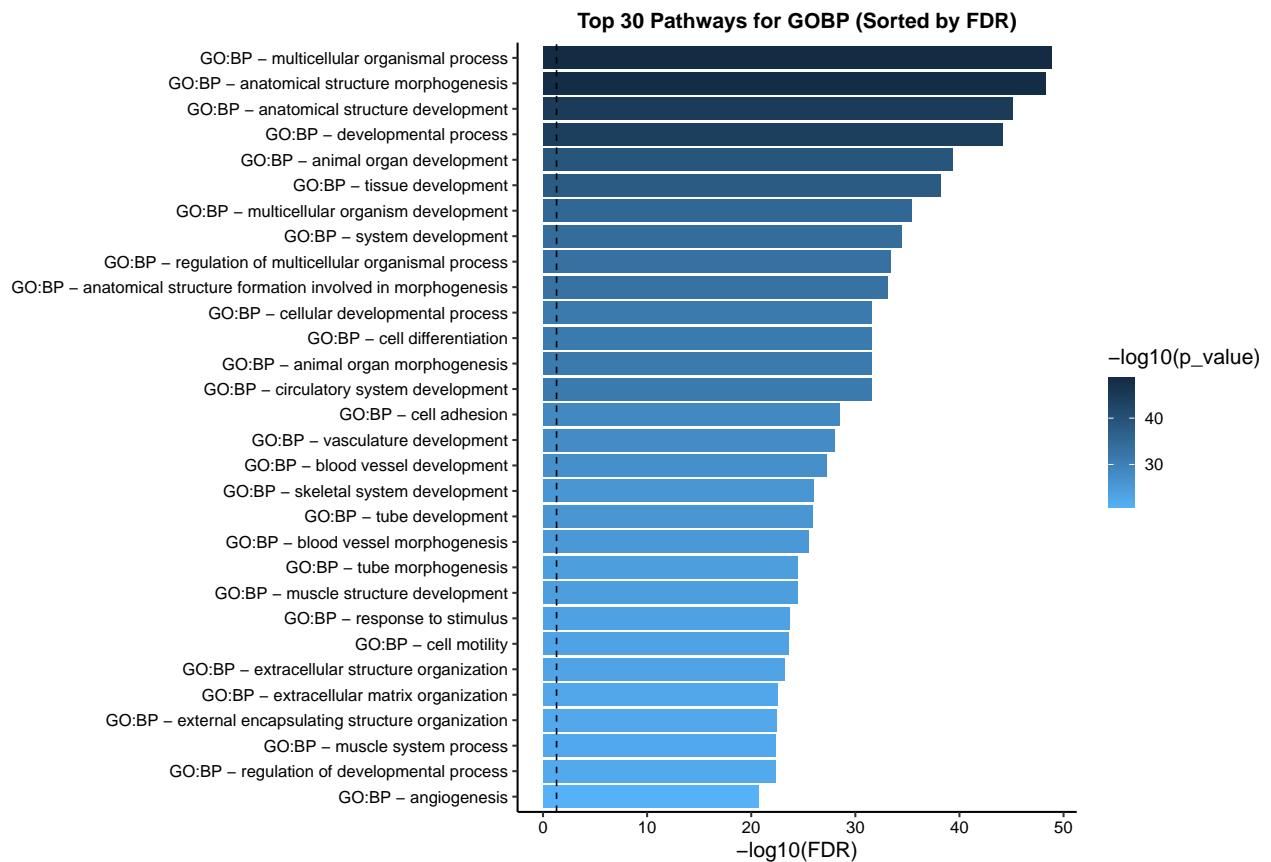
```
## [1] "Enrichment analysis for GOBP 02-DEG_1_up "
```



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

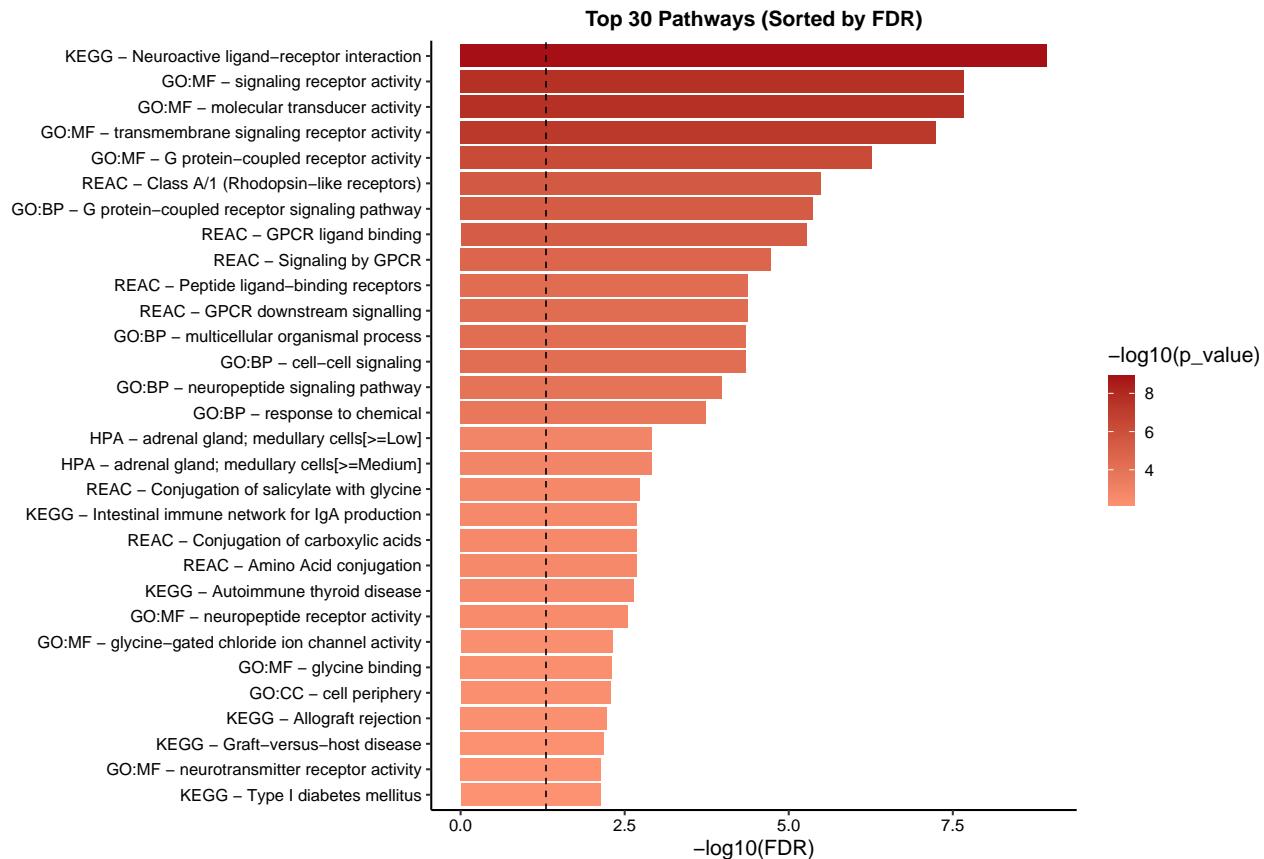


```
## [1] "Enrichment analysis for GOBP_02-DEG_1_down "
```



## (2) DEG threshold: $\log_{2}fc > 1.5$

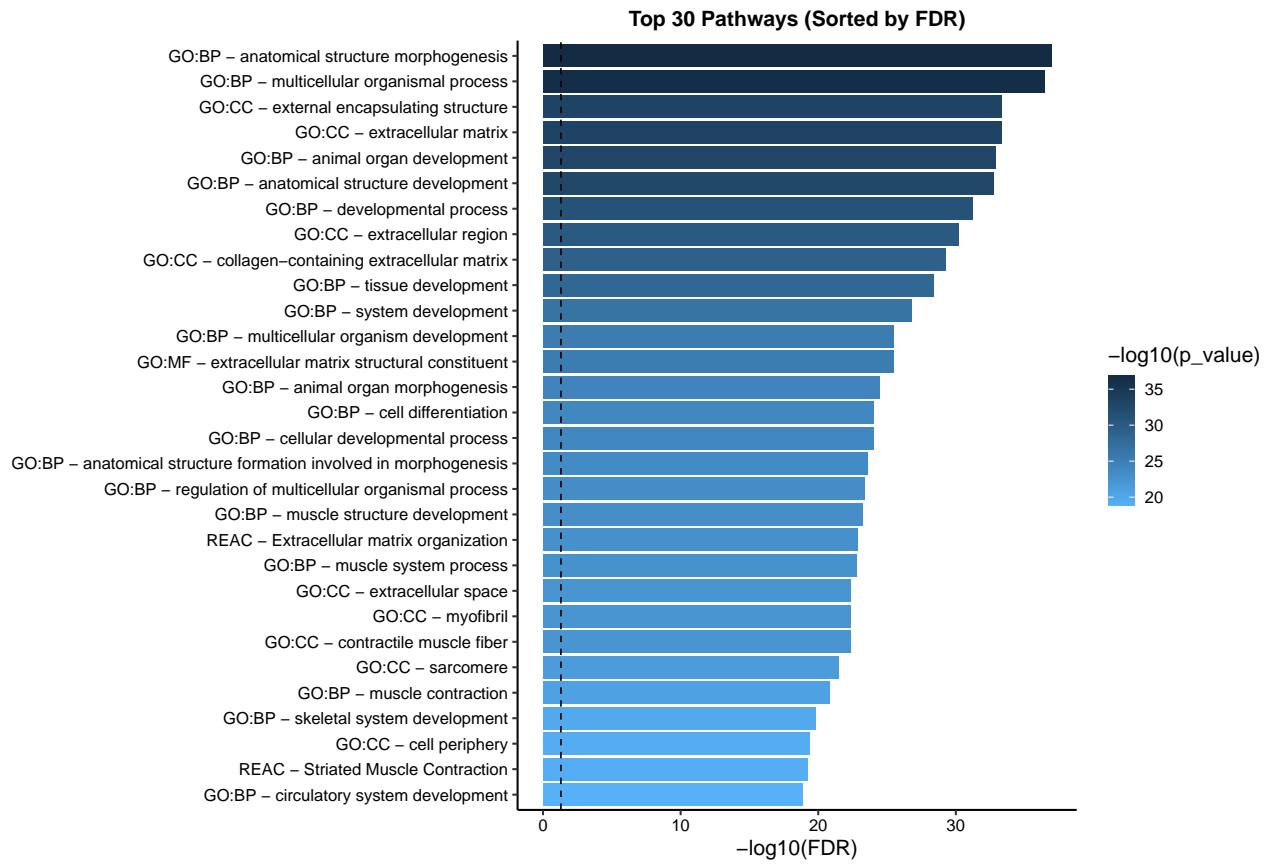
```
## [1] "Enrichment analysis for 02-DEG_1.5_up "
```



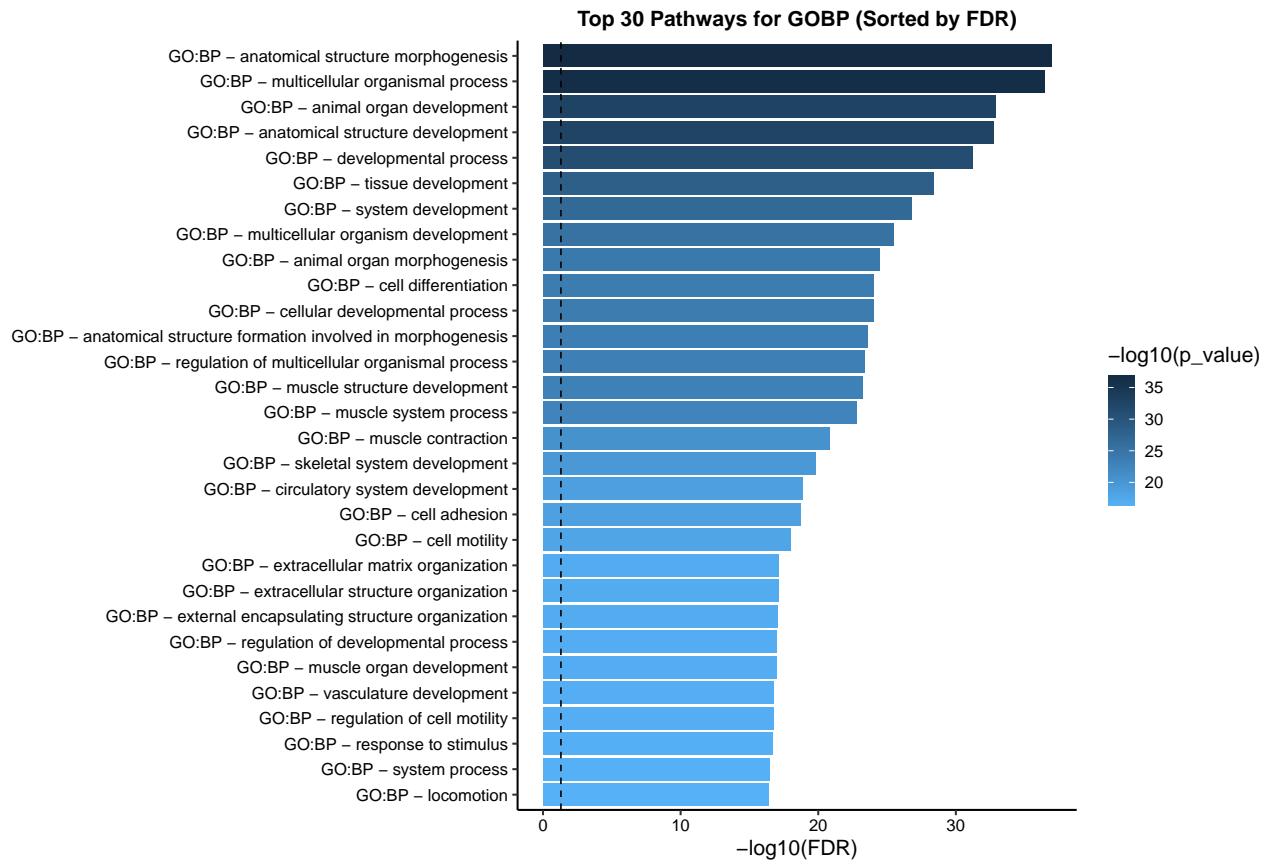
```
## [1] "Enrichment analysis for GOBP 02-DEG_1.5_up "
```



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

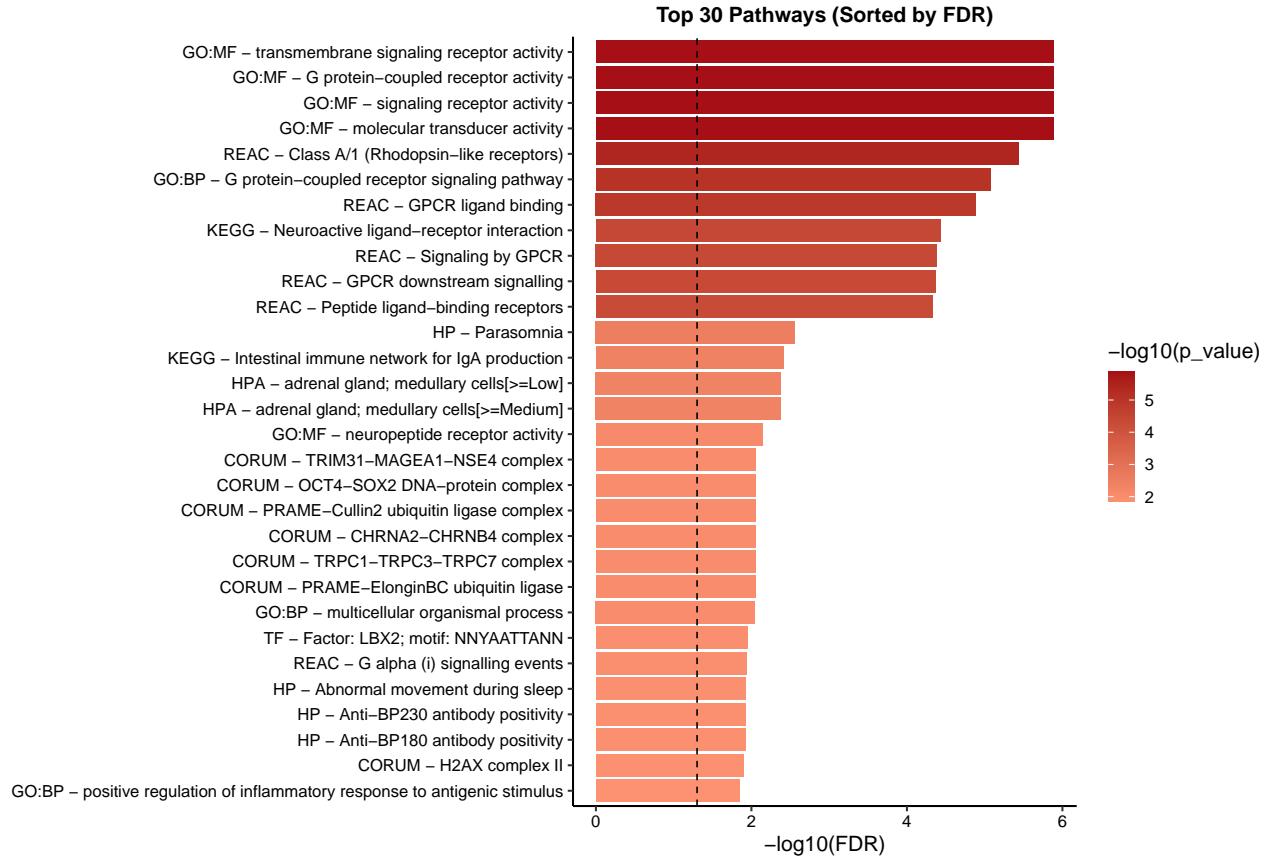


```
## [1] "Enrichment analysis for GOBP_02-DEG_1.5_down "
```

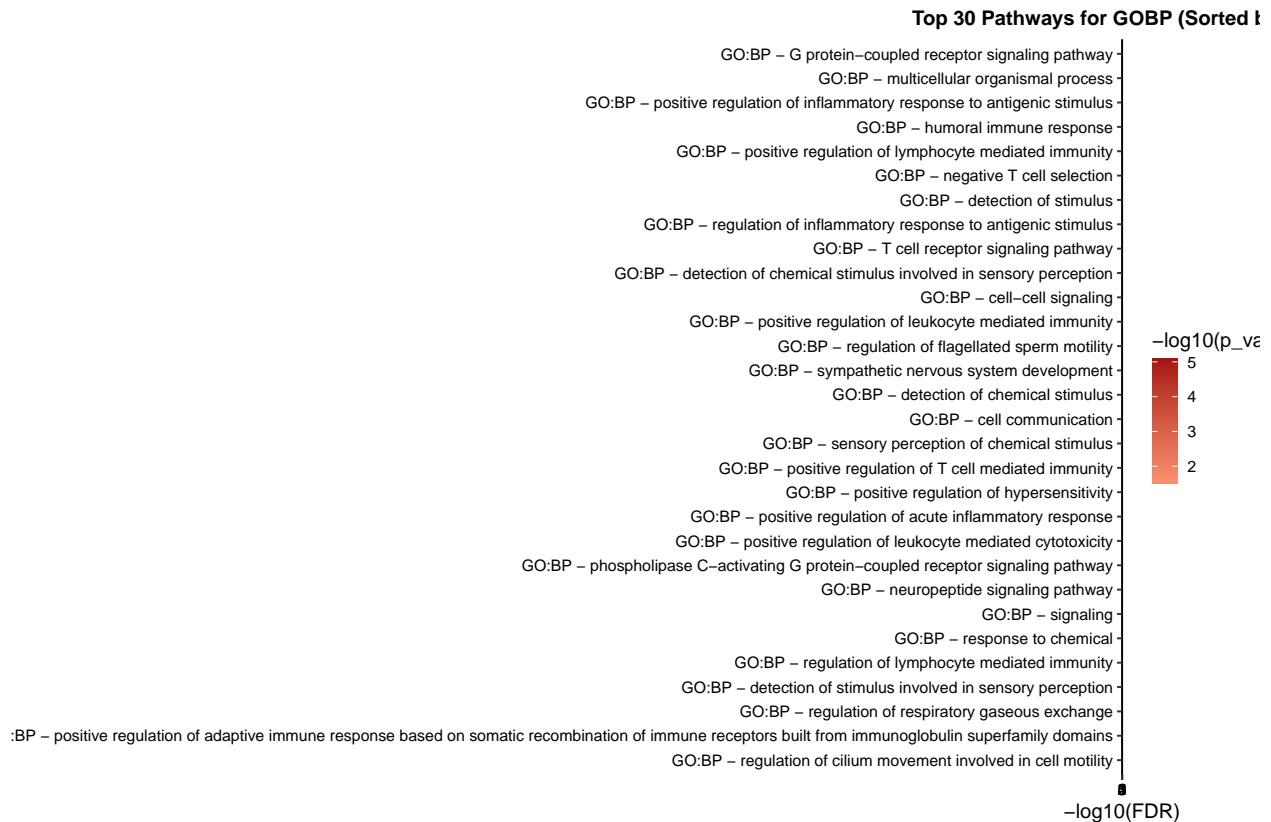


## (2) DEG threshold: $\log_{2}fc > 1.5$

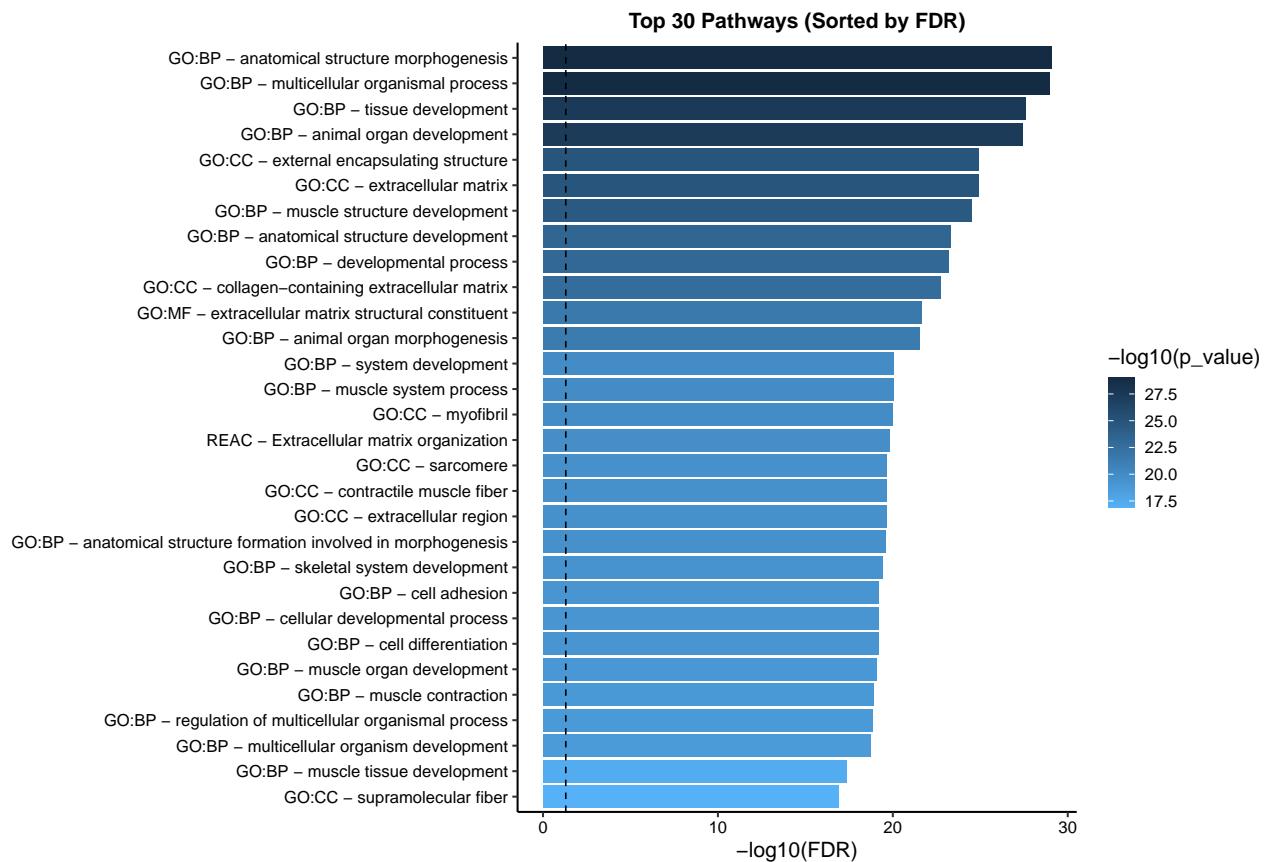
```
## [1] "Enrichment analysis for 02-DEG_2_up "
```



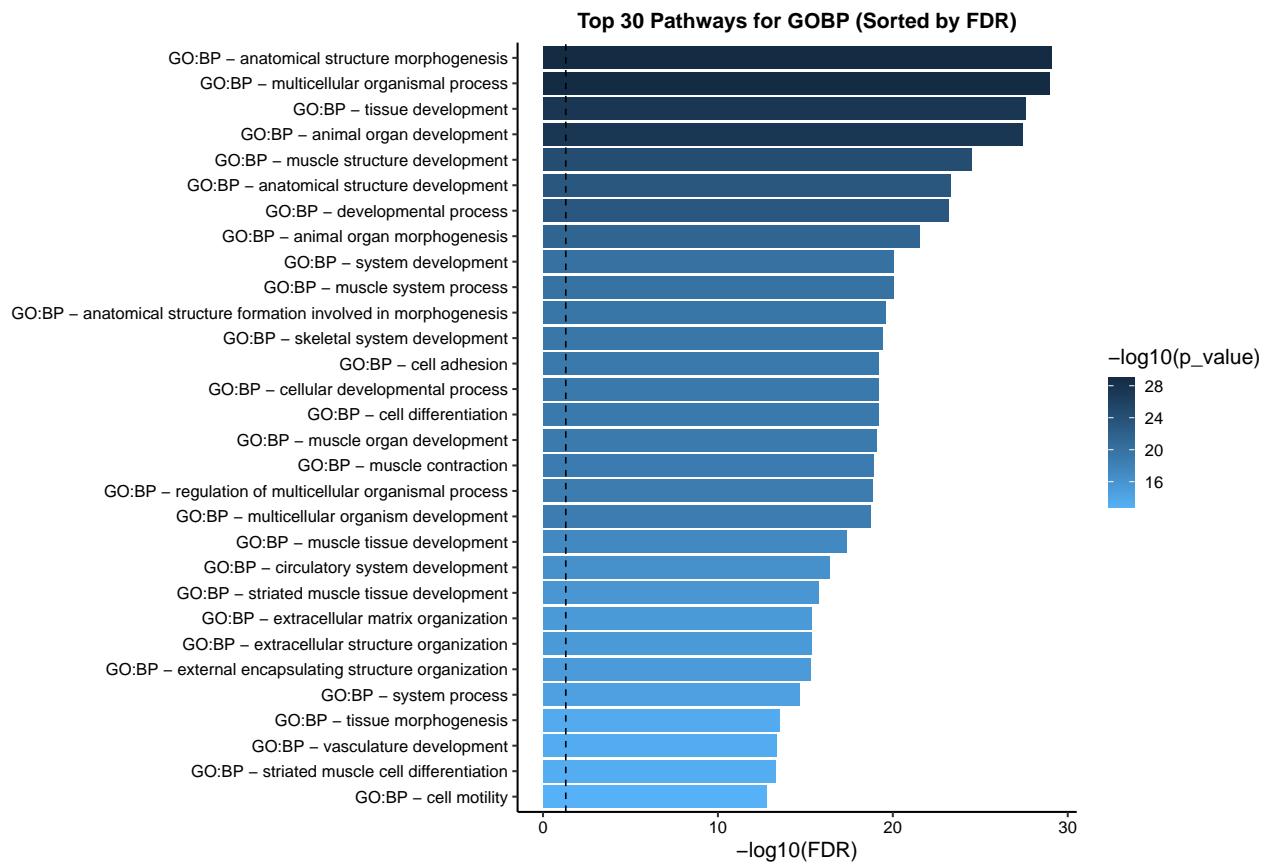
```
## [1] "Enrichment analysis for GOBP 02-DEG_2_up "
```



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



```
## [1] "Enrichment analysis for GOBP_02-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
## 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] ggrepify_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                tidyverse_2.0.0
## [51] tibble_3.2.1
## [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
## [11] deldir_2.0-4
## [13] magick_2.8.5
## [15] httpuv_1.6.15
## [17] withr_3.0.2
## [19] ggrepel_0.1.8
## [21] memoise_2.0.1
## [23] systemfonts_1.2.1
## [25] tidytree_0.4.6
## [27] pbapply_1.7-2
## [29] prettyunits_1.2.0
## [31] promises_1.3.2
## [33] restfulr_0.0.15
## [35] globals_0.16.3
## [37] rhdf5_2.48.0
## [39] UCSC.utils_1.0.0
## [41] generics_0.1.3
## [43] curl_6.2.1
## [45] ScaledMatrix_1.12.0
## [47] polyclip_1.10-7
## [49] SparseArray_1.4.8
## [51] evaluate_1.0.3
## [53] BiocFileCache_2.12.0
## [55] irlba_2.3.5.1
## [57] filelock_1.0.3
## [59] reticulate_1.40.0
## [61] magrittr_2.0.3
## [63] later_1.4.1
## [65] ggtree_3.12.0
## [67] spatstat.geom_3.3-5
## [69] scattermore_1.2
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## [73] pillar_1.10.1
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## [77] RSpectra_0.16-2
## [79] tensor_1.5
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## [83] abind_1.4-8
## [85] gridGraphics_0.5-1
## [87] locfit_1.5-9.11
## [89] bit_4.5.0.1
## [91] textshaping_1.0.0
## [93] BiocSingular_1.20.0
## [95] mime_0.12
## [97] Rcpp_1.0.14
## [99] sparseMatrixStats_1.16.0
## [101] Rttf2pt1_1.3.12
## [103] here_1.0.1
## [105] listenv_0.9.1
## [107] statmod_1.5.0
## [109] tweenr_2.0.3
## [111] tools_4.4.0
## [113] RSQLite_2.3.9
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parallelly_1.42.0
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qvalue_2.36.0
xfun_0.51
survival_3.8-3
gson_0.1.0
ragg_1.3.3
zoo_1.8-12
R.oo_1.27.0
KEGGREST_1.44.1
httr_1.4.7
rhdf5filters_1.16.0
fitdistrplus_1.2-2
rstudioapi_0.17.1
miniUI_0.1.1.1
DOSE_3.30.5
zlibbioc_1.50.0
ggraph_2.2.1
GenomeInfoDbData_1.2.12
xtable_1.8-4
S4Arrays_1.4.1
hms_1.1.3
colorspace_2.1-1
ROCR_1.0-11
spatstat.data_3.1-4
lmtest_0.9-40
viridis_0.6.5
lattice_0.22-6
future.apply_1.11.3
shadowtext_0.1.4
RcppAnnoy_0.0.22
nlme_3.1-167
beachmat_2.20.0
stringi_1.8.4
GenomicAlignments_1.40.0
crayon_1.5.3
BiocIO_1.14.0
emdbook_1.3.13
graphlayouts_1.2.2
fastmatch_1.1-6
codetools_0.2-20
plotly_4.10.4
splines_4.4.0
fastDummies_1.7.5
dbplyr_2.5.0
blob_1.2.4
fs_1.6.5
ggplotify_0.1.2
tzdb_0.4.0
pkgconfig_2.0.3
cachem_1.1.0
viridisLite_0.4.2
numDeriv_2016.8-1.1

```

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