GSVA for mutil Group

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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 `../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'
result_folder = result_folder_all
```

3. Visualization for reuslt

(1) Sample information - PCA plot

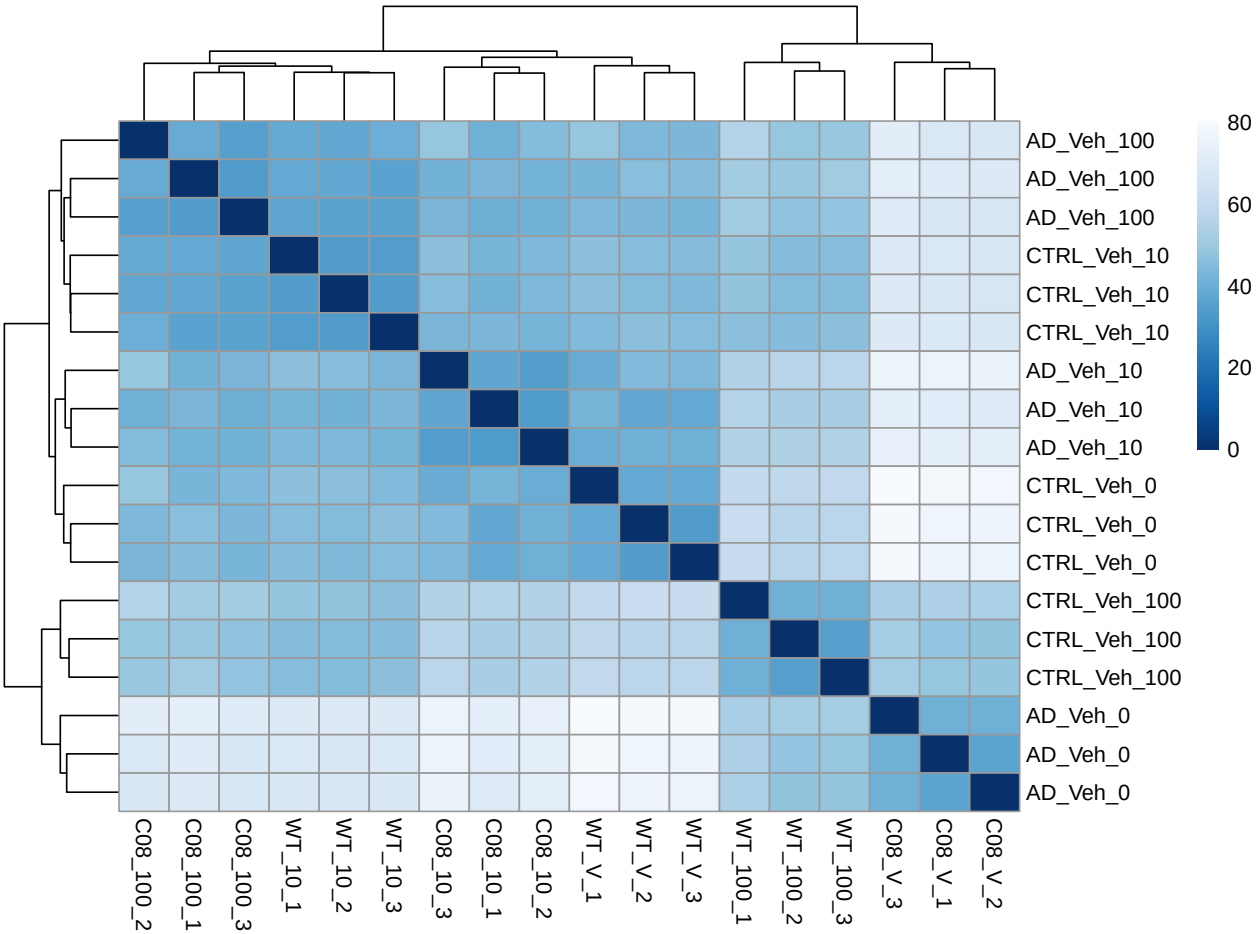
```
figure_folder = result_folder
# do PCA for counts data
dds_obj <- DESeqDataSetFromMatrix(countData = counts,
                                  colData = condition_list,
                                  design = ~ group)
vsd.obj <- varianceStabilizingTransformation(dds_obj, blind = TRUE)
pcaData <- plotPCA(vsd.obj,  intgroup = c("group"), returnData = T)
percentVar <- round(100 * attr(pcaData, "percentVar"))


p <-ggplot(pcaData, aes(PC1, PC2, color=group)) +
  geom_point(size=3) +
  labs(x = paste0("PC1: ",percentVar[1],"% variance"),
       y = paste0("PC2: ",percentVar[2],"% variance"),
  ) +
  stat_ellipse(level = 0.95)+
  theme_bw() +
  # theme_classic()+
  theme(text = element_text(family = "Arial", colour = "black")) +
  # scale_color_manual(values = assigned_colors) +
  ggrepel::geom_text_repel(aes(label = name), color = "black")


ggsave("./results/01-Sample_info/01_sample_PCA_plot.pdf", p,width = 8, height = 6, units = "in", dpi = 3
ggsave("./results/01-Sample_info/01_sample_PCA_plot.png", p,width = 8, height = 6, units = "in", dpi = 3
```

(2) Sample information - Distance heatmap

```
# Now apply variance stabilizing transformation
sampleDists <- dist(t(assay(vsd.obj)))
sampleDistMatrix <- as.matrix( sampleDists )
rownames(sampleDistMatrix) <- paste( vsd.obj$group )
colors <- colorRampPalette( rev(RColorBrewer::brewer.pal(9, "Blues")) )(255)
p <- pheatmap::pheatmap(sampleDistMatrix,
                        clustering_distance_rows = sampleDists,
                        clustering_distance_cols = sampleDists,
                        col = colors)
```




```
 ggsave("./results/01-Sample_info/02_sample_distance_heatmap.pdf", p,width = 8, height = 6, units = "in
ggsave("./results/01-Sample_info/02_sample_distance_heatmap.png",
       p, width = 8, height = 6, units = "in", dpi = 300)
```

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, "./results/02-GSVA/01_GSVA_matrix.csv")
#
#



# plot the heatmap for the GSVA result
pathway_list <- read.csv(here::here("data", "ref", "focus-pathway_2024_10_03.csv"))

box_plot_folder<- file.path(result_folder,"04-GSVA","Boxplot")
# create the folder
dir.create(box_plot_folder, showWarnings = FALSE)

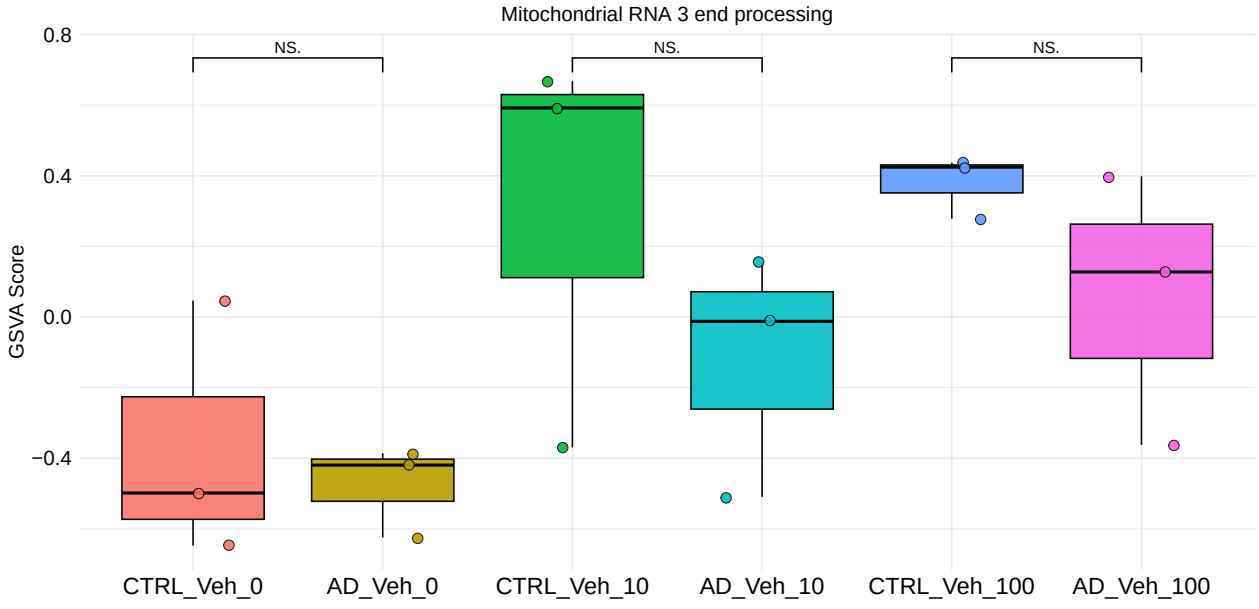
gsva_matrix <- read.csv("./results/02-GSVA/01_GSVA_matrix.csv", row.names = 1)
colnames(gsva_matrix) <- sub("^X", "", colnames(gsva_matrix))
condition_list_label <- condition_list
condition_list_label$group <- factor(
  condition_list_label$group,
  # levels = c("CTRL_Veh_0", "CTRL_Veh_10",  "CTRL_Veh_100",
  #            "AD_Veh_0", "AD_Veh_10", "AD_Veh_100")
  levels = c("CTRL_Veh_0", "AD_Veh_0",
             "CTRL_Veh_10",   "AD_Veh_10",
             "CTRL_Veh_100", "AD_Veh_100")
)


# plot for the focus pathway
for (i in 1:20){
  pathway_name <- pathway_list$pathway[i]
  print(pathway_name)
  p<-plot_gsva_boxplot_mutil_3(gsva_matrix,
                    condition_list_label =condition_list_label,
                    pathway_name =  pathway_name,
                    figure_folder = file.path(result_folder,"02-GSVA","Boxplot-pair"),
                    file_name = paste0("GSVA_", pathway_name),
                    fig.height = 6, fig.width = 12,
                    reference_group_1="CTRL_Veh_0" , compare_group_1="AD_Veh_0",
                    reference_group_2="CTRL_Veh_10", compare_group_2="AD_Veh_10",
                    reference_group_3="CTRL_Veh_100", compare_group_3="AD_Veh_100")
  print(p)
```

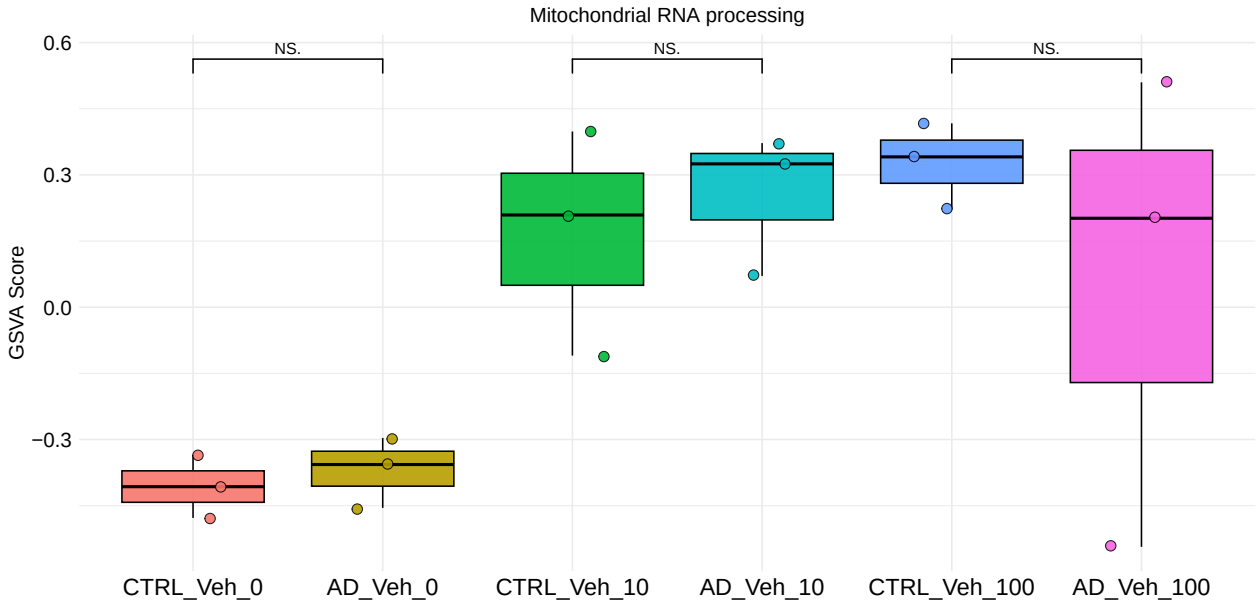
```
}
```

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

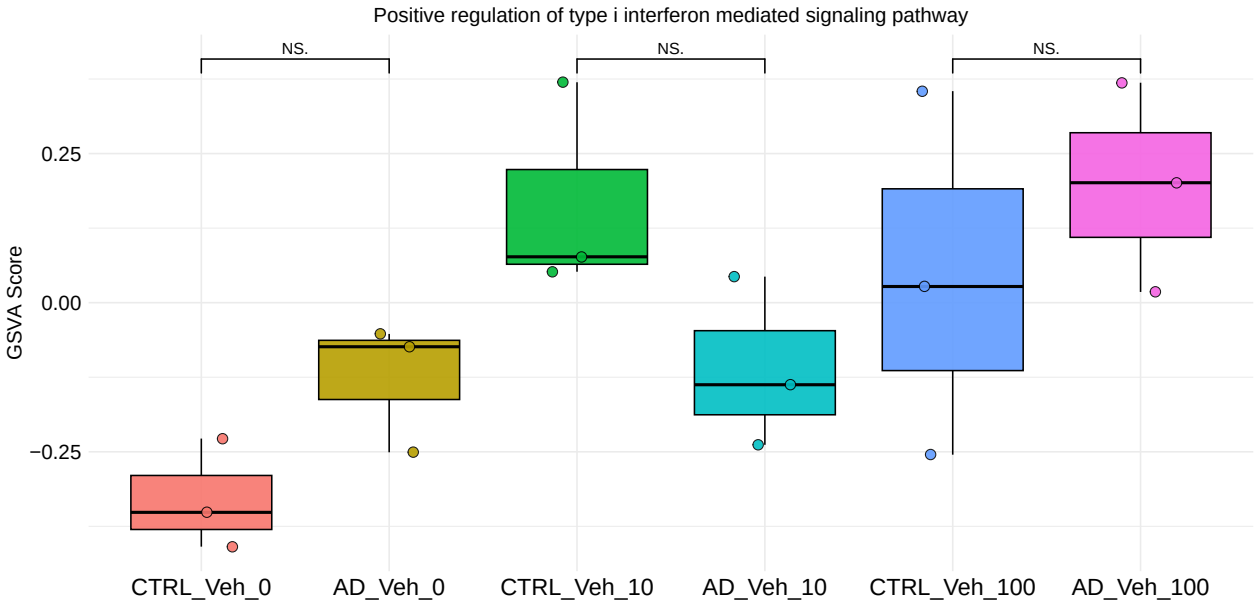
```
## [1] "GOBP_MITOCHONDRIAL_RNA_PROCESSING"
```



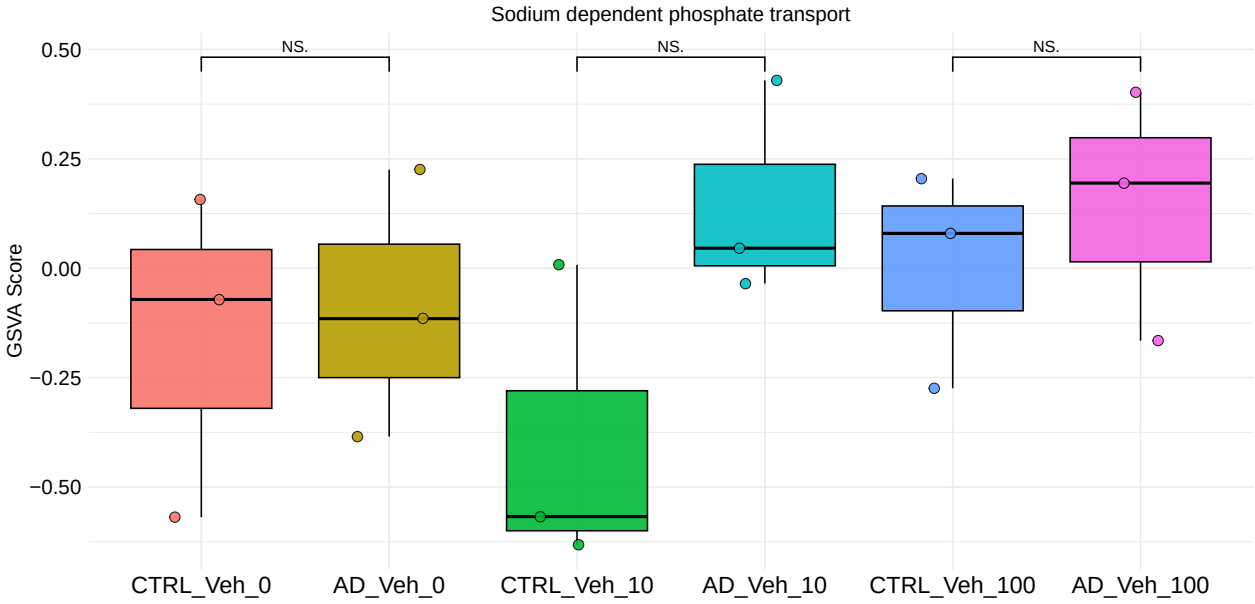

```
## [1] "GOBP_POSITIVE_REGULATION_OF_TYPE_I_INTERFERON_MEDIATED_SIGNALING_PATHWAY"
```



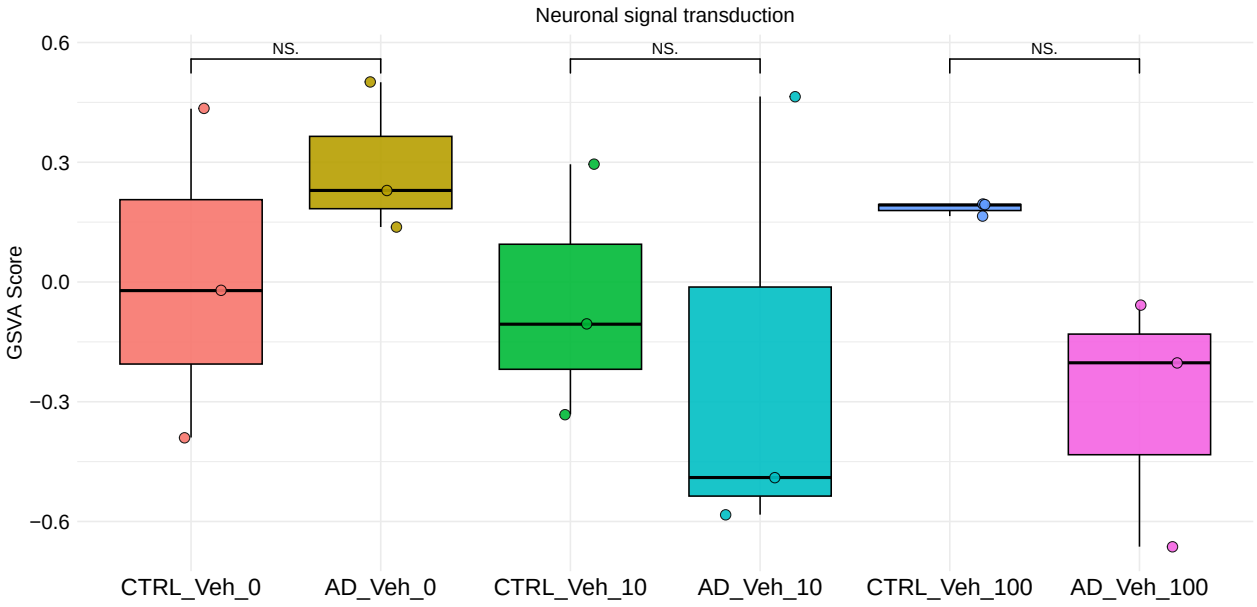

```
## [1] "GOBP_SODIUM_DEPENDENT_PHOSPHATE_TRANSPORT"
```



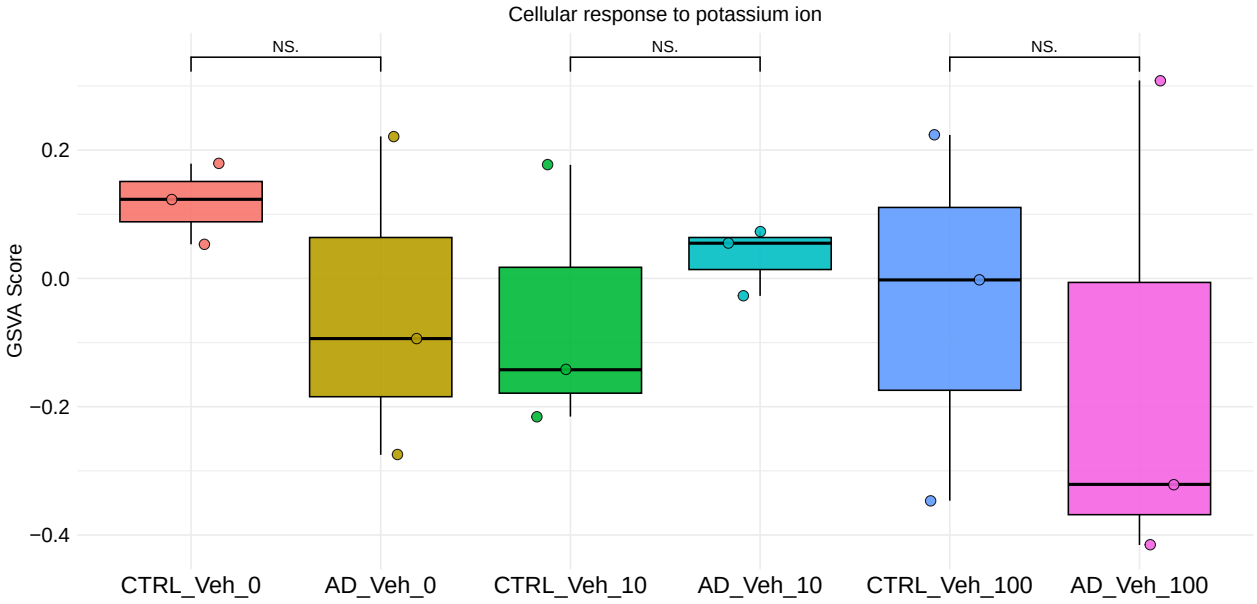

```
## [1] "GOBP_NEURONAL_SIGNAL_TRANSDUCTION"
```



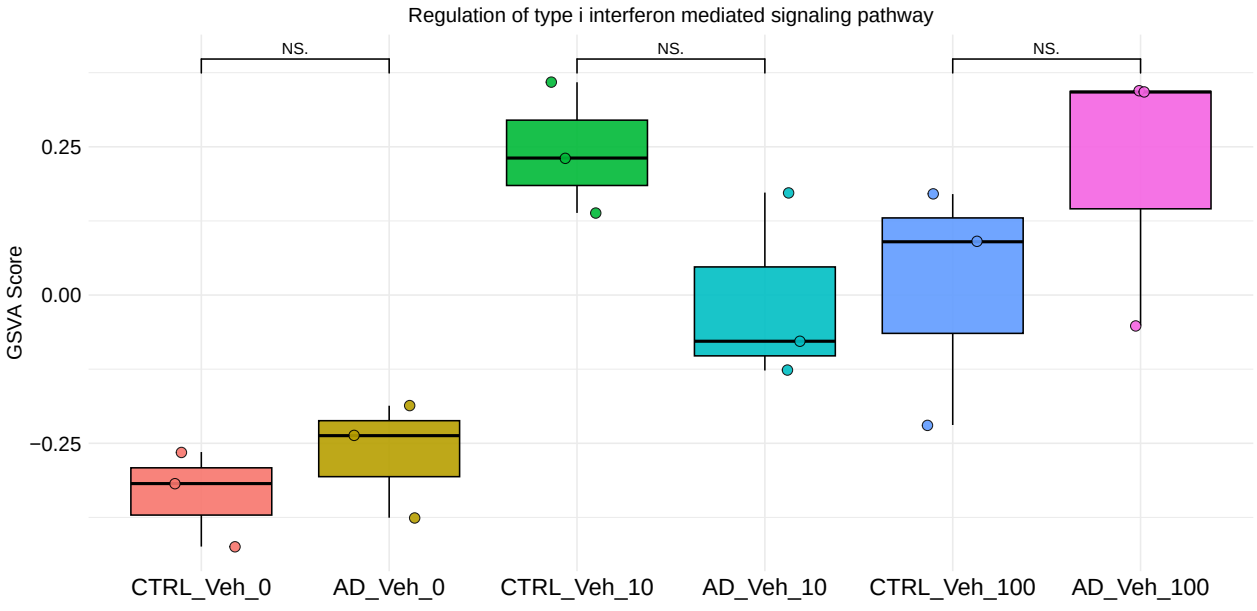

```
## [1] "GOBP_CELLULAR_RESPONSE_TO_POTASSIUM_ION"
```



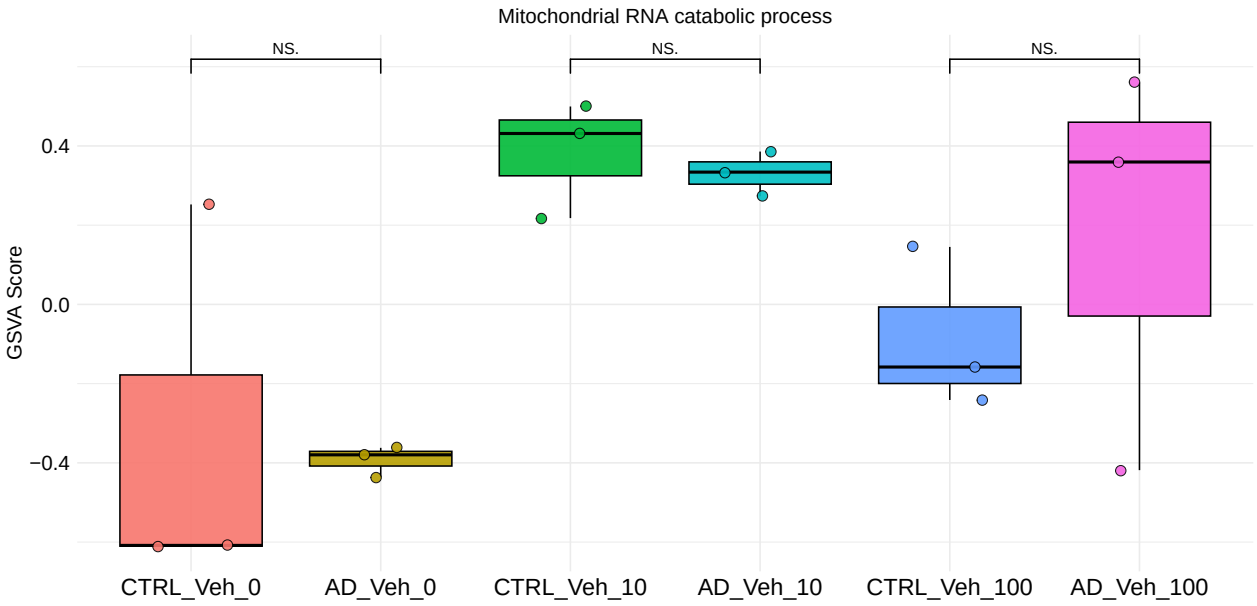

```
## [1] "GOBP_REGULATION_OF_TYPE_I_INTERFERON_MEDIATED_SIGNALING_PATHWAY"
```



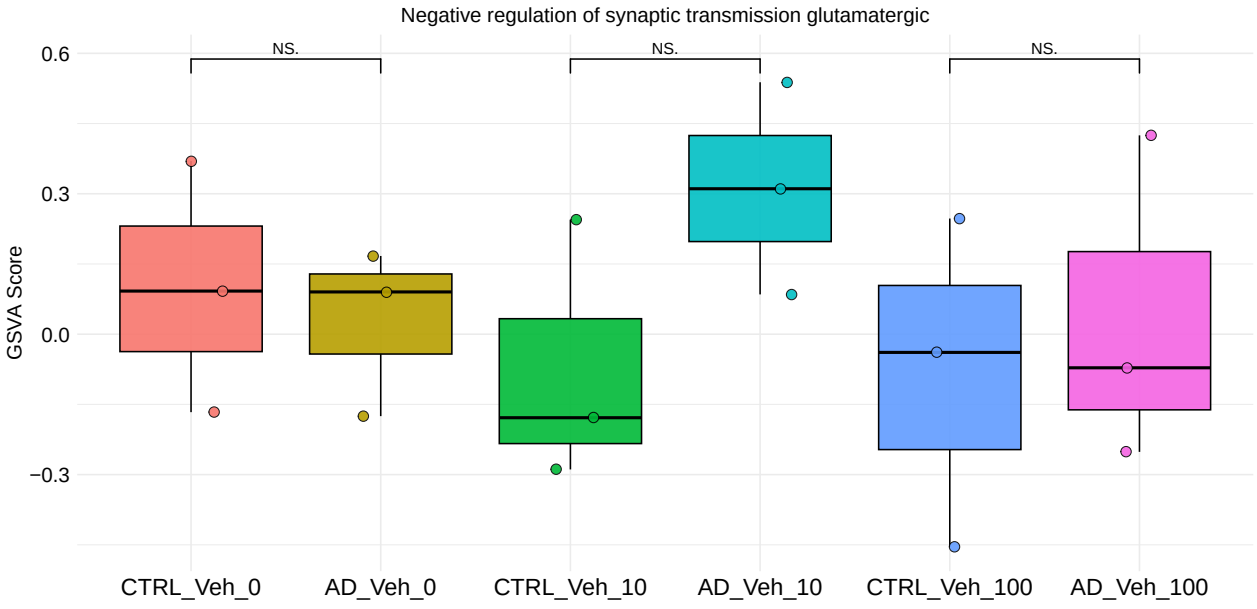

```
## [1] "GOBP_MITOCHONDRIAL_RNA_CATABOLIC_PROCESS"
```



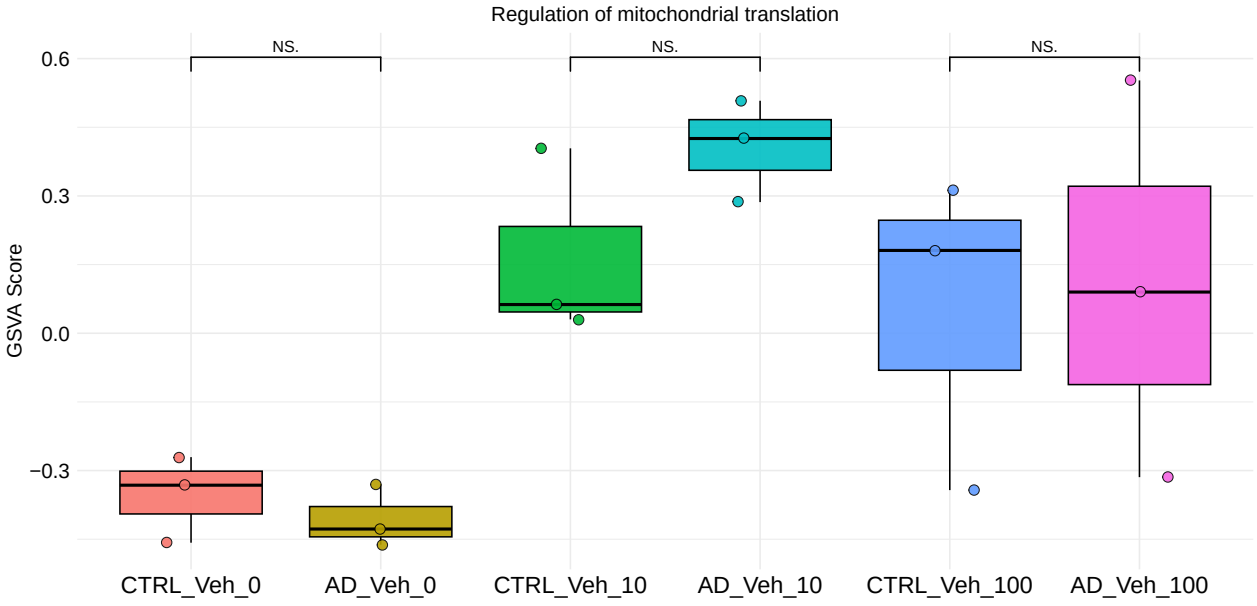

```
## [1] "GOBP_NEGATIVE_REGULATION_OF_SYNAPTIC_TRANSMISSION_GLUTAMATERGIC"
```



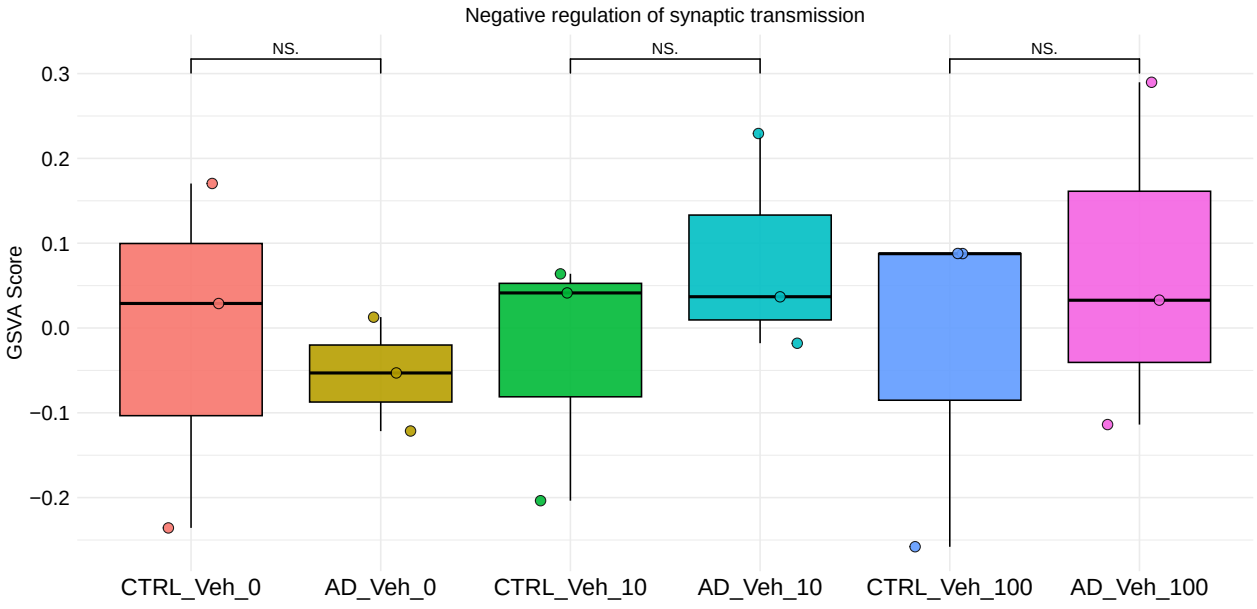

```
## [1] "GOBP_REGULATION_OF_MITOCHONDRIAL_TRANSLATION"
```



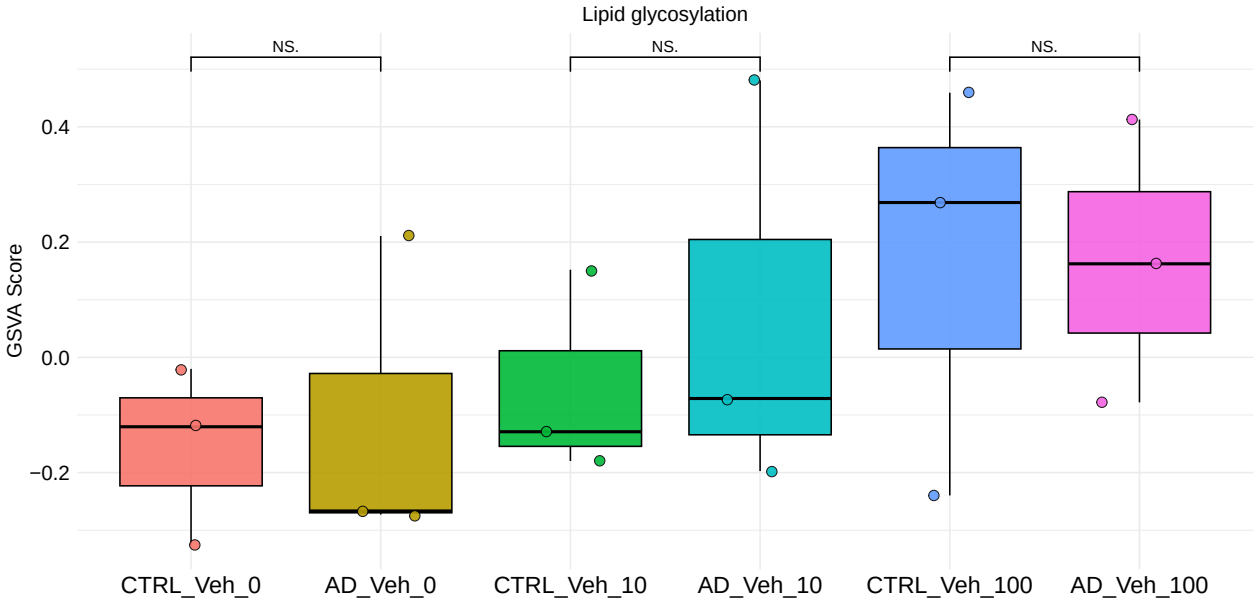

```
## [1] "GOBP_NEGATIVE_REGULATION_OF_SYNAPTIC_TRANSMISSION"
```



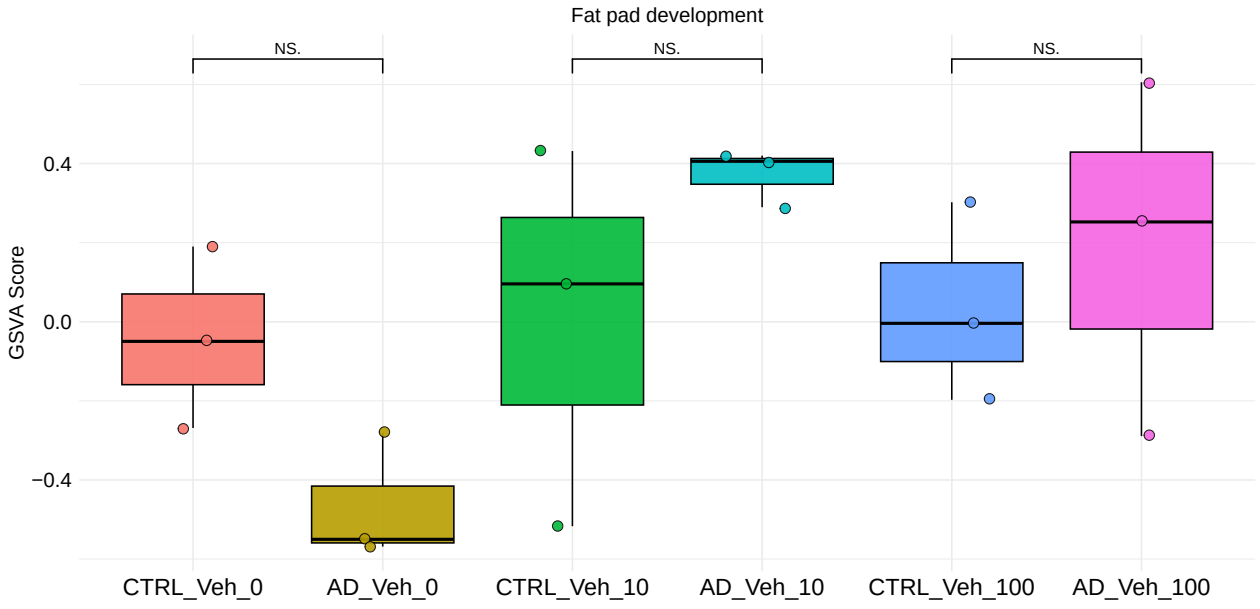

```
## [1] "GOBP_LIPID_GLYCOSYLATION"
```



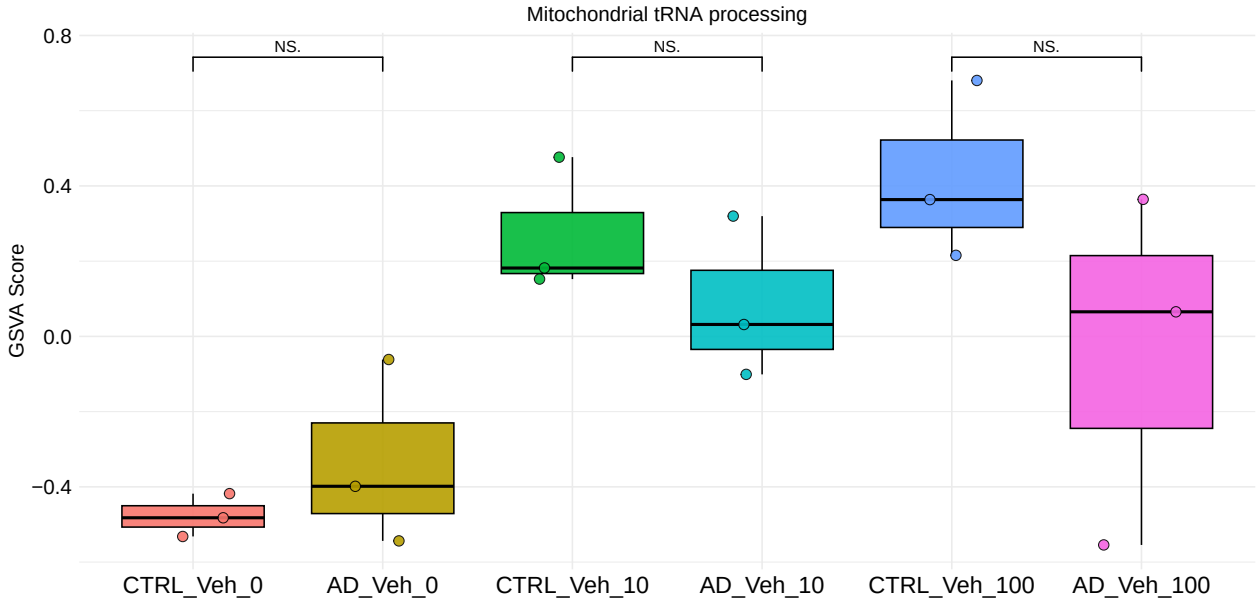

```
## [1] "GOBP_FAT_PAD_DEVELOPMENT"
```



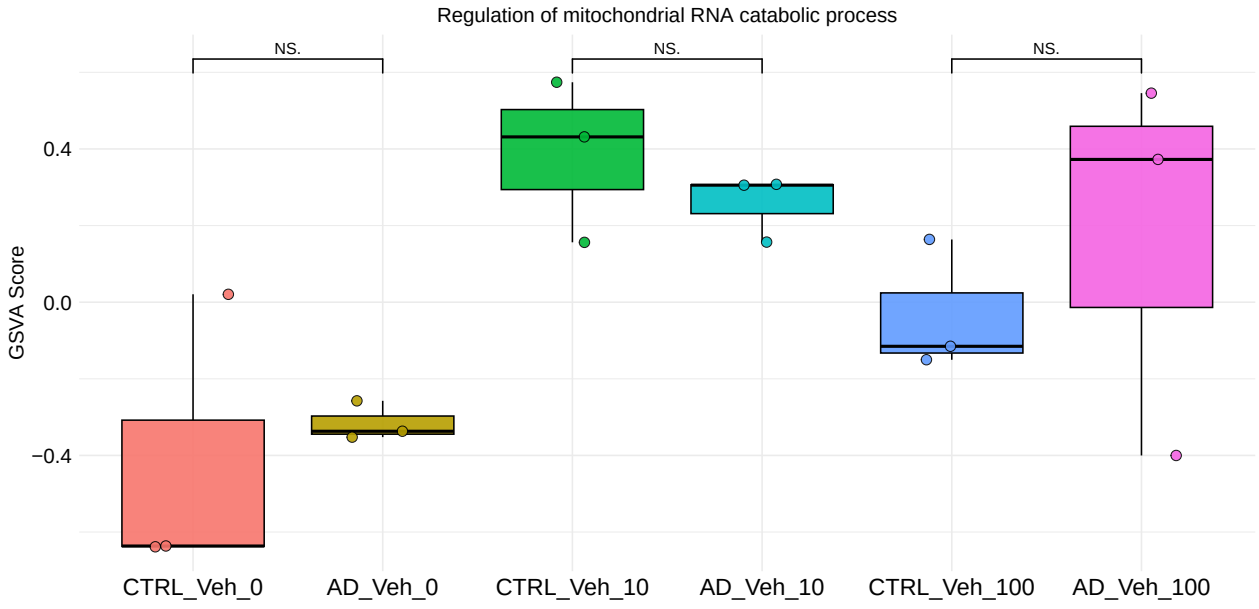

```
## [1] "GOBP_MITOCHONDRIAL_TRNA_PROCESSING"
```



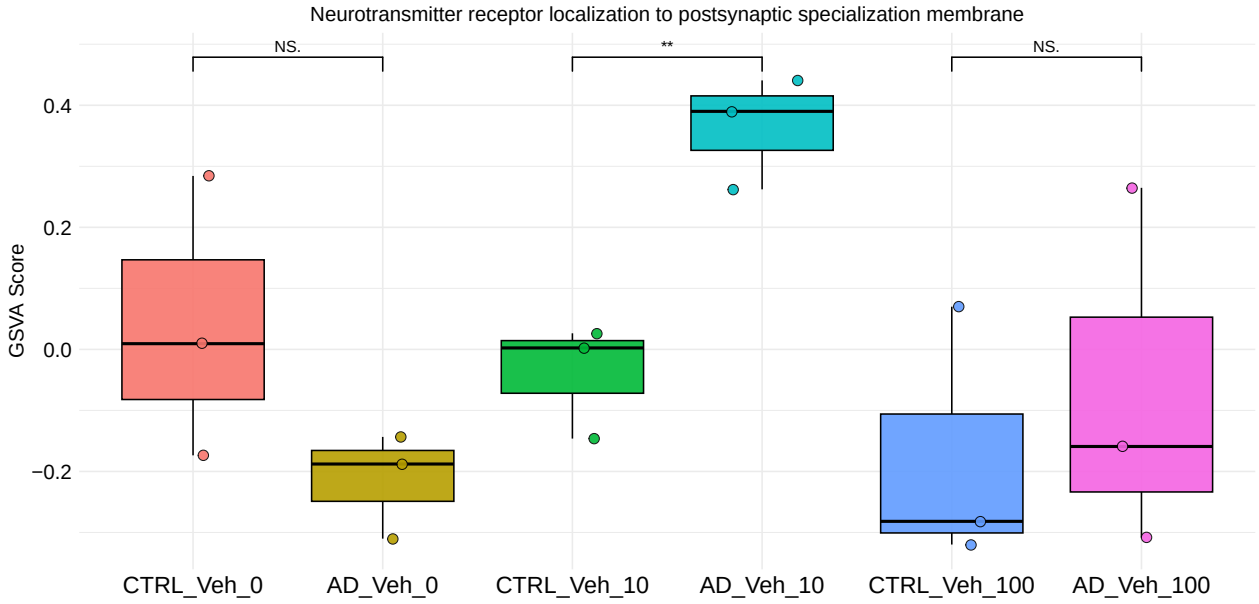

```
## [1] "GOBP_REGULATION_OF_MITOCHONDRIAL_RNA_CATABOLIC_PROCESS"
```



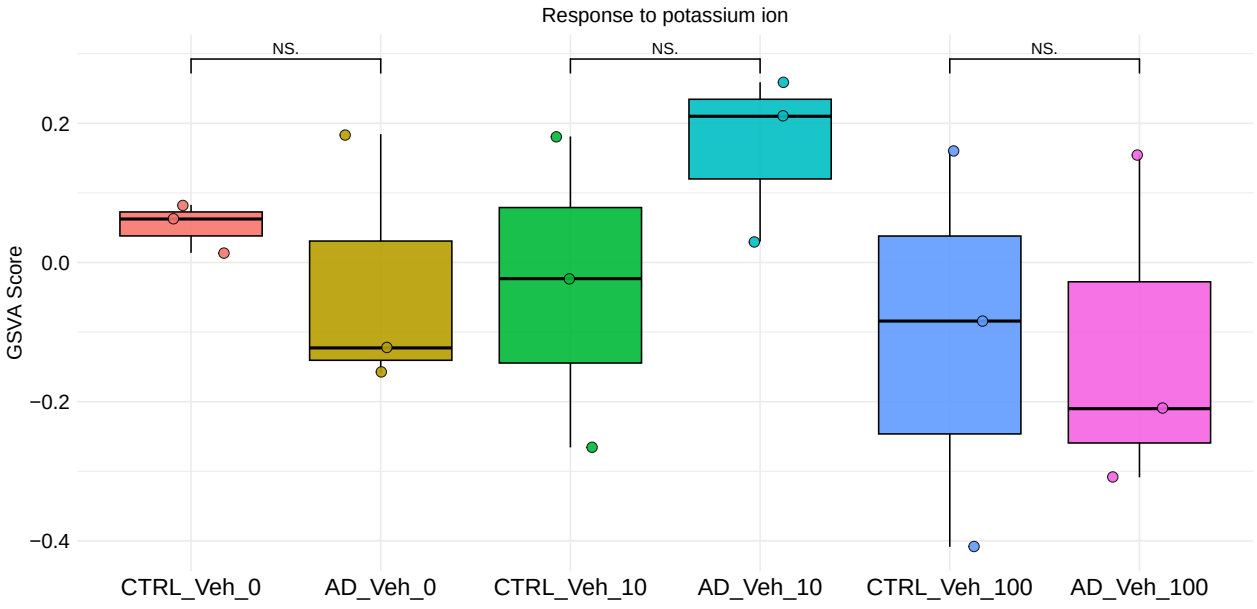

```
## [1] "GOBP_NEUROTRANSMITTER_RECEPTOR_LOCALIZATION_TO_POSTSYNAPTIC_SPECIALIZATION_MEMBRANE"
```



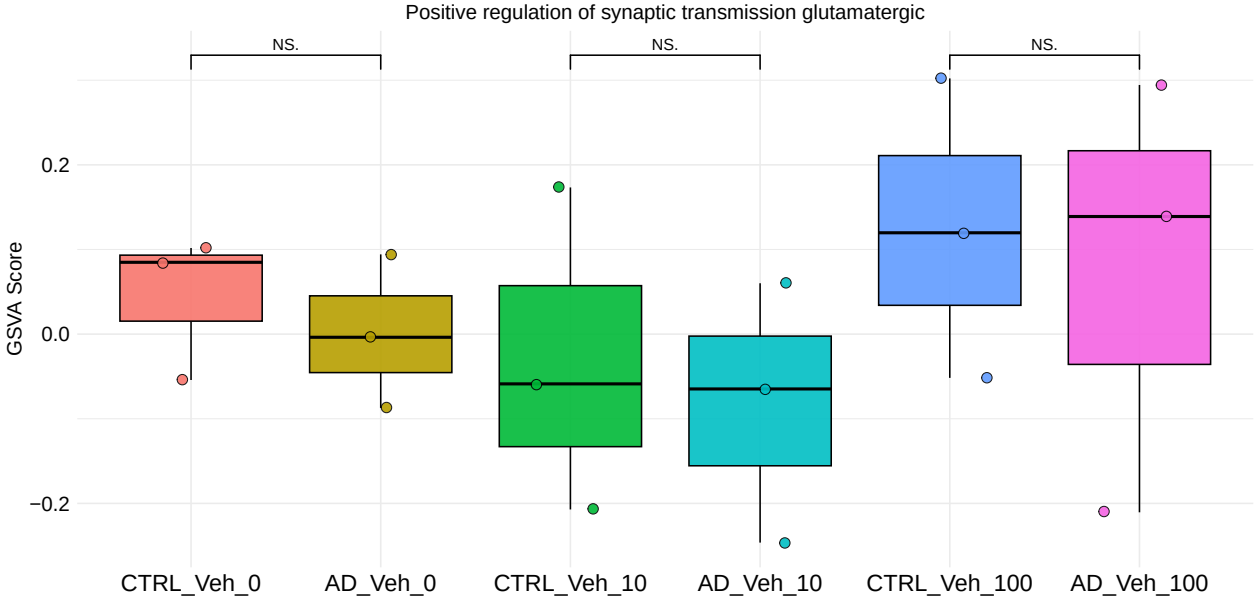

```
## [1] "GOBP_RESPONSE_TO_POTASSIUM_ION"
```



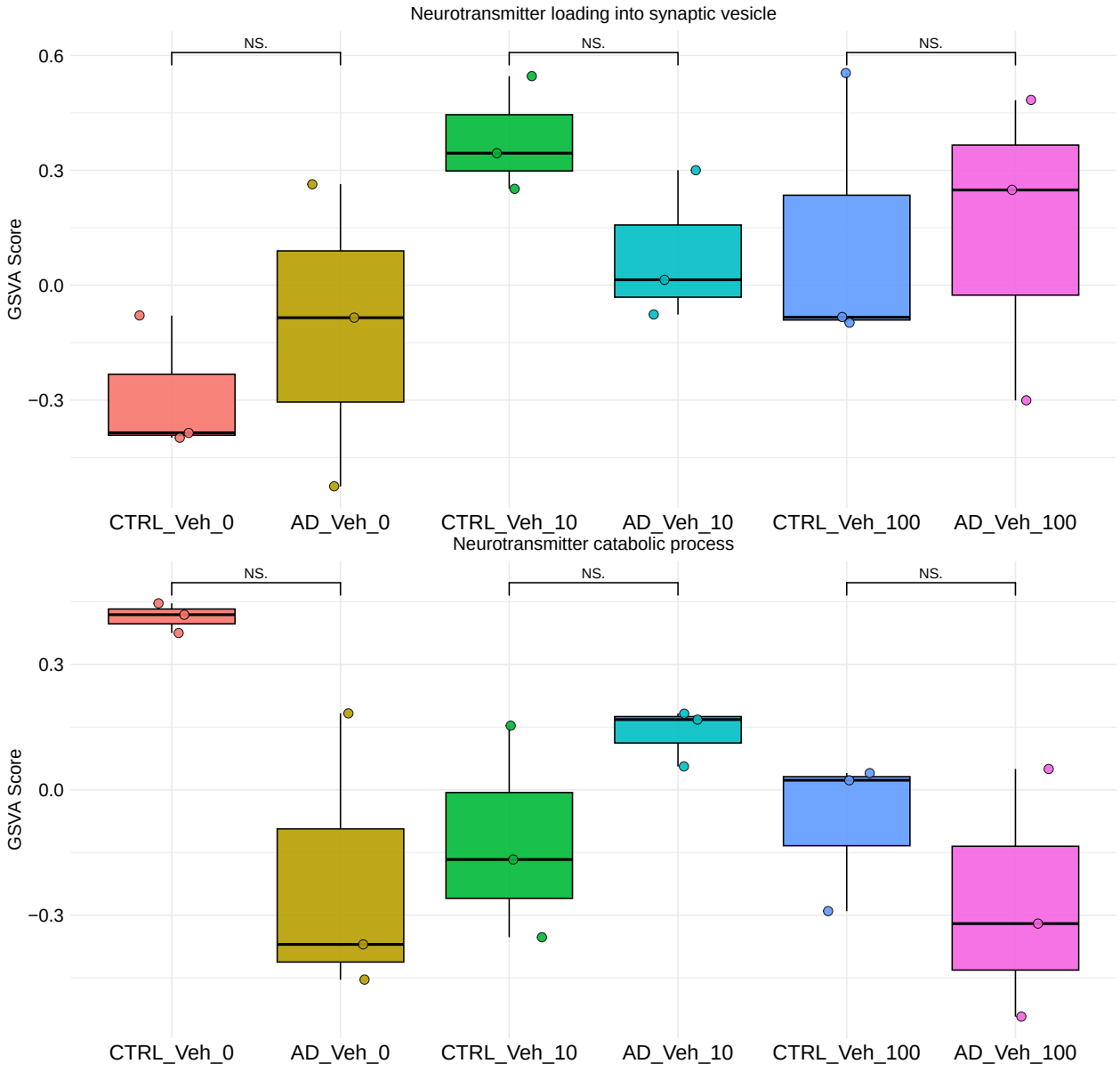

```
## [1] "GOBP_POSITIVE_REGULATION_OF_SYNAPTIC_TRANSMISSION_GLUTAMATERGIC"
```




```
## [1] "GOBP_NEUROTRANSMITTER_LOADING_INTO_SYNAPTIC_VESICLE"
```




```
## [1] "GOBP_NEUROTRANSMITTER_CATABOLIC_PROCESS"
```



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
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## [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
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