GSVA for mutil Group

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Contents

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.

2. Visualization for Result

(1) Sample Information - PCA Plot

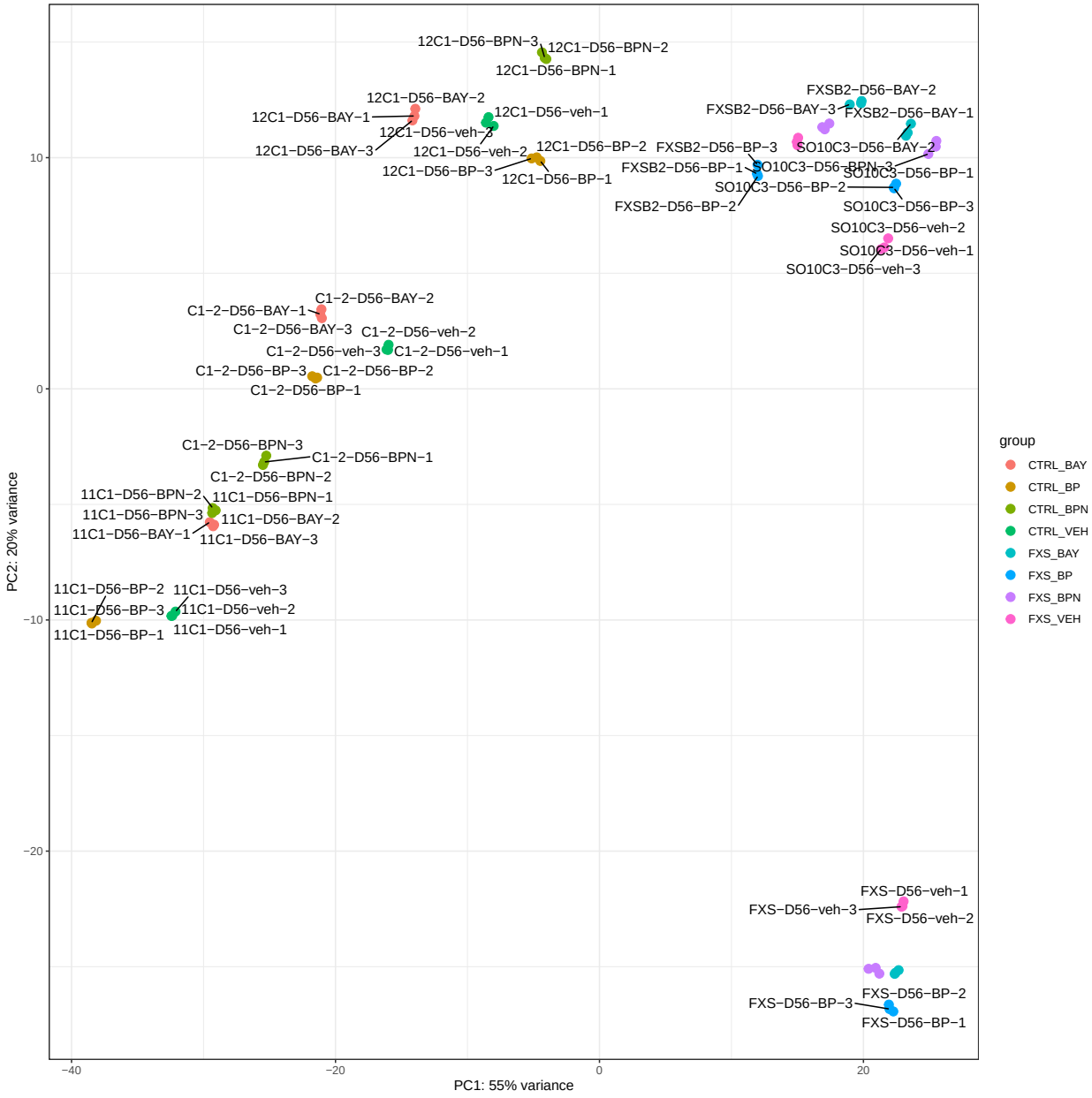


Figure 1: PCA Plot with individual label

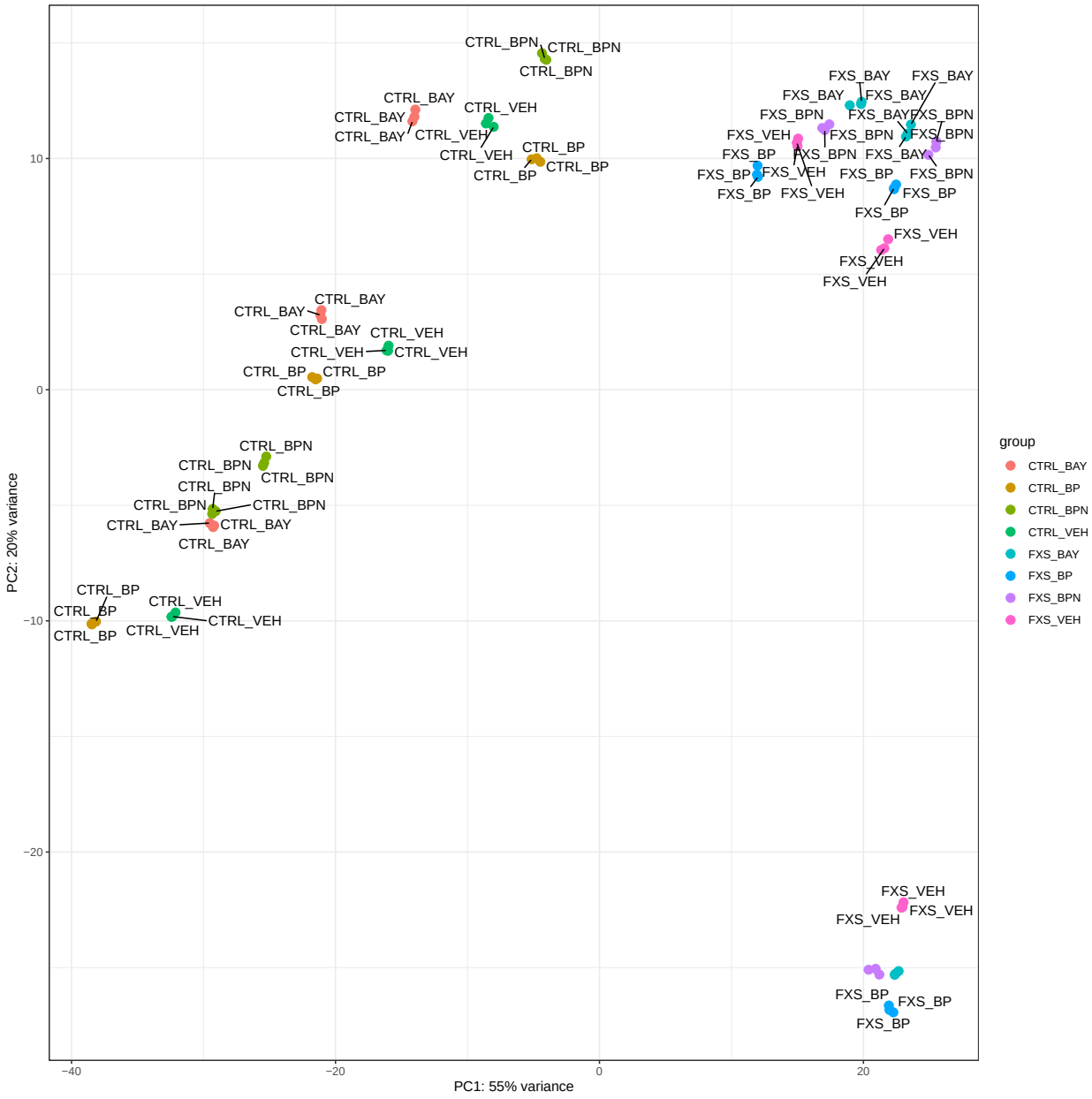


Figure 2: PCA Plot with group label

(2) Sample Information - Distance Heatmap

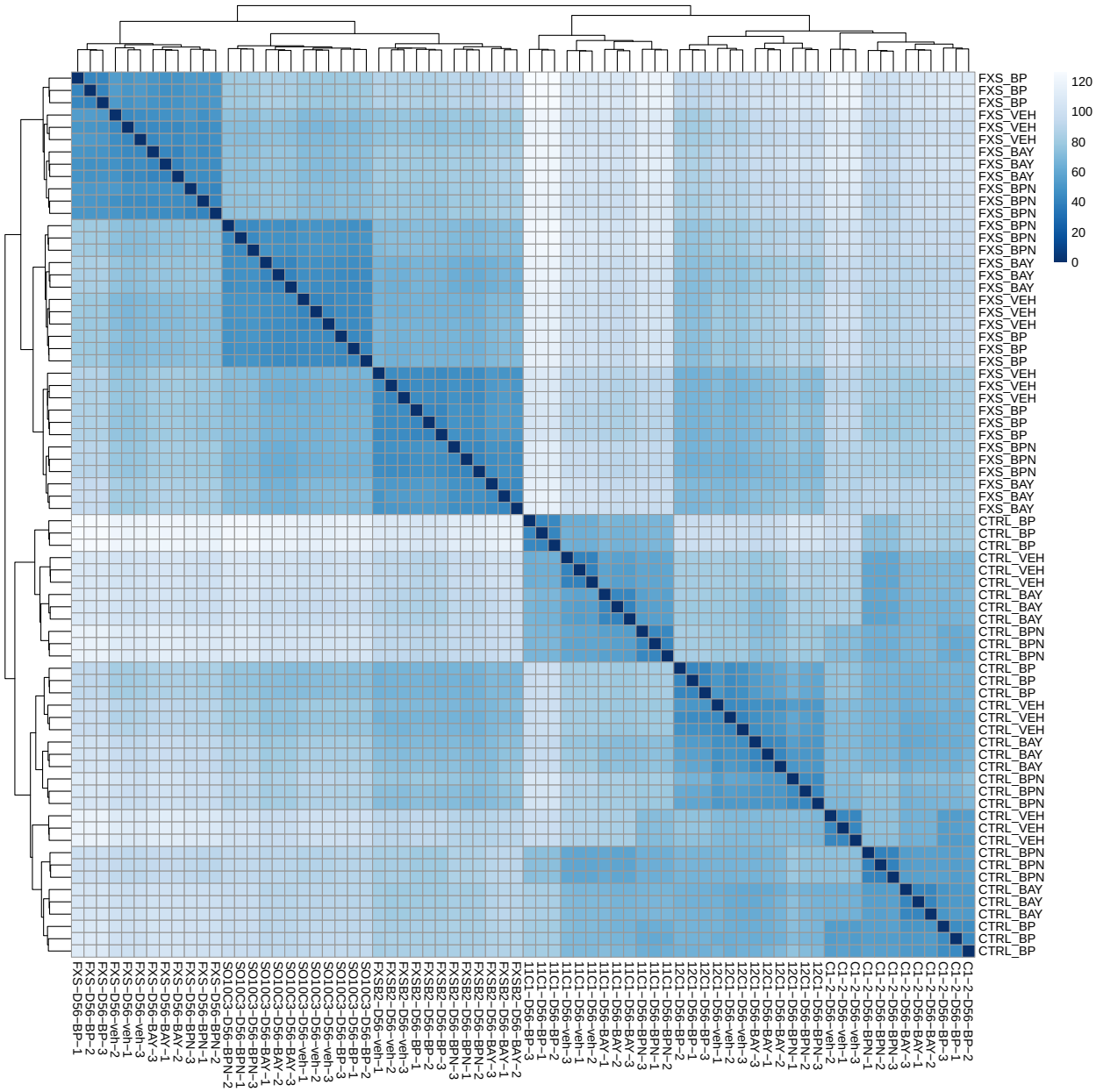


Figure 3: Sample Information Distance Heatmap

3. GSVA analysis with Mix effect model.

(1) Main Method: Mix-Effects Model with no Interaction

We model **GSVA scores** using a **mixed-effects model**, incorporating **fixed effects** for disease status (FXS) and drug treatment, and a **random intercept** for individual-level variability:

$$Y_{ij} = \beta_0 + \beta_1 X_{\text{FXS}} + \beta_2 X_{\text{Drug\_BAY}} + \beta_3 X_{\text{Drug\_BPN}} + \beta_4 X_{\text{Drug\_BP}} + b_i + \varepsilon_{ij}$$

Model Components

- Y_{ij}: GSVA score for individual i under condition j.
- **Fixed Effects:**
  - X_{FXS}: Disease indicator (1 for **FXS**, 0 for **CTRL**).
  - $X_{\text{Drug\_BAY}}, X_{\text{Drug\_BPN}}, X_{\text{Drug\_BP}}$: Drug treatment indicators (vehicle is reference).
- **Random intercept**
  - $b_i \sim \mathcal{N}(0, \tau^2)$ for individual i, capturing baseline variability.
  - $\varepsilon_{ij} \sim \mathcal{N}(0, \sigma^2)$: Residual error term.

This model allows us to estimate the effects of disease and drug treatments on GSVA scores while controlling for repeated measures within individuals.

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: Y ~ X_FXS + X_Drug_BAY + X_Drug_BPN + X_Drug_BP + (1 | Individual)
##    Data: df_pathway
##
## REML criterion at convergence: -11.1
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.98065 -0.50074 -0.00603  0.59646  2.34519
##
## Random effects:
##  Groups     Name        Variance Std.Dev.
##  Individual (Intercept) 0.01094  0.1046
##  Residual               0.03649  0.1910
## Number of obs: 72, groups:  Individual, 6
##
## Fixed effects:
##             Estimate Std. Error       df t value Pr(>|t|)
## (Intercept) -0.12855    0.07862  6.98778  -1.635   0.1461
## X_FXS        0.22634    0.09655  4.00000   2.344   0.0790 .
## X_Drug_BAY   0.06840    0.06367 63.00000   1.074   0.2868
## X_Drug_BPN  -0.09013    0.06367 63.00000  -1.416   0.1618
## X_Drug_BP   -0.20056    0.06367 63.00000  -3.150   0.0025 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##            (Intr) X_FXS  X_D_BA X_D_BPN
## X_FXS      -0.614
## X_Drug_BAY -0.405  0.000
## X_Drug_BPN -0.405  0.000  0.500
## X_Drug_BP  -0.405  0.000  0.500  0.500
```

(2) Main Method: Mix-Effects Model with Interaction

We model **GSVA scores** using a **mixed-effects model**, incorporating **fixed effects** for disease status (FXS), drug treatments, and their **interactions**, along with a **random intercept** for individual-level variability:

$$\begin{aligned} Y_{ij} = \beta_0 &+ \beta_1 X_{\text{FXS}} + \beta_2 X_{\text{Drug\_BAY}} + \beta_3 X_{\text{Drug\_BPN}} + \beta_4 X_{\text{Drug\_BP}} \\ &+ \beta_5 (X_{\text{FXS}} \times X_{\text{Drug\_BAY}}) + \beta_6 (X_{\text{FXS}} \times X_{\text{Drug\_BPN}}) + \beta_7 (X_{\text{FXS}} \times X_{\text{Drug\_BP}}) \\ &+ \ b_i + \varepsilon_{ij} \end{aligned}$$

Model Components

- Y_{ij}: GSVA score for individual i under condition j.
- **Fixed Effects:**
  - X_{FXS}: Disease indicator (1 for **FXS**, 0 for **CTRL**).
  - $X_{\text{Drug\_BAY}}, X_{\text{Drug\_BPN}}, X_{\text{Drug\_BP}}$: Drug treatment indicators (vehicle is the reference).
  - **Interaction Terms:**
    - $X_{\text{FXS}} \times X_{\text{Drug\_BAY}}$: Interaction between FXS and BAY treatment.
    - $X_{\text{FXS}} \times X_{\text{Drug\_BPN}}$: Interaction between FXS and BPN treatment.
    - $X_{\text{FXS}} \times X_{\text{Drug\_BP}}$: Interaction between FXS and BP treatment.
- **Random Intercept:**
  - $b_i \sim \mathcal{N}(0, \tau^2)$: Individual-specific random intercept capturing baseline variability.
- **Error Term:**
  - $\varepsilon_{ij} \sim \mathcal{N}(0, \sigma^2)$: Residual error term.

This model allows us to estimate not only the **main effects** of disease and drug treatments but also **how drug responses differ between FXS and CTRL individuals**. Interaction terms capture whether the drug effect is **modified by disease status**, which is essential for understanding differential pathway responses.

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula:
## Y ~ X_FXS + X_Drug_BAY + X_Drug_BPN + X_Drug_BP + X_FXS * X_Drug_BAY +
##     X_FXS * X_Drug_BPN + X_FXS * X_Drug_BP + (1 | Individual)
##    Data: df_pathway
##
## REML criterion at convergence: -12.2
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -1.5751 -0.7045 -0.0373  0.4715  2.9583
##
## Random effects:
##  Groups     Name        Variance Std.Dev.
##  Individual (Intercept) 0.01121  0.1059
##  Residual               0.03325  0.1824
## Number of obs: 72, groups:  Individual, 6
##
## Fixed effects:
##                  Estimate Std. Error       df t value Pr(>|t|)
## (Intercept)      -0.19869    0.08621  9.93526  -2.305   0.0441 *
## X_FXS             0.36661    0.12192  9.93526   3.007   0.0133 *
## X_Drug_BAY        0.08612    0.08596 60.00000   1.002   0.3205
## X_Drug_BPN        0.07042    0.08596 60.00000   0.819   0.4159
## X_Drug_BP        -0.09829    0.08596 60.00000  -1.143   0.2574
## X_FXS:X_Drug_BAY -0.03544    0.12157 60.00000  -0.291   0.7717
## X_FXS:X_Drug_BPN -0.32111    0.12157 60.00000  -2.641   0.0105 *
## X_FXS:X_Drug_BP  -0.20454    0.12157 60.00000  -1.682   0.0977 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##               (Intr) X_FXS  X_D_BA X_D_BPN X_Dr_BP X_FXS:X_D_BA X_FXS:X_D_BPN
## X_FXS         -0.707
## X_Drug_BAY    -0.499  0.353
## X_Drug_BPN    -0.499  0.353  0.500
## X_Drug_BP     -0.499  0.353  0.500  0.500
## X_FXS:X_D_BA   0.353 -0.499 -0.707 -0.354  -0.354
## X_FXS:X_D_BPN  0.353 -0.499 -0.354 -0.707  -0.354   0.500
## X_FXS:X_Dr_BP  0.353 -0.499 -0.354 -0.354  -0.707   0.500        0.500
```

Hypothesis Testing: Comparing differece with FXS after Drug and CTRL

Combined Effect Hypothesis Testing

To interpret the effect of each drug within the **FXS background**, we tested whether the **combined effect** of disease and drug treatment is significantly different from CTRL. Specifically, we evaluated the null hypothesis:

$$H_0 : \beta_{\text{FXS}} + \beta_{\text{Drug}} + \beta_{\text{FXS:Drug}} = 0$$

This tests whether the **net drug effect in the FXS group** is equivalent to the CTRL baseline (i.e., no rescue effect).

```
##
## Linear hypothesis test:
## X_FXS  + X_Drug_BAY  + X_FXS:X_Drug_BAY = 0
##
## Model 1: restricted model
## Model 2: Y ~ X_FXS + X_Drug_BAY + X_Drug_BPN + X_Drug_BP + X_FXS * X_Drug_BAY +
##     X_FXS * X_Drug_BPN + X_FXS * X_Drug_BP + (1 | Individual)
##
##   Df  Chisq Pr(>Chisq)
## 1
## 2  1 11.715  0.0006201 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

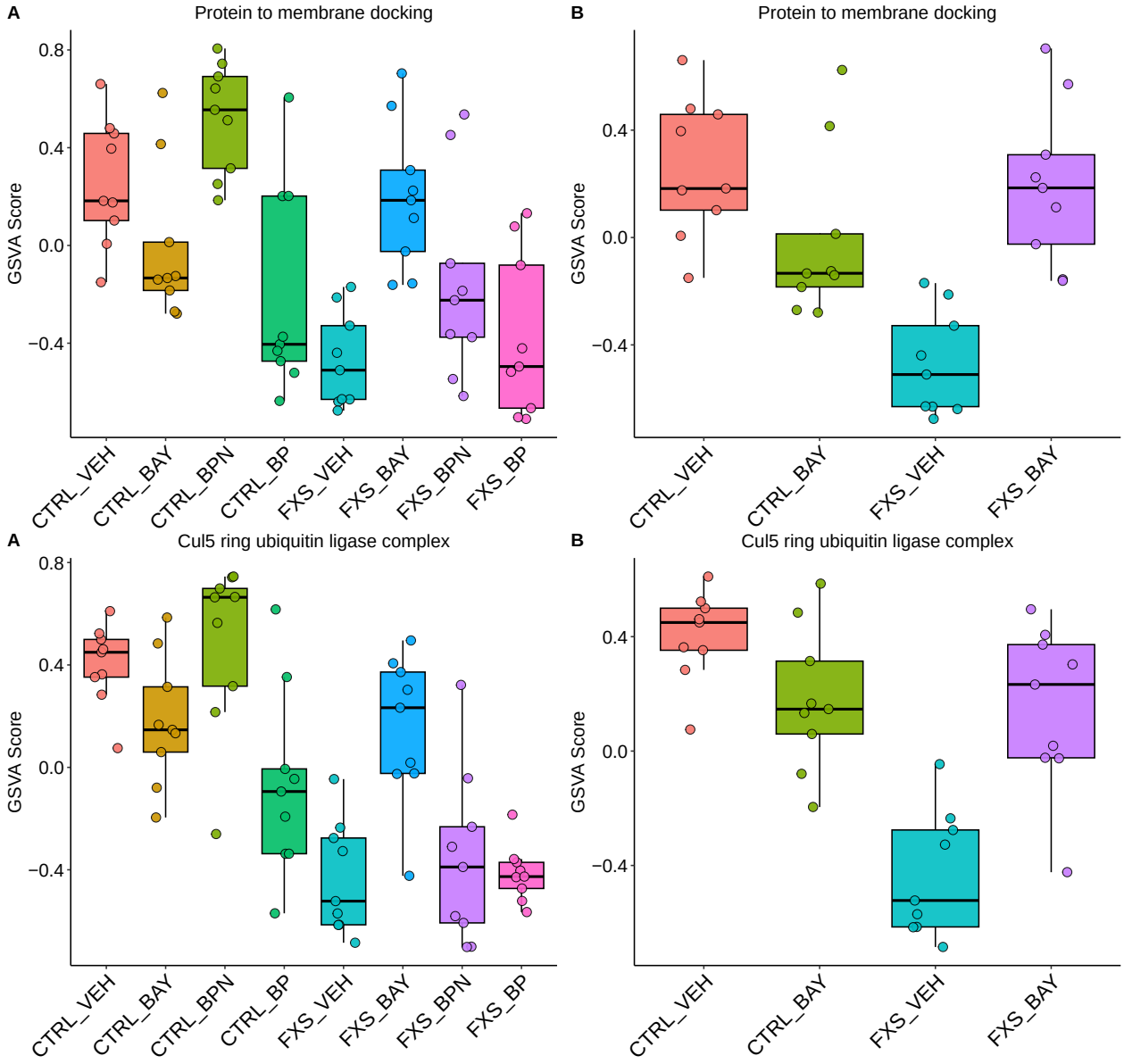
```
##
## Linear hypothesis test:
## X_FXS  + X_Drug_BPN  + X_FXS:X_Drug_BPN = 0
##
## Model 1: restricted model
## Model 2: Y ~ X_FXS + X_Drug_BAY + X_Drug_BPN + X_Drug_BP + X_FXS * X_Drug_BAY +
##     X_FXS * X_Drug_BPN + X_FXS * X_Drug_BP + (1 | Individual)
##
##   Df Chisq Pr(>Chisq)
## 1
## 2  1 0.904     0.3417
```

```
##
## Linear hypothesis test:
## X_FXS  + X_Drug_BP  + X_FXS:X_Drug_BP = 0
##
## Model 1: restricted model
## Model 2: Y ~ X_FXS + X_Drug_BAY + X_Drug_BPN + X_Drug_BP + X_FXS * X_Drug_BAY +
##     X_FXS * X_Drug_BPN + X_FXS * X_Drug_BP + (1 | Individual)
##
##   Df  Chisq Pr(>Chisq)
## 1
## 2  1 0.2737     0.6008
```

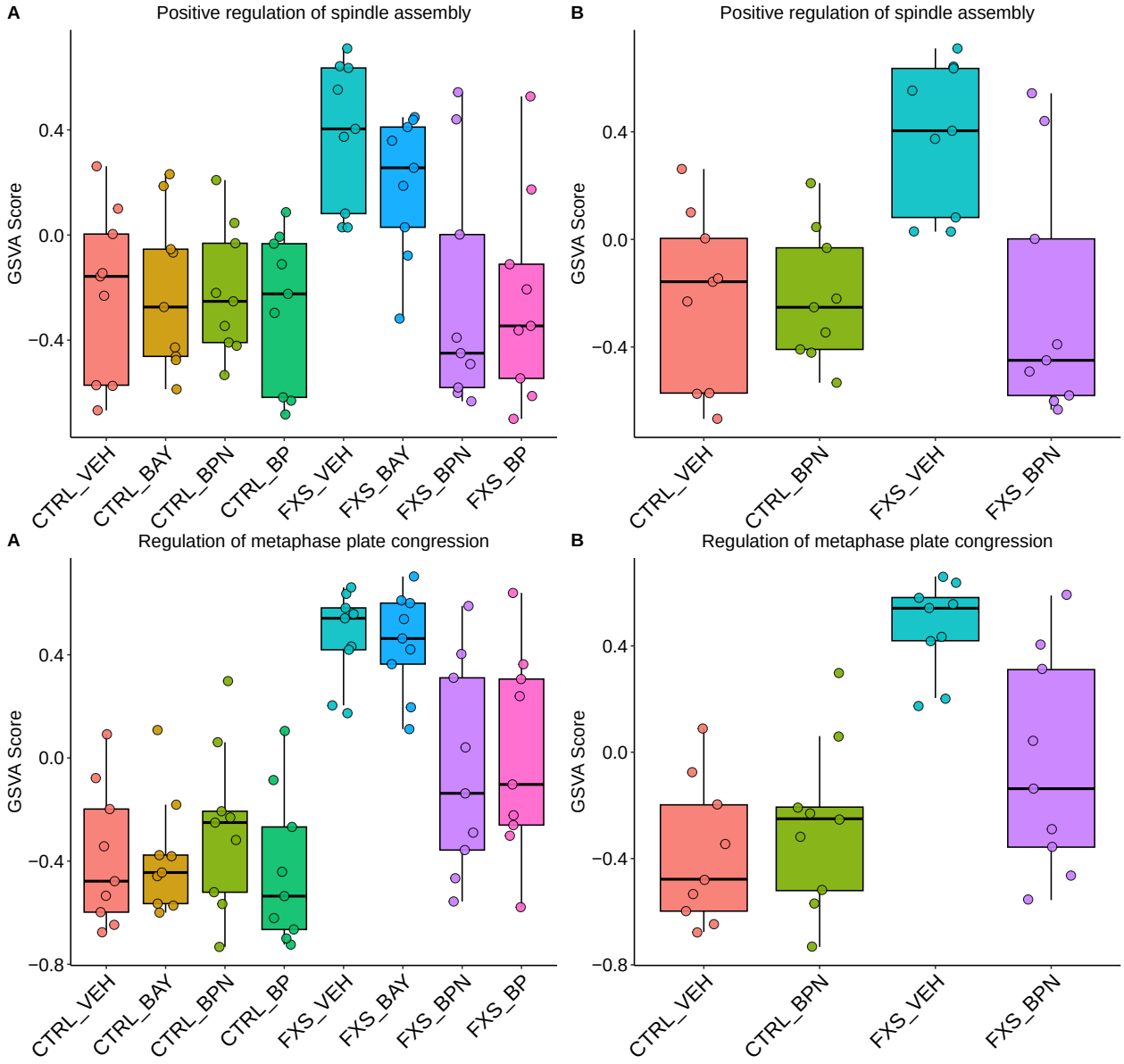
(3) Pathway Analysis: Full Rescue, Partial Rescue, and Side Effects

a. BAY Treatment

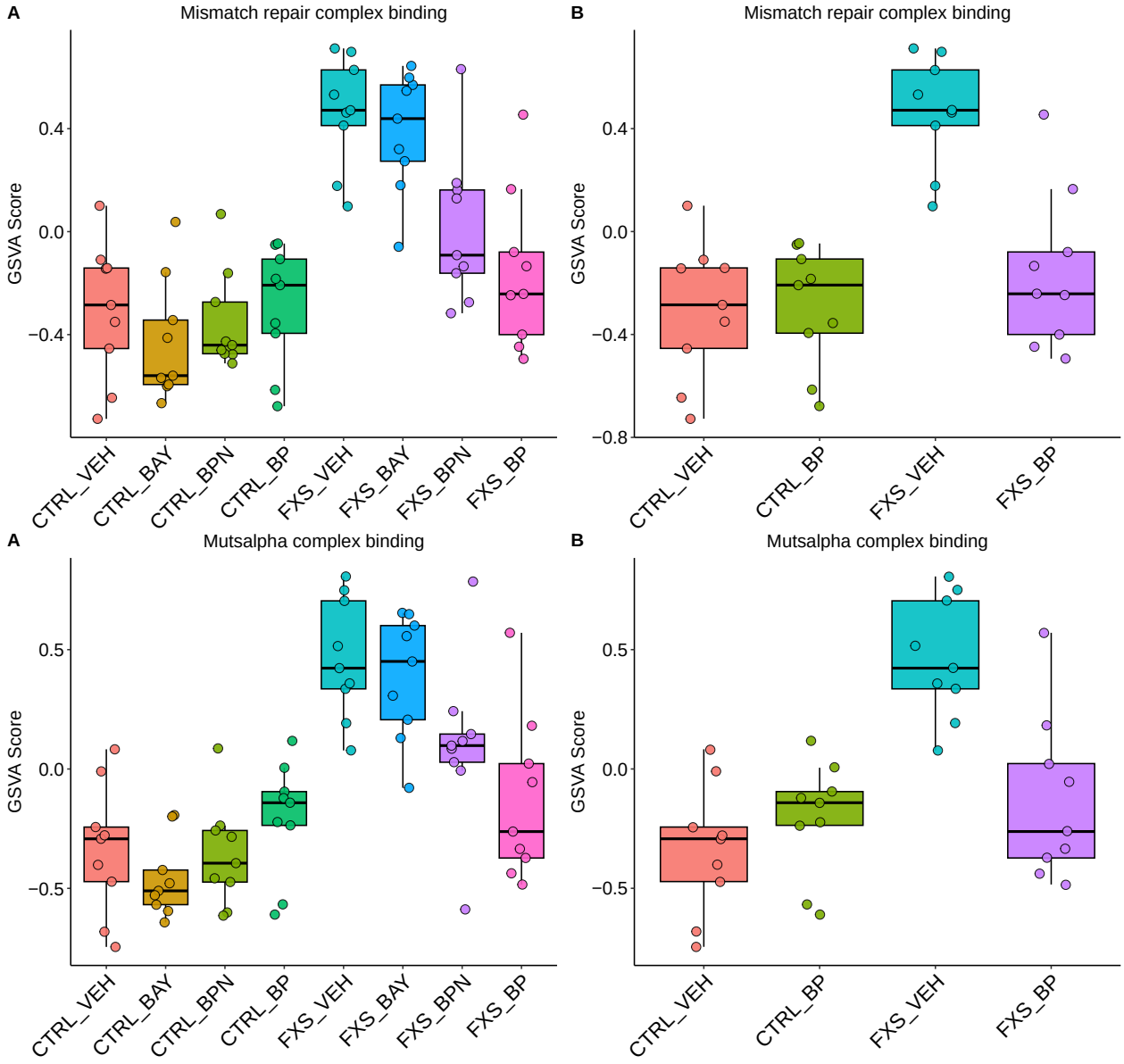
```
## [1] 717  18
```



b. BPN Treatment

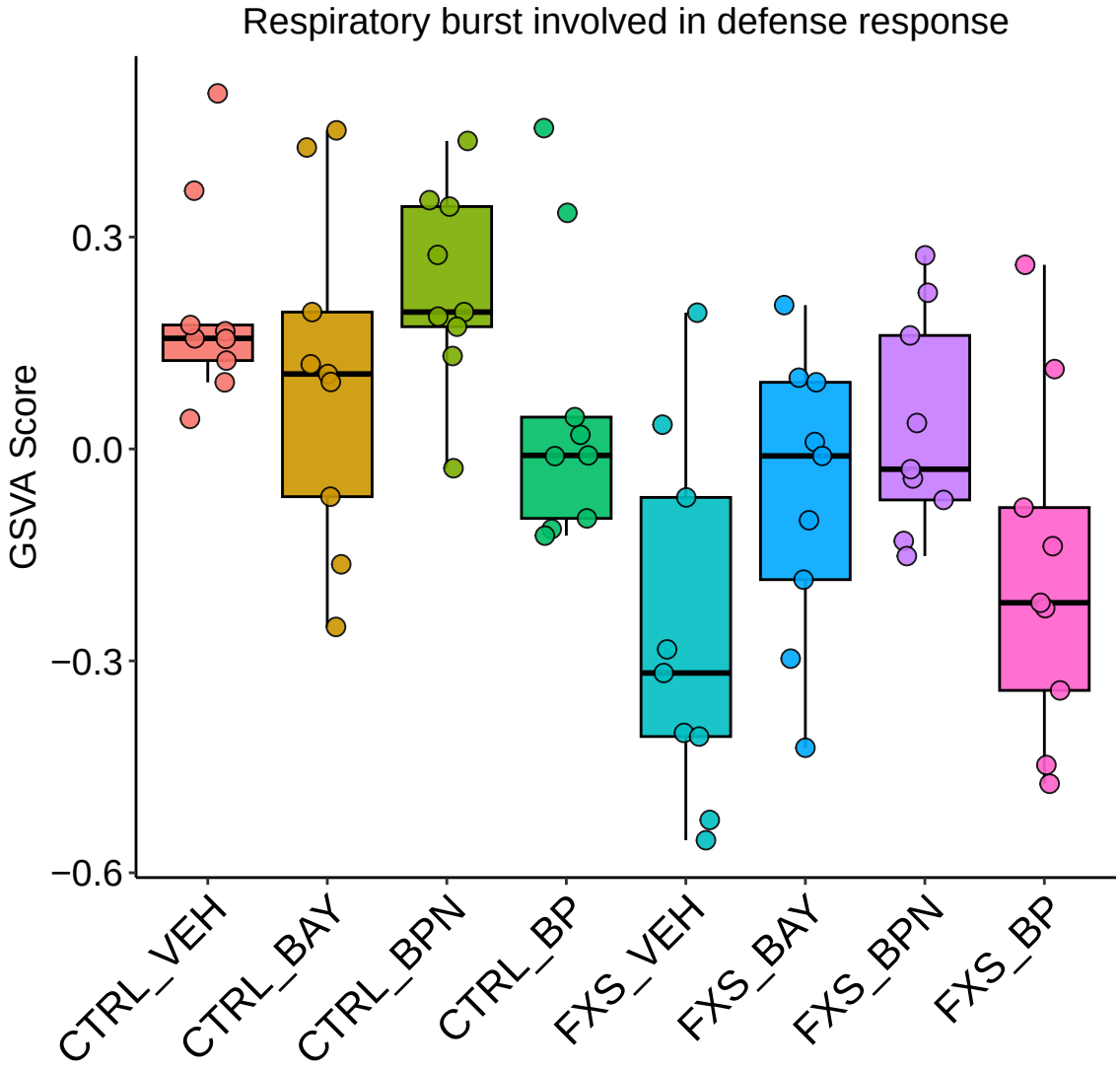


c. BP Treatment

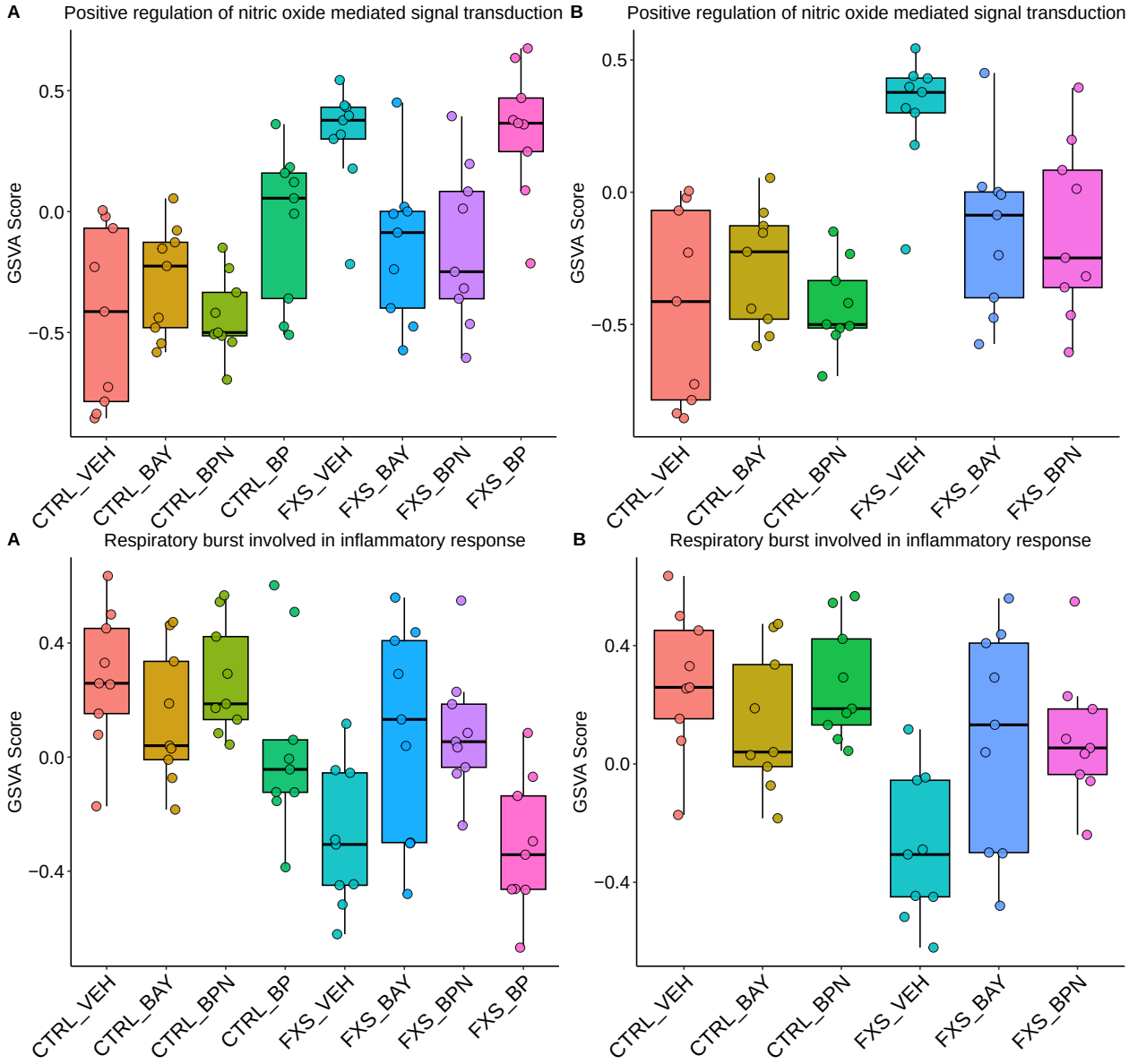


(4) Common Rescue Pathways

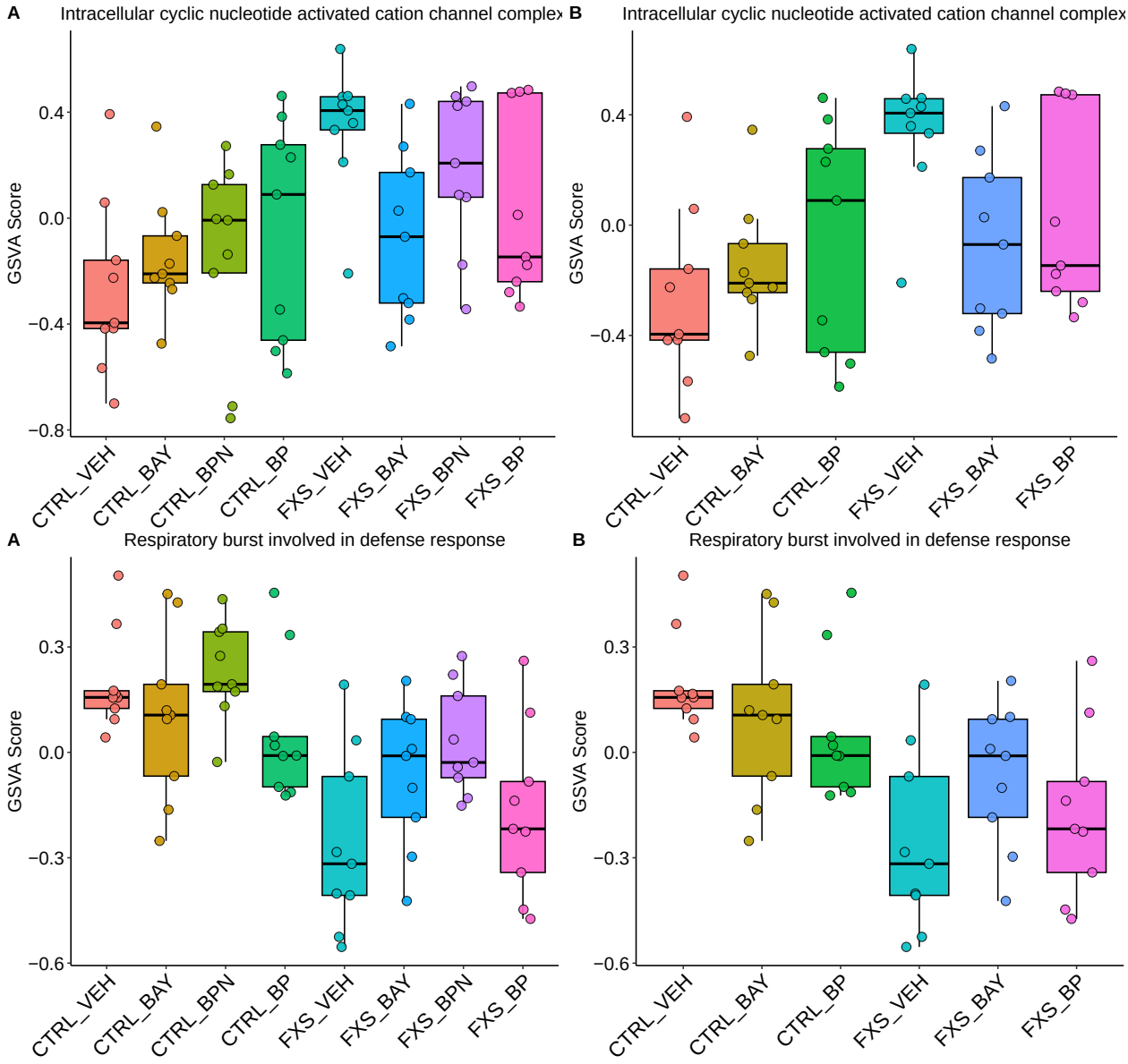
All 3 drugs.



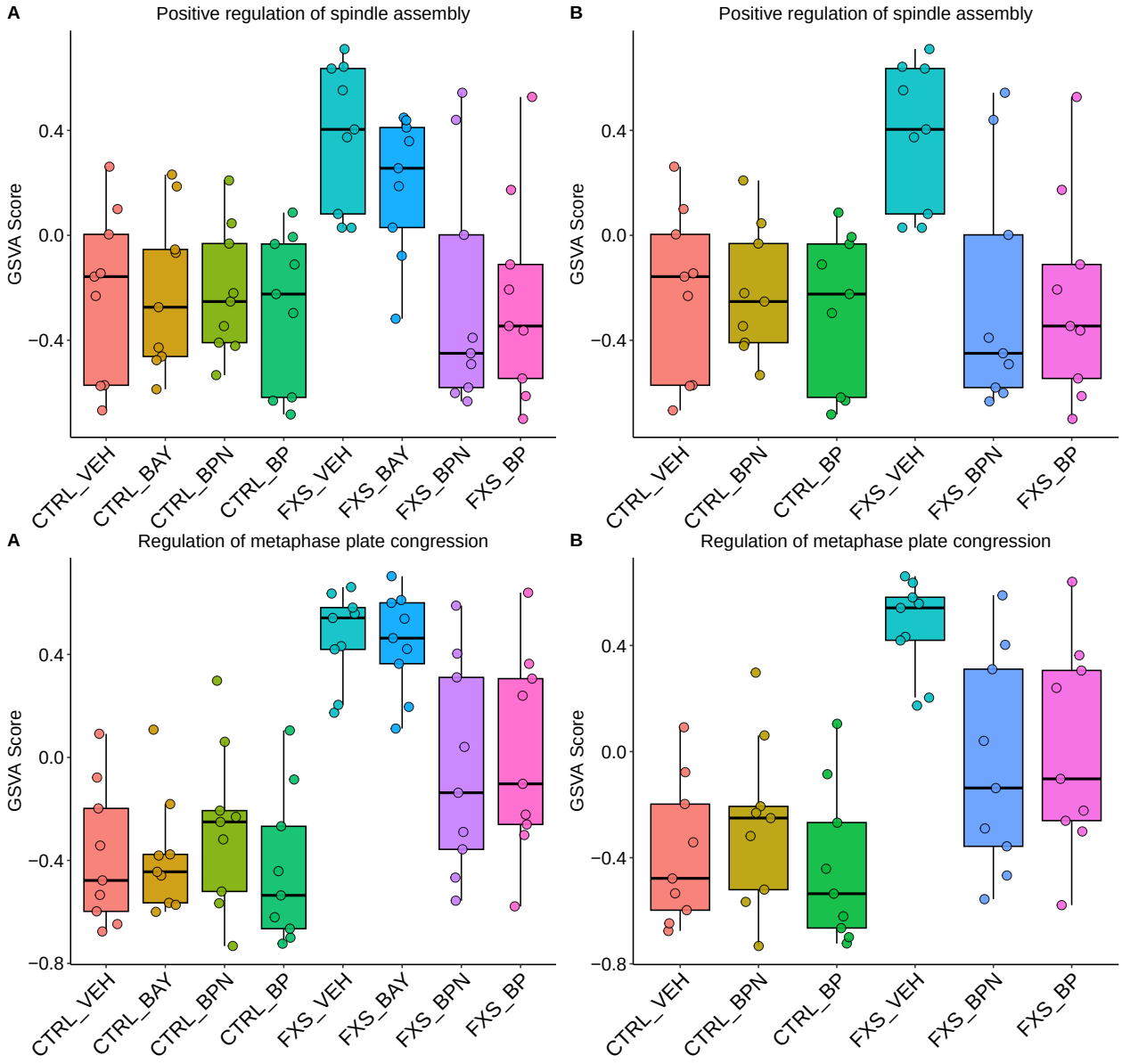
Overlap 2 drugs BAY and BPN

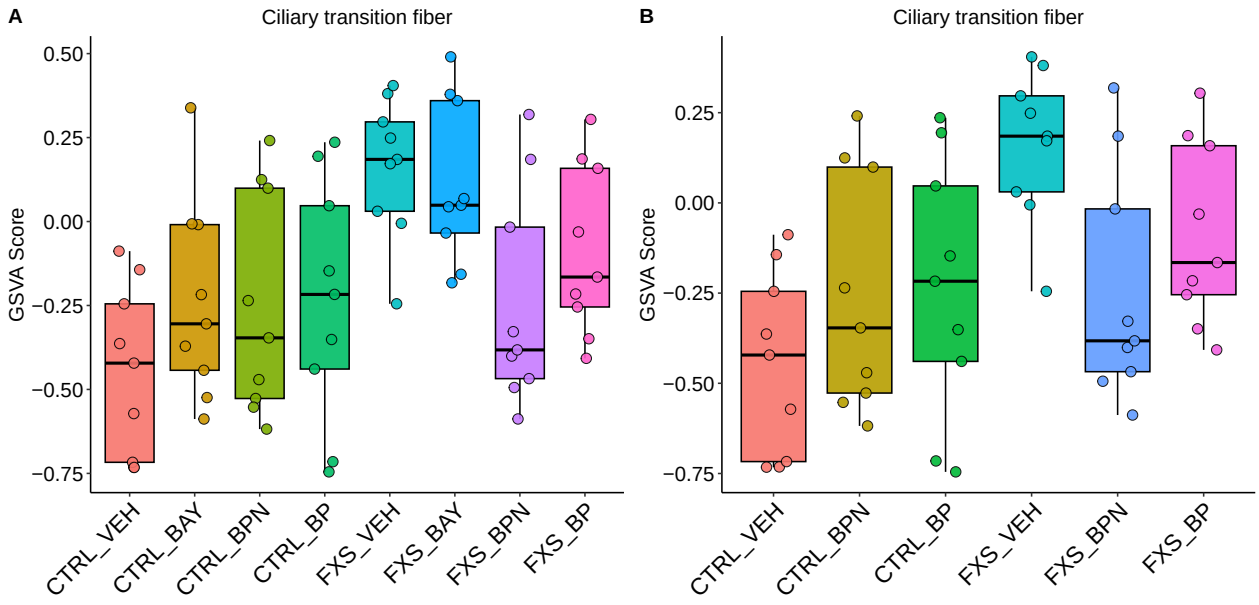


BAY and BP


A
Intracellular cyclic nucleotide activated cation channel complex
B
Intracellular cyclic nucleotide activated cation channel complex
GSVA Score
0.4
0.0
−0.4
−0.8
CTRL_VEH
CTRL_BAY
CTRL_BPN
CTRL_BP
FXS_VEH
FXS_BAY
FXS_BPN
FXS_BP
A
Respiratory burst involved in defense response
B
Respiratory burst involved in defense response
GSVA Score
0.3
0.0
−0.3
−0.6
CTRL_VEH
CTRL_BAY
CTRL_BPN
CTRL_BP
FXS_VEH
FXS_BAY
FXS_BPN
FXS_BP

BPN and BP


A
Positive regulation of spindle assembly
GSVA Score
0.4
0.0
−0.4
CTRL_VEH
CTRL_BAY
CTRL_BPN
CTRL_BP
FXS_VEH
FXS_BAY
FXS_BPN
FXS_BP
B
Positive regulation of spindle assembly
GSVA Score
0.4
0.0
−0.4
CTRL_VEH
CTRL_BPN
CTRL_BP
FXS_VEH
FXS_BPN
FXS_BP
A
Regulation of metaphase plate congression
GSVA Score
0.4
0.0
−0.4
−0.8
CTRL_VEH
CTRL_BAY
CTRL_BPN
CTRL_BP
FXS_VEH
FXS_BAY
FXS_BPN
FXS_BP
B
Regulation of metaphase plate congression
GSVA Score
0.4
0.0
−0.4
−0.8
CTRL_VEH
CTRL_BPN
CTRL_BP
FXS_VEH
FXS_BPN
FXS_BP


A
Ciliary transition fiber
GSVA Score
0.50
0.25
0.00
−0.25
−0.50
−0.75
CTRL_VEH
CTRL_BAY
CTRL_BPN
CTRL_BP
FXS_VEH
FXS_BAY
FXS_BPN
FXS_BP
B
Ciliary transition fiber
GSVA Score
0.25
0.00
−0.25
−0.50
−0.75
CTRL_VEH
CTRL_BPN
CTRL_BP
FXS_VEH
FXS_BPN
FXS_BP

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] 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              Signac_1.14.0
## [33] Seurat_5.2.1                SeuratObject_5.0.2
## [35] sp_2.2-0                    cowplot_1.1.3
## [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] lmerTest_3.1-3              lme4_1.1-36
## [45] Matrix_1.7-2                car_3.1-3
## [47] carData_3.0-5               knitr_1.49
## [49] lubridate_1.9.4             forcats_1.0.0
## [51] stringr_1.5.1               dplyr_1.1.4
## [53] purrr_1.0.4                 readr_2.1.5
## [55] tidyr_1.3.1                 tibble_3.2.1
## [57] ggplot2_3.5.1               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] Formula_1.2-5              prettyunits_1.2.0
##  [31] KEGGREST_1.44.1            promises_1.3.2
##  [33] httr_1.4.7                 restfulr_0.0.15
##  [35] rhdf5filters_1.16.0        globals_0.16.3
##  [37] fitdistrplus_1.2-2         rhdf5_2.48.0
##  [39] rstudioapi_0.17.1          UCSC.utils_1.0.0
##  [41] miniUI_0.1.1.1             generics_0.1.3
##  [43] DOSE_3.30.5                curl_6.2.1
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##  [47] ggraph_2.2.1               polyclip_1.10-7
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##  [55] hms_1.1.3                  irlba_2.3.5.1
##  [57] colorspace_2.1-1           filelock_1.0.3
##  [59] ROCR_1.0-11                reticulate_1.40.0
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##  [63] lmtest_0.9-40              later_1.4.1
##  [65] viridis_0.6.5              ggtree_3.12.0
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##  [69] future.apply_1.11.3        scattermore_1.2
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##  [73] pillar_1.10.1              nlme_3.1-167
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##  [77] RSpectra_0.16-2            stringi_1.8.4
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##  [83] crayon_1.5.3               abind_1.4-8
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##  [87] emdbook_1.3.13             locfit_1.5-9.11
##  [89] graphlayouts_1.2.2         bit_4.5.0.1
##  [91] fastmatch_1.1-6            textshaping_1.0.0
##  [93] codetools_0.2-20           BiocSingular_1.20.0
##  [95] plotly_4.10.4              mime_0.12
##  [97] splines_4.4.0              Rcpp_1.0.14
##  [99] fastDummies_1.7.5          sparseMatrixStats_1.16.0
## [101] dbplyr_2.5.0               Rttf2pt1_1.3.12
## [103] blob_1.2.4                 here_1.0.1
## [105] fs_1.6.5                   listenv_0.9.1
## [107] Rdpack_2.6.2               ggplotify_0.1.2
## [109] statmod_1.5.0              tzdb_0.4.0
## [111] tweenr_2.0.3               pkgconfig_2.0.3
```

```
## [113] tools_4.4.0                 cachem_1.1.0
## [115] rbibutils_2.3               RSQLite_2.3.9
## [117] viridisLite_0.4.2           DBI_1.2.3
## [119] numDeriv_2016.8-1.1         fastmap_1.2.0
## [121] rmarkdown_2.29              scales_1.3.0
## [123] grid_4.4.0                  ica_1.0-3
## [125] Rsamtools_2.20.0            coda_0.19-4.1
## [127] dotCall64_1.2               RANN_2.6.2
## [129] farver_2.1.2                reformulas_0.4.0
## [131] tidygraph_1.3.1             scatterpie_0.2.4
## [133] yaml_2.3.10                 cli_3.6.4
## [135] lifecycle_1.0.4             uwot_0.2.2
## [137] mvtnorm_1.3-3               timechange_0.3.0
## [139] gtable_0.3.6                rjson_0.2.23
## [141] ggridges_0.5.6              progressr_0.15.1
## [143] ape_5.8-1                   jsonlite_1.9.0
## [145] RcppHNSW_0.6.0              bitops_1.0-9
## [147] bit64_4.6.0-1               Rtsne_0.17
## [149] yulab.utils_0.2.0           spatstat.utils_3.1-2
## [151] bdsmatrix_1.3-7             GOSemSim_2.30.2
## [153] spatstat.univar_3.1-1       R.utils_2.12.3
## [155] lazyeval_0.2.2              shiny_1.10.0
## [157] htmltools_0.5.8.1           enrichplot_1.24.4
## [159] GO.db_3.19.1                sctransform_0.4.1
## [161] rappdirs_0.3.3              tinytex_0.55
## [163] glue_1.8.0                  spam_2.11-1
## [165] httr2_1.1.0                 XVector_0.44.0
## [167] RCurl_1.98-1.16             rprojroot_2.0.4
## [169] treeio_1.28.0               gridExtra_2.3
## [171] boot_1.3-31                 extrafontdb_1.0
## [173] igraph_2.1.4                R6_2.6.1
## [175] SingleCellExperiment_1.26.0 labeling_0.4.3
## [177] RcppRoll_0.3.1              cluster_2.1.8
## [179] bbmle_1.0.25.1              Rhdf5lib_1.26.0
## [181] aplot_0.2.4                 nloptr_2.1.1
## [183] DelayedArray_0.30.1         tidyselect_1.2.1
## [185] ggforce_0.4.2               xml2_1.3.6
## [187] future_1.34.0               rsvd_1.0.5
## [189] munsell_0.5.1               KernSmooth_2.23-26
## [191] htmlwidgets_1.6.4           fgsea_1.30.0
## [193] rlang_1.1.5                 spatstat.sparse_3.1-0
## [195] spatstat.explore_3.3-4
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