

WGCNA

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1. Read the count data

In this section, we will read the clean count data from the `synaptosomes_miRNA` folder. The data is stored in the format of `.csv`. We will read the data and filter the columns based on the condition list. The final table will be stored in `results/02-DEG-Vehicle/DESeq2_results.csv`.

2. Normalization to RPKMs

```
## [1] 41057
```

2. WGCNA analysis

```
## Allowing multi-threading with up to 8 threads.
```

```
## pickSoftThreshold: will use block size 1524.
```

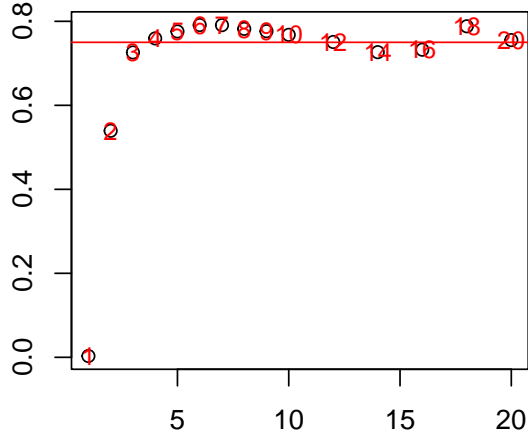
```
## pickSoftThreshold: calculating connectivity for given powers...
```

```
## ..working on genes 1 through 1524 of 29339
## ..working on genes 1525 through 3048 of 29339
## ..working on genes 3049 through 4572 of 29339
## ..working on genes 4573 through 6096 of 29339
## ..working on genes 6097 through 7620 of 29339
## ..working on genes 7621 through 9144 of 29339
## ..working on genes 9145 through 10668 of 29339
## ..working on genes 10669 through 12192 of 29339
## ..working on genes 12193 through 13716 of 29339
## ..working on genes 13717 through 15240 of 29339
## ..working on genes 15241 through 16764 of 29339
## ..working on genes 16765 through 18288 of 29339
## ..working on genes 18289 through 19812 of 29339
## ..working on genes 19813 through 21336 of 29339
## ..working on genes 21337 through 22860 of 29339
## ..working on genes 22861 through 24384 of 29339
## ..working on genes 24385 through 25908 of 29339
## ..working on genes 25909 through 27432 of 29339
## ..working on genes 27433 through 28956 of 29339
## ..working on genes 28957 through 29339 of 29339
```

##	Power	SFT.R.sq	slope	truncated.R.sq	mean.k.	median.k.	max.k.
## 1	1	0.00307	-0.0914	0.384	6940.0	6850.00	10300
## 2	2	0.53900	-0.9810	0.684	2660.0	2400.00	5480
## 3	3	0.72600	-1.2300	0.841	1320.0	1060.00	3530
## 4	4	0.75900	-1.3200	0.889	767.0	561.00	2520
## 5	5	0.77600	-1.3700	0.920	495.0	346.00	1910
## 6	6	0.79100	-1.4100	0.946	344.0	249.00	1500
## 7	7	0.79100	-1.4600	0.961	253.0	181.00	1220
## 8	8	0.78200	-1.4900	0.962	194.0	131.00	1020
## 9	9	0.77600	-1.5100	0.966	153.0	97.00	861
## 10	10	0.76800	-1.5300	0.963	125.0	73.10	740
## 11	12	0.75100	-1.5300	0.949	88.3	43.50	564
## 12	14	0.72700	-1.4800	0.918	66.9	27.20	445
## 13	16	0.73200	-1.3800	0.883	53.4	17.50	361
## 14	18	0.78800	-1.1700	0.843	44.4	11.70	299
## 15	20	0.75500	-1.1400	0.758	38.1	8.07	267

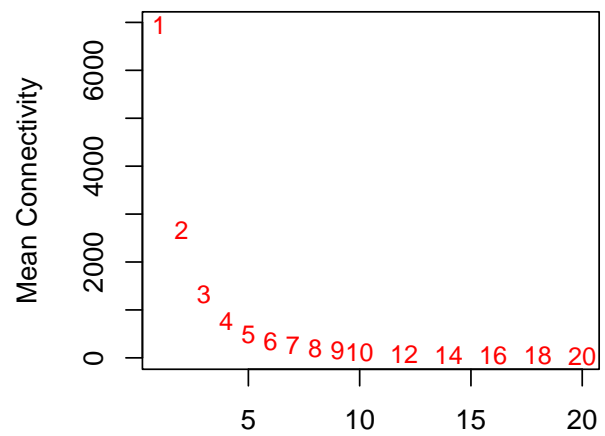
Scale Free Topology Model Fit, signed R^2

Scale independence



Soft Threshold (power)

Mean connectivity



Soft Threshold (power)

```
## Calculating module eigengenes block-wise from all genes
##   Flagging genes and samples with too many missing values...
##   ..step 1
##   ...pre-clustering genes to determine blocks..
##   Projective K-means:
##   ..k-means clustering..
##   ..merging smaller clusters...
## Block sizes:
## gBlocks
##   1   2   3   4   5   6   7
## 4963 4905 4732 4641 4470 3532 2096
## ..Working on block 1 .
##   TOM calculation: adjacency..
##   ..will use 8 parallel threads.
##   Fraction of slow calculations: 0.000000
##   ..connectivity..
##   ..matrix multiplication (system BLAS)..
##   ..normalization..
##   ..done.
##   ..saving TOM for block 1 into file ER-block.1.RData
##   ....clustering..
##   ....detecting modules..
##   ....calculating module eigengenes..
##   ....checking kME in modules..
##   ..removing 25 genes from module 1 because their KME is too low.
##   ..removing 21 genes from module 2 because their KME is too low.
##   ..removing 1 genes from module 3 because their KME is too low.
##   ..removing 19 genes from module 4 because their KME is too low.
##   ..removing 7 genes from module 5 because their KME is too low.
## ..Working on block 2 .
##   TOM calculation: adjacency..
##   ..will use 8 parallel threads.
##   Fraction of slow calculations: 0.000000
##   ..connectivity..
##   ..matrix multiplication (system BLAS)..
```

```

##      ..normalization..
##      ..done.
##      ..saving TOM for block 2 into file ER-block.2.RData
##      ....clustering..
##      ....detecting modules..
##      ....calculating module eigengenes..
##      ....checking kME in modules..
##          ..removing 25 genes from module 1 because their KME is too low.
##          ..removing 26 genes from module 2 because their KME is too low.
##          ..removing 9 genes from module 3 because their KME is too low.
##          ..removing 60 genes from module 4 because their KME is too low.
##          ..removing 20 genes from module 5 because their KME is too low.
##          ..removing 13 genes from module 6 because their KME is too low.
##          ..removing 2 genes from module 7 because their KME is too low.
##          ..removing 1 genes from module 9 because their KME is too low.
##          ..removing 2 genes from module 10 because their KME is too low.
##      ..Working on block 3 .
##          TOM calculation: adjacency..
##          ..will use 8 parallel threads.
##          Fraction of slow calculations: 0.000000
##          ..connectivity..
##          ..matrix multiplication (system BLAS)..
##          ..normalization..
##          ..done.
##          ..saving TOM for block 3 into file ER-block.3.RData
##          ....clustering..
##          ....detecting modules..
##          ....calculating module eigengenes..
##          ....checking kME in modules..
##              ..removing 52 genes from module 1 because their KME is too low.
##              ..removing 21 genes from module 2 because their KME is too low.
##              ..removing 8 genes from module 3 because their KME is too low.
##              ..removing 22 genes from module 4 because their KME is too low.
##              ..removing 12 genes from module 5 because their KME is too low.
##              ..removing 1 genes from module 6 because their KME is too low.
##      ..Working on block 4 .
##          TOM calculation: adjacency..
##          ..will use 8 parallel threads.
##          Fraction of slow calculations: 0.000000
##          ..connectivity..
##          ..matrix multiplication (system BLAS)..
##          ..normalization..
##          ..done.
##          ..saving TOM for block 4 into file ER-block.4.RData
##          ....clustering..
##          ....detecting modules..
##          ....calculating module eigengenes..
##          ....checking kME in modules..
##              ..removing 1 genes from module 1 because their KME is too low.
##              ..removing 4 genes from module 2 because their KME is too low.
##              ..removing 17 genes from module 3 because their KME is too low.
##              ..removing 15 genes from module 4 because their KME is too low.
##              ..removing 6 genes from module 5 because their KME is too low.
##      ..Working on block 5 .

```

```

## TOM calculation: adjacency..
## ..will use 8 parallel threads.
## Fraction of slow calculations: 0.000000
## ..connectivity..
## ..matrix multiplication (system BLAS)..
## ..normalization..
## ..done.
## ..saving TOM for block 5 into file ER-block.5.RData
## ....clustering..
## ....detecting modules..
## ....calculating module eigengenes..
## ....checking kME in modules..
## ..removing 18 genes from module 1 because their KME is too low.
## ..removing 8 genes from module 2 because their KME is too low.
## ..removing 32 genes from module 3 because their KME is too low.
## ..removing 25 genes from module 4 because their KME is too low.
## ..removing 10 genes from module 5 because their KME is too low.
## ..removing 22 genes from module 6 because their KME is too low.
## ..removing 7 genes from module 7 because their KME is too low.
## ..removing 4 genes from module 9 because their KME is too low.
## ..Working on block 6 .
## TOM calculation: adjacency..
## ..will use 8 parallel threads.
## Fraction of slow calculations: 0.000000
## ..connectivity..
## ..matrix multiplication (system BLAS)..
## ..normalization..
## ..done.
## ..saving TOM for block 6 into file ER-block.6.RData
## ....clustering..
## ....detecting modules..
## ....calculating module eigengenes..
## ....checking kME in modules..
## ..removing 13 genes from module 1 because their KME is too low.
## ..removing 14 genes from module 2 because their KME is too low.
## ..removing 8 genes from module 3 because their KME is too low.
## ..removing 10 genes from module 4 because their KME is too low.
## ..removing 5 genes from module 5 because their KME is too low.
## ..removing 11 genes from module 6 because their KME is too low.
## ..Working on block 7 .
## TOM calculation: adjacency..
## ..will use 8 parallel threads.
## Fraction of slow calculations: 0.000000
## ..connectivity..
## ..matrix multiplication (system BLAS)..
## ..normalization..
## ..done.
## ..saving TOM for block 7 into file ER-block.7.RData
## ....clustering..
## ....detecting modules..
## ....calculating module eigengenes..
## ....checking kME in modules..
## ..removing 28 genes from module 1 because their KME is too low.
## ..removing 22 genes from module 2 because their KME is too low.

```

```

##      ..removing 15 genes from module 3 because their KME is too low.
##      ..removing 7 genes from module 4 because their KME is too low.
##      ..reassigning 23 genes from module 1 to modules with higher KME.
##      ..reassigning 1 genes from module 2 to modules with higher KME.
##      ..reassigning 4 genes from module 3 to modules with higher KME.
##      ..reassigning 8 genes from module 4 to modules with higher KME.
##      ..reassigning 6 genes from module 7 to modules with higher KME.
##      ..reassigning 5 genes from module 8 to modules with higher KME.
##      ..reassigning 1 genes from module 9 to modules with higher KME.
##      ..reassigning 2 genes from module 10 to modules with higher KME.
##      ..reassigning 2 genes from module 17 to modules with higher KME.
##      ..reassigning 16 genes from module 18 to modules with higher KME.
##      ..reassigning 1 genes from module 19 to modules with higher KME.
##      ..reassigning 1 genes from module 20 to modules with higher KME.
##      ..reassigning 3 genes from module 21 to modules with higher KME.
##      ..reassigning 1 genes from module 22 to modules with higher KME.
##      ..reassigning 10 genes from module 24 to modules with higher KME.
##      ..reassigning 1 genes from module 25 to modules with higher KME.
##      ..reassigning 1 genes from module 26 to modules with higher KME.
##      ..reassigning 2 genes from module 27 to modules with higher KME.
##      ..reassigning 1 genes from module 28 to modules with higher KME.
##      ..reassigning 1 genes from module 29 to modules with higher KME.
##      ..reassigning 4 genes from module 30 to modules with higher KME.
##      ..reassigning 4 genes from module 32 to modules with higher KME.
##      ..reassigning 2 genes from module 33 to modules with higher KME.
##      ..reassigning 2 genes from module 35 to modules with higher KME.
##      ..reassigning 1 genes from module 37 to modules with higher KME.
##      ..reassigning 3 genes from module 39 to modules with higher KME.
##      ..reassigning 1 genes from module 40 to modules with higher KME.
##      ..reassigning 2 genes from module 41 to modules with higher KME.
##      ..reassigning 1 genes from module 42 to modules with higher KME.
##      ..reassigning 4 genes from module 45 to modules with higher KME.
##      ..reassigning 1 genes from module 46 to modules with higher KME.
##      ..reassigning 1 genes from module 47 to modules with higher KME.
##      ..merging modules that are too close..
##      mergeCloseModules: Merging modules whose distance is less than 0.15
##      Calculating new MEs...

## pdf
##      2

## pdf
##      2

## mergedColors
##      black          blue          brown          cyan          darkgreen
##      863            2131          1892            713            578
##      darkgrey      darkmagenta  darkolivegreen  darkorange    darkorange2
##      559            393          422            530            63
##      darkred       darkturquoise  floralwhite     green          greenyellow
##      585            565          90             1014           797
##      grey          grey60         ivory           lightcyan      lightcyan1
##      649            645          113            675            130
##      lightgreen    lightsteelblue1  lightyellow     magenta        mediumpurple3
##      619            131          596            817            132

```

```
##      midnightblue      orange      orangered4      paleturquoise      pink
##          709          553          138          461          862
##          plum1          purple          red          royalblue      saddlebrown
##          221          812          872          588          484
##          salmon      sienna3      skyblue      skyblue3      steelblue
##          713          365          493          243          477
##          tan      turquoise      violet      white      yellow
##          794          2269          445          503          1299
##      yellowgreen
##          336
```

```
## [1] 46
```

```
## [1] 20 1
```

```
## # A tibble: 46 x 6
```

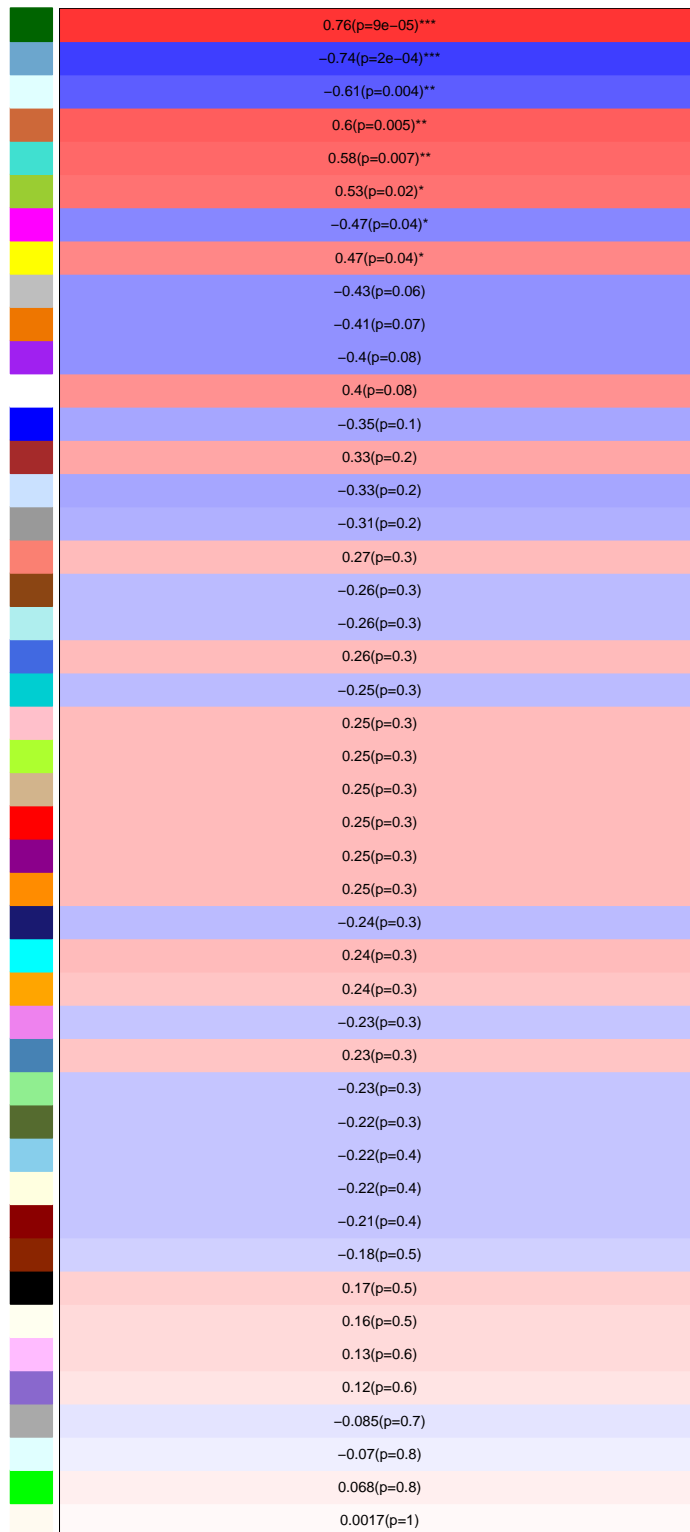
```
##      module      estimate1 estimate2 statistic  p.value  p.adj
##      <chr>      <dbl>      <dbl>      <dbl>      <dbl>      <dbl>
## 1 MEdarkgreen    0.171    -0.171      5.01 0.000267 0.00613
## 2 MESkyblue3    -0.166     0.166     -4.70 0.000198 0.00613
## 3 MElightcyan   -0.137     0.137     -3.29 0.00622 0.0945
## 4 MESienna3      0.135    -0.135      3.21 0.0103 0.0945
## 5 MEturquoise    0.130    -0.130      3.03 0.00963 0.0945
## 6 MEyellowgreen  0.119    -0.119      2.67 0.0167 0.128
## 7 MEmagenta     -0.105     0.105     -2.25 0.0428 0.281
## 8 MEyellow       0.104    -0.104      2.23 0.0522 0.300
## 9 MEgrey        -0.0955    0.0955     -2.00 0.0672 0.343
## 10 MEdarkorange2 -0.0918    0.0918     -1.91 0.0878 0.362
```

```
## # i 36 more rows
```

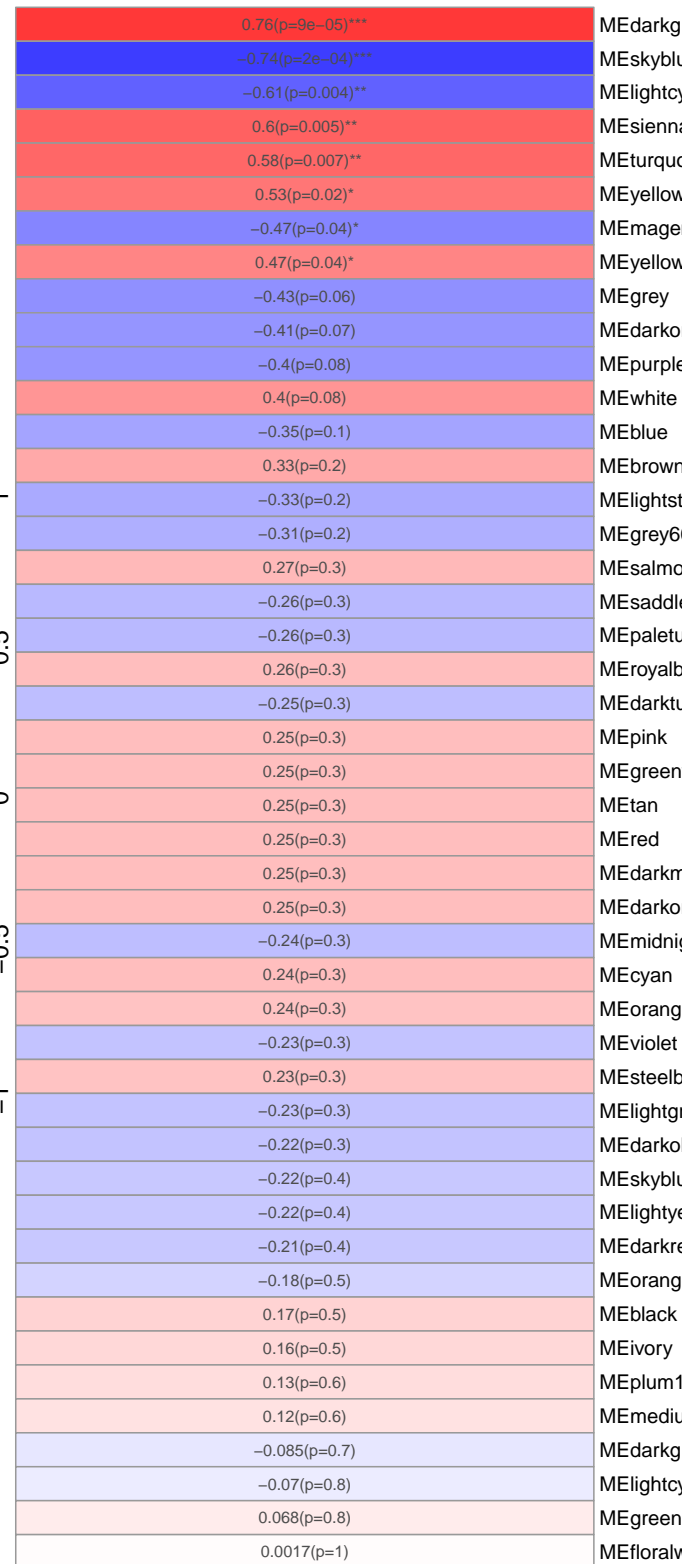
```
##      module      cor      p.value      p.adj
## 1 MEdarkgreen  0.763017163 9.120366e-05 0.004092132
## 2 MESkyblue3 -0.742397164 1.779188e-04 0.004092132
## 3 MElightcyan -0.612752771 4.073717e-03 0.055855466
## 4 MESienna3 0.603338127 4.856997e-03 0.055855466
## 5 MEturquoise 0.580705603 7.258383e-03 0.066777122
## 6 MEyellowgreen 0.532385610 1.567041e-02 0.120139796
## 7 MEmagenta -0.468957550 3.699505e-02 0.221308378
## 8 MEyellow 0.465745322 3.848841e-02 0.221308378
## 9 MEgrey -0.426964586 6.044480e-02 0.307464511
## 10 MEdarkorange2 -0.410764169 7.200601e-02 0.307464511
## 11 MEpurple -0.404106264 7.720868e-02 0.307464511
## 12 MEwhite 0.400418465 8.020813e-02 0.307464511
## 13 MEblue -0.351588067 1.284804e-01 0.451509231
## 14 MEbrown 0.331529608 1.533101e-01 0.451509231
## 15 MElightsteelblue1 -0.326398022 1.601630e-01 0.451509231
## 16 MEgrey60 -0.308872567 1.851531e-01 0.451509231
## 17 MESalmon 0.268686387 2.520224e-01 0.451509231
## 18 MESaddlebrown -0.262116510 2.642497e-01 0.451509231
## 19 MEpaleturquoise -0.261004985 2.663547e-01 0.451509231
## 20 MERoyalblue 0.258636733 2.708748e-01 0.451509231
## 21 MEdarkturquoise -0.254673912 2.785449e-01 0.451509231
## 22 MEpink 0.253740020 2.803719e-01 0.451509231
## 23 MEgreenyellow 0.251552001 2.846815e-01 0.451509231
## 24 MEtan 0.250041560 2.876803e-01 0.451509231
## 25 MERed 0.249998292 2.877665e-01 0.451509231
```

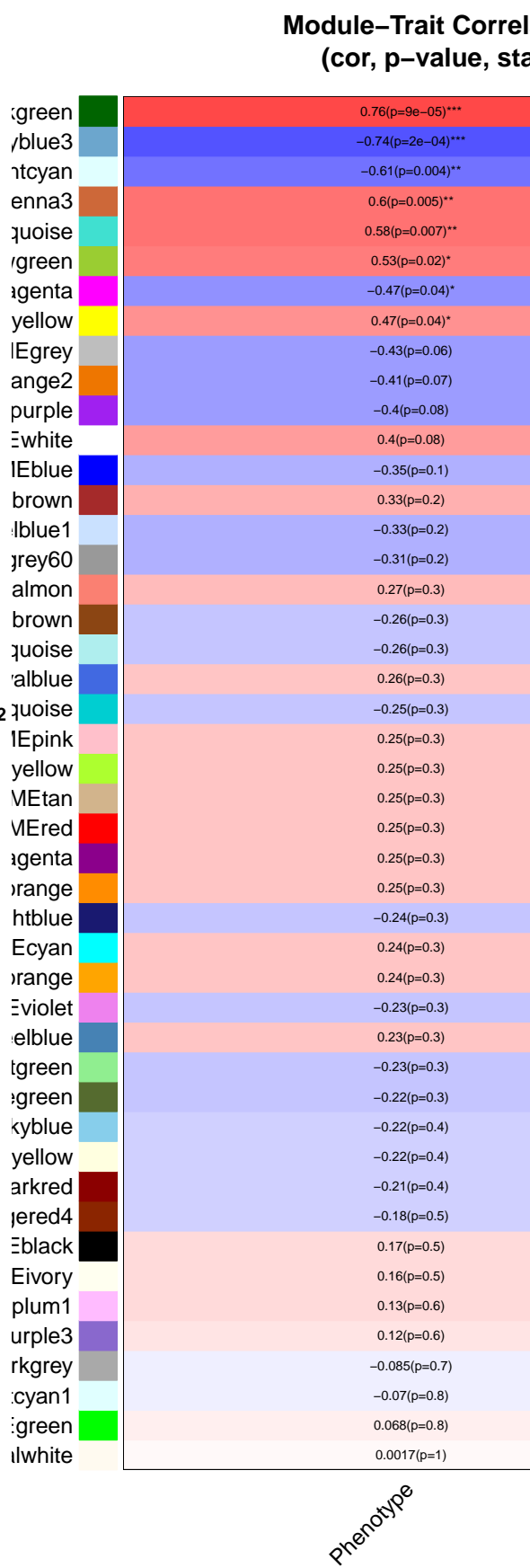
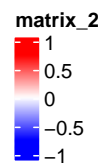
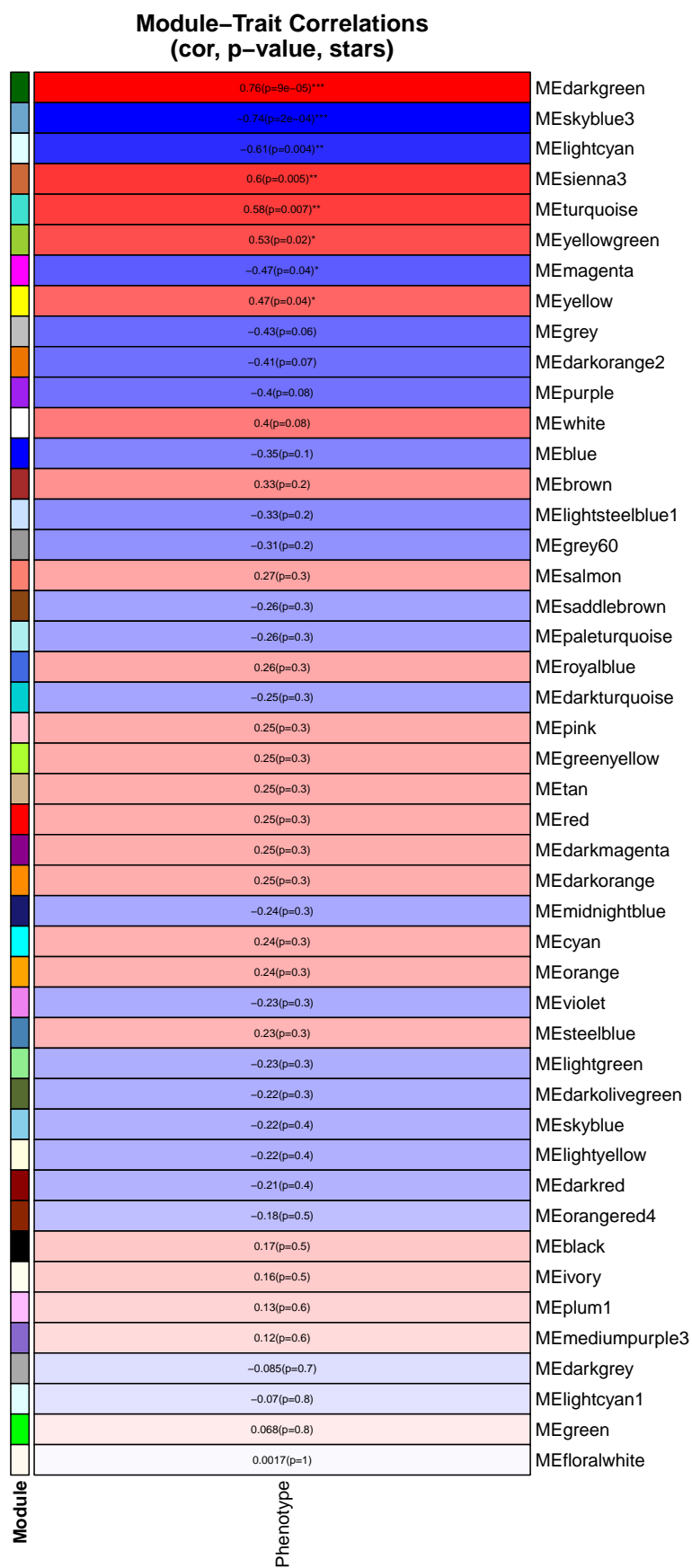
## 26	MEdarkmagenta	0.247208029	2.933582e-01	0.451509231
## 27	MEdarkorange	0.245654117	2.965009e-01	0.451509231
## 28	MEmidnightblue	-0.241168271	3.056882e-01	0.451509231
## 29	MEcyan	0.240228923	3.076336e-01	0.451509231
## 30	MEorange	0.235415353	3.177199e-01	0.451509231
## 31	MEviolet	-0.232832509	3.232126e-01	0.451509231
## 32	MEsteelblue	0.227845329	3.339771e-01	0.451509231
## 33	MElightgreen	-0.225589372	3.389150e-01	0.451509231
## 34	MEdarkolivegreen	-0.222356168	3.460660e-01	0.451509231
## 35	MEskyblue	-0.219662716	3.520897e-01	0.451509231
## 36	MElightyellow	-0.219100348	3.533551e-01	0.451509231
## 37	MEdarkred	-0.213230072	3.667192e-01	0.455921106
## 38	MEorangered4	-0.177914445	4.529874e-01	0.548353167
## 39	MEblack	0.172148624	4.679945e-01	0.551993568
## 40	MEivory	0.158963051	5.032363e-01	0.578721802
## 41	MEplum1	0.134877074	5.707476e-01	0.640350971
## 42	MEmediumpurple3	0.115053003	6.290875e-01	0.689000637
## 43	MEdarkgrey	-0.084602482	7.228679e-01	0.773300498
## 44	MElightcyan1	-0.069621440	7.705448e-01	0.793402197
## 45	MEgreen	0.067876270	7.761543e-01	0.793402197
## 46	MEfloralwhite	0.001700947	9.943215e-01	0.994321484

**Module-Trait Correlations
(cor, p-value, stars)**



**Module-Trait Correlations
(cor, p-value, stars)**





```
## pdf
## 2

## pdf
## 2

## [1] "Processing module: MEdarkgreen"
## [1] "Processing module: MELightcyan"
## [1] "Processing module: MEmagenta"
## [1] "Processing module: MESienna3"
## [1] "Processing module: MESkyblue3"
## [1] "Processing module: METurquoise"
## [1] "Processing module: MEyellow"
## [1] "Processing module: MEyellowgreen"
```

Session information

```
## R version 4.4.0 (2024-04-24)
## Platform: aarch64-apple-darwin20
## Running under: macOS 15.4
##
## 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] grid      parallel stats4      stats      graphics  grDevices  utils
## [8] datasets  methods   base
##
## other attached packages:
## [1] broom_1.0.8                countToFPKM_1.0
## [3] circlize_0.4.16            ComplexHeatmap_2.20.0
## [5] GSEABase_1.66.0            graph_1.82.0
## [7] annotate_1.82.0            XML_3.99-0.18
## [9] extrafont_0.19             ggsignif_0.6.4
## [11] patchwork_1.3.0            decoupleR_2.10.0
## [13] GSVA_1.52.3                BiocParallel_1.38.0
## [15] edgeR_4.2.2                limma_3.60.6
## [17] GenomicFeatures_1.56.0     biomaRt_2.60.1
## [19] gprofiler2_0.2.3           RColorBrewer_1.1-3
## [21] data.table_1.17.2          org.Hs.eg.db_3.19.1
## [23] AnnotationDbi_1.66.0       clusterProfiler_4.12.6
## [25] ggfortify_0.4.17           pheatmap_1.0.12
## [27] EnhancedVolcano_1.22.0     ggrepel_0.9.6
## [29] apeglm_1.26.1              DESeq2_1.44.0
## [31] SummarizedExperiment_1.34.0 Biobase_2.64.0
## [33] MatrixGenerics_1.16.0     matrixStats_1.5.0
## [35] reshape2_1.4.4            Matrix_1.7-3
## [37] Signac_1.14.0              Seurat_5.3.0
## [39] SeuratObject_5.1.0         sp_2.2-0
## [41] WGCNA_1.73                 fastcluster_1.3.0
## [43] dynamicTreeCut_1.63-1     rtracklayer_1.64.0
## [45] GenomicRanges_1.56.2      GenomeInfoDb_1.40.1
## [47] IRanges_2.38.1            S4Vectors_0.42.1
## [49] BiocGenerics_0.50.0       knitr_1.50
## [51] lubridate_1.9.4           forcats_1.0.0
## [53] stringr_1.5.1             dplyr_1.1.4
## [55] purrr_1.0.4               readr_2.1.5
## [57] tidyr_1.3.1               tibble_3.2.1
## [59] ggplot2_3.5.2             tidyverse_2.0.0
##
## loaded via a namespace (and not attached):
## [1] SpatialExperiment_1.14.0    R.methodsS3_1.8.2
```

##	[3]	dichromat_2.0-0.1	progress_1.2.3
##	[5]	nnet_7.3-20	goftest_1.2-3
##	[7]	HDF5Array_1.32.1	Biostrings_2.72.1
##	[9]	vctrs_0.6.5	spatstat.random_3.3-3
##	[11]	shape_1.4.6.1	digest_0.6.37
##	[13]	png_0.1-8	deldir_2.0-4
##	[15]	parallelly_1.44.0	magick_2.8.6
##	[17]	MASS_7.3-65	httpuv_1.6.16
##	[19]	foreach_1.5.2	qvalue_2.36.0
##	[21]	withr_3.0.2	xfun_0.52
##	[23]	ggfun_0.1.8	survival_3.8-3
##	[25]	memoise_2.0.1	gson_0.1.0
##	[27]	systemfonts_1.2.3	ragg_1.4.0
##	[29]	GlobalOptions_0.1.2	tidytree_0.4.6
##	[31]	zoo_1.8-14	pbapply_1.7-2
##	[33]	R.oo_1.27.1	Formula_1.2-5
##	[35]	prettyunits_1.2.0	KEGGREST_1.44.1
##	[37]	promises_1.3.2	httr_1.4.7
##	[39]	restfulr_0.0.15	rhdf5filters_1.16.0
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##	[43]	rhdf5_2.48.0	rstudioapi_0.17.1
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##	[47]	generics_0.1.4	DOSE_3.30.5
##	[49]	base64enc_0.1-3	curl_6.2.2
##	[51]	zlibbioc_1.50.0	ScaledMatrix_1.12.0
##	[53]	ggraph_2.2.1	polyclip_1.10-7
##	[55]	GenomeInfoDbData_1.2.12	SparseArray_1.4.8
##	[57]	xtable_1.8-4	doParallel_1.0.17
##	[59]	evaluate_1.0.3	S4Arrays_1.4.1
##	[61]	BiocFileCache_2.12.0	preprocessCore_1.66.0
##	[63]	hms_1.1.3	irlba_2.3.5.1
##	[65]	colorspace_2.1-1	filelock_1.0.3
##	[67]	ROCR_1.0-11	reticulate_1.42.0
##	[69]	spatstat.data_3.1-6	magrittr_2.0.3
##	[71]	lmtest_0.9-40	later_1.4.2
##	[73]	viridis_0.6.5	ggtree_3.12.0
##	[75]	lattice_0.22-7	spatstat.geom_3.3-6
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##	[79]	shadowtext_0.1.4	cowplot_1.1.3
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##	[83]	pillar_1.10.2	nlme_3.1-168
##	[85]	iterators_1.0.14	beachmat_2.20.0
##	[87]	compiler_4.4.0	RSpectra_0.16-2
##	[89]	stringi_1.8.7	tensor_1.5
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##	[93]	crayon_1.5.3	abind_1.4-8
##	[95]	BiocIO_1.14.0	gridGraphics_0.5-1
##	[97]	emdbbook_1.3.13	locfit_1.5-9.12
##	[99]	graphlayouts_1.2.2	bit_4.6.0
##	[101]	fastmatch_1.1-6	textshaping_1.0.1
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## [147] cli_3.6.5                    lifecycle_1.0.4
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## [199] ggforce_0.4.2                future_1.49.0
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## [203] htmlwidgets_1.6.4           fgsea_1.30.0
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## [207] spatstat.explore_3.4-2       Cairo_1.6-2

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