# Visualizing Bile Acids Proteomics results

```
mq <- read_tsv(here::here("normalyzer_limma_de_results_annotated.tsv"))
```

```
## Rows: 5705 Columns: 35
## -- Column specification --------------------------------------------------------
## Delimiter: "\t"
## chr  (4): Majority protein IDs, Protein IDs, Protein names, Gene names
## dbl (31): Cc-Lc_PValue, Cm-Lm_PValue, Cc-Lc_AdjPVal, Cm-Lm_AdjPVal, Cc-Lc_lo...
##
## i Use `spec()` to retrieve the full column specification for this data.
## i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

```
meta <- read_tsv(here::here("norm_meta.tsv"))
```

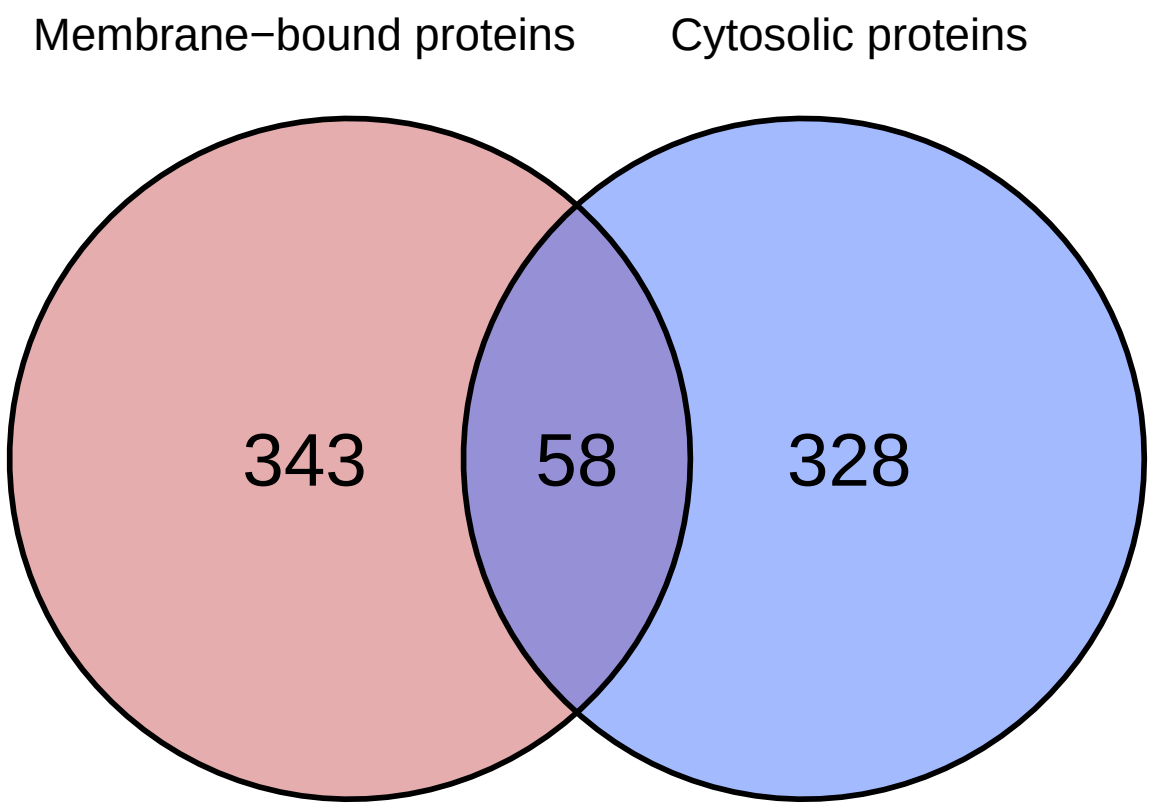
```
## Rows: 24 Columns: 2
## -- Column specification --------------------------------------------------------
## Delimiter: "\t"
## chr (2): sample, group
##
## i Use `spec()` to retrieve the full column specification for this data.
## i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

```
mq_res <- mq %>%
  select(contains("Log"), contains("Adj"), contains("Majority"), contains("Gene"))
mq_mat <- mq %>%
  select(`Gene names`, `Majority protein IDs`, matches("(C|L)[1-6](c|m)"))
#Convert the results table into a list and unify the columns names.
#Invert the foldChanges, so that comparisons are L-C instead of C-L.
#Reduce Gene names to just one gene for analysis
mq_res_list <- list(select(mq_res, -contains("Cc-Lc")),
                    select(mq_res, -contains("Cm-Lm"))) %>%
  set_names(nm = c("Membrane", "Cyto")) %>%
  map(rename_with, ~str_remove(.,"C(c|m)-L(c|m)_") ,matches("C(c|m)-L(c|m)_")) %>%
  map(mutate, across(contains("log2"), ~ -.x)) %>%
  map(separate, `Gene names`, into = letters [1:2], extra = "drop", sep = ";") %>%
  map(mutate, Genes = case_when(
    str_detect(a, "orf") & !is.na(b) & str_detect(b, "orf", negate = T) ~ b,
    TRUE ~ a
  ), Gene_rows = Genes)
```

```
## Warning: Expected 2 pieces. Missing pieces filled with `NA` in 5473 rows [3, 4, 6, 7, 8, 10, 11, 12,
## Expected 2 pieces. Missing pieces filled with `NA` in 5473 rows [3, 4, 6, 7, 8, 10, 11, 12, 13, 14,
```

## Venn diagram

```
#Filter results to only include DA proteins
da_list <- mq_res_list %>%
  map(filter, AdjPVal < 0.05, abs(log2FoldChange) > 1)
#Pull just the Protein IDs for DA proteins
da_genes <- da_list %>% map(pull, `Majority protein IDs`) %>%
  set_names(nm = c("Membrane-bound proteins", "Cytosolic proteins"))
ggvenn::ggvenn(da_genes, text_size = 10, set_name_size = 6,
               fill_color = c("indianred", "royalblue1"), show_percentage = F)
```



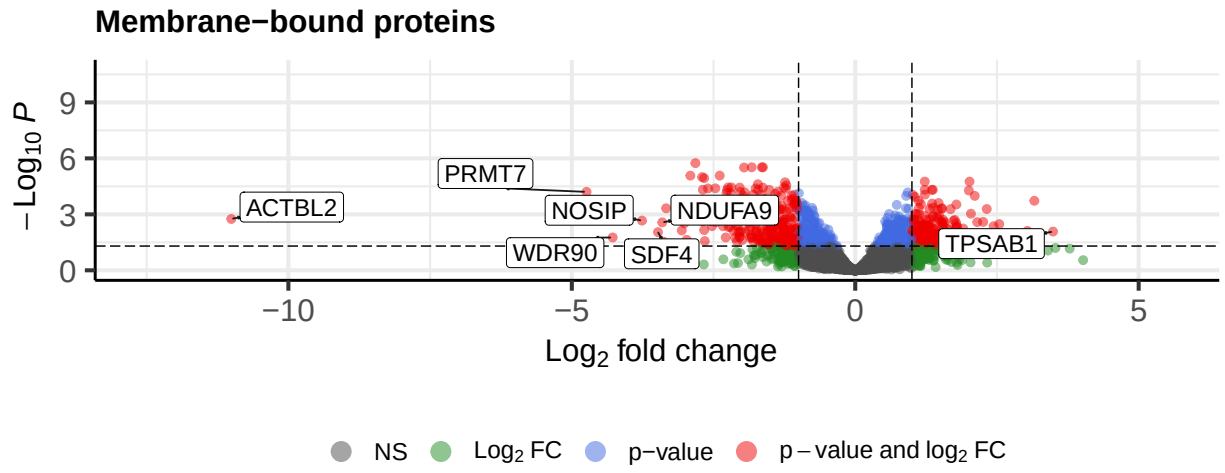

```
#ggsave(here::here("Venn diagram DA proteins.png"), units = "in", dpi = 720, width = 8, height = 6)
```

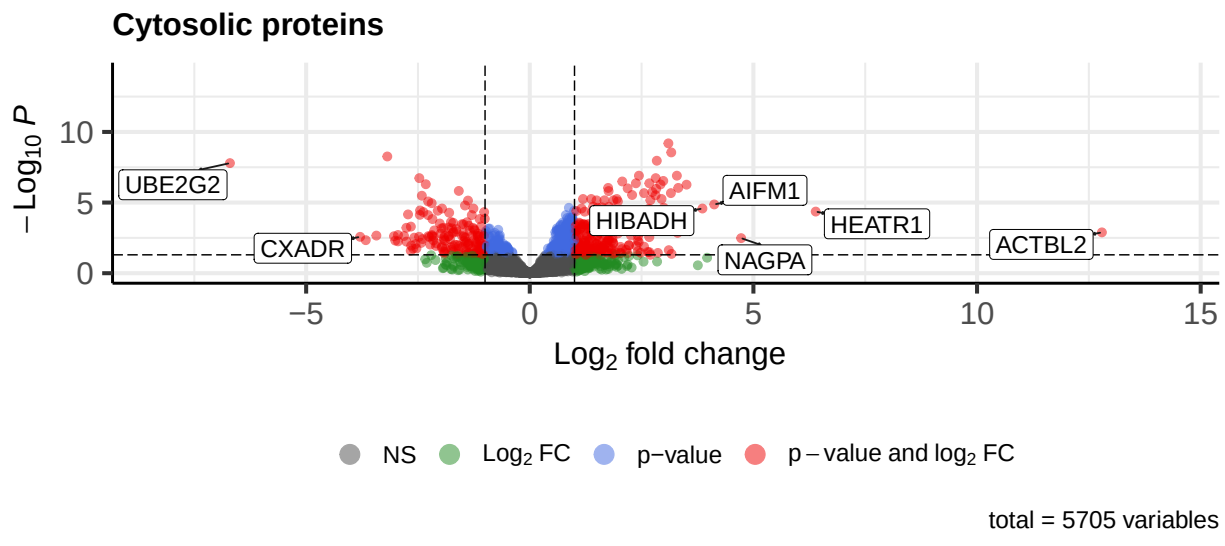
Get identities of common DE proteins

```
common_top <- da_genes %>%
  reduce(intersect)
mq_intersect_top <- mq_res %>%
  filter(`Majority protein IDs` %in% common_top)
#write_csv(mq_intersect_top, here::here("Shared DE proteins.csv"))
```

## Volcano plots

```
df <- mq_res_list[[1]]
rows <- map(mq_res_list, pull, var = Gene_rows)
titles <- names(da_genes)
top_hits <- mq_res_list %>%
  map(filter, AdjPVal < 0.05) %>%
  map(arrange, desc(abs(log2FoldChange))) %>%
  map(slice, 1:7) %>%
  map(pull, var = Gene_rows)
plot_list <- list(df = mq_res_list, rows = rows, title = titles, selectLab = top_hits)
#Create a custom volcano plot function
customvolcano <- function(df, rows, title, selectLab="") {
  print(EnhancedVolcano(toptable = df,lab = rows, x = "log2FoldChange", y = "AdjPVal",
                pCutoff = 0.05, FCcutoff = 1, legendPosition = 'bottom',
                title = title, subtitle = NULL,
                selectLab = selectLab, drawConnectors = T, boxedLabels = T))
}
#pdf(here::here("Volcano plots top 7 hits labelled.pdf"), width = 10)
pwalk(plot_list, customvolcano)
```






```
#dev.off()
```

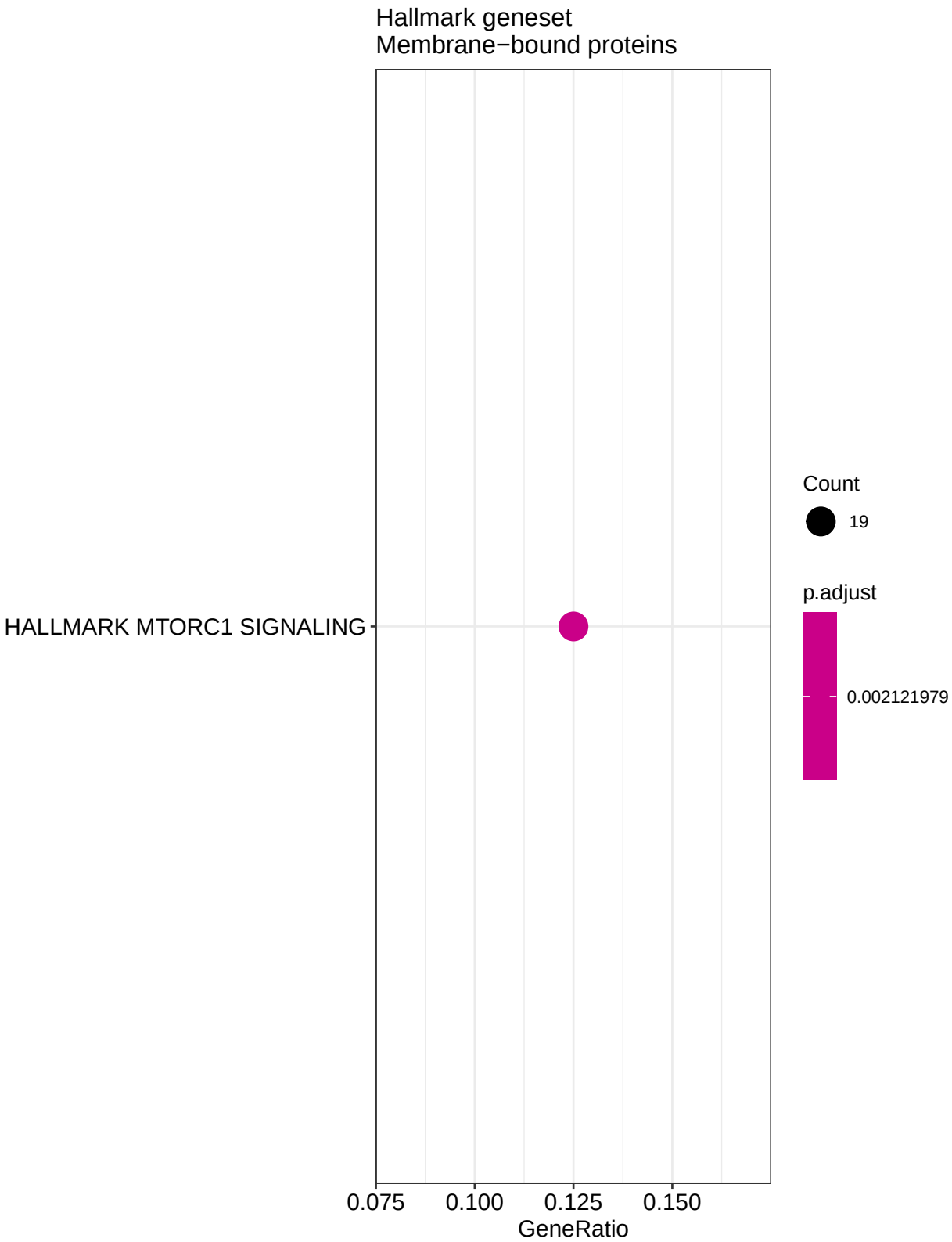
## Functional enrichment

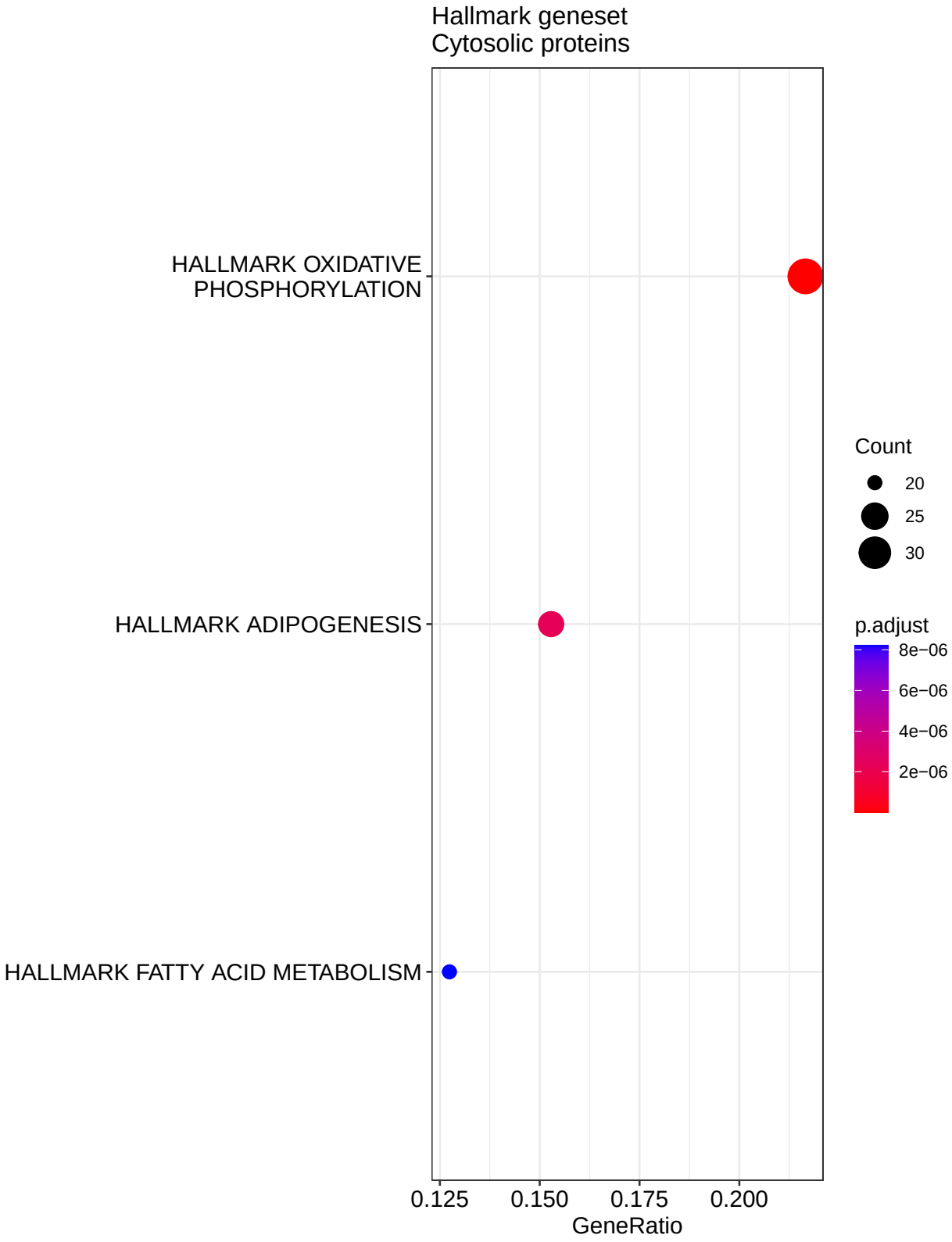
```
set.seed(711)
#Get the whole human genome as universe
human_genom <- org.Hs.egSYMBOL
mapped_genes <- mappedRkeys(human_genom)
h <- read.gmt(list.files(path = here::here("."),pattern = "h.all"))
c2_cp <- read.gmt(list.files(path = here::here("."),pattern = "c2.cp"))
c2_noID <- c2_cp %>%
  mutate(term = str_remove(term, "[:alpha:]+_"))
#Set up lists of genes for analysis
#Pull just the gene names for DA proteins
da_symbols <- da_list %>% map(pull, `Genes`) %>%
  set_names(nm = c("Membrane-bound proteins", "Cytosolic proteins"))
enrich_h_list <- map(da_symbols, ~enricher(.,
                     TERM2GENE = h,
                     universe = mapped_genes,
                     pAdjustMethod = "BH",
                     pvalueCutoff = 0.05,
                     qvalueCutoff = 0.05))
enrich_c2_list <- map(da_symbols, ~enricher(.,
                     TERM2GENE = c2_noID,
                     universe = mapped_genes,
                     pAdjustMethod = "BH",
                     pvalueCutoff = 0.05,
                     qvalueCutoff = 0.05))
ego_bp <- map(da_symbols, ~enrichGO(gene = .,
                OrgDb         = org.Hs.eg.db,
                universe = mapped_genes,
                keyType       = 'SYMBOL',
```

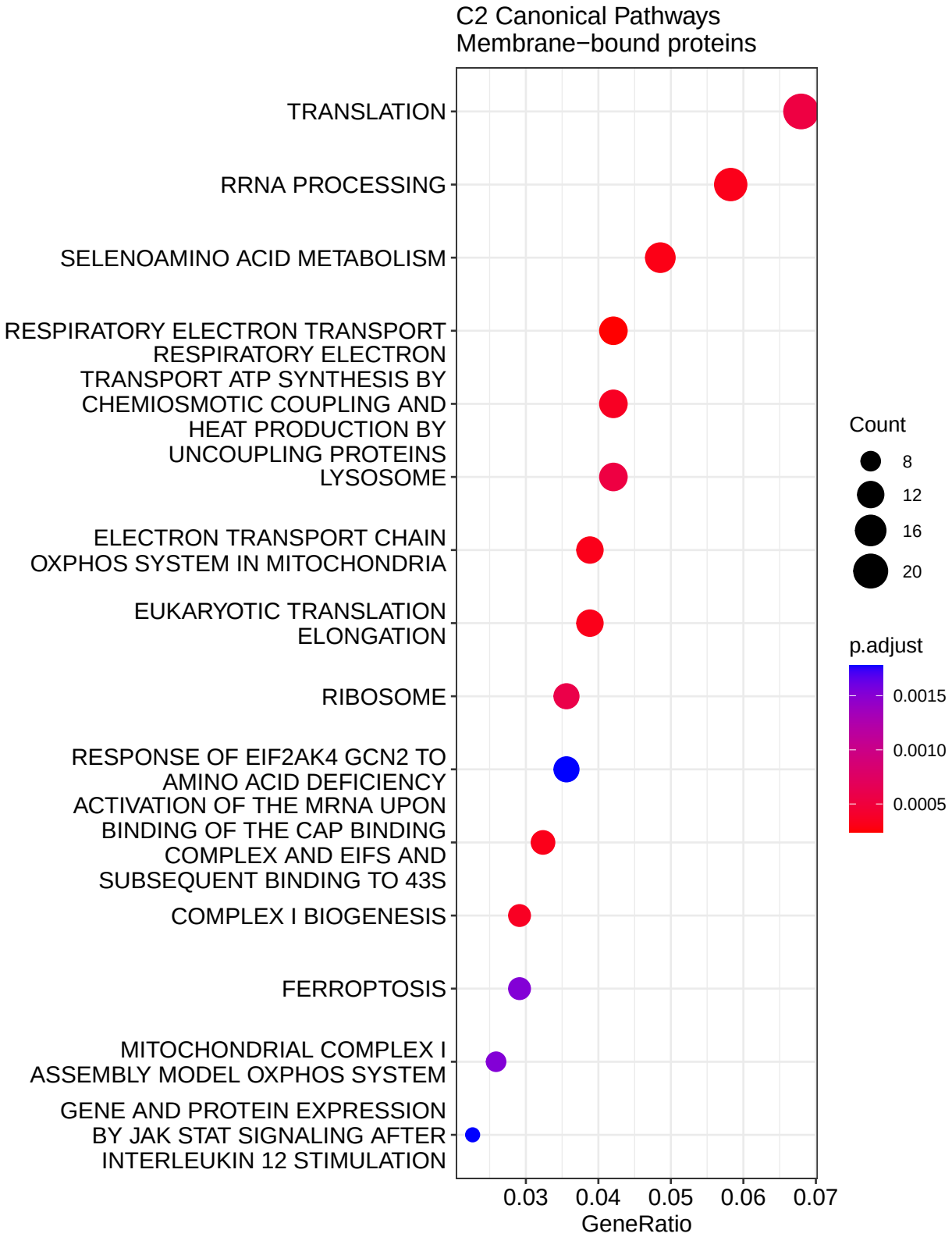
```
                ont           = "BP",
                pAdjustMethod = "BH",
                pvalueCutoff  = 0.05,
                qvalueCutoff  = 0.05))
ego_bp <- map(ego_bp,enrichplot::pairwise_termsim)
ego_bp2 <- ego_bp %>%
  map(~clusterProfiler::simplify(., cutoff=0.4, by="p.adjust", select_fun=min))
```

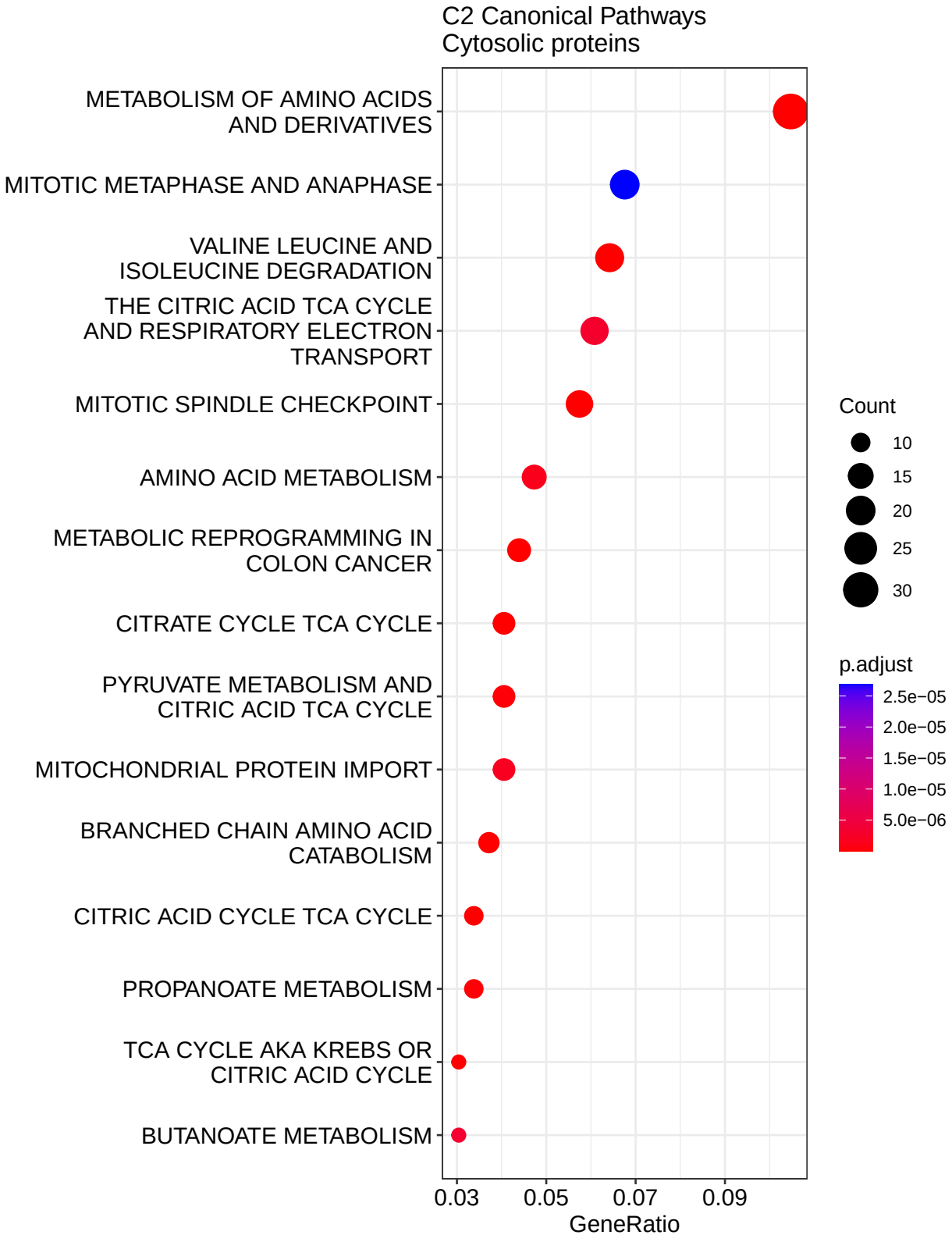
Plot the functional enrichment results

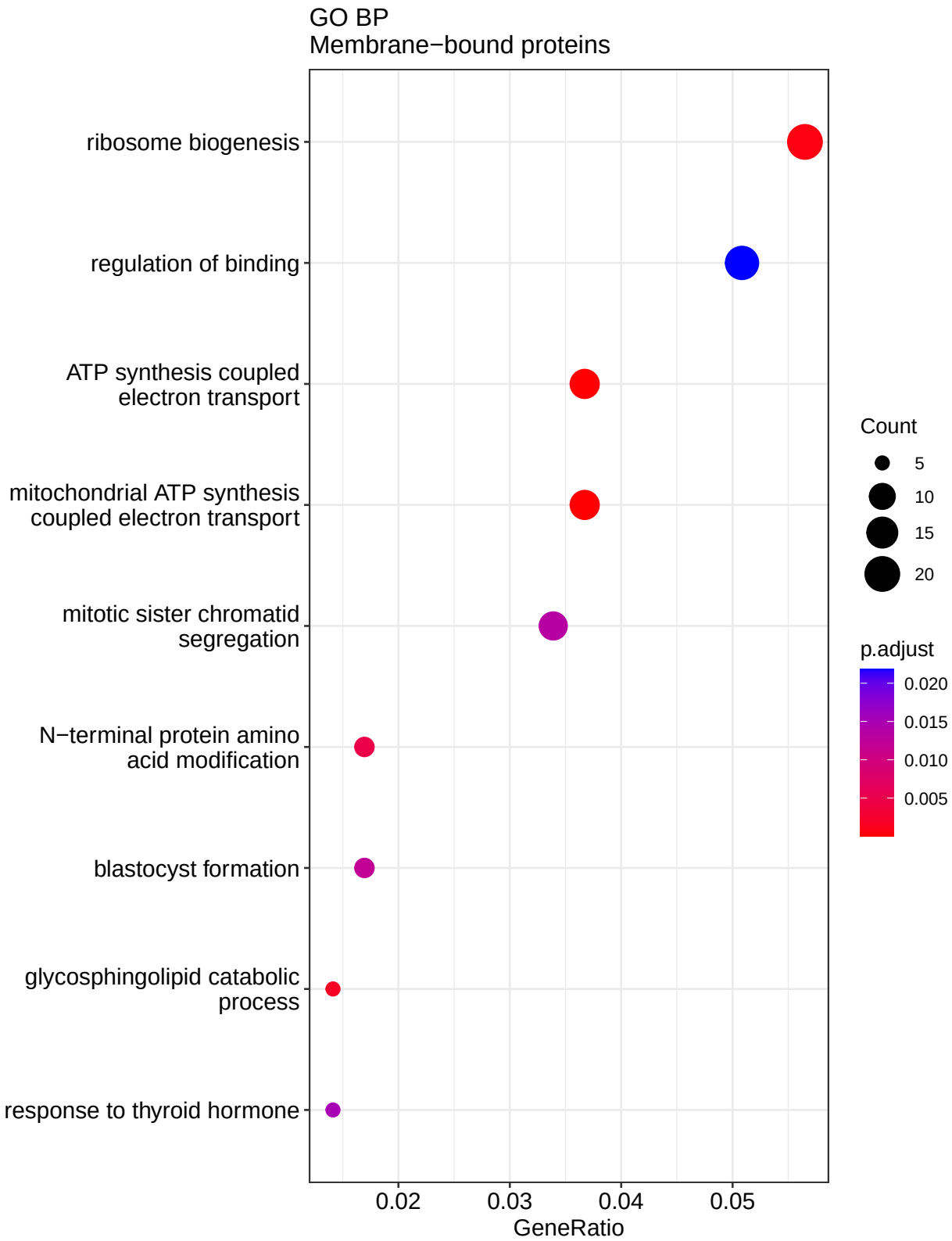
```
dot_titles <- rep(c("Hallmark geneset", "C2 Canonical Pathways", "GO BP"), each = 2) %>%
  str_c(names(da_genes), sep = "\n")
#pdf(here::here("Functional enrichment analyses.pdf"), height = 9, width = 7)
walk2(c(enrich_h_list, enrich_c2_list, ego_bp2), dot_titles,
      ~print(dotplot(.x, showCategory = 15) + ggtitle(.y)))
```

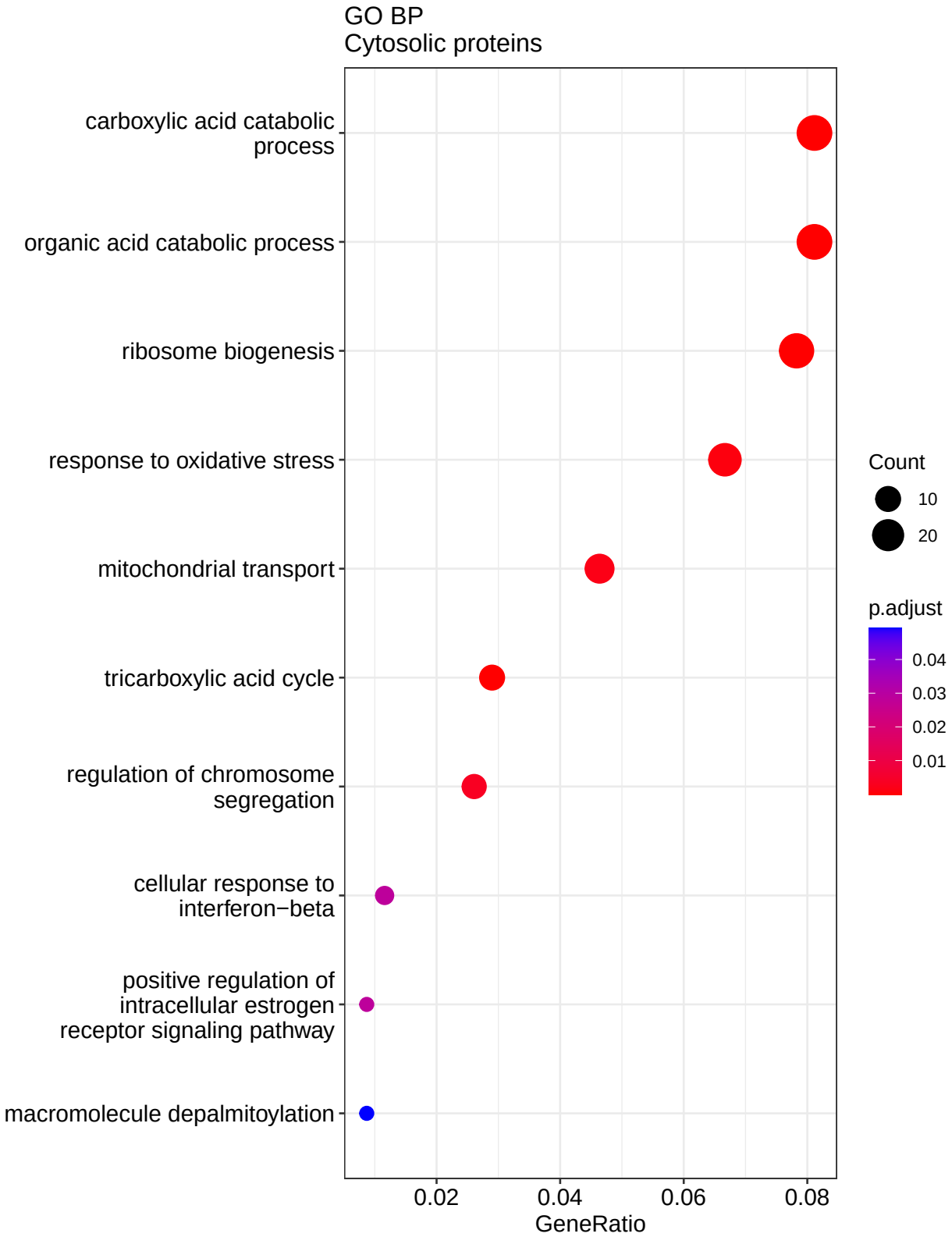

Hallmark geneset
Membrane−bound proteins
HALLMARK MTORC1 SIGNALING
0.075
0.100
0.125
0.150
GeneRatio
Count
19
p.adjust
0.002121979


Hallmark geneset
Cytosolic proteins
HALLMARK OXIDATIVE PHOSPHORYLATION
HALLMARK ADIPOGENESIS
HALLMARK FATTY ACID METABOLISM
0.125
0.150
0.175
0.200
GeneRatio
Count
20
25
30
p.adjust
8e−06
6e−06
4e−06
2e−06


C2 Canonical Pathways
Membrane−bound proteins
TRANSLATION
RRNA PROCESSING
SELENOAMINO ACID METABOLISM
RESPIRATORY ELECTRON TRANSPORT
RESPIRATORY ELECTRON TRANSPORT ATP SYNTHESIS BY CHEMIOSMOTIC COUPLING AND HEAT PRODUCTION BY UNCOUPLING PROTEINS
LYSOSOME
ELECTRON TRANSPORT CHAIN OXPHOS SYSTEM IN MITOCHONDRIA
EUKARYOTIC TRANSLATION ELONGATION
RIBOSOME
RESPONSE OF EIF2AK4 GCN2 TO AMINO ACID DEFICIENCY
ACTIVATION OF THE MRNA UPON BINDING OF THE CAP BINDING COMPLEX AND EIFS AND SUBSEQUENT BINDING TO 43S
COMPLEX I BIOGENESIS
FERROPTOSIS
MITOCHONDRIAL COMPLEX I ASSEMBLY MODEL OXPHOS SYSTEM
GENE AND PROTEIN EXPRESSION BY JAK STAT SIGNALING AFTER INTERLEUKIN 12 STIMULATION
0.03
0.04
0.05
0.06
0.07
GeneRatio
Count
8
12
16
20
p.adjust
0.0015
0.0010
0.0005


C2 Canonical Pathways
Cytosolic proteins
METABOLISM OF AMINO ACIDS AND DERIVATIVES
MITOTIC METAPHASE AND ANAPHASE
VALINE LEUCINE AND ISOLEUCINE DEGRADATION
THE CITRIC ACID TCA CYCLE AND RESPIRATORY ELECTRON TRANSPORT
MITOTIC SPINDLE CHECKPOINT
AMINO ACID METABOLISM
METABOLIC REPROGRAMMING IN COLON CANCER
CITRATE CYCLE TCA CYCLE
PYRUVATE METABOLISM AND CITRIC ACID TCA CYCLE
MITOCHONDRIAL PROTEIN IMPORT
BRANCHED CHAIN AMINO ACID CATABOLISM
CITRIC ACID CYCLE TCA CYCLE
PROPANOATE METABOLISM
TCA CYCLE AKA KREBS OR CITRIC ACID CYCLE
BUTANOATE METABOLISM
0.03
0.05
0.07
0.09
GeneRatio
Count
10
15
20
25
30
p.adjust
2.5e−05
2.0e−05
1.5e−05
1.0e−05
5.0e−06


GO BP
Membrane−bound proteins
ribosome biogenesis
regulation of binding
ATP synthesis coupled electron transport
mitochondrial ATP synthesis coupled electron transport
mitotic sister chromatid segregation
N−terminal protein amino acid modification
blastocyst formation
glycosphingolipid catabolic process
response to thyroid hormone
0.02
0.03
0.04
0.05
GeneRatio
Count
5
10
15
20
p.adjust
0.020
0.015
0.010
0.005


GO BP
Cytosolic proteins
carboxylic acid catabolic process
organic acid catabolic process
ribosome biogenesis
response to oxidative stress
mitochondrial transport
tricarboxylic acid cycle
regulation of chromosome segregation
cellular response to interferon−beta
positive regulation of intracellular estrogen receptor signaling pathway
macromolecule depalmitoylation
0.02
0.04
0.06
0.08
GeneRatio
Count
10
20
p.adjust
0.04
0.03
0.02
0.01

```
#dev.off()
```

## Session Info

```
sessionInfo()
```

```
## R version 4.1.2 (2021-11-01)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 19044)
##
## Matrix products: default
##
## locale:
## [1] LC_COLLATE=English_Canada.1252  LC_CTYPE=English_Canada.1252
## [3] LC_MONETARY=English_Canada.1252 LC_NUMERIC=C
## [5] LC_TIME=English_Canada.1252
##
## attached base packages:
## [1] stats4    stats     graphics  grDevices utils     datasets  methods
## [8] base
##
## other attached packages:
##  [1] readxl_1.4.0           forcats_0.5.1          stringr_1.4.0
##  [4] dplyr_1.0.9            purrr_0.3.4            readr_2.1.2
##  [7] tidyr_1.2.0            tibble_3.1.7           tidyverse_1.3.1
## [10] org.Hs.eg.db_3.14.0    AnnotationDbi_1.56.2   IRanges_2.28.0
## [13] S4Vectors_0.32.4       Biobase_2.54.0         BiocGenerics_0.40.0
## [16] EnhancedVolcano_1.12.0 ggrepel_0.9.1          ggplot2_3.3.6
## [19] clusterProfiler_4.2.2
##
## loaded via a namespace (and not attached):
##   [1] shadowtext_0.1.2       backports_1.4.1        fastmatch_1.1-3
##   [4] plyr_1.8.7             igraph_1.3.2           lazyeval_0.2.2
##   [7] splines_4.1.2          BiocParallel_1.28.3    GenomeInfoDb_1.30.1
##  [10] digest_0.6.29          yulab.utils_0.0.5      htmltools_0.5.2
##  [13] GOSemSim_2.20.0        viridis_0.6.2          GO.db_3.14.0
##  [16] fansi_1.0.2            magrittr_2.0.3         memoise_2.0.1
##  [19] tzdb_0.3.0             Biostrings_2.62.0      graphlayouts_0.8.0
##  [22] modelr_0.1.8           extrafont_0.18         vroom_1.5.7
##  [25] extrafontdb_1.0        enrichplot_1.14.2      colorspace_2.0-3
##  [28] rvest_1.0.2            blob_1.2.3             haven_2.4.3
##  [31] xfun_0.29              crayon_1.5.1           RCurl_1.98-1.7
##  [34] jsonlite_1.8.0         scatterpie_0.1.7       ape_5.6-2
##  [37] glue_1.6.2             polyclip_1.10-0        gtable_0.3.0
##  [40] zlibbioc_1.40.0        XVector_0.34.0         proj4_1.0-11
##  [43] Rttf2pt1_1.3.10        maps_3.4.0             scales_1.2.0
##  [46] DOSE_3.20.1            DBI_1.1.3              Rcpp_1.0.8.3
##  [49] viridisLite_0.4.0      gridGraphics_0.5-1     tidytree_0.3.9
##  [52] bit_4.0.4              httr_1.4.3             fgsea_1.20.0
##  [55] RColorBrewer_1.1-3     ellipsis_0.3.2         pkgconfig_2.0.3
```

```
##  [58] farver_2.1.0          dbplyr_2.2.1          here_1.0.1
##  [61] utf8_1.2.2            labeling_0.4.2        ggplotify_0.1.0
##  [64] tidyselect_1.1.2      rlang_1.0.3           reshape2_1.4.4
##  [67] cellranger_1.1.0      munsell_0.5.0         tools_4.1.2
##  [70] cachem_1.0.6          downloader_0.4        cli_3.3.0
##  [73] generics_0.1.3        RSQLite_2.2.14        broom_1.0.0
##  [76] evaluate_0.15         fastmap_1.1.0         yaml_2.2.2
##  [79] ggtree_3.2.1          fs_1.5.2              knitr_1.39
##  [82] bit64_4.0.5           tidygraph_1.2.1       KEGGREST_1.34.0
##  [85] ggraph_2.0.5          nlme_3.1-153          ash_1.0-15
##  [88] ggrastr_1.0.1         aplot_0.1.6           ggvenn_0.1.9
##  [91] xml2_1.3.3            DO.db_2.9             compiler_4.1.2
##  [94] rstudioapi_0.13       beeswarm_0.4.0        png_0.1-7
##  [97] reprex_2.0.1          treeio_1.18.1         tweenr_1.0.2
## [100] stringi_1.7.6         highr_0.9             ggalt_0.4.0
## [103] lattice_0.20-45       Matrix_1.3-4          vctrs_0.4.1
## [106] pillar_1.8.0          lifecycle_1.0.1       data.table_1.14.2
## [109] bitops_1.0-7          patchwork_1.1.1       qvalue_2.26.0
## [112] R6_2.5.1              KernSmooth_2.23-20    gridExtra_2.3
## [115] vipor_0.4.5           MASS_7.3-54           assertthat_0.2.1
## [118] rprojroot_2.0.3       withr_2.5.0           GenomeInfoDbData_1.2.7
## [121] parallel_4.1.2        hms_1.1.1             grid_4.1.2
## [124] ggfun_0.0.6           rmarkdown_2.14        ggforce_0.3.3
## [127] lubridate_1.8.0       ggbeeswarm_0.6.0
```