SIB - Enrichment Analysis

Exercise 1

```
library(clusterProfiler)
library(enrichplot)
library(pathview)
library(org.Hs.eg.db)
library(ggplot2)
library(ggrepel)
library(msigdbr)

library(tidyverse) # for bonus code/dplyr/pipe

# set seed
set.seed(1234)
```

```
# Import DE table:
NK_vs_Th <- read.csv("data/NK_vs_Th_diff_gene_exercise_1.csv",
  header = T
)
# Look at the structure of the data.frame:
head(NK_vs_Th)
```

```
  ensembl_gene_id   symbol      logFC         t      P.Value        p.adj
1 ENSG00000000003   TSPAN6 -5.6436044 -4.672128 0.0000426000 7.358019e-04
2 ENSG00000000419     DPM1 -0.1818981 -1.101831 0.2780198240 5.176076e-01
3 ENSG00000000457    SCYL3  0.4969874  1.491035 0.1448690710 3.449889e-01
4 ENSG00000000460 C1orf112  1.1217991  1.445899 0.1570598770 3.630935e-01
5 ENSG00000000938      FGR 10.6706873  7.212342 0.0000000198 1.718657e-06
6 ENSG00000000971      CFH -3.4129277 -2.788887 0.0084803000 4.610083e-02
```

```
# Search for a gene symbol in the data.frame, eg NCAM1 (CD56)
NK_vs_Th[which(NK_vs_Th$symbol == "NCAM1"), ]
```

```
     ensembl_gene_id symbol    logFC        t  P.Value        p.adj
7624 ENSG00000149294  NCAM1 12.19755 6.992219 3.81e-08 2.845553e-06
```

Search for 2 genes in the data.frame, CPS1 and GZMB, and verify the effect of adjustment on their p-values

```
genes <- c("CPS1", "GZMB")
NK_vs_Th |>
  filter(symbol %in% genes) |>
  select(symbol, P.Value, p.adj)
```

```
  symbol     P.Value        p.adj
1   CPS1 0.044963086 1.565113e-01
2   GZMB 0.000000629 2.402609e-05
```

CPS1 is not significant, while GZMB is significant.

```
# Import the adaptive immune response gene set (gmt file)
adaptive <- clusterProfiler::read.gmt("data/GOBP_ADAPTIVE_IMMUNE_RESPONSE.v7.5.1.gmt")
nrow(adaptive) # 719
```

```
[1] 719
```

```
length(which(NK_vs_Th$symbol %in% adaptive$gene)) # 513
```

```
[1] 513
```

```
upregulated_th <- subset(
  NK_vs_Th,
  NK_vs_Th$p.adj <= 0.05 & NK_vs_Th$logFC < 0
)

not_significant_genes <- subset(
  NK_vs_Th,
  NK_vs_Th$p.adj > 0.05
```

```
)

summary_upregulated <- summary(upregulated_th$symbol %in% adaptive$gene)
summary_not_significant <- summary(not_significant_genes$symbol %in% adaptive$gene)
```

```
contingency_table <- matrix(, nrow = 2, ncol = 2)
contingency_table[[1]] <- summary_upregulated[[3]] # up, in gene set
contingency_table[[2]] <- summary_upregulated[[2]] # up, not in gene set
contingency_table[[3]] <- summary_not_significant[[3]] # down, in gene set
contingency_table[[4]] <- summary_not_significant[[2]] # down, not in gene set

# Convert to numeric
contingency_table <- apply(contingency_table, 2, as.numeric)

# Add rows and columns
colnames(contingency_table) <- c("up", "down")
rownames(contingency_table) <- c("in_set", "not_in_set")
```

```
fisher.test(contingency_table)
```

```
    Fisher's Exact Test for Count Data

data:  contingency_table
p-value < 2.2e-16
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
 3.697701 5.654348
sample estimates:
odds ratio
  4.580549
```

The odds ratio tells us how different the two proportions are.

If the confidence interval does not include 1, then p-value is small. We can reject null hypothesis thatthe odds ratio is equal to 1.

There are more genes that are upregulated in the gene set than the genes that are not upregulated in the gene set.

```r
# Test 3 gene sets among the genes up-regulated in NK cells,
# with enricher()
# First, obtain the genes up-regulated in NK:

nk_up_genes <- subset(NK_vs_Th, NK_vs_Th$logFC > 0 & NK_vs_Th$p.adj <= 0.05)$symbol

# Import 2 other gene sets, 1 un-related to immune cells:
hair <- read.gmt("data/GOBP_HAIR_CELL_DIFFERENTIATION.v7.5.1.gmt")
dim(hair)
```

```
[1] 47  2
```

```r
cell_active <- read.gmt("data/GOBP_CELL_ACTIVATION.v7.5.1.gmt")
dim(cell_active)
```

```
[1] 1095    2
```

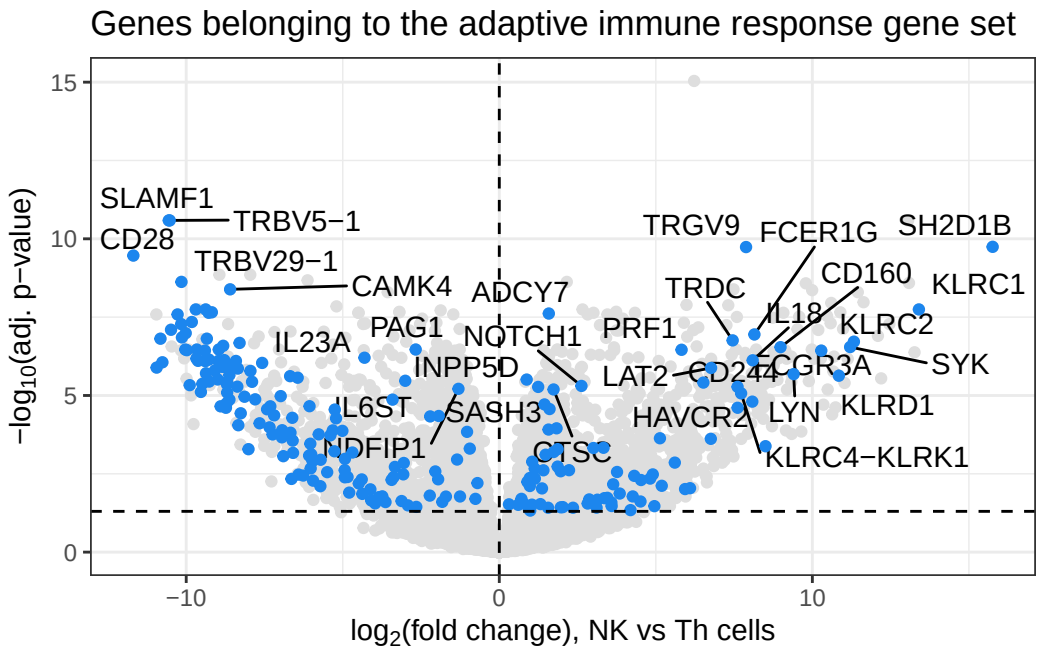
```r
# Combine the 3 gene sets into a single data.frame for the TERM2GENE argument:
genesets3 <- rbind(adaptive, hair, cell_active)

hyper_3genesets <- enricher(
  gene = nk_up_genes,
  universe = NK_vs_Th$symbol,
  TERM2GENE = genesets3,
  maxGSSize = 1000
)
hyper_3genesets@result
```

```
                                                      ID
GOBP_CELL_ACTIVATION                     GOBP_CELL_ACTIVATION
GOBP_HAIR_CELL_DIFFERENTIATION GOBP_HAIR_CELL_DIFFERENTIATION
GOBP_ADAPTIVE_IMMUNE_RESPONSE   GOBP_ADAPTIVE_IMMUNE_RESPONSE
                                                 Description GeneRatio
GOBP_CELL_ACTIVATION                     GOBP_CELL_ACTIVATION   173/200
GOBP_HAIR_CELL_DIFFERENTIATION GOBP_HAIR_CELL_DIFFERENTIATION     5/200
GOBP_ADAPTIVE_IMMUNE_RESPONSE   GOBP_ADAPTIVE_IMMUNE_RESPONSE    82/200
                                BgRatio      pvalue    p.adjust      qvalue
GOBP_CELL_ACTIVATION           896/1138 0.001505054 0.004515163 0.003168535
GOBP_HAIR_CELL_DIFFERENTIATION  34/1138 0.741306145 0.912609682 0.640427847
GOBP_ADAPTIVE_IMMUNE_RESPONSE  513/1138 0.912609682 0.912609682 0.640427847
```

```
GOBP_CELL_ACTIVATION           FGR/CD38/SKAP2/ITGAL/TYROBP/RUNX3/NR1H3/SLAMF7/IFNGR1/STAP1/HI
GOBP_HAIR_CELL_DIFFERENTIATION
GOBP_ADAPTIVE_IMMUNE_RESPONSE
                               Count
GOBP_CELL_ACTIVATION             173
GOBP_HAIR_CELL_DIFFERENTIATION     5
GOBP_ADAPTIVE_IMMUNE_RESPONSE     82
```

```r
sig_genes <- subset(NK_vs_Th, NK_vs_Th$symbol %in% adaptive$gene &
  NK_vs_Th$p.adj <= 0.05)
sig_genes_label <- subset(sig_genes, sig_genes$p.adj <= 0.00001)

ggplot(NK_vs_Th, aes(
  x = logFC,
  y = -log10(p.adj)
)) +
  geom_point(color = "grey87") +
  ggtitle("Genes belonging to the adaptive immune response gene set") +
  theme_bw() +
  geom_text_repel(
    data = sig_genes_label,
    aes(
      x = logFC,
      y = -log10(p.adj), label = symbol
    ),
    max.overlaps = 20
  ) +
  geom_point(data = sig_genes, col = "dodgerblue2") +
  theme(legend.position = "none") +
  scale_x_continuous(name = expression("log"[2] * "(fold change), NK vs Th cells")) +
  scale_y_continuous(name = expression("-" * "log"[10] * "(adj. p-value)")) +
  geom_hline(yintercept = -log10(0.05), linetype = "dashed") +
  geom_vline(xintercept = 0, linetype = "dashed")
```

```
Warning: ggrepel: 51 unlabeled data points (too many overlaps). Consider
increasing max.overlaps
```



Exercise 2 - Gene set enrichment analysis (GSEA)

```
gl <- NK_vs_Th$t
names(gl) <- make.names(NK_vs_Th$symbol, unique = T)
gl <- gl[order(gl, decreasing = T)]

GO_NK_Th <- gseGO(gl,
  ont = "BP",
  OrgDb = org.Hs.eg.db,
  keyType = "SYMBOL",
  minGSSize = 30,
  eps = 0,
  seed = T
)
```

```
preparing geneSet collections...

GSEA analysis...

Warning in preparePathwaysAndStats(pathways, stats, minSize, maxSize, gseaParam, : There are
The order of those tied genes will be arbitrary, which may produce unexpected results.
```

```
leading edge analysis...

done...
```

```
GO_NK_Th
```

```
#
# Gene Set Enrichment Analysis
#
#...@organism    Homo sapiens
#...@setType     BP
#...@keytype     SYMBOL
#...@geneList    Named num [1:20485] 19 13.1 12.1 12 10.7 ...
 - attr(*, "names")= chr [1:20485] "GHSR" "MLC1" "SH2D1B" "TRGV9" ...
#...nPerm
#...pvalues adjusted by 'BH' with cutoff <0.05
#...351 enriched terms found
'data.frame':   351 obs. of  11 variables:
 $ ID             : chr  "GO:0002181" "GO:0042254" "GO:0022613" "GO:0042273" ...
 $ Description    : chr  "cytoplasmic translation" "ribosome biogenesis" "ribonucleoprotein c
 $ setSize        : int  145 299 436 69 98 57 210 340 111 242 ...
 $ enrichmentScore: num  -0.808 -0.551 -0.491 -0.715 -0.648 ...
 $ NES            : num  -3.38 -2.52 -2.36 -2.67 -2.58 ...
 $ pvalue         : num  1.68e-48 6.30e-24 2.34e-22 1.48e-14 3.84e-14 ...
 $ p.adjust       : num  4.85e-45 9.08e-21 2.25e-19 1.07e-11 2.21e-11 ...
 $ qvalue         : num  3.77e-45 7.05e-21 1.75e-19 8.27e-12 1.72e-11 ...
 $ rank           : num  1385 3723 3746 2698 2371 ...
 $ leading_edge   : chr  "tags=58%, list=7%, signal=54%" "tags=38%, list=18%, signal=32%" "ta
 $ core_enrichment: chr  "EIF2S2/EIF3M/RPL21/RPS28/EIF4A2/YBX1/FAU/PKM/RPS9/CNBP/RPL37/RPL37A
#...Citation
 T Wu, E Hu, S Xu, M Chen, P Guo, Z Dai, T Feng, L Zhou, W Tang, L Zhan, X Fu, S Liu, X Bo, a
 clusterProfiler 4.0: A universal enrichment tool for interpreting omics data.
 The Innovation. 2021, 2(3):100141
```

```
# Class is gseaResult
class(GO_NK_Th)
```

```
[1] "gseaResult"
attr(,"package")
[1] "DOSE"
```

```r
# Is the adaptive immune response gene set significant?
GO_NK_Th[GO_NK_Th@result$Description == "adaptive immune response", ] # yes
```

```
                   ID              Description setSize enrichmentScore
GO:0002250 GO:0002250 adaptive immune response     423      -0.3652034
                  NES       pvalue     p.adjust       qvalue rank
GO:0002250 -1.743904 1.001619e-08 1.804167e-06 1.400949e-06 1623
                           leading_edge
GO:0002250 tags=23%, list=8%, signal=22%

GO:0002250 HFE/CD3E/CLU/PDCD1LG2/ADGRE1/JAK3/LEF1/IL18BP/ITK/CD80/ALCAM/TRAV34/AIRE/IGHM/BTLA
```

```r
# How many gene sets are down- or up-regulated?
count_gene_sets <- function(gsea, p_value) {
  up <- summary(gsea@result$p.adjust < p_value & gsea@result$NES > 0)
  down <- summary(gsea@result$p.adjust < p_value & gsea@result$NES < 0)

  return(list(upregulated = up, downregulated = down))
}

# 290 upregulated, 61 downregulated
count_gene_sets(GO_NK_Th, 0.05)
```

```
$upregulated
   Mode   FALSE    TRUE
logical      61     290

$downregulated
   Mode   FALSE    TRUE
logical     290      61
```

```r
GO_NK_Th_simplify <- clusterProfiler::simplify(GO_NK_Th)
GO_NK_Th_simplify@result[GO_NK_Th_simplify@result$Description == "adaptive immune response",
```

```
                   ID              Description setSize enrichmentScore
GO:0002250 GO:0002250 adaptive immune response     423      -0.3652034
                  NES       pvalue     p.adjust       qvalue rank
GO:0002250 -1.743904 1.001619e-08 1.804167e-06 1.400949e-06 1623
                           leading_edge
GO:0002250 tags=23%, list=8%, signal=22%
```

GO:0002250 HFE/CD3E/CLU/PDCD1LG2/ADGRE1/JAK3/LEF1/IL18BP/ITK/CD80/ALCAM/TRAV34/AIRE/IGHM/BTLA

```
unlist(strsplit(
  GO_NK_Th@result[GO_NK_Th@result$Description == "adaptive immune response", 11],
  "\\/"
))
```

```
 [1] "HFE"        "CD3E"       "CLU"        "PDCD1LG2"   "ADGRE1"     "JAK3"
 [7] "LEF1"       "IL18BP"     "ITK"        "CD80"       "ALCAM"      "TRAV34"
[13] "AIRE"       "IGHM"       "BTLA"       "CR1"        "C1QBP"      "CD3G"
[19] "CTSL"       "TRAJ42"     "TRBV16"     "TNF"        "CEACAM1"    "GPR183"
[25] "CD27"       "CCR6"       "ICOSLG"     "TRDV1"      "CCR2"       "CD6"
[31] "TRBD1"      "MCOLN2"     "TRAV14DV4"  "IL2"        "CR2"        "TRAV22"
[37] "CD70"       "PDCD1"      "MALT1"      "EBAG9"      "TRAV30"     "CTLA4"
[43] "TRAV23DV6"  "TNFRSF13C"  "KDM5D"      "TRAV40"     "TRAV18"     "IL6R"
[49] "CD3D"       "TRAJ3"      "TRAV39"     "TRBC2"      "SAMSN1"     "IL7R"
[55] "TRAV19"     "SUSD4"      "TRAV20"     "CD84"       "TRAV10"     "TRAV21"
[61] "TRBV13"     "TRAV41"     "TRAV29DV5"  "NDFIP1"     "TRAV36DV7"  "THEMIS"
[67] "TRBV18"     "TRAT1"      "SOCS3"      "IL6ST"      "TRBV9"      "TRAV24"
[73] "TRAV3"      "TRAV27"     "TRAV4"      "TRAV6"      "TRAV2"      "TRAV5"
[79] "JUNB"       "TRBV19"     "TRAV35"     "TRBV30"     "FOXP3"      "TRAV16"
[85] "IL23A"      "TRBV2"      "TRBV14"     "PAG1"       "CD4"        "TRAV25"
[91] "SIT1"       "TRAV17"     "CD40LG"     "CAMK4"      "TRAC"       "CD28"
[97] "SLAMF1"
```

```
GO_NK_Th@geneSets$`GO:0002250`
```

```
  [1] "ADA"         "ADCY7"       "AGER"        "JAG1"        "AHR"
  [6] "ALCAM"       "ALOX15"      "ANXA1"       "AIRE"        "ARG1"
 [11] "ARG2"        "ASCL2"       "B2M"         "BCL3"        "BCL6"
 [16] "TNFRSF17"    "CEACAM1"     "PRDM1"       "BMX"         "BTK"
 [21] "C1QBP"       "SERPING1"    "C1QA"        "C1QB"        "C1QC"
 [26] "C1R"         "C1S"         "C2"          "C3"          "C4A"
 [31] "C4B"         "C4BPA"       "C4BPB"       "C5"          "C6"
 [36] "C7"          "C8A"         "C8B"         "C8G"         "C9"
 [41] "CAMK4"       "CD1A"        "CD1B"        "CD1C"        "CD1D"
 [46] "CD1E"        "CD3D"        "CD3E"        "CD3G"        "CD247"
 [51] "CD4"         "CD6"         "CD7"         "CD8A"        "CD8B"
 [56] "CD8B2"       "CD19"        "CD27"        "CD28"        "CD80"
 [61] "CD86"        "CD40"        "CD40LG"      "CD70"        "CD74"
```

```
 [66] "CD79A"       "CD79B"       "CD81"        "CTSC"        "CLC"
 [71] "CLU"         "CCR6"        "CR1"         "CR1L"        "CR2"
 [76] "CSF2RB"      "CSK"         "CTLA4"       "CTSH"        "CTSL"
 [81] "CTSS"        "CX3CR1"      "CD55"        "GPR183"      "EMP2"
 [86] "ADGRE1"      "EPHB2"       "ERCC1"       "PTK2B"       "FCER1A"
 [91] "FCER1G"      "FCER2"       "FCGR1A"      "FCGR1BP"     "FCGR2B"
 [96] "FCGR3A"      "FGA"         "FGB"         "FGL1"        "FOXJ1"
[101] "MTOR"        "FUT7"        "FYN"         "GATA3"       "GNL1"
[106] "MSH6"        "GZMM"        "NCKAP1L"     "HFE"         "HLA-A"
[111] "HLA-B"       "HLA-C"       "HLA-DMA"     "HLA-DMB"     "HLA-DOA"
[116] "HLA-DOB"     "HLA-DPA1"    "HLA-DPB1"    "HLA-DQA1"    "HLA-DQA2"
[121] "HLA-DQB1"    "HLA-DQB2"    "HLA-DRA"     "HLA-DRB1"    "HLA-DRB3"
[126] "HLA-DRB4"    "HLA-DRB5"    "HLA-E"       "HLA-F"       "HLA-G"
[131] "HLA-H"       "MR1"         "HLX"         "HMGB1"       "HPRT1"
[136] "HPX"         "HRAS"        "HSPD1"       "ICAM1"       "CFI"
[141] "IFNA1"       "IFNA2"       "IFNA4"       "IFNA5"       "IFNA6"
[146] "IFNA7"       "IFNA8"       "IFNA10"      "IFNA13"      "IFNA14"
[151] "IFNA16"      "IFNA17"      "IFNA21"      "IFNB1"       "IFNG"
[156] "IFNW1"       "IGHA1"       "IGHA2"       "IGHD"        "IGHE"
[161] "IGHG1"       "IGHG2"       "IGHG3"       "IGHG4"       "IGHM"
[166] "JCHAIN"      "IGKC"        "IGLC1"       "IGLC2"       "IGLC3"
[171] "IGLC6"       "IGLL1"       "IL1B"        "IL1R1"       "IL2"
[176] "IL2RB"       "IL4"         "IL4R"        "IL6"         "IL6R"
[181] "IL6ST"       "IL7R"        "IL9"         "IL9R"        "IL10"
[186] "IL12A"       "IL12B"       "IL12RB1"     "IL13RA2"     "IL17A"
[191] "IL18"        "INPP5D"      "IRF1"        "IRF4"        "IRF7"
[196] "ITK"         "JAK1"        "JAK2"        "JAK3"        "JUNB"
[201] "KCNJ8"       "KLRC1"       "KLRC2"       "KLRD1"       "LAG3"
[206] "LAIR1"       "LIG4"        "LTA"         "LY9"         "LYN"
[211] "SH2D1A"      "SMAD7"       "MBL2"        "CD46"        "MEF2C"
[216] "MICB"        "MLH1"        "MPL"         "MSH2"        "MYD88"
[221] "NBN"         "NFKB2"       "NOTCH1"      "P2RX7"       "PDCD1"
[226] "PHB1"        "PIK3CD"      "PIK3CG"      "PLA2G4A"     "PMS2"
[231] "PPP3CB"      "PRF1"        "PRKCB"       "PRKCD"       "PKN1"
[236] "PRKCQ"       "PRKCZ"       "PSG9"        "PTPN6"       "PTPRC"
[241] "PVR"         "NECTIN2"     "RAB27A"      "RAG1"        "RAP1GAP"
[246] "RELB"        "TRIM27"      "RORA"        "RORC"        "CCL19"
[251] "XCL1"        "SIPA1"       "SLAMF1"      "SLC11A1"     "SPN"
[256] "STAT3"       "STAT4"       "STAT6"       "SUPT6H"      "SYK"
[261] "ADAM17"      "MAP3K7"      "TAP1"        "TAP2"        "TRA"
[266] "TRAV6"       "TRB"         "TRGC1"       "TRGC2"       "TRGV1"
[271] "TRGV2"       "TRGV3"       "TRGV4"       "TRGV5"       "TRGV8"
[276] "TRGV9"       "TRGV10"      "TRGV11"      "TEC"         "TFE3"
```

```
[281] "TFRC"         "TGFB1"        "TLR4"         "TNF"          "TNFAIP3"
[286] "TNFRSF1B"     "TP53BP1"      "TRAF2"        "TRAF6"        "TSC1"
[291] "TNFSF4"       "TXK"          "TYK2"         "UNG"          "WAS"
[296] "LAT2"         "NSD2"         "ZAP70"        "ZP3"          "FZD5"
[301] "TFEB"         "KDM5D"        "EOMES"        "STX7"         "SKAP1"
[306] "TNFSF13"      "TNFRSF14"     "RIPK2"        "FADD"         "TNFRSF11A"
[311] "IL18R1"       "CD84"         "BCL10"        "TNFSF18"      "SOCS3"
[316] "RNF8"         "EXO1"         "EBAG9"        "IL1RL1"       "SLC22A13"
[321] "IL27RA"       "SOCS5"        "THOC1"        "PARP3"        "IL18BP"
[326] "EBI3"         "LILRB2"       "TCIRG1"       "CLEC4M"       "BTN3A3"
[331] "RAPGEF3"      "MAD2L2"       "CLEC10A"      "BATF"         "CXCL13"
[336] "CD226"        "TNFSF13B"     "MASP2"        "TRAF3IP2"     "LILRB1"
[341] "ARID5A"       "MALT1"        "LILRB5"       "LILRB4"       "LILRA1"
[346] "LILRB3"       "LILRA3"       "RIPK3"        "RAPGEF4"      "BTN3A2"
[351] "BTN3A1"       "CD160"        "DUSP10"       "TREX1"        "ZBTB1"
[356] "KLRK1"        "PAXIP1"       "SWAP70"       "RAP1GAP2"     "RFTN1"
[361] "ICOSLG"       "SIRT1"        "TNFRSF13B"    "CLCF1"        "IL17RA"
[366] "PRKD2"        "TMEM98"       "LAT"          "LAMP3"        "SIT1"
[371] "TNFRSF21"     "IGKV1-5"      "IGHV8-51-1"   "IGHV7-81"     "IGHV6-1"
[376] "IGHV5-10-1"   "IGHV5-51"     "IGHV4-38-2"   "IGHV4-61"     "IGHV4-59"
[381] "IGHV4-39"     "IGHV4-34"     "IGHV4-31"     "IGHV4-30-4"   "IGHV4-28"
[386] "IGHV4-4"      "IGHV3-38-3"   "IGHV3-74"     "IGHV3-73"     "IGHV3-72"
[391] "IGHV3-66"     "IGHV3-64"     "IGHV3-53"     "IGHV3-49"     "IGHV3-48"
[396] "IGHV3-43"     "IGHV3-38"     "IGHV3-35"     "IGHV3-33"     "IGHV3-30"
[401] "IGHV3-23"     "IGHV3-21"     "IGHV3-20"     "IGHV3-16"     "IGHV3-15"
[406] "IGHV3-13"     "IGHV3-11"     "IGHV3-9"      "IGHV3-7"      "IGHV2-70"
[411] "IGHV2-26"     "IGHV2-5"      "IGHV1-69-2"   "IGHV1-38-4"   "IGHV1-69"
[416] "IGHV1-58"     "IGHV1-45"     "IGHV1-24"     "IGHV1-18"     "IGHV1-8"
[421] "IGHV1-3"      "IGHJ1"        "IGHD1-1"      "TRDV3"        "TRDV2"
[426] "TRDV1"        "TRDJ1"        "TRDD1"        "TRDC"         "TRBV30"
[431] "TRBV29-1"     "TRBV28"       "TRBV27"       "TRBV25-1"     "TRBV24-1"
[436] "TRBV23-1"     "TRBV20-1"     "TRBV19"       "TRBV18"       "TRBV17"
[441] "TRBV16"       "TRBV14"       "TRBV13"       "TRBV12-5"     "TRBV12-4"
[446] "TRBV12-3"     "TRBV11-3"     "TRBV11-2"     "TRBV11-1"     "TRBV10-3"
[451] "TRBV10-2"     "TRBV10-1"     "TRBV9"        "TRBV7-9"      "TRBV7-8"
[456] "TRBV7-7"      "TRBV7-6"      "TRBV7-4"      "TRBV7-3"      "TRBV7-2"
[461] "TRBV7-1"      "TRBV6-9"      "TRBV6-8"      "TRBV6-7"      "TRBV6-6"
[466] "TRBV6-5"      "TRBV6-4"      "TRBV6-3"      "TRBV6-1"      "TRBV5-8"
[471] "TRBV5-7"      "TRBV5-6"      "TRBV5-5"      "TRBV5-4"      "TRBV5-3"
[476] "TRBV5-1"      "TRBV4-3"      "TRBV4-2"      "TRBV4-1"      "TRBV3-1"
[481] "TRBV2"        "TRBJ2-7"      "TRBJ2-6"      "TRBJ2-5"      "TRBJ2-4"
[486] "TRBJ2-3"      "TRBJ2-2"      "TRBJ2-1"      "TRBJ1-6"      "TRBJ1-5"
[491] "TRBJ1-4"      "TRBJ1-3"      "TRBJ1-2"      "TRBJ1-1"      "TRBD1"
```

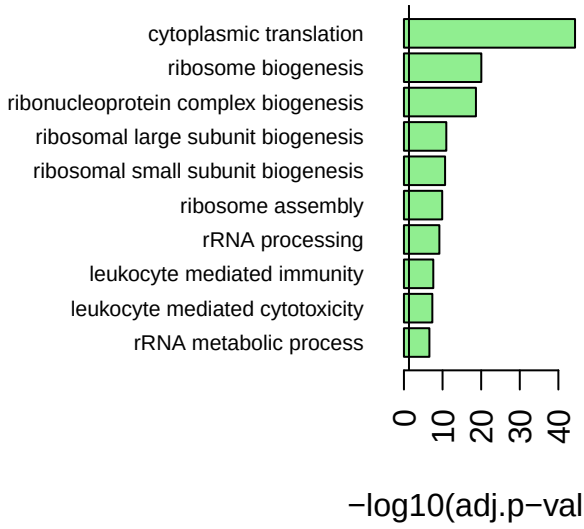
```
[496] "TRBC2"       "TRBC1"       "TRAV41"      "TRAV40"      "TRAV39"
[501] "TRAV38-2DV8" "TRAV38-1"    "TRAV36DV7"   "TRAV35"      "TRAV34"
[506] "TRAV30"      "TRAV29DV5"   "TRAV27"      "TRAV26-2"    "TRAV26-1"
[511] "TRAV25"      "TRAV24"      "TRAV23DV6"   "TRAV22"      "TRAV21"
[516] "TRAV20"      "TRAV19"      "TRAV18"      "TRAV17"      "TRAV16"
[521] "TRAV14DV4"   "TRAV13-2"    "TRAV13-1"    "TRAV12-3"    "TRAV12-2"
[526] "TRAV12-1"    "TRAV10"      "TRAV9-2"     "TRAV9-1"     "TRAV8-6"
[531] "TRAV8-4"     "TRAV8-3"     "TRAV8-2"     "TRAV8-1"     "TRAV7"
[536] "TRAV5"       "TRAV4"       "TRAV3"       "TRAV2"       "TRAV1-2"
[541] "TRAV1-1"     "TRAJ42"      "TRAJ31"      "TRAJ3"       "TRAC"
[546] "IGLV11-55"   "IGLV10-54"   "IGLV9-49"    "IGLV8-61"    "IGLV7-46"
[551] "IGLV7-43"    "IGLV6-57"    "IGLV5-52"    "IGLV5-48"    "IGLV5-45"
[556] "IGLV5-39"    "IGLV5-37"    "IGLV4-69"    "IGLV4-60"    "IGLV4-3"
[561] "IGLV3-32"    "IGLV3-27"    "IGLV3-25"    "IGLV3-22"    "IGLV3-21"
[566] "IGLV3-19"    "IGLV3-16"    "IGLV3-12"    "IGLV3-10"    "IGLV3-9"
[571] "IGLV3-1"     "IGLV2-33"    "IGLV2-23"    "IGLV2-18"    "IGLV2-14"
[576] "IGLV2-11"    "IGLV2-8"     "IGLV1-51"    "IGLV1-50"    "IGLV1-47"
[581] "IGLV1-44"    "IGLV1-40"    "IGLV1-36"    "IGLJ1"       "IGLC7"
[586] "IGKV6D-41"   "IGKV6D-21"   "IGKV3D-20"   "IGKV3D-15"   "IGKV3D-11"
[591] "IGKV3D-7"    "IGKV2D-30"   "IGKV2D-29"   "IGKV2D-28"   "IGKV2D-26"
[596] "IGKV2D-24"   "IGKV1D-43"   "IGKV1D-42"   "IGKV1D-39"   "IGKV1D-37"
[601] "IGKV1D-33"   "IGKV1D-17"   "IGKV1D-13"   "IGKV1D-12"   "IGKV1D-8"
[606] "IGKV6-21"    "IGKV5-2"     "IGKV4-1"     "IGKV3-20"    "IGKV3-15"
[611] "IGKV3-7"     "IGKV2-40"    "IGKV2-30"    "IGKV2-29"    "IGKV2-28"
[616] "IGKV2-24"    "IGKV1-39"    "IGKV1-37"    "IGKV1-27"    "IGKV1-17"
[621] "IGKV1-16"    "IGKV1-13"    "IGKV1-12"    "IGKV1-9"     "IGKV1-8"
[626] "IGKV1-6"     "IGKJ1"       "DBNL"        "PYCARD"      "CD274"
[631] "TBX21"       "CD209"       "IL21R"       "TRAT1"       "CLEC4A"
[636] "FOXP3"       "EXOSC3"      "ZBTB7B"      "KMT5B"       "LEF1"
[641] "C1RL"        "TLR8"        "IL23A"       "CYRIB"       "CD244"
[646] "ERAP1"       "IL2ORB"      "TREM2"       "TREM1"       "SASH3"
[651] "SHLD2"       "RC3H2"       "TRPM4"       "LAX1"        "LIME1"
[656] "RNF125"      "SUSD4"       "AKIRIN2"     "RIF1"        "OTUB1"
[661] "PAG1"        "CTNNBL1"     "IFNK"        "DUSP22"      "HMCES"
[666] "OTUD7B"      "ENTPD7"      "MCOLN1"      "IGHV7-4-1"   "IGHV3-30-3"
[671] "AICDA"       "SLAMF7"      "HMHB1"       "BACH2"       "MYO1G"
[676] "SAMSN1"      "NOD2"        "ERAP2"       "CARD9"       "SEMA4A"
[681] "NFKBIZ"      "DCLRE1C"     "CLEC7A"      "LILRA6"      "ULBP3"
[686] "VTCN1"       "BTNL8"       "ATAD5"       "SVEP1"       "ZC3H12A"
[691] "ULBP2"       "ULBP1"       "PDCD1LG2"    "PRR7"        "NDFIP1"
[696] "FBXO38"      "UNC93B1"     "FCRL4"       "JAM3"        "FCAMR"
[701] "SLA2"        "SANBR"       "LOXL3"       "CRACR2A"     "KMT5C"
[706] "NFKBID"      "HAVCR2"      "ORAI1"       "IGHV3-30-5"  "SIGLEC10"
```

```
[711] "KLHL6"       "IL33"        "RSAD2"       "CLEC6A"      "IL17F"
[716] "NLRP3"       "SLAMF6"      "TNFRSF13C"   "SH2D1B"      "EXOSC6"
[721] "SLC15A4"     "RNF19B"      "RAET1E"      "RC3H1"       "IL23R"
[726] "SHLD1"       "BTLA"        "RAET1L"      "DENND1B"     "RNF168"
[731] "CLEC4C"      "APLF"        "UNC13D"      "GAPT"        "MARCHF8"
[736] "IL27"        "MCOLN2"      "ZNF683"      "IL4I1"       "NLRP10"
[741] "CLEC4D"      "IFNE"        "CLEC4G"      "RAET1G"      "NCR3LG1"
[746] "THEMIS"      "MIR21"       "EIF2AK4"     "TARM1"       "SCART1"
[751] "CCR2"        "C17orf99"    "IGLL5"       "MICA"        "KLRC4-KLRK1"
[756] "IGHV2-70D"   "IGHV1-69D"   "IGHV3-64D"   "IGHV3-43D"   "SHLD3"
```

```r
GO_enrich <- enrichGO(
  gene = nk_up_genes,
  OrgDb = org.Hs.eg.db,
  keyType = "SYMBOL",
  ont = "MF", # ont="MF" is the default
  minGSSize = 30, universe = NK_vs_Th$symbol
)
```

Exercise 3 - Visualization of enrichment results

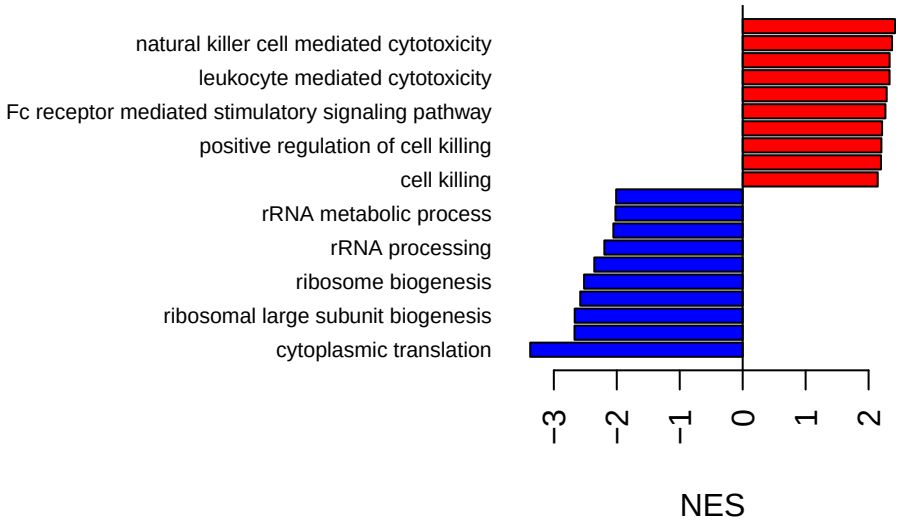
```r
par(mar = c(5, 20, 3, 3))
barplot(rev(-log10(GO_NK_Th@result$p.adjust[1:10])),
  horiz = T, names = rev(GO_NK_Th@result$Description[1:10]),
  las = 2, xlab = "-log10(adj.p-value)",
  cex.names = 0.7,
  col = "lightgreen"
)
abline(v = -log10(0.05))
```



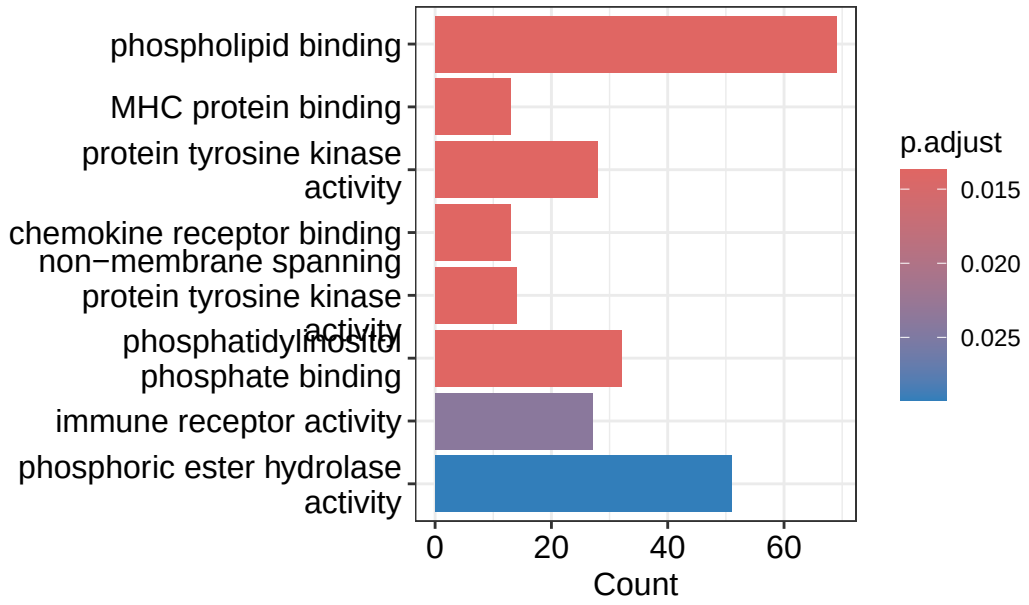

```
sorted_GO_NK_Th <- GO_NK_Th@result[order(GO_NK_Th@result$NES, decreasing = F), ]
sorted_GO_NK_Th$colors <- ifelse(sorted_GO_NK_Th$NES > 0, "red", "blue")

# Get the indices of the vector
bottom_values <- tail(seq_along(sorted_GO_NK_Th$NES), 10)

par(mar = c(5, 15, 3, 3)) # Make the figure canvas larger
barplot(sorted_GO_NK_Th$NES[c(1:10, bottom_values:nrow(sorted_GO_NK_Th))],
  horiz = T, names = sorted_GO_NK_Th$Description[c(1:10, bottom_values:nrow(sorted_GO_NK_Th))
  las = 2, xlab = "NES",
  cex.names = 0.7,
  col = sorted_GO_NK_Th$color[c(1:10, (nrow(sorted_GO_NK_Th) - 9):nrow(sorted_GO_NK_Th))]
)
```

```
Warning in bottom_values:nrow(sorted_GO_NK_Th): numerical expression has 10
elements: only the first used

Warning in bottom_values:nrow(sorted_GO_NK_Th): numerical expression has 10
elements: only the first used
```

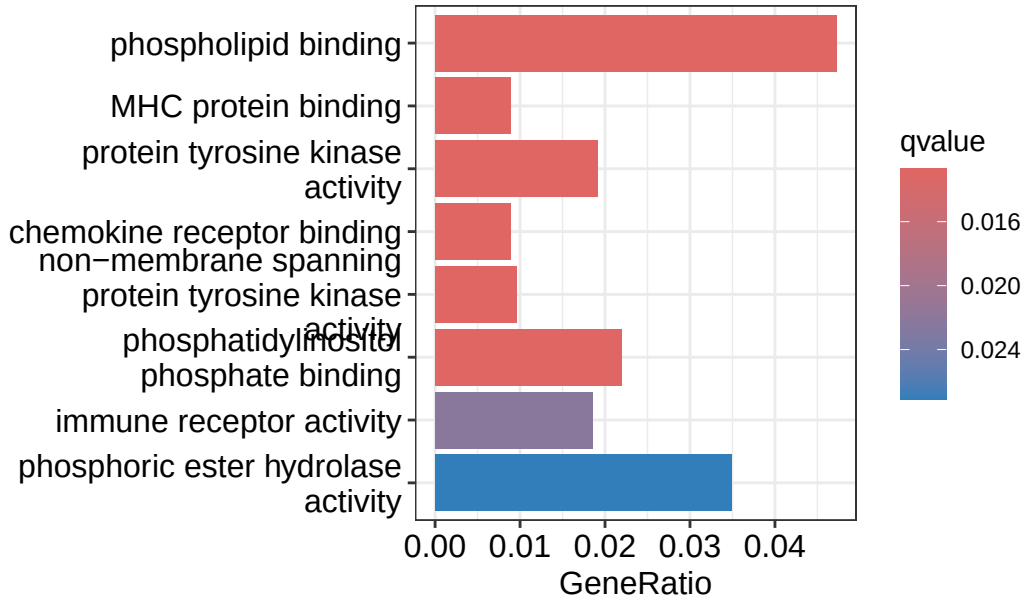
```
abline(v = 0)
```



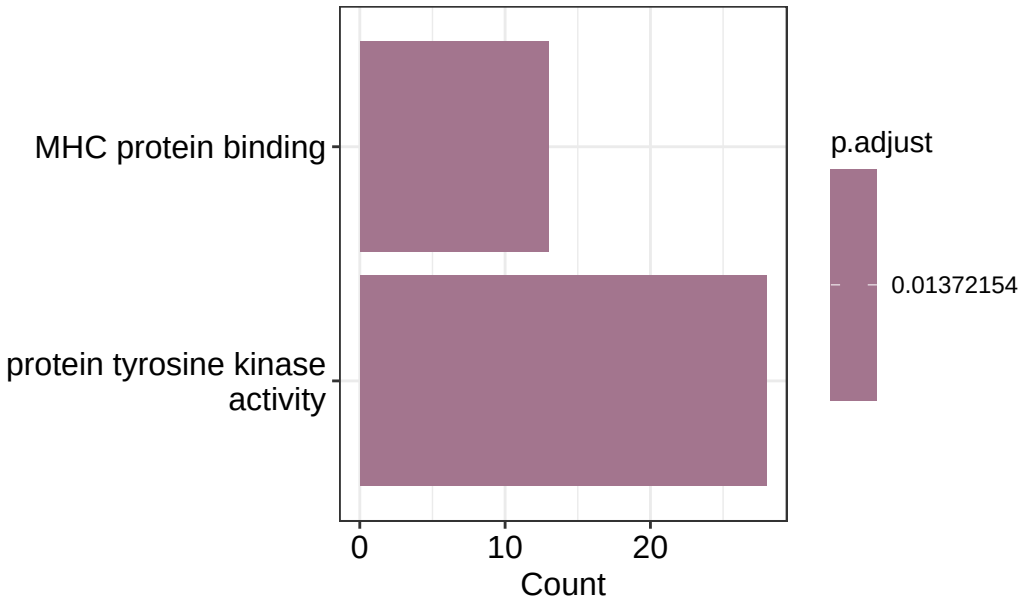

```
# Use the GO_enrich analysis performed above, of the over-representation analysis
# of genes up-regulated in NK cells:
# barplot() can be directly used on enrichResult objects: but not on gseaResult objects
graphics::barplot(GO_enrich)
```




```
graphics::barplot(GO_enrich, color = "qvalue", x = "GeneRatio")
```



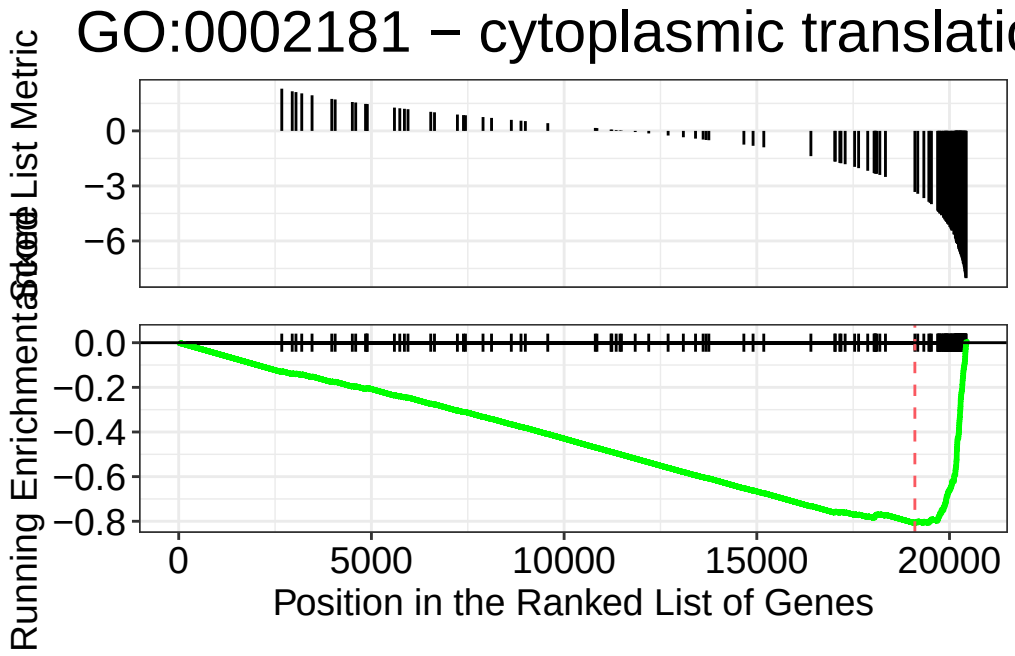

```
# Select only 2 out of the significant gene sets:
ego_selection <- GO_enrich[GO_enrich@result$ID == "GO:0042287" | GO_enrich@result$ID == "GO:0
barplot(ego_selection)
```



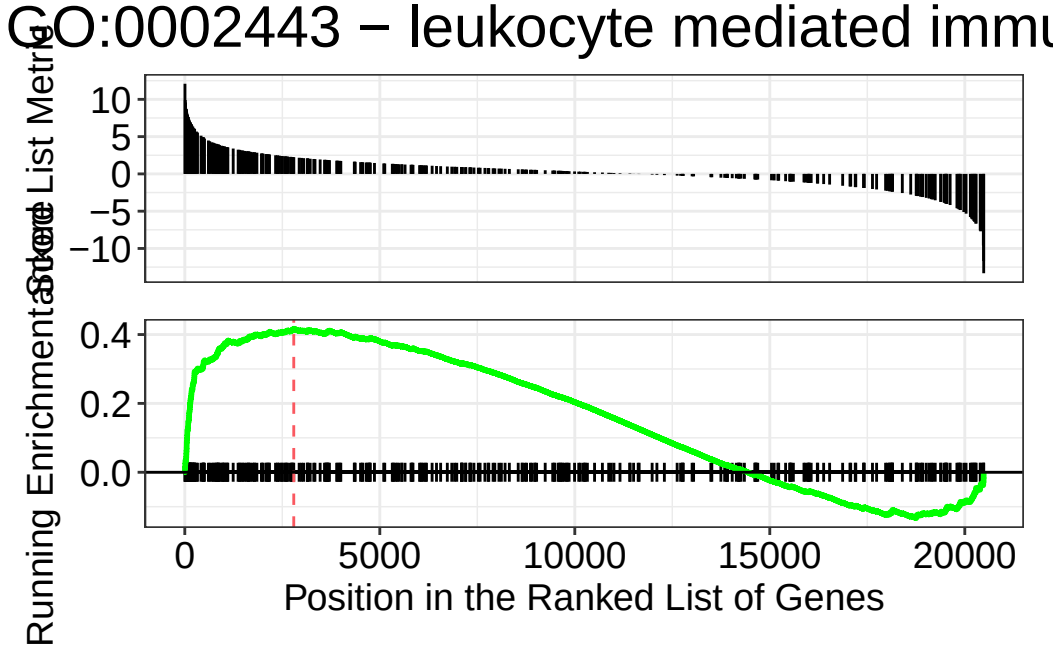

```
# Barcode plot
# You need the ID of the GO gene set to plot:
GO_NK_Th@result[1:10, 1:6]
```

```
                  ID                          Description setSize
GO:0002181 GO:0002181              cytoplasmic translation     145
GO:0042254 GO:0042254                  ribosome biogenesis     299
GO:0022613 GO:0022613 ribonucleoprotein complex biogenesis     436
GO:0042273 GO:0042273   ribosomal large subunit biogenesis      69
GO:0042274 GO:0042274   ribosomal small subunit biogenesis      98
GO:0042255 GO:0042255                    ribosome assembly      57
GO:0006364 GO:0006364                      rRNA processing     210
GO:0002443 GO:0002443          leukocyte mediated immunity     340
GO:0001909 GO:0001909      leukocyte mediated cytotoxicity     111
GO:0016072 GO:0016072               rRNA metabolic process     242
           enrichmentScore       NES       pvalue
GO:0002181      -0.8083663 -3.375135 1.684522e-48
GO:0042254      -0.5505406 -2.519660 6.298394e-24
GO:0022613      -0.4906673 -2.357458 2.339798e-22
GO:0042273      -0.7146074 -2.668647 1.478487e-14
GO:0042274      -0.6484741 -2.580517 3.835801e-14
GO:0042255      -0.7361084 -2.671760 2.555521e-13
GO:0006364      -0.5004485 -2.197559 1.614149e-12
GO:0002443       0.4169045  1.955354 6.943557e-11
GO:0001909       0.5720092  2.333365 1.381646e-10
GO:0016072      -0.4491031 -2.021848 8.811745e-10
```

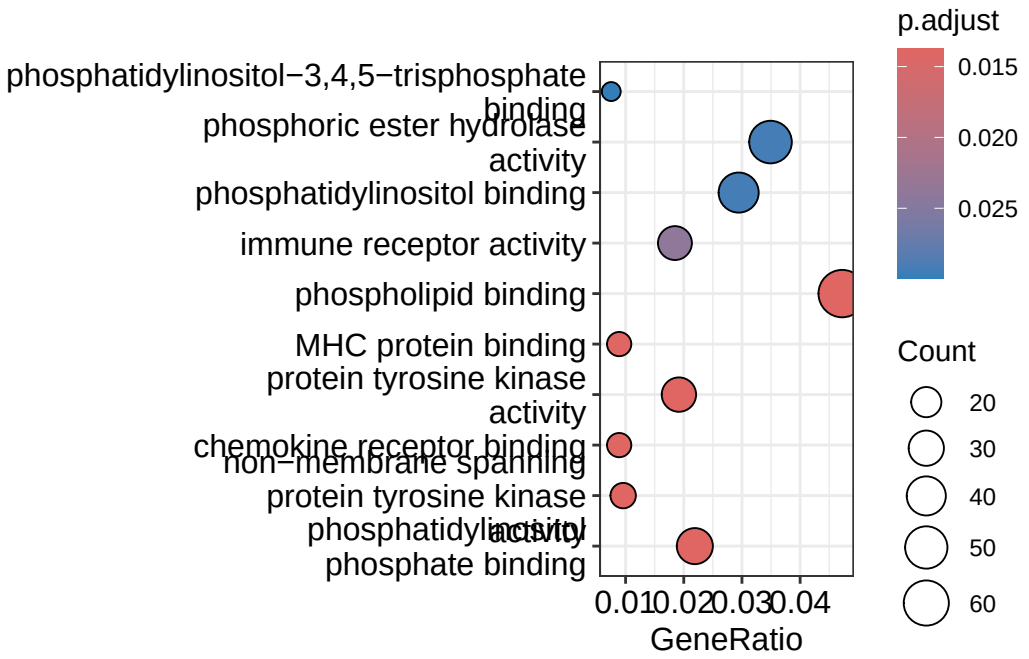
```r
# For a gene set that is down-regulated in NK cells:
gseaplot(GO_NK_Th,
  geneSetID = "GO:0002181",
  title = "GO:0002181 - cytoplasmic translation"
)
```



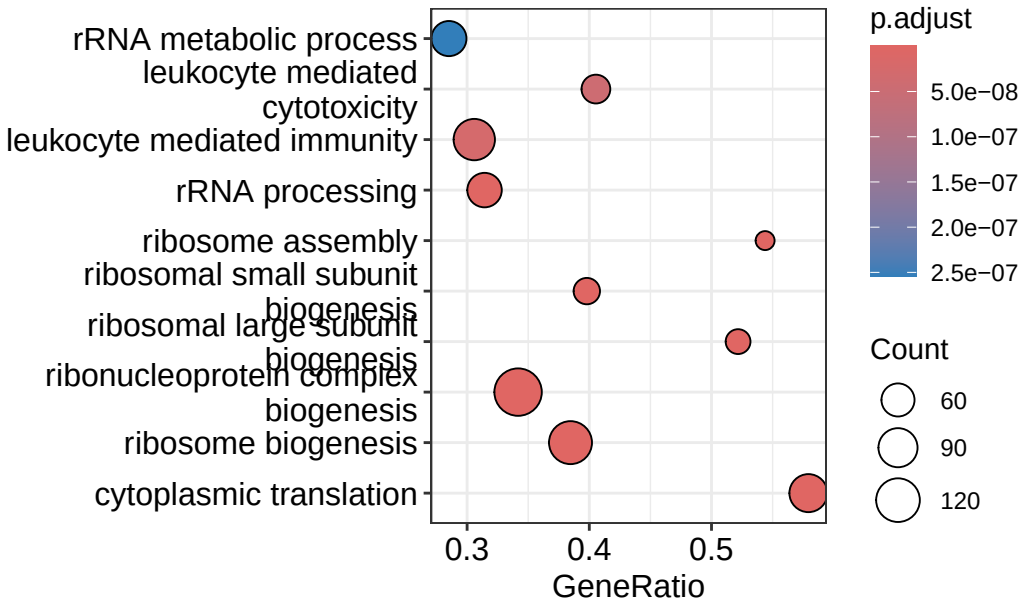

```
# And one that is up-regulated in NK cells
gseaplot(GO_NK_Th,
  geneSetID = "GO:0002443",
  title = "GO:0002443 - leukocyte mediated immunity"
)
```



```
enrichplot::dotplot(GO_enrich, orderBy = "p.adjust")
```

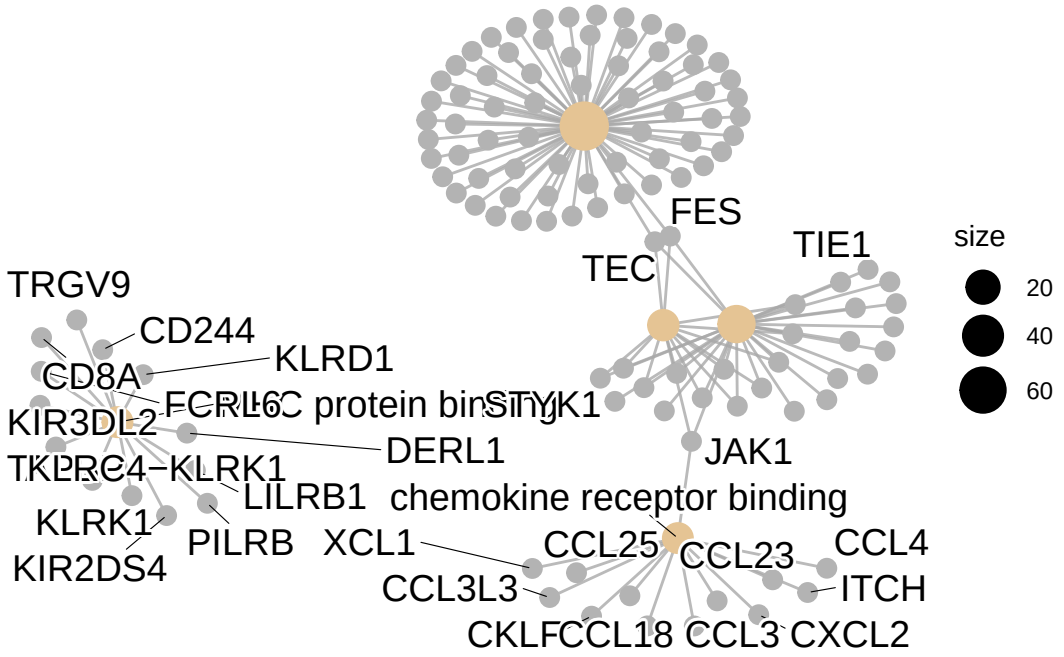



```
enrichplot::dotplot(GO_NK_Th, orderBy = "p.adjust")
```



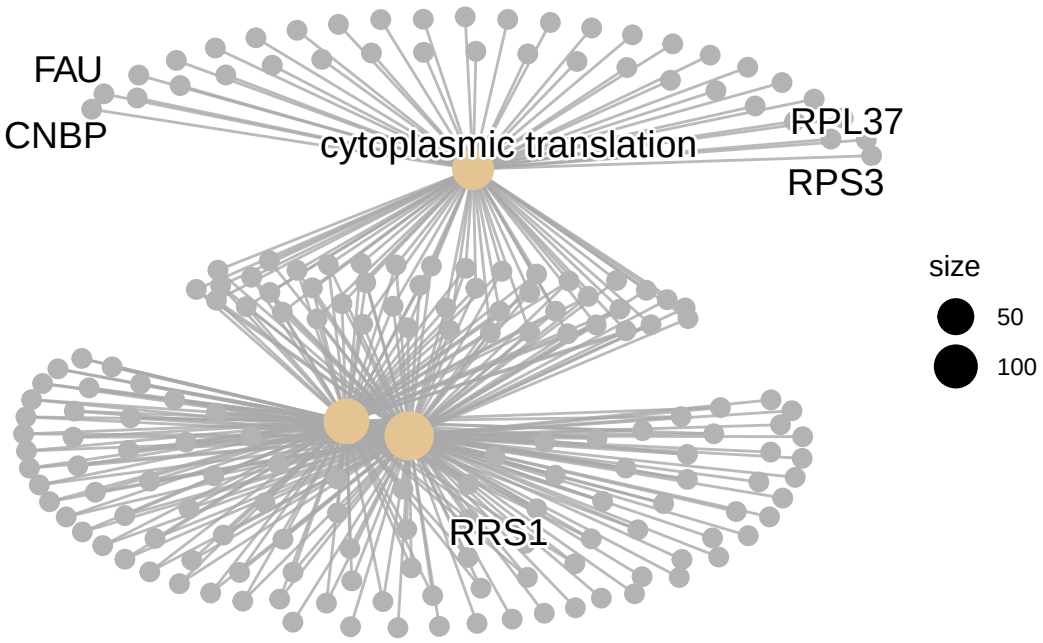
```
cnetplot(GO_enrich, categorySize = "pvalue")
```

```
Warning: ggrepel: 95 unlabeled data points (too many overlaps). Consider
increasing max.overlaps
```



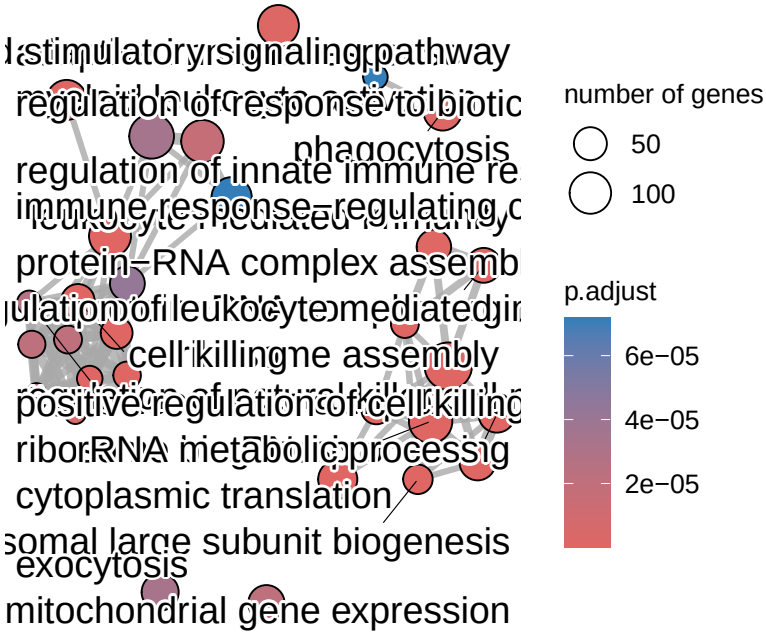

```
cnetplot(GO_NK_Th, showCategory = 3)
```

```
Warning: ggrepel: 185 unlabeled data points (too many overlaps). Consider
increasing max.overlaps
```



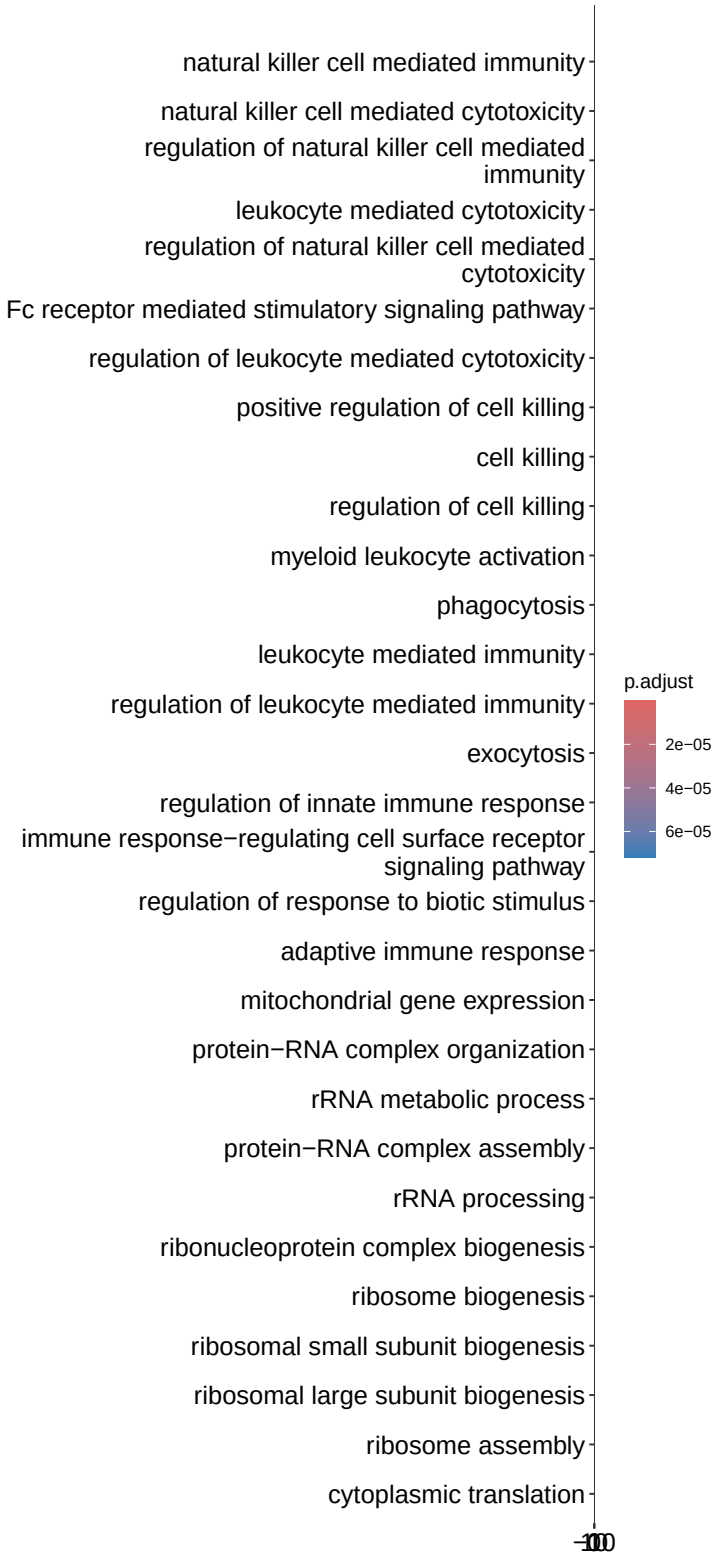

```
ego2 <- pairwise_termsim(GO_NK_Th)
emapplot(ego2, color = "p.adjust")
```

```
Warning: ggrepel: 7 unlabeled data points (too many overlaps). Consider
increasing max.overlaps
```



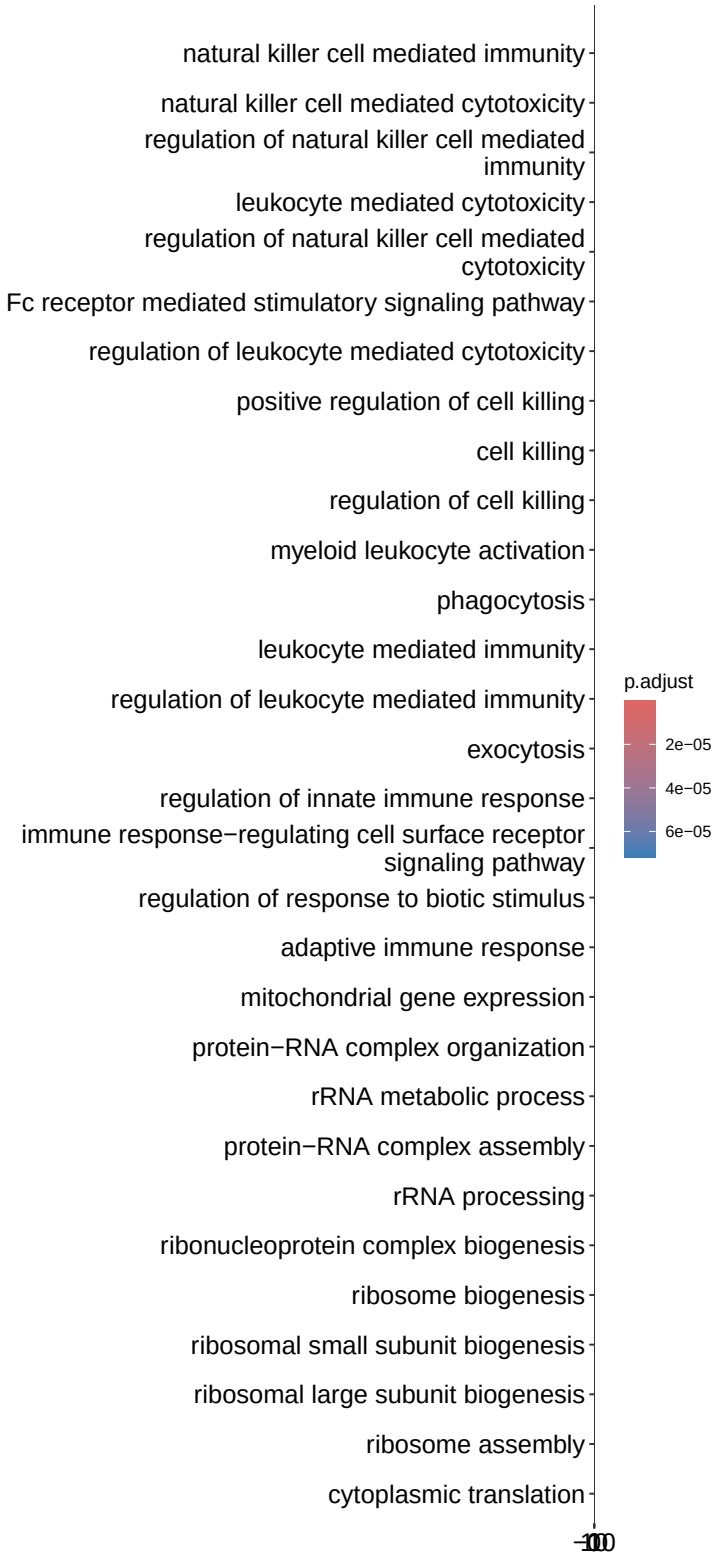
```r
# Wrap lenght of labels
label_format <- 50

# Distribution of t-statistic for genes included in significant gene sets or in selected gene
ridgeplot(GO_NK_Th, label_format = label_format)
```

```
Picking joint bandwidth of 0.787
```


natural killer cell mediated immunity
natural killer cell mediated cytotoxicity
regulation of natural killer cell mediated immunity
leukocyte mediated cytotoxicity
regulation of natural killer cell mediated cytotoxicity
Fc receptor mediated stimulatory signaling pathway
regulation of leukocyte mediated cytotoxicity
positive regulation of cell killing
cell killing
regulation of cell killing
myeloid leukocyte activation
phagocytosis
leukocyte mediated immunity
regulation of leukocyte mediated immunity
exocytosis
regulation of innate immune response
immune response−regulating cell surface receptor signaling pathway
regulation of response to biotic stimulus
adaptive immune response
mitochondrial gene expression
protein−RNA complex organization
rRNA metabolic process
protein−RNA complex assembly
rRNA processing
ribonucleoprotein complex biogenesis
ribosome biogenesis
ribosomal small subunit biogenesis
ribosomal large subunit biogenesis
ribosome assembly
cytoplasmic translation
p.adjust
2e−05
4e−05
6e−05

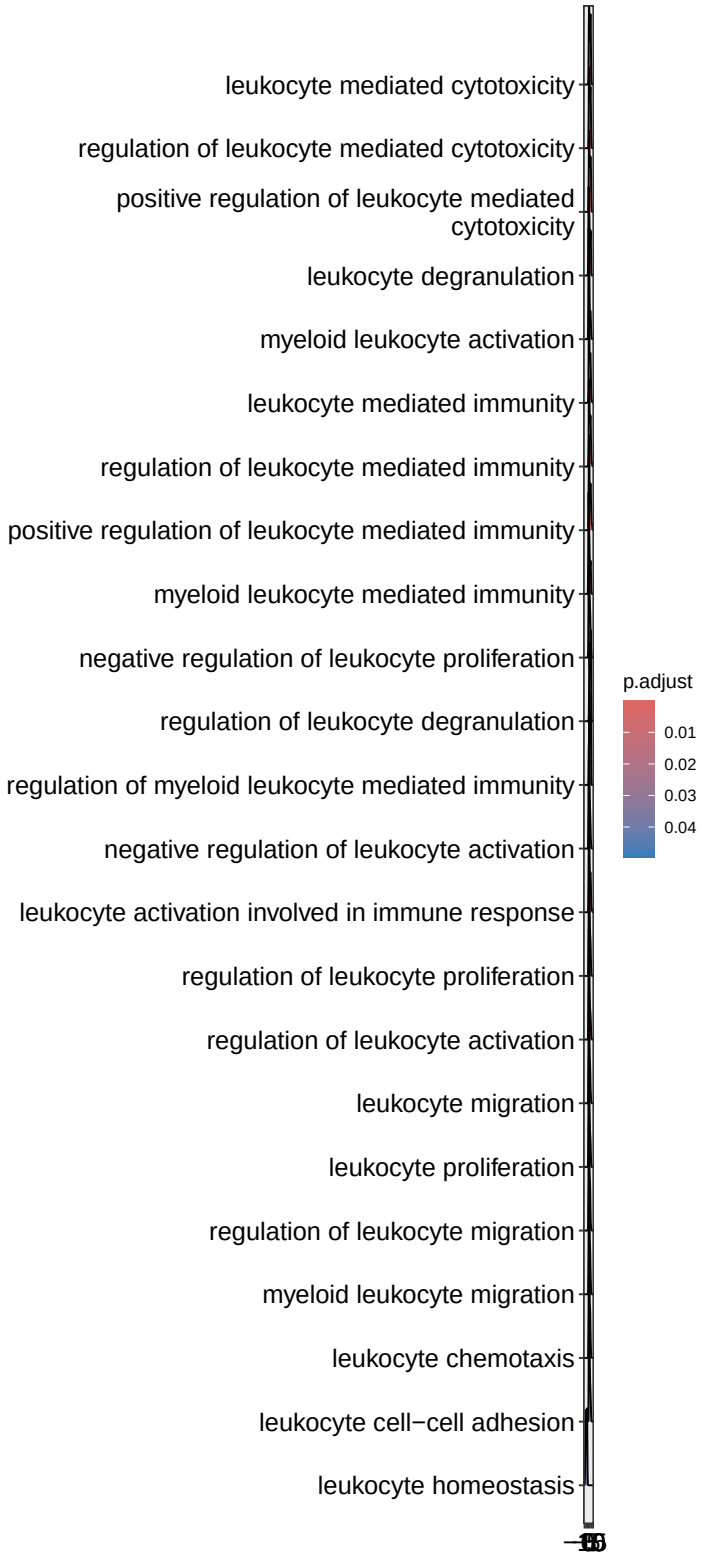
```
# What is the difference with core_enrichment =F?
ridgeplot(GO_NK_Th, core_enrichment = FALSE, label_format = label_format)
```

```
Picking joint bandwidth of 0.975
```


natural killer cell mediated immunity
natural killer cell mediated cytotoxicity
regulation of natural killer cell mediated immunity
leukocyte mediated cytotoxicity
regulation of natural killer cell mediated cytotoxicity
Fc receptor mediated stimulatory signaling pathway
regulation of leukocyte mediated cytotoxicity
positive regulation of cell killing
cell killing
regulation of cell killing
myeloid leukocyte activation
phagocytosis
leukocyte mediated immunity
regulation of leukocyte mediated immunity
exocytosis
regulation of innate immune response
immune response−regulating cell surface receptor signaling pathway
regulation of response to biotic stimulus
adaptive immune response
mitochondrial gene expression
protein−RNA complex organization
rRNA metabolic process
protein−RNA complex assembly
rRNA processing
ribonucleoprotein complex biogenesis
ribosome biogenesis
ribosomal small subunit biogenesis
ribosomal large subunit biogenesis
ribosome assembly
cytoplasmic translation
p.adjust
2e−05
4e−05
6e−05

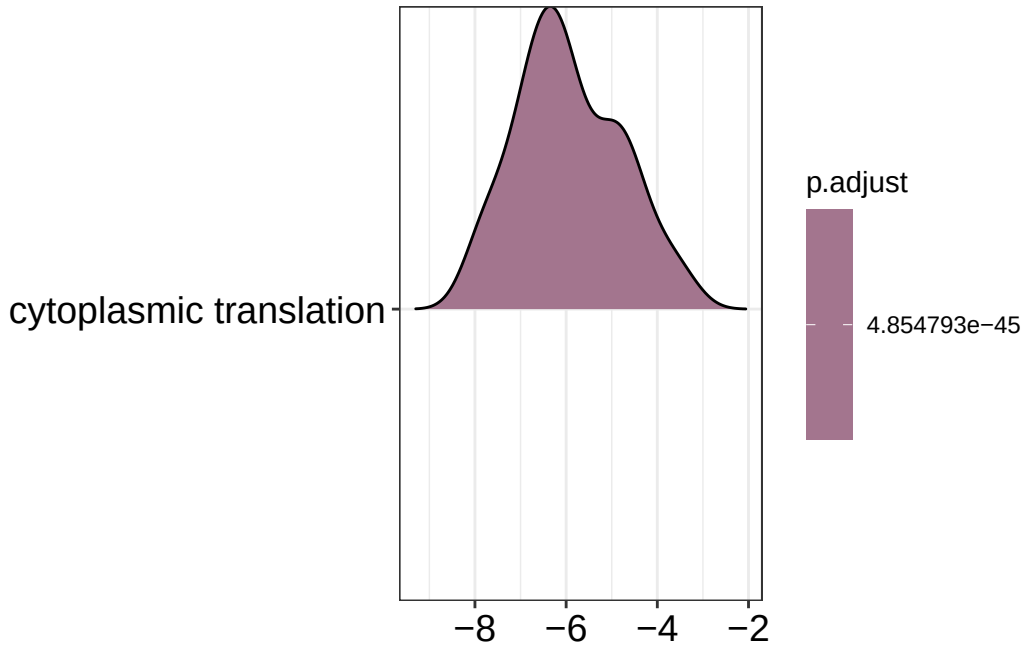
```r
# Select which GO terms to show in the ridge plot:
GO_NK_Th_selection_1 <- GO_NK_Th[GO_NK_Th$ID == "GO:0002181", asis = TRUE]
GO_NK_Th_selection_3 <- GO_NK_Th[
  GO_NK_Th$ID %in% c(
    "GO:0002181", "GO:0022613",
    "GO:0042254"
  ),
  asis = TRUE
]

# Terms that contain the keyword "leukocyte"
GO_NK_Th_selection <- GO_NK_Th[grep("leukocyte", GO_NK_Th@result$Description), asis = TRUE]
ridgeplot(GO_NK_Th_selection, label_format = label_format)
```

```
Picking joint bandwidth of 0.839
```


leukocyte mediated cytotoxicity
regulation of leukocyte mediated cytotoxicity
positive regulation of leukocyte mediated cytotoxicity
leukocyte degranulation
myeloid leukocyte activation
leukocyte mediated immunity
regulation of leukocyte mediated immunity
positive regulation of leukocyte mediated immunity
myeloid leukocyte mediated immunity
negative regulation of leukocyte proliferation
regulation of leukocyte degranulation
regulation of myeloid leukocyte mediated immunity
negative regulation of leukocyte activation
leukocyte activation involved in immune response
regulation of leukocyte proliferation
regulation of leukocyte activation
leukocyte migration
leukocyte proliferation
regulation of leukocyte migration
myeloid leukocyte migration
leukocyte chemotaxis
leukocyte cell−cell adhesion
leukocyte homeostasis
p.adjust
0.01
0.02
0.03
0.04

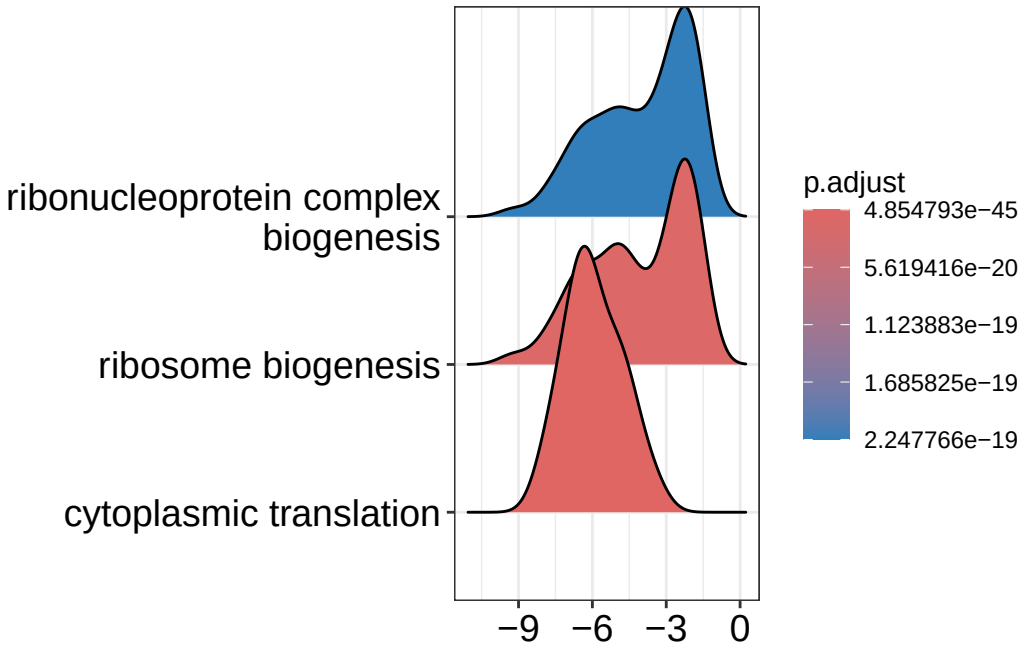
```
ridgeplot(GO_NK_Th_selection_1)
```

```
Picking joint bandwidth of 0.423
```




```
ridgeplot(GO_NK_Th_selection_3)
```

```
Picking joint bandwidth of 0.589
```



Exercise 4 - Enrichment of other collections of gene sets

```
keytypes(org.Hs.eg.db)
```

```
 [1] "ACCNUM"       "ALIAS"        "ENSEMBL"      "ENSEMBLPROT"  "ENSEMBLTRANS"
 [6] "ENTREZID"     "ENZYME"       "EVIDENCE"     "EVIDENCEALL"  "GENENAME"
[11] "GENETYPE"     "GO"           "GOALL"        "IPI"          "MAP"
[16] "OMIM"         "ONTOLOGY"     "ONTOLOGYALL"  "PATH"         "PFAM"
[21] "PMID"         "PROSITE"      "REFSEQ"       "SYMBOL"       "UCSCKG"
[26] "UNIPROT"
```

```
# convert from= "ENSEMBL" to "SYMBOL" and "ENTREZID"
gene_convert <- bitr(as.character(NK_vs_Th$ensembl_gene_id),
  fromType = "ENSEMBL",
  toType = c("SYMBOL", "ENTREZID"), OrgDb = "org.Hs.eg.db"
)
```

```
'select()' returned 1:many mapping between keys and columns
```

```
Warning in bitr(as.character(NK_vs_Th$ensembl_gene_id), fromType = "ENSEMBL", :
18.73% of input gene IDs are fail to map...
```

```r
# Check the format of the data frame obtained after conversion:
head(gene_convert)
```

```
          ENSEMBL SYMBOL ENTREZID
1 ENSG00000000003 TSPAN6     7105
2 ENSG00000000419   DPM1     8813
3 ENSG00000000457  SCYL3    57147
4 ENSG00000000460  FIRRM    55732
5 ENSG00000000938    FGR     2268
6 ENSG00000000971    CFH     3075
```

```r
dim(gene_convert)
```

```
[1] 16794     3
```

```r
# Create a vector of genes that are coded with the EntrezID:
# use the sorted gene list gl previously created:
gl_kegg <- cbind(SYMBOL = names(gl), t = gl)

# merge with converted gene symbols to combine both:
# by default the data frames are merged on the columns with names they both have
gl_kegg <- merge(gl_kegg, gene_convert)
head(gl_kegg)
```

```
  SYMBOL           t         ENSEMBL ENTREZID
1   A1BG  1.129187394 ENSG00000121410        1
2    A2M -0.382294217 ENSG00000175899        2
3 A4GALT  0.808365644 ENSG00000128274    53947
4   AAAS  0.749990903 ENSG00000094914     8086
5   AACS  2.172253591 ENSG00000081760    65985
6  AADAT  3.038354213 ENSG00000109576    51166
```

```r
gl_kegg_list <- as.numeric(as.character(gl_kegg$t))
names(gl_kegg_list) <- as.character(gl_kegg$ENTREZID)
gl_kegg_list <- sort(gl_kegg_list, decreasing = T)

# run GSEA of KEGG (please note that requires internet connection to download the KEGG annota
KEGG_NK_Th <- gseKEGG(gl_kegg_list,
  organism = "hsa", "ncbi-geneid",
  minGSSize = 30,
```

```
  eps = 0,
  seed = T
)
```

```
Reading KEGG annotation online: "https://rest.kegg.jp/link/hsa/pathway"...

Reading KEGG annotation online: "https://rest.kegg.jp/list/pathway/hsa"...

Reading KEGG annotation online: "https://rest.kegg.jp/conv/ncbi-geneid/hsa"...

preparing geneSet collections...

GSEA analysis...

Warning in preparePathwaysAndStats(pathways, stats, minSize, maxSize, gseaParam, : There are
The order of those tied genes will be arbitrary, which may produce unexpected results.

Warning in preparePathwaysAndStats(pathways, stats, minSize, maxSize,
gseaParam, : There are duplicate gene names, fgsea may produce unexpected
results.

leading edge analysis...

done...
```

```
# What does it contain?
str(KEGG_NK_Th)
```

```
Formal class 'gseaResult' [package "DOSE"] with 13 slots
  ..@ result     :'data.frame': 24 obs. of  11 variables:
  .. ..$ ID             : chr [1:24] "hsa03010" "hsa05171" "hsa04650" "hsa04666" ...
  .. ..$ Description    : chr [1:24] "Ribosome" "Coronavirus disease - COVID-19" "Natural ki
  .. ..$ setSize        : int [1:24] 130 185 98 86 163 181 93 77 118 220 ...
  .. ..$ enrichmentScore: num [1:24] -0.813 -0.678 0.62 0.525 0.426 ...
  .. ..$ NES            : num [1:24] -3.46 -3.02 2.49 2.07 1.87 ...
  .. ..$ pvalue         : num [1:24] 3.32e-46 5.30e-32 2.03e-12 1.23e-06 2.91e-06 ...
  .. ..$ p.adjust       : num [1:24] 8.90e-44 7.11e-30 1.81e-10 8.21e-05 1.56e-04 ...
  .. ..$ qvalue         : num [1:24] 7.27e-44 5.81e-30 1.48e-10 6.71e-05 1.27e-04 ...
```

```
.. ..$ rank           : num [1:24] 1852 1168 1873 1924 2216 ...
.. ..$ leading_edge   : chr [1:24] "tags=72%, list=13%, signal=63%" "tags=48%, list=8%, sig
.. ..$ core_enrichment: chr [1:24] "63875/140032/51121/64983/6139/9553/51021/51116/6133/510
..@ organism   : chr "hsa"
..@ setType    : chr "KEGG"
..@ geneSets   :List of 365
.. ..$ hsa00010: chr [1:67] "10327" "124" "125" "126" ...
.. ..$ hsa00020: chr [1:30] "1431" "1737" "1738" "1743" ...
.. ..$ hsa00030: chr [1:31] "132158" "2203" "221823" "226" ...
.. ..$ hsa00040: chr [1:36] "10327" "10720" "10941" "231" ...
.. ..$ hsa00051: chr [1:34] "197258" "2203" "226" "229" ...
.. ..$ hsa00052: chr [1:32] "130589" "231" "2538" "2548" ...
.. ..$ hsa00053: chr [1:30] "10327" "10720" "10941" "217" ...
.. ..$ hsa00061: chr [1:18] "109703458" "197322" "2180" "2181" ...
.. ..$ hsa00062: chr [1:27] "10449" "10965" "11332" "117145" ...
.. ..$ hsa00071: chr [1:43] "10449" "10455" "113612" "124" ...
.. ..$ hsa00100: chr [1:20] "1056" "10682" "120227" "1591" ...
.. ..$ hsa00120: chr [1:17] "10005" "10858" "10998" "1109" ...
.. ..$ hsa00130: chr [1:12] "10229" "154807" "1728" "2677" ...
.. ..$ hsa00140: chr [1:63] "100861540" "10720" "10941" "1109" ...
.. ..$ hsa00190: chr [1:138] "100532726" "10063" "101927180" "10312" ...
.. ..$ hsa00220: chr [1:23] "100526760" "1373" "137362" "162417" ...
.. ..$ hsa00230: chr [1:128] "100" "100526794" "10201" "102157402" ...
.. ..$ hsa00232: chr [1:6] "10" "1544" "1548" "1549" ...
.. ..$ hsa00240: chr [1:58] "100526794" "10201" "115024" "124583" ...
.. ..$ hsa00250: chr [1:37] "122622" "1373" "137362" "158" ...
.. ..$ hsa00260: chr [1:41] "102724560" "10993" "113675" "124908081" ...
.. ..$ hsa00270: chr [1:52] "102724560" "1036" "10768" "10993" ...
.. ..$ hsa00280: chr [1:48] "10449" "11112" "1629" "1738" ...
.. ..$ hsa00290: chr [1:4] "10993" "113675" "586" "587"
.. ..$ hsa00310: chr [1:63] "10157" "10919" "11105" "123688" ...
.. ..$ hsa00330: chr [1:50] "112483" "112817" "112849" "113451" ...
.. ..$ hsa00340: chr [1:22] "10841" "131669" "138199" "144193" ...
.. ..$ hsa00350: chr [1:36] "124" "125" "126" "127" ...
.. ..$ hsa00360: chr [1:16] "137362" "1644" "218" "221" ...
.. ..$ hsa00380: chr [1:42] "11185" "121278" "125061" "130013" ...
.. ..$ hsa00400: chr [1:6] "137362" "259307" "2805" "2806" ...
.. ..$ hsa00410: chr [1:31] "18" "1806" "1807" "1892" ...
.. ..$ hsa00430: chr [1:17] "102724197" "1036" "124975" "2326" ...
.. ..$ hsa00440: chr [1:6] "10390" "5130" "56994" "5833" ...
.. ..$ hsa00450: chr [1:17] "10587" "11185" "114112" "118672" ...
.. ..$ hsa00470: chr [1:6] "1610" "27165" "2744" "63826" ...
.. ..$ hsa00480: chr [1:59] "102724197" "10314" "119391" "124975" ...
```

```
.. ..$ hsa00500: chr [1:40] "11181" "124905666" "124905668" "128966568" ...
.. ..$ hsa00510: chr [1:54] "10195" "10905" "11253" "11282" ...
.. ..$ hsa00511: chr [1:18] "10825" "129807" "175" "23324" ...
.. ..$ hsa00512: chr [1:36] "100528030" "10331" "10610" "11226" ...
.. ..$ hsa00513: chr [1:42] "10195" "10905" "11253" "11282" ...
.. ..$ hsa00514: chr [1:47] "100528030" "10585" "11226" "11227" ...
.. ..$ hsa00515: chr [1:23] "10329" "10585" "10690" "11041" ...
.. ..$ hsa00520: chr [1:38] "10007" "10020" "1118" "132789" ...
.. ..$ hsa00524: chr [1:5] "2645" "3098" "3099" "3101" ...
.. ..$ hsa00531: chr [1:19] "10855" "138050" "23553" "2588" ...
.. ..$ hsa00532: chr [1:21] "10090" "11285" "113189" "126792" ...
.. ..$ hsa00533: chr [1:14] "10164" "10678" "2530" "2683" ...
.. ..$ hsa00534: chr [1:24] "11285" "126792" "2131" "2132" ...
.. ..$ hsa00541: chr [1:16] "10020" "123956252" "140838" "1727" ...
.. ..$ hsa00561: chr [1:65] "10327" "10554" "10555" "1056" ...
.. ..$ hsa00562: chr [1:73] "10423" "113026" "138429" "200576" ...
.. ..$ hsa00563: chr [1:30] "10026" "128869" "23556" "27315" ...
.. ..$ hsa00564: chr [1:103] "100137049" "10162" "10390" "1040" ...
.. ..$ hsa00565: chr [1:50] "100137049" "10390" "11145" "122618" ...
.. ..$ hsa00590: chr [1:63] "100137049" "102724197" "10728" "11145" ...
.. ..$ hsa00591: chr [1:30] "100137049" "11145" "123745" "151056" ...
.. ..$ hsa00592: chr [1:26] "100137049" "11145" "123745" "151056" ...
.. ..$ hsa00600: chr [1:54] "10558" "10715" "10825" "123099" ...
.. ..$ hsa00601: chr [1:28] "10317" "10331" "10402" "10678" ...
.. ..$ hsa00603: chr [1:16] "10317" "10690" "127550" "2523" ...
.. ..$ hsa00604: chr [1:15] "256435" "2583" "27090" "2720" ...
.. ..$ hsa00620: chr [1:47] "10327" "10873" "124" "125" ...
.. ..$ hsa00630: chr [1:31] "112817" "124908081" "125061" "132158" ...
.. ..$ hsa00640: chr [1:32] "160287" "1629" "1738" "18" ...
.. ..$ hsa00650: chr [1:27] "116285" "123876" "142827" "18" ...
.. ..$ hsa00670: chr [1:39] "100528021" "102724560" "10588" "10768" ...
.. ..$ hsa00730: chr [1:15] "122481" "158067" "203" "204" ...
.. ..$ hsa00740: chr [1:8] "5167" "5169" "52" "53" ...
.. ..$ hsa00750: chr [1:6] "29968" "316" "493911" "55163" ...
.. ..$ hsa00760: chr [1:38] "100526794" "10135" "133686" "22933" ...
.. ..$ hsa00770: chr [1:21] "1806" "1807" "217" "219" ...
.. ..$ hsa00780: chr [1:3] "3141" "54995" "686"
.. ..$ hsa00785: chr [1:20] "11019" "116285" "124908081" "1629" ...
.. ..$ hsa00790: chr [1:28] "10243" "121278" "1719" "200895" ...
.. ..$ hsa00830: chr [1:68] "100861540" "10170" "10720" "10901" ...
.. ..$ hsa00860: chr [1:46] "10720" "10941" "124454" "1352" ...
.. ..$ hsa00900: chr [1:23] "100529261" "10269" "10654" "116150" ...
.. ..$ hsa00910: chr [1:17] "11238" "1373" "23632" "2746" ...
```

```
  .. ..$ hsa00920: chr [1:10] "10380" "23474" "4357" "54928" ...
  .. ..$ hsa00970: chr [1:66] "10056" "10352" "10667" "118672" ...
  .. ..$ hsa00980: chr [1:79] "10720" "107987478" "107987479" "10941" ...
  .. ..$ hsa00982: chr [1:73] "10720" "107987478" "107987479" "10941" ...
  .. ..$ hsa00983: chr [1:81] "10" "10201" "1066" "10720" ...
  .. ..$ hsa01040: chr [1:27] "10965" "11332" "122970" "201562" ...
  .. ..$ hsa01100: chr [1:1570] "10" "100" "10005" "10007" ...
  .. ..$ hsa01200: chr [1:116] "10873" "10993" "113675" "124908081" ...
  .. ..$ hsa01210: chr [1:33] "100526760" "137362" "1431" "162417" ...
  .. ..$ hsa01212: chr [1:57] "10449" "109703458" "126129" "1374" ...
  .. ..$ hsa01230: chr [1:75] "100526760" "102724560" "10993" "113675" ...
  .. ..$ hsa01232: chr [1:85] "100" "100526794" "10201" "102157402" ...
  .. ..$ hsa01240: chr [1:154] "10201" "102157402" "10229" "10243" ...
  .. ..$ hsa01250: chr [1:37] "10020" "140838" "197258" "23483" ...
  .. ..$ hsa01320: chr [1:2] "9060" "9061"
  .. ..$ hsa01521: chr [1:80] "10000" "10018" "110117499" "1950" ...
  .. ..$ hsa01522: chr [1:99] "10000" "1019" "1026" "1027" ...
  .. ..$ hsa01523: chr [1:30] "10057" "10257" "113235" "1147" ...
  .. ..$ hsa01524: chr [1:75] "10000" "1026" "1029" "110117499" ...
  .. .. [list output truncated]
  ..@ geneList   : Named num [1:14284] 19 13.1 12.1 12 10.7 ...
  .. ..- attr(*, "names")= chr [1:14284] "2693" "23209" "117157" "6983" ...
  ..@ keytype    : chr "ncbi-geneid"
  ..@ permScores : num[0 , 0 ]
  ..@ params     :List of 6
  .. ..$ pvalueCutoff : num 0.05
  .. ..$ eps          : num 0
  .. ..$ pAdjustMethod: chr "BH"
  .. ..$ exponent     : num 1
  .. ..$ minGSSize    : num 30
  .. ..$ maxGSSize    : num 500
  ..@ gene2Symbol: chr(0)
  ..@ readable   : logi FALSE
  ..@ termsim    : num[0 , 0 ]
  ..@ method     : chr(0)
  ..@ dr         : list()
```

```
# How many gene sets are up-regulated?
sum(KEGG_NK_Th@result$NES > 0) # 17
```

```
[1] 17
```

```
#|
grep_kegg_description <- function(pattern) {
  return(grep(pattern, tolower((KEGG_NK_Th@result$Description))))
}
```

```
# Is their an immune-related gene set significant?
grep_kegg_description("immune")
```

```
integer(0)
```

```
# Is their an NK gene set significant?
grep_kegg_description("natural killer") # 3
```

```
[1] 3
```

```
# What is the total number of built-in KEGG gene sets?
length(KEGG_NK_Th@geneSets) # 265
```

```
[1] 365
```

```
KEGG_NK_Th[grep_kegg_description("natural killer"), ] |>
  select(ID, Description) # hsa04650
```

```
                ID                                   Description
hsa04650 hsa04650 Natural killer cell mediated cytotoxicity
```

```
KEGG_NK_Th@geneSets$hsa04650
```

```
  [1] "100132285" "100507436" "100528032" "102723407" "10451"     "10870"
  [7] "110117499" "117157"    "124905743" "135250"    "1437"      "154064"
 [13] "2185"      "2207"      "2214"      "2215"      "22914"     "2534"
 [19] "25759"     "259197"    "27040"     "2885"      "3002"      "3105"
 [25] "3106"      "3107"      "3133"      "3135"      "3265"      "3383"
 [31] "3384"      "3439"      "3440"      "3441"      "3442"      "3443"
 [37] "3444"      "3445"      "3446"      "3447"      "3448"      "3449"
 [43] "3451"      "3452"      "3454"      "3455"      "3456"      "3458"
 [49] "3459"      "3460"      "353091"    "355"       "356"       "3683"
 [55] "3689"      "369"       "3802"      "3803"      "3804"      "3805"
```

```
 [61] "3806"      "3808"      "3809"      "3810"      "3811"      "3812"
 [67] "3821"      "3822"      "3823"      "3824"      "3845"      "3932"
 [73] "3937"      "399694"    "4068"      "4277"      "4772"      "4773"
 [79] "4893"      "5058"      "51744"     "5290"      "5291"      "5293"
 [85] "5295"      "5296"      "5335"      "53358"     "5336"      "5530"
 [91] "5532"      "5533"      "5534"      "5535"      "5551"      "5578"
 [97] "5579"      "5582"      "5594"      "5595"      "5604"      "5605"
[103] "57292"     "5777"      "5781"      "5879"      "5880"      "5881"
[109] "5894"      "637"       "6452"      "6464"      "6654"      "6655"
[115] "673"       "6850"      "7124"      "7305"      "7409"      "7410"
[121] "7462"      "7535"      "79465"     "80328"     "80329"     "836"
[127] "8503"      "8743"      "8795"      "8797"      "919"       "9436"
[133] "9437"      "962"
```

```
# pathview map with non-significant genes in grey:
# set log fold change of non-significant genes to 0:
NK_vs_Th$logFC_0 <- ifelse(NK_vs_Th$p.adj > 0.05, 0, NK_vs_Th$logFC)

# create named vector of fold change values:
genePW <- NK_vs_Th$logFC_0
names(genePW) <- NK_vs_Th$symbol

# Create pathview map for Ribosome = hsa03010
pathview(
  gene.data = genePW,
  pathway.id = "hsa03010",
  species = "hsa",
  gene.idtype = "SYMBOL"
)
```

```
'select()' returned 1:many mapping between keys and columns
```

```
[1] "Note: 4806 of 20411 unique input IDs unmapped."
```

```
'select()' returned 1:1 mapping between keys and columns
```

```
Info: Working in directory /var/home/artur/Documents/10-19_PhD/11_Education/11.22-sib-enrichm
```

```
Info: Writing image file hsa03010.pathview.png
```

```r
# Create pathview map of Natural killer cell mediated cytotoxicity = hsa04650
pathview(
  gene.data = genePW,
  pathway.id = "hsa04650",
  species = "hsa",
  gene.idtype = "SYMBOL"
)
```

```
'select()' returned 1:many mapping between keys and columns
```

```
[1] "Note: 4806 of 20411 unique input IDs unmapped."
```

```
'select()' returned 1:1 mapping between keys and columns
```

```
Info: Working in directory /var/home/artur/Documents/10-19_PhD/11_Education/11.22-sib-enrichr
```

```
Info: Writing image file hsa04650.pathview.png
```

```r
# Import hallmark, convert to term2gene and run GSEA:
term2gene_h <- msigdbr(species = "Homo sapiens", category = "H")
# Or alternatively:
# term2gene_h<-read.gmt("h.all.v2023.2.Hs.symbols.gmt")

head(term2gene_h)
```

```
# A tibble: 6 x 15
  gs_cat gs_subcat gs_name              gene_symbol entrez_gene ensembl_gene
  <chr>  <chr>     <chr>                <chr>             <int> <chr>
1 H      ""        HALLMARK_ADIPOGENESIS ABCA1                19 ENSG00000165029
2 H      ""        HALLMARK_ADIPOGENESIS ABCB8             11194 ENSG00000197150
3 H      ""        HALLMARK_ADIPOGENESIS ACAA2             10449 ENSG00000167315
4 H      ""        HALLMARK_ADIPOGENESIS ACADL                33 ENSG00000115361
5 H      ""        HALLMARK_ADIPOGENESIS ACADM                34 ENSG00000117054
6 H      ""        HALLMARK_ADIPOGENESIS ACADS                35 ENSG00000122971
# i 9 more variables: human_gene_symbol <chr>, human_entrez_gene <int>,
#   human_ensembl_gene <chr>, gs_id <chr>, gs_pmid <chr>, gs_geoid <chr>,
#   gs_exact_source <chr>, gs_url <chr>, gs_description <chr>
```

```r
length(unique(term2gene_h$gs_name)) # 50
```

```
[1] 50
```

```r
# Run GSEA with the function that allows to use custom gene sets,
# provide the named vector of t statistics
h_NK_vs_Th <- GSEA(gl,
  TERM2GENE = term2gene_h[, c("gs_name", "gene_symbol")],
  eps = 0,
  seed = T
)
```

```
preparing geneSet collections...
```

```
GSEA analysis...
```

```
Warning in preparePathwaysAndStats(pathways, stats, minSize, maxSize, gseaParam, : There are
The order of those tied genes will be arbitrary, which may produce unexpected results.
```

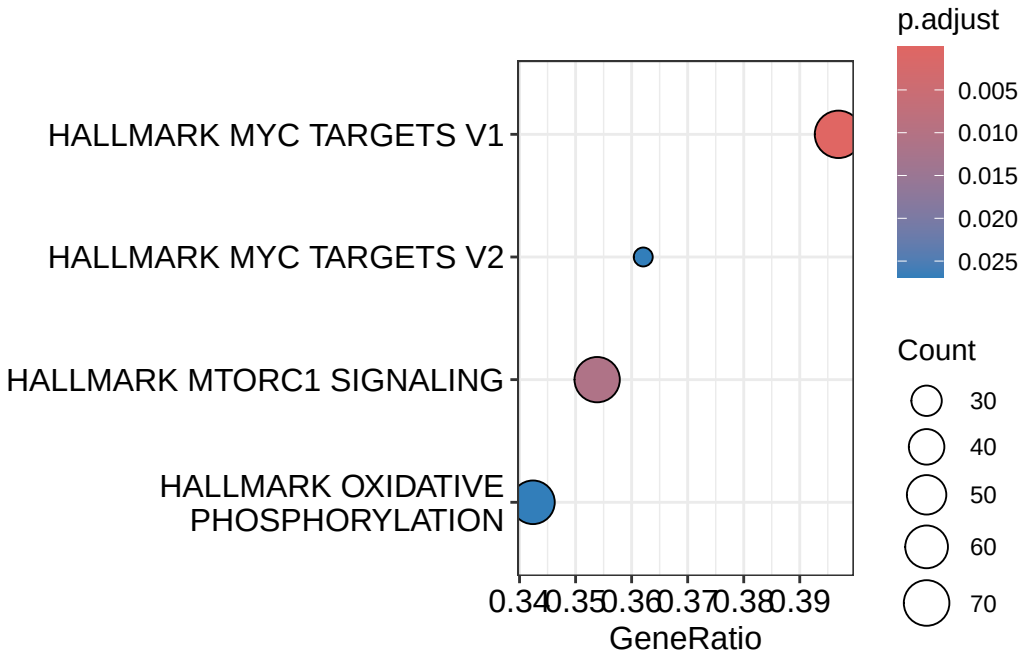
```
leading edge analysis...
```

```
done...
```

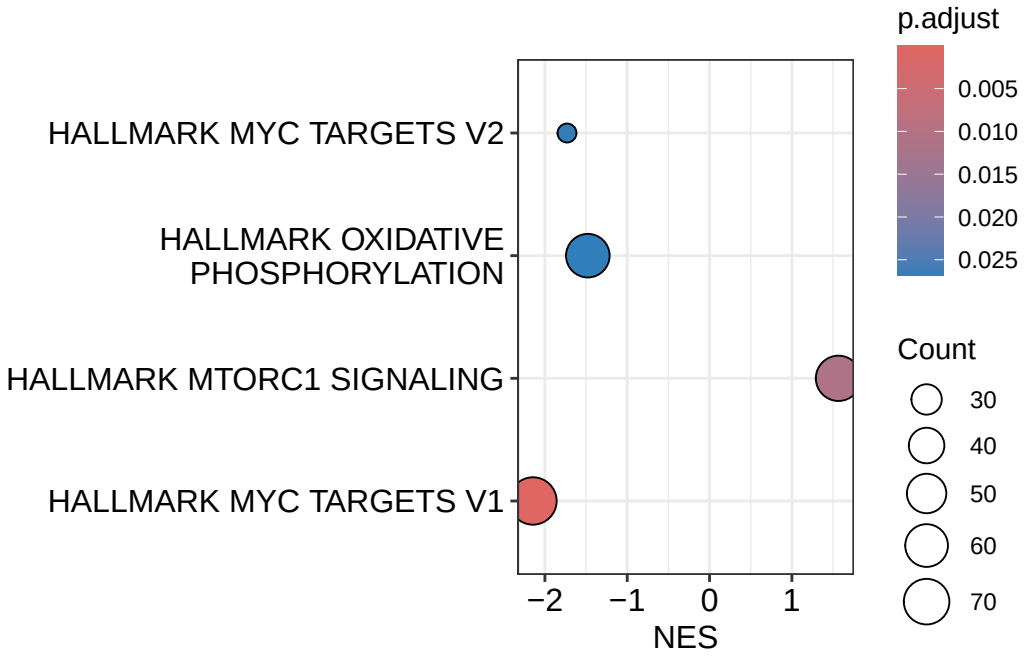
```r
# Number of significant gene sets:
length(which(h_NK_vs_Th@result$p.adjust <= 0.05))
```

```
[1] 4
```

```r
# A dotplot with geneRatio or NES on the x-axis:
dotplot(h_NK_vs_Th)
```

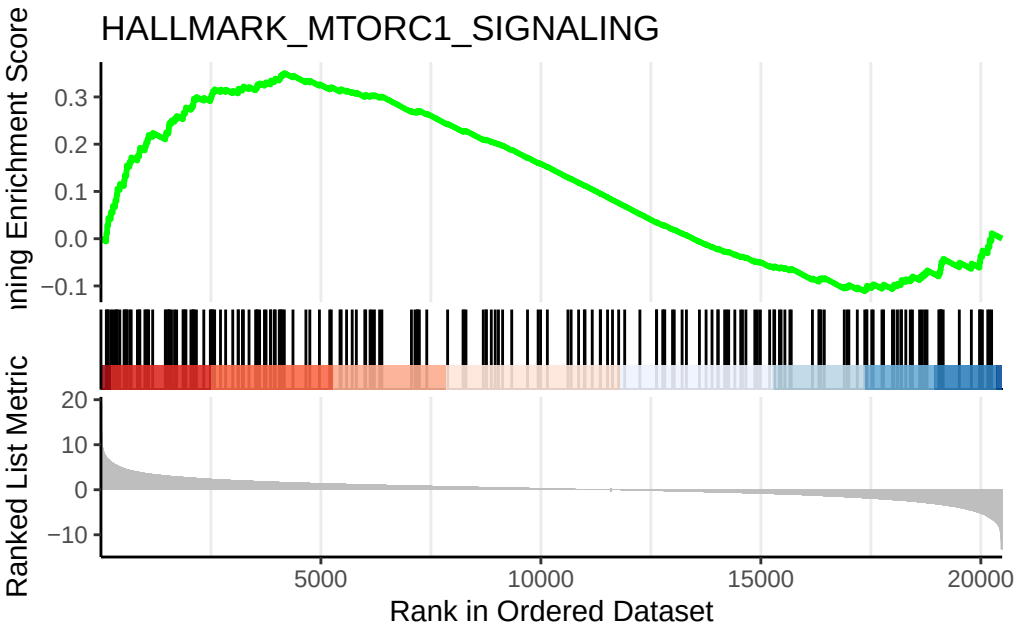



```
dotplot(h_NK_vs_Th, x = "NES", orderBy = "p.adjust")
```




```
# A barcode plot:
gseaplot2(h_NK_vs_Th,
```

```
  geneSetID = "HALLMARK_MTORC1_SIGNALING",
  title = "HALLMARK_MTORC1_SIGNALING"
)
```



Extra exercises

```
# Read in Reactome genes
reactome_gene_sets <- msigdbr(category = "C2", subcategory = "CP:REACTOME")

# Run GSEA with Reactome database
reactome_NK_vs_Th <- GSEA(gl,
  minGSSize = 30,
  TERM2GENE = reactome_gene_sets[, c("gs_name", "gene_symbol")],
  eps = 0, seed = TRUE
)
```

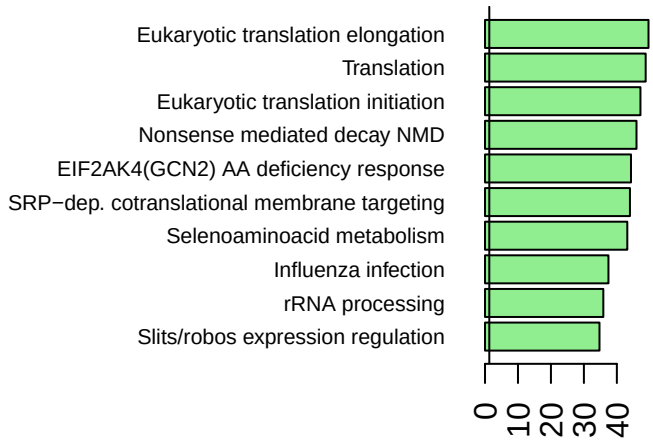
```
preparing geneSet collections...

GSEA analysis...

Warning in preparePathwaysAndStats(pathways, stats, minSize, maxSize, gseaParam, : There are
The order of those tied genes will be arbitrary, which may produce unexpected results.
```

```
leading edge analysis...


done...
```

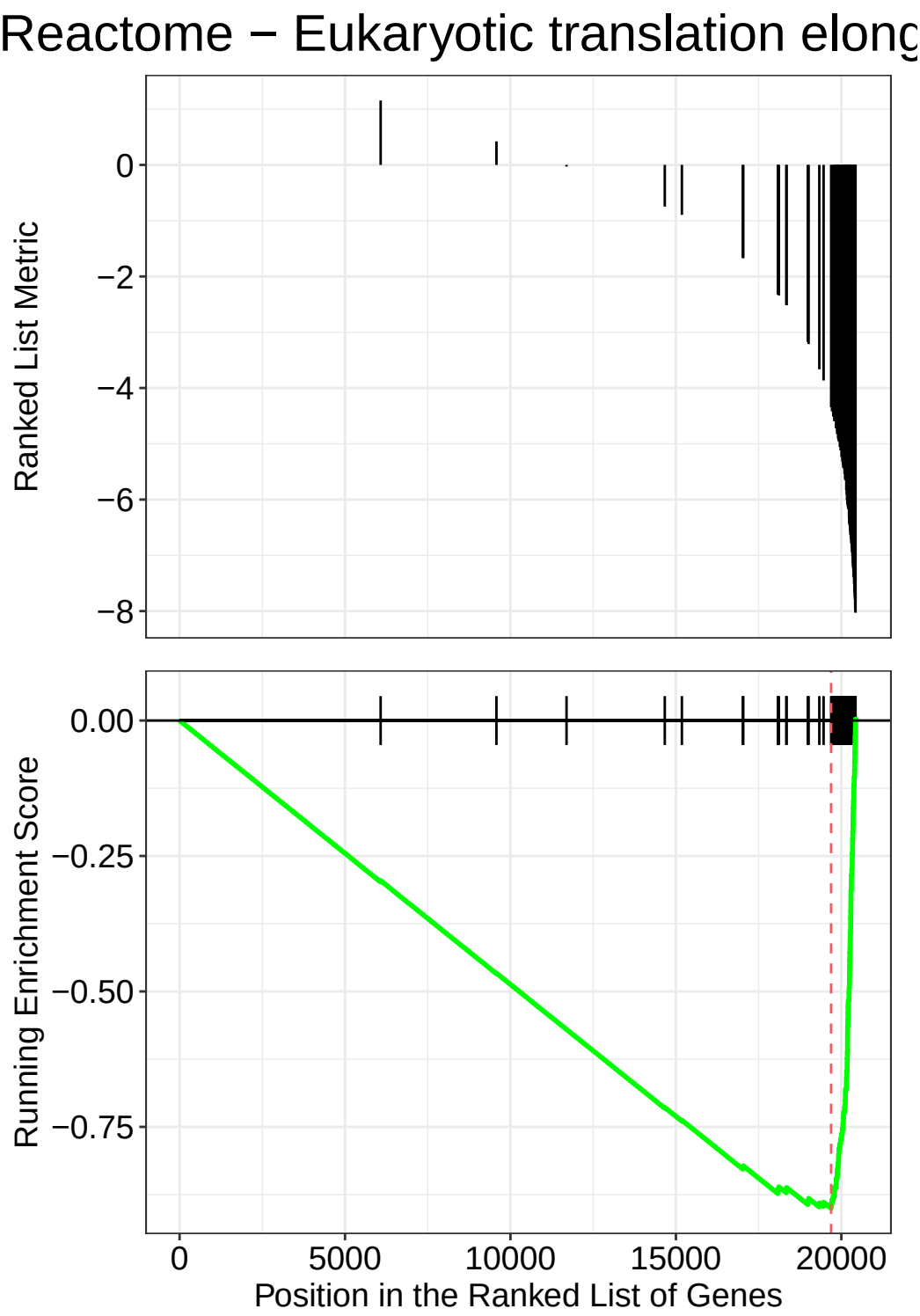
```r
# Count number of significant gene sets
reactome_significant <- length(which(reactome_NK_vs_Th@result$p.adjust < 0.05))
print(paste("Number of significant gene sets with Reactome database is", reactome_significant
```

```
[1] "Number of significant gene sets with Reactome database is 53"
```

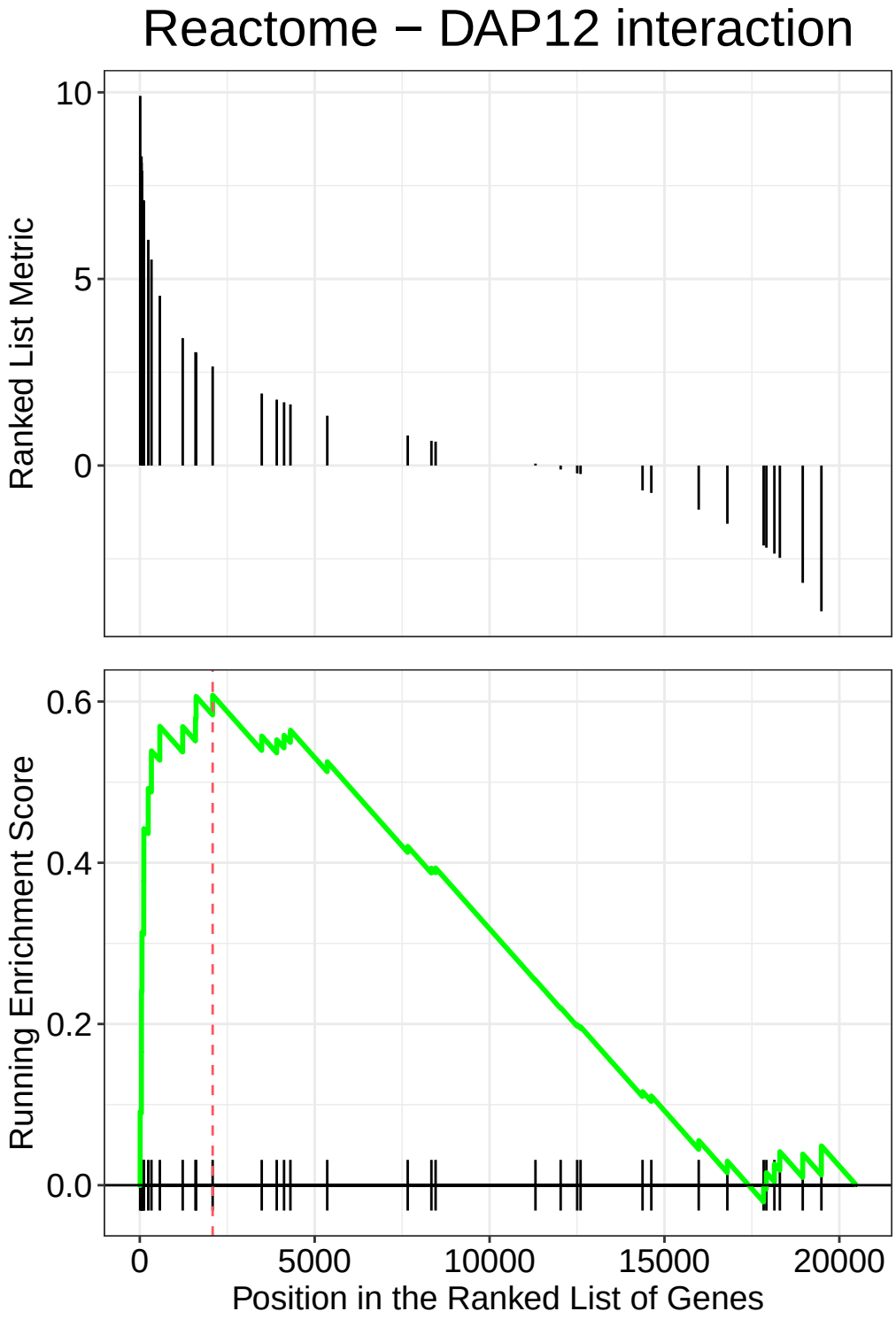
```r
par(mar = c(5, 20, 3, 3) + 0.1)
# Recode long labels
reactome_NK_vs_Th@result$Description_short <-
  reactome_NK_vs_Th@result$Description |>
  case_match(
    "REACTOME_REGULATION_OF_EXPRESSION_OF_SLITS_AND_ROBOS" ~ "Slits/robos expression regulati
    "REACTOME_RRNA_PROCESSING" ~ "rRNA processing",
    "REACTOME_INFLUENZA_INFECTION" ~ "Influenza infection",
    "REACTOME_SELENOAMINO_ACID_METABOLISM" ~ "Selenoaminoacid metabolism",
    "REACTOME_SRP_DEPENDENT_COTRANSLATIONAL_PROTEIN_TARGETING_TO_MEMBRANE" ~ "SRP-dep. cotra
    "REACTOME_RESPONSE_OF_EIF2AK4_GCN2_TO_AMINO_ACID_DEFICIENCY" ~ "EIF2AK4(GCN2) AA deficie
    "REACTOME_NONSENSE_MEDIATED_DECAY_NMD" ~ "Nonsense mediated decay NMD",
    "REACTOME_EUKARYOTIC_TRANSLATION_INITIATION" ~ "Eukaryotic translation initiation",
    "REACTOME_TRANSLATION" ~ "Translation",
    "REACTOME_EUKARYOTIC_TRANSLATION_ELONGATION" ~ "Eukaryotic translation elongation"
  )

# Bar plot
barplot(rev(-log10(reactome_NK_vs_Th@result$p.adjust[1:10])),
  horiz = TRUE, names = rev(reactome_NK_vs_Th@result$Description_short[1:10]),
  las = 2, xlab = "-log10(adj.p-value)",
  cex.names = 0.7,
  col = "lightgreen"
)
abline(v = -log10(0.05))
```




```
gseaplot(reactome_NK_vs_Th,
  geneSetID = "REACTOME_EUKARYOTIC_TRANSLATION_ELONGATION",
  title = "Reactome - Eukaryotic translation elongation"
)
```


Reactome − Eukaryotic translation elong
Ranked List Metric
0
−2
−4
−6
−8
Running Enrichment Score
0.00
−0.25
−0.50
−0.75
0
5000
10000
15000
20000
Position in the Ranked List of Genes

```
gseaplot(reactome_NK_vs_Th,
  geneSetID = "REACTOME_DAP12_INTERACTIONS",
  title = "Reactome - DAP12 interaction"
)
```


Reactome − DAP12 interaction
Ranked List Metric
10
5
0
Running Enrichment Score
0.6
0.4
0.2
0.0
0
5000
10000
15000
20000
Position in the Ranked List of Genes