Class 14: RNASeq mini-project

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Background

Pathway analysis (also known as gene set analysis or over-representation analysis), aims to reduce the complexity of interpreting gene lists via mapping the listed genes to known (i.e. annotated) biological pathways, processes and functions.

Data Import

```
library(DESeq2)
```

Let's read the `count` data and `metadata` abouot this experiment setup from the supplied CSV files

```r
metaFile <- "GSE37704_metadata.csv"
countFile <- "GSE37704_featurecounts.csv"
```

Sanity check

```r
# Import metadata and take a peek
colData = read.csv(metaFile, row.names=1)
head(colData)
```

```
              condition
SRR493366 control_sirna
SRR493367 control_sirna
SRR493368 control_sirna
SRR493369      hoxa1_kd
SRR493370      hoxa1_kd
SRR493371      hoxa1_kd
```

```r
# Import countdata and take a look
countData = read.csv(countFile, row.names=1)
head(countData)
```

```
                length SRR493366 SRR493367 SRR493368 SRR493369 SRR493370
ENSG00000186092    918         0         0         0         0         0
ENSG00000279928    718         0         0         0         0         0
ENSG00000279457   1982        23        28        29        29        28
ENSG00000278566    939         0         0         0         0         0
ENSG00000273547    939         0         0         0         0         0
ENSG00000187634   3214       124       123       205       207       212
                SRR493371
ENSG00000186092         0
ENSG00000279928         0
ENSG00000279457        46
ENSG00000278566         0
ENSG00000273547         0
ENSG00000187634       258
```

Q. Complete the code below to remove the troublesome first column from countData

```r
# We need to remove the odd first $length col
countData <- as.matrix(countData[,-1])
head(countData)
```

```
                 SRR493366 SRR493367 SRR493368 SRR493369 SRR493370 SRR493371
ENSG00000186092         0         0         0         0         0         0
ENSG00000279928         0         0         0         0         0         0
ENSG00000279457        23        28        29        29        28        46
ENSG00000278566         0         0         0         0         0         0
ENSG00000273547         0         0         0         0         0         0
ENSG00000187634       124       123       205       207       212       258
```

```r
colnames(countData)
```

```
[1] "SRR493366" "SRR493367" "SRR493368" "SRR493369" "SRR493370" "SRR493371"
```

```r
all(colnames(countData)==colData$id)
```

```
[1] TRUE
```

> Q. Complete the code below to filter countData to exclude genes (i.e. rows) where we have 0 read count across all samples (i.e. columns).

```r
# Filter count data where you have 0 read count across all samples.
countData = countData[rowSums(countData) > 0, ]
head(countData)
```

```
                 SRR493366 SRR493367 SRR493368 SRR493369 SRR493370 SRR493371
ENSG00000279457        23        28        29        29        28        46
ENSG00000187634       124       123       205       207       212       258
ENSG00000188976      1637      1831      2383      1226      1326      1504
ENSG00000187961       120       153       180       236       255       357
ENSG00000187583        24        48        65        44        48        64
ENSG00000187642         4         9        16        14        16        16
```

Setup DESeq object

```r
# Setup the DESeqDataSet object required for the DESeq() function
dds = DESeqDataSetFromMatrix(countData=countData,
                             colData=colData,
                             design=~condition)
```

```
Warning in DESeqDataSet(se, design = design, ignoreRank): some variables in
design formula are characters, converting to factors
```

Run DESeq analysis pipeline

```r
# Run the DESeq pipeline

dds = DESeq(dds)
```

```
estimating size factors

estimating dispersions

gene-wise dispersion estimates

mean-dispersion relationship

final dispersion estimates

fitting model and testing
```

```r
dds
```

```
class: DESeqDataSet
dim: 15975 6
metadata(1): version
assays(4): counts mu H cooks
rownames(15975): ENSG00000279457 ENSG00000187634 ... ENSG00000276345
  ENSG00000271254
rowData names(22): baseMean baseVar ... deviance maxCooks
colnames(6): SRR493366 SRR493367 ... SRR493370 SRR493371
colData names(2): condition sizeFactor
```

Extract the results

```r
# Get results for the HoxA1 knockdown versus control siRNA
res = results(dds)
```

> **Q**. Call the summary() function on your results to get a sense of how many genes are up or down-regulated at the default 0.1 p-value cutoff.

```r
summary(res)
```

```
out of 15975 with nonzero total read count
adjusted p-value < 0.1
LFC > 0 (up)       : 4349, 27%
LFC < 0 (down)     : 4396, 28%
outliers [1]       : 0, 0%
low counts [2]     : 1237, 7.7%
(mean count < 0)
[1] see 'cooksCutoff' argument of ?results
[2] see 'independentFiltering' argument of ?results
```

```r
head(res)
```

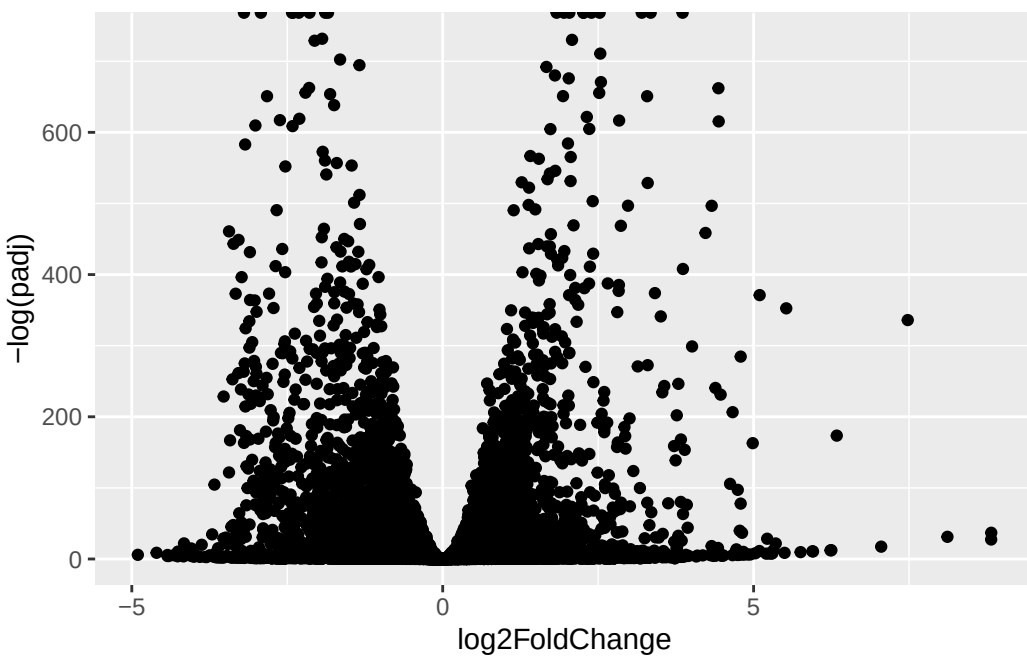
```
log2 fold change (MLE): condition hoxa1 kd vs control sirna
Wald test p-value: condition hoxa1 kd vs control sirna
DataFrame with 6 rows and 6 columns
                 baseMean log2FoldChange     lfcSE       stat      pvalue
                <numeric>      <numeric> <numeric>  <numeric>   <numeric>
ENSG00000279457   29.9136      0.1792571 0.3248215   0.551863 5.81042e-01
ENSG00000187634  183.2296      0.4264571 0.1402658   3.040350 2.36304e-03
ENSG00000188976 1651.1881     -0.6927205 0.0548465 -12.630156 1.43993e-36
ENSG00000187961  209.6379      0.7297556 0.1318599   5.534326 3.12428e-08
ENSG00000187583   47.2551      0.0405765 0.2718928   0.149237 8.81366e-01
ENSG00000187642   11.9798      0.5428105 0.5215598   1.040744 2.97994e-01
                      padj
                  <numeric>
ENSG00000279457 6.86555e-01
ENSG00000187634 5.15718e-03
ENSG00000188976 1.76553e-35
ENSG00000187961 1.13413e-07
ENSG00000187583 9.19031e-01
ENSG00000187642 4.03379e-01
```

Data viz

Volcano Plot

```
library(ggplot2)

ggplot(res) +
  aes(log2FoldChange, -log(padj)) +
  geom_point()
```

```
Warning: Removed 1237 rows containing missing values or values outside the scale range
(`geom_point()`).
```



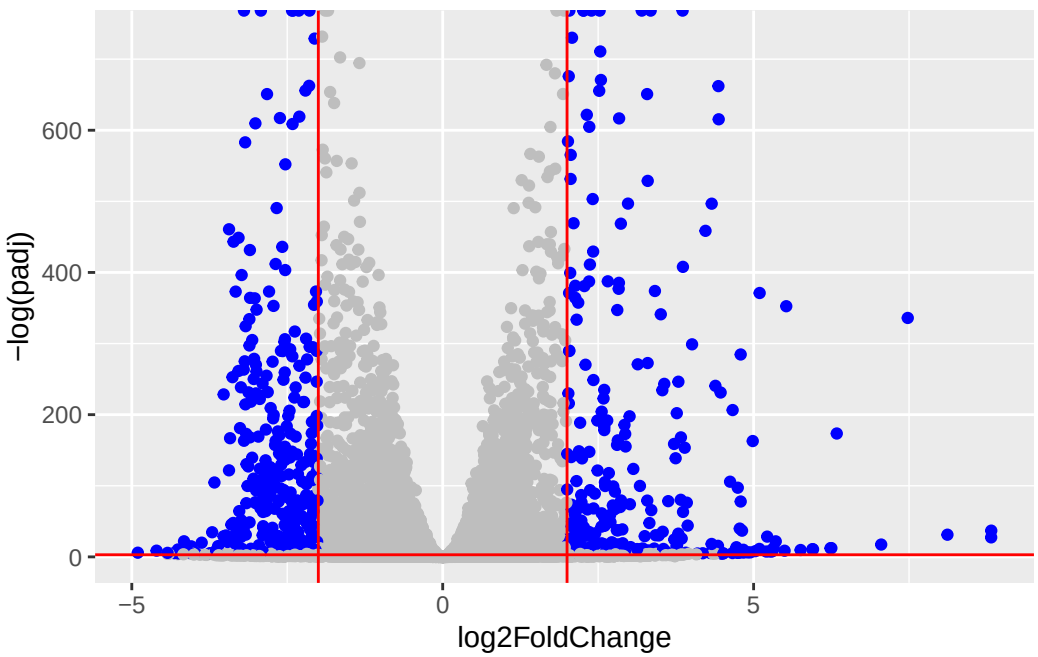
Q. Improve this plot by completing the below code, which adds color, axis labels and cutoff lines:

```
# Make a color vector for all genes
mycols <- rep("gray", nrow(res))

# Color blue the genes with fold change above 2
mycols[ abs(res$log2FoldChange) > 2 ] <- "blue"
```

```
# Color gray those with adjusted p-value more than 0.01
mycols[ res$padj > 0.01 ] <- "gray"
```

```
ggplot(res) +
  aes(log2FoldChange, -log(padj)) +
  geom_point(col=mycols) +
  geom_vline(xintercept=c(-2,+2), col="red") +
  geom_hline(yintercept = -log(0.05), col="red")
```

```
Warning: Removed 1237 rows containing missing values or values outside the scale range
(`geom_point()`).
```



Add Annotation Data

Q. Use the mapIDs() function multiple times to add SYMBOL, ENTREZID and GENENAME annotation to our results by completing the code below.

```
# Load packages
library("AnnotationDbi")
library("org.Hs.eg.db")
```

```r
columns(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"
```

```r
# Add symbol annotation
res$symbol = mapIds(org.Hs.eg.db,
                    keys=row.names(res),
                    keytype="ENSEMBL",
                    column="SYMBOL",
                    multiVals="first")
```

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

```r
# Add entrezid annotation
res$entrez = mapIds(org.Hs.eg.db,
                    keys=row.names(res),
                    keytype="ENSEMBL",
                    column="ENTREZID",
                    multiVals="first")
```

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

```r
# Add genename annotation
res$name =   mapIds(org.Hs.eg.db,
                    keys=row.names(res),
                    keytype="ENSEMBL",
                    column="GENENAME",
                    multiVals="first")
```

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

```r
head(res, 10)
```

```
log2 fold change (MLE): condition hoxa1 kd vs control sirna
Wald test p-value: condition hoxa1 kd vs control sirna
DataFrame with 10 rows and 9 columns
                   baseMean log2FoldChange     lfcSE       stat      pvalue
                  <numeric>      <numeric> <numeric>  <numeric>   <numeric>
ENSG00000279457   29.913579      0.1792571 0.3248215   0.551863 5.81042e-01
ENSG00000187634  183.229650      0.4264571 0.1402658   3.040350 2.36304e-03
ENSG00000188976 1651.188076     -0.6927205 0.0548465 -12.630156 1.43993e-36
ENSG00000187961  209.637938      0.7297556 0.1318599   5.534326 3.12428e-08
ENSG00000187583   47.255123      0.0405765 0.2718928   0.149237 8.81366e-01
ENSG00000187642   11.979750      0.5428105 0.5215598   1.040744 2.97994e-01
ENSG00000188290  108.922128      2.0570638 0.1969053  10.446970 1.51281e-25
ENSG00000187608  350.716868      0.2573837 0.1027266   2.505522 1.22271e-02
ENSG00000188157 9128.439422      0.3899088 0.0467164   8.346302 7.04333e-17
ENSG00000237330    0.158192      0.7859552 4.0804729   0.192614 8.47261e-01
                      padj      symbol      entrez                   name
                  <numeric> <character> <character>            <character>
ENSG00000279457 6.86555e-01          NA          NA                     NA
ENSG00000187634 5.15718e-03      SAMD11      148398 sterile alpha motif ..
ENSG00000188976 1.76553e-35       NOC2L       26155 NOC2 like nucleolar ..
ENSG00000187961 1.13413e-07      KLHL17      339451 kelch like family me..
ENSG00000187583 9.19031e-01     PLEKHN1       84069 pleckstrin homology ..
ENSG00000187642 4.03379e-01       PERM1       84808 PPARGC1 and ESRR ind..
ENSG00000188290 1.30538e-24        HES4       57801 hes family bHLH tran..
ENSG00000187608 2.37452e-02       ISG15        9636 ISG15 ubiquitin like..
ENSG00000188157 4.21970e-16        AGRN      375790                  agrin
ENSG00000237330          NA      RNF223      401934 ring finger protein ..
```

> Q. Finally for this section let's reorder these results by adjusted p-value and save them to a CSV file in your current project directory.

```r
# reorder these results by adjusted p-value and save them to a CSV file
res = res[order(res$pvalue), ]
write.csv(res, file = "deseq_results.csv")
```

Pathway Analysis

Now we can load the packages and setup the KEGG data-sets we need

KEGG, GO, and REACTOME

```r
# Run in your R console (i.e. not your Rmarkdown doc!)
#BiocManager::install( c("pathview", "gage", "gageData") )

# For old versions of R only (R < 3.5.0)!
#source("http://bioconductor.org/biocLite.R")
#biocLite( c("pathview", "gage", "gageData") )
```

```r
library(pathview)
```

```
##############################################################################
Pathview is an open source software package distributed under GNU General
Public License version 3 (GPLv3). Details of GPLv3 is available at
http://www.gnu.org/licenses/gpl-3.0.html. Particullary, users are required to
formally cite the original Pathview paper (not just mention it) in publications
or products. For details, do citation("pathview") within R.

The pathview downloads and uses KEGG data. Non-academic uses may require a KEGG
license agreement (details at http://www.kegg.jp/kegg/legal.html).
##############################################################################
```

```r
library(gage)
```

```r
library(gageData)

data(kegg.sets.hs)
data(sigmet.idx.hs)

# Focus on signaling and metabolic pathways only
kegg.sets.hs = kegg.sets.hs[sigmet.idx.hs]

# Examine the first 3 pathways
head(kegg.sets.hs, 3)
```

```
$`hsa00232 Caffeine metabolism`
[1] "10"   "1544" "1548" "1549" "1553" "7498" "9"

$`hsa00983 Drug metabolism - other enzymes`
```

```
 [1] "10"     "1066"   "10720"  "10941"  "151531" "1548"   "1549"   "1551"
 [9] "1553"   "1576"   "1577"   "1806"   "1807"   "1890"   "221223" "2990"
[17] "3251"   "3614"   "3615"   "3704"   "51733"  "54490"  "54575"  "54576"
[25] "54577"  "54578"  "54579"  "54600"  "54657"  "54658"  "54659"  "54963"
[33] "574537" "64816"  "7083"   "7084"   "7172"   "7363"   "7364"   "7365"
[41] "7366"   "7367"   "7371"   "7372"   "7378"   "7498"   "79799"  "83549"
[49] "8824"   "8833"   "9"      "978"

$`hsa00230 Purine metabolism`
  [1] "100"    "10201"  "10606"  "10621"  "10622"  "10623"  "107"    "10714"
  [9] "108"    "10846"  "109"    "111"    "11128"  "11164"  "112"    "113"
 [17] "114"    "115"    "122481" "122622" "124583" "132"    "158"    "159"
 [25] "1633"   "171568" "1716"   "196883" "203"    "204"    "205"    "221823"
 [33] "2272"   "22978"  "23649"  "246721" "25885"  "2618"   "26289"  "270"
 [41] "271"    "27115"  "272"    "2766"   "2977"   "2982"   "2983"   "2984"
 [49] "2986"   "2987"   "29922"  "3000"   "30833"  "30834"  "318"    "3251"
 [57] "353"    "3614"   "3615"   "3704"   "377841" "471"    "4830"   "4831"
 [65] "4832"   "4833"   "4860"   "4881"   "4882"   "4907"   "50484"  "50940"
 [73] "51082"  "51251"  "51292"  "5136"   "5137"   "5138"   "5139"   "5140"
 [81] "5141"   "5142"   "5143"   "5144"   "5145"   "5146"   "5147"   "5148"
 [89] "5149"   "5150"   "5151"   "5152"   "5153"   "5158"   "5167"   "5169"
 [97] "51728"  "5198"   "5236"   "5313"   "5315"   "53343"  "54107"  "5422"
[105] "5424"   "5425"   "5426"   "5427"   "5430"   "5431"   "5432"   "5433"
[113] "5434"   "5435"   "5436"   "5437"   "5438"   "5439"   "5440"   "5441"
[121] "5471"   "548644" "55276"  "5557"   "5558"   "55703"  "55811"  "55821"
[129] "5631"   "5634"   "56655"  "56953"  "56985"  "57804"  "58497"  "6240"
[137] "6241"   "64425"  "646625" "654364" "661"    "7498"   "8382"   "84172"
[145] "84265"  "84284"  "84618"  "8622"   "8654"   "87178"  "8833"   "9060"
[153] "9061"   "93034"  "953"    "9533"   "954"    "955"    "956"    "957"
[161] "9583"   "9615"
```

```r
foldchanges = res$log2FoldChange
names(foldchanges) = res$entrez
head(foldchanges)
```

```
     1266     54855      1465      2034      2150      6659
-2.422719  3.201955 -2.313738 -1.888019  3.344508  2.392288
```

Now, let's run the gage pathway analysis.

```r
# Get the results
keggres = gage(foldchanges, gsets=kegg.sets.hs)
```

Now lets look at the object returned from `gage()`.

```r
attributes(keggres)
```

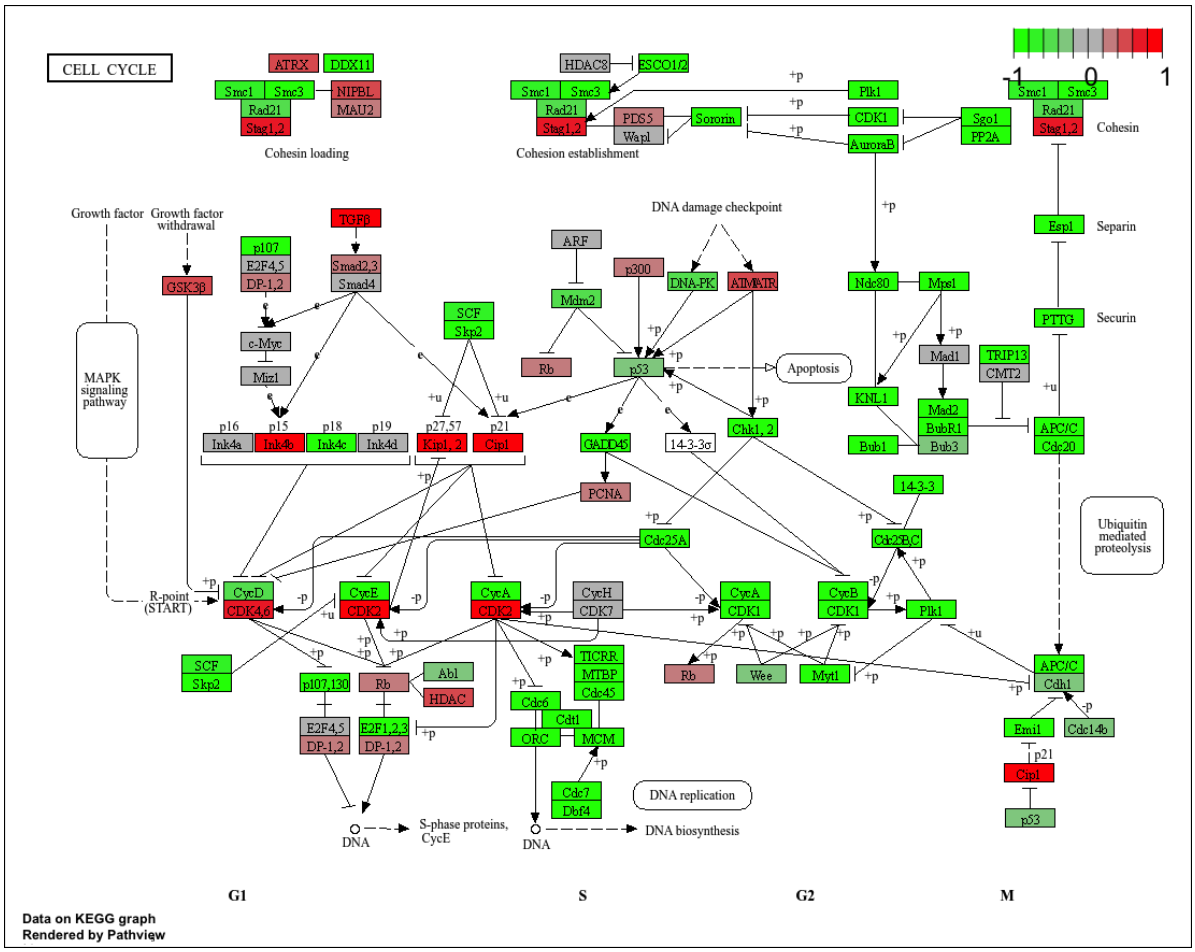
```
$names
[1] "greater" "less"    "stats"
```

```r
# Look at the first few down (less) pathways
head(keggres$less)
```

```
                                          p.geomean stat.mean        p.val
hsa04110 Cell cycle                    8.995727e-06 -4.378644 8.995727e-06
hsa03030 DNA replication               9.424076e-05 -3.951803 9.424076e-05
hsa03013 RNA transport                 1.375901e-03 -3.028500 1.375901e-03
hsa03440 Homologous recombination      3.066756e-03 -2.852899 3.066756e-03
hsa04114 Oocyte meiosis                3.784520e-03 -2.698128 3.784520e-03
hsa00010 Glycolysis / Gluconeogenesis 8.961413e-03 -2.405398 8.961413e-03
                                            q.val set.size         exp1
hsa04110 Cell cycle                    0.001448312      121 8.995727e-06
hsa03030 DNA replication               0.007586381       36 9.424076e-05
hsa03013 RNA transport                 0.073840037      144 1.375901e-03
hsa03440 Homologous recombination      0.121861535       28 3.066756e-03
hsa04114 Oocyte meiosis                0.121861535      102 3.784520e-03
hsa00010 Glycolysis / Gluconeogenesis 0.212222694       53 8.961413e-03
```

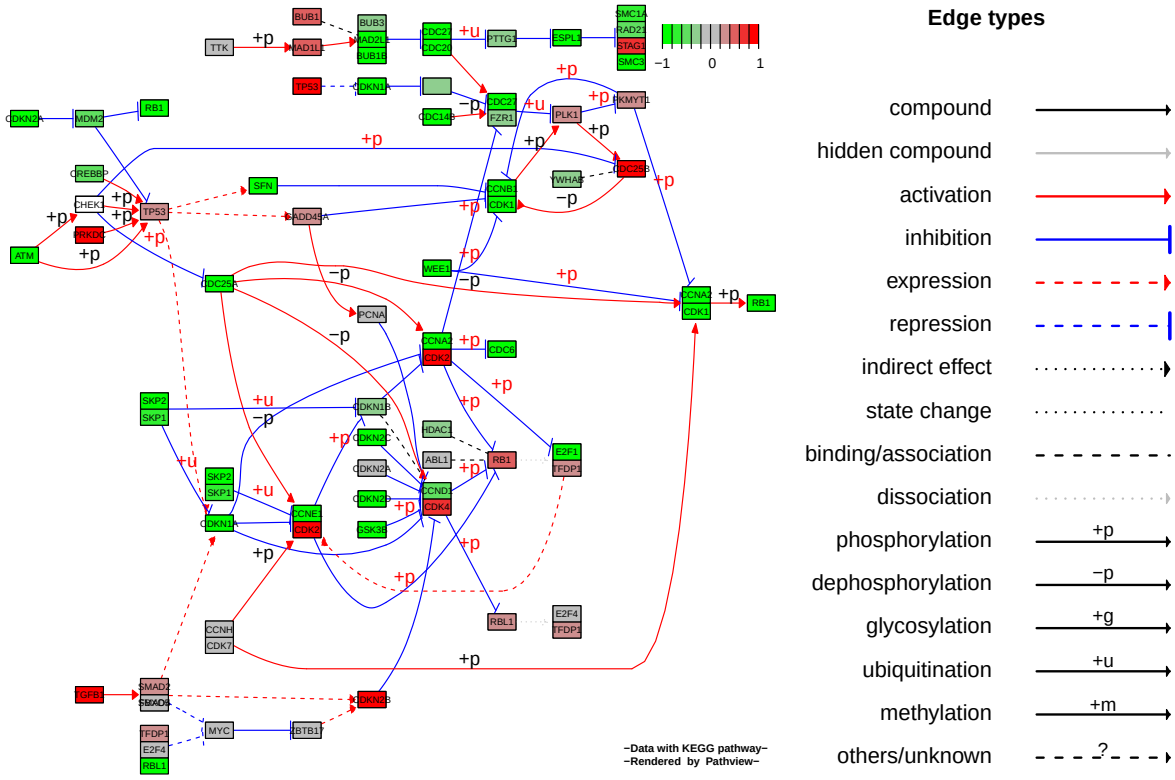
```r
pathview(gene.data=foldchanges, pathway.id="hsa04110")
```

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

Info: Working in directory /Users/christeenapano/BIMM 143 /class14

Info: Writing image file hsa04110.pathview.png
```




```
# A different PDF based output of the same data
pathview(gene.data=foldchanges, pathway.id="hsa04110", kegg.native=FALSE)
```

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

Warning: reconcile groups sharing member nodes!

     [,1] [,2]
[1,] "9"  "300"
[2,] "9"  "306"

Info: Working in directory /Users/christeenapano/BIMM 143 /class14

Info: Writing image file hsa04110.pathview.pdf
```



Now, let's process our results a bit more to automagicaly pull out the top 5 upregulated pathways, then further process that just to get the pathway IDs needed by the pathview() function. We'll use these KEGG pathway IDs for pathview plotting below.

```
## Focus on top 5 upregulated pathways here for demo purposes only
keggrespathways <- rownames(keggres$greater)[1:5]

# Extract the 8 character long IDs part of each string
keggresids = substr(keggrespathways, start=1, stop=8)
keggresids
```

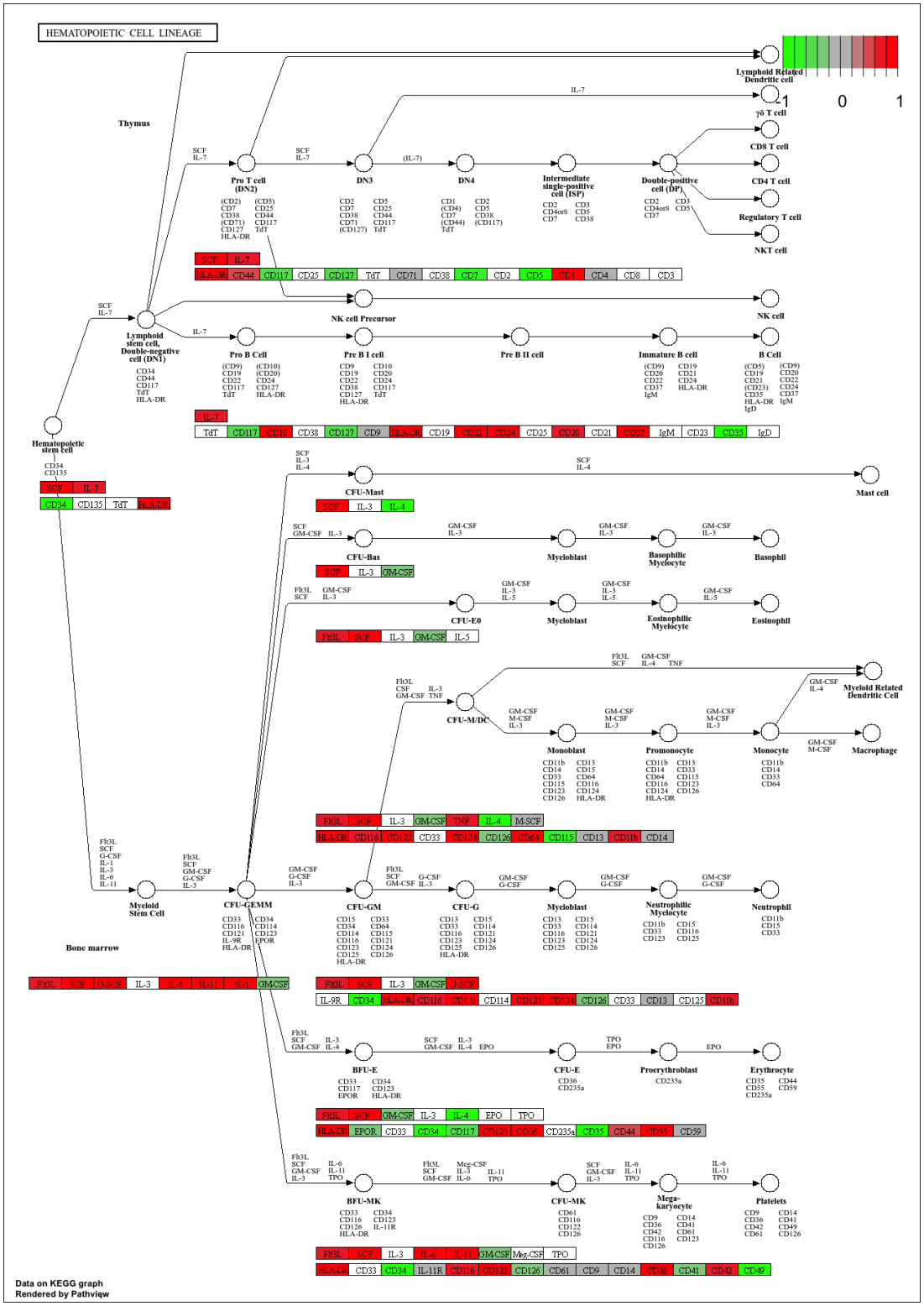
```
[1] "hsa04640" "hsa04630" "hsa00140" "hsa04142" "hsa04330"
```

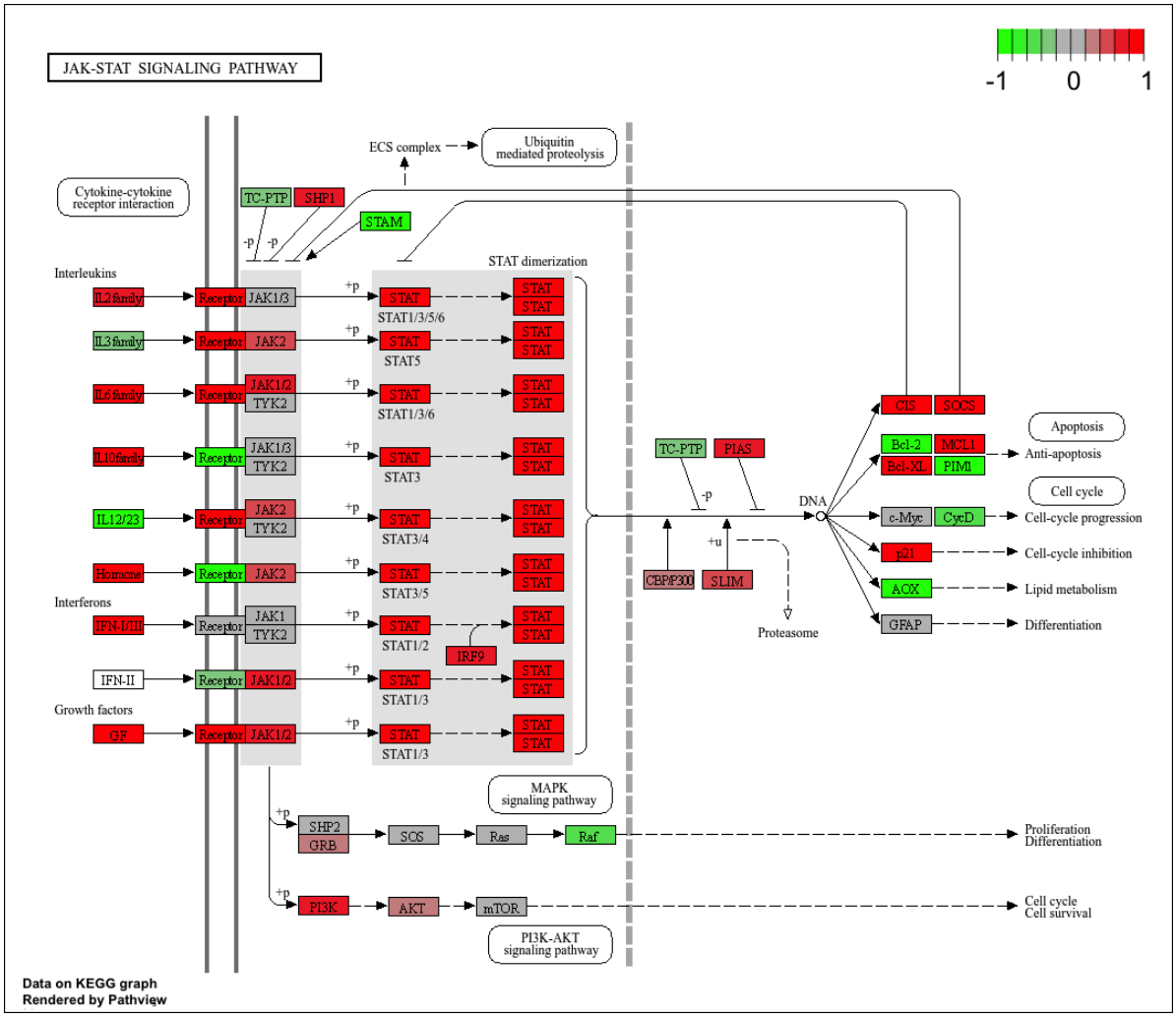
lets pass these IDs in keggresids to the pathview() function to draw plots for all the top 5 pathways.

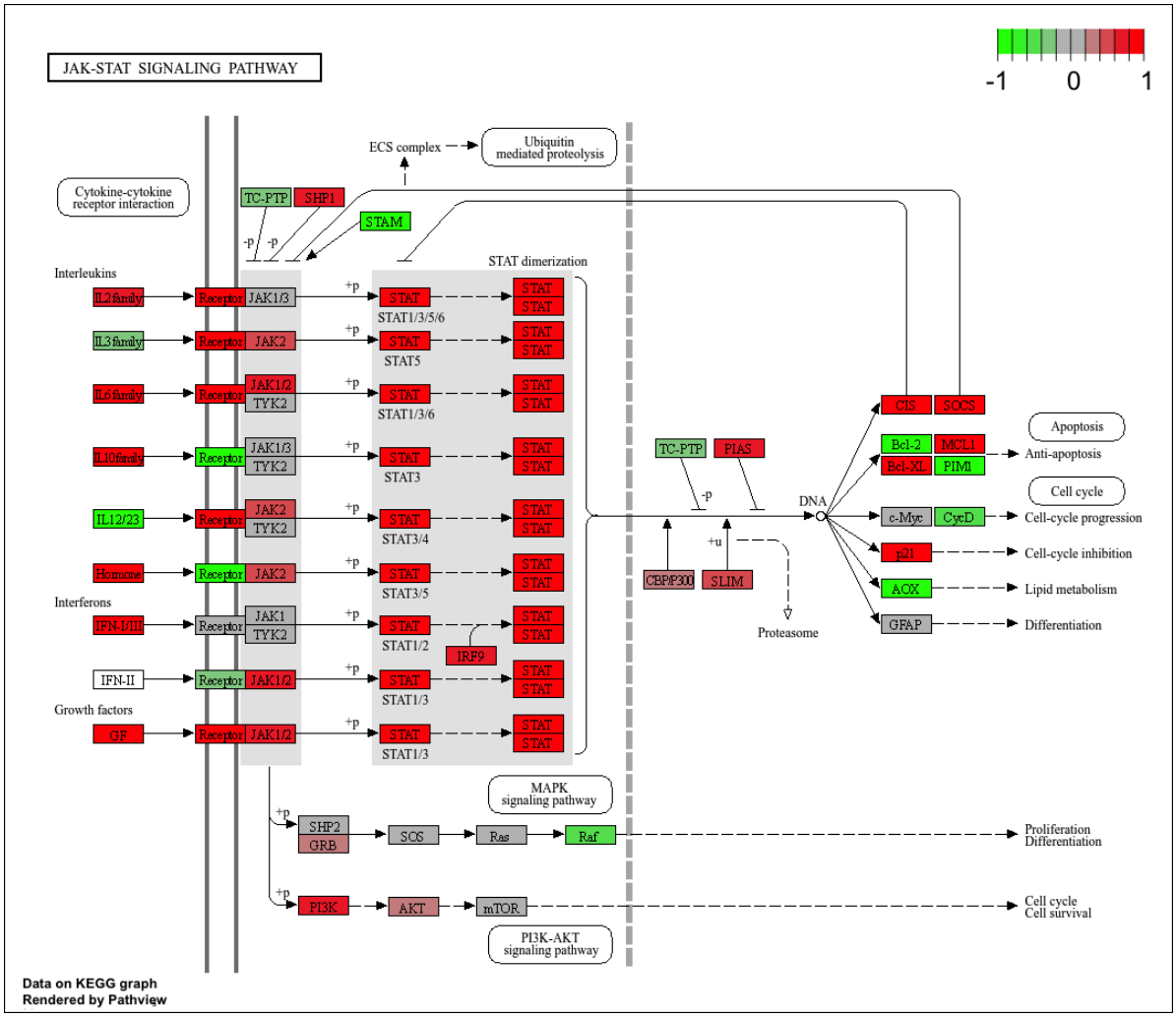
```
pathview(gene.data=foldchanges, pathway.id=keggresids, species="hsa")
```

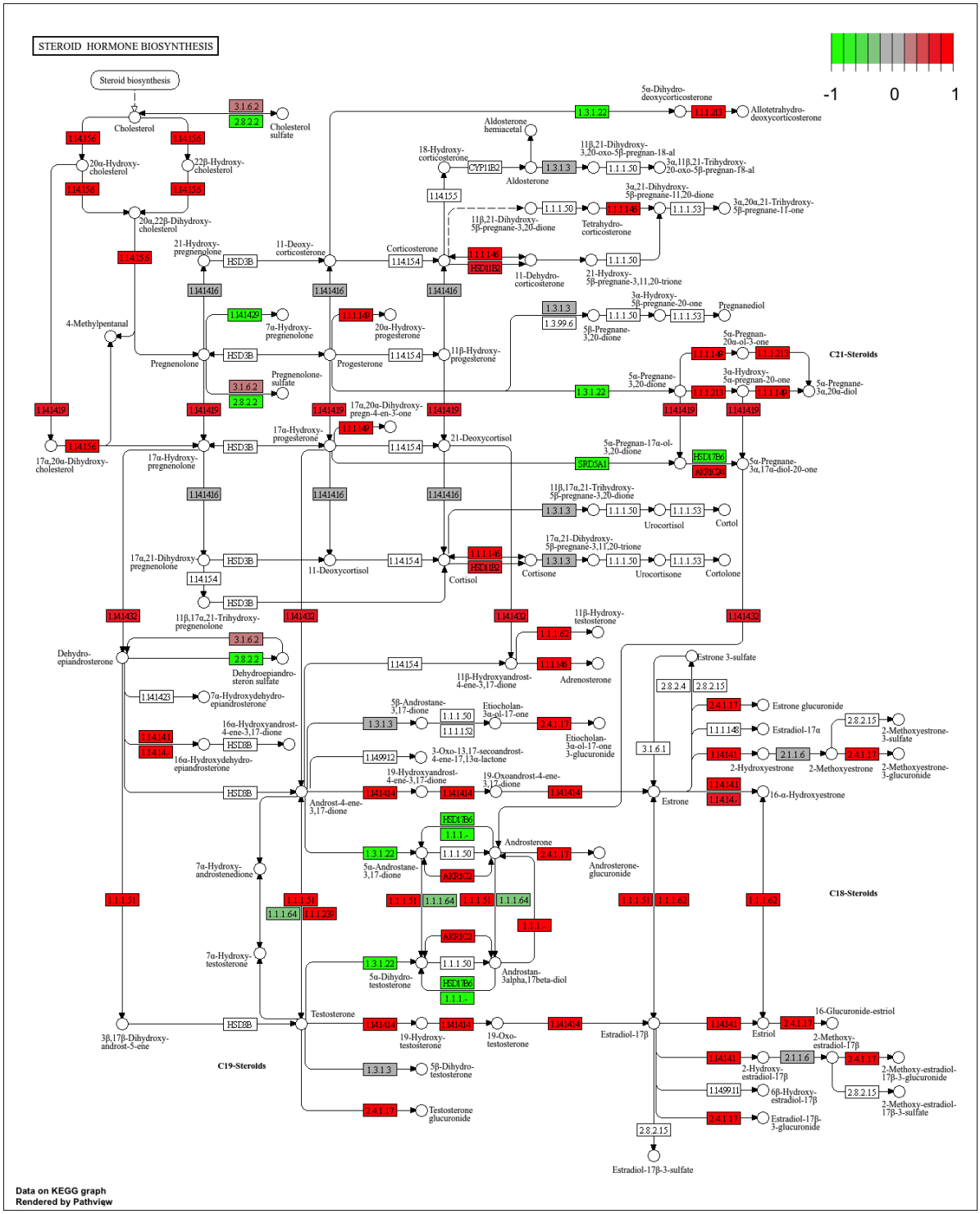
```
'select()' returned 1:1 mapping between keys and columns
```

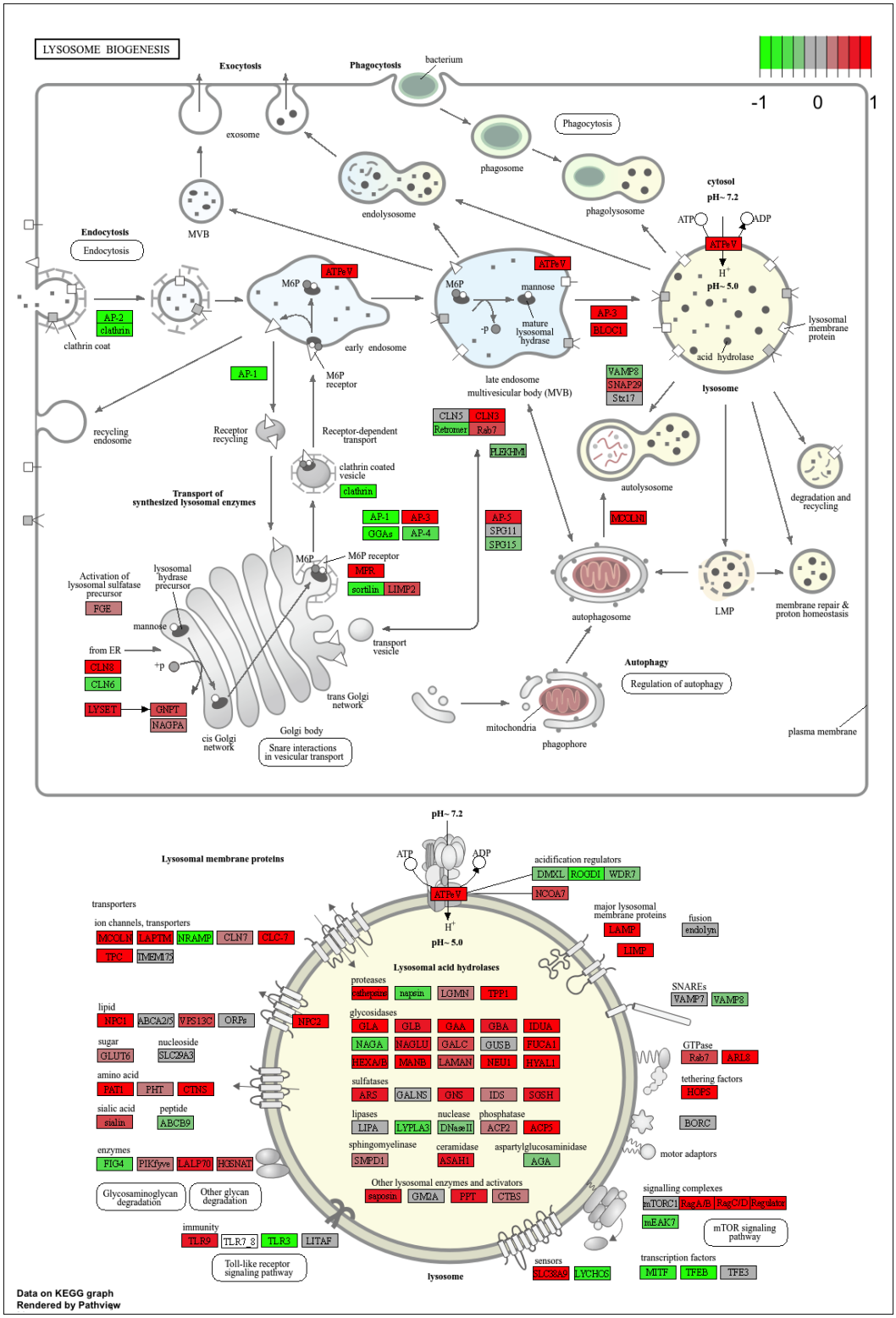
```
Info: Working in directory /Users/christeenapano/BIMM 143 /class14

Info: Writing image file hsa04640.pathview.png

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

Info: Working in directory /Users/christeenapano/BIMM 143 /class14

Info: Writing image file hsa04630.pathview.png

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

Info: Working in directory /Users/christeenapano/BIMM 143 /class14

Info: Writing image file hsa00140.pathview.png

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

Info: Working in directory /Users/christeenapano/BIMM 143 /class14

Info: Writing image file hsa04142.pathview.png

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

Info: Working in directory /Users/christeenapano/BIMM 143 /class14

Info: Writing image file hsa04330.pathview.png
```


HEMATOPOIETIC CELL LINEAGE
Lymphoid Related Dendritic cell
γδ T cell
CD8 T cell
CD4 T cell
Regulatory T cell
NKT cell
Thymus
Pro T cell (DN2)
DN3
DN4
Intermediate single-positive cell (ISP)
Double-positive cell (DP)
NK cell Precursor
NK cell
Lymphoid stem cell, Double-negative cell (DN1)
Pro B Cell
Pre B I cell
Pre B II cell
Immature B cell
B Cell
Hematopoietic stem cell
CFU-Mast
Mast cell
CFU-Bas
Myeloblast
Basophilic Myelocyte
Basophil
CFU-E0
Eosinophilic Myelocyte
Eosinophil
CFU-M/DC
Myeloid Related Dendritic Cell
Monoblast
Promonocyte
Monocyte
Macrophage
Myeloid Stem Cell
CFU-GEMM
CFU-GM
CFU-G
Neutrophilic Myelocyte
Neutrophil
Bone marrow
BFU-E
CFU-E
Proerythroblast
Erythrocyte
BFU-MK
CFU-MK
Mega-karyocyte
Platelets
Data on KEGG graph
Rendered by Pathview

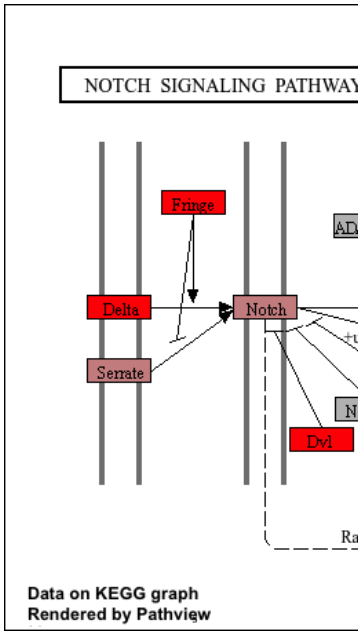

JAK-STAT SIGNALING PATHWAY
-1
0
1
ECS complex
Ubiquitin mediated proteolysis
Cytokine-cytokine receptor interaction
TC-PTP
SHP1
STAM
-p
-p
STAT dimerization
Interleukins
IL2family
IL3family
IL6family
IL10family
IL12/23
Hormone
Interferons
IFN-I/III
IFN-II
Growth factors
GF
Receptor
JAK1/3
JAK2
JAK1/2
TYK2
JAK1/3
TYK2
JAK2
TYK2
JAK2
JAK1
TYK2
JAK1/2
JAK1/2
+p
STAT
STAT1/3/5/6
STAT5
STAT1/3/6
STAT3
STAT3/4
STAT3/5
STAT1/2
STAT1/3
STAT1/3
IRF9
TC-PTP
PIAS
-p
+u
CBP/P300
SLIM
Proteasome
DNA
CIS
SOCS
Bcl-2
MCL1
Bcl-XL
PIM1
c-Myc
CycD
p21
AOX
GFAP
Apoptosis
Anti-apoptosis
Cell cycle
Cell-cycle progression
Cell-cycle inhibition
Lipid metabolism
Differentiation
MAPK signaling pathway
SHP2
GRB
SOS
Ras
Raf
Proliferation
Differentiation
PI3K
AKT
mTOR
Cell cycle
Cell survival
PI3K-AKT signaling pathway
Data on KEGG graph
Rendered by Pathview


JAK-STAT SIGNALING PATHWAY
-1
0
1
ECS complex
Ubiquitin mediated proteolysis
Cytokine-cytokine receptor interaction
TC-PTP
SHP1
STAM
-p
-p
STAT dimerization
Interleukins
IL2family
IL3family
IL6family
IL10family
IL12/23
Hormone
Interferons
IFN-I/III
IFN-II
Growth factors
GF
Receptor
JAK1/3
JAK2
JAK1/2
TYK2
JAK1/3
TYK2
JAK2
TYK2
JAK2
JAK1
TYK2
JAK1/2
JAK1/2
+p
STAT
STAT1/3/5/6
STAT5
STAT1/3/6
STAT3
STAT3/4
STAT3/5
STAT1/2
STAT1/3
STAT1/3
IRF9
TC-PTP
PIAS
-p
+u
CBP/P300
SLIM
Proteasome
DNA
CIS
SOCS
Bcl-2
MCL1
Bcl-XL
PIM1
c-Myc
CycD
p21
AOX
GFAP
Apoptosis
Anti-apoptosis
Cell cycle
Cell-cycle progression
Cell-cycle inhibition
Lipid metabolism
Differentiation
MAPK signaling pathway
SHP2
GRB
SOS
Ras
Raf
Proliferation
Differentiation
PI3K
AKT
mTOR
Cell cycle
Cell survival
PI3K-AKT signaling pathway
Data on KEGG graph
Rendered by Pathview


STEROID HORMONE BIOSYNTHESIS
-1
0
1
Steroid biosynthesis
Cholesterol
Cholesterol sulfate
20α-Hydroxy-cholesterol
22β-Hydroxy-cholesterol
20α,22β-Dihydroxy-cholesterol
21-Hydroxy-pregnenolone
11-Deoxy-corticosterone
Corticosterone
11-Dehydro-corticosterone
Aldosterone hemiacetal
Aldosterone
18-Hydroxy-corticosterone
5α-Dihydro-deoxycorticosterone
Allotetrahydro-deoxycorticosterone
11β,21-Dihydroxy-3,20-oxo-5β-pregnan-18-al
3α,11β,21-Trihydroxy-20-oxo-5β-pregnan-18-al
3α,21-Dihydroxy-5β-pregnane-11,20-dione
3α,20α,21-Trihydroxy-5β-pregnane-11-one
11β,21-Dihydroxy-5β-pregnane-3,20-dione
Tetrahydro-corticosterone
21-Hydroxy-5β-pregnane-3,11,20-trione
3α-Hydroxy-5β-pregnane-20-one
Pregnanediol
5β-Pregnane-3,20-dione
4-Methylpentanal
Pregnenolone
7α-Hydroxy-pregnenolone
20α-Hydroxy-progesterone
Progesterone
11β-Hydroxy-progesterone
Pregnenolone-sulfate
5α-Pregnan-20α-ol-3-one
5α-Pregnan-3,20-dione
3α-Hydroxy-5α-pregnan-20-one
5α-Pregnane-3α,20α-diol
C21-Sterolds
17α,20α-Dihydroxy-pregn-4-en-3-one
17α,20α-Dihydroxy-cholesterol
17α-Hydroxy-pregnenolone
17α-Hydroxy-progesterone
21-Deoxycortisol
5α-Pregnan-17α-ol-3,20-dione
5α-Pregnane-3α,17α-diol-20-one
11β,17α,21-Trihydroxy-5β-pregnane-3,20-dione
Urocortisol
Cortol
17α,21-Dihydroxy-pregnenolone
11-Deoxycortisol
Cortisol
17α,21-Dihydroxy-5β-pregnane-3,11,20-trione
Cortisone
Urocortisone
Cortolone
11β,17α,21-Trihydroxy-pregnenolone
11β-Hydroxy-testosterone
Dehydro-epiandrosterone
Dehydroepiandro-steron sulfate
11β-Hydroxyandrost-4-ene-3,17-dione
Adrenosterone
Estrone 3-sulfate
7α-Hydroxydehydro-epiandrosterone
5β-Androstane-3,17-dione
Etiocholan-3α-ol-17-one
Etiocholan-3α-ol-17-one 3-glucuronide
Estrone glucuronide
Estradiol-17α
2-Methoxyestrone-3-sulfate
16α-Hydroxyandrost-4-ene-3,17-dione
16α-Hydroxydehydro-epiandrosterone
3-Oxo-13,17-secoandrost-4-ene-17,13α-lactone
2-Hydroxyestrone
2-Methoxyestrone
2-Methoxyestrone-3-glucuronide
19-Hydroxyandrost-4-ene-3,17-dione
19-Oxoandrost-4-ene-3,17-dione
Androst-4-ene-3,17-dione
Estrone
16-α-Hydroxyestrone
Androsterone
5α-Androstane-3,17-dione
Androsterone-glucuronide
7α-Hydroxy-androstenedione
C18-Sterolds
7α-Hydroxy-testosterone
5α-Dihydro-testosterone
Androstan-3alpha,17beta-diol
Testosterone
3β,17β-Dihydroxy-androst-5-ene
19-Hydroxy-testosterone
19-Oxo-testosterone
Estradiol-17β
16-Glucuronide-estriol
Estriol
2-Methoxy-estradiol-17β
2-Hydroxy-estradiol-17β
2-Methoxy-estradiol-17β-3-glucuronide
6β-Hydroxy-estradiol-17β
2-Methoxy-estradiol-17β-3-sulfate
Estradiol-17β-3-glucuronide
C19-Sterolds
5β-Dihydro-testosterone
Testosterone glucuronide
Estradiol-17β-3-sulfate
Data on KEGG graph
Rendered by Pathview


LYSOSOME BIOGENESIS
-1
0
1
Exocytosis
Phagocytosis
bacterium
exosome
Phagocytosis
phagosome
endolysosome
phagolysosome
MVB
cytosol
pH~ 7.2
ATP
ADP
ATPeV
H+
pH~ 5.0
Endocytosis
Endocytosis
AP-2
clathrin
clathrin coat
M6P
ATPeV
early endosome
M6P
mannose
-P
mature lysosomal hydrase
late endosome
multivesicular body (MVB)
AP-3
BLOC1
lysosomal membrane protein
acid hydrolase
lysosome
AP-1
M6P receptor
VAMP8
SNAP29
Stx17
recycling endosome
Receptor recycling
Receptor-dependent transport
CLN5
CLN3
Retromer
Rab7
PLEKHM1
clathrin coated vesicle
clathrin
autolysosome
degradation and recycling
Transport of synthesized lysosomal enzymes
AP-1
AP-3
GGAs
AP-4
AP-3
SPG11
SPG15
MCOLN1
M6P
M6P receptor
MPR
sortilin
LIMP2
Activation of lysosomal sulfatase precursor
FGE
lysosomal hydrase precursor
mannose
autophagosome
LMP
membrane repair & proton homeostasis
from ER
CLN8
CLN6
+p
transport vesicle
LYSET
GNPT
NAGPA
trans Golgi network
Autophagy
Regulation of autophagy
mitochondria
phagophore
cis Golgi network
Golgi body
Snare interactions in vesicular transport
plasma membrane

pH~ 7.2
ATP
ADP
ATPeV
H+
pH~ 5.0
Lysosomal membrane proteins
acidification regulators
DMXL
ROGDI
WDR7
HCOA7
transporters
ion channels, transporters
MCOLN
LAPTM
NRAMP
CLN7
CLC-7
TPC
TMEM175
major lysosomal membrane proteins
LAMP
LIMP
fusion
endolyn
Lysosomal acid hydrolases
proteases
cathepsins
napsin
LGMN
TPP1
SNAREs
VAMP7
VAMP8
lipid
NPC1
ABCA2/5
VPS13C
ORPs
NPC2
glycosidases
GLA
GLB
GAA
GBA
IDUA
NAGA
NAGLU
GALC
GUSB
FUCA1
HEXA/B
MANB
LAMAN
NEU1
HYAL1
sugar
GLUT6
nucleoside
SLC29A3
GTPase
Rab7
ARL8
amino acid
PAT1
PHT
CTNS
sulfatases
ARS
GALNS
GNS
IDS
SGSH
tethering factors
HOPS
sialic acid
sialin
peptide
ABCB9
lipases
LIPA
LYPLA3
nuclease
DNaseII
phosphatase
ACP2
ACP5
BORC
enzymes
FIG4
PIKfyve
LALP70
HGSNAT
sphingomyelinase
SMPD1
ceramidase
ASAH1
aspartylglucosaminidase
AGA
motor adaptors
Glycosaminoglycan degradation
Other glycan degradation
Other lysosomal enzymes and activators
saposin
GM2A
PPT
CTBS
signalling complexes
mTORC1
RagA/B
RagC/D
Regulator
mEAK7
mTOR signaling pathway
immunity
TLR9
TLR7_8
TLR3
LITAF
Toll-like receptor signaling pathway
lysosome
sensors
SLC38A9
LYCHOS
transcription factors
MITF
TFEB
TFE3
Data on KEGG graph
Rendered by Pathview


NOTCH SIGNALING PATHWAY
Fringe
Delta
Notch
Serrate
Dvl
Data on KEGG graph
Rendered by Pathview

> Q. Can you do the same procedure as above to plot the pathview figures for the top 5 down-regulated pathways?

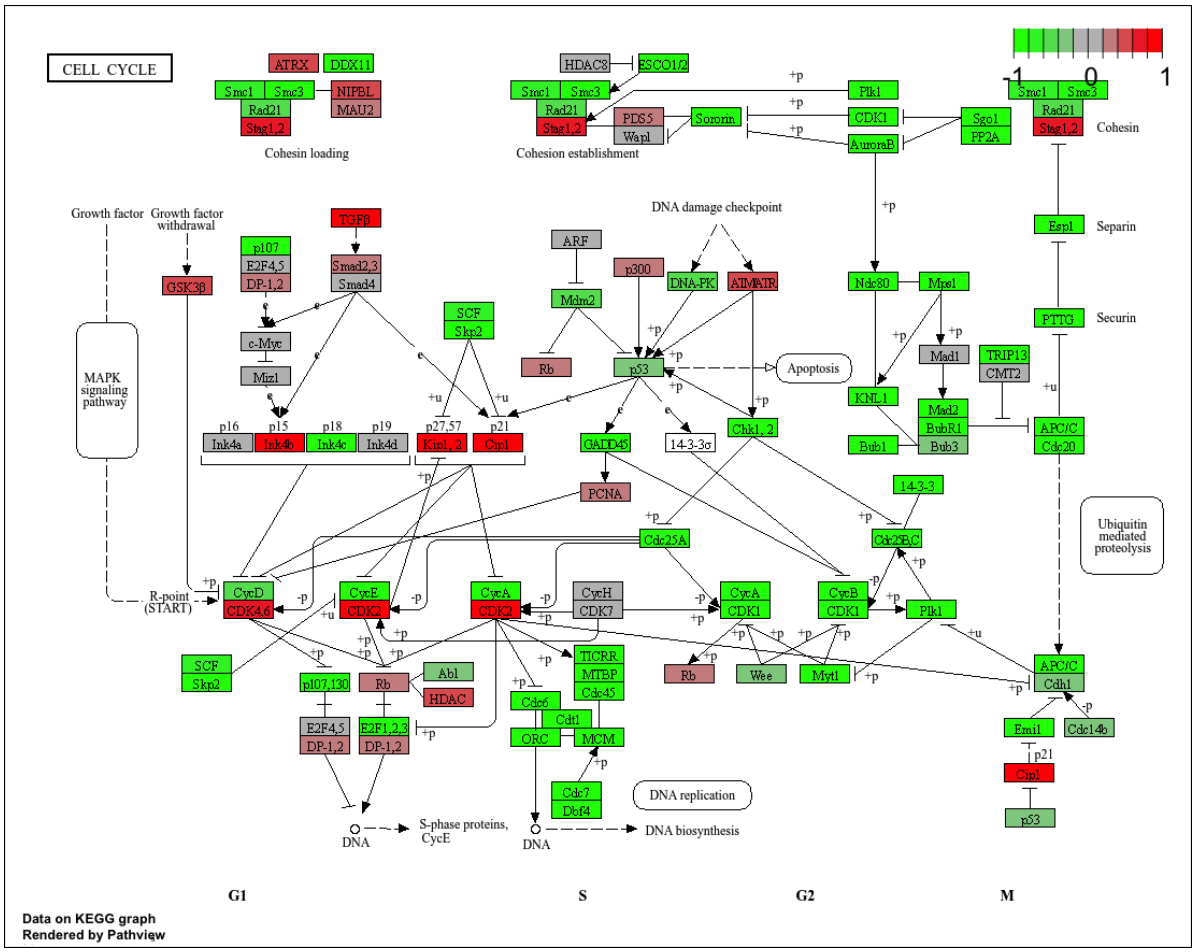
```
## Focus on top 5 drownregulated pathways here for demo purposes only
keggrespathways.down <- rownames(keggres$less)[1:5]

# Extract the 8 character long IDs part of each string
keggresids.down = substr(keggrespathways.down, start=1, stop=8)
keggresids.down
```

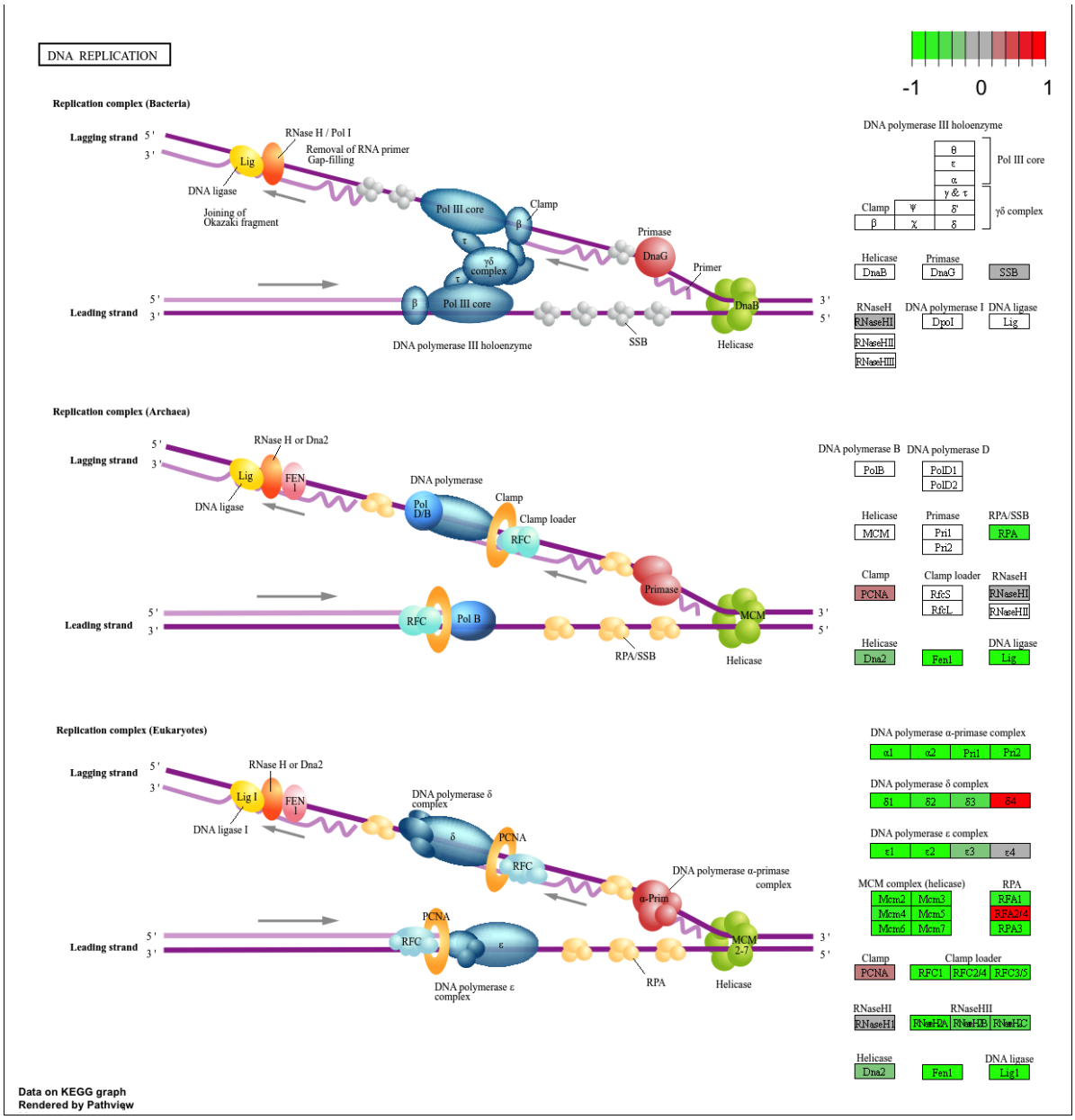
```
[1] "hsa04110" "hsa03030" "hsa03013" "hsa03440" "hsa04114"
```

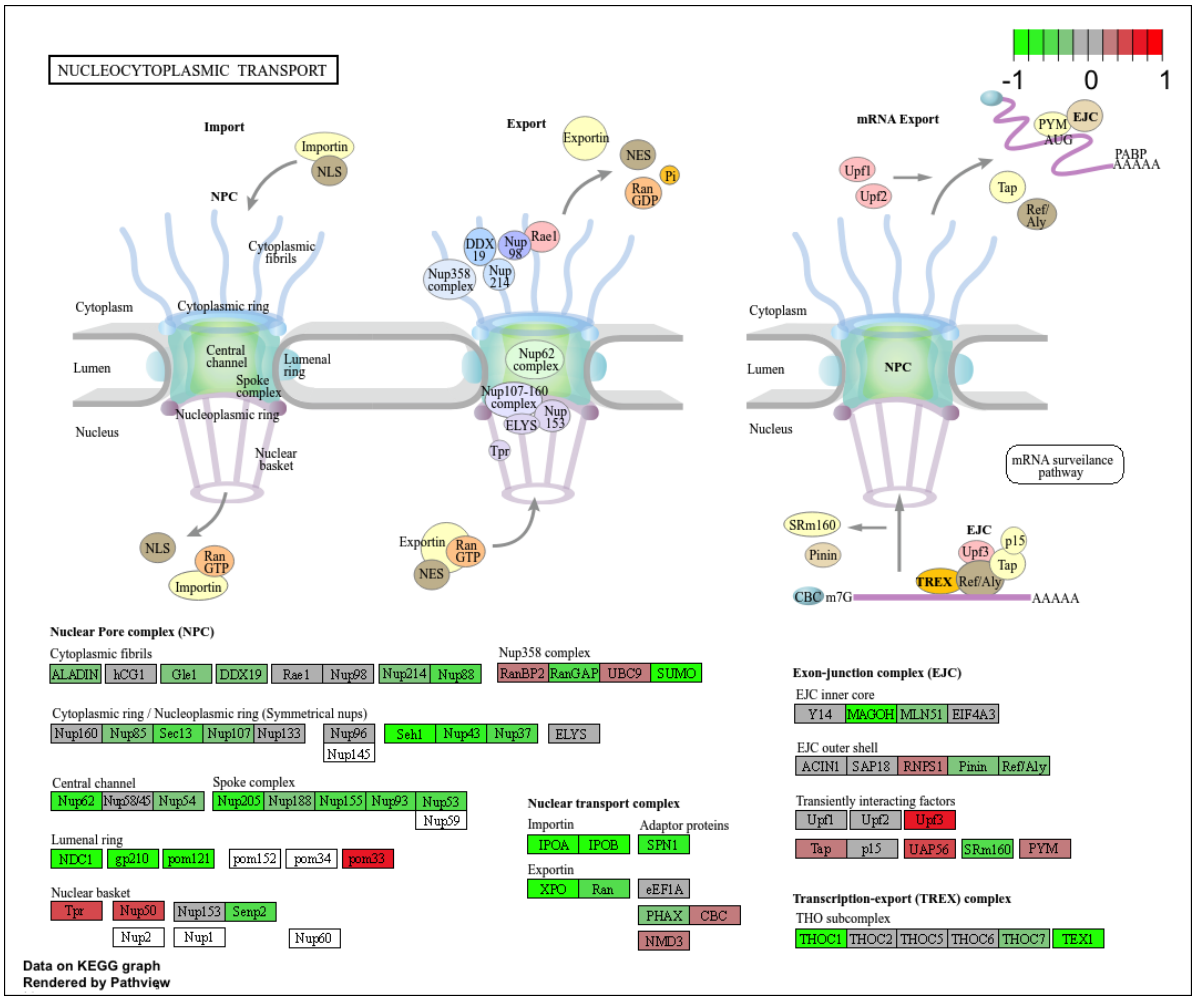
```
# Plot pathview figures for top 5 downregulated pathways
pathview(gene.data=foldchanges, pathway.id=keggresids.down, species="hsa")
```

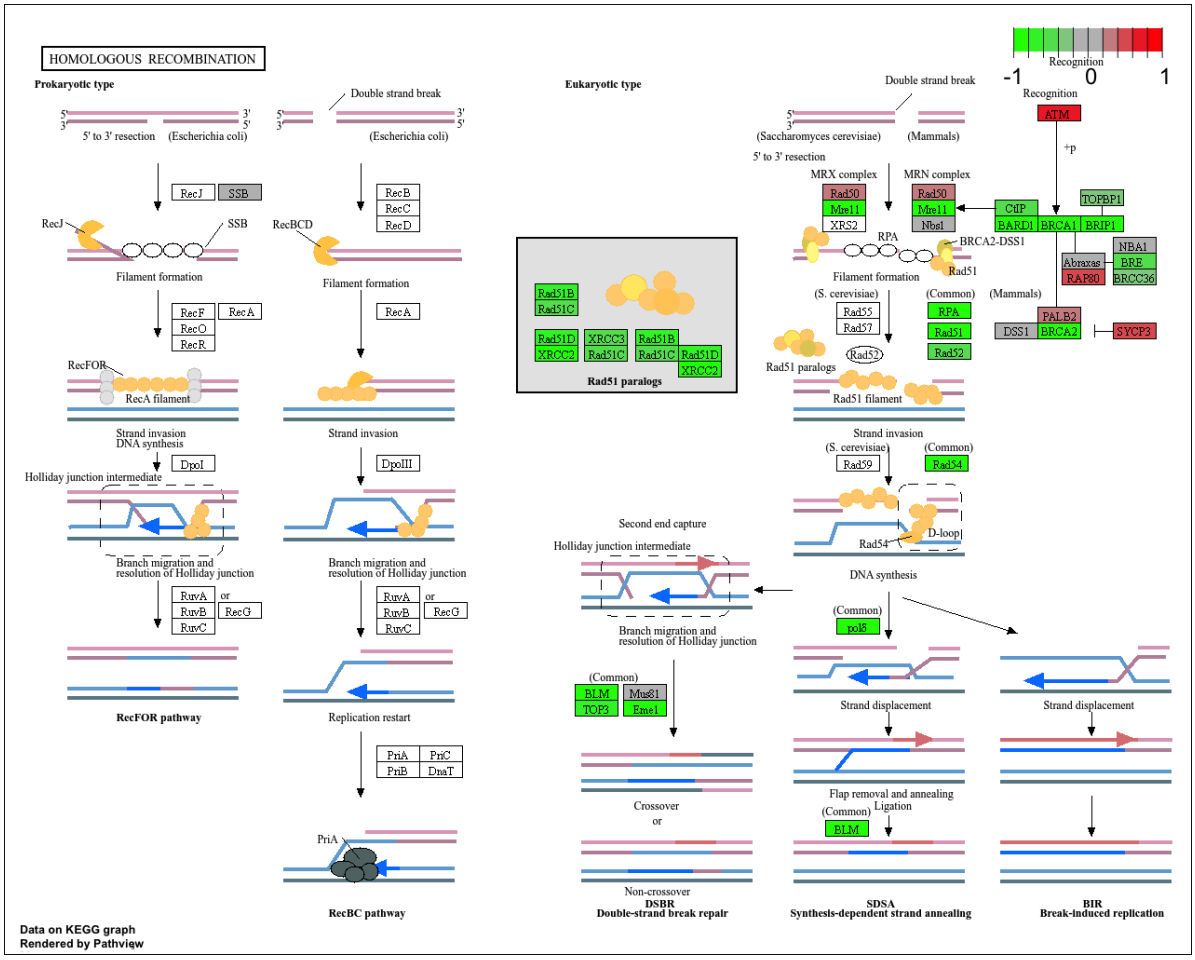
```
'select()' returned 1:1 mapping between keys and columns

Info: Working in directory /Users/christeenapano/BIMM 143 /class14

Info: Writing image file hsa04110.pathview.png

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

Info: Working in directory /Users/christeenapano/BIMM 143 /class14

Info: Writing image file hsa03030.pathview.png

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

Info: Working in directory /Users/christeenapano/BIMM 143 /class14

Info: Writing image file hsa03013.pathview.png

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

Info: Working in directory /Users/christeenapano/BIMM 143 /class14

Info: Writing image file hsa03440.pathview.png
```

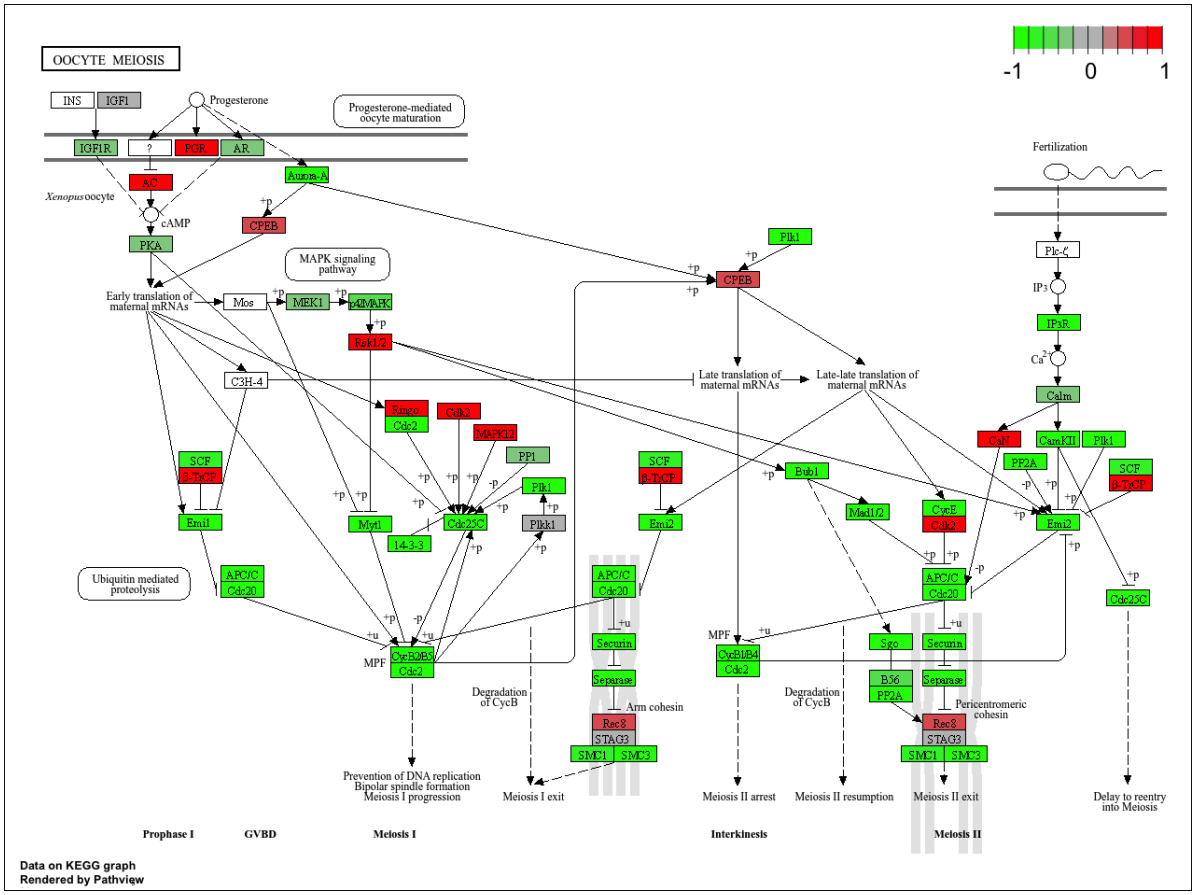
```
'select()' returned 1:1 mapping between keys and columns

Info: Working in directory /Users/christeenapano/BIMM 143 /class14

Info: Writing image file hsa04114.pathview.png
```




DNA REPLICATION
-1
0
1
Replication complex (Bacteria)
Lagging strand
5'
3'
RNase H / Pol I
Removal of RNA primer
Gap-filling
Lig
DNA ligase
Joining of
Okazaki fragment
Pol III core
Clamp
β
τ
γδ
complex
Primase
DnaG
Primer
Leading strand
DNA polymerase III holoenzyme
SSB
DnaB
Helicase
DNA polymerase III holoenzyme
θ
ε
α
γ & τ
Pol III core
Clamp
ψ
δ'
γδ complex
β
χ
δ
Helicase
DnaB
Primase
DnaG
SSB
RNaseH
RNaseHI
RNaseHII
RNaseHIII
DNA polymerase I
DpoI
DNA ligase
Lig
Replication complex (Archaea)
RNase H or Dna2
FEN
1
DNA polymerase
Pol
D/B
Clamp loader
RFC
Pol B
MCM
RPA/SSB
DNA polymerase B
PolB
DNA polymerase D
PolD1
PolD2
Helicase
MCM
Primase
Pri1
Pri2
RPA/SSB
RPA
Clamp
PCNA
Clamp loader
RfcS
RfcL
RNaseH
RNaseHI
RNaseHIII
Helicase
Dna2
Fen1
DNA ligase
Lig
Replication complex (Eukaryotes)
Lig I
DNA ligase I
DNA polymerase δ
complex
δ
PCNA
DNA polymerase α-primase
complex
α-Prim
ε
DNA polymerase ε
complex
MCM
2-7
RPA
DNA polymerase α-primase complex
α1
α2
Pri1
Pri2
DNA polymerase δ complex
δ1
δ2
δ3
δ4
DNA polymerase ε complex
ε1
ε2
ε3
ε4
MCM complex (helicase)
Mcm2
Mcm3
Mcm4
Mcm5
Mcm6
Mcm7
RPA
RFA1
RFA2/4
RFA3
Clamp
PCNA
Clamp loader
RFC1
RFC2/4
RFC3/5
RNaseHI
RNaseHI
RNaseHIII
RNaseHIA
RNaseHIB
RNaseHIC
Helicase
Dna2
Fen1
DNA ligase
Lig1
Data on KEGG graph
Rendered by Pathview


NUCLEOCYTOPLASMIC TRANSPORT
-1
0
1
Import
Importin
NLS
NPC
Cytoplasmic fibrils
Cytoplasm
Cytoplasmic ring
Central channel
Lumenal ring
Spoke complex
Lumen
Nucleoplasmic ring
Nucleus
Nuclear basket
NLS
Ran GTP
Importin
Export
Exportin
NES
Pi
Ran GDP
Rae1
DDX 19
Nup 98
Nup358 complex
Nup 214
Nup62 complex
Nup107-160 complex
ELYS
Nup 153
Tpr
Exportin
Ran GTP
NES
mRNA Export
EJC
PYM
AUG
PABP
AAAAA
Upf1
Upf2
Tap
Ref/ Aly
Cytoplasm
Lumen
NPC
Nucleus
mRNA surveilance pathway
SRm160
Pinin
EJC
p15
Upf3
Tap
TREX
Ref/Aly
CBC
m7G
AAAAA
Nuclear Pore complex (NPC)
Cytoplasmic fibrils
ALADIN
hCG1
Gle1
DDX19
Rae1
Nup98
Nup214
Nup88
Nup358 complex
RanBP2
RanGAP
UBC9
SUMO
Cytoplasmic ring / Nucleoplasmic ring (Symmetrical nups)
Nup160
Nup85
Sec13
Nup107
Nup133
Nup96
Nup145
Seh1
Nup43
Nup37
ELYS
Central channel
Nup62
Nup58/45
Nup54
Spoke complex
Nup205
Nup188
Nup155
Nup93
Nup53
Nup59
Lumenal ring
NDC1
gp210
pom121
pom152
pom34
pom33
Nuclear basket
Tpr
Nup50
Nup153
Senp2
Nup2
Nup1
Nup60
Nuclear transport complex
Importin
IPOA
IPOB
Adaptor proteins
SPN1
Exportin
XPO
Ran
eEF1A
PHAX
CBC
NMD3
Exon-junction complex (EJC)
EJC inner core
Y14
MAGOH
MLN51
EIF4A3
EJC outer shell
ACIN1
SAP18
RNPS1
Pinin
Ref/Aly
Transiently interacting factors
Upf1
Upf2
Upf3
Tap
p15
UAP56
SRm160
PYM
Transcription-export (TREX) complex
THO subcomplex
THOC1
THOC2
THOC5
THOC6
THOC7
TEX1
Data on KEGG graph
Rendered by Pathview


HOMOLOGOUS RECOMBINATION
Prokaryotic type
Eukaryotic type
Recognition
-1
0
1
Double strand break
5' to 3' resection
(Escherichia coli)
RecJ
SSB
RecBCD
RecB
RecC
RecD
Filament formation
RecF
RecO
RecR
RecA
RecFOR
RecA filament
Strand invasion
DNA synthesis
DpoI
DpoIII
Holliday junction intermediate
Branch migration and
resolution of Holliday junction
RuvA
RuvB
RuvC
or
RecG
RecFOR pathway
Replication restart
PriA
PriB
PriC
DnaT
RecBC pathway
(Saccharomyces cerevisiae)
(Mammals)
MRX complex
MRN complex
Rad50
Mre11
XRS2
Nbs1
ATM
+p
CtIP
BARD1
BRCA1
BRIP1
TOPBP1
RPA
BRCA2-DSS1
Rad51
NBA1
Abraxas
BRE
RAP80
BRCC36
(S. cerevisiae)
(Common)
Rad55
Rad57
Rad52
Rad51
PALB2
DSS1
BRCA2
SYCP3
Rad51 paralogs
Rad51 filament
Rad59
Rad54
D-loop
DNA synthesis
pol δ
Second end capture
Holliday junction intermediate
BLM
TOP3
Mus81
Eme1
Crossover
or
Non-crossover
DSBR
Double-strand break repair
Strand displacement
Flap removal and annealing
Ligation
SDSA
Synthesis-dependent strand annealing
BIR
Break-induced replication
Data on KEGG graph
Rendered by Pathview



Gene Ontology

```
data(go.sets.hs)
data(go.subs.hs)

# Focus on Biological Process subset of GO
gobpsets = go.sets.hs[go.subs.hs$BP]

gobpres = gage(foldchanges, gsets=gobpsets)

lapply(gobpres, head)
```

```
$greater
                                          p.geomean stat.mean        p.val
GO:0007156 homophilic cell adhesion       8.519724e-05  3.824205 8.519724e-05
```

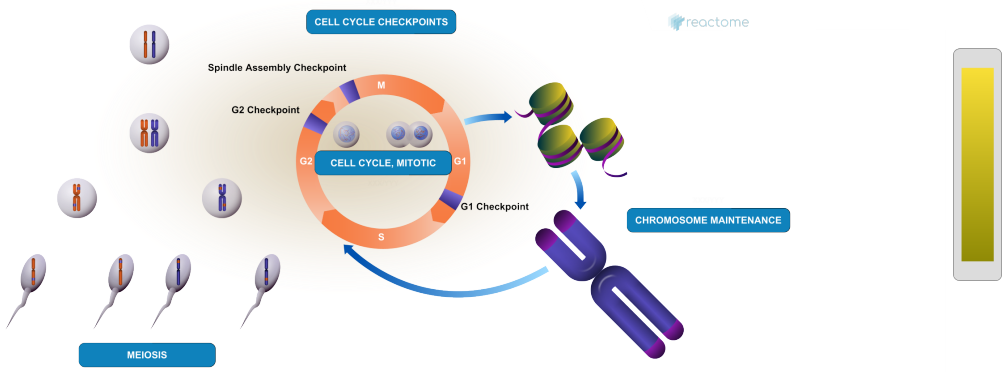
```
GO:0002009 morphogenesis of an epithelium 1.396681e-04  3.653886 1.396681e-04
GO:0048729 tissue morphogenesis           1.432451e-04  3.643242 1.432451e-04
GO:0007610 behavior                       1.925222e-04  3.565432 1.925222e-04
GO:0060562 epithelial tube morphogenesis  5.932837e-04  3.261376 5.932837e-04
GO:0035295 tube development               5.953254e-04  3.253665 5.953254e-04
                                              q.val set.size         exp1
GO:0007156 homophilic cell adhesion       0.1951953      113 8.519724e-05
GO:0002009 morphogenesis of an epithelium 0.1951953      339 1.396681e-04
GO:0048729 tissue morphogenesis           0.1951953      424 1.432451e-04
GO:0007610 behavior                       0.1967577      426 1.925222e-04
GO:0060562 epithelial tube morphogenesis  0.3565320      257 5.932837e-04
GO:0035295 tube development               0.3565320      391 5.953254e-04

$less
                                            p.geomean stat.mean        p.val
GO:0048285 organelle fission             1.536227e-15 -8.063910 1.536227e-15
GO:0000280 nuclear division              4.286961e-15 -7.939217 4.286961e-15
GO:0007067 mitosis                       4.286961e-15 -7.939217 4.286961e-15
GO:0000087 M phase of mitotic cell cycle 1.169934e-14 -7.797496 1.169934e-14
GO:0007059 chromosome segregation        2.028624e-11 -6.878340 2.028624e-11
GO:0000236 mitotic prometaphase          1.729553e-10 -6.695966 1.729553e-10
                                               q.val set.size         exp1
GO:0048285 organelle fission             5.841698e-12      376 1.536227e-15
GO:0000280 nuclear division              5.841698e-12      352 4.286961e-15
GO:0007067 mitosis                       5.841698e-12      352 4.286961e-15
GO:0000087 M phase of mitotic cell cycle 1.195672e-11      362 1.169934e-14
GO:0007059 chromosome segregation        1.658603e-08      142 2.028624e-11
GO:0000236 mitotic prometaphase          1.178402e-07       84 1.729553e-10

$stats
                                          stat.mean     exp1
GO:0007156 homophilic cell adhesion        3.824205 3.824205
GO:0002009 morphogenesis of an epithelium  3.653886 3.653886
GO:0048729 tissue morphogenesis            3.643242 3.643242
GO:0007610 behavior                        3.565432 3.565432
GO:0060562 epithelial tube morphogenesis   3.261376 3.261376
GO:0035295 tube development                3.253665 3.253665
```

Reactome

```r
sig_genes <- res[res$padj <= 0.05 & !is.na(res$padj), "symbol"]
print(paste("Total number of significant genes:", length(sig_genes)))
```

```
[1] "Total number of significant genes: 8147"
```

```r
write.table(sig_genes, file="significant_genes.txt", row.names=FALSE, col.names=FALSE, quote=
```



> Q. What pathway has the most significant "Entities p-value"? Do the most significant pathways listed match your previous KEGG results? What factors could cause differences between the two methods?

The pathway that has the most significant "Entities p-value" is the Cell Cycle. KEGG and reactome analysis show that the cell cycle is the most significantly downregulated and upregulated pathway, confirming this. However, the factors that could cause the differences between the two methods could be caused due to the differing contents of each database and their methods of generating pathways independently. These databases use different algorithims and calculate significance in different ways. This is why using different tools is important for complete and accurate analysis.

Save our results

```
write.csv(res, file="myresults_annotated.csv")
```