# Class 13 RNA-Seq Analysis Mini Project

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## Section 1. Differential Expression Analysis

```
library(DESeq2)
```

```
## Loading required package: S4Vectors
```

```
## Loading required package: stats4
```

```
## Loading required package: BiocGenerics
```

```
##
## Attaching package: 'BiocGenerics'
```

```
## The following objects are masked from 'package:stats':
##
##     IQR, mad, sd, var, xtabs
```

```
## The following objects are masked from 'package:base':
##
##     anyDuplicated, aperm, append, as.data.frame, basename, cbind,
##     colnames, dirname, do.call, duplicated, eval, evalq, Filter, Find,
##     get, grep, grepl, intersect, is.unsorted, lapply, Map, mapply,
##     match, mget, order, paste, pmax, pmax.int, pmin, pmin.int,
##     Position, rank, rbind, Reduce, rownames, sapply, setdiff, sort,
##     table, tapply, union, unique, unsplit, which.max, which.min
```

```
##
## Attaching package: 'S4Vectors'
```

```
## The following objects are masked from 'package:base':
##
##     expand.grid, I, unname
```

```
## Loading required package: IRanges
```

```
## Loading required package: GenomicRanges
```

```
## Loading required package: GenomeInfoDb
```

```
## Loading required package: SummarizedExperiment
```

```
## Loading required package: MatrixGenerics
```

```
## Loading required package: matrixStats
```

```
##
## Attaching package: 'MatrixGenerics'
## The following objects are masked from 'package:matrixStats':
##
##     colAlls, colAnyNAs, colAnys, colAvgsPerRowSet, colCollapse,
##     colCounts, colCummaxs, colCummins, colCumprods, colCumsums,
##     colDiffs, colIQRDiffs, colIQRs, colLogSumExps, colMadDiffs,
##     colMads, colMaxs, colMeans2, colMedians, colMins, colOrderStats,
##     colProds, colQuantiles, colRanges, colRanks, colSdDiffs, colSds,
##     colSums2, colTabulates, colVarDiffs, colVars, colWeightedMads,
##     colWeightedMeans, colWeightedMedians, colWeightedSds,
##     colWeightedVars, rowAlls, rowAnyNAs, rowAnys, rowAvgsPerColSet,
##     rowCollapse, rowCounts, rowCummaxs, rowCummins, rowCumprods,
##     rowCumsums, rowDiffs, rowIQRDiffs, rowIQRs, rowLogSumExps,
##     rowMadDiffs, rowMads, rowMaxs, rowMeans2, rowMedians, rowMins,
##     rowOrderStats, rowProds, rowQuantiles, rowRanges, rowRanks,
##     rowSdDiffs, rowSds, rowSums2, rowTabulates, rowVarDiffs, rowVars,
##     rowWeightedMads, rowWeightedMeans, rowWeightedMedians,
##     rowWeightedSds, rowWeightedVars
```

```
## Loading required package: Biobase
```

```
## Welcome to Bioconductor
##
##     Vignettes contain introductory material; view with
##     'browseVignettes()'. To cite Bioconductor, see
##     'citation("Biobase")', and for packages 'citation("pkgname")'.
```

```
##
## Attaching package: 'Biobase'
```

```
## The following object is masked from 'package:MatrixGenerics':
##
##     rowMedians
```

```
## The following objects are masked from 'package:matrixStats':
##
##     anyMissing, rowMedians
```

Importing the metadata:

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

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
```

Importing the countdata:

```
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
```

**Q1**. Complete the code below to remove the troublesome first column from `countData`

```
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
```

**Q2**. 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).

```
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
```

## Running DESeq2

Setting up DESeq:

```
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
```

```
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
```

Getting results for the HoxA1 Knockdown vs Control siRNA:

```r
res = results(dds, contrast=c("condition", "hoxa1_kd", "control_sirna"))
```

```r
res
```

```
## log2 fold change (MLE): condition hoxa1_kd vs control_sirna
## Wald test p-value: condition hoxa1 kd vs control sirna
## DataFrame with 15975 rows and 6 columns
##                  baseMean log2FoldChange     lfcSE       stat      pvalue
##                 <numeric>      <numeric> <numeric>  <numeric>   <numeric>
## ENSG00000279457   29.9136      0.1792571 0.3248216   0.551863 5.81042e-01
## ENSG00000187634  183.2296      0.4264571 0.1402658   3.040350 2.36304e-03
## ENSG00000188976 1651.1881     -0.6927205 0.0548465 -12.630158 1.43990e-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
## ...                   ...            ...       ...        ...         ...
## ENSG00000273748  35.30265       0.674387  0.303666   2.220817 2.63633e-02
## ENSG00000278817   2.42302      -0.388988  1.130394  -0.344117 7.30758e-01
## ENSG00000278384   1.10180       0.332991  1.660261   0.200565 8.41039e-01
## ENSG00000276345  73.64496      -0.356181  0.207716  -1.714752 8.63908e-02
## ENSG00000271254 181.59590      -0.609667  0.141320  -4.314071 1.60276e-05
##                        padj
##                   <numeric>
## ENSG00000279457 6.86555e-01
## ENSG00000187634 5.15718e-03
## ENSG00000188976 1.76549e-35
## ENSG00000187961 1.13413e-07
## ENSG00000187583 9.19031e-01
## ...                     ...
## ENSG00000273748 4.79091e-02
## ENSG00000278817 8.09772e-01
## ENSG00000278384 8.92654e-01
## ENSG00000276345 1.39762e-01
## ENSG00000271254 4.53648e-05
```

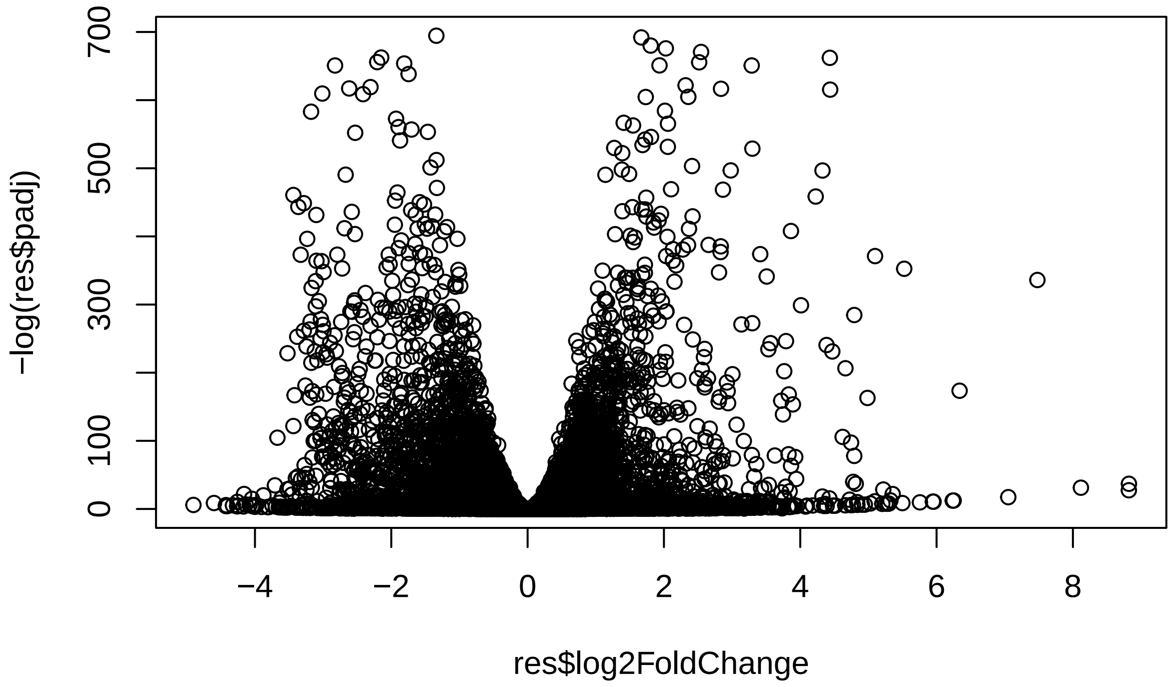
**Q3**. Call the **summary()** function on your results to get a sense of how many genes are up or down-regulated at the default 0.1 p-value cutoff.

```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
```

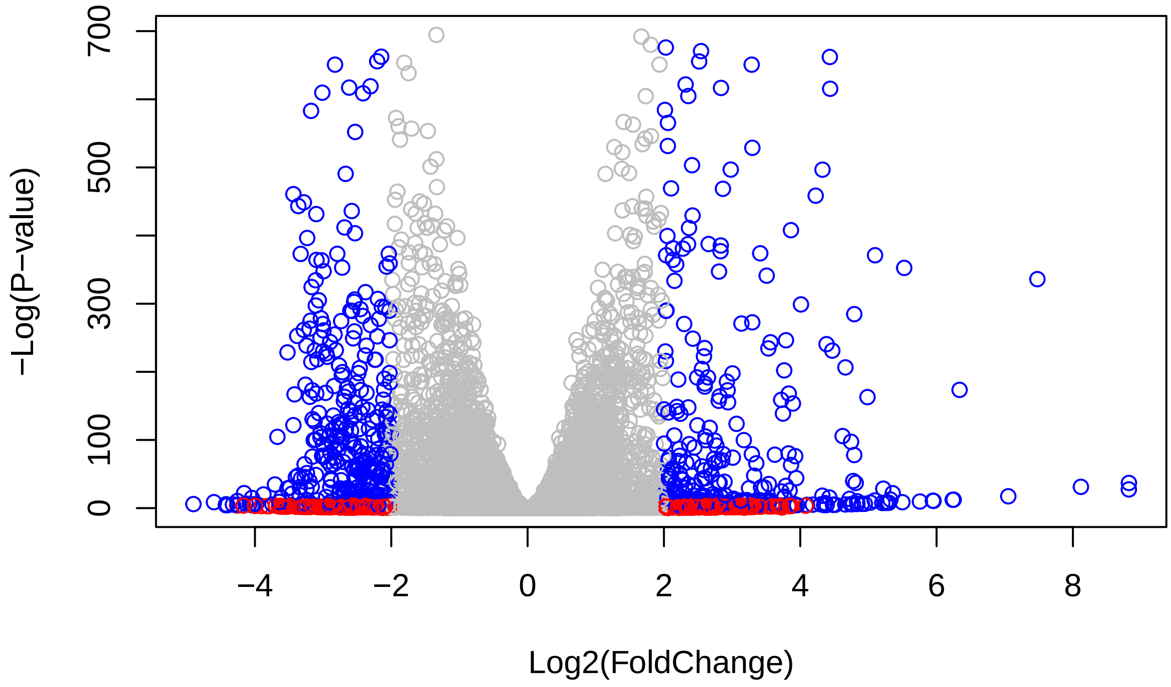
### Volcano Plot

```
plot( res$log2FoldChange, -log(res$padj) )
```



**Q4**. Improve this plot by completing the below code, which adds color and axis labels

```
mycols <- rep("gray", nrow(res) )
mycols[ abs(res$log2FoldChange) > 2 ] <- "red"
inds <- (res$pvalue < 0.01) & (abs(res$log2FoldChange) > 2 )
mycols[ inds ] <- "blue"
plot( res$log2FoldChange, -log(res$padj), col=mycols, xlab="Log2(FoldChange)", ylab="-Log(P-value)" )
```



## Adding Gene Annotation

**Q5**. Use the **mapIDs()** function multiple times to add SYMBOL, ENTREZID and GENENAME annotation to our results by completing the code below.

```
library("AnnotationDbi")
library("org.Hs.eg.db")
```

```
##
```

```
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"
```

```
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
```

```
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
```

```
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
```

```
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 0.3248216   0.551863 5.81042e-01
## ENSG00000187634  183.229650      0.4264571 0.1402658   3.040350 2.36304e-03
## ENSG00000188976 1651.188076     -0.6927205 0.0548465 -12.630158 1.43990e-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 1.51282e-25
## ENSG00000187608  350.716868      0.2573837 0.1027266   2.505522 1.22271e-02
## ENSG00000188157 9128.439422      0.3899088 0.0467163   8.346304 7.04321e-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 1.76549e-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 4.21963e-16        AGRN      375790                  agrin
## ENSG00000237330          NA      RNF223      401934 ring finger protein ..
```

**Q6**. 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.

```
res = res[order(res$pvalue),]
write.csv(res,file="deseq_results.csv")
```

## Section 2. Pathway Analysis

Installing packages:

```r
#BiocManager::install( 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)
kegg.sets.hs = kegg.sets.hs[sigmet.idx.hs]
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"
```

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

```
##      1266     54855      1465     51232      2034      2317 
## -2.422719  3.201955 -2.313738 -2.059631 -1.888019 -1.649792
```

Running **gage** pathway analysis:

```
keggres = gage(foldchanges, gsets=kegg.sets.hs)
```

```
attributes(keggres)
```

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

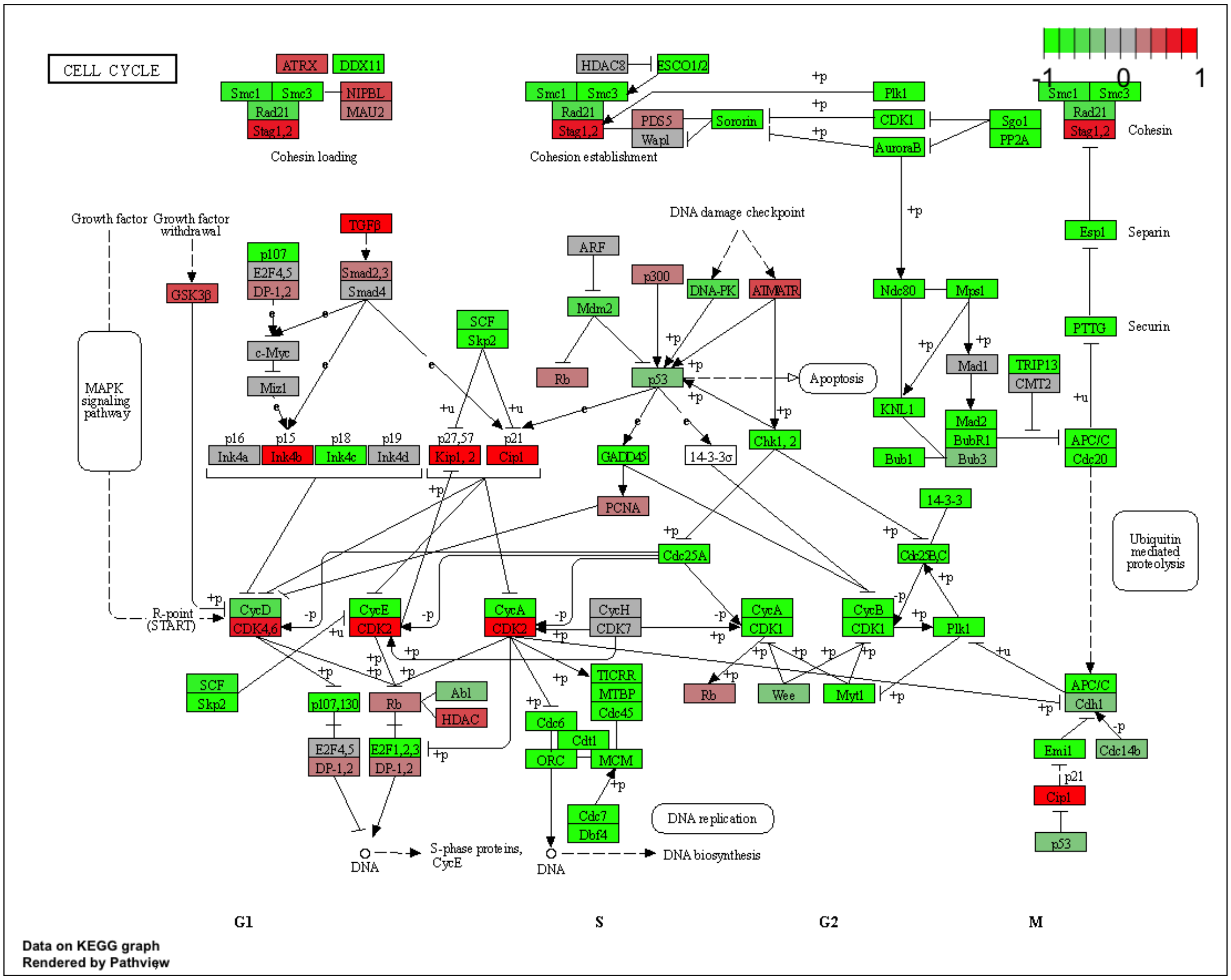
```
head(keggres$less)
```

```
##                                          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
```

Trying out `pathview()`:

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

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

## Info: Working in directory /Users/jessicadiaz-vigil/Desktop/BIMM 143/class13

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



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

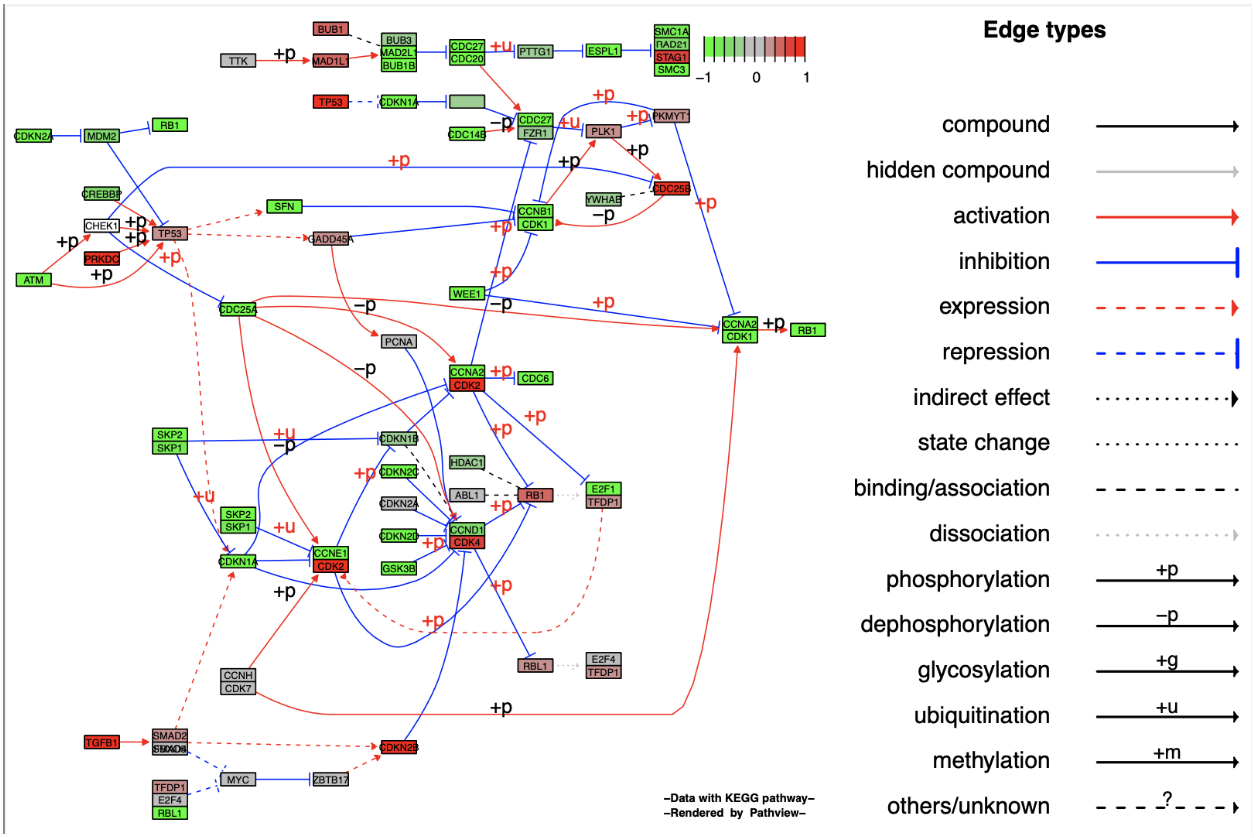
```
## 'select()' returned 1:1 mapping between keys and columns
```

```
## Warning: reconcile groups sharing member nodes!
```

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

```
## Info: Working in directory /Users/jessicadiaz-vigil/Desktop/BIMM 143/class13
```

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

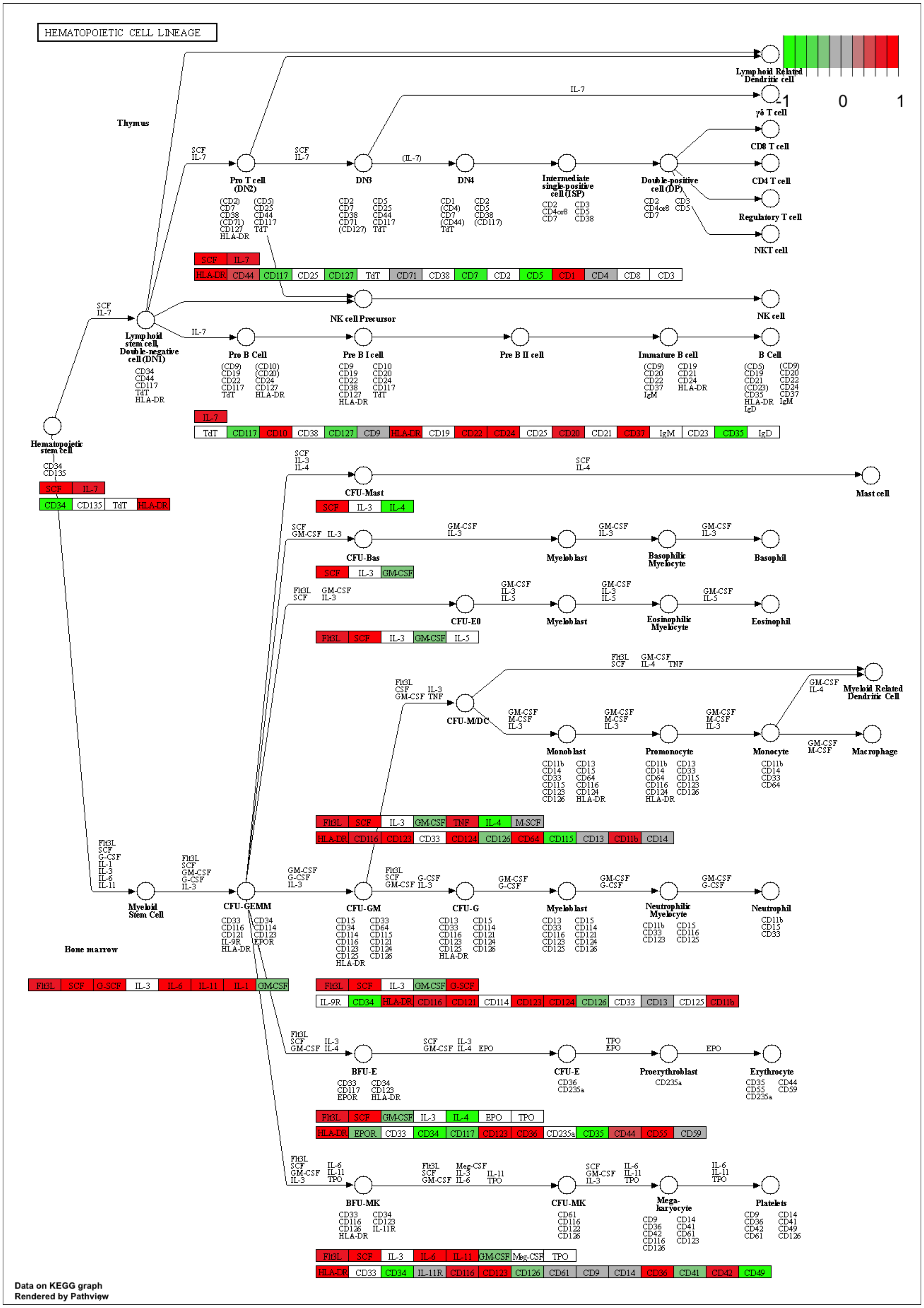


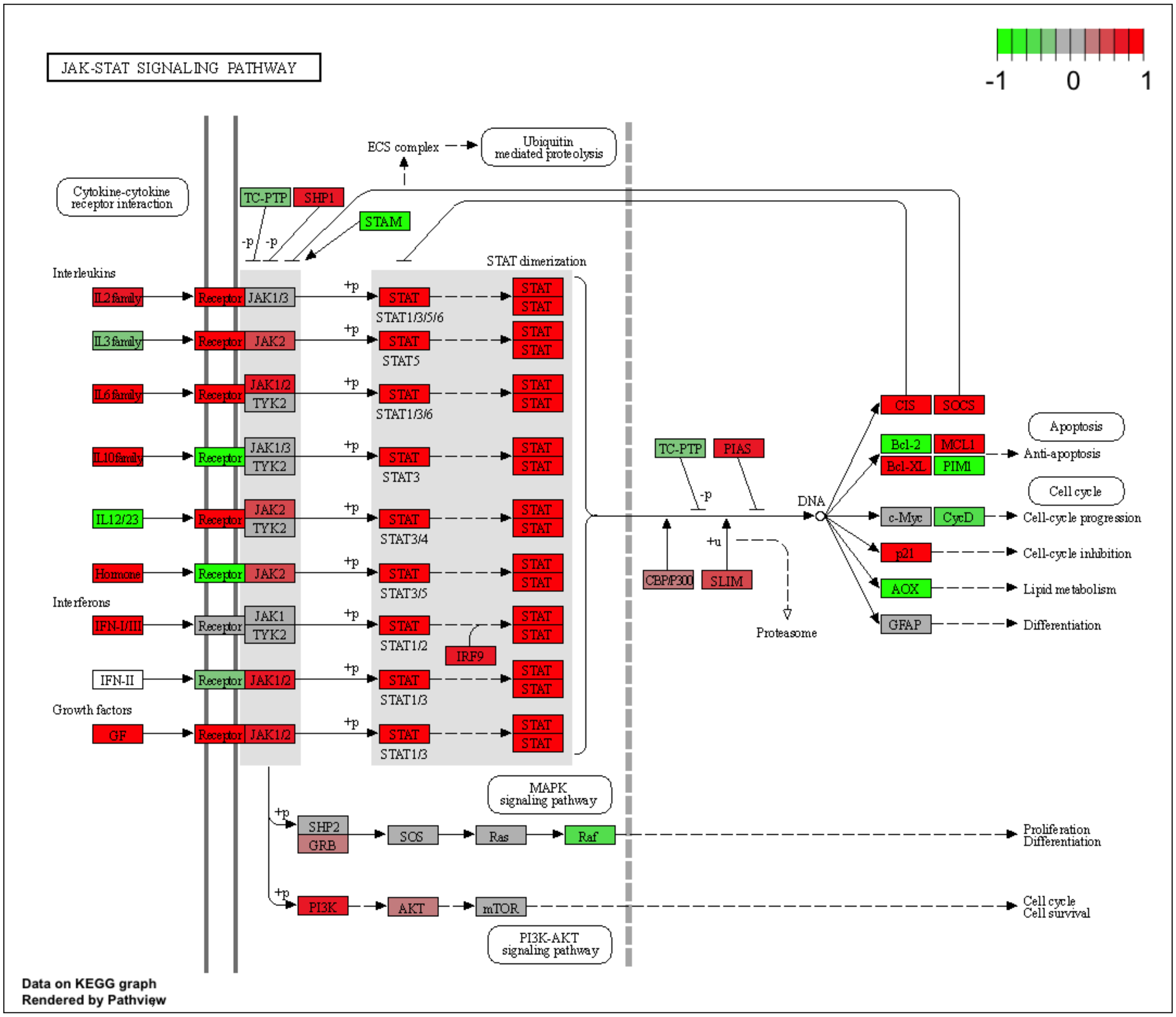
```
keggrespathways <- rownames(keggres$greater)[1:5]
keggresids = substr(keggrespathways, start=1, stop=8)
keggresids
```

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

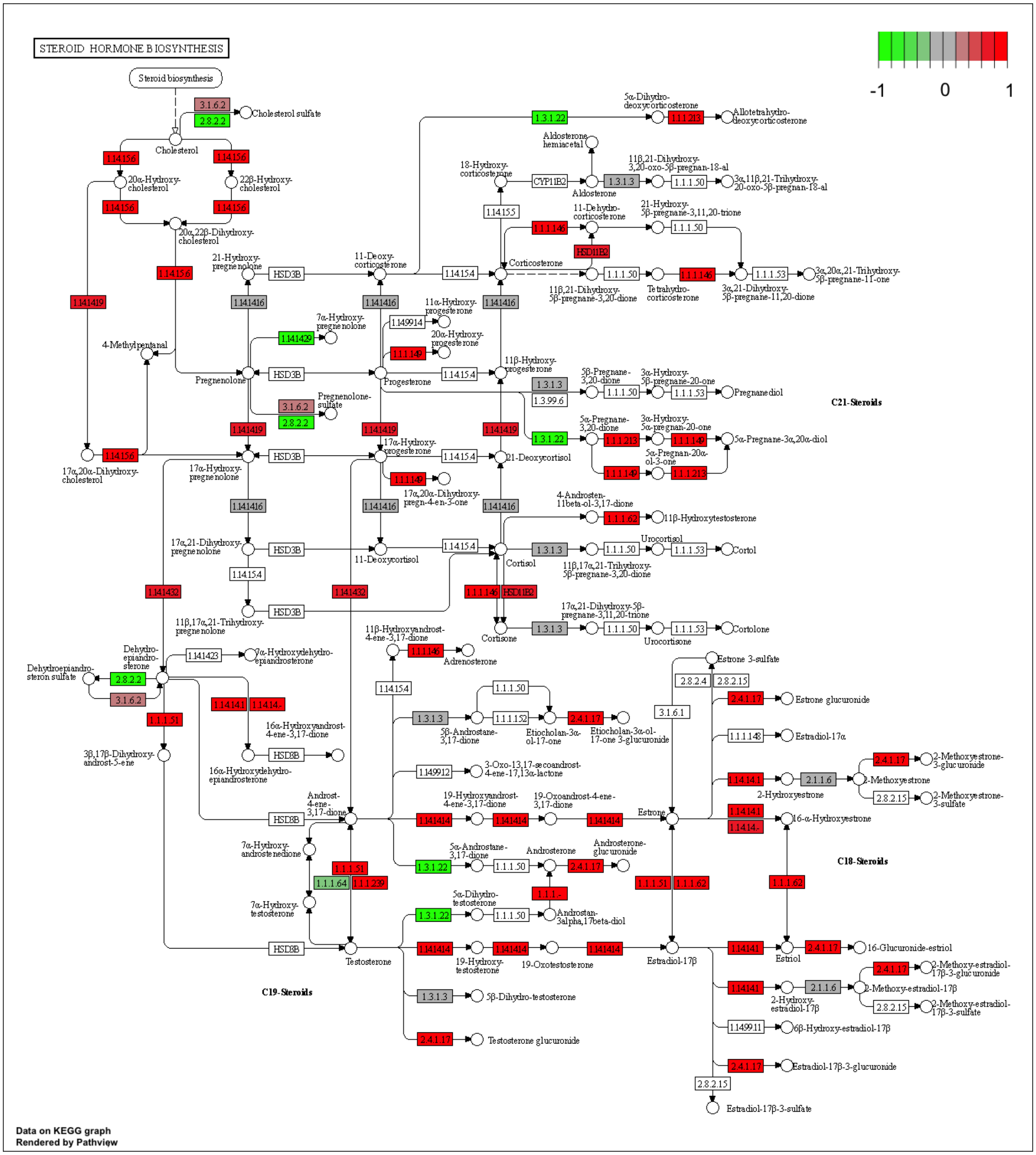
```
pathview(gene.data=foldchanges, pathway.id=keggresids, species="hsa")
```

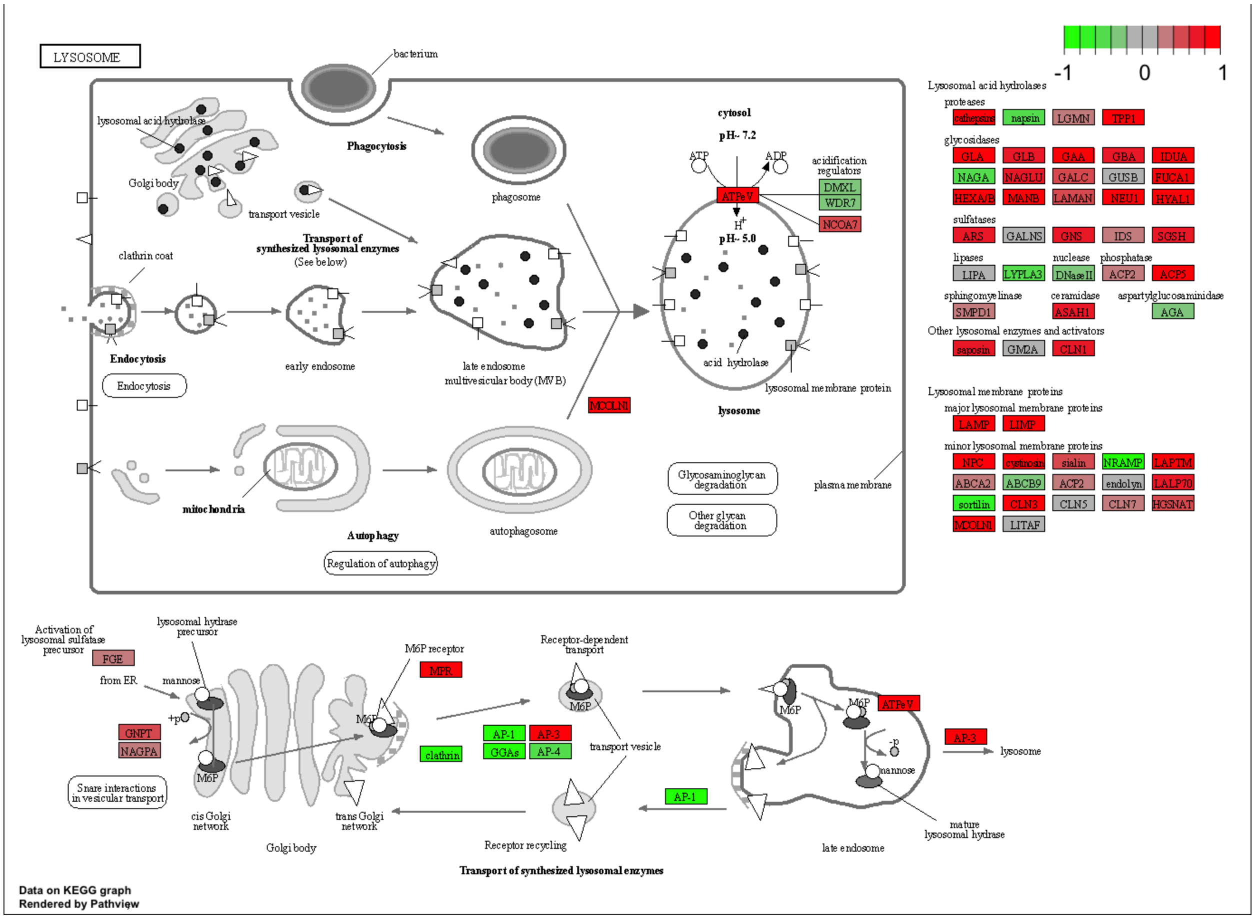
```
## 'select()' returned 1:1 mapping between keys and columns

## Info: Working in directory /Users/jessicadiaz-vigil/Desktop/BIMM 143/class13

## Info: Writing image file hsa04640.pathview.png

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

## Info: Working in directory /Users/jessicadiaz-vigil/Desktop/BIMM 143/class13

## Info: Writing image file hsa04630.pathview.png

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

## Info: Working in directory /Users/jessicadiaz-vigil/Desktop/BIMM 143/class13

## Info: Writing image file hsa00140.pathview.png

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

## Info: Working in directory /Users/jessicadiaz-vigil/Desktop/BIMM 143/class13

## Info: Writing image file hsa04142.pathview.png

## Info: some node width is different from others, and hence adjusted!

## 'select()' returned 1:1 mapping between keys and columns
## Info: Working in directory /Users/jessicadiaz-vigil/Desktop/BIMM 143/class13

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

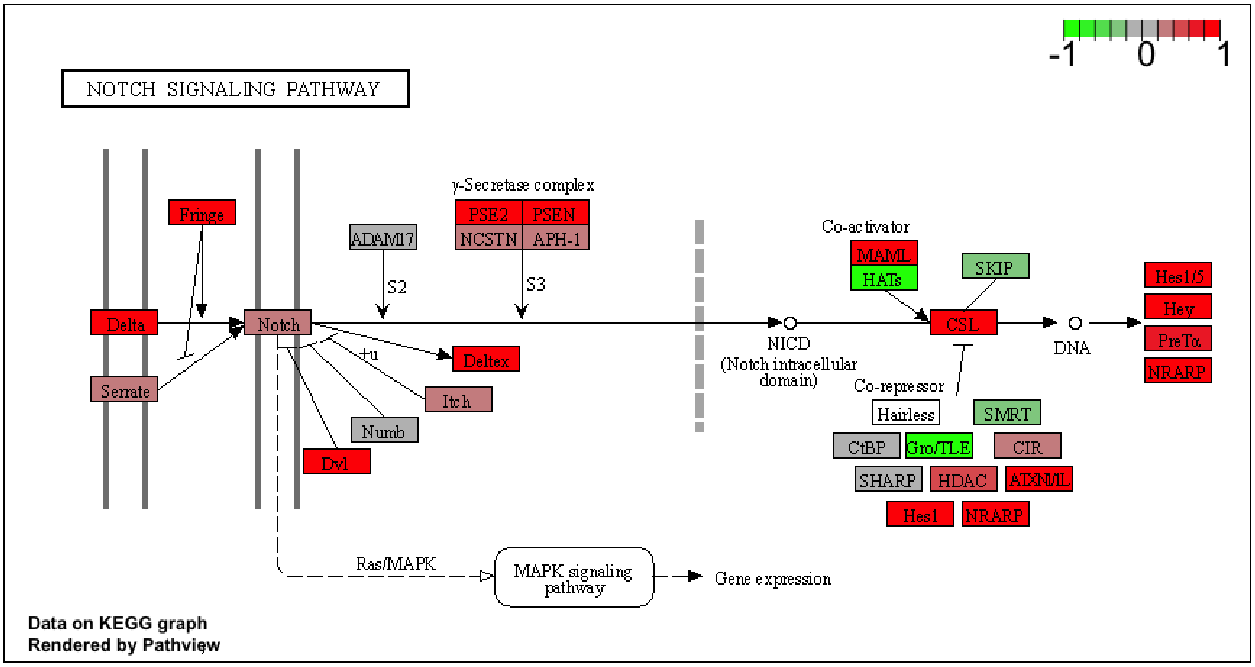

HEMATOPOIETIC CELL LINEAGE
-1
0
1
Thymus
Lymphoid Related Dendritic cell
IL-7
γδ T cell
SCF IL-7
Pro T cell (DN2)
DN3
(IL-7)
DN4
Intermediate single-positive cell (ISP)
Double-positive cell (DP)
CD8 T cell
CD4 T cell
Regulatory T cell
NKT cell
(CD2) CD7 CD38 (CD71) CD127 HLA-DR
(CD5) CD25 CD44 CD117 TdT
CD2 CD7 CD38 CD71 (CD127)
CD5 CD25 CD44 CD117 TdT
CD1 (CD4) CD7 (CD44) TdT
CD2 CD5 CD38 (CD117)
CD2 CD4or8 CD7
CD3 CD5 CD38
CD2 CD4or8 CD7
CD3 CD5
SCF
IL-7
HLA-DR
CD44
CD117
CD25
CD127
TdT
CD71
CD38
CD7
CD2
CD5
CD1
CD4
CD8
CD3
NK cell Precursor
NK cell
Lymphoid stem cell, Double-negative cell (DN1)
CD34 CD44 CD117 TdT HLA-DR
Pro B Cell
Pre B I cell
Pre B II cell
Immature B cell
B Cell
(CD9) CD19 CD22 CD117 TdT
(CD10) (CD20) CD24 CD127 HLA-DR
CD9 CD19 CD22 CD38 CD127 HLA-DR
CD10 CD20 CD24 CD117 TdT
(CD9) CD20 CD22 CD37 IgM
CD19 CD21 CD24 HLA-DR
(CD5) CD19 CD21 (CD23) CD35 HLA-DR IgD
(CD9) CD20 CD22 CD24 CD37 IgM
IL-7
TdT
CD117
CD10
CD38
CD127
CD9
HLA-DR
CD19
CD22
CD24
CD25
CD20
CD21
CD37
IgM
CD23
CD35
IgD
Hematopoietic stem cell
CD34 CD135
SCF
IL-7
CD34
CD135
TdT
HLA-DR
SCF IL-3 IL-4
CFU-Mast
SCF IL-4
Mast cell
SCF
IL-3
IL-4
SCF GM-CSF IL-3
CFU-Bas
GM-CSF IL-3
Myeloblast
Basophilic Myelocyte
Basophil
SCF
IL-3
GM-CSF
Flt3L SCF
GM-CSF IL-3
CFU-E0
GM-CSF IL-3 IL-5
Myeloblast
Eosinophilic Myelocyte
GM-CSF IL-5
Eosinophil
Flt3L
SCF
IL-3
GM-CSF
IL-5
Flt3L SCF
GM-CSF IL-4 TNF
Flt3L CSF GM-CSF
IL-3 TNF
CFU-M/DC
Myeloid Related Dendritic Cell
GM-CSF IL-4
GM-CSF M-CSF IL-3
Monoblast
Promonocyte
Monocyte
GM-CSF M-CSF
Macrophage
CD11b CD14 CD33 CD115 CD123 CD126
CD13 CD15 CD64 CD116 CD124 HLA-DR
CD11b CD14 CD64 CD116 CD124 HLA-DR
CD13 CD33 CD115 CD123 CD126
CD11b CD14 CD33 CD64
Flt3L
SCF
IL-3
GM-CSF
TNF
IL-4
M-SCF
HLA-DR
CD116
CD123
CD33
CD124
CD126
CD64
CD115
CD13
CD11b
CD14
Flt3L SCF G-CSF IL-1 IL-3 IL-6 IL-11
Myeloid Stem Cell
Flt3L SCF GM-CSF G-CSF IL-3
CFU-GEMM
CD33 CD116 CD121 IL-9R HLA-DR
CD34 CD114 CD123 EPOR
Bone marrow
Flt3L
SCF
G-SCF
IL-3
IL-6
IL-11
IL-1
GM-CSF
GM-CSF G-CSF IL-3
CFU-GM
Flt3L GM-CSF
G-CSF IL-3
CFU-G
GM-CSF G-CSF
Myeloblast
Neutrophilic Myelocyte
Neutrophil
CD15 CD34 CD114 CD116 CD123 CD125 HLA-DR
CD33 CD64 CD115 CD121 CD124 CD126
CD13 CD33 CD116 CD123 CD125 HLA-DR
CD15 CD114 CD121 CD124 CD126
CD13 CD33 CD116 CD123 CD125
CD15 CD114 CD121 CD124 CD126
CD11b CD33 CD123
CD15 CD116 CD125
CD11b CD15 CD33
Flt3L
SCF
IL-3
GM-CSF
G-SCF
IL-9R
CD34
HLA-DR
CD116
CD121
CD114
CD123
CD124
CD126
CD33
CD13
CD125
CD11b
Flt3L SCF GM-CSF
IL-3 IL-4
BFU-E
SCF GM-CSF
IL-3 IL-4
EPO
CFU-E
TPO EPO
Proerythroblast
EPO
Erythrocyte
CD33 CD117 EPOR
CD34 CD123 HLA-DR
CD36 CD235a
CD235a
CD35 CD55 CD235a
CD44 CD59
Flt3L
SCF
GM-CSF
IL-3
IL-4
EPO
TPO
HLA-DR
EPOR
CD33
CD34
CD117
CD123
CD36
CD235a
CD35
CD44
CD55
CD59
Flt3L SCF GM-CSF IL-3
IL-6 IL-11 TPO
BFU-MK
Flt3L SCF GM-CSF
Meg-CSF IL-3 IL-6
IL-11 TPO
CFU-MK
SCF GM-CSF IL-3
IL-6 IL-11 TPO
Mega-karyocyte
IL-6 IL-11 TPO
Platelets
CD33 CD116 CD126 HLA-DR
CD34 CD123 IL-11R
CD61 CD116 CD122 CD126
CD9 CD36 CD42 CD116 CD126
CD14 CD41 CD61 CD123
CD9 CD36 CD42 CD61
CD14 CD41 CD49 CD126
Flt3L
SCF
IL-3
IL-6
IL-11
GM-CSF
Meg-CSF
TPO
HLA-DR
CD33
CD34
IL-11R
CD116
CD123
CD126
CD61
CD9
CD14
CD36
CD41
CD42
CD49
Data on KEGG graph
Rendered by Pathview



Data on KEGG graph
Rendered by Pathview


STEROID HORMONE BIOSYNTHESIS
Steroid biosynthesis
-1
0
1
Cholesterol
Cholesterol sulfate
20α-Hydroxy-cholesterol
22β-Hydroxy-cholesterol
20α,22β-Dihydroxy-cholesterol
4-Methylpentanal
17α,20α-Dihydroxy-cholesterol
21-Hydroxy-pregnenolone
Pregnenolone
Pregnenolone-sulfate
7α-Hydroxy-pregnenolone
17α-Hydroxy-pregnenolone
17α,21-Dihydroxy-pregnenolone
11β,17α,21-Trihydroxy-pregnenolone
11-Deoxy-corticosterone
Progesterone
11α-Hydroxy-progesterone
20α-Hydroxy-progesterone
17α-Hydroxy-progesterone
17α,20α-Dihydroxy-pregn-4-en-3-one
11-Deoxycortisol
18-Hydroxy-corticosterone
Corticosterone
11β-Hydroxy-progesterone
21-Deoxycortisol
Cortisol
Cortisone
5α-Dihydro-deoxycorticosterone
Allotetrahydro-deoxycorticosterone
Aldosterone hemiacetal
Aldosterone
11β,21-Dihydroxy-3,20-oxo-5β-pregnan-18-al
3α,11β,21-Trihydroxy-20-oxo-5β-pregnan-18-al
11-Dehydro-corticosterone
21-Hydroxy-5β-pregnane-3,11,20-trione
11β,21-Dihydroxy-5β-pregnane-3,20-dione
Tetrahydro-corticosterone
3α,21-Dihydroxy-5β-pregnane-11,20-dione
3α,20α,21-Trihydroxy-5β-pregnane-11-one
5β-Pregnane-3,20-dione
3α-Hydroxy-5β-pregnane-20-one
Pregnanediol
5α-Pregnane-3,20-dione
3α-Hydroxy-5α-pregnan-20-one
5α-Pregnane-3α,20α-diol
5α-Pregnan-20α-ol-3-one
C21-Steroids
4-Androsten-11beta-ol-3,17-dione
11β-Hydroxytestosterone
Urocortisol
Cortol
11β,17α,21-Trihydroxy-5β-pregnane-3,20-dione
17α,21-Dihydroxy-5β-pregnane-3,11,20-trione
Urocortisone
Cortolone
11β-Hydroxyandrost-4-ene-3,17-dione
Adrenosterone
Dehydroepiandrosteron sulfate
Dehydro-epiandro-sterone
7α-Hydroxydehydro-epiandrosterone
16α-Hydroxyandrost-4-ene-3,17-dione
16α-Hydroxydehydro-epiandrosterone
3β,17β-Dihydroxy-androst-5-ene
Androst-4-ene-3,17-dione
7α-Hydroxy-androstenedione
7α-Hydroxy-testosterone
Testosterone
C19-Steroids
5β-Androstane-3,17-dione
Etiocholan-3α-ol-17-one
Etiocholan-3α-ol-17-one 3-glucuronide
3-Oxo-13,17-secoandrost-4-ene-17,13α-lactone
19-Hydroxyandrost-4-ene-3,17-dione
19-Oxoandrost-4-ene-3,17-dione
5α-Androstane-3,17-dione
Androsterone
Androsterone-glucuronide
5α-Dihydro-testosterone
Androstan-3alpha,17beta-diol
19-Hydroxy-testosterone
19-Oxotestosterone
5β-Dihydro-testosterone
Testosterone glucuronide
Estrone 3-sulfate
Estrone glucuronide
Estradiol-17α
2-Hydroxyestrone
2-Methoxyestrone
2-Methoxyestrone-3-glucuronide
2-Methoxyestrone-3-sulfate
Estrone
16-α-Hydroxyestrone
C18-Steroids
Estradiol-17β
Estriol
16-Glucuronide-estriol
2-Hydroxy-estradiol-17β
2-Methoxy-estradiol-17β
2-Methoxy-estradiol-17β-3-glucuronide
2-Methoxy-estradiol-17β-3-sulfate
6β-Hydroxy-estradiol-17β
Estradiol-17β-3-glucuronide
Estradiol-17β-3-sulfate
3.1.6.2
2.8.2.2
1.14.15.6
1.14.14.19
1.14.14.16
1.14.14.29
HSD3B
1.14.15.4
1.14.15.5
1.14.99.14
1.1.1.149
1.3.1.22
1.1.1.213
CYP11B2
1.3.1.3
1.1.1.50
1.1.1.146
HSD11B2
1.1.1.53
1.3.99.6
1.1.1.62
1.14.14.32
1.14.14.23
1.14.14.1
1.14.14.-
1.1.1.51
1.1.1.64
1.1.1.239
1.1.1.152
2.4.1.17
1.14.99.12
1.14.14.14
1.1.1.-
2.8.2.4
2.8.2.15
3.1.6.1
1.1.1.148
2.1.1.6
1.14.99.11
Data on KEGG graph
Rendered by Pathview





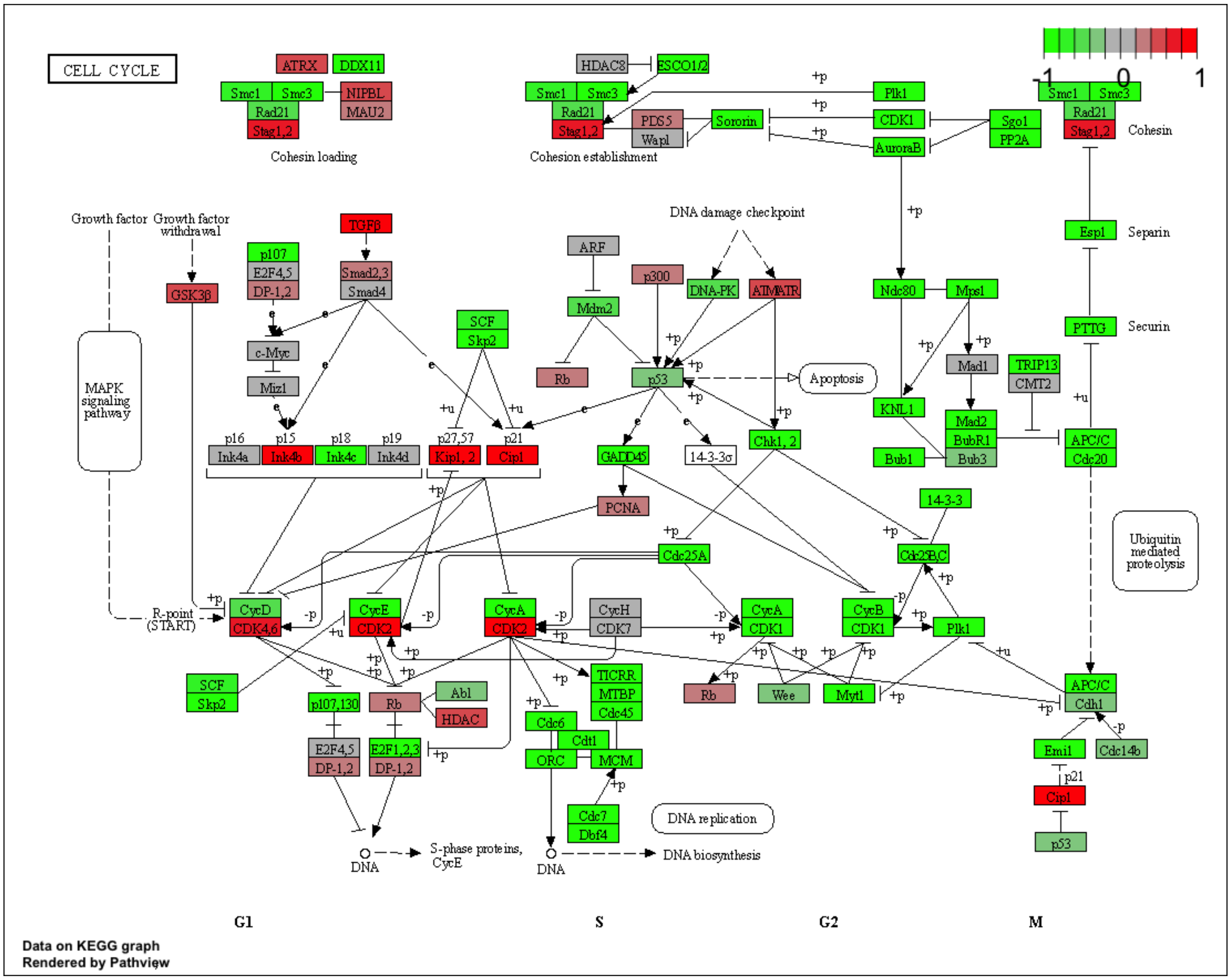
**Q7**. Can you do the same procedure as above to plot the pathview figures for the top 5 down-reguled pathways?

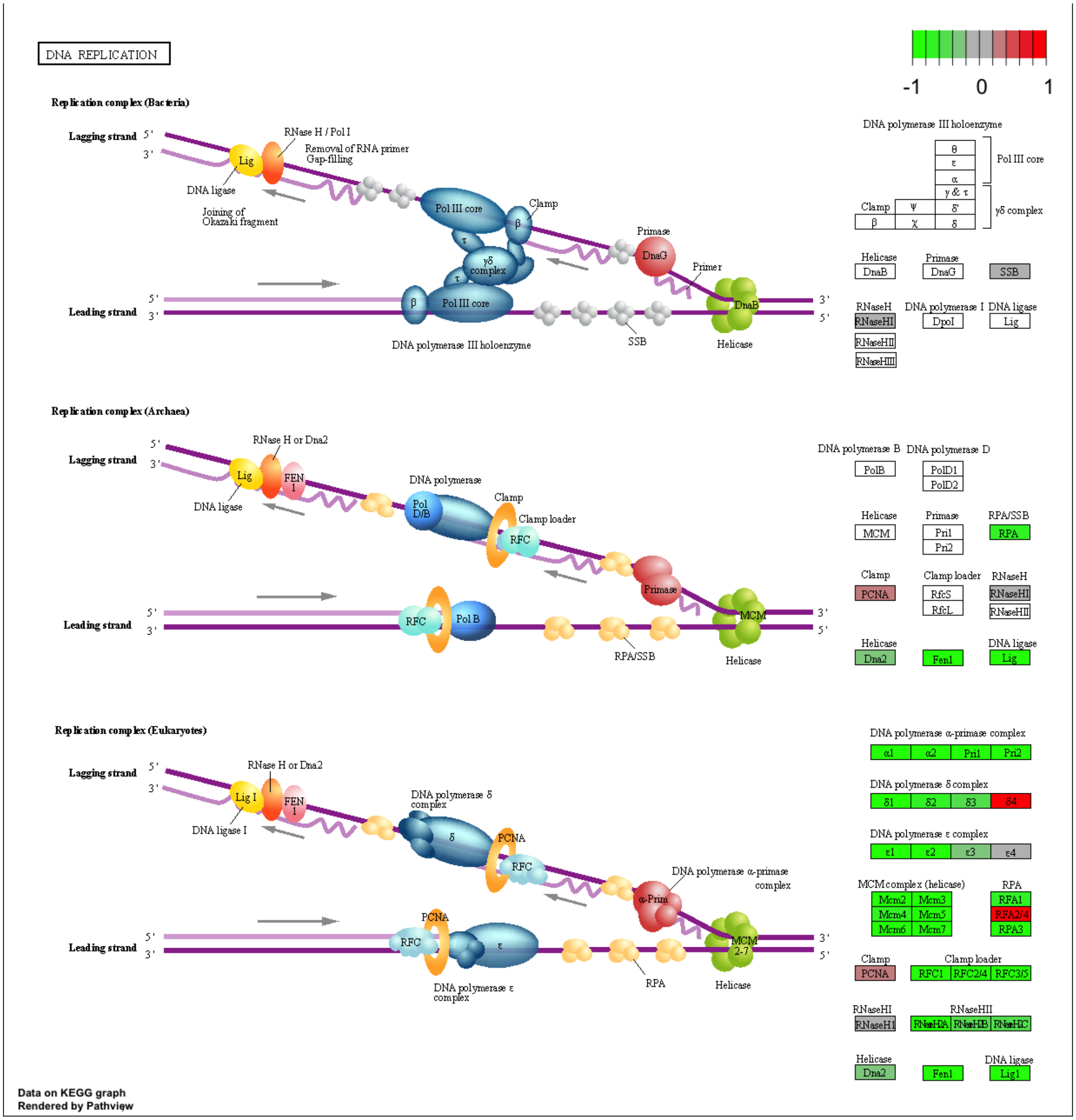
```r
keggrespathways1 <- rownames(keggres$less)[1:5]
keggresids1 = substr(keggrespathways1, start=1, stop=8)
keggresids1
```

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

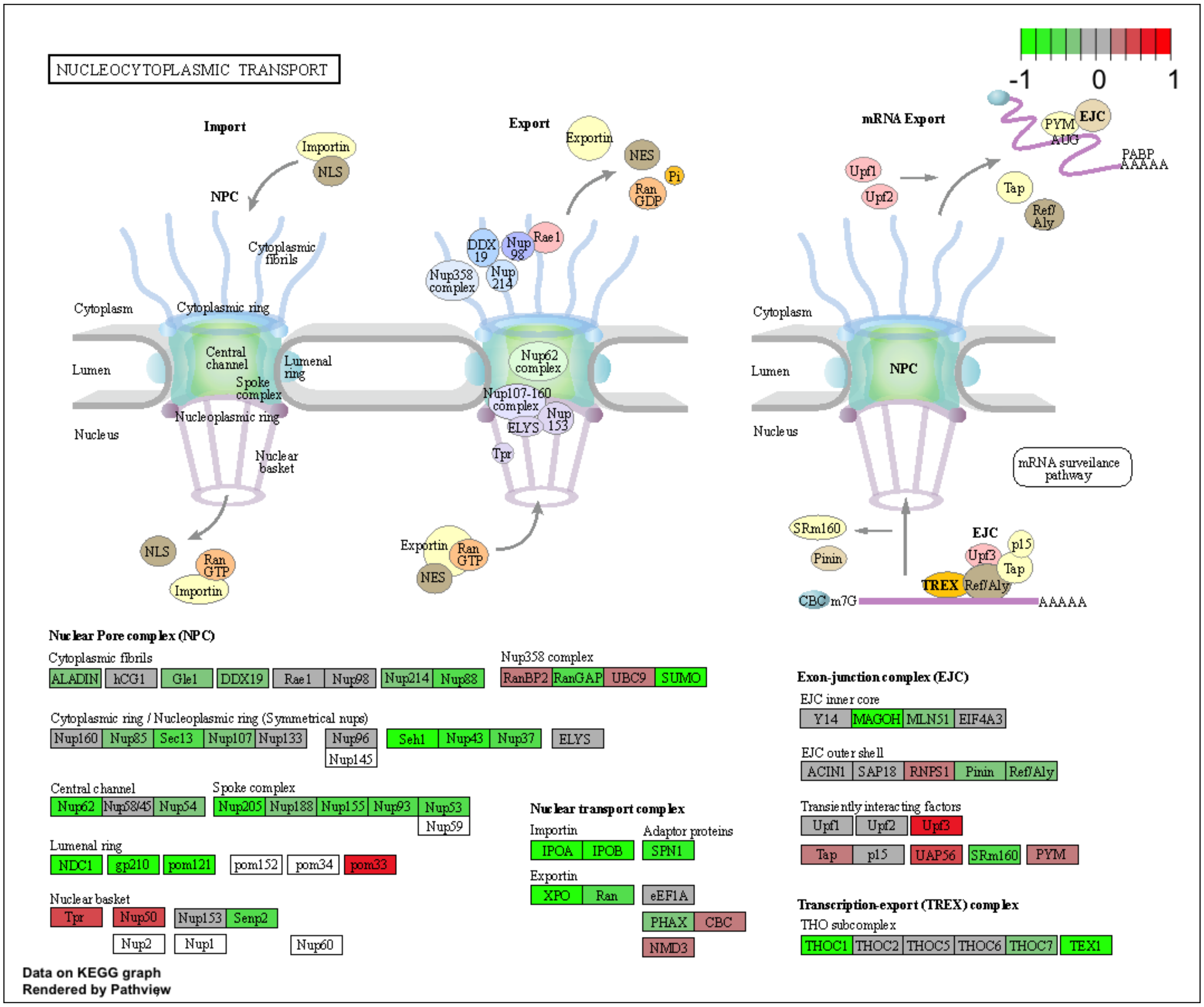
```r
pathview(gene.data=foldchanges, pathway.id=keggresids1, species="hsa")
```

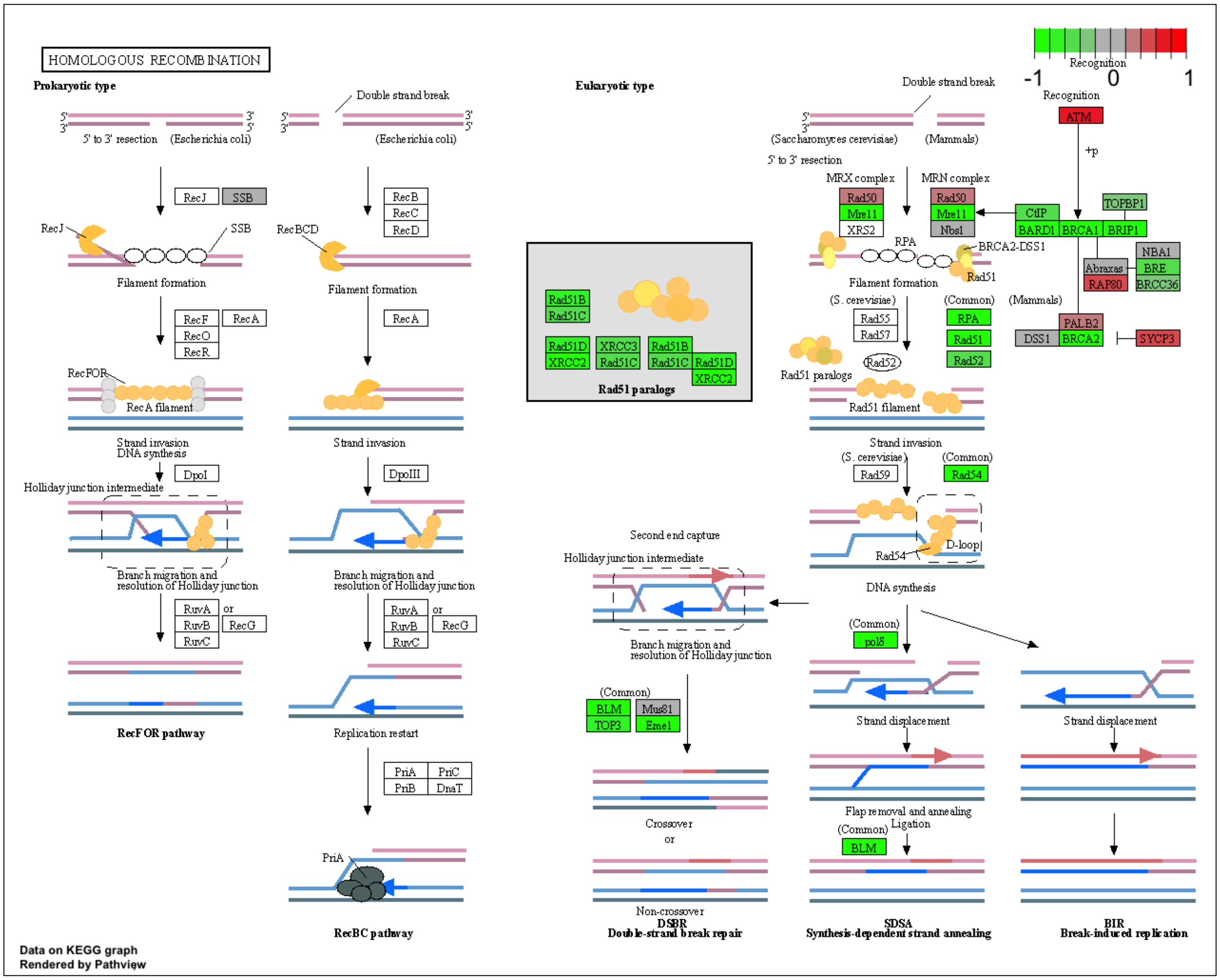
```
## 'select()' returned 1:1 mapping between keys and columns

## Info: Working in directory /Users/jessicadiaz-vigil/Desktop/BIMM 143/class13

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

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

## Info: Working in directory /Users/jessicadiaz-vigil/Desktop/BIMM 143/class13

## Info: Writing image file hsa03030.pathview.png

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

## Info: Working in directory /Users/jessicadiaz-vigil/Desktop/BIMM 143/class13

## Info: Writing image file hsa03013.pathview.png

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

## Info: Working in directory /Users/jessicadiaz-vigil/Desktop/BIMM 143/class13

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

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

## Info: Working in directory /Users/jessicadiaz-vigil/Desktop/BIMM 143/class13

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

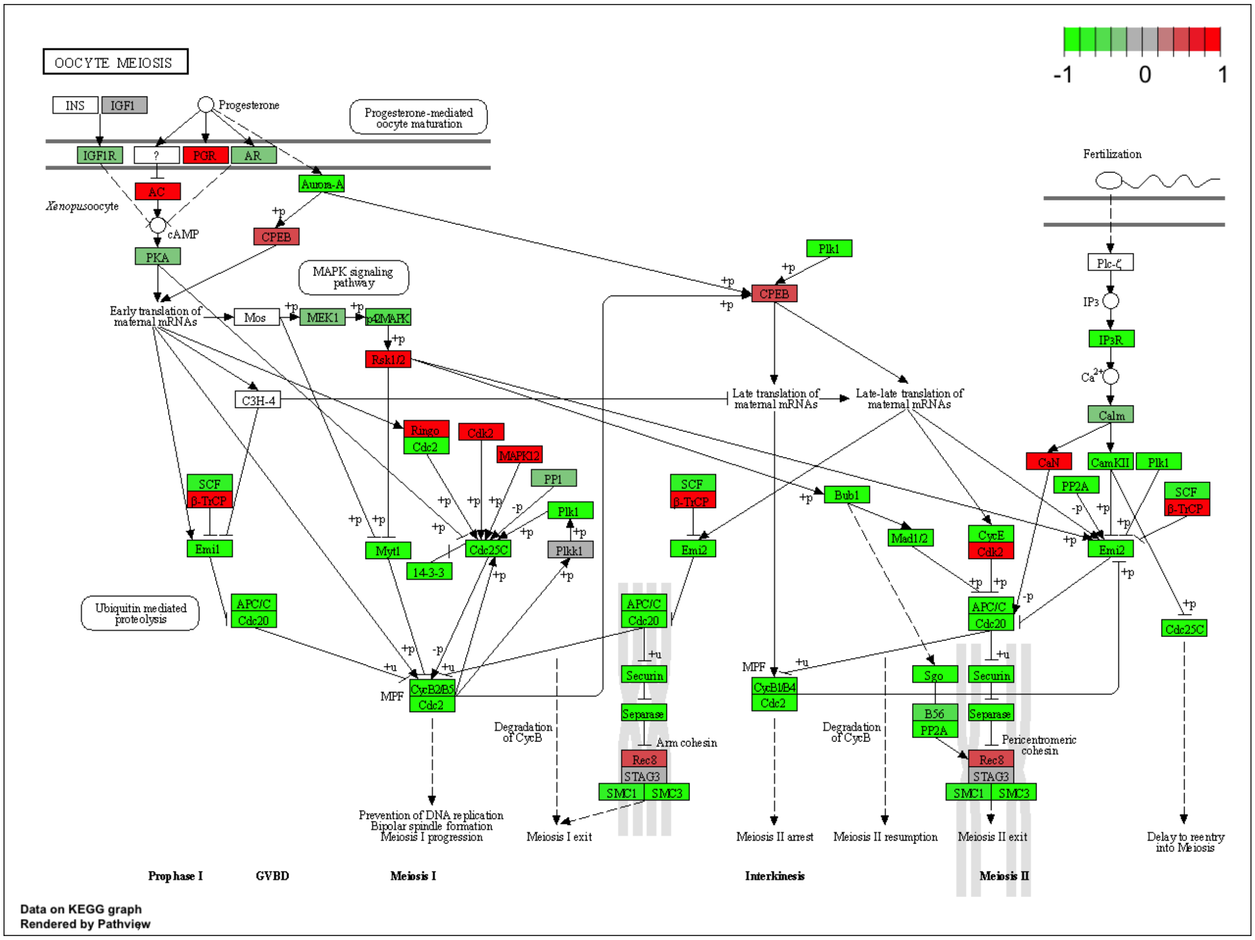

CELL CYCLE
ATRX
DDX11
Smc1
Smc3
NIPBL
Rad21
MAU2
Stag1,2
Cohesin loading
HDAC8
ESCO1/2
Smc1
Smc3
Rad21
Stag1,2
PDS5
Wapl
Sororin
Cohesion establishment
+p
Plk1
CDK1
AuroraB
Sgo1
PP2A
-1
0
1
Smc1
Smc3
Rad21
Stag1,2
Cohesin
Espl
Separin
PTTG
Securin
Growth factor
Growth factor withdrawal
GSK3β
TGFβ
p107
E2F4,5
DP-1,2
Smad2,3
Smad4
c-Myc
Miz1
MAPK signaling pathway
SCF
Skp2
ARF
Mdm2
Rb
p300
DNA damage checkpoint
DNA-PK
ATM/ATR
p53
Apoptosis
GADD45
14-3-3σ
Chk1, 2
Ndc80
Mps1
KNL1
Mad1
TRIP13
CMT2
Mad2
BubR1
Bub3
Bub1
APC/C
Cdc20
p16
p15
p18
p19
p27,57
p21
Ink4a
Ink4b
Ink4c
Ink4d
Kip1, 2
Cip1
PCNA
14-3-3
Cdc25A
Cdc25B,C
Ubiquitin mediated proteolysis
R-point (START)
CycD
CDK4,6
CycE
CDK2
CycA
CDK2
CycH
CDK7
CycA
CDK1
CycB
CDK1
Plk1
SCF
Skp2
p107,130
Rb
Abl
HDAC
TICRR
MTBP
Cdc45
Rb
Wee
Myt1
APC/C
Cdh1
E2F4,5
DP-1,2
E2F1,2,3
DP-1,2
Cdc6
Cdt1
ORC
MCM
Emi1
Cdc14b
p21
Cip1
p53
Cdc7
Dbf4
DNA replication
DNA
S-phase proteins, CycE
DNA
DNA biosynthesis
G1
S
G2
M
Data on KEGG graph
Rendered by Pathview



Data on KEGG graph
Rendered by Pathview


NUCLEOCYTOPLASMIC TRANSPORT
-1
0
1
Import
Importin
NLS
NPC
Cytoplasmic fibrils
Cytoplasm
Cytoplasmic ring
Central channel
Lumen
Lumenal ring
Spoke complex
Nucleoplasmic ring
Nucleus
Nuclear basket
NLS
Ran GTP
Importin
Export
Exportin
NES
Pi
Ran GDP
DDX 19
Nup 98
Rae1
Nup358 complex
Nup 214
Nup62 complex
Nup107-160 complex
Nup 153
ELYS
Tpr
Exportin
Ran GTP
NES
mRNA Export
PYM
EJC
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
-1
0
1
Recognition
Double strand break
5'
3'
5' to 3' resection
(Escherichia coli)
RecJ
SSB
Filament formation
RecF
RecO
RecR
RecA
RecFOR
RecA filament
Strand invasion
DNA synthesis
DpoI
Holliday junction intermediate
Branch migration and resolution of Holliday junction
RuvA
RuvB
RuvC
or
RecG
RecFOR pathway
RecB
RecC
RecD
RecBCD
DpoIII
Replication restart
PriA
PriB
PriC
DnaT
RecBC pathway
Rad51B
Rad51C
Rad51D
XRCC2
XRCC3
Rad51 paralogs
(Saccharomyces cerevisiae)
(Mammals)
MRX complex
MRN complex
Rad50
Mre11
XRS2
Nbs1
ATM
+p
CtIP
TOPBP1
BARD1
BRCA1
BRIP1
NBA1
Abraxas
BRE
RAP80
BRCC36
RPA
BRCA2-DSS1
Rad51
(S. cerevisiae)
(Common)
Rad55
Rad57
Rad52
PALB2
DSS1
BRCA2
SYCP3
Rad51 filament
Rad59
Rad54
D-loop
Second end capture
DNA synthesis
polδ
BLM
TOP3
Mus81
Eme1
Crossover
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



# Section 3. Gene Ontology (GO)

```
data(go.sets.hs)
data(go.subs.hs)
gobpsets = go.sets.hs[go.subs.hs$BP]
gobpres = gage(foldchanges, gsets=gobpsets, same.dir=TRUE)
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                         2.195494e-04  3.530241 2.195494e-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                         0.2243795      427 2.195494e-04
## GO:0060562 epithelial tube morphogenesis    0.3711390      257 5.932837e-04
## GO:0035295 tube development                 0.3711390      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                        3.530241 3.530241
## GO:0060562 epithelial tube morphogenesis   3.261376 3.261376
## GO:0035295 tube development                3.253665 3.253665
```

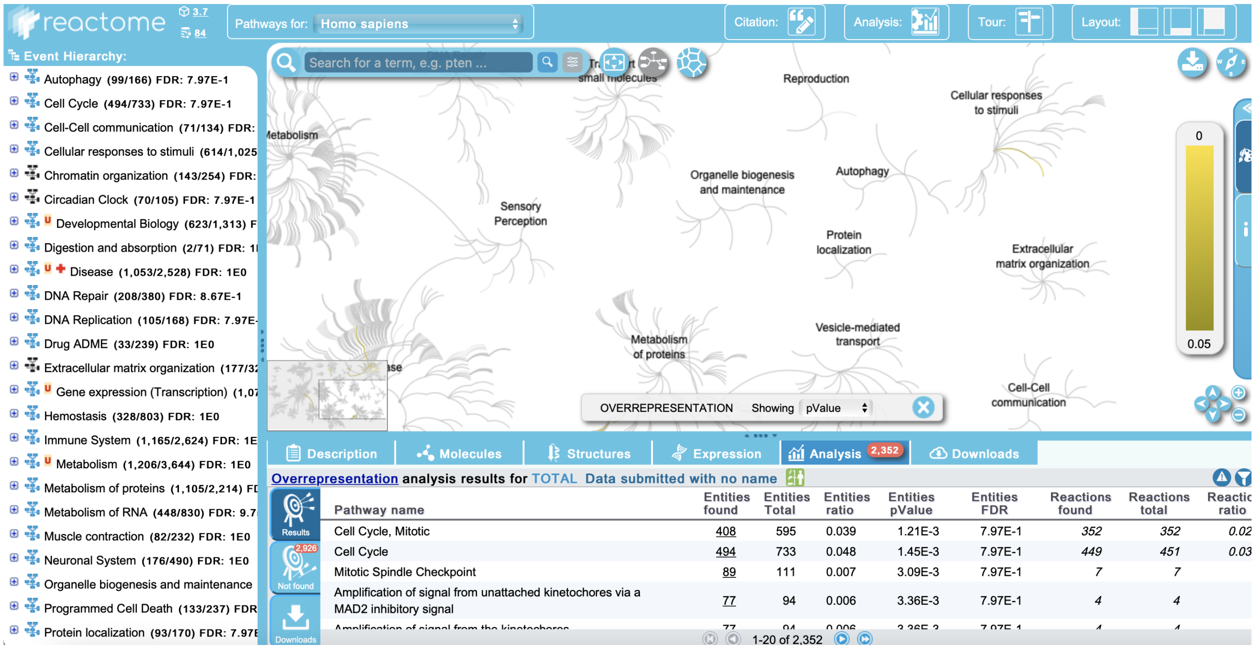
## Section 4. Reactome Analysis

```
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"
```

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

**Q8**: 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?



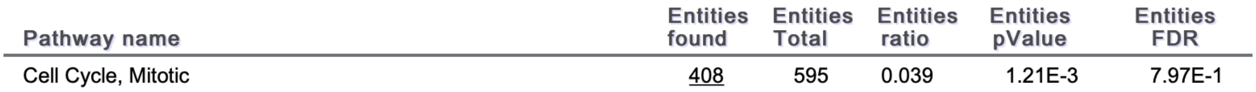
Event Hierarchy:

- Autophagy (99/166) FDR: 7.97E-1
- Cell Cycle (494/733) FDR: 7.97E-1
- Cell-Cell communication (71/134) FDR:
- Cellular responses to stimuli (614/1,025
- Chromatin organization (143/254) FDR:
- Circadian Clock (70/105) FDR: 7.97E-1
- Developmental Biology (623/1,313) F
- Digestion and absorption (2/71) FDR: 1
- Disease (1,053/2,528) FDR: 1E0
- DNA Repair (208/380) FDR: 8.67E-1
- DNA Replication (105/168) FDR: 7.97E-
- Drug ADME (33/239) FDR: 1E0
- Extracellular matrix organization (177/32
- Gene expression (Transcription) (1,07
- Hemostasis (328/803) FDR: 1E0
- Immune System (1,165/2,624) FDR: 1E
- Metabolism (1,206/3,644) FDR: 1E0
- Metabolism of proteins (1,105/2,214) FD
- Metabolism of RNA (448/830) FDR: 9.7
- Muscle contraction (82/232) FDR: 1E0
- Neuronal System (176/490) FDR: 1E0
- Organelle biogenesis and maintenance
- Programmed Cell Death (133/237) FDR
- Protein localization (93/170) FDR: 7.97E

Overrepresentation analysis results for TOTAL Data submitted with no name

| Pathway name | Entities found | Entities Total | Entities ratio | Entities pValue | Entities FDR | Reactions found | Reactions total | Reactic ratio |
|---|---|---|---|---|---|---|---|---|
| Cell Cycle, Mitotic | 408 | 595 | 0.039 | 1.21E-3 | 7.97E-1 | 352 | 352 | 0.02 |
| Cell Cycle | 494 | 733 | 0.048 | 1.45E-3 | 7.97E-1 | 449 | 451 | 0.03 |
| Mitotic Spindle Checkpoint | 89 | 111 | 0.007 | 3.09E-3 | 7.97E-1 | 7 | 7 | |
| Amplification of signal from unattached kinetochores via a MAD2 inhibitory signal | 77 | 94 | 0.006 | 3.36E-3 | 7.97E-1 | 4 | 4 | |
| Amplification of signal from the kinetochores | 77 | 94 | 0.006 | 3.36E-3 | 7.97E-1 | 4 | 4 | |

1-20 of 2,352



| Pathway name | Entities found | Entities Total | Entities ratio | Entities pValue | Entities FDR |
|---|---|---|---|---|---|
| Cell Cycle, Mitotic | 408 | 595 | 0.039 | 1.21E-3 | 7.97E-1 |

Cell Cycle, Mitotic is the pathway with the most significant "Entities p-value". These do not exactly match the listed match your previous KEGG results. Factors could cause differences between the two methods are that the KEGG database is rarely updated unlike the reactome website.