Class 14: RNASeq Mini-project

Michael McClellan (PID: A169692395)

Table of contents

Tidy counts to match metadata **3**

Remove zero count genes **3**

Setup DESeq object for analysis **4**

Run DESeq analysis **5**

Add Gene Annotation **6**

Save my results to a CSV file **8**

Result Visualization **9**

Pathway Analysis **10**

Gene Ontology **28**

Reactome analysis online 30

Here we will perform a complete RNASeq analysis from counts to pathways and biological interpretation.

The data for for hands-on session comes from GEO entry: GSE37704, which is associated with the following publication:

Trapnell C, Hendrickson DG, Sauvageau M, Goff L et al. "Differential analysis of gene regulation at transcript resolution with RNA-seq". Nat Biotechnol 2013 Jan;31(1):46-53. PMID: 23222703.

The authors report on differential analysis of lung fibroblasts in response to loss of the developmental transcription factor HOXA1.

```
library(DESeq2)
library(AnnotationDbi)
library(org.Hs.eg.db)
library(pathview)
library(gage)
library(gageData)
```

Data import

```
colData <- read.csv("GSE37704_metadata.csv", row.names = 1)
countData <- read.csv("GSE37704_featurecounts.csv", row.names = 1)
```

```
head(colData)
```

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

```
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
```

Tidy counts to match metadata

Check the correspondence of colData rows and countData columns.

```
rownames(colData)
```

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

```
colnames(countData)
```

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

Remove the troublesome first column so we match the metadata

```
counts <- countData[, -1]
```

```
head(counts)
```

```
                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
```

```
all (rownames(colData) == colnames(counts))
```

```
[1] TRUE
```

Remove zero count genes

We will have rows in `counts` for genes that we can not say anything about because they have zero expression in the particular tissue we are looking at.

```
head(counts)
```

```
                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
```

If the `rowSums()` is zero then a given gene (i.e. row) has no data and we should exclude these genes from further consideration.

```
to.keep <- rowSums(counts) != 0
cleancounts <- counts[to.keep, ]
```

Q. How many genes do we have left?

```
nrow(cleancounts)
```

```
[1] 15975
```

Setup DESeq object for analysis

```
dds <- DESeqDataSetFromMatrix(countData=cleancounts,
                              colData = colData,
                              design = ~condition)
```

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

Run DESeq analysis

```
dds <- DESeq(dds)
```

```
estimating size factors

estimating dispersions

gene-wise dispersion estimates

mean-dispersion relationship

final dispersion estimates

fitting model and testing
```

#Extract the results

```
res <- results(dds)
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 0.3248216   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 1.43989e-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 0.5215599   1.040744 2.97994e-01
                      padj
                 <numeric>
ENSG00000279457 6.86555e-01
ENSG00000187634 5.15718e-03
ENSG00000188976 1.76549e-35
ENSG00000187961 1.13413e-07
ENSG00000187583 9.19031e-01
ENSG00000187642 4.03379e-01
```

Add Gene Annotation

```
head(rownames(res))
```

```
[1] "ENSG00000279457" "ENSG00000187634" "ENSG00000188976" "ENSG00000187961"
[5] "ENSG00000187583" "ENSG00000187642"
```

```
res$symbol <- mapIds(x=org.Hs.eg.db,
                     keys=rownames(res),
                     keytype="ENSEMBL",
                     column="SYMBOL")
```

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

```
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 7 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 1.43989e-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 0.5215599   1.040744 2.97994e-01
                      padj      symbol
                 <numeric> <character>
ENSG00000279457 6.86555e-01          NA
ENSG00000187634 5.15718e-03      SAMD11
ENSG00000188976 1.76549e-35       NOC2L
ENSG00000187961 1.13413e-07      KLHL17
ENSG00000187583 9.19031e-01     PLEKHN1
ENSG00000187642 4.03379e-01       PERM1
```

```
res$entrez <- mapIds(x=org.Hs.eg.db,
                     keys=rownames(res),
                     keytype="ENSEMBL",
                     column="ENTREZID")
```

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

```
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 8 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 1.43989e-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 0.5215599   1.040744 2.97994e-01
                      padj      symbol      entrez
                 <numeric> <character> <character>
ENSG00000279457 6.86555e-01          NA          NA
ENSG00000187634 5.15718e-03      SAMD11      148398
ENSG00000188976 1.76549e-35       NOC2L       26155
ENSG00000187961 1.13413e-07      KLHL17      339451
ENSG00000187583 9.19031e-01     PLEKHN1       84069
ENSG00000187642 4.03379e-01       PERM1       84808
```

```
res$name <- mapIds(x=org.Hs.eg.db,
                   keys=rownames(res),
                   keytype="ENSEMBL",
                   column="GENENAME")
```

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

```
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 9 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 1.43989e-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 0.5215599   1.040744 2.97994e-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..
```

```
res = res[order(res$pvalue),]
head(res)
```

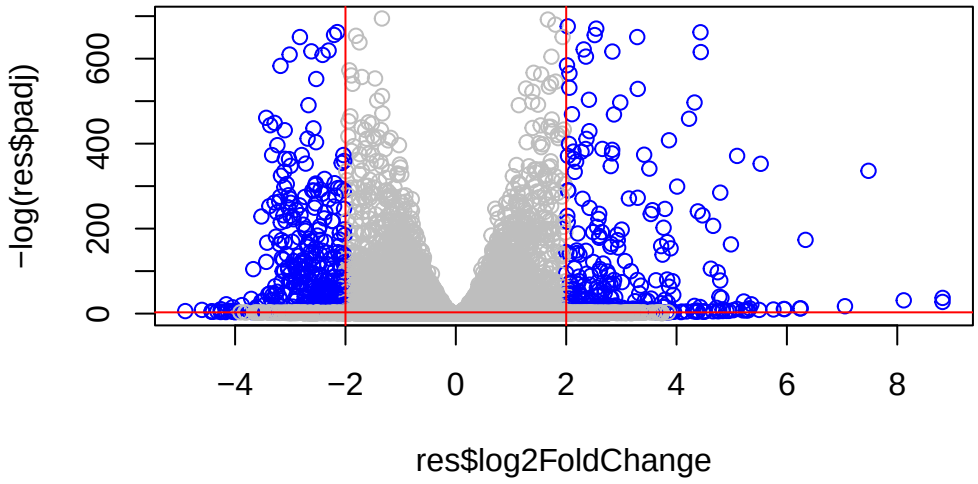
```
log2 fold change (MLE): condition hoxa1 kd vs control sirna
Wald test p-value: condition hoxa1 kd vs control sirna
DataFrame with 6 rows and 9 columns
                 baseMean log2FoldChange     lfcSE      stat    pvalue
                <numeric>      <numeric> <numeric> <numeric> <numeric>
ENSG00000117519   4483.63       -2.42272 0.0600016  -40.3776         0
ENSG00000183508   2053.88        3.20196 0.0724172   44.2154         0
ENSG00000159176   5692.46       -2.31374 0.0575534  -40.2016         0
ENSG00000150938   7442.99       -2.05963 0.0538449  -38.2512         0
ENSG00000116016   4423.95       -1.88802 0.0431680  -43.7366         0
ENSG00000136068   3796.13       -1.64979 0.0439354  -37.5504         0
                     padj      symbol      entrez                  name
                <numeric> <character> <character>           <character>
ENSG00000117519         0        CNN3        1266             calponin 3
ENSG00000183508         0      TENT5C       54855 terminal nucleotidyl..
ENSG00000159176         0       CSRP1        1465 cysteine and glycine..
ENSG00000150938         0       CRIM1       51232 cysteine rich transm..
ENSG00000116016         0       EPAS1        2034 endothelial PAS doma..
ENSG00000136068         0        FLNB        2317              filamin B
```

Save my results to a CSV file

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

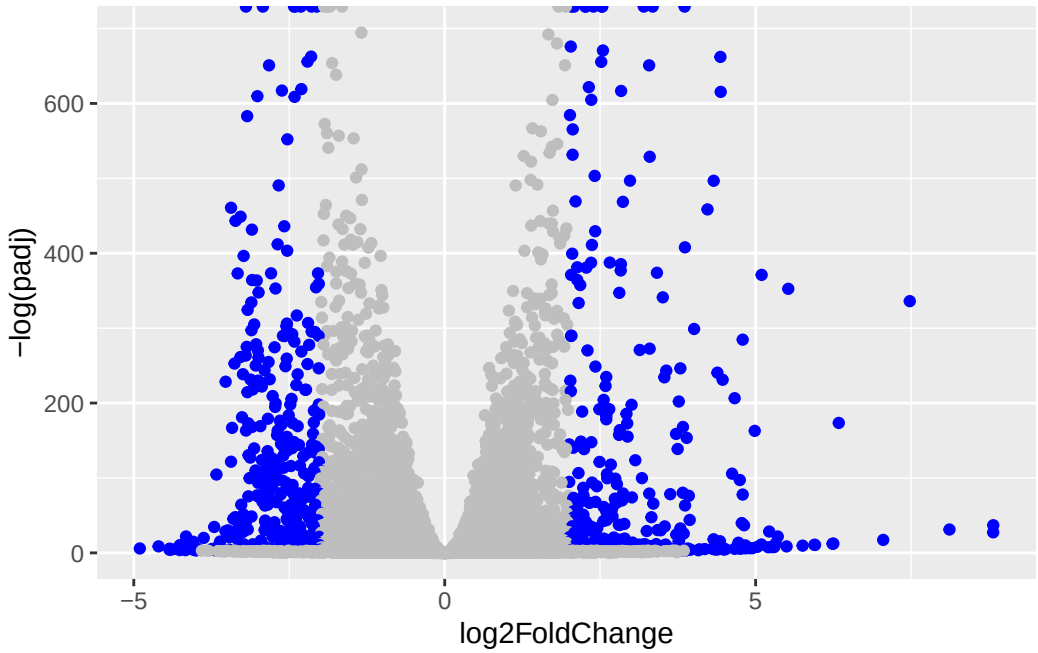
Result Visualization

```
mycols <- rep("gray", nrow(res))
mycols[res$log2FoldChange <= -2] <- "blue"
mycols[res$log2FoldChange >= 2] <- "blue"

mycols[res$padj >= 0.05] <- "gray"

plot(res$log2FoldChange, -log(res$padj), col=mycols)
abline(v=-2, col="red")
abline(v=+2, col="red")
abline(h=-log(0.05), col="red")
```




```
library(ggplot2)

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

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



Pathway Analysis

```
data(kegg.sets.hs)
data(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"

$`hsa01100 Metabolic pathways`
   [1] "10"        "100"       "10007"     "100137049" "10020"     "10026"
```

```
  [7] "100510686" "10063"     "10157"     "10170"     "10195"     "10201"
 [13] "10229"     "10312"     "10317"     "10327"     "10331"     "1036"
 [19] "10380"     "10390"     "1040"      "10400"     "10402"     "10423"
 [25] "10449"     "10476"     "10554"     "10555"     "10558"     "1056"
 [31] "10588"     "10606"     "10621"     "10622"     "10623"     "10632"
 [37] "10654"     "1066"      "10678"     "10682"     "10690"     "10714"
 [43] "10720"     "10768"     "10797"     "10826"     "10841"     "10855"
 [49] "10873"     "10901"     "10905"     "10941"     "10975"     "10993"
 [55] "10998"     "11019"     "11041"     "1109"      "11112"     "11128"
 [61] "1119"      "1120"      "11226"     "11227"     "11232"     "112483"
 [67] "11253"     "11282"     "11285"     "113026"    "11320"     "11343"
 [73] "113451"    "113612"    "114805"    "1152"      "1158"      "1159"
 [79] "1160"      "116285"    "117248"    "119548"    "120227"    "121278"
 [85] "122481"    "122622"    "123099"    "123745"    "123876"    "124"
 [91] "124454"    "124975"    "125"       "125061"    "125965"    "125981"
 [97] "126"       "126328"    "126792"    "127"       "127124"    "128"
[103] "128869"    "129607"    "129642"    "130"       "130013"    "131"
[109] "1312"      "131669"    "132"       "132158"    "1327"      "132789"
[115] "1329"      "1337"      "1339"      "1340"      "134147"    "1345"
[121] "1349"      "1350"      "1351"      "135152"    "1352"      "1353"
[127] "1355"      "1371"      "1373"      "137964"    "138050"    "138429"
[133] "139596"    "140838"    "1431"      "144193"    "144245"    "145226"
[139] "146664"    "1491"      "15"        "1503"      "150763"    "151056"
[145] "151531"    "1537"      "154141"    "1543"      "1544"      "1548"
[151] "1549"      "155066"    "1551"      "1553"      "1555"      "1557"
[157] "1558"      "1559"      "1562"      "1571"      "1573"      "157506"
[163] "1576"      "1577"      "1579"      "158"       "1581"      "1582"
[169] "1583"      "1584"      "1585"      "1586"      "1588"      "1589"
[175] "159"       "1593"      "1594"      "1595"      "160287"    "1603"
[181] "1606"      "1607"      "1608"      "160851"    "1609"      "1610"
[187] "1621"      "162417"    "162466"    "1629"      "1633"      "1635"
[193] "1638"      "1644"      "1650"      "166929"    "168391"    "169355"
[199] "170712"    "171568"    "1716"      "1717"      "1718"      "1719"
[205] "1723"      "1737"      "1738"      "1743"      "1757"      "178"
[211] "1786"      "1787"      "1788"      "1789"      "1798"      "18"
[217] "1806"      "1807"      "1841"      "1854"      "189"       "1890"
[223] "1892"      "191"       "192134"    "1962"      "197258"    "199857"
[229] "201595"    "2023"      "2026"      "2027"      "203"       "204"
[235] "205"       "2053"      "2058"      "210"       "211"       "212"
[241] "2131"      "2132"      "2134"      "2135"      "2137"      "216"
[247] "217"       "218"       "2180"      "2181"      "2182"      "2184"
[253] "219"       "2194"      "220"       "2203"      "221"       "221223"
[259] "221823"    "222"       "2222"      "2224"      "223"       "2235"
```

```
[265] "224"      "226"      "2271"     "22845"    "22856"    "229"
[271] "22928"    "22929"    "22934"    "22978"    "230"      "23057"
[277] "231"      "23193"    "23236"    "23305"    "23382"    "23396"
[283] "23417"    "23475"    "23483"    "23498"    "23530"    "23545"
[289] "23553"    "23556"    "2356"     "23600"    "23649"    "23761"
[295] "239"      "240"      "242"      "245972"   "245973"   "246"
[301] "246721"   "247"      "248"      "249"      "250"      "251"
[307] "2523"     "2524"     "2525"     "2526"     "2527"     "2528"
[313] "2529"     "2530"     "2531"     "253558"   "2538"     "2539"
[319] "254531"   "2548"     "256435"   "2571"     "2572"     "25796"
[325] "2581"     "2582"     "2583"     "25834"    "2584"     "2585"
[331] "2588"     "25885"    "2589"     "2590"     "25902"    "2591"
[337] "2592"     "259230"   "2593"     "259307"   "2595"     "2597"
[343] "26007"    "26035"    "2618"     "262"      "26227"    "26229"
[349] "26275"    "26279"    "2628"     "26289"    "2629"     "26290"
[355] "26301"    "2632"     "26330"    "2639"     "2643"     "2645"
[361] "2650"     "2651"     "2673"     "2678"     "2683"     "2686"
[367] "2687"     "270"      "27010"    "27034"    "27087"    "27089"
[373] "27090"    "271"      "2710"     "2712"     "27124"    "27165"
[379] "272"      "2720"     "27235"    "2729"     "2730"     "27306"
[385] "2731"     "27349"    "27430"    "2744"     "2746"     "2747"
[391] "275"      "2752"     "276"      "2762"     "277"      "278"
[397] "279"      "2799"     "28"       "280"      "2805"     "2806"
[403] "2821"     "283208"   "283871"   "284098"   "284541"   "2875"
[409] "290"      "29071"    "2937"     "2954"     "29796"    "2987"
[415] "29880"    "2990"     "29906"    "29920"    "29922"    "29925"
[421] "29926"    "29929"    "29947"    "29958"    "29968"    "30"
[427] "3028"     "3030"     "3032"     "3033"     "3034"     "3067"
[433] "3073"     "3074"     "3081"     "30814"    "30815"    "30833"
[439] "30834"    "3098"     "3099"     "31"       "3101"     "314"
[445] "3141"     "3145"     "3155"     "3156"     "3157"     "3158"
[451] "316"      "317749"   "32"       "3242"     "3251"     "326625"
[457] "3283"     "3284"     "3290"     "3291"     "3292"     "3293"
[463] "3294"     "3295"     "33"       "3340"     "3373"     "337876"
[469] "339221"   "34"       "340485"   "341392"   "3417"     "3418"
[475] "3419"     "341947"   "3420"     "3421"     "3422"     "3423"
[481] "3425"     "348158"   "349565"   "35"       "353"      "36"
[487] "3612"     "3613"     "3614"     "3615"     "3620"     "3628"
[493] "3631"     "3632"     "3633"     "3636"     "37"       "3703"
[499] "3704"     "3705"     "3706"     "3707"     "3712"     "374291"
[505] "374378"   "3795"     "38"       "383"      "384"      "387787"
[511] "39"       "3906"     "391013"   "3938"     "3939"     "3945"
[517] "3948"     "3990"     "4047"     "4048"     "4051"     "4056"
```

```
[523] "411"      "4121"     "4122"     "4124"     "4128"     "4129"
[529] "4143"     "4144"     "4190"     "4191"     "4199"     "4245"
[535] "4247"     "4248"     "4249"     "427"      "4329"     "435"
[541] "4351"     "4357"     "438"      "440"      "440138"   "440567"
[547] "441024"   "441531"   "442117"   "445"      "4507"     "4508"
[553] "4509"     "4512"     "4513"     "4514"     "4519"     "4522"
[559] "4524"     "4535"     "4536"     "4537"     "4538"     "4539"
[565] "4540"     "4541"     "4548"     "4594"     "4597"     "4598"
[571] "4669"     "4694"     "4695"     "4696"     "4697"     "4698"
[577] "47"       "4700"     "4701"     "4702"     "4704"     "4705"
[583] "4706"     "4707"     "4708"     "4709"     "471"      "4710"
[589] "4711"     "4712"     "4713"     "4714"     "4715"     "4716"
[595] "4717"     "4718"     "4719"     "4720"     "4722"     "4723"
[601] "4724"     "4725"     "4726"     "4728"     "4729"     "4731"
[607] "48"       "4830"     "4831"     "4832"     "4833"     "4837"
[613] "4842"     "4843"     "4846"     "4860"     "4907"     "493911"
[619] "4942"     "4952"     "4953"     "4967"     "498"      "50"
[625] "5009"     "501"      "5033"     "5048"     "50484"    "50487"
[631] "5049"     "5050"     "5051"     "5053"     "506"      "50614"
[637] "50617"    "50700"    "50814"    "509"      "5091"     "5095"
[643] "5096"     "51"       "51004"    "5105"     "51056"    "5106"
[649] "51074"    "51082"    "51084"    "51102"    "51109"    "51144"
[655] "51166"    "51179"    "51181"    "51196"    "51227"    "51251"
[661] "51268"    "513"      "5130"     "51301"    "51380"    "51382"
[667] "514"      "51477"    "51478"    "515"      "51540"    "516"
[673] "5160"     "51601"    "51604"    "51606"    "5161"     "5162"
[679] "5167"     "5169"     "517"      "51703"    "51727"    "51728"
[685] "51733"    "51763"    "518"      "51805"    "51809"    "5198"
[691] "521"      "5211"     "5213"     "5214"     "522"      "5223"
[697] "5224"     "5226"     "523"      "5230"     "5232"     "5236"
[703] "525"      "526"      "527"      "5277"     "5279"     "528"
[709] "5281"     "5283"     "5286"     "5287"     "5288"     "5289"
[715] "529"      "5297"     "5298"     "5313"     "5315"     "5319"
[721] "5320"     "5321"     "5322"     "533"      "5330"     "5331"
[727] "5332"     "5333"     "5335"     "53354"    "5336"     "5337"
[733] "5338"     "534"      "535"      "53630"    "537"      "5372"
[739] "5373"     "539"      "53947"    "5406"     "5407"     "5408"
[745] "5409"     "54107"    "54187"    "5422"     "5424"     "5425"
[751] "5426"     "5427"     "5428"     "5430"     "5431"     "5432"
[757] "5433"     "5434"     "54344"    "5435"     "5436"     "54363"
[763] "5437"     "5438"     "5439"     "5440"     "5441"     "5444"
[769] "5445"     "5446"     "54480"    "54490"    "54575"    "54576"
[775] "54577"    "54578"    "54579"    "54600"    "54657"    "54658"
```

```
 [781] "54659"    "54675"    "5471"     "54802"    "548596"   "548644"
 [787] "549"      "54947"    "54963"    "54965"    "5498"     "54988"
 [793] "54995"    "55163"    "55191"    "55224"    "55229"    "55256"
 [799] "55276"    "55300"    "55301"    "55304"    "55312"    "55361"
 [805] "5538"     "55454"    "55500"    "55512"    "55568"    "5557"
 [811] "5558"     "55627"    "55650"    "55703"    "55750"    "55753"
 [817] "55790"    "55808"    "55821"    "55902"    "55907"    "56052"
 [823] "5625"     "56267"    "5631"     "5634"     "56474"    "56623"
 [829] "56624"    "56655"    "56848"    "56894"    "56895"    "56898"
 [835] "56901"    "56913"    "56922"    "56953"    "56994"    "570"
 [841] "57016"    "57026"    "57134"    "5723"     "5730"     "5740"
 [847] "5742"     "5743"     "57452"    "574537"   "57678"    "57804"
 [853] "57818"    "57834"    "5805"     "5831"     "5832"     "5833"
 [859] "58510"    "5859"     "586"      "5860"     "587"      "593"
 [865] "594"      "5980"     "60490"    "60495"    "6120"     "6184"
 [871] "6185"     "622"      "6240"     "6241"     "6296"     "6303"
 [877] "6307"     "6309"     "6342"     "635"      "6389"     "6390"
 [883] "6391"     "63917"    "6392"     "64087"    "64131"    "64132"
 [889] "64409"    "64425"    "6448"     "64579"    "64600"    "646625"
 [895] "6470"     "6472"     "6476"     "64768"    "6480"     "64802"
 [901] "64816"    "6482"     "6483"     "6484"     "6487"     "6489"
 [907] "64902"    "65220"    "65263"    "654364"   "6609"     "661"
 [913] "6610"     "6611"     "6652"     "6675"     "6677"     "669"
 [919] "6697"     "6713"     "6718"     "6723"     "683"      "686"
 [925] "6888"     "6898"     "6916"     "6999"     "7054"     "7083"
 [931] "7084"     "7086"     "7108"     "7166"     "7167"     "7173"
 [937] "7263"     "7264"     "729020"   "7298"     "7299"     "7306"
 [943] "7357"     "7358"     "7360"     "7363"     "7364"     "7365"
 [949] "7366"     "7367"     "7368"     "7371"     "7372"     "7378"
 [955] "7381"     "7384"     "7385"     "7386"     "7388"     "7389"
 [961] "7390"     "7498"     "7841"     "790"      "79053"    "79087"
 [967] "7915"     "79178"    "7923"     "79369"    "7941"     "79586"
 [973] "79611"    "79623"    "79646"    "79695"    "79717"    "79796"
 [979] "79799"    "79814"    "79868"    "79888"    "7991"     "80025"
 [985] "80055"    "80142"    "80146"    "80201"    "80270"    "80308"
 [991] "80339"    "80347"    "8050"     "81490"    "81579"    "81616"
 [997] "81849"    "81888"    "8277"     "8309"     "8310"     "83440"
[1003] "83549"    "8372"     "8382"     "8394"     "8395"     "8398"
[1009] "8399"     "84002"    "84076"    "84172"    "84245"    "84265"
[1015] "84274"    "84284"    "84532"    "84618"    "84620"    "84647"
[1021] "84649"    "84693"    "847"      "84701"    "84706"    "84720"
[1027] "84735"    "84803"    "84812"    "84890"    "84920"    "84992"
[1033] "8509"     "8513"     "8525"     "8526"     "8527"     "8529"
```

```
[1039] "85365"     "8540"      "85465"     "8560"      "8564"      "8566"
[1045] "8608"      "8611"      "8612"      "8613"      "8630"      "8639"
[1051] "8659"      "8681"      "8692"      "8693"      "8694"      "8702"
[1057] "8703"      "8704"      "8705"      "8706"      "8707"      "8708"
[1063] "873"       "8733"      "874"       "875"       "8760"      "8789"
[1069] "8790"      "8801"      "8802"      "8803"      "8813"      "8818"
[1075] "8821"      "883"       "8833"      "8854"      "8867"      "8869"
[1081] "8871"      "8877"      "8879"      "8942"      "8972"      "8974"
[1087] "89869"     "8992"      "9"         "90423"     "9060"      "9061"
[1093] "9091"      "9114"      "91373"     "9162"      "91734"     "9197"
[1099] "9245"      "92483"     "9249"      "9296"      "93034"     "93183"
[1105] "9331"      "9348"      "9374"      "9377"      "9380"      "9388"
[1111] "94005"     "9453"      "9468"      "9487"      "9488"      "9489"
[1117] "95"        "9514"      "9517"      "952"       "9533"      "9536"
[1123] "9550"      "9551"      "9563"      "9588"      "9615"      "978"
[1129] "9791"      "9942"      "9945"
```

```
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
```

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

```
attributes(keggres)
```

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

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

```
                                                  p.geomean stat.mean
hsa04110 Cell cycle                             8.995727e-06 -4.378644
hsa03030 DNA replication                        9.424076e-05 -3.951803
hsa05130 Pathogenic Escherichia coli infection 1.405864e-04 -3.765330
hsa03013 RNA transport                          1.375901e-03 -3.028500
hsa03440 Homologous recombination               3.066756e-03 -2.852899
hsa04114 Oocyte meiosis                         3.784520e-03 -2.698128
```

```
                                                      p.val       q.val
hsa04110 Cell cycle                                8.995727e-06 0.001889103
hsa03030 DNA replication                           9.424076e-05 0.009841047
hsa05130 Pathogenic Escherichia coli infection 1.405864e-04 0.009841047
hsa03013 RNA transport                             1.375901e-03 0.072234819
hsa03440 Homologous recombination                  3.066756e-03 0.128803765
hsa04114 Oocyte meiosis                            3.784520e-03 0.132458191
                                                   set.size         exp1
hsa04110 Cell cycle                                     121 8.995727e-06
hsa03030 DNA replication                                 36 9.424076e-05
hsa05130 Pathogenic Escherichia coli infection           53 1.405864e-04
hsa03013 RNA transport                                  144 1.375901e-03
hsa03440 Homologous recombination                        28 3.066756e-03
hsa04114 Oocyte meiosis                                 102 3.784520e-03
```

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

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

Info: Working in directory /Users/michael/Desktop/BIMM143/class14

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

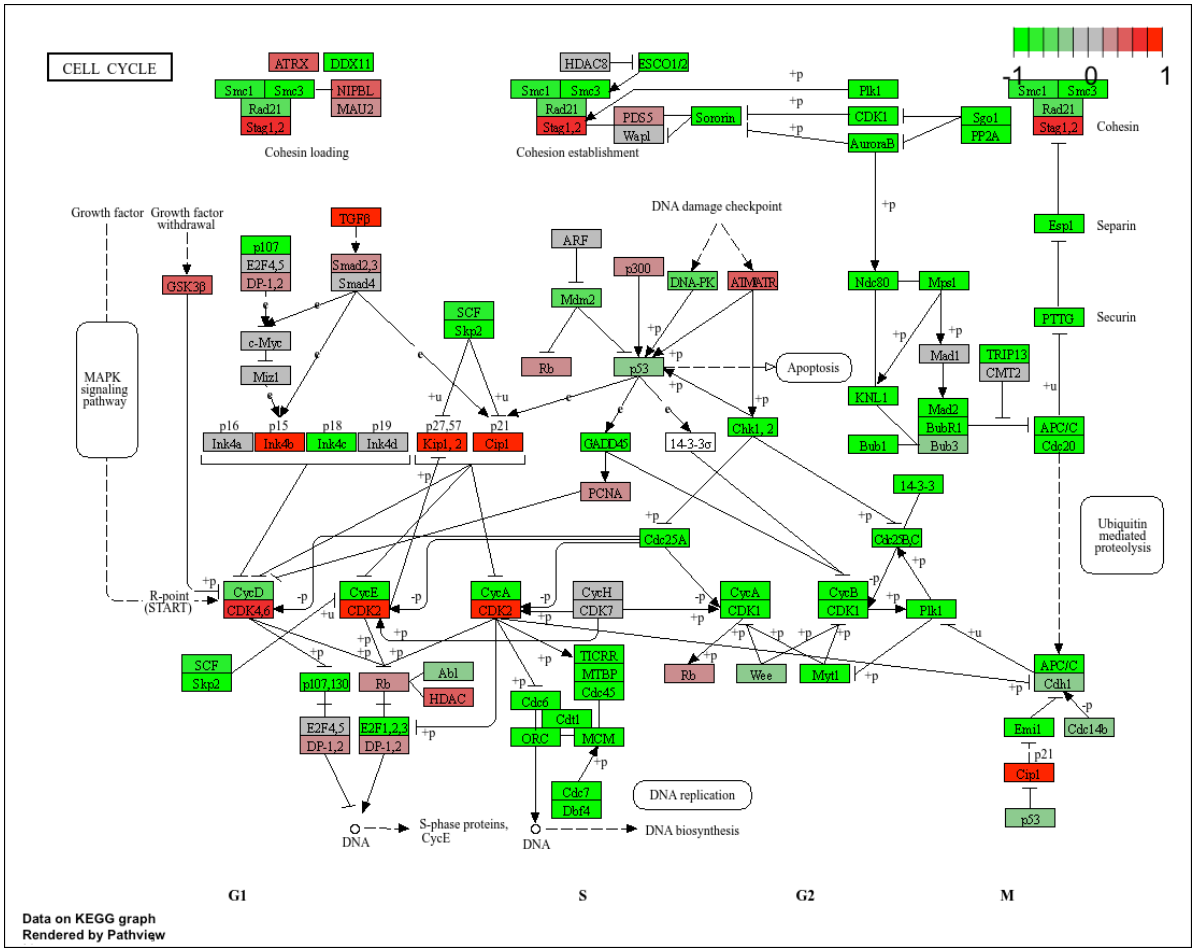


Figure 1: hsa04110

```
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/michael/Desktop/BIMM143/class14
```

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

```
## 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] "hsa04060" "hsa05323" "hsa05146" "hsa05332" "hsa04640"
```

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

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

Info: Working in directory /Users/michael/Desktop/BIMM143/class14

Info: Writing image file hsa04060.pathview.png

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

Info: Working in directory /Users/michael/Desktop/BIMM143/class14

Info: Writing image file hsa05323.pathview.png

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

Info: Working in directory /Users/michael/Desktop/BIMM143/class14

Info: Writing image file hsa05146.pathview.png

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

Info: Working in directory /Users/michael/Desktop/BIMM143/class14

Info: Writing image file hsa05332.pathview.png
```

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

Info: Working in directory /Users/michael/Desktop/BIMM143/class14

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

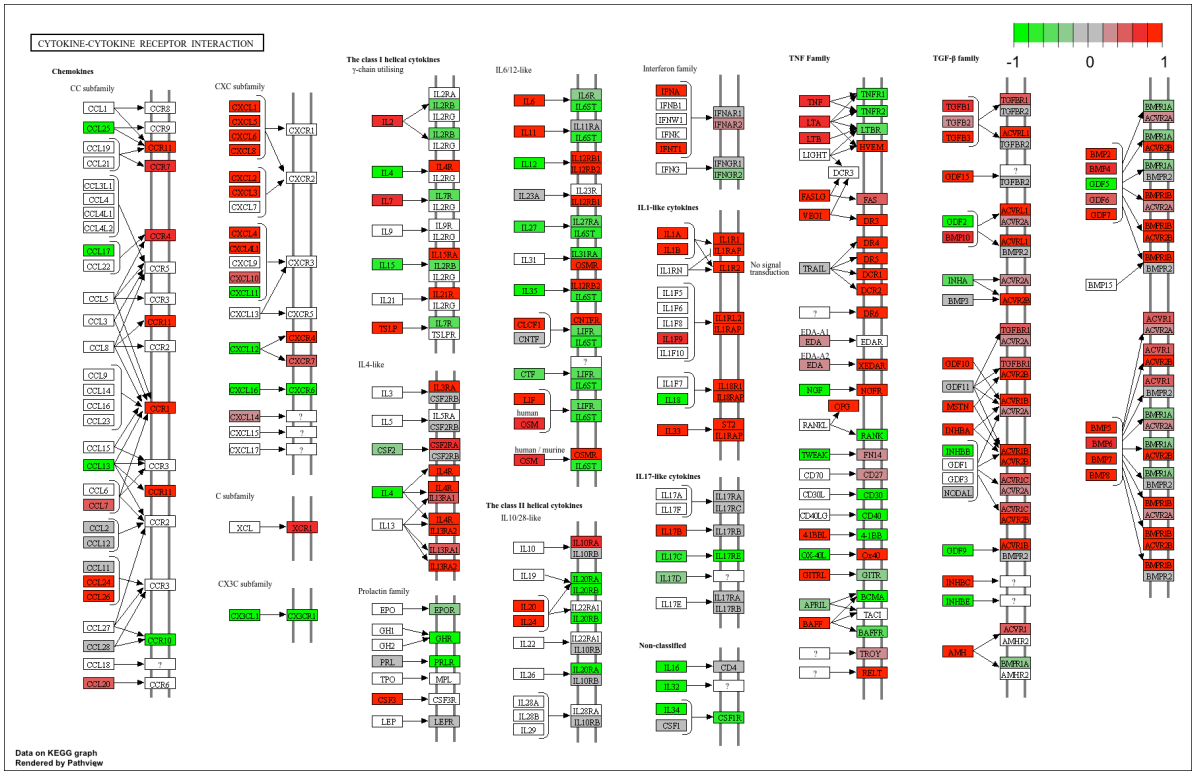
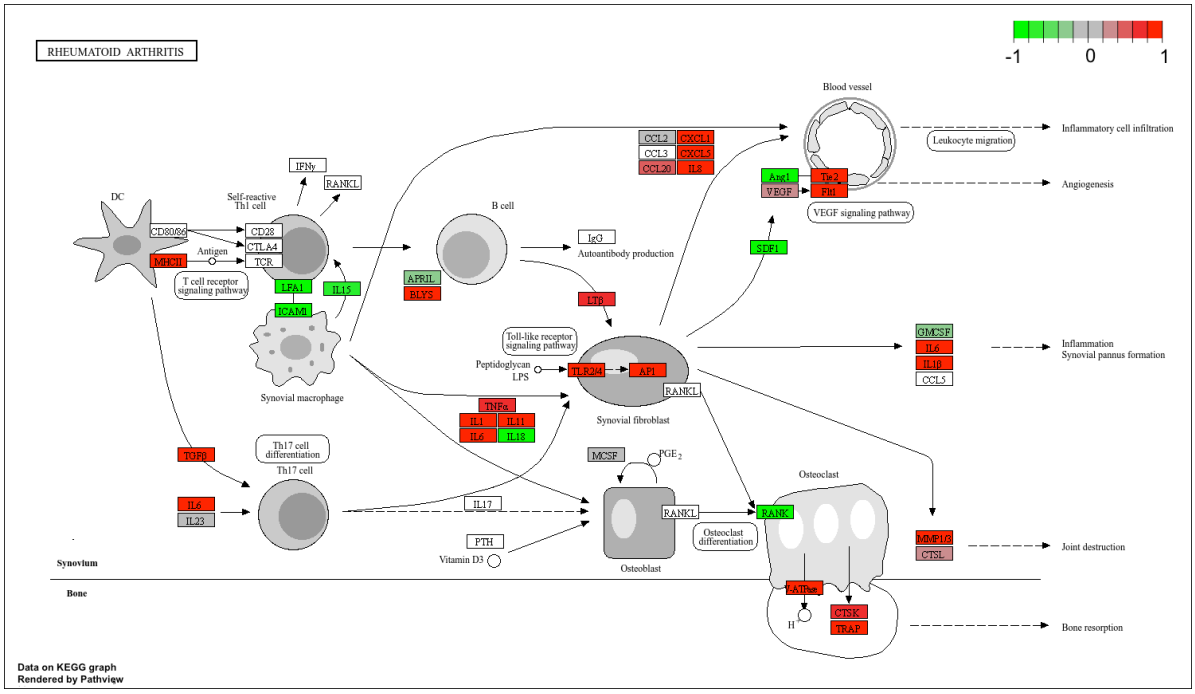


Figure 2: hsa04060


RHEUMATOID ARTHRITIS
-1
0
1
Blood vessel
Inflammatory cell infiltration
Leukocyte migration
Angiogenesis
VEGF signaling pathway
DC
Self-reactive Th1 cell
IFNγ
RANKL
CD80/86
CD28
CTLA4
TCR
MHCII
Antigen
T cell receptor signaling pathway
LFA1
IL15
ICAM1
Synovial macrophage
B cell
APRIL
BLYS
IgG
Autoantibody production
LTB
CCL2
CXCL1
CCL3
CXCL5
CCL20
IL8
Ang1
Tie2
VEGF
Flt
SDF1
Toll-like receptor signaling pathway
Peptidoglycan
LPS
TLR2/4
AP1
RANKL
Synovial fibroblast
GMCSF
IL6
IL1β
CCL5
Inflammation
Synovial pannus formation
TNFα
IL1
IL11
IL6
IL18
TGFβ
Th17 cell differentiation
Th17 cell
IL6
IL23
IL17
MCSF
PGE2
Osteoclast
RANK
RANKL
Osteoclast differentiation
MMP1/3
CTSL
Joint destruction
PTH
Vitamin D3
Osteoblast
Synovium
Bone
V-ATPase
H
CTSK
TRAP
Bone resorption
Data on KEGG graph
Rendered by Pathview

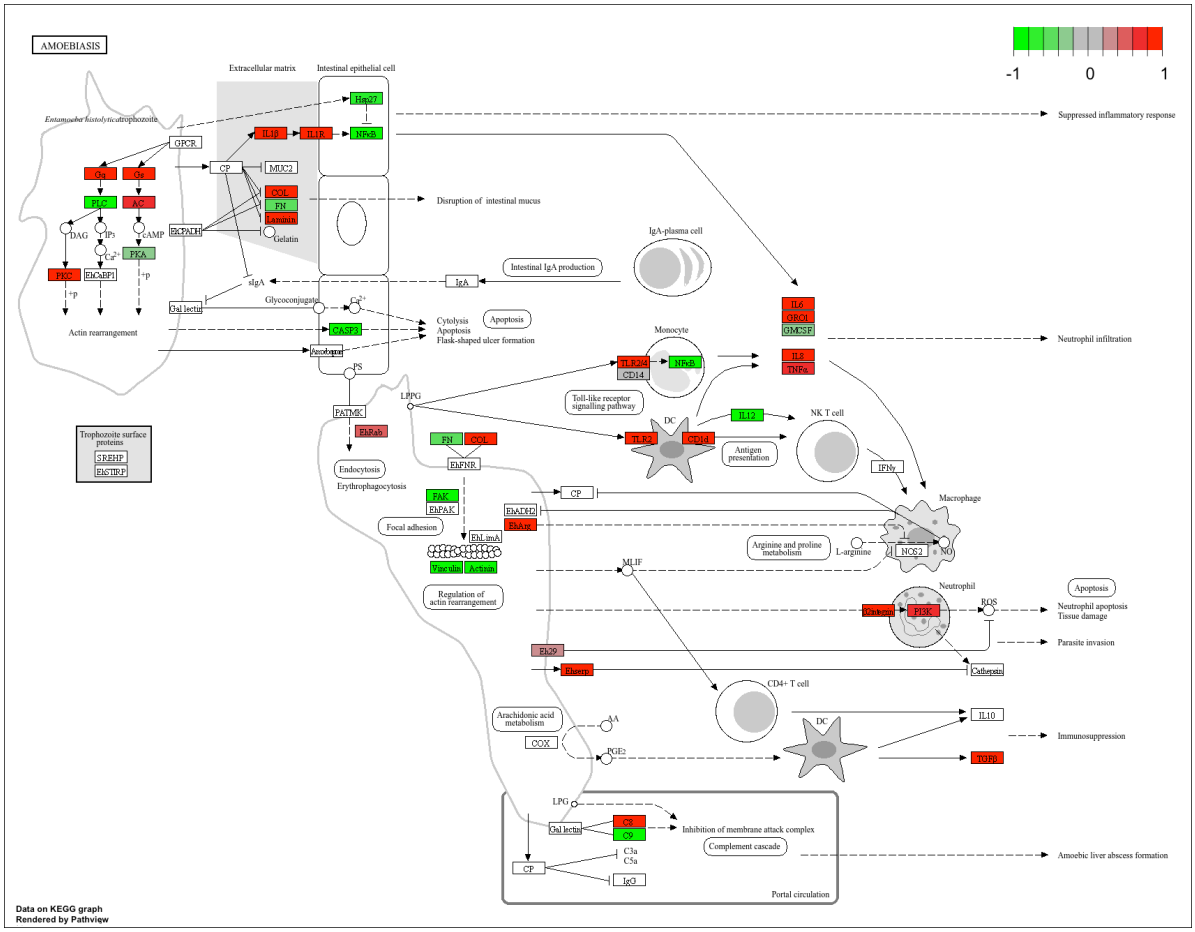
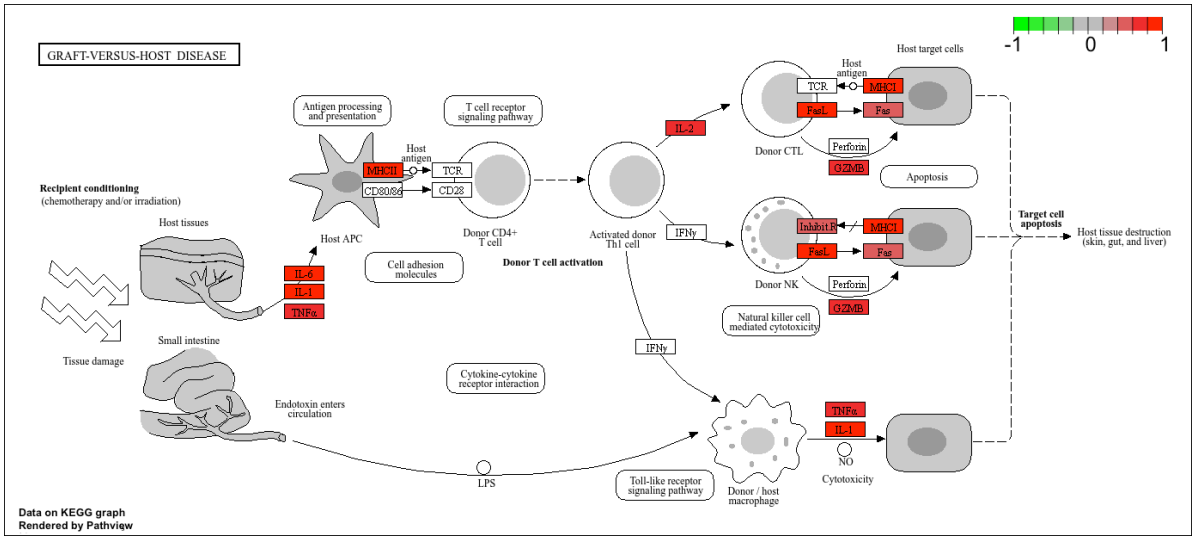


Figure 3: hsa05146


GRAFT-VERSUS-HOST DISEASE
-1
0
1
Host target cells
Host antigen
TCR
MHCI
FasL
Fas
Donor CTL
Perforin
GZMB
Apoptosis
Antigen processing and presentation
T cell receptor signaling pathway
IL-2
Host antigen
MHCII
TCR
CD80/86
CD28
Donor CD4+ T cell
Activated donor Th1 cell
IFNγ
Recipient conditioning
(chemotherapy and/or irradiation)
Host tissues
Host APC
Cell adhesion molecules
Donor T cell activation
IL-6
IL-1
TNFα
Inhibit R
Donor NK
Natural killer cell mediated cytotoxicity
Target cell apoptosis
Host tissue destruction (skin, gut, and liver)
Tissue damage
Small intestine
Cytokine-cytokine receptor interaction
Endotoxin enters circulation
LPS
Toll-like receptor signaling pathway
Donor / host macrophage
NO
Cytotoxicity
Data on KEGG graph
Rendered by Pathview

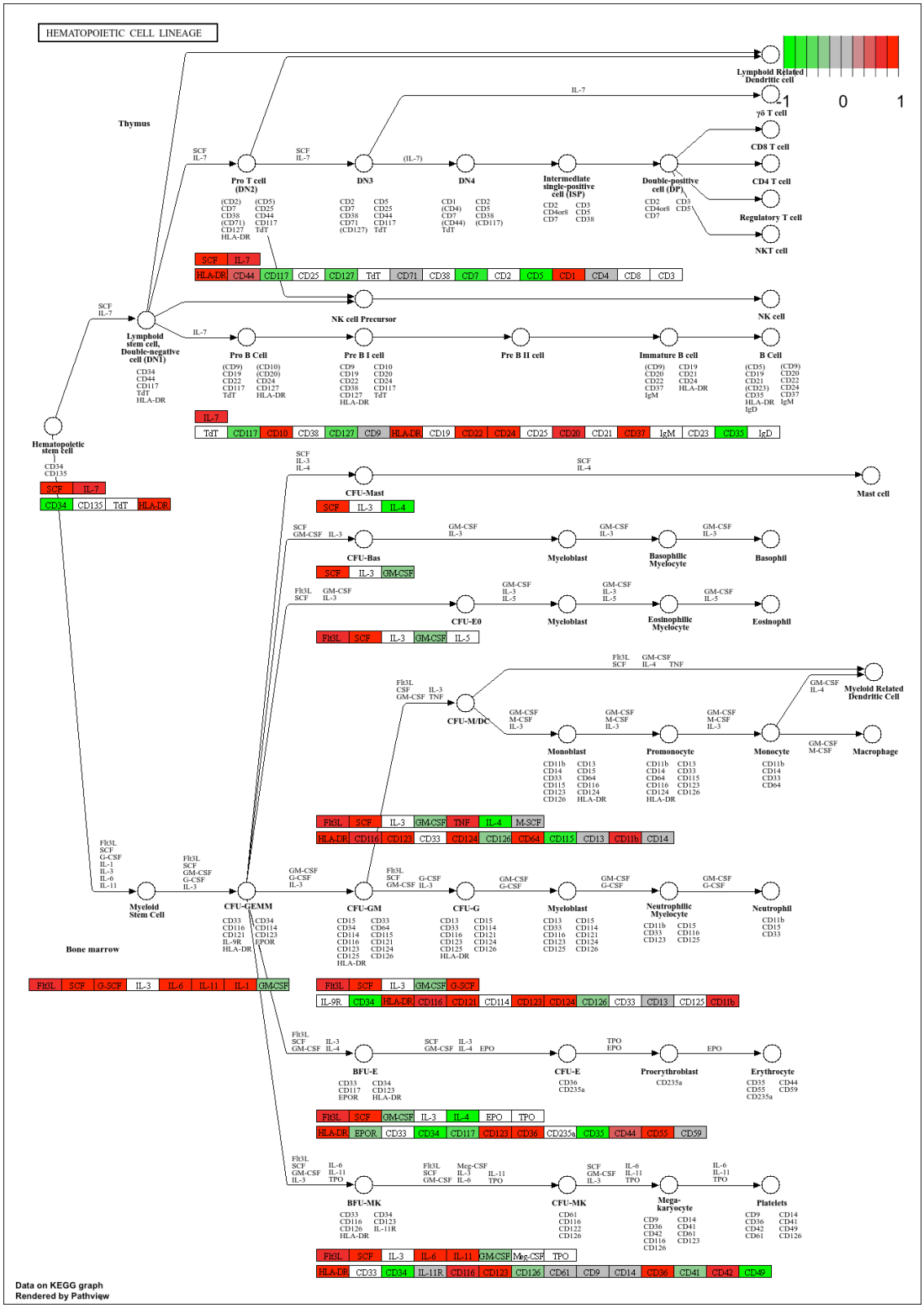


Figure 4: hsa0460

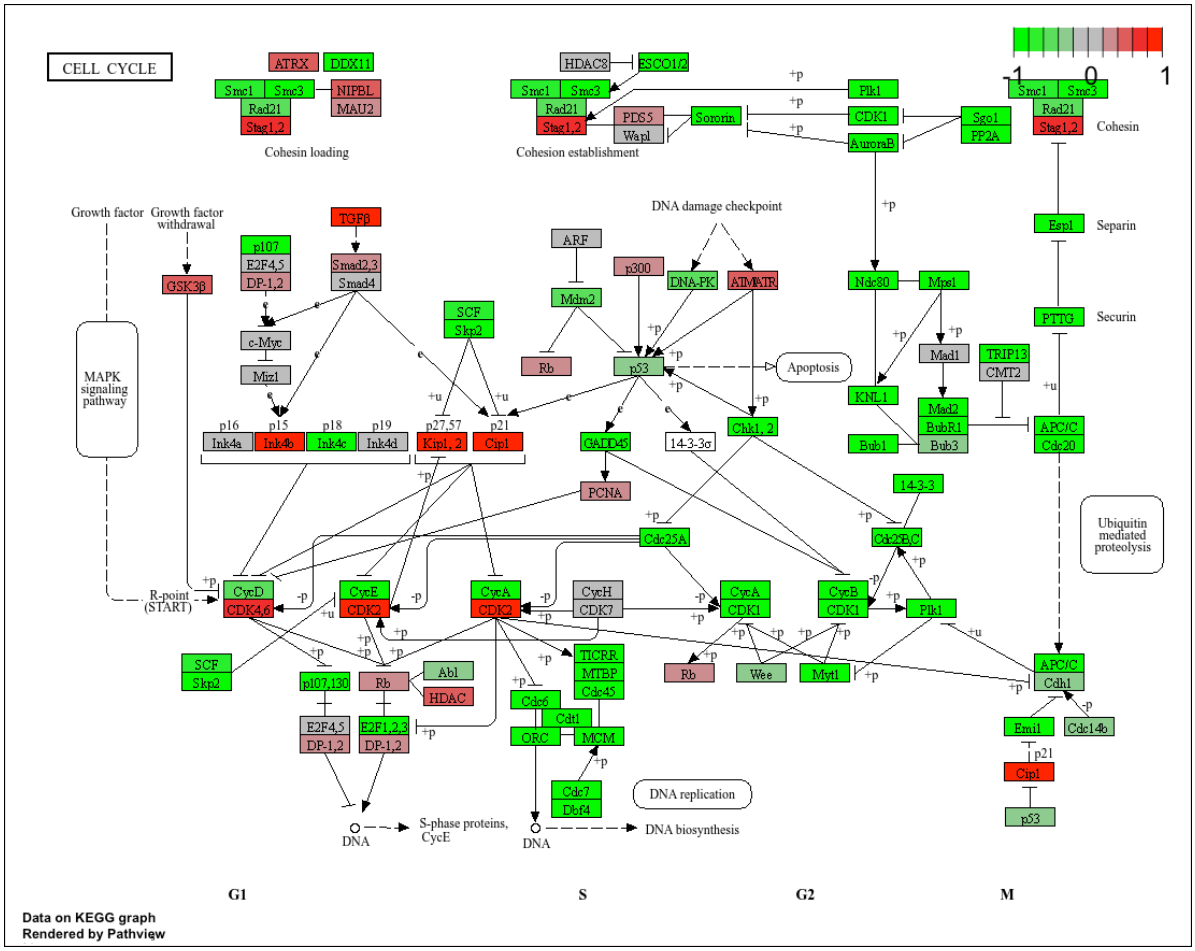
```
keggrespathways <- rownames(keggres$less)[1:5]

# Extract the 8 character long IDs part of each string
keggresids_2 = substr(keggrespathways, start=1, stop=8)
keggresids_2
```

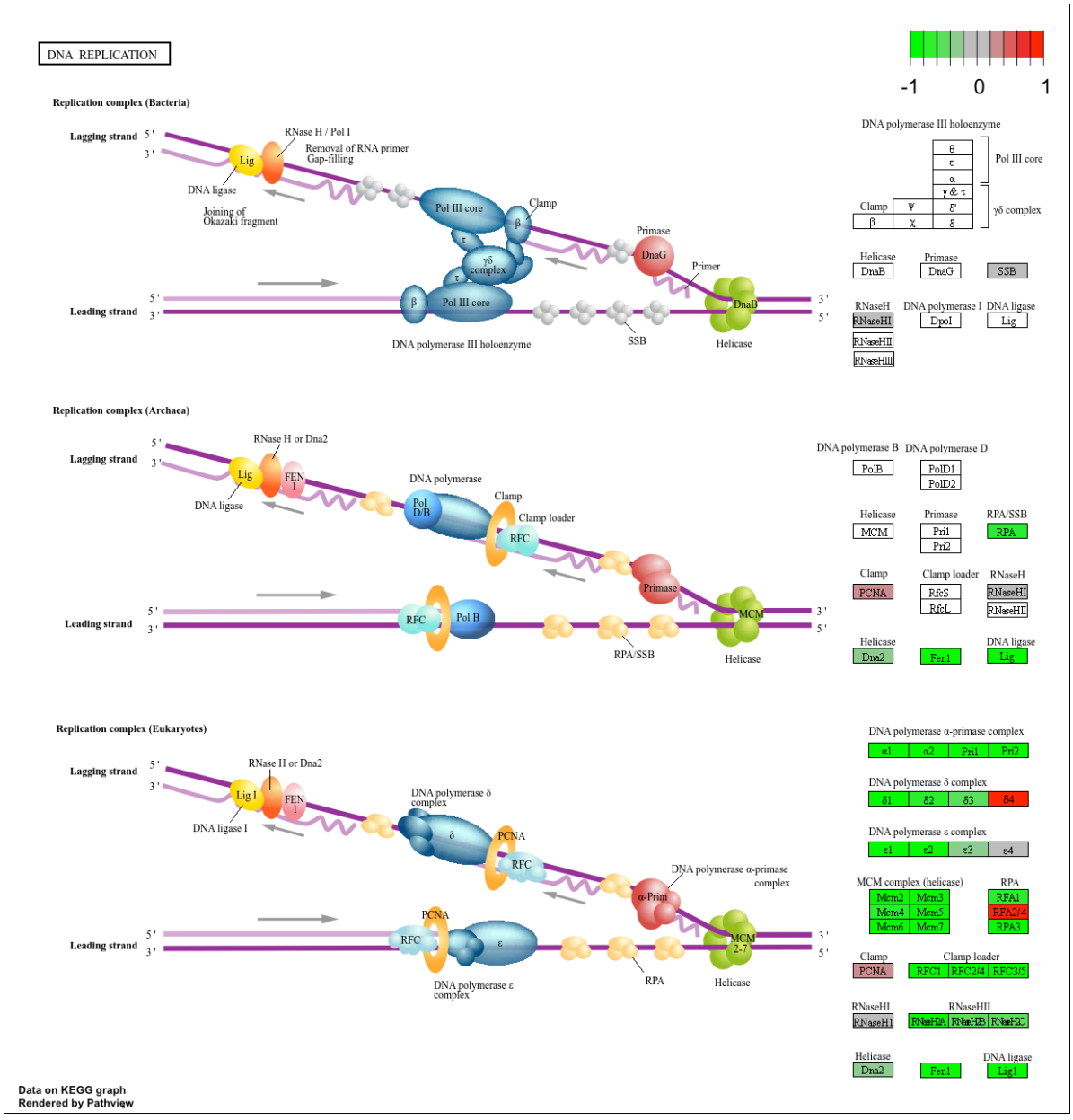
```
[1] "hsa04110" "hsa03030" "hsa05130" "hsa03013" "hsa03440"
```

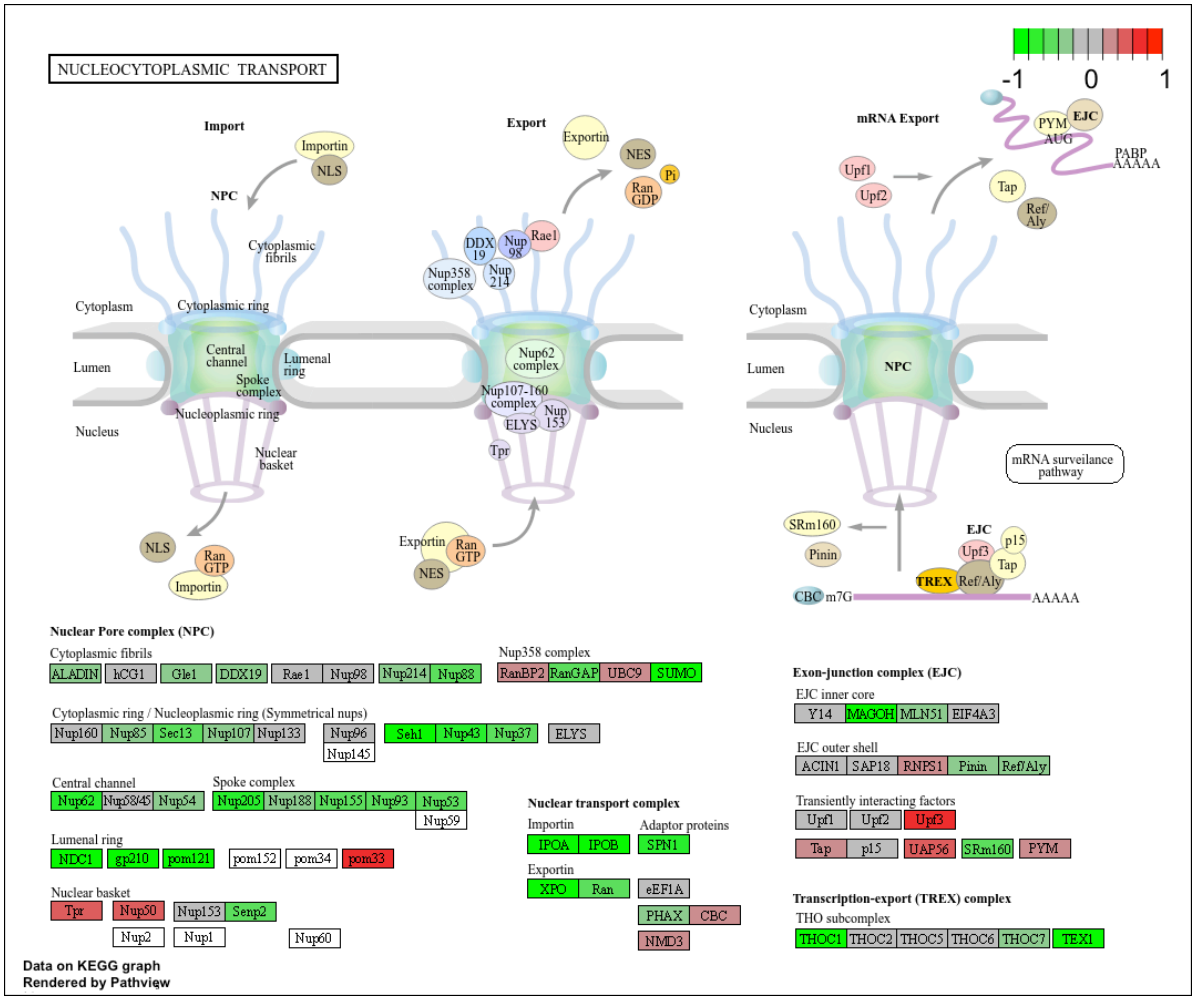
```
pathview(gene.data=foldchanges, pathway.id=keggresids_2, species="hsa")
```

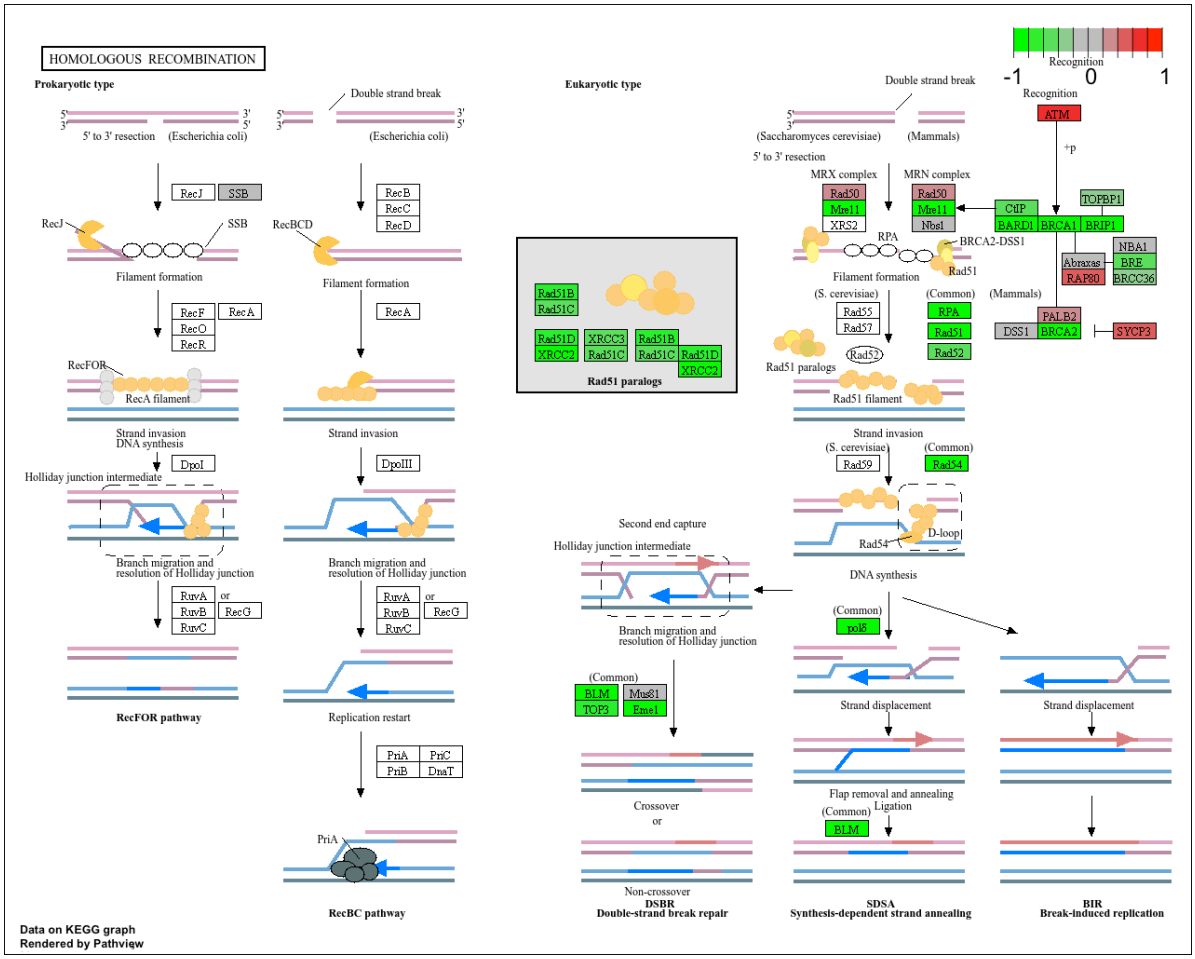
```
'select()' returned 1:1 mapping between keys and columns

Info: Working in directory /Users/michael/Desktop/BIMM143/class14

Info: Writing image file hsa04110.pathview.png

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

Info: Working in directory /Users/michael/Desktop/BIMM143/class14

Info: Writing image file hsa03030.pathview.png

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

Info: Working in directory /Users/michael/Desktop/BIMM143/class14

Info: Writing image file hsa05130.pathview.png

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

Info: Working in directory /Users/michael/Desktop/BIMM143/class14

Info: Writing image file hsa03013.pathview.png

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

Info: Working in directory /Users/michael/Desktop/BIMM143/class14
```

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




DNA REPLICATION
-1
0
1
Replication complex (Bacteria)
Lagging strand
Leading strand
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
DnaB
SSB
Helicase
DNA polymerase III holoenzyme
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
Lig
FEN
1
DNA ligase
DNA polymerase
Pol
D/B
Clamp
Clamp loader
RFC
Primase
MCM
RPA/SSB
Helicase
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
RNaseHII
Helicase
Dna2
Fen1
DNA ligase
Lig
Replication complex (Eukaryotes)
RNase H or Dna2
Lig I
FEN
1
DNA ligase I
DNA polymerase δ
complex
δ
PCNA
RFC
DNA polymerase α-primase
complex
α-Prim
MCM
2-7
RPA
Helicase
DNA polymerase ε
complex
ε
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
RNaseH1
RNaseHII
RNaseHIII
RNaseH2A
RNaseH2B
RNaseH2C
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
Lumen
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
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
Upf3
p15
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



Gene Ontology

```
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                       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
```

```
head(gobpres$less, 5)
```

```
                                            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
                                                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
```

Reactome analysis online

We need to take a little file of our significant genes that we can upload to the reactom webpage:

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

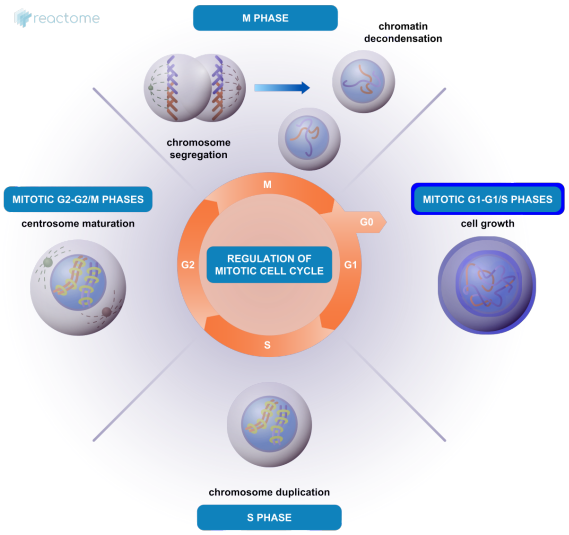
```
[1] "Total number of significant genes: 8147"
```

```
sig_genes[6]
```

```
ENSG00000136068
         "FLNB"
```

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

Then, to perform pathway analysis online go to the Reactome website (https://reactome.org/PathwayBrowser/# Select "choose file" to upload your significant gene list. Then, select the parameters "Project to Humans", then click "Analyze".


reactome
M PHASE
chromatin decondensation
chromosome segregation
MITOTIC G2-G2/M PHASES
centrosome maturation
MITOTIC G1-G1/S PHASES
cell growth
M
G0
G2
REGULATION OF MITOTIC CELL CYCLE
G1
S
chromosome duplication
S PHASE