ribosome_heatmaps_adipogenesis

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Changes:

- Using DE calculated from only 4 time points (Pro -> D0 -> D3 -> D15)

From previous revisions:

- printing lists of gene to annotate the terms/heatmaps

```
library(biomaRt)
library(ComplexHeatmap)
```

```
## Loading required package: grid
```

```
## ========================================
## ComplexHeatmap version 2.16.0
## Bioconductor page: http://bioconductor.org/packages/ComplexHeatmap/
## Github page: https://github.com/jokergoo/ComplexHeatmap
## Documentation: http://jokergoo.github.io/ComplexHeatmap-reference
##
## If you use it in published research, please cite either one:
## - Gu, Z. Complex Heatmap Visualization. iMeta 2022.
## - Gu, Z. Complex heatmaps reveal patterns and correlations in multidimensional
##     genomic data. Bioinformatics 2016.
##
##
## The new InteractiveComplexHeatmap package can directly export static
## complex heatmaps into an interactive Shiny app with zero effort. Have a try!
##
## This message can be suppressed by:
##   suppressPackageStartupMessages(library(ComplexHeatmap))
## ========================================
```

```
knitr::opts_chunk$set(echo = TRUE, dev = c("pdf"), fig.path = "ribosome_heatmaps_adipogenesis/", fig.di
```

```
rpkm = read.delim( "../03limma/adipogenesis_rpkm_tmm_means.tab", header=T) #rpkm table
head(rpkm); dim(rpkm)
```

```
##              Geneid Length gene_name
## 1 ENSG00000000003   4535    TSPAN6
## 2 ENSG00000000005   1610      TNMD
## 3 ENSG00000000419   1207      DPM1
## 4 ENSG00000000457   6883     SCYL3
## 5 ENSG00000000460   5967   C1orf112
## 6 ENSG00000000938   3474       FGR
##                                                 description day.2.D1G.bulk
## 1                                             tetraspanin 6    3.601999275
## 2                                               tenomodulin    0.007936845
## 3 dolichyl-phosphate mannosyltransferase subunit 1, catalytic   62.130005013
## 4                                       SCY1 like pseudokinase 3    1.426204636
## 5                             chromosome 1 open reading frame 112    1.906747475
## 6            FGR proto-oncogene, Src family tyrosine kinase    0.005503088
##   day.2.D2A.bulk day0.D1G.bulk day0.D2A.bulk day1.D1G.bulk day1.D2A.bulk
## 1      6.76071437    2.69543757    6.28332533    2.21416364    6.40180026
## 2      0.00000000    0.00000000    0.02464032    0.03748234    0.25958703
## 3     41.18788108   46.57260499   38.24198388   62.18075805   44.14029328
## 4      1.33753816    1.58269525    1.83452213    1.67277113    1.78269269
## 5      1.65219243    0.36937923    0.56555920    0.35661924    0.73777550
## 6      0.01975517    0.06610962    0.03066300    0.02233808    0.04456323
##   day15.D1G.bulk day15.D1G.floating day15.D2A.bulk day15.D2A.floating
## 1      9.1422795         15.7180139      8.3310883         15.8591145
## 2      0.5985436          0.5635616      1.9493616          2.5943861
## 3     35.7545717         30.0118693     36.1959969         43.8454420
## 4      1.7310483          1.5638762      1.6389382          1.9744310
## 5      0.5774263          0.8213003      0.4421079          0.7067799
## 6      0.6295882          3.7540597      1.9196028          3.2901662
##   day3.D1G.bulk day3.D2A.bulk day9.D1G.bulk day9.D2A.bulk
## 1    2.76755264    7.6279970    2.3380573    8.8221868
## 2    0.06034418    0.1242015    0.3061671    2.7625195
## 3   56.03395361   46.3859709   49.9509805   45.7146885
## 4    1.98222151    1.9524608    2.0051649    2.0390762
## 5    0.39508980    0.5258706    0.3640591    0.6355628
## 6    0.16554146    0.1578544    0.3397660    0.3845655
```

```
## [1] 21174    18
```

```r
combpval = read.delim("../03limma/nucleolus_adipogenesis_DE.tab", header=T)
head(combpval)
```

```
##              Geneid Length gene_name
## 1 ENSG00000151726   6284    ACSL1
## 2 ENSG00000099194   5362      SCD
## 3 ENSG00000042445   4005    RETSAT
## 4 ENSG00000056998   3655     GYG2
## 5 ENSG00000076555  14505    ACACB
## 6 ENSG00000101938   3920    CHRDL1
##                                         description      logFC   AveExpr        t
## 1 acyl-CoA synthetase long chain family member 1   12.916437  8.810891 50.67161
## 2                          stearoyl-CoA desaturase   16.490127 11.807874 43.63744
## 3                                 retinol saturase    8.195969  7.100134 36.32129
## 4                                     glycogenin 2   10.028582  4.980263 36.39842
```

```
## 5                        acetyl-CoA carboxylase beta  14.871196   7.351638 33.29351
## 6                               chordin like 1  14.207558   5.954537 32.38451
##         P.Value    adj.P.Val        B
## 1 8.291877e-37 1.755722e-32 68.92770
## 2 2.897205e-34 3.067271e-30 64.45829
## 3 2.309287e-31 9.779370e-28 59.33478
## 4 2.133268e-31 9.779370e-28 58.59981
## 5 7.038353e-30 2.474282e-26 55.41491
## 6 1.960845e-29 4.613214e-26 54.48242
```

```r
sig = merge(rpkm, combpval[c("gene_name","adj.P.Val")])
head(sig); dim(sig)
```

```
##   gene_name          Geneid Length
## 1      A1BG ENSG00000121410   4006
## 2  A1BG-AS1 ENSG00000268895   2793
## 3       A2M ENSG00000175899   6384
## 4   A2M-AS1 ENSG00000245105   2816
## 5    A4GALT ENSG00000128274   3407
## 6      ABAT ENSG00000183044   9744
##                                    description day.2.D1G.bulk
## 1                        alpha-1-B glycoprotein     0.01815337
## 2                             A1BG antisense RNA 1     0.90975838
## 3                        alpha-2-macroglobulin     0.21349869
## 4                             A2M antisense RNA 1     0.29287176
## 5 alpha 1,4-galactosyltransferase (P blood group)     4.67272390
## 6                 4-aminobutyrate aminotransferase     0.81520901
##   day.2.D2A.bulk day0.D1G.bulk day0.D2A.bulk day1.D1G.bulk day1.D2A.bulk
## 1      0.1047458    0.05978501    0.06987478    0.04544531     0.0815610
## 2      1.3179450    0.90779680    1.23126472    1.87323332     2.0247272
## 3      0.8046160    7.33195064   14.48690784    6.17424097    12.9542154
## 4      0.2337510    0.49255301    0.29768876    0.40976779     0.4652485
## 5      2.0682521    8.79899844    2.55743385    4.76230682     1.3129724
## 6      0.7232442    1.13859664    0.99927577    1.94422327     1.6450301
##   day15.D1G.bulk day15.D1G.floating day15.D2A.bulk day15.D2A.floating
## 1     0.03912984          0.0185444     0.03159112         0.01945774
## 2     1.27934477          1.0516270     1.39386219         0.74776720
## 3    55.90851651         39.4762646    73.55229005        69.76889731
## 4     0.80400184          1.1234145     0.59104284         0.81515811
## 5     4.78652768          9.4369909     4.47173184         2.50902053
## 6     1.99799798          2.1548674     2.29141846         2.42183547
##   day3.D1G.bulk day3.D2A.bulk day9.D1G.bulk day9.D2A.bulk    adj.P.Val
## 1    0.04925457    0.05550053    0.02259233    0.05723545 4.206849e-01
## 2    1.72406789    1.63700087    1.06522748    1.13733587 1.941349e-01
## 3    7.65875953   10.30956590    9.76357919   14.88341115 2.466237e-18
## 4    0.46771549    0.33457401    0.48169174    0.48900749 2.002885e-06
## 5    6.63361018    2.40230131    4.70604627    0.93162324 1.130921e-01
## 6    2.28435365    1.54204016    0.79823994    0.69142124 1.171812e-08
```

```
## [1] 20590    19
```

```
#formatting
sig = sig[sig$adj.P.Val < 0.01,]
sig$adj.P.Val = NULL
#discard duplicate rownames
sig = sig[!duplicated(sig$gene_name),]
dim(sig)
```

```
## [1] 11109    18
```

```
rownames(sig) = sig$gene_name
sig$gene_name = NULL
```

remove floating adipocytes

```
sig = sig[!grepl("floating", colnames(sig))]
head(sig)
```

```
##                        Geneid Length
## A2M            ENSG00000175899   6384
## A2M-AS1        ENSG00000245105   2816
## ABAT           ENSG00000183044   9744
## ABBA01000935.2 ENSG00000283544    838
## ABCA1          ENSG00000165029  11350
## ABCA2          ENSG00000107331  11514
##                                                        description
## A2M                                           alpha-2-macroglobulin
## A2M-AS1                                            A2M antisense RNA 1
## ABAT                                   4-aminobutyrate aminotransferase
## ABBA01000935.2 primase, DNA, polypeptide 2 (58kDa) (PRIM2) pseudogene
## ABCA1                           ATP binding cassette subfamily A member 1
## ABCA2                           ATP binding cassette subfamily A member 2
##                day.2.D1G.bulk day.2.D2A.bulk day0.D1G.bulk day0.D2A.bulk
## A2M                0.2134987      0.8046160      7.331951    14.4869078
## A2M-AS1            0.2928718      0.2337510      0.492553     0.2976888
## ABAT               0.8152090      0.7232442      1.138597     0.9992758
## ABBA01000935.2     2.4410337      1.8296952      1.196099     1.3088228
## ABCA1              4.1563001     10.1799243      5.787744    10.7448188
## ABCA2              1.5115265      0.6382086      3.833307     0.9471078
##                day1.D1G.bulk day1.D2A.bulk day15.D1G.bulk day15.D2A.bulk
## A2M                6.1742410    12.9542154     55.9085165    73.5522900
## A2M-AS1            0.4097678     0.4652485      0.8040018     0.5910428
## ABAT               1.9442233     1.6450301      1.9979980     2.2914185
## ABBA01000935.2     0.6836294     0.7696220      0.3267697     0.3416815
## ABCA1             12.9278962    25.8857655     27.3567395    26.2857860
## ABCA2              2.7418260     0.6593513      1.9377047     3.7777824
##                day3.D1G.bulk day3.D2A.bulk day9.D1G.bulk day9.D2A.bulk
## A2M                7.6587595    10.3095659     9.7635792    14.8834112
## A2M-AS1            0.4677155     0.3345740      0.4816917     0.4890075
## ABAT               2.2843537     1.5420402      0.7982399     0.6914212
## ABBA01000935.2     0.4290992     0.5077503      0.8565011     0.5494230
## ABCA1             12.3424820    28.8494728     18.7174119    29.2122896
## ABCA2              3.4219837     1.2736018      4.4296567     1.3636709
```

4

```
sig = sig[grepl("day(.2|0|3|15)", colnames(sig))]
#separate by donor
d1 = sig[grep("D1G", colnames(sig), value=T)]
head(d1)
```

```
##                 day.2.D1G.bulk day0.D1G.bulk day15.D1G.bulk day3.D1G.bulk
## A2M                 0.2134987      7.331951     55.9085165      7.6587595
## A2M-AS1             0.2928718      0.492553      0.8040018      0.4677155
## ABAT                0.8152090      1.138597      1.9979980      2.2843537
## ABBA01000935.2      2.4410337      1.196099      0.3267697      0.4290992
## ABCA1               4.1563001      5.787744     27.3567395     12.3424820
## ABCA2               1.5115265      3.833307      1.9377047      3.4219837
```

```
d2 = sig[grep("D2A", colnames(sig), value=T)]
head(d2)
```

```
##                 day.2.D2A.bulk day0.D2A.bulk day15.D2A.bulk day3.D2A.bulk
## A2M                 0.8046160     14.4869078     73.5522900     10.3095659
## A2M-AS1             0.2337510      0.2976888      0.5910428      0.3345740
## ABAT                0.7232442      0.9992758      2.2914185      1.5420402
## ABBA01000935.2      1.8296952      1.3088228      0.3416815      0.5077503
## ABCA1              10.1799243     10.7448188     26.2857860     28.8494728
## ABCA2               0.6382086      0.9471078      3.7777824      1.2736018
```

Get GO terms

```
mart <- biomaRt::useMart(biomart = "ensembl",
  dataset = "hsapiens_gene_ensembl",
  host = "https://jan2019.archive.ensembl.org")

cyt_ribosome = biomaRt::getBM(c("external_gene_name", "ensembl_gene_id","go_linkage_type"),
           filters = "go",
           values = c("GO:0022625","GO:0022627"),
           mart = mart)
length(unique(cyt_ribosome$ensembl_gene_id)) #120 cytosolic ribosome genes
```

```
## [1] 120
```

```
translation = getBM(c("external_gene_name", "ensembl_gene_id"),
           filters = "go",
           values = "GO:0006412",
           mart = mart)
nrow(translation)
```

```
## [1] 378
```

```
norp_trans = translation[!grepl("^M?RP", translation$external_gene_name),]
nrow(norp_trans) #165 non RP translation genes
```

```
## [1] 213
```

```
ribogen = biomaRt::getBM(c("external_gene_name", "ensembl_gene_id","go_linkage_type"),
            filters = "go",
            values = c("GO:0042254"),
            mart = mart)
length(unique(ribogen$ensembl_gene_id)) #104
```

```
## [1] 104
```

```
norp_ribogen = ribogen[!grepl("^M?RP", ribogen$external_gene_name),]
length(unique(norp_ribogen$ensembl_gene_id)) #96
```

```
## [1] 96
```

Check the gene lists

```
summary(translation$external_gene_name %in% rownames(sig))
```

```
##    Mode   FALSE    TRUE
## logical    191     187
```

```
summary(ribogen$external_gene_name %in% rownames(sig))
```

```
##    Mode   FALSE    TRUE
## logical    333     275
```

Donor 1

heatmap formatting

```
colnames(d1) = gsub(".D1G.bulk","", colnames(d1))
d1 = d1[c("day.2","day0","day3","day15")]

#create zscores of log transformed rpkms
zs = t(scale(t(log2(d1+1))))
 # before log scaling the median is always about -0.2, meaning theres a negative bias to the values
summary(zs) # the median is more variable between timepoints upon log scaling
```

```
##      day.2               day0               day3               day15
## Min.   :-1.4996364   Min.   :-1.49360   Min.   :-1.49784   Min.   :-1.499764
## 1st Qu.:-0.7525029   1st Qu.:-0.59169   1st Qu.:-0.64610   1st Qu.:-0.972699
## Median : 0.0000832   Median :-0.11097   Median :-0.10636   Median :-0.128307
## Mean   : 0.0670357   Mean   :-0.04112   Mean   :-0.02994   Mean   : 0.004022
## 3rd Qu.: 0.9457481   3rd Qu.: 0.48140   3rd Qu.: 0.58149   3rd Qu.: 1.097580
## Max.   : 1.5000000   Max.   : 1.49998   Max.   : 1.49928   Max.   : 1.500000
```

```
#create vector for ordering
#order = as.vector(zs[,"day15"] - zs[,"day.2"])
order = apply(zs, 1, cor, y=1:ncol(zs)) #uses pearson correlation
head(order)
```

```
##            A2M       A2M-AS1          ABAT ABBA01000935.2          ABCA1
##      0.9517668     0.9234575     0.8937626     -0.9643199      0.9818398
##          ABCA2
##      0.1559002
```

Cytosolic ribosome components with significantly different expression cross the timecourse.

```
summary(rownames(zs) %in% cyt_ribosome$external_gene_name) #72 genes
```

```
##     Mode    FALSE     TRUE
## logical    11035       74
```

```
Heatmap(as.matrix(zs[rownames(zs) %in% cyt_ribosome$external_gene_name,]),
        cluster_columns = F,
        name="Relative FPKM", column_title = "Cytosolic ribosome",
        col=circlize::colorRamp2(c(-2,0,2),c(rgb(0.2,0.3,0.6),rgb(1,1,1),rgb(0.9,0.1,0.1))),
         row_names_gp = gpar(fontsize = 7),
         show_row_names = FALSE,
        row_dend_reorder = order[rownames(zs) %in% cyt_ribosome$external_gene_name])
```

Cytosolic ribosome
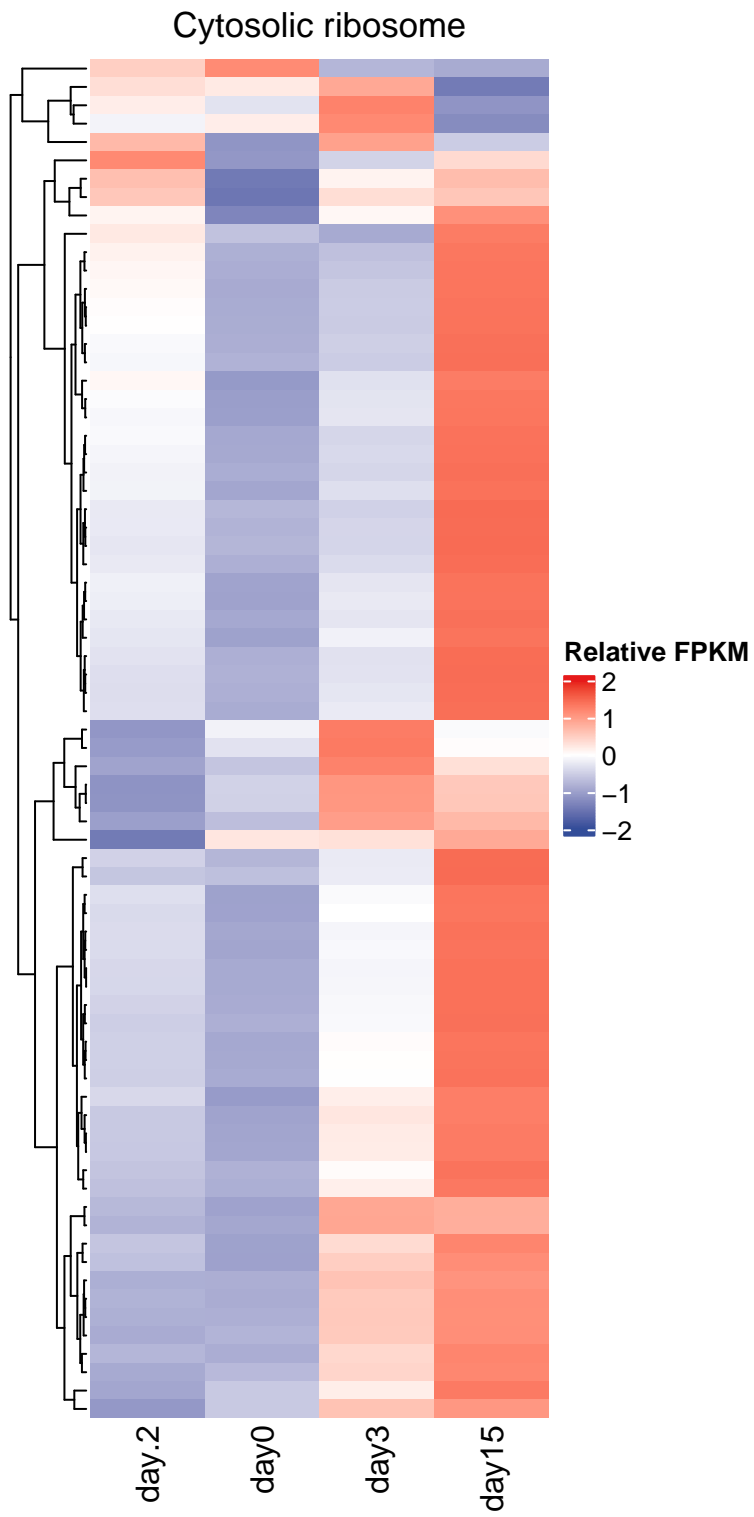
Translation terms

And with splits

```
Heatmap(as.matrix(zs[rownames(zs) %in% translation$external_gene_name,]),
        cluster_columns = F,
        name="Relative FPKM",
        column_title = "Translation",
        col=circlize::colorRamp2(c(-2,0,2),c(rgb(0.2,0.6,1),rgb(0,0,0),rgb(0.95,0.3,0.3))),
        show_row_names = F, row_split=4)
```
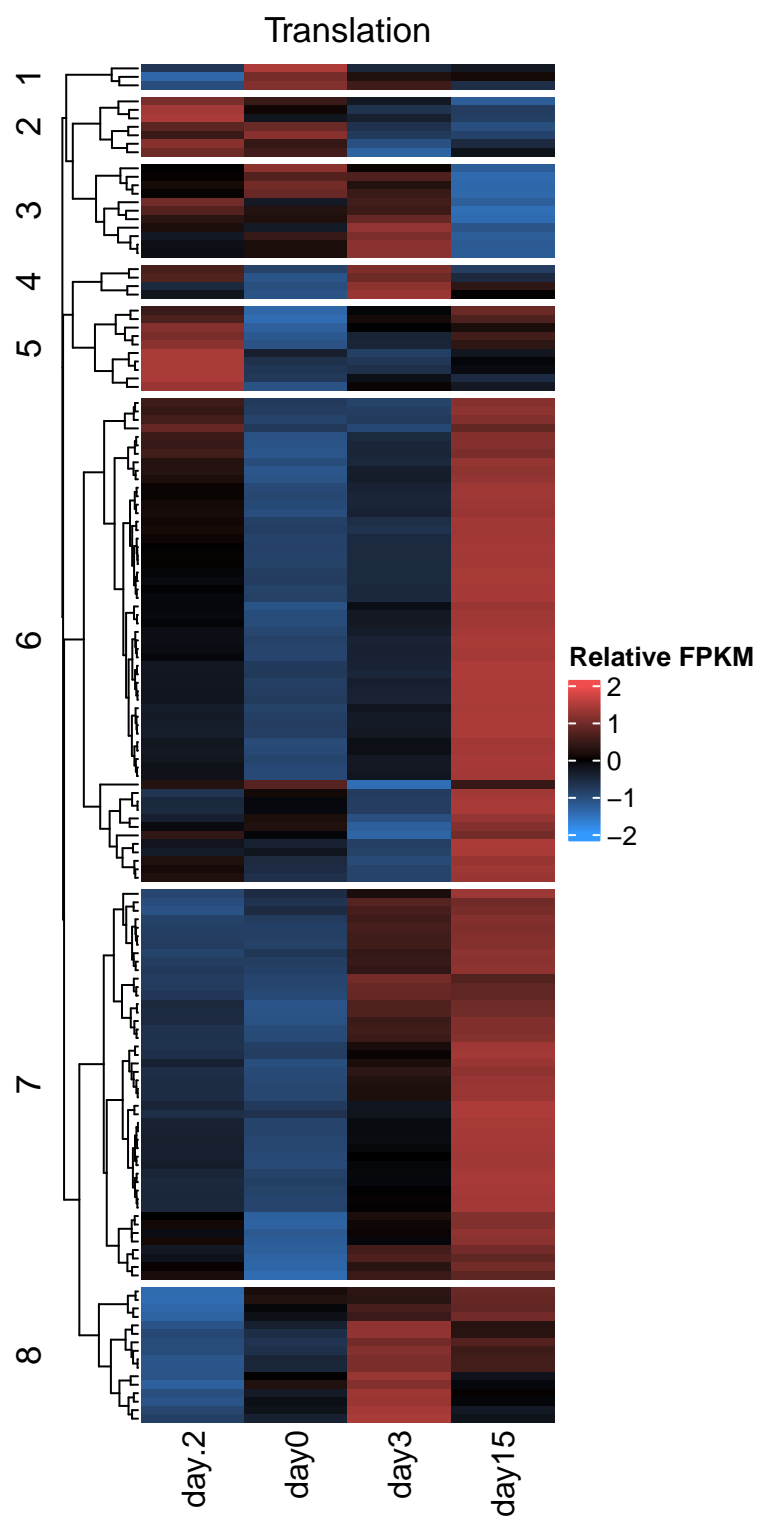
```
Heatmap(as.matrix(zs[rownames(zs) %in% translation$external_gene_name,]),
        cluster_columns = F,
        name="Relative FPKM",
        column_title = "Translation",
        col=circlize::colorRamp2(c(-2,0,2),c(rgb(0.2,0.6,1),rgb(0,0,0),rgb(0.95,0.3,0.3))),
```
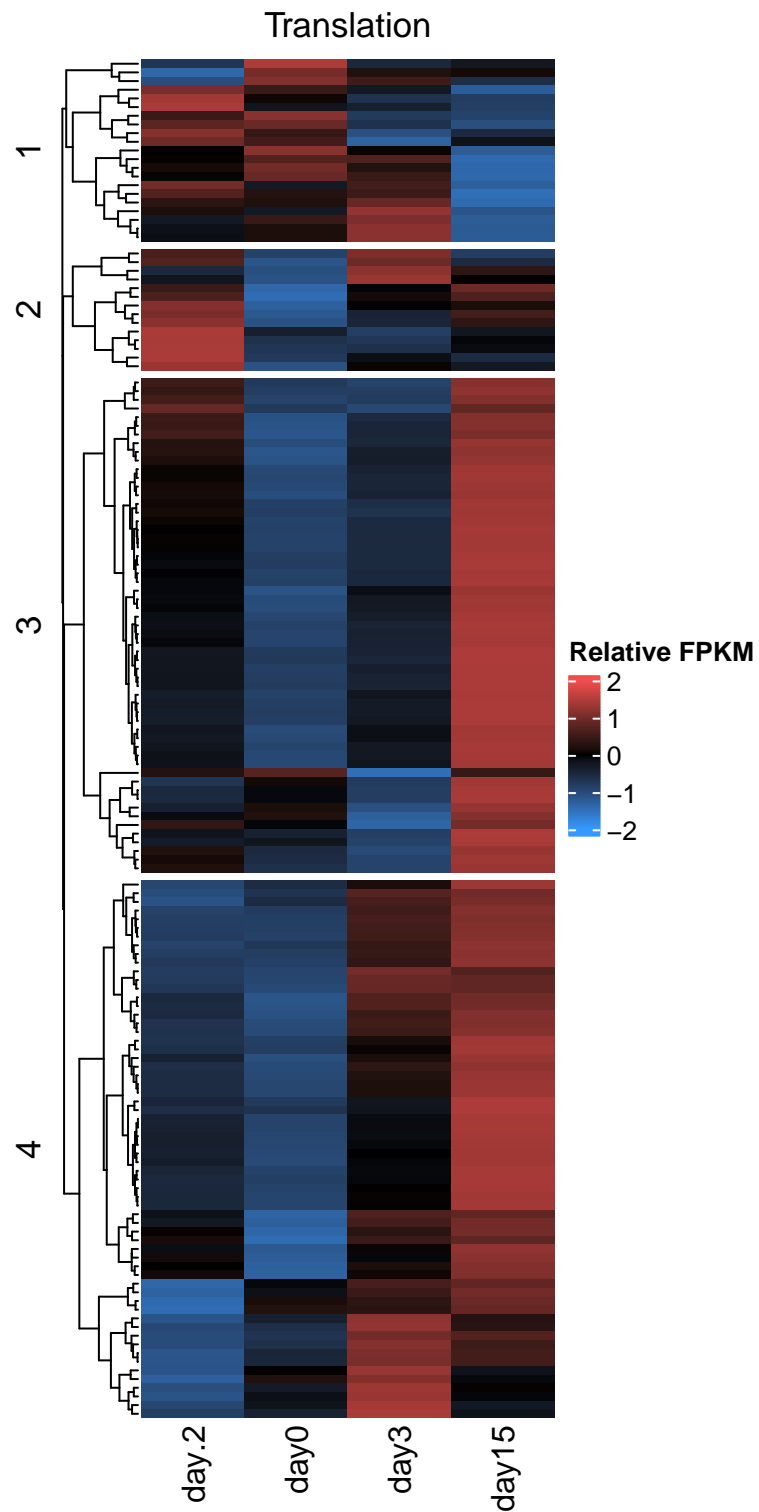
```
show_row_names = F, row_split=8)
```


Translation

Figure3a Lower panel

```
Heatmap(as.matrix(zs[rownames(zs) %in% translation$external_gene_name,]),
        cluster_columns = F,
        name="Relative FPKM",
        column_title = "Translation",
        col=circlize::colorRamp2(c(-2,0,2),c(rgb(0.2,0.6,1),rgb(0,0,0),rgb(0.95,0.3,0.3))),
        show_row_names = T, row_split=4, row_names_gp = gpar(fontsize = 4),
        row_dend_reorder = order[rownames(zs) %in% translation$external_gene_name])
```
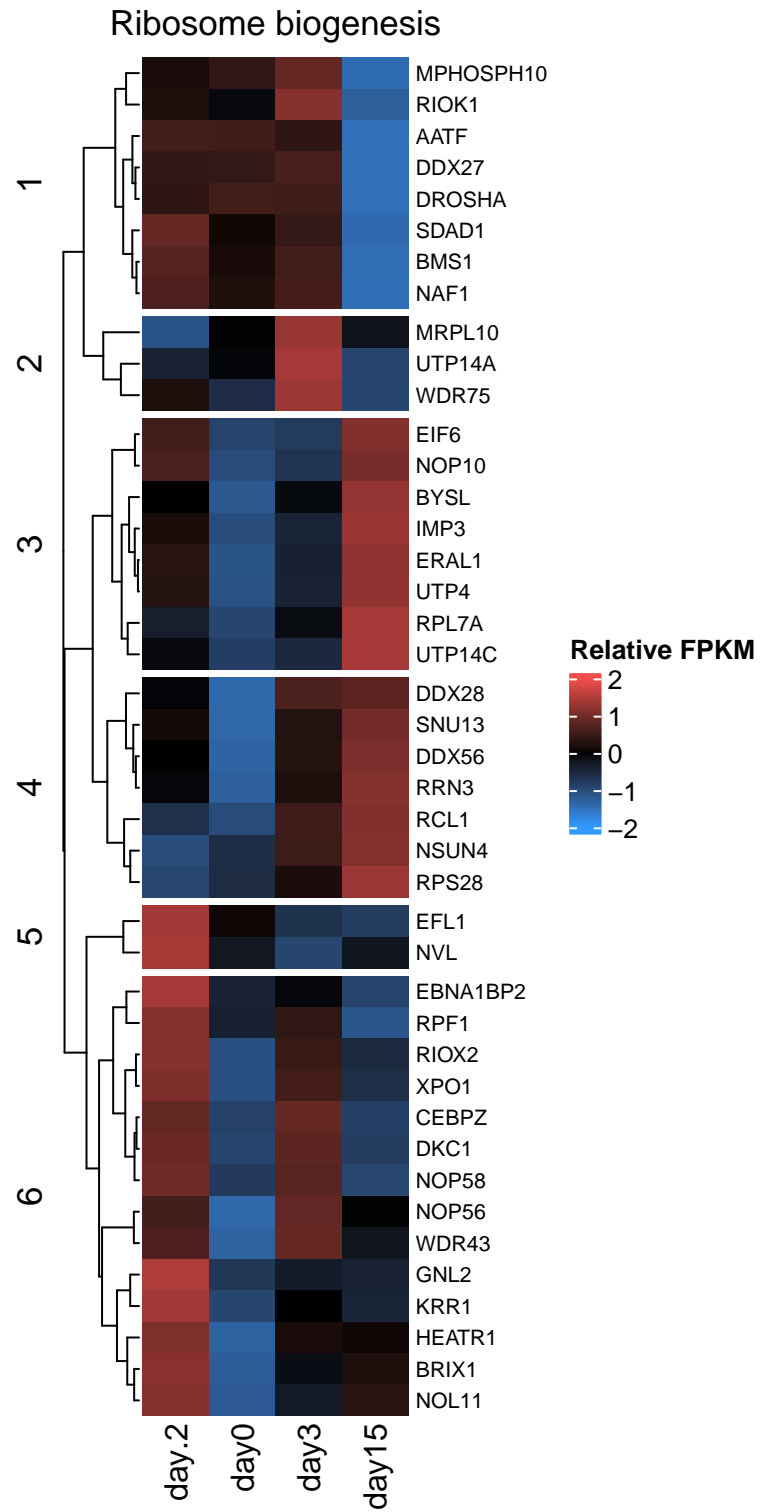
Translation

Ribosome biogenesis

```
Heatmap(as.matrix(zs[rownames(zs) %in% ribogen$external_gene_name,]),
        cluster_columns = F,
        name="Relative FPKM",
        column_title = "Ribosome biogenesis",
        height=0.25*length(unique(translation$external_gene_name)),
        width=0.5*ncol(d1),
         col=circlize::colorRamp2(c(-2,0,2),c(rgb(0.2,0.6,1),rgb(0,0,0),rgb(0.95,0.3,0.3))),
         row_names_gp = gpar(fontsize = 8), row_split=6,
        row_dend_reorder = order[rownames(zs) %in% ribogen$external_gene_name])
```

Ribosome biogenesis

Figure3a Upper panel

```r
Heatmap(as.matrix(zs[rownames(zs) %in% ribogen$external_gene_name,]),
        cluster_columns = F,
        name="Relative FPKM",
        column_title = "Ribosome biogenesis",
        height=0.25*length(unique(translation$external_gene_name)),
        width=0.5*ncol(d1),
         col=circlize::colorRamp2(c(-2,0,2),c(rgb(0.2,0.6,1),rgb(0,0,0),rgb(0.95,0.3,0.3))),
         row_names_gp = gpar(fontsize = 8), row_split=3,
        row_dend_reorder = order[rownames(zs) %in% ribogen$external_gene_name])
```

Ribosome biogenesis
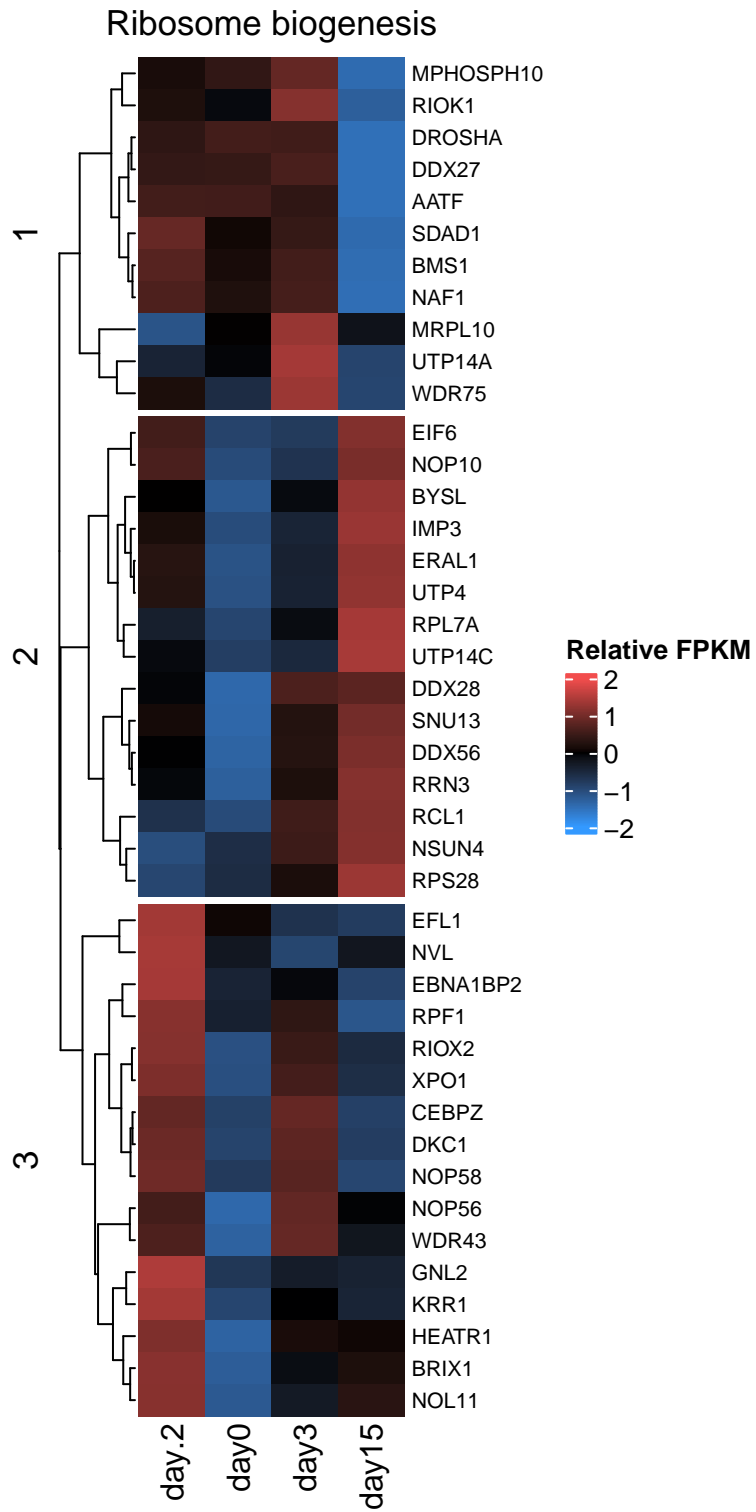
Donor 2

heatmap formatting

```r
colnames(d2) = gsub(".D2A.bulk","", colnames(d2))
d2 = d2[c("day.2","day0","day3","day15")]

#create zscores
zs13 = t(scale(t(log2(d2+1))))
head(zs13)
```

```
##                      day.2       day0       day3       day15
## A2M             -1.2620822  0.1461689 -0.05976171  1.1756750
## A2M-AS1         -0.8671337 -0.4100500 -0.15649903  1.4336827
## ABAT            -1.0442342 -0.5202807  0.32671966  1.2377953
## ABBA01000935.2   1.1225184  0.5441208 -0.66742539 -0.9992138
## ABCA1           -0.9099448 -0.8166744  0.94826986  0.7783493
## ABCA2           -0.8334671 -0.4668576 -0.13785871  1.4381834
```

```r
# create order vector
#order13 = as.vector(zs13[,"day15"] - zs13[,"day.2"])
order13 = apply(zs13, 1, cor, y=1:ncol(zs13))
```

Cytosolic ribosome components with significantly different expression cross the timecourse. Day 9 and floating adipocytes share high expression of many of these genes.

```r
summary(rownames(zs13) %in% cyt_ribosome$external_gene_name) #72 genes
```
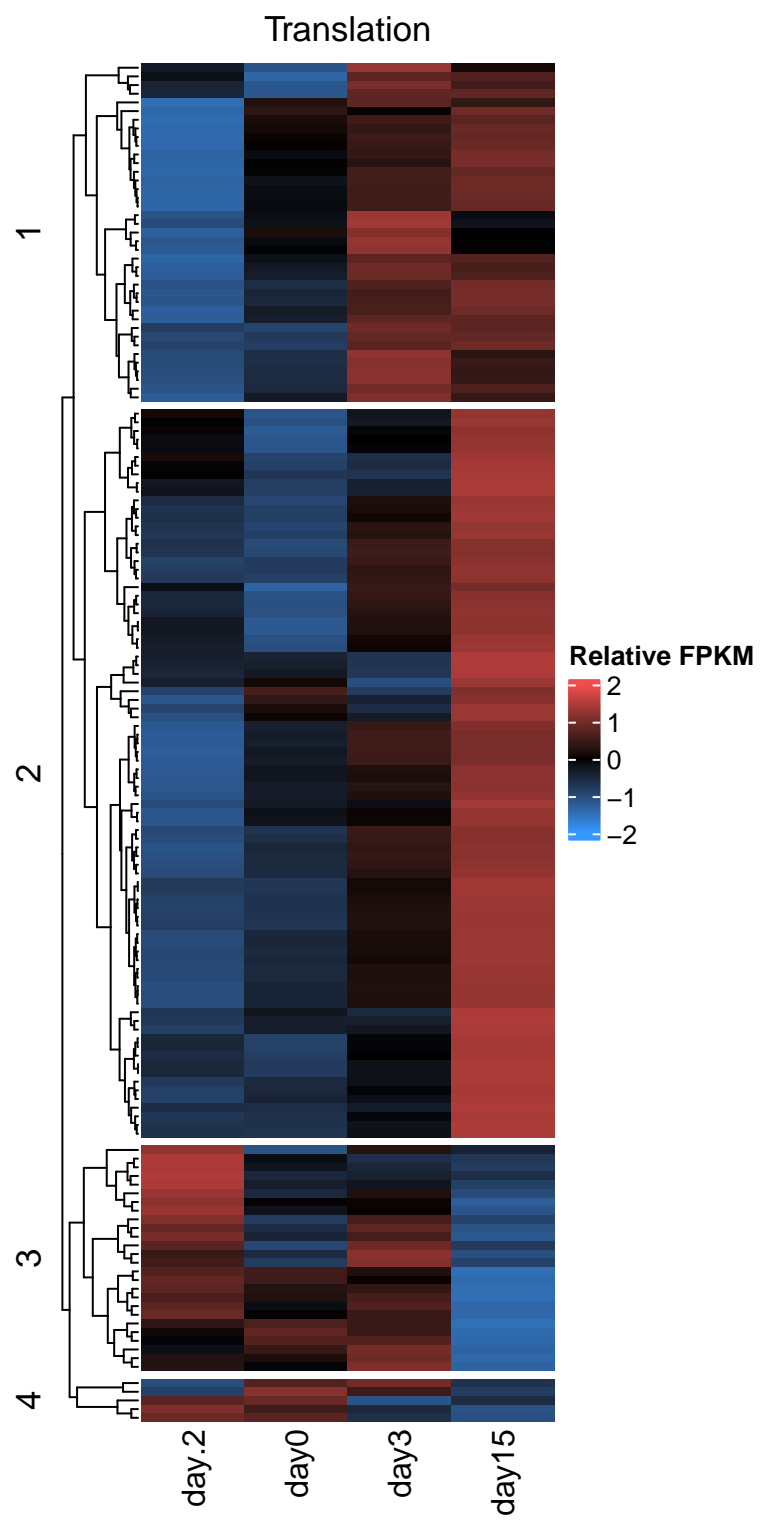
```
##    Mode   FALSE    TRUE
## logical   11035      74
```

```r
Heatmap(as.matrix(zs13[rownames(zs13) %in% cyt_ribosome$external_gene_name,]),
        cluster_columns = F,
        name="Relative FPKM", column_title = "Cytosolic ribosome",
        height=0.5*length(unique(cyt_ribosome$external_gene_name)),
        width=0.5*ncol(d1),
        col=circlize::colorRamp2(c(-2,0,2),c(rgb(0.2,0.3,0.6),rgb(1,1,1),rgb(0.9,0.1,0.1))),
        row_dend_reorder = order13[rownames(zs13) %in% cyt_ribosome$external_gene_name],
        show_row_names = F)
```
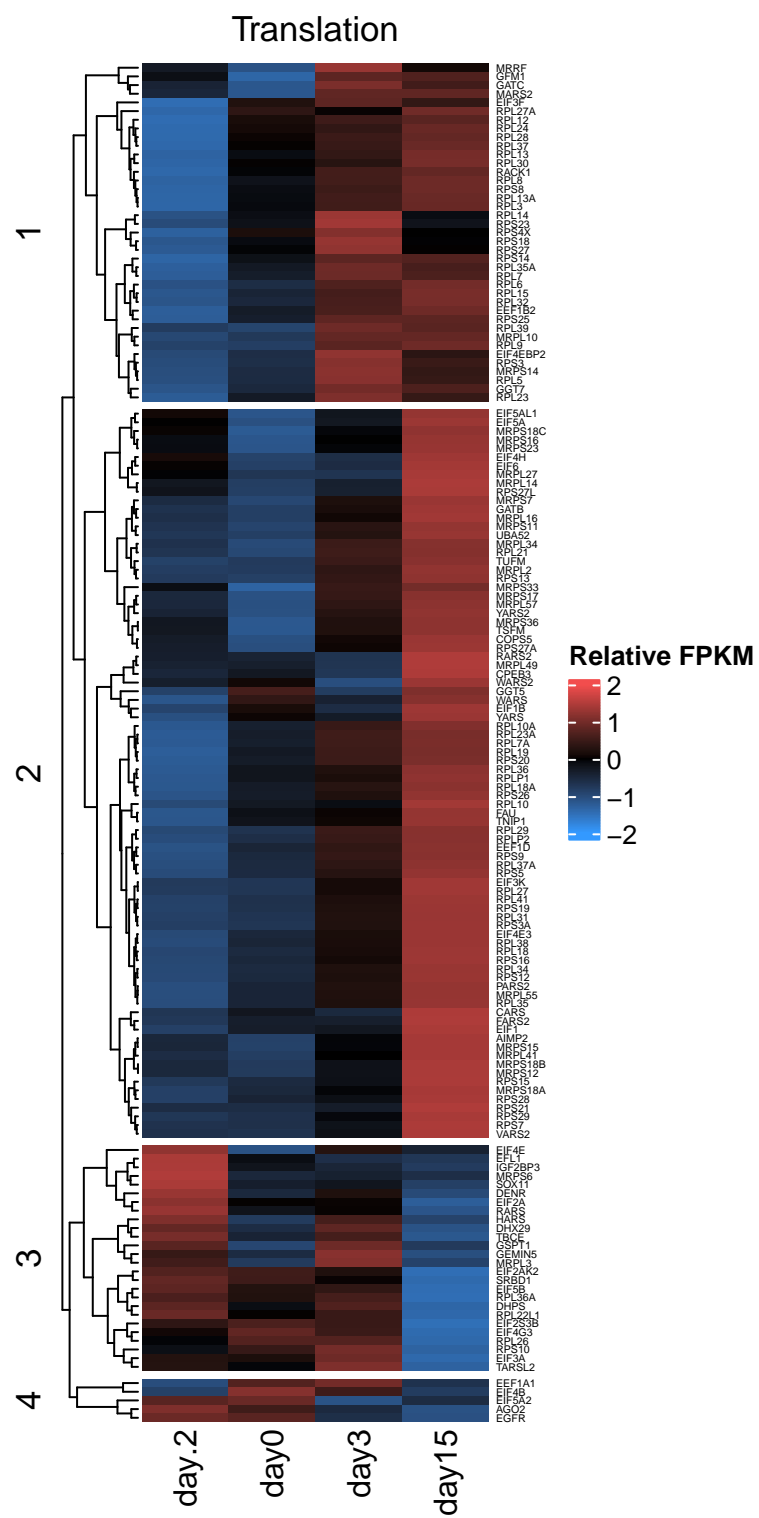
Cytosolic ribosome

day.2 day0 day3 day15

Relative FPKM
2
1
0
−1
−2
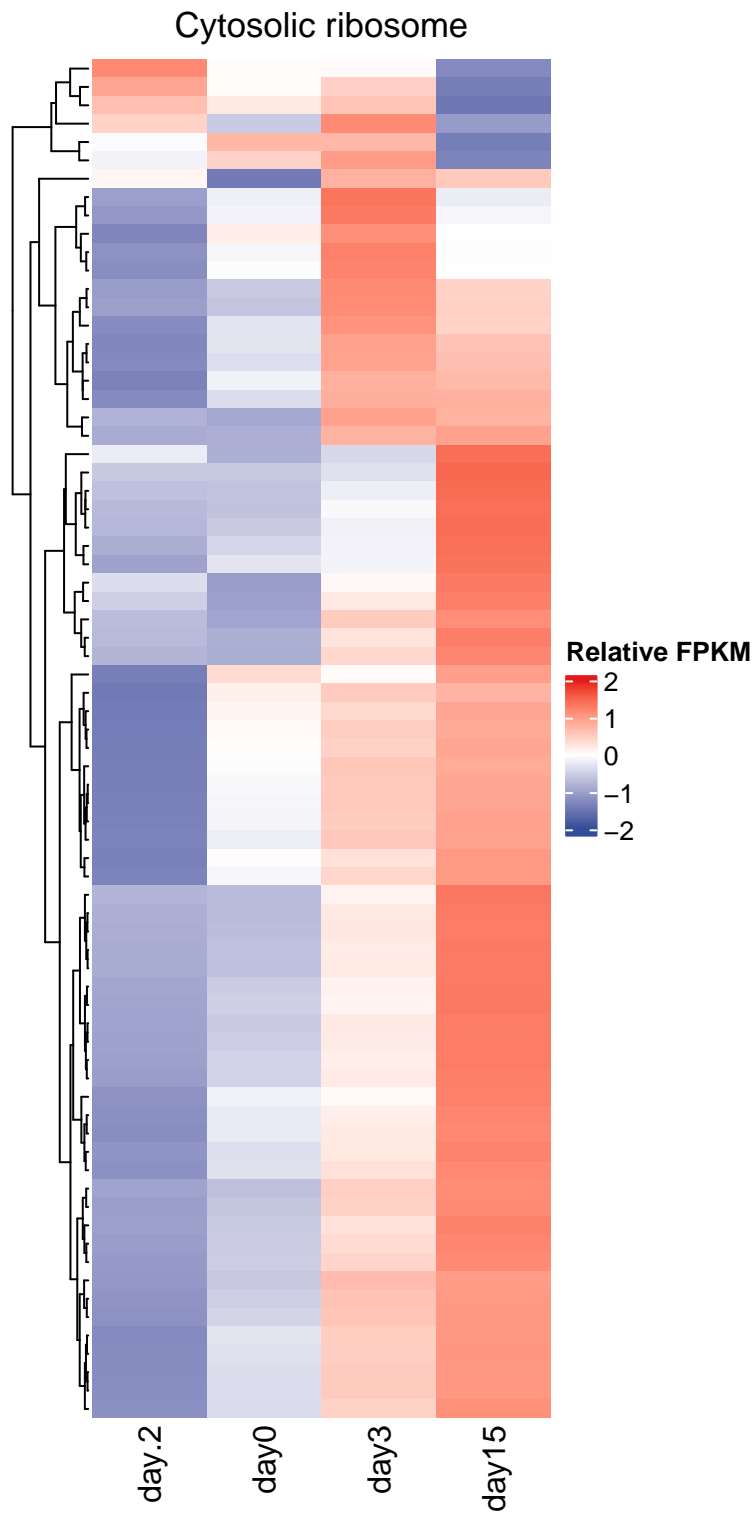
D2 Translation Heatmaps

```r
Heatmap(as.matrix(zs13[rownames(zs13) %in% translation$external_gene_name,]),
        cluster_columns = F,
        name="Relative FPKM",
        column_title = "Translation",
        col=circlize::colorRamp2(c(-2,0,2),c(rgb(0.2,0.6,1),rgb(0,0,0),rgb(0.95,0.3,0.3)))  ,
        show_row_names = F, row_split = 4,
        row_dend_reorder = order13[rownames(zs13) %in% translation$external_gene_name])
```
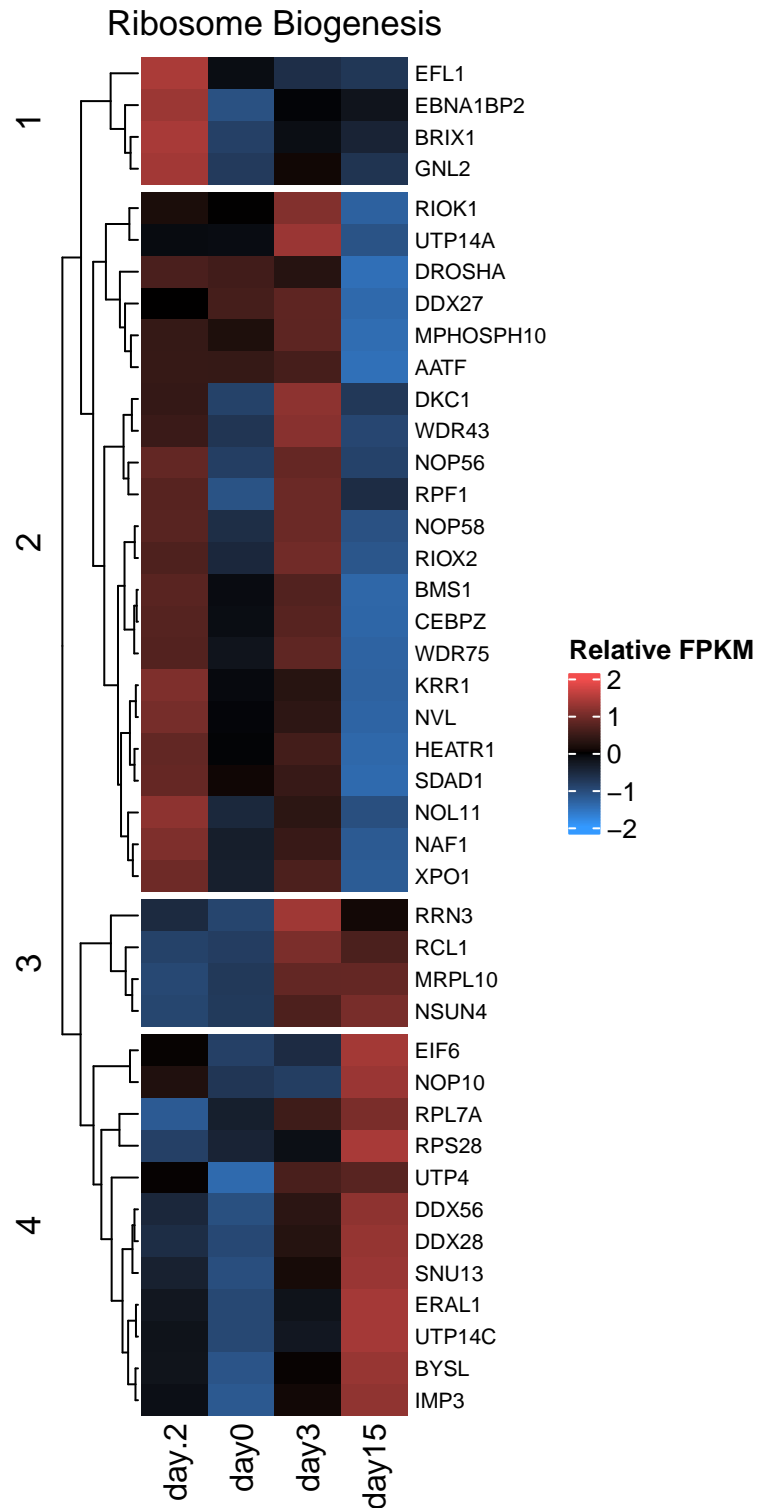
Translation

Supp Figure 6a lower panel

```
Heatmap(as.matrix(zs13[rownames(zs13) %in% translation$external_gene_name,]),
        cluster_columns = F,
        name="Relative FPKM",
        column_title = "Translation",
        col=circlize::colorRamp2(c(-2,0,2),c(rgb(0.2,0.6,1),rgb(0,0,0),rgb(0.95,0.3,0.3)))  ,
        show_row_names = T, row_split = 4, row_names_gp = gpar(fontsize=4),
        row_dend_reorder = order13[rownames(zs13) %in% translation$external_gene_name])
```
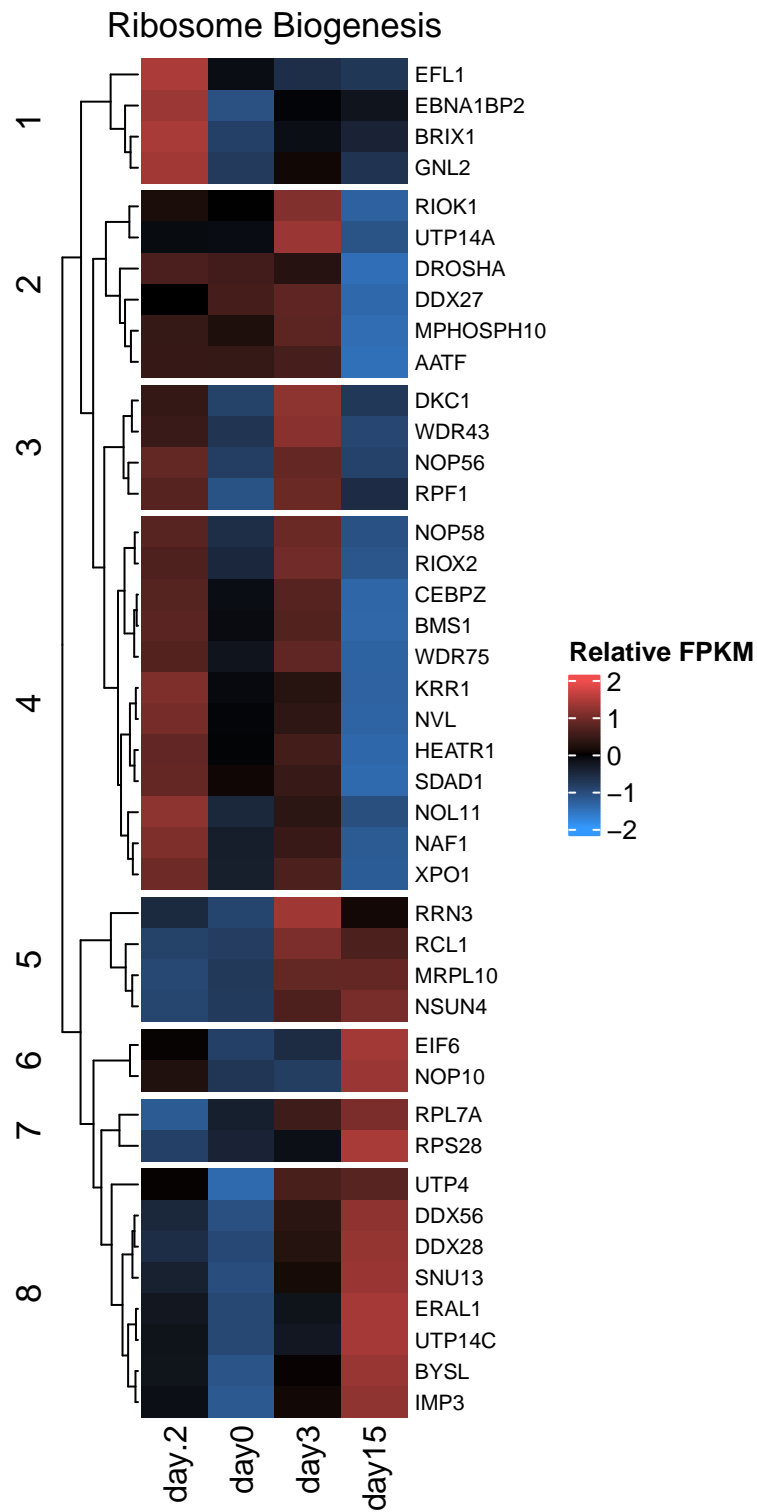
Translation

D2 Ribosome Biogenesis Heatmaps

```r
Heatmap(as.matrix(zs13[rownames(zs13) %in% ribogen$external_gene_name,]),
        cluster_columns = F,
        name="Relative FPKM",
        column_title = "Ribosome Biogenesis",
        col=circlize::colorRamp2(c(-2,0,2),c(rgb(0.2,0.6,1),rgb(0,0,0),rgb(0.95,0.3,0.3))),
        show_row_names = T, row_names_gp = gpar(fontsize=8),
        row_dend_reorder = order13[rownames(zs13) %in% ribogen$external_gene_name],
        row_split=4)
```

Ribosome Biogenesis


```
Heatmap(as.matrix(zs13[rownames(zs13) %in% ribogen$external_gene_name,]),
        cluster_columns = F,
        name="Relative FPKM",
        column_title = "Ribosome Biogenesis",
        col=circlize::colorRamp2(c(-2,0,2),c(rgb(0.2,0.6,1),rgb(0,0,0),rgb(0.95,0.3,0.3)))),
```

```
show_row_names = T, row_names_gp = gpar(fontsize=8),
row_dend_reorder = order13[rownames(zs13) %in% ribogen$external_gene_name],
row_split=8)
```


Ribosome Biogenesis

Supp Figure 6a upper panel

```
Heatmap(as.matrix(zs13[rownames(zs13) %in% ribogen$external_gene_name,]),
        cluster_columns = F,
        name="Relative FPKM",
        column_title = "Ribosome Biogenesis",
        col=circlize::colorRamp2(c(-2,0,2),c(rgb(0.2,0.6,1),rgb(0,0,0),rgb(0.95,0.3,0.3))),
        show_row_names = T, row_names_gp = gpar(fontsize=8),
        row_dend_reorder = order13[rownames(zs13) %in% ribogen$external_gene_name],
        row_split=2)
```

Ribosome Biogenesis