

Figure 3 Heatmap

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Setup

Set directory

```
setwd("D:/Krueger_Lab/Publications/miR181_paper/Figure3")
```

Load packages

```
library(ComplexHeatmap)
```

```
## Loading required package: grid
## =====
## ComplexHeatmap version 2.15.2
## 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))
## =====
library(ggplot2)
library(circlize)

## =====
## circlize version 0.4.15
## CRAN page: https://cran.r-project.org/package=circlize
## Github page: https://github.com/jokergoo/circlize
## Documentation: https://jokergoo.github.io/circlize\_book/book/
##
## If you use it in published research, please cite:
## Gu, Z. circlize implements and enhances circular visualization
##   in R. Bioinformatics 2014.
##
## This message can be suppressed by:
```

```

## suppressPackageStartupMessages(library(circlize))
## =====

library(dplyr)

##
## Attaching package: 'dplyr'

## The following objects are masked from 'package:stats':
##
## filter, lag

## The following objects are masked from 'package:base':
##
## intersect, setdiff, setequal, union

library(eulerr)
library(xlsx)

Load data

#Ribo
RNA <- read.csv("D:/Krueger_Lab/Publications/miR181_paper_v07122022/Figure_3/RNA_masterframe.csv")
RPF <- read.csv("D:/Krueger_Lab/Publications/miR181_paper_v07122022/Figure_3/RPF_masterframe.csv")
#ms
ms <- as.data.frame(read.xlsx("D:/Krueger_Lab/R/ECDF plots/Kreuger_analysis_ms.xlsx",
                             sheetName = "Analysis"))
#adjust upper case lower case of gene names of MS data:
#function
capFirst <- function(s) {
  paste(toupper(substring(s, 1, 1)), substring(s, 2), sep = "")
}
ms$Gene.Symbol <- tolower(ms$Gene.Symbol)
ms$Gene.Symbol <- capFirst(ms$Gene.Symbol)
names(ms)[names(ms) == 'Gene.Symbol'] <- 'GeneName'
head(ms$GeneName)

## [1] "Ckb"      "Gnb4"      "Ccm2"      "Rnpep"      "Aldh1b1" "Macf1"

#diff clip
eclipGR <- readRDS("D:/Krueger_Lab/miReCLIP/Mirco/DifferentialBinding/BsDifferentialResult.rds")
eclip <- as.data.frame(eclipGR)

## Loading required package: GenomicRanges
## Loading required package: stats4
## Loading required package: BiocGenerics
##
## Attaching package: 'BiocGenerics'

## The following objects are masked from 'package:dplyr':
##
## combine, intersect, setdiff, union

## 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

## Loading required package: S4Vectors

##
## Attaching package: 'S4Vectors'

## The following objects are masked from 'package:dplyr':
##
## first, rename

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

## Loading required package: IRanges

##
## Attaching package: 'IRanges'

## The following objects are masked from 'package:dplyr':
##
## collapse, desc, slice

## The following object is masked from 'package:grDevices':
##
## windows

## Loading required package: GenomeInfoDb

tframe <- eclip[eclip$res.log2FoldChange < 0 & eclip$res.padj <= 0.05,]
head(tframe)
```

##	seqnames	start	end	width	strand	scoreSum	scoreMean	scoreMax	WT	KO
## 40	chr1	15829574	15829580	7	+	18.32669	18.32669	18.32669	1	0
## 114	chr1	43509974	43509980	7	+	41.39330	41.39330	41.39330	1	0
## 204	chr1	64576272	64576278	7	+	49.28560	49.28560	49.28560	1	0
## 212	chr1	64601677	64601683	7	+	22.84493	22.84493	22.84493	0	1
## 251	chr1	82896040	82896046	7	+	31.07340	31.07340	31.07340	1	0
## 254	chr1	85650006	85650012	7	+	64.77470	64.77470	64.77470	1	0
##	geneID	geneName	region			gene_id	counts.bs.1_KO			
## 40	ENSMUSG00000025925	Terf1	intron	ENSMUSG00000025925.14			2			
## 114	ENSMUSG00000066877	Nck2	intron	ENSMUSG00000066877.11			1			
## 204	ENSMUSG00000025958	Creb1	utr3	ENSMUSG00000025958.14			5			
## 212	ENSMUSG00000025958	Creb1	utr3	ENSMUSG00000025958.14			8			
## 251	ENSMUSG00000026159	Agfg1	utr3	ENSMUSG00000026159.13			2			
## 254	ENSMUSG00000026222	Sp100	utr5	ENSMUSG00000026222.16			8			
##	counts.bs.2_KO	counts.bs.3_KO	counts.bs.4_WT	counts.bs.5_WT	counts.bs.6_WT					
## 40	0	0	12	4	2					
## 114	1	0	13	6	6					
## 204	3	2	13	13	8					
## 212	8	7	38	13	13					
## 251	1	0	10	8	14					

```
## 254          4          1          20          22          11
## counts.bg.1_K0 counts.bg.2_K0 counts.bg.3_K0 counts.bg.4_WT counts.bg.5_WT
## 40          398          580          396          623          466
## 114         4883         7795         4297         8365         6486
## 204         2524         2754         1831         3265         2963
## 212         2524         2754         1831         3265         2963
## 251          881         1074          671         1474         1202
## 254         1376         2007         1142         2498         1935
## counts.bg.6_WT res.baseMean res.log2FoldChange res.lfcSE res.stat
## 40          254          34.60655          -3.220330 1.0850775 14.64585
## 114         3484         220.01721          -3.535134 1.0657093 20.82672
## 204         1788         134.36861          -1.583008 0.5372789 10.00932
## 212         1788         180.26615          -1.281178 0.3808920 12.29309
## 251          657          77.88992          -3.082714 0.8749484 21.32573
## 254          949         127.11799          -1.800166 0.4592001 18.56607
## res.pvalue res.padj BsID
## 40 1.297197e-04 0.0067674589 ENSMUSG000000025925.bs1
## 114 5.027663e-06 0.0004944622 ENSMUSG000000066877.bs21
## 204 1.557503e-03 0.0396302933 ENSMUSG000000025958.bs3
## 212 4.546375e-04 0.0170974938 ENSMUSG000000025958.bs11
## 251 3.874960e-06 0.0004061979 ENSMUSG000000026159.bs7
## 254 1.641154e-05 0.0012883428 ENSMUSG000000026222.bs1
```

#Translational efficiency

```
TEframe <- read.csv("D:/Krueger_Lab/Publications/miR181_paper_v07122022/Supporting scripts/deltaTE/TE_m2")
head(TEframe)
```

```
## X baseMean log2FoldChange lfcSE stat pvalue padj
## 1 1 2354.3220 -0.07560025 0.1336485 -0.56566464 0.571621774 0.9277760
## 2 2 579.2399 -0.06784576 0.1617420 -0.41946900 0.674873409 0.9512329
## 3 3 20.3333 -0.02453968 0.5874974 -0.04176985 0.966682171 0.9964083
## 4 4 1154.1664 -0.05314227 0.1359683 -0.39084299 0.695913297 0.9542063
## 5 5 635.5639 0.36967194 0.1364473 2.70926532 0.006743239 0.2886809
## 6 6 378.2069 0.10567650 0.1653495 0.63910971 0.522751566 0.9143531
## Gene gene_symbol
## 1 ENSMUSG000000000001.4 Gnai3
## 2 ENSMUSG0000000000028.15 Cdc45
## 3 ENSMUSG0000000000037.17 Scml2
## 4 ENSMUSG0000000000056.7 Narf
## 5 ENSMUSG0000000000078.7 Klf6
## 6 ENSMUSG0000000000085.16 Scmh1
```

colour pattern

```
#colours
farbeneg <- "#b4b4b4"
farbe1 <- "#0073C2FF"
farbe2 <- "#EFC000FF"
farbe3 <- "#CD534CFF"
farbe4 <- "#7AA6DCFF"
farbe5 <- "#868686FF"
farbe6 <- "#003C67FF"
farbe7 <- "#8F7700FF"
farbe8 <- "#3B3B3BFF"
farbe9 <- "#A73030FF"
farbe10 <- "#4A6990FF"
```

```

farbe11 <- "#FF6F00FF"
farbe12 <- "#C71000FF"
farbe13 <- "#008EA0FF"
farbe14 <- "#8A4198FF"
farbe15 <- "#5A9599FF"
farbe16 <- "#FF6348FF"

```

```

RNA$pcol <- "#b56504"
RNA$ncol <- "#027d73"
RPF$pcol <- "#c4c404"
RPF$ncol <- "#8d0391"

```

Venn diagram to figure out the dataset for the joint heatmaps

create column for heatmap

Make columns to identify genes included in both datasets and that are significant

```

mID <- RNA[RNA$LFCandPADJSig %in% c("Significant up", "Significant down") & RNA$Gene %in%
      RPF[RPF$LFCandPADJSig %in% c("Significant up", "Significant down"), "Gene"], "Gene"]

```

```

RNA$mID <- "One dataset"
RNA$mID[RNA$Gene %in% mID] <- "Both datasets"
RPF$mID <- "One dataset"
RPF$mID[RPF$Gene %in% mID] <- "Both datasets"

```

```
head(RNA)
```

```

##      X                Gene  baseMean log2FoldChange      lfcSE      stat
## 1 1  ENSMUSG000000104197.1 2009.89774      2.741830 0.10245760 26.76063
## 2 2  ENSMUSG000000004110.17 1028.70696     -2.019241 0.09914247 -20.36706
## 3 3  ENSMUSG000000027669.14  648.18701      1.905815 0.10065041 18.93499
## 4 4  ENSMUSG000000098206.1 4004.62856      2.148076 0.12889738 16.66501
## 5 5  ENSMUSG00000004552.16  516.71005     -2.252195 0.13648248 -16.50172
## 6 6  ENSMUSG000000069306.5   68.07176      2.763638 0.19108029 14.46323
##      pvalue      padj    WT_1411    WT_1601    WT_1710    KO_1411
## 1 9.288051e-158 1.183948e-153 487.52165 474.5874 405.8464624 3338.7077
## 2 3.277594e-92 2.088974e-88 1576.25174 1737.4574 1739.7225816 400.4560
## 3 5.872559e-80 2.495250e-76 248.88185 252.5740 252.2109307 1048.3637
## 4 2.354521e-62 7.503269e-59 1449.25030 1259.2746 985.7539896 5751.8330
## 5 3.566111e-61 9.091443e-58 872.62279 1026.4751 777.9463918 148.2821
## 6 2.068594e-47 4.394729e-44 2.04841 0.0000 0.8880667 123.7258
##      KO_1601    KO_1710    LFCandPADJSig  gene_symbol      mID
## 1 3428.5263 3924.1970 Significant up      Gm37632 Both datasets
## 2 357.0428 361.3113 Significant down      Cacna1e Both datasets
## 3 1043.6635 1043.4281 Significant up      Gnb4 Both datasets
## 4 6644.9630 7936.6965 Significant up A430106G13Rik Both datasets
## 5 112.9110 162.0230 Significant down      Ctse Both datasets
## 6 146.4791 135.2892 Significant up      Hist1h4m Both datasets

```

```
head(RPF)
```

```

##      X                Gene  baseMean log2FoldChange      lfcSE      stat
## 1 1  ENSMUSG000000000001.4 3788.57802      0.05462417 0.05957247 0.9169365
## 2 2  ENSMUSG000000000028.15 1086.36203     -0.12956555 0.07692175 -1.6843811

```

```
## 3 3 ENSMUSG00000000037.17 19.45454 -0.06042604 0.15784031 -0.3828302
## 4 4 ENSMUSG00000000056.7 1454.64304 0.05456272 0.08781406 0.6213438
## 5 5 ENSMUSG00000000078.7 850.27809 0.65975317 0.07971920 8.2759636
## 6 6 ENSMUSG00000000085.16 337.25837 -0.06342545 0.12383411 -0.5121808
##      pvalue      padj    WT_1411    WT_1601    WT_1710    KO_1411
## 1 3.591759e-01 6.850504e-01 3687.40632 3612.48756 3862.10415 3905.91865
## 2 9.210804e-02 3.386014e-01 1091.79093 1147.15325 1182.54053 1081.59050
## 3 7.018457e-01 8.852986e-01 13.71269 22.17027 29.07887 28.37993
## 4 5.343734e-01 8.038393e-01 1561.28714 1359.98786 1348.39777 1344.36759
## 5 1.274210e-16 2.314117e-14 649.06709 668.27536 622.50312 1054.26168
## 6 6.085245e-01 8.452822e-01 325.18653 326.21972 396.33417 351.07019
##      KO_1601    KO_1710    LFCandPADJSig gene_symbol      mID
## 1 3984.05486 3679.496580 Not significant      Gnai3 One dataset
## 2 1030.01306 985.083926 Not significant      Cdc45 One dataset
## 3 17.83572 5.549769 Not significant      Scml2 One dataset
## 4 1559.88270 1553.935207 Not significant      Narf One dataset
## 5 1022.58151 1084.979761 Significant up      Klf6 One dataset
## 6 350.02608 274.713546 Not significant      Scmh1 One dataset
```

plot with venn

Make Venn diagrams for the significant genes

List

```
vlist <- list(RNA[RNA$LFCandPADJSig %in% c("Significant up", "Significant down"), "Gene"],
              RPF[RPF$LFCandPADJSig %in% c("Significant up", "Significant down"), "Gene"])
names(vlist) <- c("RNA", "RPF")

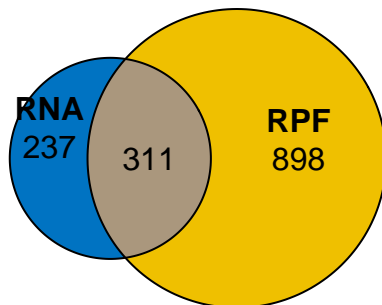
str(vlist)
```

```
## List of 2
## $ RNA: chr [1:548] "ENSMUSG000000104197.1" "ENSMUSG000000004110.17" "ENSMUSG000000027669.14" "ENSMUSG000000000000.0"
## $ RPF: chr [1:1209] "ENSMUSG000000000078.7" "ENSMUSG000000000168.9" "ENSMUSG000000000184.12" "ENSMUSG000000000000.0"

plot
```

```
plot(euler(vlist, shape="ellipse"), quantities = T, fills=c(farbe1, farbe2), main = "Overlap of differentially regulated genes")
```

differentially regul:



```
pdf("Venn_Overlap of differentially regulated genes.pdf", width = 3, height = 3)
plot(euler(vlist, shape="ellipse"), quantities = T, fills=c(farbe1, farbe2), main = "Overlap of differentially regulated genes")
dev.off()
```

```
## pdf
## 2
```

Heatmap scaled together

Combine experiments into one df and scale

```
head(RNA)
```

```
##      X      Gene baseMean log2FoldChange      lfcSE      stat
## 1 1 ENSMUSG000000104197.1 2009.89774      2.741830 0.10245760 26.76063
## 2 2 ENSMUSG00000004110.17 1028.70696      -2.019241 0.09914247 -20.36706
## 3 3 ENSMUSG000000027669.14  648.18701      1.905815 0.10065041 18.93499
## 4 4 ENSMUSG000000098206.1 4004.62856      2.148076 0.12889738 16.66501
## 5 5 ENSMUSG00000004552.16  516.71005      -2.252195 0.13648248 -16.50172
## 6 6 ENSMUSG000000069306.5   68.07176      2.763638 0.19108029 14.46323
##      pvalue      padj      WT_1411      WT_1601      WT_1710      KO_1411
## 1 9.288051e-158 1.183948e-153 487.52165 474.5874 405.8464624 3338.7077
## 2 3.277594e-92 2.088974e-88 1576.25174 1737.4574 1739.7225816 400.4560
## 3 5.872559e-80 2.495250e-76 248.88185 252.5740 252.2109307 1048.3637
## 4 2.354521e-62 7.503269e-59 1449.25030 1259.2746 985.7539896 5751.8330
## 5 3.566111e-61 9.091443e-58 872.62279 1026.4751 777.9463918 148.2821
## 6 2.068594e-47 4.394729e-44 2.04841 0.0000 0.8880667 123.7258
##      KO_1601      KO_1710      LFCandPADJSig      gene_symbol      mID
## 1 3428.5263 3924.1970 Significant up      Gm37632 Both datasets
## 2 357.0428 361.3113 Significant down      Cacna1e Both datasets
## 3 1043.6635 1043.4281 Significant up      Gnb4 Both datasets
## 4 6644.9630 7936.6965 Significant up A430106G13Rik Both datasets
## 5 112.9110 162.0230 Significant down      Ctse Both datasets
## 6 146.4791 135.2892 Significant up      Hist1h4m Both datasets
```

```
RNAhframe <- RNA[RNA$Gene %in% mID, c(16, 9:14)]
RPFhframe <- RPF[RPF$Gene %in% mID, c(16, 9:14)]
```

```
colnames(RNAhframe) <- c("Gene", "WT1 RNA", "WT2 RNA", "WT3 RNA", "KO1 RNA", "KO2 RNA", "KO3 RNA")
colnames(RPFhframe) <- c("Gene", "WT1 RPF", "WT2 RPF", "WT3 RPF", "KO1 RPF", "KO2 RPF", "KO3 RPF")
```

```
head(RNAhframe)
```

```
##      Gene      WT1 RNA      WT2 RNA      WT3 RNA      KO1 RNA      KO2 RNA      KO3 RNA
## 1      Gm37632 487.52165 474.5874 405.8464624 3338.7077 3428.5263 3924.1970
## 2      Cacna1e 1576.25174 1737.4574 1739.7225816 400.4560 357.0428 361.3113
## 3      Gnb4 248.88185 252.5740 252.2109307 1048.3637 1043.6635 1043.4281
## 4 A430106G13Rik 1449.25030 1259.2746 985.7539896 5751.8330 6644.9630 7936.6965
## 5      Ctse 872.62279 1026.4751 777.9463918 148.2821 112.9110 162.0230
## 6      Hist1h4m 2.04841 0.0000 0.8880667 123.7258 146.4791 135.2892
```

```
head(RPFhframe)
```

```
##      Gene      WT1 RPF      WT2 RPF      WT3 RPF      KO1 RPF      KO2 RPF      KO3 RPF
## 16      Ccnd2 1744.12295 1558.88688 1980.59384 1293.9144 1432.06001 1104.40395
## 34      Ccm2 2743.19000 2397.55662 2641.86879 1770.0665 2025.84099 1823.09898
## 161     Gtf2h4 2547.29450 2770.01720 2432.93175 1552.4870 1418.68322 1603.88312
## 238     Rmnd5a 5937.59264 5952.40147 6067.78995 3991.0584 3981.08224 3857.08917
## 376     Cacnb3 67.25746 67.77769 74.31266 25.2266 25.26728 19.42419
```

```
## 452 Cacna1e 534.14173 627.73543 611.73317 145.0530 121.13429 122.09491
heatframe <- left_join(RNAhframe, RPFhframe, by="Gene")
rownames(heatframe) <- heatframe$Gene
heatframe <- heatframe[, -1]
heatframe <- heatframe[!(rownames(heatframe)=="") & !is.na(rownames(heatframe)),]
head(heatframe)
```

```
##           WT1 RNA  WT2 RNA      WT3 RNA  KO1 RNA  KO2 RNA  KO3 RNA
## Gm37632      487.52165 474.5874 405.8464624 3338.7077 3428.5263 3924.1970
## Cacna1e     1576.25174 1737.4574 1739.7225816 400.4560 357.0428 361.3113
## Gnb4         248.88185 252.5740 252.2109307 1048.3637 1043.6635 1043.4281
## A430106G13Rik 1449.25030 1259.2746 985.7539896 5751.8330 6644.9630 7936.6965
## Ctse         872.62279 1026.4751 777.9463918 148.2821 112.9110 162.0230
## Hist1h4m       2.04841  0.0000  0.8880667 123.7258 146.4791 135.2892
##           WT1 RPF  WT2 RPF  WT3 RPF  KO1 RPF  KO2 RPF  KO3 RPF
## Gm37632       37.87313  31.67182  38.77182 293.2592 330.7041 285.8131
## Cacna1e       534.14173 627.73543 611.73317 145.0530 121.1343 122.0949
## Gnb4          286.00743 282.51262 308.02058 1026.9329 1038.1878 943.4607
## A430106G13Rik 171.08207 156.45878 175.55019 491.9187 534.3286 471.7303
## Ctse          4012.59285 3587.78354 3646.70514 466.6921 526.1539 513.3536
## Hist1h4m       29.38433  20.90340  28.00187 3535.9285 4264.9675 3698.9208
```

scale

```
heat_scaled = t(scale(t(heatframe)))
colnames(heat_scaled) <- c(rep("WT", 3), rep("miR-181-KO", 3), rep("WT", 3), rep("miR-181-KO", 3))
head(as.data.frame(heat_scaled))
```

```
##           WT      WT      WT miR-181-KO miR-181-KO miR-181-KO
## Gm37632     -0.3998356 -0.4084225 -0.4540584 1.4930180 1.5526471 1.8817144
## Cacna1e      1.4161464 1.6750556 1.6786937 -0.4722763 -0.5420014 -0.5351459
## Gnb4        -1.0122475 -1.0028800 -1.0038011 1.0161500 1.0042252 1.0036278
## A430106G13Rik -0.2526860 -0.3193752 -0.4153921 1.2576956 1.5712206 2.0246716
## Ctse         -0.3001186 -0.1971680 -0.3634715 -0.7848129 -0.8084815 -0.7756181
## Hist1h4m     -0.5802978 -0.5814903 -0.5809733 -0.5094641 -0.4962184 -0.5027326
##           WT      WT      WT miR-181-KO miR-181-KO miR-181-KO
## Gm37632     -0.6983496 -0.7024666 -0.6977530 -0.5288031 -0.5039442 -0.5337465
## Cacna1e     -0.2575663 -0.1072472 -0.1329482 -0.8824742 -0.9208895 -0.9193467
## Gnb4        -0.9180547 -0.9269215 -0.8622042 0.9617771 0.9903324 0.7499964
## A430106G13Rik -0.7013751 -0.7065084 -0.6998066 -0.5887484 -0.5738608 -0.5958353
## Ctse         1.8009996 1.5167375 1.5561650 -0.5717481 -0.5319591 -0.5405244
## Hist1h4m     -0.5643844 -0.5693215 -0.5651892 1.4769271 1.9013324 1.5718120
```

cluster without heatmap (this is not done yet)

```
# heat_ks <- kmeans(heat_scaled, centers = 5)
# heat_k_names <- as.data.frame(heat_ks$cluster)
# merge(heat_scaled, heat_k_names, by=0)
```

make heatmaps

make annotations for the heatmap


```
ha1 <- HeatmapAnnotation(Genotype=colnames(heat_scaled),col = list(Genotype= c("WT"=farbel, "miR-181-KO"
```

make annotation heatmaps as seperate heatmaps

ms

```
mshmat <- data.frame(row.names = rownames(heatframe), rownames(heatframe))
colnames(mshmat) <- "GeneName"
mshmat <- left_join(mshmat, ms[,c("GeneName", "Log2.FC.")], by="GeneName")
mshma <- as.data.frame(mshmat[, "Log2.FC."])
rownames(mshma) <- mshmat$GeneName
colnames(mshma) <- "Log2FC in MS"
head(mshma)
```

```
##           Log2FC in MS
## Gm37632             NA
## Cacna1e             NA
## Gnb4                1.572372
## A430106G13Rik       NA
## Ctse                NA
## Hist1h4m            NA
```

```
hmms <- Heatmap(mshma, show_row_dend = F, show_column_dend = F, show_row_names = F, colorRamp2(c(-0.5, 0.5),
name = "Mass spec")
```

```
## Warning: The input is a data frame-like object, convert it to a matrix.
```

Targets

```
targethmt <- data.frame(row.names = rownames(heatframe), rownames(heatframe))
colnames(targethmt) <- "GeneName"
targethmt$Target <- "Non-target"
targethmt[targethmt$GeneName %in% tframe$geneName, "Target"] <- "Target"

targethm <- as.data.frame(targethmt[, "Target"])
rownames(targethm) <- targethmt$GeneName
colnames(targethm) <- "miR-181 target"
head(targethm)
```

```
##           miR-181 target
## Gm37632       Non-target
## Cacna1e       Non-target
## Gnb4          Non-target
## A430106G13Rik Non-target
## Ctse          Target
## Hist1h4m      Non-target
```

```
hmtarget <- Heatmap(targethm, show_row_dend = F, show_column_dend = F, show_row_names = F, col = c(farbel, "miR-181 target")
```

```
## Warning: The input is a data frame-like object, convert it to a matrix.
```

```
## Warning: Note: not all columns in the data frame are numeric. The data frame
## will be converted into a character matrix.
```

TE annotation

```
TEsel = TEframe[unique(TEframe$gene_symbol) %in% rownames(heatframe), c("log2FoldChange")]
names(TEsel) = TEframe[unique(TEframe$gene_symbol) %in% rownames(heatframe), c("gene_symbol")]

TEsel = TEsel[order(factor(names(TEsel), levels = rownames(heat_scaled)))]

#set maximum
TElim = 0.5

TEsel[TEsel > TElim] = TElim
TEsel[TEsel < -TElim] = -TElim

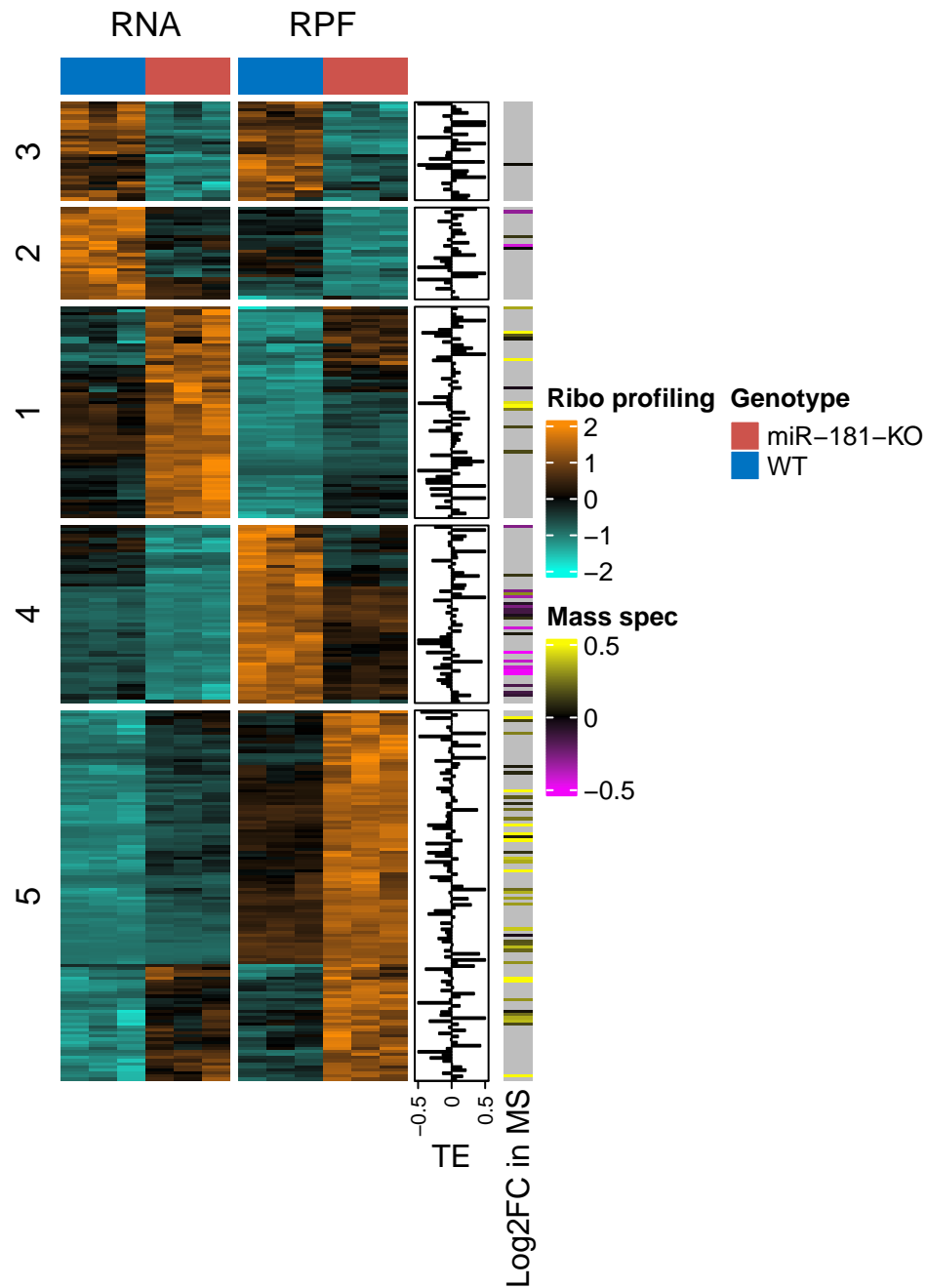
haTE = rowAnnotation(TE = anno_barplot(TEsel, ylim = c(-TElim,TElim)))
```

plot

```
set.seed(666)

HRNA <- Heatmap(heat_scaled, show_row_dend = F, show_column_dend = F, show_row_names = F, show_column_names = F,
  column_order=1:12, top_annotation = c(ha1), column_split = c(rep("RNA",6), rep("RPF",6)),
  name = "Ribo profiling", right_annotation = haTE)

HRNA + hmms
```



Heatmap scald seperately

scale

```
RNA2hframe <- RNAhframe
rownames(RNA2hframe) <- RNA2hframe$Gene
RNA2hframe <- RNA2hframe[, -1]
head(RNA2hframe)
```

```
## WT1 RNA WT2 RNA WT3 RNA KO1 RNA KO2 RNA KO3 RNA
```

```
## Gm37632      487.52165  474.5874  405.8464624 3338.7077 3428.5263 3924.1970
## Cacna1e     1576.25174 1737.4574 1739.7225816 400.4560 357.0428 361.3113
## Gnb4        248.88185  252.5740  252.2109307 1048.3637 1043.6635 1043.4281
## A430106G13Rik 1449.25030 1259.2746  985.7539896 5751.8330 6644.9630 7936.6965
## Ctse        872.62279 1026.4751  777.9463918 148.2821 112.9110 162.0230
## Hist1h4m     2.04841   0.0000   0.8880667 123.7258 146.4791 135.2892
```

```
RPF2hframe <- RPFhframe
rownames(RPF2hframe) <- RPF2hframe$Gene
RPF2hframe <- RPF2hframe[,-1]
head(RPF2hframe)
```

```
##          WT1 RPF    WT2 RPF    WT3 RPF    KO1 RPF    KO2 RPF    KO3 RPF
## Ccnd2    1744.12295 1558.88688 1980.59384 1293.9144 1432.06001 1104.40395
## Ccm2     2743.19000 2397.55662 2641.86879 1770.0665 2025.84099 1823.09898
## Gtf2h4   2547.29450 2770.01720 2432.93175 1552.4870 1418.68322 1603.88312
## Rmnd5a   5937.59264 5952.40147 6067.78995 3991.0584 3981.08224 3857.08917
## Cacnb3    67.25746  67.77769  74.31266  25.2266  25.26728  19.42419
## Cacna1e  534.14173 627.73543 611.73317 145.0530 121.13429 122.09491
```

```
RNA_scaled = as.data.frame(t(scale(t(RNA2hframe))))
colnames(RNA_scaled) <- c(rep("WT", 3), rep("miR-181-KO", 3))
head(RNA_scaled)
```

```
##          WT          WT          WT miR-181-KO miR-181-KO miR-181-KO
## Gm37632   -0.8881526 -0.8956985 -0.9358019  0.7752263  0.8276264  1.1168002
## Cacna1e    0.7594651  0.9830633  0.9862052 -0.8714075 -0.9316233 -0.9257027
## Gnb4       -0.9182414 -0.9097510 -0.9105859  0.9202455  0.9094371  0.9088957
## A430106G13Rik -0.8190839 -0.8799774 -0.9676499  0.5600372  0.8463151  1.2603587
## Ctse       0.8486754  1.2155368  0.6229191 -0.8785180 -0.9628606 -0.8457527
## Hist1h4m   -0.8940090 -0.9217461 -0.9097210  0.7536002  1.0616980  0.9101779
```

```
RPF_scaled = as.data.frame(t(scale(t(RPF2hframe))))
colnames(RPF_scaled) <- c(rep("WT", 3), rep("miR-181-KO", 3))
head(RPF_scaled)
```

```
##          WT          WT          WT miR-181-KO miR-181-KO miR-181-KO
## Ccnd2    0.7151707 0.1267205 1.4663815 -0.7150330 -0.2761778 -1.3170618
## Ccm2     1.2148270 0.3908552 0.9732826 -1.1050482 -0.4952952 -0.9786214
## Gtf2h4   0.8317081 1.2073894 0.6388049 -0.8462999 -1.0719957 -0.7596067
## Rmnd5a   0.8681965 0.8814091 0.9843593 -0.8685120 -0.8774128 -0.9880401
## Cacnb3   0.8070374 0.8273071 1.0819261 -0.8305928 -0.8290080 -1.0566697
## Cacna1e  0.6815571 1.0485294 0.9857860 -0.8440244 -0.9378073 -0.9340408
```

now combine the tables

```
RNA_scaled$gene.symbol <- rownames(RNA_scaled)
colnames(RNA_scaled) <- c(rep("WT_RNA", 3), rep("miR-181-KO_RNA", 3), "gene.symbol")
RPF_scaled$gene.symbol <- rownames(RPF_scaled)
colnames(RPF_scaled) <- c(rep("WT_RPF", 3), rep("miR-181-KO_RPF", 3), "gene.symbol")
```

```
SepScaleframe <- merge(RNA_scaled,
                        RPF_scaled, by="gene.symbol")
```

```
## Warning in merge.data.frame(RNA_scaled, RPF_scaled, by = "gene.symbol"): column
```

```
## names 'WT_RNA', 'WT_RNA', 'miR-181-KO_RNA', 'miR-181-KO_RNA' are duplicated in
## the result

SepScaleframe = SepScaleframe[!(SepScaleframe$gene.symbol == ""),]

rownames(SepScaleframe) <- SepScaleframe$gene.symbol
SepScaleframe <- SepScaleframe[,-1]
colnames(SepScaleframe) <- c(rep("WT", 3), rep("miR-181-KO", 3), rep("WT", 3), rep("miR-181-KO", 3))
head(SepScaleframe)
```

```
##           WT           WT           WT miR-181-KO miR-181-KO
## 2010016I18Rik -0.2852270 -1.1619350 -0.9454200  0.1187576  1.3311953
## 2510009E07Rik -0.8990716 -0.8810933 -0.9549938  0.8714430  0.8718551
## 4930523C07Rik -0.5093680 -0.8683728 -1.2011361  0.4487555  0.8731558
## 5830411N06Rik  0.7512700  0.1727972  1.4868718 -0.7829998 -0.4556643
## 9930111J21Rik2 1.0142836  0.8153492  0.9035324 -0.9110550 -0.9110550
## A430035B10Rik  1.5087331  0.7002925  0.2304264 -1.1538509 -0.7487766
##           miR-181-KO           WT           WT           WT miR-181-KO
## 2010016I18Rik  0.9426291 -1.1351577 -0.6698709 -0.8295780  1.2851310
## 2510009E07Rik  0.9918607 -0.8886151 -0.9452000 -0.9026435  0.8877953
## 4930523C07Rik  1.2569655 -0.9653176 -0.9695535 -0.7732223  1.0190113
## 5830411N06Rik -1.1722748  1.4113550 -0.0066162  0.9717884 -1.0438427
## 9930111J21Rik2 -0.9110550  0.9061925  0.9021519  0.9301430 -0.9128291
## A430035B10Rik -0.5368244  1.4080276  0.6025263  0.5038469 -1.2628822
##           miR-181-KO miR-181-KO
## 2010016I18Rik  0.5960406  0.7534351
## 2510009E07Rik  0.9755001  0.8731633
## 4930523C07Rik  1.0253338  0.6637484
## 5830411N06Rik -0.4589426 -0.8737418
## 9930111J21Rik2 -0.9128291 -0.9128291
## A430035B10Rik -0.7319144 -0.5196042
```

make heatmaps

make annotations for the heatmap

```
ha3 <- HeatmapAnnotation(Genotype=colnames(SepScaleframe),col = list(Genotype= c("WT"=farbe1, "miR-181-KO"=farbe2)))
ha4 <- HeatmapAnnotation(Experiment=c(rep("RNA",6), rep("RPF",6)),col = list(Experiment= c("RNA"=farbe1, "RPF"=farbe2)))
```

TE annotation

```
TEsel2 = TEframe[unique(TEframe$gene_symbol) %in% rownames(heatframe), c("log2FoldChange")]
names(TEsel2) = TEframe[unique(TEframe$gene_symbol) %in% rownames(heatframe), c("gene_symbol")]

TEsel2 = TEsel2[order(factor(names(TEsel2), levels = rownames(SepScaleframe)))]

TEsel2[TEsel2 > TELim] = TELim
TEsel2[TEsel2 < -TElim] = -TElim

haTE2 = rowAnnotation(TE = anno_barplot(TEsel2, ylim = c(-TElim,TElim)))
```

plot

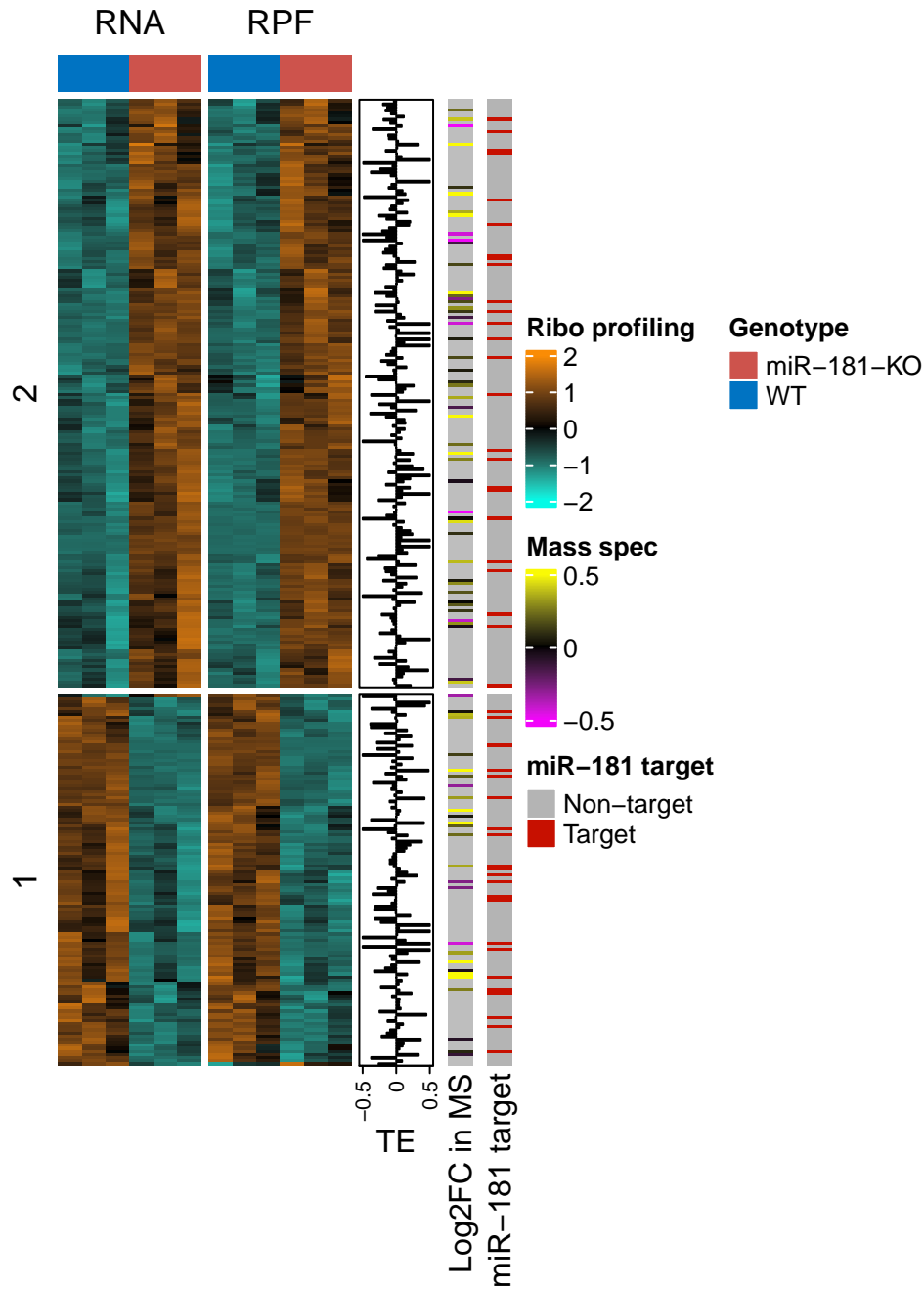
```
set.seed(666)
HRNAsep <- Heatmap(SepScaleframe, show_row_dend = F, show_column_dend = F, show_row_names = F, show_column_names = "Ribo profiling", right_annotation = haTE2)
```

```
## Warning: The input is a data frame-like object, convert it to a matrix.
```

```
HRNAsep + hmms + hmtarget
```

```
## Warning: Row names of heatmap 2 are not consistent with the main heatmap (1). It  
## may lead to wrong conclusion of your data. Please double check.
```

```
## Warning: Row names of heatmap 3 are not consistent with the main heatmap (1). It  
## may lead to wrong conclusion of your data. Please double check.
```



Heatmap (x-mean)/mean

different scale that kathi proposed

```
kheatframe <- (heatframe - rowMeans(heatframe))/rowMeans(heatframe)
head(kheatframe)
```

```
##           WT1 RNA   WT2 RNA   WT3 RNA   KO1 RNA   KO2 RNA   KO3 RNA
## Gm37632    -0.5526462 -0.5645148 -0.6275920  2.0636249  2.1460432  2.6008746
## Cacna1e     1.2695844  1.5016982  1.5049598 -0.4233987 -0.4859078 -0.4797618
## Gnb4        -0.6158363 -0.6101373 -0.6106977  0.6182106  0.6109557  0.6105923
## A430106G13Rik -0.3318564 -0.4194403 -0.5455407  1.6517508  2.0635079  2.6590320
## Ctse       -0.3394868 -0.2230316 -0.4111501 -0.8877611 -0.9145345 -0.8773601
## Hist1h4m    -0.9979493 -1.0000000 -0.9991109 -0.8761352 -0.8533564 -0.8645588
##           WT1 RPF   WT2 RPF   WT3 RPF   KO1 RPF   KO2 RPF
## Gm37632    -0.9652473 -0.97093769 -0.9644227 -0.7309030 -0.6965433
## Cacna1e    -0.2309098 -0.09614785 -0.1191889 -0.7911438 -0.8255834
## Gnb4       -0.5585308 -0.56392526 -0.5245522  0.5851309  0.6025035
## A430106G13Rik -0.9211265 -0.92786827 -0.9190666 -0.7732122 -0.7536601
## Ctse       2.0372466  1.71569622  1.7602957 -0.6467474 -0.6017391
## Hist1h4m   -0.9705827 -0.97907312 -0.9719667  2.5399000  3.2697578
##           KO3 RPF
## Gm37632    -0.7377356
## Cacna1e    -0.8242002
## Gnb4       0.4562867
## A430106G13Rik -0.7825195
## Ctse      -0.6114279
## Hist1h4m   2.7030753
```

make heatmaps

make annotations for the heatmap

```
ha5 <- HeatmapAnnotation(Genotype=colnames(kheatframe),col = list(Genotype= c("WT"=farbe1, "miR-181-KO"=farbe2)))
ha6 <- HeatmapAnnotation(Experiment=c(rep("RNA",6), rep("RPF",6)),col = list(Experiment= c("RNA"=farbe1, "RPF"=farbe2)))
```

TE annotation

```
TEsel3 = TEframe[unique(TEframe$gene_symbol) %in% rownames(heatframe), c("log2FoldChange")]
names(TEsel3) = TEframe[unique(TEframe$gene_symbol) %in% rownames(heatframe), c("gene_symbol")]
```

```
TEsel3 = TEsel3[order(factor(names(TEsel3), levels = rownames(kheatframe)))]
```

```
TEsel3[TEsel3 > TELim] = TELim
TEsel3[TEsel3 < -TElim] = -TElim
```

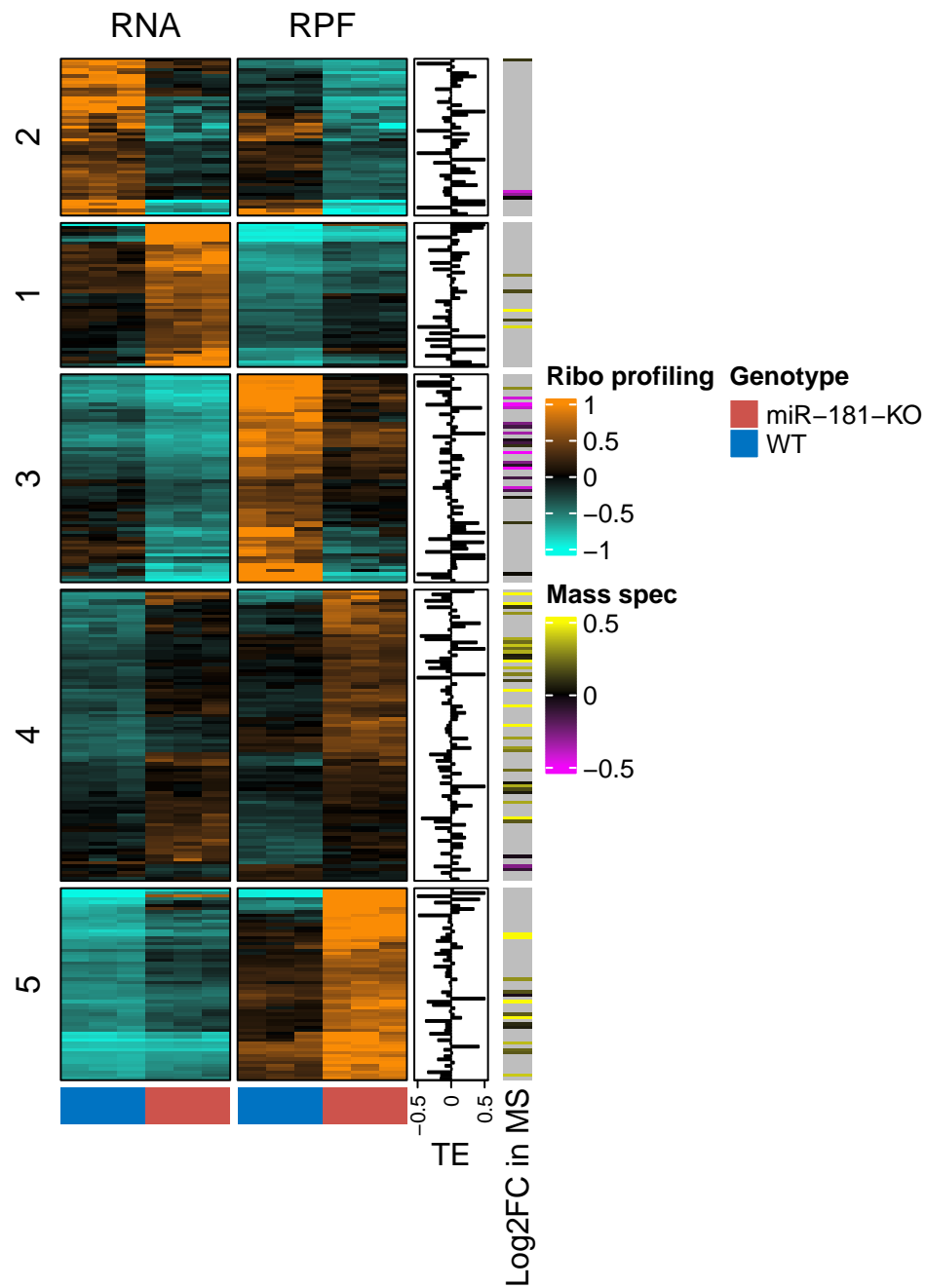
```
haTE3 = rowAnnotation(TE = anno_barplot(TEsel3, ylim = c(-TElim,TElim)))
```

plot

```
set.seed(666)
HRNAk <- Heatmap(kheatframe, show_row_dend = F, show_column_dend = F, show_row_names = F, show_column_names = F,
  name = "Ribo profiling", right_annotation = haTE3,
  border = TRUE
)
```

```
## Warning: The input is a data frame-like object, convert it to a matrix.
```

```
HRNAk + hmms
```



```
#session info
```

```
sessionInfo()
```

```
## R version 4.2.3 (2023-03-15 ucrt)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 19044)
##
## Matrix products: default
```



```

##
## locale:
## [1] LC_COLLATE=German_Germany.utf8 LC_CTYPE=German_Germany.utf8
## [3] LC_MONETARY=German_Germany.utf8 LC_NUMERIC=C
## [5] LC_TIME=German_Germany.utf8
##
## attached base packages:
## [1] stats4      grid      stats      graphics  grDevices  utils      datasets
## [8] methods     base
##
## other attached packages:
## [1] GenomicRanges_1.50.2 GenomeInfoDb_1.34.9 IRanges_2.32.0
## [4] S4Vectors_0.36.2     BiocGenerics_0.44.0  xlsx_0.6.5
## [7] eulerr_7.0.0          dplyr_1.1.1          circlize_0.4.15
## [10] ggplot2_3.4.1         ComplexHeatmap_2.15.2
##
## loaded via a namespace (and not attached):
## [1] shape_1.4.6           GetoptLong_1.0.5      tidysselect_1.2.0
## [4] xfun_0.37             rJava_1.0-6          colorspace_2.1-0
## [7] vctrs_0.6.1           generics_0.1.3        htmltools_0.5.4
## [10] yaml_2.3.7            utf8_1.2.3           rlang_1.1.0
## [13] pillar_1.9.0          glue_1.6.2           withr_2.5.0
## [16] RColorBrewer_1.1-3    GenomeInfoDbData_1.2.9 matrixStats_0.63.0
## [19] foreach_1.5.2         lifecycle_1.0.3      zlibbioc_1.44.0
## [22] munsell_0.5.0         gtable_0.3.3         GlobalOptions_0.1.2
## [25] codetools_0.2-19     evaluate_0.20         knitr_1.42
## [28] fastmap_1.1.1         doParallel_1.0.17    parallel_4.2.3
## [31] fansi_1.0.4           xlsxjars_0.6.1       highr_0.10
## [34] Rcpp_1.0.10           scales_1.2.1         magick_2.7.4
## [37] XVector_0.38.0        rjson_0.2.21         png_0.1-8
## [40] digest_0.6.31         polyclip_1.10-4      clue_0.3-64
## [43] bitops_1.0-7          cli_3.6.0            tools_4.2.3
## [46] magrittr_2.0.3        RCurl_1.98-1.10      tibble_3.2.1
## [49] cluster_2.1.4         crayon_1.5.2         pkgconfig_2.0.3
## [52] polylabelr_0.2.0      rmarkdown_2.21       rstudioapi_0.14
## [55] iterators_1.0.14      R6_2.5.1             compiler_4.2.3

```