find_MMsat4

Nikita Verheyden

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Setup

```
setwd("D:/Krueger_Lab/Publications/miR181_paper/Figure2/MMsat4")
```

packages

```
library(AnnotationHub)
```

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

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

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

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

```
## Loading required package: BiocFileCache
```

```
## Loading required package: dbplyr
```

```
library(dplyr)
```

```
##
## Attaching package: 'dplyr'
```

```
## The following objects are masked from 'package:dbplyr':
##
##     ident, sql
```

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

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

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

```
library(ggplot2)
library(ggpubr)
library(rtracklayer)
```

```
## Loading required package: GenomicRanges
## Loading required package: stats4
## 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
##
## Attaching package: 'rtracklayer'
## The following object is masked from 'package:AnnotationHub':
##
##     hubUrl
```

data

```
RNA <- read.csv("D:/Krueger_Lab/Publications/miR181_paper/Figure3/RNA_masterframe.csv")
RPF <- read.csv("D:/Krueger_Lab/Publications/miR181_paper/Figure3/RPF_masterframe.csv")

#load the gtf file to compare genes
gff23 <- import.gff3("D:/Krueger_Lab/Ribo_Profiling/run15112022M23/ref_genome/gencode.vM23.annotation.g

#target data
tject <- readRDS("D:/Krueger_Lab/Publications/miR181_paper/Figure1/mir181_binding_sites__venn_types/mir
names(tject) <- 1:length(tject$geneName)
tframe <- as.data.frame(tject)
head(tframe)
```

```
##   seqnames   start     end width strand scoreSum scoreMean scoreMax
## 1     chr1 6245651 6245657     7      +  9.52553  4.762765  6.00678
## 2     chr1 6248341 6248347     7      + 92.68921 23.172303 48.76900
## 3     chr1 6248857 6248863     7      + 14.07133  7.035665  7.04425
## 4     chr1 6248918 6248924     7      + 38.91451 12.971503 20.65080
## 5     chr1 7170481 7170487     7      + 66.92218 13.384436 25.84490
## 6     chr1 9899605 9899611     7      + 25.15963  6.289907  8.61019
##         geneType geneName             geneID region BS_ID          mir_IP
## 1 protein_coding   Rb1cc1 ENSMUSG00000025907    cds     5 mmu-miR-181a-5p
## 2 protein_coding   Rb1cc1 ENSMUSG00000025907    cds     8 mmu-miR-181a-5p
## 3 protein_coding   Rb1cc1 ENSMUSG00000025907    cds    10 mmu-miR-181a-5p
## 4 protein_coding   Rb1cc1 ENSMUSG00000025907    cds    11 mmu-miR-181a-5p
## 5 protein_coding   Pcmtd1 ENSMUSG00000051285   utr3    19 mmu-miR-181a-5p
## 6 protein_coding     Sgk3 ENSMUSG00000025915   utr3    23 mmu-miR-181a-5p
##   n_mir181 n_mir181a n_mir181b n_mir181c n_mir181d               set WT KO
## 1        1         1         0         0         0 ago_bs_mir181_chi  1  1
## 2        5         5         0         0         0 ago_bs_mir181_chi  1  1
## 3        6         6         0         0         0 ago_bs_mir181_chi  1  0
## 4        6         6         0         0         0 ago_bs_mir181_chi  1  1
## 5        4         4         0         0         0 ago_bs_mir181_chi  1  1
## 6        1         1         0         0         0 ago_bs_mir181_chi NA NA
##            geneID.2 geneName.1 region.1 counts.bs.1_KO counts.bs.2_KO
## 1 ENSMUSG00000025907     Rb1cc1      cds              4             3
## 2 ENSMUSG00000025907     Rb1cc1      cds             28            32
## 3 ENSMUSG00000025907     Rb1cc1      cds             13            11
## 4 ENSMUSG00000025907     Rb1cc1      cds             15            15
## 5 ENSMUSG00000051285     Pcmtd1     utr3             12            22
## 6               <NA>       <NA>     <NA>             NA            NA
##   counts.bs.3_KO counts.bs.4_WT counts.bs.5_WT counts.bs.6_WT
## 1              3              3             10              3
## 2             27             46             41             20
## 3              4             22             13             12
## 4             10             33             20             18
## 5             14             16             20              9
## 6             NA             NA             NA             NA
##            geneID.1 counts.bg.1_KO counts.bg.2_KO counts.bg.3_KO
## 1 ENSMUSG00000025907           1609           1973           1250
## 2 ENSMUSG00000025907           1609           1973           1250
## 3 ENSMUSG00000025907           1609           1973           1250
## 4 ENSMUSG00000025907           1609           1973           1250
## 5 ENSMUSG00000051285           1355           1706           1064
## 6               <NA>             NA             NA             NA
##   counts.bg.4_WT counts.bg.5_WT counts.bg.6_WT resBs.baseMean
## 1           2638           2231           1352       92.10645
## 2           2638           2231           1352      281.53271
## 3           2638           2231           1352      145.51107
## 4           2638           2231           1352      186.74162
## 5           1654           1348            755      151.36245
## 6             NA             NA             NA             NA
##   resBs.log2FoldChange resBs.lfcSE resBs.stat resBs.pvalue resBs.padj
## 1           -0.1093039   0.5923673 0.03419066    0.8533018  0.9652601
## 2            0.2749428   0.2351157 1.35874137    0.2437557  0.6729889
## 3           -0.1805519   0.3623758 0.25017050    0.6169550  0.8961239
## 4           -0.2606282   0.3062717 0.73169661    0.3923338  0.7868678
```

```
## 5            0.1466485   0.3122905 0.22052922    0.6386370  0.9013566
## 6                   NA          NA         NA           NA         NA
##   resBg.baseMean resBg.log2FoldChange resBg.lfcSE resBg.stat resBg.pvalue
## 1             NA                   NA          NA         NA           NA
## 2             NA                   NA          NA         NA           NA
## 3             NA                   NA          NA         NA           NA
## 4             NA                   NA          NA         NA           NA
## 5             NA                   NA          NA         NA           NA
## 6             NA                   NA          NA         NA           NA
##   resBg.padj tpm.counts.bg.1_KO tpm.counts.bg.2_KO tpm.counts.bg.3_KO
## 1         NA           133.7259           117.9980           129.8669
## 2         NA           133.7259           117.9980           129.8669
## 3         NA           133.7259           117.9980           129.8669
## 4         NA           133.7259           117.9980           129.8669
## 5         NA           248.6210           225.2505           244.0445
## 6         NA                 NA                 NA                 NA
##   tpm.counts.bg.4_WT tpm.counts.bg.5_WT tpm.counts.bg.6_WT
## 1           139.8635           146.2855           163.5360
## 2           139.8635           146.2855           163.5360
## 3           139.8635           146.2855           163.5360
## 4           139.8635           146.2855           163.5360
## 5           193.5994           195.1330           201.6149
## 6                 NA                 NA                 NA
##                   BS_ID.1 tpm_support_KO tpm_support_WT tpm_supported  down
## 1  ENSMUSG00000025907.bs5              3              3          TRUE FALSE
## 2  ENSMUSG00000025907.bs8              3              3          TRUE FALSE
## 3 ENSMUSG00000025907.bs10              3              3          TRUE FALSE
## 4 ENSMUSG00000025907.bs11              3              3          TRUE FALSE
## 5  ENSMUSG00000051285.bs4              3              3          TRUE FALSE
## 6                    <NA>             NA             NA            NA    NA
```

```
#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"

RNApcol <- "#b56504"
RNAncol <- "#027d73"
RPFpcol <- "#c4c404"
RPFncol <- "#8d0391"
```

Get annotation

```
# ah = AnnotationHub()
# query(ah, c("RepeatMasker", "Mus musculus"))
# repeat_masker <- ah[["AH99012"]]

# load the downloaded data
repeat_masker <- readRDS("D:/Krueger_Lab/Publications/miR181_paper/Figure2/MMsat4/repeat_masker.rds")
repeat_masker[repeat_masker$repName == "MMSAT4"]
```

```
## GRanges object with 1693 ranges and 11 metadata columns:
##                   seqnames              ranges strand |   swScore  milliDiv
##                      <Rle>           <IRanges>  <Rle> | <integer> <numeric>
##      [1]              chr1   75624925-75625061      + |       347       293
##      [2]              chr1   75625261-75625377      + |       228       292
##      [3]              chr1   75625502-75625561      + |       257       233
##      [4]              chr1 116745797-116745936      + |       357       314
##      [5]              chr1 116745987-116746488      + |       600       228
##      ...               ...                 ...    ... .       ...       ...
##   [1689] chr9_KB469738_fix         24254-24454      - |       304       289
##   [1690] chr9_KB469738_fix         24588-24803      - |       440       281
##   [1691] chr9_KB469738_fix         25005-25142      - |       399       270
##   [1692] chr9_KB469738_fix         25257-25488      - |       389       275
##   [1693] chrY_JH792832_fix       349767-349817      + |       230       180
##           milliDel  milliIns   genoLeft     repName    repClass   repFamily
##          <numeric> <numeric>  <integer> <character> <character> <character>
##      [1]        29        29 -119846910      MMSAT4   Satellite   Satellite
##      [2]        34        34 -119846594      MMSAT4   Satellite   Satellite
##      [3]         0         0 -119846410      MMSAT4   Satellite   Satellite
##      [4]         7         0  -78726035      MMSAT4   Satellite   Satellite
##      [5]        48        22  -78725483      MMSAT4   Satellite   Satellite
##      ...       ...       ...        ...         ...         ...         ...
##   [1689]        24        16    -186187      MMSAT4   Satellite   Satellite
##   [1690]         0         0    -185838      MMSAT4   Satellite   Satellite
##   [1691]         7         7    -185499      MMSAT4   Satellite   Satellite
##   [1692]        21        21    -185153      MMSAT4   Satellite   Satellite
##   [1693]         0        20    -194372      MMSAT4   Satellite   Satellite
##           repStart    repEnd   repLeft
##          <integer> <integer> <integer>
##      [1]        32       168         0
##      [2]        32       148       -20
##      [3]        31        90       -78
##      [4]        27       167        -1
##      [5]         1       514         0
##      ...       ...       ...       ...
##   [1689]         0       202         1
##   [1690]         0       216         1
##   [1691]         0       168        31
##   [1692]         0       232         1
##   [1693]       115       164        -4
##   -------
##   seqinfo: 239 sequences (1 circular) from mm10 genome
```

find overlaps

```
# same strand
MMSAT4 <- repeat_masker[repeat_masker$repName == "MMSAT4"]

OLgenes <- as.data.frame(subsetByOverlaps(gff23, MMSAT4))


# opposite strand
antiMMSAT4 <- MMSAT4
strand(antiMMSAT4) <- ifelse(strand(MMSAT4) == '+', '-', '+')

antiOLgenes <- as.data.frame(subsetByOverlaps(gff23, antiMMSAT4))

dim(OLgenes)
```

```
## [1] 3806   28
```

```
dim(antiOLgenes)
```

```
## [1] 140  28
```

```
RNA$MMsat4 <- "No_MMsat4"
RNA$MMsat4[RNA$gene_symbol %in% OLgenes$gene_name] <- "MMsat4"
RNA$MMsat4[RNA$gene_symbol %in% antiOLgenes$gene_name] <- "anti-MMsat4"
table(RNA$MMsat4)
```

```
##
## anti-MMsat4      MMsat4   No_MMsat4
##          12         238       13051
```

```
RPF$MMsat4 <- "No_MMsat4"
RPF$MMsat4[RPF$gene_symbol %in% OLgenes$gene_name] <- "MMsat4"
RPF$MMsat4[RPF$gene_symbol %in% antiOLgenes$gene_name] <- "anti-MMsat4"
table(RPF$MMsat4)
```

```
##
## anti-MMsat4      MMsat4   No_MMsat4
##          12         225       11132
```

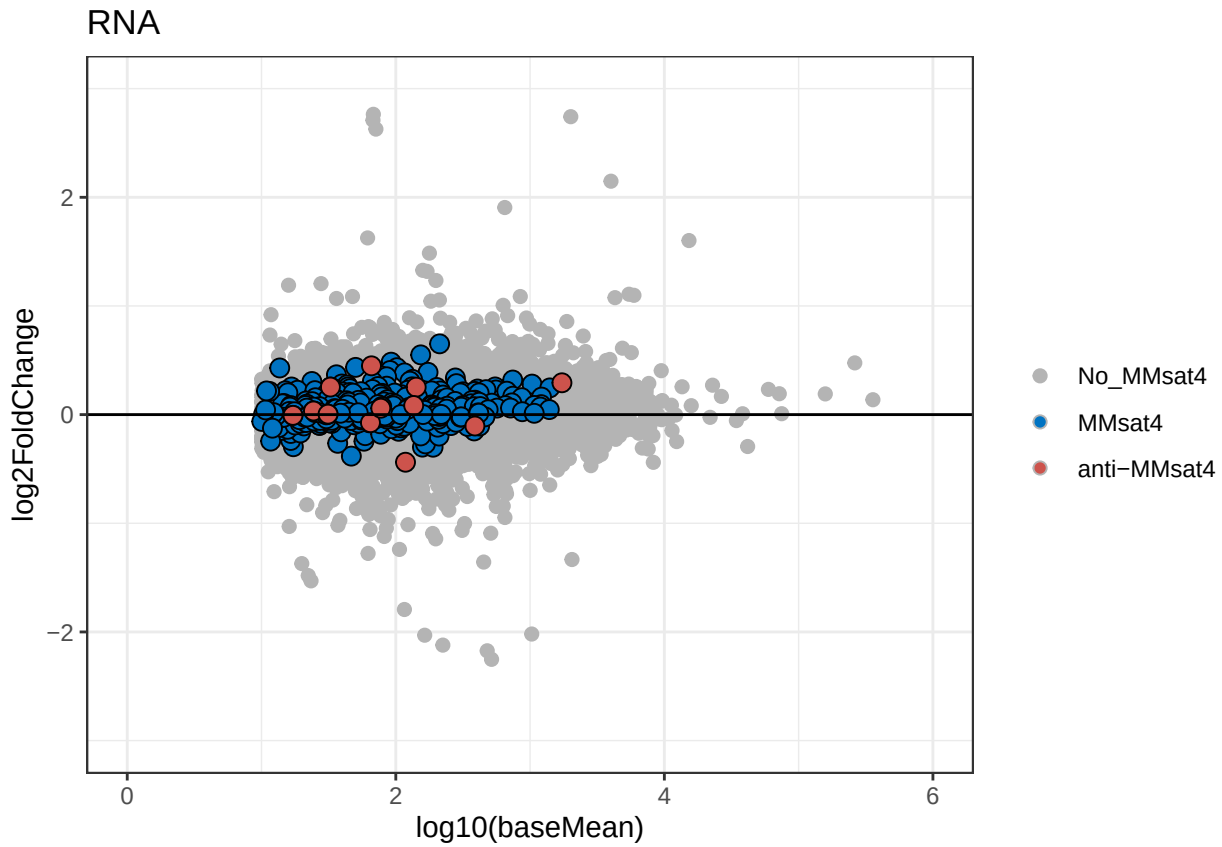
plot into MA plots

```
#RNA
RNAMA <- ggplot(RNA, aes(x=log10(baseMean), y=log2FoldChange, fill=factor(MMsat4, levels = c("No_MMsat4"
  geom_point(shape=21, size=2, colour=farbeneg) +
  scale_fill_manual(values = c(farbeneg, farbe1, farbe3))+
  coord_cartesian(ylim = c(-3,3), xlim = c(0,6))+
  geom_point(data=RNA[RNA$gene_symbol %in% OLgenes$gene_name,], aes(x=log10(baseMean), y=log2FoldChange
  geom_point(data=RNA[RNA$gene_symbol %in% antiOLgenes$gene_name,], aes(x=log10(baseMean), y=log2FoldCha
  geom_hline(yintercept = 0, colour= "black") +
  theme_bw() +
  theme(legend.title = element_blank()) +
  ggtitle("RNA")

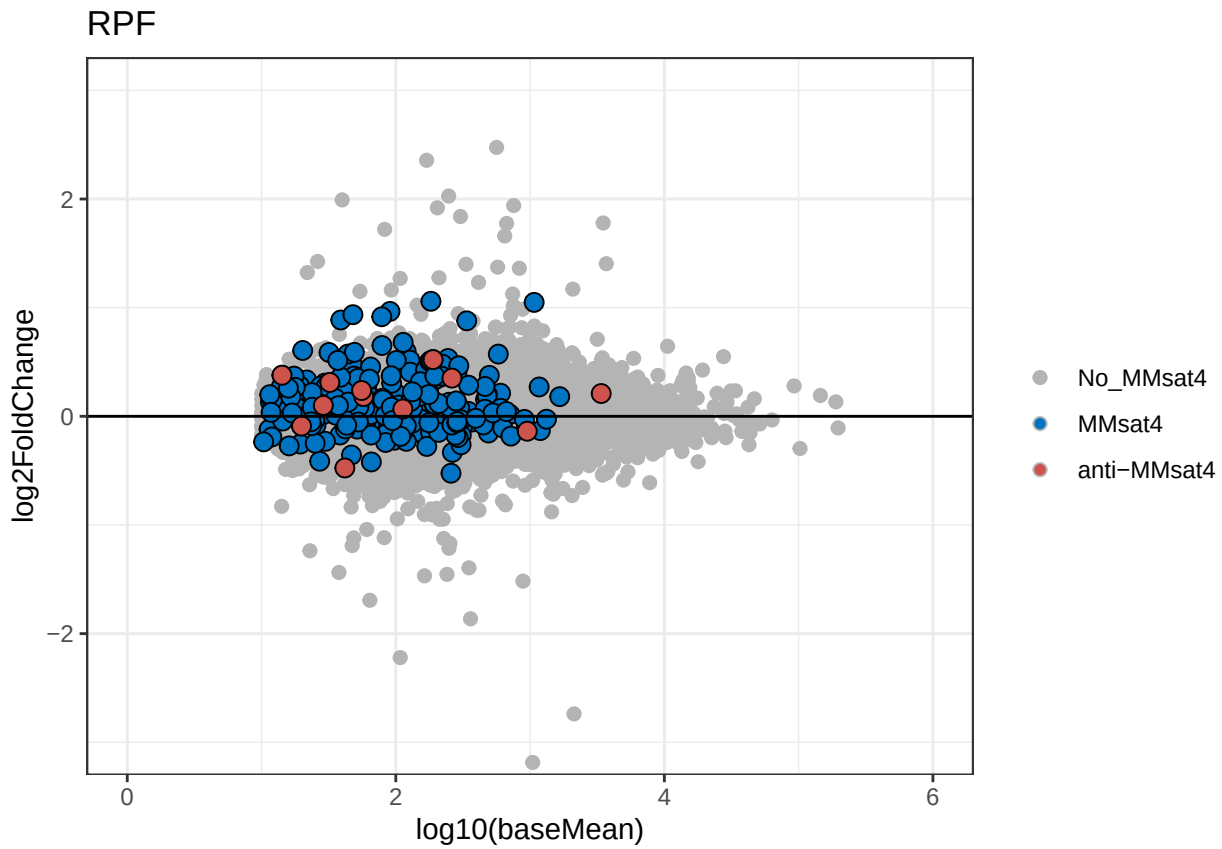
#RPF
```

```
RPFMA <- ggplot(RPF, aes(x=log10(baseMean), y=log2FoldChange, fill=factor(MMsat4, levels = c("No_MMsat4
  geom_point(shape=21, size=2, colour=farbeneg) +
  scale_fill_manual(values = c(farbeneg, farbe1, farbe3))+
  coord_cartesian(ylim = c(-3,3), xlim = c(0,6))+
  geom_point(data=RPF[RPF$gene_symbol %in% OLgenes$gene_name,], aes(x=log10(baseMean), y=log2FoldChange
  geom_point(data=RPF[RPF$gene_symbol %in% antiOLgenes$gene_name,], aes(x=log10(baseMean), y=log2FoldCha
  geom_hline(yintercept = 0, colour= "black") +
  theme_bw() +
  theme(legend.title = element_blank()) +
  ggtitle("RPF")

RNAMA
```




```
RPFMA
```

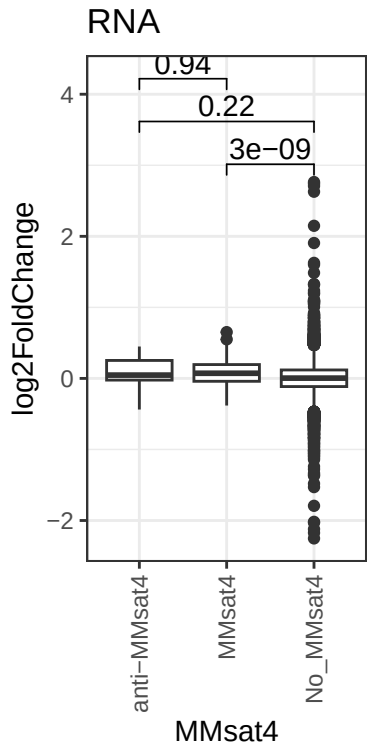



```
my_comparisons <- list( c("No_MMsat4", "MMsat4"), c("No_MMsat4", "anti-MMsat4"), c("MMsat4", "anti-MMsa

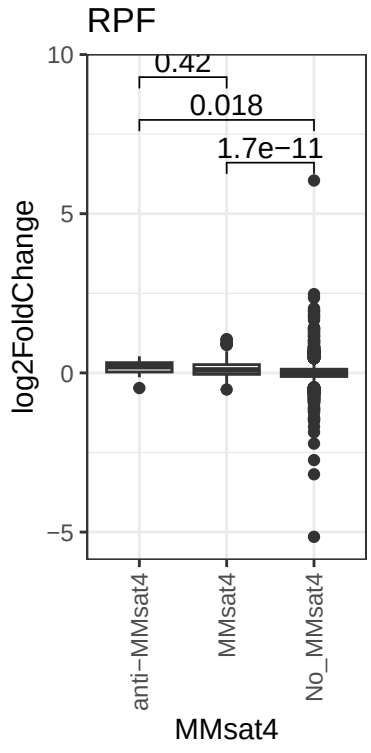
RNAbox <- ggplot(RNA, aes(x=MMsat4, y=log2FoldChange)) +
  geom_boxplot() +
  stat_compare_means(comparisons = my_comparisons) +
  theme_bw() +
  theme(axis.text.x = element_text(angle = 90, hjust = 1, vjust = 0.5)) +
  ggtitle("RNA")

RPFbox <- ggplot(RPF, aes(x=MMsat4, y=log2FoldChange)) +
  geom_boxplot() +
  stat_compare_means(comparisons = my_comparisons) +
  theme_bw() +
  theme(axis.text.x = element_text(angle = 90, hjust = 1, vjust = 0.5)) +
  ggtitle("RPF")

RNAbox
```


RNA
0.94
0.22
3e−09
4
2
0
−2
log2FoldChange
anti−MMsat4
MMsat4
No_MMsat4
MMsat4

```
RPFbox
```


RPF
0.42
0.018
1.7e−11
10
5
0
−5
log2FoldChange
anti−MMsat4
MMsat4
No_MMsat4
MMsat4

ecdf plot with targets vs MMsat4

```
#RNA
RNA$tvsmmsat4 <- "Non-target"
RNA$tvsmmsat4[RNA$gene_symbol %in% OLgenes$gene_name] <- "MMsat4"
RNA$tvsmmsat4[RNA$gene_symbol %in% tframe$geneName] <- "miR-181 target"
RNA$tvsmmsat4[RNA$gene_symbol %in% tframe$geneName & RNA$gene_symbol %in% OLgenes$gene_name] <- "both"
table(RNA$tvsmmsat4)
```

```
##
##           both miR-181 target         MMsat4     Non-target
##            103           3441            141           9616
```

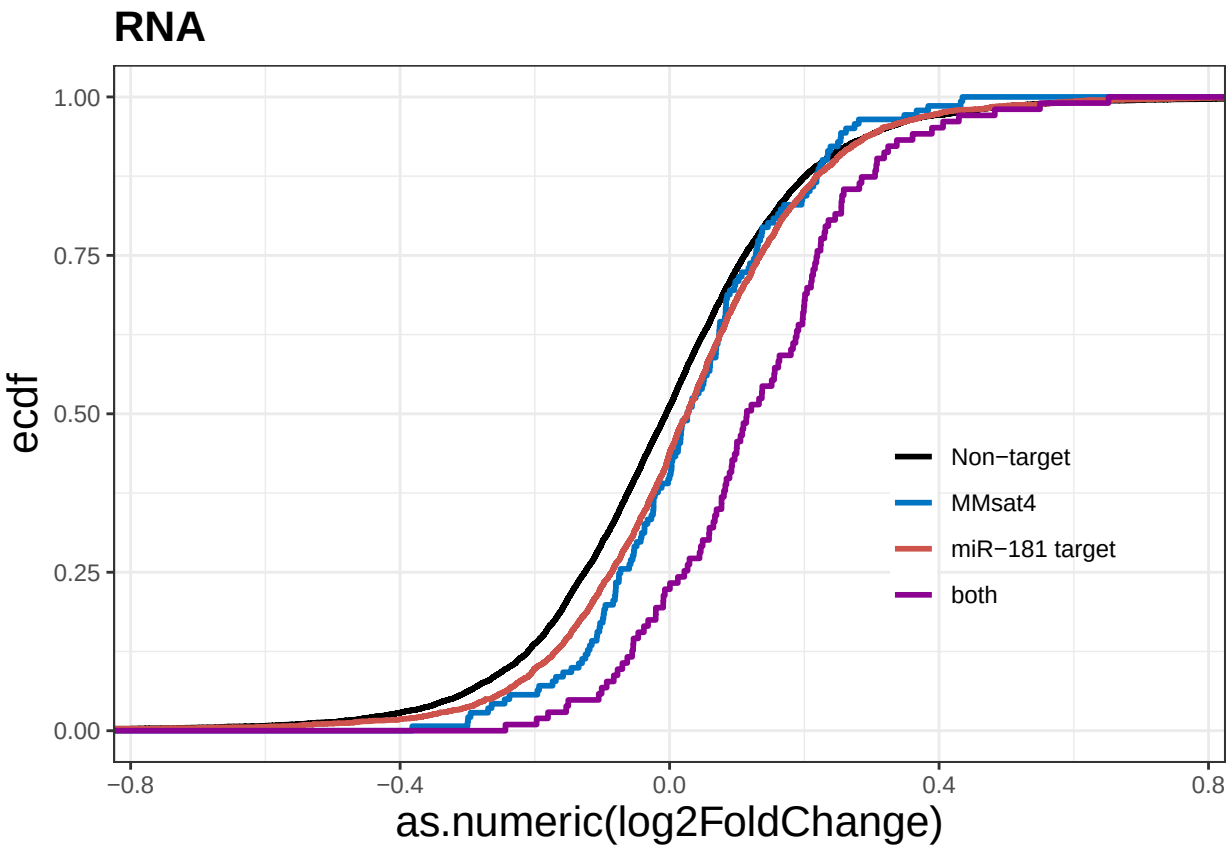
```
#RPF
RPF$tvsmmsat4 <- "Non-target"
RPF$tvsmmsat4[RPF$gene_symbol %in% OLgenes$gene_name] <- "MMsat4"
RPF$tvsmmsat4[RPF$gene_symbol %in% tframe$geneName] <- "miR-181 target"
RPF$tvsmmsat4[RPF$gene_symbol %in% tframe$geneName & RPF$gene_symbol %in% OLgenes$gene_name] <- "both"
table(RPF$tvsmmsat4)
```

```
##
##           both miR-181 target         MMsat4     Non-target
##            100           3405            131           7733
```

```
#RNA
tolECDFRNA <- ggplot(RNA, aes(as.numeric(log2FoldChange), colour=factor(tvsmmsat4, levels = c("Non-targ
  stat_ecdf(geom="step", size=1) +
  scale_colour_manual(values = c("black", farbe1, farbe3, RPFncol)) +
  coord_cartesian(xlim = c(-0.75, 0.75)) + theme_bw() +
  theme(legend.position = c(0.8, 0.35), legend.title = element_blank(),
        legend.background = element_rect(colour = "transparent", fill="transparent"),
        axis.title=element_text(size=16),plot.title = element_text(size=16, face = "bold")) +
  ggtitle("RNA")
```

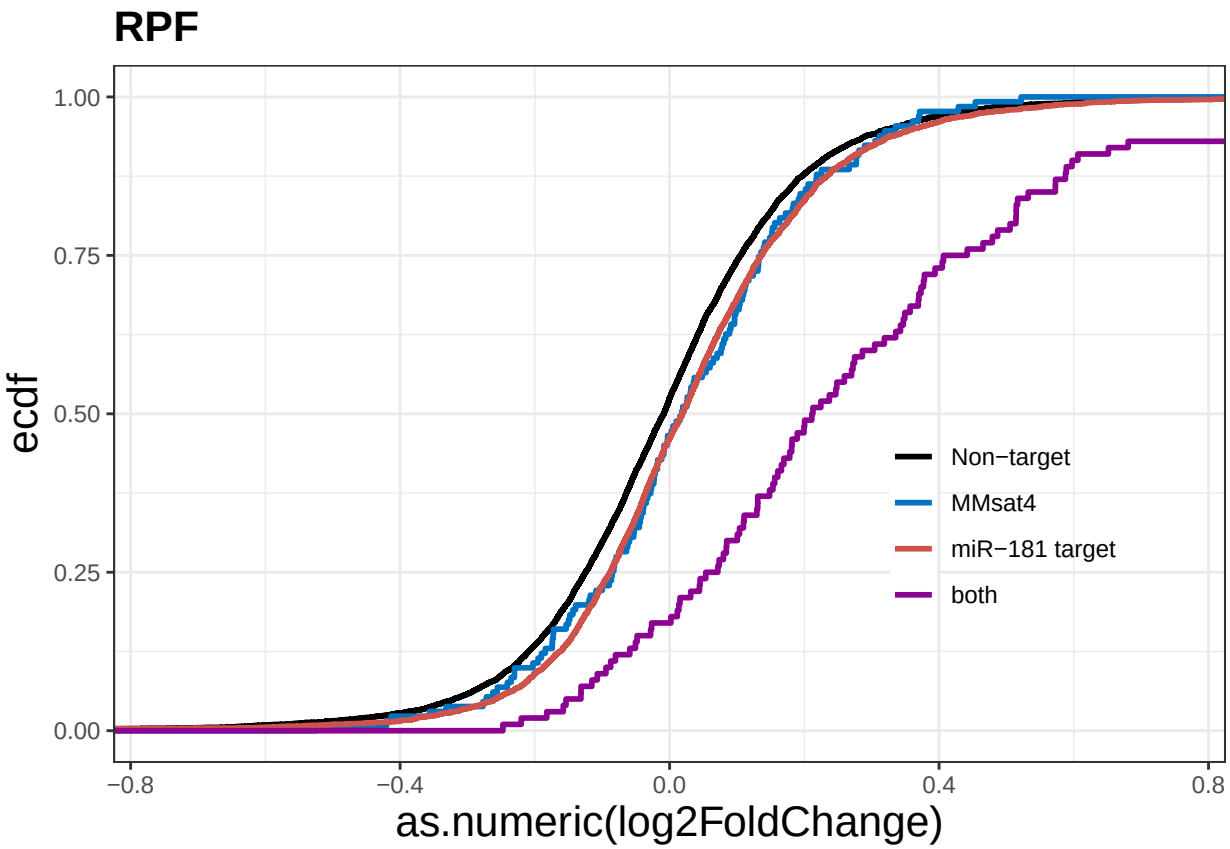
```
## Warning: Using `size` aesthetic for lines was deprecated in ggplot2 3.4.0.
## i Please use `linewidth` instead.
## This warning is displayed once every 8 hours.
## Call `lifecycle::last_lifecycle_warnings()` to see where this warning was
## generated.
```

```
tolECDFRNA
```




```
#RPF
tolECDFRPF <- ggplot(RPF, aes(as.numeric(log2FoldChange), colour=factor(tvsmmsat4, levels = c("Non-targ
  stat_ecdf(geom="step", size=1) +
  scale_colour_manual(values = c("black", farbe1, farbe3, RPFncol)) +
  coord_cartesian(xlim = c(-0.75, 0.75)) + theme_bw() +
  theme(legend.position = c(0.8, 0.35), legend.title = element_blank(),
        legend.background = element_rect(colour = "transparent", fill="transparent"),
        axis.title=element_text(size=16),plot.title = element_text(size=16, face = "bold")) +
  ggtitle("RPF")

tolECDFRPF
```



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 19045)
##
## 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    stats     graphics  grDevices utils     datasets  methods
## [8] base
##
## other attached packages:
##  [1] rtracklayer_1.58.0   GenomicRanges_1.50.2 GenomeInfoDb_1.34.9
##  [4] IRanges_2.32.0       S4Vectors_0.36.2     ggpubr_0.6.0
##  [7] ggplot2_3.4.2        dplyr_1.1.2          AnnotationHub_3.6.0
## [10] BiocFileCache_2.6.1  dbplyr_2.3.2         BiocGenerics_0.44.0
##
```

```
## loaded via a namespace (and not attached):
##  [1] bitops_1.0-7                  matrixStats_0.63.0
##  [3] bit64_4.0.5                   filelock_1.0.2
##  [5] httr_1.4.6                    tools_4.2.3
##  [7] backports_1.4.1               utf8_1.2.3
##  [9] R6_2.5.1                      DBI_1.1.3
## [11] colorspace_2.1-0              withr_2.5.0
## [13] tidyselect_1.2.0              bit_4.0.5
## [15] curl_5.0.0                    compiler_4.2.3
## [17] cli_3.6.0                     Biobase_2.58.0
## [19] DelayedArray_0.23.2           labeling_0.4.2
## [21] scales_1.2.1                  rappdirs_0.3.3
## [23] digest_0.6.31                 Rsamtools_2.14.0
## [25] rmarkdown_2.21                XVector_0.38.0
## [27] pkgconfig_2.0.3               htmltools_0.5.4
## [29] MatrixGenerics_1.10.0         highr_0.10
## [31] fastmap_1.1.1                 rlang_1.1.0
## [33] rstudioapi_0.14               RSQLite_2.3.1
## [35] shiny_1.7.4                   farver_2.1.1
## [37] BiocIO_1.8.0                  generics_0.1.3
## [39] BiocParallel_1.32.6           car_3.1-2
## [41] RCurl_1.98-1.12               magrittr_2.0.3
## [43] GenomeInfoDbData_1.2.9        Matrix_1.5-3
## [45] Rcpp_1.0.10                   munsell_0.5.0
## [47] fansi_1.0.4                   abind_1.4-5
## [49] lifecycle_1.0.3               yaml_2.3.7
## [51] carData_3.0-5                 SummarizedExperiment_1.28.0
## [53] zlibbioc_1.44.0               grid_4.2.3
## [55] blob_1.2.4                    parallel_4.2.3
## [57] promises_1.2.0.1              crayon_1.5.2
## [59] lattice_0.20-45               Biostrings_2.66.0
## [61] KEGGREST_1.38.0               knitr_1.42
## [63] pillar_1.9.0                  rjson_0.2.21
## [65] ggsignif_0.6.4                codetools_0.2-19
## [67] XML_3.99-0.14                 glue_1.6.2
## [69] BiocVersion_3.16.0            evaluate_0.21
## [71] BiocManager_1.30.20           png_0.1-8
## [73] vctrs_0.6.2                   httpuv_1.6.11
## [75] gtable_0.3.3                  purrr_1.0.1
## [77] tidyr_1.3.0                   cachem_1.0.8
## [79] xfun_0.39                     mime_0.12
## [81] xtable_1.8-4                  broom_1.0.4
## [83] restfulr_0.0.15               rstatix_0.7.2
## [85] later_1.3.1                   tibble_3.2.1
## [87] GenomicAlignments_1.34.1      AnnotationDbi_1.60.2
## [89] memoise_2.0.1                 ellipsis_0.3.2
## [91] interactiveDisplayBase_1.36.0
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