fig3 v2

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Setup

dir

```
setwd("D:/Krueger_Lab/Publications/miR181_paper/Figure3/Fig3_2")
```

packages

```
source("D:/Krueger_Lab/Publications/miR181_paper/Supporting_scripts/themes/theme_paper.R")
library(ggplot2)
library(rtracklayer)
```

```
## Loading required package: GenomicRanges
## Loading required package: stats4
## Loading required package: BiocGenerics
##
## Attaching package: 'BiocGenerics'
## The following objects are masked from 'package:stats':
##
##     IQR, mad, sd, var, xtabs
## The following objects are masked from 'package:base':
##
##     anyDuplicated, aperm, append, as.data.frame, basename, cbind,
##     colnames, dirname, do.call, duplicated, eval, evalq, Filter, Find,
##     get, grep, grepl, intersect, is.unsorted, lapply, Map, mapply,
##     match, mget, order, paste, pmax, pmax.int, pmin, pmin.int,
##     Position, rank, rbind, Reduce, rownames, sapply, setdiff, sort,
##     table, tapply, union, unique, unsplit, which.max, which.min
## Loading required package: S4Vectors
##
## Attaching package: 'S4Vectors'
## The following objects are masked from 'package:base':
##
##     expand.grid, I, unname
## Loading required package: IRanges
##
## Attaching package: 'IRanges'
```

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

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

```
library(dplyr)
```

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

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

```
## The following object is masked from 'package:GenomeInfoDb':
##
##     intersect
```

```
## The following objects are masked from 'package:IRanges':
##
##     collapse, desc, intersect, setdiff, slice, union
```

```
## The following objects are masked from 'package:S4Vectors':
##
##     first, intersect, rename, setdiff, setequal, union
```

```
## 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
```

data

```
#Ribo profiling
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

#targets
larget <- readRDS("D:/Krueger_Lab/Publications/miR181_paper/Figure3/mir181_bs_with_seeds.rds")
largetframe <- as.data.frame(larget)

#targets with introns and other
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
```

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

colours

```
#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"
```

inspect targetdata

We're keeping all of those targets for now but will analyze the in ecdf plots

```
table(largetframe$set)
```

```
##
##                 ago_bs_mir181_chi ago_bs_mir181_chi&mir181_enriched
##                              5815                               1082
##                   mir181_enriched
##                              3576
```

```
colnames(largetframe)
```

```
##  [1] "seqnames"                          "start"
##  [3] "end"                               "width"
##  [5] "strand"                            "scoreSum"
##  [7] "scoreMean"                         "scoreMax"
##  [9] "geneType"                          "geneName"
## [11] "geneID"                            "region"
## [13] "mir_IP"                            "n_mir181"
## [15] "n_mir181a"                         "n_mir181b"
## [17] "n_mir181c"                         "n_mir181d"
## [19] "set"                               "mir181BS_ID"
## [21] "WT"                                "KO"
## [23] "geneID.2"                          "geneName.1"
## [25] "region.1"                          "counts.bs.1_KO"
## [27] "counts.bs.2_KO"                    "counts.bs.3_KO"
## [29] "counts.bs.4_WT"                    "counts.bs.5_WT"
## [31] "counts.bs.6_WT"                    "geneID.1"
## [33] "counts.bg.1_KO"                    "counts.bg.2_KO"
## [35] "counts.bg.3_KO"                    "counts.bg.4_WT"
## [37] "counts.bg.5_WT"                    "counts.bg.6_WT"
## [39] "resBs.baseMean"                    "resBs.log2FoldChange"
## [41] "resBs.lfcSE"                       "resBs.stat"
## [43] "resBs.pvalue"                      "resBs.padj"
## [45] "resBg.baseMean"                    "resBg.log2FoldChange"
## [47] "resBg.lfcSE"                       "resBg.stat"
## [49] "resBg.pvalue"                      "resBg.padj"
## [51] "tpm.counts.bg.1_KO"                "tpm.counts.bg.2_KO"
## [53] "tpm.counts.bg.3_KO"                "tpm.counts.bg.4_WT"
## [55] "tpm.counts.bg.5_WT"                "tpm.counts.bg.6_WT"
## [57] "BS_ID"                             "tpm_support_KO"
## [59] "tpm_support_WT"                    "tpm_supported"
## [61] "down"                              "all_seeds_200down"
## [63] "first_seed_200down.start"          "first_seed_200down.end"
## [65] "first_seed_200down.width"          "first_seed_200down.type"
## [67] "first_seed_200down.wobble"         "seed_repetitions.200down"
## [69] "seed_repetitions.200down.wobble"   "all_seeds_200up"
```

```
## [71] "first_seed_200up.start"          "first_seed_200up.end"
## [73] "first_seed_200up.width"          "first_seed_200up.type"
## [75] "first_seed_200up.wobble"         "seed_repetitions.200up"
## [77] "seed_repetitions.200up.wobble"
```

```
#remove duplicates but kep NAs (and ask melina where the NAs come from)
largetframe <- largetframe[!(duplicated(largetframe$BS_ID) & !is.na(largetframe$BS_ID)), ]

## adjust type column
# Add "No seed" as a level to the factor column
largetframe$first_seed_200down.type <- factor(largetframe$first_seed_200down.type, levels = c(levels(la

# Replace NAs with "No seed"
largetframe$first_seed_200down.type[is.na(largetframe$first_seed_200down.type)] <- "No seed"
table(largetframe$first_seed_200down.type)
```

```
##
##    seed_8mer seed_7mer_m8 seed_7mer_a1    seed_6mer      No seed
##          245         1354          321         1054         7490
```

```
## adjust wobble column
largetframe$first_seed_200down.wobble[is.na(largetframe$first_seed_200down.wobble)] <- "No seed"
```

ECDF plots

each code chunk is a split of the main target table that is then used for a specific ecdf plot

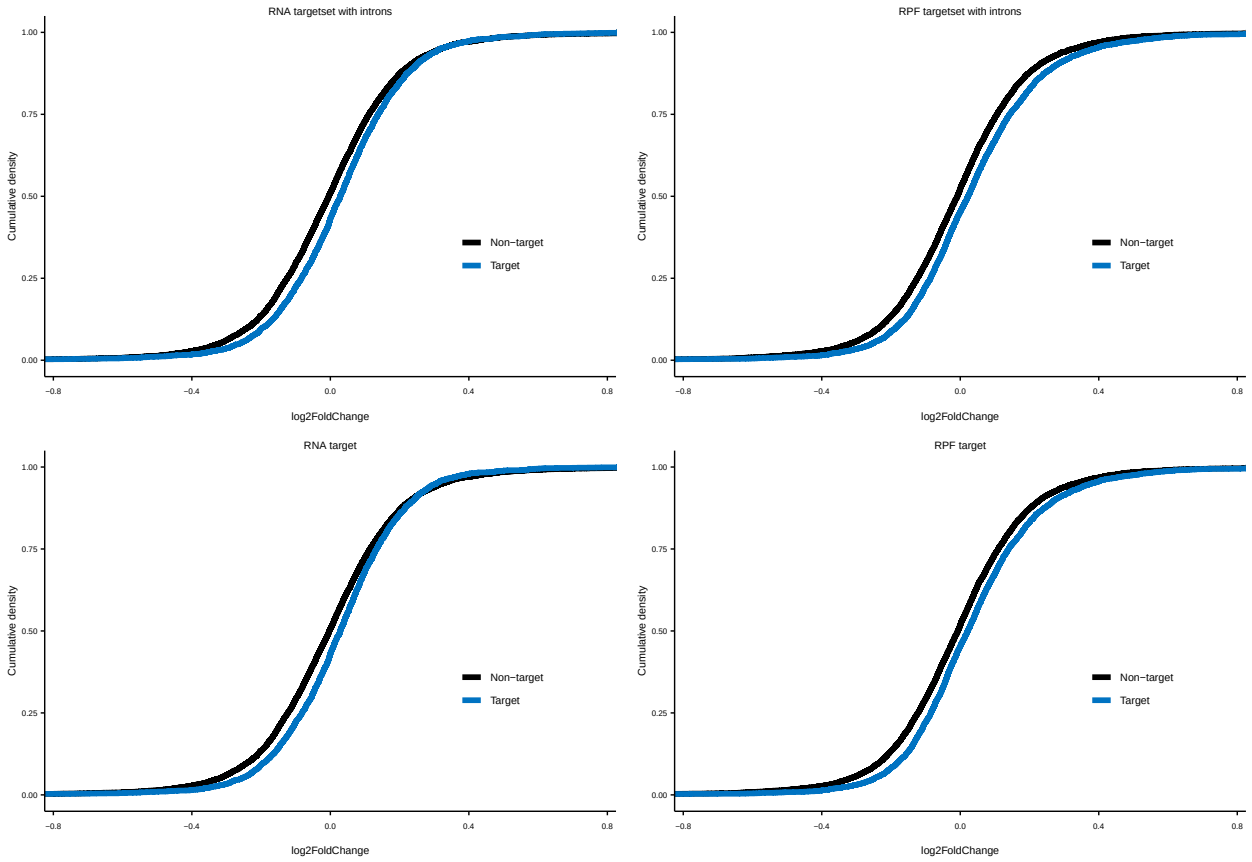
targetfiles

```
#old target with introns
#RNA
RNA$oldtarg <- "Non-target"
RNA$oldtarg[RNA$gene_symbol %in% tframe$geneName] <- "Target"
#RPF
RPF$oldtarg <- "Non-target"
RPF$oldtarg[RPF$gene_symbol %in% tframe$geneName] <- "Target"

#giant frame
#RNA
RNA$target <- "Non-target"
RNA$target[RNA$gene_symbol %in% largetframe$geneName] <- "Target"
#RPF
RPF$target <- "Non-target"
RPF$target[RPF$gene_symbol %in% largetframe$geneName] <- "Target"


#ECDF
#old targets with introns
#RNA
targetoldECDFRNA <- ggplot(RNA, aes(as.numeric(log2FoldChange), colour=factor(oldtarg, levels = c("Non-
  stat_ecdf(geom="step", linewidth=2) +
  scale_colour_manual(values = c("black", farbe1)) +
  coord_cartesian(xlim = c(-0.75, 0.75)) +
  theme_paper() +
```

```
  scale_y_continuous("Cumulative density") + scale_x_continuous("log2FoldChange") +
  ggtitle("RNA targetset with introns")
```

```
## Warning: The `size` argument of `element_line()` is deprecated as of ggplot2 3.4.0.
## i Please use the `linewidth` argument instead.
## This warning is displayed once every 8 hours.
## Call `lifecycle::last_lifecycle_warnings()` to see where this warning was
## generated.
```

```
targetoldECDFRNA

#RPF
targetoldECDFRPF <- ggplot(RPF, aes(as.numeric(log2FoldChange), colour=factor(oldtarg, levels = c("Non-
  stat_ecdf(geom="step", linewidth=2) +
  scale_colour_manual(values = c("black", farbe1)) +
  coord_cartesian(xlim = c(-0.75, 0.75)) +
  theme_paper() +
  scale_y_continuous("Cumulative density") + scale_x_continuous("log2FoldChange") +
  ggtitle("RPF targetset with introns")

targetoldECDFRPF

#targets
#RNA
targetECDFRNA <- ggplot(RNA, aes(as.numeric(log2FoldChange), colour=factor(target, levels = c("Non-targ
  stat_ecdf(geom="step", linewidth=2) +
  scale_colour_manual(values = c("black", farbe1)) +
  coord_cartesian(xlim = c(-0.75, 0.75)) +
  theme_paper() +
  scale_y_continuous("Cumulative density") + scale_x_continuous("log2FoldChange") +
  ggtitle("RNA target")

targetECDFRNA

#RPF
targetECDFRPF <- ggplot(RPF, aes(as.numeric(log2FoldChange), colour=factor(target, levels = c("Non-targ
  stat_ecdf(geom="step", linewidth=2) +
  scale_colour_manual(values = c("black", farbe1)) +
  coord_cartesian(xlim = c(-0.75, 0.75)) +
  theme_paper() +
  scale_y_continuous("Cumulative density") + scale_x_continuous("log2FoldChange") +
  ggtitle("RPF target")

targetECDFRPF
```



datasets

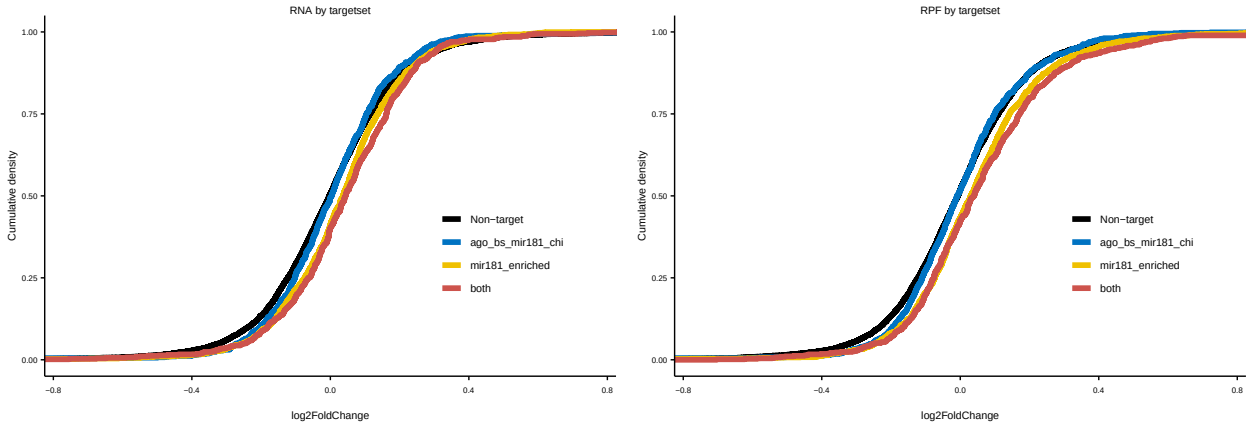
```
#RNA
RNA$targetset <- "Non-target"
RNA$targetset[RNA$gene_symbol %in% largetframe[largetframe$set == "ago_bs_mir181_chi", "geneName"]] <- "
RNA$targetset[RNA$gene_symbol %in% largetframe[largetframe$set == "mir181_enriched", "geneName"]] <- "mi
RNA$targetset[RNA$gene_symbol %in% largetframe[largetframe$set == "ago_bs_mir181_chi&mir181_enriched", "

table(RNA$targetset)
```

```
##
## ago_bs_mir181_chi              both   mir181_enriched        Non-target
##               783               666              1522             10330
```

```
#RPF
RPF$targetset <- "Non-target"
RPF$targetset[RPF$gene_symbol %in% largetframe[largetframe$set == "ago_bs_mir181_chi", "geneName"]] <- "
RPF$targetset[RPF$gene_symbol %in% largetframe[largetframe$set == "mir181_enriched", "geneName"]] <- "mi
RPF$targetset[RPF$gene_symbol %in% largetframe[largetframe$set == "ago_bs_mir181_chi&mir181_enriched", "

table(RPF$targetset)
```

```
##
## ago_bs_mir181_chi              both   mir181_enriched        Non-target
##               782               666              1509              8412
```

```
# ecdf plots
#RNA
setECDFRNA <- ggplot(RNA, aes(as.numeric(log2FoldChange), colour=factor(targetset, levels = c("Non-targ
  stat_ecdf(geom="step", linewidth=2) +
  scale_colour_manual(values = c("black", farbe1, farbe2, farbe3)) +
  coord_cartesian(xlim = c(-0.75, 0.75)) +
  theme_paper() +
  scale_y_continuous("Cumulative density") + scale_x_continuous("log2FoldChange") +
  ggtitle("RNA by targetset")

setECDFRNA

#RPF
setECDFRPF <- ggplot(RPF, aes(as.numeric(log2FoldChange), colour=factor(targetset, levels = c("Non-targ
  stat_ecdf(geom="step", linewidth=2) +
  scale_colour_manual(values = c("black", farbe1, farbe2, farbe3)) +
  coord_cartesian(xlim = c(-0.75, 0.75)) +
  theme_paper() +
  scale_y_continuous("Cumulative density") + scale_x_continuous("log2FoldChange") +
  ggtitle("RPF by targetset")

setECDFRPF
```



modify dataset to only include working targetsets

only use miR181_enriched und both

```
largetframe <- largetframe[largetframe$set %in% c("mir181_enriched", "ago_bs_mir181_chi&mir181_enriched
```

region (single targets)

```
#get number of binding sites per gene to be able to sort for singles
bsnum <- as.data.frame(table(largetframe$geneName))
colnames(bsnum) <- c("geneName", "BS_number")

#RNA
RNA$region_single <- "Non-target"
RNA$region_single[RNA$gene_symbol %in% largetframe[largetframe$region == "utr5", "geneName"]] <- "5'UTR
RNA$region_single[RNA$gene_symbol %in% largetframe[largetframe$region == "cds", "geneName"]] <- "CDS"
```

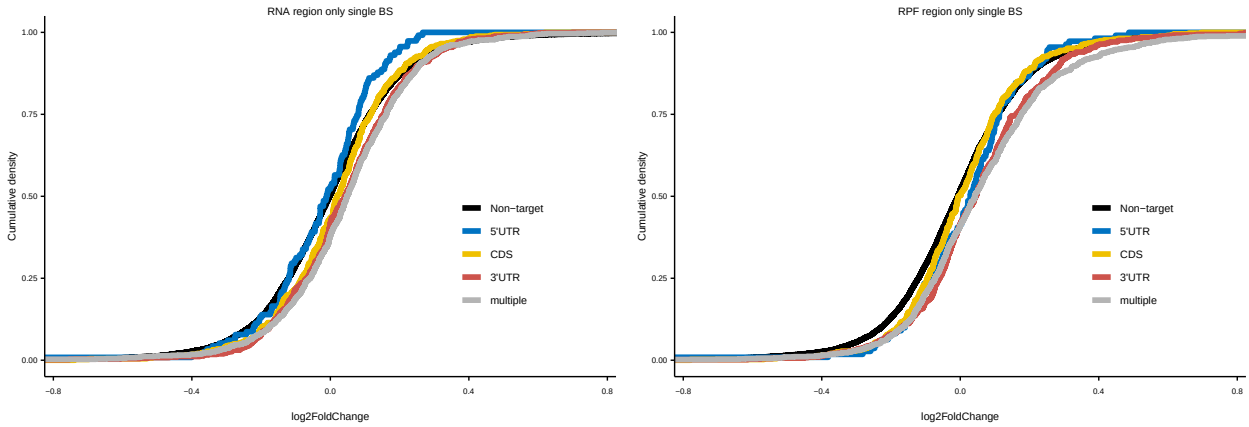
```
RNA$region_single[RNA$gene_symbol %in% largetframe[largetframe$region == "utr3", "geneName"]] <- "3'UTR
RNA$region_single[RNA$gene_symbol %in% bsnum[bsnum$BS_number > 1, "geneName"]] <- "multiple"

table(RNA$region_single)
```

```
##
##      3'UTR      5'UTR        CDS   multiple Non-target
##        618        115        436       1019      11113
```

```
#RPF
RPF$region_single <- "Non-target"
RPF$region_single[RPF$gene_symbol %in% largetframe[largetframe$region == "utr5", "geneName"]] <- "5'UTR
RPF$region_single[RPF$gene_symbol %in% largetframe[largetframe$region == "cds", "geneName"]] <- "CDS"
RPF$region_single[RPF$gene_symbol %in% largetframe[largetframe$region == "utr3", "geneName"]] <- "3'UTR
RPF$region_single[RPF$gene_symbol %in% bsnum[bsnum$BS_number > 1, "geneName"]] <- "multiple"

table(RPF$region_single)
```

```
##
##      3'UTR      5'UTR        CDS   multiple Non-target
##        616        111        434       1014       9194
```

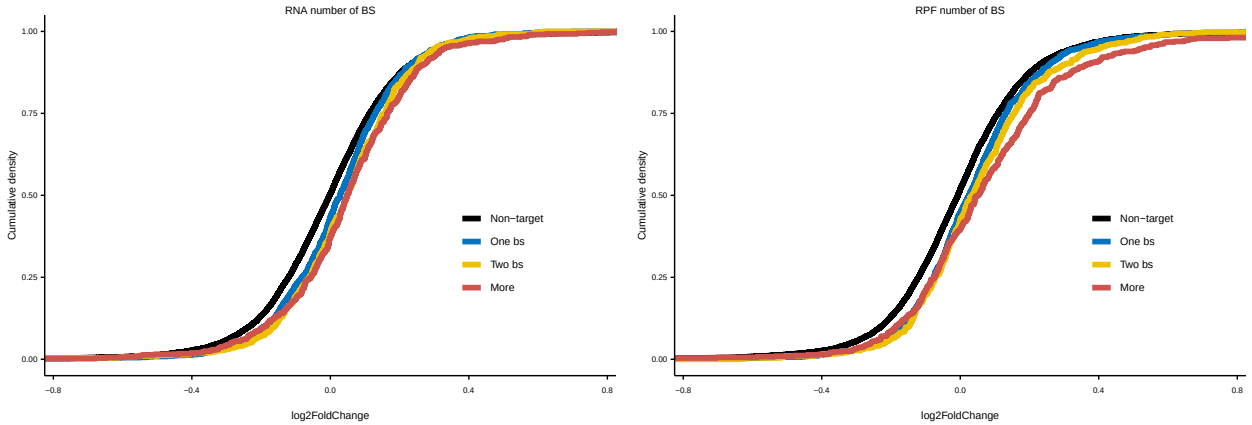
```
# ECDF plots
#RNA
regsingECDFRNA <- ggplot(RNA, aes(as.numeric(log2FoldChange), colour=factor(region_single, levels = c("
  stat_ecdf(geom="step", linewidth=2) +
  scale_colour_manual(values = c("black", farbe1, farbe2, farbe3, farbeneg)) +
  coord_cartesian(xlim = c(-0.75, 0.75)) +
  theme_paper() +
  scale_y_continuous("Cumulative density") + scale_x_continuous("log2FoldChange") +
  ggtitle("RNA region only single BS")

regsingECDFRNA

#RPF
regsingECDFRPF <- ggplot(RPF, aes(as.numeric(log2FoldChange), colour=factor(region_single, levels = c("
  stat_ecdf(geom="step", linewidth=2) +
  scale_colour_manual(values = c("black", farbe1, farbe2, farbe3, farbeneg)) +
  coord_cartesian(xlim = c(-0.75, 0.75)) +
  theme_paper() +
  scale_y_continuous("Cumulative density") + scale_x_continuous("log2FoldChange") +
  ggtitle("RPF region only single BS")

regsingECDFRPF
```



number of target sites

```
colnames(bsnum) <- c("gene_symbol", "BS_number")

#RNA
RNAnum <- left_join(RNA, bsnum, by="gene_symbol")
RNAnum$BS_number[is.na(RNAnum$BS_number)] <- "Non-target"
RNAnum$BS_num_plot <- ifelse(RNAnum$BS_number == "Non-target", "Non-target",
                             ifelse(RNAnum$BS_number == 1, "One bs",
                                    ifelse(RNAnum$BS_number == 2, "Two bs", "More")))


#RPF
RPFnum <- left_join(RPF, bsnum, by="gene_symbol")
RPFnum$BS_number[is.na(RPFnum$BS_number)] <- "Non-target"
RPFnum$BS_num_plot <- ifelse(RPFnum$BS_number == "Non-target", "Non-target",
                             ifelse(RPFnum$BS_number == 1, "One bs",
                                    ifelse(RPFnum$BS_number == 2, "Two bs", "More")))

#ecdf plots
#RNA
numECDFRNA <- ggplot(RNAnum, aes(as.numeric(log2FoldChange), colour=factor(BS_num_plot, levels = c("Non
  stat_ecdf(geom="step", linewidth=2) +
  scale_colour_manual(values = c("black", farbe1, farbe2, farbe3)) +
  coord_cartesian(xlim = c(-0.75, 0.75)) +
  theme_paper() +
  scale_y_continuous("Cumulative density") + scale_x_continuous("log2FoldChange") +
  ggtitle("RNA number of BS")

numECDFRNA

#RPF
numECDFRPF <- ggplot(RPFnum, aes(as.numeric(log2FoldChange), colour=factor(BS_num_plot, levels = c("Non
  stat_ecdf(geom="step", linewidth=2) +
  scale_colour_manual(values = c("black", farbe1, farbe2, farbe3)) +
  coord_cartesian(xlim = c(-0.75, 0.75)) +
  theme_paper() +
  scale_y_continuous("Cumulative density") + scale_x_continuous("log2FoldChange") +
  ggtitle("RPF number of BS")
```

```
numECDFRPF
```



MMsat4

```
mmsat4frame <- as.data.frame(subsetByOverlaps(gff23, MMSAT4))

#RNA
RNA$tvsmmsat4 <- "Non-target"
RNA$tvsmmsat4[RNA$gene_symbol %in% mmsat4frame$gene_name] <- "MMsat4"
RNA$tvsmmsat4[RNA$gene_symbol %in% tframe$geneName] <- "miR-181 target"
RNA$tvsmmsat4[RNA$gene_symbol %in% tframe$geneName & RNA$gene_symbol %in% mmsat4frame$gene_name] <- "bo
table(RNA$tvsmmsat4)
```

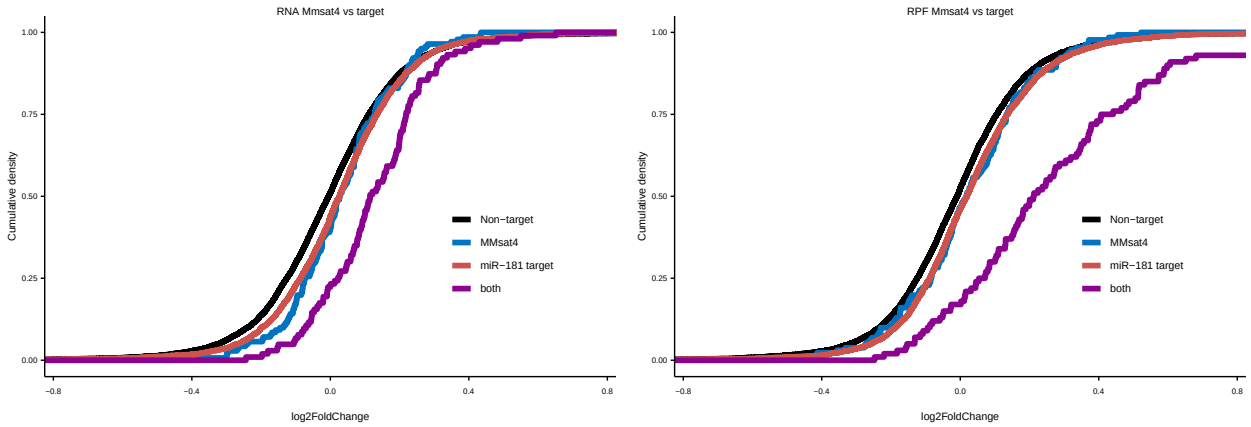
```
##
##           both miR-181 target         MMsat4     Non-target
##            103            3441            141           9616
```

```
#RPF
RPF$tvsmmsat4 <- "Non-target"
RPF$tvsmmsat4[RPF$gene_symbol %in% mmsat4frame$gene_name] <- "MMsat4"
RPF$tvsmmsat4[RPF$gene_symbol %in% tframe$geneName] <- "miR-181 target"
RPF$tvsmmsat4[RPF$gene_symbol %in% tframe$geneName & RPF$gene_symbol %in% mmsat4frame$gene_name] <- "bo
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", linewidth=2) +
  scale_colour_manual(values = c("black", farbe1, farbe3, RPFncol)) +
  coord_cartesian(xlim = c(-0.75, 0.75)) +
  theme_paper() +
  scale_y_continuous("Cumulative density") + scale_x_continuous("log2FoldChange") +
  ggtitle("RNA Mmsat4 vs target")

tolECDFRNA
```

```
#RPF
tolECDFRPF <- ggplot(RPF, aes(as.numeric(log2FoldChange), colour=factor(tvsmmsat4, levels = c("Non-targ
  stat_ecdf(geom="step", linewidth=2) +
  scale_colour_manual(values = c("black", farbe1, farbe3, RPFncol)) +
  coord_cartesian(xlim = c(-0.75, 0.75)) +
  theme_paper() +
  scale_y_continuous("Cumulative density") + scale_x_continuous("log2FoldChange") +
  ggtitle("RPF Mmsat4 vs target")

tolECDFRPF
```



Mmsat4 in 3UTR and CDS

Here we took all targets (genes) that also contain a MMsat4 element and split them by 3'UTR or UTR. The location of the MMsat4 within the gene is not considered in this analysis.

```
rframe <- tframe[tframe$geneName %in% mmsat4frame$gene_name,]

#RNA
RNA$regMMsat4 <- "Non-target"
RNA$regMMsat4[RNA$gene_symbol %in% rframe[rframe$region == "cds","geneName"]] <- "cds"
RNA$regMMsat4[RNA$gene_symbol %in% rframe[rframe$region == "utr3","geneName"]] <- "utr3"
RNA$regMMsat4[RNA$gene_symbol %in% rframe[rframe$region == "utr3","geneName"] &
                RNA$gene_symbol %in% rframe[rframe$region == "cds","geneName"]] <- "both"

table(RNA$regMMsat4)
```

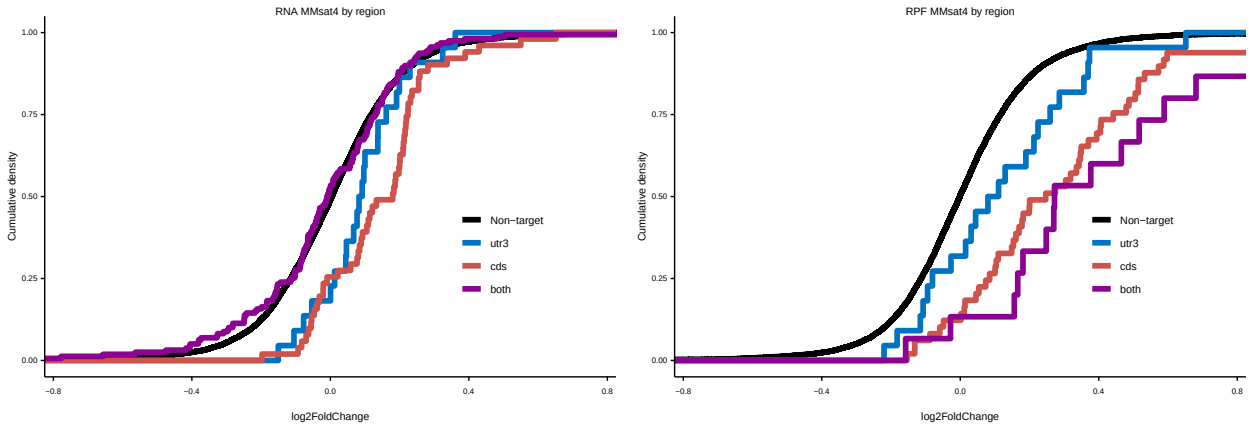
```
##
##       both        cds Non-target       utr3
##        159         51      13069         22
```

```
#RPF
RPF$regMMsat4 <- "Non-target"
RPF$regMMsat4[RPF$gene_symbol %in% rframe[rframe$region == "cds","geneName"]] <- "cds"
RPF$regMMsat4[RPF$gene_symbol %in% rframe[rframe$region == "utr3","geneName"]] <- "utr3"
RPF$regMMsat4[RPF$gene_symbol %in% rframe[rframe$region == "utr3","geneName"] &
                RPF$gene_symbol %in% rframe[rframe$region == "cds","geneName"]] <- "both"

table(RPF$regMMsat4)
```

```
##
```

```
##        both         cds Non-target        utr3
##          15          49       11283          22
```

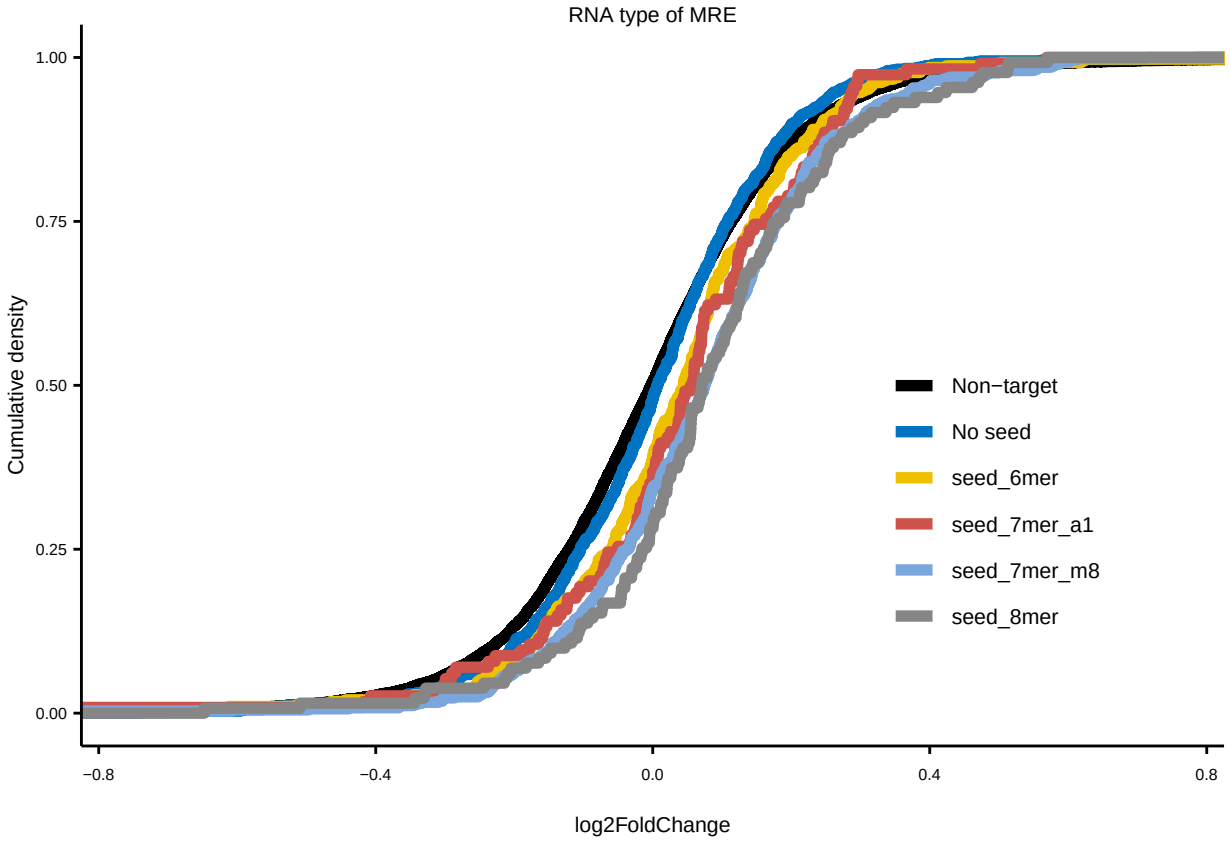
```
#ecdf plots
#RNA
posECDFRNA <- ggplot(RNA, aes(as.numeric(log2FoldChange), colour=factor(regMMsat4, levels = c("Non-targe
  stat_ecdf(geom="step", linewidth=2) +
  scale_colour_manual(values = c("black", farbe1, farbe3, RPFncol)) +
  coord_cartesian(xlim = c(-0.75, 0.75)) +
  theme_paper() +
  scale_y_continuous("Cumulative density") + scale_x_continuous("log2FoldChange") +
  ggtitle("RNA MMsat4 by region")

posECDFRNA

#RPF
posECDFRPF <- ggplot(RPF, aes(as.numeric(log2FoldChange), colour=factor(regMMsat4, levels = c("Non-targe
  stat_ecdf(geom="step", linewidth=2) +
  scale_colour_manual(values = c("black", farbe1, farbe3, RPFncol)) +
  coord_cartesian(xlim = c(-0.75, 0.75)) +
  theme_paper() +
  scale_y_continuous("Cumulative density") + scale_x_continuous("log2FoldChange") +
  ggtitle("RPF MMsat4 by region")

posECDFRPF
```



by type of MRE (wobble and non wobble combined)

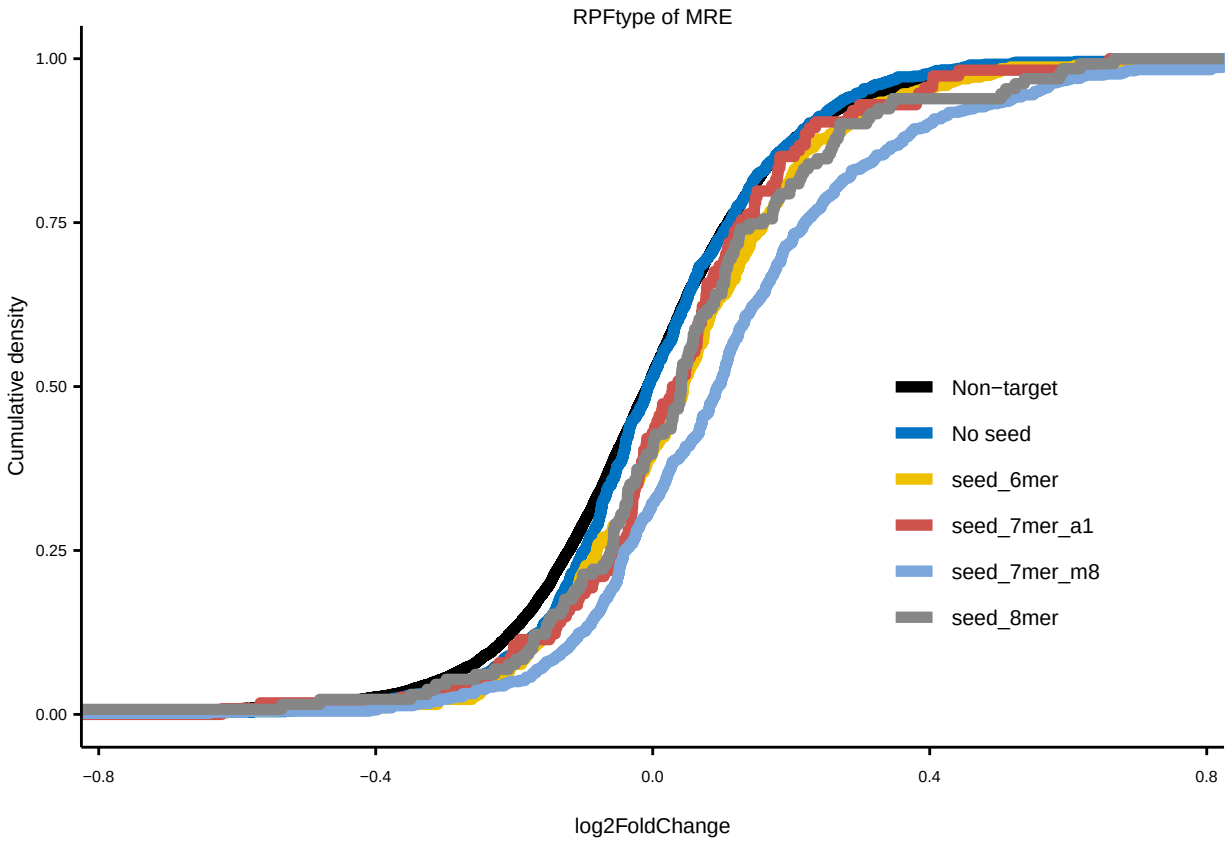
```
#RNA
RNA$MREtype <- "Non-target"
RNA$MREtype[RNA$gene_symbol %in% largetframe[largetframe$first_seed_200down.type == "No seed","geneName
RNA$MREtype[RNA$gene_symbol %in% largetframe[largetframe$first_seed_200down.type == "seed_6mer","geneNa
RNA$MREtype[RNA$gene_symbol %in% largetframe[largetframe$first_seed_200down.type == "seed_7mer_a1","gen
RNA$MREtype[RNA$gene_symbol %in% largetframe[largetframe$first_seed_200down.type == "seed_7mer_m8","gen
RNA$MREtype[RNA$gene_symbol %in% largetframe[largetframe$first_seed_200down.type == "seed_8mer","geneNa

#RPF
RPF$MREtype <- "Non-target"
RPF$MREtype[RPF$gene_symbol %in% largetframe[largetframe$first_seed_200down.type == "No seed","geneName
RPF$MREtype[RPF$gene_symbol %in% largetframe[largetframe$first_seed_200down.type == "seed_6mer","geneNa
```

```
RPF$MREtype[RPF$gene_symbol %in% largetframe[largetframe$first_seed_200down.type == "seed_7mer_a1","gene
RPF$MREtype[RPF$gene_symbol %in% largetframe[largetframe$first_seed_200down.type == "seed_7mer_m8","gene
RPF$MREtype[RPF$gene_symbol %in% largetframe[largetframe$first_seed_200down.type == "seed_8mer","geneNa

# ecdf plots
#RNA
typeECDFRNA <- ggplot(RNA, aes(as.numeric(log2FoldChange),
                               colour=factor(MREtype, levels = c("Non-target", "No seed", "seed_6mer", "
  stat_ecdf(geom="step", linewidth=2) +
  scale_colour_manual(values = c("black", farbe1, farbe2, farbe3, farbe4, farbe5)) +
  coord_cartesian(xlim = c(-0.75, 0.75)) +
  theme_paper() +
  scale_y_continuous("Cumulative density") + scale_x_continuous("log2FoldChange") +
  ggtitle("RNA type of MRE")

typeECDFRNA
```




```
#RPF
typeECDFRPF <- ggplot(RPF, aes(as.numeric(log2FoldChange),
                               colour=factor(MREtype, levels = c("Non-target", "No seed", "seed_6mer", "
  stat_ecdf(geom="step", linewidth=2) +
  scale_colour_manual(values = c("black", farbe1, farbe2, farbe3, farbe4, farbe5)) +
  coord_cartesian(xlim = c(-0.75, 0.75)) +
  theme_paper() +
  scale_y_continuous("Cumulative density") + scale_x_continuous("log2FoldChange") +
  ggtitle("RPFtype of MRE")
```

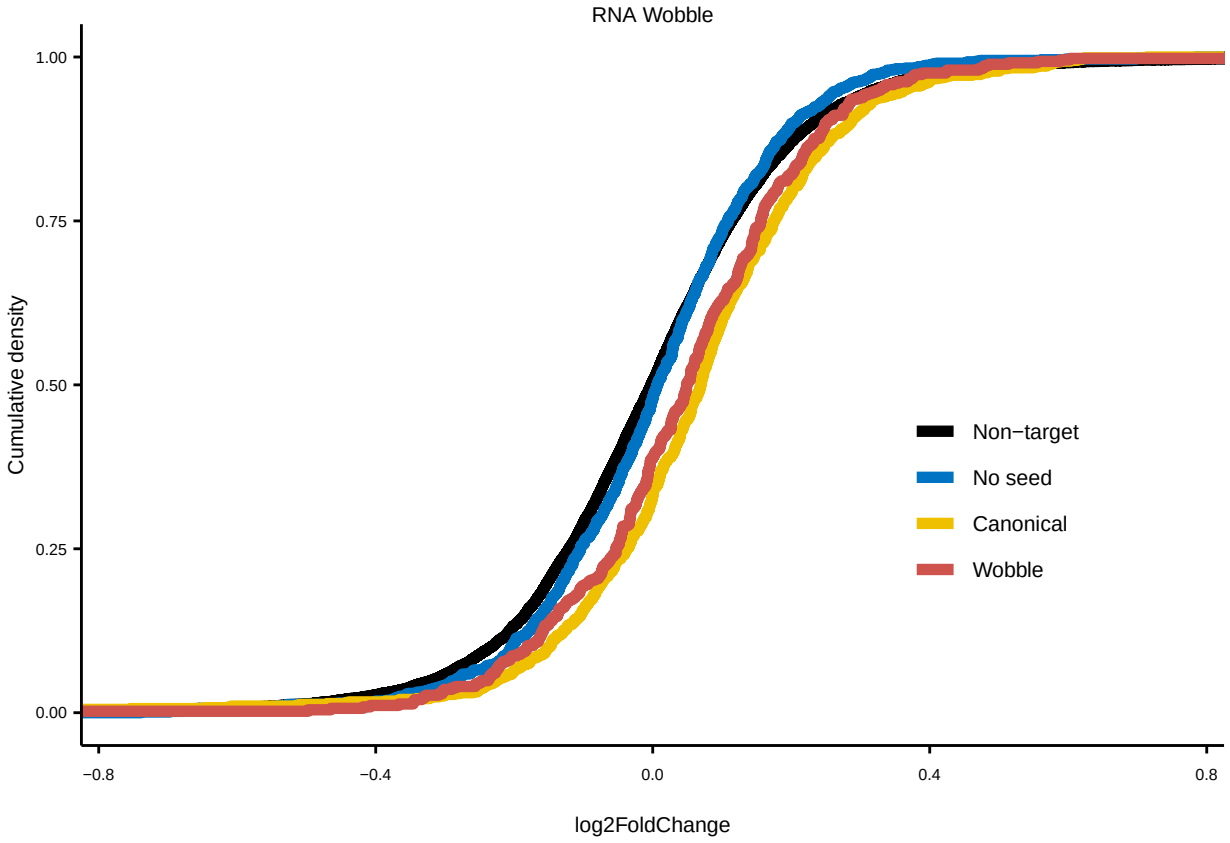
```
typeECDFRPF
```



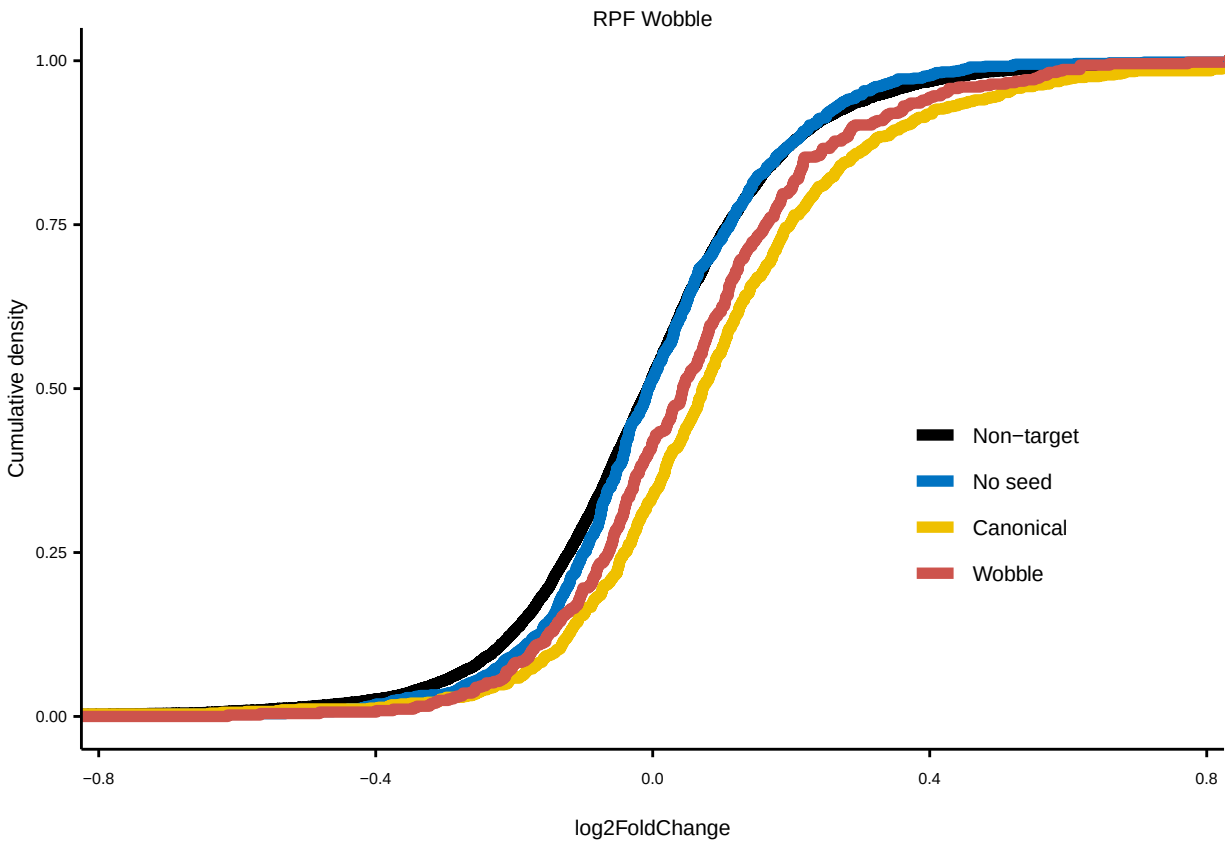
divide by wobble and non wobble in seed

```
#RNA
RNA$wobble <- "Non-target"
RNA$wobble[RNA$gene_symbol %in% largetframe[largetframe$first_seed_200down.wobble == "No seed","geneNam
RNA$wobble[RNA$gene_symbol %in% largetframe[largetframe$first_seed_200down.wobble == FALSE,"geneName"]]
RNA$wobble[RNA$gene_symbol %in% largetframe[largetframe$first_seed_200down.wobble == TRUE,"geneName"]] +

#RPF
RPF$wobble <- "Non-target"
RPF$wobble[RPF$gene_symbol %in% largetframe[largetframe$first_seed_200down.wobble == "No seed","geneNam
RPF$wobble[RPF$gene_symbol %in% largetframe[largetframe$first_seed_200down.wobble == FALSE,"geneName"]]
RPF$wobble[RPF$gene_symbol %in% largetframe[largetframe$first_seed_200down.wobble == TRUE,"geneName"]] +


#ECDF plots
#RNA
wobbleECDFRNA <- ggplot(RNA, aes(as.numeric(log2FoldChange),
                                 colour=factor(wobble, levels = c("Non-target", "No seed", "Canonical", "W
  stat_ecdf(geom="step", linewidth=2) +
  scale_colour_manual(values = c("black", farbe1, farbe2, farbe3)) +
  coord_cartesian(xlim = c(-0.75, 0.75)) +
  theme_paper() +
```

```
  scale_y_continuous("Cumulative density") + scale_x_continuous("log2FoldChange") +
  ggtitle("RNA Wobble")

wobbleECDFRNA
```



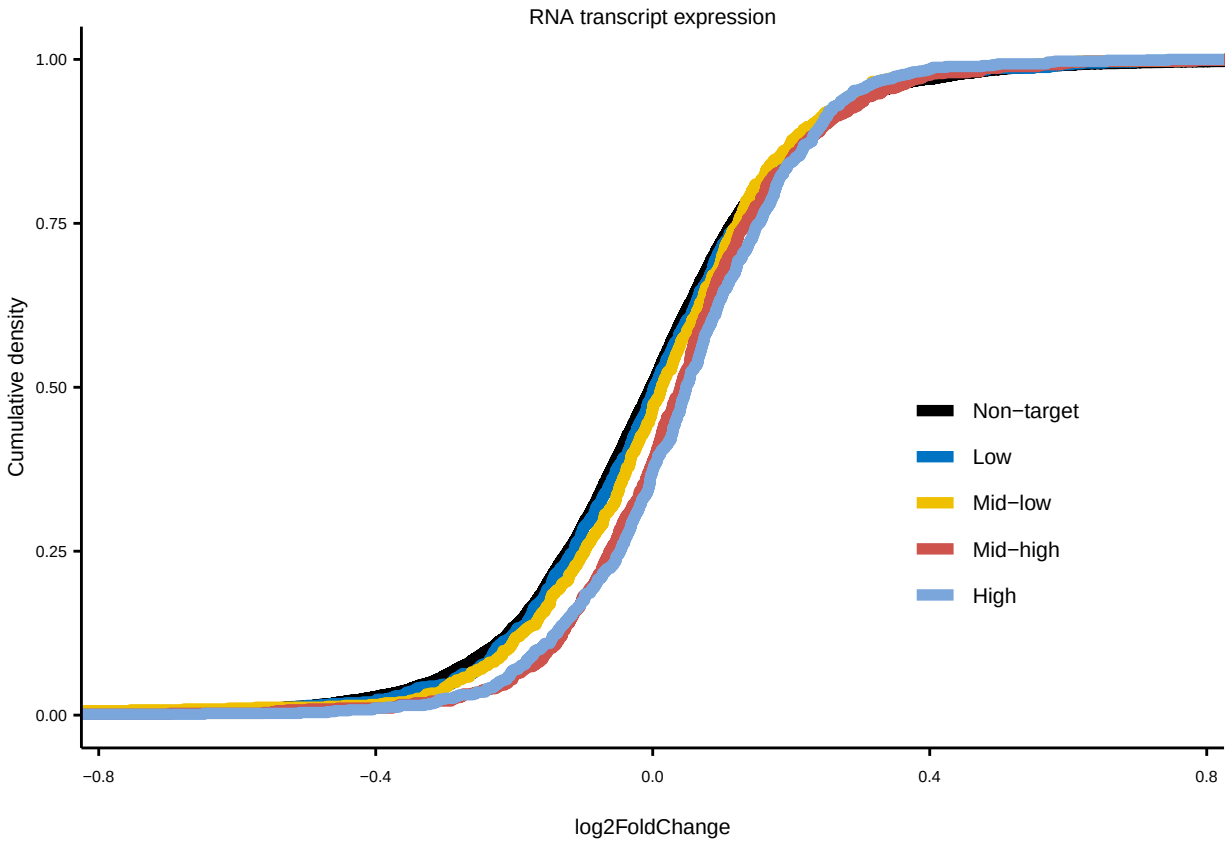

```
#RPF
wobbleECDFRPF <- ggplot(RPF, aes(as.numeric(log2FoldChange),
                            colour=factor(wobble, levels = c("Non-target", "No seed", "Canonical", "W
  stat_ecdf(geom="step", linewidth=2) +
  scale_colour_manual(values = c("black", farbe1, farbe2, farbe3)) +
  coord_cartesian(xlim = c(-0.75, 0.75)) +
  theme_paper() +
  scale_y_continuous("Cumulative density") + scale_x_continuous("log2FoldChange") +
  ggtitle("RPF Wobble")

wobbleECDFRPF
```



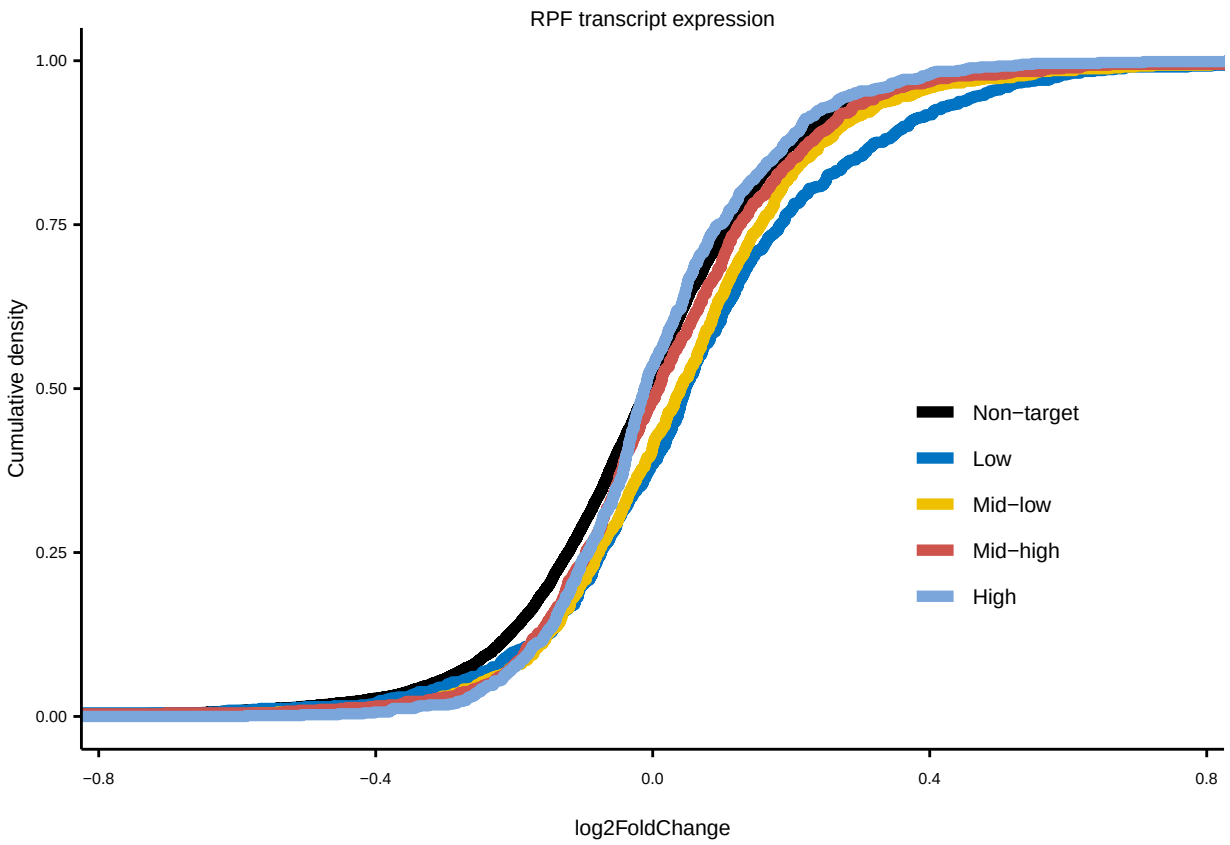
mRNA target expression levels (10% bins or quartiles)

```
#RNA
tRNA <- RNA[RNA$target == "Target",]
tRNA <- tRNA[order(tRNA$baseMean, decreasing = T),]
RNA$expressionlvl <- "Non-target"
RNA$expressionlvl[RNA$gene_symbol %in% tRNA[1:(0.25*length(tRNA$gene_symbol)), "gene_symbol"]] <- "High
RNA$expressionlvl[RNA$gene_symbol %in% tRNA[(0.25*length(tRNA$gene_symbol)):(0.5*length(tRNA$gene_symbol
RNA$expressionlvl[RNA$gene_symbol %in% tRNA[(0.5*length(tRNA$gene_symbol)):(0.75*length(tRNA$gene_symbol
RNA$expressionlvl[RNA$gene_symbol %in% tRNA[(0.75*length(tRNA$gene_symbol)):length(tRNA$gene_symbol), "

#RPF
tRPF <- RPF[RPF$target == "Target",]
tRPF <- tRPF[order(tRPF$baseMean, decreasing = T),]
RPF$expressionlvl <- "Non-target"
RPF$expressionlvl[RPF$gene_symbol %in% tRPF[1:(0.25*length(tRPF$gene_symbol)), "gene_symbol"]] <- "High
RPF$expressionlvl[RPF$gene_symbol %in% tRPF[(0.25*length(tRPF$gene_symbol)):(0.5*length(tRPF$gene_symbol
RPF$expressionlvl[RPF$gene_symbol %in% tRPF[(0.5*length(tRPF$gene_symbol)):(0.75*length(tRPF$gene_symbol
RPF$expressionlvl[RPF$gene_symbol %in% tRPF[(0.75*length(tRPF$gene_symbol)):length(tRPF$gene_symbol), "


#ECDF plots
#RNA
expECDFRNA <- ggplot(RNA, aes(as.numeric(log2FoldChange),
                              colour=factor(expressionlvl, levels = c("Non-target", "Low", "Mid-low", "
```

```
  stat_ecdf(geom="step", linewidth=2) +
  scale_colour_manual(values = c("black", farbe1, farbe2, farbe3, farbe4)) +
  coord_cartesian(xlim = c(-0.75, 0.75)) +
  theme_paper() +
  scale_y_continuous("Cumulative density") + scale_x_continuous("log2FoldChange") +
  ggtitle("RNA transcript expression")

expECDFRNA
```




```
#RPF
expECDFRPF <- ggplot(RPF, aes(as.numeric(log2FoldChange),
                              colour=factor(expressionlvl, levels = c("Non-target", "Low", "Mid-low", "
  stat_ecdf(geom="step", linewidth=2) +
  scale_colour_manual(values = c("black", farbe1, farbe2, farbe3, farbe4)) +
  coord_cartesian(xlim = c(-0.75, 0.75)) +
  theme_paper() +
  scale_y_continuous("Cumulative density") + scale_x_continuous("log2FoldChange") +
  ggtitle("RPF transcript expression")

expECDFRPF
```

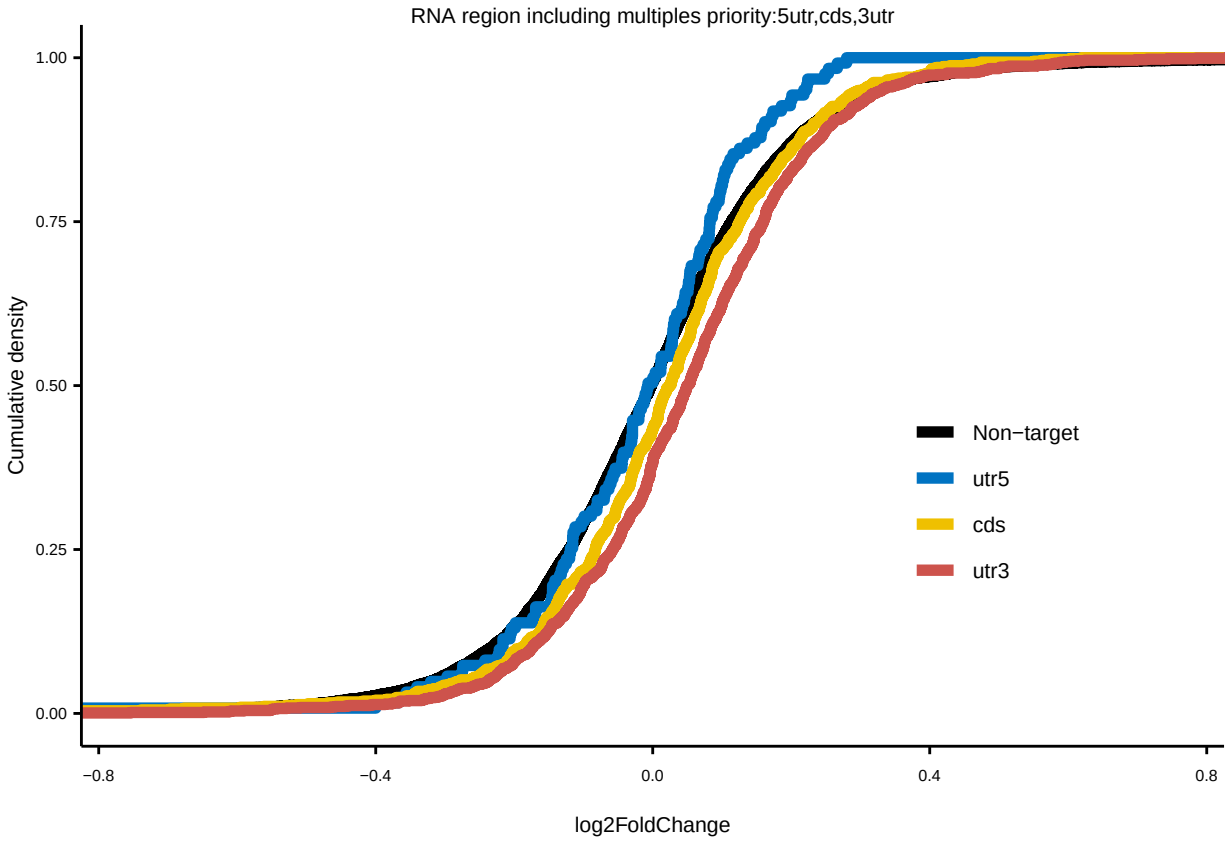


Region with multiple sites ordered by "importance"

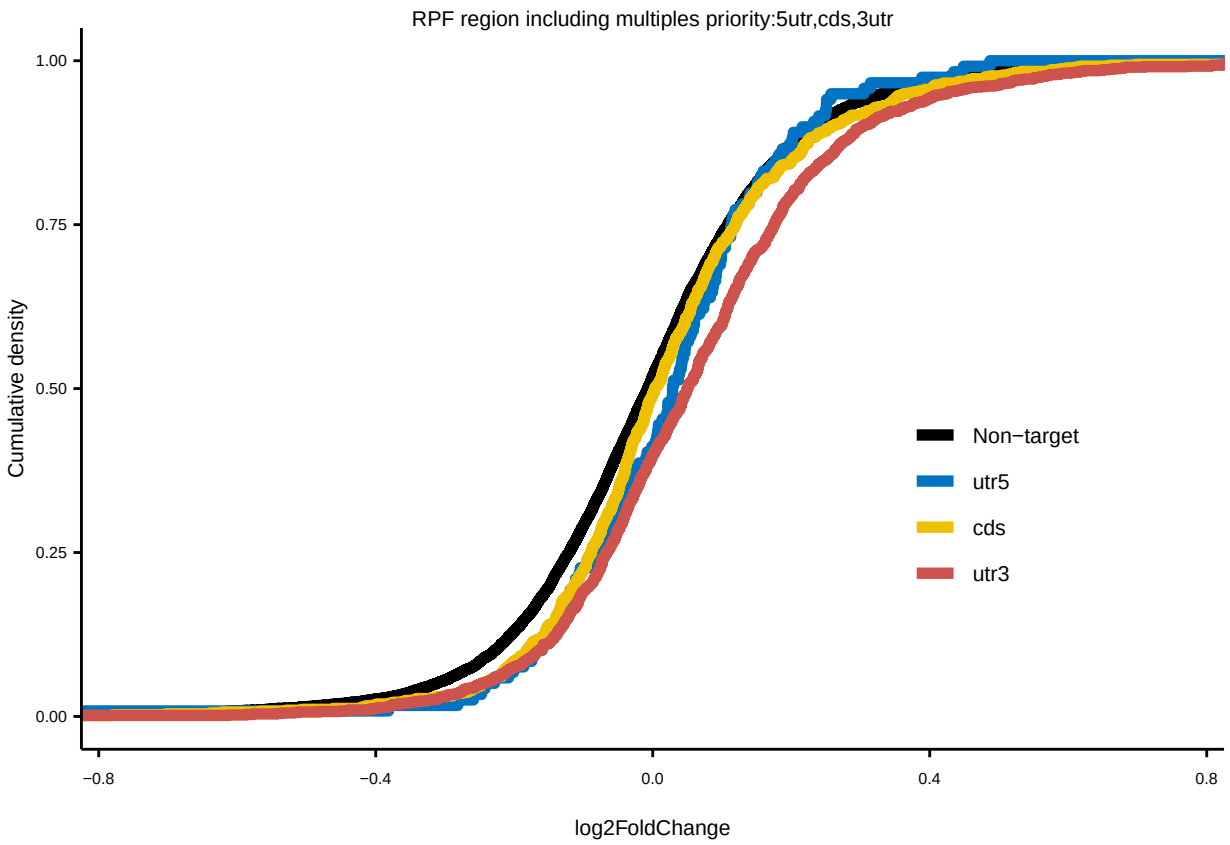
they were sorted in the following order: 5utr cds 3UTR

```
RNA$region_multiple <- "Non-target"
RNA$region_multiple[RNA$gene_symbol %in% largetframe[largetframe$region == "utr5", "geneName"]] <- "utr5
RNA$region_multiple[RNA$gene_symbol %in% largetframe[largetframe$region == "cds", "geneName"]] <- "cds"
RNA$region_multiple[RNA$gene_symbol %in% largetframe[largetframe$region == "utr3", "geneName"]] <- "utr3

RPF$region_multiple <- "Non-target"
RPF$region_multiple[RPF$gene_symbol %in% largetframe[largetframe$region == "utr5", "geneName"]] <- "utr5
RPF$region_multiple[RPF$gene_symbol %in% largetframe[largetframe$region == "cds", "geneName"]] <- "cds"
RPF$region_multiple[RPF$gene_symbol %in% largetframe[largetframe$region == "utr3", "geneName"]] <- "utr3

# ECDF plots

#RNA
regionmultiECDFRNA <- ggplot(RNA, aes(as.numeric(log2FoldChange),
                             colour=factor(region_multiple, levels = c("Non-target", "utr5", "cds", "u
  stat_ecdf(geom="step", linewidth=2) +
  scale_colour_manual(values = c("black", farbe1, farbe2, farbe3)) +
  coord_cartesian(xlim = c(-0.75, 0.75)) +
  theme_paper() +
  scale_y_continuous("Cumulative density") + scale_x_continuous("log2FoldChange") +
  ggtitle("RNA region including multiples priority:5utr,cds,3utr")
```

```
regionmultiECDFRNA
```




```
#RPF
regionmultiECDFRPF <- ggplot(RPF, aes(as.numeric(log2FoldChange),
                             colour=factor(region_multiple, levels = c("Non-target", "utr5", "cds", "u
  stat_ecdf(geom="step", linewidth=2) +
  scale_colour_manual(values = c("black", farbe1, farbe2, farbe3)) +
  coord_cartesian(xlim = c(-0.75, 0.75)) +
  theme_paper() +
  scale_y_continuous("Cumulative density") + scale_x_continuous("log2FoldChange") +
  ggtitle("RPF region including multiples priority:5utr,cds,3utr")

regionmultiECDFRPF
```




```
head(largetframe)
```

```
##      seqnames    start      end width strand  scoreSum  scoreMean  scoreMax
## 5816     chr1  6240149  6240155     7      +  1.539866  0.7699330  0.976837
## 5817     chr1  6244793  6244799     7      +  1.069113  0.5345565  0.607505
## 5818     chr1  6249310  6249316     7      + 11.351500  3.7838332  8.571320
## 5819     chr1  6270741  6270747     7      + 30.310850 10.1036167 17.150800
## 5820     chr1  7163305  7163311     7      +  9.214680  3.0715600  5.533180
## 5821     chr1 33740116 33740122     7      +  7.169806  1.4339612  5.002240
##            geneType geneName            geneID region mir_IP n_mir181
## 5816 protein_coding   Rb1cc1 ENSMUSG00000025907    cds   <NA>       NA
## 5817 protein_coding   Rb1cc1 ENSMUSG00000025907    cds   <NA>       NA
## 5818 protein_coding   Rb1cc1 ENSMUSG00000025907    cds   <NA>       NA
## 5819 protein_coding   Rb1cc1 ENSMUSG00000025907   utr3   <NA>       NA
## 5820 protein_coding   Pcmtd1 ENSMUSG00000051285   utr3   <NA>       NA
## 5821 protein_coding    Rab23 ENSMUSG00000004768   utr3   <NA>       NA
##      n_mir181a n_mir181b n_mir181c n_mir181d            set mir181BS_ID WT KO
## 5816        NA        NA        NA        NA mir181_enriched        7118 NA NA
## 5817        NA        NA        NA        NA mir181_enriched        7119 NA NA
## 5818        NA        NA        NA        NA mir181_enriched        7120  1  1
## 5819        NA        NA        NA        NA mir181_enriched        7122 NA NA
## 5820        NA        NA        NA        NA mir181_enriched        7123 NA NA
## 5821        NA        NA        NA        NA mir181_enriched        7125 NA NA
##               geneID.2 geneName.1 region.1 counts.bs.1_KO counts.bs.2_KO
## 5816              <NA>       <NA>     <NA>             NA             NA
## 5817              <NA>       <NA>     <NA>             NA             NA
```

```
## 5818 ENSMUSG00000025907     Rb1cc1      cds              6              9
## 5819               <NA>       <NA>     <NA>             NA             NA
## 5820               <NA>       <NA>     <NA>             NA             NA
## 5821               <NA>       <NA>     <NA>             NA             NA
##      counts.bs.3_KO counts.bs.4_WT counts.bs.5_WT counts.bs.6_WT
## 5816             NA             NA             NA             NA
## 5817             NA             NA             NA             NA
## 5818              7             12              7              9
## 5819             NA             NA             NA             NA
## 5820             NA             NA             NA             NA
## 5821             NA             NA             NA             NA
##                geneID.1 counts.bg.1_KO counts.bg.2_KO counts.bg.3_KO
## 5816               <NA>             NA             NA             NA
## 5817               <NA>             NA             NA             NA
## 5818 ENSMUSG00000025907           1609           1973           1250
## 5819               <NA>             NA             NA             NA
## 5820               <NA>             NA             NA             NA
## 5821               <NA>             NA             NA             NA
##      counts.bg.4_WT counts.bg.5_WT counts.bg.6_WT resBs.baseMean
## 5816             NA             NA             NA             NA
## 5817             NA             NA             NA             NA
## 5818           2638           2231           1352       121.5778
## 5819             NA             NA             NA             NA
## 5820             NA             NA             NA             NA
## 5821             NA             NA             NA             NA
##      resBs.log2FoldChange resBs.lfcSE resBs.stat resBs.pvalue resBs.padj
## 5816                   NA          NA         NA           NA         NA
## 5817                   NA          NA         NA           NA         NA
## 5818            0.2188539   0.4259348  0.2620372     0.608724  0.8940161
## 5819                   NA          NA         NA           NA         NA
## 5820                   NA          NA         NA           NA         NA
## 5821                   NA          NA         NA           NA         NA
##      resBg.baseMean resBg.log2FoldChange resBg.lfcSE resBg.stat resBg.pvalue
## 5816             NA                   NA          NA         NA           NA
## 5817             NA                   NA          NA         NA           NA
## 5818             NA                   NA          NA         NA           NA
## 5819             NA                   NA          NA         NA           NA
## 5820             NA                   NA          NA         NA           NA
## 5821             NA                   NA          NA         NA           NA
##      resBg.padj tpm.counts.bg.1_KO tpm.counts.bg.2_KO tpm.counts.bg.3_KO
## 5816         NA                 NA                 NA                 NA
## 5817         NA                 NA                 NA                 NA
## 5818         NA           133.7259            117.998           129.8669
## 5819         NA                 NA                 NA                 NA
## 5820         NA                 NA                 NA                 NA
## 5821         NA                 NA                 NA                 NA
##      tpm.counts.bg.4_WT tpm.counts.bg.5_WT tpm.counts.bg.6_WT
## 5816                 NA                 NA                 NA
## 5817                 NA                 NA                 NA
## 5818           139.8635           146.2855            163.536
## 5819                 NA                 NA                 NA
## 5820                 NA                 NA                 NA
## 5821                 NA                 NA                 NA
##                        BS_ID tpm_support_KO tpm_support_WT tpm_supported  down
```

```
## 5816                   <NA>             NA             NA             NA    NA
## 5817                   <NA>             NA             NA             NA    NA
## 5818 ENSMUSG00000025907.bs14              3              3           TRUE FALSE
## 5819                   <NA>             NA             NA             NA    NA
## 5820                   <NA>             NA             NA             NA    NA
## 5821                   <NA>             NA             NA             NA    NA
##      all_seeds_200down first_seed_200down.start first_seed_200down.end
## 5816     NA, NA, ....                       NA                     NA
## 5817     NA, NA, ....                       NA                     NA
## 5818     NA, NA, ....                       NA                     NA
## 5819     NA, NA, ....                       NA                     NA
## 5820     NA, NA, ....                       NA                     NA
## 5821     34, 39, ....                       34                     39
##      first_seed_200down.width first_seed_200down.type first_seed_200down.wobble
## 5816                       NA                No seed                   No seed
## 5817                       NA                No seed                   No seed
## 5818                       NA                No seed                   No seed
## 5819                       NA                No seed                   No seed
## 5820                       NA                No seed                   No seed
## 5821                        6               seed_6mer                     FALSE
##      seed_repetitions.200down seed_repetitions.200down.wobble all_seeds_200up
## 5816                       NA                              NA    c(70, 70....
## 5817                       NA                              NA    NA, NA, ....
## 5818                       NA                              NA    NA, NA, ....
## 5819                       NA                              NA    NA, NA, ....
## 5820                       NA                              NA    NA, NA, ....
## 5821                        1                               0    NA, NA, ....
##      first_seed_200up.start first_seed_200up.end first_seed_200up.width
## 5816                     70                   76                     7
## 5817                     NA                   NA                    NA
## 5818                     NA                   NA                    NA
## 5819                     NA                   NA                    NA
## 5820                     NA                   NA                    NA
## 5821                     NA                   NA                    NA
##      first_seed_200up.type first_seed_200up.wobble seed_repetitions.200up
## 5816          seed_7mer_a1                   FALSE                     1
## 5817                  <NA>                      NA                    NA
## 5818                  <NA>                      NA                    NA
## 5819                  <NA>                      NA                    NA
## 5820                  <NA>                      NA                    NA
## 5821                  <NA>                      NA                    NA
##      seed_repetitions.200up.wobble
## 5816                             0
## 5817                            NA
## 5818                            NA
## 5819                            NA
## 5820                            NA
## 5821                            NA
```

Export

"D:/Krueger_Lab/Publications/miR181_paper/Figure4"

```
#target

pdf("D:/Krueger_Lab/Publications/miR181_paper/Figure3/Fig3_2/targetoldintronECDFRNA.pdf", width=2, heigh
targetoldECDFRNA
dev.off()
```

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

```
pdf("D:/Krueger_Lab/Publications/miR181_paper/Figure3/Fig3_2/targetoldintronECDFRPF.pdf", width=2, heigh
targetoldECDFRPF
dev.off()
```

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

```
pdf("D:/Krueger_Lab/Publications/miR181_paper/Figure3/Fig3_2/targetECDF_RNA.pdf", width=2, height = 2)
targetECDFRNA
dev.off()
```

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

```
pdf("D:/Krueger_Lab/Publications/miR181_paper/Figure3/Fig3_2/targetECDF_RPF.pdf", width=2, height = 2)
targetECDFRPF
dev.off()
```

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

```
#set

pdf("D:/Krueger_Lab/Publications/miR181_paper/Figure3/Fig3_2/setECDF_RNA.pdf", width=2, height = 2)
setECDFRNA
dev.off()
```

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

```
pdf("D:/Krueger_Lab/Publications/miR181_paper/Figure3/Fig3_2/setECDF_RPF.pdf", width=2, height = 2)
setECDFRPF
dev.off()
```

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

```
#region single targets

pdf("D:/Krueger_Lab/Publications/miR181_paper/Figure3/Fig3_2/regsingECDF_RNA.pdf", width=2, height = 2)
regsingECDFRNA
dev.off()
```

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

```
pdf("D:/Krueger_Lab/Publications/miR181_paper/Figure3/Fig3_2/regsingECDF_RPF.pdf", width=2, height = 2)
regsingECDFRPF
dev.off()
```

```
## pdf
```

```
##   2
```

```
#number

pdf("D:/Krueger_Lab/Publications/miR181_paper/Figure3/Fig3_2/numECDF_RNA.pdf", width=2, height = 2)
numECDFRNA
dev.off()
```

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

```
pdf("D:/Krueger_Lab/Publications/miR181_paper/Figure3/Fig3_2/numECDF_RPF.pdf", width=2, height = 2)
numECDFRPF
dev.off()
```

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

```
# MMsat4 vs target

pdf("D:/Krueger_Lab/Publications/miR181_paper/Figure3/Fig3_2/MMsat4vsTargetECDF_RNA.pdf", width=2, heigh
tolECDFRNA
dev.off()
```

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

```
pdf("D:/Krueger_Lab/Publications/miR181_paper/Figure3/Fig3_2/MMsat4vsTargetECDF_RPF.pdf", width=2, heigh
tolECDFRPF
dev.off()
```

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

```
# region with MMsat4 cds and 3'utr

pdf("D:/Krueger_Lab/Publications/miR181_paper/Figure3/Fig3_2/MMsat4byRegionECDF_RNA.pdf", width=2, heigh
posECDFRNA
dev.off()
```

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

```
pdf("D:/Krueger_Lab/Publications/miR181_paper/Figure3/Fig3_2/MMsat4byRegionECDF_RPF.pdf", width=2, heigh
posECDFRPF
dev.off()
```

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

```
# MRE type

pdf("D:/Krueger_Lab/Publications/miR181_paper/Figure3/Fig3_2/MRtypeECDF_RNA.pdf", width=2, height = 2)
typeECDFRNA
dev.off()
```

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

```
pdf("D:/Krueger_Lab/Publications/miR181_paper/Figure3/Fig3_2/MRtypeECDF_RPF.pdf", width=2, height = 2)
typeECDFRPF
```

```
dev.off()
```

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

```
# Wobble

pdf("D:/Krueger_Lab/Publications/miR181_paper/Figure3/Fig3_2/wobbleECDF_RNA.pdf", width=2, height = 2)
wobbleECDFRNA
dev.off()
```

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

```
pdf("D:/Krueger_Lab/Publications/miR181_paper/Figure3/Fig3_2/wobbleECDF_RPF.pdf", width=2, height = 2)
wobbleECDFRPF
dev.off()
```

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

```
# transcript expression

pdf("D:/Krueger_Lab/Publications/miR181_paper/Figure3/Fig3_2/expresssionECDF_RNA.pdf", width=2, height =
expECDFRNA
dev.off()
```

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

```
pdf("D:/Krueger_Lab/Publications/miR181_paper/Figure3/Fig3_2/expresssionECDF_RPF.pdf", width=2, height =
expECDFRPF
dev.off()
```

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

```
# transcript expression

pdf("D:/Krueger_Lab/Publications/miR181_paper/Figure3/Fig3_2/regiomultiECDF_RNA.pdf", width=2, height =
regionmultiECDFRNA
dev.off()
```

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

```
pdf("D:/Krueger_Lab/Publications/miR181_paper/Figure3/Fig3_2/regiomultiECDF_RPF.pdf", width=2, height =
regionmultiECDFRPF
dev.off()
```

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

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] dplyr_1.1.2          rtracklayer_1.58.0   GenomicRanges_1.50.2
## [4] GenomeInfoDb_1.34.9  IRanges_2.32.0       S4Vectors_0.36.2
## [7] BiocGenerics_0.44.0  ggplot2_3.4.2
##
## loaded via a namespace (and not attached):
##  [1] lattice_0.20-45             tidyr_1.3.0
##  [3] Rsamtools_2.14.0            Biostrings_2.66.0
##  [5] digest_0.6.31               utf8_1.2.3
##  [7] R6_2.5.1                    backports_1.4.1
##  [9] evaluate_0.21               highr_0.10
## [11] pillar_1.9.0                zlibbioc_1.44.0
## [13] rlang_1.1.0                 rstudioapi_0.14
## [15] car_3.1-2                   Matrix_1.5-3
## [17] rmarkdown_2.21              labeling_0.4.2
## [19] BiocParallel_1.32.6         RCurl_1.98-1.12
## [21] munsell_0.5.0               DelayedArray_0.23.2
## [23] broom_1.0.4                 compiler_4.2.3
## [25] xfun_0.39                   pkgconfig_2.0.3
## [27] htmltools_0.5.4             tidyselect_1.2.0
## [29] SummarizedExperiment_1.28.0 tibble_3.2.1
## [31] GenomeInfoDbData_1.2.9      codetools_0.2-19
## [33] matrixStats_0.63.0          XML_3.99-0.14
## [35] fansi_1.0.4                 crayon_1.5.2
## [37] withr_2.5.0                 ggpubr_0.6.0
## [39] GenomicAlignments_1.34.1    bitops_1.0-7
## [41] grid_4.2.3                  gtable_0.3.3
## [43] lifecycle_1.0.3             magrittr_2.0.3
## [45] scales_1.2.1                cli_3.6.0
## [47] carData_3.0-5               farver_2.1.1
## [49] XVector_0.38.0              ggsignif_0.6.4
## [51] generics_0.1.3              vctrs_0.6.2
## [53] rjson_0.2.21                restfulr_0.0.15
## [55] tools_4.2.3                 Biobase_2.58.0
## [57] glue_1.6.2                  purrr_1.0.1
## [59] MatrixGenerics_1.10.0       abind_1.4-5
## [61] parallel_4.2.3              fastmap_1.1.1
## [63] yaml_2.3.7                  colorspace_2.1-0
## [65] rstatix_0.7.2               knitr_1.42
## [67] BiocIO_1.8.0
```