Bound genes and gene regions of MREs

Melina Klostermann

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farbe2 <- "#EFC000FF"</pre>

farbe4 <- "#7AA6DCFF"
farbe5 <- "#868686FF"
farbe6 <- "#003C67FF"
farbe7 <- "#8F7700FF"</pre>

 $\texttt{farbe3} \ \texttt{<-} \ \texttt{"\#CD534CFF"} \ \textit{\#miR181KO} \ farbe$

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1 Libraries and settings	
# # libraries #	
library(tidyverse)	
library(GenomicRanges)	
library(colorspace)	
library(eulerr)	
library(gghalves)	
#	
# settings	
#	
<pre>out <- "/Users/melinaklostermann/Documents/projects/AgoCLIP_miR181/R_github/miR181_pape</pre>	
<pre>source("/Users/melinaklostermann/Documents/projects/R_general_functions/theme_paper.R")</pre>	
<pre>source("/Users/melinaklostermann/Documents/projects/AgoCLIP_miR181/mirko_files/mirECLIP</pre>	//DifferentialBi
# fambon	
# farben farbeneg <- "#B4B4B4"	
farbe1 <- "#0073C2FF" #WT farbe	

```
farbe8 <- "#3B3B3BFF"
farbe9 <- "#A73030FF"
farbe10 <- "#4A6990FF"
farbe11 <- "#FF6F00FF"
farbe12 <- "#C71000FF"
farbe13 <- "#008EA0FF"
farbe14 <- "#8A4198FF"
farbe15 <- "#5A9599FF"
farbe16 <- "#FF6348FF"</pre>
```

2 What was done?

• the genetype and gene region of the mir 181 binding sites (union) are ploted (Figure 2XX)

3 Files

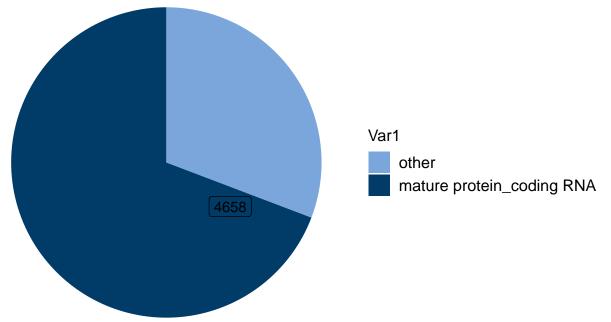
```
# ------
# MREs
# ------
mir181_bs <- readRDS("/Users/melinaklostermann/Documents/projects/AgoCLIP_miR181/R_github/miR181_paper/s
names(mir181_bs) <- 1:NROW(mir181_bs)
mir181_bs <- as.data.frame(mir181_bs)

mir181_enriched_set <- mir181_bs %>%
    as.data.frame(.) %>%
    subset(set %in% c("ago_bs_mir181_chi&mir181_enriched", "mir181_enriched"))
```

4 Characterise MRE

4.1 mir181 bound genes - Figure 2A

```
mir181_enriched_set <- mutate(mir181_enriched_set, geneType =</pre>
                         case_when(geneType != "protein_coding" ~ "other",
                                    region == "intron" ~ "other",
                                    region == "outside" ~ "other",
                                    is.na(region) ~ "other",
                                    T ~ "mature protein_coding RNA"))
gene_type_df <- table(mir181_enriched_set$geneType) %>%
  as.data.frame(.)
p <- ggplot(gene_type_df, aes(y=Freq, x="", fill=Var1)) +</pre>
     geom_col()+
     coord_polar(theta="y") +
     xlim(c(2, 4)) +
  geom_label(data = gene_type_df %>% subset(gene_type_df == "mature protein_coding RNA"), aes(y=Freq, x
             position = position_stack(vjust = 0.5),
             show.legend = FALSE) +
  scale fill manual(values = c (farbe6, farbe4)) +
  theme_paper() +
```



```
ggsave(p, filename = pasteO(out, "Figure2A_bound_gene_types_miR181_BS", Sys.Date(), ".pdf"), width = un
```

4.1.1 Remove non protein-coding binding sites

For all further analyses we removed binding sites on non protein-coding RNAs.

```
mir181_enriched_set <- subset(mir181_enriched_set, geneType == "mature protein_coding RNA")
```

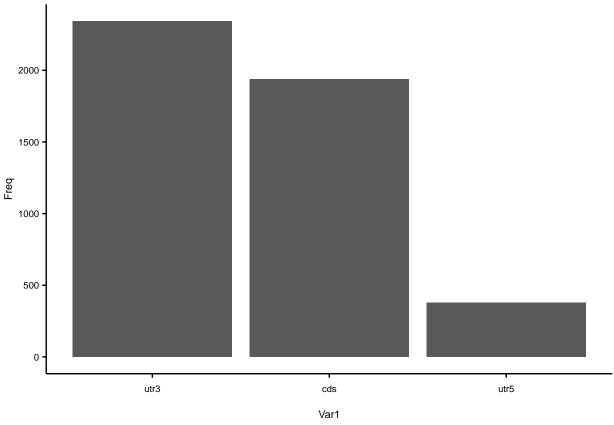
4.2 mir181 bound regions - Figure 2B

```
gene_region_df <- table(mir181_enriched_set$region) %>%
   as.data.frame(.) %>%
   arrange(desc(Freq))

gene_region_df$Var1 <- factor(gene_region_df$Var1, levels = gene_region_df$Var1)

p <- ggplot(gene_region_df %>% subset(Var1 != "outside"), aes(y=Freq, x=Var1)) +
        geom_col()+
   theme_paper()

p
```



```
ggsave(p, filename = paste0(out, "Figure2B_bound_gene_regions_miR181_BS", Sys.Date(), ".pdf"), width = sum(gene_region_df$Freq)
```

[1] 4658

4.2.1 Remove binding sites in introns or outside of any gene region

For all further analyses we removed binding sites in introns or in a gene regions with no region annotation.

5 Save filtered BS

```
saveRDS(mir181_bs, paste0(out, "mir181_bs_afterFigure2B.rds"))
```

6 Session Info

```
sessionInfo()
```

```
## R version 4.2.2 (2022-10-31)
## Platform: x86_64-apple-darwin17.0 (64-bit)
## Running under: macOS Big Sur ... 10.16
##
## Matrix products: default
          /Library/Frameworks/R.framework/Versions/4.2/Resources/lib/libRblas.0.dylib
## BLAS:
## LAPACK: /Library/Frameworks/R.framework/Versions/4.2/Resources/lib/libRlapack.dylib
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
## attached base packages:
                           graphics grDevices utils
## [1] stats4
                 stats
                                                         datasets methods
## [8] base
##
## other attached packages:
## [1] gghalves_0.1.4
                             eulerr_7.0.0
                                                  colorspace_2.1-0
## [4] GenomicRanges 1.50.2 GenomeInfoDb 1.34.9
                                                  IRanges 2.32.0
## [7] S4Vectors_0.36.2
                             BiocGenerics_0.44.0
                                                  lubridate_1.9.2
## [10] forcats 1.0.0
                             stringr 1.5.0
                                                  dplyr 1.1.2
## [13] purrr_1.0.1
                             readr_2.1.4
                                                  tidyr_1.3.0
## [16] tibble 3.2.1
                             ggplot2_3.4.2
                                                  tidyverse_2.0.0
## [19] knitr_1.43
## loaded via a namespace (and not attached):
## [1] tidyselect_1.2.0
                               xfun 0.39
                                                       carData 3.0-5
## [4] vctrs_0.6.3
                               generics_0.1.3
                                                      htmltools_0.5.5
## [7] yaml_2.3.7
                               utf8_1.2.3
                                                      rlang_1.1.1
## [10] pillar_1.9.0
                               ggpubr_0.6.0
                                                       glue_1.6.2
## [13] withr_2.5.0
                               GenomeInfoDbData_1.2.9 lifecycle_1.0.3
                                                       ggsignif_0.6.4
## [16] zlibbioc_1.44.0
                               munsell_0.5.0
## [19] gtable_0.3.3
                               ragg_1.2.5
                                                       evaluate_0.21
## [22] labeling_0.4.2
                               tzdb_0.4.0
                                                       fastmap_1.1.1
## [25] fansi_1.0.4
                               highr_0.10
                                                      broom_1.0.5
## [28] Rcpp 1.0.10
                               backports_1.4.1
                                                       scales 1.2.1
## [31] XVector_0.38.0
                               abind_1.4-5
                                                      systemfonts_1.0.4
## [34] farver 2.1.1
                               textshaping 0.3.6
                                                      hms 1.1.3
## [37] digest_0.6.31
                               stringi_1.7.12
                                                      rstatix_0.7.2
## [40] grid_4.2.2
                               cli_3.6.1
                                                       tools 4.2.2
## [43] bitops_1.0-7
                               magrittr_2.0.3
                                                      RCurl_1.98-1.12
## [46] car 3.1-2
                               pkgconfig 2.0.3
                                                      timechange 0.2.0
## [49] rmarkdown 2.22
                               rstudioapi_0.14
                                                      R6_2.5.1
## [52] compiler_4.2.2
```