AGO targetome

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1 Libraries and settings

2 What was done?

- Overview of the detected chimeric reads in all conditions.
- Chimeric reads are assigned to AGO-binding sites (chimeric AGO sites).
- Co-ocurrence of miRs in the same AGO binding site (fisher-test heatmap).

We obtain chimeric reads from 4 different conditions: - AGO eCLIP (IP_WT) - AGO eCLIP with mir181a KO and mir181b KO (IP_KO) - AGO eCLIP with mir181 enrichment (IP_mir181_WT) - AGO eCLIP with mir181 enrichment and with mir181a KO and mir181b KO (IP_mir181_KO)

3 Files

```
# ----------------------------------------
# AGO binding sites
# ----------------------------------------
ago_bs <- readRDS("/Users/melinaklostermann/Documents/projects/AgoCLIP_miR181/R_github/miR181_paper/Met


# ----------------------------------------
# chimeric reads
# ----------------------------------------
mir_crosslinks <- list.files("/Users/melinaklostermann/Documents/projects/AgoCLIP_miR181/pipe_output_22
```

```
                              pattern = "*.bed", recursive = TRUE, full.names = T) %>% map(~import.bed(.
names(mir_crosslinks) <- list.files("/Users/melinaklostermann/Documents/projects/AgoCLIP_miR181/pipe_ou
                              pattern = "*.bed")

# ----------------------------------------
# mir181 expression imgen
# ----------------------------------------
mir_imgen <- read.csv("/Users/melinaklostermann/Documents/projects/AgoCLIP_miR181/files_public_mir_data/
```

4 Chimeric reads

These are the chimeric reads that were isolated during the read processing via raccon (link TODO)

4.1 Number of chimeric reads per sample

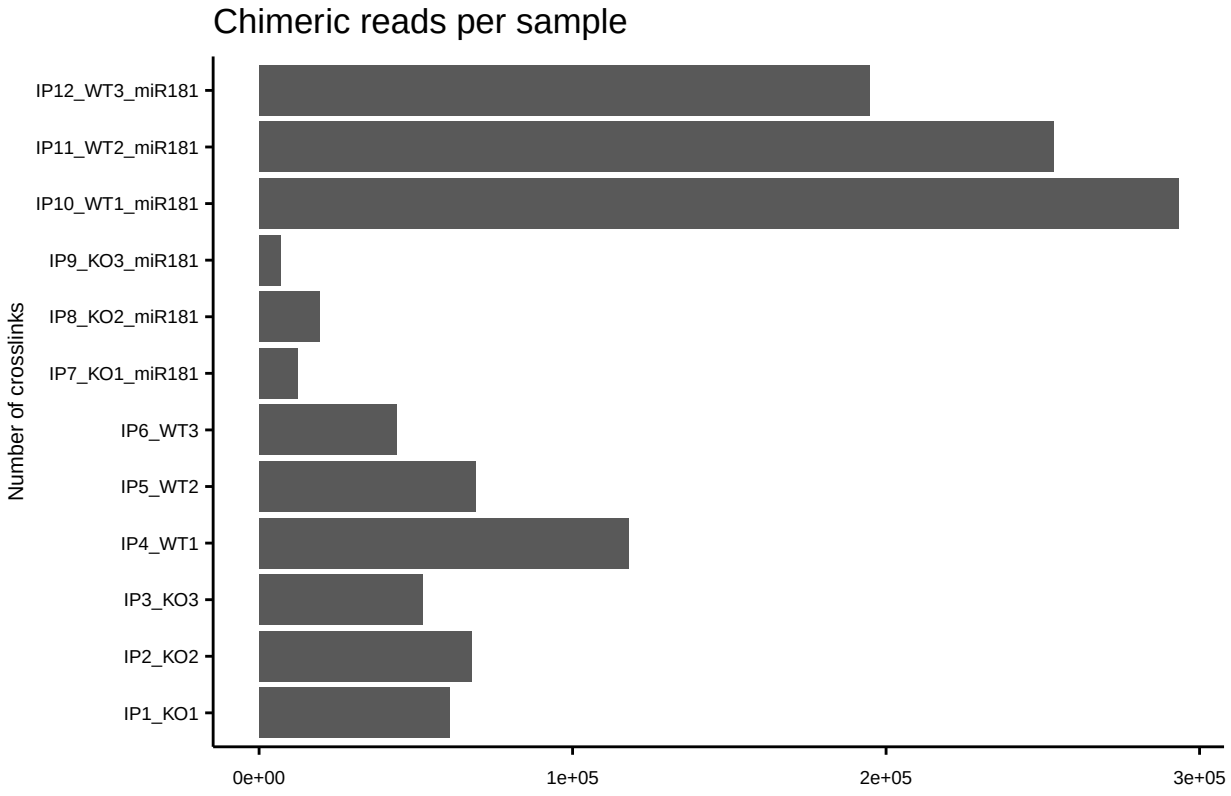
```
# clean files
mir_crosslinks <- map(mir_crosslinks, ~ as.data.frame(.x) %>%
                        mutate(strand = Strand, Strand = NULL))

sample_names <- c("Inp1_KO1", "Inp2_KO2", "Inp3_KO3",
                  "Inp4_WT1", "Inp5_WT2", "Inp6_WT3",
                  "IP1_KO1", "IP2_KO2", "IP3_KO3",
                  "IP4_WT1", "IP5_WT2", "IP6_WT3",
                  "IP7_KO1_miR181", "IP8_KO2_miR181", "IP9_KO3_miR181",
                  "IP10_WT1_miR181", "IP11_WT2_miR181", "IP12_WT3_miR181"
                  )

mir_crosslinks <- map( sample_names , ~bind_rows(mir_crosslinks[grepl(names(mir_crosslinks), pattern =

names(mir_crosslinks) <- sample_names


table_num_crosslinks <- map_dfr(mir_crosslinks, ~c(number_of_crosslinks = NROW(.x)))
table_num_crosslinks$sample <- sample_names

kable(table_num_crosslinks[,c(2,1)], format.args = list(big.mark = ",")) %>%
  kable_material(c("striped", "hover")) %>%
  scroll_box(width = "100%", height = "500px")
```

4.2 FigureS1C Barchart chimeric reads per sample

```
gg_df <- table_num_crosslinks %>% subset(sample %in% c("IP1_KO1", "IP2_KO2", "IP3_KO3",
                  "IP4_WT1", "IP5_WT2", "IP6_WT3",
                  "IP7_KO1_miR181", "IP8_KO2_miR181", "IP9_KO3_miR181",
                  "IP10_WT1_miR181", "IP11_WT2_miR181", "IP12_WT3_miR181"))

p <- ggplot(gg_df, aes(x = factor(sample, levels = sample), y = number_of_crosslinks))+
  geom_col()+
  coord_flip()+
  ylab("")+
  xlab("Number of crosslinks")
```

sample	number_of_crosslinks
Inp1_KO1	946
Inp2_KO2	737
Inp3_KO3	717
Inp4_WT1	854
Inp5_WT2	951
Inp6_WT3	698
IP1_KO1	60,789
IP2_KO2	67,639
IP3_KO3	52,100
IP4_WT1	117,849
IP5_WT2	69,074
IP6_WT3	43,983
IP7_KO1_miR181	12,186
IP8_KO2_miR181	19,264
IP9_KO3_miR181	6,832
IP10_WT1_miR181	293,149
IP11_WT2_miR181	253,502
IP12_WT3_miR181	194,628

```
p + ggtitle("Chimeric reads per sample")
```




```
p <- p + theme_paper()
```

```
ggsave(p, filename = paste0(out, "FigureS1C_Barchart_chimeric_reads_", Sys.Date(), ".pdf"), width = uni
```

4.3 Detected mirs per sample

```
detected_mirs <- map(mir_crosslinks, ~.x %>%
                       group_by(`Name`) %>%
                       summarise(n = sum(Score), .groups= "keep") )

detected_mirs <- map(detected_mirs, ~arrange(.x, desc(n)))
```

4.4 Detected mirs per condition

```
condition_regex <- list("Inp.+KO", "Inp.+WT",
                  "IP.+KO[1-3]+$",  "IP.+WT[1-3]+$",
                  "IP.+KO.+_miR181", "IP.+_WT.+_miR181" )
condition_names <- list("Inp_KO", "Inp_WT",
                  "IP_KO",  "IP_WT",
                  "IP_KO_miR181", "IP_WT_miR181" )


mir_crosslinks_per_cond <- map(condition_regex,
                   ~bind_rows(mir_crosslinks[grepl(names(mir_crosslinks), pattern =.x)] ))

names(mir_crosslinks_per_cond ) <- condition_names
```

```
detected_mirs_per_cond <- map(mir_crosslinks_per_cond, ~.x %>%
                       group_by(`Name`) %>%
                       summarise(n = sum(Score), .groups= "keep",
                                 mean = n/3) )

detected_mirs_per_cond <- map(detected_mirs_per_cond , ~arrange(.x, desc(n)))

detected_mirs_per_cond_top_10 <- map(detected_mirs_per_cond, ~.x[1:10,] %>%
                             arrange(., n))
```

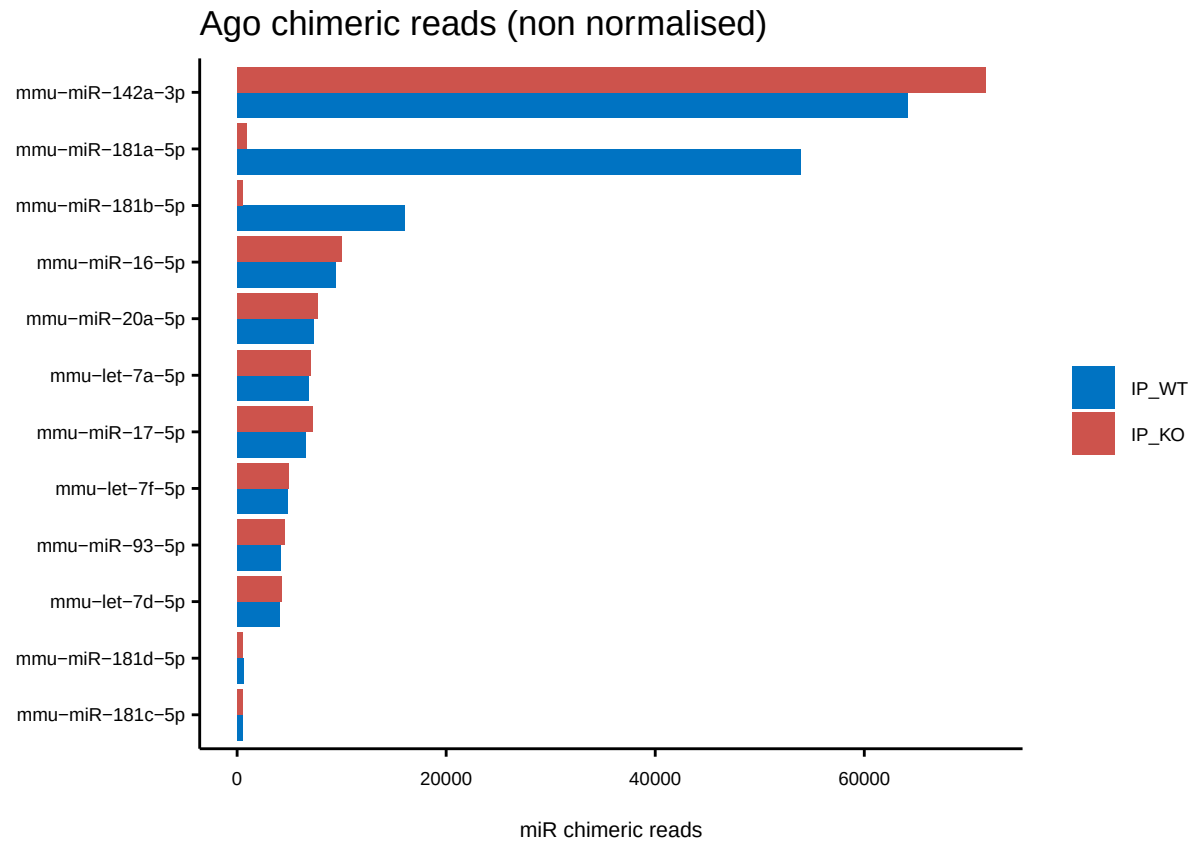
4.5 Supplementary Table XX

```
xlsx::write.xlsx(x = as.data.frame(detected_mirs_per_cond$IP_WT), file = paste0(out,"detected_miR_IP_WT
xlsx::write.xlsx(x = as.data.frame(detected_mirs_per_cond$IP_KO), file = paste0(out,"detected_miR_IP_KO
xlsx::write.xlsx(x = as.data.frame(detected_mirs_per_cond$IP_WT_miR181), file = paste0(out,"detected_mi
xlsx::write.xlsx(x = as.data.frame(detected_mirs_per_cond$IP_KO_miR181), file = paste0(out,"detected_mi
```

4.5.1 Figure1E Barchart IP_WT/KO topb mirs & Figure1G IP_mir181_WT/KO

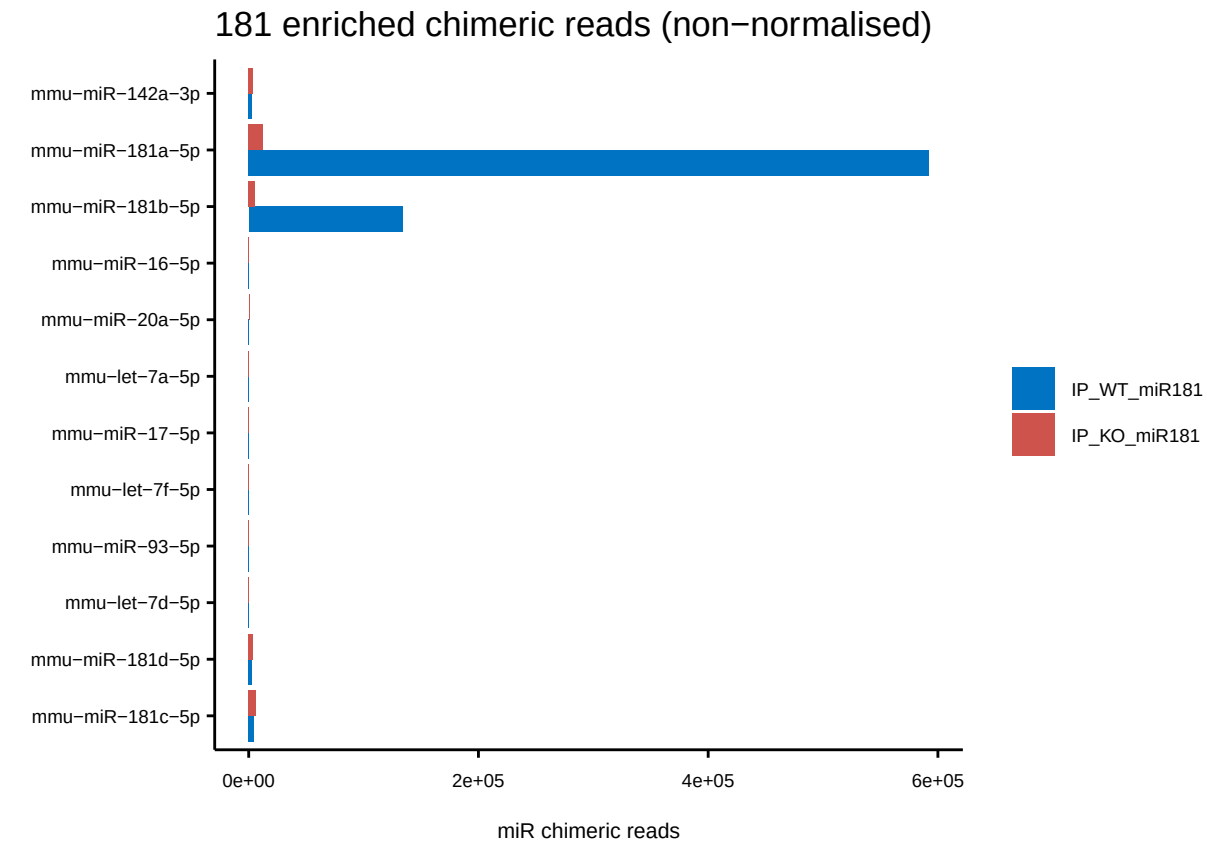
```
#####################
# different version of barcharts for paper
#####################

# Barchart of AGO IP
#####################
```

```r
# make one df with all conditions
conditions_of_samples_list <- rep(condition_names, each =3)
mirs <- pmap(list(x=detected_mirs, y=as.list(sample_names), z= conditions_of_samples_list),
                              function(x,y,z) mutate(x, Sample = y,
                                                     condition = z)) %>%
  map_dfr(~.x)

# get conditions
mirs_ago_wt_ko <- mirs %>% subset(condition %in% c("IP_WT", "IP_KO"))

# calculate relative amount per condition
mirs_ago_wt_ko <- mirs_ago_wt_ko %>%
  mutate( n_total = case_when(condition == "IP_WT" ~ sum(detected_mirs_per_cond$IP_WT$n),
                              condition == "IP_KO" ~ sum(detected_mirs_per_cond$IP_KO$n))) %>%
  group_by(condition, Name) %>%
  mutate(
    n_per_cond_rel = sum(n)/n_total,
    sum = sum(n))

# select top 10 from wt condition
mirs_t10_ago_wt_ko <- mirs_ago_wt_ko %>% subset(Name %in% c(detected_mirs_per_cond_top_10$IP_WT$Name, "
                                                    arrange(desc(condition), n_per_cond_rel)



p1 <- ggplot(mirs_t10_ago_wt_ko, aes(x = factor(Name, levels = unique(Name)), y = sum, fill = factor(co
   geom_col( stat="identity",position = "dodge")+
      #scale_x_discrete(guide = guide_axis(angle = 45)) +
  scale_fill_manual(values = c(farbe1, farbe3))+
  xlab("") +
  ylab("miR chimeric reads")+
  coord_flip()

p1 + ggtitle("Ago chimeric reads (non normalised)")
```




```
# Barchart of 181 IP
######################

# get conditions
mirs_181_wt_ko <- mirs %>% subset(condition %in% c("IP_WT_miR181", "IP_KO_miR181"))

# calculate relative amount per condition
mirs_181_wt_ko <- mirs_181_wt_ko %>%
  mutate( n_total = case_when(condition == "IP_WT_miR181" ~ sum(detected_mirs_per_cond$IP_WT_miR181$n),
                              condition == "IP_KO_miR181" ~ sum(detected_mirs_per_cond$IP_KO_miR181$n)))
  group_by(condition, Name) %>%
  mutate(
    n_per_cond_rel = sum(n)/n_total,
    sum = sum(n))

# select top 10 from wt condition
mirs_t10_181_wt_ko <- mirs_181_wt_ko %>% subset(Name %in% c(detected_mirs_per_cond_top_10$IP_WT$Name, "
                                                arrange(desc(condition), n_per_cond_rel)



p3 <- ggplot(mirs_t10_181_wt_ko, aes(x = factor(Name, levels = unique(mirs_t10_ago_wt_ko$Name)), y = su
   geom_col( stat="identity",position = "dodge")+
      #scale_x_discrete(guide = guide_axis(angle = 45)) +
  scale_fill_manual(values = c(farbe1, farbe3))+
```

```
  xlab("") +
  ylab("miR chimeric reads")+
  coord_flip()

p3 + ggtitle("181 enriched chimeric reads (non-normalised)")
```




```
# save for paper
p1 <- p1 + theme_paper()
p3 <- p3 + theme_paper()

ggsave(p1, filename = paste0(out, "Figure1E_Barchart_IP_WT_KO_top_mirs_", Sys.Date(), ".pdf"), width = 
ggsave(p3, filename = paste0(out, "Figure1G_Barchart_IP_WT_KO_181enriched_top_mirs_", Sys.Date(), ".pdf
```

5 Assign chimeric reads to binding sites

We assign chimeric reads that are in a window of 10nt before the binding site until 10nt after the binding site to the respective binding site.

```
# use region of bs +-10nt for overlaps
ago_bs_10 <- ago_bs + 10

# find overlaps of mirt and AGO bs
idx <- findOverlaps(ago_bs_10,
                    makeGRangesFromDataFrame(mir_crosslinks_per_cond$IP_WT, keep.extra.columns = T))

# make a data frame from the ago bs
```

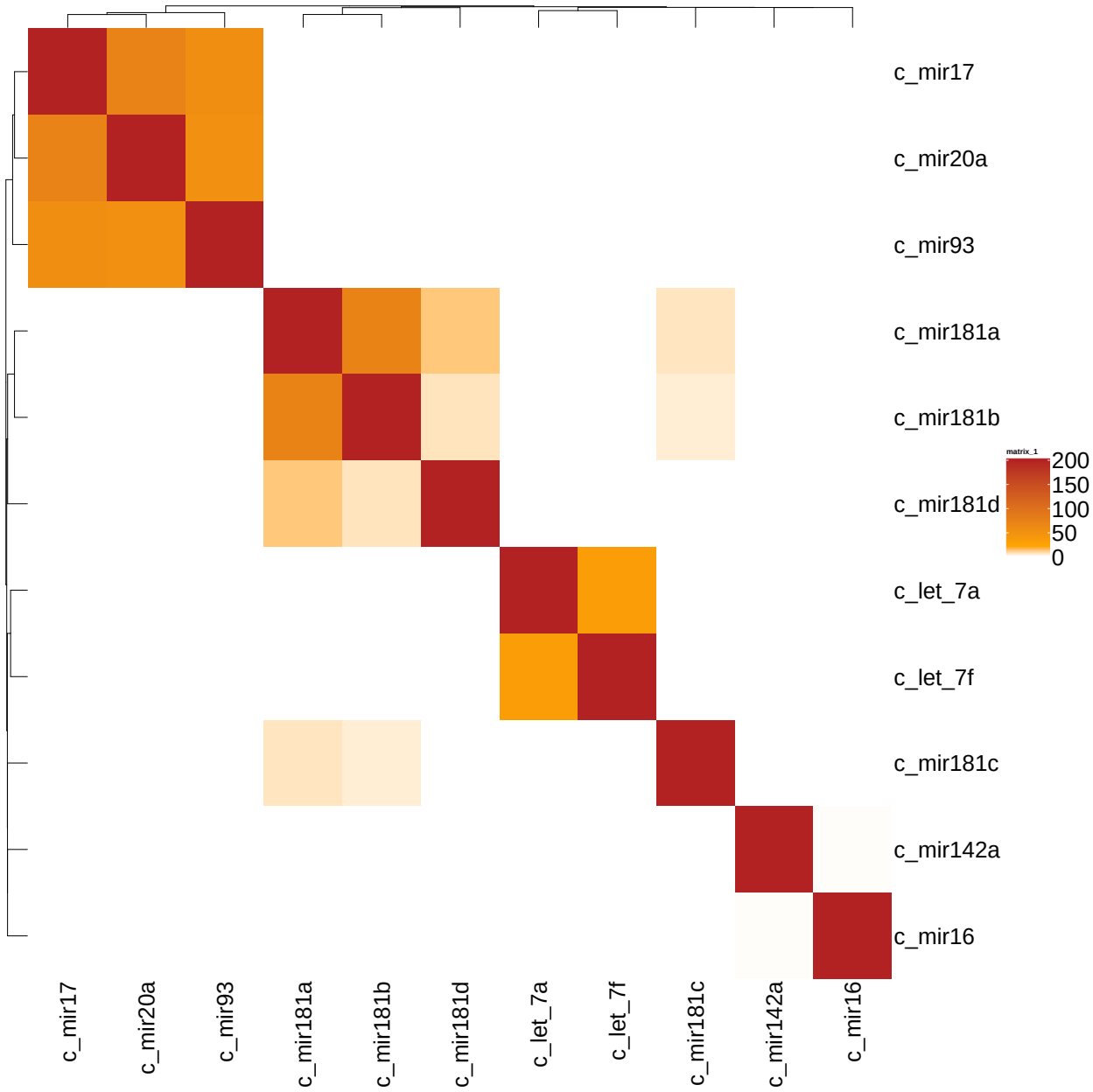
```
names(ago_bs)<- 1:NROW(ago_bs)
ago_bs <- as.data.frame(ago_bs)

# add mir info to ago bs
ago_bs_chi <- cbind(ago_bs[queryHits(idx),], mir_IP_WT = mir_crosslinks_per_cond$IP_WT[subjectHits(idx)

ago_bs_chi <- ago_bs_chi %>% tidyr::nest(mir_IP_WT)
```

5.1 Enriched sharing of binding sites by two miRs

Ago binding sites can contain more than one miR. Here we look at which miR sharing is enriched. The heatmap shows the r p-values of fisher-tests after bh adjustment.

```
# get co-occurences of top 10 mirs
t <- ago_bs_chi %>% mutate(n_different_mirs = map(data, ~length(unique(.x$mir_IP_WT))) %>% unlist(.),
                  c_mir181a = map(data, ~ "mmu-miR-181a-5p" %in% .x$mir_IP_WT) %>% unlist(.),
                  c_mir181b = map(data, ~ "mmu-miR-181b-5p" %in% .x$mir_IP_WT) %>% unlist(.),
                  c_mir142a = map(data, ~ "mmu-miR-142a-3p" %in% .x$mir_IP_WT) %>% unlist(.),
                  c_mir16 = map(data, ~ "mmu-miR-16-5p" %in% .x$mir_IP_WT) %>% unlist(.),
                  c_mir20a = map(data, ~ "mmu-miR-20a-5p" %in% .x$mir_IP_WT) %>% unlist(.),
                  c_let_7a = map(data, ~ "mmu-let-7a-5p" %in% .x$mir_IP_WT) %>% unlist(.),
                  c_mir17 = map(data, ~ "mmu-miR-17-5p" %in% .x$mir_IP_WT) %>% unlist(.),
                  c_mir181c = map(data, ~ "mmu-miR-181c-5p" %in% .x$mir_IP_WT) %>% unlist(.),
                  c_mir181d = map(data, ~ "mmu-miR-181d-5p" %in% .x$mir_IP_WT) %>% unlist(.),
                  c_let_7f = map(data, ~ "mmu-let-7f-5p" %in% .x$mir_IP_WT) %>% unlist(.),
                  c_mir93 = map(data, ~ "mmu-miR-93-5p" %in% .x$mir_IP_WT) %>% unlist(.)
                  )


# function for fisher test
fisher_fun <- function(v){
  overlap_m <- eulerr::euler(data.frame(m[,v[[1]]], m[, v[[2]]] ))

  plot(overlap_m , quantities = TRUE, shape = "ellipse")


  v <- overlap_m$original.values
  v <- matrix(c(v[3], v[1], v[2], length(m[,1])), ncol = 2)

  f <- fisher.test(v)
  f$p.value
}

# make matix of top 10 miRs
m <- as.matrix(t[ ,grepl(colnames(t), pattern = "c_")])
# calc p-value and adj p-value from pairwise fisher tests
p.values <- combn(x = 1:ncol(m), m = 2, fisher_fun)
p.adj <- p.adjust(p.values)

# make plotable matirx
n <- ncol(m)
mat <- `dimnames<-`(matrix(0,n,n), list(colnames(m), colnames(m)))
mat[lower.tri(mat, diag = F)] <- as.vector(p.adj)
```

```
mat <- t(mat) +mat
mat <- -log10(mat)

mat[mat==Inf] <- 200
mat[mat<log10(0.01)] <- 0

# plot with ComplexHeatmap
col_fun = colorRamp2(c(0, 20, 200), c("white",  "orange", "firebrick"))

lgd = list(grid_width = unit(2, "cm"), grid_height= unit(100, "cm"), labels_gp = gpar(fontsize = 30))

h <- Heatmap(mat, col = col_fun,
         row_names_gp = gpar(fontsize = 30),
        row_names_max_width = unit(10, "cm"),
         column_names_gp = gpar(fontsize = 30),
        column_names_max_height = unit(10, "cm"),
        heatmap_legend_param = lgd)
h
```




```
pdf(file = paste0(out, "Figure1F_co-occurence_heatmap", Sys.Date(), ".pdf"), width = 11, height = 10)
h
dev.off()
```

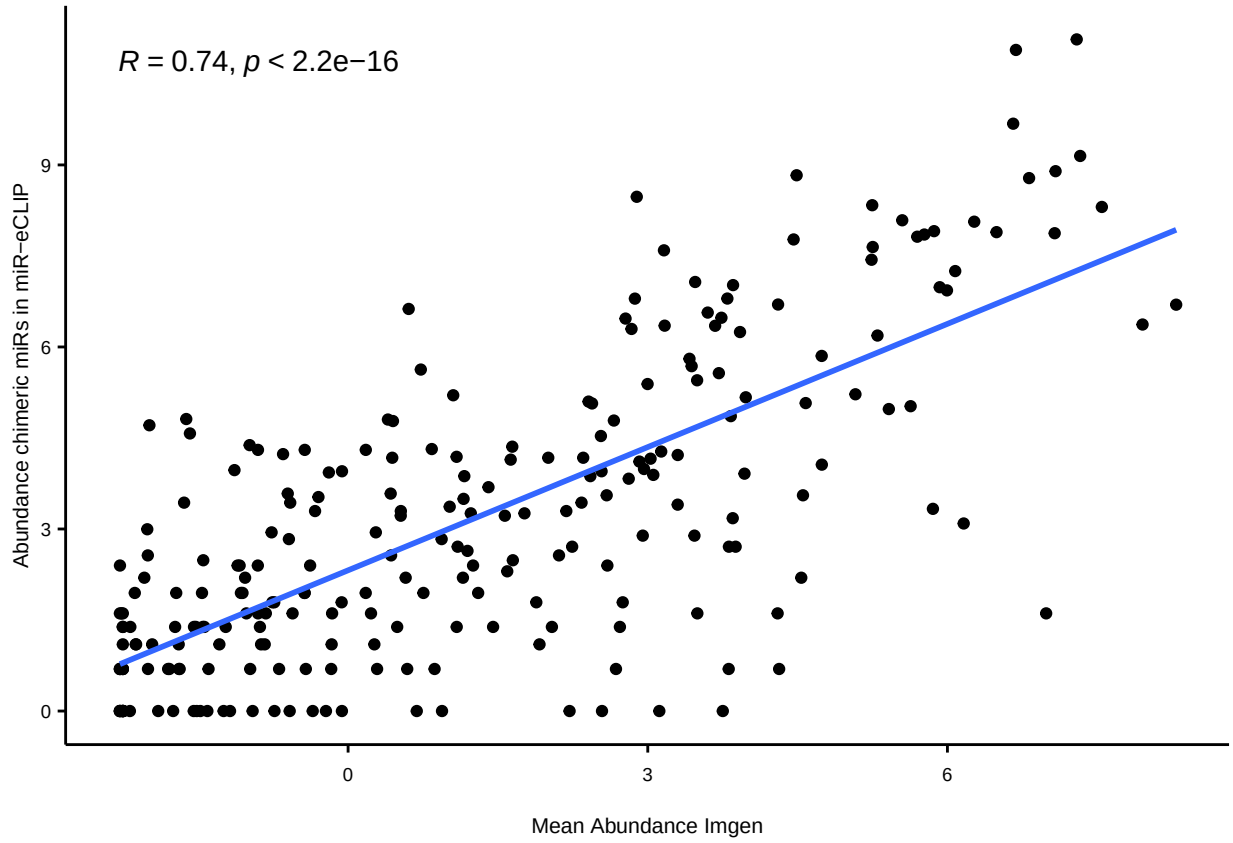
```
## pdf
##   2
```

6 Commarison to mir181 quantification from imgen

Here we compare detected miR amounts to the publicity available quantification from Immgen.

```
comp_df <- table(mir_crosslinks_per_cond$IP_WT$Name) %>% as.data.frame(.) %>%
  rowwise(.)%>%
  mutate(Var1 = substr(Var1, 5, nchar(as.character(Var1))))
```

```
mir_imgen <- mir_imgen %>% full_join(comp_df, by = c(ID_REF = "Var1"))

p4 <- ggplot(mir_imgen, aes(x = log(mean), y = log(Freq)))+
  geom_point()+
  geom_smooth(method =lm,se=F)+
  stat_cor()+
  xlab("Mean Abundance Imgen")+
  ylab("Abundance chimeric miRs in miR-eCLIP")

p4
```




```
p4 <- p4 + theme_paper()

ggsave(p4, filename = paste0(out, "Figure1D_Comparison_miR_expression_Imgen", Sys.Date(), ".pdf"), widt
```

7 Save files

```
saveRDS(mir_crosslinks_per_cond, file = paste0(out, "mir_chimeric_crosslinks.rds"))
```

8 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
## BLAS:   /Library/Frameworks/R.framework/Versions/4.2/Resources/lib/libRblas.0.dylib
## 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:
## [1] grid      stats4    stats     graphics  grDevices utils     datasets
## [8] methods   base
##
## other attached packages:
##  [1] ggpubr_0.5.0                        circlize_0.4.15
##  [3] BSgenome.Mmusculus.UCSC.mm10_1.4.3 BSgenome_1.66.2
##  [5] Biostrings_2.66.0                  XVector_0.38.0
##  [7] ComplexHeatmap_2.14.0              kableExtra_1.3.4
##  [9] rtracklayer_1.58.0                 GenomicRanges_1.50.2
## [11] GenomeInfoDb_1.34.7                IRanges_2.32.0
## [13] S4Vectors_0.36.1                   BiocGenerics_0.44.0
## [15] purrr_1.0.1                        dplyr_1.0.10
## [17] ggplot2_3.4.0                      knitr_1.42
##
## loaded via a namespace (and not attached):
##  [1] colorspace_2.1-0            ggsignif_0.6.4
##  [3] rjson_0.2.21                GlobalOptions_0.1.2
##  [5] clue_0.3-63                 rstudioapi_0.14
##  [7] farver_2.1.1                fansi_1.0.4
##  [9] xml2_1.3.3                  codetools_0.2-18
## [11] splines_4.2.2               doParallel_1.0.17
## [13] polyclip_1.10-4             Rsamtools_2.14.0
## [15] Cairo_1.6-0                 rJava_1.0-6
## [17] broom_1.0.3                 cluster_2.1.4
## [19] png_0.1-8                   compiler_4.2.2
## [21] httr_1.4.4                  backports_1.4.1
## [23] assertthat_0.2.1            Matrix_1.5-3
## [25] fastmap_1.1.0               cli_3.6.0
## [27] htmltools_0.5.4             tools_4.2.2
## [29] gtable_0.3.1                glue_1.6.2
## [31] GenomeInfoDbData_1.2.9      Rcpp_1.0.10
## [33] carData_3.0-5               Biobase_2.58.0
## [35] eulerr_7.0.0                vctrs_0.5.2
## [37] svglite_2.1.1               nlme_3.1-161
## [39] iterators_1.0.14            xfun_0.36
## [41] polylabelr_0.2.0            stringr_1.5.0
## [43] xlsxjars_0.6.1              rvest_1.0.3
## [45] lifecycle_1.0.3             restfulr_0.0.15
## [47] rstatix_0.7.1               XML_3.99-0.13
## [49] xlsx_0.6.5                  zlibbioc_1.44.0
## [51] scales_1.2.1                ragg_1.2.5
## [53] MatrixGenerics_1.10.0       parallel_4.2.2
```

```
## [55] SummarizedExperiment_1.28.0 RColorBrewer_1.1-3
## [57] yaml_2.3.7                  stringi_1.7.12
## [59] highr_0.10                  BiocIO_1.8.0
## [61] foreach_1.5.2               BiocParallel_1.32.5
## [63] shape_1.4.6                 rlang_1.0.6
## [65] pkgconfig_2.0.3             systemfonts_1.0.4
## [67] matrixStats_0.63.0          bitops_1.0-7
## [69] evaluate_0.20               lattice_0.20-45
## [71] GenomicAlignments_1.34.0    labeling_0.4.2
## [73] tidyselect_1.2.0            magrittr_2.0.3
## [75] R6_2.5.1                    magick_2.7.3
## [77] generics_0.1.3              DelayedArray_0.24.0
## [79] DBI_1.1.3                   pillar_1.8.1
## [81] withr_2.5.0                 mgcv_1.8-41
## [83] abind_1.4-5                 RCurl_1.98-1.9
## [85] tibble_3.1.8                crayon_1.5.2
## [87] car_3.1-1                   utf8_1.2.2
## [89] rmarkdown_2.20              GetoptLong_1.0.5
## [91] digest_0.6.31               webshot_0.5.4
## [93] tidyr_1.3.0                 textshaping_0.3.6
## [95] munsell_0.5.0               viridisLite_0.4.1
```