

# Figure2 RAPseq

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### 0.1 ENV settings

```

###  

# @Description: Figure2  

# @Description: Adapted from https://github.com/IonutAtanasoai1/RAPseq  

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# @Date: 1 Sep 2024 ( 16:39:06 )  

###  

rm(list = ls())  

setwd("/Users/liqun/Desktop/Projects/RAPseq/0_RAPSeq/")

```

## 0.2 R libraries

```

library(ChIPpeakAnno)  

## Loading required package: IRanges  

## 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  

## Loading required package: stats4  

##  

## Attaching package: 'S4Vectors'  

## The following object is masked from 'package:utils':  

##  

##     findMatches  

## The following objects are masked from 'package:base':  

##  

##     expand.grid, I, unname  

## Loading required package: GenomicRanges  

## Loading required package: GenomeInfoDb  

## Warning: package 'GenomeInfoDb' was built under R version 4.3.3  

library(Biostrings)  

## Warning: package 'Biostrings' was built under R version 4.3.3  

## Loading required package: XVector  

##  

## Attaching package: 'Biostrings'  

## The following object is masked from 'package:base':  

##  

##     strsplit  

library(edgeR)

```

```

## Loading required package: limma
##
## Attaching package: 'limma'
## The following object is masked from 'package:BiocGenerics':
##
##      plotMA
library(ggfortify)
## Loading required package: ggplot2
library(tidyverse)
## -- Attaching core tidyverse packages ----- tidyverse 2.0.0 --
## v dplyr     1.1.4     v readr     2.1.5
## v forcats   1.0.0     v stringr   1.5.1
## v lubridate 1.9.3     v tibble    3.2.1
## v purrr     1.0.2     v tidyrr    1.3.1
## -- Conflicts ----- tidyverse_conflicts() --
## x lubridate::within%() masks IRanges::within%()
## x dplyr::collapse()   masks Biostrings::collapse(), IRanges::collapse()
## x dplyr::combine()   masks BiocGenerics::combine()
## x purrr::compact()   masks XVector::compact()
## x dplyr::desc()     masks IRanges::desc()
## x tidyrr::expand()   masks S4Vectors::expand()
## x dplyr::filter()   masks stats::filter()
## x dplyr::first()    masks S4Vectors::first()
## x dplyr::lag()      masks stats::lag()
## x ggplot2::Position() masks BiocGenerics::Position(), base::Position()
## x purrr::reduce()   masks GenomicRanges::reduce(), IRanges::reduce()
## x dplyr::rename()   masks S4Vectors::rename()
## x lubridate::second() masks S4Vectors::second()
## x lubridate::second<-() masks S4Vectors::second<_()
## x dplyr::slice()    masks XVector::slice(), IRanges::slice()
## i Use the conflicted package (<http://conflicted.r-lib.org/>) to force all conflicts to become errors
library(scales)
##
## Attaching package: 'scales'
##
## The following object is masked from 'package:purrr':
##
##      discard
##
## The following object is masked from 'package:readr':
##
##      col_factor
library(ggplot2)
library(dendextend)
##
## -----
## Welcome to dendextend version 1.17.1
## Type citation('dendextend') for how to cite the package.
##
## Type browseVignettes(package = 'dendextend') for the package vignette.
## The github page is: https://github.com/talgalili/dendextend/
##
## Suggestions and bug-reports can be submitted at: https://github.com/talgalili/dendextend/issues

```

```

## You may ask questions at stackoverflow, use the r and dendextend tags:
##   https://stackoverflow.com/questions/tagged/dendextend
##
## To suppress this message use: suppressPackageStartupMessages(library(dendextend))
## -----
##
##
## Attaching package: 'dendextend'
##
## The following object is masked from 'package:Biostrings':
##
##      nnodes
##
## The following object is masked from 'package:stats':
##
##      cutree
library(dichromat)
library(reshape2)
##
## Attaching package: 'reshape2'
##
## The following object is masked from 'package:tidyverse':
##
##      smiths
library(dplyr)
library(stringr)
library(GenomicRanges)
library(GenomicFeatures)
## Warning: package 'GenomicFeatures' was built under R version 4.3.3
## Loading required package: AnnotationDbi
## Loading required package: Biobase
## Welcome to Bioconductor
##
##      Vignettes contain introductory material; view with
##      'browseVignettes()'. To cite Bioconductor, see
##      'citation("Biobase")', and for packages 'citation("pkgname")'.
##
##
## Attaching package: 'AnnotationDbi'
##
## The following object is masked from 'package:dplyr':
##
##      select
library(gplots)
##
## Attaching package: 'gplots'
##
## The following object is masked from 'package:IRanges':
##
##      space
##
## The following object is masked from 'package:S4Vectors':
##

```

```

##      space
##
## The following object is masked from 'package:stats':
##
##      lowess
library(clusterProfiler)
## Warning: package 'clusterProfiler' was built under R version 4.3.3
##
## clusterProfiler v4.10.1 For help: https://yulab-smu.top/biomedical-knowledge-mining-book/
##
## If you use clusterProfiler in published research, please cite:
## T Wu, E Hu, S Xu, M Chen, P Guo, Z Dai, T Feng, L Zhou, W Tang, L Zhan, X Fu, S Liu, X Bo, and G Yu.
##
## Attaching package: 'clusterProfiler'
##
## The following object is masked from 'package:AnnotationDbi':
##
##      select
##
## The following object is masked from 'package:purrr':
##
##      simplify
##
## The following object is masked from 'package:XVector':
##
##      slice
##
## The following object is masked from 'package:IRanges':
##
##      slice
##
## The following object is masked from 'package:S4Vectors':
##
##      rename
##
## The following object is masked from 'package:stats':
##
##      filter
library(org.Hs.eg.db)
##
library(org.Dr.eg.db)
##
library(tidyr)
library(gtools)
library(ggrepel)
library(ggbeeswarm)
library(ReactomePA)
## ReactomePA v1.46.0 For help: https://yulab-smu.top/biomedical-knowledge-mining-book/
##
## If you use ReactomePA in published research, please cite:
## Guangchuang Yu, Qing-Yu He. ReactomePA: an R/Bioconductor package for reactome pathway analysis and
library(VennDiagram)
## Loading required package: grid

```

```

## 
## Attaching package: 'grid'
##
## The following object is masked from 'package:Biostrings':
## 
##     pattern
##
## Loading required package: futile.logger
##
## Attaching package: 'futile.logger'
##
## The following object is masked from 'package:gtools':
## 
##     scat
##
## 
## Attaching package: 'VennDiagram'
##
## The following object is masked from 'package:dendextend':
## 
##     rotate
library(LSD)
library(gridExtra)
##
## Attaching package: 'gridExtra'
##
## The following object is masked from 'package:Biobase':
## 
##     combine
##
## The following object is masked from 'package:dplyr':
## 
##     combine
##
## The following object is masked from 'package:BiocGenerics':
## 
##     combine
library(UpSetR)
library(ggpubr)
##
## Attaching package: 'ggpubr'
##
## The following object is masked from 'package:VennDiagram':
## 
##     rotate
##
## The following object is masked from 'package:dendextend':
## 
##     rotate
library(GenomicScores)
##
## Attaching package: 'GenomicScores'
##

```

```

## The following object is masked from 'package:utils':
##
##      citation
library(phastCons7way.UCSC.hg38)
## Warning: replacing previous import 'utils::findMatches' by
## 'S4Vectors::findMatches' when loading 'phastCons7way.UCSC.hg38'
library(phastCons100way.UCSC.hg38)
## Warning: replacing previous import 'utils::findMatches' by
## 'S4Vectors::findMatches' when loading 'phastCons100way.UCSC.hg38'
library(corrplot)
## Warning: package 'corrplot' was built under R version 4.3.3
## corrplot 0.94 loaded
library(ggforce)
library(idr)
library(GGally)
## Registered S3 method overwritten by 'GGally':
##   method from
##   +.gg  ggplot2
library(eulerr)
## Registered S3 method overwritten by 'eulerr':
##   method from
##   plot.venn gplots
##
## Attaching package: 'eulerr'
##
## The following object is masked from 'package:gplots':
##
##      venn
library(ggseqlogo)
library(robustbase)
## Warning: package 'robustbase' was built under R version 4.3.3
##
## Attaching package: 'robustbase'
##
## The following object is masked from 'package:Biobase':
##
##      rowMedians
library(zoo)
##
## Attaching package: 'zoo'
##
## The following objects are masked from 'package:base':
##
##      as.Date, as.Date.numeric

```

### 0.3 Colors settings

```

acqua_greens <- c("#04493F", "#02655B", "#08756A", "#0C8074", "#179286", "#25A296", "#3EB3A7", "#5FCBC1"
acqua_blues  <- c("#02465B", "#015666", "#0C6476", "#157589", "#1E8498", "#4397A8", "#4FB0C3", "#70C2D2"
greys <- c("#202020", "#404040", "#606060", "#808080", "#AOAOAO", "#COCOCO", "#EOEOEO", "#FFFFFF")
reds <- c("#C10303", "#D83535", "#E95F5F", "#F08686", "#FAAEAE")

```

```

pinks <- c("#660000", "#990000", "#CC0000", "#FF0000", "#FF6666", "#FF9999", "#FFCECE")
yellows <- c( "#9C8803", "#A59213", "#B09D1F", "#BAA82F", "#C6B441", "#D0BF53", "#DACP69", "#E4D782", "#F0F5B7")
oranges <- c( "#A15000", "#CE7012", "#E47B12", "#F07D09", "#FF8B15", "#FFA141", "#FFCC99")

```

## 0.4 Motifs settings

```

## the following lines reconstructs the sequence motif logos from PWM published by the relative studies
## the RAP and CLIP PWM are computed by DREME with the parameters specified in the manuscript methods section
## For YBX3 in order to obtain the ACAHC motif, one background 4 mers (GAAC, masked to NNNN) needed to be added
## The RBFOX2 GMAUG motif is recomputed by weighing the abundance of GCAUG and GAAUG with the binding site length
## de novo motif discovery was performed only for RAPseq and eCLIPseq
## in figure 2A the logos for RBNS were drawn based on the logos published on the ENCODE Project website

```

### 0.4.1 eCLIP

```

#####
# eCLIP-seq data #####
# https://www.encodeproject.org
# HNRNPA1, accession ENCFF797GSK
# HNRNPC, accession ENCFF440R0Z
# PTBP1, accession ENCFF726SQU
# RBFOX2, accession ENCFF871NYM
# YBX3, accession ENCFF185OEI

# annotated data
PATH_eCLIP_ann <- "./Data/eCLIP/annotated/"
PEAKS_eCLIP_ann <- list.files(path = PATH_eCLIP_ann)
Names_eCLIP_ann <- unlist(str_split(PEAKS_eCLIP_ann, "\\.p"))[grep("txt", unlist(str_split(PEAKS_eCLIP_ann, "\\.p")))]
CLIPs <- list()
for (i in 1:length(Names_eCLIP_ann)){
  CLIPs[[i]] <- as.data.frame(read.table(paste(PATH_eCLIP_ann, PEAKS_eCLIP_ann[i], sep = ""), header = T))
}
names(CLIPs) <- Names_eCLIP_ann

# PFM
PATH_eCLIP_PFM <- "./Data/eCLIP/PFMs/"
PEAKS_eCLIP_PFM <- list.files(path = PATH_eCLIP_PFM)
Names_eCLIP <- unlist(str_split(PEAKS_eCLIP_PFM, "\\."))
eCLIPseq <- list()
for (i in 1:length(Names_eCLIP)){
  eCLIPseq[[i]] <- t(as.data.frame(read.table(paste(PATH_eCLIP_PFM, PEAKS_eCLIP_PFM[i], sep = ""), stringsAsFactors = F)))
  rownames(eCLIPseq[[i]]) <- c("A", "C", "G", "U")
  colnames(eCLIPseq[[i]]) <- NULL
}

names(eCLIPseq) <- Names_eCLIP
eCLIPseq$HNRNPA1 <- cbind(eCLIPseq$HNRNPA1, c(0,0,0,0), c(0,0,0,0), c(0,0,0,0))
eCLIPseq$HNRNPC <- cbind(eCLIPseq$HNRNPC, c(0,0,0,0), c(0,0,0,0))

```

```

eCLIPseq$PTBP1 <- cbind(eCLIPseq$PTBP1, c(0,0,0,0))
eCLIPseq$RBFOX2 <- cbind(eCLIPseq$RBFOX2, c(0,0,0,0), c(0,0,0,0))
eCLIPseq$YBX3 <- cbind(eCLIPseq$YBX3, c(0,0,0,0), c(0,0,0,0))

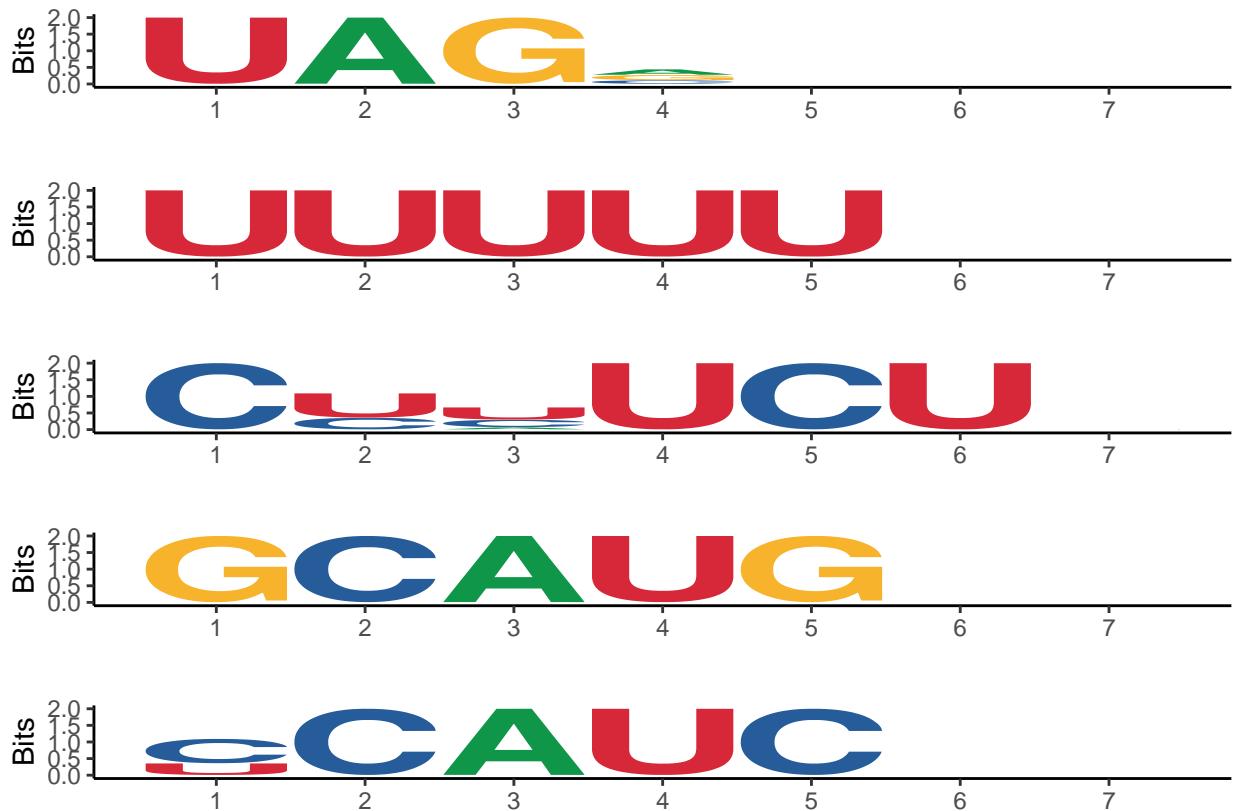
logos_eCLIPseq <- list()

for (i in 1:length(Names_eCLIP)){
  logos_eCLIPseq[[i]] <- ggplot() + geom_logo(eCLIPseq[[Names_eCLIP[i]]], font="helvetica_bold") + theme_minimal()
}

## Warning: The `<scale>` argument of `guides()` cannot be `FALSE`. Use "none" instead as
## of ggplot2 3.3.4.
## i The deprecated feature was likely used in the ggseqlogo package.
## Please report the issue at <https://github.com/omarwagih/ggseqlogo/issues>.
## This warning is displayed once every 8 hours.
## Call `lifecycle::last_lifecycle_warnings()` to see where this warning was
## generated.

names(logos_eCLIPseq) <- Names_eCLIP
eCLIP_logo <- ggarrange(logos_eCLIPseq$HNRNPA1, logos_eCLIPseq$HNRNPC, logos_eCLIPseq$PTBP1, logos_eCLIPseq$YBX3)
eCLIP_logo

```



#### 0.4.2 RNA Compete

```

#####
# Ref: A compendium of RNA-binding motifs for decoding gene regulation
# Link: https://hugheslab.ccbr.utoronto.ca/supplementary-data/RNAcompete_eukarya/
# HNRNPA1: https://hugheslab.ccbr.utoronto.ca/supplementary-data/RNAcompete_eukarya/Experiment_reports/
# HNRNPC: https://hugheslab.ccbr.utoronto.ca/supplementary-data/RNAcompete_eukarya/Experiment_reports/
# PTBP1: https://hugheslab.ccbr.utoronto.ca/supplementary-data/RNAcompete_eukarya/Experiment_reports/
# https://hugheslab.ccbr.utoronto.ca/supplementary-data/RNAcompete_eukarya/Experiment_reports

PATH_RNACompete <- "./Data/RNAcompete/"
PEAKS_RNACompete <- list.files(path = PATH_RNACompete)
Names_RNACompete <- unlist(str_split(PEAKS_RNACompete, "\\.")) [grep("txt", unlist(str_split(PEAKS_RNACompete, "\\.")))]
RNACompete <- list()
for (i in 1:length(Names_RNACompete)){
  RNACompete[[i]] <- as.data.frame(read.table(paste(PATH_RNACompete, PEAKS_RNACompete[i], sep = ""), stringsAsFactors = FALSE))
  RNACompete[[i]] <- t(RNACompete[[i]][,-1])
}
names(RNACompete) <- Names_RNACompete

logos_plots_RNACompete <- list()
for (i in 1:length(Names_RNACompete)){
  logos_plots_RNACompete[[i]] <- ggplot() + geom_logo(RNACompete[[Names_RNACompete[i]]]), font="helvetica")
}
names(logos_plots_RNACompete) <- Names_RNACompete
RNA_Compete_logo <- ggarrange(logos_plots_RNACompete$HNRNPA1, logos_plots_RNACompete$HNRNPC, logos_plots_RNACompete$PTBP1)

```



### 0.4.3 RBNS

```
# Ref: Sequence, Structure, and Context Preferences of Human RNA Binding Proteins
# Link: https://ars.els-cdn.com/content/image/1-s2.0-S1097276518303514-mmc4.xlsx
# Link: https://www.encodeproject.org/encore-matrix/?type=Experiment&status=released&internal_tags=ENCODE
# HNRNPC: https://www.encodeproject.org/experiments/ENCSR569UIU/
# https://www.encodeproject.org/documents/35943eb4-837d-4f96-a705-46eec6838f5d/@@download/attach
# RBFOX2: https://www.encodeproject.org/experiments/ENCSR441HLP/
# https://www.encodeproject.org/documents/2403659a-974e-4312-900f-45d0c8e3566d/@@download/attach
```

### 0.4.4 RAPSeq

```
##### RAP SEQ #####
PATH_RAP_ann <- "./Data/RAPseq/annotated/"
PEAKS_RAP_ann <- list.files(path = PATH_RAP_ann)
Names_RAP <- unlist(str_split(PEAKS_RAP_ann, "\\\\.p"))[grep("txt", unlist(str_split(PEAKS_RAP_ann, "\\\\.p")))
RAPS <- list()
for (i in 1:length(Names_RAP)){
  print(paste(PATH_RAP_ann, PEAKS_RAP_ann[i], sep = ""))
  RAPs[[i]] <- as.data.frame(read.table(paste(PATH_RAP_ann, PEAKS_RAP_ann[i], sep = ""), header = T, stringsAsFactors = F))
}
## [1] "./Data/RAPseq/annotated/HNRNPA1.peaks.txt"
## [1] "./Data/RAPseq/annotated/HNRNPC.peaks.txt"
## [1] "./Data/RAPseq/annotated/hsHuRHUMAN.peaks.txt"
## [1] "./Data/RAPseq/annotated/HuRPTBP1.peaks.txt"
## [1] "./Data/RAPseq/annotated/IGF2BP1.peaks.txt"
## [1] "./Data/RAPseq/annotated/PTBP1.peaks.txt"
## [1] "./Data/RAPseq/annotated/RBFOX2.peaks.txt"
## [1] "./Data/RAPseq/annotated/YBX3.peaks.txt"
names(RAPs) <- Names_RAP

### A C G U #####
#GCATG <- RAPs[["RBFOX2"]][grep("GAATG", str_sub(RAPs[["RBFOX2"]]$positive_fa, 80, 120), invert=T), ]
GCATG <- RAPs[["RBFOX2"]][grep("GCATG", str_sub(RAPs[["RBFOX2"]]$positive_fa, 80, 120)), "BS"]
GCATG <- rep("GCATG", round(sqrt(length(GCATG))*median(GCATG)) )

#GAATG <- RAPs[["RBFOX2"]][grep("GCATG", str_sub(RAPs[["RBFOX2"]]$positive_fa, 80, 120), invert=T), ]
GAATG <- RAPs[["RBFOX2"]][grep("GAATG", str_sub(RAPs[["RBFOX2"]]$positive_fa, 80, 120)), "BS"]
GAATG <- rep("GAATG", round(sqrt(length(GAATG))*median(GAATG)) )
GMATG <- c(GCATG, GAATG)
GMATG <- DNAStringSet(GMATG)
GMATG <- consensusMatrix(DNAStringSet(GMATG))[1:4,]
rownames(GMATG) <- c("A", "C", "G", "U")
GMAUG <- GMATG
GMAUG <- cbind(GMAUG, c(0, 0, 0, 0), c(0, 0, 0, 0))

GMAUG_logo <- ggplot() + geom_logo(GMAUG, font="helvetica_bold") + theme_classic() + ylim(0, 2)

PATH_RAP_PFM <- "./Data/RAPseq/PFMs/"
PEAKS_RAP_PFM <- list.files(path = PATH_RAP_PFM)
Names_RAP_PFM <- unlist(str_split(PEAKS_RAP_PFM, "\\\\."))[grep("txt", unlist(str_split(PEAKS_RAP_PFM, "\\\\.p")))]
```

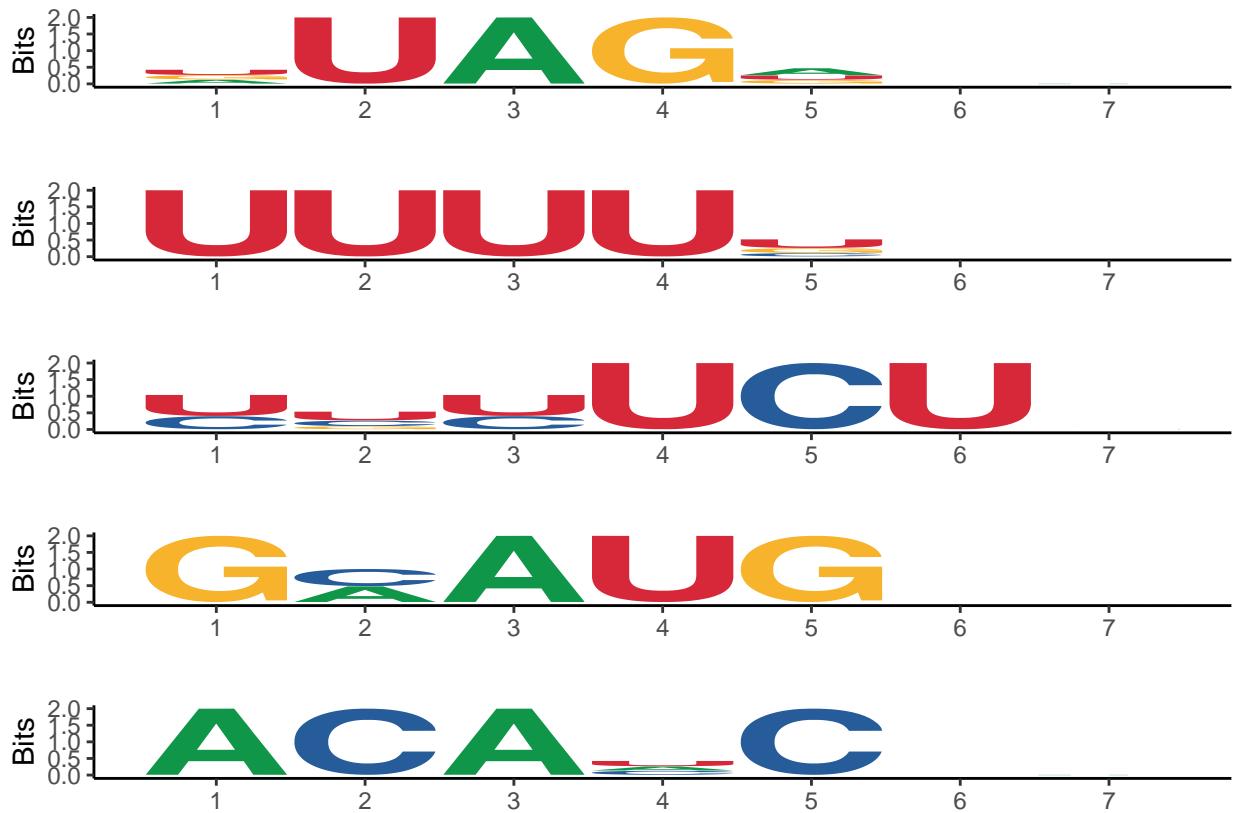
```

RAPseq <- list()

for (i in 1:length(Names_RAP_PFMs)){
  RAPseq[[i]] <- t(as.data.frame(read.table(paste(PATH_RAP_PFM, PEAKS_RAP_PFM[i], sep = ""), stringsAsFactors = TRUE)))
  rownames(RAPseq[[i]]) <- c("A", "C", "G", "U")
  colnames(RAPseq[[i]]) <- NULL
}

names(RAPseq) <- Names_RAP_PFM
RAPseq$HNRNPA1 <- cbind(RAPseq$HNRNPA1, c(0,0,0,0), c(0,0,0,0))
RAPseq$HNRNPC <- cbind(RAPseq$HNRNPC, c(0,0,0,0), c(0,0,0,0))
RAPseq$PTBP1 <- cbind(RAPseq$PTBP1, c(0,0,0,0))
RAPseq$YBX3 <- cbind(RAPseq$YBX3, c(0,0,0,0), c(0,0,0,0))
logos_RAPseq <- list()
for (i in 1:length(Names_RAP_PFM)){
  logos_RAPseq[[i]] <- ggplot() + geom_logo(RAPseq[[Names_RAP_PFM[i]]], font="helvetica_bold") + theme_minimal()
}
names(logos_RAPseq) <- Names_RAP_PFM
RAP_logo <- ggarrange(logos_RAPseq$HNRNPA1, logos_RAPseq$HNRNPC, logos_RAPseq$PTBP1, GMAUG_logo, logos_RAPseq$YBX3)
RAP_logo

```



## 0.5 Figures settings

### 0.5.1 Figure 2A

```
# RAP_logo, eCLIP_logo, RNA_Compete_logo and HTRSELEX_logo
# RBNS data were from ENCODE Project
p_motifLOGO <- ggarrange(RAP_logo,eCLIP_logo, RNA_Compete_logo, NULL, ncol=4)
ggsave(filename = "./Figure/Figure2A_motifLogo.pdf", plot = p_motifLOGO, width = 11.5, height = 7.8)
```

### 0.5.2 Figure 2B

```
# uracil content
RAPS[["HNRNPC"]]\$Us <- str_count(str_sub(RAPS[["HNRNPC"]]\$positive_fa,80,120),"T") / (120-80)
CLIPs[["HNRNPC"]]\$Us <- str_count(str_sub(CLIPs[["HNRNPC"]]\$positive_fa,43,83),"T") / (83-43)

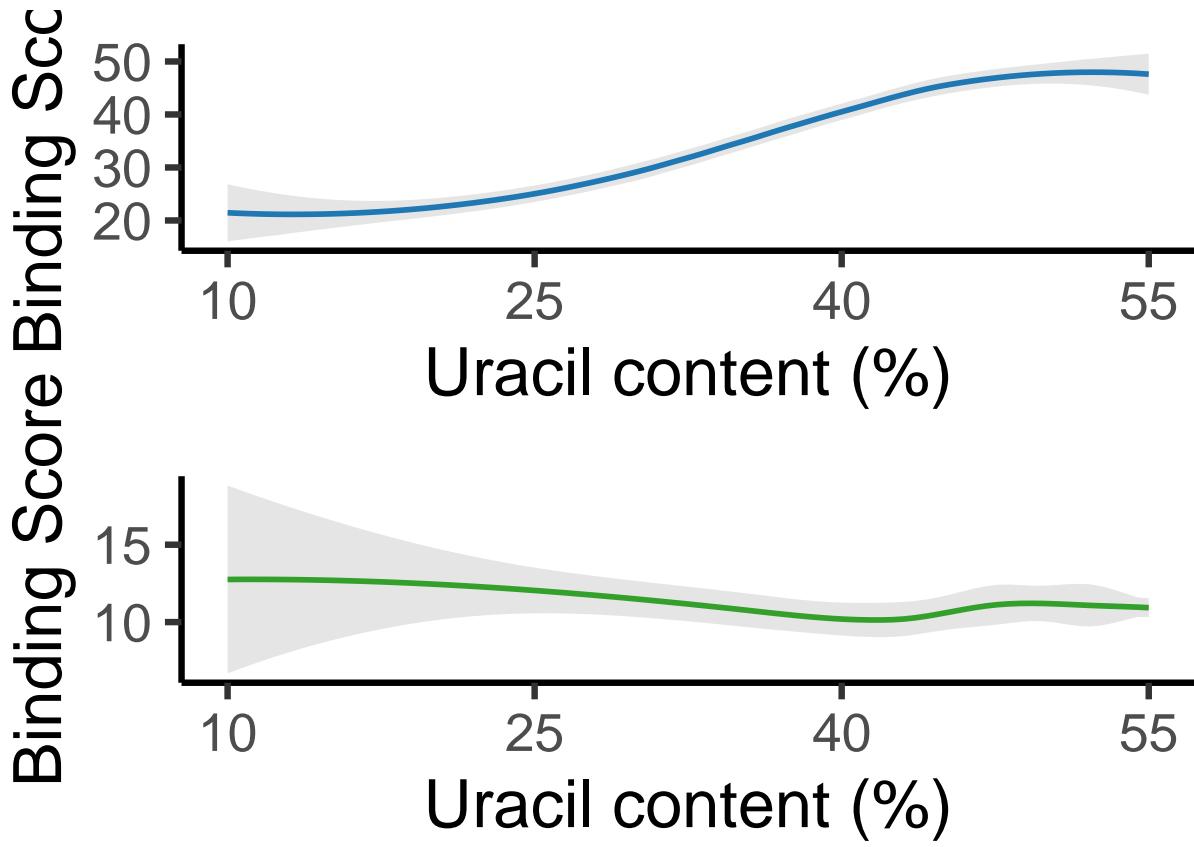
#
RAP_Us_data <- RAPS[["HNRNPC"]][,c("Us","BS","Mean_FCI")]
colnames(RAP_Us_data) <- c("Us","BS","MeanFC")
RAP_Us_data\$Assay <- rep("RAP",nrow(RAP_Us_data))
CLIP_Us_data <- CLIPs[["HNRNPC"]][,c("Us","BS","Mean_FC")]
colnames(CLIP_Us_data) <- c("Us","BS","MeanFC")
CLIP_Us_data\$Assay <- rep("CLIP",nrow(CLIP_Us_data))

HNRNPC_Us_data <- rbind(RAP_Us_data,CLIP_Us_data)
HNRNPC_Us_data\$Us <- HNRNPC_Us_data\$Us*100
HNRNPC_Us_data\$Us[HNRNPC_Us_data\$Us < 10] <- 10
HNRNPC_Us_data\$Us[HNRNPC_Us_data\$Us > 55] <- 55

fig_bindingScore_rap <- ggplot2::ggplot(data=HNRNPC_Us_data[HNRNPC_Us_data\$Assay == "RAP",], aes(x=Us,
  stat_smooth(method="loess", color="#1f78b4", fill=greys[6], level = 0.99) +
  theme_classic2(base_size = 25) +
  ylab("Binding Score") +
  xlab("Uracil content (%)") +
  scale_x_continuous(breaks = c(10,25,40,55))

fig_bindingScore_clip <- ggplot2::ggplot(data=HNRNPC_Us_data[HNRNPC_Us_data\$Assay == "CLIP",], aes(x=Us,
  stat_smooth(method="loess", color="#33a02c", fill=greys[6], level = 0.99) +
  theme_classic2(base_size = 25) +
  ylab("Binding Score") +
  xlab("Uracil content (%)") +
  scale_x_continuous(breaks = c(10,25,40,55))

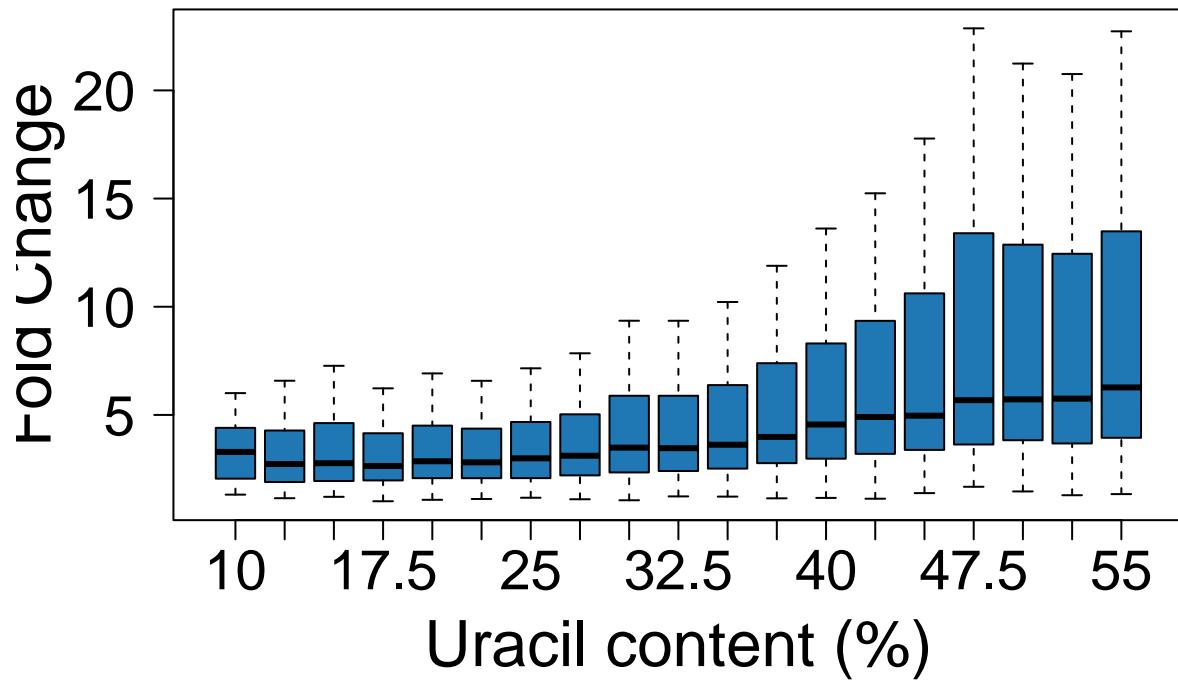
p_bindingScore_Us <- ggarrange(fig_bindingScore_rap, fig_bindingScore_clip, ncol=1)
## `geom_smooth()` using formula = 'y ~ x'
## `geom_smooth()` using formula = 'y ~ x'
p_bindingScore_Us
```



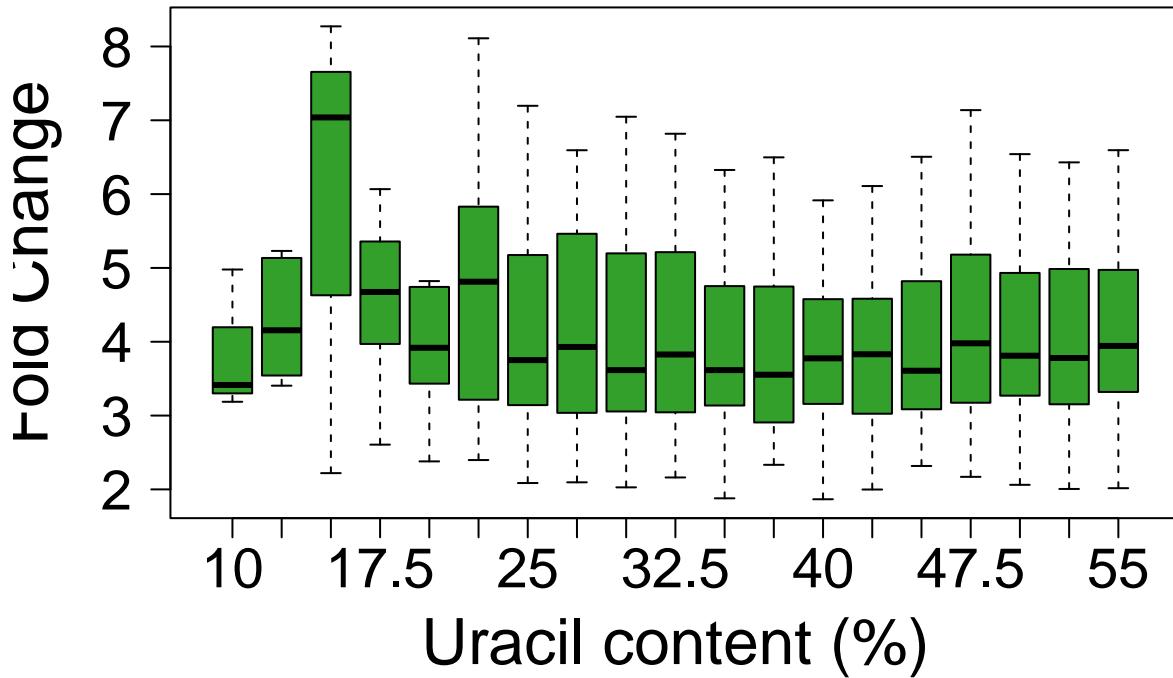
```

ggsave(filename = "./Figure/Figure2B_BindingScoreUracilContent_LOSSES.pdf", plot = p_bindingScore_Us, 
#pdf("./Figure/Figure2B_UracilContentBoxplot_RAP.pdf", width = 11.5, height = 7.8)
boxplot(data=HNRNPC_Us_data[HNRNPC_Us_data$Assay == "RAP",], MeanFC~Us, outline=F, range=1, notch=F, colo

```



```
#dev.off()  
  
#pdf("./Figure/Figure2B_UracilContentBoxplot_CLIP.pdf", width = 11.5, height = 7.8)  
boxplot(data=HNRPUs_data[HNRPUs_data$Assay == "CLIP",], MeanFC~Us, outline=F, range=1, notch=F, c
```



```
#dev.off()
```

### 0.5.3 Figure 2C

```
## DeepTools count matrices (bin size = 10nt) are read in
## DeepTools was used with the following command to generate the per nt count matrices
## computeMatrix reference-point -S rep1.bw -R rep1.bed -a 100 -b 100 -bs 10
## RAPseq
PATH_RAP_Coverage <- "./Data/RAPseq/Coverage/10nt/"
PEAKS_RAP_Coverage <- list.files(path = PATH_RAP_Coverage)
Names_RAP_Coverage <- unlist(str_split(PEAKS_RAP_Coverage, "\\\\.matr"))[grep("txt", unlist(str_split(PEAKS_RAP_Coverage, "\\\\.matr")))]
RAPseq_Coverage <- list()

for (i in 1:length(Names_RAP_Coverage)){
  RAPseq_Coverage[[i]] <- as.data.frame(read.table(paste(PATH_RAP_Coverage, PEAKS_RAP_Coverage[i], sep = "."))
}
names(RAPseq_Coverage) <- Names_RAP_Coverage

#par(mfrow=c(4,5), bty="n")
Names_protein <- c("HNRNPA1", "HNRNPC", "PTBP1", "RBFOX2", "YBX3")
# coverage normalized RPM
for (i in 1:length(Names_protein)) {

  rep1_name <- paste (Names_protein[i], ".rep1", sep = "")
```

```

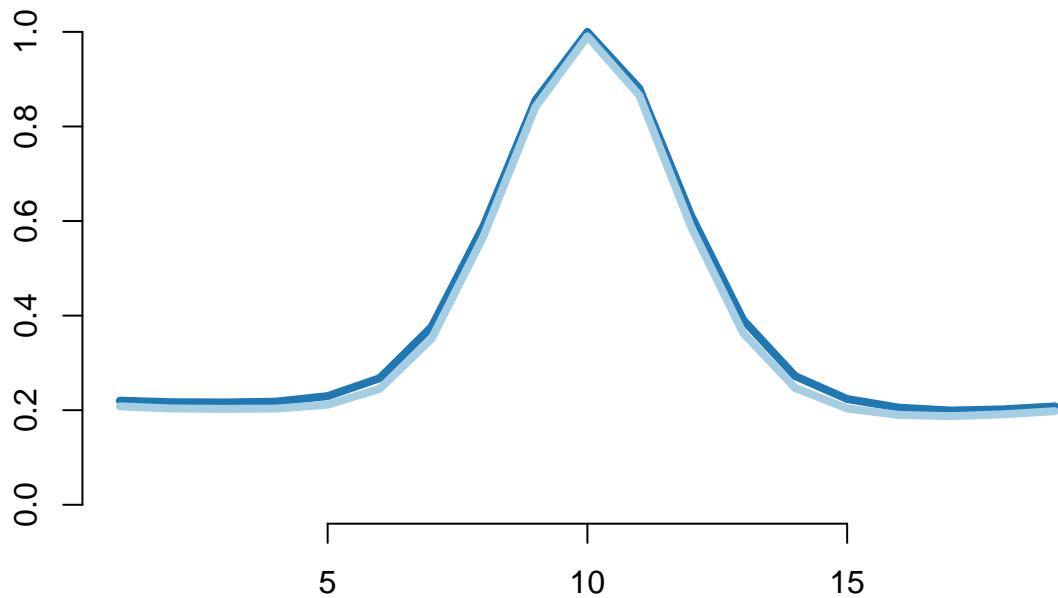
rep2_name <- paste (Names_protein[i] , ".rep2" , sep="")

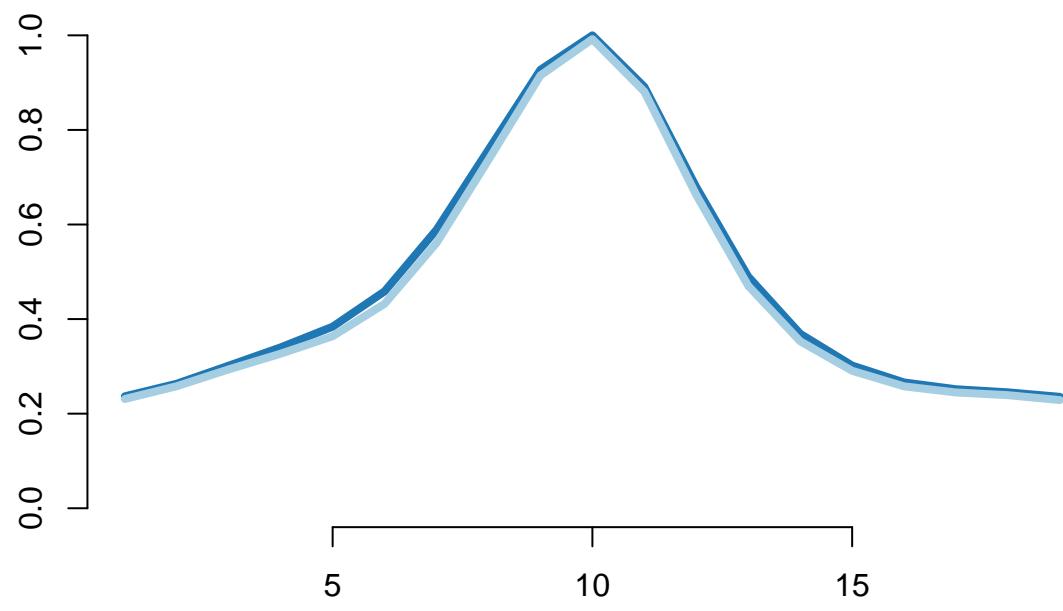
mean_rep1 <- rollapply(colMeans(as.matrix(RAPseq_Coverage[[rep1_name]])), width=2, FUN = mean)
mean_rep2 <- rollapply(colMeans(as.matrix(RAPseq_Coverage[[rep2_name]])), width=2, FUN = mean)

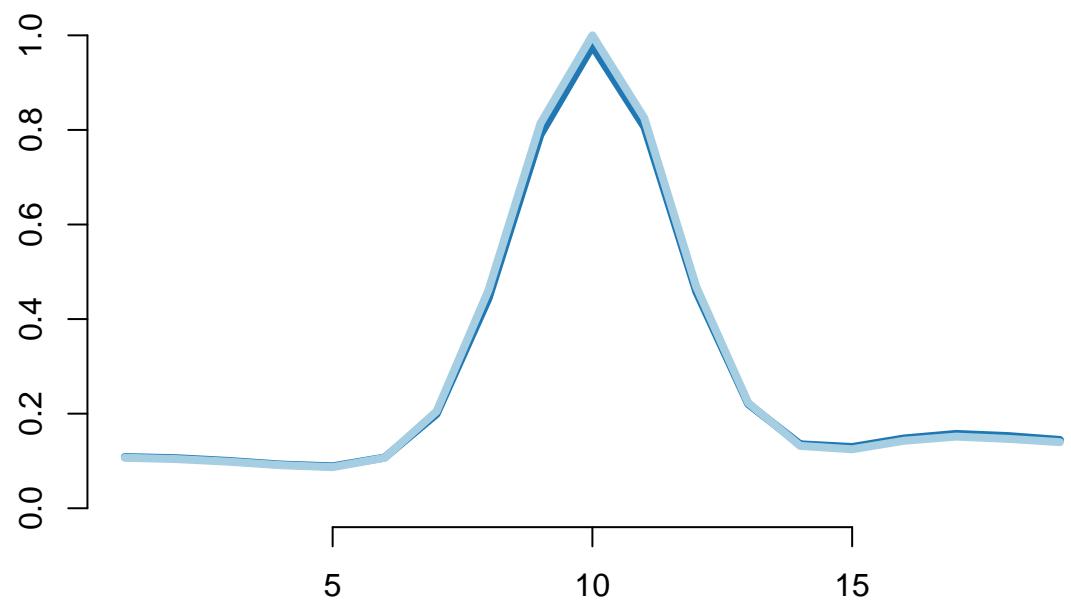
normalize_rep1 <- mean_rep1/max(c(mean_rep1,mean_rep2))
normalize_rep2 <- mean_rep2/max(c(mean_rep1,mean_rep2))

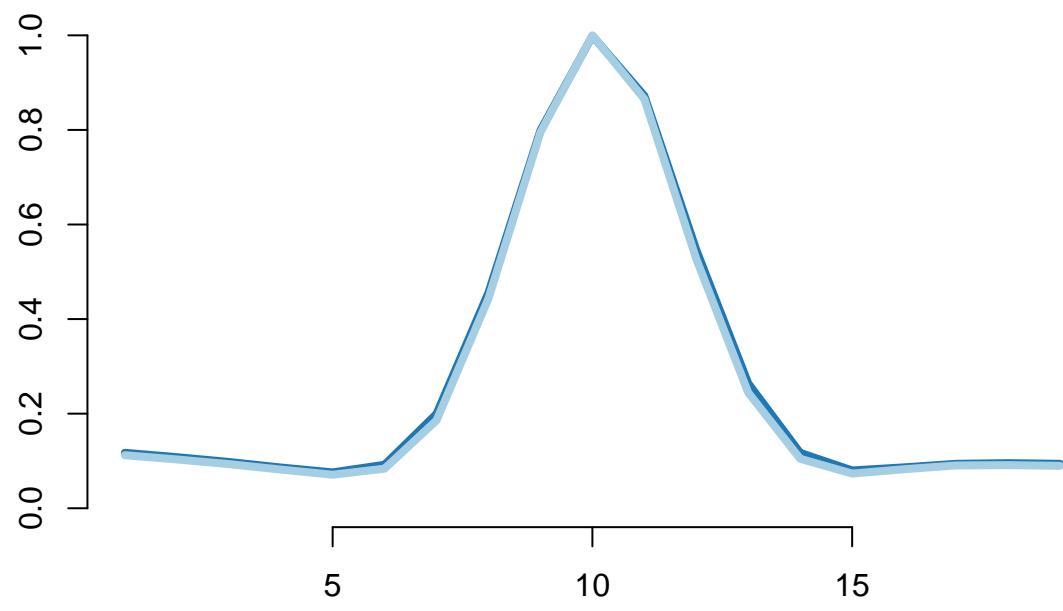
plot(normalize_rep1, type="l", col="#1f78b4", lwd=4, ylab=NA, xlab=NA, ylim=c(0,1), las=1, axes=FALSE)
axis(side=1, labels=T)
axis(side=2, labels=T)
points(normalize_rep2,type="l",col="#a6cee3", lwd=4)
}

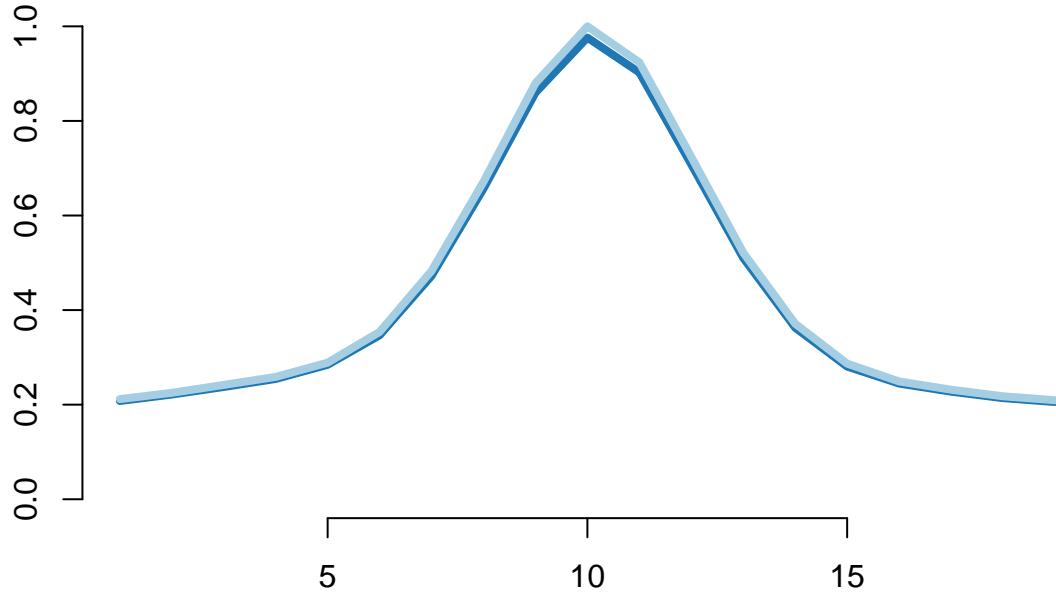
```











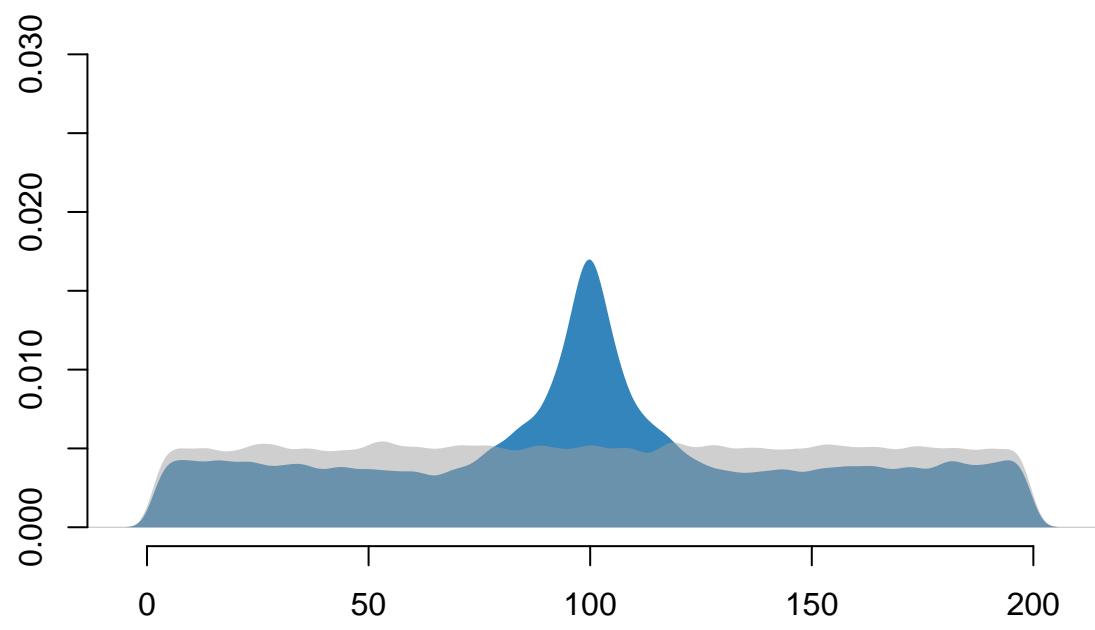
```

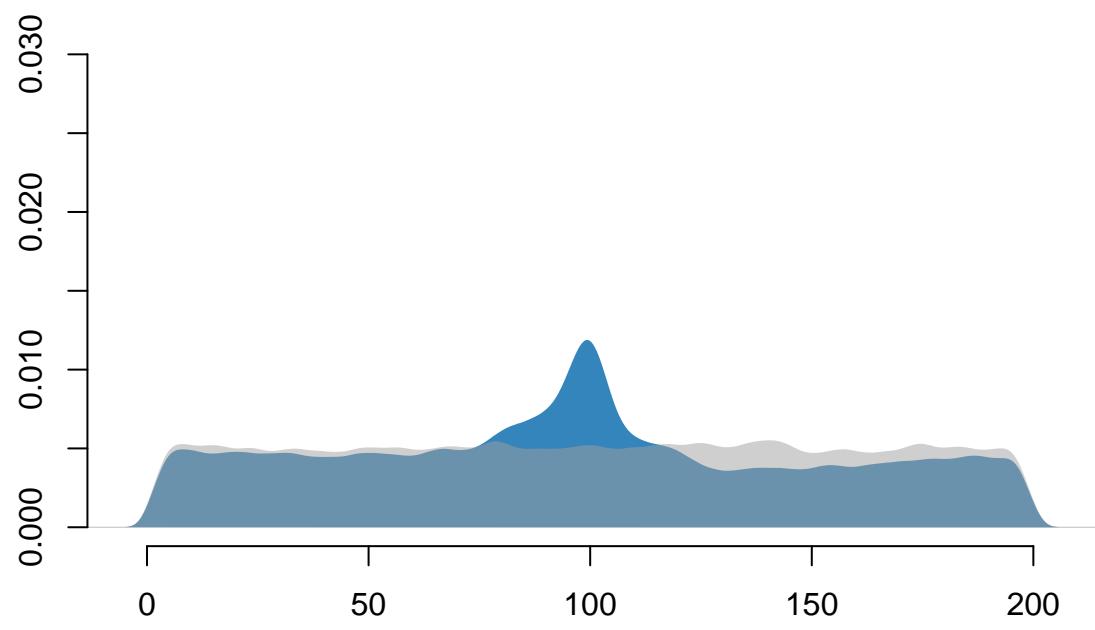
# motif density
RAPs_motifdensity <- RAPs
RAPs_motifdensity[["PTBP1"]]\$positive_fa <- gsub("T", "Y", RAPs_motifdensity[["PTBP1"]]\$positive_fa)
RAPs_motifdensity[["PTBP1"]]\$positive_fa <- gsub("C", "Y", RAPs_motifdensity[["PTBP1"]]\$positive_fa)
RAPs_motifdensity[["PTBP1"]]\$negative_fa <- gsub("T", "Y", RAPs_motifdensity[["PTBP1"]]\$negative_fa)
RAPs_motifdensity[["PTBP1"]]\$negative_fa <- gsub("C", "Y", RAPs_motifdensity[["PTBP1"]]\$negative_fa)

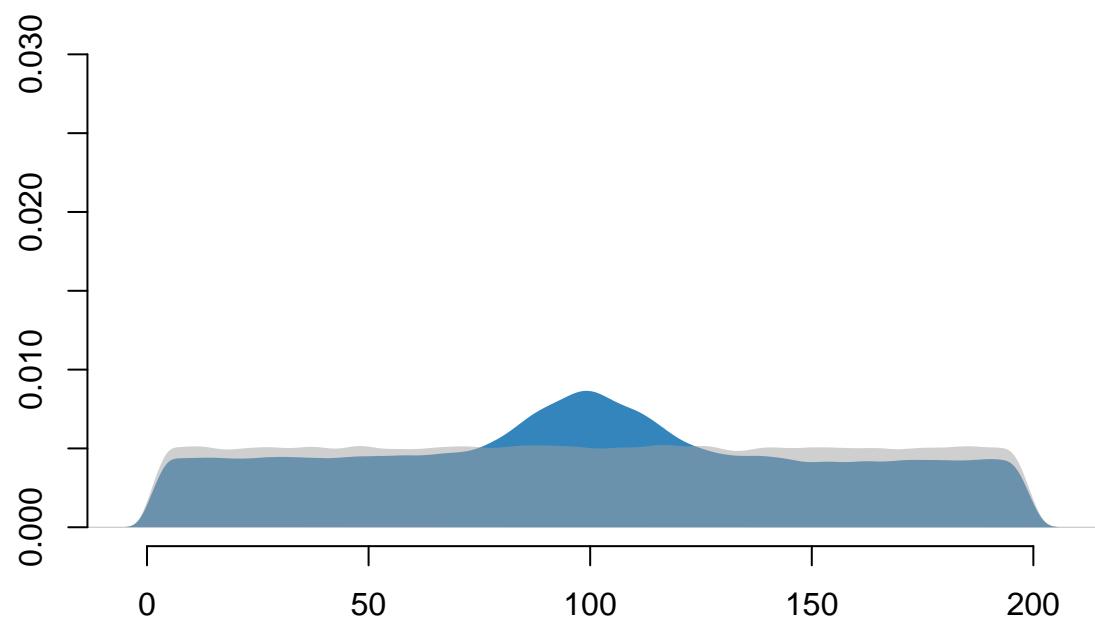
Motifs <- c("TAG", "TTTT", "YYYY", "GCATG|GAATG", "AACATC|AACACAC|AACACC")

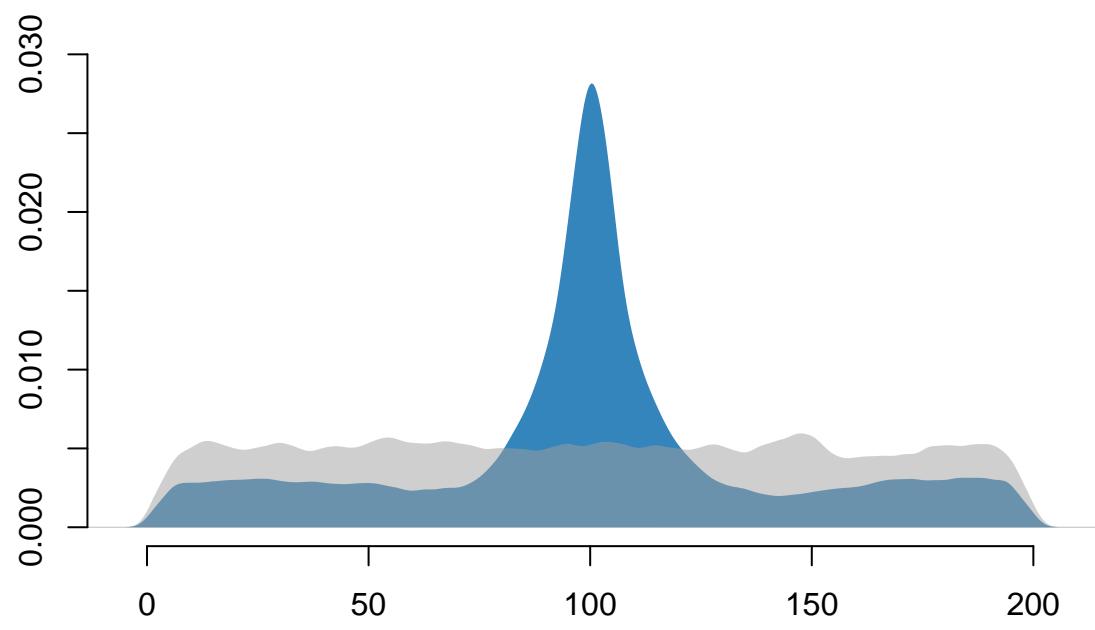
for (i in 1:length(Names_protein)){
  signal <- density(unlist(str_locate_all(RAPs_motifdensity[[Names_protein[i]]]\$positive_fa,Motifs[i])))
  noise <- density(unlist(str_locate_all(RAPs_motifdensity[[Names_protein[i]]]\$negative_fa,Motifs[i])))
  plot(signal, col=NA, main=NA, xlab=NA, ylab=NA, ylim=c(0,0.03), axes=FALSE)
  axis(side=1, labels=T)
  axis(side=2, labels=T)
  polygon(signal, col=alpha("#1f78b4", 0.9),border = NA)
  polygon(noise, col=alpha("#AOAOAO", 0.5),border = NA)
}

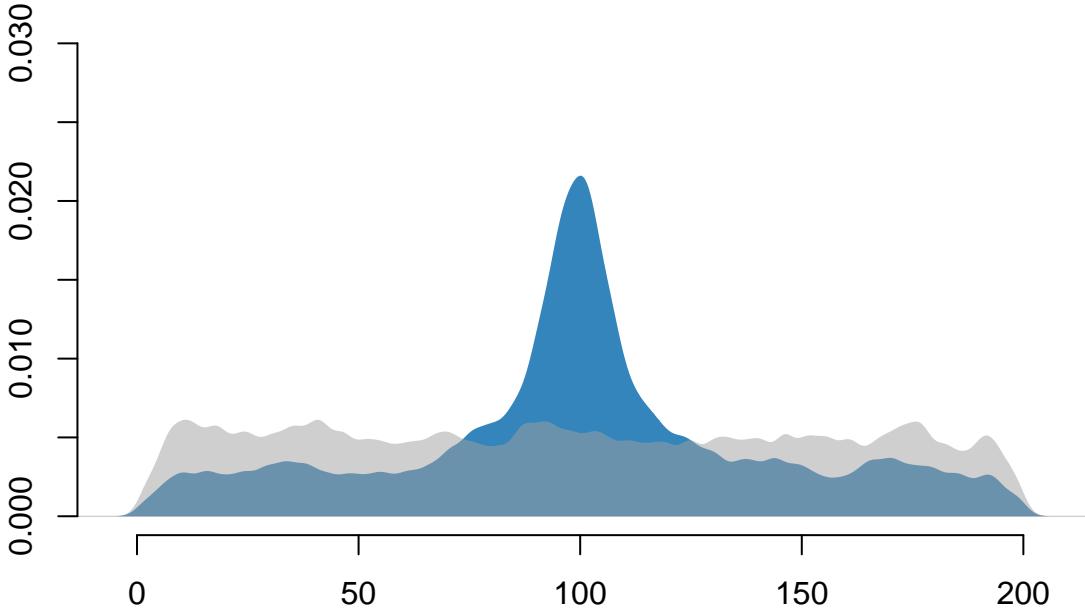
```











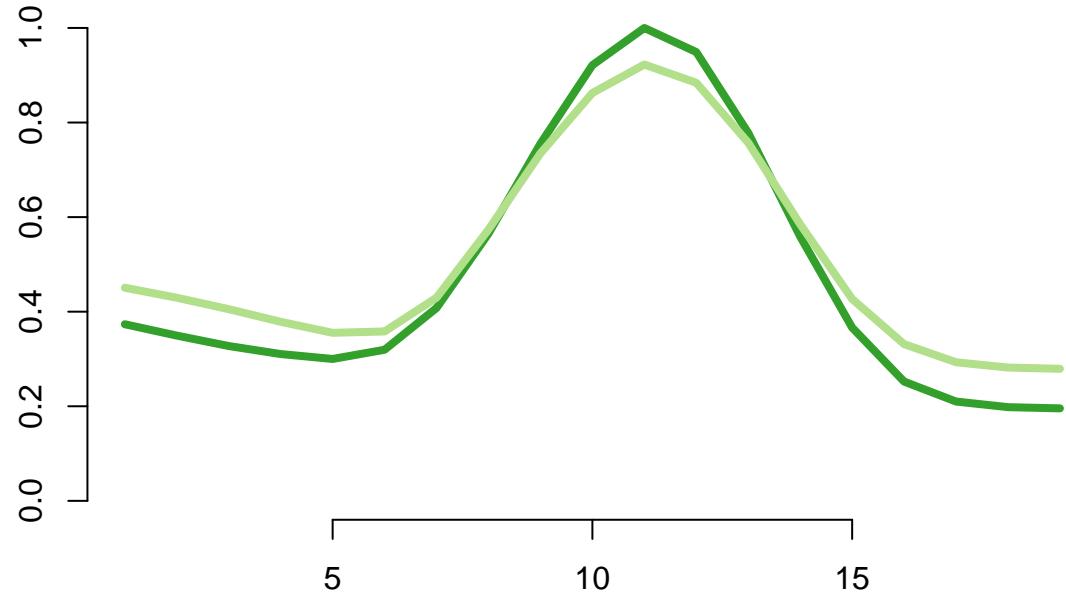
```

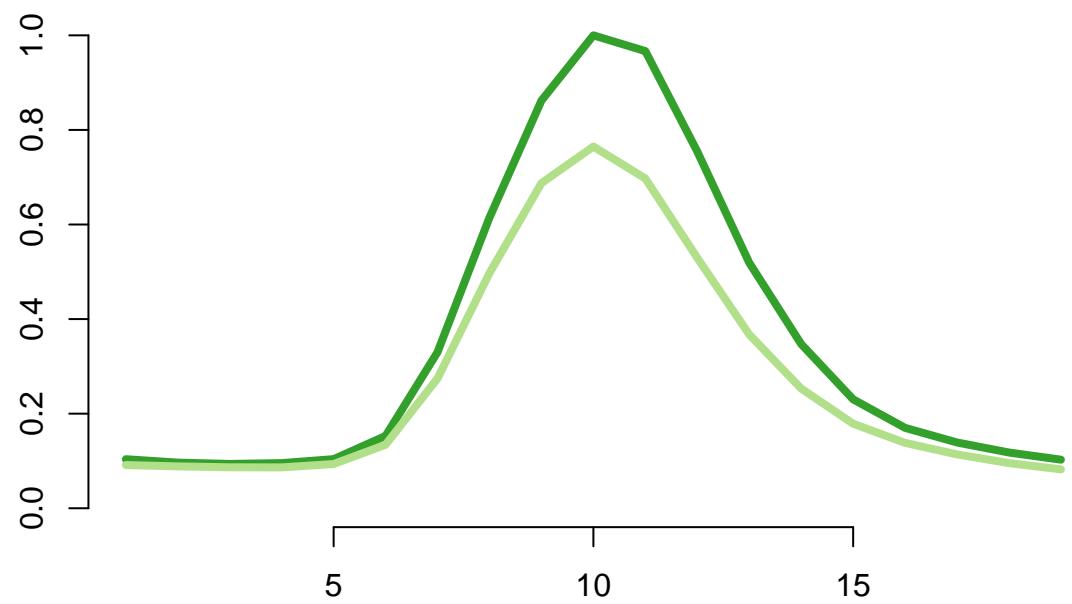
# eCLIP
## DeepTools count matrices (bin size = 10nt) are read in
## DeepTools was used with the following command to generate the per nt count matrices
## computeMatrix reference-point -S rep1.bw -R rep1.bed -a 100 -b 100 -bs 10
PATH_eCLIP_Coverage <- "./Data/eCLIP/Coverage/10nt/"
PEAKS_eCLIP_Coverage <- list.files(path = PATH_eCLIP_Coverage)
Names_eCLIP_Coverage <- unlist(str_split(PEAKS_eCLIP_Coverage, "\\matr"))[grep("txt", unlist(str_split(PEAKS_eCLIP_Coverage, "\\matr")))]
CLIPseq_Coverage <- list()
for (i in 1:length(Names_eCLIP_Coverage)){
  CLIPseq_Coverage[[i]] <- as.data.frame(read.table(paste(PATH_eCLIP_Coverage, PEAKS_eCLIP_Coverage[i], sep = "."))
}
names(CLIPseq_Coverage) <- Names_eCLIP_Coverage
Names_eCLIP_Coverage <- c("HNRNPA1_eCLIP", "HNRNPC_eCLIP", "PTBP1_eCLIP", "RBFOX2_eCLIP", "YBX3_eCLIP")
# coverage normalized RPM
for (i in 1:length(Names_eCLIP_Coverage)){
  rep1_name <- paste(Names_eCLIP_Coverage[i], ".rep1", sep = "")
  rep2_name <- paste(Names_eCLIP_Coverage[i], ".rep2", sep = "")

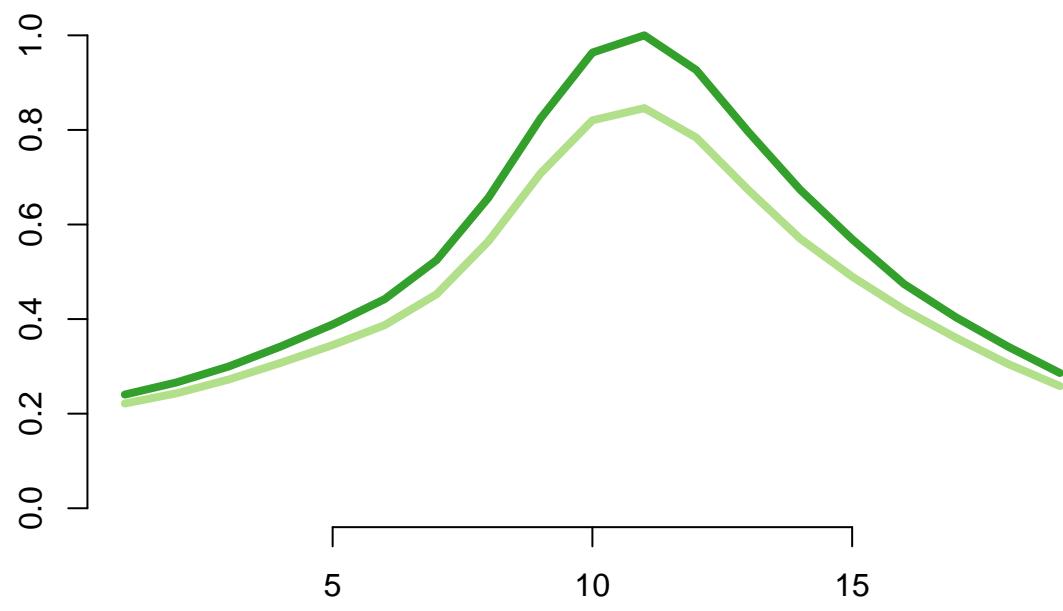
  rep1_mean <- rollapply(colMeans(as.matrix(CLIPseq_Coverage[[rep1_name]])), width=2, FUN = mean)
  rep2_mean <- rollapply(colMeans(as.matrix(CLIPseq_Coverage[[rep2_name]])), width=2, FUN = mean)

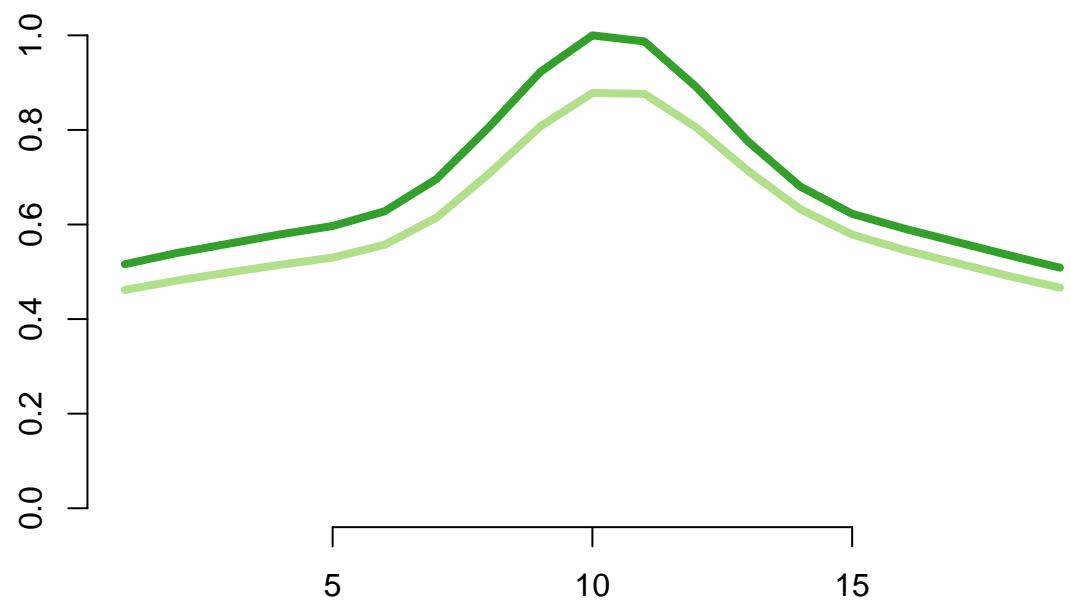
  x <- rep1_mean/max(c(rep1_mean, rep2_mean))
  y <- rep2_mean/max(c(rep1_mean, rep2_mean))
}
```

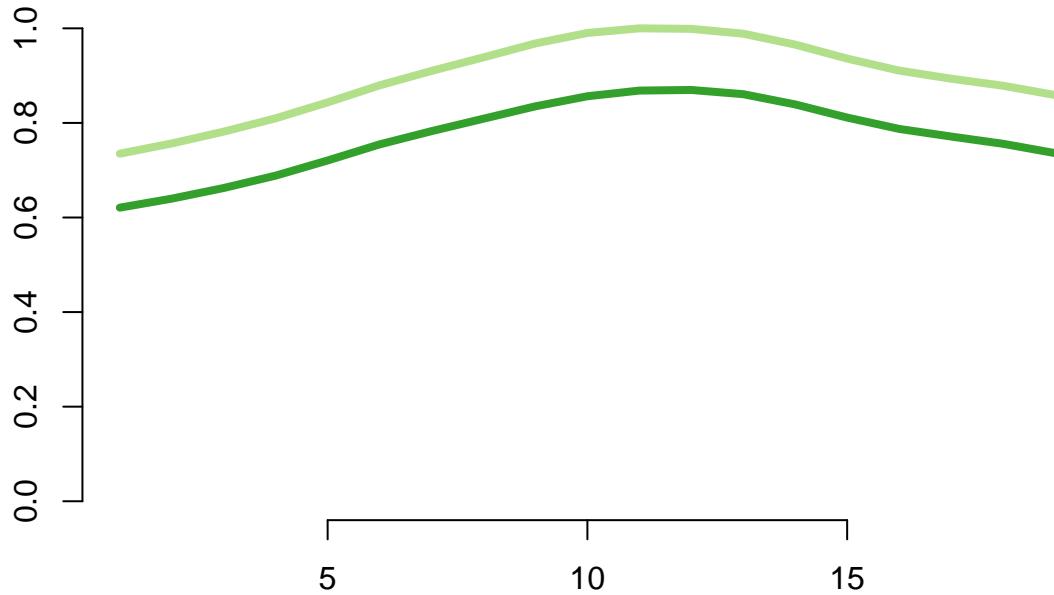
```
plot(x,type="l",col="#33a02c", lwd=4, ylab=NA, xlab=NA, ylim=c(0,1), las=1, axes=FALSE)
axis(side=1, labels=T)
axis(side=2, labels=T)
points(y,type="l",col="#b2df8a", lwd=4)
}
```











```

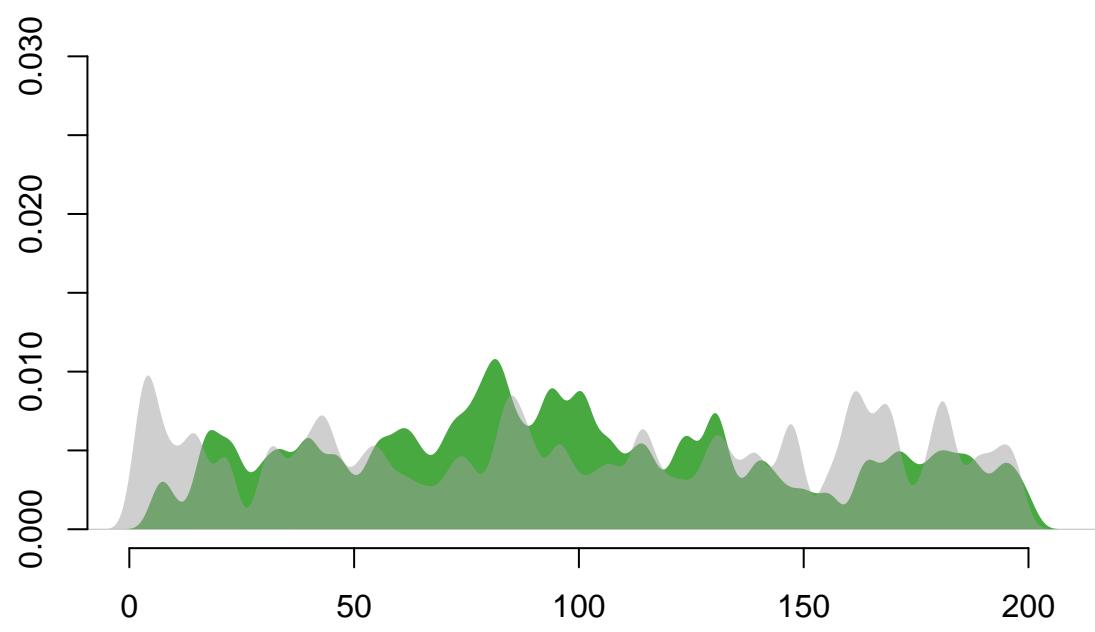
# motif density
CLIPs[["PTBP1"]]$positive_fa <- gsub("T", "Y", CLIPs[["PTBP1"]]$positive_fa)
CLIPs[["PTBP1"]]$positive_fa <- gsub("C", "Y", CLIPs[["PTBP1"]]$positive_fa)
CLIPs[["PTBP1"]]$negative_fa <- gsub("T", "Y", CLIPs[["PTBP1"]]$negative_fa)
CLIPs[["PTBP1"]]$negative_fa <- gsub("C", "Y", CLIPs[["PTBP1"]]$negative_fa)

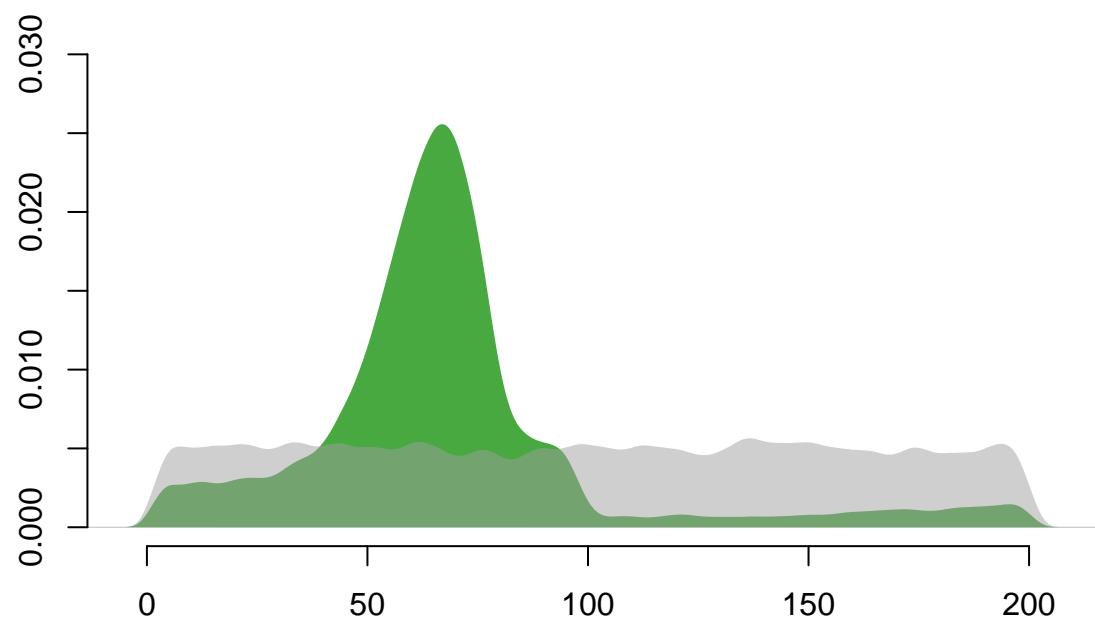
Motifs <- c("TAG", "TTTT", "YYYY", "GCATG", "CCATC|TCATC")
Names_protein <- c("HNRNPA1", "HNRNPC", "PTBP1", "RBFOX2", "YBX3")
for (i in 1:length(Names_protein)) {

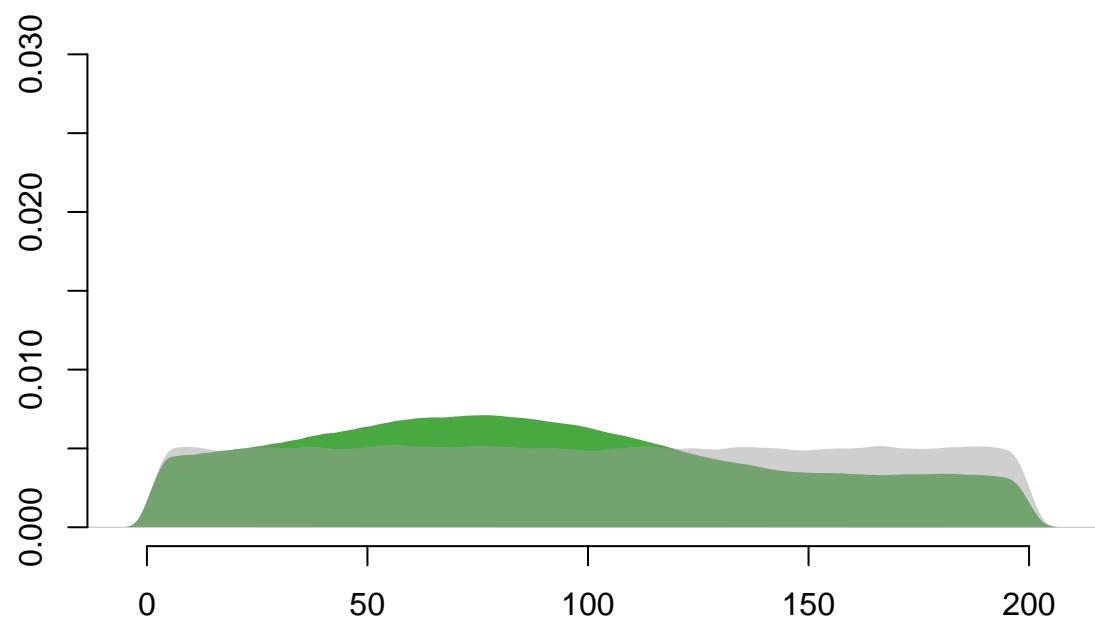
  signal <- density(unlist(str_locate_all(CLIPs[[Names_protein[i]]]$positive_fa,Motifs[i])),bw=2)
  noise <- density(unlist(str_locate_all(CLIPs[[Names_protein[i]]]$negative_fa,Motifs[i])),bw=2)

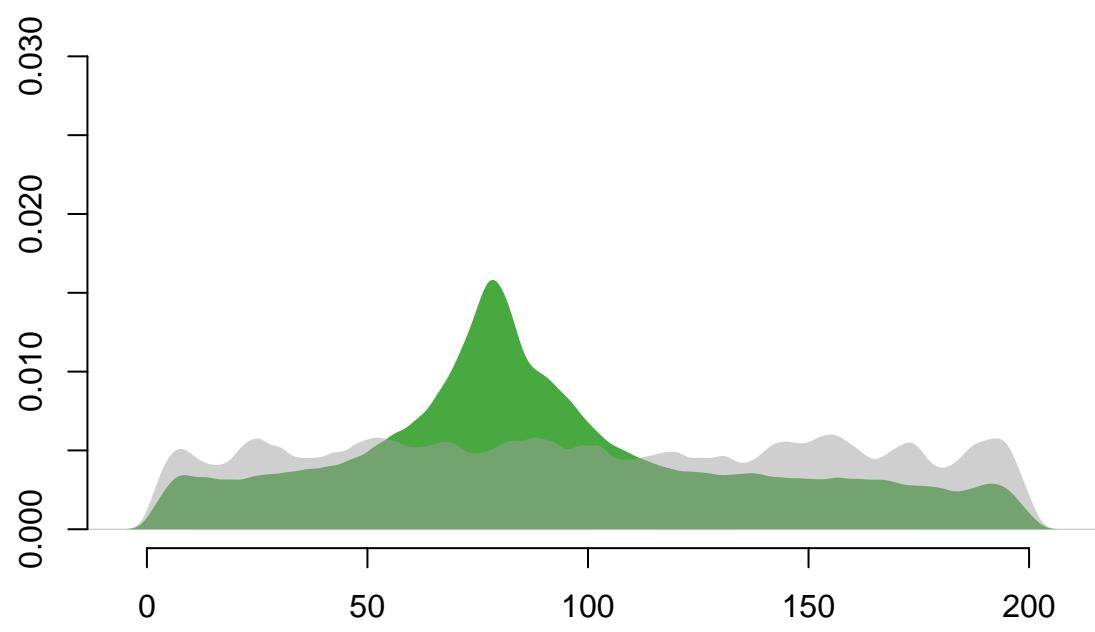
  plot(signal, col=NA, main=NA, xlab=NA, ylab=NA, ylim=c(0,0.03), axes=FALSE)
  axis(side=1, labels=T)
  axis(side=2, labels=T)
  polygon(signal, col=alpha("#33a02c", 0.9),border = NA)
  polygon(noise, col=alpha("#AOAOAO", 0.5),border = NA)
}

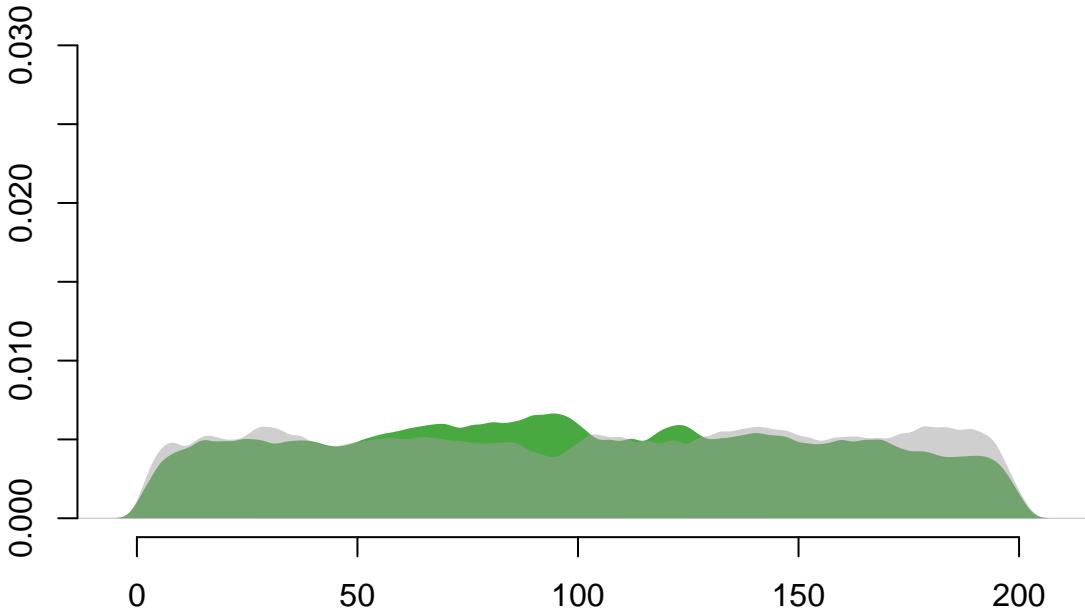
```











#### 0.5.4 Figure 2E

```

Names_protein <- c("HNRNPA1", "HNRNPC", "PTBP1", "RBF0X2", "YBX3")

#pdf("./Figure/Figure2E_similarity.pdf", width = 11, height = 11)
par(mfrow=c(5,3), mar=c(1,2,1,1))
set.seed(123)

for(i in Names_protein){
  # RAPSeq
  raps_rep1 <- log2(RAPs[[i]]$FCH_rep1)
  raps_rep2 <- log2(RAPs[[i]]$FCH_rep2)
  plot(raps_rep1, raps_rep2, xlim=c(0,10), ylim=c(0,10), xlab=NA, ylab=NA, bty="n", xaxt="n", yaxt="n",
    axis(1, at = c(0, 5, 10), lwd=1.5, labels = T)
  axis(2, at = c(0, 5, 10), lwd=1.5, labels = T)
  # spearman
  text_ann <- "rho ="
  raps_spearman <- round(cor(raps_rep1,raps_rep2, method = "spearman"), 2)
  text_ann <- paste(text_ann, raps_spearman, sep=" ")
  text(3,9.5,labels = raps_spearman, cex=1.5)

  # RAP sampling
  raps_sample <- RAPs[[i]][sample(1:nrow(RAPs[[i]]),(nrow(CLIPs[[i]]))),]
  raps_sample_rep1 <- log2(raps_sample$FCH_rep1)
}

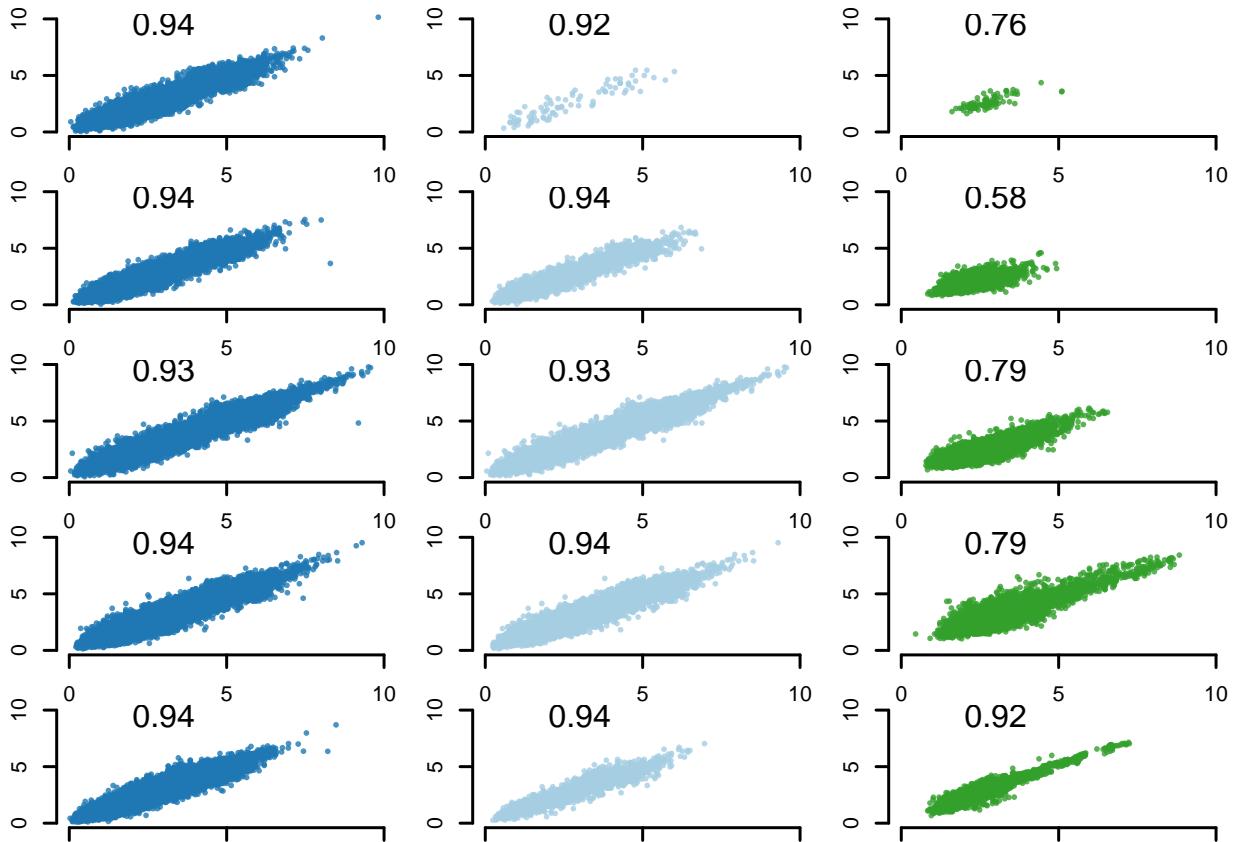
```

```

raps_sample_rep2 <- log2(raps_sample$FCH_rep2)
plot(raps_sample_rep1,raps_sample_rep2,xlim=c(0,10),ylim=c(0,10), xlab=NA, ylab=NA, bty="n", xaxt="n"
axis(1, at = c(0, 5, 10), lwd=1.5, labels = T)
axis(2, at = c(0, 5, 10), lwd=1.5, labels = T)
raps_sample_spearman <- round(cor(raps_sample_rep1,raps_sample_rep2, method = "spearman"), 2)
text_ann <- paste(text_ann, raps_sample_spearman, sep=" ")
text(3,9.5,labels = raps_sample_spearman, cex=1.5)

# eCLIP
eCLIP_rep1 <- log2(CLIPs[[i]]$FC_rep1)
eCLIP_rep2 <- log2(CLIPs[[i]]$FC_rep2)
plot(eCLIP_rep1, eCLIP_rep2, xlim=c(0,10),ylim=c(0,10), xlab=NA, ylab=NA, bty="n", xaxt="n", yaxt="n"
axis(1, at = c(0, 5, 10), lwd=1.5, labels = T)
axis(2, at = c(0, 5, 10), lwd=1.5, labels = T)
eCLIP_spearman <- round(cor(eCLIP_rep1,eCLIP_rep2, method = "spearman"), 2)
text_ann <- paste(text_ann, eCLIP_spearman,sep=" ")
text(3,9.5, labels = eCLIP_spearman, cex=1.5)
}

```



```
#dev.off()
```

### 0.5.5 Figure 2F

```

# spearman
RAP_spearman <- c()
CLIP_spearman <- c()
Names_protein <- c("HNRNPA1", "HNRNPC", "PTBP1", "RBFOX2", "YBX3")
for(i in Names_protein){
  raps_rep1 <- RAPs[[i]]$FCH_rep1
  raps_rep2 <- RAPs[[i]]$FCH_rep2
  RAP_spearman <- c(RAP_spearman, cor(raps_rep1, raps_rep2, method="spearman"))

  clip_rep1 <- CLIPs[[i]]$FC_rep1
  clip_rep2 <- CLIPs[[i]]$FC_rep2
  CLIP_spearman <- c(CLIP_spearman, cor(clip_rep1,clip_rep2,method="spearman"))
}

# merging
cors <- c(RAP_spearman,CLIP_spearman)
assay <- c(rep("aRAP",5),rep("eCLIP",5))

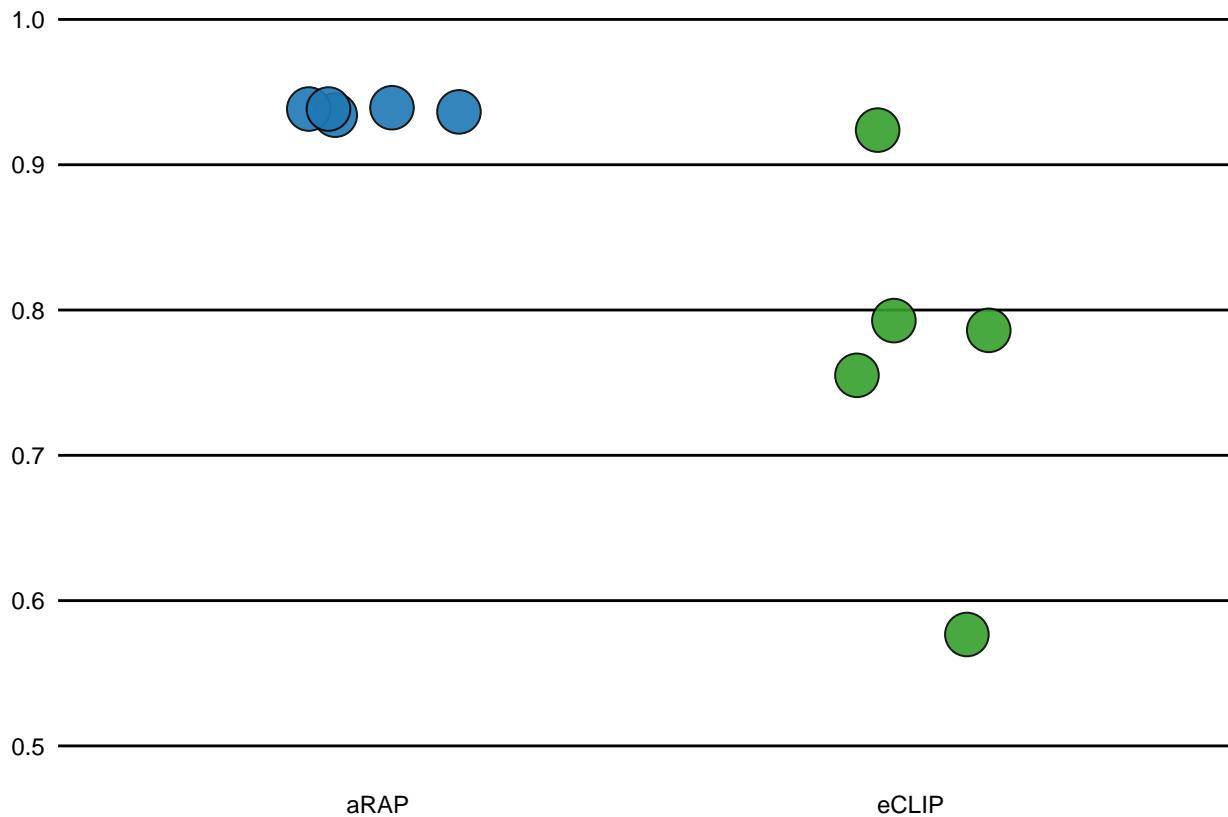
# data frame
data_cors <- data.frame(cors,assay)
data_cors$cors <- as.numeric(data_cors$cors)
data_cors$assay <- as.factor(data_cors$assay)

wilcox.test(data_cors[data_cors$assay == "aRAP",]$cors, data_cors[data_cors$assay == "eCLIP",]$cors)
##
## Wilcoxon rank sum exact test
##
## data: data_cors[data_cors$assay == "aRAP", ]$cors and data_cors[data_cors$assay == "eCLIP", ]$cors
## W = 25, p-value = 0.007937
## alternative hypothesis: true location shift is not equal to 0

# plot
set.seed(1)
#pdf("./Figure/Figure2_F.pdf", width = 2.9, height = 5.3)
ggplot(data=data_cors,aes(x=assay,y=cors,fill=assay)) +
  geom_dotplot(binaxis = "y",
               stackdir='center',
               binwidth = .005,
               dotsize = 6,
               position = position_jitter(height=NULL, width = 0.15),
               alpha = 0.9
  ) +
  theme(panel.background = element_blank(),
        panel.grid.major.x = element_blank(),
        panel.grid.minor.x = element_blank(),
        panel.grid.major.y = element_line(color="black"),
        axis.ticks = element_blank(),
        axis.text = element_text(color="black"),
        legend.position = "none",
        axis.title = element_blank()
  ) +

```

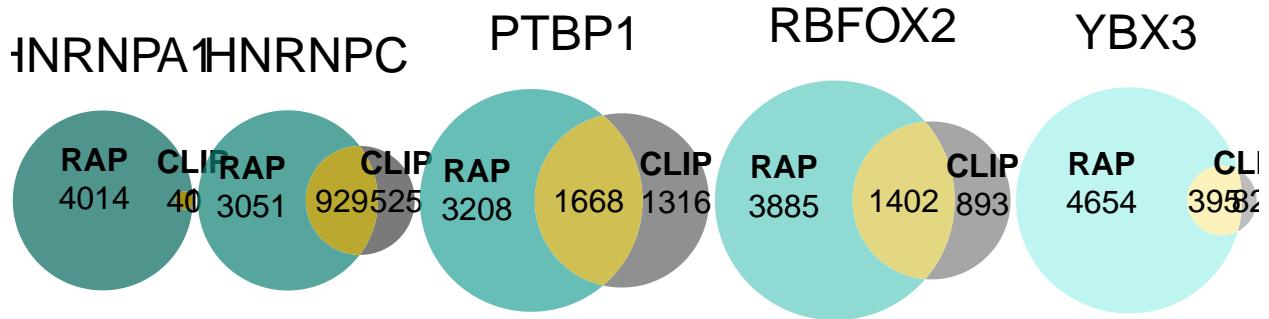
```
scale_fill_manual(values = c("#1f78b4", "#33a02c") ) +
ylim(0.5,1)
```



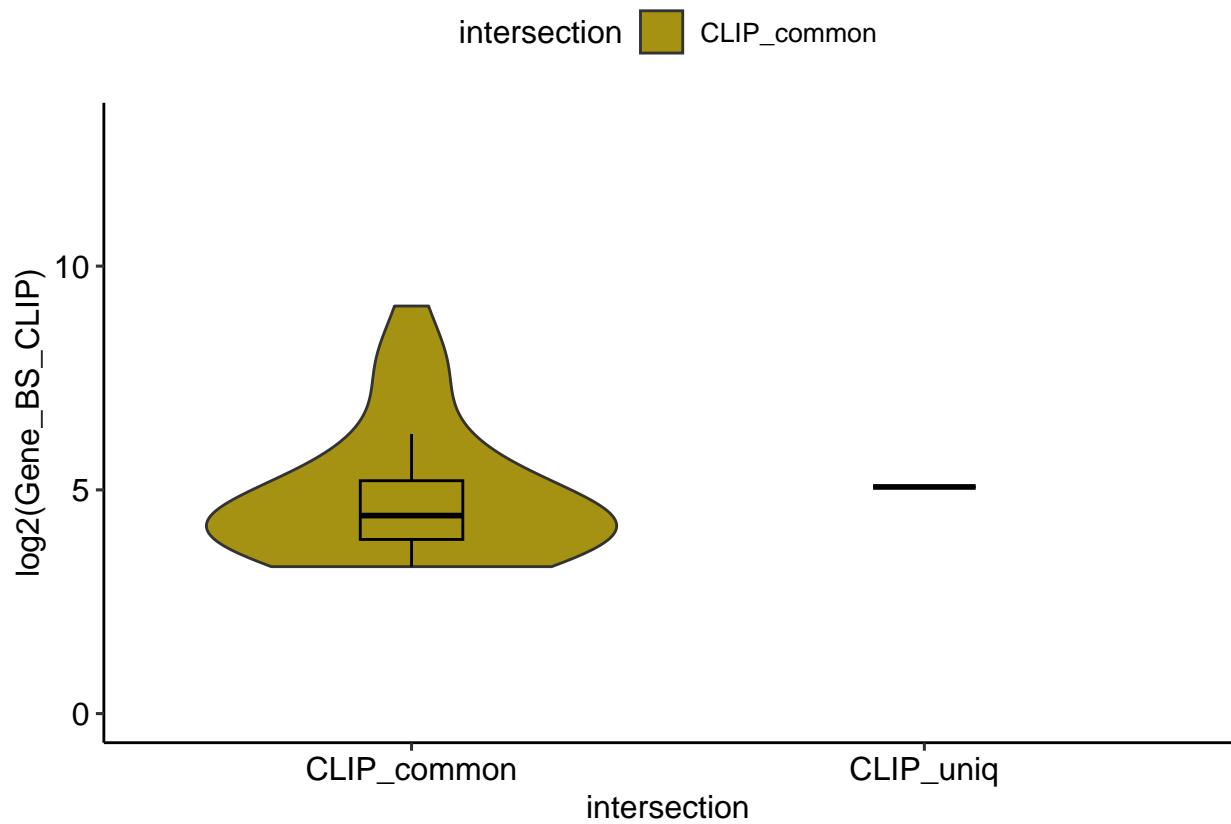
```
#dev.off()
```

### 0.5.6 Figure 2G

```
# left
venns <- list()
plot_area <- c()
Names_protein <- c("HNRNPA1", "HNRNPC", "PTBP1", "RBFOX2", "YBX3")
for (i in 1:length(Names_protein)){
  common_RAPs_CLIPs <- length(intersect(unique(RAPs[[Names_protein[i]]]$gene_ID), unique(CLIPs[[Names_protein[i]]]$gene_ID)))
  uniq_RAP <- length(unique(RAPs[[Names_protein[i]]]$gene_ID)) - common_RAPs_CLIPs
  uniq_CLIP <- length(unique(CLIPs[[Names_protein[i]]]$gene_ID)) - common_RAPs_CLIPs
  plot_area <- c(plot_area, length(unique(RAPs[[Names_protein[i]]]$gene_ID)) + common_RAPs_CLIPs)
  v <- euler(c(RAP=uniq_RAP, CLIP=uniq_CLIP, "RAP&CLIP"=common_RAPs_CLIPs))
  venns[[i]] <- plot(v, fills=c(alpha(acqua_greens[i*2],0.7),alpha(greys[i],0.7), yellows[i*2]), main="")
}
grid.arrange(grobs = venns, widths=plot_area)
```



```
# right
# HNRNPA1_pval
RAPs_HNRNPA1 <- unique(RAPs[["HNRNPA1"]][,c("gene_ID", "Gene_BS")])
CLIPs_HNRNPA1 <- unique(CLIPs[["HNRNPA1"]][,c("gene_ID", "Gene_BS_CLIP")])
RAPs_CLIPs_common_HNRNPA1 <- CLIPs_HNRNPA1[grep(paste(intersect(CLIPs_HNRNPA1$gene_ID, RAPs_HNRNPA1$gene_ID), collapse = ","), CLIPs_HNRNPA1$intersection) <- rep("CLIP_common", nrow(RAPs_CLIPs_common_HNRNPA1))]
CLIPs_uniq_HNRNPA1 <- CLIPs_HNRNPA1[grep(paste(setdiff(CLIPs_HNRNPA1$gene_ID, RAPs_HNRNPA1$gene_ID), collapse = ","), CLIPs_HNRNPA1$intersection) <- rep("CLIP_uniq", nrow(CLIPs_uniq_HNRNPA1))]
CLIPS_common_uniq_HNRNPA1 <- rbind(RAPs_CLIPs_common_HNRNPA1, CLIPs_uniq_HNRNPA1)
plot_HNRNPA1 <- ggplot( data = CLIPS_common_uniq_HNRNPA1, aes( x=intersection,y=log2(Gene_BS_CLIP) ) ) +
  geom_violin(aes(fill = intersection), bw=0.85, width=0.8) +
  scale_fill_manual(values = c(yellows[2],greys[5])) +
  geom_boxplot(color="black", fill=NA, width = 0.2, outlier.shape = NA) +
  theme_pubr() +
  coord_cartesian(ylim=c(0,13))
plot_HNRNPA1
## Warning: Groups with fewer than two datapoints have been dropped.
## i Set `drop = FALSE` to consider such groups for position adjustment purposes.
```

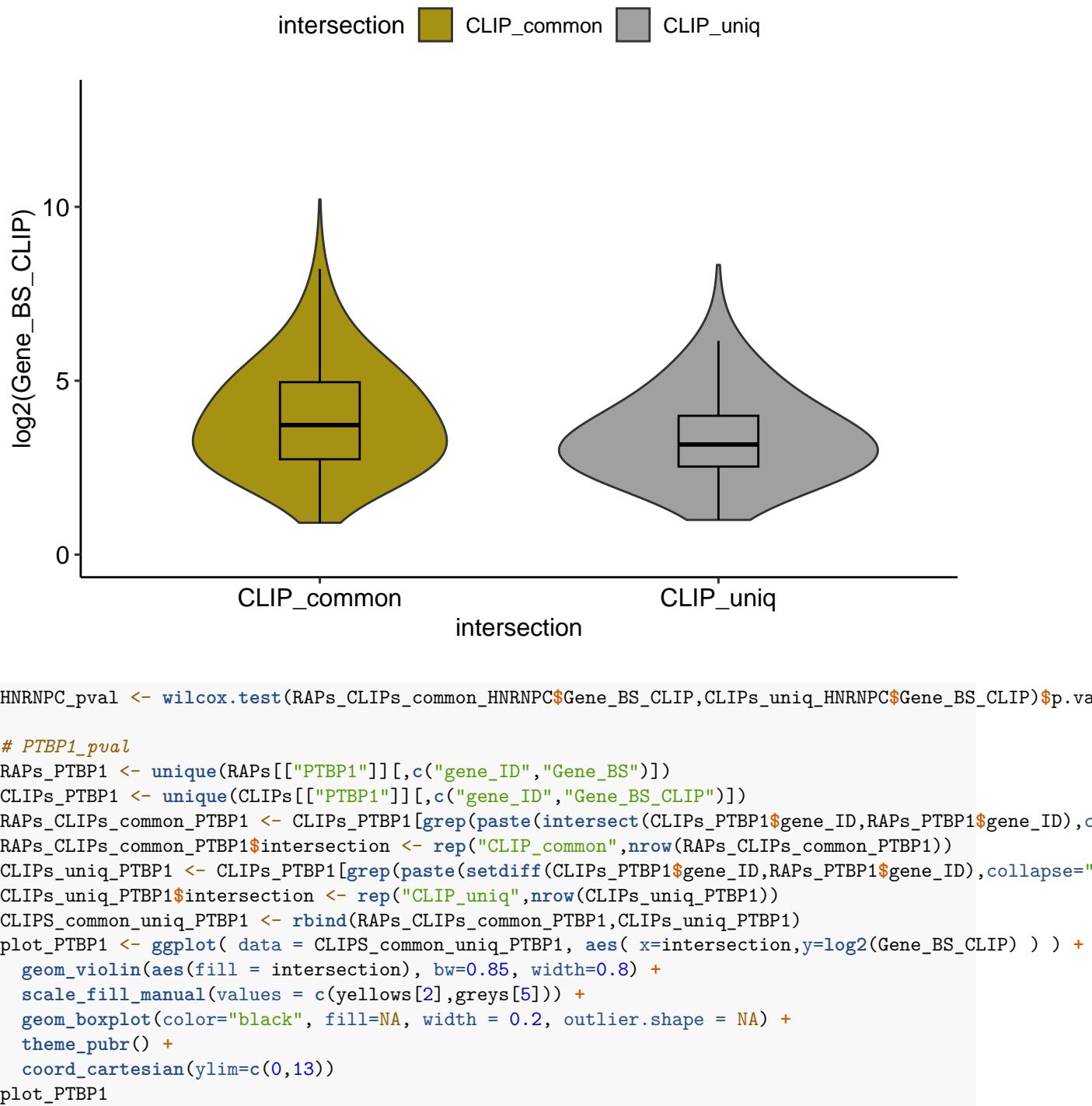


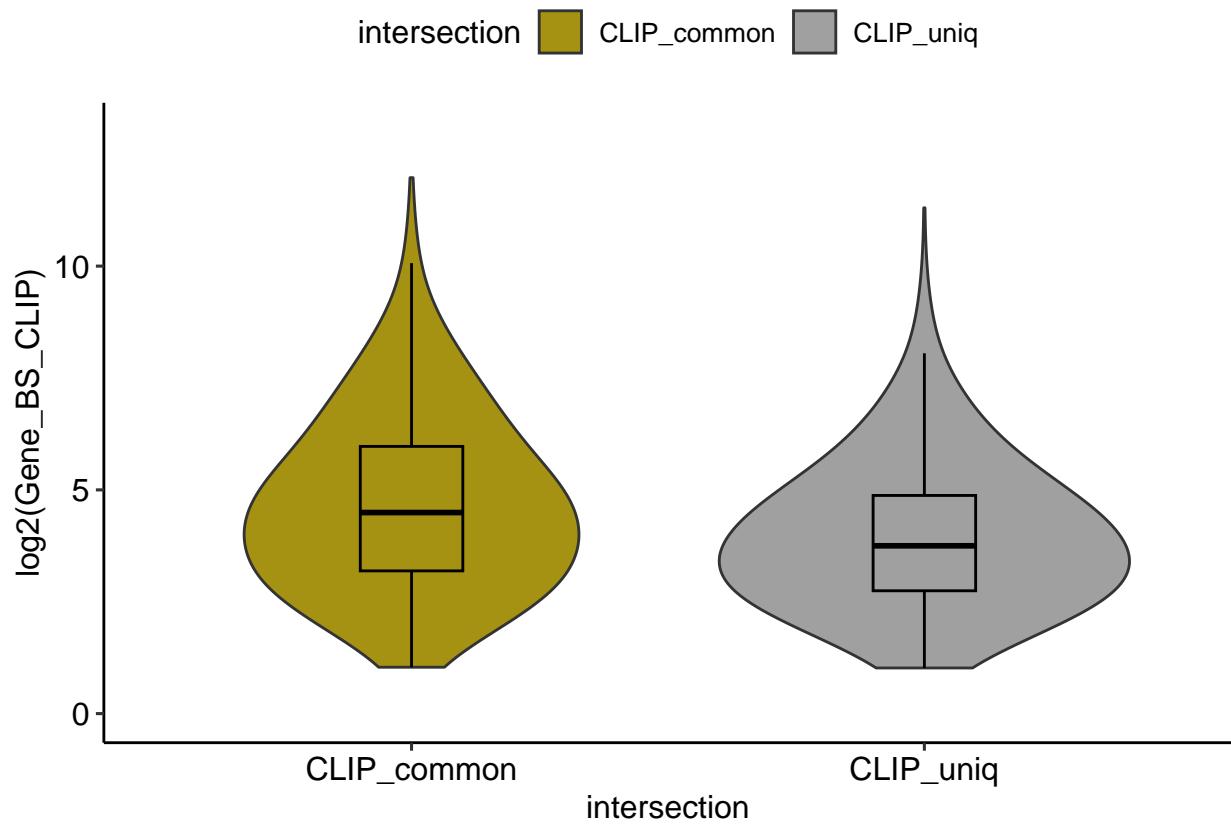
```

HNRNPA1_pval <- wilcox.test(RAPs_CLIPs_common_HNRNPA1$Gene_BS_CLIP, CLIPs_uniq_HNRNPA1$Gene_BS_CLIP)$p.value

# HNRNPC_pval
RAPs_HNRNPC <- unique(RAPs[["HNRNPC"]][,c("gene_ID", "Gene_BS")])
CLIPs_HNRNPC <- unique(CLIPs[["HNRNPC"]][,c("gene_ID", "Gene_BS_CLIP")])
RAPs_CLIPs_common_HNRNPC <- CLIPs_HNRNPC[grep(paste(intersect(CLIPs_HNRNPC$gene_ID, RAPs_HNRNPC$gene_ID), "CLIP_common"), nrow(RAPs_CLIPs_common_HNRNPC))]
CLIPs_uniq_HNRNPC <- CLIPs_HNRNPC[grep(paste(setdiff(CLIPs_HNRNPC$gene_ID, RAPs_HNRNPC$gene_ID), collapse="CLIP_uniq"), nrow(CLIPs_uniq_HNRNPC))]
CLIPs_common_uniq_HNRNPC <- rbind(RAPs_CLIPs_common_HNRNPC, CLIPs_uniq_HNRNPC)
plot_HNRNPC <- ggplot( data = CLIPs_common_uniq_HNRNPC, aes( x=intersection, y=log2(Gene_BS_CLIP) ) ) +
  geom_violin(aes(fill = intersection), bw=0.85, width=0.8) +
  scale_fill_manual(values = c(yellows[2], greys[5])) +
  geom_boxplot(color="black", fill=NA, width = 0.2, outlier.shape = NA) +
  theme_pubr() +
  coord_cartesian(ylim=c(0,13))
plot_HNRNPC

```



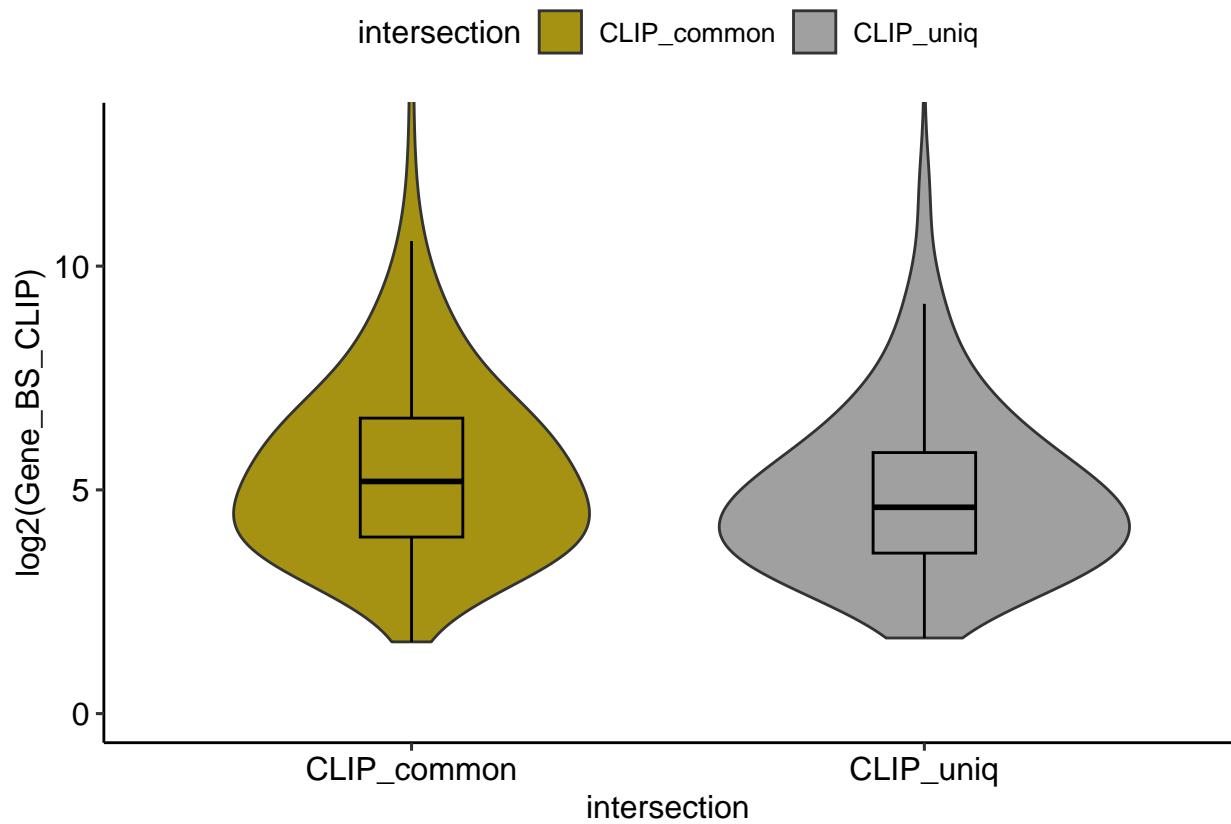


```

PTBP1_pval <- wilcox.test(RAPs_CLIPs_common_PTBP1$Gene_BS_CLIP, CLIPs_uniq_PTBP1$Gene_BS_CLIP)$p.value

# RBFOX2_pval
RAPs_RBFOX2 <- unique(RAPs[["RBFOX2"]][,c("gene_ID", "Gene_BS")])
CLIPs_RBFOX2 <- unique(CLIPs[["RBFOX2"]][,c("gene_ID", "Gene_BS_CLIP")])
RAPs_CLIPs_common_RBFOX2 <- CLIPs_RBFOX2[grep(paste(intersect(CLIPs_RBFOX2$gene_ID, RAPs_RBFOX2$gene_ID),
RAPs_CLIPs_common_RBFOX2$intersection <- rep("CLIP_common", nrow(RAPs_CLIPs_common_RBFOX2))
CLIPs_uniq_RBFOX2 <- CLIPs_RBFOX2[grep(paste(setdiff(CLIPs_RBFOX2$gene_ID, RAPs_RBFOX2$gene_ID), collapse=""),
CLIPs_uniq_RBFOX2$intersection <- rep("CLIP_uniq", nrow(CLIPs_uniq_RBFOX2))
CLIPs_common_uniq_RBFOX2 <- rbind(RAPs_CLIPs_common_RBFOX2, CLIPs_uniq_RBFOX2)
plot_RBFOX2 <- ggplot( data = CLIPs_common_uniq_RBFOX2, aes( x=intersection, y=log2(Gene_BS_CLIP) ) ) +
  geom_violin(aes(fill = intersection), bw=0.85, width=0.8) +
  scale_fill_manual(values = c(yellows[2], greys[5])) +
  geom_boxplot(color="black", fill=NA, width = 0.2, outlier.shape = NA) +
  theme_pubr() +
  coord_cartesian(ylim=c(0,13))
plot_RBFOX2

```

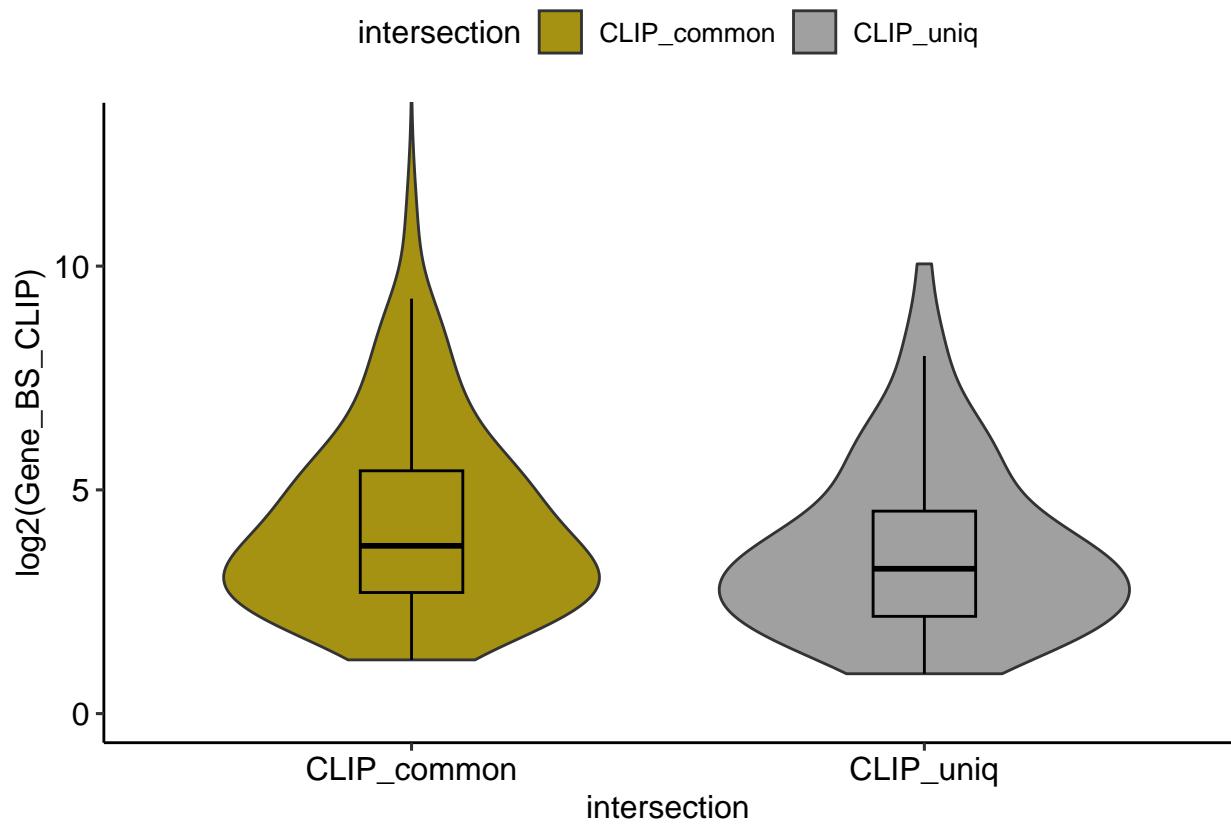


```

RBFOX2_pval <- wilcox.test(RAPs_CLIPs_common_RBFOX2$Gene_BS_CLIP, CLIPs_uniq_RBFOX2$Gene_BS_CLIP)$p.value

# YBX3_pval
RAPs_YBX3 <- unique(RAPs[["YBX3"]][,c("gene_ID", "Gene_BS")])
CLIPs_YBX3 <- unique(CLIPs[["YBX3"]][,c("gene_ID", "Gene_BS_CLIP")])
RAPs_CLIPs_common_YBX3 <- CLIPs_YBX3[grep(paste(intersect(CLIPs_YBX3$gene_ID, RAPs_YBX3$gene_ID), collapse="|"), CLIPs_YBX3$intersection) <- rep("CLIP_common", nrow(RAPs_CLIPs_common_YBX3))
CLIPs_uniq_YBX3 <- CLIPs_YBX3[grep(paste(setdiff(CLIPs_YBX3$gene_ID, RAPs_YBX3$gene_ID), collapse="|"), CLIPs_YBX3$intersection) <- rep("CLIP_uniq", nrow(CLIPs_uniq_YBX3))
CLIPs_common_uniq_YBX3 <- rbind(RAPs_CLIPs_common_YBX3, CLIPs_uniq_YBX3)
plot_YBX3 <- ggplot( data = CLIPs_common_uniq_YBX3, aes( x=intersection, y=log2(Gene_BS_CLIP) ) ) +
  geom_violin(aes(fill = intersection), bw=0.85, width=0.8) +
  scale_fill_manual(values = c(yellows[2], greys[5])) +
  geom_boxplot(color="black", fill=NA, width = 0.2, outlier.shape = NA) +
  theme_pubr() +
  coord_cartesian(ylim=c(0,13))
plot_YBX3

```



```

YBX3_pval <- wilcox.test(RAPs_CLIPs_common_YBX3$Gene_BS_CLIP,CLIPs_uniq_YBX3$Gene_BS_CLIP)$p.value

# all
print("Wilcoxon rank sum test p values")
## [1] "Wilcoxon rank sum test p values"
print(paste("HNRNPA1",HNRNPA1_pval, sep=" "))
## [1] "HNRNPA1 0.585365853658537"
print(paste("HNRNPC",HNRNPC_pval,sep=" "))
## [1] "HNRNPC 5.79007340906723e-11"
print(paste("PTBP1",PTBP1_pval,sep=" "))
## [1] "PTBP1 1.1011492584217e-25"
print(paste("RBFOX2",RBFOX2_pval,sep=" "))
## [1] "RBFOX2 1.3676840269862e-12"
print(paste("YBX3",YBX3_pval,sep=" "))
## [1] "YBX3 0.00451598791860509"

p_figure_violin_2G <- ggarrange(plot_HNRNPA1,plot_HNRNPC,plot_PTBP1,plot_RBFOX2,plot_YBX3,nrow=5, common_y_labels = TRUE)
## Warning: Groups with fewer than two datapoints have been dropped.
## i Set `drop = FALSE` to consider such groups for position adjustment purposes.
## Groups with fewer than two datapoints have been dropped.
## i Set `drop = FALSE` to consider such groups for position adjustment purposes.
ggsave(filename = "./Figure/Figure2G_Violin.pdf", plot = p_figure_violin_2G, width = 4.7, height = 12.6)

CLIPS_common_uniq_HNRNPA1 <- CLIPS_common_uniq_HNRNPA1[,c("gene_ID", "intersection", "Gene_BS_CLIP")]
CLIPS_common_uniq_HNRNPC <- CLIPS_common_uniq_HNRNPC[,c("gene_ID", "intersection", "Gene_BS_CLIP")]
CLIPS_common_uniq_PTBP1 <- CLIPS_common_uniq_PTBP1[,c("gene_ID", "intersection", "Gene_BS_CLIP")]

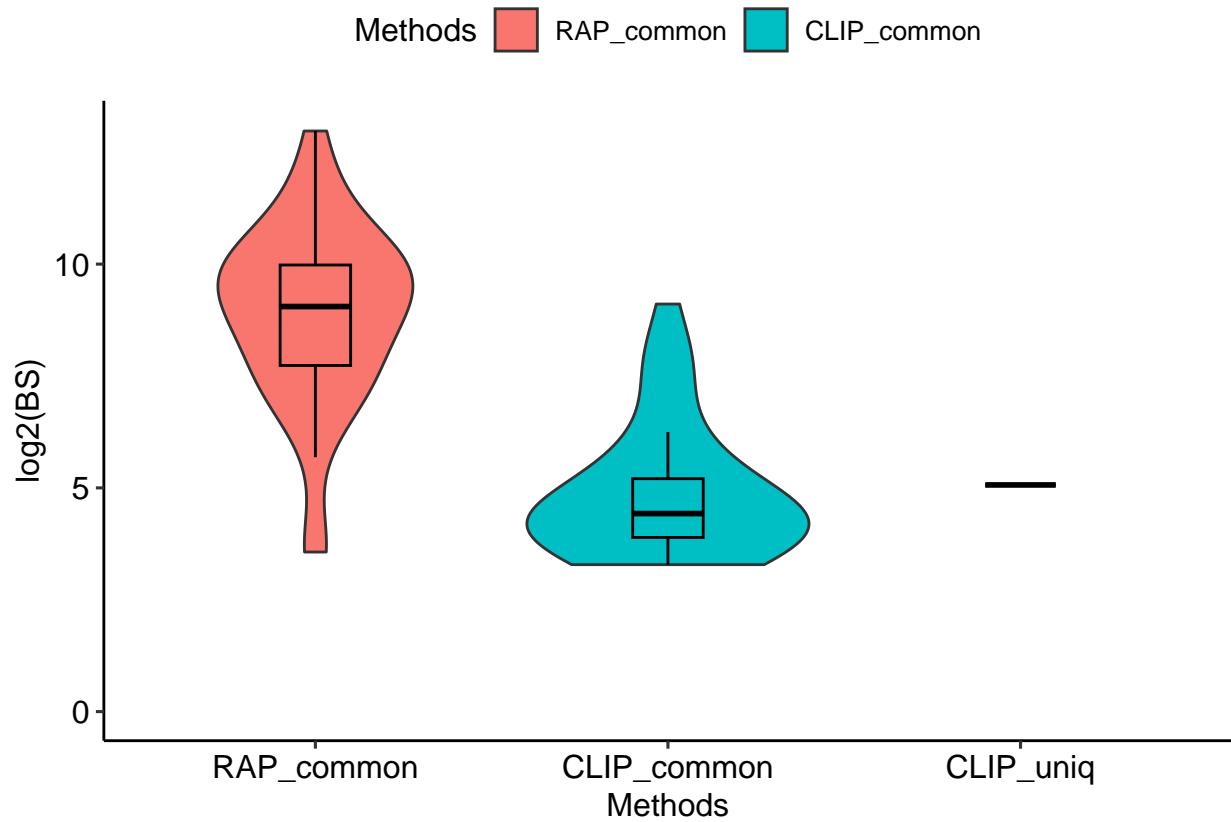
```

```

CLIPS_common_uniq_RBFOX2 <- CLIPS_common_uniq_RBFOX2[,c("gene_ID", "intersection", "Gene_BS_CLIP")]
CLIPS_common_uniq_YBX3 <- CLIPS_common_uniq_YBX3[,c("gene_ID", "intersection", "Gene_BS_CLIP")]

# HNRNPA1
HNRNPA1_CLIPS_RAPS_Qun <- merge(RAPs_HNRNPA1, CLIPs_HNRNPA1, by = "gene_ID")
colnames(HNRNPA1_CLIPS_RAPS_Qun) <- c("gene_ID", "RAP_common", "Gene_BS_CLIP")
HNRNPA1_CLIPS_RAPS_Qun_long <- pivot_longer(
  HNRNPA1_CLIPS_RAPS_Qun,
  cols = -gene_ID,
  names_to = "Methods",
  values_to = "BS"
)
#CLIPS_common_uniq_HNRNPA1 <- CLIPS_common_uniq_HNRNPA1[,c("gene_ID", "intersection", "Gene_BS_CLIP")]
colnames(CLIPS_common_uniq_HNRNPA1) <- c("gene_ID", "Methods", "BS")
data_HNRNPA1_comparision_Qun <- rbind(HNRNPA1_CLIPS_RAPS_Qun_long, CLIPS_common_uniq_HNRNPA1)
data_HNRNPA1_comparision_Qun <- data_HNRNPA1_comparision_Qun[data_HNRNPA1_comparision_Qun$Methods != "G"]
data_HNRNPA1_comparision_Qun$Methods <- factor(data_HNRNPA1_comparision_Qun$Methods, levels = c("RAP_common", "BS"))
plot_HNRNPA1 <- ggplot( data = data_HNRNPA1_comparision_Qun, aes( x=Methods,y=log2(BS) ) ) +
  geom_violin(aes(fill = Methods), bw=0.85, width=0.8) +
  #scale_fill_manual(values = c(yellows[2],greys[5])) +
  geom_boxplot(color="black", fill=NA, width = 0.2, outlier.shape = NA) +
  theme_pubr() +
  coord_cartesian(ylim=c(0,13))
plot_HNRNPA1
## Warning: Groups with fewer than two datapoints have been dropped.
## i Set `drop = FALSE` to consider such groups for position adjustment purposes.

```

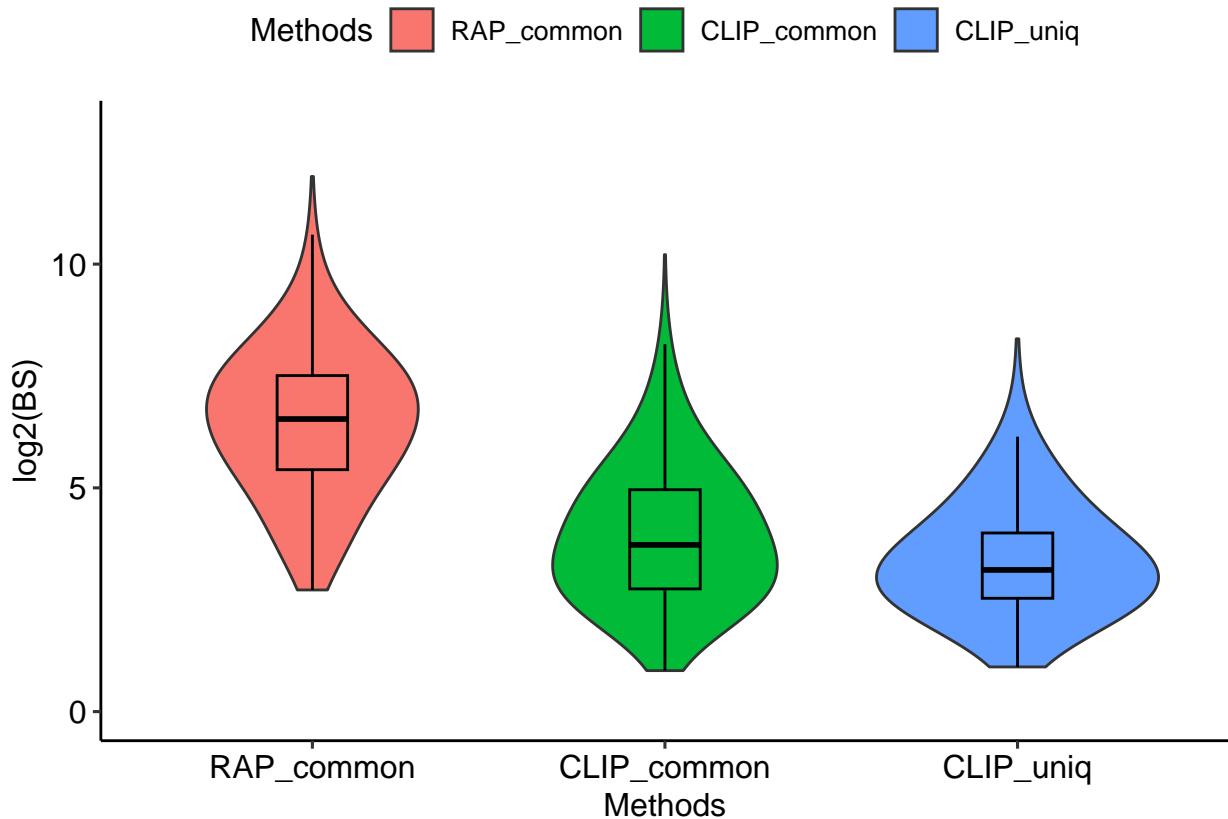


```

HNRNPA1_pval <- wilcox.test(HNRNPA1_CLIPS_RAPS_Qun$RAP_common,HNRNPA1_CLIPS_RAPS_Qun$Gene_BS_CLIP)$p.value

# HNRNPC
HNRNPC_CLIPS_RAPS_Qun <- merge(RAPs_HNRNPC, CLIPs_HNRNPC, by = "gene_ID")
colnames(HNRNPC_CLIPS_RAPS_Qun) <- c("gene_ID", "RAP_common", "Gene_BS_CLIP")
HNRNPC_CLIPS_RAPS_Qun_long <- pivot_longer(
  HNRNPC_CLIPS_RAPS_Qun,
  cols = -gene_ID,
  names_to = "Methods",
  values_to = "BS"
)
#CLIPS_common_uniq_HNRNPC <- CLIPS_common_uniq_HNRNPC[,c("gene_ID", "intersection", "Gene_BS_CLIP")]
colnames(CLIPS_common_uniq_HNRNPC) <- c("gene_ID", "Methods", "BS")
data_HNRNPC_comparision_Qun <- rbind(HNRNPC_CLIPS_RAPS_Qun_long, CLIPS_common_uniq_HNRNPC)
data_HNRNPC_comparision_Qun <- data_HNRNPC_comparision_Qun[data_HNRNPC_comparision_Qun$Methods != "Gene_BS_CLIP"]
data_HNRNPC_comparision_Qun$Methods <- factor(data_HNRNPC_comparision_Qun$Methods, levels = c("RAP_common", "CLIP_common"))
plot_HNRNPC <- ggplot( data = data_HNRNPC_comparision_Qun, aes( x=Methods,y=log2(BS) ) ) +
  geom_violin(aes(fill = Methods), bw=0.85, width=0.8) +
  #scale_fill_manual(values = c(yellows[2],greys[5])) +
  geom_boxplot(color="black", fill=NA, width = 0.2, outlier.shape = NA) +
  theme_pubr() +
  coord_cartesian(ylim=c(0,13))
plot_HNRNPC

```

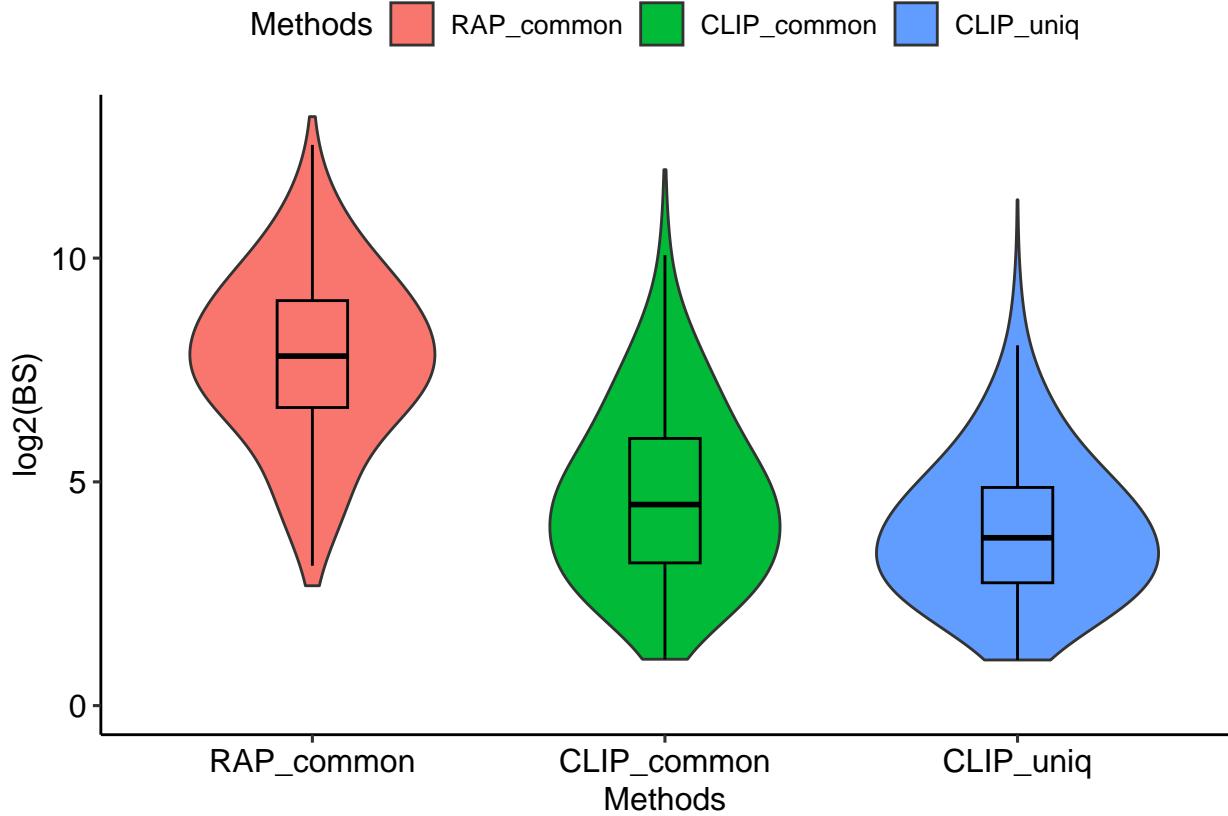


```

HNRNPC_pval <- wilcox.test(HNRNPC_CLIPS_RAPS_Qun$RAP_common,HNRNPC_CLIPS_RAPS_Qun$Gene_BS_CLIP)$p.value

# PTBP1
PTBP1_CLIPS_RAPS_Qun <- merge(RAPs_PTBP1, CLIPs_PTBP1, by = "gene_ID")
colnames(PTBP1_CLIPS_RAPS_Qun) <- c("gene_ID", "RAP_common", "Gene_BS_CLIP")
PTBP1_CLIPS_RAPS_Qun_long <- pivot_longer(
  PTBP1_CLIPS_RAPS_Qun,
  cols = -gene_ID,
  names_to = "Methods",
  values_to = "BS"
)
#CLIPS_common_uniq_PTBP1 <- CLIPS_common_uniq_PTBP1[,c("gene_ID", "intersection", "Gene_BS_CLIP")]
colnames(CLIPS_common_uniq_PTBP1) <- c("gene_ID", "Methods", "BS")
data_PTBP1_comparision_Qun <- rbind(PTBP1_CLIPS_RAPS_Qun_long, CLIPS_common_uniq_PTBP1)
data_PTBP1_comparision_Qun <- data_PTBP1_comparision_Qun[data_PTBP1_comparision_Qun$Methods != "Gene_BS"]
data_PTBP1_comparision_Qun$Methods <- factor(data_PTBP1_comparision_Qun$Methods, levels = c("RAP_common", "CLIP_common", "CLIP_uniq"))
plot_PTBP1 <- ggplot( data = data_PTBP1_comparision_Qun, aes( x=Methods,y=log2(BS) ) ) +
  geom_violin(aes(fill = Methods), bw=0.85, width=0.8) +
  #scale_fill_manual(values = c(yellows[2],greys[5])) +
  geom_boxplot(color="black", fill=NA, width = 0.2, outlier.shape = NA) +
  theme_pubr() +
  coord_cartesian(ylim=c(0,13))
plot_PTBP1

```

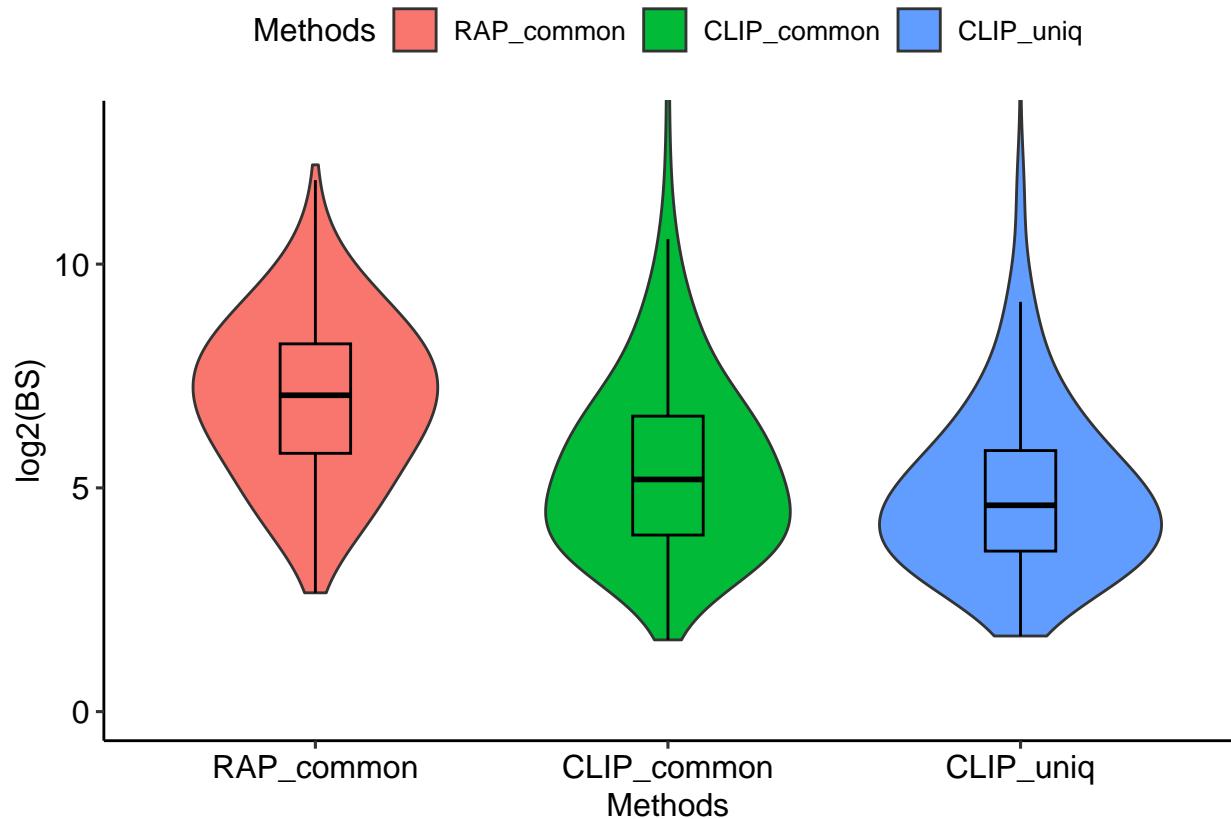


```

PTBP1_pval <- wilcox.test(PTBP1_CLIPS_RAPS_Qun$RAP_common, PTBP1_CLIPS_RAPS_Qun$Gene_BS_CLIP)$p.value

# RBFOX2
RBFOX2_CLIPS_RAPS_Qun <- merge(RAPs_RBFOX2, CLIPs_RBFOX2, by = "gene_ID")
colnames(RBFOX2_CLIPS_RAPS_Qun) <- c("gene_ID", "RAP_common", "Gene_BS_CLIP")
RBFOX2_CLIPS_RAPS_Qun_long <- pivot_longer(
  RBFOX2_CLIPS_RAPS_Qun,
  cols = -gene_ID,
  names_to = "Methods",
  values_to = "BS"
)
#CLIPS_common_uniq_RBFOX2 <- CLIPS_common_uniq_RBFOX2[,c("gene_ID", "intersection", "Gene_BS_CLIP")]
colnames(CLIPS_common_uniq_RBFOX2) <- c("gene_ID", "Methods", "BS")
data_RBFOX2_comparision_Qun <- rbind(RBFOX2_CLIPS_RAPS_Qun_long, CLIPS_common_uniq_RBFOX2)
data_RBFOX2_comparision_Qun <- data_RBFOX2_comparision_Qun[data_RBFOX2_comparision_Qun$Methods != "Gene"]
data_RBFOX2_comparision_Qun$Methods <- factor(data_RBFOX2_comparision_Qun$Methods, levels = c("RAP_common", "CLIP_common", "CLIP_uniq"))
plot_RBFOX2 <- ggplot( data = data_RBFOX2_comparision_Qun, aes( x=Methods,y=log2(BS) ) ) +
  geom_violin(aes(fill = Methods), bw=0.85, width=0.8) +
  #scale_fill_manual(values = c(yellows[2], greys[5])) +
  geom_boxplot(color="black", fill=NA, width = 0.2, outlier.shape = NA) +
  theme_pubr() +
  coord_cartesian(ylim=c(0,13))
plot_RBFOX2

```

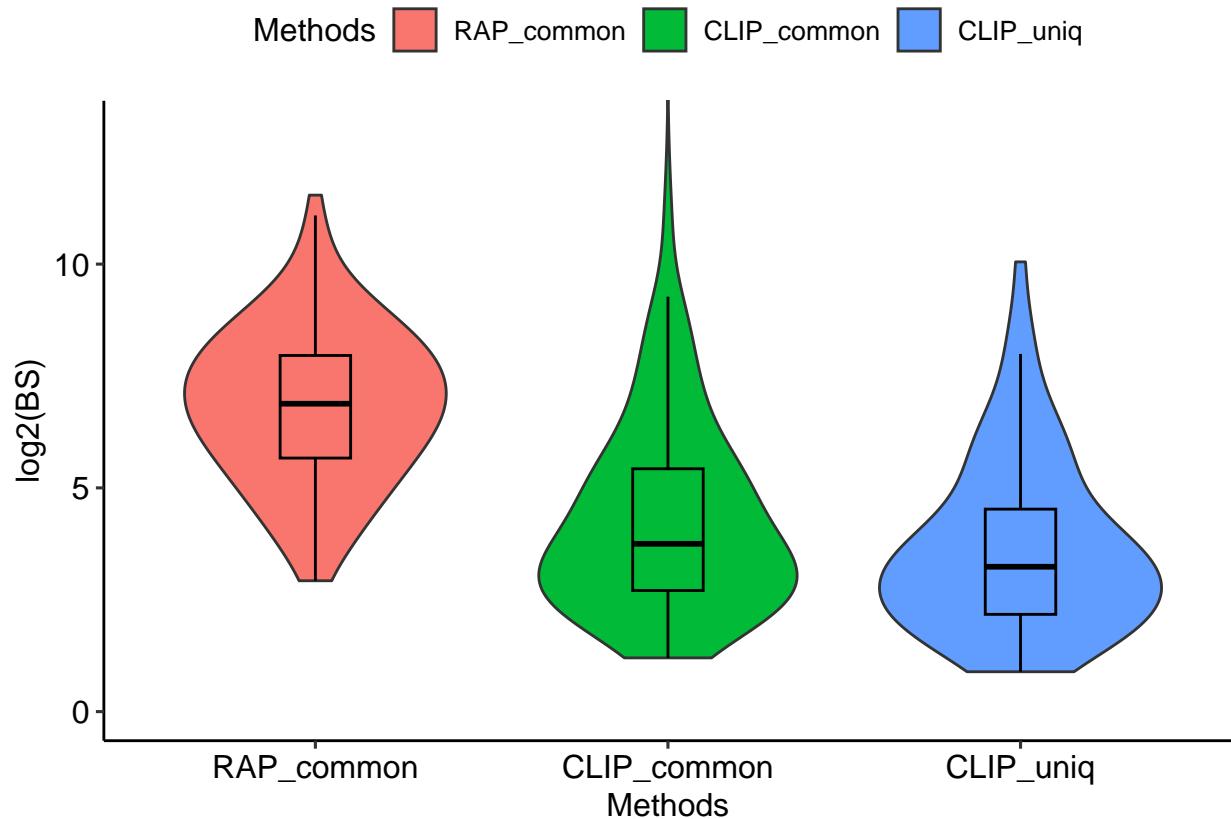


```

RBFOX2_pval <- wilcox.test(RBFOX2_CLIPS_RAPS_Qun$RAP_common,RBFOX2_CLIPS_RAPS_Qun$Gene_BS_CLIP)$p.value

# YBX3
YBX3_CLIPS_RAPS_Qun <- merge(RAPs_YBX3, CLIPs_YBX3, by = "gene_ID")
colnames(YBX3_CLIPS_RAPS_Qun) <- c("gene_ID", "RAP_common", "Gene_BS_CLIP")
YBX3_CLIPS_RAPS_Qun_long <- pivot_longer(
  YBX3_CLIPS_RAPS_Qun,
  cols = -gene_ID,
  names_to = "Methods",
  values_to = "BS"
)
#CLIPS_common_uniq_YBX3 <- CLIPS_common_uniq_YBX3[,c("gene_ID", "intersection", "Gene_BS_CLIP")]
colnames(CLIPS_common_uniq_YBX3) <- c("gene_ID", "Methods", "BS")
data_YBX3_comparision_Qun <- rbind(YBX3_CLIPS_RAPS_Qun_long, CLIPS_common_uniq_YBX3)
data_YBX3_comparision_Qun <- data_YBX3_comparision_Qun[data_YBX3_comparision_Qun$Methods != "Gene_BS_CLIP"]
data_YBX3_comparision_Qun$Methods <- factor(data_YBX3_comparision_Qun$Methods, levels = c("RAP_common",
plot_YBX3 <- ggplot( data = data_YBX3_comparision_Qun, aes( x=Methods,y=log2(BS) ) ) +
  geom_violin(aes(fill = Methods), bw=0.85, width=0.8) +
  #scale_fill_manual(values = c(yellows[2],greys[5])) +
  geom_boxplot(color="black", fill=NA, width = 0.2, outlier.shape = NA) +
  theme_pubr() +
  coord_cartesian(ylim=c(0,13))
plot_YBX3

```



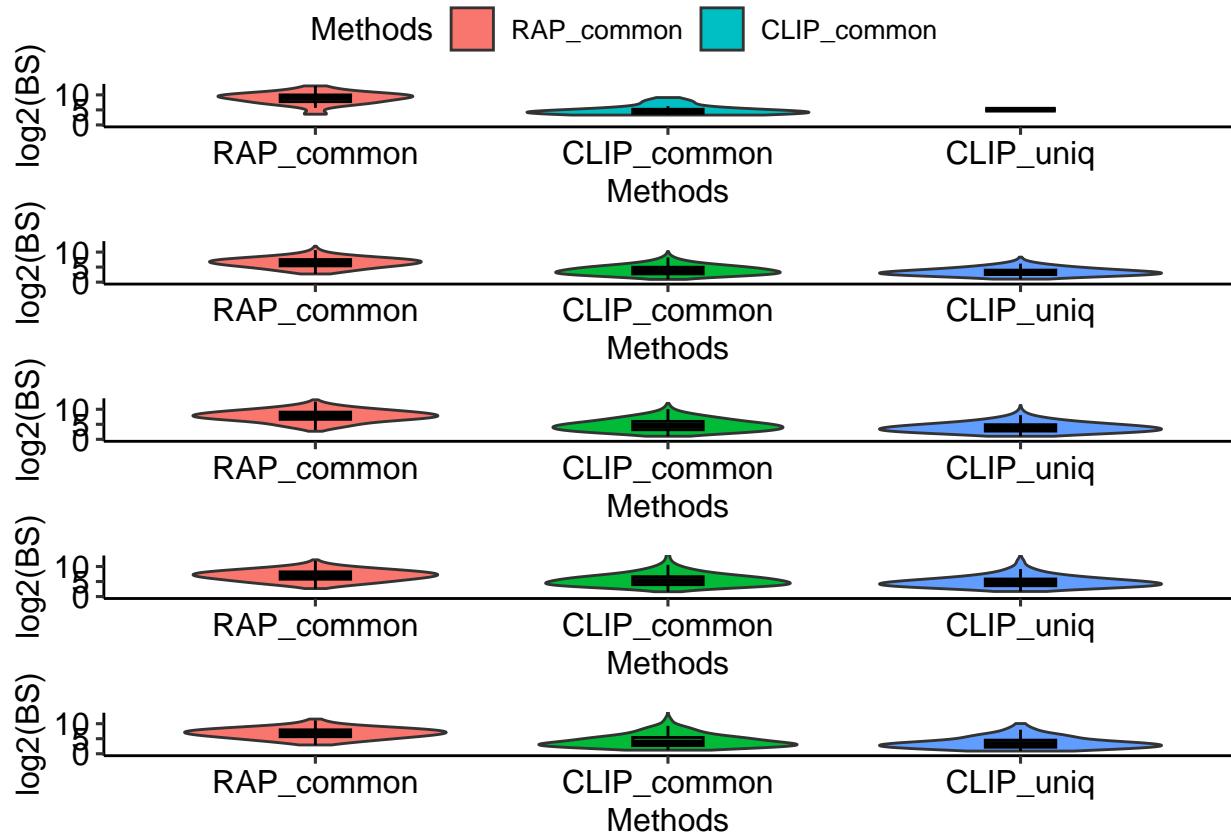
```

YBX3_pval <- wilcox.test(YBX3_CLIPS_RAPS_Qun$RAP_common,YBX3_CLIPS_RAPS_Qun$Gene_BS_CLIP)$p.value

print("Wilcoxon rank sum test p values")
## [1] "Wilcoxon rank sum test p values"
print(paste("HNRNPA1",HNRNPA1_pval, sep=" "))
## [1] "HNRNPA1 4.04267121005842e-13"
print(paste("HNRNPC",HNRNPC_pval,sep=" "))
## [1] "HNRNPC 4.22620979968423e-171"
print(paste("PTBP1",PTBP1_pval,sep=" "))
## [1] "PTBP1 6.42758024429207e-294"
print(paste("RBFOX2",RBFOX2_pval,sep=" "))
## [1] "RBFOX2 1.79421281391685e-98"
print(paste("YBX3",YBX3_pval,sep=" "))
## [1] "YBX3 5.32399130122868e-58"

p_figure_violin_2G <- ggarrange(plot_HNRNPA1,plot_HNRNPC,plot_PTBP1,plot_RBFOX2,plot_YBX3,nrow=5, common
## Warning: Groups with fewer than two datapoints have been dropped.
## i Set `drop = FALSE` to consider such groups for position adjustment purposes.
## Groups with fewer than two datapoints have been dropped.
## i Set `drop = FALSE` to consider such groups for position adjustment purposes.
p_figure_violin_2G

```



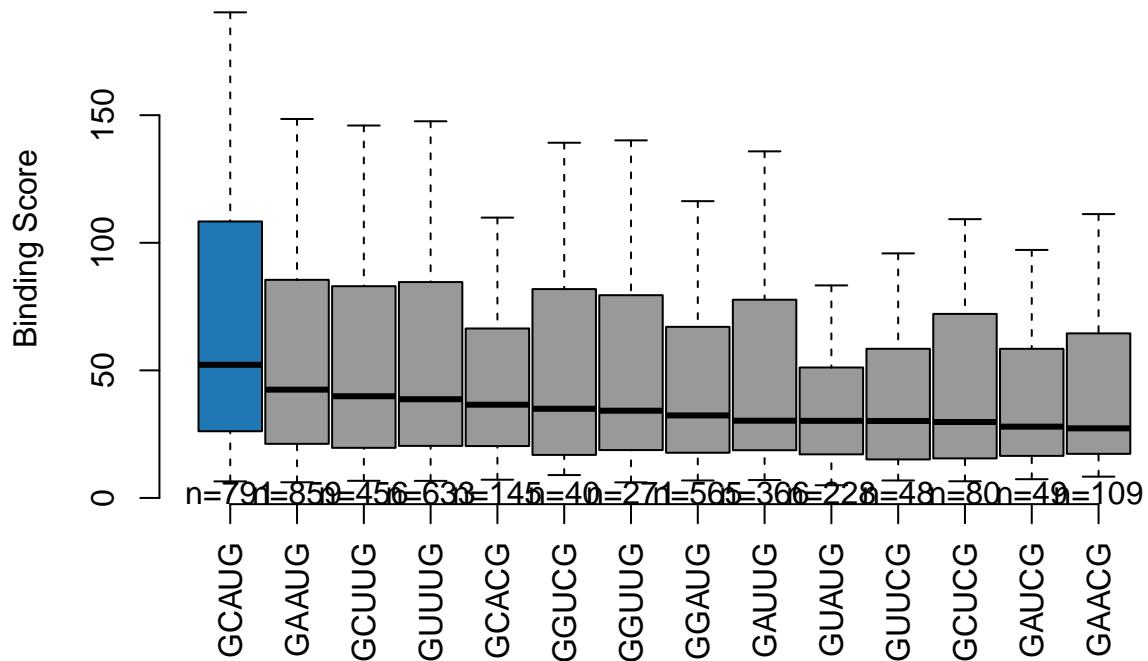
```
ggsave(filename = "./Figure/Figure2G_Violin_Qun.pdf", plot = p_figure_violin_2G, width = 4.7, height = 1)
```

### 0.5.7 Figure S2B

```
GNWYG <- c("GCATG", "GAATG", "GGATG", "GTTTG", "GCTTG", "GATTG", "GGTTG", "GAACG", "GCACG", "GTATG", "GATCG", "GCT")
GNWYG_scores <- list()
medians <- c()
for (i in 1:length(GNWYG)){
  RAPs_GNWYG <- RAPs[["RBFOX2"]][grep(GNWYG[i], str_sub(RAPs[["RBFOX2"]]$positive_fa, 50, 150)),]
  GNWYG_subset <- GNWYG[grep(GNWYG[i], GNWYG, invert=T)]
  GNWYG_scores[[i]] <- RAPs_GNWYG[grep(paste(GNWYG_subset, collapse = "|"), str_sub(RAPs_GNWYG$positive_fa, 50, 150))]]
  medians <- c(medians, median(GNWYG_scores[[i]])))
}

GNWYG <- gsub("T", "U", GNWYG)
names(GNWYG_scores) <- GNWYG

#pdf("./Figure/Figure2/FigureS2B.pdf", width = 11, height = 7)
par(bty="n")
boxplot2(GNWYG_scores[order(-medians)], outline=F, horizontal=F, las=3, ylab="Binding Score", range=1, cex.lab=1.5, cex.axis=1.5, cex=1.5)
```



```
#dev.off()

GCAUG <- GNWYG_scores$GCAUG
GNWYG_scores <- GNWYG_scores[-1]

for (i in names(GNWYG_scores)){
  anno_text_0 <- paste("GCAUG vs", i)
  test_compare <- wilcox.test(GCAUG, GNWYG_scores[[i]])$p.value
  anno_text <- paste("P-value =", test_compare)
  anno_text <- paste(anno_text_0, anno_text, sep=" ")
  print(anno_text)
}
## [1] "GCAUG vs GAAUG P-value = 0.000187642717483513"
## [1] "GCAUG vs GGAUG P-value = 1.75027346808954e-14"
## [1] "GCAUG vs GCUUG P-value = 1.34540298133905e-05"
## [1] "GCAUG vs GUUUG P-value = 4.53503535262265e-05"
## [1] "GCAUG vs GACCG P-value = 3.61588355304033e-09"
## [1] "GCAUG vs GGUUG P-value = 4.84423104579228e-06"
## [1] "GCAUG vs GAAACG P-value = 2.25248427316068e-06"
## [1] "GCAUG vs GCACG P-value = 0.000236877780284992"
## [1] "GCAUG vs GUAUG P-value = 3.30289723362946e-13"
## [1] "GCAUG vs GAUCG P-value = 0.00021981847552523"
## [1] "GCAUG vs GCUCG P-value = 4.55398275989635e-05"
## [1] "GCAUG vs GUUCG P-value = 0.000457210969649777"
## [1] "GCAUG vs GGUCG P-value = 0.0813437382604083"
```

### 0.5.8 Figure S2C

```

GNWYG <- c("GCATG", "GAATG", "GGATG", "GTTTG", "GCTTG", "GATTG", "GGTTG", "GAACG", "GCACG", "GTATG", "GATCG", "GCT
GNWYG <- GNWYG[order(-medians)]

#pdf("./Figure/Figure2/FigureS2C.pdf", width = 10, height = 8)
par(mfrow=c(3,5), mar=c(2,3.5,2,0.5))

for (i in GNWYG){
  ymax <- round(nrow(RAPs[["RBFOX2"]][grep(i,str_sub(RAPs[["RBFOX2"]]$positive_fa,50,150)),]) / 2)

  hist(unlist(str_locate_all(RAPs[["RBFOX2"]]$positive_fa,i))-100, breaks=40, main=gsub("T","U",i), yla
axis(side = 1, at = c(-100,-50,0,50,100), labels = c("-100",NA,"0",NA,"100"))
x <- nrow(RAPs[["RBFOX2"]][grep(i,str_sub(RAPs[["RBFOX2"]]$positive_fa,80,120)),])
n <- nrow(RAPs[["RBFOX2"]][grep(i,RAPs[["RBFOX2"]]$positive_fa),])

  hist(unlist(str_locate_all(RAPs[["HNRNPA1"]]$positive_fa,i))-100, breaks=40, type="l",col=alpha("#ff7f
n_neg <- nrow(RAPs[["HNRNPA1"]][grep(i,RAPs[["HNRNPA1"]]$positive_fa),])
x_neg <- nrow(RAPs[["HNRNPA1"]][grep(i,str_sub(RAPs[["HNRNPA1"]]$positive_fa,80,120)),])
p <- x_neg / n_neg
bt <- binom.test(x,n,p, alternative = "greater")
btp <- bt$p.value
if ( btp <= 0.001 ) {
  to_add <- "***"
} else if ( btp <= 0.01 ) {
  to_add <- "**"
} else if ( btp <= 0.05 ) {
  to_add <- "*"
} else {
  to_add <- "n.s."
}
text(50,ymax/10*9,labels = to_add, cex=2.5, col = yellows[1])

  hist(unlist(str_locate_all(RAPs[["HNRNPC"]]$positive_fa,i))-100, breaks=40, type="l",col=alpha("#6a3d
n_neg <- nrow(RAPs[["HNRNPC"]][grep(i,RAPs[["HNRNPC"]]$positive_fa),])
x_neg <- nrow(RAPs[["HNRNPC"]][grep(i,str_sub(RAPs[["HNRNPC"]]$positive_fa,80,120)),])
p <- x_neg / n_neg
bt <- binom.test(x,n,p, alternative = "greater")
btp <- bt$p.value
if ( btp <= 0.001 ) {
  to_add <- "***"
} else if ( btp <= 0.01 ) {
  to_add <- "**"
} else if ( btp <= 0.05 ) {
  to_add <- "*"
} else {
  to_add <- "n.s."
}
text(50,ymax/10*7.75,labels = to_add, cex=2.5, col = yellows[2])

```

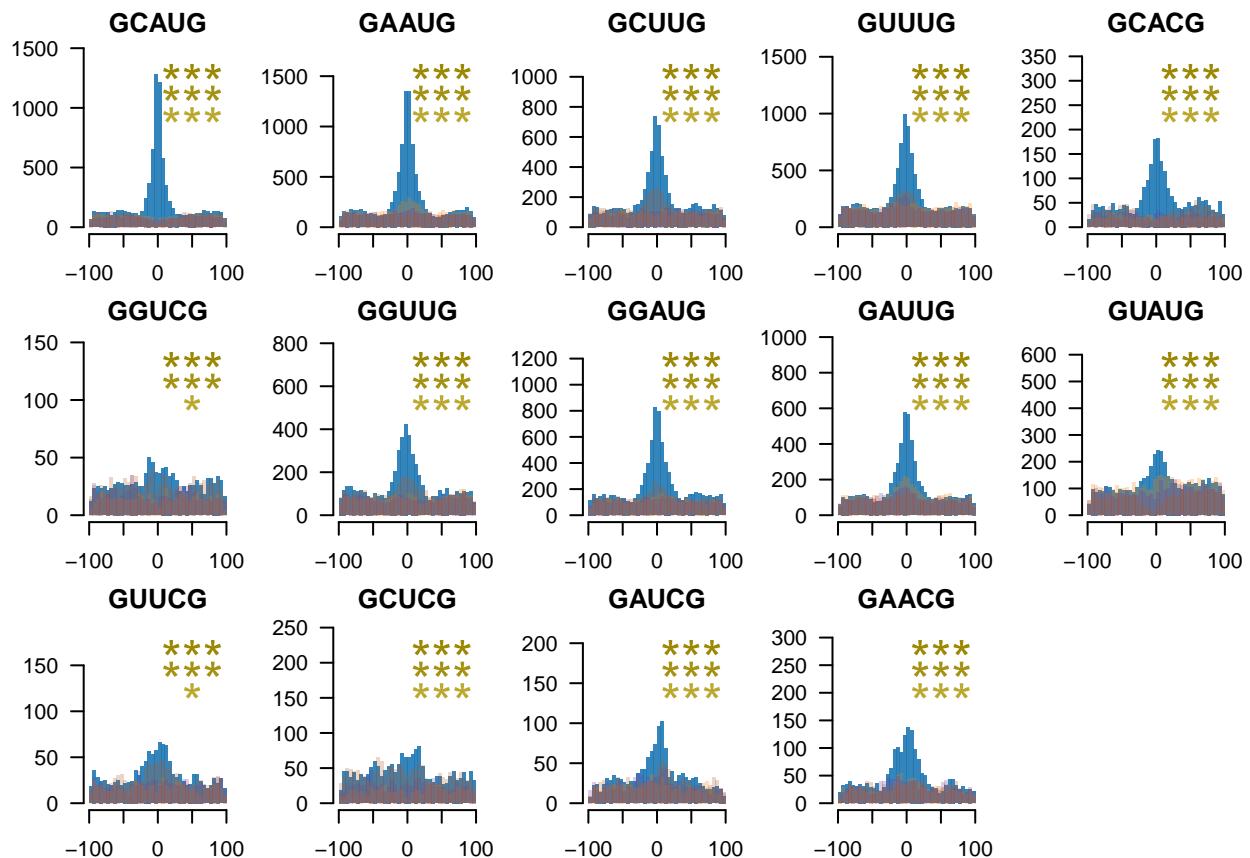
```

hist(unlist(str_locate_all(RAPs[["PTBP1"]]$positive_fa,i))-100, breaks=40, type="l", col=alpha("#b1592d"))
n_neg <- nrow(RAPs[["PTBP1"]][grep(i,RAPs[["PTBP1"]]$positive_fa),])
x_neg <- nrow(RAPs[["PTBP1"]][grep(i,str_sub(RAPs[["PTBP1"]]$positive_fa,80,120)),])
p <- x_neg / n_neg
#bt <- binom.test(x,n,p, alternative = "greater")
bt <- binom.test(x,n,p)
btp <- bt$p.value
if ( btp <= 0.001 ) {
  to_add <- "***"
} else if ( btp <= 0.01 ) {
  to_add <- "**"
} else if ( btp <= 0.05 ) {
  to_add <- "*"
} else {
  to_add <- "n.s."
}
text(50,ymax/10*6.5,labels = to_add, cex=2.5, col = yellows[4])

}

#dev.off()

```



### 0.5.9 Figure S2D

```

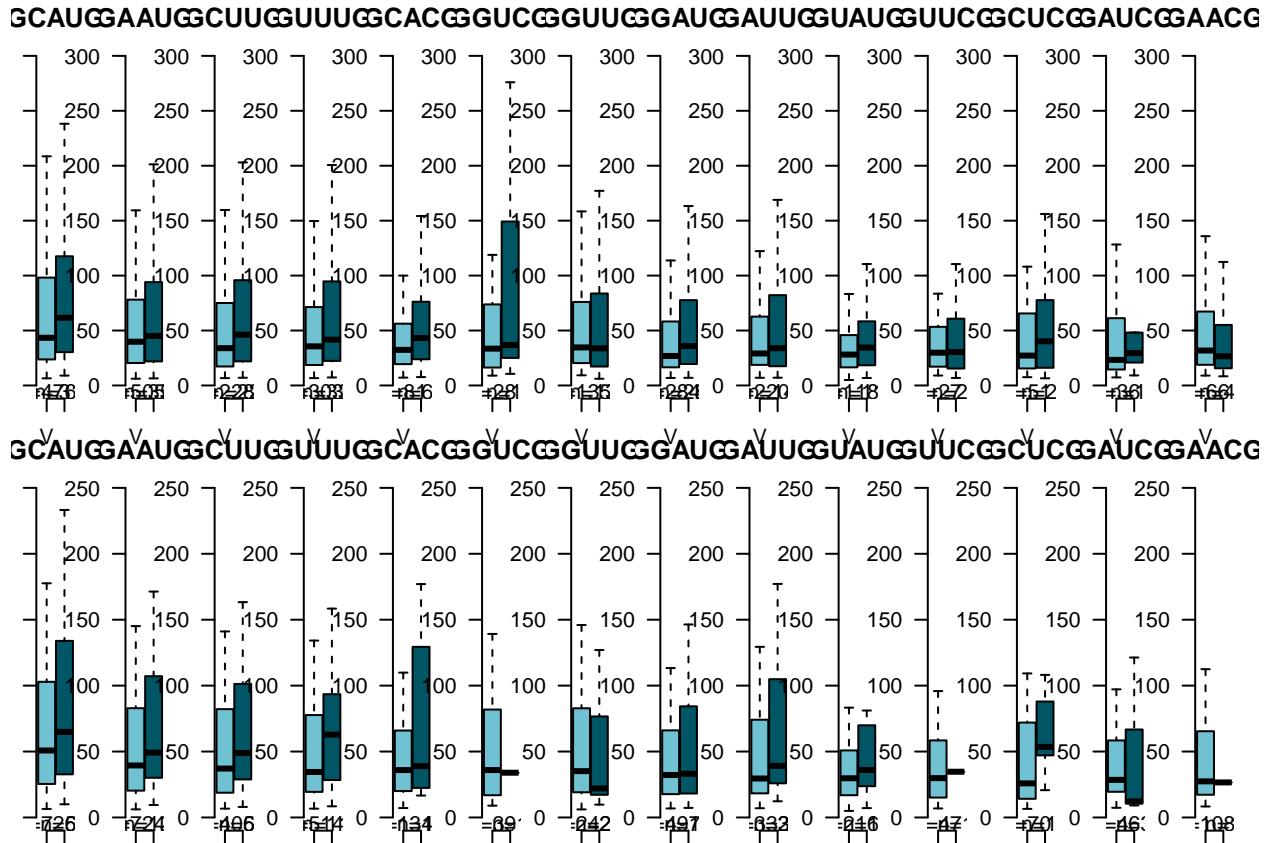
GNWYG <- c("GCATG", "GAATG", "GGATG", "GTTTG", "GCTTG", "GATTG", "GGTTG", "GAACG", "GCACG", "GTATG", "GATCG", "GCT
GNWYG <- GNWYG[order(-medians)]

#pdf("./Figure/Figure2/FigureS2D.pdf", width = 18, height = 5.3)
par(mar=c(1,1,2,1),mfrow=c(2,14),bty="n")

for (i in GNWYG){
  RAPs_GNWYG <- RAPs[["RBFOX2"]][grep(i,str_sub(RAPs[["RBFOX2"]]$positive_fa,50,150)),]
  GNWYG_subset <- GNWYG[grep(i,GNWYG,invert=T)]
  RAPs_GNWYG <- RAPs_GNWYG[grep(paste(GNWYG_subset,collapse = "|"),str_sub(RAPs_GNWYG$positive_fa,50,150))]
  aaa <- paste("T",i,sep="")
  TGNWYG <- RAPs_GNWYG[grep(aaa,str_sub(RAPs_GNWYG$positive_fa,50,150)),]$BS
  VGNWYG <- RAPs_GNWYG[grep(aaa,str_sub(RAPs_GNWYG$positive_fa,50,150), invert = T),]$BS
  NGNWYG <- list(VGNWYG, TGNWYG)
  names(NGNWYG) <- c("V","U")
  boxplot2(NGNWYG, outline=F, ylab=NA, main=gsub("T","U",i), cex.lab=2, las=1, boxwex=0.95,ylim=c(0,300))
}

for (i in GNWYG){
  RAPs_GNWYG <- RAPs[["RBFOX2"]][grep(i,str_sub(RAPs[["RBFOX2"]]$positive_fa,50,150)),]
  GNWYG_subset <- GNWYG[grep(i,GNWYG,invert=T)]
  RAPs_GNWYG <- RAPs_GNWYG[grep(paste(GNWYG_subset,collapse = "|"),str_sub(RAPs_GNWYG$positive_fa,50,150))]
  RAPs_GNWYG$GNWYG_subset <- str_count(str_sub(RAPs_GNWYG$positive_fa,50,150),i)
  RAPs_GNWYG$GNWYG_subset[RAPs_GNWYG$GNWYG_subset>2] <- 2
  RAPs_GNWYG$GNWYG_subset <- factor(as.character(RAPs_GNWYG$GNWYG_subset),levels=c("1","2"))
  boxplot2(data=RAPs_GNWYG,BS~GNWYG_subset, outline=F, ylab=NA,main=gsub("T","U",i), cex.lab=2, las=1, boxwex=0.95,ylim=c(0,300))
}

```



```
#dev.off()
```

### 0.5.10 Figure S2E

```
## the following lines exclude canonical GCAUG as part of the GNWYG to demonstrate that GNWYG acts independently
#pdf("./Figure/Figure2/FigureS2E.pdf", width = 8, height = 5)
par(mfrow=c(1,2),pty="n")
GNWYG <- c("GAATG", "GGATG", "GTTTG", "GCTTG", "GATTG", "GGTTG", "GAACG", "GCACG", "GTATG", "GATCG", "GCTCG", "GTTA")
RAPS_GNWYG_count <- str_count(str_sub(RAPS[["RBFOX2"]])$positive_fa, 50, 150), paste(GNWYG, collapse=" | "))
RAPS_GNWYG_Mean_FCI <- log2(RAPS[["RBFOX2"]][RAPS_GNWYG_count>0, "Mean_FCI"])

RAPS_GNWYG_count[RAPS_GNWYG_count>4] <- 4
RAPS_GNWYG_count <- RAPS_GNWYG_count[RAPS_GNWYG_count>0]
boxplot2(RAPS_GNWYG_Mean_FCI~RAPS_GNWYG_count, outline=F, boxwex=0.95, ylab="Fold Change (log2)", ylim=c(0, 10))

x <- RAPS_GNWYG_Mean_FCI[RAPS_GNWYG_count == 1]
y <- RAPS_GNWYG_Mean_FCI[RAPS_GNWYG_count == 2]
rst <- wilcox.test(x,y)$p.value
if ( rst <= 0.001 ) {
  to_add <- "***"
} else if ( rst <= 0.01 ) {
```

```

    to_add <- "***"
} else if ( rst <= 0.05 ) {
  to_add <- "*"
} else {
  a <- "P ="
  b <- round(rst,3)
  to_add <- paste(a,b,sep=" ")
}
text(1.5,7.25,labels = to_add, cex=1.5)

x <- RAPs_GNWYG_Mean_FCI[RAPs_GNWYG_count == 2]
y <- RAPs_GNWYG_Mean_FCI[RAPs_GNWYG_count == 3]
rst <- wilcox.test(x,y)$p.value
if ( rst <= 0.001 ) {
  to_add <- "***"
} else if ( rst <= 0.01 ) {
  to_add <- "**"
} else if ( rst <= 0.05 ) {
  to_add <- "*"
} else {
  a <- "P ="
  b <- round(rst,3)
  to_add <- paste(a,b,sep=" ")
}
text(2.5,7.5,labels = to_add, cex=1.5)

x <- RAPs_GNWYG_Mean_FCI[RAPs_GNWYG_count == 3]
y <- RAPs_GNWYG_Mean_FCI[RAPs_GNWYG_count == 4]
rst <- wilcox.test(x,y)$p.value
if ( rst <= 0.001 ) {
  to_add <- "***"
} else if ( rst <= 0.01 ) {
  to_add <- "**"
} else if ( rst <= 0.05 ) {
  to_add <- "*"
} else {
  a <- "P ="
  b <- round(rst,3)
  to_add <- paste(a,b,sep=" ")
}
text(3.5,7.75,labels = to_add, cex=1.5)

CLIPs_GNWYG_count <- str_count(str_sub(CLIPs[["RBFOX2"]])$positive_fa,20,120),paste(GNWYG,collapse="|"))
CLIPs_GNWYG_MeanFC <- CLIPs[["RBFOX2"]][CLIPs_GNWYG_count>0,"MeanFC"]
CLIPs_GNWYG_count[CLIPs_GNWYG_count>4] <- 4
CLIPs_GNWYG_count <- CLIPs_GNWYG_count[CLIPs_GNWYG_count>0]
boxplot2(CLIPs_GNWYG_MeanFC~CLIPs_GNWYG_count,outline=F,boxwex=0.95, ylab="Fold Change (log2)", ylim=c(0,10))

x <- CLIPs_GNWYG_MeanFC[CLIPs_GNWYG_count == 1]
y <- CLIPs_GNWYG_MeanFC[CLIPs_GNWYG_count == 2]
rst <- wilcox.test(x,y)$p.value
if ( rst <= 0.001 ) {
  to_add <- "***"
}

```

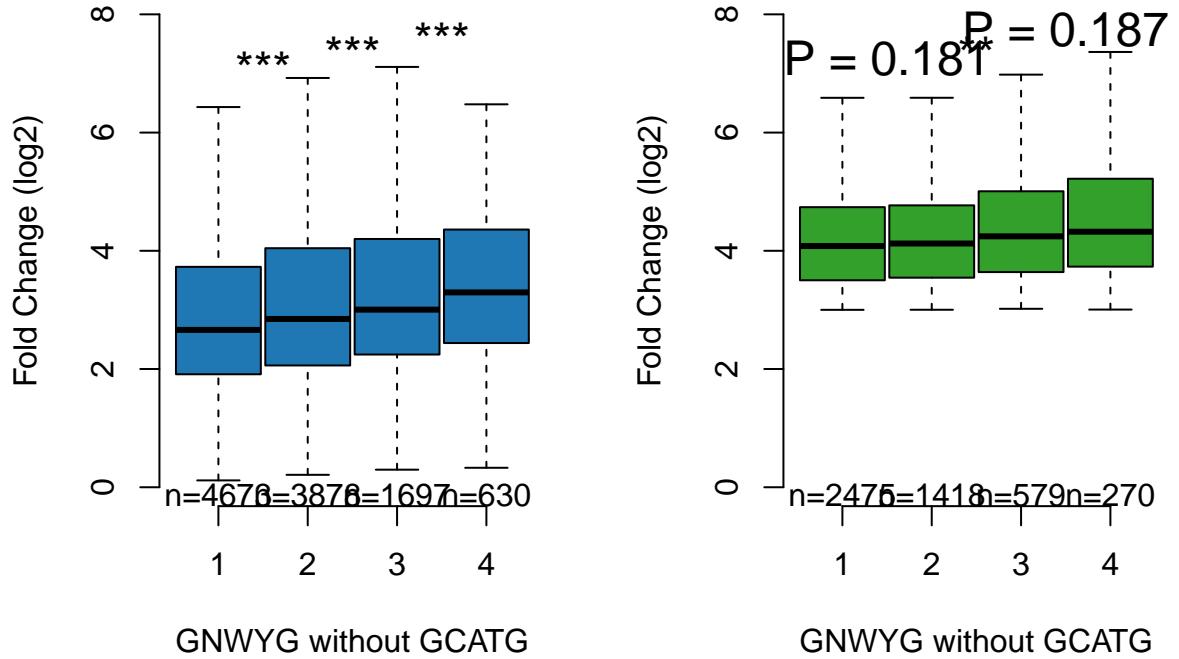
```

} else if ( rst <= 0.01 ) {
  to_add <- "***"
} else if ( rst <= 0.05 ) {
  to_add <- "*"
} else {
  a <- "P ="
  b <- round(rst,3)
  to_add <- paste(a,b,sep=" ")
}
text(1.5,7.25,labels = to_add, cex=1.5)

x <- CLIPs_GNWYG_MeanFC[CLIPs_GNWYG_count == 2]
y <- CLIPs_GNWYG_MeanFC[CLIPs_GNWYG_count == 3]
rst <- wilcox.test(x,y)$p.value
if ( rst <= 0.001 ) {
  to_add <- "****"
} else if ( rst <= 0.01 ) {
  to_add <- "***"
} else if ( rst <= 0.05 ) {
  to_add <- "*"
} else {
  a <- "P ="
  b <- round(rst,3)
  to_add <- paste(a,b,sep=" ")
}
text(2.5,7.5,labels = to_add, cex=1.5)

x <- CLIPs_GNWYG_MeanFC[CLIPs_GNWYG_count == 3]
y <- CLIPs_GNWYG_MeanFC[CLIPs_GNWYG_count == 4]
rst <- wilcox.test(x,y)$p.value
if ( rst <= 0.001 ) {
  to_add <- "****"
} else if ( rst <= 0.01 ) {
  to_add <- "***"
} else if ( rst <= 0.05 ) {
  to_add <- "*"
} else {
  a <- "P ="
  b <- round(rst,3)
  to_add <- paste(a,b,sep=" ")
}
text(3.5,7.75,labels = to_add, cex=1.5)

```



```
#dev.off()
```

### 0.5.11 Figure S2F

```

AACAHC <- c("AACATC", "AACACC", "AACAAC")
ACAHC <- c("ACATC", "ACACC", "ACAAC")
CAHC <- c("CATC", "CACC", "CAAC")

AACAHC_scores <- list()
for (i in 1:3){
  RAPs_AACAHC <- RAPs[["YBX3"]][grep(AACAHC[i], str_sub(RAPs[["YBX3"]]$positive_fa, 50, 150)),]
  RAPs_AACAHC_target <- CAHC[-i]
  AACAHC_scores[[i]] <- RAPs_AACAHC[grep(paste(RAPs_AACAHC_target, collapse = "|"), str_sub(RAPs_AACAHC$positive_fa, 50, 150)))]
}
names(AACAHC_scores) <- AACAHC

ACAHC_scores <- list()
for (i in 1:3){
  RAPs_ACAHC <- RAPs[["YBX3"]][grep(ACAHC[i], str_sub(RAPs[["YBX3"]]$positive_fa, 50, 150), invert=T),]
  RAPs_ACAHC <- RAPs_ACAHC[grep(ACAHC[i], str_sub(RAPs_ACAHC$positive_fa, 50, 150)))]
  RAPs_ACAHC_target <- CAHC[-i]
  ACAHC_scores[[i]] <- RAPs_ACAHC[grep(paste(RAPs_ACAHC_target, collapse = "|"), str_sub(RAPs_ACAHC$positive_fa, 50, 150)))]
}
```

```

    ACAHC_scores[[i]] <- RAPs_ACAHC$BS
}
names(ACAHC_scores) <- ACAHC

CAHC_scores <- list()
for (i in 1:3){
  RAPs_CAHC <- RAPs[["YBX3"]][grep(AACAHC[i],str_sub(RAPs[["YBX3"]])$positive_fa,50,150), invert=T,]
  RAPs_CAHC <- RAPs_CAHC[grep(ACAHC[i],str_sub(RAPs_CAHC$positive_fa,50,150), invert=T,]
  RAPs_CAHC <- RAPs_CAHC[grep(CAHC[i],str_sub(RAPs_CAHC$positive_fa,50,150)),]
  RAPs_CAHC_target <- CAHC[-i]
  CAHC_scores[[i]] <- RAPs_CAHC[grep(paste(RAPs_CAHC_target,collapse = " | "),str_sub(RAPs_CAHC$positive_fa,50,150)),]
  CAHC_scores[[i]] <- RAPs_CAHC$BS
}
names(CAHC_scores) <- CAHC

ALL_CAHCs_scores <- c(AACAHC_scores[1],ACAHC_scores[1],CAHC_scores[1], AACAHC_scores[2],ACAHC_scores[2])

#pdf("./Figure/Figure2/FigureS2F.pdf", width = 8, height = 5)
par(bty="n")

boxplot2(ALL_CAHCs_scores,outline=F, range=1, col= c("#1f78b4", "#6baed6", "#c6dbef"), las=3, ylim=c(0,8))

x <- ALL_CAHCs_scores$AACATC
y <- ALL_CAHCs_scores$ACATC
rst <- wilcox.test(x,y)$p.value
if ( rst <= 0.001 ) {
  to_add <- "***"
} else if ( rst <= 0.01 ) {
  to_add <- "**"
} else if ( rst <= 0.05 ) {
  to_add <- "*"
} else {
  to_add <- "ns"
}
text(1.5,77.5,labels = to_add, cex=1.5)

x <- ALL_CAHCs_scores$ACATC
y <- ALL_CAHCs_scores$CATC
rst <- wilcox.test(x,y)$p.value
if ( rst <= 0.001 ) {
  to_add <- "***"
} else if ( rst <= 0.01 ) {
  to_add <- "**"
} else if ( rst <= 0.05 ) {
  to_add <- "*"
} else {
  to_add <- "ns"
}
text(2.5,75,labels = to_add, cex=1.5)

```

```

x <- ALL_CAHCs_scores$AACACC
y <- ALL_CAHCs_scores$ACACC
rst <- wilcox.test(x,y)$p.value
if ( rst <= 0.001 ) {
  to_add <- "***"
} else if ( rst <= 0.01 ) {
  to_add <- "**"
} else if ( rst <= 0.05 ) {
  to_add <- "*"
} else {
  to_add <- "ns"
}
text(4.5,77.5,labels = to_add, cex=1.5)

x <- ALL_CAHCs_scores$ACACC
y <- ALL_CAHCs_scores$CACC
rst <- wilcox.test(x,y)$p.value
if ( rst <= 0.001 ) {
  to_add <- "***"
} else if ( rst <= 0.01 ) {
  to_add <- "**"
} else if ( rst <= 0.05 ) {
  to_add <- "*"
} else {
  to_add <- "ns"
}
text(5.5,75,labels = to_add, cex=1.5)

x <- ALL_CAHCs_scores$AACAC
y <- ALL_CAHCs_scores$ACAC
rst <- wilcox.test(x,y)$p.value
if ( rst <= 0.001 ) {
  to_add <- "***"
} else if ( rst <= 0.01 ) {
  to_add <- "**"
} else if ( rst <= 0.05 ) {
  to_add <- "*"
} else {
  to_add <- "ns"
}
text(7.5,77.5,labels = to_add, cex=1.5)

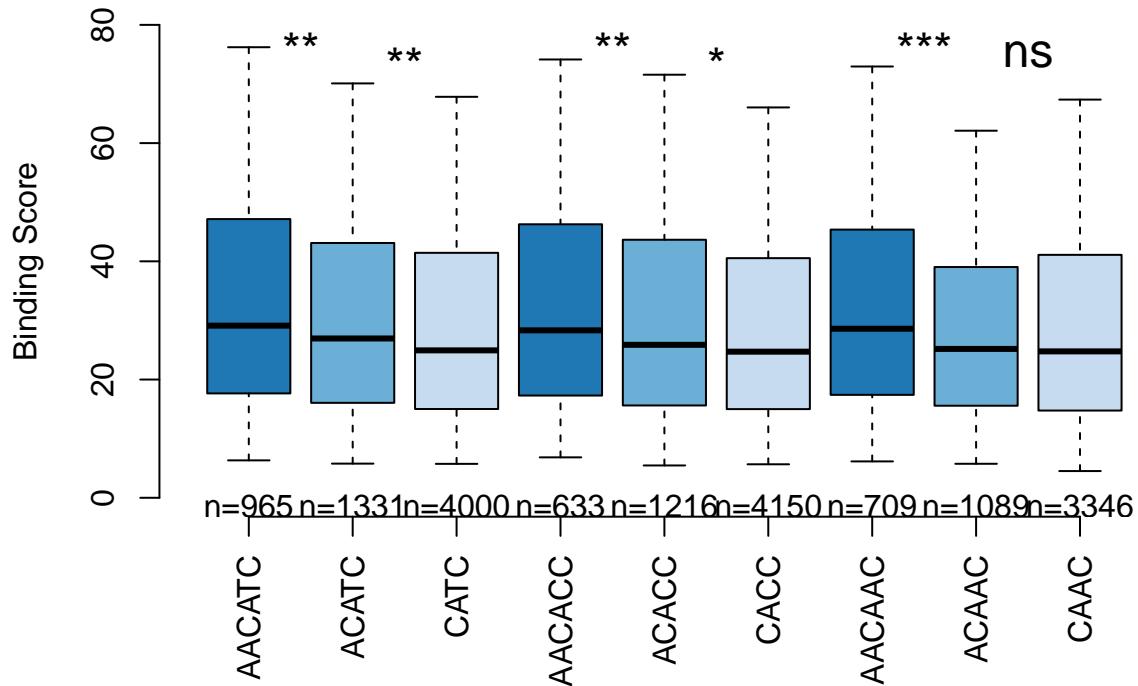
x <- ALL_CAHCs_scores$ACAC
y <- ALL_CAHCs_scores$CAAC
rst <- wilcox.test(x,y)$p.value
if ( rst <= 0.001 ) {
  to_add <- "***"
} else if ( rst <= 0.01 ) {
  to_add <- "**"
} else if ( rst <= 0.05 ) {
  to_add <- "*"
} else {

```

```

    to_add <- "ns"
}
text(8.5,75,labels = to_add, cex=1.5)

```



```
#dev.off()
```

### 0.5.12 Figure S2G

```

Names_protein <- c("HNRNPA1", "HNRNPC", "PTBP1", "RBFOX2", "YBX3", "IGF2BP1")

# CLIPs
CLIPs_RBFOX2_feature_count <- table(CLIPs[["RBFOX2"]][, "feature"])

for (i in Names_protein){
  CLIPs_RBFOX2_feature_tmp <- table(CLIPs[[i]][, "feature"])/nrow(CLIPs[[i]])
  CLIPs_RBFOX2_feature_count <- rbind(CLIPs_RBFOX2_feature_count, CLIPs_RBFOX2_feature_tmp)
}
CLIPs_RBFOX2_feature_count <- CLIPs_RBFOX2_feature_count[-1,]
CLIPs_RBFOX2_feature_count <- t(CLIPs_RBFOX2_feature_count)
colnames(CLIPs_RBFOX2_feature_count) <- Names_protein

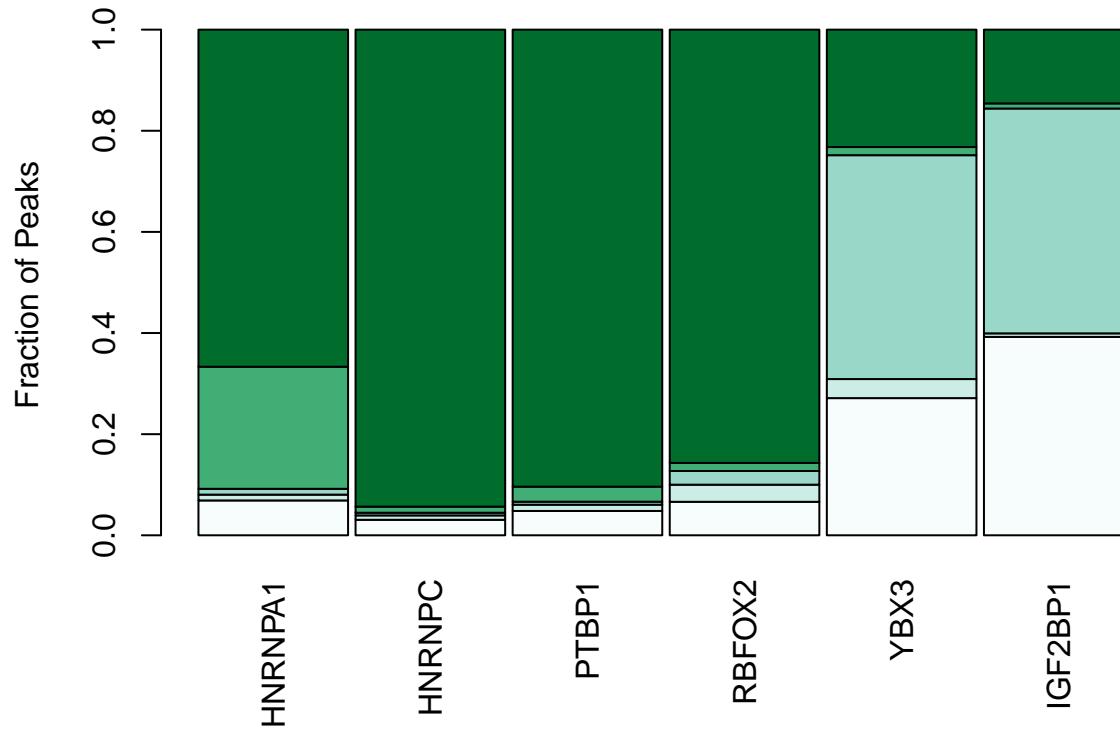
```

```

# RAPs
RAPs_RBFOX2_feature_count <- table(RAPs[["RBFOX2"]][, "feature"])
for (i in Names_protein){
  print(i)
  RAPs_RBFOX2_feature_tmp <- table(RAPs[[i]][, "feature"])/nrow(RAPs[[i]])
  RAPs_RBFOX2_feature_count <- rbind(RAPs_RBFOX2_feature_count, RAPs_RBFOX2_feature_tmp)
}
## [1] "HNRNPA1"
## [1] "HNRNPC"
## [1] "PTBP1"
## [1] "RBFOX2"
## [1] "YBX3"
## [1] "IGF2BP1"
RAPs_RBFOX2_feature_count <- RAPs_RBFOX2_feature_count[-1,]
RAPs_RBFOX2_feature_count <- t(RAPs_RBFOX2_feature_count)
colnames(RAPs_RBFOX2_feature_count) <- Names_protein

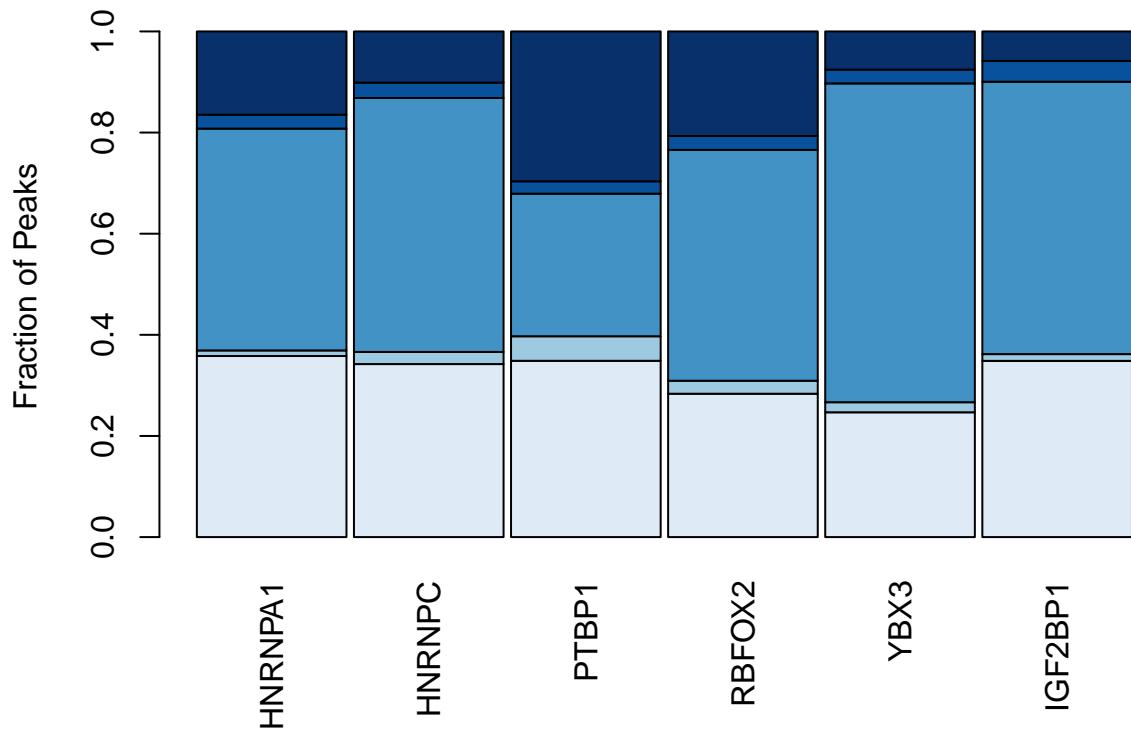
barplot(CLIPs_RBFOX2_feature_count, col=c("#f7fcfd", "#ccece6", "#99d8c9", "#41ae76", "#006d2c"), las=3)

```

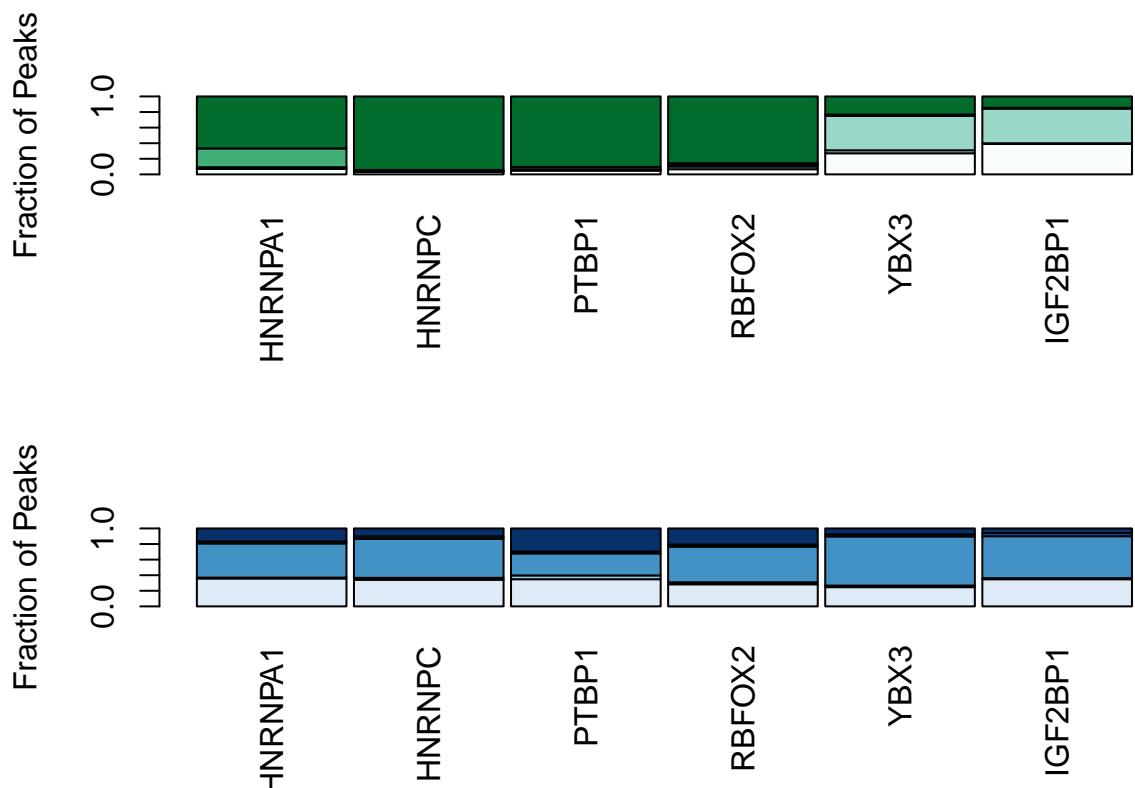


#### 0.5.12.1 include exon

```
barplot(RAPs_RBFOX2_feature_count, col=c("#deebf7", "#9ecae1", "#4292c6", "#08519c", "#08306b"), las=3, spc
```



```
#pdf("./Figure/Figure2/FigureS2G.pdf", width = 13.9, height = 13.7)
par(mfrow=c(2,1))
barplot(CLIPs_RBFOX2_feature_count, col=c("#f7fcfd", "#ccece6", "#99d8c9", "#41ae76", "#006d2c"), las=3)
barplot(RAPs_RBFOX2_feature_count, col=c("#deebf7", "#9ecae1", "#4292c6", "#08519c", "#08306b"), las=3, spc
```



```
#dev.off()
```

```
Names_protein <- c("HNRNPA1", "HNRNPC", "PTBP1", "RBFOX2", "YBX3", "IGF2BP1")

# CLIPs
CLIPs_RBFOX2_feature_count <- table(CLIPs[["RBFOX2"]][CLIPs[["RBFOX2"]]$feature != "exon", "feature"])

for (i in Names_protein){
  CLIPs_RBFOX2_feature_tmp <- table(CLIPs[[i]][CLIPs[[i]]$feature != "exon", "feature"])/nrow(CLIPs[[i]])
  CLIPs_RBFOX2_feature_count <- rbind(CLIPs_RBFOX2_feature_count, CLIPs_RBFOX2_feature_tmp)
}
CLIPs_RBFOX2_feature_count <- CLIPs_RBFOX2_feature_count[-1,]
CLIPs_RBFOX2_feature_count <- t(CLIPs_RBFOX2_feature_count)
colnames(CLIPs_RBFOX2_feature_count) <- Names_protein

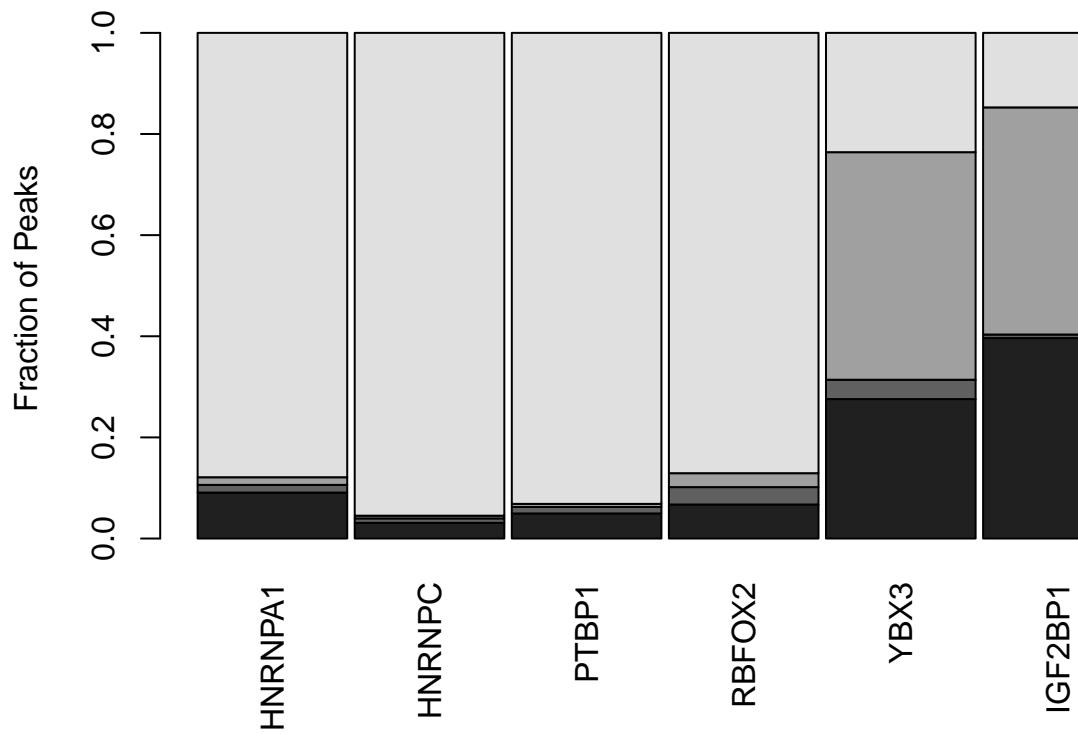
# RAPs
RAPs_RBFOX2_feature_count <- table(RAPs[["RBFOX2"]][RAPs[["RBFOX2"]]$feature != "exon", "feature"])
for (i in Names_protein){
  print(i)
  RAPs_RBFOX2_feature_tmp <- table(RAPs[[i]][RAPs[[i]]$feature != "exon", "feature"])/nrow(RAPs[[i]][RAPs[[i]]$feature != "exon", "feature"])
  RAPs_RBFOX2_feature_count <- rbind(RAPs_RBFOX2_feature_count, RAPs_RBFOX2_feature_tmp)
}
## [1] "HNRNPA1"
```

```

## [1] "HNRNPC"
## [1] "PTBP1"
## [1] "RBFOX2"
## [1] "YBX3"
## [1] "IGF2BP1"
RAPs_RBFOX2_feature_count <- RAPs_RBFOX2_feature_count[-1,]
RAPs_RBFOX2_feature_count <- t(RAPs_RBFOX2_feature_count)
colnames(RAPs_RBFOX2_feature_count) <- Names_protein

barplot(CLIPs_RBFOX2_feature_count, col=greys[c(1,3,5,7)], las=3, space=0.05, ylab="Fraction of Peaks")

```

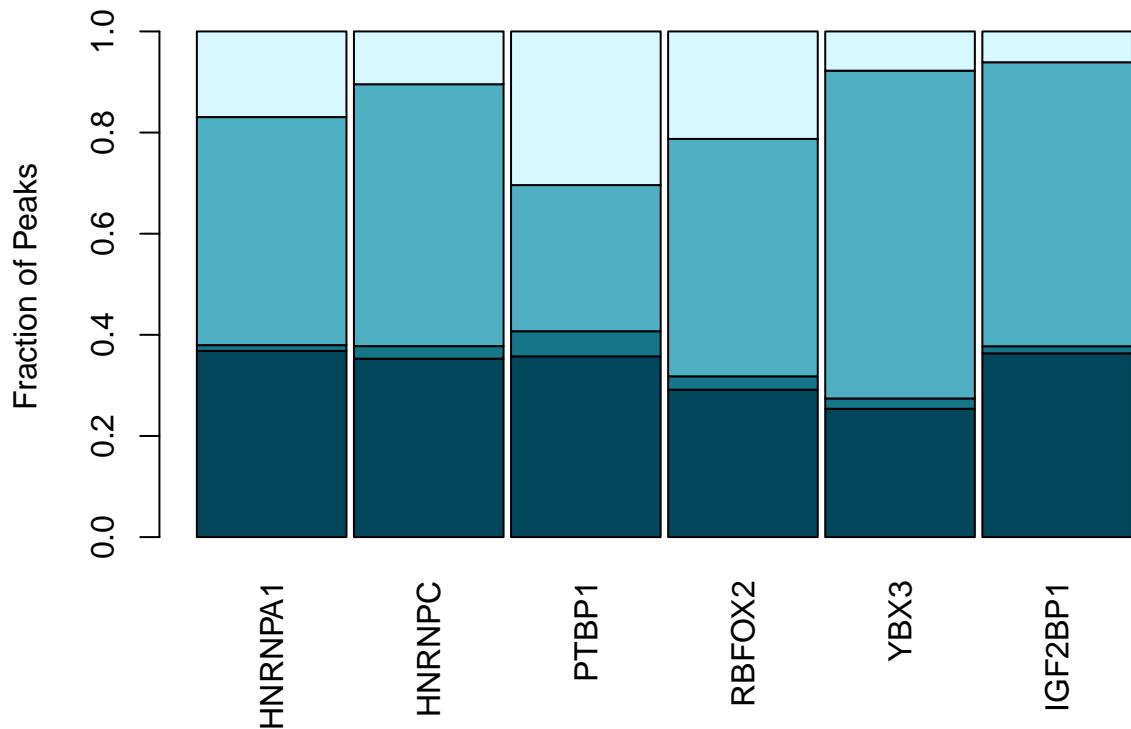


#### 0.5.12.2 exclude exome

```

barplot(RAPs_RBFOX2_feature_count, col=acqua_blues[c(1,4,7,11)], las=3, space=0.05, ylab="Fraction of P

```



```
par(mfrow=c(2,1))
barplot(CLIPs_RBFOX2_feature_count, col=greys[c(1,3,5,7)], las=3, space=0.05, ylab="Fraction of Peaks")
barplot(RAPs_RBFOX2_feature_count, col=acqua_blues[c(1,4,7,11)], las=3, space=0.05, ylab="Fraction of Peaks")
```

