Figure 5 YTHDF1

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This markdown show how to generate Figure 5

Loading packages and required data: Input in TPM related to RAPseq and T7RAPseq, YTHDF1 metafile with binding sites of RAPseq Input and T7 input, generated in the script "YTHDF1_metafile_annotation

```
library(eulerr)
library(ggplot2)
library(reshape2)
library(gplots)
library(tidyverse)
library(ggpubr)
library(dendextend)
library(dplyr)
library(gtools)
library(corrplot)
library(clusterProfiler)
library(org.Hs.eg.db)
library(rrvgo)
library(LSD)
library(stringr)

Inputs <- read.table(file =
"/Users/riccardomosca/Desktop/RAPseq_PAPER/Inputs_TPM.txt",
    stringsAsFactors = F, header = T)

YTHDF1 <- read.table(file =
"/Users/riccardomosca/Desktop/RAPseq_PAPER/PEAKs/ANNOTATED/T7_Fig5/YTHDF1/YTH
DF1_T7_scored_annotated.txt",
    header = T, stringsAsFactors = F)
head(Inputs)
```

```
##            Geneid       tpm     tpmT7
## 1 ENSG00000000003 11.479072 12.808744
## 2 ENSG00000000419 38.450198 36.641031
## 3 ENSG00000000460  2.275249  2.251449
## 4 ENSG00000001036 37.303358 35.645586
## 5 ENSG00000001084  6.370261  6.188329
## 6 ENSG00000001167  9.356073  8.646681
```

```
head(YTHDF1)
```

```
##    chr   start     end                peak_ID         RBPs strand
## 1 chr1 1387212 1387220 chr1_1387202_1387251_- YTHDF1_final      -
## 2 chr1 1440085 1440093 chr1_1440065_1440110_+ YTHDF1_final      +
## 3 chr1 2308290 2308298 chr1_2308269_2308373_+ YTHDF1_final      +
## 4 chr1 6224723 6224731 chr1_6224711_6224765_- YTHDF1_final      -
## 5 chr1 6342574 6342582 chr1_6342564_6342591_- YTHDF1_final      -
## 6 chr1 6550420 6550428 chr1_6550398_6550459_- YTHDF1_final      -
##   YTHDF1_rep1_signal YTHDF1_rep2_signal halo_signal input_signal
## 1                 38                 37           8            4
## 2                 42                 42          11           11
## 3                 42                 45           2            7
## 4                 91                101           2            6
## 5                 73                 45          10            6
## 6                 30                 36           7            6
##   T7YTHDF1_rep1_signal T7YTHDF1_rep2_signal T7halo_signal T7input_signal
## 1                    6                    5             9              2
## 2                   15                   14            10             10
## 3                    3                    3             6             10
## 4                    5                    4             3              6
## 5                    5                    6             4              1
## 6                   12                    9            11              3
##
pos_fa
## 1
CACTTGCGCTGAGAGCACACCCGGGGGTCAAAGGGCAGCCACCGGGGGTCAAAGGGCAGCCATCAGGTACTCCCCAG
GGAAGGGCTTGCGGCCACCAGTCACTGCAACCCCGCCTCACCTCCGATGCCTGCTGTGCCCAGGGTGGTCCCGCTCA
TAGCGACGGCCTGTGCGTTCATACGACCTCGAGCGCTCTCGTCGCT
## 2
GCGGGACCCCGCAGCAGCCCCGGCCCCATCCCCGCCCAGAGCCGGGCGTCGTGTGGGTCCGTGGGTGATAATTGAGA
GCGTCAGACCCAGGACTGTTCAGGGAGGAGCCCCGGTCAGACTCCCACGTGTGAAGACCGGGCCCCAAGTGGCAAGG
GCTGGCCTGGGGCGGGCAGCTTGGGTCCTGGACGTTGATAGGAAGC
## 3
TTCTAACACCCGCGGCAGCTGCACGCGCTCACAGAAGGTGGAGGTTACTTGCCCAGGTACAGACGACCTCGGGGCAG
TGACGAGCAAAGACCAGAGACTGCTGAGCCCTCGCATCTGGGTGGCGGAATTGCCTGCGGGGTTTTGCCCTTGGTTT
ACTGAGGGGGGTCTTGGTTGCTGCTGAAGCCCCCCACCCCTTCTAA
## 4
TTCGGTTAAATGAAAGGTTGTAAATGCATTTTTACCGCTGATAAGAAGGGGTACCTGCTACCCCTTTCTGCTGTGGA
GTGTTGCTGAGGACAGAGTCCATCTCTCCCAGCGAGTCCTGGTTATCCTTTACTTACACTCTGGCCTCGGGGGCAGT
GGCGTGTGGCCTCGGTCCTCCCCAGGTAACTCTGGAGGGCGCTGTG
## 5
GGACCTTGCTGTGTTGCCCAGGCAGGAATGCAATGGCTATTCACAGGCACAATCATGCTCACTGCAGCCTCAAACTC
CTCCTGGGCTCAAGCGATCCTCCAGCCTCAGCCTCCTGAGTAGCTGCGACTACAGGCACATGCAACCACACCTAACT
TTTTTTTAGAAAATTACTTTCAATTGCTAGCCAGGTGGAATTGAAC
## 6
CTGGCCCTAAAAATTATGAATTAGCCTTATTACAGTAGGCCCTCAGTAAATTACCCAGTTCTTTTCATTACCAAGGT
TAAGATGAGATTTGAGCAAATTCCGGGCTTCCCTTTTCAGTTCCTTCTTGCTTTTCTCAGGCTGTGAGTAGTGAAGT
GCATGGATACTCAAGCAAGAGTGGGTATACACAGAGAAGATGTCTT
##
neg_fa
## 1
```

```
AGAAACAAGTTCGTAGAGCGAGATAAGGCCCCTCTACAGGTCAAACAGCGACGCAAGCAGACTGTAACTTCCGGCTC
CCAGCTGGGACCCAGAACTTACACAGACATCACAGAAATGCAGATGGGGCTGGACGCGGGGGCTCACGCCTGTGAGC
CCAGCACTTCGGGAGGCCGAGGAGGGCGGATCACAAAGTCAATACA
## 2
AGGGGGCTGGGGCCTCTGCAGGGCCATTCCCACCGCAGTCTCTGCCACGGCCCGGAGCCCTGGGCCCAGGGCCACGC
GGGCTCCCTCTGCTGGCAAAGCCACACCCTGCACCGCATGGGGTCCACTGCCCCTTTCCCCACGTGGACTCTGCGTG
ACCCCAGGAACTGCAGCATTGAGGTGGTCTCAGTCCCTCCCTCAGG
## 3
ACAGGTGCGAGTCCGTTTCTTTTCAGCAGAAGGGGGAAGAGGTGTCCGCTGTGTGGGCTGCTGACTCCTCTGTGTGT
GAGGCCCTTCATCTAAGTGATTGTGTATTCAGTTTAATTCTCATTATATTTCTATACTGAAAGAAGATTTTTAACGA
AGGGAAAAACAACAGCAATAACATTCATATCTCTGGAGCAGCTAAC
## 4
TTACACCACAGCTGCCTGGGCCTCTGCCTCCTAAACCCACAGCCTCTGCTTCAGCACGAGATACAGTGCCACAAAAC
TCAGCTTCCGAAATGCCCCTTCCAGCAGATTACCTCCACGCTCAGAGAGCTCCAGAGACTGAACCCCACCCCCTGAG
TCTGGGTTTCCAACCCCAGAAGAGCAGCACTTCGCCCACTTCCCCA
## 5
CGATGCTTCCCCATTCTGGTTTGCACGGGCTTGTCCCCCAGCAAACCCTGCCCTACTCACAACAGGTCACAGTAAAG
CGATGCCTGGAACTGTCTCTTTCACCCCCAAGTCAATGTATGTGGCTATGGCGCCACCCATTGGCCAAAGGCTGTTA
GGGCAGGGCTGCACAGAGCCAGGCAAGAATTAGGCACCGCCTCTGG
## 6
ATCCCAGAACTCTGGGAGAAGGAGGTGGGGGGATCACTTGAGTGAGGCCAGGAGTTTGAGACCAGCCTGGATAACAT
GGCAAACCCCCCTGCACCCCACCCCCGCCACCACCATCTCTACTAAAATTACAAAAATTAGCTGGGCATGTGCCTGT
AATCCCAGCTACTCCAGAGGCTGAGGCATGAGAATCGCTTGAATCC
##    FCH_rep1  FCH_rep2  FCI_rep1  FCI_rep2 FCH_T7rep1 FCH_T7rep2 FCI_T7rep1
## 1  4.750000  4.625000  9.500000  9.250000  0.6666667  0.5555556  3.0000000
## 2  3.818182  3.818182  3.818182  3.818182  1.5000000  1.4000000  1.5000000
## 3 21.000000 22.500000  6.000000  6.428571  0.5000000  0.5000000  0.3000000
## 4 45.500000 50.500000 15.166667 16.833333  1.6666667  1.3333333  0.8333333
## 5  7.300000  4.500000 12.166667  7.500000  1.2500000  1.5000000  5.0000000
## 6  4.285714  5.142857  5.000000  6.000000  1.0909091  0.8181818  4.0000000
##   FCI_T7rep2 Mean_FCs_rep1 Mean_FCs_rep2 Mean_FCs_T7rep1 Mean_FCs_T7rep2
## 1  2.5000000      7.125000      6.937500        1.833333        1.527778
## 2  1.4000000      3.818182      3.818182        1.500000        1.400000
## 3  0.3000000     13.500000     14.464286        0.400000        0.400000
## 4  0.6666667     30.333333     33.666667        1.250000        1.000000
## 5  6.0000000      9.733333      6.000000        3.125000        3.750000
## 6  3.0000000      4.642857      5.571429        2.545455        1.909091
##     BS_rep1   BS_rep2 BS_T7rep1 BS_T7rep2        BS     BS_T7  Mean_FCH
## 1  30.79374  29.72998 3.6666667 2.7612367  30.26186 3.2139517  4.687500
## 2  17.02692  17.02692 4.6311943 4.2000000  17.02692 4.4155971  3.818182
## 3  60.20233  65.87887 0.5287712 0.5287712  63.04060 0.5287712 21.750000
## 4 168.02115 191.44552 2.2591937 1.5849625 179.73333 1.9220781 48.000000
## 5  50.89384  27.32753 5.6479841 7.5000000  39.11068 6.5739921  5.900000
## 6  18.57143  23.66702 7.1459943 4.6952785  21.11923 5.9206364  4.714286
##    Mean_FCI Mean_FCH_T7 Mean_FCI_T7    MeanFC MeanFC_T7            gene_ID
## 1  9.375000   0.6111111        2.75  7.031250  1.680556 ENSG00000221978.12
## 2  3.818182   1.4500000        1.45  3.818182  1.450000 ENSG00000179403.12
## 3  6.214286   0.5000000        0.30 13.982143  0.400000 ENSG00000157933.10
## 4 16.000000   1.5000000        0.75 32.000000  1.125000 ENSG00000116237.16
## 5  9.833333   1.3750000        5.50  7.866667  3.437500 ENSG00000097021.20
```

```
## 6  5.500000   0.9545455        3.50  5.107143  2.227273 ENSG00000162408.11
##   feature gene_name      gene_type
## 1    3UTR     CCNL2 protein_coding
## 2    3UTR      VWA1 protein_coding
## 3    3UTR       SKI protein_coding
## 4    3UTR      ICMT protein_coding
## 5  intron     ACOT7 protein_coding
## 6     CDS      NOL9 protein_coding
```

Figure 5B

```
fits <- summary(lm(Inputs$tpm~Inputs$tpmT7))
R2 <- as.character(round(fits$adj.r.squared,2))
R2 <- paste("R2 = ", R2, sep="")
spearman <- as.character(round(cor(Inputs$tpmT7,Inputs$tpm, method =
"spearman"),2))
spearman <- paste("Spearman = ", spearman, sep="")
pearson <- as.character(round(cor(Inputs$tpmT7,Inputs$tpm, method =
"pearson"),2))
pearson <- paste("Pearson = ", pearson, sep="")
N <- paste("n = ", nrow(Inputs), sep="")

TPM_RAP <- Inputs$tpm
TPM_T7RAP <- Inputs$tpmT7



layout.matrix <- matrix(c(2, 1, 0, 3), nrow = 2, ncol = 2)
layout(mat = layout.matrix, heights = c(0.5, 2),  widths = c(2, 0.5))
# scatterplot
par(mar = c(5, 5, 0, 0))
heatscatter(log2(TPM_T7RAP),log2(TPM_RAP), colpal=c("black","#9F9F9F"), alpha
= 80, cex=0.8, bty="l", las=1, xlim=c(0,18), ylim=c(0,18), main="", pch=16)
text(x=4.4,y=16,labels=spearman)
text(x=3.9,y=15,labels=pearson)
text(x=2.5,y=14,labels=R2)
text(x=2.6,y=13,labels=N)
# density plot T7RAPseq Input
par(mar = c(0.5, 5, 0.5, 0))
d1 <- density(log2(TPM_T7RAP), bw=0.2)
plot(d1$x,d1$y, main=NA, bty="n", xlab=NA, type="l", ylab="density",
ylim=c(0,0.3), bty="n", xlim=c(0,18), las=1)
abline(v=median(log2(TPM_T7RAP)))
text(x=5,y=0.29,labels=round(median(log2(TPM_T7RAP)),1))
# density plot RAPseq Input
par(mar = c(5, 0.5, 0, 0.5))
d2 <- density(log2(TPM_RAP), bw=0.2)
plot(d2$y,d2$x, main=NA, bty="n", ylab=NA, type="l", xlab="density",
xlim=c(0,0.3), bty="n", ylim=c(0,18), las=2)
```

```
abline(h=median(log2(TPM_RAP)))
text(x=0.275,y=5,labels=round(median(log2(TPM_RAP)),1))
```

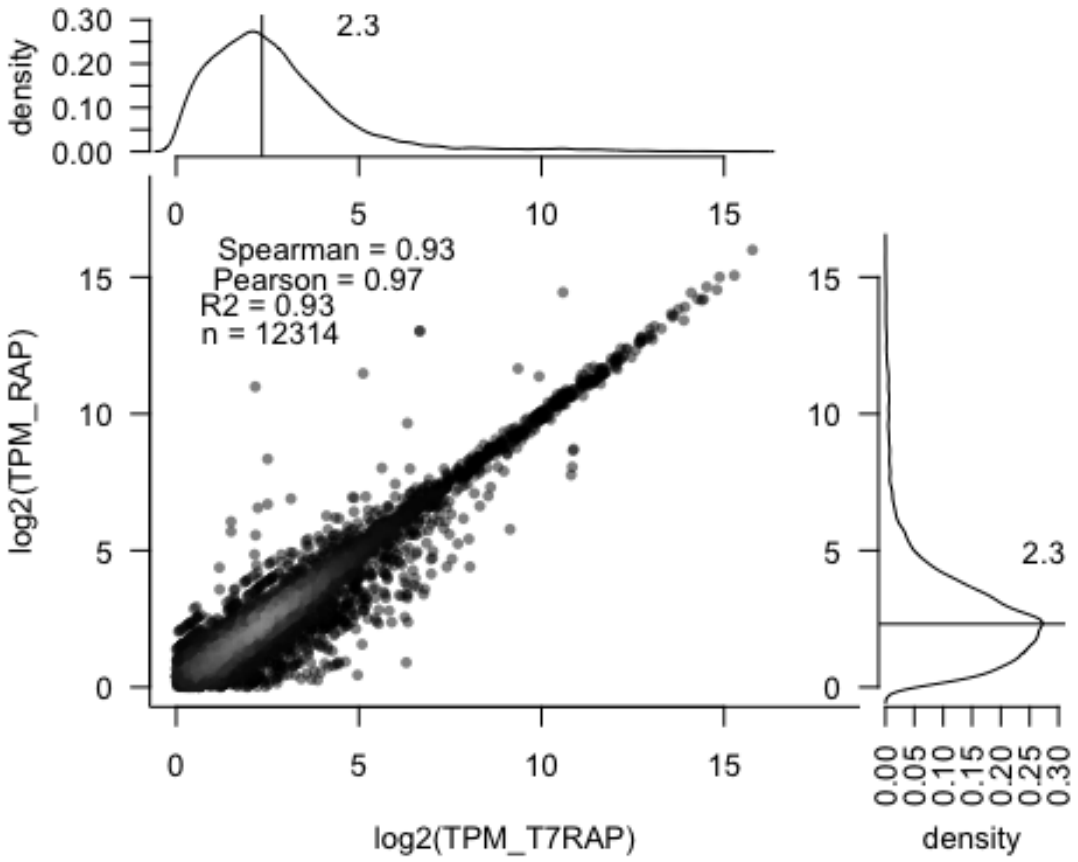


Figure 5C

```
# Only the RAPseq YTHDF1 binding sites are considered and compared with the
# signal on the T7RAPseq
YTHDF1$T7input_signal[YTHDF1$T7Input_signal == 0] <-
min(YTHDF1[YTHDF1$T7Input_signal !=
    0, "T7Input_signal"])
YTHDF1$T7halo_signal[YTHDF1$T7halo_signal == 0] <-
min(YTHDF1[YTHDF1$T7halo_signal !=
    0, "T7halo_signal"])




RAP_enrichments <- YTHDF1$peak_ID
T7RAP_enrichments <- YTHDF1[YTHDF1$T7YTHDF1_rep1_signal/YTHDF1$T7input_signal
> 1 &
    YTHDF1$T7YTHDF1_rep2_signal/YTHDF1$T7input_signal > 1 &
```

```
YTHDF1$T7YTHDF1_rep1_signal/YTHDF1$T7halo_signal >
    1 & YTHDF1$T7YTHDF1_rep2_signal/YTHDF1$T7halo_signal > 1, "peak_ID"]


AB <- list(RAP_enrichments, T7RAP_enrichments)
names(AB) <- c("with modif", "without modif")
v <- euler(AB, shape = "circle")


plot(v, fills = c("#93ADD0", "#8CBDA0"), quantities = TRUE, edges = F)
```

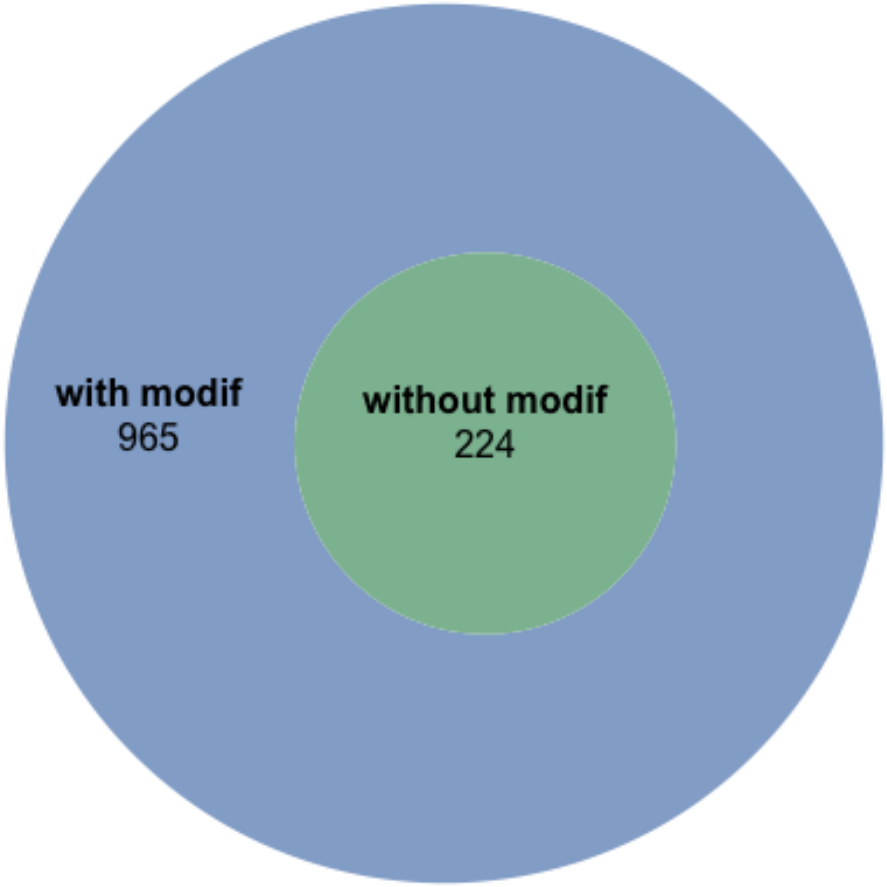
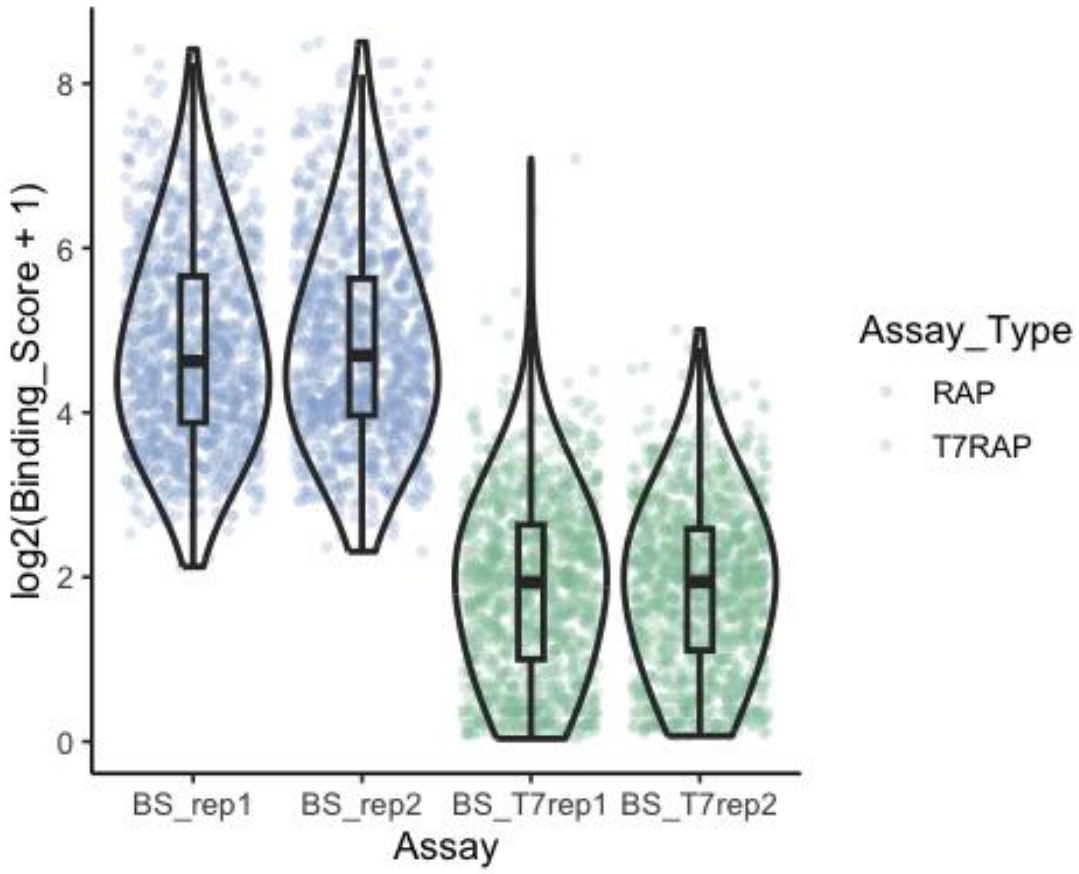


Figure 5D

```
FCs <- YTHDF1[, c("BS_rep1", "BS_rep2", "BS_T7rep1", "BS_T7rep2")]
FCs <- melt(FCs, value.name = "Binding_Score", variable.name = "Assay")
RAP <- rep("RAP", nrow(FCs)/2)
T7RAP <- rep("T7RAP", nrow(FCs)/2)
FCs$Assay_Type <- c(RAP, T7RAP)


plot <- ggplot(data = FCs, aes(x = Assay, y = log2(Binding_Score + 1))) +
geom_jitter(aes(color = Assay_Type),
```

```
    pch = 16, alpha = 0.25) + scale_color_manual(values = c("#93ADD0",
"#8ABEA0")) +
    theme_classic(base_size = 12.5)

Fig5D <- plot + geom_violin(trim = T, bw = 0.75, scale = "width", lwd = 1,
fill = NA) +
    geom_boxplot(outlier.shape = NA, width = 0.15, lwd = 1, fill = NA)

Fig5D
```




```
list <- list(BS_T71 = YTHDF1$BS_T7rep1, BS_T72 = YTHDF1$BS_T7rep2)

# run wilcox.test() for each, comparing to the overlap vector
wt <- lapply(names(list), function(nm) {
    y <- list[[nm]]
    test <- wilcox.test(YTHDF1$BS_rep1, y)
    data.frame(comparison = paste0("BS1_vs_", nm), W =
as.numeric(test$statistic),
        p.value = test$p.value)
})

# combine into one df
wt_df <- do.call(rbind, wt)
```

Figure 5E

```
# Considering all the different combinations of the DRACH motif of YTHDF1 (X.
# Wang et al., Cell, 2015)
DRACH <- c("GGACT", "GAACT", "GGACA", "GAACA", "GGACC", "GAACC", "AAACT",
"AGACT",
    "AAACA", "AGACA", "AAACC", "AGACC", "TGACT", "TAACT")



COUNTS <- c()
for (i in DRACH) {
    COUNTS <- c(COUNTS, length(grep(i, str_sub(YTHDF1$pos_fa, 85, 115))))  #
30 nucleotide window
}

names(COUNTS) <- DRACH
COUNTS <- COUNTS[order(COUNTS)]

NEG_COUNTS <- c()
for (i in names(COUNTS)) {
    NEG_COUNTS <- c(NEG_COUNTS, length(grep(i, str_sub(YTHDF1$neg_fa, 85,
115))))
}
names(NEG_COUNTS) <- names(COUNTS)

FCs <- COUNTS/NEG_COUNTS
FCs <- FCs[order(FCs)]
names(FCs) <- gsub("T", "U", names(FCs))

barplot(height = FCs, horiz = T, las = 1, xlab = "Counts: Bound sites /
Control sites",
    col = "#93ADD0", xlim = c(0, 10), space = 0.1)
abline(v = c(0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11), lty = 2, lwd = 1)
```

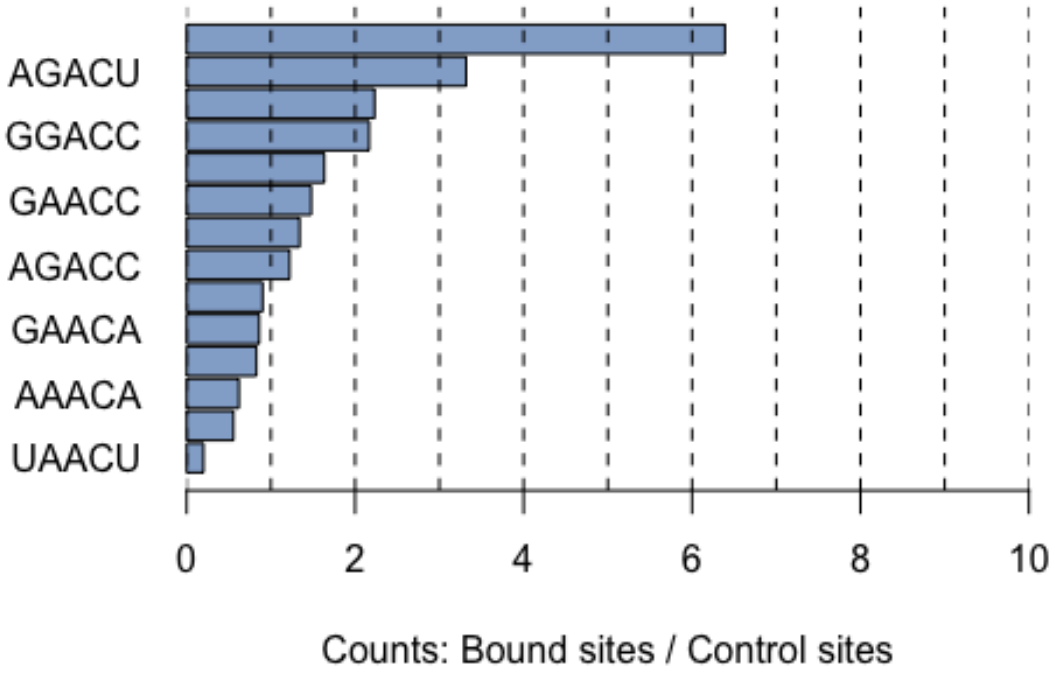
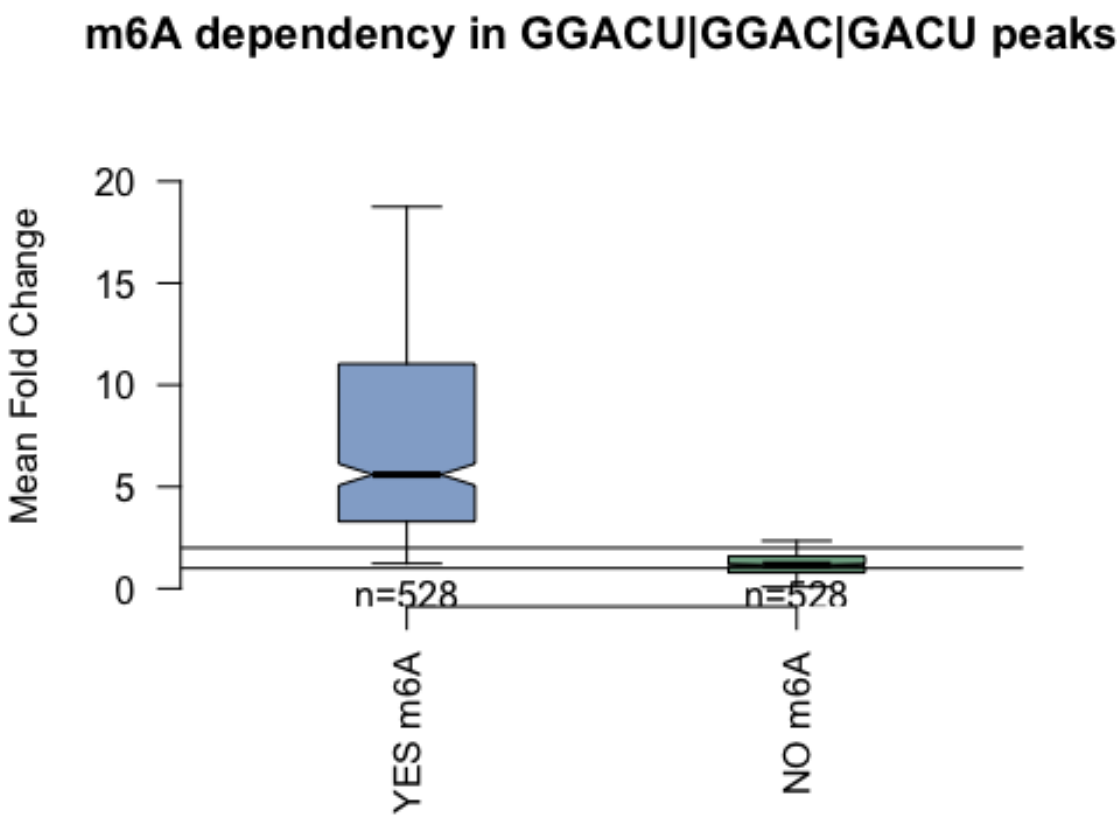


Figure 5F

```
# Most rapresented motif
Top_motif <- YTHDF1[grep("GGACT|GGAC|GACT",str_sub(YTHDF1$pos_fa,70,130)),]

BS_Top_motif <- list(log2( Top_motif$MeanFC+1  ),log2( Top_motif$MeanFC_T7+1
))
BS_Top_motif<- list(Top_motif$MeanFC,Top_motif$MeanFC_T7)

names(BS_Top_motif) <- c("YES m6A","NO m6A")



par(bty="n")
boxplot2(BS_Top_motif, outline=F, range=1, boxwex=0.35, notch=T, las=2,
ylab="Mean Fold Change", ylim=c(0,22), lty=1, main="m6A dependency in
GGACU|GGAC|GACU peaks", col=c("#93ADD0","#84B297"))
abline(h=c(1,2))
```




```
wt <- wilcox.test(BS_Top_motif$`YES m6A`, BS_Top_motif$`NO m6A`)
wt <- wt$p.value
```

Figure 5G

```
YTHDF1$positive_fa_check <- str_sub(YTHDF1$pos_fa, 85, 115)
YTHDF1$negative_fa_check <- str_sub(YTHDF1$neg_fa)
STRING_posT7 <- as.character(paste(YTHDF1$positive_fa_check, collapse =
"NN"))
STRING_negT7 <- as.character(paste(YTHDF1$negative_fa_check, collapse =
"NN"))

# Computing the counts of the most rapresented motif in k=5
k = 5
bases <- c("A", "C", "T", "G")
kmers <- unite(as.data.frame(permutations(n = 4, r = k, v = bases,
repeats.allowed = T)),
    col = kmers, sep = "")

kmers <- cbind(kmers, str_count(STRING_posT7, kmers[, 1]))
kmers <- cbind(kmers, str_count(STRING_negT7, kmers[, 1]))
colnames(kmers) <- c("K", "pos", "neg")
```

```
kmers$pos_fraction <- kmers$pos/sum(kmers$pos)
kmers$neg_fraction <- kmers$neg/sum(kmers$neg)
kmers <- kmers[kmers$pos > 10, ]
kmers$Enr <- kmers$pos_fraction/kmers$neg_fraction
kmers$RBP <- rep("YTHDF1", nrow(kmers))
kmers$K_length <- rep("5", nrow(kmers))
kmers <- kmers[(kmers$pos_fraction - kmers$neg_fraction) > 0, ]
kmers$Norm_enr <- kmers$pos_fraction * kmers$Enr
kmers5 <- kmers

noGGACT <- kmers5[grep("GGACT", kmers5$K, invert = T), ]
GGACT <- kmers5[grep("GGACT", kmers5$K), ]
GGACT$motif <- "GGACT"
GGAC <- noGGACT[grep("GGAC", noGGACT$K), ]
GGAC$motif <- rep("GGAC", nrow(GGAC))
GACT <- noGGACT[grep("GACT", noGGACT$K), ]
GACT$motif <- rep("GACT", nrow(GACT))
noMotif <- noGGACT[grep("GACT|GGAC|GGACT", noGGACT$K, invert = T), ]
noMotif$motif <- rep("None", nrow(noMotif))
kmers5 <- rbind(GGACT, GGAC, GACT, noMotif)



# Computing the counts of the most rapresented mtoif in k=6
k = 6
bases <- c("A", "C", "T", "G")
kmers <- unite(as.data.frame(permutations(n = 4, r = k, v = bases,
repeats.allowed = T)),
    col = kmers, sep = "")

kmers <- cbind(kmers, str_count(STRING_posT7, kmers[, 1]))
kmers <- cbind(kmers, str_count(STRING_negT7, kmers[, 1]))
colnames(kmers) <- c("K", "pos", "neg")

kmers$pos_fraction <- kmers$pos/sum(kmers$pos)
kmers$neg_fraction <- kmers$neg/sum(kmers$neg)
kmers <- kmers[kmers$pos > 10, ]
kmers$Enr <- kmers$pos_fraction/kmers$neg_fraction
kmers$RBP <- rep("YTHDF1", nrow(kmers))
kmers$K_length <- rep("6", nrow(kmers))
kmers <- kmers[(kmers$pos_fraction - kmers$neg_fraction) > 0, ]
kmers$Norm_enr <- kmers$pos_fraction * kmers$Enr
kmers6 <- kmers

noGGACT <- kmers6[grep("GGACT", kmers6$K, invert = T), ]
GGACT <- kmers6[grep("GGACT", kmers6$K), ]
GGACT$motif <- rep("GGACT", nrow(GGACT))
GGAC <- noGGACT[grep("GGAC", noGGACT$K), ]
GGAC$motif <- rep("GGAC", nrow(GGAC))
```

```r
GACT <- noGGACT[grep("GACT", noGGACT$K), ]
GACT$motif <- rep("GACT", nrow(GACT))
noMotif <- noGGACT[grep("GACT|GGAC|GGACT", noGGACT$K, invert = T), ]
noMotif$motif <- rep("None", nrow(noMotif))
kmers6 <- rbind(GGACT, GGAC, GACT, noMotif)



# Computing the counts of the most rapresented mtoif in k=6
k = 7
bases <- c("A", "C", "T", "G")
kmers <- unite(as.data.frame(permutations(n = 4, r = k, v = bases,
repeats.allowed = T)),
    col = kmers, sep = "")

kmers <- cbind(kmers, str_count(STRING_posT7, kmers[, 1]))
kmers <- cbind(kmers, str_count(STRING_negT7, kmers[, 1]))
colnames(kmers) <- c("K", "pos", "neg")

kmers$pos_fraction <- kmers$pos/sum(kmers$pos)
kmers$neg_fraction <- kmers$neg/sum(kmers$neg)
kmers <- kmers[kmers$pos > 10, ]
kmers$Enr <- kmers$pos_fraction/kmers$neg_fraction
kmers$RBP <- rep("YTHDF1", nrow(kmers))
kmers$K_length <- rep("7", nrow(kmers))
kmers <- kmers[(kmers$pos_fraction - kmers$neg_fraction) > 0, ]
kmers$Norm_enr <- kmers$pos_fraction * kmers$Enr
kmers7 <- kmers

noGGACT <- kmers7[grep("GGACT", kmers7$K, invert = T), ]
GGACT <- kmers7[grep("GGACT", kmers7$K), ]
GGACT$motif <- rep("GGACT", nrow(GGACT))
GGAC <- noGGACT[grep("GGAC", noGGACT$K), ]
GGAC$motif <- rep("GGAC", nrow(GGAC))
GACT <- noGGACT[grep("GACT", noGGACT$K), ]
GACT$motif <- rep("GACT", nrow(GACT))
noMotif <- noGGACT[grep("GACT|GGAC|GGACT", noGGACT$K, invert = T), ]
noMotif$motif <- rep("None", nrow(noMotif))
kmers7 <- rbind(GGACT, GGAC, GACT, noMotif)




kmers <- rbind(kmers5, kmers6, kmers7)
kmers$K_length <- factor(kmers$K_length, levels = c("5", "6", "7"))



plot <- ggplot() + geom_jitter(data = kmers[kmers$motif != "None", ], aes(x =
```

```
K_length,
    y = Norm_enr, size = log2(Norm_enr + 2), color = motif), width = 0.15,
alpha = 0.75) +
    scale_color_manual(values = c("#9ECAE1", "#DEEBF7", "#08306B")) + ylim(0,
0.06)


Fig5G <- plot + geom_jitter(data = kmers[kmers$motif == "None", ], aes(x =
K_length,
    y = Norm_enr, size = log2(Norm_enr + 2)), width = 0.25, alpha = 0.25,
color = "grey75") +
    theme(panel.background = element_blank(), panel.grid.major =
element_line(size = 0.25,
        linetype = 2, color = alpha("grey40", 0.5)), panel.grid.minor =
element_line(size = 0.1,
        linetype = 2, color = alpha("grey40", 0.5)), axis.text =
element_text(size = 20,
        color = "black"), panel.border = element_rect(fill = NA, color =
"black",
        size = 1), axis.ticks.length = unit(3, "mm"), axis.ticks =
element_line(size = 0.75,
        color = "black"))


Fig5G
```

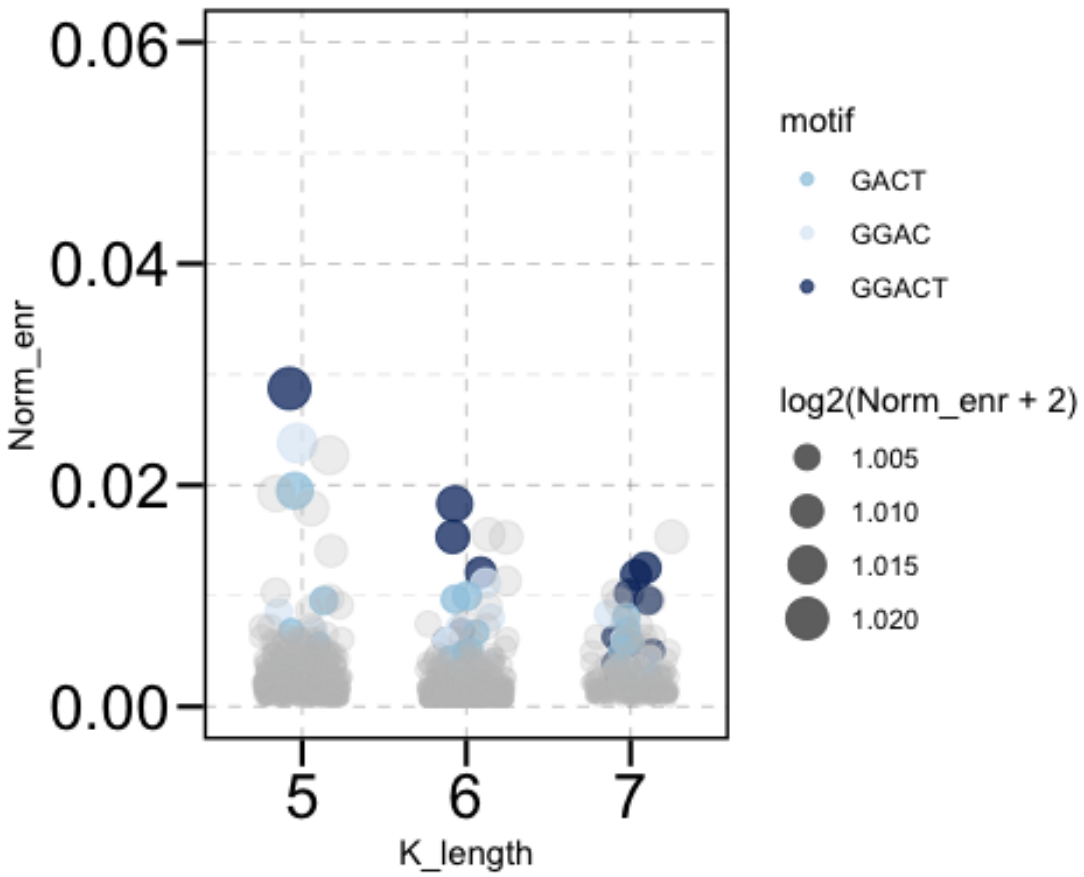
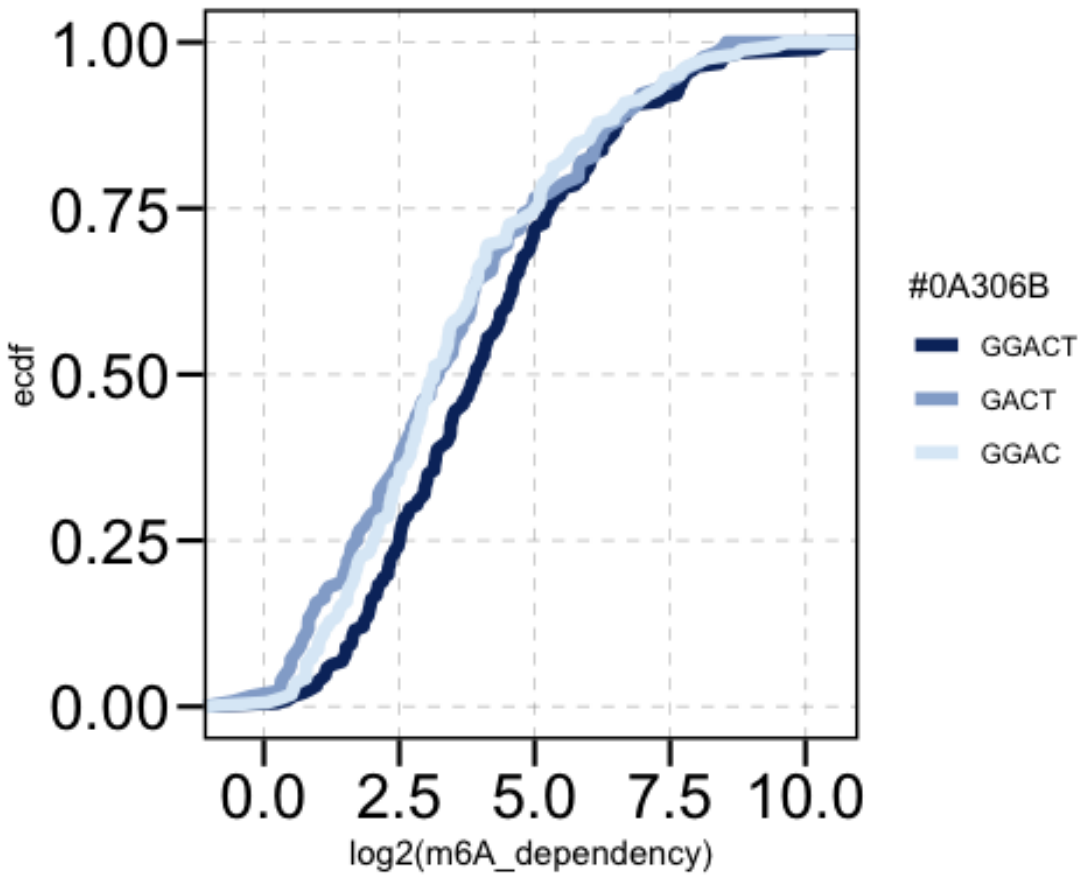


Figure 5H

```
YTHDF1$BS_T7[YTHDF1$BS_T7 == 0] <- min(YTHDF1$BS_T7[YTHDF1$BS_T7 != 0])
YTHDF1$m6A_dependency <- YTHDF1$BS/YTHDF1$BS_T7
YTHDF1$positive_fa_check <- str_sub(YTHDF1$pos_fa,70,130)

GGACT <-
YTHDF1[grep("GGACT",YTHDF1$positive_fa_check),c("peak_ID","positive_fa_check"
,"m6A_dependency","MeanFC","BS", "MeanFC_T7","BS_T7")]
GGACT$motif <- rep("GGACT",nrow(GGACT))


noGGACT <- YTHDF1[grep("GGACT",YTHDF1$positive_fa_check, invert =
T),c("peak_ID","positive_fa_check","m6A_dependency","MeanFC","BS",
"MeanFC_T7","BS_T7")]
noGGACT$motif <- rep("noGGACT",nrow(noGGACT))



GACT <- noGGACT[grep("GACT",noGGACT$positive_fa_check),]
GACT <- GACT[grep("GGAC",GACT$positive_fa,invert = T),]
GACT$motif <- rep("GACT",nrow(GACT))
```

```
GGAC <- noGGACT[grep("GGAC",noGGACT$positive_fa_check),]
GGAC <- GGAC[grep("GACT",GGAC$positive_fa,invert = T),]
GGAC$motif <- rep("GGAC",nrow(GGAC))




all_4mers <- noGGACT[grep("GACT|GGAC",noGGACT$positive_fa_check),]
unique_4mers <- rbind(GACT,GGAC)

two_or_more_4mers <-
all_4mers[grep(paste(setdiff(all_4mers$Peak_ID,unique_4mers$Peak_ID),collapse
= "|"),all_4mers$Peak_ID),]
two_or_more_4mers$motif <- rep("2orMORE", nrow(two_or_more_4mers))

YTH_motifs <- rbind(GGACT,unique_4mers,two_or_more_4mers)
YTH_motifs$motif <- factor(YTH_motifs$motif, levels =
c("GGACT","GACT","GGAC"))



Fig5H <- ggplot(data=YTH_motifs) +
  stat_ecdf(aes(log2(m6A_dependency), color=motif), geom = "line", lwd=2) +
  theme(panel.background = element_blank(),
        panel.grid.major = element_line(size = 0.25, linetype = 2,
color=alpha("grey50",0.5)),
        panel.grid.minor = element_blank(),
        axis.text = element_text(size=20,color = "black"),
        panel.border = element_rect(fill=NA,color="black", size = 1),
        axis.ticks.length = unit(3,"mm"),
        axis.ticks = element_line(size = 0.75, color = "black")) +
  scale_color_manual(values = c("#0A306B","#93ACD1",
"#DDEBF7"),c("#0A306B","#93ACD1", "#DDEBF7"))


Fig5H
```




```
list <- list(
  GACU = YTH_motifs[YTH_motifs$motif == "GACT","m6A_dependency"],
  GGAC  = YTH_motifs[YTH_motifs$motif == "GGAC","m6A_dependency"]
)

# run wilcox.test() for each, comparing to the overlap vector
wt <- lapply(names(list), function(nm) {
  y   <- list[[nm]]
  test <- wilcox.test(YTH_motifs[YTH_motifs$motif ==
"GGACT","m6A_dependency"], y)
  data.frame(
    comparison = paste0("GGACU_vs_", nm),
    W          = as.numeric(test$statistic),
    p.value    = test$p.value
  )
})

# combine into one df
wt_df <- do.call(rbind, wt)
```

Figure 5I

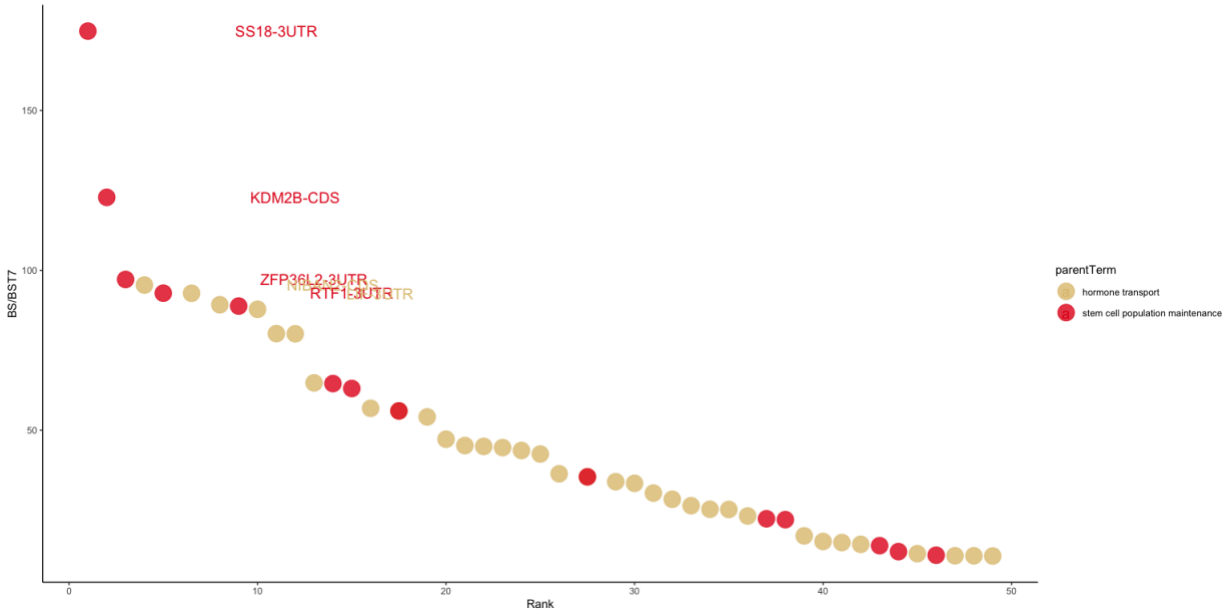
```r
# Extract unique gene IDs from the 'gene_ID' column
gene_IDs <- YTHDF1 %>%
    mutate(gene_ID = strsplit(gene_ID, "\\.") %>%
        lapply(., function(x) x[1]) %>%
        unlist())
gene_IDs <- unique(gene_IDs)

expressed_genes <- Inputs %>%
    mutate(Geneid = strsplit(Geneid, "\\.") %>%
        lapply(., function(x) x[1]) %>%
        unlist())

expressed_genes <- unique(c(expressed_genes$Geneid, gene_IDs$gene_ID))

ALL_GOs <- enrichGO(gene = gene_IDs$gene_ID, universe = expressed_genes,
keyType = "ENSEMBL",
    OrgDb = org.Hs.eg.db, ont = "ALL", pAdjustMethod = "BH", pvalueCutoff =
0.05,
    qvalueCutoff = 0.05, minGSSize = 15, readable = TRUE)
ALL_GO <- as.data.frame(ALL_GOs)

# Molecular function
MF <- ALL_GO[ALL_GO$ONTOLOGY == "MF", ]
# Collapse child GOs into parent GOs
simMatrix <- calculateSimMatrix(MF$ID, orgdb = "org.Hs.eg.db", ont = "MF",
method = "Rel")
scores <- setNames(-log10(MF$qvalue), MF$ID)
reducedTerms_MF <- reduceSimMatrix(simMatrix, scores, threshold = 0.7, orgdb
= "org.Hs.eg.db")
MFs <- reducedTerms_MF[, c("go", "parentTerm")]
MFs$Ont <- rep("GO:MF", nrow(MFs))
colnames(MFs) <- c("ID", "parentTerm", "Ont")




# Cellular component
CC <- ALL_GO[ALL_GO$ONTOLOGY == "CC", ]
# Collapse child GOs into parent GOs
simMatrix <- calculateSimMatrix(CC$ID, orgdb = "org.Hs.eg.db", ont = "CC",
method = "Rel")
scores <- setNames(-log10(CC$qvalue), CC$ID)
reducedTerms_CC <- reduceSimMatrix(simMatrix, scores, threshold = 0.7, orgdb
= "org.Hs.eg.db")
CCs <- reducedTerms_CC[, c("go", "parentTerm")]
CCs$Ont <- rep("GO:CC", nrow(CCs))
colnames(CCs) <- c("ID", "parentTerm", "Ont")
```

```r
# Biological process
BP <- ALL_GO[ALL_GO$ONTOLOGY == "BP", ]
# Collapse child GOs into parent GOs
simMatrix <- calculateSimMatrix(BP$ID, orgdb = "org.Hs.eg.db", ont = "BP",
method = "Rel")
scores <- setNames(-log10(BP$qvalue), BP$ID)
reducedTerms_BP <- reduceSimMatrix(simMatrix, scores, threshold = 0.7, orgdb
= "org.Hs.eg.db")
BPs <- reducedTerms_BP[, c("go", "parentTerm")]
BPs$Ont <- rep("GO:BP", nrow(BPs))
colnames(BPs) <- c("ID", "parentTerm", "Ont")




Parent_GOs <- rbind(BPs, CCs, MFs)
Child_GOs <- as.data.frame(ALL_GOs)
Child_GOs <- Child_GOs[, c("ID", "p.adjust", "geneID")]

merged_GO <- merge(Parent_GOs, Child_GOs, by = "ID")

# Initial dummy setup to allow rbind to work
ontology_vector <- c("O", "O")
parent_term_vector <- c("pT", "pT")
fdr_vector <- c("pp", "pp")
gene_vector <- c("gg", "gg")

# Initialize the result data frame
go_gene_mapping <- data.frame(ontology_vector, parent_term_vector,
fdr_vector, gene_vector)
colnames(go_gene_mapping) <- c("Ontology", "parentTerm", "FDR", "gene_name")

# Loop over each parent term
for (term in unique(merged_GO$parentTerm)) {

    gene_vector <- paste(merged_GO[grep(term, merged_GO$parentTerm),
"geneID"], collapse = "/")
    gene_vector <- unique(unlist(str_split(gene_vector, "\\/")))

    fdr_vector <- rep(median(merged_GO[grep(term, merged_GO$parentTerm),
"p.adjust"]),
        length(gene_vector))
    parent_term_vector <- rep(term, length(gene_vector))

    ontology_vector <- unique(merged_GO[grep(term, merged_GO$parentTerm),
"Ont"])
```

```r
    ontology_vector <- rep(ontology_vector, length(gene_vector))

    current_block <- data.frame(ontology_vector, parent_term_vector,
fdr_vector,
        gene_vector)
    colnames(current_block) <- c("Ontology", "parentTerm", "FDR",
"gene_name")

    go_gene_mapping <- rbind(go_gene_mapping, current_block)
}

# Remove the initial dummy rows
go_gene_mapping <- go_gene_mapping[-c(1, 2), ]


# Extract BS values and merge with GO mapping
BS_values <- YTHDF1[, c("gene_name", "feature", "BS", "BS_T7")]
GO_BS_value <- merge(BS_values, go_gene_mapping, by = "gene_name")
GO_BS_value$BS_T7[GO_BS_value$BS_T7 == 0] <-
min(GO_BS_value$BS_T7[GO_BS_value$BS_T7 !=
    0])


# Summarize total BS (binding sites) per parent GO term
bs_per_pathway <- GO_BS_value[, c("parentTerm", "BS")]
bs_per_pathway <- bs_per_pathway %>%
    group_by(parentTerm) %>%
    summarize(Pathway_BS = sum(BS))
bs_per_pathway <- as.data.frame(bs_per_pathway)

# Summarize total BS_T7 per parent GO term
bs_t7_per_pathway <- GO_BS_value[, c("parentTerm", "BS_T7")]
bs_t7_per_pathway <- bs_t7_per_pathway %>%
    group_by(parentTerm) %>%
    summarize(Pathway_BST7 = sum(BS_T7))
bs_t7_per_pathway <- as.data.frame(bs_t7_per_pathway)

# Merge BS and BS_T7 summaries
merged_bs_data <- merge(bs_per_pathway, bs_t7_per_pathway, by = "parentTerm")

# Add Ontology info (only unique pairings)
binding_site_counts <- unique(GO_BS_value[, c("parentTerm", "Ontology")])
merged_bs_data <- merge(merged_bs_data, binding_site_counts, by =
"parentTerm")

# Calculate m6A dependency score
merged_bs_data$m6A_dependency <-
merged_bs_data$Pathway_BS/merged_bs_data$Pathway_BST7
```

```r
# Count how many genes (binding sites) per parentTerm
binding_site_counts <-
as.data.frame(table(as.character(GO_BS_value$parentTerm)))
colnames(binding_site_counts) <- c("parentTerm", "Binding_Sites")
merged_bs_data <- merge(merged_bs_data, binding_site_counts, by =
"parentTerm")

# Merge FDR values
fdr_info <- unique(GO_BS_value[, c("parentTerm", "FDR")])
merged_bs_data <- merge(merged_bs_data, fdr_info, by = "parentTerm")


# Filter top 10 pathways by m6A dependency
merged_bs_data$FDR <- as.numeric(merged_bs_data$FDR)
top_pathways <- merged_bs_data %>%
    top_n(10, m6A_dependency)

# Remove duplicates and order factor levels by dependency
top_pathways <- top_pathways[!duplicated(top_pathways$parentTerm), ]
top_pathways$parentTerm <- factor(top_pathways$parentTerm, levels =
top_pathways[order(top_pathways$m6A_dependency),
    "parentTerm"])




term_selected <- unique(GO_BS_value[grep("stem cell population
maintenance|hormone transport",
    GO_BS_value$parentTerm), c("gene_name", "parentTerm")])
term_selected <- merge(term_selected, YTHDF1, by = "gene_name")
term_selected <- unique(term_selected[, c("gene_name", "parentTerm",
"peak_ID", "BS",
    "BS_T7", "MeanFC", "MeanFC_T7", "feature", "YTHDF1_rep1_signal",
"YTHDF1_rep2_signal")])
term_selected$Signal <- (term_selected$YTHDF1_rep1_signal +
term_selected$YTHDF1_rep2_signal)/2
term_selected$BS_T7[term_selected$BS_T7 < 1] <- 1
term_selected$m6A_dependency_sites <- term_selected$BS/term_selected$BS_T7
term_selected <- term_selected[term_selected$m6A_dependency_sites >= 10, ]
term_selected$Rank <- rank(-term_selected$m6A_dependency_sites)
term_selected <- term_selected[order(term_selected$Rank), ]
term_selected$COLOR <- gsub("hormone transport", "#DFC27D",
term_selected$parentTerm)
term_selected$COLOR <- gsub("stem cell population maintenance", "#E31A1C",
term_selected$COLOR)
```

```r
# The best two for each term are showed in the figure, LIF belong to both, so
# the stem cell population maintenance one is deleted
term_selected <- term_selected %>%
    filter(!(gene_name == "LIF" & parentTerm == "stem cell population
maintenance"))
top6 <- head(term_selected, 6)



plot <- ggplot(data = term_selected, aes(x = Rank, y =
(m6A_dependency_sites), color = parentTerm)) +
    geom_point(size = 7, alpha = 0.8) + theme_classic() + ylab("BS/BST7")

Fig5I <- plot + geom_text(data = top6, aes(x = Rank + 10, y =
(m6A_dependency_sites),
    color = parentTerm, label = paste(gene_name, feature, sep = "-")), size =
5) +
    scale_color_manual(values = c("#DFC27D", "#E31A1C"))

Fig5I
```




```r
# In the manuscript only the top 2 for each class are showed.

sessionInfo()

## R version 4.3.2 (2023-10-31)
## Platform: aarch64-apple-darwin20 (64-bit)
## Running under: macOS Sonoma 14.5
##
## Matrix products: default
```

```
## BLAS:   /Library/Frameworks/R.framework/Versions/4.3-
arm64/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.3-
arm64/Resources/lib/libRlapack.dylib;  LAPACK version 3.11.0
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## time zone: Europe/Stockholm
## tzcode source: internal
##
## attached base packages:
## [1] stats4    stats     graphics  grDevices utils     datasets  methods
## [8] base
##
## other attached packages:
##  [1] LSD_4.1-0              rrvgo_1.14.2           org.Hs.eg.db_3.18.0
##  [4] AnnotationDbi_1.64.1   IRanges_2.36.0         S4Vectors_0.40.2
##  [7] Biobase_2.62.0         BiocGenerics_0.48.1    clusterProfiler_4.10.1
## [10] corrplot_0.95          gtools_3.9.5           dendextend_1.19.0
## [13] ggpubr_0.6.0           lubridate_1.9.4        forcats_1.0.0
## [16] stringr_1.5.1          dplyr_1.1.4            purrr_1.0.4
## [19] readr_2.1.5            tidyr_1.3.1            tibble_3.2.1
## [22] tidyverse_2.0.0        gplots_3.2.0           reshape2_1.4.4
## [25] ggplot2_3.5.2          eulerr_7.0.2
##
## loaded via a namespace (and not attached):
##   [1] RColorBrewer_1.1-3      rstudioapi_0.17.1       jsonlite_2.0.0
##   [4] umap_0.2.10.0           magrittr_2.0.3          farver_2.1.2
##   [7] rmarkdown_2.29          fs_1.6.6                zlibbioc_1.48.2
##  [10] vctrs_0.6.5             memoise_2.0.1           RCurl_1.98-1.17
##  [13] askpass_1.2.1           ggtree_3.10.1           rstatix_0.7.2
##  [16] htmltools_0.5.8.1       broom_1.0.8             Formula_1.2-5
##  [19] gridGraphics_0.5-1      KernSmooth_2.23-26      plyr_1.8.9
##  [22] cachem_1.1.0            igraph_2.1.4            mime_0.13
##  [25] lifecycle_1.0.4         pkgconfig_2.0.3         gson_0.1.0
##  [28] Matrix_1.6-5            R6_2.6.1                fastmap_1.2.0
##  [31] shiny_1.10.0            GenomeInfoDbData_1.2.11 digest_0.6.37
##  [34] aplot_0.2.5             enrichplot_1.22.0       colorspace_2.1-1
##  [37] patchwork_1.3.0         RSpectra_0.16-2         RSQLite_2.3.11
##  [40] labeling_0.4.3          timechange_0.3.0        httr_1.4.7
##  [43] polyclip_1.10-7         abind_1.4-8             compiler_4.3.2
##  [46] bit64_4.6.0-1           withr_3.0.2             backports_1.5.0
##  [49] BiocParallel_1.36.0     carData_3.0-5           viridis_0.6.5
##  [52] DBI_1.2.3               ggforce_0.4.2           ggsignif_0.6.4
##  [55] MASS_7.3-60.0.1         openssl_2.3.2           HDO.db_0.99.1
##  [58] caTools_1.18.3          tools_4.3.2             scatterpie_0.2.4
##  [61] ape_5.8-1               httpuv_1.6.16           glue_1.8.0
##  [64] promises_1.3.2          nlme_3.1-168            GOSemSim_2.28.1
##  [67] polylabelr_0.3.0        shadowtext_0.1.4        grid_4.3.2
```

```
##  [70] gridBase_0.4-7          fgsea_1.28.0            generics_0.1.3
##  [73] gtable_0.3.6            tzdb_0.5.0              data.table_1.17.0
##  [76] hms_1.1.3               xml2_1.3.8              tidygraph_1.3.1
##  [79] car_3.1-3               XVector_0.42.0          ggrepel_0.9.6
##  [82] pillar_1.10.2           yulab.utils_0.2.0       later_1.4.2
##  [85] splines_4.3.2           tweenr_2.0.3            treeio_1.26.0
##  [88] lattice_0.22-7          bit_4.6.0               tidyselect_1.2.1
##  [91] GO.db_3.18.0            tm_0.7-16               Biostrings_2.70.3
##  [94] knitr_1.50              gridExtra_2.3           NLP_0.3-2
##  [97] xfun_0.52               graphlayouts_1.2.2      pheatmap_1.0.12
## [100] stringi_1.8.7           lazyeval_0.2.2          ggfun_0.1.8
## [103] yaml_2.3.10             evaluate_1.0.3          codetools_0.2-20
## [106] wordcloud_2.6           ggraph_2.2.1            qvalue_2.34.0
## [109] ggplotify_0.1.2         cli_3.6.5               reticulate_1.42.0
## [112] xtable_1.8-4            treemap_2.4-4           dichromat_2.0-0.1
## [115] Rcpp_1.0.14             GenomeInfoDb_1.38.8     png_0.1-8
## [118] parallel_4.3.2          blob_1.2.4              DOSE_3.28.2
## [121] bitops_1.0-9            slam_0.1-55             viridisLite_0.4.2
## [124] tidytree_0.4.6          scales_1.4.0            crayon_1.5.3
## [127] rlang_1.1.6             cowplot_1.1.3           fastmatch_1.1-6
## [130] KEGGREST_1.42.0         formatR_1.14
```