Figure 3 - Combined analysis of cRBP & ncRBP targets

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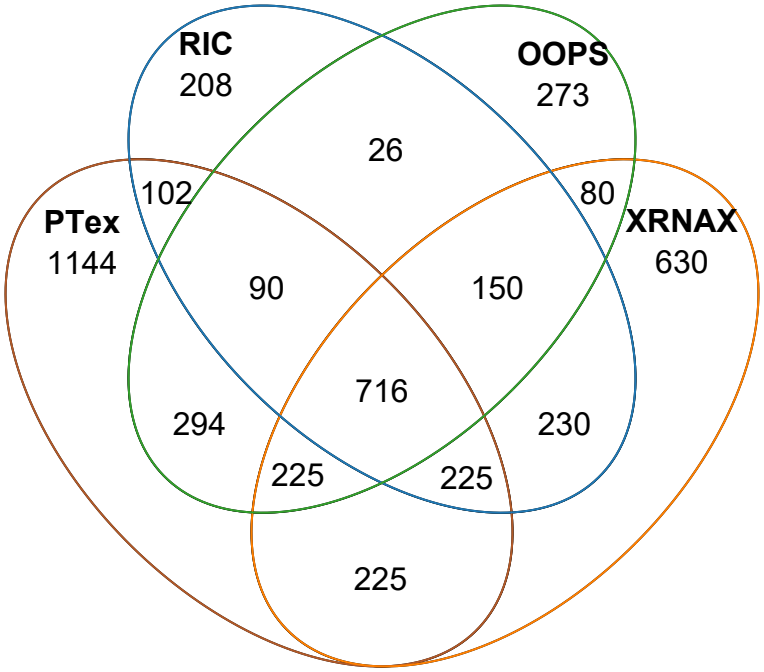
```
# source and library import
source('code/00_helper_functions.R')
library(tidyverse)
library(org.Hs.eg.db)
library(eulerr)
library(g3viz)
library(patchwork)
library(ComplexUpset)
library(clusterProfiler)
library(rrvgo)
library(ComplexHeatmap)
library(circlize)
library(fgsea)
library(ggrepel)
```

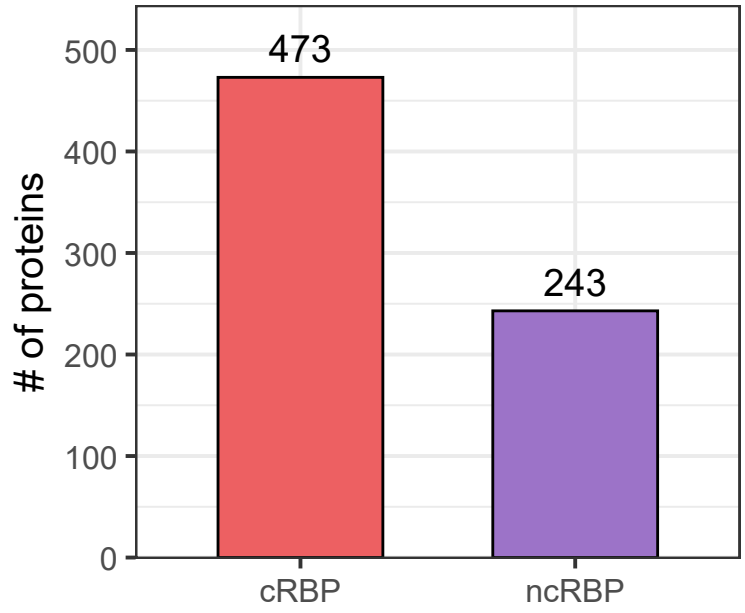
Figure S3A - RBPome overlaps

```
# RBPomes
RBPomes <- list(
  PTex = read.csv(file = "data/RBPomes/PTex.csv", stringsAsFactors = F, header = T, sep=";"),
  XRNAX = read.csv(file = "data/RBPomes/XRNAX_table.csv", stringsAsFactors = F, header = T, sep=";"),
  RIC = read.csv(file = "data/RBPomes/RIC_table.csv", stringsAsFactors = F, header = T, sep=";"),
  OOPS = read.csv(file = "data/RBPomes/OOPS.csv", stringsAsFactors = F, header = T, sep=";")
)

# extract RBPs
RBPomes$PTex <- RBPomes$PTex %>%
  pull(Gene.name) %>%
  unique()
RBPomes$XRNAX <- RBPomes$XRNAX %>%
  pull(Gene.name) %>%
  unique()
RBPomes$RIC <- RBPomes$RIC[rowSums(RBPomes$RIC[,seq(4,length(RBPomes$RIC))])>0,] %>%
  pull(UNIQUE) %>%
  unique()
RBPomes$OOPS <- AnnotationDbi::select(org.Hs.eg.db, keys=RBPomes$OOPS$Uniprot_ID, columns = c('UNIPROT', 'SYMBOL'), keytype="UNIPROT") %>%
  pull(SYMBOL) %>%
  unique()

# plot venn diagram
plot(venn(RBPomes), fills = FALSE, edges=c("#B15928", "#FF7F00", "#1F78B4", "#33A02C"), quantities=TRUE)
```




```r
# overlap of all 4 RBPome datasets
overlap <- table(unlist(RBPomes))[table(unlist(RBPomes))==4] %>%
  names()

# resources for canonical RBPs
RBDs <- read.table(file = "Data/RBPomes/RBDs_in_RBP_Census.txt", stringsAsFactors = F, header = T, sep="\t")
RBP_Census <- read.csv(file = "Data/RBPomes/Canonical_RBPs_Gerstberger_et_al.csv", stringsAsFactors = F, header = T, sep=";")
RBPDB <- read.table(file = "Data/RBPomes/RBPDB.utoronto.txt", stringsAsFactors = F, header = T, sep="\t")

pattern <- paste(RBDs$Pfam.RNA.binding.domains, collapse = "|")
RBP_Census <- RBP_Census[grepl(pattern,RBP_Census$domains.count.) | grepl("established",RBP_Census$supporting.evidence....pubmed.ID.),] %>%
  pull(gene.name) %>%
  unique()

RBPDB <- RBPDB$Annotation.ID %>%
  AnnotationDbi::select(org.Hs.eg.db, keys=., columns = c('ENSEMBL','SYMBOL'), keytype="ENSEMBL") %>%
  pull(SYMBOL) %>%
  unique()

# remove high confident RBPs with RBDs based on published annotations
ncRBPs_unfilt <- setdiff(overlap, unique(c(RBP_Census, RBPDB)))

ncRBPs_unfilt_ids <- AnnotationDbi::select(org.Hs.eg.db, keys=ncRBPs_unfilt, columns = c('SYMBOL','UNIPROT','GENENAME'), keytype="SYMBOL") %>%
  dplyr::rename(gene_name = SYMBOL, uniprot = UNIPROT, Description = GENENAME)

# extract Pfam domains and filter ncRBPs
ncRBPs <- lapply(ncRBPs_unfilt_ids$uniprot, uniprot2pfam) %>%
  bind_rows() %>%
  merge(ncRBPs_unfilt_ids, by="uniprot") %>%
  # dplyr::slice(grep(paste(unique(RBDs$Pfam.RNA.binding.domains), collapse = "|"), .$hmm.name, invert = T))
  filter(!grepl(paste(unique(.$gene_name[grepl(pattern, hmm.name)]), collapse = "|"), gene_name))

# manual curation to remove splicing factors, elongation factors, etc
ncRBPs <- ncRBPs %>%
  filter(!grepl(c("TCERG1|AQR|PRPF40A|PSIP1|GTF2F1|ERH|TCOF1|PRPF4B|TMA16|SCAF11|EIF3|ERH|NVL|RNH1|RPRD2|TCEA1|ZNF598"), gene_name)) %>%
  pull(gene_name) %>%
  unique()

# get canonical RBPs
cRBPs <- setdiff(overlap, ncRBPs)

# plot cRBPs vs ncRBPs
df <- data.frame(
  Category = c("cRBP", "ncRBP"),
```

```
  Count = c(length(cRBPs), length(ncRBPs))
)

ggplot(df, aes(x = Category, y = Count, fill = Category)) +
  geom_bar(stat = "identity", width = 0.6, color='black', show.legend = FALSE) +
  geom_text(aes(label = Count), vjust = -0.5, size = 5) +
  scale_fill_manual(values = c("cRBP" = "#ED6062", "ncRBP" = "#9C73C8")) +
  scale_y_continuous(expand =expansion(mult = c(.002, .15))) +
  labs(
    title = "",
    x = "",
    y = "# of proteins"
  ) +
  theme_bw(base_size = 15)
```



Load RAPseq peaks

```
all_RBP_peaks <- lapply(list.files("data/peaks/"), function(x) {
  read.table(paste("data/peaks/",x, sep=""), header = T, stringsAsFactors = F, sep="\t")
}) %>%
  bind_rows() %>%
  mutate(RBP = gsub("_final","",RBP)) %>%
  mutate(RBP = str_to_upper(RBP))

# split canonical and noncanonical RBP peaks
cRBP_peaks <- all_RBP_peaks %>%
  filter(str_detect(RBP, "RBFOX|HSHUR|PTBP|IGF2BP|HNRNP|YBX|IRP1|YTHDF1")) %>%
  filter(!str_detect(RBP, "HURPTBP1|HSHURFISH|IGF2BP1R|IGF2BP2B|IGF2BP3R|IGF2BP3I")) %>%
  mutate(RBP = str_replace(RBP, "HSHURHUMAN", "HUR")) %>%
  mutate(RBP = str_replace(RBP, "IGF2BP1WT", "IGF2BP1")) %>%
  mutate(RBP = str_replace(RBP, "IGF2BP3WT", "IGF2BP3"))

ncRBP_peaks <- all_RBP_peaks %>%
  filter(!str_detect(RBP, "RBFOX|HUR|PTBP|IGF2BP|HNRNP|YBX|IRP1|YTHDF1"))

RBP_peaks <- rbind(cRBP_peaks %>% mutate(RBP_type = 'cRBP'),
                   ncRBP_peaks %>% mutate(RBP_type = 'ncRBP'))
```

Figure S3B - RBP binding sites

```
RBP_BS <- RBP_peaks %>%
  group_by(RBP) %>%
  summarise(n_sites = n(), RBP_type) %>%
  distinct() %>%
  arrange(desc(n_sites))

ggplot(RBP_BS, aes(x=RBP_type, y=n_sites, fill=RBP_type)) +
  geom_boxplot(color = "black", outlier.shape = NA) +
  scale_fill_manual(values = c("cRBP" = "#ED6062", "ncRBP" = "#9C73C8")) +
  labs(
    x = "",
    y = "# of Peaks"
  ) +
  ylim(0, 15000) +
  theme_classic(base_size = 14) +
  theme(
    axis.text.x = element_text(size = 10, angle = 90, hjust = 1, vjust = 0.5),
    plot.margin = margin(10, 10, 10, 10)
  )
```

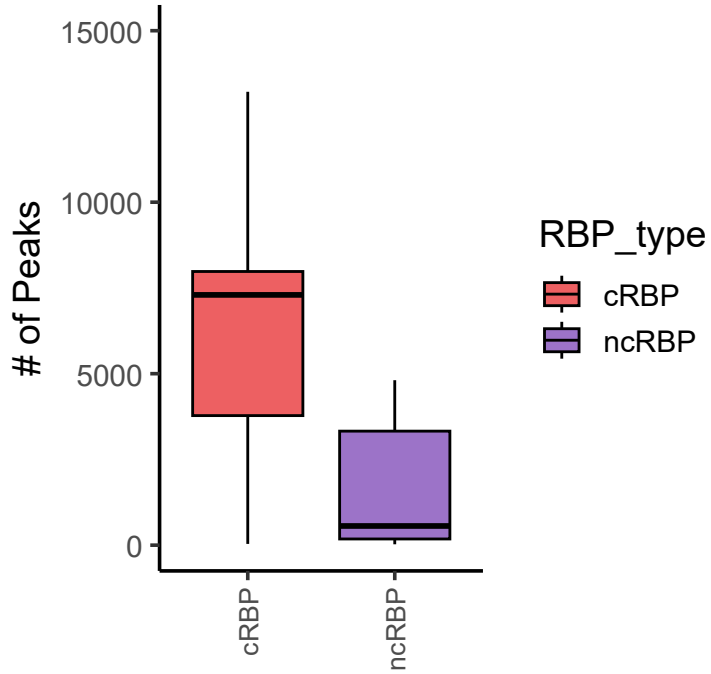
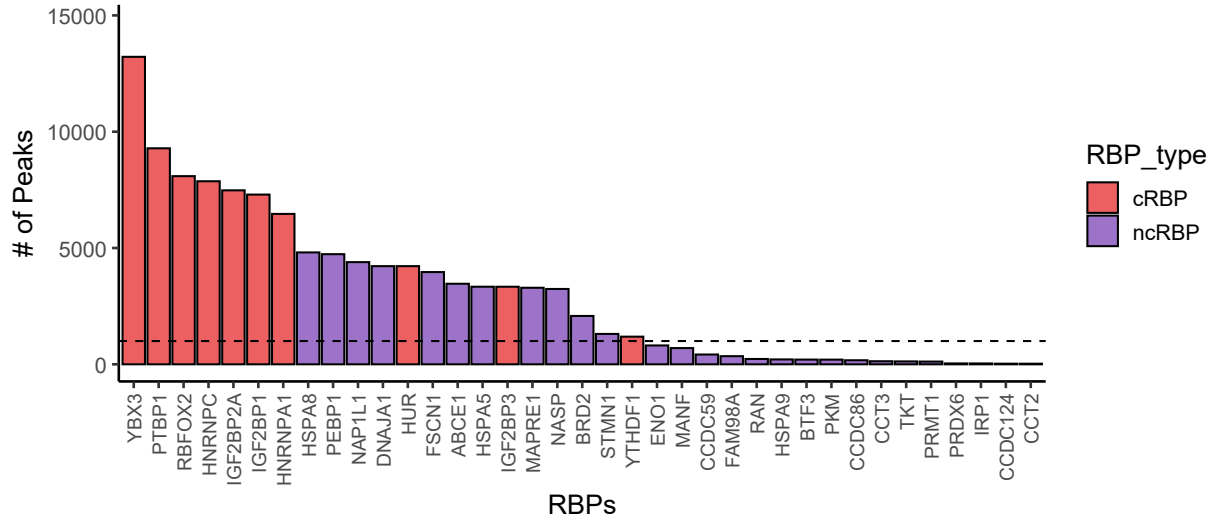


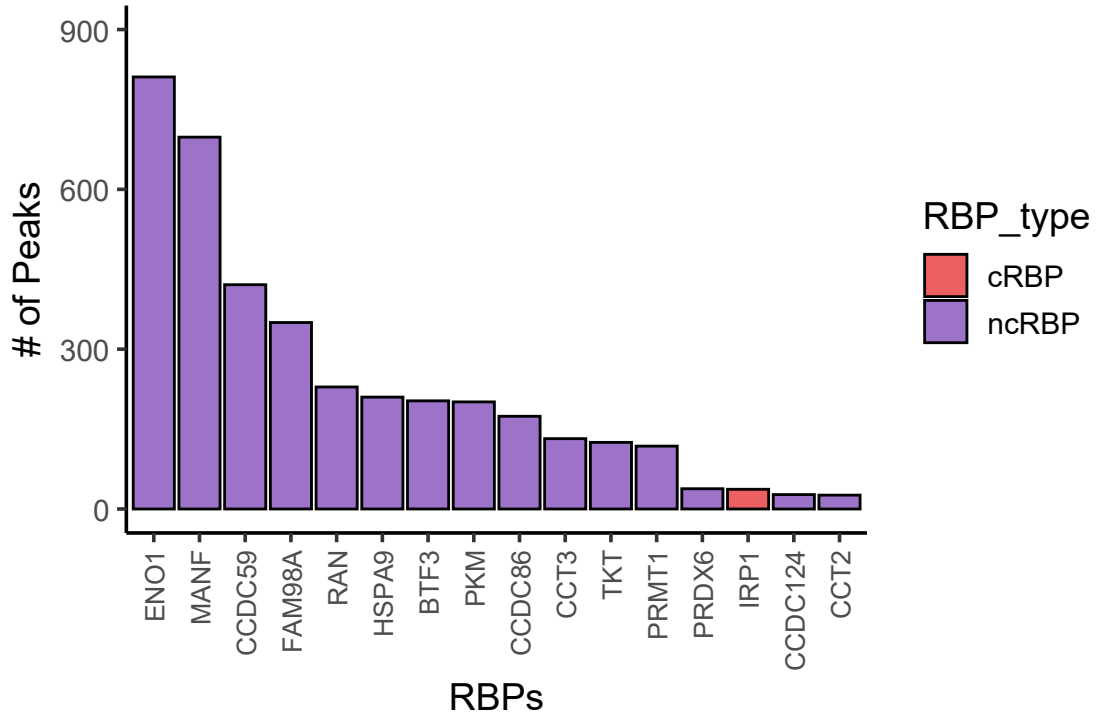
Figure S3C - RBP binding sites per RBP

```
# get binding sites for each RBP
RBP_BS <- RBP_peaks %>%
  group_by(RBP) %>%
  summarise(n_sites = n(), RBP_type) %>%
  distinct() %>%
  arrange(desc(n_sites))
## canonical RBP
cRBP_BS <- cRBP_peaks %>%
  group_by(RBP) %>%
  summarise(n_sites = n()) %>%
  arrange(desc(n_sites))
## noncanonical RBP
```

```r
ncRBP_BS <- ncRBP_peaks %>%
  group_by(RBP) %>%
  summarise(n_sites = n()) %>%
  arrange(desc(n_sites))

y_lim <- max(RBP_BS$n_sites)

p <- ggplot(RBP_BS, aes(x = reorder(RBP, -n_sites), y = n_sites, fill = RBP_type)) +
  geom_bar(stat = "identity", color = "black") +
  geom_hline(yintercept = 1000, linetype = "dashed", color = "black") +
  # geom_text(aes(label = n_sites),
  #           vjust = -0.5,
  #           size = 3) +
  scale_fill_manual(values = c("cRBP" = "#ED6062", "ncRBP" = "#9C73C8")) +
  labs(
    x = "RBPs",
    y = "# of Peaks"
  ) +
  ylim(0, y_lim + 1500) +
  theme_classic(base_size = 14) +
  theme(
    axis.text.x = element_text(size = 10, angle = 90, hjust = 1, vjust = 0.5),
    plot.margin = margin(10, 10, 10, 10)
  )

p
```




```r
df <- RBP_BS %>%
  filter(n_sites<1000)

p <- ggplot(df, aes(x = reorder(RBP, -n_sites), y = n_sites, fill = RBP_type)) +
  geom_bar(stat = "identity", color = "black") +
  scale_fill_manual(values = c("cRBP" = "#ED6062", "ncRBP" = "#9C73C8")) +
  labs(
    x = "RBPs",
    y = "# of Peaks"
  ) +
  scale_y_continuous(limits = c(0, 900), breaks = seq(0, 900, by = 300)) +
  theme_classic(base_size = 14) +
  theme(
    axis.text.x = element_text(size = 10, angle = 90, hjust = 1, vjust = 0.5),
    plot.margin = margin(10, 10, 10, 10)
  )

p
```




```
# filter RBPs with les than 1000 peaks
cRBP_names <- cRBP_BS %>%
  pull(RBP)
cRBP_filt_names <- cRBP_BS %>%
  filter(n_sites >= 1000) %>%
  pull(RBP)
cRBP_filt_peaks <- cRBP_peaks %>%
  filter(RBP %in% cRBP_filt_names)

ncRBP_names <- ncRBP_BS %>%
  pull(RBP)
ncRBP_filt_names <- ncRBP_BS %>%
  filter(n_sites >= 1000) %>%
  pull(RBP)
ncRBP_filt_peaks <- ncRBP_peaks %>%
  filter(RBP %in% ncRBP_filt_names)

RBP_filt_names <- c(cRBP_filt_names, ncRBP_filt_names)
RBP_filt_peaks <- rbind(cRBP_filt_peaks %>% mutate(RBP_type = 'cRBP'),
                        ncRBP_filt_peaks %>% mutate(RBP_type = 'ncRBP'))
```

Figure 3A - RBP target distribution

```
# feature_colors <- brewer.pal(8, "Set3")
feature_colors <- c("intron" = "#08306B",
                    "exon" = "#08519C",
                    "CDS" = "#4292C6",
                    "5UTR" = "#9ECAE1",
                    "3UTR" = "#DEEBF7")

# get uniquely targeted genes
cRBP_filt_genes <- cRBP_filt_peaks %>%
  group_by(RBP) %>%
  summarise(n_genes = n_distinct(gene_name)) %>%
  arrange(desc(n_genes))

ncRBP_filt_genes <- ncRBP_filt_peaks %>%
  group_by(RBP) %>%
  summarise(n_genes = n_distinct(gene_name)) %>%
```

```
  arrange(desc(n_genes))

# get genomic feature distribution
cRBP_filt_features <- cRBP_filt_peaks %>%
  group_by(RBP, feature) %>%
  summarise(n = n()) %>%
  mutate(percentage = n / sum(n) * 100) %>%
  ungroup() %>%
  mutate(RBP = factor(RBP, levels = rev(cRBP_filt_genes$RBP)),
         feature = factor(feature, levels = rev(names(feature_colors))))

ncRBP_filt_features <- ncRBP_filt_peaks %>%
  group_by(RBP, feature) %>%
  summarise(n = n()) %>%
  mutate(percentage = n / sum(n) * 100) %>%
  ungroup() %>%
  mutate(RBP = factor(RBP, levels = rev(ncRBP_filt_genes$RBP)),
         feature = factor(feature, levels = rev(names(feature_colors))))

# get log2FC to Halo
cRBP_filt_FC <- cRBP_filt_peaks %>%
  mutate(log2_FC = log2(Mean_FCH)) %>%
  mutate(RBP = factor(RBP, levels = rev(cRBP_filt_genes$RBP)))

ncRBP_filt_FC <- ncRBP_filt_peaks %>%
  mutate(log2_FC = log2(Mean_FCH)) %>%
  mutate(RBP = factor(RBP, levels = rev(ncRBP_filt_genes$RBP)))

# define maximum x-axis limits
max_genes <- max(cRBP_filt_genes$n_genes,ncRBP_filt_genes$n_genes)
max_FC <- max(c(max(cRBP_filt_FC$log2_FC), max(ncRBP_filt_FC$log2_FC)))


# plot
plot_cRBP_genes <- ggplot(cRBP_filt_genes, aes(x = n_genes, y = reorder(RBP, n_genes))) +
  geom_bar(stat = "identity", fill = '#ED6062', width = 0.8, color = "black") +
  geom_text(aes(label = n_genes),
            hjust = -0.2,
            size = 3.5) +
  labs(
    x = NULL,
    y = "Canonical RBPs"
  ) +
  # xlim(0, max_genes + 1500) +
  scale_x_continuous(limits = c(0, max_genes + 1500), breaks = c(0, 3000, 6000)) +
  theme_classic(base_size = 12) +
  theme(
    axis.text.y = element_text(size = 10),
    axis.text.x = element_blank(),
    axis.ticks.x = element_blank(),
    plot.margin = margin(10, 10, 10, 10)
  )
plot_ncRBP_genes <- ggplot(ncRBP_filt_genes, aes(x = n_genes, y = reorder(RBP, n_genes))) +
  geom_bar(stat = "identity", fill = '#9C73C8', width = 0.8, color = "black") +
  geom_text(aes(label = n_genes),
            hjust = -0.2,
            size = 3.5) +
  labs(
    x = "# of Target Genes",
    y = "Noncanonical RBPs"
  ) +
  # xlim(0, max_genes + 1500) +
  scale_x_continuous(limits = c(0, max_genes + 1500), breaks = c(0, 3000, 6000)) +
  theme_classic(base_size = 12) +
  theme(
    axis.text.y = element_text(size = 10),
    plot.margin = margin(10, 10, 10, 10)
  )
plot_cRBP_features <- ggplot(cRBP_filt_features,
                             aes(x = percentage, y = RBP, fill = feature)) +
  geom_bar(stat = "identity", position = "fill", width = 0.8, color="black") +
  scale_fill_manual(values = feature_colors) +
  labs(
    x = NULL,
    y = NULL,
    fill = "Feature"
  ) +
  theme_classic(base_size = 12) +
  theme(
    legend.position="none",
```

```
    axis.text.y = element_blank(),
    axis.text.x = element_blank(),
    axis.ticks.y = element_blank(),
    axis.ticks.x = element_blank(),
    plot.margin = margin(10, 10, 10, 10)
  )
plot_ncRBP_features <- ggplot(ncRBP_filt_features,
                              aes(x = percentage, y = RBP, fill = feature)) +
  geom_bar(stat = "identity", position = "fill", width = 0.8, color="black") +
  scale_fill_manual(values = feature_colors) +
  labs(
    x = "Feature Distribution (%)",
    y = NULL,
    fill = "Feature"
  ) +
  theme_classic(base_size = 12) +
  theme(
    legend.position="none",
    axis.text.y = element_blank(),
    axis.ticks.y = element_blank(),
    # axis.ticks.x = element_blank(),
    plot.margin = margin(10, 10, 10, 10)
  )
plot_cRBP_FC <- ggplot(cRBP_filt_FC, aes(x = log2_FC, y = RBP)) +
  geom_boxplot(fill = "#ED6062", color = "black", outlier.shape = NA) +
  labs(
    x = NULL,
    y = NULL
  ) +
  xlim(0, max_FC) +
  scale_x_continuous(breaks = c(0, 4, 8)) +
  theme_classic(base_size = 12) +
  theme(
    axis.text.y = element_blank(),
    axis.text.x = element_blank(),
    axis.ticks.x = element_blank(),
    plot.margin = margin(10, 10, 10, 10)
  )
plot_ncRBP_FC <- ggplot(ncRBP_filt_FC, aes(x = log2_FC, y = RBP)) +
  geom_boxplot(fill = "#9C73C8", color = "black", outlier.shape = NA) +
  labs(
    x = "log2(FC to Halo)",
    y = NULL
  ) +
  xlim(0, max_FC) +
  scale_x_continuous(breaks = c(0, 4, 8)) +
  theme_classic(base_size = 12) +
  theme(
    axis.text.y = element_blank(),
    axis.text.x = element_text(size = 10),
    plot.margin = margin(10, 10, 10, 10)
  )


combined_plot <- (plot_cRBP_genes | plot_cRBP_features | plot_cRBP_FC) / (plot_ncRBP_genes | plot_ncRBP_features | plot_ncRBP_FC)

print(combined_plot)
```

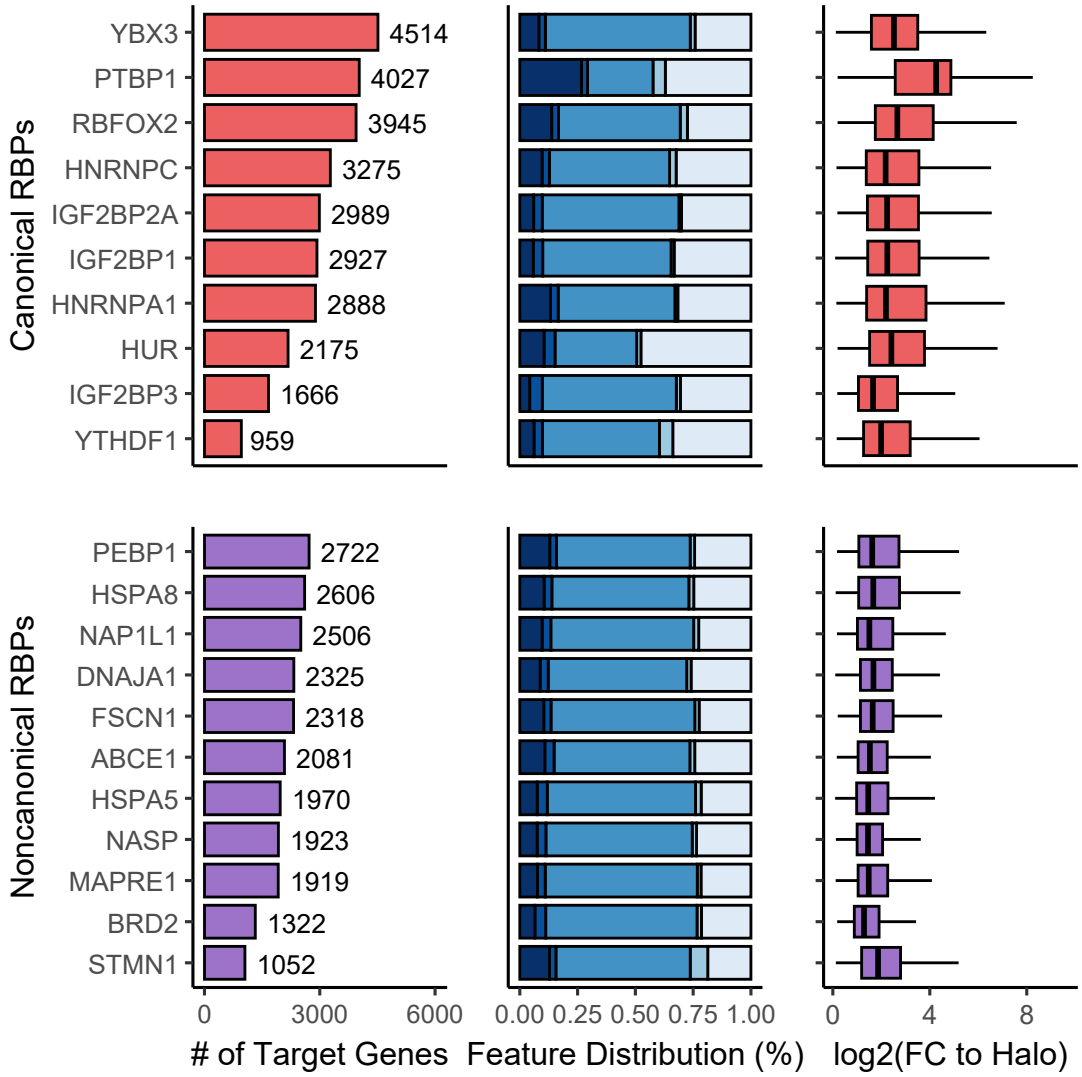


Figure S3D - RBP binding sites per gene

```
RBP_BS_per_gene_mean <- RBP_peaks %>%
  group_by(RBP,gene_name) %>%
  summarise(n_sites = n(), RBP_type) %>%
  distinct() %>%
  group_by(RBP) %>%
  summarise(mean_sites_per_gene = mean(n_sites), RBP_type) %>%
  distinct() %>%
  arrange(desc(mean_sites_per_gene))

p <- ggplot(RBP_BS_per_gene_mean, aes(x=RBP_type, y=mean_sites_per_gene, fill=RBP_type)) +
  geom_boxplot(color = "black", outlier.shape = NA) +
  scale_fill_manual(values = c("cRBP" = "#ED6062", "ncRBP" = "#9C73C8")) +
  labs(
    x = "",
    y = "Mean # of Peaks per Gene"
  ) +
  ylim(0, 3) +
  theme_classic(base_size = 14) +
  theme(
```

```
    axis.text.x = element_text(size = 10, angle = 90, hjust = 1, vjust = 0.5),
    plot.margin = margin(10, 10, 10, 10)
  )

p
```

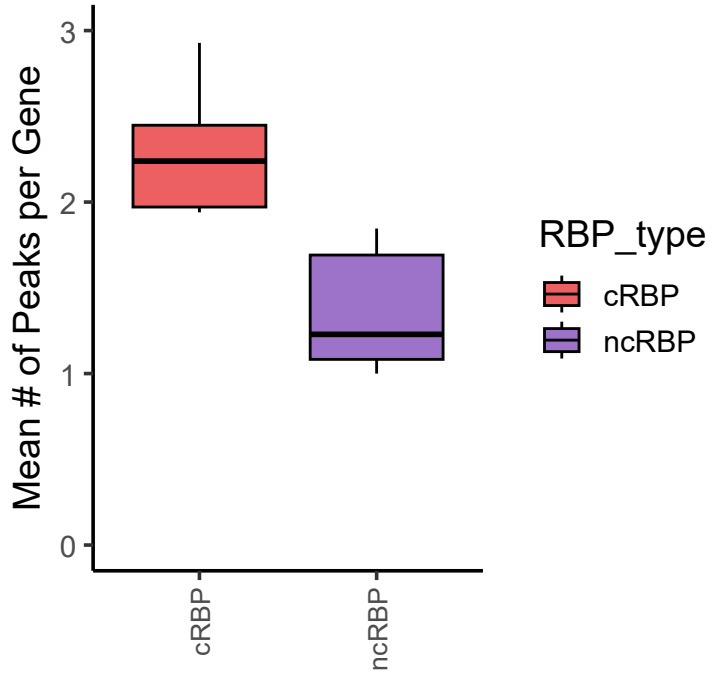
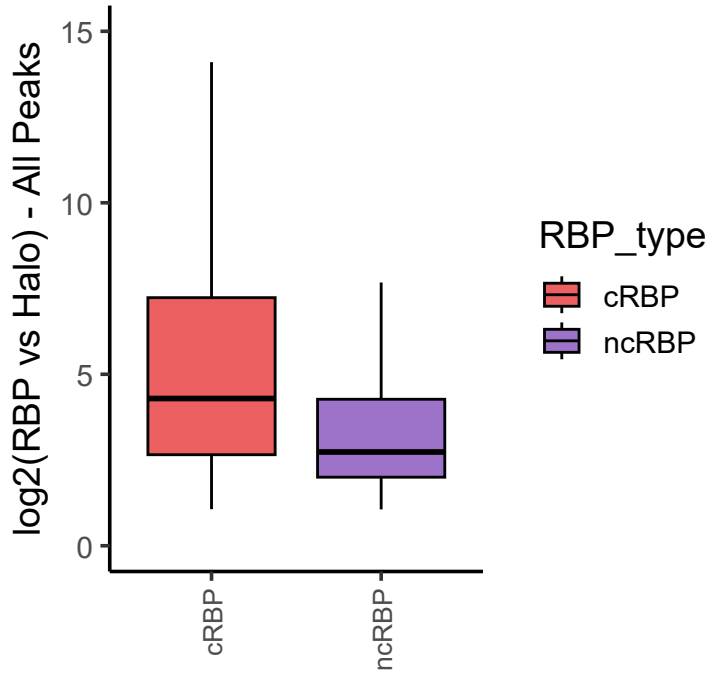


Figure S3E - RBP binding strength (downsampled)

```
RBP_FCH <- RBP_peaks

p <- ggplot(RBP_FCH, aes(x=RBP_type, y=Mean_FCH, fill=RBP_type)) +
  geom_boxplot(color = "black", outlier.shape = NA) +
  scale_fill_manual(values = c("cRBP" = "#ED6062", "ncRBP" = "#9C73C8")) +
  labs(
    x = "",
    y = "log2(RBP vs Halo) - All Peaks"
  ) +
  ylim(0, 15) +
  theme_classic(base_size = 14) +
  theme(
    axis.text.x = element_text(size = 10, angle = 90, hjust = 1, vjust = 0.5),
    plot.margin = margin(10, 10, 10, 10)
  )

p
```




```
RBP_FCH <- RBP_peaks %>%
  group_by(RBP_type) %>%
  sample_n(42601)

p <- ggplot(RBP_FCH, aes(x=RBP_type, y=Mean_FCH, fill=RBP_type)) +
  geom_boxplot(color = "black", outlier.shape = NA) +
  scale_fill_manual(values = c("cRBP" = "#ED6062", "ncRBP" = "#9C73C8")) +
  labs(
    x = "",
    y = "log2(RBP vs Halo) - Downsampled"
  ) +
  ylim(0, 15) +
  theme_classic(base_size = 14) +
  theme(
    axis.text.x = element_text(size = 10, angle = 90, hjust = 1, vjust = 0.5),
    plot.margin = margin(10, 10, 10, 10)
  )

p
```

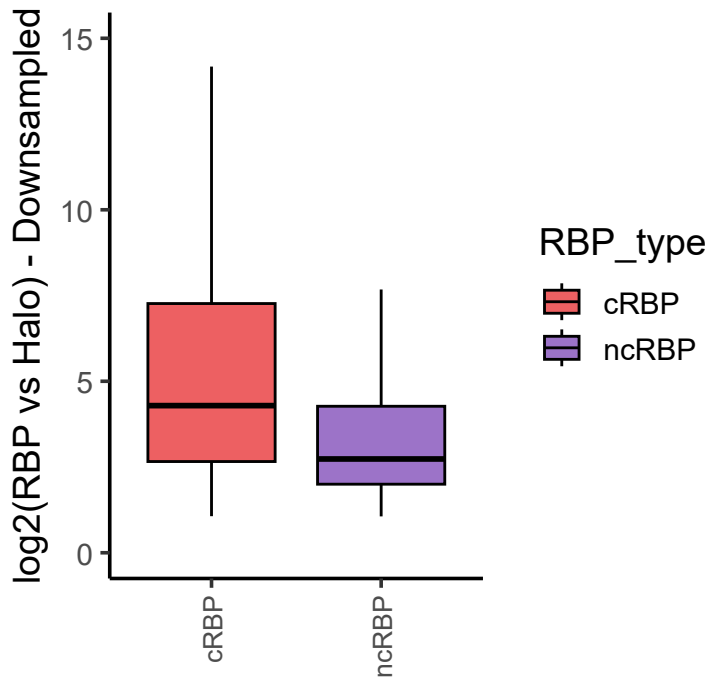
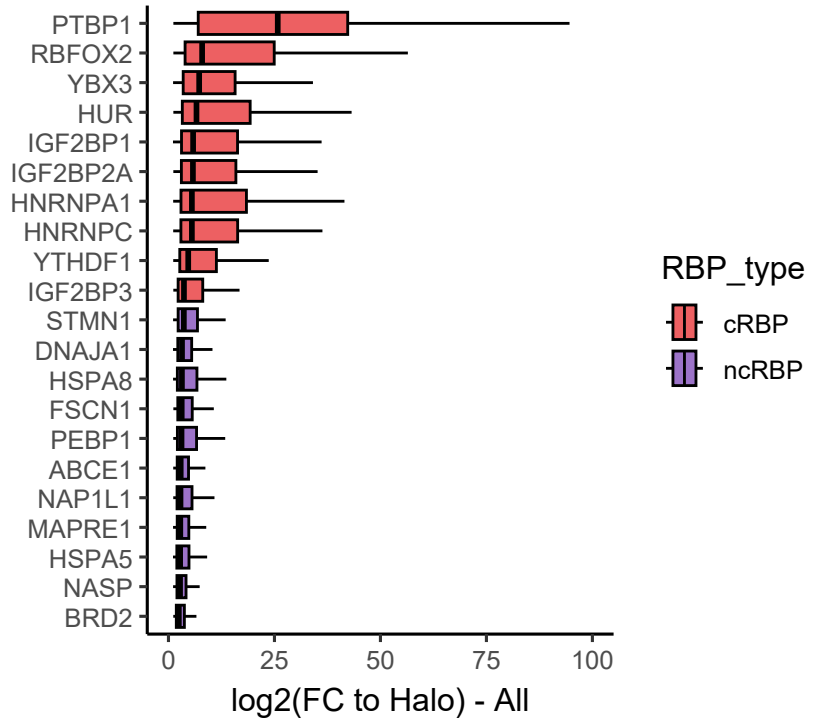
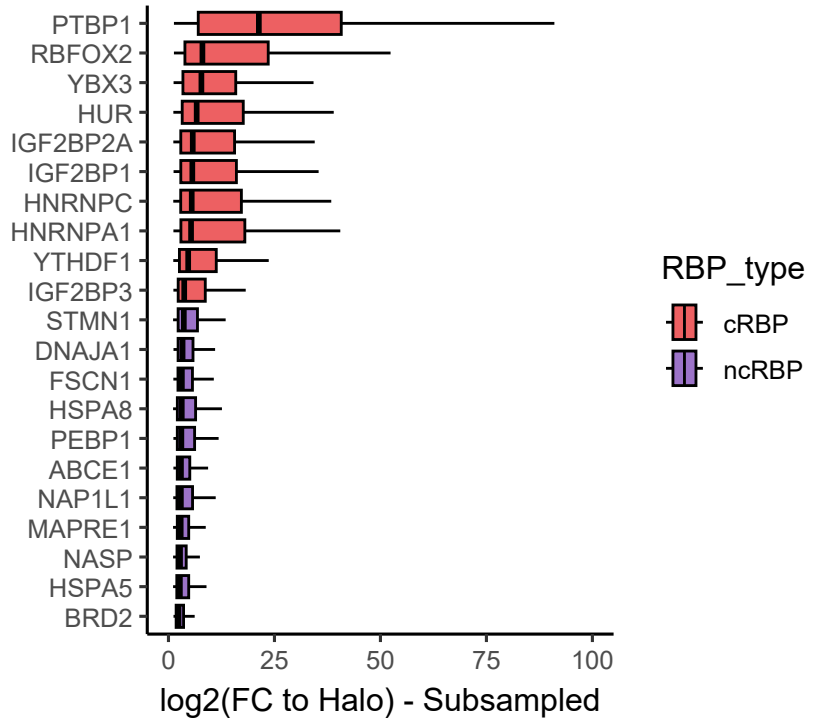


Figure S3F - Binding strength per RBP (downsampled)

```
RBP_filt_sorted_median_FCH <- RBP_filt_peaks %>%
  group_by(RBP) %>%
  summarise(median_FCH = median(Mean_FCH)) %>%
  arrange(desc(median_FCH)) %>%
  pull(RBP)

df <- RBP_filt_peaks %>%
  mutate(RBP = factor(RBP, levels = rev(RBP_filt_sorted_median_FCH)))

p <- ggplot(df, aes(x = Mean_FCH, y = RBP, fill = RBP_type)) +
  geom_boxplot(color = "black", outlier.shape = NA) +
  scale_fill_manual(values = c("cRBP" = "#ED6062", "ncRBP" = "#9C73C8")) +
  labs(
    x = "log2(FC to Halo) - All",
    y = NULL
  ) +
  xlim(0, 100) +
  theme_classic(base_size = 12) +
  theme(
    plot.margin = margin(10, 10, 10, 10)
  )

p
```

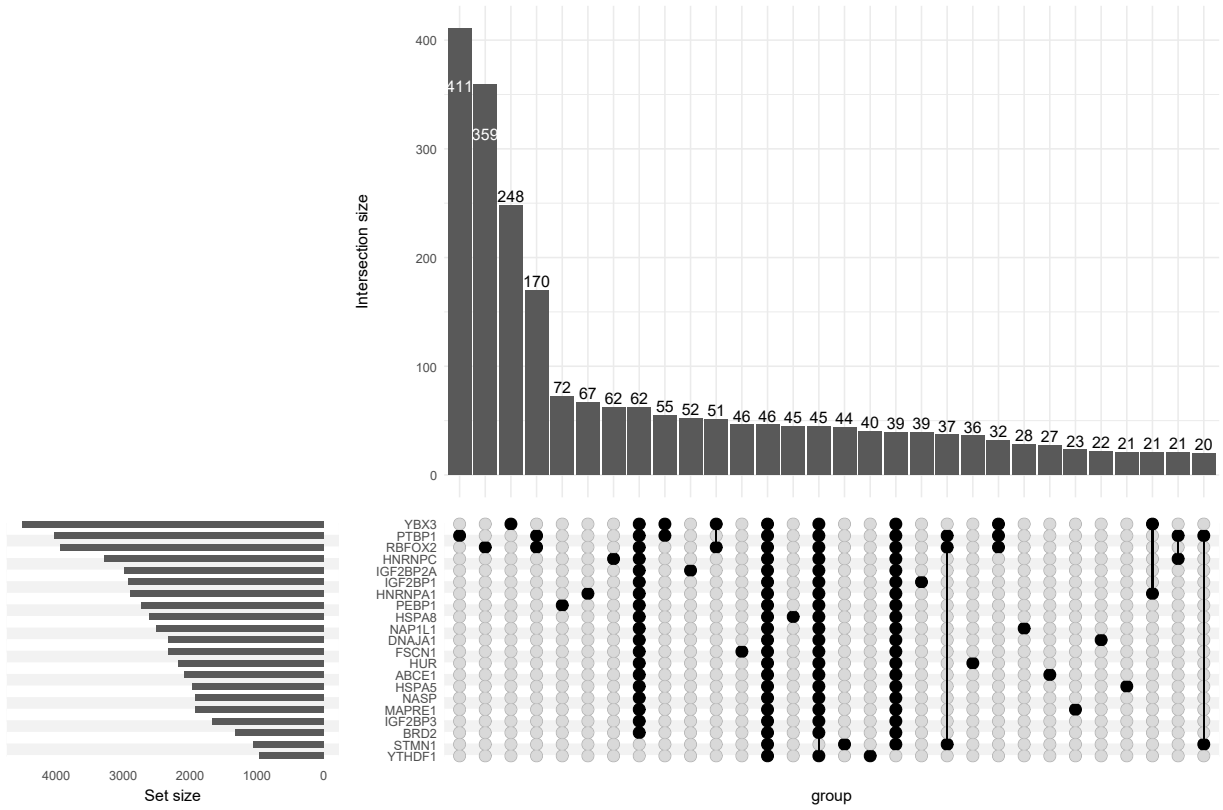



```r
df <- RBP_filt_peaks %>%
  group_by(RBP) %>%
  sample_n(1000)

new_order <- df %>%
  group_by(RBP) %>%
  summarise(median_FCH = median(Mean_FCH)) %>%
  arrange(desc(median_FCH)) %>%
  pull(RBP)

df <- df %>%
  mutate(RBP = factor(RBP, levels = rev(new_order)))

p <- ggplot(df, aes(x = Mean_FCH, y = RBP, fill = RBP_type)) +
  geom_boxplot(color = "black", outlier.shape = NA) +
  scale_fill_manual(values = c("cRBP" = "#ED6062", "ncRBP" = "#9C73C8")) +
  labs(
    x = "log2(FC to Halo) - Subsampled",
    y = NULL
  ) +
  xlim(0, 100) +
  theme_classic(base_size = 12) +
  theme(
    plot.margin = margin(10, 10, 10, 10)
  )

p
```



Overlap analysis of RBP targets

```
RBP_targets <- lapply(setNames(RBP_filt_names,RBP_filt_names), function(x){
  RBP_filt_peaks %>%
    filter(RBP == x) %>%
    pull(gene_name) %>%
    unique()
})

RBP_targets_all <- RBP_targets %>%
  unlist() %>%
  unique()

RBP_targets_overlap <- lapply(setNames(RBP_filt_names,RBP_filt_names), function(x){
  overlap <- c(x = RBP_targets_all %in% RBP_targets[[x]])
}) %>%
  bind_cols(.) %>%
  mutate(target = RBP_targets_all) %>%
  column_to_rownames(var = "target") %>%
  mutate(n_overlaps = rowSums(.),
         RBP_binders = apply(., 1, function(x) paste0(names(x)[x], collapse = '_')))

# plotting intersections with at lest 20 gene targets
upset(RBP_targets_overlap,
      RBP_filt_names,
      min_size = 20)
```



Generate RBP target network

```
RBP_targets_overlap_more20 <- RBP_targets_overlap$RBP_binders %>%
  table(.) %>%
  sort(decreasing = T) %>%
  as.data.frame() %>%
  filter(Freq>=20) %>%
  pull('.') %>%
  as.character()

RBP_targets_overlap_more20 <- setNames(RBP_targets_overlap_more20,RBP_targets_overlap_more20)

RBP_targets_overlap_more20_sets <- lapply(RBP_targets_overlap_more20, function(x){
  RBP_targets_overlap %>%
    rownames_to_column(var = "target") %>%
    filter(RBP_binders == x) %>%
    pull(target)
})

# establish nodes and edges of RBP target overlaps network
nodes <- data.frame(node_description = RBP_filt_names,
                    node_type = "RBP",
                    node_size = 100,
                    id = RBP_filt_names) %>%
  bind_rows(data.frame(node_description = names(RBP_targets_overlap_more20_sets),
                       node_type = "RBP_overlap",
                       node_size = lapply(RBP_targets_overlap_more20_sets, length) %>% unlist()) %>%
              mutate(id = paste(node_description,node_size, sep="_"))
  )

edges <- nodes %>%
  filter(node_type == "RBP_overlap") %>%
  mutate(source = id) %>%
  add_column(target = .$node_description, .after = 'source') %>%
  separate_rows(target, sep="_") %>%
```

```
  mutate(interaction = 'link') %>%
  dplyr::select(source, interaction, target)

# export node and edge tables for Cytoscape
write.table(nodes, file = "results/RBP_target_network_nodes.txt", sep="\t", row.names = F, col.names = T, quote = F)
write.table(edges, file = "results/RBP_target_network_edges.txt", sep="\t", row.names = F, col.names = T, quote = F)
```

Figure 3D - Analysis of RBP target network subsets

GO enrichment analysis of RBP target subsets

```
gene_targets_subsets <- RBP_targets_overlap_more20_sets[c("PTBP1", "RBFOX2", "YBX3", "STMN1", "PTBP1_RBFOX2", "YBX3_PTBP1", "YBX3_RBFOX2", "PTBP1_

input <- read.table(file="data/INPUTs.TPMs.txt", stringsAsFactors = F, header=T)
input_genes <- input %>%
  filter(TPM_RAP >= 1) %>%
  pull(gene_ID) %>%
  gsub("\\..*","",.) %>%
  unique() %>%
  mapIds(x=org.Hs.eg.db, keys=., column='SYMBOL', keytype = 'ENSEMBL') %>%
  unique()
RBP_bound_genes <- RBP_filt_peaks %>%
  pull(gene_name) %>%
  unique()
gene_background <- unique(c(input_genes, RBP_bound_genes))

# GO enrichment
gene_targets_subsets_GO <- lapply(setNames(names(gene_targets_subsets),names(gene_targets_subsets)), function(x) {
  res <- enrichGO(gene = gene_targets_subsets[[x]],
                  universe = gene_background,
                  keyType = "SYMBOL",
                  OrgDb = org.Hs.eg.db,
                  ont = "ALL",
                  pAdjustMethod = "BH",
                  pvalueCutoff = 0.1,
                  qvalueCutoff = 0.1,
                  minGSSize = 10,
                  maxGSSize = 100,
                  readable = TRUE)
  res@result <- res@result %>%
    add_column(subset_name = x, .before = 1)
  res
})
gene_targets_subsets_GO_combined <- lapply(gene_targets_subsets_GO, function(x) x@result) %>%
  bind_rows()

# split by BP, MF and CC
gene_targets_subsets_GO_combined_BP <- gene_targets_subsets_GO_combined %>%
  filter(ONTOLOGY == "BP")
gene_targets_subsets_GO_combined_MF <- gene_targets_subsets_GO_combined %>%
  filter(ONTOLOGY == "MF")
gene_targets_subsets_GO_combined_CC <- gene_targets_subsets_GO_combined %>%
  filter(ONTOLOGY == "CC")

# collapse GO terms
## BP
sim_mtx <- calculateSimMatrix(unique(gene_targets_subsets_GO_combined_BP$ID),
                              orgdb="org.Hs.eg.db",
                              ont="BP",
                              method="Rel")
sim_mtx_reduced <- reduceSimMatrix(sim_mtx,
                                   threshold=0.9,
                                   orgdb="org.Hs.eg.db")
gene_targets_subsets_GO_combined_BP <- gene_targets_subsets_GO_combined_BP %>%
  left_join(sim_mtx_reduced, by=c("ID"="go")) %>%
  dplyr::filter(!is.na(parent))
gene_targets_subsets_GO_combined_BP_reduced <- gene_targets_subsets_GO_combined_BP %>%
  dplyr::group_by(parentTerm) %>%
  dplyr::summarise(ONTOLOGY, geneID = paste0(geneID, collapse="/")) %>%
  dplyr::mutate(geneID = unlist(lapply(geneID, function(x) paste0(unique(unlist(strsplit(x, split="/"))), collapse="/" )))) %>%
  dplyr::filter(!duplicated(parentTerm))

## MF
```

```
sim_mtx <- calculateSimMatrix(unique(gene_targets_subsets_GO_combined_MF$ID),
                              orgdb="org.Hs.eg.db",
                              ont="MF",
                              method="Rel")
sim_mtx_reduced <- reduceSimMatrix(sim_mtx,
                                   threshold=0.9,
                                   orgdb="org.Hs.eg.db")
gene_targets_subsets_GO_combined_MF <- gene_targets_subsets_GO_combined_MF %>%
  left_join(sim_mtx_reduced, by=c("ID"="go")) %>%
  dplyr::filter(!is.na(parent))
gene_targets_subsets_GO_combined_MF_reduced <- gene_targets_subsets_GO_combined_MF %>%
  dplyr::group_by(parentTerm) %>%
  dplyr::summarise(ONTOLOGY, geneID = paste0(geneID, collapse="/")) %>%
  dplyr::mutate(geneID = unlist(lapply(geneID, function(x) paste0(unique(unlist(strsplit(x, split="/"))), collapse="/" )))) %>%
  dplyr::filter(!duplicated(parentTerm))

## CC
sim_mtx <- calculateSimMatrix(unique(gene_targets_subsets_GO_combined_CC$ID),
                              orgdb="org.Hs.eg.db",
                              ont="CC",
                              method="Rel")
sim_mtx_reduced <- reduceSimMatrix(sim_mtx,
                                   threshold=0.9,
                                   orgdb="org.Hs.eg.db")
gene_targets_subsets_GO_combined_CC <- gene_targets_subsets_GO_combined_CC %>%
  left_join(sim_mtx_reduced, by=c("ID"="go")) %>%
  dplyr::filter(!is.na(parent))
gene_targets_subsets_GO_combined_CC_reduced <- gene_targets_subsets_GO_combined_CC %>%
  dplyr::group_by(parentTerm) %>%
  dplyr::summarise(ONTOLOGY, geneID = paste0(geneID, collapse="/")) %>%
  dplyr::mutate(geneID = unlist(lapply(geneID, function(x) paste0(unique(unlist(strsplit(x, split="/"))), collapse="/" )))) %>%
  dplyr::filter(!duplicated(parentTerm))

# merge collapsed enriched GO terms
gene_targets_subsets_GO_combined_reduced <- bind_rows(gene_targets_subsets_GO_combined_BP_reduced,
                                    gene_targets_subsets_GO_combined_MF_reduced,
                                    gene_targets_subsets_GO_combined_CC_reduced) %>%
  dplyr::mutate(ONTOLOGY = factor(ONTOLOGY, levels = c("BP", "MF", "CC"))) %>%
  dplyr::arrange(ONTOLOGY, parentTerm, .locale = "en")
```

Binding strength of RBP targets

```
# binding strength of RBPs per gene target
GO_enriched_genes <- gene_targets_subsets_GO_combined_reduced %>%
  pull(geneID) %>%
  str_split("\\/") %>%
  unlist() %>%
  unique()

df <- RBP_filt_peaks %>%
  filter(gene_name %in% GO_enriched_genes) %>%
  select(RBP, gene_name, Gene_BS) %>%
  filter(!duplicated(.)) %>%
  pivot_wider(names_from = RBP, values_from = Gene_BS, values_fill = 0.1) %>%
  mutate(gene_name = factor(gene_name, levels=GO_enriched_genes)) %>%
  arrange(gene_name) %>%
  column_to_rownames(var = "gene_name") %>%
  select("YBX3","PTBP1","RBFOX2","STMN1")

p <- list()

p[[1]] <- Heatmap(log2(df+1),
        name = "Gene_BS",
        col = circlize::colorRamp2(c(0, max((log2(df)))), c("#BEBEBE", "#000000")),
        cluster_rows = F,
        cluster_columns = F,
        row_names_side = "left",
        column_names_side = "bottom",
        width = unit(ncol(df)*4, "mm"),
        height = unit(nrow(df)*4, "mm"),
        cell_fun = function(j, i, x, y, width, height, fill) {
            grid.rect(x, y, width, height,
                      gp = gpar(col = "white", fill=NA,lwd = 1))
        },
        border = T)
```

Expression of RBP target genes in liver cancer stages

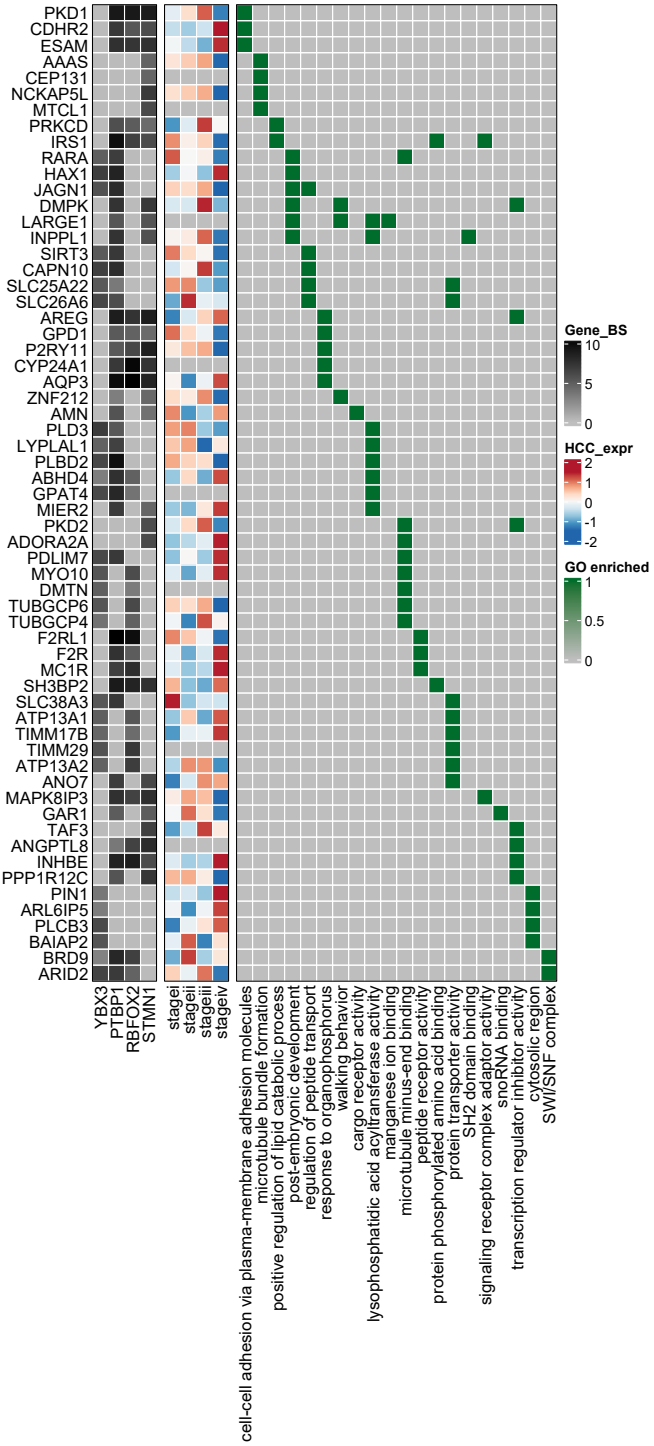
```
# median expression of gene targets in liver cancer stages (TCGA cohort)
TCGA_annot <- read.table(file="data/TCGA/Human__TCGA_LIHC__MS__Clinical__Clinical__01_28_2016__BI__Clinical__Firehose.tsi.txt",
                         stringsAsFactors = F,
                         sep = "\t",
                         header=T) %>%
  pivot_longer(cols = -attrib_name, names_to = "patient_id", values_to = "value") %>%
  pivot_wider(names_from = attrib_name, values_from = value) %>%
  dplyr::filter(!is.na(pathologic_stage)) %>%
  mutate(pathologic_stage = factor(pathologic_stage, levels = c("stagei", "stageii", "stageiii", "stageiv"))) %>%
  arrange(pathologic_stage)

TCGA_RNAseq <- read.table(file="data/TCGA/Human__TCGA_LIHC__UNC__RNAseq__HiSeq_RNA__01_28_2016__BI__Gene__Firehose_RSEM_log2.cct",
                         stringsAsFactors = F,
                         sep = "\t",
                         header=T) %>%
  column_to_rownames("attrib_name") %>%
  dplyr::select(TCGA_annot$patient_id[TCGA_annot$patient_id %in% colnames(.)])


TCGA_RNAseq_scaled_median_by_stage <- groupTransform(TCGA_RNAseq,
                TCGA_annot$pathologic_stage[TCGA_annot$patient_id %in% colnames(TCGA_RNAseq)],
                function(x) apply(x, 1, median)) %>%
  scaleData(., method = "zscore") %>%
  rownames_to_column(var = "geneID")


GO_enriched_genes <- gene_targets_subsets_GO_combined_reduced %>%
  pull(geneID) %>%
  str_split("\\/") %>%
  unlist() %>%
  unique()

df <- data.frame(geneID = GO_enriched_genes) %>%
  left_join(TCGA_RNAseq_scaled_median_by_stage, by=c("geneID"="geneID")) %>%
  column_to_rownames("geneID")

divergent_pal <- c( "#2166AC", "#4393C3", "#92C5DE", "#D1E5F0", "#F7F7F7", "#FDDBC7", "#F4A582", "#D6604D", "#B2182B")

p[[2]] <- Heatmap(df,
        name = "HCC_expr",
        col = divergent_pal,
        cluster_rows = F,
        cluster_columns = F,
        row_names_side = "left",
        column_names_side = "bottom",
        width = unit(ncol(df)*4, "mm"),
        height = unit(nrow(df)*4, "mm"),
        cell_fun = function(j, i, x, y, width, height, fill) {
            grid.rect(x, y, width, height,
                      gp = gpar(col = "white", fill=NA,lwd = 1))
        },
        border = T)
```

Presence of gene targets in enriched GO terms

```
# presence of gene targets in enriched GO terms
GO_enriched_genes <- gene_targets_subsets_GO_combined_reduced %>%
  pull(geneID) %>%
  str_split("\\/") %>%
  unlist() %>%
  unique()

df <- gene_targets_subsets_GO_combined_reduced %>%
  select(parentTerm, geneID) %>%
  separate_longer_delim(col = geneID, delim = "/") %>%
  pivot_wider(names_from = parentTerm, values_from = parentTerm,
              values_fn = length, values_fill = 0) %>%
  group_by(geneID) %>%
  summarise_all(sum) %>%
  mutate(geneID = factor(geneID, levels=GO_enriched_genes)) %>%
  arrange(geneID) %>%
  column_to_rownames('geneID')
```

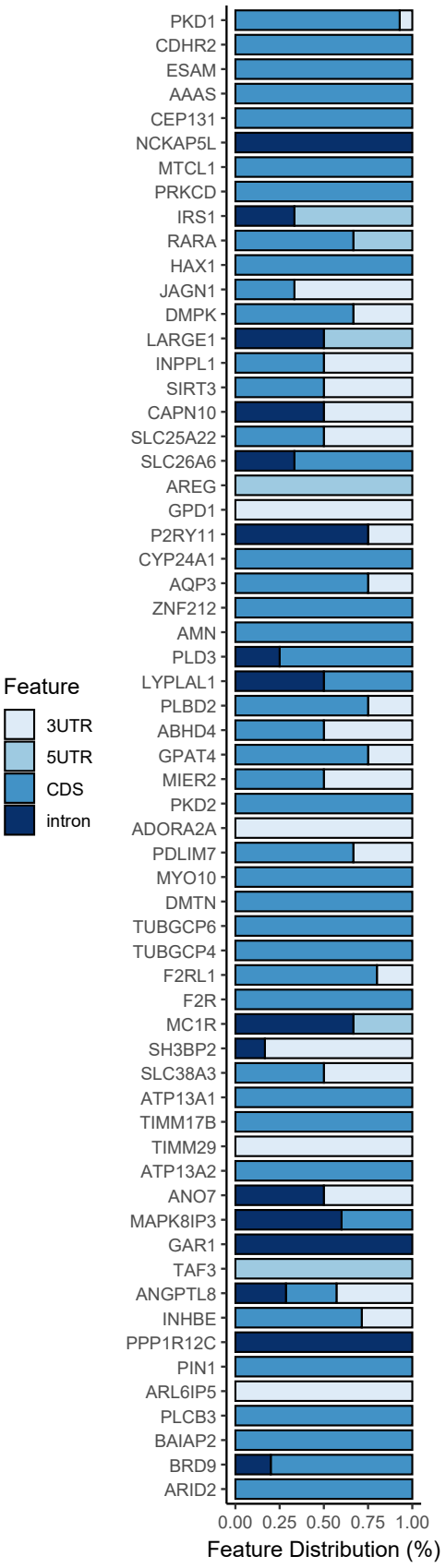
```
p[[4]] <- Heatmap(df,
        name = "GO enriched",
        col = circlize::colorRamp2(c(0, 1), c("#BEBEBE", "#006D2C")),
        cluster_rows = F,
        cluster_columns = F,
        row_names_side = "left",
        column_names_side = "bottom",
        width = unit(ncol(df)*4, "mm"),
        height = unit(nrow(df)*4, "mm"),
        cell_fun = function(j, i, x, y, width, height, fill) {
            grid.rect(x, y, width, height,
                      gp = gpar(col = "white", fill=NA,lwd = 1))
        },
        border = T)
```

```
p[[1]] + p[[2]] + p[[4]]
```


PKD1
CDHR2
ESAM
AAAS
CEP131
NCKAP5L
MTCL1
PRKCD
IRS1
RARA
HAX1
JAGN1
DMPK
LARGE1
INPPL1
SIRT3
CAPN10
SLC25A22
SLC26A6
AREG
GPD1
P2RY11
CYP24A1
AQP3
ZNF212
AMN
PLD3
LYPLAL1
PLBD2
ABHD4
GPAT4
MIER2
PKD2
ADORA2A
PDLIM7
MYO10
DMTN
TUBGCP6
TUBGCP4
F2RL1
F2R
MC1R
SH3BP2
SLC38A3
ATP13A1
TIMM17B
TIMM29
ATP13A2
ANO7
MAPK8IP3
GAR1
TAF3
ANGPTL8
INHBE
PPP1R12C
PIN1
ARL6IP5
PLCB3
BAIAP2
BRD9
ARID2
YBX3
PTBP1
RBFOX2
STMN1
stagei
stageii
stageiii
stageiv
cell-cell adhesion via plasma-membrane adhesion molecules
microtubule bundle formation
positive regulation of lipid catabolic process
post-embryonic development
regulation of peptide transport
response to organophosphorus
walking behavior
cargo receptor activity
lysophosphatidic acid acyltransferase activity
manganese ion binding
microtubule minus-end binding
peptide receptor activity
protein phosphorylated amino acid binding
protein transporter activity
SH2 domain binding
signaling receptor complex adaptor activity
snoRNA binding
transcription regulator inhibitor activity
cytosolic region
SWI/SNF complex
Gene_BS
10
5
0
HCC_expr
2
1
0
-1
-2
GO enriched
1
0.5
0

Distribution of RBP target sites in target genes

```
# distributions of RBP target sites in target genes
feature_colors <- c("intron" = "#08306B",
                    "exon" = "#08519C",
                    "CDS" = "#4292C6",
                    "5UTR" = "#9ECAE1",
                    "3UTR" = "#DEEBF7")

GO_enriched_genes <- gene_targets_subsets_GO_combined_reduced %>%
  pull(geneID) %>%
  str_split("\\/") %>%
  unlist() %>%
  unique()

df <- RBP_filt_peaks %>%
  filter(gene_name %in% GO_enriched_genes) %>%
  group_by(gene_name,feature) %>%
  summarise(n_sites = n()) %>%
  mutate(percent = n_sites/sum(n_sites)*100) %>%
  mutate(gene_name = factor(gene_name, levels=rev(GO_enriched_genes))) %>%
  mutate(feature = factor(feature, levels=rev(names(feature_colors))))

p[[5]] <- ggplot(df, aes(x = percent, y = gene_name, fill = feature)) +
  geom_bar(stat = "identity", position = "fill", width = 0.8, color="black") +
  scale_fill_manual(values = feature_colors) +
  labs(
    x = "Feature Distribution (%)",
    y = NULL,
    fill = "Feature"
  ) +
  theme_classic(base_size = 12) +
  theme(
    legend.position="left",
    plot.margin = margin(10, 10, 10, 10)
  )

p[[5]]
```


PKD1
CDHR2
ESAM
AAAS
CEP131
NCKAP5L
MTCL1
PRKCD
IRS1
RARA
HAX1
JAGN1
DMPK
LARGE1
INPPL1
SIRT3
CAPN10
SLC25A22
SLC26A6
AREG
GPD1
P2RY11
CYP24A1
AQP3
ZNF212
AMN
PLD3
LYPLAL1
PLBD2
ABHD4
GPAT4
MIER2
PKD2
ADORA2A
PDLIM7
MYO10
DMTN
TUBGCP6
TUBGCP4
F2RL1
F2R
MC1R
SH3BP2
SLC38A3
ATP13A1
TIMM17B
TIMM29
ATP13A2
ANO7
MAPK8IP3
GAR1
TAF3
ANGPTL8
INHBE
PPP1R12C
PIN1
ARL6IP5
PLCB3
BAIAP2
BRD9
ARID2
0.00 0.25 0.50 0.75 1.00
Feature Distribution (%)
Feature
3UTR
5UTR
CDS
intron

Load RNAseq data from siRNA KD experiments

```
# from Sondergaard et al. 2022
RBPKDs_Sondergaard2022 <- lapply(setNames(readxl::excel_sheets("data/RBPKD/table_s7_rbp_kd_rnaseq_deg.xlsx"),readxl::excel_sheets("data/RBPKD/table
  readxl::read_excel("data/RBPKD/table_s7_rbp_kd_rnaseq_deg.xlsx", sheet = x) %>%
    dplyr::rename(ensembl_gene_id = ensembl_ID,
                  external_gene_name = gene_name,
                  log2FoldChange = logFC,
                  padj = FDR,
                  gene_biotype = gene_type)
})
names(RBPKDs_Sondergaard2022) <- gsub("(.+)_DEG", "\\1", names(RBPKDs_Sondergaard2022))

# from ENCODE Consortium (Van Nostrand et al. 2020)
RBPKDs_ENCODE_DESeq2 <- readRDS("data/RBPKD/RBPKDs_ENCODE_filt_DESeq2.rds")
```

Figure S3G - Enrichment of RBP targets in RBPKD datasets by binding site distribution

```
rbp_names <- list(
  "YBX3" = "YBX3",
  "PTBP1" = "PTBP1",
  "RBFOX2" = "RBFOX2",
  "STMN1" = "STMN1"
)

feature_names <- list(
  "exon" = "exon",
  "intron" = "intron",
  "5UTR" = "5UTR",
  "3UTR" = "3UTR",
  "CDS" = "CDS",
  "all" = c("exon", "intron", "5UTR", "3UTR", "CDS")
)

rbp_targets <- lapply(rbp_names, function(x){
  lapply(feature_names, function(y){
    RBP_filt_peaks %>%
      filter(RBP == x & feature %in% y) %>%
      pull(gene_name) %>%
      unique()
  })
})

# gene background
input <- read.table(file="Data/INPUTs.TPMs.txt", stringsAsFactors = F, header=T)
input_genes <- input %>%
  filter(TPM_RAP >= 1) %>%
  pull(gene_ID) %>%
  gsub("\\..*","",.) %>%
  unique() %>%
  mapIds(x=org.Hs.eg.db, keys=., column='SYMBOL', keytype = 'ENSEMBL') %>%
  unique()
RBP_bound_genes <- RBP_filt_peaks %>%
  pull(gene_name) %>%
  unique()
gene_background <- unique(c(input_genes, RBP_bound_genes))
random_sets_n100 <- lapply(setNames(seq(1,100), paste0("set_", seq(1,100))), function(x){
  sample(x = gene_background, size = 100)
})

genes_rnk_kd <- list(
  "YBX3" = RBPKDs_ENCODE_DESeq2$HepG2_RNAi$HepG2_RNAi_YBX3_ENCFF571NNW %>%
    dplyr::filter(!is.na(pvalue)) %>%
    dplyr::mutate(external_gene_name = mapIds(x=org.Hs.eg.db, keys=ensembl_gene_id, column='SYMBOL', keytype = 'ENSEMBL')) %>%
    dplyr::filter(!is.na(external_gene_name)) %>%
    dplyr::mutate(neg_log10Pval = -log10(pvalue)*sign(log2FoldChange)) %>%
    dplyr::mutate(neg_log10Pval = ifelse(is.na(neg_log10Pval), 0, neg_log10Pval)) %>%
    dplyr::arrange(dplyr::desc(neg_log10Pval)),
  "PTBP1" = RBPKDs_ENCODE_DESeq2$HepG2_RNAi$HepG2_RNAi_PTBP1_ENCFF044UQQ %>%
    dplyr::filter(!is.na(pvalue)) %>%
    dplyr::mutate(external_gene_name = mapIds(x=org.Hs.eg.db, keys=ensembl_gene_id, column='SYMBOL', keytype = 'ENSEMBL')) %>%
    dplyr::filter(!is.na(external_gene_name)) %>%
```

```
    dplyr::mutate(neg_log10Pval = -log10(pvalue)*sign(log2FoldChange)) %>%
    dplyr::mutate(neg_log10Pval = ifelse(is.na(neg_log10Pval), 0, neg_log10Pval)) %>%
    dplyr::arrange(dplyr::desc(neg_log10Pval)),
  "RBFOX2" = RBPKDs_ENCODE_DESeq2$HepG2_RNAi$HepG2_RNAi_RBFOX2_ENCFF166HLT %>%
    dplyr::filter(!is.na(pvalue)) %>%
    dplyr::mutate(external_gene_name = mapIds(x=org.Hs.eg.db, keys=ensembl_gene_id, column='SYMBOL', keytype = 'ENSEMBL')) %>%
    dplyr::filter(!is.na(external_gene_name)) %>%
    dplyr::mutate(neg_log10Pval = -log10(pvalue)*sign(log2FoldChange)) %>%
    dplyr::mutate(neg_log10Pval = ifelse(is.na(neg_log10Pval), 0, neg_log10Pval)) %>%
    dplyr::arrange(dplyr::desc(neg_log10Pval)),
  "STMN1" = RBPKDs_Sondergaard2022$STMN1 %>%
    dplyr::filter(!is.na(external_gene_name)) %>%
    dplyr::mutate(neg_log10Pval = -log10(PValue)*sign(log2FoldChange)) %>%
    dplyr::mutate(neg_log10Pval = ifelse(is.na(neg_log10Pval), 0, neg_log10Pval)) %>%
    dplyr::arrange(dplyr::desc(neg_log10Pval))
)

# run GSEA
gsea_res <- lapply(rbp_names, function(x) {
  print(x)
  runGSEA(gene.rnk = setNames(genes_rnk_kd[[x]]$neg_log10Pval,
                              genes_rnk_kd[[x]]$external_gene_name),
          gene.sets = rbp_targets[[x]],
          min.size = 1,
          max.size = 5000,
          nproc = 1)
})
```

```
## [1] "YBX3"
```

```
##   |                                                                      |
##
## [1] "PTBP1"
```

```
##   |                                                                      |
##
## [1] "RBFOX2"
```

```
##   |                                                                      |
##
## [1] "STMN1"
```

```
##   |                                                                      |
```

```
gsea_res_rand_n100 <- lapply(rbp_names, function(x) {
  print(x)
  runGSEA(gene.rnk = setNames(genes_rnk_kd[[x]]$neg_log10Pval,
                              genes_rnk_kd[[x]]$external_gene_name),
          gene.sets = random_sets_n100,
          min.size = 1,
          max.size = 5000,
          nproc = 1)
})
```

```
## [1] "YBX3"
```

```
##   |                                                                      |
##
## [1] "PTBP1"
```

```
##   |                                                                      |
##
## [1] "RBFOX2"
```

```
##   |                                                                      |
##
## [1] "STMN1"
```

```
##   |                                                                      |
```

```r
df <- lapply(rbp_names, function(x) {
  gsea_res_NES = gsea_res[[x]]$all %>%
    dplyr::mutate(abs_NES = abs(NES),
                  neg_log10pval = -log10(pval)) %>%
    dplyr::select(pathway, abs_NES, neg_log10pval) %>%
    dplyr::add_row(pathway = "rand_n100", abs_NES = mean(abs(gsea_res_rand_n100[[x]]$all$NES)), neg_log10pval = mean(-log10(gsea_res_rand_n100[[x]
    filter(!pathway %in% c("all")) %>%
    dplyr::mutate(pathway = factor(pathway, levels = c("intron", "exon", "CDS", "5UTR", "3UTR", "rand_n100")))
  if(!"exon" %in% unique(gsea_res_NES$pathway)){
    gsea_res_NES <- gsea_res_NES %>%
      add_row(pathway = "exon", abs_NES = 0, neg_log10pval = 0)
  }
  gsea_res_NES
})

lab_colors <- c("intron" = "#08306B",
                "exon" = "#08519C",
                "CDS" = "#4292C6",
                "5UTR" = "#9ECAE1",
                "3UTR" = "#DEEBF7",
                "rand_n100" = "#CCCCCC")

p <- list()
rbp <- "YBX3"
p[[1]] <- ggplot(df[[rbp]], aes(x = pathway, y = abs_NES, fill = pathway)) +
  geom_bar(stat = "identity", width = 0.8, color="black") +
  labs(
    x = "Genomic feature",
    y = "|Norm. enrich. score|"
  ) +
  scale_fill_manual(values = lab_colors) +
  ylim(0, 2) +
  ggtitle(rbp) +
  theme_classic(base_size = 14)

rbp <- "PTBP1"
p[[2]] <- ggplot(df[[rbp]], aes(x = pathway, y = abs_NES, fill = pathway)) +
  geom_bar(stat = "identity", width = 0.8, color="black") +
  labs(
    x = "Genomic feature",
    y = "|Norm. enrich. score|"
  ) +
  scale_fill_manual(values = lab_colors) +
  ylim(0, 2) +
  ggtitle(rbp) +
  theme_classic(base_size = 14)

rbp <- "RBFOX2"
p[[3]] <- ggplot(df[[rbp]], aes(x = pathway, y = abs_NES, fill = pathway)) +
  geom_bar(stat = "identity", width = 0.8, color="black") +
  labs(
    x = "Genomic feature",
    y = "|Norm. enrich. score|"
  ) +
  scale_fill_manual(values = lab_colors) +
  ylim(0, 2) +
  ggtitle(rbp) +
  theme_classic(base_size = 14)

rbp <- "STMN1"
p[[4]] <- ggplot(df[[rbp]], aes(x = pathway, y = abs_NES, fill = pathway)) +
  geom_bar(stat = "identity", width = 0.8, color="black") +
  labs(
    x = "Genomic feature",
    y = "|Norm. enrich. score|"
  ) +
  scale_fill_manual(values = lab_colors) +
  ylim(0, 2) +
  ggtitle(rbp) +
  theme_classic(base_size = 14)

combined_plot <- (p[[1]] | p[[2]]) / (p[[3]] | p[[4]])

print(combined_plot)
```

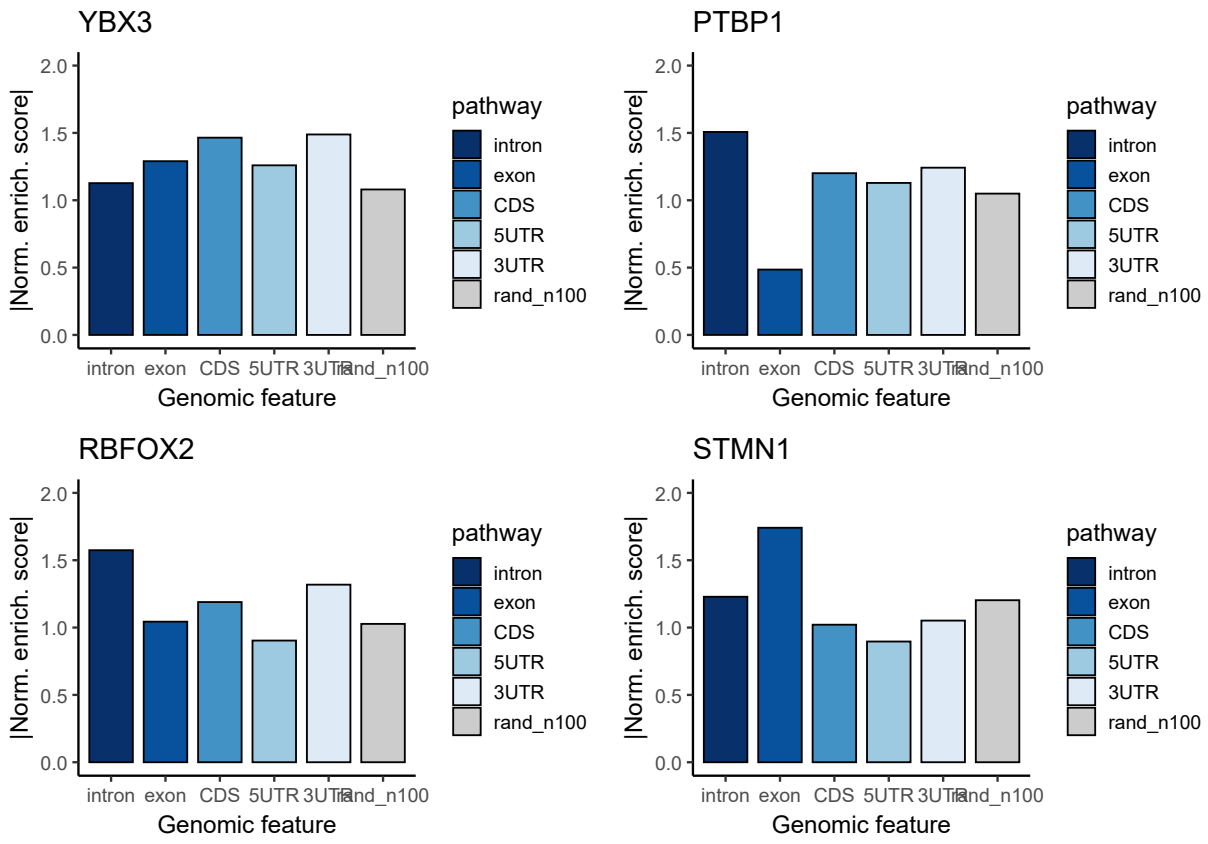


Figure 3C - Enrichment of RBP target subsets in RBPKD datasets

```
# target subsets for PTBP1, RBFOX2, YBX3, STMN1. Both shared and unique.
gene_targets_subsets <- RBP_targets_overlap_more20_sets[c("YBX3",
                                                          "YBX3_PTBP1",
                                                          "YBX3_RBFOX2",
                                                          "YBX3_PTBP1_RBFOX2",
                                                          "YBX3_HNRNPA1",
                                                          "PTBP1",
                                                          "PTBP1_RBFOX2",
                                                          "PTBP1_STMN1",
                                                          "PTBP1_RBFOX2_STMN1",
                                                          "PTBP1_HNRNPC",
                                                          "RBFOX2",
                                                          "STMN1")]

input <- read.table(file="Data/INPUTs.TPMs.txt", stringsAsFactors = F, header=T)
input_genes <- input %>%
  filter(TPM_RAP >= 1) %>%
  pull(gene_ID) %>%
  gsub("\\..*","",.) %>%
  unique() %>%
  mapIds(x=org.Hs.eg.db, keys=., column='SYMBOL', keytype = 'ENSEMBL') %>%
  unique()
RBP_bound_genes <- RBP_filt_peaks %>%
  pull(gene_name) %>%
  unique()
gene_background <- unique(c(input_genes, RBP_bound_genes))

genes_rnk_kd <- list(
  "YBX3" = RBPKDs_ENCODE_DESeq2$HepG2_RNAi$HepG2_RNAi_YBX3_ENCFF571NNW %>%
    dplyr::filter(!is.na(pvalue)) %>%
    dplyr::mutate(external_gene_name = mapIds(x=org.Hs.eg.db, keys=ensembl_gene_id, column='SYMBOL', keytype = 'ENSEMBL')) %>%
    dplyr::filter(!is.na(external_gene_name)) %>%
    dplyr::mutate(neg_log10Pval = -log10(pvalue)*sign(log2FoldChange)) %>%
```

```
    dplyr::mutate(neg_log10Pval = ifelse(is.na(neg_log10Pval), 0, neg_log10Pval)) %>%
    dplyr::arrange(dplyr::desc(neg_log10Pval)),
  "PTBP1" = RBPKDs_ENCODE_DESeq2$HepG2_RNAi$HepG2_RNAi_PTBP1_ENCFF044UQQ %>%
    dplyr::filter(!is.na(pvalue)) %>%
    dplyr::mutate(external_gene_name = mapIds(x=org.Hs.eg.db, keys=ensembl_gene_id, column='SYMBOL', keytype = 'ENSEMBL')) %>%
    dplyr::filter(!is.na(external_gene_name)) %>%
    dplyr::mutate(neg_log10Pval = -log10(pvalue)*sign(log2FoldChange)) %>%
    dplyr::mutate(neg_log10Pval = ifelse(is.na(neg_log10Pval), 0, neg_log10Pval)) %>%
    dplyr::arrange(dplyr::desc(neg_log10Pval)),
  "RBFOX2" = RBPKDs_ENCODE_DESeq2$HepG2_RNAi$HepG2_RNAi_RBFOX2_ENCFF166HLT %>%
    dplyr::filter(!is.na(pvalue)) %>%
    dplyr::mutate(external_gene_name = mapIds(x=org.Hs.eg.db, keys=ensembl_gene_id, column='SYMBOL', keytype = 'ENSEMBL')) %>%
    dplyr::filter(!is.na(external_gene_name)) %>%
    dplyr::mutate(neg_log10Pval = -log10(pvalue)*sign(log2FoldChange)) %>%
    dplyr::mutate(neg_log10Pval = ifelse(is.na(neg_log10Pval), 0, neg_log10Pval)) %>%
    dplyr::arrange(dplyr::desc(neg_log10Pval)),
  "STMN1" = RBPKDs_Sondergaard2022$STMN1 %>%
    dplyr::filter(!is.na(external_gene_name)) %>%
    dplyr::mutate(neg_log10Pval = -log10(PValue)*sign(log2FoldChange)) %>%
    dplyr::mutate(neg_log10Pval = ifelse(is.na(neg_log10Pval), 0, neg_log10Pval)) %>%
    dplyr::arrange(dplyr::desc(neg_log10Pval))
)

# run GSEA
gsea_res <- lapply(rbp_names, function(x) {
  print(x)
  runGSEA(gene.rnk = setNames(genes_rnk_kd[[x]]$neg_log10Pval,
                              genes_rnk_kd[[x]]$external_gene_name),
          gene.sets = gene_targets_subsets,
          min.size = 1,
          max.size = 5000,
          nproc = 1)
})
```

```
## [1] "YBX3"
```

```
## [1] "PTBP1"
```

```
## [1] "RBFOX2"
```

```
##   |                                                                      |
##
## [1] "STMN1"
```

```
##   |                                                                      |
```

```
df <- lapply(rbp_names, function(x) {
  gsea_res_NES = gsea_res[[x]]$all %>%
    dplyr::mutate(abs_NES = abs(NES),
                  neg_log10pval = -log10(pval)) %>%
    dplyr::select(pathway, abs_NES, neg_log10pval) %>%
    dplyr::mutate(pathway = factor(pathway, levels = c("YBX3",
                                                       "YBX3_PTBP1",
                                                       "YBX3_RBFOX2",
                                                       "YBX3_PTBP1_RBFOX2",
                                                       "YBX3_HNRNPA1",
                                                       "PTBP1",
                                                       "PTBP1_RBFOX2",
                                                       "PTBP1_STMN1",
                                                       "PTBP1_RBFOX2_STMN1",
                                                       "PTBP1_HNRNPC",
                                                       "RBFOX2",
                                                       "STMN1")))
  gsea_res_NES
})

p <- list()
rbp <- "YBX3"
p[[1]] <- ggplot(df[[rbp]], aes(x = pathway, y = abs_NES)) +
  geom_bar(stat = "identity", width = 0.8, color="black", fill="#ED6062") +
  labs(
    x = "",
    y = "|Norm. enrich. score|"
  ) +
  ylim(0, 2) +
```

```
  ggtitle(rbp) +
  theme_classic(base_size = 14) +
  theme(axis.text.x = element_blank())

rbp <- "PTBP1"
p[[2]] <- ggplot(df[[rbp]], aes(x = pathway, y = abs_NES)) +
  geom_bar(stat = "identity", width = 0.8, color="black", fill="#ED6062") +
  labs(
    x = "",
    y = "|Norm. enrich. score|"
  ) +
  ylim(0, 2) +
  ggtitle(rbp) +
  theme_classic(base_size = 14) +
  theme(axis.text.x = element_blank())

rbp <- "RBFOX2"
p[[3]] <- ggplot(df[[rbp]], aes(x = pathway, y = abs_NES)) +
  geom_bar(stat = "identity", width = 0.8, color="black", fill="#ED6062") +
  labs(
    x = "",
    y = "|Norm. enrich. score|"
  ) +
  ylim(0, 2) +
  ggtitle(rbp) +
  theme_classic(base_size = 14) +
  theme(axis.text.x = element_blank())

rbp <- "STMN1"
p[[4]] <- ggplot(df[[rbp]], aes(x = pathway, y = abs_NES)) +
  geom_bar(stat = "identity", width = 0.8, color="black", fill="#9C73C8") +
  labs(
    x = "RBP Target Subset",
    y = "|Norm. enrich. score|"
  ) +
  ylim(0, 2) +
  ggtitle(rbp) +
  theme_classic(base_size = 14) +
  theme(axis.text.x = element_text(angle = 90, hjust = 1, vjust = 0.5))

combined_plot <- p[[1]] / p[[2]] / p[[3]] / p[[4]]

print(combined_plot)
```

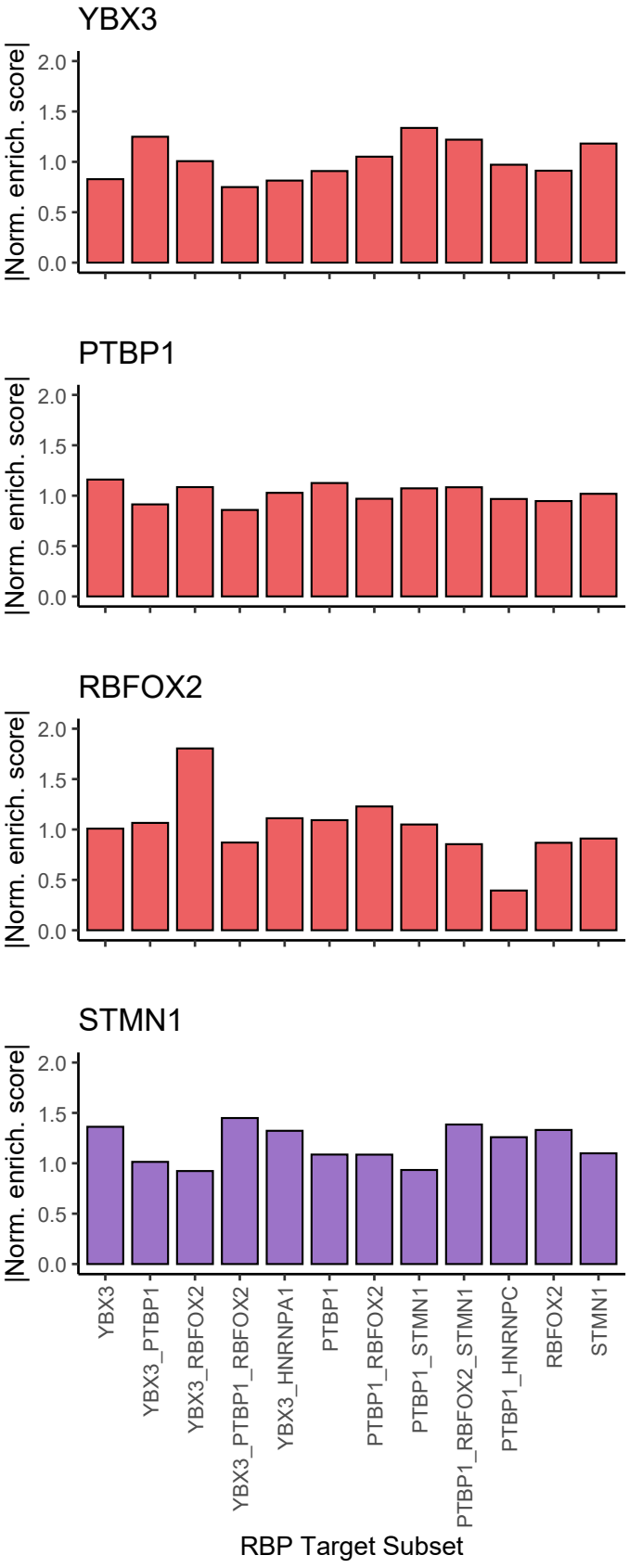

YBX3
PTBP1
RBFOX2
STMN1
|Norm. enrich. score|
2.0
1.5
1.0
0.5
0.0
YBX3
YBX3_PTBP1
YBX3_RBFOX2
YBX3_PTBP1_RBFOX2
YBX3_HNRNPA1
PTBP1
PTBP1_RBFOX2
PTBP1_STMN1
PTBP1_RBFOX2_STMN1
PTBP1_HNRNPC
RBFOX2
STMN1
RBP Target Subset

Figure S3H - Volcano plots of RBP target genes in RBPKD datasets

```
RBP_diff_expr <- list(
  "YBX3" = RBPKDs_ENCODE_DESeq2$HepG2_RNAi$HepG2_RNAi_YBX3_ENCFF571NNW %>%
    dplyr::filter(!is.na(padj)) %>%
    dplyr::mutate(external_gene_name = mapIds(x=org.Hs.eg.db, keys=ensembl_gene_id, column='SYMBOL', keytype = 'ENSEMBL')) %>%
    dplyr::filter(!is.na(external_gene_name)) %>%
    dplyr::mutate(padj = ifelse(is.na(padj), 1, padj)) %>%
    dplyr::mutate(neg_log10Pval = -log10(padj)) %>%
    dplyr::mutate(is_target = external_gene_name %in% RBP_targets[["YBX3"]]) %>%
    dplyr::mutate(is_target_in_subset = external_gene_name %in% intersect(RBP_targets[["YBX3"]], unlist(RBP_targets_overlap_more20_sets))) %>%
    dplyr::arrange(neg_log10Pval),

  "PTBP1" = RBPKDs_ENCODE_DESeq2$HepG2_RNAi$HepG2_RNAi_PTBP1_ENCFF044UQQ %>%
    dplyr::filter(!is.na(padj)) %>%
    dplyr::mutate(external_gene_name = mapIds(x=org.Hs.eg.db, keys=ensembl_gene_id, column='SYMBOL', keytype = 'ENSEMBL')) %>%
    dplyr::filter(!is.na(external_gene_name)) %>%
    dplyr::mutate(padj = ifelse(is.na(padj), 1, padj)) %>%
    dplyr::mutate(neg_log10Pval = -log10(padj)) %>%
    dplyr::mutate(is_target = external_gene_name %in% RBP_targets[["PTBP1"]]) %>%
    dplyr::mutate(is_target_in_subset = external_gene_name %in% intersect(RBP_targets[["PTBP1"]], unlist(RBP_targets_overlap_more20_sets))) %>%
    dplyr::arrange(neg_log10Pval),
  "RBFOX2" = RBPKDs_ENCODE_DESeq2$HepG2_RNAi$HepG2_RNAi_RBFOX2_ENCFF166HLT %>%
    dplyr::filter(!is.na(padj)) %>%
    dplyr::mutate(external_gene_name = mapIds(x=org.Hs.eg.db, keys=ensembl_gene_id, column='SYMBOL', keytype = 'ENSEMBL')) %>%
    dplyr::filter(!is.na(external_gene_name)) %>%
    dplyr::mutate(padj = ifelse(is.na(padj), 1, padj)) %>%
    dplyr::mutate(neg_log10Pval = -log10(padj)) %>%
    dplyr::mutate(is_target = external_gene_name %in% RBP_targets[["RBFOX2"]]) %>%
    dplyr::mutate(is_target_in_subset = external_gene_name %in% intersect(RBP_targets[["RBFOX2"]], unlist(RBP_targets_overlap_more20_sets))) %>%
    dplyr::arrange(neg_log10Pval),
  "STMN1" = RBPKDs_Sondergaard2022$STMN1 %>%
    dplyr::filter(!is.na(external_gene_name)) %>%
    dplyr::mutate(padj = ifelse(is.na(padj), 1, padj)) %>%
   dplyr::mutate(neg_log10Pval = -log10(padj)) %>%
    dplyr::mutate(is_target = external_gene_name %in% RBP_targets[["STMN1"]]) %>%
    dplyr::mutate(is_target_in_subset = external_gene_name %in% intersect(RBP_targets[["STMN1"]],
                                                                          unlist(RBP_targets_overlap_more20_sets))) %>%
    dplyr::arrange(neg_log10Pval)
)

p <- list()
# YBX volcano plot
rbp <- "YBX3"
df <- RBP_diff_expr[[rbp]] %>%
  dplyr::mutate(is_target = ifelse(is_target, "Target", "Non-target")) %>%
  dplyr::filter(is_target == 'Target' | external_gene_name == rbp) %>%
  dplyr::mutate(is_target_in_subset = ifelse(is_target_in_subset, "Target in subset", "Non-target in subset")) %>%
  dplyr::mutate(is_target_in_subset = ifelse(padj<0.05 & abs(log2FoldChange)>log2(1.5), is_target_in_subset, "Non significant")) %>%
  dplyr::mutate(is_target_in_subset = ifelse(external_gene_name == rbp, rbp, is_target_in_subset)) %>%
  dplyr::mutate(is_target_in_subset = factor(is_target_in_subset, levels = c("Non significant", "Non-target in subset", "Target in subset", rbp))) %>%
  dplyr::arrange(is_target_in_subset)
to_label <- c(df %>% filter(external_gene_name == rbp) %>% pull(external_gene_name),
              df %>% filter(is_target_in_subset == "Non-target in subset" & padj<0.05 & abs(log2FoldChange)>log2(1.5)) %>% dplyr::arrange(padj) %>
              df %>% filter(is_target_in_subset == "Target in subset" & padj<0.05 & abs(log2FoldChange)>log2(1.5)) %>% pull(external_gene_name)) %
  unique()
df <- df %>%
  mutate(label = ifelse(external_gene_name %in% to_label, external_gene_name, ""))

paste0(rbp, ":")
```

```
## [1] "YBX3:"
```

```
table(df$is_target_in_subset)
```

```
##
##      Non significant Non-target in subset     Target in subset
##                 4067                   39                    6
##                 YBX3
##                    1
```

```
p[[1]] <- ggplot(df, aes(x = log2FoldChange, y = neg_log10Pval, color = is_target_in_subset)) +
  geom_point(size = 2) +
  scale_alpha_manual(values = c("Target in subset" = 0.8, "Non-target in subset" = 0.3, rbp = 1)) +
  scale_color_manual(values = c("Target in subset" = "#e31a1c", "Non-target in subset" = "#fb9a99", rbp = "#000000", "Non significant" = "#BFBFBF"
```

```
  geom_hline(yintercept = -log10(0.05), linetype = "dashed", color = "black") +
  geom_vline(xintercept = c(-log2(1.5), log2(1.5)), linetype = "dashed", color = "black") +
  geom_text_repel(aes(label = label), box.padding = 0.5, point.padding = 0.5, segment.color = "black", max.overlaps = Inf) +
  labs(
    x = "log2 Fold Change",
    y = "-log10(padj)"
  ) +
  ggtitle(rbp) +
  theme_bw(base_size = 14) +
  theme(panel.grid = element_blank(),
        legend.position = "none")

# PTBP1 volcano plot
rbp <- "PTBP1"
df <- RBP_diff_expr[[rbp]] %>%
  dplyr::mutate(is_target = ifelse(is_target, "Target", "Non-target")) %>%
  dplyr::filter(is_target == 'Target' | external_gene_name == rbp) %>%
  dplyr::mutate(is_target_in_subset = ifelse(is_target_in_subset, "Target in subset", "Non-target in subset")) %>%
  dplyr::mutate(is_target_in_subset = ifelse(padj<0.05 & abs(log2FoldChange)>log2(1.5), is_target_in_subset, "Non significant")) %>%
  dplyr::mutate(is_target_in_subset = ifelse(external_gene_name == rbp, rbp, is_target_in_subset)) %>%
  dplyr::mutate(is_target_in_subset = factor(is_target_in_subset, levels = c("Non significant", "Non-target in subset", "Target in subset", rbp))) %>%
  dplyr::arrange(is_target_in_subset)
to_label <- c(df %>% filter(external_gene_name == rbp) %>% pull(external_gene_name),
              df %>% filter(is_target_in_subset == "Non-target in subset" & padj<0.05 & abs(log2FoldChange)>log2(1.5)) %>% dplyr::arrange(padj) %>
              df %>% filter(is_target_in_subset == "Target in subset" & padj<0.05 & abs(log2FoldChange)>log2(1.5)) %>% dplyr::arrange(padj) %>% sl
  unique()
df <- df %>%
  mutate(label = ifelse(external_gene_name %in% to_label, external_gene_name, ""))

paste0(rbp, ":")
```

```
## [1] "PTBP1:"
```

```
table(df$is_target_in_subset)
```

```
##
##      Non significant Non-target in subset     Target in subset
##                 3169                  406                  114
##                PTBP1
##                    1
```

```
p[[2]] <- ggplot(df, aes(x = log2FoldChange, y = neg_log10Pval, color = is_target_in_subset)) +
  geom_point(size = 2) +
  scale_alpha_manual(values = c("Target in subset" = 0.8, "Non-target in subset" = 0.3, rbp = 1)) +
  scale_color_manual(values = c("Target in subset" = "#e31a1c", "Non-target in subset" = "#fb9a99", rbp = "#000000", "Non significant" = "#BFBFBF"
  geom_hline(yintercept = -log10(0.05), linetype = "dashed", color = "black") +
  geom_vline(xintercept = c(-log2(1.5), log2(1.5)), linetype = "dashed", color = "black") +
  geom_text_repel(aes(label = label), box.padding = 0.5, point.padding = 0.5, segment.color = "black", max.overlaps = Inf) +
  labs(
    x = "log2 Fold Change",
    y = "-log10(padj)"
  ) +
  ggtitle(rbp) +
  theme_bw(base_size = 14) +
  theme(panel.grid = element_blank(),
        legend.position = "none")

# RBFOX2 volcano plot
rbp <- "RBFOX2"
df <- RBP_diff_expr[[rbp]] %>%
  dplyr::mutate(is_target = ifelse(is_target, "Target", "Non-target")) %>%
  dplyr::filter(is_target == 'Target' | external_gene_name == rbp) %>%
  dplyr::mutate(is_target_in_subset = ifelse(is_target_in_subset, "Target in subset", "Non-target in subset")) %>%
  dplyr::mutate(is_target_in_subset = ifelse(padj<0.05 & abs(log2FoldChange)>log2(1.5), is_target_in_subset, "Non significant")) %>%
  dplyr::mutate(is_target_in_subset = ifelse(external_gene_name == rbp, rbp, is_target_in_subset)) %>%
  dplyr::mutate(is_target_in_subset = factor(is_target_in_subset, levels = c("Non significant", "Non-target in subset", "Target in subset", rbp))) %>%
  dplyr::arrange(is_target_in_subset)
to_label <- c(df %>% filter(external_gene_name == rbp) %>% pull(external_gene_name),
              df %>% filter(is_target_in_subset == "Non-target in subset" & padj<0.05 & abs(log2FoldChange)>log2(1.5)) %>% dplyr::arrange(padj) %>
              df %>% filter(is_target_in_subset == "Target in subset" & padj<0.05 & abs(log2FoldChange)>log2(1.5)) %>% pull(external_gene_name)) %
  unique()
df <- df %>%
  mutate(label = ifelse(external_gene_name %in% to_label, external_gene_name, ""))

paste0(rbp, ":")
```

```
## [1] "RBFOX2:"
```

```r
table(df$is_target_in_subset)
```

```
##
##      Non significant Non-target in subset     Target in subset
##                 3624                   12                    3
##               RBFOX2
##                    1
```

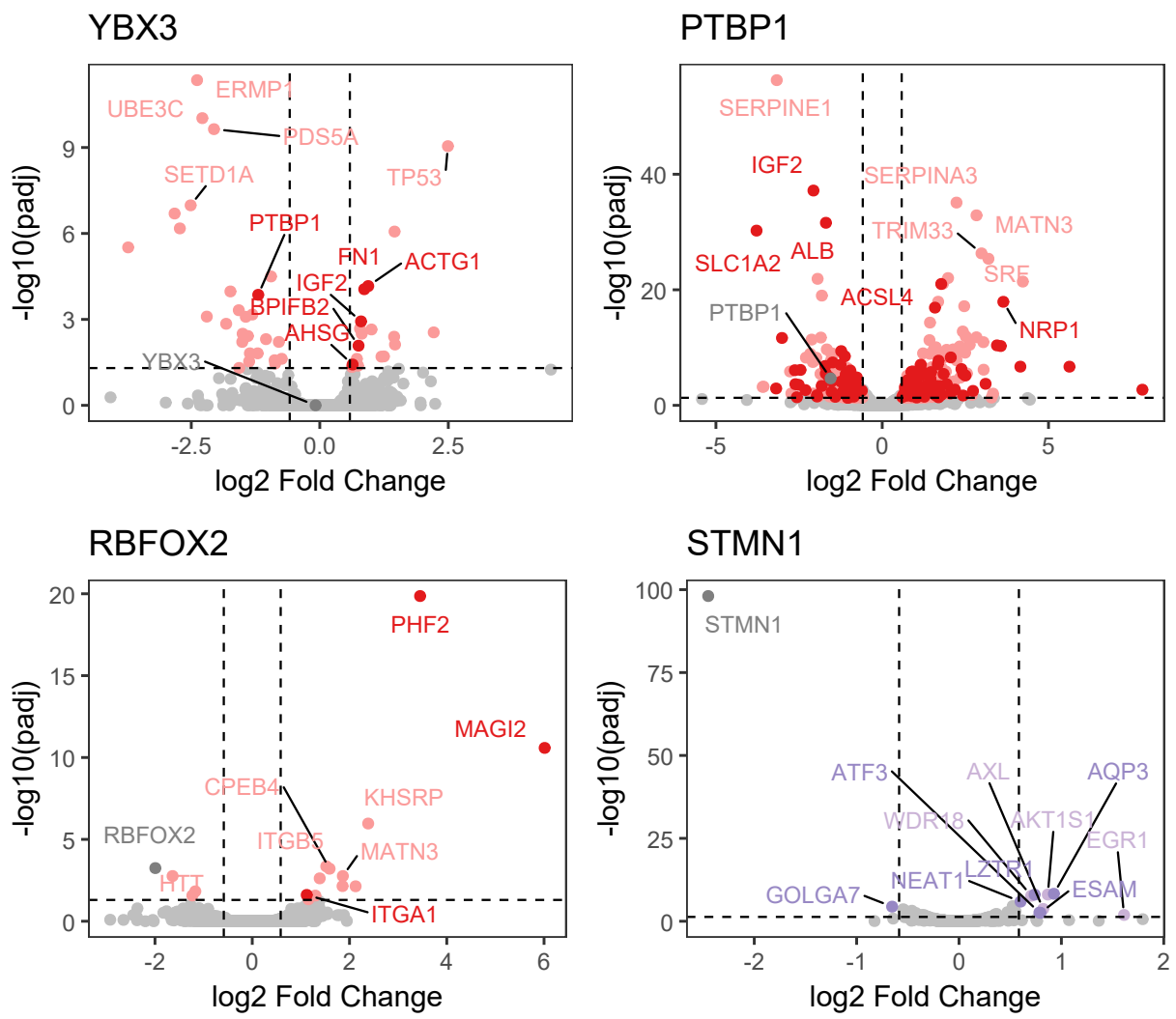
```r
p[[3]] <- ggplot(df, aes(x = log2FoldChange, y = neg_log10Pval, color = is_target_in_subset)) +
  geom_point(size = 2) +
  scale_alpha_manual(values = c("Target in subset" = 0.8, "Non-target in subset" = 0.3, rbp = 1)) +
  scale_color_manual(values = c("Target in subset" = "#e31a1c", "Non-target in subset" = "#fb9a99", rbp = "#000000", "Non significant" = "#BFBFBF"
  geom_hline(yintercept = -log10(0.05), linetype = "dashed", color = "black") +
  geom_vline(xintercept = c(-log2(1.5), log2(1.5)), linetype = "dashed", color = "black") +
  geom_text_repel(aes(label = label), box.padding = 0.5, point.padding = 0.5, segment.color = "black", max.overlaps = Inf) +
  labs(
    x = "log2 Fold Change",
    y = "-log10(padj)"
  ) +
  ggtitle(rbp) +
  theme_bw(base_size = 14) +
  theme(panel.grid = element_blank(),
        legend.position = "none")

# STMN1 volcano plot
rbp <- "STMN1"
df <- RBP_diff_expr[[rbp]] %>%
  dplyr::mutate(is_target = ifelse(is_target, "Target", "Non-target")) %>%
  dplyr::filter(is_target == 'Target' | external_gene_name == rbp) %>%
  dplyr::mutate(is_target_in_subset = ifelse(is_target_in_subset, "Target in subset", "Non-target in subset")) %>%
  dplyr::mutate(is_target_in_subset = ifelse(padj<0.05 & abs(log2FoldChange)>log2(1.5), is_target_in_subset, "Non significant")) %>%
  dplyr::mutate(is_target_in_subset = ifelse(external_gene_name == rbp, rbp, is_target_in_subset)) %>%
  dplyr::mutate(is_target_in_subset = factor(is_target_in_subset, levels = c("Non significant", "Non-target in subset", "Target in subset", rbp))) 
  dplyr::arrange(is_target_in_subset)
to_label <- c(df %>% filter(external_gene_name == rbp) %>% pull(external_gene_name),
              df %>% filter(is_target_in_subset == "Non-target in subset" & padj<0.05 & abs(log2FoldChange)>log2(1.5)) %>% dplyr::arrange(padj) %>
              df %>% filter(is_target_in_subset == "Target in subset" & padj<0.05 & abs(log2FoldChange)>log2(1.5)) %>% pull(external_gene_name)) %
  unique()
df <- df %>%
  mutate(label = ifelse(external_gene_name %in% to_label, external_gene_name, ""))

paste0(rbp, ":")
```

```
## [1] "STMN1:"
```

```r
table(df$is_target_in_subset)
```

```
##
##      Non significant Non-target in subset     Target in subset
##                  980                    4                    6
##                STMN1
##                    1
```

```r
p[[4]] <- ggplot(df, aes(x = log2FoldChange, y = neg_log10Pval, color = is_target_in_subset)) +
  geom_point(size = 2) +
  scale_alpha_manual(values = c("Target in subset" = 0.8, "Non-target in subset" = 0.3, rbp = 1)) +
  scale_color_manual(values = c("Target in subset" = "#9787c4", "Non-target in subset" = "#cab2d6", rbp = "#000000", "Non significant" = "#BFBFBF"
  geom_hline(yintercept = -log10(0.05), linetype = "dashed", color = "black") +
  geom_vline(xintercept = c(-log2(1.5), log2(1.5)), linetype = "dashed", color = "black") +
  geom_text_repel(aes(label = label), box.padding = 0.5, point.padding = 0.5, segment.color = "black", max.overlaps = Inf) +
  labs(
    x = "log2 Fold Change",
    y = "-log10(padj)"
  ) +
  ggtitle(rbp) +
  theme_bw(base_size = 14) +
  theme(panel.grid = element_blank(),
        legend.position = "none")

combined_plot <- (p[[1]] | p[[2]]) / (p[[3]] | p[[4]])

print(combined_plot)
```



SessionInfo

```
utils::sessionInfo()
```

```
## R version 4.4.1 (2024-06-14 ucrt)
## Platform: x86_64-w64-mingw32/x64
## Running under: Windows 10 x64 (build 19045)
##
## Matrix products: default
##
##
## locale:
## [1] LC_COLLATE=English_United States.utf8
## [2] LC_CTYPE=English_United States.utf8
## [3] LC_MONETARY=English_United States.utf8
## [4] LC_NUMERIC=C
## [5] LC_TIME=English_United States.utf8
##
## time zone: Europe/Stockholm
## tzcode source: internal
##
## attached base packages:
```

```
## [1] grid      stats4    stats     graphics  grDevices utils     datasets
## [8] methods   base
##
## other attached packages:
##  [1] ggrepel_0.9.6          fgsea_1.30.0           circlize_0.4.16
##  [4] ComplexHeatmap_2.20.0  rrvgo_1.16.0           clusterProfiler_4.12.6
##  [7] ComplexUpset_1.3.3     patchwork_1.3.1        g3viz_1.2.0
## [10] eulerr_7.0.2           org.Hs.eg.db_3.19.1    AnnotationDbi_1.66.0
## [13] IRanges_2.38.1         S4Vectors_0.42.1       Biobase_2.64.0
## [16] BiocGenerics_0.50.0    lubridate_1.9.4        forcats_1.0.0
## [19] stringr_1.5.1          dplyr_1.1.4            purrr_1.0.4
## [22] readr_2.1.5            tidyr_1.3.1            tibble_3.3.0
## [25] ggplot2_3.5.2          tidyverse_2.0.0
##
## loaded via a namespace (and not attached):
##   [1] RColorBrewer_1.1-3      shape_1.4.6.1           rstudioapi_0.17.1
##   [4] jsonlite_2.0.0          umap_0.2.10.0           magrittr_2.0.3
##   [7] farver_2.1.2            rmarkdown_2.29          GlobalOptions_0.1.2
##  [10] fs_1.6.6                zlibbioc_1.50.0         vctrs_0.6.5
##  [13] memoise_2.0.1           askpass_1.2.1           ggtree_3.12.0
##  [16] tinytex_0.57            htmltools_0.5.8.1       cellranger_1.1.0
##  [19] gridGraphics_0.5-1      htmlwidgets_1.6.4       plyr_1.8.9
##  [22] httr2_1.1.2             cachem_1.1.0            igraph_2.1.4
##  [25] iterators_1.0.14        mime_0.13               lifecycle_1.0.4
##  [28] pkgconfig_2.0.3         Matrix_1.7-3            R6_2.6.1
##  [31] fastmap_1.2.0           gson_0.1.0              clue_0.3-66
##  [34] GenomeInfoDbData_1.2.12 shiny_1.11.0            digest_0.6.37
##  [37] aplot_0.2.7             enrichplot_1.24.4       colorspace_2.1-1
##  [40] RSpectra_0.16-2         RSQLite_2.4.1           labeling_0.4.3
##  [43] timechange_0.3.0        httr_1.4.7              polyclip_1.10-7
##  [46] compiler_4.4.1          doParallel_1.0.17       bit64_4.6.0-1
##  [49] withr_3.0.2             BiocParallel_1.38.0     viridis_0.6.5
##  [52] DBI_1.2.3               ggforce_0.5.0           R.utils_2.13.0
##  [55] MASS_7.3-65             openssl_2.3.3           rappdirs_0.3.3
##  [58] rjson_0.2.23            tools_4.4.1             ape_5.8-1
##  [61] scatterpie_0.2.5        httpuv_1.6.16           R.oo_1.27.1
##  [64] glue_1.8.0              promises_1.3.3          nlme_3.1-168
##  [67] GOSemSim_2.30.2         polylabelr_0.3.0        shadowtext_0.1.4
##  [70] gridBase_0.4-7          cluster_2.1.8.1         reshape2_1.4.4
##  [73] generics_0.1.4          gtable_0.3.6            tzdb_0.5.0
##  [76] R.methodsS3_1.8.2       data.table_1.17.6       hms_1.1.3
##  [79] xml2_1.3.8              tidygraph_1.3.1         XVector_0.44.0
##  [82] foreach_1.5.2           pillar_1.10.2           yulab.utils_0.2.0
##  [85] later_1.4.2             splines_4.4.1           tweenr_2.0.3
##  [88] treeio_1.28.0           lattice_0.22-7          bit_4.6.0
##  [91] tidyselect_1.2.1        GO.db_3.19.1            tm_0.7-16
##  [94] Biostrings_2.72.1       knitr_1.50              gridExtra_2.3
##  [97] NLP_0.3-2               xfun_0.52               graphlayouts_1.2.2
## [100] matrixStats_1.5.0       pheatmap_1.0.13         stringi_1.8.7
## [103] UCSC.utils_1.0.0        lazyeval_0.2.2          ggfun_0.1.9
## [106] yaml_2.3.10             evaluate_1.0.4          codetools_0.2-20
## [109] wordcloud_2.6           ggraph_2.2.1            qvalue_2.36.0
## [112] ggplotify_0.1.2         cli_3.6.5               reticulate_1.42.0
## [115] xtable_1.8-4            readxl_1.4.5            treemap_2.4-4
## [118] Rcpp_1.0.14             GenomeInfoDb_1.40.1     png_0.1-8
## [121] parallel_4.4.1          blob_1.2.4              DOSE_3.30.5
## [124] slam_0.1-55             viridisLite_0.4.2       tidytree_0.4.6
## [127] scales_1.4.0            crayon_1.5.3            GetoptLong_1.0.5
## [130] rlang_1.1.6             cowplot_1.1.3           fastmatch_1.1-6
## [133] KEGGREST_1.44.1
```