

# Motif enrichment

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```
#Load libraries
library(tidyverse)
library(ggplot2)
library(gridExtra)
library(grid)
library(ggpubr)
```

```
#read data
```

```
# TC motif
```

```
TC_ESharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TC motif/TC E30S.tsv",sep = "\t",head=1)
TC_ESharp<-TC_ESharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #252
```

```
TC_EBroad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TC motif/TC E30B.tsv",sep = "\t",head=1)
TC_EBroad<-TC_EBroad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #421
```

```
TC_SSharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TC motif/TC S30S.tsv",sep = "\t",head=1)
TC_SSharp<-TC_SSharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #282
```

```
TC_SBroad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TC motif/TC S30B.tsv",sep = "\t",head=1)
TC_SBroad<-TC_SBroad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #432
```

```
TC_E37Sharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TC motif/TC E37S.tsv",sep = "\t",head=1)
TC_E37Sharp<-TC_E37Sharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #247
```

```
TC_E37Broad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TC motif/TC E37B.tsv",sep = "\t",head=1)
TC_E37Broad<-TC_E37Broad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
```

```

mutate(motif="TC")%>%
mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #346

TC_S37Sharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TC motif/TC S37S.tsv",sep = "\t",header=TRUE)
TC_S37Sharp<-TC_S37Sharp%>%filter(p.value<0.0005)%>%
mutate(position_to_TSS=start-100)%>%
mutate(motif="TC")%>%
mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #221

TC_S37Broad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TC motif/TC S37B.tsv",sep = "\t",header=TRUE)
TC_S37Broad<-TC_S37Broad%>%filter(p.value<0.0005)%>%
mutate(position_to_TSS=start-100)%>%
mutate(motif="TC")%>%
mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #388

#GG motif
GG_ESharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/GG motif/GG E30S.tsv",sep = "\t",header=TRUE)
GG_ESharp<-GG_ESharp%>%filter(p.value<0.0005)%>%
mutate(position_to_TSS=start-100)%>%
mutate(motif="GG")%>%
mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #23

GG_EBroad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/GG motif/GG E30B.tsv",sep = "\t",header=TRUE)
GG_EBroad<- GG_EBroad%>%filter(p.value<0.0005)%>%
mutate(position_to_TSS=start-100)%>%
mutate(motif="GG")%>%
mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #27

GG_SSharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/GG motif/GG S30S.tsv",sep = "\t",header=TRUE)
GG_SSharp<-GG_SSharp%>%filter(p.value<0.0005)%>%
mutate(position_to_TSS=start-100)%>%
mutate(motif="GG")%>%
mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #21

GG_SBroad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/GG motif/GG S30B.tsv",sep = "\t",header=TRUE)
GG_SBroad<-GG_SBroad%>%filter(p.value<0.0005)%>%
mutate(position_to_TSS=start-100)%>%
mutate(motif="GG")%>%
mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #64

GG_E37Sharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/GG motif/GG E37S.tsv",sep = "\t",header=TRUE)
GG_E37Sharp<- GG_E37Sharp%>%filter(p.value<0.0005)%>%
mutate(position_to_TSS=start-100)%>%
mutate(motif="GG")%>%
mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #23

GG_E37Broad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/GG motif/GG E37B.tsv",sep = "\t",header=TRUE)
GG_E37Broad<-GG_E37Broad%>%filter(p.value<0.0005)%>%
mutate(position_to_TSS=start-100)%>%
mutate(motif="GG")%>%
mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #30

GG_S37Sharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/GG motif/GG S37S.tsv",sep = "\t",header=TRUE)

```

```

GG_S37Sharp<-GG_S37Sharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="GG")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #13

GG_S37Broad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/GG motif/GG S37B.tsv",sep = "\t",h
GG_S37Broad<-GG_S37Broad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="GG")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #49

#TATA motif
TATA_ESharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TATA motif/TATA E30S.tsv",sep = "\
TATA_ESharp<- TATA_ESharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TATA")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #46

TATA_EBroad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TATA motif/TATA E30B.tsv",sep = "\
TATA_EBroad<- TATA_EBroad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TATA")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #58

TATA_SSharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TATA motif/TATA S30S.tsv",sep = "\
TATA_SSharp<- TATA_SSharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TATA")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #45

TATA_SBroad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TATA motif/TATA S30B.tsv",sep = "\
TATA_SBroad<- TATA_SBroad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TATA")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #77

TATA_E37Sharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TATA motif/TATA E37S.tsv",sep = \
TATA_E37Sharp<- TATA_E37Sharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TATA")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #43

TATA_E37Broad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TATA motif/TATA E37B.tsv",sep = \
TATA_E37Broad<- TATA_E37Broad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TATA")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #67

TATA_S37Sharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TATA motif/TATA S37S.tsv",sep = \
TATA_S37Sharp<- TATA_S37Sharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TATA")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #36

```

```

TATA_S37Broad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TATA motif/TATA S37B.tsv",sep = "\t",head=1)
TATA_S37Broad<- TATA_S37Broad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TATA")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #91

#AC motif
AC_ESharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/AC motif/AC E30S.tsv",sep = "\t",head=1)
AC_ESharp<- AC_ESharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="AC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #9

AC_EBroad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/AC motif/AC E30B.tsv",sep = "\t",head=1)
AC_EBroad<- AC_EBroad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="AC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #61

AC_SSharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/AC motif/AC S30S.tsv",sep = "\t",head=1)
AC_SSharp<- AC_SSharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="AC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #13

AC_SBroad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/AC motif/AC S30B.tsv",sep = "\t",head=1)
AC_SBroad<- AC_SBroad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="AC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #78

AC_E37Sharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/AC motif/AC E37S.tsv",sep = "\t",head=1)
AC_E37Sharp<- AC_E37Sharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="AC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #19

AC_E37Broad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/AC motif/AC E37B.tsv",sep = "\t",head=1)
AC_E37Broad<- AC_E37Broad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="AC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #57

AC_S37Sharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/AC motif/AC S37S.tsv",sep = "\t",head=1)
AC_S37Sharp<- AC_S37Sharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="AC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #16

AC_S37Broad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/AC motif/AC S37B.tsv",sep = "\t",head=1)
AC_S37Broad<- AC_S37Broad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%

```

```

mutate(motif="AC")%>%
mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #52

#TTAC motif
TTAC_ESharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TTAC motif/TTAC E30S.tsv",sep = "\t")
TTAC_ESharp<- TTAC_ESharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TTAC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #11

TTAC_EBroad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TTAC motif/TTAC E30B.tsv",sep = "\t")
TTAC_EBroad<- TTAC_EBroad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TTAC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #50

TTAC_SSharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TTAC motif/TTAC S30S.tsv",sep = "\t")
TTAC_SSharp<- TTAC_SSharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TTAC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #15

TTAC_SBroad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TTAC motif/TTAC S30B.tsv",sep = "\t")
TTAC_SBroad<- TTAC_SBroad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TTAC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #48

TTAC_E37Sharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TTAC motif/TTAC E37S.tsv",sep = "\t")
TTAC_E37Sharp<- TTAC_E37Sharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TTAC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #15

TTAC_E37Broad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TTAC motif/TTAC E37B.tsv",sep = "\t")
TTAC_E37Broad<- TTAC_E37Broad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TTAC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #37

TTAC_S37Sharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TTAC motif/TTAC S37S.tsv",sep = "\t")
TTAC_S37Sharp<- TTAC_S37Sharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TTAC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #11

```

```

TTAC_S37Broad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/TTAC motif/TTAC S37B.tsv",sep = "\t",header = 1)
TTAC_S37Broad<- TTAC_S37Broad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="TTAC")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+)::")[,2]) #47

#GA motif
GA_ESharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/GA motif/GA E30S.tsv",sep = "\t",header = 1)
GA_ESharp<- GA_ESharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="GA")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+)::")[,2]) #12

GA_EBroad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/GA motif/GA E30B.tsv",sep = "\t",header = 1)
GA_EBroad<- GA_EBroad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="GA")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+)::")[,2]) #39

GA_SSharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/GA motif/GA S30S.tsv",sep = "\t",header = 1)
GA_SSharp<- GA_SSharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="GA")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+)::")[,2]) #10

GA_SBroad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/GA motif/GA S30B.tsv",sep = "\t",header = 1)
GA_SBroad<- GA_SBroad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="GA")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+)::")[,2]) #33

GA_E37Sharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/GA motif/GA E37S.tsv",sep = "\t",header = 1)
GA_E37Sharp<- GA_E37Sharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="GA")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+)::")[,2]) #11

GA_E37Broad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/GA motif/GA E37B.tsv",sep = "\t",header = 1)
GA_E37Broad<- GA_E37Broad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="GA")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+)::")[,2]) #27

GA_S37Sharp <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/GA motif/GA S37S.tsv",sep = "\t",header = 1)
GA_S37Sharp<- GA_S37Sharp%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%

```

```

mutate(motif="GA")%>%
mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #6

GA_S37Broad <- read.table("D:/PhD/TSS cluster/H99/TRASS_d17/MEME all/GA motif/GA S37B.tsv",sep = "\t",h
GA_S37Broad<- GA_S37Broad%>%filter(p.value<0.0005)%>%
  mutate(position_to_TSS=start-100)%>%
  mutate(motif="GA")%>%
  mutate(Id=str_match(sequence_name,"(\\b.+):")[,2]) #36

#Position relative to TSS
#position of motif in Expo Sharp cluster
ESharp_motif<-bind_rows(TC_ESharp,GG_ESharp,TATA_ESharp,AC_ESharp,TTAC_ESharp,GA_ESharp)

#remove gene as identify in Percentage small set All cluster
gene_reject_E30<-read.delim("D:/PhD/TSS cluster/H99/TRASS_d17/gene_reject_E30.txt")
ESharp_motif<-ESharp_motif%>%filter(!(Id%in%gene_reject_E30$gene_reject_E30))

neworder <- c("AC","TTAC","GG","GA","TATA","TC")
table(ESharp_motif$motif)

##
##   AC   GA   GG TATA   TC TTAC
##    9   10   21   41  241   11

ESharp_motif<-ESharp_motif%>%
  mutate(motif=factor(motif,levels=neworder))%>%
  arrange(motif)

Sharp_plot<-ESharp_motif%>%ggplot(aes(x=position_to_TSS))+
  geom_density()+
  facet_wrap(vars(motif),nrow=1)+
  scale_y_continuous(limits = c(0,0.09))+
  theme_bw()+
  theme(panel.spacing = unit(0.5, "cm"))+
  theme(plot.title = element_text(size=12,vjust=4),
        axis.title.x.bottom = element_blank(),
        axis.title.y.left = element_blank(),
        axis.text.x.bottom = element_text(size=11),
        axis.text.y.left = element_text(size=11))+
  labs(title="SHARP")

#Position of motif in Expo Broad cluster

EBroad_motif<-bind_rows(TC_EBroad,GG_EBroad,TATA_EBroad,AC_EBroad,TTAC_EBroad,GA_EBroad)

#remove gene as identify in Percentage small set All cluster
EBroad_motif<-EBroad_motif%>%filter(!(Id%in%gene_reject_E30$gene_reject_E30))

table(EBroad_motif$motif)

```



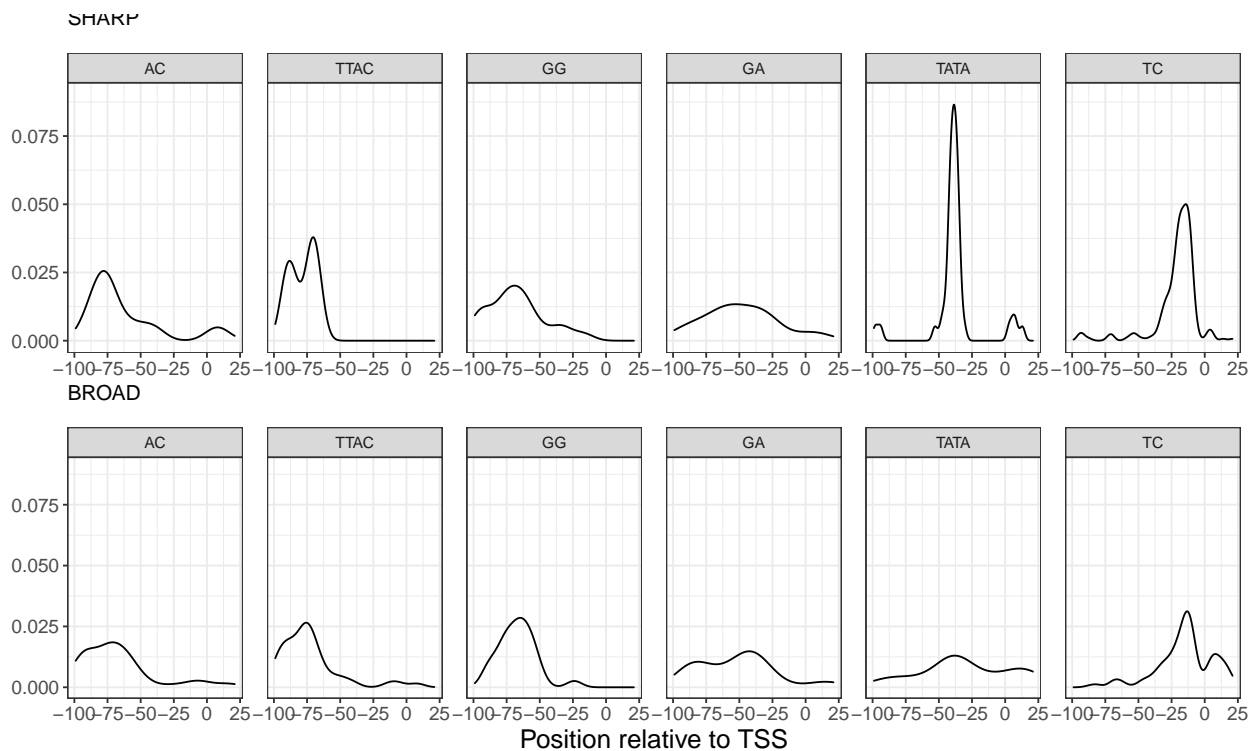
```
##
##   AC   GA   GG TATA   TC TTAC
##   60   39   27  55  392  47
```

```
neworder <- c("AC","TTAC","GG","GA","TATA","TC")
```

```
EBroad_motif<-EBroad_motif%>%
  mutate(motif=factor(motif,levels=neworder))%>%
  arrange(motif)
```

```
Broad_plot<-EBroad_motif%>%ggplot(aes(x=position_to_TSS))+
  geom_density()+
  facet_wrap(vars(motif),nrow=1)+
  scale_y_continuous(limits = c(0,0.09))+
  xlab("Position relative to TSS")+
  theme_bw()+
  theme(panel.spacing = unit(0.5, "cm"))+
  theme(plot.title = element_text(size=12,vjust=4),
        axis.title.x.bottom = element_text(size=15),
        axis.title.y.left = element_blank(),
        axis.text.x.bottom = element_text(size=11),
        axis.text.y.left = element_text(size=11))+
  labs(title="BROAD")
```

```
grid.arrange(Sharp_plot,Broad_plot, nrow=2)
```





```
All_condition_clusters<-read.table("D:/PhD/TSS_cluster/H99/TRASS_d17/All_condition_clusters.txt",sep="\n")
All_condition_clusters<-All_condition_clusters%>%arrange(condition,Cluster_Shape)
```

```
#Percentage of motif: need to remove left, right
```

```
Expo_30<- All_condition_clusters%>%
  filter(condition=="EXPO 30",Cluster_Shape!="NA")%>%
  mutate(TC_box = Id %in% c(TC_ESharp$Id,TC_EBroad$Id),
         GG_box = Id %in% c(GG_ESharp$Id,GG_EBroad$Id),
         TATA_box = Id %in% c(TATA_ESharp$Id,TATA_EBroad$Id),
         AC_box = Id %in% c(AC_ESharp$Id,AC_EBroad$Id),
         TTAC_box = Id %in% c(TTAC_EBroad$Id,TTAC_ESharp$Id),
         GA_box = Id %in% c(GA_ESharp$Id,GA_EBroad$Id))
```

```
Summary<-Expo_30%>%group_by(Cluster_Shape)%>%
  dplyr::summarise(nTC=sum(TC_box),perTC=100*nTC/n(),
                  nGG=sum(GG_box),perGG=100*nGG/n(),
                  nTATA=sum(TATA_box),perTATA=100*nTATA/n(),
                  nAC=sum(AC_box),perAC=100*nAC/n(),
                  nTTAC=sum(TTAC_box),perTTAC=100*nTTAC/n(),
                  nGA=sum(GA_box),perGA=100*nGA/n())
```

```
Summary
```

```
## # A tibble: 2 x 13
##   Cluste-1  nTC perTC  nGG perGG nTATA perTATA  nAC perAC nTTAC perTTAC  nGA
##   <chr>    <int> <dbl> <int> <dbl> <int>   <dbl> <int> <dbl> <int>   <dbl> <int>
## 1 Broad      154  40.7   23  6.08   44   11.6   53  14.0   48   12.7   32
## 2 Sharp       64  50.4   15  11.8   31   24.4    9  7.09   11    8.66   11
## # ... with 1 more variable: perGA <dbl>, and abbreviated variable name
## #   1: Cluster_Shape
```

```
Expo_30%>%dplyr::summarise(nTATA_all=sum(TATA_box),
                          perTATA_all=100*nTATA_all/n())
```

```
##   nTATA_all perTATA_all
## 1         75    14.85149
```

```
#Change the name of levels
```

```
Expo_30<-Expo_30%>%
  mutate(TC_box=if_else(TC_box==TRUE,"TC box","no TC box"),
         GG_box=if_else(GG_box==TRUE,"GG box",'no GG box'),
         TATA_box=if_else(TATA_box==TRUE,"TATA box","no TATA box"),
         AC_box=if_else(AC_box==TRUE,"AC box",'no AC box'),
         TTAC_box=if_else(TTAC_box==TRUE,"TTAC box","no TTAC box"),
         GA_box=if_else(GA_box==TRUE,"GA box",'no GA box')
  )
```

```
#chi-squared test
```

```
#TATA box
```

```
TATA_table<-table(Expo_30$Cluster_Shape,Expo_30$TATA_box)
TATA_table
```

```
##
```

```
##           no TATA box TATA box
##   Broad           334      44
##   Sharp           96      31
```

```
chisq.test(TATA_table)
```

```
##
##   Pearson's Chi-squared test with Yates' continuity correction
##
## data:  TATA_table
## X-squared = 11.268, df = 1, p-value = 0.0007885
```

```
#GG box
GG_table<-table(Expo_30$Cluster_Shape,Expo_30$GG_box)
GG_table
```

```
##
##           GG box no GG box
##   Broad      23      355
##   Sharp     15      112
```

```
chisq.test(GG_table)
```

```
##
##   Pearson's Chi-squared test with Yates' continuity correction
##
## data:  GG_table
## X-squared = 3.6945, df = 1, p-value = 0.05459
```

```
#TC box
TC_table<-table(Expo_30$Cluster_Shape,Expo_30$TC_box)
TC_table
```

```
##
##           no TC box TC box
##   Broad      224      154
##   Sharp      63       64
```

```
chisq.test(TC_table)
```

```
##
##   Pearson's Chi-squared test with Yates' continuity correction
##
## data:  TC_table
## X-squared = 3.2278, df = 1, p-value = 0.0724
```

```
#TTACbox
TTAC_table<-table(Expo_30$Cluster_Shape,Expo_30$TTAC_box)
TTAC_table
```

```
##
##          no TTAC box TTAC box
##   Broad      330      48
##   Sharp      116      11
```

```
chisq.test(TTAC_table)
```

```
##
##   Pearson's Chi-squared test with Yates' continuity correction
##
## data:  TTAC_table
## X-squared = 1.1357, df = 1, p-value = 0.2866
```

```
#AC box
AC_table<-table(Expo_30$Cluster_Shape,Expo_30$AC_box)
AC_table
```

```
##
##          AC box no AC box
##   Broad      53      325
##   Sharp       9      118
```

```
chisq.test(AC_table)
```

```
##
##   Pearson's Chi-squared test with Yates' continuity correction
##
## data:  AC_table
## X-squared = 3.6251, df = 1, p-value = 0.05692
```

```
#GA box
GA_table<-table(Expo_30$Cluster_Shape,Expo_30$GA_box)
GA_table
```

```
##
##          GA box no GA box
##   Broad      32      346
##   Sharp      11      116
```

```
chisq.test(GA_table)
```

```
##
##   Pearson's Chi-squared test with Yates' continuity correction
##
## data:  GA_table
## X-squared = 2.2311e-29, df = 1, p-value = 1
```

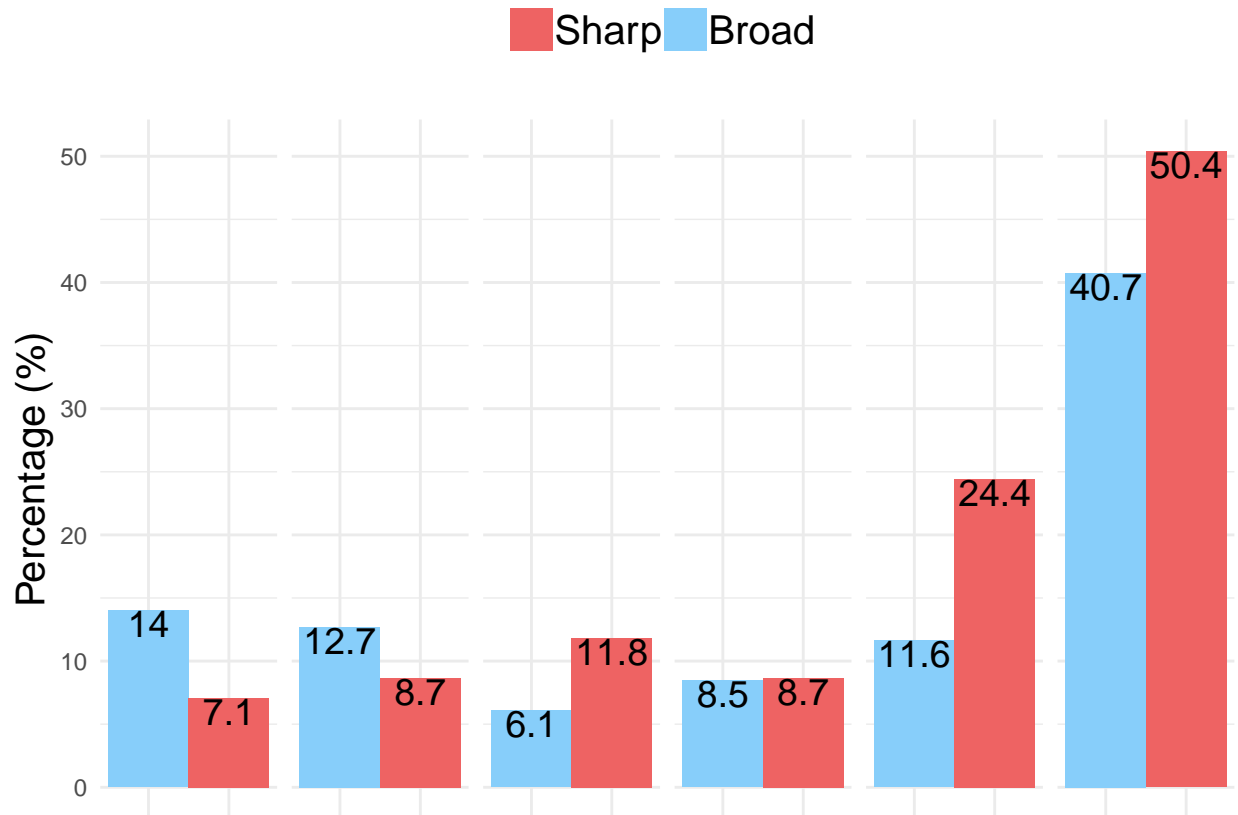
```

Summary_Expo30_long<-Summary%>%
  pivot_longer(cols=c("perTC","perGG","perTATA","perAC","perTTAC","perGA"),
               names_to="Motif",
               values_to="Percentage")

#control order of facet_wrap
neworder<-c("perAC","perTTAC","perGG","perGA","perTATA","perTC")
Summary_Expo30_long<- Summary_Expo30_long%>%
  mutate(Motif=factor(Motif,levels=neworder))%>%
  arrange(Motif)

Summary_Expo30_long%>%ggplot(aes(x=Cluster_Shape,y=Percentage,fill=Cluster_Shape))+
  geom_col(width = 1)+
  scale_fill_manual(
    values =c("indianred2","lightskyblue"),
    breaks = c("Sharp","Broad"),
    labels = c("Sharp", "Broad ")
  )+
  geom_text(aes(label = round(Percentage,1)), size = 5, hjust = 0.5, vjust = 1, position = "stack")+
  theme_minimal()+
  ylab("Percentage (%)")+
  theme(axis.title.x = element_blank(),
        axis.text.x.bottom = element_blank(),
        axis.title.y.left = element_text(size=15),
        legend.position = "top",
        legend.title = element_text(size=0),
        legend.text = element_text(size=15))+
  facet_wrap(vars(Motif),#labeller = labeller(Motif = c("perTC"="TC box",
#                                     "perGG" = "GG box",
#                                     "perTATA" = "TATA box",
#                                     "perAC" = "AC box",
#                                     "perTTAC"="TTAC box",
#                                     "perGA"="GA box"))),
  nrow=1) +
  theme(strip.text.x = element_text(size=0))

```



```
#Relationship between motif and gene expression
```

```
#Load gene expression data
```

```
txt_files = list.files("D:/PhD/TSS cluster/H99/regulated genes/",pattern = "\\..txt");
txt_files
```

```
## [1] "FlucovsWT.complete.txt" "FlucovsWT.down.txt"
## [3] "FlucovsWT.up.txt"      "SDSvsWT.complete.txt"
## [5] "SDSvsWT.down.txt"      "SDSvsWT.up.txt"
## [7] "WT37vsWT.complete.txt" "WT37vsWT.down.txt"
## [9] "WT37vsWT.up.txt"       "WTST30vsWT.complete.txt"
## [11] "WTST30vsWT.down.txt"   "WTST30vsWT.up.txt"
```

```
setwd("D:/PhD/TSS cluster/H99/regulated genes/")
```

```
# read multiple txt files at the same time
```

```
List <- lapply(txt_files,read.table,sep="\t",header=T,fill=TRUE)
```

```
#change name of files
```

```
newnames <- gsub('\\..', '_', txt_files)
```

```
newnames1 <- gsub('\\_txt', '', newnames)
```

```
newnames1
```

```
## [1] "FlucovsWT_complete" "FlucovsWT_down" "FlucovsWT_up"
## [4] "SDSvsWT_complete" "SDSvsWT_down" "SDSvsWT_up"
## [7] "WT37vsWT_complete" "WT37vsWT_down" "WT37vsWT_up"
## [10] "WTST30vsWT_complete" "WTST30vsWT_down" "WTST30vsWT_up"
```

```
#Assign the names to List
names(List) <- newnames1
```

```
fluconazol<-rbind(List$FlucovsWT_down,List$FlucovsWT_up)
sds <-rbind(List$SDSvsWT_down,List$SDSvsWT_up)
stat<- rbind(List$WTST30vsWT_down,List$WTST30vsWT_up)
T37 <- rbind(List$WT37vsWT_down,List$WT37vsWT_up)
```

```
#GE change or not
```

```
Expo_30<-Expo_30%>%
  mutate(Fluconazol= Id %in% fluconazol$Id,
         SDS= Id %in% sds$Id,
         STAT=Id %in% stat$Id,
         t37= Id %in% T37$Id)
```

```
# Assign Score of gene expression change = Sum of change
```

```
Expo_30<-Expo_30%>%
  mutate(Sum_Change = Fluconazol+SDS+STAT+t37)
Expo_30%>%group_by(TATA_box)%>%
  dplyr::summarise(mean_GE_change = mean(Sum_Change))
```

```
## # A tibble: 2 x 2
##   TATA_box    mean_GE_change
##   <chr>          <dbl>
## 1 no TATA box      0.970
## 2 TATA box        1.41
```

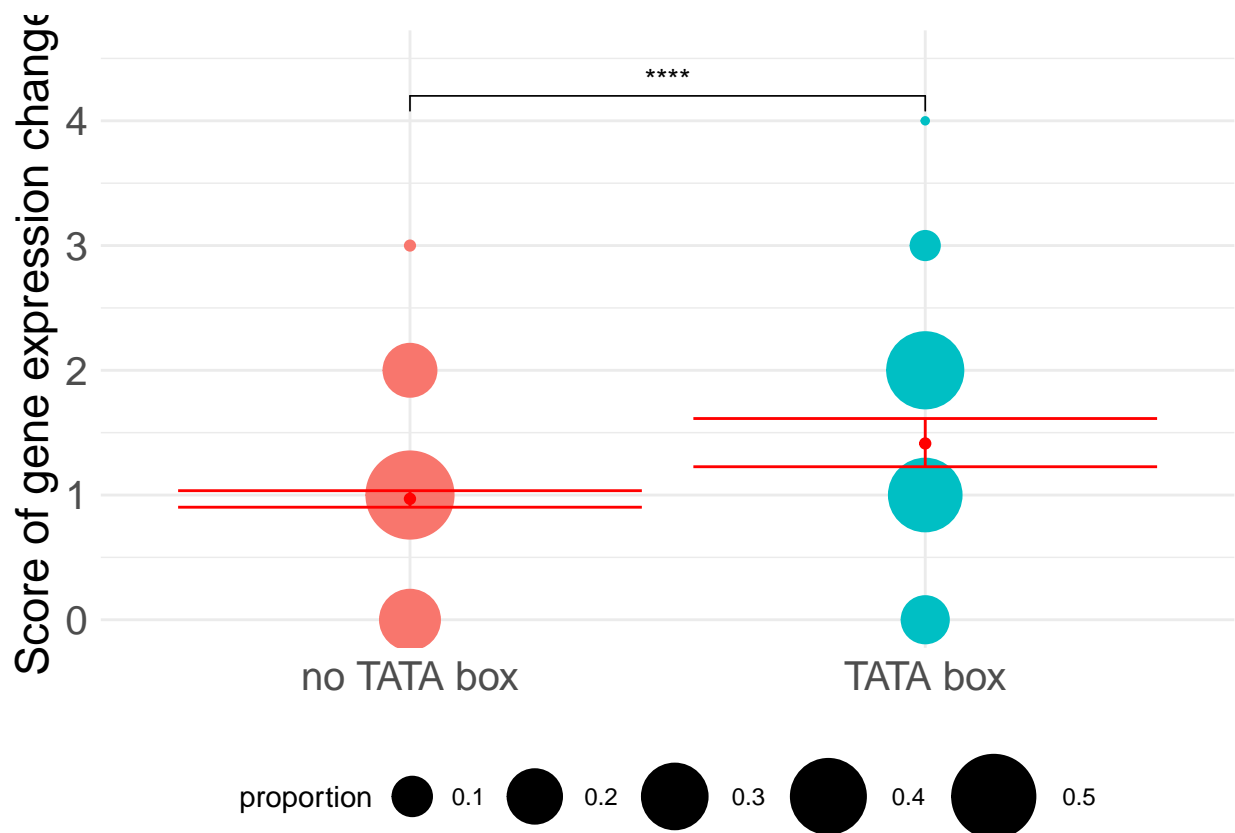
```
head(Expo_30)
```

```
##      Chr start_max end_max      Id score strand condition Cluster_Shape
## 1     1     53175   53305 CNAG_00016     0      +   EXPO 30      Broad
## 2     1     73431   73561 CNAG_00024     0      +   EXPO 30      Broad
## 5     1    177227  177357 CNAG_00065     0      +   EXPO 30      Broad
## 7     1    255924  256054 CNAG_00092     0      +   EXPO 30      Broad
## 353   1    259681  259811 CNAG_00093     0      -   EXPO 30      Broad
## 8     1    301034  301164 CNAG_00109     0      +   EXPO 30      Broad
##      TC_box  GG_box  TATA_box  AC_box  TTAC_box  GA_box Fluconazol
## 1 no TC box no GG box no TATA box no AC box  TTAC box no GA box  FALSE
## 2   TC box no GG box no TATA box no AC box no TTAC box no GA box  TRUE
## 5   TC box no GG box no TATA box no AC box no TTAC box no GA box  FALSE
## 7   TC box no GG box no TATA box   AC box no TTAC box no GA box  FALSE
## 353 no TC box no GG box no TATA box no AC box no TTAC box no GA box  FALSE
## 8 no TC box no GG box no TATA box no AC box no TTAC box no GA box  FALSE
##      SDS  STAT  t37 Sum_Change
## 1  TRUE  TRUE FALSE         2
## 2 FALSE  TRUE FALSE         2
## 5 FALSE  TRUE FALSE         1
## 7 FALSE FALSE FALSE         0
## 353 TRUE FALSE FALSE         1
## 8 FALSE FALSE FALSE         0
```

```

#proportion plot (geom_count)
Expo_30%>%
  ggplot(aes(x=TATA_box,y=Sum_Change,colour=TATA_box))+
  geom_count(aes(size=.prop..))+
  stat_summary(fun.data = mean_cl_boot, geom = "errorbar", colour = "red") +
  stat_summary(fun = mean, geom = "point", colour = "red")+
  stat_compare_means(method = "wilcox.test",
                    method.args = list(var.equal = TRUE),
                    comparisons=list(c("no TATA box","TATA box")),label="p.signif")+
  ylab("Score of gene expression change")+
  theme_minimal()+
  scale_colour_discrete(guide = "none")+      #mute the color=TATA box legend
  scale_size_continuous(name="proportion",range = c(1,15), breaks=seq(0,0.5,by=0.1))+
  ylim(0,4.5)+
  theme(axis.title.x = element_blank(),
        axis.text.x.bottom = element_text(size=15),
        axis.text.y.left = element_text(size=15),
        axis.title.y.left = element_text(size=17,hjust=0.5),
        plot.title = element_blank(),
        legend.position = "bottom")

```



```

#Load data from DeSEQ2
complete_txt_files = list.files("D:/PhD/TSS cluster/H99/regulated genes/",pattern = "\\complete.txt")
setwd("D:/PhD/TSS cluster/H99/regulated genes/")
List <- lapply(complete_txt_files,read.table,sep="\t",header=T,fill=TRUE)

```



```
complete_txt_files
```

```
## [1] "FlucovsWT.complete.txt" "SDSvsWT.complete.txt"  
## [3] "WT37vsWT.complete.txt" "WTST30vsWT.complete.txt"
```

```
newnames <- gsub('\\\\.', '_', complete_txt_files)  
newnames1 <- gsub('\\\\_txt', '', newnames)  
names(List)<-newnames1  
FlucovsWT_complete<-List$FlucovsWT_complete  
SDSvsWT_complete<-List$SDSvsWT_complete  
STATvsWT_complete<-List$WTST30vsWT_complete  
t37vsWT_complete<-List$WT37vsWT_complete
```

```
WT_sum_flu=sum(FlucovsWT_complete$WT)  
FlucovsWT_complete<-FlucovsWT_complete%>%  
  mutate(abs_scale_LFC=abs(scale(log2FoldChange)))%>%  
  mutate(normalized_WT=1000000*WT/WT_sum_flu)
```

```
WT_sum_SDS=sum(SDSvsWT_complete$WT)  
SDSvsWT_complete<-SDSvsWT_complete%>%  
  mutate(abs_scale_LFC=abs(scale(log2FoldChange)))%>%  
  mutate(normalized_WT=1000000*WT/WT_sum_SDS)
```

```
WT_sum_STAT=sum(STATvsWT_complete$WT)  
STATvsWT_complete<-STATvsWT_complete%>%  
  mutate(abs_scale_LFC=abs(scale(log2FoldChange)))%>%  
  mutate(normalized_WT=1000000*WT/WT_sum_STAT)
```

```
WT_sum_t37=sum(t37vsWT_complete$WT)  
t37vsWT_complete<-t37vsWT_complete%>%  
  mutate(abs_scale_LFC=abs(scale(log2FoldChange)))%>%  
  mutate(normalized_WT=1000000*WT/WT_sum_t37)
```

```
#Expression mean and change level
```

```
Fluco<-FlucovsWT_complete%>%select(c(Id,normalized_WT,Fluco,abs_scale_LFC))%>%  
  rename(WTa=normalized_WT)%>%  
  rename(Fluconazol_abs_scale_LFC=abs_scale_LFC)  
SDS<-SDSvsWT_complete%>%select(c(Id,normalized_WT,SDS,abs_scale_LFC))%>%  
  rename(WTb=normalized_WT)%>%  
  rename(SDS_abs_scale_LFC=abs_scale_LFC)  
STAT<-STATvsWT_complete%>%select(c(Id,normalized_WT,WTST30,abs_scale_LFC))%>%  
  rename(WTc=normalized_WT)%>%  
  rename(STAT_abs_scale_LFC=abs_scale_LFC)  
temp<-t37vsWT_complete%>%select(c(Id,normalized_WT,WT37,abs_scale_LFC))%>%  
  rename(WTd=normalized_WT)%>%  
  rename(temp_abs_scale_LFC=abs_scale_LFC)
```

```
Mean_vs_change<-merge(Fluco,SDS,by="Id")%>%merge(STAT,by="Id")%>%merge(temp,by="Id")%>%filter(Id!="__no__")
```

```
Mean_vs_change<-Mean_vs_change%>%mutate(log_expression_mean=log((WTa+WTb+WTc+WTd+Fluco+SDS+WTST30+WT37),  
  mutate(avg_abs_LFC=(Fluconazol_abs_scale_LFC+SDS_abs_scale_LFC+STAT_abs_scale_LFC+temp_abs_scale_LFC),  
  mutate(mean_log_expression_WT= (log2(WTa)+log2(WTb)+log2(WTc)+log2(WTd))/4)
```

```

#Take gene from Expo_30
Mean_vs_change <- Mean_vs_change %>%
  right_join(Expo_30[,c("Id", "Cluster_Shape", "TATA_box", "TC_box", "GG_box", "TTAC_box", "AC_box", "GA_box")],
    by="Id")

Mean_vs_change<-Mean_vs_change%>%
  mutate(mean_log_expression_WT=round(mean_log_expression_WT,3))%>%
  mutate(avg_abs_LFC=round(avg_abs_LFC,3))
head(Mean_vs_change)

```

```

##           Id           WTa Fluco Fluconazol_abs_scale_LFC           WTb   SDS
## 1 CNAG_00016  111.1171  2927                0.4281820  111.10492  2609
## 2 CNAG_00024 1019.7123 15778                1.5816172 1019.62845 25317
## 3 CNAG_00057  684.5586 24066                1.5222895  684.85818 11138
## 4 CNAG_00060   38.3943  1037                0.5252611   38.39808  1454
## 5 CNAG_00065  412.0681  7659                0.8889449  412.05160 10535
## 6 CNAG_00092  169.4630  4373                0.3520930  169.42980  6036
##   SDS_abs_scale_LFC           WTc WTST30 STAT_abs_scale_LFC           Wtd  WT37
## 1      0.6466970  111.14964   1025      0.9226299 110.30411  2565
## 2      0.4698233 1019.93146   8055      1.1637873 996.45989 31286
## 3      1.8184855  684.44777  13796      0.2982973 715.13170 24029
## 4      0.8744170   38.34281    698      0.1371652  40.55686  1253
## 5      0.3769646  411.97855   4356      0.7085080 415.78191 10623
## 6      0.6798559  169.39510   2205      0.3851624 171.97952  4686
##   temp_abs_scale_LFC log_expression_mean avg_abs_LFC mean_log_expression_WT
## 1      0.5583391                7.086913      0.639                6.796
## 2      0.2029380                9.264967      0.855                9.994
## 3      0.3752363                9.156386      1.004                9.419
## 4      0.1625007                6.353868      0.425                5.262
## 5      0.3192313                8.378646      0.573                8.687
## 6      0.1574818                7.717589      0.394                7.404
##   Cluster_Shape   TATA_box   TC_box   GG_box   TTAC_box   AC_box   GA_box
## 1      Broad no TATA box no TC box no GG box   TTAC box no AC box no GA box
## 2      Broad no TATA box   TC box no GG box no TTAC box no AC box no GA box
## 3      Sharp no TATA box no TC box no GG box no TTAC box no AC box no GA box
## 4      Sharp no TATA box no TC box no GG box no TTAC box no AC box no GA box
## 5      Broad no TATA box   TC box no GG box no TTAC box no AC box no GA box
## 6      Broad no TATA box   TC box no GG box no TTAC box   AC box no GA box

```

```

#Relationship between TATA box and gene expression change
#Box plot for mean expression
p1<-Mean_vs_change%>%
  ggplot(aes(x=TATA_box,y=mean_log_expression_WT,fill=TATA_box))+
  scale_fill_manual(values=c("no TATA box"="palevioletred2","TATA box"="seagreen2"),)+
  geom_violin()+
  geom_boxplot(width=0.1,notch=T)+
  stat_compare_means(comparisons=list(c("TATA box","no TATA box")),
    label="p.signif",label.y = 14, bracket.size = 1)+ #wilcoxon test
  ylab("Log Expression Average")+
  theme_minimal()+
  ylim(0,15)+
  theme(axis.title.x = element_blank(),
    axis.text.x = element_text(size=20),

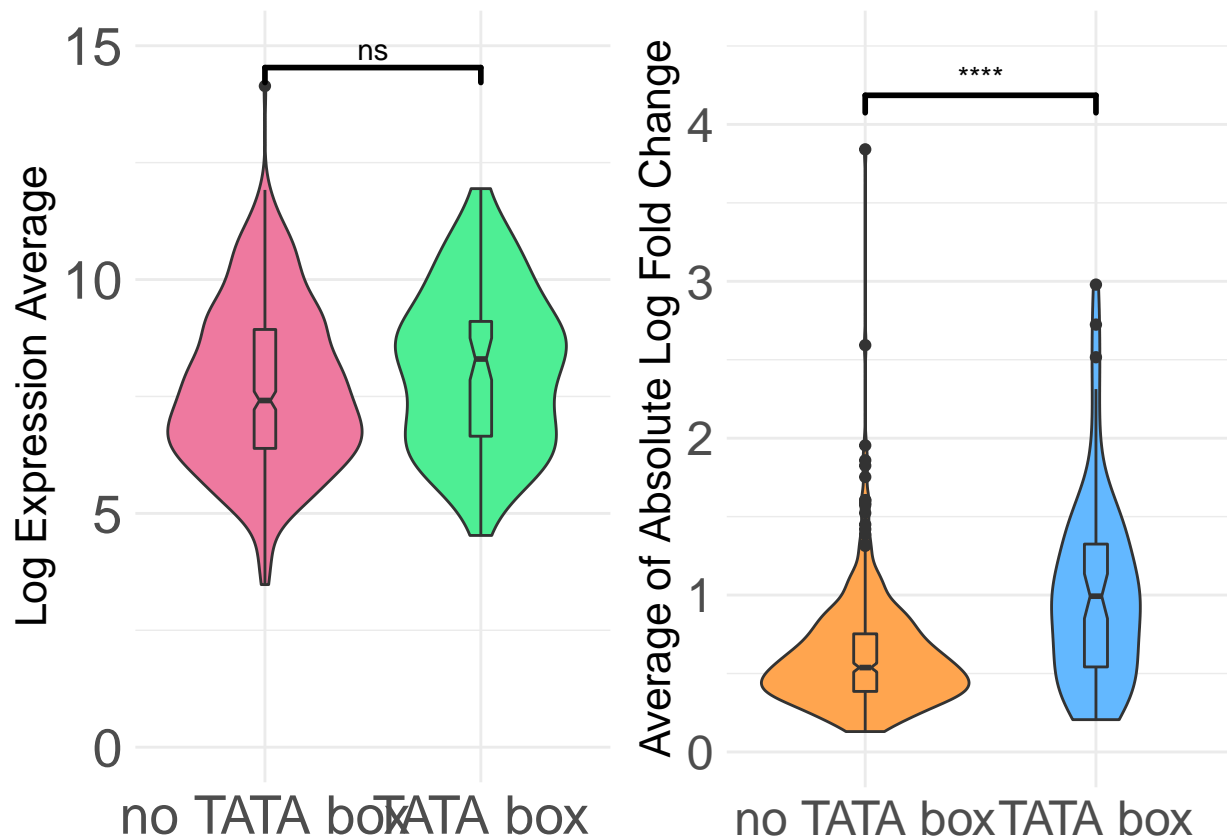
```

```
axis.title.y = element_text(size=16),
axis.text.y = element_text(size=20),
legend.position = "none")
```

*#Box\_plot for mean of absolute LFC*

```
p2<-Mean_vs_change%>%
  ggplot(aes(x=TATA_box,y=avg_abs_LFC,fill=TATA_box))+
  geom_violin()+
  geom_boxplot(width=0.1,notch=T)+
  scale_fill_manual(values=c("no TATA box"="tan1","TATA box"="steelblue1"),)+
  stat_compare_means(comparisons=list(c("TATA box","no TATA box")), #wilcoxon test
    label="p.signif",label.y = 4, bracket.size = 1
  )+
  ylab("Average of Absolute Log Fold Change")+
  ylim(c(0,4.5))+
  theme_minimal()+
  theme(axis.title.x = element_blank(),
    axis.text.x = element_text(size=18),
    axis.title.y = element_text(size=16),
    axis.text.y = element_text(size=18),
    legend.position = "none")
```

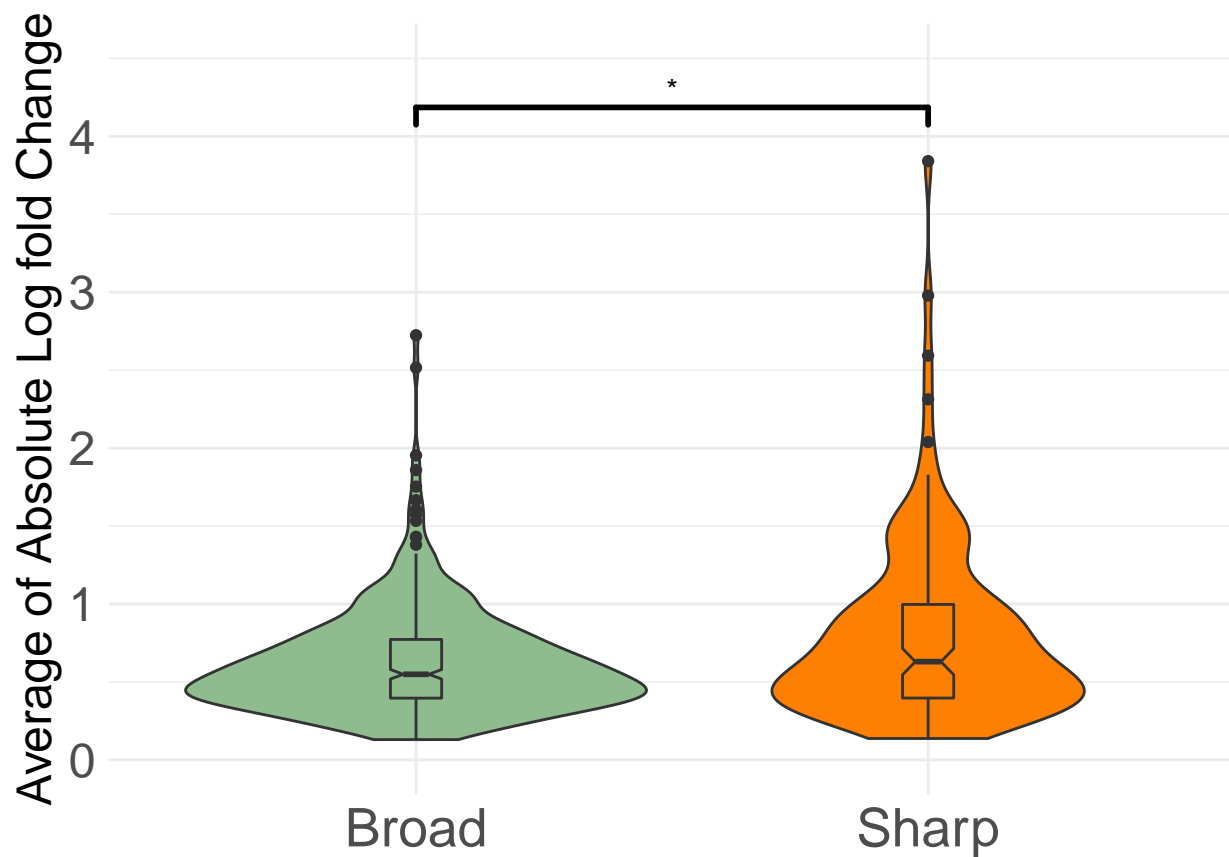
```
ggarrange(p1, p2,nrow=1,ncol=2)
```



```

#CLUSTER SHAPE
#Both TATA box and no TATA box
pBoth_LFC<-Mean_vs_change%>%
  ggplot(aes(x=Cluster_Shape,y=avg_abs_LFC,fill=Cluster_Shape))+
  geom_violin()+
  geom_boxplot(width=0.1,notch=T)+
  scale_fill_manual(
    values =c("darkorange1","darkseagreen"),
    breaks = c("Sharp","Broad"),
    labels = c("Sharp Clusters", "Broad Cluster")
  )+
  stat_compare_means(comparisons=list(c("Sharp","Broad")),label="p.signif",label.y = 4, bracket.size = 1)+
  theme_minimal()+
  theme(legend.position = "none")+
  theme(axis.title.x = element_blank(),
        axis.text.x = element_text(size=20),
        axis.title.y=element_text(size=18),
        axis.text.y.left = element_text(size=18))+
  ylim(c(0,4.5))+
  ylab("Average of Absolute Log fold Change")+
  theme(plot.title = element_text(hjust = 0.5,size=22))
pBoth_LFC

```



```

#Stratification for TATA box-containing genes and non TATA box genes
#TATA box

```

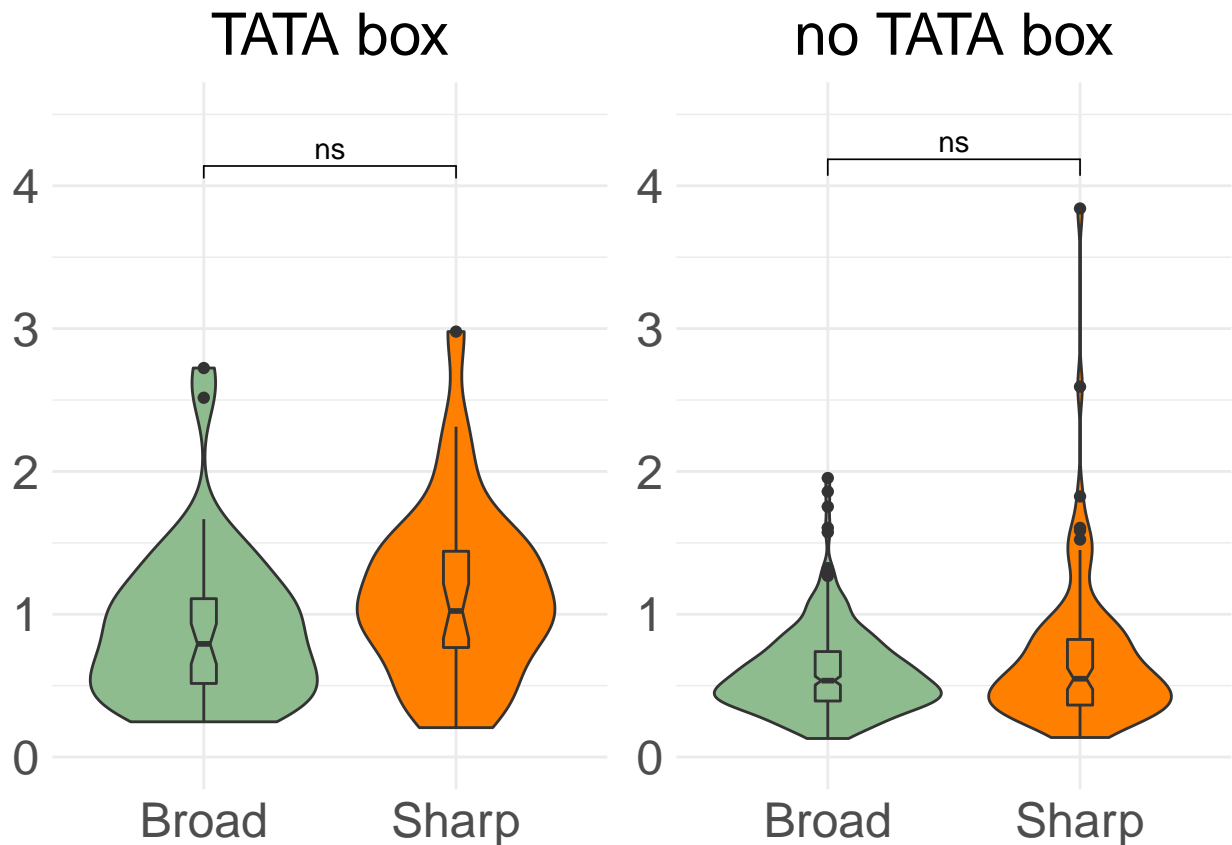
```

p_TATA_LFC<-Mean_vs_change%>%
  filter(TATA_box=="TATA box")%>%
  ggplot(aes(x=Cluster_Shape,y=avg_abs_LFC,fill=Cluster_Shape))+
  geom_violin()+
  geom_boxplot(width=0.1,notch=T)+
  scale_fill_manual(
    values =c("darkorange1","darkseagreen"),
    breaks = c("Sharp","Broad"),
    labels = c("Sharp Clusters", "Broad Cluster")
  )+
  stat_compare_means(comparisons=list(c("Sharp","Broad")),label="p.signif",label.y = 4,method = "t.test")
  theme_minimal()+
  theme(legend.position = "none")+
  theme(axis.title.x = element_blank(),
        axis.text.x = element_text(size=18),
        axis.title.y=element_blank(),
        axis.text.y.left = element_text(size=18))+
  ylim(c(0,4.5))+
  labs(title = "TATA box")+
  theme(plot.title = element_text(hjust = 0.5,size=22))

#non TATA
p_nonTATA_LFC<-Mean_vs_change%>%
  filter(TATA_box=="no TATA box")%>%
  ggplot(aes(x=Cluster_Shape,y=avg_abs_LFC,fill=Cluster_Shape))+
  geom_violin()+
  geom_boxplot(width=0.1,notch=T)+
  scale_fill_manual(
    values =c("darkorange1","darkseagreen"),
    breaks = c("Sharp","Broad"),
    labels = c("Sharp Clusters", "Broad Cluster")
  )+
  stat_compare_means(comparisons=list(c("Sharp","Broad")),label="p.signif",label.y = 4,method = "t.test")
  theme_minimal()+
  theme(legend.position = "none")+
  theme(axis.title.x = element_blank(),
        axis.text.x = element_text(size=18),
        axis.title.y=element_blank(),
        axis.text.y.left = element_text(size=18))+
  ylim(c(0,4.5))+
  labs(title = "no TATA box")+
  theme(plot.title = element_text(hjust = 0.5,size=22))

ggarrange(p_TATA_LFC,p_nonTATA_LFC,nrow = 1,ncol = 2)

```



```
#TATA box: do stratification with Broad and Sharp cluster
#Only Sharp
p_sharp_LFC<-Mean_vs_change%>%
  filter(Cluster_Shape=="Sharp")%>%
  ggplot(aes(x=TATA_box,y=avg_abs_LFC,fill=TATA_box))+
  geom_violin()+
  geom_boxplot(width=0.1,notch = T)+
  scale_fill_manual(values=c("no TATA box"="tan1","TATA box"="steelblue1"))+
  stat_compare_means(comparisons=list(c("no TATA box","TATA box")),label="p.signif",label.y = 4)+
  theme_minimal()+
  theme(legend.position = "none")+
  theme(axis.title.x = element_blank(),
        axis.text.x = element_text(size=18),
        axis.title.y.left = element_blank(),
        axis.text.y.left = element_text(size=18))+
  ylim(0,4.5)+
  labs(title = "SHARP")+
  theme(plot.title = element_text(hjust = 0.5,size=22))

#Broad
p_Broad_LFC<-Mean_vs_change%>%
  filter(Cluster_Shape=="Broad")%>%
  ggplot(aes(x=TATA_box,y=avg_abs_LFC,fill=TATA_box))+
  geom_violin()+
  geom_boxplot(width=0.1,notch=T)+
```

```

scale_fill_manual(values = c("no TATA box"="tan1","TATA box"="steelblue1"))+
stat_compare_means(comparisons=list(c("no TATA box","TATA box")),label="p.signif",label.y = 4)+
theme_minimal()+
ylim(0,4.5)+
theme(legend.position = "none")+
theme(axis.title.x = element_blank(),
      axis.text.x = element_text(size=18),
      axis.title.y.left = element_blank(),
      axis.text.y.left = element_text(size=18))+
labs(title = "BROAD")+
theme(plot.title = element_text(hjust = 0.5,size=22))

ggarrange(p_sharp_LFC,p_Broad_LFC,nrow = 1,ncol = 2)

```

