histone_stats H3K4me3

```
library(tidyr)
library(dplyr)
```

```
##
## Attaching package: 'dplyr'
```

```
## The following objects are masked from 'package:stats':
##
##     filter, lag
```

```
## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union
```

```
library(ggplot2)
library(ggpubr)
library(ggrepel)
library(here); i_am("R/Figure3/Fig3C_histonestats_H3K4me3.Rmd")
```

```
## here() starts at /projects/imb-pkbphil/sp/rnaseq/six_donor_trans/splicing_paper
```

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```

```
histone = "H3K4me3"
window = "1000:2000"
tss_sets = c("beige","white","not_sig")

enrich_tables = list()
for (set in tss_sets){
  file = here("31_leafcutter/histone_profile", histone, paste0("window", window), paste0(set,".",window
  annot = read.delim(file, quote="'")
  colnames(annot)[grep("chr", colnames(annot))] = "chr"
  annot$tss_set = set
  enrich_tables[[set]] = annot
}
str(enrich_tables)
```

```
## List of 3
##  $ beige  :'data.frame': 209 obs. of  6 variables:
##   ..$ chr       : chr [1:209] "chr1" "chr1" "chr1" "chr1" ...
##   ..$ start     : int [1:209] 6613856 14923129 23798401 45337954 55213361 87128764 113756062 1137578
##   ..$ end       : int [1:209] 6616857 14926130 23801402 45340955 55216362 87131765 113759063 1137608
##   ..$ White_Adi.: num [1:209] 3.73 2.09 2.14 2.02 3.8 ...
```

```
##   ..$ Beige_Adi.: num [1:209] 4.54 2.55 2.36 2.92 4.59 ...
##   ..$ tss_set   : chr [1:209] "beige" "beige" "beige" "beige" ...
##  $ white  :'data.frame': 213 obs. of  6 variables:
##   ..$ chr       : chr [1:213] "chr1" "chr1" "chr1" "chr1" ...
##   ..$ start     : int [1:213] 6612730 11801676 14944918 17633256 23798780 26431281 33347884 55213363
##   ..$ end       : int [1:213] 6615731 11804677 14947919 17636257 23801781 26434282 33350885 55216364
##   ..$ White_Adi.: num [1:213] 4.591 2.003 0.861 -0.35 2.365 ...
##   ..$ Beige_Adi.: num [1:213] 5.447 1.773 0.637 -0.488 2.537 ...
##   ..$ tss_set   : chr [1:213] "white" "white" "white" "white" ...
##  $ not_sig:'data.frame': 23636 obs. of  6 variables:
##   ..$ chr       : chr [1:23636] "chr1" "chr1" "chr1" "chr1" ...
##   ..$ start     : int [1:23636] 27369 493475 496975 496975 497174 499606 500872 500872 512412 515251
##   ..$ end       : int [1:23636] 30370 496476 499976 499976 500175 502607 503873 503873 515413 518252
##   ..$ White_Adi.: num [1:23636] 0 0.0087 0 0 0 ...
##   ..$ Beige_Adi.: num [1:23636] -0.0124 0 0 0 0 ...
##   ..$ tss_set   : chr [1:23636] "not_sig" "not_sig" "not_sig" "not_sig" ...
```

```
annot <- do.call(rbind, enrich_tables)
table(annot$tss_set)
```

```
##
##   beige not_sig   white
##     209   23636     213
```

```
head(annot); nrow(annot)
```

```
##          chr    start      end White_Adi. Beige_Adi. tss_set
## beige.1 chr1  6613856  6616857   3.727283   4.543351   beige
## beige.2 chr1 14923129 14926130   2.089665   2.546589   beige
## beige.3 chr1 23798401 23801402   2.138708   2.357445   beige
## beige.4 chr1 45337954 45340955   2.018657   2.918385   beige
## beige.5 chr1 55213361 55216362   3.796409   4.585399   beige
## beige.6 chr1 87128764 87131765   1.176095   1.777729   beige
```

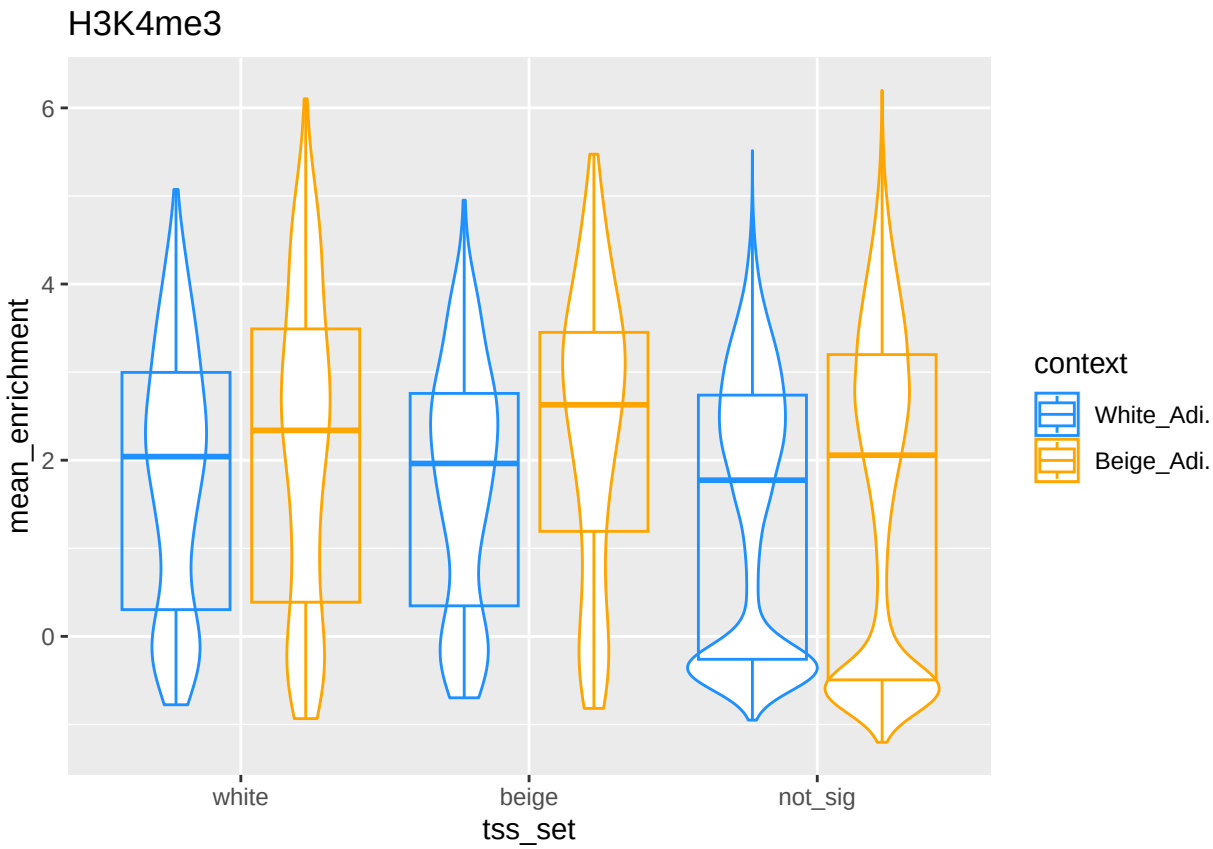
```
## [1] 24058
```

```
long = pivot_longer(annot, grep("Adi.",colnames(annot)), names_to = "context", values_to = "mean_enrichm
long$context = factor(long$context, levels=c("White_Adi.","Beige_Adi."))
long$tss_set = factor(long$tss_set, levels=c("white","beige","not_sig"))
```

```
ggplot(long,
       aes(x=tss_set, y=mean_enrichment, color=context)) +geom_violin()+
    geom_boxplot(fill=NA, position=position_dodge(0.9)) +scale_color_manual(values= c("dodgerblue","ora
```

```
## Warning: Removed 12 rows containing non-finite values (`stat_ydensity()`).
```

```
## Warning: Removed 12 rows containing non-finite values (`stat_boxplot()`).
```

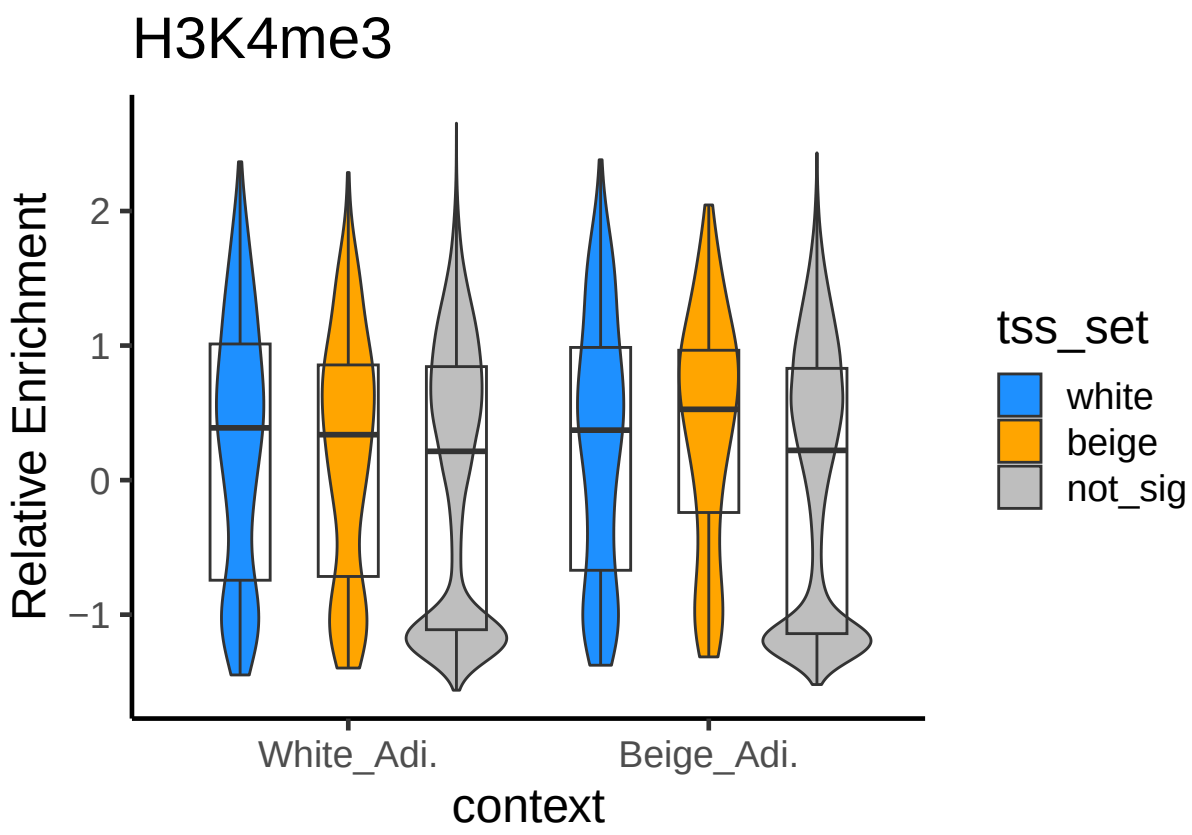



```
relative = long %>% group_by(context) %>% mutate(zscore = scale(mean_enrichment), group=paste0(context,
relative$group = factor(relative$group, levels=paste0(rep(levels(relative$context),each=3), levels(rela

ggplot(relative,
       aes(fill=tss_set, y=zscore, x=context, group=group)) + geom_violin() +
    geom_boxplot(fill=NA, width=0.5, position= position_dodge(0.9)) + scale_fill_manual(values= c("dodg
    theme_classic(base_size=18) + ylab("Relative Enrichment") + ggtitle(histone)
```

```
## Warning: Removed 12 rows containing non-finite values (‘stat_ydensity()‘).
```

```
## Warning: Removed 12 rows containing non-finite values (‘stat_boxplot()‘).
```



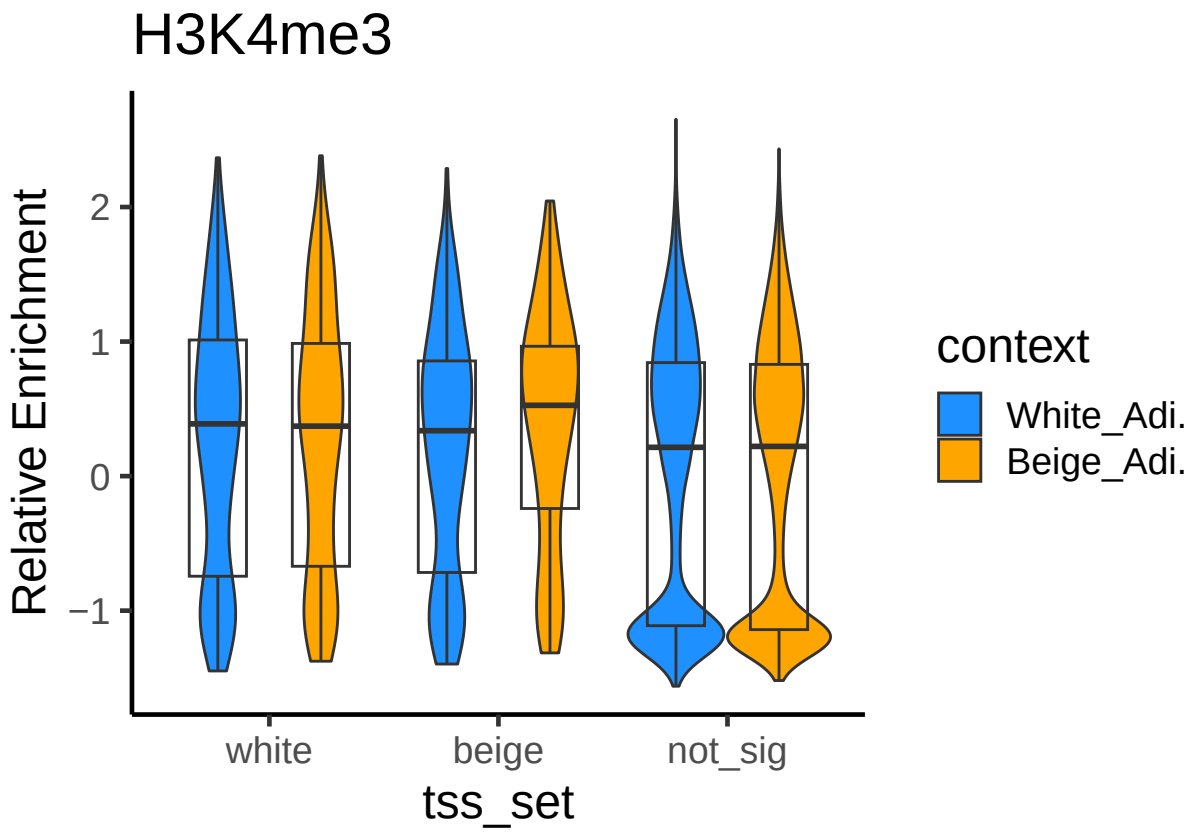

```
ggsave(here("31_leafcutter/histone_profile", histone, paste0(histone, "_violin_plot.pdf")))
```

```
## Saving 6.5 x 4.5 in image
```

```
## Warning: Removed 12 rows containing non-finite values (`stat_ydensity()`).
## Removed 12 rows containing non-finite values (`stat_boxplot()`).
```

```
ggplot(relative,
       aes(x=tss_set, y=zscore, fill=context, group=group)) + geom_violin() +
    geom_boxplot(fill=NA, width=0.5, position= position_dodge(0.9))+scale_fill_manual(values= c("dodgerb
  theme_classic(base_size=18) + ylab("Relative Enrichment")+ ggtitle(histone)
```

```
## Warning: Removed 12 rows containing non-finite values (`stat_ydensity()`).
## Removed 12 rows containing non-finite values (`stat_boxplot()`).
```




```
summary(aov(zscore ~tss_set+context, data=relative))
```

```
##                Df Sum Sq Mean Sq F value   Pr(>F)
## tss_set         2     63  31.252   31.29 2.63e-14 ***
## context         1      0   0.000    0.00        1
## Residuals   48100  48039   0.999
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## 12 observations deleted due to missingness
```

```
#print(kruskal.test(zscore ~ tss_set * context, data=relative)) #cannot run a kruskal wallis with inter

compare_means(zscore ~ tss_set, data=relative, method="wilcox.test", group.by="context")
```

```
## # A tibble: 6 x 9
##   context     .y.    group1 group2            p   p.adj p.format p.signif method
##   <fct>       <chr>  <chr>  <chr>         <dbl>   <dbl> <chr>    <chr>    <chr>
## 1 White_Adi. zscore white  beige       5.50e-1 5.5 e-1 0.54990  ns       Wilco~
## 2 White_Adi. zscore white  not_sig     1.91e-4 7.6 e-4 0.00019  ***      Wilco~
## 3 White_Adi. zscore beige  not_sig     5.10e-3 1.5 e-2 0.00510  **       Wilco~
## 4 Beige_Adi. zscore white  beige       1.64e-1 3.3 e-1 0.16386  ns       Wilco~
## 5 Beige_Adi. zscore white  not_sig     3.32e-5 1.7 e-4 3.3e-05  ****     Wilco~
## 6 Beige_Adi. zscore beige  not_sig     6.54e-9 3.90e-8 6.5e-09  ****     Wilco~
```

```r
summary(aov(zscore ~tss_set*context, data=relative))
```

```
##                     Df Sum Sq Mean Sq F value   Pr(>F)
## tss_set              2     63  31.252  31.293 2.62e-14 ***
## context              1      0   0.000   0.000    1.000
## tss_set:context      2      4   2.014   2.016    0.133
## Residuals        48098  48035   0.999
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## 12 observations deleted due to missingness
```

```r
compare_means(zscore ~ context, data=relative, method="t.test", group.by="tss_set") # <- not significan
```

```
## # A tibble: 3 x 9
##   tss_set .y.    group1     group2          p p.adj p.format p.signif method
##   <fct>   <chr>  <chr>      <chr>       <dbl> <dbl> <chr>    <chr>    <chr>
## 1 beige   zscore White_Adi. Beige_Adi. 0.0248 0.074 0.025    *        T-test
## 2 white   zscore White_Adi. Beige_Adi. 0.851  1     0.851    ns       T-test
## 3 not_sig zscore White_Adi. Beige_Adi. 0.838  1     0.838    ns       T-test
```