Figure S3

Sergio Garcia-Moreno Alcantara (sga@stowers.org)

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Aim

The aim of this analysis is to investigate whether there are differences in the occupancy of the SAGA core module at different promoter types in the ovaries

Enviroment setup

Set working directory and load required libraries and lab functions

```
setwd("/n/projects/sga/analysis/SAGA/saga_publication/")
options(knitr.figure_dir = "plots/figure_s3/")

# Standard packages
library(GenomicAlignments)
library(GenomicRanges)
library(Biostrings)
library(BSgenome.Dmelanogaster.UCSC.dm6)
library(TxDb.Dmelanogaster.UCSC.dm6.ensGene)
library(dplyr)
library(reshape2)
library(plyranges)
library(CAGEr)
library(magrittr)
library(ggplot2)
library(cowplot)
library(ggseqlogo)
library(gridExtra)
library(ggpubr)


# Lab sources
source("./shared_code/granges_common.r")
source("./shared_code/metapeak_common.r")
source("./shared_code/knitr_common.r")
```

Analysis

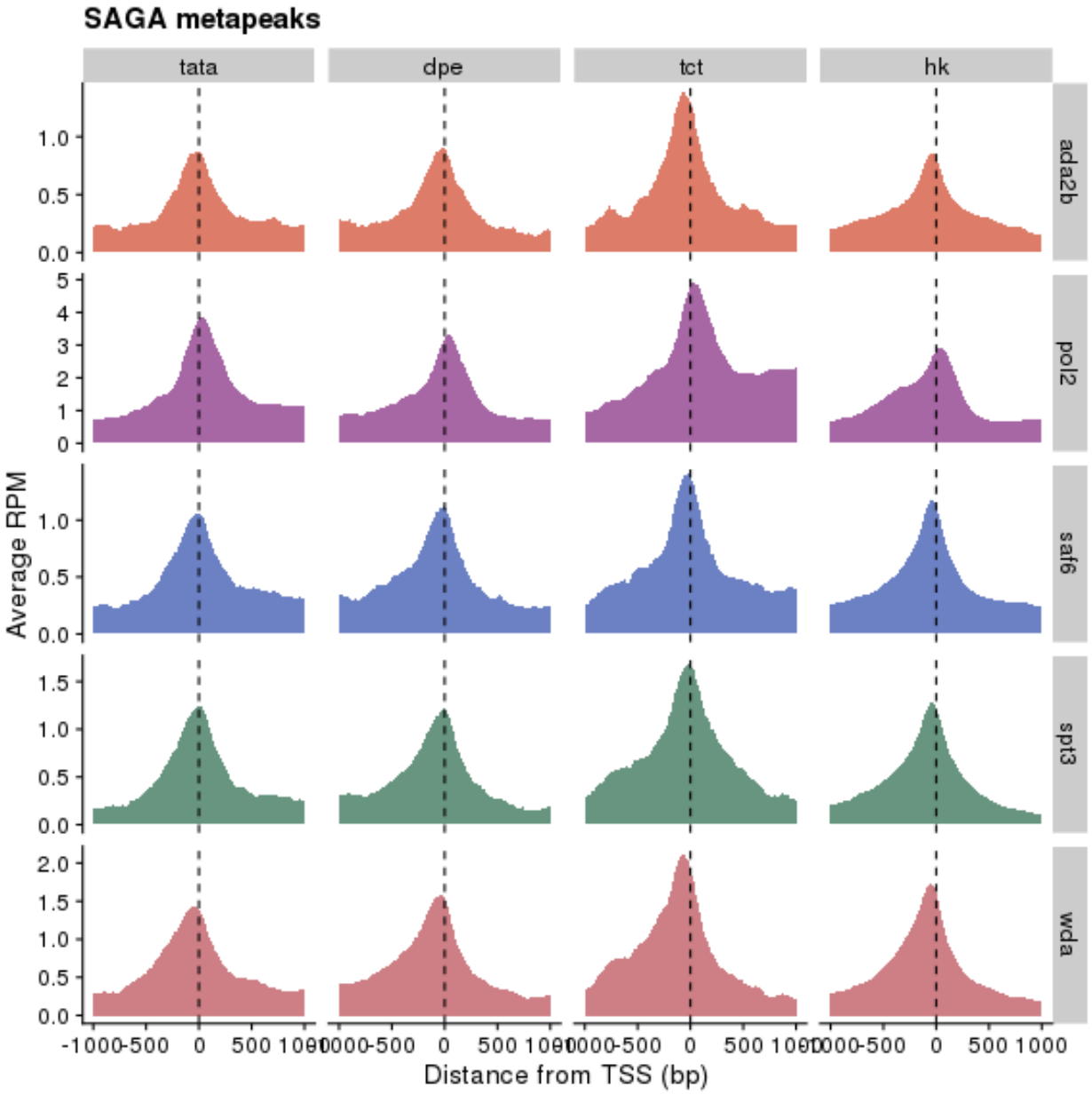
1. Loading samples and necessary data sets

```
sample_list <- read.csv("./chipseq_samples.csv", sep = ";")

load_bigwig <- function(sample_list) {

    bw_path <- function(path) {
        path = path
    }
    sample_list %>%
        mutate(list = purrr::map(as.character(path), bw_path)) %>%
        pull(list)
}

bw_list <- load_bigwig(sample_list)
names(bw_list) <- sample_list$short_name

tss <- get(load("./rdata/dm6_mrna_ovaries_tss.RData"))

motif_list_ovaries <- get(load("./rdata/motif_list_ovaries.RData"))
```

2. Generate a metapeak plot for each SAGA core subunits at the different promoter types

```
## Calculate the average signal per factor per base pair at different promoter
## types

promoter_type_metapeak_df <- mclapply(names(motif_list_ovaries), function(x) {
    motif <- motif_list_ovaries[[x]]
    mclapply(names(bw_list), function(y) {
        bw <- bw_list[[y]]
        standard_metapeak(motif, bw, 1000, 1001, paste(x, "at", y), NA)
    }, mc.cores = 10)
}, mc.cores = 10) %>%
    do.call(c, .) %>%
    bind_rows()

promoter_type_metapeak_df %<>%
    mutate(., factor = gsub(".* ", "", promoter_type_metapeak_df$sample_name)) %<>%
    mutate(., motif = gsub(" .*", "", promoter_type_metapeak_df$sample_name))

## Setting the plotting order

sample_levels <- c("ada2b", "spt3", "saf6", "wda", "pol2")
motif_levels <- c("tata", "dpe", "tct", "hk")

## Create a plotting function

plot_func <- function(df, name, color) {
```

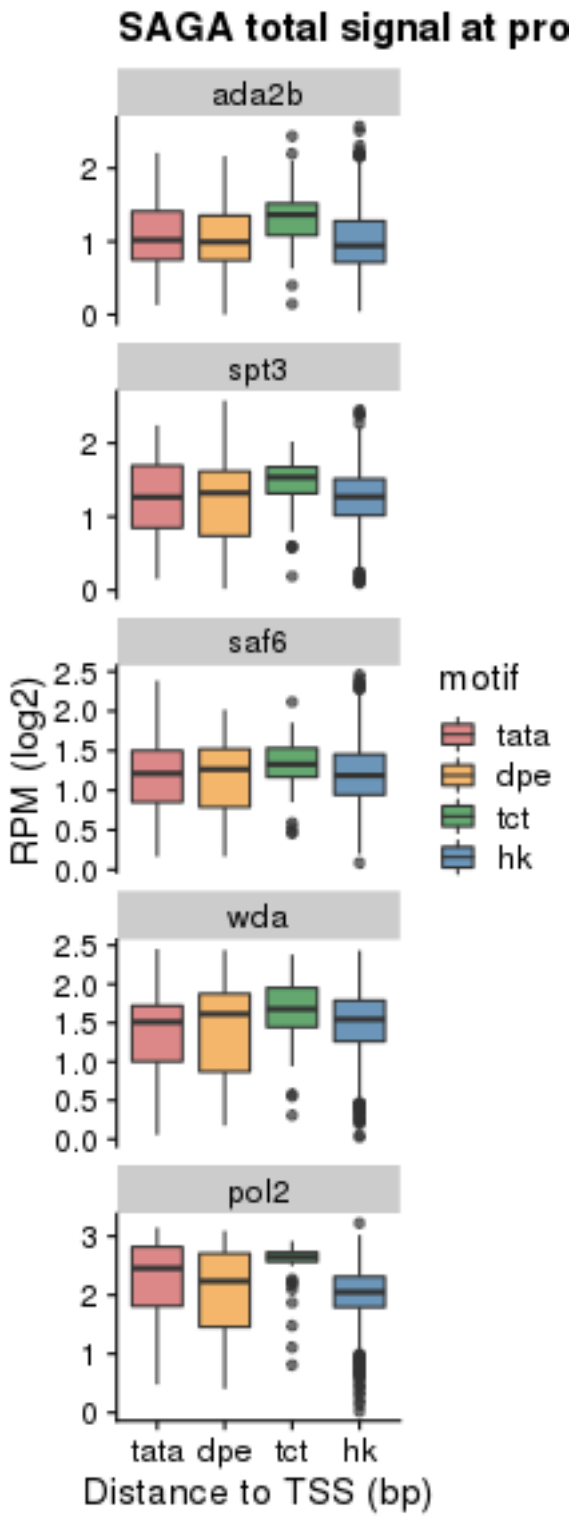
```
    df$motif <- factor(df$motif, levels = c(motif_levels))
    ggplot(df, aes(x = tss_distance, y = reads)) + geom_area(aes(fill = factor),
        alpha = 0.6, show.legend = F) + scale_fill_manual(values = color) + geom_vline(xintercept = 0,
        linetype = 2) + facet_grid(factor ~ motif, scales = "free") + ggtitle(name) +
        theme_cowplot() + theme(plot.title = element_text(size = 15, face = "bold")) +
        xlab("Distance from TSS (bp)") + ylab("Average RPM")
}

plot_func(promoter_type_metapeak_df, "SAGA metapeaks", c("#C62606", "#6D0068", "#0B2E9B",
    "#044F2A", "#AB2A35"))
```



3. Plot the signal distribution of each SAGA core subunit at the different promoter types

Signal was calculated as max height of the peaks for each promoter in 500 bp window centered at the TSS

```
# Make a data frame containing transcript ID and total signal per gene and
# promoter type

sig_df <- mclapply(names(motif_list_ovaries), function(x) {

    motif <- motif_list_ovaries[[x]]
    mclapply(names(bw_list), function(y) {
        bw <- bw_list[[y]]

        df <- data.frame(fb_t_id = motif$fb_t_id, signal = regionMaxs(resize(motif,
            501, "center"), bw), sample = y, motif = x)
        df
    }, mc.cores = 5)
}, mc.cores = 5) %>%
    do.call(c, .) %>%
    bind_rows()

sig_df$motif <- factor(sig_df$motif, levels = motif_levels)
sig_df$sample <- factor(sig_df$sample, levels = sample_levels)

sig_df <- filter(sig_df, signal >= 0)

boxplot <- ggplot(sig_df, aes(motif, log2(signal + 1), fill = motif)) + geom_boxplot(alpha = 0.7) +
    theme_cowplot() + scale_fill_manual(values = c("indianred3", "#EE962B", "#228232",
    "#2C699B")) + ggtitle("SAGA total signal at promoter types", ) + facet_wrap(~sample,
    scales = "free_y", ncol = 1) + theme(plot.title = element_text(size = 15, face = "bold")) +
    xlab("Distance to TSS (bp)") + ylab("RPM (log2)")

boxplot
```


SAGA total signal at pro
ada2b
spt3
saf6
wda
pol2
RPM (log2)
motif
tata
dpe
tct
hk
tata dpe tct hk
Distance to TSS (bp)

Session Info

```
sessionInfo()
```

```
## R version 4.1.0 (2021-05-18)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: CentOS Linux 7 (Core)
##
## Matrix products: default
## BLAS:   /n/apps/CentOS7/install/r-4.1.0/lib64/R/lib/libRblas.so
## LAPACK: /n/apps/CentOS7/install/r-4.1.0/lib64/R/lib/libRlapack.so
##
## locale:
##  [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C
##  [3] LC_TIME=en_US.UTF-8        LC_COLLATE=en_US.UTF-8
##  [5] LC_MONETARY=en_US.UTF-8    LC_MESSAGES=en_US.UTF-8
##  [7] LC_PAPER=en_US.UTF-8       LC_NAME=C
##  [9] LC_ADDRESS=C               LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] stats4    parallel  stats     graphics  grDevices utils     datasets
## [8] methods   base
##
## other attached packages:
##  [1] digest_0.6.27
##  [2] pander_0.6.3
##  [3] data.table_1.14.0
##  [4] lattice_0.20-44
##  [5] ggpubr_0.4.0
##  [6] gridExtra_2.3
##  [7] ggseqlogo_0.1
##  [8] cowplot_1.1.1
##  [9] ggplot2_3.3.3
## [10] magrittr_2.0.1
## [11] CAGEr_1.34.0
## [12] MultiAssayExperiment_1.18.0
## [13] plyranges_1.12.0
## [14] reshape2_1.4.4
## [15] dplyr_1.0.6
## [16] TxDb.Dmelanogaster.UCSC.dm6.ensGene_3.12.0
## [17] GenomicFeatures_1.44.0
## [18] AnnotationDbi_1.54.0
## [19] BSgenome.Dmelanogaster.UCSC.dm6_1.4.1
## [20] BSgenome_1.60.0
## [21] rtracklayer_1.52.0
## [22] GenomicAlignments_1.28.0
## [23] Rsamtools_2.8.0
## [24] Biostrings_2.60.1
## [25] XVector_0.32.0
## [26] SummarizedExperiment_1.22.0
## [27] Biobase_2.52.0
## [28] MatrixGenerics_1.4.0
```

```
## [29] matrixStats_0.59.0
## [30] GenomicRanges_1.44.0
## [31] GenomeInfoDb_1.28.0
## [32] IRanges_2.26.0
## [33] S4Vectors_0.30.0
## [34] BiocGenerics_0.38.0
##
## loaded via a namespace (and not attached):
##   [1] VGAM_1.1-5             colorspace_2.0-1       ggsignif_0.6.1
##   [4] rjson_0.2.20           rio_0.5.26             ellipsis_0.3.2
##   [7] som_0.3-5.1            farver_2.1.0           bit64_4.0.5
##  [10] fansi_0.5.0            splines_4.1.0          cachem_1.0.5
##  [13] knitr_1.33             broom_0.7.6            cluster_2.1.2
##  [16] dbplyr_2.1.1           png_0.1-7              compiler_4.1.0
##  [19] httr_1.4.2             backports_1.2.1        assertthat_0.2.1
##  [22] Matrix_1.3-4           fastmap_1.1.0          formatR_1.11
##  [25] htmltools_0.5.1.1      prettyunits_1.1.1      tools_4.1.0
##  [28] gtable_0.3.0           glue_1.4.2             GenomeInfoDbData_1.2.6
##  [31] rappdirs_0.3.3         Rcpp_1.0.6             carData_3.0-4
##  [34] cellranger_1.1.0       vctrs_0.3.8            nlme_3.1-152
##  [37] xfun_0.23              stringr_1.4.0          openxlsx_4.2.3
##  [40] lifecycle_1.0.0        restfulr_0.0.13        formula.tools_1.7.1
##  [43] gtools_3.9.2           rstatix_0.7.0          XML_3.99-0.6
##  [46] beanplot_1.2           stringdist_0.9.6.3     zlibbioc_1.38.0
##  [49] MASS_7.3-54            scales_1.1.1           hms_1.1.0
##  [52] yaml_2.2.1             curl_4.3.1             memoise_2.0.0
##  [55] biomaRt_2.48.0         reshape_0.8.8          stringi_1.6.2
##  [58] RSQLite_2.2.7          highr_0.9              BiocIO_1.2.0
##  [61] permute_0.9-5          filelock_1.0.2         zip_2.2.0
##  [64] BiocParallel_1.26.0    operator.tools_1.6.3   rlang_0.4.11
##  [67] pkgconfig_2.0.3        bitops_1.0-7           evaluate_0.14
##  [70] purrr_0.3.4            labeling_0.4.2         bit_4.0.4
##  [73] tidyselect_1.1.1       plyr_1.8.6             R6_2.5.0
##  [76] generics_0.1.0         DelayedArray_0.18.0    DBI_1.1.1
##  [79] haven_2.4.1            foreign_0.8-81         pillar_1.6.1
##  [82] withr_2.4.2            mgcv_1.8-36            abind_1.4-5
##  [85] KEGGREST_1.32.0        RCurl_1.98-1.3         tibble_3.1.2
##  [88] crayon_1.4.1           car_3.0-10             KernSmooth_2.23-20
##  [91] utf8_1.2.1             BiocFileCache_2.0.0    rmarkdown_2.8
##  [94] progress_1.2.2         readxl_1.3.1           grid_4.1.0
##  [97] blob_1.2.1             vegan_2.5-7            forcats_0.5.1
## [100] tidyr_1.1.3            munsell_0.5.0
```