Figure 5 The Inr strongly contributes to the degree of Pol II pausing

Wanqing Shao(was@stowers.org)

Contents

Description

To test if initiator (Inr) sequence can contribute to paused Pol II stability, we took a stably paused promoter *dve*, changed its upstream sequence or its upstream sequence together with the Inr to that of a TATA promoter *Act5C*. To further test if Inr can contribute to Pol II pausing independent of TATA box,, we took the stably paused promoter *dve* and replaced its Inr sequence with that of a TATA promoter *Act5C*.

Enviroment setup

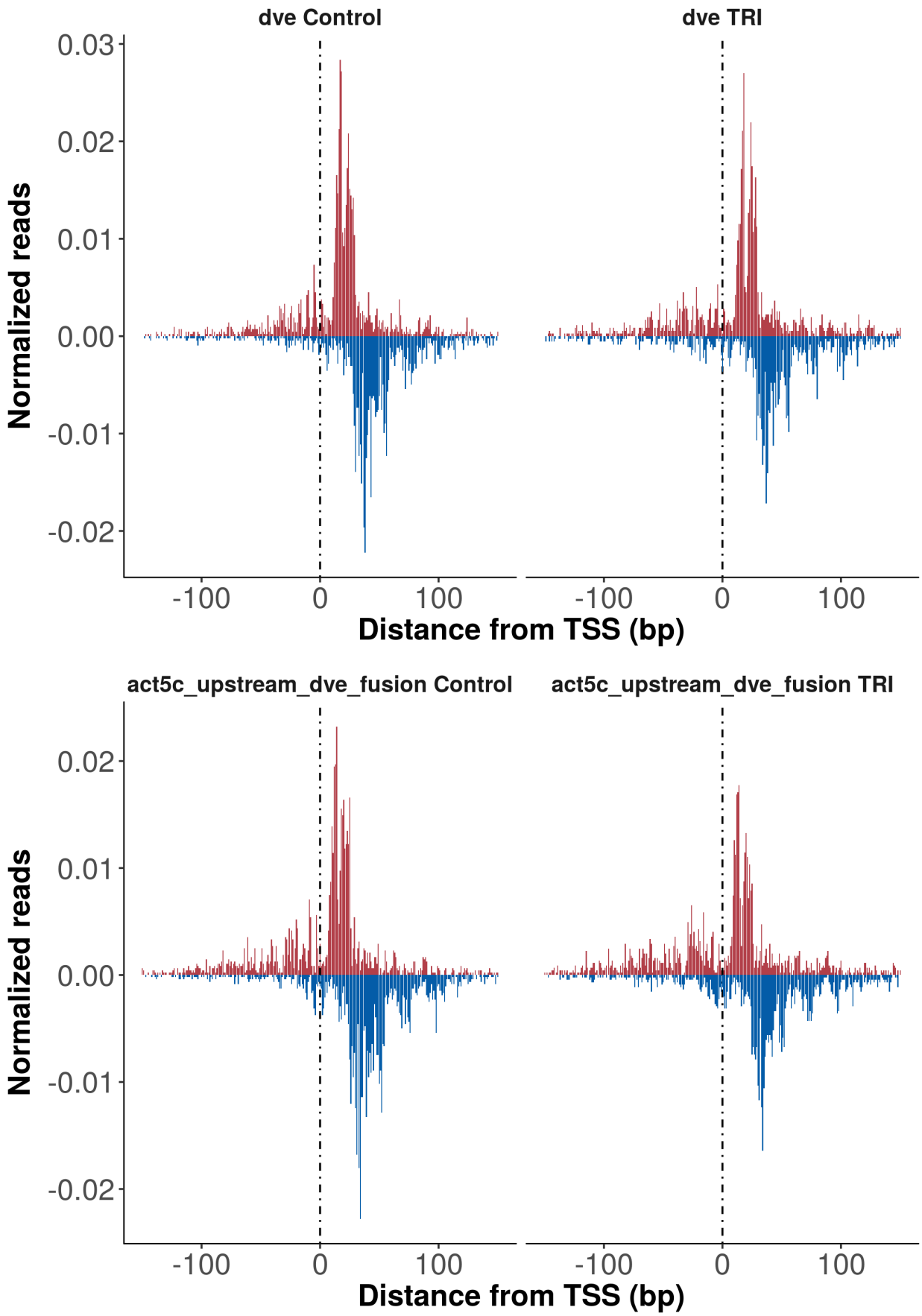
```
library(GenomicRanges, warn.conflicts=F)
library(magrittr)
library(Rmisc)

setwd("/data/analysis_code")
options(knitr.figure_dir =
        "Figure5_Inr_contributes_to_the_degree_of_Pol II_pausing"
        )

source("shared_code/knitr_common.r")
source("shared_code/ggplot_common.r")
source("shared_code/granges_common.r")
source("shared_code/metapeak_common.r")
source("shared_code/sample_common.r")
```

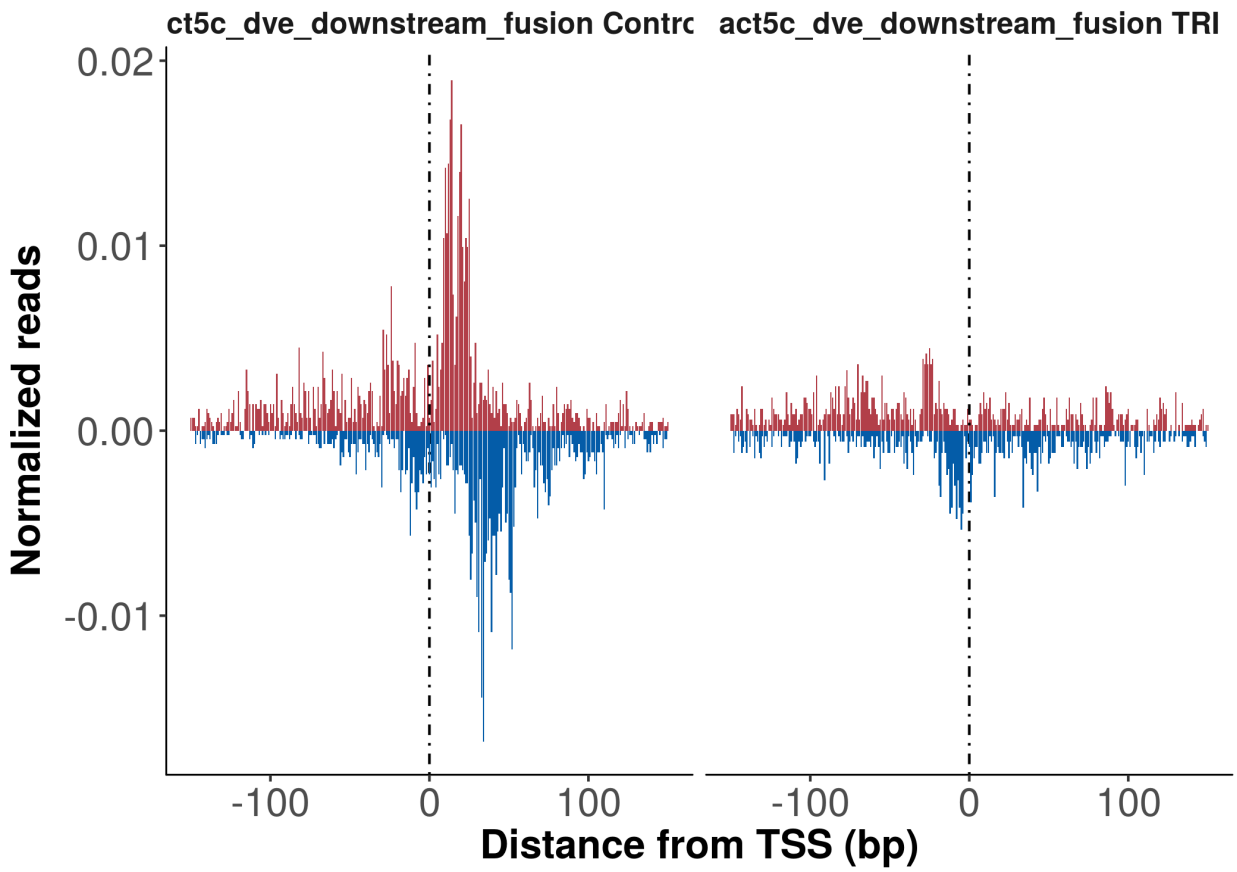
Analysis

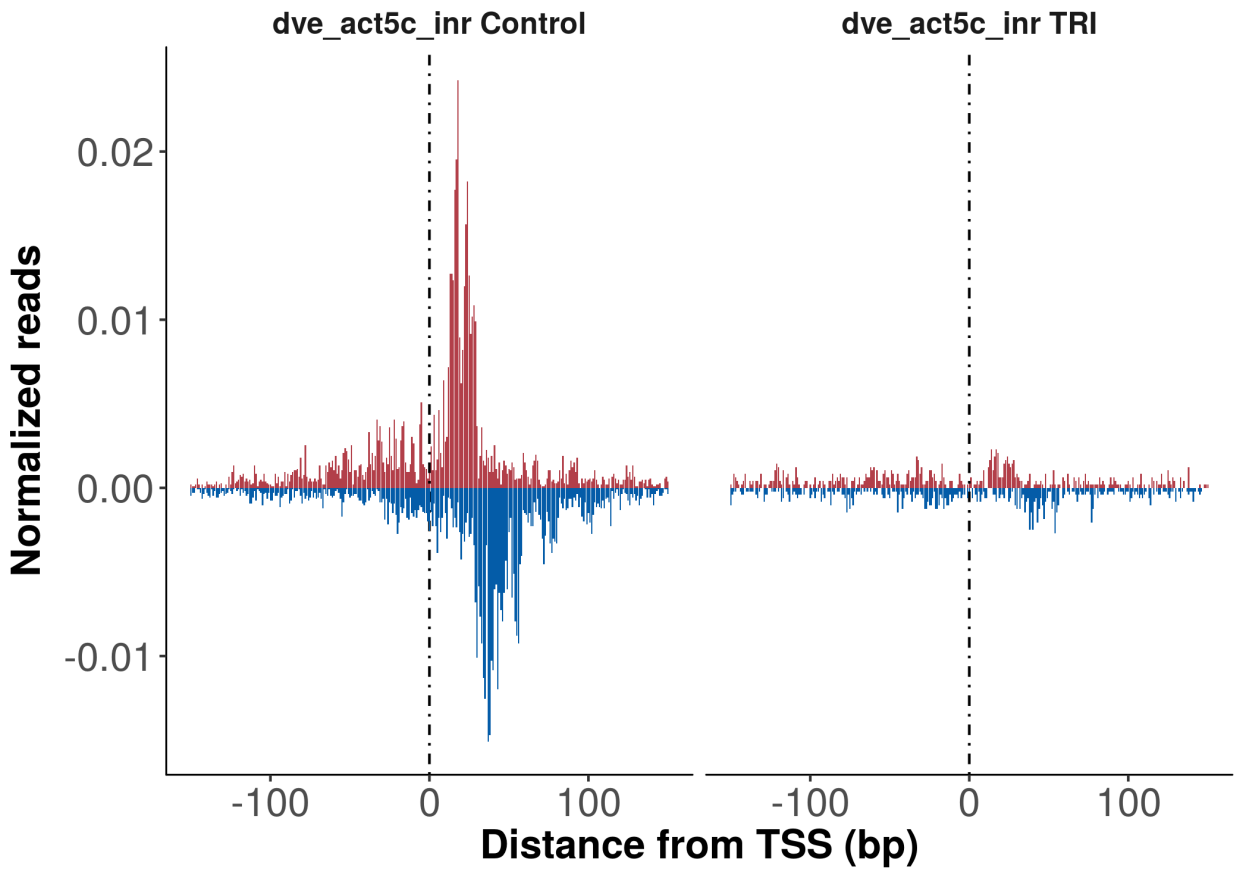
Pol II signal changes after Inr replacement

```
plasmid_annotations <- import("./plasmid_annotation.bed")

get_exo_metapeak <- function(sample, upstream=100, downstream=101,
                             smooth=NA, endogeneous = F, dps_sample_path=NULL){

  gene <- as.character(subset(sample_list, sample_name == sample)$gene)
    chromosome <- as.character(subset(sample_list, sample_name == sample )$chromosome)

    if(endogeneous ==F){

        sample_path <- load_bigwig(sample)
        region <- plasmid_annotations[seqnames(plasmid_annotations) == chromosome &
                                      plasmid_annotations$name == gene] %>%
                  resize(., 1, "start")
        seqlevels(region) <- chromosome
      metapeak <- exo_metapeak(region, sample_path,
                               upstream=upstream, downstream=downstream,
                               sample_name=gene, smooth=smooth)
      metapeak$sample <- paste(metapeak$sample_name, metapeak$strand)
      metapeak

    }else{

      region <- genome_annotations[grep(gene, genome_annotations$name, ignore.case = T)]
      seqlevels(region) <- as.character(seqnames(region))
      metapeak <- exo_metapeak(region, dps_sample_path,
                               upstream=upstream, downstream=downstream,
                               sample_name=gene, smooth=smooth)
      metapeak$sample <- paste(metapeak$sample_name, metapeak$strand)
      metapeak

    }
}


compare_dmso_and_tri <- function(dmso, tri, name,  plotting = T){

  plasmid_dmso <- get_exo_metapeak(dmso, upstream=150, downstream = 151)
  plasmid_tri <- get_exo_metapeak(tri, upstream=150, downstream = 151)

  plasmid_tri$reads <- plasmid_tri$reads / sum(abs(plasmid_dmso$reads))
  plasmid_dmso$reads <- plasmid_dmso$reads / sum(abs(plasmid_dmso$reads))


  plasmid_dmso$sample_name <- paste(name, "Control")
  plasmid_tri$sample_name <- paste(name, "TRI")

  if(plotting){
    metapeak <- rbind(plasmid_dmso, plasmid_tri)
    metapeak.p <- subset(metapeak, strand == "+")
    metapeak.n <- subset(metapeak, strand == "-")

  plot <- ggplot(metapeak.p, aes(x = tss_distance, y = reads, fill = strand))+
```

```
          geom_bar(fill="#B23F49", stat="identity") +
          geom_vline(xintercept =0, linetype = "dotdash")+
          geom_bar(data=metapeak.n, aes(x=tss_distance, y=reads),
                   fill="#045CA8", stat="identity")+
          xlab("Distance from TSS (bp)")+ ylab("Normalized reads")+
          facet_wrap(facets = "sample_name", ncol =2 )
  print(plot)
  }

  dmso_sig <- subset(plasmid_dmso, tss_distance >0 & tss_distance <= 80)$reads %>%
              abs() %>% sum()
  tri_sig <-  subset(plasmid_tri, tss_distance >0 & tss_distance <= 80)$reads%>%
              abs() %>% sum()

  sig_df <- data.frame(condition = c("dmso", "tri"),
                       paused_polii = c(dmso_sig, tri_sig),
                       name = name)
  sig_df$paused_pol_norm <- sig_df$paused_polii / sig_df$paused_polii[1]
  sig_df
}


name_list <-c("dve", "act5c_upstream_dve_fusion", "act5c_dve_downstream_fusion", "dve_act5c_inr")

pol_sig_rep1 <- mapply(compare_dmso_and_tri,
       paste0("reporter_dmso_1h_dps_", name_list, "_rpb3_chipnexus_rep1"),
       paste0("reporter_triptolide_1h_dps_", name_list, "_rpb3_chipnexus_rep1"),
       name_list,list(T), SIMPLIFY = F, USE.NAMES =F)  %>% do.call(rbind, .)
```

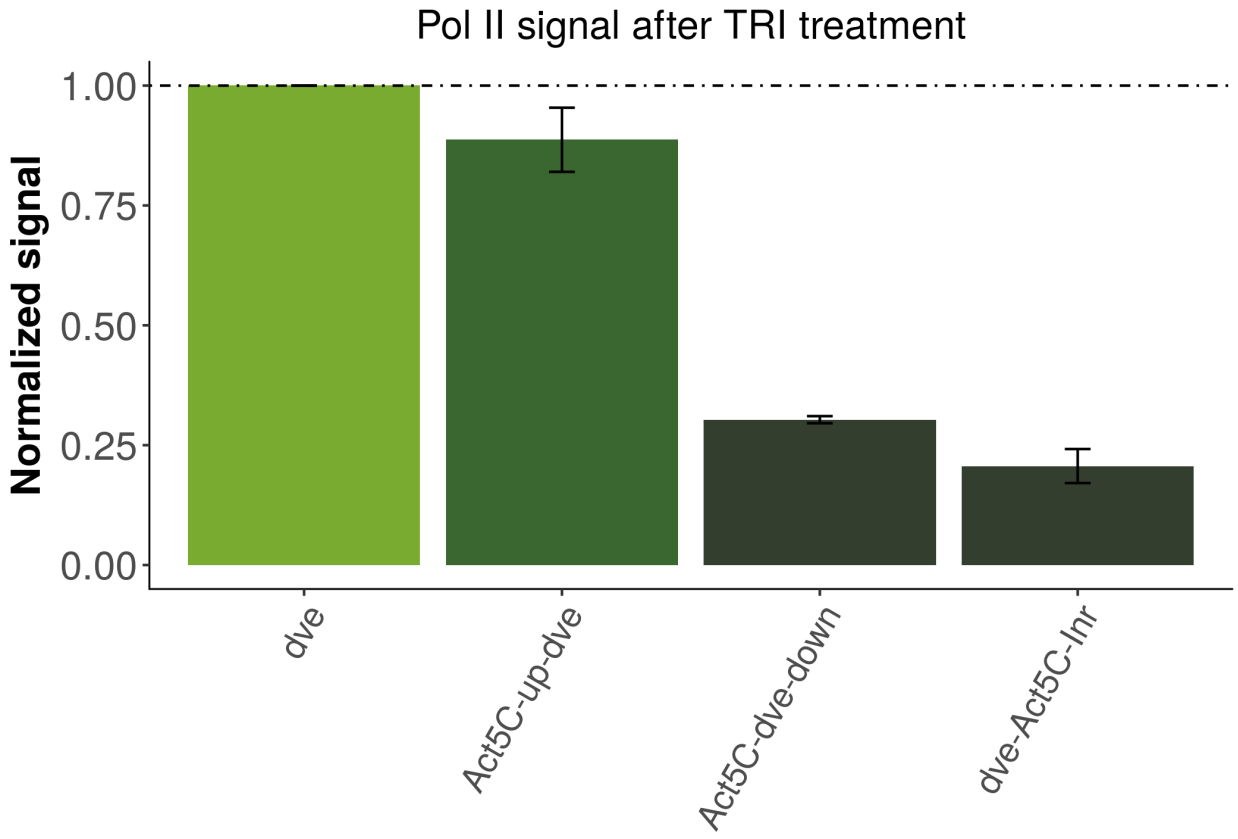

dve Control
dve TRI
act5c_upstream_dve_fusion Control
act5c_upstream_dve_fusion TRI
Normalized reads
0.03
0.02
0.01
0.00
-0.01
-0.02
-100
0
100
Distance from TSS (bp)


ct5c_dve_downstream_fusion Contrc
act5c_dve_downstream_fusion TRI
Normalized reads
0.02
0.01
0.00
-0.01
-100
0
100
-100
0
100
Distance from TSS (bp)




```
pol_sig_rep2 <- mapply(compare_dmso_and_tri,
       paste0("reporter_dmso_1h_dps_", name_list, "_rpb3_chipnexus_rep2"),
       paste0("reporter_triptolide_1h_dps_", name_list, "_rpb3_chipnexus_rep2"),
       name_list, list(F), SIMPLIFY = F, USE.NAMES =F)  %>% do.call(rbind, .)

pol_sig_rep3 <- mapply(compare_dmso_and_tri,
       paste0("reporter_dmso_1h_dps_", name_list[1:3], "_rpb3_chipnexus_rep3"),
       paste0("reporter_triptolide_1h_dps_", name_list[1:3], "_rpb3_chipnexus_rep3"),
       name_list[1:3], list(F), SIMPLIFY = F, USE.NAMES =F)  %>% do.call(rbind, .)

pol_sig_rep4 <- mapply(compare_dmso_and_tri,
       paste0("reporter_dmso_1h_dps_", name_list[1:2], "_rpb3_chipnexus_rep4"),
       paste0("reporter_triptolide_1h_dps_", name_list[1:2], "_rpb3_chipnexus_rep4"),
       name_list[1:2], list(F), SIMPLIFY = F, USE.NAMES =F)  %>% do.call(rbind, .)

process_pol_sig <- function(df, control_n = 1){
  df_sub <- subset(df, condition == "tri")
  df_sub$paused_pol_norm <- df_sub$paused_pol_norm /  df_sub$paused_pol_norm[control_n]
  df_sub
}


sig_df <- lapply(list(pol_sig_rep1, pol_sig_rep2, pol_sig_rep3, pol_sig_rep4),
                 process_pol_sig) %>% do.call(rbind, .)
```

```
summary_df <- summarySE(sig_df, measurevar="paused_pol_norm",
                         groupvars=c("name", "condition"))

ggplot(summary_df, aes(x=name, y=paused_pol_norm)) +
  geom_bar(stat= "identity", position = "dodge",
          fill =  c("#78AB30", "#3A662F", "#333E2F", "#333E2F")) +
  geom_errorbar(aes(ymin=paused_pol_norm-se, ymax=paused_pol_norm+se),
                width=.1, position=position_dodge(.9)) +
  ggtitle("Pol II signal after TRI treatment")+
  ylab("Normalized signal")+
  scale_x_discrete(labels=c("dve", "Act5C-up-dve",
                            "Act5C-dve-down", "dve-Act5C-Inr")) +
  xlab("")+
  geom_hline(yintercept = 1, lty = 4) +
  theme(axis.text.x = element_text(size=14, angle = 60, hjust = 1))
```



Session Info

This analysis was performed with the following R/Bioconductor session:

```
sessionInfo()
```

```
## R version 3.4.4 (2018-03-15)
## Platform: x86_64-pc-linux-gnu (64-bit)
```

```
## Running under: Ubuntu 16.04.4 LTS
##
## Matrix products: default
## BLAS: /usr/lib/libblas/libblas.so.3.6.0
## LAPACK: /usr/lib/lapack/liblapack.so.3.6.0
##
## 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] parallel  stats4    stats     graphics  grDevices utils     datasets
## [8] methods   base
##
## other attached packages:
##  [1] reshape2_1.4.3       rtracklayer_1.38.3   ggplot2_2.2.1
##  [4] pander_0.6.1         Rmisc_1.5            plyr_1.8.4
##  [7] lattice_0.20-35      magrittr_1.5         GenomicRanges_1.30.3
## [10] GenomeInfoDb_1.14.0  IRanges_2.12.0       S4Vectors_0.16.0
## [13] BiocGenerics_0.24.0
##
## loaded via a namespace (and not attached):
##  [1] Rcpp_0.12.17               compiler_3.4.4
##  [3] pillar_1.2.3               XVector_0.18.0
##  [5] bitops_1.0-6               tools_3.4.4
##  [7] zlibbioc_1.24.0            digest_0.6.15
##  [9] evaluate_0.10.1            tibble_1.4.2
## [11] gtable_0.2.0               rlang_0.2.1
## [13] Matrix_1.2-14              DelayedArray_0.4.1
## [15] yaml_2.1.19                GenomeInfoDbData_1.0.0
## [17] stringr_1.3.1              knitr_1.20
## [19] Biostrings_2.46.0          rprojroot_1.3-2
## [21] grid_3.4.4                 Biobase_2.38.0
## [23] XML_3.98-1.11              BiocParallel_1.12.0
## [25] rmarkdown_1.10             matrixStats_0.53.1
## [27] GenomicAlignments_1.14.2   backports_1.1.2
## [29] scales_0.5.0               Rsamtools_1.30.0
## [31] htmltools_0.3.6            SummarizedExperiment_1.8.1
## [33] colorspace_1.3-2           labeling_0.3
## [35] stringi_1.2.3              RCurl_1.95-4.10
## [37] lazyeval_0.2.1             munsell_0.5.0
```