CUT&RUN LPM WT vs KO - Figures

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2025-09-05 21:23:42 +0200

Contents

Introduction

To test whether MafB also regulates mac identity in primary RTM, we performed CUT&RUN for MafB on LPMs from Mafbfl/fl or Lyz2CreMafbfl/fl mice. CUT&RUN was performed with a CUTANA ChIC/CUT&RUN Kit (EpiCypher, 141048) according to manufacturer's instructions, with modifications. BMDMs from Mafbfl/fl or Lyz2CreMafbfl/fl mice were washed with ice-cold PBS and fixed with 0.1 % formaldehyde in PBS for 2 min at room temperature. Fixation was quenched by adding glycine (Merck, 104691000) to 0.125 M. For each CUT&RUN sample, 1 x 10^6 fixed cells and 0.5 µg antibodies were added:

IgG (EpiCypher, 13-0042) MafB2 (Cell Signaling Technology, 41019)

CUT&RUN libraries were prepared with a CUTANA™ CUT&RUN Library Prep Kit (EpiCypher, 141001) according to manufacturer's instructions. These libraries were sequenced on an NovaSeq 6000 (Illumina) sequencer on an S4 flow cell at 10 million reads per sample.

Load Packages

```
suppressMessages({
    library(ggplot2)
    library(colorRamp2)
    library(ComplexHeatmap)
    library(readxl)
    library(futile.logger)
    library(grid)
    library(rtracklayer)
    library(dplyr)
    library(VennDiagram)
    library(Rtoolbox)
})
```

```
## Warning: package 'colorRamp2' was built under R version 4.4.3
```

Figure S6A

```
MafB2_peaks_rep1 <- read.table("MafB2_WT_R1.seacr.peaks.stringent.bed",
    header = FALSE, sep = "\t")

MafB2_peaks_rep2 <- read.table("MafB2_WT_R2.seacr.peaks.stringent.bed",
    header = FALSE, sep = "\t")

MafB2_peaks_consensus <- read.table("MafB2_WT.seacr.consensus.peak_counts.bed",
    header = FALSE, sep = "\t")
```

```
# peaks rep1
nrow(MafB2_peaks_rep1)
```

```
## [1] 24776
```

```
# peaks rep2
nrow(MafB2_peaks_rep2)
```

```
## [1] 15978
```

```
# consensus peaks
nrow(MafB2_peaks_consensus)
```

```
## [1] 28717
```

```
# shared peaks
length(which(MafB2_peaks_consensus[, 10] == 2))
```

```
## [1] 11000
```

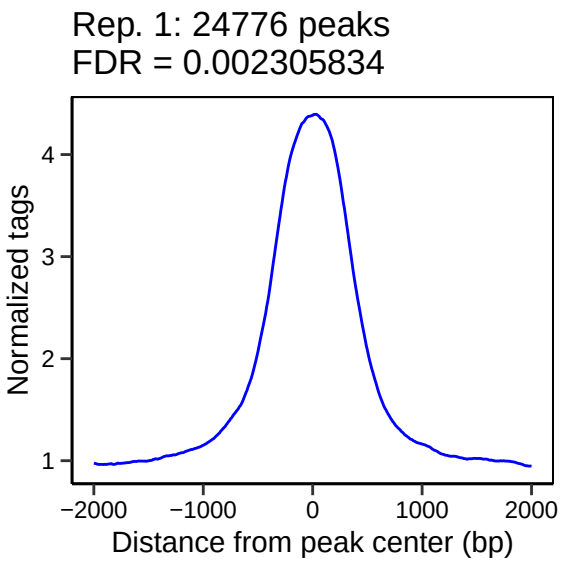
Rep. 1

```
annotatePeaks.pl MafB2_WT_R1.seacr.peaks.stringent.bed mm10 -size 4000 -hist 10 -d MafB2_WT_R1/ > MafB2_
```

```
MafB2_WT_R1_hist <- read.table("MafB2_WT_R1_hist.txt", header = TRUE,
    sep = "\t")

colnames(MafB2_WT_R1_hist)[colnames(MafB2_WT_R1_hist) == "Distance.from.Center..cmd.annotatePeaks.pl.Ma
```

n peaks : 24776 FDR = 0.00230583412634511

```
ggplot(data = MafB2_WT_R1_hist, aes(x = Distance.from.Center,
    y = MafB2_WT_R1..Coverage)) + geom_line(show.legend = FALSE,
    colour = "blue") + ggtitle("Rep. 1: 24776 peaks \nFDR = 0.002305834") +
    xlab("Distance from peak center (bp)") + ylab("Normalized tags") +
    theme_classic() + theme(axis.text.x = element_text(color = "black"),
    axis.text.y = element_text(color = "black"), axis.ticks.length = unit(0.15,
        "cm"), panel.border = element_rect(fill = NA, color = "black",
        linetype = "solid"))
```




```
ggsave("Figure_S6E_Rep1.pdf", width = 3, height = 3)
```

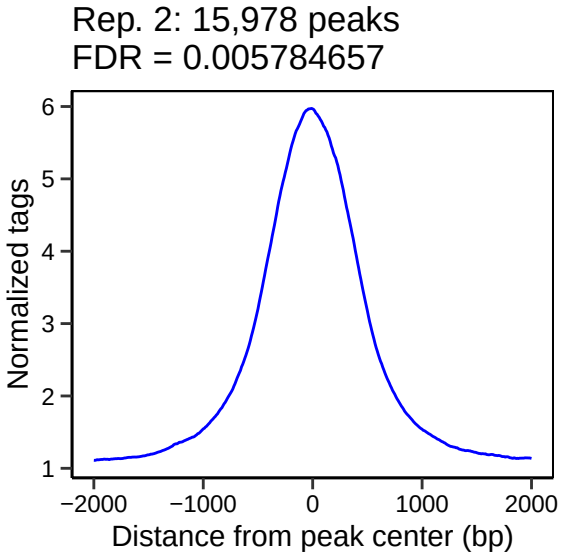
Rep. 2

```
annotatePeaks.pl MafB2_WT_R2.seacr.peaks.stringent.bed mm10 -size 4000 -hist 10 -d MafB2_WT_R2/ > MafB2_
```

```
MafB2_WT_R2_hist <- read.table("MafB2_WT_R2_hist.txt", header = TRUE,
    sep = "\t")

colnames(MafB2_WT_R2_hist)[colnames(MafB2_WT_R2_hist) == "Distance.from.Center..cmd.annotatePeaks.pl.Ma
```

n peaks : 15978 FDR = 0.0057846571008926

```
ggplot(data = MafB2_WT_R2_hist, aes(x = Distance.from.Center,
    y = MafB2_WT_R2..Coverage)) + geom_line(show.legend = FALSE,
    colour = "blue") + ggtitle("Rep. 2: 15,978 peaks \nFDR = 0.005784657") +
    xlab("Distance from peak center (bp)") + ylab("Normalized tags") +
    theme_classic() + theme(axis.text.x = element_text(color = "black"),
    axis.text.y = element_text(color = "black"), axis.ticks.length = unit(0.15,
        "cm"), panel.border = element_rect(fill = NA, color = "black",
        linetype = "solid"))
```




```
ggsave("Figure_S6E_Rep2.pdf.pdf", width = 3, height = 3)
```

Figure S6F

```
samtools view -c -F 4 MafB2_WT_R1.target.markdup.sorted.bam
bedtools intersect -a MafB2_WT_R1.target.markdup.sorted.bam -b MafB2_WT_R1.seacr.peaks.stringent.bed -b

samtools view -c -F 4 MafB2_WT_R2.target.markdup.sorted.bam
bedtools intersect -a MafB2_WT_R2.target.markdup.sorted.bam -b MafB2_WT_R2.seacr.peaks.stringent.bed -b
```

Figure S6G

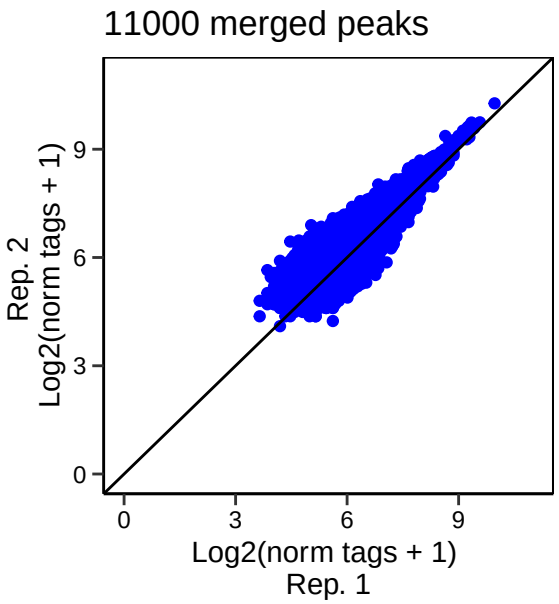
```
MafB2_peaks_merged <- MafB2_peaks_consensus[MafB2_peaks_consensus[,
    10] == 2, ]

split_scores <- strsplit(as.character(MafB2_peaks_merged$V6),
    ",")
```

```
means <- sapply(split_scores, function(x) {
    if (all(is.na(x)))
        return(NA_real_)
    nums <- as.numeric(x)
    mean(nums, na.rm = TRUE)
})

MafB2_peaks_merged$V4 <- means

write.table(MafB2_peaks_merged, "MafB2_peaks_merged.bed", sep = "\t",
    quote = FALSE, row.names = FALSE, col.names = FALSE)
```

```
annotatePeaks.pl MafB2_peaks_merged.bed mm10 -d MafB2_WT_R1/ MafB2_WT_R2/ MafB2_KO_R1/ MafB2_KO_R2/ > Ma
```

```
MafB2_counts <- read.table("MafB2_counts.txt", header = TRUE,
    sep = "\t")

colnames(MafB2_counts)[colnames(MafB2_counts) == "PeakID..cmd.annotatePeaks.pl.MafB2_peaks_merged.bed.mm

colnames(MafB2_counts)[colnames(MafB2_counts) == "MafB2_WT_R1..Tag.Count.in.given.bp..5198073.0.Total..r

colnames(MafB2_counts)[colnames(MafB2_counts) == "MafB2_WT_R2..Tag.Count.in.given.bp..5587453.0.Total..r

colnames(MafB2_counts)[colnames(MafB2_counts) == "MafB2_KO_R1..Tag.Count.in.given.bp..6219029.0.Total..r

colnames(MafB2_counts)[colnames(MafB2_counts) == "MafB2_KO_R2..Tag.Count.in.given.bp..6260374.0.Total..r
```

```
ggplot(data = MafB2_counts, aes(x = log2(MafB2_WT_R1 + 1), y = log2(MafB2_WT_R2 +
    1))) + geom_point(show.legend = FALSE, colour = "blue") +
    geom_abline(slope = 1) + ggtitle("11000 merged peaks") +
    xlab("Log2(norm tags + 1) \nRep. 1") + ylab("Rep. 2 \nLog2(norm tags + 1)") +
    xlim(0, 11) + ylim(0, 11) + theme_classic() + theme(axis.text.x = element_text(color = "black"),
    axis.text.y = element_text(color = "black"), axis.ticks.length = unit(0.15,
        "cm"), panel.border = element_rect(fill = NA, color = "black",
        linetype = "solid"))
```




```
ggsave("Figure_S6G.pdf", width = 3, height = 3.225)
```

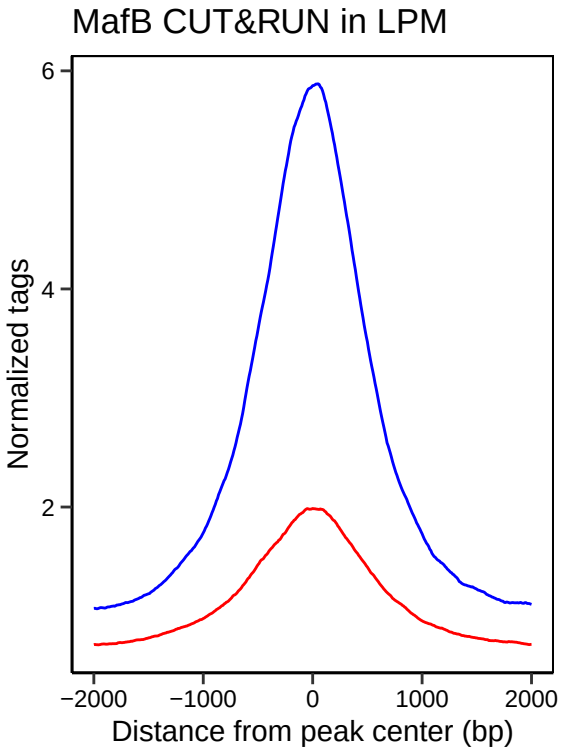
Figure 6I

Histogram

```
annotatePeaks.pl MafB2_peaks_merged.bed mm10 -size 4000 -hist 10 -d MafB2_WT_R1/ MafB2_WT_R2/ MafB2_KO_
```

```
MafB2_hist <- read.table("MafB2_hist.txt", header = TRUE, sep = "\t")

colnames(MafB2_hist)[colnames(MafB2_hist) == "Distance.from.Center..cmd.annotatePeaks.pl.MafB2_peaks_me

MafB2_hist$MafB2_WT <- rowMeans(MafB2_hist[, c("MafB2_WT_R1..Coverage",
    "MafB2_WT_R2..Coverage")])

MafB2_hist$MafB2_KO <- rowMeans(MafB2_hist[, c("MafB2_KO_R1..Coverage",
    "MafB2_KO_R2..Coverage")])
```

```
ggplot(data = MafB2_hist, aes(x = Distance.from.Center, y = MafB2_WT)) +
    geom_line(show.legend = FALSE, colour = "blue") + geom_line(show.legend = FALSE,
    colour = "red", aes(x = Distance.from.Center, y = MafB2_KO)) +
    ggtitle("MafB CUT&RUN in LPM") + xlab("Distance from peak center (bp)") +
    ylab("Normalized tags") + theme_classic() + theme(axis.text.x = element_text(color = "black"),
    axis.text.y = element_text(color = "black"), axis.ticks.length = unit(0.15,
        "cm"), panel.border = element_rect(fill = NA, color = "black",
        linetype = "solid"))
```




```
ggsave("Figure_6I_WTvsKO_Hist.pdf", width = 3, height = 4)
```

Heatmap

```
annotatePeaks.pl MafB2_peaks_merged.bed mm10 -size 4000 -hist 25 -ghist -d MafB2_WT_R1/ MafB2_WT_R2/ Ma
```

```
MafB2_heatmap <- read.table("MafB2_heatmap.txt", header = TRUE,
    check.names = FALSE, sep = "\t")

MafB2_WT_R1_heatmap <- MafB2_heatmap[, 2:162]
MafB2_WT_R2_heatmap <- MafB2_heatmap[, 163:323]
MafB2_WT_heatmap <- (MafB2_WT_R1_heatmap + MafB2_WT_R2_heatmap)/2

MafB2_KO_R1_heatmap <- MafB2_heatmap[, 324:484]
MafB2_KO_R2_heatmap <- MafB2_heatmap[, 485:645]
MafB2_KO_heatmap <- (MafB2_KO_R1_heatmap + MafB2_KO_R2_heatmap)/2
```

WT

```
col_fun = colorRamp2(c(0, 10), c("white", "red"))

pdf("Figure_6I_heatmap_WT.pdf", width = 2, height = 4)
Heatmap(MafB2_WT_heatmap, col_fun, cluster_columns = F, cluster_rows = F,
    heatmap_legend_param = list(title = "Tags"), use_raster = TRUE,
    raster_quality = 10, column_title = "Mafbfl/fl")
```

```
## Warning: The input is a data frame-like object, convert it to a matrix.

## 'magick' package is suggested to install to give better rasterization.
##
## Set 'ht_opt$message = FALSE' to turn off this message.
```

```
dev.off()
```

```
## pdf
##   2
```

KO

```
col_fun = colorRamp2(c(0, 10), c("white", "red"))

pdf("Figure_6I_heatmap_KO.pdf", width = 2, height = 4)
Heatmap(MafB2_KO_heatmap, col_fun, cluster_columns = F, cluster_rows = F,
    heatmap_legend_param = list(title = "Tags"), use_raster = TRUE,
    raster_quality = 10, , column_title = "Lyz2CreMafbfl/fl")
```

```
## Warning: The input is a data frame-like object, convert it to a matrix.

## 'magick' package is suggested to install to give better rasterization.
##
## Set 'ht_opt$message = FALSE' to turn off this message.
```

```
dev.off()
```

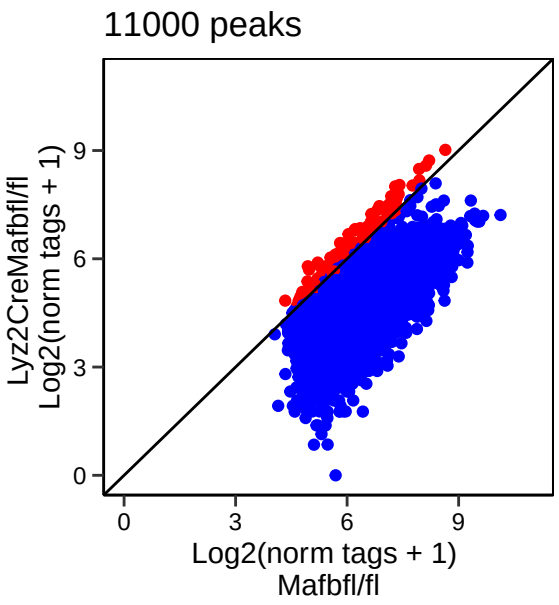
```
## pdf
##   2
```

Figure 6J

```
MafB2_counts$MafB2_WT <- rowMeans(MafB2_counts[, c("MafB2_WT_R1",
    "MafB2_WT_R2")])
MafB2_counts$MafB2_KO <- rowMeans(MafB2_counts[, c("MafB2_KO_R1",
    "MafB2_KO_R2")])

MafB2_counts$log2_MafB2_WT <- log2(MafB2_counts$MafB2_WT + 1)
MafB2_counts$log2_MafB2_KO <- log2(MafB2_counts$MafB2_KO + 1)
MafB2_counts$below_diag <- MafB2_counts$log2_MafB2_KO < MafB2_counts$log2_MafB2_WT
```

```
ggplot(data = MafB2_counts, aes(x = log2_MafB2_WT, y = log2_MafB2_KO)) +
    geom_point(aes(color = below_diag), show.legend = FALSE) +
    scale_color_manual(values = c(`FALSE` = "red", `TRUE` = "blue")) +
    geom_abline(slope = 1) + ggtitle("11000 peaks") + xlab("Log2(norm tags + 1) \nMafbfl/fl") +
```

```
    ylab("Lyz2CreMafbfl/fl \nLog2(norm tags + 1)") + xlim(0,
    11) + ylim(0, 11) + theme_classic() + theme(axis.text.x = element_text(color = "black"),
    axis.text.y = element_text(color = "black"), axis.ticks.length = unit(0.15,
        "cm"), panel.border = element_rect(fill = NA, color = "black",
        linetype = "solid"))
```




```
ggsave("Figure_6J.pdf", width = 3, height = 3.225)
```

Figure 6K

```
annotatePeaks.pl MafB2_peaks.bed genome.fa -gtf genes.gtf > MafB2_peaks_annot.txt
```

```
MafB2_peaks_annot <- read.table("MafB2_peaks_annot.txt", header = TRUE,
    sep = "\t")
```

```
MafB2_peaks_annot$Annotation.not.detailed <- sub(" .*", "", MafB2_peaks_annot$Annotation)

table(MafB2_peaks_annot$Annotation.not.detailed)
```

```
##
##         exon   Intergenic       intron promoter-TSS          TTS
##          565         3165         3891         2986          251
```

Figure 6L

```r
MafB2_BMDM_peaks <- read.table("MafB2_BMDM_peaks.bed", header = FALSE,
    sep = "\t")
MafB2_peaks <- read.table("MafB2_peaks.bed", header = FALSE,
    sep = "\t")

nrow(MafB2_BMDM_peaks)
```

```
## [1] 7242
```

```r
nrow(MafB2_peaks)
```

```
## [1] 10858
```

```r
bedtools window -a MafB2_peaks.bed -b MafB2_BMDM_peaks.bed -w 100 > MafB2_overlap.bed
```

```r
MafB2_overlap <- read.table("MafB2_overlap.bed", header = FALSE,
    sep = "\t")

nrow(MafB2_overlap)
```

```
## [1] 5133
```

```r
pdf(file = "Figure_6L_Venn.pdf", width = 4, height = 4)
draw.pairwise.venn(area1 = 7242, area2 = 10858, cross.area = 5133,
    category = c("BMDM", "LPM"), fill = c("red", "green"))
```

```
## (polygon[GRID.polygon.822], polygon[GRID.polygon.823], polygon[GRID.polygon.824], polygon[GRID.polyg
```

```r
grid.newpage()
```

Figure 6M

```r
# Helper function to display Venn diagram
display_venn <- function(x, ...) {
    grid.newpage()
    venn_object <- venn.diagram(x, filename = NULL, ...)
    grid.draw(venn_object)
}
```

```r
MafB2_LPM_genes <- MafB2_peaks_annot[MafB2_peaks_annot[, "Distance.to.TSS"] >=
    -2000 & MafB2_peaks_annot[, "Distance.to.TSS"] <= 2000, ]

MafB2_LPM_genes <- unique(MafB2_LPM_genes$Gene.Name)
MafB2_LPM_genes <- na.omit(MafB2_LPM_genes)
```

```r
MafB2_BMDM_peaks_annot <- read.table("MafB2_BMDM_peaks_annot.txt",
    header = TRUE, sep = "\t")

MafB2_BMDM_genes <- MafB2_BMDM_peaks_annot[MafB2_BMDM_peaks_annot[,
    "Distance.to.TSS"] >= -2000 & MafB2_BMDM_peaks_annot[, "Distance.to.TSS"] <=
    2000, ]

MafB2_BMDM_genes <- unique(MafB2_BMDM_genes$Gene.Name)
MafB2_BMDM_genes <- na.omit(MafB2_BMDM_genes)
```

```r
x <- list(LPM = MafB2_LPM_genes, BMDM = MafB2_BMDM_genes)
pdf(file = "Figure_6M_Venn.pdf", width = 4, height = 4)
display_venn(x, fill = c("green", "red"))
dev.off()
```

```
## pdf
##   2
```