CUT&RUN MDM - Figures

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2025-09-06 21:35:11 +0200

Contents

Introduction

To assess whether MafB also regulates macrophage identity in humans, we performed CUT&RUN for MafB on human monocyte-derived mac (MDMs). 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 10ˆ6 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)
})
```

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

Figure S7

```
MafB2_peaks_rep1 <- read.table("MafB2_R1.seacr.peaks.stringent.bed",
    header = FALSE, sep = "\t")

MafB2_peaks_rep2 <- read.table("MafB2_R2.seacr.peaks.stringent.bed",
    header = FALSE, sep = "\t")

MafB2_peaks_consensus <- read.table("MafB2.seacr.peaks.consensus.bed",
    header = FALSE, sep = "\t")
```

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

```
## [1] 26327
```

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

```
## [1] 30718
```

```
# shared peaks
nrow(MafB2_peaks_consensus)
```

```
## [1] 24755
```

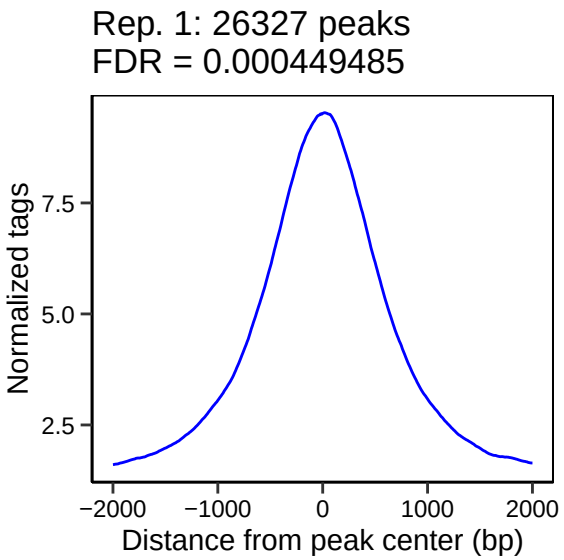
Rep. 1

```
annotatePeaks.pl MafB2_R1.seacr.peaks.stringent.bed mm10 -size 4000 -hist 10 -d MafB2_R1/ > MafB2_R1_his
```

```
MafB2_R1_hist <- read.table("MafB2_R1_hist.txt", header = TRUE,
    sep = "\t")

colnames(MafB2_R1_hist)[colnames(MafB2_R1_hist) == "Distance.from.Center..cmd.annotatePeaks.pl.MafB2_R1
```

n peaks : 26327 FDR = 0.000449484821243296

```
ggplot(data = MafB2_R1_hist, aes(x = Distance.from.Center, y = MafB2_R1..Coverage)) +
    geom_line(show.legend = FALSE, colour = "blue") + ggtitle("Rep. 1: 26327 peaks \nFDR = 0.000449485")
    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_S7G_Rep1.pdf", width = 3, height = 3)
```

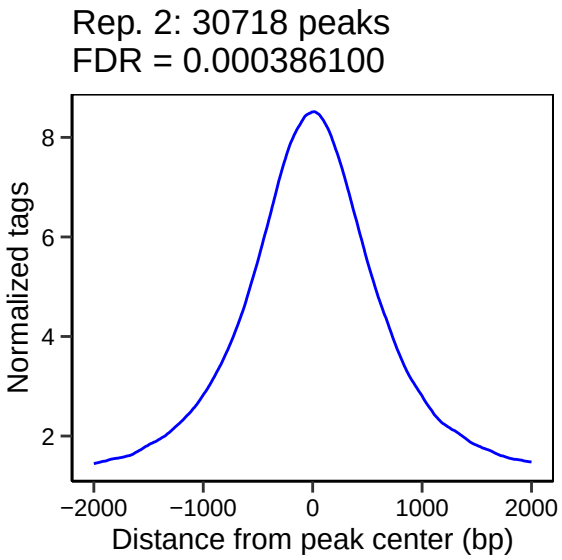
Rep 2

```
annotatePeaks.pl MafB2_R2.seacr.peaks.stringent.bed mm10 -size 4000 -hist 10 -d MafB2_R2/ > MafB2_R2_his
```

```
MafB2_R2_hist <- read.table("MafB2_R2_hist.txt", header = TRUE,
    sep = "\t")

colnames(MafB2_R2_hist)[colnames(MafB2_R2_hist) == "Distance.from.Center..cmd.annotatePeaks.pl.MafB2_R2
```

n peaks : 30718 FDR = 0.00038610038610043

```
ggplot(data = MafB2_R2_hist, aes(x = Distance.from.Center, y = MafB2_R2..Coverage)) +
    geom_line(show.legend = FALSE, colour = "blue") + ggtitle("Rep. 2: 30718 peaks \nFDR = 0.000386100")
    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_S7G_Rep2.pdf", width = 3, height = 3)
```

Figure S7H

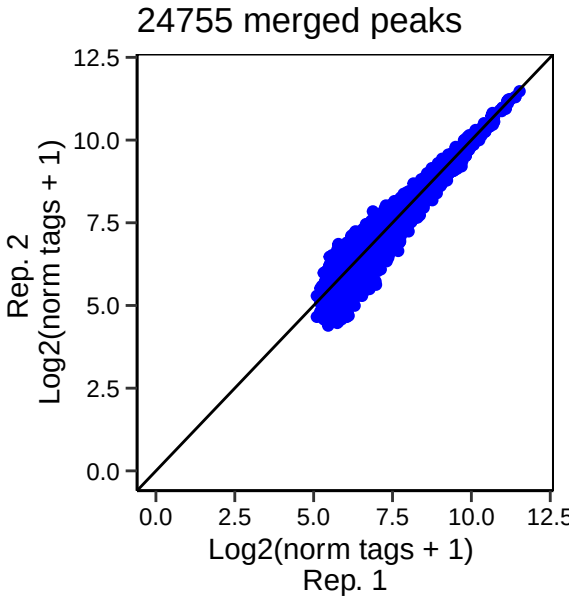
```
samtools view -c -F 4 MafB2_R1.target.markdup.sorted.bam
bedtools intersect -a MafB2_R1.target.markdup.sorted.bam -b MafB2_R1.seacr.peaks.stringent.bed -bed | w

samtools view -c -F 4 MafB2_R2.target.markdup.sorted.bam
bedtools intersect -a MafB2_R2.target.markdup.sorted.bam -b MafB2_R2.seacr.peaks.stringent.bed -bed | w
```

Figure S7I

```
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.m

colnames(MafB2_counts)[colnames(MafB2_counts) == "IgG_R1..Tag.Count.in.given.bp..4149983.0.Total..norma

colnames(MafB2_counts)[colnames(MafB2_counts) == "MafB2_R1..Tag.Count.in.given.bp..7202841.0.Total..nor
```

```
colnames(MafB2_counts)[colnames(MafB2_counts) == "MafB2_R2..Tag.Count.in.given.bp..8260258.0.Total..norm
```

```
ggplot(data = MafB2_counts, aes(x = log2(MafB2_R1 + 1), y = log2(MafB2_R2 +
    1))) + geom_point(show.legend = FALSE, colour = "blue") +
    geom_abline(slope = 1) + ggtitle("24755 merged peaks") +
    xlab("Log2(norm tags + 1) \nRep. 1") + ylab("Rep. 2 \nLog2(norm tags + 1)") +
    xlim(0, 12) + ylim(0, 12) + 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_S7I.pdf", width = 3, height = 3.225)
```

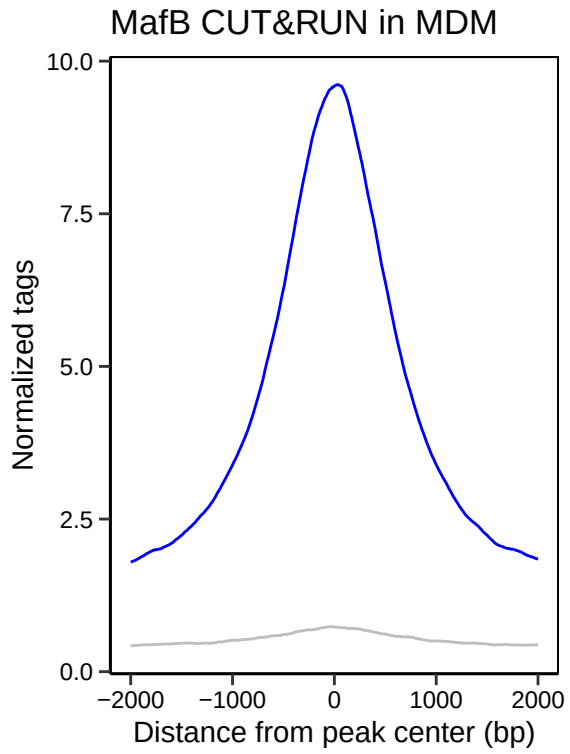
Figure 7A

Histogram

```
annotatePeaks.pl MafB2_peaks_merged.bed mm10 -size 4000 -hist 10 -d IgG_R1/ MafB2_R1/ MafB2_R2/ > MafB2_
```

```
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_mer

MafB2_hist$MafB2 <- rowMeans(MafB2_hist[, c("MafB2_R1..Coverage",
    "MafB2_R2..Coverage")])
```

```
ggplot(data = MafB2_hist, aes(x = Distance.from.Center, y = MafB2)) +
    geom_line(show.legend = FALSE, colour = "blue") + geom_line(show.legend = FALSE,
    colour = "grey", aes(x = Distance.from.Center, y = IgG_R1..Coverage)) +
    ggtitle("MafB CUT&RUN in MDM") + 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_7A_MDM_Hist.pdf", width = 3, height = 4)
```

Heatmap

```
annotatePeaks.pl MafB2_peaks_merged.bed mm10 -size 4000 -hist 25 -ghist -d IgG_R1/ MafB2_R1/ MafB2_R2/ >
```

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

IgG_R1_heatmap <- MafB2_heatmap[, 2:162]

MafB2_R1_heatmap <- MafB2_heatmap[, 163:323]
MafB2_R2_heatmap <- MafB2_heatmap[, 324:484]
MafB2_heatmap <- (MafB2_R1_heatmap + MafB2_R2_heatmap)/2
```

IgG

```
col_fun = colorRamp2(c(0, 20), c("white", "red"))

pdf("Figure_7A_heatmap_IgG.pdf", width = 2, height = 4)
Heatmap(IgG_R1_heatmap, col_fun, cluster_columns = F, cluster_rows = F,
    heatmap_legend_param = list(title = "Tags"), use_raster = TRUE,
    raster_quality = 10, , column_title = "IgG")
```

```
## 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
```

MafB

```
col_fun = colorRamp2(c(0, 20), c("white", "red"))

pdf("Figure_7A_heatmap_MafB.pdf", width = 2, height = 4)
Heatmap(MafB2_heatmap, col_fun, cluster_columns = F, cluster_rows = F,
    heatmap_legend_param = list(title = "Tags"), use_raster = TRUE,
    raster_quality = 10, column_title = "MafB2")
```

```
## 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.
```

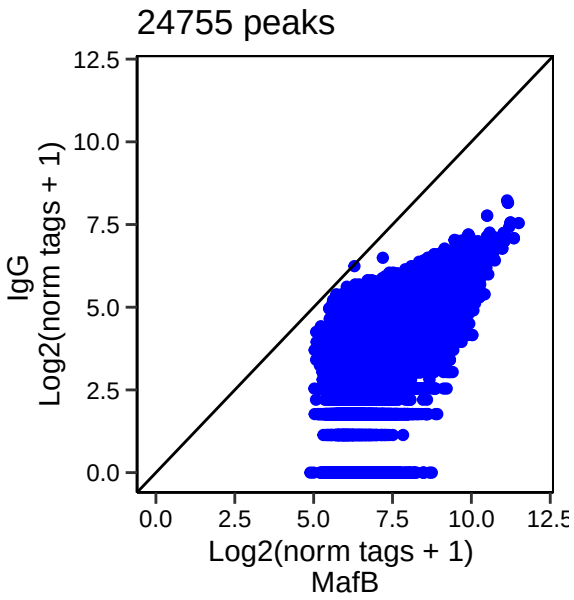
```
dev.off()
```

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

Figure 7B

```
MafB2_counts$MafB2 <- rowMeans(MafB2_counts[, c("MafB2_R1", "MafB2_R2")])

MafB2_counts$log2_IgG <- log2(MafB2_counts$IgG_R1 + 1)
MafB2_counts$log2_MafB2 <- log2(MafB2_counts$MafB2 + 1)
MafB2_counts$below_diag <- MafB2_counts$log2_IgG < MafB2_counts$log2_MafB2
```

```
ggplot(data = MafB2_counts, aes(x = log2_MafB2, y = log2_IgG)) +
    geom_point(aes(color = below_diag), show.legend = FALSE) +
    scale_color_manual(values = c(`FALSE` = "grey", `TRUE` = "blue")) +
    geom_abline(slope = 1) + ggtitle("24755 peaks") + xlab("Log2(norm tags + 1) \nMafB") +
    ylab("IgG \nLog2(norm tags + 1)") + xlim(0, 12) + ylim(0,
    12) + 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_7B.pdf", width = 3, height = 3.225)
```

```
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.m

colnames(MafB2_counts)[colnames(MafB2_counts) == "IgG_R1..Tag.Count.in.given.bp..4149983.0.Total..norma

colnames(MafB2_counts)[colnames(MafB2_counts) == "MafB2_R1..Tag.Count.in.given.bp..7202841.0.Total..nor

colnames(MafB2_counts)[colnames(MafB2_counts) == "MafB2_R2..Tag.Count.in.given.bp..8260258.0.Total..nor

MafB2_counts$MafB2 <- rowMeans(MafB2_counts[, c("MafB2_R1", "MafB2_R2")])

MafB2_counts$log2_IgG <- log2(MafB2_counts$IgG_R1 + 1)
MafB2_counts$log2_MafB2 <- log2(MafB2_counts$MafB2 + 1)
MafB2_counts$below_diag <- MafB2_counts$log2_IgG < MafB2_counts$log2_MafB2
```

```
MafB2_peaks_merged <- read.table("MafB2_peaks_merged.bed", header = F,
    sep = "\t")
MafB2_PeakID <- c(MafB2_counts[MafB2_counts[, "below_diag"] ==
```

```
    "TRUE", ]$PeakID)
MafB2_peaks <- MafB2_peaks_merged[MafB2_peaks_merged[, 4] %in%
    MafB2_PeakID, ]

write.table(MafB2_peaks, "MafB2_peaks.bed", sep = "\t", quote = FALSE,
    row.names = FALSE, col.names = FALSE)
```

Figure 7C

```
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
##         1076         5173        11663         5606         1237
```

Figure 7D

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

MafB2_BMDM_peaks$V1 <- paste0("chr", MafB2_BMDM_peaks$V1)

MafB2_BMDM_peaks <- MafB2_BMDM_peaks[, 1:4]

write.table(MafB2_BMDM_peaks, "MafB2_BMDM_peaks_for_liftover.bed",
    sep = "\t", quote = FALSE, row.names = FALSE, col.names = FALSE)
```

Convert mouse MafB2 BMDM peaks to human

```
MafB2_BMDM_peaks_human <- read.table("MafB2_BMDM_peaks_human.bed",
    header = FALSE, sep = "\t")

MafB2_BMDM_peaks_human$V1 <- sub("^chr", "", MafB2_BMDM_peaks_human$V1)

write.table(MafB2_BMDM_peaks_human, "MafB2_BMDM_peaks_human.bed",
    sep = "\t", quote = FALSE, row.names = FALSE, col.names = FALSE)
```

check overlap

```
bedtools window -a MafB2_peaks.bed -b MafB2_BMDM_peaks_human.bed -w 100 > MafB2_peaks_conserved.bed
```

```
MafB2_peaks_conserved <- read.table("MafB2_peaks_conserved.bed",
    header = FALSE, sep = "\t")

nrow(MafB2_peaks)
```

```
## [1] 24755
```

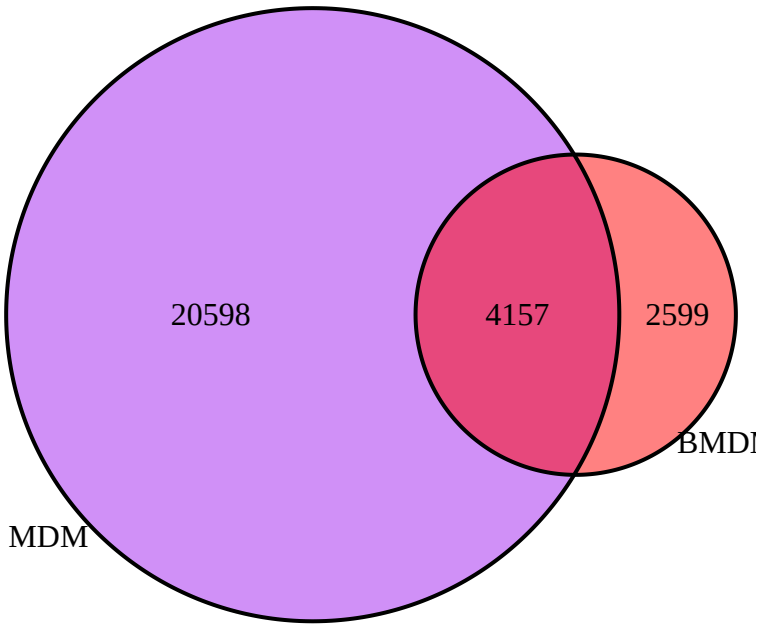
```
nrow(MafB2_BMDM_peaks_human)
```

```
## [1] 6756
```

```
nrow(MafB2_peaks_conserved)
```

```
## [1] 4157
```

```
# pdf(file = 'Figure_7D_Venn.pdf', width = 4, height = 4)
draw.pairwise.venn(area1 = 6756, area2 = 24755, cross.area = 4157,
    category = c("BMDM", "MDM"), fill = c("red", "purple"))
```




```
## (polygon[GRID.polygon.846], polygon[GRID.polygon.847], polygon[GRID.polygon.848], polygon[GRID.polyg
```

```
# grid.newpage()
```

Figure 7E

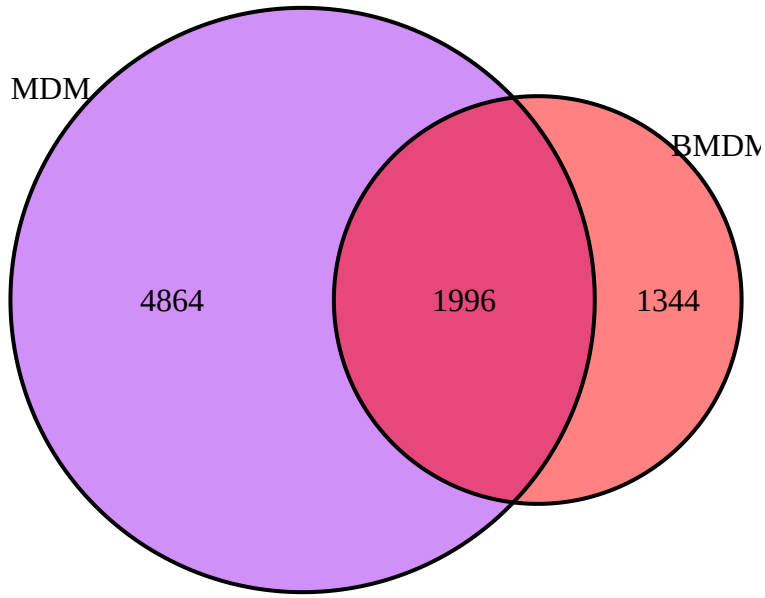
```
# Helper function to display Venn diagram
display_venn <- function(x, ...) {
    grid.newpage()
    venn_object <- venn.diagram(x, filename = NULL, ...)
    grid.draw(venn_object)
}
```

```
MafB2_genes <- MafB2_peaks_annot[MafB2_peaks_annot[, "Distance.to.TSS"] >=
    -2000 & MafB2_peaks_annot[, "Distance.to.TSS"] <= 2000, ]

MafB2_genes <- unique(MafB2_genes$Gene.Name)
MafB2_genes <- na.omit(MafB2_genes)
```

```
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)

write.table(MafB2_BMDM_genes, "MafB2_BMDM_genes.txt", sep = "\t",
    quote = FALSE, row.names = FALSE, col.names = FALSE)
```

```
MafB2_BMDM_genes_human <- read.csv("MafB2_BMDM_genes_human.csv")

MafB2_BMDM_genes_human <- na.omit(unique(MafB2_BMDM_genes_human$ortholog_name))
```

```
x <- list(MDM = MafB2_genes, BMDM = MafB2_BMDM_genes_human)
# pdf(file = 'Figure_7E_Venn.pdf', width = 4, height = 4)
display_venn(x, fill = c("purple", "red"))
```




```
# dev.off()
```