CUT&RUN BMDM WT vs KO - Figures

Domien Vanneste

2025-09-05 16:42:28 +0200

Contents

Introduction **3**

Load Packages **4**

Figure S6A **4**

Rep. 1 5

Rep. 2 5

Figure S6B **6**

Figure S6C **7**

Figure 6A **8**

Histogram 8

Heatmap 9

Figure 6B **10**

Figure 6C **11**

Figure 6D **11**

Figure 6E **13**

Figure 6F **14**

Figure 6H **15**

15

LPM 18
KC 19
IM 19
Ly6C CM 20
MHC2 CM 21
MG 22

Introduction

Despite substantial evidence pointing to MafB as an essential regulator of core mac identity, the genes it directly controls and the mechanisms underlying this regulation remain poorly understood. To address this question, we performed cleavage under targets and release using nuclease (CUT&RUN) for MafB on BMDMs from Mafbfl/fl and Lyz2CreMafbfl/fl mice. MafB and H3K27ac 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)

H3K27ac (Thermo Fisher Scientific, MA5-23516).

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)
    library(SeuratObject)
    library(Seurat)
    library(introdataviz)
})
```

```
## 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] 10549
```

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

```
## [1] 11478
```

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

```
## [1] 14436
```

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

```
## [1] 7256
```

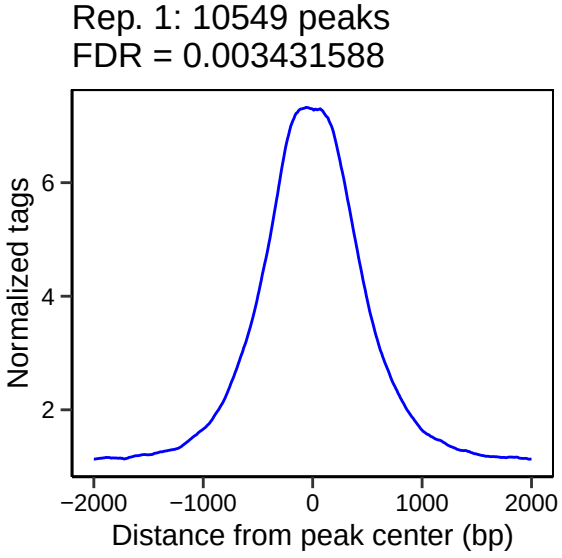
Rep. 1

```
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 : 10549 FDR = 0.00343158820941492

```
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: 10549 peaks \nFDR = 0.003431588") +
    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_S6A_Rep1.pdf", width = 3, height = 3)
```

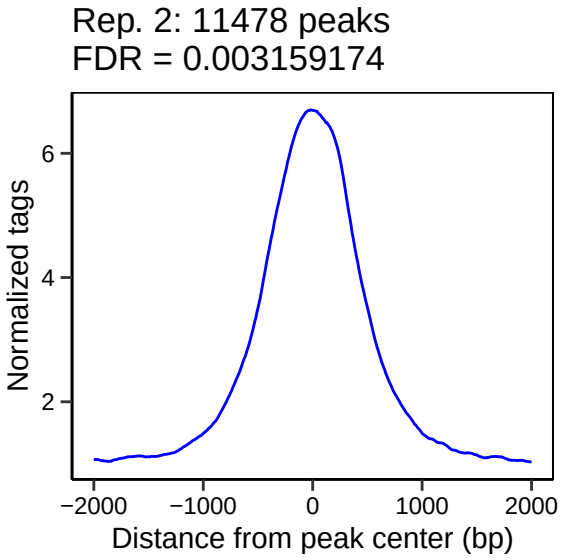
Rep. 2

```
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 : 11478 FDR = 0.00315917375455654

```
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: 11478 peaks \nFDR = 0.003159174") +
    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_S6A_Rep2.pdf.pdf", width = 3, height = 3)
```

Figure S6B

```
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
```

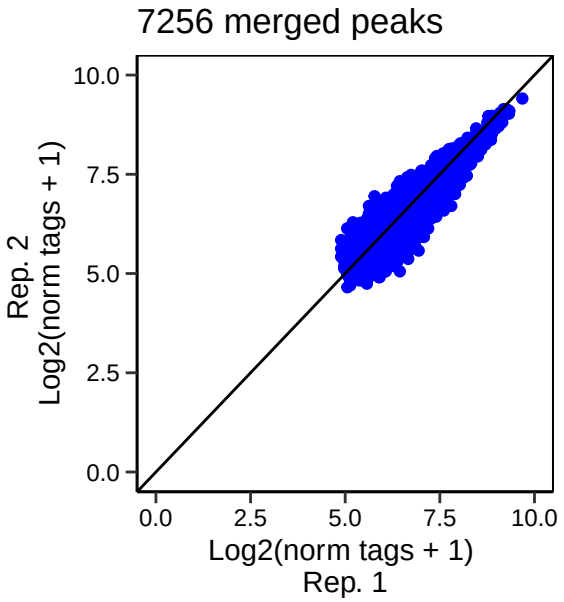
Figure S6C

```
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..5577784.0.Total..r

colnames(MafB2_counts)[colnames(MafB2_counts) == "MafB2_WT_R2..Tag.Count.in.given.bp..6205913.0.Total..r

colnames(MafB2_counts)[colnames(MafB2_counts) == "MafB2_KO_R1..Tag.Count.in.given.bp..5444540.0.Total..r

colnames(MafB2_counts)[colnames(MafB2_counts) == "MafB2_KO_R2..Tag.Count.in.given.bp..6810425.0.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("7256 merged peaks") + xlab("Log2(norm tags + 1) \nRep. 1") +
    ylab("Rep. 2 \nLog2(norm tags + 1)") + xlim(0, 10) + ylim(0,
    10) + 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_S6C.pdf", width = 3, height = 3.225)
```

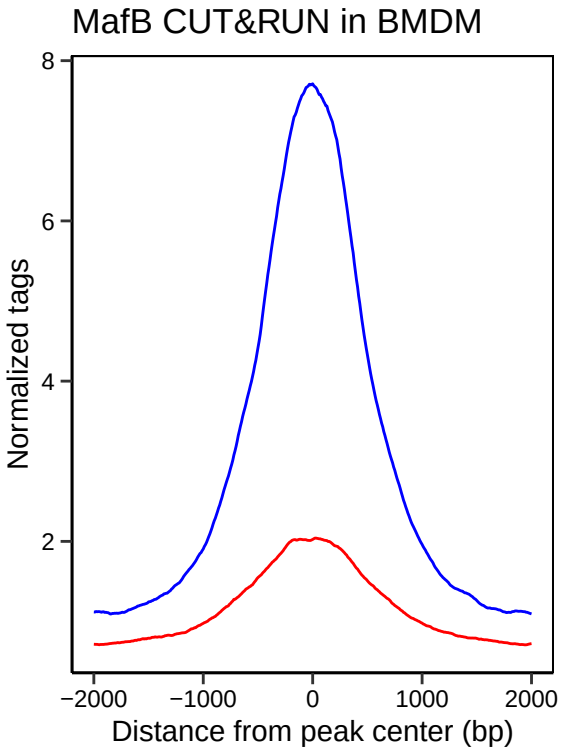
Figure 6A

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 BMDM") + 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_6A_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, 15), c("white", "red"))

pdf("Figure_6A_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, 15), c("white", "red"))

pdf("Figure_6A_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()
```

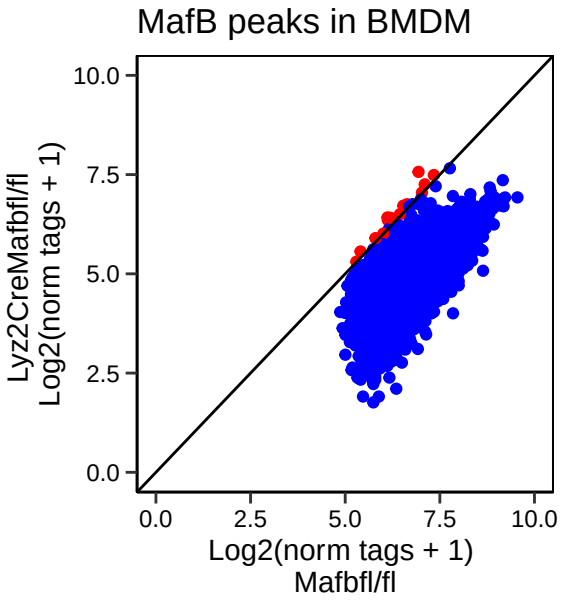
```
## pdf
##   2
```

Figure 6B

```
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("MafB peaks in BMDM") +
```

```
    xlab("Log2(norm tags + 1) \nMafbfl/fl") + ylab("Lyz2CreMafbfl/fl \nLog2(norm tags + 1)") +
    xlim(0, 10) + ylim(0, 10) + 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_6B.pdf", width = 3, height = 3.225)
```

Figure 6C

```
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)
```

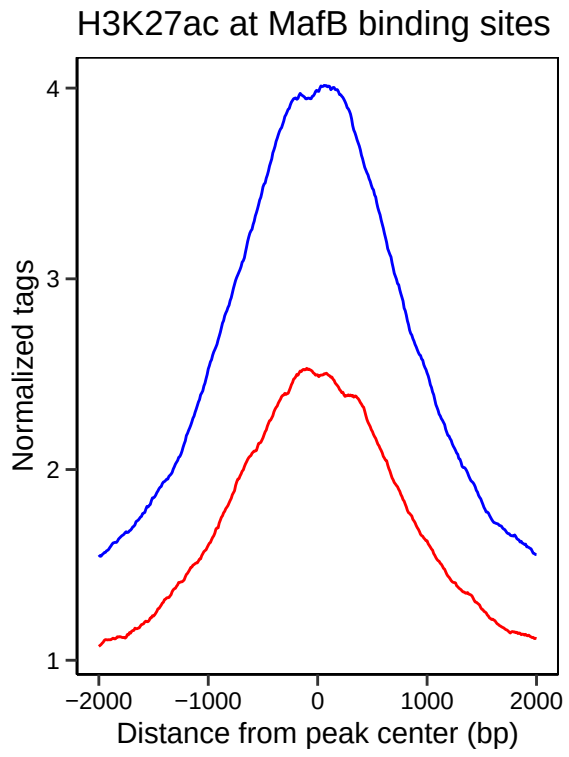
```
##
##         exon   Intergenic       intron promoter-TSS          TTS
##          472         1897         2423         2271          178
```

Figure 6D

```
annotatePeaks.pl MafB2_peaks.bed mm10 -size 4000 -hist 10 -d tag_directory_WT-H3K27Ac_R1/ tag_directory
```

```
MafB2_H3K27Ac_hist <- read.table("MafB2_H3K27Ac_hist.txt", header = TRUE,
    sep = "\t")

colnames(MafB2_H3K27Ac_hist)[colnames(MafB2_H3K27Ac_hist) ==
    "Distance.from.Center..cmd.annotatePeaks.pl.MafB2_peaks.bed.mm10..size.4000..hist.10..d.tag_directo

MafB2_H3K27Ac_hist$H3K27Ac_WT <- rowMeans(MafB2_H3K27Ac_hist[,
    c("tag_directory_WT.H3K27Ac_R1..Coverage", "tag_directory_WT.H3K27Ac_R2..Coverage")])

MafB2_H3K27Ac_hist$H3K27Ac_KO <- rowMeans(MafB2_H3K27Ac_hist[,
    c("tag_directory_KO.H3K27Ac_R1..Coverage", "tag_directory_KO.H3K27Ac_R2..Coverage")])
```

```
ggplot(data = MafB2_H3K27Ac_hist, aes(x = Distance.from.Center,
    y = H3K27Ac_WT)) + geom_line(show.legend = FALSE, colour = "blue") +
    geom_line(show.legend = FALSE, colour = "red", aes(x = Distance.from.Center,
        y = H3K27Ac_KO)) + ggtitle("H3K27ac at MafB binding sites") +
    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_6D_Hist_H3K27Ac.pdf", height = 4, width = 3)
```

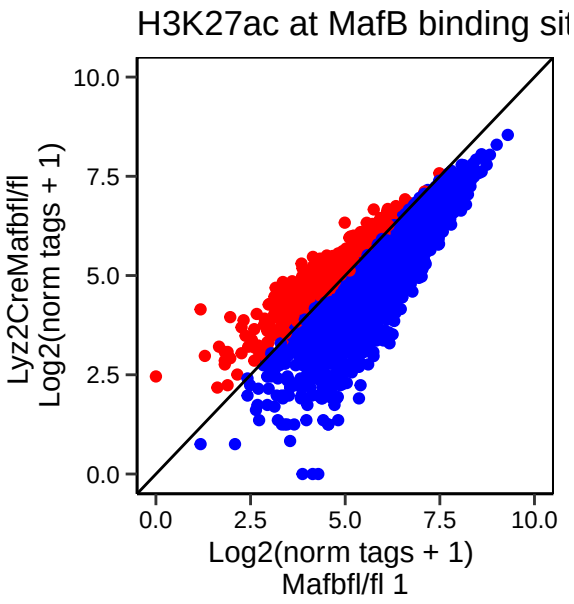
Figure 6E

```
annotatePeaks.pl MafB2_peaks.bed mm10 -d tag_directory_WT-H3K27Ac_R1/ tag_directory_WT-H3K27Ac_R2/ tag_
```

```
MafB2_H3K27Ac_counts <- read.table("MafB2_H3K27Ac_counts.txt",
    header = TRUE, sep = "\t")

colnames(MafB2_H3K27Ac_counts)[colnames(MafB2_H3K27Ac_counts) ==
    "PeakID..cmd.annotatePeaks.pl.MafB2_peaks.bed.mm10..d.tag_directory_WT.H3K27Ac_R1..tag_directory_WT

colnames(MafB2_H3K27Ac_counts)[colnames(MafB2_H3K27Ac_counts) ==
    "tag_directory_WT.H3K27Ac_R1..Tag.Count.in.given.bp..3449599.0.Total..normalization.factor...2.90..

colnames(MafB2_H3K27Ac_counts)[colnames(MafB2_H3K27Ac_counts) ==
    "tag_directory_WT.H3K27Ac_R2..Tag.Count.in.given.bp..3954896.0.Total..normalization.factor...2.53..

colnames(MafB2_H3K27Ac_counts)[colnames(MafB2_H3K27Ac_counts) ==
    "tag_directory_KO.H3K27Ac_R1..Tag.Count.in.given.bp..3206024.0.Total..normalization.factor...3.12..

colnames(MafB2_H3K27Ac_counts)[colnames(MafB2_H3K27Ac_counts) ==
    "tag_directory_KO.H3K27Ac_R2..Tag.Count.in.given.bp..3641165.0.Total..normalization.factor...2.75..
```

```
MafB2_H3K27Ac_counts$H3K27Ac_WT <- rowMeans(MafB2_H3K27Ac_counts[,
    c("H3K27Ac_WT_R1", "H3K27Ac_WT_R2")])
MafB2_H3K27Ac_counts$H3K27Ac_KO <- rowMeans(MafB2_H3K27Ac_counts[,
    c("H3K27Ac_KO_R1", "H3K27Ac_KO_R2")])

MafB2_H3K27Ac_counts$log2_H3K27Ac_WT <- log2(MafB2_H3K27Ac_counts$H3K27Ac_WT +
    1)
MafB2_H3K27Ac_counts$log2_H3K27Ac_KO <- log2(MafB2_H3K27Ac_counts$H3K27Ac_KO +
    1)
MafB2_H3K27Ac_counts$below_diag <- MafB2_H3K27Ac_counts$log2_H3K27Ac_KO <
    MafB2_H3K27Ac_counts$log2_H3K27Ac_WT
```

```
ggplot(data = MafB2_H3K27Ac_counts, aes(x = log2_H3K27Ac_WT,
    y = log2_H3K27Ac_KO)) + geom_point(aes(color = below_diag),
    show.legend = FALSE) + scale_color_manual(values = c(`FALSE` = "red",
    `TRUE` = "blue")) + geom_abline(slope = 1) + ggtitle("H3K27ac at MafB binding sites") +
    xlab("Log2(norm tags + 1) \nMafbfl/fl 1") + ylab("Lyz2CreMafbfl/fl \nLog2(norm tags + 1)") +
    xlim(0, 10) + ylim(0, 10) + 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_6E_WTvsKO_H3K27ac_Tags.pdf", height = 3.225, width = 3)
```

Figure 6F

```
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)
```

```
Mac_sign <- read_excel("Mac_sign.xlsx")

Mac_sign_genes <- Mac_sign$Gene_Symbol
```

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

```
list <- list(MafB2_target = MafB2_genes, Mac_sign = Mac_sign_genes)
pdf(file = "Figure_6F_Venn.pdf", width = 4, height = 4)
display_venn(list, fill = c("red", "blue"))
dev.off()
```

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

Figure 6H

```
MafB_Mac_genes <- intersect(MafB2_genes, Mac_sign_genes)

write.table(append(MafB_Mac_genes, "#MafB_Mac_genes", 0), file = "MafB_Mac_genes.grp",
    row.names = FALSE, col.names = FALSE, quote = FALSE)
```

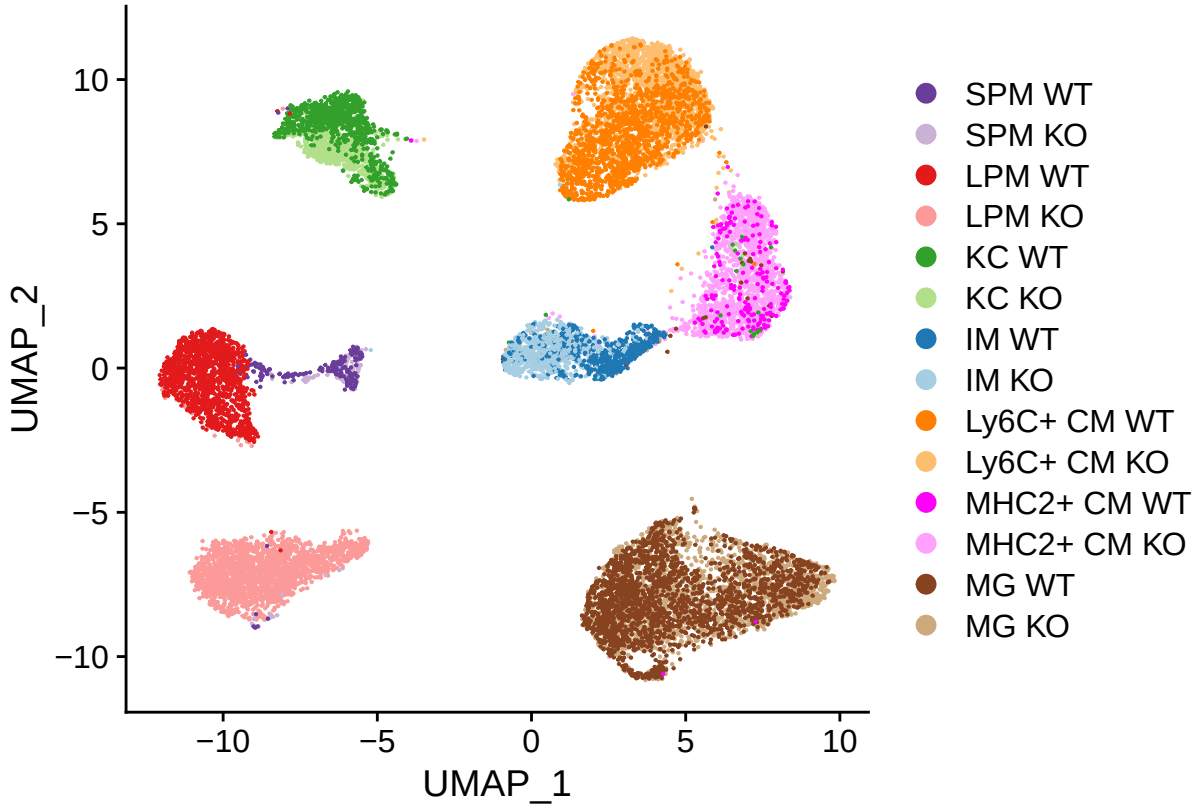
```
path = "C:/Users/domie/Documents/CUTandRUN/GSEA_MafB_Mac_genes"
replotGSEA(path = path, gene.set = "MafB_Mac_genes.grp", class.name = "")
dev.copy(pdf, "C:/Users/domie/Documents/CUTandRUN/GSEA_MafB_Mac_genes.pdf",
    width = 3, height = 3.3)
```

```
## pdf
##   4
```

```
dev.off()
```

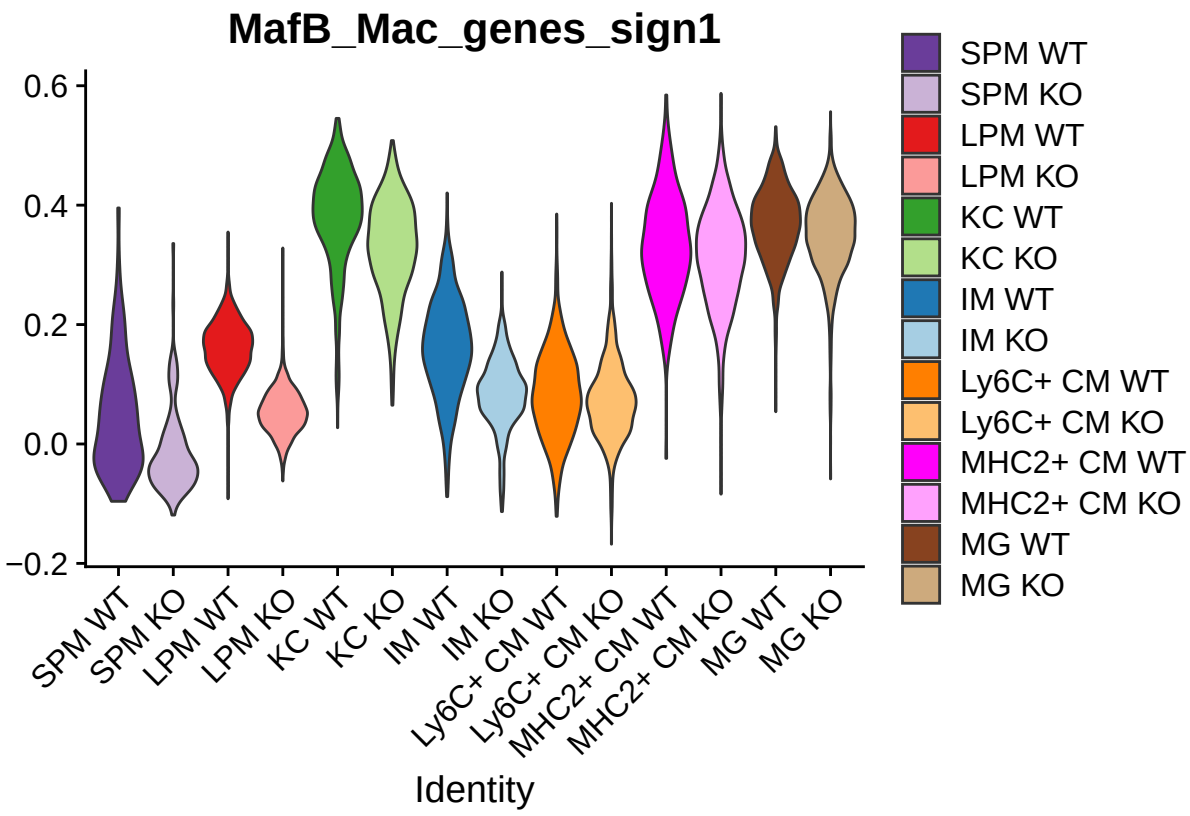
```
## pdf
##   2
```

```
sc <- readRDS("sc.rds")

pal <- c("#6A3D9A", "#CAB2D6", "#E31A1C", "#FB9A99", "#33A02C",
    "#B2DF8A", "#1F78B4", "#A6CEE3", "#FF7F00", "#FDBF6F", "#FF00FA",
    "#FFA1FD", "#87421F", "#CDAA7D")

DimPlot(sc, cols = pal)
```



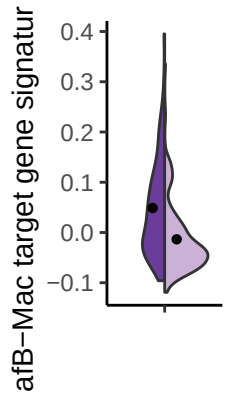

```
sc <- AddModuleScore(sc, features = list(MafB_Mac_genes), name = "MafB_Mac_genes_sign")
```

```
VlnPlot(sc, features = "MafB_Mac_genes_sign1", cols = pal, pt.size = 0)
```




```
metadata <- sc@meta.data
SPM_metadata <- metadata[metadata$cell.type == "SPM", ]
SPM_MafB <- SPM_metadata[, c("Condition", "MafB_Mac_genes_sign1")]
```

```
pal_SPM <- c("#6A3D9A", "#CAB2D6")
ggplot(SPM_MafB, aes(x = "", y = MafB_Mac_genes_sign1, fill = Condition)) +
    introdataviz::geom_split_violin() + stat_summary(fun = mean,
    geom = "point", position = position_dodge(width = 0.5), size = 1.5,
    shape = 16, color = "black") + scale_fill_manual(values = pal_SPM) +
    scale_x_discrete(name = "") + ylab("MafB-Mac target gene signature score") +
    theme_classic() + theme(legend.position = "none")
```



```
ggsave("VlnPlot_Scoring_MafB-Mac_target_SPM.pdf", width = 1.25,
    height = 2)
```

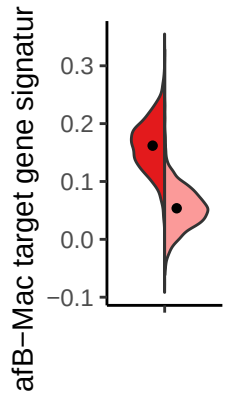
```
SPM_WT <- metadata[metadata$Condition == "SPM WT", ]
SPM_KO <- metadata[metadata$Condition == "SPM KO", ]
wilcox.test(SPM_WT$MafB_Mac_genes_sign1, SPM_KO$MafB_Mac_genes_sign1)
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  SPM_WT$MafB_Mac_genes_sign1 and SPM_KO$MafB_Mac_genes_sign1
## W = 20234, p-value = 1.043e-11
## alternative hypothesis: true location shift is not equal to 0
```

LPM

```
metadata <- sc@meta.data
LPM_metadata <- metadata[metadata$cell.type == "LPM", ]
LPM_MafB <- LPM_metadata[, c("Condition", "MafB_Mac_genes_sign1")]
```

```
pal_LPM <- c("#E31A1C", "#FB9A99")
ggplot(LPM_MafB, aes(x = "", y = MafB_Mac_genes_sign1, fill = Condition)) +
    introdataviz::geom_split_violin() + stat_summary(fun = mean,
    geom = "point", position = position_dodge(width = 0.5), size = 1.5,
    shape = 16, color = "black") + scale_fill_manual(values = pal_LPM) +
    scale_x_discrete(name = "") + ylab("MafB-Mac target gene signature score") +
    theme_classic() + theme(legend.position = "none")
```




```
ggsave("VlnPlot_Scoring_MafB-Mac_target_LPM.pdf", width = 1.25,
    height = 2)
```

```
LPM_WT <- metadata[metadata$Condition == "LPM WT", ]
LPM_KO <- metadata[metadata$Condition == "LPM KO", ]
wilcox.test(LPM_WT$MafB_Mac_genes_sign1, LPM_KO$MafB_Mac_genes_sign1)
```

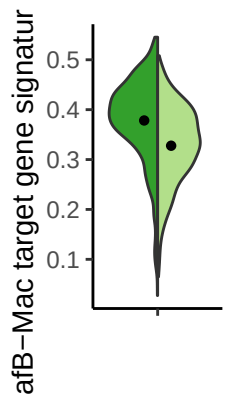
```
##
```

```
##  Wilcoxon rank sum test with continuity correction
##
## data:  LPM_WT$MafB_Mac_genes_sign1 and LPM_KO$MafB_Mac_genes_sign1
## W = 2915366, p-value < 2.2e-16
## alternative hypothesis: true location shift is not equal to 0
```

KC

```
metadata <- sc@meta.data
KC_metadata <- metadata[metadata$cell.type == "KC", ]
KC_MafB <- KC_metadata[, c("Condition", "MafB_Mac_genes_sign1")]
```

```
pal_KC <- c("#33A02C", "#B2DF8A")
ggplot(KC_MafB, aes(x = "", y = MafB_Mac_genes_sign1, fill = Condition)) +
    introdataviz::geom_split_violin() + stat_summary(fun = mean,
    geom = "point", position = position_dodge(width = 0.5), size = 1.5,
    shape = 16, color = "black") + scale_fill_manual(values = pal_KC) +
    scale_x_discrete(name = "") + ylab("MafB-Mac target gene signature score") +
    theme_classic() + theme(legend.position = "none")
```




```
ggsave("VlnPlot_Scoring_MafB-Mac_target_KC.pdf", width = 1.25,
    height = 2)
```

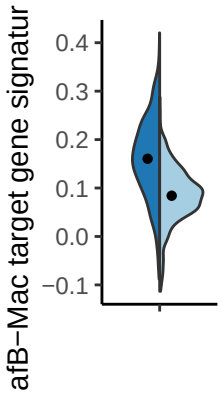
```
KC_WT <- metadata[metadata$Condition == "KC WT", ]
KC_KO <- metadata[metadata$Condition == "KC KO", ]
wilcox.test(KC_WT$MafB_Mac_genes_sign1, KC_KO$MafB_Mac_genes_sign1)
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  KC_WT$MafB_Mac_genes_sign1 and KC_KO$MafB_Mac_genes_sign1
## W = 440476, p-value < 2.2e-16
## alternative hypothesis: true location shift is not equal to 0
```

IM

```
metadata <- sc@meta.data
IM_metadata <- metadata[metadata$cell.type == "IM", ]
IM_MafB <- IM_metadata[, c("Condition", "MafB_Mac_genes_sign1")]
```

```
pal_IM <- c("#1F78B4", "#A6CEE3")
ggplot(IM_MafB, aes(x = "", y = MafB_Mac_genes_sign1, fill = Condition)) +
    introdataviz::geom_split_violin() + stat_summary(fun = mean,
    geom = "point", position = position_dodge(width = 0.5), size = 1.5,
    shape = 16, color = "black") + scale_fill_manual(values = pal_IM) +
    scale_x_discrete(name = "") + ylab("MafB-Mac target gene signature score") +
    theme_classic() + theme(legend.position = "none")
```




```
ggsave("VlnPlot_Scoring_MafB-Mac_target_IM.pdf", width = 1.25,
    height = 2)
```

```
IM_WT <- metadata[metadata$Condition == "IM WT", ]
IM_KO <- metadata[metadata$Condition == "IM KO", ]
wilcox.test(IM_WT$MafB_Mac_genes_sign1, IM_KO$MafB_Mac_genes_sign1)
```

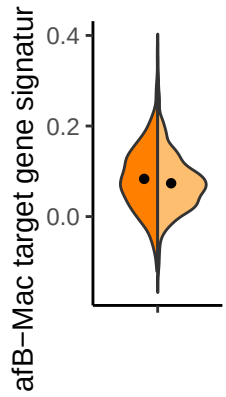
```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  IM_WT$MafB_Mac_genes_sign1 and IM_KO$MafB_Mac_genes_sign1
## W = 342906, p-value < 2.2e-16
## alternative hypothesis: true location shift is not equal to 0
```

Ly6C CM

```
metadata <- sc@meta.data
Ly6C_metadata <- metadata[metadata$cell.type == "Ly6C+ CM", ]
Ly6C_MafB <- Ly6C_metadata[, c("Condition", "MafB_Mac_genes_sign1")]
```

```
pal_Ly6C <- c("#FF7F00", "#FDBF6F")
ggplot(Ly6C_MafB, aes(x = "", y = MafB_Mac_genes_sign1, fill = Condition)) +
    introdataviz::geom_split_violin() + stat_summary(fun = mean,
    geom = "point", position = position_dodge(width = 0.5), size = 1.5,
```

```
    shape = 16, color = "black") + scale_fill_manual(values = pal_Ly6C) +
    scale_x_discrete(name = "") + ylab("MafB-Mac target gene signature score") +
    theme_classic() + theme(legend.position = "none")
```




```
ggsave("VlnPlot_Scoring_MafB-Mac_target_Ly6C_CM.pdf", width = 1.25,
    height = 2)
```

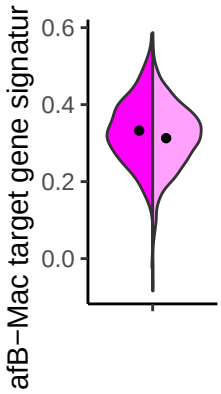
```
Ly6C_WT <- metadata[metadata$Condition == "Ly6C+ CM WT", ]
Ly6C_KO <- metadata[metadata$Condition == "Ly6C+ CM KO", ]
wilcox.test(Ly6C_WT$MafB_Mac_genes_sign1, Ly6C_KO$MafB_Mac_genes_sign1)
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  Ly6C_WT$MafB_Mac_genes_sign1 and Ly6C_KO$MafB_Mac_genes_sign1
## W = 1379681, p-value = 8.946e-06
## alternative hypothesis: true location shift is not equal to 0
```

MHC2 CM

```
metadata <- sc@meta.data
MHC2_metadata <- metadata[metadata$cell.type == "MHC2+ CM", ]
MHC2_MafB <- MHC2_metadata[, c("Condition", "MafB_Mac_genes_sign1")]
```

```
pal_MHC2 <- c("#FF00FA", "#FFA1FD")
ggplot(MHC2_MafB, aes(x = "", y = MafB_Mac_genes_sign1, fill = Condition)) +
    introdataviz::geom_split_violin() + stat_summary(fun = mean,
    geom = "point", position = position_dodge(width = 0.5), size = 1.5,
    shape = 16, color = "black") + scale_fill_manual(values = pal_MHC2) +
    scale_x_discrete(name = "") + ylab("MafB-Mac target gene signature score") +
    theme_classic() + theme(legend.position = "none")
```




```
ggsave("VlnPlot_Scoring_MafB-Mac_target_MHC2_CM.pdf", width = 1.25,
    height = 2)
```

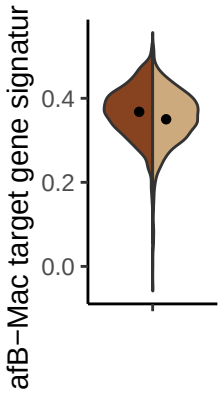
```
MHC2_WT <- metadata[metadata$Condition == "MHC2+ CM WT", ]
MHC2_KO <- metadata[metadata$Condition == "MHC2+ CM KO", ]
wilcox.test(MHC2_WT$MafB_Mac_genes_sign1, MHC2_KO$MafB_Mac_genes_sign1)
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  MHC2_WT$MafB_Mac_genes_sign1 and MHC2_KO$MafB_Mac_genes_sign1
## W = 161609, p-value = 0.01255
## alternative hypothesis: true location shift is not equal to 0
```

MG

```
metadata <- sc@meta.data
MG_metadata <- metadata[metadata$cell.type == "MG", ]
MG_MafB <- MG_metadata[, c("Condition", "MafB_Mac_genes_sign1")]
```

```
pal_MG <- c("#87421F", "#CDAA7D")
ggplot(MG_MafB, aes(x = "", y = MafB_Mac_genes_sign1, fill = Condition)) +
    introdataviz::geom_split_violin() + stat_summary(fun = mean,
    geom = "point", position = position_dodge(width = 0.5), size = 1.5,
    shape = 16, color = "black") + scale_fill_manual(values = pal_MG) +
    scale_x_discrete(name = "") + ylab("MafB-Mac target gene signature score") +
    theme_classic() + theme(legend.position = "none")
```




```
ggsave("VlnPlot_Scoring_MafB-Mac_target_MG.pdf", width = 1.25,
    height = 2)
```

```
MG_WT <- metadata[metadata$Condition == "MG WT", ]
MG_KO <- metadata[metadata$Condition == "MG KO", ]
wilcox.test(MG_WT$MafB_Mac_genes_sign1, MG_KO$MafB_Mac_genes_sign1)
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  MG_WT$MafB_Mac_genes_sign1 and MG_KO$MafB_Mac_genes_sign1
## W = 2338163, p-value = 2.299e-15
## alternative hypothesis: true location shift is not equal to 0
```