scRNA-Seq RTM WT vs KO - Signature scoring

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Contents

Introduction

To objectively examine the effects of MafB deficiency on global and subset-specific mac identity, we used the global mac signature score generated above and created RTM subset-specific scores based on the DEGs of each RTM subset compared to other RTM in our scRNA-seq data. Interestingly, global mac signature scores were all significantly lower in Mafb-deficient RTMs as compared to their wild-type counterparts, except for MHC-II+ CM. Moreover, we found that each RTM subsets from Lyz2CreMafbfl/fl mice, except for SPM and IM, exhibited a significantly lower score for RTM-subset specific signatures as compared to their wild-type controls. Fourth, we mapped the monocyte signature and found that all Mafb-deficient RTM, except Ly6C+ CM, exhibited a significantly higher monocyte score as compared to wild-type RTM. These findings collectively support a profound disruption in global mac and RTM subset-specific identities in the absence of MafB in vivo, as well as a monocyte signature suggestive of immaturity and incomplete differentiation.

We identified MafB target genes by CUT&RUN on BMDM and found that the expression of MafB target genes was reduced in all RTM subsets from Lyz2CreMafbfl/fl mice. One binding site of MafB was situated in the Csf1r FIRE enhancer. Csf1r expression was reduced in SPM, KC and IM from Lyz2CreMafbfl/fl mice.

Load packages

```
suppressMessages({
    library(Seurat)
    library(SeuratObject)
    library(limma)
    library(ggplot2)
    library(RColorBrewer)
    library(dplyr)
    library(readxl)
})
```

Load Seurat object

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

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

DimPlot(sc, cols = pal)
```
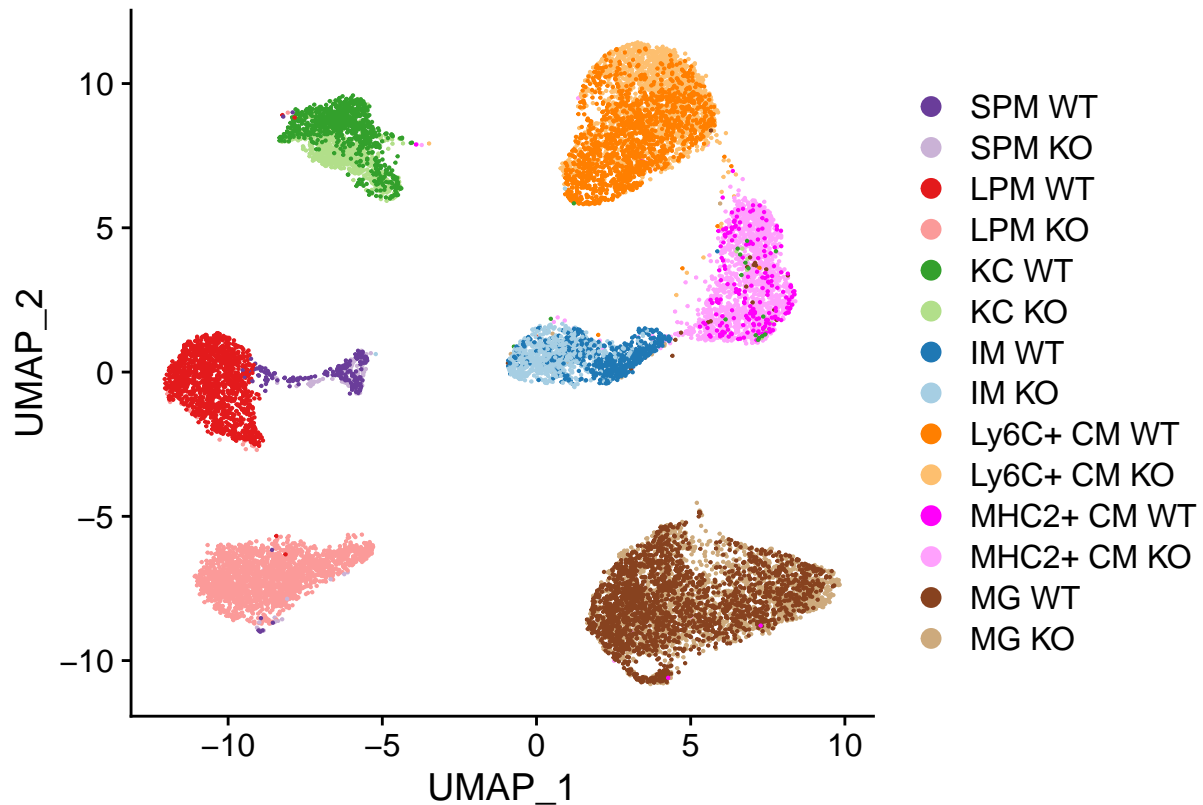
Signature scoring
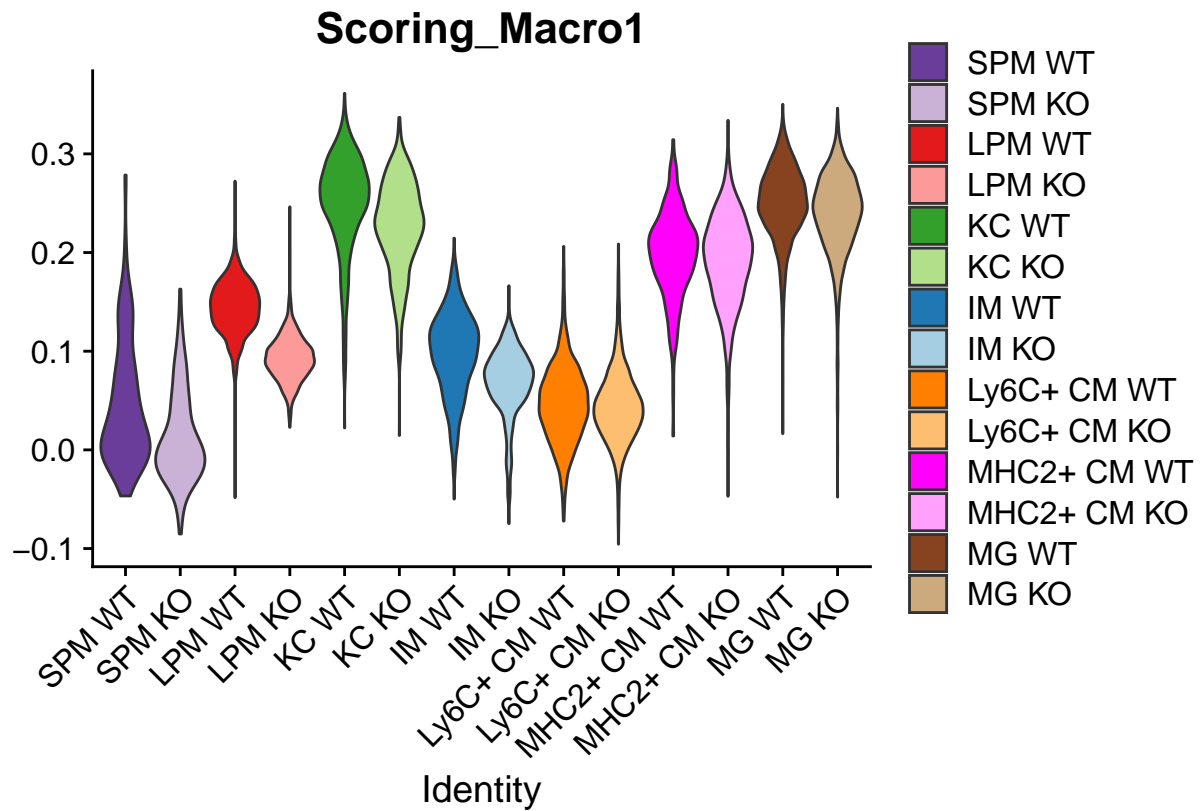
Macrophage signature score

```
MacvscMo <- read.csv("Mac_sign.csv", header = T)

Mac.genes <- list(MacvscMo$Gene_Symbol)

sc <- AddModuleScore(sc, features = Mac.genes, name = "Scoring_Macro")
```

```
## Warning: The following features are not present in the object: Gm10134, Rny3,
## Snord49b, Pvt1, Rny1, Snord61, A930039A15Rik, Vaultrc5, Rnu3b1, Snord22,
## 9830001H06Rik, Rnu12, Snord47, F630111L10Rik, Epb41l1, Snora16a, Snord15b,
## Snord16a, AW112010, B930036N10Rik, LOC100504914, Entpd4, Lphn2, Firre,
## Snord32a, G530011O06Rik, Mir29c, Snord118, Snord35b, Snord35a, Gm6762, Snord3a,
## 39692, Snord116, Snord55, Snord73b, 3222401L13Rik, Vstm2a, Snord104, Gas5,
## Lipo1, Atosa, Stxbp3a, Gm10790, Malat1, Gm12191, C1rb, Snora7a, Ccr1l1, Phxr4,
## Snord49a, Lipf, Mir99ahg, Gm9909, Gm2897, Ppp4r3c1, C4a, not searching for
## symbol synonyms
```

```
VlnPlot(sc, features = "Scoring_Macro1", cols = pal, pt.size = 0)
```

Scoring_Macro1

Legend:
- SPM WT
- SPM KO
- LPM WT
- LPM KO
- KC WT
- KC KO
- IM WT
- IM KO
- Ly6C+ CM WT
- Ly6C+ CM KO
- MHC2+ CM WT
- MHC2+ CM KO
- MG WT
- MG KO

Identity

SPM

```
pal_SPM <- c("#6A3D9A", "#CAB2D6")

VlnPlot(sc, features = "Scoring_Macro1", cols = pal_SPM, pt.size = 0,
    idents = c("SPM WT", "SPM KO")) + ggtitle("SPM") + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank())
```

SPM
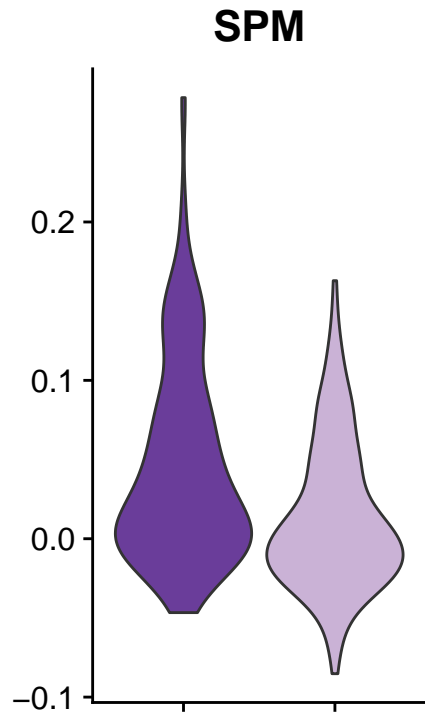

```
metadata <- sc@meta.data
SPM_WT <- metadata[metadata$Condition == "SPM WT", ]
SPM_KO <- metadata[metadata$Condition == "SPM KO", ]

wilcox.test(SPM_WT$Scoring_Macro1, SPM_KO$Scoring_Macro1)  #2.154e-07
```
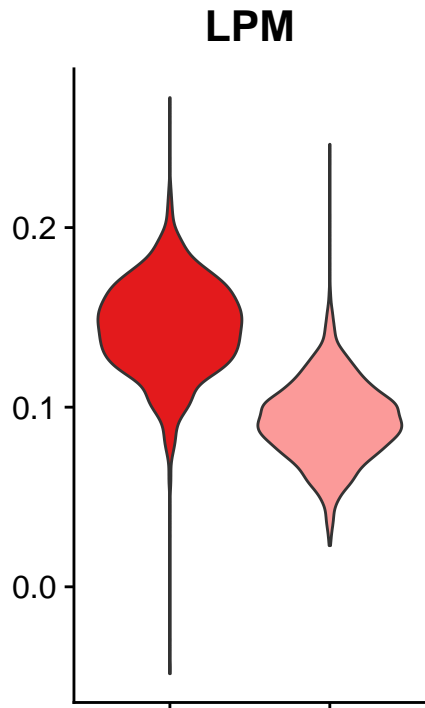
```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  SPM_WT$Scoring_Macro1 and SPM_KO$Scoring_Macro1
## W = 18790, p-value = 2.154e-07
## alternative hypothesis: true location shift is not equal to 0
```

LPM

```
pal_LPM <- c("#e31a1c", "#fb9a99")

VlnPlot(sc, features = "Scoring_Macro1", cols = pal_LPM, pt.size = 0,
    idents = c("LPM WT", "LPM KO")) + ggtitle("LPM") + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank())
```

LPM


```
LPM_WT <- metadata[metadata$Condition == "LPM WT", ]
LPM_KO <- metadata[metadata$Condition == "LPM KO", ]

wilcox.test(LPM_WT$Scoring_Macro1, LPM_KO$Scoring_Macro1)  #< 2.2e-16
```
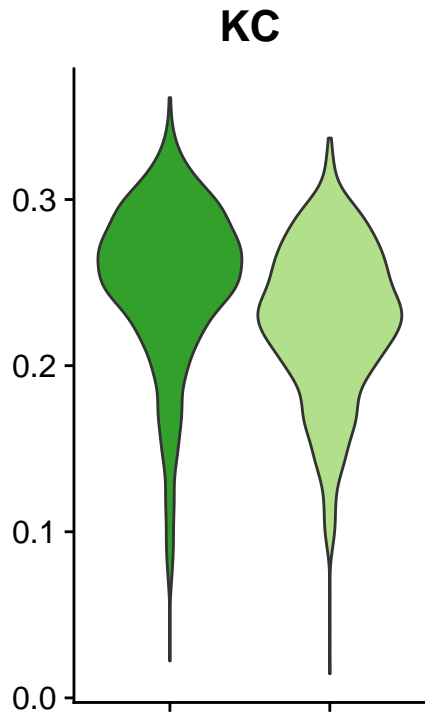
```
## 
##  Wilcoxon rank sum test with continuity correction
## 
## data:  LPM_WT$Scoring_Macro1 and LPM_KO$Scoring_Macro1
## W = 2813333, p-value < 2.2e-16
## alternative hypothesis: true location shift is not equal to 0
```

KC

```
pal_KC <- c("#33a02c", "#b2df8a")

VlnPlot(sc, features = "Scoring_Macro1", cols = pal_KC, pt.size = 0,
    idents = c("KC WT", "KC KO")) + ggtitle("KC") + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank())
```

KC

```
KC_WT <- metadata[metadata$Condition == "KC WT", ]
KC_KO <- metadata[metadata$Condition == "KC KO", ]

wilcox.test(KC_WT$Scoring_Macro1, KC_KO$Scoring_Macro1)  #< 2.2e-16
```
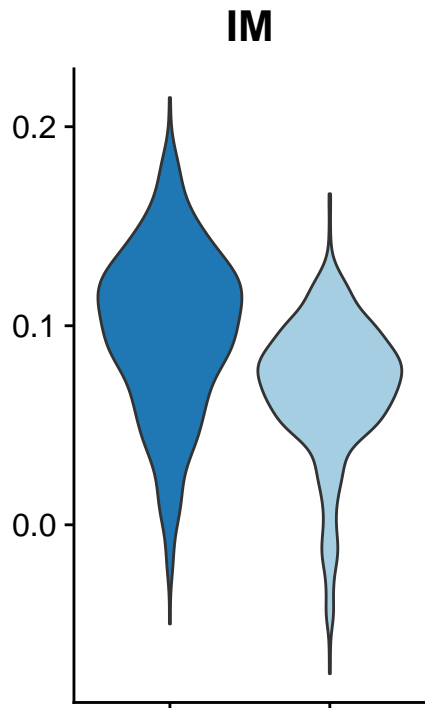
```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  KC_WT$Scoring_Macro1 and KC_KO$Scoring_Macro1
## W = 422529, p-value < 2.2e-16
## alternative hypothesis: true location shift is not equal to 0
```

IM

```
pal_IM <- c("#1f78b4", "#a6cee3")

VlnPlot(sc, features = "Scoring_Macro1", cols = pal_IM, pt.size = 0,
    idents = c("IM WT", "IM KO")) + ggtitle("IM") + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank())
```

IM


```
IM_WT <- metadata[metadata$Condition == "IM WT", ]
IM_KO <- metadata[metadata$Condition == "IM KO", ]

wilcox.test(IM_WT$Scoring_Macro1, IM_KO$Scoring_Macro1)  #< 2.2e-16
```
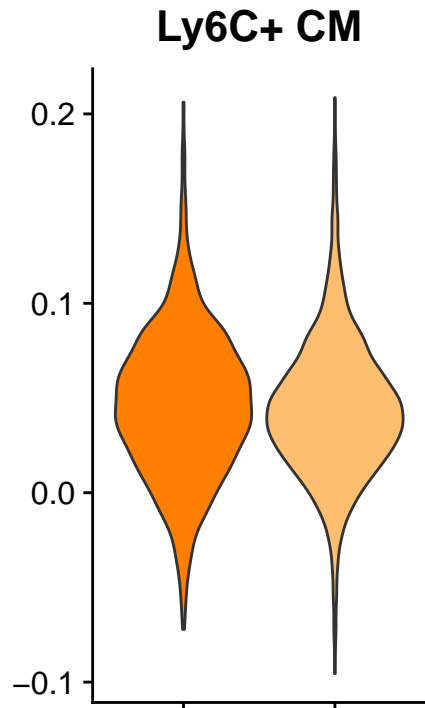
```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  IM_WT$Scoring_Macro1 and IM_KO$Scoring_Macro1
## W = 314330, p-value < 2.2e-16
## alternative hypothesis: true location shift is not equal to 0
```

Ly6C+ CM

```
pal_Ly6C <- c("#FF7F00", "#FDBF6F")

VlnPlot(sc, features = "Scoring_Macro1", cols = pal_Ly6C, pt.size = 0,
    idents = c("Ly6C+ CM WT", "Ly6C+ CM KO")) + ggtitle("Ly6C+ CM") +
    theme(legend.position = "none", axis.title.x = element_blank(),
        axis.text.x = element_blank())
```

Ly6C+ CM


```
Ly6C_WT <- metadata[metadata$Condition == "Ly6C+ CM WT", ]
Ly6C_KO <- metadata[metadata$Condition == "Ly6C+ CM KO", ]

wilcox.test(Ly6C_WT$Scoring_Macro1, Ly6C_KO$Scoring_Macro1)   #0.02762
```
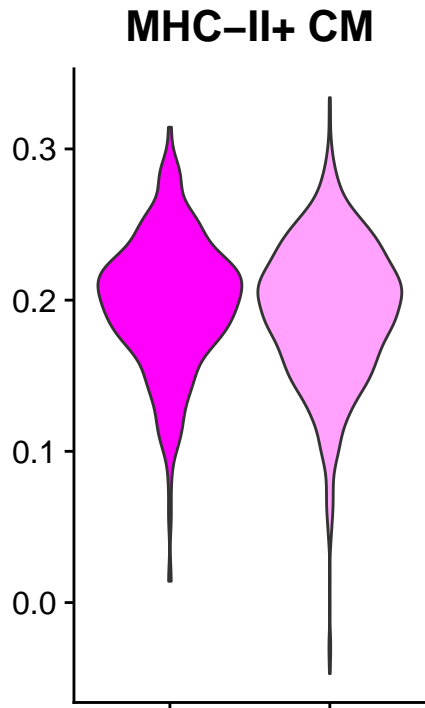
```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  Ly6C_WT$Scoring_Macro1 and Ly6C_KO$Scoring_Macro1
## W = 1321543, p-value = 0.02762
## alternative hypothesis: true location shift is not equal to 0
```

MHC-II+ CM

```
pal_MHC2 <- c("#FF00FA", "#FFA1FD")

VlnPlot(sc, features = "Scoring_Macro1", cols = pal_MHC2, pt.size = 0,
    idents = c("MHC2+ CM WT", "MHC2+ CM KO")) + ggtitle("MHC-II+ CM") +
    theme(legend.position = "none", axis.title.x = element_blank(),
        axis.text.x = element_blank())
```

MHC−II+ CM


```r
MHC2_WT <- metadata[metadata$Condition == "MHC2+ CM WT", ]
MHC2_KO <- metadata[metadata$Condition == "MHC2+ CM KO", ]

wilcox.test(MHC2_WT$Scoring_Macro1, MHC2_KO$Scoring_Macro1)  #0.1286
```
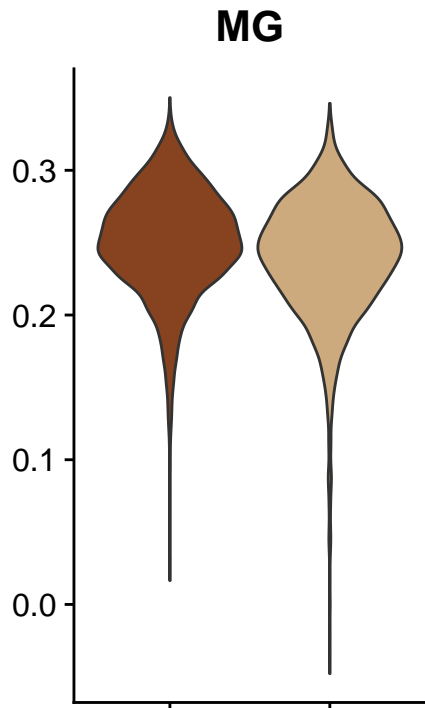
```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  MHC2_WT$Scoring_Macro1 and MHC2_KO$Scoring_Macro1
## W = 155320, p-value = 0.1286
## alternative hypothesis: true location shift is not equal to 0
```

MG

```r
pal_MG <- c("#87421F", "#CDAA7D")

VlnPlot(sc, features = "Scoring_Macro1", cols = pal_MG, pt.size = 0,
    idents = c("MG WT", "MG KO")) + ggtitle("MG") + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank())
```

MG


```
MG_WT <- metadata[metadata$Condition == "MG WT", ]
MG_KO <- metadata[metadata$Condition == "MG KO", ]

wilcox.test(MG_WT$Scoring_Macro1, MG_KO$Scoring_Macro1)  #< 2.2e-16
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  MG_WT$Scoring_Macro1 and MG_KO$Scoring_Macro1
## W = 2359869, p-value < 2.2e-16
## alternative hypothesis: true location shift is not equal to 0
```

RTM subset specific signature score

```
wt <- subset(sc, idents = c("SPM WT", "LPM WT", "KC WT", "IM WT",
    "Ly6C+ CM WT", "MHC2+ CM WT", "MG WT"))

markers <- FindAllMarkers(wt, only.pos = TRUE)
```

```
## Calculating cluster SPM WT

## Calculating cluster LPM WT

## Calculating cluster KC WT

## Calculating cluster IM WT
```

```
## Calculating cluster Ly6C+ CM WT

## Calculating cluster MHC2+ CM WT

## Calculating cluster MG WT
```

```
write.csv(markers, file = "markers.csv")
```

```
markers <- read.csv("markers.csv", header = T, row.names = 1)
```

SPM

```
SPM.markers <- filter(markers, cluster == "SPM WT")
SPM.markers <- SPM.markers[SPM.markers$p_val_adj < 0.05 & abs(SPM.markers$avg_log2FC) >
    0.25, ]
SPM.markers <- SPM.markers[order(SPM.markers$avg_log2FC, decreasing = TRUE),
    ]
SPM.markers.top100 <- head(SPM.markers$gene, 100)

write.table(SPM.markers.top100, file = "SPM_sign.txt")

SPM.markers.top100 <- list(SPM.markers.top100)


sc <- AddModuleScore(sc, features = SPM.markers.top100, name = "SPM_sign")
```
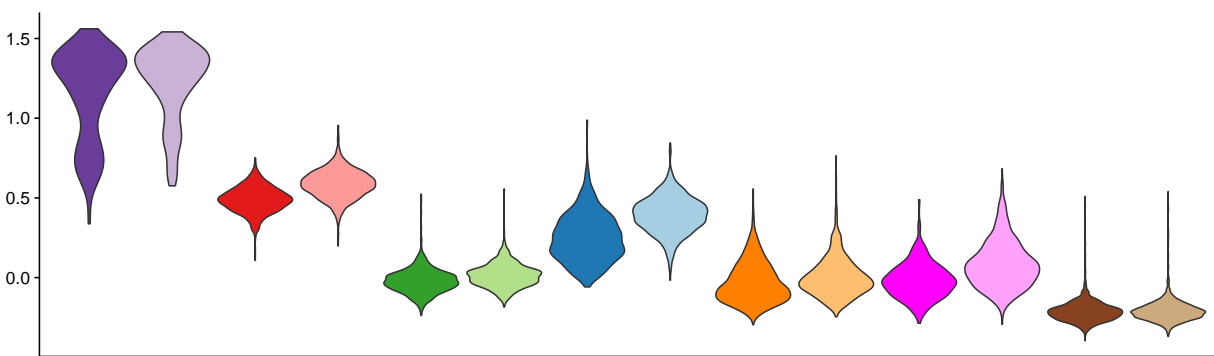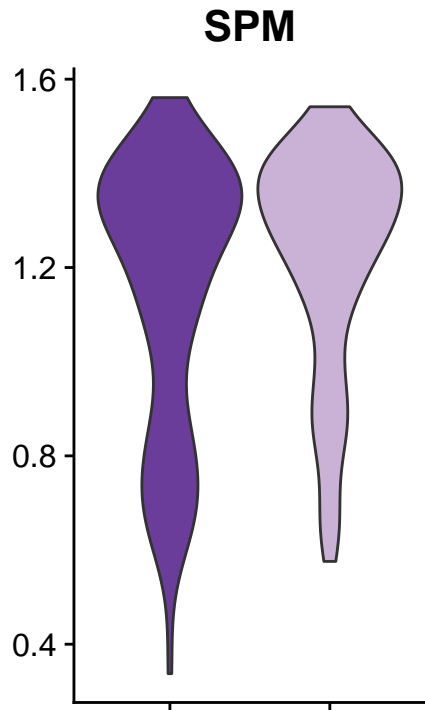
```
VlnPlot(sc, features = "SPM_sign1", cols = pal, pt.size = 0) +
    theme(legend.position = "none", axis.title.x = element_blank(),
        axis.text.x = element_blank()) + ggtitle("")
```



```
VlnPlot(sc, features = "SPM_sign1", cols = pal_SPM, pt.size = 0,
    idents = c("SPM WT", "SPM KO")) + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank()) +
    ggtitle("SPM")
```

SPM


```
metadata <- sc@meta.data
SPM_WT <- metadata[metadata$Condition == "SPM WT", ]
SPM_KO <- metadata[metadata$Condition == "SPM KO", ]

wilcox.test(SPM_WT$SPM_sign1, SPM_KO$SPM_sign1)  #0.03641
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  SPM_WT$SPM_sign1 and SPM_KO$SPM_sign1
## W = 12281, p-value = 0.03641
## alternative hypothesis: true location shift is not equal to 0
```

LPM

```
LPM.markers <- filter(markers, cluster == "LPM WT")
LPM.markers <- LPM.markers[LPM.markers$p_val_adj < 0.05 & abs(LPM.markers$avg_log2FC) >
    0.25, ]
LPM.markers <- LPM.markers[order(LPM.markers$avg_log2FC, decreasing = TRUE),
    ]
LPM.markers.top100 <- head(LPM.markers$gene, 100)

write.table(LPM.markers.top100, file = "LPM_sign.txt")

LPM.markers.top100 <- list(LPM.markers.top100)
```
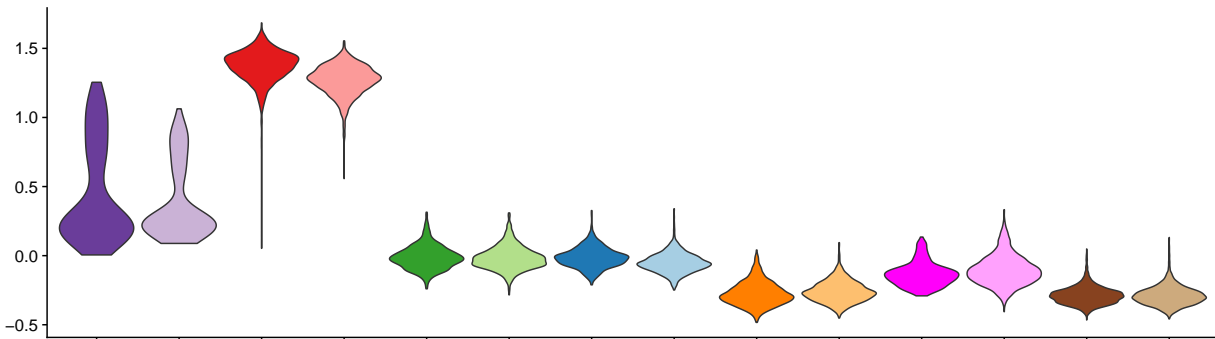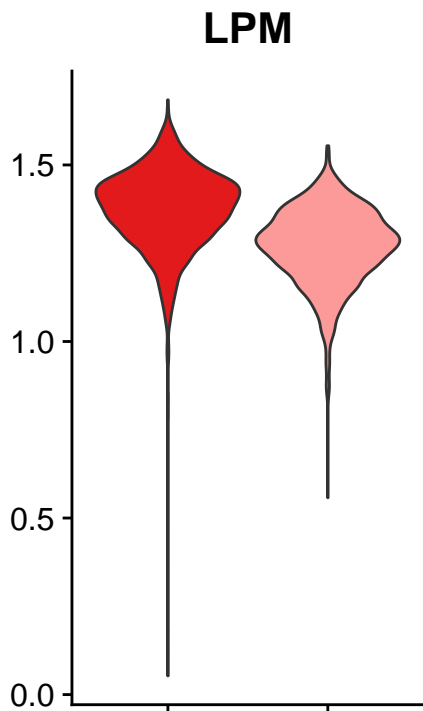
```
sc <- AddModuleScore(sc, features = LPM.markers.top100, name = "LPM_sign")
```

```
VlnPlot(sc, features = "LPM_sign1", cols = pal, pt.size = 0) +
    theme(legend.position = "none", axis.title.x = element_blank(),
        axis.text.x = element_blank()) + ggtitle("")
```



```
VlnPlot(sc, features = "LPM_sign1", cols = pal_LPM, pt.size = 0,
    idents = c("LPM WT", "LPM KO")) + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank()) +
    ggtitle("LPM")
```



```
metadata <- sc@meta.data
LPM_WT <- metadata[metadata$Condition == "LPM WT", ]
```

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```
LPM_KO <- metadata[metadata$Condition == "LPM KO", ]

wilcox.test(LPM_WT$LPM_sign1, LPM_KO$LPM_sign1)   #< 2.2e-16
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  LPM_WT$LPM_sign1 and LPM_KO$LPM_sign1
## W = 2301210, p-value < 2.2e-16
## alternative hypothesis: true location shift is not equal to 0
```

KC

```
KC.markers <- filter(markers, cluster == "KC WT")
KC.markers <- KC.markers[KC.markers$p_val_adj < 0.05 & abs(KC.markers$avg_log2FC) >
    0.25, ]
KC.markers <- KC.markers[order(KC.markers$avg_log2FC, decreasing = TRUE),
    ]
KC.markers.top100 <- head(KC.markers$gene, 100)

write.table(KC.markers.top100, file = "KC_sign.txt")

KC.markers.top100 <- list(KC.markers.top100)


sc <- AddModuleScore(sc, features = KC.markers.top100, name = "KC_sign")
```
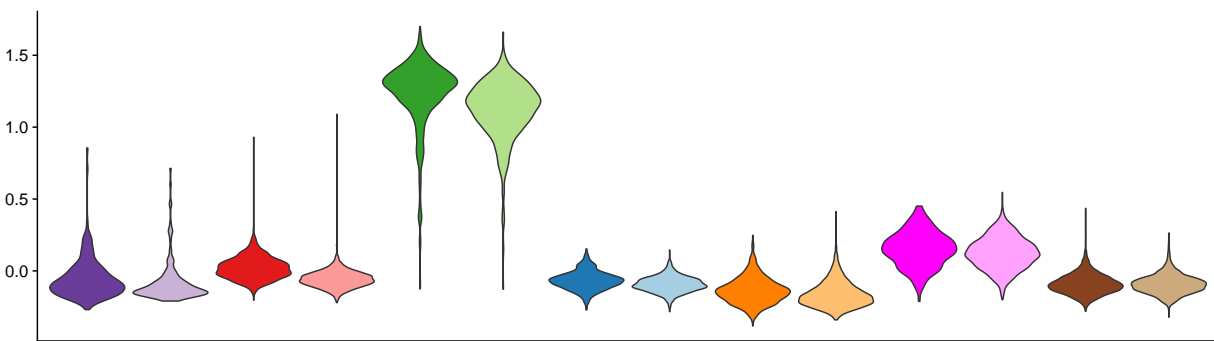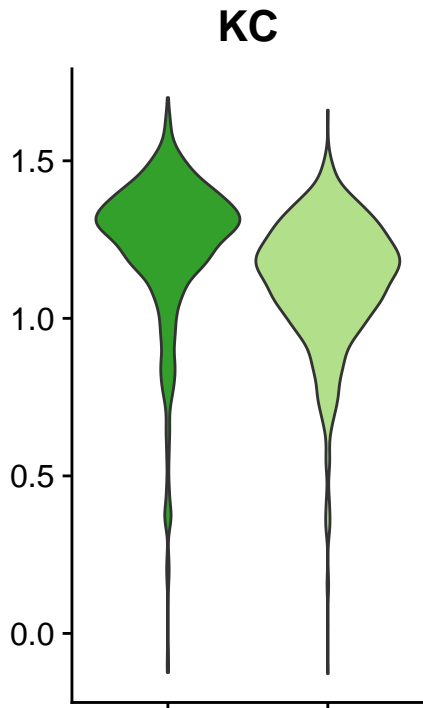
```
VlnPlot(sc, features = "KC_sign1", cols = pal, pt.size = 0) +
    theme(legend.position = "none", axis.title.x = element_blank(),
        axis.text.x = element_blank()) + ggtitle("")
```



```
VlnPlot(sc, features = "KC_sign1", cols = pal_KC, pt.size = 0,
    idents = c("KC WT", "KC KO")) + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank()) +
    ggtitle("KC")
```

KC


```
metadata <- sc@meta.data
KC_WT <- metadata[metadata$Condition == "KC WT", ]
KC_KO <- metadata[metadata$Condition == "KC KO", ]

wilcox.test(KC_WT$KC_sign1, KC_KO$KC_sign1)  #< 2.2e-16
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  KC_WT$KC_sign1 and KC_KO$KC_sign1
## W = 451181, p-value < 2.2e-16
## alternative hypothesis: true location shift is not equal to 0
```

IM

```
IM.markers <- filter(markers, cluster == "IM WT")
IM.markers <- IM.markers[IM.markers$p_val_adj < 0.05 & abs(IM.markers$avg_log2FC) >
    0.25, ]
IM.markers <- IM.markers[order(IM.markers$avg_log2FC, decreasing = TRUE),
    ]
IM.markers.top100 <- head(IM.markers$gene, 100)

write.table(IM.markers.top100, file = "IM_sign.txt")

IM.markers.top100 <- list(IM.markers.top100)
```
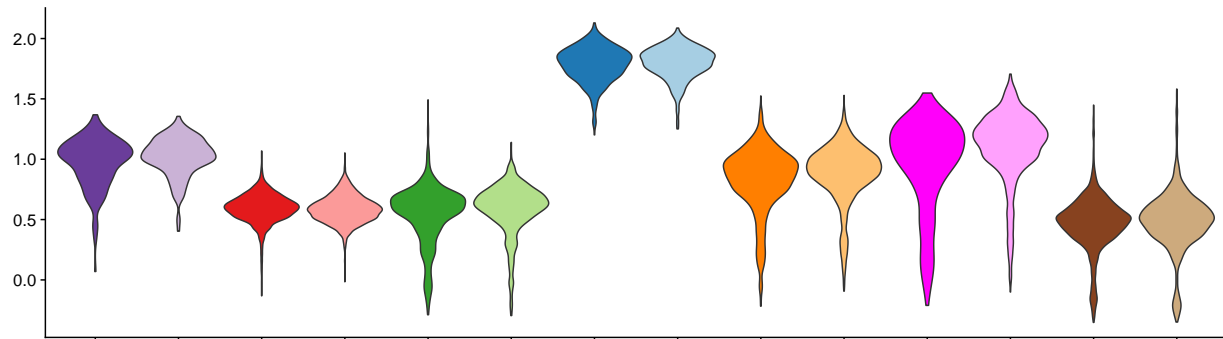
```
sc <- AddModuleScore(sc, features = IM.markers.top100, name = "IM_sign")
```

```
VlnPlot(sc, features = "IM_sign1", cols = pal, pt.size = 0) +
    theme(legend.position = "none", axis.title.x = element_blank(),
        axis.text.x = element_blank()) + ggtitle("")
```



```
VlnPlot(sc, features = "IM_sign1", cols = pal_IM, pt.size = 0,
    idents = c("IM WT", "IM KO")) + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank()) +
    ggtitle("IM")
```

IM


```
metadata <- sc@meta.data
IM_WT <- metadata[metadata$Condition == "IM WT", ]
```
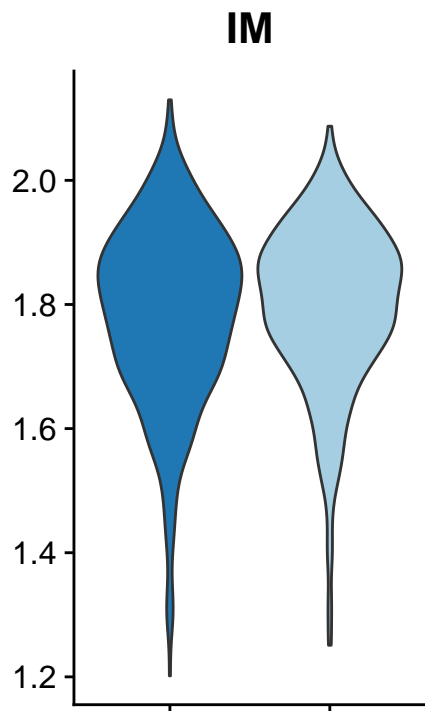
```
IM_KO <- metadata[metadata$Condition == "IM KO", ]

wilcox.test(IM_WT$IM_sign1, IM_KO$IM_sign1)   #0.1182
```

```
##
##   Wilcoxon rank sum test with continuity correction
##
## data:  IM_WT$IM_sign1 and IM_KO$IM_sign1
## W = 208587, p-value = 0.1182
## alternative hypothesis: true location shift is not equal to 0
```

Ly6C+ CM

```
Ly6C.markers <- filter(markers, cluster == "Ly6C+ CM WT")
Ly6C.markers <- Ly6C.markers[Ly6C.markers$p_val_adj < 0.05 &
    abs(Ly6C.markers$avg_log2FC) > 0.25, ]
Ly6C.markers <- Ly6C.markers[order(Ly6C.markers$avg_log2FC, decreasing = TRUE),
    ]
Ly6C.markers.top100 <- head(Ly6C.markers$gene, 100)

write.table(Ly6C.markers.top100, file = "Ly6C_sign.txt")

Ly6C.markers.top100 <- list(Ly6C.markers.top100)


sc <- AddModuleScore(sc, features = Ly6C.markers.top100, name = "Ly6C_sign")
```
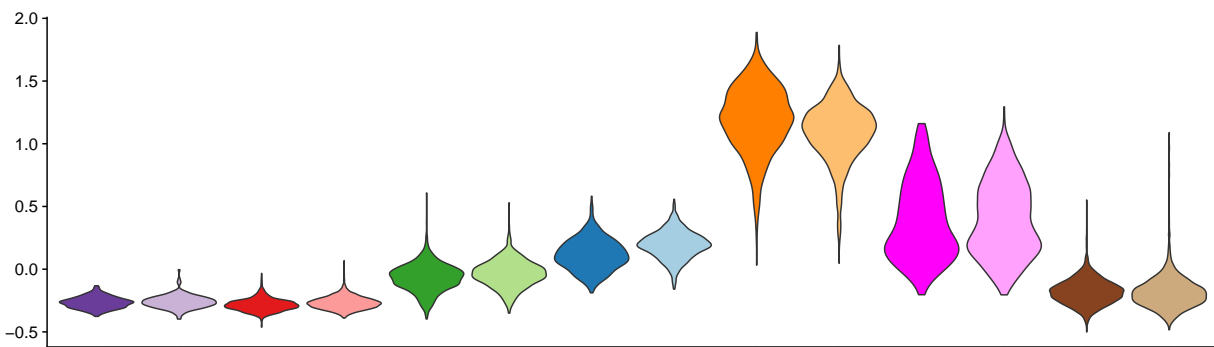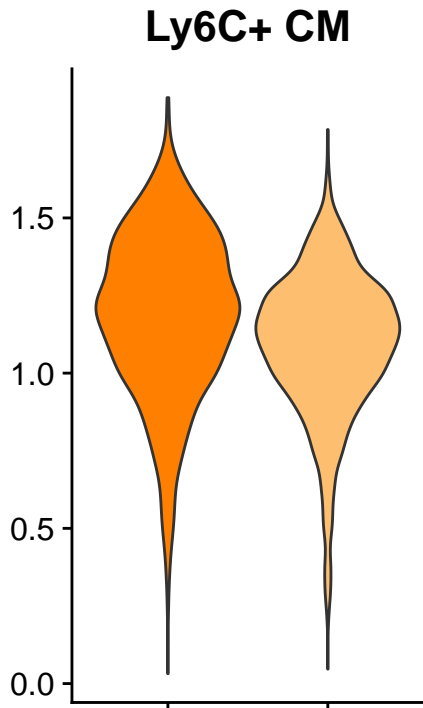
```
VlnPlot(sc, features = "Ly6C_sign1", cols = pal, pt.size = 0) +
    theme(legend.position = "none", axis.title.x = element_blank(),
        axis.text.x = element_blank()) + ggtitle("")
```



```
VlnPlot(sc, features = "Ly6C_sign1", cols = pal_Ly6C, pt.size = 0,
    idents = c("Ly6C+ CM WT", "Ly6C+ CM KO")) + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank()) +
    ggtitle("Ly6C+ CM")
```

Ly6C+ CM


```r
metadata <- sc@meta.data
Ly6C_CM_WT <- metadata[metadata$Condition == "Ly6C+ CM WT", ]
Ly6C_CM_KO <- metadata[metadata$Condition == "Ly6C+ CM KO", ]

wilcox.test(Ly6C_CM_WT$Ly6C_sign1, Ly6C_CM_KO$Ly6C_sign1)  #< 2.2e-16
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  Ly6C_CM_WT$Ly6C_sign1 and Ly6C_CM_KO$Ly6C_sign1
## W = 1556987, p-value < 2.2e-16
## alternative hypothesis: true location shift is not equal to 0
```

MHC-II+ CM

```r
MHC2.markers <- filter(markers, cluster == "MHC2+ CM WT")
MHC2.markers <- MHC2.markers[MHC2.markers$p_val_adj < 0.05 &
    abs(MHC2.markers$avg_log2FC) > 0.25, ]
MHC2.markers <- MHC2.markers[order(MHC2.markers$avg_log2FC, decreasing = TRUE),
    ]
MHC2.markers.top100 <- head(MHC2.markers$gene, 100)

write.table(MHC2.markers.top100, file = "MHC2_sign.txt")

MHC2.markers.top100 <- list(MHC2.markers.top100)
```
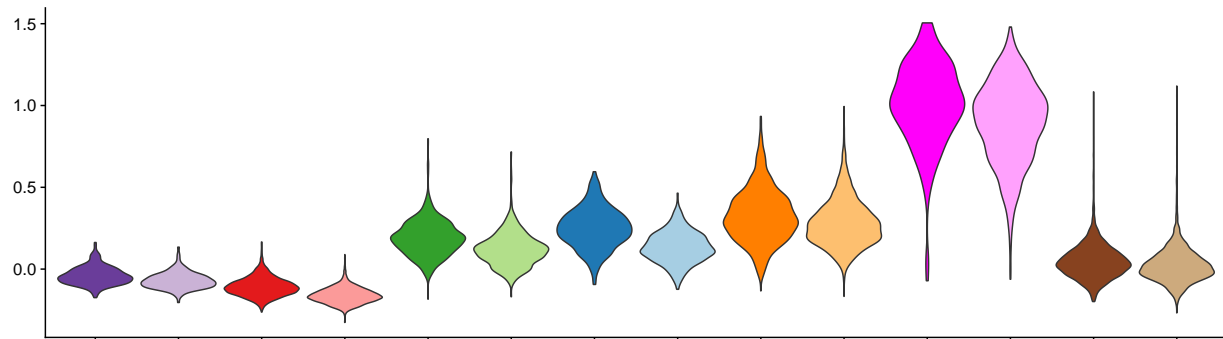
```
sc <- AddModuleScore(sc, features = MHC2.markers.top100, name = "MHC2_sign")
```

```
VlnPlot(sc, features = "MHC2_sign1", cols = pal, pt.size = 0) +
    theme(legend.position = "none", axis.title.x = element_blank(),
        axis.text.x = element_blank()) + ggtitle("")
```



```
VlnPlot(sc, features = "MHC2_sign1", cols = pal_MHC2, pt.size = 0,
    idents = c("MHC2+ CM WT", "MHC2+ CM KO")) + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank()) +
    ggtitle("MHC-II+ CM")
```
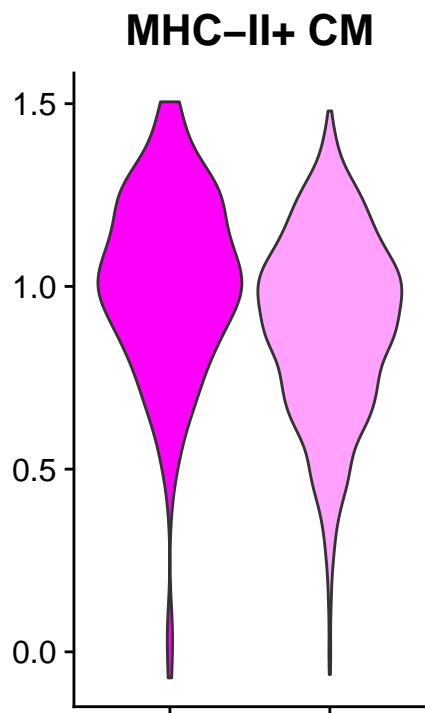


```
metadata <- sc@meta.data
MHC2_CM_WT <- metadata[metadata$Condition == "MHC2+ CM WT", ]
```

```
MHC2_CM_KO <- metadata[metadata$Condition == "MHC2+ CM KO", ]

wilcox.test(MHC2_CM_WT$MHC2_sign1, MHC2_CM_KO$MHC2_sign1)   #2.388e-09
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  MHC2_CM_WT$MHC2_sign1 and MHC2_CM_KO$MHC2_sign1
## W = 183967, p-value = 2.388e-09
## alternative hypothesis: true location shift is not equal to 0
```

MG

```
MG.markers <- filter(markers, cluster == "MG WT")
MG.markers <- MG.markers[MG.markers$p_val_adj < 0.05 & abs(MG.markers$avg_log2FC) >
    0.25, ]
MG.markers <- MG.markers[order(MG.markers$avg_log2FC, decreasing = TRUE),
    ]
MG.markers.top100 <- head(MG.markers$gene, 100)

write.table(MG.markers.top100, file = "MG_sign.txt")

MG.markers.top100 <- list(MG.markers.top100)


sc <- AddModuleScore(sc, features = MG.markers.top100, name = "MG_sign")
```
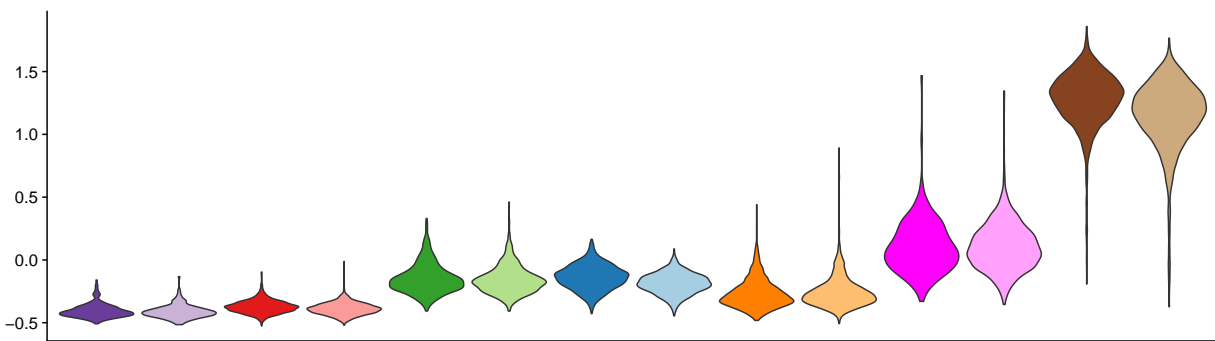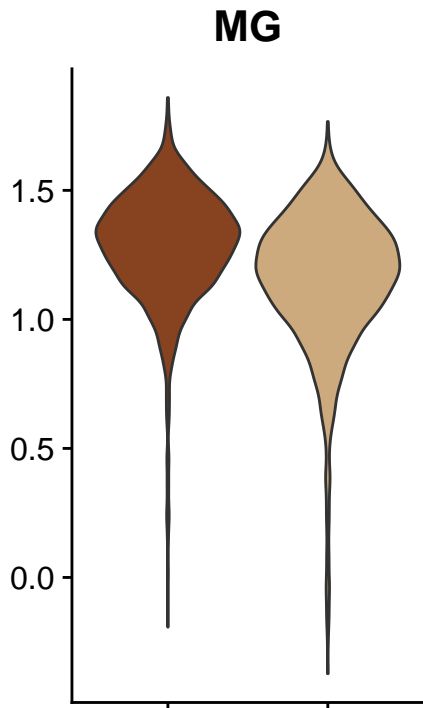
```
VlnPlot(sc, features = "MG_sign1", cols = pal, pt.size = 0) +
    theme(legend.position = "none", axis.title.x = element_blank(),
        axis.text.x = element_blank()) + ggtitle("")
```



```
VlnPlot(sc, features = "MG_sign1", cols = pal_MG, pt.size = 0,
    idents = c("MG WT", "MG KO")) + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank()) +
    ggtitle("MG")
```

MG


```
metadata <- sc@meta.data
MG_WT <- metadata[metadata$Condition == "MG WT", ]
MG_KO <- metadata[metadata$Condition == "MG KO", ]

wilcox.test(MG_WT$MG_sign1, MG_KO$MG_sign1)   #< 2.2e-16
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  MG_WT$MG_sign1 and MG_KO$MG_sign1
## W = 2688279, p-value < 2.2e-16
## alternative hypothesis: true location shift is not equal to 0
```

Monocyte signature score

```
cMovsMac <- read.csv("Mo_sign.csv", header = T)

cMo.genes <- list(cMovsMac$Gene_Symbol)

sc <- AddModuleScore(sc, features = cMo.genes, name = "Scoring_Mono")
```

```
## Warning: The following features are not present in the object: Ly6a2,
## Serpinb10, E430024C06Rik, LOC625360, Hbb-b2, Hbb-b1, Mx1, Pira1, Morrbid, Mx2,
## Dleu2, Pira11, Mirt1, Mir223hg, Gm12250, 9330175E14Rik, Aim1, Oas1b, G6pd2,
## AI607873, 38961, Ugt1a9, 4930562F07Rik, Trex1, Prss34, Phospho1, Gm10759,
## 9430025C20Rik, Gvin3, 4930518I15Rik, Gm10099, Gas5, Mir15a, H2-T10,
```

```
## 2610524H06Rik, 4930426L09Rik, Mir17hg, Pgam2, Phf11, Gm10551, not searching for
## symbol synonyms
```
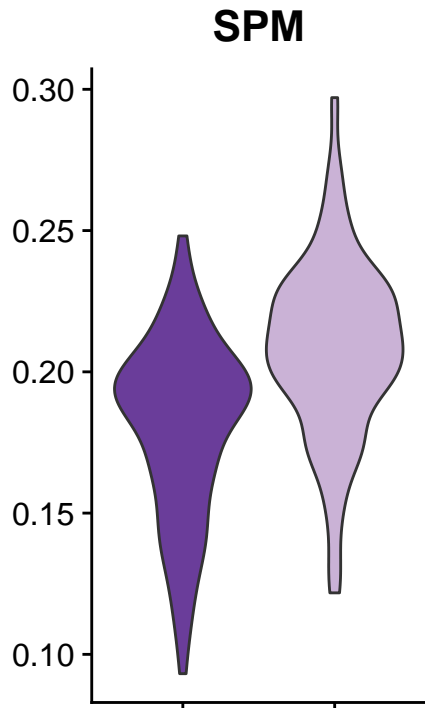
```
VlnPlot(sc, features = "Scoring_Mono1", cols = pal, pt.size = 0)
```


SPM
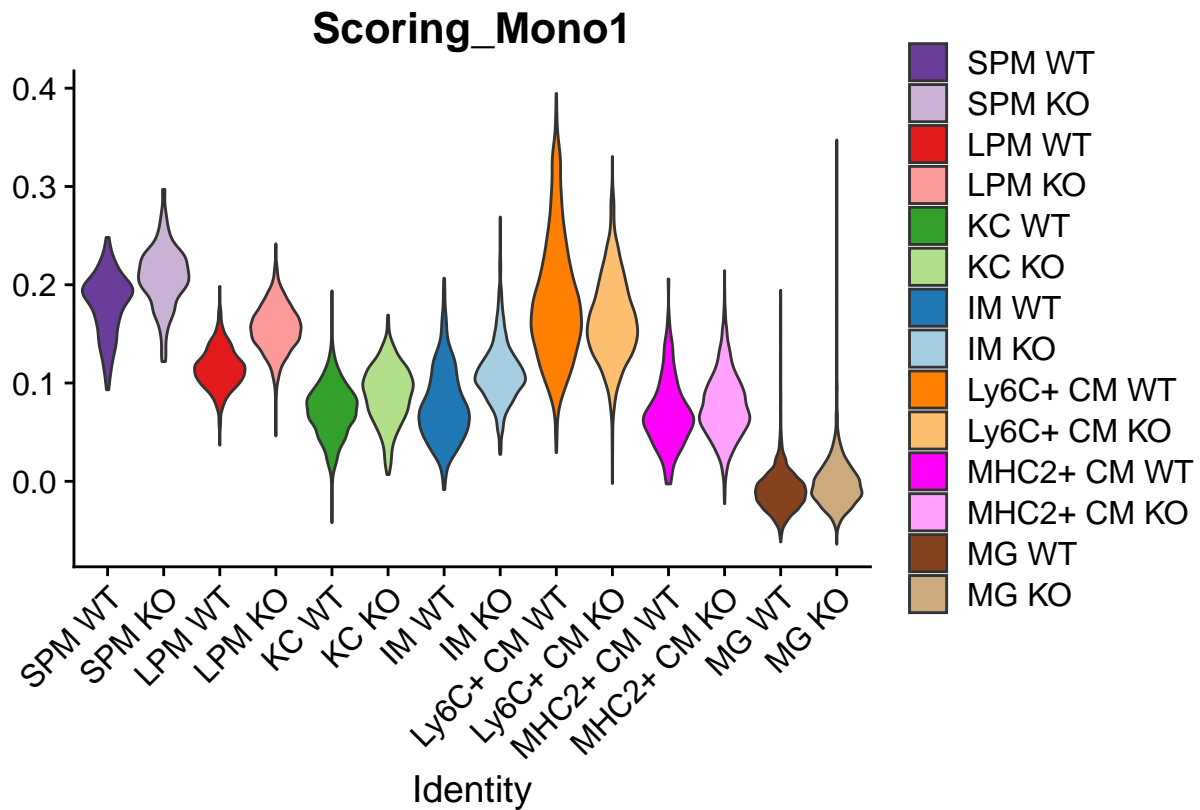
```
pal_SPM <- c("#6A3D9A", "#CAB2D6")

VlnPlot(sc, features = "Scoring_Mono1", cols = pal_SPM, pt.size = 0,
    idents = c("SPM WT", "SPM KO")) + ggtitle("SPM") + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank())
```

SPM


```
metadata <- sc@meta.data
SPM_WT <- metadata[metadata$Condition == "SPM WT", ]
SPM_KO <- metadata[metadata$Condition == "SPM KO", ]

wilcox.test(SPM_WT$Scoring_Mono1, SPM_KO$Scoring_Mono1)  #1.307e-14


##
##  Wilcoxon rank sum test with continuity correction
##
## data:  SPM_WT$Scoring_Mono1 and SPM_KO$Scoring_Mono1
## W = 7262, p-value = 1.307e-14
## alternative hypothesis: true location shift is not equal to 0
```
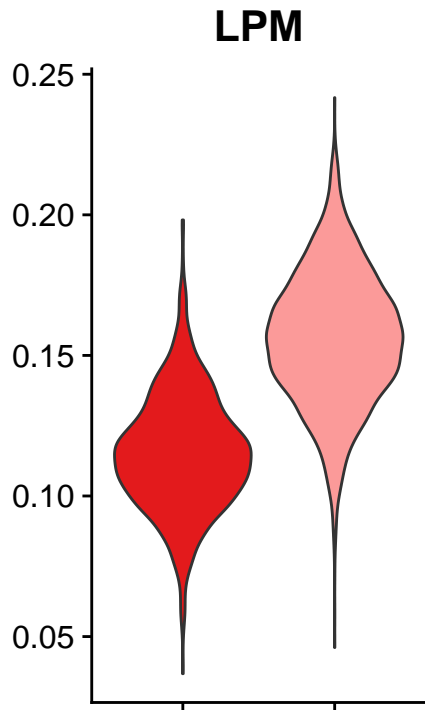
LPM

```
pal_LPM <- c("#e31a1c", "#fb9a99")

VlnPlot(sc, features = "Scoring_Mono1", cols = pal_LPM, pt.size = 0,
    idents = c("LPM WT", "LPM KO")) + ggtitle("LPM") + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank())
```

LPM


```
LPM_WT <- metadata[metadata$Condition == "LPM WT", ]
LPM_KO <- metadata[metadata$Condition == "LPM KO", ]

wilcox.test(LPM_WT$Scoring_Mono1, LPM_KO$Scoring_Mono1)  #< 2.2e-16
```
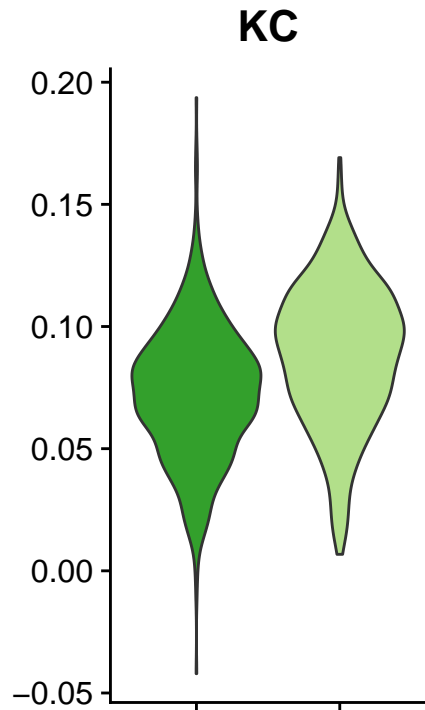
```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  LPM_WT$Scoring_Mono1 and LPM_KO$Scoring_Mono1
## W = 292555, p-value < 2.2e-16
## alternative hypothesis: true location shift is not equal to 0
```

KC

```
pal_KC <- c("#33a02c", "#b2df8a")

VlnPlot(sc, features = "Scoring_Mono1", cols = pal_KC, pt.size = 0,
    idents = c("KC WT", "KC KO")) + ggtitle("KC") + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank())
```

KC


```
KC_WT <- metadata[metadata$Condition == "KC WT", ]
KC_KO <- metadata[metadata$Condition == "KC KO", ]

wilcox.test(KC_WT$Scoring_Mono1, KC_KO$Scoring_Mono1)  #< 2.2e-16
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  KC_WT$Scoring_Mono1 and KC_KO$Scoring_Mono1
## W = 213858, p-value < 2.2e-16
## alternative hypothesis: true location shift is not equal to 0
```

IM

```
pal_IM <- c("#1f78b4", "#a6cee3")

VlnPlot(sc, features = "Scoring_Mono1", cols = pal_IM, pt.size = 0,
    idents = c("IM WT", "IM KO")) + ggtitle("IM") + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank())
```

IM


```r
IM_WT <- metadata[metadata$Condition == "IM WT", ]
IM_KO <- metadata[metadata$Condition == "IM KO", ]

wilcox.test(IM_WT$Scoring_Mono1, IM_KO$Scoring_Mono1)  #< 2.2e-16
```
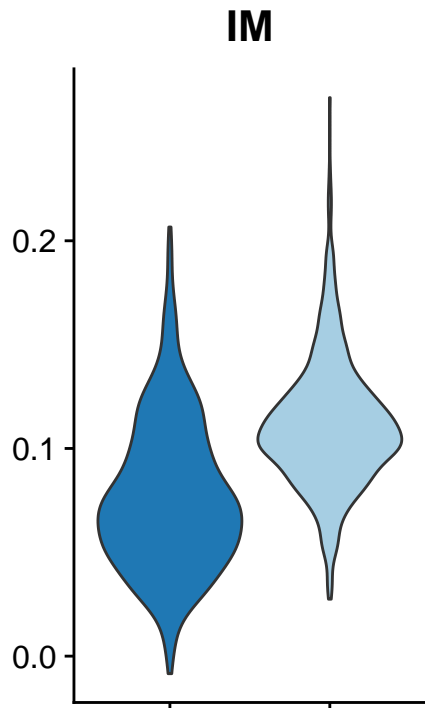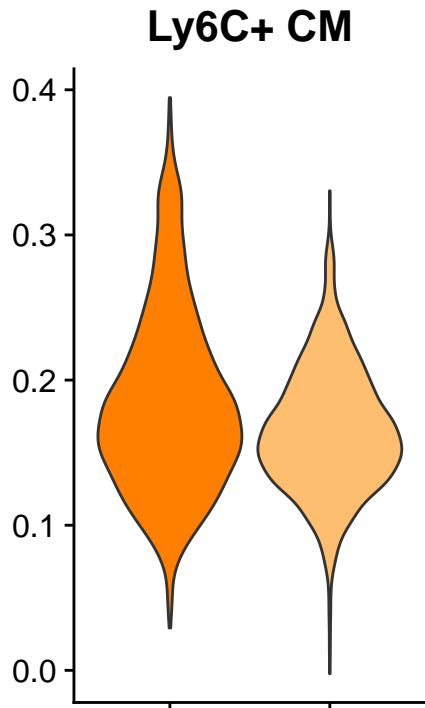
```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  IM_WT$Scoring_Mono1 and IM_KO$Scoring_Mono1
## W = 98864, p-value < 2.2e-16
## alternative hypothesis: true location shift is not equal to 0
```

Ly6C+ CM

```r
pal_Ly6C <- c("#FF7F00", "#FDBF6F")

VlnPlot(sc, features = "Scoring_Mono1", cols = pal_Ly6C, pt.size = 0,
    idents = c("Ly6C+ CM WT", "Ly6C+ CM KO")) + ggtitle("Ly6C+ CM") +
    theme(legend.position = "none", axis.title.x = element_blank(),
        axis.text.x = element_blank())
```

Ly6C+ CM


```
Ly6C_WT <- metadata[metadata$Condition == "Ly6C+ CM WT", ]
Ly6C_KO <- metadata[metadata$Condition == "Ly6C+ CM KO", ]

wilcox.test(Ly6C_WT$Scoring_Mono1, Ly6C_KO$Scoring_Mono1)   #1.205e-14
```
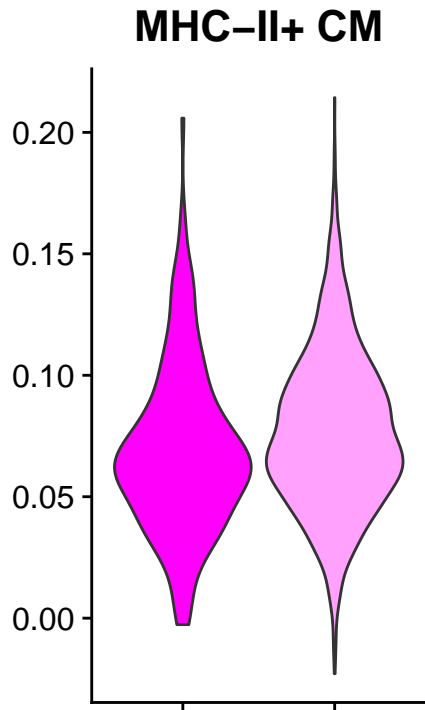
```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  Ly6C_WT$Scoring_Mono1 and Ly6C_KO$Scoring_Mono1
## W = 1464721, p-value = 1.205e-14
## alternative hypothesis: true location shift is not equal to 0
```

MHC-II+ CM

```
pal_MHC2 <- c("#FF00FA", "#FFA1FD")

VlnPlot(sc, features = "Scoring_Mono1", cols = pal_MHC2, pt.size = 0,
    idents = c("MHC2+ CM WT", "MHC2+ CM KO")) + ggtitle("MHC-II+ CM") +
    theme(legend.position = "none", axis.title.x = element_blank(),
        axis.text.x = element_blank())
```

MHC−II+ CM


```
MHC2_WT <- metadata[metadata$Condition == "MHC2+ CM WT", ]
MHC2_KO <- metadata[metadata$Condition == "MHC2+ CM KO", ]

wilcox.test(MHC2_WT$Scoring_Mono1, MHC2_KO$Scoring_Mono1)  #0.0005466
```
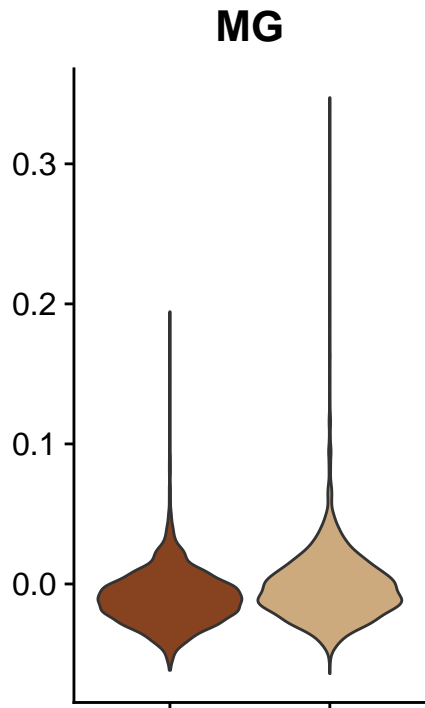
```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  MHC2_WT$Scoring_Mono1 and MHC2_KO$Scoring_Mono1
## W = 123279, p-value = 0.0005466
## alternative hypothesis: true location shift is not equal to 0
```

MG

```
pal_MG <- c("#87421F", "#CDAA7D")

VlnPlot(sc, features = "Scoring_Mono1", cols = pal_MG, pt.size = 0,
    idents = c("MG WT", "MG KO")) + ggtitle("MG") + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank())
```

MG


```r
MG_WT <- metadata[metadata$Condition == "MG WT", ]
MG_KO <- metadata[metadata$Condition == "MG KO", ]

wilcox.test(MG_WT$Scoring_Mono1, MG_KO$Scoring_Mono1)  #< 2.2e-16
```

```
## 
##  Wilcoxon rank sum test with continuity correction
## 
## data:  MG_WT$Scoring_Mono1 and MG_KO$Scoring_Mono1
## W = 1634349, p-value < 2.2e-16
## alternative hypothesis: true location shift is not equal to 0
```

Embrionically dervived mac and Monocyte dervived mac signature score

KC

```r
Microarray_KC_EMvsMo <- read_excel("Microarray_KC_EMvsMo.xlsx")
Microarray_KC_EMvsMo <- na.omit(Microarray_KC_EMvsMo)
Microarray_KC_EMvsMo <- Microarray_KC_EMvsMo[Microarray_KC_EMvsMo$adj.P.Val <
    0.05 & abs(Microarray_KC_EMvsMo$logFC) > 1, ]

write.csv(Microarray_KC_EMvsMo, file = "Microarray_KC_EMvsMo.csv")

EM_KC_sign <- Microarray_KC_EMvsMo[Microarray_KC_EMvsMo$logFC >
    1, ]$Gene.symbol
```

```
EM_KC_sign <- list(EM_KC_sign)

sc <- AddModuleScore(sc, features = EM_KC_sign, name = "EM_KC_sign")
```

Warning: The following features are not present in the object: Snurf///Snrpn,
## not searching for symbol synonyms

```
Mo_KC_sign <- Microarray_KC_EMvsMo[Microarray_KC_EMvsMo$logFC <
    -1, ]$Gene.symbol

Mo_KC_sign <- list(Mo_KC_sign)

sc <- AddModuleScore(sc, features = Mo_KC_sign, name = "Mo_KC_sign")
```

Warning: The following features are not present in the object:
LOC100038947///Sirpb1b///Sirpb1a, Ccdc109b, not searching for symbol synonyms

```
VlnPlot(sc, features = "EM_KC_sign1", cols = pal_KC, pt.size = 0,
    idents = c("KC WT", "KC KO")) + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank()) +
    ggtitle("KC")
```
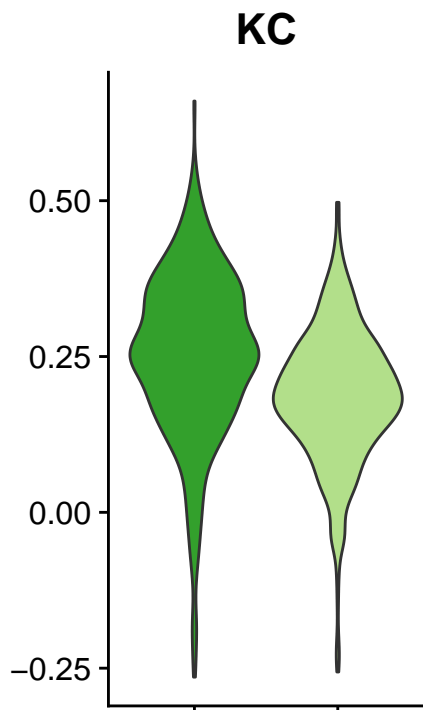


```
metadata <- sc@meta.data
KC_WT <- metadata[metadata$Condition == "KC WT", ]
KC_KO <- metadata[metadata$Condition == "KC KO", ]

wilcox.test(KC_WT$EM_KC_sign1, KC_KO$EM_KC_sign1)  #< 2.2e-16
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  KC_WT$EM_KC_sign1 and KC_KO$EM_KC_sign1
## W = 427683, p-value < 2.2e-16
## alternative hypothesis: true location shift is not equal to 0
```

```
VlnPlot(sc, features = "Mo_KC_sign1", cols = pal_KC, pt.size = 0,
    idents = c("KC WT", "KC KO")) + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank()) +
    ggtitle("KC")
```
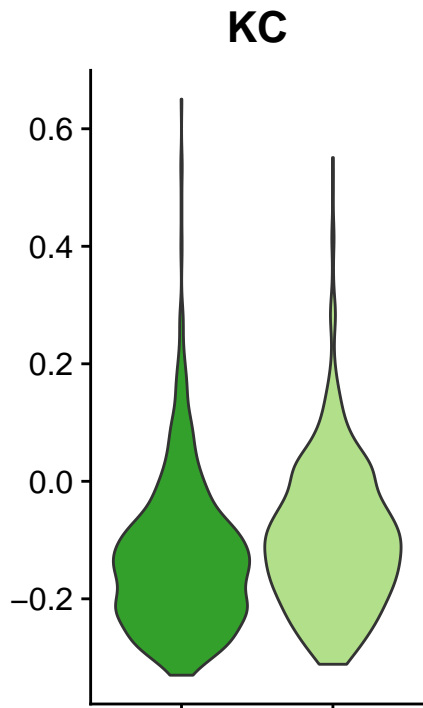


```
wilcox.test(KC_WT$Mo_KC_sign1, KC_KO$Mo_KC_sign1)  #P= 1.116e-10
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  KC_WT$Mo_KC_sign1 and KC_KO$Mo_KC_sign1
## W = 259572, p-value = 1.116e-10
## alternative hypothesis: true location shift is not equal to 0
```

IM

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

```
Bulk_RNAseq_IM_EMvsMo <- Bulk_RNAseq_IM_EMvsMo[Bulk_RNAseq_IM_EMvsMo$padj.y <
```

```
    0.05 & abs(Bulk_RNAseq_IM_EMvsMo$log2FoldChange.y) > 1, ]

write.csv(Bulk_RNAseq_IM_EMvsMo, file = "Bulk_RNAseq_IM_EMvsMo.csv")

EM_IM_sign <- Bulk_RNAseq_IM_EMvsMo[Bulk_RNAseq_IM_EMvsMo$log2FoldChange.y <
    -1, ]$Gene

EM_IM_sign <- list(EM_IM_sign)

sc <- AddModuleScore(sc, features = EM_IM_sign, name = "EM_IM_sign")
```

```
## Warning: The following features are not present in the object: H2.M2, not
## searching for symbol synonyms
```

```
Mo_IM_sign <- Bulk_RNAseq_IM_EMvsMo[Bulk_RNAseq_IM_EMvsMo$log2FoldChange.y >
    1, ]$Gene

Mo_IM_sign <- list(Mo_IM_sign)

sc <- AddModuleScore(sc, features = Mo_IM_sign, name = "Mo_IM_sign")
```
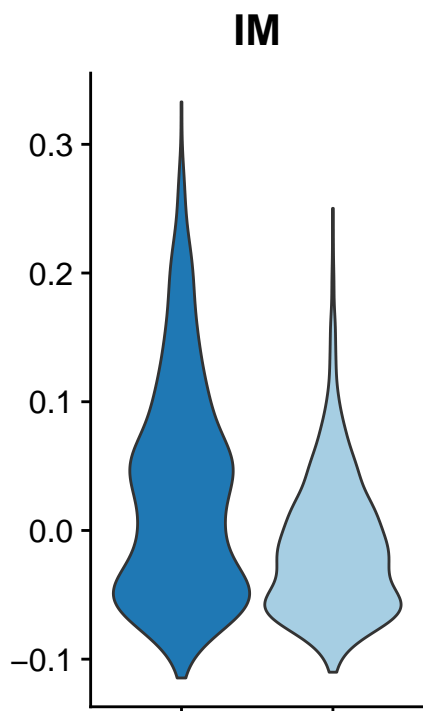
```
VlnPlot(sc, features = "EM_IM_sign1", cols = pal_IM, pt.size = 0,
    idents = c("IM WT", "IM KO")) + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank()) +
    ggtitle("IM")
```

```
metadata <- sc@meta.data
IM_WT <- metadata[metadata$Condition == "IM WT", ]
IM_KO <- metadata[metadata$Condition == "IM KO", ]

wilcox.test(IM_WT$EM_IM_sign1, IM_KO$EM_IM_sign1)  #< 2.2e-16
```
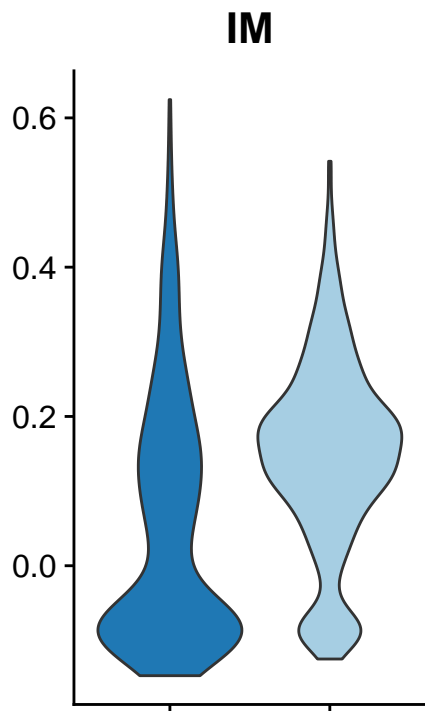
```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  IM_WT$EM_IM_sign1 and IM_KO$EM_IM_sign1
## W = 284975, p-value < 2.2e-16
## alternative hypothesis: true location shift is not equal to 0
```

```
VlnPlot(sc, features = "Mo_IM_sign1", cols = pal_IM, pt.size = 0,
    idents = c("IM WT", "IM KO")) + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank()) +
    ggtitle("IM")
```



```
wilcox.test(IM_WT$Mo_IM_sign1, IM_KO$Mo_IM_sign1)  #< 2.2e-16
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  IM_WT$Mo_IM_sign1 and IM_KO$Mo_IM_sign1
## W = 138648, p-value < 2.2e-16
## alternative hypothesis: true location shift is not equal to 0
```

MG

```r
Bulk_RNAseq_MG_EMvsMo <- read_excel("Bulk_RNAseq_MG_EMvsMo.xlsx",
    na = "NA")

Bulk_RNAseq_MG_EMvsMo <- Bulk_RNAseq_MG_EMvsMo[Bulk_RNAseq_MG_EMvsMo$p.value.45.1.45.2.nt <
    0.05 & abs(Bulk_RNAseq_MG_EMvsMo$FC.45.1.45.2.nt) > 1, ]

Bulk_RNAseq_MG_EMvsMo$diff <- Bulk_RNAseq_MG_EMvsMo$average.45.1.nt -
    Bulk_RNAseq_MG_EMvsMo$average.45.2.nt

EM_MG_sign <- Bulk_RNAseq_MG_EMvsMo[Bulk_RNAseq_MG_EMvsMo$diff <
    0, ]$Gene.Name

EM_MG_sign <- intersect(rownames(sc), EM_MG_sign)

EM_MG_sign <- head(EM_MG_sign, 100)

write.table(EM_MG_sign, file = "EM_MG_sign.txt")

EM_MG_sign <- list(EM_MG_sign)

sc <- AddModuleScore(sc, features = EM_MG_sign, name = "EM_MG_sign")

Mo_MG_sign <- Bulk_RNAseq_MG_EMvsMo[Bulk_RNAseq_MG_EMvsMo$diff >
    0, ]$Gene.Name

Mo_MG_sign <- intersect(rownames(sc), Mo_MG_sign)

Mo_MG_sign <- head(Mo_MG_sign, 100)

write.table(Mo_MG_sign, file = "Mo_MG_sign.txt")

Mo_MG_sign <- list(Mo_MG_sign)

sc <- AddModuleScore(sc, features = Mo_MG_sign, name = "Mo_MG_sign")
```
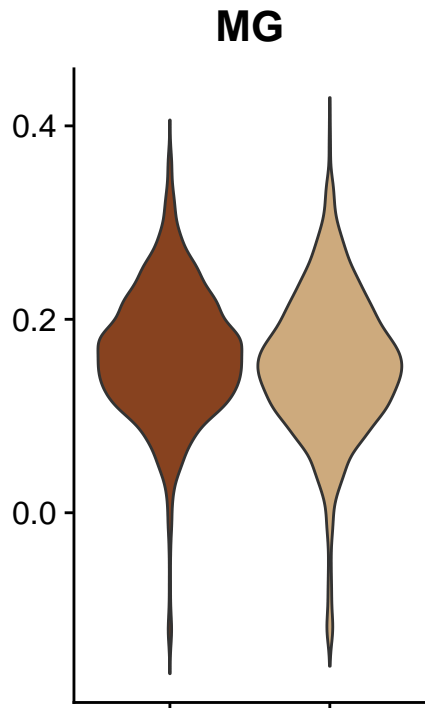
```r
VlnPlot(sc, features = "EM_MG_sign1", cols = pal_MG, pt.size = 0,
    idents = c("MG WT", "MG KO")) + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank()) +
    ggtitle("MG")
```

MG


```r
metadata <- sc@meta.data
MG_WT <- metadata[metadata$Condition == "MG WT", ]
MG_KO <- metadata[metadata$Condition == "MG KO", ]

mean(MG_WT$EM_MG_sign1)
```

```
## [1] 0.1635021
```

```r
mean(MG_KO$EM_MG_sign1)
```

```
## [1] 0.1522682
```

```r
wilcox.test(MG_WT$EM_MG_sign1, MG_KO$EM_MG_sign1)   #p-value = 4.694e-07
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  MG_WT$EM_MG_sign1 and MG_KO$EM_MG_sign1
## W = 2230982, p-value = 4.694e-07
## alternative hypothesis: true location shift is not equal to 0
```

```r
VlnPlot(sc, features = "Mo_MG_sign1", cols = pal_MG, pt.size = 0,
    idents = c("MG WT", "MG KO")) + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank()) +
    ggtitle("MG")
```

MG
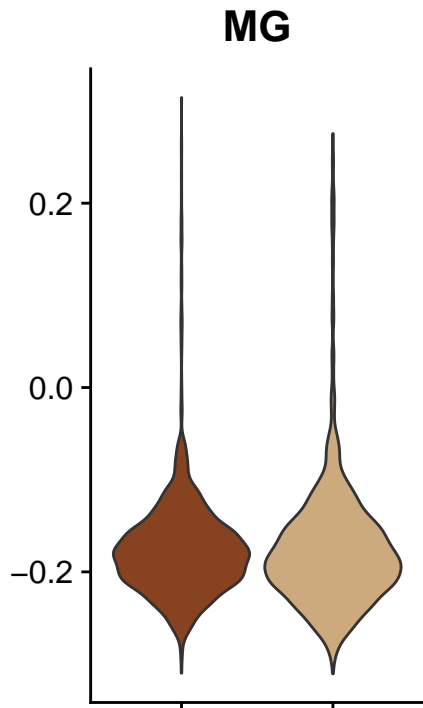

```
metadata <- sc@meta.data
MG_WT <- metadata[metadata$Condition == "MG WT", ]
MG_KO <- metadata[metadata$Condition == "MG KO", ]

mean(MG_WT$Mo_MG_sign1)
```

```
## [1] -0.1756634
```

```
mean(MG_KO$Mo_MG_sign1)
```

```
## [1] -0.1750868
```

```
wilcox.test(MG_WT$Mo_MG_sign1, MG_KO$Mo_MG_sign1)   #p-value = 0.02041
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  MG_WT$Mo_MG_sign1 and MG_KO$Mo_MG_sign1
## W = 2129965, p-value = 0.02041
## alternative hypothesis: true location shift is not equal to 0
```

MafB target gene signature score

MafB peaks were identified by CUT&RUN on BMDM

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

MafB_target_genes <- MafB_Peaks$`Gene Name`

MafB_target_genes <- unique(MafB_target_genes)


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

MafB_target_genes <- intersect(MafB_target_genes, rownames(sc))

sc <- AddModuleScore(sc, features = list(MafB_target_genes),
    name = "Mafb_sign")

VlnPlot(sc, features = "Mafb_sign1", cols = pal, pt.size = 0) +
    theme(legend.position = "none", axis.title.x = element_blank(),
        axis.text.x = element_blank()) + ggtitle("")
```
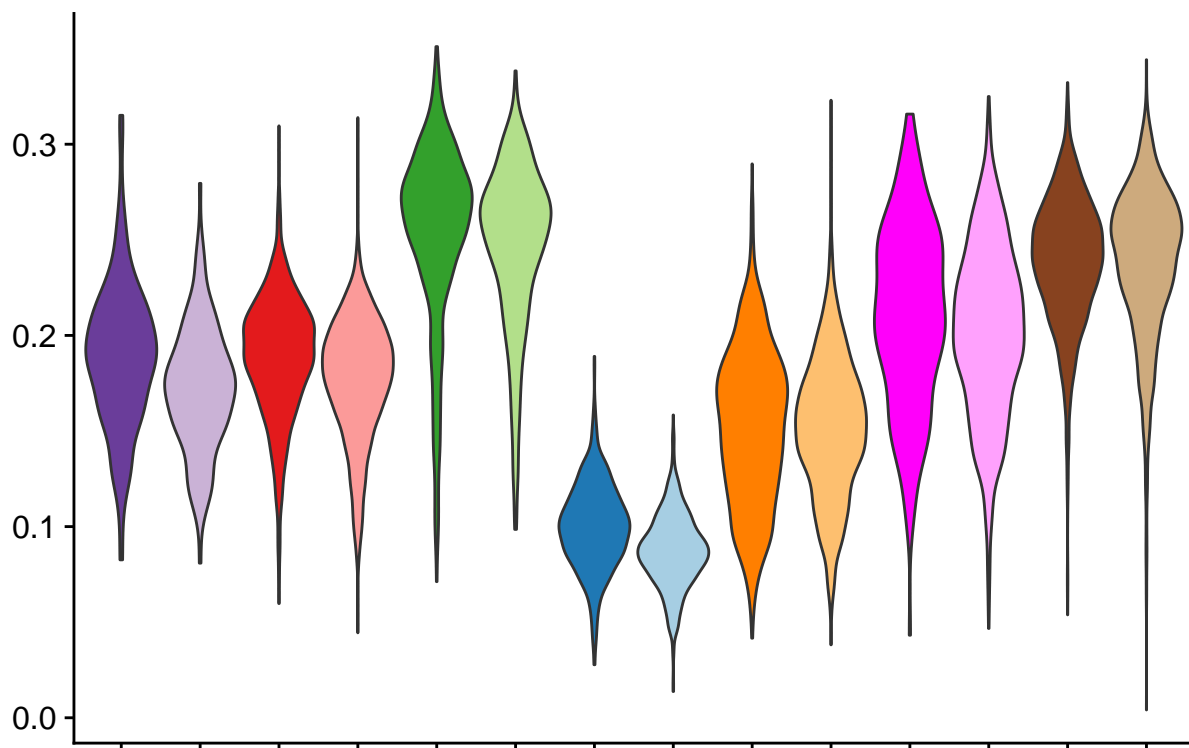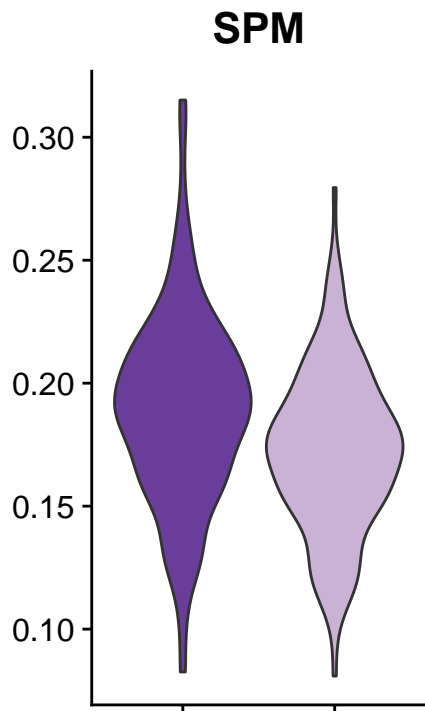

SPM

```r
pal_SPM <- c("#6A3D9A", "#CAB2D6")

VlnPlot(sc, features = "Mafb_sign1", cols = pal_SPM, pt.size = 0,
    idents = c("SPM WT", "SPM KO")) + ggtitle("SPM") + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank())
```
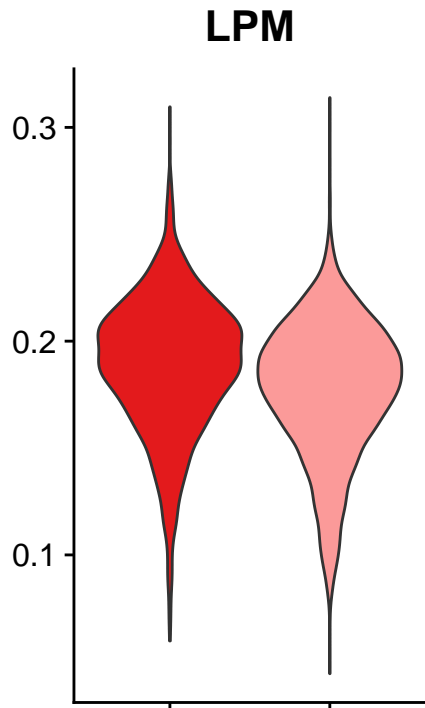


```r
metadata <- sc@meta.data
SPM_WT <- metadata[metadata$Condition == "SPM WT", ]
SPM_KO <- metadata[metadata$Condition == "SPM KO", ]

wilcox.test(SPM_WT$Mafb_sign1, SPM_KO$Mafb_sign1)
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  SPM_WT$Mafb_sign1 and SPM_KO$Mafb_sign1
## W = 18086, p-value = 1.091e-05
## alternative hypothesis: true location shift is not equal to 0
```

LPM

```r
pal_LPM <- c("#e31a1c", "#fb9a99")

VlnPlot(sc, features = "Mafb_sign1", cols = pal_LPM, pt.size = 0,
    idents = c("LPM WT", "LPM KO")) + ggtitle("LPM") + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank())
```

LPM


```r
LPM_WT <- metadata[metadata$Condition == "LPM WT", ]
LPM_KO <- metadata[metadata$Condition == "LPM KO", ]

wilcox.test(LPM_WT$Mafb_sign1, LPM_KO$Mafb_sign1)
```
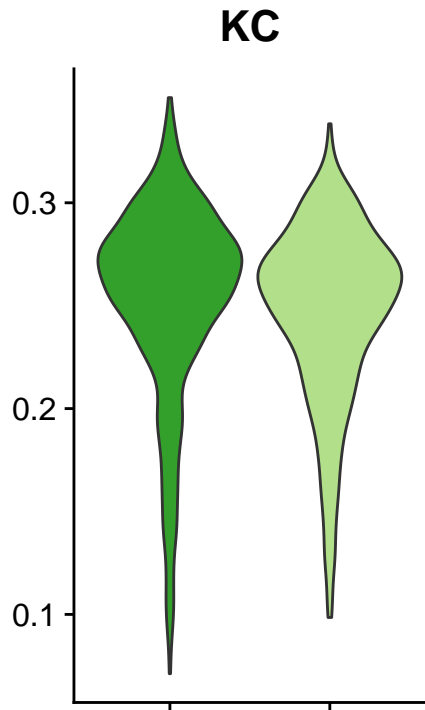
```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  LPM_WT$Mafb_sign1 and LPM_KO$Mafb_sign1
## W = 1828381, p-value < 2.2e-16
## alternative hypothesis: true location shift is not equal to 0
```

KC

```r
pal_KC <- c("#33a02c", "#b2df8a")

VlnPlot(sc, features = "Mafb_sign1", cols = pal_KC, pt.size = 0,
    idents = c("KC WT", "KC KO")) + ggtitle("KC") + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank())
```

KC


```r
KC_WT <- metadata[metadata$Condition == "KC WT", ]
KC_KO <- metadata[metadata$Condition == "KC KO", ]

wilcox.test(KC_WT$Mafb_sign1, KC_KO$Mafb_sign1)
```
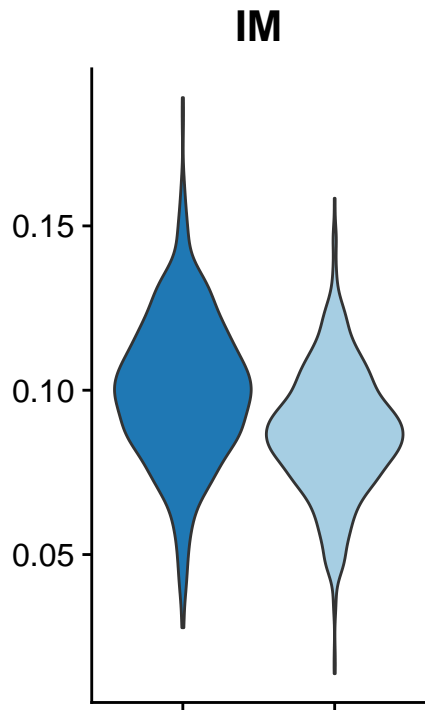
```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  KC_WT$Mafb_sign1 and KC_KO$Mafb_sign1
## W = 361619, p-value = 3.979e-06
## alternative hypothesis: true location shift is not equal to 0
```

IM

```r
pal_IM <- c("#1f78b4", "#a6cee3")

VlnPlot(sc, features = "Mafb_sign1", cols = pal_IM, pt.size = 0,
    idents = c("IM WT", "IM KO")) + ggtitle("IM") + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank())
```

IM


```r
IM_WT <- metadata[metadata$Condition == "IM WT", ]
IM_KO <- metadata[metadata$Condition == "IM KO", ]

wilcox.test(IM_WT$Mafb_sign1, IM_KO$Mafb_sign1)
```
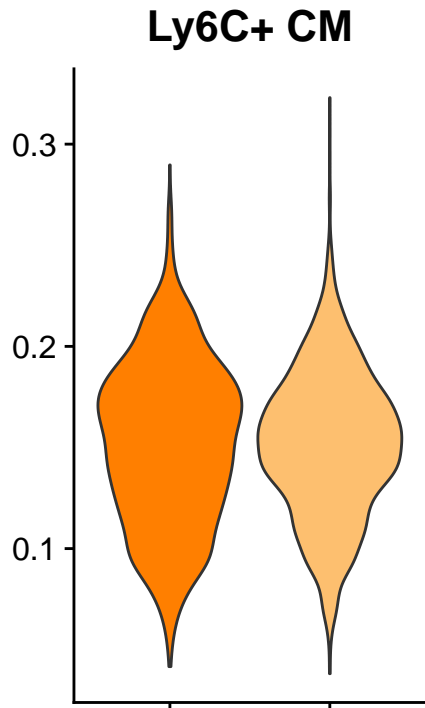
```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  IM_WT$Mafb_sign1 and IM_KO$Mafb_sign1
## W = 295620, p-value < 2.2e-16
## alternative hypothesis: true location shift is not equal to 0
```

Ly6C+ CM

```r
pal_Ly6C <- c("#FF7F00", "#FDBF6F")

VlnPlot(sc, features = "Mafb_sign1", cols = pal_Ly6C, pt.size = 0,
    idents = c("Ly6C+ CM WT", "Ly6C+ CM KO")) + ggtitle("Ly6C+ CM") +
    theme(legend.position = "none", axis.title.x = element_blank(),
        axis.text.x = element_blank())
```

Ly6C+ CM


```
Ly6C_WT <- metadata[metadata$Condition == "Ly6C+ CM WT", ]
Ly6C_KO <- metadata[metadata$Condition == "Ly6C+ CM KO", ]

wilcox.test(Ly6C_WT$Mafb_sign1, Ly6C_KO$Mafb_sign1)
```
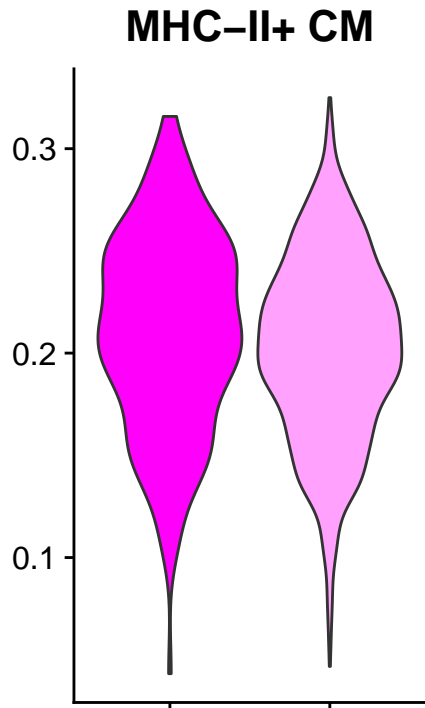
```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  Ly6C_WT$Mafb_sign1 and Ly6C_KO$Mafb_sign1
## W = 1274171, p-value = 0.7049
## alternative hypothesis: true location shift is not equal to 0
```

MHC-II+ CM

```
pal_MHC2 <- c("#FF00FA", "#FFA1FD")

VlnPlot(sc, features = "Mafb_sign1", cols = pal_MHC2, pt.size = 0,
    idents = c("MHC2+ CM WT", "MHC2+ CM KO")) + ggtitle("MHC-II+ CM") +
    theme(legend.position = "none", axis.title.x = element_blank(),
        axis.text.x = element_blank())
```

MHC−II+ CM


```r
MHC2_WT <- metadata[metadata$Condition == "MHC2+ CM WT", ]
MHC2_KO <- metadata[metadata$Condition == "MHC2+ CM KO", ]

wilcox.test(MHC2_WT$Mafb_sign1, MHC2_KO$Mafb_sign1)
```
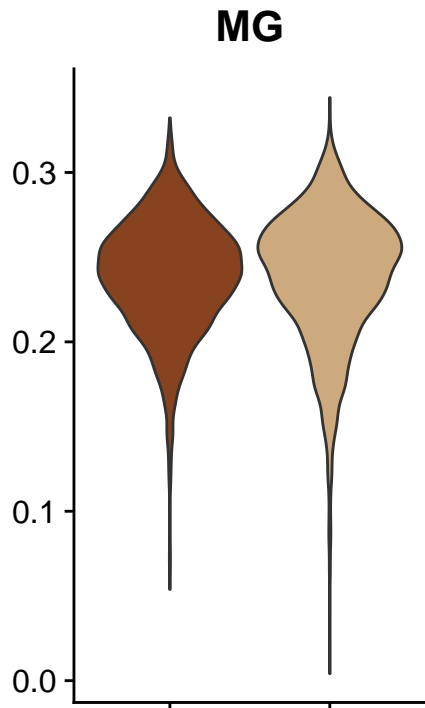
```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  MHC2_WT$Mafb_sign1 and MHC2_KO$Mafb_sign1
## W = 161016, p-value = 0.01621
## alternative hypothesis: true location shift is not equal to 0
```

MG

```r
pal_MG <- c("#87421F", "#CDAA7D")

VlnPlot(sc, features = "Mafb_sign1", cols = pal_MG, pt.size = 0,
    idents = c("MG WT", "MG KO")) + ggtitle("MG") + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank())
```
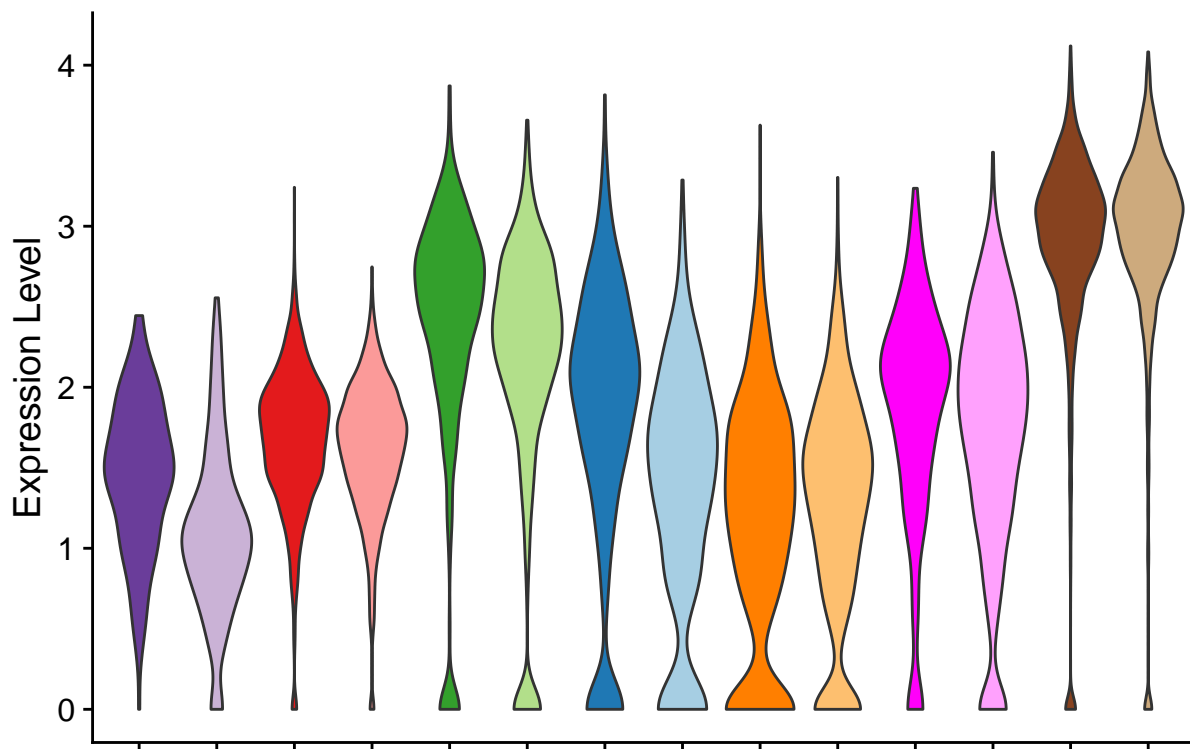
MG


```
MG_WT <- metadata[metadata$Condition == "MG WT", ]
MG_KO <- metadata[metadata$Condition == "MG KO", ]

wilcox.test(MG_WT$Mafb_sign1, MG_KO$Mafb_sign1)
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  MG_WT$Mafb_sign1 and MG_KO$Mafb_sign1
## W = 1985671, p-value = 0.1173
## alternative hypothesis: true location shift is not equal to 0
```
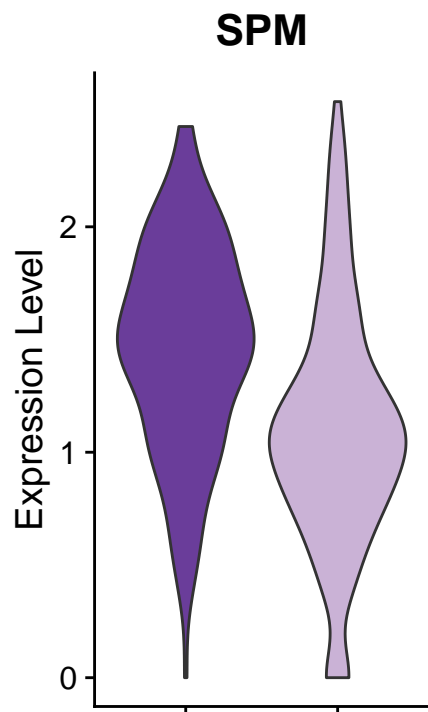
Csf1r expression

```
VlnPlot(sc, features = "Csf1r", cols = pal, pt.size = 0) + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank()) +
    ggtitle("")
```
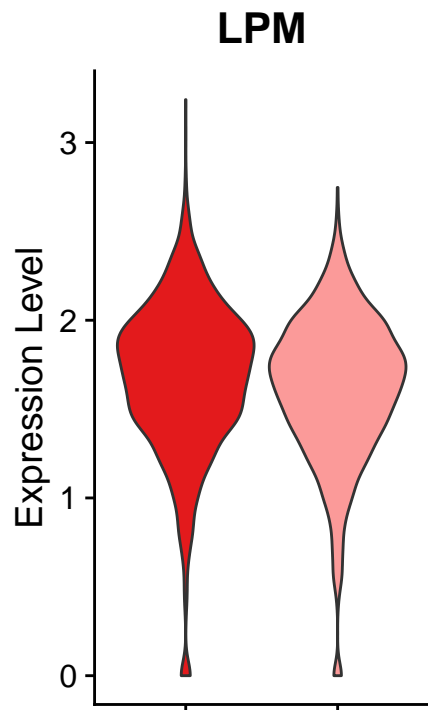
SPM

```
pal_SPM <- c("#6A3D9A", "#CAB2D6")

VlnPlot(sc, features = "Csf1r", cols = pal_SPM, pt.size = 0,
    idents = c("SPM WT", "SPM KO")) + ggtitle("SPM") + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank())
```
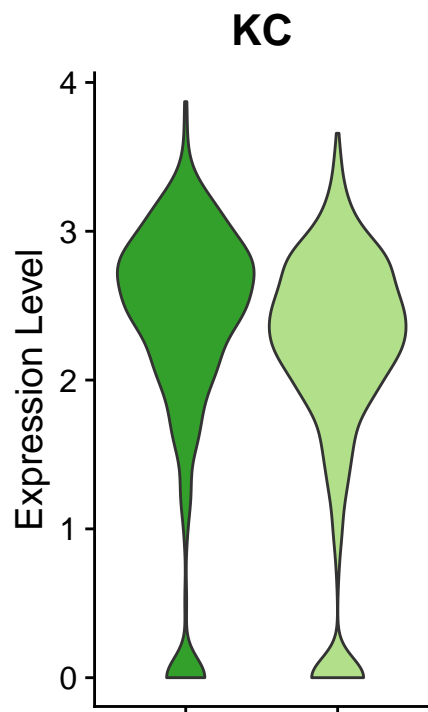
SPM

LPM

```
pal_LPM <- c("#e31a1c", "#fb9a99")

VlnPlot(sc, features = "Csf1r", cols = pal_LPM, pt.size = 0,
    idents = c("LPM WT", "LPM KO")) + ggtitle("LPM") + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank())
```

LPM

KC

```
pal_KC <- c("#33a02c", "#b2df8a")

VlnPlot(sc, features = "Csf1r", cols = pal_KC, pt.size = 0, idents = c("KC WT",
    "KC KO")) + ggtitle("KC") + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank())
```

KC

IM

```r
pal_IM <- c("#1f78b4", "#a6cee3")

VlnPlot(sc, features = "Csf1r", cols = pal_IM, pt.size = 0, idents = c("IM WT",
    "IM KO")) + ggtitle("IM") + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank())
```

IM

Ly6C+ CM

```r
pal_Ly6C <- c("#FF7F00", "#FDBF6F")

VlnPlot(sc, features = "Csf1r", cols = pal_Ly6C, pt.size = 0,
    idents = c("Ly6C+ CM WT", "Ly6C+ CM KO")) + ggtitle("Ly6C+ CM") +
    theme(legend.position = "none", axis.title.x = element_blank(),
        axis.text.x = element_blank())
```
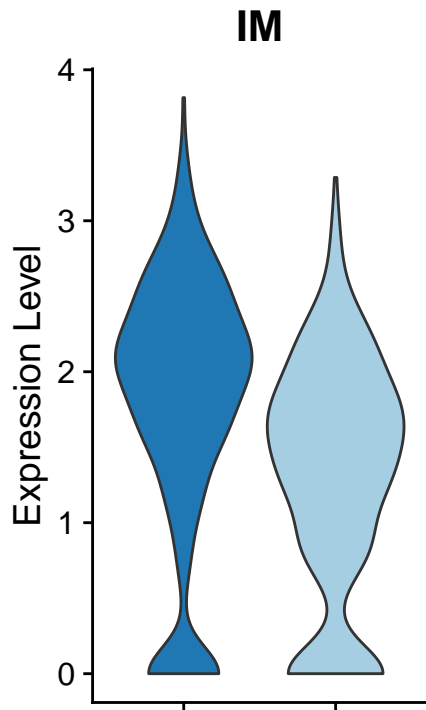
Ly6C+ CM

MHC-II+ CM

```r
pal_MHC2 <- c("#FF00FA", "#FFA1FD")

VlnPlot(sc, features = "Csf1r", cols = pal_MHC2, pt.size = 0,
    idents = c("MHC2+ CM WT", "MHC2+ CM KO")) + ggtitle("MHC-II+ CM") +
    theme(legend.position = "none", axis.title.x = element_blank(),
        axis.text.x = element_blank())
```

MHC−II+ CM

MG

```
pal_MG <- c("#87421F", "#CDAA7D")

VlnPlot(sc, features = "Csf1r", cols = pal_MG, pt.size = 0, idents = c("MG WT",
    "MG KO")) + ggtitle("MG") + theme(legend.position = "none",
    axis.title.x = element_blank(), axis.text.x = element_blank())
```
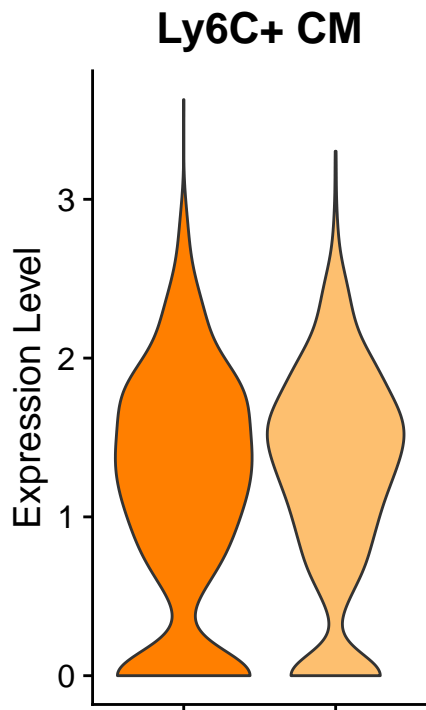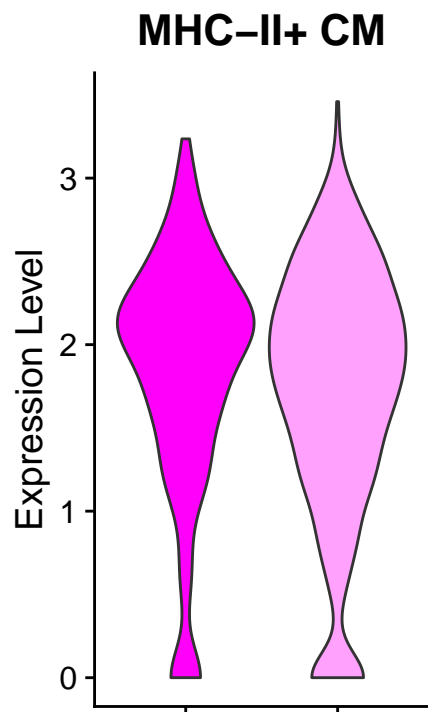
MG


```
sessionInfo()
```
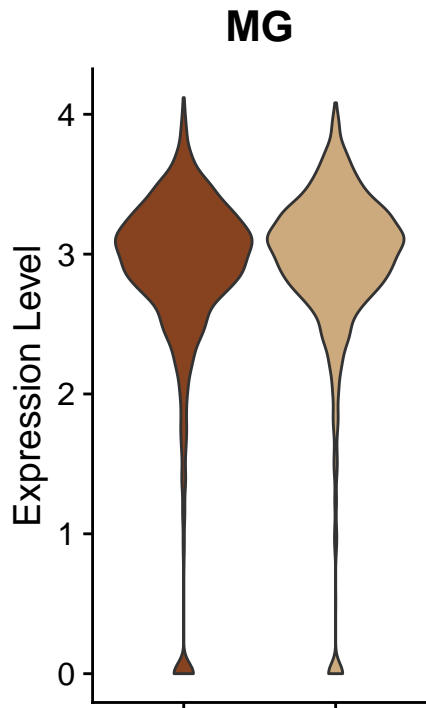
```
## R version 4.2.3 (2023-03-15 ucrt)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 19045)
##
## Matrix products: default
##
## locale:
## [1] LC_COLLATE=Dutch_Netherlands.utf8  LC_CTYPE=Dutch_Netherlands.utf8
## [3] LC_MONETARY=Dutch_Netherlands.utf8 LC_NUMERIC=C
## [5] LC_TIME=Dutch_Netherlands.utf8
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
## [1] readxl_1.4.3       dplyr_1.1.4        RColorBrewer_1.1-3 ggplot2_3.4.4
## [5] limma_3.54.2       SeuratObject_4.1.3 Seurat_4.3.0
##
## loaded via a namespace (and not attached):
##   [1] Rtsne_0.17         colorspace_2.1-0   deldir_2.0-2
##   [4] ellipsis_0.3.2     ggridges_0.5.5     rstudioapi_0.15.0
##   [7] spatstat.data_3.0-3 farver_2.1.1      leiden_0.4.3.1
##  [10] listenv_0.9.0      ggrepel_0.9.4      fansi_1.0.6
##  [13] codetools_0.2-19   splines_4.2.3      knitr_1.45
##  [16] polyclip_1.10-6    spam_2.10-0        jsonlite_1.8.8
##  [19] ica_1.0-3          cluster_2.1.4      png_0.1-8
##  [22] uwot_0.1.16        shiny_1.8.0        sctransform_0.4.1
```

```
##  [25] spatstat.sparse_3.0-3 compiler_4.2.3        httr_1.4.7
##  [28] Matrix_1.6-4          fastmap_1.1.1         lazyeval_0.2.2
##  [31] cli_3.6.2             later_1.3.2           formatR_1.14
##  [34] htmltools_0.5.7       tools_4.2.3           dotCall64_1.1-1
##  [37] igraph_1.6.0          gtable_0.3.5          glue_1.6.2
##  [40] RANN_2.6.1            reshape2_1.4.4        Rcpp_1.0.11
##  [43] scattermore_1.2       cellranger_1.1.0      vctrs_0.6.5
##  [46] spatstat.explore_3.2-5 nlme_3.1-162         progressr_0.14.0
##  [49] lmtest_0.9-40         spatstat.random_3.2-2 xfun_0.39
##  [52] stringr_1.5.1         globals_0.16.2        mime_0.12
##  [55] miniUI_0.1.1.1        lifecycle_1.0.4       irlba_2.3.5.1
##  [58] goftest_1.2-3         future_1.33.1         MASS_7.3-58.2
##  [61] zoo_1.8-12            scales_1.3.0          promises_1.2.1
##  [64] spatstat.utils_3.0-4  parallel_4.2.3        yaml_2.3.7
##  [67] reticulate_1.34.0     pbapply_1.7-2         gridExtra_2.3
##  [70] stringi_1.8.3         highr_0.10            rlang_1.1.2
##  [73] pkgconfig_2.0.3       matrixStats_1.2.0     evaluate_0.23
##  [76] lattice_0.20-45       ROCR_1.0-11           purrr_1.0.2
##  [79] tensor_1.5            labeling_0.4.3        patchwork_1.1.3
##  [82] htmlwidgets_1.6.4     cowplot_1.1.2         tidyselect_1.2.1
##  [85] parallelly_1.36.0     RcppAnnoy_0.0.21      plyr_1.8.9
##  [88] magrittr_2.0.3        R6_2.5.1              generics_0.1.3
##  [91] withr_3.0.1           pillar_1.9.0          fitdistrplus_1.1-11
##  [94] survival_3.5-3        abind_1.4-5           sp_2.1-2
##  [97] tibble_3.2.1          future.apply_1.11.1   KernSmooth_2.23-20
## [100] utf8_1.2.4            spatstat.geom_3.2-7   plotly_4.10.3
## [103] rmarkdown_2.25        grid_4.2.3            data.table_1.14.10
## [106] digest_0.6.31         xtable_1.8-4          tidyr_1.3.0
## [109] httpuv_1.6.13         munsell_0.5.1         viridisLite_0.4.2
```