PAPER TITLE TO BE DEFINED (in common.yaml)

8-SCENIC analysis

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Abstract

Lung interstitium macrophages (IMs) are non-alveolar resident tissue macrophages which contribute to the lung homeostasis. These cells were reported to be heterogeneous by our group and other teams, which contains two main distinct subpopulations: CD206+ IMs and CD206- IMs. However, the exact origin of IMs and the transcriptional programs that control IM differentiation remains unclear. In recent report, we analyzed the refilled IMs in the course of time after induced IM depletion with single-cell RNA sequencing (10X Genomics Chromium) and bulk RNA sequencing.

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1 Description

2 Prepare data

2.1 Load Seurat objects

The 3D file store 3D embedding for UMAP and TSNE.

```
suppressMessages({
  library(Seurat)
  library(SCENIC)
  library(AUCell)
  library(RcisTarget)
  library(SCopeLoomR)
  library(ggplot2)})

obj <- readRDS("../6-Merge_two_experiments/immune_imdtr3.seuratObject.Rds"
   )
```

2.2 Filter only the cells in classic monocytes, patrolling monocytes and interstitial macrophages

```
obj <- subset(obj, subset = cell.type2 %in% c("Classical_Mono",
                                              "MHCII_IM",
                                              "CD206_IM",
                                              "Transit"))
obj$cell.type2 <- as.character(obj$cell.type2)
```

2.3 Prepare input data for SCENIC.

```
obj.sce <- as.SingleCellExperiment(obj) # we indeed don't need this.
dir.create("data")
saveRDS(obj.sce, file="data/IM-DTR3_3D.sce.Rds")
```

2.3.1 Matrix construction:

```
exprMat <- as.matrix(obj@assays$RNA@data)
exprMat[1:3,1:3]
```

```
dir.create("int")
saveRDS(exprMat, file="int/exprMat.Rds")
```

2.4 Cell info/phenodata

Cell information:

```
cellInfo <- data.frame(CellType = obj@meta.data$cell.type2, nFeature_RNA =
    obj@meta.data$nFeature_RNA, nCount_RNA = obj@meta.data$nCount_RNA)
rownames(cellInfo) <- colnames(obj)
head(cellInfo)
```

Save data for later:

```
saveRDS(cellInfo, file="int/cellInfo.Rds")
```

Color to assign to the variables:

```
colVars <- list(CellType=c("Classical_Mono"="#A6CEE3",
                           "Patrolling_Mono"="#1F78B4",
                           "MHCII_IM"="#B2DF8A",
                           "CD206_IM"="#7E893D",
                           "chemoattractant"="#FB9A99",
                           "Transit"="#E31A1C",
                           "CCR7_IM"="#FDBF6F"))
colVars$CellType <- colVars$CellType[intersect(names(colVars$CellType),
   cellInfo$CellType)]
plot.new(); legend(0,1, fill=colVars$CellType, legend=names(colVars$
   CellType))
```

3 Initialize settings

```
scenicOptions <- initializeScenic(org="mgi",
                                  dbDir="./Data/Scenic_database/cisTarget_
                                     databases/",  # this should be
                                     prepared before, see SCENIC package
                                     instructions
                                  nCores=22 # use appropriate core number
                                  )
  # NOTE: nCores is the number of cores used for calculating. For GENIE3
     analysis is time-consuming and calculation-consuming, make this
     nCores maximum.
```

```
scenicOptions@inputDatasetInfo$cellInfo <- "int/cellInfo.Rds"
scenicOptions@inputDatasetInfo$colVars <- "int/colVars.Rds"
# update scenicOptions object.
saveRDS(scenicOptions, file="int/scenicOptions.Rds")
```

4 Co-expression network

4.1 Gene filter/selection

1. Filter by the total number of reads per gene. minCountsPerGene: By default it keeps only the genes with at least 6 UMI counts across all samples (e.g. the total number the gene would have, if it was expressed with a value of 3 in 1% of the cells). ***NOTE: here we use value of 3 in 0.5% cells as our data is bigger (avoid removing too many useful genes)***
2. Filter by the number of cells in which the gene is detected** (e.g. >0 UMI, or >1 log2(TPM)). minSamples: detected in at least 1% of the cells

```
genesKept <- geneFiltering(exprMat, scenicOptions=scenicOptions,
                           minCountsPerGene=3*.005*ncol(exprMat),
                           minSamples=ncol(exprMat)*.01)
exprMat_filtered <- exprMat[genesKept, ]
dim(exprMat_filtered)
```

```
saveRDS(exprMat_filtered, file = "int/exprMat_filtered.Rds")
saveRDS(genesKept, file = "int/geneKept.Rds")
```

4.2 Correlation

```
source("/home/qiang/Desktop/velocyto/Script/runCorrelation.R") # package does not contain this function.
runCorrelation(exprMat_filtered, scenicOptions)
```

4.3 GENIE3

This step is time consuming.

Run GENIE3:

```
# here you should run the following codes:
runGenie3(exprMat_filtered, scenicOptions)
```

5 Build and score the GRN

- Build the gene regulatory network:
  1. Get co-expression modules
  2. Get regulons (with RcisTarget): TF motif analysis)
- Identify cell states:
  3. Score GRN (regulons) in the cells (with AUCell)
  4. Cluster cells according to the GRN activity

Prepare setting:

```
scenicOptions@settings$verbose <- TRUE
scenicOptions@settings$seed <- 123 # this is important to have a reproducible result, because the algothritm is built on Ramdom Forest model.
scenicOptions@settings$nCores <- 10 # when run with 22 core, runSCENIC_2_createRegulons will overflow memory.
```

Run the remaining steps using the wrapper functions:

```
runSCENIC_1_coexNetwork2modules(scenicOptions)
runSCENIC_2_createRegulons(scenicOptions)
runSCENIC_3_scoreCells(scenicOptions, exprMat_filtered_log)
```

6 Demonstration of SCENIC results with Seurat

```
regulonAUC <- readRDS("int/3.4_regulonAUC.Rds")
```

Load regulon AUC score:

```
regulonAUC <- readRDS("../../../IM_DTR_exp2/analyses/20210505_SCENIC_only_IM_DIfferentiation/int/3.4_regulonAUC.Rds")
```

Extract the useful information:

```
regulonAUC.mat <- regulonAUC@assays@data@listData$AUC
dim(regulonAUC.mat)
```

```
## [1]   398 10566
```

Now take the top 50 most variable Regulons:

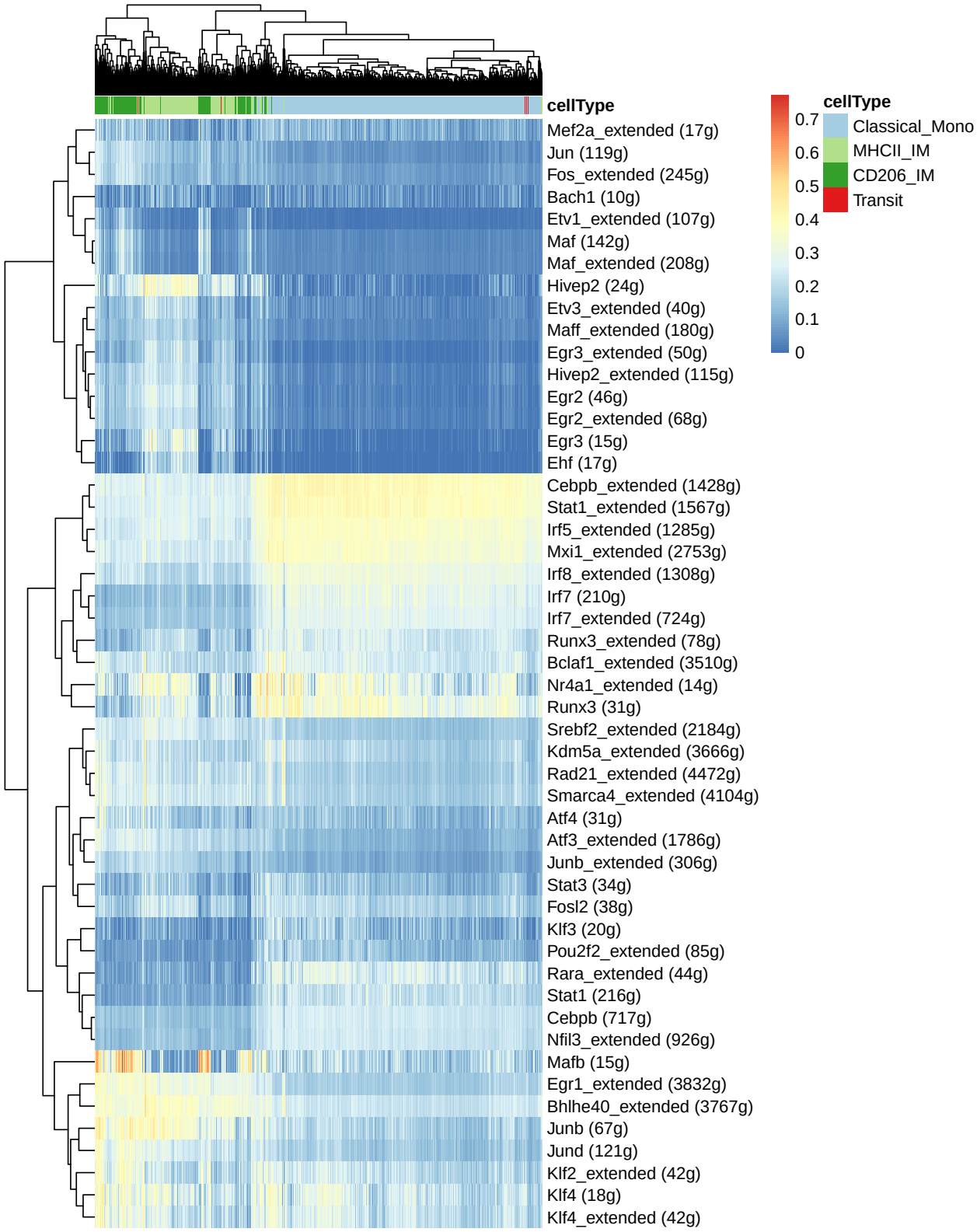
```
suppressMessages(library(genefilter))
rv <- rowVars(regulonAUC.mat)
idx <- order(-rv)[1:50]
```

```
sce <- readRDS("./data/IM-DTR3_3D.sce.Rds")
```

```
suppressMessages({
  library(pheatmap)
  library(RColorBrewer)
  library(SingleCellExperiment)
})

pal <- brewer.pal(length(levels(sce@colData$ident)), name = "Paired")

cellInfo.cellType <- data.frame(cellType = sce@colData$ident)
rownames(cellInfo.cellType) <- colnames(sce)
  annotationColors <- list(cellType = c(`Classical_Mono`="#A6CEE3",
                                        `MHCII_IM`="#B2DF8A",
                                        `CD206_IM`="#33A02C",
                                        `Transit` = "#E31A1C"))
pheatmap(regulonAUC.mat[idx,], show_colnames = FALSE, annotation_col =
   cellInfo.cellType, annotation_colors = annotationColors)
```




```
suppressMessages(library(ComplexHeatmap))
```

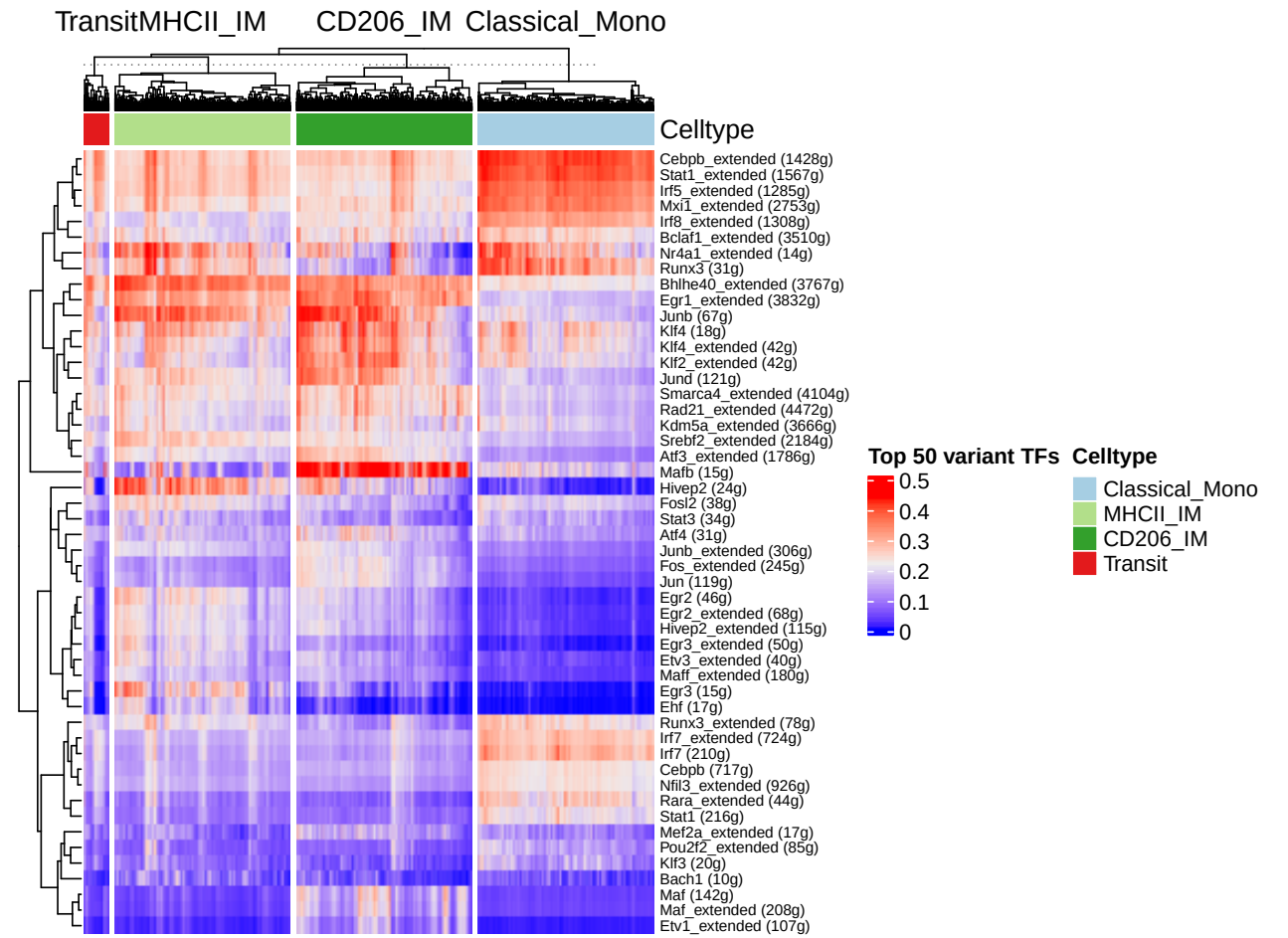
1

Randomly select 1500 cells in monocytes and IMs:

```
set.seed(41)
cellnames.15k <- unlist(sapply(unique(sce$cell.type2), function(x) {
  cellnames <- colnames(sce)[sce$cell.type2== x ]
  if (length(cellnames)>1500) {
    cellnames <- cellnames[sample(1:length(cellnames), 1500)]
  }
  return(cellnames)
} ))
```

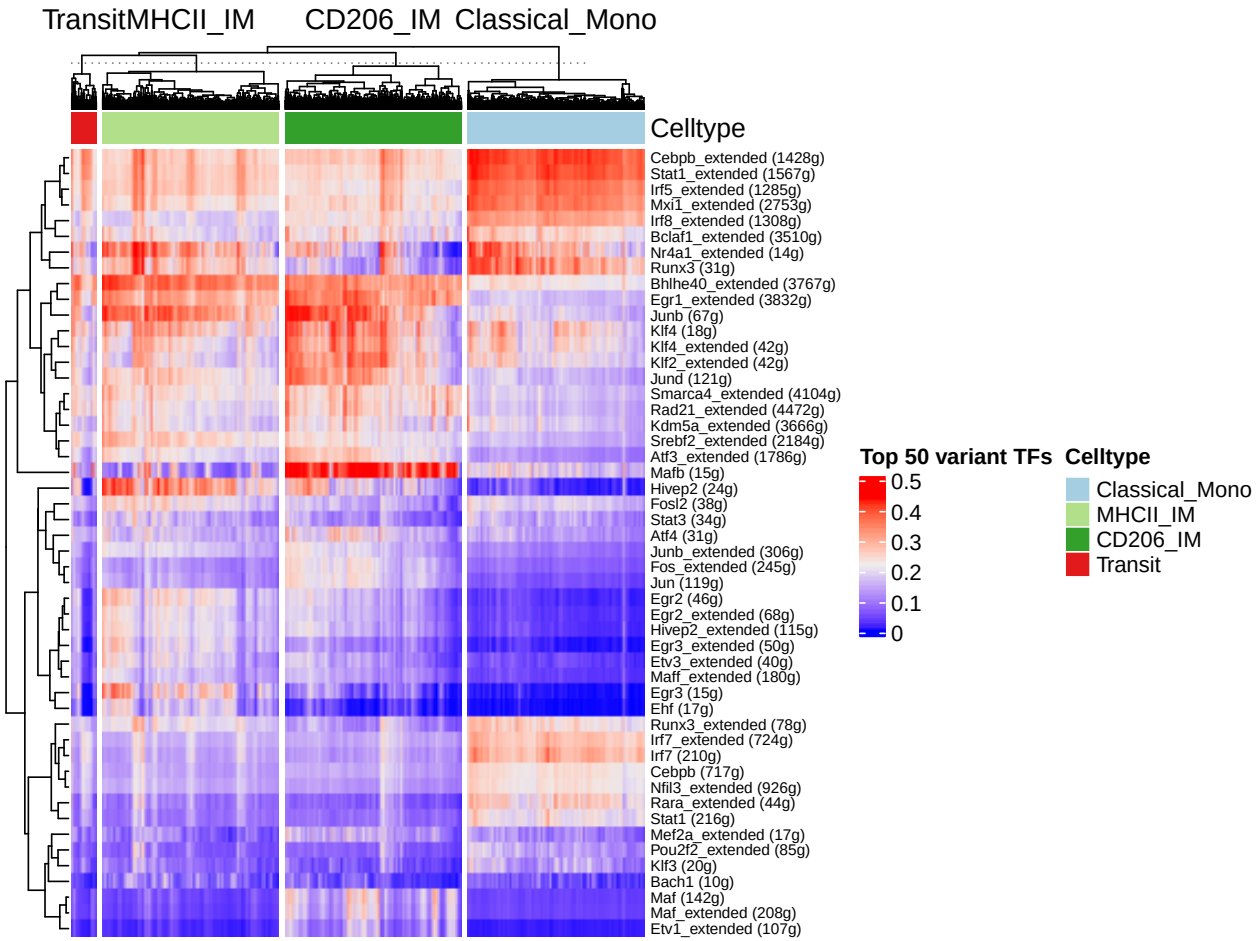
```
top50.15k.mat <- regulonAUC.mat[idx,cellnames.15k]
sce.15k <- sce[, cellnames.15k]
```

```
hp <- Heatmap(top50.15k.mat, name = "Top␣50␣variant␣TFs",
        show_row_names=TRUE, show_column_names = FALSE,
        row_names_gp = gpar ( fontsize = 7),
        column_split = factor(sce.15k$cell.type2),
        top_annotation = HeatmapAnnotation(Celltype=sce.15k$cell.type2,
                                           col = list(Celltype =
                                  c(`Classical_Mono`="#A6CEE3",
                                    `MHCII_IM`="#B2DF8A",
                                    `CD206_IM`="#33A02C",
                                    `Transit` = "#E31A1C")))
        )
hp <- draw(hp)
```


TransitMHCII_IM
CD206_IM
Classical_Mono
Celltype
Cebpb_extended (1428g)
Stat1_extended (1567g)
Irf5_extended (1285g)
Mxi1_extended (2753g)
Irf8_extended (1308g)
Bclaf1_extended (3510g)
Nr4a1_extended (14g)
Runx3 (31g)
Bhlhe40_extended (3767g)
Egr1_extended (3832g)
Junb (67g)
Klf4 (18g)
Klf4_extended (42g)
Klf2_extended (42g)
Jund (121g)
Smarca4_extended (4104g)
Rad21_extended (4472g)
Kdm5a_extended (3666g)
Srebf2_extended (2184g)
Atf3_extended (1786g)
Mafb (15g)
Hivep2 (24g)
Fosl2 (38g)
Stat3 (34g)
Atf4 (31g)
Junb_extended (306g)
Fos_extended (245g)
Jun (119g)
Egr2 (46g)
Egr2_extended (68g)
Hivep2_extended (115g)
Egr3_extended (50g)
Etv3_extended (40g)
Maff_extended (180g)
Egr3 (15g)
Ehf (17g)
Runx3_extended (78g)
Irf7_extended (724g)
Irf7 (210g)
Cebpb (717g)
Nfil3_extended (926g)
Rara_extended (44g)
Stat1 (216g)
Mef2a_extended (17g)
Pou2f2_extended (85g)
Klf3 (20g)
Bach1 (10g)
Maf (142g)
Maf_extended (208g)
Etv1_extended (107g)
Top 50 variant TFs
0.5
0.4
0.3
0.2
0.1
0
Celltype
Classical_Mono
MHCII_IM
CD206_IM
Transit

```
hp
```

1




```
pdf(file = "../Figures/HeatMap_top50_TF_SCENIC_in_15kcells.pdf",
    width = 8, height = 6)
hp
dev.off()
```

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

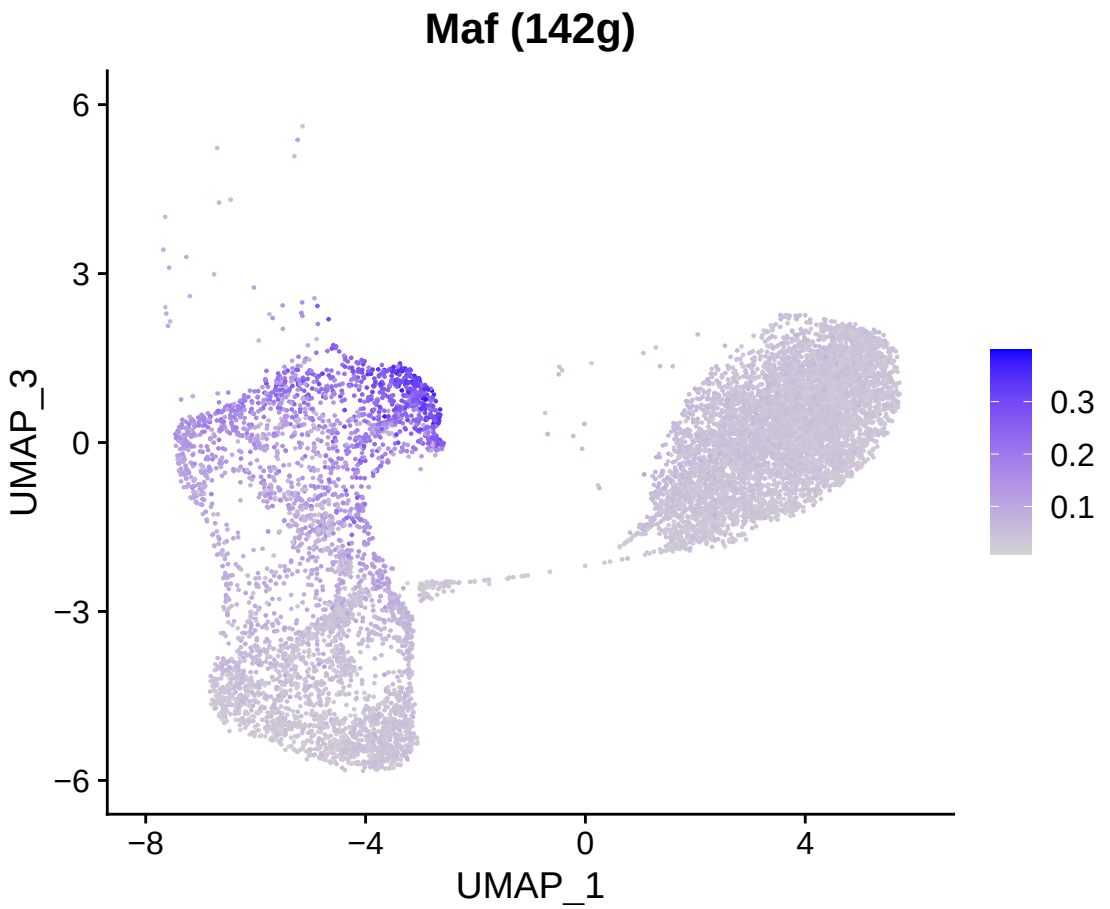
7 Make plots

Let's show the expression and activity of each TF in the pop:

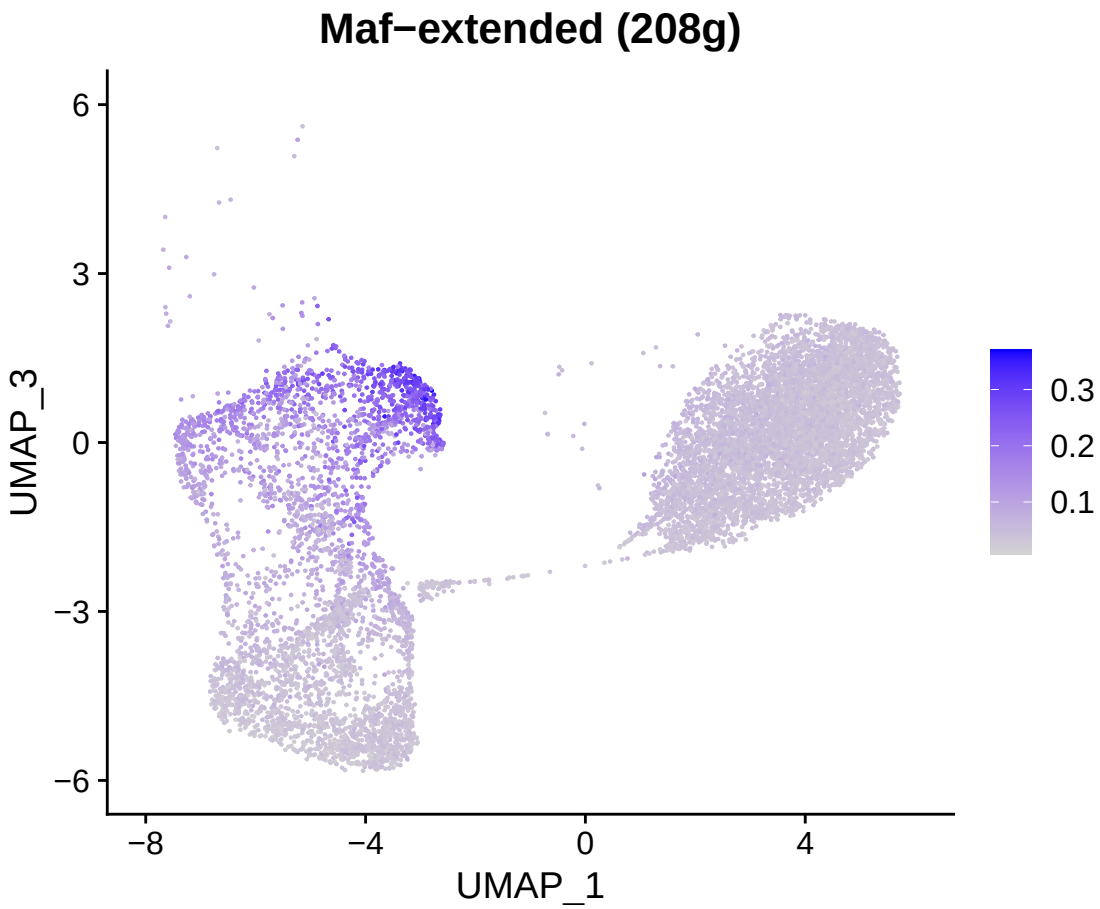
7.1 Show TF activity

```
obj[["scenic"]] <- CreateAssayObject(counts = regulonAUC.mat)
DefaultAssay(obj) <- "scenic"
```

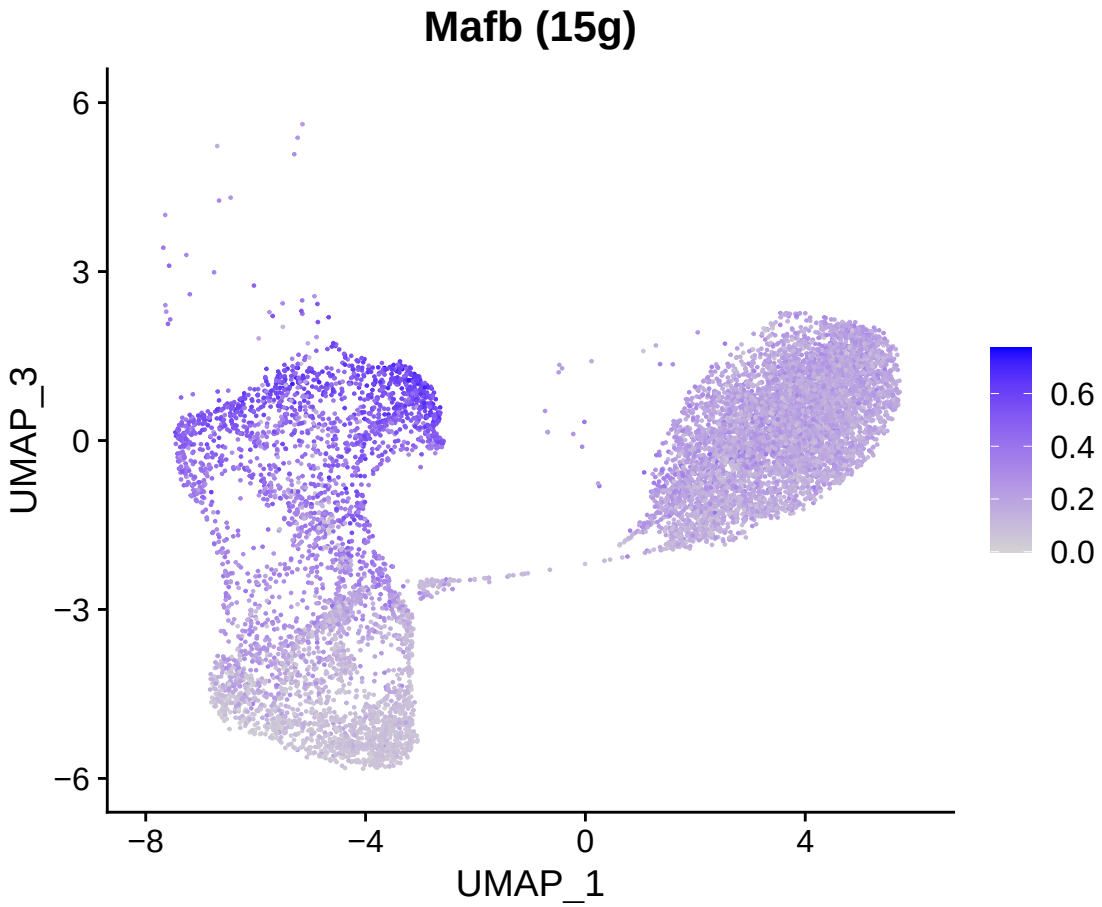
```
  FeaturePlot(obj, features = "Maf␣(142g)", dims = c(1,3))
```



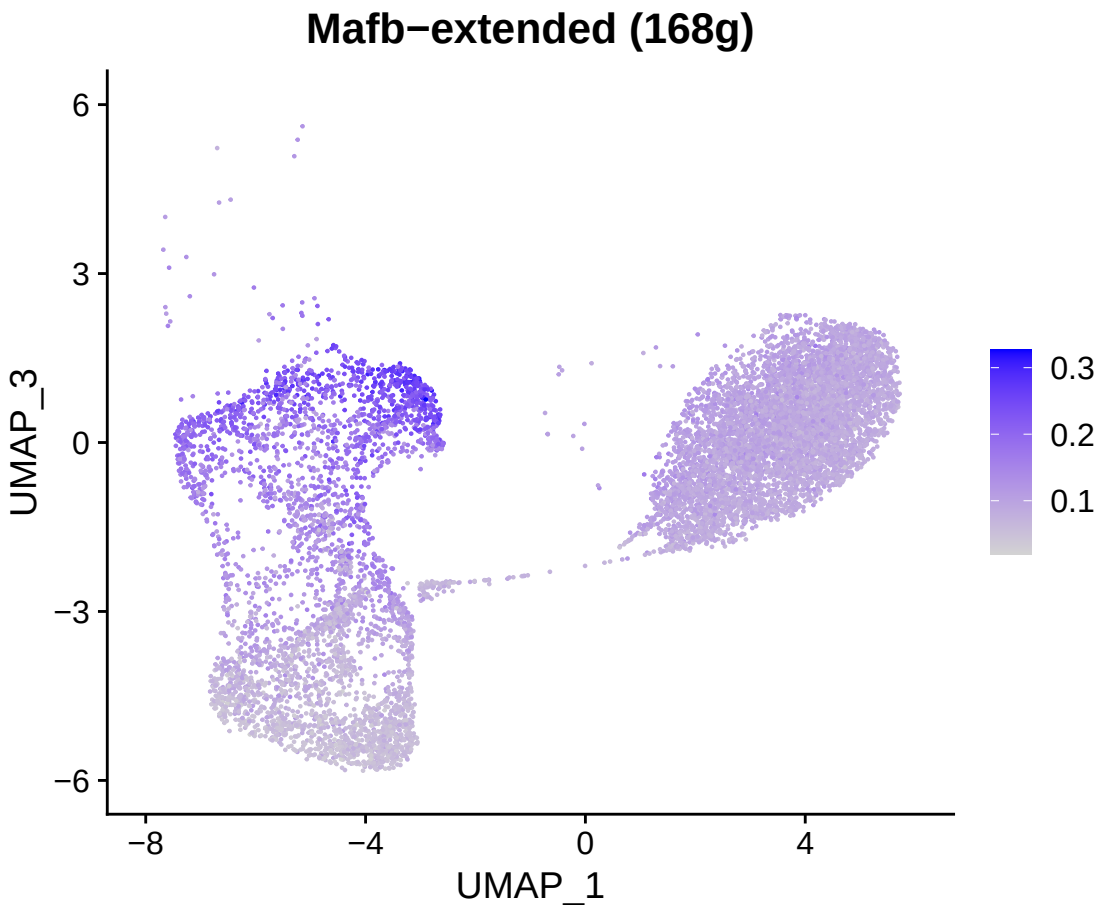

```
FeaturePlot(obj, features = "Maf-extended␣(208g)", dims = c(1,3))
```



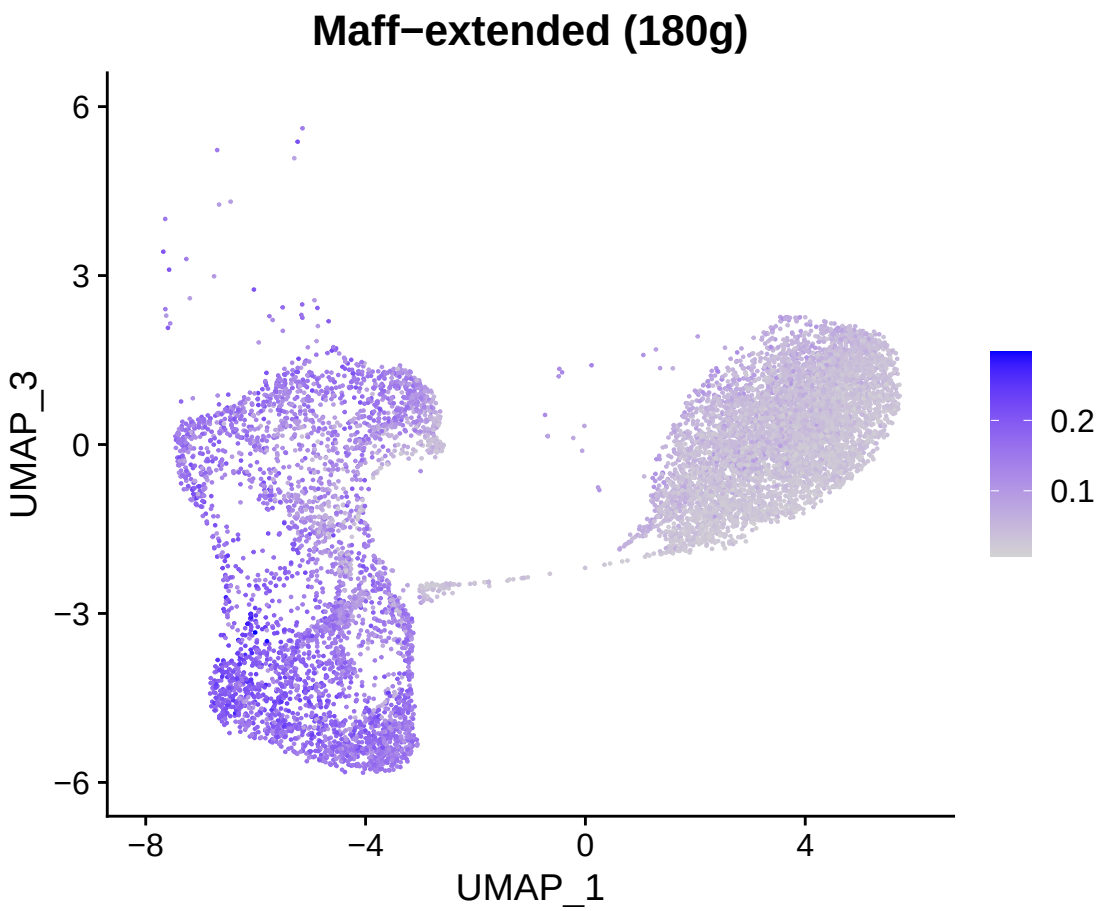

```
FeaturePlot(obj, features = "Mafb␣(15g)", dims = c(1,3))
```



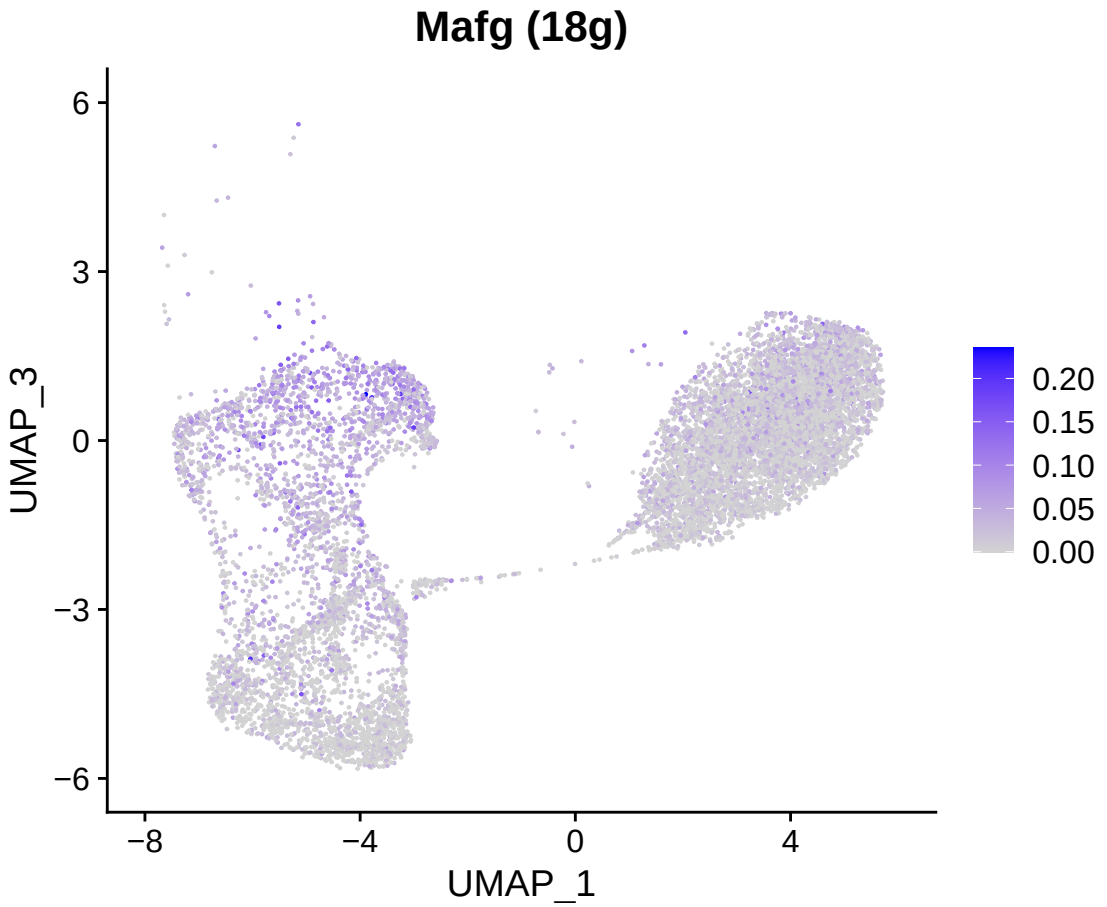

```
FeaturePlot(obj, features = "Mafb-extended (168g)", dims = c(1,3))
```



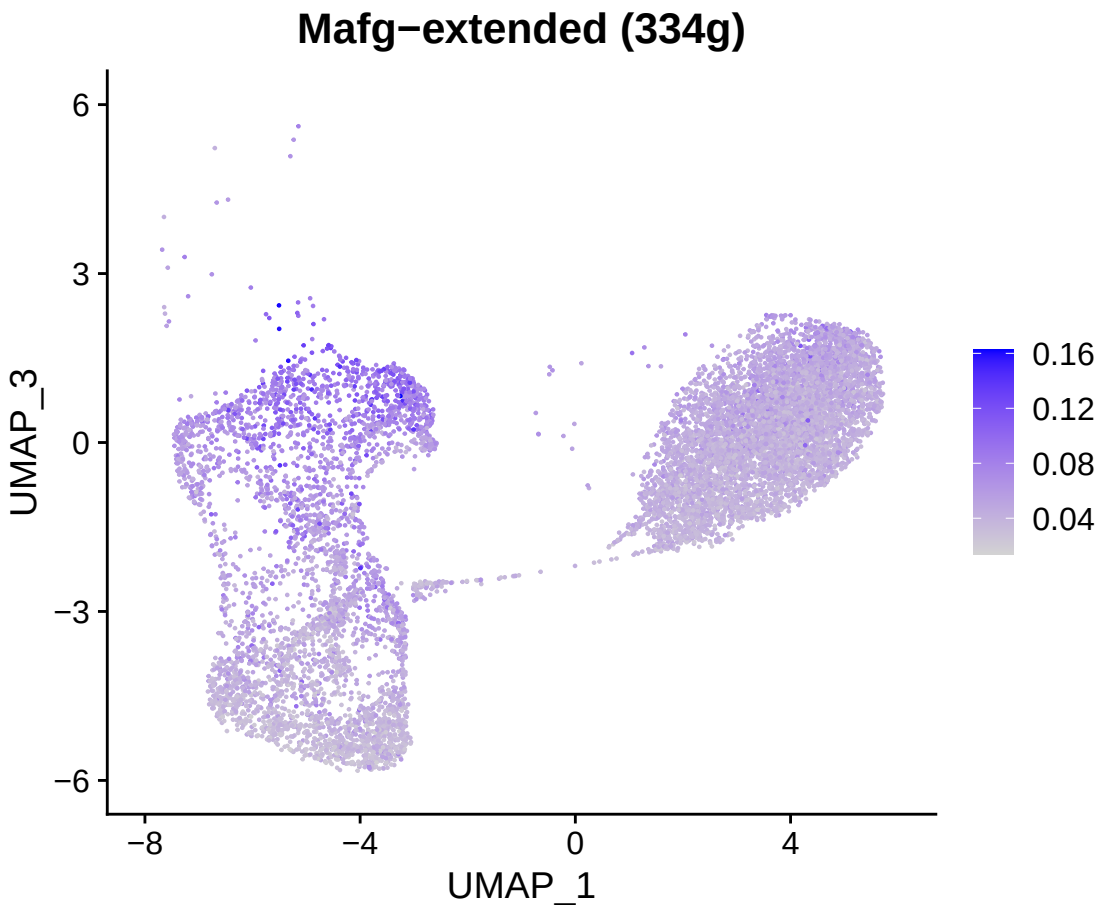

```
FeaturePlot(obj, features = "Maff-extended␣(180g)", dims = c(1,3))
```



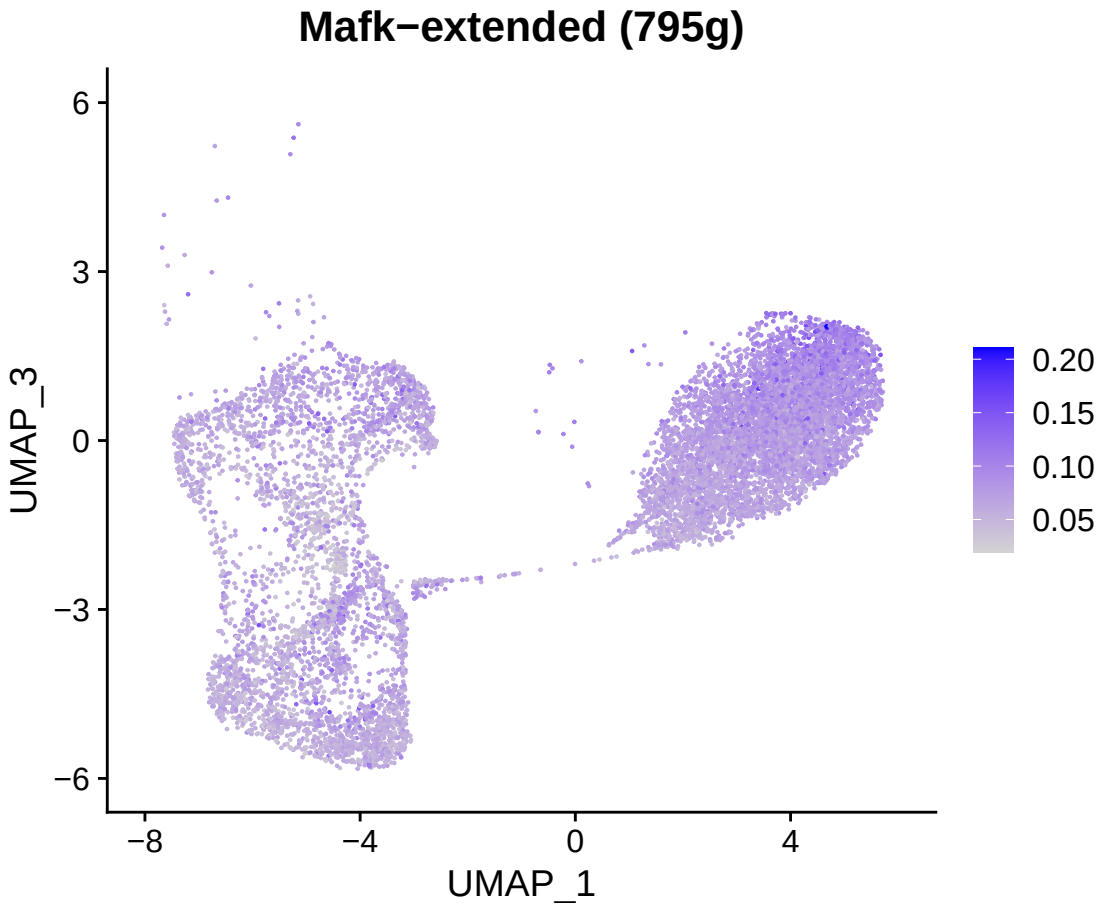

```
FeaturePlot(obj, features = "Mafg␣(18g)", dims = c(1,3))
```




```
FeaturePlot(obj, features = "Mafg-extended␣(334g)", dims = c(1,3))
```

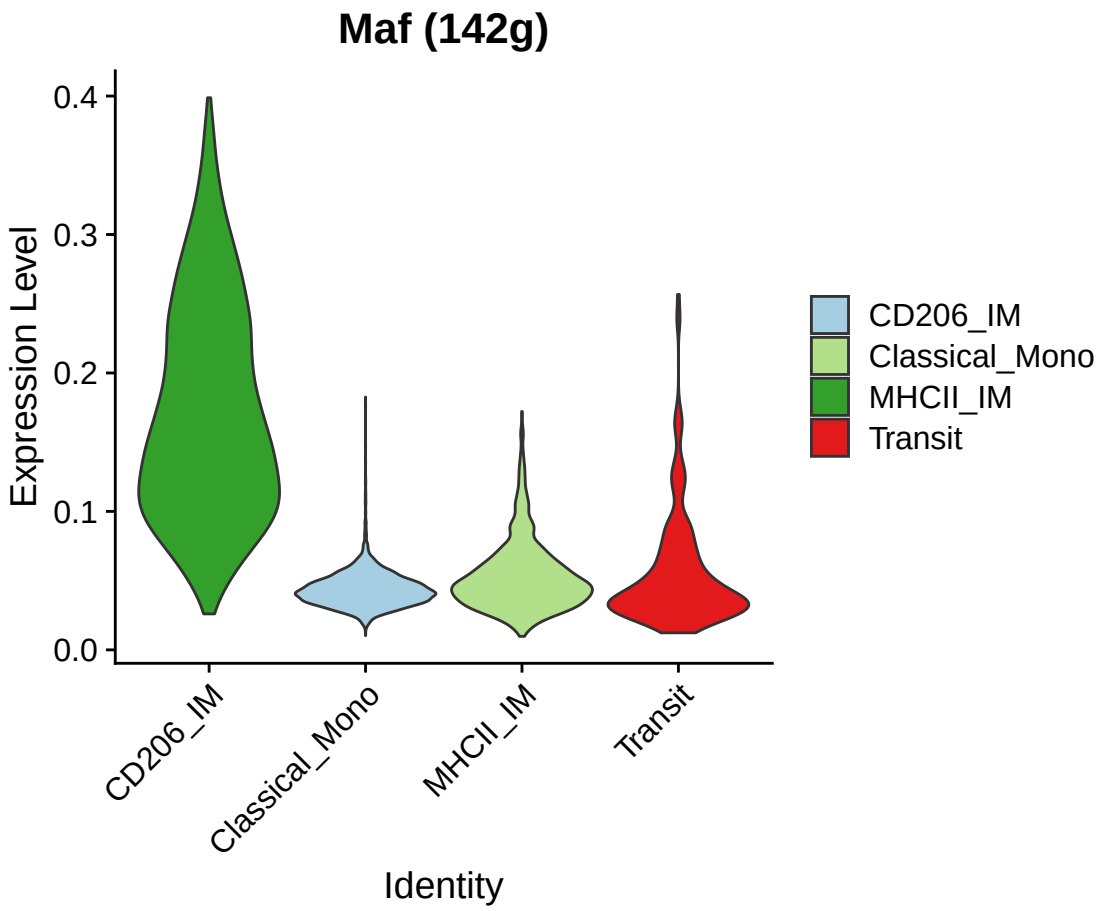



```
FeaturePlot(obj, features = "Mafk-extended␣(795g)", dims = c(1,3))
```

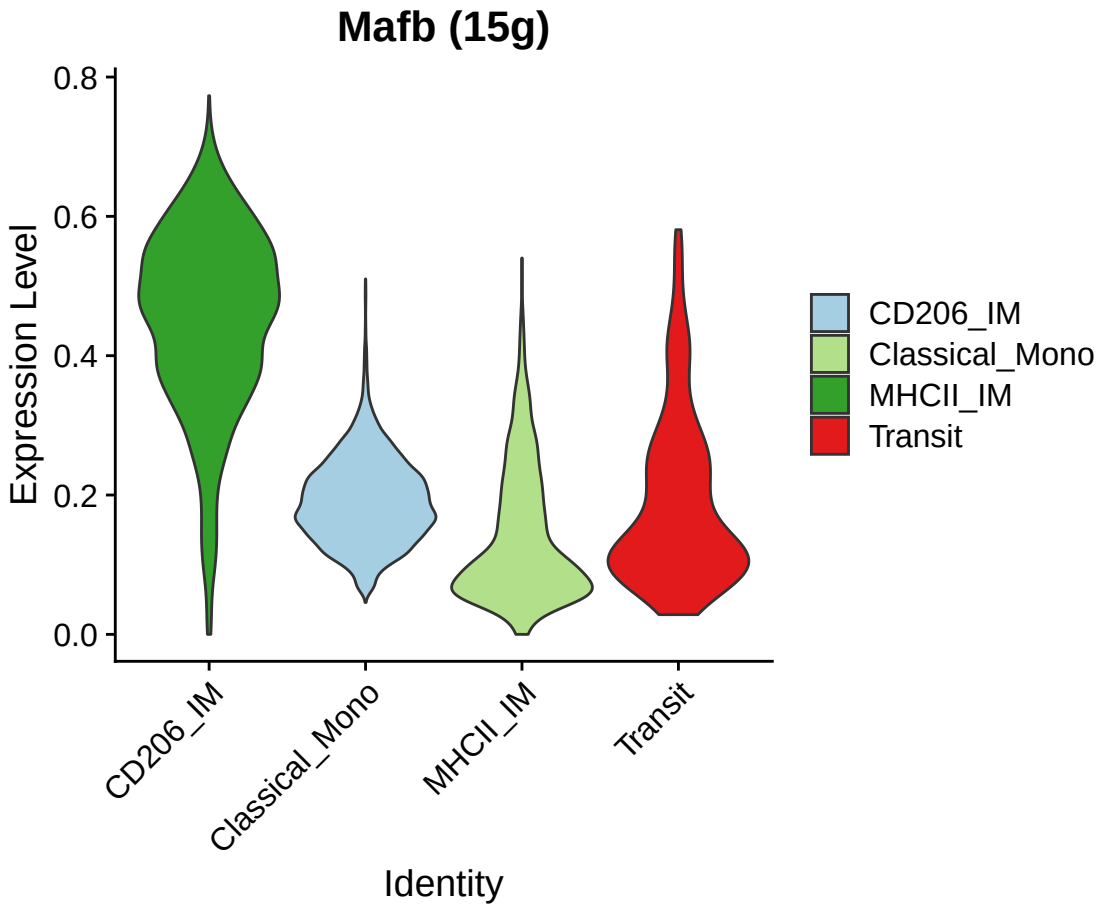



```
pal2 <- c(`Classical_Mono`="#A6CEE3",                                    1
                                        `MHCII_IM`="#B2DF8A",            2
                                        `CD206_IM`="#33A02C",            3
                                        `Transit` = "#E31A1C")           4
```

```
  VlnPlot(obj, features = "Maf␣(142g)", group.by  = "cell.type2", pt.size   1
     = 0, cols = pal2)
```

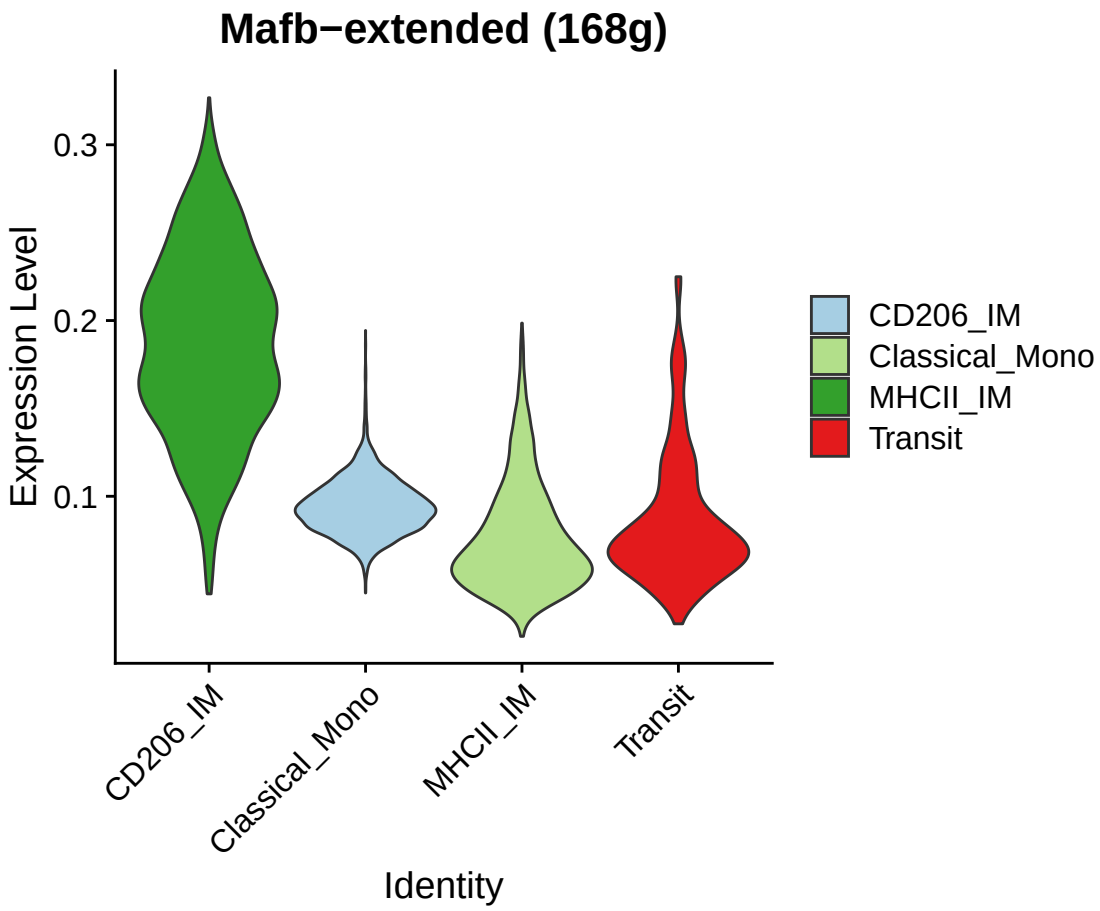



```
VlnPlot(obj, features = "Mafb␣(15g)", group.by  = "cell.type2", pt.size
   = 0, cols = pal2)
```




```
ggsave(filename = "../Figures/VlnPlot_Mafb_TF_activity_in_IM_
   differentiation.pdf",
       height = 5, width = 6)
```

```
  VlnPlot(obj, features = "Mafb-extended␣(168g)", group.by  = "cell.type2"
     , pt.size = 0, cols = pal2)
```



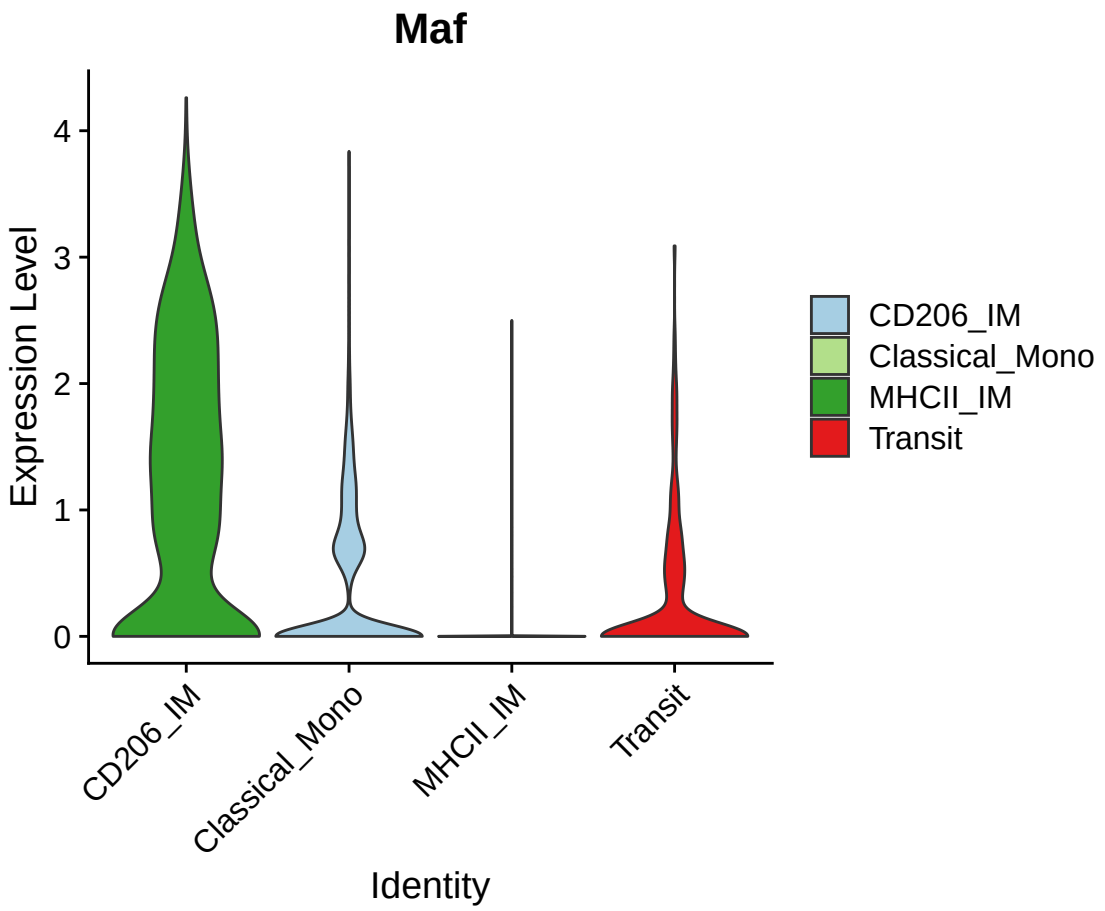
7.2 Show TF expression

```
DefaultAssay(obj) <- "RNA"
```

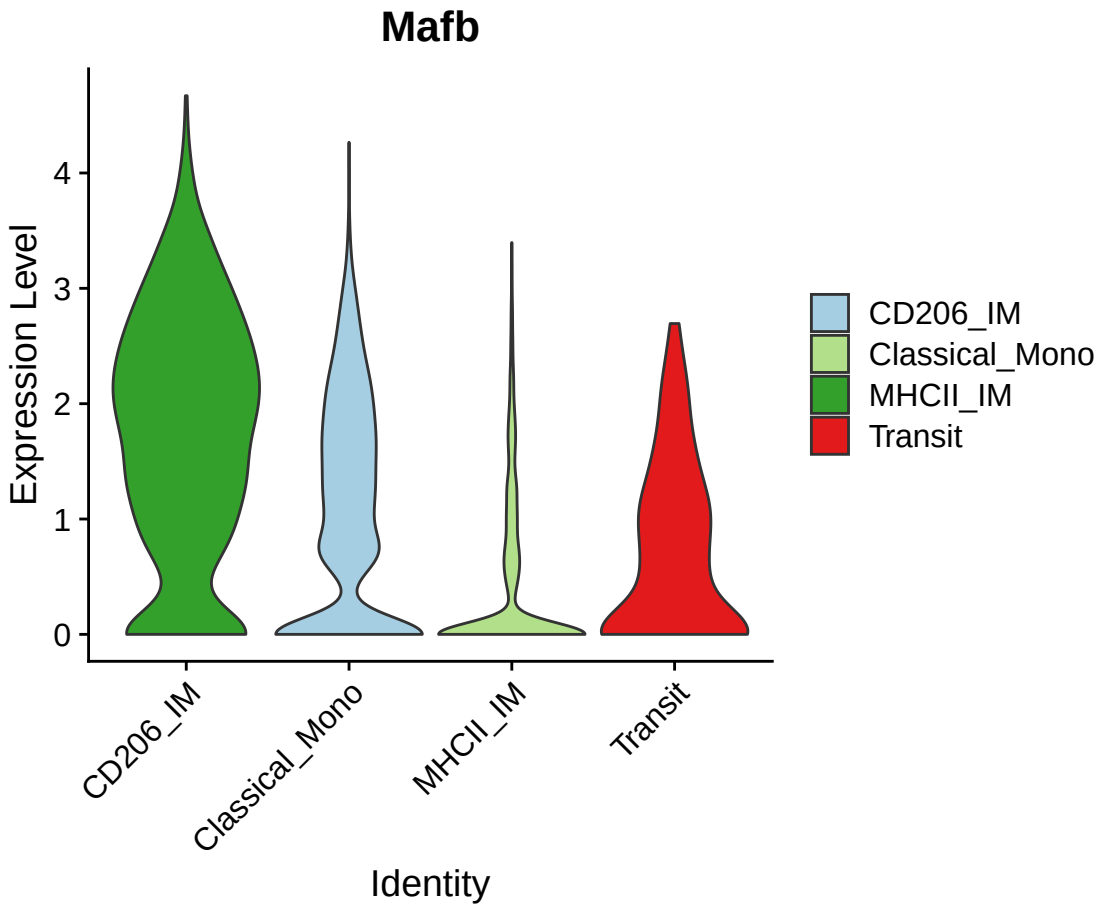
1

```
  VlnPlot(obj, features = "Maf", group.by  = "cell.type2", pt.size = 0,
     cols = pal2)
```

1




```
VlnPlot(obj, features = "Mafb", group.by  = "cell.type2", pt.size = 0,
    cols = pal2)
```




```
ggsave(filename = "../Figures/VlnPlot_Mafb_expression_in_IM_
   differentiation.pdf",
       height = 5, width = 6)
```

Save data

```
saveRDS(obj, file = "./only_IM_differentiation.with_SCENIC.seuratObject.
   Rds")
```

8 Session information

R sesssion:

```
sessionInfo()
```

```
## R version 4.0.3 (2020-10-10)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 20.04.3 LTS
##
## Matrix products: default
## BLAS:   /usr/lib/x86_64-linux-gnu/openblas-pthread/libblas.so.3
## LAPACK: /usr/lib/x86_64-linux-gnu/openblas-pthread/liblapack.so.3
##
## locale:
```

```
##  [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C                                 10
##  [3] LC_TIME=en_GB.UTF-8        LC_COLLATE=en_US.UTF-8                       11
##  [5] LC_MONETARY=en_GB.UTF-8    LC_MESSAGES=en_US.UTF-8                      12
##  [7] LC_PAPER=en_GB.UTF-8       LC_NAME=C                                    13
##  [9] LC_ADDRESS=C               LC_TELEPHONE=C                               14
## [11] LC_MEASUREMENT=en_GB.UTF-8 LC_IDENTIFICATION=C                          15
##                                                                              16
## attached base packages:                                                      17
##  [1] grid      parallel  stats4    stats     graphics  grDevices utils      18
##  [8] datasets  methods   base                                               19
##                                                                              20
## other attached packages:                                                     21
##  [1] ComplexHeatmap_2.6.2        SingleCellExperiment_1.12.0                 22
##  [3] SummarizedExperiment_1.20.0 Biobase_2.50.0                              23
##  [5] GenomicRanges_1.42.0        GenomeInfoDb_1.26.7                         24
##  [7] IRanges_2.24.1              S4Vectors_0.28.1                            25
##  [9] BiocGenerics_0.36.1         MatrixGenerics_1.2.1                        26
## [11] matrixStats_0.61.0          RColorBrewer_1.1-2                          27
## [13] pheatmap_1.0.12             genefilter_1.72.1                           28
## [15] ggplot2_3.3.5               SCopeLoomR_0.10.4                           29
## [17] RcisTarget_1.10.0           AUCell_1.13.3                               30
## [19] SCENIC_1.2.4                SeuratObject_4.0.4                          31
## [21] Seurat_4.0.5                                                            32
##                                                                              33
## loaded via a namespace (and not attached):                                   34
##   [1] circlize_0.4.13        systemfonts_1.0.3      plyr_1.8.6               35
##   [4] igraph_1.2.9           lazyeval_0.2.2         GSEABase_1.52.1          36
##   [7] splines_4.0.3          listenv_0.8.0          feather_0.3.5            37
##  [10] scattermore_0.7        digest_0.6.29          htmltools_0.5.2          38
##  [13] magick_2.7.3           fansi_0.5.0            magrittr_2.0.1           39
##  [16] memoise_2.0.1          tensor_1.5             cluster_2.1.0            40
##  [19] ROCR_1.0-11            globals_0.14.0         annotate_1.68.0          41
##  [22] R.utils_2.11.0         spatstat.sparse_2.0-0  colorspace_2.0-2         42
##  [25] blob_1.2.2             ggrepel_0.9.1          textshaping_0.3.6        43
##  [28] xfun_0.28              dplyr_1.0.7            crayon_1.4.2             44
##  [31] RCurl_1.98-1.5         jsonlite_1.7.2         graph_1.68.0             45
##  [34] spatstat.data_2.1-0    survival_3.2-7         zoo_1.8-9                46
##  [37] glue_1.5.1             polyclip_1.10-0        gtable_0.3.0             47
##  [40] zlibbioc_1.36.0        XVector_0.30.0         leiden_0.3.9             48
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9 References