

Monocytes can Proliferate in Vacant Tissue Niches prior to Differentiation into Macrophages

8-SCENIC analysis

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Abstract

Resident tissue macrophages (RTM) are differentiated immune cells populating distinct niches and exhibiting important tissue-supportive functions. RTM maintenance is thought to depend either on monocyte engraftment and differentiation, or on the self-renewal of mature RTM. Here, we discovered that monocytes can re-enter cell cycle and proliferate locally before their differentiation into RTM. We developed a mouse model of inducible lung interstitial macrophage (IM) depletion in which the vacant niche is repopulated by BM-derived monocytes giving rise to fully differentiated IM subsets. By performing time-course single-cell RNA-sequencing analyses of myeloid cells during niche refilling, we found that few Ly6C+ classical monocytes could self-renew locally in a CSF1R-dependent manner. We further showed that the transcription factor MafB restricted such proliferation and was essential to mediate RTM specification and identity in our model. Our data provide evidence that, in the mononuclear phagocyte system, self-renewal is not merely restricted to myeloid progenitor cells and mature macrophages, but is also a tightly regulated capability of mature monocytes developing into RTM *in vivo*.

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1 Description

To predict the potential active transcription factors (TF), Ly6C+ cMo, transit cells, CD206- and CD206+ IM were subjected to SCENIC analysis using SCENIC package (Aibar et al., 2017). The normalized counts, nFeature_RNA, nCount_RNA in merged Seurat object were used for the initial SCENIC analysis. The genes expressed with a value of 3 in 0.5% of the cells and detected in 1% of the cells were kept for following SCENIC analysis. Co-expression network analysis was made with GENIE3 in the SCENIC package. To represent the SCENIC results, the results of 3.4_regulonsAUC were added to the metadata of Seurat object so that regulon AUC scores could be plot using FeaturePlot function. The top 50 regulons with highest variance were showed in the heatmap with their Z-scores.

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$cell.type2, nFeature_RNA = 1
  obj@meta.data$nFeature_RNA, nCount_RNA = obj@meta.data$nCount_RNA)
rownames(cellInfo) <- colnames(obj) 2
head(cellInfo) 3
```

Save data for later:

```
saveRDS(cellInfo, file="int/cellInfo.Rds") 1
```

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$CellType, legend=names(colVars$ 1
  CellType)) 2
  3
  4
  5
  6
  7
  8
  9
```

3 Initialize settings

```
scenicOptions <- initializeScenic(org="mgi",
  dbDir=".~/Data/Scenic_database/cisTarget_ 1
  databases/", # this should be
  prepared before, see SCENIC package
  instructions
  nCores=22 # use appropriate core number 2
  )
# NOTE: nCores is the number of cores used for calculating. For GENIE3
  analysis is time-consuming and calculation-consuming, make this
  nCores maximum. 3
  4
  5
```

```
scenicOptions$inputDatasetInfo$cellInfo <- "int/cellInfo.Rds" 1
scenicOptions$inputDatasetInfo$colVars <- "int/colVars.Rds" 2
# update scenicOptions object.
saveRDS(scenicOptions, file="int/scenicOptions.Rds") 3
  4
```

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 algorithm is built on Random 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") 1
```

Load regulon AUC score:

```
regulonAUC <- readRDS("../.../IM_DTR_exp2/analyses/20210505_SCENIC_only_IM_DIFFERENTIATION/int/3.4_regulonAUC.Rds") 1  
1
```

Extract the useful information:

```
regulonAUC.mat <- regulonAUC@assays@data@listData$AUC 1  
dim(regulonAUC.mat) 2
```

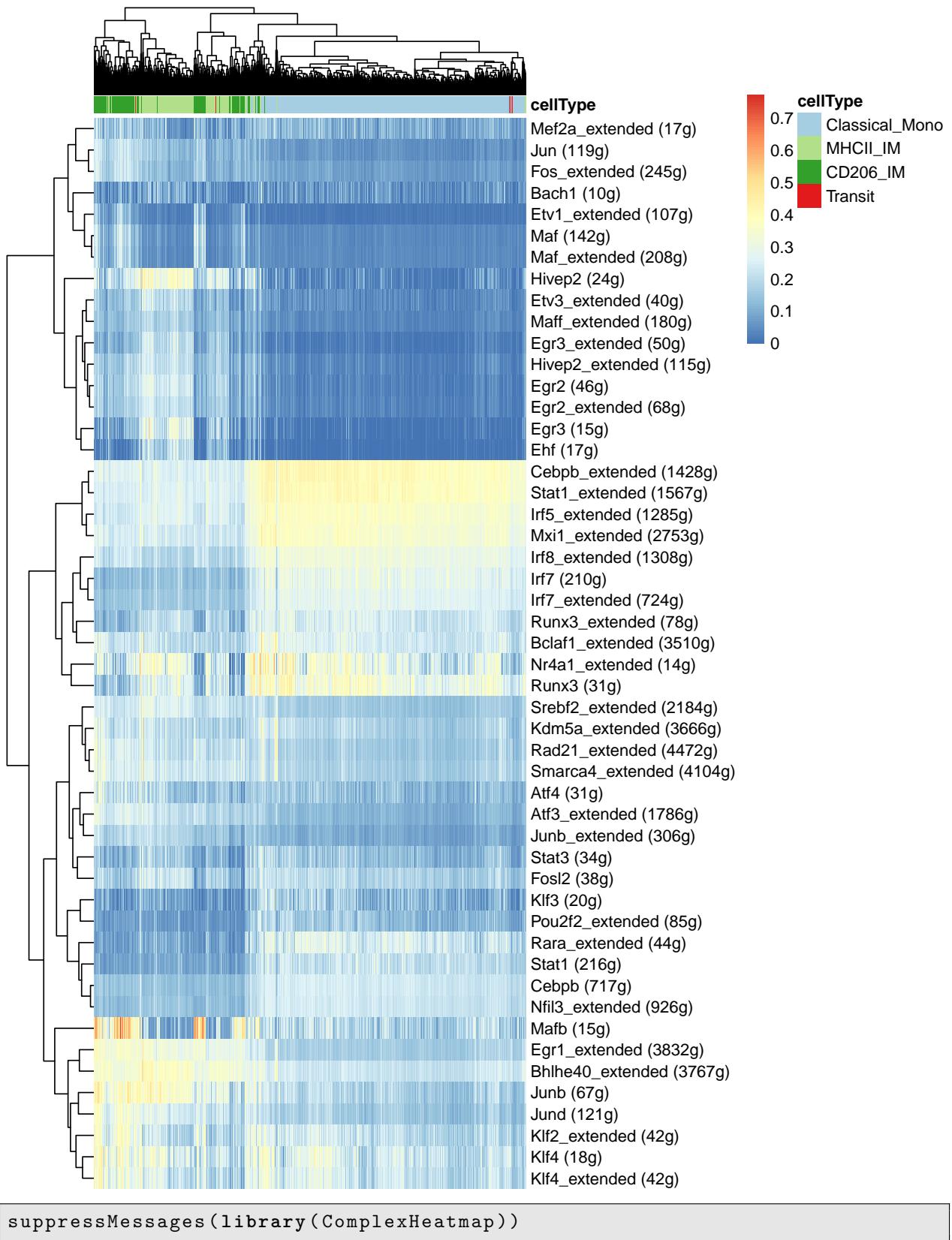
```
## [1] 398 10566 1
```

Now take the top 50 most variable Regulons:

```
suppressMessages(library(genefilter)) 1  
rv <- rowVars(regulonAUC.mat) 2  
idx <- order(-rv)[1:50] 3
```

```
sce <- readRDS("./data/IM-DTR3_3D.sce.Rds") 1
```

```
suppressMessages({ 1  
  library(pheatmap) 2  
  library(RColorBrewer) 3  
  library(SingleCellExperiment) 4  
) 5  
  
pal <- brewer.pal(length(levels(sce@colData$ident)), name = "Paired") 6  
7  
cellInfo.cellType <- data.frame(cellType = sce@colData$ident) 8  
rownames(cellInfo.cellType) <- colnames(sce) 9  
annotationColors <- list(cellType = c(`Classical_Mono`="#A6CEE3", 10  
  `MHCII_IM`="#B2DF8A", 11  
  `CD206_IM`="#33A02C", 12  
  `Transit` = "#E31A1C")) 13  
14  
pheatmap(regulonAUC.mat[idx,], show_colnames = FALSE, annotation_col = 15  
  cellInfo.cellType, annotation_colors = annotationColors)
```



Randomly select 1500 cells in monocytes and IMs:

```

1 set.seed(41)
2 cellnames.15k <- unlist(sapply(unique(sce$cell.type2), function(x) {
3   cellnames <- colnames(sce)[sce$cell.type2== x ]
4   if (length(cellnames)>1500) {
5     cellnames <- cellnames[sample(1:length(cellnames), 1500)]
6   }
7   return(cellnames)
8 }) )

```

```

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

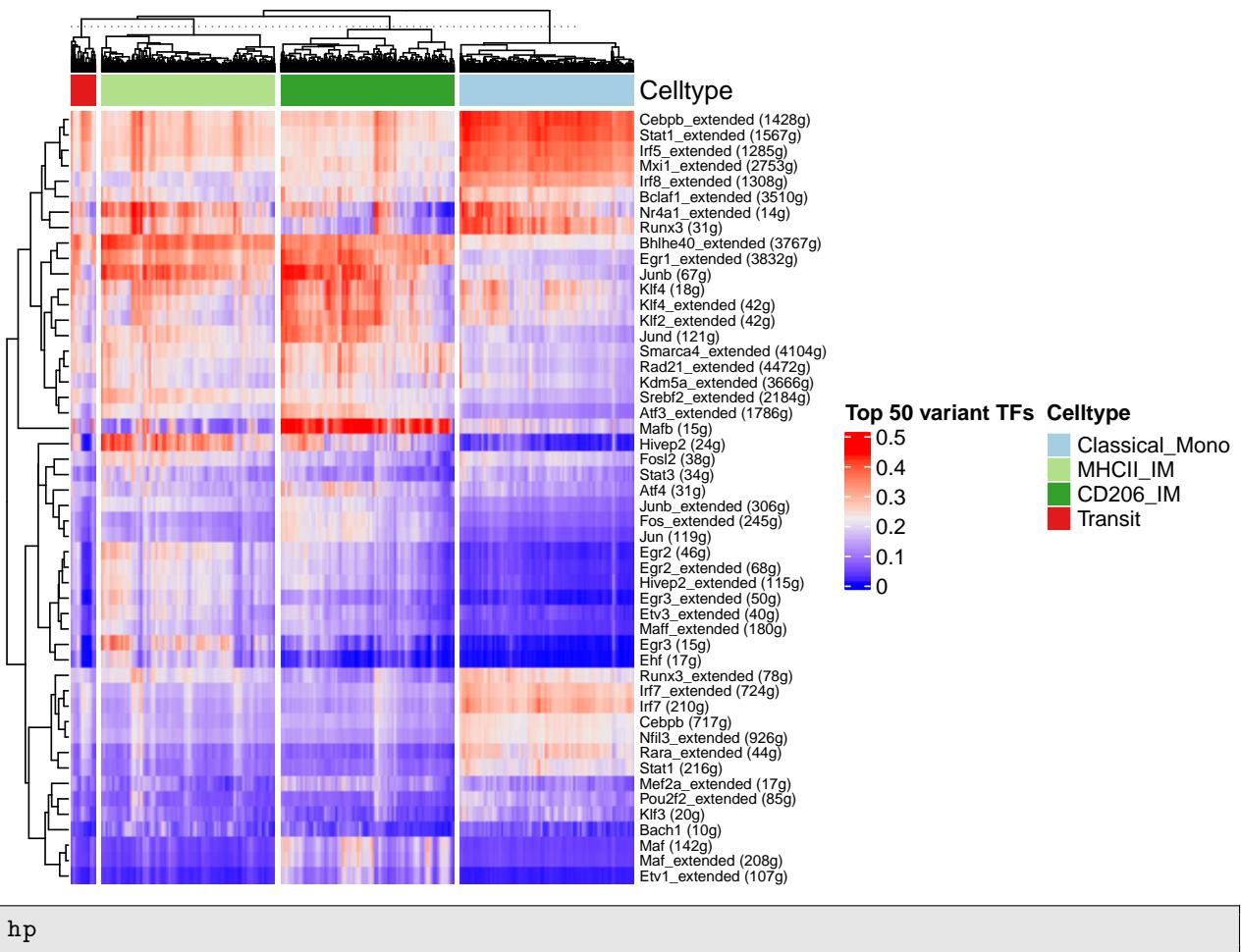
```

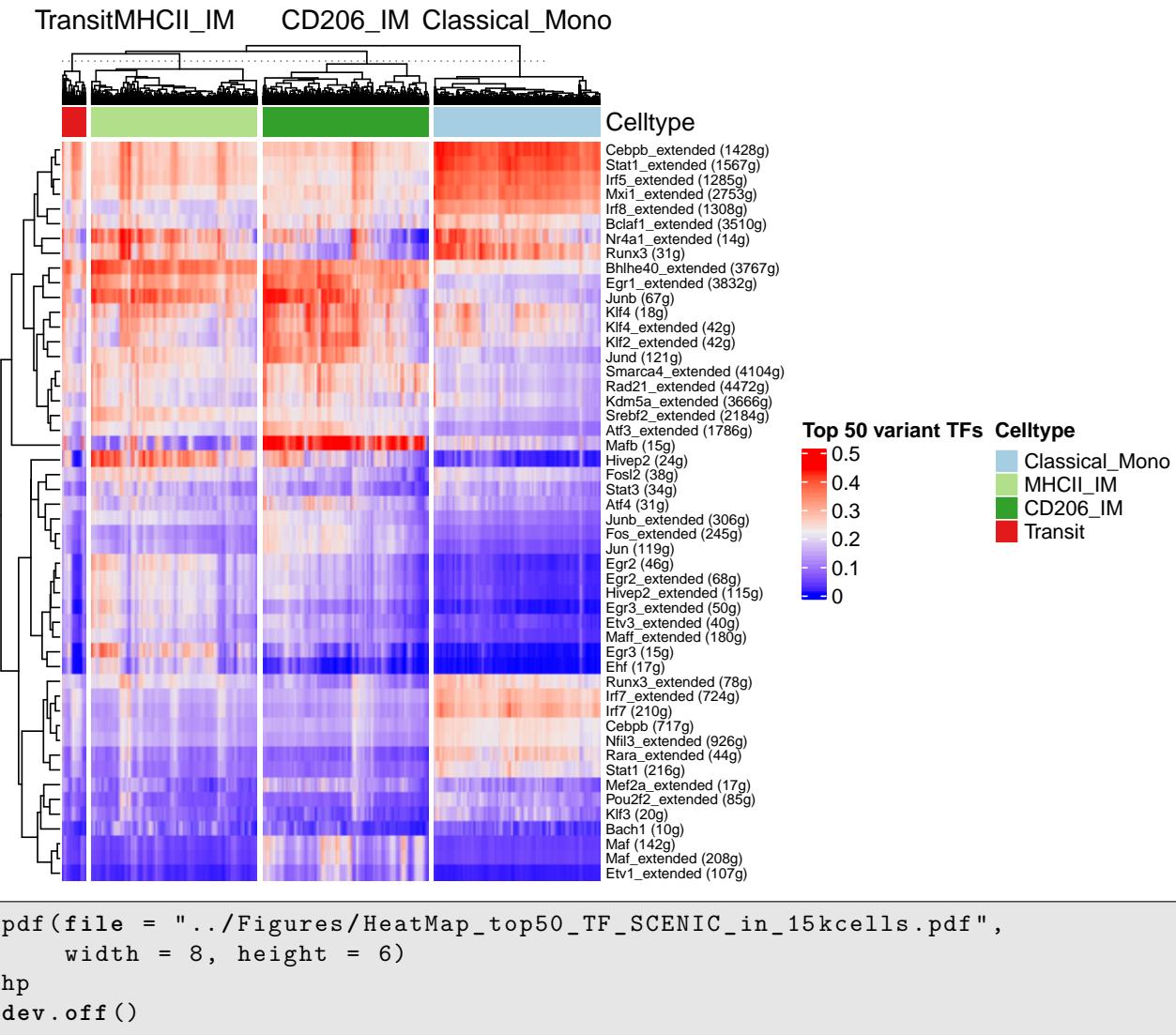
```

1 hp <- Heatmap(top50.15k.mat, name = "Top_50_variant_TFs",
2   show_row_names=TRUE, show_column_names = FALSE,
3   row_names_gp = gpar ( fontsize = 7),
4   column_split = factor(sce.15k$cell.type2),
5   top_annotation = HeatmapAnnotation(Celltype=sce.15k$cell.type2,
6                                     col = list(Celltype =
7                                       c(`Classical_Mono`="#A6CEE3",
8                                         `MHCII_IM`="#B2DF8A",
9                                         `CD206_IM`="#33A02C",
10                                         `Transit` = "#E31A1C"))))
11 )
12 hp <- draw(hp)

```

TransitMHCII_IM CD206_IM Classical_Mono





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"

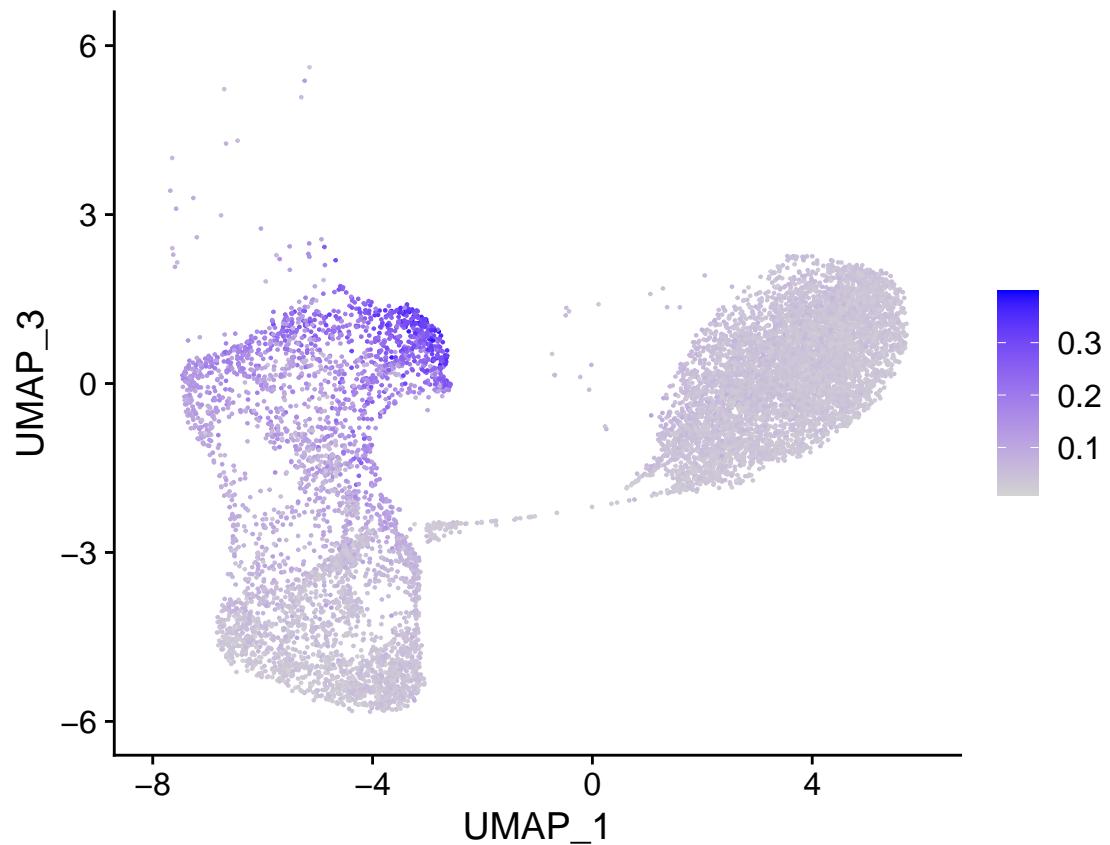
```

```

FeaturePlot(obj, features = "Maf_(142g)", dims = c(1,3))

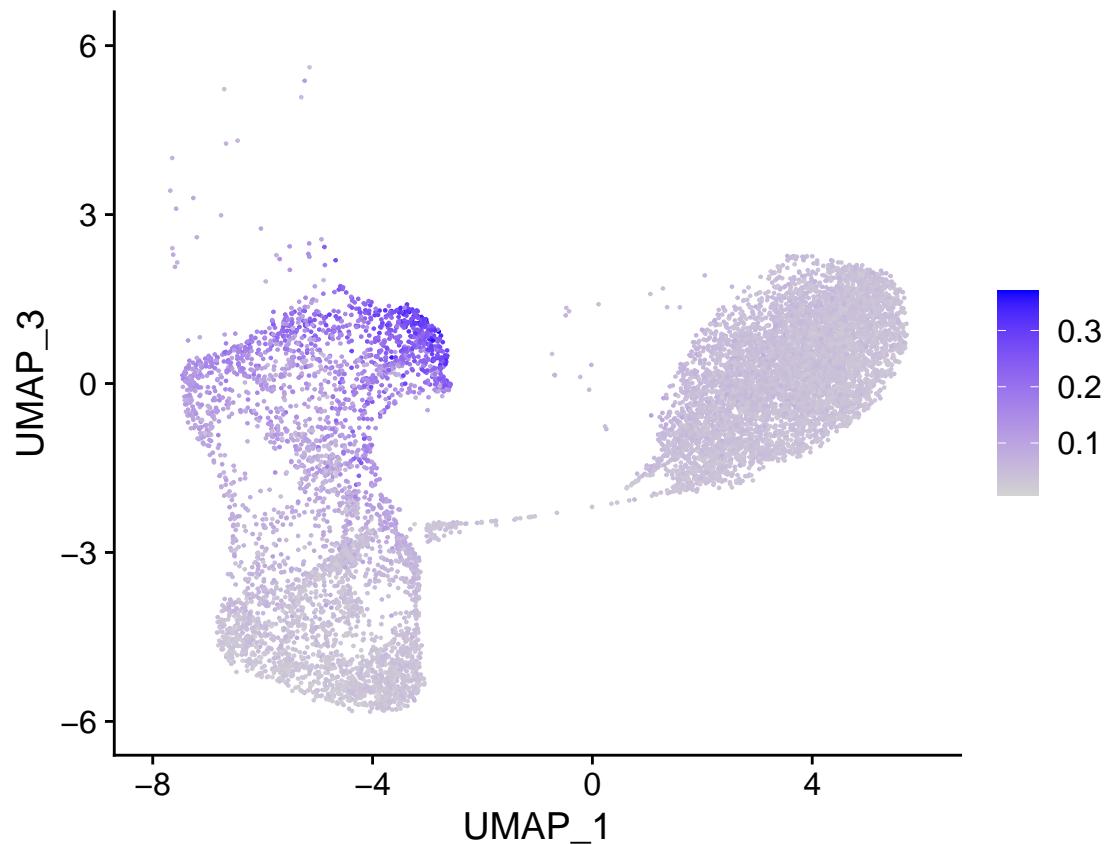
```

Maf (142g)



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

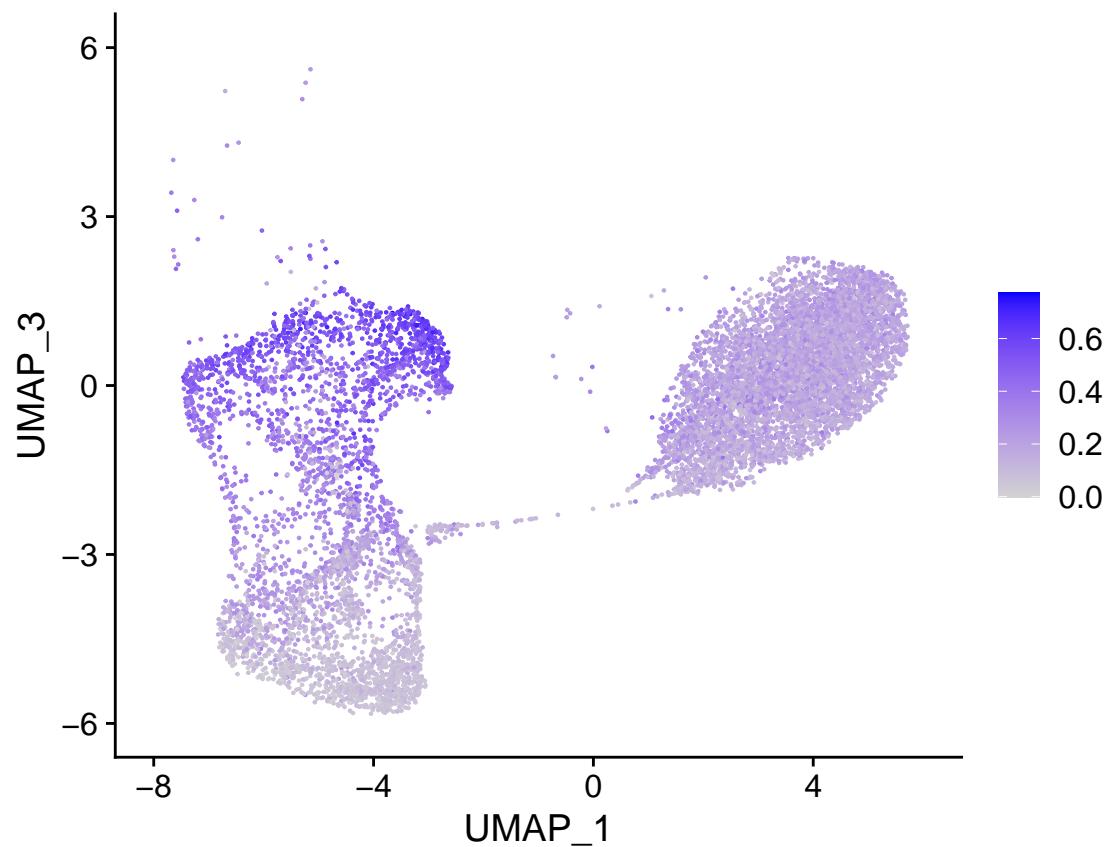
Maf-extended (208g)



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

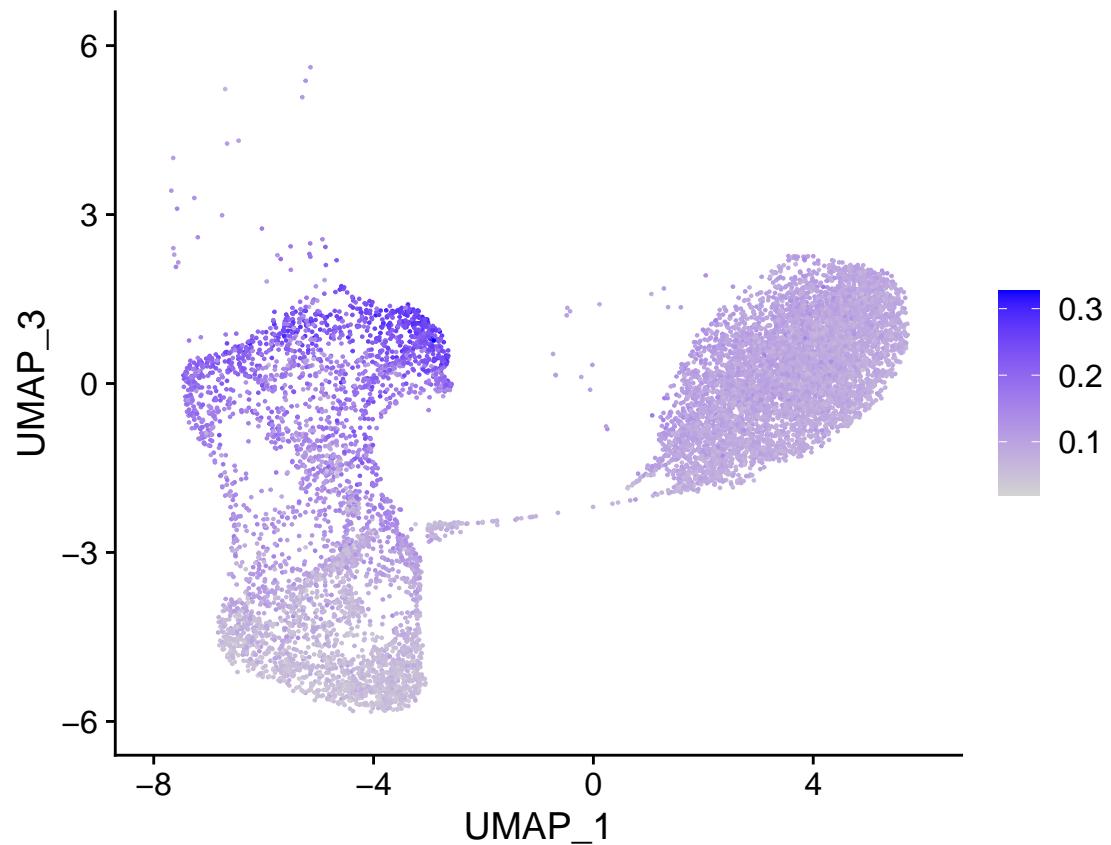
1

Mafb (15g)



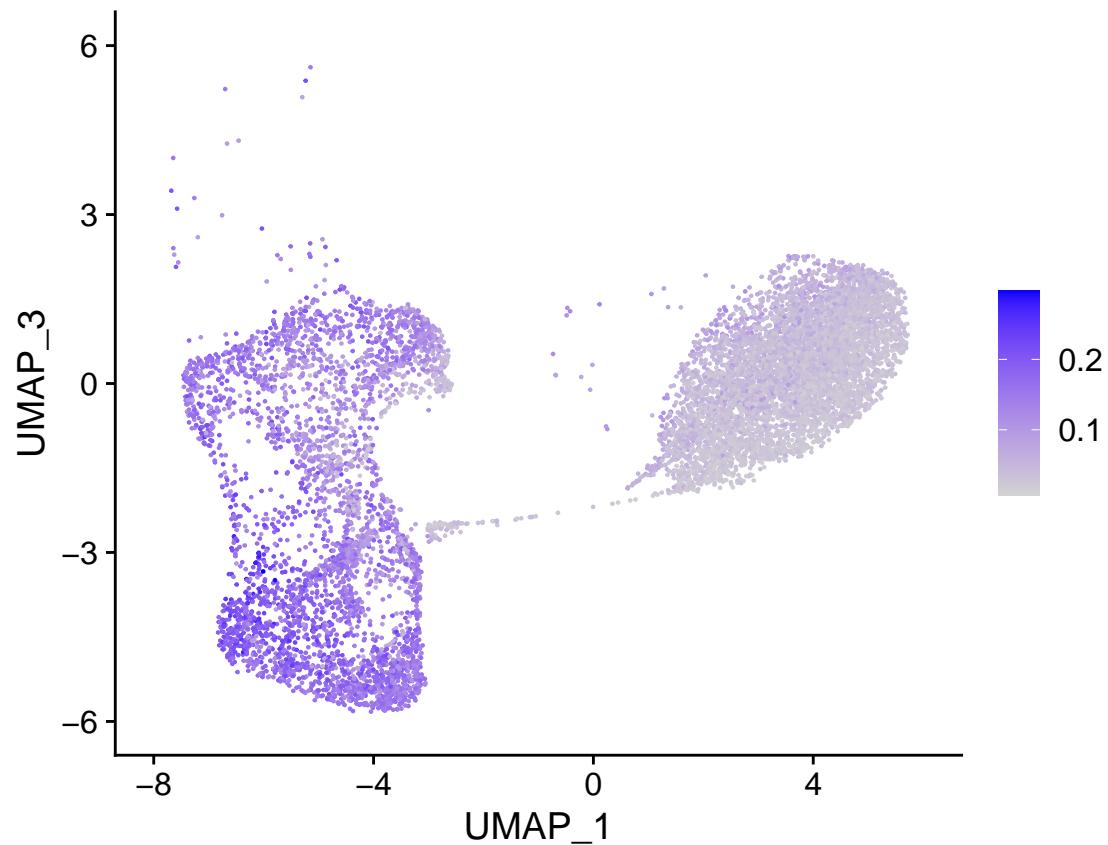
```
FeaturePlot(obj, features = "Mafb-extended_U(168g)", dims = c(1,3))
```

Mafb–extended (168g)



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

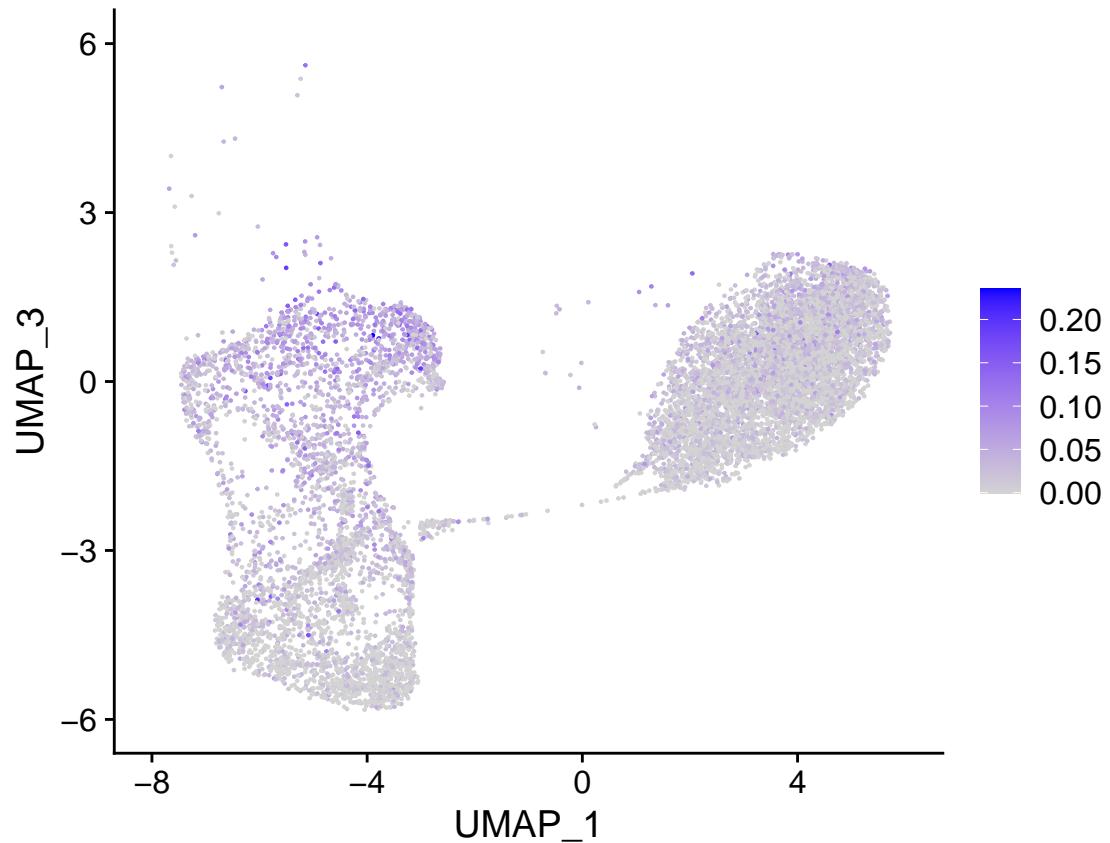
Maff–extended (180g)



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

1

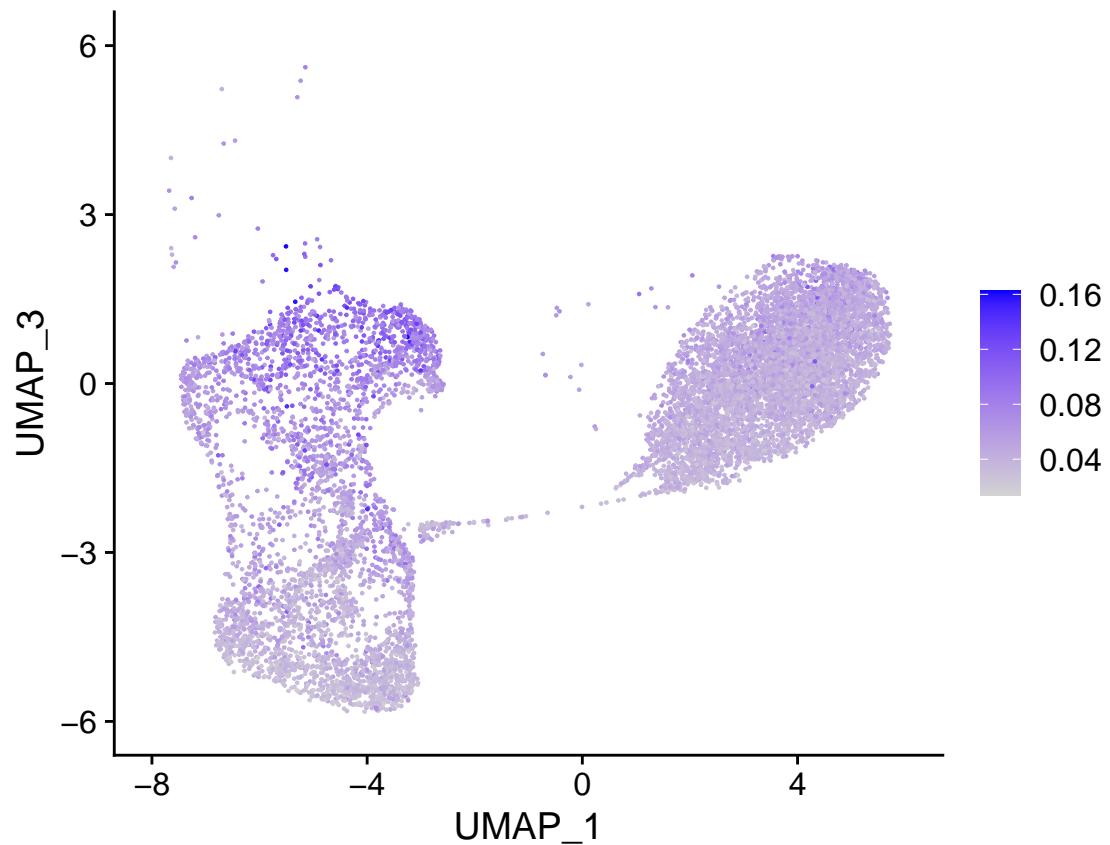
Mafg (18g)



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

1

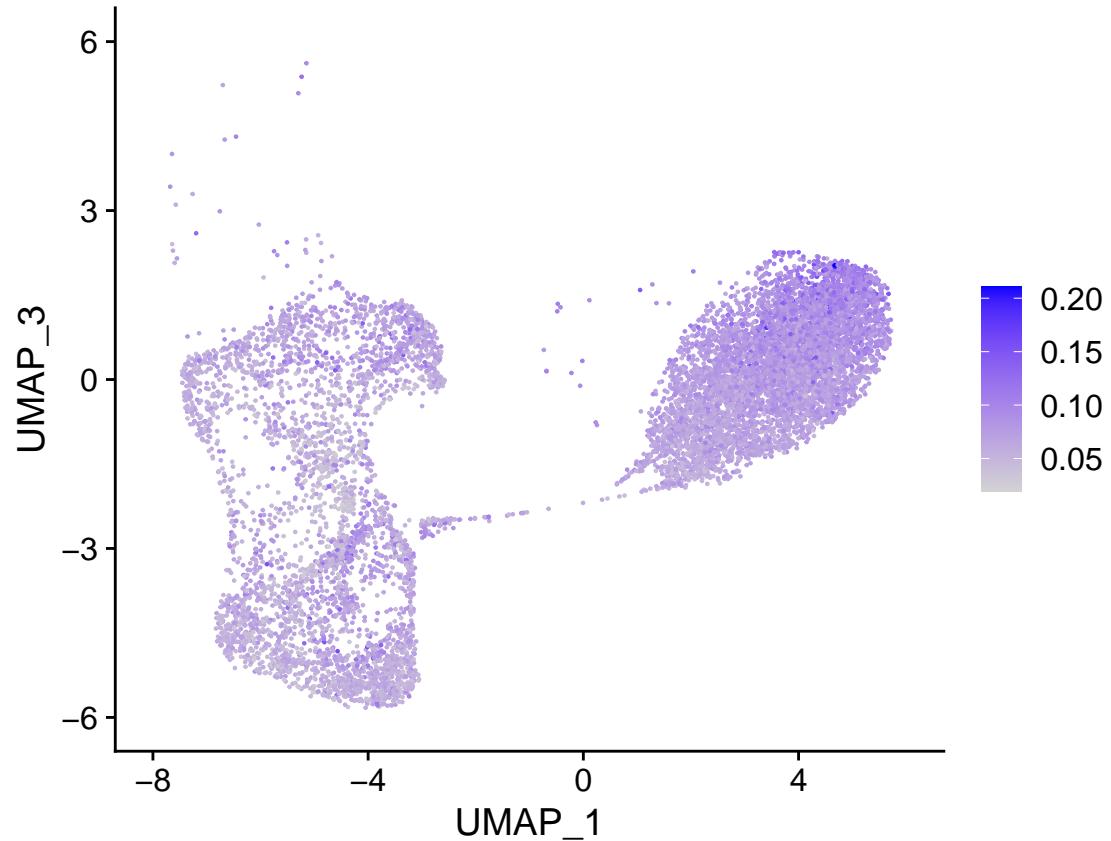
Mafg-extended (334g)



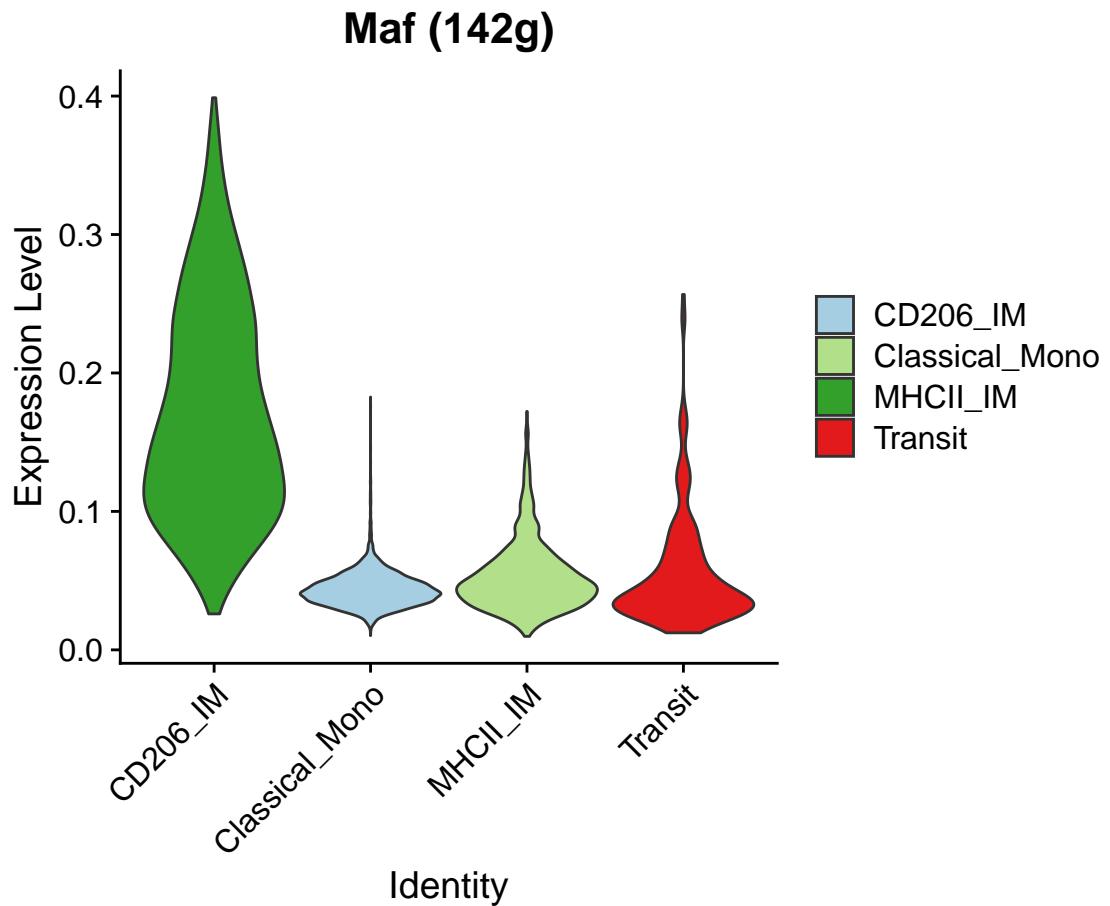
```
FeaturePlot(obj, features = "Mafk-extended (795g)", dims = c(1,3))
```

1

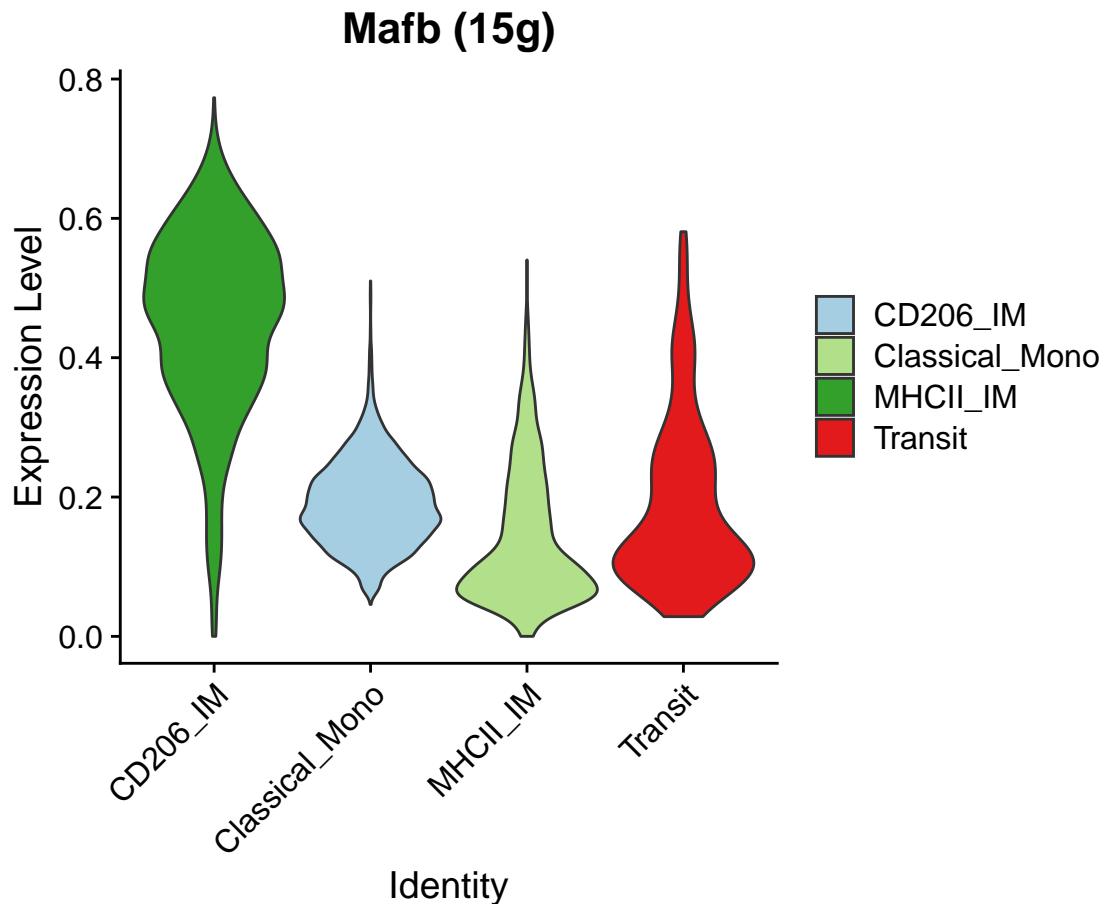
Mafk-extended (795g)



```
pal2 <- c(`Classical_Mono` = "#A6CEE3",  
           `MHCII_IM` = "#B2DF8A",  
           `CD206_IM` = "#33A02C",  
           `Transit` = "#E31A1C")  
  
VlnPlot(obj, features = "Mafk(142g)", group_by = "cell.type2", pt.size  
= 0, cols = pal2)
```

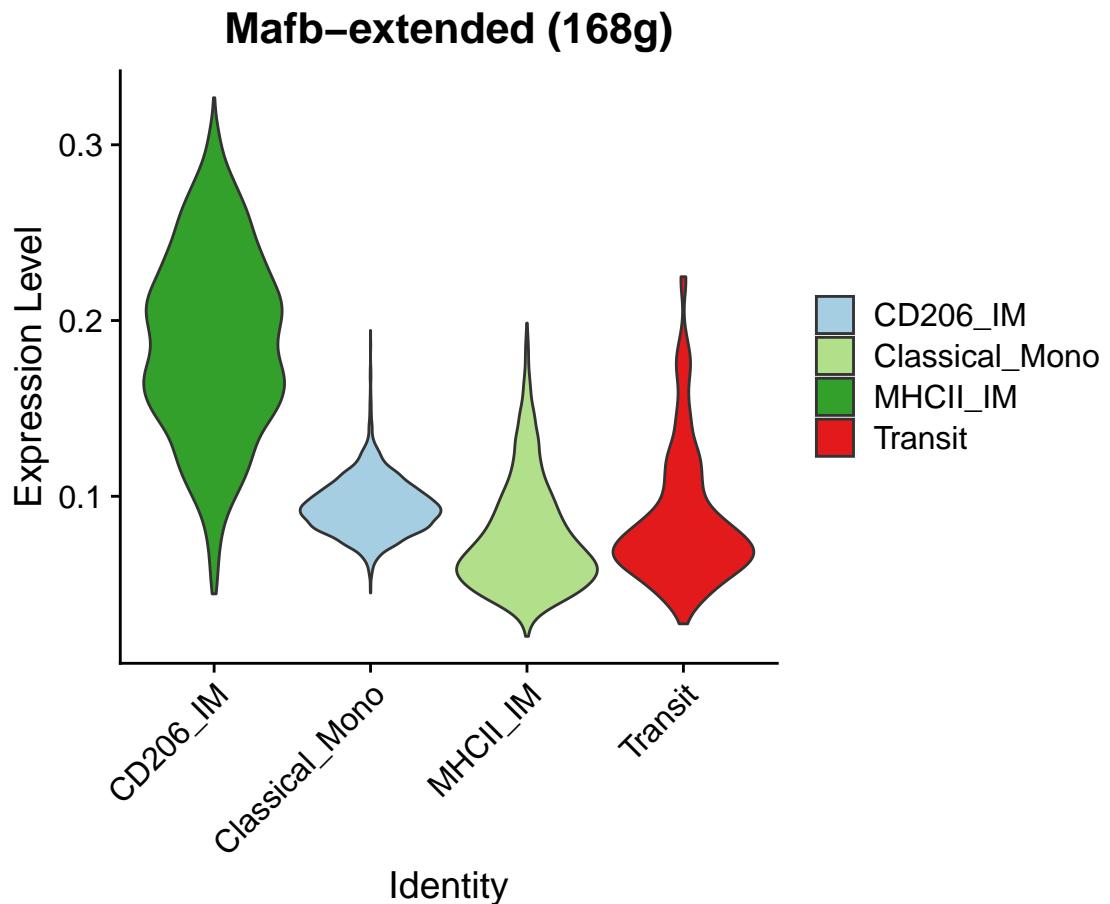


```
VlnPlot(obj, features = "Mafb\u222a(15g)", group.by = "cell.type2", pt.size = 0, cols = pal2)
```



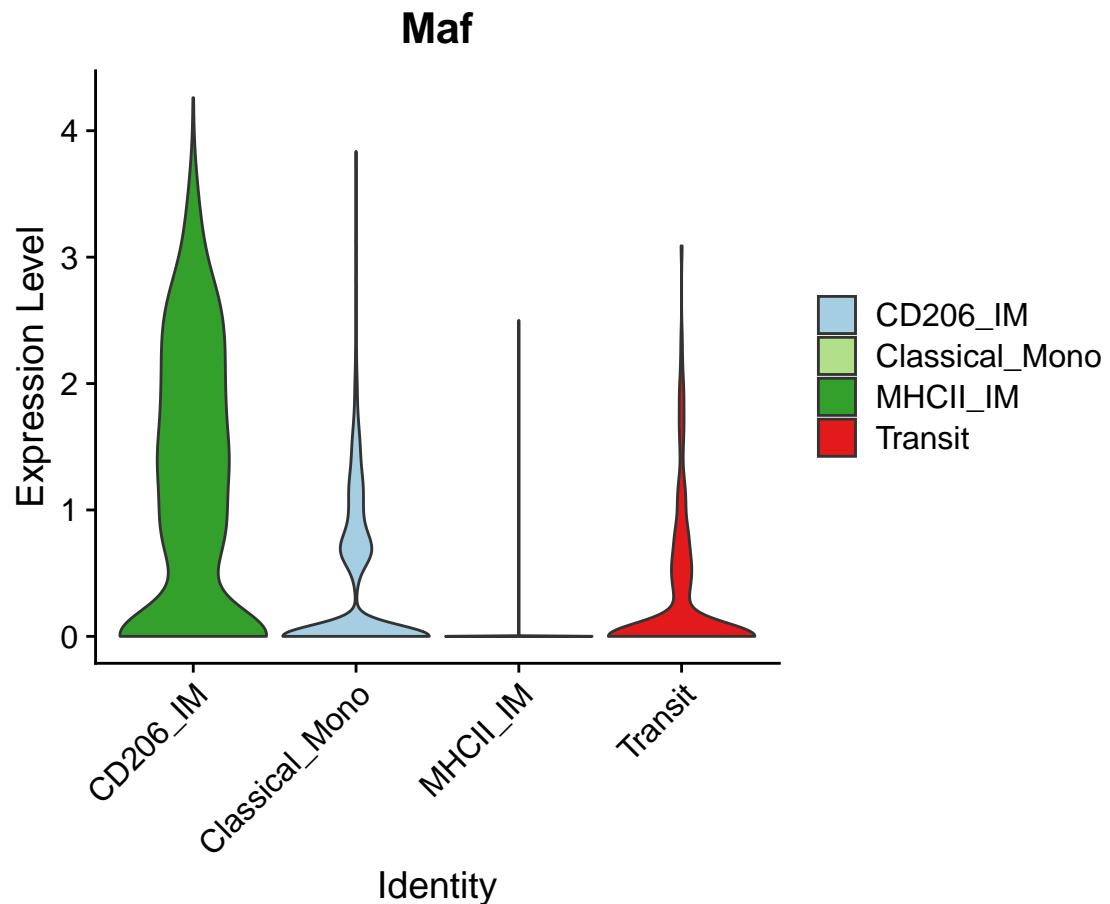
```
1 ggsave(filename = "../Figures/VlnPlot_Mafb_TF_activity_in_IM_
2 differentiation.pdf",
height = 5, width = 6)
```

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

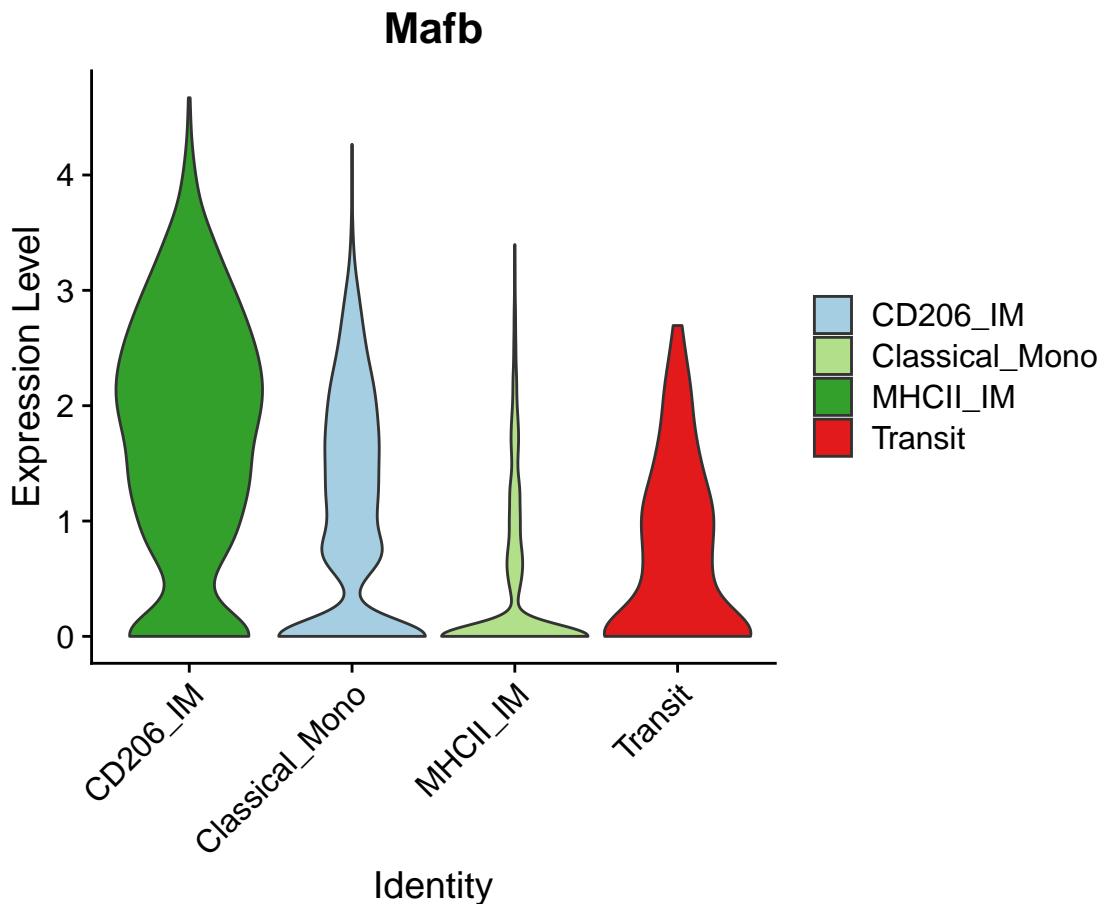


7.2 Show TF expression

```
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) 1
```



```
ggsave(filename = "../Figures/VlnPlot_Mafb_expression_in_IM_
differentiation.pdf",
height = 5, width = 6) 1
2
```

Save data

```
saveRDS(obj, file = "./only_IM_differentiation.with_SCENIC.seuratObject.
Rds") 1
2
```

8 Session information

R sesssion:

```
sessionInfo() 1

## 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
2
3
4
5
6
7
8
9
```

## [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	plyr_1.8.6	35
## [4] lazyeval_0.2.2	GSEABase_1.52.1	36
## [7] listenenv_0.8.0	feather_0.3.5	37
## [10] digest_0.6.29	htmltools_0.5.2	38
## [13] fansi_0.5.0	magrittr_2.0.1	39
## [16] tensor_1.5	cluster_2.1.0	40
## [19] globals_0.14.0	annotate_1.68.0	41
## [22] spatstat.sparse_2.0-0	colorspace_2.0-2	42
## [25] ggrepel_0.9.1	xfun_0.28	43
## [28] crayon_1.4.2	RCurl_1.98-1.5	44
## [31] graph_1.68.0	spatstat.data_2.1-0	45
## [34] zoo_1.8-9	glue_1.5.1	46
## [37] gtable_0.3.0	zlibbioc_1.36.0	47
## [40] leiden_0.3.9	GetoptLong_1.0.5	48
## [43] shape_1.4.6	future.apply_1.8.1	49
## [46] scales_1.1.1	DBI_1.1.1	50
## [49] Rcpp_1.0.7	viridisLite_0.4.0	51
## [52] clue_0.3-60	reticulate_1.22	52
## [55] bit_4.0.4	htmlwidgets_1.5.4	53
## [58] ellipsis_0.3.2	ica_1.0-2	54
## [61] pkgconfig_2.0.3	XML_3.99-0.8	55
## [64] uwot_0.1.11	deldir_1.0-6	56
## [67] labeling_0.4.2	tidyselect_1.1.1	57
## [70] reshape2_1.4.4	later_1.3.0	58
.52.0		
## [73] munsell_0.5.0	tools_4.0.3	59
## [76] generics_0.1.1	RSSQLite_2.2.9	60
## [79] evaluate_0.14	stringr_1.4.0	61
## [82] yaml_2.2.1	goftest_1.2-3	62

## [85] bit64_4.0.5	fitdistrplus_1.1-6	purrr_0.3.4	63
## [88] RANN_2.6.1	pbapply_1.5-0	future_1.23.0	64
## [91] nlme_3.1-153	mime_0.12	R.oo_1.24.0	65
## [94] hdf5r_1.3.5	compiler_4.0.3	plotly_4.10.0	66
## [97] png_0.1-7	spatstat.utils_2.2-0	tibble_3.1.6	67
## [100] stringi_1.7.6	highr_0.9	lattice_0.20-41	68
## [103] Matrix_1.3-4	vctrs_0.3.8	pillar_1.6.4	69
## [106] lifecycle_1.0.1	GlobalOptions_0.1.2	spatstat.geom_2.3-0	70
## [109] lmtest_0.9-39	RcppAnnoy_0.0.19	data.table_1.14.2	71
## [112] cowplot_1.1.1	bitops_1.0-7	irlba_2.3.5	72
## [115] httpuv_1.6.3	patchwork_1.1.1	R6_2.5.1	73
## [118] promises_1.2.0.1	KernSmooth_2.23-20	gridExtra_2.3	74
## [121] parallelly_1.29.0	codetools_0.2-18	MASS_7.3-53	75
## [124] assertthat_0.2.1	rjson_0.2.20	withr_2.4.3	76
## [127] sctransform_0.3.2	GenomeInfoDbData_1.2.4	hms_1.1.1	77
## [130] mgcv_1.8-33	rpart_4.1-15	tidyr_1.1.4	78
## [133] rmarkdown_2.11	Cairo_1.5-12.2	Rtsne_0.15	79
## [136] shiny_1.7.1			80

References

Aibar, S., González-Blas, C.B., Moerman, T., Huynh-Thu, V.A., Imrichova, H., Hulselmans, G., Rambow, F., Marine, J.C., Geurts, P., Aerts, J., et al. (2017). SCENIC: Single-cell regulatory network inference and clustering. *Nature Methods* 2017 14:11 1083–1086.