Mafb-restricted local monocyte proliferation precedes lung interstitial macrophage differentiation

0-Microarray data 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 rely on either monocyte engraftment and differentiation, or RTM self-renewal. Here, we developed an inducible mouse model of lung interstitial macrophage (IM) niche depletion and repopulation to investigate IM development in vivo. Using time-course single-cell RNA-sequencing analyses, bone marrow chimeras and gene targeting, we found that engrafted Ly6C+ classical monocytes could self-renew locally in a CSF1R-dependent manner before their differentiation into RTM. We further showed that the switch from monocyte proliferation towards IM subset specification was controlled by MafB, while c-Maf specifically regulated the identity of the CD206+ IM subset. Our data shed new light on the transcriptional regulation of IM development and 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.

Contents

1 Description

In order to build a lung interstitial macrophage (IM) specific mouse model, we need to find genes specific to this cell type comparing to other myeloid cell types in the lung. That's why we collected published microarray data and compared the expression profile of them.

The following published microarray data were used.

Sample name	Source	IDENTIFIER (Raw data)
Classical Monocytes MHCII+ in blood	ImmGen	GEO: GSM605868, GSM605870, GSM605871
Classical Monocytes MHCII- in bone marrow	ImmGen	GEO: GSM854329, GSM854330, GSM854331
Classical Monocytes MHCII- in blood	ImmGen	GEO: GSM605872, GSM605873, GSM605874
Nonclassical Monocytes, MHCII+	ImmGen	GEO: GSM605878, GSM605879
Nonclassical Monocytes in bone marrow	ImmGen	GEO: GSM854332, GSM854333, GSM854334
Nonclassical Monocytes in blood	ImmGen	GEO: GSM605884, GSM605885
Nonclassical Monocytes, MHCII int	ImmGen	GEO: GSM605886, GSM605887, GSM605888, GSM605889, GSM605890
Lung CD11b+ CD24- macrophage	ImmGen	GEO: GSM854271, GSM854272
Small Intestinal Lamina Propria CD11c-hi CD103- CD11b+ MF	ImmGen	GEO: GSM854262, GSM854263, GSM854264, GSM854265, GSM854266, GSM854267, GSM854268
Bone marrow macrophages	ImmGen	GEO: GSM854317, GSM854318, GSM854319
Spleen Red Pulp macrophages	ImmGen	GEO: GSM605853, GSM605854, GSM605855
Peritoneal macrophage steady state	ImmGen	GEO: GSM854294, GSM854295, GSM854296
Peritoneal cavity macrophages steady state	ImmGen	GEO: GSM605850, GSM605851, GSM605852
Medullary macrophages from skin draining lymph nodes	ImmGen	GEO: GSM854322, GSM854323
Central nervous system microglia	ImmGen	GEO: GSM854326, GSM854327, GSM854328
CD103+ migratory DC, Mediastinal LN CD103+ DC	ImmGen	GEO: GSM854243, GSM854244, GSM854245
CD11b+ migratory DC, Mediastinal LN CD11b+ DC	ImmGen	GEO: GSM854255, GSM854256, GSM854257
Lung CD103+ dendritic cells	ImmGen	GEO: GSM538231, GSM538232, GSM538233, GSM854241, GSM854242
Lung MHCII+ CD11c+ CD103- CD11b+ CD24+ dendritic cells	ImmGen	GEO: GSM854269, GSM854270
Lung IM, Ly6C+ cMo and AM	[1]	EMBL-EBI: E-MTAB-5012

2 Load packages and data

The gene expression activity matrix for each samples were generated in Gene Expression Commons (https://gexc.riken.jp).[2]

```
# packages
library(ComplexHeatmap)
library(RColorBrewer)
library(circlize)

# data
dir.files <- "./downloaded_data"
files.list <- list.files(dir.files, pattern = "Gene_Expression_Activity.
   csv", full.names = T)
```

```
names.list <- sub(basename(files.list), pattern = "_in_Gene_Expression_Activity.csv",
    replacement = "")
```

read csv files and bind tables to one:

```
expr.table <- data.frame(read.csv(files.list[1]), row.names = 1)
expr.table <- expr.table[, -2]
n.rep <- length(2:ncol(expr.table))

names.rep <- paste(rep(names.list[1]), 1:n.rep, sep = "_")
colnames(expr.table)[2:ncol(expr.table)] <- names.rep

for (i in 2:length(files.list)) {
    tb <- read.csv(files.list[i])
    tb <- tb[, 4:ncol(tb)]
    n <- ncol(tb)
    repname <- paste(rep(names.list[i]), 1:n, sep = "_")
    colnames(tb) <- repname

    expr.table <- cbind(expr.table, tb)
    n.rep <- append(n.rep, n)
    names.rep <- append(names.rep, repname)
}
meta.sample <- data.frame(sampleName = names.list, n.rep = n.rep)
head(expr.table)
```

```
## # A tibble: 6 x 70
##   Gene.Symbol GEXC_AMs_1 GEXC_AMs_2 GEXC_AMs_3 `GEXC_DC_Lu_CD~` `GEXC_DC_Lu_CD~`
##   <chr>            <dbl>      <dbl>      <dbl>            <dbl>            <dbl>
## 1 ---               12.2       25.8      -0.23             64.1             75.2
## 2 ---               17.3       33.8     -15.0              40.0             60.2
## 3 ---               -2.6       30.2      -9.45             45.3             59.4
## 4 ---               23.3       44.9      16.6              63.8             65.3
## 5 ---               16.4       61.3     -11.1              22.4             43.5
## 6 ---               19.8       44.8      14.6              75.4             82.6
## # ... with 64 more variables: `GEXC_DC_Lu_CD103+_3` <dbl>,
## #   `GEXC_DC_Lu_CD103+_4` <dbl>, `GEXC_DC_Lu_CD103+_5` <dbl>,
## #   `GEXC_DC_Lu_CD24+_1` <dbl>, `GEXC_DC_Lu_CD24+_2` <dbl>,
## #   `GEXC_DCLuLN_CD103+_1` <dbl>, `GEXC_DCLuLN_CD103+_2` <dbl>,
## #   `GEXC_DCLuLN_CD103+_3` <dbl>, `GEXC_DCLuLN_CD11b+_1` <dbl>,
## #   `GEXC_DCLuLN_CD11b+_2` <dbl>, `GEXC_DCLuLN_CD11b+_3` <dbl>,
## #   `GEXC_L+WT_1` <dbl>, `GEXC_L+WT_2` <dbl>, `GEXC_L+WT_3` <dbl>, ...
```

3 Data preparration

3.1 Make a list with genes to show in heatmap

The gene list IMvs(AM&DC).csv is calculated in ImmGen Datasets. We compared IM microarray data to both MA and DC and get the DE genes.

```
probset.DE <- read.csv("./IMvs(AM&DC).csv")
probset.toShow <- unique(as.character(probset.DE$ProbeSet_ID))

# the table is ordered by ratio, so take the top 100:
probset.toShow <- probset.toShow[1:100]
```

Then base on the intensity in IM, we choose the only top 50 probsets.

Take the to 50 probsets with highest intensity:

```
probset.DE <- probset.DE[order(probset.DE$Mean_A, decreasing = TRUE), ]
probset.top50 <- as.character(probset.DE[1:50, "ProbeSet_ID"])
```

```
probset.toShow <- intersect(probset.top50, probset.toShow)
```

subset expr.table

```
expr.table.toShow <- expr.table[probset.toShow, ]
genes.toShow <- unique(expr.table.toShow$Gene.Symbol)
length(genes.toShow)
```

```
## [1] 50
```

As genes are unique to each probset, we can use gene symbols as rownames.

```
rownames(expr.table.toShow) <- expr.table.toShow$Gene.Symbol
rownames(expr.table.toShow)
```

```
##  [1] "C1qa"              "Cxcl10"            "C1qb"
##  [4] "Mmp12 or Mmp1b"    "C1qc"              "Ptgs2"
##  [7] "C3ar1"             "Ccl4"              "Itgam"
## [10] "Cx3cr1"            "Cd72 or Tesk1"     "Col14a1"
## [13] "Mmp13"             "Pla2g7"            "Mafb"
## [16] "Rasgrp1"           "Abca9"             "Ms4a4a"
## [19] "Ephx1"             "Hpgd"              "Ecm1"
## [22] "Cxcl13"            "Lifr"              "Ccl2"
## [25] "Ms4a6b"            "Hpgds"             "Plk2"
## [28] "---"               "Stab1"             "Itga9"
## [31] "Ifnb1"             "Cmklr1"            "H2-M2"
## [34] "Blnk"              "Emr4"              "Dnajb4"
## [37] "Ccl7"              "Ms4a7"             "Tmem119"
## [40] "Clec10a"           "Retnla"            "Tm4sf19"
## [43] "Olfr111"           "Abcc3"             "Ifit1"
## [46] "Gbp3"              "St3gal6 or Dcbld2" "Rtp4"
## [49] "Maf"               "St8sia6"
```

Remove the row without annotation:

```
expr.table.toShow <- expr.table.toShow[expr.table.toShow$Gene.Symbol != "
   ---", ]
```

3.2 Make metadata table

```
data.frame(meta.sample$sampleName, order = 1:nrow(meta.sample))
```

```
## # A tibble: 22 x 2
##    meta.sample.sampleName  order
##    <chr>                   <int>
##  1 GEXC_AMs                    1
##  2 GEXC_DC_Lu_CD103+           2
##  3 GEXC_DC_Lu_CD24+            3
##  4 GEXC_DCLuLN_CD103+          4
##  5 GEXC_DCLuLN_CD11b+          5
##  6 GEXC_L+WT                   6
##  7 GEXC_LMIsW                  7
##  8 GEXC_MF_BM                  8
##  9 GEXC_MF_CNS                 9
## 10 GEXC_MF_Lu_CD11b+_CD24-    10
## # ... with 12 more rows
```

```
meta.sample$cellType3 <- c("Mac␣Alv␣Lu", #1
                           "DC␣CD103+␣Lu", #2
                           "DC␣CD24+␣Lu", #3
                           "DC␣CD103+␣LuLN", #4
                           "DC␣CD11b+␣LuLN", #5
                           "Mo␣Ly6C+␣Lu", #6
                           "Mac␣Int␣Lu", #7
                           "Mac␣BM", #8
                           "Mac␣CNS", #9
                           "Mac␣Int␣Lu", #10
                           "Mac␣F4/80hi␣PC", #11
                           "Mac␣F4/80lo␣PC", #12
                           "Mac␣SI", #13
                           "Mac␣SLN", #14
                           "Mac␣SP", #15
                           "Mo␣Ly6C-␣MHCII-␣BL", #16
                           "Mo␣Ly6C-␣MHCII+␣BL", #17
                           "Mo␣Ly6C-␣MHCIIint␣BL", #18
                           "Mo␣Ly6C+␣MHCII-␣BL", #19
                           "Mo␣Ly6C+␣MHCII+␣BL", #20
                           "Mo␣Ly6C-␣MHCII-␣BM", #21
                           "Mo␣Ly6C+␣MHCII-␣BM" #22
             )

meta.sample$cellType <- c("aMac", #1
             rep("DC", 4),  # 2-5
             "Mo", # 6
             "iMac", #7
             rep("Mac", 2), # 8-9
             "iMac", #10
```

```
              rep("Mac", 5), # 11-15
              rep("Mo", 7) # 16-22
              )
meta.sample$organ <- c(rep("Lu", 3), #1-3
           rep("LuLN", 2), #4-5
           rep("Lu", 2), #6-7
           "BM", #8
           "CNS", #9
           "Lu", #10
           rep("PC",2), #11-12
           "SI", #13
           "SLN", #14
           "SP", #15
           rep("BL",5 ),
           rep("BM", 2)

           )
meta.sample$cellType2 <- c(
              "Mac", #1
              rep("DC", 4),  # 2-5
              "Mo", # 6
              "Mac", #7
              rep("Mac", 2), # 8-9
              "Mac", #10
              rep("Mac", 5), # 11-15
              rep("Mo", 7) # 16-22
              )
meta.sample$organ2 <- c(
            "Lu-Alv", #1
            rep("Lu", 2), #2-3
           rep("LuLN", 2), #4-5
           "Lu", #6
           "Lu-Int", #7
           "BM", #8
           "CNS", #9
           "Lu-Int", #10
           rep("PC",2), #11-12
           "SI", #13
           "SLN", #14
           "SP", #15
           rep("BL",5 ),
           rep("BM", 2)

           )
```

```
meta.table <- data.frame(CellType = rep(meta.sample$cellType, meta.sample$
   n.rep),
    OrganType = rep(meta.sample$organ, meta.sample$n.rep), CellType2 = rep
       (meta.sample$cellType2,
        meta.sample$n.rep), OrganType2 = rep(meta.sample$organ2, meta.
           sample$n.rep),
    cellType3 = rep(meta.sample$cellType3, meta.sample$n.rep), row.names =
        names.rep)
```

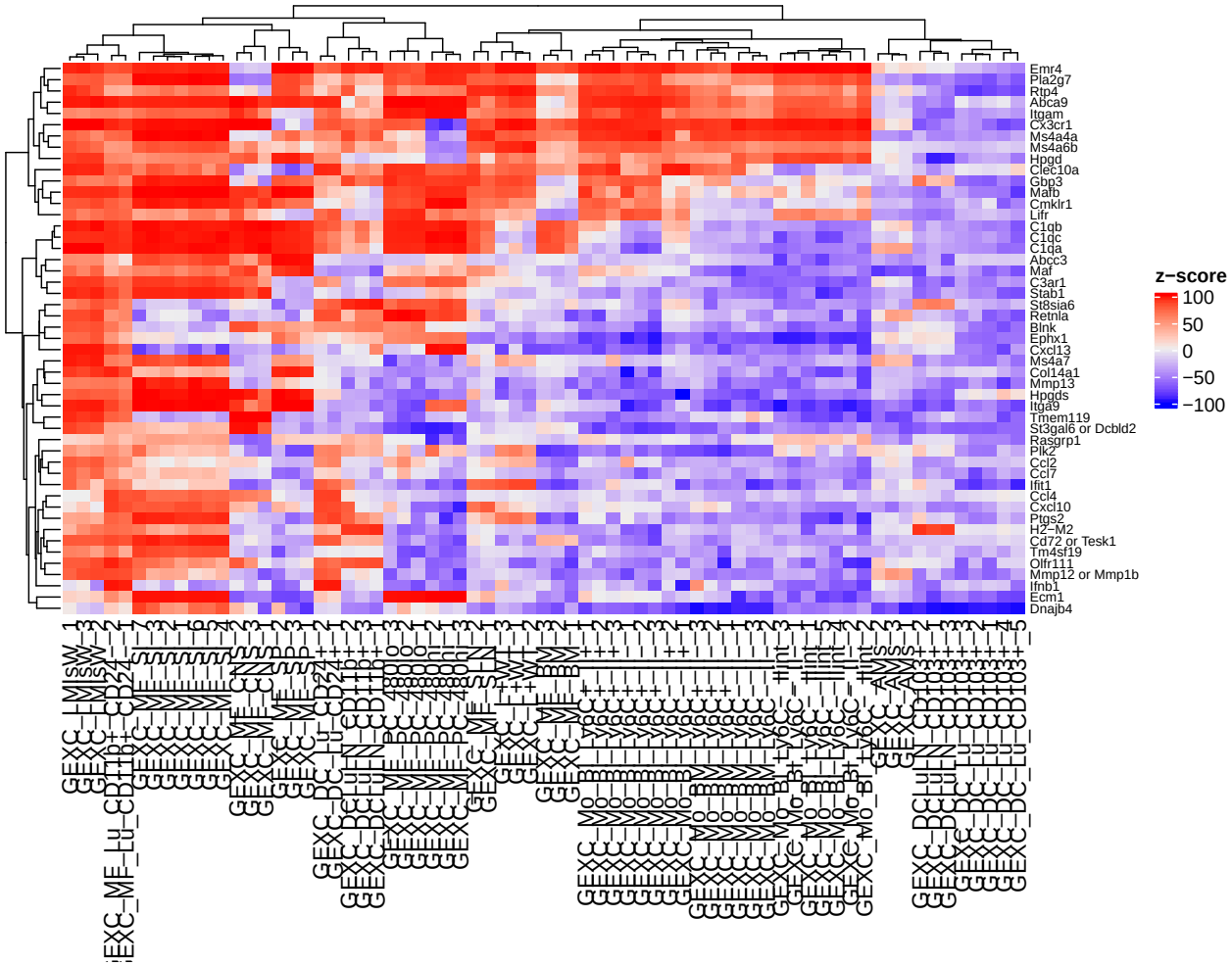
```
HeatmapAnnotation(Cell_type = meta.table$CellType, Organ_type = meta.table
   $OrganType)
```

```
## A HeatmapAnnotation object with 2 annotations
##   name: heatmap_annotation_0
##   position: column
##   items: 69
##   width: 1npc
##   height: 10.3514598035146mm
##   this object is subsetable
##   23.1191666666667mm extension on the right
##
##        name annotation_type color_mapping height
##   Cell_type discrete vector        random    5mm
##  Organ_type discrete vector        random    5mm
```

4 Make heatmaps

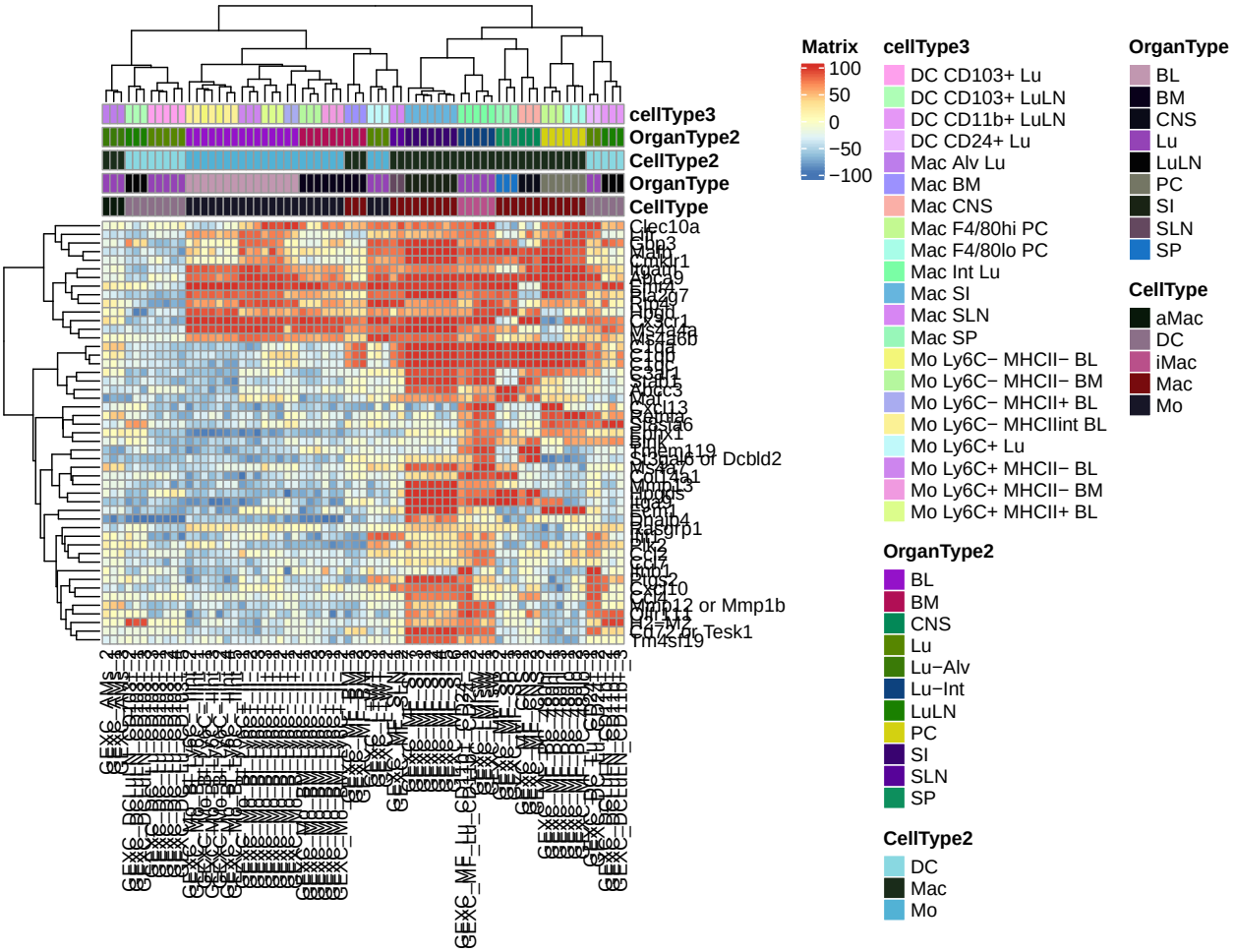
Use Heatmap:

```
Heatmap(
  as.matrix(expr.table.toShow[2:ncol(expr.table.toShow)]),
  # use_raster = FALSE, # use FALSE to export to vector image.
  name                          = "z-score",
  # col                           = colorRamp2(seq(from=-2,to=2,length=11),
     rev(brewer.pal(11, "Spectral"))),
  # show_row_names                = TRUE,
  # show_column_names             = FALSE,
  row_names_gp                  = gpar(fontsize = 7),

  # row_title_rot                 = 0,
  # cluster_rows                  = TRUE,
  # cluster_row_slices            = FALSE,
  #cluster_columns               = FALSE
)
```



Using pheatmap.

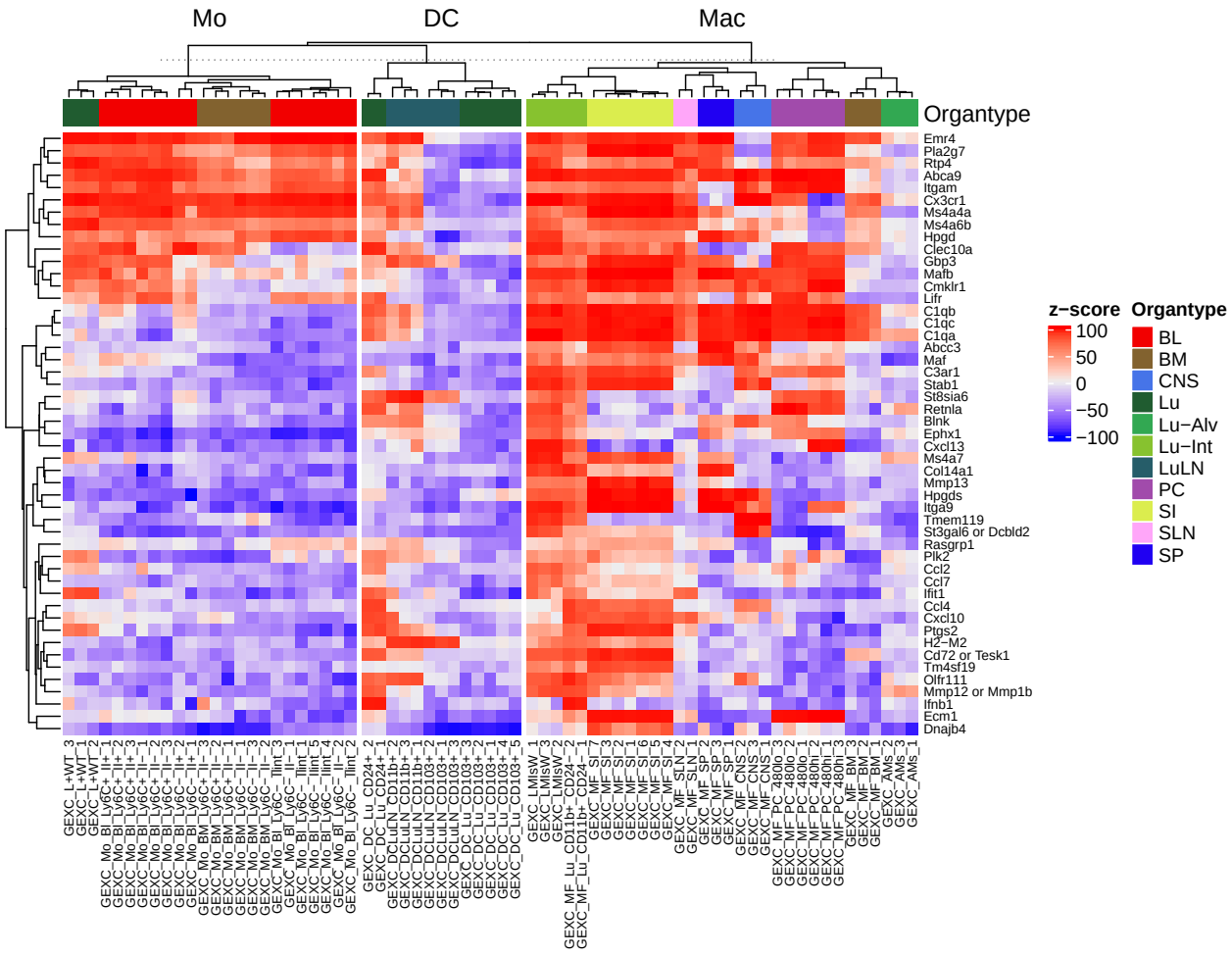
```
pheatmap(
  as.matrix(expr.table.toShow[2:ncol(expr.table.toShow)]), annotation_col
     = meta.table
  # use_raster = FALSE, # use FALSE to export to vector image.
  #name                          = "z-score",
  # col                           = colorRamp2(seq(from=-2,to=2,length=11),
     rev(brewer.pal(11, "Spectral"))),
  # show_row_names                = TRUE,
  # show_column_names             = FALSE,
  #row_names_gp                  = gpar(fontsize = 7),

  # row_title_rot                 = 0,
  # cluster_rows                  = TRUE,
  # cluster_row_slices            = FALSE,
  #cluster_columns               = FALSE
)
```



Using Heatmap with annotations.

```
hp <- Heatmap(
  as.matrix(expr.table.toShow[2:ncol(expr.table.toShow)]),
  # use_raster = FALSE, # use FALSE to export to vector image.
  name                          = "z-score",
  # col                           = colorRamp2(seq(from=-2,to=2,length=11),
     rev(brewer.pal(11, "Spectral"))),
  # show_row_names                = TRUE,
  # show_column_names             = FALSE,
  row_names_gp                  = gpar(fontsize = 7),
  column_names_gp               = gpar(fontsize = 7),

  #column_split = meta.table$CellType2,
  column_split = factor(meta.table$CellType2, levels = c("Mac", "Mo", "DC"
     )),
  top_annotation = HeatmapAnnotation(Organtype=meta.table$OrganType2,
                                     col = list(Organtype = c(`Lu-Alv`="
                                        #32a852",
                                          `Lu-Int`="#87c22f",
                                          Lu="#205c30",
                                          LuLN="#265d69",
                                          BM="#82622f",
                                          CNS="#4674e8",
```

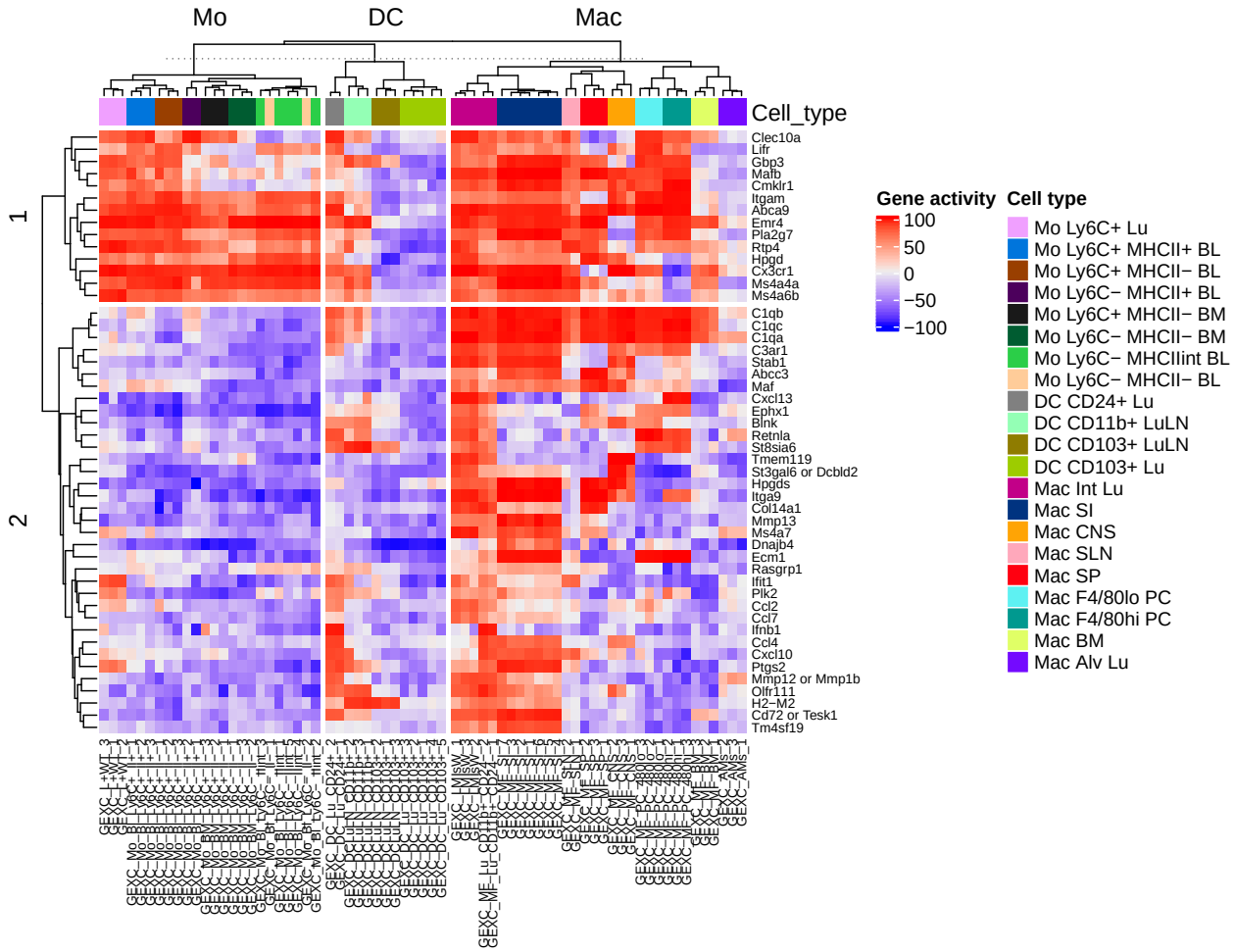
```
                                                PC="#a14bab",
                                                SI="#dbed4e",
                                                SLN="#ffa6f9",
                                                SP="#2000f2",
                                                BL="#f20000")) )

)

p <- draw(hp)
```



Change colors and annotations

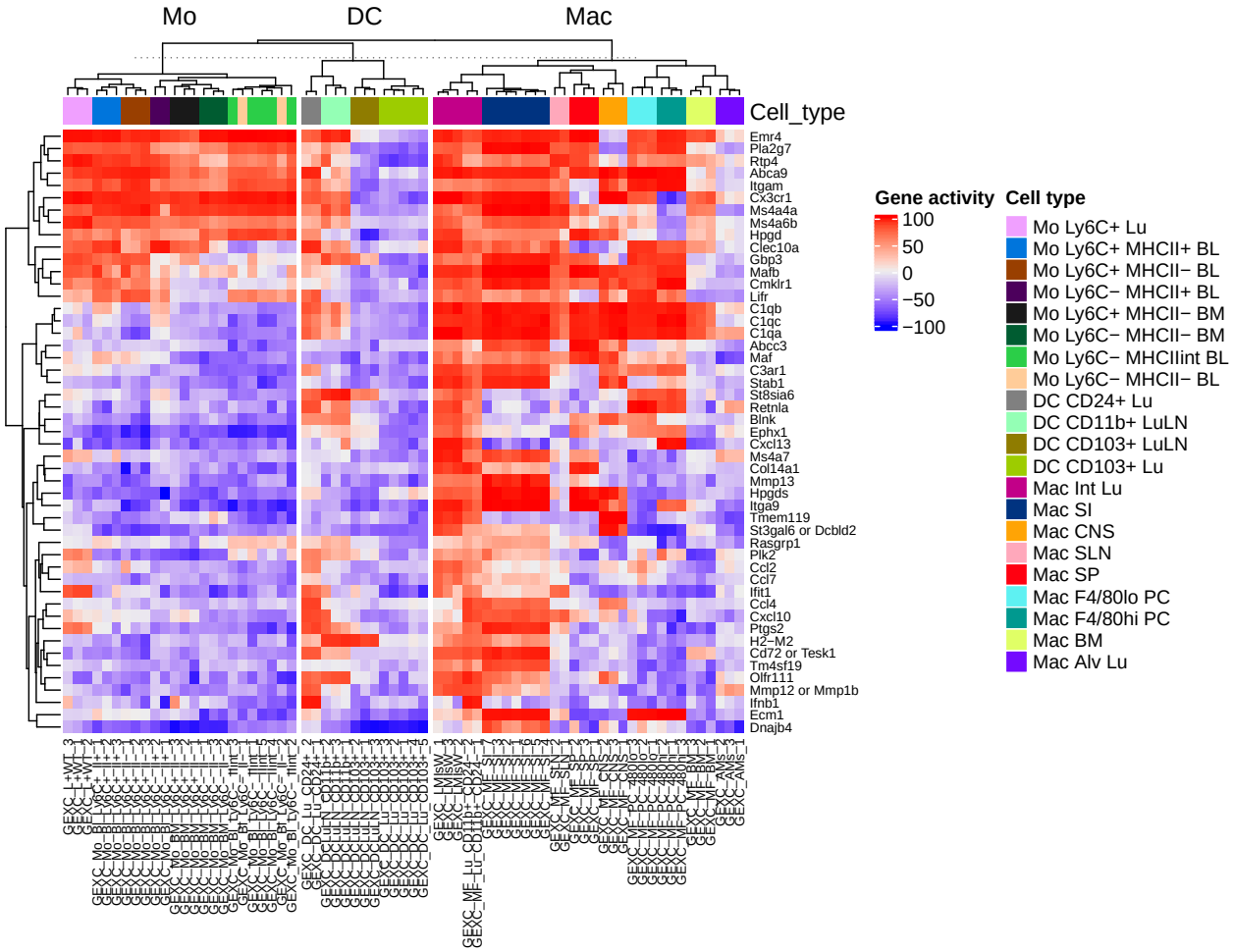
```
col.cellType3 <- read.csv("../0-Microarrays/colors_celltype3.csv", sep = "
   \t", header = FALSE, row.names = 1)
colors.cellType3 <- as.character(col.cellType3$V2)
names(colors.cellType3) <- rownames(col.cellType3)
genes.toSplit <- rownames(expr.table.toShow)
genes.toSplit <- genes.toSplit %in% c("Tmem119", "Cx3cr1")

# the one with row split:
hp2 <- Heatmap(
  as.matrix(expr.table.toShow[2:ncol(expr.table.toShow)]),
  # use_raster = FALSE, # use FALSE to export to vector image.
```

```
  name                              = "Gene activity",                                  
  row_names_gp                      = gpar(fontsize = 7),                               
  column_names_gp                   = gpar(fontsize = 7),                               
  column_split = factor(meta.table$CellType2, levels = c("Mac", "Mo", "DC"
     )),
  row_split = 2,                                                                        
  top_annotation = HeatmapAnnotation(Cell_type=meta.table$cellType3,                    
                    col = list(                                                         
                                Cell_type = colors.cellType3 ),                         
                    annotation_legend_param = list(                                     
                                Cell_type = list(title = "Cell type",                   
                                                 at = names(colors.
                                                    cellType3),
                                                 labels = names(colors.
                                                    cellType3))) )
                                                                                        
)                                                                                       
                                                                                        
p2 <- draw(hp2)                                                                         
```




```
# the one WITHOUT row split:                                                          
hp3 <- Heatmap(                                                                       
  as.matrix(expr.table.toShow[2:ncol(expr.table.toShow)]),                            
```

```
  # use_raster = FALSE, # use FALSE to export to vector image.
  name                              = "Gene activity",
  row_names_gp                      = gpar(fontsize = 7),
  column_names_gp                   = gpar(fontsize = 7),
  column_split = factor(meta.table$CellType2, levels = c("Mac", "Mo", "DC"
     )),
  #row_split = 2,
  top_annotation = HeatmapAnnotation(Cell_type=meta.table$cellType3,
                    col = list(
                                Cell_type = colors.cellType3 ),
                    annotation_legend_param = list(
                                Cell_type = list(title = "Cell type",
                                                 at = names(colors.
                                                    cellType3),
                                                 labels = names(colors.
                                                    cellType3))) )

)

p3 <- draw(hp3)
```



5 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
##  [3] LC_TIME=en_GB.UTF-8        LC_COLLATE=en_US.UTF-8
##  [5] LC_MONETARY=en_GB.UTF-8    LC_MESSAGES=en_US.UTF-8
##  [7] LC_PAPER=en_GB.UTF-8       LC_NAME=C
##  [9] LC_ADDRESS=C               LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_GB.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] grid      stats     graphics  grDevices utils     datasets  methods
## [8] base
##
## other attached packages:
## [1] circlize_0.4.14      RColorBrewer_1.1-2   ComplexHeatmap_2.6.2
##
## loaded via a namespace (and not attached):
##  [1] Rcpp_1.0.8          highr_0.9           pillar_1.7.0
##  [4] compiler_4.0.3      formatR_1.11        tools_4.0.3
##  [7] digest_0.6.29       clue_0.3-60         evaluate_0.15
## [10] lifecycle_1.0.1     tibble_3.1.6        pkgconfig_2.0.3
## [13] png_0.1-7           rlang_1.0.1         cli_3.2.0
## [16] rstudioapi_0.13     magick_2.7.3        yaml_2.3.5
## [19] parallel_4.0.3      xfun_0.29           fastmap_1.1.0
## [22] cluster_2.1.0       stringr_1.4.0       knitr_1.37
## [25] vctrs_0.3.8         GlobalOptions_0.1.2 S4Vectors_0.28.1
## [28] IRanges_2.24.1      stats4_4.0.3        glue_1.6.1
## [31] GetoptLong_1.0.5    fansi_1.0.2         rmarkdown_2.11
## [34] magrittr_2.0.2      matrixStats_0.61.0  ellipsis_0.3.2
## [37] htmltools_0.5.2     BiocGenerics_0.36.1 shape_1.4.6
## [40] colorspace_2.0-3    utf8_1.2.2          stringi_1.7.6
## [43] crayon_1.5.0        rjson_0.2.21        Cairo_1.5-14
```

References

1. Sabatel, C. *et al.* Exposure to Bacterial CpG DNA Protects from Airway Allergic Inflammation by Expanding Regulatory Lung Interstitial Macrophages. *Immunity* **46**, 457–473 (2017).

2. Seita, J. *et al.* Gene Expression Commons: An Open Platform for Absolute Gene Expression Profiling. *PLoS ONE* **7**, 40321 (2012).