A lung Tgf-beta-signaling-mediated endothelial-interstitial macrophage axis prevents age-related abnormalities

10_TGFbIIR_KO_IM

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Abstract

Lung interstitial macrophages (IMs) are monocyte-derived parenchymal macrophages whose homeostatic and tissue-supportive functions remain unclear. While recent progress has been made about the diversity and transcriptional regulation of lung IMs, the microenvironmental signals responsible for their development from monocytes and for their functional specification remain unidentified. Here we found, in mice, that lung endothelial cell-derived Tgf-beta1 specifically triggered a core Tgf-beta receptor-dependent IM signature in bone marrow-derived monocytes and macrophages (Macs). In vivo, myeloid-specific ablation of Tgf-beta receptor signaling severely impaired monocyte-to-IM development, resulting in the accumulation of perivascular monocytes, decreased IM numbers and a loss of IM-intrinsic identity. Of note, monocyte-to-IM development was similarly impaired in the absence of endothelial-specific Tgf-beta1. Functionally, lungs from mice selectively lacking Tgf-beta receptor in IMs exhibited spatial changes in monocyte and IM niche occupancies, a severe disruption in their immunoregulatory environment, and prematurely developed fibrosis, hyperinflation, increased compliance and decreased elastance, changes classically associated with aging. Our work identifies a novel endothelial-IM axis involving Tgf-beta1 - Tgf-beta receptor interactions that shapes IM development and identity and thereby sustains lung tissue integrity, thus providing foundations for IM-targeted interventions in the context of lung aging and other chronic inflammatory disorders.

Contents

1 Description

Datasets from two samples were first merged and analysed together for normalization, dimension reduction and clustering analysis using `Seurat` package *(1)*. Analyses of differential expression (DE) were made with `FindMarkers` function. The following parameters were used to define significant DE genes in each subset:

- Adjusted p value < 0.05
- Absolute log fold change > 0.5

The significant DE genes in Ly6C+ monocytes were subjected to gene ontology (GO) enrichment analysis using `clusterProfiler` package *(2)* and annotation in `org.Mm.eg.db` package (version 3.18.0).

2 Load data and packages

```
suppressMessages(library(Seurat))
suppressMessages(library(ggplot2))
```

```
cd45pos_ctl <- readRDS("../4_scRNAseq_initiation/cd45pos_WT.seuratObject.
   rds")

cd45pos_ko <- readRDS("../4_scRNAseq_initiation/cd45pos_KO.seuratObject.
   rds")
```

3 Merge to analyze TGFbRII-KO and control samples together

3.1 Prepare metadata

```
cd45pos_ctl$genotype <- "TGFbRII␣F/F"
cd45pos_ko$genotype <- "TGFbRII␣F/F␣Lyz2-Cre"
```

Now merge:

```
cd45pos <- merge(cd45pos_ctl, cd45pos_ko)
```

3.2 Normalize and process PCA

```
cd45pos <- NormalizeData(cd45pos)
cd45pos <- FindVariableFeatures(cd45pos, selection.method = "vst",
   nfeatures = 2000)
cd45pos <- ScaleData(cd45pos, features = rownames(cd45pos))
cd45pos <- RunPCA(cd45pos, features = VariableFeatures(cd45pos))
PCAPlot(cd45pos, group.by = "genotype")
```

3.3 Non-linear dimension reduction

```
# For TSNE:
cd45pos <- RunTSNE(cd45pos, dims = 1:12)

# For UMAP:
cd45pos.for3d <- RunUMAP(cd45pos, dims = 1:12, n.components = 3L)
```

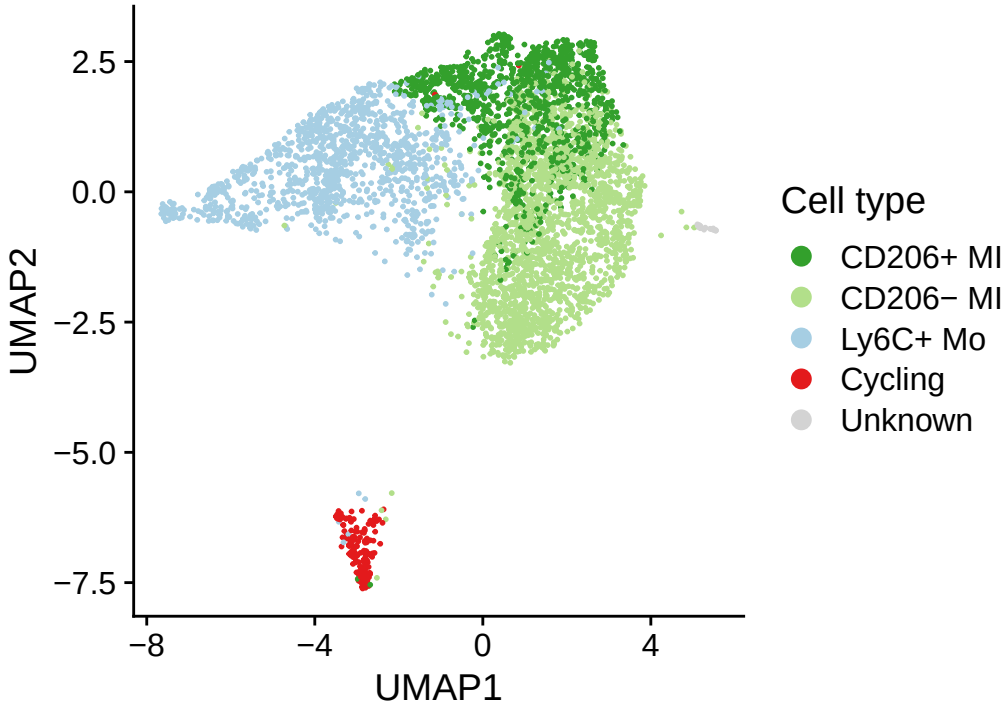
3.4 Clustering

```
cd45pos.for3d <- FindNeighbors(cd45pos.for3d, dims = 1:10)
cd45pos.for3d <- FindClusters(cd45pos.for3d, resolution = 0.15)
Idents(cd45pos.for3d) <- "RNA_snn_res.0.15"

celltype.2 <- as.character(cd45pos.for3d$RNA_snn_res.0.15)
celltype.2[celltype.2 == "3"] <- "2"
celltype.2 <- factor(celltype.2)
levels(celltype.2) <- c("CD206- MI", "Ly6C+ Mo", "CD206+ MI", "Cycling", "
   Unknown")

cd45pos.for3d[["cell.type.2"]] <- celltype.2
```

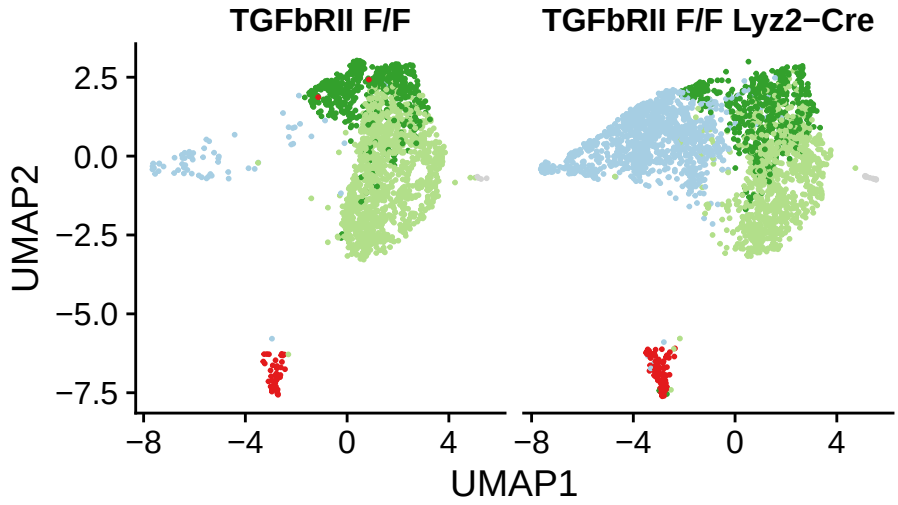
```
celltype.1 <- as.character(celltype.2)
celltype.1[celltype.1 %in% c("CD206- MI", "CD206+ MI")] <- "MI"
celltype.1[celltype.1 %in% c("Ly6C+ Mo")] <- "Mo"
celltype.1 <- factor(celltype.1)

cd45pos.for3d[["cell.type.1"]] <- celltype.1
```

3.5 Plotting cell subsets and validate by gene expression

```
pal2 <- c(`CD206- MI` = "#B2DF8A", `Ly6C+ Mo` = "#A6CEE3", `CD206+ MI` = "
   #33A02C",
    Cycling = "#E31A1C", Unknown = "lightgrey")

# change the order in cell type list for plotting cd45pos.for3d$cell.type
   .2 <-
# cd45pos.for3d$RNA_snn_res.0.15
levels(cd45pos.for3d$cell.type.2) <- c("CD206- MI", "Ly6C+ Mo", "CD206+ MI
   ", "Cycling",
    "Unknown")
cd45pos.for3d$cell.type.2 <- factor(cd45pos.for3d$cell.type.2, c("CD206+
   MI", "CD206- MI",
    "Ly6C+ Mo", "Cycling", "Unknown"))

DimPlot(cd45pos.for3d, group.by = "cell.type.2", cols = pal2, reduction =
   "umap") +
    theme(aspect.ratio = 1, plot.title = element_blank()) + labs(x = "
       UMAP1", y = "UMAP2",
    color = "Cell type")
```



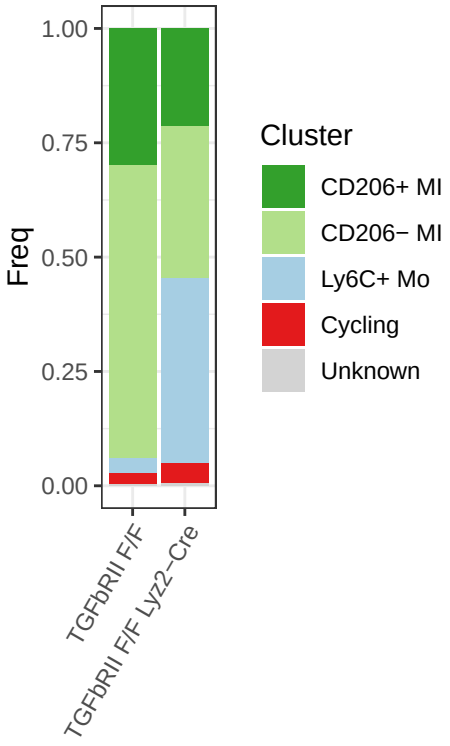

```
DimPlot(cd45pos.for3d, group.by = "cell.type.2", cols = pal2, split.by = "
    genotype",
     reduction = "umap") + theme(aspect.ratio = 1, plot.title = element_
        blank()) +
     labs(x = "UMAP1", y = "UMAP2", color = "Cell type") + guides(color = "
        none")
```



Frequencies of each cell type in KO and control:

```
source("../../R/SeuratFreqTable.R")
```

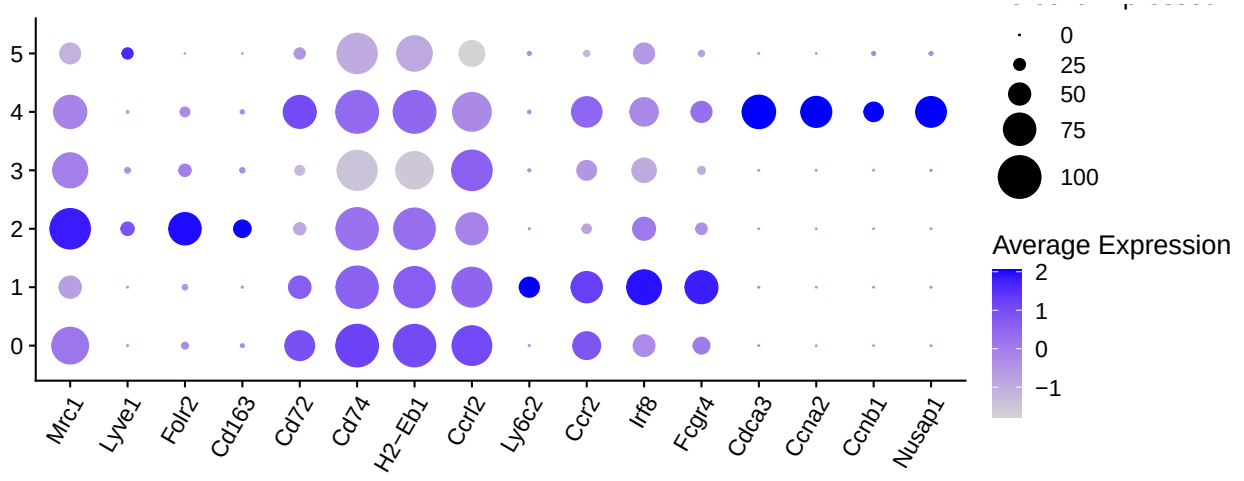
```
source("../../R/barChart.R")


freq.celltype.list <- list('TGFbRII F/F' = Seurat2CellFreqTable(subset(
    cd45pos.for3d,
     subset = genotype == "TGFbRII␣F/F"), slotName = "cell.type.2"), '
        TGFbRII F/F Lyz2-Cre' = Seurat2CellFreqTable(subset(cd45pos.for3d,
     subset = genotype == "TGFbRII␣F/F␣Lyz2-Cre"), slotName = "cell.type.2"
        ))

barChart(freq.celltype.list) + labs(fill = "Cluster") + scale_fill_manual(
    values = pal2) +
     theme(axis.text.x = element_text(angle = 60, vjust = 1, hjust = 1),
        axis.title.x = element_blank())
```




```
library(dplyr)
Idents(cd45pos.for3d) <- "cell.type.2"
cd45pos.for3d <- JoinLayers(cd45pos.for3d)
all_cluster.markers <- FindAllMarkers(cd45pos.for3d, verbose = FALSE)
top20 <- all_cluster.markers %>%
    group_by(cluster) %>%
    top_n(n = 20, wt = avg_log2FC)
```

```
DotPlot(cd45pos.for3d, features = c("Mrc1", "Lyve1", "Folr2", "Cd163", "
    Cd72", "Cd74",
     "H2-Eb1", "Ccrl2", "Ly6c2", "Ccr2", "Irf8", "Fcgr4", "Cdca3", "Ccna2",
         "Ccnb1",
     "Nusap1"), dot.scale = 10) + theme(axis.text.x = element_text(angle =
        60, vjust = 1,
```

```
    hjust = 1), axis.title.x = element_blank(), axis.title.y = element_
        blank())
```



4 Compare transcriptome of subsets, between TGFbRII-KO and Control

4.1 DE expression in subsets between TGFbRII-KO and Control

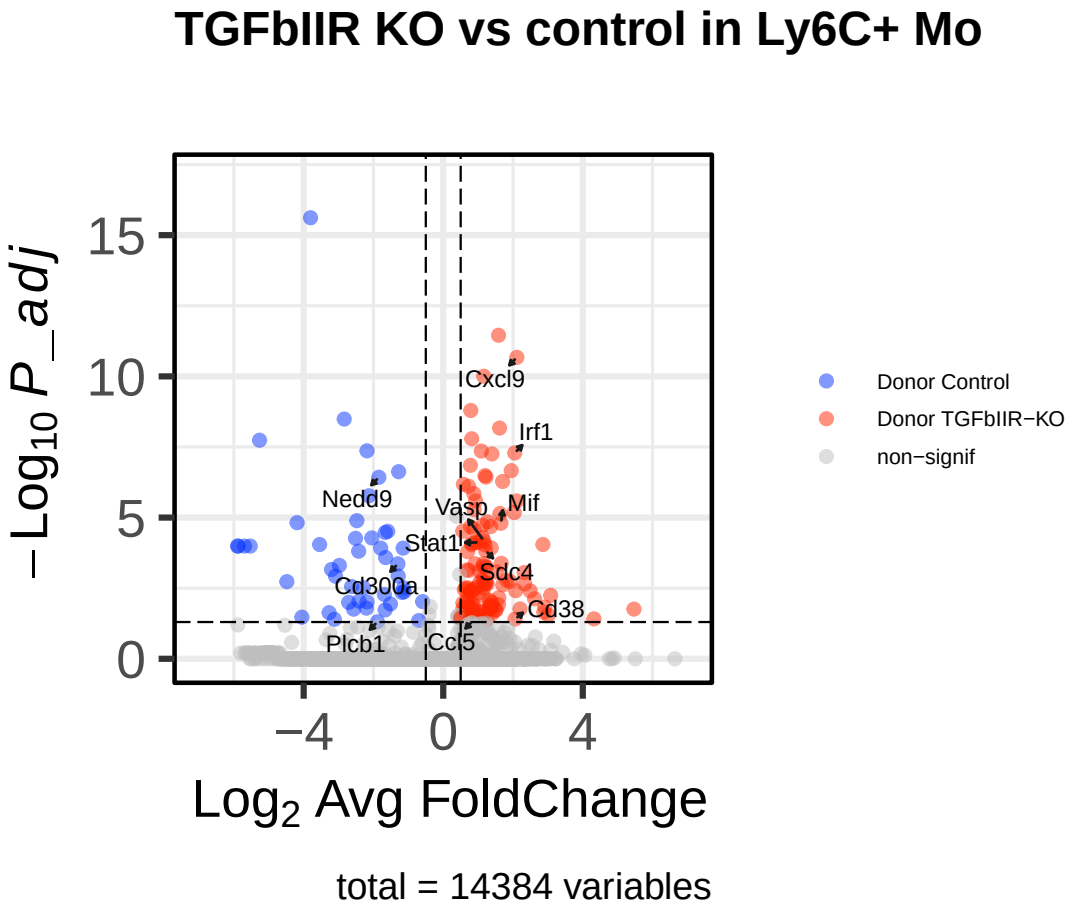
```
Idents(cd45pos.for3d) <- "cell.type.2"
cd45pos.for3d <- JoinLayers(cd45pos.for3d)
```

```
de.cd206im.KO_vs_Ctrl <- FindMarkers(cd45pos.for3d, ident.1 = "TGFbRII␣F/F
    ␣Lyz2-Cre",
     ident.2 = "TGFbRII␣F/F", group.by = "genotype", subset.ident = "CD206+
        ␣MI", verbose = FALSE,
     random.seed = 1, min.pct = 0, min.cells.feature = 0, min.cells.group =
         0, logfc.threshold = 0)

de.mhc2im.KO_vs_Ctrl <- FindMarkers(cd45pos.for3d, ident.1 = "TGFbRII␣F/F␣
    Lyz2-Cre",
     ident.2 = "TGFbRII␣F/F", group.by = "genotype", subset.ident = "CD206-
        ␣MI", verbose = FALSE,
     random.seed = 1, min.pct = 0, min.cells.feature = 0, min.cells.group =
         0, logfc.threshold = 0)

de.extMo.KO_vs_Ctrl <- FindMarkers(cd45pos.for3d, ident.1 = "TGFbRII␣F/F␣
    Lyz2-Cre",
     ident.2 = "TGFbRII␣F/F", group.by = "genotype", subset.ident = "Ly6C+␣
        Mo", verbose = FALSE,
     random.seed = 1, min.pct = 0, min.cells.feature = 0, min.cells.group =
         0, logfc.threshold = 0)

de.cycling.KO_vs_Ctrl <- FindMarkers(cd45pos.for3d, ident.1 = "TGFbRII␣F/F
    ␣Lyz2-Cre",
```

```
    ident.2 = "TGFbRII␣F/F", group.by = "genotype", subset.ident = "
       Cycling", verbose = FALSE,
    random.seed = 1, min.pct = 0, min.cells.feature = 0, min.cells.group =
        0, logfc.threshold = 0)
```

4.2 Volcano plot show DE genes in LyC6+ monocytes comparing Tgfbr2-KO to control

```
library(EnhancedVolcano)
# library(RColorBrewer)
de.res <- de.extMo.KO_vs_Ctrl
cols <- c("#FF2600", "#0433FF")


de.res$Gene <- rownames(de.res)
keyvals <- rep("grey", nrow(de.res))
names(keyvals) <- rep("non-signif", nrow(de.res))

keyvals[which(de.res$avg_log2FC > 0.5 & de.res$p_val_adj < 0.05)] <- cols
   [1]
names(keyvals)[which(de.res$avg_log2FC > 0.5 & de.res$p_val_adj < 0.05)]
   <- "Donor␣TGFbIIR-KO"

keyvals[which(de.res$avg_log2FC < -0.5 & de.res$p_val_adj < 0.05)] <- cols
   [2]
names(keyvals)[which(de.res$avg_log2FC < -0.5 & de.res$p_val_adj < 0.05)]
   <- "Donor␣Control"

plot.vol <- EnhancedVolcano(de.res, subtitle = "", lab = rownames(de.res),
    x = "avg_log2FC",
    y = "p_val_adj", xlab = bquote(~Log[2] ~ "Avg␣FoldChange"), ylab =
       bquote(~-Log[10] ~
        italic(P_adj)), xlim = c(-7, 7), ylim = c(0, -log10(1e-17)),
           selectLab = c("Nedd9",
        "Cd300a", "Plcb1", "Irf1", "Cxcl9", "Stat1", "Vasp", "Sdc4", "Ccl5
           ", "Mif",
        "Cd38"), labSize = 3, pCutoff = 0.05, FCcutoff = 0.5, colAlpha =
           0.5, colCustom = keyvals,
    legendLabSize = 8, legendIconSize = 2, border = "full", legendPosition
        = "right",
    axisLabSize = 20, title = "TGFbIIR␣KO␣vs␣control␣in␣Ly6C+␣Mo",
       drawConnectors = TRUE,
    widthConnectors = 0.5)

plot.vol
```



4.3 Summary of significant DE genes:

```
threshold.padj <- 0.05
threshold.lfc <- 0.5

sigDE.celltype2.KOvsCtrl <- list(cd206im = de.cd206im.KO_vs_Ctrl[abs(de.
   cd206im.KO_vs_Ctrl$avg_log2FC) >
    threshold.lfc & de.cd206im.KO_vs_Ctrl$p_val_adj < threshold.padj, ],
       mhc2im = de.mhc2im.KO_vs_Ctrl[abs(de.mhc2im.KO_vs_Ctrl$avg_log2FC)
       >
    threshold.lfc & de.mhc2im.KO_vs_Ctrl$p_val_adj < threshold.padj, ],
       extMo = de.extMo.KO_vs_Ctrl[abs(de.extMo.KO_vs_Ctrl$avg_log2FC) >
    threshold.lfc & de.extMo.KO_vs_Ctrl$p_val_adj < threshold.padj, ],
       cycling = de.cycling.KO_vs_Ctrl[abs(de.cycling.KO_vs_Ctrl$avg_
       log2FC) >
    threshold.lfc & de.cycling.KO_vs_Ctrl$p_val_adj < threshold.padj, ])

sapply(sigDE.celltype2.KOvsCtrl, nrow)
```

```
## cd206im  mhc2im   extMo cycling
##     553     166     167      17
```

All significant up-regulated genes in KO are named "KO_UP":

```
sig.symbol.KO_UP <- lapply(sigDE.celltype2.KOvsCtrl, function(x) {
    rownames(x)[x$avg_log2FC > 0]
})

names(sig.symbol.KO_UP) <- paste(names(sig.symbol.KO_UP), "KO_UP", sep = "
    _")
```

All significant down-regulated genes in KO are named "KO_DN":

```
sig.symbol.KO_DN <- lapply(sigDE.celltype2.KOvsCtrl, function(x) {
    rownames(x)[x$avg_log2FC < -0]
})

names(sig.symbol.KO_DN) <- paste(names(sig.symbol.KO_DN), "KO_DN", sep = "
    _")
```

Here's list for enrichment analyses:

```
sig.symbol.celltype2 <- c(sig.symbol.KO_UP, sig.symbol.KO_DN)
str(sig.symbol.celltype2)
```

```
## List of 8
##  $ cd206im_KO_UP: chr [1:248] "Ifitm3" "Cfp" "Clec10a" "H2-K1" ...
##  $ mhc2im_KO_UP : chr [1:119] "Lyz1" "Ifitm3" "Ifi27l2a" "Fcgr4" ...
##  $ extMo_KO_UP  : chr [1:120] "H2-Q7" "Cxcl9" "Aif1" "Pfn1" ...
##  $ cycling_KO_UP: chr [1:15] "Psmb9" "Ifitm3" "H2-D1" "Fcgr4" ...
##  $ cd206im_KO_DN: chr [1:305] "Trem2" "Lyz2" "Pmepa1" "Skil" ...
##  $ mhc2im_KO_DN : chr [1:47] "Lyz2" "Trem2" "Mpp7" "Tlr2" ...
##  $ extMo_KO_DN  : chr [1:47] "Fam20a" "Ace" "Slc25a27" "Ifitm6" ...
##  $ cycling_KO_DN: chr [1:2] "Emp1" "Trem2"
```

The significance is defined as: threshold.padj <- 0.05 threshold.lfc <- 0.5

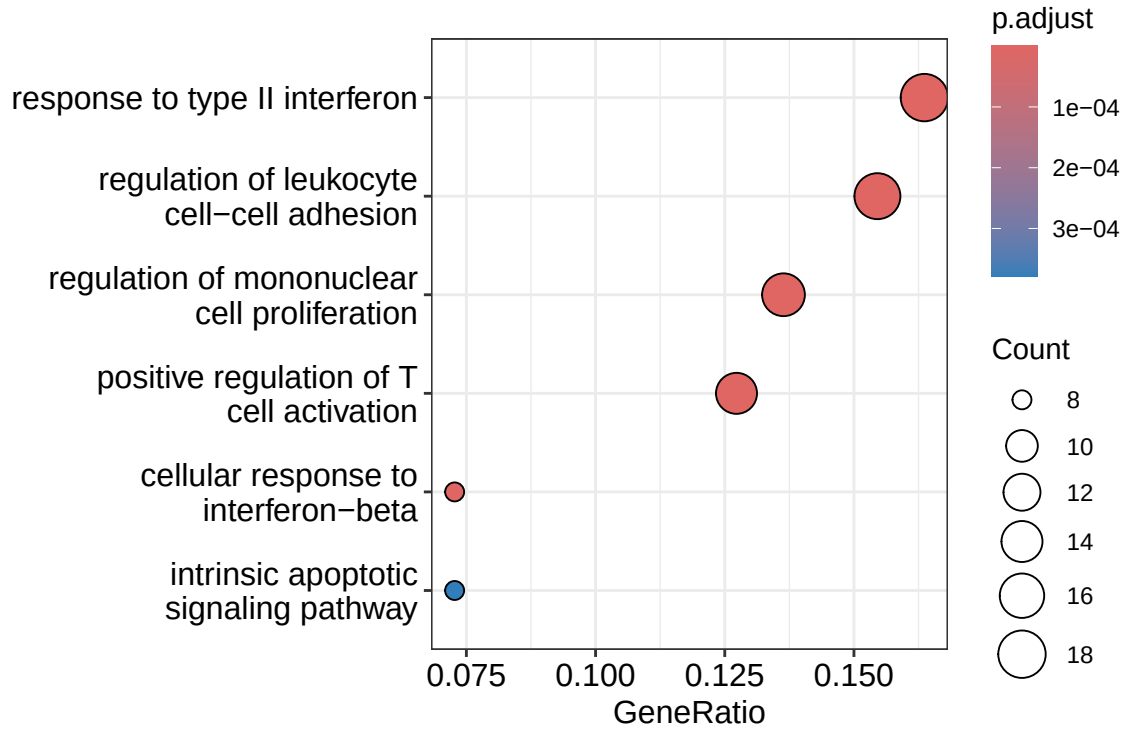
4.4 GO enrichment analysis with DE genes in Ly6C+ monocytes

```
library(org.Mm.eg.db)
library(clusterProfiler)
sig.symbol.extMo <- sig.symbol.celltype2[c("extMo_KO_DN", "extMo_KO_UP")]

extMo.KO_UP.GO_BP <- enrichGO(gene = sig.symbol.extMo$extMo_KO_UP, OrgDb =
    org.Mm.eg.db,
    keyType = "SYMBOL", ont = "BP", pAdjustMethod = "BH", pvalueCutoff =
       0.05, qvalueCutoff = 0.05)
```

With KO_DN genes:

```
p1 <- dotplot(extMo.KO_UP.GO_BP, showCategory = c("response␣to␣type␣II␣
   interferon",
    "regulation␣of␣leukocyte␣cell-cell␣adhesion", "regulation␣of␣
       mononuclear␣cell␣proliferation",
```

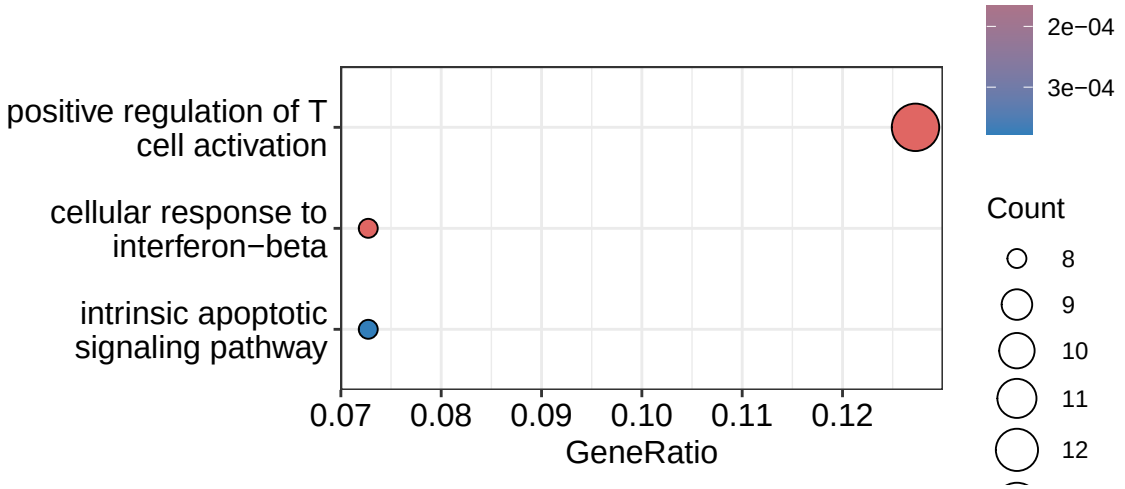
```
    "positive regulation of T cell activation", "cellular response to
       interferon-beta",
    "intrinsic apoptotic signaling pathway"))
p1
```



With KO_DN genes:

```
extMo.KO_DN.GO_BP <- enrichGO(gene = sig.symbol.extMo$extMo_KO_UP, OrgDb =
    org.Mm.eg.db,
    keyType = "SYMBOL", ont = "BP", pAdjustMethod = "BH", pvalueCutoff =
       0.05, qvalueCutoff = 0.05)
```

```
p2 <- dotplot(extMo.KO_DN.GO_BP, showCategory = c("positive regulation of
   T cell activation",
    "cellular response to interferon-beta", "intrinsic apoptotic signaling
       pathway"))
p2
```



5 Session information

```
sessionInfo()
```

```
## R version 4.3.3 (2024-02-29)
## Platform: aarch64-apple-darwin20 (64-bit)
## Running under: macOS 15.1.1
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/4.3-arm64/Resources/
   lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.3-arm64/Resources/
   lib/libRlapack.dylib;  LAPACK version 3.11.0
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## time zone: Europe/Paris
## tzcode source: internal
##
## attached base packages:
## [1] stats4    stats     graphics  grDevices utils     datasets  methods
## [8] base
##
## other attached packages:
##  [1] clusterProfiler_4.10.1 org.Mm.eg.db_3.18.0    AnnotationDbi_1.64.1
##  [4] IRanges_2.36.0         S4Vectors_0.40.2       Biobase_2.62.0
##  [7] BiocGenerics_0.48.1    EnhancedVolcano_1.20.0 ggrepel_0.9.6
## [10] RColorBrewer_1.1-3     ggplot2_3.5.1          Seurat_5.1.0
## [13] SeuratObject_5.0.2     sp_2.1-4
##
## loaded via a namespace (and not attached):
##   [1] RcppAnnoy_0.0.22        splines_4.3.3           later_1.4.1
##   [4] ggplotify_0.1.2         bitops_1.0-9            tibble_3.2.1
##   [7] polyclip_1.10-7         fastDummies_1.7.4       lifecycle_1.0.4
##  [10] globals_0.16.3          lattice_0.22-6          MASS_7.3-60.0.1
```

```
##  [13] magrittr_2.0.3          plotly_4.10.4           rmarkdown_2.29
##  [16] yaml_2.3.10             httpuv_1.6.15           sctransform_0.4.1
##  [19] spam_2.11-0             spatstat.sparse_3.1-0   reticulate_1.40.0
##  [22] cowplot_1.1.3           pbapply_1.7-2           DBI_1.2.3
##  [25] abind_1.4-8             zlibbioc_1.48.2         Rtsne_0.17
##  [28] purrr_1.0.2             ggraph_2.2.1            RCurl_1.98-1.16
##  [31] yulab.utils_0.1.8       tweenr_2.0.3
   GenomeInfoDbData_1.2.11
##  [34] enrichplot_1.22.0       irlba_2.3.5.1           listenv_0.9.1
##  [37] spatstat.utils_3.1-1    tidytree_0.4.6          goftest_1.2-3
##  [40] RSpectra_0.16-2         spatstat.random_3.3-2   fitdistrplus_1
   .2-1
##  [43] parallelly_1.40.1       leiden_0.4.3.1          codetools_0.2-20
##  [46] ggforce_0.4.2           DOSE_3.28.2             tidyselect_1.2.1
##  [49] aplot_0.2.3             farver_2.1.2            viridis_0.6.5
##  [52] matrixStats_1.4.1       spatstat.explore_3.3-3  jsonlite_1.8.9
##  [55] tidygraph_1.3.1         progressr_0.15.1        ggridges_0.5.6
##  [58] survival_3.7-0          tools_4.3.3             treeio_1.26.0
##  [61] ica_1.0-3               Rcpp_1.0.13-1           glue_1.8.0
##  [64] gridExtra_2.3           xfun_0.49               qvalue_2.34.0
##  [67] GenomeInfoDb_1.38.8     dplyr_1.1.4             withr_3.0.2
##  [70] formatR_1.14            fastmap_1.2.0           fansi_1.0.6
##  [73] digest_0.6.37           gridGraphics_0.5-1      R6_2.5.1
##  [76] mime_0.12               colorspace_2.1-1        scattermore_1.2
##  [79] GO.db_3.18.0            tensor_1.5              spatstat.data_3
   .1-4
##  [82] RSQLite_2.3.9           utf8_1.2.4              tidyr_1.3.1
##  [85] generics_0.1.3          data.table_1.16.4       graphlayouts_1
   .2.1
##  [88] httr_1.4.7              htmlwidgets_1.6.4       scatterpie_0.2.4
##  [91] uwot_0.1.16             pkgconfig_2.0.3         gtable_0.3.6
##  [94] blob_1.2.4              lmtest_0.9-40           XVector_0.42.0
##  [97] shadowtext_0.1.4        htmltools_0.5.8.1       dotCall64_1.2
## [100] fgsea_1.28.0            scales_1.3.0            png_0.1-8
## [103] spatstat.univar_3.1-1   ggfun_0.1.8             knitr_1.49
## [106] rstudioapi_0.17.1       reshape2_1.4.4          nlme_3.1-166
## [109] zoo_1.8-12              cachem_1.1.0            stringr_1.5.1
## [112] KernSmooth_2.23-24      parallel_4.3.3          miniUI_0.1.1.1
## [115] HDO.db_0.99.1           pillar_1.9.0            grid_4.3.3
## [118] vctrs_0.6.5             RANN_2.6.2              promises_1.3.2
## [121] xtable_1.8-4            cluster_2.1.7           evaluate_1.0.1
## [124] cli_3.6.3               compiler_4.3.3          rlang_1.1.4
## [127] crayon_1.5.3            future.apply_1.11.3     labeling_0.4.3
## [130] plyr_1.8.9              fs_1.6.5                stringi_1.8.4
## [133] viridisLite_0.4.2       deldir_2.0-4            BiocParallel_1
   .36.0
## [136] munsell_0.5.1           Biostrings_2.70.3       lazyeval_0.2.2
## [139] spatstat.geom_3.3-4     GOSemSim_2.28.1         Matrix_1.6-5
## [142] RcppHNSW_0.6.0          patchwork_1.3.0         bit64_4.5.2
## [145] future_1.34.0           KEGGREST_1.42.0         shiny_1.9.1
## [148] ROCR_1.0-11             igraph_2.1.2            memoise_2.0.1
## [151] ggtree_3.10.1           fastmatch_1.1-4         bit_4.5.0.1
## [154] gson_0.1.0              ape_5.8
```

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