

# 3-Comparison-Condition

Joan Abinet

2025-06-25 15:30:07 +0200

## Contents

|                                |          |
|--------------------------------|----------|
| <b>Introduction</b>            | <b>2</b> |
| <b>Loading packages</b>        | <b>2</b> |
| <b>Loading data</b>            | <b>2</b> |
| Volcano_plot in gCap . . . . . | 2        |
| <b>DE per cluster</b>          | <b>3</b> |

## Introduction

Differentially expressed genes (DEGs) between the MarNeu depleted and control conditions were identified for each cluster using the FindMarkers function from the Seurat package. Genes with an adjusted p-value < 0.05 and an average log fold change (logFC) > 0.25 or < -0.25 were considered significantly differentially expressed between the two conditions.

## Loading packages

```
suppressMessages({
  library(dplyr)
  library(Seurat)
  library(patchwork)
  library(ggplot2)
  library(formatR)
  library(SingleR)
  library(dittoSeq)
  library(ComplexHeatmap)
  library(ggrepel)
  library(xlsx)
})
```

## Loading data

```
neutro_depletion <- readRDS("../06-Neutrophils_Processing/neutro_depleted_Part1.rds")
```

## Volcano\_plot in gCap

```
Markers_depletion <- FindMarkers(neutro_depletion, ident.1 = "PBS", ident.2 = c("acxcr2",
  "aLy6G"), min.pct = 0.25, group.by = "orig.ident", logfc.threshold = 0)

# Markers_depletion <- Markers_depletion[Markers_depletion$p_val_adj
# < 0.05,] write.xlsx(Markers_depletion, 'plot/DE_gene_neutro.xlsx',
# sheetName = 'Whole', col.names = T, append = T)

Markers_depletion$category <- ifelse(Markers_depletion$p_val_adj < 0.05 &
  abs(Markers_depletion$avg_log2FC) > 0.25, "overexpressed", ifelse(Markers_depletion$p_val_adj <
  0.05 & Markers_depletion$avg_log2FC < 0.25, "significant", "no significant"))

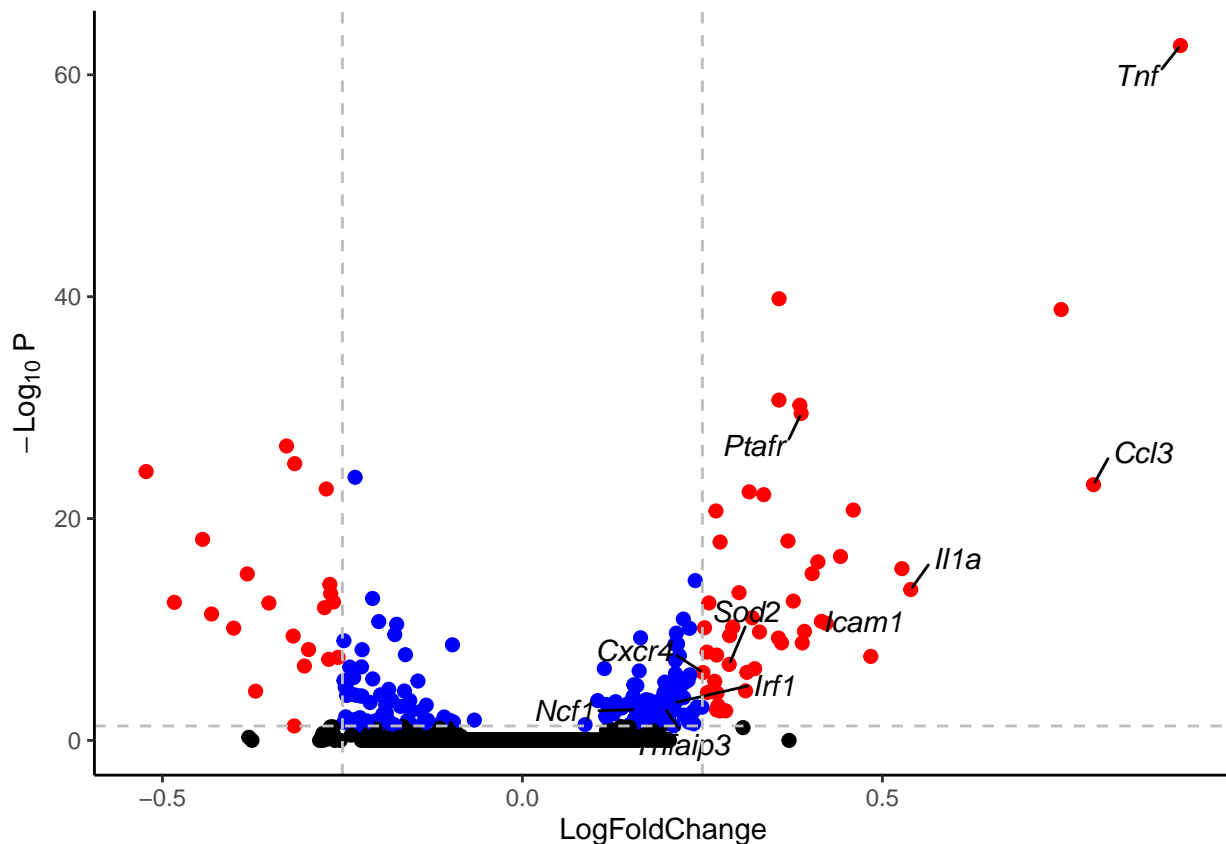
Markers_depletion <- Markers_depletion[order(Markers_depletion$avg_log2FC,
  decreasing = T), ]

gene_to_highlight <- c("Tnf", "Ccl3", "Il1a", "Icam1", "Ptafr", "Sod2",
  "Cxcr4", "Tlr2", "Irf1", "Tnfaip3", "Ncf1")
Markers_depletion$label <- NA
Markers_depletion$label <- rownames(Markers_depletion)

ggplot(data = Markers_depletion, aes(x = avg_log2FC, y = -log10(p_val_adj),
  col = category)) + geom_point(size = 2, alpha = 1) + theme_classic() +
```

```
scale_color_manual(values = c("black", "red", "blue")) + geom_text_repel(data = Markers_depletion[r
gene_to_highlight, ], size = 4, aes(label = gene_to_highlight, fontface = "italic"),
colour = "black", force = 5, box.padding = 0.5) + NoLegend() + ylab(expression(-Log[10] *
" P")) + xlab("LogFoldChange") + theme() + geom_hline(yintercept = -log10(0.05),
linetype = "dashed", col = "grey") + geom_vline(xintercept = c(-0.25,
0.25), linetype = "dashed", col = "grey")
```

```
## Warning: ggrepel: 1 unlabeled data points (too many overlaps). Consider
## increasing max.overlaps
```



```
# ggsave('plot/Volcano_neutro.pdf', width = 8, height = 5)
```

## DE per cluster

```
for (Cluster in unique(neutro_depletion$seurat_clusters)) {

  subset_neutro <- subset(neutro_depletion, seurat_clusters %in% Cluster)

  Markers_cond <- FindMarkers(subset_neutro, ident.1 = "PBS", ident.2 = c("acxcr2",
    "aLy6G"), min.pct = 0.1, group.by = "orig.ident", logfc.threshold = 0.25)

  Markers_cond <- Markers_cond[Markers_cond$p_val_adj < 0.05, ]
  # write.xlsx(Markers_cond, 'plot/DE_gene_neutro.xlsx', sheetName
  # = Cluster, col.names = T, append = T)

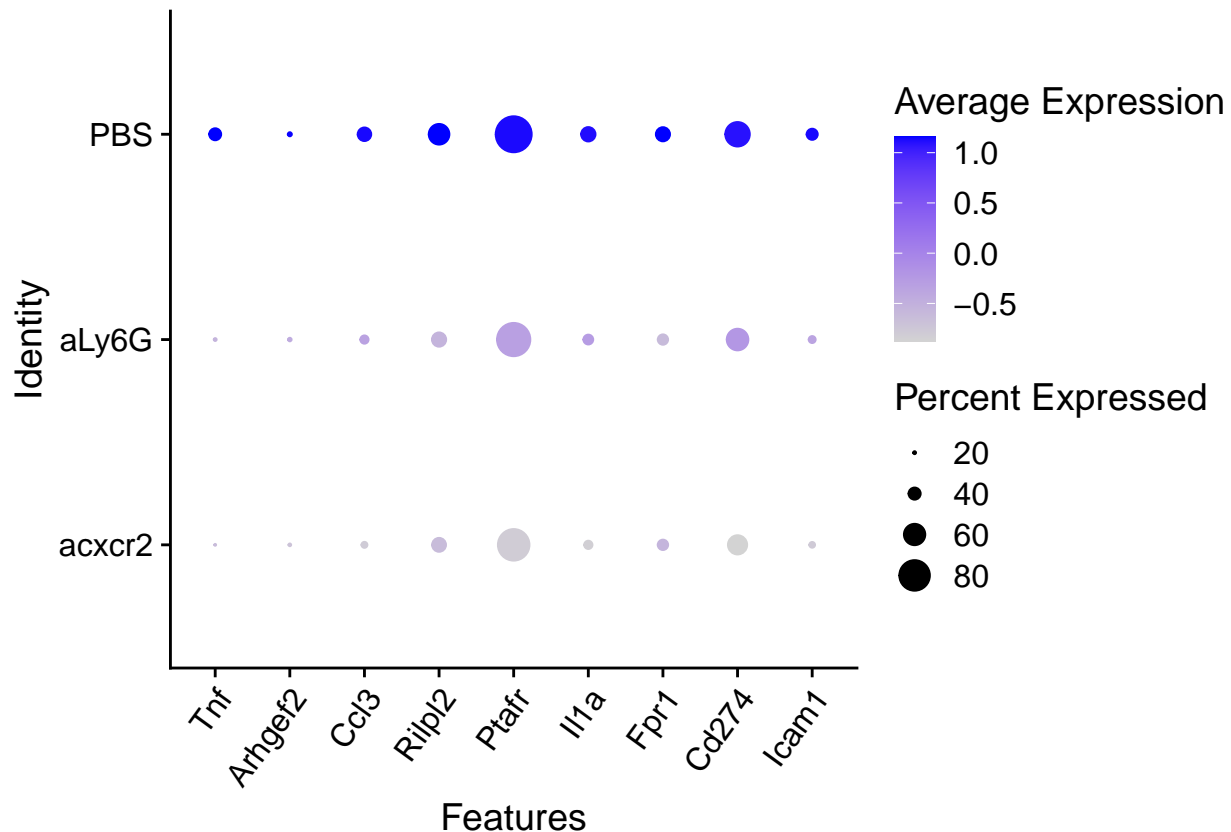
  print(paste0("The number of DEG for ", Cluster, " is ", length(rownames(Markers_cond))))
}
```

```
}
```

```
## [1] "The number of DEG for 1 is 27"
## [1] "The number of DEG for 0 is 58"
## [1] "The number of DEG for 3 is 0"
## [1] "The number of DEG for 2 is 41"
## [1] "The number of DEG for 6 is 0"
## [1] "The number of DEG for 5 is 1"
## [1] "The number of DEG for 4 is 0"
## [1] "The number of DEG for 7 is 0"
```

```
DotPlot(neutro_depletion, features = c("Tnf", "Arhgef2", "Ccl3", "Rilpl2",
  "Ptafr", "Il1a", "Fpr1", "Cd274", "Icam1"), group.by = "orig.ident") +
  theme(axis.text.x = element_text(angle = 54, hjust = 1))
```

```
## Warning: Scaling data with a low number of groups may produce misleading
## results
```



```
# ggsave('plot/dotplot_gene_neutro.pdf', width = 7, height = 4)
```

```
sessionInfo()
```

```
## R version 4.3.3 (2024-02-29)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 24.04.2 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/libopenblas-p-r0.3.26.so; LAPACK version 3.12.0
```

```

##
## locale:
## [1] LC_CTYPE=en_US.UTF-8          LC_NUMERIC=C
## [3] LC_TIME=fr_BE.UTF-8          LC_COLLATE=en_US.UTF-8
## [5] LC_MONETARY=fr_BE.UTF-8      LC_MESSAGES=en_US.UTF-8
## [7] LC_PAPER=fr_BE.UTF-8         LC_NAME=fr_BE.UTF-8
## [9] LC_ADDRESS=fr_BE.UTF-8       LC_TELEPHONE=fr_BE.UTF-8
## [11] LC_MEASUREMENT=fr_BE.UTF-8   LC_IDENTIFICATION=fr_BE.UTF-8
##
## time zone: Europe/Brussels
## tzcode source: system (glibc)
##
## attached base packages:
## [1] grid      stats4      stats      graphics  grDevices  utils      datasets
## [8] methods  base
##
## other attached packages:
## [1] xlsx_0.6.5                ggrepel_0.9.3
## [3] ComplexHeatmap_2.16.0     dittoSeq_1.12.0
## [5] SingleR_2.2.0             SummarizedExperiment_1.30.2
## [7] Biobase_2.60.0            GenomicRanges_1.52.0
## [9] GenomeInfoDb_1.36.0       IRanges_2.34.0
## [11] S4Vectors_0.38.1          BiocGenerics_0.46.0
## [13] MatrixGenerics_1.12.2     matrixStats_1.0.0
## [15] formatR_1.14              ggplot2_3.4.2
## [17] patchwork_1.1.2           SeuratObject_4.1.3
## [19] Seurat_4.3.0              dplyr_1.1.2
##
## loaded via a namespace (and not attached):
## [1] RcppAnnoy_0.0.21          splines_4.3.3
## [3] later_1.3.1              bitops_1.0-7
## [5] tibble_3.2.1             polyclip_1.10-4
## [7] lifecycle_1.0.3          doParallel_1.0.17
## [9] globals_0.16.2           lattice_0.22-5
## [11] MASS_7.3-60.0.1          magrittr_2.0.3
## [13] limma_3.56.2             plotly_4.10.2
## [15] rmarkdown_2.23           yaml_2.3.7
## [17] httpuv_1.6.11            sctransform_0.3.5
## [19] spam_2.9-1               sp_2.2-0
## [21] spatstat.sparse_3.0-2     reticulate_1.30
## [23] cowplot_1.1.1            pbapply_1.7-2
## [25] RColorBrewer_1.1-3       abind_1.4-5
## [27] zlibbioc_1.46.0          Rtsne_0.16
## [29] purrr_1.0.1              RCurl_1.98-1.12
## [31] xlsxjars_0.6.1           circlize_0.4.15
## [33] GenomeInfoDbData_1.2.10  irlba_2.3.5.1
## [35] listenv_0.9.0            spatstat.utils_3.0-3
## [37] pheatmap_1.0.12          goftest_1.2-3
## [39] spatstat.random_3.1-5    fitdistrplus_1.1-11
## [41] parallelly_1.36.0        DelayedMatrixStats_1.22.1
## [43] leiden_0.4.3             codetools_0.2-19
## [45] DelayedArray_0.26.3      tidyselect_1.2.0
## [47] shape_1.4.6              farver_2.1.1
## [49] ScaledMatrix_1.8.1       spatstat.explore_3.2-1

```

|                              |                             |
|------------------------------|-----------------------------|
| ## [51] jsonlite_1.8.7       | GetoptLong_1.0.5            |
| ## [53] ellipsis_0.3.2       | progressr_0.13.0            |
| ## [55] ggribges_0.5.4       | survival_3.5-8              |
| ## [57] iterators_1.0.14     | foreach_1.5.2               |
| ## [59] tools_4.3.3          | ica_1.0-3                   |
| ## [61] Rcpp_1.0.11          | glue_1.6.2                  |
| ## [63] gridExtra_2.3        | xfun_0.39                   |
| ## [65] withr_2.5.0          | fastmap_1.1.1               |
| ## [67] fansi_1.0.4          | digest_0.6.33               |
| ## [69] rsvd_1.0.5           | R6_2.5.1                    |
| ## [71] mime_0.12            | colorspace_2.1-0            |
| ## [73] scattermore_1.2      | tensor_1.5                  |
| ## [75] spatstat.data_3.0-1  | utf8_1.2.3                  |
| ## [77] tidyr_1.3.0          | generics_0.1.3              |
| ## [79] data.table_1.14.8    | httr_1.4.6                  |
| ## [81] htmlwidgets_1.6.2    | S4Arrays_1.2.1              |
| ## [83] uwot_0.1.16          | pkgconfig_2.0.3             |
| ## [85] rJava_1.0-6          | gtable_0.3.3                |
| ## [87] lmtest_0.9-40        | SingleCellExperiment_1.22.0 |
| ## [89] XVector_0.40.0       | htmltools_0.5.5             |
| ## [91] dotCall64_1.0-2      | clue_0.3-64                 |
| ## [93] scales_1.2.1         | png_0.1-8                   |
| ## [95] knitr_1.43           | rstudioapi_0.14             |
| ## [97] reshape2_1.4.4       | rjson_0.2.21                |
| ## [99] nlme_3.1-164         | zoo_1.8-12                  |
| ## [101] GlobalOptions_0.1.2 | stringr_1.5.0               |
| ## [103] KernSmooth_2.23-22  | parallel_4.3.3              |
| ## [105] miniUI_0.1.1.1      | pillar_1.9.0                |
| ## [107] vctrs_0.6.3         | RANN_2.6.1                  |
| ## [109] promises_1.2.0.1    | BiocSingular_1.16.0         |
| ## [111] beachmat_2.16.0     | xtable_1.8-4                |
| ## [113] cluster_2.1.6       | evaluate_0.21               |
| ## [115] cli_3.6.1           | compiler_4.3.3              |
| ## [117] rlang_1.1.1         | crayon_1.5.2                |
| ## [119] future.apply_1.11.0 | labeling_0.4.2              |
| ## [121] plyr_1.8.8          | stringi_1.8.4               |
| ## [123] viridisLite_0.4.2   | deldir_1.0-9                |
| ## [125] BiocParallel_1.34.2 | munsell_0.5.0               |
| ## [127] lazyeval_0.2.2      | spatstat.geom_3.2-4         |
| ## [129] Matrix_1.6-1        | sparseMatrixStats_1.12.0    |
| ## [131] future_1.33.0       | shiny_1.7.4.1               |
| ## [133] highr_0.10          | ROCR_1.0-11                 |
| ## [135] igraph_1.5.0.1      |                             |