

3-Comparison-Condition

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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

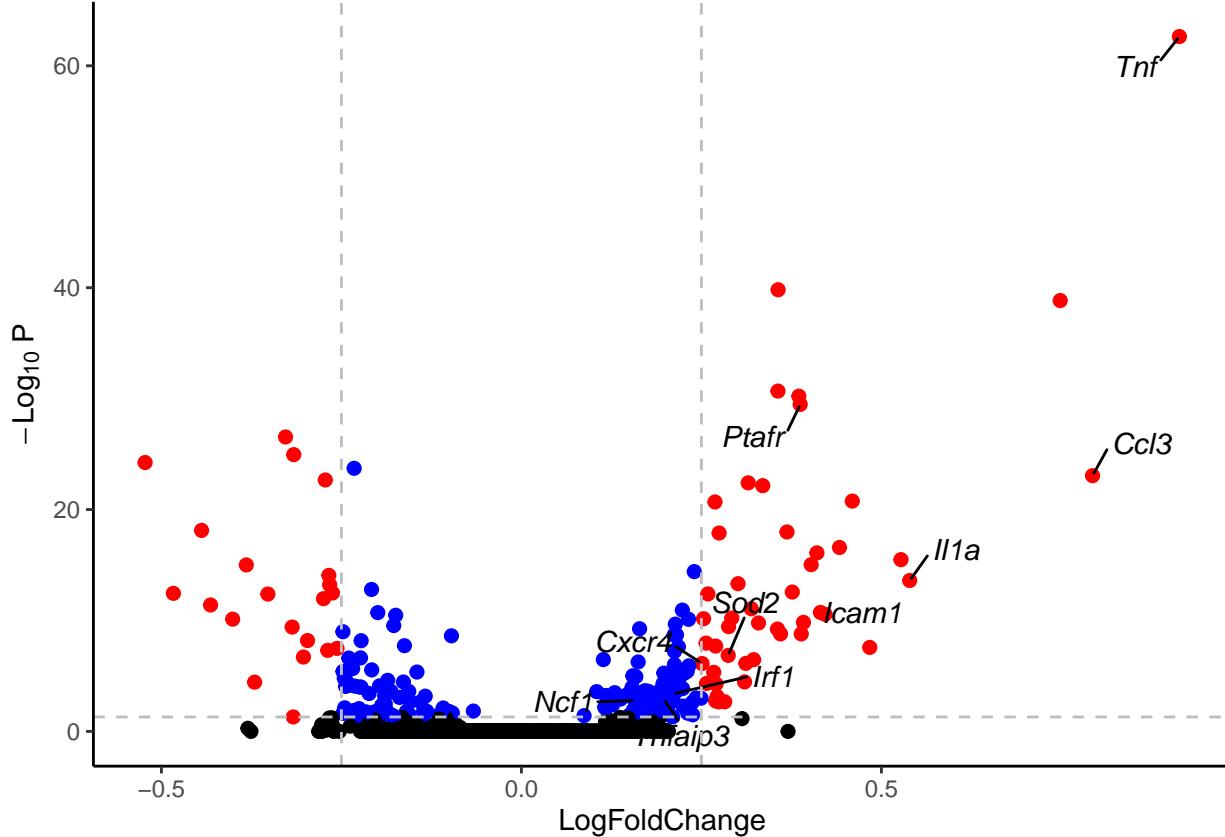
```
Markers_depletion <- FindMarkers(neutro_depletion, ident.1 = "PBS", ident.2 = c("acxcr2",  
  "aLy6G"), min.pct = 0.25, group.by = "orig.ident", logfc.threshold = 0)  
  
# Markers_depletion <- Markers_depletion[Markers_depletion$p_val_adj  
# < 0.05,] write.xlsx(Markers_depletion, 'plot/DE_gene_neutro.xlsx',  
# 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, "significative", "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[re
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))))
}
```

```

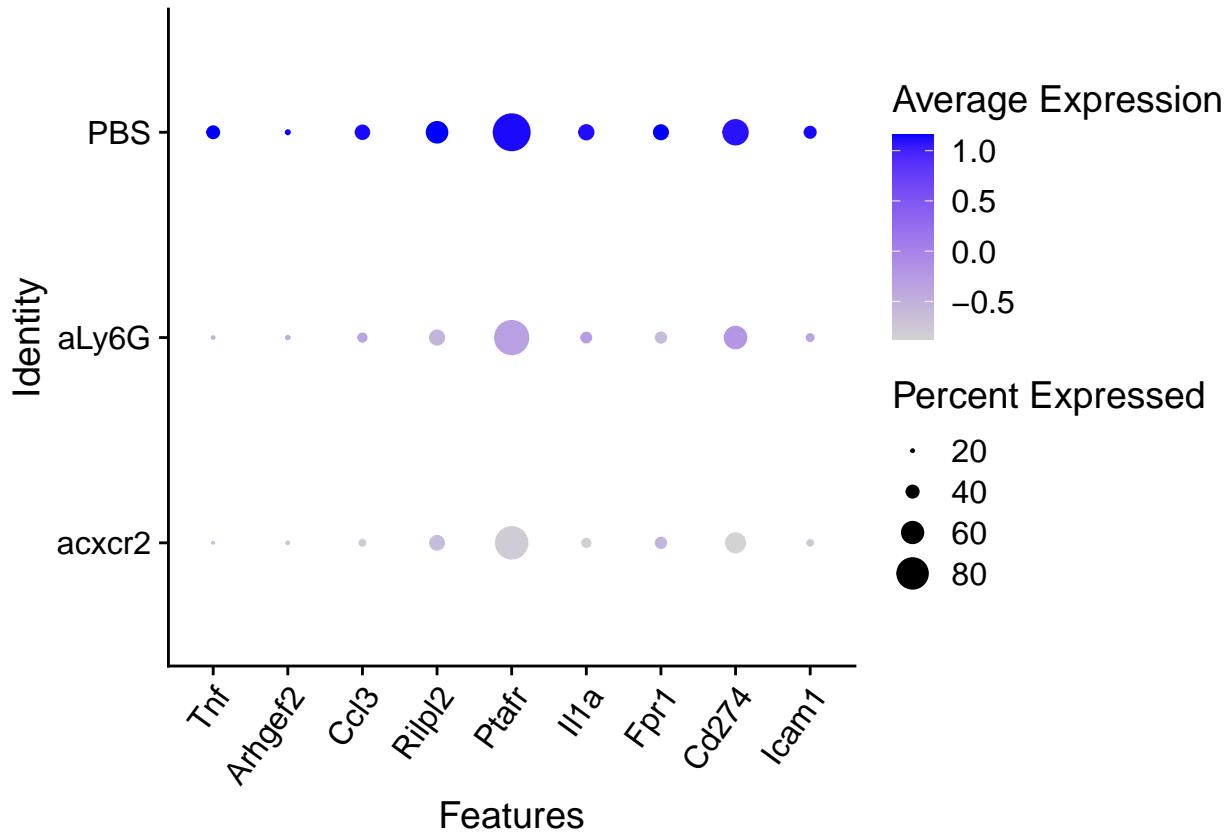
}

## [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-pthread/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] purrrr_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
## [53] ellipsis_0.3.2
## [55] ggridges_0.5.4
## [57] iterators_1.0.14
## [59] tools_4.3.3
## [61] Rcpp_1.0.11
## [63] gridExtra_2.3
## [65] withr_2.5.0
## [67] fansi_1.0.4
## [69] rsvd_1.0.5
## [71] mime_0.12
## [73] scattermore_1.2
## [75] spatstat.data_3.0-1
## [77] tidyR_1.3.0
## [79] data.table_1.14.8
## [81] htmlwidgets_1.6.2
## [83] uwot_0.1.16
## [85] rJava_1.0-6
## [87] lmtest_0.9-40
## [89] XVector_0.40.0
## [91] dotCall164_1.0-2
## [93] scales_1.2.1
## [95] knitr_1.43
## [97] reshape2_1.4.4
## [99] nlme_3.1-164
## [101] GlobalOptions_0.1.2
## [103] KernSmooth_2.23-22
## [105] miniUI_0.1.1.1
## [107] vctrs_0.6.3
## [109] promises_1.2.0.1
## [111] beachmat_2.16.0
## [113] cluster_2.1.6
## [115] cli_3.6.1
## [117] rlang_1.1.1
## [119] future.apply_1.11.0
## [121] plyr_1.8.8
## [123] viridisLite_0.4.2
## [125] BiocParallel_1.34.2
## [127] lazyeval_0.2.2
## [129] Matrix_1.6-1
## [131] future_1.33.0
## [133] highr_0.10
## [135] igraph_1.5.0.1

## [51] GetoptLong_1.0.5
## [53] progressr_0.13.0
## [55] survival_3.5-8
## [57] foreach_1.5.2
## [59] ica_1.0-3
## [61] glue_1.6.2
## [63] xfun_0.39
## [65] fastmap_1.1.1
## [67] digest_0.6.33
## [69] R6_2.5.1
## [71] colorspace_2.1-0
## [73] tensor_1.5
## [75] utf8_1.2.3
## [77] generics_0.1.3
## [79] httr_1.4.6
## [81] S4Arrays_1.2.1
## [83] pkgconfig_2.0.3
## [85] gtable_0.3.3
## [87] SingleCellExperiment_1.22.0
## [89] htmltools_0.5.5
## [91] clue_0.3-64
## [93] png_0.1-8
## [95] rstudioapi_0.14
## [97] rjson_0.2.21
## [99] zoo_1.8-12
## [101] stringr_1.5.0
## [103] parallel_4.3.3
## [105] pillar_1.9.0
## [107] RANN_2.6.1
## [109] BiocSingular_1.16.0
## [111] xtable_1.8-4
## [113] evaluate_0.21
## [115] compiler_4.3.3
## [117] crayon_1.5.2
## [119] labeling_0.4.2
## [121] stringi_1.8.4
## [123] deldir_1.0-9
## [125] munsell_0.5.0
## [127] spatstat.geom_3.2-4
## [129] sparseMatrixStats_1.12.0
## [131] shiny_1.7.4.1
## [133] ROCR_1.0-11

```