

2-Cluster characterization

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Introduction

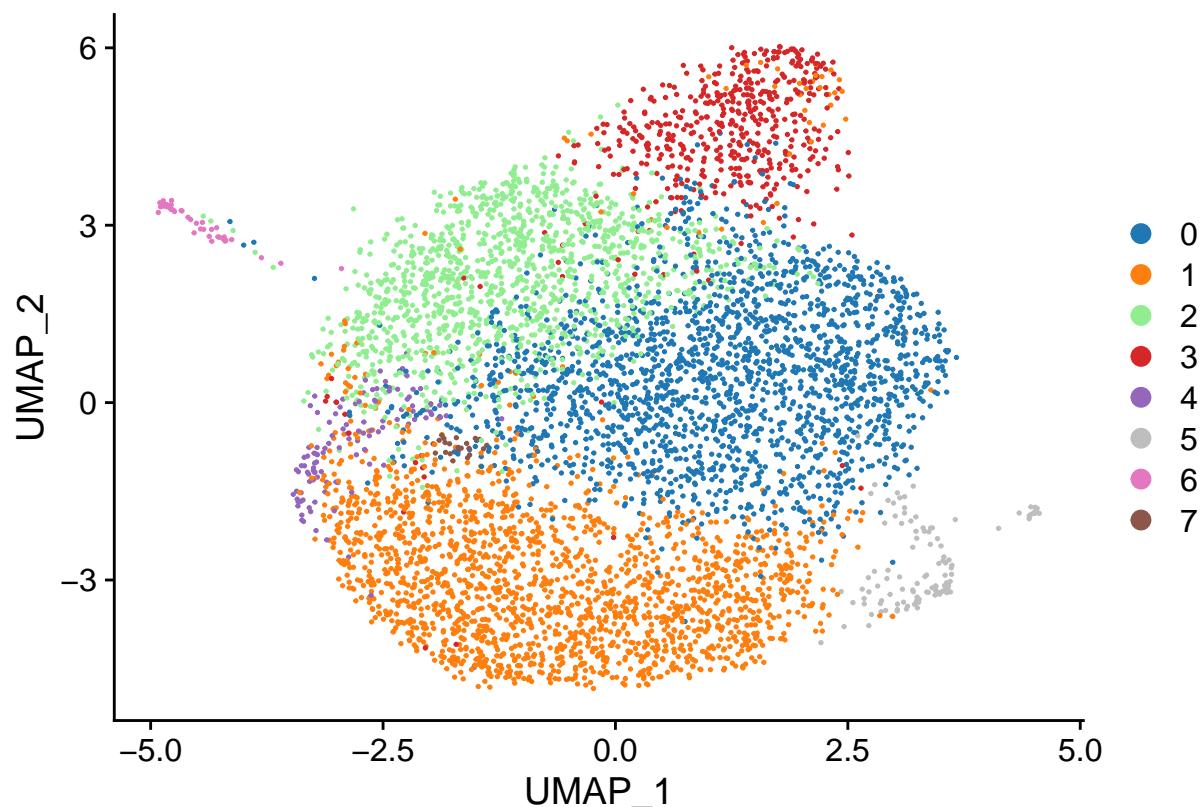
A total of 8 clusters of neutrophils were obtained using the FindClusters function (15 Principal Components were included and a resolution of 0.25 was selected) and the differentially expressed genes (DEGs) were calculated using the FindAllMarkers function (Seurat package).

Loading packages

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

Loading data

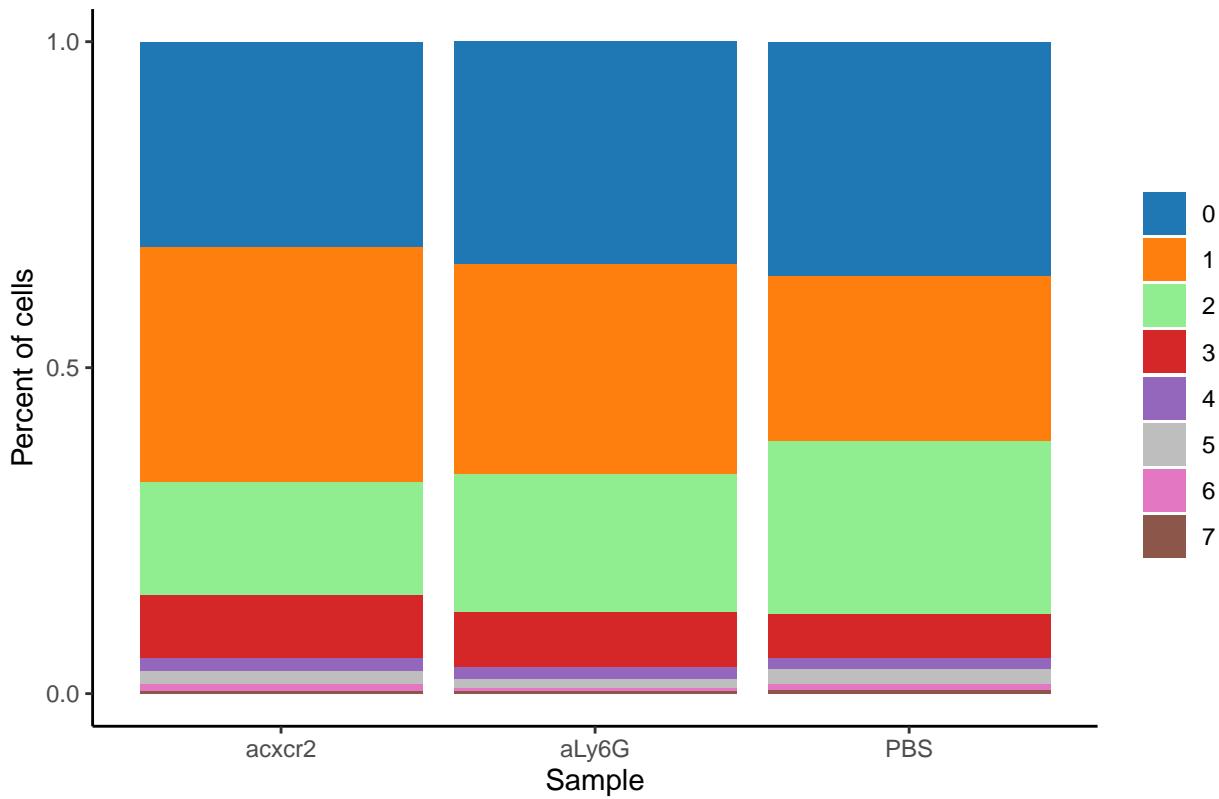
```
neutro_depletion <- readRDS("../06-Neutrophils_Processing/neutro_depleted_Part1.rds")  
  
color_neut <- c("#1f77b4", "#ff7f0e", "lightgreen", "#d62728", "#9467bd",  
  "grey", "#e377c2", "#8c564b")  
DimPlot(neutro_depletion, cols = color_neut)
```



```
# ggsave('plot/umap.pdf', width = 6, height = 4)
```

Cluster Frequency

```
dittoBarPlot(neutro_depletion, "seurat_clusters", group.by = "orig.ident",
  main = "", x.labels = c("acxcr2", "aLy6G", "PBS"), x.labels.rotate = F,
  xlab = "Sample", color.panel = color_neut)
```



```
# ggsave('plot/cluster_freq.pdf', width = 10, height = 6)
```

Visualisation

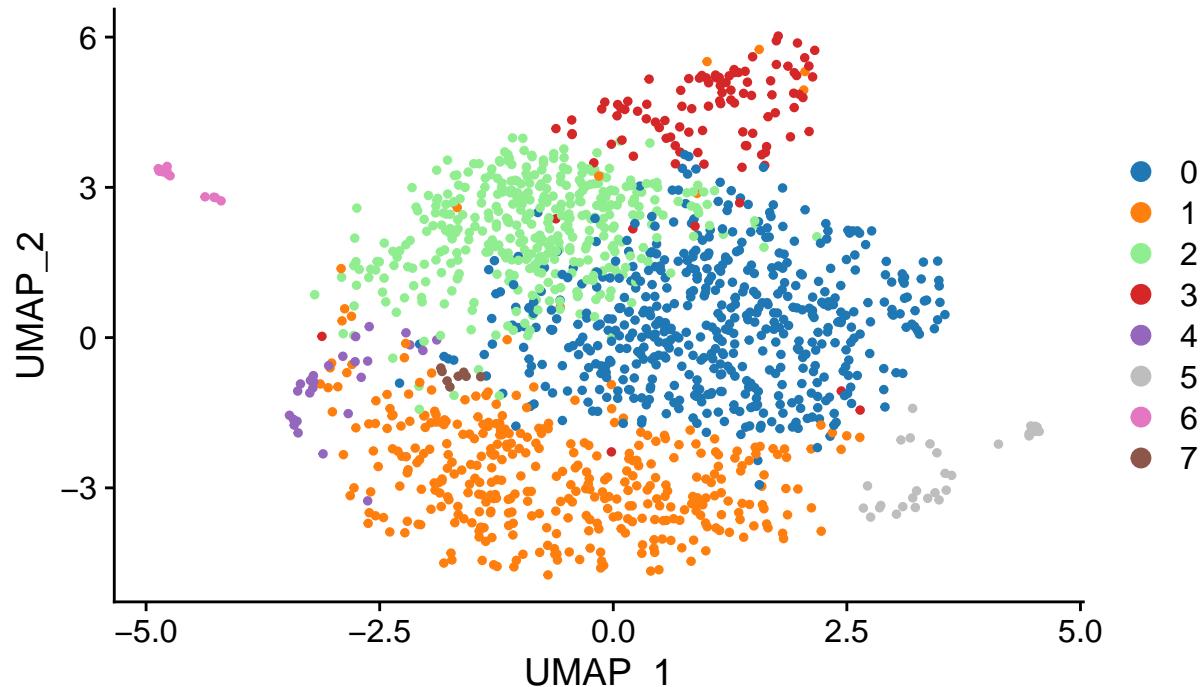
Neutro PBS

```
neutro_depletion_PBS <- subset(neutro_depletion, orig.ident %in% "PBS")

DimPlot(neutro_depletion_PBS, reduction = "umap", cols = color_neut, group.by = "seurat_clusters") +
  plot_annotation(title = "Neutrophils - PBS", theme = theme(plot.title = element_text(size = 16,
    hjust = 0.5)))
```

Neutrophils – PBS

seurat_clusters



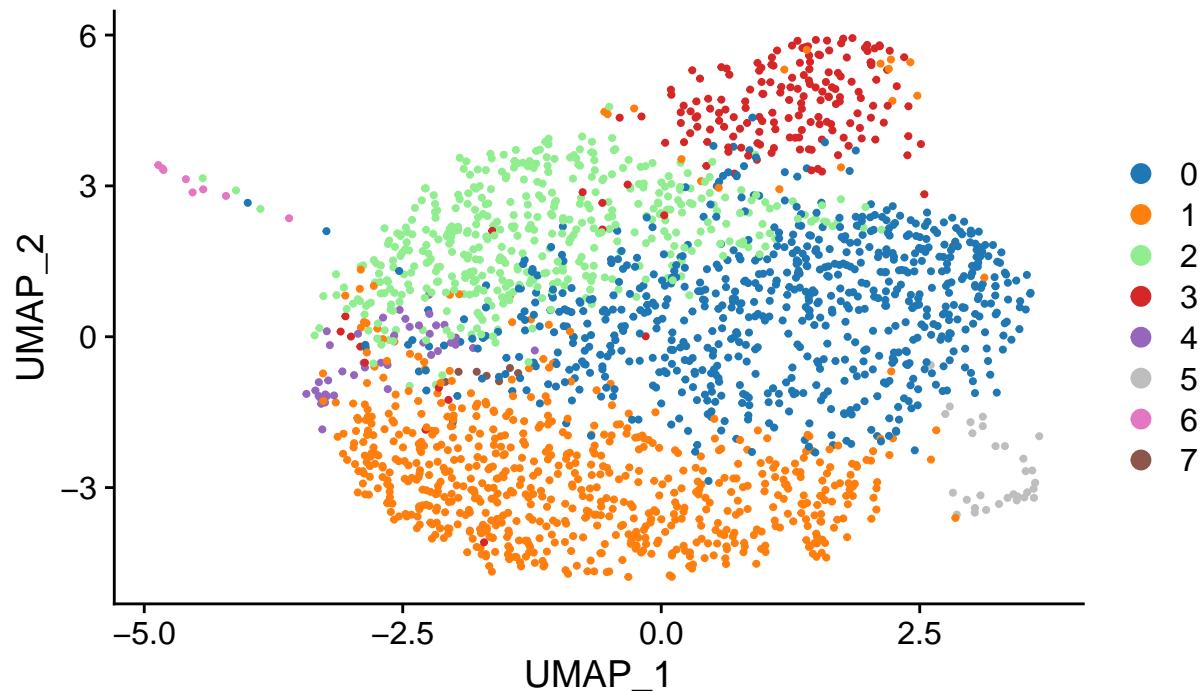
```
# ggsave('plot/UMAP_PBS.pdf', width = 6, height = 4)
```

Neutro post depletion by aLy6g

```
neutro_depletion_aLy6G <- subset(neutro_depletion, orig.ident %in% "aLy6G")  
  
DimPlot(neutro_depletion_aLy6G, reduction = "umap", cols = color_neut,  
group.by = "seurat_clusters") + plot_annotation(title = "Neutrophils - aLy6G",  
theme = theme(plot.title = element_text(size = 16, hjust = 0.5)))
```

Neutrophils – aLy6G

seurat_clusters



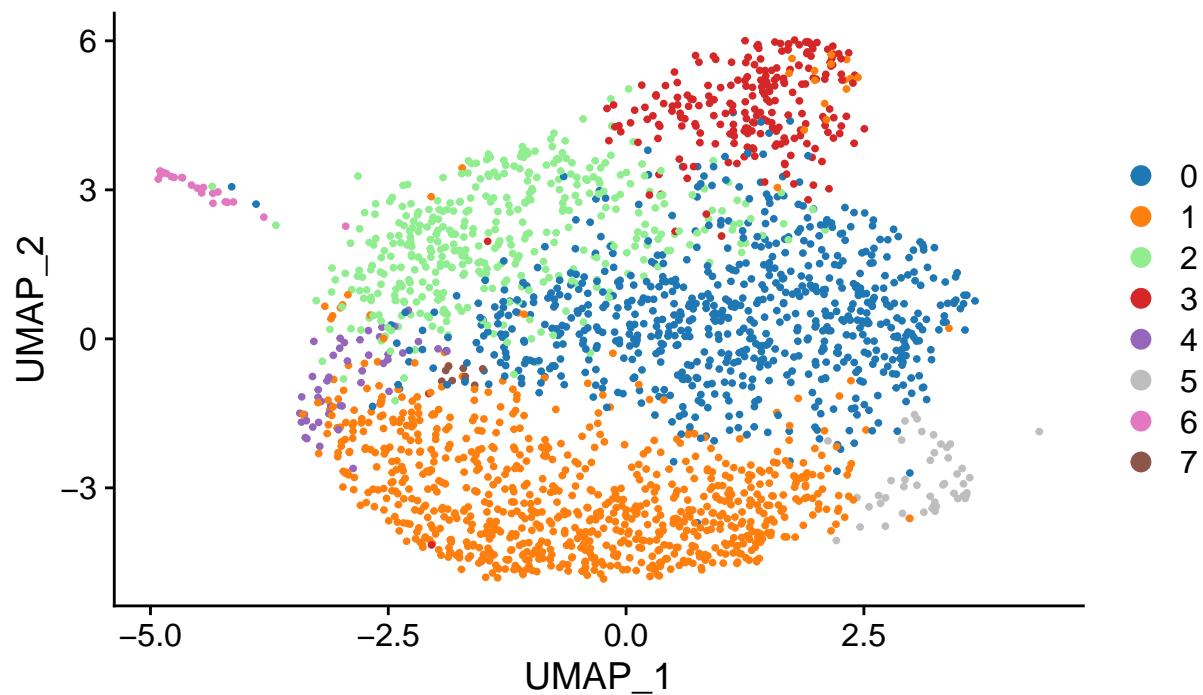
```
# ggsave('plot/UMAP_aLy6G.pdf', width = 6, height = 4)
```

Neutro post depletion by aCXCR2

```
neutro_depletion_acxcr2 <- subset(neutro_depletion, orig.ident %in% "acxcr2")  
  
DimPlot(neutro_depletion_acxcr2, reduction = "umap", cols = color_neut,  
group.by = "seurat_clusters") + plot_annotation(title = "Neutrophils - acxcr2",  
theme = theme(plot.title = element_text(size = 16, hjust = 0.5)))
```

Neutrophils – acxcr2

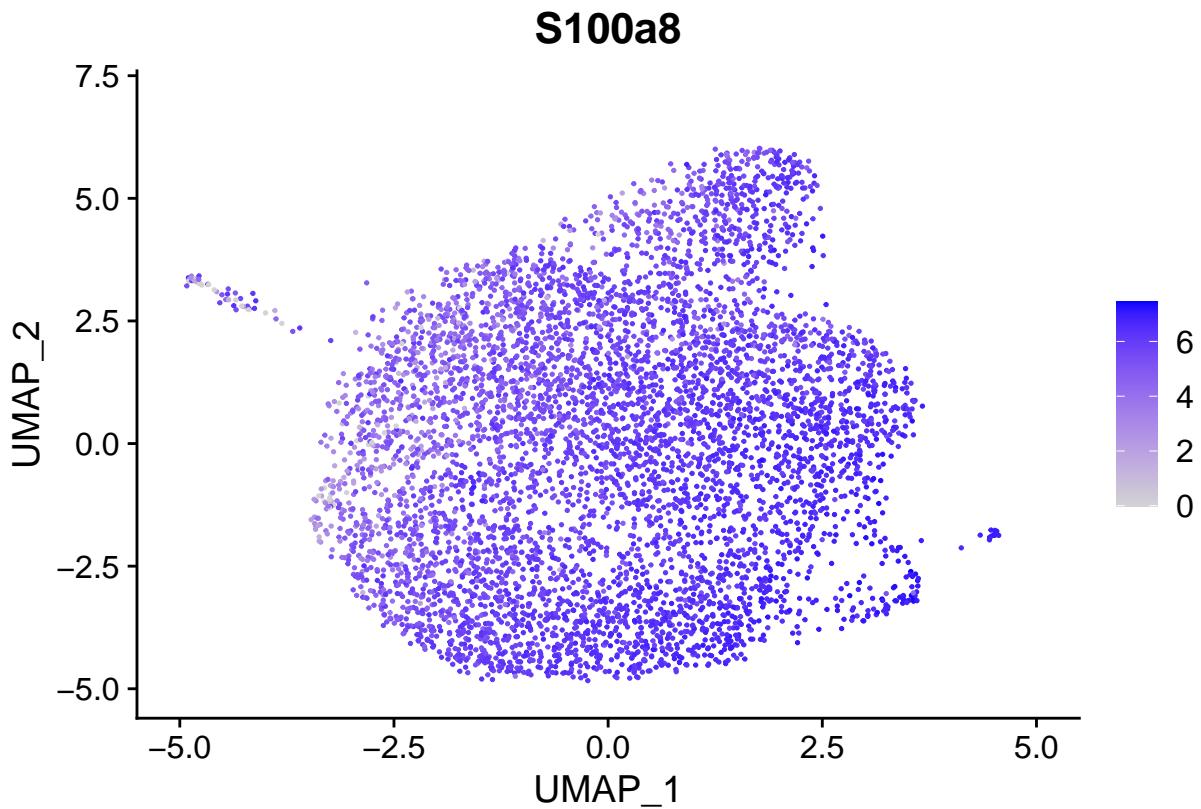
seurat_clusters



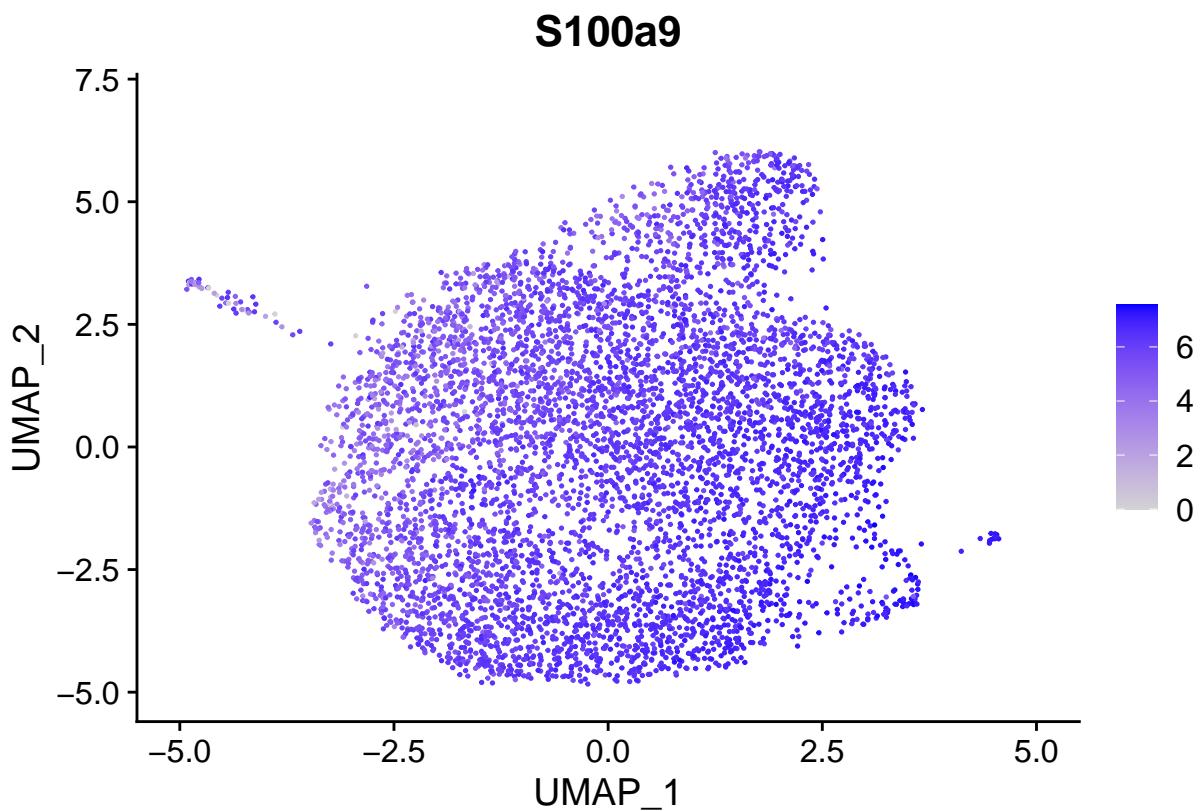
```
# ggsave('plot/UMAP_acxcr2.pdf', width = 6, height = 4)
```

Feature Plot

```
FeaturePlot(neutro_depletion, features = c("S100a8"))
```

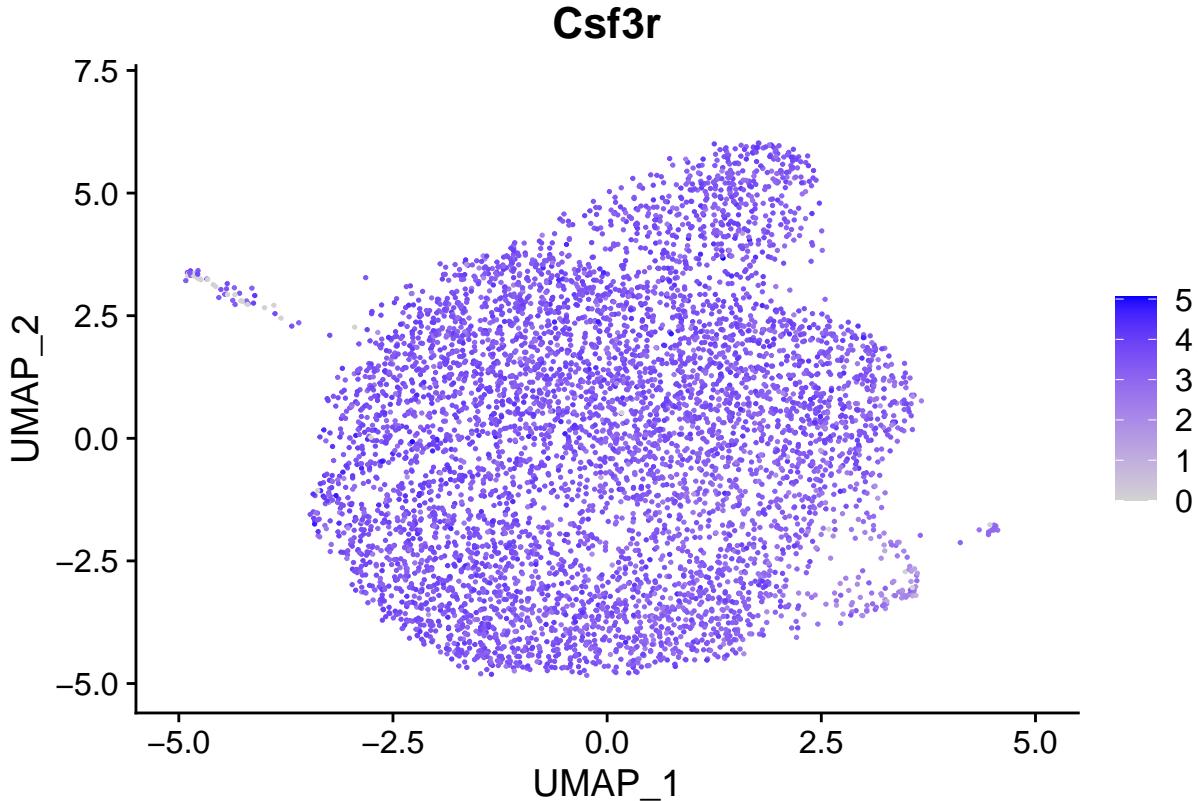


```
# ggsave('plot/Feature_S100a8.pdf', width = 6, height = 4)  
FeaturePlot(neutro_depletion, features = c("S100a8"))
```



```
# ggsave('plot/Feature_S100a9r.pdf', width = 6, height = 4)
```

```
FeaturePlot(neutro_depletion, features = c("Csf3r"))
```



```
# ggsave('plot/Feature_Csf3r.pdf', width = 6, height = 4)
```

Heatmap DE gene per cluster

```
clusters.markers <- FindAllMarkers(neutro_depletion, only.pos = TRUE, min.pct = 0.25,
logfc.threshold = 0.25)

clusters.markers %>%
  group_by(cluster) %>%
  slice_max(n = 10, order_by = avg_log2FC) -> top10

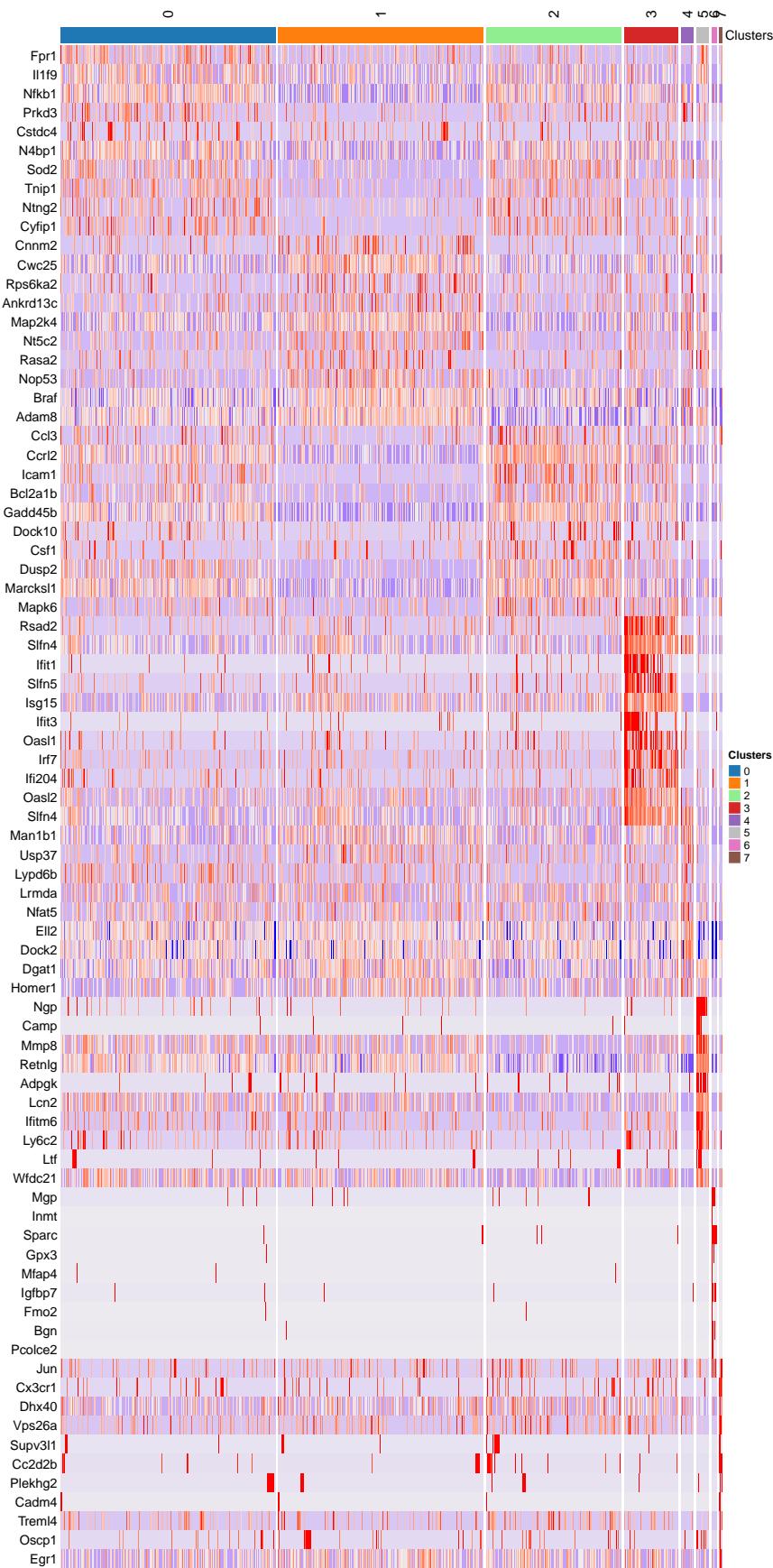
mat <- as.matrix(GetAssayData(object = neutro_depletion, slot = "data")[as.character(top10$gene),
])

df <- as.data.frame(neutro_depletion$seurat_clusters)
colnames(df) <- "Clusters"
color_df <- list(Clusters = c(`0` = "#1f77b4", `1` = "#ff7f0e", `2` = "lightgreen",
`3` = "#d62728", `4` = "#9467bd", `5` = "grey", `6` = "#e377c2", `7` = "#8c564b"))

Heatmap <- Heatmap(t(scale(t(mat))), show_column_names = F, column_split = neutro_depletion$seurat_clusters,
cluster_column_slices = F, cluster_rows = F, top_annotation = HeatmapAnnotation(df = df,
col = color_df), use_raster = F, show_heatmap_legend = F, show_column_dend = F,
column_title_rot = 90, row_names_side = "left")
```

```
# tidyHeatmap::save_pdf(Heatmap, 'plot/Heatmap_cluster_neutro.pdf',
# width = 25, height = 35, units ='cm')
```

```
Heatmap
```



```

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-p0.3.26.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=C
## [9] LC_ADDRESS=C                 LC_TELEPHONE=C
## [11] LC_MEASUREMENT=fr_BE.UTF-8   LC_IDENTIFICATION=C
##
## 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] ComplexHeatmap_2.16.0      dittoSeq_1.12.0
## [3] SingleR_2.2.0             SummarizedExperiment_1.30.2
## [5] Biobase_2.60.0            GenomicRanges_1.52.0
## [7] GenomeInfoDb_1.36.0        IRanges_2.34.0
## [9] S4Vectors_0.38.1           BiocGenerics_0.46.0
## [11] MatrixGenerics_1.12.2     matrixStats_1.0.0
## [13] formatR_1.14               ggplot2_3.4.2
## [15] patchwork_1.1.2            SeuratObject_4.1.3
## [17] 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] circlize_0.4.15              GenomeInfoDbData_1.2.10
## [33] ggrepel_0.9.3                irlba_2.3.5.1

```

```

## [35] listenv_0.9.0
## [37] pheatmap_1.0.12
## [39] spatstat.random_3.1-5
## [41] parallelly_1.36.0
## [43] leiden_0.4.3
## [45] DelayedArray_0.26.3
## [47] shape_1.4.6
## [49] ScaledMatrix_1.8.1
## [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] Cairo_1.6-2
## [75] tensor_1.5
## [77] utf8_1.2.3
## [79] generics_0.1.3
## [81] httr_1.4.6
## [83] S4Arrays_1.2.1
## [85] pkgconfig_2.0.3
## [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] magick_2.7.5
## [117] compiler_4.3.3
## [119] crayon_1.5.2
## [121] labeling_0.4.2
## [123] stringi_1.8.4
## [125] deldir_1.0-9
## [127] munsell_0.5.0
## [129] spatstat.geom_3.2-4
## [131] sparseMatrixStats_1.12.0
## [133] shiny_1.7.4.1
## [135] ROCR_1.0-11
spatstat.utils_3.0-3
goftest_1.2-3
fitdistrplus_1.1-11
DelayedMatrixStats_1.22.1
codetools_0.2-19
tidyselect_1.2.0
farver_2.1.1
spatstat.explore_3.2-1
GetoptLong_1.0.5
progressr_0.13.0
survival_3.5-8
foreach_1.5.2
ica_1.0-3
glue_1.6.2
xfun_0.39
fastmap_1.1.1
digest_0.6.33
R6_2.5.1
colorspace_2.1-0
scattermore_1.2
spatstat.data_3.0-1
tidyR_1.3.0
data.table_1.14.8
htmlwidgets_1.6.2
uwot_0.1.16
gttable_0.3.3
SingleCellExperiment_1.22.0
htmltools_0.5.5
clue_0.3-64
png_0.1-8
rstudioapi_0.14
rjson_0.2.21
zoo_1.8-12
stringr_1.5.0
parallel_4.3.3
pillar_1.9.0
RANN_2.6.1
BiocSingular_1.16.0
xtable_1.8-4
evaluate_0.21
cli_3.6.1
rlang_1.1.1
future.apply_1.11.0
plyr_1.8.8
viridisLite_0.4.2
BiocParallel_1.34.2
lazyeval_0.2.2
Matrix_1.6-1
future_1.33.0
highr_0.10
igraph_1.5.0.1

```