3-Perturbation Score

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Contents

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(dittoSeq)
    library(ggrepel)
    library(xlsx)
})
```

Loading annotated Cell

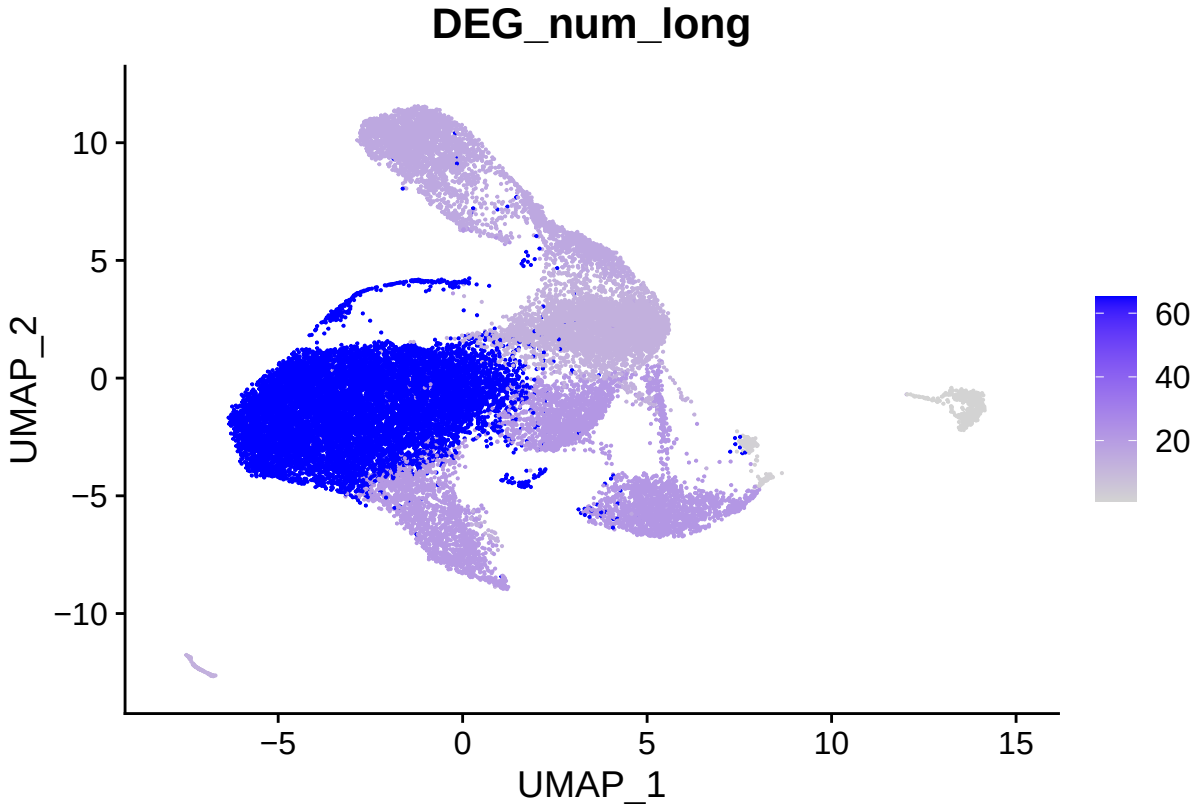
```
endothelial_cells <- readRDS("../02-Clustering_Endo/endothelial_cells_annotated.rds")
```

Perturbation score Day 14

calculating number of gene

```
## [1] "The number of DEG for 1 general capillaries is 65"
## [1] "The number of DEG for 3 pulmonary veins is 22"
## [1] "The number of DEG for 7 other is 13"
## [1] "The number of DEG for 2 aerocyte capilaries is 16"
## [1] "The number of DEG for 5 arteries is 21"
## [1] "The number of DEG for 6 lymphaptics is 1"
## [1] "The number of DEG for 4 systemic veins is 2"
```

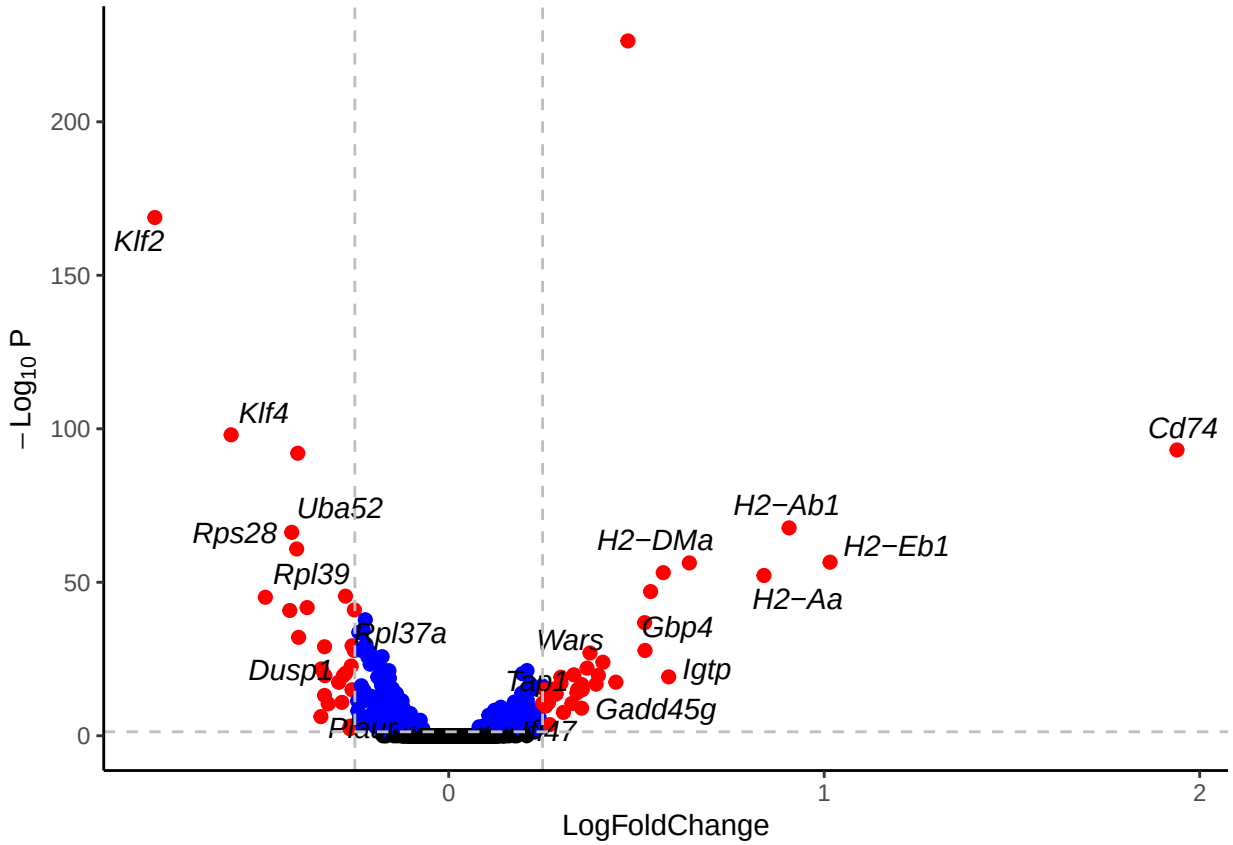
Feature Plot DEG number



Volcano_plot in gCap

```
endothelial_cells_subset <- subset(endothelial_cells, CellType %in%
    "1 general capillaries")

Markers_j14 <- FindMarkers(endothelial_cells_subset, ident.1 = "PBS",
    ident.2 = c("aCXCR2-D14", "aLy6G-D14"), min.pct = 0.25, group.by = "Condition",
    logfc.threshold = 0)

Markers_j14$category <- ifelse(Markers_j14$p_val_adj < 0.05 & abs(Markers_j14$avg_log2FC) >
    0.25, "overexpressed", ifelse(Markers_j14$p_val_adj < 0.05 & Markers_j14$avg_log2FC <
    0.25, "significative", "no significant"))

Markers_j14 <- Markers_j14[order(Markers_j14$avg_log2FC), ]

Markers_j14$label <- NA
# order <- c(1,2,5,6,12,14,15,19,27)
gene_to_highlight <- c("Klf2", "Klf4", "Uba52", "Rps28", "Dusp1",
    "Plaur", "Rpl39", "Rpl37a", "Ifi47", "Tap1", "Gadd45g", "Wars",
    "Gbp4", "Igtp", "H2-DMa", "H2-Aa", "H2-Ab1", "H2-Eb1", "Cd74")
Markers_j14$label <- ifelse(rownames(Markers_j14) %in% gene_to_highlight,
    rownames(Markers_j14), NA)

ggplot(data = Markers_j14, aes(x = avg_log2FC, y = -log10(p_val_adj),
    col = category, label = label)) + geom_point(size = 2, alpha = 1) +
    theme_classic() + scale_color_manual(values = c("black", "red",
```

```
    "blue")) + geom_text_repel(data = na.omit(Markers_j14), size = 4,
    aes(label = gene_to_highlight, fontface = "italic"), colour = "black",
    force = 4) + 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")
```




```
# ggsave('Plot/Volcano_gcap_J14.pdf', width = 8, height = 5)
```

Perturbation score Day 3

calculating number of gene

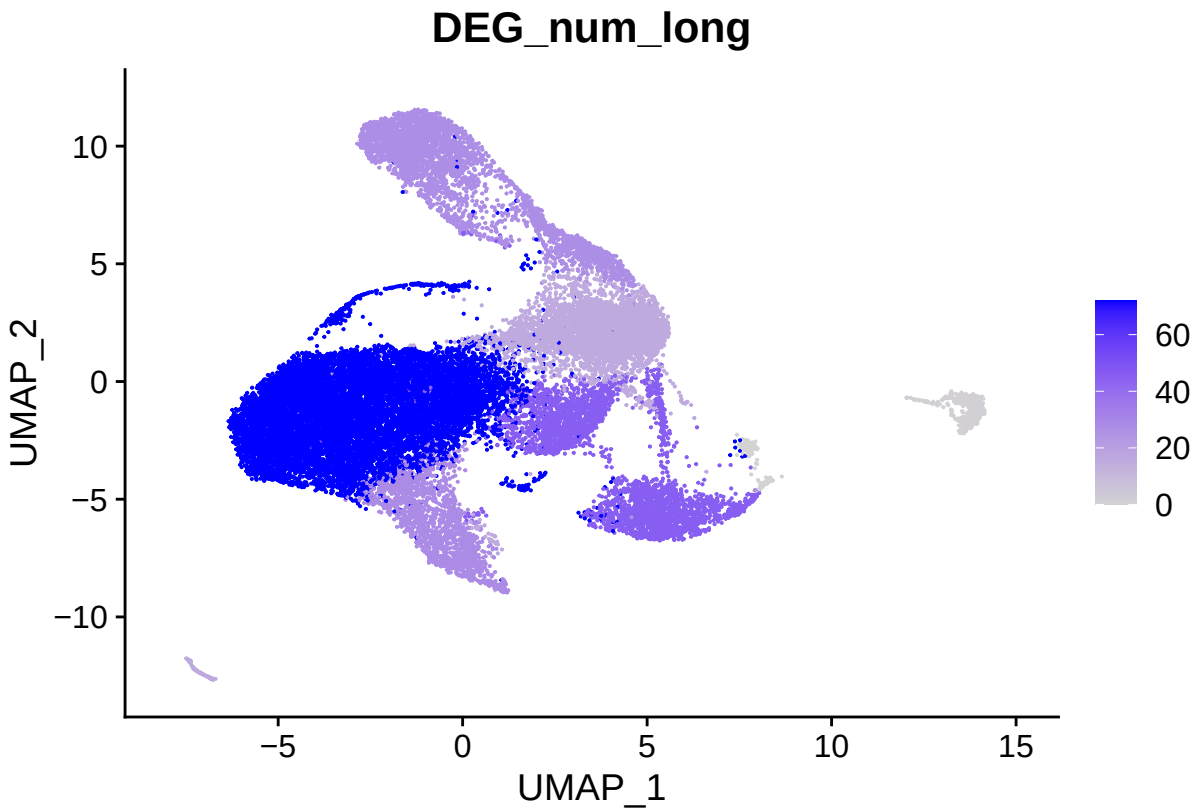
```
for (celltype in unique(endothelial_cells$CellType)) {

    endothelial_cells_subset <- subset(endothelial_cells, CellType %in%
        celltype)

    Markers_short <- FindMarkers(endothelial_cells_subset, ident.1 = "PBS",
        ident.2 = c("aCXCR2-D3", "aLy6G-D3"), min.pct = 0.1, group.by = "Condition",
        logfc.threshold = 0.25)

    Markers_short <- Markers_short[Markers_short$p_val_adj < 0.05,
        ]
    # write.xlsx(Markers_long, 'Plot/DE_gene_J3.xlsx', sheetName
    # = celltype, col.names = T, append = T)
```

```
    print(paste0("The number of DEG for ", celltype, " is ", length(rownames(Markers_long))))

}
```

```
## [1] "The number of DEG for 1 general capillaries is 2"
## [1] "The number of DEG for 3 pulmonary veins is 2"
## [1] "The number of DEG for 7 other is 2"
## [1] "The number of DEG for 2 aerocyte capilaries is 2"
## [1] "The number of DEG for 5 arteries is 2"
## [1] "The number of DEG for 6 lymphaptics is 2"
## [1] "The number of DEG for 4 systemic veins is 2"
```

Feature Plot DEG number

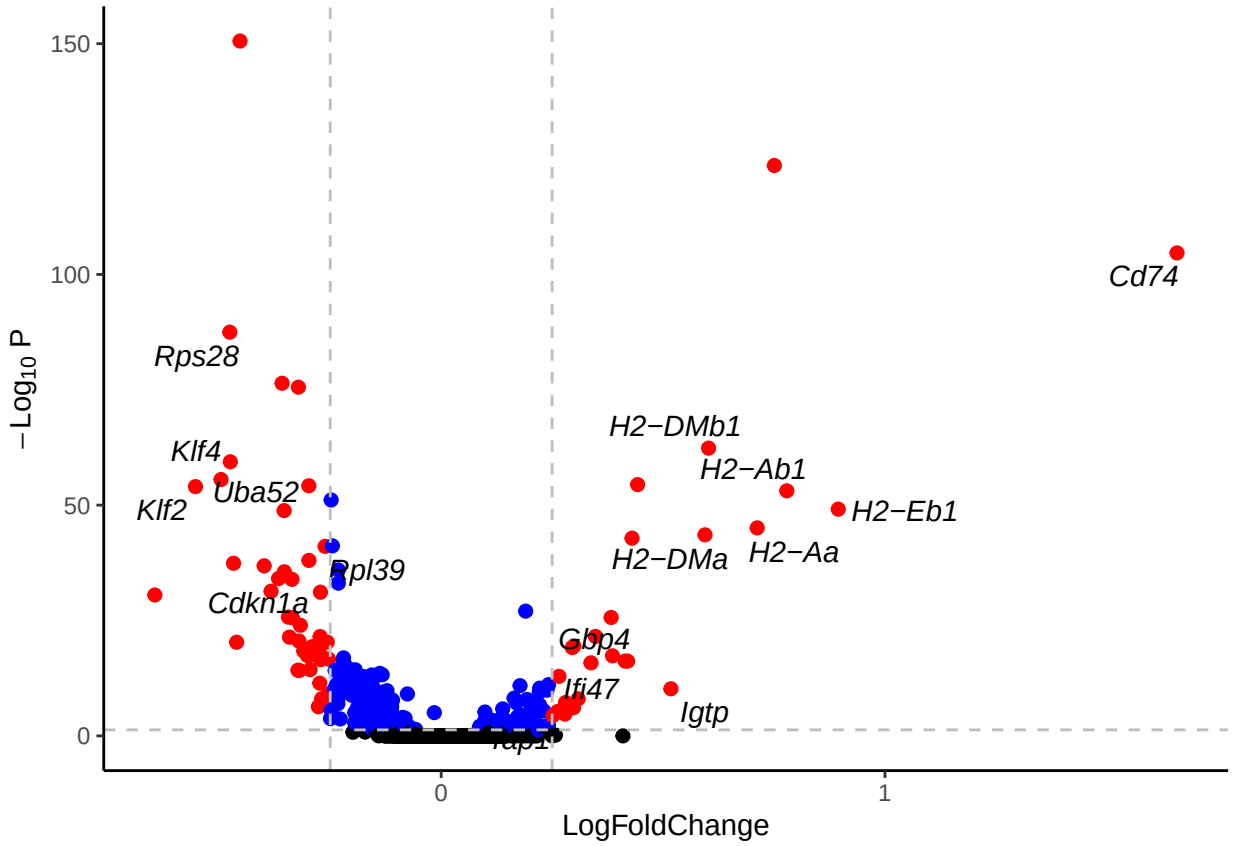
```
endothelial_cells$DEG_num_long <- endothelial_cells$CellType
levels(endothelial_cells$DEG_num_long) <- c(72, 27, 46, 0, 28, 1,
    16)

levels_as_integers <- as.integer(levels(endothelial_cells$DEG_num_long))
endothelial_cells$DEG_num_long <- levels_as_integers[as.integer(endothelial_cells$DEG_num_long)]

FeaturePlot(endothelial_cells, features = "DEG_num_long")
```




```
# ggsave('Plot/DEG_number_short.pdf', width = 8, height = 5)
```

Volcano_plot in gCap

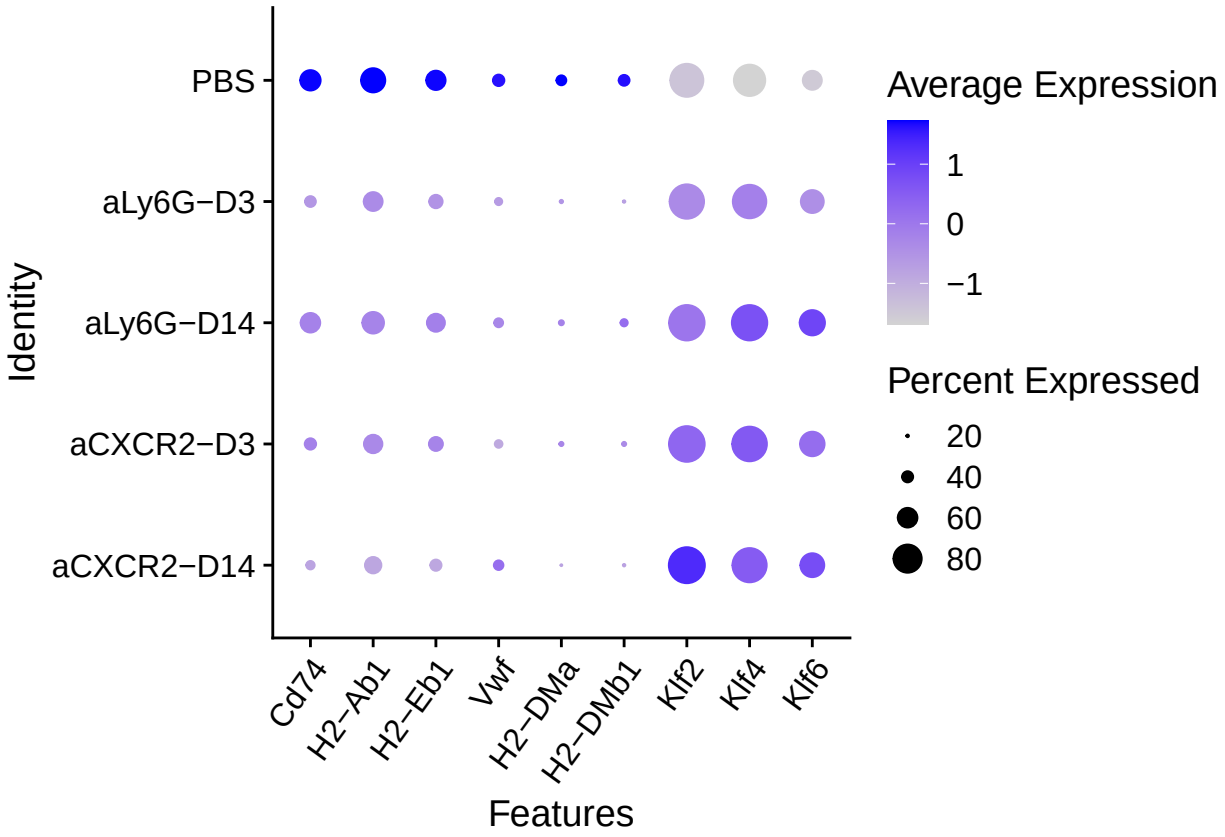
```
endothelial_cells_subset <- subset(endothelial_cells, CellType %in%
    "1 general capillaries")

Markers_j3 <- FindMarkers(endothelial_cells_subset, ident.1 = "PBS",
    ident.2 = c("aCXCR2-D3", "aLy6G-D3"), min.pct = 0.25, group.by = "Condition",
    logfc.threshold = 0)

Markers_j3$category <- ifelse(Markers_j3$p_val_adj < 0.05 & abs(Markers_j3$avg_log2FC) >
    0.25, "overexpressed", ifelse(Markers_j3$p_val_adj < 0.05 & Markers_j3$avg_log2FC <
    0.25, "significative", "no significant"))

Markers_j3 <- Markers_j3[order(Markers_j3$avg_log2FC), ]

gene_display <- c("Klf2", "Klf4", "Rps28", "Uba52", "Cdkn1a", "Rpl39",
    "Tap1", "Ifi47", "Gbp4", "Igtp", "H2-Dma", "H2-Dmb1", "H2-Aa",
    "H2-Ab1", "H2-Eb1", "Cd74")
Markers_j3$label <- NA
Markers_j3$label[c(2:4, 13, 17, 40, 3207, 3209, 3223, 3226:3229, 3231:3233)] <- rownames(Markers_j3)[c(
    13, 17, 40, 3207, 3209, 3223, 3226:3229, 3231:3233)]

order <- c(2:4, 13, 17, 40, 3207, 3209, 3223, 3226:3229, 3231:3233)

Markers_j3 <- Markers_j3[order(Markers_j3$avg_log2F), ]

ggplot(data = Markers_j3, aes(x = avg_log2FC, y = -log10(p_val_adj),
    col = category, label = label)) + geom_point(size = 2, alpha = 1) +
    theme_classic() + scale_color_manual(values = c("black", "red",
    "blue")) + geom_text_repel(data = Markers_j3[order, ], size = 4,
    aes(label = Markers_j3$label[order], fontface = "italic"), colour = "black",
    force = 4) + 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")
```




```
# ggsave('Plot/Volcano_gcap_J3.pdf', width = 8, height = 5)
```

Dotplot of perturbated genes in gCap

```
DotPlot(endothelial_cells_subset, features = c("Cd74", "H2-Ab1", "H2-Eb1",
    "Vwf", "H2-DMa", "H2-DMb1", "Klf2", "Klf4", "Klf6"), group.by = "Condition") +
    theme(axis.text.x = element_text(angle = 54, hjust = 1))
```




```
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/libopenblasp-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] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
## [1] xlsx_0.6.5         ggrepel_0.9.3      dittoSeq_1.12.0    formatR_1.14
## [5] ggplot2_3.4.2      patchwork_1.1.2    SeuratObject_4.1.3 Seurat_4.3.0
## [9] dplyr_1.1.2
```

```
##
## loaded via a namespace (and not attached):
##   [1] RColorBrewer_1.1-3          rstudioapi_0.14
##   [3] jsonlite_1.8.7              magrittr_2.0.3
##   [5] spatstat.utils_3.0-3        farver_2.1.1
##   [7] rmarkdown_2.23              zlibbioc_1.46.0
##   [9] vctrs_0.6.3                 ROCR_1.0-11
##  [11] spatstat.explore_3.2-1      RCurl_1.98-1.12
##  [13] S4Arrays_1.2.1              htmltools_0.5.5
##  [15] sctransform_0.3.5           parallelly_1.36.0
##  [17] KernSmooth_2.23-22          htmlwidgets_1.6.2
##  [19] ica_1.0-3                   plyr_1.8.8
##  [21] plotly_4.10.2               zoo_1.8-12
##  [23] igraph_1.5.0.1              mime_0.12
##  [25] lifecycle_1.0.3             pkgconfig_2.0.3
##  [27] Matrix_1.6-1                R6_2.5.1
##  [29] fastmap_1.1.1               GenomeInfoDbData_1.2.10
##  [31] MatrixGenerics_1.12.2       fitdistrplus_1.1-11
##  [33] future_1.33.0               shiny_1.7.4.1
##  [35] digest_0.6.33               colorspace_2.1-0
##  [37] S4Vectors_0.38.1            tensor_1.5
##  [39] irlba_2.3.5.1               GenomicRanges_1.52.0
##  [41] labeling_0.4.2              progressr_0.13.0
##  [43] fansi_1.0.4                 spatstat.sparse_3.0-2
##  [45] httr_1.4.6                  polyclip_1.10-4
##  [47] abind_1.4-5                 compiler_4.3.3
##  [49] withr_2.5.0                 highr_0.10
##  [51] MASS_7.3-60.0.1             DelayedArray_0.26.3
##  [53] tools_4.3.3                 lmtest_0.9-40
##  [55] httpuv_1.6.11               future.apply_1.11.0
##  [57] goftest_1.2-3               glue_1.6.2
##  [59] nlme_3.1-164                promises_1.2.0.1
##  [61] grid_4.3.3                  Rtsne_0.16
##  [63] cluster_2.1.6               reshape2_1.4.4
##  [65] generics_0.1.3              gtable_0.3.3
##  [67] spatstat.data_3.0-1         tidyr_1.3.0
##  [69] data.table_1.14.8           XVector_0.40.0
##  [71] sp_2.2-0                    utf8_1.2.3
##  [73] BiocGenerics_0.46.0         spatstat.geom_3.2-4
##  [75] RcppAnnoy_0.0.21            RANN_2.6.1
##  [77] pillar_1.9.0                stringr_1.5.0
##  [79] limma_3.56.2                spam_2.9-1
##  [81] later_1.3.1                 rJava_1.0-6
##  [83] splines_4.3.3               lattice_0.22-5
##  [85] survival_3.5-8              deldir_1.0-9
##  [87] tidyselect_1.2.0            SingleCellExperiment_1.22.0
##  [89] miniUI_0.1.1.1              pbapply_1.7-2
##  [91] knitr_1.43                  gridExtra_2.3
##  [93] IRanges_2.34.0              SummarizedExperiment_1.30.2
##  [95] scattermore_1.2             stats4_4.3.3
##  [97] xfun_0.39                   Biobase_2.60.0
##  [99] matrixStats_1.0.0           pheatmap_1.0.12
## [101] stringi_1.8.4               lazyeval_0.2.2
## [103] yaml_2.3.7                  xlsxjars_0.6.1
```

```
## [105] evaluate_0.21              codetools_0.2-19
## [107] tibble_3.2.1               cli_3.6.1
## [109] uwot_0.1.16                xtable_1.8-4
## [111] reticulate_1.30            munsell_0.5.0
## [113] Rcpp_1.0.11                GenomeInfoDb_1.36.0
## [115] globals_0.16.2             spatstat.random_3.1-5
## [117] png_0.1-8                  parallel_4.3.3
## [119] ellipsis_0.3.2             dotCall64_1.0-2
## [121] bitops_1.0-7               listenv_0.9.0
## [123] viridisLite_0.4.2          scales_1.2.1
## [125] ggridges_0.5.4             crayon_1.5.2
## [127] leiden_0.4.3               purrr_1.0.1
## [129] rlang_1.1.1                cowplot_1.1.1
```