# 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)
    library(viridis)
})
```

# Loading annotated Cell

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

# Perturbation score Day 14

## calculating number of gene

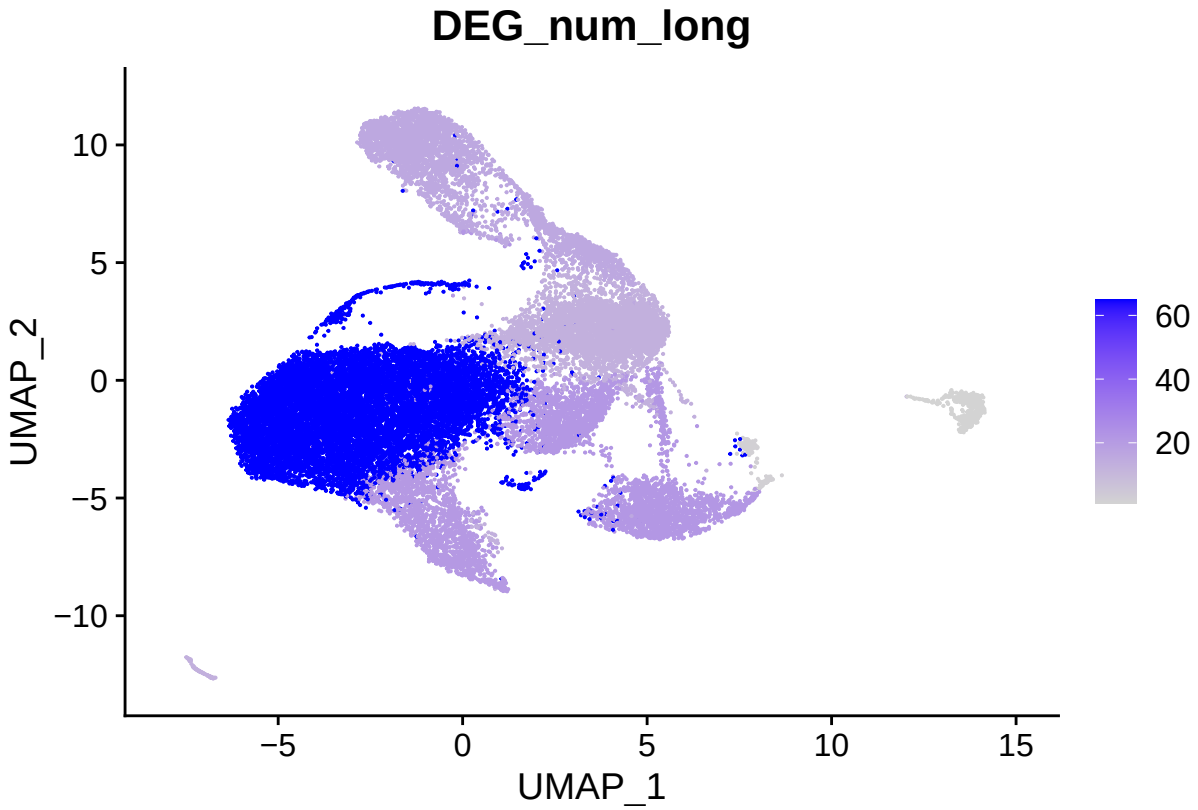
```
for (celltype in unique(endothelial_cells$CellType)) {

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

    Markers_long <- FindMarkers(sub_endothelial_cells, ident.1 = "PBS",
        ident.2 = c("aCXCR2-D14", "aLy6G-D14"), min.pct = 0.1, group.by = "Condition",
        logfc.threshold = 0.25)

    Markers_long <- Markers_long[Markers_long$p_val_adj < 0.05, ]
    # write.xlsx(Markers_long[order(Markers_long$avg_log2FC,
    # decreasing = T),], 'Plot/DE_gene_J14.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 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

```
endothelial_cells$DEG_num_long <- endothelial_cells$CellType
levels(endothelial_cells$DEG_num_long) <- c(65, 16, 22, 2, 21, 1,
    13)

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_long.pdf', width = 8, height = 5)
```

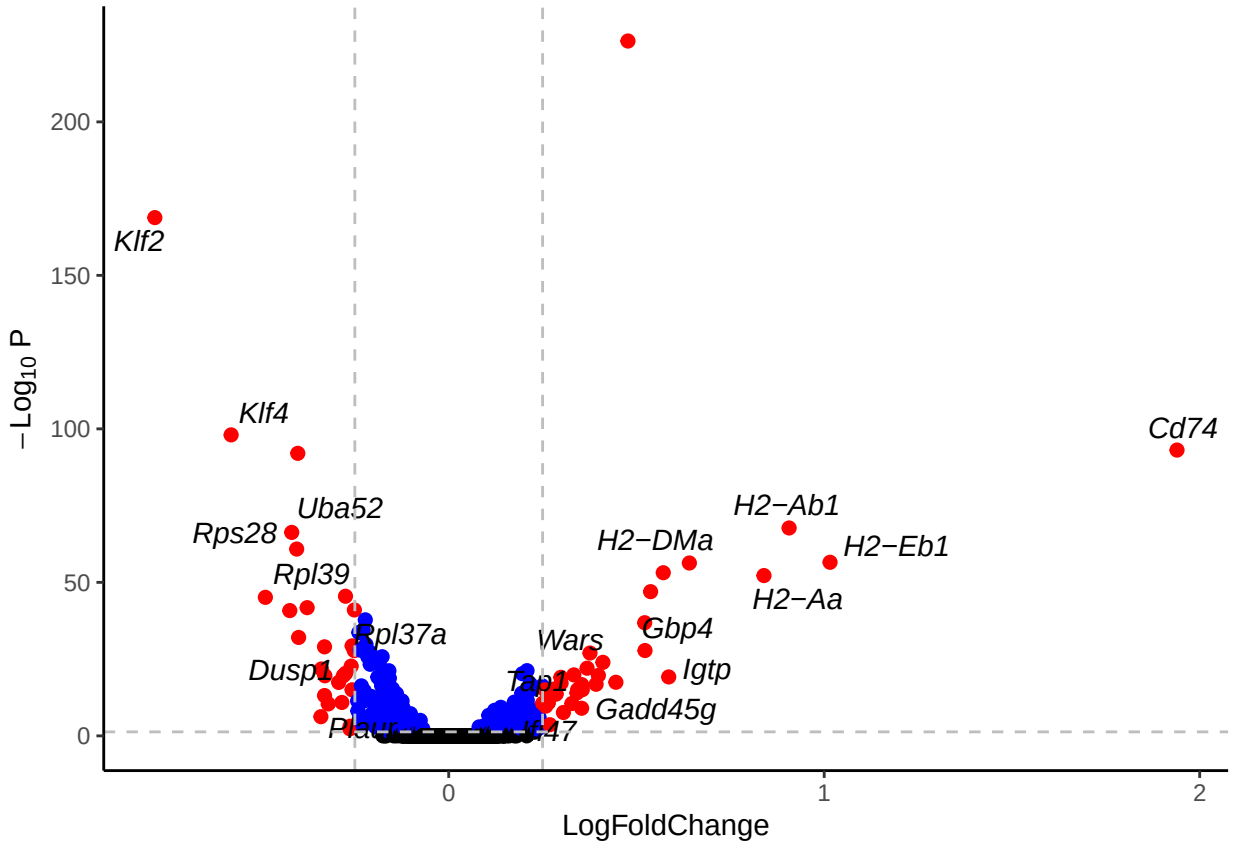
## Create VolcanoPlot

```
create_volcanoplt <- function(subtype, list_gene_name, Day) {

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

    Markers_j14 <- FindMarkers(endothelial_cells_subset, ident.1 = "PBS",
        ident.2 = c(paste0("aCXCR2-", Day), paste0("aLy6G-", Day)),
        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$avg_log2FC), ]
    Markers_j14$Gene <- rownames(Markers_j14)

    # 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)) + geom_point(size = 2, alpha = 1) + theme_classic() +
        scale_color_manual(values = c("black", "red", "blue")) + geom_text_repel(data = filter(Markers_
        Gene %in% list_gene_name), size = 4, aes(label = list_gene_name,
        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")

}
```

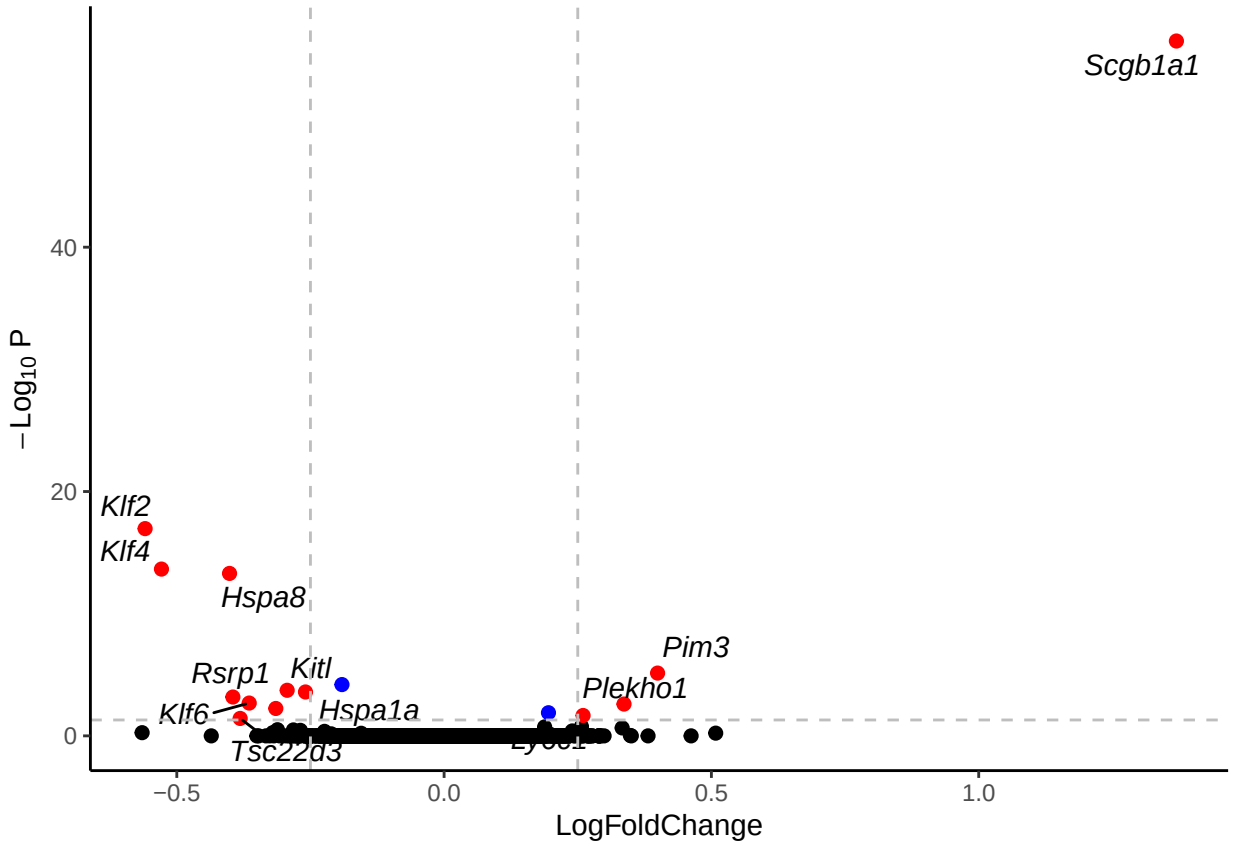
### Volcano_plot in gCap

```
list_gene_name <- c("Klf2", "Klf4", "Uba52", "Rps28", "Dusp1", "Plaur",
    "Rpl39", "Rpl37a", "Ifi47", "Tap1", "Gadd45g", "Wars", "Gbp4",
    "Igtp", "H2-DMa", "H2-Aa", "H2-Ab1", "H2-Eb1", "Cd74")

create_volcanoplt("1 general capillaries", list_gene_name, "D14")
```




### Volcano_plot in aCap

```
gene_to_highlight <- c("Klf2", "Klf4", "Hspa8", "Rsrp1", "Tsc22d3",
    "Klf6", "Dnajb1", "Kitl", "Hspa1a", "Ly6c1", "Plekho1", "Pim3",
    "Scgb1a1")

create_volcanoplt("2 aerocyte capilaries", gene_to_highlight, "D14")
```




# Perturbation score Day 3

### calculating number of gene

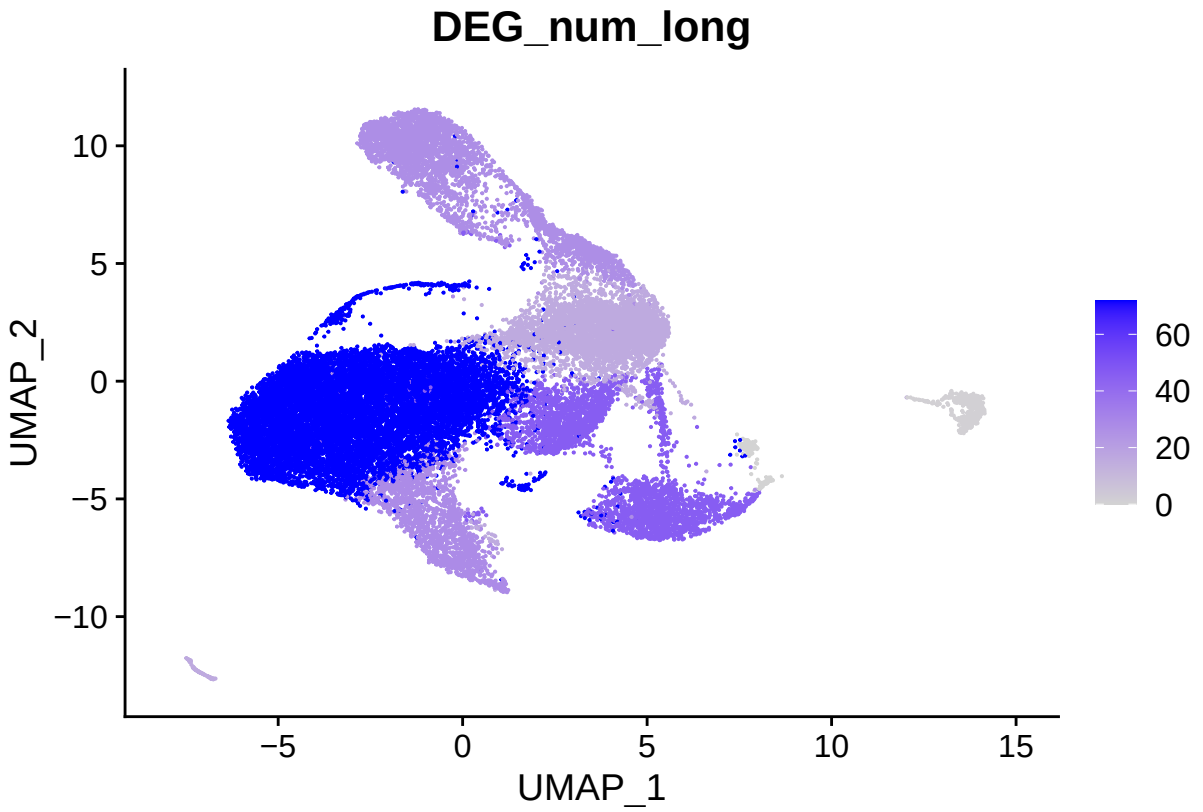
```
for (celltype in unique(endothelial_cells$CellType)) {

    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$p_val_adj < 0.05,
        ]
    # write.xlsx(Markers_short[order(Markers_short$avg_log2FC,
    # decreasing = T),], 'Plot/DE_gene_J3.xlsx', sheetName =
    # celltype, col.names = T, append = T)

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

}
```

```
## [1] "The number of DEG for 1 general capillaries is 72"
## [1] "The number of DEG for 3 pulmonary veins is 46"
## [1] "The number of DEG for 7 other is 16"
```

```
## [1] "The number of DEG for 2 aerocyte capilaries is 27"
## [1] "The number of DEG for 5 arteries is 28"
## [1] "The number of DEG for 6 lymphaptics is 1"
## [1] "The number of DEG for 4 systemic veins is 0"
```

### Feature Plot DEG number

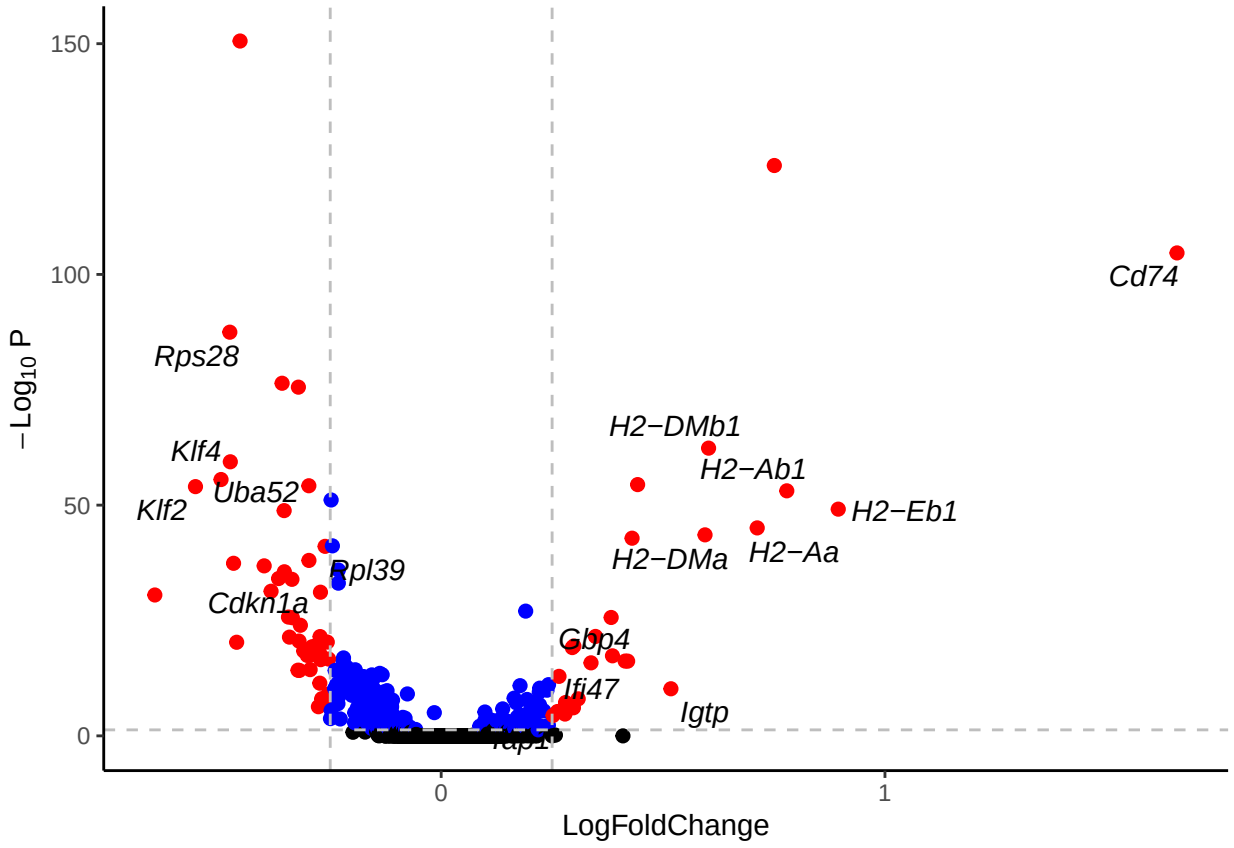
```
endothelial_cells$DEG_num_long <- endothelial_cells$CellType
levels(endothelial_cells$DEG_num_long) <- c(72, 27, 46, 0, 28, 1,
    16)

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)
```

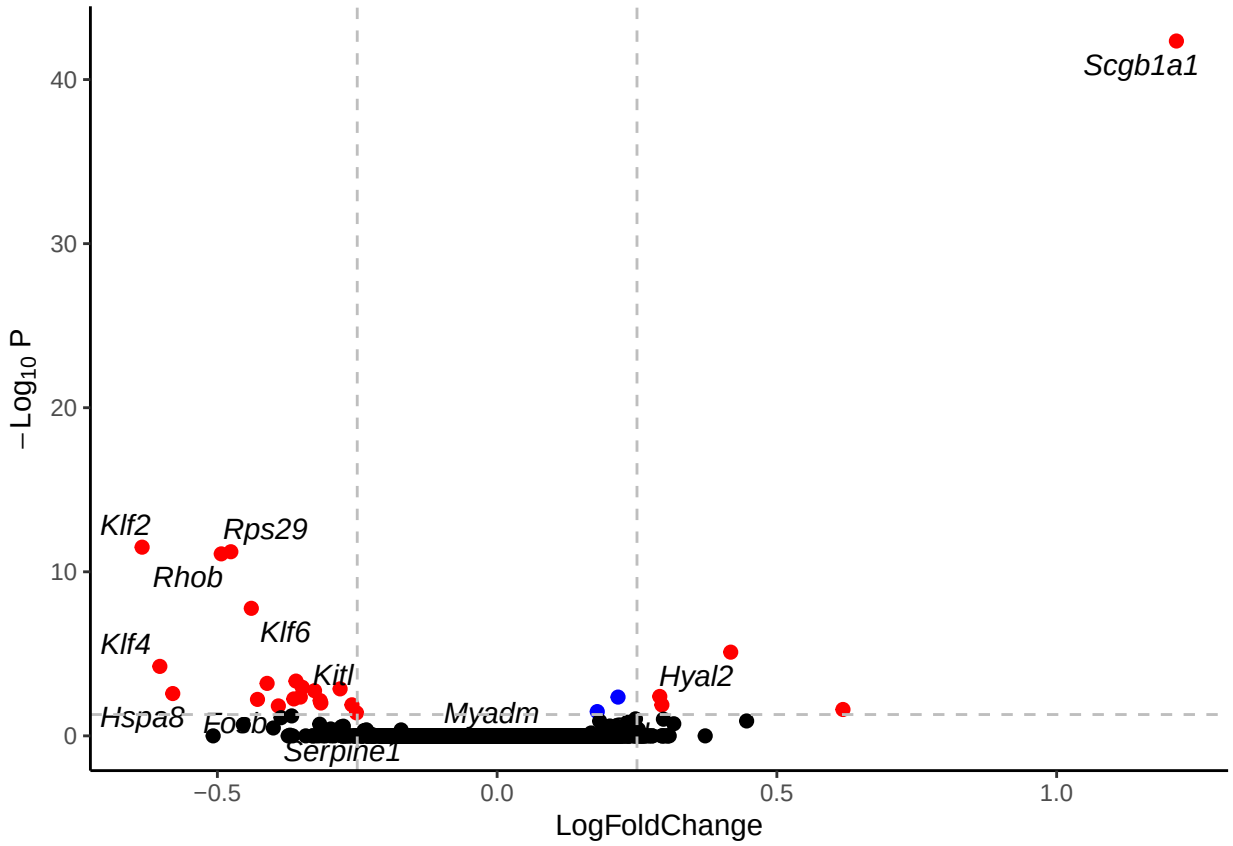
### Volcano_plot in gCap

```
gene_display <- c("Klf2", "Klf4", "Rps28", "Uba52", "Cdkn1a", "Rpl39",
    "Tap1", "Ifi47", "Gbp4", "Igtp", "H2-DMa", "H2-DMb1", "H2-Aa",
    "H2-Ab1", "H2-Eb1", "Cd74")
create_volcanoplt("1 general capillaries", gene_display, "D3")
```



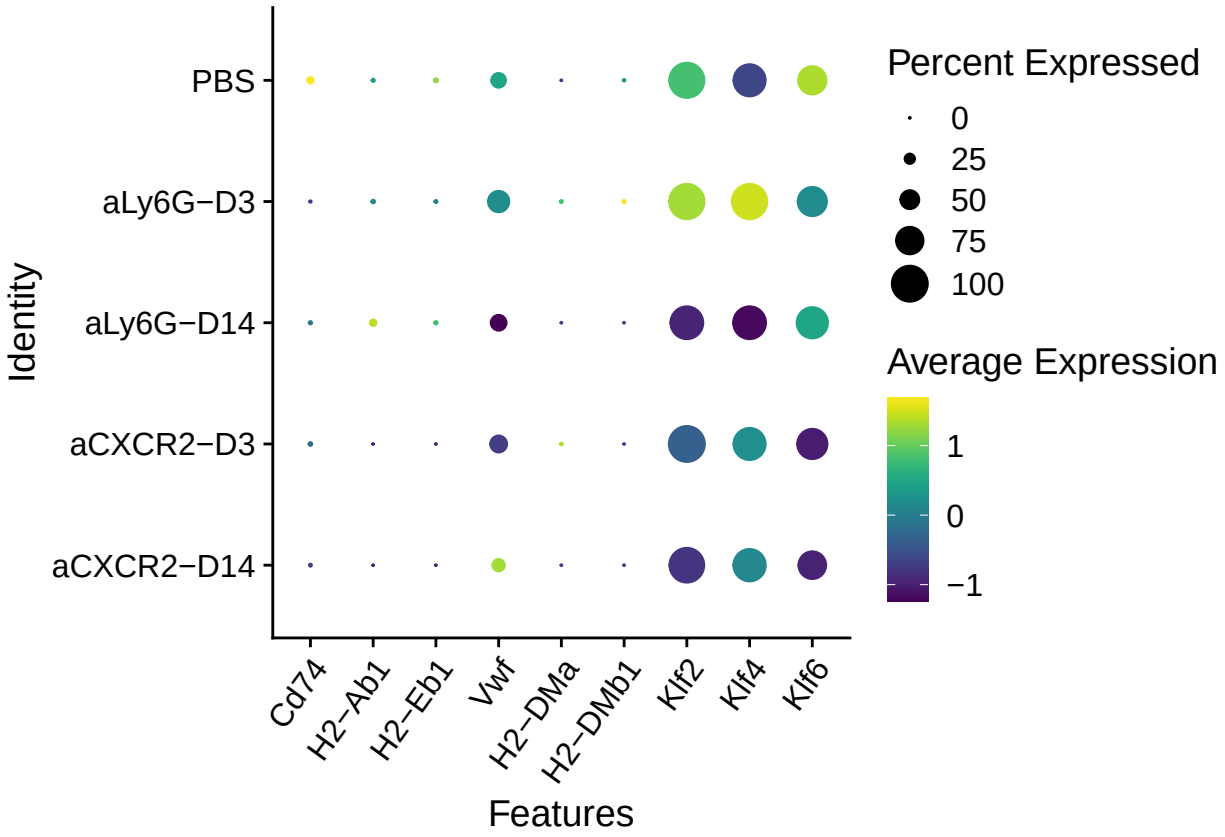

### Volcano_plot in aCap

```
gene_to_highlight <- c("Klf2", "Klf4", "Hspa8", "Rhob", "Rps29", "Klf6",
    "Fosb", "Kitl", "Serpine1", "Myadm", "Clu", "Hyal2", "Scgb1a1")

create_volcanoplt("2 aerocyte capilaries", gene_to_highlight, "D3")
```




## 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)) + scale_color_gradientn(colours = viridis(5
```



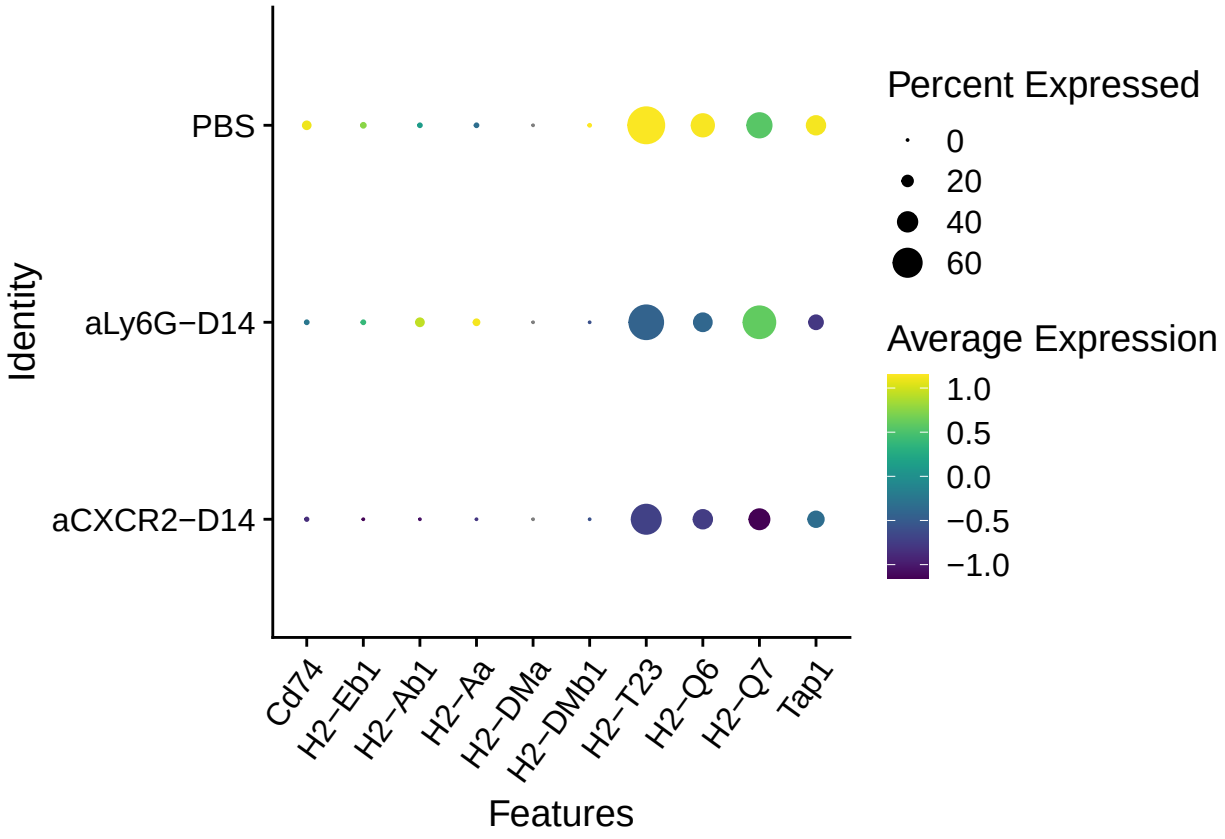

## Dotplot of mechanosensing and shear response genes

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

    endothelial_cells_subset <- subset(endothelial_cells, CellType %in%
        celltype)
    endothelial_cells_subset <- subset(endothelial_cells_subset, Condition %in%
        c("aCXCR2-D14", "aLy6G-D14", "PBS"))

    DotPlot(endothelial_cells_subset, features = c("Klf2", "Klf4",
        "Txnip", "Dusp1", "Atf3", "Fosb", "Per1", "Plaur", "Emp1",
        "S1pr1"), group.by = "Condition") + theme(axis.text.x = element_text(angle = 45,
        hjust = 1)) + scale_color_gradientn(colours = viridis(50))
    # ggsave(paste0('new_plot/dotplot_',celltype,'.pdf'), width
    # = 9, height = 6)

}
```

## Antigen presentation

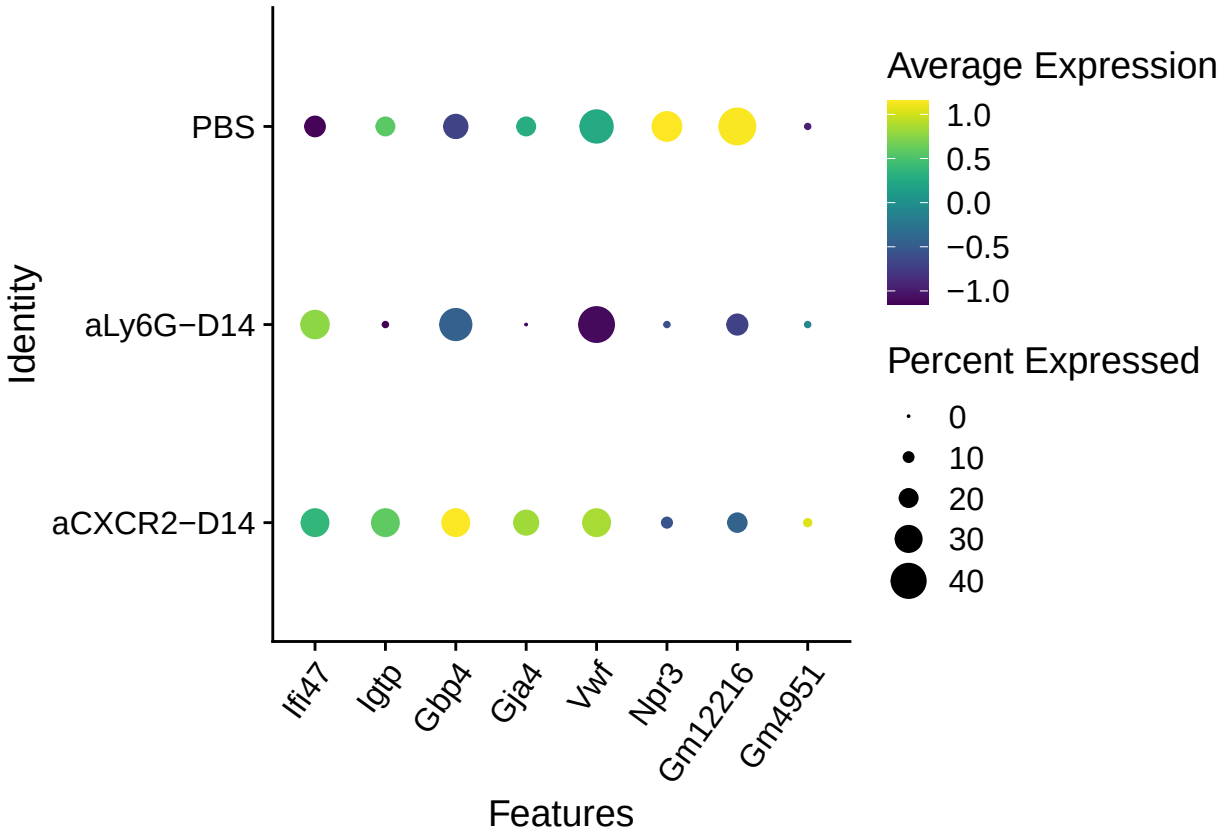
```
Antigen_presentation <- c("Cd74", "H2-Eb1", "H2-Ab1", "H2-Aa", "H2-DMa",
    "H2-DMb1", "H2-T23", "H2-Q6", "H2-Q7", "Tap1")

DotPlot(endothelial_cells_subset, features = Antigen_presentation,
    group.by = "Condition") + theme(axis.text.x = element_text(angle = 54,
```

```
    hjust = 1)) + scale_color_gradientn(colours = viridis(50))
```




## Interferon response - Endothelial - Other : feature upregulated in PBS

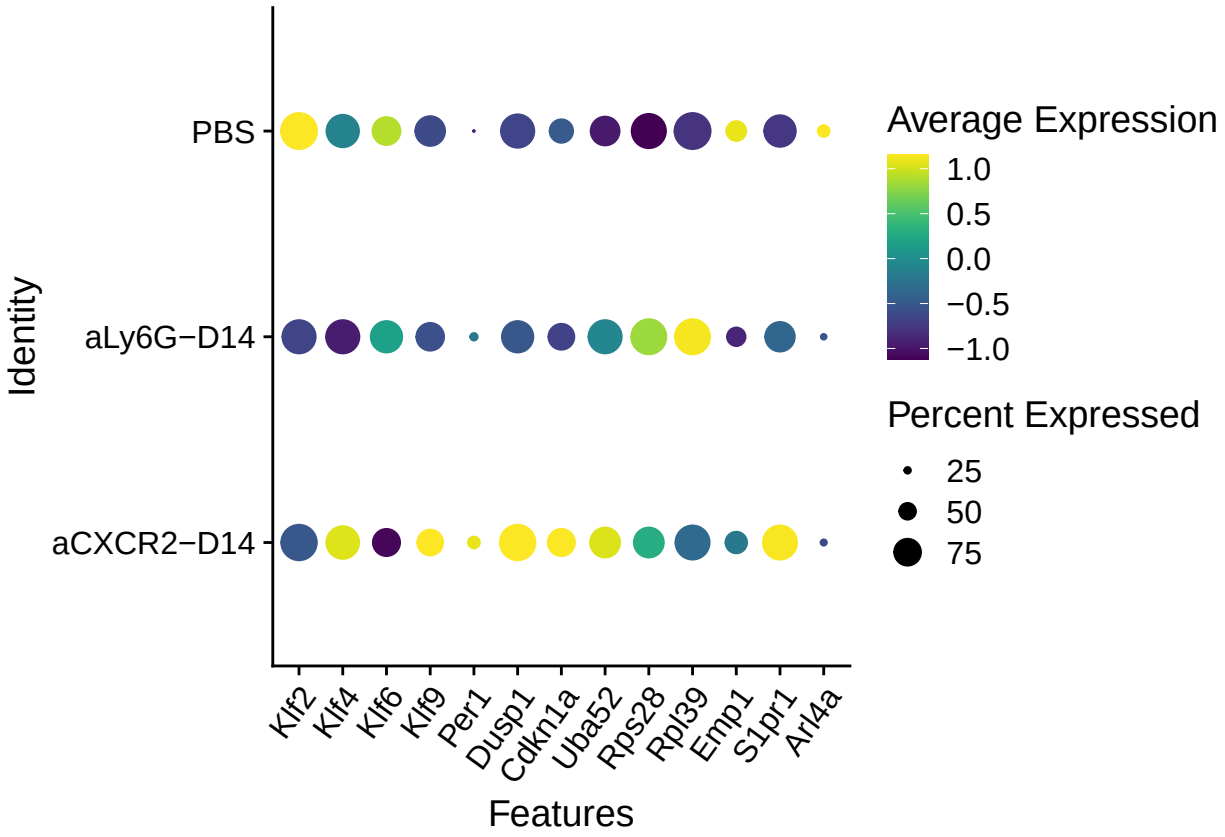
```
# Interferon response - Endothelial - Other : feature
# upregulated in PBS
feature_receptor <- c("Ifi47", "Igtp", "Gbp4", "Gja4", "Vwf", "Npr3",
    "Gm12216", "Gm4951")

DotPlot(endothelial_cells_subset, features = feature_receptor, group.by = "Condition") +
    theme(axis.text.x = element_text(angle = 54, hjust = 1)) + scale_color_gradientn(colours = viridis(
```




```
# ggsave('new_plot/dotplot_gcap_upregulate.pdf', width = 8,
# height = 5)
```

## Shear-responsive TFS - Negative regulation apoptosis - Ribosomal - Other : feature downregulated in PBS

```
feature_ligand <- c("Klf2", "Klf4", "Klf6", "Klf9", "Per1", "Dusp1",
    "Cdkn1a", "Uba52", "Rps28", "Rpl39", "Emp1", "S1pr1", "Arl4a")

DotPlot(endothelial_cells_subset, features = feature_ligand, group.by = "Condition") +
    theme(axis.text.x = element_text(angle = 54, hjust = 1)) + scale_color_gradientn(colours = viridis(5
```




```
# ggsave('new_plot/dotplot_gcap_downregulate.pdf', width = 8,
# height = 5)
```

```
sessionInfo()
```

```
## R version 4.3.3 (2024-02-29)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 24.04.3 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] viridis_0.6.3      viridisLite_0.4.2  xlsx_0.6.5         ggrepel_0.9.3
##  [5] dittoSeq_1.12.0    formatR_1.14       ggplot2_3.4.2      patchwork_1.1.2
##  [9] SeuratObject_4.1.3 Seurat_4.3.0       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] scales_1.2.1               ggridges_0.5.4
## [125] crayon_1.5.2               leiden_0.4.3
## [127] purrr_1.0.1                rlang_1.1.1
## [129] cowplot_1.1.1
```