

Scoring Ly6G Macs Score

Abinet Joan

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Contents

Loading packages	1
Loading Data	1
Renaming clusters	1
Heatmap Differential Expression Analysis	2
Annotation par patients	4
scoring for Ly6G Macs	4
Saving Data	6

Loading packages

```
suppressMessages(library(dplyr))
suppressMessages(library(Seurat))
suppressMessages(library(patchwork))
suppressMessages(library(ggplot2))
suppressMessages(library(formatR))
suppressMessages(library(RColorBrewer))
suppressMessages(library(dittoSeq))
```

Loading Data

```
cells.combined <- readRDS("../9.1-Loading_BAL_samples/cells.combined_Part1.rds")
```

Renaming clusters

```
cells.combined$Merge_annotation <- cells.combined$seurat_clusters
cells.combined$Merge_annotation <- droplevels(cells.combined$Merge_annotation)

levels(cells.combined$Merge_annotation) <- c("C1 - AM", "C2 - AM", "C3 - AM", "C8 - Mono", "C12 - LT", "C13 - LT", "C9 - Mo-Mac", "C11 - Neutro", "C14 - LT", "C15 - LT", "C16 - LT", "C19 - Epith", "C10 - Mo-DC", "C4 - AM", "C5 - AM", "C6 - AM", "C18 - Mast", "C17 - DC", "C7 - AM")
```

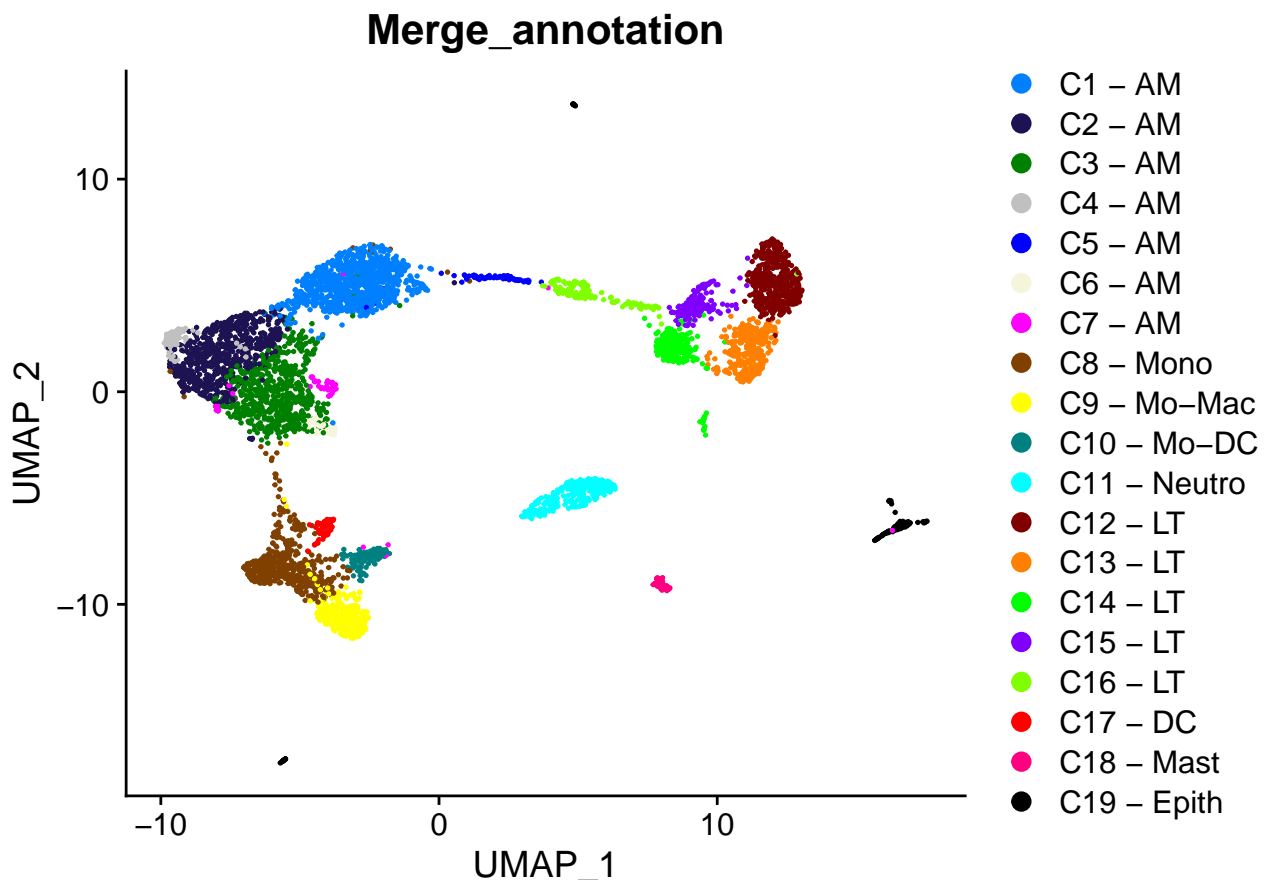
```

cells.combined$Merge_annotation <- factor(cells.combined$Merge_annotation, levels = c("C1
- AM", "C2 - AM", "C3 - AM", "C4 - AM", "C5 - AM", "C6 - AM", "C7 - AM", "C8 - Mono", "C9
- Mo-Mac", "C10 - Mo-DC", "C11 - Neutro", "C12 - LT", "C13 - LT", "C14 - LT", "C15 - LT",
"C16 - LT", "C17 - DC", "C18 - Mast", "C19 - Epith"))

color_clusters <- c("#0080FF", "#1d1352", "#008000", "#C0C0C0", "#0000FF", "beige",
"#FF00FF", "#804000", "#FFFF00", "#008080", "#00FFFF", "#800000", "#FF8000", "#00FF00",
"#8000FF", "#80FF00", "#FF0000", "#FF0080", "#000000")

DimPlot(cells.combined, reduction = "umap", group.by = "Merge_annotation", cols =
color_clusters)

```



Heatmap Differential Expression Analysis

```

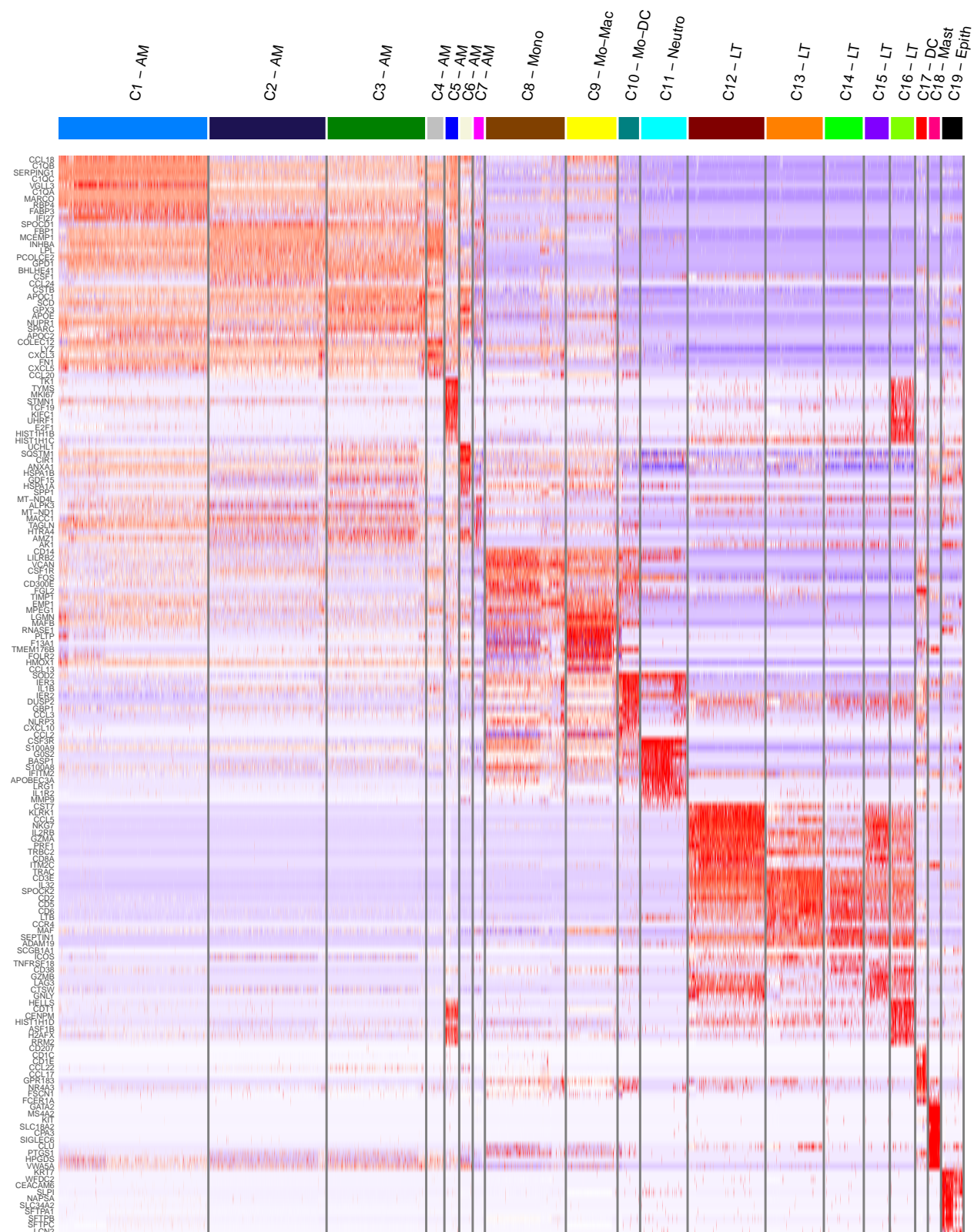
Idents(cells.combined) <- "Merge_annotation"
Balf.10x <- FindAllMarkers(cells.combined, only.pos = T, min.pct = 0.25)

Balf.10x %>%
  group_by(cluster) %>%
  top_n(n = 10, wt = avg_log2FC) -> top10

Heatmap <- DoHeatmap(cells.combined, features = top10$gene, group.colors =
color_clusters, angle = 80) + NoLegend() + scale_fill_gradientn(colors = c("blue",
"white", "red"))

```

Heatmap

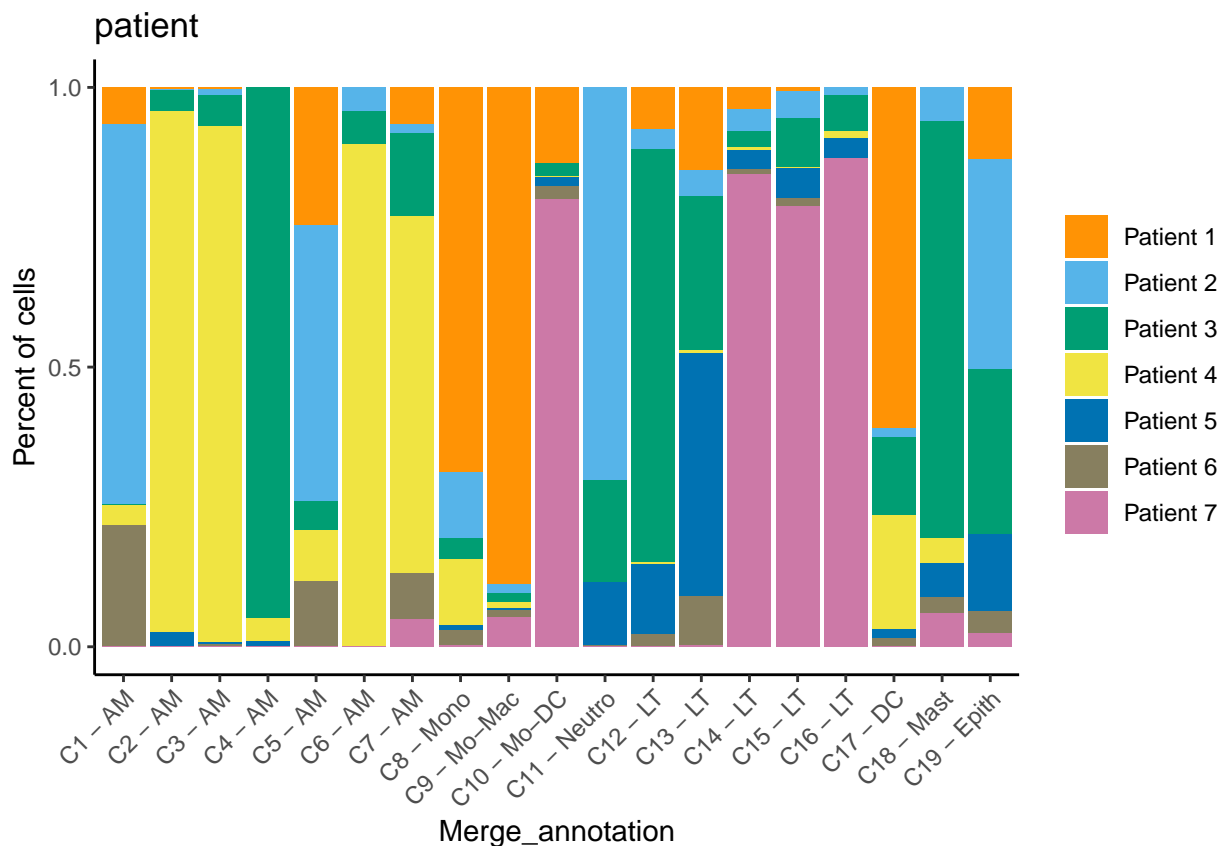


Annotation par patients

```
my_palette <- c("#ff9305", "#56B4E9", "#009E73", "#F0E442", "#0072B2",
               "#877f5f", "#CC79A7")

cells.combined$patient <- cells.combined$orig.ident
cells.combined$patient <- as.factor(cells.combined$patient)
levels(cells.combined$patient) <- c("Patient 1", "Patient 1", "Patient 4", "Patient 6",
  "Patient 2", "Patient 3", "Patient 5", "Patient 7")

labels_order <- c(1,12,13,14,15,16,17,18,19,2,3,4,5,6,7,8,9,10,11)
dittoBarPlot(cells.combined, "patient", group.by = "Merge_annotation", x.reorder =
labels_order, color.panel = my_palette)
```



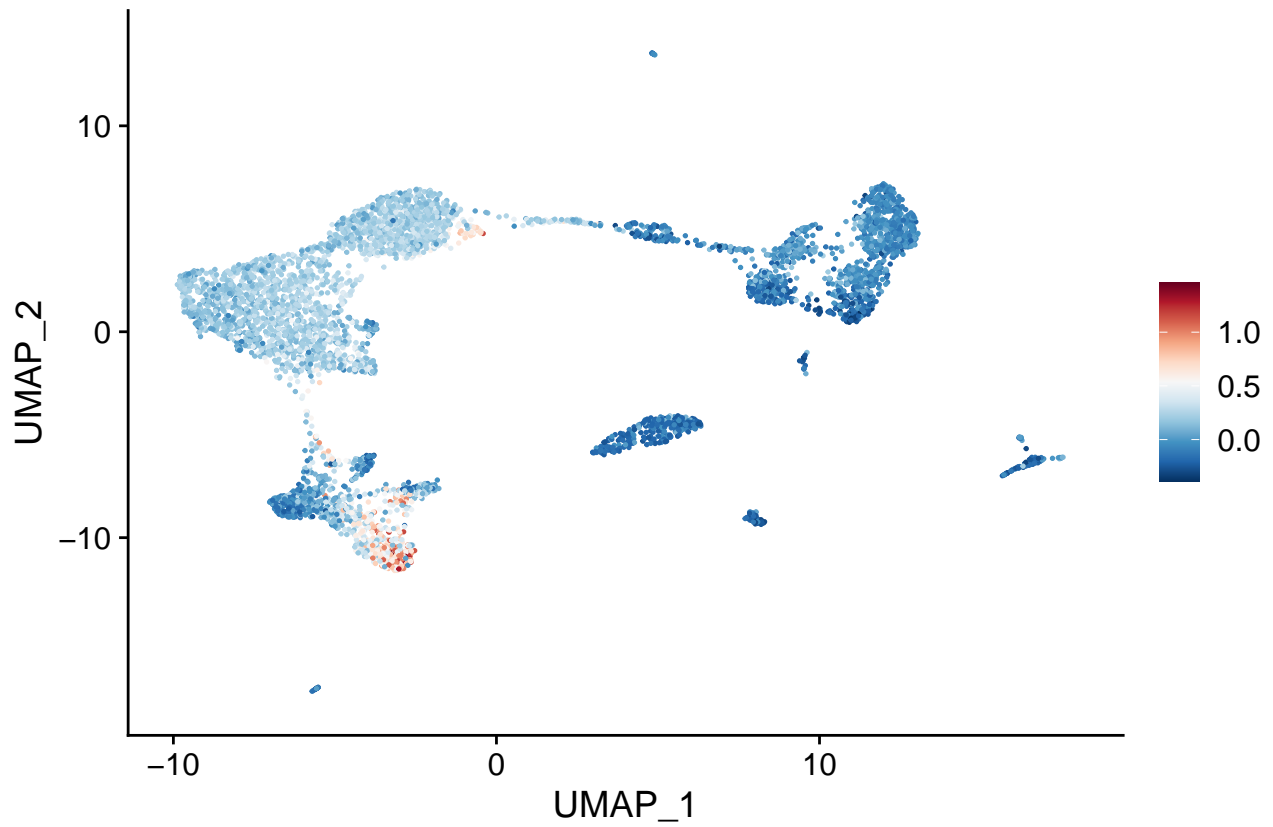
```
#ggsave("barplot_patient.pdf", width = 8, height = 5)
```

scoring for Ly6G Macs

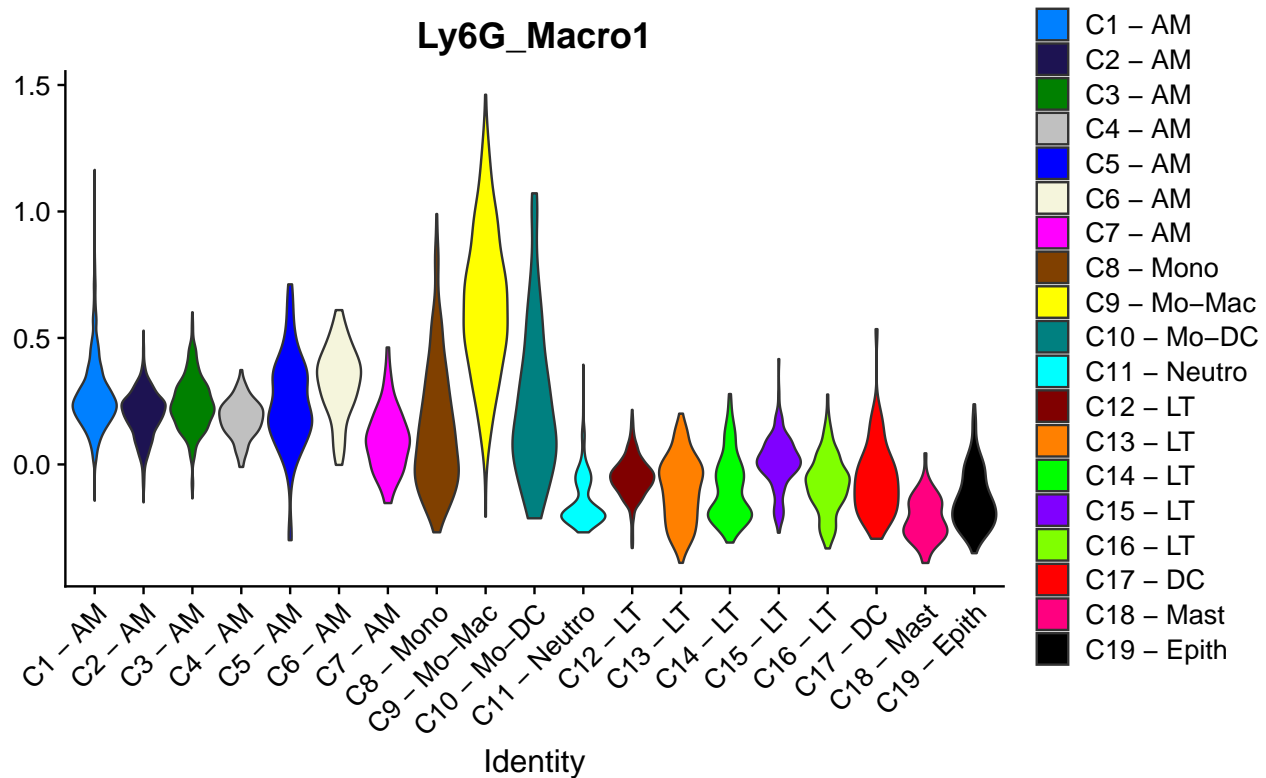
```
DefaultAssay(cells.combined) <- "RNA"
top_motro_human <- c("SPP1", "ARG1", "CCL7", "CXCL9", "CCL13", "LGMN", "CCL8",
  "CTSB", "SAA3P", "CCL2", "FABP5", "CTSL", "GATM", "CTSB", "CCL5", "PDPN", "MSR1")

cells.combined <- AddModuleScore(cells.combined,
  features = list(top_motro_human),
  name="Ly6G_Macro")
```

```
FeaturePlot(cells.combined, features = "Ly6G_Macro1") +  
  scale_colour_gradientn(colours = rev(brewer.pal(n = 11, name = "RdBu")))+  
  ggtitle("")
```



```
VlnPlot(cells.combined, features = "Ly6G_Macro1", group.by = "Merge_annotation", cols =  
color_clusters, pt.size = 0)
```



Saving Data

```
saveRDS(cells.combined, "cells.combined_Part2.rds")
```

```
sessionInfo()
```

```
## R version 4.3.3 (2024-02-29)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 22.04.4 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-p0.3.20.so; LAPACK version 3.10.0
##
## 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] stats      graphics  grDevices  utils      datasets  methods    base
```

```

##
## other attached packages:
## [1] dittoSeq_1.12.0      RColorBrewer_1.1-3 formatR_1.14      ggplot2_3.4.2
## [5] patchwork_1.1.2      SeuratObject_4.1.3 Seurat_4.3.0      dplyr_1.1.2
##
## loaded via a namespace (and not attached):
## [1] rstudioapi_0.14      jsonlite_1.8.7
## [3] magrittr_2.0.3       spatstat.utils_3.0-3
## [5] farver_2.1.1         rmarkdown_2.23
## [7] zlibbioc_1.46.0      vctrs_0.6.3
## [9] ROCR_1.0-11          spatstat.explore_3.2-1
## [11] RCurl_1.98-1.12      S4Arrays_1.0.4
## [13] htmltools_0.5.5      sctransform_0.3.5
## [15] parallelly_1.36.0    KernSmooth_2.23-22
## [17] htmlwidgets_1.6.2    ica_1.0-3
## [19] plyr_1.8.8           plotly_4.10.2
## [21] zoo_1.8-12           igraph_1.5.0.1
## [23] mime_0.12            lifecycle_1.0.3
## [25] pkgconfig_2.0.3      Matrix_1.6-1
## [27] R6_2.5.1             fastmap_1.1.1
## [29] GenomeInfoDbData_1.2.10 MatrixGenerics_1.12.2
## [31] fitdistrplus_1.1-11  future_1.33.0
## [33] shiny_1.7.4.1        digest_0.6.33
## [35] colorspace_2.1-0     S4Vectors_0.38.1
## [37] tensor_1.5           irlba_2.3.5.1
## [39] GenomicRanges_1.52.0 labeling_0.4.2
## [41] progressr_0.13.0     fansi_1.0.4
## [43] spatstat.sparse_3.0-2 httr_1.4.6
## [45] polyclip_1.10-4      abind_1.4-5
## [47] compiler_4.3.3       withr_2.5.0
## [49] highr_0.10           MASS_7.3-60
## [51] DelayedArray_0.26.3  tools_4.3.3
## [53] lmtest_0.9-40        httpuv_1.6.11
## [55] future.apply_1.11.0  goftest_1.2-3
## [57] glue_1.6.2           nlme_3.1-163
## [59] promises_1.2.0.1     grid_4.3.3
## [61] Rtsne_0.16           cluster_2.1.6
## [63] reshape2_1.4.4       generics_0.1.3
## [65] gtable_0.3.3         spatstat.data_3.0-1
## [67] tidyr_1.3.0          data.table_1.14.8
## [69] XVector_0.40.0       sp_2.0-0
## [71] utf8_1.2.3           BiocGenerics_0.46.0
## [73] spatstat.geom_3.2-4  RcppAnnoy_0.0.21
## [75] ggrepel_0.9.3        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          splines_4.3.3
## [83] lattice_0.22-5       survival_3.5-8
## [85] deldir_1.0-9         tidyselect_1.2.0
## [87] SingleCellExperiment_1.22.0 miniUI_0.1.1.1
## [89] pbapply_1.7-2        knitr_1.43
## [91] gridExtra_2.3         IRanges_2.34.0
## [93] SummarizedExperiment_1.30.2 scattermore_1.2
## [95] stats4_4.3.3         xfun_0.39

```

## [97] Biobase_2.60.0	matrixStats_1.0.0
## [99] pheatmap_1.0.12	stringi_1.7.12
## [101] lazyeval_0.2.2	yaml_2.3.7
## [103] evaluate_0.21	codetools_0.2-19
## [105] tibble_3.2.1	cli_3.6.1
## [107] uwot_0.1.16	xtable_1.8-4
## [109] reticulate_1.30	munsell_0.5.0
## [111] Rcpp_1.0.11	GenomeInfoDb_1.36.0
## [113] globals_0.16.2	spatstat.random_3.1-5
## [115] png_0.1-8	parallel_4.3.3
## [117] ellipsis_0.3.2	dotCall64_1.0-2
## [119] bitops_1.0-7	listenv_0.9.0
## [121] viridisLite_0.4.2	scales_1.2.1
## [123] ggridges_0.5.4	crayon_1.5.2
## [125] leiden_0.4.3	purrr_1.0.1
## [127] rlang_1.1.1	cowplot_1.1.1