# Slingshot Trajectory

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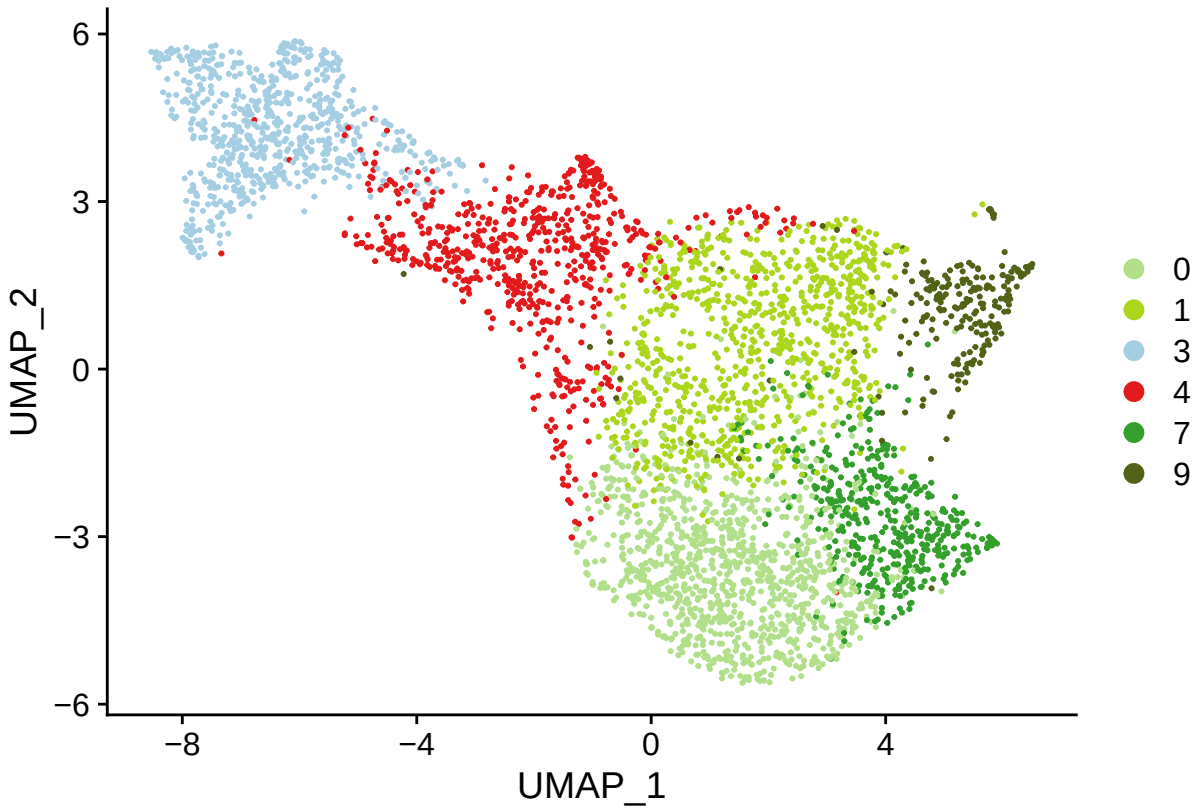
## Loading Package

```
suppressMessages(library(dplyr))
suppressMessages(library(Seurat))
suppressMessages(library(patchwork))
suppressMessages(library(slingshot))
suppressMessages(library(ggplot2))
suppressMessages(library(grDevices))
suppressMessages(library(scales))
suppressMessages(library(tradeSeq))
suppressMessages(library(viridisLite))
```

## Slingshot trajectory

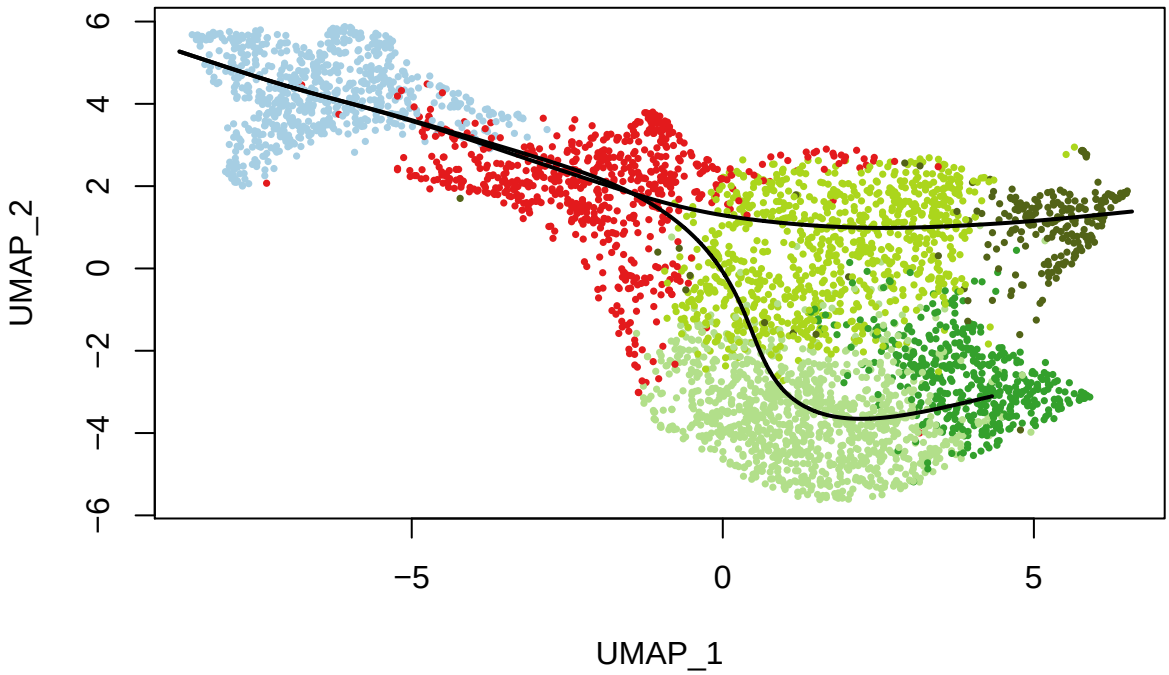
### Loading UMAP and subseting clusters

```
myeloid_cells_clustered <-
readRDS("../3-Visualisation_Clustering/Myeloid_cells_Final.rds")

myeloid_cells_pseudotime <- subset(myeloid_cells_clustered, seurat_clusters %in%
c(0,1,3,4,7,9))
myeloid_cells_pseudotime <- RunUMAP (myeloid_cells_pseudotime , dims = 1:12)

colors <- c("#B2DF8A","#ABD61C","#A6CEE3", "#E31A1C", "#33A02C", "#526317")

DimPlot(myeloid_cells_pseudotime, cols = colors, reduction = "umap")
```




### Run slingshot

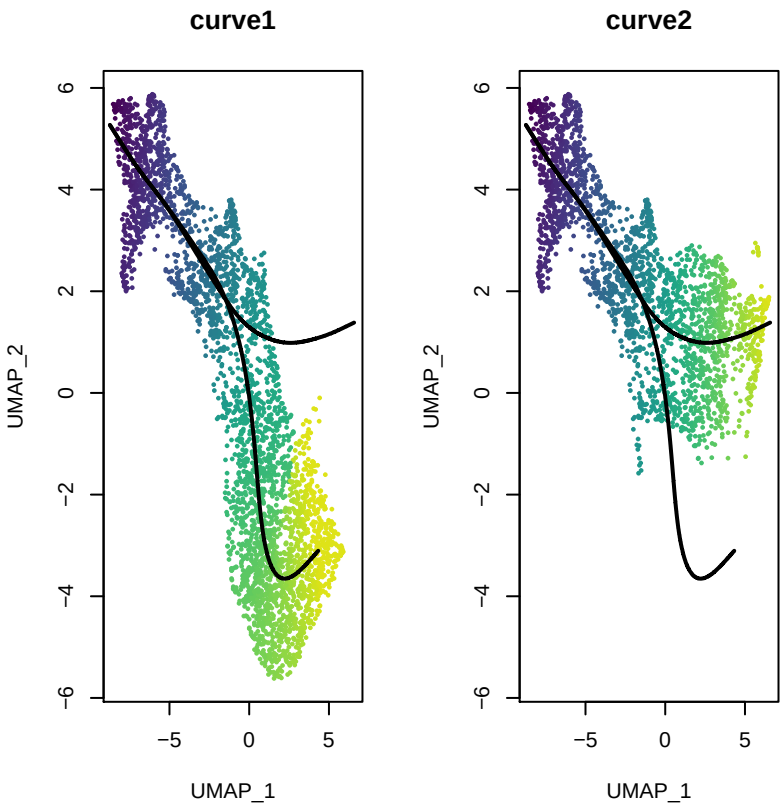
```
p <- Embeddings(myeloid_cells_pseudotime, "umap")

sds <- slingshot(Embeddings(myeloid_cells_pseudotime, "umap"),
myeloid_cells_pseudotime$seurat_clusters)
# the two step of slingshot can be run separately using getlineages and getcurves

myeloid_cells_pseudotime$color <- myeloid_cells_pseudotime$seurat_clusters
levels(myeloid_cells_pseudotime$color) <-c("#B2DF8A","#ABD61C","#1F78B4","#A6CEE3",
"#E31A1C","#E3751C","#600078", "#33A02C", "#FDBF6F", "#526317",  "#D4AAC6","#784620")

#pdf("Trajectory_slingshot.pdf", width = 19/3, height = 14/3)
plot(reducedDim(sds), col = as.vector(myeloid_cells_pseudotime$color), pch = 16, cex =
0.5)
lines(sds, lwd = 2, col = 'black')
```




```
#dev.off()
```

### Visualizing lineage

```
nc <- 3
pt <- slingPseudotime(sds)
nms <- colnames(pt)
nr <- ceiling(length(nms)/nc)
pal <- viridis(100, end = 0.95)
par(mfrow = c(nr, nc))

y <- 1
for (i in nms) {
  colors <- pal[cut(pt[,i], breaks = 100)]
  #pdf(paste0("Trajectory_",y,".pdf"), width = 19/3, height = 14/3)
  plot(reducedDim(sds), col = colors, pch = 16, cex = 0.5, main = i)
  lines(sds, lwd = 2, col = 'black')
  #dev.off()
  y <- y+1
}
```

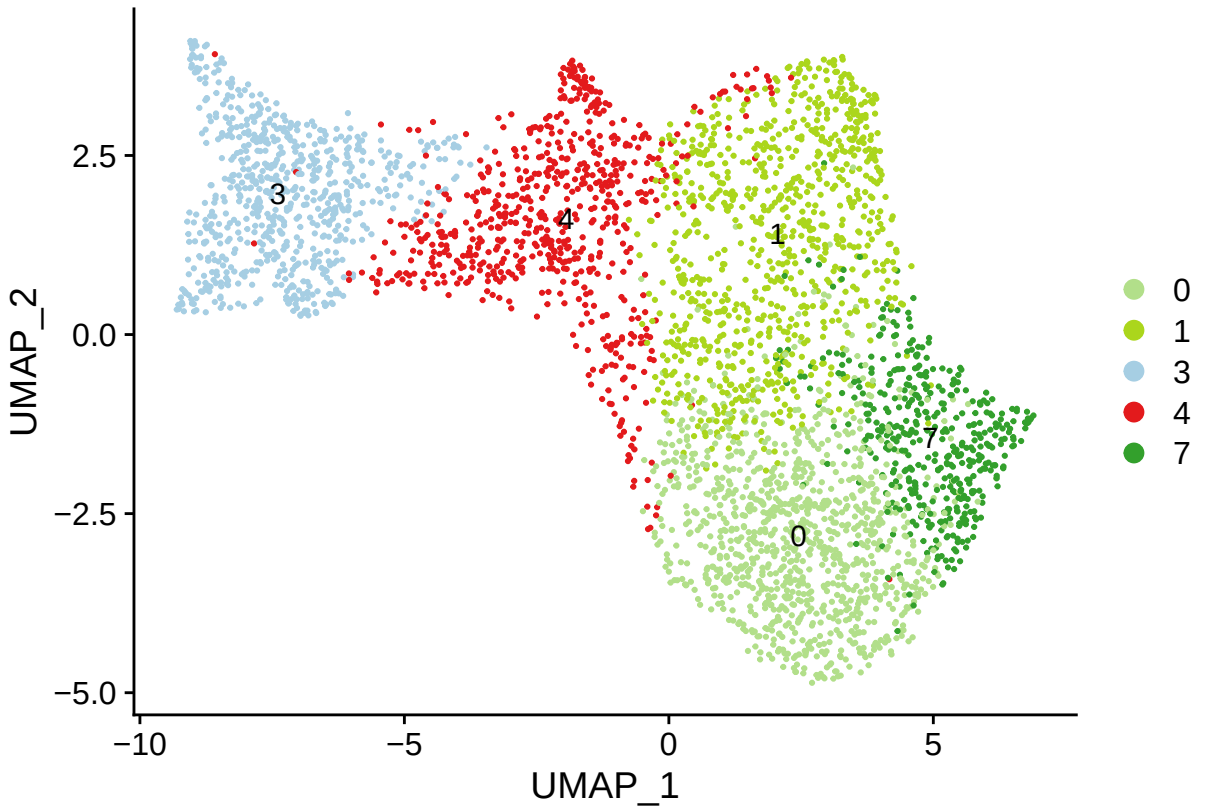



Since the dying cells of cluster 9 cause huge differences in gene expression, it hides smaller differences in the trajectory between cluster 4 and 1, and between cluster 4 and 0. So cluster 9 will be removed.

# Slingshot without the Dying cells

## Loading UMAP and subseting clusters

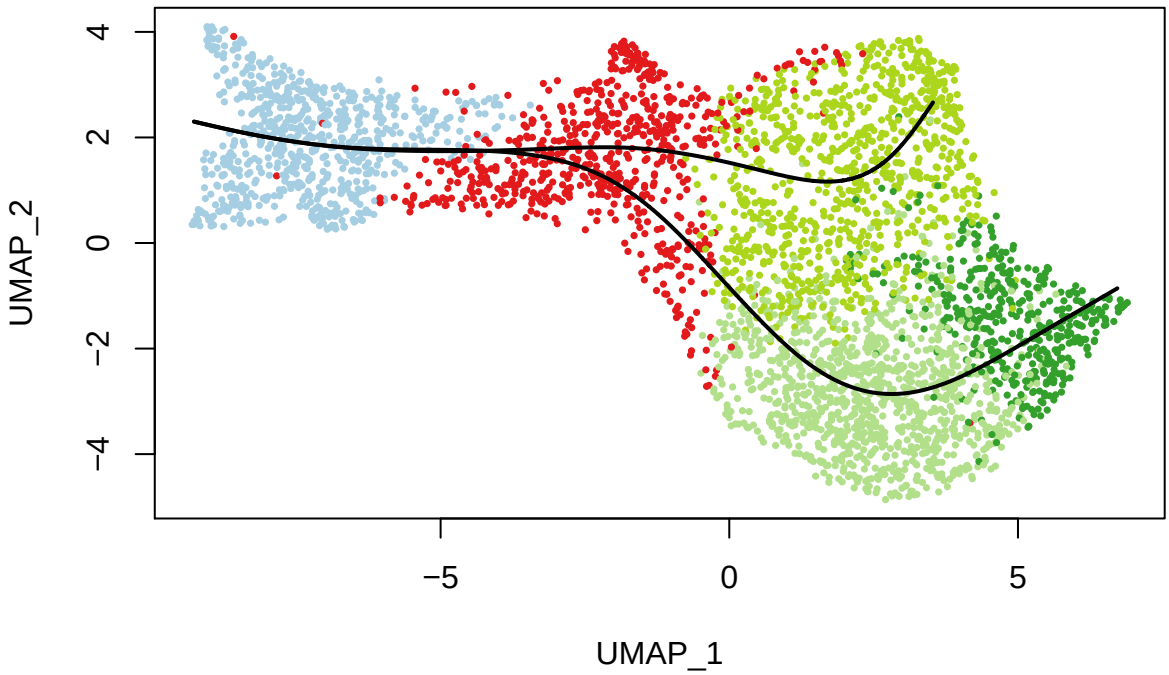
```
myeloid_cells_pseudotime <- subset(myeloid_cells_clustered, seurat_clusters %in%
c(0,1,3,4,7))
myeloid_cells_pseudotime <- RunUMAP (myeloid_cells_pseudotime , dims = 1:12)

colors <- c("#B2DF8A","#ABD61C","#A6CEE3", "#E31A1C", "#33A02C")
DimPlot(myeloid_cells_pseudotime, reduction = "umap", label = T, cols = colors)
```




### Pseudotime by slingshot

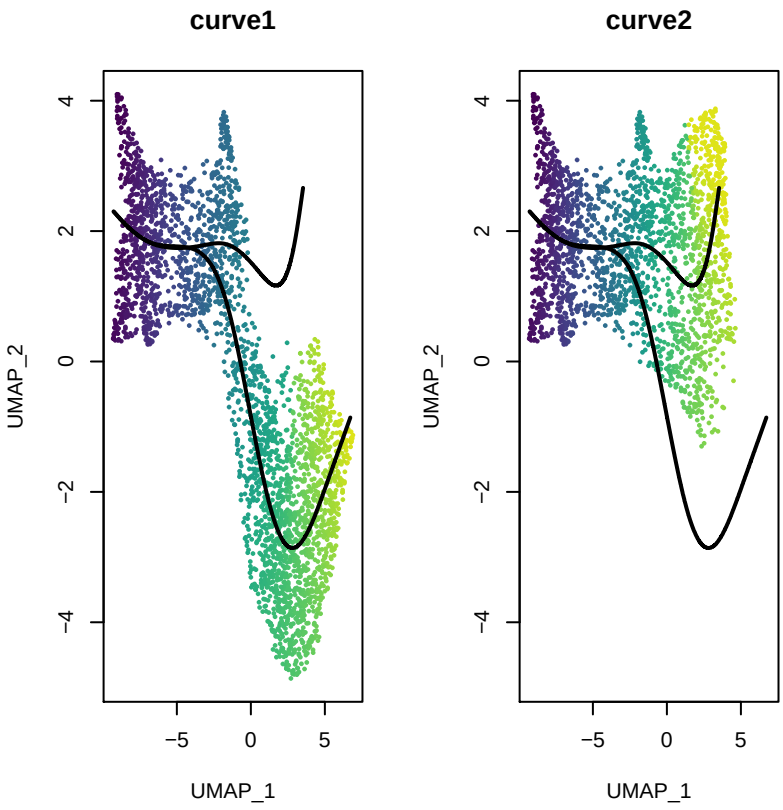
The Parameter have been modify to obtain a similar trajectory

```
p <- Embeddings(myeloid_cells_pseudotime, "umap")

myeloid_cells_pseudotime$color <- myeloid_cells_pseudotime$seurat_clusters
levels(myeloid_cells_pseudotime$color) <-c("#B2DF8A","#ABD61C","#1F78B4","#A6CEE3",
"#E31A1C","#E3751C","#600078", "#33A02C", "#FDBF6F", "#526317",  "#D4AAC6","#784620")

sds2 <- getLineages(Embeddings(myeloid_cells_pseudotime, "umap"),
myeloid_cells_pseudotime$seurat_clusters, start.clus = "3", end.clus = "1")
sds2 <- getCurves(sds2)

#pdf("Trajectory_slingshot_moddif.pdf", width = 19/3, height = 14/3)
plot(reducedDim(sds2), col = as.vector(myeloid_cells_pseudotime$color), pch = 16, cex =
0.5)
lines(sds2, lwd = 2, col = 'black')
```




```
#dev.off()
```

```
nc <- 3
pt <- slingPseudotime(sds2)
nms <- colnames(pt)
nr <- ceiling(length(nms)/nc)
pal <- viridis(100, end = 0.95)
par(mfrow = c(nr, nc))
for (i in nms) {
  colors <- pal[cut(pt[,i], breaks = 100)]
  plot(reducedDim(sds2), col = colors, pch = 16, cex = 0.5, main = i)
  lines(sds2, lwd = 2, col = 'black')
}
```




# TradeSeq

## Decide the number of knots (step exclusive to tradeseq)

```
CountMat <- myeloid_cells_pseudotime@assays$RNA@counts

icMat <- evaluateK(counts = CountMat, sds = sds, k = 3:10,
                   nGenes = 200, verbose = T)
```

## Launching tradeSeq

```
pseudotime <- slingPseudotime(sds, na = FALSE)
cellWeights <- slingCurveWeights(sds)

sce_slingshot <- fitGAM(counts = CountMat, pseudotime = pseudotime, cellWeights =
cellWeights)
```

## Save the results

```
saveRDS(sce_slingshot,"sce_slingshot.rds")
```

```
sessionInfo()
```

```
## R version 4.0.3 (2020-10-10)
## Platform: x86_64-pc-linux-gnu (64-bit)
```

```
## Running under: Ubuntu 20.04.6 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/liblapack.so.3
##
## 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
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
##  [1] viridisLite_0.4.1  tradeSeq_1.4.0     scales_1.2.1       ggplot2_3.4.0
##  [5] slingshot_1.8.0    princurve_2.1.6    patchwork_1.1.2    SeuratObject_4.1.3
##  [9] Seurat_4.3.0       dplyr_1.0.10
##
## loaded via a namespace (and not attached):
##   [1] VGAM_1.1-7                  plyr_1.8.8
##   [3] igraph_1.4.1                lazyeval_0.2.2
##   [5] sp_1.6-0                    splines_4.0.3
##   [7] BiocParallel_1.24.1         densityClust_0.3.2
##   [9] listenv_0.9.0               scattermore_0.8
##  [11] fastICA_1.2-3               GenomeInfoDb_1.26.7
##  [13] digest_0.6.31               htmltools_0.5.4
##  [15] viridis_0.6.2               fansi_1.0.4
##  [17] magrittr_2.0.3              tensor_1.5
##  [19] cluster_2.1.0               ROCR_1.0-11
##  [21] limma_3.46.0                globals_0.16.2
##  [23] matrixStats_0.63.0          docopt_0.7.1
##  [25] spatstat.sparse_3.0-0       colorspace_2.1-0
##  [27] ggrepel_0.9.2               xfun_0.37
##  [29] crayon_1.5.2                sparsesvd_0.2-2
##  [31] RCurl_1.98-1.10             jsonlite_1.8.4
##  [33] progressr_0.13.0            spatstat.data_3.0-0
##  [35] survival_3.2-7              zoo_1.8-11
##  [37] ape_5.6-2                   glue_1.6.2
##  [39] polyclip_1.10-4             gtable_0.3.1
##  [41] zlibbioc_1.36.0             XVector_0.30.0
##  [43] leiden_0.4.3                DelayedArray_0.16.3
##  [45] future.apply_1.10.0         SingleCellExperiment_1.12.0
##  [47] BiocGenerics_0.36.1         abind_1.4-5
##  [49] pheatmap_1.0.12             edgeR_3.32.1
##  [51] DBI_1.1.3                   spatstat.random_3.1-3
##  [53] miniUI_0.1.1.1              Rcpp_1.0.10
##  [55] xtable_1.8-4                reticulate_1.27
##  [57] stats4_4.0.3                htmlwidgets_1.6.1
##  [59] httr_1.4.5                  FNN_1.1.3.1
##  [61] RColorBrewer_1.1-3          ellipsis_0.3.2
```

```
##  [63] ica_1.0-3                   farver_2.1.1
##  [65] pkgconfig_2.0.3             uwot_0.1.14
##  [67] deldir_1.0-6                locfit_1.5-9.4
##  [69] utf8_1.2.3                  labeling_0.4.2
##  [71] tidyselect_1.2.0            rlang_1.0.6
##  [73] reshape2_1.4.4              later_1.3.0
##  [75] munsell_0.5.0               tools_4.0.3
##  [77] cli_3.6.0                   generics_0.1.3
##  [79] ggridges_0.5.4              evaluate_0.20
##  [81] stringr_1.5.0               fastmap_1.1.1
##  [83] yaml_2.3.7                  goftest_1.2-3
##  [85] knitr_1.42                  fitdistrplus_1.1-8
##  [87] DDRTree_0.1.5               purrr_1.0.1
##  [89] RANN_2.6.1                  pbapply_1.7-0
##  [91] future_1.32.0               nlme_3.1-162
##  [93] mime_0.12                   monocle_2.18.0
##  [95] slam_0.1-50                 compiler_4.0.3
##  [97] rstudioapi_0.14             plotly_4.10.1
##  [99] png_0.1-8                   spatstat.utils_3.0-1
## [101] tibble_3.1.8                stringi_1.7.12
## [103] highr_0.10                  lattice_0.20-41
## [105] Matrix_1.5-3                HSMMSingleCell_1.10.0
## [107] vctrs_0.5.2                 pillar_1.8.1
## [109] lifecycle_1.0.3             combinat_0.0-8
## [111] spatstat.geom_3.0-6         lmtest_0.9-40
## [113] RcppAnnoy_0.0.20            data.table_1.14.8
## [115] cowplot_1.1.1               bitops_1.0-7
## [117] irlba_2.3.5.1               httpuv_1.6.9
## [119] GenomicRanges_1.42.0        R6_2.5.1
## [121] promises_1.2.0.1            KernSmooth_2.23-20
## [123] gridExtra_2.3               IRanges_2.24.1
## [125] parallelly_1.34.0           codetools_0.2-19
## [127] MASS_7.3-53                 assertthat_0.2.1
## [129] SummarizedExperiment_1.20.0 withr_2.5.0
## [131] qlcMatrix_0.9.7             sctransform_0.3.5
## [133] S4Vectors_0.28.1            GenomeInfoDbData_1.2.4
## [135] mgcv_1.8-33                 parallel_4.0.3
## [137] grid_4.0.3                  tidyr_1.2.1
## [139] rmarkdown_2.19              MatrixGenerics_1.2.1
## [141] Rtsne_0.16                  spatstat.explore_3.0-5
## [143] Biobase_2.50.0              shiny_1.7.4
```