

# PAPER TITLE TO BE DEFINED (in common.yaml)

8-Monocle analysis and pseudotime estimation

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## Abstract

Lung interstitium macrophages (IMs) are non-alveolar resident tissue macrophages which contribute to the lung homeostasis. These cells were reported to be heterogeneous by our group and other teams, which contains two main distinct subpopulations: CD206+ IMs and CD206- IMs. However, the exact origin of IMs and the transcriptional programs that control IM differentiation remains unclear. In recent report, we analyzed the refilled IMs in the course of time after induced IM depletion with single-cell RNA sequencing (10X Genomics Chromium) and bulk RNA sequencing.

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## 1 Description

## 2 Load packages and data

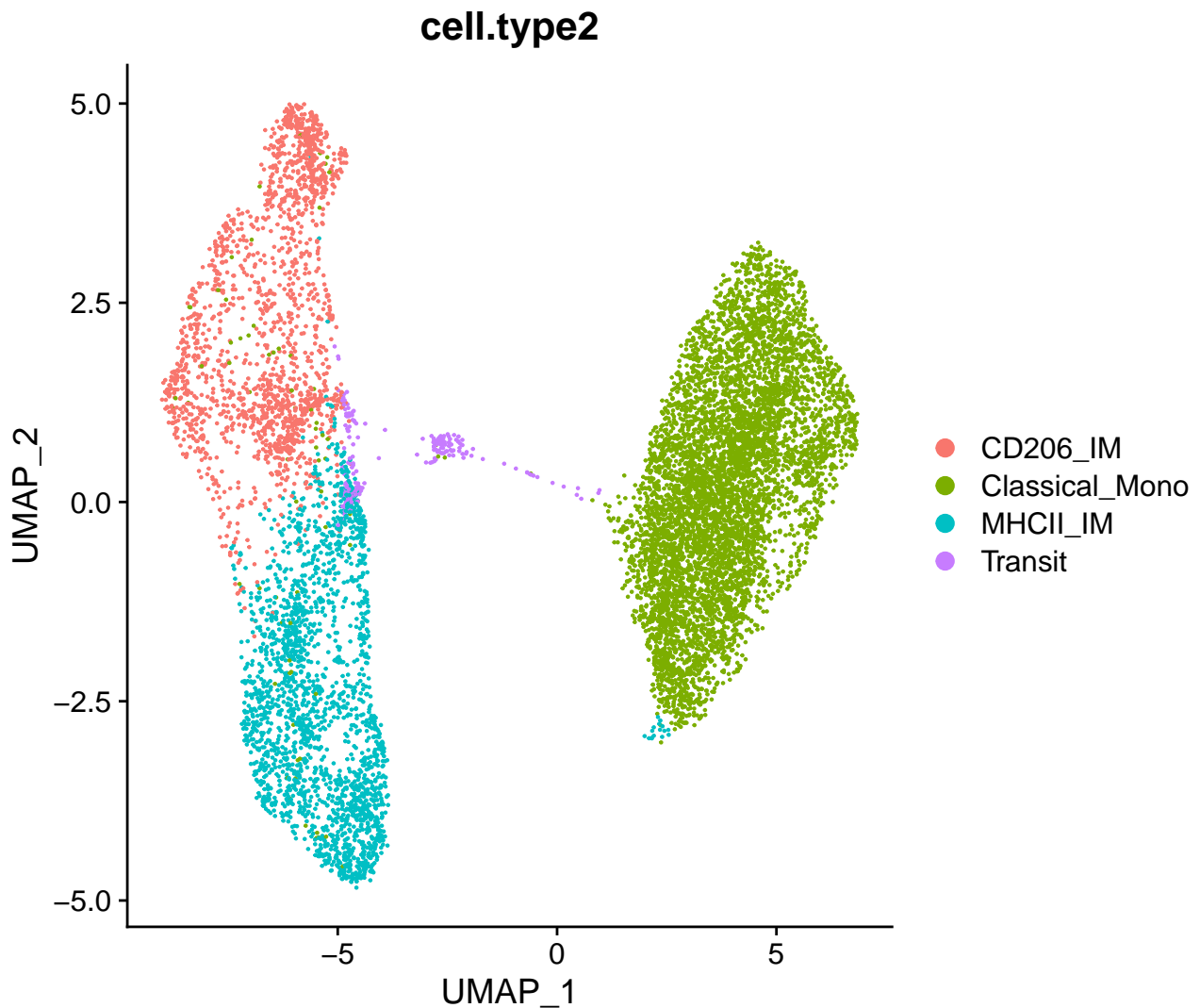
```
suppressMessages(library(Seurat))  
suppressMessages(library(SeuratWrappers))  
suppressMessages(library(monocle3))
```

Load data

```
IM_DTR3 <- readRDS(file = "../8-SCENIC_analysis/only_IM_differentiation.  
with_SCENIC.seuratObject.Rds")
```

Re-calculate UMAP

```
IM_DTR3 <- RunUMAP(IM_DTR3, dims = 1:8, n.components = 3L)  
DimPlot(IM_DTR3, group.by = "cell.type2")
```



### 3 Create monocle object

```
DefaultAssay(IM_DTR3) <- "RNA" 1

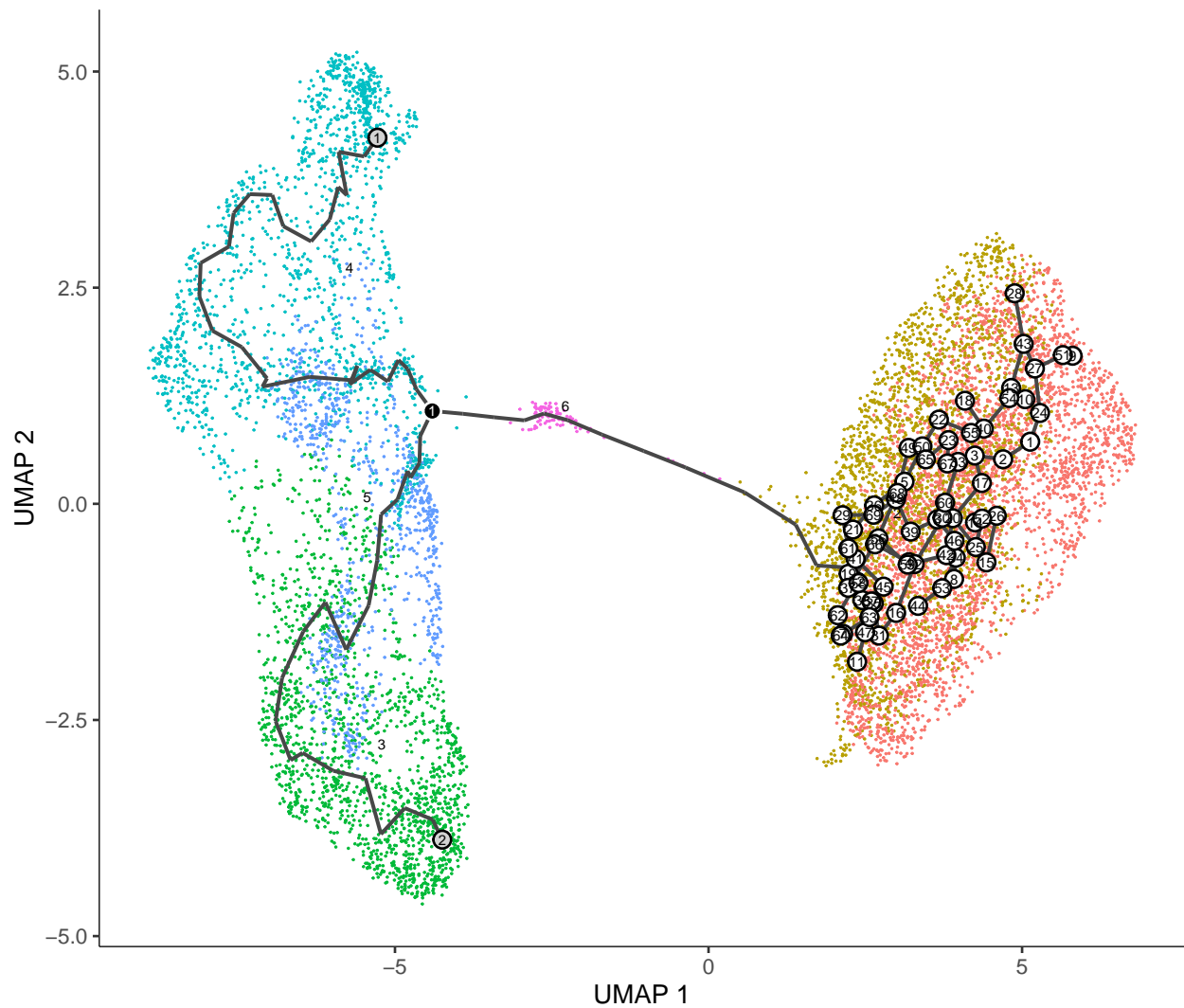
IM_DTR3.cds <- as.cell_data_set(IM_DTR3) 1
IM_DTR3.cds <- estimate_size_factors(IM_DTR3.cds) 2
IM_DTR3.cds <- estimate_size_factors(IM_DTR3.cds) 3
IM_DTR3.cds@rowRanges@elementMetadata@listData[["gene_short_name"]] <- 4
  rownames(IM_DTR3[["RNA"]]) 5
```

#### 3.1 Clustering, pseudotime estimation and trajectory analysis

Since `cluster_cells` uses random seed, the results could be slightly different. To recapitulate the exact results showed in report, please download the cds object: `only_IM_differentiation.with_Pseudotime.cds`

```
IM_DTR3.cds <- cluster_cells(cds = IM_DTR3.cds, reduction_method = "UMAP", 1
  resolution=0.51e-3, random_seed = 41)
IM_DTR3.cds <- learn_graph(IM_DTR3.cds, use_partition = FALSE) 2

plot_cells(IM_DTR3.cds) 1
```



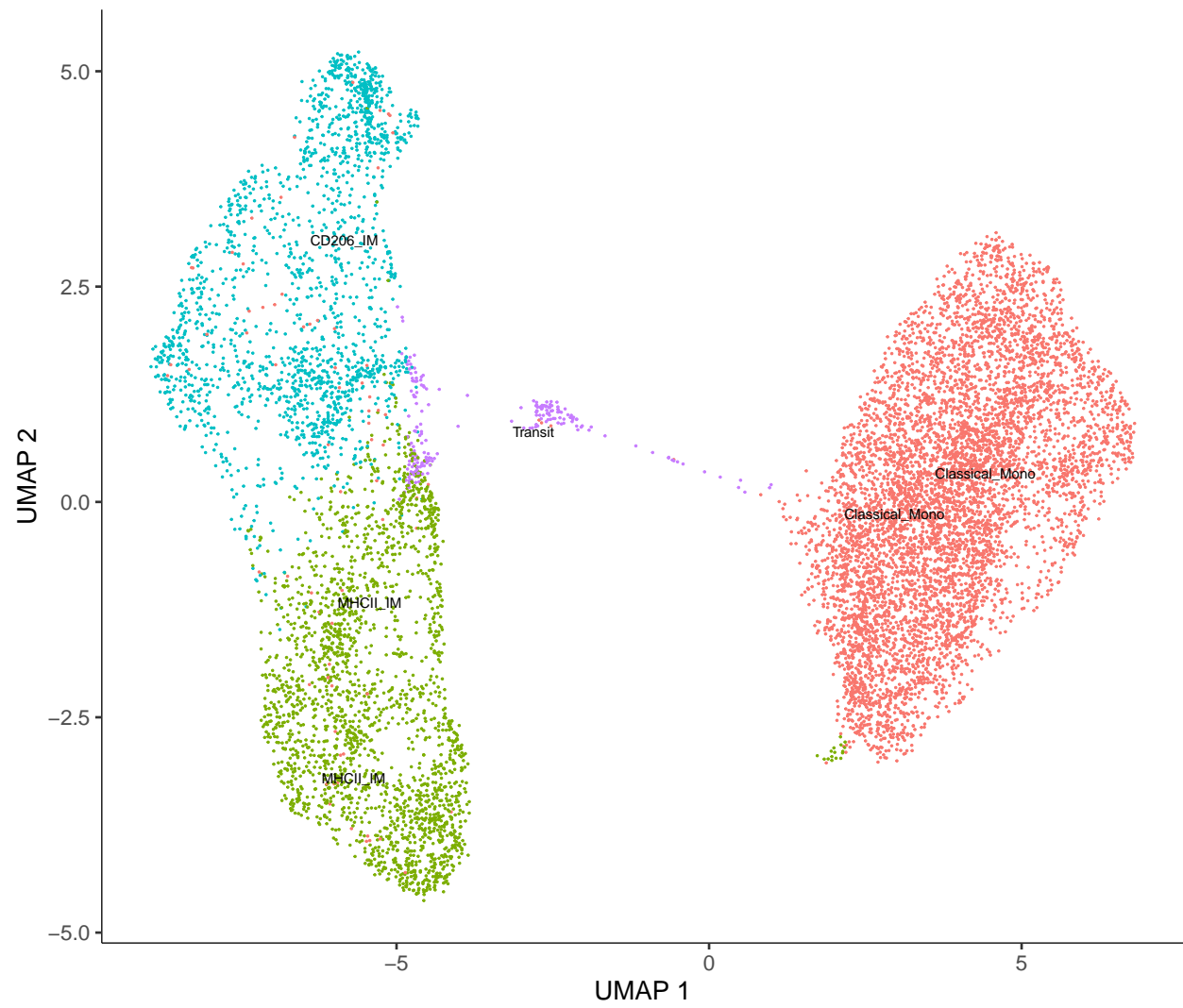
```
all_cells <- choose_cells(IM_DTR3.cds, return_list = TRUE) 1
not_mono <- choose_graph_segments(IM_DTR3.cds, return_list = TRUE) 2
root <- setdiff(all_cells, not_mono$cells) 3
```

```
IM_DTR3.cds <- order_cells(IM_DTR3.cds, reduction_method = "UMAP", root_ 1
  cells = root)
```

### 3.2 Plot pseudotime across subsets

Plot with recalculated UMAP and cell types:

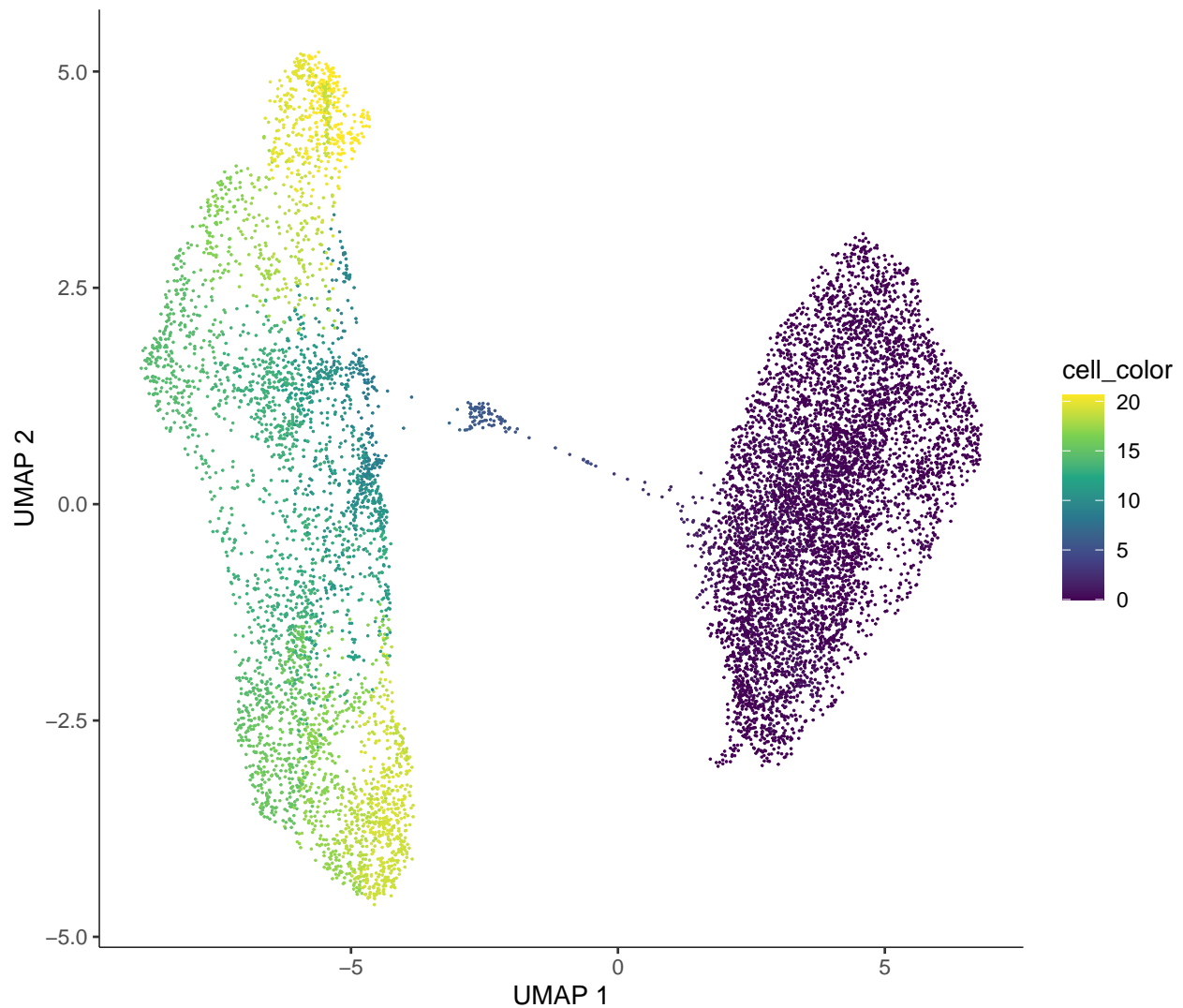
```
library(ggplot2) 1
plot_cells( 2
  cds = IM_DTR3.cds, 3
  color_cells_by = "cell.type2", 4
  show_trajectory_graph = FALSE 5
) 6
```



Pseudotime and

```
plot_cells(
  cds = IM_DTR3.cds,
  color_cells_by = "pseudotime",
  show_trajectory_graph = FALSE
)& scale_color_viridis_c()
```

1  
2  
3  
4  
5



To visualize in 3D plot:

```
library(plotly)
p1 <- plot_cells_3d(IM_DTR3.cds, color_cells_by="pseudotime", show_
  trajectory_graph = TRUE)

# p1
# plot will not show in rendered PDF.
```

### 3.3 3D Plots with trajectories across cell types

```
pal2 <- c(`Classical_Mono`="#A6CEE3",
  `MHCII_IM`="#B2DF8A",
  `CD206_IM`="#33A02C",
  `Transit` = "#E31A1C")

p2 <- plot_cells_3d(IM_DTR3.cds, color_cells_by="cell.type2", show_
  trajectory_graph = TRUE, color_palette = pal2, alpha = 0.6)

# p2
```

```
# plot will not show in rendered PDF. 9
```

```
saveRDS(IM_DTR3.cds, file = "./only_IM_differentiation.with_Pseudotime.cds 1
.Rds")
```

Add pseudotime to seurat object

```
IM_DTR3 <- AddMetaData(object = IM_DTR3, metadata = IM_DTR3.cds@principal_ 1
graph_aux@listData$UMAP$pseudotime, col.name = "pseudotime")
```

Save seurat object

```
saveRDS(IM_DTR3, file = "./only_IM_differentiation.with_SCENIC.with_ 1
Pseudotime.seuratObject.Rds")
```

In the next step, we will only analysis the IM differentiation. Subsetting differentiating cells

```
# Choose the nodes from beginning of Transit cells to both CD206+ and 1
CD206- IMs. 2
Mono_to_IM <- choose_graph_segments(IM_DTR3.cds, return_list = TRUE) 3
Mono_to_IM.cds <- IM_DTR3.cds[,IM_DTR3.cds@colData@rownames %in% Mono_to_ 4
IM$cells]
```

```
saveRDS(Mono_to_IM.cds, file = "Mono_to_IM.cds") 1
```

## 4 Session information

R session:

```
sessionInfo() 1
```

```
## R version 4.0.3 (2020-10-10) 1
## Platform: x86_64-pc-linux-gnu (64-bit) 2
## Running under: Ubuntu 20.04.3 LTS 3
## 4
## Matrix products: default 5
## BLAS: /usr/lib/x86_64-linux-gnu/openblas-pthread/libblas.so.3 6
## LAPACK: /usr/lib/x86_64-linux-gnu/openblas-pthread/liblapack.so.3 7
## 8
## locale: 9
## [1] LC_CTYPE=en_US.UTF-8 LC_NUMERIC=C 10
## [3] LC_TIME=en_GB.UTF-8 LC_COLLATE=en_US.UTF-8 11
## [5] LC_MONETARY=en_GB.UTF-8 LC_MESSAGES=en_US.UTF-8 12
## [7] LC_PAPER=en_GB.UTF-8 LC_NAME=C 13
## [9] LC_ADDRESS=C LC_TELEPHONE=C 14
## [11] LC_MEASUREMENT=en_GB.UTF-8 LC_IDENTIFICATION=C 15
## 16
## attached base packages: 17
## [1] stats4 parallel stats graphics grDevices utils 18
datasets 19
## [8] methods base
```

```

##
## other attached packages:
## [1] ggplot2_3.3.5                monocle3_1.0.0
## [3] SingleCellExperiment_1.12.0 SummarizedExperiment_1.20.0
## [5] GenomicRanges_1.42.0        GenomeInfoDb_1.26.7
## [7] IRanges_2.24.1              S4Vectors_0.28.1
## [9] MatrixGenerics_1.2.1        matrixStats_0.61.0
## [11] Biobase_2.50.0              BiocGenerics_0.36.1
## [13] SeuratWrappers_0.3.0        SeuratObject_4.0.4
## [15] Seurat_4.0.5
##
## loaded via a namespace (and not attached):
## [1] Rtsne_0.15                    colorspace_2.0-2          deldir_1.0-6
## [4] ellipsis_0.3.2               ggribes_0.5.3            XVector_0.30.0
## [7] spatstat.data_2.1-0          farver_2.1.0              leiden_0.3.9
## [10] listenv_0.8.0                remotes_2.4.2             ggrepel_0.9.1
## [13] RSpectra_0.16-0             fansi_0.5.0              codetools_0.2-18
## [16] splines_4.0.3                R.methodsS3_1.8.1        knitr_1.36
## [19] polyclip_1.10-0             jsonlite_1.7.2           ica_1.0-2
## [22] cluster_2.1.0               png_0.1-7                 R.oo_1.24.0
## [25] uwot_0.1.10.9000            shiny_1.7.1               sctransform_0.3.2
## [28] spatstat.sparse_2.0-0        BiocManager_1.30.16      compiler_4.0.3
## [31] httr_1.4.2                   assertthat_0.2.1         Matrix_1.3-4
## [34] fastmap_1.1.0               lazyeval_0.2.2           later_1.3.0
## [37] htmltools_0.5.2             tools_4.0.3              rsvd_1.0.5
## [40] igraph_1.2.9                 GenomeInfoDbData_1.2.4   gtable_0.3.0
## [43] glue_1.5.1                   RANN_2.6.1               reshape2_1.4.4
## [46] dplyr_1.0.7                  Rcpp_1.0.7               scattermore_0.7
## [49] vctrs_0.3.8                  nlme_3.1-153             lmtest_0.9-39
## [52] xfun_0.28                    stringr_1.4.0            globals_0.14.0
## [55] mime_0.12                     miniUI_0.1.1.1           lifecycle_1.0.1
## [58] irlba_2.3.3                  goftest_1.2-3            future_1.23.0
## [61] zlibbioc_1.36.0             MASS_7.3-53              zoo_1.8-9
## [64] scales_1.1.1                 spatstat.core_2.3-2      promises_1.2.0.1
## [67] spatstat.utils_2.2-0         RColorBrewer_1.1-2       yaml_2.2.1
## [70] reticulate_1.22             pbapply_1.5-0            gridExtra_2.3
## [73] rpart_4.1-15                 stringi_1.7.6            highr_0.9
## [76] bitops_1.0-7                 rlang_0.4.12             pkgconfig_2.0.3
## [79] evaluate_0.14                lattice_0.20-41          ROCR_1.0-11
## [82] purrr_0.3.4                  tensor_1.5               labeling_0.4.2
## [85] patchwork_1.1.1             htmlwidgets_1.5.4        cowplot_1.1.1
## [88] tidyselect_1.1.1            parallelly_1.29.0        RcppAnnoy_0.0.19
## [91] plyr_1.8.6                   magrittr_2.0.1           R6_2.5.1
## [94] generics_0.1.1              DelayedArray_0.16.3      DBI_1.1.1
## [97] withr_2.4.3                  pillar_1.6.4             mgcv_1.8-33
## [100] fitdistrplus_1.1-6          RCurl_1.98-1.5           survival_3.2-7
## [103] abind_1.4-5                  tibble_3.1.6             future.apply_1.8.1
## [106] crayon_1.4.2                 KernSmooth_2.23-20       utf8_1.2.2
## [109] spatstat.geom_2.3-0         plotly_4.10.0            rmarkdown_2.11
## [112] viridis_0.6.2               grid_4.0.3               data.table_1.14.2
## [115] digest_0.6.29               xtable_1.8-4             tidyr_1.1.4
## [118] httpuv_1.6.3                 R.utils_2.11.0           munsell_0.5.0
## [121] viridisLite_0.4.0

```



## 5 References