Monocytes can Proliferate in Vacant Tissue Niches prior to Differentiation into Macrophages

8-Monocle analysis and pseudotime estimation

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Abstract

Resident tissue macrophages (RTM) are differentiated immune cells populating distinct niches and exhibiting important tissue-supportive functions. RTM maintenance is thought to depend either on monocyte engraftment and differentiation, or on the self-renewal of mature RTM. Here, we discovered that monocytes can re-enter cell cycle and proliferate locally before their differentiation into RTM. We developed a mouse model of inducible lung interstitial macrophage (IM) depletion in which the vacant niche is repopulated by BM-derived monocytes giving rise to fully differentiated IM subsets. By performing time-course single-cell RNA-sequencing analyses of myeloid cells during niche refilling, we found that few Ly6C+ classical monocytes could self-renew locally in a CSF1R-dependent manner. We further showed that the transcription factor MafB restricted such proliferation and was essential to mediate RTM specification and identity in our model. Our data provide evidence that, in the mononuclear phagocyte system, self-renewal is not merely restricted to myeloid progenitor cells and mature macrophages, but is also a tightly regulated capability of mature monocytes developing into RTM in vivo.

Contents

1 Description

To evaluate trajectory-based DE analysis during IM development in IM-DTR mice, Ly6C+ cMo, transit cells, CD206- and CD206+ IM were subjected to Monocle (Trapnell et al., 2014) analysis. The Monocle CDS object was built with counts and metadata from Seurat object and converted using SeuratWrappers package. Cells were clustered with cluster_cells function using calculated UMAP coordination and resolution of 0.51E-3. The trajectories along pseudotime were built using learn_graph and order_cells functions. The DE genes across trajectory were calculated using Moran's I test (graph_test function) and only the genes with q_value of 0 and Morans_I over 0.25 were kept as significant DE genes and subjected to further analyses.

Here we build up Monocle object with data and metadata in Seurat project.

2 Load packages and data

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

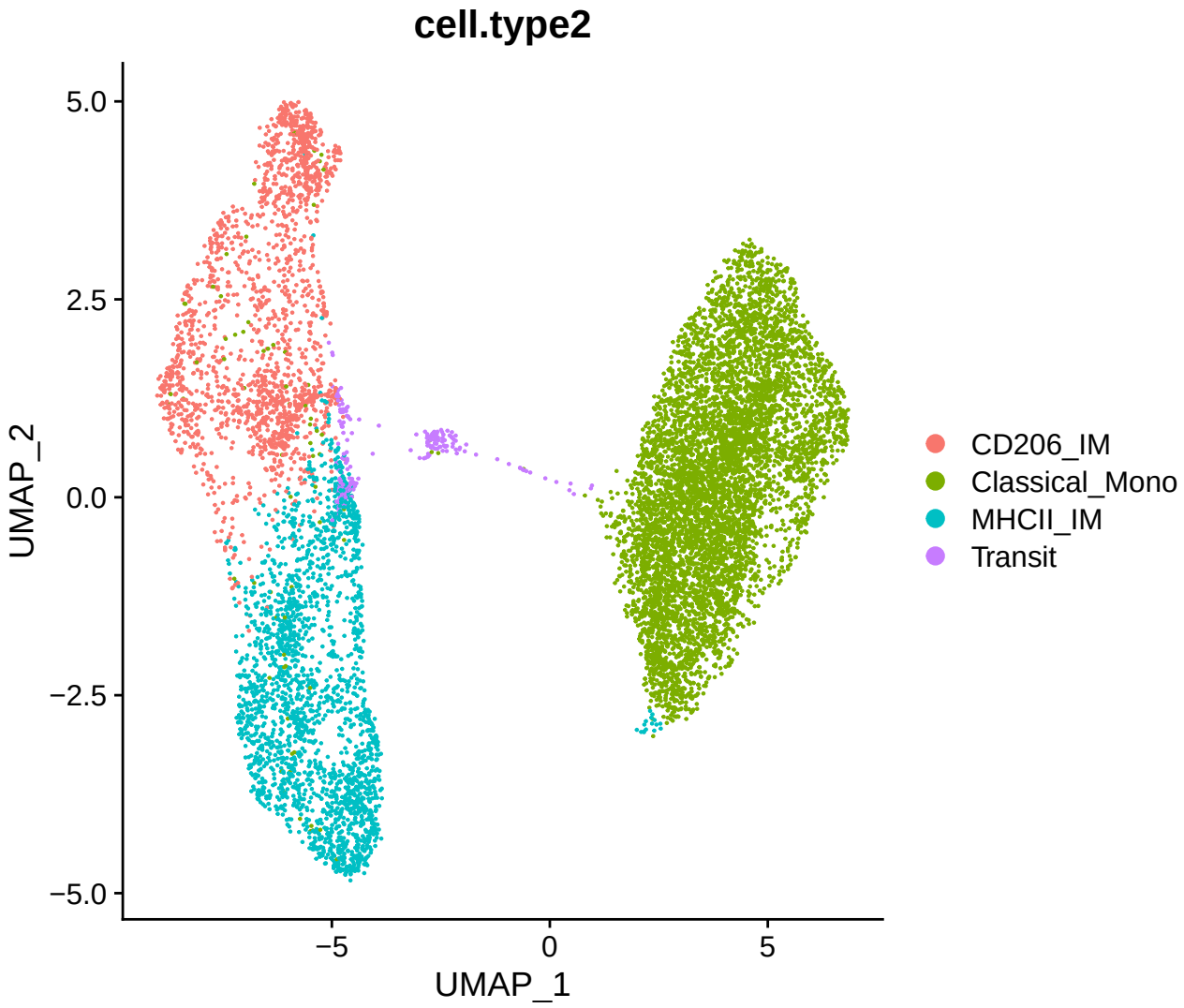
Load

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

2.1 Re-calculate UMAP

As a subset of population was used in the following analysis, we re calculated 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"
```

```
IM_DTR3.cds <- as.cell_data_set(IM_DTR3)

IM_DTR3.cds <- estimate_size_factors(IM_DTR3.cds)

IM_DTR3.cds@rowRanges@elementMetadata@listData[["gene_short_name"]] <-
   rownames(IM_DTR3[["RNA"]])
```

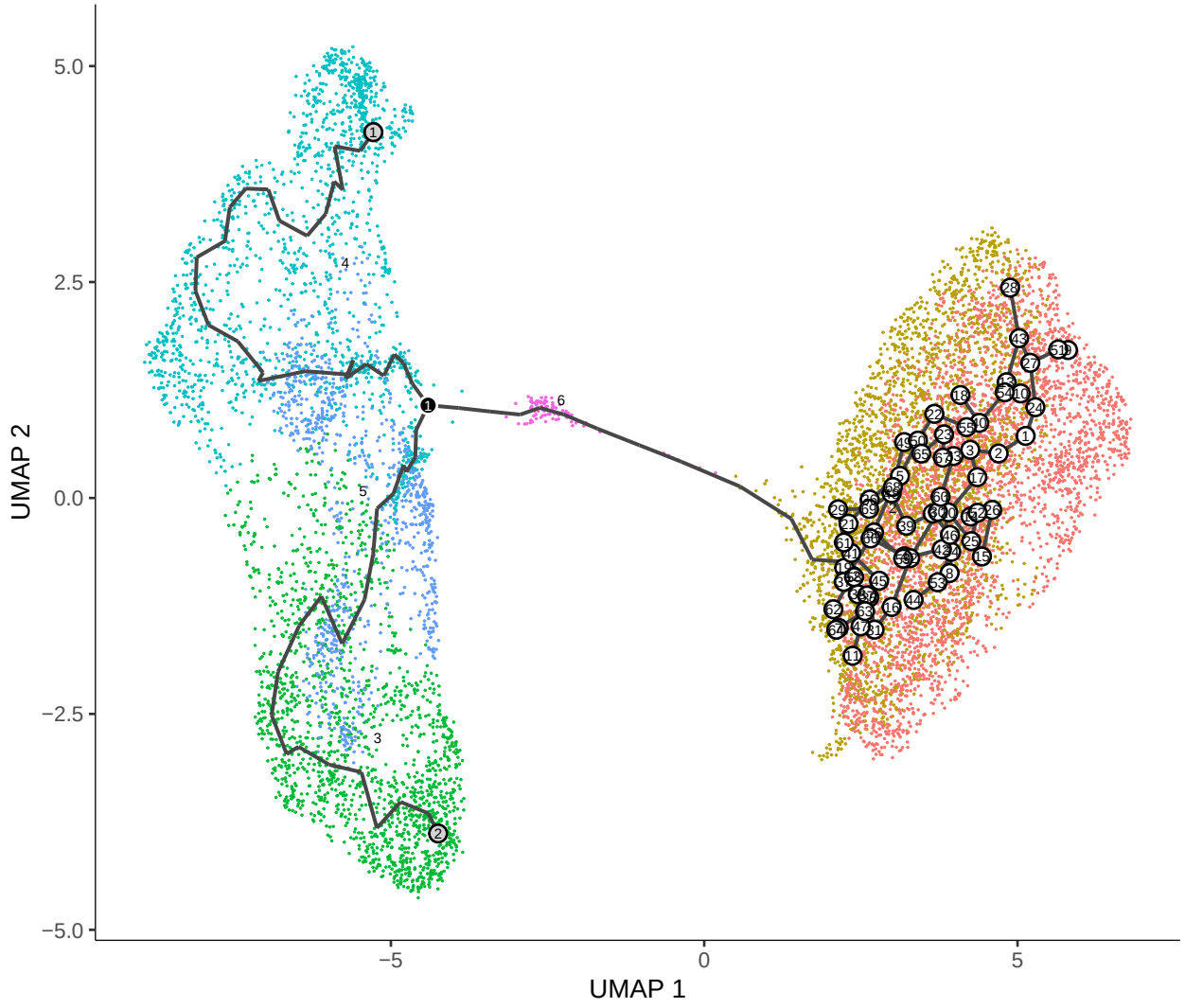
3.1 Clusterting, pseudotime estimation and trajectory analysis

Since cluster_cells uses randam 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",
    resolution=0.51e-3, random_seed = 41)
```

```
IM_DTR3.cds <- learn_graph(IM_DTR3.cds, use_partition = FALSE)
```

```
plot_cells(IM_DTR3.cds)
```




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

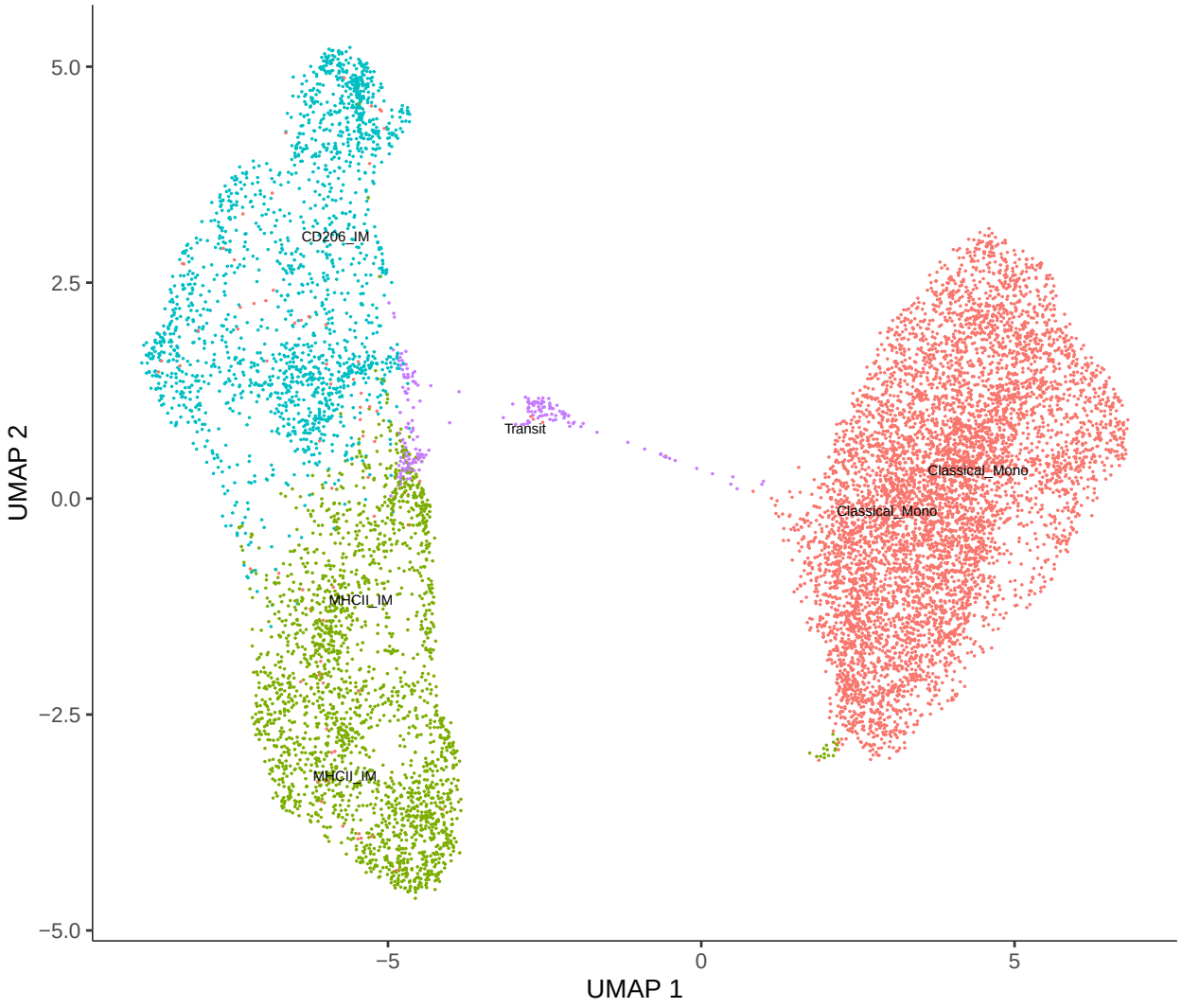
```
IM_DTR3.cds <- order_cells(IM_DTR3.cds, reduction_method = "UMAP", root_
    cells = root)
```

3.2 Plot pseudotime across subsets

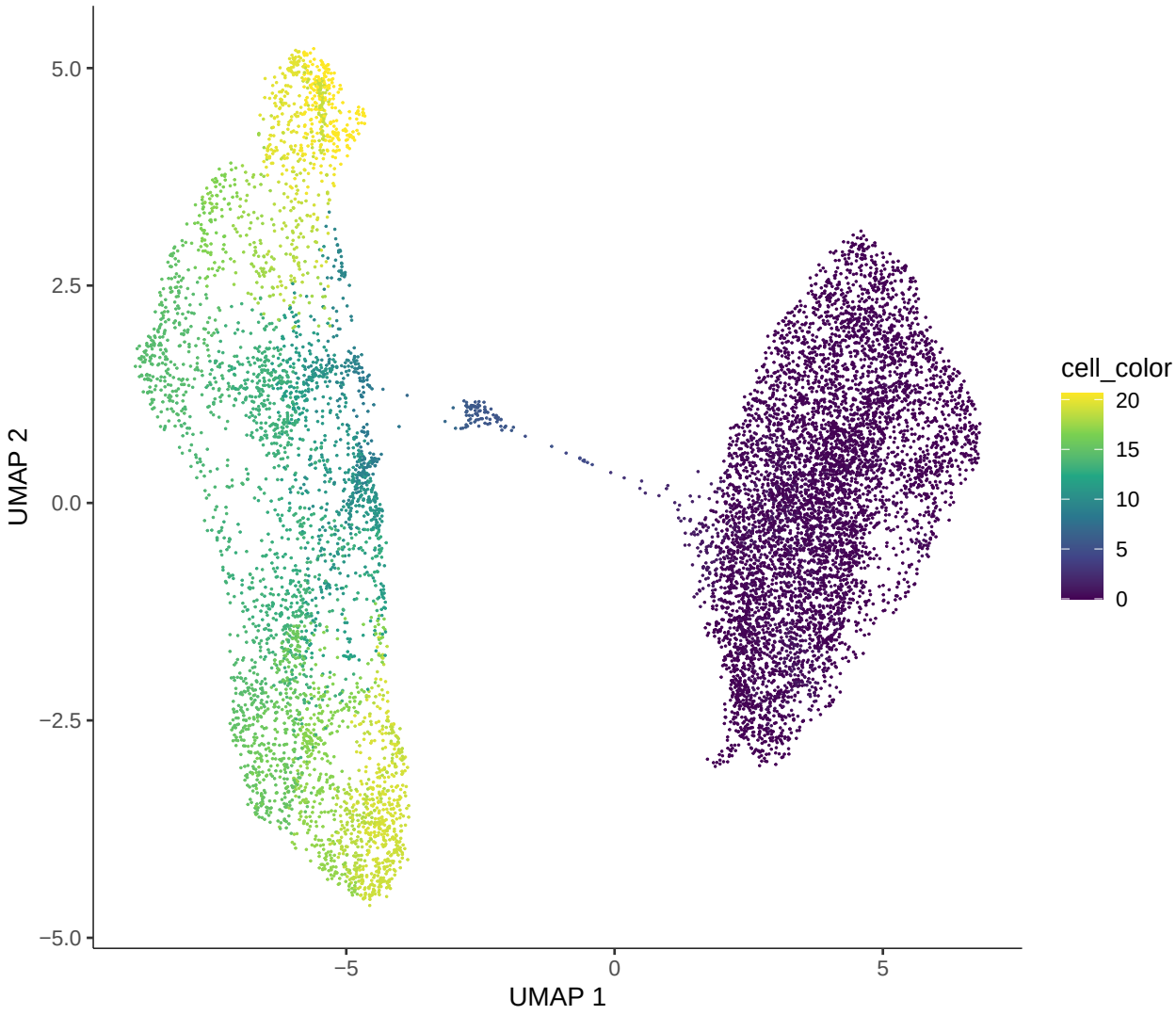
Plot with relaculated UMAP and cell types:

```
library(ggplot2)
plot_cells(
  cds = IM_DTR3.cds,
  color_cells_by = "cell.type2",
```

```
  show_trajectory_graph = FALSE
)
```




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



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.
```

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

Add pseodotime to seurat object

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

Save seurat object

```
saveRDS(IM_DTR3, file = "./only_IM_differentiation.with_SCENIC.with_Pseudotime.seuratObject.Rds")
```

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

```
# Choose the nodes from beginning of Transit cells to both CD206+ and CD206- IMs.

Mono_to_IM <- choose_graph_segments(IM_DTR3.cds, return_list = TRUE)
Mono_to_IM.cds <- IM_DTR3.cds[,IM_DTR3.cds@colData@rownames %in% Mono_to_IM$cells]
```

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

4 Session information

R sesssion:

```
sessionInfo()
```

```
## R version 4.0.3 (2020-10-10)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 20.04.3 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/liblapack.so.3
##
## locale:
##  [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C
##  [3] LC_TIME=en_GB.UTF-8        LC_COLLATE=en_US.UTF-8
##  [5] LC_MONETARY=en_GB.UTF-8    LC_MESSAGES=en_US.UTF-8
##  [7] LC_PAPER=en_GB.UTF-8       LC_NAME=C
##  [9] LC_ADDRESS=C               LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_GB.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] stats4    parallel  stats     graphics  grDevices utils
   datasets
## [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          ggridges_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_0.1.11             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_2.3.5             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
```

References

Trapnell, C., Cacchiarelli, D., Grimsby, J., Pokharel, P., Li, S., Morse, M., Lennon, N.J., Livak, K.J., Mikkelsen, T.S., and Rinn, J.L. (2014). The dynamics and regulators of cell fate decisions are revealed by pseudotemporal ordering of single cells. Nature Biotechnology 2014 32:4 *32*, 381–386.