

MafB-restricted local monocyte proliferation precedes lung interstitial macrophage differentiation

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 rely on either monocyte engraftment and differentiation, or RTM self-renewal. Here, we developed an inducible mouse model of lung interstitial macrophage (IM) niche depletion and repopulation to investigate IM development in vivo. Using time-course single-cell RNA-sequencing analyses, bone marrow chimeras and gene targeting, we found that engrafted Ly6C+ classical monocytes could self-renew locally in a CSF1R-dependent manner before their differentiation into RTM. We further showed that the switch from monocyte proliferation towards IM subset specification was controlled by MafB, while c-Maf specifically regulated the identity of the CD206+ IM subset. Our data shed new light on the transcriptional regulation of IM development and 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	2
2	Load packages and data	2
2.1	Re-calculate UMAP	2
3	Create Monocle object	3
3.1	Clustering, pseudotime estimation and trajectory analysis	3
3.2	Plot pseudotime across subsets	4
3.3	3D Plots with trajectories across cell types	6
4	Session information	7
	References	9

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 Monocle¹ 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)) 1
suppressMessages(library(SeuratWrappers)) 2
suppressMessages(library(monocle3)) 3
```

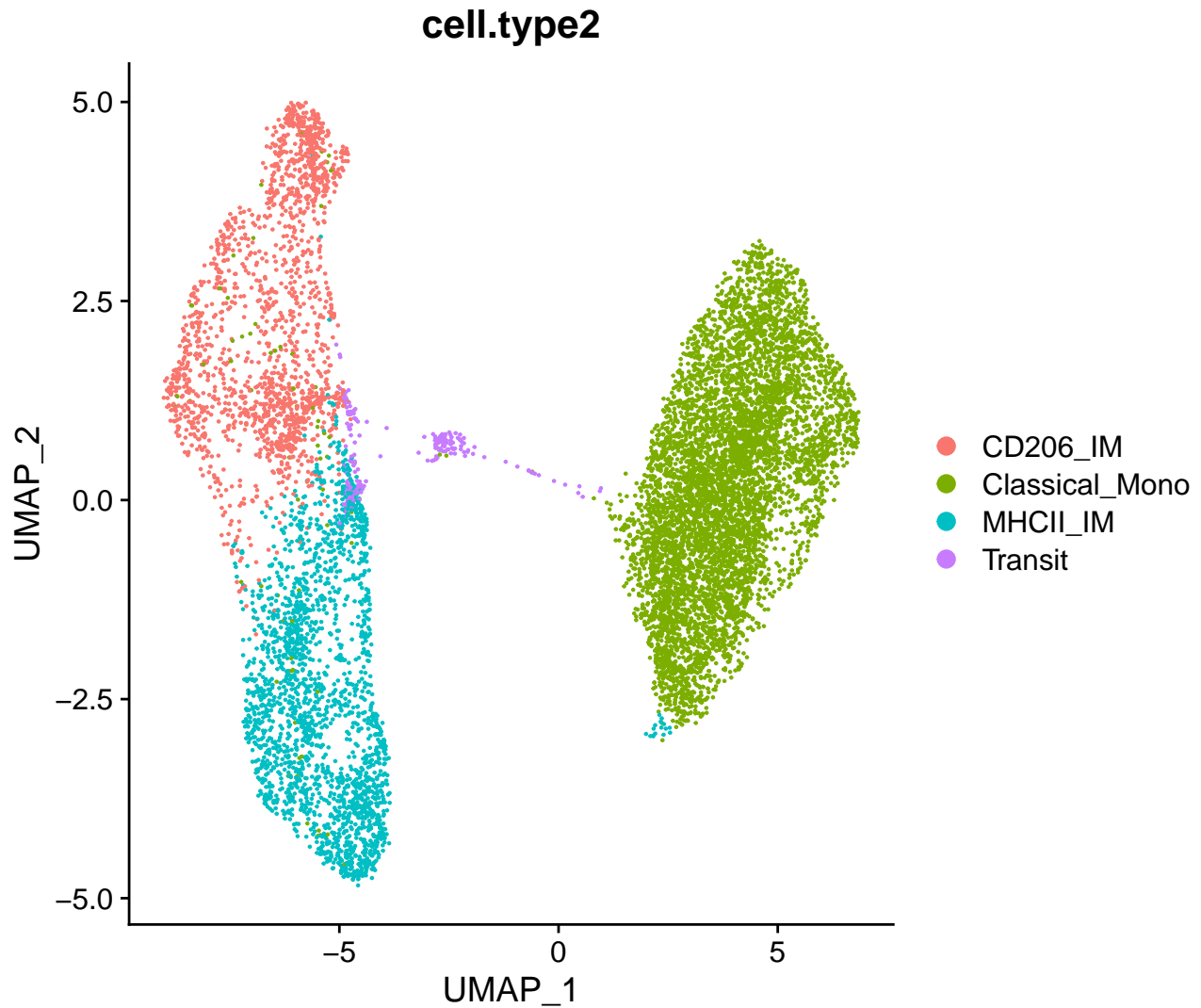
Load

```
IM_DTR3 <- readRDS(file = "../8-SCENIC_analysis/only_IM_differentiation. 1
  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) 1
DimPlot(IM_DTR3, group.by = "cell.type2") 2
```



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@rowRanges@elementMetadata@listData[["gene_short_name"]] <- 3  
  rownames(IM_DTR3[["RNA"]]) 4  
  5
```

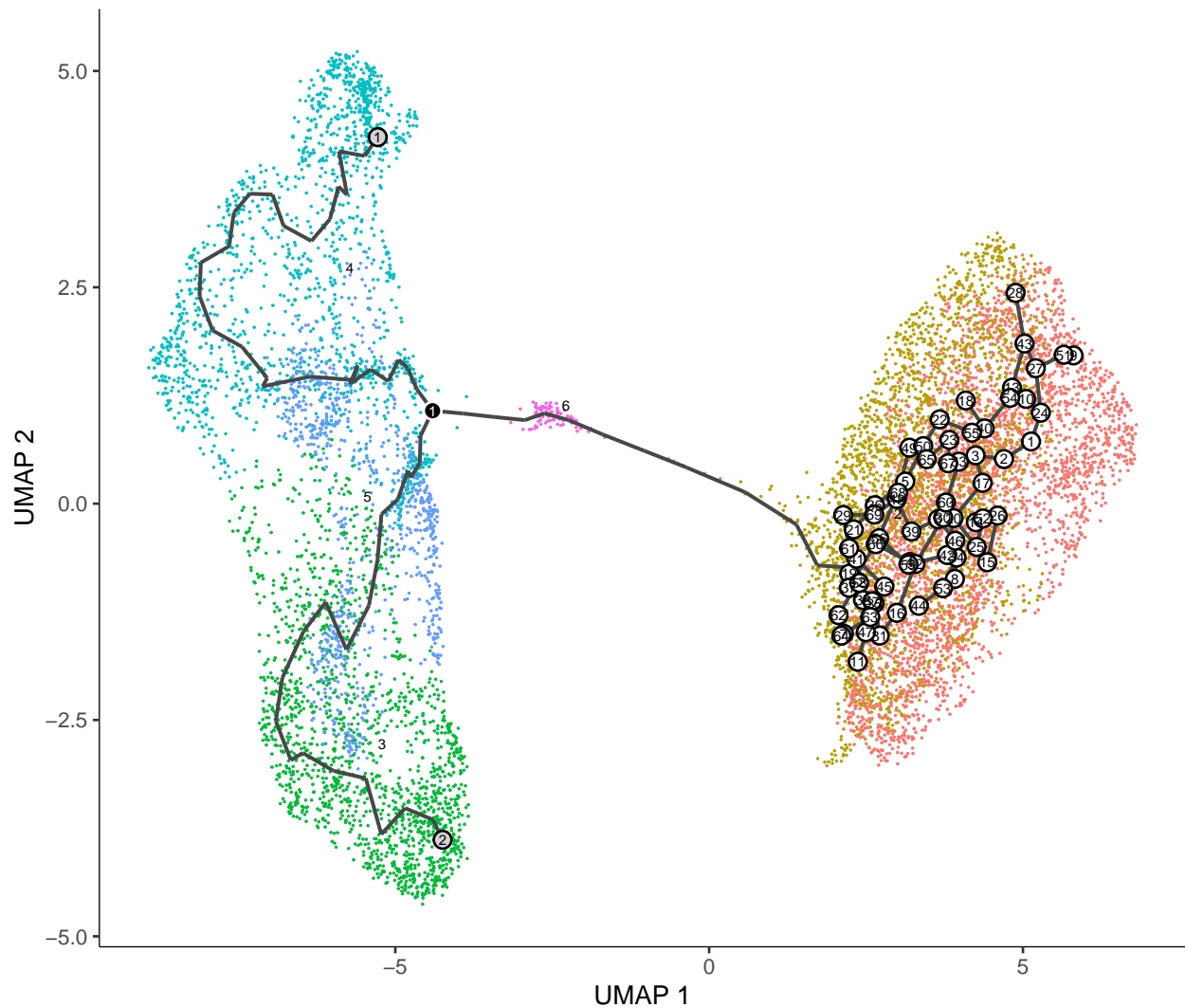
3.1 Clustering, pseudotime estimation and trajectory analysis

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)
```

```
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)
```

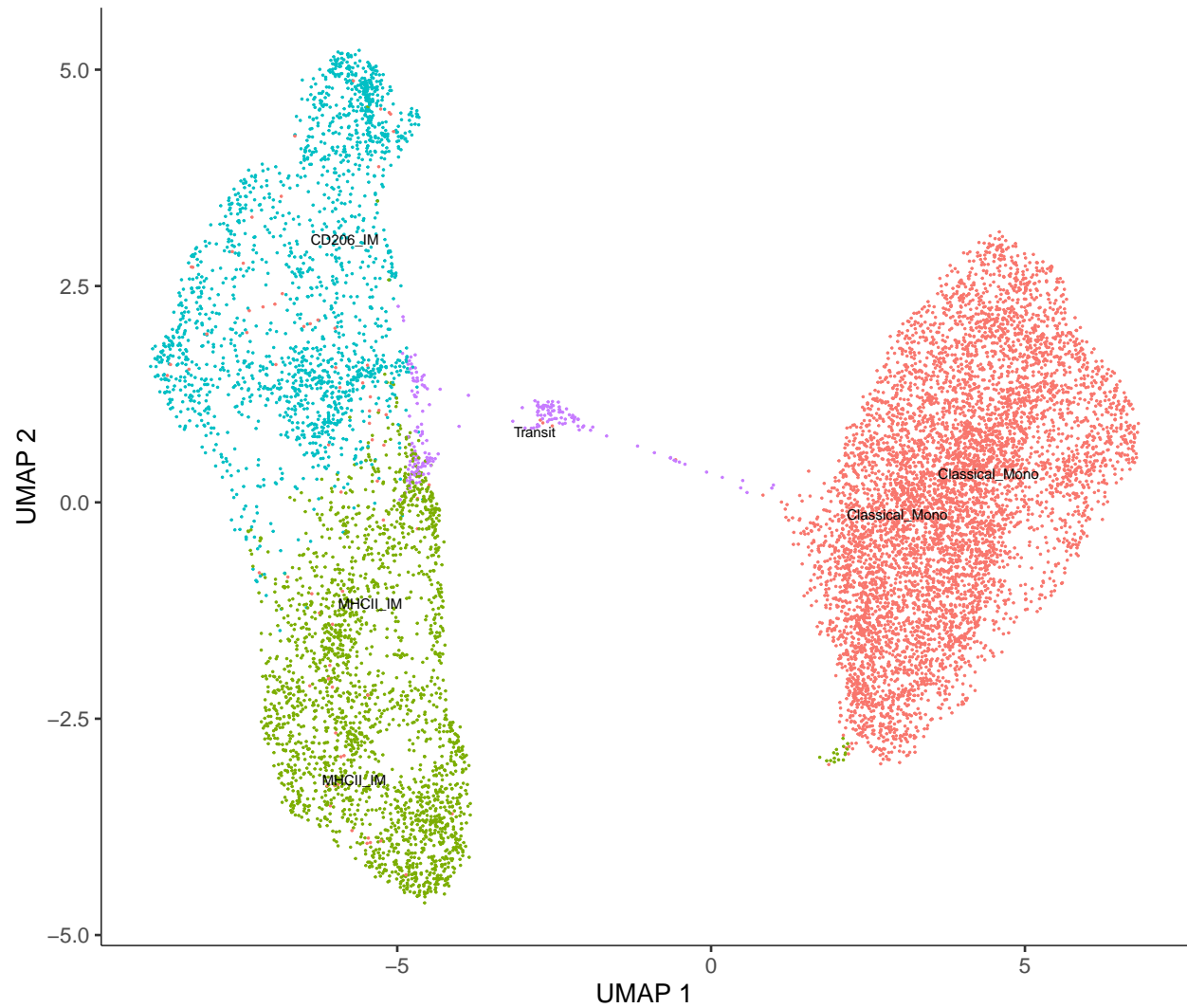
```
plot_cells(
```

```
  cds = IM_DTR3.cds,
```

```
  color_cells_by = "cell.type2",
```

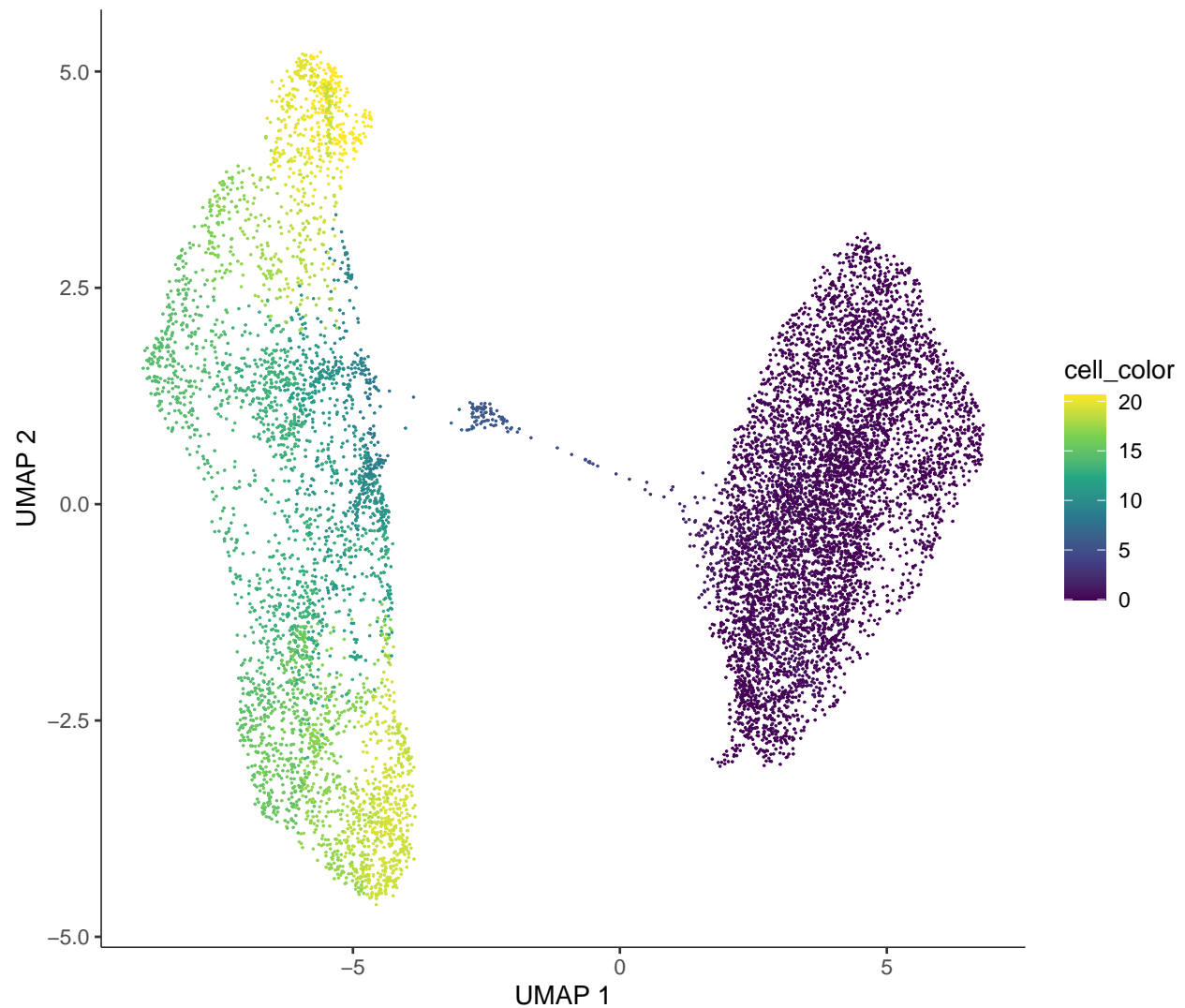
```
show_trajectory_graph = FALSE
)
```

5
6



```
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_4.1.0
##
## loaded via a namespace (and not attached):
## [1] plyr_1.8.6                    igraph_1.2.11                lazyeval_0.2.2
## [4] splines_4.0.3                listenv_0.8.0                scattermore_0.8
## [7] digest_0.6.29                htmltools_0.5.2              viridis_0.6.2
## [10] fansi_1.0.2                  magrittr_2.0.2               tensor_1.5
## [13] cluster_2.1.0                ROCR_1.0-11                  remotes_2.4.2
## [16] globals_0.14.0              R.utils_2.11.0               spatstat.sparse_2
## [19] colorspace_2.0-3             ggrepel_0.9.1                xfun_0.29
## [22] dplyr_1.0.8                  crayon_1.5.0                 RCurl_1.98-1.6
## [25] jsonlite_1.7.3              spatstat.data_2.1-2          survival_3.2-7
## [28] zoo_1.8-9                   glue_1.6.1                   polyclip_1.10-0
## [31] gtable_0.3.0                 zlibbioc_1.36.0              XVector_0.30.0
## [34] leiden_0.3.9                 DelayedArray_0.16.3          future.apply_1.8.1
## [37] abind_1.4-5                  scales_1.1.1                 DBI_1.1.2
## [40] spatstat.random_2.1-0        miniUI_0.1.1.1               Rcpp_1.0.8
## [43] viridisLite_0.4.0           xtable_1.8-4                 reticulate_1.24
## [46] spatstat.core_2.4-0          rsvd_1.0.5                   htmlwidgets_1.5.4
## [49] httr_1.4.2                   RColorBrewer_1.1-2           ellipsis_0.3.2
## [52] ica_1.0-2                    farver_2.1.0                 pkgconfig_2.0.3
## [55] R.methodsS3_1.8.1           uwot_0.1.11                  deldir_1.0-6
## [58] utf8_1.2.2                   labeling_0.4.2                tidyselect_1.1.1
## [61] rlang_1.0.1                  reshape2_1.4.4               later_1.3.0
## [64] munsell_0.5.0                tools_4.0.3                   cli_3.2.0
## [67] generics_0.1.2              ggribges_0.5.3               evaluate_0.15
## [70] stringr_1.4.0                fastmap_1.1.0                yaml_2.3.5
## [73] goftest_1.2-3                knitr_1.37                    fitdistrplus_1.1-6
## [76] purrr_0.3.4                  RANN_2.6.1                   pbapply_1.5-0
## [79] future_1.24.0                nlme_3.1-155                 mime_0.12
## [82] R.oo_1.24.0                  compiler_4.0.3                rstudioapi_0.13
## [85] plotly_4.10.0                png_0.1-7                     spatstat.utils_2
## [88] tibble_3.1.6                 stringi_1.7.6                 highr_0.9
## [91] RSpectra_0.16-0             lattice_0.20-41               Matrix_1.4-0
## [94] vctrs_0.3.8                  pillar_1.7.0                  lifecycle_1.0.1
## [97] BiocManager_1.30.16         spatstat.geom_2.3-2           lmtest_0.9-39
## [100] RcppAnnoy_0.0.19            data.table_1.14.2            cowplot_1.1.1
## [103] bitops_1.0-7                irlba_2.3.5                   httpuv_1.6.5
## [106] patchwork_1.1.1              R6_2.5.1                      promises_1.2.0.1
## [109] KernSmooth_2.23-20          gridExtra_2.3                 parallelly_1.30.0
## [112] codetools_0.2-18            MASS_7.3-53                   assertthat_0.2.1
## [115] withr_2.4.3                  sctransform_0.3.3             GenomeInfoDbData_1
## [118]

```


##	[118]	mgcv_1.8-33	grid_4.0.3	rpart_4.1-15	71
##	[121]	tidyr_1.2.0	rmarkdown_2.11	Rtsne_0.15	72
##	[124]	shiny_1.7.1			73

References

1. Trapnell, C. *et al.* The dynamics and regulators of cell fate decisions are revealed by pseudotemporal ordering of single cells. *Nature Biotechnology* 2014 32:4 **32**, 381–386 (2014).