

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

3-scRNAseq initiation for Exp1: IMs in Day 4 post-depletion

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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*.

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# 1 Description

To compare lung monocytes and IM from untreated IM-DTR mice (group “No treatment”) with those from IM-DTR mice treated with 50 ng DT i.p. 96 h before (group “DT96h”), 5 mice from each group was sacrificed and lung single-cell-susensions were obtained after enzymatic digestion. CD11b+ cells were enriched by MACS using CD11b MicroBeads (Miltenyi Biotec, 130-049-601). Lung monocytes and IM were then FACS-sorted and the 10x Genomics platform (Single Cell 3’ Solution) was used for scRNA-seq. Lung monocytes and IM were FACS-sorted separately and IM were then enriched in the final single cell suspension to reach a monocyte/IM ratio of 5:5.

The raw scRNAseq data were mapped and counted in the analysis 2. Here we check the sample quality and build up Seurat object from the counts matrix using Seurat package (Hao et al., 2021).

## 2 Load packages and data

```
library(Seurat)
library(ggpubr)
source("../R/seurat.setup.R")
dir.10x <- "Counts/scRNAseq" # 10X output directory
```

1  
2  
3  
4

## 3 QC

### 3.1 Sample: CPlus\_NGS20\_Q147

```
CPlus_NGS20_Q147 <- seurat.setup(path.10x = file.path(dir.10x, "CPlus_"
  NGS20_Q147/outs/filtered_feature_bc_matrix/"), project = "IM-DTR",
  dimensionality = 1:20, mt.percentage = 10, human = FALSE)
```

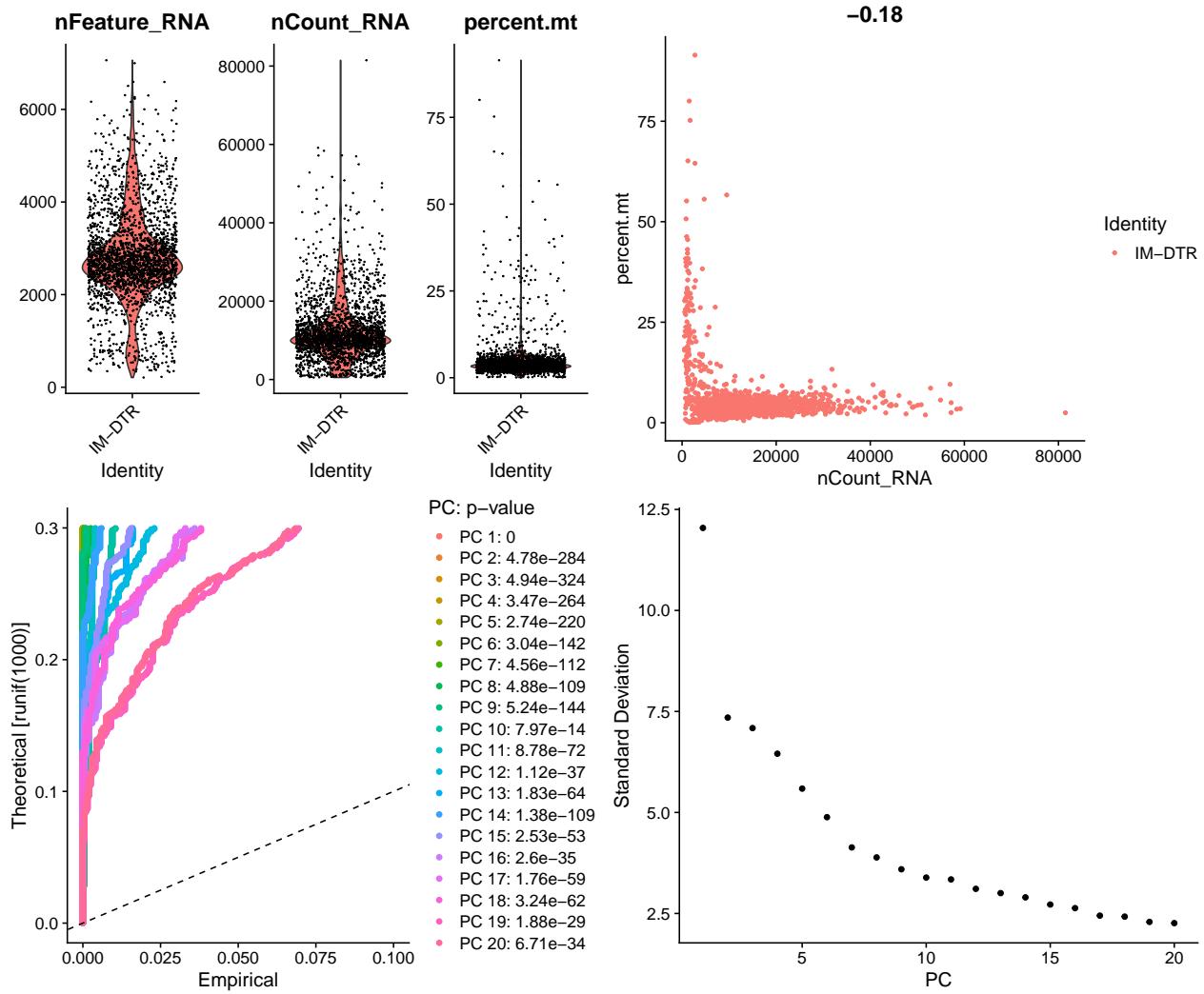
1  
2  
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4  
5  
6  
7  
8  
9

```
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
## Number of nodes: 2269
## Number of edges: 79406
## Running Louvain algorithm...
## Maximum modularity in 10 random starts: 0.8569
## Number of communities: 8
## Elapsed time: 0 seconds
```

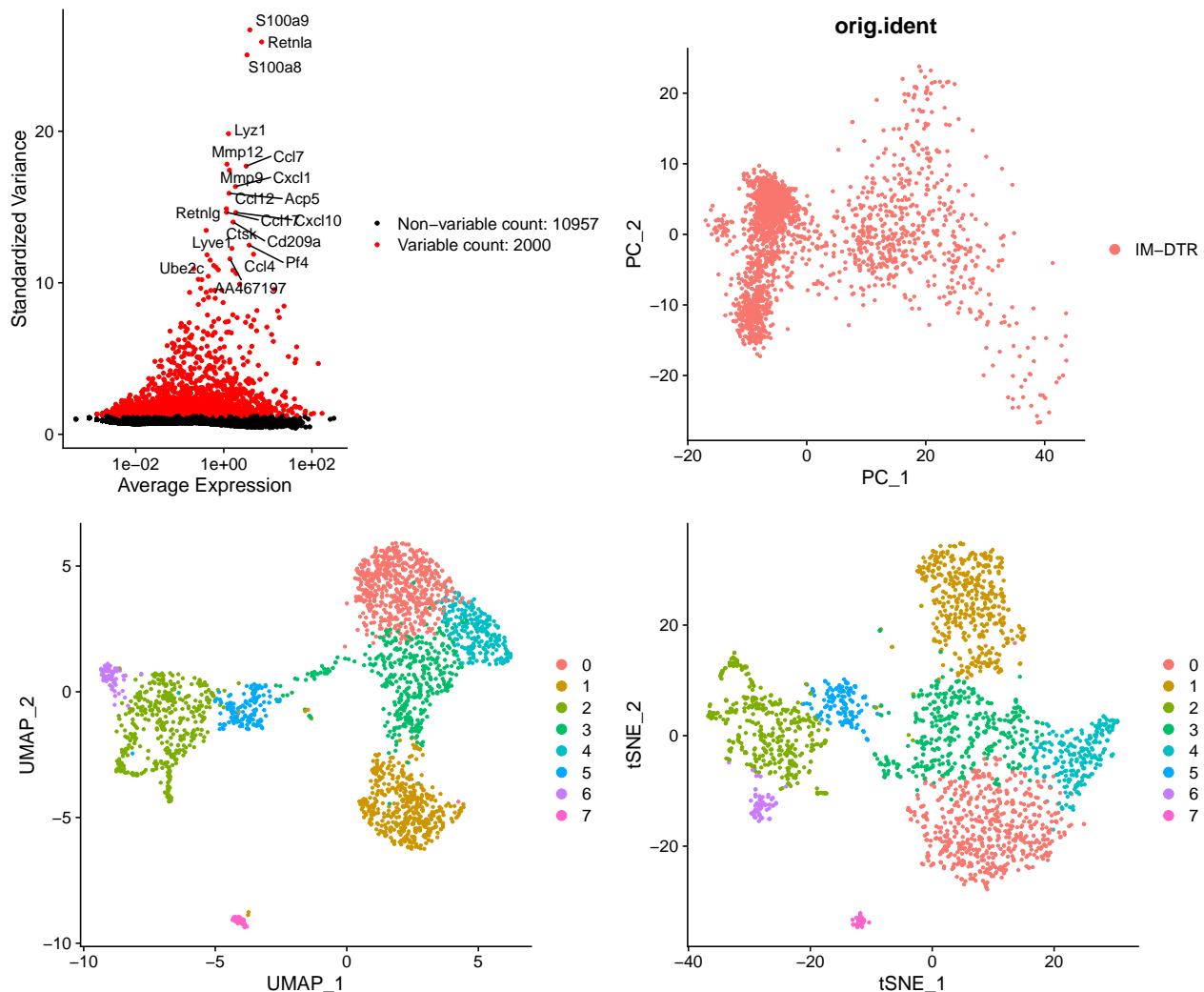
1  
2  
3  
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8  
9

```
ggarrange(CPlus_NGS20_Q147$plots$feature_vln, CPlus_NGS20_Q147$plots$RNA_
  mt.pct_scatter, CPlus_NGS20_Q147$plots$JackStrawPlot, CPlus_NGS20_Q147$plots$ElbowPlot,
  ncol = 2, nrow = 2)
```

1  
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7  
8  
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```
ggarrange(CPlus_NGS20_Q147$plots$variable_features, CPlus_NGS20_Q147$plots $PCA_plot, CPlus_NGS20_Q147$plots$UMAP_plot, CPlus_NGS20_Q147$plots$ TSNE_plot, ncol = 2, nrow = 2) 1
```

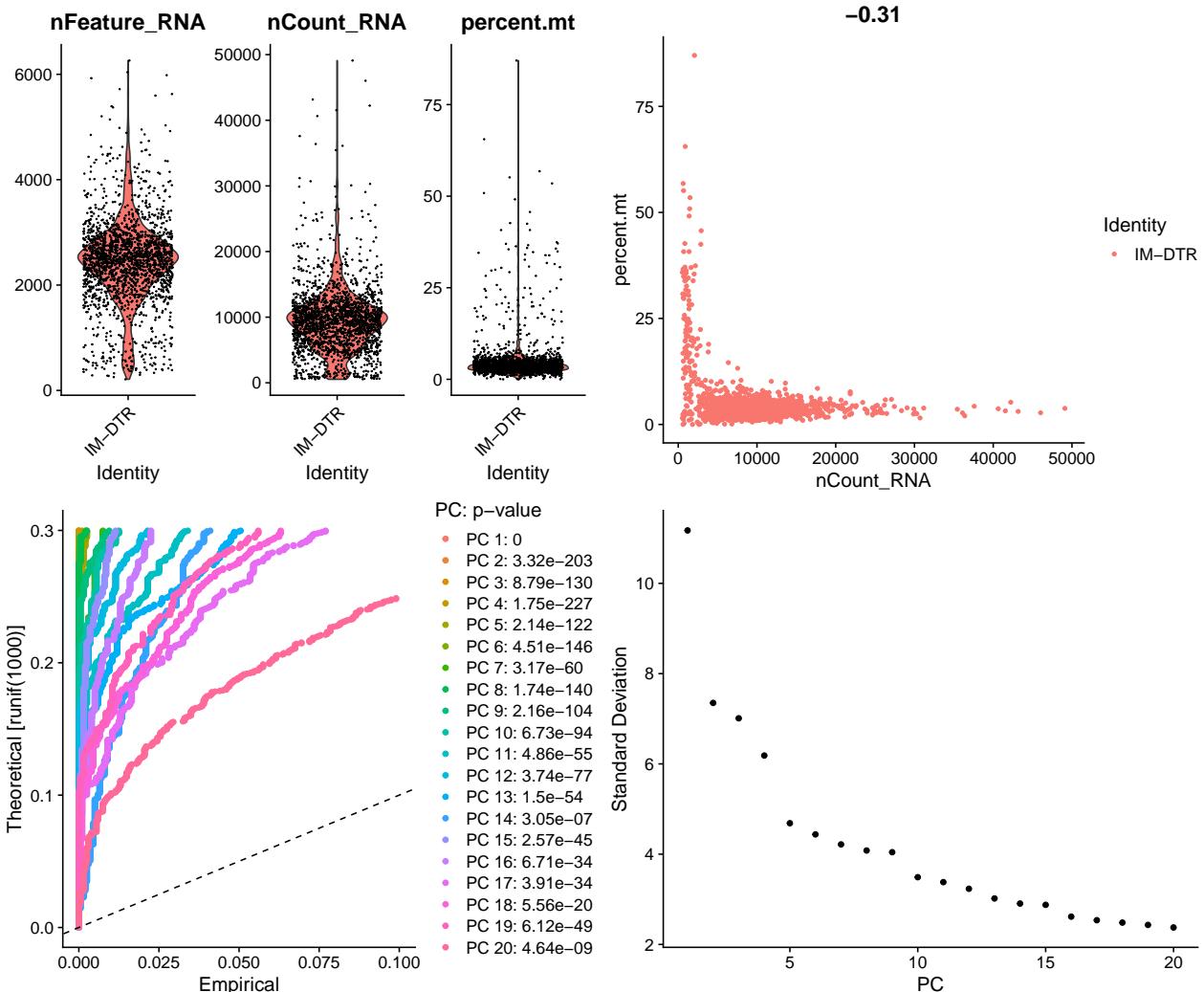


### 3.2 Sample: Plusplus\_NGS20\_Q148

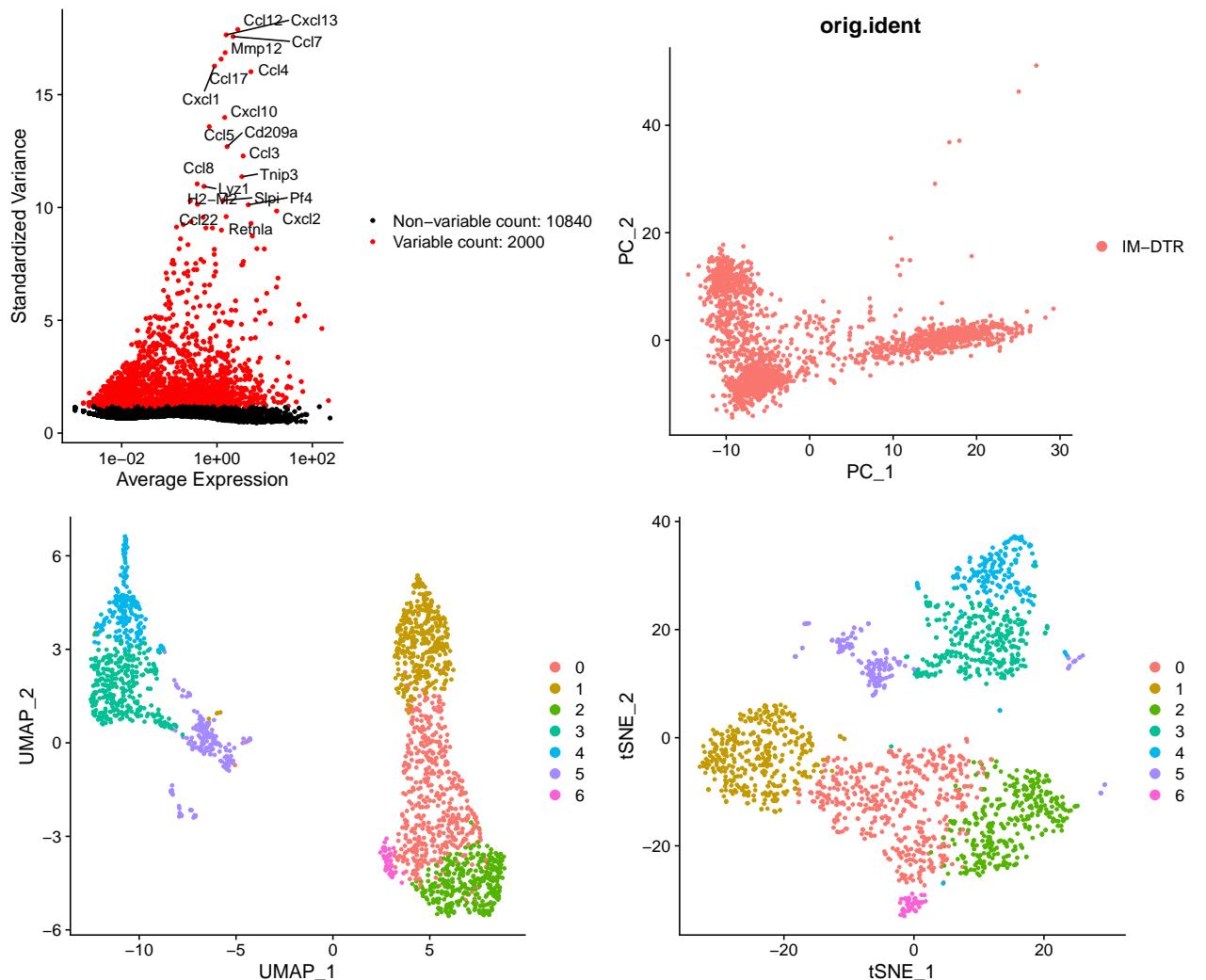
```
Plusplus_NGS20_Q148 <- seurat.setup(file.path(dir.10x, "Plusplus_NGS20_Q148/outs/filtered_feature_bc_matrix/"), project = "IM-DTR",
dimensionality = 1:20, mt.percentage = 10, human = FALSE)
```

```
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
## Number of nodes: 1900
## Number of edges: 66575
## Running Louvain algorithm...
## Maximum modularity in 10 random starts: 0.8576
## Number of communities: 7
## Elapsed time: 0 seconds
```

```
ggarrange(Plusplus_NGS20_Q148$plots$feature_vln, Plusplus_NGS20_Q148$plots$RNA_mt.pct_scatter, Plusplus_NGS20_Q148$plots$JackStrawPlot, Plusplus_NGS20_Q148$plots$ElbowPlot, ncol = 2, nrow = 2)
```



```
ggarrange(Plusplus_NGS20_Q148$plots$variable_features, Plusplus_NGS20_Q148
$plots$PCA_plot, Plusplus_NGS20_Q148$plots$UMAP_plot, Plusplus_NGS20_
Q148$plots$TSNE_plot, ncol = 2, nrow = 2)
```



## 4 Save Seurat objects for further analyses

```
# save data for next use:
saveRDS(CPlus_NGS20_Q147$seuratObject, file = "./CPlus_NGS20_Q147.
    seuratObject.rds")
saveRDS(Plusplus_NGS20_Q148$seuratObject, file = "./Plusplus_NGS20_Q148.
    seuratObject.rds")
```

## 5 Session information

sessionInfo()	1
## 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	1 2 3 4 5 6

```

## LAPACK: /usr/lib/x86_64-linux-gnu/openblas-pthread/liblapack.so.3    7
##
## locale:                                         8
##   [1] LC_CTYPE=en_US.UTF-8           LC_NUMERIC=C          9
##   [3] LC_TIME=en_GB.UTF-8          LC_COLLATE=en_US.UTF-8 10
##   [5] LC_MONETARY=en_GB.UTF-8      LC_MESSAGES=en_US.UTF-8 11
##   [7] LC_PAPER=en_GB.UTF-8         LC_NAME=C            12
##   [9] LC_ADDRESS=C                 LC_TELEPHONE=C       13
##  [11] LC_MEASUREMENT=en_GB.UTF-8  LC_IDENTIFICATION=C 14
##
## attached base packages:                      15
## [1] stats      graphics   grDevices  utils      datasets   methods   base 16
##
## other attached packages:                     17
## [1] dplyr_1.0.7      ggpubr_0.4.0      ggplot2_3.3.5 18
##     SeuratObject_4.0.4
## [5] Seurat_4.0.5
##
## loaded via a namespace (and not attached): 19
##   [1] Rtsne_0.15          colorspace_2.0-2    ggsignif_0.6.3 20
##   [4] deldir_1.0-6        ellipsis_0.3.2     ggridges_0.5.3 21
##   [7] spatstat.data_2.1-0 farver_2.1.0      leiden_0.3.9   22
##  [10] listenv_0.8.0       ggrepel_0.9.1     RSpectra_0.16-0 23
##  [13] fansi_0.5.0        codetools_0.2-18   splines_4.0.3   24
##  [16] knitr_1.36         polyclip_1.10-0   jsonlite_1.7.2 25
##  [19] broom_0.7.10      ica_1.0-2        cluster_2.1.0 26
##  [22] png_0.1-7         uwot_0.1.11      shiny_1.7.1    27
##  [25] sctransform_0.3.2  spatstat.sparse_2.0-0 compiler_4.0.3 28
##  [28] httr_1.4.2         backports_1.4.0    assertthat_0.2.1 29
##  [31] Matrix_1.3-4       fastmap_1.1.0     lazyeval_0.2.2 30
##  [34] later_1.3.0        hmltools_0.5.2    tools_4.0.3    31
##  [37] igraph_1.2.9       gtable_0.3.0      glue_1.5.1    32
##  [40] RANN_2.6.1         reshape2_1.4.4    Rcpp_1.0.7    33
##  [43] carData_3.0-4      scattermore_0.7   vctrs_0.3.8   34
##  [46] nlme_3.1-153       lmtest_0.9-39    xfun_0.28    35
##  [49] stringr_1.4.0      globals_0.14.0   mime_0.12    36
##  [52] miniUI_0.1.1.1    lifecycle_1.0.1   irlba_2.3.5   37
##  [55] rstatix_0.7.0      goftest_1.2-3    future_1.23.0 38
##  [58] MASS_7.3-53        zoo_1.8-9       scales_1.1.1  39
##  [61] spatstat.core_2.3-2 promises_1.2.0.1 spatstat.utils_2.2-0 40
##  [64] parallel_4.0.3      RColorBrewer_1.1-2 yaml_2.2.1    41
##  [67] reticulate_1.22    pbapply_1.5-0     gridExtra_2.3 42
##  [70] rpart_4.1-15       stringi_1.7.6    highr_0.9    43
##  [73] rlang_0.4.12       pkgconfig_2.0.3   matrixStats_0.61.0 44
##  [76] evaluate_0.14      lattice_0.20-41  ROCR_1.0-11   45
##  [79] purrr_0.3.4        tensor_1.5       labeling_0.4.2 46
##  [82] patchwork_1.1.1    htmlwidgets_1.5.4 cowplot_1.1.1 47
##  [85] tidyselect_1.1.1   parallely_1.29.0 RcppAnnoy_0.0.19 48
##  [88] plyr_1.8.6         magrittr_2.0.1    R6_2.5.1    49
##  [91] generics_0.1.1     DBI_1.1.1       pillar_1.6.4  50
##  [94] withr_2.4.3       mgcv_1.8-33     fitdistrplus_1.1-6 51
##  [97] survival_3.2-7    abind_1.4-5     tibble_3.1.6  52
## [100] future.apply_1.8.1 crayon_1.4.2    car_3.0-12   53
## [103] KernSmooth_2.23-20 utf8_1.2.2     spatstat.geom_2.3-0 54

```

## [106] plotly_4.10.0	rmarkdown_2.11	grid_4.0.3	60
## [109] data.table_1.14.2	digest_0.6.29	xtable_1.8-4	61
## [112] tidyverse_1.1.4	httpuv_1.6.3	msnseal_0.5.0	62
## [115] viridisLite_0.4.0			63

## References

Hao, Y., Hao, S., Andersen-Nissen, E., III, W.M.M., Zheng, S., Butler, A., Lee, M.J., Wilk, A.J., Darby, C., Zagar, M., et al. (2021). Integrated analysis of multimodal single-cell data. *Cell*.