

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

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

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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

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 regulate 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.

The IMs in Day 4 post-depletion were compared to those without depletion. Results showed that refilled IMs had a lower ratio of CD206+ IM vs CD206- subpopulation comparing to IMs without depletion, but they shared high similarity to each other, indicating that the de novo IM population had been established before Day 4 post-depletion.

## 2 Load packages and data

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

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## 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)
```

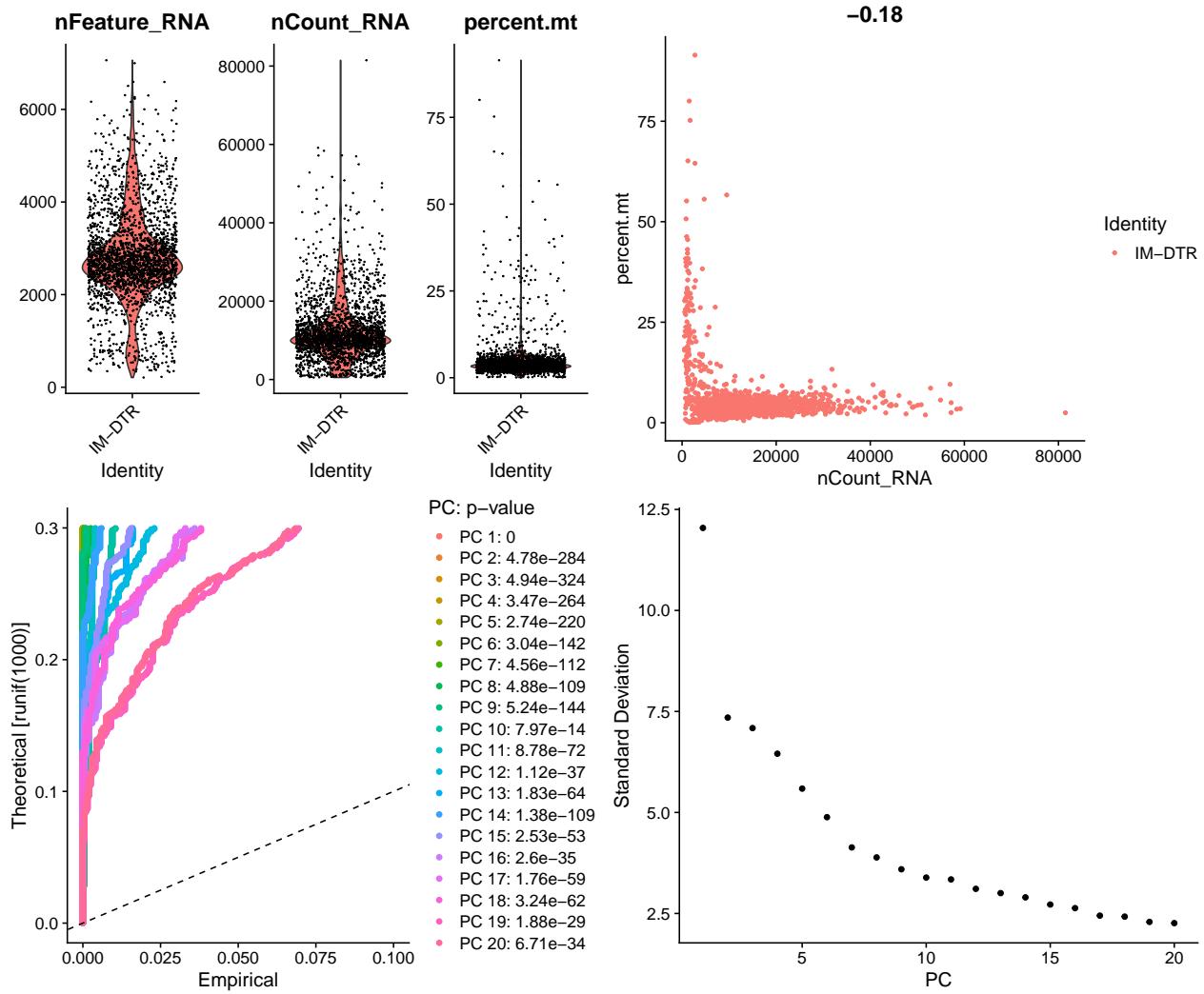
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```
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
## Number of nodes: 2269
## Number of edges: 79406
##
## Running Louvain algorithm...
## Maximum modularity in 10 random starts: 0.8569
## Number of communities: 8
## Elapsed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

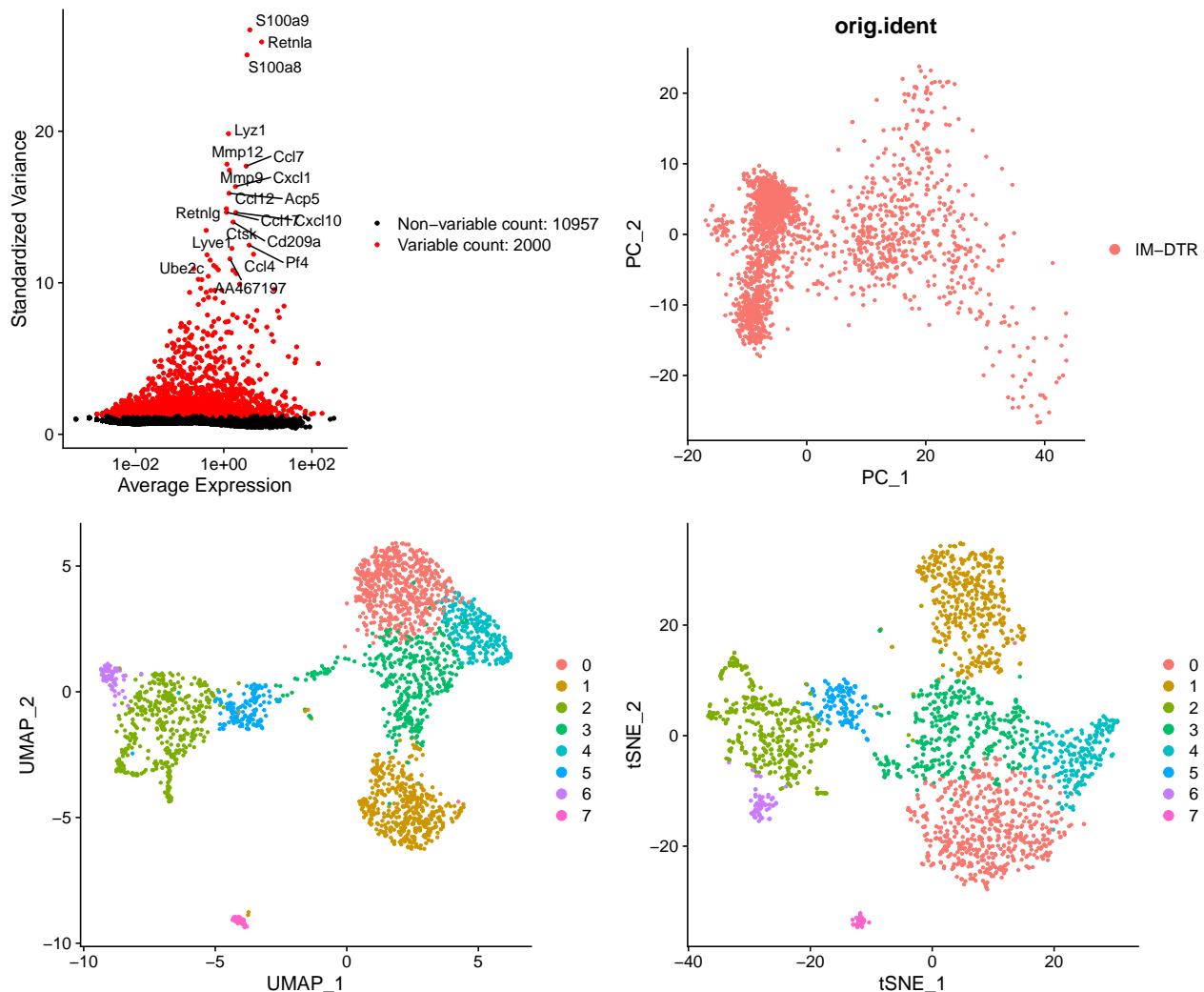
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```
ggarrange(CPlus_NGS20_Q147$plots$feature_vln, CPlus_NGS20_Q147$plots$RNA_
mt.pct_scatter, CPlus_NGS20_Q147$plots$JackStrawPlot, CPlus_NGS20_Q147$plots$ElbowPlot, ncol = 2, nrow = 2)
```

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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
```

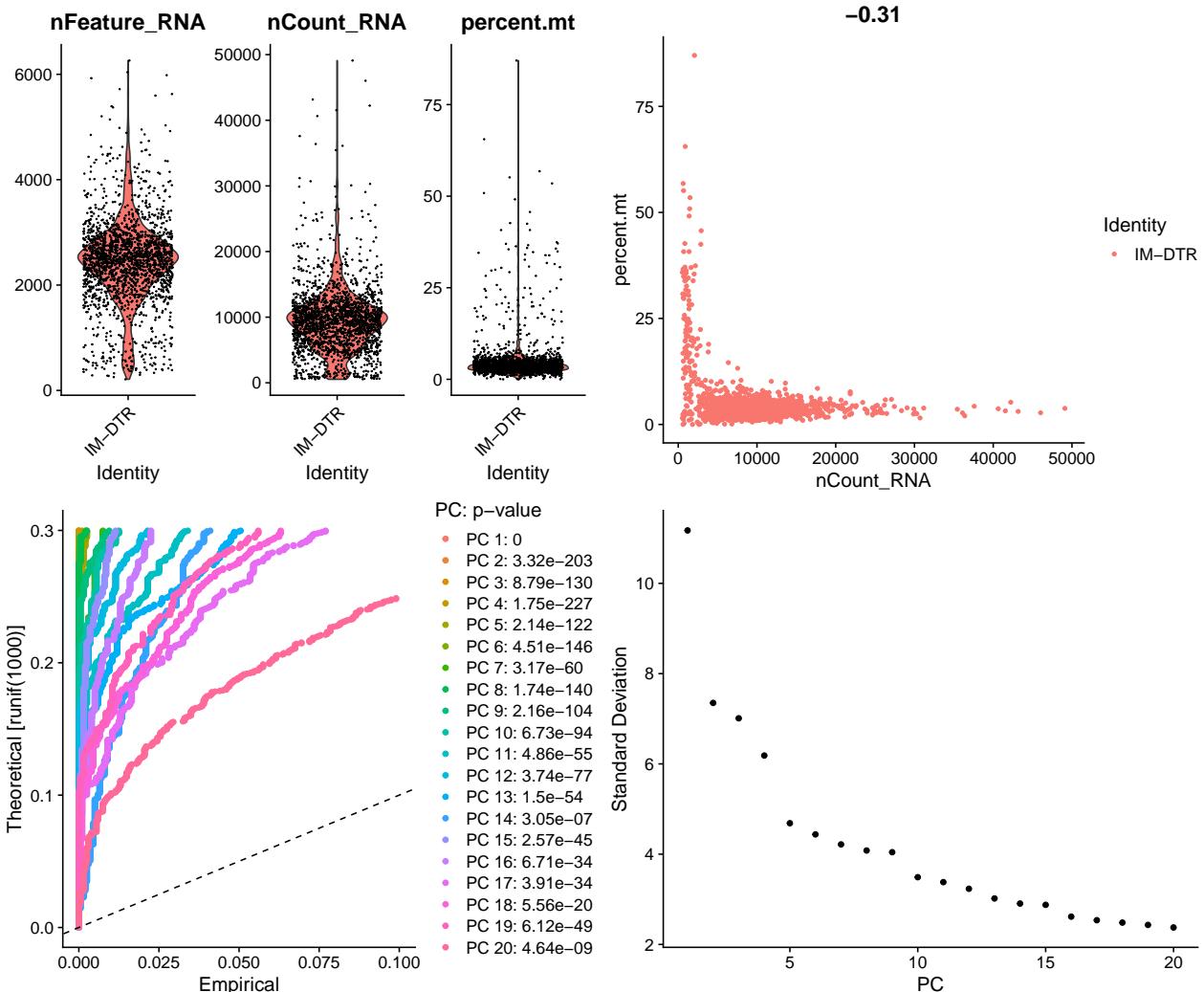


### 3.2 Sample: Plusplus\_NGS20\_Q148

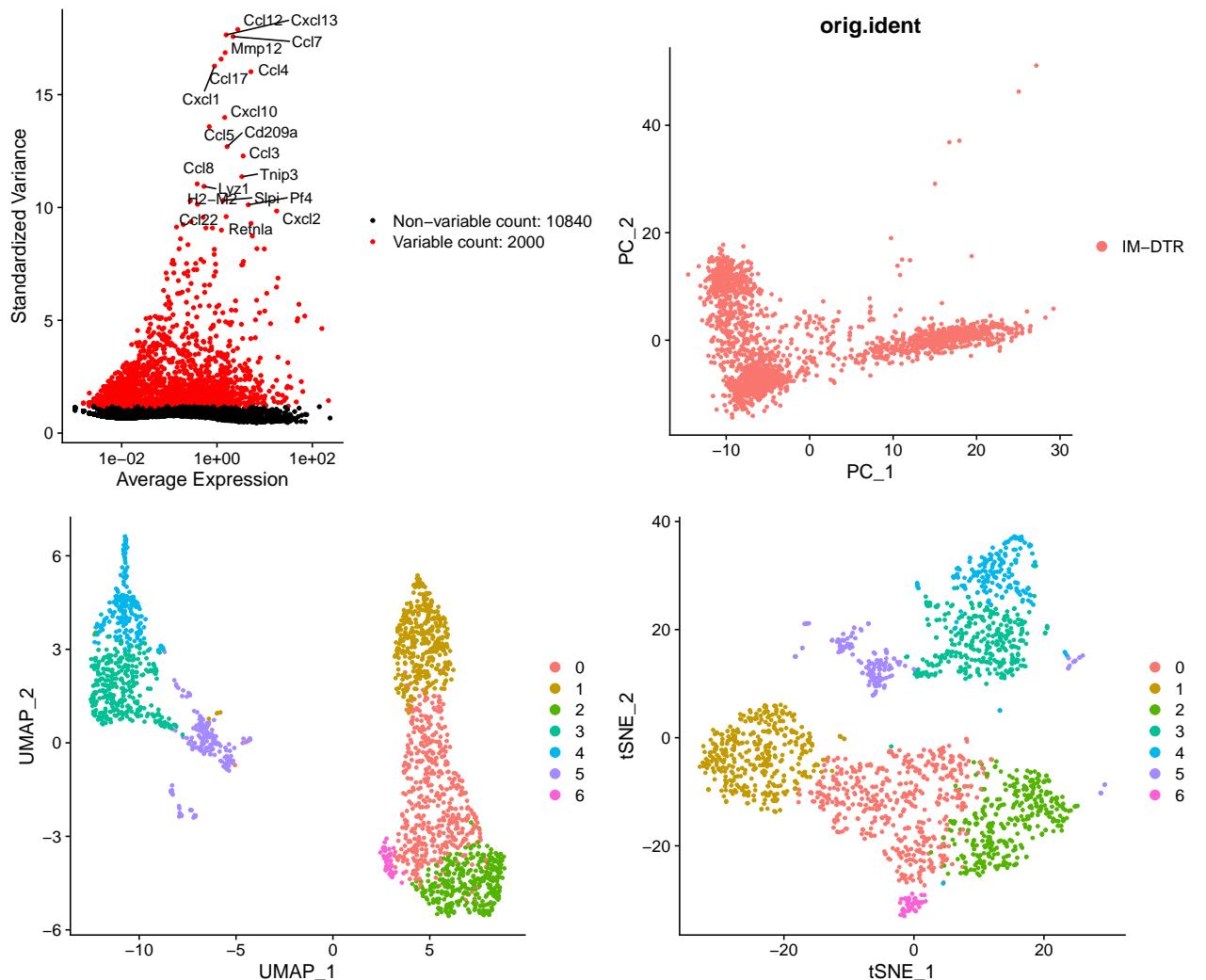
```
Plusplus_NGS20_Q148 <- seurat.setup(file.path(dir.10x, "Plusplus_NGS20_Q148/outs/filtered_feature_bc_matrix/"), project = "IM-DTR",
dimensionality = 1:20, mt.percentage = 10, human = FALSE)
```

```
## 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_4.0.2
## [5] Seurat_4.0.3
##
## loaded via a namespace (and not attached): 19
##   [1] Rtsne_0.15          colorspace_2.0-2    ggsignif_0.6.2 20
##   [4] deldir_0.2-10       rio_0.5.27        ellipsis_0.3.2 21
##   [7] ggridges_0.5.3      spatstat.data_2.1-0 farver_2.1.0   22
##  [10] leiden_0.3.9        listenv_0.8.0      ggrepel_0.9.1   23
##  [13] RSpectra_0.16-0     fansi_0.5.0       codetools_0.2-18 24
##  [16] splines_4.0.3        knitr_1.33       polyclip_1.10-0 25
##  [19] jsonlite_1.7.2      broom_0.7.9       ica_1.0-2       26
##  [22] cluster_2.1.0       png_0.1-7        uwot_0.1.10.9000 27
##  [25] shiny_1.6.0          sctransform_0.3.2  spatstat.sparse_2.0-0 28
##  [28] compiler_4.0.3       httr_1.4.2       backports_1.2.1 29
##  [31] assertthat_0.2.1     Matrix_1.3-4      fastmap_1.1.0   30
##  [34] lazyeval_0.2.2       later_1.2.0      htmltools_0.5.1.1 31
##  [37] tools_4.0.3          igraph_1.2.6      gtable_0.3.0    32
##  [40] glue_1.4.2           RANN_2.6.1       reshape2_1.4.4   33
##  [43] Rcpp_1.0.7           carData_3.0-4     scattermore_0.7 34
##  [46] cellranger_1.1.0     vctrs_0.3.8       nlme_3.1-152   35
##  [49] lmtest_0.9-38        xfun_0.24        stringr_1.4.0   36
##  [52] globals_0.14.0        openxlsx_4.2.4    future_1.21.0   37
##  [55] miniUI_0.1.1.1      lifecycle_1.0.0   scales_1.1.1   38
##  [58] rstatix_0.7.0        goftest_1.2-2    promises_1.2.0.1 39
##  [61] MASS_7.3-53          zoo_1.8-9       promises_1.2.0.1 40
##  [64] spatstat.core_2.3-0   hms_1.1.0        RColorBrewer_1.1-2 41
##  [67] spatstat.utils_2.2-0  parallel_4.0.3   reticulate_1.20 42
##  [70] curl_4.3.2           yaml_2.2.1       rpart_4.1-15   43
##  [73] pbapply_1.4-3        gridExtra_2.3    zip_2.2.0      44
##  [76] stringi_1.7.3       highr_0.9        rpart_4.1-15   45
##  [79] rlang_0.4.11         pkgconfig_2.0.3  labeling_0.4.2   46
##  [82] evaluate_0.14        lattice_0.20-41 matrixStats_0.60.0 47
##  [85] purrr_0.3.4          tensor_1.5       cowplot_1.1.1   48
##  [88] patchwork_1.1.1      htmlwidgets_1.5.3 RcppAnnoy_0.0.19 49
##  [91] tidyselect_1.1.1     parallely_1.27.0 haven_2.4.3    50
##  [94] plyr_1.8.6           magrittr_2.0.1    R6_2.5.0       51
##  [97] generics_0.1.0       DBI_1.1.1       withr_2.4.2    52
## [100] foreign_0.8-81      pillar_1.6.2     survival_3.2-7 53
## [103] mgcv_1.8-33          fitdistrplus_1.1-5

```

## [106] abind_1.4-5	tibble_3.1.3	future.apply_1.7.0	60
## [109] crayon_1.4.1	car_3.0-11	KernSmooth_2.23-20	61
## [112] utf8_1.2.2	spatstat.geom_2.2-2	plotly_4.9.4.1	62
## [115] rmarkdown_2.9	readxl_1.3.1	grid_4.0.3	63
## [118] data.table_1.14.0	forcats_0.5.1	digest_0.6.27	64
## [121] xtable_1.8-4	tidyverse_1.1.3	httpuv_1.6.1	65
## [124] munsell_0.5.0	viridisLite_0.4.0		66

## 6 References