

A lung Tgf-beta-signaling-mediated endothelial-interstitial macrophage axis prevents age-related abnormalities

4-Data preparation for single-cell RNA sequencing analysis

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Abstract

Lung interstitial macrophages (IMs) are monocyte-derived parenchymal macrophages whose homeostatic and tissue-supportive functions remain unclear. While recent progress has been made about the diversity and transcriptional regulation of lung IMs, the microenvironmental signals responsible for their development from monocytes and for their functional specification remain unidentified. Here we found, in mice, that lung endothelial cell-derived Tgf-beta1 specifically triggered a core Tgf-beta receptor-dependent IM signature in bone marrow-derived monocytes and macrophages (Macs). In vivo, myeloid-specific ablation of Tgf-beta receptor signaling severely impaired monocyte-to-IM development, resulting in the accumulation of perivascular monocytes, decreased IM numbers and a loss of IM-intrinsic identity. Of note, monocyte-to-IM development was similarly impaired in the absence of endothelial-specific Tgf-beta1. Functionally, lungs from mice selectively lacking Tgf-beta receptor in IMs exhibited spatial changes in monocyte and IM niche occupancies, a severe disruption in their immunoregulatory environment, and prematurely developed fibrosis, hyperinflation, increased compliance and decreased elastance, changes classically associated with aging. Our work identifies a novel endothelial-IM axis involving Tgf-beta1 - Tgf-beta receptor interactions that shapes IM development and identity and thereby sustains lung tissue integrity, thus providing foundations for IM-targeted interventions in the context of lung aging and other chronic inflammatory disorders.

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1 Description

Lung CD45.1-CD45.2+Ly6G-SiglecF-CD11b+SSClloCD64+ cells were FACS-sorted from lung single-cell suspensions pooled from 5 chimeric IMDTR mice in each of groups as the same in bulk RNA sequencing analysis. For each sample, an aliquot of Trypan blue-treated cells was examined under the microscope for counting, viability and aggregate assessment following FACS sorting. Viability was above 90% for all samples and no aggregates were observed. Cells from each group were then labelled with BioLegend TotalSeq anti-mouse hashtags (TotalSeq-B0301 for control group and TotalSeq-B0303 for Tgfb2-KO group) before being pooled. Pooled cells were centrifuged and pellet was resuspended in calcium- and magnesium-free PBS containing 0.4 mg ml⁻¹ UltraPure BSA (Thermo Fisher Scientific). For library preparation, approximately 10 000 cells were loaded into the Chromium Controller, in which they were partitioned, their polyA RNAs captured and barcoded using Chromium Single Cell 3' GEM, Library & Gel Bead Kit v3 (10X Genomics). The cDNAs were amplified and libraries compatible with Illumina sequencers were generated using Chromium Single Cell 3' GEM, Library & Gel Bead Kit v3 (10X Genomics). For Hash Tag Oligonucleotide (HTO) library, HTO additive primer v2 were added to the mix at the cDNA amplification step. The libraries were sequenced on an Illumina NovaSeq sequencer on an SP100 cell flow (read 1, 28 cy; read 2, 76 cy; index 1, 10 cy; index 2, 10 cy) at a depth of 50,000 reads per cell.

The Cell Ranger (v8.0) application (10x Genomics) was then used to demultiplex the BCL files into FASTQ files (cellranger mkfastq), to perform alignment (to Cell Ranger human genome references 3.0.2 GRCm38/build 97), filtering, UMI counting and to produce gene-barcode matrices for each sample (cellranger multi).

The counts data are analysed in this step using **Seurat** package (1).

2 Demultiplexing (de-hashtag) of raw data

The fastq data were demultiplexed and de-hashtagged with **cellranger multi** command in **cellranger** (v8.0).

3 Load data and Seurat package

The counts data were generated for two samples: *Tgfb2-KO* (termed **KO** in the following codes) and *control* (**WT** in the following codes).

```
library(Seurat)
library(ggpubr)
source("../R/seurat.setup.R") # a wrapped function to make Seurat
    object and show QC results.
project = "TGFb_IM"
```

3.1 Sample WT:

```
cd45pos_WT <- seurat.setup(path.10x = "../counts/counts_CD45pos/outs/
per_sample_outs/CD45pos_WT/count/sample_filtered_feature_bc_matrix/",
project = project, dimensionality = 1:20, mt.percentage = 10, human =
FALSE)
```

```

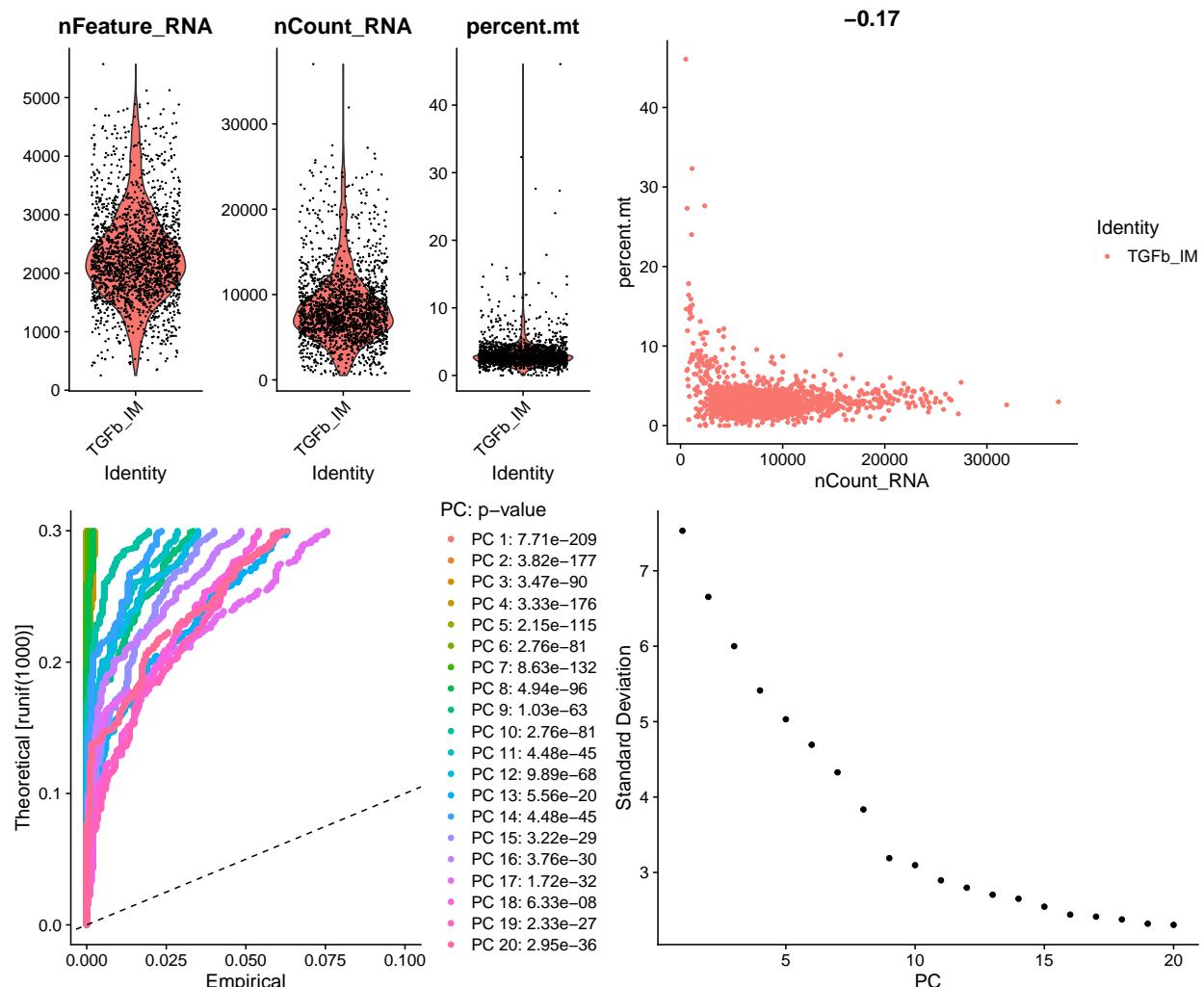
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck 1
## 2
## Number of nodes: 1903 3
## Number of edges: 64730 4
## 5
## Running Louvain algorithm... 6
## Maximum modularity in 10 random starts: 0.8109 7
## Number of communities: 8 8
## Elapsed time: 0 seconds 9

```

```

ggarrange(cd45pos_WT$plots$feature_vln, cd45pos_WT$plots$RNA_mt.pct_
scatter, cd45pos_WT$plots$JackStrawPlot,
cd45pos_WT$plots$ElbowPlot, ncol = 2, nrow = 2) 1
2

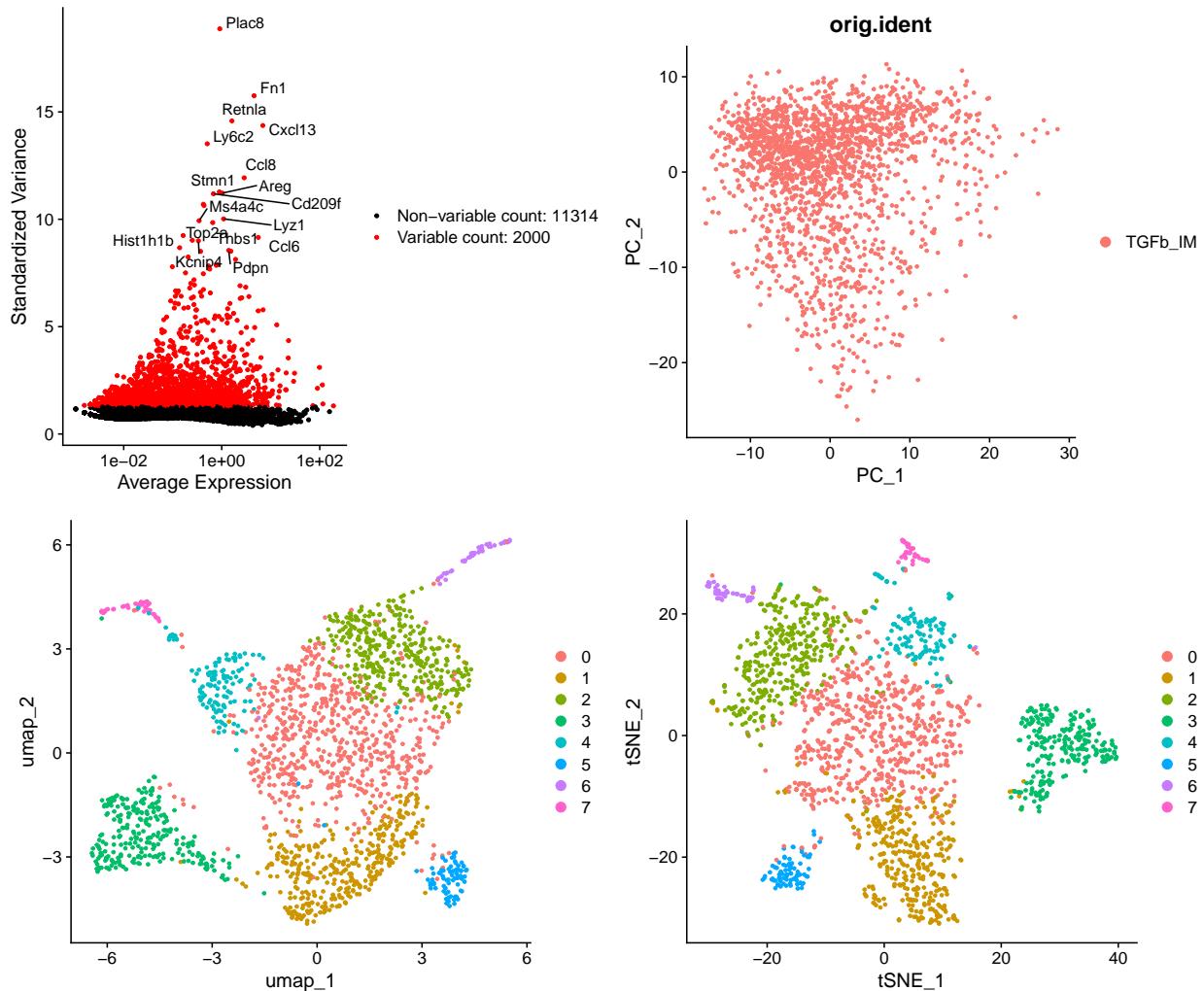
```



```

ggarrange(cd45pos_WT$plots$variable_features, cd45pos_WT$plots$PCA_plot,
cd45pos_WT$plots$UMAP_plot,
cd45pos_WT$plots$TSNE_plot, ncol = 2, nrow = 2) 1
2

```



3.2 Sample KO

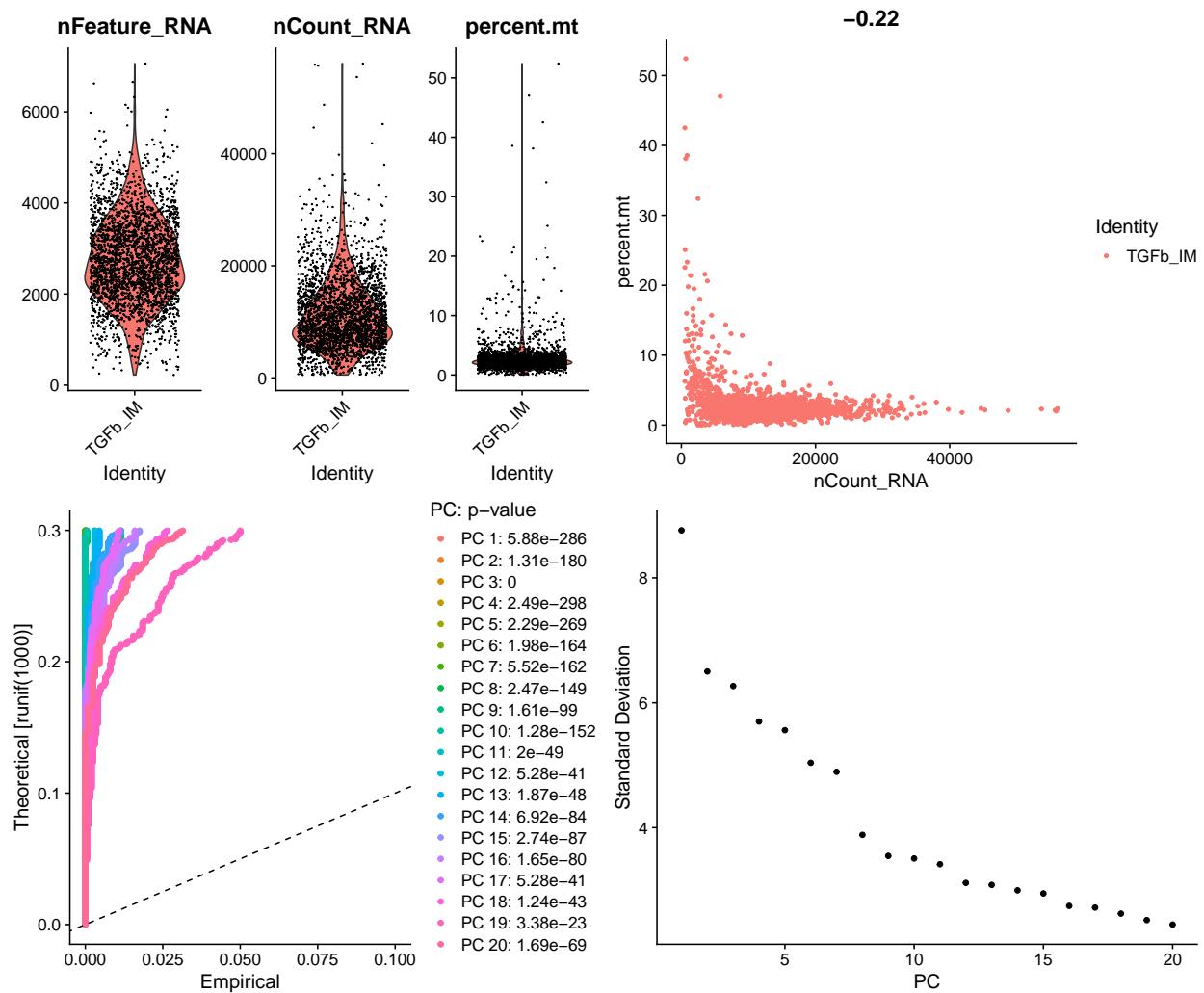
```
cd45pos_KO <- seurat.setup(path.10x = "../../counts/counts_CD45pos/out/
per_sample_outs/CD45pos_KO/count/sample_filtered_feature_bc_matrix/",
project = project, dimensionality = 1:20, mt.percentage = 10, human =
FALSE)
```

```
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
## 
## Number of nodes: 2686
## Number of edges: 89611
## 
## Running Louvain algorithm...
## Maximum modularity in 10 random starts: 0.8191
## Number of communities: 9
## Elapsed time: 0 seconds
```

```
ggarrange(cd45pos_KO$plots$feature_vln, cd45pos_KO$plots$RNA_mt.pct_
scatter, cd45pos_KO$plots$JackStrawPlot,
```

```
cd45pos_KO$plots$ElbowPlot , ncol = 2 , nrow = 2)
```

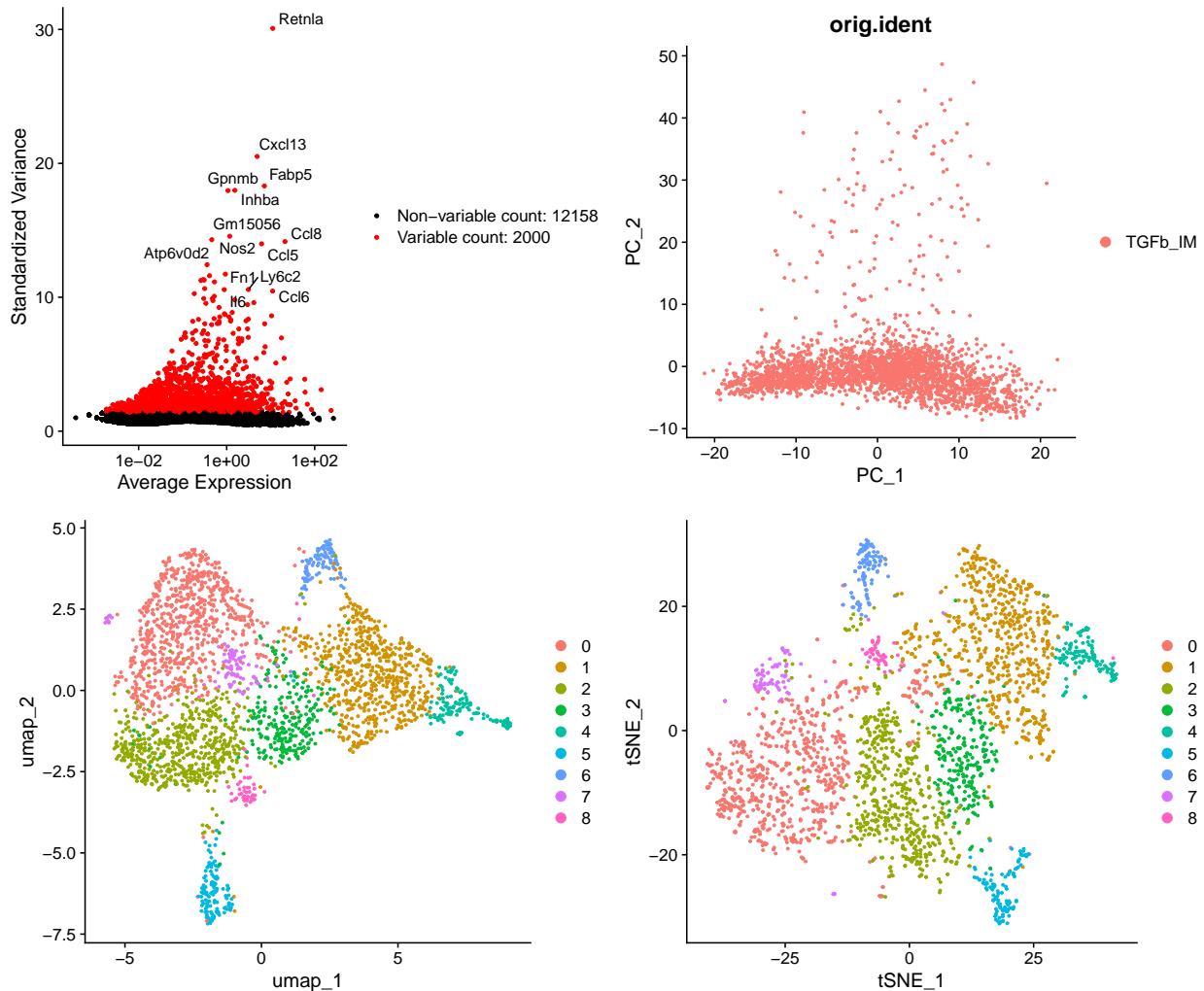
2



```
ggarrange(cd45pos_KO$plots$variable_features , cd45pos_KO$plots$PCA_plot ,  
cd45pos_KO$plots$UMAP_plot ,  
cd45pos_KO$plots$TSNE_plot , ncol = 2 , nrow = 2)
```

1

2



4 Save Seurat objects for further analyses

```
# save data for next use:
saveRDS(cd45pos_WT$seuratObject, file = "./cd45pos_WT.seuratObject.rds") 1
saveRDS(cd45pos_KO$seuratObject, file = "./cd45pos_KO.seuratObject.rds") 2
3
```

5 Session information

sessionInfo()	1
## R version 4.3.3 (2024-02-29) ## Platform: aarch64-apple-darwin20 (64-bit) ## Running under: macOS 15.1.1 ## ## Matrix products: default	1 2 3 4 5

```

## BLAS: /Library/Frameworks/R.framework/Versions/4.3-arm64/Resources/
## lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.3-arm64/Resources/
## lib/libRlapack.dylib; LAPACK version 3.11.0
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
## 
## time zone: Europe/Paris
## tzcode source: internal
##
## attached base packages:
## [1] stats      graphics   grDevices utils      datasets   methods    base
## 
## other attached packages:
## [1] dplyr_1.1.4       ggpubr_0.6.0       ggplot2_3.5.1     Seurat_5
## .1.0
## [5] SeuratObject_5.0.2 sp_2.1-4
## 
## loaded via a namespace (and not attached):
## [1] RColorBrewer_1.1-3      rstudioapi_0.17.1    jsonlite_1.8.9
## [4] magrittr_2.0.3          spatstat.utils_3.1-1 farver_2.1.2
## [7] rmarkdown_2.29           vctrs_0.6.5          ROCR_1.0-11
## [10] spatstat.explore_3.3-3 rstatix_0.7.2        htmltools_0.5.8.1
## [13] broom_1.0.7            Formula_1.2-5       sctransform_0.4.1
## [16] parallelly_1.40.1      KernSmooth_2.23-24  htmlwidgets_1.6.4
## [19] ica_1.0-3              plyr_1.8.9           plotly_4.10.4
## [22] zoo_1.8-12             igraph_2.1.2         mime_0.12
## [25] lifecycle_1.0.4        pkgconfig_2.0.3      Matrix_1.6-5
## [28] R6_2.5.1               fastmap_1.2.0       fitdistrplus_1.2-1
## [31] future_1.34.0          shiny_1.9.1          digest_0.6.37
## [34] colorspace_2.1-1       patchwork_1.3.0     tensor_1.5
## [37] RSpecSpectra_0.16-2    irlba_2.3.5.1       labeling_0.4.3
## [40] progressr_0.15.1      fansi_1.0.6          spatstat.sparse_3
## .1-0
## [43] httr_1.4.7             polyclip_1.10-7     abind_1.4-8
## [46] compiler_4.3.3         withr_3.0.2          backports_1.5.0
## [49] carData_3.0-5          fastDummies_1.7.4   R.utils_2.12.3
## [52] ggsignif_0.6.4         MASS_7.3-60.0.1     tools_4.3.3
## [55] lmtest_0.9-40          httpuv_1.6.15       future.apply_1.11.3
## [58] goftest_1.2-3          R.oo_1.27.0          glue_1.8.0
## [61] nlme_3.1-166           promises_1.3.2     grid_4.3.3
## [64] Rtsne_0.17              cluster_2.1.7       reshape2_1.4.4
## [67] generics_0.1.3          gtable_0.3.6         spatstat.data_3.1-4
## [70] R.methodsS3_1.8.2      tidyR_1.3.1          data.table_1.16.4
## [73] car_3.1-3              utf8_1.2.4           spatstat.geom_3.3-4
## [76] RcppAnnoy_0.0.22       ggrepel_0.9.6        RANN_2.6.2
## [79] pillar_1.9.0            stringr_1.5.1       spam_2.11-0
## [82] RcppHNSW_0.6.0          later_1.4.1          splines_4.3.3
## [85] lattice_0.22-6          survival_3.7-0      deldir_2.0-4
## [88] tidyselect_1.2.1         miniUI_0.1.1.1      pbapply_1.7-2
## [91] knitr_1.49              gridExtra_2.3         scattermore_1.2
## [94] xfun_0.49               matrixStats_1.4.1    stringi_1.8.4
## [97] lazyeval_0.2.2          yaml_2.3.10          evaluate_1.0.1

```

## [100] codetools_0.2-20	tibble_3.2.1	cli_3.6.3	56
## [103] uwot_0.1.16	xtable_1.8-4	reticulate_1.40.0	57
## [106] munsell_0.5.1	Rcpp_1.0.13-1	globals_0.16.3	58
## [109] spatstat.random_3.3-2 .1-1	png_0.1-8	spatstat.univar_3	59
## [112] parallel_4.3.3	dotCall64_1.2	listenv_0.9.1	60
## [115] viridisLite_0.4.2	scales_1.3.0	ggridges_0.5.6	61
## [118] leiden_0.4.3.1	purrr_1.0.2	rlang_1.1.4	62
## [121] cowplot_1.1.3	formatR_1.14		63

References

- Y. Hao, T. Stuart, M. H. Kowalski, S. Choudhary, P. Hoffman, A. Hartman, A. Srivastava, G. Molla, S. Madad, C. Fernandez-Granda, R. Satija, Dictionary learning for integrative, multimodal and scalable single-cell analysis. *Nature Biotechnology* (2023), doi:10.1038/s41587-023-01767-y.