

3-scVelo analysis

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1 Description

For each sample, the counts for unspliced- and ambiguous transcripts were calculated from CellRanger output using velocyto command-line tool (<http://velocyto.org>)[1] and saved in loom files. The single-cell RNA velocities were estimated using scVelo toolkit (<https://scvelo.readthedocs.io>)[2]. Briefly, the loom files were used as input for scVelo analysis. Genes with minimum 20 of both unspliced and spliced counts and on the top list of 2000 genes were filtered, normalized and log transformed (scv.pp.filter_and_normalize with default parameters). Thirty principal components (PCs) and 30 neighbors obtained from euclidean distances in PCA space were used for computing first-/second-order moments for each cell. We used generalized dynamical modeling to recover the full splicing kinetics of spliced genes and the single-cell RNA velocities were plotted with the same cluster labels and embedding as used in previous analyses.

2 scVelo analysis with dynamical model

For the details in the estimation of single-cell RNA velocity using dynamical model, refer to the original report[2]:

Bergen, V., Lange, M., Peidli, S., Wolf, F. A. & Theis, F. J. Generalizing RNA velocity to transient cell states through dynamical modeling. Nat. Biotechnol. (2020) doi:10.1038/s41587-020-0591-3.

The following codes were used to calculate scRNA velocity and presenting with the existing embedding and labels.

```
# python below
import scvelo as scv
scv.settings.verbosity = 3 # show errors(0), warnings(1), info(2), hints
                           (3)
scv.settings.presenter_view = True # set max width size for presenter
                                   view
scv.set_figure_params('scvelo') # for beautified visualization

# load data
ldata_basal = scv.read("./KO_Hp.loom")

# Preprocess the Data
scv.pp.filter_and_normalize(ldata_basal, min_shared_counts=20, n_top_genes
                           =2000)
scv.pp.moments(ldata_basal, n_pcs=30, n_neighbors=30)

# Estimate RNA velocity with dynamical model
scv.tl.recover_dynamics(ldata_basal)
scv.tl.velocity(ldata_basal, mode='dynamical')
scv.tl.velocity_graph(ldata_basal)
scv.pl.velocity_embedding_stream(ldata_basal, basis='umap_cell_embeddings'
                                 , color='seurat_clusters',
                                 figsize=(10,10), components='1,2',
                                 palette=["#2E359A", "#FC990E", "#720DOD"
                                         ],
                                 linewidth=1.4,
                                 title="scVelo_analysis", save="KO_Hp.png"
                                 )
```

3 Results

3.1 For “KO_Hp”

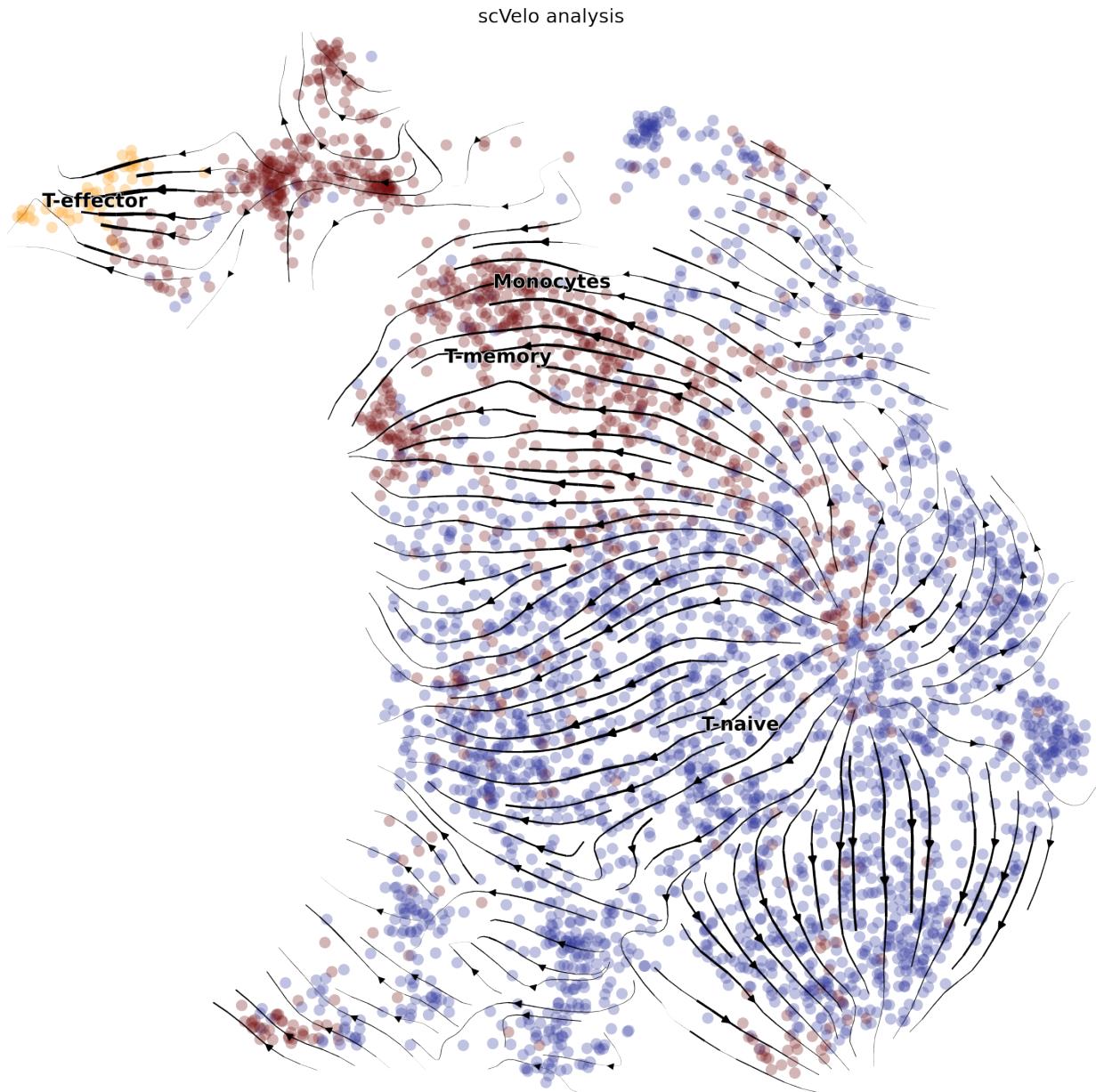


Figure 1: scVelo results for “KO_Hp” grouped sample

scVelo analysis

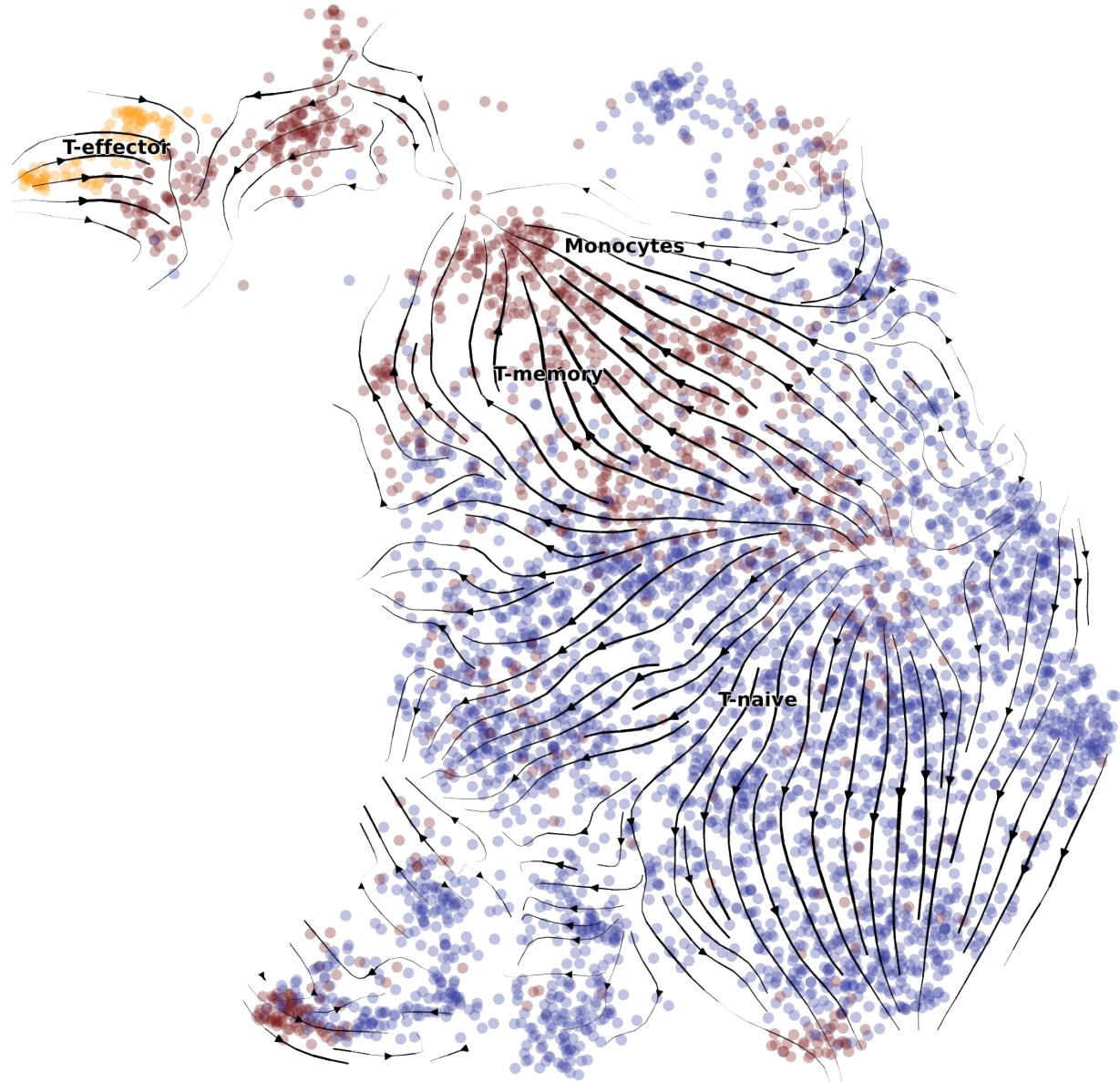


Figure 2: scVelo results for “KO_Hp” grouped sample

scVelo analysis



Figure 3: scVelo results for “KO_Hp” grouped sample

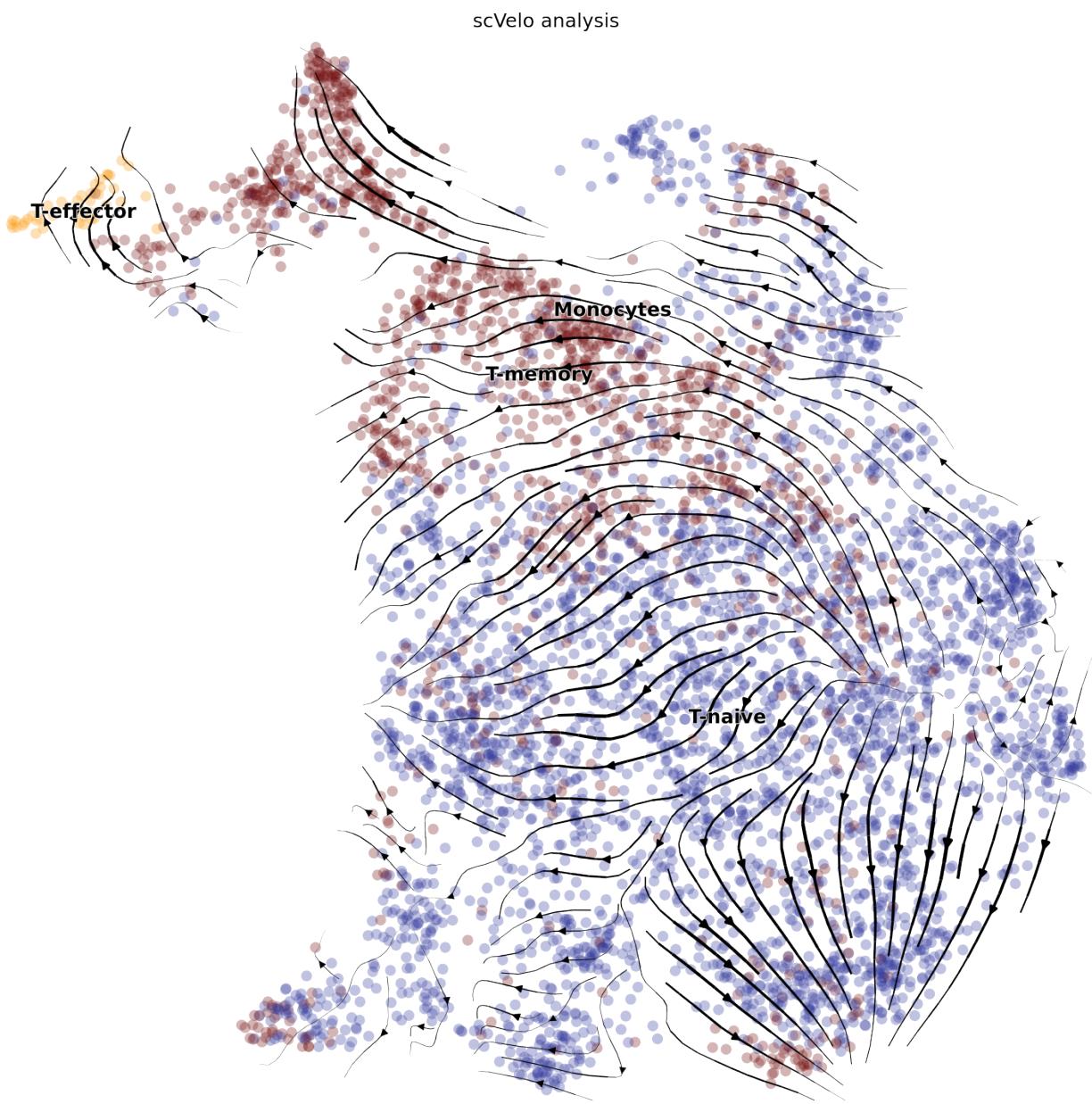


Figure 4: scVelo results for “KO_Hp” grouped sample

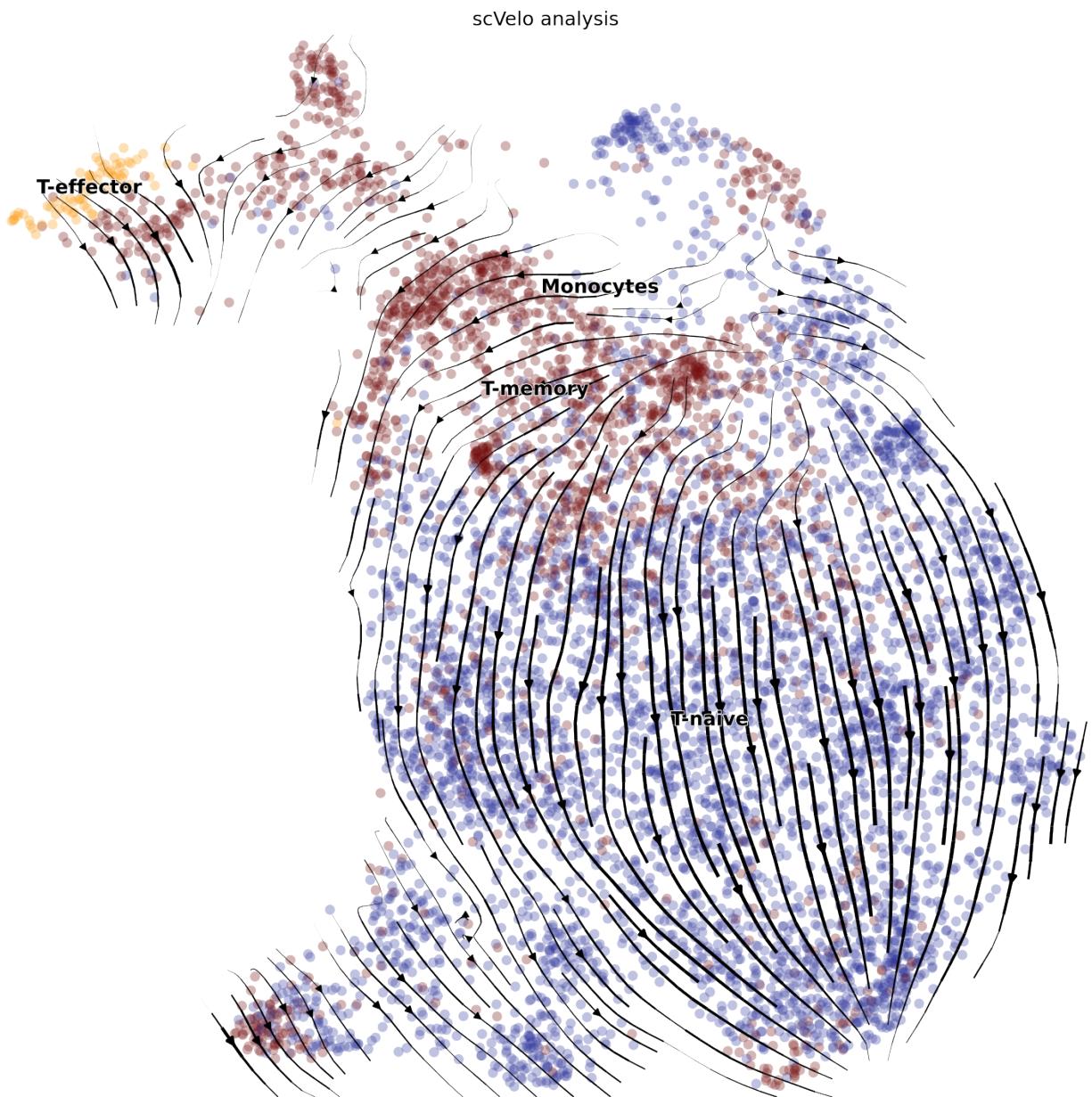


Figure 5: scVelo results for “KO_Hp” grouped sample

- 3.2 For “KO_IL4C”
- 3.3 For “KO_Naive”
- 3.4 For “WT_Hp”
- 3.5 For “WT_IL4C”
- 3.6 For “WT_Naive”

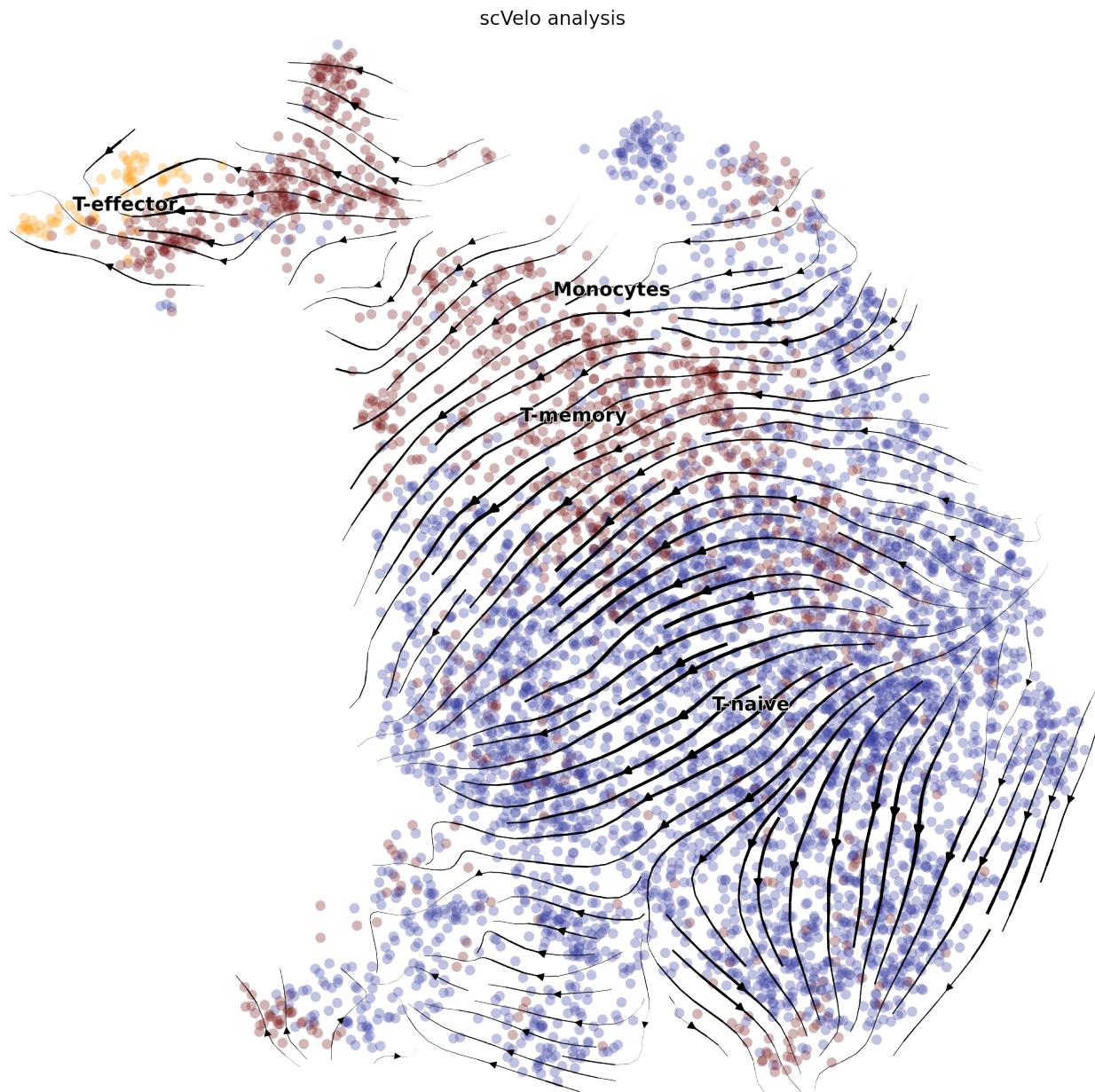


Figure 6: scVelo results for “KO_Hp” grouped sample

scVelo analysis

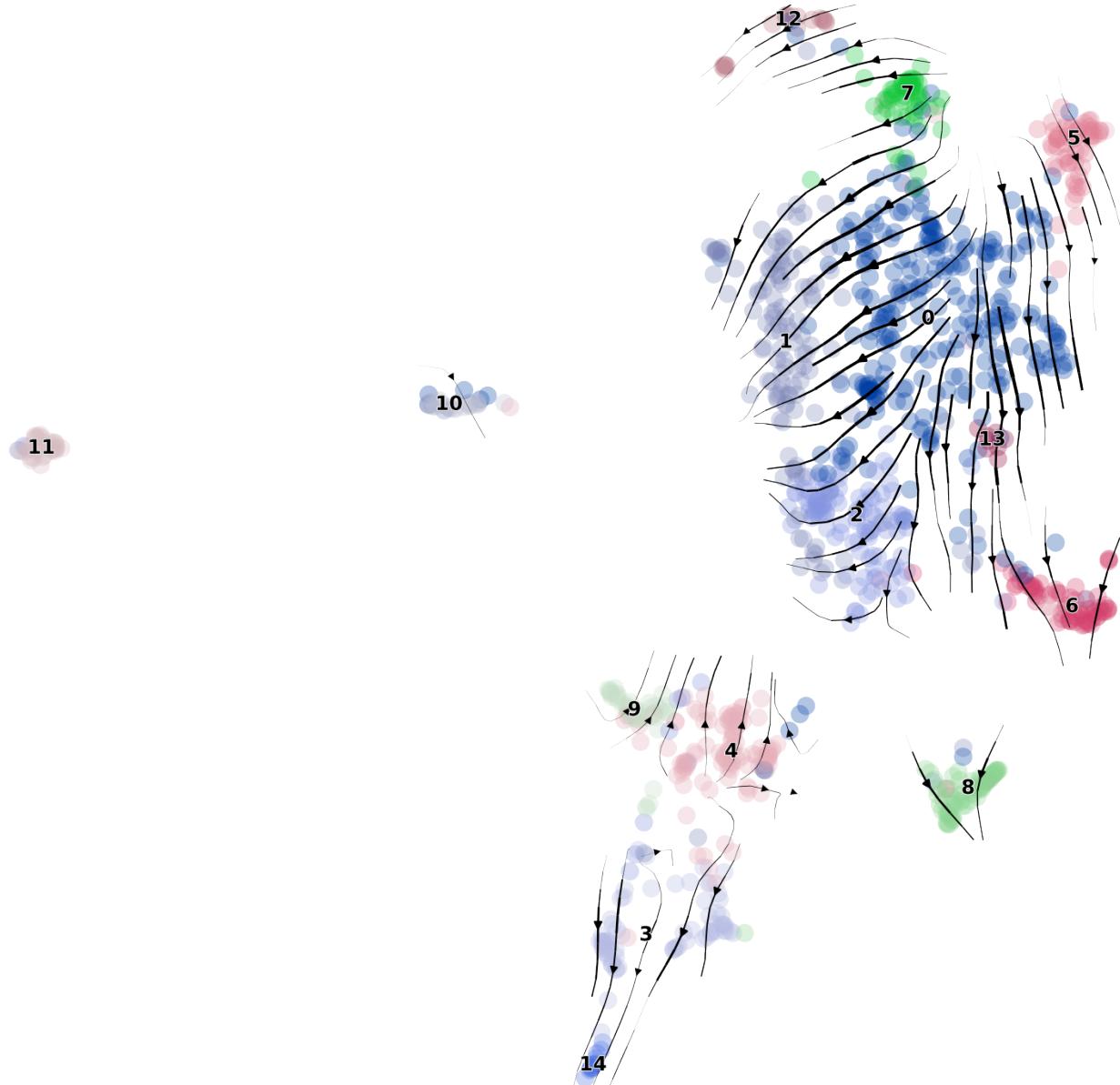


Figure 7: scVelo results for “KO_Hp_zoom” grouped sample

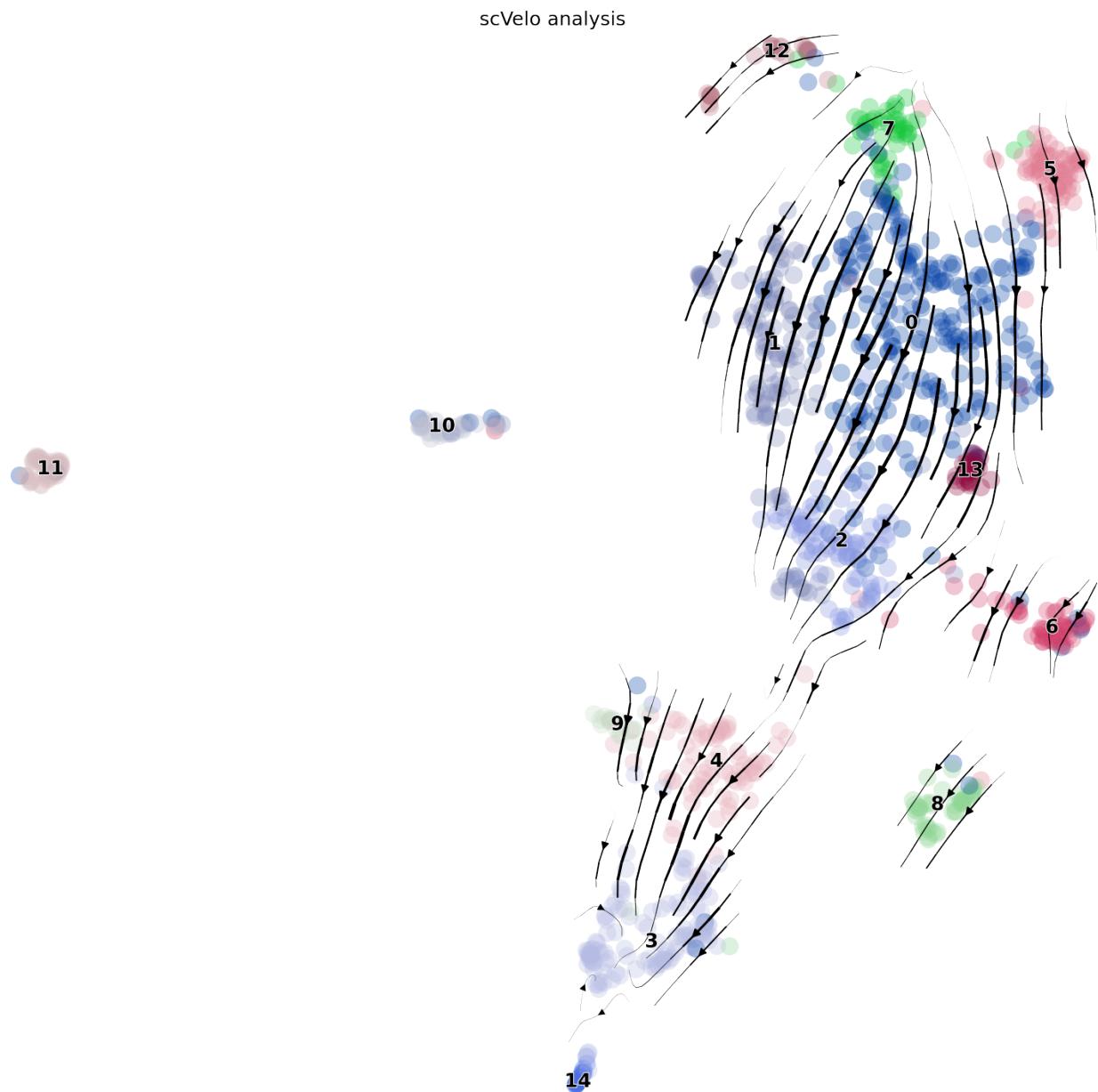


Figure 8: scVelo results for “KO_IL4C_zoom” grouped sample

3.7 For “KO_Hp_zoom”

3.8 For “KO_IL4C_zoom”

3.9 For “KO_Naive_zoom”

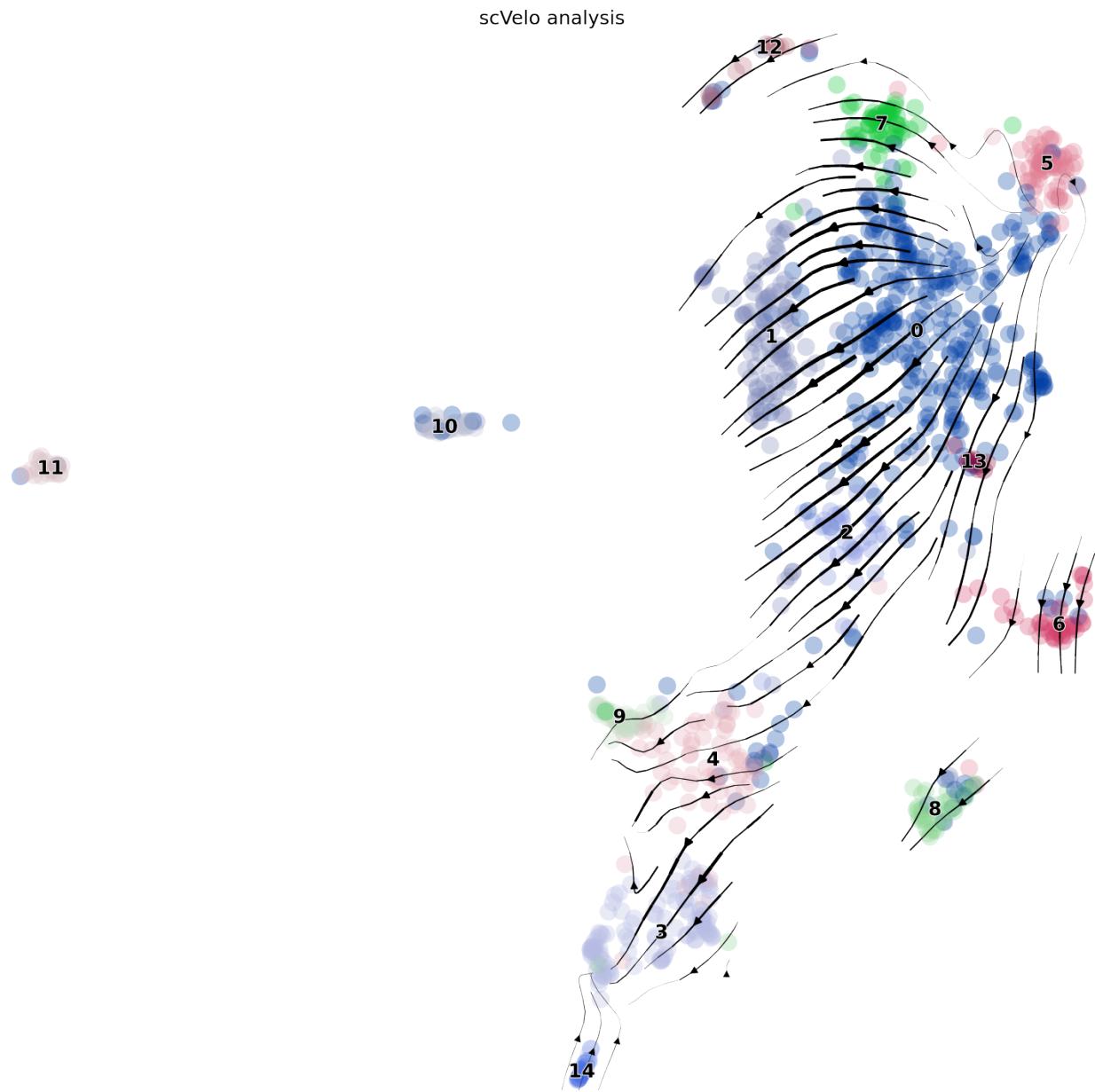


Figure 9: scVelo results for “KO_Naive_zoom” grouped sample

scVelo analysis

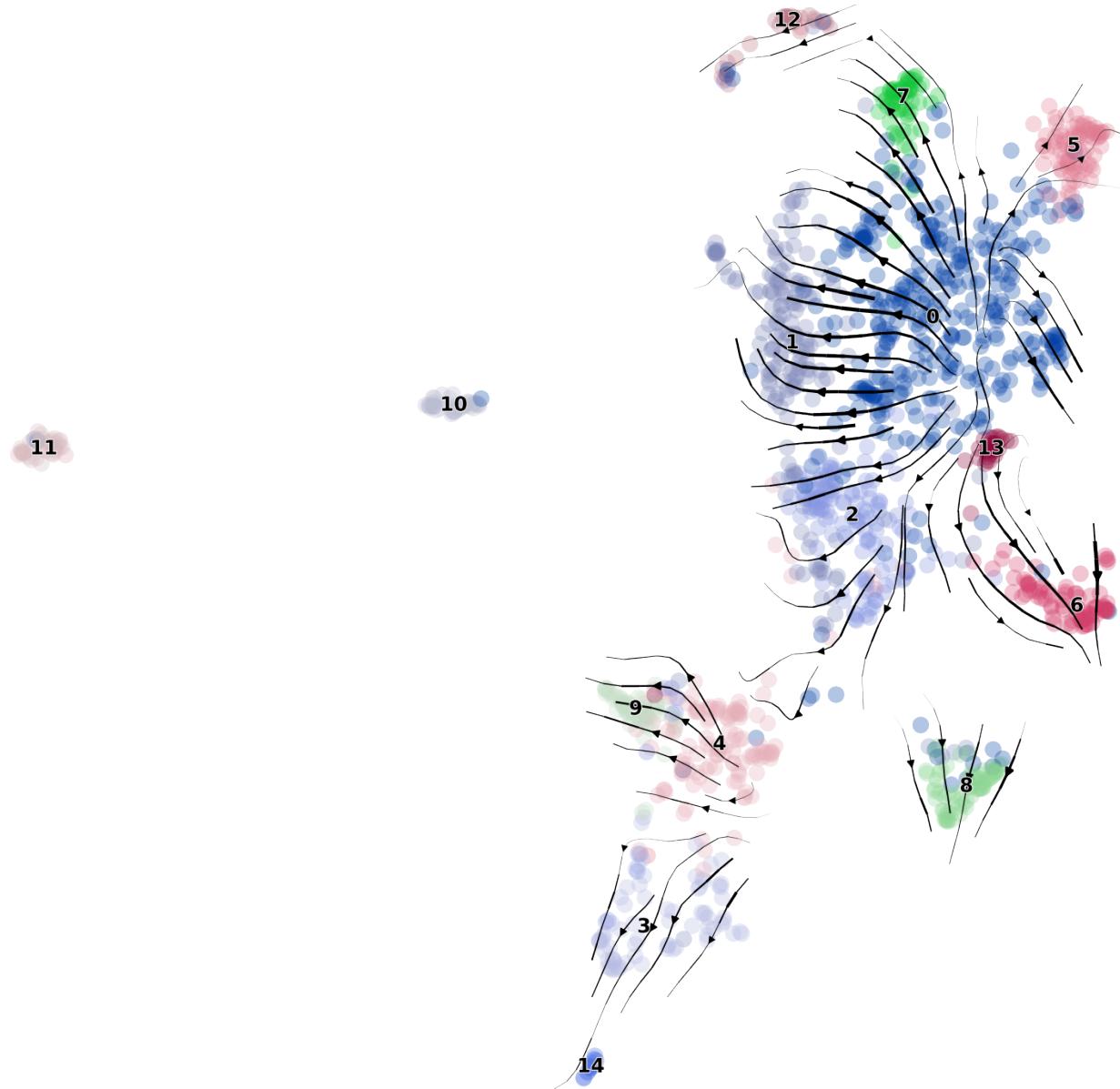


Figure 10: scVelo results for “WT_Hp_zoom” grouped sample

scVelo analysis

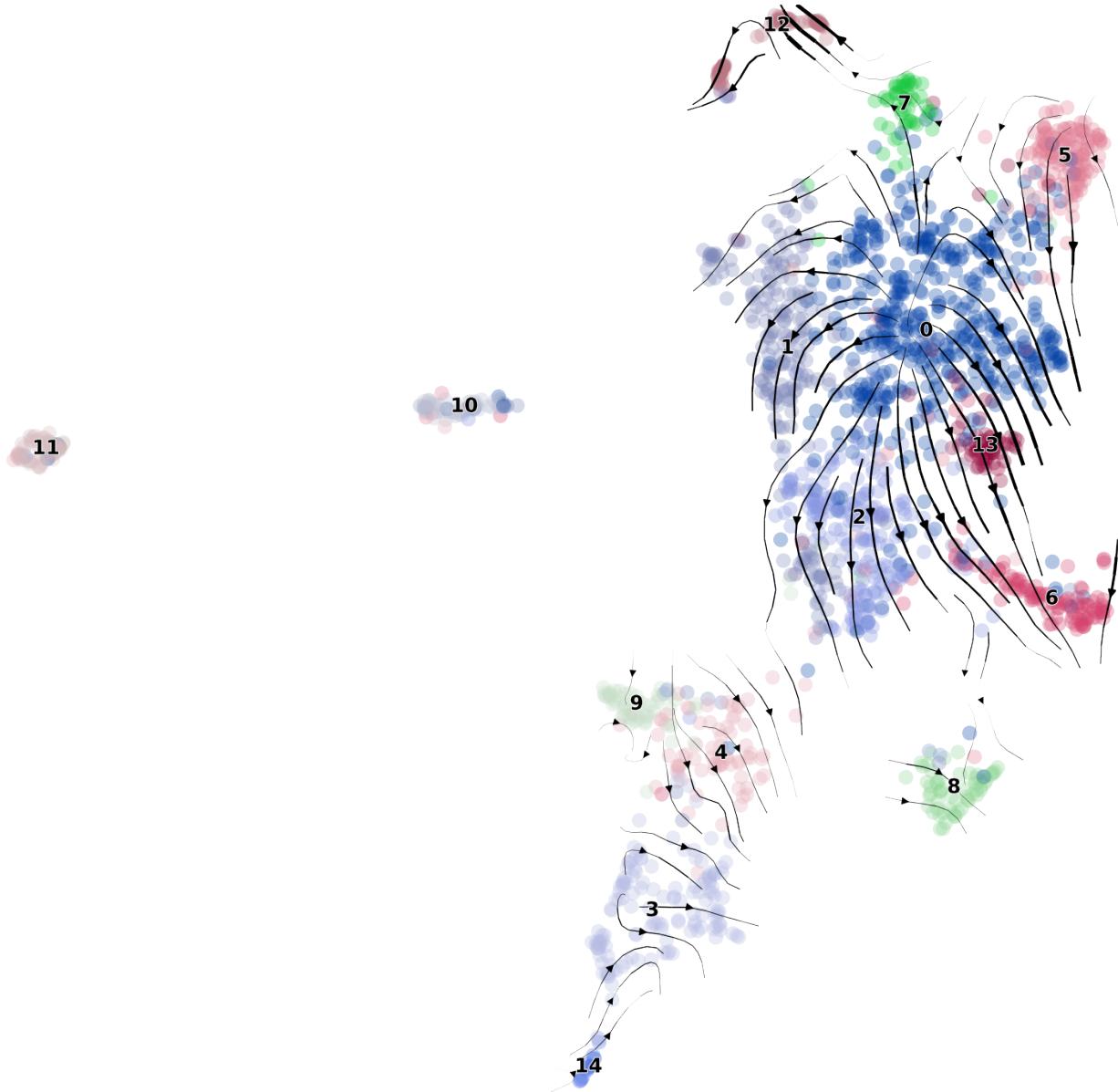


Figure 11: scVelo results for “WT_IL4C_zoom” grouped sample

scVelo analysis

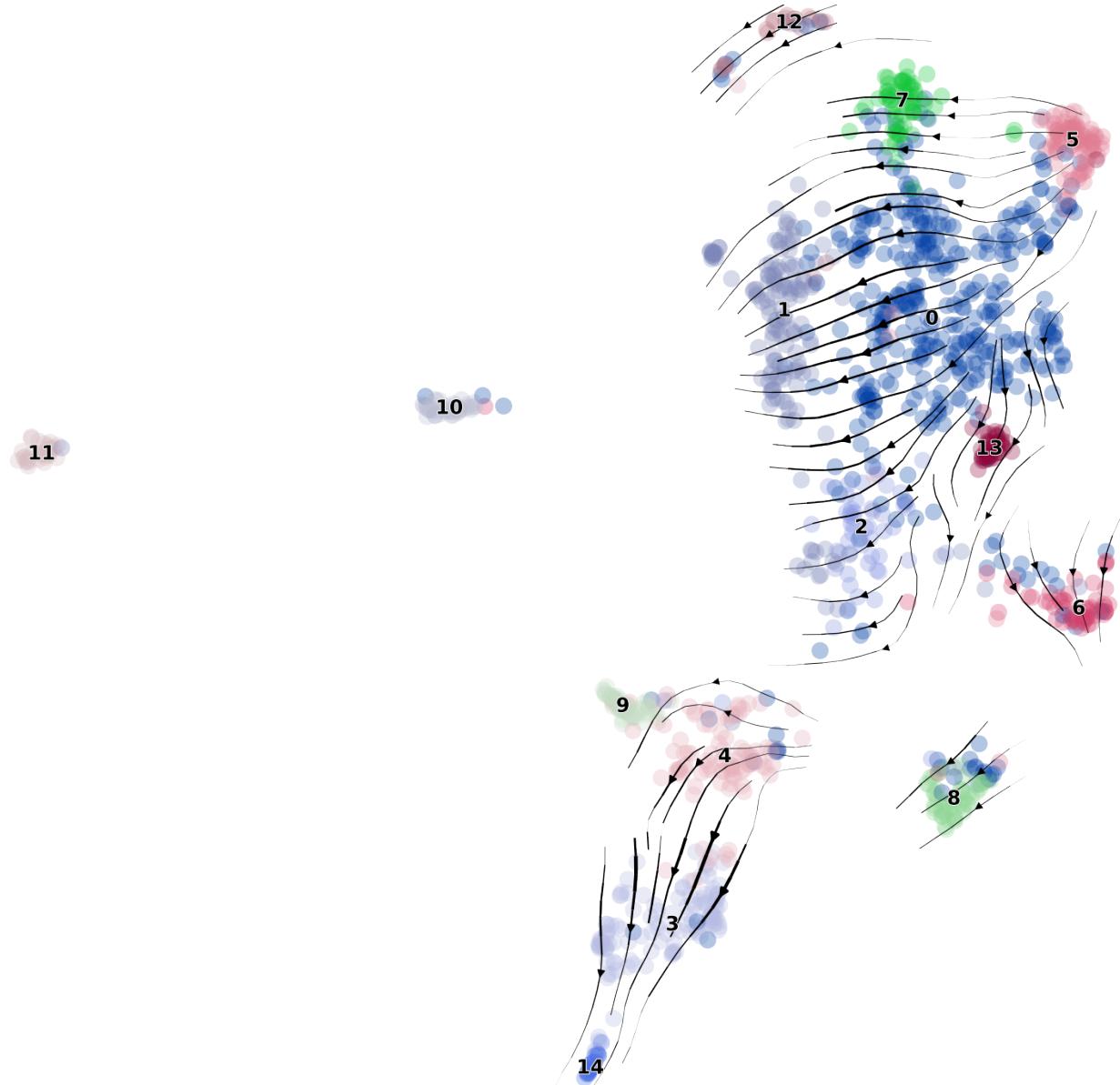


Figure 12: scVelo results for “WT_Naive_zoom” grouped sample

- 3.10 For “WT_Hp_zoom”
- 3.11 For “WT_IL4C_zoom”
- 3.12 For “WT_Naive_zoom”

4 Session information

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] stats graphics grDevices utils datasets methods base	18
##	19
## loaded via a namespace (and not attached):	20
## [1] Rcpp_1.0.7 lattice_0.20-41 png_0.1-7 fansi_0.5.0	21
## [5] crayon_1.4.1 digest_0.6.27 utf8_1.2.2 grid_4.0.3	22
## [9] jsonlite_1.7.2 lifecycle_1.0.0 magrittr_2.0.1 evaluate_0	23
.14	
## [13] pillar_1.6.2 stringi_1.7.3 rlang_0.4.11 Matrix_1.3-4	24
## [17] reticulate_1.20 vctrs_0.3.8 ellipsis_0.3.2 rmarkdown_2	25
.9	
## [21] tools_4.0.3 stringr_1.4.0 xfun_0.24 yaml_2.2.1	26
## [25] compiler_4.0.3 pkgconfig_2.0.3 htmltools_0.5.1.1 knitr_1.33	27
## [29] tibble_3.1.3	28

References

1. La Manno G, Soldatov R, Zeisel A, Braun E, Hochgerner H, Petukhov V, Lidschreiber K, Kastriti ME, Lönnberg P, Furlan A, Fan J, Borm LE, Liu Z, Bruggen D van, Guo J, He X, Barker R, Sundström E, Castelo-Branco G, Cramer P, Adameyko I, Linnarsson S, Kharchenko PV. RNA velocity of single cells. *Nature* 2018;
2. Bergen V, Lange M, Peidli S, Wolf FA, Theis FJ. Generalizing RNA velocity to transient cell states through dynamical modeling. *Nature Biotechnology* 2020;