

Supplementary of “TIVelo: RNA velocity estimation leveraging cluster-level trajectory inference”

Muyang Ge¹, Jishuai Miao¹, Ji Qi¹, Xiaocheng Zhou¹, and Zhixiang Lin^{1,*}

¹Department of Statistics, The Chinese University of Hong Kong, Shatin, Hong Kong SAR, China

*Correspondence: zhixianglin@cuhk.edu.hk

Supplementary Figures

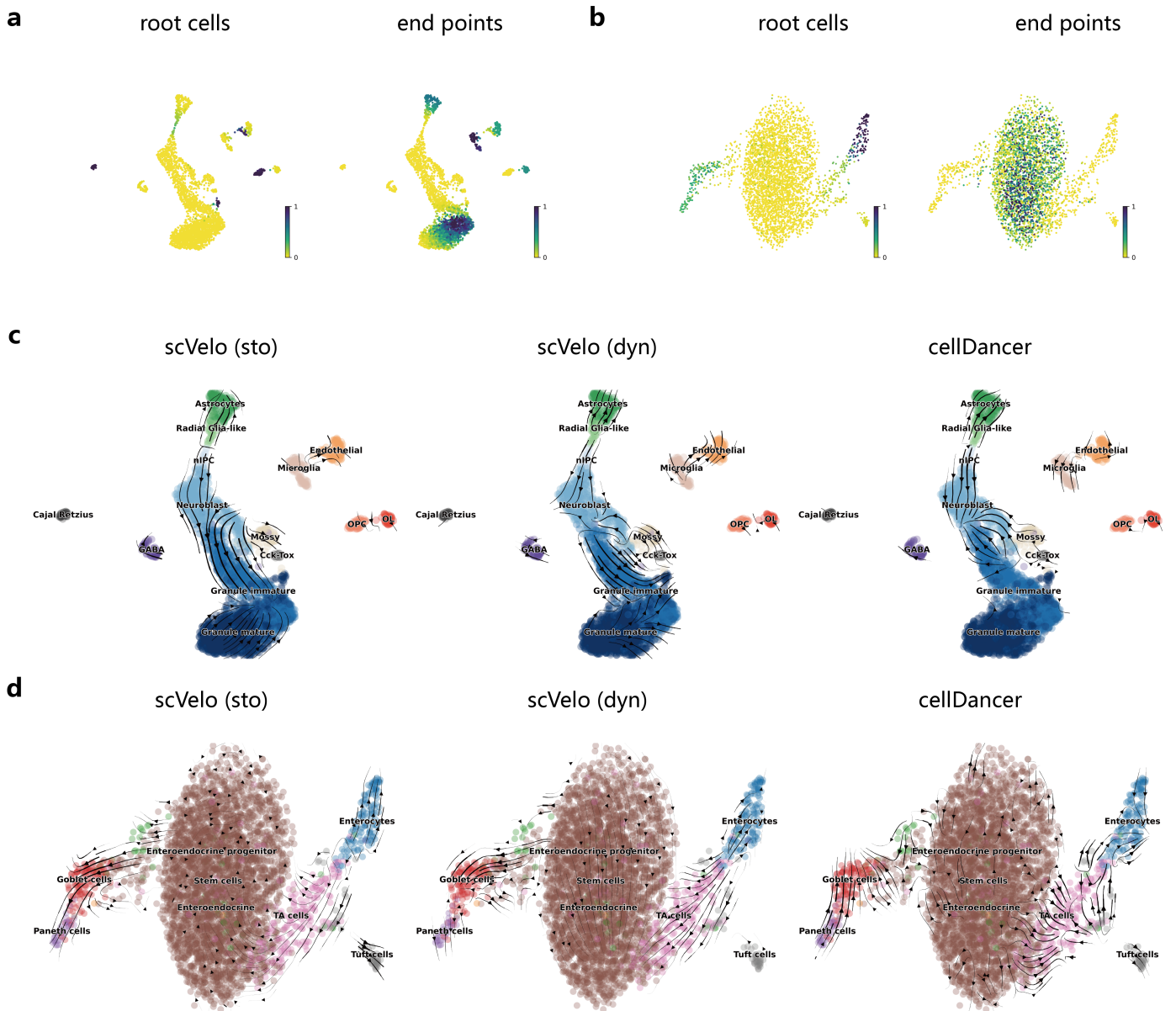


Fig.S1 | Supplementary results from dentate gyrus development and intestinal organoid. a. The root cells and end points scores inferred from scVelo on dentate gyrus development. **b.** The root cells and end points scores inferred from scVelo on intestinal organoid. **c.** Comparative velocity stream plots of dentate gyrus development produced by scVelo (stochastic mode), scVelo (dynamical mode) and cellDancer. **d.** Comparative velocity stream plots of intestinal organoid produced by scVelo (stochastic mode), scVelo (dynamical mode) and cellDancer.

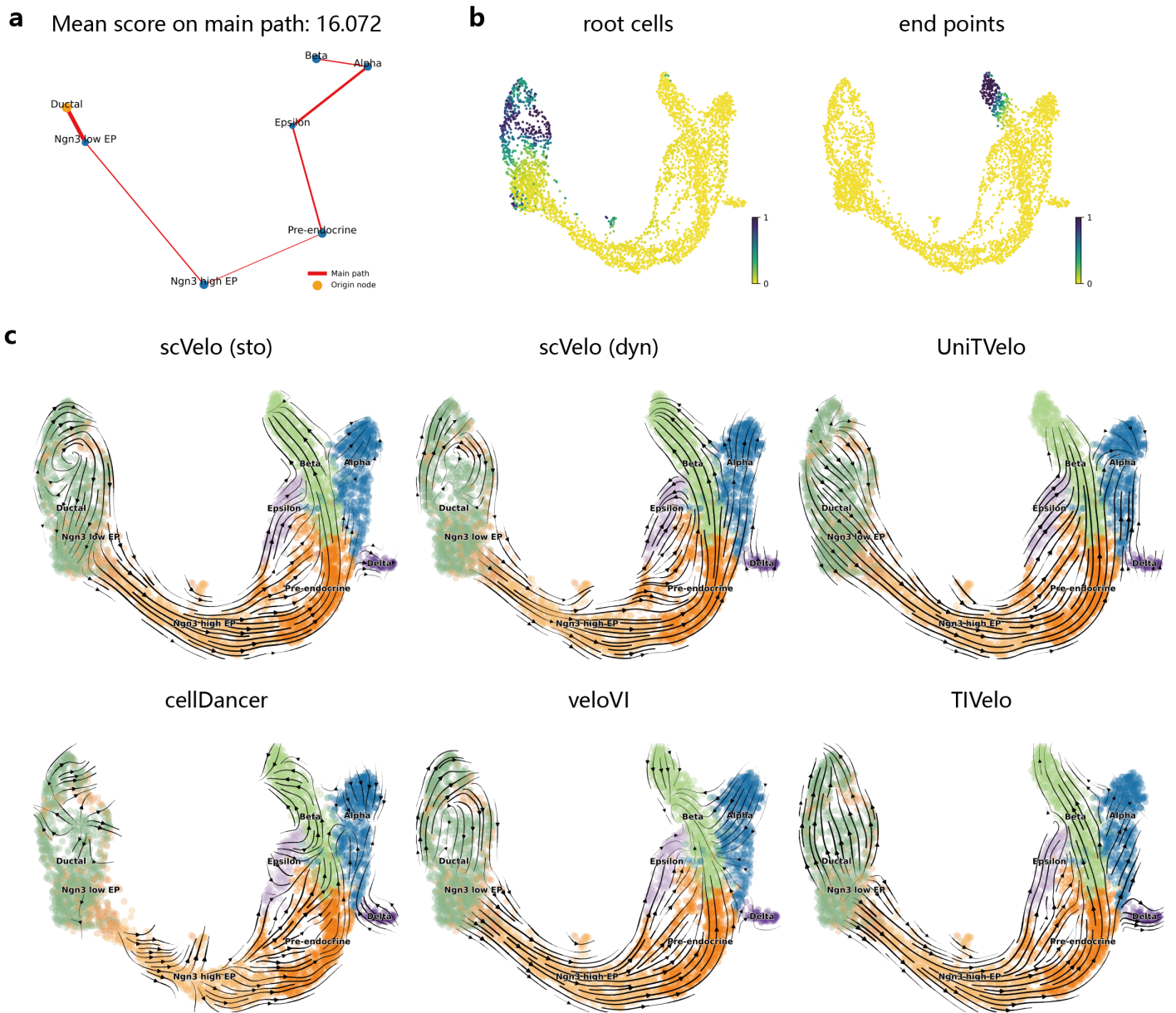


Fig.S2 | Evaluation of TIVelo's performance on pancreatic endocrinogenesis. **a.** The cluster graph after graph pruning and main path selection. The cluster Ductal is selected as the origin node. The mean orientation score along the main path is 16.072. **b.** The root cells and end points scores inferred from scVelo. **c.** Comparative velocity stream plots of pancreatic endocrinogenesis produced by scVelo (stochastic mode), scVelo (dynamical mode), UniTVelo, cellDancer, veloVI and TIVelo.

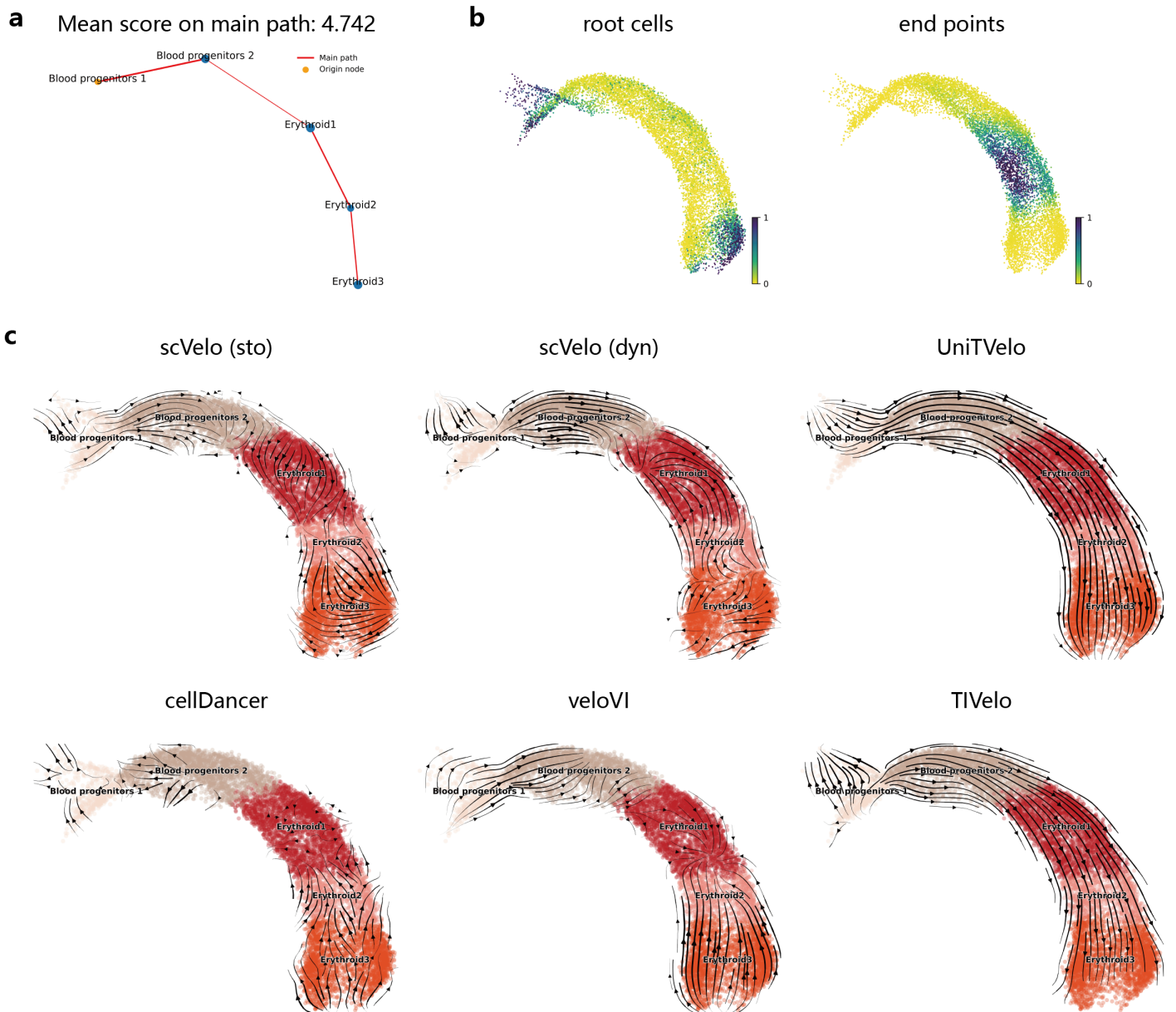


Fig.S3 | Evaluation of TIVelo's performance on mouse gastrulation (erythroid). **a.** The cluster graph after graph pruning and main path selection. The cluster Blood Progenitors 1 is selected as the origin node. The mean orientation score along the main path is 4.742. **b.** The root cells and end points scores inferred from scVelo. **c.** Comparative velocity stream plots of mouse gastrulation produced by scVelo (stochastic mode), scVelo (dynamical mode), UniTVelo, cellDancer, veloVI and TIVelo.

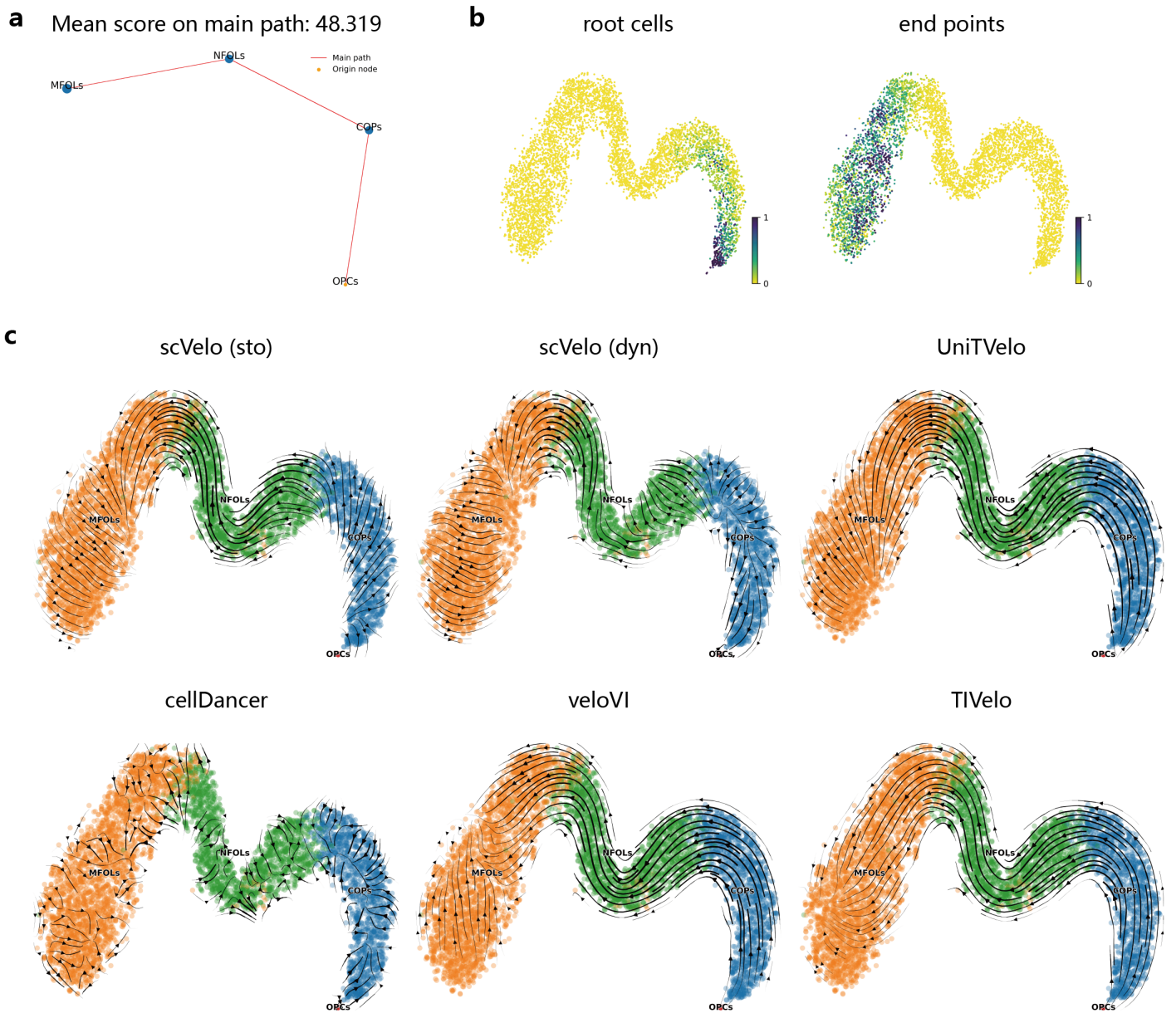


Fig.S4 | Evaluation of TIVelo's performance on mouse hindbrain (oligo). **a.** The cluster graph after graph pruning and main path selection. The cluster OPCs is selected as the origin node. The mean orientation score along the main path is 48.319. **b.** The root cells and end points scores inferred from scVelo. **c.** Comparative velocity stream plots of mouse hindbrain (oligo) produced by scVelo (stochastic mode), scVelo (dynamical mode), UniTVelo, cellDancer, veloVI and TIVelo.

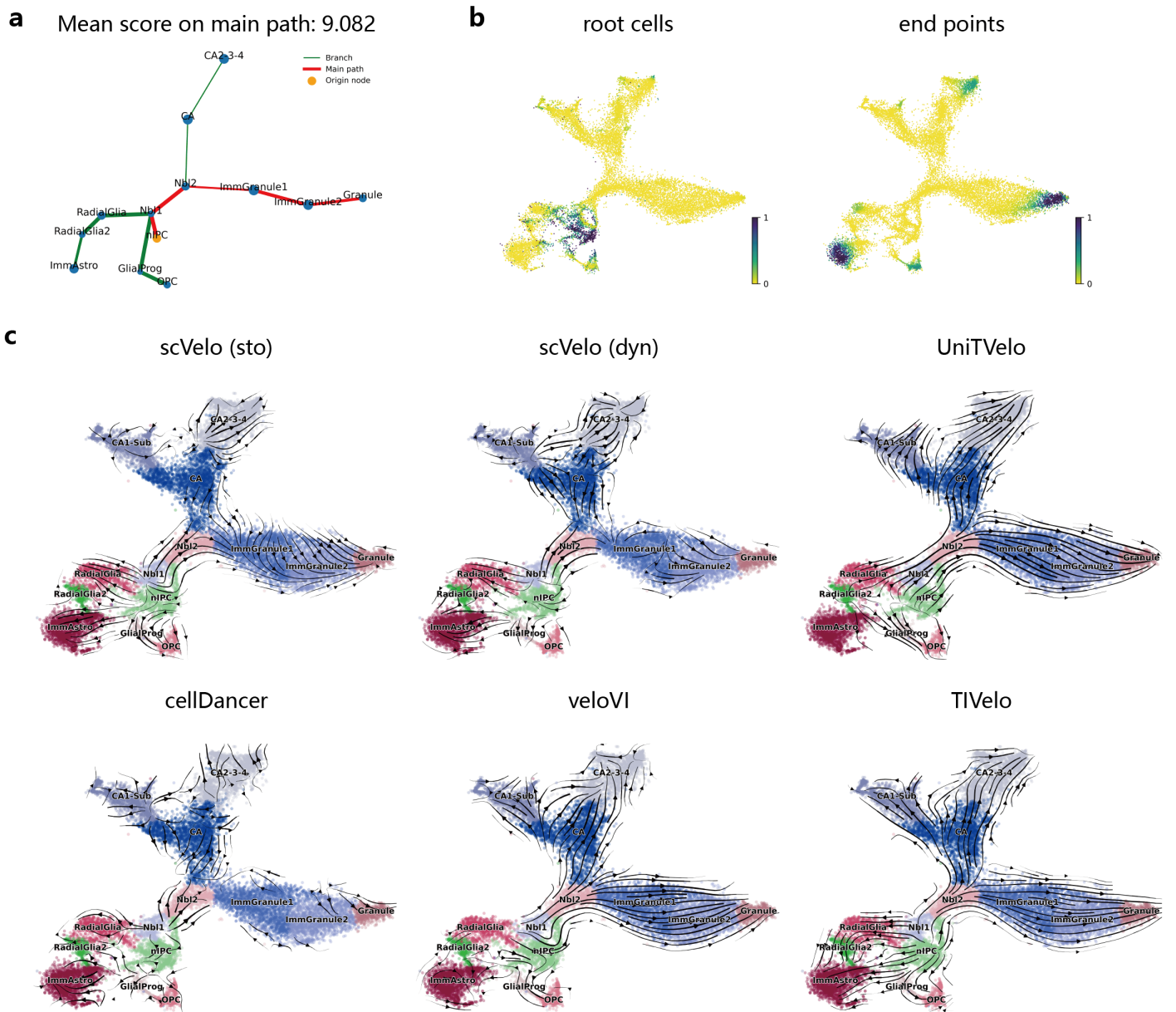


Fig.S5 | Evaluation of TIVelo's performance on dentate gyrus development 2. **a.** The cluster graph after graph pruning and main path selection. The cluster nIPC is selected as the origin node. The mean orientation score along the main path is 9.082. **b.** The root cells and end points scores inferred from scVelo. **c.** Comparative velocity stream plots of dentate gyrus development 2 produced by scVelo (stochastic mode), scVelo (dynamical mode), UniTVelo, cellDancer, veloVI and TIVelo.

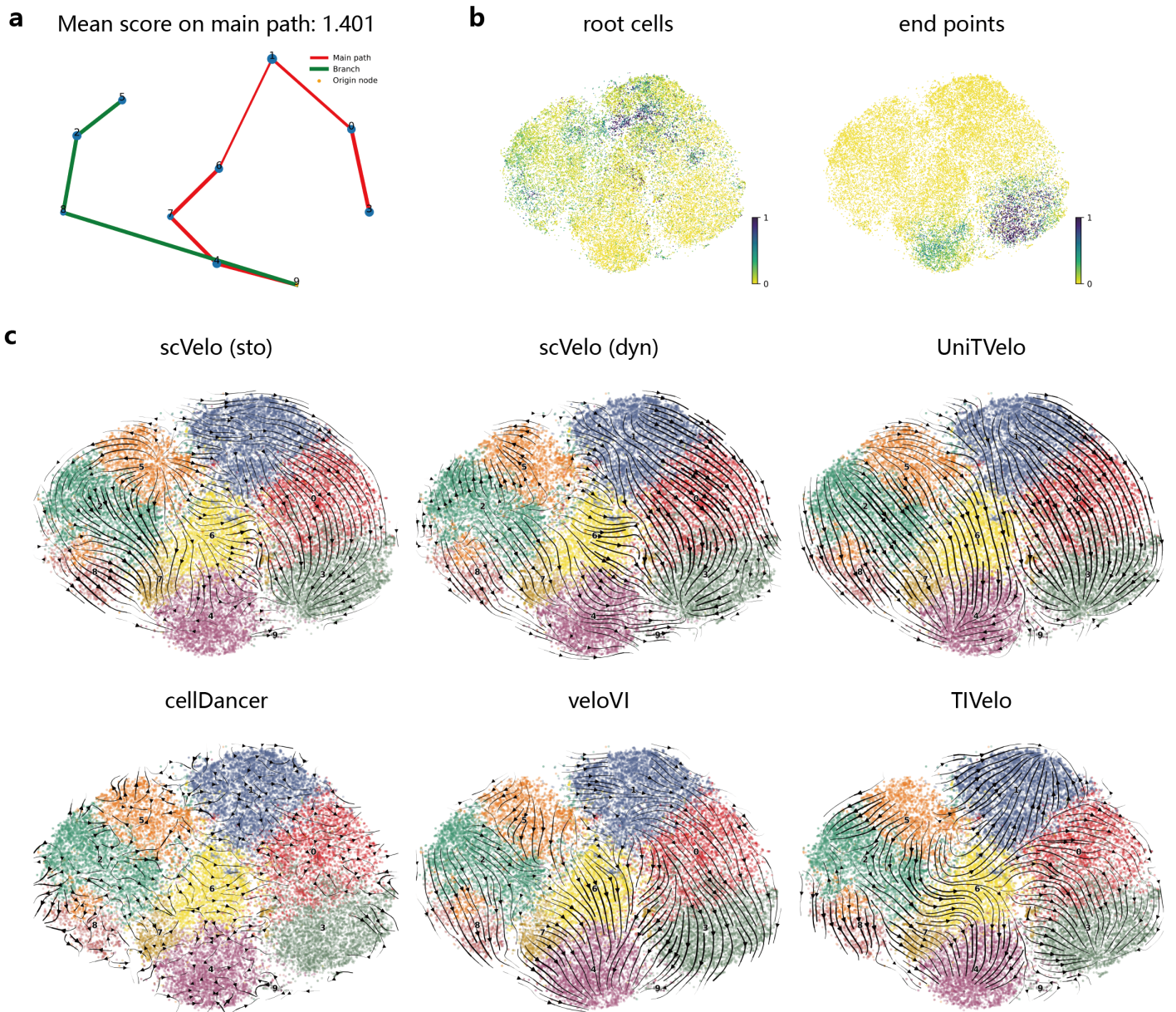


Fig.S6 | Evaluation of TIVelo's performance on mouse embryonic fibroblast reprogramming.

a. The cluster graph after graph pruning and main path selection. The cluster 9 is selected as the origin node. The mean orientation score along the main path is 1.401. The mean orientation score along the branch in the graph is -0.972. Since this branch connects to the main path both by their endpoints, the origin node is reset as cluster 5 (Methods; Fig.S14). **b.** The root cells and end points scores inferred from scVelo. **c.** Comparative velocity stream plots of mouse embryonic fibroblast reprogramming produced by scVelo (stochastic mode), scVelo (dynamical mode), UniTVelo, cellDancer, veloVI and TIVelo.

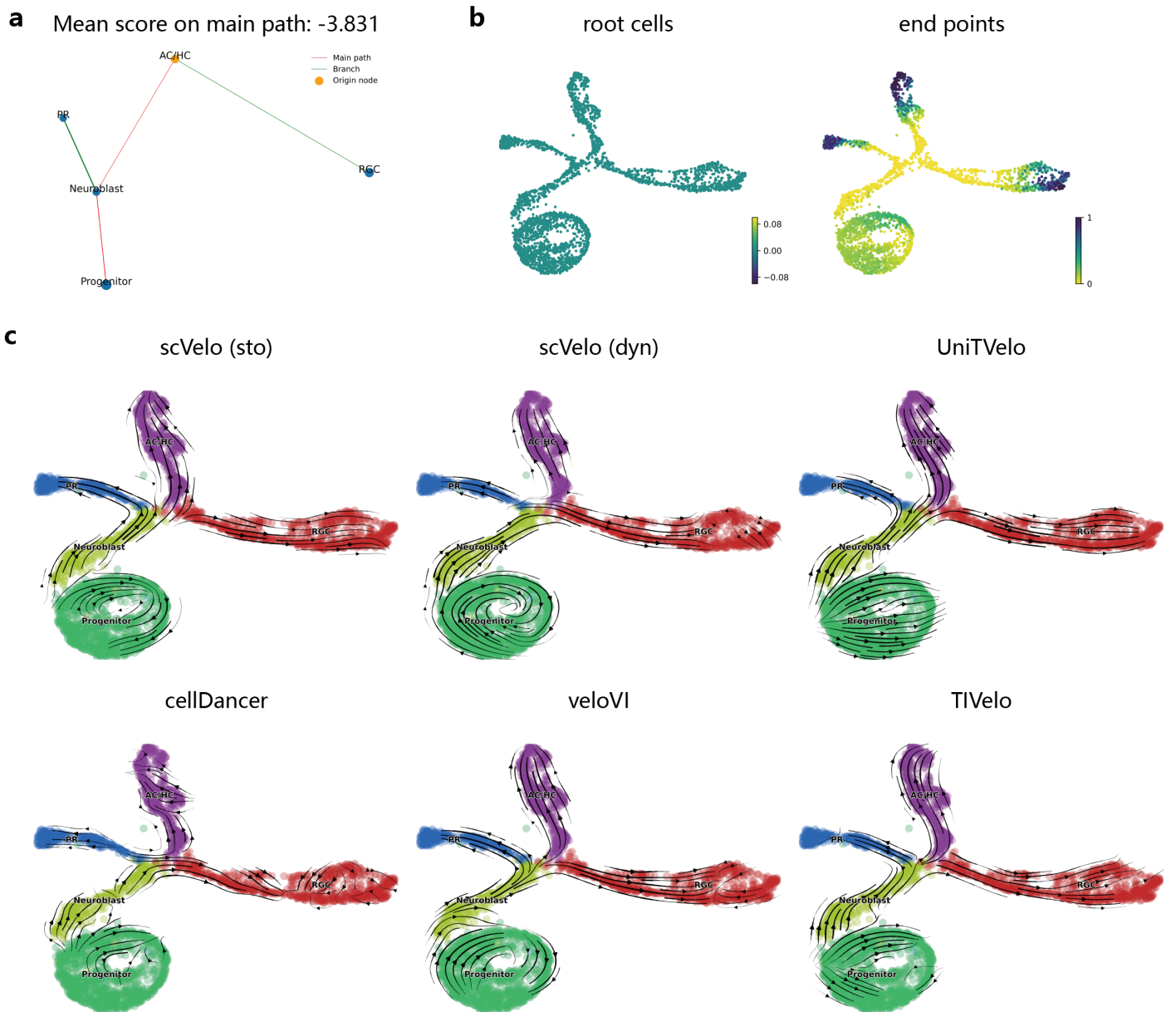


Fig. S7 | Evaluation of TIVelo's performance on mouse retina development. **a.** The cluster graph after graph pruning and main path selection. The cluster AC/HC is selected as the origin node. The mean orientation score along the main path is -3.831. The origin node is reset as cluster Progenitor. **b.** The root cells and end points scores inferred from scVelo. **c.** Comparative velocity stream plots of mouse retina development produced by scVelo (stochastic mode), scVelo (dynamical mode), UniTVelo, cellDancer, veloVI and TIVelo.

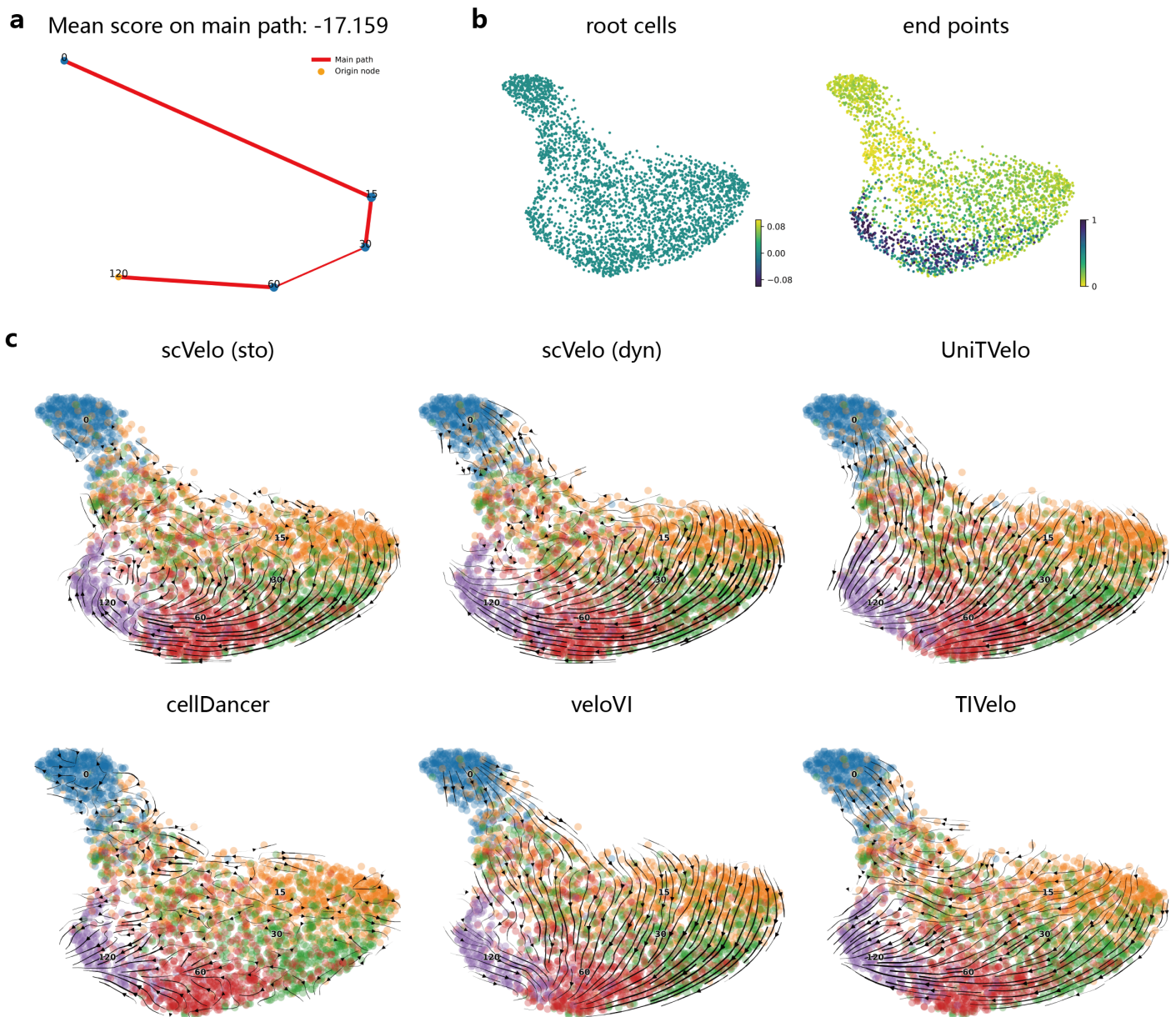


Fig.S8 | Evaluation of TIVelo's performance on scNT-seq neuron KCl stimulation. **a.** The cluster graph after graph pruning and main path selection. The cluster 120 is selected as the origin node. The mean orientation score along the main path is -17.159. The origin node is reset as cluster 0. **b.** The root cells and end points scores inferred from scVelo. **c.** Comparative velocity stream plots of scNT-seq neuron KCl stimulation produced by scVelo (stochastic mode), scVelo (dynamical mode), UniTVelo, cellDancer, veloVI and TIVelo.

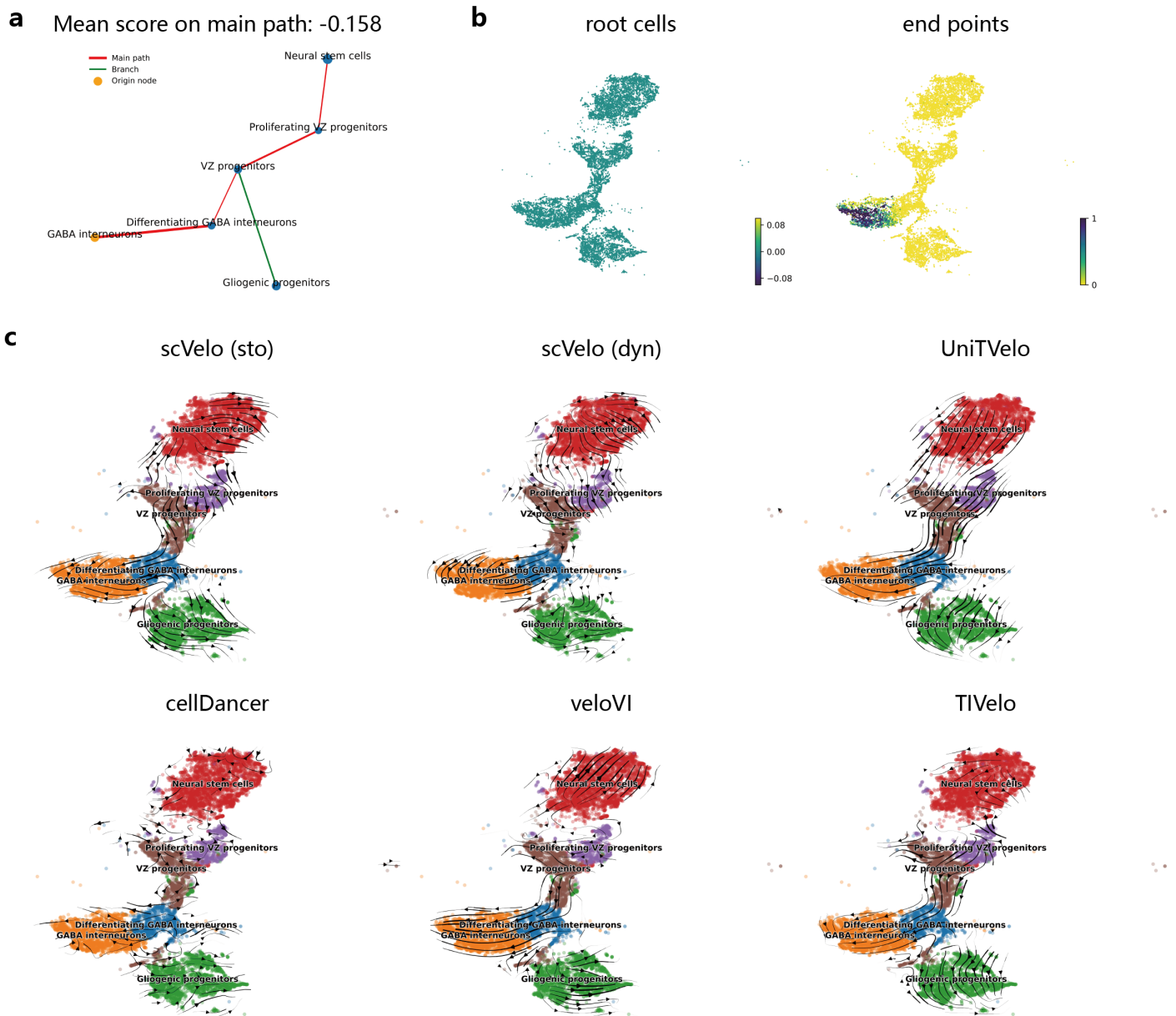
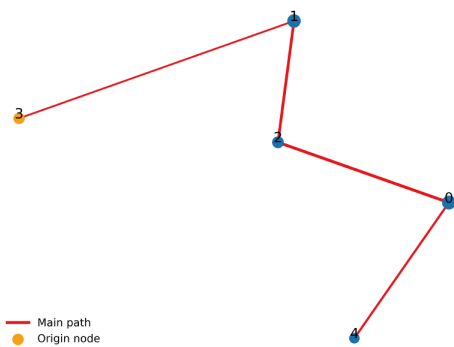
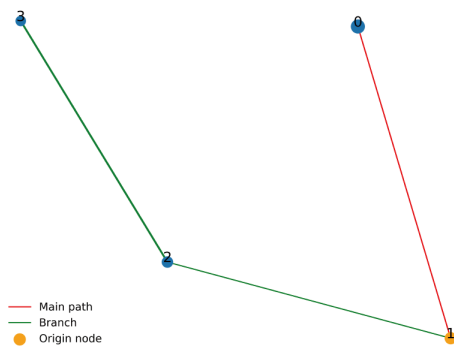


Fig. S9 | Evaluation of TIVelo's performance on mouse hindbrain (GABA, Glial). **a.** The cluster graph after graph pruning and main path selection. The cluster GABA interneurons is selected as the origin node. The mean orientation score along the main path is -0.158. The origin node is reset as cluster Neural stem cells. **b.** The root cells and end points scores inferred from scVelo. **c.** Comparative velocity stream plots of mouse hindbrain (GABA, Glial) produced by scVelo (stochastic mode), scVelo (dynamical mode), UniTVelo, cellDancer, veloVI and TIVelo.

a Mean score on main path: 7.965



b Mean score on main path: -1.610



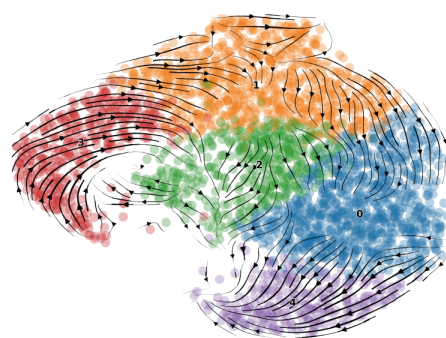
c scVelo (sto)



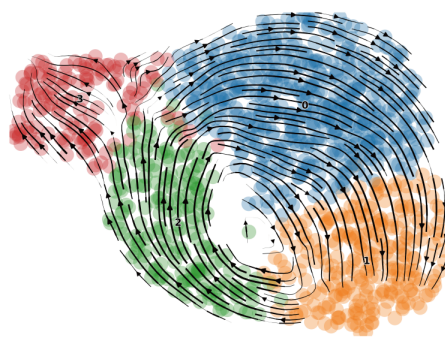
scVelo (dyn)



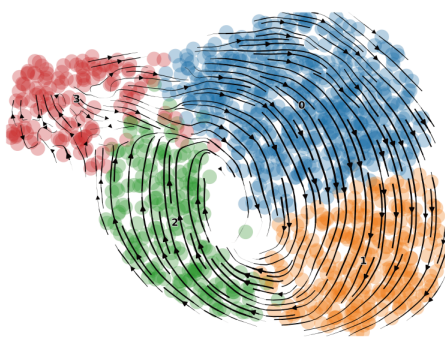
cellDancer



d scVelo (sto)



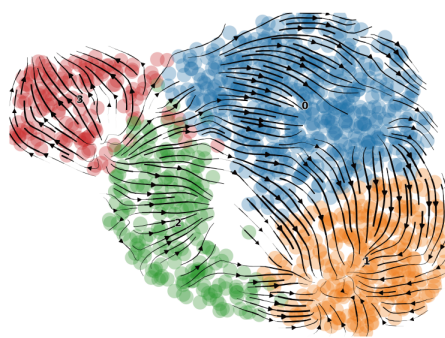
scVelo (dyn)



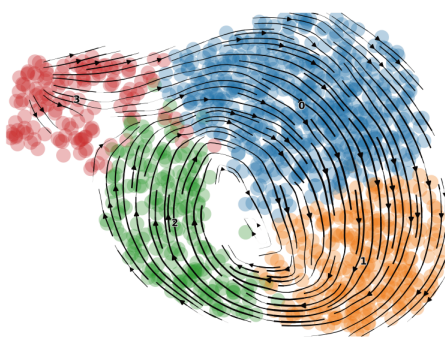
UniTVelo



cellDancer



veloVI



TIVelo

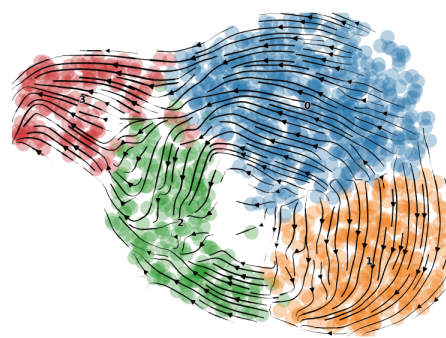


Fig.S10 | Supplementary results on 2 datasets with cell-cycle scores. **a.** The cluster graph after graph pruning and main path selection on FUCCI-RPE1. The cluster 3 is selected as the origin node. The mean orientation score along the main path is 7.965. **b.** The cluster graph after graph pruning and main path selection on FUCCI-U2OS. The cluster 1 is selected as the origin node. The mean orientation score along the main path is -1.610. The origin node is reset as cluster 0. **c.** Comparative velocity stream plots of FUCCI-RPE1 produced by scVelo (stochastic mode), scVelo (dynamical mode) and cellDancer. **d.** Comparative velocity stream plots of FUCCI-U2OS produced by scVelo (stochastic mode), scVelo (dynamical mode), UniTVelo, cellDancer, veloVI and TIVelo. For FUCCI-U2OS, the velocity stream shows a anticlockwise cycling pattern, as in the result from veloVI . TIVelo successfully predicts the velocity direction on the cluster level (cluster $0 \rightarrow 1 \rightarrow 2 \rightarrow 3$). However, TIVelo can only deal cluster graphs with tree structure, and cannot predict the development from cluster 3 back to 1. This leads to some reversed velocity stream from cluster 0 to cluster 2 and 3. Despite of this, TIVelo achieves highest velocity sign accuracy (Fig.5e), since the trajectory cluster $0 \rightarrow 1 \rightarrow 2$ is correctly inferred.

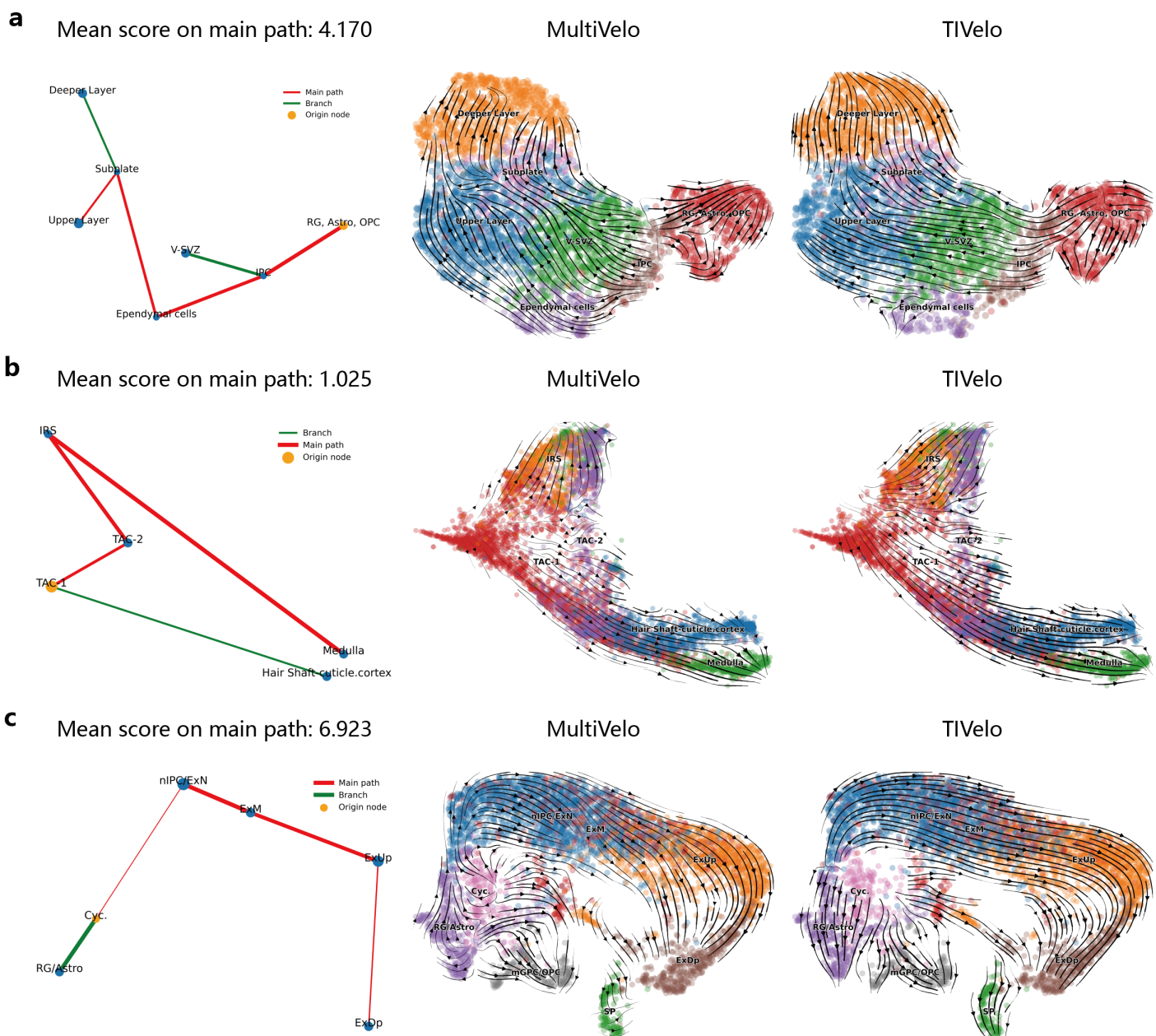


Fig. S11 | Supplementary results on three single-cell multi-omics datasets. **a.** Left: The cluster graph after graph pruning and main path selection on embryonic mouse brain. Middle and right: Comparative velocity stream plots of embryonic mouse brain produced by MultiVelo and TIVelo. **b.** Left: The cluster graph after graph pruning and main path selection on SHARE-seq mouse skin. Middle and right: Comparative velocity stream plots of SHARE-seq mouse skin produced by MultiVelo and TIVelo. **c.** Left: The cluster graph after graph pruning and main path selection on developing human brain. Middle and right: Comparative velocity stream plots of developing human brain produced by MultiVelo and TIVelo.

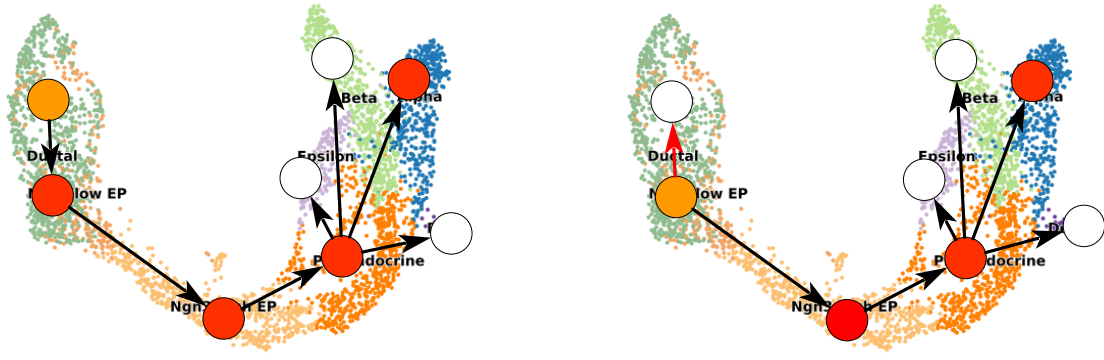


Fig.S12 | The significance of selecting origin node in TIVelo. Left: If a root or end cluster is selected as the origin node (orange node), and the direction on the main path (orange & red nodes) is correctly inferred, the developmental direction of the entire graph can be correctly determined. Right: If an intermediate cluster is selected as the origin node, even with correctly inferred direction along the main path, there still exist some edges on which the inferred direction is incorrect (red arrow).

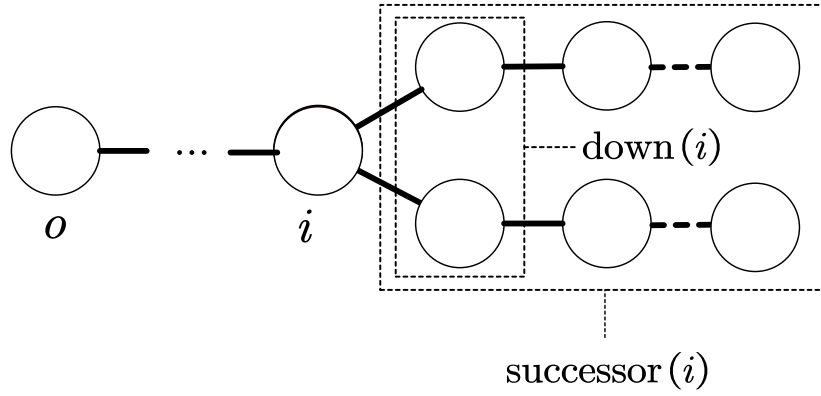


Fig.S13 | Defining downstream and successor nodes. Downstream nodes for node i refers to nodes that directly connected to i , excluding any nodes located on the path from origin node o to node i . Successor nodes for node i refers to nodes that directly or indirectly connected to i , except those on the path between the origin node o and i .

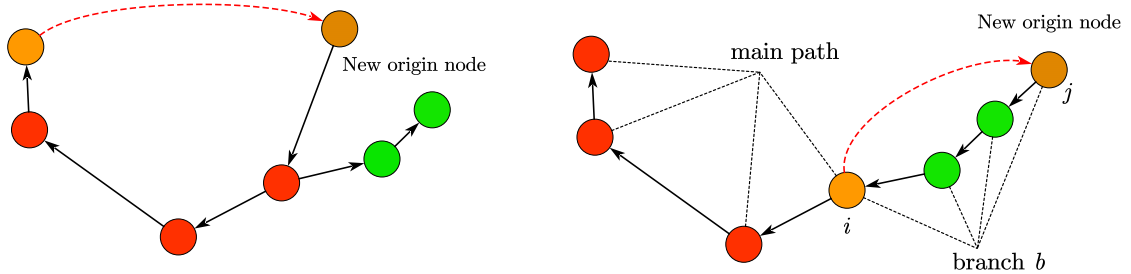


Fig.S14 | Rules for inferring orientation and setting new origin node. Left: If $\frac{1}{G} \sum_g S_g < 0$, the inferred direction along the main path (orange & red nodes) is considered incorrect, and a new origin node should be set as the other end of the main path. Right: If an endpoint i of one branch b is also an endpoint of the main path, and the corrected direction on the main path is aligned with the corrected direction on branch b , the origin node (orange node) will be redefined as the other endpoint j of branch b (orange node, shaded).