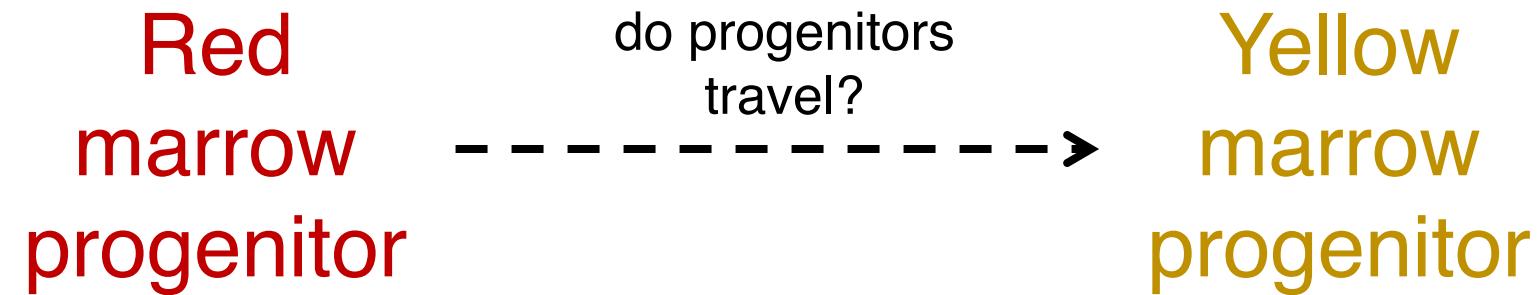


RNA velocity analysis of the BM-32 samples

Zinger Yang Loureiro

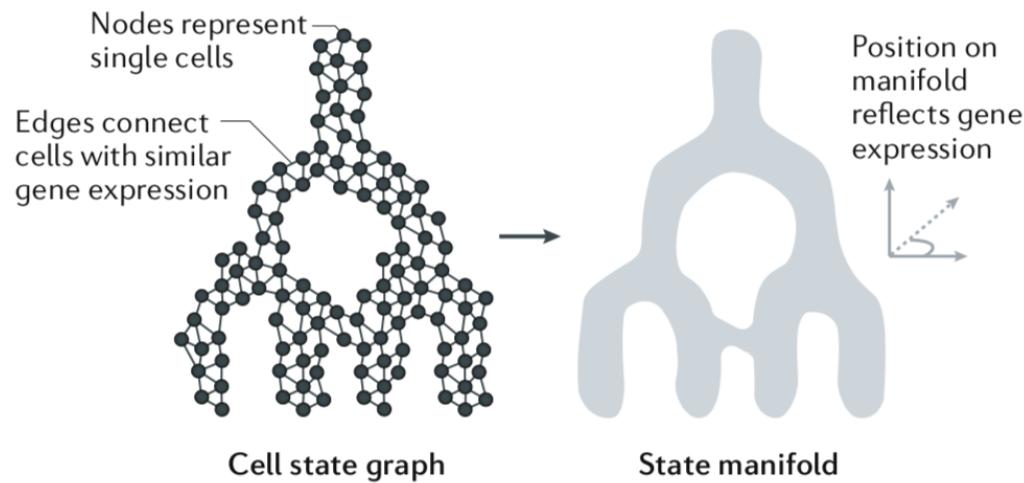
October 12, 2021

Trajectory analysis of scRNA-seq results may provide further insight into the cell movement, development



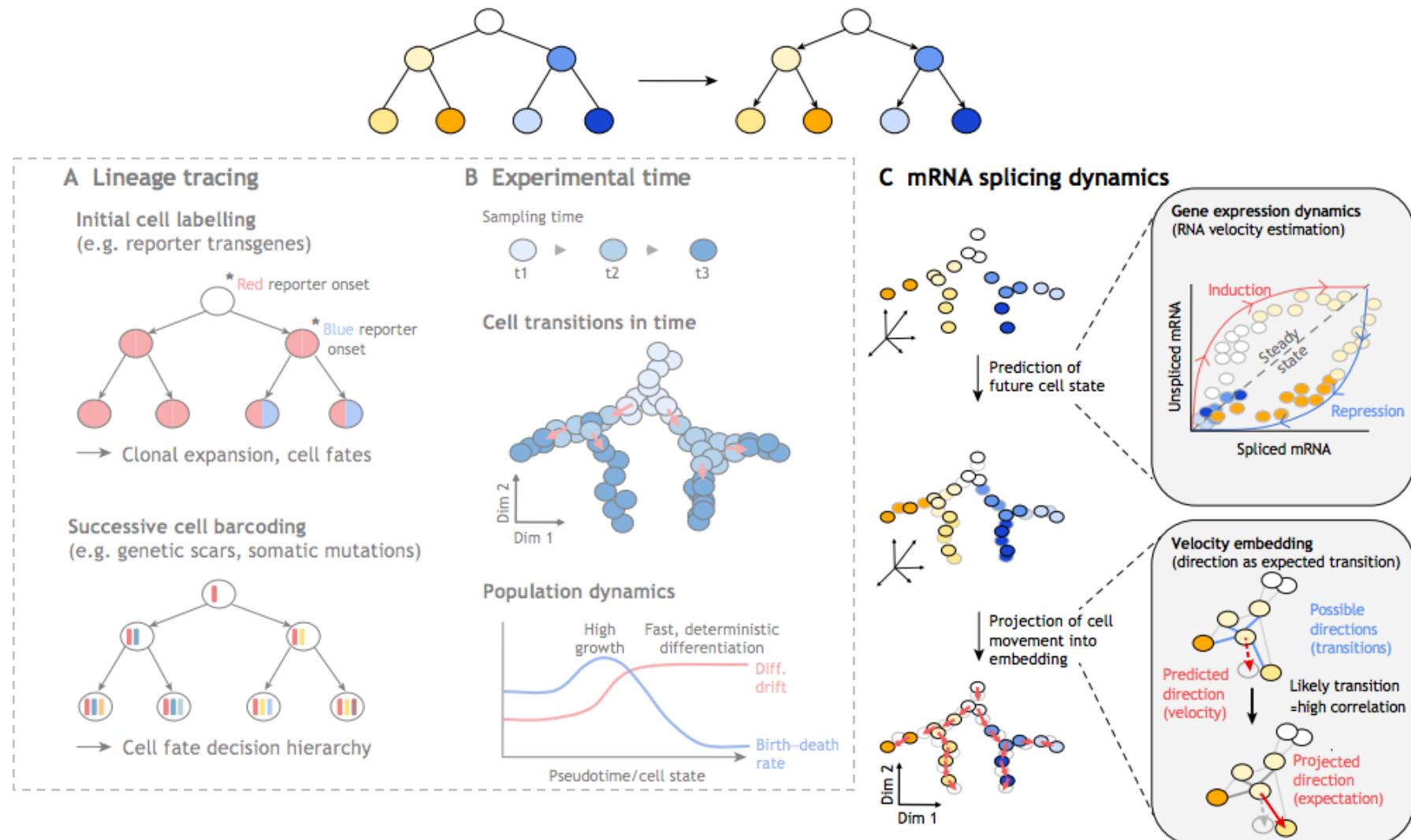
Trajectory inferences

- Trajectory inference methods interpret single-cell data as a snapshot of a continuous process. This process is reconstructed by finding paths through cellular space that minimize transcriptional changes between neighbouring cells

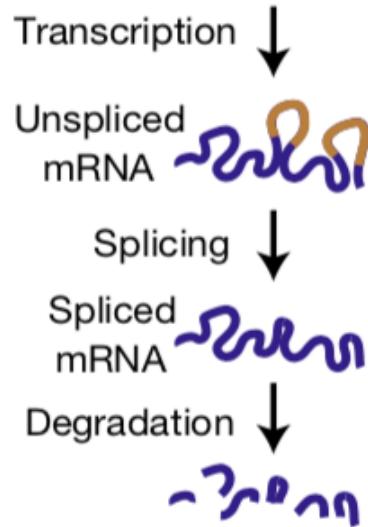


*“Two-dimensional representations... can be misleading, as they distort high-dimensional structures upon ‘flattening’ them, and in some cases algorithms force tree-like visual layouts that may further distort the original structure.... **The dynamics predicted from cell state snapshots should thus be considered hypotheses**”*

Approaches for inferring directionality and dynamics in trajectories

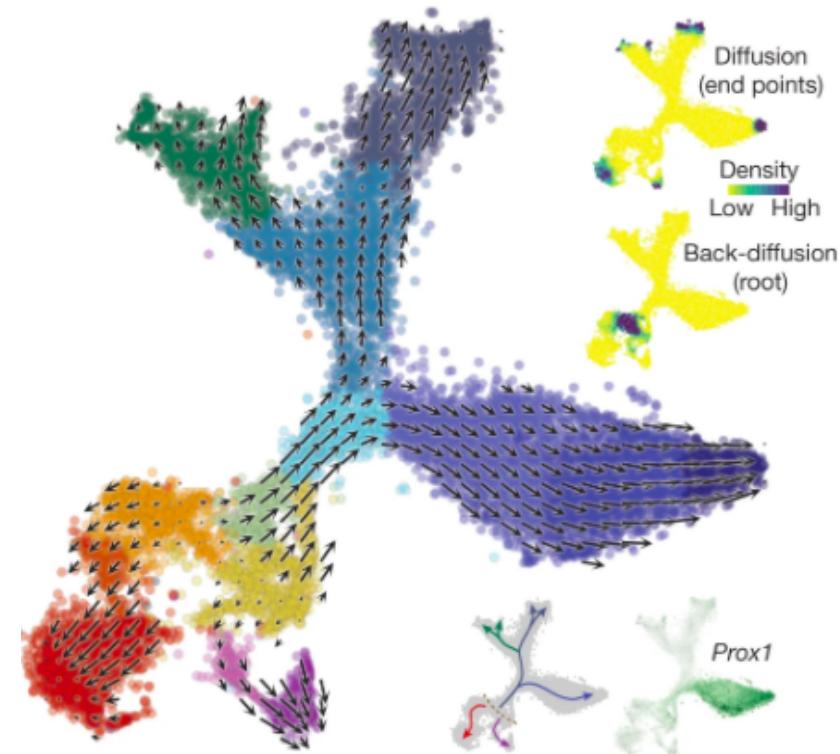


RNA velocity exploits underlying molecular kinetics to predict gene expression changes in cells



The relative abundance of nascent (unspliced) and mature (spliced) mRNA can be exploited to estimate the rates of gene splicing and degradation

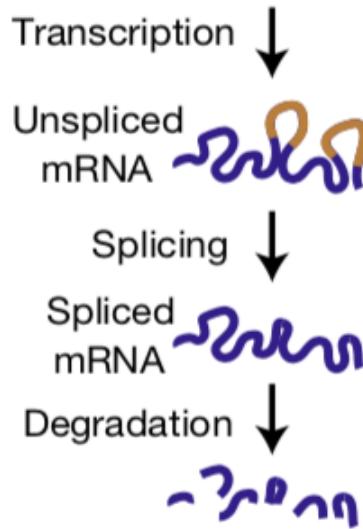
mRNA abundance over time (dS/dt) is the *velocity* of gene expression



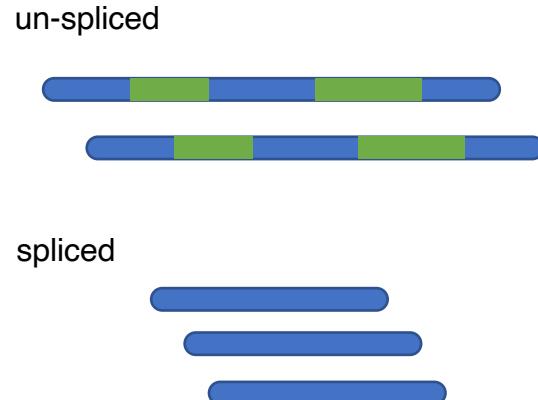
RNA velocity field projected onto the *t*-SNE plot. Arrows show the local average velocity evaluated on a regular grid.

The balance of un-spliced and spliced mRNA abundance is an indicator of the future state of mature mRNA abundance, and thus the future state of the cell.

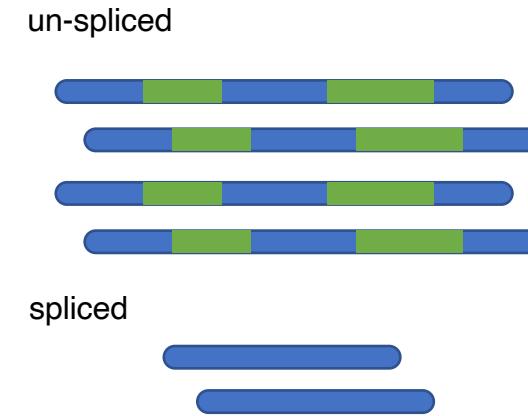
Model of transcription dynamic



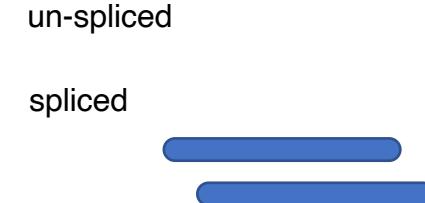
**Steady state
(equilibrium of
un-spliced vs spliced)**



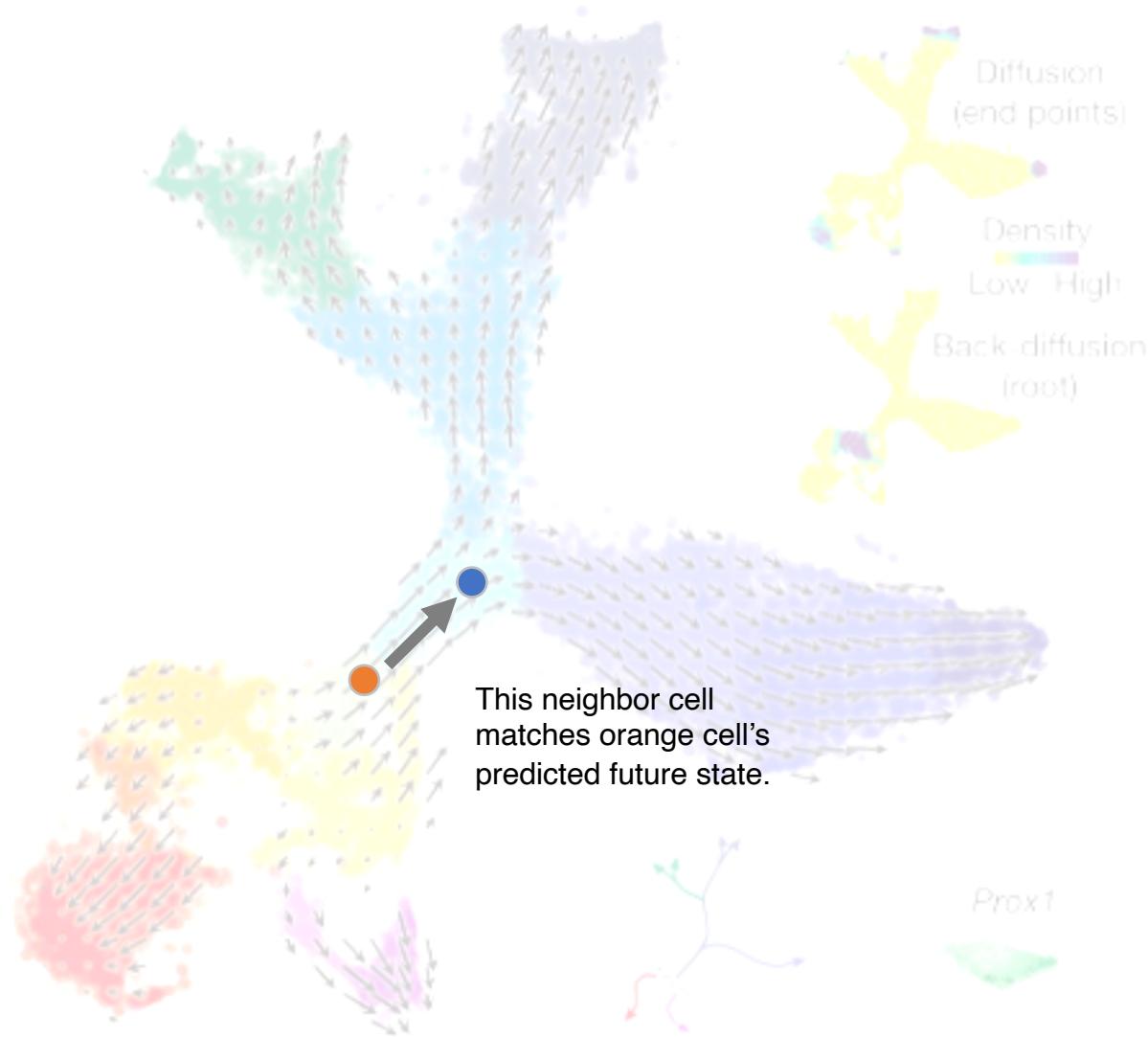
Induction: toward an increase in expression



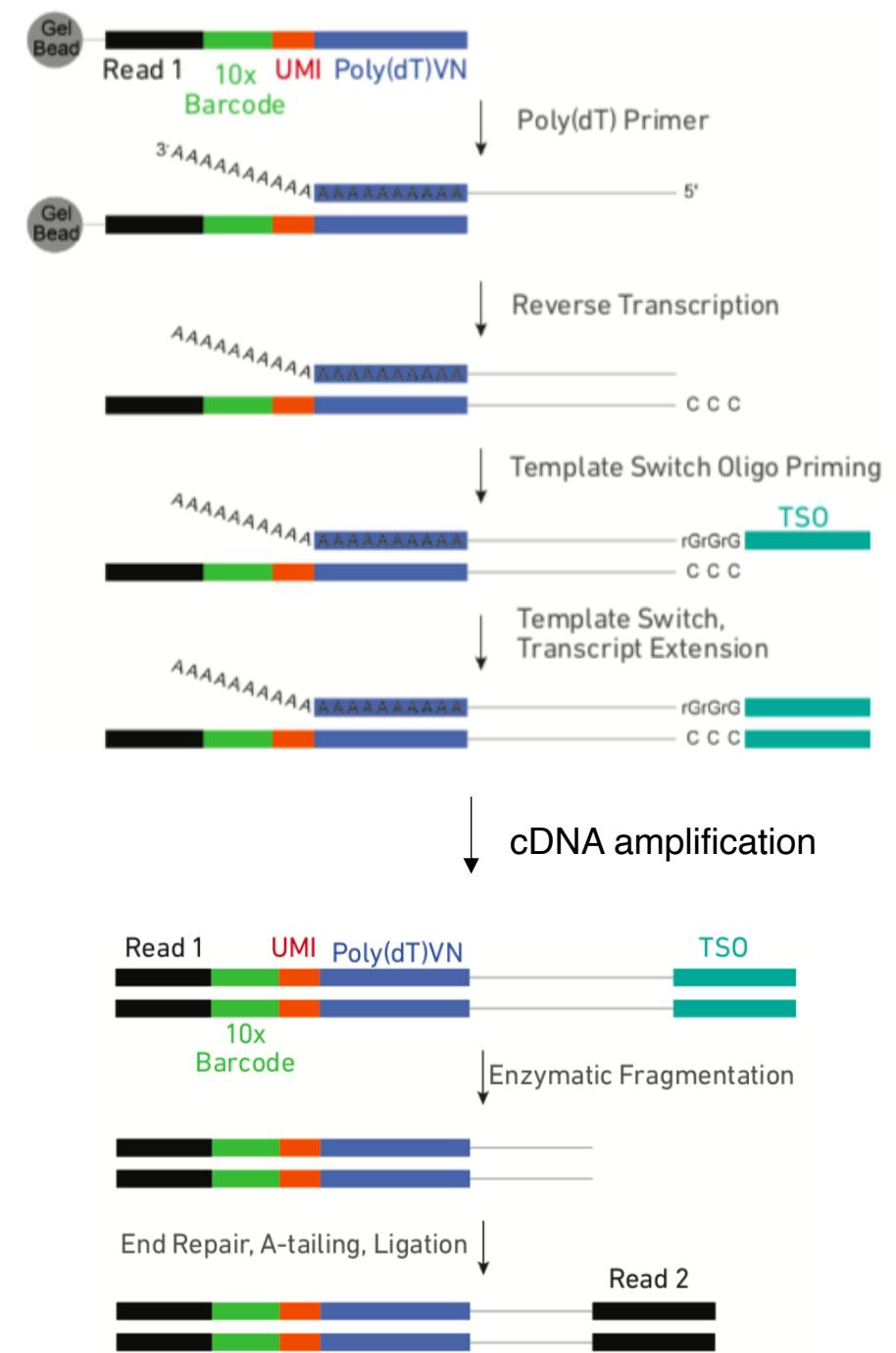
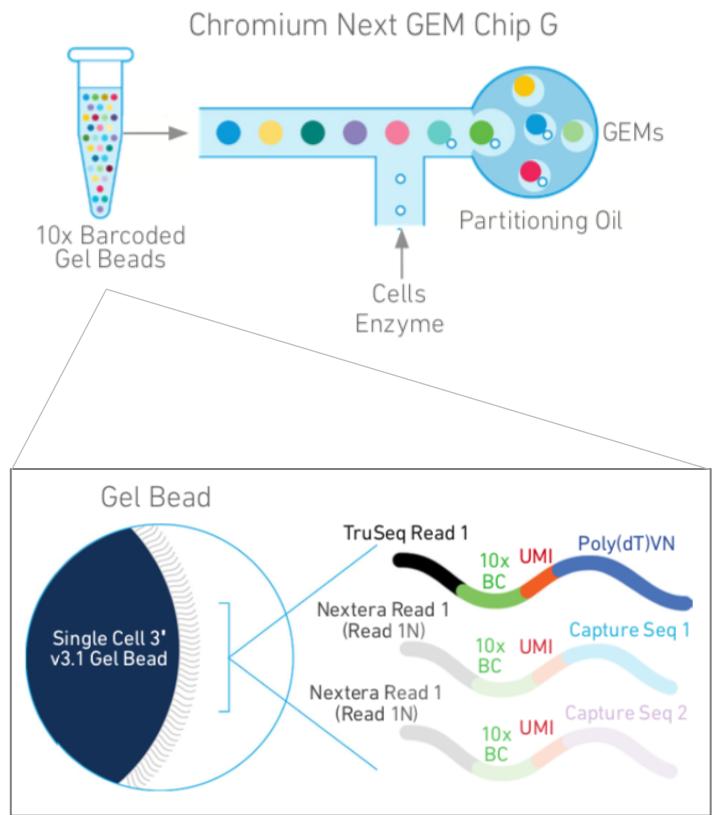
Repression: toward a decrease in expression



The similarity of the extrapolated cell states to cells in local neighborhood could be visualized as local averaged vector fields



10X genomic workflow sequences only the mRNA 3' ends. Thus, only limited splicing information is used in the velocity analysis.



Analysis of the BM-32 samples

1. Re-alignment of sequencing results to capture both spliced and un-spliced information (HPCC)
2. Velocity analysis and visualization (R)
 - BMY
 - BMR
 - BMS
 - BMY+BMR

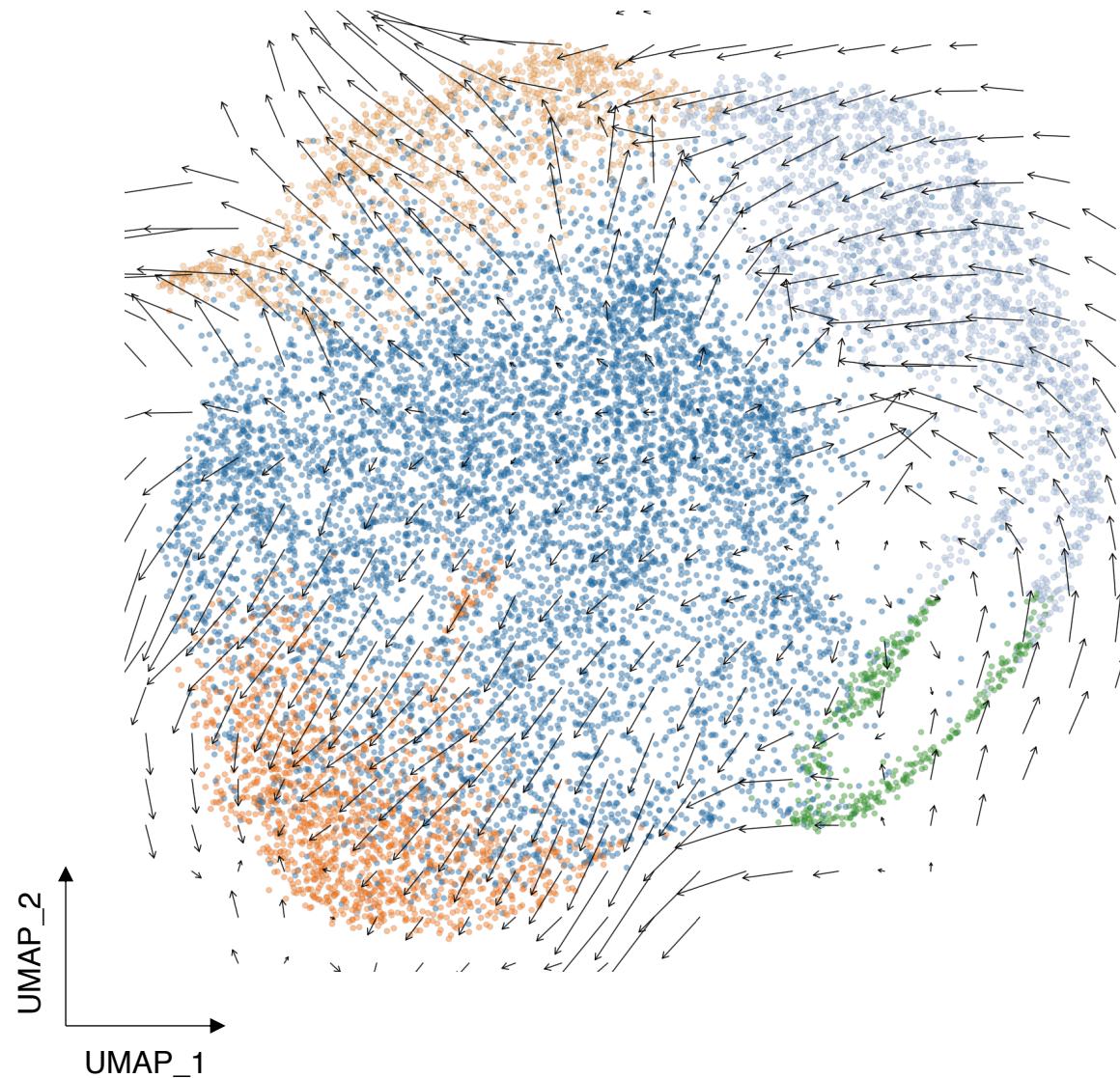
* Analysis included only cells defined in Iteration 3, min.feature=2000 analysis.

** BMY + BMR analysis was reaching the limit of my laptop workstation.

grid.n: number of grid points along each axis

BMR-32

grid.n = 25



grid.n = 50

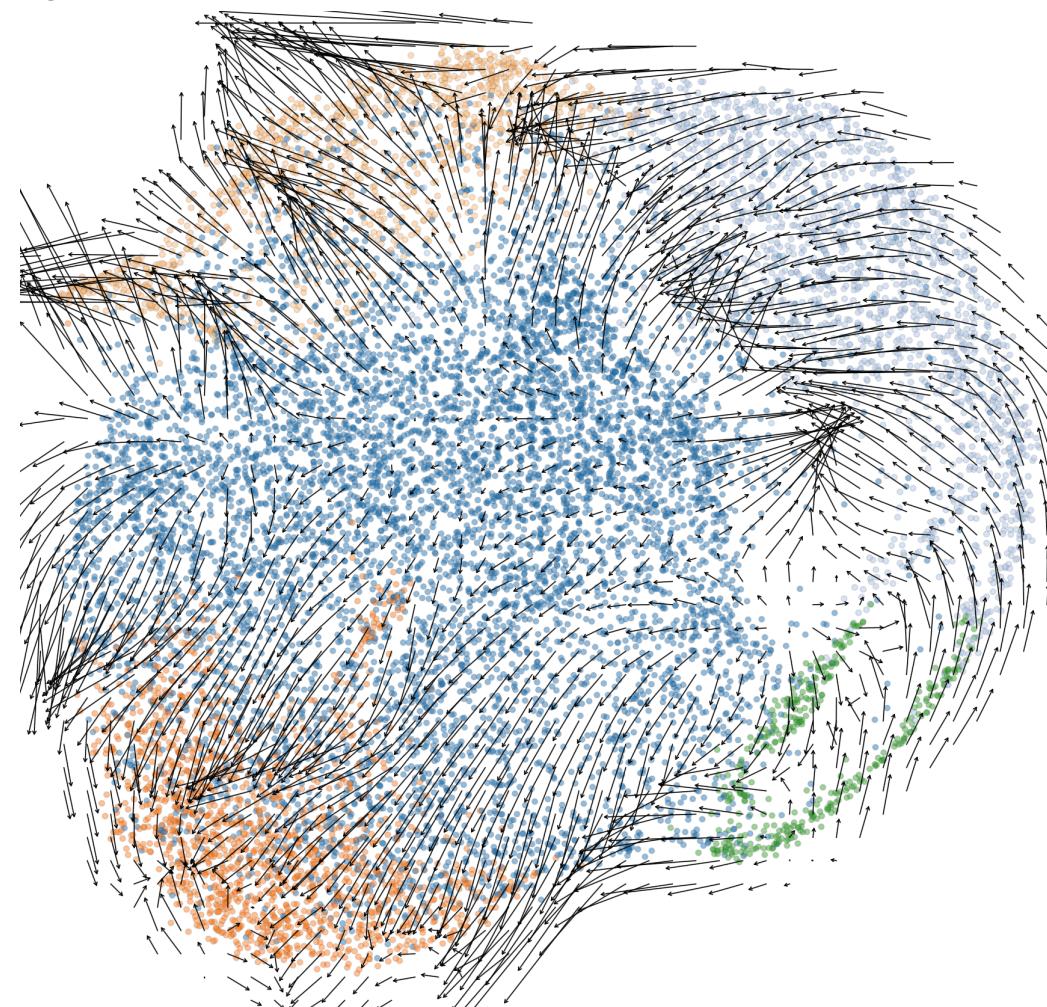
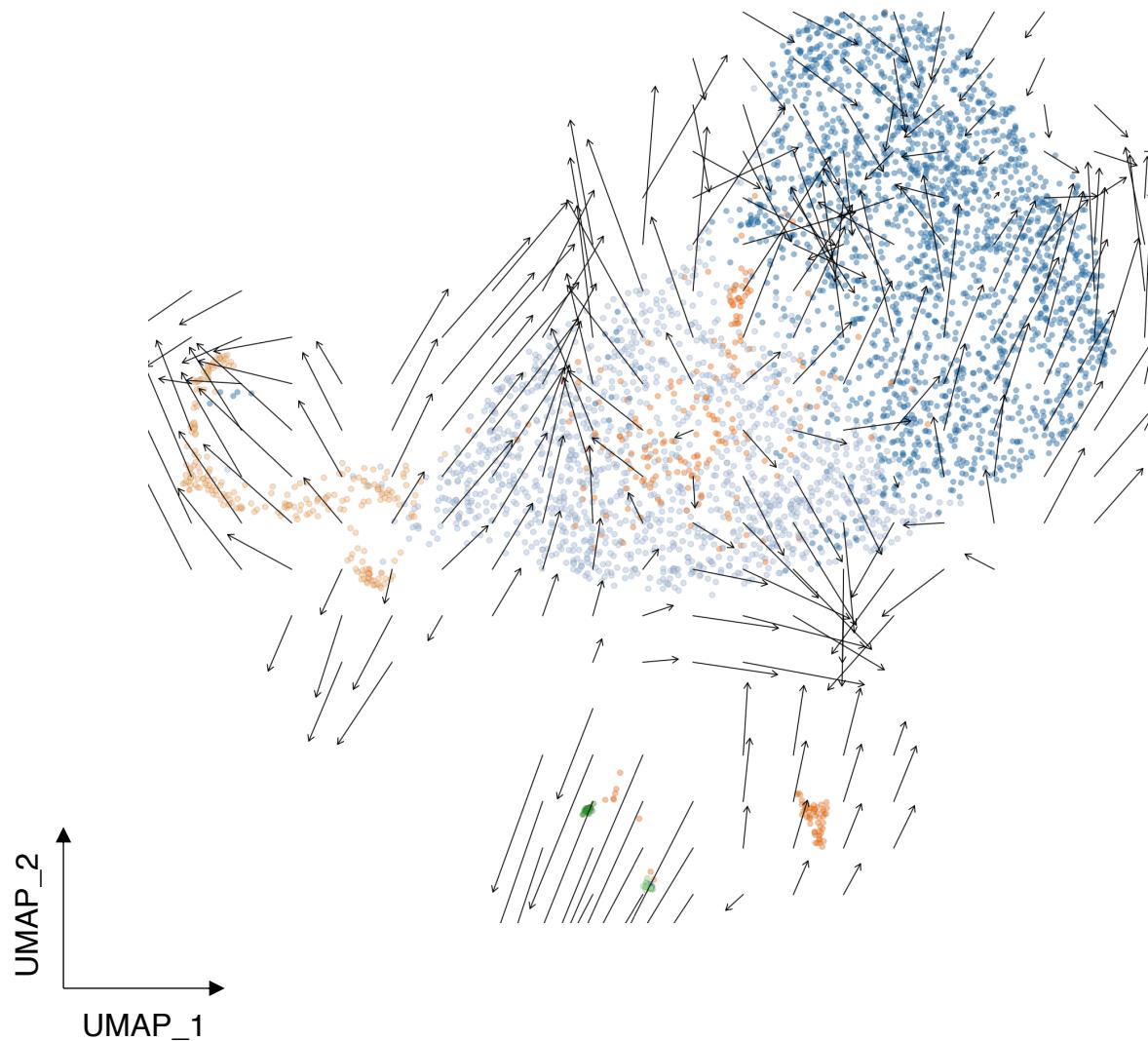


Figure PDF with higher resolution rendering is available in the Dropbox Iteration 4 folder

grid.n: number of grid points along each axis

EMY-32

grid.n = 25



grid.n = 50

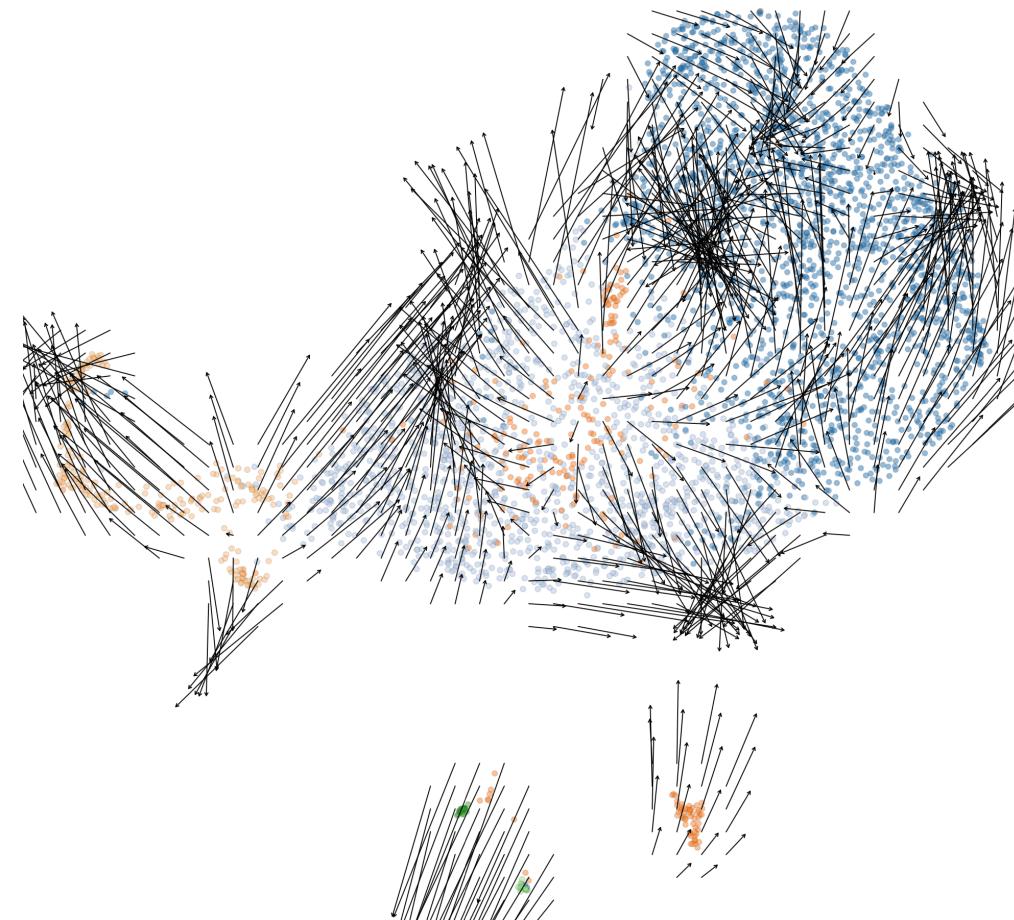
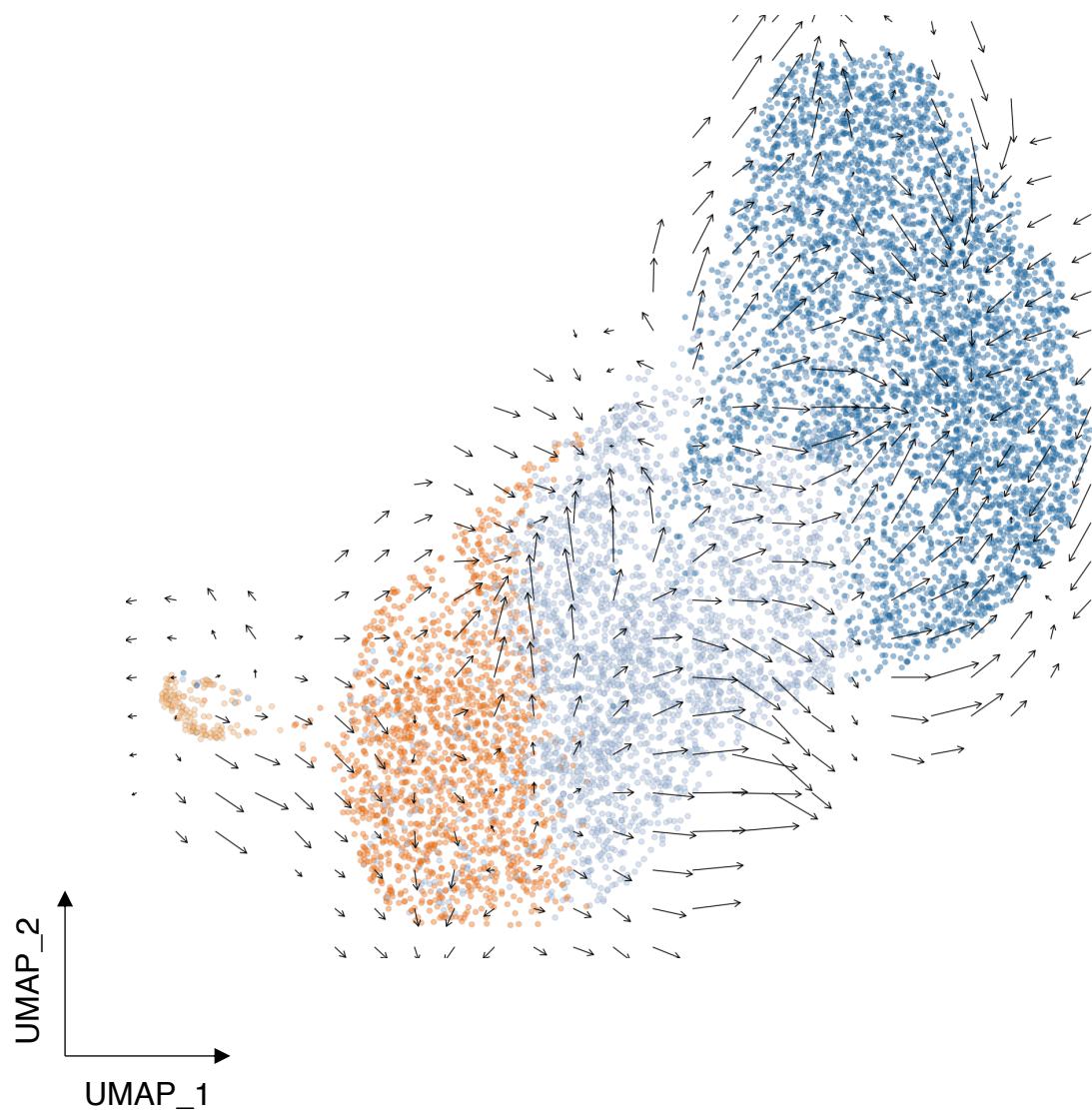


Figure PDF with higher resolution rendering is available in the Dropbox Iteration 4 folder

grid.n: number of grid points along each axis

BMS-32

grid.n = 25



grid.n = 50

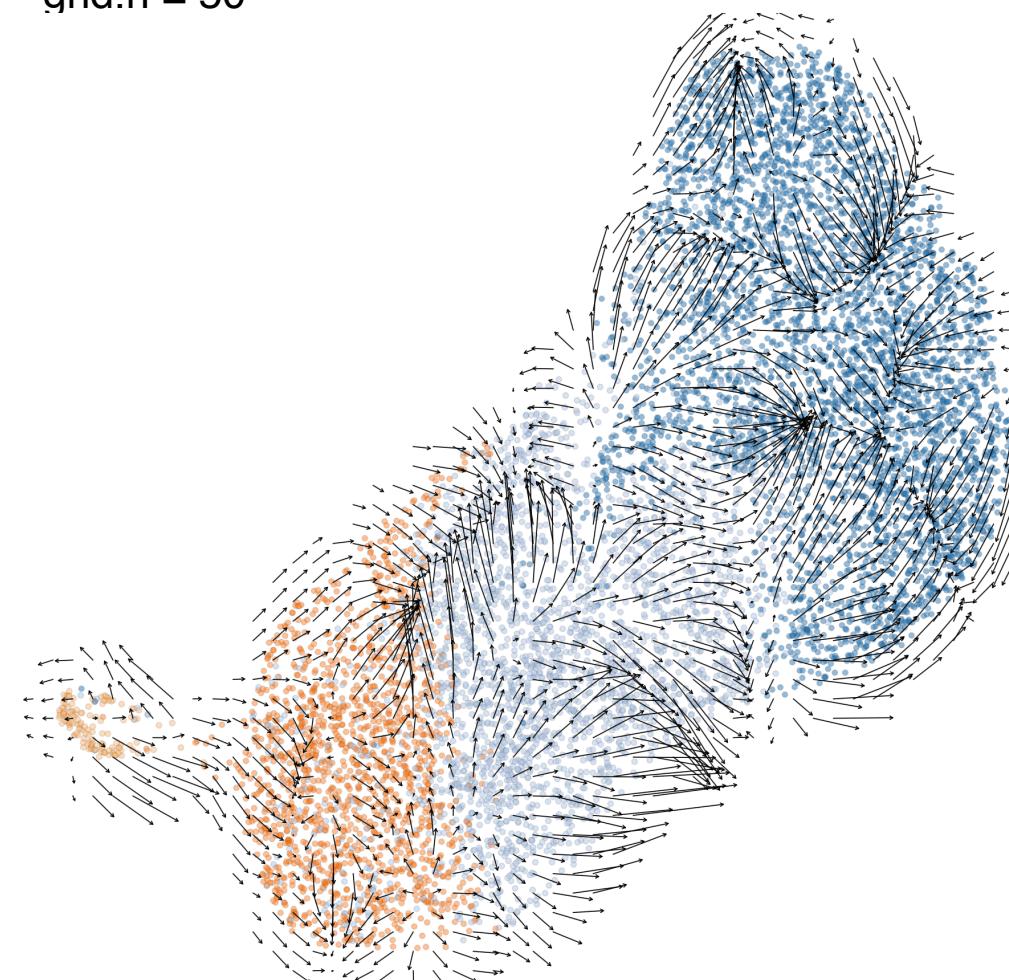
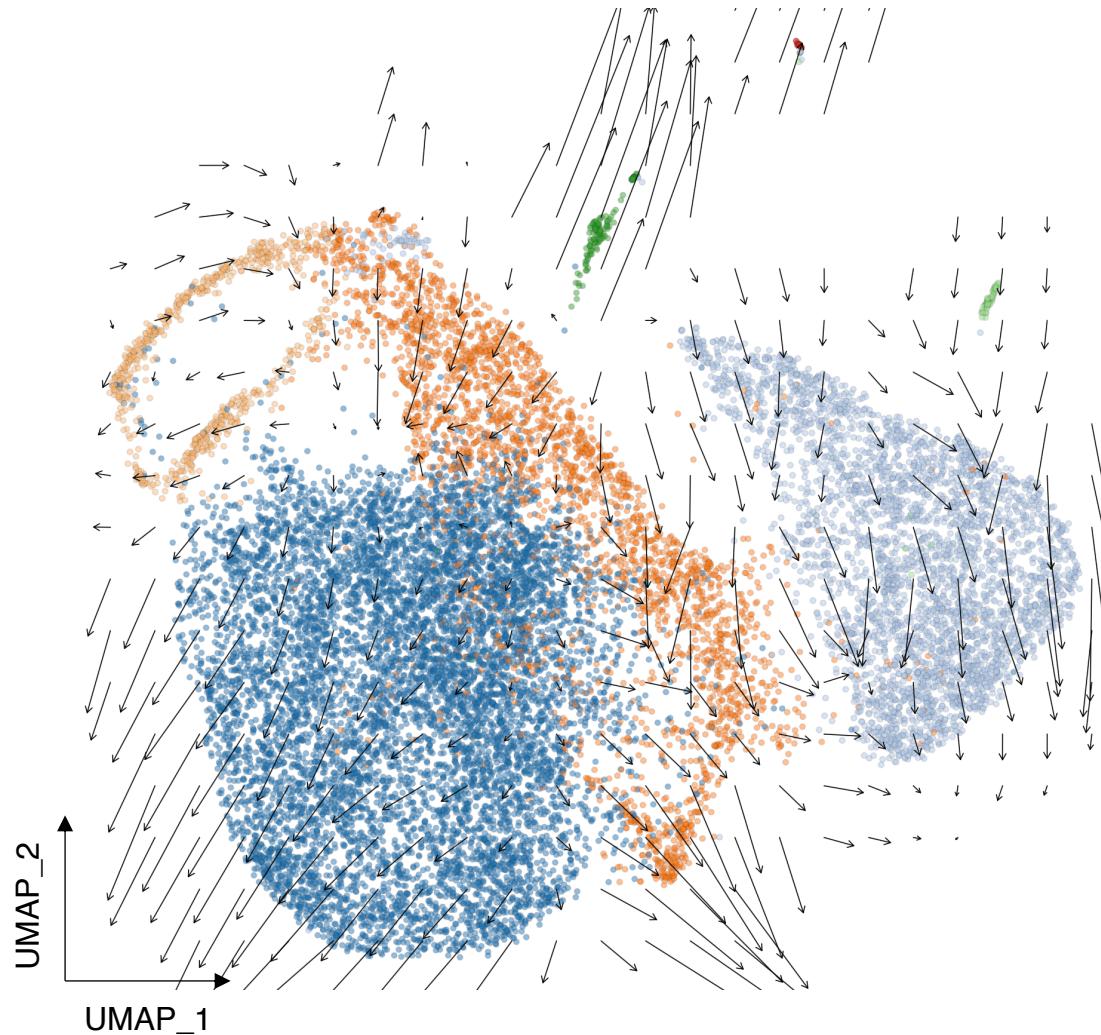


Figure PDF with higher resolution rendering is available in the Dropbox Iteration 4 folder

BMY+BMR

grid.n = 25



grid.n = 50

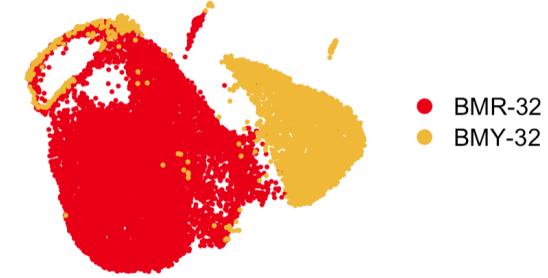
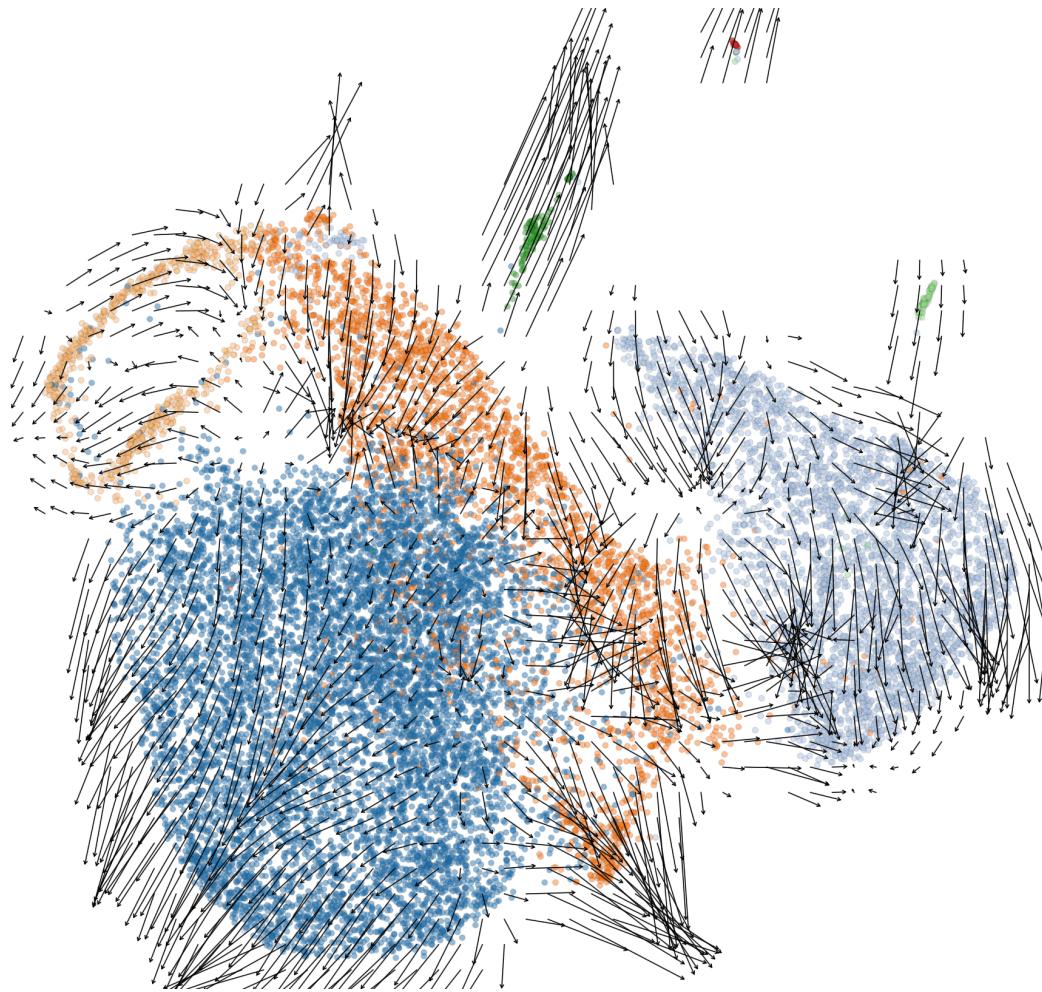
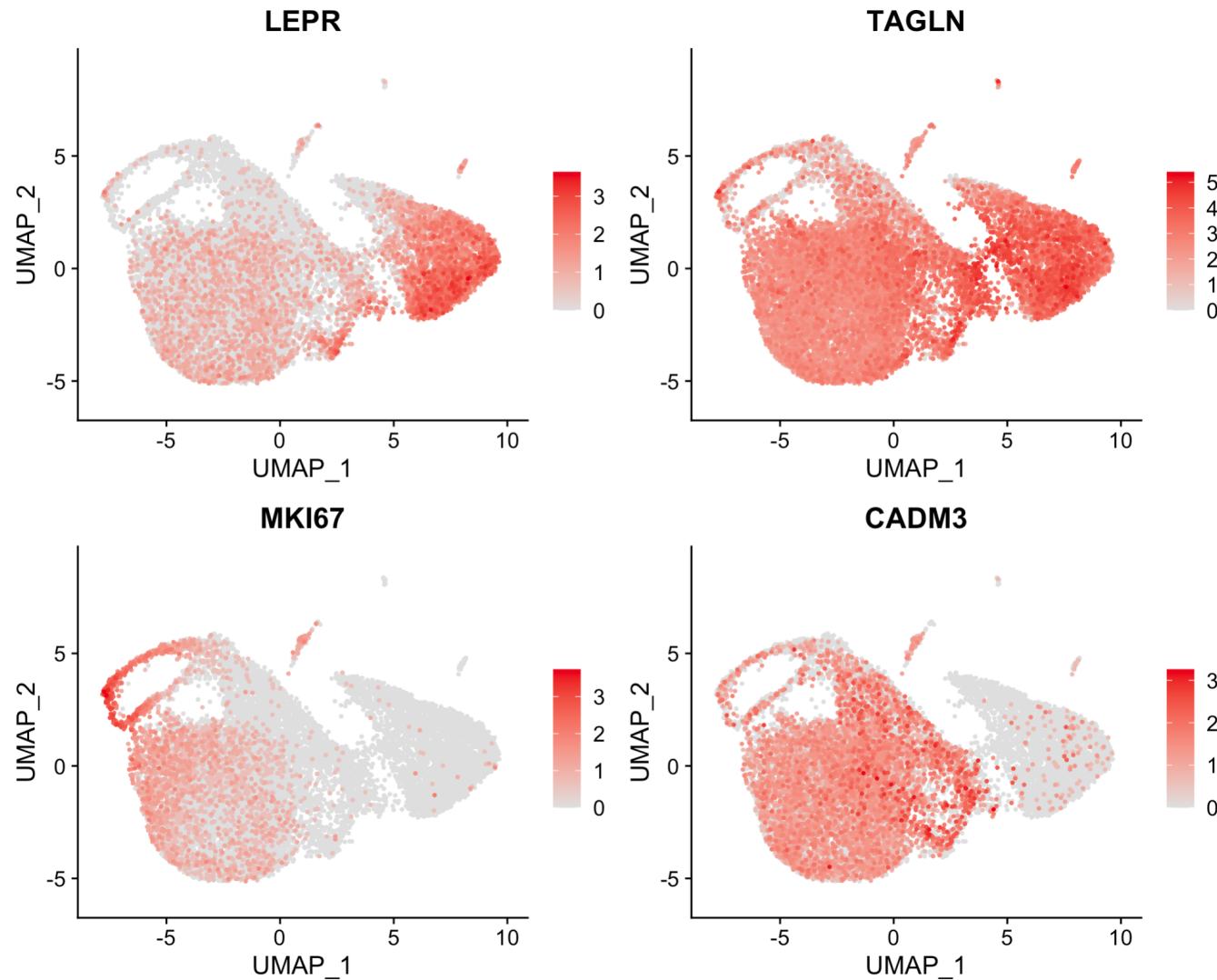
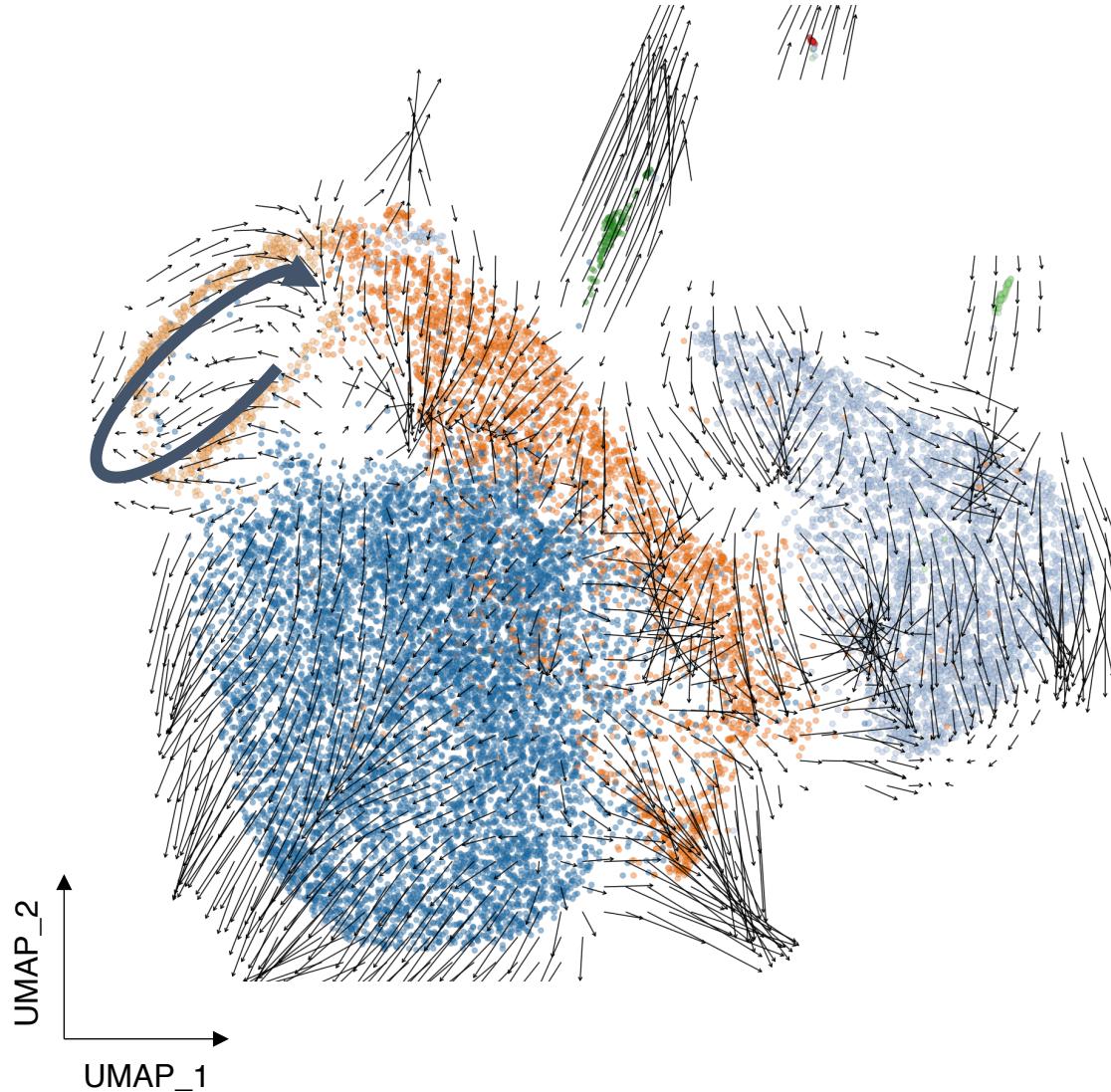


Figure PDF with higher resolution rendering is available in the Dropbox Iteration 4 folder

Selected genes in current UMAP projection



Cell cycle?



Discussion

- The BMR-BMY32 Velocity analysis produces a different projection, retains similar shape as Iteration 3 analysis.
 - The processing of velocity analysis limited to cells & genes with detected splicing. It is possible to integrate with the previously processed results if necessary.
- The trajectory prediction does not provide a clear answer to relationship between BMR and BMY.
- Arrow length are scaled / can be adjusted. All plots here were rendered using identical settings.