

TIME-COURSE SINGLE-CELL TRANSCRIPTOMIC ANALYSIS OF CONTROLLED NEURAL CONVERSION OF HUMAN iPS CELLS



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ABSTRACT

Recent progress in epigenetic reprogramming leveraged the development of new strategies in regenerative medicine, disease modeling and stem cell therapy. One key translational challenge in the field is to differentiate induced pluripotent stem cells (iPSC) in a controlled, scalable, and reproducible fashion into distinct functional cell types at high purity under chemically defined conditions. Neural induction of pluripotent cells can be achieved by small molecule inhibition of specific pathways and previous studies used microarray and bulk RNA-Seq strategies to characterize this process. However, such datasets cannot resolve how individual cells exit the pluripotent state and commit toward specific multipotent progenitor states, owing to the heterogeneous and asynchronous nature of the differentiation process. Identification and characterization of subpopulations and their lineage dynamics during differentiation is essential to define cell type signatures and optimize protocols. Here, we applied single-cell transcriptomics to analyze the transition from pluripotency to neural lineage entry by blocking BMP and TGF-beta pathways separately and in combination. By estimating gene expression correlations in differentiating cells across seven days, we identified critical state transitions. We constructed an unsupervised pseudotime model and clustered cells by nonparametric tSNE to identify significantly differentially expressed genes between differentiating subpopulations. Dissection of critical transition points suggests the existence of a stepwise regulatory process towards neural commitment in relationship to cell cycle states.

METHODS

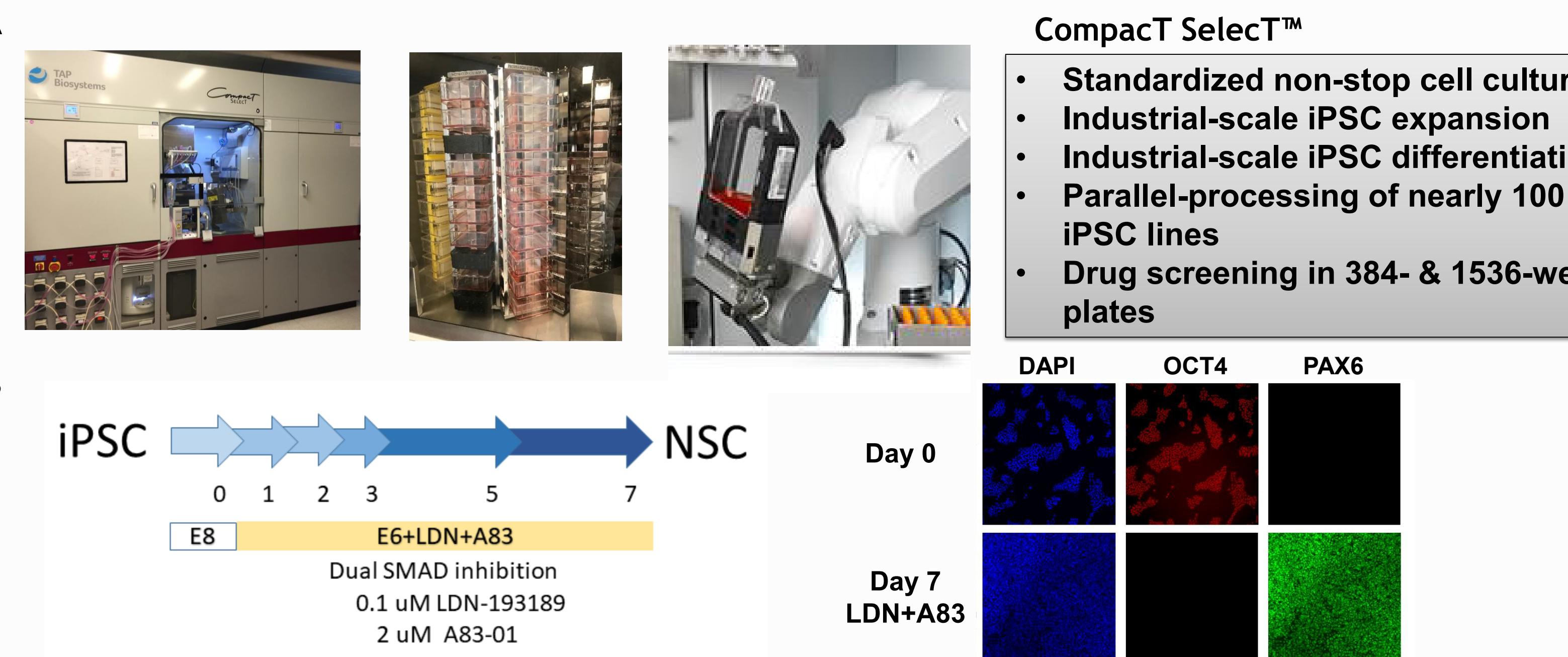


Figure 1. Fully automated and efficient neural stem cell (NSC) differentiation of induced pluripotent stem cells (iPSCs).

(A) Automated cell culture using CompacTSelect™ reduces human bias/technical variation in daily cell culture work and produces high quality iPSCs for translational research. (B) Highly efficient neural conversion of iPSCs by inhibition of TGF-beta and BMP signaling ("dual SMAD inhibition"). Immunostaining for OCT4 (also known as POU5F1; red) and PAX6 (green) expression of cells at Day 0 and Day 7 documents highly efficient neural differentiation. Cells were collected at different time points during the differentiation process for transcriptomics analysis using droplet-based single-cell RNA sequencing (scRNA-Seq) technology (ddSEQ and Illumina NextSeq 500).

RESULTS

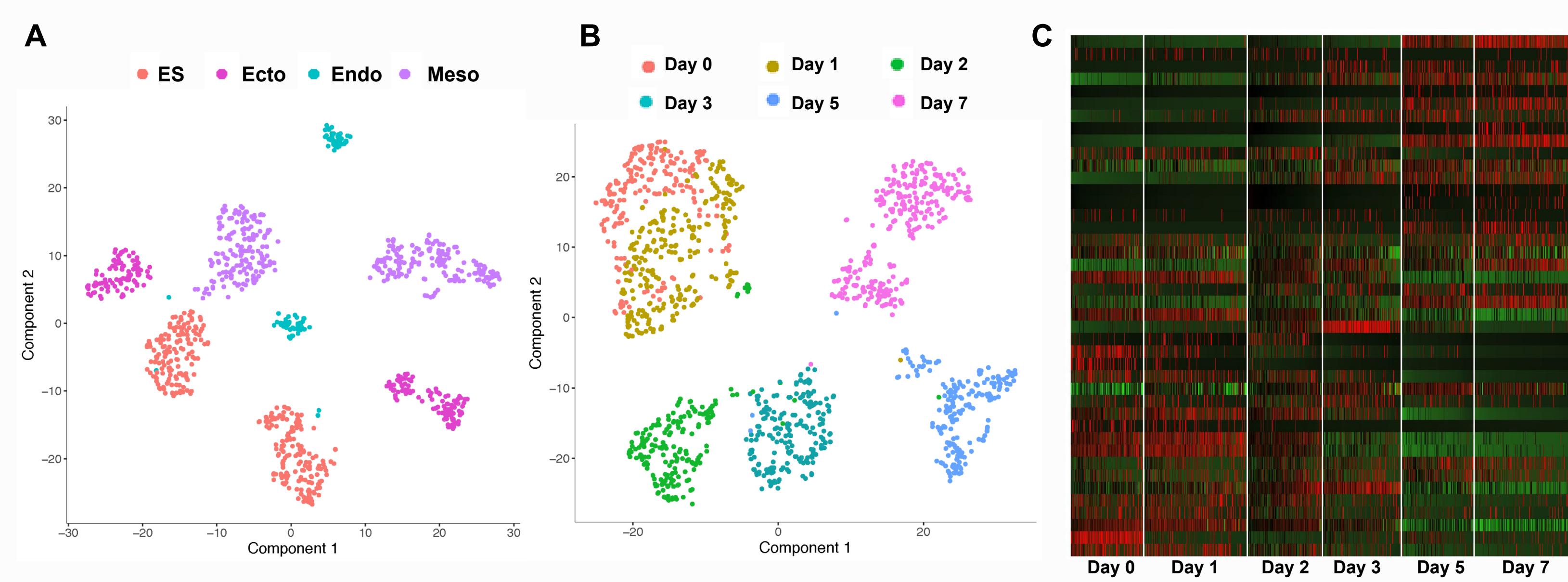


Figure 2. Single-cell RNA Sequencing (scRNA-Seq) of iPSCs and controlled differentiation into the three primary germ layers (ectoderm, mesoderm, endoderm).

(A) tSNE plot (dimensionality reduction algorithm) reveals distinct subpopulations of iPSCs and differentiated cells representing ectoderm, endoderm and mesoderm lineages generated under chemically defined culture conditions. Each dot represents a single cell and is colored according to the differentiation state. (B) Time-course scRNA-Seq analysis of neural differentiation. (C) Heatmap of population dynamics changes during differentiation (Day 0-7) displaying expression profiles of lineage-specific genes at single cell resolution.

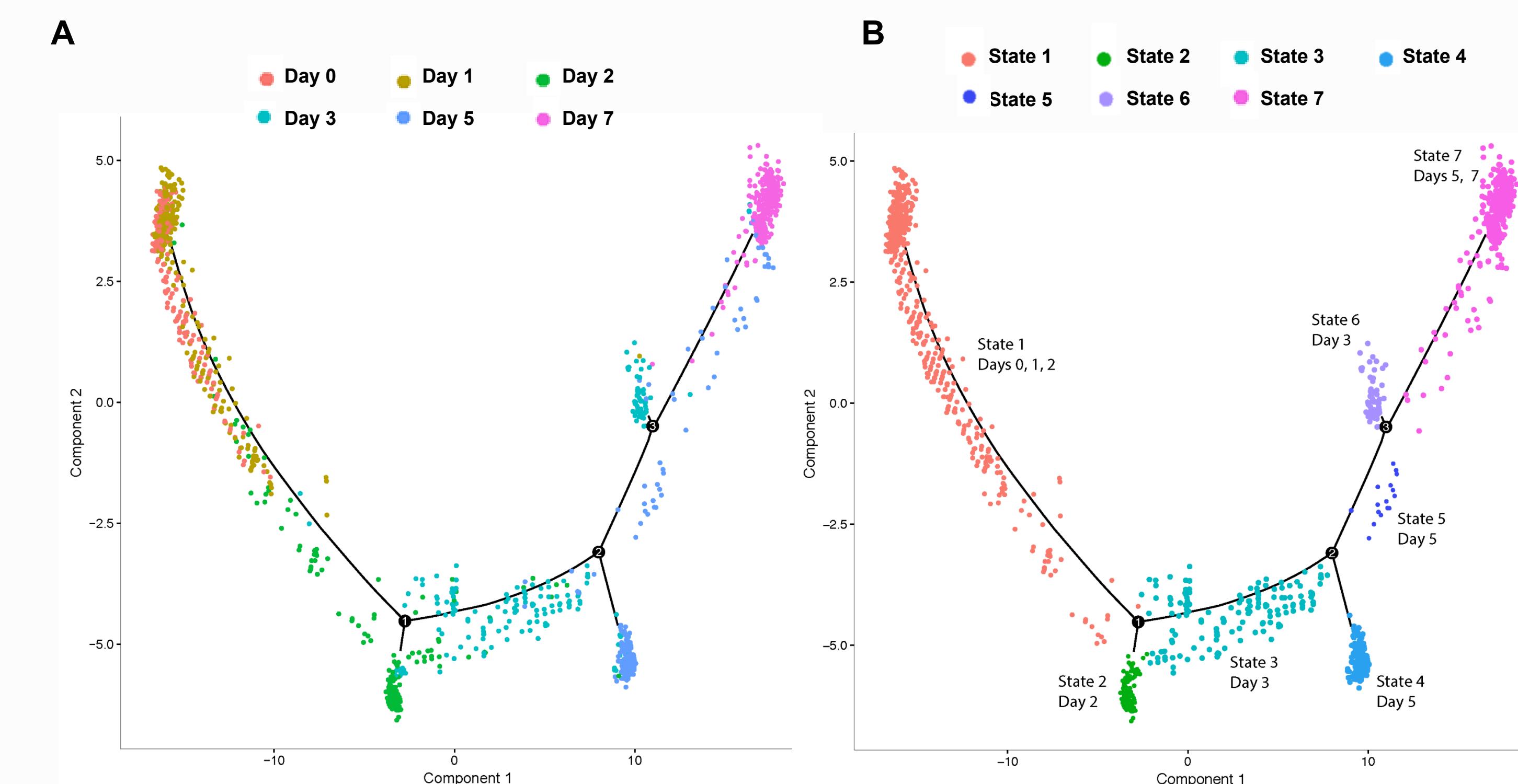


Figure 3. Pseudo-temporal ordering of time-course scRNA-Seq data reveals transition states and branching events during neural differentiation.

(A) Unsupervised pseudo-time ordering (Monocle¹) of all cells during the differentiation time-course allows detection of differentiation trajectory and branch points during cell fate commitment. (B) Trajectory analysis allows reconstructing data-driven assignment of transition and branching states during differentiation to discover critical regulators and pathways related to cell fate decision making process.

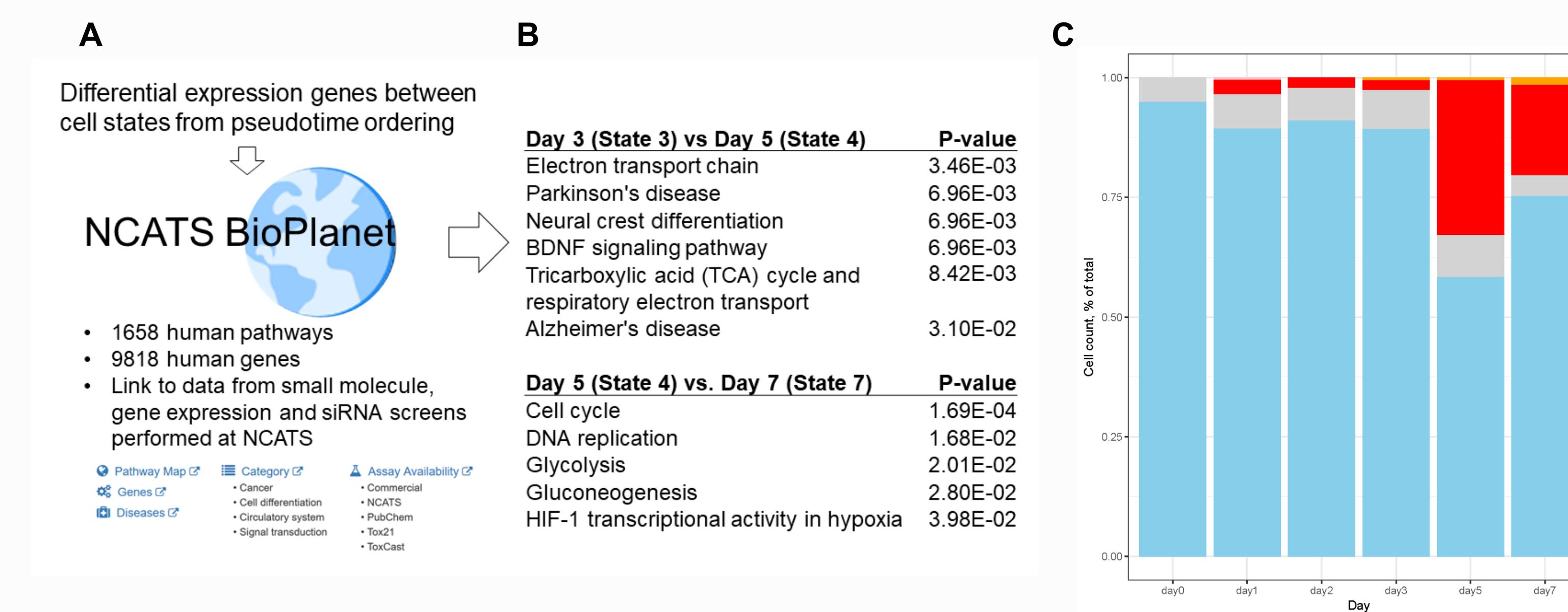


Figure 4. Identification of signaling pathways of cell fate commitment using BioPlanet and single-cell transcriptome analysis. (A) The NCATS BioPlanet² is a newly developed integrated platform for comprehensive pathways analysis. (B) Pathway analysis by BioPlanet using differential expression genes identified from pseudo-time trajectory between different transition and branching states. Glycolysis identified as a critical biological function for State 4 (Blue/Day 5) compared to State 7 (Pink/Day 5 and Day 7). (C) Analysis of cell cycle status³ reveals a significant increase in G2/M phase toward the late stages of differentiation. The cell cycle phase of each single cell was determined by scoring for signature gene sets reflecting the five phases of cell cycle.

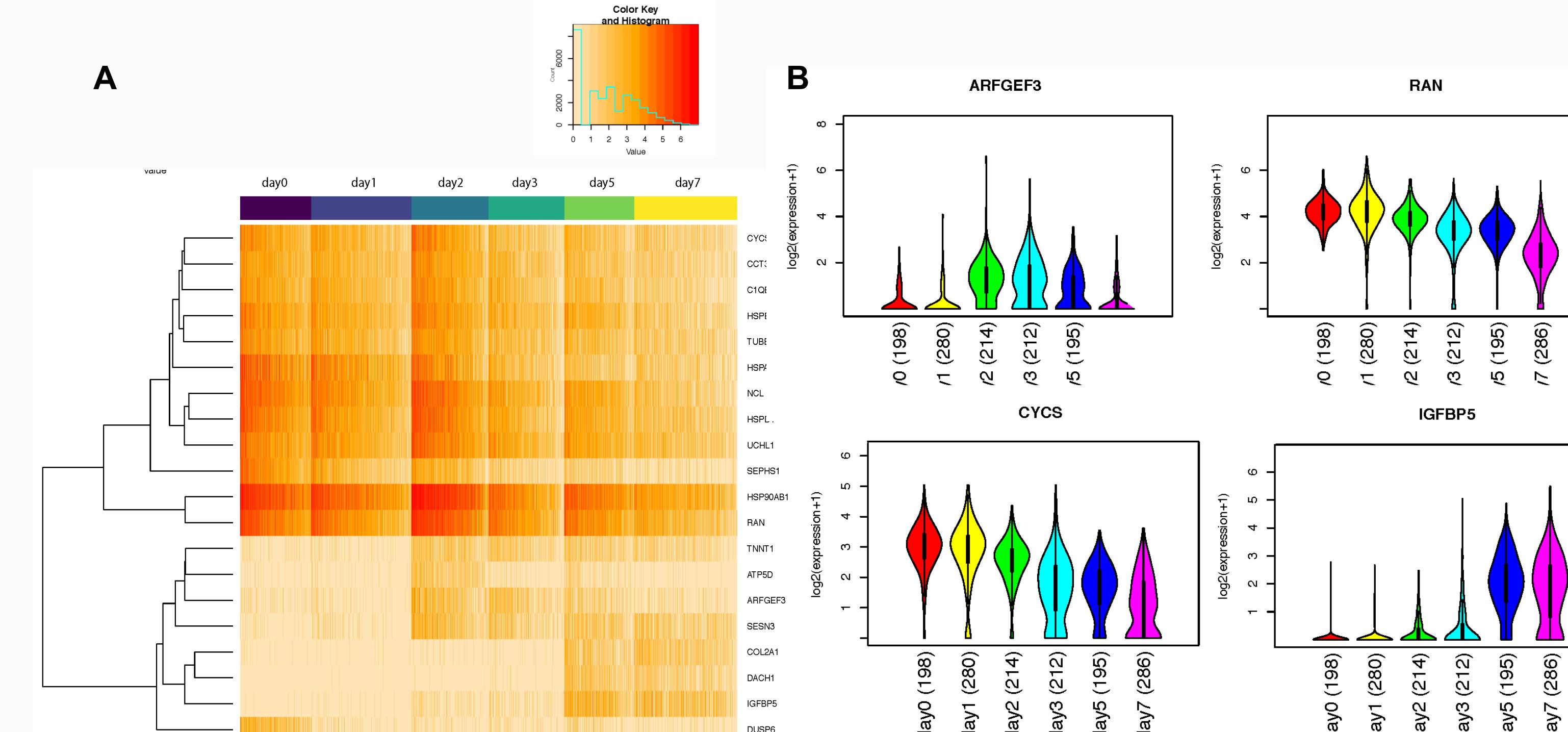


Figure 5. Detection of genes with dynamic changes over differentiation time-course using SCPattern⁴. (A) Heatmap of gene expression profiles of Top 20 genes for each single cell during differentiation. (B) Illustration of representative genes which are potentially important in regulating the differentiation process.

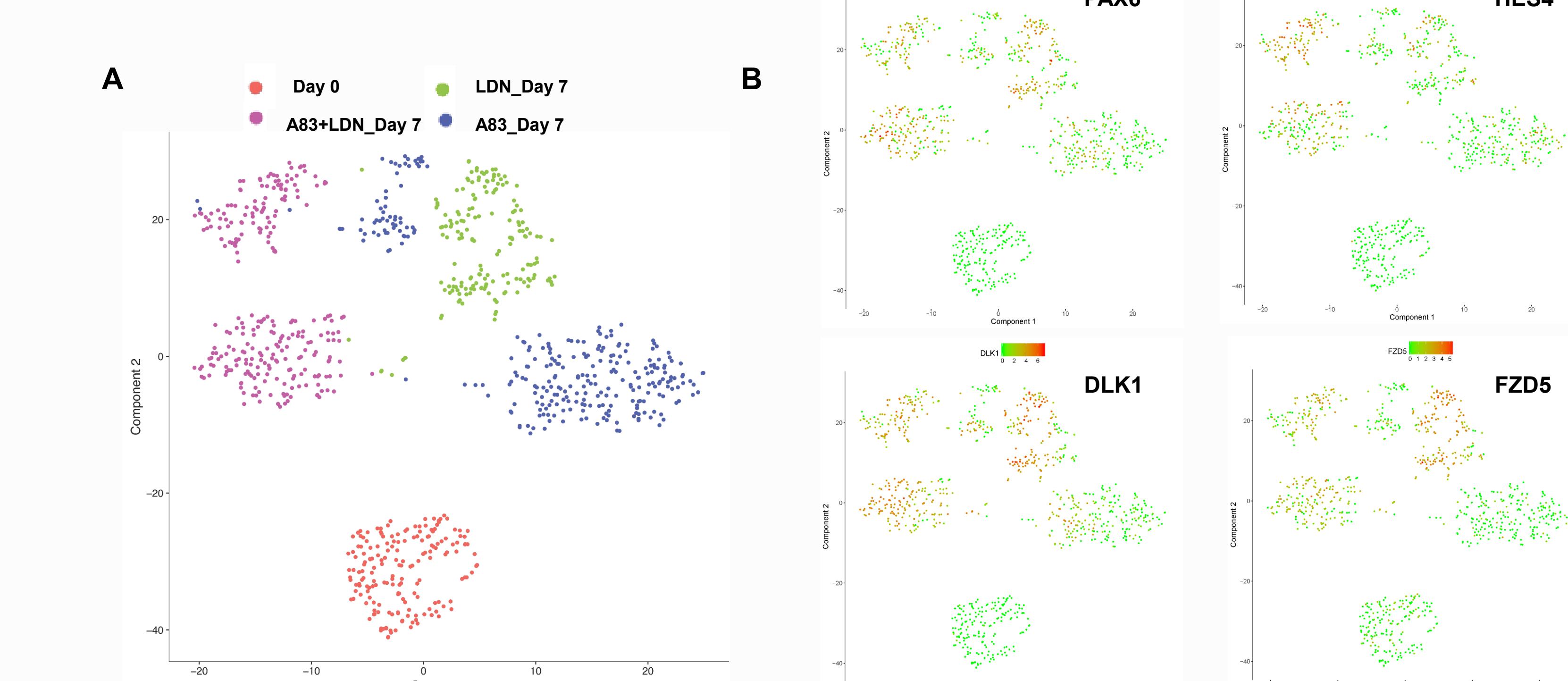


Figure 6. Principal component analysis of iPSCs and differentiated cells under different culture conditions. (A) iPSCs differentiated using A83-01 only (TGF-beta inhibition) or LDN-193189 only (BMP inhibition) show distinct transcriptomes compared to combination treatment ("dual SMAD inhibition"). (B) iPSCs differentiated by A83-01 only show lower expression of neural transcription factor PAX6 as compared to other conditions.

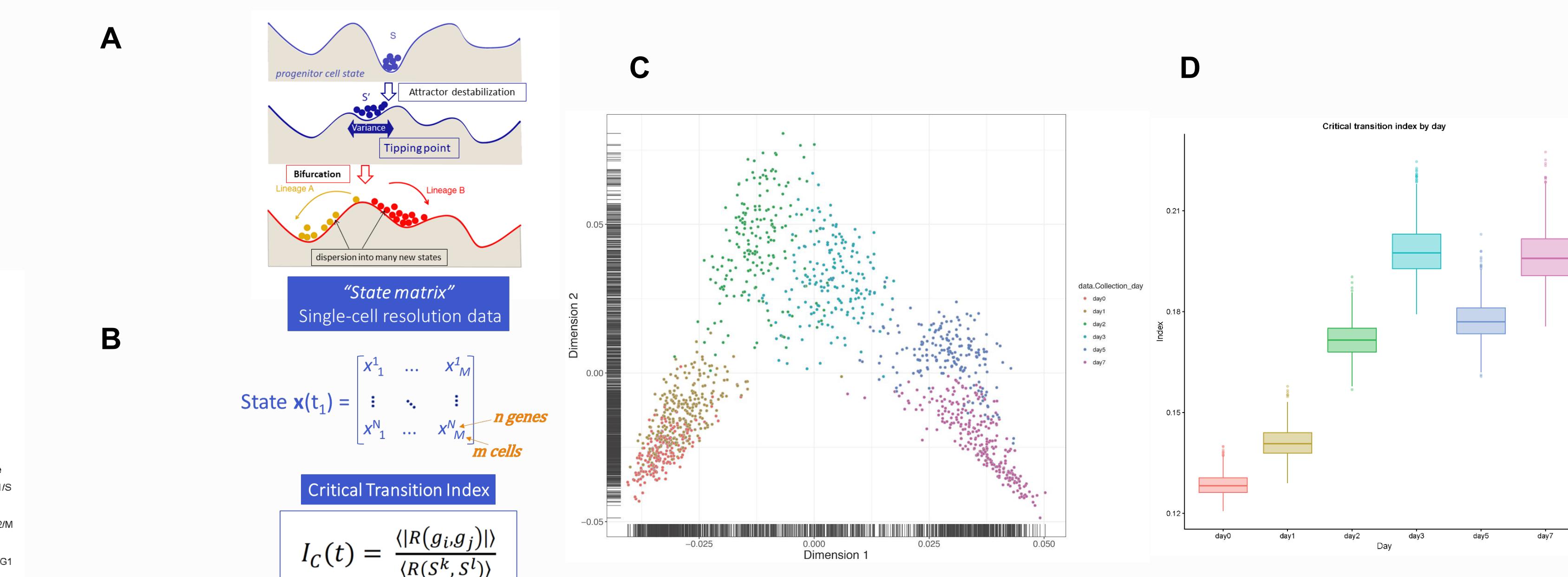


Figure 7. Identification of critical transitions to delineate regulators of neural lineage commitment. (A) Phenomenological description of critical transitions in cellular systems: Representation of cellular states before and after critical transitions or tipping points. Upon perturbation, the attractor state is destabilized and the cellular system moves towards a tipping point. This critical transition is marked by increase in gene-to-gene correlations and decrease in cell-to-cell correlations. After critical transition, the cells move to new stable attractor states. (B) Diffusion maps (non-linear dimension reduction method) was used to reconstruct and visualize the cell state trajectories in gene expression state space for 1,273 single cells based on gene expression. Each dot is a single cell and color represents the day of collection during differentiation. (C) A quantitative signature of critical transitions was derived from principles of non-linear dynamical systems. An increase in critical transition index (I_c) a destabilized state governed by underlying gene regulatory networks. (D) A significant increase in I_c value at Day 3 and Day 7 marks impending state transitions, allowing the identification of potential key regulators of lineage commitment.

SUMMARY

- Demonstrate highly efficient and quality controlled automation system for iPSC culture and differentiation.
- Time-course scRNA-Seq experiment reconstructs the neuronal progenitor differentiation process and identify key signaling pathways for cell fate decision-making, including glycolysis, TCA cycle, hypoxia and BDNF signaling.
- SCPatter identifies genes with dynamic changes through differentiation course.
- Critical transition analysis reveal an important time window during differentiation and identify key regulators for cell fate decision.

Reference :

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