

Spherical Manifolds Capture Drug-Induced Changes in Tumor Cell Cycle Behavior

Olivia Wen^{1,2}, Samuel C. Wolff^{2,3}, Wayne Stallaert⁶, Didong Li⁴, Jeremy E. Purvis^{2,3,†} & Tarek M. Zikry^{2,4,5,†}

¹Department of Biology, ²Computational Medicine Program, ³Department of Genetics,

⁴Department of Biostatistics, ⁵School of Data Science and Society,

University of North Carolina at Chapel Hill, NC

⁶Department of Computational and Systems Biology, University of Pittsburgh, PA

†Corresponding Authors: jeremy_purvis@med.unc.edu, tarek@unc.edu

CDK4/6 inhibitors such as palbociclib block cell cycle progression and improve outcomes for many ER+/HER2- breast cancer patients. Unfortunately, many patients are initially resistant to the drug or develop resistance over time in part due to heterogeneity among individual tumor cells. To better understand these mechanisms of resistance, we used multiplex, single-cell imaging to profile cell cycle proteins in ER+ breast tumor cells under increasing palbociclib concentrations. We then applied spherical principal component analysis (SPCA), a dimensionality reduction method that leverages the inherently cyclical nature of the high-dimensional imaging data, to look for changes in cell cycle behavior in resistant cells. SPCA characterizes data as a hypersphere and provides a framework for visualizing and quantifying differences in cell cycles across treatment-induced perturbations. The hypersphere representations revealed shifts in the mean cell state and population heterogeneity. SPCA validated expected trends of CDK4/6 inhibitor response such as decreased expression of proliferation markers (Ki67, pRB), but also revealed potential mechanisms of resistance including increased expression of cyclin D1 and CDK2. Understanding the molecular mechanisms that allow treated tumor cells to evade arrest is critical for identifying targets of future therapies. Ultimately, we seek to further SPCA as a tool of precision medicine, targeting treatments by individual tumors, and extending this computational framework to interpret other cyclical biological processes represented by high-dimensional data.

Keywords: Manifold learning; Dimensionality reduction; ER+/HER2- Cancer.

1. Introduction

Despite promising results of CDK4/6 inhibitors for treating ER+/HER2- breast cancer, 10–20% of patients show initial drug resistance, and all patients develop resistance over time.¹ Resistance is thought to arise from the heterogeneity of molecular states in individual tumor cells, and one potential source of this cell-to-cell heterogeneity is the cell cycle. In recent years, single-cell studies have revealed that the cell cycle can show remarkable flexibility.² For example, individual tumor cells may progress through cell cycle phases with variable durations, or show altered expression levels of core cell cycle regulators.^{3–5} The ability of cells to upregulate or downregulate certain protein signaling pathways is referred to as cell cycle plasticity.

Supplementary figures

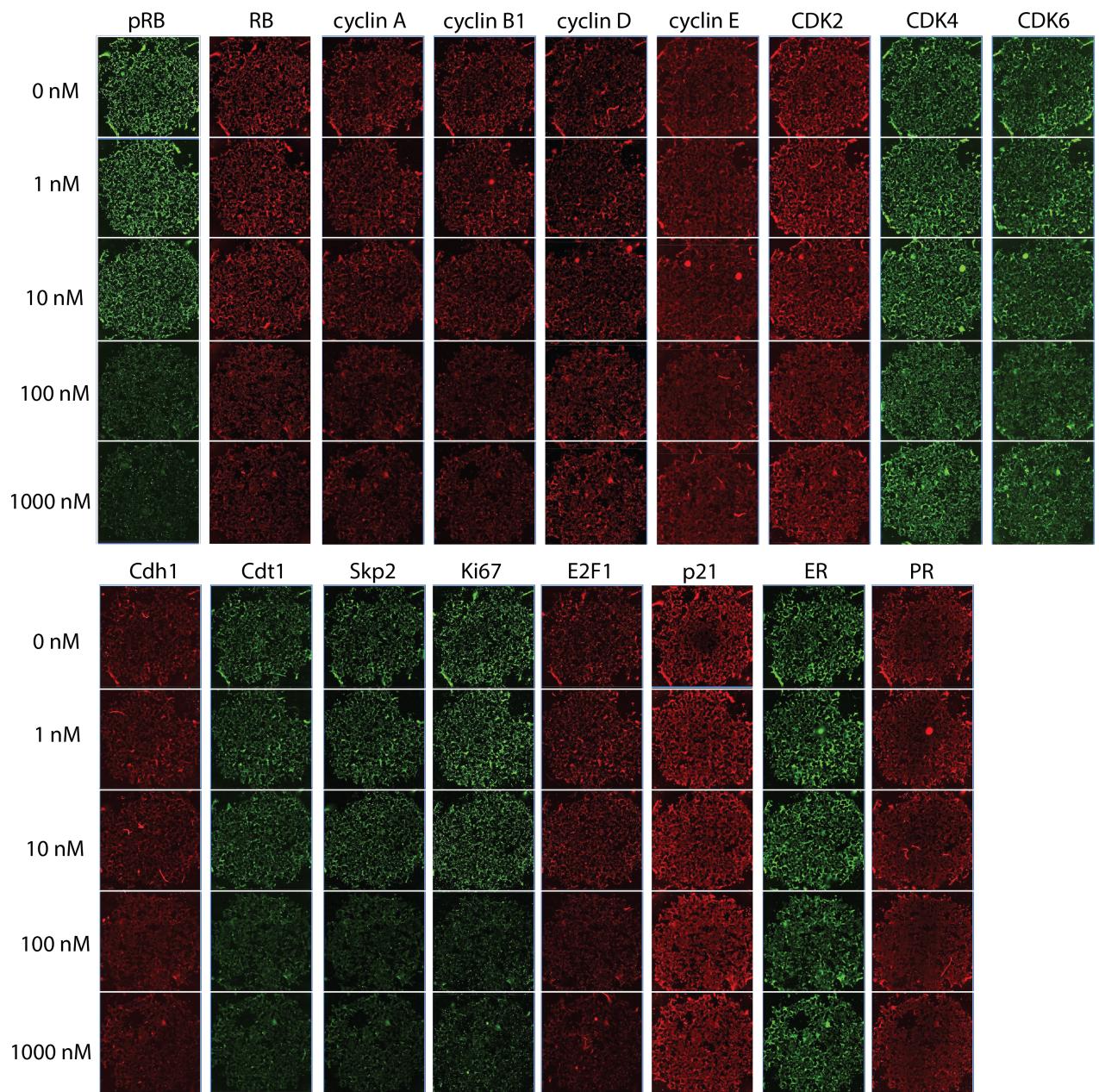


Fig. S1. Plate images of T47D tumor cells showing protein expression (immunofluorescence) across increasing doses of palbociclib.

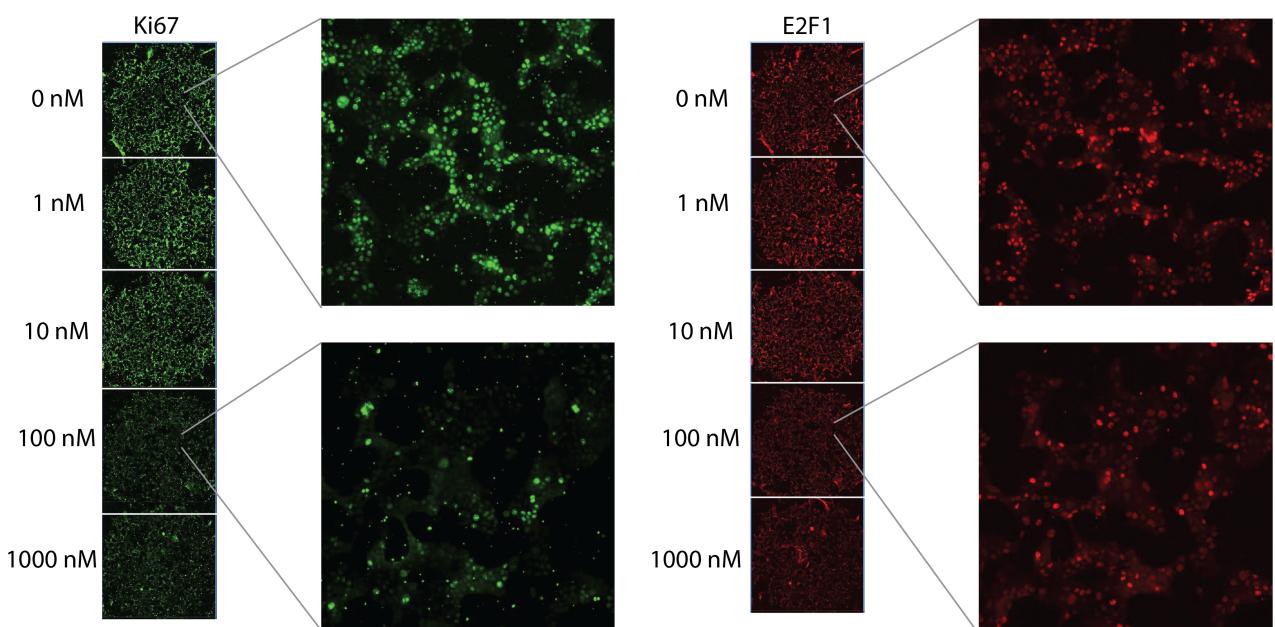


Fig. S2. Close-up single-cell views of T47D tumor cell responses to increasing levels of CDK4/6 inhibition. Decreasing population expression of Ki67 protein is visualized from decreasing immunofluorescence with higher palbociclib.

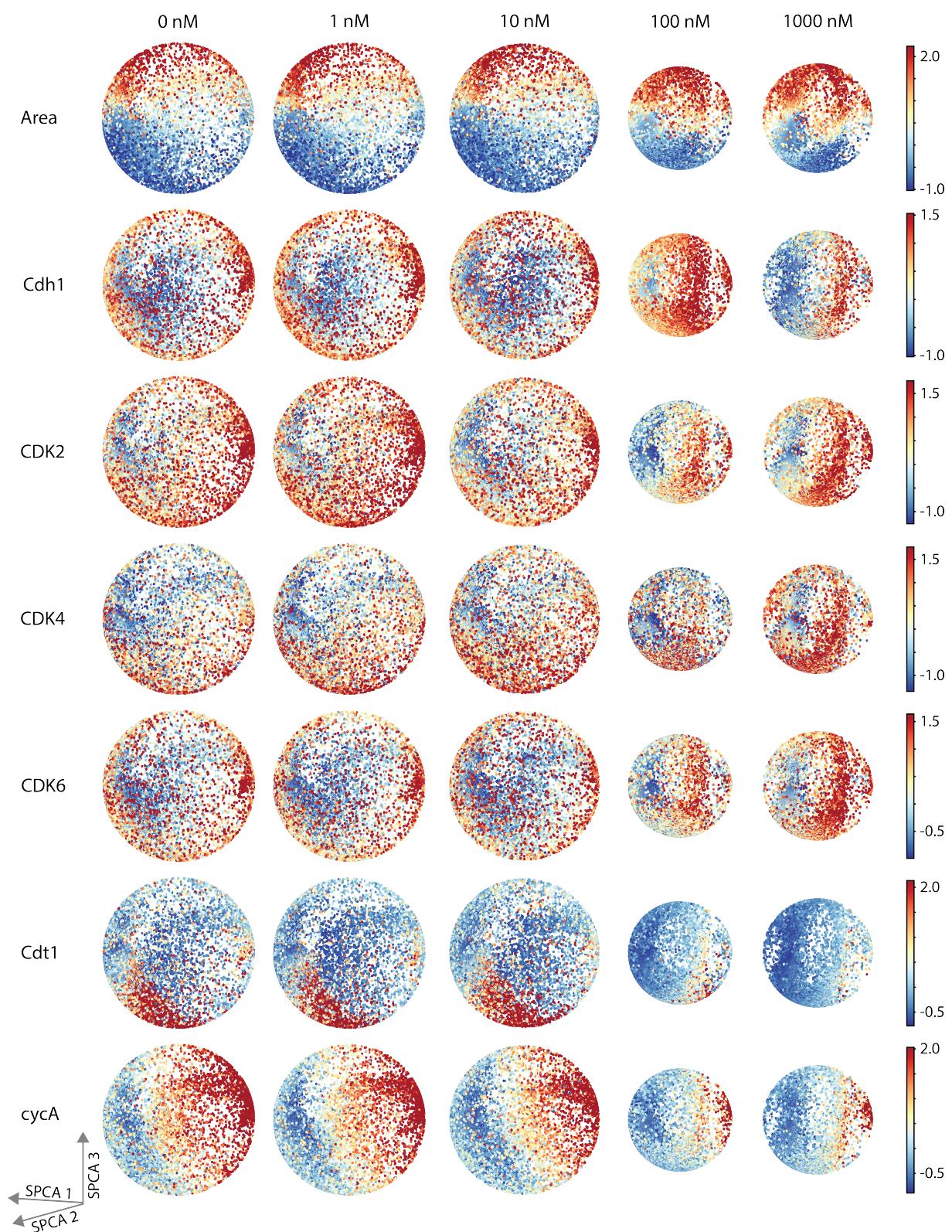


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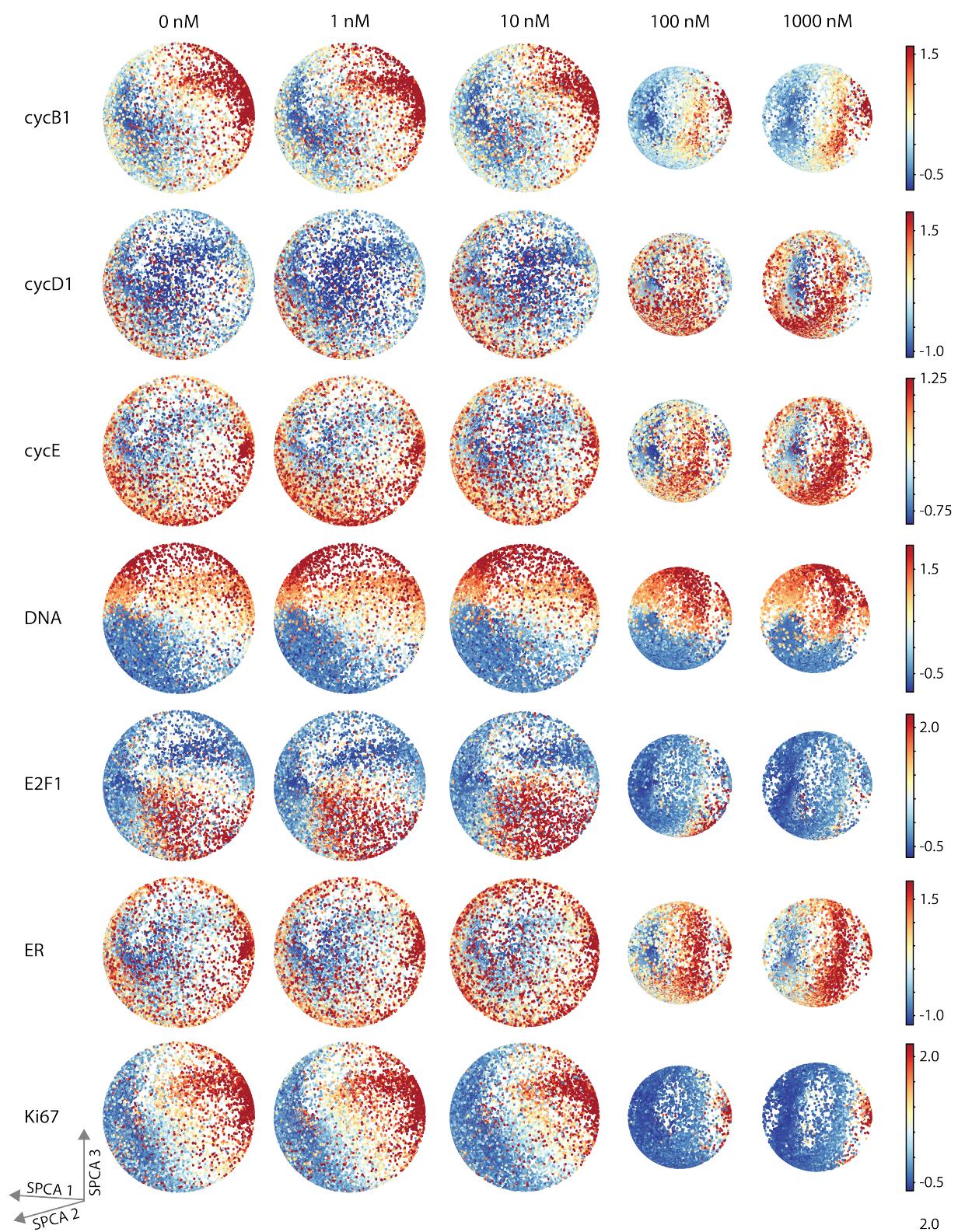


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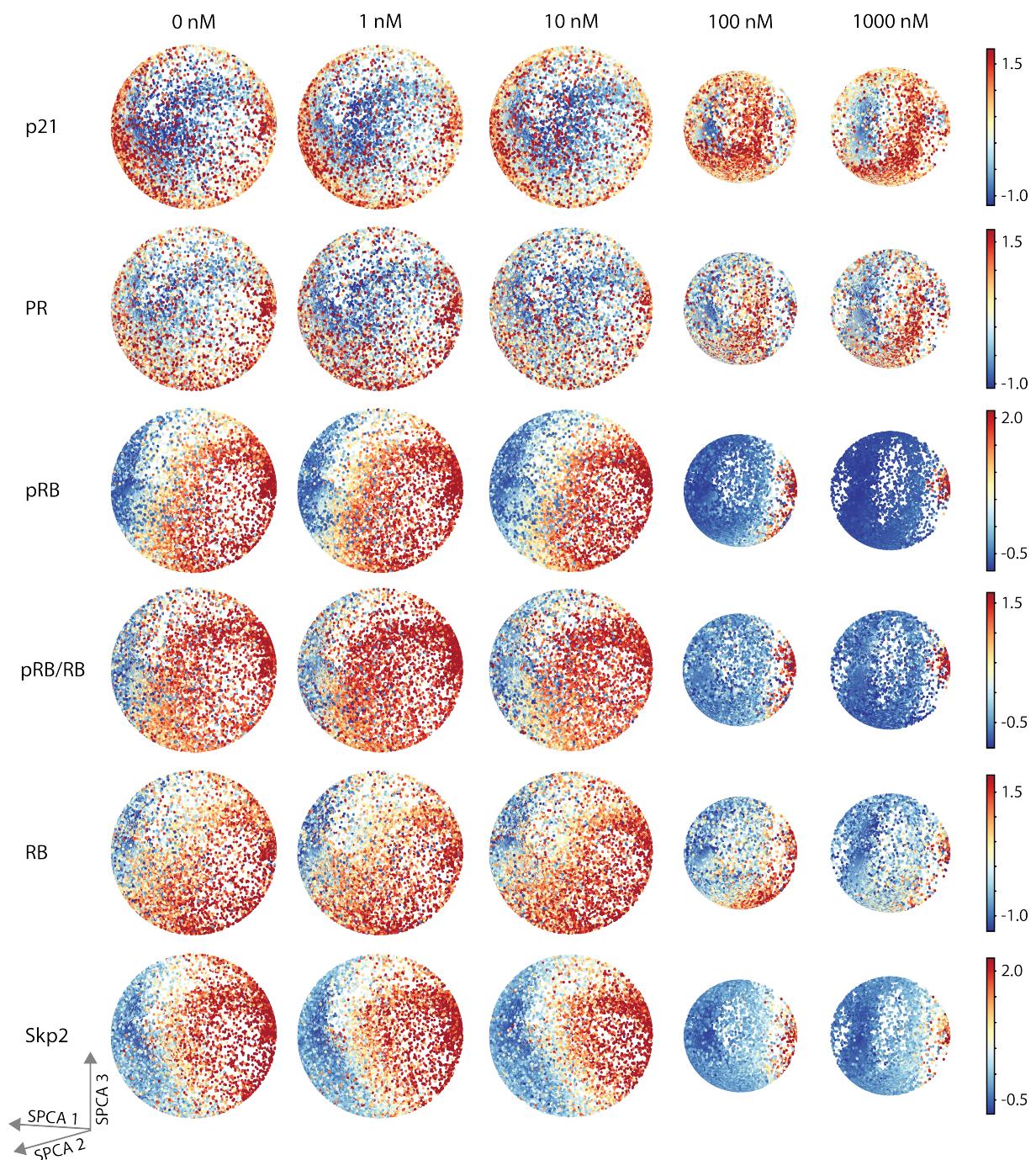


Fig. S3. SPCA captures shifts in cell cycle regulators across treatment conditions. Cells were projected onto three-dimensional spherical manifolds identified by SPCA into a shared space for each treatment condition. Points are colored according to normalized median expression level for each proteomic feature. These are the full plots first seen in Fig. 2B.

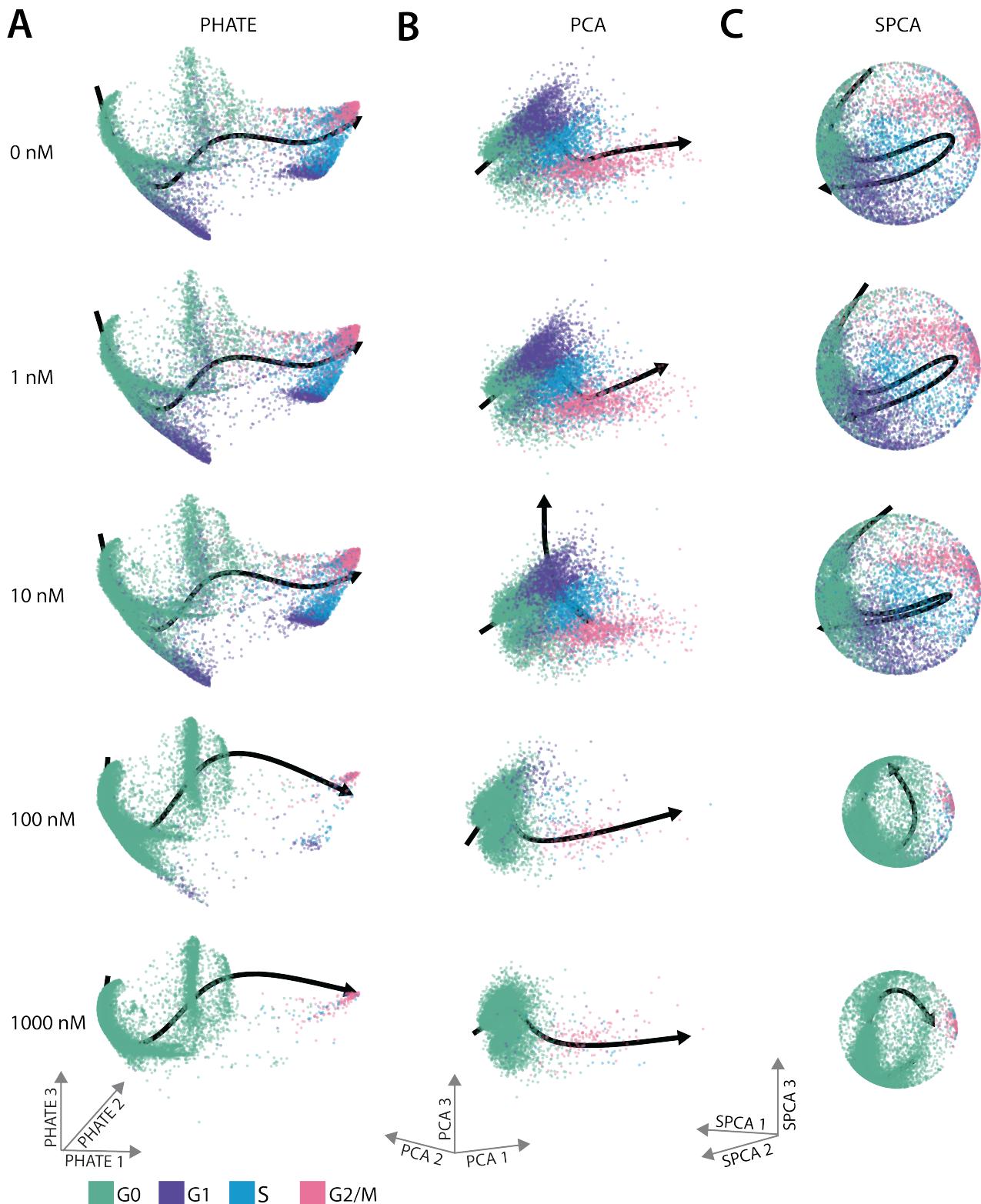


Fig. S4. Slingshot trajectories identify cyclical cell cycle paths. PHATE, PCA, and SPCA were applied to cells in each treatment condition. Data points were projected into three dimensions and colored according to cell cycle phase label. Cell cycle trajectories were identified by Slingshot and overlaid atop (A) PHATE, (B) PCA, and (C) SPCA manifold structures. These plots show all treatment conditions for the plots seen in Fig. 3A-C.

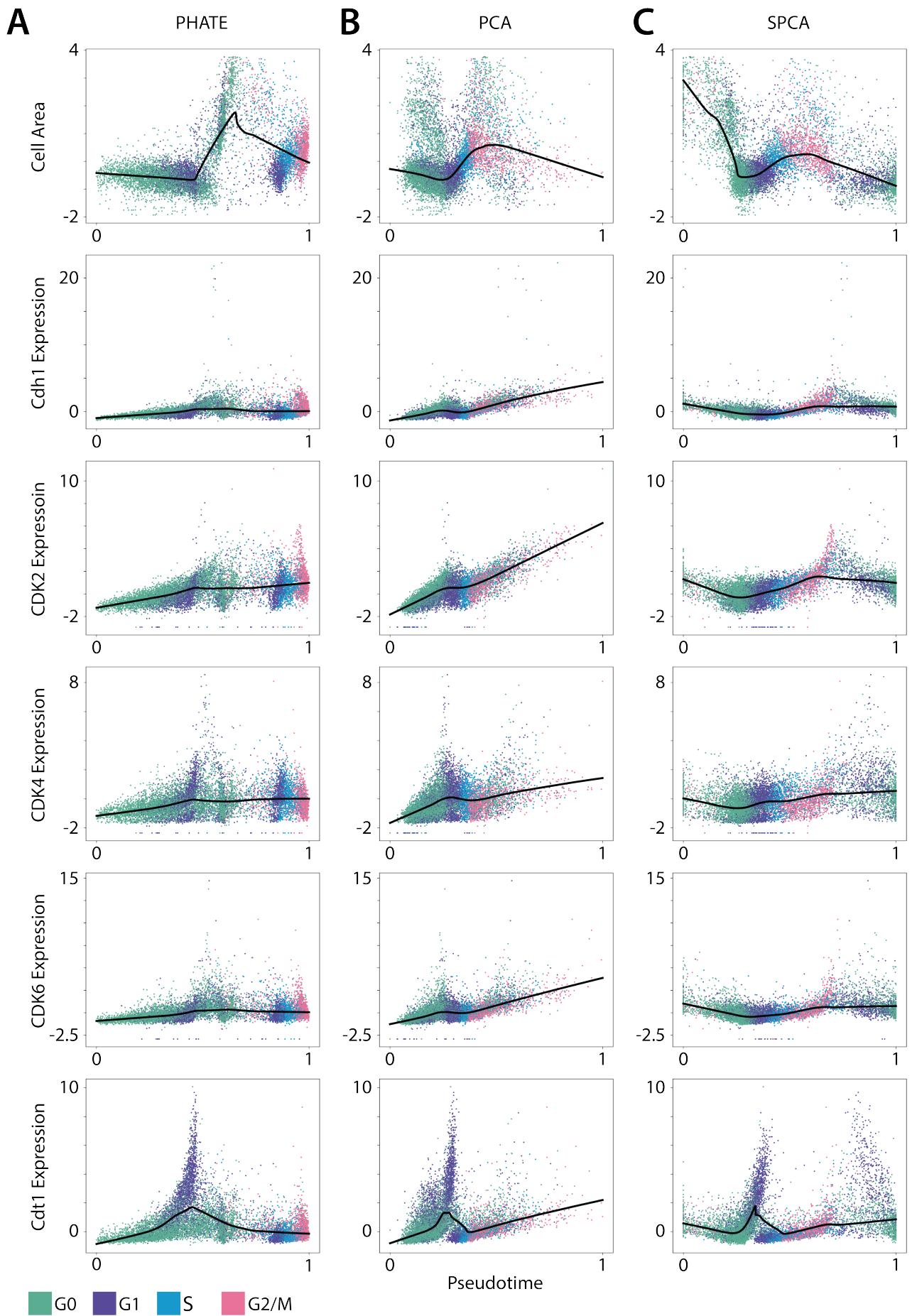


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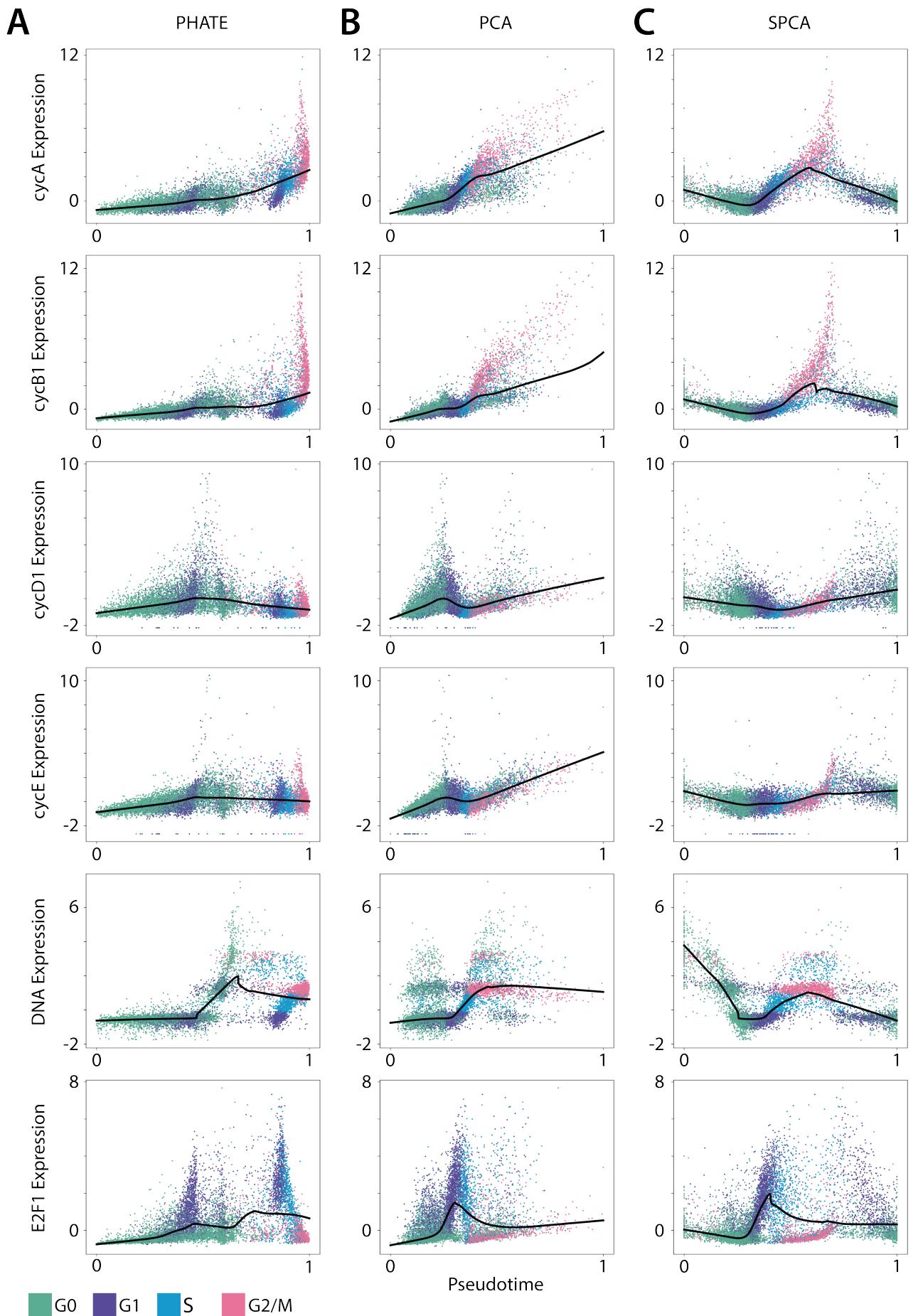


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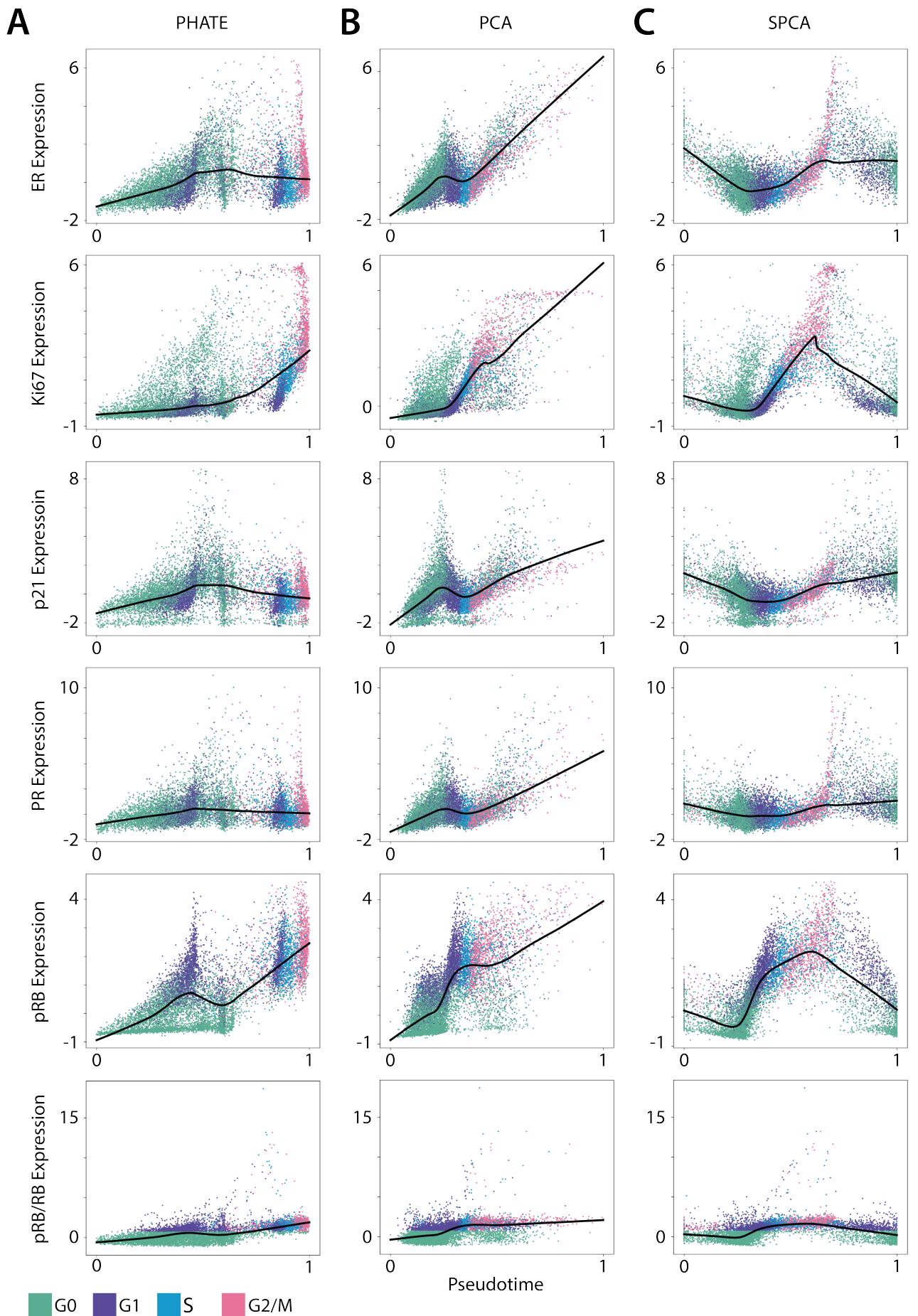


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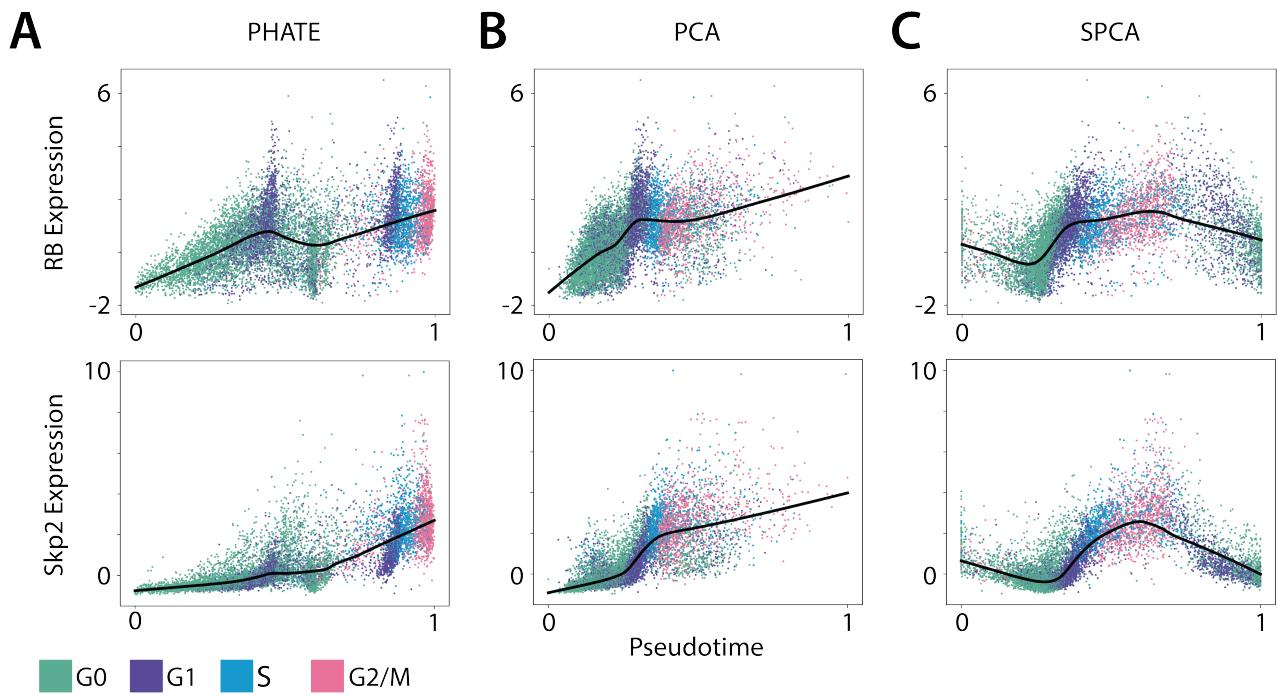


Fig. S5. SPCA recapitulates cyclical protein expression trends. PHATE, PCA, and SPCA were performed on untreated cells (0 nM palbociclib). Each cell was plotted according to its feature expression and normalized Slingshot pseudotime, and colored according to its cell cycle phase label. To identify a smooth temporal trajectory, A LOESS curve (black line) was fit through the points for all (A) PHATE, (B) PCA, and (C) SPCA plots. These plots show the full feature profiles for the plots seen in Fig. 3D.

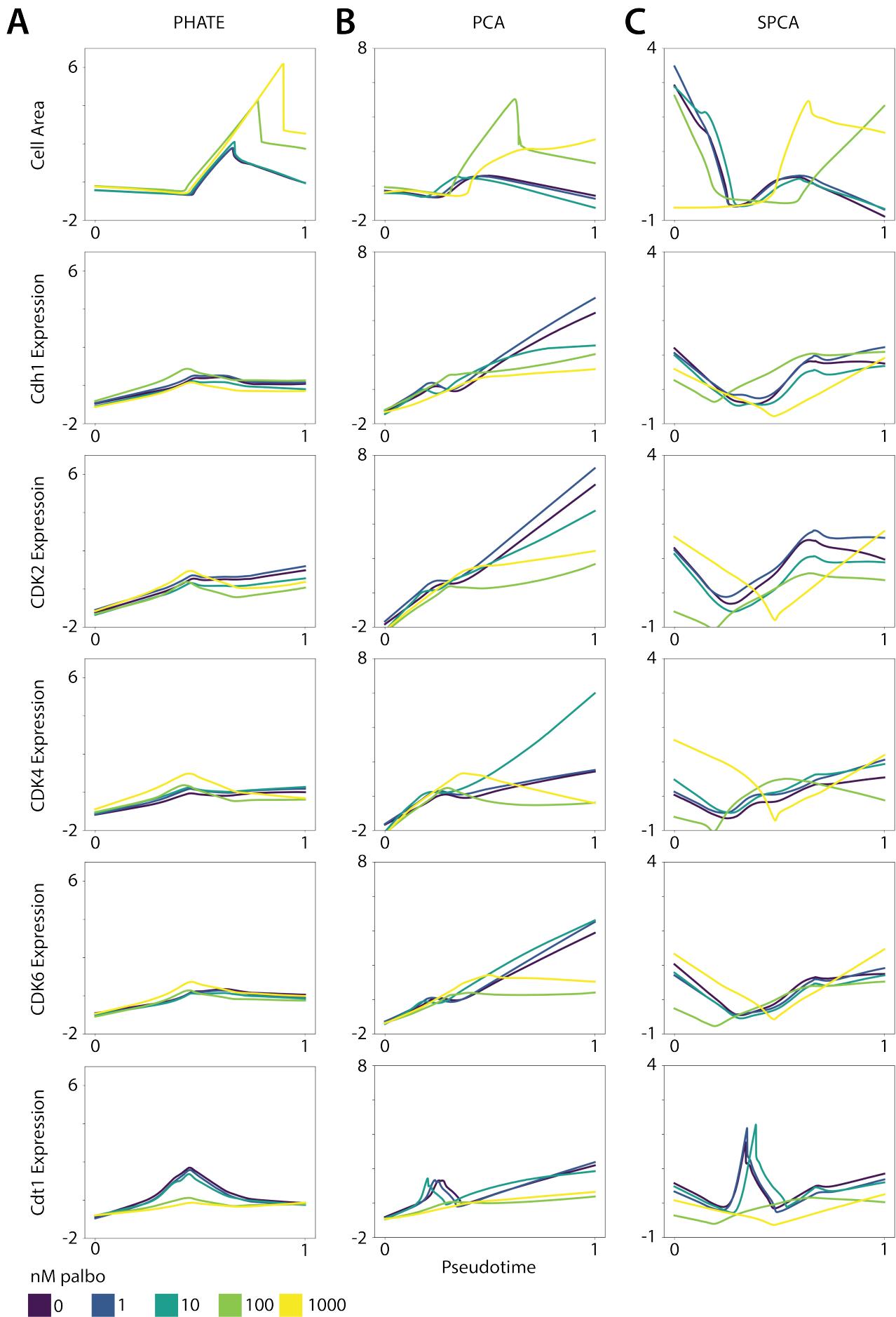


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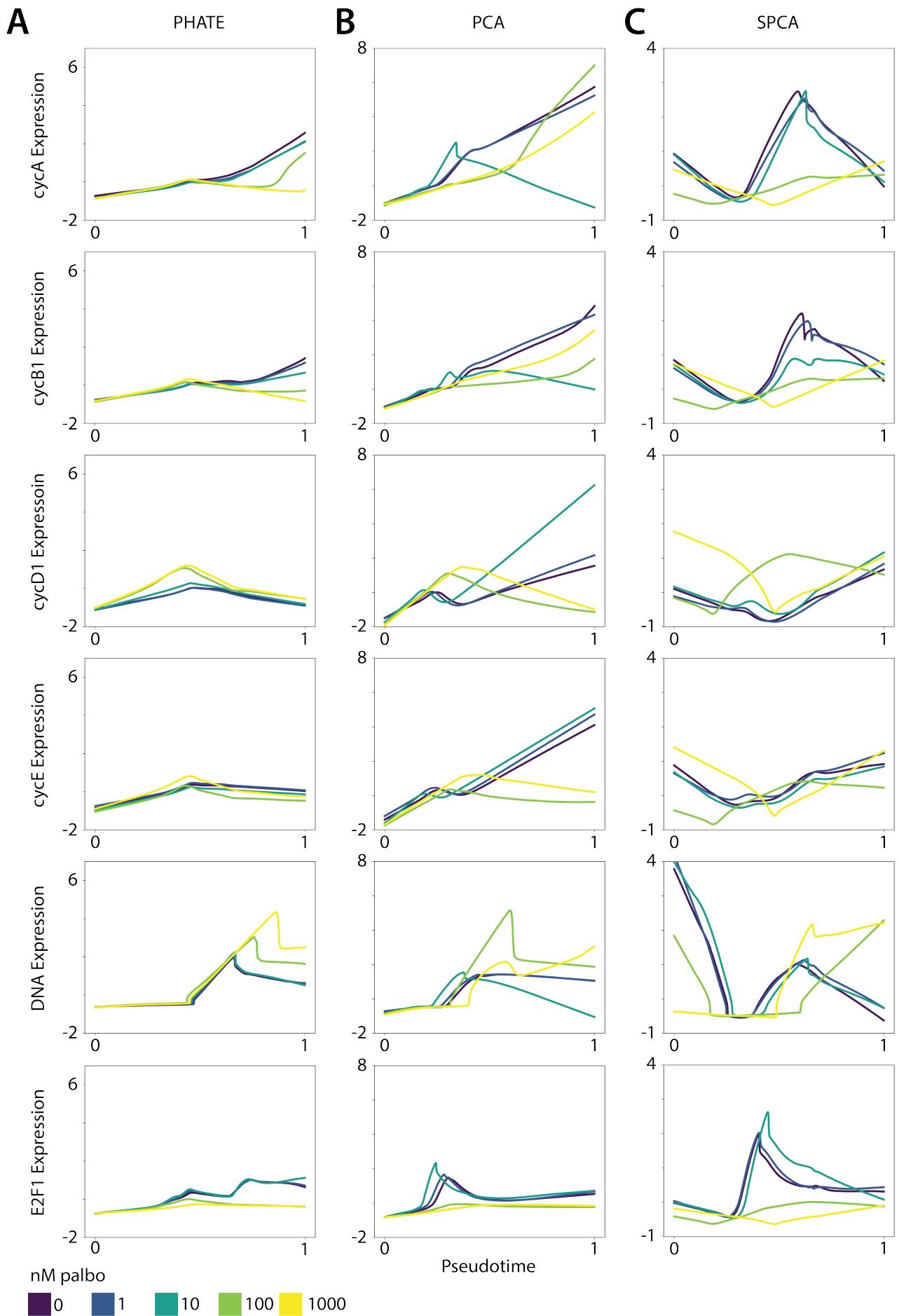


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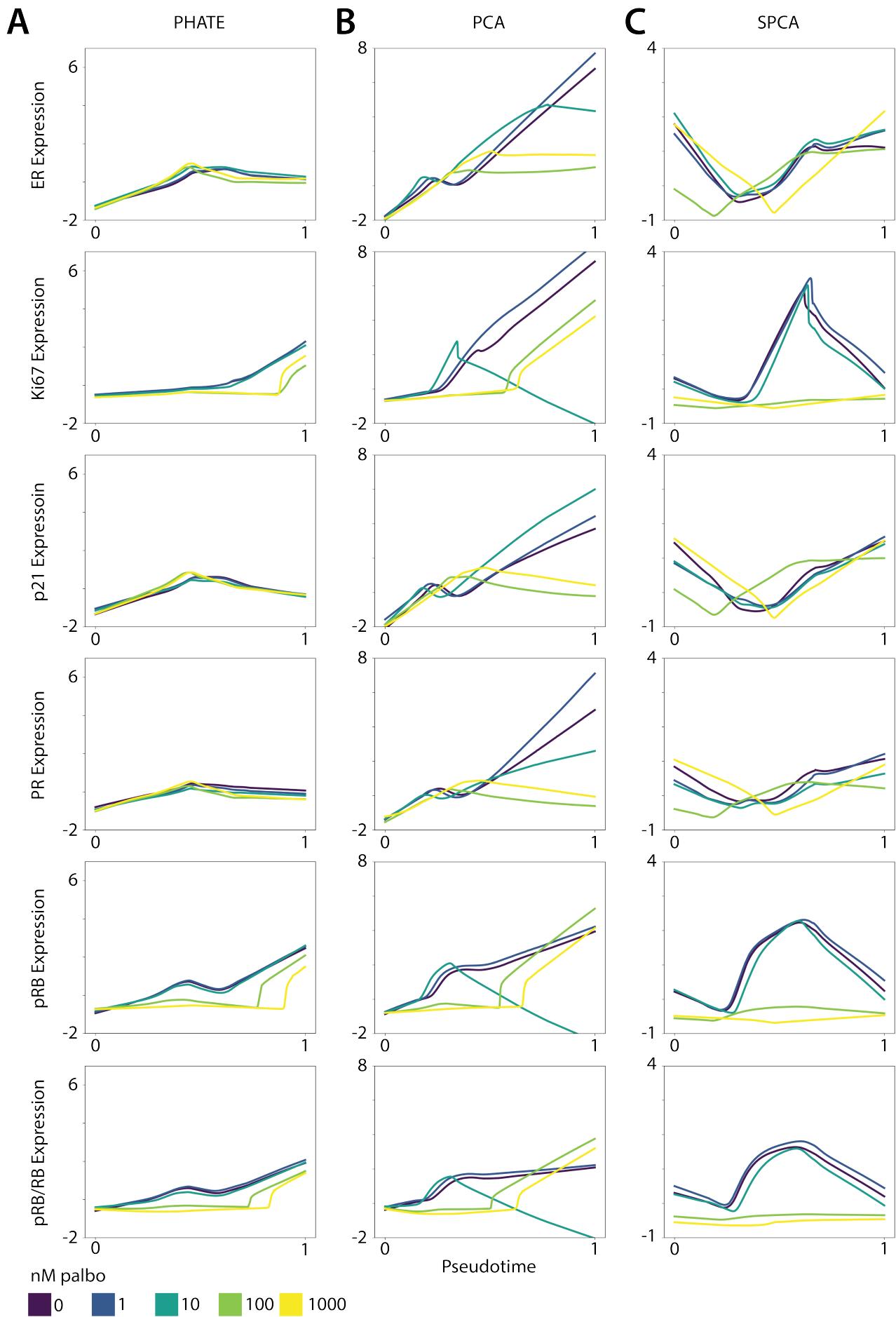


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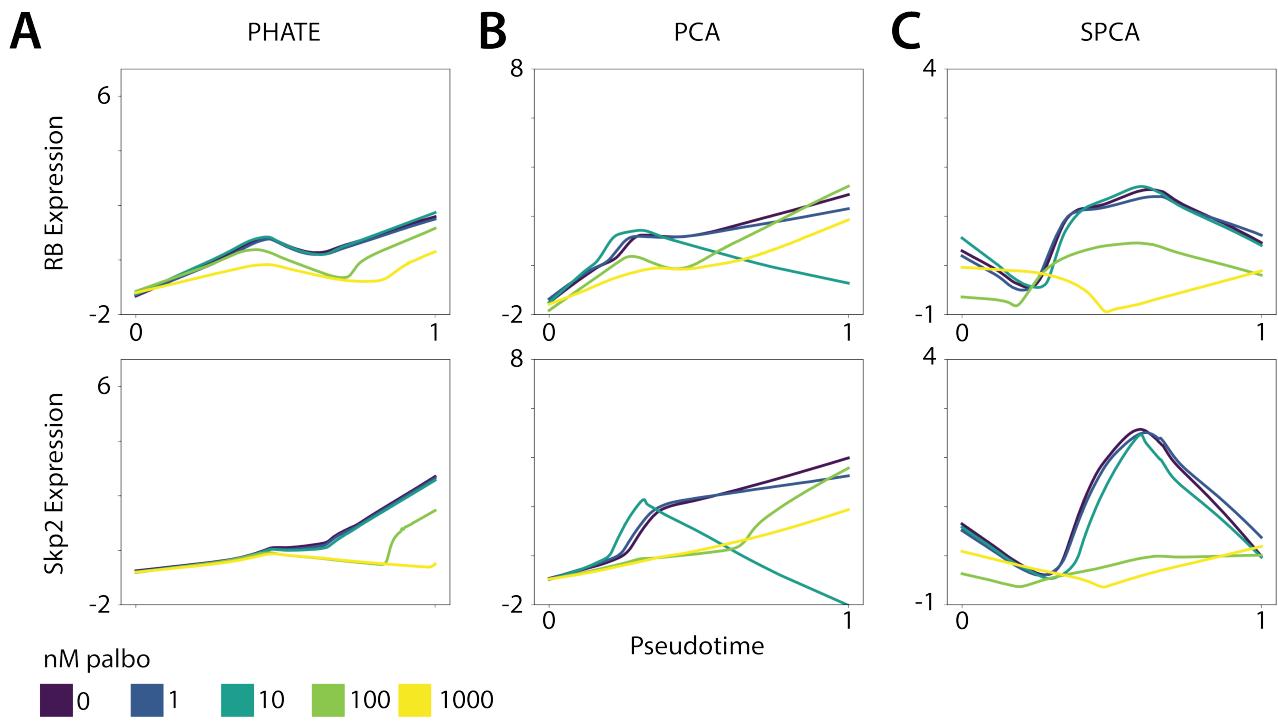


Fig. S6. SPCA captures dose-dependent shifts across treatment conditions. LOESS curves were fit through points plotted according to protein expression levels and Slingshot pseudotime found using (A) PHATE, (B) PCA, and (C) SPCA. Five curves were identified and colored according to treatment condition. These plots show the full feature profiles for all methods first shown for SPCA in Fig. 4D.

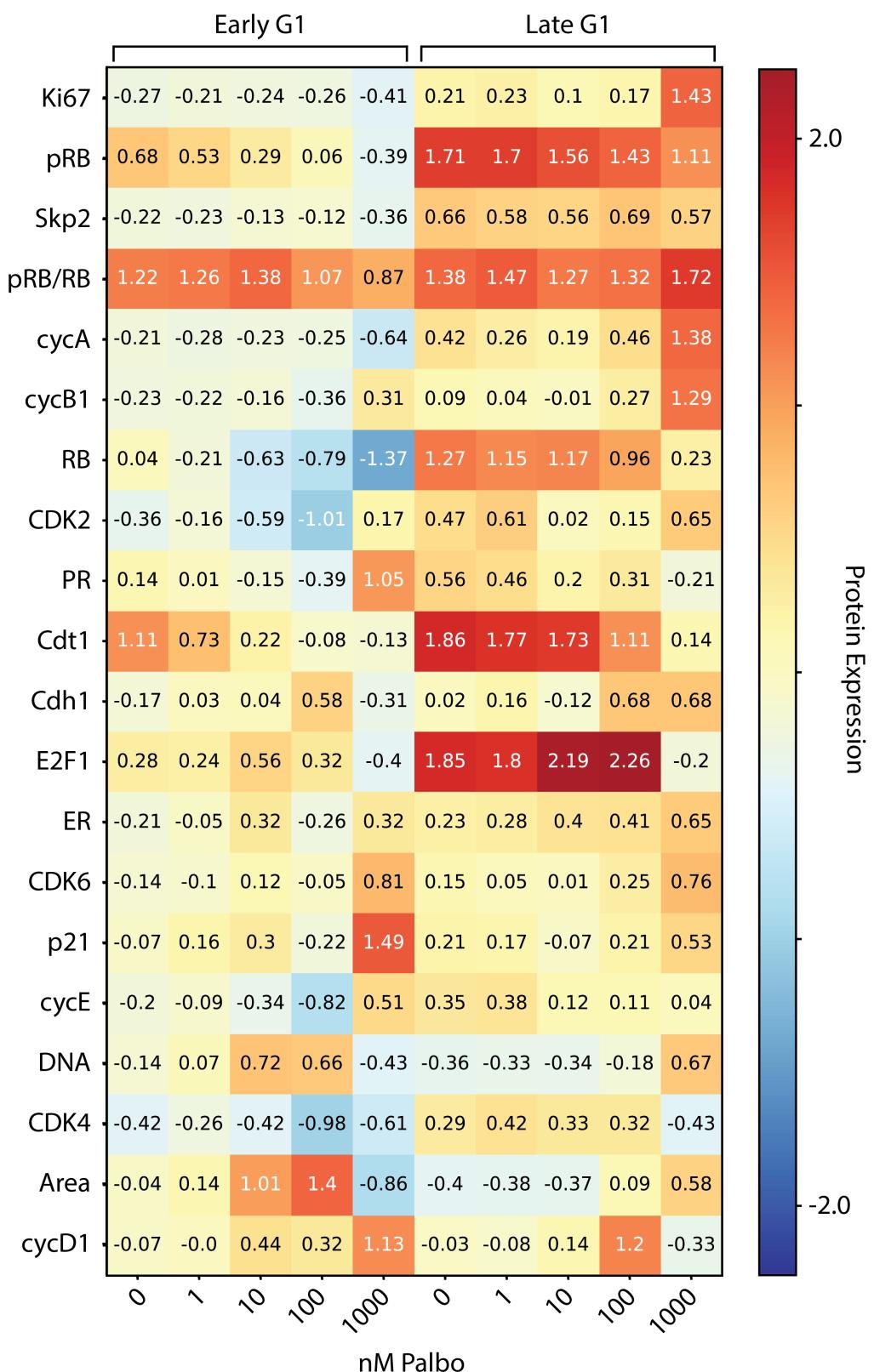


Fig. S7. **SPCA and Slingshot identify differences in G1 cells.** G1 cells were separated according to median normalized pseudotime. Mean expression levels for each cell cycle feature are represented in each row of the heatmap.