

Supplementary Material for “Spherical Manifolds Capture Drug-Induced Changes in Tumor Cell Cycle Behavior”

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Supplement summary

In this supplement, we present supporting information to the methods and results in the main text. Specifically, we detail specific antibodies used in the iterative indirect immunofluorescence imaging (4i) process in the table below along with the 4i images and single-cell views of the data. Additionally, we provide full plots for each proteomic feature and palbociclib dose first shown in Figures 2-5 in the main text.

Supplementary tables

Table S1. Features labeled in the 4i protocol by round. Columns indicate which secondary antibody was utilized to visualize the primary antibodies listed. DAR-488 is the green channel, DAM-555 is red, and DAG-647 is far red in Fig. S1, S2 below.

Round	DAR-488	DAM-555	DAG-647
1	pRB	RB	p21
2	ER	PR	cyclin B1
3	CDK4	cyclin E	CDK2
4	CDK6	cyclin D1	
5	Ki67	E2F1	
6	Cdt1	cyclin A	
7	Skp2	Cdh1	

Supplementary figures

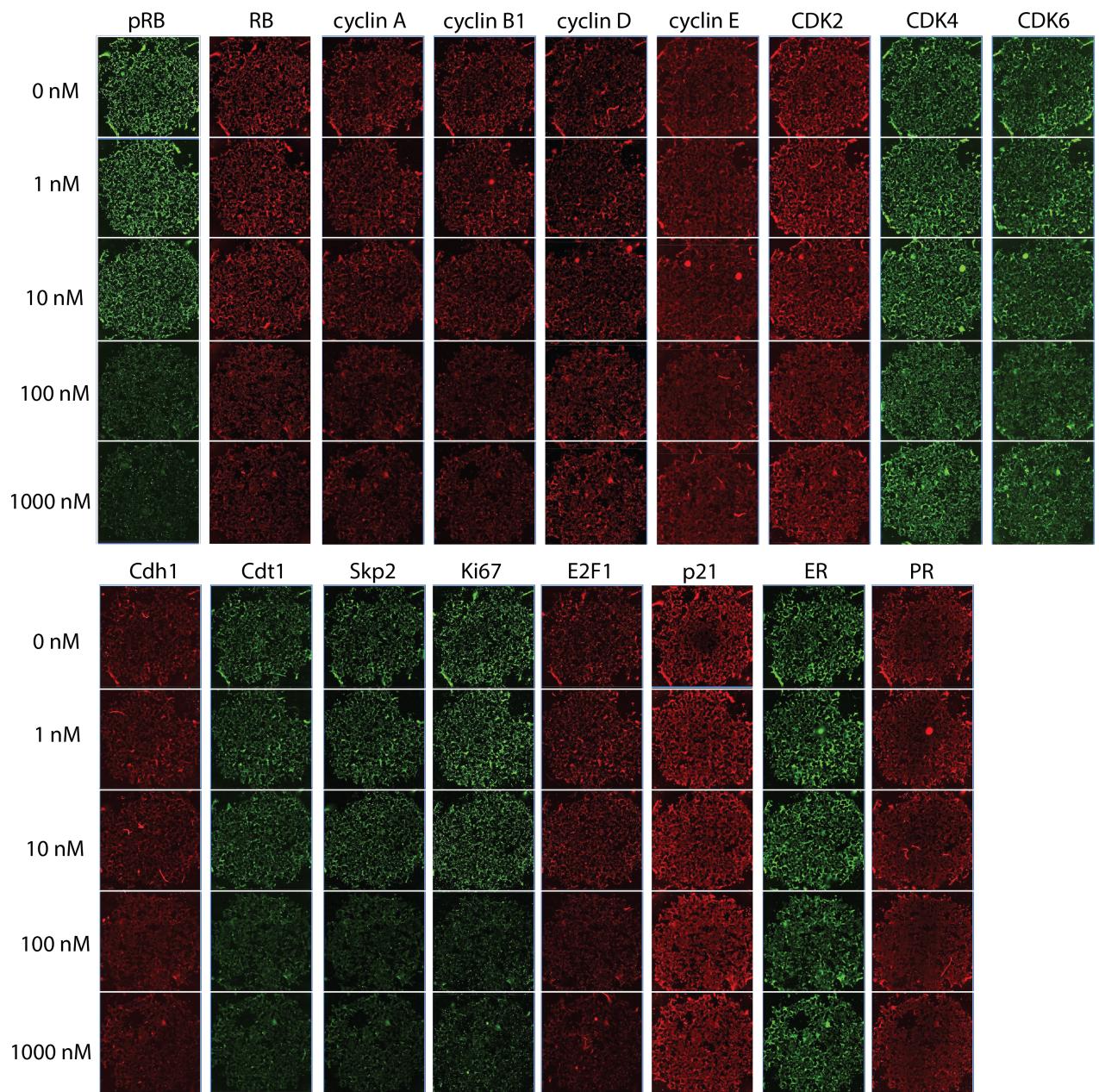


Fig. S1. Plate images of T47D tumor cells showing protein measurements (immunofluorescence) across increasing doses of palbociclib.

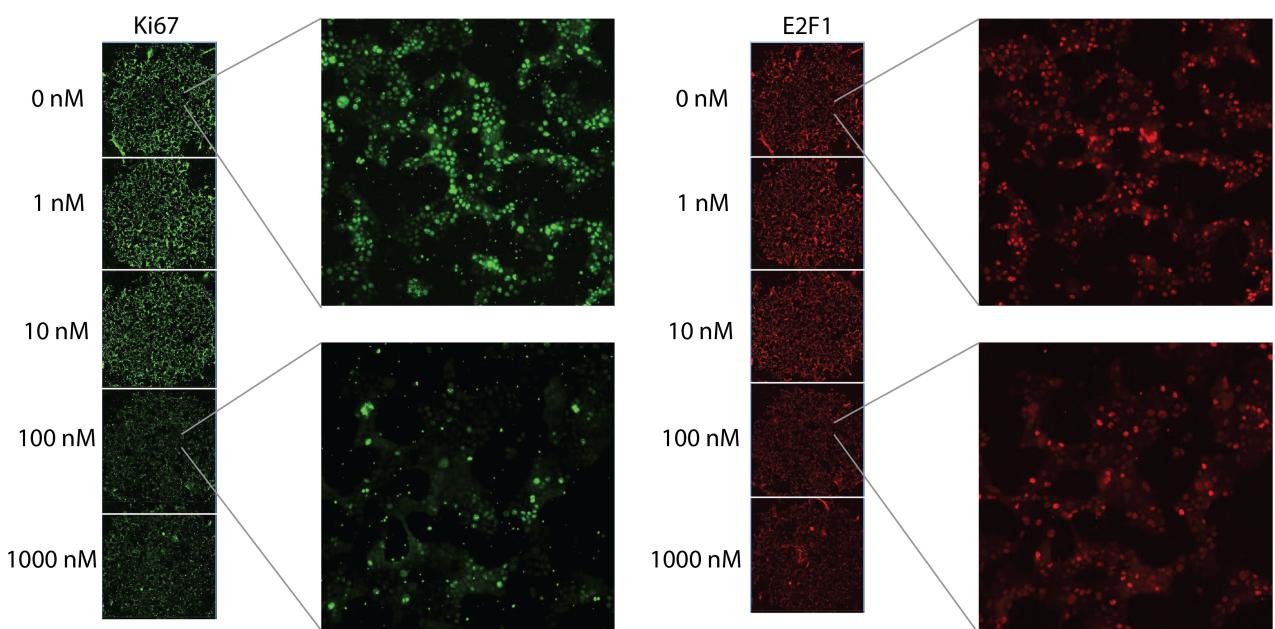


Fig. S2. Close-up single-cell views of T47D tumor cell responses to increasing levels of CDK4/6 inhibition. Decreasing population expression of Ki67 protein is visualized from decreasing immunofluorescence with higher palbociclib.

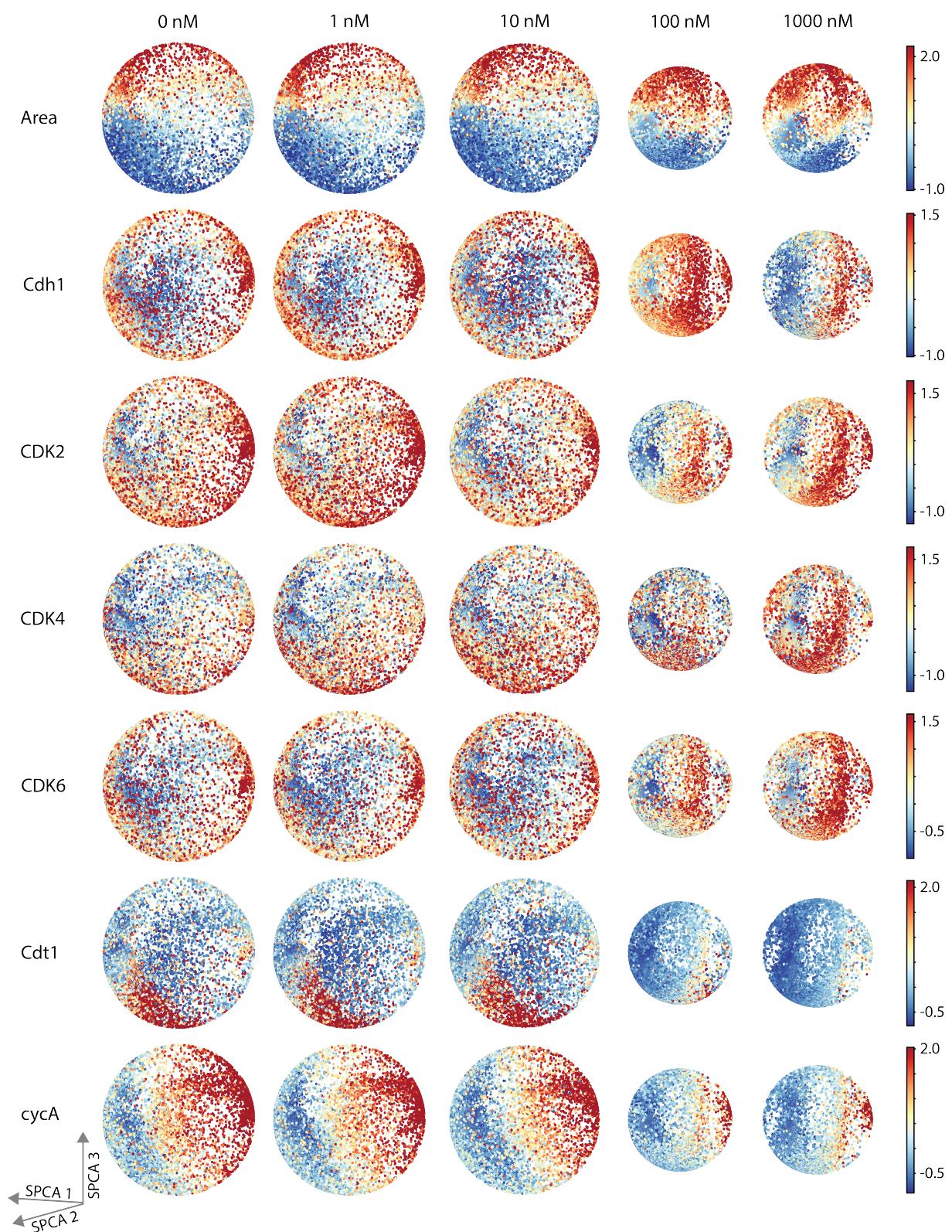


Fig. S3. Continued on next page.

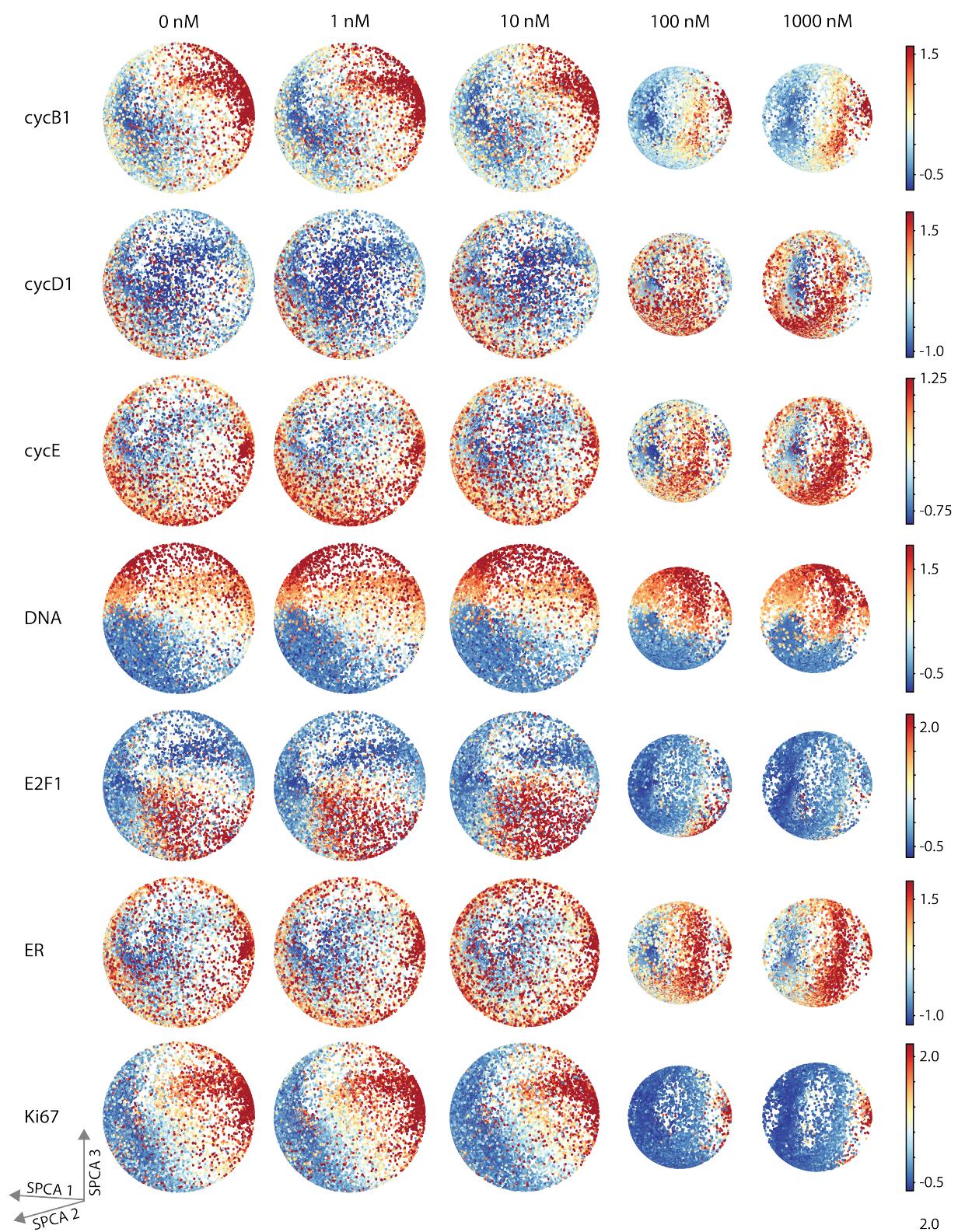


Fig. S3. Continued on next page.

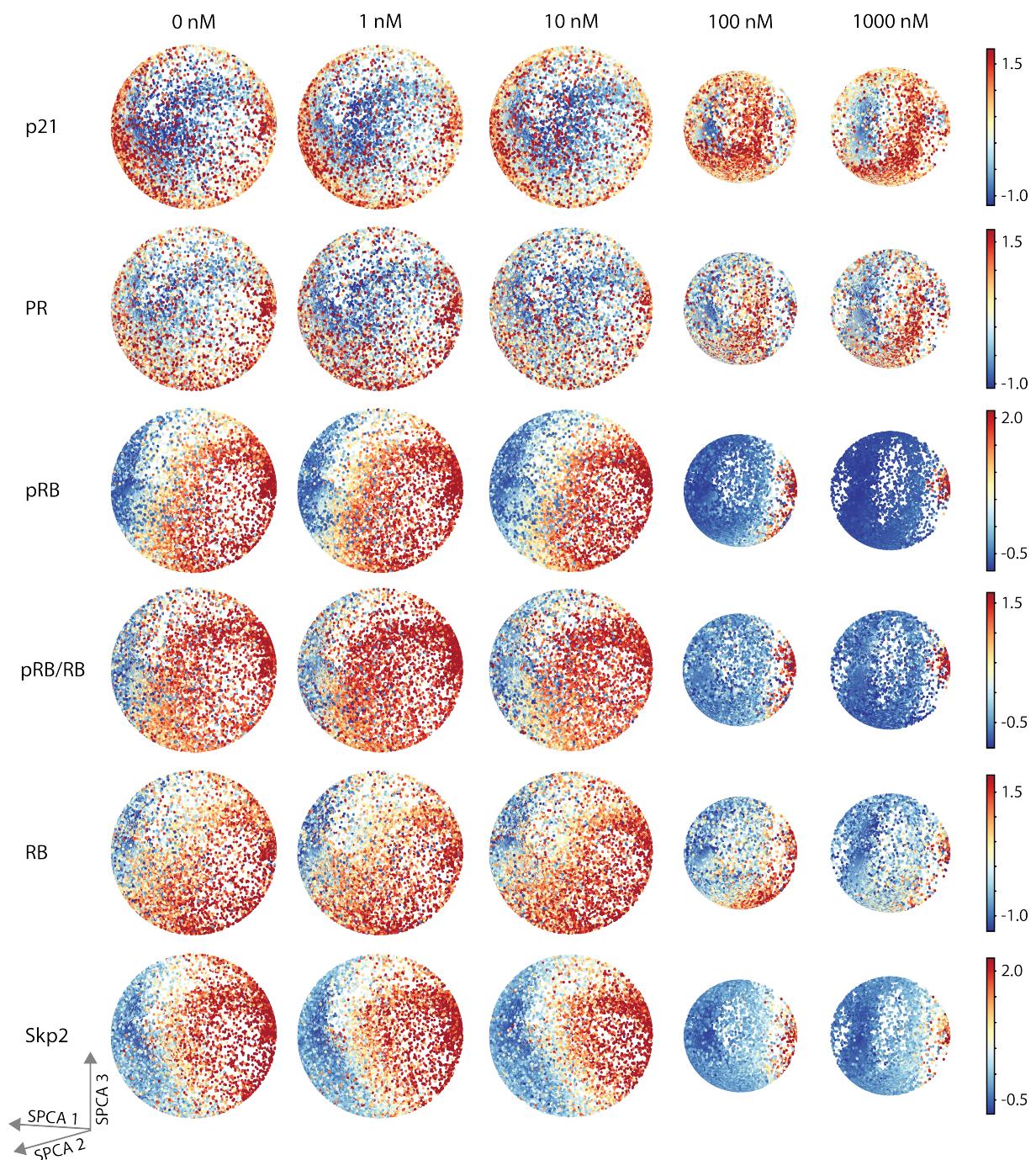


Fig. S3. SPCA captures shifts in cell cycle regulators across treatment conditions. Cells were projected onto three-dimensional spherical manifolds identified by SPCA into a shared space for each treatment condition. Points are colored according to normalized median expression level for each proteomic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

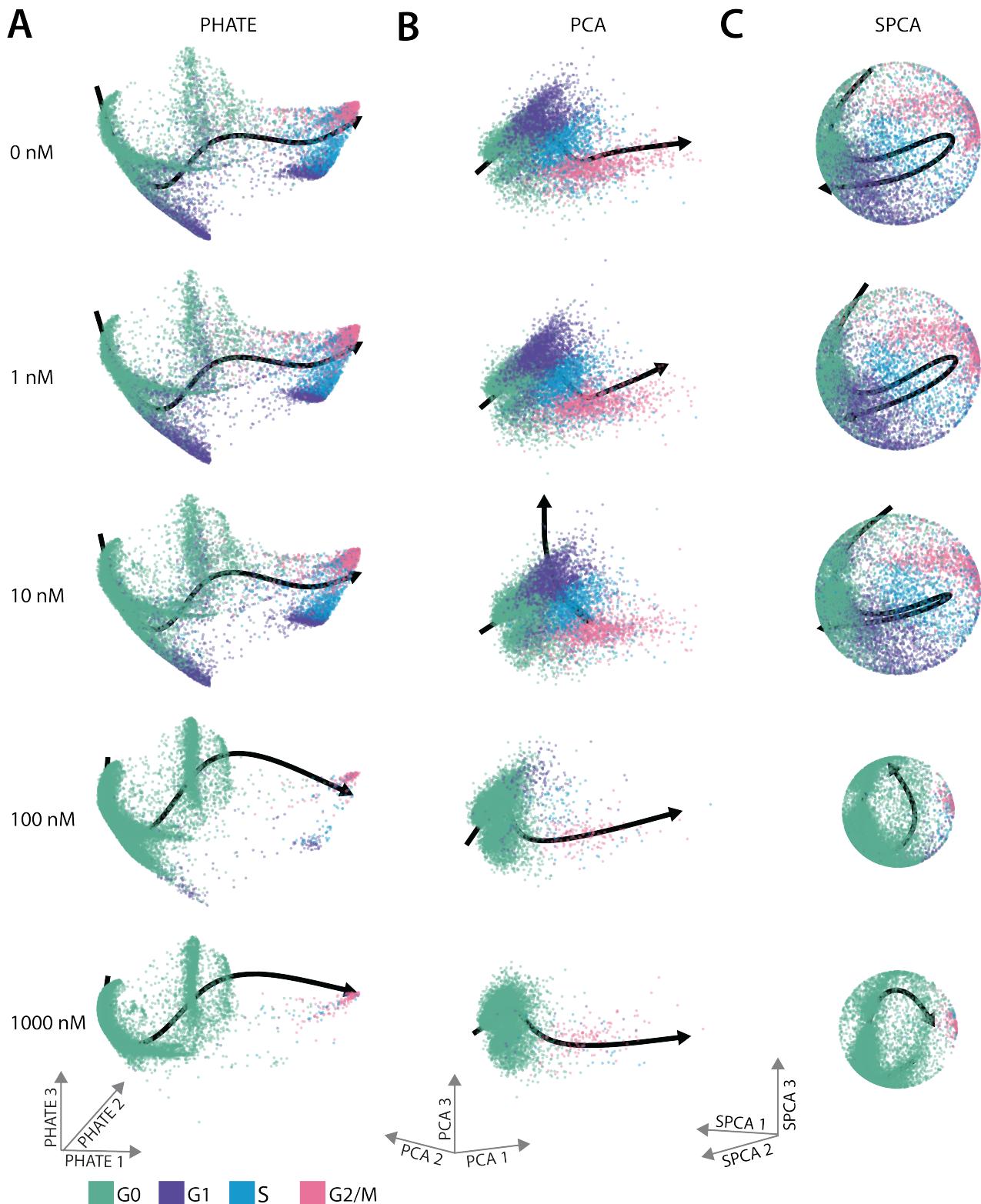


Fig. S4. Slingshot trajectories identify cyclical cell cycle paths. PHATE, PCA, and SPCA were applied to cells in each treatment condition. Data points were projected into three dimensions and colored according to cell cycle phase label. Cell cycle trajectories were identified by Slingshot and overlaid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

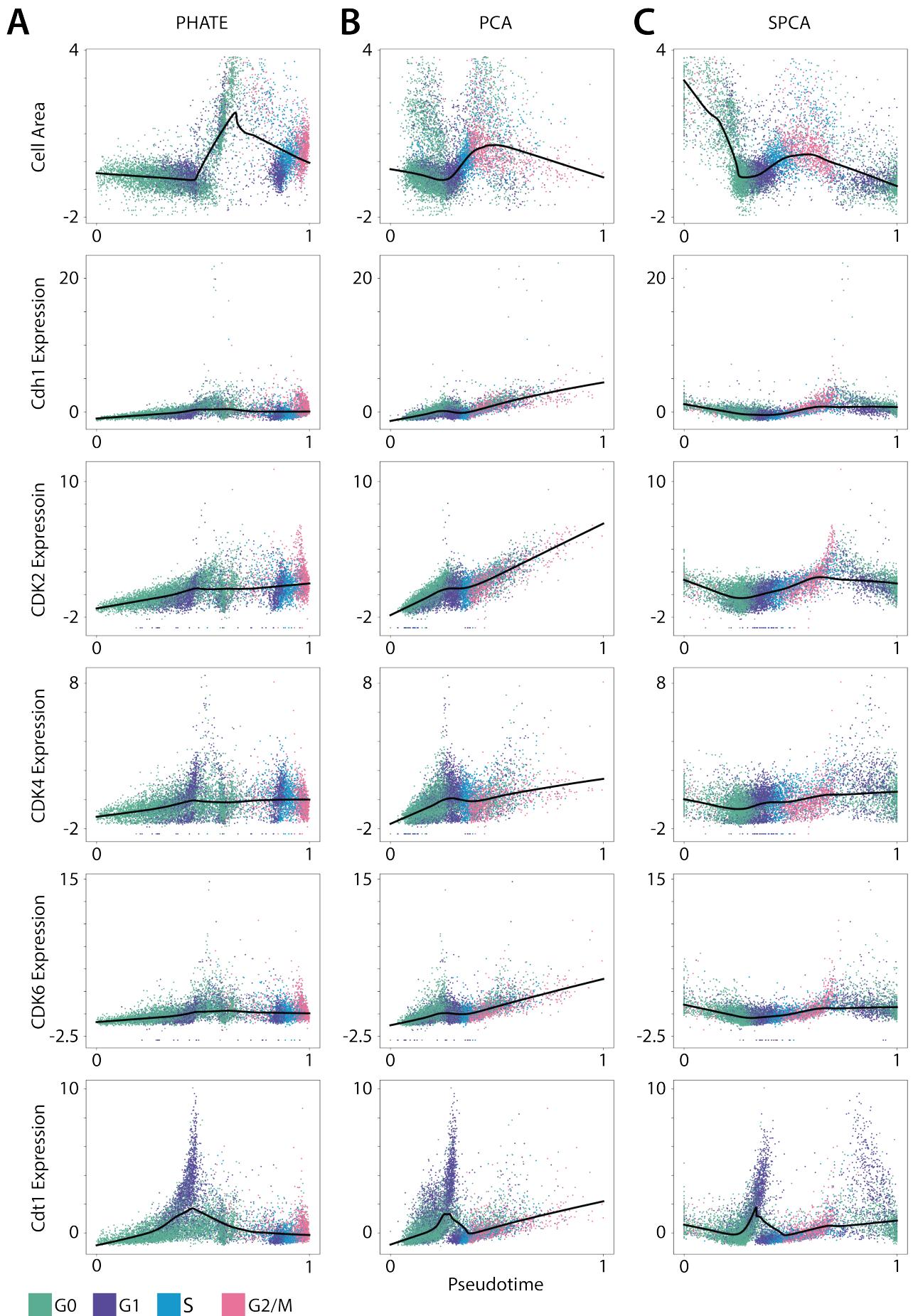


Fig. S5. Continued on next page.

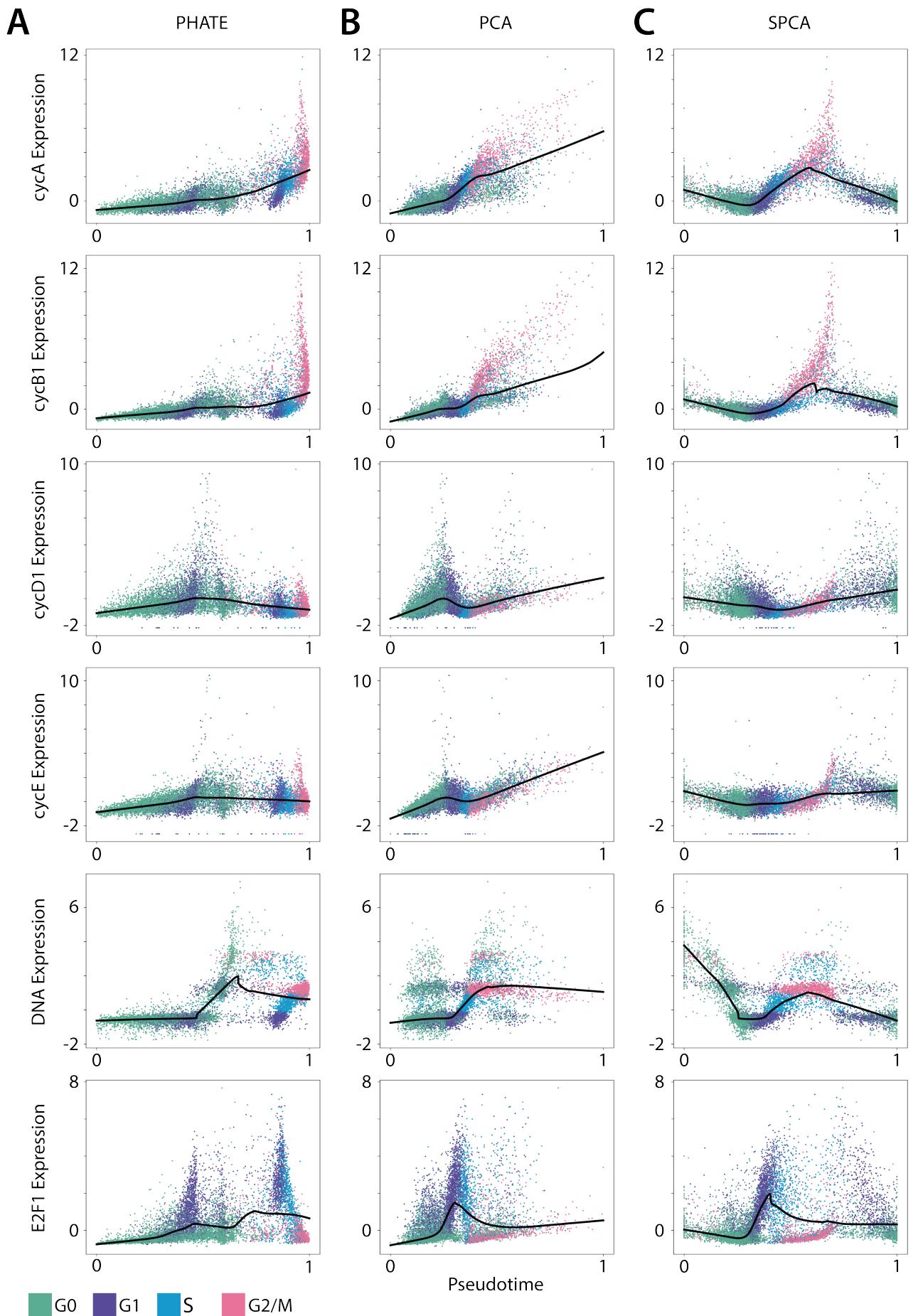


Fig. S5. Continued on next page.

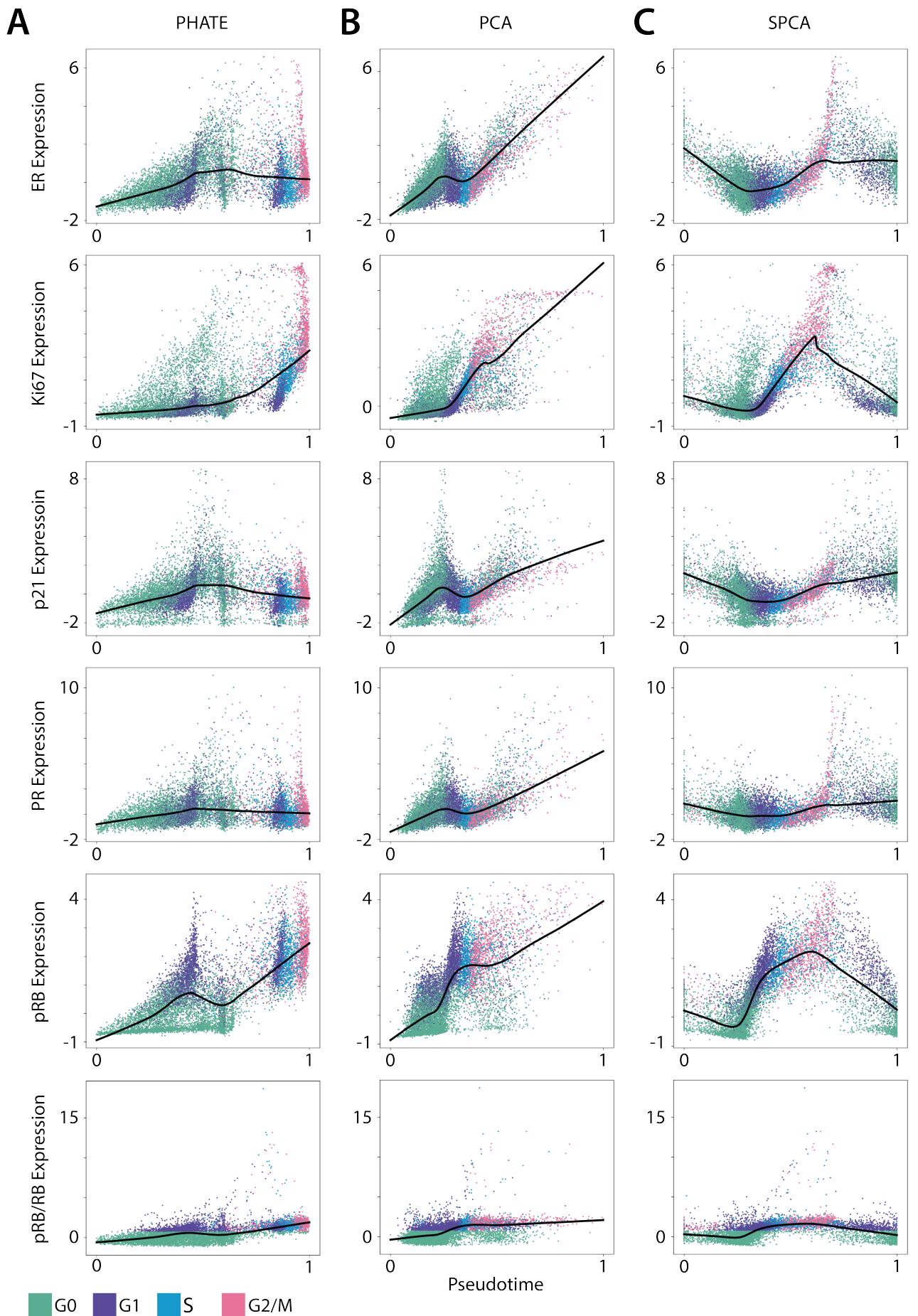


Fig. S5. Continued on next page.

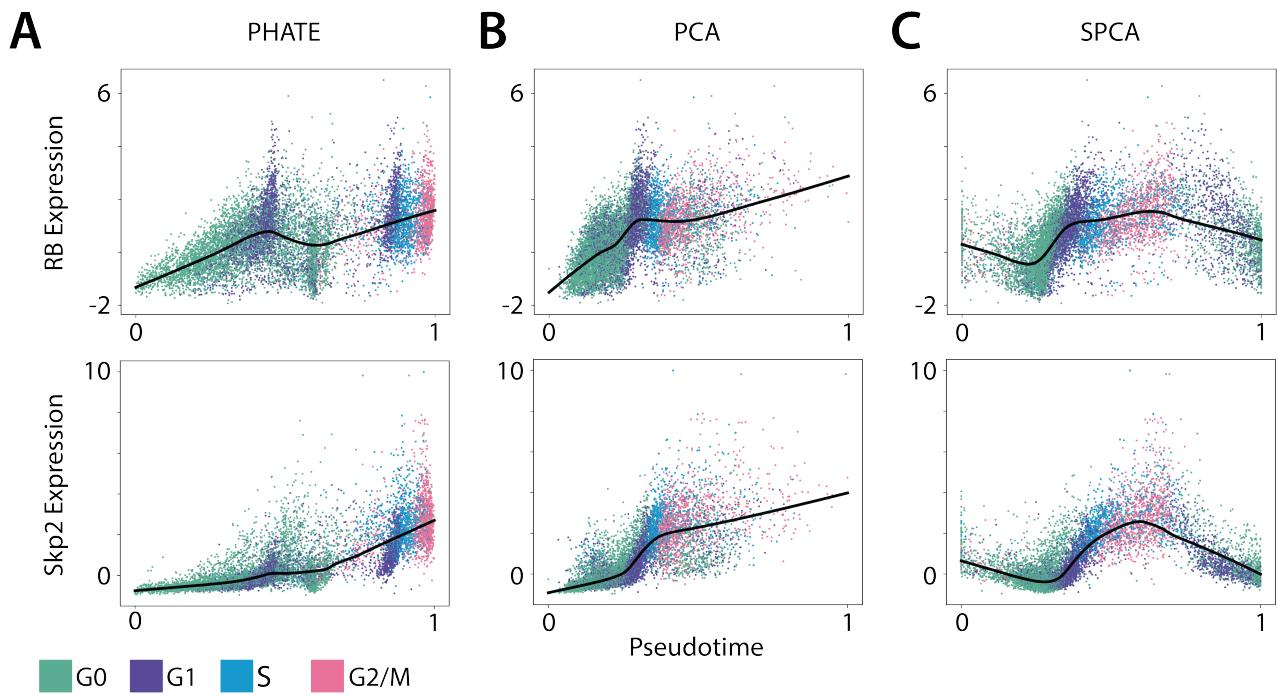


Fig. S5. SPCA recapitulates cyclical protein expression level trends. PHATE, PCA, and SPCA were performed on untreated cells (0 nM palbociclib). Each cell was plotted according to its feature expression and normalized Slingshot pseudotime, and colored according to its cell cycle phase label. To identify 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.

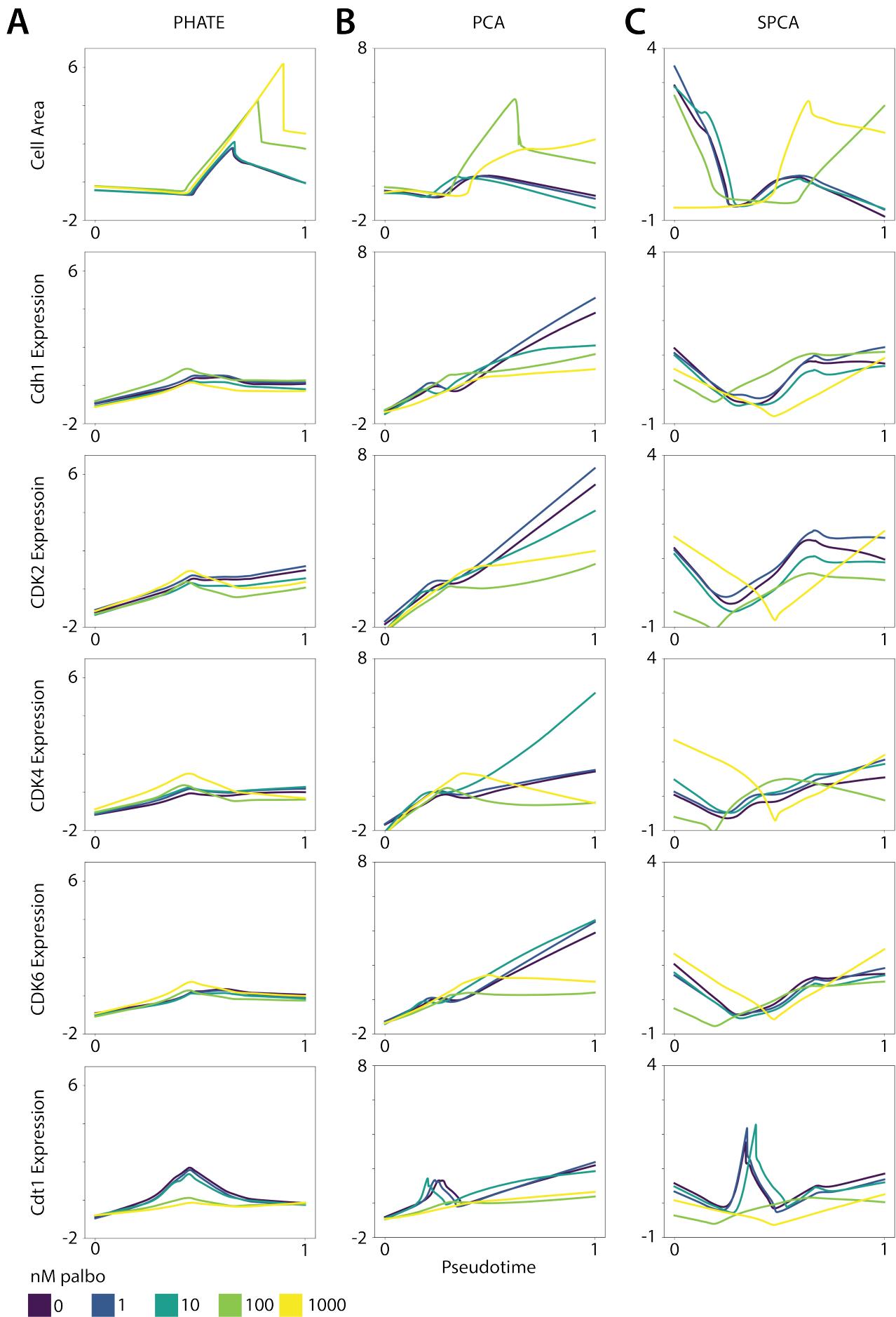


Fig. S6. Continued on next page.

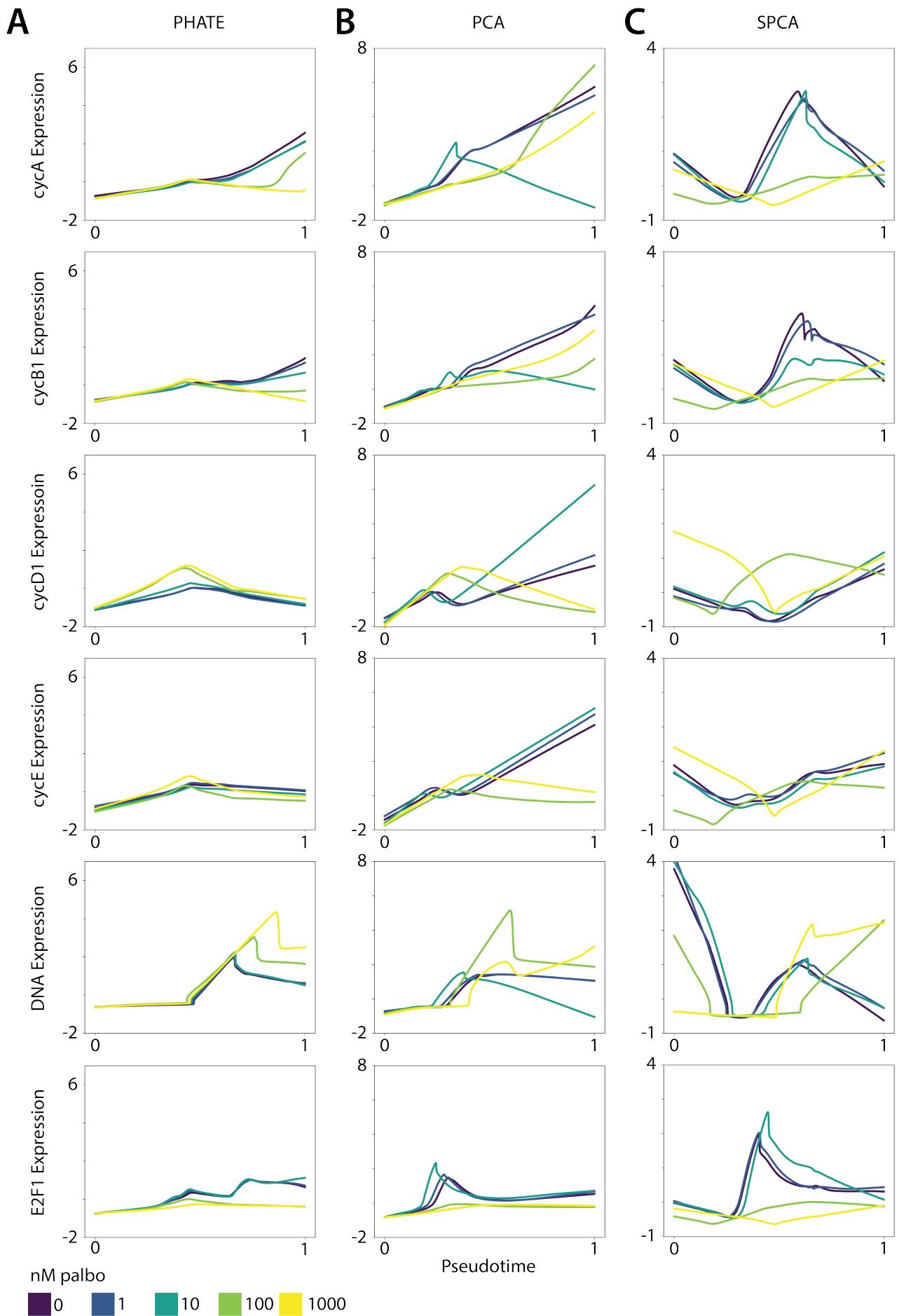


Fig. S6. Continued on next page.

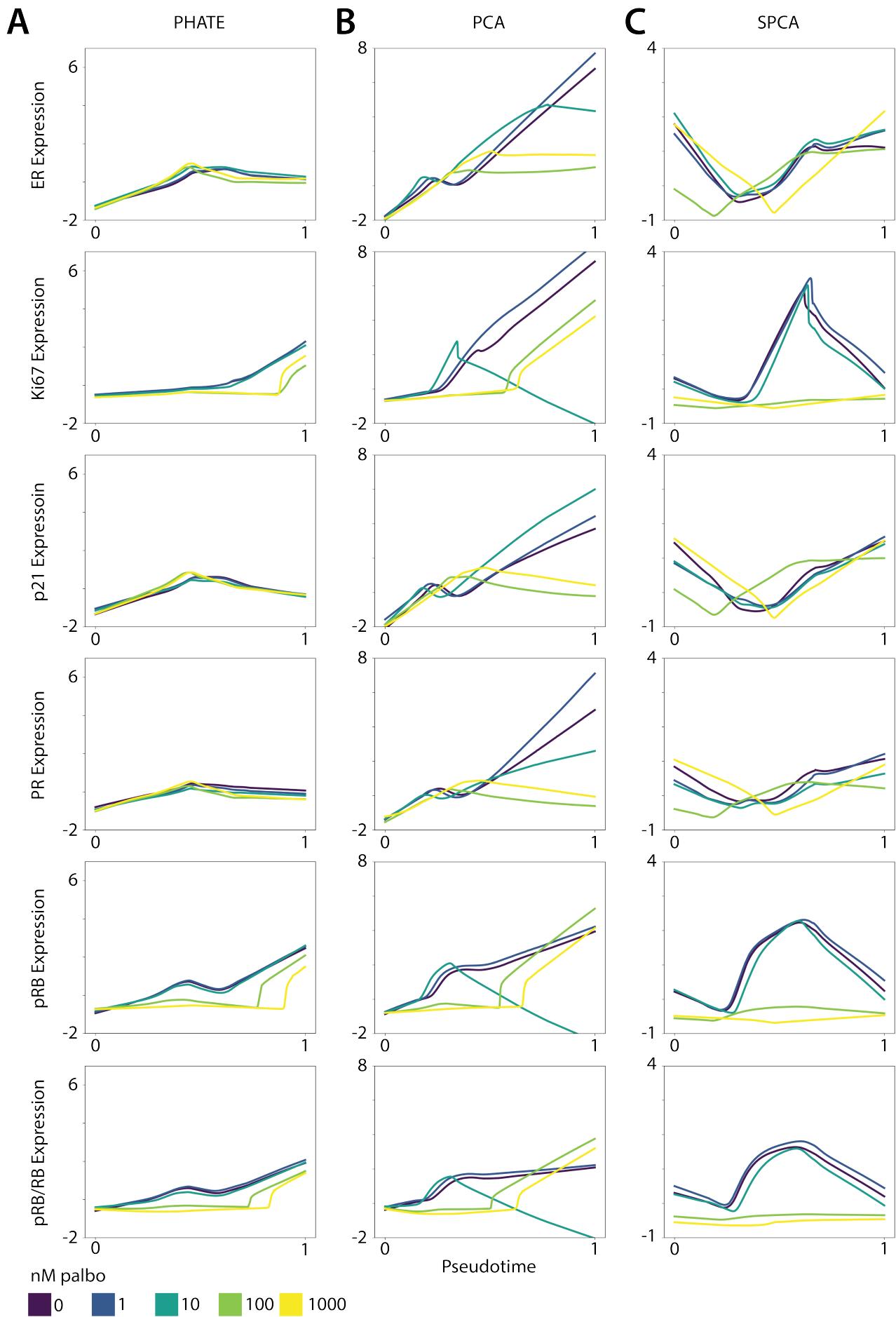


Fig. S6. Continued on next page.

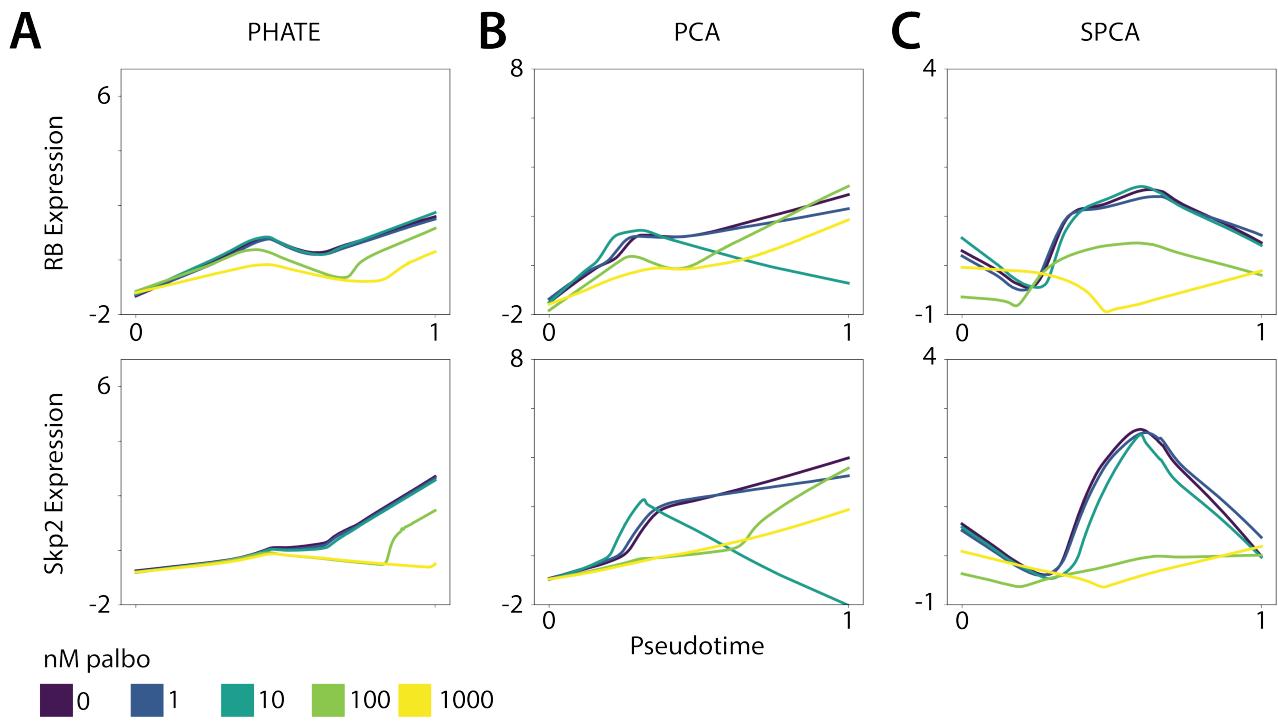


Fig. S6. SPCA captures dose-dependent shifts across treatment conditions. LOESS curves were fit through points plotted according to protein expression levels and Slingshot pseudotime found using (A) PHATE, (B) PCA, and (C) SPCA. Five curves were identified and colored according to treatment condition.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

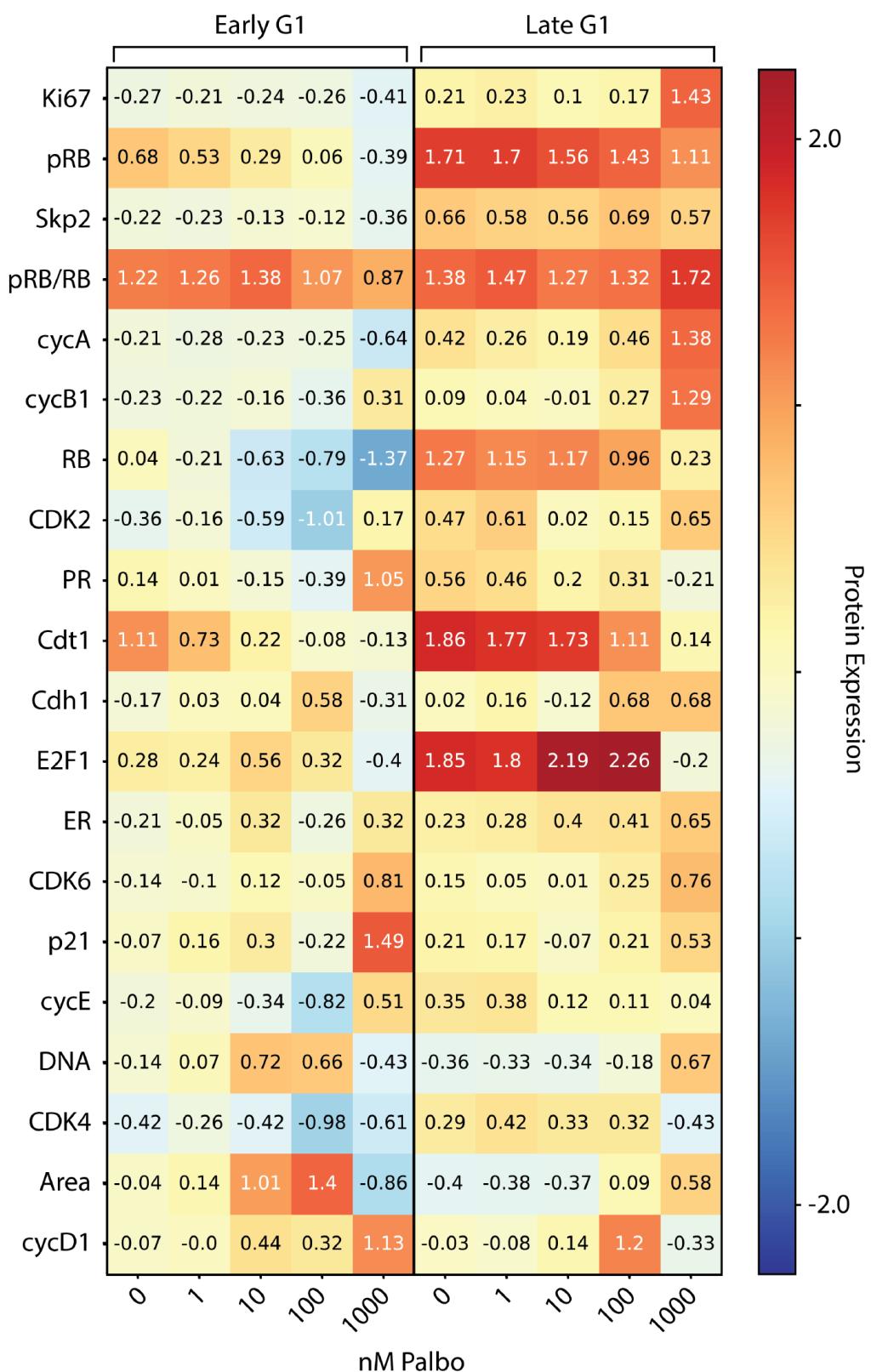


Fig. S7. **SPCA and Slingshot identify differences in G1 cells.** G1 cells were separated according to median normalized pseudotime. Mean expression levels for each cell cycle feature are represented in each row of the heatmap.