Supplemental figures legends

Fig. S1. Cell cycle distributions of MD-MB-231 cells were verified by flow cytometry (FC) analysis. A. Cell cycle distribution of human breast cancer cells (MDA-MB-231) were measured by both FC and the microscopy assay. The number of analyzed cells is indicated in each panel. **B.** Fractions of cells in each phase of the cell cycle quantified with our microscopy-based assay and compared to FC.

Fig. S2. Cell cycle analysis applied to a wide range of cell types. To demonstrate the versatility of our method, seven additional types of cells were analyzed. These cells include human pancreatic normal epithelial cells (HPNE), human breast normal epithelial cells (MCF10A), patient-derived pancreatic cancer ductal adenocarcinoma cells that metastasized to the liver, *LMNA*$^{+/+}$ mouse embryonic fibroblasts (MEFs), *LMNA*$^{-/-}$ MEFs, metastatic breast cancer cells (MD-MB-231), and patient-derived pancreatic cancer ductal adenocarcinoma cells from the primary tumor. At least 1,000 cells were analyzed for each cell line.

Fig. S3. Alteration of cell cycle causes long term effects on cell properties. A and B. Asynchronized cell cycle distribution is recovered after 24h release of from synchronizing drug. Orange represents thymidine treatment (A); purple represents nocodazole treatment (B). **C and D.** Mean changes in nuclear size and cell size induced 0h, 6h, and 24h after release of thymidine (C, orange) and nocodazole (D, purple) compared to control cells (blue). ***: $P < 0.0001$ (one-way ANOVA). **E**. Cell-cycle distributions of control cells and cells treated with Cdk4/6 inhibitor IV. Blue represents control. Black represents Cdk4/6 inhibitor IV treatment. **F**. Mean changes in nuclear size and cell size induced 0h, 6h, and 24h after removing Cdk4/6 inhibitor IV. Three biological repeats on different cells were analyzed for a total of >3,000 cells for each tested condition.

Fig. S4. Lamin deficiency enriches cells in the G_0/G_1 phase. A. Cell cycle distributions of *LMNA*$^{-/-}$ MEFs and Lamin A/C KD c2c12 cells compared to *LMNA*$^{+/+}$ MEFs and control c2c12 cells transfected with a scrambled construct, respectively, obtained by the microscopy assay. **B.** Quantitative analysis demonstrates significant ($P<0.0001$) increase in the G_0/G_1 fractions and decrease in the G_2/M fractions of both *LMNA*$^{-/-}$ MEFs and Lamin A/C KD c2c12 cells compared to control cells. **C.** Relative contributions to population-averaged changes in nuclear size of *LMNA*$^{-/-}$ MEFs and Lamin A/C KD c2c12 cells show only ~75%

contribution from direct intrinsic nuclear size changes. The rest of ~25% is contributed from re-distribution of cell cycle and the second order term. At least 1,000 cells were analyzed in three biological repeats (different cells are analyzed each time) for a total of >3,000 cells for each cell line.