Hep3B cell line (ATCC HB-8064) was maintained in EMEM with 10% FBS at 37 °C in a humidified atmosphere (air supplemented with 5% CO2) and passaged with 0.05% trypsin at a subcultivation ratio of 1:3. Hepatocytes (ATCC CRL-11233) were grown in flasks coated with BSA, fibronectin, and bovine collagen type I;the culture medium used was BEGM (gentamycin, amphotericin, and epinephrine were discarded from the BEGM bullet kit) with 5 ng/mL epidermal growth factor, 70 ng/mL phosphatidylethanolamine, and 10% FBS at 37 °C in a humidified atmosphere (air supplemented with 5% CO2) and passaged with 0.05% trypsin at a subcultivation ratio of 1:3. 1 x 10^6 cells were exposed to siRNA loaded in SP94-targeted DOPC protocells for various periods of time at 37 °C. siRNA payload comprised a siRNA cocktail specific for cyclins A2, B1, D1, and E, and the total siRNA concentration was maintained at ~125 pM. After incubation cells were harvested by gentle shaking in 5 mM EDTA for 30 min at 37 °C, centrifuged (1000 rpm, 1 min) to remove excess protocells, and stained with Alexa Fluor 488-labeled annexin V and propidium iodide per the manufacturer's instructions. The numbers of viable (double-negative) and nonviable (single- or double-positive) cells were determined via flow cytometry (FACSCalibur). Voltages were established using (1) untreated, unlabeled Hep3B (100% of cells were contained within the lower left quadrant, spanning from 10^0 to 10^2 fluorescence units on the FL-1 and FL-2 axes); (2) Hep3B transfected with the cyclin-specific siRNA cocktail using Lipofectamine RNAiMAX and singly stained with Alexa Fluor 488-labeled annexin V (96% of cells were contained within the lower right quadrant, spanning from 10^2 to 10^4 FUs on the FL-1 axis and 10^0 to 10^2 FUs on the FL-2 axis); and (3) Hep3B transfected with the cyclin-specific siRNA cocktail using Lipofectamine RNAiMAX and singly stained with propidium iodide (98% of cells were contained within the upper right quadrant, spanning from 10^0 to 10^2 FUs on the FL-1 axis and 10^2 to 10^4 FUs on the FL-2 axis). Cells were transfected according to Invitrogen's “reverse transfection” protocol with an initial cell concentration of 5 x 10^5 (seeded in 60 mm plates), a final siRNA concentration of 50 nM, and a total incubation time of 72 h.