

Hep3B cell line (ATCC HB-8064) was maintained in EMEM with 10% FBS at 37 °C in a humidified atmosphere (air supplemented with 5% CO<sub>2</sub>) 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% CO<sub>2</sub>) and passaged with 0.05% trypsin at a subcultivation ratio of 1:3.  $1 \times 10^6$  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  $5 \times 10^5$  (seeded in 60 mm plates), a final siRNA concentration of 50 nM, and a total incubation time of 72 h.