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. $1 \times 10^6$ Hep3B 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 at 37 °C for 48 h cells were washed three times with cold 1 x PBS to remove excess protocells. In order to determine the percentage of proliferating Hep3B, protocell-treated cells were incubated with 10 μM BrdU (in complete growth medium) for 12 h at 37 C, harvested by gentle shaking in 5 mM EDTA for 30 min at 37 C, and fixed with 4% formaldehyde for 30 min at 4 C. Cells were then washed three times in 1 x PBS with 0.1% Triton X-100, incubated in 1 N HCl for 10 min on ice, incubated in 2 N HCl for 10 min at room temperature and then 20 min at 37 C, incubated in 0.1 M borate for 12 min at room temperature, and washed three times in 1PBS with 0.1%Triton X-100. Cells were blocked in 1 x PBS with 0.1% Triton X-100, 1 M glycine, and 5% goat serum for 1 h at room temperature and then incubated with an Alexa Fluor 488-labeleled mouse monoclonal antibody to BrdU (1:100 dilution in 1 x PBS with 1% BSA) overnight at 4 °C. Cells were washed three times with 1 x PBS, and the number of cells positive for BrdU incorporation was determined using a FACSCalibur flow cytometer. Cells were considered positive if their mean fluorescence intensities (MFI) were 100 fluorescence units (FU) greater than the MFI of unlabeled cells. In order to determine the percentage of G0/G1 and G2/M arrested Hep3B, protocell treated cells were harvested by gentle shaking in 5mM EDTA for 30 min at 37 C, incubated with 1 μg/mL of Hoechst 33342 for 15 min at 37 °C, washed three times with 1PBS, and immediately analyzed using a MoFlo high-performance cell sorter (Dako-Cytomation; Carpinteria, CA, USA) equipped with Dako-Cytomation's SUMMIT software, version 4.3.01. Cells were detected using a 488 nm Innova 90 laser (Coherent Inc.; Santa Clara, CA,USA), and a gate was placed on the forward scatter-side scatter plot that excluded cellular debris. Hoechst 33342 was excited with a 355 nm Innova 90 laser, and emission intensity was collected in the FL-6 channel (450/65 filter/bandpass). Single cells were gated using width and area parameters; the area parameter histogram was used to determine the percentage of gated cells in G0/G1, S, and G2/M phases. Data were acquired with the side scatter channel in log mode and all other channels in linear mode.