Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

A photo-triggerable aptamer nanoswitcher for spatiotemporal controllable siRNA delivery

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Materials and Methods

Materials

PLK1 against siRNA, siRNA-AS1411 chimera, and Cy5-labeled oligonucleotide were synthesized by Guangzhou RiboBio Co., Ltd. (Guangzhou, Guangdong, China). The sequences of these oligonucleotides were shown in Table S1. In order to enhance their stability, several bases of their sequences were modified with methoxy group at the 2' site hydroxyl groups. Dulbecco's Modified Eagle's Medium (DMEM), Opti-MEM, fetal bovine serum (FBS), penicillin, streptomycin, trypsin, and TRIzol® were purchased from Life Technologies (a Thermo Fisher Scientific company, Molecular Probes, Eugene, OR, USA). Cell culture plates and serological pipettes were from Corning Inc. (NY, USA). Reverse transcription kit and real-time PCR kit (UltraSYBR Mixture) were purchased from Promega Corporation (Fitchburg, Wisconsin, USA) and Beijing ComWin Biotech Co.,Ltd. (Beijing, China), respectively. RNA*later*® was purchased from Sigma-Aldrich (St Louis, MO, USA). Optimal cutting temperature (OCT) compound was from Sakura Finetek USA, Inc. (Torracne, CA90501, USA). DAPI (4', 6-diamidino-2-phenylindole, for staining nuclei) was from Zhongshan Goldenbridge (Beijing, China). Fluorescein isothiocyanate-labeled phalloidin (for staining F actin) and hyaluronidase were supplied by Sigma-Aldrich, USA. All of the chemicals used were of analytical grade and were used without further purification.

RNase resistance of siRNA

Fetal bovine serum(FBS) was used to investigate the stability of siPLK1 two different serum/siRNA incubation solutions, with 90% and 50% (v/v) serum respectively. Ten microliter of siRNA (20 μM) and 90 μL of fetal bovine serum were mixed to prepare 100 μL of serum/siRNA incubation solution (with 90% serum). Meanwhile, 10 μl of siRNA (20 μM), 50 μL of fetal bovine serum and 40 μL of 1×PBS were mixed to obtain another 100 μL incubation solution (with 50% serum). Then these solutions were incubated at 37 °C, followed by collection of 10 μL of sample at each desired time point and immediately freezing at -20 °C until gel electrophoresis, in which all samples were separated in 12% native polyacrylamide gels for 80 min at constant

voltage of 100 V (the current was approximately 40 mA). Finally, gels were stained with Sybr Gold for 20 min, exposed by Champ Gel (Beijing Sage Creation Science Co, Ltd., Beijing, China) to show the locations of the siRNA.

Preparation of siRNA-Ap/OliP

siRNA-Ap/OliP stock solution was prepared by adding OliP to a solution (PBS, 150 mM NaCl, pH 7.4) of siRNA-Ap with a final molar ratio of Aptamer (A1411): OliP of 1:1. The solution was annealed and stored at 4 °C overnight to allow full hybridization.

Circular dichroism (CD) spectrometer

CD was introduced to investigate the changes in oligonucleotides conformation due to different treatments. CD spectra were performed by J-810 Spectropolarimeter (JASCO, Tokyo, Japan). OliP(-hv), OliP(+hv), siRNA-Ap, siRNA-Ap&OliP (-hv), siRNA-Ap&OliP (+hv) with a final concentration of 4 μM were respectively scanned in the range of 180-400 nm with a speed of 10 nm/min at 25 °C. The sample buffer was used as a control, and all samples were determined three times. Data analysis was performed by OriginPro 2018 software(OriginLab, USA).

Gel electrophoresis

siRNA-Ap, siRNA-Ap&OliP (-hv), siRNA-Ap&OliP (+hv) with a final concentration of 4 μM were respectively separated in 12% native polyacrylamide gels for 70 min at constant voltage of 100 V (the current was approximately 40 mA). Finally, gels were stained with Sybr Gold for 20 min, exposed by Champ Gel (Beijing Sage Creation Science Co, Ltd., Beijing, China) to show the locations.

Cell culture

4T1 cells, a breast cancer cell line derived from the mammary gland tissue of a mouse BALB/c, were cultured with RPMI-1640 medium supplemented with 100 units/mL penicillin G, 100 μg/mL streptomycin, and 10% (wt/wt) FBS (all from Life Technology) at 37 °C in a humidified atmosphere of 5% CO_2.

FACS assay

4T1 cells were plated in a 6 well (2 x 10^5 per well) plate one day before transfection.

Solutions of free siRNA, siRNA-Ap and siRNA-Ap/OliP diluted in 1 mL Opti-MEM were transfected to cells at the transfection concentration of 200 nM at 37 °C. Light irradiation (365 nm light at 5 mW/cm^2 for 5 min) was performed immediately for groups of siRNA-Ap (+hv) and siRNA-Ap/OliP (+hv). These two groups of cells were seeded at separated plats that are different to groups of free siRNA, siRNA-Ap and siRNA-Ap/OliP without light triggering. After incubation for 2 h, the cells were washed with 1×PBS (2 × 1 mL per well) and treated with 0.25% trypsin with EDTA for 5 min (Life Technologies). The cells were then washed three times with 1 ml of PBS to remove residual free nanoparticles, suspended in PBS, and subsequently introduced into a FACS Calibur flow cytometer (Becton Dickinson, San Jose, CA, USA).

Confocal assay

Confocal microscopy was used to record the subcellular localization of siRNA-Ap, siRNA-Ap&OliP (+hv) and siRNA-Ap&OliP (-hv). Here, 4T1 cells were plated in 6-well plates at 2×10^5 cells per well one day before transfection. Then siRNA-Ap, siRNA-Ap&OliP (+hv) and siRNA-Ap&OliP (-hv) were transfected at 200 nM, and light irradiation was performed for siRNA-Ap&OliP (+hv)-treated cells. Three or five hours later, distributions of the nucleic acids were examined and recorded in living cells by using Zeiss confocal microscopy (LSM700, Carl Zeiss, Germany). Meanwhile, lysotracker Green DND-26 (Invitrogen, Carlsbad, CA) was used to indicate the endosome/lysosome organelles, and hoechst 33342 was used to stain cell nuclei. Imaging processing programs were coded in Interactive Data Language.

Real-time PCR

Cells were seeded in 6-well plates at 2×10^5 cells per well one day before transfection and incubated in RPMI-1640 medium to approximately 60 % confluence. siRNA-Ap, siRNA-Ap&OliP (+hv) and siRNA-Ap&OliP (-hv) were transfected at 200 nM in Opti-MEM at 37 °C. Twenty-four hours later, total RNA of the cells was harvested with Trizol according to the manufacturer's protocol. Then, cDNA was prepared by two-step reverse transcription. First, 1 μg total RNA was incubated for 10 min at 70 °C, followed by immediately transferring onto ice. Then 2 μl of 10× reaction buffer, 4 μl of

$MgCl_2$ (25 mM), 2 μl of dNTP (10 mM), 0.5 μl of RNase inhibitor, and 1 μl (15U) of AMV reverse-transcription enzyme, 1 μl of oligo dT, and desired volume of ddH_2O were added into the precooled tube (20 μl in total), followed by a reaction at 42 °C for 30 min and 95 °C for 5 min, respectively. Then the cDNA solution was diluted 5 times by adding 80 μl ddH_2O. A real-time PCR reaction system (10 μl of 2× UltraSYBR Mixture, 0.5 μl of forward primer (10 μM), 0.5 μl of reverse primer (10 μM), 5 μl of cDNA solution, and 4 μl of ddH_2O) was prepared and first hot-started for 10 min at 95 °C before 40 cycles of 30 s at 95 °C, 30 s at 59 °C and 30 s at 72 °C. After the melting procedure was completed, the samples were stored at 4 °C. The expression level of RRM2 was analyzed by the Ct (cycle threshold) values using standard protocol.

***In vivo* imaging and cryosectioning**

Animals were purchased from Vital River Laboratory Animal Technology Co., Ltd. and maintained in Peking University Laboratory Animal Center (an AAALAC-accredited and specific pathogen free (SPF) experimental animal facility). All procedures involving experimental animals were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) of Peking University.

Female BALB/C mice, 6-8 weeks and weighing 18-22 g, was used to establish tumor-bearing murine model. 4T1 cells (5×10^6 cells per mouse in 100 μl PBS solution) were injected under the fat pad of the second right breast of the mice. When the tumor had increased to 300 mm^3, mice were divided five groups with 2 animals per group. Solutions of PBS, naked siRNA, siRNA-Ap, siRNA-Ap&OliP (+hv) and siRNA-Ap&OliP (-hv) were intravenously injected into the mice, respectively. The dose calculated by the mass of siRNA, without including the mass of aptamer and OliP, was approximate 0.5 mg/kg. Light triggering was performed at 0.5 hour post injection at the tumor site for the mice of siRNA-Ap&OliP (+hv) (365 nm light at 200 mW/cm^2 for 5 min). Two hour post injection, all mice were sacrificed by cervical dislocation, and the major organs (liver, heart, spleen, lung, and kidneys) or tumors were isolated

and imaged using a Kodak in vivo imaging system (Kodak In-Vivo Imaging System FX Pro, Carestream Health, USA). Then the tissues were placed on Omnisette tissue cassettes, embedded in OCT, and frozen on a foam floater that float on liquid nitrogen contained in a pre-chilled Dewar flask for ~1 min until the OCT turned white and opaque. Subsequently, the specimens were cut into 10 μm sections on a cryostat. Each section was picked up on a glass slides, stained by DAPI for viewing the nucleus and by phalloidin to visualize F actin in order to display the rough outline of the cell. Finally, cryosections were examined under the confocal microscope.

Statistical analysis

The data were expressed as the mean ± SD. The statistical variance was calculated by a *t*-test; $p < 0.05$ was considered statistically significant.

Table S1. Sequences of studied oligonucleotides.

Olgio ID	Sense (5'-3')	Antisense (5'-3')
siPLK1-Ap	UmGAAGAAGAUmCmACmCmCmUmCmCmUmUmAdTdTdTdTdTdTdTdTdTdGdGdTdGdGdTdGdGdTdGdGdTdTdGdTdGdGdTdGdGdTdGdG	UAAGGAGGGUGAUCUUCUUCAdTdT
Cy5-siPLK1-Ap	Cy5-UmGAAGAAGAUmCmACmCmCmUmCmCmUmUmAdTdTdTdTdTdTdTdTdTdGdGdTdGdGdTdGdGdTdGdGdTdTdGdTdGdGdTdGdGdTdGdG	UAAGGAGGGUGAUCUUCUUCAdTdT
Cy5-siPLK1	Cy5-UmGAAGAAGAUmCmACmCmCmUmCmCmUmUmAdTdT	UAAGGAGGGUGAUCUUCUUCAdTdT
AS1411	dGdGdT dGdGdT dGdGdT dGdGdT dTdGdT dGdGdT dGdGdT dGdGdT dGdG	
OliP	dCdCdA dCdCdA// dCdCdA dCdCdA// dCdAdA dCdCdA dC	

Note: ‘m’ means the nucleotide was modified with 2’-OMe. dN means the nucleotide is deoxynucleotide. // indicated the photo-labile 1-(2-nitrophenyl)ethyl bonds (Figure S1).

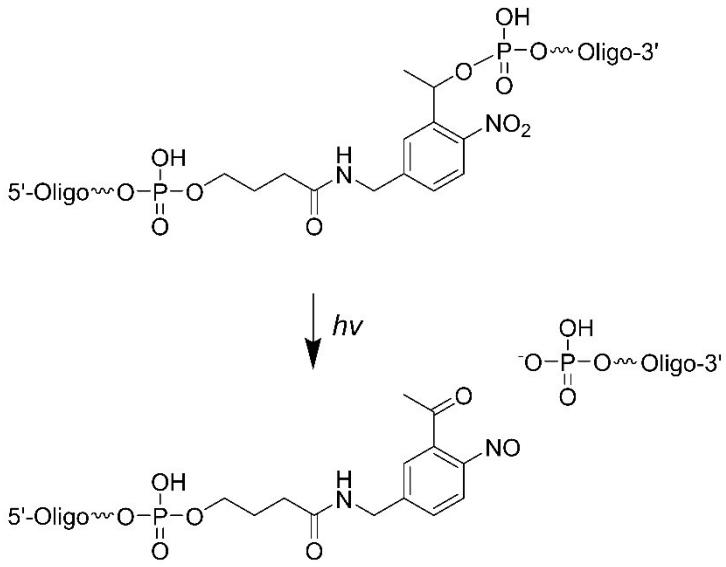

Figure S1 Chemical structures of the photo-cleavage of the oligonucleotide.

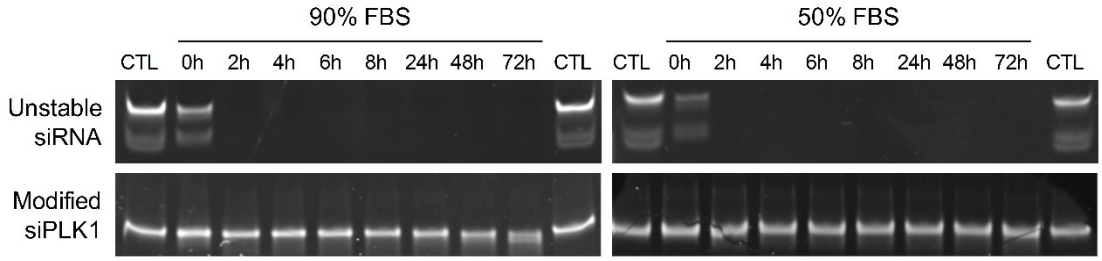

Figure S2 Stability evaluation of chemically-modified siPLK1

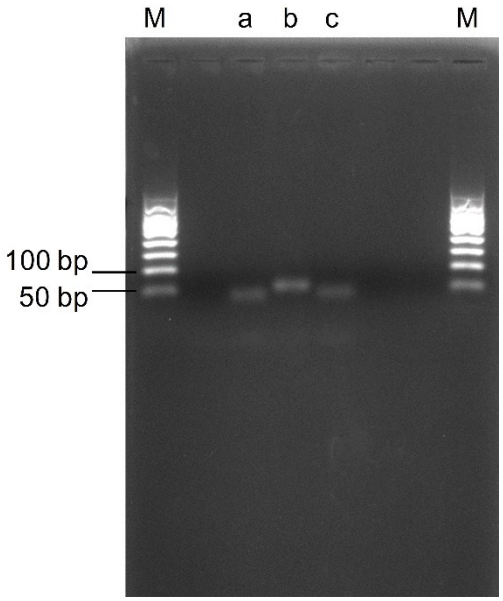

Figure S3. Gel electrophoresis of siRNA-Ap&OliP cleavage by UV irradiation over time. a, siRNA-Ap; b, siRNA-Ap&OliP without irradiation treatment; c, siRNA-Ap&OliP with irradiation treatment. M, DNA ladder.

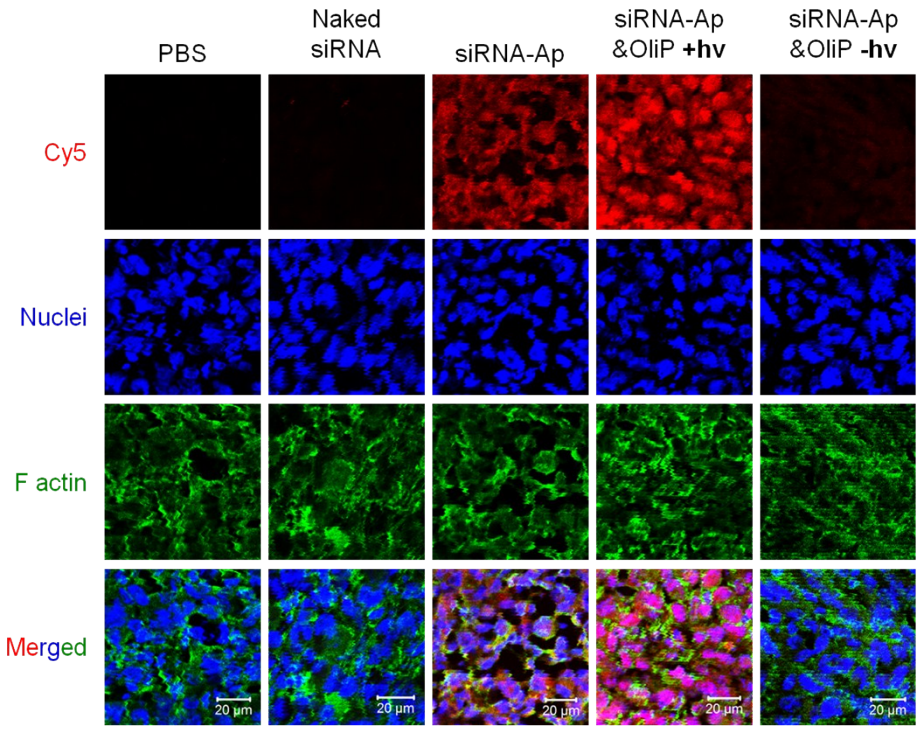

Figure S4 Confocal images with higher magnification than tumor cryosections showed in Figure 5c. The nuclei and F actin were stained with DAPI (in blue) and FITC-labelled phalloidin (in green), respectively. siRNA was labelled with Cy5 (in red). Scale bar, 20 μm.

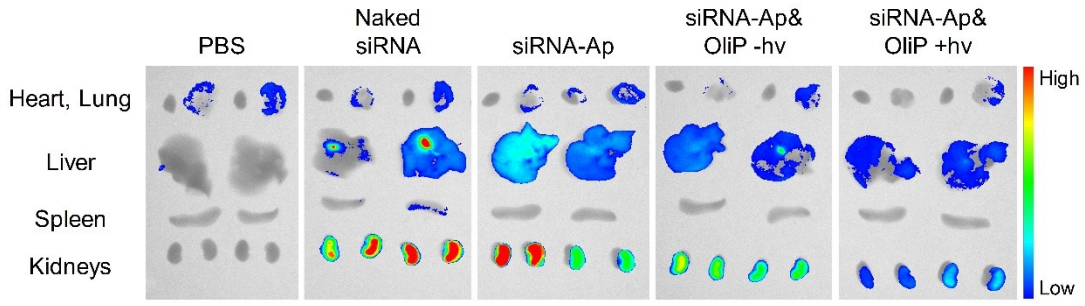

Figure S5 Fluorescence detection of isolated main organs of mice at the end observation point.