Supporting Information

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SI Materials and Methods

S1. General Information. All of the chemicals used were of analytical grade and were used without further purification. The labeled DNAs were synthesized and purified by Integrated DNA Technologies, Inc. and Gene Link, Inc. The sequences were as follows: A_{1411} aptamer: 5′-GGT GGT GGT GGT TGT GGT GGT GGT GG, OliP: 5′-CCA CCA// CCA CCA// CAA CCA C (// represents photocleavable bonds), Oli: 5′-CCA CCA CCA CCA CAA CCA C. Fluorescent spectra were recorded on a FluoroMax-P fluorimeter (HORIBA Jobin Yvon Inc.). Flow cytometry was performed on a BD FACSAria II (BD Bioscience). Fluorescence microscopy was conducted on an Inverted Olympus ×71 microscope. In vivo deep tissue imaging was conducted on an Olympus FV1000 multiphoton laser scanning confocal microscope. In vivo fluorescence imaging of whole body and organs was performed with an IVIS imaging system (IVIS Spectrum; Caliper Life Sciences).

S2. Preparation of A_{1411}/OliP Bioprobe. A_{1411}/OliP stock solution was prepared by adding OliP to a solution (PBS, 150 mM NaCl, pH 7.4) of DNA aptamers labeled with a dye with a final ratio of A_{1411}: OliP of 1:1. The solution was annealed and stored at 4 °C overnight to allow full hybridization.

S3. FRET Test. The FRET pair was prepared by hybridizing aptamer (labeled with Cy3 at the 3 terminus) with OliP [labeled with quencher (Iowa Black RQ) at the 5 terminus]. A 40 nM solution of the FRET pair in 150 mM NaCl PBS buffer with 0.05% Tween-20 added (pH 7.4) was irradiated with UV light (365 nm, 5 mW/cm^2) for a specified period. Fluorescence spectra were recorded on a fluorometer with excitation at 540 nm.

S4. Analysis of Cellular Uptake by Fluorescence Microscopy. 4T1 cells (American Type Culture Collection) were grown in chamber slides (Nunc Lab-Tek; Thermo Scientific) in RPMI-1640 medium supplemented with 100 units/mL aqueous penicillin G, 100 μg/mL streptomycin, and 10% (wt/wt) FBS (all from Life Technology) at concentrations to allow 70% confluence in 24 h (i.e., 2×10^4 cells/cm^2). On the day of experimentation, the medium was replaced with Opti-MEM medium (500 μL) containing 0.5 μM Cy3-labeled A_{1411} or A_{1411}/OliP probes with or without light triggering (365 nm light at 5 mW/cm^2 for 5 min). After incubation for 2 h, 4T1 cells were washed with prewarmed PBS (3 × 200 μL) and fixed with 4% (wt/wt) paraformaldehyde. Actin and nuclei were stained with Alexa Fluor 488 Phalloidin and DAPI, respectively, according to the manufacturer's instructions (Life Technologies). Fluorescence images were taken on an Inverted Olympus ×71 microscope.

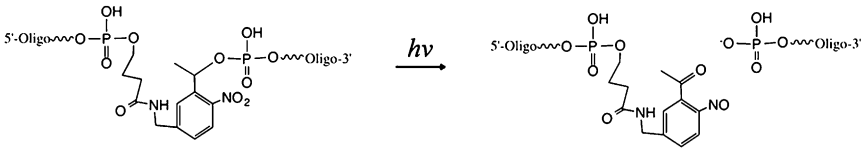
S5. Analysis of Cellular Uptake by Flow Cytometry. 4T1 cells were grown in 12-well plates in RPMI-1640 medium, supplemented with 100 units/mL aqueous penicillin G, 100 μg/mL streptomycin, and 10% (wt/wt) FBS (all from Life Technologies) at concentrations to allow 70% confluence in 24 h (i.e., 2×10^4 cells/cm^2). On the day of experimentation, the medium was replaced with Opti-MEM medium (500 μL) containing 0.5 μM Cy3-labeled A_{1411} or A_{1411}/OliP probes with or without light triggering (365 nm light at 5 mW/cm^2 for 5 min). After incubation for 2 h, the cells were washed with PBS (2 × 500 μL per well) and treated with 0.25% trypsin with EDTA for 5 min (Life Technologies). After the cells were washed with PBS (2 × 1 mL), they were fixed with 4% (wt/wt) formaldehyde for 10 min at room temperature, washed with PBS (2 × 1 mL), and stored in 1 mL PBS with 1 wt% BSA solution at 4 °C for flow cytometry analysis.

S6. The 4T1 Tumor Model and in Vivo Imaging. Immunodeficient 6- to 8-wk-old nu/nu nude mice were purchased from Charles River Laboratories and maintained under pathogen-free conditions for all animal studies. The study protocol was reviewed and approved by the Massachusetts Institute of Technology Committee on Animal Care. For s.c. 4T1 tumor models, 4T1 cells were injected in the dorsal aspect of the neck with 1×10^6 cells /0.2 mL in 1:1 (vol/vol) PBS and Matrigel (BD Biosciences). The mice were fed alfalfa-free food for at least 1 wk before the study to minimize the autofluorescence (1). For orthotopic 4T1 tumors, 1×10^5 cells/0.1 mL in HBSS were injected into mouse mammary fat pads. Tumor length and width were measured with calipers, and the tumor volume was calculated using the following equation: tumor volume = length × width × width/2. Mice whose tumors reached ∼100–200 mm^3 were used in subsequent experiments. The mice were injected i.v. with Cy5-labeled A_{1411} or A_{1411}/OliP at an aptamer dose of 250 nmol/kg. In light-triggering experiments, the tumor site was illuminated by UV light for 3 min (365 nm, 200 mW/cm^2) immediately after injection. [The minimum time to dehybridize A_{1411}/OliP when irradiating through mouse skin with 365 nm light at 200 mW/cm^2 was 2 min (Fig. S5). However, in vivo imaging (Fig. S6*A*) showed that irradiation for 3 min was more effective than irradiation for 2 min in enhancing aptamer retention in the tumor after injection of A_{1411}/OliP. This duration of irradiation was then used in experiments where aptamers were injected intravenously. Irradiation time had no effect on the retention of aptamer in tumors after injection of the A_{1411} (Fig. S6*B*).] Mice were anesthetized with isoflurane and imaged at specified time intervals postinjection. Whole-body fluorescence imaging was performed with an IVIS imaging system (IVIS Spectrum; Caliper Life Sciences) with excitation and emission wavelengths of 640 and 680 nm, respectively. At 2 h postinjection, mice were killed and organs were immediately collected and imaged. Data were processed using Living Image software from Caliper Life Sciences. For intravital microscopy, the orthotopic 4T1 tumor model was used. Mice were anesthetized by isoflurane, and orthotopic 4T1 tumors on the mammary fat pads were exposed for microscopy by brief skin-snap surgery (2, 3). The intratumoral collagen matrix was imaged by second harmonic generation (4). Upon administration of A_{1411} or A_{1411}/OliP, mice were injected i.v. with 0.1 mL 2.5 wt% FITC dextran solution (molecular mass ∼70 kDa; Life Technologies) to demarcate the vasculature for microscopy.

S7. Immunostaining. For fluorescent immunostaining, tumor tissues were excised, snap-frozen, embedded in Optimum Cutting Temperature Compound (Sakura Finetek USA) and 5- to 10-μm histological sections were obtained. Samples were fixed in cold acetone and incubated ("blocked") for 1 h at room temperature in PBS buffer containing 1% BSA. Samples were incubated with rabbit anti-mouse CD-31 (1:50; Abcam) for 12 h. The corresponding secondary antibody Alexa Fluor 594 goat anti-rat IgG (1:500; Life Technologies) was added and incubated for 1 h at room temperature. The slides were washed three times with PBS and mounted in Vectashield mounting medium containing DAPI (Vector Laboratories). Imaging was performed with an Inverted Olympus ×71 microscope.

S8. Statistical Analysis. Data that were reasonably normally distributed were described with means and SDs and compared with *t* tests. Otherwise, data were presented as median ± quartiles and differences between groups were assessed with a Mann–Whitney *u* test. All data analyses were performed using Origin 8 software.

1. Inoue Y, Izawa K, Kiryu S, Tojo A, Ohtomo K (2008) Diet and abdominal autofluorescence detected by in vivo fluorescence imaging of living mice. *Mol Imaging* 7(1):21–27.
2. van de Ven AL, Kim P, Ferrari M, Yun SH (2013) Real-time intravital microscopy of individual nanoparticle dynamics in liver and tumors of live mice. *Nat Protocols Exchange*, 10.1038/protex.2013.1049.
3. Jain RK, Munn LL, Fukumura D (2012) Mammary fat pad tumor preparation in mice. *Cold Spring Harbor Protocols* 2012(10):1115–1116.
4. Brown E, et al. (2003) Dynamic imaging of collagen and its modulation in tumors in vivo using second-harmonic generation. *Nat Med* 9(6):796–800.



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

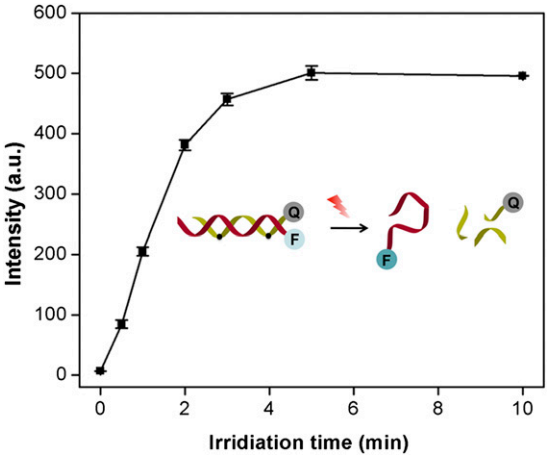


Fig. S1. Effect of irradiation time on fluorescence intensity (λ_{ex} = 545 nm; λ_{em} = 562 nm) of the Cy3-A_{1411}/OliP FRET pair (irradiated by 365 nm light at 5 mW/cm^2). Data are means ± SD, n = 4.

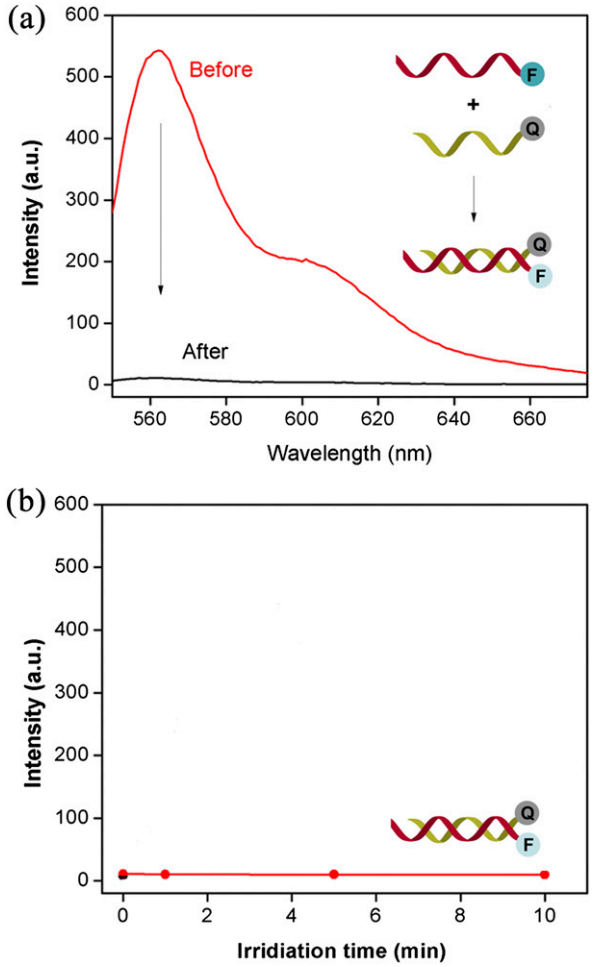


Fig. S2. (*A*) Fluorescence spectra of Cy3-A_{1411} before and after hybridization with a quencher-labeled complementary sequence without photo-cleavable bonds (Q-Oli). (*Inset*) Schematic illustration of the hybridization. (*B*) Effect of duration of irradiation on fluorescence intensity of the Cy3-A_{1411}/Q-Oli FRET hybrid. Compare with Fig. S1.

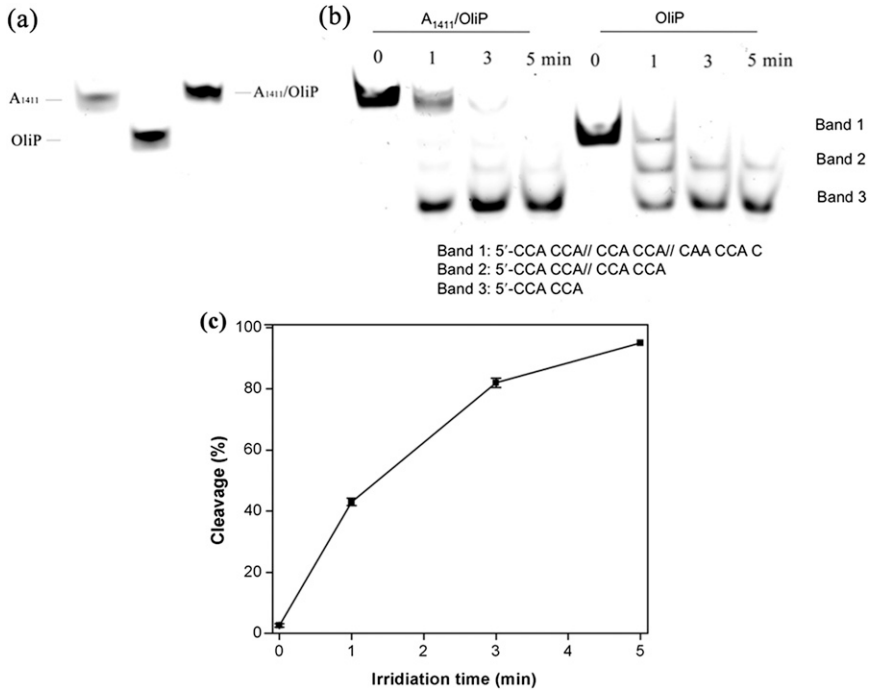


Fig. S3. Polyacrylamide gel electrophoresis of A_{1411}/OliP cleavage by UV irradiation over time. (*A*) Fluorescence images of a representative polyacrylamide gel of fluorescein isothiocyanate (FITC)-A_{1411}, FITC-OliP, and A_{1411}/FITC-OliP, indicating that OliP was hybridized with A_{1411}. (*B*) Fluorescence images of a representative gel of A_{1411}/FITC-OliP and FITC-OliP irradiated by UV light at 5 mW/cm^2 for different lengths of time. Note that the breakdown products of the irradiation of FITC-OliP have the same migration characteristics as the breakdown products of A_{1411}/FITC-OliP. (*C*) Quantitation of the photo-cleavage of A_{1411}/FITC-OliP from the data in *B*. % cleavage is the intensity of the cleaved band (band 3, corresponding to 5′-CCA CCA) as a percentage of the sum of the fluorescence of all bands in a lane. Data are means ± SD, $n = 4$. The FITC-labeled strand was visualized using a STORM 840 optical scanner.

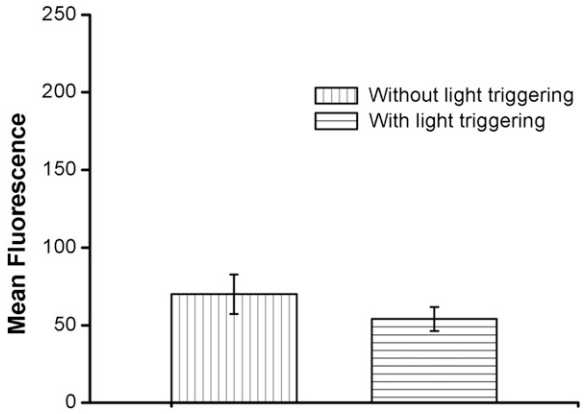


Fig. S4. Flow cytometric quantification of fluorescence of 4T1 cells treated with Cy3-A_{1411}/Oli with or without light triggering (365 nm, 5 mW/cm^2 for 5 min). Data are means ± SD, $n = 4$.

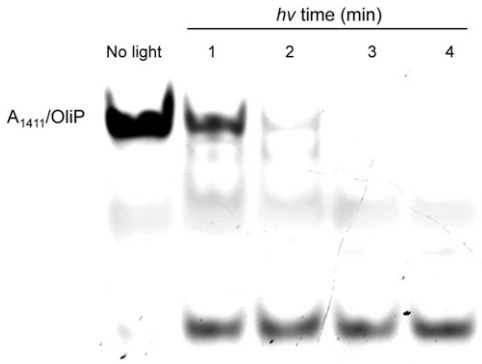


Fig. S5. Polyacrylamide gel electrophoretogram of A_{1411}/OliP cleavage after irradiation through mouse skin (to mimic irradiation in vivo) for varying lengths of time with 365 nm light at 200 mW/cm^2. The majority of A_{1411}/OliP was cleaved in 2 min.

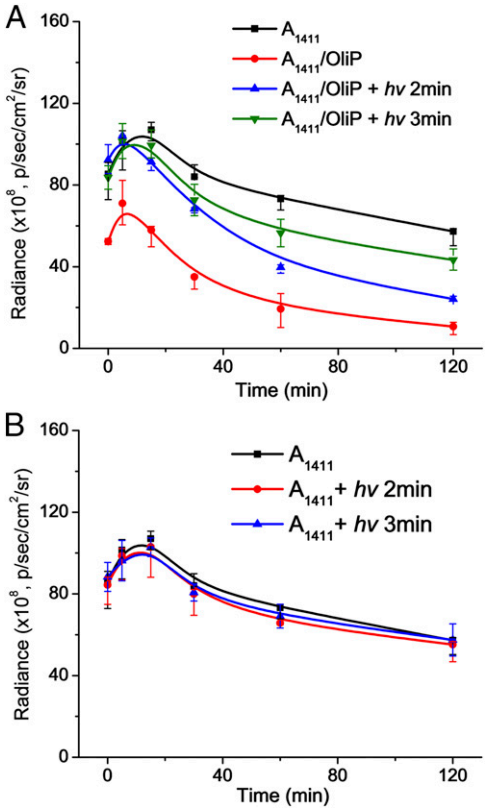


Fig. S6. Effect of photo-triggering time on the intratumoral accumulation of (*A*) A_{1411}/OliP and (*B*) A_{1411}. The 4T1 tumor-bearing mice were injected i.v. with Cy5-labeled A_{1411} or A_{1411}/OliP with subsequent irradiation at the tumor site (365 nm light at 200 mW/cm^2 for different lengths of time). The Cy5 fluorescence was derived from integration of the photoradiance (p/s/cm^2/sr). $n = 5$. Numeric data are medians ± quartiles.

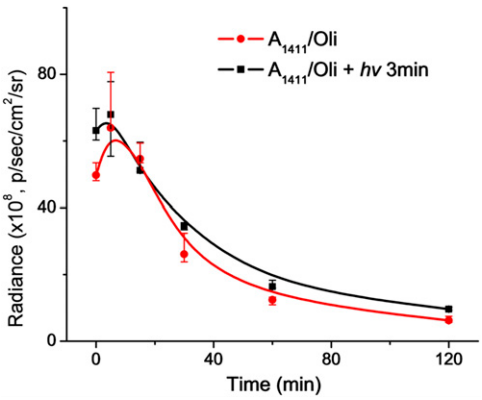


Fig. S7. Effect of UV irradation on the intratumoral accumulation of A_{1411}/Oli. The Cy5 fluorescence was derived from integration of the photoradiance (p/s/cm^2/sr). $n = 5$. Numeric data are medians ± quartiles.

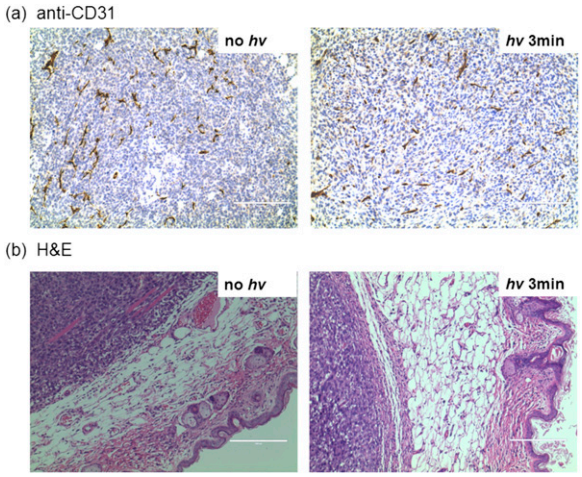


Fig. S8. Representative (of five animals) (*A*) anti-CD31 (staining blood vessels) and (*B*) H&E-stained tissue sections of 4T1 tumors 2 h after irradiation (200 mW/cm^2, 3 min) or not.

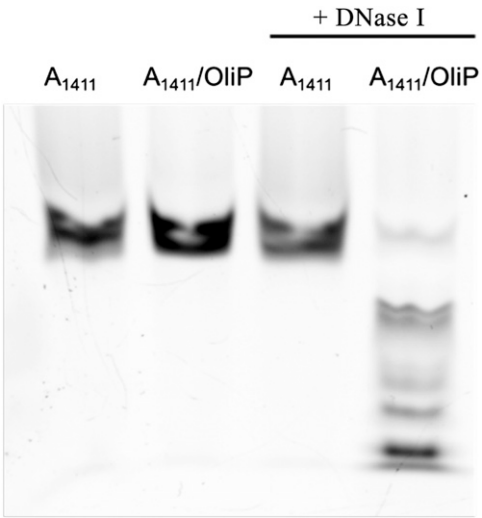


Fig. S9. Polyacrylamide gel electrophoresis of the degradation of A_{1411} and A_{1411}/OliP by DNase I in 20 min. A_{1411} and A_{1411}/OliP (200 nM) were incubated with DNase I (0.1 U/μL) at 37 °C for 20 min then analyzed by gel electrophoresis. A_{1411} was not degraded, whereas A_{1411}/OliP was.