Supporting Information

A DNA conformational nanoswitch for amplification of both living cells low abundance proteins imaging and photodynamic therapy

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1. Material and experimental methodology

1.1. Materials

Anti-MUC1 antibodies, Alpha fetoprotein (AFP), mucin-1 (MUC1), carcinoembryonic (CEA), vascular epidermal growth factor (VEGF) were obtained from Sangon Biotech (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM) were obtained from Gibco (Shanghai, China). The MUC1 inhibitor GO-201 ($C_{133}H_{233}N_{61}O_{31}S_2 \cdot xC_2HF_3O_2$), dopamine hydrochloride (DA), Penicillin-Streptomycin and Foetal bovine serum (FBS) were obtained from Sigma-Aldrich (Shanghai, China). Cell apoptosis kit, Singlet Oxygen detection kit (SOSG), cell reactive oxygen species detection kit, MUC1 ELISA fluorescent kit, and cell dead and alive staining kit, were acquired from Beyotime Biotechnology Co.,Ltd. (Shanghai, China). Human lung cancer cell (A549), human normal lung epithelial cell (BEAS-2B), human nasopharyngeal carcinoma cell (CNE-2), nasopharyngeal normal cells (NP69), breast cancer (MCF-7), and normal mammary epithelial (MCF-10A) cells were supplied by were supplied by the Committee on Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Nucleic acid chains were synthesized by Sangon Biotech (Shanghai, China). The sequences of these nucleic acid chains are provided in Table S1.

1.2. Preparation of the PDA nanoparticles (PDA NPs), PDA/P/H1 nanoprbe and nanoswitch

First, dissolve 500 mg of dopamine hydrochloride in 10 mL of tris buffer, and stir the solution at room temperature for 24 h. After this, the solution is carefully washed by centrifugation (10000 r/min, 8 min) with distilled water three times. Finally, after drying (60 ℃), you obtain the PDA NPs.

To prepare the nanoswitch, 100 μL of a PDA NPs solution (0.8 mg/mL), 100 μL of a mixture containing P (1000 nM), H1 (1000 nM), and H2 (1000 nM), and 300 μL of buffer were mixed in a microcentrifuge tube and incubated at room temperature for 30 min. The resulting PDA@H1 nanoswitch was then stored at 4°C for future use.

The preparation method for PDA/P/H1 nanoprbe and nanoswitch was the same, except that the 100 μL of P (1000 nM), H1 (1000 nM) and H2 (1000 nM) mixture was replaced with the 100 μL of P (1000 nM) and H1 (1000 nM) mixture.

1.3. Gel electrophoresis and fluorescence detection in vitro

Polyacrylamide gel electrophoresis validated the successful reaction between the DNA probe and the MUC1 target strand. Sample one: P (1.0 μM); Sample two H1 (1.0 μM); Sample three: H2 (1.0 μM); Sample four: the mixture of H1 (1.0 μM), 1 H2 (1.0 μM) and P (1.0 μM); Sample five: the mixture of P (1.0 μM), target strand (1.0 μM); Sample six: the mixture of P (1.0 μM), target strand (1.0 μM) and H1 (1.0 μM); Sample eight: the mixture of P (1.0 μM), target strand (1.0 μM), H1 (1.0 μM) and H2 (1.0 μM). All samples were prepared in Tris-HCl buffer (pH 7.4) containing 100 mM NaCl, 10 mM KCl, and 10 mM $MgCl_2$, and incubated at 37 °C for 100 min. For analysis, 10 μL of each sample was loaded onto a 15% polyacrylamide gel, and electrophoresis was conducted in 1× TBE buffer (pH 7.9) at 80 V for 90 min. The gels were then stained with ethidium bromide and imaged using a digital camera.

In a standard experiment, 10 μL of MUC1 or other proteins were added to a Tris-HCl buffer containing 160 μg/mL nanoswitch. The mixture was incubated at 37°C for 120 min before measuring fluorescence. Ce6 fluorescence was excited at 405 nm,

with both the excitation and emission slits set to 10 nm. All experiments were conducted in triplicate, with five replicates per sample.

For the detection of $^1O_2$, the sample preparation was similar to the MUC1 testing, except that SOSG was incorporated into the sample. After sample preparation, the samples were subjected to 10 min of laser irradiation before fluorescence determination.

1.4. Fluorescence imaging of cells

MCF-7 cells were treated with the 160 μg/mL nanoswitch, and then incubated for various time intervals. Following incubation, fluorescence imaging was performed to visualize the cellular fluorescence.

The 160 μg/mL nanoswitch, or the 160 μg/mL NP were individually added to MCF-7 cells, MCF-10A cells, A549 cells, BEAS-2B cells, CNE-2 cells, and NP69 cells. After 10 h incubation, fluorescence imaging was performed to visualize the cellular fluorescence.

MCF-7 and MCF-10A cells are combined and co-cultured for 24 h. After this incubation period, the 160 μg/mL nanoswitch was added to the cells, which are then further incubated for 10 h. Fluorescence imaging is subsequently performed to observe the cells.

After treating MCF-7 cells with GO-201 for 24 h, the cells were separately incubated with the 160 μg/mL nanoswitch for an additional 10 h. Fluorescence imaging was then performed to observe the cells.

After incubating MCF-7 cells with the 160 μg/mL nanoswitch for 10 h, the cells were irradiated with a 660 nm laser for 10 min. The other groups were not exposed to the laser. Finally, the cells were stained with a Reactive Oxygen Species Assay Kit for fluorescence imaging.

After incubating T24 cells with the 160 μg/mL nanoswitch for 10 h, the cells were irradiated with a 660 nm laser for 10 min, while the other groups were not exposed to the laser. Finally, the cells were stained with calcein AM and propidium iodide (PI) for imaging.

The excitation and emission wavelengths were as follows: calcein AM at 488 nm and 495–540 nm; DCFH-DA at 488 nm and 495–540 nm; PI at 543 nm and 550–620 nm; and Ce6 at 405 nm and 630–730 nm.

1.5. Flow cytometry

MCF-7, A549, CNE-2, MCF-10A, BEAS-2B, and NP69 cells were incubated with the 160 μg/mL nanoswitch, or the 160 μg/mL PDA/P/H1 nanoprbe for 10 h. After incubation, the cells were collected and the fluorescence intensity of Ce6 was measured using flow cytometry.

MCF-7, A549, CNE-2, MCF-10A, BEAS-2B, and NP69 cells were incubated with the 160 μg/mL nanoswitch, or the 160 μg/mL PDA/P/H1 nanoprbe for 10 h. The cells were then irradiated with a 660 nm laser for 10 min. Finally, the cell apoptosis rate was measured after treating the cells with an apoptosis assay kit.

1.6. Western blotting (WB)

First, MCF-7 cells were treated with a MUC1 inhibitor for 24 h. Total cellular proteins were then extracted by incubating the cells in lysis buffer. The protein concentration was quantified using the BCA Protein Assay Kit. The proteins were separated by SDS-PAGE and transferred onto a PVDF membrane. The membrane was blocked for 2 h at room temperature with 10% (w/v) non-fat dry milk powder in TBST buffer (20 mM Tris-HCl, 137 mM NaCl, 0.1% Tween-20, pH 7.4). After blocking, it was incubated overnight at 4°C with anti-MUC1 antibodies (1:1000), and anti-β-actin antibody was used as a loading control. The membrane was then incubated with a horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody (1:2000) for 2 h at room temperature. Protein detection was performed using ECL reagents and analyzed with the imaging system and Image Lab software.

The determination of MUC1 protein expression levels in group MCF-7, A549, CNE-2, MCF-10A, BEAS-2B, and NP69 cells followed the same experimental steps as described above, except that the cells were not treated with the inhibitor.

1.7. MTT test for toxicity

The MTT assay was performed to evaluate toxicity. Cells were first incubated with the 160 μg/mL nanoswitch, or the 160 μg/mL PDA/P/H1 nanoprbe alone, for 10 h. Following incubation, one set of cells was irradiated with a 660 nm laser for 10 min, while the other groups were not exposed to the laser. Subsequently, 20 μL of MTT solution was added to the medium and incubated for 6 h. The culture medium was

then removed, and 100 μL of DMSO was added to each well to dissolve the formazan crystals. The plates were gently shaken to ensure even distribution of the dissolved formazan crystals. The absorbance of each well was then measured at 490 nm using an enzyme-labeled instrument.

2. Supporting Tables

Table S1. Sequences of oligonucleotides used in this work.

Oligonucleotides	Sequences
P	TGATCCTTTGGATACCCTGGTGAGGTAGTAGGTTGTATA GTTCTCACCAGGGTATCC
H1	CTAGCTAGCGCAGTAGGT-Ce6- TGTATAGATCAAAGTATCTAT-Ce6- ACAACCTACTACCTCA
H2	TTGATCTATACAACCTACTACCTTCTGAGGTAGTAGGTT GTATA ACTGCAGATCG
Target	CCAGGGTATCCAAAGGATCAACTGC

The blue part is the MUC1 recognition sequence.

3. Supporting Figures

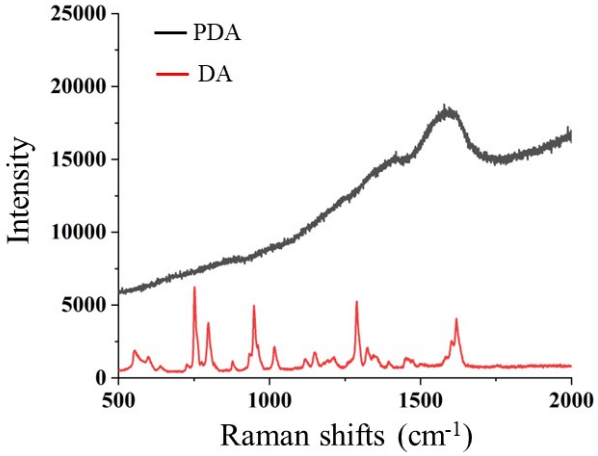

Fig. S1. The Raman spectra of dopamine (DA) and PDA.

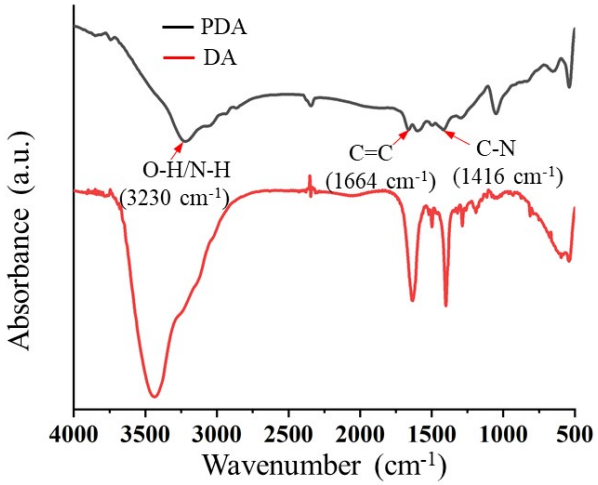

Fig.S2. The FT-IR spectrum of dopamine and PDA.

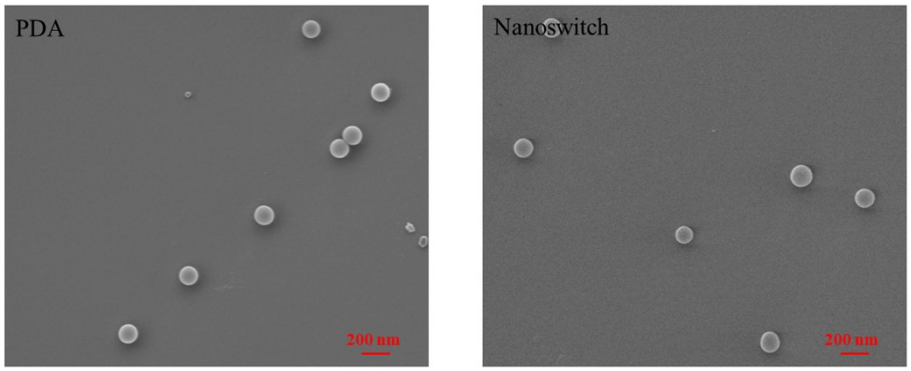

Fig.S3. SEM imaging of PDA and nanoswitch.

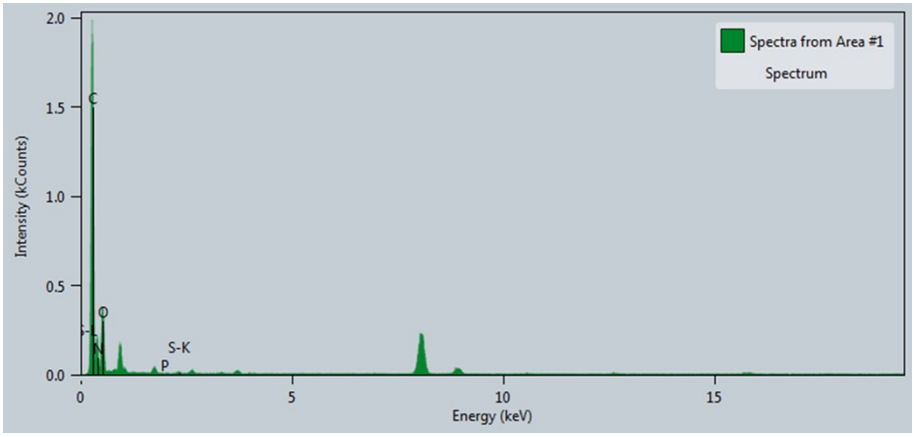

Fig.S4. Energy Dispersive X-Ray Spectroscopy of nanoswitch.

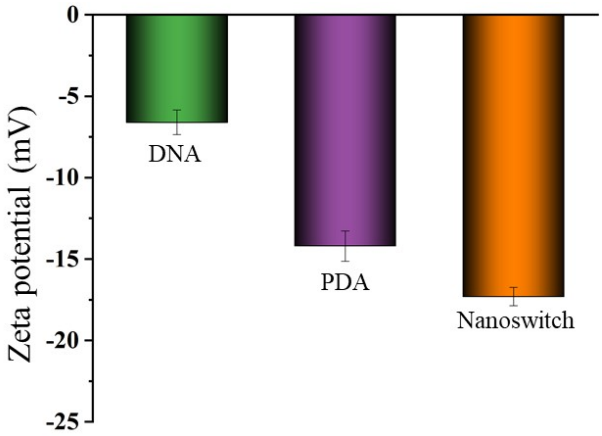

Fig.S5. Zeta potential analysis of DNA, PDA and nanoswitch. Error bars were derived from N=5 experiments.

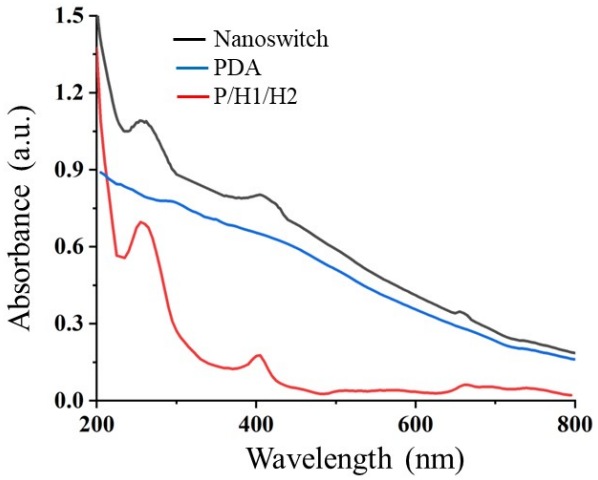

Fig. S6. The UV-vis spectrum of the P/H1/H2, PDA and nanoswitch.

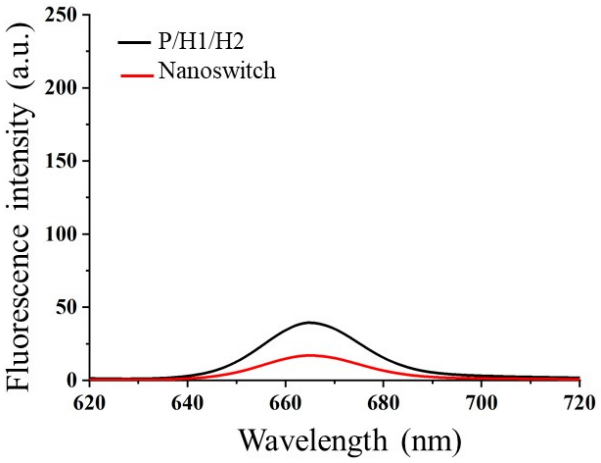

Fig. S7. Fluorescence spectrum of P/H1/H2 and nanoswitch

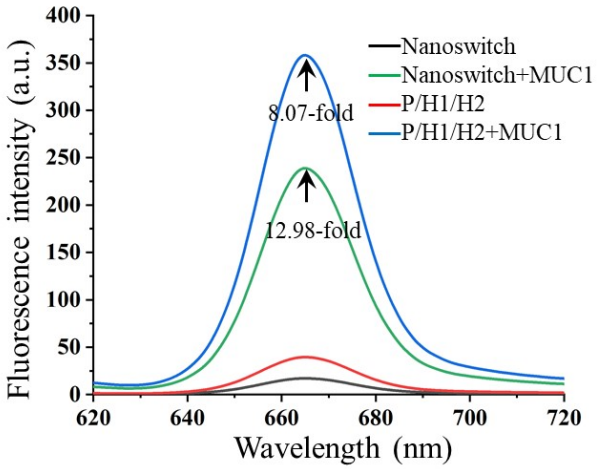

Fig. S8. Fluorescence spectrum of MUC1 analysed by nanoswitch or P/H1/H2.

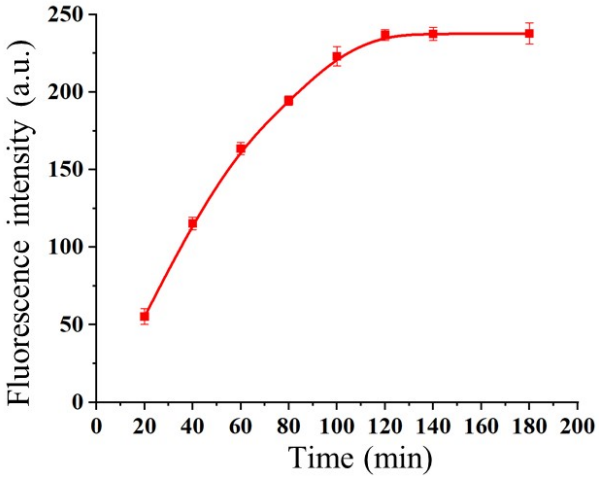

Fig. S9. Fluorescence intensity of nanoswitch with MUC1 (20 nM) during the reaction time. Error bars were derived from five experiments.

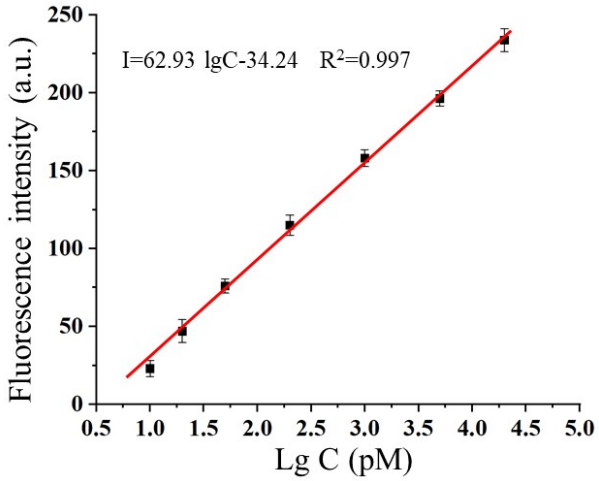

Fig. S10. (A) Calibration curves corresponding to the nanoswitch. Error bars were derived from five experiments.

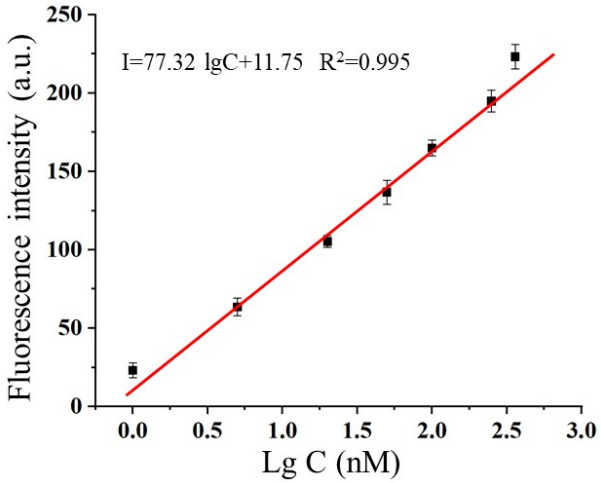

Fig. S11. Calibration curves corresponding to the NP. Error bars were derived from five experiments.

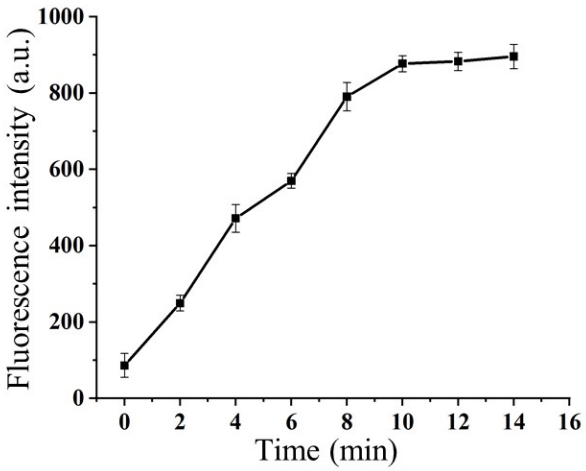

Fig. S12. SOSG fluorescence intensity of the mixture of nanoswitch incubated with MUC1 at different time after laser irradiation. Error bars were derived from five experiments.

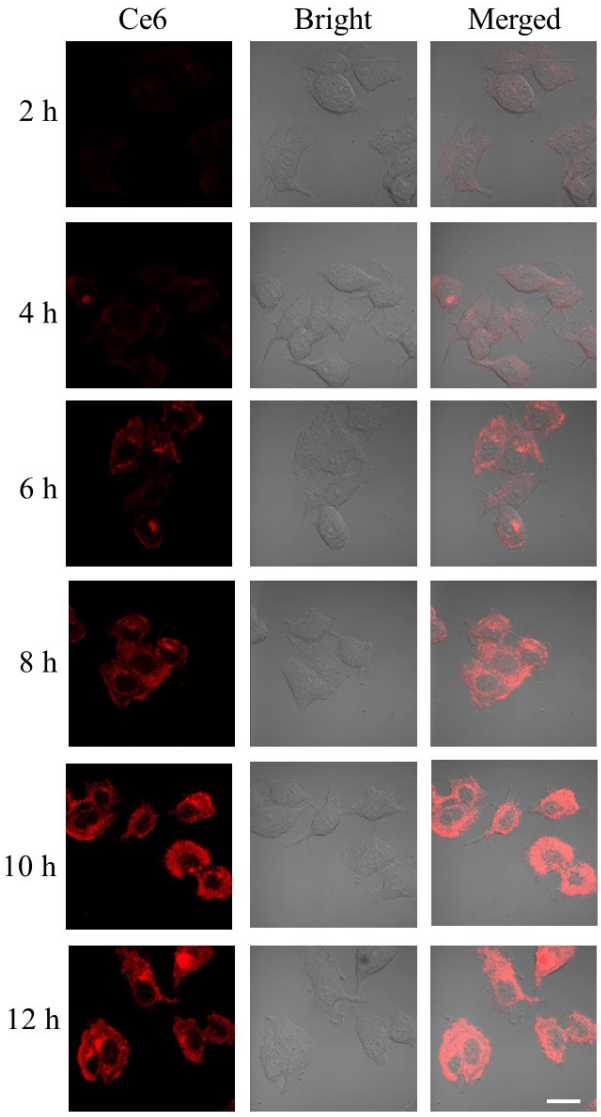

Fig. S13. Fluorescence imaging of MCF-7 cells after incubation with the nanoswitch (160 μg/mL) for different time. Scale bar: 20 μm.

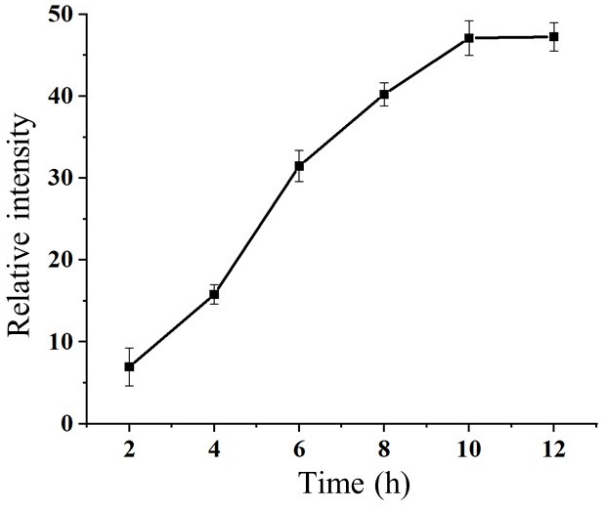

Fig. S14. Relative intensity of fluorescence of MCF-7 cells after incubation with the nanoswitch for different time. Error bars were derived from five experiments.

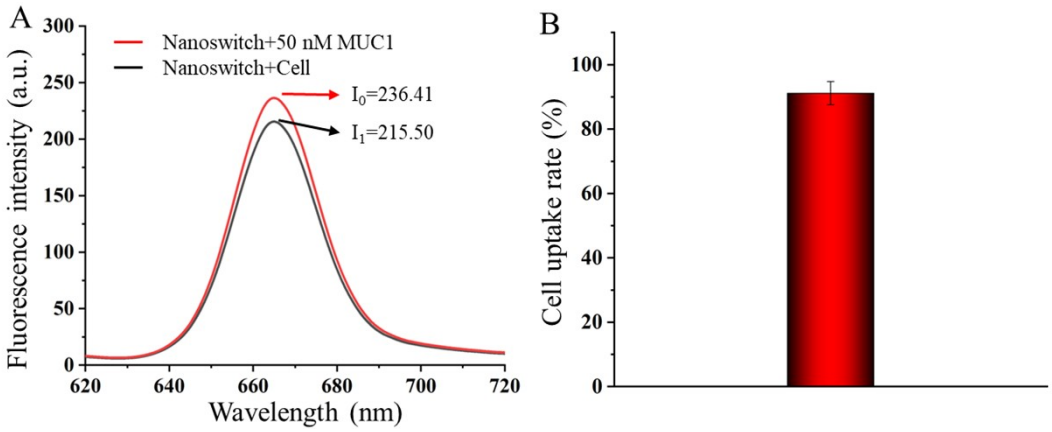

Fig. S15. Fluorescence spectrum of the nanoswitch treated with MUC1 or cells.

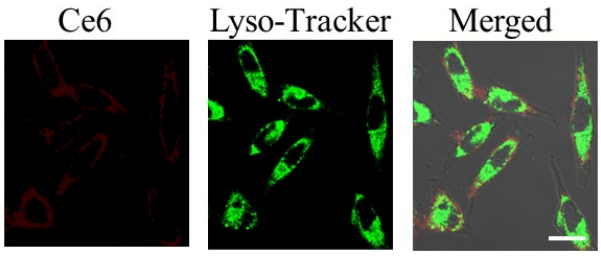

Fig. S16. Fluorescence imaging of A549 cells after incubation with the nanoswitch for 4 h. Scale bar: 20 μm.

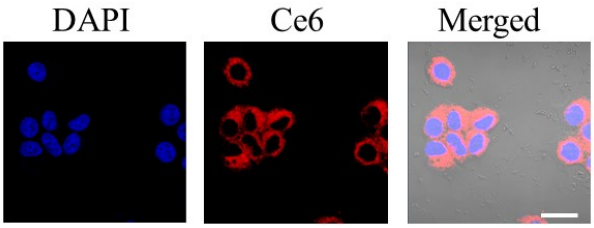

Fig. S17. Fluorescence imaging of MCF-7 cells after incubation with the nanoswitch for 10 h. Scale bar: 20 μm.

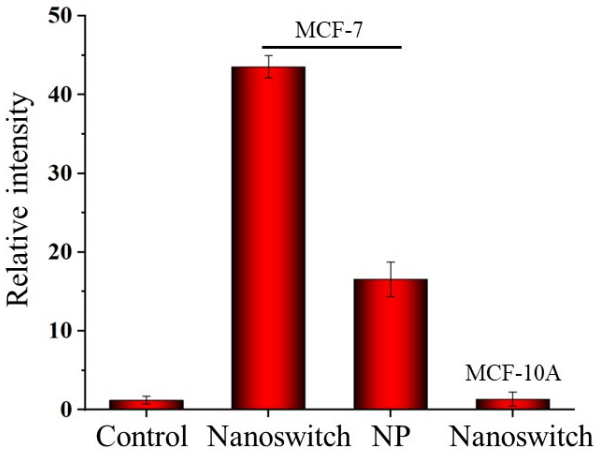

Fig. S18. Relative intensity of fluorescence of different cells after incubation with the nanoswitch (160 μg/mL) or NP (160 μg/mL) for 10 h. Error bars were derived from five experiments.

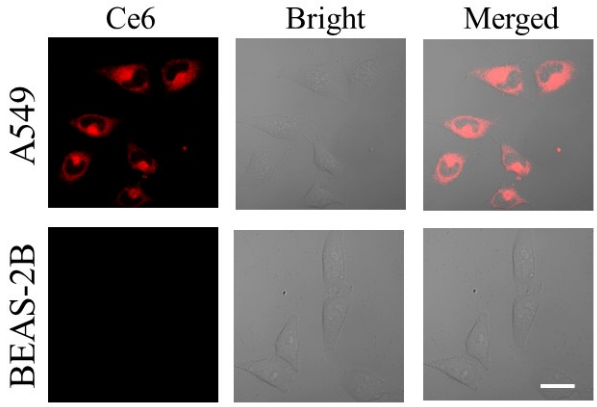

Fig. S19. Fluorescence imaging of A549 cells and BEAS-2B cells treated with the nanoswitch. Scale bar: 20 μm.

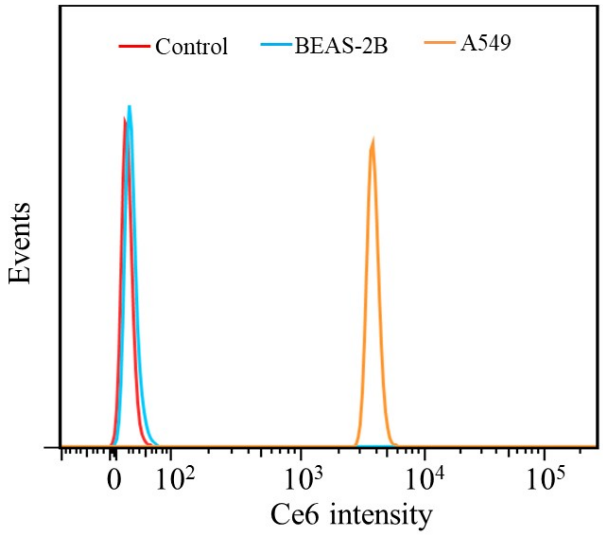

Fig. S20. Flow cytometry analysis of Ce6 fluorescence in A549 cells and BEAS-2B cells treated with the nanoswitch.

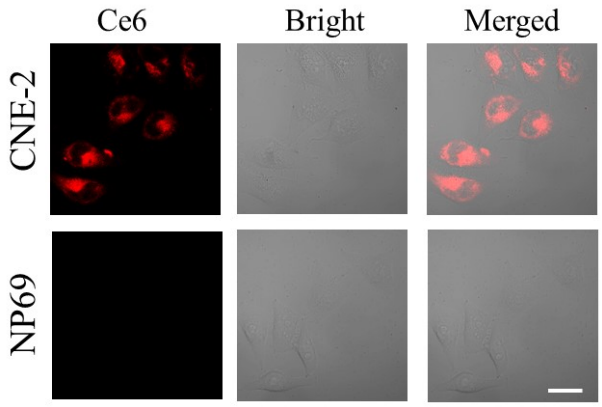

Fig. S21. Fluorescence imaging of CNE-2 cells and NP69 cells treated with the nanoswitch. Scale bar: 20 μm.

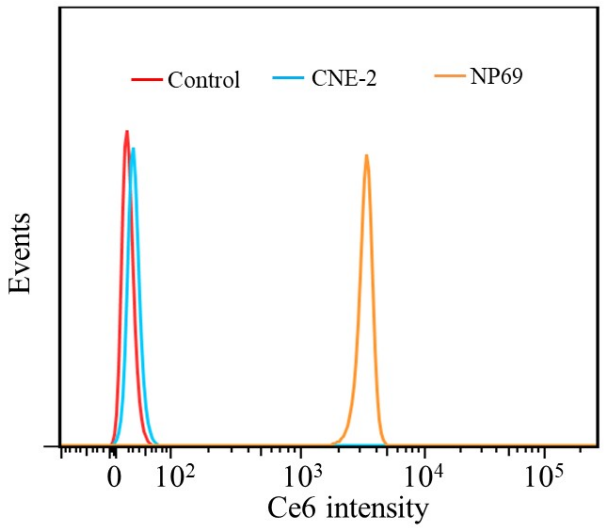

Fig. S22. Flow cytometry analysis of Ce6 fluorescence in CNE-2 cells and NP69 cells treated with the nanoswitch.

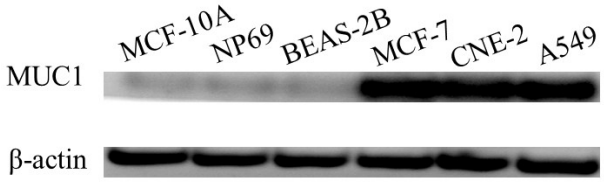

Fig. S23. Expression of MUC1 in different cells

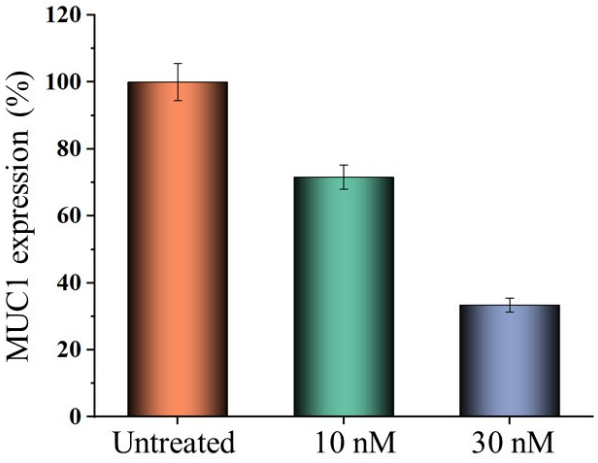

Fig. S24. The expression of MUC1 in cells treated with different concentrations of inhibitors. Error bars were derived from five experiments.

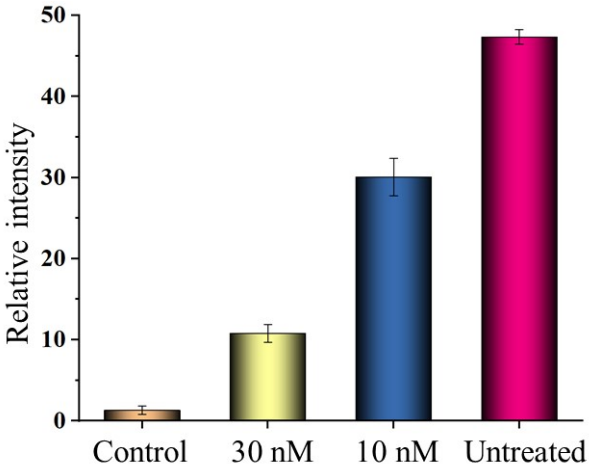

Fig. S25. The relative fluorescence intensity of cells treated with inhibitors and then incubated with nanoswitch for 10 h. Error bars were derived from five experiments.

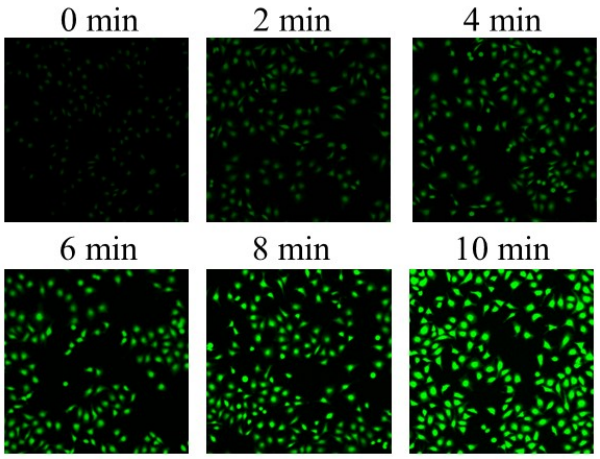

Fig. S26. Fluorescence imaging of reactive oxygen species at different time after laser irradiation of nanoswitch treated cells.

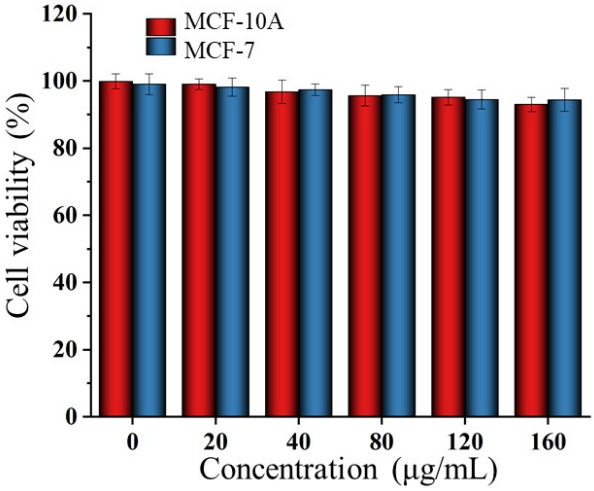

Fig. S27. Cell viability treated with different concentrations of nanoswitch. Error bars were derived from five experiments.

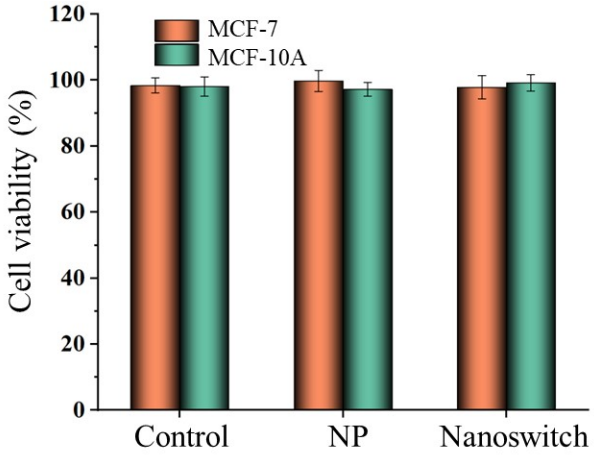

Fig. S28. Cell viability treated with nanoswitch or NP without laser irradiation. Error bars were derived from five experiments.