Supporting Information

Light and endogenous enzyme triggered plasmonic antennas for accurate subcellular molecular imaging with enhanced spatial resolution

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Materials

Adenosine 5'-triphosphate (ATP) disodium salt hydrate, cytidine 5'-triphosphate disodium salt (CTP), guanosine 5'-triphosphate sodium salt hydrate (GTP), uridine 5'-triphosphate tris salt (UTP), Alendronic acid (ADA), 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC), cetyltrimethylammonium bromide (CTAB), methanol (MeOH), anhydrous gadolinium chloride ($GdCl_3$, 99.99%), oleic acid (OA), cyclohexene, ammonium fluoride (NH_4F), neodymium chloride ($NdCl_3$, 99.9%), sodium hydroxide, 25%glutaraldehyde ($OHC(CH_2)_3CHO$) ammonia solution, hydrogen peroxide solution, potassium iodide, hydrogen tetrachloroaurate trihydrate, ascorbic acid, silver nitrate, sodiumborohydride, 1-octadecene (ODE), (3-aminopropyl)triethoxysilane (APTES), cetyltrimethylammonium chloride (CTAC), sodium citrate, N-hydroxylsuccinimide sodium salt (NHS), anhydrous ytterbium chloride ($YbCl_3$, 99.99%) and anhydrous thulium chloride ($TmCl_3$, 99.99%) were purchased from Sigma-Aldrich (USA). Human apurinic/apyrimidinic endonuclease1 (APE1), 7-nitroindole-2-carboxylicacid (NCA), mitochondrion staining kit and all oligonucleotides shown in **Table S1** were obtained from Sangon Biological Engineering Technology.

Instruments

The pictures of native PAGE were obtained by bioimaging system (VILBER QuantumCX5, France). Fluorescence spectra were collected using an F-7000 fluorescence spectrometer (Hitachi, Japan). The transmission electron microscopic (TEM) was performed on a JEM-2100 (Hitachi, Japan). Dynamic light scattering (DLS) and zeta potentials were obtained by Nano-ZS Zetasizer ZEN3600 (Malvern, U.K.). Confocal images were acquired using a TCS SP8 STED 3x (Leica).

Synthesis of UCNPs ($NaGdF_4$: Yb,Tm@$NaGdF_4$: Yb,Nd)

UCNPs ($NaGdF_4$: Yb,Tm@$NaGdF_4$: Yb,Nd were synthesized according to our previous work.[S1] Firstly, $GdCl_3$ (0.60 mmol), $TmCl_3$ (0.02 mmol) and $YbCl_3$ (1.38 mmol) were mixed in a three-

necked flask containing 12 mL oleic acid and 30 mL octadecene and heated up to 150 °C under vacuum condition for 30 min. After cooling naturally, 20 mL of methanol solution containing NaOH (200 mg) and NH_4F (296 mg) was added slowly, heated up to 50°C and stirred for 0.5 h. Subsequently, the temperature was raised to 110°C under N_2 for 15 min to remove methanol. After cooling naturally, rapidly heated up to 300 °C for 90 min and cooled naturally. Then, 40 mL of acetone was added and centrifuged at 13000 rpm for 10 min and washed three times using ethanol, and re-dispersed in 20 mL of cyclohexane. Thus, the core of the UCNPs ($NaGdF_4$:Yb,Tm) were obtained. Next, $GdCl_3$ (0.96 mmol), $NdCl_3$ (0.48 mmol) and $YbCl_3$ (0.16 mmol) were mixed in a three-necked flask containing 12 mL oleic acid and 30 mL octadecene and heated up to 150 °C for 30 min under vacuum condition. After cooling naturally, followed by the addition of 20 mL of the above synthesized core of the UCNPs ($NaGdF_4$:Yb,Tm) and then heated to 110 °C to evaporate cyclohexane. 20 mL of methanol solution containing NaOH (200 mg) and NH_4F (296 mg) was added slowly after cooling down to room temperature, heated to 50°C and stirred for 0.5 h, temperature was raised to 110°C under N_2 for 15 min. After cooling naturally, rapidly heated up to 300 °C for 1.5 h and cooled naturally. The resulting $NaGdF_4$: Yb,Tm@$NaGdF_4$: Yb,Nd UCNPs were precipitated by the addition of 40 mL acetone, centrifuged at 13000 rpm for 10 min and washed three times using ethanol, and re-dispersed in 20 mL of cyclohexane, Thus, oleate-capped core-shell UCNPs ($NaGdF_4$:Yb,Tm@$NaGdF_4$:Yb,Nd) were obtained. Finally, 20 mL of the as-prepared oleate-capped core-shell UCNPs ($NaGdF_4$:Yb,Tm@$NaGdF_4$:Yb,Nd) was added to the mixtures of 12 mL of ethanol and 8 mL of water containing 100 mg alendronic acid (ADA), followed by the addition of HCI (1.0 M) to adjust the pH to 4.0 and stirred overnight. After centrifugation, the precipitates were dispersed in DMF. Afterwards, 120μL NHS-IRDye® 800CW (100μM) and DMAP (0.1 mg/mL) were added to the above obtained core-shell UCNPs DMF solutions (2 mg/mL) and reacted at room

temperature for 6 h. At last, OA-free core-shell UCNPs were obtained after centrifuging at 13000 rpm for 10 min and washed three times with water.

Synthesis of Au NBPs

The Au NBPs were synthesized according to our previous work.[S1] First, 750 μL of $HAuCl_4 \cdot 3H_2O$ (0.01 M) and 1500 μL of sodium citrate (0.01M) were added to 57.75 mL of deionized water and stirred at 30°C for 10 min, Then, 900 μL of freshly prepared ice $NaBH_4$ (0.01 M) was added and stirred continuously for 15 min. Finally, the seed solution was obtained after aged in a water bath at 30 °C for 2 h. Then, 7.5 mL of $HAuCl_4 \cdot 3H_2O$ (0.01 M), 1.5 mL of $AgNO_3$ solution(0.01 M), 3.0 mL of HCl (1.0 M) and 1.2 mL of ascorbic acid (0.1 M) solution were added to 150 mL of CTAB (0.1 M) solution to obtain growth solution, 6.0 mL of seed solution was added to the growth solution, and it was left to stand in a 30 ℃ water bath for 12 h to obtain a crude product. 165.0 mL of crude product was centrifuged at 12,000 rpm for 10 min to obtain a precipitate, which was then re-dispersed and dissolved with 120.0 mL of 0.08 M CTAC. Then 11.25 mL of 0.1 M ascorbic acid (0.1 M) and 37.5 mL of $AgNO_3$ (0.01 M solution were added, and it was left to stand in a water bath at 65 °C for 6 h. Following centrifugation at 12,000 rpm for 10 min, the pellet was re-dissolved in 112.5 mL of CTAB (0.1 M) solution and in a 30 °C water bath overnight. Subsequently, the black Au/Ag nanorods at the bottom were re-dispersed in 75.0 mL of deionized water, Immediately, 1.2 mL of 25% ammonia water and 0.6 mL of 30% hydrogen peroxide were added, mixed well, and allowed to stand for 3 h. Finally, it was centrifuged at 10,000 rpm for 15 min, washed twice with deionized water, and the precipitate was dispersed in CTAB (0.1 M) solution for later use.

Cell culture

HeLa and L02 cells were used in all cell experiments. The cells were cultured in Dulbecoo's

modification of Eagle's medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin in a 5% CO_2, 37 °C incubator.

MTT assays

First, cells were treated with LP and EP for 24h, followed by the addition of 20 μL of MTT solution (5 mg/mL) and incubated at 37°C for 4 h. Finally, the supernatants were aspirated and then 150 μL of DMSO was added to each well. The absorbance values of the wells at 490 nm were recorded to evaluate the relative viabilities of cells.

Co localization experiment

The first batch of HeLa cells were seeded into confocal dish for 12 h and then cultured with 1mL DMEM medium containing 20 μL LP for 2 h, 3 h, 4 h and 5h at 37 °C, respectively. Afterward, the cells were washed with PBS solution (10 mM, pH 7.4) for three times and then incubated with 30 μL MitoTracker Green in 1 mL of fresh DMEM medium for 30 min to stain mitochondria. Second batch of HeLa cells were seeded into confocal dish for 12 h and then cultured with 1mL DMEM medium containing 20 μL EP for 2 h, 3 h, 4 h and 5h at 37 °C, respectively. Afterward, the cells were washed with PBS solution (10 mM, pH 7.4) for three times and then incubated with 30 μL MitoTracker Green in 1 mL of fresh DMEM medium for 30 min to stain mitochondria. All fluorescence images were analyzed by Image J.

Calculation about relative ratio of Cy5.5 covalently linked to Au NBPs@SiO_2

The relative ratio of Cy5.5 covalently linked to Au NBP@SiO_2 can be calculated from the absorption spectra according to the formular:

$$N = c \cdot V \cdot N_A \quad (1)$$

$$A = \varepsilon \cdot b \cdot c \quad (2)$$

$$N = (A \cdot V \cdot N_A) / (\varepsilon \cdot b) \quad (3)$$

The number of Cy5.5 covalently linked to Au NBP@SiO_2 NPs ($N_{Cy5.5}$):

$$N_{Cy5.5} = [(A \cdot V)_{Cy5.5\text{-original}} - (A \cdot V)_{Cy5.5\text{-supernatant}}] \cdot N_A / \varepsilon_{Cy5.5} \cdot b \quad (4)$$

The number of Au NBP@SiO_2 NPs in the solution for linking Cy5.5 (N_{NP}):

$$N_{NP} = (A \cdot V)_{NP} \cdot N_A / \varepsilon_{NP} \cdot b \quad (5)$$

Therefore, the relative ratio of Cy 5.5 covalently linked to Au NBP@SiO_2 =the number of conjugated Cy 5.5 /the number of Au NBP@SiO_2 NPs ($N_{Cy5.5}$ /N_{NP})

$$N_{Cy5.5} / N_{NP} = [(A \cdot V)_{Cy5.5\text{-original}} - (A \cdot V)_{Cy5.5\text{-supernatant}}] \cdot \varepsilon_{NP} / (A \cdot V)_{NP} \cdot \varepsilon_{Cy5.5} \quad (6)$$

Table S1 Detailed DNA and RNA sequences information

Name	Sequence (5'-3')
L1	CGACTAGAAGTTCATCTTCATCTCTCGTTTTGACCTGGGGGAGTATTGCGGAGGAAGGT
L2	COOH-GCAATACTCC/iPC-Link/CCCAGGTCAAAACGAG
L3	BHQ-3- CGACTAGAAGTTCATCTTCATCTCTC/iAP-Site/GTTTTGTC
L4	COOH-GACAAAACGAGAGATGAAGATGAACTTCTAGTCG-Cy5.5

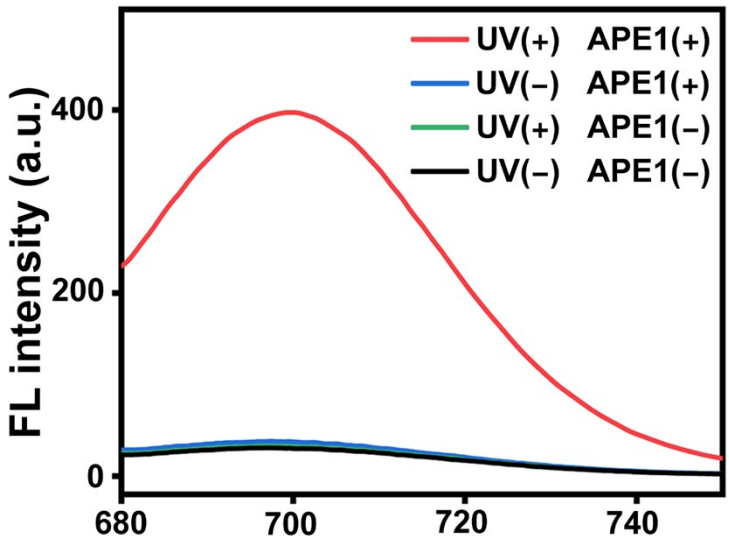

Fig. S1 Fluorescent responses of EP to ATP (5 mM) with or without UV and APE1 activation.

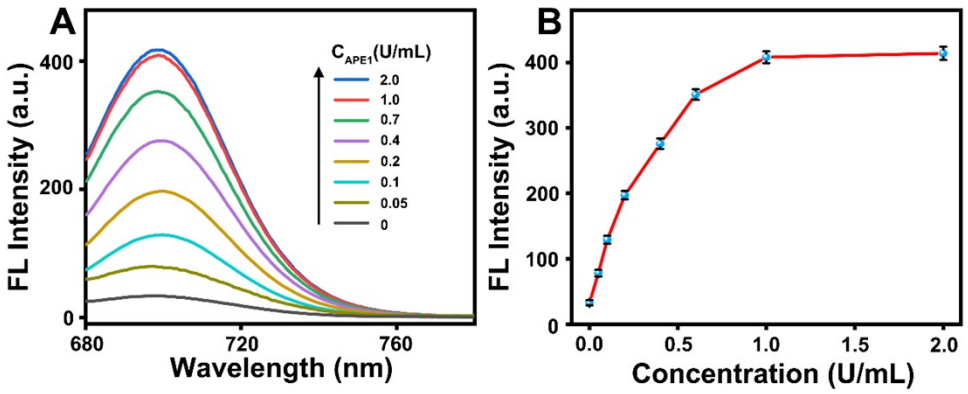

Fig. S2 (A) Fluorescence spectra of free LP and free EP responding to different concentrations of APE1 in the presence of target ATP (5 mM). (B) Fluorescence intensity corresponding to panel A.

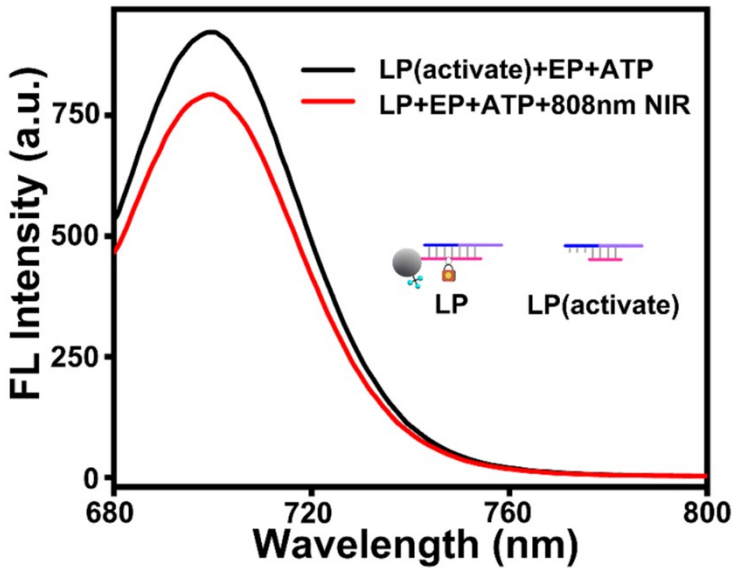

Fig. S3 Fluorescence spectrum of the LP+EP probe in the presence of target ATP with 808nm NIR irradiation (red line). Fluorescence spectrum of the LP(activate)+EP probe in the presence of target ATP (black line).

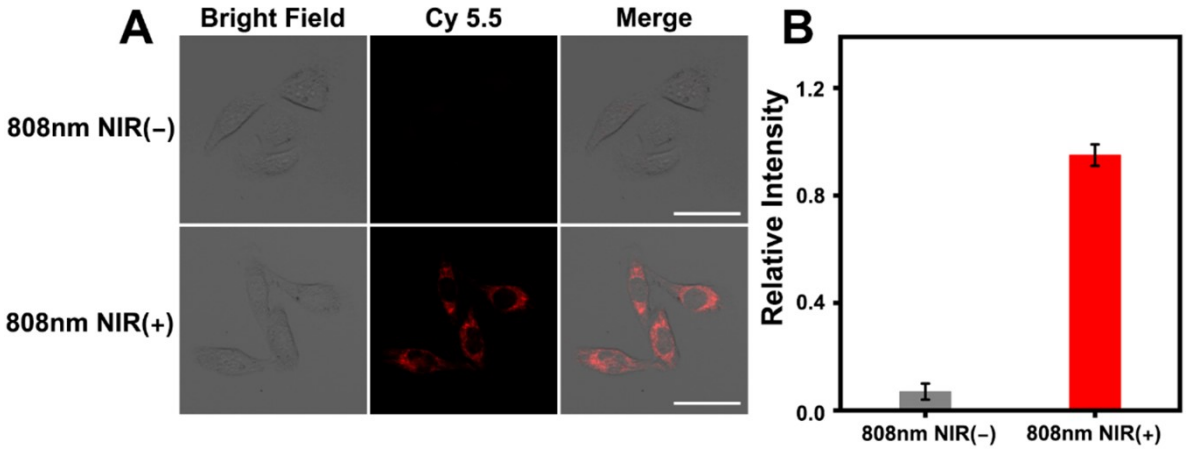

Fig. S4 (A) Confocal fluorescence images of HeLa cells treated with LP and EP with or without subsequent NIR irradiation. (B) Quantification of the fluorescence data corresponding to panel A. (scale bar: 50 μm)

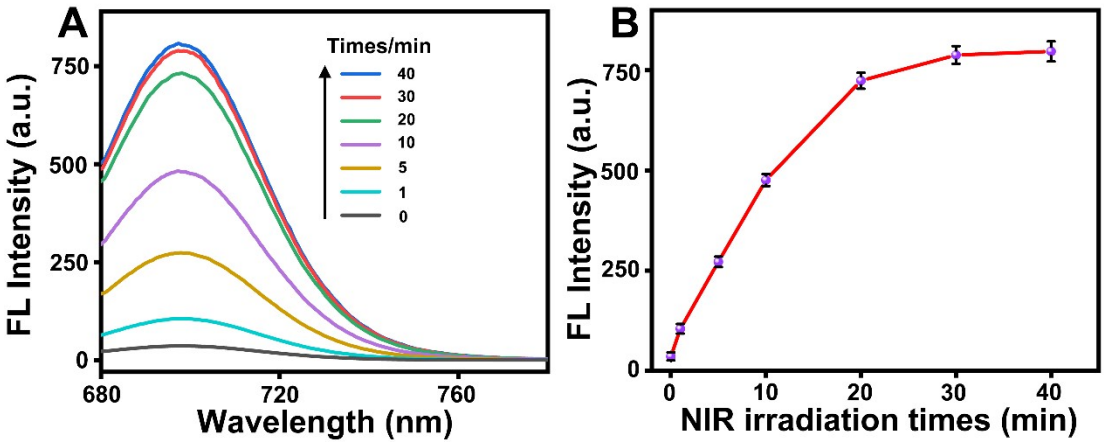

Fig. S5 (A) Fluorescence spectra of LP and EP in response to target ATP (5 mM) with different NIR irradiation times. (B) Fluorescence intensity corresponding to panel A.

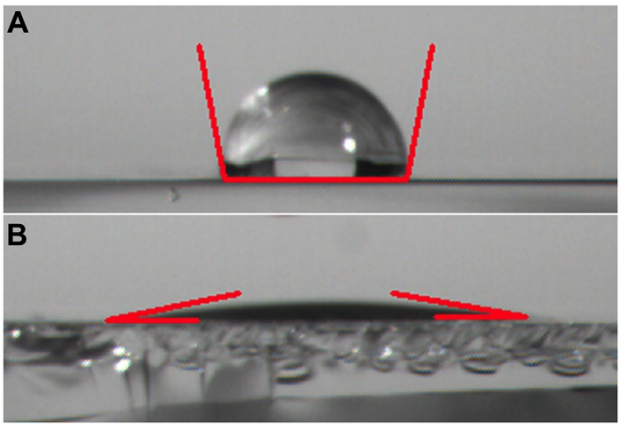

Fig. S6 Contact angles of (A) UCNPs and (B) OA-free UCNPs.

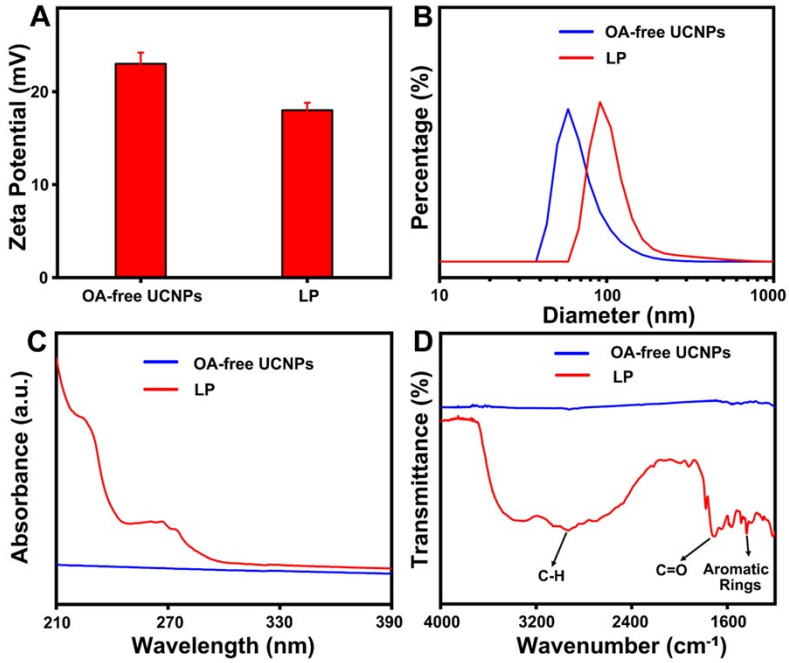

Fig. S7 (A) Zeta Potential, (B) DLS, (C) absorption spectra and (D) FT-IR spectra of OA-free UCNPs and LP.

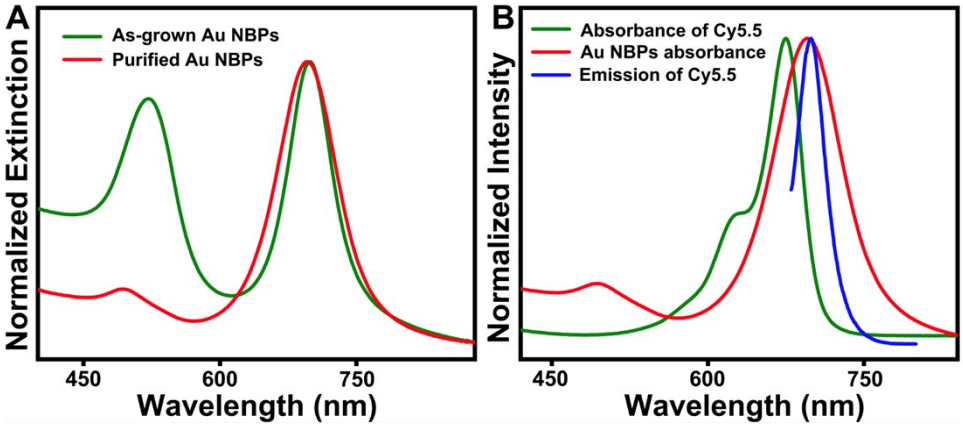

Fig. S8 (A) Absorption spectra of the As-grown (green line) and purified Au NBPs (red line). (B) Emission (blue line) and absorption (green line) spectra of Cy5.5, and the absorption spectra of Au NBPs (red line).

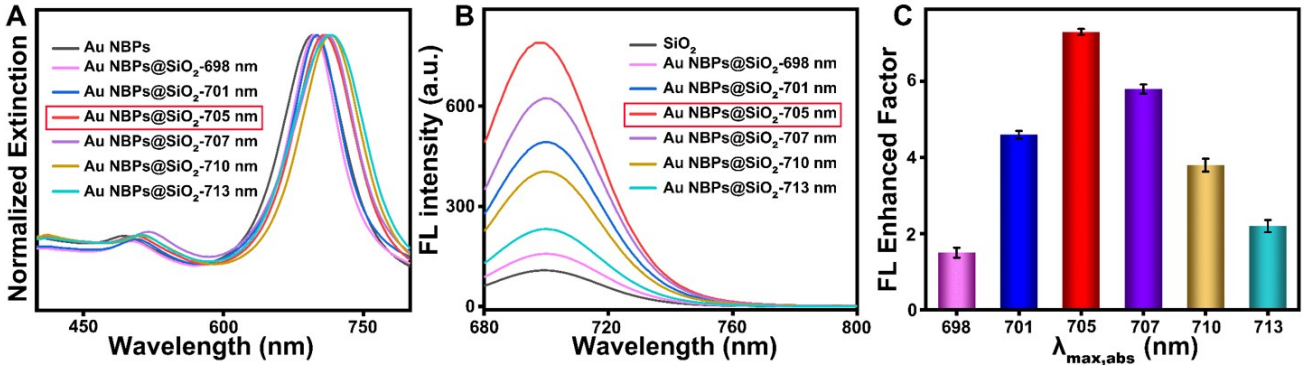

Fig. S9 (A) Absorption spectra of Au NBPs and with different silica shell thickness. (B) Fluorescence spectra of the Au NBPs@SiO_2 with different silica shell thicknesses. (C) Fluorescence enhanced factor of the Au NBPs@SiO_2 with different silica shell thickness.

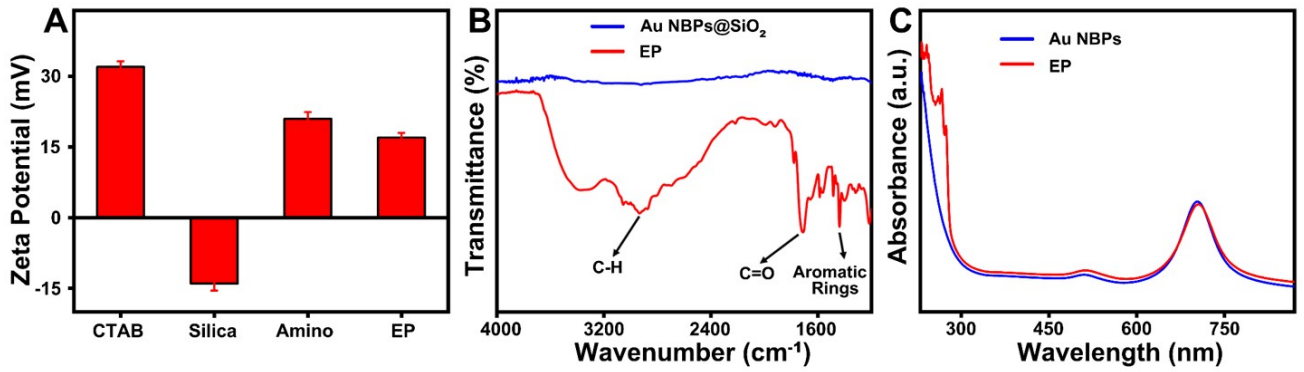

Fig. S10 (A) Zeta Potentials, (B) FT-IR spectra and (C) absorption spectrum of Au NBPs and EP, respectively.

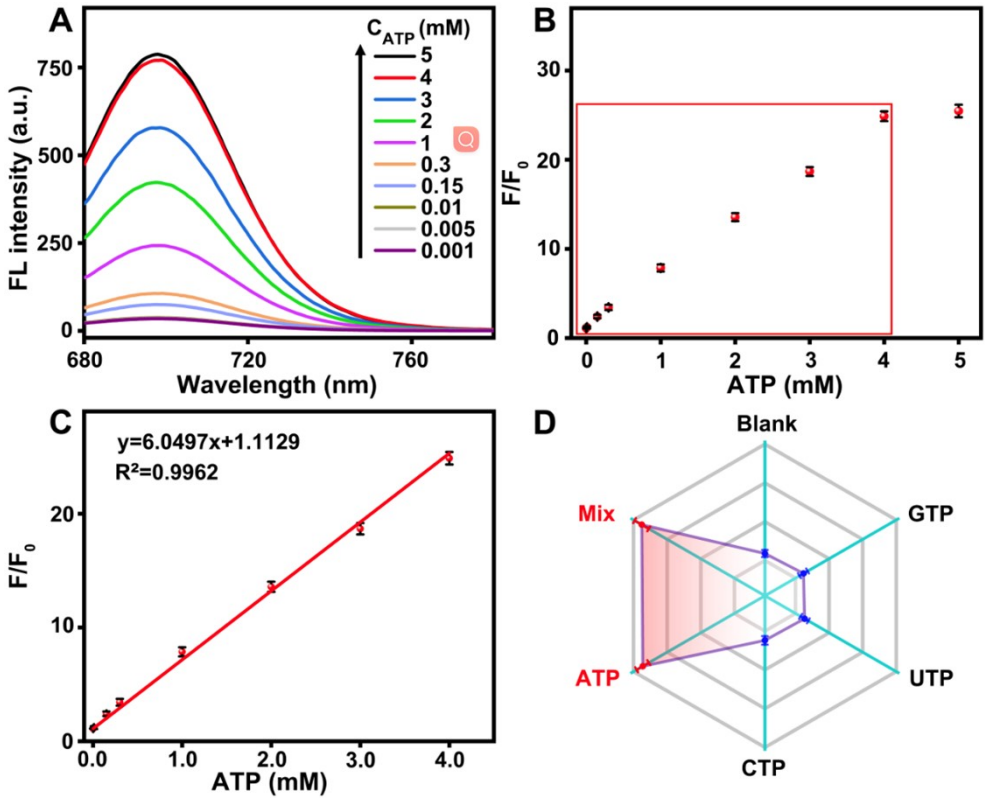

Fig. S11 (A) Fluorescence spectra of LP and EP response to increasing concentration of ATP (0.001−5 mM) in the presence of 808 nm NIR and APE1 (1U/mL). (B) Relationship between the fluorescence intensities and ATP concentrations. (C) Linear correlation between fluorescence intensities and ATP concentrations. (D) Selectivity of the LP and EP toward ATP (5 mM) against GTP (5 mM), UTP (5 mM) and CTP (5 mM), respectively.

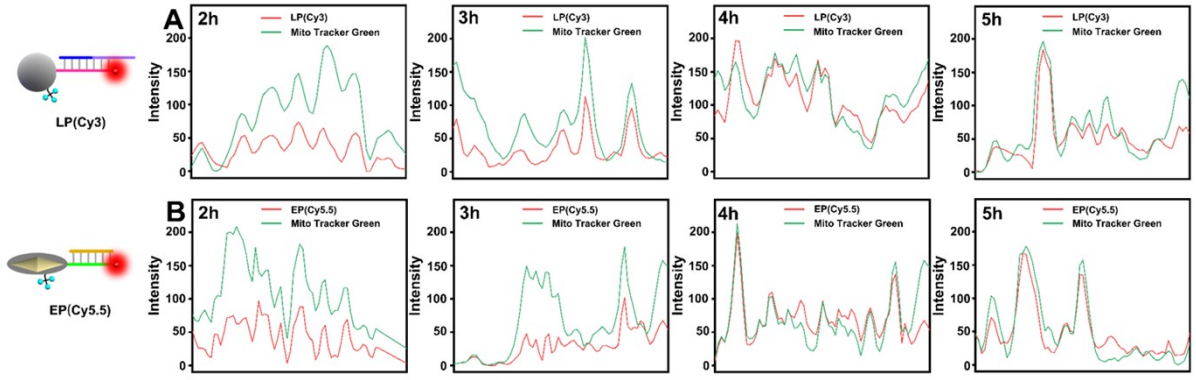

Fig. S12 (A) Intensity profiles of LP(Cy3) and Mito Tracker green along the white line in the HeLa cells in Fig. 4A, respectively. (B) Intensity profiles of EP(Cy5.5) and Mito Tracker green along the white line in the HeLa cells in Fig. 4 B, respectively.

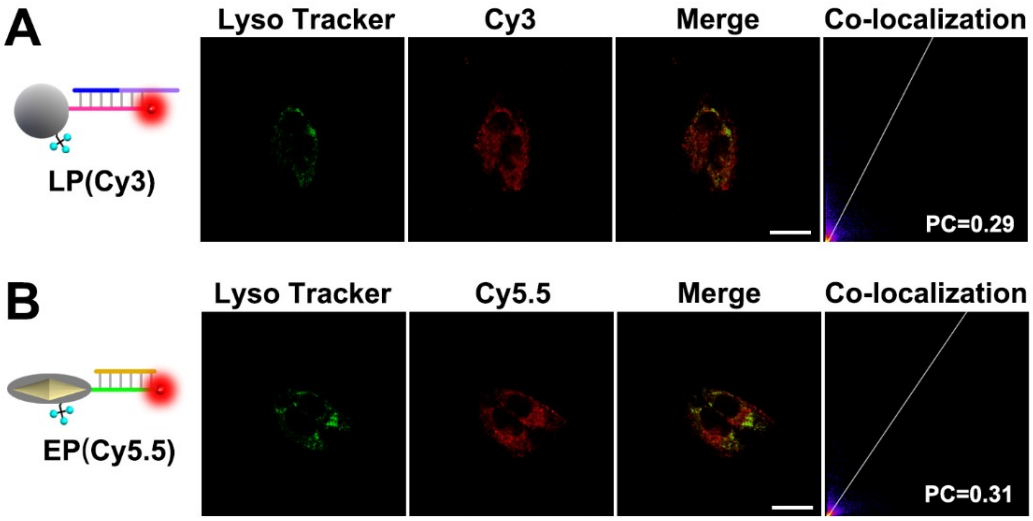

Fig. S13 (A) Confocal imaging of HeLa cells incubated with Lyso Tracker, LP(Cy3). (scale bar: 25 μm) (B)Confocal imaging of HeLa cells incubated with Lyso Tracker, EP(Cy5.5). (scale bar: 25 μm)

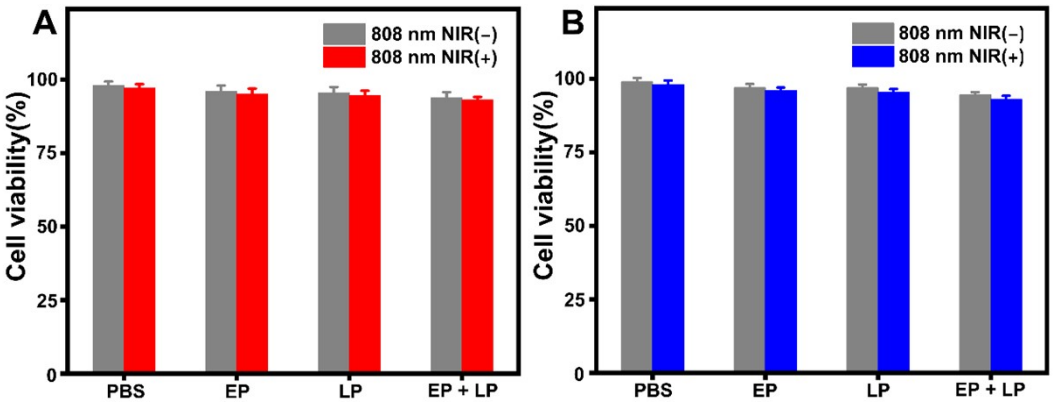

Fig. S14 (A) Cell viability of HeLa cells incubated with PBS (control) and other different treatment with or without 808 nm NIR irradiation. (B) Cell viability of L02 cells incubated with PBS (control) and other different treatment with or without 808 nm NIR irradiation.

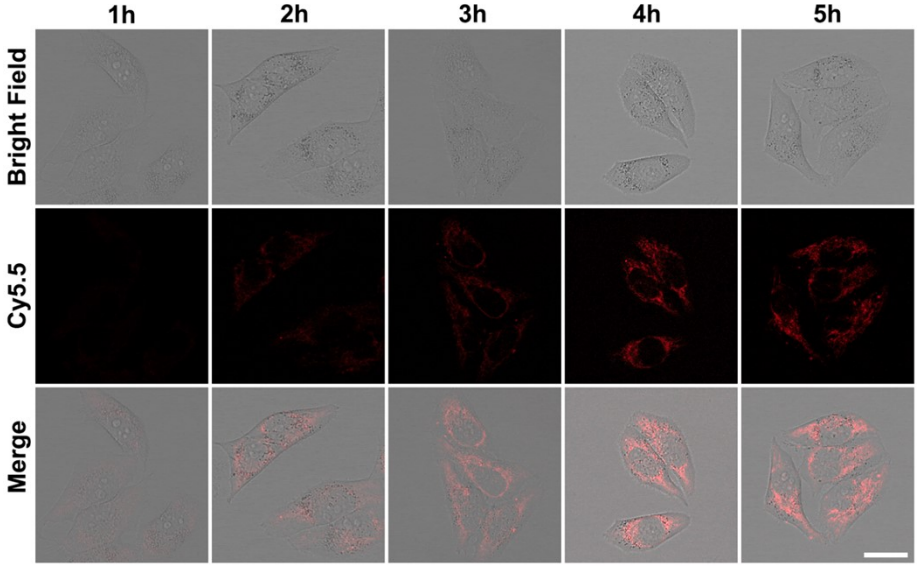

Fig. S15 Confocal fluorescence images of Hela cells incubated with LP and EP for 4 h followed by irradiation with 808 NIR for 30 min, and then incubated for different times. (Scale bar: 50 μm)

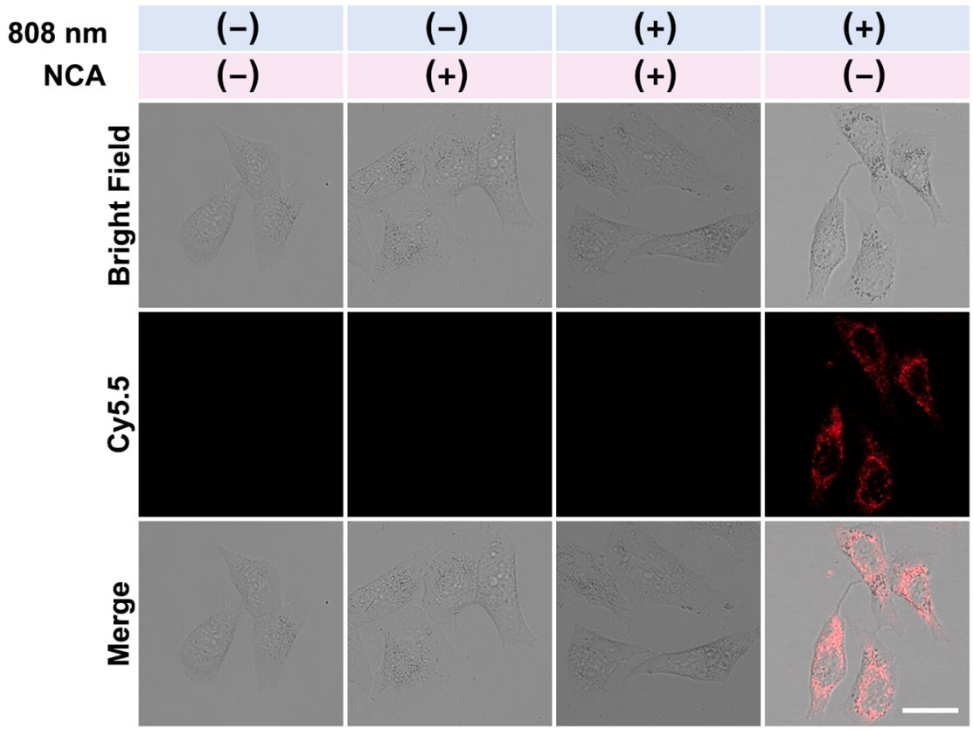

Fig. S16 Confocal images of HeLa cells incubated with LP and EP under different treatments. (Scale bar: 50 μm)

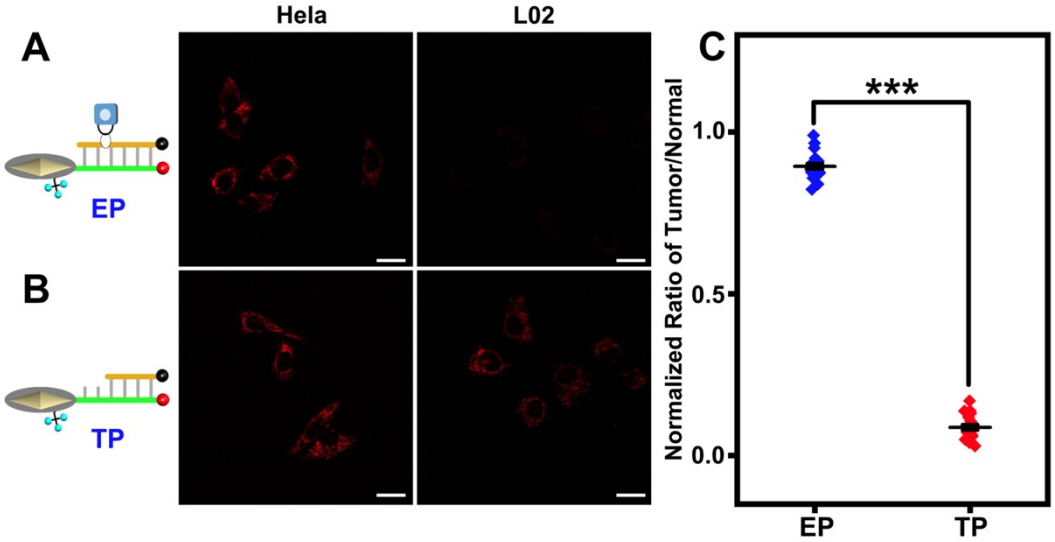

Fig. S17 Confocal fluorescence images of Hela and L02 cells treated with LP and (A) EP, (B) TP, respectively. (C) Quantification of normalized fluorescence ratio (Tumor/Normal) corresponding to panel A and panel B, respectively. ***P<0.001.

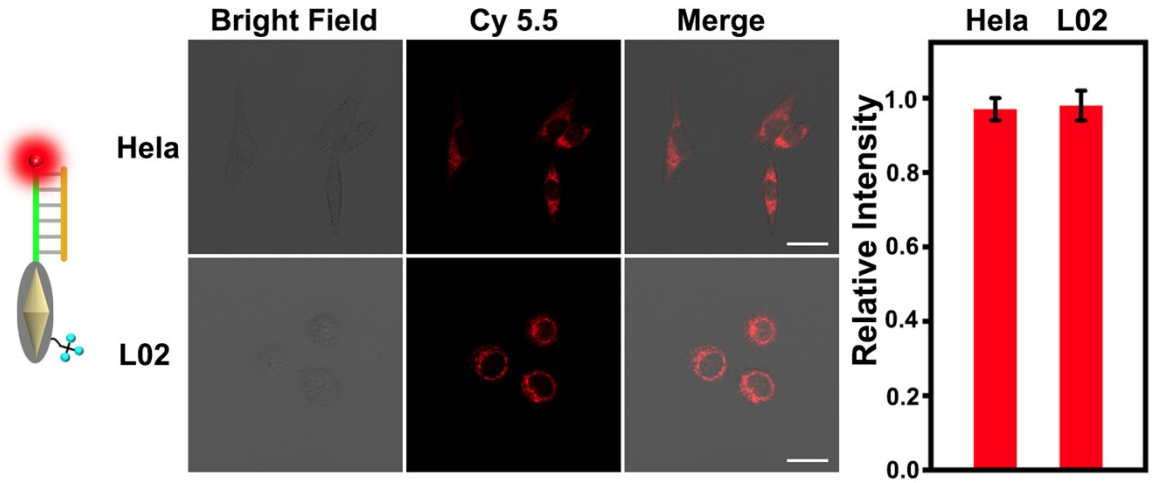

Fig. S18 Confocal fluorescence images of Hela and L02 cells treated with the designed EP without BHQ-3 quencher. (Scale bar:50 μm)

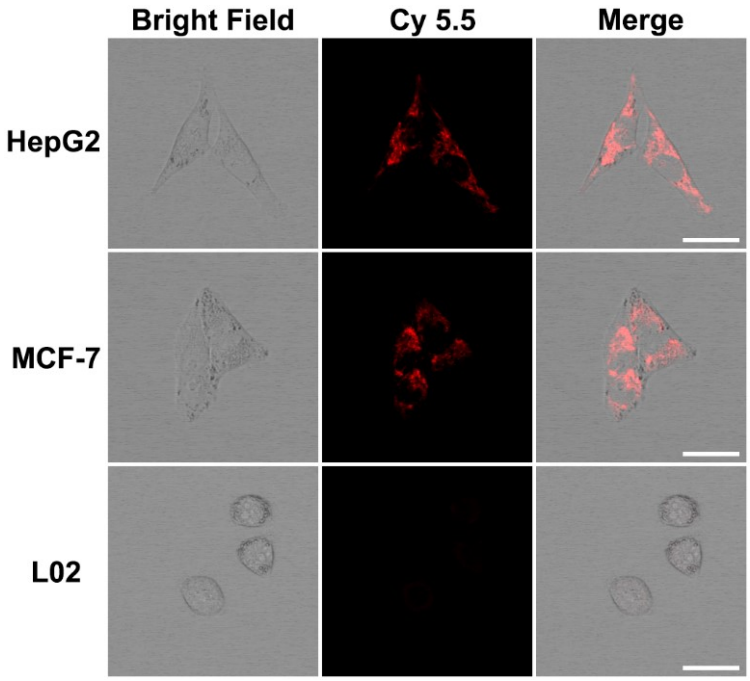

Fig. S19 Confocal fluorescence images of HepG2, MCF-7 and L02 cells treated with EP and LP, respectively. (scale bar: 50 μm)

Reference

[S1] Li D, Zhao T, Chen J, et al. Spatiotemporally controlled ultrasensitive molecular imaging using a DNA computation-mediated DNAzyme platform. *Anal. Chem.*, **2022**, *94*, 14467-14474.