Supporting Information

Engineering of ATP-Powered Photosensitizer for Targeted Recycling Activatable Imaging of MicroRNA and Controllable Cascade Amplification Photodynamic Therapy

Yizhong Shen,[a] Tingting Wu,[a] Qian Tian,[b] Yu Mao,[a] Junjie Hu,[b] Xiliang Luo,[c] Yingwang Ye,[a]* Hong-Yuan Chen,[b] and Jing-Juan Xu[b]*

[a] *Engineering Research Center of Bio-Process, Ministry of Education, School of Food & Biological Engineering, Hefei University of Technology, Hefei, 230009, China*

[b] *State Key Laboratory of Analytical Chemistry for Life Sciences, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210023, China*

[c] *Key Laboratory of Optic-electric Sensing and Analytical Chemistry for Life Science, Ministry of Education, College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, China*

Corresponding Author:

* E-mail: yeyw04@mails.gucas.ac.cn (Y. Ye); E-mail: xujj@nju.edu.cn (J.-J. Xu);

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SUPPLEMENTARY FIGURES

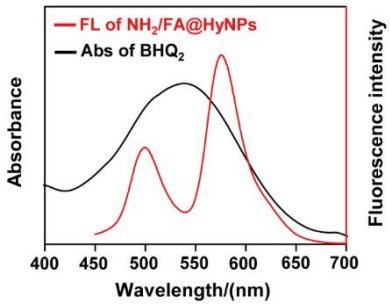

Figure S1. Absorption spectrum of BHQ_2, and fluorescence spectrum of NH_2/FA@HyNPs. The good overlap between the fluorescence spectrum of NH_2/FA@HyNPs and absorption spectrum of BHQ_2 indicates the occurrence of fluorescence resonance energy transfer (FRET).[1,2]

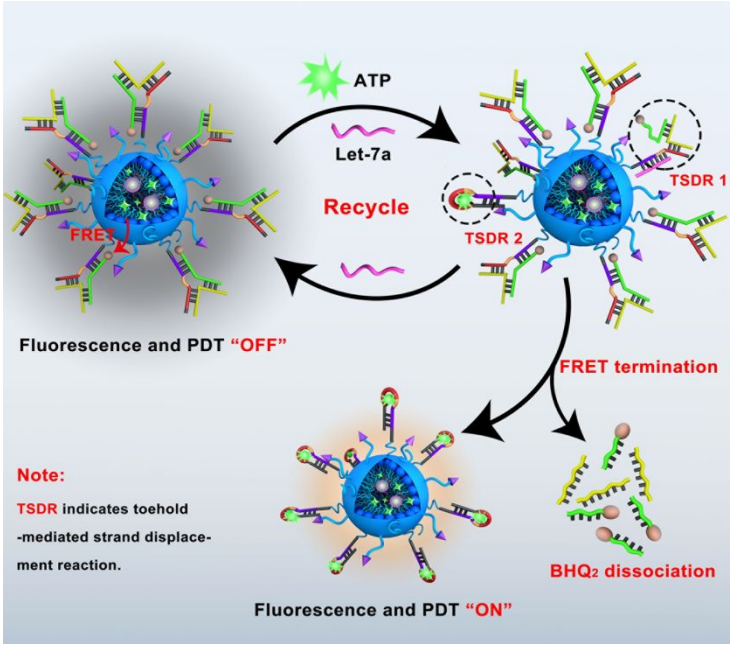

Figure S2. The process of Let-7a miRNA-activatable Y-motif/FA@HyNPs in the presence of ATP in detail, which could be realizing target-triggered recycling cascade amplification imaging of trace Let-7a miRNA, and imaging-guided site-specific PDT.

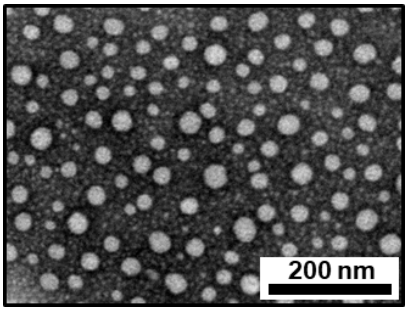

Figure S3. TEM image of NH_2/FA@HyNPs negatively stained with uranyl acetate.

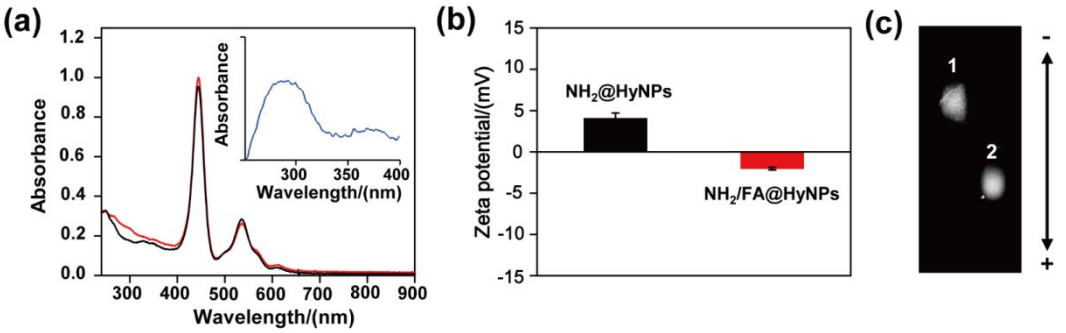

Figure S4. Testify the existence of FA in NH_2/FA@HyNPs nanoparticles. (a) UV-vis absorption spectra of NH_2/FA@HyNPs (red curve) and NH_2@HyNPs (black curve) in PBS buffer (pH = 7.4, 1X). Inset is the difference spectrum of 4.3 μM NH_2/FA@HyNPs with 4.3 μM NH_2@HyNPs in D.I. water shows the characteristic peak at ~ 280 nm, which is in consistence with free FA. (b) Zeta potentials of NH_2/FA@HyNPs and NH_2@HyNPs in PBS buffer (pH = 7.4, 1X). The negative zeta potential of NH_2/FA@HyNPs (-2.0 ± 0.5 mV) compared to that of NH_2@HyNPs (4.1 ± 0.6 mV) was ascribed to the incorporation of FA in NH_2@HyNPs. (c) Agarose gel electrophoresis (1.0%) of (1) NH_2@HyNPs and (2) NH_2/FA@HyNPs.

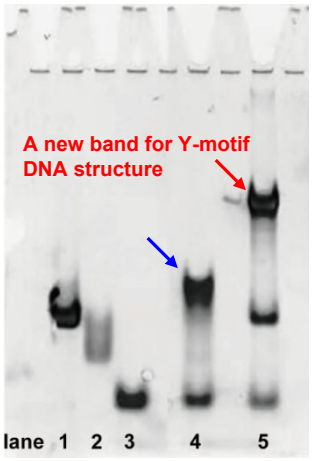

Figure S5. Polyacrylamide hydrogel electrophoresis images of lane (1) Y_a' single strand DNA, (2) Y_b single strand DNA, (3) Y_c' single strand DNA, (4) the mixture of Y_b and Y_c' after incubation at 37 °C for 1.0 h, and (5) the mixture of Y_a', Y_b and Y_c' after incubation at 37 °C for 1.0 h. In the lane 4, a new band (Blue arrow indicator) was observed after incubation of Y_b with Y_c' by comparing with the lane 2 and lane 3, revealing the successful assembly of Y_b with Y_c'. At this moment, the Y_a' was added into the mixture of Y_b and Y_c' at 37 °C for incubation 1.0 h, a new band (Red arrow indicator) in lane 5 was appeared, which was completely different from the bands in other lanes including Y_a', Y_b, Y_c', and the mixture of Y_b and Y_c' after incubation at 37 °C for 1.0 h, implying the formation of the Y-motif DNA structure.

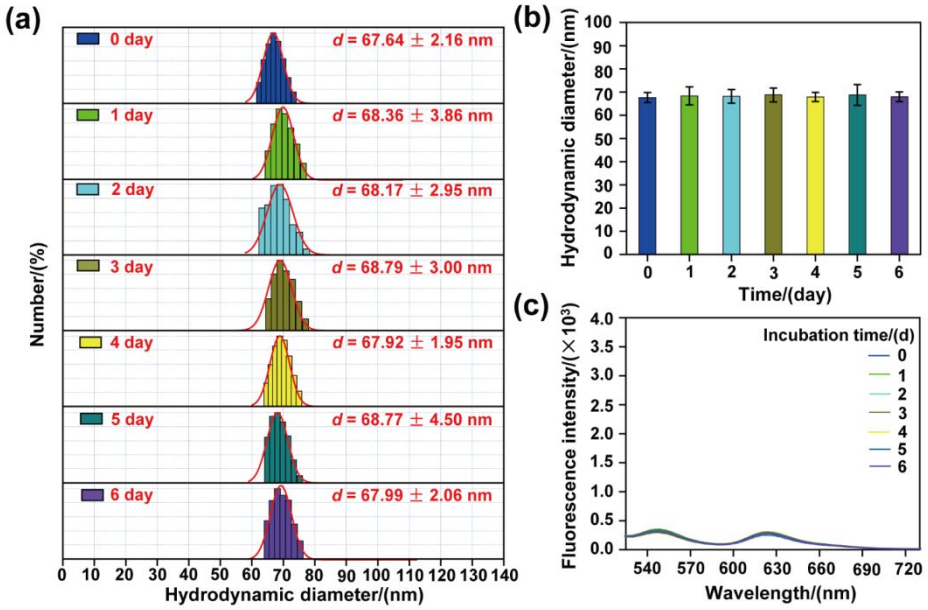

Figure S6. Size stability test of Y-motif/FA@HyNPs in PBS buffer (pH = 7.4, 1X). (a) DLS of Y-motif/FA@HyNPs in PBS buffer (pH = 7.4, 1X) for 6 days. (b) Comparison the hydrodynamic diameter of Y-motif/FA@HyNPs in PBS buffer (pH = 7.4, 1X) for 0, 1, 2, 3, 4, 5, and 6 days. (c) Fluorescence spectra of Y-motif/FA@HyNPs in PBS buffer (pH = 7.4, 1X) for 0, 1, 2, 3, 4, 5, and 6 days. (EX: 443 nm). The negligible change in either hydrodynamic diameter or fluorescence spectra of Y-motif/FA@HyNPs incubation in PBS buffer revealed that the Y-motif/FA@HyNPs possessed a good stability in aqueous solution.

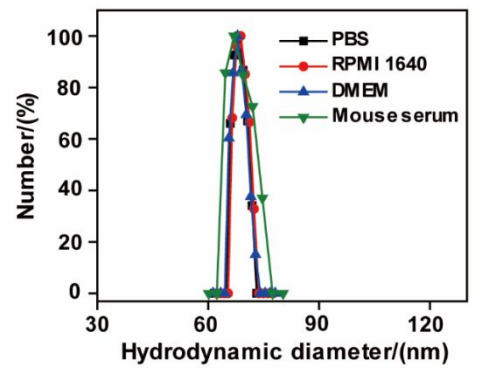

Figure S7. DLS analysis of Y-motif/FA@HyNPs upon incubation in PBS buffer (pH = 7.4, 1X), two different cell culture media and mouse serum at 37 °C for 24.0 h. The hydrodynamic diameter of Y-motif/FA@HyNPs was around 67.82 ± 2.40 nm, 69.05 ± 3.29 nm, 67.83 ± 2.64 nm, and 67.61 ± 2.19 nm for incubation with PBS, RPMI 1640, DMEM, and mouse serum, respectively. The negligible change in hydrodynamic diameter of Y-motif/FA@HyNPs in different culture medium indicated that Y-motif/FA@HyNPs owned a nice stability and the possibility for use in living cells.

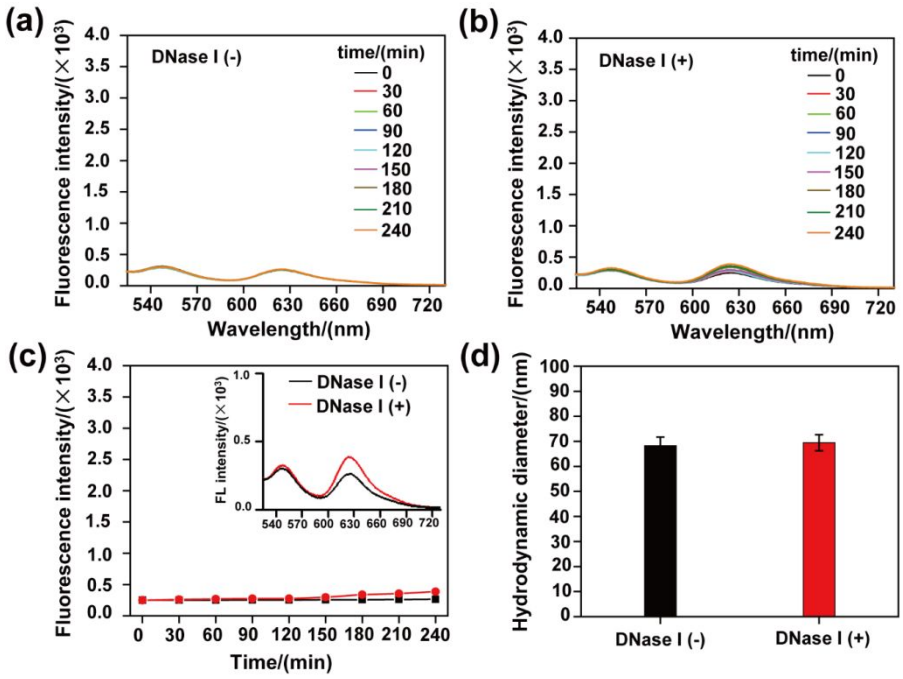

Figure S8. Determination of the nuclease stability of Y-motif/FA@HyNPs. (a) Time-dependent fluorescence spectra of Y-motif/FA@HyNPs was incubated without or (b) with DNase I at 37 °C. (c) Plots between the fluorescence intensity of Y-motif/FA@HyNPs at ~ 627 nm and incubation time without or with DNase I at 37 °C. Inset: the corresponding fluorescence spectra of the mixture after incubation with an excitation at 443 nm. (d) Size stability test of Y-motif/FA@HyNPs incubated without or with DNase I for 240 min at 37 °C. The negligible change in hydrodynamic diameter of Y-motif/FA@HyNPs incubation without or with DNase I suggested that Y-motif/FA@HyNPs owned a well stability under the complicated physiological conditions.

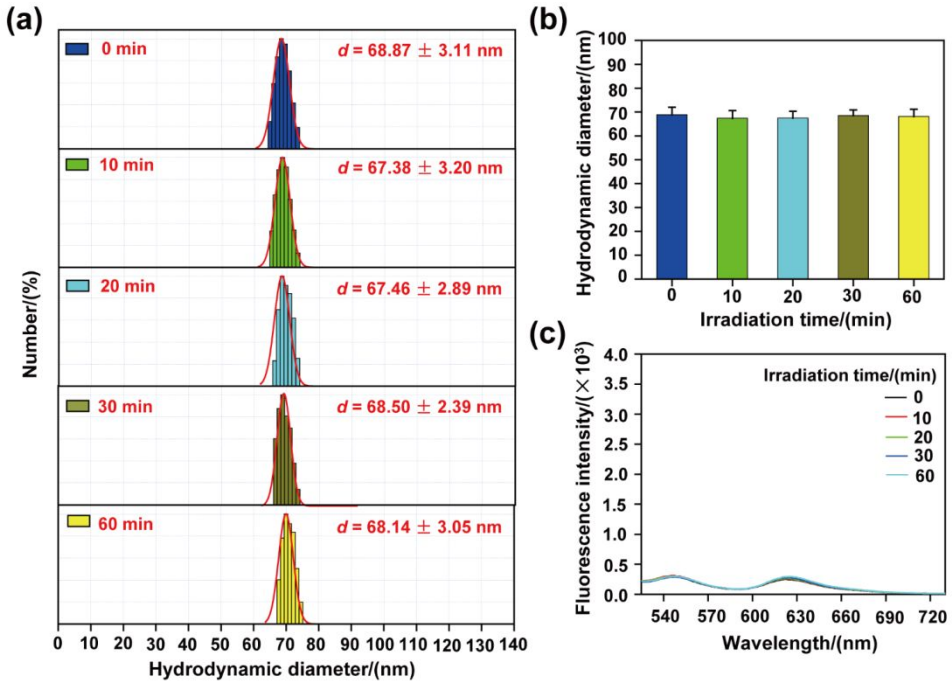

Figure S9. Testify the effect of light dose on the stability of the Y-motif/FA@HyNPs in PBS buffer (pH = 7.4, 1X). (a) DLS analysis of Y-motif/FA@HyNPs in PBS buffer (pH = 7.4, 1X) before and after irradiation with white light (400 nm long pass filter) at the power of 20.0 mW/cm^2 for 0, 10, 20, 30, and 60 min. (b) Comparison the hydrodynamic diameter of Y-motif/FA@HyNPs in PBS buffer (pH = 7.4, 1X) before and after irradiation with white light (400 nm long pass filter) at the power of 20.0 mW/cm^2 for 0, 10, 20, 30, and 60 min. (c) Fluorescence spectra of Y-motif/FA@HyNPs in PBS buffer (pH = 7.4, 1X) before and after irradiation with white light (400 nm long pass filter) at the power of 20 mW/cm^2 for 0, 10, 20, 30, and 60 min. (EX: 443 nm). The negligible change in either hydrodynamic diameter or fluorescence spectra under the different light dose uncovered that Y-motif/FA@HyNPs had a good stability under light irradiation.

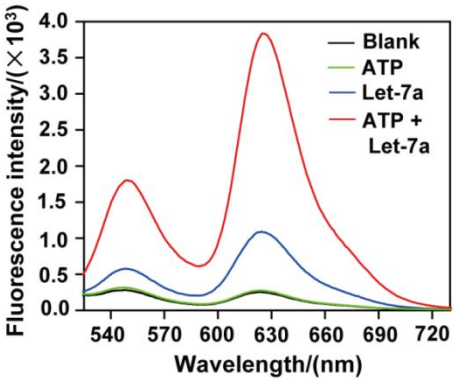

Figure S10. The fluorescence spectra of 2.0 μM Y-motif/FA@HyNPs before and after incubation with 10.0 mM ATP, 1.4 nM Let-7a miRNA, and 10.0 mM ATP + 1.4 nM Let-7a miRNA in PBS buffer (pH = 7.4, 1X) at 37.0 °C for 15.0 min.

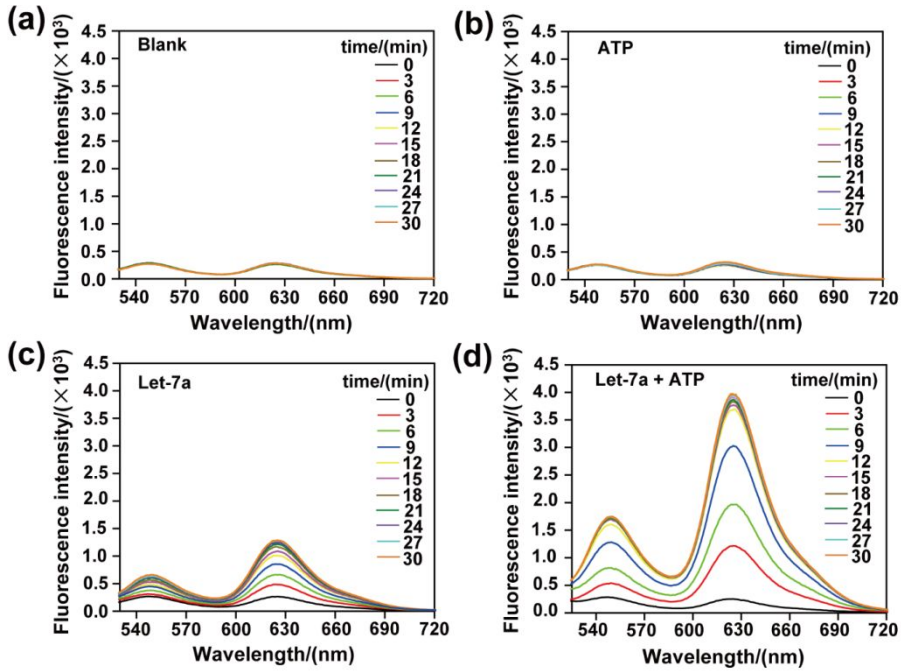

Figure S11. Time-dependent fluorescence spectra of 2.0 μM Y-motif/FA@HyNPs (a) before or after incubation with (b) 10.0 mM ATP, (c) 1.4 nM Let-7a, (d) 1.4 nM Let-7a + 10.0 mM ATP in PBS buffer (pH = 7.4, 1X) at 37 °C for 15.0 min. The excitation wavelength was selected at 443 nm.

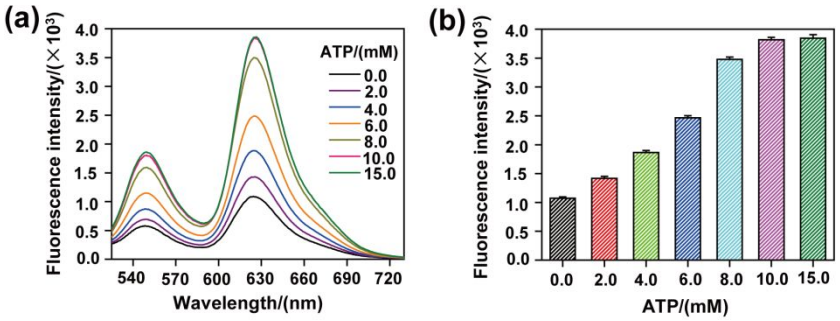

Figure S12. Effect of ATP concentrations on the fluorescence activation efficiency of Let-7a-triggered Y-motif/FA@HyNPs in PBS buffer (pH = 7.4, 1X) at 37 °C for 15.0 min. (a) Fluorescence spectra of 1.4 nM Let-7a-triggerred 2.0 μM Y-motif/FA@HyNPs response to various concentrations of ATP ranging from 0 to 15.0 mM. (b) Fluorescence intensity at 627 nm of 2.0 μM Y-motif/FA@HyNPs incubation with 1.4 nM Let-7a and various concentrations of ATP ranging from 0 to 15.0 mM. The excitation wavelength was selected at 443 nm.

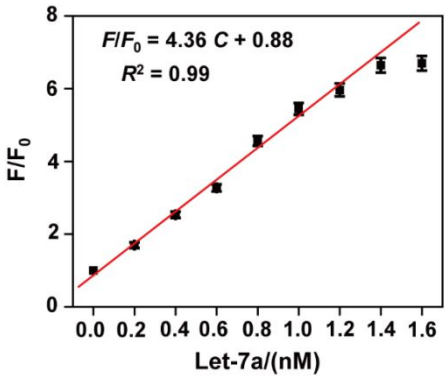

Figure S13. The relationship corresponding to the fluorescence intensity of 2.0 μM Y-motif/FA@HyNPs incubation with 10.0 mM ATP at 555 nm vs. the concentrations of Let-7a within the range of 0-1.6 nM in PBS buffer (pH = 7.4, 1X) at 37 °C for 15.0 min.

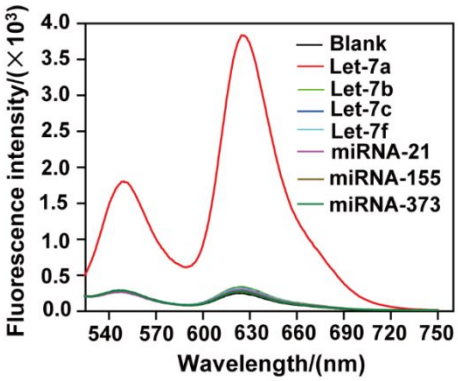

Figure S14. Fluorescence spectra of 2.0 μM Y-motif/FA@HyNPs with the assistance of 10.0 mM ATP toward Let-7a in PBS buffer (pH = 7.4, 1X) at 37.0 °C for 15.0 min. The concentrations of all miRNA sequences were 1.4 nM, and the fluorescence responses of Y-motif/FA@HyNPs to all miRNA sequences were performed in the same conditions.

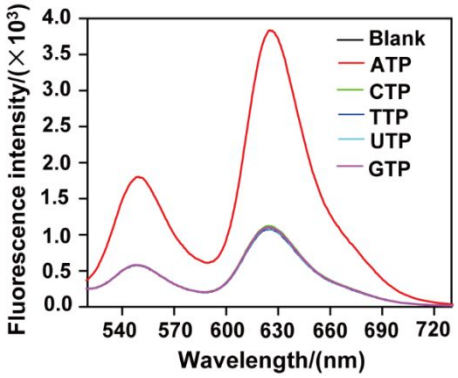

Figure S15. Fluorescence spectra of different thymidine triphosphate analogues on 2.0 μM Y-motif/FA@HyNPs for response to 1.4 nM Let-7a in PBS buffer (pH = 7.4, 1X) at 37.0 °C for 15.0 min. The concentrations of ATP and its analogues were 10.0 mM.

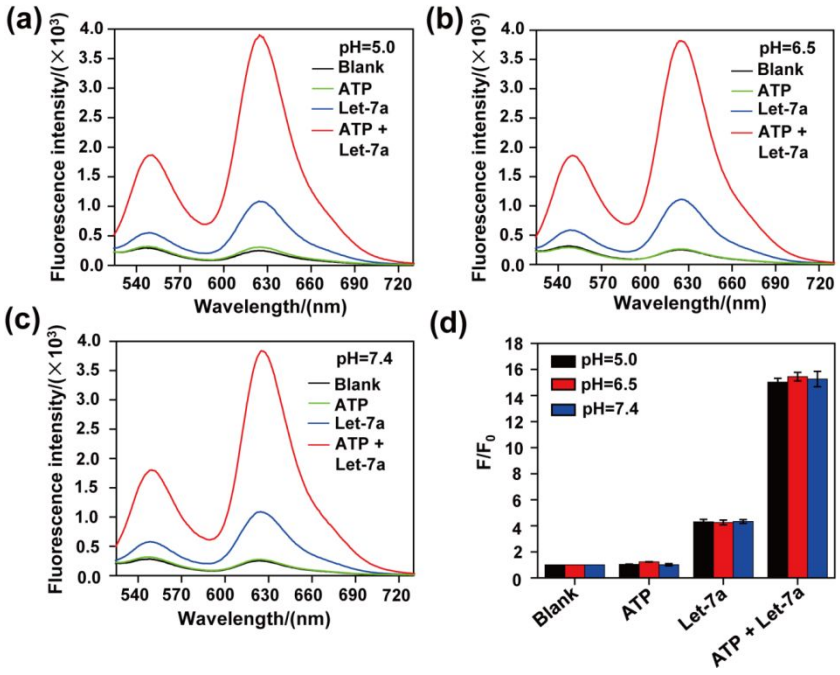

Figure S16. Investigation of the effect of pH on the Y-motif/FA@HyNPs-assisted with ATP for response to Let-7a miRNA at low level. (a-c) The fluorescence spectra of 2.0 μM Y-motif/FA@HyNPs before and after incubation with 10. 0 mM ATP, 14.0 nM Let-7a, and 10. 0 mM ATP + 14.0 nM Let-7a in PBS buffer (1 X) at pH = 5.0, pH = 6.0, and pH = 7.4, respectively. The fluorescence emission was collected upon the excitation of 443 nm. (d) Quantification of the fluorescence intensity of Y-motif/FA@HyNPs at 627 nm at different pH of Figure S16 (a-c).

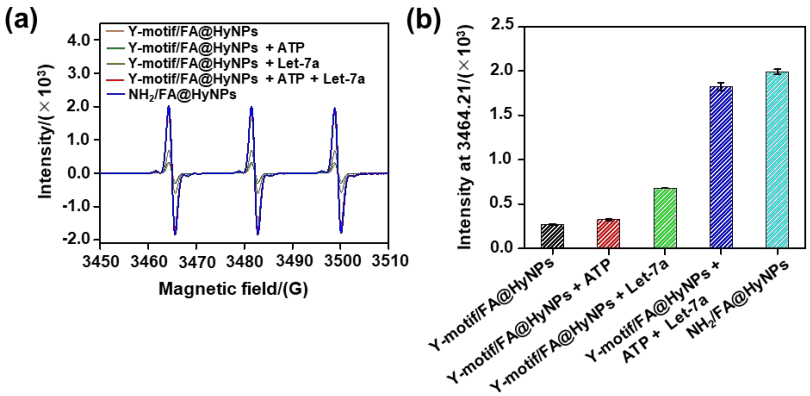

Figure S17. (a) ESR spectra of 20.0 mM TEMP incubation with 2.0 μM NH_2/FA@HyNPs, 2.0 μM Y-motif/FA@HyNPs, 2.0 μM Y-motif/FA@HyNPs + 10.0 mM ATP, 2.0 μM Y-motif/FA@HyNPs + 1.4 nM Let-7a, and 2.0 μM Y-motif/FA@HyNPs + 10.0 mM ATP + 1.4 nM Let-7a upon light irradiation (LED, 20.0 mW/cm^2, 400 nm long pass filter) for 180 s, and (b) ESR intensity at 3464.21 G of the indicated treatments as shown in Figure S17a.

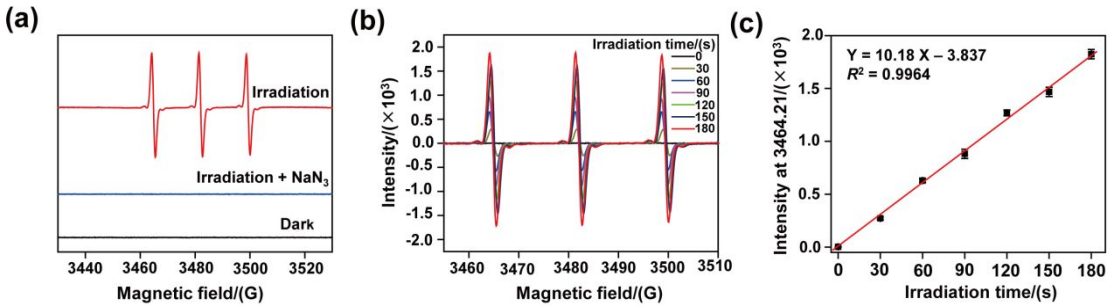

Figure S18. Investigation of the $^1O_2$ cascade amplification capacity of Let-7a miRNA-activatable Y-motif/FA@HyNPs using ATP as a power in PBS buffer (pH = 7.4, 1X). (a) ESR spectra of 20.0 mM TMEP incubation with 2.0 μM Y-motif/FA@HyNPs + 10.0 mM ATP + 1.4 nM Let-7a + Dark, 2.0 μM Y-motif/FA@HyNPs + 10.0 mM ATP + 1.4 nM Let-7a + Light, and 2.0 μM Y-motif/FA@HyNPs + 10.0 mM ATP + 1.4 nM Let-7a + 10.0 mM NaN_3 + Light in PBS buffer (pH = 7.4, 1X). (b) Time-dependent ESR signals of 20.0 mM TMEP incubation with 2.0 μM Y-motif/FA@HyNPs + 10.0 mM ATP + 1.4 nM Let-7a in PBS buffer (pH = 7.4, 1X) upon irradiation with a LED light (400 nm long pass filter) at the power of 20.0 mW/cm^2 for 0, 30, 60, 90, 120, 150, and 180 s, respectively. (c) The relationship of ESR intensities at 3464.21 G *vs* irradiation time of Figure S18b.

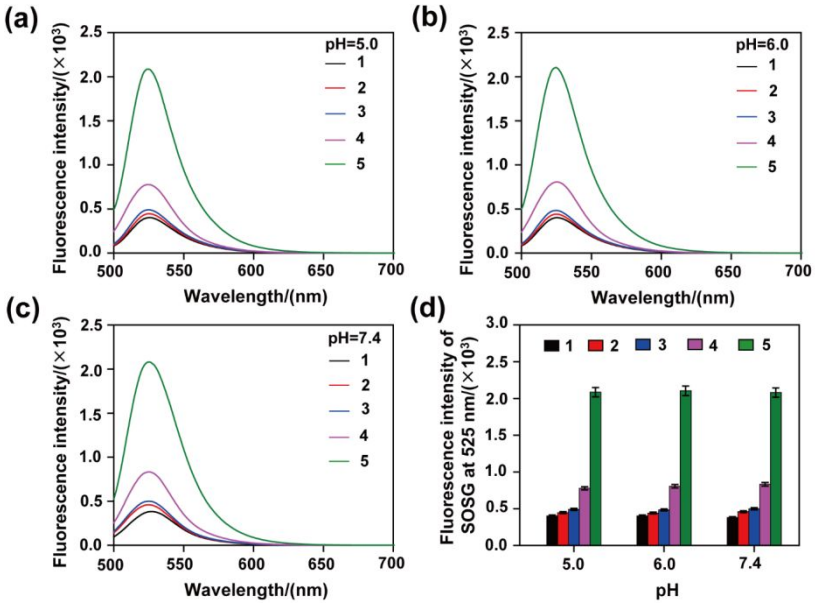

Figure S19. Testify the effect of pH on the $^1O_2$ cascade amplification capacity of Let-7a miRNA-activatable Y-motif/FA@HyNPs using ATP as a power in PBS buffer (pH = 7.4, 1X) at 37.0 °C. (a-c) The fluorescence spectra of 10.0 μM SOSG incubation with (1) 2.0 μM Y-motif/FA@HyNPs + 10.0 mM ATP + 1.4 nM Let-7a + Dark, (2) 2.0 μM Y-motif/FA@HyNPs + irradiation, (3) 2.0 μM Y-motif/FA@HyNPs + 10.0 mM ATP + irradiation, (4) 2.0 μM Y-motif/FA@HyNPs + 1.4 nM Let-7a + irradiation, and (5) 2.0 μM Y-motif/FA@HyNPs + 10.0 mM ATP + 1.4 nM Let-7a + irradiation in PBS buffer at pH = 5.0, pH = 6.0, and pH = 7.4 upon a LED light irradiation (20.0 mW/cm^2, 400 nm long pass filter) for 3.0 min, respectively. (d) Quantification of the fluorescence intensity of SOSG at 525 nm at different pH of Figure S19(a-c).

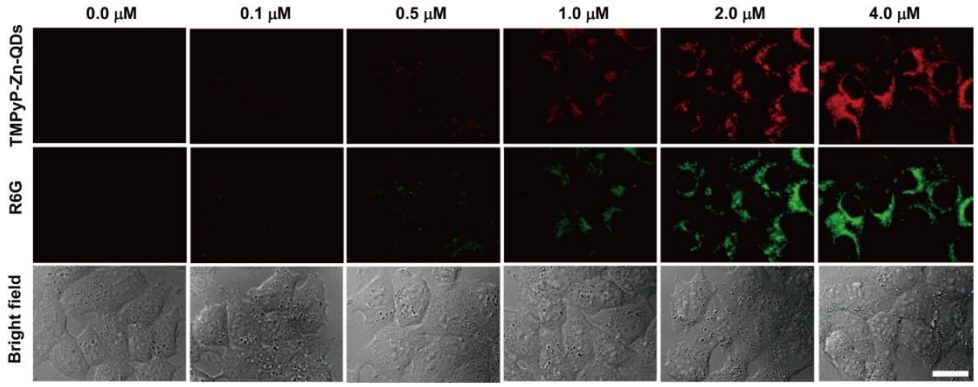

Figure S20. Fluorescence imaging of MCF-7 cells incubated with 0.0, 0.1, 0.5, 1.0, 2.0, and 4.0 μM Y-motif/FA@HyNPs at 37.0 °C for 2.0 h, respectively. Scale bar: 20 μm.

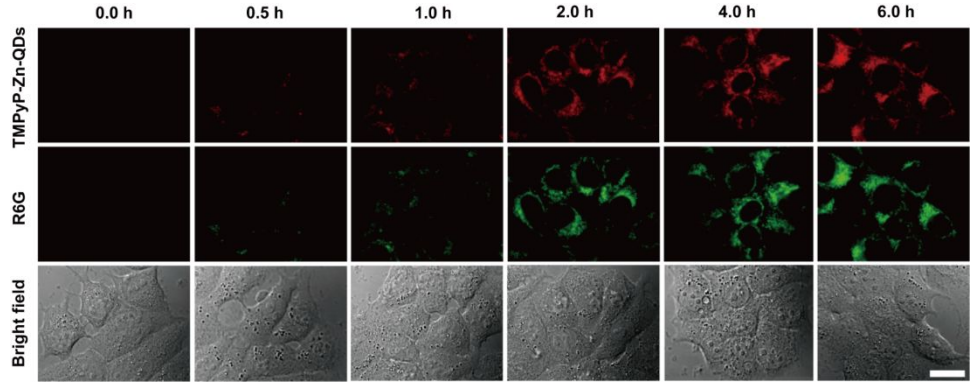

Figure S21. Fluorescence imaging of MCF-7 cells incubated with 2.0 μM Y-motif/FA@HyNPs at 37.0 °C for 0.0, 0.5, 1.0, 2.0, 4.0, and 6.0 h, respectively. Scale bar: 20 μm.

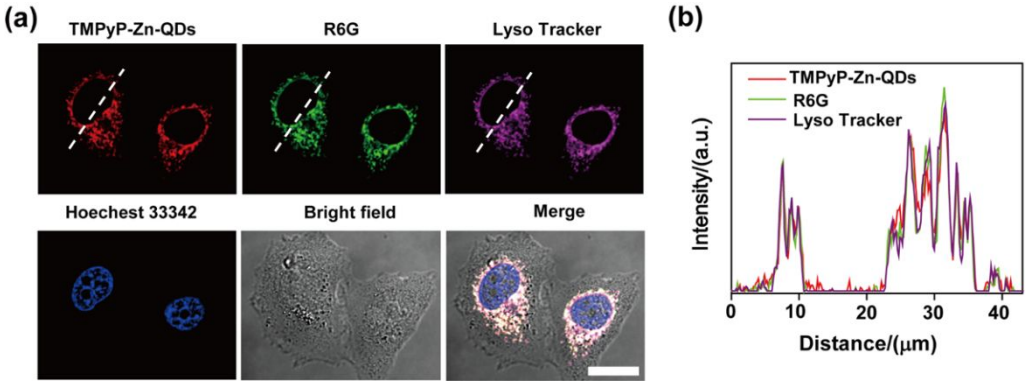

Figure S22. Colocalization assay for studying the distribution of Y-motif/FA@HyNPs in living MCF-7 cells. (a) Co-staining imaging of Y-motif/FA@HyNPs-loaded MCF-7 cells with a Lysotracker red and Hoechst 33342. Scale bar: 20 μm. (b) Quantification of fluorescent intensity of the line scanning profiles in the indicated images of Figure 22a.

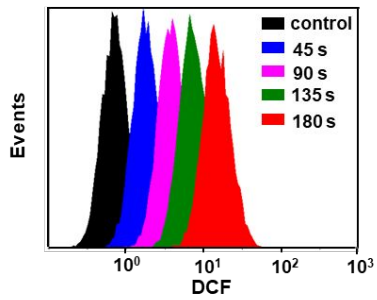

Figure S23. Effect of irradiation time on the intracellular $^1O_2$ levels of Y-motif/FA@HyNPs-treated MCF-7 cells by flow cytometric assay. MCF-7 cells were first cultured with 2.0 μM Y-motif/FA@HyNPs at 37.0 °C for 2.0 h, and further cultured with 20.0 μM DCFH-DA for another 20.0 min. Afterward, the MCF-7 cells were exposed to light irradiation (LED, 400 nm long pass filter, 20.0 mW/cm^2) for 0 (control), 45, 90, 135, and 180 s, respectively.

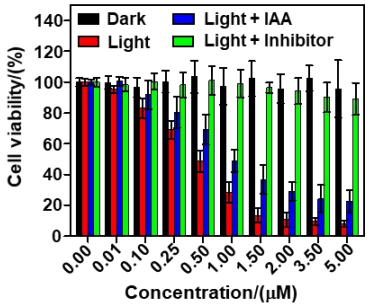

Figure S24. Cytotoxicity of Y-motif/FA@HyNPs to MCF-7 cells in the absence and presence of 200.0 nM Let-7a inhibitor or 100.0 mM IAA with and without light irradiation (LED, 400 nm long pass filter, 20.0 mW/cm^2) for 180 s.

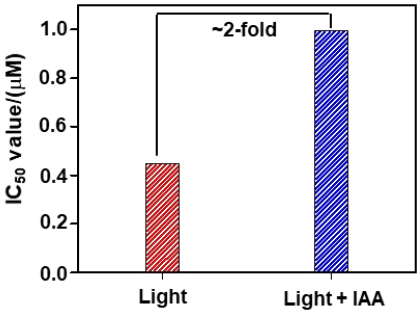

Figure S25. Quantification analysis of the IC_{50} value of Y-motif/FA@HyNPs in the absence and presence of IAA to MCF-7 cells upon light irradiation (LED, 400 nm long pass filter, 20.0 mW/cm^2) for 180 s.

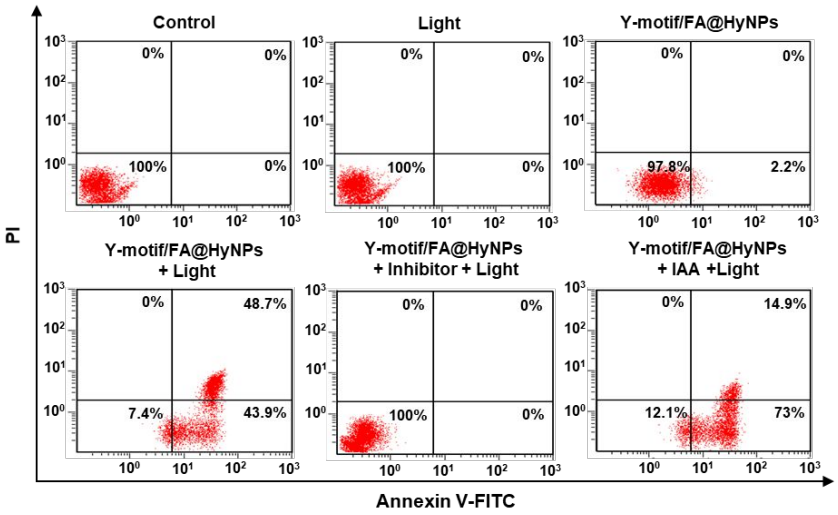

Figure S26. Cellular apoptosis assay of MCF-7 cells after the indicated treatments by staining with Annexin V-FITC/PI. MCF-7 cells were first treated without or with 2.0 μM Y-motif/FA@HyNPs in the absence and presence of 200.0 nM Let-7a inhibitor or 100.0 mM IAA, and then exposed to light irradiation (LED, 400 nm long pass filter, 20.0 mW/cm^2) for 180 s.

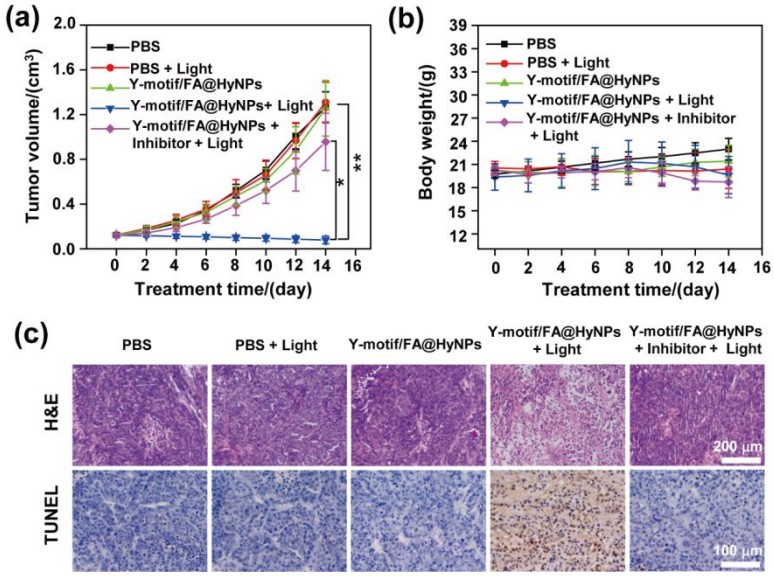

Figure S27. *In vivo* PDT efficacy of Y-motif/FA@HyNPs in MCF-7 tumor-bearing nude mice. (a) Tumor volume and (b) body weight of MCF-7 tumor-bearing nude mice during the 14 days evaluation period with the five indicated treatments. All data were mean ± SD (n = 5, *P < 0.05, **P < 0.01). (c) H&E (Top) and TUNEL (bottom) staining images of MCF-7 tumor tissue slices resected from nude mice after the five indicated treatments.

Movie S1. Real-time images of the morphology of blank MCF-7 cells upon light irradiation. The result shows that over 170 s continuous light irradiation cannot cause morphological change of blank MCF-7 cells, indicating that the effect of light dosage on death viability is negligible.

Movie S2. Real-time images of the morphology of Y-motif/FA@HyNPs-treated MCF-7 cells upon light irradiation. The result shows that only ~ 50 s continuous irradiation can cause obvious collapse of Y-motif/FA@HyNPs-treated MCF-7 cells, and with significant blebs generation indicative of remarkable cell death, demonstrating that Y-motif/FA@HyNPs exhibits an excellent PDT activity.

Movie S3. Real-time images of the morphology of Let-7a miRNA inhibitor and Y-motif/FA@HyNPs-treated MCF-7 cells upon light irradiation. The result shows that over 170 s continuous irradiation can cause negligible morphological change of Let-7a miRNA inhibitor and Y-motif/FA@HyNPs-treated MCF-7 cells, implying that PDT activity of Y-motif/FA@HyNPs can be specifically activated by endogenous Let-7a miRNA.

Movie S4. Real-time images of the morphology of IAA and Y-motif/FA@HyNPs-treated MCF-7 cells upon light irradiation. The result shows that over ~ 120 s continuous irradiation can cause collapse of IAA and Y-motif/FA@HyNPs-treated MCF-7 cells. Meanwhile, the collapse extent of IAA and Y-motif/FA@HyNPs-treated MCF-7 cells is obviously weaker than that of Y-motif/FA@HyNPs-treated MCF-7 cells after over 170 s continuous light irradiation. These results reveal that intracellular ATP as a power can assist the PDT activity of endogenous Let-7a miRNA-triggered Y-motif/FA@HyNPs to achieve cascade amplification in living tumor cells.

SUPPLEMENTARY EXPERIMENTS

Materials and Regents. The main chemical and biological reagents used in this article were obtained from commercial suppliers and used without further purification, unless otherwise indicated. In detail, tellurium (Te) powder, sodium borohydride ($NaBH_4$), *N*-acetyl-L-cysteine (NAC), $Zn(CH_3COO)_2$, and $CdCl_2 \cdot 2.5H_2O$ were purchased from Aladdin Reagent Co. Ltd. (Shanghai, China). Lipids such as 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-*N*-[amino(polyethylene glycol)-2000] (DSPE-PEG_{2000}-NH_2), 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-*N*-[folate(poly ethylene glycol)-2000] (DSPE-PEG_{2000}-FA), and 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-2000] (DSPE-PEG_{2000}-OMe) were purchased from Avanti Polar Lipids Inc. (Alabaster, AL, USA). 5,10,15,20-tetrakis(1-methyl 4-pyridinio) porphyrin tetra(p-toluenesulfonate) (TMPyP), Tris-(hydroxymethyl)aminomethane (Tris), 2',7'-dichlorfluorescein-diacetate (DCFH-DA), Rhodamine 6G (R6G), vitamin C (VC), and 2,2,6,6-tetramethylpiperidine (TEMP) were obtained from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). Singlet oxygen sensor green (SOSG), lipofectamine3000 transfection reagent, and LysoTracker@Red DND-99 were purchased from Invitrogen (Carlsbad, CA, USA). DNase I endonuclease (DNase I), Hoechst 33342, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) kit, Annexin V-FITC/propidium iodide (PI) cell apoptosis kit, Iodoacetic acid (IAA), PBS buffer (pH 7.4, 1X), human breast cancer MCF-7 cells, and normal immortalized human mammary epithelial MCF-10A cells were purchased from KeyGen Biotech. Co. Ltd. (Nanjing, China). All aqueous solutions were prepared using D.I. water (≥18 MΩ, Milli-Q, Millipore). Oligonucleotide sequences used in this article were synthesized from

Shanghai Sangon Biological Engineering Technology & Services Co. Ltd. (Shanghai. China), and presented in Table S1.

Table S1 Oligonucleotide sequences used in this article.

Name	Sequence from 5' to 3'
Y_a	CGT ACT AGT AGG CAA GCT ACT TAC CTG GGG GAG TAT TGC GGA GGA AGG TAA CTA TAC AAC CTA CTA GTA CG-(COOH)
Y_b	CAC GGC GAA TGA GTC GAA CCT TCC TCC GCA ATA CTC CCC C
Y_c	(BHQ_2)-TCT ACT AGT AGG TTG TAC ATT CGC CGT G
Y_a'	CGT ACT AGT AGG CAA GCT ACT TAC CTG GGG GAG TAT TGC GGA GGA AGG TAA CTA TAC AAC CTA CTA GTA CG
Y_c'	TCT ACT AGT AGG TTG TAC ATT CGC CGT G
Let-7a	UGA GGU AGU AGG UUG UAU AGU U
Let-7a inhibitor	mAmAmCmUmAmUmAmCmAmAmCmCmUmAmCmUmAmCmCmUmCmA
Let-7b	UGA GGU AGU AGG UUG UGU GGU U
Let-7c	UGA GGU AGU AGG UUG UAU GGU U
Let-7f	UGA GGU AGU AGA UUG UAU AGU U
miRNA-21	UAG CUU AUC AGA CUG AUG UUG A
miRNA-155	UUA AUG CUA AUC GUG AUA GGG GU
MiRNA-373	GAA GUG UUC GAU UUU GGG GUG U

Note: (1) The “m” in the sequence of Let-7a inhibitor indicated the 2'-O-methyl.

(2) The blue letters in Let-7b, Let-7c, and Let-7f presented the mutant bases in the Let-7a.

Instruments. Fluorescence spectra were measured on the F-2700 spectrophotometer (Hitachi Company, Tokyo, Japan). Absorption spectra were record on the UV-3600 UV-VIS-NIR spectrophotometer (Shimadzu Company, Japan). Transmission electron microscopic (TEM) images were observed on a JEM-2100 transmission electron microscope (JEOL, Ltd., Japan). Dynamic light scattering (DLS)

and Zeta potentials measurements were measured on a 90 Plus/BI-MAS equipment (Brook haven, USA). EPR spectra were recorded on the Bruker EMX-10/12 X-band variable-temperature apparatus (USA). Gel electrophoresis was conducted on the DYCP-31BN Electrophoresis Analyzer (Liuyi Instrument Company, China) and imaged on the Biorad ChemDoc XRS (Bio-Rad, USA). Flow cytometric analysis were performed on a Coulter FC-500 flow cytometer (Beckman-Coulter, USA). Cytotoxicity assay was carried out on the Hitachi/Roche System Cobas 6000 (680, Bio-Rad, USA). The images of cells were observed on the IX73 optical microscope (Olympus, Japan). In vivo image acquisition was conducted on the IVIS Lumina XR III *in vivo* imaging system (PerkinElmer, USA).

Preparation of the Core-Shell NAC-capped CdTe/ZnS QDs. Aqueous core-shell NAC-capped CdTe/ZnS QDs were prepared by referring to the previously reported literatures.[3,4] Firstly, 0.75 mmol Te powder and excessive amount of $NaBH_4$ were dissolved in D.I. water, and followed by stirring under an ice/water bath to generate the sodium hydrogen telluride (NaHTe) aqueous solution. Meanwhile, a mixed aqueous solution containing 1.5 mmol of $CdCl_2$, 2.25 mmol NAC, and 85 mL D.I. water was heated to 95 °C under N_2 flow and magnetic stirring. After that, the NaHTe aqueous solution was rapidly trasferred into the hot and oxygen-free Cd^{2+} solution under the powerful stirring and N_2 flow, and the resulting solution was then kept powerful stirring at 95 °C for another 1.0 h to obtained the orange CdTe core solution. Afterward, 1.33 mmol NAC and 0.667 mmol $Zn(CH_3COO)_2$ were rapidly added to the orange CdTe core solution, and followed by powerful stirring under N_2 flow at 95 °C for another 3 h. Then the salmon-pink and core-shell NAC-CdTe/ZnS QDs were synthesized, which were purified by means of precipitation using absolute ethanol. After centrifugation, the precipitate was resuspended in D.I. water to obtain the core-shell NAC-capped

CdTe/ZnS QDs stock solution, whose concentration was calculated using $\varepsilon = 1.11\times10^5$ $L\cdot mol^{-1}\cdot cm^{-1}$, as estimated by an empirical formula based on the first absorbance feature (λ = 536 nm).[5]

Fabrication of the Nanocomplexes TMPyP-Zn-QDs. Firstly, 34.0 μL of 29.4 μM core-shell NAC-capped CdTe/ZnS QDs and 3.4 μL of 20 mM TMPyP were added to 462.6 μL D.I. water. Afterward, the mixed solution was sonicated at room temperature for 5 min, and following by sharply stirred at room temperature for another 30 min to promote the extensive complexion. At this moment, the mixed solution was added to a 10 KD Millipore, centrifuged (4000 rmp), and washed with D.I. water for three times. Finally, the nano-photosensitizer of TMPyP-Zn-QDs were prepared, whose concentration was determined by TMPyP.

Synthesis of the Nanophotosensitizer of NH_2/FA@HyNPs. The nanophotosensitizer of NH_2/FA@HyNPs were prepared according to our previously reported method with minor modifications.[6] In details, 0.9 mg of 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-*N*-[amino(polyethylene glycol)-2000] (DSPE-PEG_{2000}-NH_2), 0.1 mg of 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-*N*-[folate(poly ethylene glycol)-2000] (DSPE-PEG_{2000}-FA), and 1.1 mg of 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-2000] (DSPE-PEG_{2000}-OMe) were firstly dissolved in 1.5 mL mixture solution containing 1.35 mL CH_2Cl_2 and 0.15 mL CH_3OH. Subsequently, the above solution was rapidly transferred into a brown sample bottle containing 1.5 mL aqueous solution of 20 μg R6G and 45.3 μM TMPyP-Zn-QDs, and the resulting mixture was sonicated at room temperature for 5 min. At this time, a white emulsion was obtained, which was placed in a magnetic stirrer for vigorous stirring under dark overnight. After that, the white emulsion was purified to remove the organic solvents by vacuum, and the purified aqueous solution

was transferred into a 10 KD Millipore, centrifuged (4000 rpm), and washed with D.I. water three times to afford FA and -NH_2 bifunctional NH_2/FA@HyNPs nanoparticles. The concentration of NH_2/FA@HyNPs nanoparticles was determined by the organic molecule of TMPyP.

Construction of Y-motif/FA@HyNPs Theranostic Nanoprobe. A mixed solution of 15.0 nmol Y_a DNA strand, 200.0 nmol 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDC), and 200.0 nmol N-hydroxysuccinimide (NHS) were added to 2.0 mL Tris-HCl buffer (pH 7.4, 10.0 mM $MgCl_2$), and incubated at 37.0 ºC for 60.0 min. Then, 8.0 nmol FA/NH_2@HyNPs nanoparticles was added to above mixture solution, and the resulting solution was placed in a shaking table for gently shaking 4.0 h at 37.0 ºC. Afterward, the mixture solution was then transferred into a 50.0 KD Millipore, centrifuged (8000 rpm), and washed with Tris-HCl buffer (pH 7.4, 10.0 mM $MgCl_2$) for three times to obtain Y_a DNA strand attached Ya/FA@HyNPs nanoprobe. The concentration of Y_a/FA@HyNPs nanoprobe was also determined by the organic molecule of TMPyP.

To prepare Y-motif/FA@HyNPs nanoprobe, a mixed solution of 8.0 nmol Ya/FA@HyNPs nanoprobe, 15.0 nmol Y_b DNA strand, and 15.0 nmol Y_c DNA strand were added to 2.0 mL Tris-HCl buffer (pH 7.4, 10.0 mM $MgCl_2$), and incubated at 95.0 ºC for 5.0 min. After that, the mixture solution was sequentially cooled down to 65.0 ºC, 60.0 ºC, 55.0 ºC, 50.0 ºC, and 45.0 ºC (each step for 5.0 min), and then the mixed solution was placed in a shaking table for gently shaking overnight at 37.0 ºC. Finally, the mixed solution was transferred into a 50.0 KD Millipore, centrifuged (8000 rpm), and washed with Tris-HCl buffer (pH 7.4, 10.0 mM $MgCl_2$) for three times to obtain purified Y-motif/FA@HyNPs nanoprobe, and the concentration of Y-motif/FA@HyNPs nanoprobe was also determined by the organic molecule of TMPyP.

Evaluation the $^1O_2$ Generation Ability of Y-motif/FA@HyNPs in Aqueous Solution. The $^1O_2$ generation ability of Y-motif/FA@HyNPs in aqueous solution was identified by two independent methods. First, singlet oxygen sensor green (SOSG) was employed as a fluorescence indicator to evaluate the generation ability of $^1O_2$ of Y-motif/FA@HyNPs in aqueous solution. In brief, 10.0 μM SOSG was mixed with 2.0 μM NH_2/FA@HyNPs, 2.0 μM Y-motif/FA@HyNPs, 2.0 μM Y-motif/FA@HyNPs + 10.0 mM ATP, 2.0 μM Y-motif/FA@HyNPs + 1.4 nM Let-7a, 2.0 μM Y-motif/FA@HyNPs+ 10.0 mM ATP + 1.4 nM Let-7a, and 2.0 μM Y-motif/FA@HyNPs+ 10.0 mM ATP + 1.4 nM Let-7a + 100.0 nM Let-7a inhibitor in PBS buffer (pH = 7.4, 1X), respectively. Afterward, all of these solutions were irradiated with or without a white light (LED lamp, 400 nm long pass filter) at 20.0 mW/cm^2 for 180.0 s, respectively. Then, the fluorescence emission of SOSG was excited with an excitation wavelength of 488 nm. The $^1O_2$ generation ability of different solutions were evaluated by comparing the SOSG fluorescence enhancement with the background at 525 nm. Additionally, the electron spin resonance (ESR) spectra were also used to evaluate the generation ability of $^1O_2$ of Y-motif/FA@HyNPs in aqueous solution. In brief, 20.0 mM 2,2,6,6-tetramethylpiperidine (a $^1O_2$ scavenger, TEMP) was mixed with 2.0 μM NH_2/FA@HyNPs, 2.0 μM Y-motif/FA@HyNPs, 2.0 μM Y-motif/FA@HyNPs + 10.0 mM ATP, 2.0 μM Y-motif/FA@HyNPs + 1.4 nM Let-7a, and 2.0 μM Y-motif/FA@HyNPs+ 10.0 mM ATP + 1.4 nM Let-7a in PBS buffer (pH = 7.4, 1X), respectively. Afterward, all of these solutions were irradiated with a white light (LED lamp, 400 nm long pass filter) at 20.0 mW/cm^2 for 180 s, respectively. Then, the ESR spectra of TEMP were observed using an electron paramagnetic resonance spectrometer (EMX-10/12, Bruker, Germany). The $^1O_2$ generation ability of different solutions were evaluated by comparing the ESR intensity of TEMP at 3464.21 G.

Cell Culture and Incubation Conditions. MCF-7 and MCF-10A cells were obtained from Nanjing Keygen Biotech Co. Ltd. and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS, V/V, Gibco), 100.0 μg/mL streptomycin, and 100 units/mL penicillin at 37.0 °C in 5% CO_2 incubator. The culture medium was changed every other day and the cells were subcultured after reaching confluence.

Fluorescence Imaging of Endogenous Let-7a in Living Tumor Cells Using Y-motif/FA@HyNPs. MCF-7 cells and MCF-10A cells at the density of ~ 100 k were inoculated into 35.0 mm confocal dishes, and then cultured in CO_2 incubator at 37.0 °C for 24.0 h. Before fluorescence imaging studies, the cells were incubated with 2.0 μM Y-motif/FA@HyNPs for 2.0 h at 37.0 °C, and then washed with PBS buffer (pH = 7.4, 1X) for three times, which were followed to achieve the epifluorescence images using an IX73 optical microscope. For colocalization studies, MCF-7 cells were incubated with 2.0 μM Y-motif/FA@HyNPs for 2.0 h at 37.0 °C, and washed with PBS buffer (pH = 7.4, 1X) for three times, which were further incubated with 1.0 μM Lyso-Tracker@Red DND-99 and 2.0 μg/mL Hoechst 33342 at 37.0 °C for another 20.0 min. Afterward, the medium was removed, and the cells were washed with PBS buffer (pH = 7.4, 1X) for three times, which were followed to collect the epifluorescence images using an IX73 optical microscope. R6G in Y-motif/FA@HyNPs was excited at 530 to 550 nm, and emission from 570 to 600 nm. TMPyP-Zn-QDs in Y-motif/FA@HyNPs was excited at 400 to 440 nm, and emission from 600 to 650 nm. Lyso-Tracker@Red DND-99 was excited at 540 to 580 nm, and the emission was collected from 600 to 650 nm. Hoechst 33342

was excited from 340 to 390 nm, and the emission was collected from 420 to 460 nm.

Controllable Cascade Amplification $^1O_2$ Imaging in Living Tumor Cells. MCF-7 cells at the density of ~ 100 k were seeded into 35.0 mm confocal dishes and cultured in CO_2 incubator at 37.0 °C for 24.0 h. After that, the cells were washed with PBS buffer (pH = 7.4, 1X) three times and then treated with the five following ways: (1) incubation with 2.0 μM Y-motif/FA@HyNPs in dark at 37.0 °C for 2.0 h, and then incubation with 30.0 μM DCFH-DA for another 20.0 min; (2) incubation with 2.0 μM Y-motif/FA@HyNPs at 37.0 °C for 2.0 h, followed incubation with 30.0 μM DCFH-DA for another 20.0 min, and then irradiation with white light (LED lamp, 400 nm long pass filter) at a power of 20.0 mW/cm^2 for 180 s; (3) incubation with 2.0 μM Y-motif/FA@HyNPs at 37.0 °C for 2.0 h, followed incubation with 30.0 μM DCFH-DA + 2.5 mM VC for another 20.0 min, and then irradiation with white light (LED lamp, 400 nm long pass filter) at a power of 20.0 mW/cm^2 for 180 s; (4) transfection with 200.0 nM Let-7a inhibitor using lipofectamine3000 reagent at 37.0 °C for 6.0 h, followed incubation with 2.0 μM Y-motif/FA@HyNPs at 37.0 °C for 2.0 h, then incubation with 30.0 μM DCFH-DA for another 20.0 min, and last irradiation with white light (LED lamp, 400 nm long pass filter) at a power of 20.0 mW/cm^2 for 180 s; (5) incubation with 2.0 μM Y-motif/FA@HyNPs + 100.0 mM IAA at 37.0 °C for 2.0 h, followed incubation with 30.0 μM DCFH-DA for another 20.0 min, and then irradiation with white light (LED lamp, 400 nm long pass filter) at a power of 20.0 mW/cm^2 for 180 s. After above operations, all MCF-7 cells were washed with PBS buffer (pH 7.4, 1X) for three times, and further incubation with

1.0 mL DMEM for fluorescence imaging using an IX73 optical microscope. DCF was excited at 470 to 495 nm, and emission from 510 to 530 nm. R6G in Y-motif/FA@HyNPs was excited at 530 to 550 nm, and emission from 570 to 600 nm. TMPyP-Zn-QDs in Y-motif/FA@HyNPs was excited at 400 to 440 nm, and emission from 600 to 650 nm.

MTT Assay. The cytotoxicity of Y-motif/FA@HyNPs was investigated by means of MTT assay. In detail, MCF-7 cells were seeded on four 96-well plates at an original density of 5×10^3 cells per well. After incubation at 37.0 °C for 24.0 h, all cells were washed with PBS buffer (pH = 7.4, 1X) for three times, and treated with four ways. (1) To explore the dark toxicity of Y-motif/FA@HyNPs, MCF-7 cells in one 96-well plates were further cultured with 100.0 μL DMEM medium containing serial concentrations of Y-motif/FA@HyNPs at 37.0 °C for 24.0 h. (2) To explore the phototoxicity of Y-motif/FA@HyNPs, MCF-7 cells in another 96-well plates were further cultured with 100.0 μL DMEM medium containing serial concentrations of Y-motif/FA@HyNPs at 37.0 °C for 24.0 h, and then were irradiated by a white light (LED lamp, 400 nm long pass filter) at a power of 20.0 mW/cm^2 for 180 s. (3) To explore the behavior of Let-7a-activatable phototoxicity of Y-motif/FA@HyNPs, MCF-7 cells in third 96-well plates were transfected with 200.0 nM Let-7a inhibitor using lipofectamine3000 reagent at 37.0 °C for 6.0 h, and then were cultured with 100 μL DMEM medium containing serial concentrations of Y-motif/FA@HyNPs at 37.0 °C for 24.0 h, followed irradiation with a white light (LED lamp, 400 nm long pass filter) at a power of 20.0 mW/cm^2 for 180 s. (4) To explore the behavior of ATP-powered phototoxicity of Y-motif/FA@HyNPs, MCF-7 cells in fourth 96-well plates were further cultured with 100.0 μL DMEM medium containing serial concentrations of Y-motif/FA@HyNPs and 100.0 mM IAA at 37.0 °C for 24.0 h, and then were irradiated by a white light

(LED lamp, 400 nm long pass filter) at 20.0 mW/cm^2 for 180 s. After that, all of MCF-7 cells in four 96-well plates were cultured with 50.0 μL PBS buffer containing 1 mg/mL MTT at 37.0 °C for 4.0 h, and then the PBS buffer in per well was removed carefully, followed by addition of 150 μL DMSO to observe the produced blue formazan. Finally, the optical density (OD) of each well at 490 nm was measured using a Bio-Rad microplate reader. The relative cell viability was calculated as $(OD/OD_{control})\times100\%$. Each value was averaged from three independent experiments.

Flow Cytometric Analysis of Cell Apoptosis. MCF-7 cells were seeded on a 6-well plate at an original density of 200 k cells per well. After incubation at 37.0 °C for 24.0 h, the cells were washed with PBS buffer (pH 7.4, 1X) for three times, and treated with the following six ways: (1) incubation with 1.0 mL fresh DMEM at 37.0 °C for 24.0 h, which was selected as control; (2) irradiation by a white light (LED lamp, 400 nm long pass filter) at 20.0 mW/cm^2 for 180 s; (3) incubation with 1.0 mL fresh DMEM containing 2.0 μM Y-motif/FA@HyNPs at 37.0 °C for 2.0 h; (4) incubation with 1.0 mL fresh DMEM containing 2.0 μM Y-motif/FA@HyNPs at 37.0 °C for 2.0 h, and then irradiation by a white light (LED lamp, 400 nm long pass filter) at 20.0 mW/cm^2 for 180 s; (5) transfection with 200 nM Let-7a inhibitor using lipofectamine3000 reagent at 37.0 °C for 6.0 h, and then incubation with 1.0 mL fresh DMEM containing 2.0 μM Y-motif/FA@HyNPs at 37.0 °C for 2.0 h, followed by irradiation with a white light (LED lamp, 400 nm long pass filter) at 20.0 mW/cm^2 for 180 s; (6) incubation with 1.0 mL fresh DMEM containing 2.0 μM Y-motif/FA@HyNPs + 100.0 mM IAA at 37.0 °C for 2.0 h, and then irradiation by a white light (LED lamp, 400 nm long pass filter) at 20.0 mW/cm^2 for 180 s. Afterward, all cells were washed with PBS buffer (pH 7.4, 1X) for three times, and the cells were trypsinized with 1.0 mL trypsin. After that, the cells were centrifuged and suspended in PBS. At this moment, 5.0 μL Annexin V-

FITC and 5.0 μL propidium iodide (PI) according to the manufacturer's protocol were added and incubation 15.0 min for staining. Subsequently, the apoptosis assay of MCF-7 cells was performed on Coulter FC-500 flow cytometer using FITC and PI channel. The cell number for each flow cytometry analysis was ~ 1×10^4, and the data was processed using FlowJo software.

Animals and Tumor Model. Female BALB/c mice (4-5 weeks old) were obtained from the Model Animal Research Center (MARC) of Nanjing University (Nanjing, China), and performed in agreement with the regulations of the Institutional Animal Care and Use Committee (IACUC) of Nanjing University. MCF-7 tumor model was created through subcutaneous injection of MCF-7 cells (~ 2.0×10^6) into the selected positions of the nude mice.

Immunohistochemistry Analysis. Immunohistochemistry experiments were investigated on MCF-7 tumor-bearing female nude mice. In detail, five groups of mice were treated with group 1 (PBS only), group 2 (PBS + light irradiation), group 3 (Y-motif/FA@HyNPs only), group 4 (Y-motif/FA@HyNPs + light irradiation), and group 5 (Y-motif/FA@HyNPs + Let-7a inhibitor + light irradiation). For group 1 and 2, 100.0 μL PBS was directly injected into tumor in each mouse. For group 3 and 4, 100.0 μL of 200.0 μM Y-motif/FA@HyNPs was directly injected into tumor in each mouse. After injection of 3.0 h, mice in groups 2 and 4 were irradiated with a xenon lamp (400 nm long pass filter, 120 mW/cm^2) for three consecutive exposures of 10 min each, with a 20-min interval. For group 5, 100.0 μL of 20.0 mM Let-7a inhibitor by means of lipofectamine3000 transfection reagent was first injected into tumor in each mouse. After injection of 12.0 h, 100.0 μL of 200.0 μM Y-motif/FA@HyNPs was also injected into tumor in each mouse. After injection of another 3.0 h, these mice in group 5 were irradiated with a xenon lamp (400 nm long pass filter, 120 mW/cm^2) for three

consecutive exposures of 10 min each, with a 20-min interval. After these mice above were treated with 24.0 h, the tumors from the five groups were cryosectioned at 10-μm thickness onto slices, followed with H&E and TUNNE staining by the manufacturer's protocols. Finally, all images of tumor slice were observed on an IX73 optical microscope (Olympus, Japan).

Statistical Analysis. All the data were presented as mean ± SD unless otherwise stated. Statistical significance was performed using two-tailed Student's t-test, which was set at $P < 0.05$ (*$P < 0.05$, **$P < 0.01$).

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