Electronic Supplementary Information (ESI)

Photocaged FRET Nanoflares for Intracellular MicroRNA Imaging

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Experimental section

Reagents and instruments

Trisodium citrate, sodium chloride, phosphate and chloroauric acid ($HAuCl_4 \cdot 4HO_2$) were obtained from Sinopharm Chemical Reagent Co., Ltd. (China). MTS and lipofectamine™ 3000 transfection reagent purchased from Thermo Fisher Scientific Co., Ltd (U.S.A.). Hoechst 33258 was ordered from Sangon Biotechnology Co., Ltd (Shanghai, China). Loading buffer was purchased from TaKaRa Bio Inc. (Dalian, China). SYBR Gold was purchased from Invitrogen (U.S.A.). All other reagents were analytically grade. MCF-7, A-549 and SMMC-7721 cell lines were obtained from Cell Bank of the Committee on Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). All aqueous solutions were prepared using ultrapure water (≥18 MΩ, Milli-Q, Millipore). All oligonucleotides were synthesized by Sangon Biotechnology Co., Ltd (Shanghai, China). These sequences are listed as **Table S1.**

Gel imaging was obtained from multifunctional molecular imaging analysis system Azure C600 (America). The UV–vis absorption measured on a Biospec-nano UV–vis spectrophotometer (Shimadzu, Japan). JEM-2100 transmission electron microscope (JEOL Ltd., Japan) was used for transmission electron microscopic (TEM) images of AuNPs. The all fluorescence spectra were performed on near infrared steady state fluorescence spectroscopy system (QM40-NIR, PTI Inc.,

America). The confocal microscope imaging was recorded by Nikon A1 confocal laser scanning microscopy (Japan). Cells incubated in a humidified HF90 CO_2 incubator (Shanghai Lishen Scientific Equipment Co. Ltd.). MTS assays were obtained from a M1000 microplate reader (TECAN Inc., America). All buffer pH measurements were performed with Orion 3 Star pH meter (Thermo Scientific, USA). Centrifuge was performed with Beckman Coulter Allegra 25R centrifuge (Brea, CA, USA).

Polyacrylamide gel electrophoresis

For UV light cleavage feasibility of inhibitor strand containing PC-linker (P), it was pretreated with 365 nm UV light irradiation for 0 min, 5 min, 15 min and 25 min. For feasibility verification of photocaged FRET nanoflares, S: H (1:1), S: H: P (1:1:1) and S: T (1:1) were heated to 80^{o}C for 10 min and then cooled down room temperature to form stable complex, respectively. Subsequently, three groups of S: H: P (1:1:1) complex were treated with UV light irradiation for 5 min, 1 μm target, UV light irradiation 5 min and 1 μm target, respectively. After that samples were incubated at 37^{o}C for 1 h in 10 mM PBS (137 mM NaCl, 10 mM Phosphate, 2.72 mM KCl, pH 7.4). All of the concentrations of samples were 1 μM. 10 μL samples were mixed with 2 μL 6×loading buffer and 2 μL 100×SYBR Gold before pointing samples. Gels run at a constant voltage of 95 V for 1 or 2 h at 0^{o}C. After that the results were analysed by Azure C600 imaging system.

Synthesis and characterization of AuNPs

The method of sodium citrate reduction universally used for preparation of 13 nm AuNPs. Before preparation, all the glassware soaked in aqua regia (HCl/HNO_3 3:1) for at least 8 h. After that washed with deionized H_2O for three times and dried in drying box. Subsequently, 100 mL 0.01% $HAuCl_4$ was heated and uniformly stirred until to boiling in 250 mL conical flask. Next, we rapidly added 3.5 mL trisodium citrate (1%) into vortex center and observed the color change of the solution until turn to steady claret. Finally, micro boiling 15 min and removing the heating source to room temperature under stirring condition. The synthesized AuNPs were stored at 4^{o}C for preparation use. Concentration measurement of AuNPs was obtained by UV absorption at 519 nm ($\varepsilon = 2.7\times10^{8}$ L mol^{-1} cm^{-1}). The pattern and size was determined on transmission electron microscopy images and showed about 13 nm ± 2 nm uniform spherical shapes in **Fig. S3**

Preparation of photocaged FRET nanoflares

First, fresh S, H and P were mixed and heated to 80^{o}C for 10 min, then cooled down to room temperature for forming stable complex. Next, 13 nm AuNPs were added to the complex and incubated for 16 h at 4^{o}C. After that 0.2 M PB (Na_2HPO_4 and NaH_2PO_4; pH =7.4) was added to the mixture and final concentration was 0.01 M PB, followed by 4 M NaCl was slowly added to mixture drop by drop and final concentration was 0.1 M, 0.2 M and 0.3 M every eight-hours. Modified AuNPs were centrifuged for 30 min at 13000 rpm and resuspended in 10 mM PBS for three times to discard excessive DNA strands, and then resuspended in required buffer and stocked at 4^{o}C for use in the following experiments. The concentration of photocaged FRET nanoflares were determined by measuring their UV absorption at 524 nm ($\varepsilon = 2.7\times10^{8}$ L mol^{-1} cm^{-1}).

Fluorescence feasibility and experiments condition analysis

The design of inhibitor strands might affect sensing performance of photocaged FRET nanoflares. Different inhibitor strands (PC-1, PC-2, PC-3, PC-4, PC-5) were designed. Subsequently, each corresponding photocged FRET nanoflares were divided into three groups and treated with target, 5 min UV light irradiation + target, without treatment as control group. For the fluorescence feasibility of photocaged FRET nanoflares, samples were treated with only target, only 5 min UV light irradiation, 5 min UV light irradiation + target and control group without treatment. Meanwhile, studied on the FRET signal change with target concentration, samples were divided into two groups. Group one without UV light irradiation were incubated with different concentration targets (0 nM, 10 nM, 25 nM, 50 nM, 75 nM, 100 nM) at 37^{o}C for 1 h in 10 mM PBS, respectively. Group two were pretreated with 5 min UV light irradiation and then incubated with various concentration targets (0 nM, 10 nM, 25 nM, 50 nM, 75 nM, 100 nM) at 37^{o}C for 1 h in 10 mM PBS, respectively. For light irradiation time analysis, by samples were pretreated with different light irradiation time (0 min, 1 min, 2 min, 3 min, 4 min, 5 min, 6 min, 7 min, 8 min, 9 min and 10 min) and incubated with certain concentration target at 37^{o}C for 1 h in 10 mM PBS. At the same time, without targets treatment as control group.

To obtain the optimal experiments condition, the ratio S, H and P were first studied. The ratio of S to P was kept 1:2 and changed the ratio of S to H (2:1, 1:1, 1:1.2, 1:2). Corresponding samples were treated with only target, 5 min UV light irradiation and target, without treatment, respectively. Similarly, fixing the ratio of S to H was 1:1.2 and changed the ratio of S to P (2:1,

1:1, 1:1.2, 1:2), according to the above methods. All samples were incubated at 37°C for 1 h in 10 mM PBS. Subsequently, the fluorescence was monitored on steady-state near infrared fluorescence spectrometer (QM40-NIR) exciting at 530 nm and recording emission from 550 nm to 750 nm, excitation /emission slit width was 10 nm. All experiments were repeated at least three times.

Sensitivity and selectivity analysis

Same concentration photocaged FRET nanoflares were pretreated with and without UV light irradiation and then incubated with various target concentrations (0 nM, 10 nM, 25 nM, 50 nM, 75 nM, 100 nM, 150 nM, 200 nM, 250 nM) in 10 mM PBS for 1 h at 37°C. The specificity and one-base identification of photocaged FRET nanoflares were investigated by adding the 100 nM let-7a target and 200 nM other negative control miRNAs (homologous family let-7e, let-7i and let-7d; non-homologous family miRNA-200b and miRNA-429). At the same time, the photocaged FRET nanoflares also was pretreated with UV light irradiation, followed by incubated with no target, one-base mismatched target and target, respectively in 10 mM PBS for 1 h at 37°C. All the fluorescence of samples were monitored on QM40-NIR exciting at 530 nm and recording emission from 550 nm to 750 nm, excitation /emission slit width was 10 nm. All experiments were repeated at least three times.

Cell culture and UV light irradiation toxicity analysis

A549 cells (human lung cancer cell line) and SMMC-7721 cells (human liver cancer cell line) were grown in RPMI 1640 medium and MCF-7 (human breast cancer cell line) was cultured in Dulbecco's Modified Eagle Medium (DMEM) medium with 10% fetal bovine serum (FBS) and 150 μL streptomycin/penicillin antibiotics solution. Followed by they were incubated at 37°C in humidified HF90 cell incubator containing 5% CO_2. According to a certain density, MCF-7 cells suspension dispersed in a 96-well microtiter plates maintaining a total volume of 100 μL $well^{-1}$. Subsequently, the plate was incubated in CO_2 cell incubator for 24 h at 37°C. After that discarding the original medium, adding 100 μL fresh medium in every well and MCF-7 cells were treated with various UV light irradiation time (0 min, 1 min, 2 min, 3 min, 4 min, 5 min……10 min). Followed by further incubated for 12 h. Then discarded old medium, 10 μL MTS solution and 90 μL fresh medium were added to each well for 4 h. The absorption was measured at 490 nm with a M1000 microplate reader.

Light-activated feasibility and colocalization imaging analysis in cells

To test photocaged FRET nanoflares was intracellular UV light-activated, after photocaged FRET nanoflares incubated with cells for 3 h and discarded the old medium, washed three times with PBS for discarding non-internalized probe and added fresh medium. Subsequently, with 5 min UV light exposed to cells for activating probe or without UV light irradiation and further incubated for hours in CO_2 cell incubator. For colocalization imaging analysis, the same methods as above were conducted. Before imaging, the old medium was removed, added 5 μL (1 mg/mL) hoechst 33258 to the total volume of 200 μL and further incubated for 30 min. Then all confocal microscopy imaging were performed on Nikon A1 confocal laser scanning microscopy (CLSM). The Cy3 and Cy5 fluorescence emission channels were recorded under an exictation laser at 561 nm. The fluorescence images data were processed by software of Nikon A1 Analysis.

Optimizing incubation time of UV light-initiated FRET nanoflares with cells

According to the above methods, MCF-7 cells were plated on 35-mm confocal incubation dishes for 24 h. Then photocaged FRET nanoflares were incubated with MCF-7 cell at 37°C in 5% CO_2 cell incubator for 3 h internalization, respectively. After that the old medium was discarded and washed three times with PBS. Fresh medium was added and 5 min UV light irradiation exposed on cells. Subsequently, UV light activation FRET nanoflares continue to incubate with cells for various times (1 h, 1.5 h, 2 h, 2.5 h). Finally, the fluorescence imaging was performed on Nikon A1 confocal laser scanning microscopy (CLSM). The Cy3 and Cy5 fluorescence emission channels were recorded at 561 nm exictation. The fluorescence images data were processed by software of Nikon A1Analysis.

Sensing of miRNA expression in different types cell lines

MCF-7 cells, A549 cells and SMMC-7721 cells were respectively grown on 35-mm confocal incubation dishes for 1-2 days. First, the same concentration photocaged FRET nanoflares were respectively incubated with three cell lines at 37°C in CO_2 cells incubator for 3 h internalization. Next, old medium was discarded, washed three times with PBS and replenished fresh medium. Final, UV light irradiation exposed on cells and further incubated for 2 h at 37°C in CO_2 cells incubator. The fluorescence imaging was conducted by Nikon A1 confocal laser scanning microscopy (CLSM). The relative ratio intensity was measured by Image J.

Monitoring the intracellular miRNA changes in concentration

First, miRNA mimics or anti-miRNA entered into MCF-7 cells by liposome transfection to regulate miRNA concentration, respectively and without treatment MCF-7 cells served as a control group. Second, after incubation for 3 h, cells were washed three times with PBS and further incubated with same concentration photocaged FRET nanoflares for 3 h internalization. Third, cells were washed three times with PBS, replenished fresh medium, exposed on UV light irradiation and continued to incubate for 2 h. The fluorescence microscope imaging was performed on Nikon A1 confocal laser scanning microscopy (CLSM) at 561 nm exictation. The fluorescence image data was analyzed by software of Nikon A1Analysis.

QRT-PCR

The total cellular RNA was extracted from three different cell lines using Trizol reagent (Sangon Co. Ltd., Shanghai, China) according to the manufacturer's instructions. 1 mL Trizol reagent added into about 10^6 cells until total lysis and stored -20^{o}C for use as the following experiment. To quantify the relative expression of intracellular mature let-7a, the following steps were conducted, 1 μL revert aid premium reverse transcriptase to made total miRNA reverse-transcribed to cDNA, qPCR analysis was performed with SG Fast qPCR Master Mix (2×) (BBI) on ABI Stepone plus qRT-PCR system. U6 was used as internal control, all primers involved in these assays as the following.

let-7a F：5’-ACACTCCAGCTGGGTGAGGTAGTAGGTTG -3’

All R：TGGTGTCGTGGAGTCG

U6 forward primer: 5’-CTCGCTTCGGCAGCACA-3’

U6 revers primer: 5’-AACGCTTCACGAATTTGCGT-3’

Table S1. All oligonucleotides sequences used in experiment section are listed as following (from 5' to 3').

S'	CTGCACAGCGAACTATACAACCTACTACCTCAGAGTCAG
H'	ATGACTCTGAGGTAGTAGGTTGTCAGAGTCAT
P	ATAGTTCG//CTGTGCAG
T	TGAGGTAGTAGGTTGTATAGTT
S	SH-AAAAAAAAAACTGCACAGCGAACTATACAACCTACTACCTCAGAGTCAG
H	Cy3-ATGACTCTGAGGTAGTAGGTTGTCAGAGTCAT-Cy5
PC-1	GTTG//CTCCAGAC
S-1	SH-AAAAAAAAAAGTCTGGAGCAACACAACCTACTACCTCAGAGTCAG
PC-2	ATAGTTG//CTCCAGAC
S-2	SH-AAAAAAAAAAGTCTGGAGCAACTATACAACCTACTACCTCAGAGTCAG
PC-3	ATAGTTCG//CTGTGCAG
S-3	SH-AAAAAAAAAAACTGCACAGCGAACTATACAACCTACTACCTCAGAGTCAG
PC-4	ATAGTTCG//CTCCAGAC
S-4	SH-AAAAAAAAAAGTCTGGAGCGAACTATACAACCTACTACCTCAGAGTCAG
PC-5	ATAGTTCG//CTCCAGACG
S-5	SH-AAAAAAAAAAACGTCTGGAGCGAACTATACAACCTACTACCTCAGAGTCAG
let-7e T	TGAGGTAGGAGGTTGTATAGTT
let-7i T	TGAGGTAGTAGTTTGTGCTGTT
let-7d T	AGAGGTAGTAGGTTGCATAGTT
miRNA-429T	TAATACTGTCTGGTAAAACCGT
miRNA-200bT	TAATACTGCCTGGTAATGATGAC
mismatched T	TGAGGTAGGAGGTTGTATAGTT
anti-let7a	A*A*C*TATACAACCTACTACCT*C*A*
let7a mimics	T*G*A*GGTAGTAGGTTGTATAG*T*T*

Red font represents one-base mismatched site, "//" denoted PC-linker group, S' and H' represented purified by PAGE for gel electrophoresis analysis, "*" represents phosphorothioate modification.

Supporting Figures：

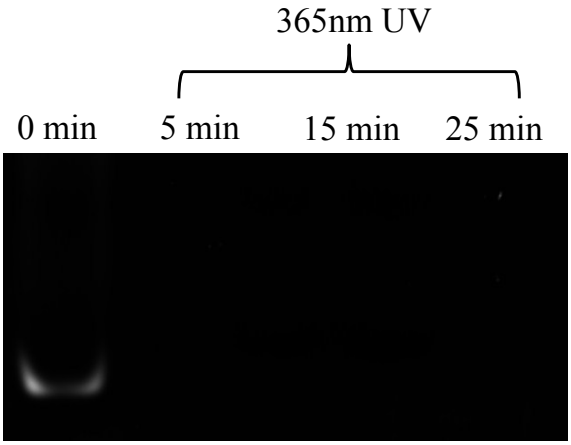

Fig. S1 Study on cleavage performance of photocleavable linker (PC-linker) group by 18% PAGE

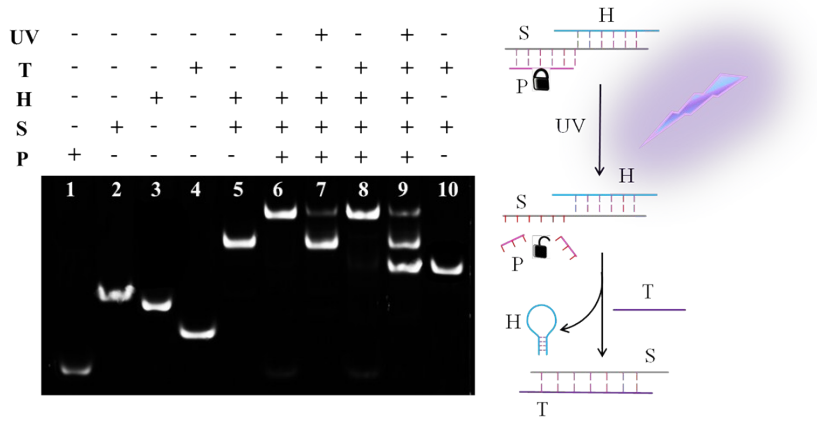

Fig. S2 Verification of UV light activated FRET nanoflares for miRNA detection by PAGE. All samples were 1 μM and "-" denotes without it, "+" denotes with it. It run at 95 V for 2 h in 1×TBE buffer.

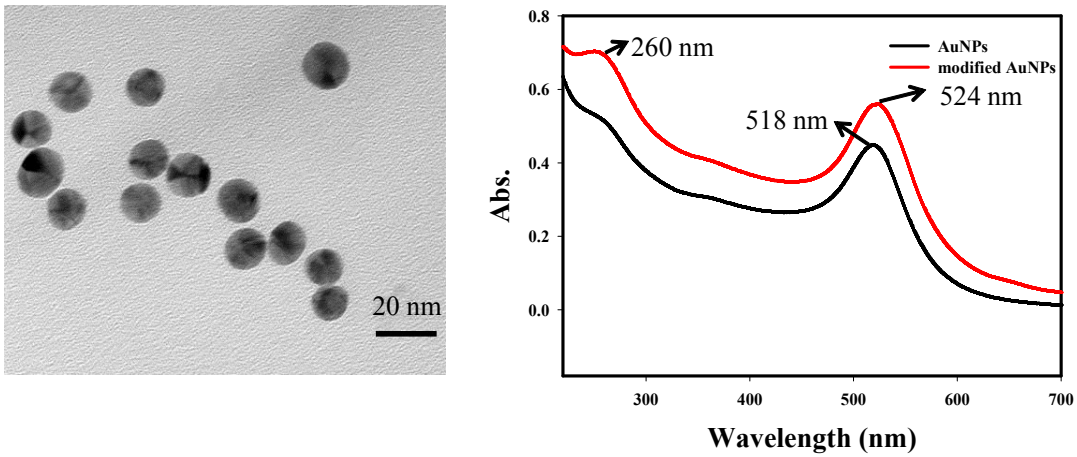

Fig. S3 The characterization of AuNPs, modified AuNPs by TME imaging and absorption spectrum methods.

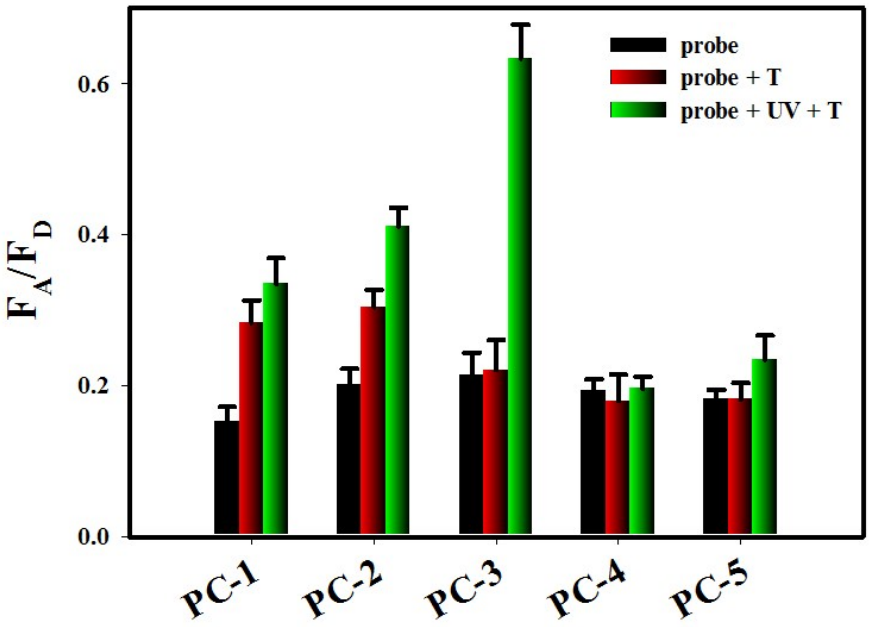

Fig. S4 Study on the length of inhibitor strand by fluorescence analysis. Different length inhibitor strands were designed and corresponding photocaged FRET nanoflares were treated with UV light and miRNA targets. Error bars represented three independent repeated experiments.

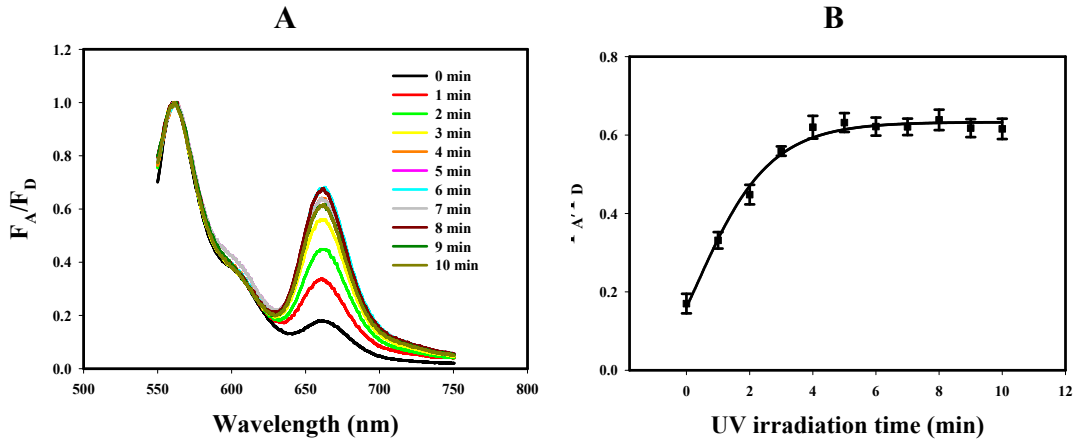

Fig. S5 Sensing performance of photocaged FRET nanoflares with different UV irradiation times (A) Fluorescence spectra of photocaged FRET nanoflares responded to different UV irradiation times in the presence of miRNA targets. (B) Responding curve of probe as a function of different length UV irradiation times. Error bars represented three independent repeated experiments.

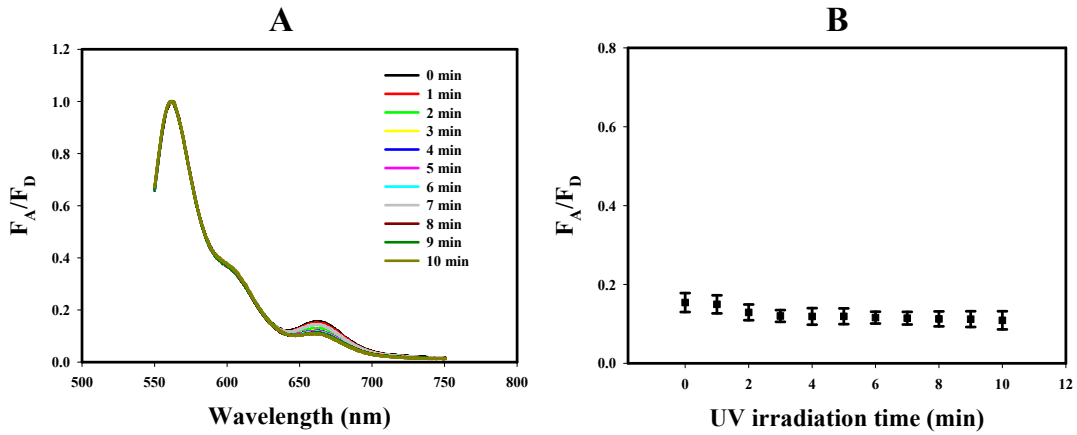

Fig. S6 The influence of different UV irradiation times on photocaged FRET nanoflares in the absence of miRNA targets (A) Fluorescence spectra of photocaged FRET nanoflares responding to different UV irradiation times in the absence of miRNA targets. (B) Responding curve of probe as a function of different length UV irradiation without miRNA targets. Error bars represented three independent repeated experiments.

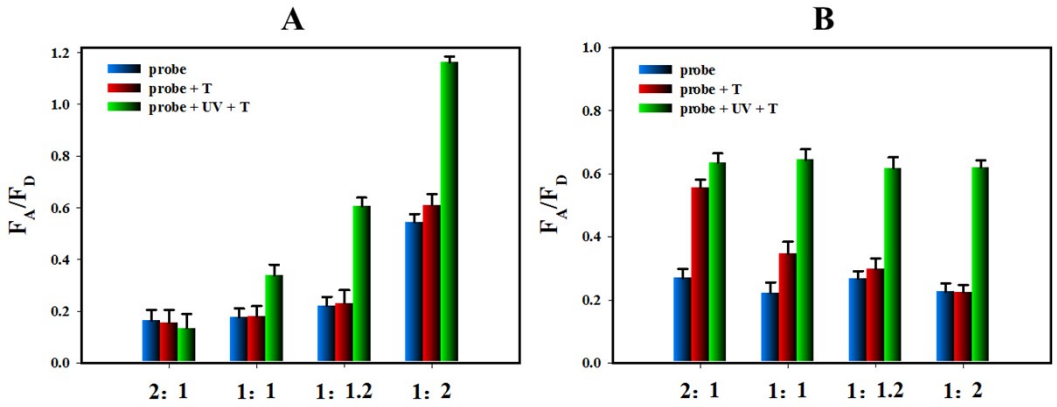

Fig. S7 Optimization of the experiment condition (A) Investigating the ratio of recognition sequence (R) to hairpin strand (H) and fixing the ratio of recognition sequence (R) to inhibitor strand (P) 1:2. (B) Optimization of the ratio of recognition sequence (R) to inhibitor strand (P) and keeping the ratio of recognition sequence (R) to hairpin strand (H) 1:1.2. Error bars represented three independent repeated experiments.

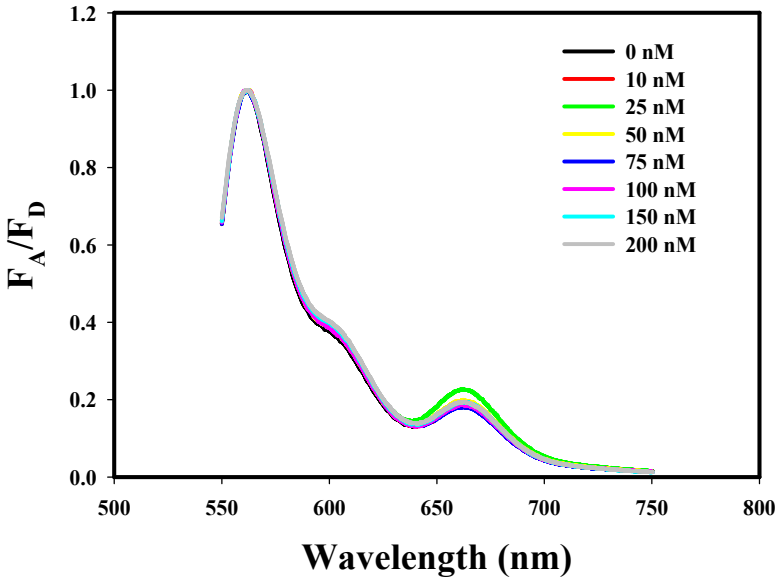

Fig. S8 Fluorescence spectra of photocaged FRET nanoflares responding to various concentration miRNA targets in the absence of 365 nm light irradiation.

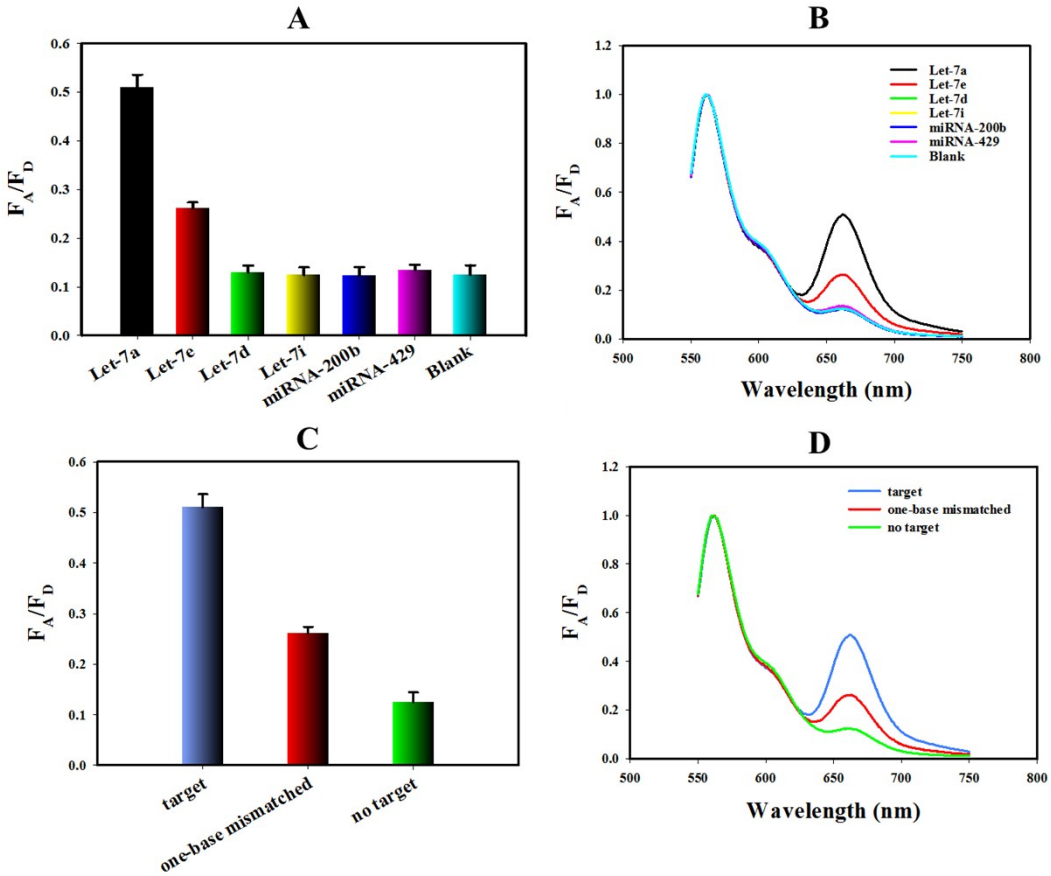

Fig. S9 Specificity and one-base mismatched test of photocaged FRET nanoflares (A) Histogram of photocaged FRET nanoflares over homologous family miRNA targets (let-7e, let-7d and let-7i) and non-homologous family miRNA targets (miRNA-200b and miRNA-429). (B) Corresponding fluorescence spectra of photocaged FRET nanoflares over different miRNA targets. (C) Histogram of photocaged FRET nanoflares treated with perfect matched, one-base mismatched and no miRNA targets. (D) Corresponding fluorescence spectra of photocaged FRET nanoflares treated with perfect matched, one-base mismatched and no miRNA targets. Error bars represented three independent repeated experiments.

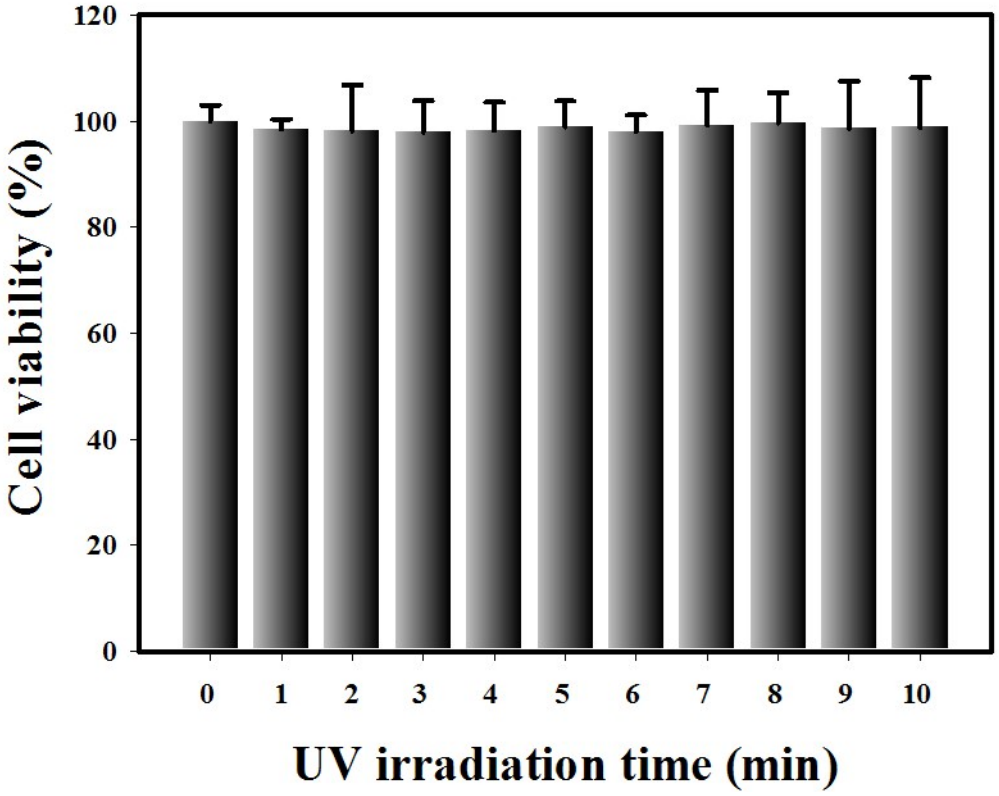

Fig. S10 Cell viability assay of MCF-7 cells treated with different UV irradiation times. MCF-7 cells were treated with various 365 nm light irradiation time and then cultured for 24 h in CO_2 incubator. Error bars represented three independent repeated experiments.

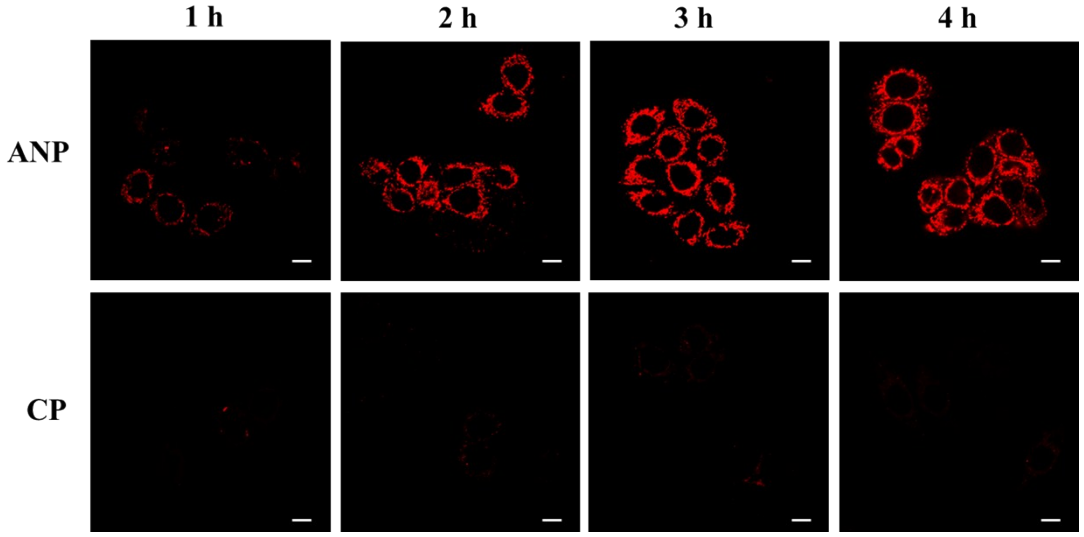

Fig. S11. The cellular uptake time of ANP (activated nanoprobe, single-dye Cy5 labeled DNA modified AuNPs) and CP (control probe, single-dye Cy5 labeled DNA strand). ANP and CP were treated with MCF-7 cells for 1 h, 2 h, 3 h and 4 h respectively. Scale bar is 10 μm.

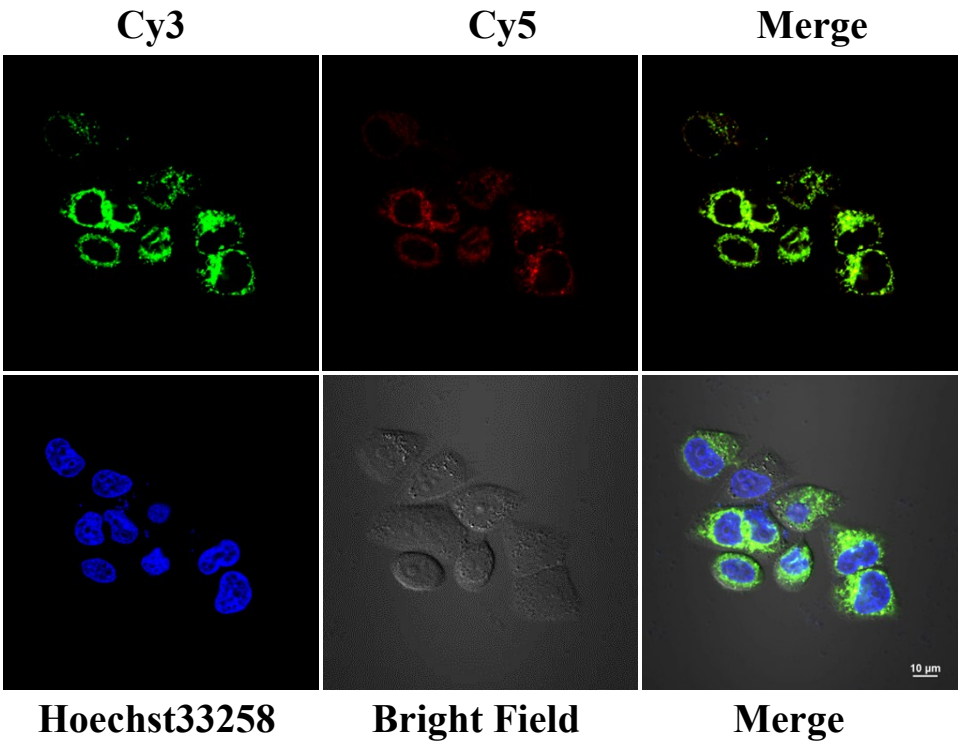

Fig. S12 The distribution of photocaged FRET nanoflares inside MCF-7 cells. MCF-7 cells were pretreated with photocaged FRET nanoflares and incubated with Hoechst 33258 (nucleus stain) for 30 min before confocal microscopy imaging. Scale bar is 10 μm.

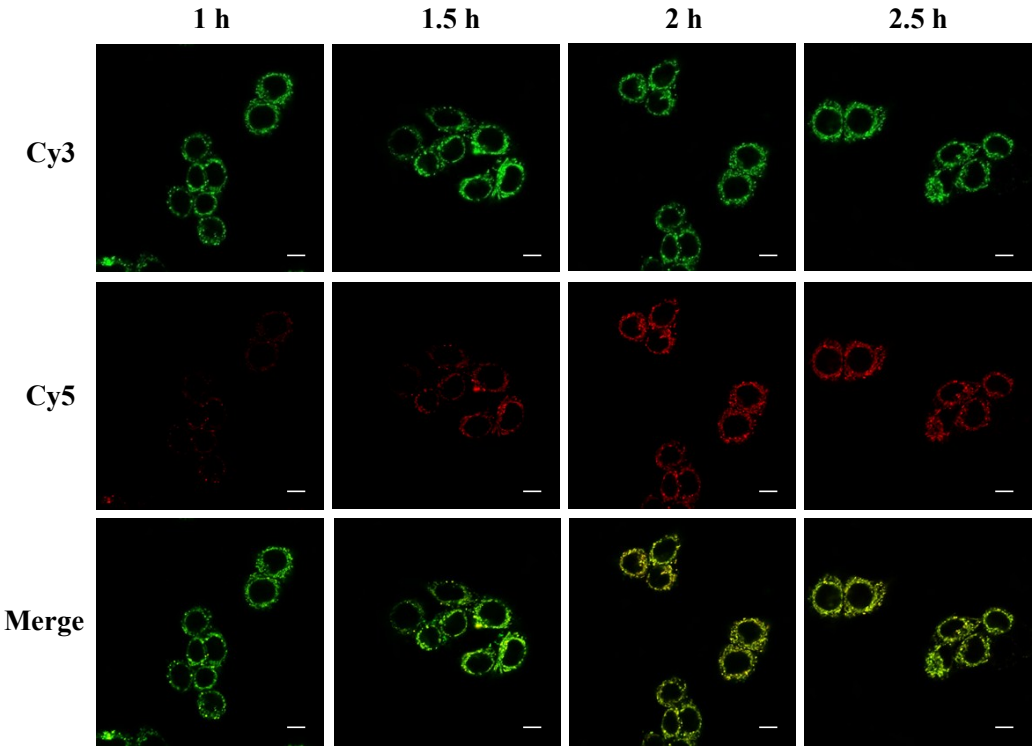

Fig. S13 Confocal fluorescence images of MCF-7 cells for various times. MCF-7 cells were pretreated with photocaged FRET nanoflares for 3 h and washed for three times with PBS and UV irradiation 5 min, after that further incubated for 1 h, 1.5 h, 2 h and 2.5 h for confocal fluorescence imaging. Scale bar is 10 μm.

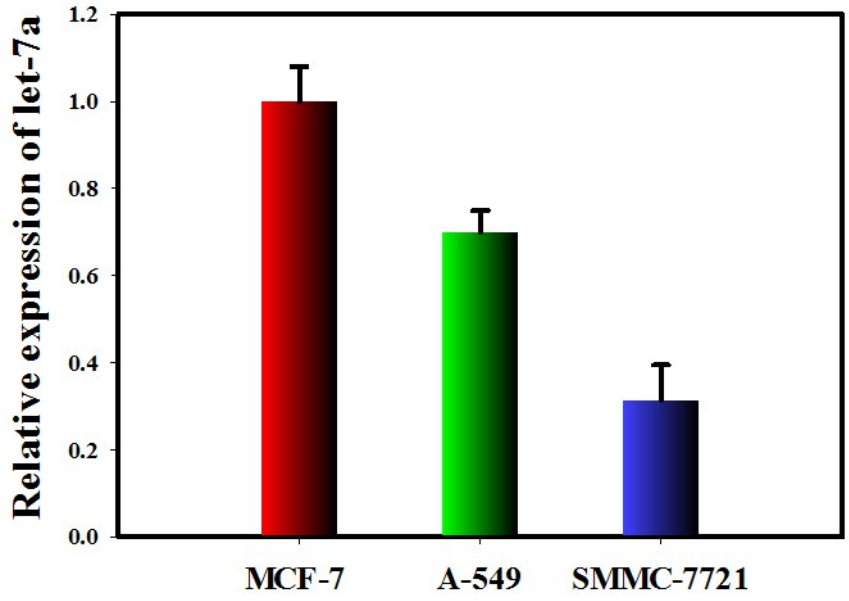

Fig. S14 qRT-PCR measured the relative expressions of let-7a miRNA in three cell lines. Error bars represented three independent repeated experiments.

Table S2. Comparison of our platform to the reported photoactivatable probes for miRNA imaging

Probe's names	Design	Output	False positive	Time	Amplification	Refs
NIR light gated nanodevice based on DNA beacon	Simple, flexibility	Single signal	high	~	No	1
Photocaged nanoparticle sensor based on entropy-driven	Complex design	Single signal	high	3 h	Yes	2
NIR-assisted entropy-driven DNA system	Complex design	Single signal	high	10 h	Yes	3
Photocontrolled DNA walking machine	Complex design	Single signal	high	1 h	Yes	4
Photocaged FRET nanoflares	Simple, flexibility	FRET Ratiometric signal	low	1 h	No	Our work

References

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