Electronic Supplementary Information (ESI)

Biomineralized Zeolitic Imidazolate Framework-8 Nanoparticles Enable Polymerase/Endonuclease Synergistic Amplification Reaction in Living Cells for Sensitive MicroRNA Imaging

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Materials and Reagents. Zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$, 99%), 2-Methylimidazole (2-MIM, 99%), and calcimycin were purchased from Sigma-Aldrich (St. Louis, MO, USA). LysoTracker®Red DND-99, 2,5'-Bi-1H-benzimidazole (Hoechst 33342) was acquired from Thermo Fisher Scientific (Massachusetts, USA). Bovine Serum Albumin (BSA), Fluorescein isothiocyanate (FITC) and the Deoxynucleotide Triphosphates (dNTP) Mixture were supplied by Sangon Biotech Co., Ltd. (Shanghai, China). MicrOFF hsa-miR-21-5p inhibitor and micrON hsa-miR-21-5p mimic were obtained from RiboBio Company (Guangzhou, China). Klenow Fragment (3'→5' exo^-) Polymerase, Nicking endonuclease (Nt.BbvCI), and the corresponding buffer were purchased from New England Biolabs Ltd. (Beijing, China). Cell Counting Kit-8 (CCK-8) reagent was provided by Dojindo Laboratories (Kumamoto, Japan). All oligonucleotides were custom-synthesized by Sangon Biotech (Shanghai, China) Co. Ltd. and the detailed sequences were listed in **Table S1**. All other reagents were analytical reagents and supplied by Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All solutions were prepared using ultrapure water (Resistivity > 18.2 $M\Omega \cdot cm^{-1}$), which was treated by a Millipore Milli-Q water purification system (Billerica, MA, USA).

Apparatus. Fluorescence spectra were carried out by using spectrofluorometer FS5 (Edinburgh, UK). Transmission electron microscopy (TEM) images of nanoparticles for shape and size analysis were performed on Tecnai G2 F20 S-TWIN transmission electron microscope (FEI, America) with an accelerating voltage of 200 kV. Malvern Zetasizer Nano ZS90 (Malvern, England) was used for dynamic light scattering (DLS) analysis and zeta potential measurement of different functional nanoparticles. Powder X-ray diffraction (PXRD) patterns reflecting the crystal structure of nanoparticles were performed on XRD-6100 (Shimadzu, Japan) with Cu-Ka radiation at a 2θ range of 5-50°. Fourier transform infrared spectra (FTIR) were tested on IR Affinity-1 spectrometer (Shimadzu, Japan) scanned from 400 to 4000 cm^{-1}. UV 2450 (Shimadzu, Japan) was employed to get UV-vis absorption spectra for measurement of protein encapsulation efficiency in different nanoparticles. All fluorescent images were obtained by using 60× oil dipping objective on the Nikon Ti-E+ A1R MP confocal

laser scanning fluorescence microscope (Nikon, Japan).

Synthesis of Biomineralized ZIF-8 NPs Encapsulating Different Proteins. Proteins@ZIF-8 nanoparticles were prepared by using the similar procedure as described in our previous work. In order to construct effective protein-encapsulating ZIF-8 nanoparticles, we selected bovine serum protein (BSA), an enzyme stabilizer, as the model protein to initiate our synthesis. The first step in this process was to add 33.3 μL 5 mg/mL BSA or FITC labeled BSA (FITC-BSA) was dispersed in 416.7 μL contained 1.4 mmol 2-MIM aqueous solution. After vigorously stirring at 25 °C for 30 min, 50 μL of 0.02 mmol $Zn(NO_3)_2$ aqueous solution was quickly added to above solution for another 10 min at 25 °C under vigorous stirring. The resulting nanoparticles were centrifuged at 6500 rpm (the corresponding RCF was 10282 *g*) for 10 min, and washed with ethanol twice.

To establish intracellular polymerase/endonuclease synergistic amplification reaction (PESAR) system, three proteins-encapsulated ZIF-8 nanoparticle was prepared as follow: the protein mixture containing 25 μL 50000 U/mL Klenow fragment polymerase, 100 μL 10000 U/mL nicking enzyme Nt.BbvCI and 27.1 μL 5 mg/mL BSA, were dispersed in 297.9 μL contained 1.4 mmol 2-MIM and 50 μL of 0.02 mmol $Zn(NO_3)_2$ aqueous solution for synthesis of biomineralized nanoparticles (KleNtB@ZIF-8 NPs) in the same way. The final amount of the product was about 3 mg. The resulting nanoparticles were redissolved at a concentration of ~6 mg/mL and stored at 4 °C for future use.

Determination of Protein Encapsulation Efficiency. To determine the amount of protein encapsulated in ZIF-8 NPs, KleNtB@ZIF-8 nanoparticles were dissolved in 1mL 5 M hydrochloric acid for 5 min to release the loaded protein. The resulting solution was centrifuged by ultrafiltration tube (Millipore, molecular weight cut-off was 10KD) at 7000 rpm for 15 min, and washed twice by ultrapure water. The concentration of protein was determined by the absorbance measured at 280 nm in the UV-Vis absorption spectra. UV 2450 (Shimadzu, Japan) was used to collect the ultraviolet-visible absorption spectra with a wavelength interval of 1 nm. The ratio of the absorbance of the encapsulated protein mixture in KleNtB@ZIF-8 NPs to the

absorbance of the natural protein added to the synthetic experimental solution was used to calculate the protein encapsulation efficiency. Since the protein mixtures are fully homogeneous during the processing of biomineralization, the encapsulating efficiency of each protein is similar. Therefore, the loading amount of different enzymes and BSA was calculated by the equation:

$$\text{The loading amount} = c \times V \times EE\ (\%) / \text{the product amount}$$

Where c is the concentration or activity of different proteins according to manufacturer's instructions (NEB), V is the volume of different protein solutions, EE (%) is encapsulation efficiency.The product amount was about 3 mg in one typical synthesis.

Protein pH-responsive Release and Protection. To study the pH-triggered protein release, FITC-BSA was chosen as a model protein, and the releasing assay was carried out as follow: 2 mg FITC-BSA@ZIF-8 NPs were dispersed in 5 mL PBS buffer with different pH value (pH 5.5 or 7.4) at 37 °C under magnetic agitation. Then, the resulting FITC-BSA@ZIF-8 NPs dispersion was collected at different time intervals, and centrifuged at 15000 rpm (the corresponding RCF was 15619 *g*) for 10 min (In this situation, the biomineralized ZIF-8 NPs could be degraded at an acidic environment, and a greater centrifugal speed was required to collect the particles). The fluorescence intensity of the obtained supernatant was measured on FS5 fluorescence spectrometer (Edinburgh, UK).

The protein protection assay was investigated by using miR-21 initiated PESAR system to recover the fluorescent signal of molecular beacon (MB). In this analysis, 0.5 mL of 20 mg/mL proteinase mixtures (trypsin/α-chymostrypsin) was added to 0.5 mL of 6 mg/mL KleNtB@ZIF-8 NPs (the Klenow fragment polymerase and nicking enzyme in ZIF-8 NPs were ~1093 U and ~874 U, respectively) in PBS buffer and incubated at 37°C for 4 h followed by centrifugation at 15000 rpm for 15 min. The obtained precipitate was washed twice and resuspended in 0.5 mL PBS (pH 5.5) for 2 h. Then, the protein was collected by ultrafiltration tube at 15000 rpm for 10 min. The resulting protein mixture was diluted to 1 mL for further utilizing.

Then, the resulting protein mixture was used to drive the PESAR system in a 100 μL reaction solution as follows: 1 μL dNTP (10 mM), 10 μL 10×buffer (500 mM NaCl, 100 mM Tris-HCl, 100 mM $MgCl_2$, 1 mg/mL dithiothreitol), 2 μL DNA template strand (1 μM), 10 μL MB (1 μM), 1.8 μL as-prepared mixture containing Klenow fragment polymerase (the final concentration was ~20 U/mL) and nicking enzyme (the final concentration was ~16 U/mL), 2 μL of miR-21s (a DNA substitute for target miR-21, 1 μM) and 73.2 μL sterile water. After the solution was incubated at 37°C for 2 h, the fluorescence signal was collected at 500 ~ 600 nm under an excitation wavelength of 488 nm with a FS5 fluorescence spectrometer.

Preparation of the KleNtB@ZIF-8/TM NPs and Determination of the DNA Loading Efficiency. The KleNtB@ZIF-8/TM NPs were prepared as follow: 15 μL of 6 mg/mL KleNtB@ZIF-8 NPs were mixed with 4 μL of 5 μM Template, 20 μL of 5 μM MB, and 61 μL PBS at room temperature for 1 h to generate nanocomplex (KleNtB@ZIF-8/TM NPs). The resulting nanoparticles could be centrifuged at 15000 rpm (the corresponding RCF was 15619 *g*) for 10 min, and washed with PBS twice to remove excess nucleic acid. To determine the DNA loading efficiency, the Template and MB were labeled with Cy3 and FAM, respectively. The fluorescence intensity of the total probes before incubation and the fluorescence intensity of supernatant after centrifugation were determined by the spectrofluorometer FS5 instrument. The amount of adsorbed probes could be calculated by subtracting the amount of probes in supernatant from the total amount of probes. The ratio of the amount of adsorbed probes to the total amount of probes was used to estimate the DNA loading efficiency.

In Vitro Fluorescence Detection of miR-21. The PESAR system used for the detection of miRNA were performed as followed: In a typical procedure, 100 μL reaction solution containing 1 μL dNTP (10 mM), 10 μL of 10×buffer (500 mM NaCl, 100 mM Tris-HCl, 100 mM $MgCl_2$, 1 mg/mL dithiothreitol), 2 μL DNA template strand (1 μM), 10 μL molecular beacon (1 μM), 2 μL Klenow fragment polymerase (1000 U/mL), 2 μL nicking enzyme (500 U/mL), 71 μL water and 2 μL various concentrations of miR-21s was incubated at 37 °C for 2 h.

The fluorescence spectra were performed on the Edinburgh FS5 fluorescence spectrometer at room temperature. The emission spectra was obtained from the wavelength of 500 nm to 600 nm, with an excitation wavelength of 488 nm. We chose the fluorescence intensity at the emission wavelength of 520 nm as the optical experiment condition to evaluate the performance of this system. Both the excitation and emission slits width were set at 3 nm.

Nondenaturating Polyacrylamide Gel Electrophoresis (PAGE). For visualization of the polymerizing/nicking enzymatic amplification reaction, A standard 10 μL reaction solution containing 1 μL dNTP (1 mM), 1 μL of 10×buffer (500 mM NaCl, 100 mM Tris-HCl, 100 mM $MgCl_2$, 1 mg/mL dithiothreitol), 1 μL DNA template strand (Template, 200 nM), 1 μL Klenow fragment polymerase (200 U/mL), 1 μL nicking enzyme (100 U/mL), 1 μL miR-21s (200 nM) and 4 μL water, was incubated at 37 °C for 2 h. Finally, the mixture was incubated with 2 μL of 6×loading buffer and analyzed by a 20% nondenaturating polyacrylamide gel.

Cell culture. The A549, Hela, L02 cell lines were cultured in high glucose Dulbecco's modified Eagle' s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% streptomycin/penicillin. All cell lines were maintained in a humidified atmosphere incubator in the presence of 5% CO_2 at 37°C. Before the fluorescence imaging, the cells were seeded on a 35 mm Petri dish with a 10 mm well in 2 mL of culture medium at 37 °C for 24 h.

Fluorescence Imaging. Fluorescence imaging of intracellular co-delivery of protein and DNA was performed as follows: 90 μg/mL of FITC-BSA@ZIF-8 NPs absorbing Cy5 labeled DNA probe was incubated with A549 cells at 37 °C for 3 h. Then the cells were stained with Hoechst 33342 and washed by cold PBS three times before fluorescence imaging.

Fluorescence imaging of intracellular localization for the delivered protein was performed as follows: The A549 cells were incubated with 90 μg/mL FITC-BSA@ZIF-8 NPs at 37 °C for 3 h. Following, the cells were stained with10 μg/mL Hoechst 33342 and 100 nM LysoTracker Red DND-99 respectively, and washed with cold PBS for three times before fluorescence imaging.

Fluorescence imaging of intracellular miR-21 in different cells was performed as follows: before treated with biomineralized nanoparticles, the cells were cultured with PBS containing 10 mM $Mg^{2+}$and 5 μM calcium ionophore at 37 °C for 30 min. Then, 15 μL of 6 mg/mL KleNtB@ZIF-8 NPs were mixed with 4 μL of 5 μM Template and 20 μL of 5 μM MB to generate nanocomplex (KleNtB@ZIF-8/TM NPs) in 100 μL PBS at room temperature for 1 h. Slight ultrasound was required for mixing thoroughly. Subsequently, 100 μL above solution was added to 900 μL DMEM culture medium and incubated with different cells at 37 °C for 3 h. The cells were washed three times with cold PBS and the nuclei were stained with Hoechst 33342 before fluorescence imaging.

For exploring the ability of nanocomplex to monitor the undulate expression level of miR-21, miR-21 mimic (micrONhsa-miR-21-5p mimic) and miR-21 inhibitor (MicrOFFhsa-miR-21-5p inhibitor) were transfected into Hela cells by using the lipofectamine 3000 transfection reagent. Subsequently, the fluorescence imaging of the cells incubated with nanoprobe was the same as the mentioned above.

Determination of Cell Viability. The Cell Counting Kit-8 (CCK-8) assay was used to determine cell viability and evaluate the cytotoxicity of KleNtB@ZIF-8/TM NPs according to the manufacturer's protocol. A549 cells were seeded in a 96-well plate (~ 8000 per well), and incubated with KleNtB@ZIF-8/TM NPs of a given concentration (90, 110, 130 μg/ml) in 100 μL of culture medium at 37 ^{o}C for different times (0, 3, 6, 9, 12, 24 h). After washing three times with cold PBS, the cells were incubated with 100 μL of DMEM fresh culture medium including 10% CCK-8 reagents for another 3 h. The absorbances were recorded at 450 nm by using an ELx800™ microplate reader.

Flow Cytometry. Cell lines (A549, Hela, L02) were seeded on 35 mm diameter cell culture dishes (~ 5×10^4 cells) and incubated at 37 °C for 24 h. These cells were processed in the corresponding way as mentioned above for the fluorescence imaging of the cells. After the incubation, the cells were washed three times with 1× PBS (pH 7.4) and treated with 100 μL of 0.25% trypsin for 1 min, and centrifuged at 1500 rpm for 3 min to collect cells. Then the cells were washed with 500 μL cold PBS three

times and redispersed in 500 μl PBS for measurement by flow cytometry assay in the FITC channel of Cyto FLEX flow cytometer (Beckman, USA).

Fluorescence Quantitative Reverse Transcription PCR assay. For the expression level of miR-21, Trizol Mini Kit was used to extract total cellular RNA from A549, Hela and L02 cell lines. cDNA samples were prepared using Maxima Reverse Transcriptase according to the protocol. The total reaction system for qPCR analysis was 20 μL including 10 μL of SybrGreen qPCR Master Mix, 0.4 μL of primer F (10μM), 0.4 μL of primer R (10μM), 7.2 μL of ddH2O and 2 μL of Template (cDNA). qPCR was performed in ABI Stepone plus, under reaction condition that an initial 95 °C for 3 min, then 95 °C for 5 s and 60 °C for 30 s for 45 cycles. U6 RNA was used as a control to quantify the relative level of miR-21. All data related to the expression of miR-21 were evaluated by normalizing the expression of U6 and using $2^{-\Delta\Delta Ct}$ method.

Table S1. DNA and RNA sequences used in this work.

Name	Sequences (5'-3')
Template	TCTAGGAAGTGATACGCTGAGGTCAACATCAGTCTGATAAGCTA
MB	**FAM**-GCTCGTCTAGGAAGTGATACGCTGACGAGC-**BHQ1**
FAM-MB	**FAM**-GCTCGTCTAGGAAGTGATACGCTGACGAGC
Cy5-MB	Cy5-GCTCGTCTAGGAAGTGATACGCTGACGAGC
Cy3-Template	Cy3-TCTAGGAAGTGATACGCTGAGGTCAACATCAGTCTGATAAGCTA
miR-21	UAGCUUAUCAGACUGAUGUUGA
miR-21s	TAGCTTATCAGACTGATGTTGA
miR-155s	TTAATGCTAATCGTGATAGGGGT
miR-26as	TTCAAGTAATCCAGGATAGGCT
miR-31s	TAGCAGCACGTAAATATTGGCG
MT1	TAGCTTATCAGACTAATGTTGA
MT2	TAGCTTATCAGACTCATCTTGA
MT3	TAGCTTTTCAGTCTGAAGTTGA
U6 forward	CTCGCTTCGGCAGCACA
U6 reverse	AACGCTTCACGAATTTGCGT
miR-21 foward	ACACTCCAGCTGGGTAGCTTATCAGACTGA
miR-21 reverse	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCAACATC

Table S2. Comparison between other miR-21 detection methods for intracellular imaging and PESAR system.

Methods	Detection limit	Application	Ref.
Hairpin-fuelled catalytic nanobeacons	67 pM	linear	S1
Au nanoparticles with EDC	8 pM	linear	S2
UCNP-based CHA	1.02 nM	linear	S3
APE1-powered DNA circuit	0.2 nM	linear	S4
RCA with phi29 polymerase	65 pM	linear	S5
DSN-powered stochastic RNA walker	1.67 fM	exponential	S6
Exonuclease III-assisted amplification	3.2 pM	exponential	S7
PESAR system	40 pM	linear	This work

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S2 C. P. Liang, P. Q. Ma, H. Liu, X. Guo, B. Yi and B. Ye, *Angew. Chem., Int. Ed.*, 2017, **56**, 9077−9081.

S3 H. Min, S. Li, P. Zhang, Y. Feng and D. Lin, *Anal. Chem.*, 2019, **91,** 3374−3381.

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S6 M. Xiao, X. Wang, L. Li and H. Pei, *Anal. Chem.*, 2019, **91**, 11253−11258.

S7 M. Xiong, M. Zhang, Q. Liu, C. Yang, Q. Xie, G. Ke, H. Meng, X. Zhang and W. Tan, *Chem. Commun*, 2020, **56**, 2091−2094

Fig. S1 TEM image of KleNtB@ZIF-8 NPs.

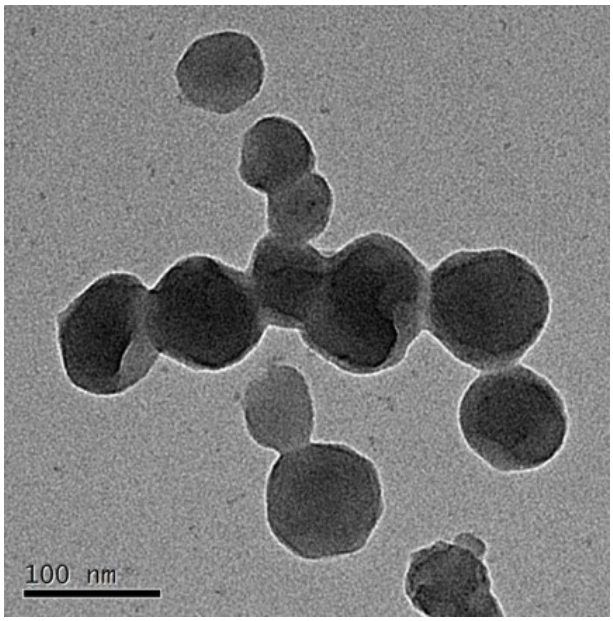


Fig. S2 XRD characterization of simulated ZIF-8, ZIF-8, KleNtB@ZIF-8 and KleNtB@ZIF-8/TM NPs.

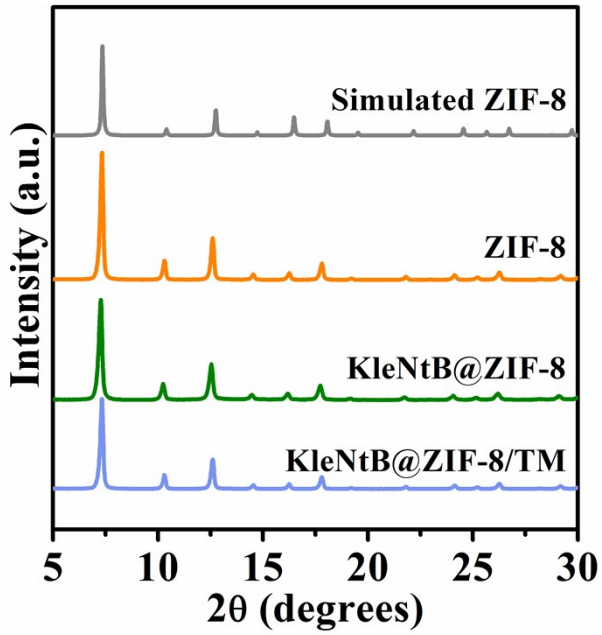


Fig. S3 DLS analysis of ZIF-8, KleNtB@ZIF-8 and KleNtB@ZIF-8/TM NPs.

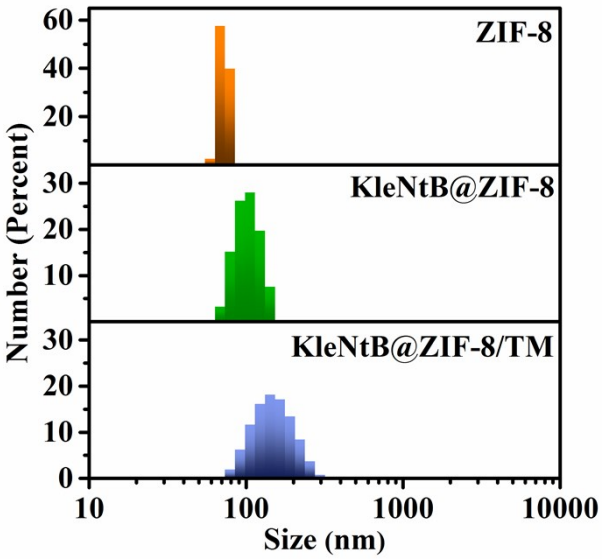


Fig. S4 Zeta potential characterization of ZIF-8, KleNtB@ZIF-8 and KleNtB@ZIF-8/TM NPs.

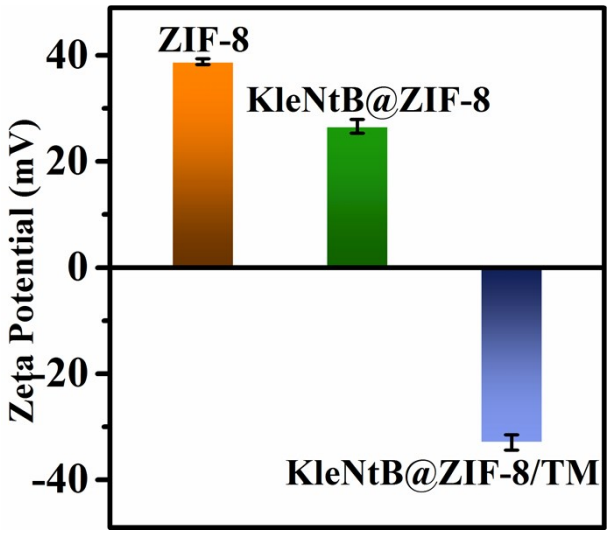


Fig. S5 FITR spectra of BSA, ZIF-8, KleNtB@ZIF-8, KleNtB@ZIF-8/TM NPs and DNA. The characteristic absorption peak of 1672 cm^{-1} in the spectra of KleNtB@ZIF-8 and KleNtB@ZIF-8/TM NPs was attributed to the amide I band (1600-1700 cm^{-1}) of encapsulated proteins. The characteristic absorption peaks at 1240 cm^{-1} and 1040 cm^{-1} corresponded to the stretching vibration of phosphodiester bond in DNA, which could be seen in the spectra of KleNtB@ZIF-8/TM NPs and DNA. The ability of ZIF-8 to encapsulate proteins and adsorb nucleic acid probes was validated.

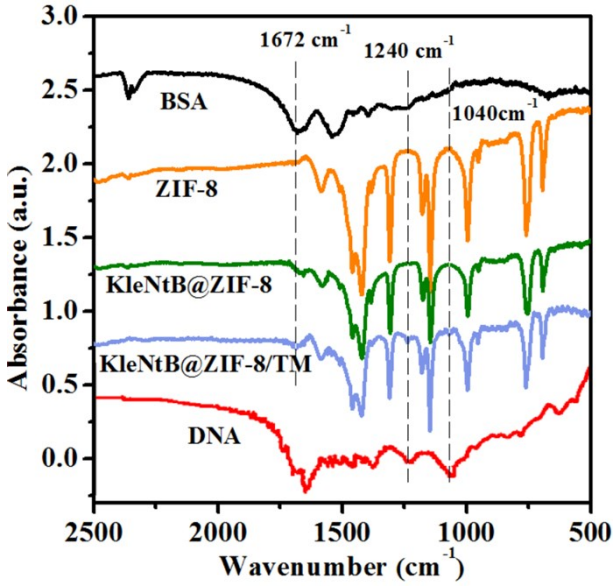


Fig. S6 UV-vis absorption spectra of protein mixture and protein mixtures released from biomineralized ZIF-8 NPs treated with 5 M hydrochloric acid.

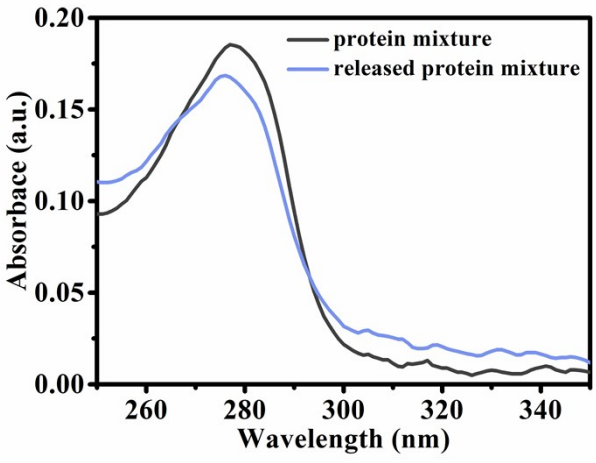


Fig. S7 Determination of the loading efficiency of the nucleic acid probe, including Cy3-labeled Template (Cy3-Tempalte), FAM-labeled MB (FITC-MB). The black curve represented the fluorescence intensity of the total probes before incubation with nanoparticles, and the red curve was the fluorescence intensity of the supernatant after incubation and centrifugation.

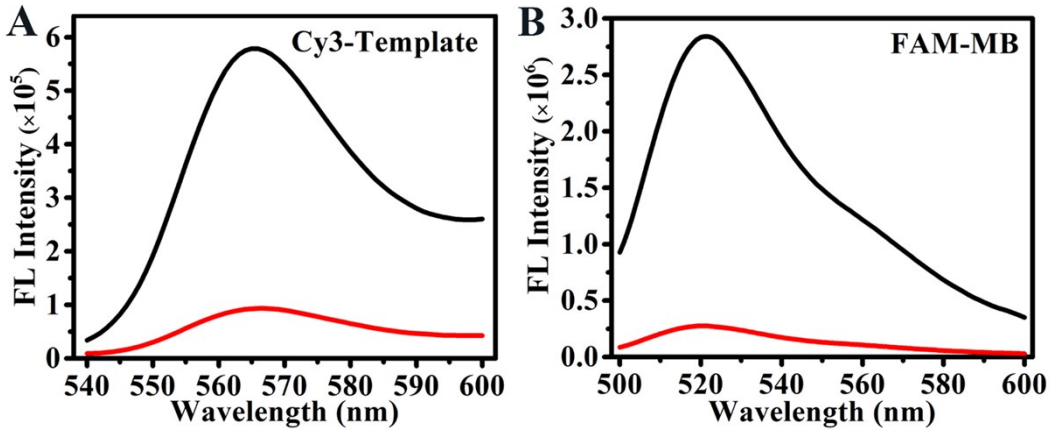


Fig. S8 The releasing kinetic curves of FITC-BSA encapsulated in biomineralized ZIF-8 NPs at pH 5.5 and 7.4 (the different time intervals were the time recorded before the centrifugation beginning). Error bars were estimated from the measurements of three parallel experiments .

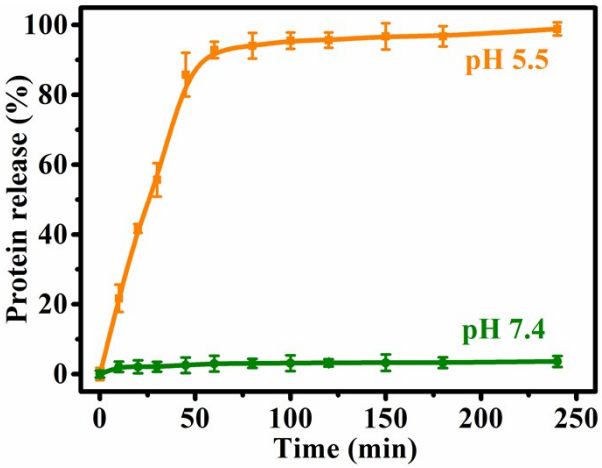


Fig. S9 Biomineralized ZIF-8 NPs can protect the encapsulated proteins from protease-mediated degradation. (A) Schematic for signal reporting principle of protein protection performance. (B) Fluorescence intensity of different PESAR systems in response to miR-21s (a-d): (a) PESAR system with free Klenow/Nt.BbvCI, (b) PESAR system with Klenow/Nt.BbvCI released from protease-treated KleNtB@ZIF-8, (c) PESAR system with protease-treated free Klenow/Nt.BbvCI, (d) PESAR system without free Klenow/Nt.BbvCI. Error bars were estimated from the measurements of five parallel experiments, ** $p < 0.01$.

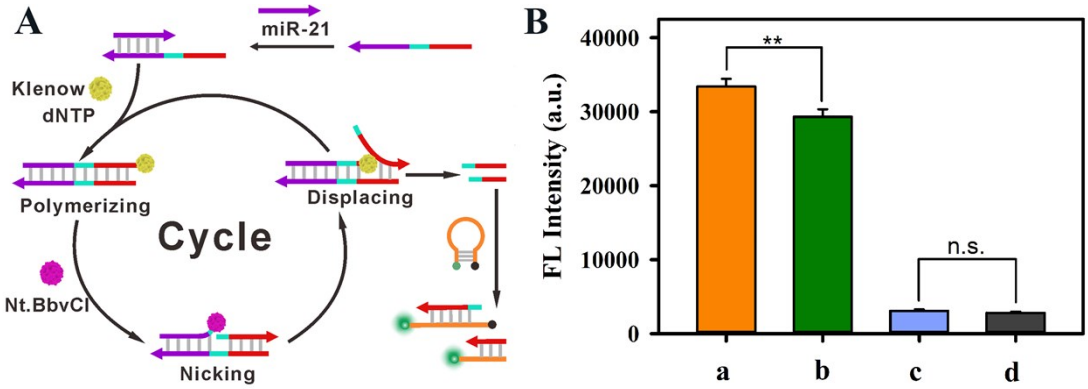


Fig. S10 Biomineralized ZIF-8 NPs can protect the absorbed nucleic acid probes against DNase I and culture medium mediated degradation. Fluorescence kinetic curves of (a) free MB treated with DNase I, (b) free MB in DMEM (10% FBS), (c) KleNtB@ZIF-8/M treated with DNase I and (d) KleNtB@ZIF-8/M treated with DNase I in DMEM (10% FBS).

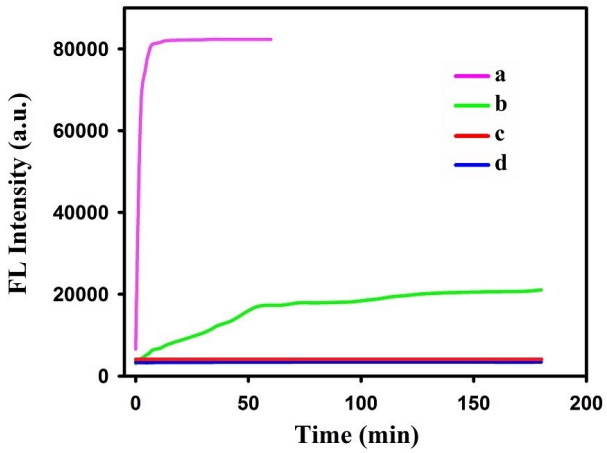


Fig. S11 Cytotoxicity assay of KleNtB@ZIF-8 NPs. Cell viability of A549 cells treated with different concentrations of KleNtB@ZIF-8/TM NPs after series of incubation time.

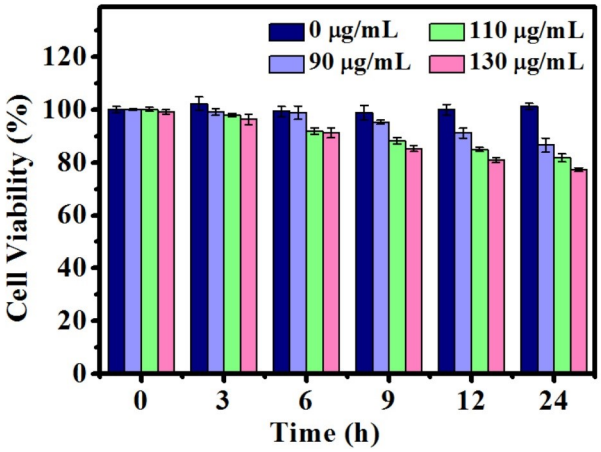


Fig. S12 Optimization of the experimental conditions. Fluorescence signal of PESAR system at different concentrations of (A) Klenow, (B) Nt.BbvCI and (C) MB. F and F_0 represent the fluorescence intensity of PESAR system obtained at the presence and absence of miR-21s, respectively. Error bars were estimated from the measurements of three parallel experiments .

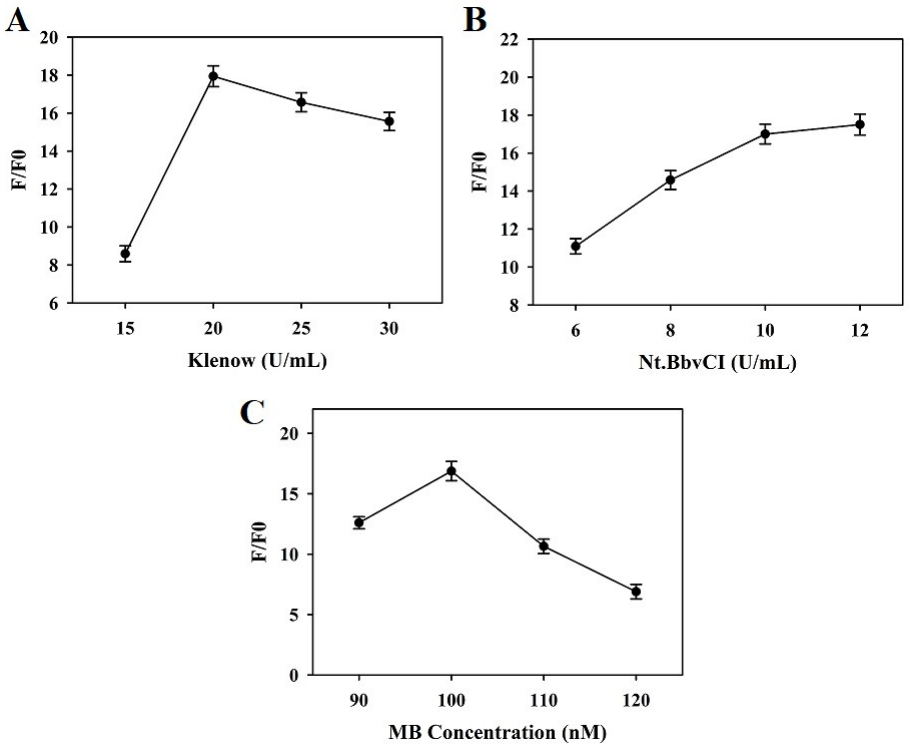


Fig. S13 Fluorescence response kinetic curves of the PESAR system obtained at the presence (red) and absence (black) of miR-21s versus the incubation time. Error bars were estimated from the measurements of three parallel experiments .

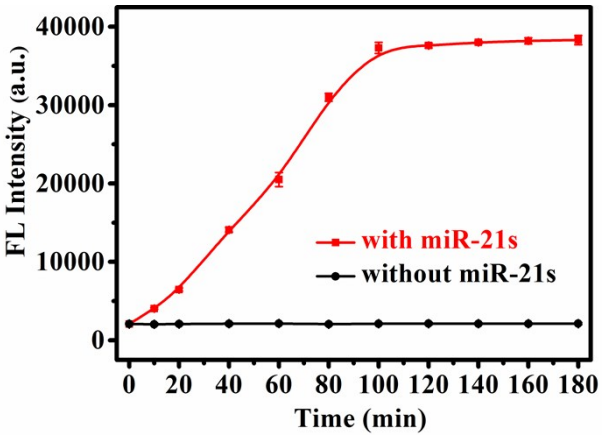


Fig. S14 Fluorescence intensity of PESAR system in response to different concentrations of miR-21s. (A) Relationship between the fluorescence intensity at 520 nm and different concentrations of miR-21s. The concentrations of miR-21s were 0, 0.01, 0.05, 0.1, 0.5, 1, 3, 5, 7, 10, 14, 17, 20 and 25nM, respectively. Inset: high-resolution relationship between the fluorescence intensity at 520 nm and concentrations of miR-21s from 0 to 1 nM. (B) linear correlation between fluorescence intensity and concentrations of miR-21s in the range of 0.1-20 nM. Error bars were estimated from the measurements of three parallel experiments.

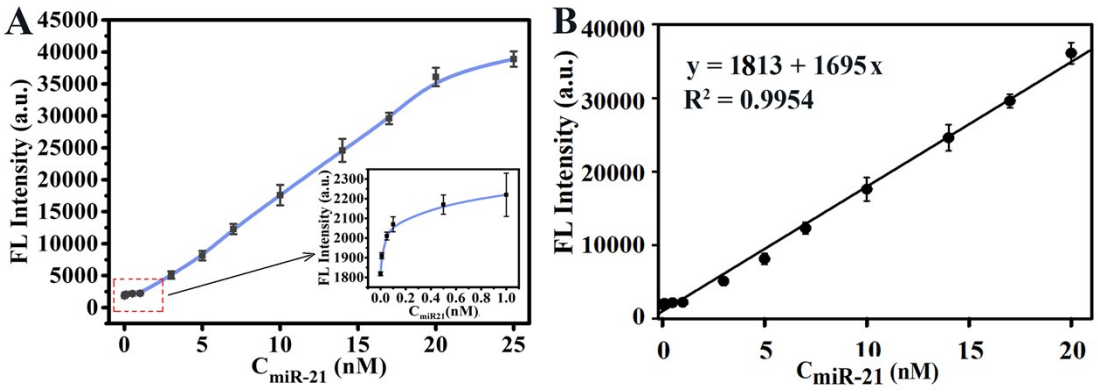


Fig. S15 Fluorescence imaging of co-delivering protein and nucleic acid probe in A549 cells, scale bar: 25 μm. A549 cells were incubated with 90 μg/mL ZIF-8 encapsulating FITC-BSA and absorbing Cy5-MB for 3 h.

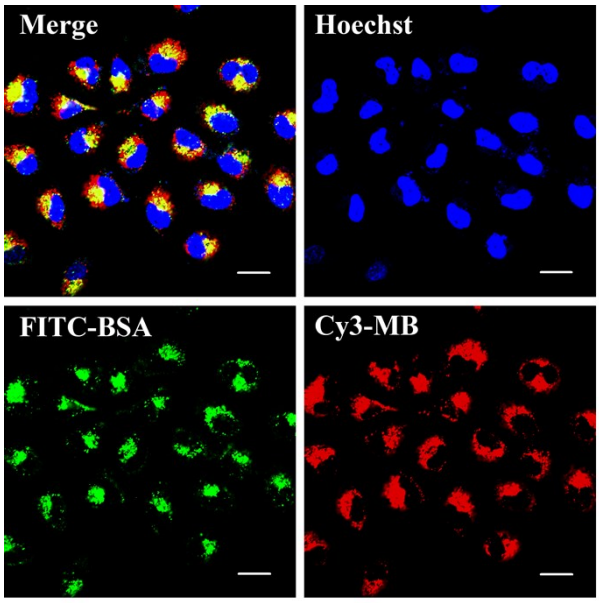


Fig. S16 CLSM images of lysosome release of biomineralized ZIF-8 NPs in A549 cells, scale bar: 25 μm.

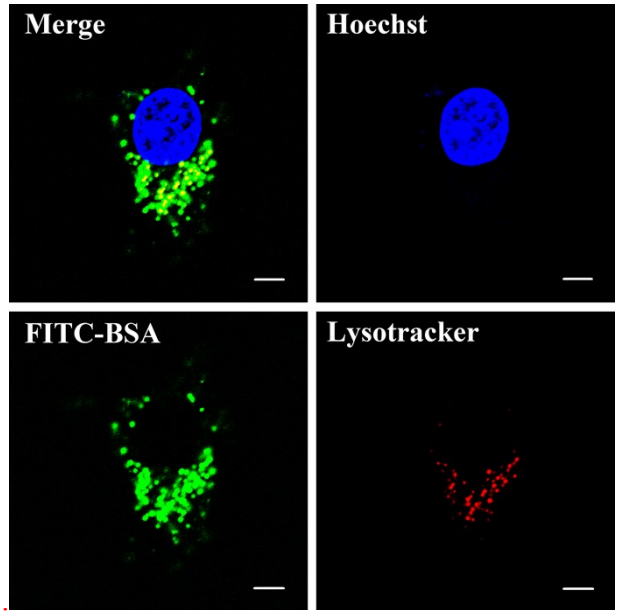


Fig. S17 The study of intracellular performance of PESAR system assisted by biomineralized ZIF-8 NPs. (A) CLSM images of A549 cells after different treatments, scale bar: 25 μm. (B) The corresponding mean fluorescence intensity in CLSM images. The fluorescence intensity was the average from six regions of interests (ROIs) inside the cells, ** $p < 0.01$. (C) The corresponding flow cytometry analysis of A549 cells treated as mentioned above. Blank was the A549 cells without any treatment.

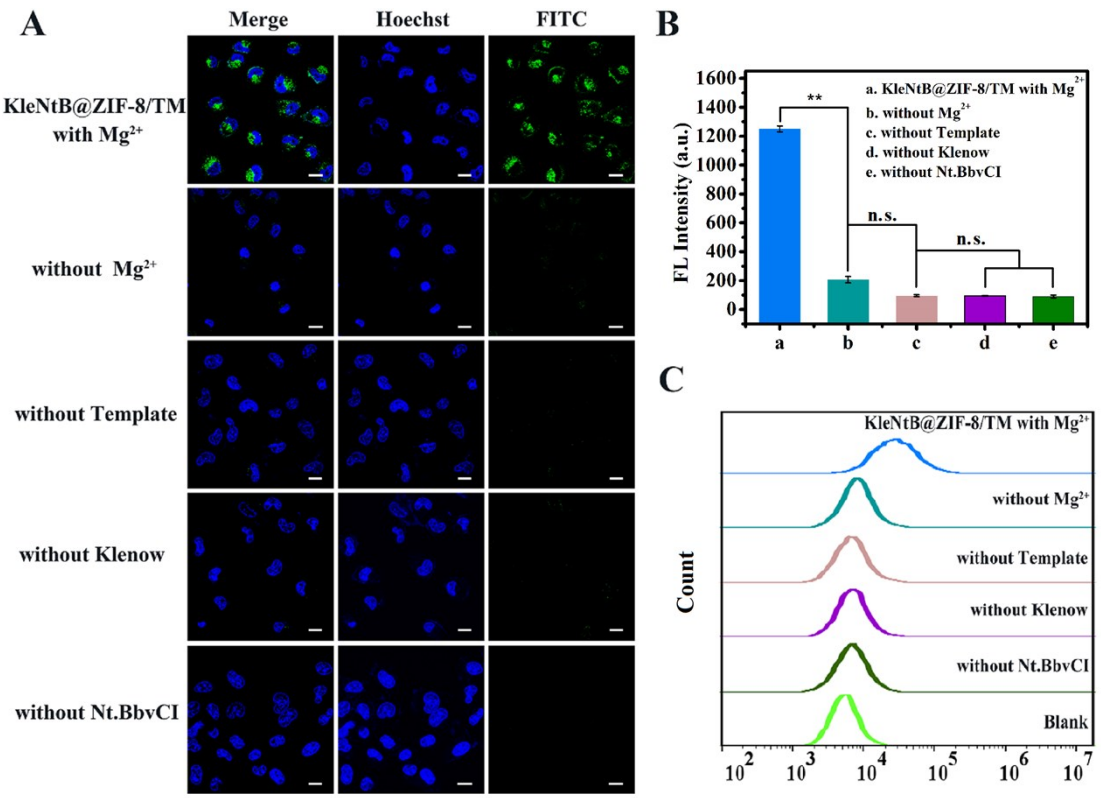
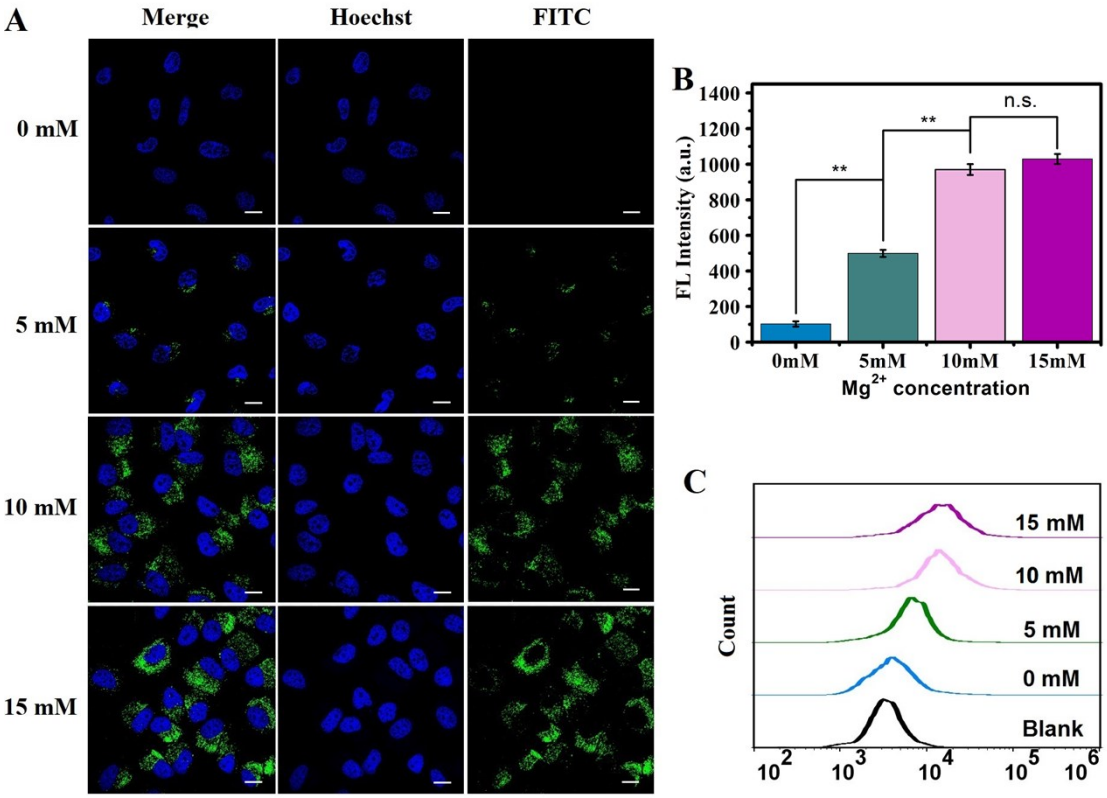


Fig. S18 The influence of Mg^{2+} concentration on the performance of the PESAR system. (A) CLSM images of A549 cells treated with different Mg^{2+} concentration, scale bar: 25 μm. (B) The corresponding mean fluorescence intensity in CLSM images. The fluorescence intensity was the average from six region of interests (ROIs) inside the cells, ** $p < 0.01$. (C) The corresponding flow cytometry analysis of A549 cells treated as mentioned above. Blank was the A549 cells without any treatment.



A549 cells were treated with increasing concentrations of Mg^{2+} (from 0 mM to 15 mM), and displayed fluorescence images with increased brightness. Moreover, the bright image of A549 cells treated with 10 mM Mg^{2+} was similar as that of A549 cells treated with 15 mM Mg^{2+}, demonstrating that the operation of PESAR system in living cells required at least 10 mM Mg^{2+}.

Fig. S19 qPCR analysis of miR-21 in different cells. (A) Real-time fluorescence curves in qPCR analysis. (B) Histograms of relative miR-21 expression levels in different cells. Error bars were estimated from the measurements of five parallel experiments, ** $p < 0.01$.

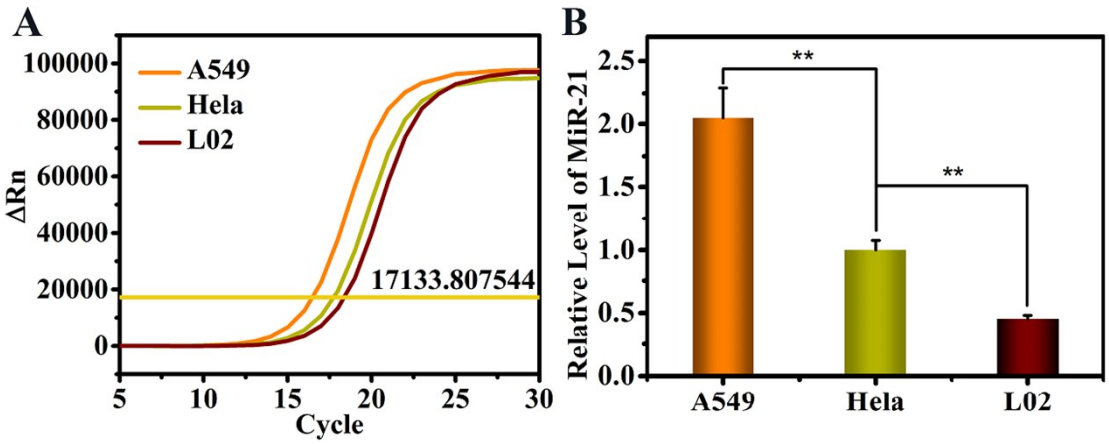


Fig. S20 qPCR analysis of miR-21 in Hela cells after treatment with different regulators. (A) Real-time fluorescence curves in qPCR analysis. (B) Histograms of relative miR-21 expression levels. Error bars were estimated from the measurements of five parallel experiments, ** $p < 0.01$.