Supporting Information

Biomineralized Metal-Organic Framework Nanoparticles Enable a Primer Exchange Reaction-Based DNA Machine to Work in Living Cells for Imaging and Gene Therapy

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Materials and Reagents. Zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$, 99%), 2-methylimidazole ($C_4H_6N_2$, 2-MIM, 99%), bisbenzimide H 33342 trihydro chloride (Hoechst 33342), and dimethylsulfoxide (DMSO) were purchased from Sigma Aldrich. Klenow (exo-) fragment polymerases (KFP) and Deoxyribonucleotides mixture (dNTPs) and were purchased from New England Biolabs (NEB, Ipswich, MA, USA). Lyso@Tracker Green was purchased from Invitrogen (Carlsbad, CA).CCK-8 reagent was purchased from Dojindo Laboratories (Kumamoto, Japan). Sepantronium bromide (YM155) was purchased from Selleck (USA). Bovine serum albumin (BSA) and fluorescein isothiocyanate (FITC) were obtained from Sangon Biological Engineering Technology (Shanghai, China). FITC-labeled BSA was produced by mixing 20 mg BSA and 2 mg FITC in l mL of 0.1 M phosphate buffer (PB, pH 8.0) at 37 °C for 4 h. To remove excess fluorescent dye, the FITC-labeled BSA was purified using dialysis against ultrapure water for 48 h and stored at 4 °C for future use. All solutions were prepared using an electric resistance of >18.2 MΩ ultrapure water, which was obtained via a Millipore Milli-Q water purification system (Billerica, MA, USA). In this work, all oligonucleotides used were HPLC Purified and synthesized by Shanghai Sangon Biological Engineering Technology and the sequences were listed in Table S1.

Apparatus. JEM-2100 transmission electron microscope was used for transmission electron microscopy (TEM) images.Dynamic light scattering (DLS) and surface charge (zeta potential) of the different nanoparticles were analyzed using a Malvern Zetasizer Nano ZS90 (Malvern, England). Wide-angle X-ray powder diffraction (XRD) patterns were conducted with the crystal phase of nanoparticles via D8-advance X-ray diffraction (Bruker, Germany) using Cu Kα radiation over the 2θ range 5-50°. Fourier transform infrared spectroscopy (FTIR) were recorded on a Bruker Vertex 70 over a KBr pellet and then scanned from 400-4000 cm^{-1} at a resolution of 1 cm^{-1}. The fluorescence spectra were measured by a FS5 fluorescence spectrometer (Hitachi, Japan).All microscopy images of cells were obtained on a Nikon ECLIPSE Ti laser scanning confocal microscope with a 60× oil dipping objective less.

Assay of Protein Encapsulation Efficiency in Protein@ZIF-8 NPs. To determine the protein encapsulation efficiency of BSA@ZIF-8 NPs, the supernatant

were collected and dissolved in hydrochloric acid to release the encapsulated BSA. And the result solutions were recovered and purified by an ultra-centrifugal filters (molecularweight cut-off: 10K, Millipore) at 7000 rpm centrifugation for 15 min and then washed with ultrapure water twice to remove free 2-MIM. The protein concentration was measured using UV-Vis adsorption spectra at 280nm. So, the absorption spectra of the supernatant solution and same amount of free BSA with ultra-centrifugal purification under same conditions were collected on a UV-2550 spectrometer (Shimadzu, Japan) with a wavelength interval of 1 nm. The encapsulation efficiency of proteins was calculated using the following formulas: the ratios of the absorbance of encapsulated proteins to the absorbance of initially added proteins for synthesis of the BSA@ZIF-8 NPs.

$$encapsulation\ efficiency = \frac{absorbance\ of\ encapsulated\ proteins}{absorbance\ of\ initially\ added\ proteins} \times 100\%$$

Protein Release from protein@ZIF-8 NPs. To research the pH-triggered protein release from ZIF-8NPs, 2.0 mg of FITC-BSA@ZIF-8NPs was dispersed in 5 mL of pH=7.4 or pH=5.5 phosphate-buffered saline at 37 °C under magneticagitation. 100 μL of FITC-BSA@ZIF-8NPs dispersion wasthen centrifuged and collected result supernatant by 15000 rpm centrifugation for 10 min at different time intervals. Then, the fluorescence intensity from the released FITC-BSA proteins in the supernatant was measured by using a FS5 fluorescence spectrophotometer. The cumulative protein release was calculated as a function of time.

Protection Analysis. The protective performance was analyzed using PER-based DNA machine cycle reaction synthesis of Bcl-2 antisense ASD (Bcl-2 ASD) to open MBs in the present of KFP, leading to a fluorescence signal. In this assay, 0.5 mL of 20 mg mL^{-1} protease mixture of trypsin/α-chymostrypsin was added to 0.5 mL of 3 mg mL^{-1} BSA+KFP@ZIF-8 NPs (the KFP concentration in NPs was ~1500 U) in phosphate buffered saline (pH 7.4) and incubated at 37 °C for 4 h. The mixture (1 mL) was centrifuged at 15000 rpm for 10 min and washed with PBS (pH 7.4)twice. Then, the obtained sediment was suspended in 0.5 mL PBS (pH 5.5) for 4 h followed by 15000 rpm centrifuging for 10 min using an ultra-centrifugal filters (molecular weight cut-off:

10K, Millipore) and washed with PBS (pH 7.4) twice.The BSA and KFP were then collected and diluted to 0.5 mL (the KFP concentration in NPs was ~3000 U/mL). Then, the PER cycle reaction was carried out as follow: A 100 μL of reaction solution was made to contain 10 μL of 10×PER reaction buffer (100 mMTris-HCl, 500 mMNaCl, 100 mM $MgCl_2$, and 10 mM DTT, pH 7.9), 1 μL above collected and dilutedsolution, 2 μL of deoxyribonucleotides mixture (dNTPs, 10 mM), 4 μL hairpin probe (HP, 0.5 μM), 4 μL primer probe (5 μM), 3 μL molecular beacon (MB, 1 μM), 72 μL sterile water and 4 μL of target survivin DNA (0.5 μM) were included to initiate the PER cycle reaction. After the mixture was incubated at 37 °C for 2 h, the fluorescence of the reaction mixture were collected at room temperature using a Hitachi FS5 fluorescence spectrometer from 650 to 720 nm under the excitation wavelength of 640 nm and the emission wavelength of 665 nm.

Experimental Section

Preparation of biomineralized nanoparticles: The biomineralized nanoparticles were determined according to our group's previously reported protocol with little modification.[1,2] Biomineralized ZIF-8 and proteins@ZIF-8 nanoparticles were prepared in a pure aqueous system. For the construction of ZIF-8 nanoparticles with caged proteins, we chose a model protein, bovine serum albumin (BSA), to start the synthesis. 0.25 mg BSA or FITC-BSA was firstly dissolved in 0.4 mL of 1.4 mmol 2-MIM aqueous solution and stirring for 30 min at 30 °C followed by adding 0.1 mL of 0.02 mmol zinc nitrate aqueous solution and stirring for 10 minat 30 °C. The resulting nanoparticles were collected by centrifugation and washed using ethanol to remove free reagents for 2 times. For primer exchange reaction (PER) cascades system, 0.25 mg of two protein mixture of Klenow Fragment (3'-5' exo-) DNA polymerase (KFP, 0.0138 mg) and BSA (0.2362 mg) was co-encapsulated in ZIF-8 nanoparticles (ZIF-8 NPs). The ZIF-8 NPs encapsulated with KFP and BSA were lyophilized and weighed to determine the amount of products acquired in above synthesis. The product amounts were around 3 mg of KFP+BSA@ZIF-8 NPs. The loading amount (LA) of KFP was calculated by the equation $LA_{(KFP)} = (c)(V)(EE)$ (%), where c is the initial concentration of KFP [4.6 mg mL^{-1} according to the manufacturer's instructions (New England BioLabs, NEB)], V is the added volume of KFP (30 μL), and EE (%) is the protein encapsulation efficiency (~93.2% determined by UV-vis absorption analysis in Figure 2E).About 0.0129 mg of KFP and 0.2201 mg of BSA were co-encapsulated in ~3 mg of ZIF-8 NPs. Those nanoparticles were dissolved in water for characterization or culture medium for cell study to a concentration of 60 μg mL^{-1} (concentrations of protein in the solution were ~4.72 μg mL^{-1} for BSA and ~30 U/mL for KFP, respectively). For pure ZIF-8 NPs, in short, 0.4 mL of 2-MIM aqueous solution (1.4 mmol) was incubated with a 0.1 mL of zinc nitrate solution (0.02 mmol) for 10 min at 30 °C. After centrifugation, washing, and resuspension, the resulting ZIF-8 NPs were also characterized.

In Vitro Response by primer exchange reaction (PER)-based DNA machine: Every 100 μL of reaction solution prepared contained 10 μL of 10 × NEBuffer 2 (100 mM

Tris-HCl, 500 mM NaCl, 100 mM $MgCl_2$, and 10 mM DTT, pH 7.9), 1 μL Klenow Fragment (3'-5' exo$^-$) DNA polymerase (KFP, 2500 U mL^{-1}), 2 μL of deoxyribonucleotides mixture (dNTPs, 10 mM), 4 μL hairpin probe (HP, 0.5 μM), 4 μL primer probe (5 μM), 3 μL molecular beacon (MB, 1 μM), 72 μL sterile water and 4 μL of a series of different concentrations of target survivin DNA were added followed by performance at 37 °C for 2 h. The fluorescence intensity (FL) of above solutions was recorded using a Hitachi FS5 fluorescence spectrometer from 650 to 720 nm ($\lambda_{ex}/\lambda_{em}$, 640/667). PER products in 10 μL of 1 × gel loading buffer were separated by native 20% Native polyacrylamide gel electrophoresis (PAGE). To demonstrate the selectivity of PER-based machine, single-base mismatched (MM1), double-base mismatched (MM2), and three-base mismatched (MM3) survivin mRNA targets, other cancer marker such as TK1 mRNA, c-myc mRNA and GalNAc-T mRNA were added instead of the fully complementary mRNA target. The fluorescence signal of fully complementary target mRNA showed a dramatic enhancement in contrast to single-, double-, or three-mismatched and other cancer marker mRNA target, which suggested the target mRNA specificity of PER-based DNA machine.

Cells Culture: The MCF-7, HeLa, and L02 cell lines were provided from the cell bank of Central Laboratory at Xiangya Hospital (Changsha, China) and maintained in the RPMI 1640 cell-specific medium containing fetal bovine serum (FBS, 10%), streptomycin (100 U mL^{-1}), and penicillin (100 U mL^{-1}). All cells were cultured at 37 °C in a humidified incubator in the present of 5% CO_2.

Intracellular co-delivery study of proteins@ZIF-8/DNA probes: To verify that the ZIF-8 NPs encapsulation had able to co-deliver proteins and DNA probes into living cells, fluorescein (FITC)-labeled BSA (FITC-BSA) was chosen as a model functional protein. We prepared the NPs encapsulated with FITC-BSA, which was mixed with Cy5-labeled MB (Cy5-MB) for 60 min to prepare nanoparticles as FITC-BSA@ZIF-8/Cy5-MB NPs. Then, the resulting nanoparticles (around 60 μg/mL) were dispersed in culture medium supplemented with 10% FBS and incubated with HeLa cells at 37 °C for 3 h. Finally, HeLa cells were incubated with 10 μg/mL Hoechst 33342 for 10 min at 37 °C followed by fluorescence imaging.

Cellular distribution study of proteins@ZIF-8/DNA probes: to investigate the intracellular localization of proteins@ZIF-8/DNA probe. HeLa cells were treated with BSA+KFP@ZIF-8/Cy5-MB NPs at 37 °C for 3 h at a final concentration of 60 μg/mL. At the end of incubation, the lysosomes and nuclei were treated with 100 nM green Lyso@tracker for 25 min and 10 μg/mL Hoechst 33342 for 10 min, respectively before imaging on confocal laser scanning microscopy (CLSM) on NIKON TI-E+A1 SI.

Confocal imaging of survivin mRNA by PER-based DNA machine: different cell lines were seeded and grown at a density of 80% in glass bottom cell culture dish for 24 h before experiment. For PER PER-based DNA machine reaction in living cells, firstly, in a 100 μL solution containing 20 μL of BSA+KFP@ZIF-8 nanoparticles (6 mg mL^{-1}), 4 μL HP (5 μM), 4 μL primer probe (50 μM) and 6 μL molecular beacon (MB, 5 μM), which was mixed for 60 min at room temperature to prepare nanoparticles denoted as BSA+KFP@ZIF-8/HP+primer+MB NPs. KFP required Mg^{2+} for activity, so cell lines were pre-treated with PBS including 10 mM Mg^{2+} and 5 μM calcimycin for 30 min at 37 °C. Subsequently, 100 μL of BSA+KFP@ZIF-8/HP+primer+MB NPs and 20 μL of dNTPs (10 mM) were added to 880 μL of RPMI 1640 culture medium and PER was performed at 37 °C for 3 h. Then, the cells were washed three times with cold PBS (pH 7.4) and incubated with 10 μg mL^{-1} Hoechst 33342 for cell nuclei counterstain for 10 min. Finally, fluorescence images of MCF-7, HeLa, and L02 cells were recorded by CLSM.

The suppression experiments were performed, where survivin mRNA expression was downregulated by YM155. After HeLa cells with 1 mL culture medium plated on culture dish 6 h, another 1 mL culture medium containing a given volume of YM155 (10 μM) was added. Then, the incubation continued overnight, the cells were washed and treated with prepared nanoparticles followed by incubation at 37 °C for 3 h. Cells were washed by cold PBS two times before CLSM.

Flow cytometry assay: the cell lines were seeded in culture dish for 24 h prior to experiment. After pre-treated with PBS including 10 mM Mg^{2+} and 5 μM calcimycin for 30 min at 37 °C, those cells were treated with the BSA+KFP@ZIF-8/HP+primer+MB NPs or the control reagents under the given conditions for 3 h and

then washed with cold PBS for three times. After treatment with 100 μL of 0.25 % trypsin for 1 min and centrifuged at 1800 rpm for 2 min, the cells were suspended in 0.5 mL PBS and measured by flow cytometry assay on a CytoFLEX™ flow cytometer.

Cytotoxicity assay of BSA+KFP@ZIF-8 NPs: the Cell Counting Kit-8 (CCK-8) assay was employed to evaluate the cytotoxicity of BSA+KFP@ZIF-8 NPs according to the manufacturer's protocol. Hela cells (~8000) were inoculated into a 96-well plate and cultured in 200 μL RPMI 1640 medium for 24 h prior to the delivery of proposed nanoparticles. After washing there with PBS buffer, the HeLa cells were incubated with BSA+KFP@ZIF-8 NPs (60, 80, 100 μg/mL) in culture medium at 37 ºC for different time. Finally, HeLa cells were cultured in 100 μL of RPMI 1640 fresh culture medium with 10 μL CCK-8 reagents for the cytotoxicity assay. After another 3 h incubation at 37 ºC, cell viability was recorded by measuring the absorption at 450 on an Elx800™ microplate reader. No treated cells are as a blank.

Intracellular Oxidative Stress Study: the intracellulargeneration of ROS was observed on a CLSM; HeLa cellswere seeded into plates with glass well bottom overnight. Then, the culture medium was replaced with freshmedium containing BSA+KFP@ZIF-8 NPs (60, 80, 100 μg/mL), respectively, and incubated at 37 °C in 5% CO_2 for another 3 hfollowed by another fresh culture medium and further sustained for 48 h incubation. Then, the cells were treated with FBS-free medium containing 10 μM of 2,7-dichlorofluorescein diacetate (DCFH-DA) at 37 °C in 5% $CO_2$for 20 min. The cells were washed with PBS three times, and the production of reactive oxygen species (ROS) was evaluatedby measuring the fluorescence of dichlorofluorescin (DCF) (λ_{ex} = 488 nm, λ_{em} = 525 nm)with a CLSM.

DNA damage: DNA damage was studied using comet assay. After treatment with BSA+KFP@ZIF-8 NPs (60, 80, 100 μg/mL) for 3 hfollowed by another fresh culture medium and further sustained for 48 h incubation, the cells were rinsed with ice-cold 1×PBS and trypsinized. Then the cells were washed once in ice-cold 1×PBS and resuspended at 1×10^5 cells mL^{-1} in ice-cold 1×PBS. 10 μL cell suspension was mixed with 100 μL molten agarose (37°C), and 75 μL of this mixture was immediately applied to a glass slide. The slide was held horizontal at 4 °C for 30 min to improve adherence.

Then the slide was immersed in cold lysis solution to lyse the cells. After 50 min at 4 °C in the dark, the slide was immersed in an alkaline solution (300 mM NaOH, 1 mM EDTA, pH>13) at room temperature in the dark to denature the DNA. After 30 min, the slide was placed on a horizontal electrophoresis unit and the unit was filled with fresh buffer (300 mM NaOH, 1 mM EDTA, pH>13) to cover the slide. Electrophoresis was conducted at 27 V (300 mA) for 40 min at 4°C in the dark. The slide was then washed gently with distilled water and immersed in 70% ethanol for 5 min. After the slide was air dried, 50 μL of Ethidium bromide (EB) working solution was applied to each circle of dried agarose. Slides were viewed using an epifluorescence Leica DMI 4000B microscope equipped with a fluorescein filter. Observations were made at a final magnification of 400×. Fifty randomly selected cells per experimental point were imaged and analyzed using CASP software (download from http://www.casp.of.pl/). Results were reported as tail moment, a parameter describing the number of migrated fragments, represented by the fluorescence intensity in the tail.

Cell apoptosis assay: Apoptosis analysis was determined using the Alexa Fluor 488-conjugated Annexin V (Annexin V) and propidium iodide (PI) detection kit according to the manufacturer's protocol. Briefly, after pre-treated with PBS iucluding 10 mM Mg^{2+} and 5 μM calcimycin for 30 min, the cells were incubated with BSA+KFP@ZIF-8 NPs (60 μg mL^{-1}), BSA@ZIF-8/HP+primer NPs (60 μg mL^{-1}), BSA+KFP@ZIF-8/primer NPs (60 μg mL^{-1}) and BSA+KFP@ZIF-8/HP NPs (60 μg mL^{-1}), respectively at 37ºC for 3 h. Afterward, the culture medium was replaced with fresh culture medium and further incubated for 48 h, the treated cells were stained with 5.0 μL Alexa Fluor 488-conjugated Annexin V, 5.0 μL propidium iodide (PI) and 10 μg mL^{-1} Hoechst 33342 at room temperature followed by CLSM imaging. In addition, the treated cells were harvested and then suspended in Annexin V binding buffer including of 5.0 μL Alexa Fluor 488-conjugated Annexin V and 5.0 μL propidium iodide (PI) for 15 min. The samples also performed on a flow cytometer to evaluate the amount of cells with green fluorescence (Alexa Fluor 488) or propidium iodide (PI) for quantitative determination of apoptosis. The apoptosis efficacy was further studied through CCK-8 assay. After different nanoparticles treatments for 3 h followed by

replacement of media for 48 hours, the cell culture medium was replaced with fresh medium containing 10% CCK-8 reagents. After cultured for 3 h, the cells were washed with PBS and measured the absorption at 450 on an Elx800™ microplate reader.

Western Blot: western blotting analysis was experimented. Briefly, after pre-treated with PBS including 10 mM Mg^{2+} and 5 μM calcimycin for 30 min, the cells were incubated in culture medium containing BSA+KFP@ZIF-8 NPs (60 μg mL^{-1}), BSA@ZIF-8/HP+primer NPs (60 μg mL^{-1}), BSA+KFP@ZIF-8/primer NPs (60 μg mL^{-1}) and BSA+KFP@ZIF-8/HP NPs (60 μg mL^{-1}),respectively at 37 ºC for 3 h. After 3 h incubation, the culture medium was replaced by another fresh culture medium and further sustained for 48 h incubation. Next, cells were lysed in 60 μL RIPA lysis buffer for 10 min and the total proteins were harvested by centrifugation for 30 min at 4 ºC. All cellular proteins were separated using sodium dodecyl sulfate-PAGE (SDS-PAGE) and followed transferring onto polyvinylidene difluoride (PVDF) membranes. Primary monoclonal antibodies and HRP-conjugated secondary antibodies were then added and incubated with the PVDF membranes. The protein expressions were detected and analyzed by a ChemiDoc XRS+ system with image Lab software (Bio-RAD).

Immunofluorescence assays: The cells were seeded on 35 mm confocal dish and allowed to adhere for 24 h. Then, cells were cultured with PBS including 10 mM Mg^{2+} and 5 μM calcimycin for 30 min and treated with BSA+KFP@ZIF-8 NPs (60 μg mL^{-1}), BSA@ZIF-8/HP+primer NPs (60 μg mL^{-1}), BSA+KFP@ZIF-8/primer NPs (60 μg mL^{-1}) and BSA+KFP@ZIF-8/HP NPs (60 μg mL^{-1}),respectively. After 3 h incubation, the culture medium was replaced with another fresh culture medium (2 mL). After 48 hour treatment, the cells were fixed in 4% paraformaldehyde for 16 min at room temperature, followed by permeabilization with 0.2% Triton X-100 for 3 minutes. Then, cells were blocked for nonspecific binding with 5% BSA for 40 min, and incubated with Bcl-2 (1:150) antibodies overnight at 4 ºC. After washing with PBS, the cells were incubated with CruzFluor™ 488-conjugated secondary antibodies (mouse anti-goat IgG-CFL 488, 1:50) for 1 h at room temperature. Prior to imaging, the cells were treated with 10 μg mL^{-1} Hoechst 33342 for 10 min at room temperature. Immunofluorescence images were collected on NIKON TI-E+A1 SI confocal

microscope. For each channel, all images were obtained with the same settings.

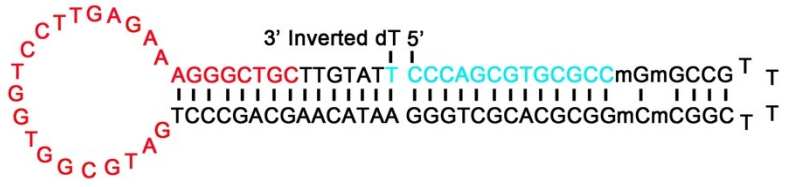
Quantitative reverse transcription-PCR (qRT-PCR) analysis: For Bcl-2 mRNA expression, total cellular RNA was extracted from HeLa and L02 cultivated with BSA+KFP@ZIF-8 NPs (60 μg mL^{-1}), BSA@ZIF-8/HP+primer NPs (60 μg mL^{-1}), BSA+KFP@ZIF-8/primer NPs (60 μg mL^{-1}) and BSA+KFP@ZIF-8/HP NPs (60 μg mL^{-1}),respectivelyusing the Trizol Mini Kit according to its manual. The cDNA samples were prepared with a Maxima Reverse Transcriptase kit (Thermo Scientific) according to the indicated protocol. The cDNA samples were diluted 10 times before use. qPCR analysis of cDNA was performed with SybrGreen qPCR Master Mix (BBI) on an ABI Stepone Plus qPCR instrument. A total volume of 20 μL reaction solution contained 10 μL SybrGreen qPCR Master Mix, 2 μL template (cDNA), 0.4 μL of forward transcription primer (10 μM), 0.4 μL of reverse transcription primer (10 μM) and 7.2 μL ddH_2O. The PCR conditions were as follows: an initial 95 °C for 3 min followed by 45 cycles of 95 °C for 5 s and 60 °C for 30 s. The endogenous β-actin RNA was used as control for quantifying the relative level of Bcl-2 mRNA. The primers used as follow: Bcl-2 forward, 5'-ACT CCT CTT CTT TCT CTG GGG G-3'; Bcl-2 reverse, 5'-CCT CTG CGA CAG CTT ATA ATG G-3'; β-actin forward, 5'-TAG TTG CGT TAC ACC CTT TCT TG-3'; β-actin reverse, 5'-TCA CCT TCA CCG TTC CAG TTT-3'. We evaluated all the data with respect to the mRNA expression by normalizing to the expression of actin and using the $2^{-\Delta\Delta Ct}$ method.

For survivin mRNA expression, total cellular RNA was extracted from MCF-7, HeLa, L02 or HeLa cells treated with YM155. All subsequent steps were the same as qPCR analysis of survivin mRNA expression. The primers used as follow: survivin forward, 5'-GCC ATT AAC CGC CAG ATT T-3'; survivin reverse, 5'-CCT CTG CGA CAG CTT ATA ATG G-3'; β-actin forward, 5'-AGT GGA TGA AGC CAG CCT C-3'; β-actin reverse, 5'-TCA CCT TCA CCG TTC CAG TTT-3'. We evaluated also all the data with respect to the mRNA expression by normalizing to the expression of actin and using the $2^{-\Delta\Delta Ct}$ method.

Table S1. The sequences are listed below as text sequences annotated with segment

Name	Sequence (5'-3')
hairpin (HP)	CCCAGCGTGCGCC**mGmG**CCGTTTTCGG**mCmC**GGCGCACG CTGGGAATACAAGCAGCCCTGATGCGGTGGTCCTTGAGAA AGGGCTGCTTGTATT-Inverted dT
$Hairpin_{\beta\text{-actin}}$ ($HP_{\beta\text{-actin}}$)	CCCAGCGTGCGCC**mGmG**CCGTTTTCGG**mCmC**GGCGCACG CTGGGAATACAATGACTACCCTCGGTGAGGATCTTCATGA GGTAGTCATTGTATT-Inverted dT
$Hairpin_{c\text{-myc}}$ ($HP_{c\text{-myc}}$)	CCCAGCGTGCGCC**mGmG**CCGTTTTCGG**mCmC**GGCGCACG CTGGGAATACAACCTCAACGGTTCCTGTTGGTGAAGCTAA CGTTGAGGTTGTATT-Inverted dT
primer	TTTTTTTTTTTTTTTTGCTTGTA
$primer_{\beta\text{-actin}}$	TTTTTTTTTTTTTTTTCATTGTA
$Primer_{c\text{-myc}}$	TTTTTTTTTTTTTTTTCCTTGTA
molecular beacon (MB)	Cy5-GGCGCACGCTGGGAATTTATTGCGCC-BHQ2
survivin DNA	GCAGCCCTTTCTCAAGGACCACCGCATCTCTAC
HP-FAM	CCCAGCGTGCGCC**mGmG**CCGTTTTCGG**mCmC**GGCGCACG CTGGGAATACAAGCAGCCCTGATGCGGTGGTCCTTGAGAA AGGGCTGCTTGTATT-FAM
Cy3-Pirmer	Cy3-TTTTTTTTTTTTTTTTGCTTGTA
Cy5-MB	Cy5-GGCGCACGCTGGGAATTTATTGCGCC
TK1 mRNA	CAGTACAAGTGCCTGGTGATCAAGTATGCCAAA
c-myc mRNA	CCTCAACGTTAGCTTCACCAACAGGAACTATGA
GalNAc-T mRNA	GCTTTCACTATCCGCATAAGACACCCGCCCAAC
single-base mismatched	GCAGCACTTTCTCAAGGACCACCGCATCTCTAC
two-base mismatched	GCAGCACTTTCTTAAGGACCACCGCATCTCTAC
three-base mismatched	GCAGCACTTTCTTAAGGACAACCGCATCTCTAC

The design of dumbbell shaped hairpin, as follow:



-Red sequences stand for hybridization strand survivin mRNA.

-Blue sequences stand for Bcl-2 antisense ASD.

-mG, mC stand for 2'-O-methyl G and 2'-O-methyl C.

-dT stand for Inverted dT.

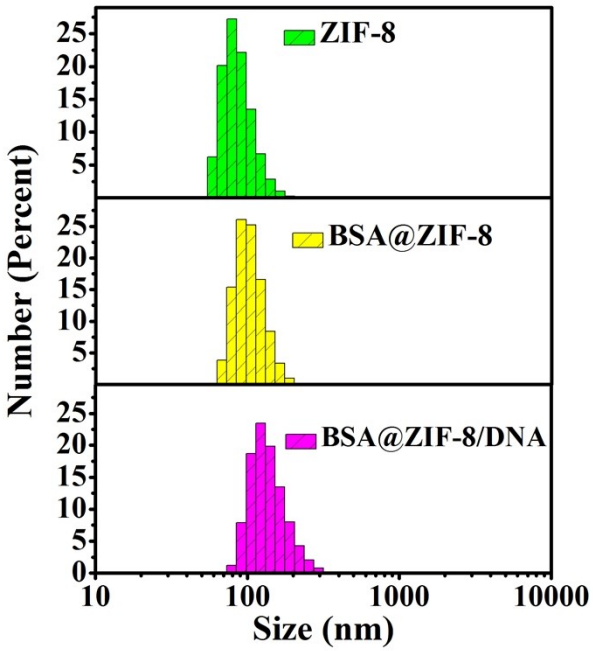


Figure S1. Dynamic light scattering (DLS) analysis of the ZIF-8 NPs, BSA@ZIF-8 NPs and BSA@ZIF-8/DNA NPs. The average hydrodynamic diameters of ZIF-8 NPs, BSA@ZIF-8 NPs and BSA@ZIF-8/DNA NPs were 80.47±19.25, 108.6±18.59 and 161.9±25.69 nm, respectively.

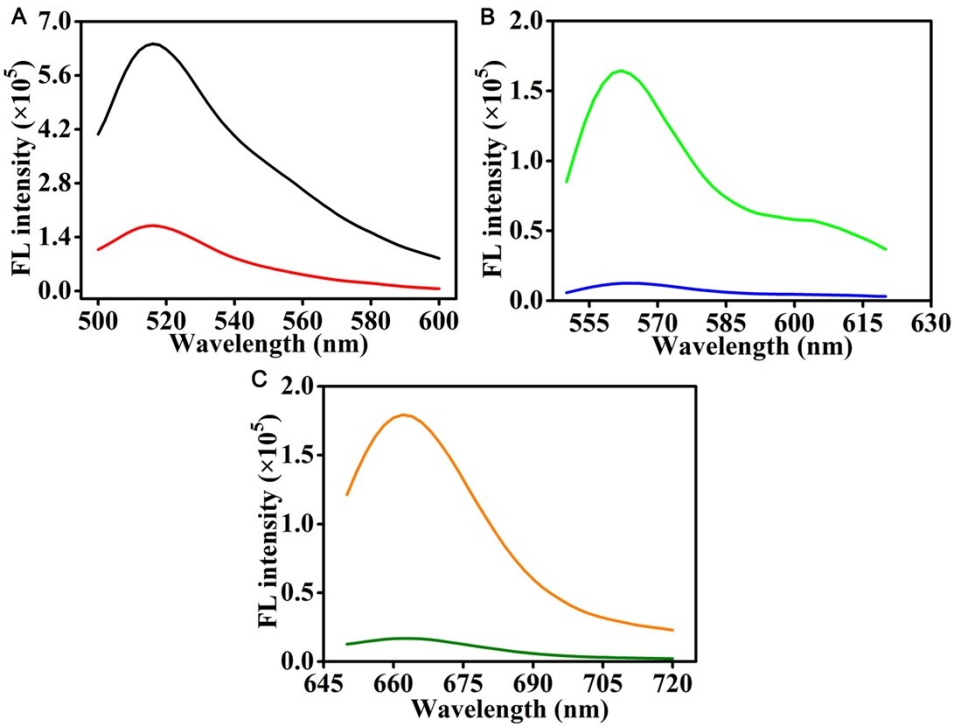


Figure S2. Determination of the loading efficiencyof the nucleic acid probe including FAM-labeled hairpin (FAM-HP, 200 nM), Cy3-labeled primer (Cy3-primer, 1 μM) and Cy5-labeled molecular beacons (Cy5-MB, 300 nM), whichwere added to the BSA+KFP@ZIF-8 nanoparticles (final concentration of 6 mg mL^{-1}) and incubated for 60 min at room temperature. After that, the nanoparticles were centrifuged, and the fluorescence intensities of the FAM-HP probes (black curve) and the supernatant (red curve) were determined (Figure S2A). The fluorescence intensities of the Cy3-primer probes (green curve) and the supernatant (blue curve) were also determined (Figure S2B). The fluorescence intensities of the Cy5-MB probes (orange curve) and the supernatant (dark blue curve) were also determined (Figure S2C).The loading efficiencies of the FAM-HP probes, Cy3-primer probes and the Cy5-MB probes were calculated to be 73.5±2.5%, 90.5±3.5% and 92.4±4.8%, respectively, corresponding to the loading capacity of ~2.46×10^{-10} mol FAM-HP, ~3.02×10^{-9} mol Cy3-primer and ~4.62×10^{-10} mol Cy5-MB probes per mg BSA@ZIF-8/DNA NPs.

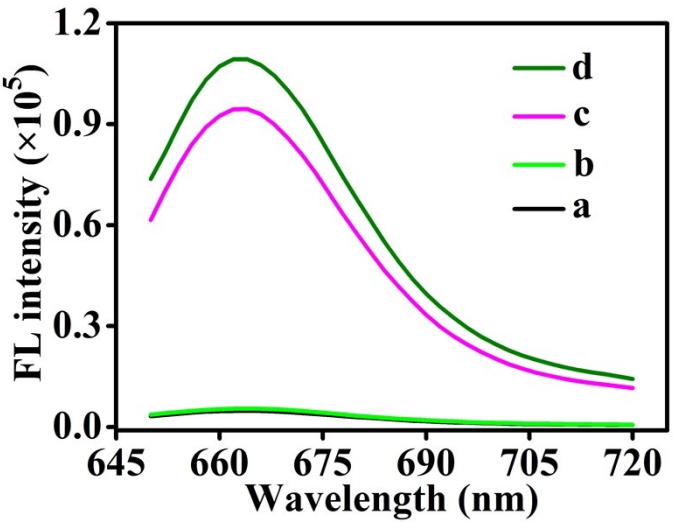


Figure S3. ZIF-8 NPs can protect the encapsulated proteins from protease-mediated degradation. (a) Primer exchange reaction (PER) in the absence of KFP; (b) PERcycle reactionin the present of protease-treatedKFP; (c) PERcycle reaction in the present of KFP released from protease-treated BSA+KFP@ZIF-8 NPs; (d) PERcycle reaction in the present of KFP.

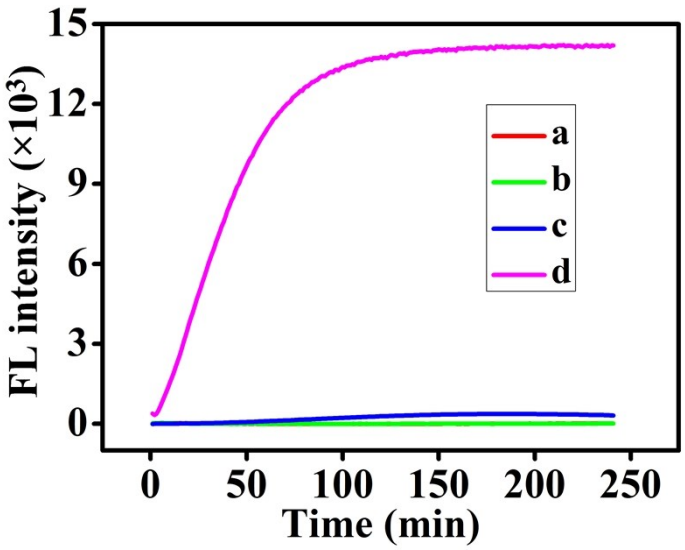


Figure S4. ZIF-8 NPs can protect the adsorbed DNA probes from the digestion by DNase I and RPMI 1640 (10% FBS). The change of the fluorescent intensity with time of the free MB probes labeled with Cy5 and BHQ2 and BSA+KFP@ZIF-8/MB NPs after addition of DNase I or RPMI 1640 (10% FBS): (a) BSA+KFP@ZIF-8/MB NPs+DNase I in buffer; (b) BSA+KFP@ZIF-8/MBNPs+RPMI 1640 (10% FBS); (c) MB+RPMI 1640 (10% FBS); (d) MB+DNase I in buffer.

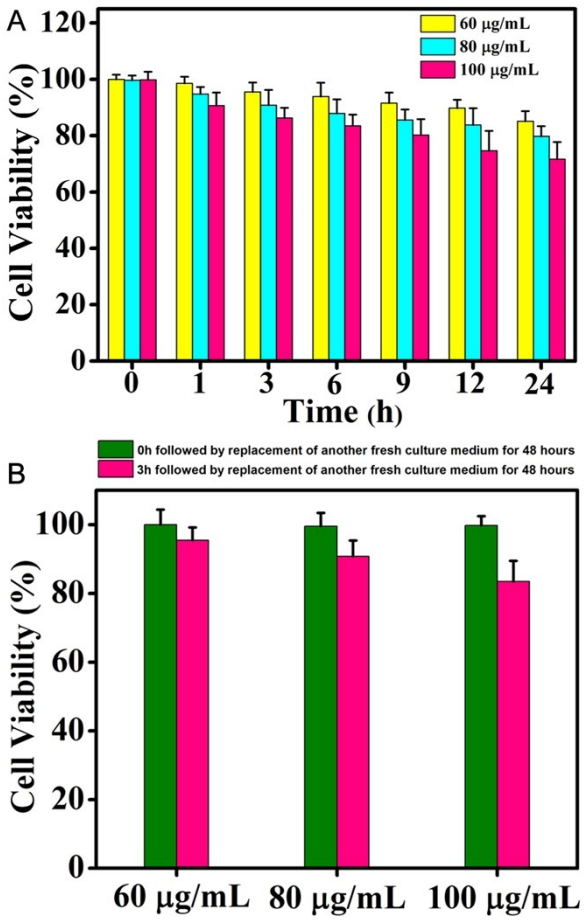


Figure S5. Cytotoxicity assay of BSA+KFP@ZIF-8 NPs. (A) The cell viability values (%) are determined by incubated HeLa cells with BSA+KFP@ZIF-8 NPs of varying concentrations (60, 80, 100 μg mL^{-1}) for 0-24h. (B) An additional cytotoxicity experiment using CCK-8 assays. The HeLa cells were treated with BSA+KFP@ZIF-8 NPs (60, 80 and 100 μg mL^{-1}) at 37 ºC for 3 h followed by replacement of another fresh culture medium for 48 hours.

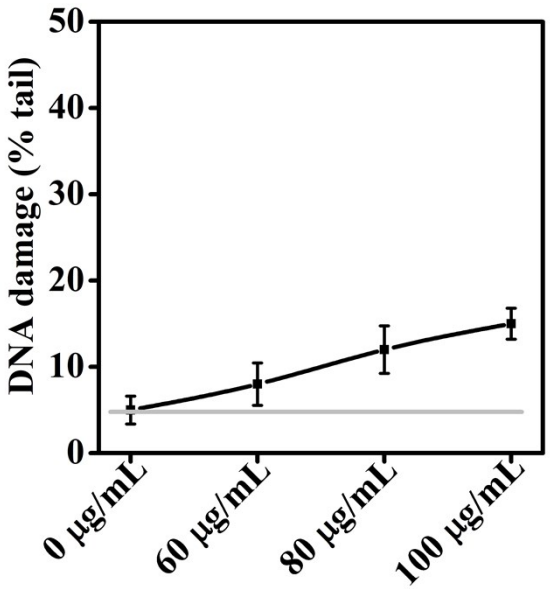


Figure S6. DNA damage in cultured HeLa cells after exposure to BSA+KFP@ZIF-8 NPs (0, 60, 80 and 100 μg mL^{-1}) at 37 ºC for 3 h followed by replacement of another fresh culture medium for 48 hours. DNA damage was measured as DNA percent in tail by the cometassay. The data demonstrated that 60 μg mL^{-1} of BSA+KFP@ZIF-8 working concentration had relatively little effect on living cells.

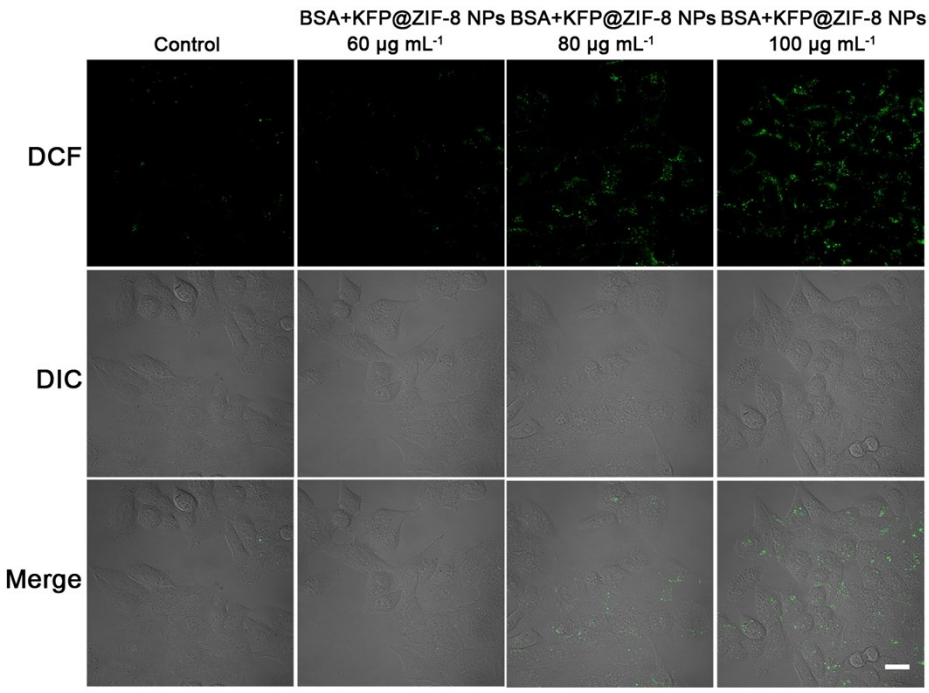


Figure S7. ROS assay in HeLa cells after incubation with different concentrations of BSA+KFP@ZIF-8 NPs. The data demonstrated that 60 μg mL^{-1} of BSA+KFP@ZIF-8 working concentration had relatively little effect on living cells. Scale bar: 20 μm.

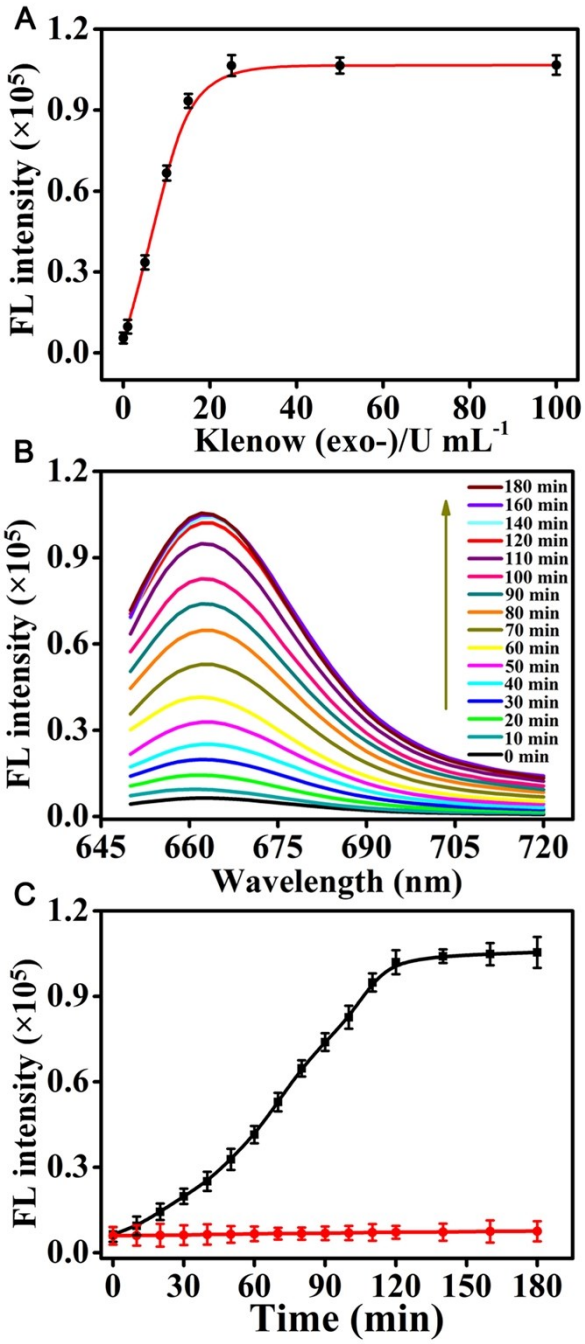


Figure S8. Optimization of the experimental conditions. (A) The effect of Klenow (exo-) fragment polymerases (KFP) concentration on the PER reaction system. (B) Fluorescence emission spectra of PER reaction systemin thepresence of survivin DNAat a series of different incubation time. (C) Plot of fluorescence peak intensities obtained in (B) in the presence (black) and absence (red) of target survivin DNA versus the incubation time. Error bars were estimated from three replicate measurements.

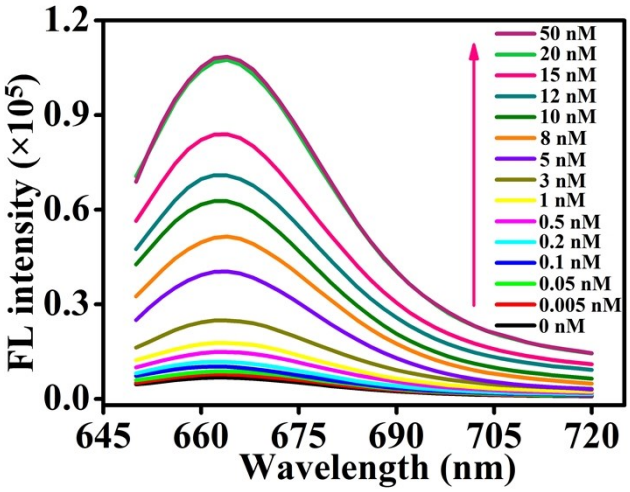


Figure S9. Fluorescence spectra of the PER based-DNA machine reaction system after incubation with varying concentrations of the survivin DNA target for 2 h.

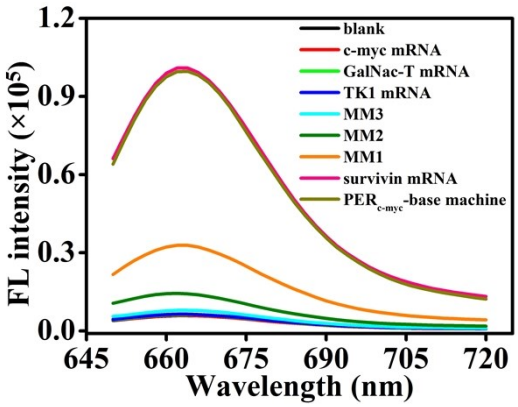


Figure S10. Fluorescence spectra of the PER-based DNA machine responsive to various different DNA molecules for 2 h.

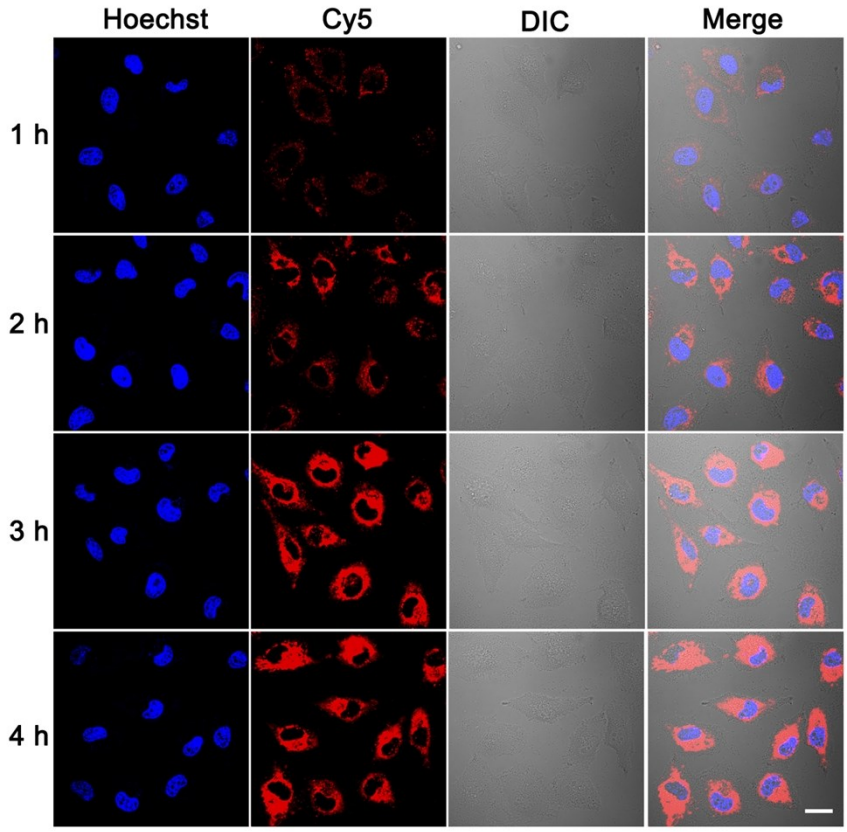


Figure S11. The time-dependent fluorescence imaging experiments were performed by incubating BSA+KFP@ZIF-8/HP+primer+MB NPs with HeLa cells for different time to monitor PER-based DNA machine work.

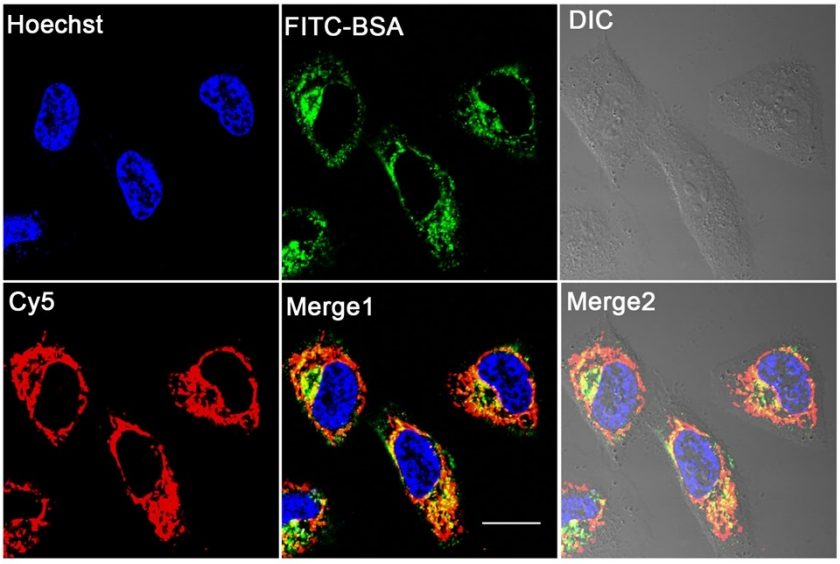


Figure S12. Confocal laser scanning microscopy (CLSM) imagesof HeLa cells after incubation with ZIF-8 NPs caged withFITC-labeled BSA and adsorbed with Cy5-labeled MBfor 3 h followed by staining with 10 μg mL^{-1} Hoechst 33342 for 10 min (blue).The excitation wavelengths were set at 488 and 650 nm for FITC-BSA and Cy5-labeled MB, and fluorescence signals were collected at 500-550 nm and 663-738 nm for FITC-BSA and Cy5-labeled MB, respectively.Scale bar: 10μm.

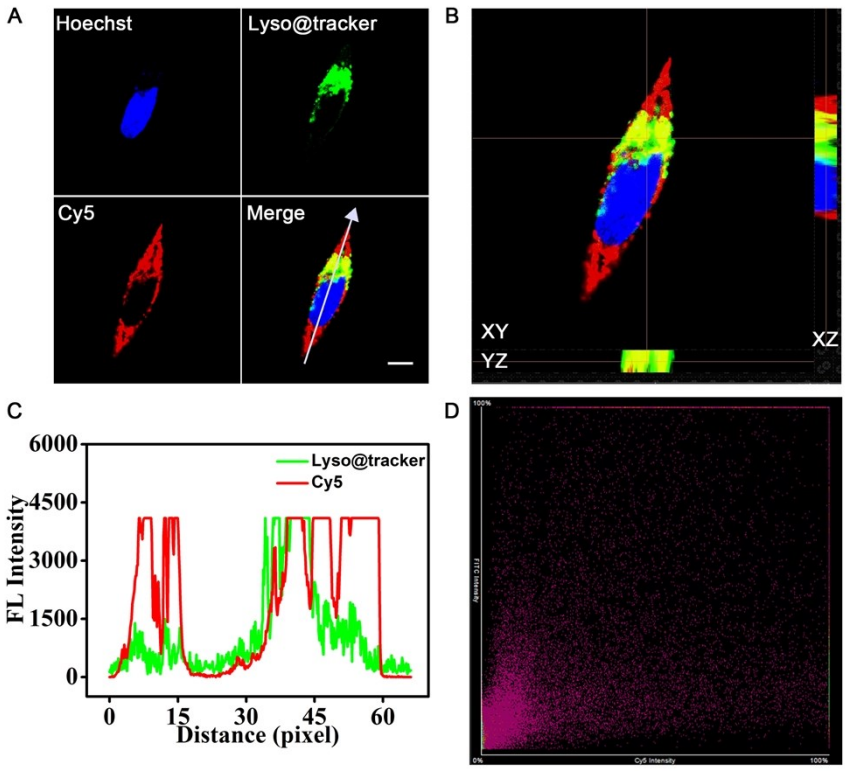


Figure S13. Endo-lysosomal release of nanoparticles in HeLa cells. (A) Confocal images of HeLa cells after incubation with 60 μg mL^{-1} BSA+KFP@ZIF-8/Cy5-MBNPs for 3 h and then with 100 nM Lyso@Tracker Green DND-26 for 25 min (green) followed by staining with 10 μg mL^{-1} Hoechst 33342 for 10 min (blue). The excitation wavelengths were set at 488 and 650 nm for Lyso@tracker and Cy5, and fluorescence signals were collected at 500-550 nm and 663-738 nm for Lyso@tracker and Cy5, respectively. Scale bar: 10μm. (B) Confocal z-stacks images with a cross-section in XY, XZ, and YZ. (C) Display fluorescence intensity curves of Lyso@tracker and Cy5 in the regions markedstraight whitelines of A. (D) Scatter plot of colocalization of green channel (Lyso@tracker) and Red channel (Cy5). The colocalization coefficient (Pearson's correlation coefficient) was 0.29.

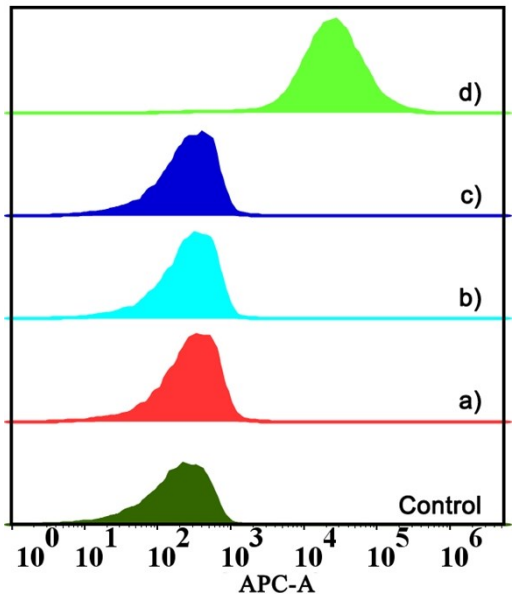


Figure S14. Flow cytometry analysis of HeLa cell spre-treatment with 10 mM $Mg^{2+}$and 5 μM calcimycin for 30 min and then treated withdifferent nanoparticles: (a) BSA+KFP@ZIF-8/primer+MB NPs (60 μg mL^{-1}); (b) BSA+KFP@ZIF-8/HP+MB NPs (60 μg mL^{-1}) (c) BSA@ZIF-8/HP+primer+MB NPs (60 μg mL^{-1}); and (d) BSA+KFP@ZIF-8/HP+primer+MB NPs (60 μg mL^{-1}). HeLa cells without any treatment served as control.

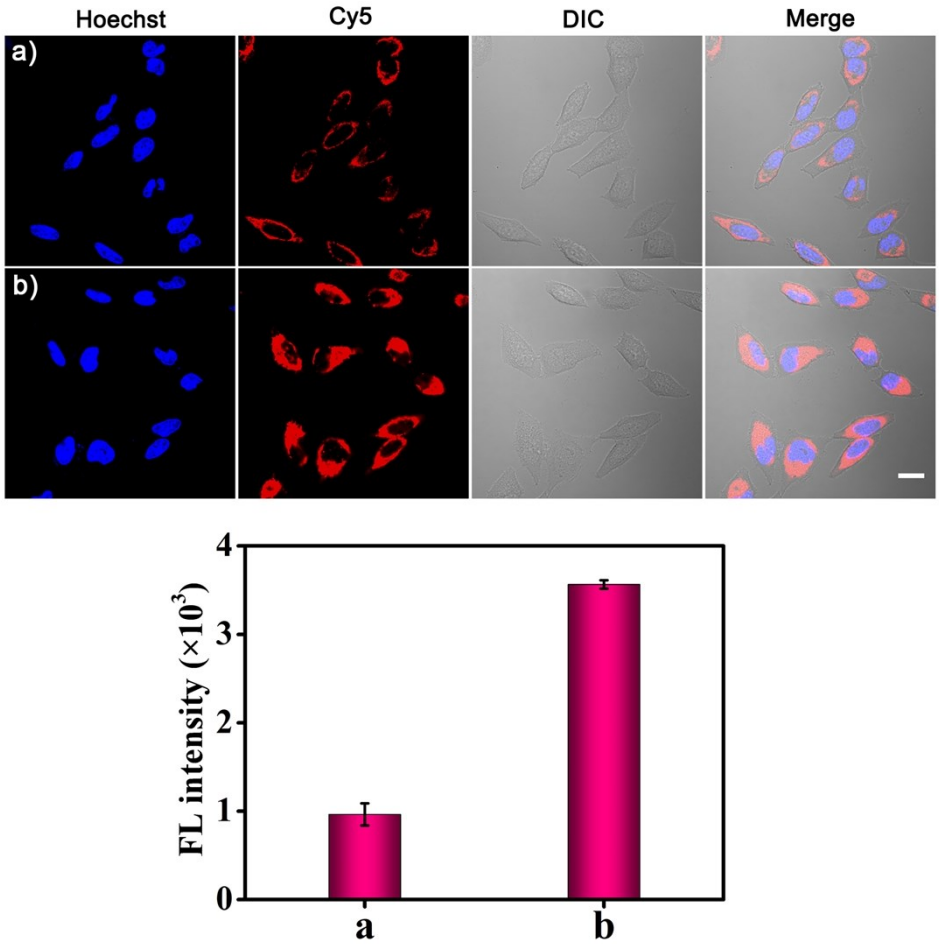


Figure S15. The effect of Mg^{2+} on the intracellular PER reaction. Upper: Confocal fluorescence imaging of HeLa cellswithout (a) and with (b) the pre-treatment with10 mM Mg^{2+} and 5 μM calcimycin for 30 min. Scale bar: 20μm.Lower:Corresponding fluorescence intensities of HeLa cells without (a) and with (b) the pre-treatment with 10 mM Mg^{2+} and 5 μMcalcimycin for 30 min.

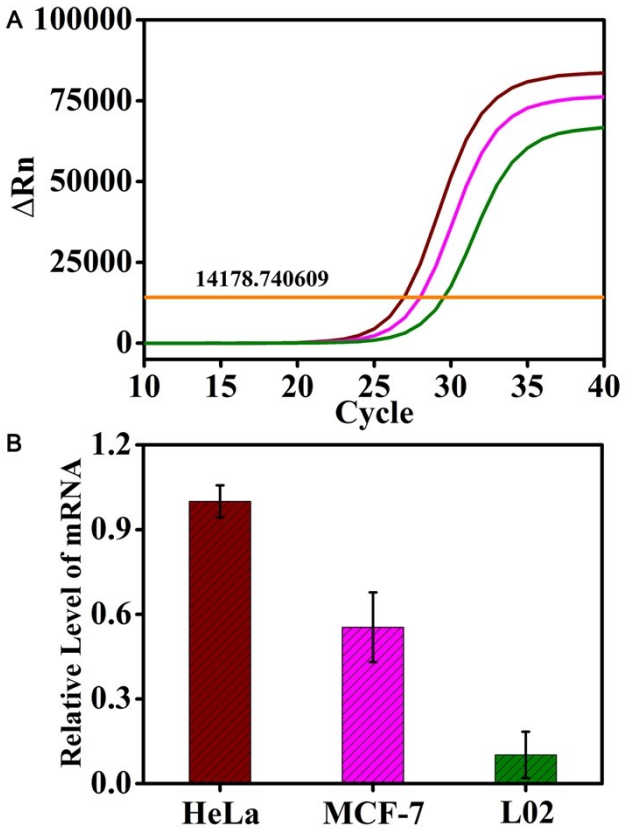


Figure S16. Quantitative reverse transcription-PCR (qRT-PCR) analysis of survivin mRNA in the three different cell lines. (A) Real-time fluorescence curves in qRT-PCR analysis of HeLa (red curve), MCF-7 (purple curve) and L02 (green curve). (B) Relative expressionlevels of survivin mRNA in three different cell lines.

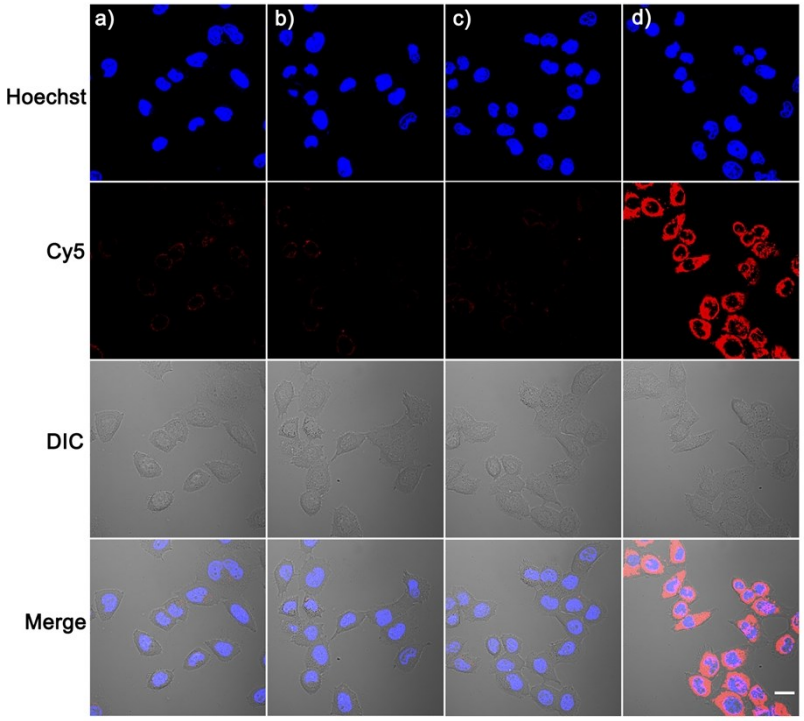


Figure S17. Confocal laser scanning microscopy (CLSM) imaging of L02 cells. The L02 cells were pretreated with 10 mM Mg^{2+} and 5 μM calcimycin for 30 min and then incubated for 3 h with different nanoparticles: (a) BSA+KFP@ZIF-8/primer$_{β\text{-actin}}$+MB NPs (60 μg mL^{-1}); (b) BSA+KFP@ZIF-8/HP$_{β\text{-actin}}$+MB NPs (60 μg mL^{-1}); (c) BSA@ZIF-8/HP$_{β\text{-actin}}$+primer$_{β\text{-actin}}$+MB NPs (60 μg mL^{-1}); and (d) BSA+KFP@ZIF-8/HP$_{β\text{-actin}}$+primer$_{β\text{-actin}}$+MB NPs (60 μg mL^{-1}). Scale bar: 20 μm.

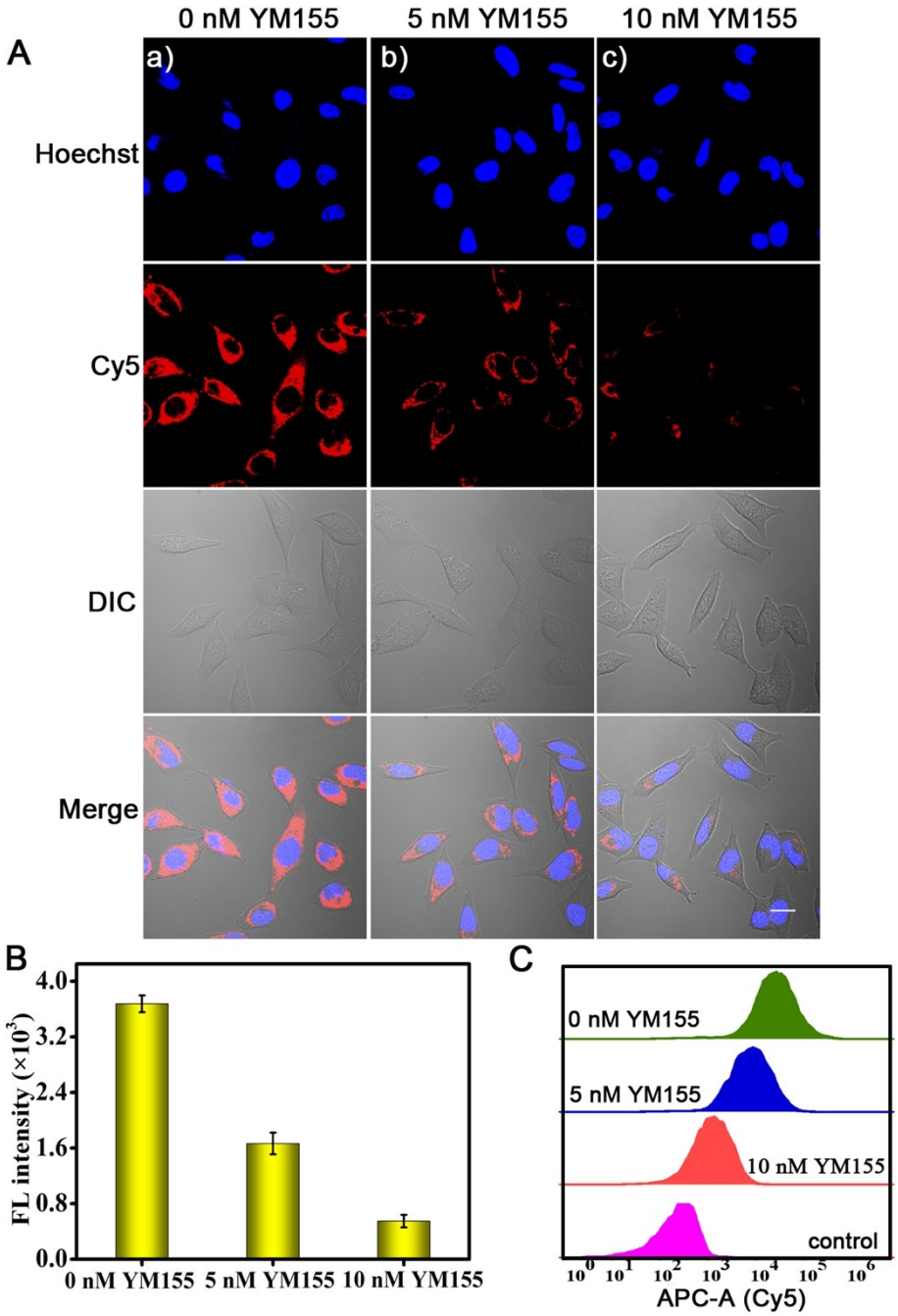


Figure S18. (A) Cellular CLSM imaging of the survivin mRNA in HeLa cells treated with (a) 0 nM, (b) 5 nM, and (c) 10 nM survivin mRNA expression repressor YM155, followed by incubation with 60 μg mL^{-1}BSA+KFP@ZIF-8/HP+primer+MB NPs at 37°C for 3 h and staining with Hoechst 33342 for 10 min. Scale bar: 20 μm. (B) Fluorescence intensity of the HeLa cells treated with different concentrations of YM155. (C) Flow cytometry analysis of HeLa cells treated with different concentrations of YM155. HeLa cells without treatment served as control.

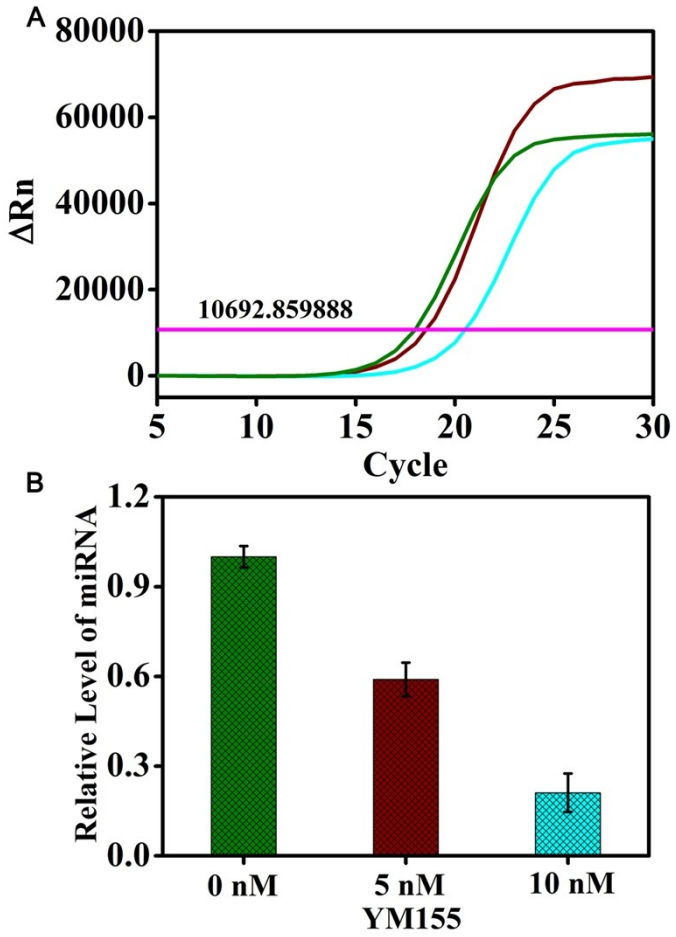


Figure S19. Expression analysis of survivin mRNA in HeLa cells treated with different concentrations of YM155. (A) Real-time fluorescence curves of qRT-PCR analysis. The YM155 concentrations were 0 nM (green curve), 5 nM (red curve) and 10 nM (light blue curve), respectively. (B) Relative expression levels of survivin mRNA in HeLa cells treated with different concentrations of YM155.

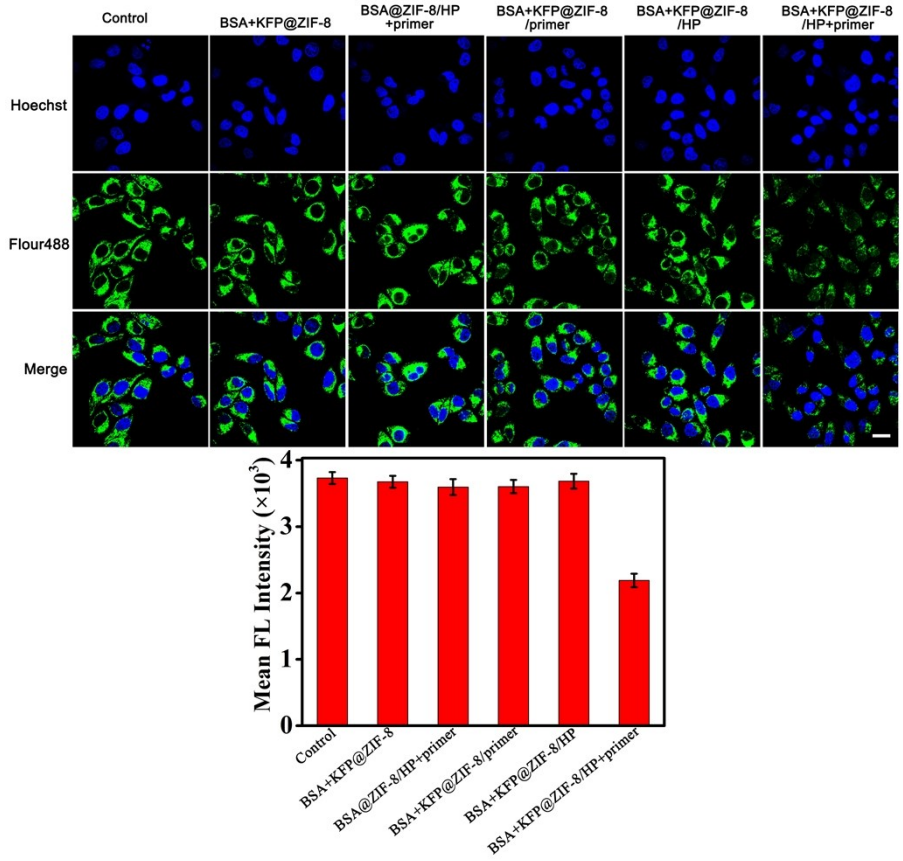


Figure S20. Immunofluorescence analysis with antibodies against Bcl-2 proteinin the fixed HeLa cells. HeLa cells were treated with the samples from left to right: BSA+KFP@ZIF-8 NPs; BSA@ZIF-8/HP+primer NPs; BSA+KFP@ZIF-8/primer NPs; BSA+KFP@ZIF-8/HP NPs. BSA+KFP@ZIF-8/HP+primer NPs. Cells without treatment served as control. Scale bar: 20 μm.

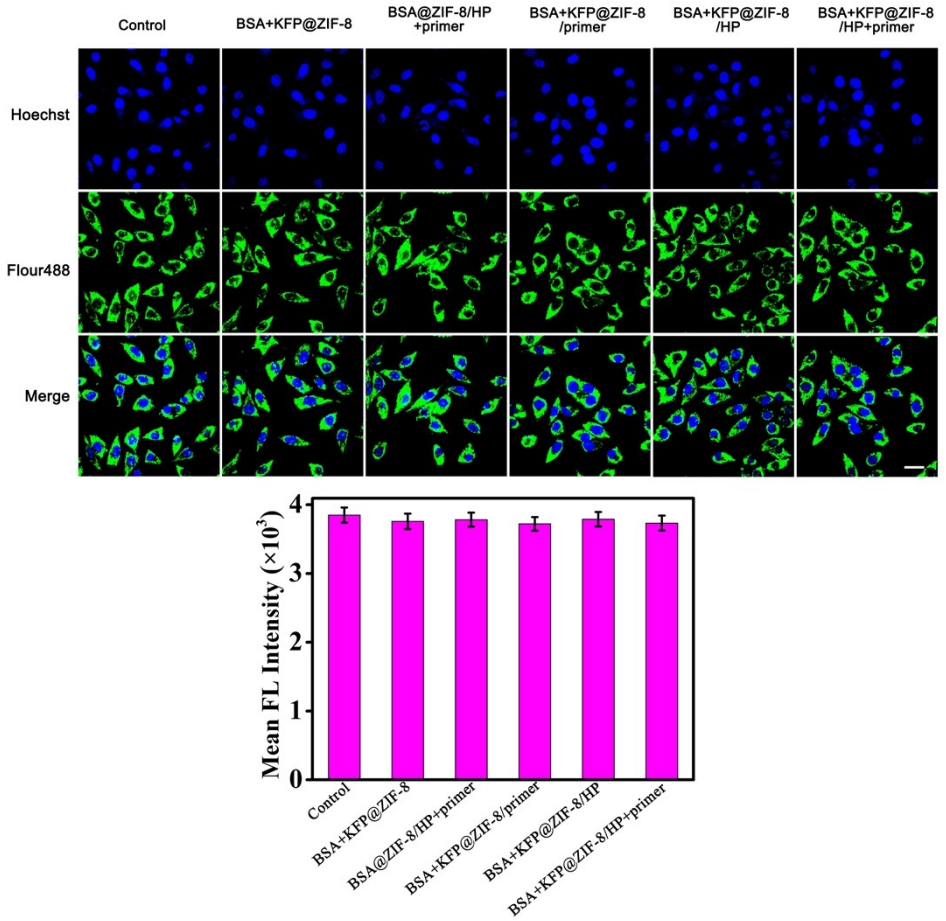


Figure S21. Immunofluorescence analysis with antibody against Bcl-2 protein in the fixed L02 cells. L02 cells were treated with the samples from left to right: BSA+KFP@ZIF-8 NPs, BSA@ZIF-8/HP+primer NPs, BSA+KFP@ZIF-8/primer NPs, BSA+KFP@ZIF-8/HP NPs, and BSA+KFP@ZIF-8/HP+primer NPs. Cells without treatment served as control. Scale bar: 20μm.

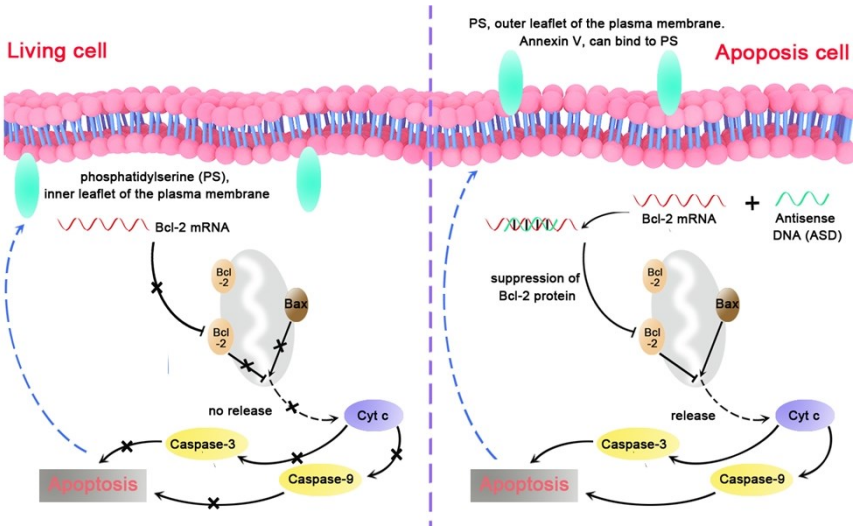


Figure S22. The bcl-2 pathway and this relates to apoptosis/annexin V induction.

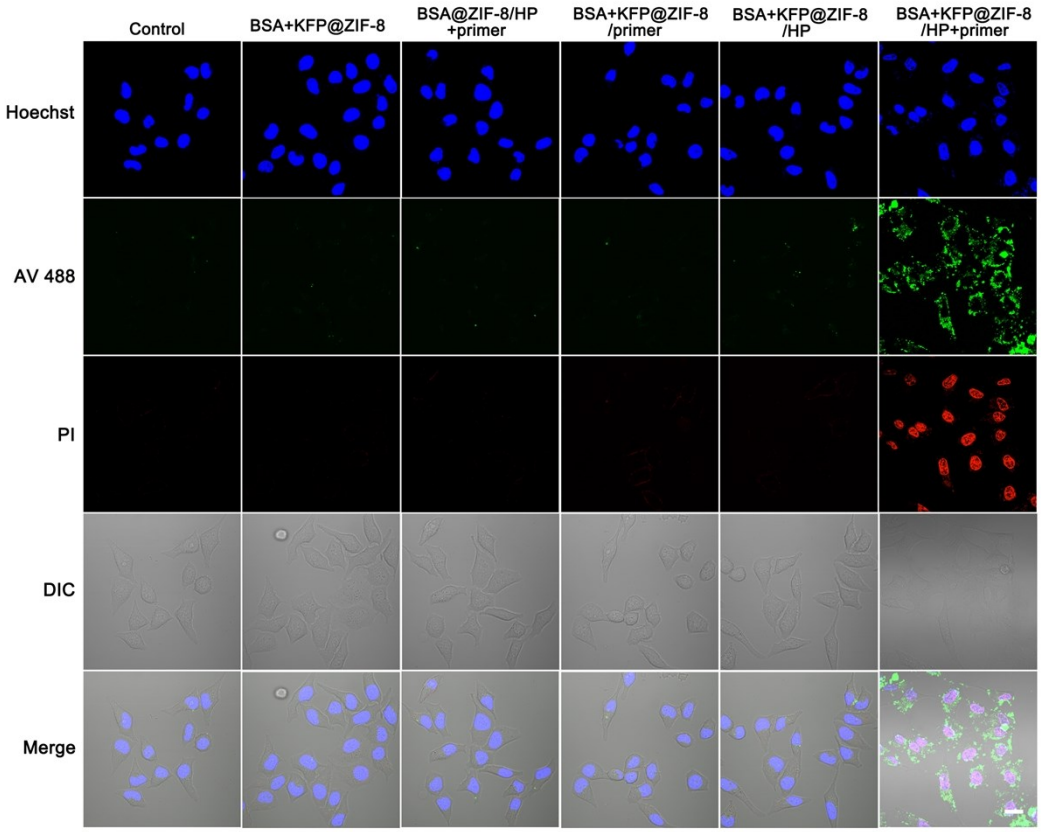


Figure S23. Cellular CLSM images of annexin V Alexa Fluor 488 (AV 488)/PI stained apoptotic cells of HeLa cells after treatment with the samples from left to right: BSA+KFP@ZIF-8 NPs, BSA@ZIF-8/HP+primer NPs, BSA+KFP@ZIF-8/primer NPs, BSA+KFP@ZIF-8/HP NPs, and BSA+KFP@ZIF-8/HP+primer NPs, respectively. Cells without treatment served as control.Scale bar: 20 μm.

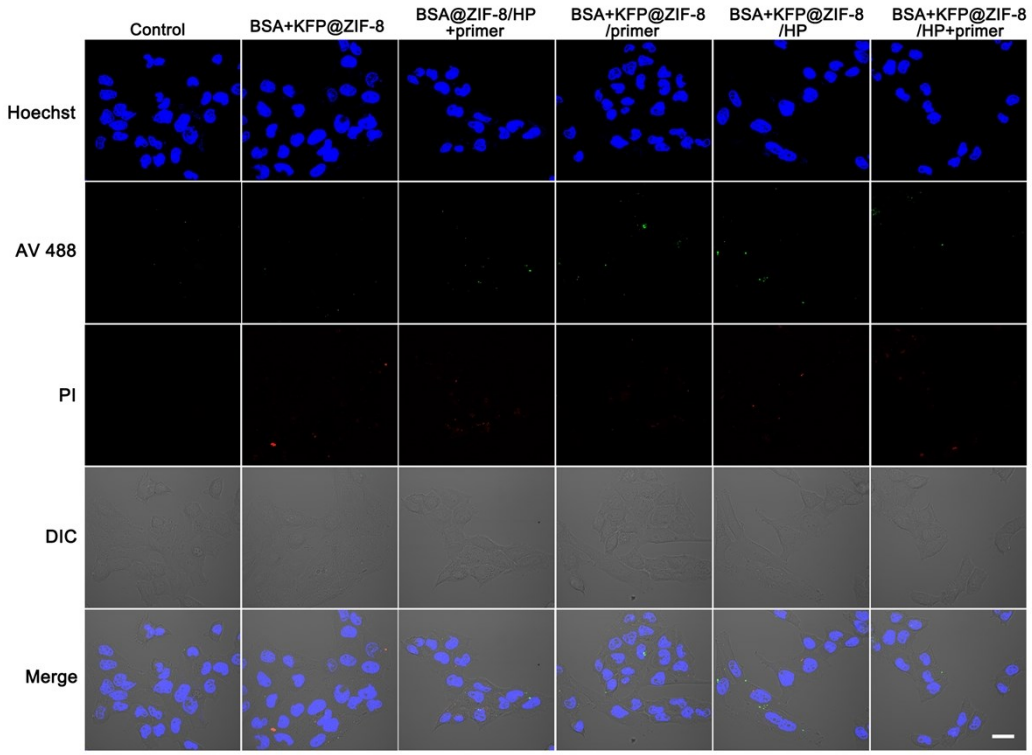


Figure S24. Cellular CLSM images of annexin V Alexa Fluor 488 (AV 488)/PI stained apoptotic cells of L02 cells after incubation with the samples from left to right: BSA+KFP@ZIF-8 NPs, BSA@ZIF-8/HP+primer NPs, BSA+KFP@ZIF-8/primer NPs, BSA+KFP@ZIF-8/HP NPs, and BSA+KFP@ZIF-8/HP+primer NPs, respectively. Cells without treatment served as control. Scale bar: 20 μm.

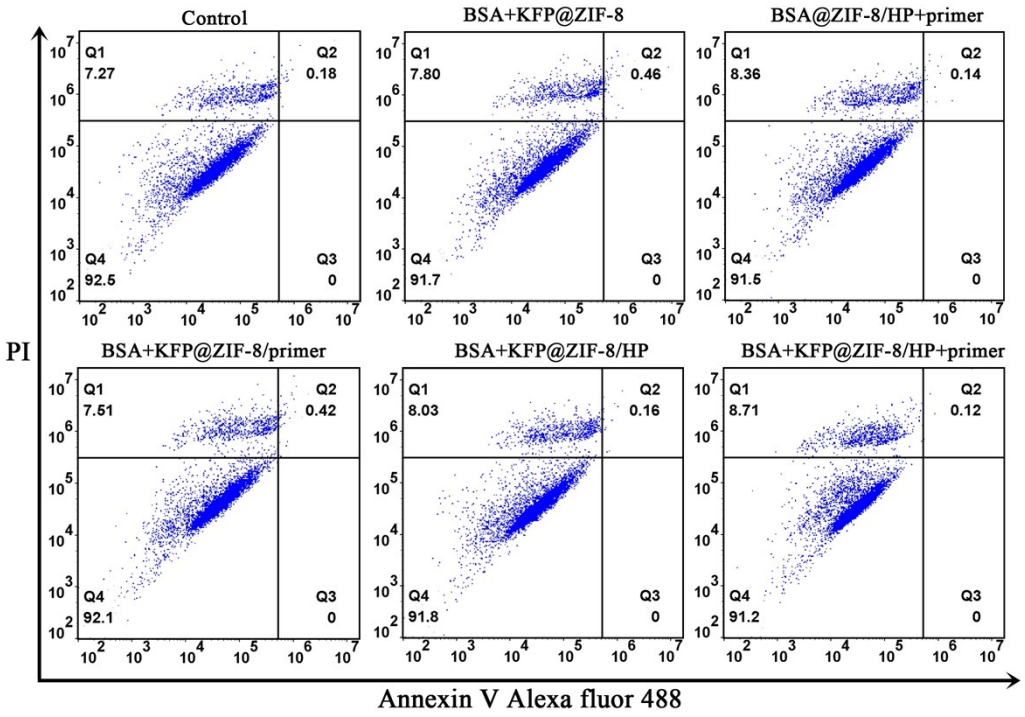


Figure S25. Flow cytometry assay of annexin V Alexa Fluor 488/PI stained apoptotic cells of L02 cells after incubated with the BSA+KFP@ZIF-8 NPs, BSA@ZIF-8/HP+primer NPs, BSA+KFP@ZIF-8/primer NPs, BSA+KFP@ZIF-8/HP NPs, and BSA+KFP@ZIF-8/HP+primer NPs, respectively. Cells without treatment served as control.

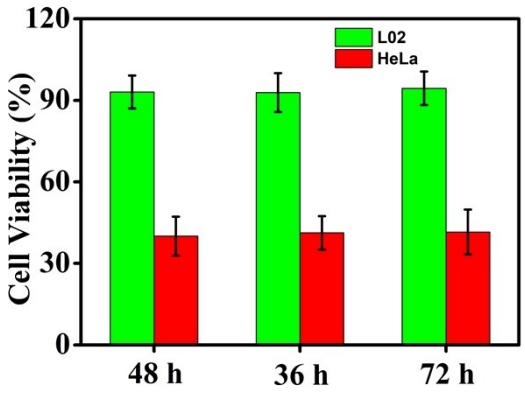


Figure S26. Cell viability of HeLa and L02 cells treated with BSA+KFP@ZIF-8/HP+primer NPs for different times.

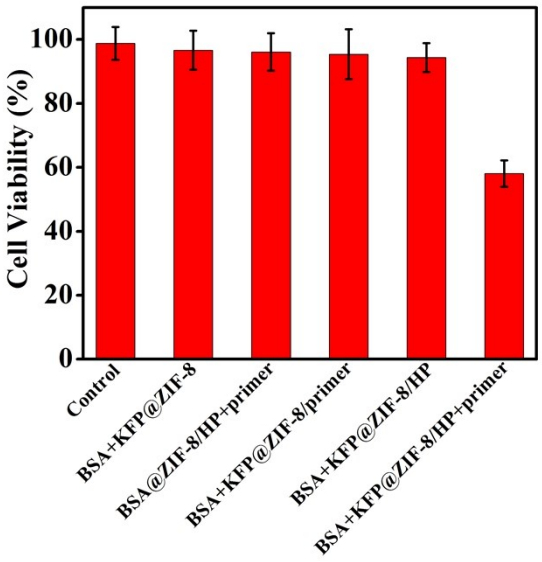


Figure S27. Cell viability of MCF-7 cells after treatment with different nanoparticles.

References

1. T. T. Chen, J. T. Yi, Y. Y. Zhao and X. Chu, J. Am. Chem. Soc., 2018, **140**, 9912-9920.

2. J. Zhang, M. Y. He, C. P. Nie, M. M. He, Q. S. Pan, C. Liu, Y. L. Hu, J. T. Yi, T. T. Chen and X. Chu, Anal. Chem., 2019, **91**, 9049-9057.