Supporting Information

Targeted imaging and targeted therapy of breast cancer cells *via* fluorescence double template-imprinted polymer coated silicon nanoparticles by epitope approach

Hai-Yan Wang,[a] Pei-Pei Cao,[b] Zheng-Ying He,[a] Xi-Wen He,[a] Wen-You Li,*[ac] Yu-Hao Li,*[b] and Yu-Kui Zhang*[ad]

[a] College of Chemistry, Research Center for Analytical Sciences, State Key Laboratory of Medicinal Chemical Biology, Tianjin Key Laboratory of Biosensing and Molecular Recognition, Nankai University, Tianjin 300071, China, wyli@nankai.edu.cn, Fax: +86-22-23502458.

[b] Key Laboratory of Tumor Microenvironment and Neurovascular Regulation, Nankai University School of Medicine, Tianjin 300071, China, liyuhao@nankai.edu.cn, Fax: +86-22-23502554.

[c] Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Tianjin 300071, China

[d] National Chromatographic Research and Analysis Center, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China, ykzhang@dicp.ac.cn., Fax: +86-411-84379779.

*Corresponding authors. E-mail: wyli@nankai.edu.cn, liyuhao@nankai.edu.cn, ykzhang@dicp.ac.cn.

Experimental section

The absolute photoluminescence quantum yield (APLQY) and fluorescence lifetime of Si NPs. The APLQY value was measured under excitation of 405 nm and calculated by the following equation:

$$\text{APLQY}(\%) = \frac{\varepsilon}{\alpha} = \frac{\int L_{emission}}{\int E_{solvent} - \int E_{sample}} \times 100\%$$

Equation S1

Where ε and α respectively represent the number of photons emitted and the total number of photons absorbed. $L_{emission}$ is the complete emission spectrum of the sample being tested. $E_{solvent}$ and E_{sample} refer to the solvent scattering and the sample scattering. The fluorescence lifetime decay curve of the sample was measured by an FLS-920 spectrophotometer at the excitation wavelength of 385 nm.

Time stability and pH effect of samples. NaOH and HCl were used to adjust the pH value of MIP's and NIP's aqueous solution from 2 to 11 for monitoring the pH effects on fluorescence emission. We measured the fluorescence intensities of MIP and MIP@DOX at the range of 0 to 4 h. Here, the concentrations of MIP, NIP and MIP@DOX were 0.5 $mg{\cdot}mL^{-1}$, and the excitation wavelength was 385 nm.

Adsorption experiments. In kinetic adsorption tests, the initial concentrations of the peptide and DOX were 0.3 $mg{\cdot}mL^{-1}$ and 0.08 $mg{\cdot}mL^{-1}$, respectively. Isothermal adsorption experiments were carried out by varying the concentrations of the peptide

and DOX from 0.01 to 0.12 $mg \cdot mL^{-1}$ during 6 hours. The amount of peptide and DOX adsorbed by MIP and NIP was calculated according to the following formula:

$$Q = (C_0 - C_S)\ V/\ m \qquad \text{Equation S2}$$

Where Q ($mg \cdot g^{-1}$) is the adsorption capacity, C_0 and C_S ($mg \cdot mL^{-1}$) are the initial concentration and treated solution concentration after incubation, V (mL) is the volume of targets solution and m (g) is the weight of MIP or NIP.

The imprinting factor (IF) was to evaluate MIP to specifically recognize the targets and calculated by the formula as follows:

$$IF = Q_{MIP}/Q_{NIP} \qquad \text{Equation S3}$$

Where Q_{MIP} and Q_{NIP} ($mg \cdot g^{-1}$) indicate the adsorption performance of MIP and NIP for templates, respectively.

The adsorption equilibrium constant (K_L) and saturation capacity (Q_m) of MIP or NIP to templates can be calculated by Langmuir equation. The Langmuir equation is as follows:

$$C_e/Q = 1/\ (Q_m \times K_L) + C_e/Q_m \qquad \text{Equation S4}$$

Where Q ($mg \cdot g^{-1}$) is the capacity of the template adsorbed by MIP or NIP at the equilibrium concentration, Q_m ($mg \cdot g^{-1}$) is the saturation capacity, C_e ($mg \cdot mL^{-1}$) is the equilibrium concentration of the template, and K_L ($mL \cdot mg^{-1}$) is the Langmuir adsorption equilibrium constant.

Cell culture. Human breast cancer cells (SK-BR-3 and MDA-MB-231), Human breast ductal carcinoma cells (BT-474) and Human umbilical vein endothelial cells (HUVEC) were obtained from the Chinese Academy of Sciences (Beijing, China). SK-BR-3 cells were incubated in DMEM medium (DMEM, Biological Industries, Israel) supplemented with 10% (v/v) fetal bovine serum (FBS, BI, Israel), 1% penicillin-streptomycin solution (PS, Life technologies, America) and 2% $NaHCO_3$. BT-474 cells were maintained with RPMI-1640 medium (1640, Biological Industries, Israel) containing 10% fetal bovine serum (FBS, BI, Israel), 1% penicillin-streptomycin solution (PS, Life technologies, America), 2% $NaHCO_3$, 1% glucose and 1% sodium pyruvate. MDA-MB-231 cells were seeded in RPMI-1640 medium (1640, Biological Industries, Israel) containing 10% fetal bovine serum (FBS, BI, Israel) and 1% penicillin-streptomycin solution (PS, Life technologies, America). HUVEC cells were maintained with DMEM medium (DMEM, Biological Industries, Israel) containing 10% fetal bovine serum (FBS, BI, Israel) and 1% penicillin-streptomycin solution (PS, Life technologies, America). All cell lines were incubated in HERAcell VIOS 150i CO_2 incubator (Thermo scientific, America) at 37°C in a 5% CO_2 atmosphere with 95% humidity.

MTT test. The cytotoxicity of MIP and NIP, the targeted therapy of MIP@DOX and NIP@DOX against SK-BR-3 cells, HUVEC cells or MDA-MB-231 cells were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. SK-BR-3 cells (1×10^5 cells per mL per well) and HUVEC cells (1×10^5 cells per mL per well) were seeded in 96-well plates with 100 μL DMEM medium

containing 10% (v/v) FBS and incubated at 37℃ with 5% CO_2 for 24 h. Subsequently, SK-BR-3, HUVEC cells or MDA-MB-231 cells were treated with different concentration of MIP or NIP (0, 20, 40, 60, 80, 100, 300, 500 μg·mL^{-1}), and MIP@DOX or NIP@DOX (0, 20, 40, 60, 80, 100 μg·mL^{-1}) for 24 h. After that plates were washed three times with PBS solution, and MTT solution was added to each well and incubated for another 3 h. The remaining MTT solution was removed, and 150 μL of DMSO was added into wells to dissolve the formazan crystals. The absorbance (OD value) of each well was measured on a microplate reader (Promega, WI, USA) at the wavelength of 560 nm and the cell viability was calculated as follows:

$$\text{Cell viability} = OD_{sample}/OD_{control} \times 100\% \qquad \text{Equation S5}$$

Trypan blue staining experiment. The trypan blue staining experiment was carried out to detect cell survival and evaluate the therapeutic effect of MIP, NIP, MIP@DOX and NIP@DOX. SK-BR-3 cells were seeded in 24-well plates at a density of 4×10^4 cells per well and these plates were maintained at 37°C under 5% CO_2 atmosphere for 24 h. Later, the cells were incubated with MIP, NIP, MIP @DOX and NIP @DOX for 20 h at 40 μg·mL^{-1}, respectively. Next, these plates were washed three times with PBS solution, and trypan blue reagent (Sigam, USA) was added to each well and incubated for 30 min. Then the excessive trypan blue was removed, and the dead cell of each well was observed under the microscope (OLYMPUS, Japan).

Monitoring the transportation pathway of DOX. SK-BR-3 cells were seeded in

24-well plates at a density of 4×10^4 cells per well and these plates were maintained at 37°C under 5% CO_2 atmosphere for 24 h, following by co-incubation with the MIP@DOX at 37°C for 30 min, 1 h, 2 h or 3 h. Then, these plates were washed three times with PBS and stained with Lyso Tracker Green DND-26 (100 nM) for 1 h. Finally, the confocal laser microscope was used to observe the intracellular localization. The DOX from MIP@DOX and Lyso Tracker Green DND-26 were excited under 488 nm and 594 nm, respectively. Emission of DOX from MIP@DOX and Lyso Tracker Green DND-26 was 500-545 nm and 575-675 nm, respectively.

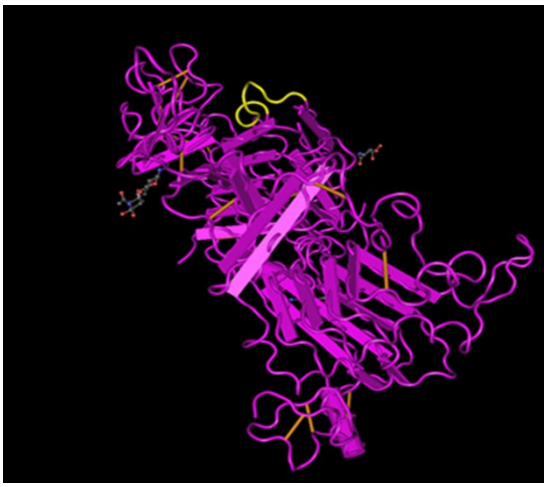

Fig. S1 The yellow part of the extracellular HER2 structure (amino acids: 22-530) is the sequence of the peptide template used for MIP.

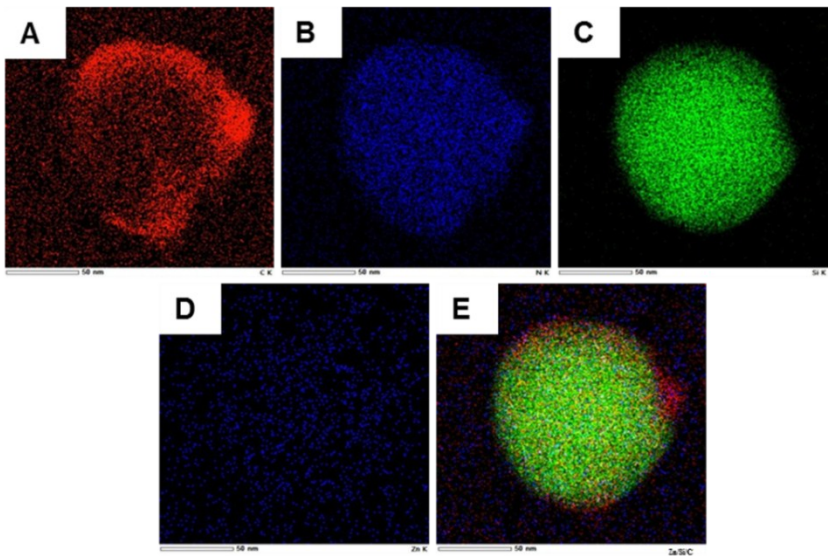

Fig. S2 EDS mapping of C (A), N (B), Si (C) and Zn (D) obtained from MIP. (E) Element overlay of Zn, Si, and C.

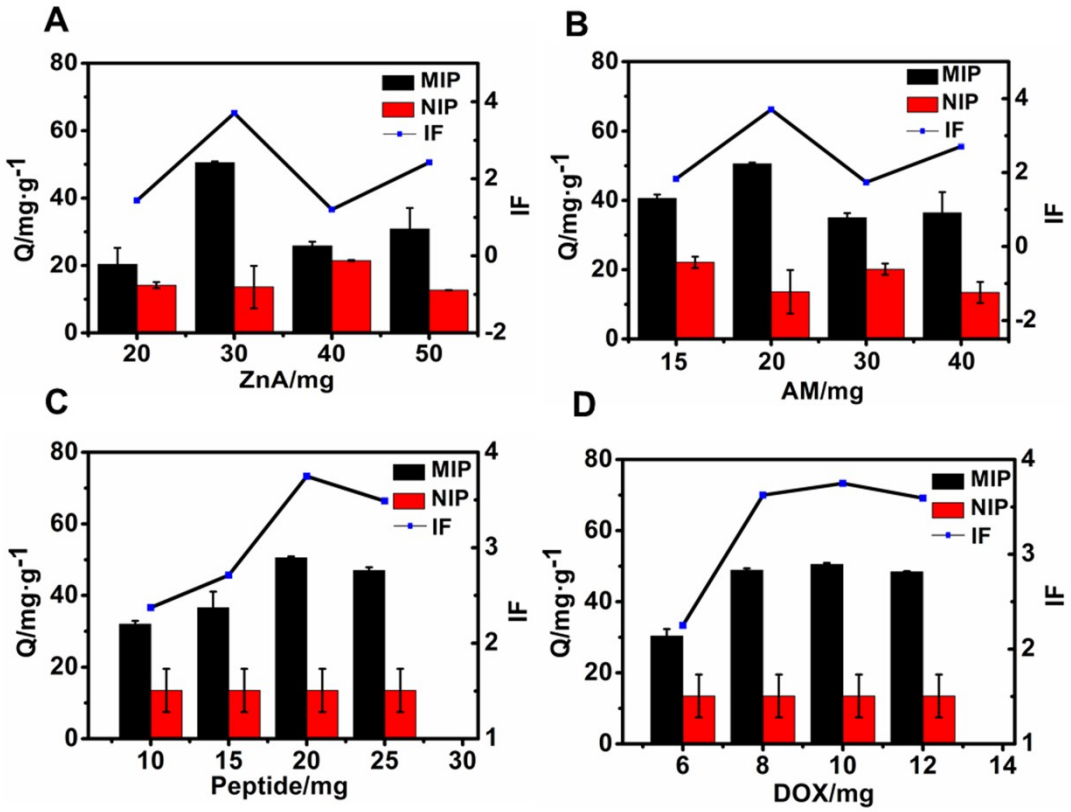

Fig. S3 The optimization of reaction conditions concerning (A) ZnA, (B) AM, (C) peptide, and (D) DOX on adsorption capacity and imprinting factor for the peptide.

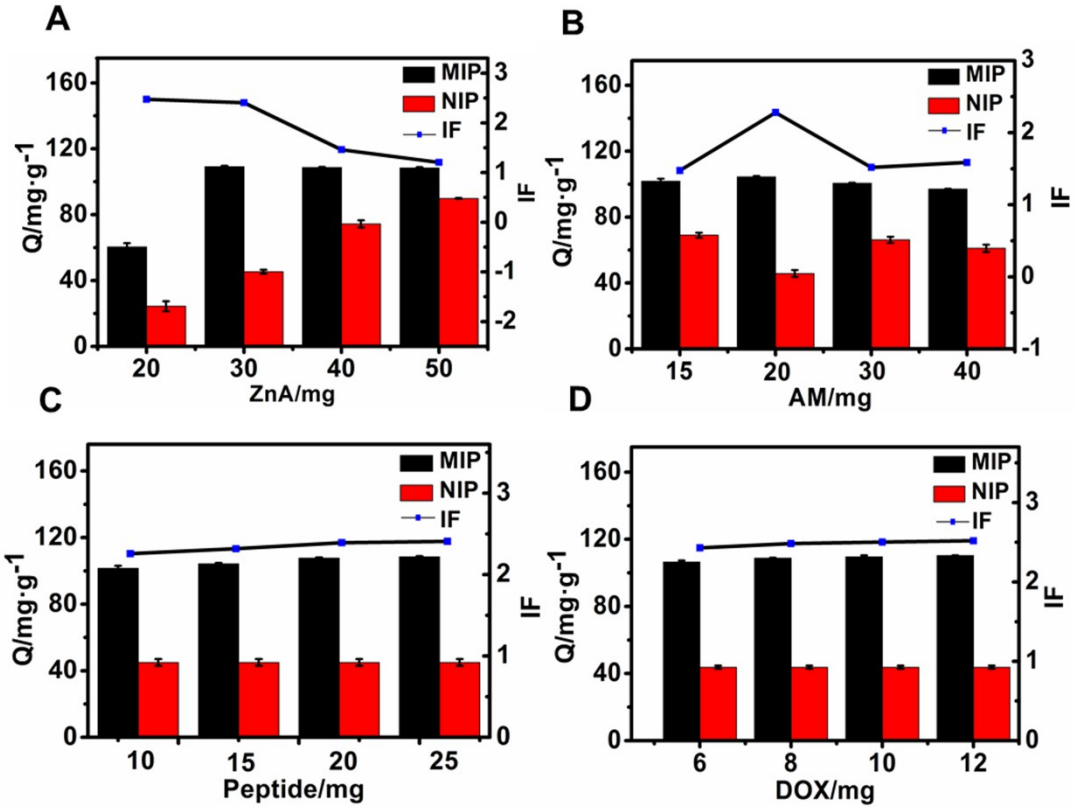

Fig. S4 The optimization of reaction conditions concerning (A) ZnA, (B) AM, (C) peptide, and (D) DOX on adsorption capacity and imprinting factor for DOX.

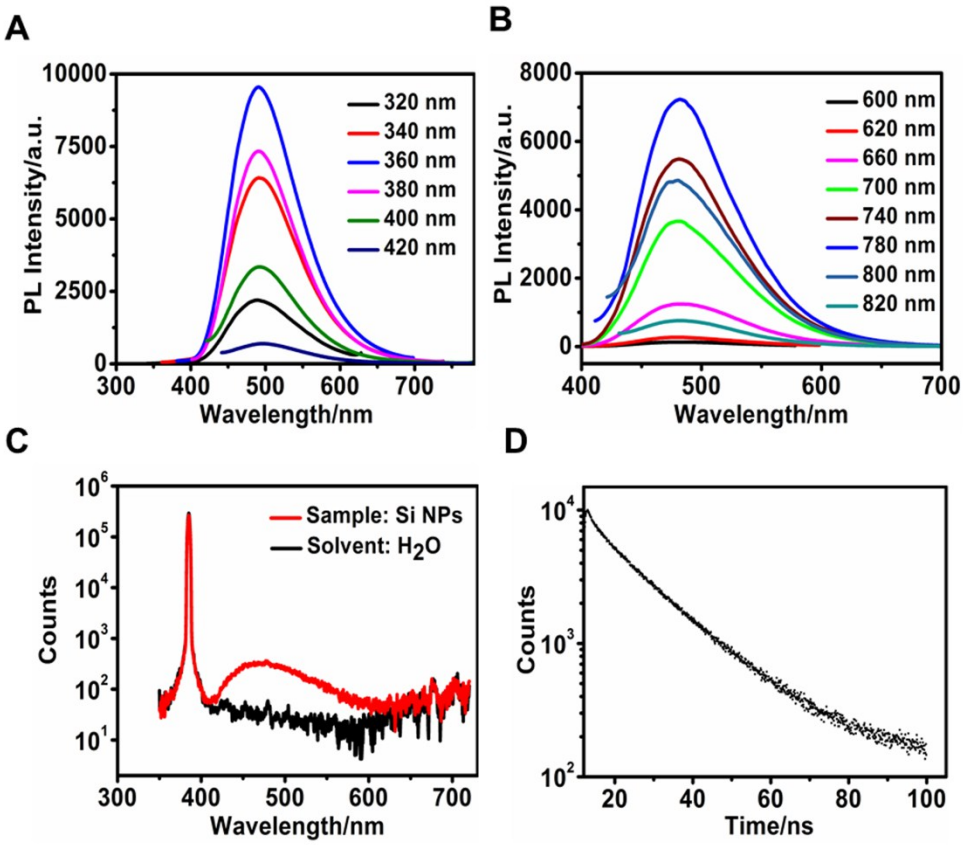

Fig. S5 Optical properties of the Si NPs aqueous solution. (A) The excitation wavelength-independent emission spectra with the excitation wavelength changed from 320 to 420 nm. (B) Up-conversion fluorescence spectra with the excitation wavelength ranged from 600 to 820 nm. (C) APLQY spectra of the as-prepared Si NPs. (D) The fluorescence lifetime spectrum of Si NPs.

Table S1. Fluorescence lifetime value and relative content of Si NPs.

Parameter	Value/ns	Relative %
τ_1	2.41	4.75
τ_2	15.67	95.25

$$\tau = 2.41*4.75\% + 15.67*95.25\% = 15.04 \text{ ns}$$

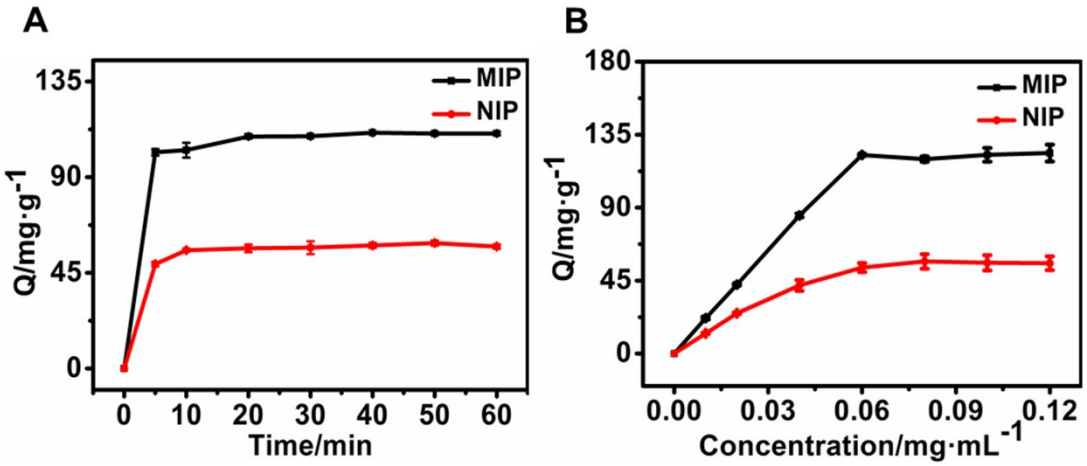

Fig. S6 Adsorption kinetics (A) and adsorption isotherms (B) of MIP and NIP toward DOX.

Table S2. Parameter values in Langmuir model of MIP and NIP for the peptide and DOX.

Samples	Q_m /mg·g^{-1}	K_L/mL·mg^{-1}	r
$MIP_{Peptide}$	55.2	0.77	0.996
$NIP_{Peptide}$	16.2	3.47	0.998
MIP_{DOX}	142.9	0.26	0.978
NIP_{DOX}	63.4	0.09	0.985

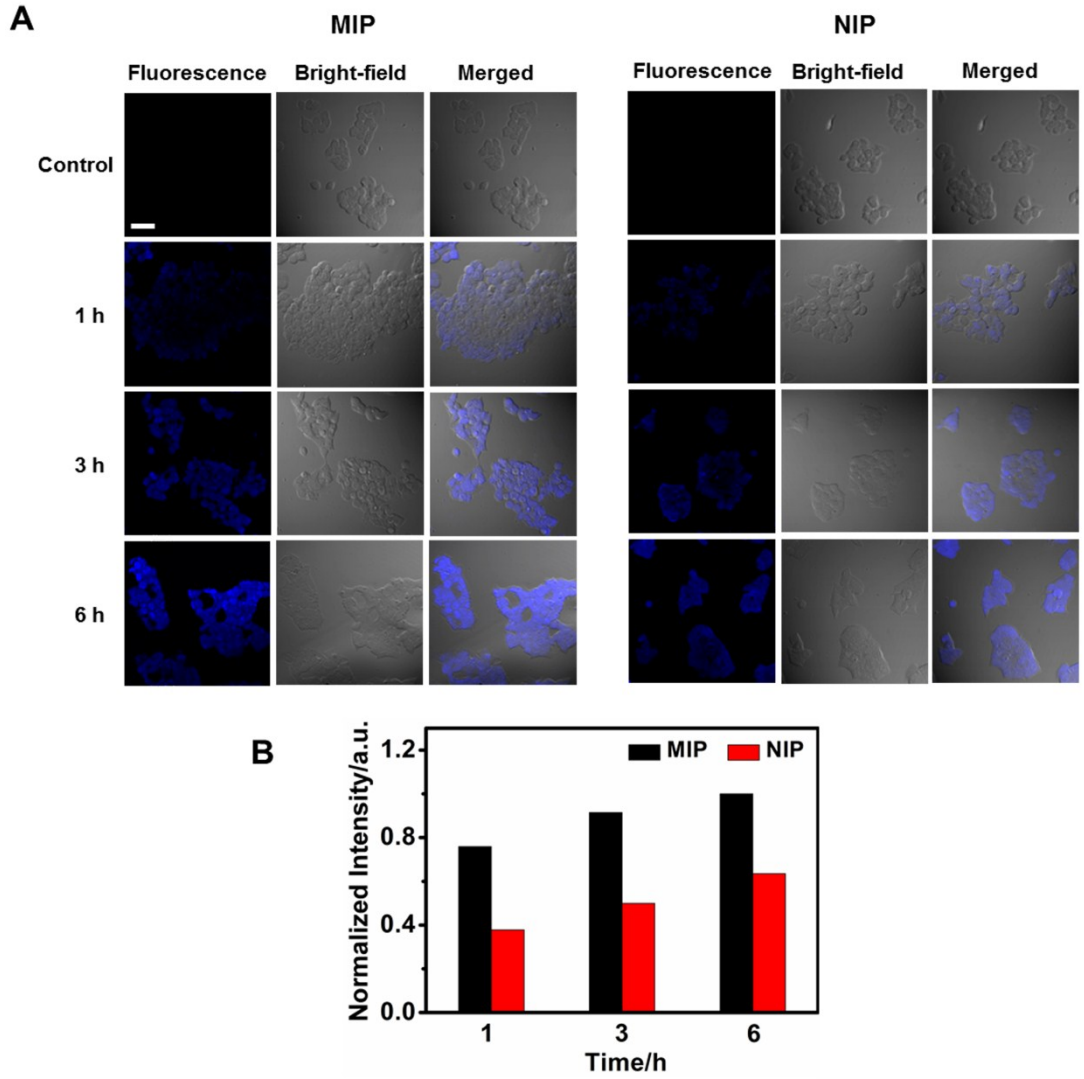

Fig. S7 (A) Confocal fluorescence images of BT-474 cells after incubating with 40 μg·mL^{-1} of MIP and NIP at certain time points (1, 3, and 6 h). Scale bar: 50 μm. (B) Normalization of mean fluorescence intensity (MFI) of Si NPs from MIP or NIP incubated with BT-474 cells at 1 h, 3 h and 6 h.

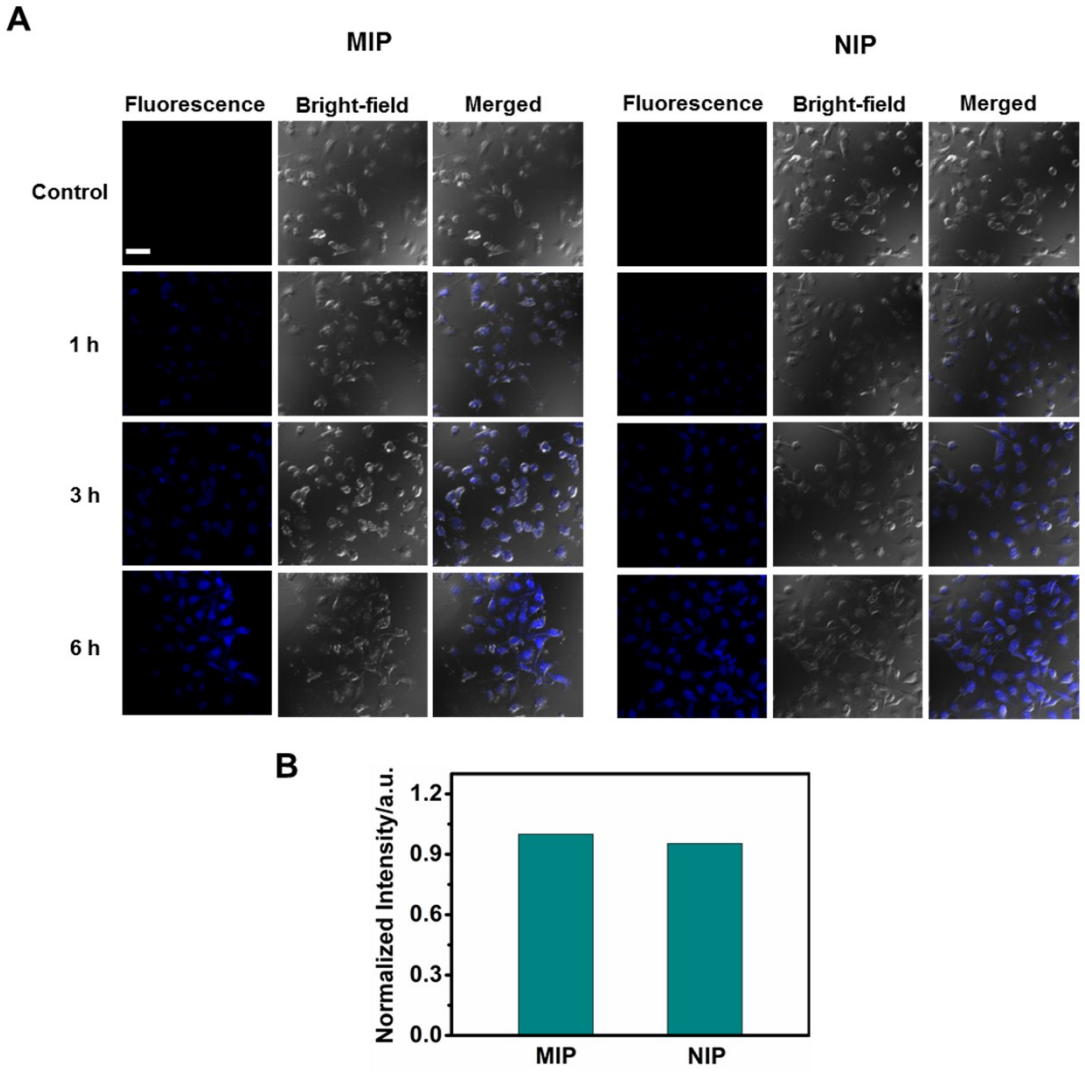

Fig. S8 (A) Confocal fluorescence images of MDA-MB-231 cells after incubating with 40 μg·mL^{-1} of MIP and NIP at certain time points (1, 3, and 6 h). Scale bar: 50 μm. (B) Normalization of mean fluorescence intensity (MFI) of Si NPs from MIP or NIP incubated with MDA-MB-231 cells for 3 h.

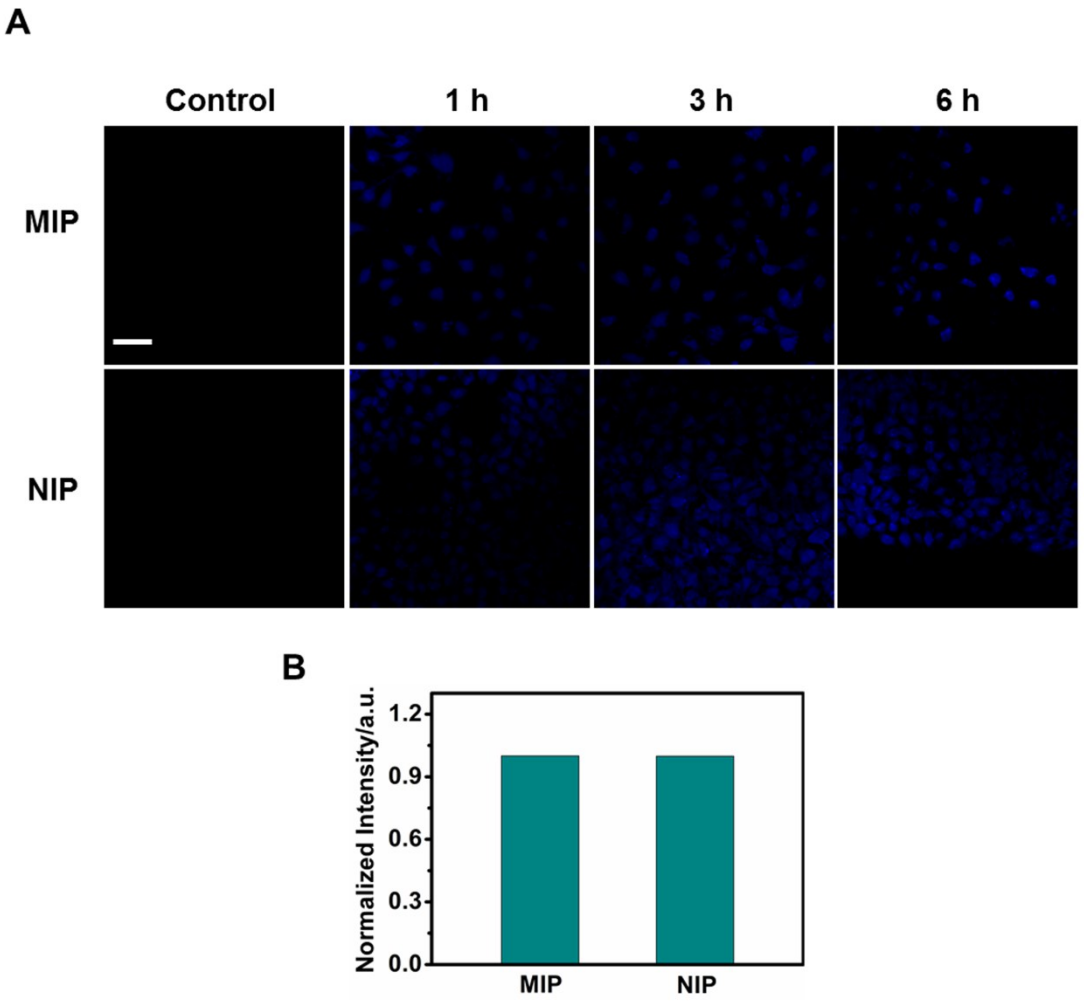

Fig. S9 (A) Confocal fluorescence images of HUVEC cells after incubating with 40 μg·mL^{-1} of MIP and NIP at certain time points (1, 3, and 6 h). Scale bar: 50 μm. (B) Normalization of mean fluorescence intensity (MFI) of Si NPs from MIP or NIP incubated with HUVEC cells for 3 h.

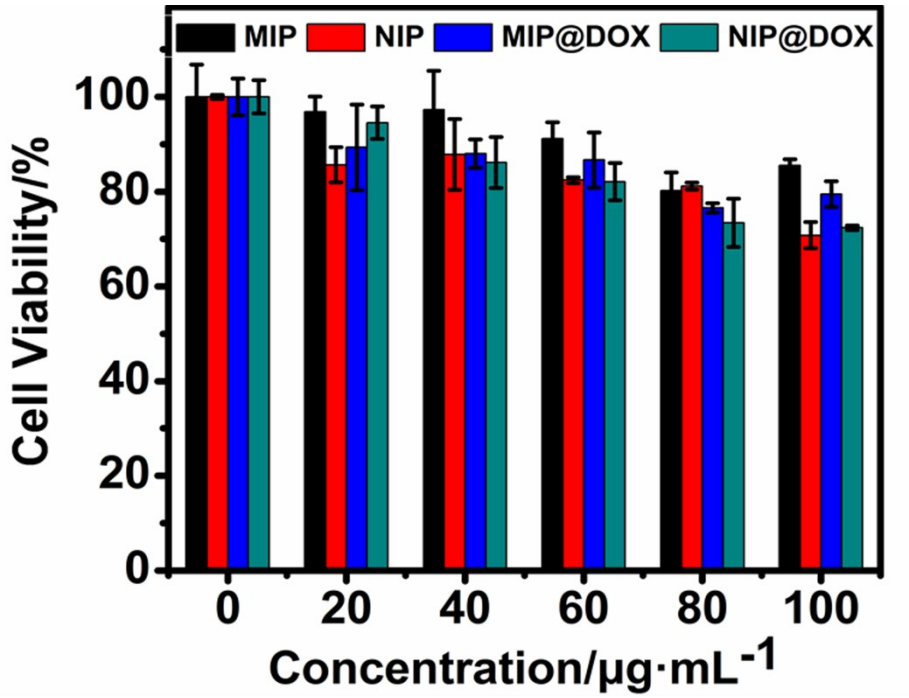

Fig. S10 Cell viability of MDA-MB-231 cells incubated with different concentrations of nanoparticles (MIP, NIP, MIP@DOX and NIP@DOX) for 24 h.