Supporting Information

Elimination of macrophage-entrapped antibiotic-resistant bacteria by a targeting metal-organic framework-based nanoplatform

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Materials

Zinc acetate dihydrate ($Zn(CH_3COOH)_2 \cdot 2H_2O$, ≥ 99%), Zinc phthalocyanine (ZnPc, 97%), imidazolate-2-carboxyaldehyde (2-ICA, $C_4H_4N_2O$, 98%), 2-Amino-2-deoxy-D-mannose hydrochloride, Methanol (AR), N,N-Dimethylformamide (DMF), Dulbecco's modified eagle's medium (DMEM), fetal bovine serum (FBS), ampicillin, 4',6-diamidino-2-phenylindole (DAPI), Rhodamine B, Sodium hydroxide (NaOH), hydrochloride (HCl, assay 37%), phosphate-buffered saline (PBS, pH = 7.4) buffer, 2′,7′-dichlorofuorescein diacetate (DCFH-DA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and dimethyl sulfoxide (DMSO) were purchased commercially and used as-received.

Instruments

The morphology of the nanocompounds was characterized by field-emission scanning electron microscopy (Hitachi S4800) and transmission electron microscopy (TEM) (FEI Tecnai G2 F20). X-ray diffractometer (XRD) measurements were performed on a Bruker D8 FOCUS using Cu Kα radiation. The UV -Vis absorption spectra were recorded using a JASCO V550 UV-Visible spectrophotometer. Fluorescence measurements were carried out on a JASC FP-6500 spectrofluorometer.

Experimental Section

Synthesis of ZnPc@ZIF-90 and ZnPc@ZIF-90-M. For the preparation of ZnPc@ZIF-90 nanoparticles, each 2 mL of imidazolate-2-carboxyaldehyde (2-ICA) (0.2 M) and $Zn(CH_3COOH)_2 \cdot 2H_2O$ (0.1 M) with 5 mg/mL ZnPc in DMF was mixed under vigorous stirring for 5 min. Then another 10 mL DMF was added for another 10

min stirring. The mixture was purified by washing with DMF and ethanol for three times and collected under centrifugation. The ZnPc@ZIF-90 nanoparticles were dried in vacuum at room temperature overnight and stored at 4℃ avoiding light. In addition, ZIF-90 was prepared without the addition of ZnPc.

For the preparation of ZnPc@ZIF-90-M, D-Mannosamine hydrochloride and ZnPc@ZIF-90 at the ratio of one-to-ten was added to 25 mL methanol in round-bottomed flask (50 mL). After mixing, the solution was further stirred at room temperature for 48 h, and particles were separated by centrifugation and washed with methanol and water to remove excess mannosamine. After that, the product was dried by lyophilization.

ROS Production Ability of ZnPc@ZIF-90-M. DCFH was utilized as a probe for measuring ROS in tubes, which was obtained by hydrolyzing DCFH-DA according to the literature. To test the generated ROS, DCFH (10 μM) was mixed with ZnPc@ZIF-90-M (100 mg mL^{-1}). Then, the mixture was irradiated with 638 ± 10 nm light at the power of 90 mW for 10 min. After the irradiation, the fluorescence intensities under 488 nm excitation (λ_{ex} of DCFH) were detected to determine the ROS generation efficiency. Furthermore, the variation tendency of fluorescence intensity of DCF at 522 nm was detected with different concentrations of ZnPc@ZIF-90-M and the irradiation time.

Planktonic Bacterial Culture and Antibacterial Experiments. Monocolony of MRSA on the Luria-Bertani (LB) agar plate were transferred to LB culture medium (20 mL) and grown at 37°C under 180 rpm rotation for 12 h. Then, the bacteria were diluted to 10^8 CFU·mL^{-1}. The 500 μL of obtained solution was treated with the as-obtained materials at different concentrations with and without 638 ± 10 nm light irradiation at the power of 90 mW for 10 min. For solid medium culture, the bacteria solution was diluted 10^{-4} times after irradiation. 100 μL of diluted bacterial solution was streaked on the tryptone agar plates by spread plate method. The plates were cultured at 37 °C for 12 h and the number of colony forming units (CFUs) was counted.

Cell Culture. Raw 264.7 mouse macrophage cells were cultured in DMEM medium containing 100 μg mL^{-1} streptomycin, 10% heat inactivated FBS, 100 U mL^{-1} penicillin,

and maintained in a humidified incubator with 5% CO_2 at 37°C for 2 days. The Raw 264.7 cells were used for bacteria invasion experiments when they reached 80% confluence.

MTT assay of macrophage. The viabilities of Raw264.7 cells were determined by MTT assay. After incubation with different concentrations of ZnPc@ZIF-90-M in 96 well plates, the macrophage cells in DMEM medium were irradiated under 638 ± 10 nm light at the power of 90 mW for 10 min or not. The treated macrophage cells were further incubated at 37°C with 5% CO_2 for 24 h. Then, they were all proceeded to run MTT assay to study their viabilities. The serum-containing medium was replaced with serum-free medium and MTT reagent (5 mg/mL) in Raw264.7 cell cultures. Raw 264.7 cells were incubated at 37°C for 4 h. The culture medium was then removed and 100 μL DMSO was added. The plate was shaked on an orbital shaker for 15 min. The sample was analyzed with the microplate reader to detect absorbance at 570 nm.

Microscopic observation of mannosyl ligand-mediated cell internalization of as-prepared nanoparticles. RB@ZIF-90 and RB@ZIF-90-M were synthesised by the modified methods of ZnPc@ZIF-90 and ZnPc@ZIF-90-M respectively, only to replace ZnPc with Rhodamine B. Rhodamine B or Rhodamine B-loaded RB@ZIF-90 or RB@ZIF-90-M (1 μg mL^{-1} of Rhodamine B, final concentration) were incubated with Raw264.7 cells (1×10^5) in 0.5 mL complete DMEM culture medium. For microscopic observation, Raw264.7 cells (1×10^5) were seeded on coverslips in 24-well tissue culture plates and cultured overnight. Rhodamine B or RB@ZIF-90 or RB@ZIF-90-M were added to distinct wells and incubated at 37°C for 4 h in 0.5 mL of fresh complete DMEM culture medium. The cells were washed twice with ice-cold PBS and fixed with fresh 4% paraformaldehyde for 10 min at room temperature. The cells were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) for the cell nucleus and FITC phalloidin (Yeasen Biotechnology (Shanghai) Co., CN) for the cytoskeleton following the manufacturer's instructions. The coverslips were mounted on glass microscope slides and the slides were visualized under a laser scanning confocal microscope.

The localization of bacteria and NPs in macrophage. Raw264.7 cells (1×10^5) were

seeded on coverslips in 24-well plate and incubated 20 h at 37°C with 5% CO_2. Then, the medium of each well was replaced with 500 μL fresh complete medium containing MRSA solution at a ratio of 20 (bacteria/macrophage). After 1 h infection, the medium was removed and the samples were washed with PBS for 3 times to remove the bacteria outside of macrophages. Then, antibiotic gentamycin (50 μg mL^{-1}) was added into infected macrophages with an incubation time of 60 min, to eliminate MRSA adhered to the surface of macrophage cells. The samples were wash with PBS for 3 times to remove the added gentamycin and dead bacteria, followed by adding 500 μL of fresh medium containing ZnPc@ZIF-90-M (100 μg mL^{-1}). After 4 h incubation at 37 °C, the medium was removed and washed with PBS for three times to remove the NPs, which was followed by the addition of DAPI (2 μg/mL) for cell nuclei labeling in 15 min incubation. Samples were then washed three times with PBS for intracellular bacteria imaging by using CLSM.

Ablation of bacteria in macrophage. To measure the intracellular bacteria ablation effect, macrophages were infected with MRSA at a ratio of 20 bacteria per macrophage. After 1 h infection, the medium was removed and washed with fresh culture medium to wash away extracellular MRSA. Fresh culture medium containing 50 μg mL^{-1} gentamycin was then added and incubated for 1 h to inhibit the growth of extracellular bacteria on the surface of Raw 264.7 cells. After that, ampicillin (100 μg mL^{-1}), ZIF-90 (100 μg mL^{-1}) or ZnPc@ZIF-90-M (100 μg mL^{-1}) were added into the culture medium and incubated for 4 h. Then, the infected macrophages were irradiated with and without 638 ± 10 nm light at the power of 90 mW for 10 min. The survival of intracellular bacteria was assessed by using plate colony counting method. Macrophages were lysed with PBS solution supplemented with 1 % Triton-X, and serial dilutions of the lysate were made in PBS. The number of the surviving intracellular bacteria was determined by plating on LB agar plates.

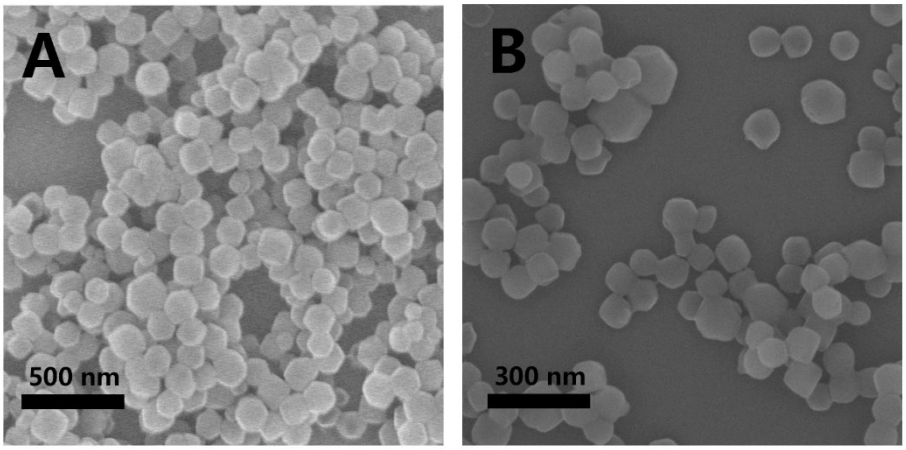

Figure S1. SEM images of ZnPc@ZIF-90 (A) and ZIF-90 (B).

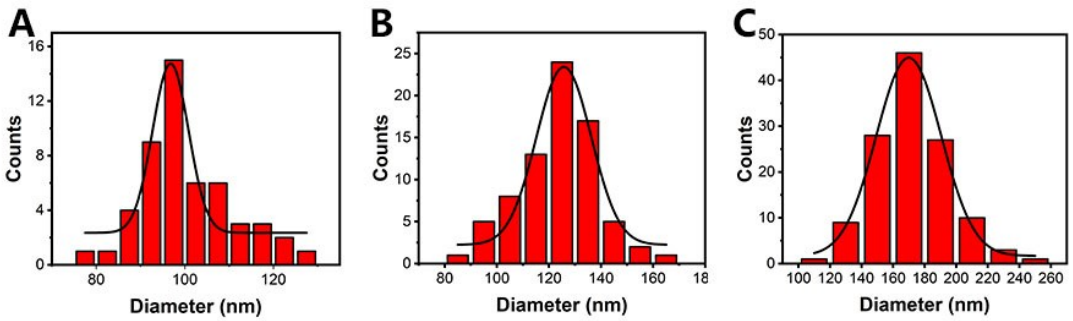

Figure S2. Size distribution histograms of ZIF-90 (A), ZnPc@ZIF-90 (B) and ZnPc@ZIF-90-M (C).

Table S1. Hydrodynamic size, PDI and Zeta potentials of ZnPc@ZIF-90-M.

	Hydrodynamic size (nm)	PDI	Zeta potentials (mV)
ZnPc@ZIF-90-M	269.9±4.8	0.022	11.1

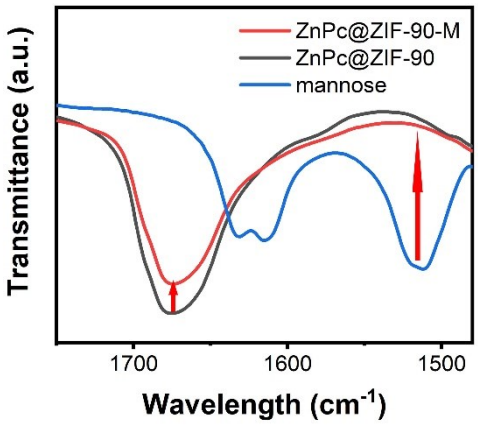

Figure S3. FT-IR spectra of ZnPc@ZIF-90, ZnPc@ZIF-90-M and Mannose.

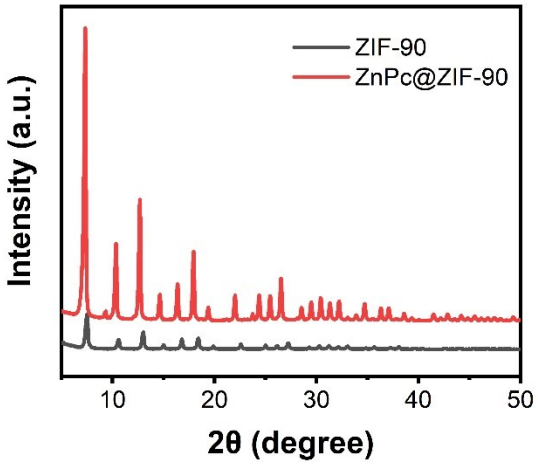

Figure S4. XRD patterns of ZIF-90 and ZnPc@ZIF-90.

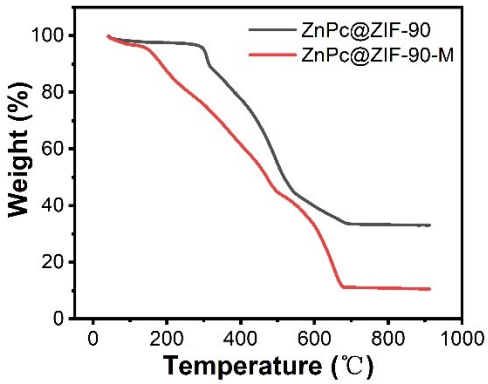

Figure S5. TGA curves of ZnPc@ZIF-90 and ZnPc@ZIF-90-M

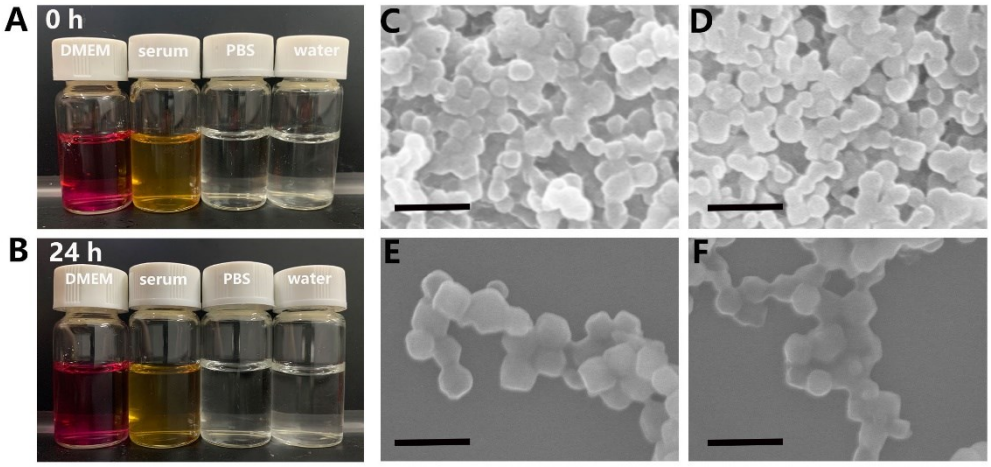

Figure S6. Photograph of ZnPc@ZIF-90-M dissolved in different solutions including DMEM, serum, PBS and water for 0 h (A) and 24 h (B). SEM images of ZnPc@ZIF-90-M dissolved in DMEM (C), serum (D), PBS (E) and water (F) after 24 h, and the scale bars represent 500 nm.

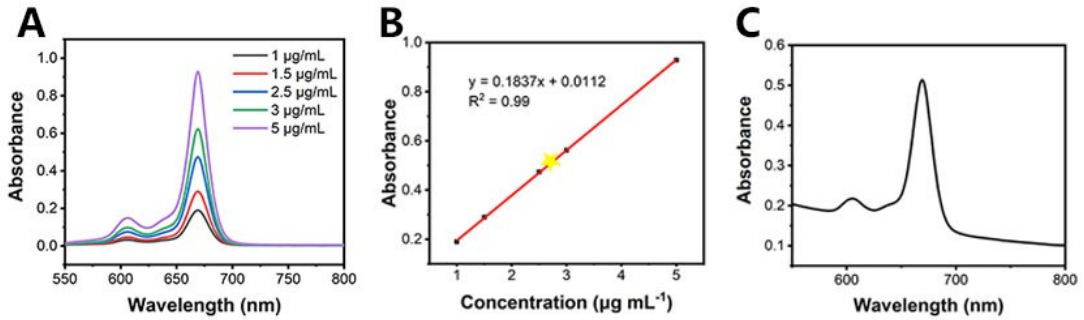

Figure S7. The loading amount of ZnPc on ZnPc@ZIF-90-M was calculated by UV-Vis spectrum. (A) Absorption spectrum of ZnPc with various concentrations from 1 μg mL^{-1} to 5 μg mL^{-1} in DMF. (B) The standard curve: the absorbance at 670 nm as a function of ZnPc concentration. The yellow star stands for the absorption at 670 nm of ZnPc@ZIF-90-M (0.5 mg mL^{-1}). (C) Absorption spectrum of ZnPc@ZIF-90-M with the concentration of 0.5 mg mL^{-1} in DMF.

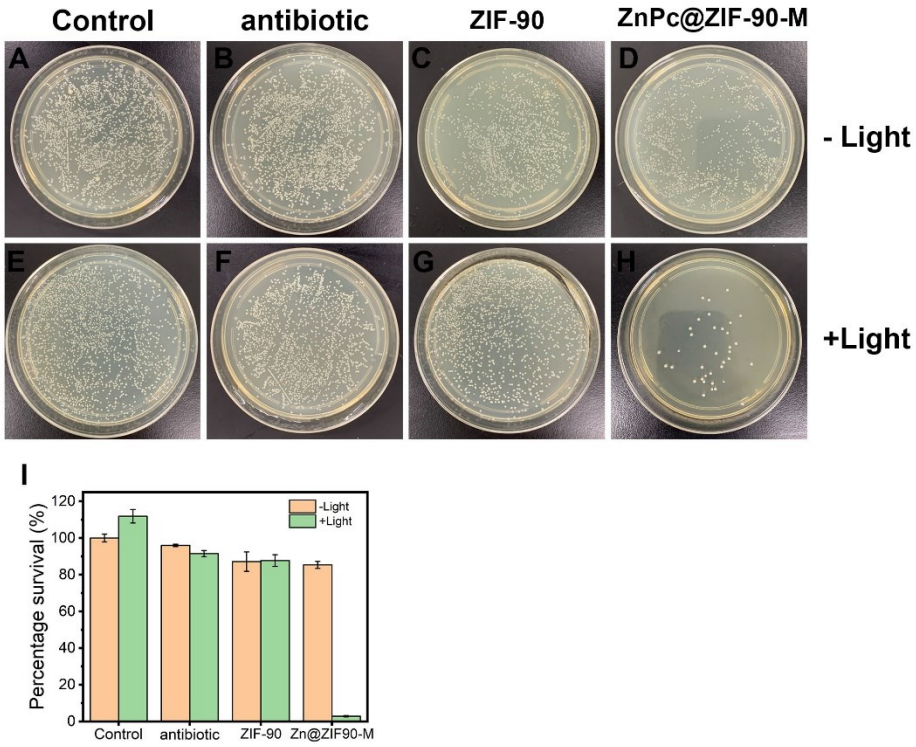

Figure S8. MRSA colonies on agar plates after treating with A) PBS, B) antibiotic, C) ZIF-90, D) ZnPc@ZIF-90-M, and E) PBS + light, F) antibiotic + light, G) ZIF-90 + light, H) ZnPc@ZIF-90-M + light. Antibiotic (100 μg mL^{-1} ampicillin), ZIF-90 (100 μg mL^{-1}), ZnPc@ZIF-90-M (100 μg mL^{-1}). (I) Percentage survival of MRSA after different treatments.

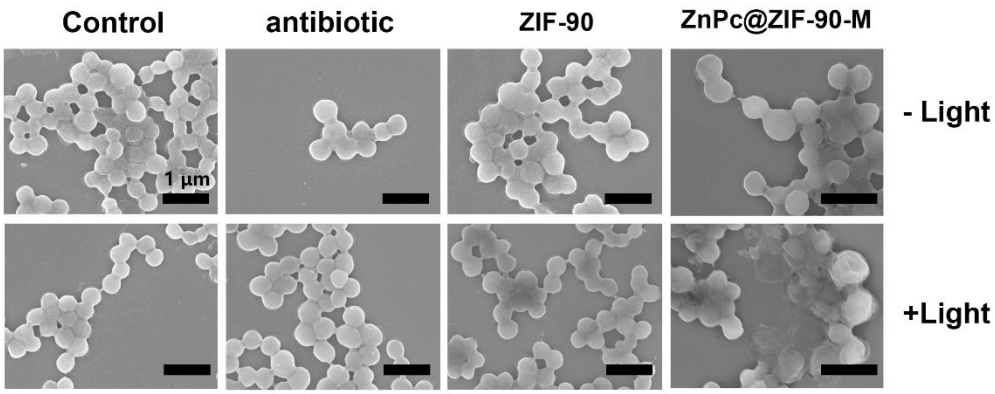

Figure S9. The SEM images of MRSA samples after different treatments. The scale bars represent 1 μm.

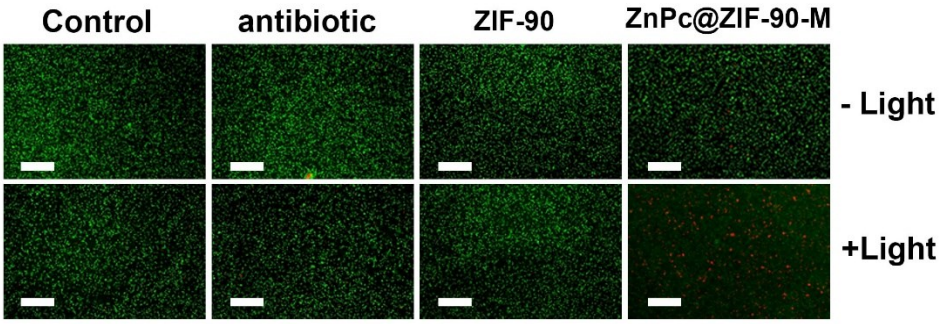

Figure S10. Live-dead fluorescence images of MRSA treated with different groups. Viable cells were stained green with FDA, and dead cells were stained red with PI. Scale bars represented 100 μm.

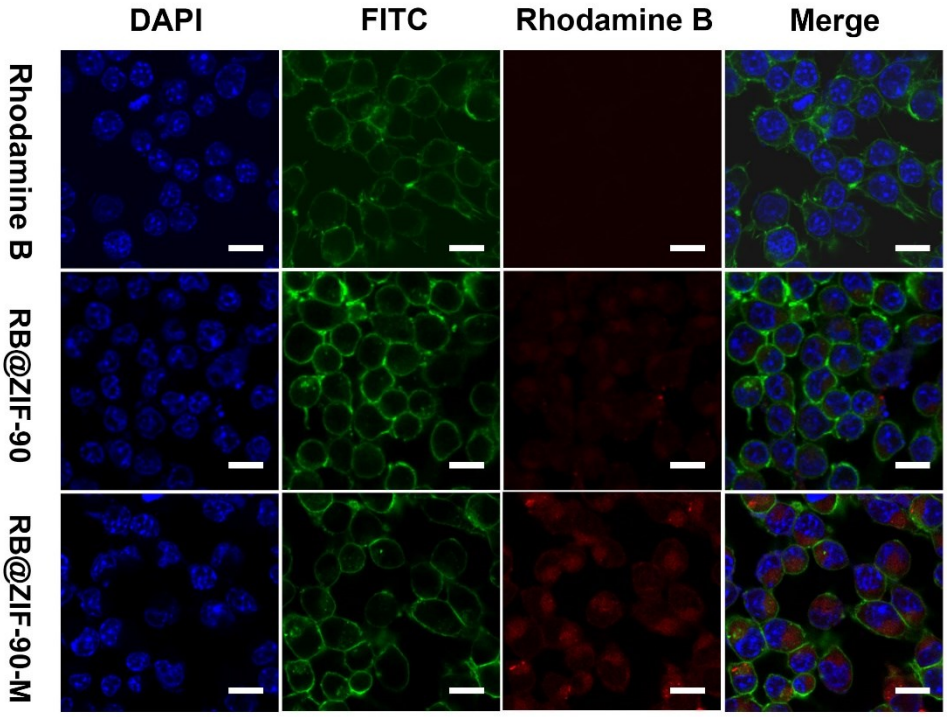

Figure S11. Confocal microscopy observation of cellular fluorescence intensity of Raw 264.7 cells incubated with Rhodamine B, RB@ZIF-90 and RB@ZIF-90-M. The scale bars represent 20 μm.

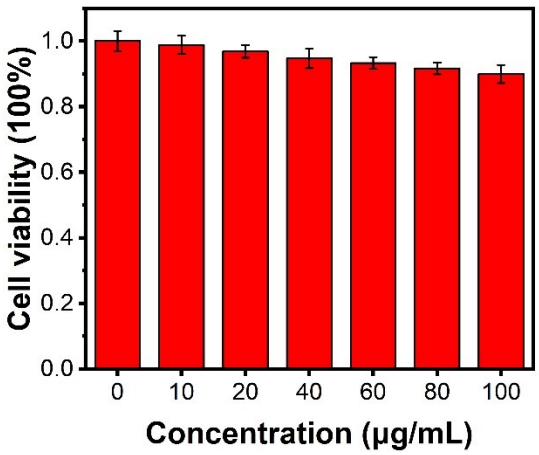

Figure S12. Cell viability of Raw 264.7 cells after incubated with ZnPc@ZIF-90-M for 24 h in the dark.