Supporting Information

Materials

3-Methyl-1H-1,2,4-triazole (Hmtz) was acquired from Bide Pharmatech Co., Ltd. (Shanghai, China). $Zn(NO_3)_2{\cdot}6H_2O$ was purchased from Xilong Chemicals (Guangdong, China). Doxorubicin·HCl (Dox) was obtained from Aladdin Reagents Co., Ltd (Shanghai, China). Dopamine-mPEG (DA-PEG) was purchased from ToYongBio Co., Ltd. DNase I was obtained from Thermo Fisher Scientific. All the chemicals were of analytical grade and used without further purification.

The sequences of oligonucleotides listed below were supplied by Sangon Biotechnology Co., Ltd. (Shanghai, China).

DNazyme: 5'-CAAGATCCAGGCTAGCTACAACGACCCGACCT-3'

Cy5-labeled DNAzyme: 5'-Cy5-CAAGATCCAGGCTAGCTACAACGACCCGACCT-3'

TAMRA-labeled DNAzyme (TAMRA-Dz): 5'-TAMRA-CAAGATCCAGGCTAGCTACAACGACCCGACCT-3'

Inactive DNA (CDz): 5'-GCGGATCGCGGCTAGCTACAACGACTAGGACT-3'

Substrate: 5'-AGGTCGGGrArUGGATCTTG-3'

FAM-labeled substrate: 5'-FAM-AGGTCGGGrArUGGATCTTG-3'

Apparatus and Characterization

Transmission electron microscopy (TEM) images were collected from a JEM-1011 transmission electron microscope (JEOL, Japan) with an accelerating voltage of 100 kV. Scanning electron microscopy (SEM) images were obtained from a Hitachi S-4800 scanning electron microscope (Hitachi Co., Japan). The ultraviolet-visible (UV-vis) and fluorescence measurements were performed on UV-3600 UV-Vis spectrometer (Shimadzu, Japan) and RF-5301PC fluorescence spectrometer (Shimadzu Co. Japan), respectively. Zeta potential analysis was conducted on a Nano-Z zeta potential analyzer (Malvern Instruments, USA). Dynamic light scattering (DLS) characterization was carried out on a 90 Plus Nanoparticle Size Analyzer (Brookhaven, USA). Confocal laser scanning microscopy (CLSM) images were collected from a TCS SP8 confocal laser microscope (Leica, Germany). Flow cytometry analysis was performed on a Cytomics FC 500 MCL instrument (Beckman Coulter, USA). Powder X-ray diffraction (PXRD) patterns were acquired from a D8 ADVANCE X-Ray powder diffractometer (Bruker AXS, Germany). The native polyacrylamide gel electrophoresis (PAGE) and western blot (WB) analysis were operated on GelDoc-XRTM with Image Lab software (Bio-Rad, USA).

Methods

Synthesis of Dz@MAF-7 NPs.

Dz@MAF-7 NPs were synthesized *via* a mild biomineralization approach. Briefly, DNAzyme (10 μL, 100 μM) was pre-incubated with Hmtz (250 μL, 800 mM) for 10 min. Then, 10 μL $NH_3{\cdot}H_2O$ (10%) and 250 μL

$Zn(NO_3)_2 \cdot 6H_2O$ aqueous solution (40 mM) was added sequentially. The mixture was stirred for 3 min at room temperature, followed by 3 cycles of washing and centrifugation (10,000 rpm, 8 min) to isolate the product.

Synthesis of Dz-Dox@MAF-7 NPs

The synthesis procedure of Dz-Dox@MAF-7 NPs was similar to that of Dz@MAF-7 NPs, except that 0.2 mg Dox was added immediately after the addition of $Zn(NO_3)_2 \cdot 6H_2O$ aqueous solution.

Synthesis of Dz-DOX@MAF-7-P NPs

To modify Dz-Dox@MAF-7 NPs with DA-PEG, 0.7 mg Dz-Dox@MAF-7 NPs were dispersed in 1 mL DA-PEG-containing aqueous solution (2 mg mL^{-1}) and stirred at room temperature for 2 h. The product was centrifuged and washed 3 times with deionized water, and finally resuspended in deionized water.

Synthesis of Dz-on-MAF-7 and TAMRA-Dz-Dox-on-MAF-7.

Nanoscale MAF-7 biocomposites were fabricated by adding $NH_3 \cdot H_2O$ (10 μL, 10%) and $Zn(NO_3)_2 \cdot 6H_2O$ (250 μL, 40 mM) to Hmtz (250 μL, 800 mM) in sequence followed by 3 min of stirring. With the extension of the reaction time (10 min) and subsequent aging for 3 h, microscale MAF-7 particles were obtained. To prepare TAMRA-Dz-Dox-on-MAF-7, the resulting microscale MAF-7 were incubated with the mixture of TAMRA-Dz (10 μL, 100 μM) and Dox (0.2 mg, 30 μL) for 3 min. After centrifugation and washing, the precipitate was re-dispersed in deionized water. Similarly, nanoscale MAF-7 was dispersed in DNAzyme-containing water to prepare Dz-on-MAF-7.

ATP-triggered cargo release

The loading capacity for Dox and TAMRA-Dz was determined by UV-Vis and fluorescence measurement, respectively. To monitor the release of Dox, 0.14 mg of Dz-Dox@MAF-7-P NPs were suspended in neutral PBS buffer containing different concentrations of ATP (400 μM, 2 mM and 10 mM) or pH 5.0 PBS. At specific time intervals, 90 μL of the solution was taken and centrifugated (12,000 rpm×15 min). Afterwards, 60 μL of supernatant was collected and injected into 96-well plates for UV-Vis measurement. The absorption spectra of Dox was recorded by a Microplate Reader (BioTek, USA).

To study the DNAzyme release behavior, TAMRA-Dz-Dox@MAF-7-P NPs were incubated with PBS buffer containing different concentrations of ATP (400 μM, 2 mM and 10 mM) for 0.5, 4, and 12 h. Next, each group was centrifuged (12,000 rpm, 15 min), and the precipitate was collected, washed and digested with NaOH aqueous solution (10 μL, 2 M) to liberate the encapsulated TAMRA-Dz for quantitative fluorescence analysis. Finally, all the samples were diluted to 100 μL with PBS (pH 7.4, 10 mM) and the fluorescence spectra ranging from 585 nm to 800 nm were collected under excitation at 560 nm.

Polyacrylamide gel electrophoresis (PAGE)

To investigate the biocatalytic activity of DNAzyme, 2 μM DNAzyme and 3 μM substrate with or without

FAM labeling were pre-incubated for 3 h at 37°C. After that, the mixtures were further treated with different concentrations of Zn^{2+} ions for 1 h, and then subjected to PAGE analysis. Meanwhile, Dz-Dox@MAF-7-P NPs (0.6, 0.9 and 1.5 mg mL^{-1}) stimulated by ATP were reacted with 3 μM substrate for 1 h, and then analysed by PAGE.

DNase I and fetal bovine serum (FBS) were introduced to verify the protective effect of MAF-7 coating on DNAzyme. Free DNAzyme and Dz@MAF-7 NPs were treated with DNase I (1 U mL^{-1}) at 37°C for 24 h, and then DNase I was denatured by heating at 80°C for 30 min. Similarly, Dz@MAF-7 NPs and Dz-on-MAF-7 NPs were treated with FBS at 37°C for 24 h followed by centrifugation and washing. Afterwards, all the precipitated samples were digested with NaOH solution (2 μL, 2 M) to release the remaining DNAzyme for subsequent PAGE assay. In the PAGE experiments, the above samples were diluted with loading buffer and loaded into 12% native polyacrylamide gels. The electrophoresis was performed in TBE buffer (89 mM Tris, 89 mM boric acid, 2.0 mM EDTA, pH 8.3). After staining in diluted GelRed™ solution for 30 min, the gels were imaged using GelDoc-XRTM with Image Lab software (Bio-Rad, USA).

Cell culture

K562 cells (human chronic myelogenous leukemia cell line), Dox-resistant K562 cells (K562/D cells) and L02 cells (normal liver cells) were obtained from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). K562 cells and K562/D cells were cultured in RPMI 1640 medium containing 10% FBS, penicillin (80 U mL^{-1}) and streptomycin (0.08 mg mL^{-1}). Particularly, for K562/D cell culture, the concentration of Dox in the culture medium was gradually elevated to 800 ng mL^{-1} to maintain the Dox resistance of K562/D cells. L02 cells were cultured in DMEM medium containing 10% FBS, penicillin (80 U mL^{-1}) and streptomycin (0.08 mg mL^{-1}). All the cells were cultured at 37°C in a 5% CO_2 atmosphere.

Confocal laser scanning microscope (CLSM)

To investigate the intracellular distribution of nanoscale MAF-7 biocomposites, K562/D cells (2×10^5 cells per well) seeded in 6-well plates were subjected to different treatments (PBS, Dox, Cy5-labeled DNAzyme, and Cy5-Dz-Dox@MAF-7 NPs). After 18 h of incubation, the cells were collected, and washed with sterile PBS, and stained with 4′,6′-diamidino-2-phenylindole (DAPI) for 10 min. Finally, the cells were washed and immersed in PBS buffer for microscopic observation using a TCS SP8 confocal laser microscope (Leica, Germany).

Flow cytometry

Flow cytomety was utilized to study the cellular uptake of Dz-Dox@MAF-7-P NPs. Briefly, K562/D cells (2×10^5 cells) were seeded in 6-well plates and incubated with Dz-Dox@MAF-7-P NPs for 0, 0.5, 1, 2 and 8 h, respectively. Then, the cells were washed 3 times and resuspended in 0.8 mL PBS. Finally, the Cytomics FC500–MCL instrument (Beckman Coulter, USA) was employed to record the intercellular fluorescence

intensity of Dox by counting at least 10,000 events.

Western blot analysis

After 48 h of treatment with PBS, DNAzyme, CDz@MAF-7-P NPs or Dz@MAF-7-P NPs, the K562/D cells were harvested, washed with cold PBS, and lysed in RIPA buffer containing protease inhibitor (Beyotime) for 20 min at 4°C. Proteins were collected in the supernatant after centrifugation (15,000 rpm, 20min, 4°C). The total proteins in each group were then quantified using BCA protein assay kits (Sangon) and diluted into the same concentration. After that, the proteins were separated with SDS-PAGE gradient gel (4%–20%) and transferred to the polyvinylidene fluoride membranes (PVDF) membrane. After blocked with 2% BSA for 1 h at room temperature, the membrane was incubated with primary antibody (ab170904, Abcam Trading Co., Ltd., Shanghai, China) at 4°C overnight. Subsequently, the membrane was incubated with HRP-labeled secondary antibody (A0208, beyotime, Shanghai, China) for 1 h at room temperature and then imaged by ECL Chemiluminescence using GelDoc-XRTM (Bio-Rad, USA) with Image Lab software.

***In vitro* Cytotoxicity assays**

MTT experiments were performed to evaluate the cytotoxicity of samples. Briefly, L02, K562/D and K562 cells (6×10^3 cells, 100 µL) were seeded in 96-well plates and treated with different formulations. After 48 h of incubation, the cells were centrifuged (1,000 rpm for 4 min) to discard the supernatant medium. 200 µL MTT solution (0.5 mg mL^{-1}) was then added to each well for another 4 h of incubation at 37°C. After that, the supernatant medium was replaced with 100 µL DMSO. The absorbance values at 490 nm were measured by a Microplate Reader (Biotek, USA).

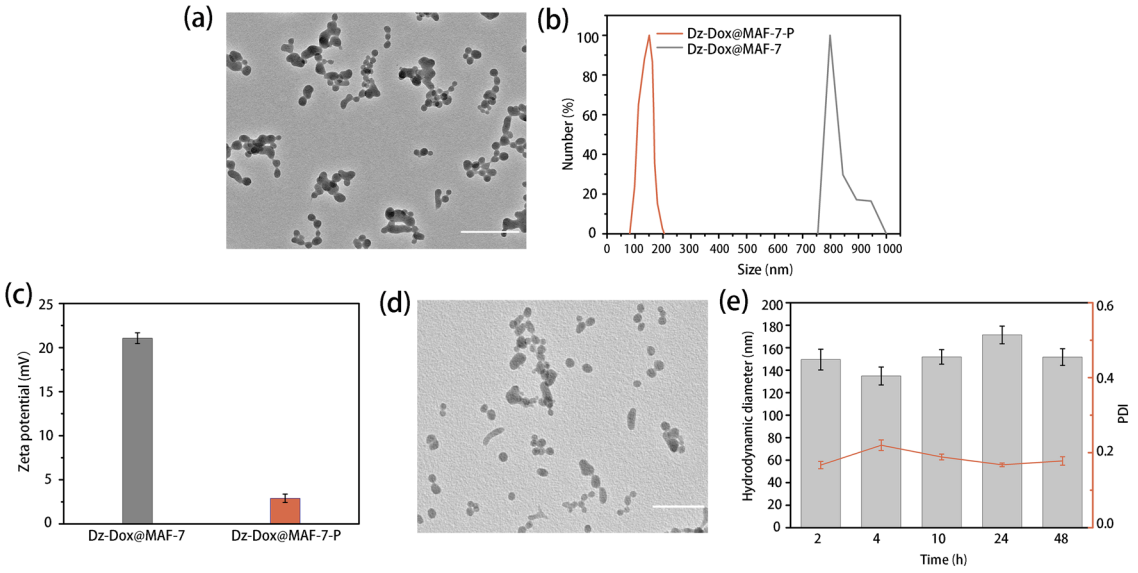

Fig. S1 (a) TEM image of Dz@MAF-7 (scale bar: 500 nm). (b) Dynamic light scattering (DLS) and (c) zeta potential of Dz-Dox@MAF-7 and Dz-Dox@MAF-7-P. (d) TEM image of Dz-Dox@MAF-7-P (scale bar: 500 nm). (e) Hydrodynamic diameter and PDI values of Dz-Dox@MAF-7-P NPs at different time points. Error

bars represent s.d. (n = 3).

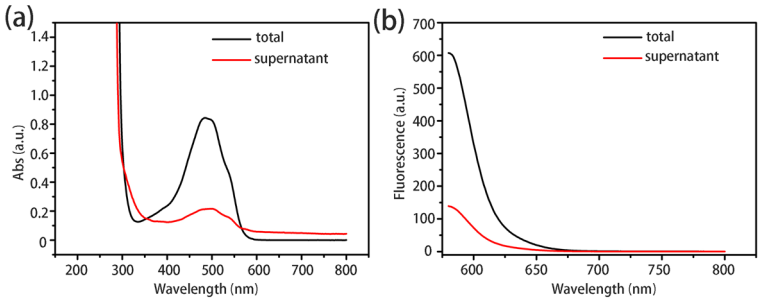

Fig. S2 (a) UV-Vis spectra of the total and supernatant Dox; (b) Fluorescence spectra of the total and supernatant TAMRA (Ex: 565 nm).

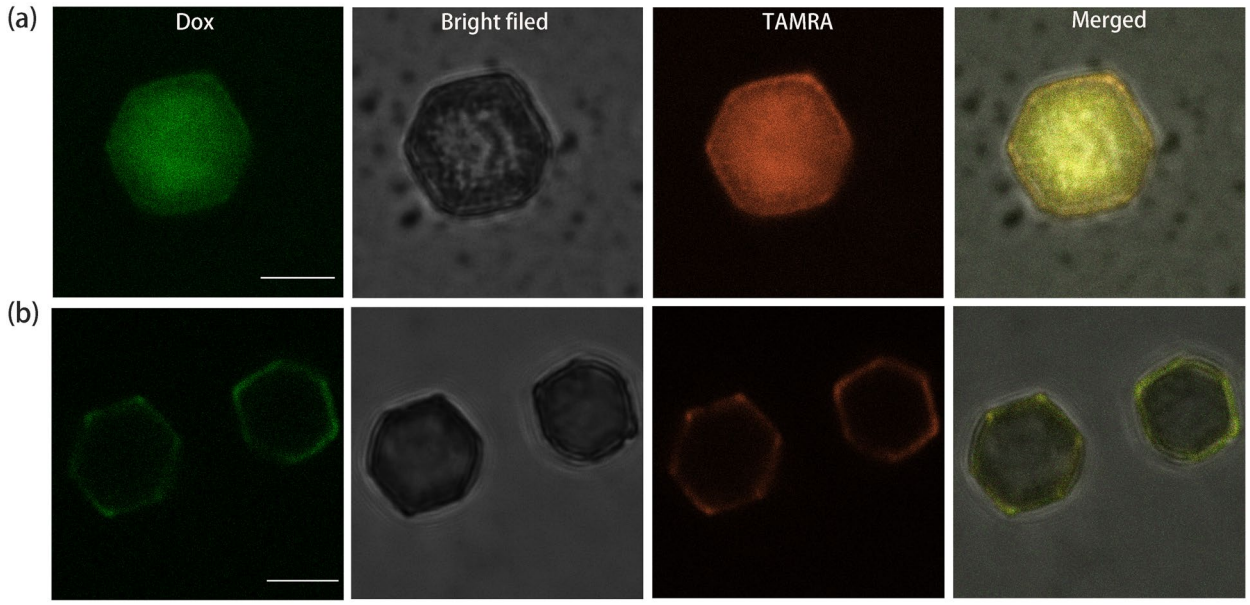

Fig. S3 CLSM images of (a) TAMRA-Dz-Dox@MAF-7 and (b) TAMRA-Dz-Dox-on-MAF-7 particles (scale bar: 5 μm).

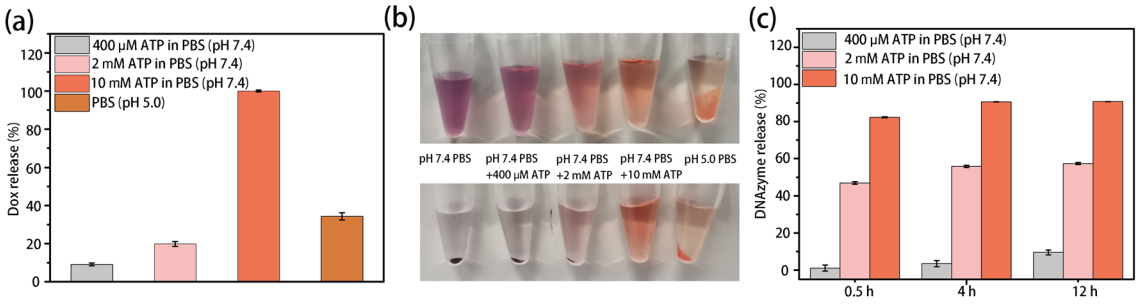

Fig. S4 (a) The release percentage of Dox in different groups at 24 h; (b) Photographs of Dz-Dox@MAF-7-P NPs under different stimuli. The bottom row displays the corresponding samples after centrifugation; (c) The release ratio of DNAzyme under various stimuli at different time points (0.5, 4 and 12 h). Error bars represent s.d. (n = 3).

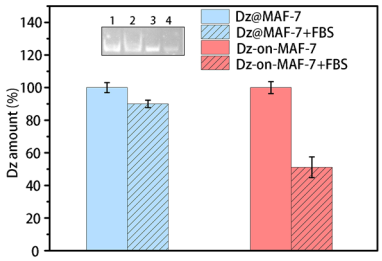

Fig. S5 The residual DNAzyme amount of Dz@MAF-7 and Dz-on-MAF-7 NPs after FBS treatment. Inserts are the corresponding PAGE images. Lane 1: Dz@MAF-7; lane 2: Dz@MAF-7+FBS; lane 3: Dz-on-MAF-7; lane 4: Dz-on-MAF-7+FBS. Error bars represent s.d. (n = 3).

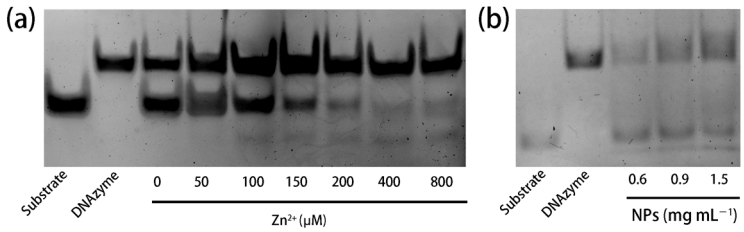

Fig. S6 PAGE analysis of (a) DNAzyme-mediated cleavage reaction under different Zn^{2+} concentrations (0, 50, 100, 150, 200, 400, 800 μM) and (b) Dz-Dox@MAF-7-P NPs-triggered substrate cleavage reaction under different concentrations (0.6, 0.9 and 1.5 mg mL^{-1}).

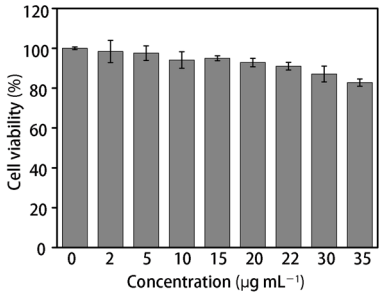

Fig. S7 Cytotoxicity of CDz@MAF-7-P NPs to K562/D cells. Error bars represent s.d. (n = 3).

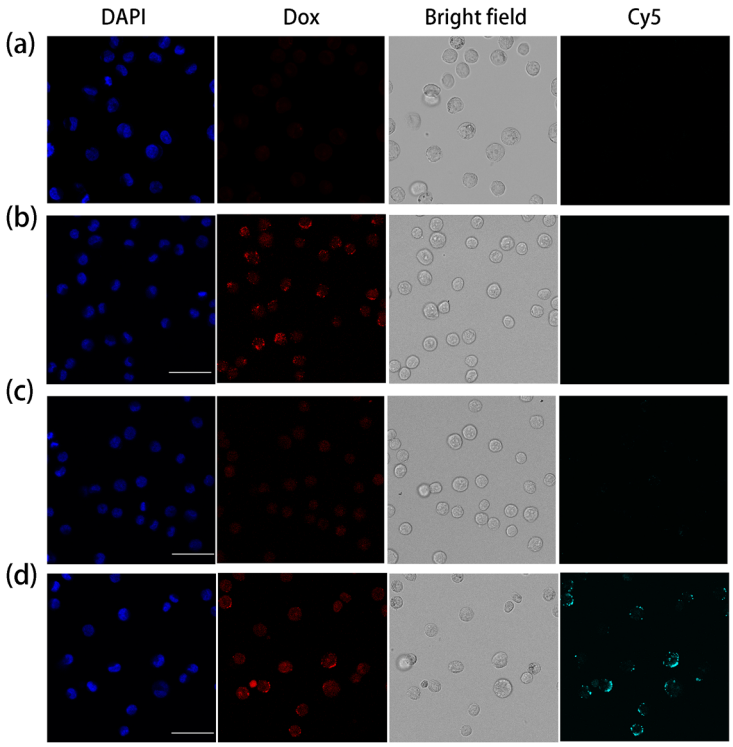

Fig. S8 CLSM images of K562/D cells after treatment with (a) PBS; (b) Dox; (c) Cy5-labeled DNAzyme; (d) Cy5-Dz-Dox@MAF-7-P (scale bar: 50 μm).

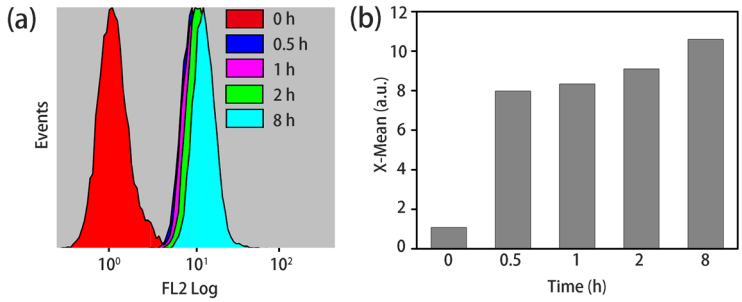

Fig. S9 (a) Flow cytometry analysis and (b) quantitative median fluorescence intensity of Dox fluorescence insides K562/D cells treated with Dz-Dox@MAF-7-P NPs for different time (0, 0.5, 1, 2 and 8 h).

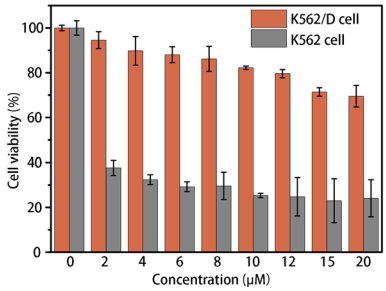

Fig. S10 Cell survival rate of K562/D and K562 cells receiving a series of doses of Dox. Error bars represent s.d. (n = 3).

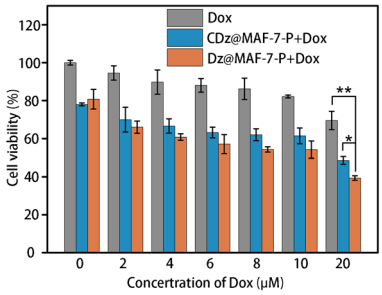

Fig. S11 Cell viability of K562/D cells treated with equivalent Dz@MAF-7-P or CDz@MAF-7-P NPs along with a series of concentrations of Dox. Error bars represent s.d. (n = 3). (*p<0.05 or **p<0.01 is analyzed by student's t-test.)

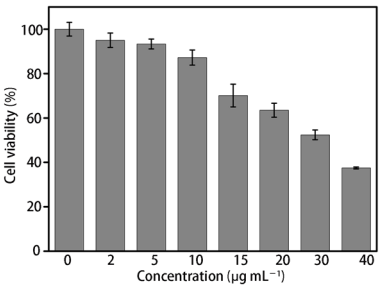

Fig. S12 Cytotoxicity of Dz-Dox@MAF-7-P NPs against K562/D cells. Error bars represent s.d. (n = 3).