Supplementary Information

DNA-spheres Decorated Magnetic Nanocomposite Based on Terminal Transfer Reaction for Versatile Target Detection and Cellular Targeted Drug Delivery

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EXPERIMENTAL SECTION

Reagents and materials. N-ethylmaleimide (NEM) was purchased from Sigma-Aldrich, China. T4 DNA ligase, and phi29 DNA polymerase were purchased from Thermo Scientific. Doxorubicin hydrochloride (DOX) and dNTP were purchased from Bio Basic Inc. Terminal transferase was purchased from New England Biolabs. Other reagents were analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. Sequences of the oligonucleotides listed in Table S1 was purchased from Sangon Biotech Co., Ltd. Shanghai China. obtained. All animal experimental procedures and techniques were approved by the Animal Ethics Committee of East China Normal University, and methods were carried out in accordance with the approved guidelines and laws.

Table S1 Oligonucleotide sequences

	Sequences (5'-3')
Primer 1	AAA AAA AAA AAA AAA TCT AAC TGC TGC GCC GCC GGG AAA ATA CTG TAC GGT TAG ATG CTG CTG C
Primer 2	Bio- TCT AAC TGC TGC GCC GCC GGG AAA ATA CTG TAC GGT TAG ATG CTG CTG C
Disulfide bond modified primer 3	TTT TTT TTT TTT TTT-/HS-SH/-TTT-Bio
Template	Phosphate- TTC CCG GCG GCG CAG CAG TTA GAT GCT GCT GCA GCG ATA CGC GTA TCG CTA TGG CAT ATC GTA CGA TAT GCC GCA GCA GCA TCT AAC CGT ACA GTA TT
Folic acid	Folate-TTT TTT TTT TTT TTT TTT

modified poly (T)

sequence

Apparatus

Fluorescent spectra were observed under an F-4600 Fluorescence Spectrophotometer (HITACHI, Japan). Bioimaging was performed using confocal laser scaning microscopy (CLSM) on a Leica TCS SP8 confocal microscope (Leica Microsystems Inc., Exton, PA) in DIC mode. Transmission electron microscopic (TEM) images were recorded transmission electron microscopy (JEM-2100, HITACHI, Japan). Ultraviolet spectra were conducted on a Cary 60 UV-Vis spectrometer (Agilent Technologies, USA). The surface charge was measured using Zeta PALS (Malvern Instruments Limited). Flow cytometry were performed on a FACScan cytometer (Cytoflex, Beckman Coulter, Inc., Suzhou China).

Preparation of DNA-SP

0.6 μL of phosphorylated linear templates (100 μM,) and 1.2 μL of primer 1 (100 μM) was mixed in 97.7 μL Dulbecco's PBS buffer (pH 7.4), annealed by heating at 95 ºC for 2 min, followed by gradual cooling to 25 ºC over 3 h in a PCR instrument. The annealed product was incubated with 0.5 μL of T4 DNA ligase (2000 U/μL) at 25 ºC for 4 h to attain a circular DNA template. For RCR, the circular DNA templates were then incubated with 40 μL of phi29 DNA polymerase (10 U/μL), 40 μL of dNTP (10 mM) and 20μL of PBS buffer at 30 ºC for 10 h. Reactions were terminated by heating at 75 ºC for 10min. Finally, DNA-SP were washed with double-distilled H_2O, precipitated by centrifugation, and stored at 4 ºC for use.

Preparation of MNP/DNA-SP. 0.6 μL of phosphorylated linear templates (100 μM) was mixed with 97.7 μL of D-PBS buffer pH 7.4), annealed by heating at 95 ºC for 2 min, followed by

gradual cooling to 55 ºC and then 1.2 μL of primer 2 (100 μM) was added to continue to cool down to 25 ºC in a PCR instrument. The annealed product was incubated with 0.5 μL of T4 DNA ligase (2000U/μL) and 10 μL of MNP at 25 ºC for 4 h to attain a circular DNA template combined with MNP. For RCA, the circular DNA templates were then incubated with 40 μL of phi29 DNA polymerase (10U/μL), 40 μL of dNTP (10 mM) and 10 μL of PBS buffer at 30 ºC for 10 h. Reactions were terminated by heating at 65 ºC for 10 min. Finally, MNP/DNA-SP were washed with double-distilled H_2O and stored at 4 ºC for future use. To load DOX into MNP/DNA-SP, DOX (1 mM) and MNP/DNA-SP were mixed and incubated at 25 ºC for 24 h, followed by magnetic separation to remove free DOX in the supernatant.

Preparation of MNP/DS-SP. 1.2 μL of phosphorylated linear templates (100 μM) was mixed with 97.7 μL of D-PBS buffer pH 7.4), annealed by heating at 95 ºC for 2 min, followed by gradual cooling to 55 ºC and then 0.6 μL of primer 1 (100 μM) was added to continue to cool down to 25 ºC in a PCR instrument. The annealed product was incubated with 0.5 μL of T4 DNA ligase (2000U/μL) at 25 ºC for 4 h to attain a circular DNA template. For RCA, the circular DNA templates were then incubated with 40 μL of phi29 DNA polymerase (10U/μL), 40 μL of dNTP (10 mM) and 10 μL of PBS buffer at 30 ºC for 10 h. Reactions were terminated by heating at 65 ºC for 10 min. 10 μL of MNP was reacted with primer 3 at 25 ºC for 1 h and then washed by magnetic separation to form MNP-primer 3. The RCA product was washed three times with PBS buffer (pH7.4) and then reacted with MNP-primer 3 to form MNP/DS-SP. Finally, MNP/DNA-SP were washed with double-distilled H_2O and stored at 4 ºC for future use. To load DOX into MNP/DS-SP, DOX (1 mM) and MNP/DS-SP were mixed and incubated at 25 ºC for 24 h, followed by magnetic separation to remove free DOX in the supernatant.

Preparation of folic acid modified MNP/DNA-SP. MNP/DNA-SP mentioned above was dispersed in D-PBS buffer in a total volume of 156 μL. Then 2 μL of dATP (100 mM), 2 μL of terminal transferase (TdT, 20 U/μL), 20 μL of $CoCl_2$ (2.5 mM) and 20 μL of TdT buffer were added to make a adenine tail at 37 ºC for 30min followed by 70 ºC for 10 min to terminate reaction. 10 μL of the folic acid (FA) modified poly (T) sequence (100 mM) were hybridized at 37 ºC for 1 h to attain the FA modified MNP/DNA-SP (MNP/FA-SP). Reactions were terminated by heating at 70 ºC for 10 min. The products were washed three times by Dulbecco's PBS buffer, and stored at 4 ºC for future use. To load DOX into MNP/DNA-SP, DOX (1 mM) and MNP/DNA-SP were mixed and incubated at 25 ºC for 24 h, followed by magnetic separation to remove free DOX in the supernatant.

Cell culture

CCRF-CEM cell, Ramos cell, HeLa cell and MCF-7 cell were cultured in DMEM culture medium containing 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humid atmosphere with 5% CO_2. The medium was replenished every other day, and the cells were subcultured after reaching confluence. Cell density was determined using a hemocytometer prior to each experiment.

Preparation of cell lysate

A 1.0 mL of 2.1×10^5 suspended cells was centrifuged at 1000 rad min^{-1} for 5 min in culture medium, washed twice with ice-cold PBS buffer (pH 7.4). After that, they were suspended again in PBS buffer in EP tube. Cell homogenates were obtained by disrupted cell suspensions for 30 min in ultrasonic disintegrater. During sonic disruption, the temperature was maintained below 4 °C with ice bath.

Thiols detection

100 μL GSH sample (or cell lysate) was added to MNP/DS-SP solution. After incubated at 37 °C, the mixture was separated with a magnetic rack. The supernatant was maintained in 25 °C for 24 h away from light. Finally, the signal was detected by fluorescence spectrophotometry.

Gel electrophoresis

The size of circular template/primer, linear template, and DNA-SP were characterized via gel electrophoresis. Agarose gels were prepared by mixing agarose (2%) with TB buffer. Gel electrophoresis was conducted at 100 V for 100 min, stained with ethidium bromide, and gel images were visualized using a UV transilluminator.

Gel electrophoresis was applyed to distinguish the resultant of circular DNA template structures from these hybridized and ligated linear template strands. Band shifts certified the variation from linear template (line 2) to circular template/primer (line 1), illustrating the prosperity of producing a circular template/primer (Fig. S1). Successful construction of DNA-SP (lane 3) was also confirmed using gel electrophoresis, as seen by the slower migration rates compared with circular template (lane 1).

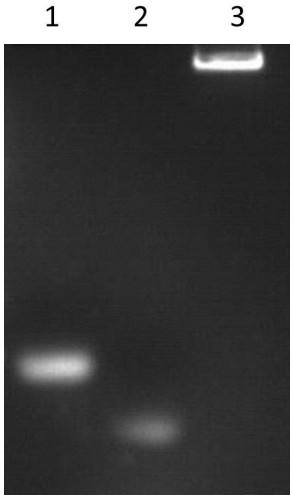

Fig.S1. Gel electrophoresis image of multiple types of linear, circular DNA templates and DNA-SP. Bandshifts were observed, circular template/primer (lane 1), linear template (lane 2) and DNA-SP (lane 3).

DNA-SP, MNP and MNP/DNA-NP were investigated by UV−vis spectra. Compared with the MNPs, an obvious peak of DNA at ~260 nm was observed with MNP/DNA-NP, indicating the successful decoration of DNA-NP on MNPs (Fig. S2A). TEM and confocal microscopy images (Fig. S2B) show that DNA-SP were spherical in shape and congruously nanometer-sized complex particles. We get the DNA-SP with the size of about ~40 nm. The surface charge of MNP and MNP/DNA-SP were determined by zeta potential measurements. The samples were diluted in double-distilled H2O and all measurements were carried out at 25 °C. The decreased potential

from −13 to −24 mV (Fig. S2C) suggested that DNA-SP had been successfully attached to the surface of MNP (MNP/DNA-SP) in a covalent manner. TEM results and EDX data ulteriorly attest the entire self-assembly process (Inset of Fig. S2D). We get the MNP/DNA-SP in a size of about 150 nm.

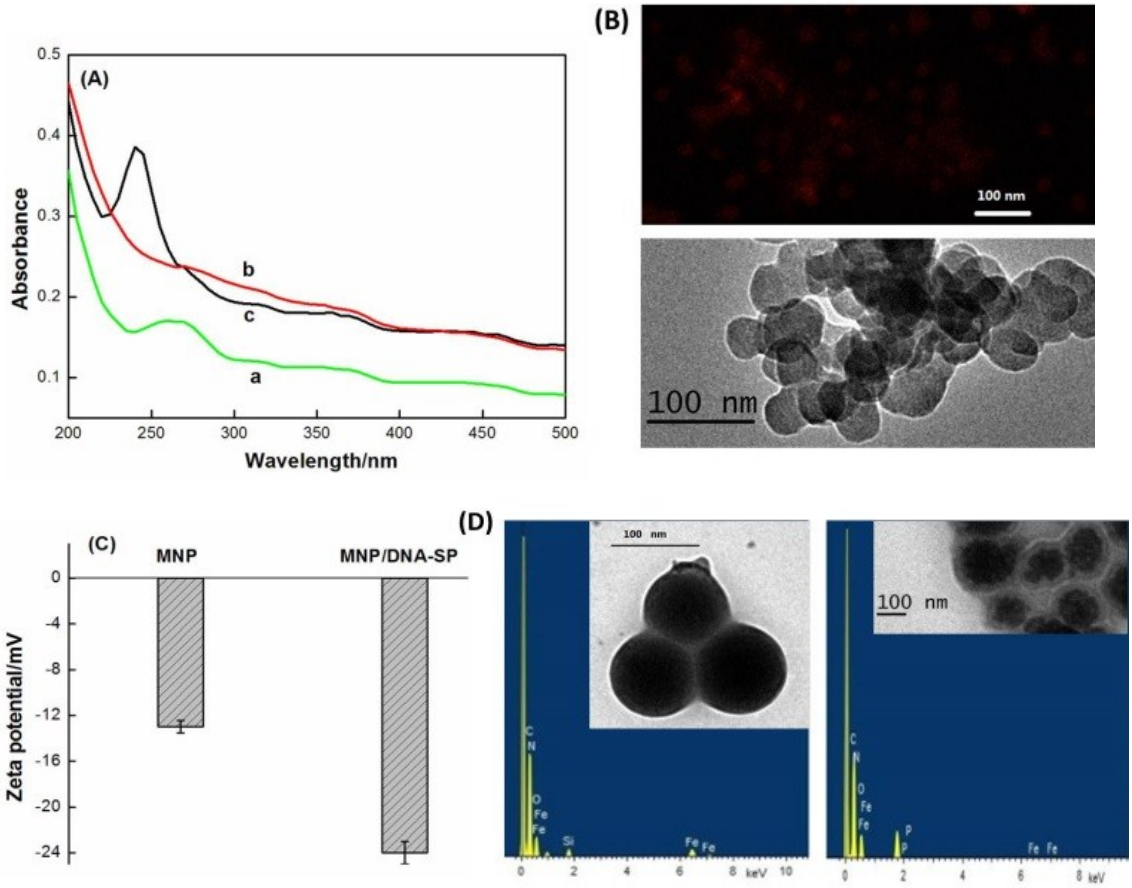

Fig. S2 (A) UV−vis spectra of (a) MNP/DNA-SP, (b) MNP and (c) DNA-SP. (B) (up) confocal microscopy of DNA-SP and (down) TEM image of DNA-SP. (C) The Zeta potential values of (a) MNP and (b) MNP/DNA-SP. (D) EDX spectra of the obtained MNPs (left) and MNP/DNA-SP (right). Inset was TEM images of MNPs and MNP/DNA-SP.

Lecture experiment was used to state the significance of magnetic separation. The magnetization curves were used to investigate magnetic properties of multifunctional MNP/DNA-SP (Fig**.** S3). The saturation magnetization values of MNPs were 11.2 emu/g, which is mildly higher than that

of the MNP/DNA-SP (9.3 emu/g) coming out from the shrouded DNA. This result shows that either remnant magnetization or coercivity of the tested materials exhibits typical properties of magnetic. The magnetic separation effect was showed in the inset image of Fig. S3 after 10 min standing. We can see that MNP were good magnetic separation. A conclusion could be drawn that the magnetic multifunctional MNP/DNA-SP will be separated easily and directly when left in an external magnetic field.

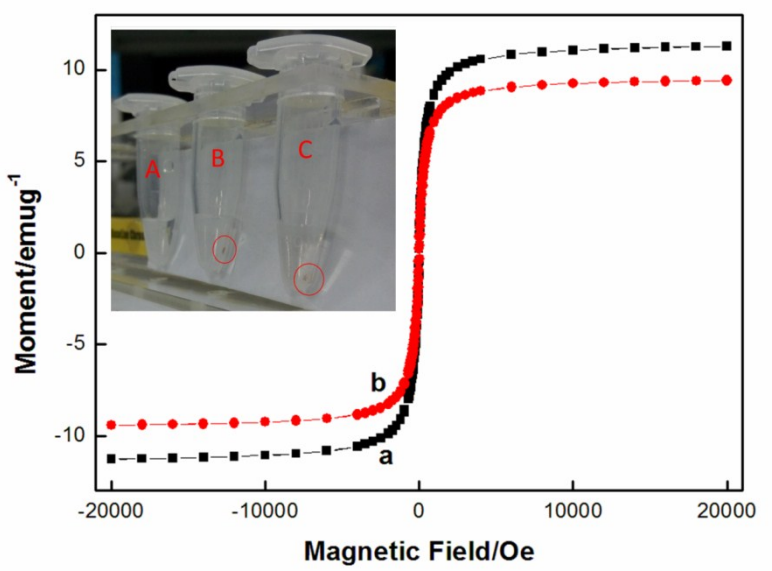

Fig.S3. Magnetization curves of (a) MNPs and (b) MNP/DNA-SP. Inset: magnetic separation effect (A) PBS buffer, (B)MNPs and (C) MNP/DNA-SP.

The assorted magnetic and fluorescence properties of MNP/DNA-SP and DNA-SP were tested simultaneously. We prepared DOX and Sybr Green I subsequently embedded them in MNP/DNA-SP and DNA-SP, respectively. Then, DOX/ MNP/DNA-SP were mingled with Sybr Green I/DNA-SP in aqueous solution, and followed separated with magnetic. The confocal microscope image of the compound solution (Fig. S4A) showed that red and green dots was concomitant, whereas only green dots was persistent after separation of DOX/MNP/DNA-SP with

the magnet (Fig. S4B), illustrating that green-emitting Sybr Green I/DNA-SP was drastically separated from the compound using a magnet.

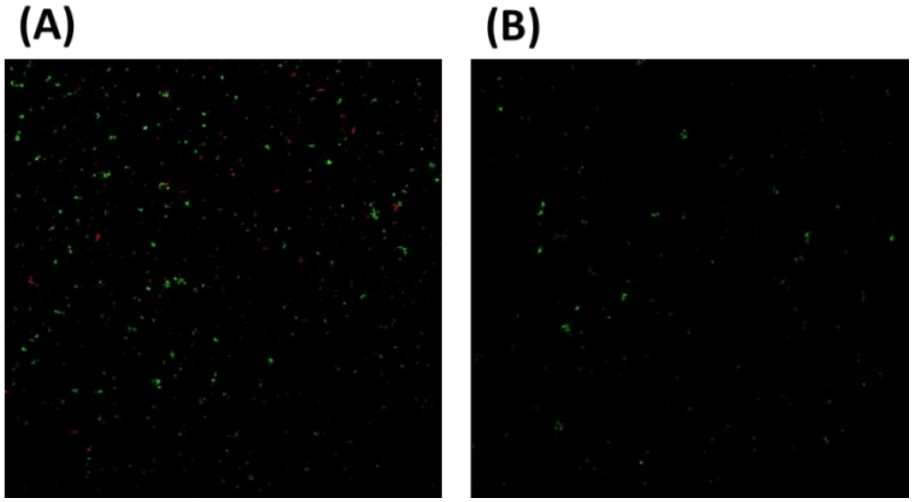

Fig. S4 Confocal microscope images from a mixture suspension of DOX/MNP/DNA-SP and Sybr green I/DNA-SP before (A) and after (B) removal of DOX/ MNP/DNA-SP by magnetic separation.

DOX drug was loaded on MNP/DNA-SP by intercalation. The data of fluorescence spectrometry demonstrated the piecemeal fluorescence quenching with increasing equivalents of MNP/DNA-SP. To load DOX into the MNP/DNA-SP, MNP/DNA-SP was dispersed into DOX solution for 48 h under mild vibration to achieve the equilibrium state. Then, the compounds were magnetic separated to take out the excrescent uncombined DOX. The upper solution was collected and then tested the fluorescence intensity. We calculated that the loading of DOX in MNP/DNA-SP is 83% on the basis of following equation: loading efficiency={(feeding amount of DOX-residual amount of DOX in supernatants) /feeding amount of DOX}×100%. This result revealed the high drug payload capacity.

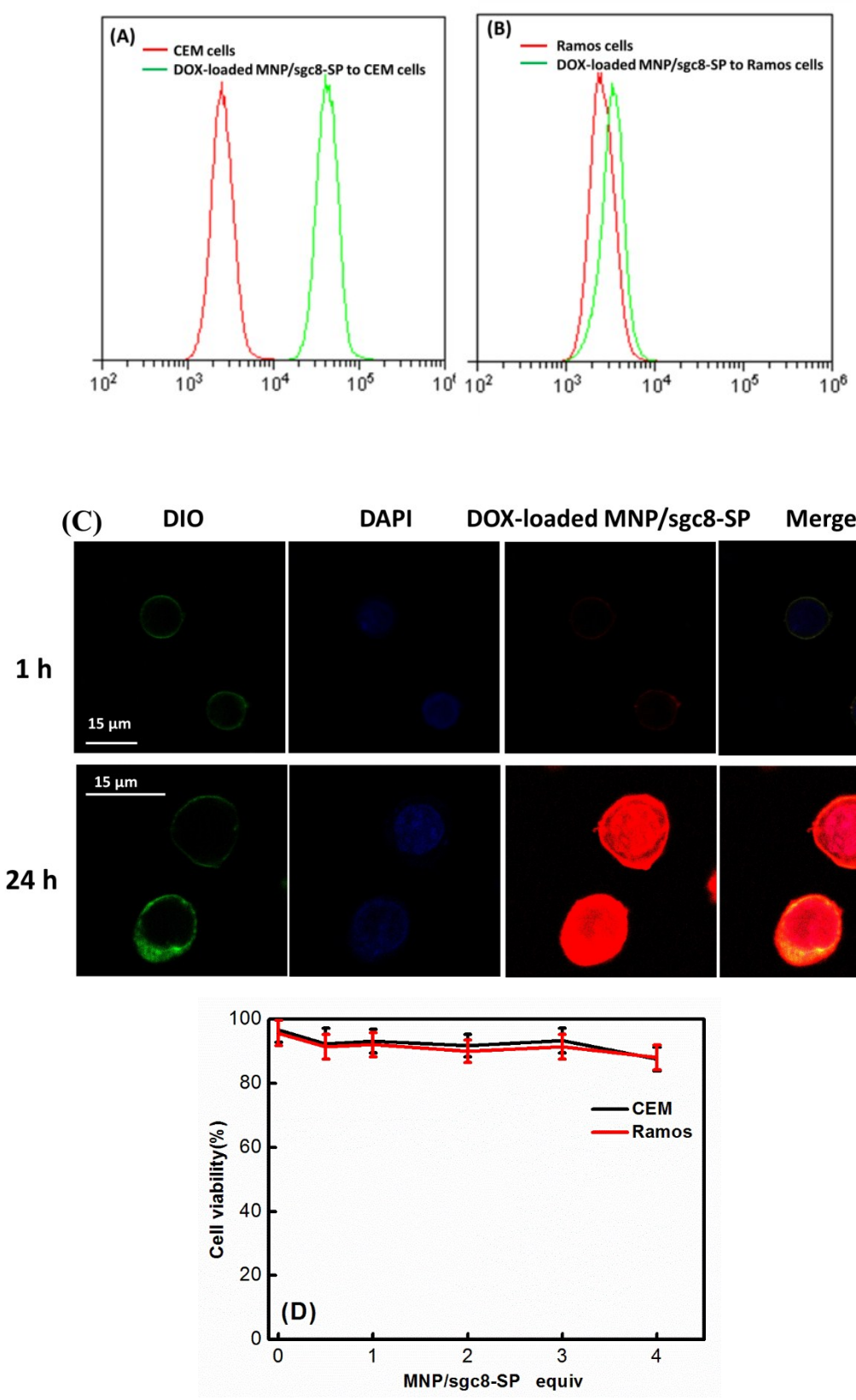

Fig. S5 (A) The selective recognition abilities of DOX-loaded MNP/sgc8-SP to target CEM cells from flow-cytometric results; (B) The selective recognition abilities of DOX-loaded

MNP/sgc8-SP not to nontarget Ramos cells from flow-cytometric results; (C) Images of CEM cells after incubation with (a) DIO, (b) DAPI, (c) DOX-loaded MNP/sgc8-SP, (d) merged images. (D) The cytotoxicity of MNP/sgc8-SP in both CEM cell and Ramos cell.

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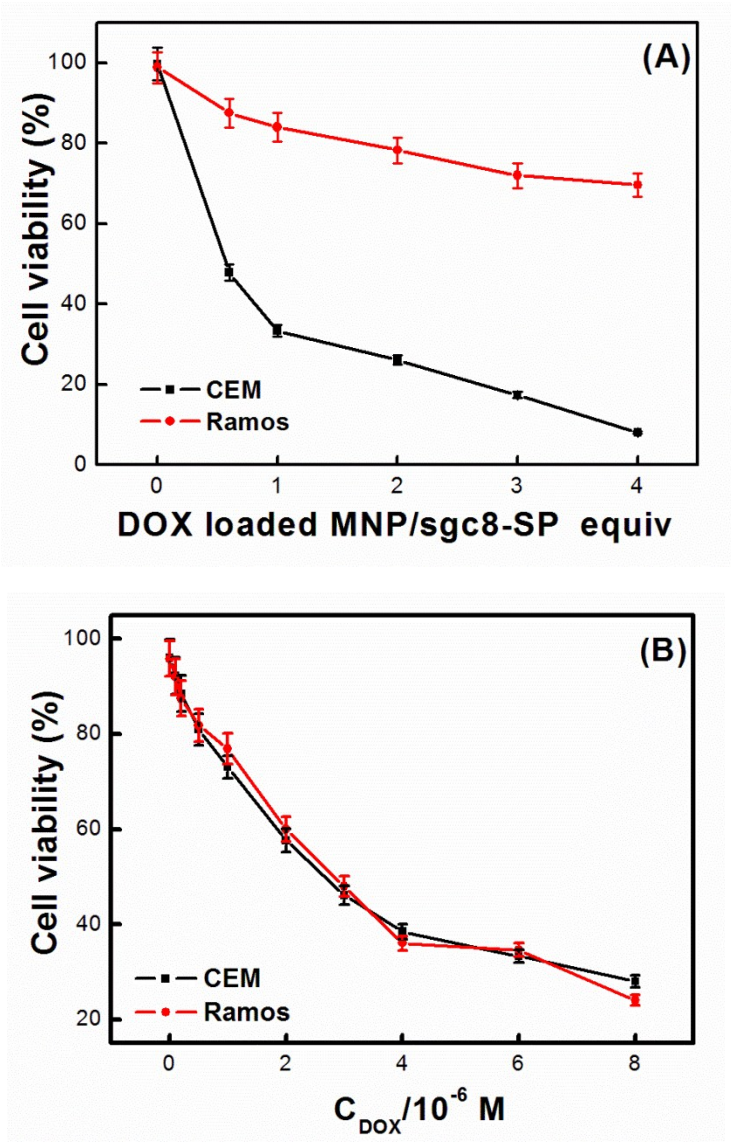
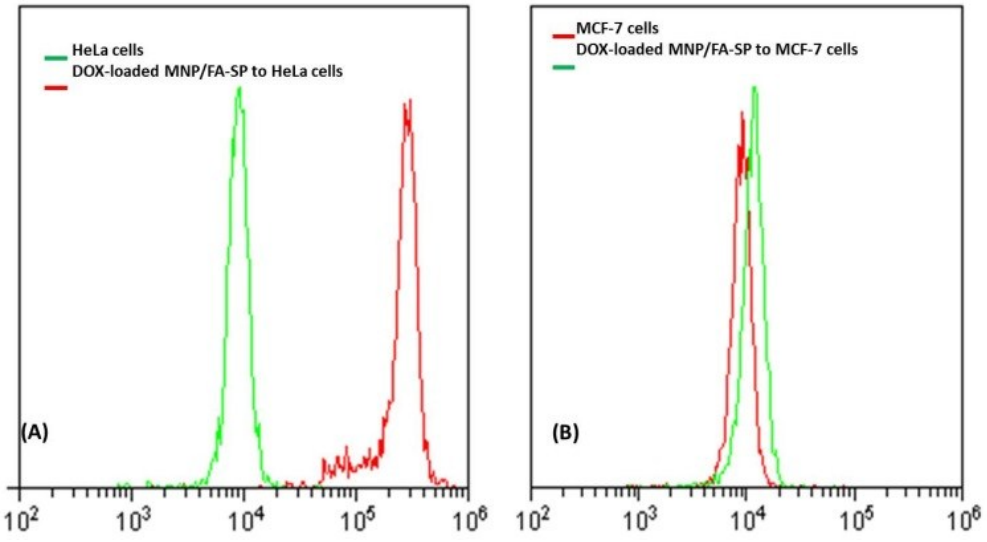
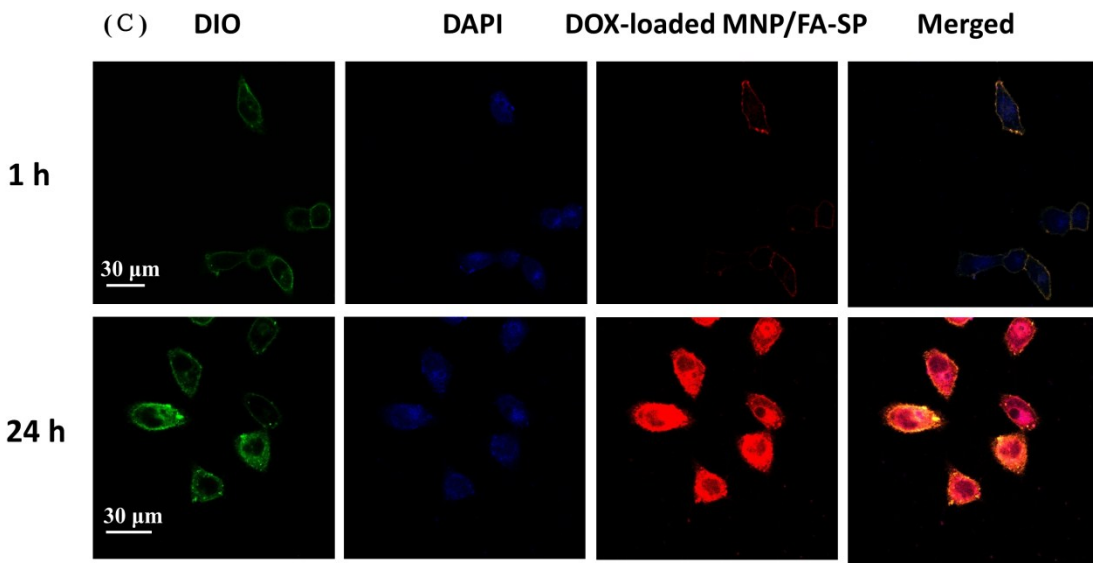


Fig.S6 Selection in recognition of cancer cell and targeted delivery of drug about DOX-loaded MNP/sgc8-SP in CEM cell and Ramos cell. (A) cytotoxicity of DOX delivered by MNP/sgc8-SP; (B) cytotoxicity of free DOX.

Both HeLa cell and MCF-7 cell showed high cell viability (above 90%) after 4 h incubation with MNP/FA-SP (Fig. S7D). So the MNP/FA-SP was basically noncytotoxic, indicating that MNP/FA-SP has excellent biocompatibility. The cytotoxicity of DOX-loaded MNP/FA-SP to HeLa cell and MCF-7 cell was also examined. For HeLa, the cell viability was ~14% with DOX-loaded MNP/FA-SP treatment, as shown in Fig. S8A. This can be attributed to the lack of FR expression on the surface of MCF-7 cells, indicating that nonspecific uptake of DOX-loaded MNP/FA-SP was minimized. The result of selective cytotoxicity about DOX delivered by MNP/FR-SP in HeLa cells is confirmed obvious, compared with nonselective cytotoxicity of free DOX in both HeLa cell and MCF-7 cell (Fig. S8B). The cytotoxicity results demonstrate that the drug delivery system has excellent selectivity.





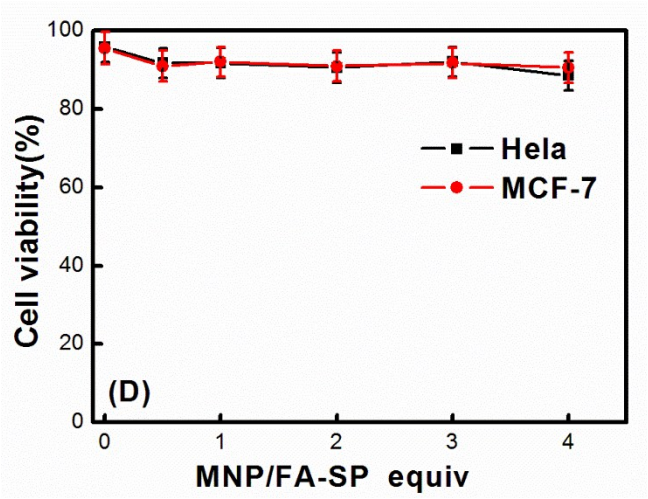
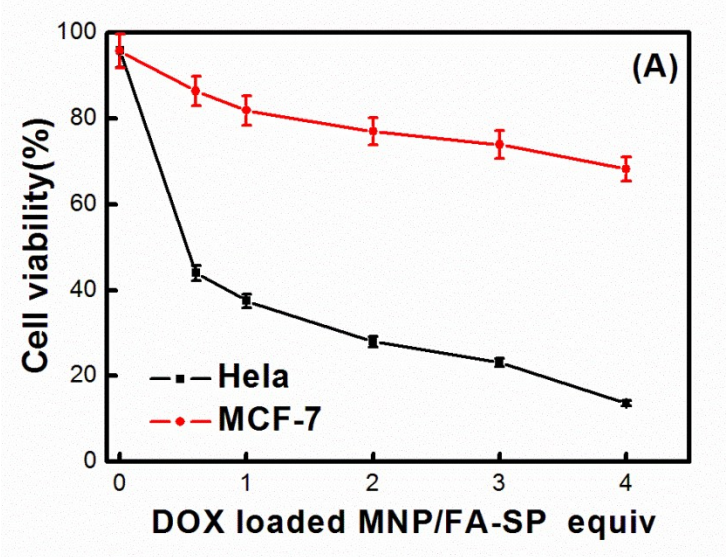


Fig. S7 (A)The selection of DOX-loaded MNP/FA-SP to HeLa cells demonstrated by flow cytometry; (B) The selection of DOX-loaded MNP/FA-SP not to MCF-7 cells demonstrated by flow cytometry; (C) Images of HeLa cells after incubation with (a) DIO, (b) DAPI, (c) DOX-loaded MNP/FA-SP, (d) merged images. (D) The cytotoxicity of MNP/FA-SP in both HeLa cell and MCF-7 cell.



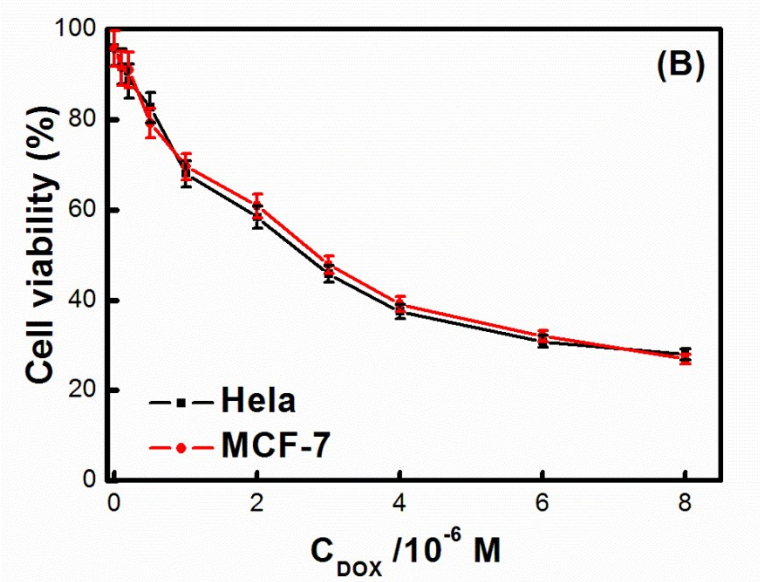


Fig.S8. Selection in recognition of cancer cell and targeted delivery of drug about DOX-loaded MNP/FA-SP in HeLa cell and MCF-7 cell. (A) cytotoxicity of DOX delivered by MNP/FA-SP; (B) cytotoxicity of free DOX.

Table S2. Recovery of Determination of Thiols in Cells.

Sample	Detected[a]	Added	Found[a]	Recovery
1	6.6×10^{-8} M	5.0×10^{-9} M	7.42×10^{-8} M	104.5%
2	2.3×10^{-8} M	4.0×10^{-8} M	5.91×10^{-8} M	93.9%
3	7.2×10^{-9} M	8.0×10^{-9} M	1.46×10^{-8} M	96.1%

[a] The average of three determinations.

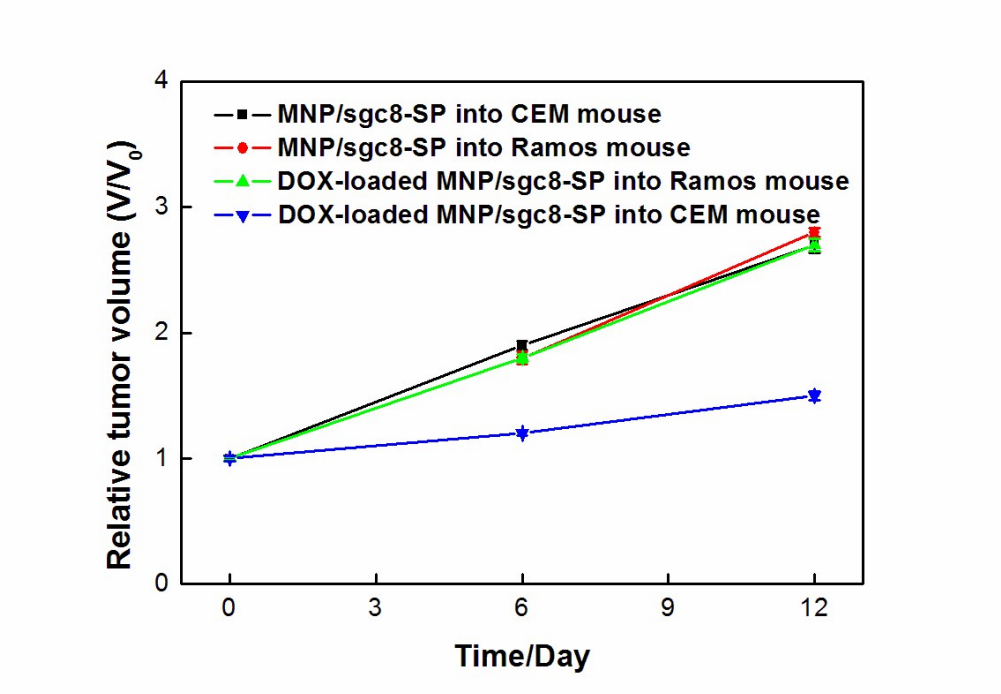


Fig. S9 Change of relative tumor volume (V/V_0) upon different treatments. (a) MNP/sgc8-SP into CEM subcutaneously mouse, (b) DOX-loaded MNP/sgc8-SP into CEM subcutaneously mouse, (c) MNP/sgc8-SP into Ramos subcutaneously mouse, (d) DOX-loaded MNP/sgc8-SP into Ramos subcutaneously mouse.