Supporting Information

Monitoring of "on-Demand" Drug Release using Dual Tumor Markers Mediated DNA-Capped Versatile Mesoporous Silica Nanoparticles

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Experimental Section

Reagents and Materials. N-cetyltrimethylammonium bromide (CTAB), sodium hydroxide, doxorubicin hydrochloride (Dox), tetraethyl orthosilicate (TEOS), (3-aminopropyl)triethoxysilane (APTES, 99%) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich. Suspension cells-acute myeloblastic leukemia (HL-60) cells, adherent cells-human cervical cancer (HeLa) cells, the normal immortalized human mammary epithelial (MCF-10A) cells, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and Hoechst 33342 staining kit were purchased from Key GEN Biotech. Dulbecco's Modified Eagles Medium (DMEM), fetal bovine serum (FBS), trypsin and CellMask™ Deep Red Plasma membrane Stain were purchased from Invitrogen. All other reagents were of analytical reagent grade. The water from Milli-Q (Millipore, Inc., Bedford, MA) was RNase-free by pretreated with diethylpyrocarbonate. The oligonucleotides were synthesized by TaKaRa Bio Inc. (Dalian China) and Shanghai Sangon Biological Engineering Technology & Services Co. (Shanghai China). The sequences are listed in Table S1.

Apparatus. Absorption spectra were measured on a UV-vis spectrophotometer (Nanodrop-2000C, Nanodrop, USA). Transmission electron micrographs (TEM and HRTEM) were measured on JEM-1011 and JEM-2100 transmission electron microscope (JEOL Ltd., Japan). Fluorescence spectra were obtained with Shimadzu fluorescence spectrophotometer (RF-5301PC, Shimadzu Co., Japan). Confocal fluorescence images of cells were performed with a Leica TCS SP5 confocal laser scanning microscopy (Leica Co., Ltd. Germany) The ζ-potential was acquired with a Malvern (Nano-Z, Malvern Instruments Ltd., Britain) instrument. The cell viability assay was performed using a Thermo Scientific Varioskan Flash (Thermo Fisher Scientific, USA).

Table S1 DNA sequences	
DNA	Sequences (5′ to 3′)
$S1_F$	$(A)_9$CCCAGCCTTCCAGCTCCTTGC-Alexa Fluor 488
Q1	BHQ1-GCAAGGAGCT
chemical synthesized target survivin mRNA strand S2	CAAGGAGCUGGAAGGCUGGG
single-base mismatch strand S3 [a]	CAAGGAGCUAGAAGGCUGGG
two-base mismatch strand S4 [a]	CAAGGAGCCAGAAGGCUGGG
$M1_F$	$(A)_9$TCAACATCAGTCTGATAAGCTAG-Cy3
Q2	BHQ1-CTAGCTTATC
chemical synthesized target miR-21 strand M2	UAGCUUAUCAGACUGAUGUUGA
single-base mismatch strand M3	UAGCUUAUCAAAACUGAUGUUGA
two-base mismatch strand M4	UAGCUUAUCCTACUGAUGUUGA
MTT assay	
S1	$(A)_9$CCCAGCCTTCCAGCTCCTTG
Q1	CAAGGAGCTGG
M1	$(A)_9$TCAACATCAGTCTGATAAGCTAG
Q2	CTAGCTTATC

a The single mismatched base and two mismatched base are highlighted in the box.

Synthesis of MSNs, DOX-loaded MSNs and DNA-DOX-loaded MSNs complexes. The mesoporous silica nanoparticles (MSNs) were synthesized according to a reference procedure.[1] Detailedly, 0.50 g N-cetyltrimethylammonium bromide (CTAB) and 2.00 M NaOH (aq) (1.75 mL) in sequence were completely dissolved into 240 mL of deionized water under vigorous stirring, followed by adjusting the solution temperature to 80 ºC. Then 2.5 mL of tetraethyl orthosilicate (TEOS) was added dropwise to the surfactant solution under vigorous stirring, hence the vigorous stirring was continued for 3 h. After reaction at 80 ºC for 3 h, the synthesized materials were

isolated via centrifugation and washed thoroughly with deionized water and ethanol. in order to remove the surfactant template, the final products were dried for 12 h at 80 ºC in vacuum and then calcined at 550 ºC for 5 h to obtain MSNs.

In the process of synthesis DOX-loaded MSNs, the extracted MSNs (0.20 g) were dispersed in ethanol (40 mL), then drug doxorubicin hydrochloride (DOX) (0.40 mM) was added and the mixture was stirred at 36 ºC under N_2 for 24 h to obtain DOX-loaded MSNs nanoparticles. After this procedure, the synthesized DOX-loaded MSNs with an excess of APTS (0.75 mL) were mixed and stirred for 5.5 h to immobilize the amine group on the surface. Finally, the synthesized materials were was filtered off, washed with ethanol, and dried under vacuum to obtain the intermediate product DOX-loaded MSNs-NH_2.

The DNA capping progress were operated according to the references.[2] Briefly, the nucleic acids in concentrations of (3×10^{-5} M) and 600 μg intermediate DOX-loaded MSNs-NH_2 were suspended in 500μL hybridization buffer (20 mM Tris-HCl, 37.5 mM $MgCl_2$ at pH 7.5) and shaken at 37 °C for 1 h. The unreacted bio-molecules were removed by three centrifugation/washing cycles and then the resulting solids (DNA-DOX-loaded MSNs) was collected carefully, dispersed in hybridization buffer and stored at 4 ºC for further use. Besides, the complexes DNA-capped MSNs were formed by the above procedure just without the loading process.

Cell culture and preparation. Human cervical cancer (HeLa) cells[3, 4], the normal immortalized human mammary epithelial (MCF-10A) cells and acute myeloblastic leukemia (HL-60) cells with or without irritant were cultured in Dulbecco's Modified Eagle Medium (DMEM) at 37 ºC in a humidified atmosphere (95% air and 5% CO_2), supplemented with 10% FBS and 100 IU mL-1 penicillin-streptomycin. Then cancer cells at the logarithmic growth phase were seeded in 6-well plates and cultured with DNA-DOX-loaded MSNs complexes in cultured medium without FBS for investigating the drug delivery performance. After incubation at 37 ºC for 6 h, the medium was removed and fresh medium was added into each well. Fluorescence measurements and fluorescence imaging were performed over a period of time (0-24 h) after transfection.

Results and Discussion

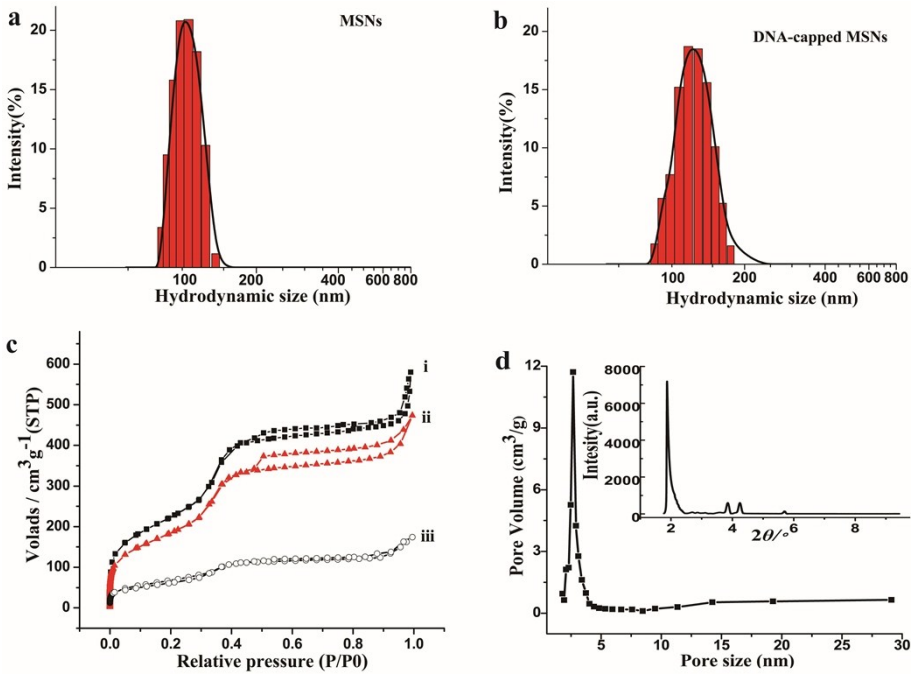

Fig. S1 DLS characterization of MSNs (a) and DNA-capped MSNs dispersed in cell culture medium (b). BET nitrogen adsorption/desorption isotherms of nanoparticles : MSNs (i), MSNs-NH_2 (ii) and DOX-loaded MSNs (iii). (d) BJH pore size distributions of MSNs, the inset is Low-angle XRD pattern of MSNs.

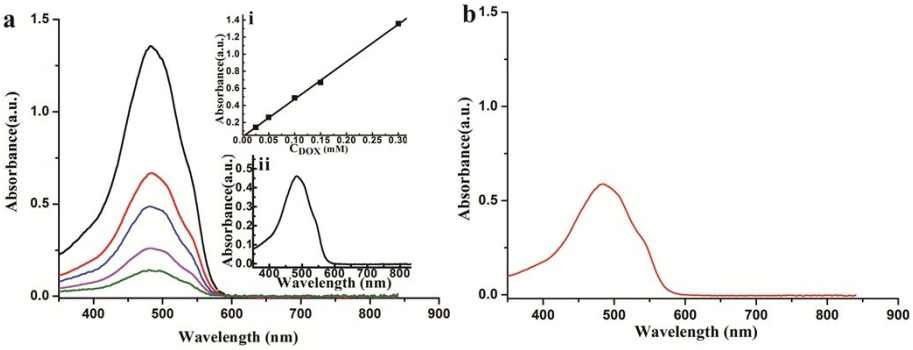

Fig. S2 (a) The absorbance intensities of different concentrations of DOX, the insert curve (i) is the linear relationship between absorbance intensity and drug concentration, the insert curve (ii) is the absorbance intensities of DOX in supernatant after the loading process. (b) The absorbance intensities of DOX trapped in the nanocarriers without endocytosed by cells.

As shown in Fig. S2a, a linear range of DOX from 0.025 to 0.3 mM was observed with I_A = 0.03519 + 4.3836 C_{DOX} (mM) (R = 0.998). After loading process, the concentration of DOX in supernatant was 0.097 mM calculated by the above linear equation. As the total amount of drug used in the process was 0.4 mM (amount of drug initially added), then the drug concentration retained in the nanocarriers was estimated to be 0.303 mM (actual loaded amount of drug). Based on the encapsulation efficiency equation[5]: EE = (actual amount of drug loaded in nanoparticles/amount of drug initially added) × 100 %, the EE could be achieved at nearly 75.8%

in this system. Additionally, in the internalization process, the absorbance intensities decrease of DOX trapped in the nanocarriers was relevant to the amount of nanocarriers internalized by cells. After incubated with cell, the concentration of DOX trapped in the nanocarriers, without endocytosed by cells, was 0.125 mM calculated by the above linear equation. Thus, the concentration of DOX trapped in the nanocarriers, which was internalized by cells, was 0.178 mM. The internalization efficiency could be achieved at 58.7% calculated by the followed equation: internalization efficiency = (amount of drug internalized by cells / actual amount of drug loaded in nanoparticles) × 100 %.

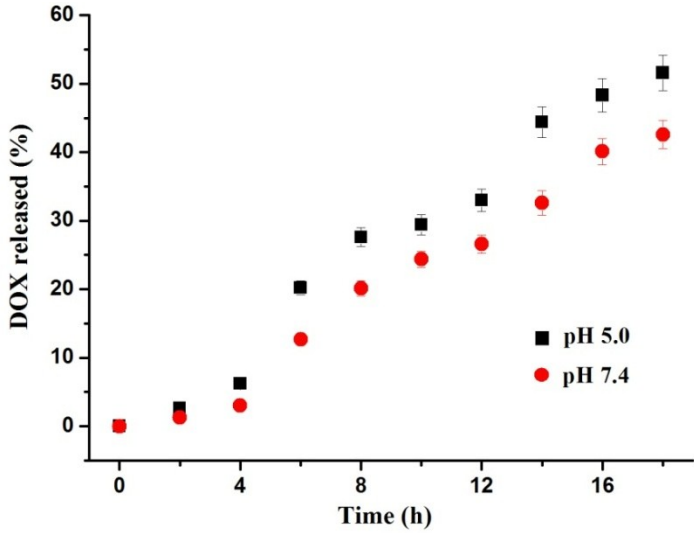

Fig. S3 Release profiles of DNA-DOX-loaded MSNs with successive addition of survivin mRNA (t=2 h) and miR-21 (t=10 h) at pH 5.0 (black squares) and 7.4 (red circle).

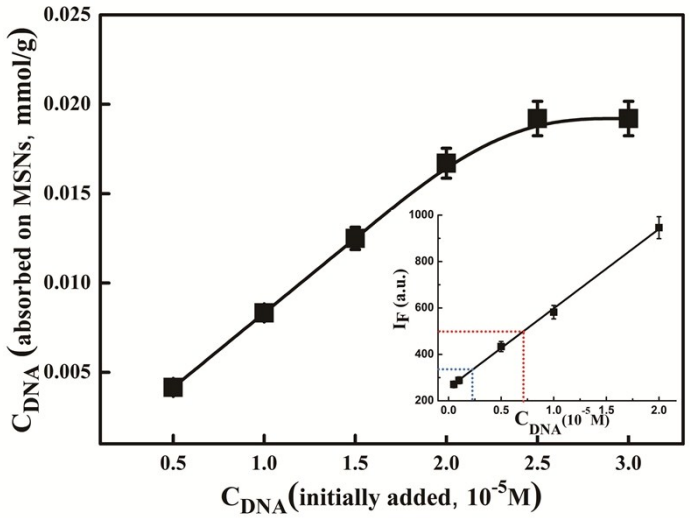

Fig. S4 The amount of DNA capped on the MSNs nanocarriers relative to concentration change, the insert curve is the linear relationship between fluorescence intensity and DNA ($S1_F$) concentration.

In order to optimize the necessary amounts of oligonucleotide in the capping process, the fluorescence intensity of DNA ($S1_F$ chosen as representative) in the supernatant after capping was detected. As shown in Fig. S3, the amount of DNA capped on MSNs increased rapidly with the increase of the initial added concentration at first and reached an equilibration step at the concentration of at 2.5×10^{-5} M. Meanwhile, at concentrations 2.5×10^{-5} M or higher, residual fluorescence value of Fluor 488 in the supernatant can be measured after centrifugation, revealing that the nanocarriers achieving its maximum capping. In order to achieving completely capping, a little higher concentration value as 3.0×10^{-5} M was used in the capping process.

Furthermore, according to the linear relation of DNA concentration and fluorescence intensity (Fig. S3, inset), the concentration of residual DNA labeled as $C_{DNA\ -upper}$ was 2×10^{-6} M (blue line) or 7×10^{-6} M (red line), corresponding to the initial added concentration 2.5×10^{-5} M or 3.0×10^{-5} M (named as $C_{DNA\text{-}total}$). Then the DNA concentration adsorbed on the MSNs was estimated to be 2.3×10^{-5} M, and the amount DNA capped on the nanocarriers was calculated as 0.0192 mmol oligonucleotide /g MSNs based on the capping process condition.

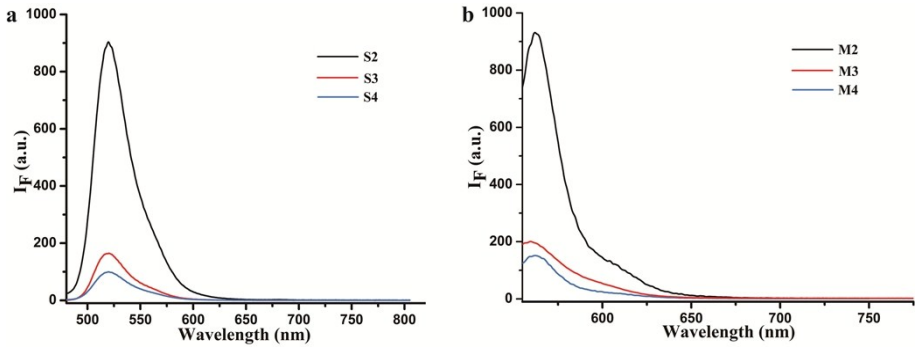

Fig. S5 Fluorescence intensity change upon addition of different oligonucleotide S2(a, black curve), single-base mismatch oligonucleotide S3(a, red curve), two-base mismatch oligonucleotide S4(a, blue curve), M2(b, black curve), single-base mismatch oligonucleotide M3(b, red curve) and two-base mismatch oligonucleotide M4(b, blue curve)to DNA-capped MSNs system.

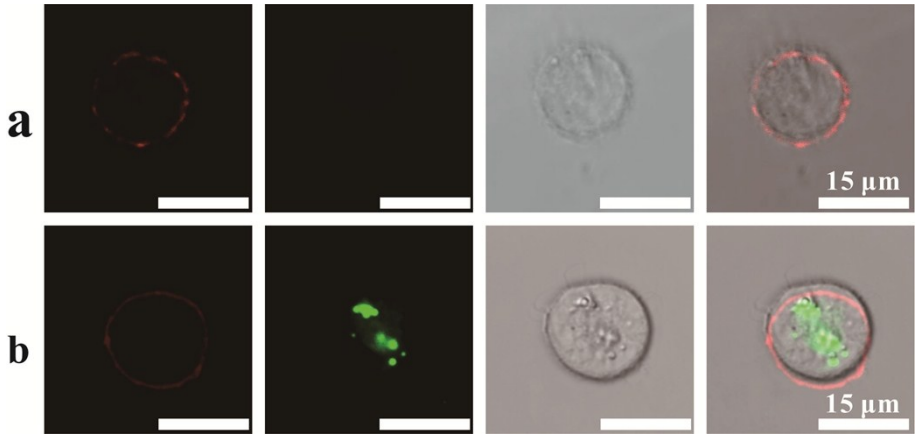

Fig. S6 Confocal fluorescence images of HL-60 cells as control (a) and HL-60 cells incubated with DNA-capped MSNs nanocarriers (b). The images from left to right represent: Plasma membrane Stain (red fluorescence, present cell membrane location), DNA-capped MSNs composite emissions from Alexa Fluor 488 (green fluorescence, present DNA-capped MSNs location), bright image and merged images respectively.

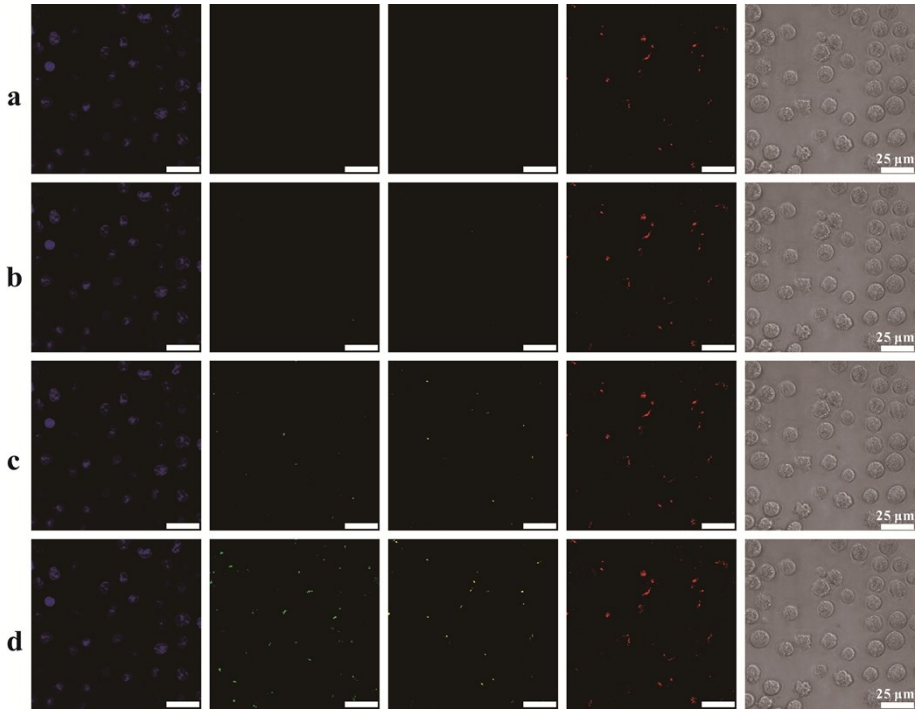

Fig. S7 CLSM tracking analysis of HL-60 cells incubated with DNA-DOX-loaded MSNs for different time: (a) 0 h, (b) 1 h, (c) 2 h, (d) 3 h. The images from left to right represent: Hoechst 33342 fluorescence field, survivin mRNA fluorescence field, miR-21 fluorescence field, drug fluorescence field and bright field.

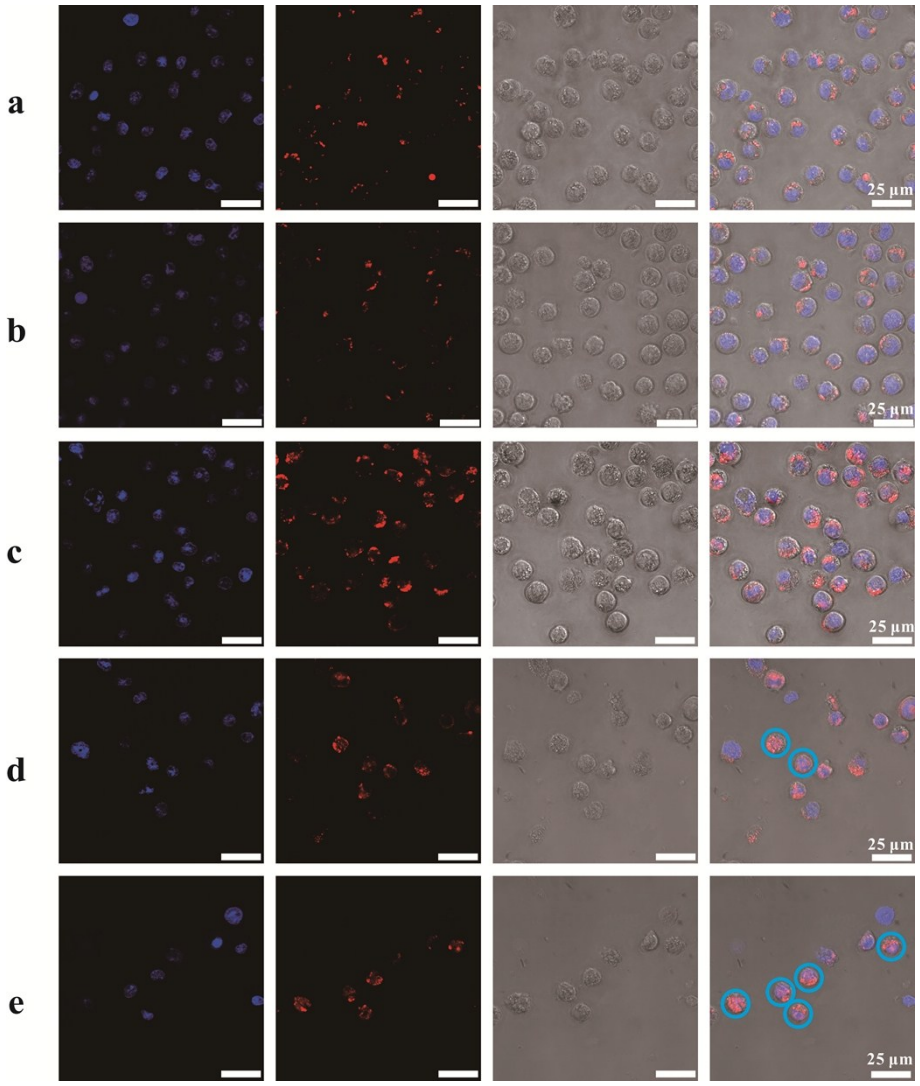

Fig. S8 CLSM images of HL-60 cells after incubation with DNA-DOX-loaded MSNs for different time: (a) 0 h, (b) 3h, (c) 6 h, (d) 12 h, (e) 24 h in Hoechst 33342 fluorescence field, drug fluorescence field, bright field and merged.

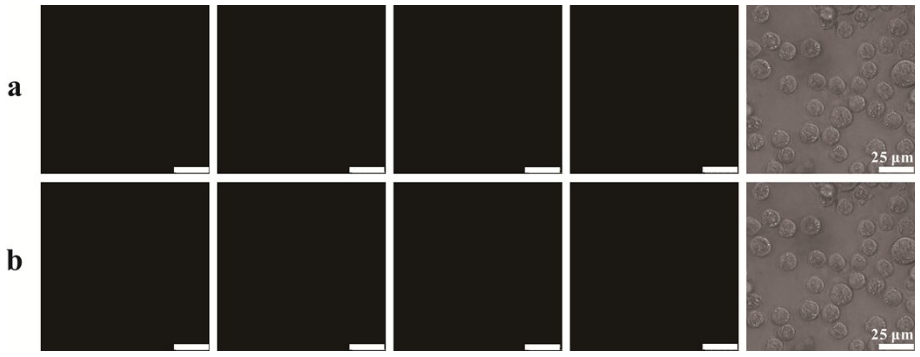

Fig. S9 CLSM images of HL-60 cells without any addition as control experiment for different time: (a) 0 h, (b) 3 h. The images from left to right represent: Hoechst 33342 fluorescence field, survivin mRNA fluorescence field, miR-21 fluorescence field, drug fluorescence field, bright field.

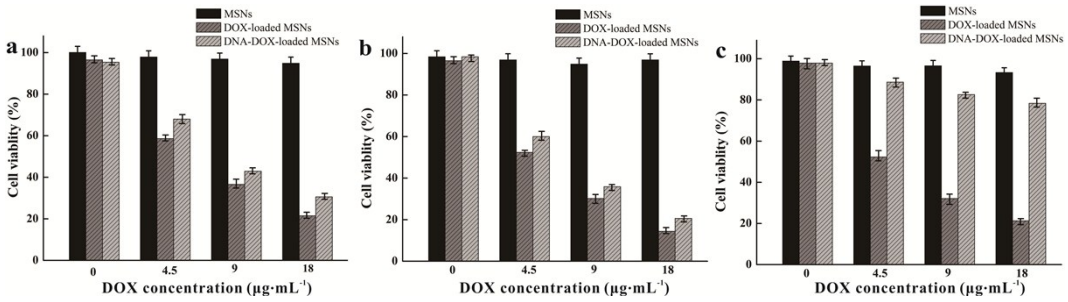

Fig. S10 Cytotoxicity assays of HL-60 cells (a), HeLa cells (b) and MCF-10A incubated with MSNs, DOX-loaded MSNs and DNA-DOX-loaded MSNs.

References

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