Supporting Information

Vitamin-responsive Mesoporous Nanocarrier with DNA Aptamer-mediated Cell Targeting

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Chemicals

Tetraethylorthosilicate (TEOS), N-cetyltrimethylammonium bromide (CTAB), and Rhodamine 640 (Rh640) were purchased from Sinopharm Chemical Reagent Co, Ltd. 3-aminopropyltriethoxysilane (APTES) was purchased from Alfa Aesar. Doxorubicin hydrochloride (DOX) was purchased from Aladin. N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide-HCl (EDC), desthiobiotin, biotin, andavidin were all obtained from Sigma-Aldrich. Hoechst 33342 was purchased from Beyotime Institute of Biotechnology. RPMI-1640 medium, penicillin streptomycin solution and fetal bovine serum were obtained from Invitrogen. DesthiobiothinTEG phosphoramidite was purchused from Glen Research. Ultra pure water (purified using a Synergy ultrapure water system (Millipore) was used as solvent in all experiments.

Cell lines

Human acute lymphoblastic leukemic CCRF-CEM cell line and human Burkitt's lymphoma Ramos cell line were kindly provided by Prof. Xiaobing Zhang in Hunan University.

Instruments

The concentrations of all DNAs were determined using the absorbance of DNA at 260 nm, as measured on a UV-2550 UV-Vis spectrometer (SHIMADZU), by calculating the absorbance of DNA at 260 nm.Fluorescence measurements were carried out on a FluoroLog-3 spectrofluorometer (Jobin Yvon). XRD patterns were recorded on a D8 Advance diffractometer (Bruker), using Cu-Kα radiation (λ = 1.5406 Å).The scan was conducted from 1°to 10°with a speed of 1°/min.Scanning electron microscopy (SEM) images were taken from a Sirion 200scanning electron microscope(FEI) with an accelerating voltage of 12 kV. Transmission electron microscopy (TEM) images were obtained on anJEM 2010 transmission electron microscope (JEOL) with a working voltage of 200 kV. The nitrogen adsorption and desorption isotherms at 78.3 K were measured using an ASAP 2010 analyzer (Micromeritics). The BET model was applied to evaluate the specific surface areas. Zeta potential was measured by a Zetasizer Nano ZS(Malvern). FT-IR spectra were measured by a Nicolet iS10FT-IR spectrometer (Thermo Scientific). Aptamers were purified by a 1260 Infinity reversed-phase high performance liquidchromatography (HPLC) using a C_{18} column (Agilent). Confocal microscopy images for cell internalization studies were taken by a Revolution XD confocal laser scanning microscope (Andor) with a 40×oil-dispersion objective. A 405 nmlaser was the excitation source for Hoechst 33342 dye, and a 488 nmlaser was used for the excitation of Rhodamine dye. The specific targeting test of aptamer and MSN-Avi-Apt toward different cell lines wascarried out on a FACSVerse cytometer (BectonDickinson). Agreen laser at 488 nm with different excitation voltages (650, 700, and 750 V) was used as the excitation source. In cytotoxicity assay, the 96-well plate was subjected toabsorption measurement at 570 nm using a Multiskan GO miroplate reader (Thermo Scientific).

Experimental

Synthesis of desthiobiotin-functionalized mesoporous silica nanoparticles (MSN-desthiobiotin): CTAB (1.00 g) was dissolved in 480 mL of ultrapure water and NaOH aqueous solution (2.00 M, 3.50 mL) was added. After the temperature of the mixture was adjusted to 80 °C, TEOS (5.00 mL) was added dropwise to the surfactant solution under vigorous stirring. The mixture was allowed to react for 2 h to give a white precipitate. This solid crude product was centrifuged, washed with ultrapure water and methanol, and dried in air to yield the as-synthesized mesoporous silica nanoparticles(denoted as MSN). To remove the surfactant template of CTAB, 1.5 g of the as-synthesized MSN was refluxed for 24 h in a methanolic solution of 9 mL of HCl (37%) in 200 mL methanol. The resulting material was centrifuged and washed with ultrapure water and methanol.1.6 g of MSN was suspended in 20 mL of toluene, and 0.5 g of APTES was added. The mixture was stirred at room temperature for 20 h and then evaporated by a rotary evaporator to obtain amine-modified MSN (denoted as MSN-NH_2). 50mg of desthiobiotin was dissolved in 3 mL of H_2O containing 50 mg of EDC and 20 mg of NHS, then suspension containing 80 mg of MSN-NH_2 was added and stirred at room temperature for 8 h. The mixture was washed with ultrapure water and dried in air to yield the desthiobiotin-modified MSN (denoted as MSN-desthiobiotin).

Synthesis of desthiobiotin-modified-aptamer: The desthiobiothin-modified DNA sequences were synthesized using an ABI3400 DNA/RNA synthesizer (Applied Biosystems). The synthesis started with acontrolled pore glass (CPG) column at 1 μmole scale. The desthiobiothin coupling was realized using the desthiobiothinTEG phosphoramidite. A proper amount of desthiobiothinTEG phosphoramidite was dissolved in dry acetonitrile in a vial connected to the synthesizer. The aptamer with FAM labeled at 3’ end was used primarily for flow cytometry. The aptamer without dye labeling was used for the cytotoxicity assay. The aptamers with and without FAM labelingwere deprotected in 3 mL of AMA solution (ammonium hydroxide: 40% aqueous methylamine = 1:1) at 65 °C for 25 min. All deprotected sequences were precipitated byadding 250 μL of 3 M NaCl and 6 mL of cold ethanol. Thenthe

precipitated aptamers were collected by centrifugationand dissolved in 400 μL of triethylammonium acetate (TEAA) for further purification by reversed-phase HPLC usinga C18 column and acetonitrile TEAA solvent. Finally, these aptamers were quantified by measuring their absorbance at 260 nm.

The sequences are as follows:

Desthiobiotin-Aptamer (5'-desthiobiotin-TTT TTT TTT TAT CTA ACT GCT GCG CCG GGA AAA TAC TGT ACG GTT AGA-3')

Desthiobiotin-Aptamer-FAM (5'-desthiobiotin-TTT TTT TTT TAT CTA ACT GCT GCG CCG GGA AAA TAC TGT ACG GTT AGA-FAM-3')

Rh640/DOX loading and capping of desthiobiotin-MSNs: The desthiobiotin-MSNs (5 mg) was added to 5 mL of Rh640 (1.00 mM) in PBS buffer (100 mM, pH = 7.4) solution. After stirring for 12 h, avidin (1 mg) was added and stirred for 2h to cap the pores on the mesoporous particles. The final mixture was then centrifuged and washed with a PBS buffer solution three times. The amount of Rh640 loaded on the MSN was determined by analyzing the supernatant solution spectrophotometrically. All the washing solutions were collected, and the loading amount was calculated from the difference in number of moles of the initial and uncapped dye. DOX was loaded by the same protocol. Avidin capped desthiobiotin-MSNs was redespersed in 1 mL of PBS buffer and desthiobiotin-modified-aptamer was added to a final concentration of 0.1 μM. The mixture was incubated for another 2 h to obtain aptamer-modified MSNs (denoted as MSN-Avi-Apt).

Biotin triggered release test: MSN-Avi-Apt (1 mg) loaded with Rh640 was dispersed in 10 mL of PBS buffer. The release kinetics study was carried out using a dialysis bag (MW = 3,500). The released Rh640 could cross the dialysis membrane, but not the MSNs. Aliquots were taken from the solution outside the dialysis membrane every 30 min. After 200 min, 0.6 mg of biotin was added into the dialysis bag. At the same time, a certain amount of biotin was added to the solution outside the dialysis bag to keep consistent concentration with the solution in the dialysis bag. The release of rhodamine dye from the pores to the buffer solution was monitored via the absorbance peak of the dye centered at 575 nm.

Internalization and co-localization study: CEM or Ramos cells with a concentration of 1 $\times 10^6$cells/mL were incubated with desired concentrations of dye-loaded MSN-Avi or dye-loaded MSN-Avi-Apt at 37°C for 2 h with 5% CO_2 atmosphere and then washed with PBS buffer. Nucleuses were stained by Hoechst 33342. Cells were imaged on a confocal fluorescence microscopy (40$\times$oil-dispersion objective). A 405 nm laser was used for the excitation of Hoechst 33342 dye, and a 488 nm laser was used for the excitation of Rhodamin dye.

Binding test. Samples containing CEM or Ramos cells with a concentration of 10^6cells/mL were incubated with desired concentrations of aptamer, MSN-Avi, or MSN-Avi-Apt at 4°C in a 200 μL volume of binding buffer for 30 min. The cells were centrifuged, washed three times with 200 μL of washing buffer and redispersed in 200 μL of binding buffer. For the analysis with flow cytometry, 10000 cells were counted in each sample.

Cytotoxicity assay: The cytotoxicity study was measured using the MTT assay in CEM and Ramos cell line. Cells were seeded into 96-well cell-culture plate at 1 $\times$ 10^5/well in 50 μL of PBS buffer. Then DOX loaded MSN-Avi, DOX loaded MSN-Avi-Apt, or DOX only (0–5 μg/mL) in 50 μL of PBS buffer was added to the test well. The resultant cell mixture was incubated at 37°C in a 5% CO_2 atmosphere for 2 h and then washed with PBS. The cells were further incubated in 100 μL of fresh medium for 48 h in a 5% CO2 atmosphere at 37°C. A standard MTT assay was followed thereafter.

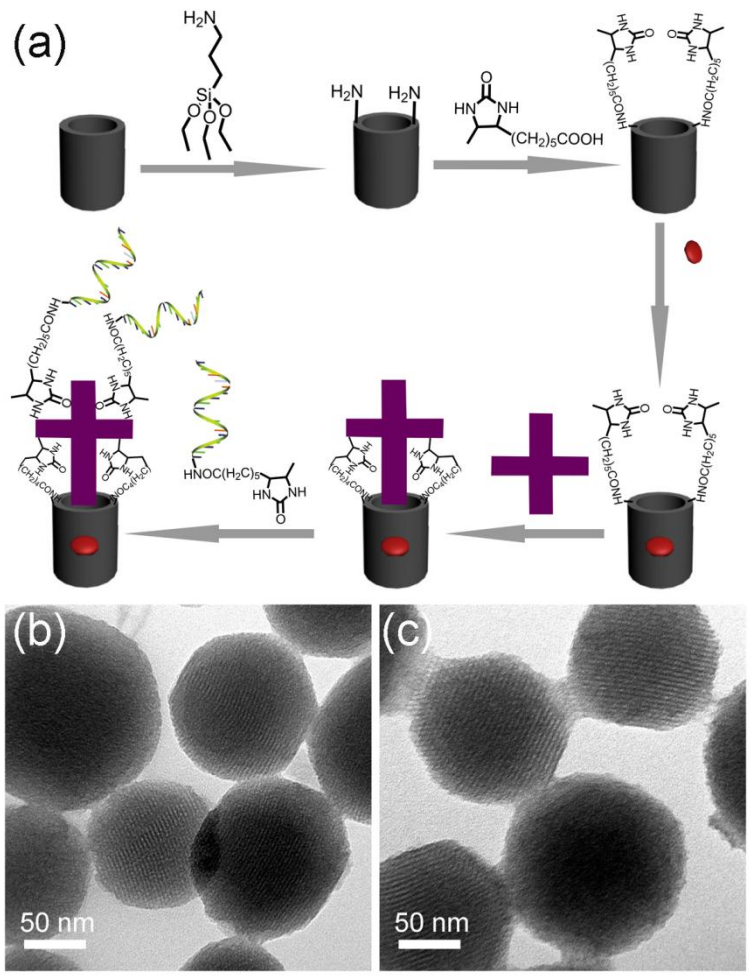

Figure S1. (a) Schematic representation of the preparion of MSN-Avi-Apt. TEM image of (b) MSN and (c) MSN-Avi-Apt.

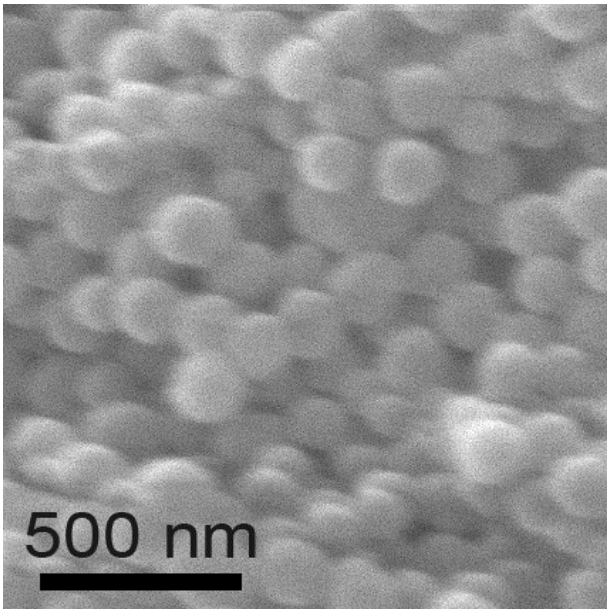

Figure S2. SEM image of MSN-Avi-Apt.

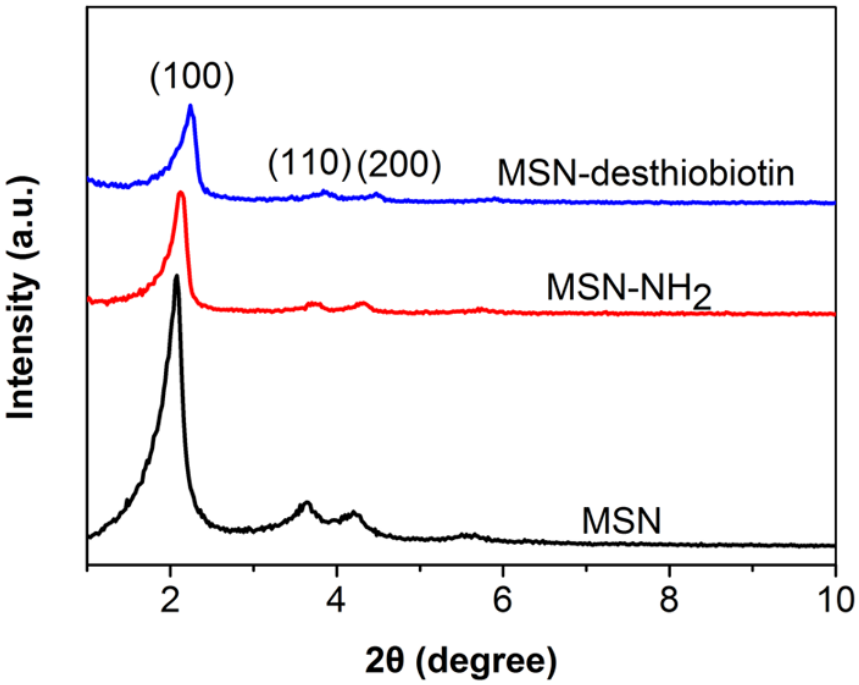

Figure S3. Small-angle X-ray (SAXRD) diffraction pattern of MSN, MSN-NH_2 and MSN-desthiobiotin.

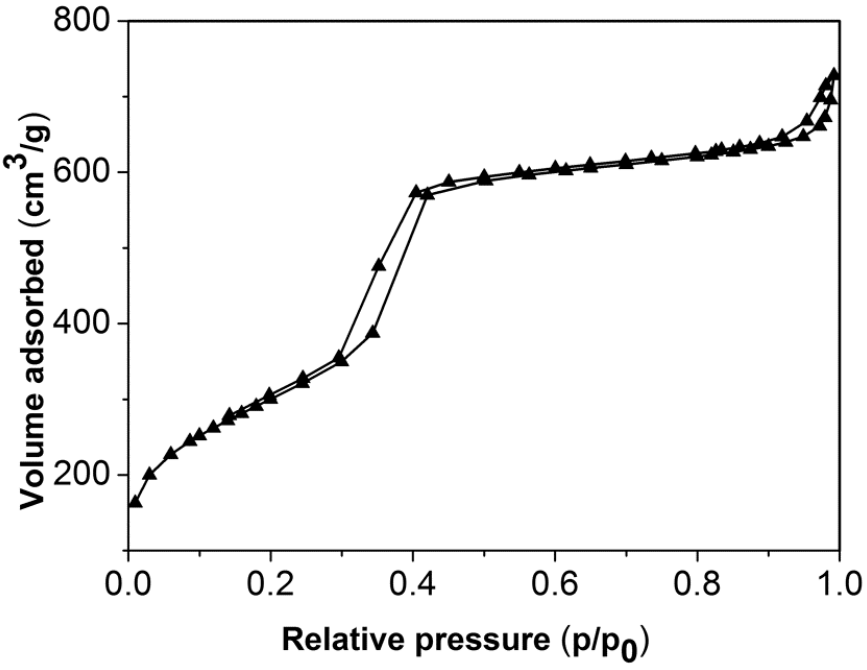

Figure S4. Nitrogen adsorption-desorption isotherms of MSNs.

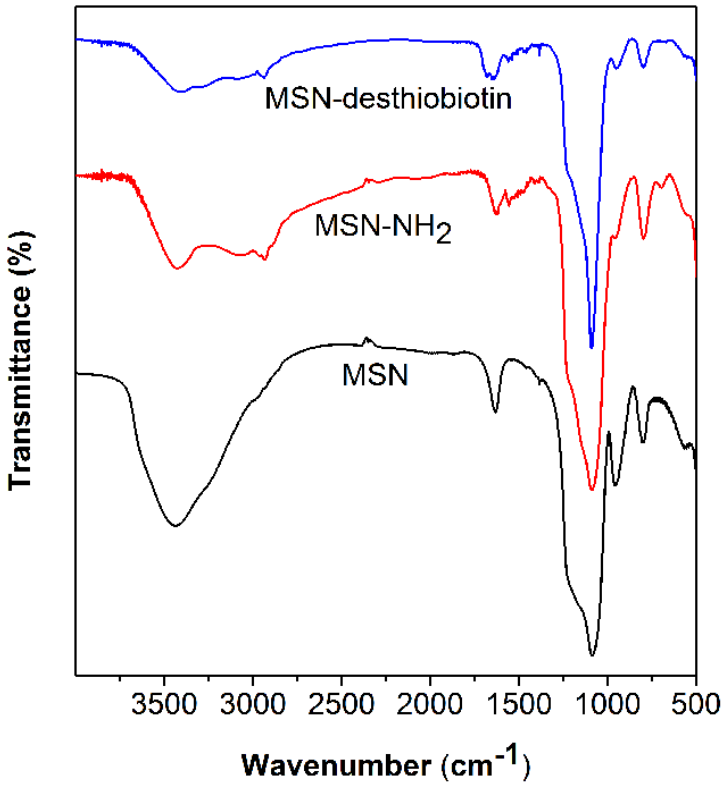

Figure S5. FTIR spectra of MSN, MSN-NH_2 and MSN-desthiobiotin.

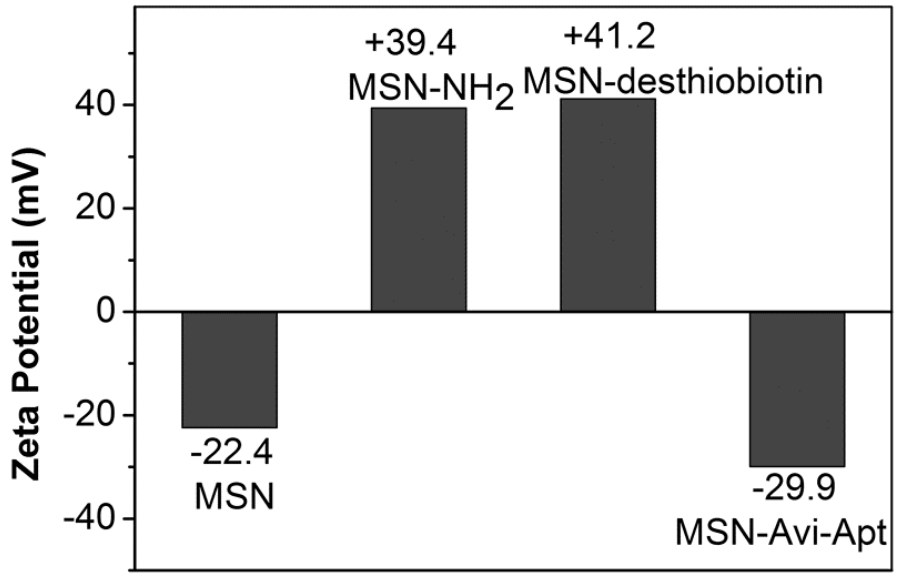

Figure S6. Zeta potential of MSN, MSN-NH_2, MSN-desthiobiotin and MSN-Avi-Apt.

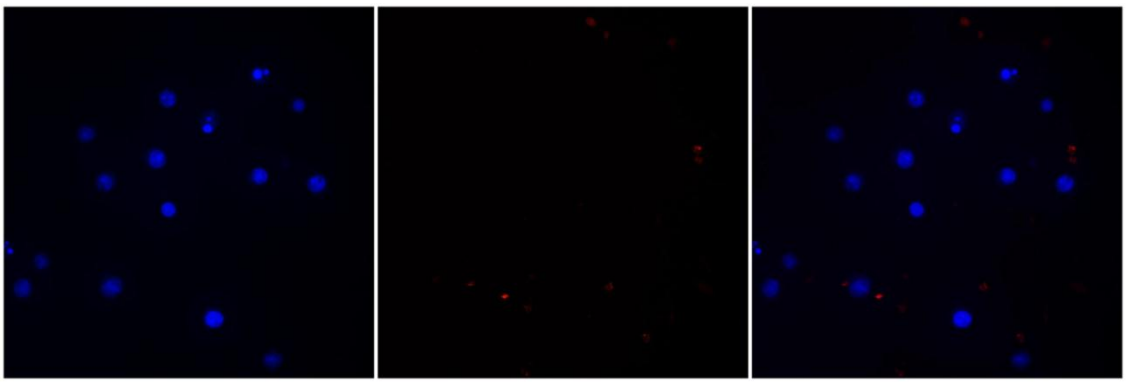

Figure S7. Confocal microscopy images of Ramos cells treated with dye-MSN-Avi.

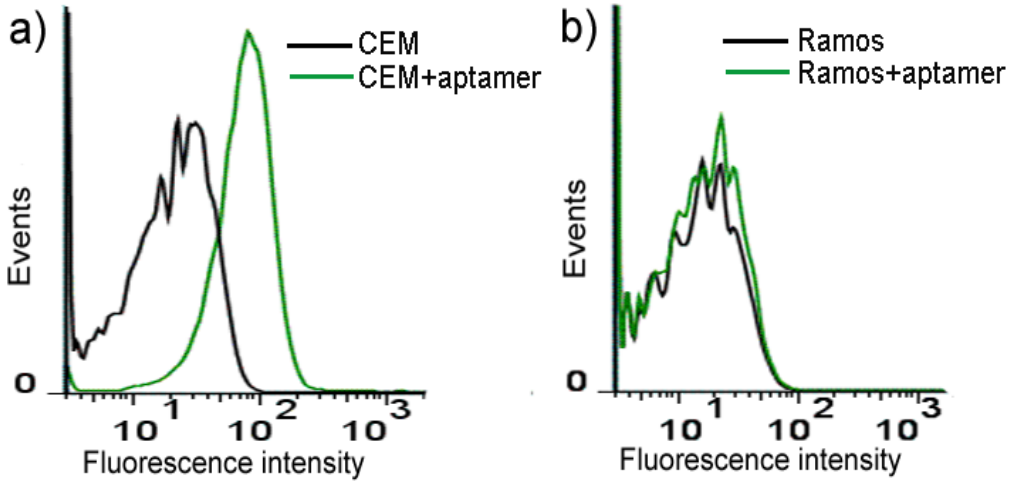

Figure S8. Flow cytometry analysis of (a) CEM cells (target cells) and (b) Ramos cells (control cells) treated with free sgc8 aptamer.

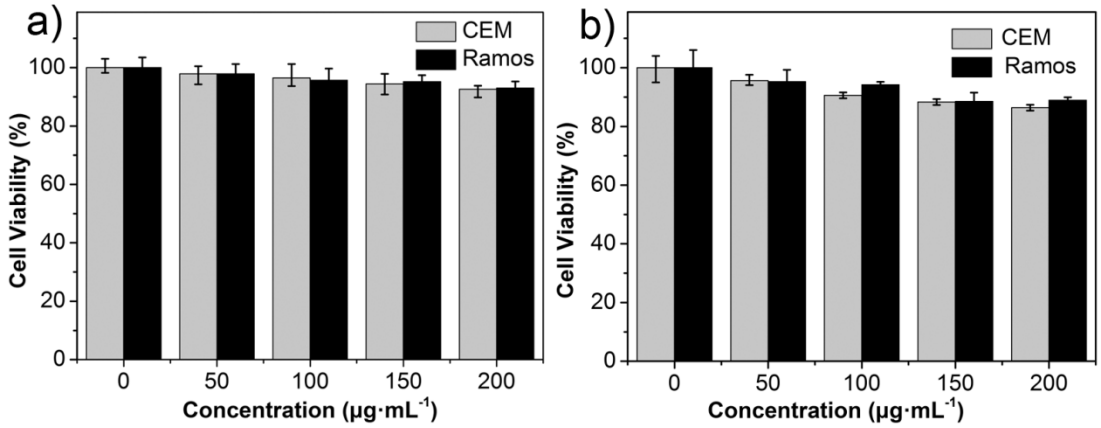

Figure S9. Cytotoxicity assay of CEM cells and Ramos cells treated with (a) MSN-Avi and (b) MSN-Avi-Apt.

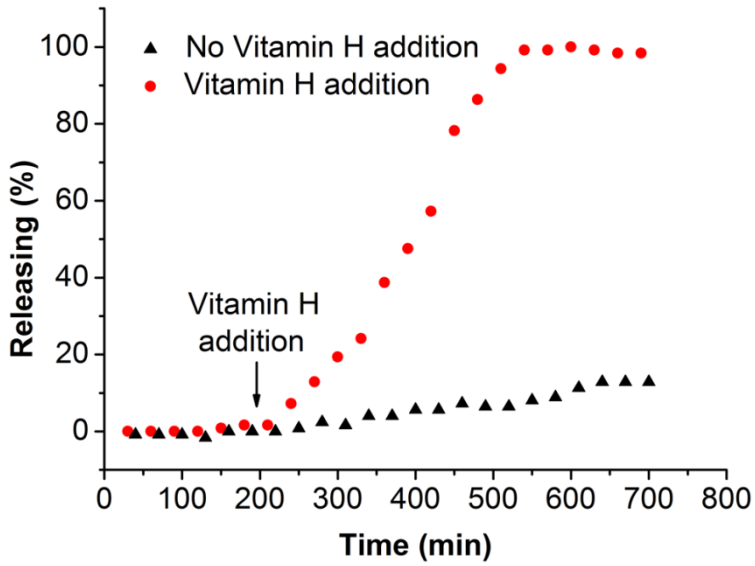

Figure S10. Release curve for MSN-Avi-Apt with and without vitamin H addition. Data have been normalized to the maximum level of DOX released in the experiment.