SUPPORTING INFORMATION

Experimental Section

Materials: 1,2-dihexadecanoyl-sn-glycero-3-phosphocholine (DPPC, MW=734.039), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC, MW=790.145) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (ammonium salt) (DSPE-PEG2000, MW=2805.497) were purchased from Avanti Polar Lipids (Alabama, USA). $FeCl_3 \cdot 6H_2O$, $FeCl_2 \cdot 4H_2O$, Oleic acid (90%), Ammonium hydroxide (25%), acetone, chloroform, Triton X-100, pareform and glycerinum were bought from the Oriental Chemical Co (Chongqing, China). DMEM/F-12 medium, fetal bovine serum (FBS, Gibco), penicillin, streptomycin and Cell Counting Assay Kit-8 were bought from the Huake Chemical Co (Chongqing, China). 5-(and-6)-carboxylfluorescein (CF), H33258 and phalloidine was supplied by Sigma (St. Louis, MO, USA).

Preparation of oleic acid-coated magnetic iron oxide NPs: Magnetic iron oxide NPs were synthesized through a chemical co-precipitation method. [1] Briefly, $FeCl_3 \cdot 6H_2O$ (21.624 g, 0.08 mol) and $FeCl_2 \cdot 4H_2O$ (7.9524 g, 0.04 mol) were dissolved in distilled water (50 mL) and stirred 1 h at room temperature. After the ferric chloride and ferrous chloride were dissolved completely, 50 mL ammonium hydroxide was rapidly added into the solution. The mixture was heated to 95 °C with vigorous agitation for 30 min. Next, 55 mL oleic acid was added into the mixture. The mixture was heated to 95 °C and reacted for 1.5 h. Along with the evaporation of ammonium hydroxide, the magnetic nanocrystals were coated by hydrophobic oleic acid upon continuous heating. After the aqueous phase was evaporated completely, 100 mL acetone was added into the mixture. The oleic acid-coated magnetic nanocrystals became flocculate rapidly. The magnetic flocculate was collected by a permanent magnet (1.4 tesla). To remove excess oleic acid, the flocculate was washed three times with 80 mL acetone and centrifuged at 800 G for 15 min at room temperature. The oleic acid-coated Fe_3O_4 magnetic NPs (OA-MNPs) were dried in vacuum at room temperature for 10 h.

Preparation of thermosensitive magnetic liposomes: Thermosensitive magnetic liposomes (TSMLs) were prepared by lipid film hydration coupled with extrusion method.[2] TSMLs were composed of DPPC/DSPC/DSPE-PEG2000 in a molar ratio of 80:15:5(total 10 mg), and dispersed into 2 mL chloroform in a round-bottomed flask. The dried OA-MNPs were dispersed in chloroform at a concentration of 0.5 mg/mL. Adding 10 mL of the OA-MNPs containing chloroform into the DPPC/DSPC/DSPE-PEG2000 containing flask, the color of solvent became brown. The solvent was evaporated at 35 °C under nitrogen stream in a rotating vacuum evaporator (IKA-RV10, IKA, Germany) for 30 min. The obtained thin film was stored overnight at 30 mbar at room temperature. The lipid film was hydrated at 60 °C using water bash with a 10 mM TRIS buffer (pH 7.4) for 15 min. The suspension was extruded 10 times through 200 nm and 30 times through 100 nm polycarbonate filters (Alabama, USA) at 55 °C. OA-MNPs were removed by gel-permeation chromatography through Sephadex column filled with Sephadex G-50 (Sigma) at a pressure of 1 atm. The pure liposomes were separated from TSMLs by a permanent magnet (1.4 tesla). The pure TSMLs can be stored at 4 °C for 2 weeks.

Drud loading::The lipid film was hydrated at 60 °C using water bash with 5 mL of 0.2 mg/mL CF solution in TRIS buffer (10 mM, pH 7.4) for 15 min. The obtained suspension was then sequentially extruded 10 times through 200 nm and 30 times through 100 nm polycarbonate filters (Alabama, USA) at 55 °C. Non-entrapped CF and OA-MNPs were removed by gel-permeation chromatography through Sephadex column filled with Sephadex G-50 (Sigma) at a pressure of 1 atm. The pure liposomes were separated from TSMLs by a permanent magnet (1.4 tesla). CF loaded liposomes were stored at 4 °C and used within 24 h.

DOX loaded TSMLs were prepared via the ammonium sulfate gradient driven loading.[3] In brief, TSMLs and DOX were dissolved in 20 mM Hepes-buffered solution containing 150 mM NaCl (pH 7.4) with a DOX/lipid (mol/mol) ratio of 0.13 and were incubated for 1 h at 38 °C. Free DOX were removed by centrifugation at 800 G for 20 min at 15 °C. The supernatant was removed and the pellet was kept in PBS buffer (10 mM, pH 7.4).

Characterization: The morphologies and particle size of OA-MNPs and TSMLs were observed by using transmission electron microscopy (Hitachi-7500, Hitachi, Japan) and scanning electron microscopy (FEI- Nova400, Philips, The Netherlands). Fourier transform infrared spectra (FTIR, model 6300, Bio-Rad Co. Ltd., USA), Magnetic hysteresis loops (VSM, Lake Shore 7410, Germany), and Thermogravimetric analysis (Micromeritics Co, USA) were employed to analyze the physical properties of OA-MNPs and TSMLs. Enzyme linked immunosorbent assay (ELISA, model 689, Bio-Rad Co. Ltd., USA) was employed to analyze cytotoxicity assay. The morphologies of cells and cell apoptisis were observed by Fluorescence microscope (TH4-200, Olympus Co, Japan) and Confocal laser scanning microscopy (CLSM, LSM 510 Metanlo, Zeiss Co., Germany).

In vitro CF and DOX release study: *In vitro* CF and DOX release was measured by fluorometry (LS50B, PerkinElmer, USA). To reduce the analytical system error, CF loaded liposomes were divided equally 10 portions and TRIS buffer (10 mM, pH 7.4) were added to 30 mL in flask. Each of 5 portions of samples were kept at different temperature. A water bath was used to control the environmental temperature. The CF/DOX loaded TSMLs were collected by a permanent magnet (1.4 tesla). 2 mL supernatant liquid was taken into PE tube and measured by fluorometry. The excitation wavelength of CF was set at 493 nm and the emission intensity was measured at wavelength of 518 nm. The excitation wavelength of DOX was set at 468 nm and the emission intensity was measured at wavelength of 590 nm.

Cell culture: Human breast cancer cells (MDA-MB-231) were kindly provided by Professor Li Yang (Chongqing University). Cells were cultured with DMEM/F-12 medium containing 10% fetal bovine serum (FBS, Gibco), 100 U/mL of penicillin and 100 μg/mL streptomycin at 37 °C under 5% CO_2 atmosphere. The culture medium was changed every 2 days.

Cytotoxicity assay: The effect of DOX and DOX-TSMLs on the proliferation of MDA-MB-231 cells was evaluated by Cell counting assay kit-8 (CCK-8). MDA-MB-231 cells were seeded into 24-well plates at an initial cell density of 2×10^4 cells/cm^2. When cell confluence reached around 60-70%, the culture medium was replaced with fresh media containing TSMLs, DOX or DOX-TSMLs at a concentration of 40 μg/mL, and cells were

incubated at 37 ℃ and 42 ℃ for another 6 h, 12 h and 24 h, respectively. Every six wells were parallel samples. Then, 10 μL of CCK-8 solution was added to each well and the cells were incubated for anther 2 h. Finally, the culture medium was collected in 96-well plates and was measured by enzyme linked immunosorbent assay (ELISA) at wavelengh of 450 nm.

Cell apoptosis assay: MDA-MB-231 cells were cultured with DOX and DOX-TSMLs in 35 mm × 12 mm cell culture dish (NEST Biotechnology Co., LTD, China) at 37 ℃ and 42 ℃ in the same manner as above mentioned. After incubation at 37 ℃ and 42 ℃ for another 6 h, 12 h and 24 h, respectively, the state of cells was photoed through fluorescence microscope. Then, after washing the plate with PBS at 3 times, cells were fixed by 2% pareform for 25 min at 4 ℃. Next, 0.2% Triton X-100 was added and kept 2 min at 4 ℃ after the plate was washed 3 times. Cytoskeleton was colored by 5 μg/mL phalloidine after the plate was washed 3 times and kept 4 ℃ overnight in the dark. Next day, cell nuclei were stained with 10 μg/mL Hoechst 33258 (Sigma) for 5 min after PBS washed. Finally, the stained samples were mounted with 90% glycerinum. Cell nuclei and skeleton were observed with a confocal laser scanning microscopy (CLSM).

Supplementary Figures

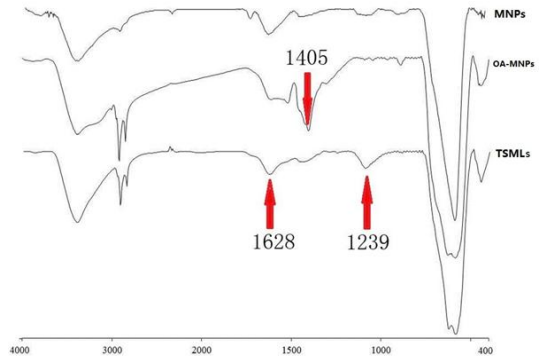

Figure S1. FITR spectra of MNPs, OA-MNPs and TSMLs.

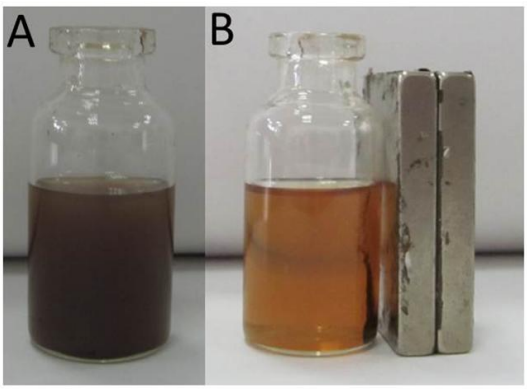

Figure S2. Optical photos of TSMLs respond to a magnetic field: A. TSMLs are black without a magnetic field; B. TSMLs are separated in a magnetic field.

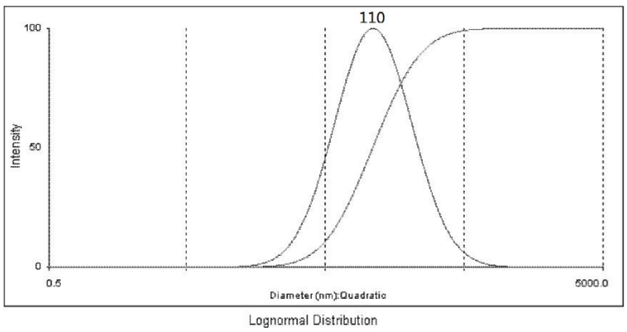

Figure S3. Dynamic light scattering spectrum of TSMLs.

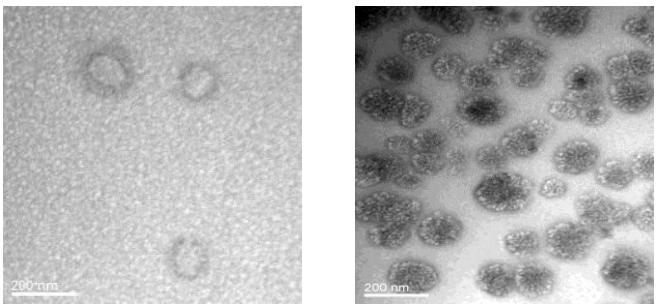

Figure S4. TEM image of (a) pure liposomes and (b) TSMLs at 42°C.

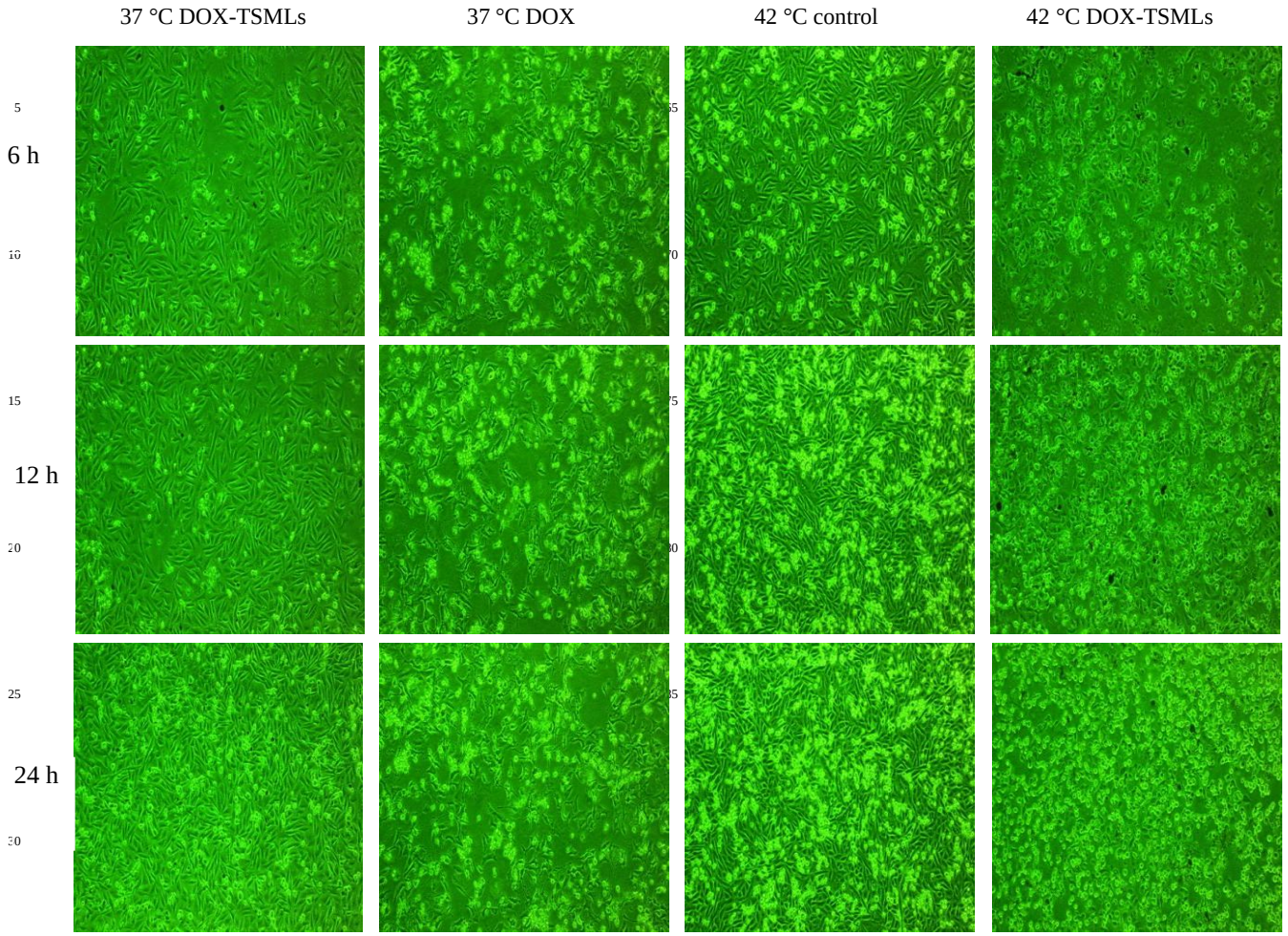

Figure S5. Cellular morphology of MDA-MB-231 treated with DOX and DOX-TSMLs at 37 °C and 42 °C by Fluorescence microscope.

References:

[1] Y. Namiki, H. Yoshida, T. Namiki, Y. Shii, A. Tsubota, S. Koido, and K. Naridi, *Nature Nanotechnology*, **2009**, 4, 598.
[2] K. Kono, T. Murakami, T. Yoshida, Y. Haba, S. Kanaoka, T. Takagishi and S. Aoshima, *Bioconjugate Chem.*, **2005**, 16, 1367.
[3] A. Agarwal, M. A. Mackey, et al. *Acs Nano*, **2011**, 5, 4919