Supporting information for

MTH1-Targeted Nanosystem for Enhanced PDT via Improving Cellular Sensitivity to Reactive Oxygen Species

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Reagents. Chlorin e6 (Ce6) and N,N dimethylformamide (DMF) were purchased from Sigma-Aldrich and used as received without further purification. MTS reagent (3-(4,5-dimethyl-thiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) was purchased from Promega Corporation. All siRNA oligonucleotides (Table S1 and Table S2) were synthesized and purified by GenePharma Biotechnology (Suzhou, China). Cetyltrimethylammonium bromide (CTAB), tetraethylorthosilicate (TEOS), aminopropyltriethoxysilane and other chemicals were of analytical grade, purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), and used without further purification. Antibody to MTH1-1 was purchased from ABcom, and antibody to GAPDH was purchased from Bioworld Technology. All solutions were prepared using ultrapure water, which was prepared through a Millipore Milli-Q water purification system (Billerica, MA, USA), with an electrical resistance >18.3 MΩ.

Characterizations of mesoporous silica nanoparticle (MSN). Dynamic light scattering (DLS) was measured on the Malvern Zetasizer Nano ZS90 (Malvern Instruments, Ltd., Worcestershire, UK). Transmission electron microscopy (TEM) was carried out on an H-7000 NAR transmission electron microscope (Hitachi) with a working voltage of 100 kV.

UV-vis and fluorescence spectrum measurements. All fluorescence measurements were carried out on a fluoromax-4 spectrofluorometer (HORIBA JobinYvon, Edison, NJ) with a 200 μL quartz cuvette. The UV-vis spectrum was measured by a UV-2450 UV-vis spectrophotometer (Shimadzu).

Synthesis of MSN. 1.00 g CTAB was dissolved into 480 mL of water first. Then 3.5 mL of sodium hydroxide aqueous solution (2.00 M) was introduced to the CTAB solution, and the temperature of the mixture was adjusted to 80℃. 5 mL of TEOS was added dropwise to the surfactant solution under vigorous stirring. The mixture was allowed to react for another 2 h to give a white precipitate. This solid crude

product was centrifuged, washed with deionized water and methanol, and dried in air to yield the as-synthesized mesoporous silica nanoparticles (denoted as MSN). To remove the surfactant template (CTAB), 1.5 g of the as-synthesized MSN was refluxed for 24 h in a methanolic solution of 9.00 mL of HCl (37.4%) in 160 mL of methanol. The resulting product was centrifuged and extensively washed with deionized water and methanol.

Synthesis of MSN-NH_2. The surface of MSNs was functionalized with amine groups by treatment with APTES. 50 mg of MSNs were dispersed in 50 mL of ethanol. The solution was then refluxed for 4 h, followed by the addition of 100 μL APTES. After centrifugation and washing with water, amine-functionalized MSNs were dried in air.

Synthesis of MSN@siRNA. For the preparation of MSN@siRNA, 500 μg of MSN-NH_2 was suspended in 500 μL of DEPC water containing siRNA for a final concentration of 1 μmol/g MSN. The mixture was slightly shaked using the shaking table for 3 hours at 4℃ before experiments.

Synthesis of Ce6@MSN. 2.5 mg of Ce6 and 5 mg of MSN-NH_2 were added into 1 mL solution (water : MeOH = 1:1). The mixture was then shaking overnight at 37℃ and kept in dark place.

Synthesis of Ce6@MSN@siRNA. 60 μL of Ce6@MSN was centrifuged and washed two times with DEPC water and then suspended into 200 μL of DEPC water containing siRNA for a final concentration of 1 μM. The mixture was slightly shaking using the shaking table for 3 hours at 4℃ before experiments.

Cell Culture and buffer. U2OS cells were purchased from ATCC (American Type Culture Collection, Manassas, VA, USA) and incubated in DMEM media supplemented with 10% (v/v) fetal bovine serum (Gibco), 100 U/mL penicillin and 100 mg/mL streptomycin at 37 °C in 5% CO_2 atmosphere. Dulbecco's Phosphate-Buffered Saline (D-PBS) without Ca^{2+} and Mg^{2+} (Invitrogen) was used to wash cells.

Flow cytometry experiments. U2OS cells (3 x 10^5) were seeded in a 30-mm dish. After 24-h incubation, the culture medium was removed. Cells were incubated with samples at desired concentrations in 1 mL of culture medium containing 5% FBS for 4 h. Then the cells were washed three times with 1 mL of D-PBS and detached with 200 μL of 0.25% EDTA. Finally, the cells were resuspended in 600 μL of D-PBS for flow cytometric analysis on the BD FACSVerse™ flow cytometer.

Confocal laser scanning microscopy experiments. U2OS cells (2.5 x 10^5) were seeded in a 35-mm confocal dish and incubated for 24 h. After washed three times with D-PBS, the cells were incubated with samples in 1 mL of culture medium containing 5% FBS for 4 h. After incubation, cells were washed twice with 1 mL of D-PBS and subjected to confocal microscope imaging using the FV1000-X81 confocal microscope (Olympus).

MTS assays. U2OS cells (3 x 10^5) were seeded in a 96-well plate and incubated for 24 h. After washing twice with D-PBS, the cells were treated with different concentrations of MSN or MSN@NC siRNA and MSN@MTH1 siRNA nanoparticles in culture medium supplemented with 5% FBS at 37 °C. Then, the culture medium was replaced with 200 μL of fresh culture medium. After 48-h incubation, the culture medium was discarded. And cells were washed with D-PBS. Finally, U2OS cells were cultured in 100 μL of fresh culture medium and 20 μL MTS reagent for the proliferation assay. After 30-min incubation at 37 °C, cell viability was monitored by measuring the absorption at 490 nm using a Synergy 2 Multi-Mode Microplate Reader (Bio-Tek, Winooski, VT).

Western blot analysis. U2OS cells (3 x 10^5) were seeded in a 30-mm dish and incubated for 24 h. Then cells were incubated with MSN@MTH1 siRNA, MSN@NC siRNA, Ce6@MSN@MTH1 siRNA or Ce6@MSN@NC siRNA in culture medium supplemented with 5% FBS at 37 °C. After 24-h incubation, the culture medium was replaced by 2 mL fresh culture medium. The other 24h later, cells were washed

with D-PBS. All subsequent steps were performed at 4°C. Cells were lysed for 20 min in 60 μL of lysis buffer (50 mM HEPES, pH 7.6, 250 mM NaCl, 0.1% NP-40, 5 mM EDTA, 1 mM PMSF, 10 mM N-ethylmaleimide, and 2 μg each of aprotinin, bestatin, and leupeptin/mL). The lysates were clarified by microcentrifugation for 20 min. The concentrations of protein were determined by NanoDrop 2000/2000c (Thermo). Total cellular proteins were separated by SDS-PAGE and transferred to nitrocellulose membranes. Protein detection was done using infrared fluorescent-conjugated secondary antibodies on a ChemiDoc XRS+ with image Lab software (Bio-RAD).

Reverse transcriptase quantitative PCR analysis. U2OS cells (3×10^5) were seeded in a 30-mm dish. After 24-h incubation, the cells were washed and incubated with MSN@MTH1 siRNA, MSN@NC siRNA, Ce6@MSN@MTH1 siRNA or Ce6@MSN@NC siRNA in culture medium supplemented with 5% FBS at 37 °C for 24 h. Then the culture medium was replaced by 2 mL fresh culture medium. After another 24-h incubation, cells were washed with D-PBS and used for the quantitative PCR analysis. Quantitative real-time PCR analysis was performed using the Power SYBR Green PCR Master kit (Applied Biosystems). The sequences of PCR primers are listed in Supplementary Table S2. Amplification was performed on 7500 real time PCR system.

Immunofluorescence analysis. U2OS cells were seeded on 30 mm confocal dish and incubated for 24 h. Then, cells were treated with MSN@MTH1 siRNA, MSN@NC siRNA, Ce6@MSN@MTH1 siRNA or Ce6@MSN@NC siRNA. After 24-h incubation, the culture medium was replaced by another 2 mL fresh culture medium. After 24-h treatment, the cells were then fixed in 4% paraformaldehyde for 15 min at room temperature, followed by permeabilization with 0.2% Triton X-100 in D-PBS for 15 min. Cells were then blocked for nonspecific binding with 5% BSA, and incubated with anti-MTH1 (1:500) antibodies overnight. The cells were then washed with D-PBS, incubated with secondary antibodies conjugated with Alexa Fluor

594 goat anti-rabbit IgG (1:200) for 60 minutes at room temperature. Then the cells were treated with DAPI. Immunofluorescence images were acquired on the FV1000-X81 confocal microscope (Olympus). For each sample, all images were acquired with the same settings.

Phototoxicity study. U2OS cells were seeded in a 96-well plate at a density of 4 x 10^3 cells per well and incubated for 24 h. After removing the culture medium, cells were incubated with different concentrations of Ce6, Ce6@MSN or Ce6@MSN@siRNA nanosystem at 37 °C for 4 h. Then the culture medium was replaced with 200 μL of fresh culture medium. For PDT, cells were irradiated with white light (4100K, 32W, USA) for 3 h. After 48-h incubation, the cell medium was replaced with 100 μL of fresh culture medium and 20 μL of MTS solution. After 30-min incubation, therapeutic efficacy of PDT on U2OS cells was assayed by measuring the absorbance at 490 nm, using a Synergy 2 Multi-Mode Microplate Reader (Bio-Tek, Winooski, VT).

Animal model. Female Balb/c mice (~20 g) were obtained from Hunan SJA Laboratory Animal Co., Ltd. and used under protocols approved by Hunan University Laboratory Animal Center. To generate the HCT116 human colon carcinoma tumor model, ~8 x 10^6 HCT116 cells in ~100 μL D-PBS were subcutaneously injected in the shoulder of each mouse. The mice were treated when the tumor volumes approached 60~70 mm^3.

In vivo PDT treatment. When the tumor size reached ~60 mm^3, HCT116 tumor-bearing mice were divided into 8 groups of four animals per group to quantify the growth rate of tumors after the following treatments: #1 D-PBS only; #2 MSN@NC siRNA; #3 MSN@MTH1 siRNA; #4 Ce6@MSN; #5 Ce6@MSN and MSN@NC siRNA; #6 Ce6@MSN and MSN@MTH1 siRNA; #7 Ce6@MSN@NC siRNA; #8 Ce6@MSN@MTH1 siRNA. The total amount of the regent for each treatment was 50 μL. The Ce6 concentration in Ce6@MSN and Ce6@MSN@siRNA were 100 μM. The siRNA concentration in

MSN@siRNA and Ce6@MSN@siRNA were 2 μM. 24 h later, the tumors were exposed to 660 nm laser (LSR660NL-FC-500) of power density at 500 mW for 10 min. The spot of laser beam was adjusted to cover the entire region of tumor. 48 h later, the tumors were exposed to 660 nm laser for 10 min again. For the groups treated without irradiation, the total amount of the regent for each treatment was also 50 μL. The Ce6 and siRNA concentration were also 100 μM and 2 μM, respectively. The size of tumors was measured by caliper every other day after the treatment. The volume of tumor (V) was calculated by the following equation: $V = A*B^2/2$, where A and B are the longer and shorter diameter (mm) of the tumor, respectively. The relative volume of tumors was evaluated by normalizing the measured values to their initial volumes. The mice were finally sacrificed to examine the histopathology of tumors by H&E staining. Slides were recorded using a Pannoramic MIDI digital slide scanner with Zeiss plan-apochromat objective (magnification: 20x, Numerical aperture: 0.8) and Hitachi (HV-F22CL) 3CCD progressive scan colour camera (resolution: 0.2325 μm/pixel).

Table S1. SiRNA oligonucleotides used in this work.

Oligonucleotides	Sequence (from 5’ to 3’)
MTH1 sense	FAM-GAC GAC AGC UAC UGG UUU CTT
MTH1 antisense	GAA ACC AGU AGC UGU CGU CTT
NC sense	FAM-UUC UCC GAA CGU GUC ACG UTT
NC antisense	ACG UGA CAC GUU CGG AGA ATT

Table S2. Primers used for reverse transcriptase quantitative PCR in this work.

Oligonucleotides	Sequence (from 5’ to 3’)
MTH1_F	GTG CAG AAC CCA GGG ACC AT
MTH1_R	GCC CAC GAA CTC AAA CAC GA
GAPDH_F	GGA GCG AGA TCC CTC CAA AAT
GAPDH_R	GGC TGT TGT CAT ACT TCT CAT GG

Table S3. Barret-Joyner-Halenda (BJH) surface area and pore volume values calculated from the N_2 adsorption-desorption isotherms for selected materials.

	Surface area / m^2g^{-1}	Pore volume / cm^3g^{-1}
MSN	1149	1.21
Ce6@MSN	534	0.41

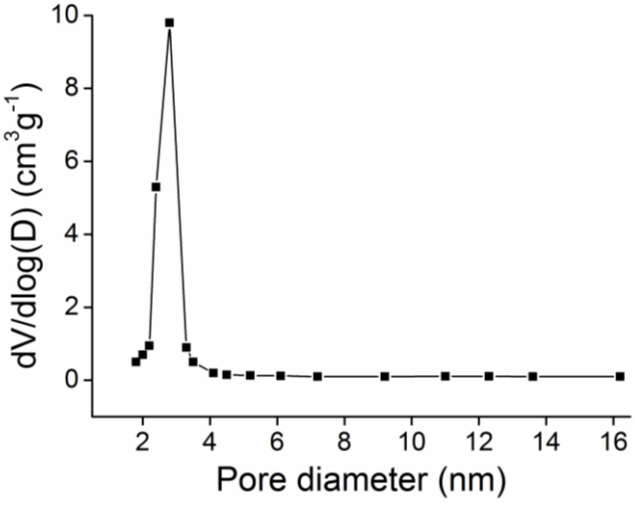

Fig. S1 BJH pore size distributions of MSN.

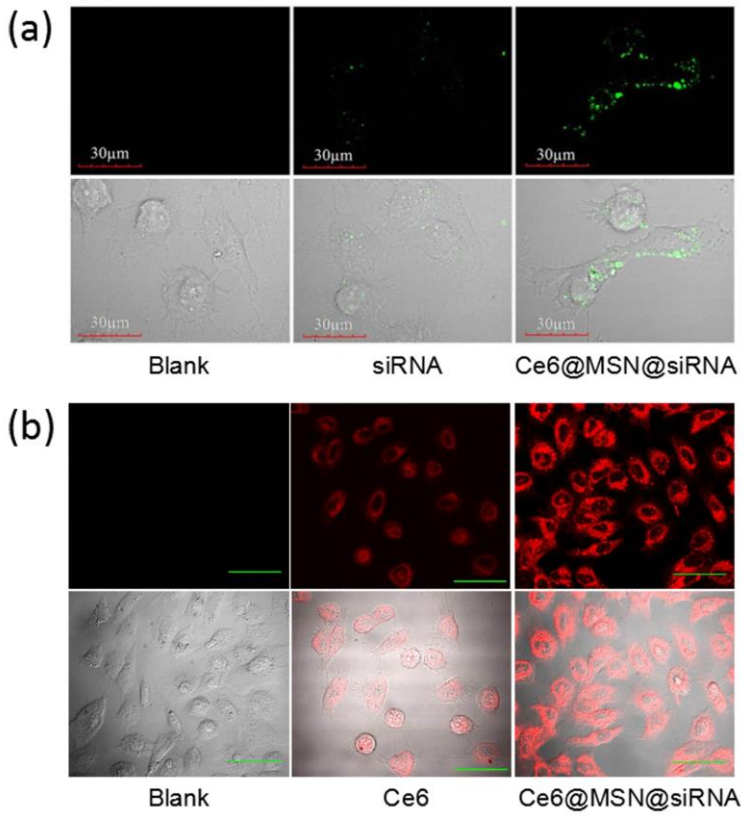

Fig. S2 (a) Confocal images of U2OS cells treated with free MTH1 siRNA or Ce6@MSN@MTH1 siRNA nanosystem. FAM ex: 488 nm, em: 500 nm~550 nm. (b) Confocal images of U2OS cells treated with free Ce6 or Ce6@MSN@MTH1 siRNA nanosyatem. Scale bar = 50 μm. Ce6 ex: 405 nm, em: 650 nm~700 nm.

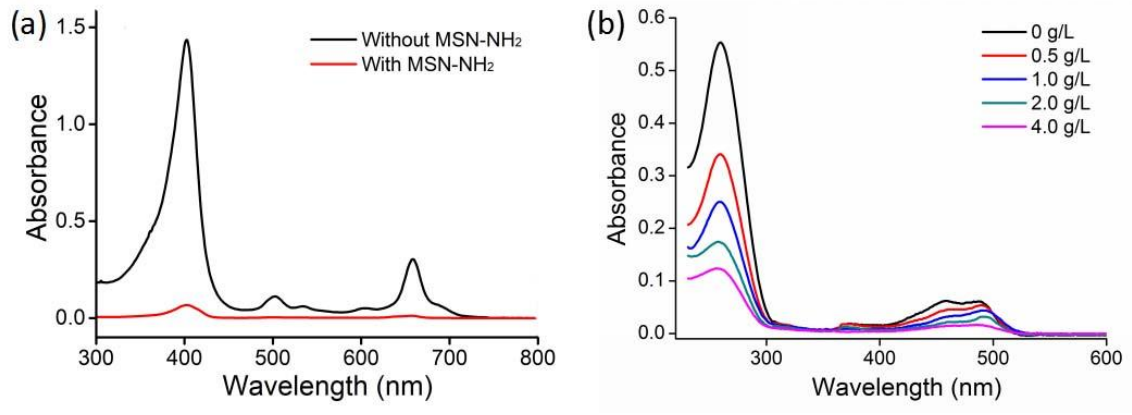

Fig. S3 (a) UV-vis spectrum of Ce6 in the supernatant before and after incubation with MSN-NH_2. (b) UV-vis spectrum of FAM-siRNA in the supernatant after mixing 1 μM siRNA with different concentrations of Ce6@MSN.

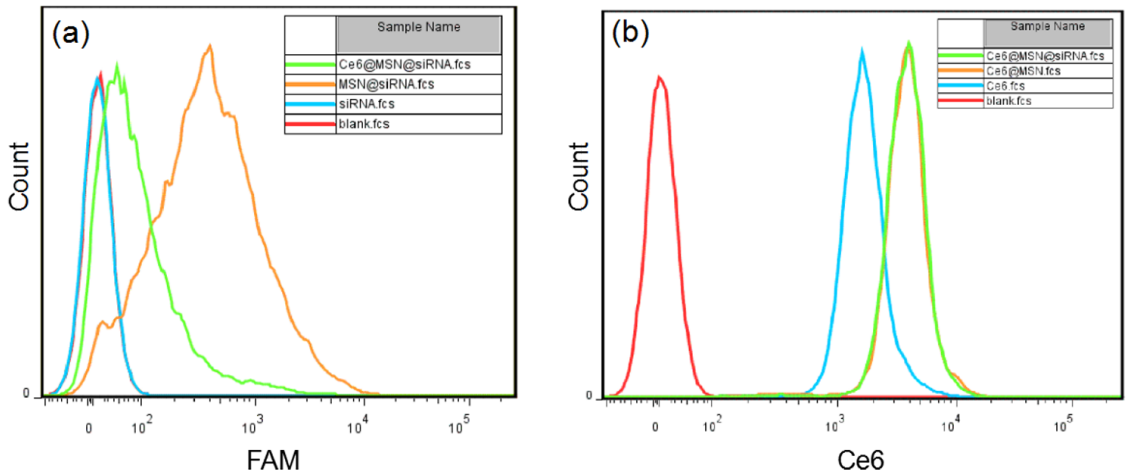

Fig. S4 (a) Flow cytometric analysis of cells treated with free siRNA, MSN@siRNA and Ce6@MSN@MTH1 siRNA. (b) Flow cytometric analysis of cells treated with free Ce6, Ce6@MSN and Ce6@MSN@MTH1 siRNA. The concentration of MSN was 1.5 g/L. FAM em: 520 nm. Ce6 em: 670 nm.

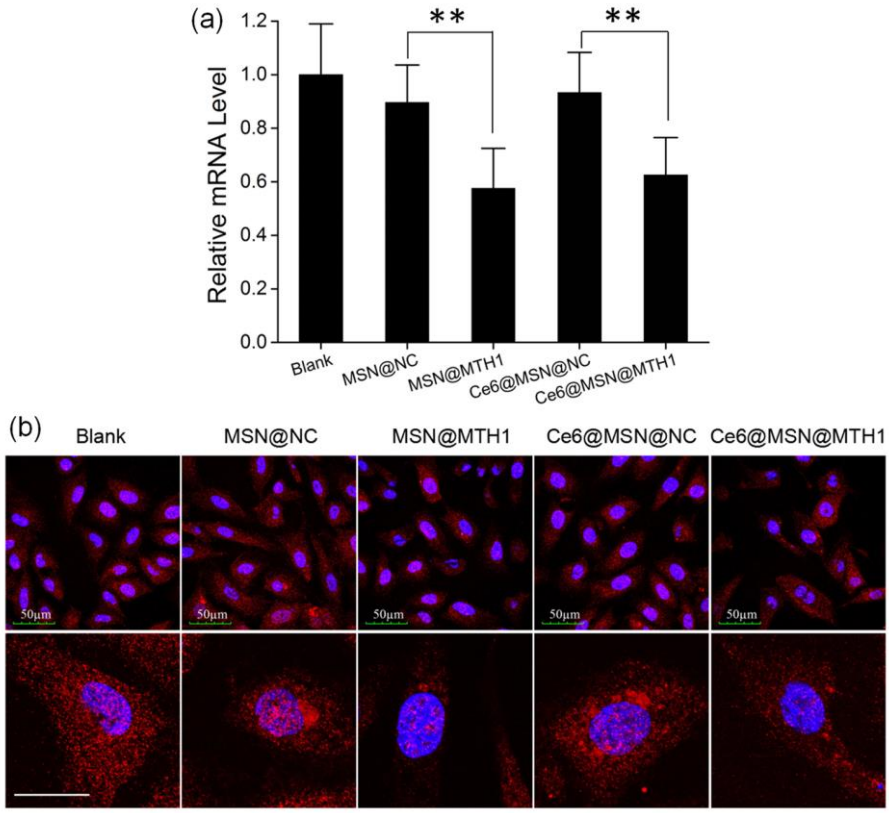

Fig. S5 (a) Reverse transcriptase quantitative PCR analysis of MTH1 expression in U2OS cells treated with MSN@NC siRNA, MSN@MTH1 siRNA, Ce6@MSN@NC siRNA and Ce6@MSN@MTH1 siRNA nanosystem. P values were calculated by t-test. **P<0.01. (b) Immunofluorescence analysis with antibodies against MTH1. U2OS cells were treated with the same systems as in (a). (Red fluorescence: Antibodies against MTH1, ex: 543 nm, em: 580 nm~650 nm; blue fluorescence: DAPI, ex: 365 nm, em: 400 nm~500 nm.). Samples in the bottom were images of samples on the top at high magnification. Scale bar = 20 μm.

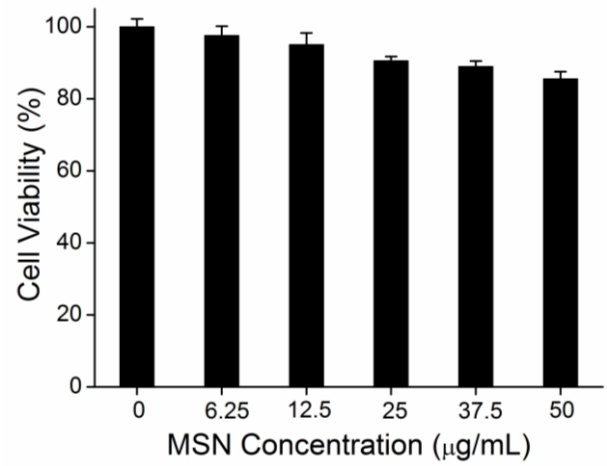

Fig. S6 Cytotoxicity of MSN. U2OS cells were treated with MSN at different concentrations as indicated.

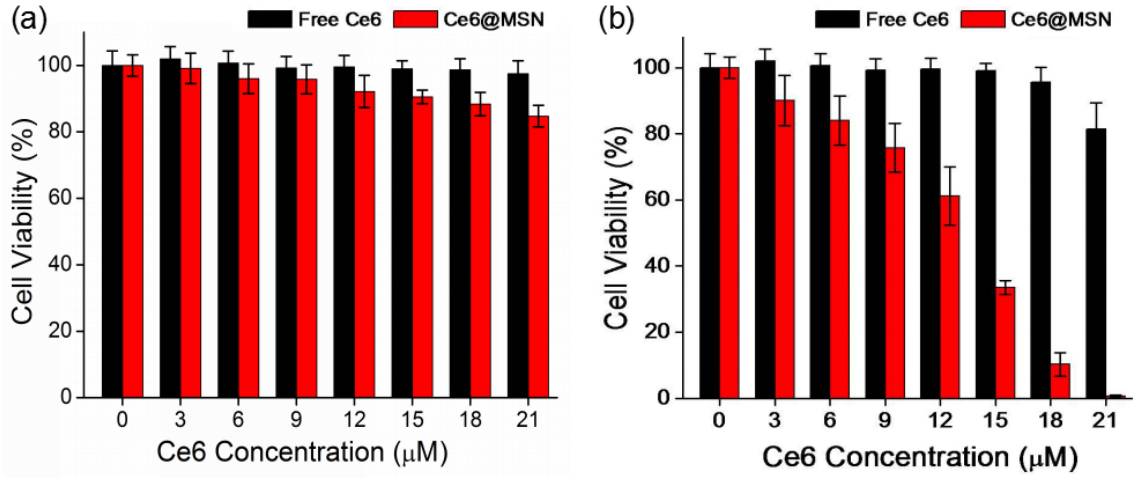

Fig. S7 Cytotoxicity of Ce6 and Ce6@MSN. U2OS cells were treated with Ce6 or Ce6@MSN with Ce6 at different concentrations as indicated in the absence (a) or presence (b) of light irradiation.

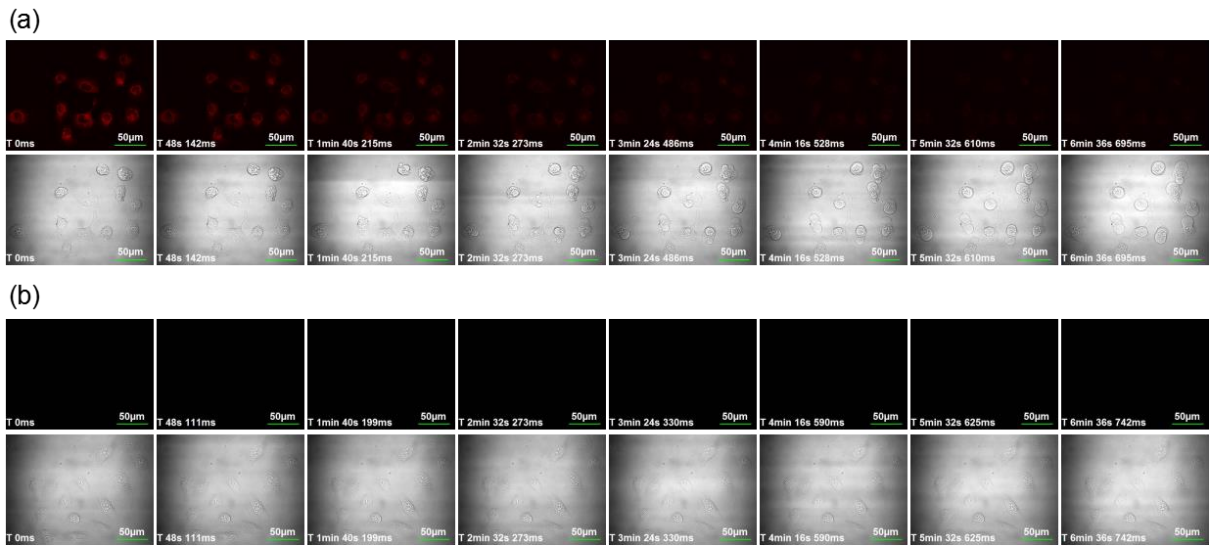

Fig. S8 Real-time fluorescence images (top) and morphology (bottom) of U2OS cells treated with Ce6@MSN (a) or untreated (b) with a laser of 405 nm (2 mW).

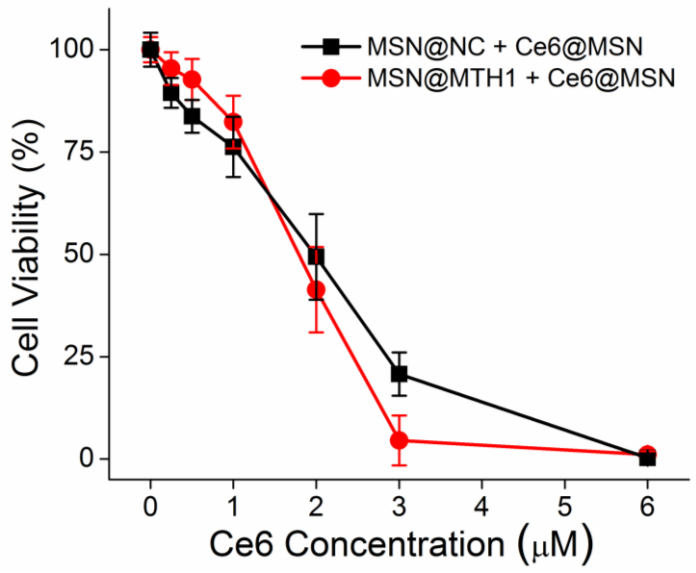

Fig. S9 HEK-293 cells were first treated with MSN@NC siRNA or MSN@MTH1 siRNA, and then treated with Ce6@MSN with Ce6 at different concentrations as indicated.

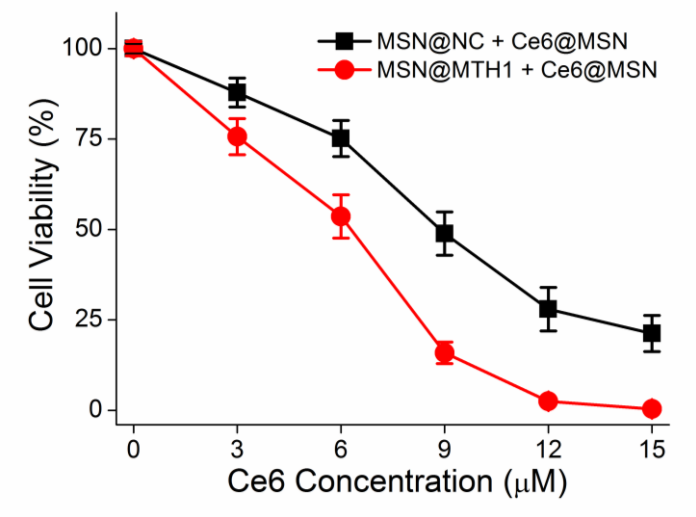

Fig. S10 HCT116 cells were first treated with MSN@NC siRNA or MSN@MTH1 siRNA, and then treated with Ce6@MSN with Ce6 at different concentrations as indicated.

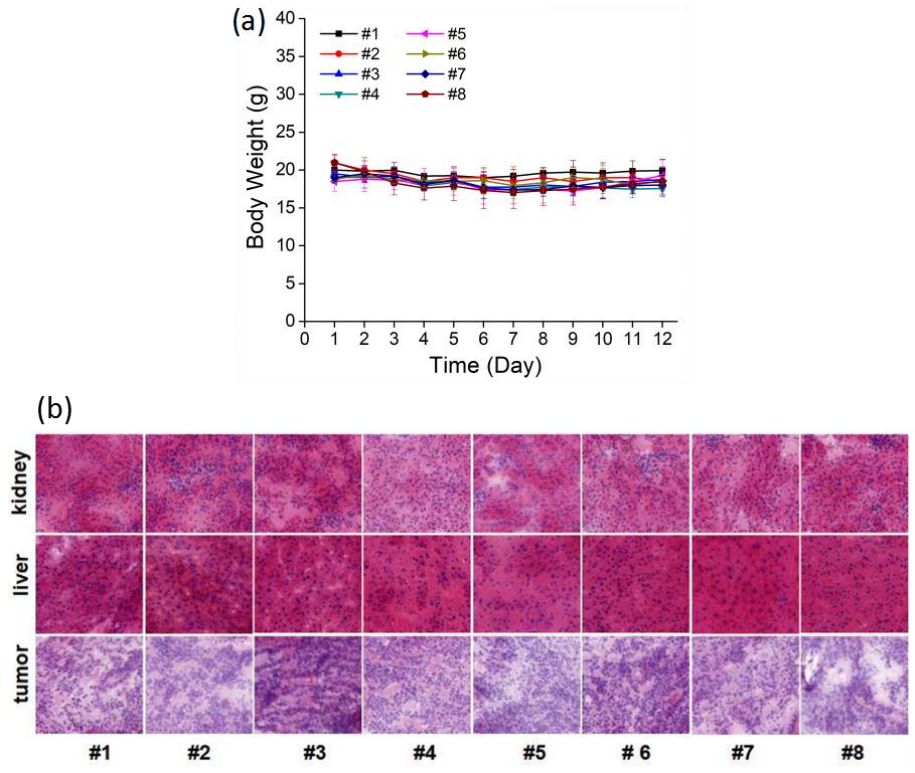

Fig. S11 (a) Body weight curves of HCT116 tumor-bearing mice for each group. (b) H&E stained images of kidney, liver and tumor collected from different groups of mice 12 days post-treatment.

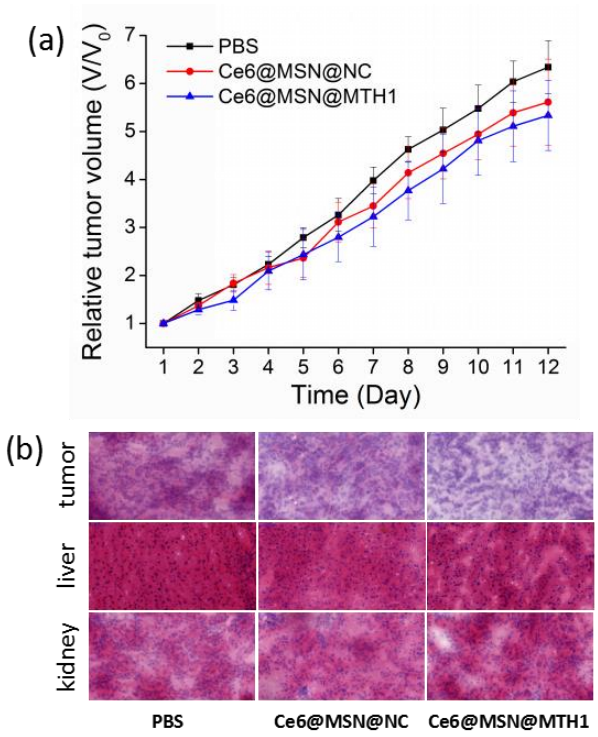

Fig. S12 (a) Tumor growth rate in each group after drug injection without irradiation. Tumor volumes were normalized to their initial size. (b) H&E stained images of tumor, liver and kidney collected from different groups of mice 12 days post-treatment.