Supporting Information

ROS-Sensitive Polymeric Nanocarriers with Red Light-Activated Size Shrinkage for Remotely Controlled Drug Release

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MATERIALS AND METHODS

Synthesis of the ROS-cleavable TK linker: In a typical reaction, a mixture of cysteamine hydrochloride (11.36 g, 100 mmol) and anhydrous acetone (15.6 g, 269 mmol) were saturated with dry hydrogen chloride and stirred at room temperature for 8 h. After the reaction, the product was filtered, washed with chloroform twice. Then, the product was dried and recrystallized from 6 M NaOH aqueous solution three times. Finally, the product was extracted with 5 × 100 mL of dichloromethane (CH_2Cl_2) to obtain the product (6.33 g, 33 mmol, 65% yield). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 2.92 (-CH_2-, 4H), 2.74 (-CH_2-, 4H), 1.62 (-CH_3, 6H).

Synthesis of the ROS-sensitive polymer TK-PPE: TK linker (1.0 g, 5.15 mmol) and triethylamine (1.56 g, 15.4 mmol) were dissolved in 10 mL dichloromethane. And then, ethyl dichlorophosphate (0.84 g, 5.15 mmol) dissolved in 10 mL CH_2Cl_2 was dropwise added into the above solution under stirring. And then, the mixture was reacted for another 24 h; hereafter, the precipitate triethylammonium chloride was removed, and the filtrate was washed three times with 1 M NaOH, 10% $NaHCO_3$ and saturated brine, respectively. Finally, the product solution was dried, precipitated into a cold diethyl ether/methanol mixture (10/1, v/v) twice to gain the ROS-sensitive polymers TK-PPE (0.67 g, 44% yield).

Synthesis of the amphiphilic polymer PEG-b-PCL: The amphiphilic polymer PEG-*b*-PCL was synthesized by ring-opening polymerization of ε-caprolactone (CL) in bulk using PEG

(M_n = 5000) as the initiator and Stannous octoate ($Sn(Oct)_2$) as the catalyst. In a typical example, the PEG (2.0 g, 0.40 mmol), CL (1.14 g, 10.0 mmol), and $Sn(Oct)_2$ (0.04 g, 0.1 mmol) were added into a flask in a glove box with H_2O and O_2 contents less than 0.1 ppm. The mixture was maintained at 100 °C for 2 h. After reaction, the crude product was dissolved in 40 mL of tetrahydrofuran (THF) and precipitated into a cold mixture of diethyl ether/methanol (10/1, v/v) twice. The precipitated product was dried under vacuum to a constant weight at room temperature with a yield of 83.7%.

Analysis of loading contents of DOX and Ce6: To determine drug loading content (DLC) and encapsulation efficiencies (EE), the TK-PPE@$NP_{Ce6/DOX}$ or the control formulations TK-PPE@NP_{DOX}/TK-NP_{Ce6} solution was lyophilized. Then, the lyophilized nanoparticles were weighted and redissolved in DMSO. The DOX and Ce6 concentrations were determined according to described methods below. The DLC and EE of DOX and Ce6 were calculated by the following equations:

$$\text{DLC (\%)} = \frac{\text{amount of DOX or Ce6 in nanoparticles}}{\text{amount of DOX and Ce6 - loaded nanoparticles}} \times 100\%$$

$$\text{EE (\%)} = \frac{\text{amount of DOX or Ce6 in nanoparticles}}{\text{amount of DOX or Ce6 added}} \times 100\%$$

Determining DOX and Ce6 concentrations: The concentration of DOX was determined by high-performance liquid chromatography (HPLC) analyses, using a Waters HPLC system equipping with a Waters 1525 binary pump, a Waters 2475 fluorescence detector, 1500 column heater, and a Symmetry C18 column. HPLC grade acetonitrile/water (50/50, v/v) with pH 2.7 adjusted by $HClO_4$ was used as the mobile phase at 30 °C with a flow rate of 1.0 mL min^{-1}. Fluorescence detector was set at 460 nm for excitation and 570 nm for emission and linked to Breeze software for data analysis. Linear calibration curves for concentrations in the range of 0.1-10.0 μg/mL were constructed using the peak areas by linear regression analysis. The concentration of DOX in the solution was calculated based the standard curve.

The concentration of Ce6 was also determined by HPLC analyses, using a Waters HPLC system consisting of a Waters 1525 binary pump, a Waters 2487 UV/visible detector, a 1500 column heater and a Symmetry C18 column. The UV/visible detector was set at 405 nm and linked to Breeze software for data analysis. HPLC grade ammonium acetate buffer (0.05 M, pH 5.5) with methanol at a ratio of 38:62 (v/v) was used as the mobile phase at 30 °C with a flow rate of 0.7 mL min^{-1}. Linear calibration curves for concentrations in the range of 1.0-32.0 μg/mL were constructed using the peak areas by linear regression analysis. The concentration of Ce6 in the solution was calculated based the standard curve.

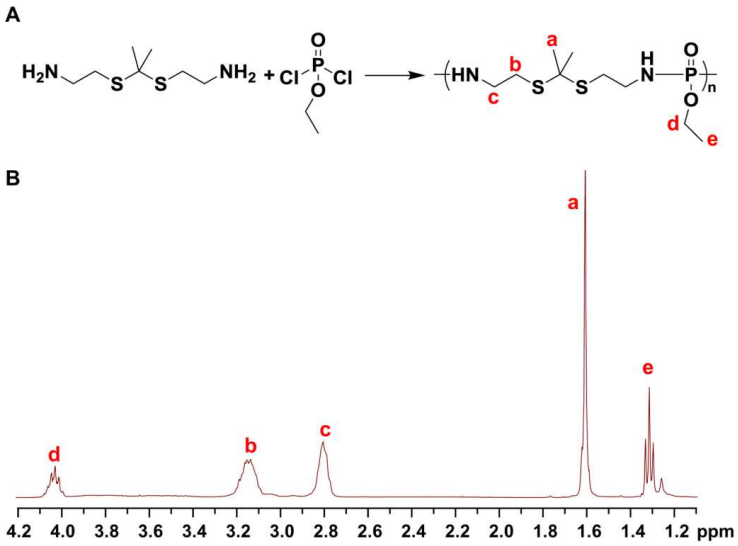

Figure S1. (A) Schematic illustration for the synthesis of ROS-responsive polymer TK-PPE. (B) ^{1}H NMR spectra of TK-PPE.

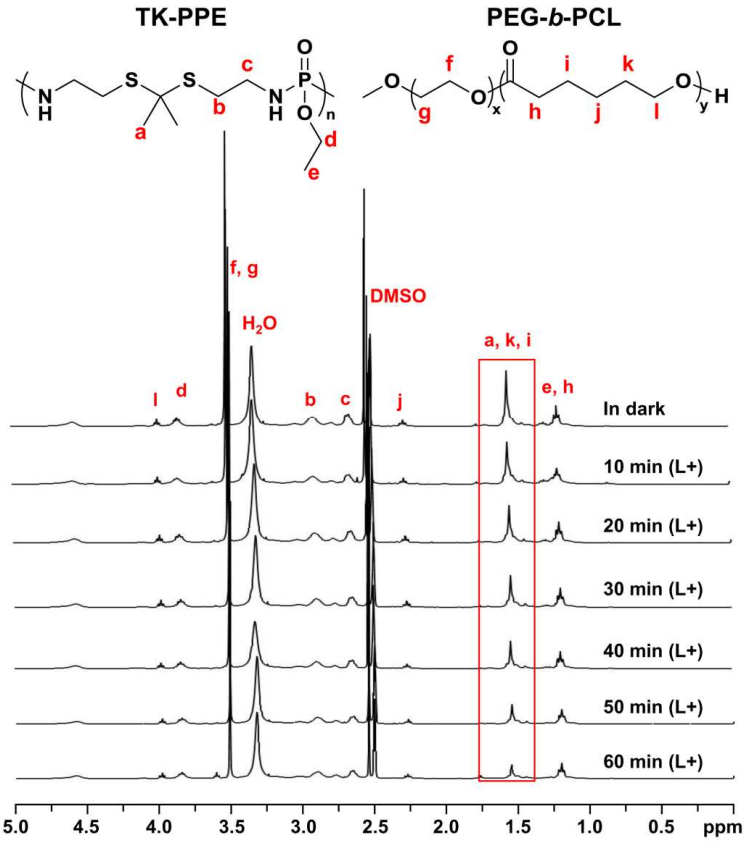

Figure S2. ^{1}H NMR spectra of TK-PPE@NP$_{Ce6}$ after 660 nm laser irradiation for different times (10, 20, 30, 40, 50 and 60 min) at a power density of 0.3 W/cm^2.

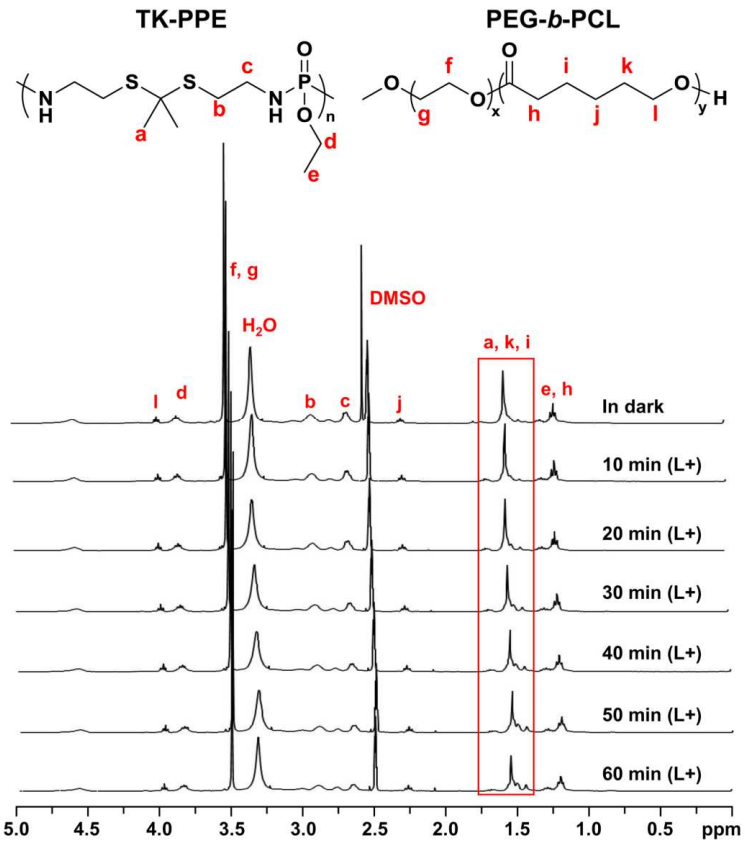

Figure S3. ^{1}H NMR spectra of TK-PPE@NP$_{Ce6}$ after 660 nm laser irradiation for different times (10, 20, 30, 40, 50 and 60 min) at a power density of 0.15 W/cm^{2}.

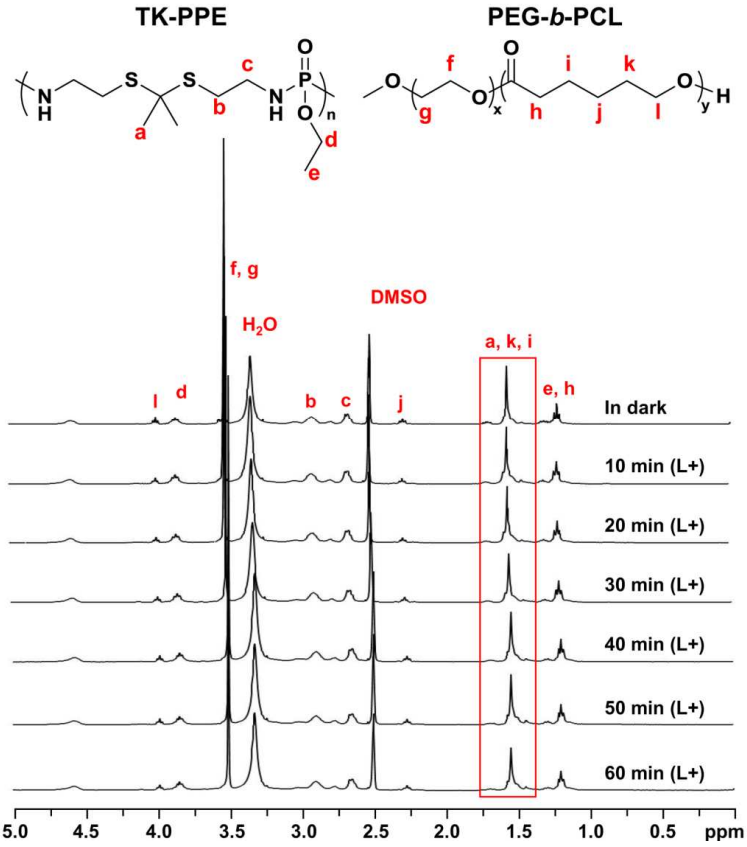

Figure S4. ^{1}H NMR spectra of TK-PPE@NP$_{Ce6}$ after 660 nm laser irradiation for different times (10, 20, 30, 40, 50 and 60 min) at a power denstiy of 0.06 W/cm^2.

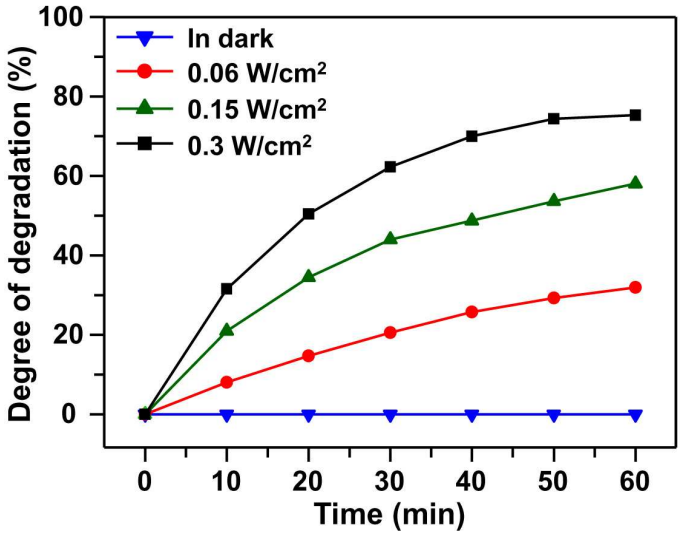

Figure S5. The degradation of TK-PPE in TK-PPE@NP$_{Ce6}$ *verses* 660-nm laser irradiation times at different power densities.

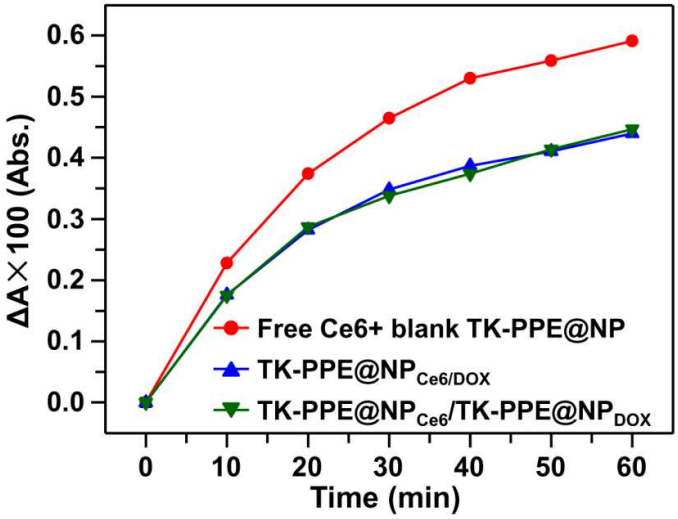

Figure S6. ROS generation by various samples under the 660 nm laser irradiation. The generation of ROS was determined by the bleaching of RNO absorbance at 440 nm.

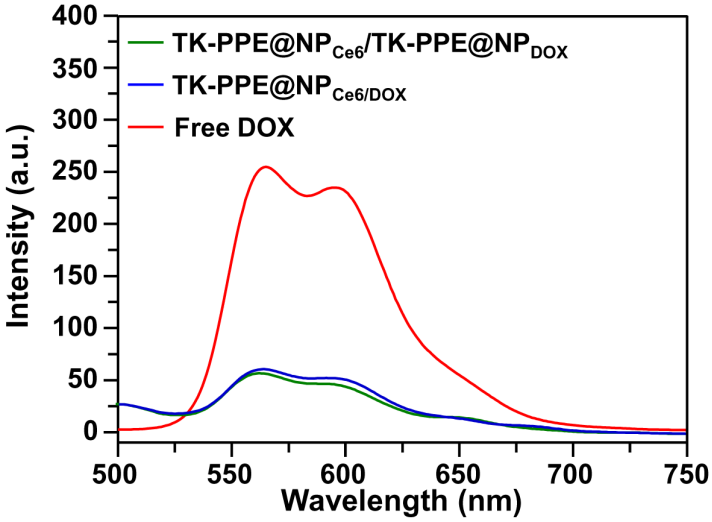

Figure S7. Fluorescent intensity of free DOX, TK-PPE@NP$_{Ce6/DOX,}$ and TK-PPE@NP$_{Ce6}$/TK-PPE@NP$_{DOX}$ at the equivalent DOX concentrations (5.0 μg/mL).

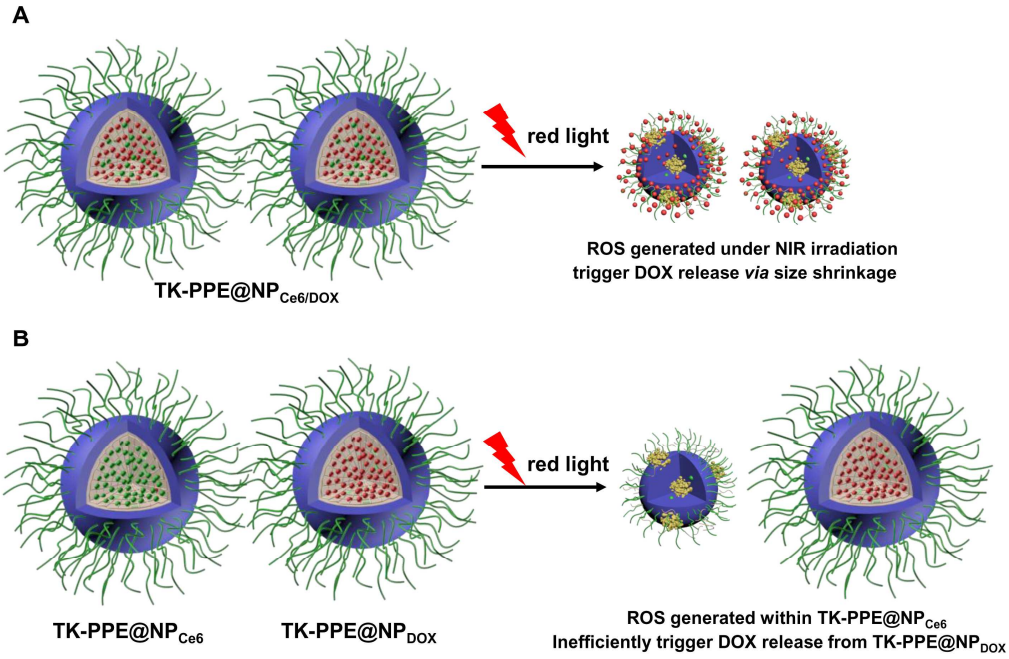

Figure S8. Schematic illustration of the mechanism of DOX release from TK-PPE@NP$_{Ce6/DOX}$ and TK-PPE@NP$_{Ce6}$/TK-PPE@NP$_{DOX}$ under 660-nm laser irradiation.

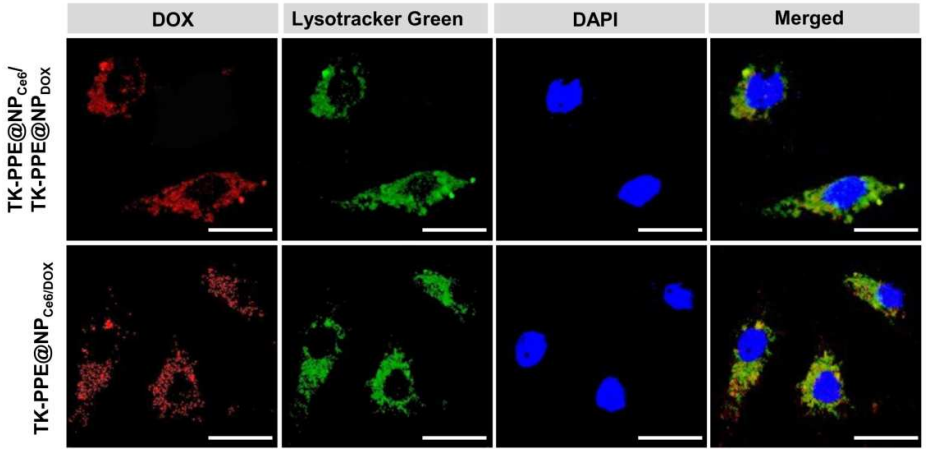

Figure S9. CLSM images of MDA-MB-231 cells without 660-nm laser irradiation. The MDA-MB-231 cells were pre-cultures with TK-PPE@$_{Ce6/DOX}$ or TK-PPE@NP$_{Ce6}$/TK-PPE@NP$_{DOX}$ for 2 h. Cell nuclei and endosomes/lysosomes were counterstained with DAPI (blue) and LysoTracker Green (Green). The scale bar is 10 μm.

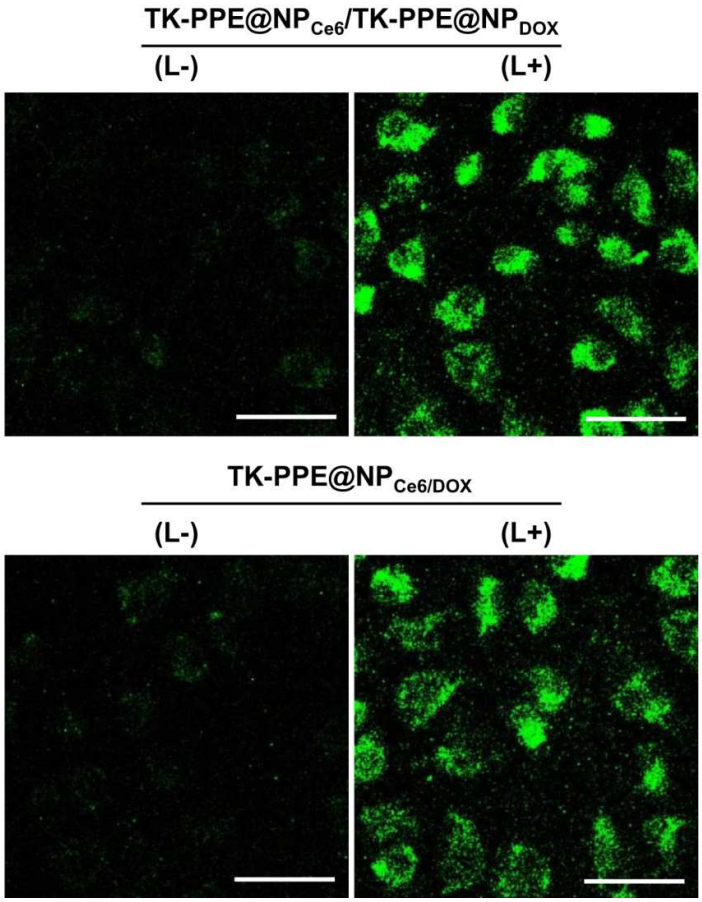

Figure S10. Fluorescence microscopy image of cells incubated with DCFH-DA and then treated with TAT-NP$_{Ce6/DOX}$ and TK-PPE@NP$_{Ce6}$/TK-PPE@NP$_{DOX}$ with or without 660-nm laser irradiation (0.3 W/cm^2, 30 min). Scale bar = 20 μM.

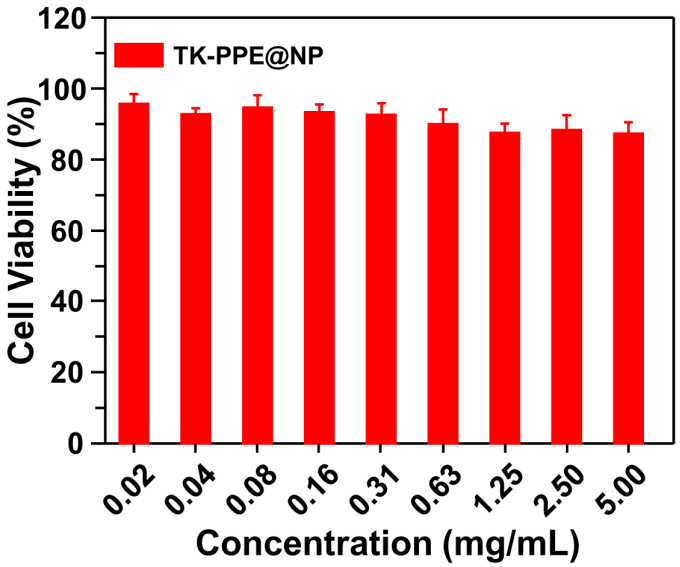

Figure S11. Relative viability of MDA-MB-231 cells after incubation with blank nanoparticle TK-PPE@NP.

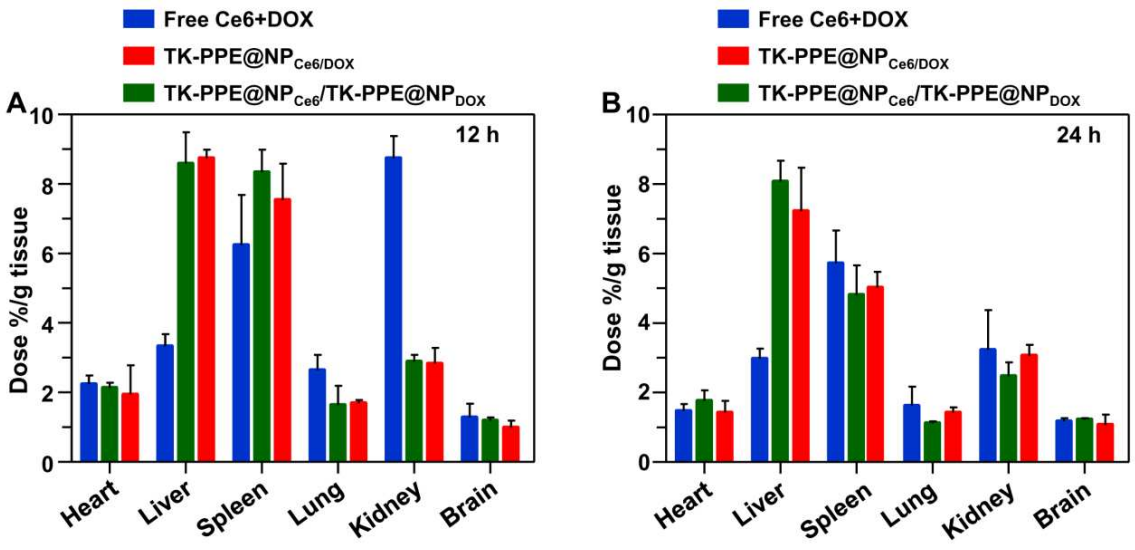

Figure S12. Biodistributions of DOX in the major organs at 12 h (A) and 24 h (B) after administration of different formulations.

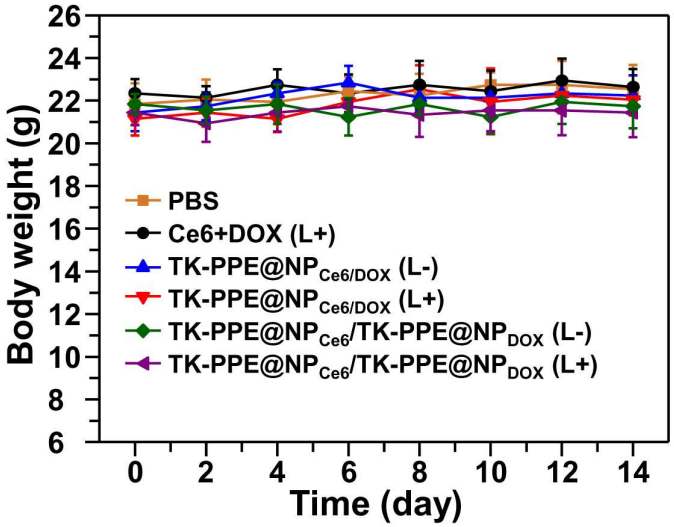

Figure S13. Body weight of mice bearing MDA-MB-231 tumor at different time points after treatment.

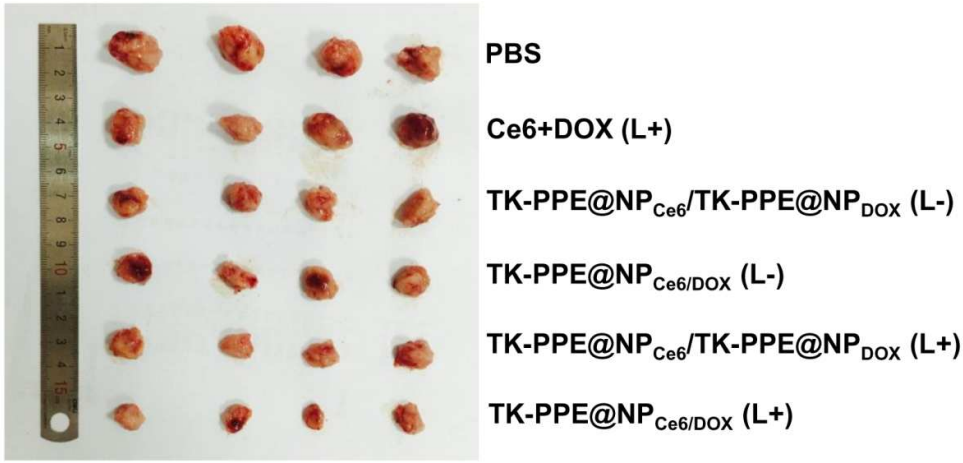

Figure S14. Tumor images of mice at the end time point of the treatment.