Supporting Information

Site-Specific Drug-Releasing Polypeptide Nanocarriers Based on Dual-pH Response for Enhanced Therapeutic Efficacy against Drug-Resistant Tumors

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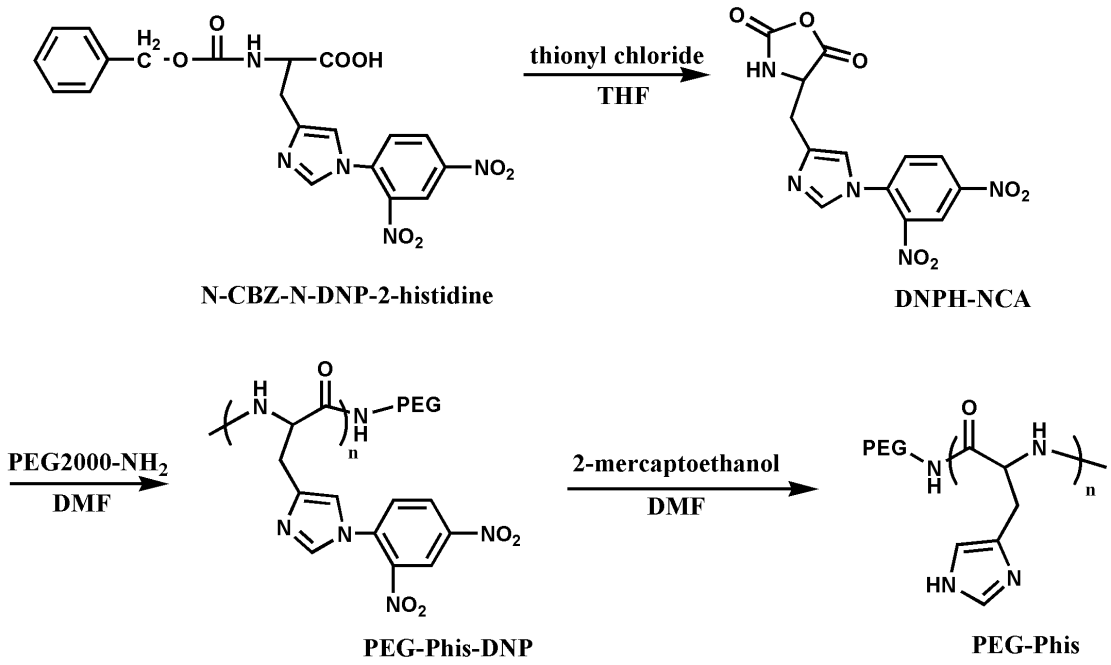

Figure S1. Illustration of the synthetic approach of PEG-Phis block polymers.

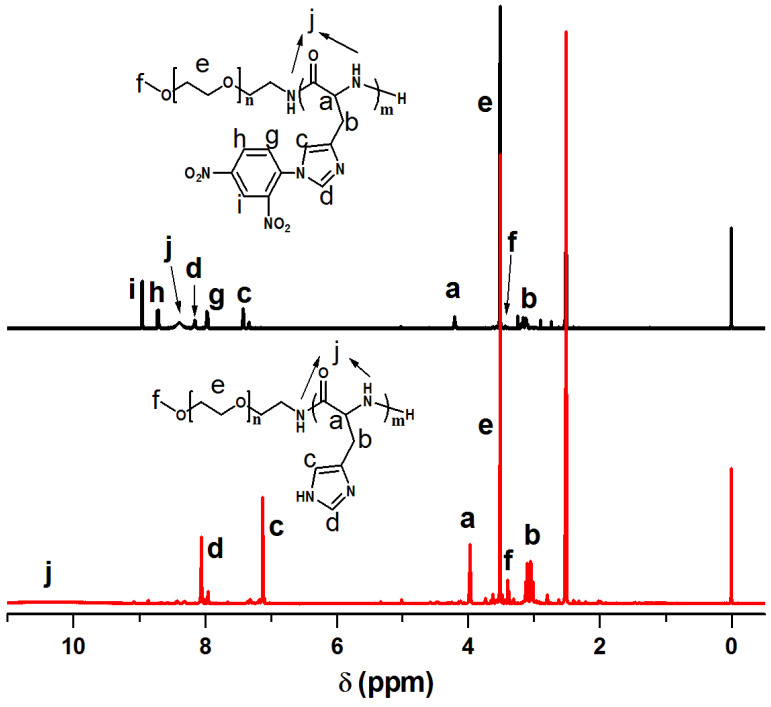

Figure S2. The NMR spectra of PEG-Phis-DNP (upper, black color) and PEG-Phis (lower, red color) in DMSO-d_6.

Table S1. The molecular weights of PEG-Phis block polymers and degree of polymerization (DP) of the polyhistidine block.

PEG2000:polyhistidine	DP by NMR	Mn*
PEG-Phis20	40	7600
PEG-Phis40	70	11800
PEG-Phis60	140	21600
PEG-Phis80	180	27200

*Calculated by NMR.

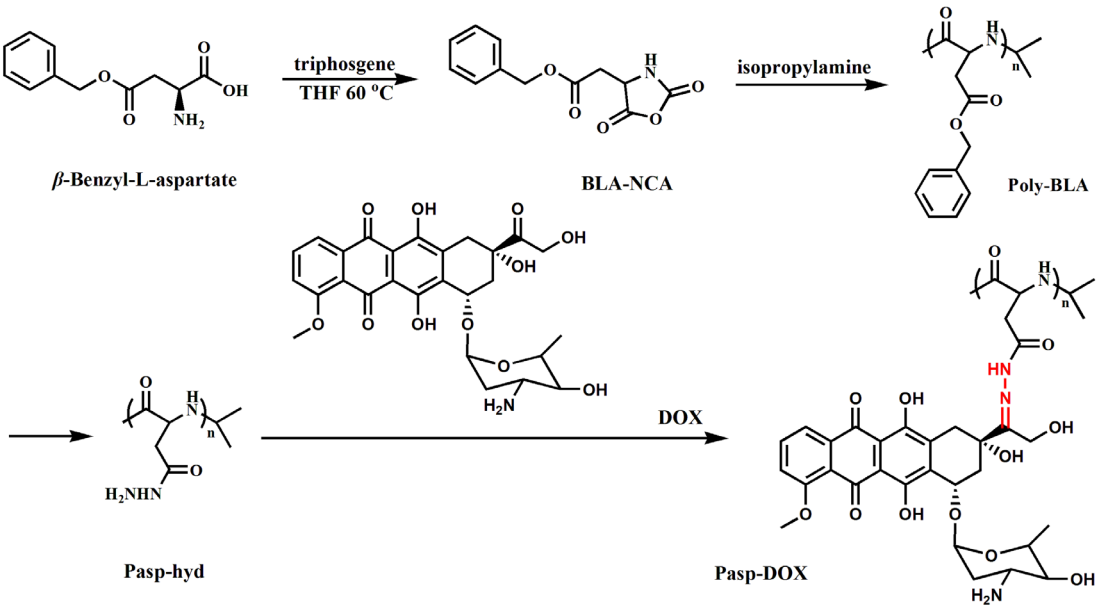

Figure S3. Illustration of the synthetic approach of Pasp-DOX. The red linker represented the hydrazone bond, which could sensitive to break at lower pH (5-6).

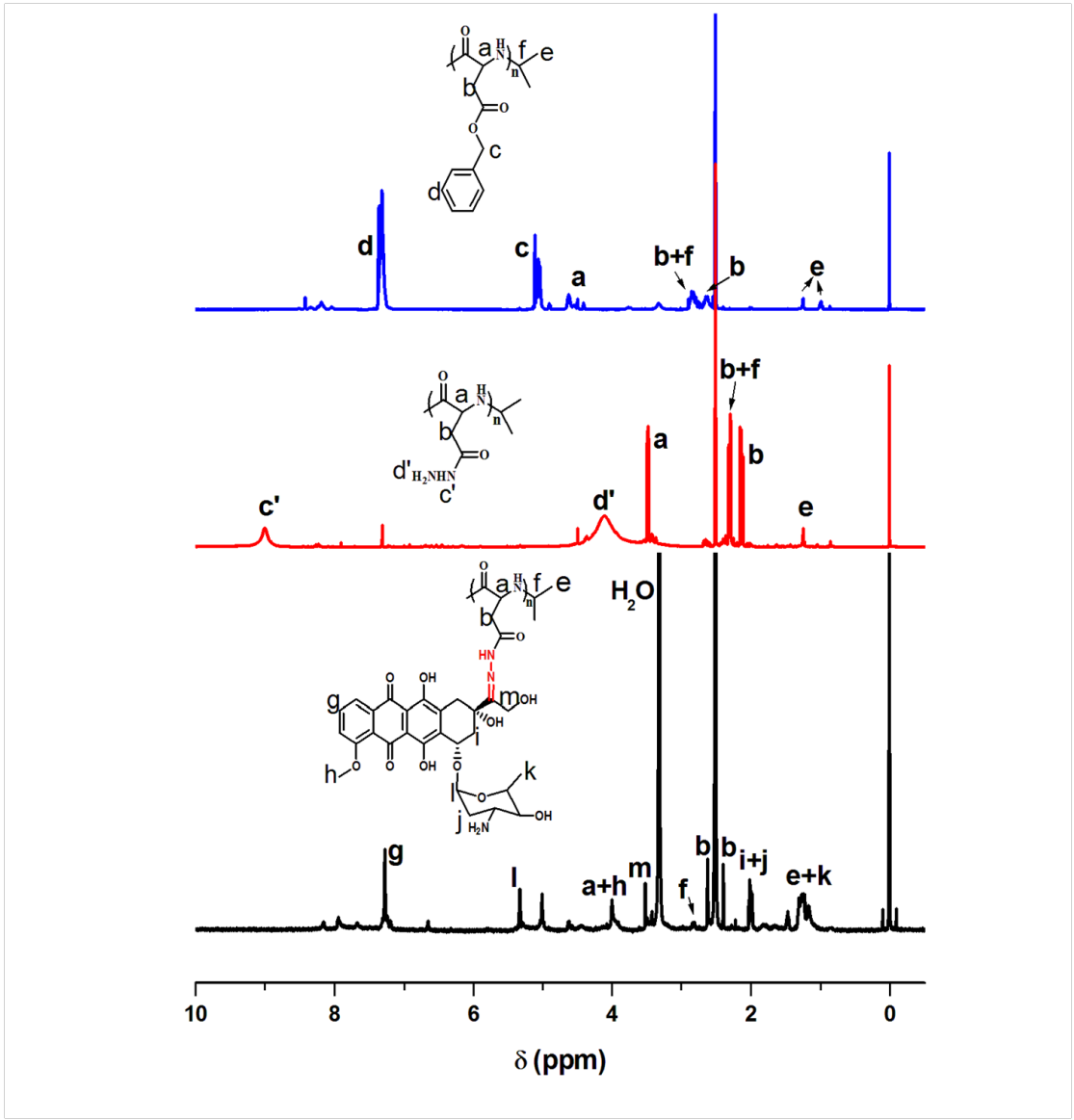

Figure S4. The NMR spectra of Pasp-BLA (upper, blue color), Pasp-hyd (middle, red color) and Pasp-DOX (lower, black color) in DMSO-d_6.

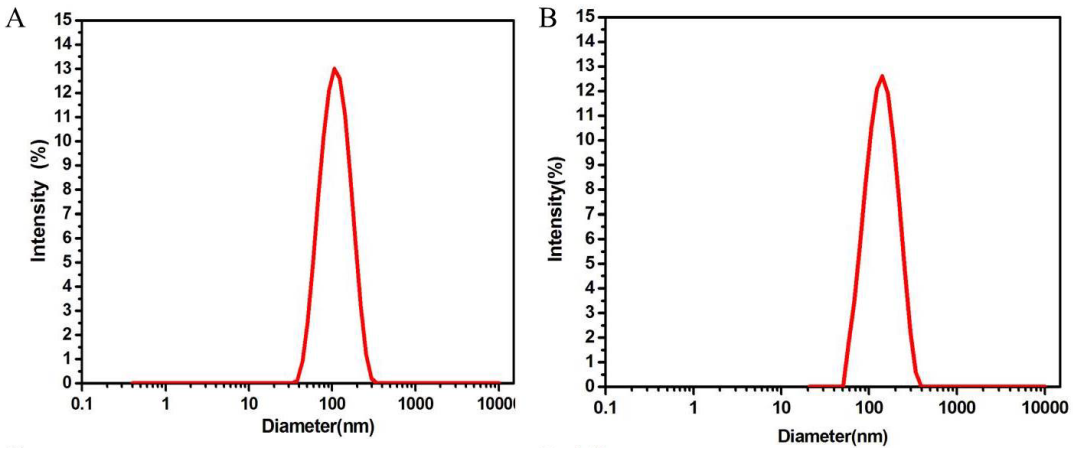

Figure S5. Dynamic light scattering analyses of the PEG-Phis60/Pasp-DOX/CA4 nanoparticles (A) and PEG-Phis40/Pasp-DOX/CA4 (B) nanoparticles.

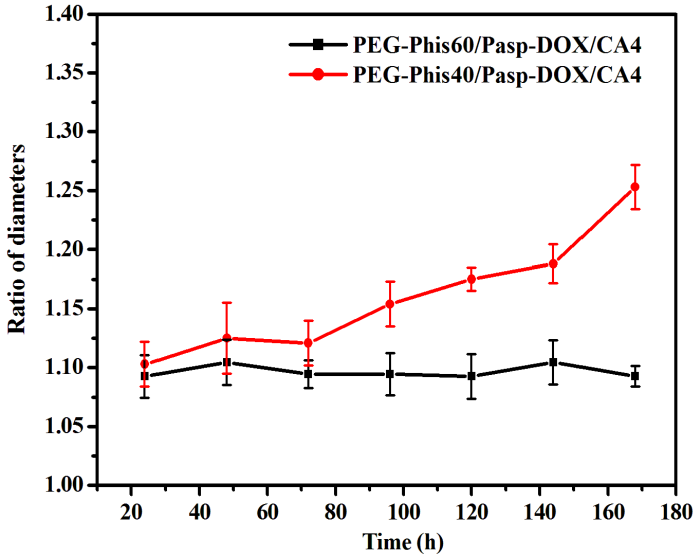

Figure S6. Dynamic light scattering analyses of the PEG-Phis60/Pasp-DOX/CA4 nanoparticles and PEG-Phis40/Pasp-DOX/CA4 nanoparticles, which was incubated with 20% FBS for 1 week at 37°C. Data are shown as the mean±S.D. of three independent experiments.

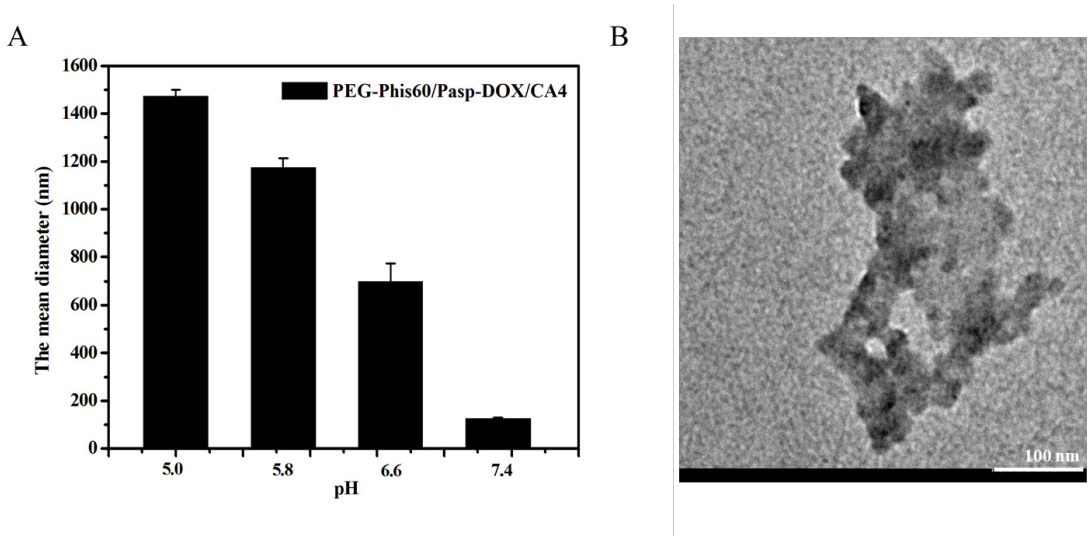

Figure S7. A) The particle size change of PEG-Phis60/Pasp-DOX/CA4 in respond to pH change at 37^{o}C for 0.5 h. Data are shown as the mean±S.D. of three independent experiments. B) Transmission electronic microscopic (TEM) images of the PEG-Phis60/Pasp-DOX/CA4 when incubated at pH 5.0 for 0.5 h. The scale bar is 100 nm.

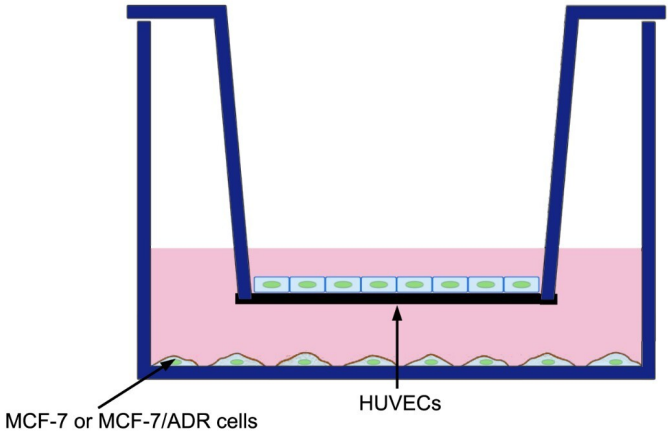

Figure S8. Schematic diagram of *in vitro* non-contact co-culture study. HUVECs were cultured in transwell chambers and then placed in the wells above MCF-7 and MCF-7/ADR cells, respectively. After incubation, the co-cultures were exposed to different treatments. The medium should be enough to exchange through the transwell.

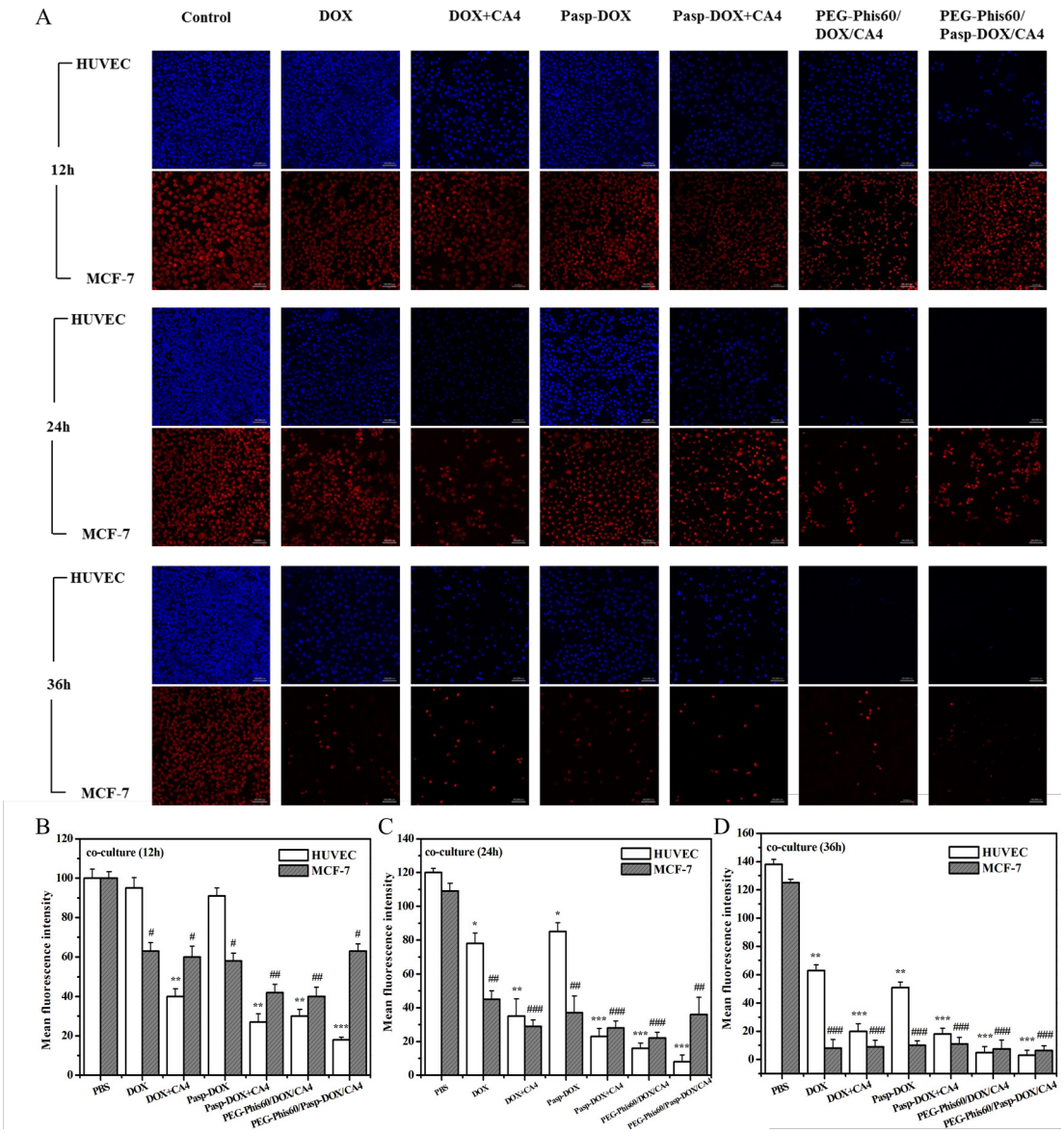

Figure S9. *In vitro* non-contact co-culture studies. (A) The co-culture cells were treated with different formulations for 12, 24, and 36 h, respectively. After treatment, the HUVECs and tumor cells MCF-7 were stained with Hoechst 33342 (blue) and propidium iodide (red), respectively. Quantitative analysis of mean fluorescence intensity of the co-culture cells after treated with different formulations for 12 h (B), 24 h (C) and 36 h (D). Results are mean±S.D. of three independent experiments. Statistical significance: *P<0.05, **P<0.005 and ***P<0.0005 *versus* the MFI of HUVEC cells after 12 h incubation. $^{\#}$P<0.05, $^{\#\#}$P<0.005 and $^{\#\#\#}$P<0.0005 *versus* the MFI of MCF-7 cells after 12 h incubation. The scale bar was 100 μm.

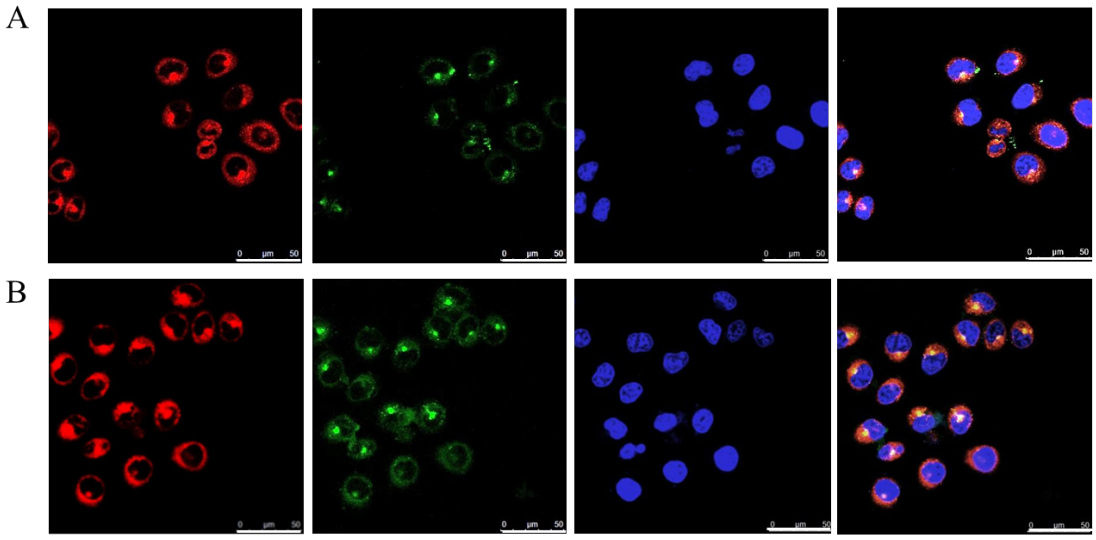

Figure S10. Confocal microscopic images of MCF-7 cells after culturing with PEG-Phis60/DOX/CA4 (A) and PEG-Phis60/Pasp-DOX/CA4 (B) for 2 h. Nuclei and lysosomes/endosomes were stained by Hoechst 33342 and LysoTracker Green, respectively. The red signal represented the DOX fluorescence from the nanoparticles (Scale bar: 50 μm).

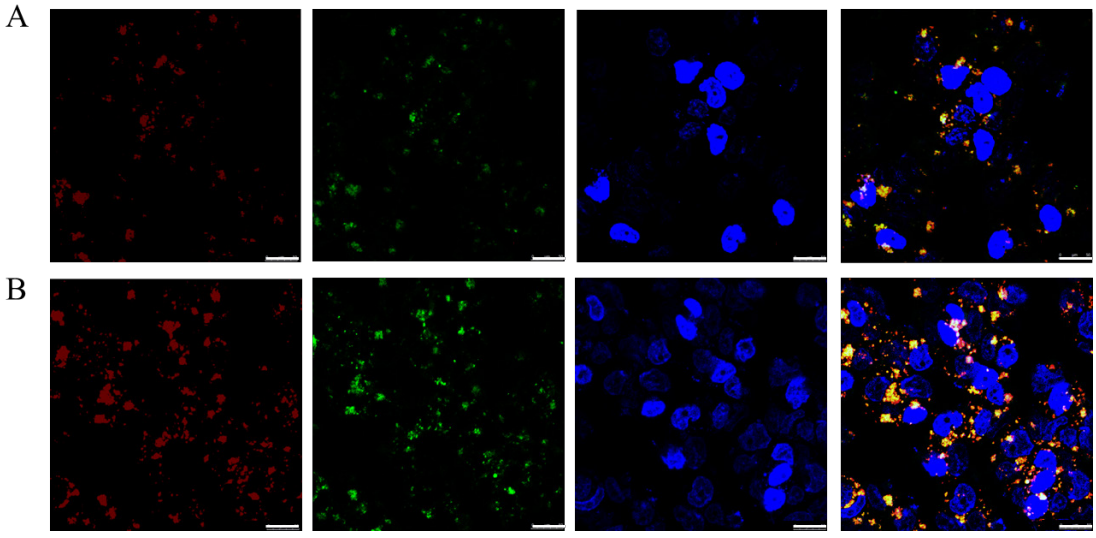

Figure S11. Confocal microscopic images of MCF-7/ADR cells after culturing with PEG-Phis60/DOX/CA4 (A) and PEG-Phis60/Pasp-DOX/CA4 (B) for 2 h. Nuclei and lysosomes/endosomes were stained by Hoechst 33342 and LysoTracker Green, respectively. The red signal represented the DOX fluorescence from the nanoparticles (Scale bar: 50 μm).

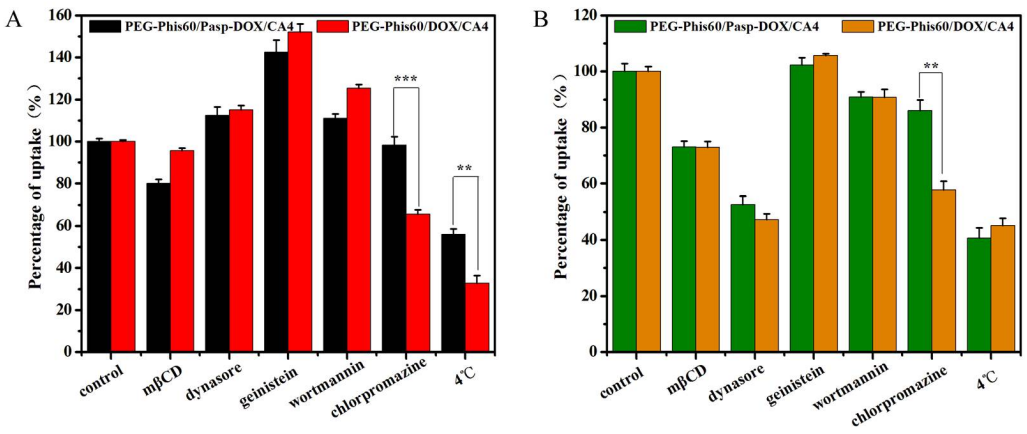

Figure S12. Elucidation of the mechanisms underlying cellular uptake of the nanoparticles. MCF-7 cells (A) and MCF-7/ADR cells (B) were treated with PEG-Phis60/DOX/CA4 and PEG-Phis60/Pasp-DOX/CA4 for 2 h in the presence of various endocytic inhibitors, and results were expressed as percentage uptake (%) of control cells which were incubated in the absence of endocytic inhibitors (n=3). Statistical significance: *P<0.05, **P<0.005 and ***P<0.0005.

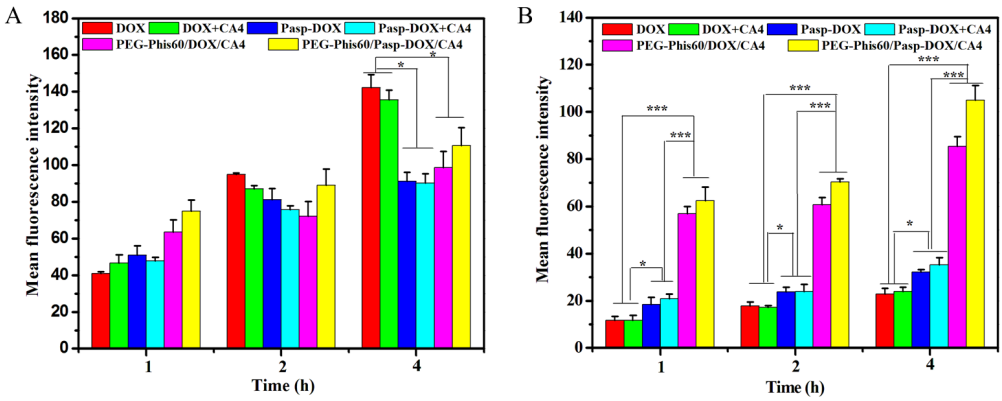

Figure S13. The accumulation of DOX in MCF-7 cells (A) and MCF-7/ADR cells (B) after incubation with PEG-Phis60/Pasp-DOX/CA4 at pH 6.6 for 1, 2 and 4 h. Data are shown as the mean±S.D. of three independent experiments. Statistical significance: *P<0.05, **P<0.005 and ***P<0.0005.

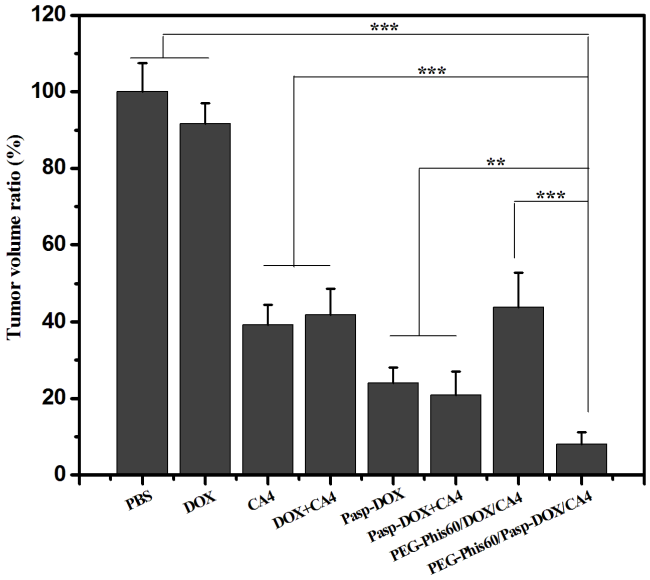

Figure S14. The tumor inhibition effect of different treatments of the nude mice bearing MCF-7/ADR tumors at the end of the *in vivo* antitumor experiment. The results were expressed as means±S.D. (n=5). Statistical significance: *P<0.05, **P<0.005 and ***P<0.0005.

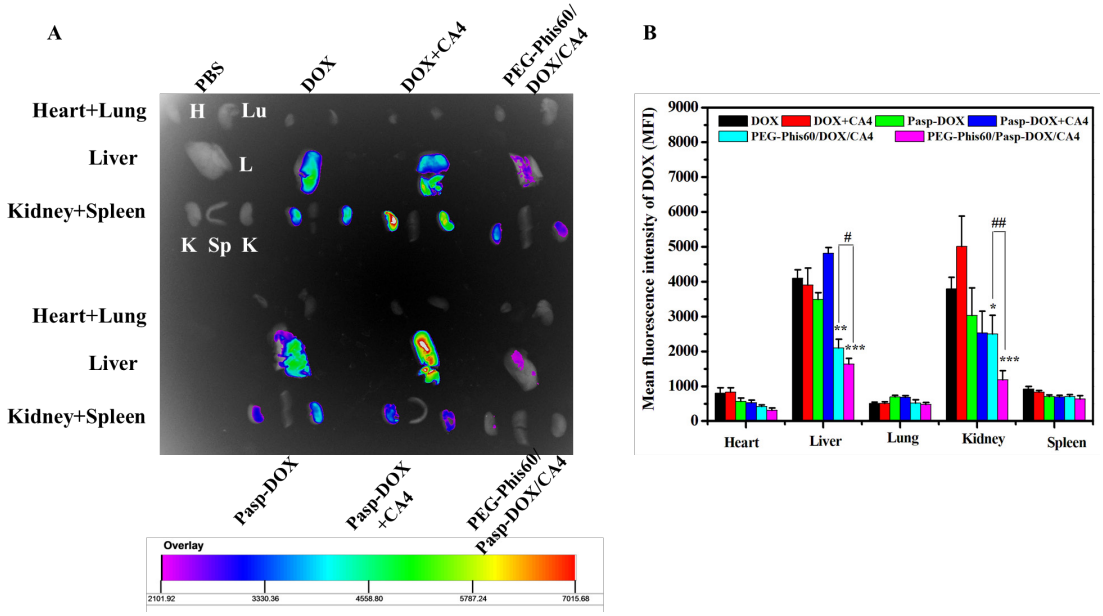

Figure S15. A) DOX accumulation in normal organs (heart (H), lung (Lu), liver (L), kidney (K) and spleen (Sp)) after *in vivo* antitumor activity with different formulations at day 16. B) Quantification of mean fluorescence intensity of DOX was calculated. Results are expressed as mean±S.D. (n=5). Statistical significance: *P<0.05, **P<0.005 and ***P<0.0005 *versus* the DOX treated group, $^{\#}$P<0.05, $^{\#\#}$P<0.005.

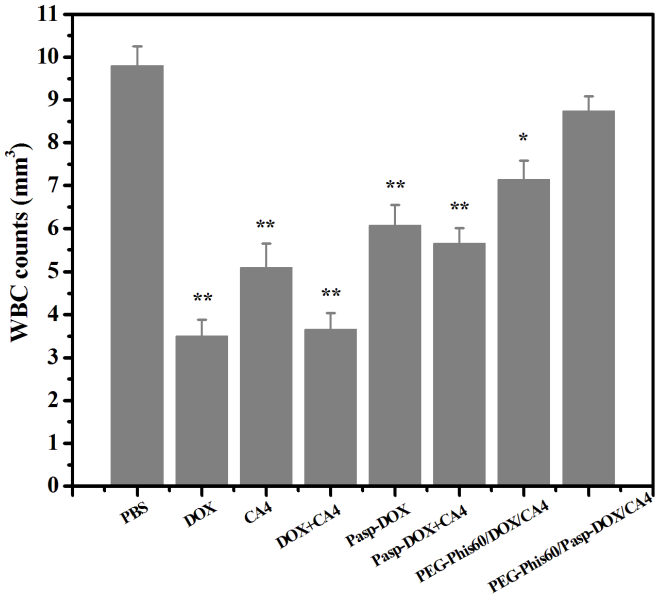

Figure S16. Effect of different treatments on the white blood cell (WBC) counts. Results are expressed as means ±S.D. (n=5). Statistical significance: *P<0.05 and **P<0.005 *versus* control.

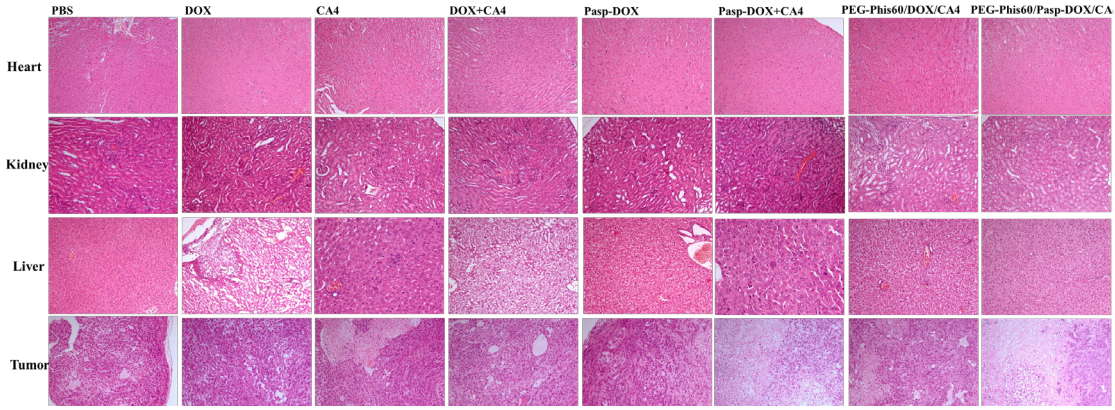

Figure S17. Hematoxylin-eosin (H&E) staining examination of heart, kidney, liver and tumor from the treated mice.