Supporting Information

Supporting information includes experimental materials; synthesis methods of mPEG-P(Asp(DIP)-co-Phe); schematic synthesis of mPEG-P(Asp(DIP)-co-Phe); CLSM images of 4T1 cells incubated with nanodrugs; cell viability of 4T1 cells incubated with B-NV and C-NVs; ROS generation in 4T1 tumor cells cultured with B-NVs and C-NVs; Live/dead cell viability assay of 4T1 cells treated with B-NVs and C-NVs; Ce6-mediated PDT aggravated hypoxia in 4T1 cells; and changes in body weight of tumor-bearing mice.

Supporting experimental section

Materials

Methoxy-ε-amino poly(ethylene glycol) (mPEG-OH, Mn = 2000 Da), anhydrous dimethylsulfoxide (DMSO) were purchased from Sigma-Aldrich. Dihydrogen porphin (Chlorin e6, Ce6) and tirapazamine (TPZ) were purchased from Aladdin. L-aspartic acid benzyl ester was purchased in Adamas. L-phenylalanine, 1,1-dioctadecyl-3,3,3,3-tetramethylindotricarbo-cyanine iodide (DiR) were purchased from J&K Scientific. Dialysis bag (MWCO: 3.5 and 14 kDa) was purchased from Shanghai Green Bird Technology Development Co., Ltd. All other reagents were of analytical grade and purchased from Guangzhou Chemical Reagent Factory. Ethyl acetate, petroleum ether (60-90 ℃), trichloromethane ($CHCl_3$) and dichloromethane were dried over CaH_2 and then distilled under ambient pressure. mPEG-OH was converted into α-methoxy-ε-amino poly(ethylene glycol) (mPEG-NH_2) as previously described.[1] N-carboxyanhydride of β-benzyl-L-aspartate (BLA-NCA) and N-carboxyanhydride of

L-phenylalanine (Phe-NCA) were synthesized according to the literature.[2, 3] DMEM high glucose medium, fetal bovine serum (FBS), streptomycin/penicillin medium, phosphate buffer (PBS, pH 7.4), 0.25% trypsin-EDTA were purchased from Gibco Co., Ltd. The MTT and DAPI reagents were purchased from Sigma-Aldrich. Paraformaldehyde was purchased from Nanjing KeyGen BIOTECH. H&E staining reagent was purchased from Shanghai Hongzi Industrial. TUNEL Apoptosis Detection Kit (FragEL™ DNA Fragmentation Detection Kit) was purchased from Merck.

Synthesis of mPEG-P(Asp(DIP)-co-Phe)

mPEG-P(BLA-co-Phe) was firstly synthesized by ring-opening polymerization of BLA-NCA and Phe-NCA with $mPEG_{2k}$-NH_2 as a macroinitiator as previously reported.[4] Briefly, 0.25 g $mPEG_{2k}$-NH_2 (0.125 mmol, 2000 $g \cdot mol^{-1}$) was vacuum-dried at 70 ºC for 2 h in a 100 mL of reaction flask. After the temperature decreased, 50 mL anhydrous CH_2Cl_2 was added. Then, 1.56 g BLA-NCA (6.25 mmol, 249 $g \cdot mol^{-1}$) and 0.48 g Phe-NCA (2.5 mmol, 191 $g \cdot mol^{-1}$) were dissolved in 5 mL anhydrous DMF and added into the reaction solution. The reaction was kept stirring at 35 ºC for 48 h and then the solution was precipitated into excessive anhydrous diethyl ether twice. The solid was filtered, washed with diethyl ether for three times, and vacuum dried to obtain mPEG-P(BLA-co-Phe). Then, 0.50 g mPEG-P(BLA-co-Phe) (0.033 mmol, 15190 $g \cdot mol^{-1}$) was dissolved in 10 mL DMSO, and 0.35 g DIP (2.43 mmol, 144 $g \cdot mol^{-1}$) was added into the solution. The reaction was conducted for 24 h

at room temperature, then the mixture was dialyzed against anhydrous methanol using dialysis bag (MWCO: 7.0 kDa) for 2 days and then concentrated by rotary evaporation. The target polymer mPEG-P(Asp(DIP)-co-Phe) was obtained after precipitation in diethyl ether.

Characterization of the polymers

^{1}H NMR spectra were obtained using an AVANCE III 400 MHz nuclear magnetic spectrometer using DMSO-d_6 as a solvent. FT-IR spectral studies were carried out using a Thermo AVATER 330 FT-IR spectrometer in the range between 4000 and 400 cm^{-1} with a resolution of 2 cm^{-1}. All powder samples were compressed into KBr pellets in the FT-IR measurements.

Supporting Figures

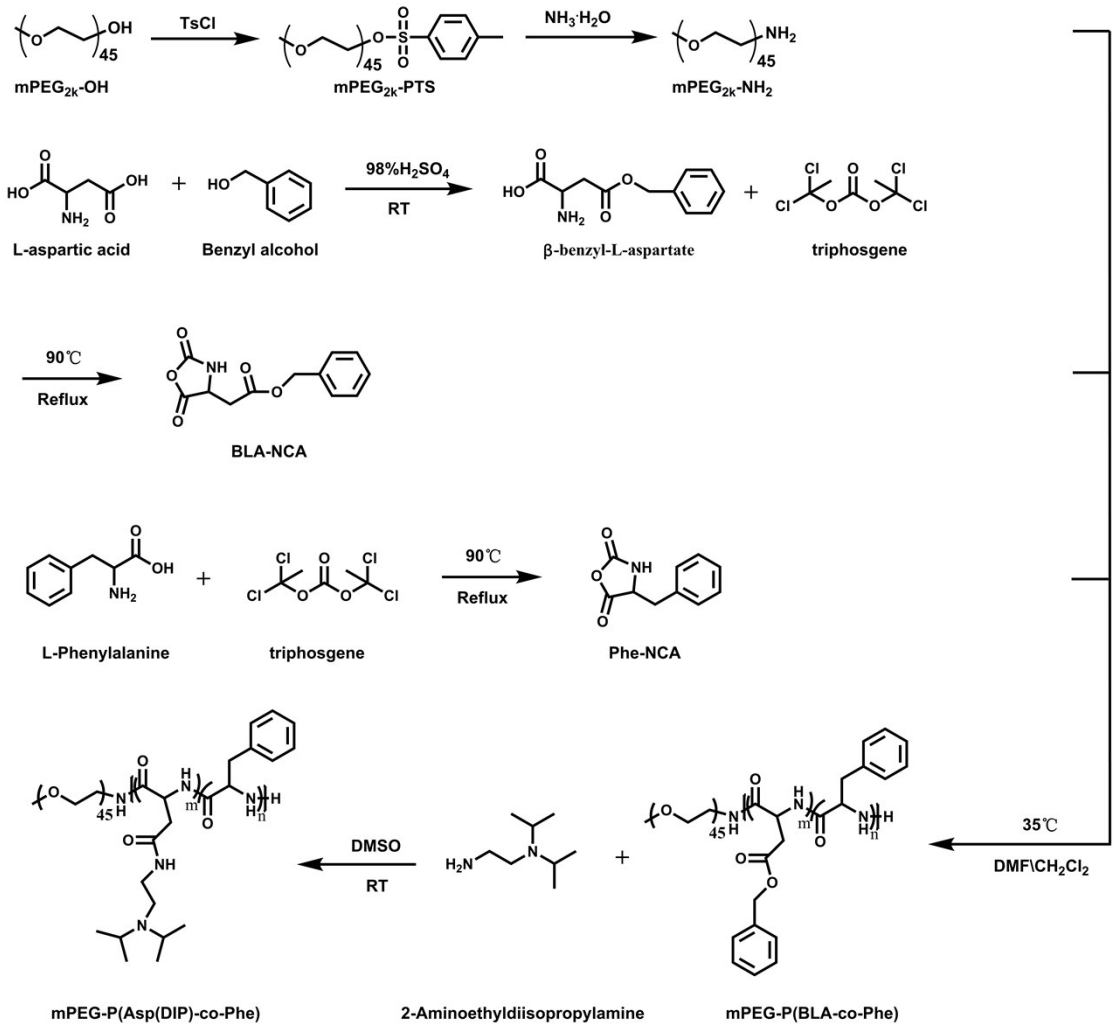

Figure S1. The synthesis of mPEG-P(Asp(DIP)-co-Phe).

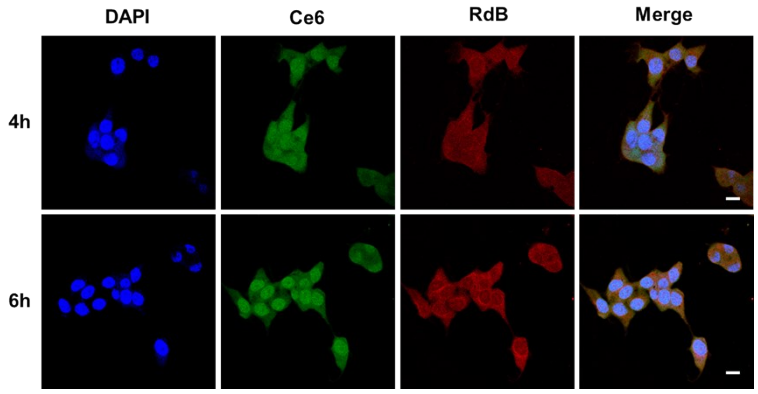

Figure S2. The CLSM images showed the fluorescence intensity and the distribution in 4T1 cells cultured with the Rhodamine B and Ce6 co-loaded nanovesicles for 4 h and 6 h. The scale bars represent 10 μm.

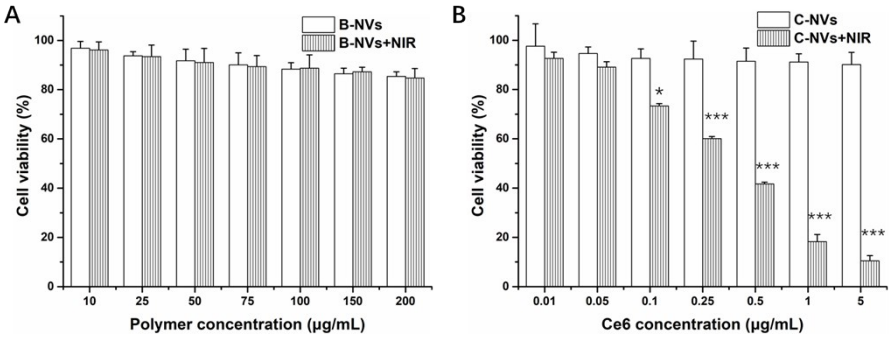

Figure S3. The cell viability of 4T1 cells after co-incubation with B-NVs (A) and C-NVs (B). The NIR irradiation was applied using a 665 nm laser at a power of 400 mW/cm^2 for 3 min if needed. *P < 0.05, ***P < 0.001 versus NIR group with the same lack-NIR concentration.

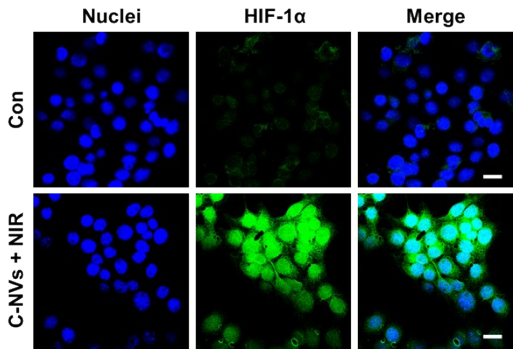

Figure S4. Ce6-mediated PDT aggravated hypoxia in 4T1 cells and induced the upregulation of HIF-1α. Scale bars represent 20 μm.

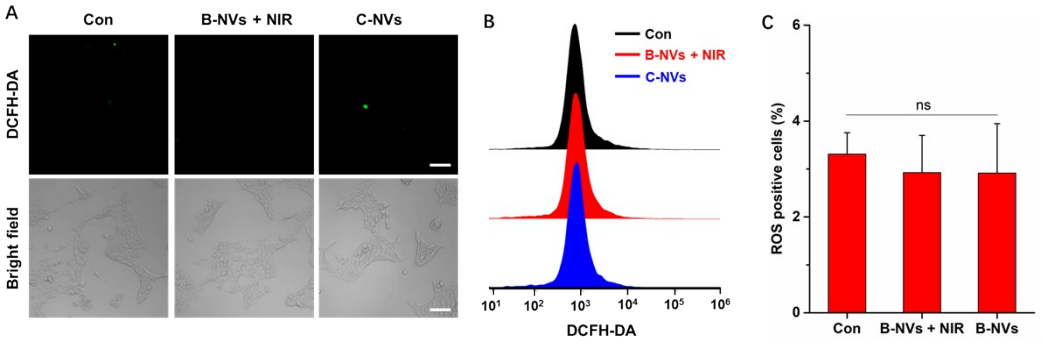

Figure S5. ROS generation in 4T1 tumor cells cultured with B-NVs and C-NVs in normoxic environment was detected by CLSM (A) and FCM (B). (C) The ROS positive cells detected by FCM. The NIR irradiation was applied using a 665 nm laser at a power of 400 mW/cm^2 for 3 min if needed. The scale bars represent 100 μm.

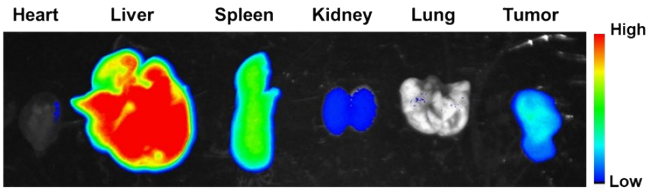

Figure S6. *Ex vivo* fluorescence imaging of excised organs and tumor at 48 h after intravenous injection.

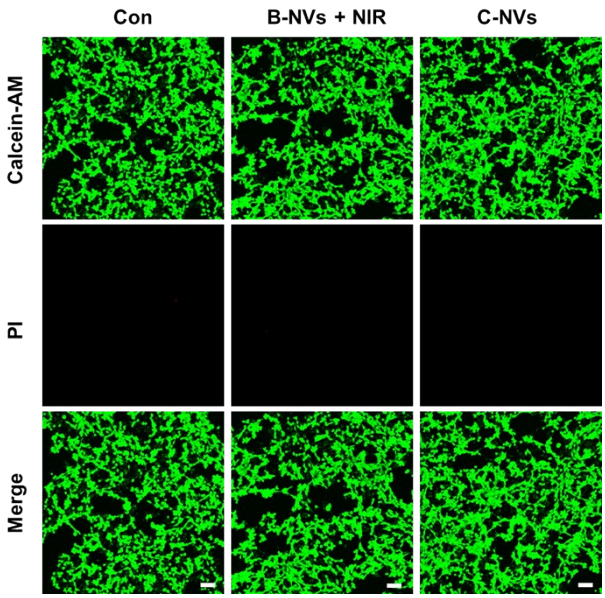

Figure S7. Live/dead cell viability assay of 4T1 cells treated with B-NVs and C-NVs in normoxic environment. Green fluorescence represents the living cells labeled with Calcein-AM and red fluorescence represents the dead cells labeled with PI. The NIR irradiation was applied using a 665 nm laser at a power of 400 mW/cm^2 for 3 min if needed. The scale bars represent 100 μm.

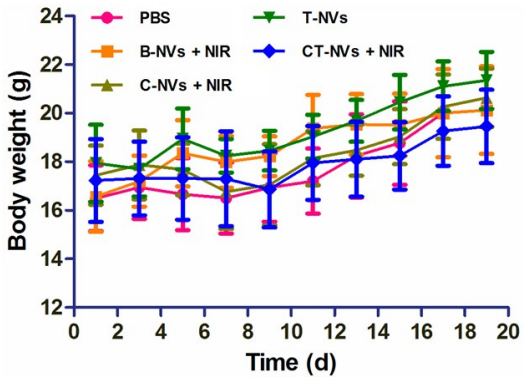

Figure S8. Changes in body weight of tumor-bearing mice during 19 days' treatment.

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References

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3 L. N. Li, C. D. Xiong and D. L. Chen, *Chinese J. Polym. Sci.*, 2016, 34, 594-605.

4 J. G. Li, T. Wang, D. L. Wu, X. Q. Zhang, J. T. Yan, S. Du, Y. F. Guo, J. T. Wang and A. F. Zhang, *Biomacromolecules*, 2008, 9, 2670-2676.