Electronic Supplementary Material (ESI) for Nanoscale.

Dual pH-sensitive liposomal system with charge-reversal and NO generation for overcoming multidrug resistance in cancer

- Supporting Information

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Table S1. The composition of PTX/DMA-L, PTX/NO/SA-L and PTX/NO/DMA-L.

Liposomes	HSPC:Cholesterol: $DSPE\text{-}PEG_{2000}$:DOTAP	Encapsulated drug	Polymer modified on the surface of liposomes
PTX/DMA-L	12:1:1:2	PTX	PEG-PLL-DMA
PTX/NO/SA-L	12:1:1:2	PTX + DETA NONOate	PEG-PLL-SA
PTX/NO/DMA-L	12:1:1:2	PTX + DETA NONOate	PEG-PLL-DMA

Fig. S1 Synthesis route of PEG-PLL-DMA and PEG-PLL-SA. PEG-PLL is synthesized by ring opening polymerization of N(ε)-benzyloxycarbonyl-L-lysine N-carboxyanhydriade (Z-Lys-NCA) using PEG_{2000}-NH_2 as the initiator, and then the protected benzyloxycarbonyl (Z) group was removed from PEG-PLL(Z). Finally, DMA or SA is grafted onto PEG-PLL to obtain PEG-PLL-DMA or PEG-PLL-SA.

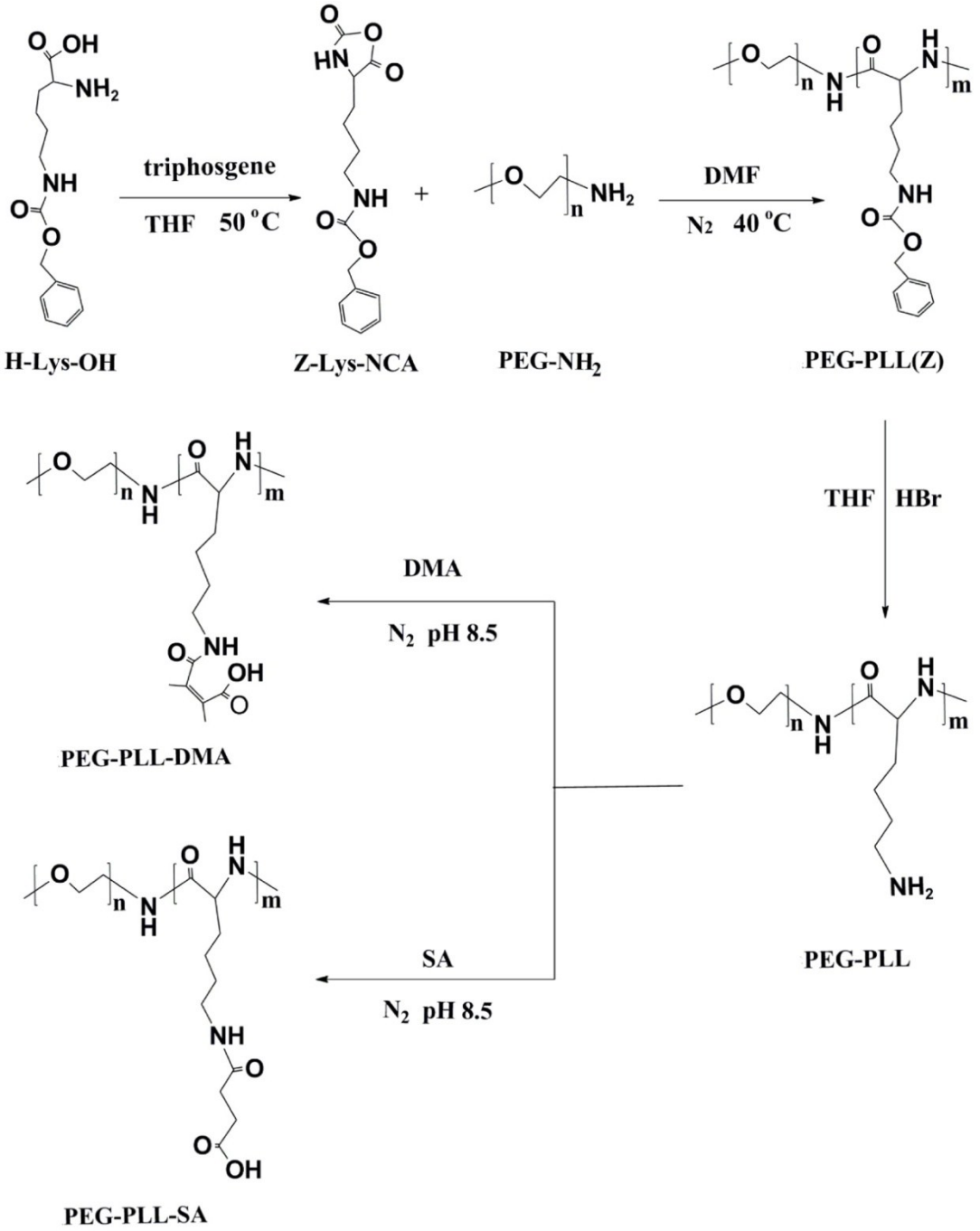

Fig. S2 ^{1}H-NMR spectra of Z-Lys-NCA, PEG-PLL(Z), PEG-PLL, PEG-PLL-DMA and PEG-PLL-SA.

Z-Lys-NCA (^{1}H NMR; 300 MHz, DMSO-d_6, d, ppm): 1.31-1.72 (-$CH_2CH_2CH_2$-CH-), 2.99 (-CH_2-NH-), 4.43 (-CH-NH-), 5.00 (-CH_2-C_6H_5), 7.26-7.37 (-C_6H_5), 9.09 (-NH-C=O-).

PEG-PLL(Z) (^{1}H NMR; 300 MHz, DMSO-d_6, d, ppm): 1.20-1.60 (-$CH_2CH_2CH_2$-CH-), 2.90 (-CH_2-NH-), 3.51 (-CH_2CH_2-O-), 4.20 (-CH-NH-), 4.98 (-CH_2-C_6H_5), 7.26-7.44 (-C_6H_5).

PEG-PLL (^{1}H NMR; 300 MHz, D_2O, d, ppm): 1.32-1.54 (-$CH_2CH_2CH_2$-CH-), 2.88 (-CH_2-NH_2), 3.51 (-CH_2CH_2-O-), 4.18 (-CH-NH-).

PEG-PLL-DMA (^{1}H NMR; 300 MHz, D_2O, d, ppm): 1.26-1.80 (-$CH_2CH_2CH_2$-CH_2-), 3.20 (-CH_2-NH_2), 3.55 (-CH_2CH_2-O-), 4.15 (-CH-NH-).

PEG-PLL-SA (^{1}H NMR; 300 MHz, D_2O, d, ppm): 1.20-1.75 (-$CH_2CH_2CH_2$-CH_2-), 3.10 (-CH_2-NH_2), 3.65 (-CH_2CH_2-O-), 4.25 (-CH-NH-).

Fig. S2

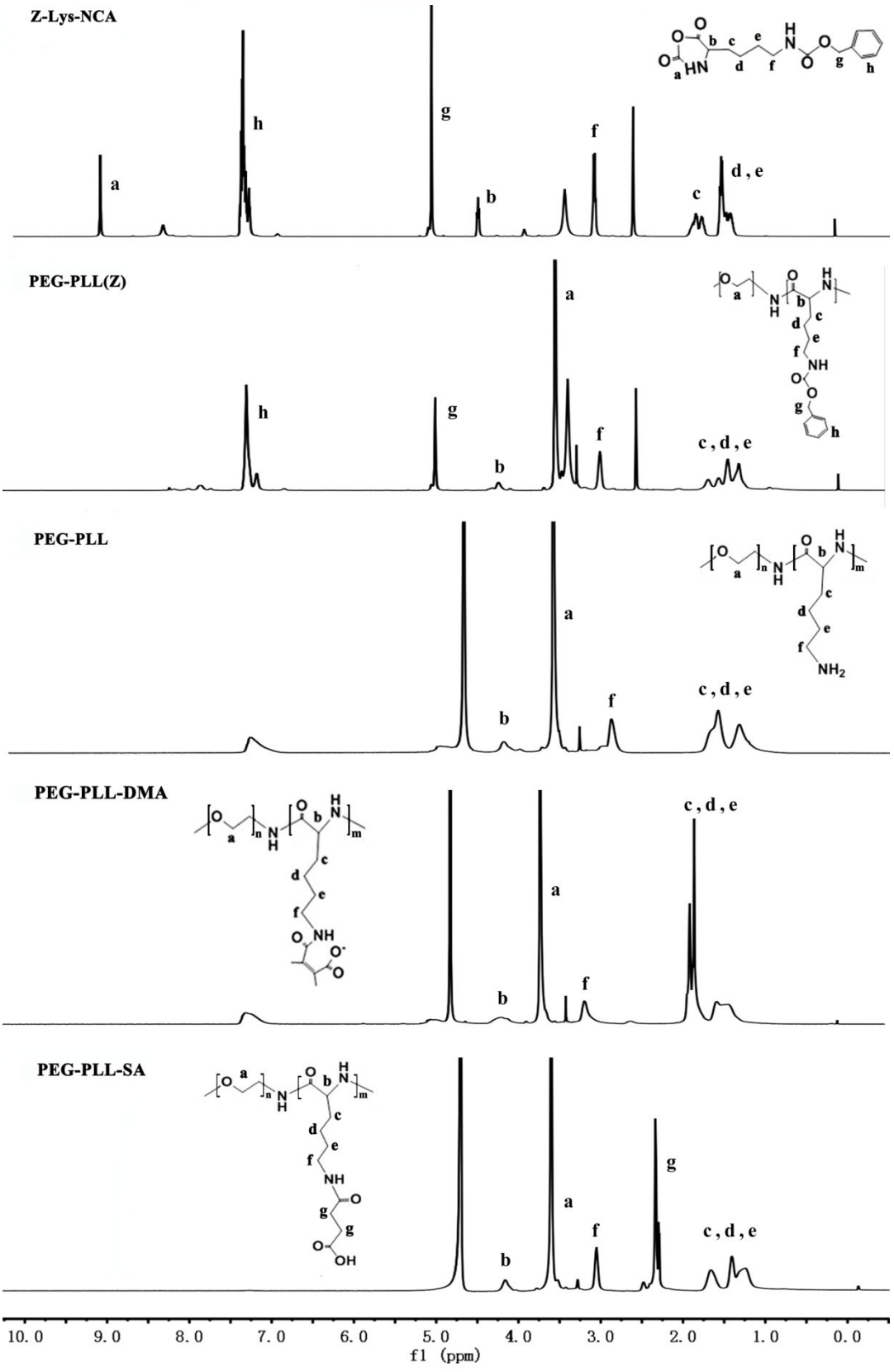

Fig.S3 FT-IR spectra of Z-Lys-OH, Z-Lys-NCA, PEG-PLL(Z), PEG-PLL, PEG-PLL-DMA and PEG-PLL-SA.

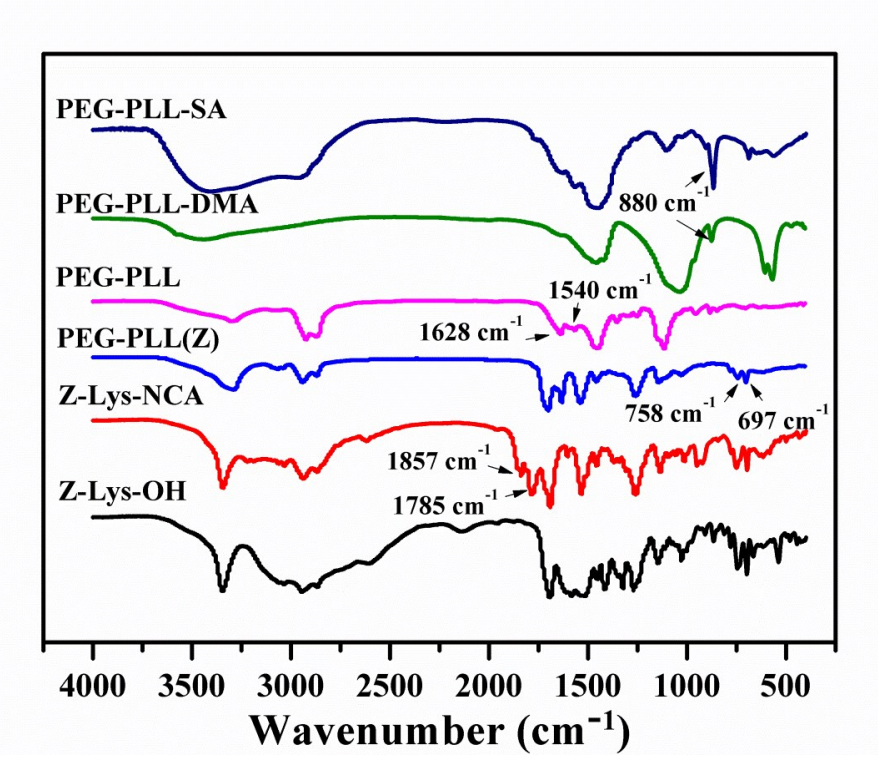

Fig. S4 (A) The average diameters and zeta potentials of PTX/NO/DMA-L in PBS (pH 7.4) at 4 °C for 7 d. The leakage ratios of PTX and DETA NONOate in PBS (pH 7.4) at 4 °C for 7 d (B) and PBS (pH 7.4) containing 50% FBS (v/v) at 37 °C for 24 h (C).

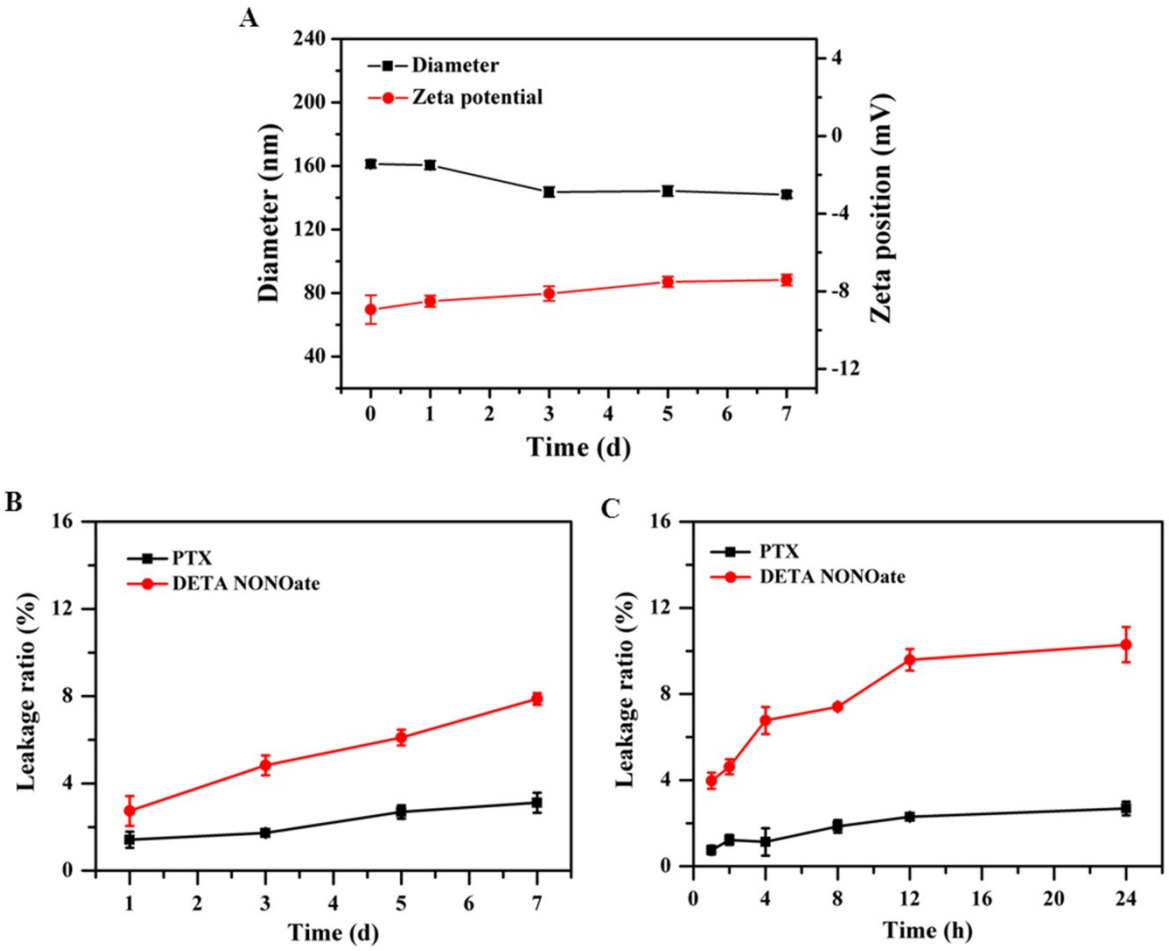

Fig. S5 UV absorption spectra of the released solution of PTX/NO/DMA-L with or without Griess reagent at different pHs. In this study, the released NO amount from the liposomes was determined by evaluating the content of nitrite/nitrate using Griess assay (1% sulfanilamide mixed with 0.1% naphthylethylenediamine dihydrochloride in 5% phosphoric acid). NO is oxidized to nitrate/nitrite easily in the aqueous solution. In acid solution, sulfanilic acid is quantitatively converted to a diazonium salt by reaction with nitrite/nitrate. The diazonium salt is then coupled to naphthylethylenediamine, forming an azo dye that can be spectrophotometrically quantitated based on its absorbance at 540 nm. The results show that the UV adsorption at 540 nm appears after the addition of Griess reagent into the released solution of PTX/NO/DMA-L. Therefore, we use the Griess reagent to assess the NO release behavior.

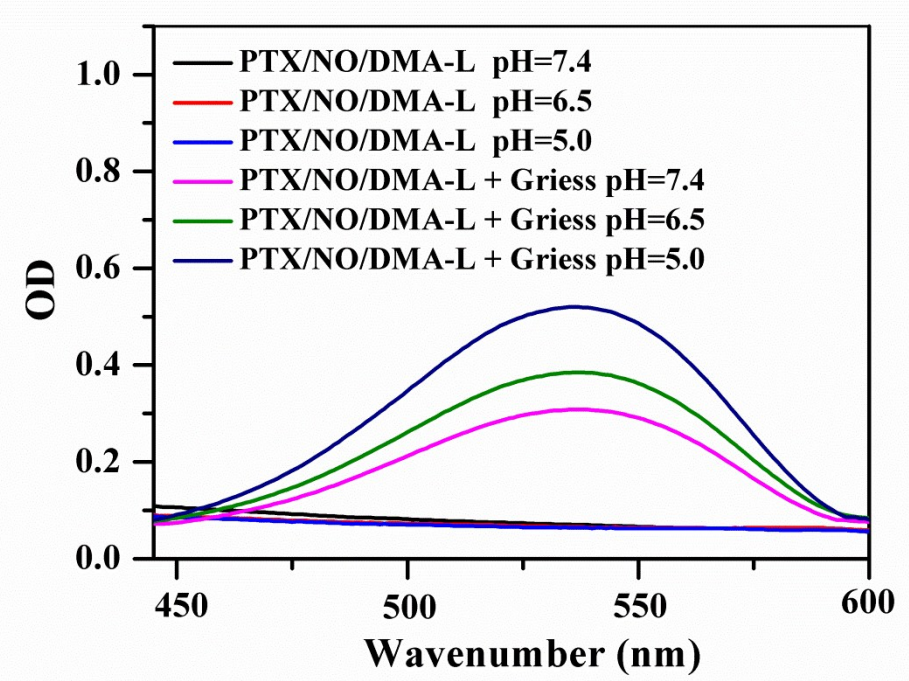

Fig. S6 Cellular uptake study. Flow cytometer measurement of A549/T cells incubated with C6/NO/DMA-L, C6/NO/SA-L, and C6/DMA-L for 1 h at pH 7.4 and 6.5, respectively.

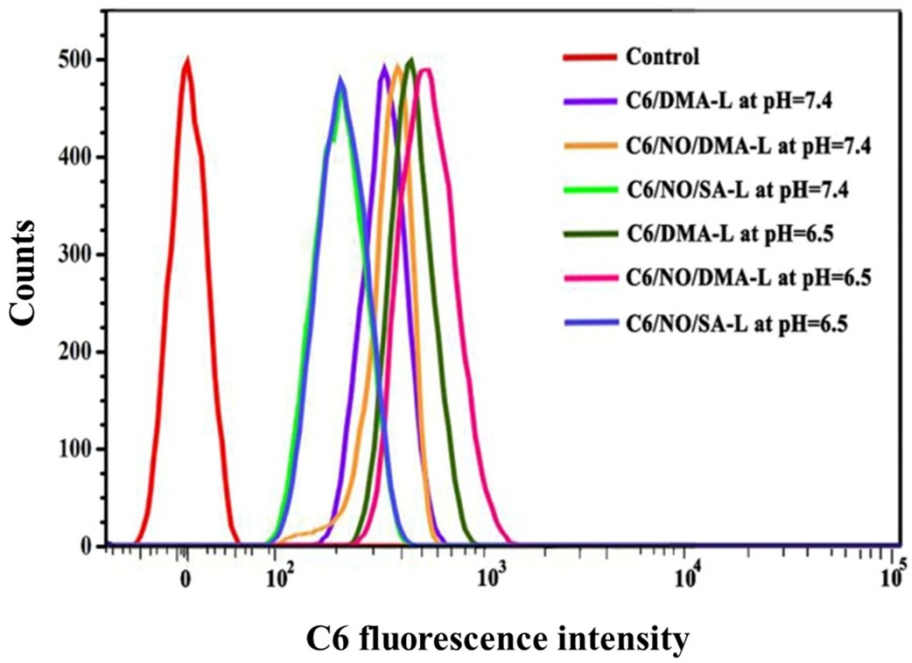

Fig. S7 Cell viability of A549/T cells treated with free DETA NONOate at various concentrations at pH 6.5. When the concentration of DETA NONOate was lower than 25 μg/mL, the cell viabilities were all above 80%. However, the cell viability was only 41% at the concentration of 50 μg/mL. Hence, we choose 10-25 μg/mL to be the DETA NONOate concentration in this study, at which is unable to kill the tumor cells directly but can inhibit the P-gp mediated drug efflux effectively.

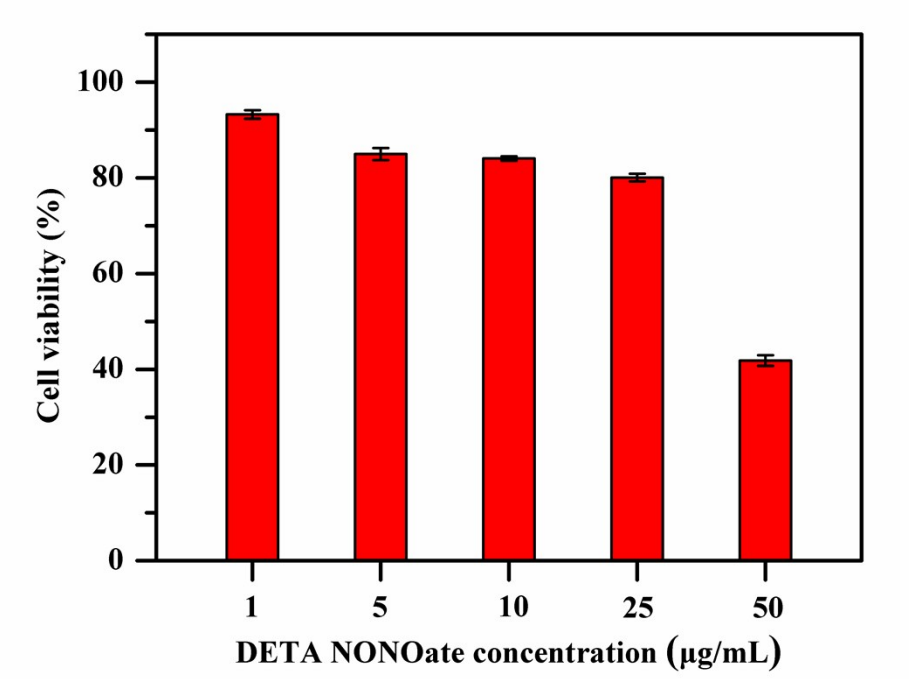

Fig. S8 Rh123 efflux assay. Flow cytometer analysis of A549/T cells incubated with free Rh123, free DETA NONOate and Rh123-loaded liposomes for 4 h followed by 1 h efflux at pH 7.4 or 6.5, respectively.

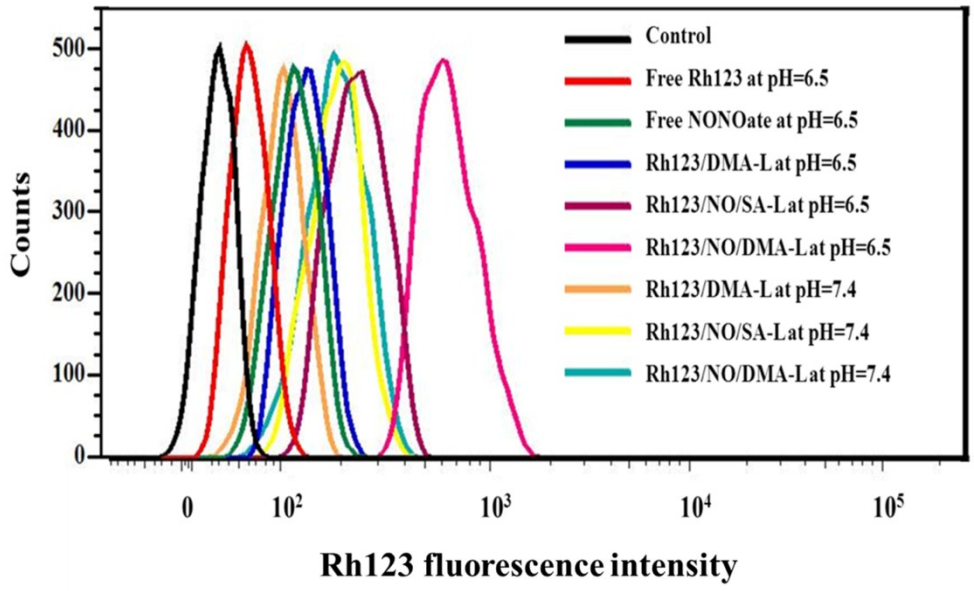

Fig. S9 P-gp expression level test. Flow cytometer analysis of A549/T cells treated with the liposomes without PTX at pH 6.5.

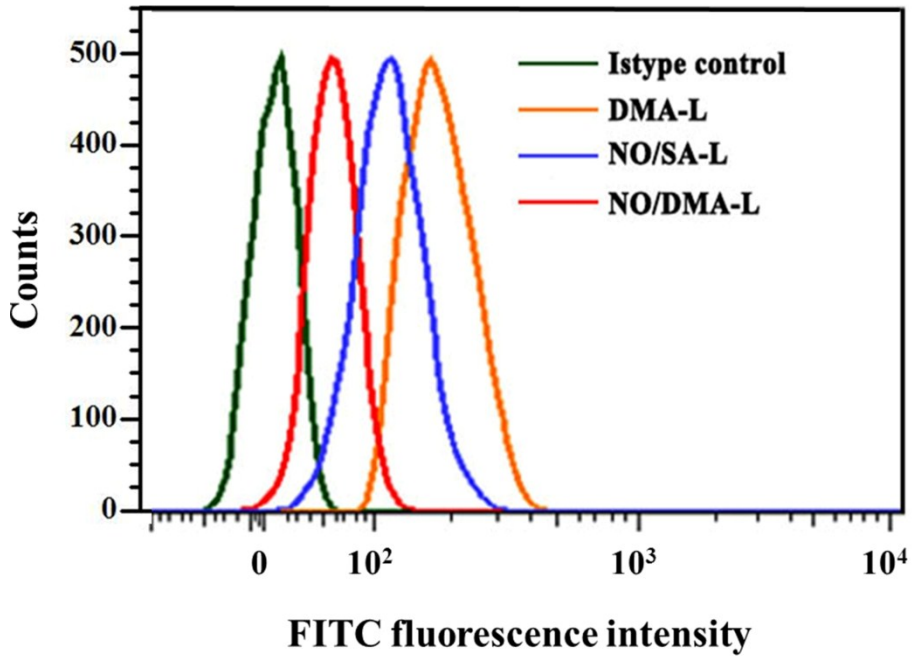

Fig. S10 H&E staining images of primary organs, and the scale bars represent 50 μm. Compared with normal saline group, no obvious inflammation reaction, structural alteration and pathological abnormalities were detected in the heart, liver, spleen, lung and kidney of the mice treated with free PTX and various liposomes, indicating the slight side effects on the major organs.