

ARTICLE

Supporting Information

PEGylated Prussian blue nanoparticles with modulating polyethyleneimine cytotoxicity and attenuating tumor hypoxia for dual-enhanced photodynamic therapy

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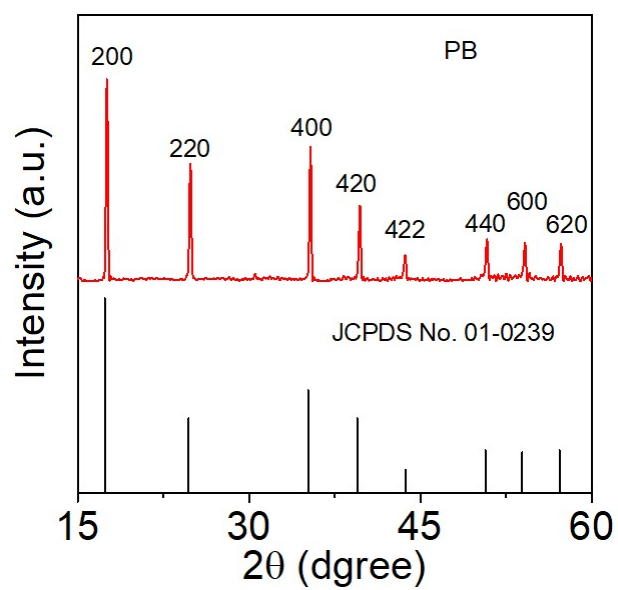


Fig. S1 XRD pattern of PB powder.

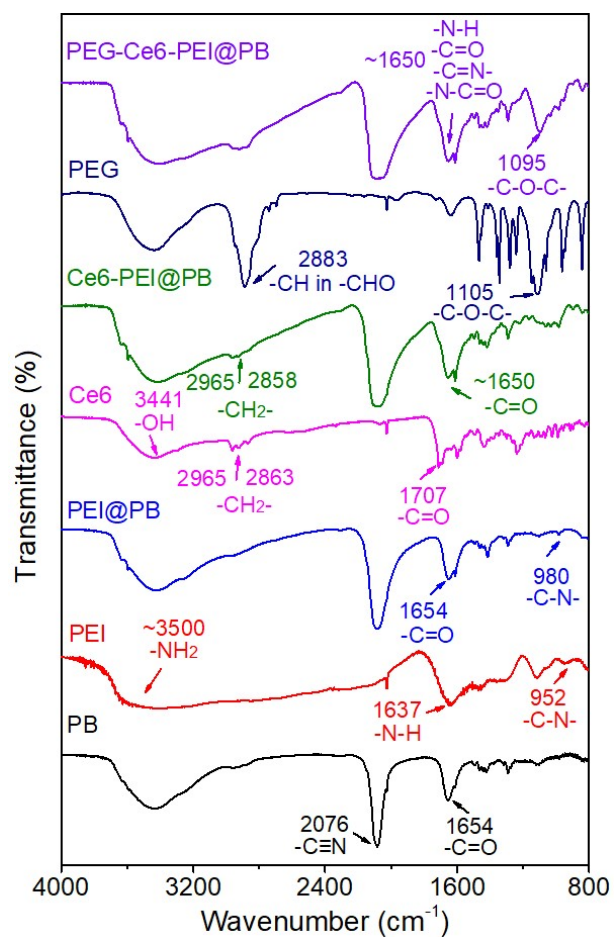


Fig. S2 FTIR spectra of PB, PEI, PEI@PB, Ce6, Ce6-PEI@PB, PEG, and PEG-Ce6-PEI@PB.

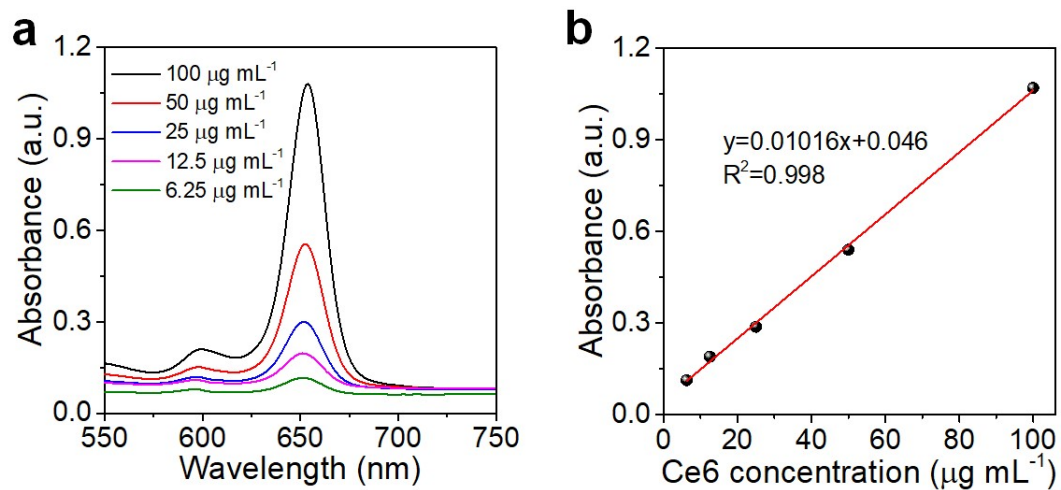


Fig. S3 The quantification curves of Ce6. (a) The UV-vis spectra of Ce6 with different concentrations. (b) The standard curve of Ce6 absorbance value at 655 nm.

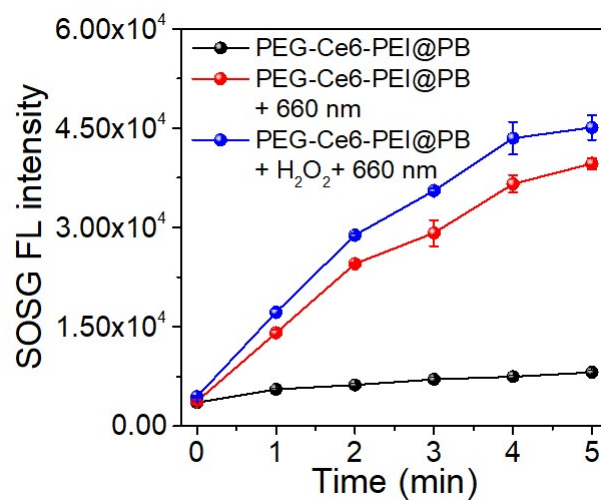


Fig. S4 Single oxygen generation of PEG-Ce6-PEI@PB with H₂O₂ upon 660 nm irradiation, as indicated by the FL intensity of SOSG. Data presented as mean \pm SD (n = 3).

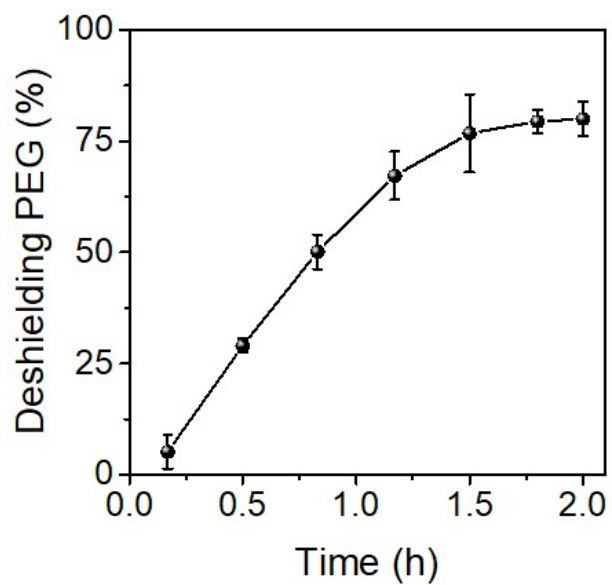


Fig. S5 PEG deshielding percentage from PEG-Ce6-PEI@PB at pH 6.8 for different durations. Data presented as mean \pm SD ($n = 3$).

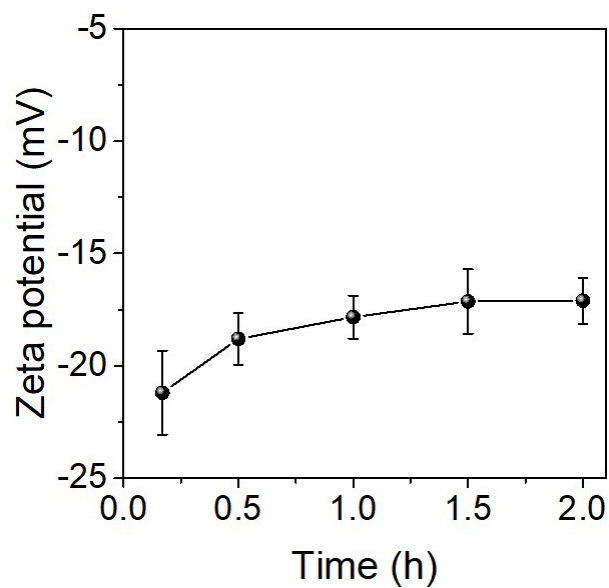


Fig. S6 Zeta potential of PEG-Ce6-PEI@PB at pH 6.8. Data presented as mean \pm SD (n = 3).

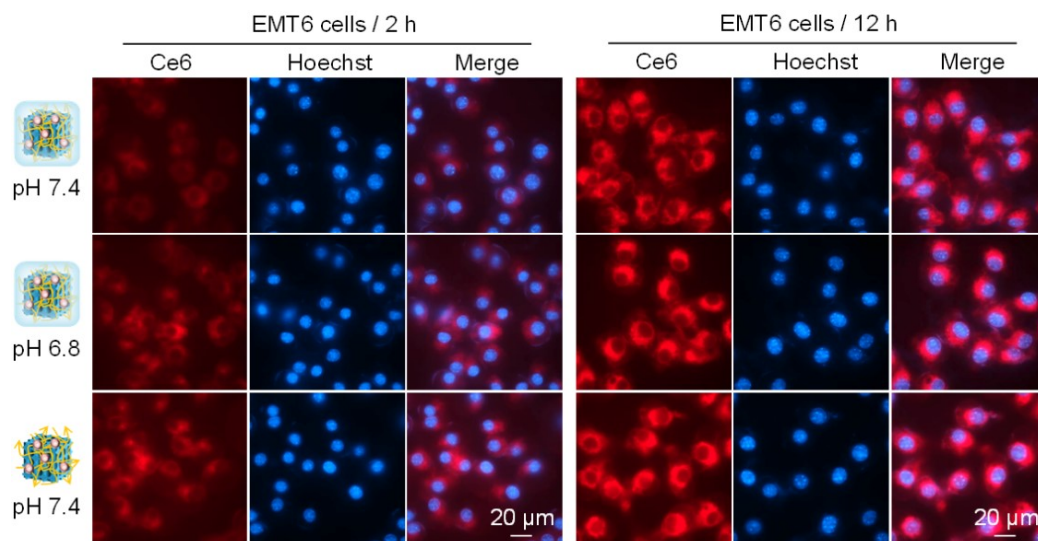


Fig. S7 PEG shedding to enhance endocytosis of PEG-Ce6-PEI@PB triggered by weak acid in EMT6 cells after incubation for 2 h and 12 h, respectively.

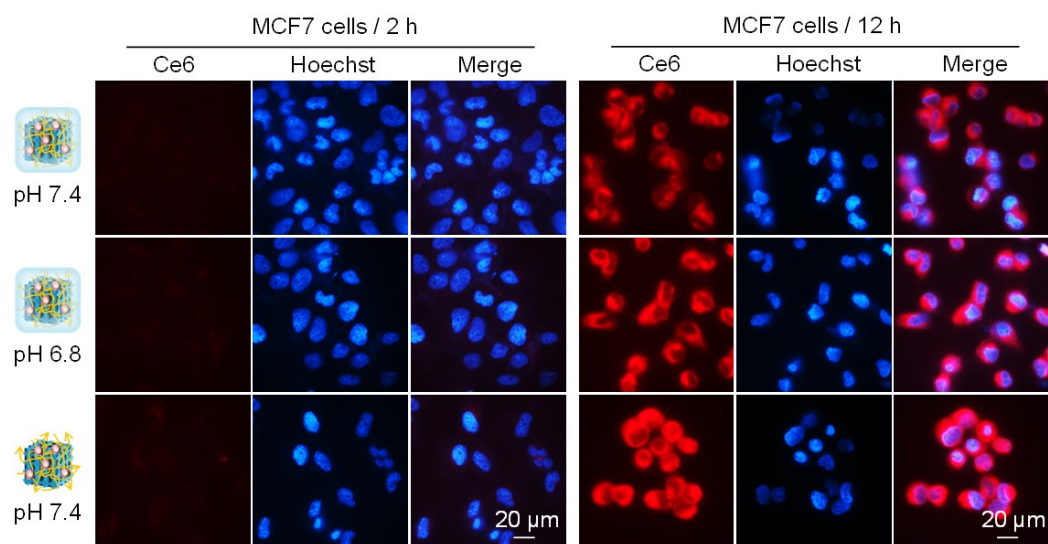


Fig. S8 PEG shedding to enhance endocytosis of PEG-Ce6-PEI@PB triggered by weak acid in MCF7 cells after incubation for 2 h and 12 h, respectively.

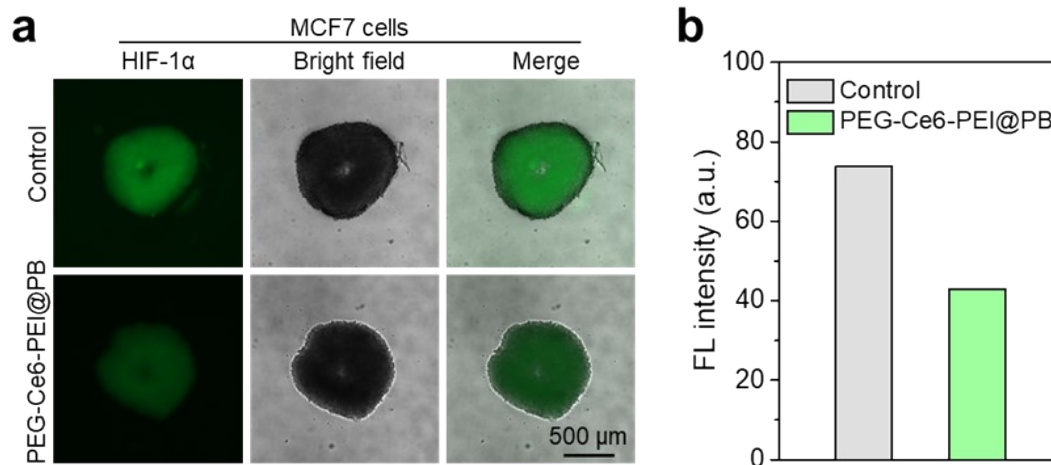


Fig. S9 The ability of PEG-Ce6-PEI@PB on attenuating hypoxia in multicellular tumor spheroids. (a) Hypoxia was assessed by immunofluorescence staining with HIF-1 α (green) after 24 h treatment. (b) Corresponding fluorescence intensity of HIF-1 α in a.

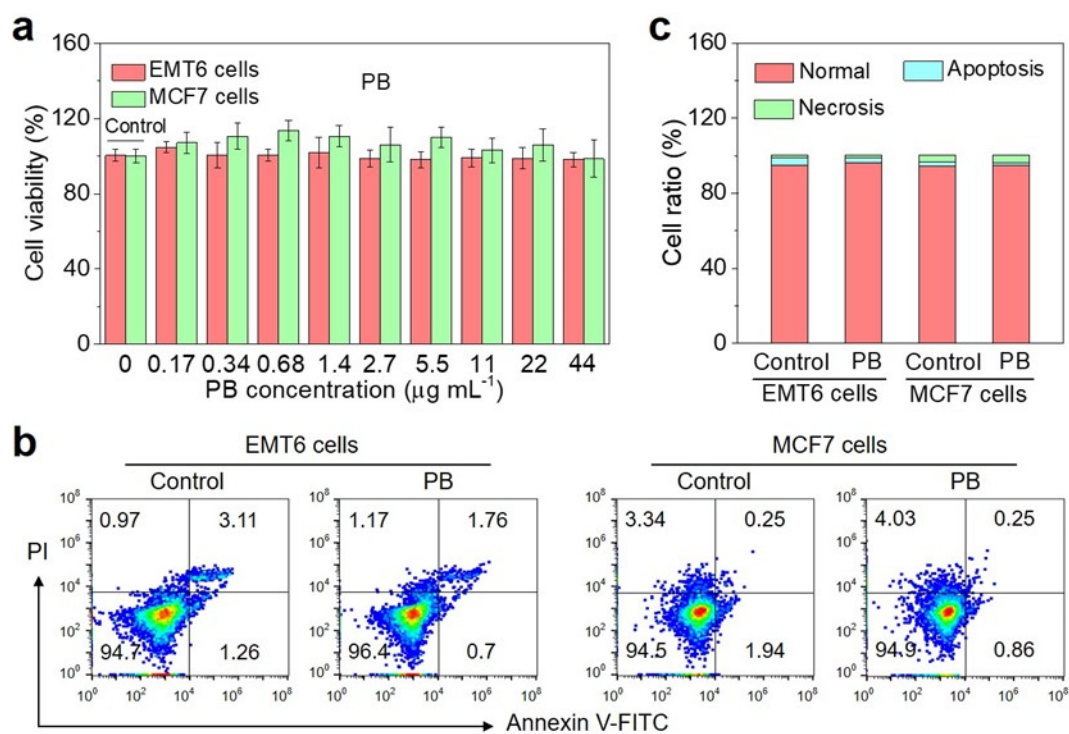


Fig. S10 *In vitro* biocompatibility of PB in EMT6 and MCF7 cells. (a) Cell viability assay of EMT6 and MCF7 cells treated with different concentrations of PB. Data presented as mean \pm SD ($n = 3$). (b) Apoptosis/necrosis analyzed by flow cytometry in EMT6 and MCF7 cells using Annexin V-FITC and PI double staining. (c) Quantitative analysis of corresponding cell apoptosis/necrosis percentages based on b.

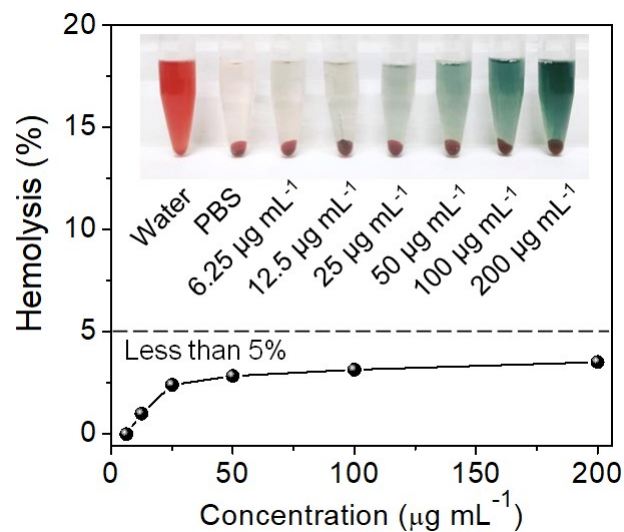


Fig. S11 Hemolysis analysis of PEG-Ce6-PEI@PB suspension at various concentrations with red blood cells incubation. Inset: Hemolysis photographs after centrifugation. Water and PBS were used as positive and negative controls, respectively.

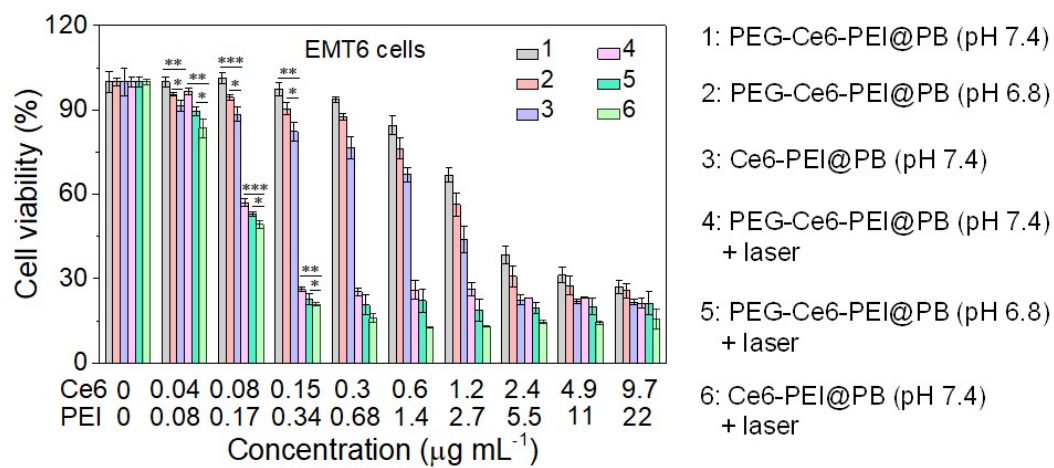


Fig. S12 Cell viability assay of EMT6 cells after incubation with PEG-Ce6-PEI@PB and Ce6-PEI@PB with and without 660 nm laser irradiation (34 mW cm^{-2} , 12 min). Data presented as mean \pm SD ($n = 3$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

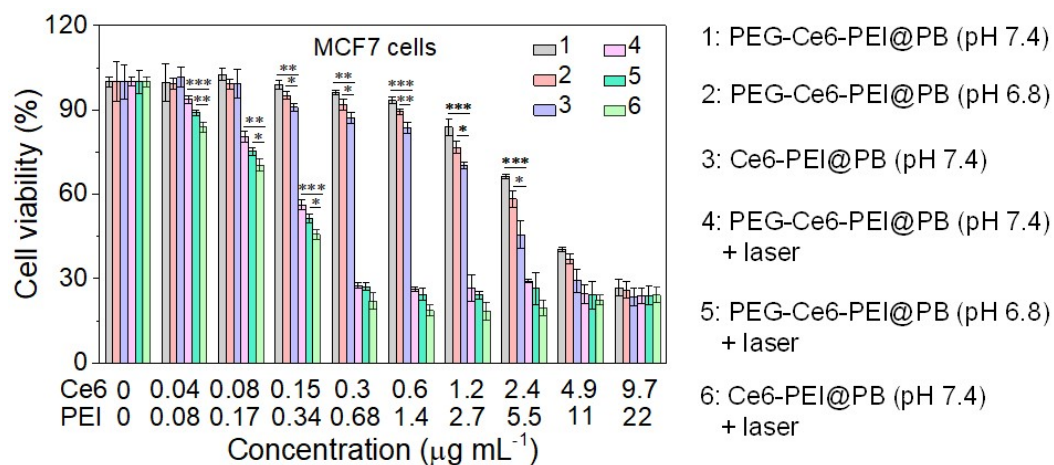


Fig. S13 Cell viability assay of MCF7 cells after incubation with PEG-Ce6-PEI@PB and Ce6-PEI@PB with and without 660 nm laser irradiation (34 mW cm^{-2} , 12 min). Data presented as mean \pm SD ($n = 3$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

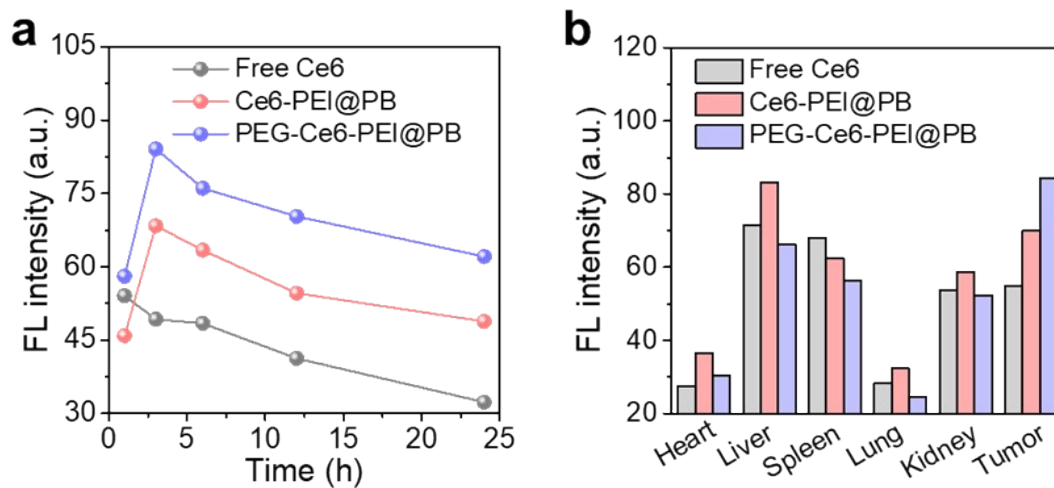


Fig. S14 *In vivo* bio-distribution of PEG-Ce6-PEI@PB. (a) Corresponding tumor-site fluorescence intensity of free Ce6, Ce6-PEI@PB, and PEG-Ce6-PEI@PB *in vivo*. (b) Corresponding fluorescence intensity of major organs and tumors *ex vivo*.

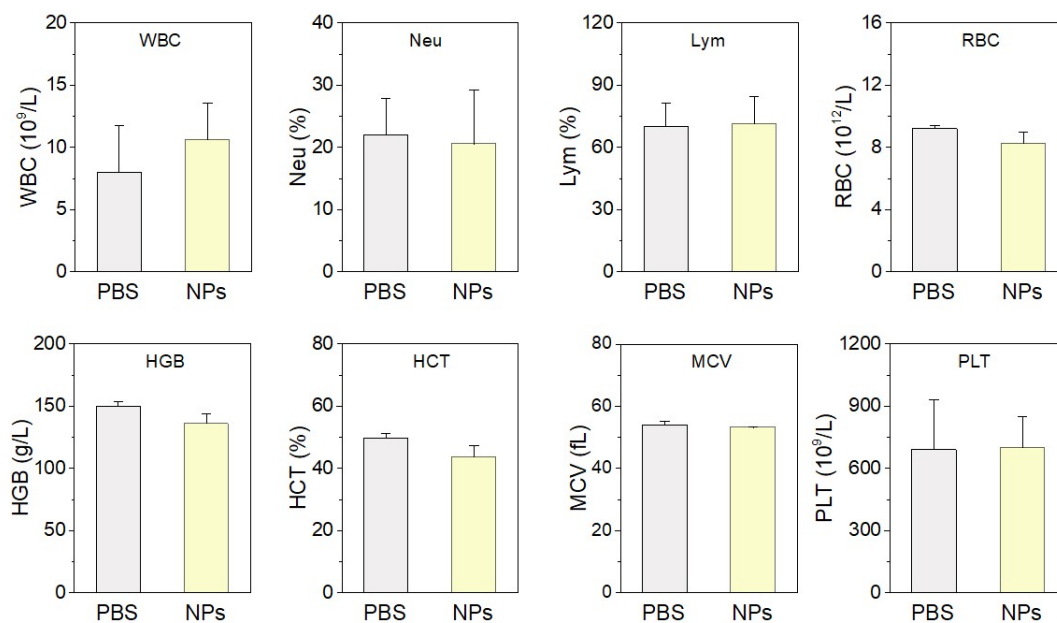


Fig. S15 Blood hematology analyses of healthy mice after intravenous injection with PEG-Ce6-PEI@PB nanoparticles. Key indicators of the white cell system: WBC = white blood cell count, Neu = neutrophil, Lym = lymphocyte. Key indicators of the red blood cell system: RBC = red blood cell count, HGB = hemoglobin, HCT = hematocrit, MCV = mean corpuscular volume. Key indicators of the platelet system: PLT = platelet. Data presented as mean \pm SD ($n = 3$).

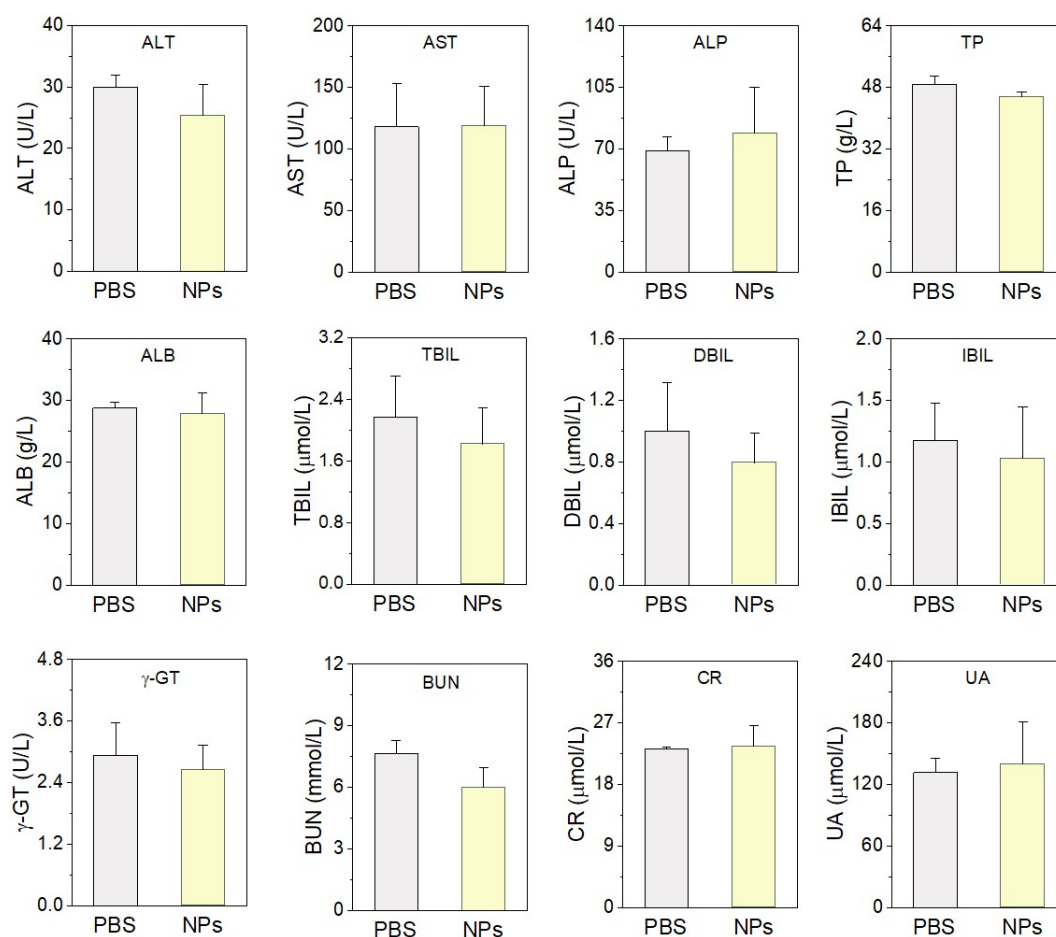


Fig. S16 Blood biochemistry analyses of healthy mice after intravenous injection with PEG-Ce6-PEI@PB nanoparticles. Liver function examination main index: ALT = alanine aminotransferase, AST = aspartate transaminase, ALP = alkaline phosphate, TP = total protein, ALB = albumin, TBIL = total bilirubin, DBIL = direct bilirubin, IBIL = indirect bilirubin, γ -GT = γ -glutamyltransferase. Renal function examination main index: BUN = blood urea nitrogen, CR = creatinine, UA = uric acid. Data presented as mean \pm SD (n = 3).

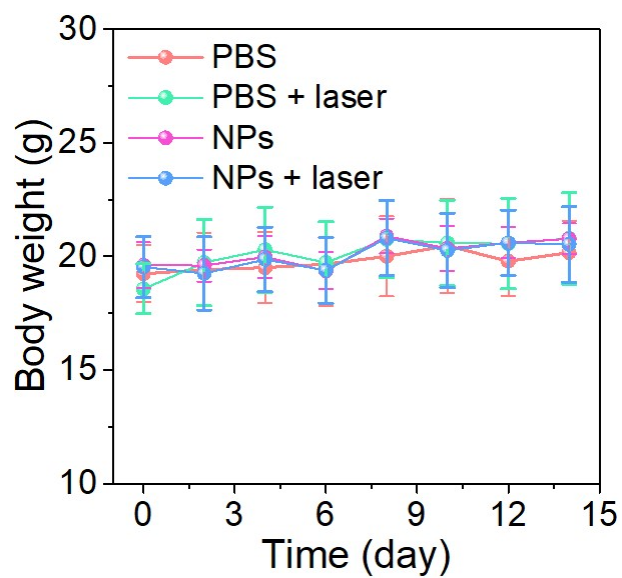


Fig. S17 Body weight changes of the tumor-bearing mice measured during the antitumor studies. Data presented as mean \pm SD (n = 5).

Table S1 IC₅₀ (half maximal inhibitory concentration) of PEI and Ce6 in several samples for EMT6 cells.

Samples	IC ₅₀ of PEI [$\mu\text{g mL}^{-1}$]	IC ₅₀ of Ce6 [$\mu\text{g mL}^{-1}$]
PEI@PB	15.161	—
Ce6@PB + laser	—	0.162
Ce6-PEI@PB + laser	0.175	0.078

Table S2 IC₅₀ of PEI and Ce6 in several samples for MCF7 cells.

Samples	IC ₅₀ of PEI [$\mu\text{g mL}^{-1}$]	IC ₅₀ of Ce6 [$\mu\text{g mL}^{-1}$]
PEI@PB	18.625	—
Ce6@PB + laser	—	0.199
Ce6-PEI@PB + laser	0.290	0.129