SUPPORTING INFORMATION

Instant ultrasound-evoked precise nanobubble explosion and deep photodynamic therapy for tumor guided by molecular imaging

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Table S1. Bubble size comparison before and after encapsulation.

Groups	Average particle size	Particles/mL
Before encap.	1.15 μm	$2.0–3.0\times10^{10}$
After encap.	0.25 μm	$1.6–3.0\times10^{7}$

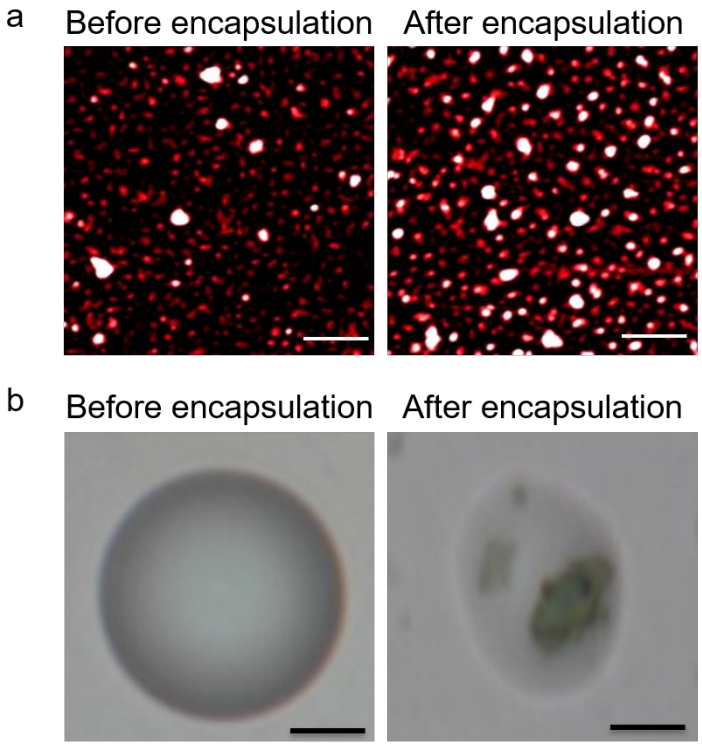

Figure S1. Ultra-high-resolution fluorescence microscope image of the microbubble before and after HPPH encapsulation respectively. (a) Scale bar = 2 μm. (b) Scale bar = 100 nm.

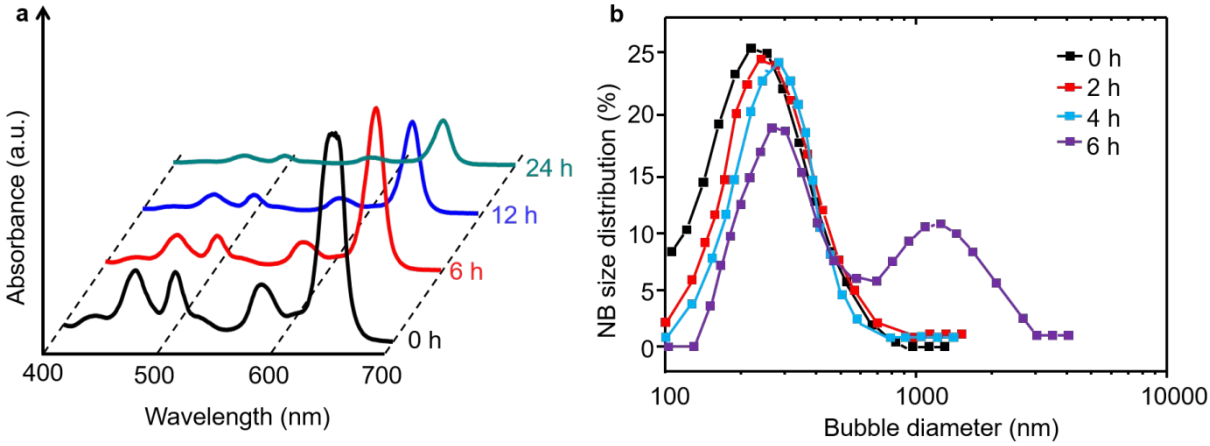

Figure S2. Stability of NBs. (a) Time-dependent UV-Vis spectra after encapsulation of HPPH. (b) Time-dependent size distribution of NBs after encapsulation of HPPH.

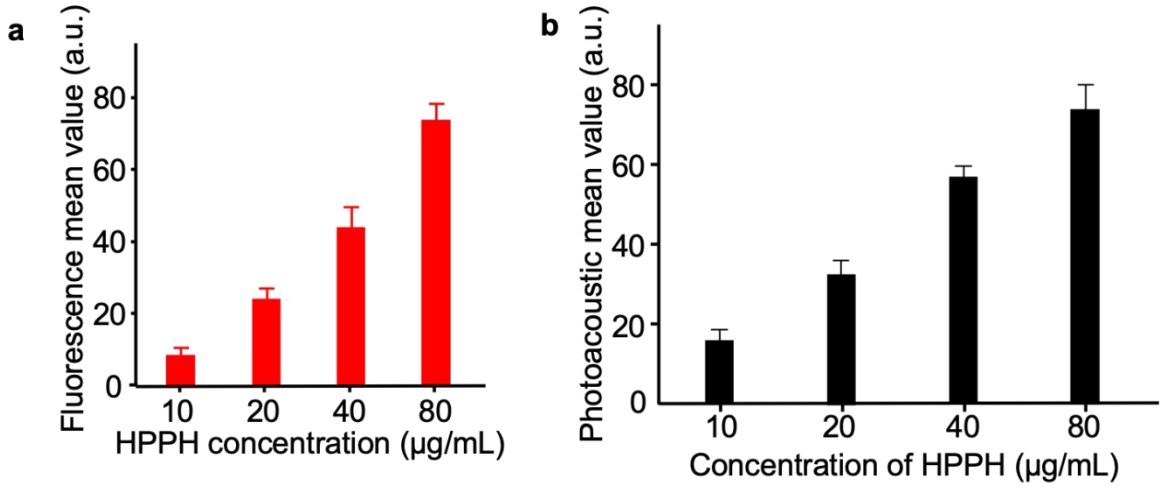

Figure S3. Corresponding Fluorescence image (a) and Photoacoustic image (b) intensities of the nanobubble with HPPH.

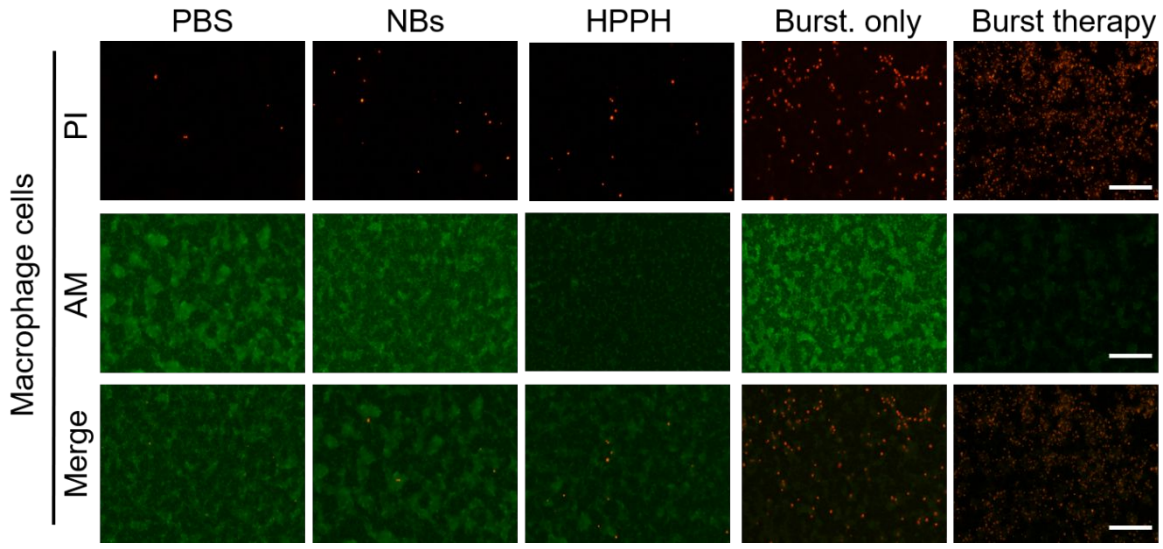

Figure S4. Confocal fluorescence images of normal cells (macrophage cell) treated with NBs and ultrasound after laser irradiation for 5 min. The scale bar is 100 μm. The confocal fluorescence images of cells treated with NBs and ultrasound after laser irradiation for 5 min.

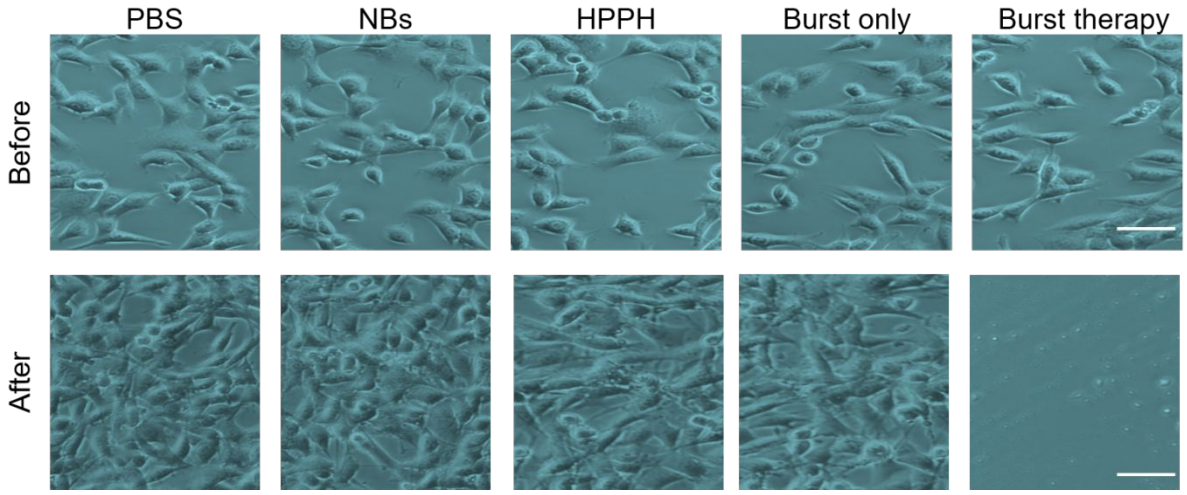

Figure S5. High-magnification microscope images of five groups of U87MG tumor cells.

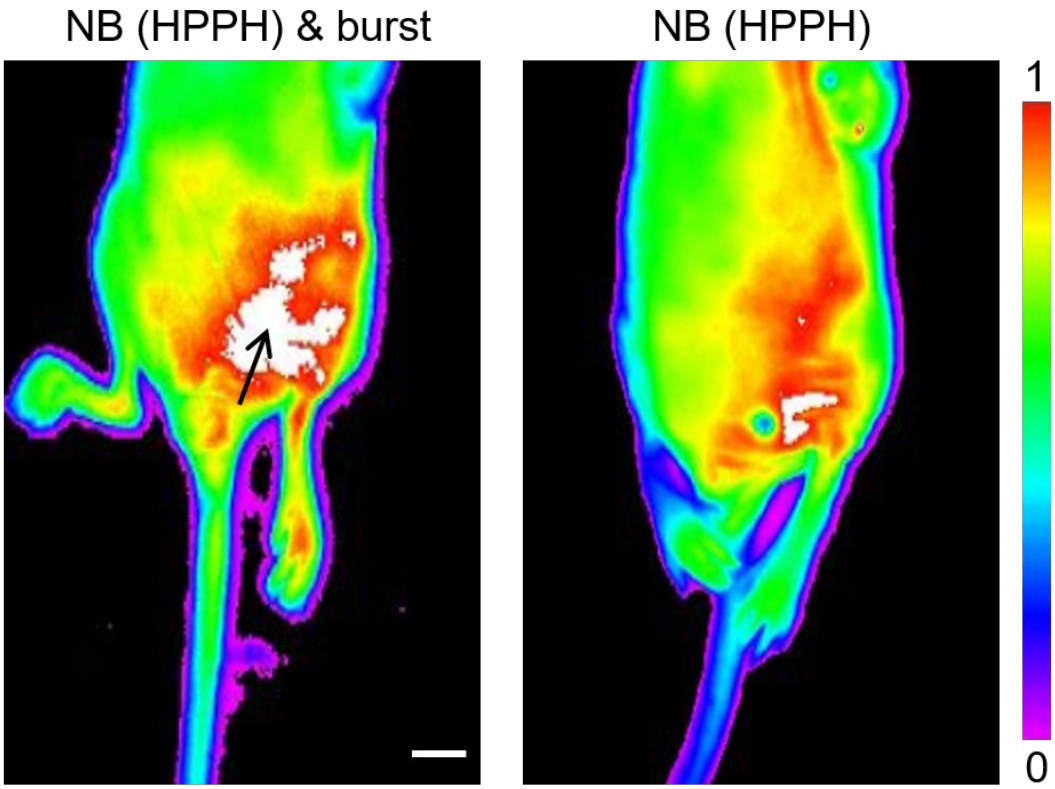

Figure S6. Fluorescence imaging of mouse tumor treated with NB(HPPH) injection. The left image was acquired on mouse tumor after 40% NBs injection with ultrasound burst. HPPH leakage after NB explosion led to significant fluorescent signal increase. The right image was acquired on mouse tumor after 40% NBs injection without ultrasound burst. Scale bar = 5 mm.

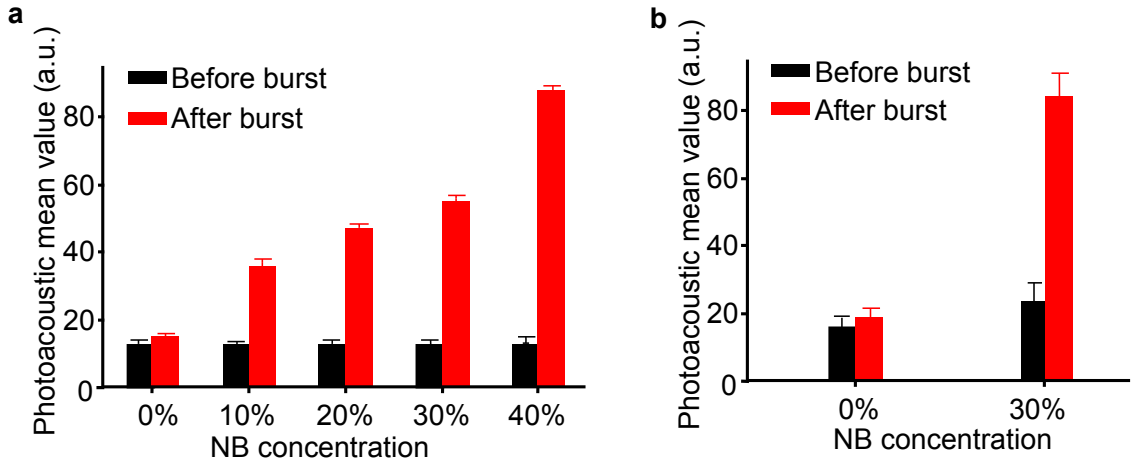

Figure S7. Quantitative Results of the photoacoustic imaging of the "burst therapy". (a) Corresponding PAT signal intensities after NBs explosion at different concentrations. (b) Corresponding PAM signal intensities in the rectangular region-of-interest (ROI) after PBS and NBs explosion.

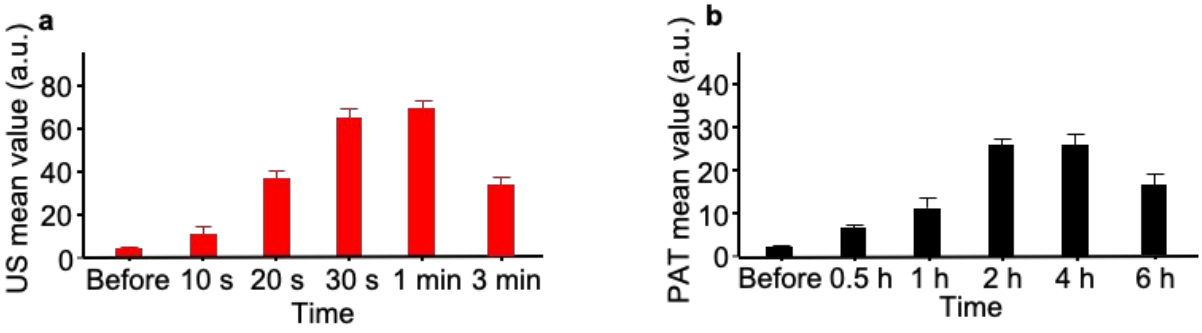

Figure S8. (a-b) Corresponding US and PAT signal intensities at different time points post injection.

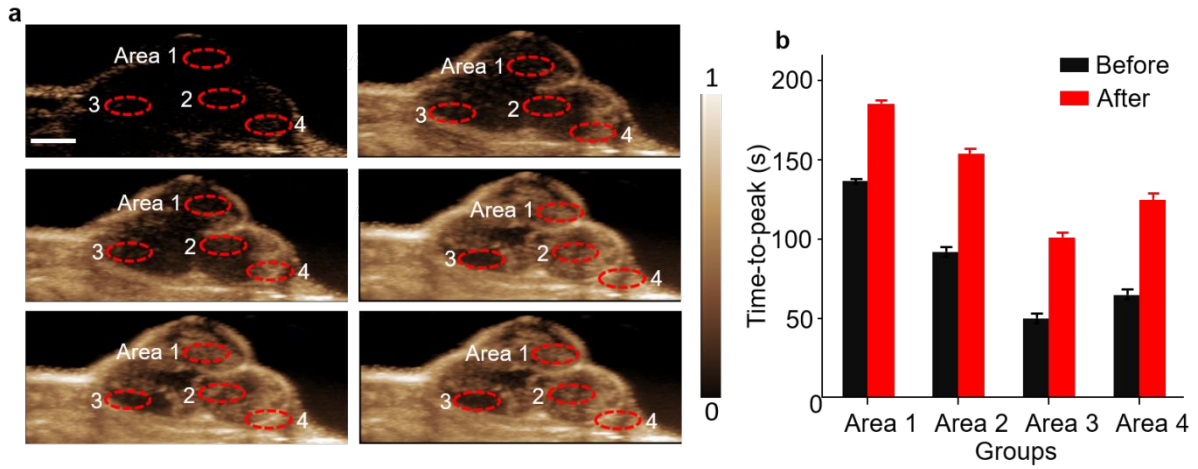

Figure S9. The mechanical impact affected the perfusion ability in tumors. (a-b) Area 1-4 showed time-to-peak (T-T-P) after reperfusion of tumor vessels. The left and right column images represent the ultrasound imaging of tumor before and after therapy, respectively. All areas show that the ultrasound peak value increased after injecting NBs and burst. The results indicate that the explosion of NBs changed the peak and T-T-P of the tumor vessels. Scale bar = 3 mm.

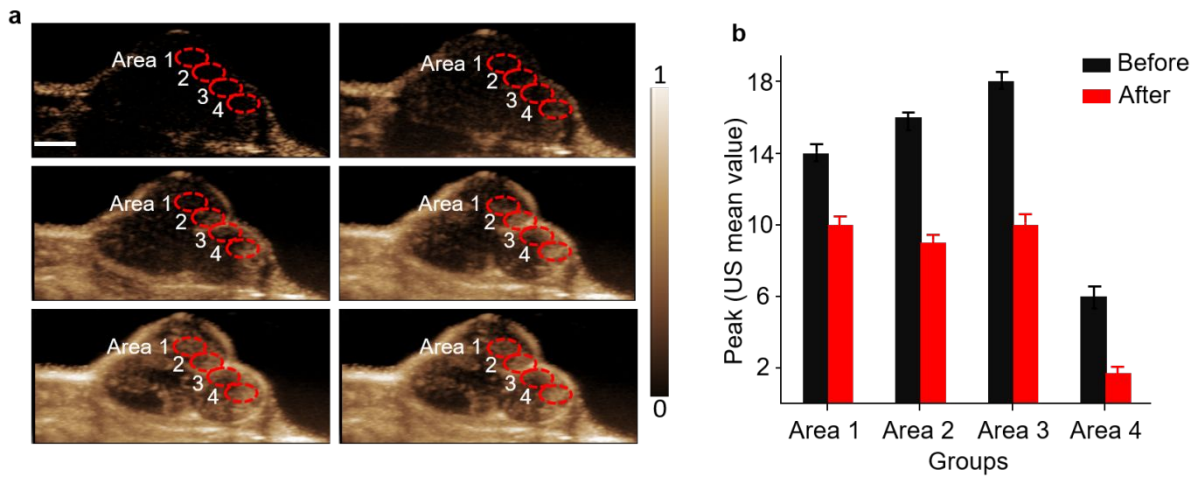

Figure S10. The mechanical impact affected the perfusion ability of the tumors. (a) Area 1-4 showed the ultrasound signal peak after reperfusion of tumor vessels. The left and right column images represent ultrasound imaging of tumor before and after therapy, respectively. (b) The 4 areas showed that the US peak value of tumor reduced after therapy. Scale bar = 3 mm.

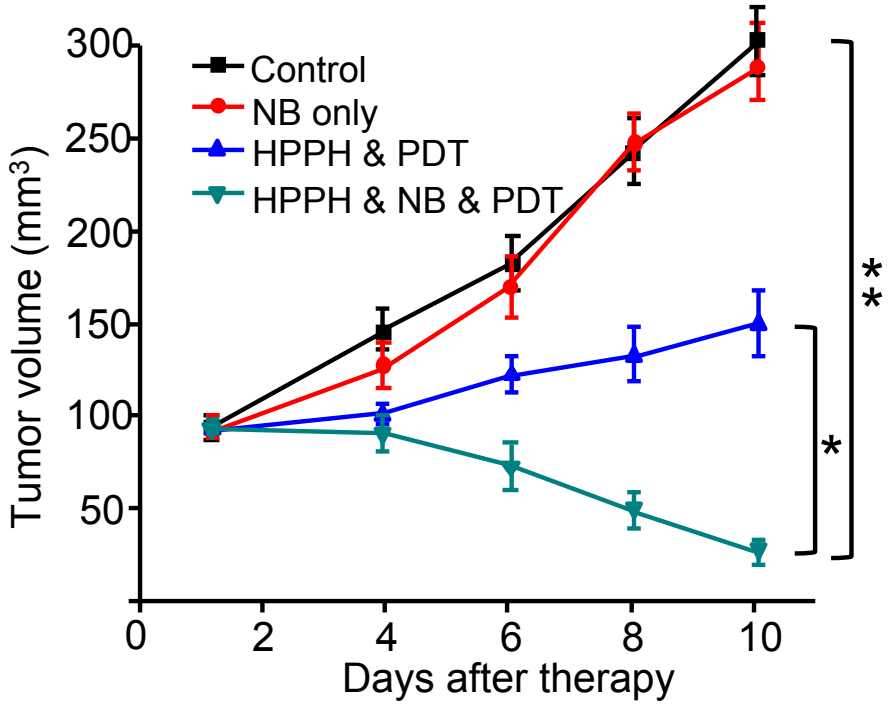

Figure S11. Graphs of the tumor volume of mice before and after treatment of four groups (control, NB only, HPPH + PDT, HPPH + NB + PDT) up to 10 days. N = 7 in each group. P values were calculated by Student's t-test (**P<0.001, *P<0.01).

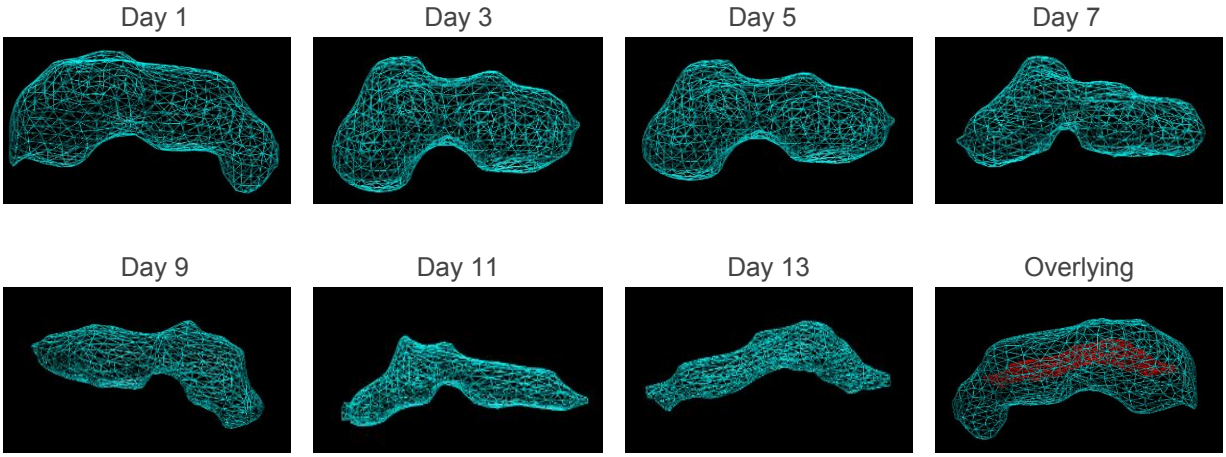

Figure S12. 3D (three-dimensional) graphs of the tumor volume by ultrasound imaging. 3D graphs of the tumor volume of mice after treatment of PDT with NB-HPPH group up to 13 days. The red line in the overlying image corresponds the tumor anatomy in Day 13.

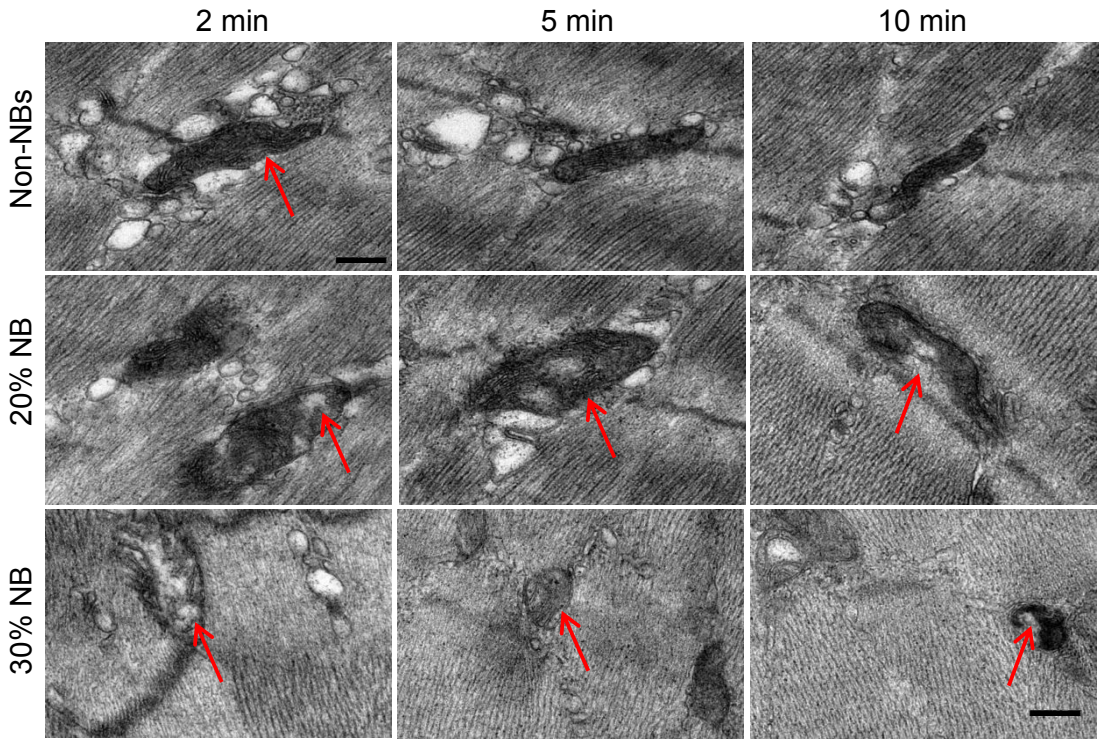

Figure S13. TEM images of mitochondrion acquired from tumor tissue after explosion therapy. TEM images show obvious destruction of the cytoplasmic membrane and mitochondrial cristae after NBs explosion indicated by red arrows. The cristae of mitochondria were partly disintegrated. Some completely became transparent density area. In the non-NBs group, it could hardly be observed with any obvious destruction. Scale bar = 1 μm.

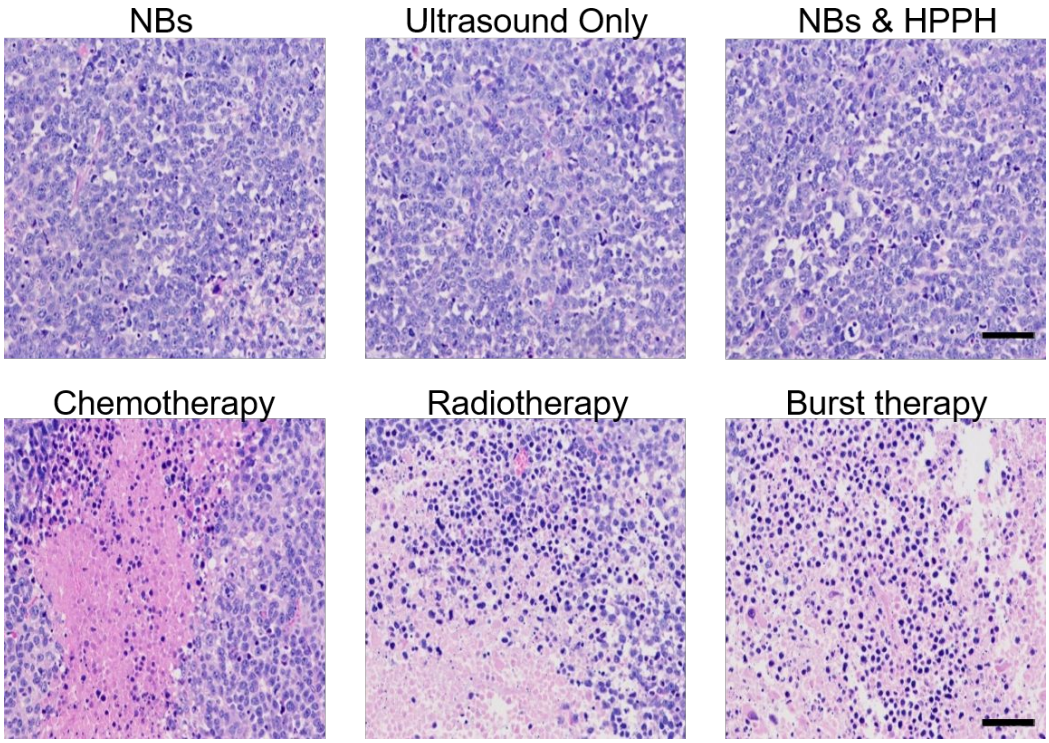

Figure S14. Representative H&E stained images of the A549 tumor after being harvested from tumor-bearing mice after various treatments. Scale bar = 100 μm.

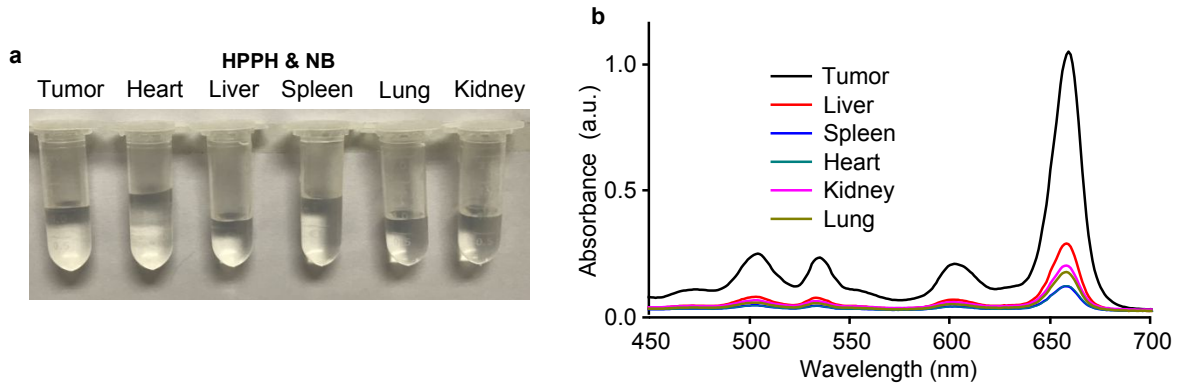

Figure S15. The biodistribution of HPPH in tumor and organs 24 h after i.v. injection of HPPH encapsulated NBs. (a) Various organs samples were removed and homogenized with 1.0 mL acetonitrile for quantitative measurement. (b) UV−vis spectra of the supernatant samples.

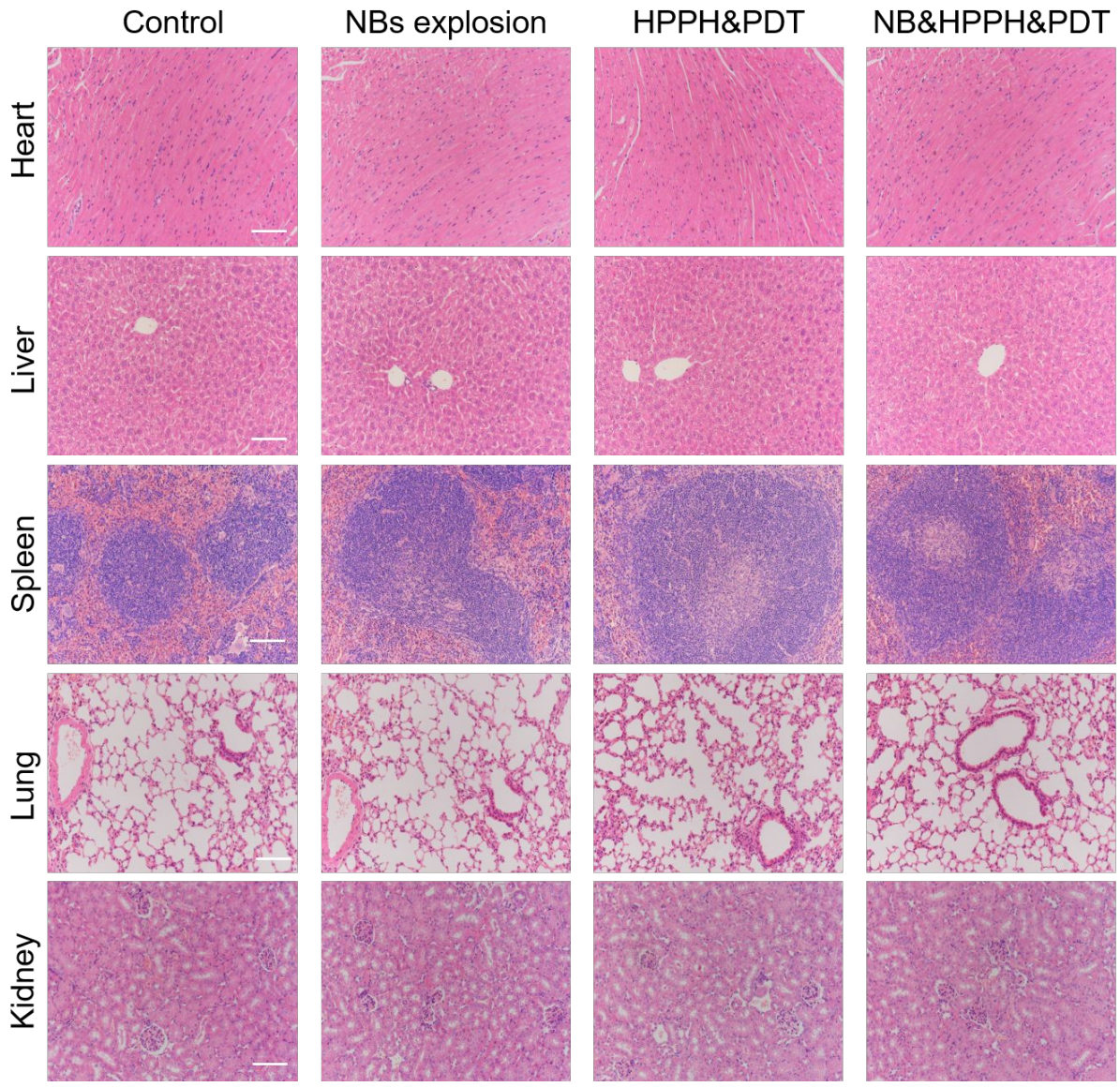

Figure S16. H&E stains of mouse heart, liver, spleen, lung, and kidney 7 days after i.v. injection of PBS (control) or NB explosion (200 μL, 20 mg mL^{-1}). Scale bar = 50 μm.

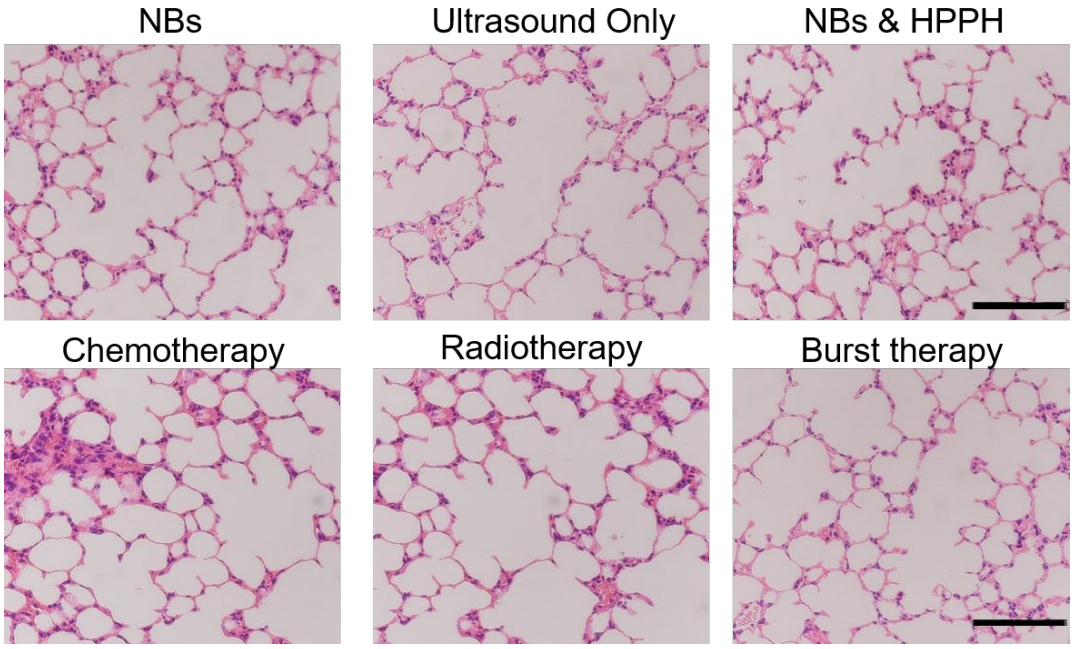

Figure S17. **Representative H&E stains images of the lung after harvested from tumor-bearing mice after various treatments.** Scale bar = 100 μm.

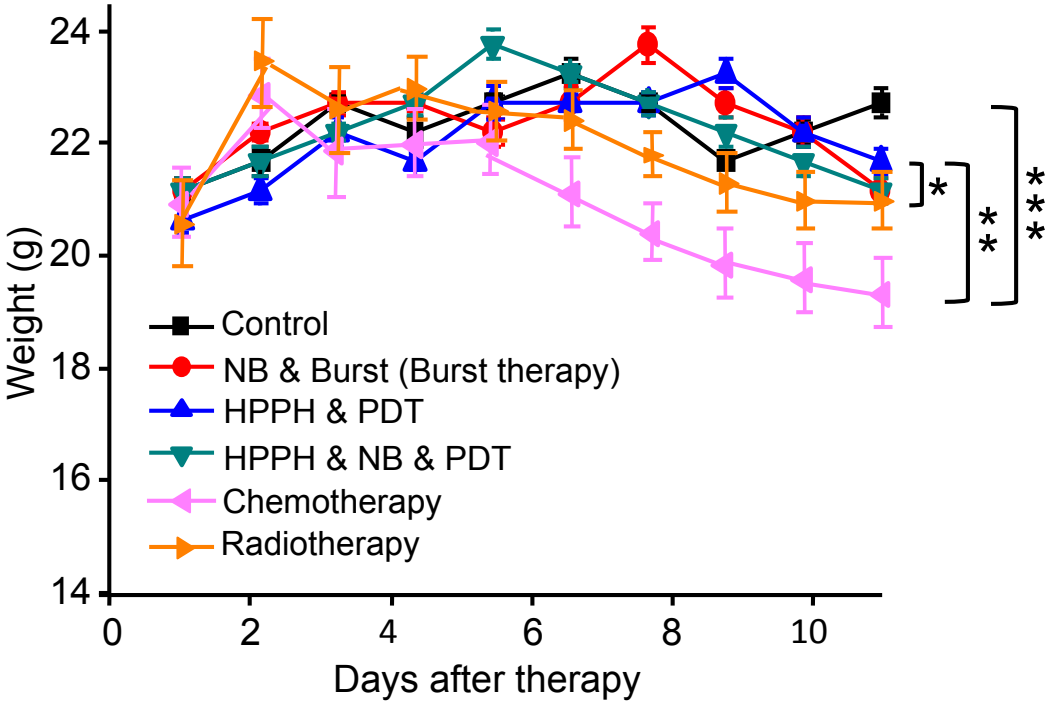

Figure S18. Time courses of the mice growth. Graphs of the body weight of mice before and after treatment of six groups (control, NB&Burst, HPPH&PDT, HPPH&NB&PDT, chemotherapy, radiotherapy) up to 10 days. N = 7 in each group. P values were calculated by Student's t-test (***P<0.005, **P<0.01 *P<0.05).

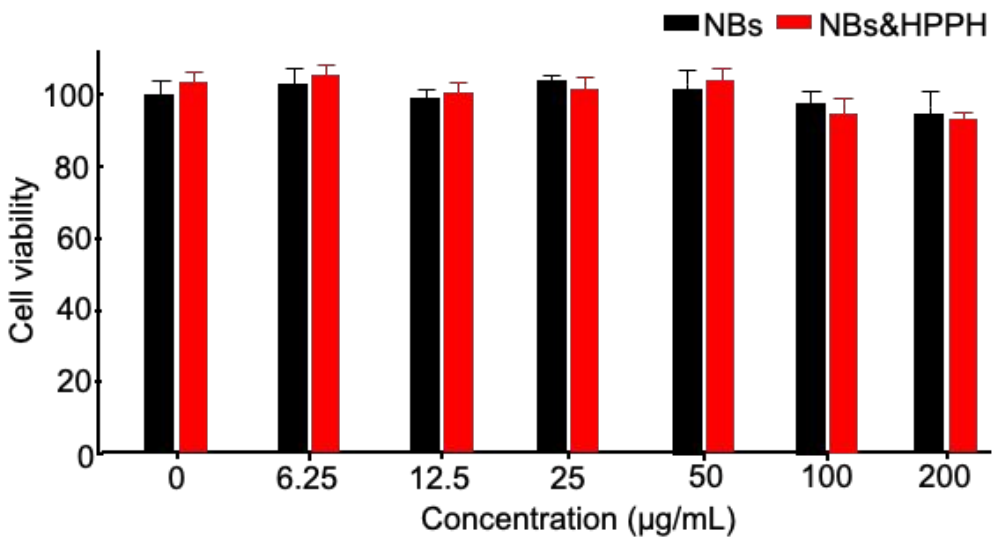

Figure S19. **The cytotoxicity experiment of NBs and HPPH for U87MG cells.** U87MG cells were used for the cytotoxicity experiment of NBs and HPPH. The cells were randomly divided into seven groups and then co-cultured with NBs and HPPH at different concentrations (0, 6.25, 12.5, 25, 50, 100 and 200 μg mL-1) for 24 h. After that, the relative cell survival rates of different groups were detected using the standard MTT assay.

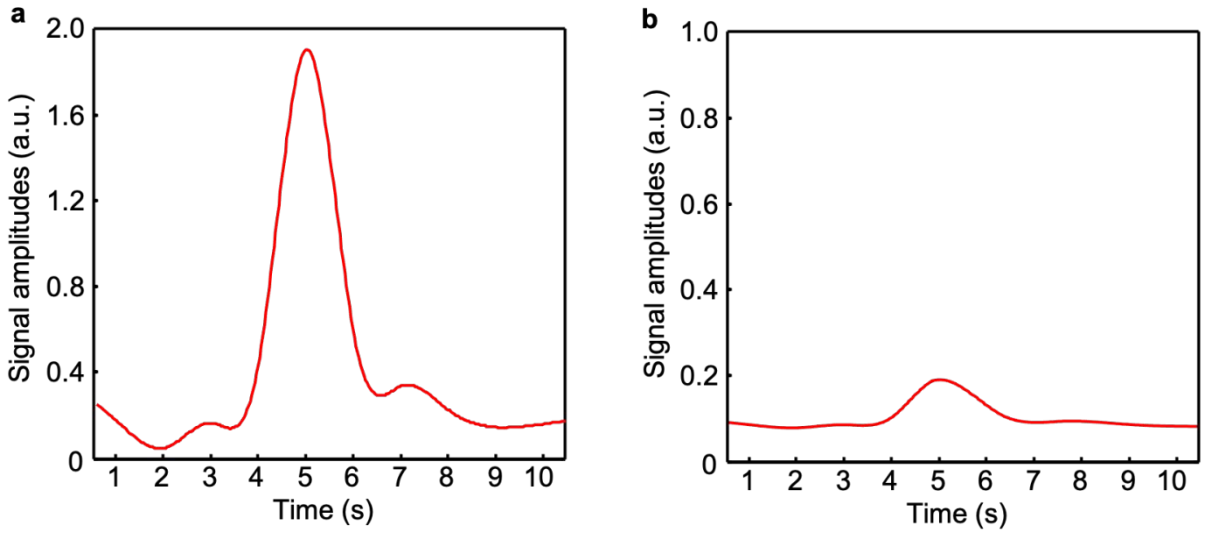

Figure S20. Ultrasound wave pressure of the NBs explosion. (a) Time domain signal curve of ultrasound wave generated by ultrasound-evoked processed NBs explosion. (b) Time domain signal curve of ultrasound wave generated by conventional MBs' cavitation.