Materials. Black phosphorus nanosheets (BP NSs) wsa purchased from Hangzhou Nanomall Technology Co., Ltd. (Hangzhou, China). $PEG\text{-}NH_2$ and $FITC\text{-}PEG\text{-}NH_2$ (average molecular weight of 2000) were purchased from Shang Hai Ponsure Biotech, Inc. (China). FITC-anti-CD3, PE-anti-CD4, APC-anti-CD8 were purchased from 4A Biotech Co., Ltd (China). 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO) wsa purchased from Macklin reagent (China). Lactic Acid Assay Kit was provided by Nanjing Jiancheng Bioengineering Institute (China). Methylene blue (MB) was obtained from Aladdin-Reagent (China). Reactive Oxygen Species Assay Kit wsa purchased from Beyotime Company (China). The ATP assay kit, Antibodies for Calreticulin (CRT) and Lyso-tracker Green was purchased from Beyotime Company (China). All of the aqueous solutions were prepared using purified deionized (DI) water purified with a purification system (Direct-Q3, Millipore, USA). The other solvents used in this work were purchased from Sinopharm Chemical Reagent (China) and Aladdin-Reagent (China).

•OH-Generation Detection

MB was used as the •OH - monitoring agent. In the experiments, 10 μL of MB stock solution (7.5 mM) was added to 2 mL of BO suspension or BP suspension (50 μg/mL), and ultrasound (US, power density = 0.75 W/cm^2, transducer frequency = 1 MHz, 30% duty cycle) was employed as the radiation source. The absorption of MB at 660 nm was recorded at various irradiation times to obtain the decay rate[1].

ESR measurements

$^1O_2$ and •OH generation was evaluated by TEMP and DMPO. 3 μL TEMP was mixed with 100 μL BO at 50 μg/mL and irradiated by US (1.0 MHz, 0.75 W/cm^2 ,1 min). 10 μL DMPO was mixed with 50 μL BP at 50 μg/mL and irradiated by US (1.0 MHz, 0.75 W/cm^2, 2 min). The signals of $^1O_2$ and •OH can be shown by the ESR spectrometer.

3PO release study

The *in vitro* 3PO release profile of BO was carried out by dialysis method. 1 mL of BO containing 0.05 mg 3PO was added into 1× PBS (pH=7.4) under horizontal shaking at 100 rpm for 3 h. To investigate the stimuli effect of US radiation on the release behavior, the release experiment of BO was initially performed with or without US radiation (1.0 MHz, 0.75 W/cm^2, 5 min). At appropriate time point, the solution was centrifuged at 10000 rpm for 10 min, 100 μL of supernatant were collected, and an UV−vis spectrophotometer was used to monitor the released 3PO content[2].

Cancer cell internalization study

4T1 cells were incubated for 0.5 h with FITC labeled BO. The 3PO concentration was 5 μg/mL. Then, cells were washed with PBS and stained with the Lyso-tracker Green (10 μM) for 10 min at 37°C. Then, cells were washed with PBS observed by fluoresence microscope (IX81).

Intracellular reactive oxygen species (ROS) generation. For determination of ROS levels *via* fluorescent imaging, 4T1 cells were incubated for 0.5 h with 5 different groups: (1) PBS; (2) US (1.0 MHz, 0.75 W/cm^2, 5 min); (3) BO; (4) BP+US; (5) BO+US. The 3PO concentration was 5 μg/mL in group 3, and 5. Then, the fluorescent dye, DCFH-DA (10 μM), was added and co-incubated for 20 min at 37°C. Then, cells in group 2, 4 and 5 were exposed to US radiation for 5 min. ROS level was determined by confocal laser scanning microscope (CLSM; Zeiss LSM 710)[3].

Intracellular ATP detection.

After seeded in 48-well plate (2 × 10^4 cells per well) for 12 h, 4T1 cells were pre-incubated incubated and treated with 5 different groups: (1) PBS; (2) US (1.0 MHz, 0.75 W/cm^2, 5 min); (3) BO; (4) BP+US; (5) BO+US. The 3PO concentration was 5 μg/mL in group 3, and 5. The cells were cultured for another 6 h. Thereafter, the supernatant was discarded and the intracellular ATP content was measured by the ATP Assay Kit according to the instructions[4].

Lactic acid reduction ability of BO.

4T1 cells were seeded into the 48-well plate (2 × 10^4 cells per well). After cultured for 12 h, the cell supernatant was displaced with the fresh culture medium and treated with 5 different groups under hypoxia (pO_2: 2%) condition: (1) PBS; (2) US (1.0 MHz, 0.75 W/cm^2, 5 min); (3) BO; (4) BP+US; (5) BO+US. After 4 h, cell supernatant samples of cells with different treatments were collected, and lactate content was detected using the Lactic Acid assay Kit.

In vitro anticancer effect of BO. The phototoxicity was measured by MTT assay. 4T1 cells were seeded in 96-well plates at a density of 5 × 10^4 cells per well and incubated for 12 h. Afterwards, cells were incubated with 5 different groups: (1) PBS; (2) US (1.0 MHz, 0.75 W/cm^2, 5 min); (3) BO; (4) BP+US; (5) BO+US. The 3PO concentration was 5 μg/mL in group 3, and 5. Then, cells in group 2, 4 and 5 were exposed to US radiation for 5 min. Then, cells were washed with PBS for three times. At the end of the incubation, 5 mg/mL MTT PBS solution was added, and the plate was incubated for another 4 h. Finally, the absorbance values of the cells were determined by using a microplate reader (Emax Precision, USA) at 570 nm. The background absorbance of the well plate was measured and subtracted. The cytotoxicity was calculated by dividing the optical density (OD) values of treated groups (T) by the OD values of the control (C) (T/C × 100%)[5]. We then used the same method to verify

the *in vitro* phototoxicity of BO with different TBP-2 concentration (0, 5 and 20 μg/mL). The above experiments were subsequently performed under hypoxic conditions.

***In vitro* surface expression of calreticulin (CRT).** For flow cytometric analysis of cell surface expose of CRT, 4T1 cells were seeded into the 12-well plate (2×10^5 cells/well) and the next day the cells were treated with different ways: (1) PBS; (2) US (1.0 MHz, 0.75 W/cm^2, 5 min); (3) BO; (4) BP+US; (5) BO+US. The 3PO concentration was 5 μg/mL in group 3, and 5. Then, cells in group 2, 4 and 5 were exposed to US radiation for 5 min. The cells were then harvested, washed twice with PBS, fixed in 0.25% paraformaldehyde for 5 min and incubated with anti-CRT antibody for 30 min. After washed with PBS for three times, the cells were incubated with Alexa Fluor-488-conjugated secondary antibody for 30 min. The cells were conducted by flow cytometric examination (FACS Caliber system, BD Biosciences, Oxford, UK.).

Extracellular release of HMGB1. 4T1 cells were seeded into the 12-well plate (2×10^5 cells/well) and the next day the cells were treated with different ways: (1) PBS; (2) US (1.0 MHz, 0.75 W/cm^2, 5 min); (3) BO; (4) BP+US; (5) BO+US. The 3PO concentration was 5 μg/mL in group 3, and 5. Then, cells in group 2, 4 and 5 were exposed to US radiation for 5 min. For quantification of released HMGB1 in medium, the medium was collected after the cells treated with materials. Then 20 μL medium was used for HMGB1 ELISA assay.

***In vivo* tumor vascular and hypoxia microenvironment study.** To investigate the In vivo tumor vascular and hypoxia TME, BALB/c mice were subcutaneous injected with 1×10^6 4T1 cells. The mice were grouped and treated when the primary tumor volume reached 200 mm^3. Tumor bearing mice were divided randomly into 5 groups (each group included 5 mice): (1) PBS; (2) US; (3) BO; (4) BP + US (5) BO + US. The equivalent 3PO dose was 3.4 mg/kg in group 3, 4 and 5. After 12 h of intravenous injection, US irradiation (power density = 0.75 W cm−2, transducer frequency = 1 MHz, 30% duty cycle, 10 min) was carried out. Immunofluorescence histochemical staining of CD31 and anti-PIMO were conduct according to the standard protocol. The cryosections were observed by a confocal laser scanning microscope (CLSM; IX81, Olympus, Japan).

***In vivo* lactic acid reduction.** When tumor size reached about 200 mm^3, the 4T1 tumor-bearing mice were randomly divided into the following 5 groups (n = 3), (1) PBS; (2) US; (3) BO; (4) BP + US (5) BO + US. The equivalent 3PO dose was 3.4 mg/kg in group 3, 4 and 5. After 12 h of intravenous injection, US irradiation (power density = 0.75 W cm^{-2}, transducer frequency = 1 MHz, 30% duty cycle, 10 min) was carried out. 48 h after different treatments, mice were sacrificed to obtained the tumor tissues. After the lysis with the tissue homogenizer

(Dragonlab, D-160), the supernatant of tumor tissues was collected and detected via the Lactic Acid assay Kit.

Evaluation of intratumoral oxidative stress. To investigate the in vivo intratumoral oxidative stress, BALB/c mice were subcutaneous injected with 1×10^6 4T1 cells. The mice were grouped and treated when the primary tumor volume reached 200 mm^3. Tumor bearing mice were divided randomly into 5 groups (each group included 5 mice): (1) PBS; (2) US; (3) BO; (4) BP + US (5) BO + US. The equivalent 3PO dose was 3.4 mg/kg in group 3, 4 and 5. After 12 h of intravenous injection, US irradiation (power density = 0.75 W cm^{-2}, transducer frequency = 1 MHz, 30% duty cycle, 10 min) was carried out. Then the fluorescent dye, DCFH-DA (10 μmol/L, 50 μL) was injected intratumorally 12 h after intravenous injection in all groups . Next, laser (532 nm, 0.5W/cm^2, and we chose four points of exposure, each of which was 5 minutes) was performed in group 2, 5 and 6. Subsequently, tumors from each group were dissected. The cryosections were observed by a confocal laser scanning microscope (CLSM; IX81, Olympus, Japan).

***In vivo* antitumor study.** To investigate the abscopal effect of the prodrug vesicles, BALB/c mice were subcutaneous injected with 1×10^6 4T1 cells into the right flank (primary tumors) and 2×10^5 4T1 cells into the left flank (abscopal tumors), respectively. The mice were grouped and treated when the primary tumor volume reached 200 mm^3. Tumor bearing mice were divided randomly into 5 groups (each group included 5 mice): (1) PBS; (2) US; (3) BO; (4) BP + US (5) BO + US. The equivalent 3PO dose was 3.4 mg/kg in group 3, 4 and 5. After 12 h of intravenous injection, US irradiation (power density = 0.75 W cm^{-2}, transducer frequency = 1 MHz, 30% duty cycle, 10 min) was carried out. The treatment was conducted every 4 days for 13 days. Mice body weight was monitored every 2 days. After 16 days treatment, all the mice were sacrificed. The blood samples from these mice (≈1 mL) were collected for blood biochemistry analysis. Five main organs (heart, liver, spleen, lung and kidney) and tumors of all mice were harvested, washed with PBS, and fixed with paraformaldehyde for histology analysis. And the tumor tissues were imaged and weighed, and fixed in 4% neutral buffered formalin, processed routinely into paraffin, and sectioned at 4 μm. Then the sections were stained with Terminal deoxynucleotidyl transferase-mediated deoxyuridinetriphosphate nick end labeling (Tunel), TNF-α and Ki-67 and finally examined by using an optical microscope (BX51, Olympus, Japan).

Immune response analysis *in vivo*.

To investigate the *in vivo* treatment-induced CRT expression, BALB/c mice were subcutaneous injected with 1×10^6 4T1 cells. The mice were grouped and treated when the primary tumor volume reached 200 mm^3. Tumor bearing mice were divided randomly into 5 groups (each group included 5 mice): (1) PBS; (2) US; (3) BO; (4) BP + US (5) BO + US. The equivalent 3PO dose was 3.4 mg/kg in group 3, 4 and 5. After 12 h of intravenous injection, US irradiation (power density = 0.75 W cm^{-2}, transducer frequency = 1 MHz, 30% duty cycle, 10 min) was carried out. Then the tumors were harvested. Treatment-induced CRT expression at the tumor site were examined by immunofluorescence.

BALB/c mice were subcutaneous injected with 5×10^6 4T1 cells into the right flank (primary tumors) and 1×10^6 4T1 cells into the left flank (abscopal tumors), respectively. The mice were grouped and treated when the primary tumor volume reached 200 mm^3. Tumor bearing mice were divided randomly into 5 groups (each group included 5 mice): (1) PBS; (2) US; (3) BO; (4) BP + US (5) BO + US. The equivalent 3PO dose was 3.4 mg/kg in group 3, 4 and 5. After 12 h of intravenous injection, US irradiation (power density = 0.75 W cm^{-2}, transducer frequency = 1 MHz, 30% duty cycle, 10 min) was carried out. The treatment was conducted every 4 days for 13 days. After 16 days treatment, all the mice were sacrificed. To determine the intratumoral infiltration of $CD8^+$ ($CD3^+CD4^-CD8^+$) and $CD4^+$ T cells ($CD3^+CD4^+CD8^-$) in abscopal tumors, T lymphocytes were stained with anti-CD3-PerCP-Cy5.5, anti-CD4-FITC, anti-CD8-PE antibodies before flow cytometry. anti-CD4-FITC, anti-CD8-PE antibodies before flow cytometry. Treatment-induced intratumoral infiltration of T cells were also examined by immunofluorescence. In addition, tumor tissues were collected and homogenized in cold saline and infiltrating IFN-γ, IL-12 and TNF-α were measured by ELISA kit.

Supplementary figures

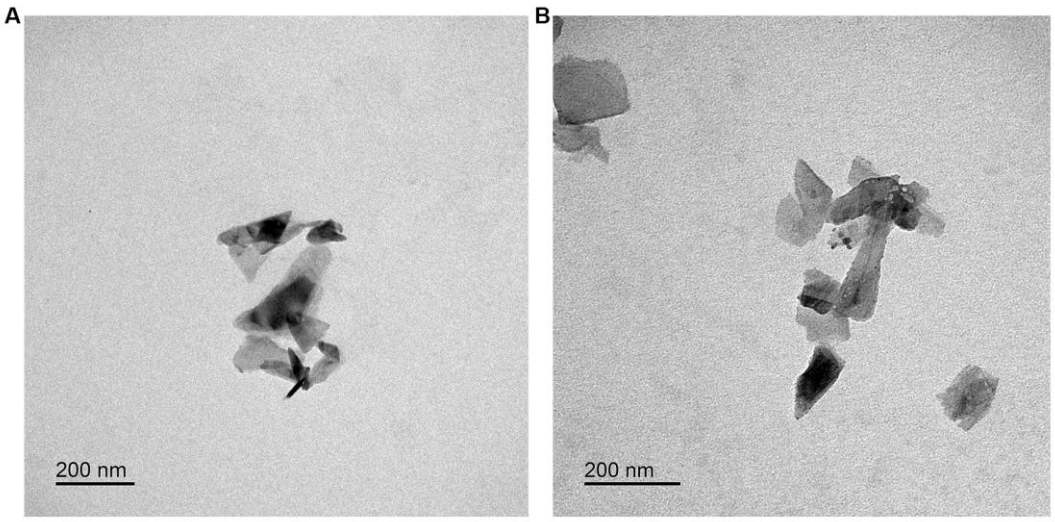

Figure S1. TEM image of (A) BP NSs and (B) BP@PEG (BP).

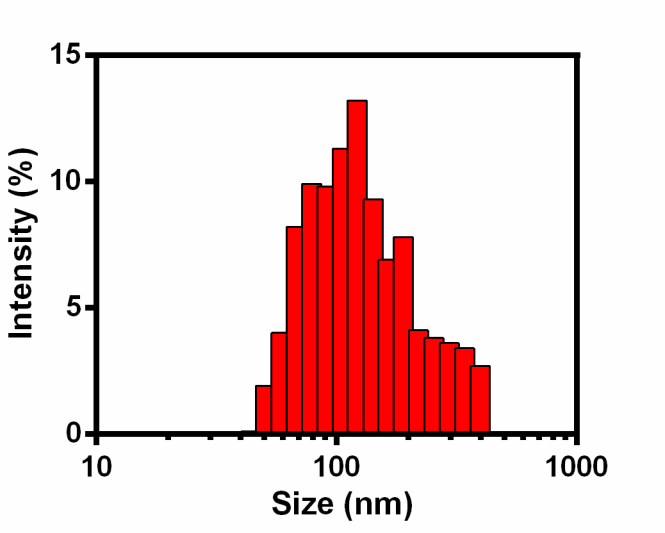

Figure S2. lateral size distribution of BO, based on statistical analysis on the TEM images.

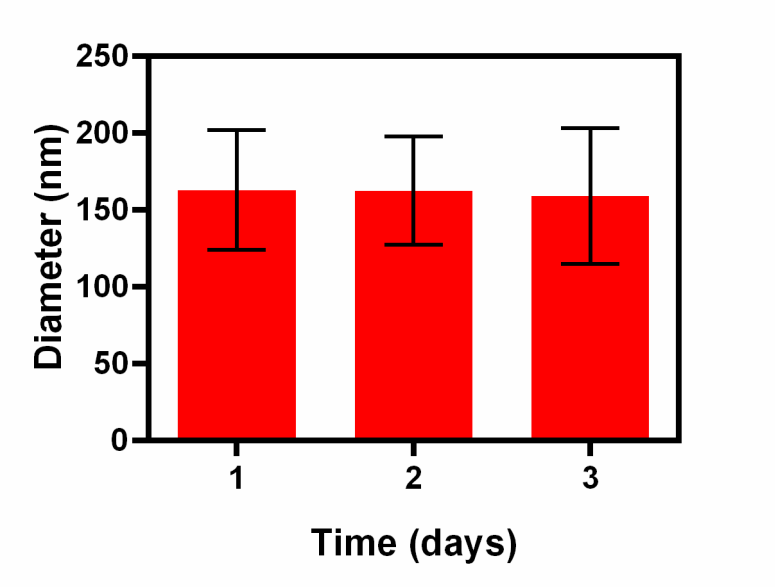

Figure S3. Lateral size distribution of BO within 3 days.

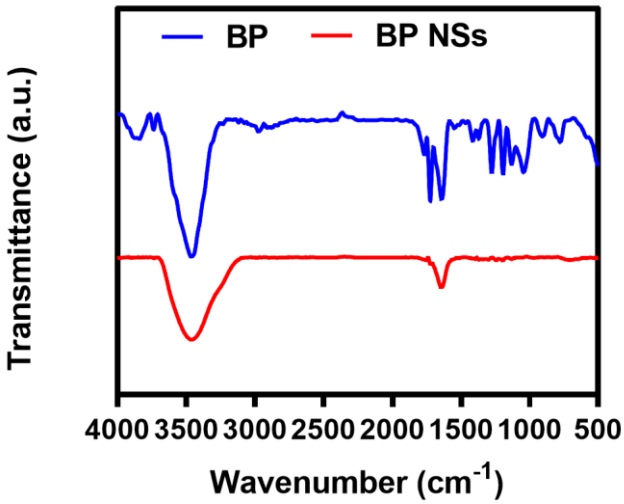

Figure S4. FTIR spectra of BP and BP NSs.

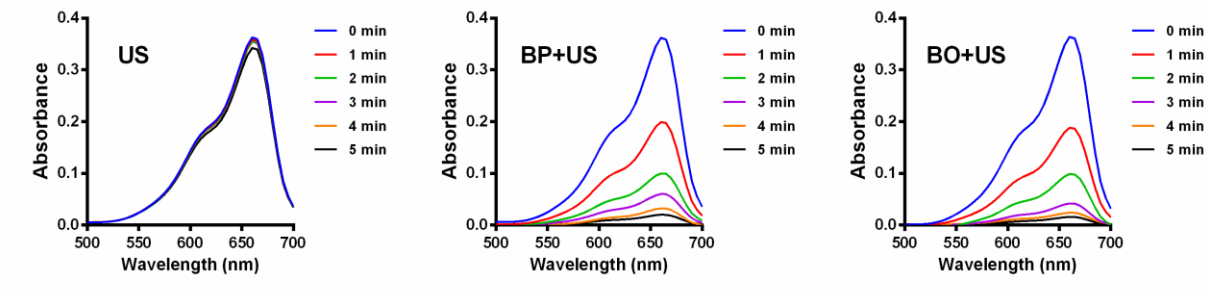

Figure S5. MB degradation profile treated with different groups.

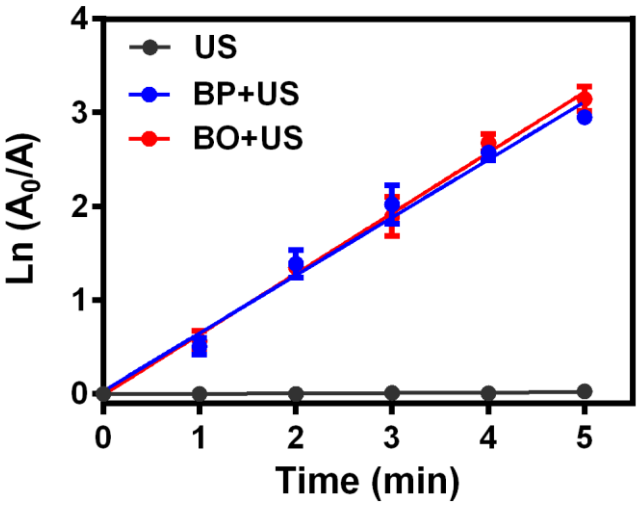

Figure S6. Rate constant for MB decomposition in the presence of different conditions.

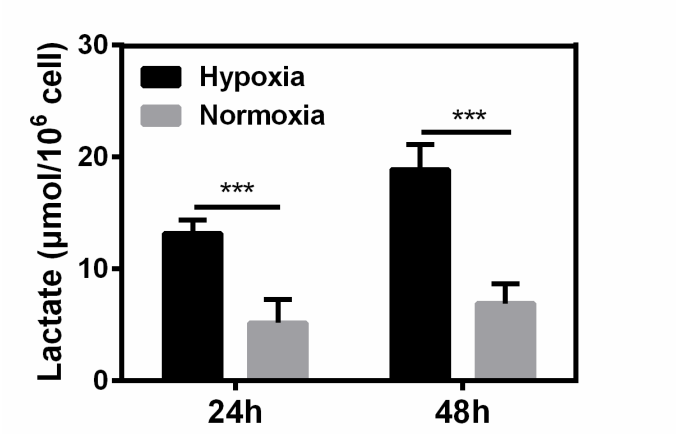

Figure S7. Lactic acid production in 4T1 cells cultured in normoxia or hypoxia were analyzed at 24 h and 48 h respectively.

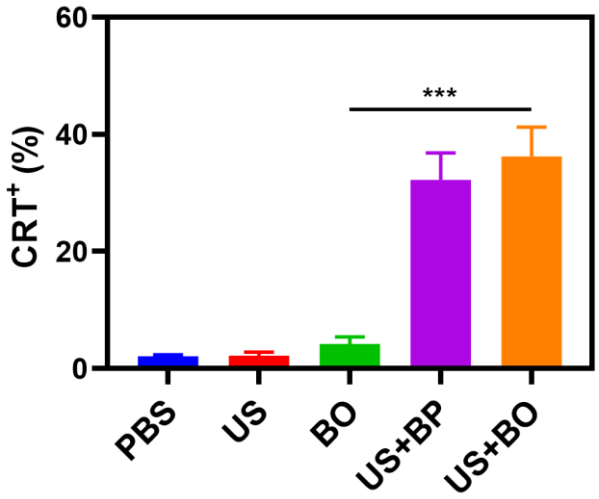

Figure S8. Flow cytometry analysis of the frequency of CRT^+ tumor cells *in vitro*.

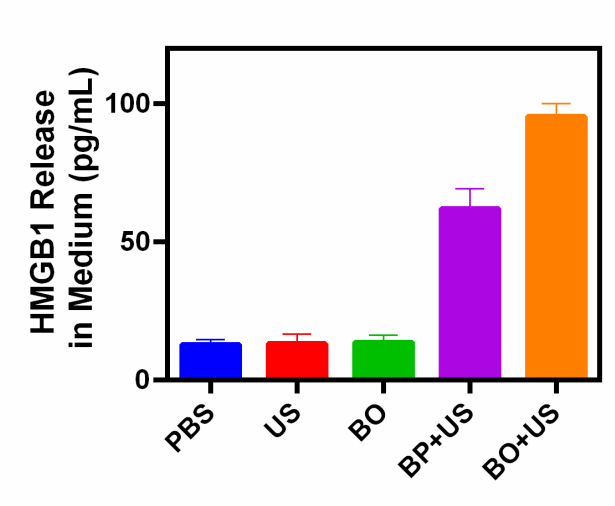

Figure S9. Quantitative examination of released HMGB1 from 4T1 tumor cells after various treatments for 24 h.

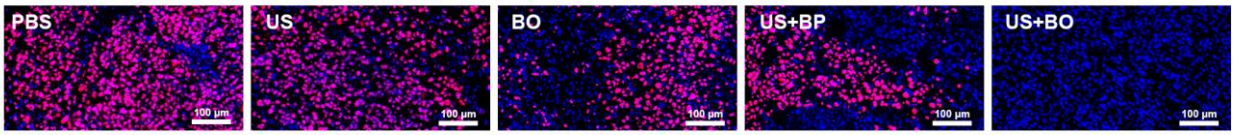

Figure S10. Tumor sections from the indicated treatment groups were stained with Ki-67.

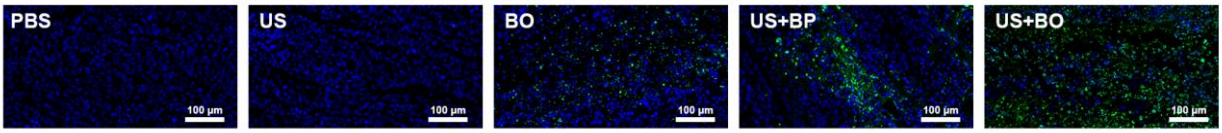

Figure S11. Tumor sections from the indicated treatment groups were stained with TUNEL.

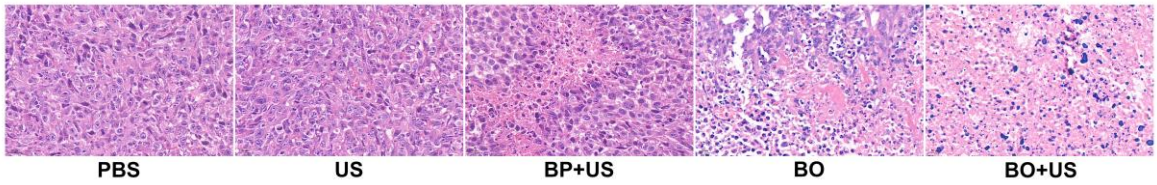

Figure S12. Tumor sections from the indicated treatment groups were stained with TUNEL.

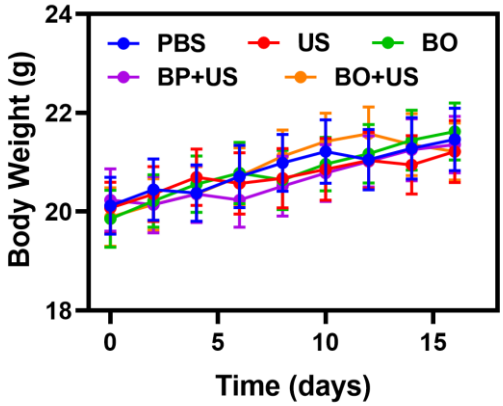

Figure S13. Changes in body weight were monitored over time in mice treated as indicated. Data are means ± SD.

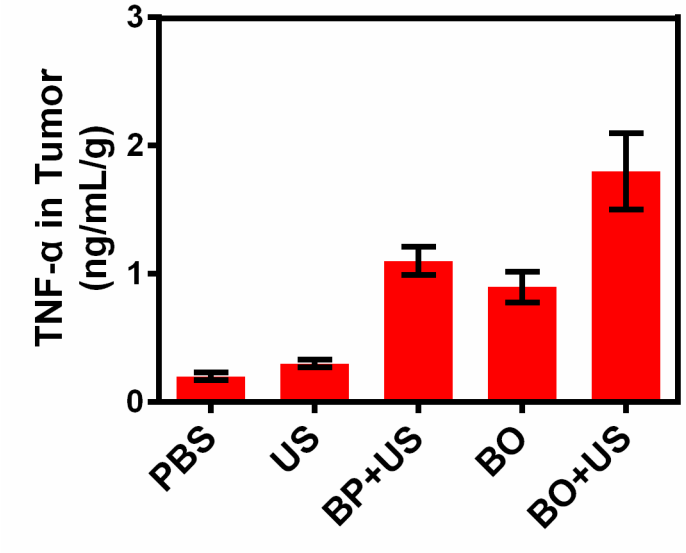

Figure S14. Cytokine secretion levels of TNF-α in the 4T1 tumor on mice with different treatments as indicated and analyzed using corresponding ELISA kits (n = 5). Data are present as mean ± SD.

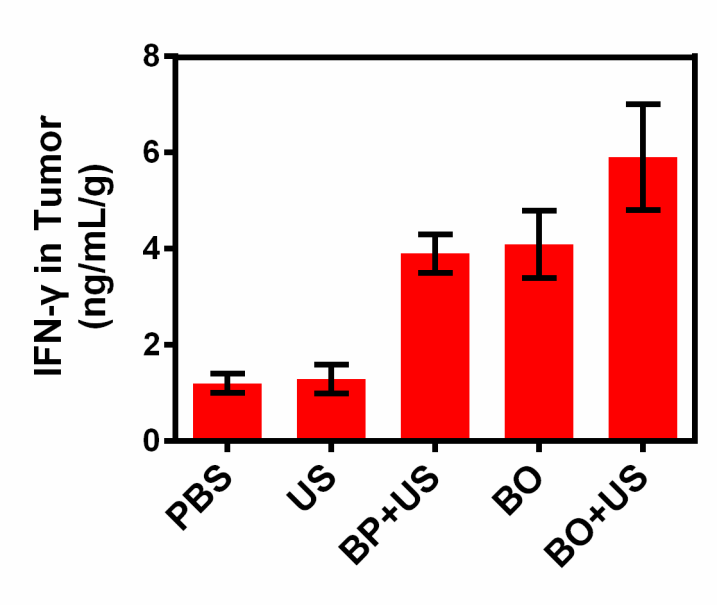

Figure S15. Cytokine secretion levels of IFN-γ in the 4T1 tumor on mice with different treatments as indicated and analyzed using corresponding ELISA kits (*n* = 5). Data are present as mean ± SD.

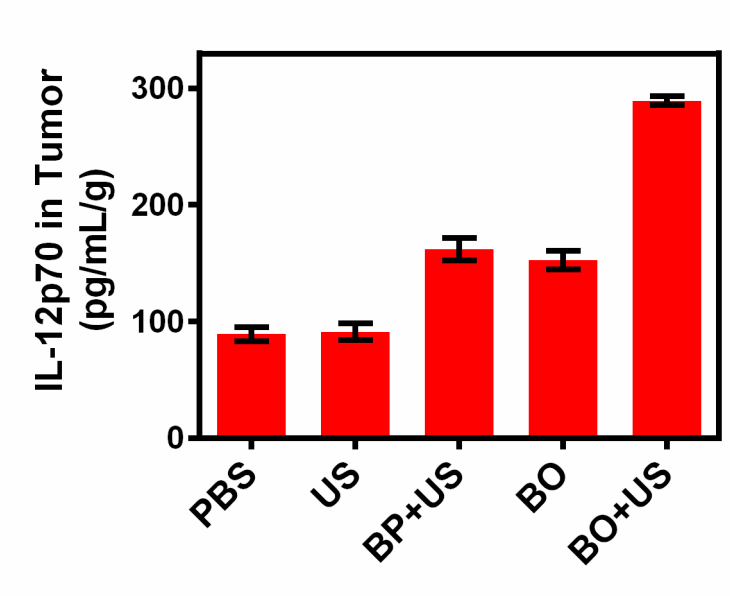

Figure S16. Cytokine secretion levels of IL-12p70 in the 4T1 tumor on mice with different treatments as indicated and analyzed using corresponding ELISA kits (*n* = 5). Data are present as mean ± SD.

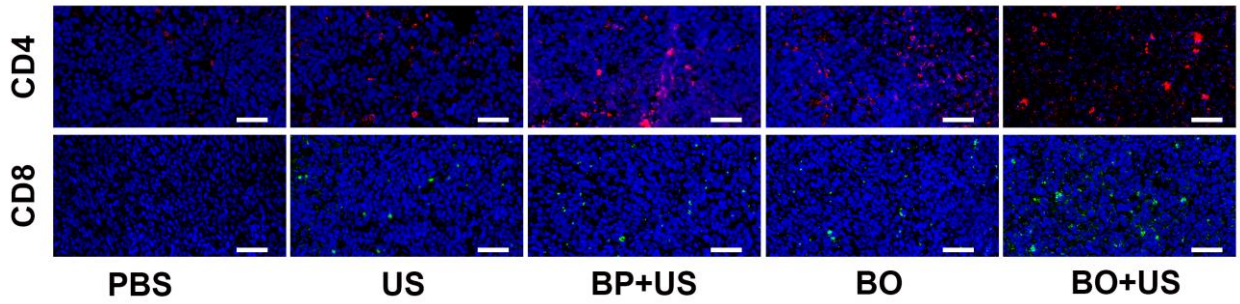

Figure S17. Representative immunofluorescence showing CD4+ and CD8+ T cells infiltrating the tumor. Scale bar: 100 μm.

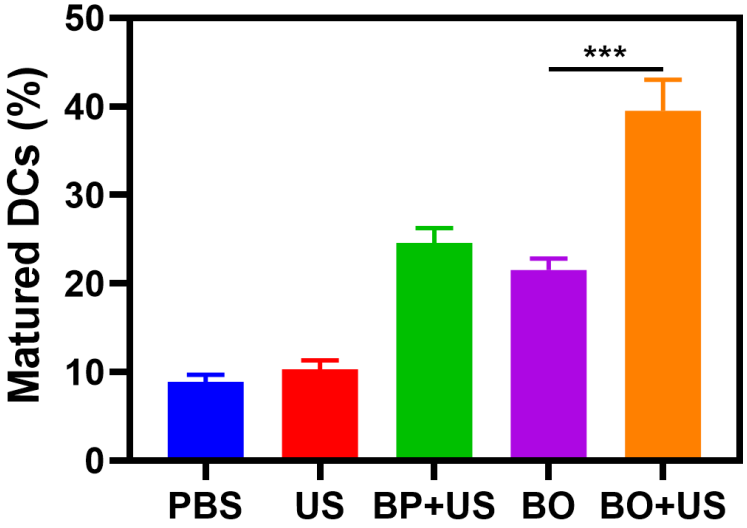

Figure S18. Semi-quantitative analysis of the frequency of mature dendritic cells in vivo.

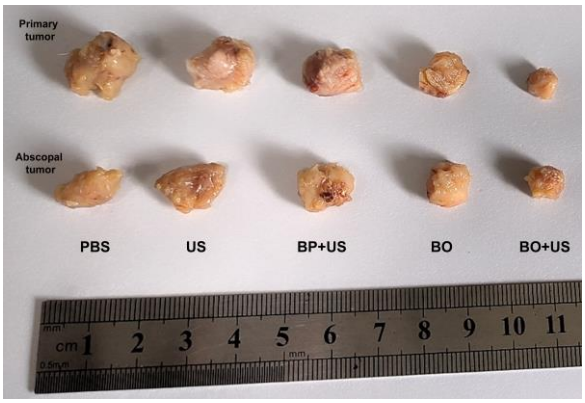

Figure S19. Representative tumor photograph of the harvested tumors after different treatments.

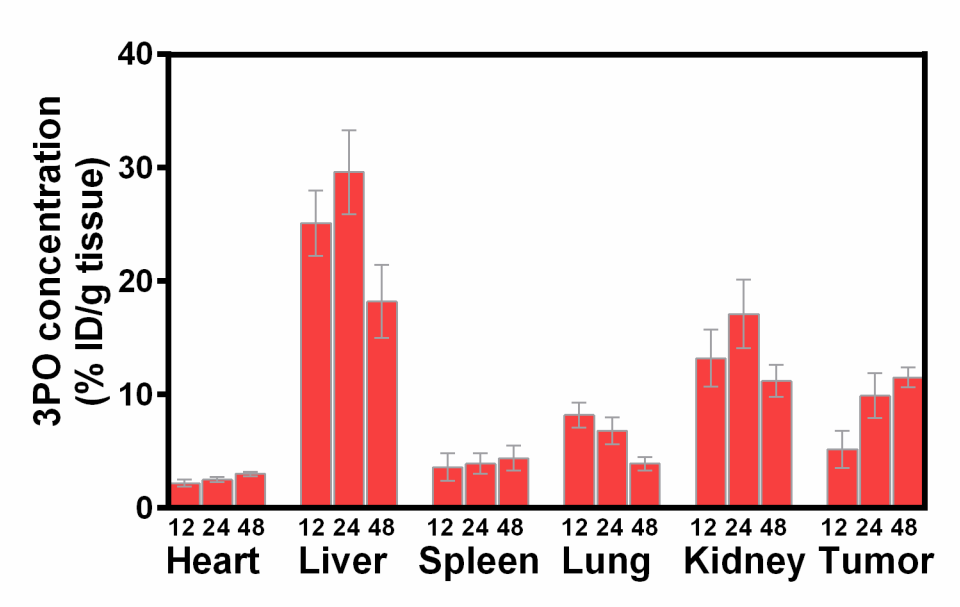

Figure S20. 3PO biodistribution in the tumor and organs at different time points after BO injection (n = 3).

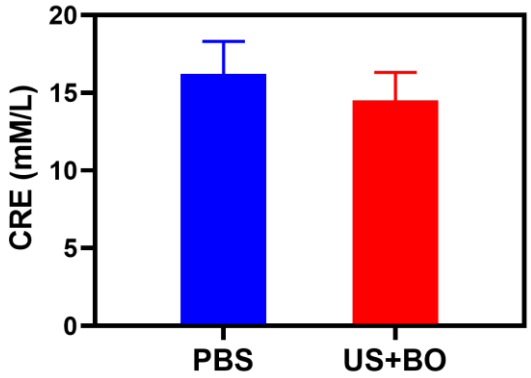

Figure S21. Blood biochemistry data including kidney function markers: CRE

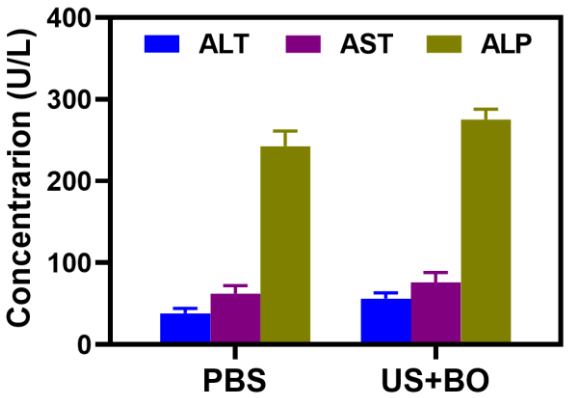

Figure S22. Blood biochemistry data including liver function markers: ALT, ALP, and AST.

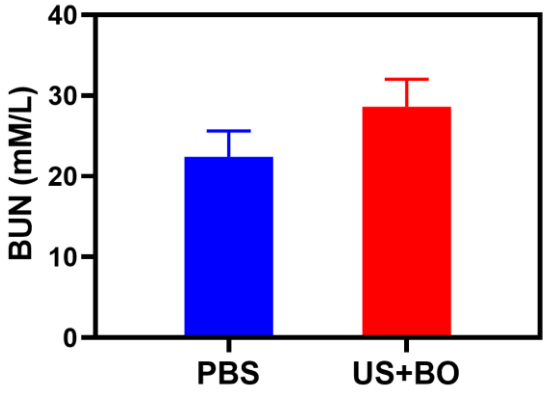

Figure S23. Blood biochemistry data including kidney function markers: BUN.

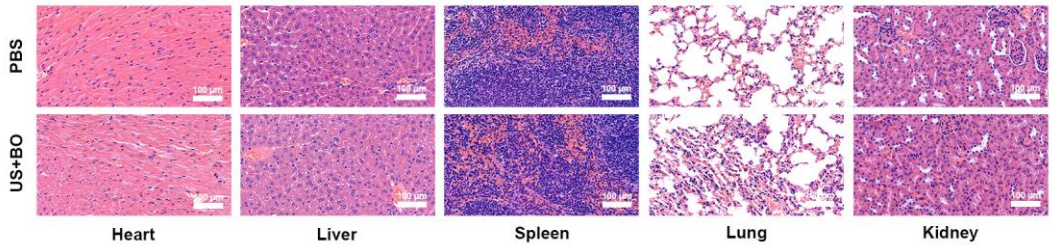

Figure S24. Histopathologic examination of the tissues including heart, liver, spleen, lung and kidney from BALB/c mice after different treatment. Scale bars = 100 μm.

References

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