Supporting Information Available

A porphysome-based photodynamic O_2 economizer for hypoxic tumor treatment by inhibiting mitochondria respiration

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Experimental Section

1. Preparation and Characterization.

The pheophorbide A (PPa)-lipid conjugate was synthesized according to the previous studies, which could self-assembly into PPa conjugated porphysome (designated as P-PPA) in aqueous phase. To fabricate the porphysome-based photodynamic O_2 economizer (designated as P-PAT), 0.111 mg of ATO dissolved in DMSO (0.222 mL) was dispersed into 2 mL of pure water, which was then mixed with 2 mg of the PPa-lipid conjugate. After untrasound for 5min, the mixed solution was subjected to the dialysis for 2 h to obtain P-PAT. The particle size and zeta potential of P-PAT was measured. The morphologies of P-PPA and P-PAT were observed by transmission scanning electron microscope (TEM). The characteristic absorption peaks of PPa, P-PPA and P-PAT were detected by UV-vis spectrum after dissolving into DMSO.

2. Reactive Oxygen Species (ROS) Detection.

ROS generated by photosensitizers was detected by fluorescence spectrum and confocal laser scanning microscopy (CLSM). First, using singlet oxygen sensor green (SOSG) as the indicator, the fluorescence spectra of ATO, P-PPA and P-PAT were recorded in the presence or absence of light irradiation. Briefly, ATO, P-PPA and P-PAT were respectively mixed with SOSG (5 μM) at the equivalent concentrations of PPa (1 mg/L) and ATO (2.5 mg/L). With or without light irradiation (80 $mW \cdot cm^{-2}$), the emission wavelength of the solution at 524 nm was recorded (excitation wavelength: 488 nm). Second, the intracellular ROS production was observed by

CLSM using 2′,7′-dichlorofluorescin diacetate (DCFH-DA) as the sensor. In brief, after being seeded and cultured for 24 h, 4T1 were incubated with ATO (2.5 mg/L), P-PPA (containing 1mg/L of PPa) or P-PAT (containing 1mg/L of PPa and 2.5 mg/L of ATO). For hypoxic groups, the cells were incubated under normoxia for 4 h and then incubated under hypoxia (1.1% O_2) for 2 h. For normoxic groups, the cells were incubated for 6 h under normoxia. Subsequently, all of the cells were washed by PBS and treated with DCFH-DA (10 μM) for 20 min. In the presence or absence of light irradiation (1.8 $J \cdot cm^{-2}$, 29.8 $mW \cdot cm^{-2}$, 10 min), the intracellular fluorescence was observed by CLSM. 4T1 cells treated with DCFH-DA only were used as the blank control.

Besides, the cellular ROS levels were also measured by using CellROX® Deep Red Reagent as the probe. Briefly, 4T1 and HeLa cells were respectively incubated with ATO (2.5 mg/L), P-PPA (containing 1mg/L of PPa) or P-PAT (containing 1mg/L of PPa and 2.5 mg/L of ATO). 6 h later, the culture medium of 4T1 and HeLa cells was replaced by the fresh medium containing CellROX® Deep Red Reagent (5 μM). For normoxic groups, 4T1 and HeLa cells were incubated under normoxia for 30 min. For hypoxic groups, 4T1 and HeLa cells were incubated under hypoxia for 30 min. In the presence or absence of light irradiation (1.8 $J \cdot cm^{-2}$, 29.8 $mW \cdot cm^{-2}$, 10 min), the intracellular fluorescence was observed by CLSM. 4T1 cells treated with CellROX® Deep Red Reagent only were used as the blank control.

3. Measurement of dissolved oxygen.

4T1 cells were incubated with ATO (2.5 mg/L), P-PPA (containing 1mg/L of PPa) or P-PAT (containing 1mg/L of PPa and 2.5 mg/L of ATO). 6 h later, the cells were washed by PBS and the fresh medium was added. After that, the electrode was immersed into the medium to measure the oxygen content. To prevent the oxygen circulation, the cell medium was sealed by paraffin.

4. Cellular Uptake and Subcellular Localization.

4T1 and HeLa cells were respectively incubated with P-PAT (containing 1mg/L of PPa and 2.5 mg/L of ATO) for 6, 12 and 24 h, respectively. Then, the cells were washed by PBS and observed by CLSM to evaluate the cellular uptake behavior. Besides, after treated with P-PAT for 6 h, 4T1 cells were further stained with Lyso Tracker Green, Mito Tracker Green and Hoechst 33342 for 30, 20 and 20 min respectively. Subsequently, the cells were washed by PBS and the intracellular fluorescence was observed by CLSM to evaluate the subcellular localization.

5. Rhodamine 123 Staining.

4T1 cells were incubated with P-PAT (containing 1mg/L of PPa and 2.5 mg/L of ATO) for 6 h. After washed by PBS, 4T1 cells were stained with rhodamine 123 (2 μM) for 20 min. Then the cells were washed by PBS again and the cellular fluorescence was observed by CLSM. 4T1 cells treated with equivalent concentrations of ATO and P-PPA were used as the control.

6. MTT Assay.

4T1 cells were seeded and cultured for 24 h in 96-well plates. After that, 4T1 cells were incubated with gradient concentrations of ATO, P-PPA or P-PAT. For hypoxic

groups, the cells were incubated under normoxia for 4 h and then incubated under hypoxia for 2 h. For normoxic groups, the cells were incubated for 6 h under normoxia. In the presence or absence of light irradiation (1.8 J·cm^{-2}, 29.8 mW·cm^{-2}, 10 min), the cells were cultured for another 18 h. Then 20 μL of MTT (5 g/L) was added into the cell medium of every well. 4 h later, the medium was replaced by 150 μL of DMSO and the absorbance of the DMSO solution in each well was measured to calculate the cell viability. To evaluate the effect of light, 4T1 cells were irradiated with light for different times. And the cell viability was detected using the similar method.

7. Cell Apoptosis Assay.

4T1 cells were seeded and cultured for 24 h in 6-well plates. After that, 4T1 cells were incubated with ATO (2.5 mg/L), P-PPA (containing 1mg/L of PPa) or P-PAT (containing 1mg/L of PPa and 2.5 mg/L of ATO). For hypoxic groups, the cells were incubated under normoxia for 4 h and then incubated under hypoxia for 2 h. For normoxic groups, the cells were incubated for 6 h under normoxia. Then the cells were irradiated for 1 min (1.8 J·cm^{-2}, 29.8 mW·cm^{-2}) or incubated in the dark. After that, 4T1 cells were washed by PBS and stained with Annexin V-FITC and PI. At last, the cells were performed for apoptosis analysis by flow cytometry.

8. Live/Dead Cell Staining Analysis.

4T1 and HeLa cells were respectively incubated with ATO (2.5 mg/L), P-PPA (containing 1mg/L of PPa) or P-PAT (containing 1mg/L of PPa and 2.5 mg/L of ATO). For hypoxic groups, the cells were incubated under normoxia for 5.5 h and

then incubated under hypoxia for 0.5 h. For normoxic groups, the cells were incubated for 6 h under normoxia. After washed by PBS, the cells were irradiated for 10 min (1.8 $J \cdot cm^{-2}$, 29.8 $mW \cdot cm^{-2}$) or incubated in the dark. After that, 4T1 and HeLa cells were stained with Annexin V-FITC/PI and the cellular fluorescence was observed by CLSM.

9. Oxygen Consumption Rate (OCR) Detection.

4T1 cells were seeded in Agligent Seahorse XF 24 cell culture microplates over night. Then 4T1 cells were incubated with ATO (2.5 mg/L), P-PPA (containing 1mg/L of PPa) or P-PAT (containing 1mg/L of PPa and 2.5 mg/L of ATO). 6 h, the cells were washed by XF cell Mito stress test assay medium for twice and incubated at 37℃ for 1 h in the absence of CO_2. Subsequently, oligomycin (1 μM)，FCCP (1 μM) and rotenone (0.5 μM) were added in sequence. The OCR of 4T1 cells was detected by using Seahorse XFe 24 Analyzer.

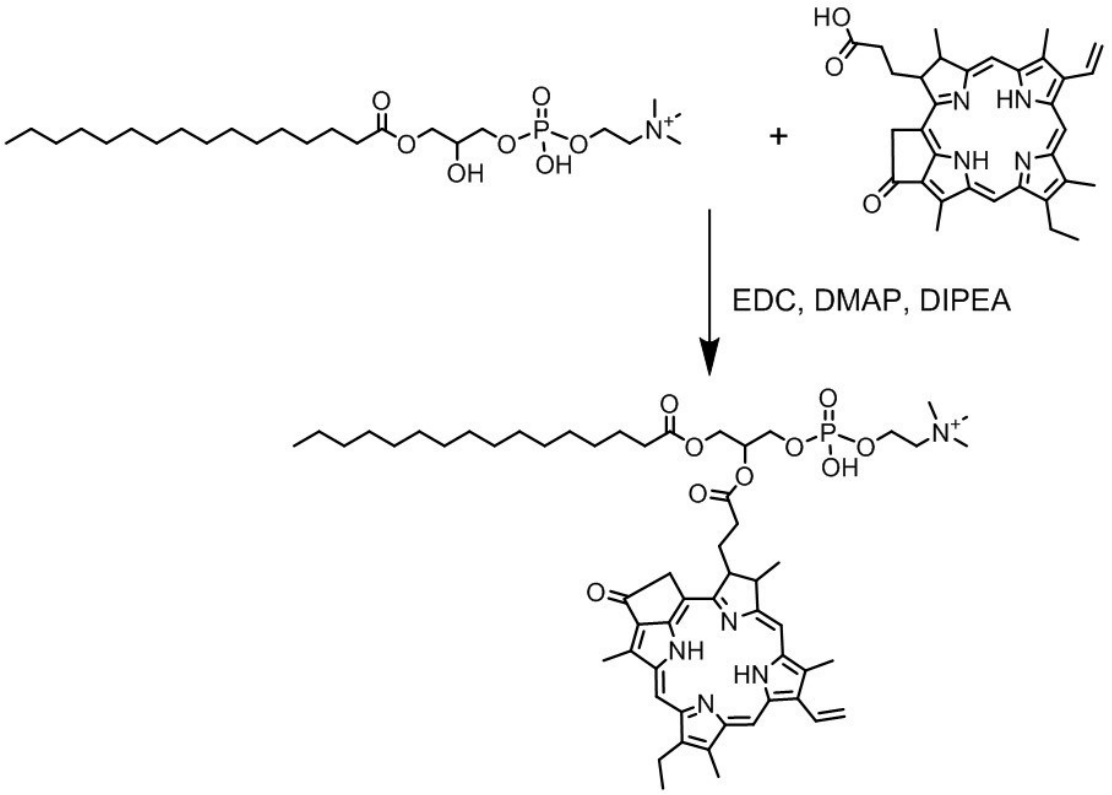

Fig. S1 Synthetic procedures of PPa-lipid conjugate.

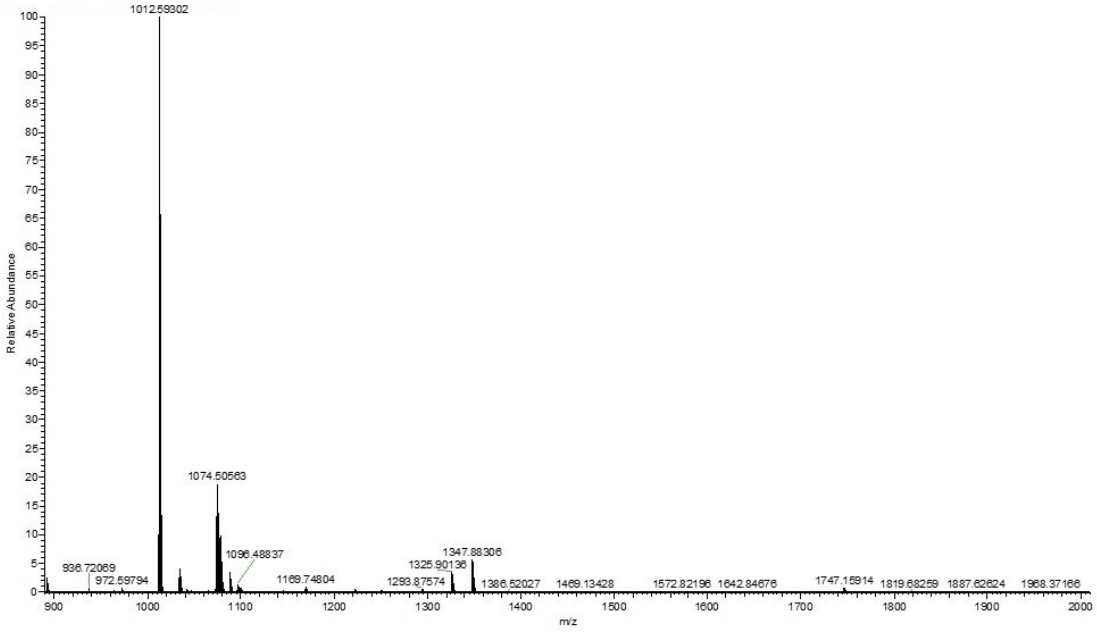

Fig. S2 ESI-MS of PPa-lipid conjugate.

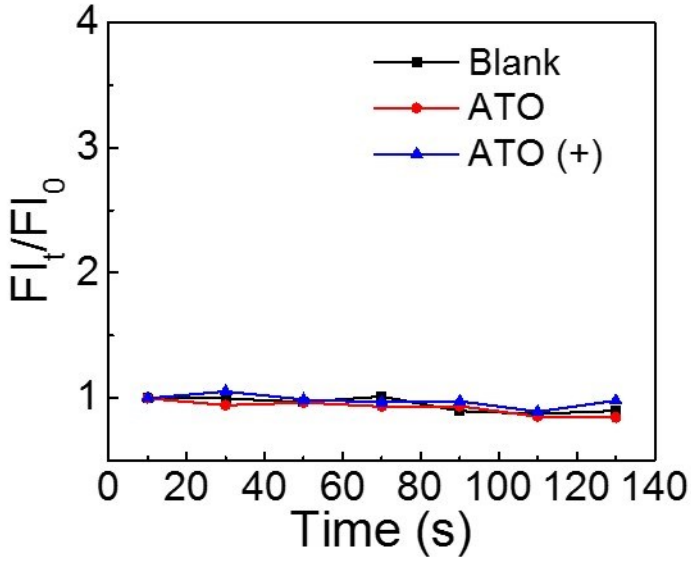

Fig. S3 The $^1O_2$ production ability of ATO in the presence or absence of light. SOSG was employed as the $^1O_2$ sensor.

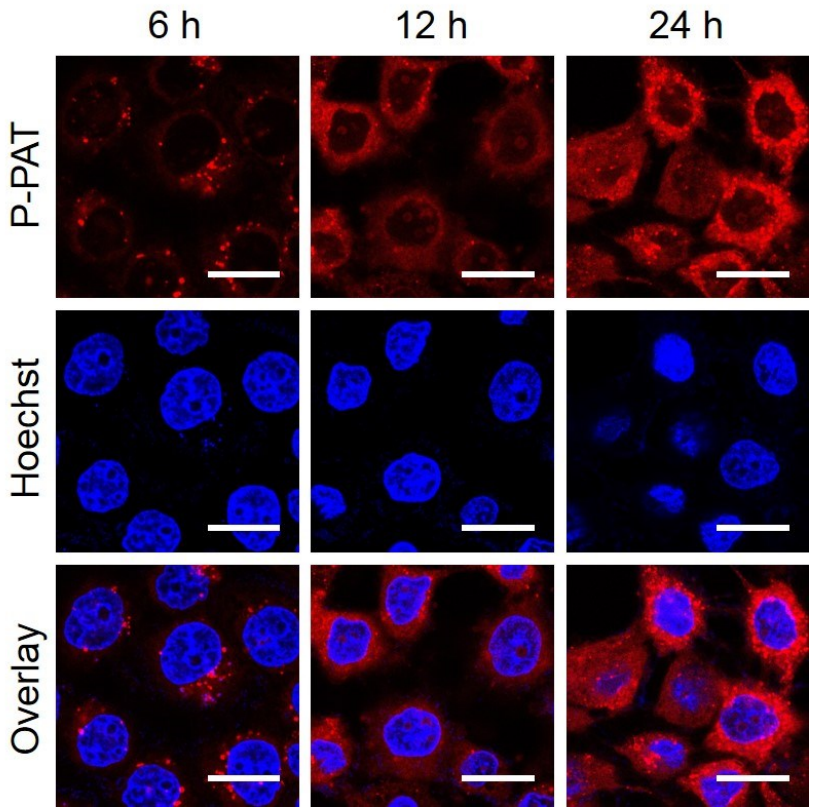

Fig. S4 CLSM images of HeLa cells after treatment with P-PAT for 6 h, 12 h and 24 h. Scale bar: 20 μm.

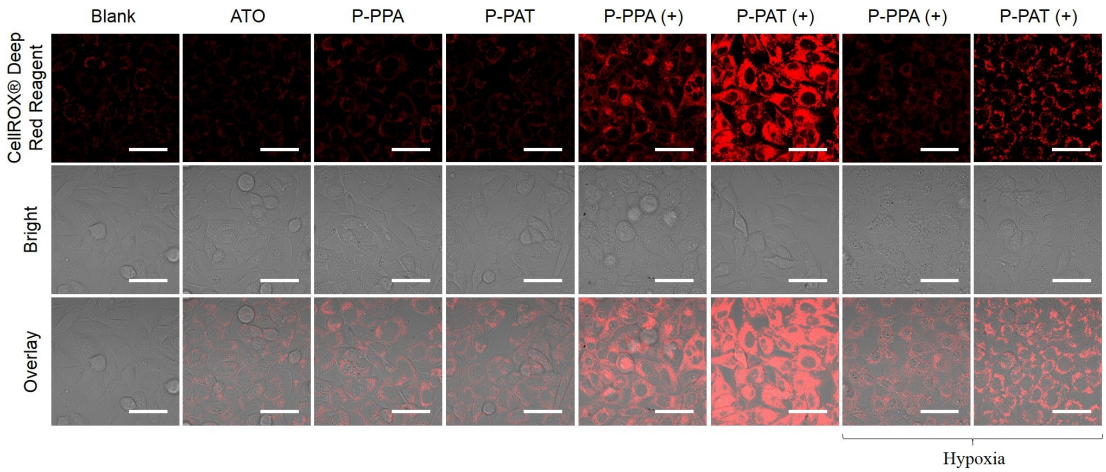

Fig. S5 CLSM images of 4T1 cells after treatment with ATO, P-PPA, P-PAT in the presence and absence of light under normoxic or hypoxic condition. CellROX® Deep Red Reagent was employed as the ROS sensor. Scale bar: 40 μm.

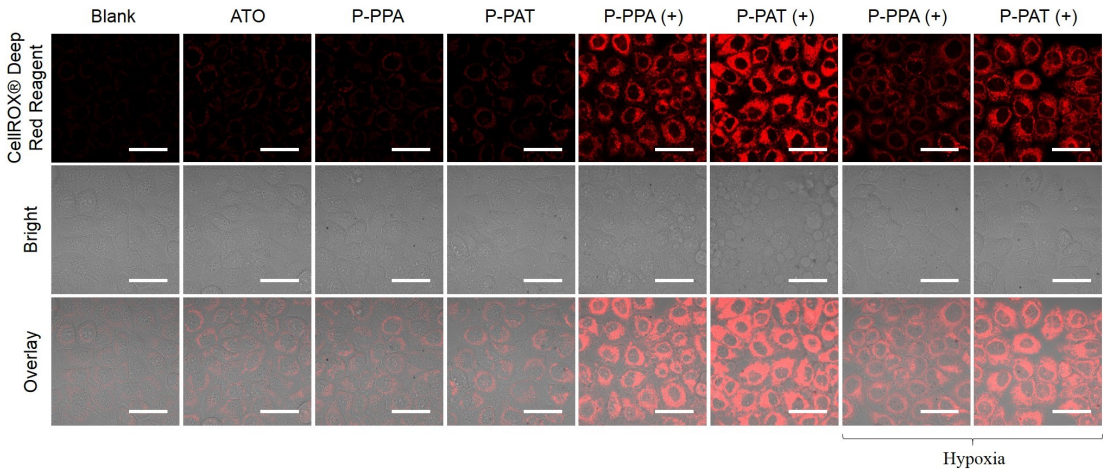

Fig. S6 CLSM images of HeLa cells after treatment with ATO, P-PPA, P-PAT in the presence and absence of light under normoxic or hypoxic condition. CellROX® Deep Red Reagent was employed as the ROS sensor. Scale bar: 40 μm.

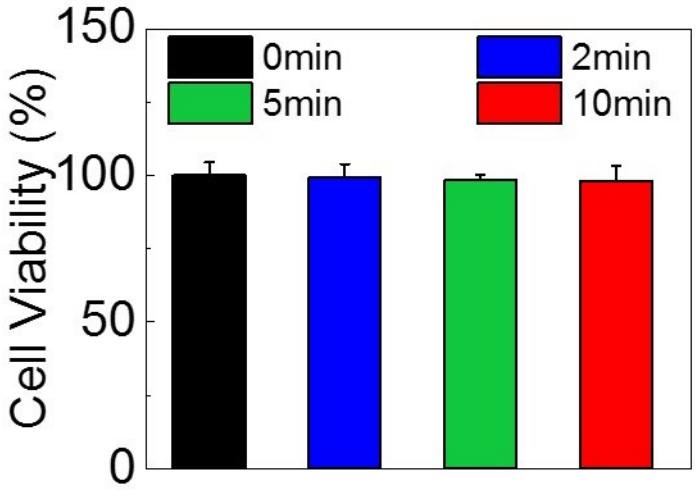

Fig. S7 Viability of 4T1 cells after treatment with light for different times.

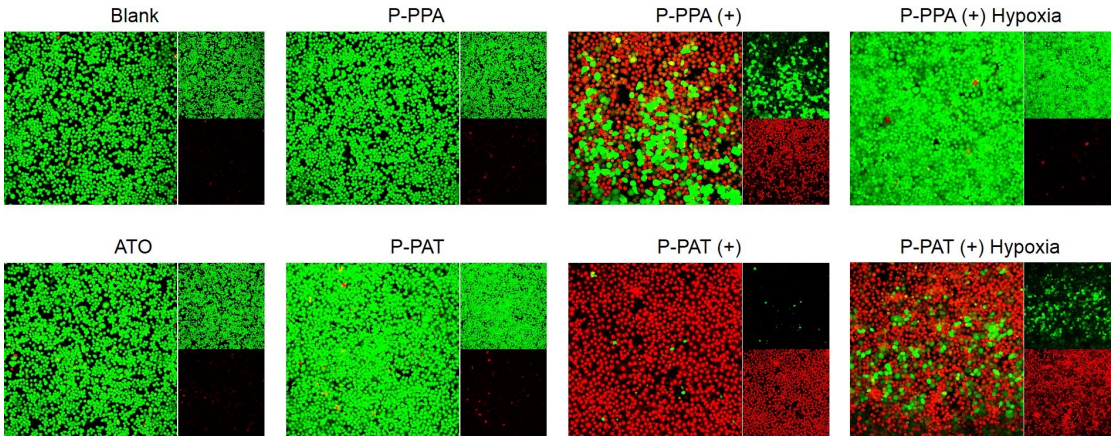

Fig. S8 Live/dead cell staining analysis of HeLa cells after treatment with P-PPA, P-PAT or ATO in the presence or absence of light under normoxic or hypoxic condition.