Electronic Supplementary Material (ESI) for Chemical Science.

Supplementary information

Biomimetically Constructing a Hypoxia activated Programmable Phototheranostics on the Molecular Level

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Materials and reagents. Chlorin e6 (Ce6, 95%), Hydrogen peroxide (H_2O_2, 30%), Dimethyl sulfoxide (DMSO, ≥99%), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-(amino-(polyethyleneglycol)-2000) (DSPE-PEG-2000), were purchased from Sigma-Aldrich. 1,3-Diphenylisobenzofuran (DPBF) was provided by Thermo Fisher Scientific Co., Ltd. Axitinib (99%) was purchased from Meilunbio Co., Ltd. RSL3 was obtained from MedChemExpress. Cell Counting Kit-8 (CCK8), BCA kit and 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA, 97%) were provided by Beyotime Biotechnology. Human cervical carcinoma cells (HeLa) cells was obtained from the American Type Culture Collection (ATCC, USA), HUVEC cells were obtained from the Peking University Health Science Center. DMEM, F12K and fetal bovine serum media were obtained from Hyclone (USA). Fertilized egg was provided by Suqian founder Trading Co., Ltd. Primary antibodies against Bax, Bcl-2, Caspase-3/cleaved Caspase-3, cleaved PARP, HIF-1α, VEGF, β-actin were purchased from Cell Signaling Technology.

Characterizations. Transmission electron microscopy (TEM) images were captured on a JEM-2100 transmission electron microscope (Hitachi Co. Ltd., Japan). Dynamic light scattering (DLS) was measured on a Zetasizer nano ZS instrument (DLS, Zetasizer nano zs90). Ultraviolet-visible (UV-vis) absorption spectra were collected using a UH4150 Spectrophotometer (Hitachi Co. Ltd., Japan). The fluorescence spectra were measured with a fluorimeter Hitachi F-4600 (Hitachi Co. Ltd., Japan). The cells were imaged through a confocal laser scanning microscope (Nikon C2, Japan). Microplate reader (Thermo Multiskan MK3, Helsinki, Finland).

Cellular Photocytotoxicity Irradiators (LED Model, PR-CPC2-660nm) and Small Animal Phototherapy Irradiators (Mix Model, PR-PDT-MIX) was provided by Shenzhen PURI Materials Technologies Co., LTD.

Preparation of Ce6-1@NP and PL-1@NP. Briefly, 8.4 mg **1** and 1 mg Ce6 (or PL) was dissolved in 1.5 mL DMSO, 94 mg DSPE-PEG (2000) were dissolved in 12 mL water. Then, DMSO mixed solution was dropped into water (DSPE-PEG) and stir for one hour at room temperature to prepare a homogeneous nanoplatform. Then dialysis in deionized water for 48 hours, change water every 6 hours. The unassembled **1** or Ce6 were extracted by centrifugation at 4000 rpm for 10 min. Finally, the solution was frozen dried into powder, and the absorbance at 323 nm and 655 nm was detected to determine the loaded **1** and Ce6 content in Ce6-**1**@NP.

Measurement of catalase-like activity (O_2 production). The catalase-like reactivity of **1** was evaluated using a reported literature protocol. [46] In brief, a clark-type polarographic oxygen electrode was used to monitor the conversion of hydrogen peroxide to oxygen. **1** was added to a solution containing H_2O_2 (100 mM) in PBS buffer (pH = 7.4), and the initial rates and total amount of oxygen produced were determined.

The generation of singlet oxygen (DPBF Assay). Under hypoxic environment, Ce6-**1**@NP, H_2O_2(10 mM) and DPBF was mixed in water (50% DMSO), then exposed to laser for 10 second, the change of UV-Vis absorption wavelength in 417 nm (DPBF) was detected every 10 second. (660 nm, 6.5 mW/cm^2).

CCK8 assay for cell toxicity. Cells were seeded in 96-well plates (10^4 cells/well), cultured at 37 °C in an atmosphere containing 5% CO_2 for 24 hours. Then cells were treated by nanoplatform (Ce6-**1**@NP etc.) with different concentration and incubated for 24 hours, the cells were then washed with PBS and sealed in an Anaero-Pouch-Bag for 6 hours to create an anoxic environment and then irradiated for 3 min with a 640 nm - 660 nm LED (30 $mW \cdot cm^{-2}$). The PBS was then replaced with fresh culture media (100 μL) and the cells were cultured for another 24 hours. CCK8 solution (10 μL in 90 μL DMEM) was added to each well after refreshing the medium, incubated for another 1 hour. Finally, the OD_{450} was measured using a microplate reader. The IC_{50} value is calculated according to the experimental results by Prism GraphPad.

Longitudinal relaxivities (r_1) test. In order to evaluate the longitudinal relaxivities (r_1) in solutions of **1** and Ce6-**1**@NP. **1** and Ce6-**1**@NP were dispersed into water respectively (0.125, 0.25, 0.5, 0.1, 0.2 mM). Then the samples were transferred into Sample Jet tubes for MRI scanning and calculated the value of r_1.

Anti-angiogenesis test in CAM model (Chick chorioallantois membrane model). The fertilized eggs were placed in a constant temperature incubator at 37.5 °C and humidity higher than 55%. The eggs were rotated three times every day. After 12 days, a window was opened at the round end of each egg to observe the survival of chick embryos and blood vessels. The survival eggs were randomly assigned to each group used for experiments. First, Photographed, and the length of blood vessels was measured, then 100 uL compounds was added to each window, after 1 hour, exposed to

laser 10 min (640 nm - 660 nm LED (30 $mW \cdot cm^{-2}$) and incubated another 24 hours. Finally, the growth of blood vessels was photographed, and the length of blood vessels was measured.

Western blotting. For obtaining protein, HeLa cells and HUVEC cells were inoculated in 10 cm Petri dishes and grew to over 80%, washed with pre-cooled PBS and centrifuged for 5 min at 2000 rpm after treated with Ce6-**1**@NP and laser. Then lysed in 100 μL of Lysis Buffer-about 30 min at 4 °C, the cells subsequently centrifuged for 10 min at 11000 rpm, protein concentrations were detected by BCA protein assay kit. Protein samples (40 μg/lane) extracted were separated by 10% or 12% SDS-PAGE and transferred onto 0.45 μm polyvinylidene difluoride (PVDF) membranes. After they were blocked with PBST containing 5% bovine serum albumin (BSA) for 2 hours at room temperature, incubated with primary antibody overnight at 4 °C, followed by three washes with PBST and incubated with diluted the enzyme horseradish peroxidase (HRP)-conjugated secondary antibodies (1:5000) for 1 hour at room temperature. After three times washed with PBST, the signals were detected by ECL reagent and quantified. β-actin was used as the loading control.

The antitumor effect *in vivo*. All animal procedures were approved by the Institutional Animal Care and Use Committee of Sino research (Beijing) Biotechnology Co., Ltd. (Protocol number: ZYZCLL20210101)., First, 5-6 weeks-old female mice were used as model candidates, 2×10^6 HeLa cells suspended in 0.1 mL DMEM were subcutaneously injected into each mouse. When tumor volume reached about 100 mm^3, they were randomized into several

groups (n = 4) and Ce6-**1**@NP were injected through caudal vein. After 24 hours, all mice exposed to laser 10 min (0.2 $W \cdot cm^{-2}$), tumor sizes were measured every 2 days, and the actual size of tumors calculated by the formula: length×$width^2/2$. After 14 days, the nude mice were sacrificed, tumor tissues and other organs were isolated for further study. Heart, liver, spleen, lung and kidney were collected to evaluate the toxicity of Ce6-**1**@NP.

Antitumor immune responses triggered study *in vivo*. Matured DCs in spleen T lymphocytes was studied on BALB/c mice. B16 tumor-bearing mice were divided into four groups, (1) Control group, (2) **1**@NP with laser group, (3) Ce6@NP with laser group, (4) Ce6-**1**@NP with laser group (**1**: 8.4 mg/kg, Ce6: 1 mg/kg). Spleen T lymphocytes was harvested from the mice post treatment for 3 days. Single-cell suspensions of the spleen T lymphocytes was prepared by gentle pressure with the homogenizer, and the cell suspensions were filtered with a cell strainer. The obtained cells were co-stained with FITC-CD4 antibody and PE-CD8 antibody (1 μL antibody/10^6 cells) at 37 °C under dark environment for 30 min and centrifuged under 3000 rpm for 10 min to remove redundant antibody. The obtained cells were dispersed in PBS and analyzed on a FACS Verse flow cytometer (Becton Dickinson, USA). The FlowJo software was used for data analysis.

***In vivo* MRI evaluation.** The HeLa tumor-bearing model was chosen for the *in vivo* MRI test. When the tumor diameter reached about 100 - 300 mm^3, two mice with similar tumor size were chosen for imaging. *In vivo* T_1-weighed MR imaging was carried out before and after intravenous injection of PBS and Ce6-**1**@NP (200 μL, 1

mg/kg based on Ce6 content), separately. Then MRI scanning were performed at different time periods. The intensity signal value of tumor site was calculated by image J software.

Statistical analysis. Statistical significance was determined by *t* tests (two-tailed unpaired) using Prism 7 (GraphPad) and origin pro 8 software. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$ and ****$P \leq 0.0001$.

Table S1. Crystal data and structure refinements.

Complex	**1** (CCDC: 2159529)
Molecular formula	$C_{86}\ H_{110}\ Cl_3\ Mn_3\ N_6\ O_{18}$
Formula wt. (g mol^{-1})	1786.96
Temperature (K)	180
Radiation (λ, Å)	0.71073
Crystal system	trigonal
Space group	*R* -3 *c*
a (Å)	22.2032(3)
b (Å)	22.2032(3)
c (Å)	30.9870(6)
α (º)	90
β (º)	90
γ (º)	120
Volume ($Å^3$)	13229.4(4)
Z	6
ρ_{calcd} (g cm^{-3})	1.346
μ (mm^{-1})	0.583
F (000)	5628
Crystal size (mm^3)	0.18×0.12×0.07
Theta range	2.493 to 29.414
Reflections collected	16002
Independent reflections	3776 [R(int) = 0.0170]
Completeness	0.927
Goodness-of-fit on F^2	1.010
Final R indices	$R1^a$ = 0.0412
[R > 2σ (I)]	wR_2^b = 0.1309
R indices (all data)	$R1^a$ = 0.0486 wR_2^b = 0.1220
Largest diff. peak and hole (e $Å^{-3}$)	0.733 and -0.372

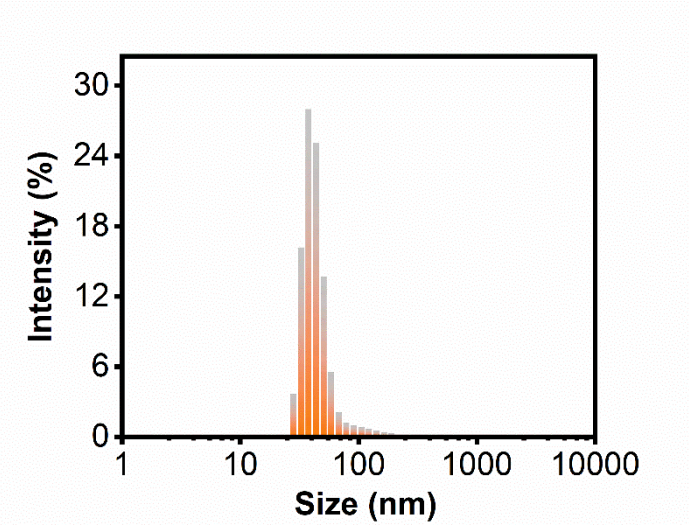

Fig. S1 Size distribution of Ce6-**1**@NP obtained by DLS.

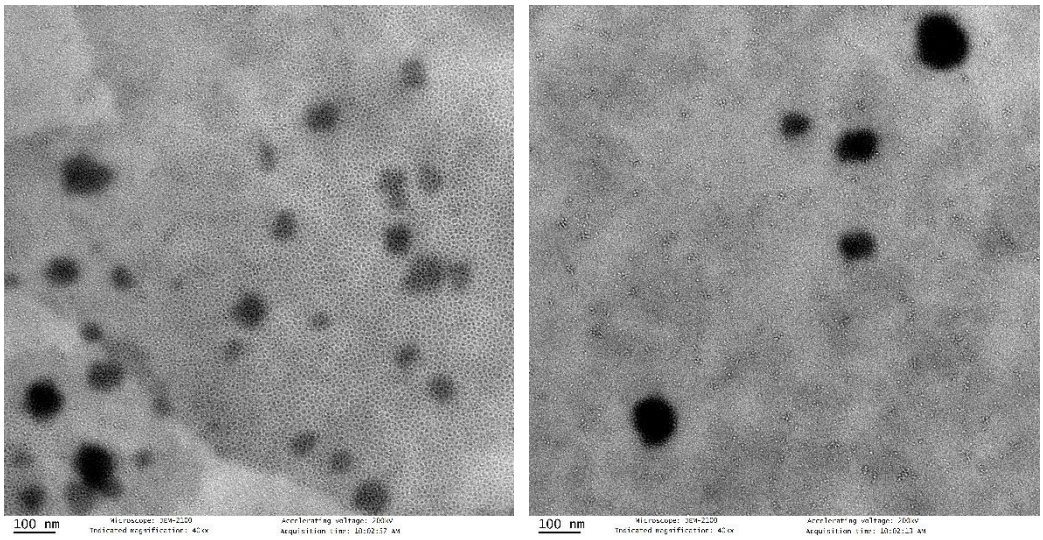

Fig. S2 TEM image of Ce6-**1**@NP.

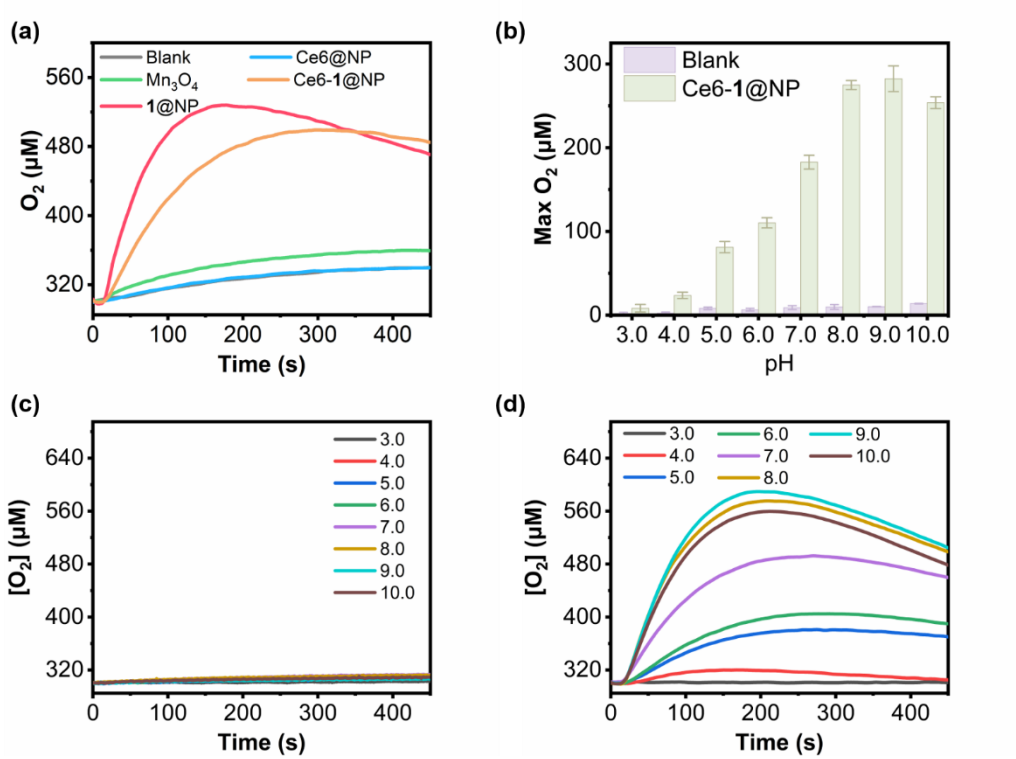

Fig. S3 (a) The Oxygen production over time via catalytic H_2O_2 (100 mM) dismutation mediated by Mn_3O_4, Ce6@NP (5 μM), **1**@NP (5 μM), Ce6-**1**@NP (5 μM) at PBS buffer (pH 7.4). **(b)** Oxygen production via catalytic dismutation of H_2O_2 (100 mM) by Ce6-1@NP (5 μM) in different pH water solution. **(c)** The Oxygen production curve over time via catalytic H_2O_2 (100 mM) dismutation mediated by blank group at different pH water solution. **(d)** The Oxygen production curve over time via catalytic H_2O_2 (100 mM) dismutation mediated by Ce6-**1**@NP (5 μM) at different pH water solution.

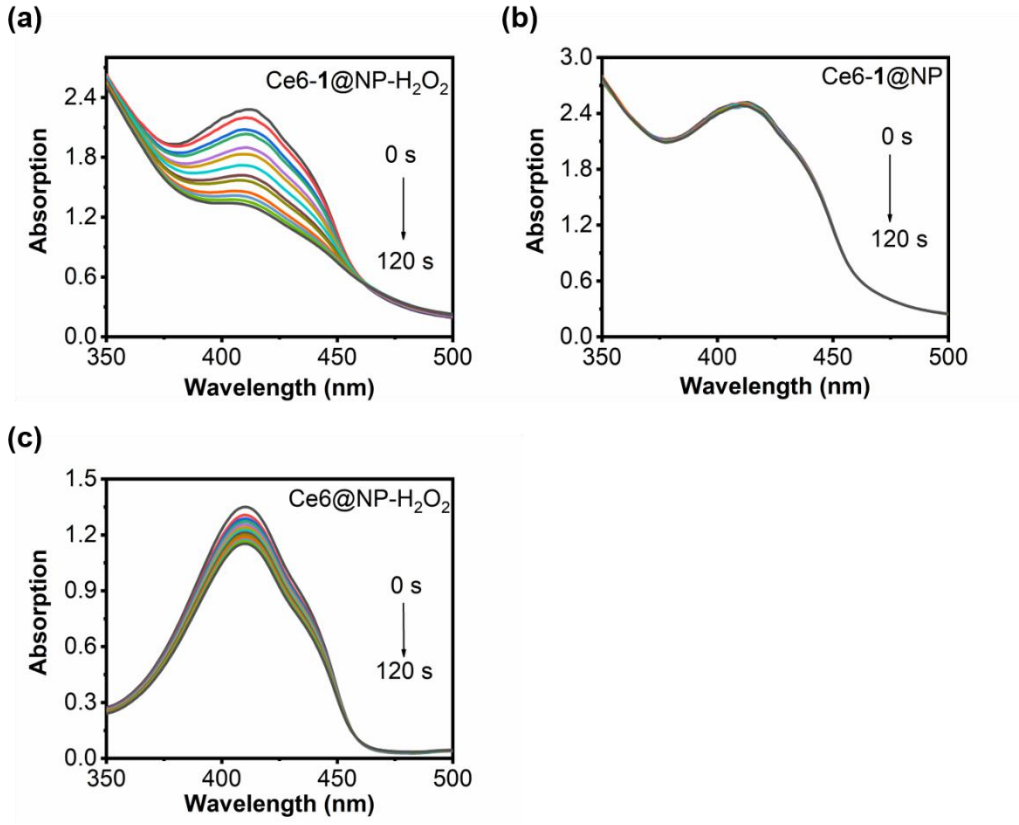

Fig. S4 UV–vis absorption spectra of DPBF. **(a)** Ce6-**1**@NP (0.1 μM) and DPBF (60 μM) in presence of H_2O_2 (10 mM) exposed to laser (660 nm, 6.5 mW/cm^2, 10 seconds) every 10 seconds under N_2 atmosphere. **(b)** Ce6-**1**@NP (0.1 μM) and DPBF (60 μM) in absence of H_2O_2 exposed to laser (660 nm, 6.5 mW/cm^2, 10 seconds) every 10 seconds under N_2 atmosphere. **(c)** Ce6@NP (0.1 μM) and DPBF (60 μM) in presence of H_2O_2 (10 mM) exposed to laser (660 nm, 6.5 mW/cm^2, 10 seconds) every 10 seconds under N_2 atmosphere.

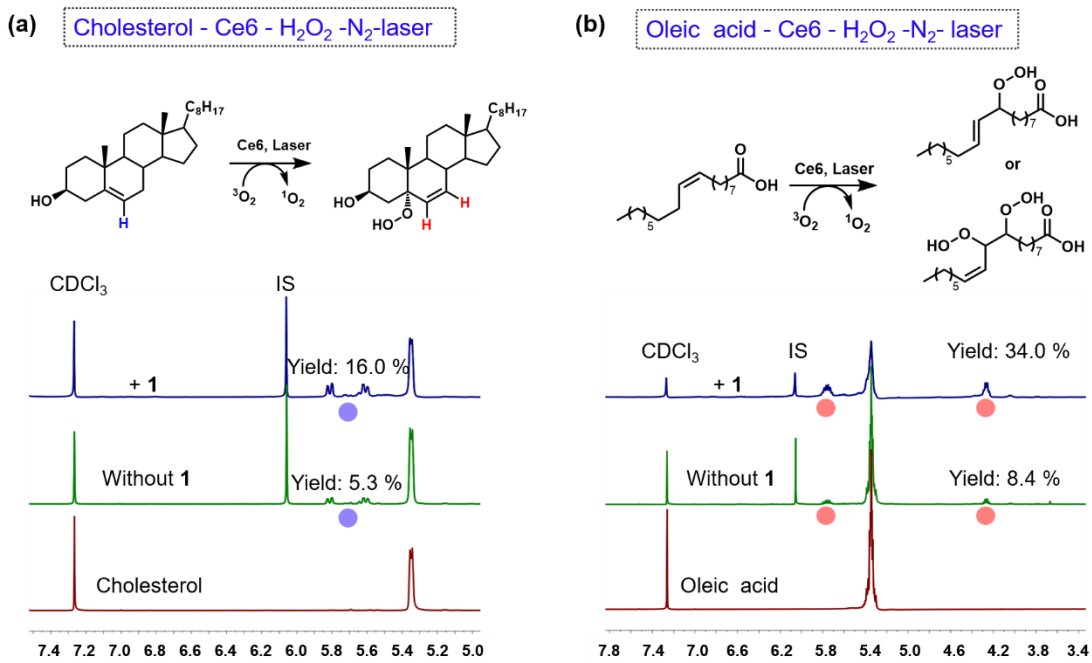

Fig. S5 ^{1}H NMR spectroscopy of **(a)** Cholesterol (20 mg) or **(b)** Oleic acid (20 mg) in $CDCl_3$. Catalyst (Ce6, 0.25 mg),**1** (1 mg), H_2O_2 (30%, 40 μL), MeCN/$CHCl_3$/H_2O (1 mL/1 mL/200 μL), N_2 bubbling, 50 mW/cm^2 660 nm Led. Conversions were determined by using tetrabromoethane as an internal standard. [1-2]

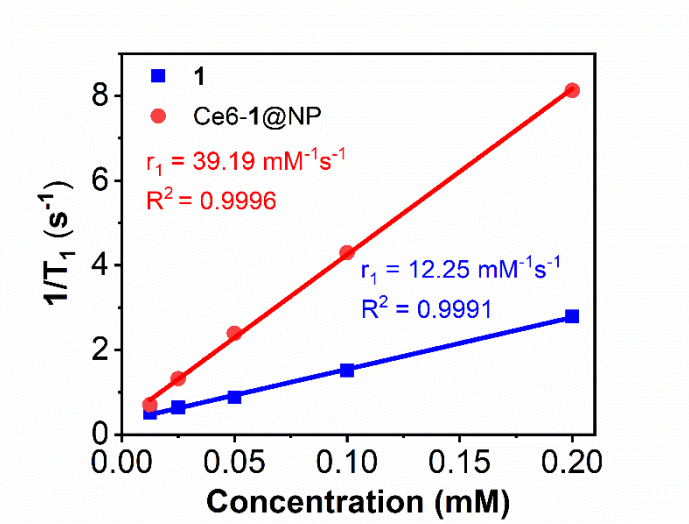

Fig. S6 The curve of longitudinal relaxation times (T_1) of **1** and Ce6-**1**@NP.

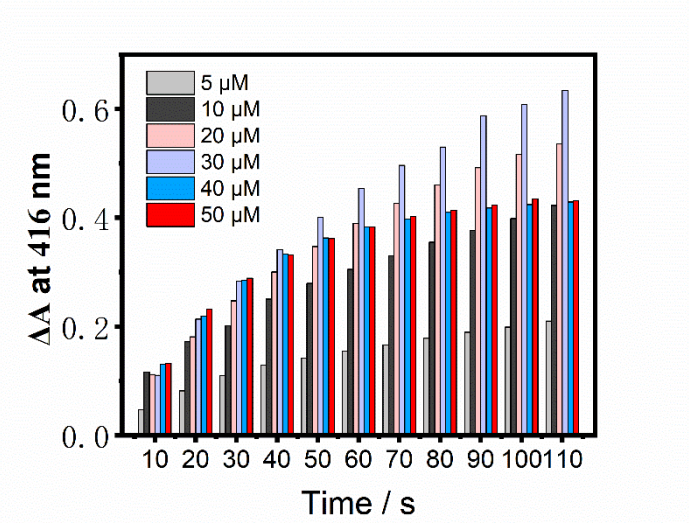

Fig. S7 The ability of Ce6 promote singlet oxygen generation with different ratio **1** under hypoxic condition detected with DPBF assay. (Concentration of Ce6: 5 μM; concentration of **1**: 5,10,20,30,40,50 μM).

Table S2. SID results and calculation formula

SID (μM)		**50%**			**Random**	
S_a	9.60	13.80	18.20	1.60	8.00	32.00
C_a	0.60	1.00	0.03	0.30	0.40	0.80
S_b	0.20	0.26	0.10	1.04	0.80	1.60
C_b	0.16	0.10	0.02	0.26	0.20	0.40
SID	0.87	0.47	0.83	0.93	0.60	0.55
Mean		0.72			0.69	
Formula			$\mathrm{SID} = \frac{C_a}{S_a} + \frac{C_b}{S_b}$			

Abbreviation: C_a and C_b represent the concentrations of **1** and Ce6 in combination therapy, and S_a and S_b refer the concentrations of **1** and Ce6 in single drug therapy. SID_{50} and SID_{arb} are the synergy indexes when the inhibition of cells reached at 50% and the arbitrary level, respectively.

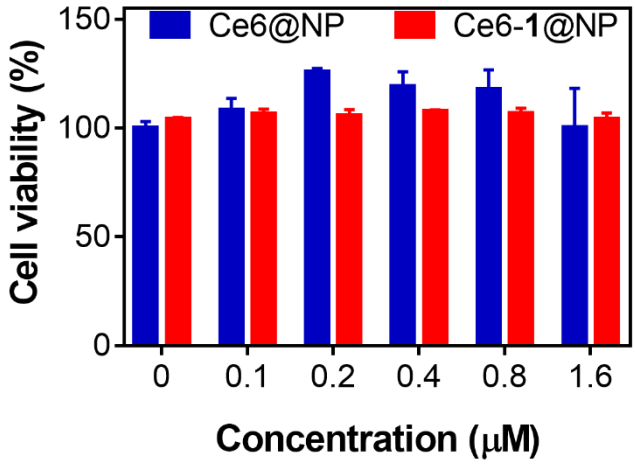

Fig. S8 Cytotoxicity of Ce6@NP, Ce6-**1**@NP in HeLa cells under no irradiation condition.

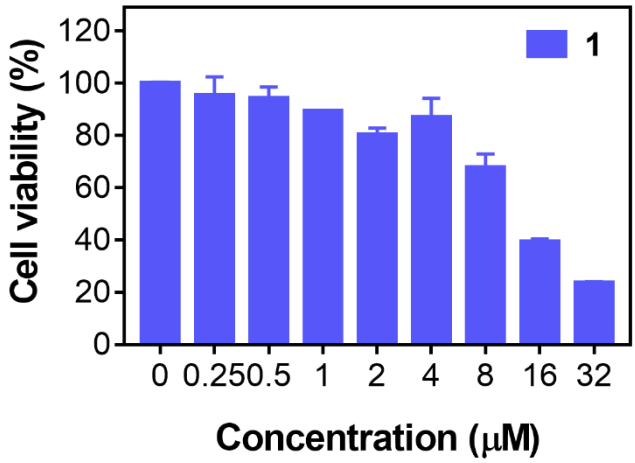

Fig. S9 Cytotoxicity of **1** under irradiation condition (660 nm, 30 mW/cm^2, 1 min).

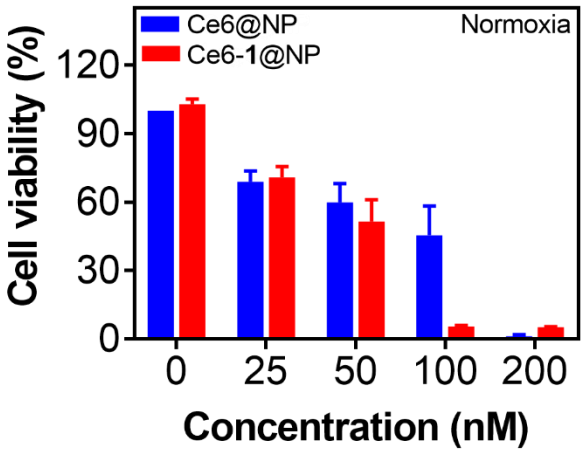

Fig. S10 Cytotoxicity of Ce6@NP, Ce6-**1**@NP in HeLa cells under irradiation and normoxia condition (660 nm, 30 mW/cm^2, 1 min).

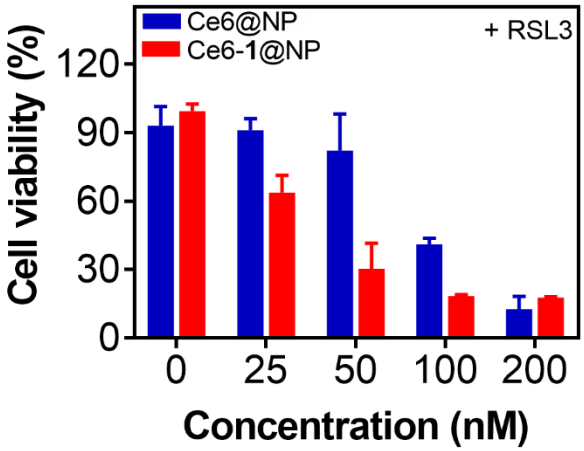

Fig. S11 Comparison of cytotoxicity of Ce6@NP and Ce6-**1**@NP added RSL3 (1.6 μM) under hypoxia condition (660 nm, 30 mW/cm^2, 1 min).

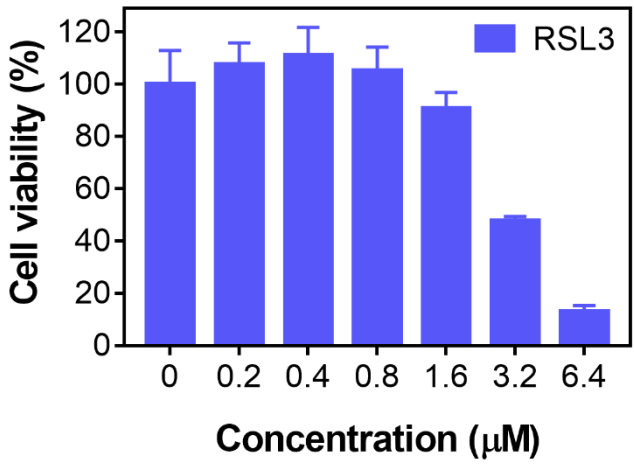

Fig. S12 Cytotoxicity of RSL3 (660 nm, 30 mW/cm^2, 1 min).

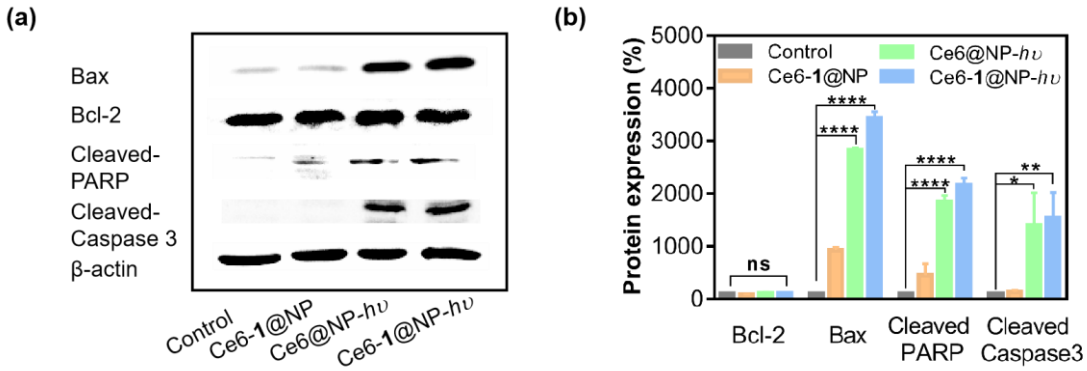

Fig. S13 (a) The expression levels of key protein in apoptosis signaling pathway by western blotting analysis. **(b)** Histogram of Bax, Bcl-2, cleaved-PARP and cleaved-Caspase 3 expression levels.

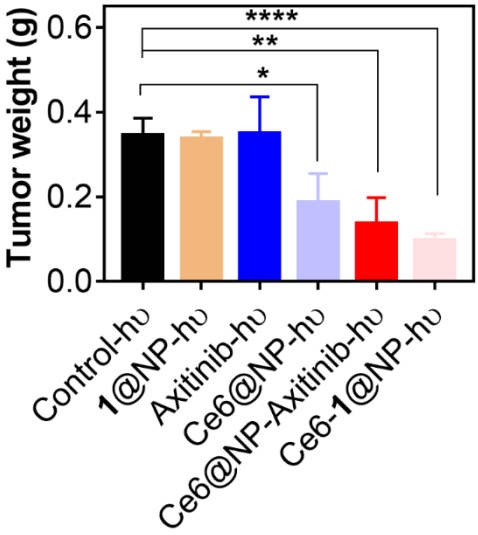

Fig. S14 Weights of tumors (Control-hv, **1**@NP-hv, Axitinib-hv, Ce6@NP-hv, Ce6-**1**@NP-hv, Ce6@NP-Axitinib-hv) after all mice harvested.

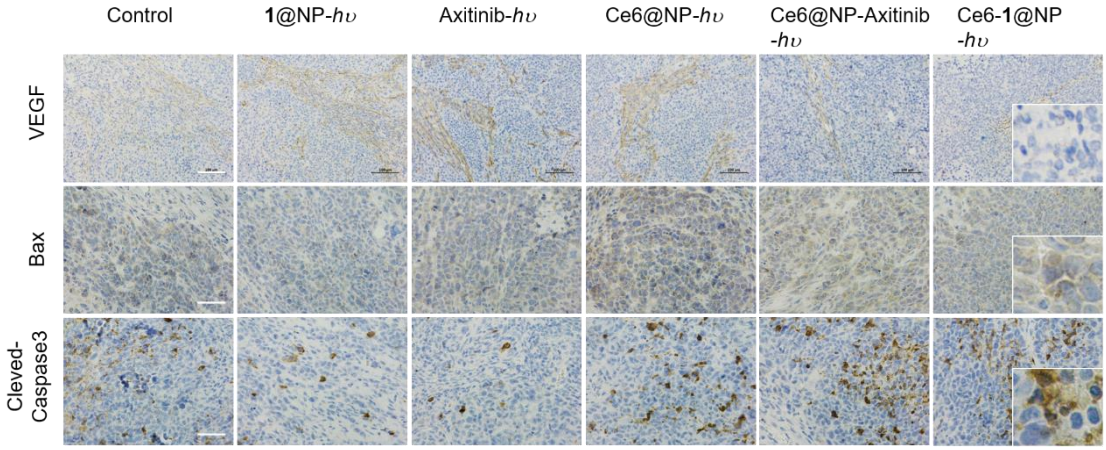

Fig. S15 Immunostaining analysis of key proteins for different groups in tumor cells (Scale bar = 100 μm).

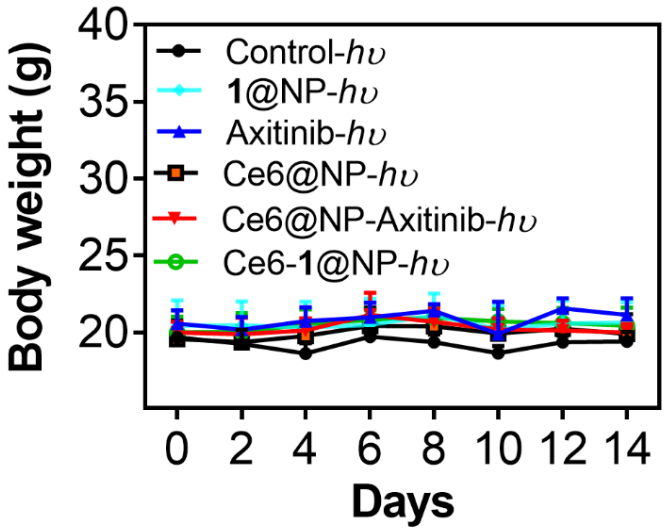

Fig. S16 The body weight of HeLa tumor-bearing mice over 14 days after receiving the nanoplatforms (Control-hv, **1**@NP-hv, Axitinib-hv, Ce6@NP-hv, Ce6-**1**@NP-hv, Ce6@NP-Axitinib-hv) treatment.

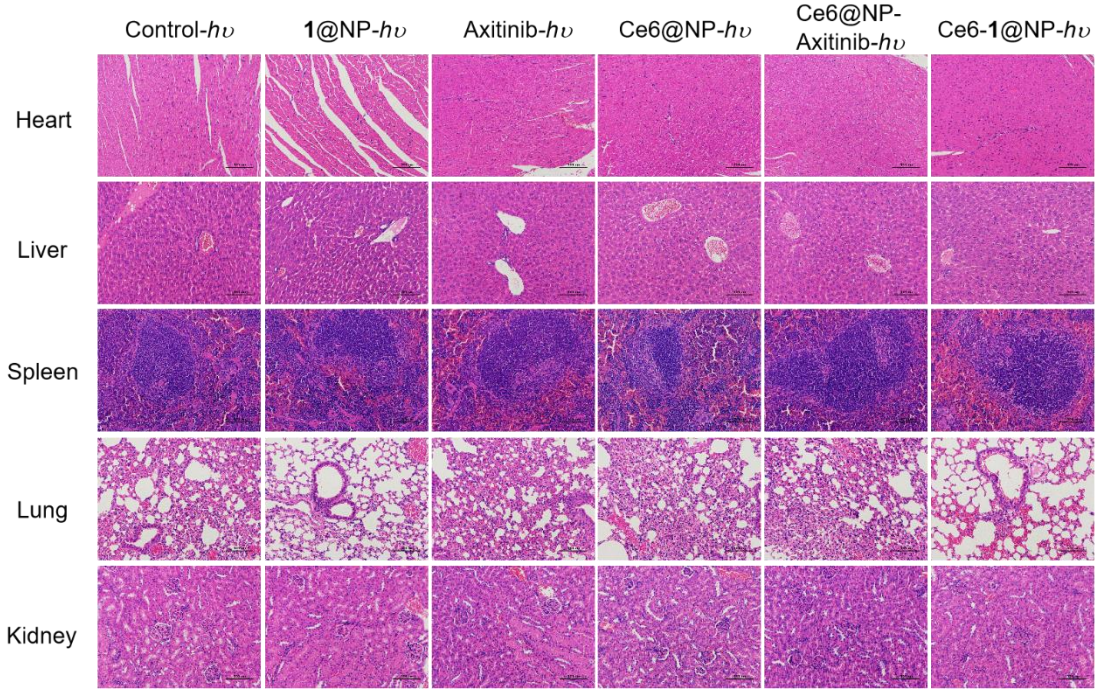

Fig. S17 Hematoxylin and Eosin (H&E) stained images of major organs taken from nanoplatforms (Control-hv, **1**@NP, Axitinib-hv, Ce6@NP-hv, Ce6-**1**@NP-hv, Ce6@NP-Axitinib-hv) injected mice after 14 days. Scale bar: 100 μm.

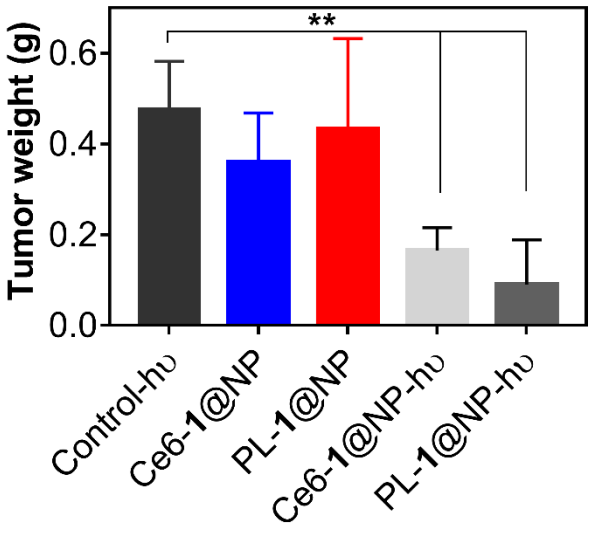

Fig. S18 Weights of tumors (Control-hv, Ce6-**1**@NP, PL-**1**@NP, Ce6-**1**@NP-hv and PL-**1**@NP-hv) after all mice harvested.

Reference

(1) X. S. Ke, Y. Y. Ning, J. Tang, J. Y. Hu, H. Y. Yin, G. X. Wang, Z. S. Yang, J. L. Jie, K. H. Liu, Z. S. Meng, Z. Y. Zhang, H. M. Su, C. Y. Shu, J. L. Zhang, *Chem. Eur. J.,* **2016**, *22*, 9676-9686.

(2) F. Julien, J. Christophe, R. Romain, J. C. Henry, *Org. Lett.,* **2005**, *7*, 23.