## Supplementary Information

## For

## Light-triggered photosynthetic engineered bacteria for enhanced-photodynamic therapy by relieving tumor hypoxic microenvironment

Chenyang Yin<sup>1,#</sup>, ZeKun Wang<sup>1,#</sup>, Chunxue Dai<sup>1</sup>, Bangjia Yang<sup>1</sup>, Weiyun Wang<sup>1</sup>, Endong Yang<sup>1</sup>, Feng Guo<sup>1</sup>, Cundong Fan<sup>2,\*</sup>, Pu Zhang<sup>3,\*</sup>, Jikui Sun<sup>4,\*</sup>, Dongdong Sun<sup>1,\*</sup>

<sup>[1]</sup> School of Life Sciences, Anhui Agricultural University, Hefei 230036, China <sup>[2]</sup> Shandong Key Laboratory of TCM Multi-Target Intervention and Disease Control, the Second Affiliated Hospital of Shandong First Medical University, Taian, Shandong 271000, China

<sup>[3]</sup> Department of Cardiology, Affiliated Taian City Central Hospital of Qingdao University, Taian, Shandong, 271000, China

<sup>[4]</sup> Department of Neurosurgery, the First Affiliated Hospital of Shandong First Medical University & Shandong Provincial Qianfoshan Hospital, Jinan, China

<sup>#</sup> These authors contributed equally to this work.

## \*Corresponding authors:

Dongdong Sun, Email: <u>sunddwj@126.com; Cundong Fan, Email:</u> tcdfan66@163.com; **Jikui Sun**, Email: jikuisun2015@163.com; **Pu Zhang**, <u>Email: zp8198423@163.com</u>



Figure S1. The TEM mapping of Au-Ce6. Scale bar: 25 nm.



Figure S2. FTIR spectra of Ce6 and Au-Ce6.



Figure S3. The standard curve for Ce6.



Figure S4. (A) Time-dependent survival of Bac@Au-Ce6 over 7 days. (B) Timedependent survival of Bac@Au-Ce6 under growth conditions at 27°C and 37°C. (C-H) Cell viability of SKOV3, HEK293, HepG2, fibroblasts, Jurkat T, and J774 cells treated with Bac@Au-Ce6 (Syne = different concentrations, 3  $\mu$ g ~ 30  $\mu$ g of Ce6 concentration). (I) Quantification of percent hemolysis. Data are mean ± SD, n = 3.



Figure S5. The growth curve of Syne in the light or dark (Mean  $\pm$  SD, n = 3).



Figure S6. Oxygen production curves of Bac@Au-Ce6 under different conditions. LED irradiation with a wavelength range of 400-750 nm (Mean ± SD, n = 3).



Figure S7. Oxygen production curves of various concentrations of Bac@Au-Ce6 treated under hypoxia with 660 nm laser irradiation (Mean  $\pm$  SD, n = 3).



Figure S8. The release percentage of Ce6 versus time in Bac@Au-Ce6 (Laser: 660 nm, 20 mW/cm<sup>2</sup>, 5 min, and 808 nm, 2 W/cm<sup>2</sup>, 5 min).



Figure S9. Cytotoxicity of Bac@Au-Ce6 on L929 and 4T1 cells (Mean ± SD, n = 3).



Figure S10. (A) Calcein AM/PI co-staining 4T1 cells in different treatments. Scale bars = 50  $\mu$ m. (B) Confocal microscopic images of 4T1 tumor cells stained by O<sub>2</sub> indicator Ru(dpp)<sub>3</sub>Cl<sub>2</sub> probe when treated with PBS, *Syne*, Au-Ce6, Bac@Au-Ce6. Scale bars = 50  $\mu$ m. (C) Confocal microscopic images of 4T1 tumor cells stained by SOSG probe when treated with PBS, *Syne*, Au-Ce6, Bac@Au-Ce6. Scale bars = 10  $\mu$ m.



Figure S11. (A) In vivo fluorescence images of 4T1 tumor-bearing mice and (B) Fluorescence images of the major organs and tumors after the injection for 8 h.



Figure S12. Bio-distribution of Au-Ce6, Bac@Au-Ce6, and Bac@Au-Ce6 in major organs and tumors within 24 h (Mean  $\pm$  SD, n = 5).



Figure S13. (A) Average tumor growth curves of all the groups (Mean  $\pm$  SD, n = 5). (B) The tumor weights at 14 d in different groups (Mean  $\pm$  SD, n = 5). (C) Representative photographs of mice and tumors from different groups after 14-day therapy. (D) The body weight of mice corresponds to different time points (Mean  $\pm$  SD, n = 5). (E) Histological analysis of tumor tissues, stained with HIF-1 $\alpha$  for hypoxia, PCNA for cell proliferation, SOSG for <sup>1</sup>O<sub>2</sub>, H&E for inflammation, TUNEL and Caspase-3 for apoptosis and (F) corresponding index quantitative analysis after 14-day therapy (Mean  $\pm$  SD, n = 5). Scale bar: 100 µm. (1): PBS as control, (2): Syne, (3): Au-Ce6, (4): Bac@Au-Ce6.



Figure S14. The body weight of mice corresponds to different time points (Mean  $\pm$  SD, n = 5).



Figure S15. The percentage of "Body weight loss" [(starting weight - body weight at day 2, 4, 6, 8, 10, 12, 14) / starting weight] × 100% (Mean  $\pm$  SD, n = 5).



Figure S16. The survival rate of mice corresponds to different time points.