Supporting Information

Dual-enzyme decorated semiconducting polymer nanoagents for second near-infrared photoactivatable ferroptosis-immunotherapy

Yue Liu,‡[a] Renjie Lu,‡[b] Meng Li,[a] Danling Cheng,[a] Fengshuo Wang,[a] Xiumei Ouyang,[c] Yitian Zhang,[c] Qin Zhang,*[d] Jingchao Li*[a] and Shaojun Peng*[c]

[a] *State Key Laboratory for Modification of Chemical Fibers and Polymer Materials, Shanghai Engineering Research Center of Nano-Biomaterials and Regenerative Medicine, College of Biological Science and Medical Engineering, Donghua University, Shanghai 201620, China. E-mail: jcli@dhu.edu.cn*

[b] *Department of Orthopedics, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai 200233, China.*

[c] *Zhuhai Institute of Translational Medicine, Zhuhai Precision Medical Center, Zhuhai People's Hospital (Zhuhai hospital affiliated with Jinan University), Zhuhai, Guangdong 519000, China. Email: henry2008_ok@126.com*

[d] *Institute of Translational Medicine, Shanghai University, Shanghai 200444, China. Email: Sabrina_1985@shu.edu.cn*

‡ *These authors contributed equally to this work.*

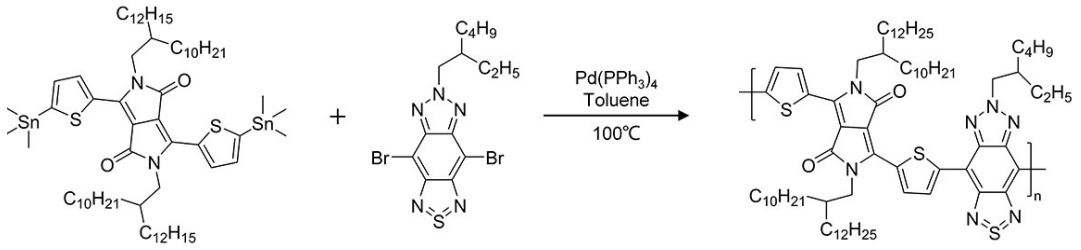

Fig. S1. Schematic of the synthetic routes of SP.

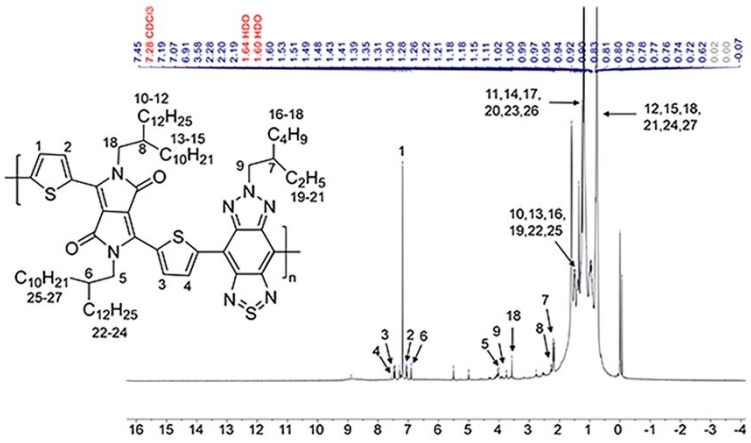

Fig. S2. ^{1}H NMR spectrum of SP in $CDCl_3$-D.

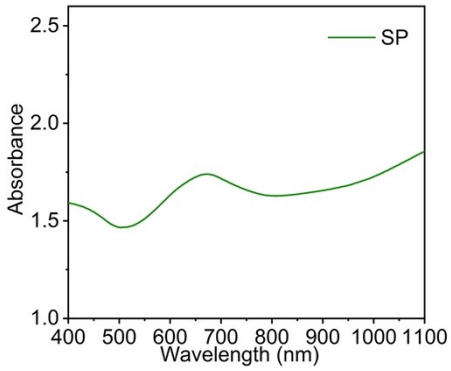

Fig. S3. Absorbance spectrum of SP (100 μg/mL).

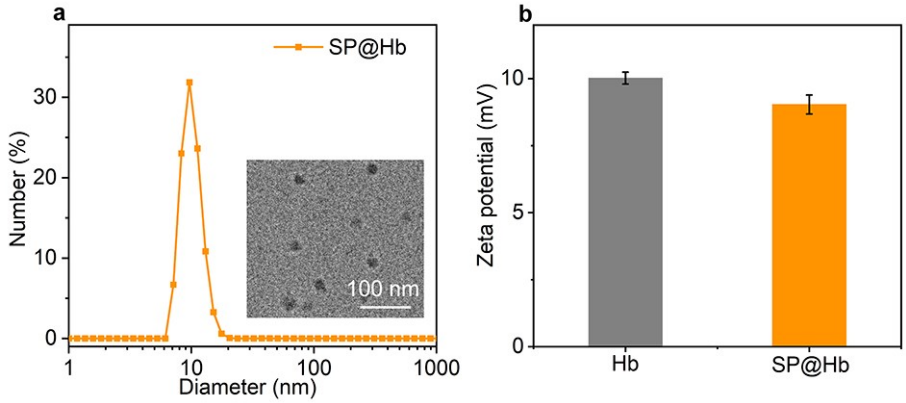

Fig. S4. (a) TEM image and hydrodynamic size of SP@Hb (5 μg/mL). (b) Zeta potentials of Hb and SP@Hb (5 μg/mL) (n = 5).

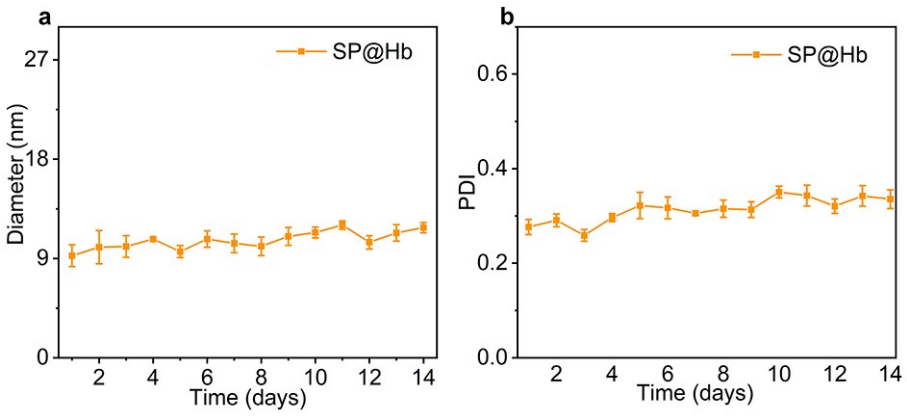

Fig. S5. (a) Hydrodynamic sizes of SP@Hb on different days in PBS (5 μg/mL) (n = 5). (b) PDI of SP@Hb on different days in PBS solution (5 μg/mL) (n = 5).

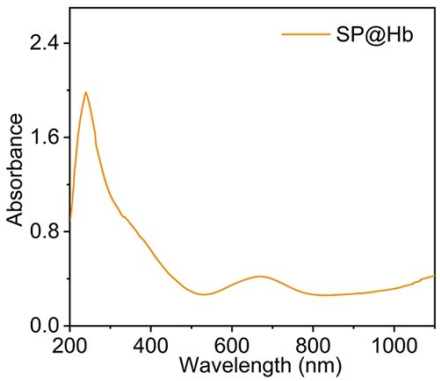

Fig. S6. Absorbance spectrum of SP@Hb (20 μg/mL).

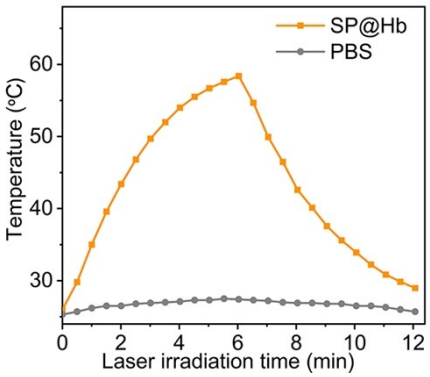

Fig. S7. Temperature curves of SP@Hb (100 μg/mL) and PBS solution under 1064 nm laser irradiation.

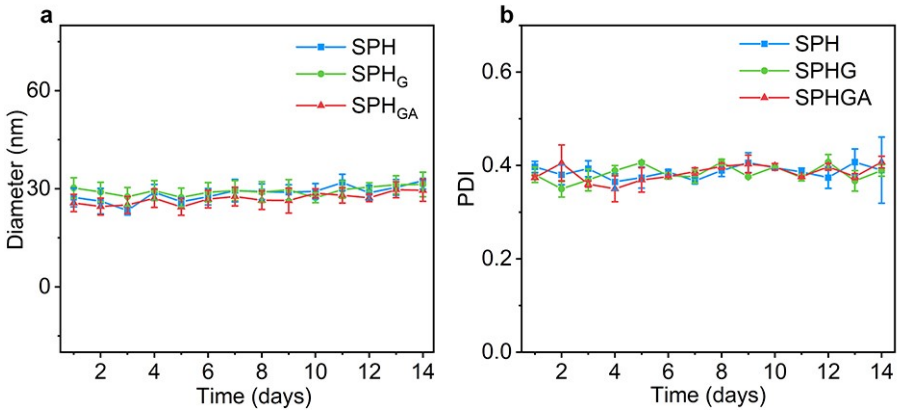

Fig. S8. (a) Hydrodynamic sizes of SPH, SPH_G, and SPH_{GA} on different days in PBS solution (5 μg/mL) (n = 5). (b) PDI of SPH, SPH_G, and SPH_{GA} on different days in PBS solution (5 μg/mL) (n = 5).

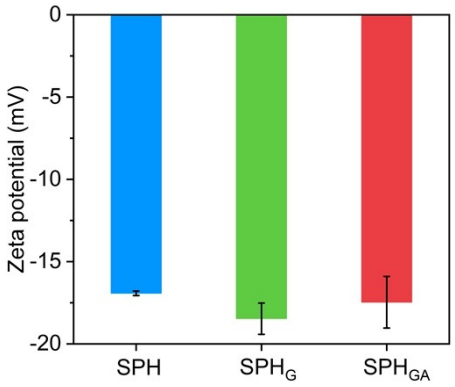

Fig. S9. Zeta potential measurements of SPH, SPH_G and SPH_{GA} (5 μg/mL) (n = 5).

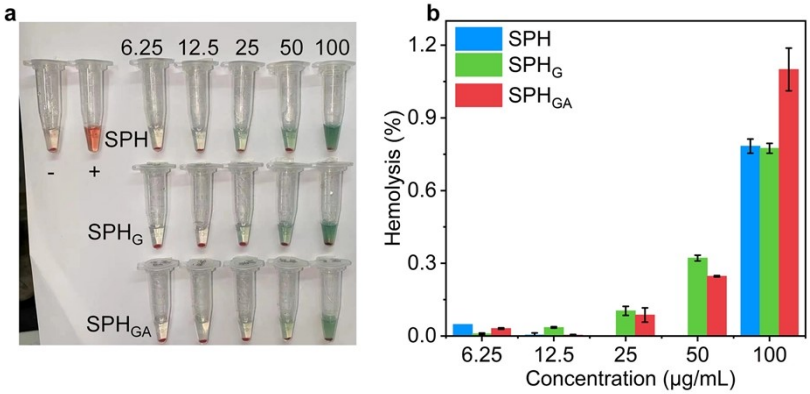

Fig. S10. (a) Hemolysis analysis of blood red cells after incubations with SPH, SPH_G and SPH_{GA} at different concentrations for 2 h. (b) Hemolysis percentages of blood red cells after treatments (n = 3).

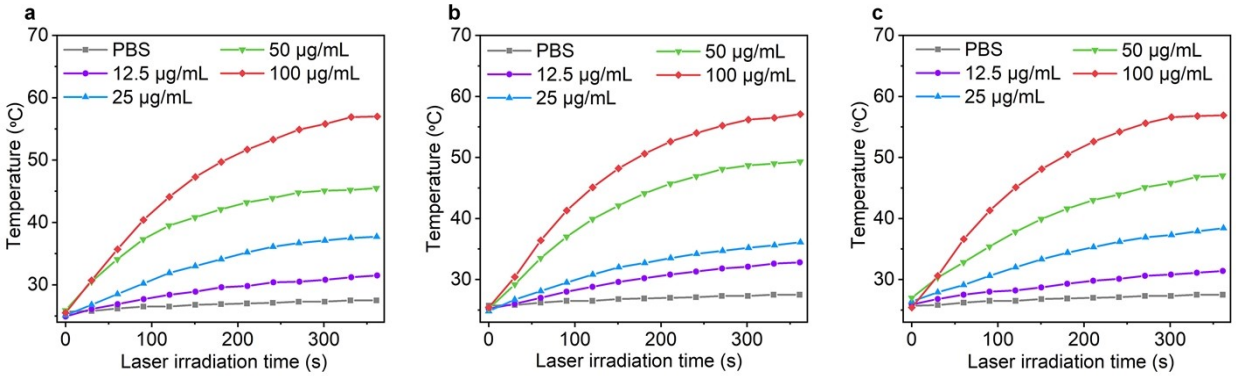

Fig. S11. Temperature change curves of (a) SPH, (b) SPH_G and (c) SPH_{GA} at different concentrations under 1064 nm laser irradiation.

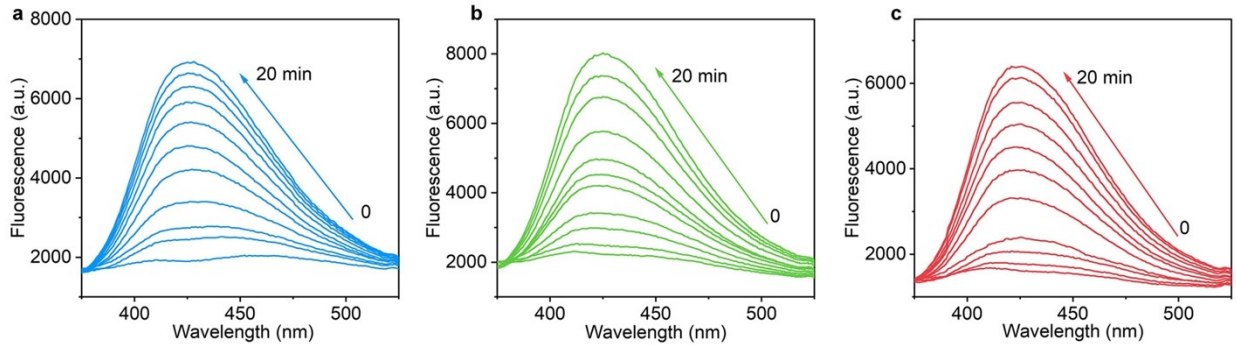

Fig. S12. Fluorescence intensity of ·OH probe for (a) SPH, (b) SPH_G and (c) SPH_{GA} under 1064 nm laser irradiation for different times (25 μg/mL).

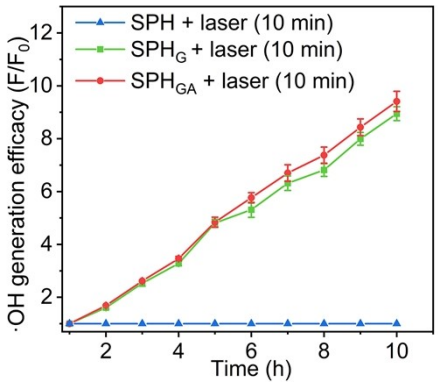

Fig. S13. Evaluation of ·OH generation for SPH, SPH_G and SPH_{GA} (150 μg/mL) after 10 min of 1064 nm laser irradiation at different incubation time (n = 3).

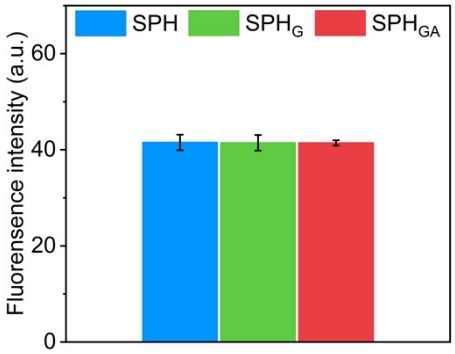

Fig. S14. Mean fluorescence intensity of red signals for SPH-, SPH$_G$- and SPH$_{GA}$-treated cells (15 μg/mL) (n = 5).

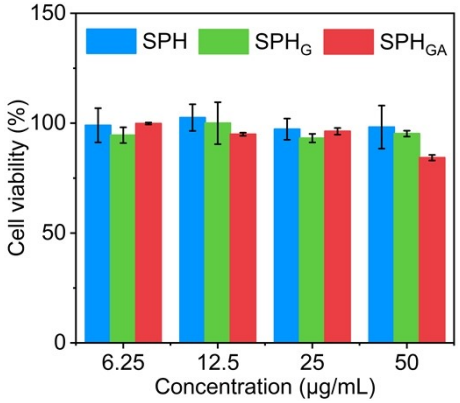

Fig. S15. Cell viability analysis of SPH-, SPH$_G$- and SPH$_{GA}$-treated 4T1 cells (n = 5).

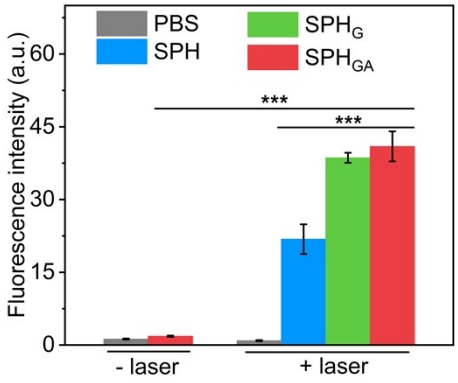

Fig. S16. Fluorescence intensity of the generated ·OH for various treated 4T1 cells (25 μg/mL) (n = 5).

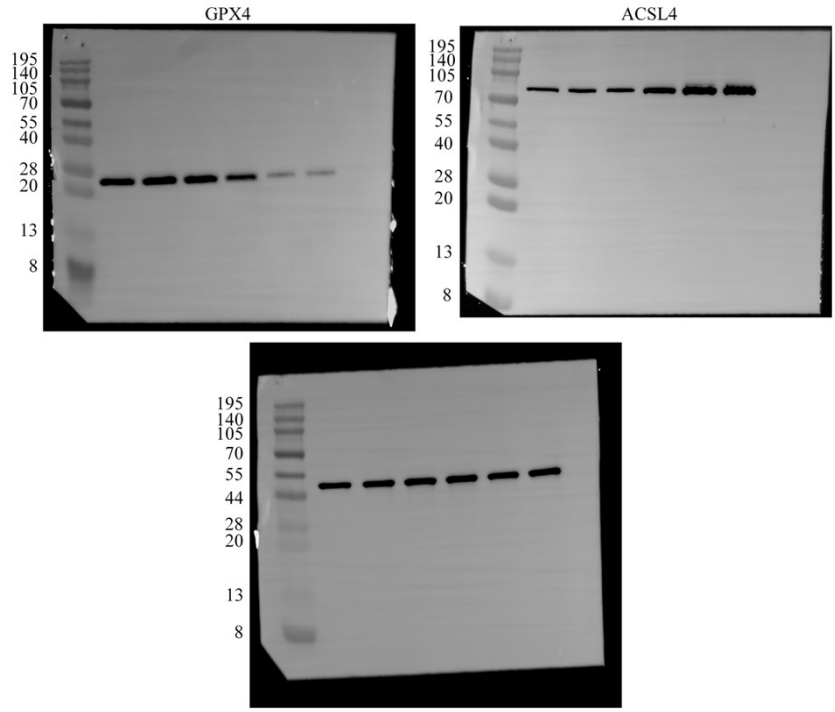

Fig. S17. The uncropped Western blot for Fig 3f.

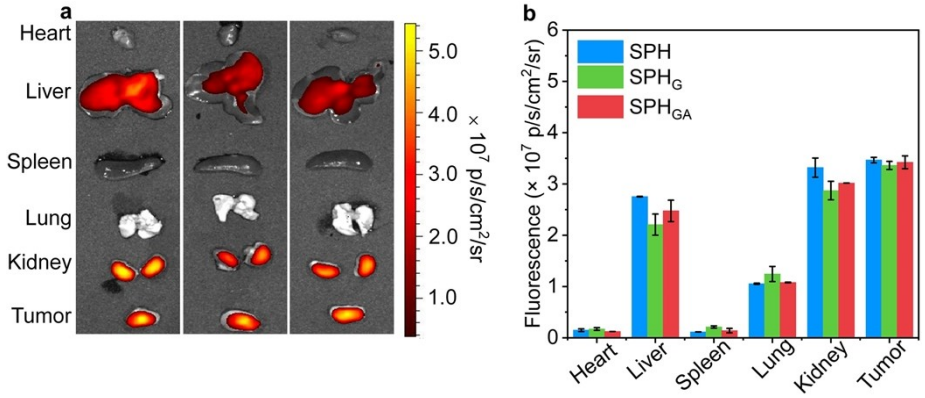

Fig. S18. (a) Fluorescence imaging of tumors, livers, kidneys, spleens, lungs and hearts. (b) Analysis of fluorescence intensity of major tissues (250 μg/mL, 200 μL) (n = 3).

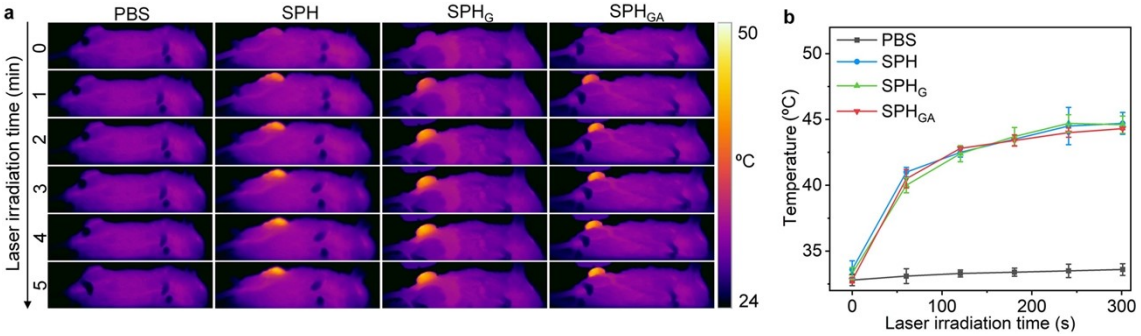

Fig. S19. (a) Thermal imaging of tumor-bearing mice under irradiation of the tumors using 1064 nm laser. (b) Temperature change curves of tumors after injection of nanoparticles (250 μg/mL, 200 μL) under laser irradiation (1.0 W/cm^2, 5 min) (n = 3).

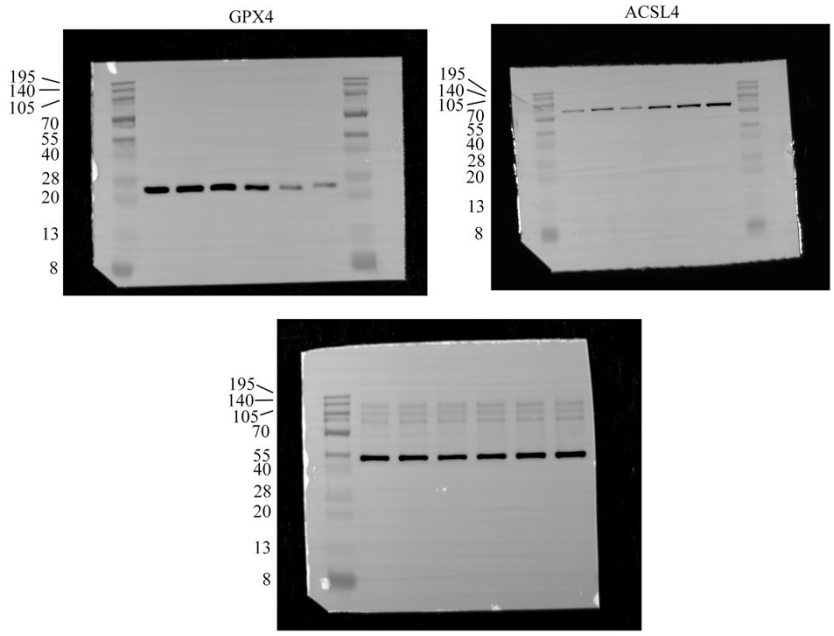

Fig. S20. The uncropped Western blot for Fig 5c.

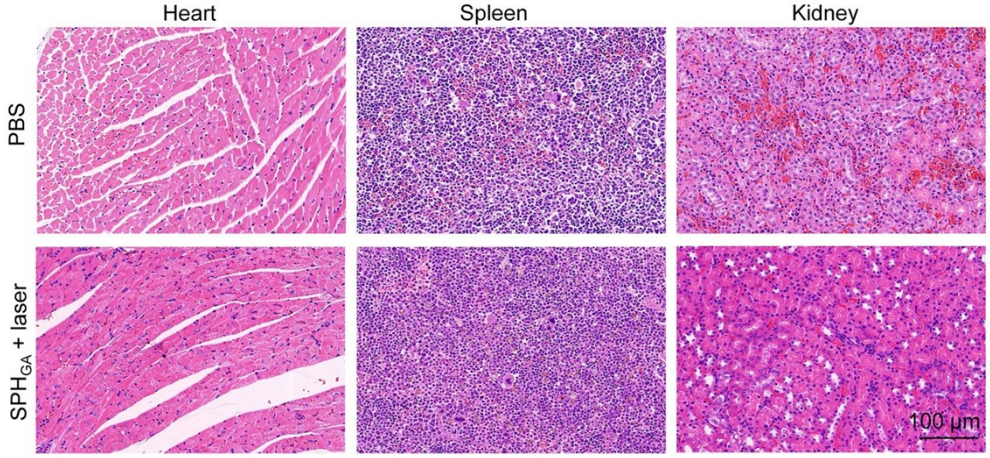

Fig. S21. H&E staining images of heart, spleen and kidney in PBS and SPH_{GA} + laser groups (250 μg/mL, 200 μL).

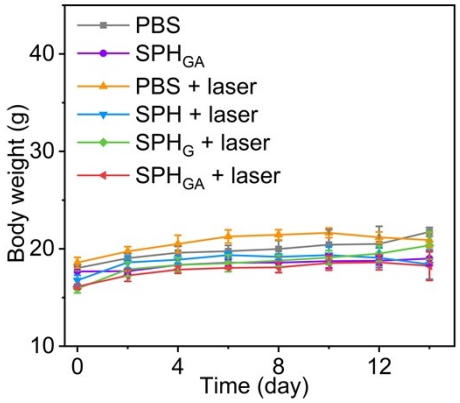

Fig. S22. Body weights of tumor-bearing mice after treatments with PBS, SPH, SPH_G and SPH_{GA} (250 μg/mL, 200 μL) and laser irradiation (1.0 W/cm^2, 10 min) (n = 5).

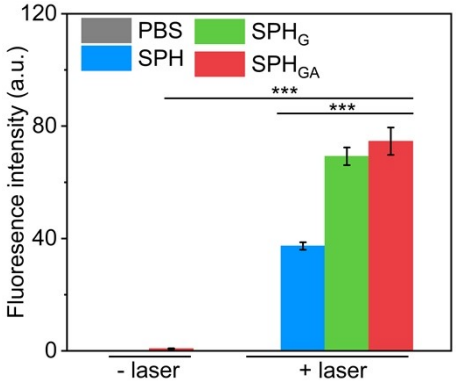

Fig. S23. Mean fluorescence intensity of CRT staining signals of primary tumors after treatments with SPH, SPH_G and SPH_{GA} (250 μg/mL, 200 μL) and laser irradiation (1.0 W/cm^2, 10 min) (n = 5).

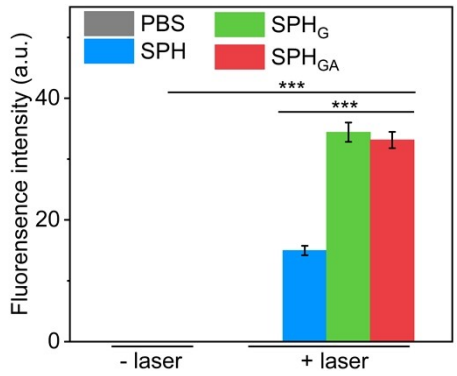

Fig. S24. Mean fluorescence intensity of HMGB1 staining signals of primary tumors after treatments with SPH, SPH_G and SPH_{GA} (250 μg/mL, 200 μL) and laser irradiation (1.0 W/cm^2, 10 min) (n = 5).

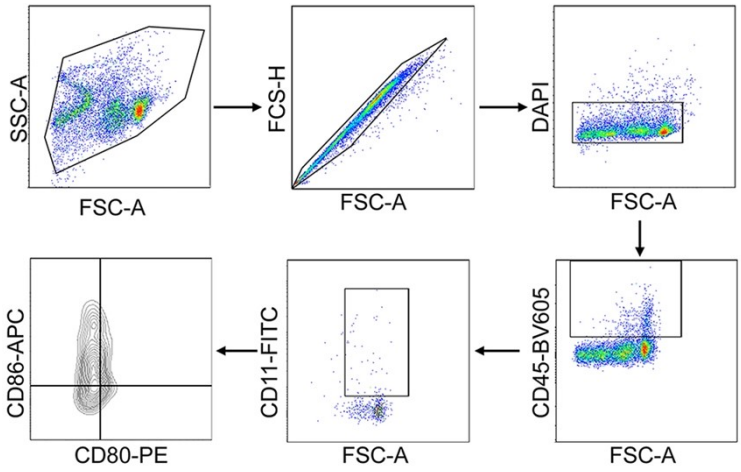

Fig. S25. Gating strategy for flow cytometry analysis of matured DCs ($CD80^+CD86^+$).

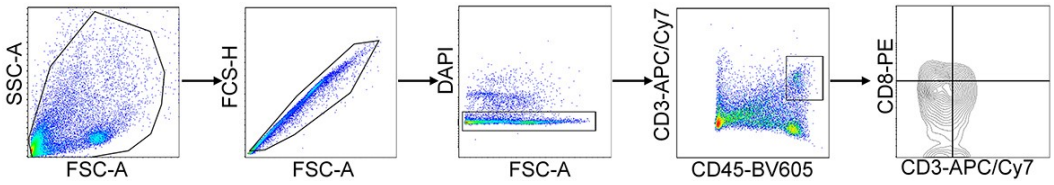

Fig. S26. Gating strategy for flow cytometry analysis of matured $CD3^+CD8^+$ T cells.

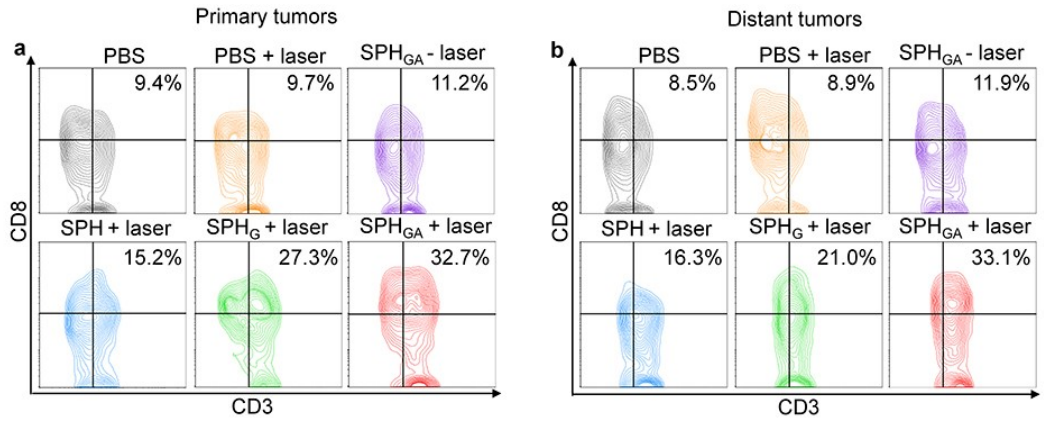

Fig. S27. Flow cytometer analysis of $CD3^+CD8^+$ T cells in (a) primary tumors and (b) distant tumors after treatments with SPH, SPH_G and SPH_{GA} (250 μg/mL, 200 μL) and laser irradiation (1.0 W/cm^2, 10 min).

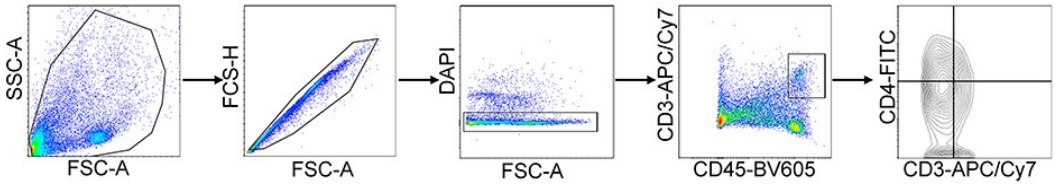

Fig. S28. Gating strategy for flow cytometry analysis of matured $CD3^+CD4^+$ T cells.

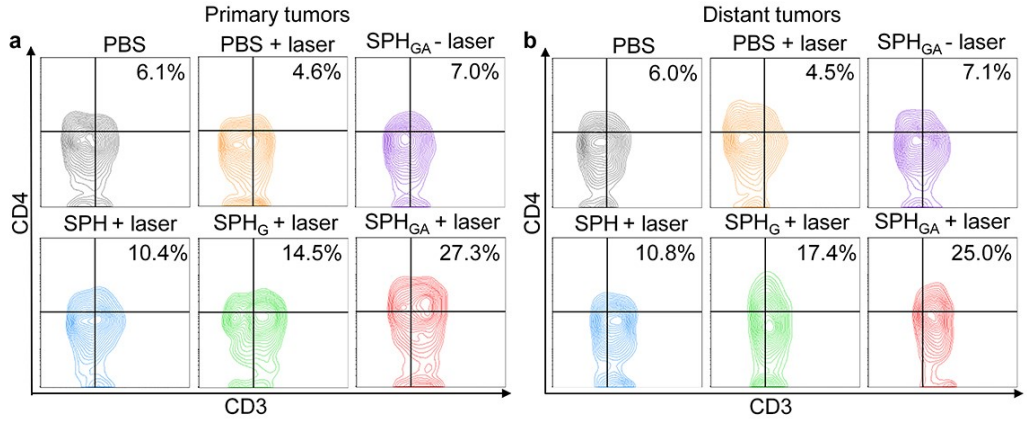

Fig. S29. Flow cytometer analysis of $CD3^+CD4^+$ T cells in (a) primary tumors and (b) distant tumors after treatments with SPH, SPH_G and SPH_{GA} (250 μg/mL, 200 μL) and laser irradiation (1.0 W/cm^2, 10 min).

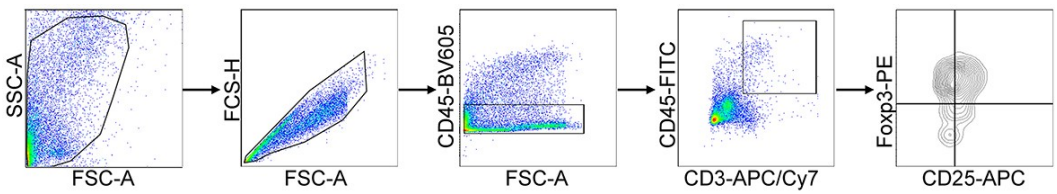

Fig. S30. Gating strategy for flow cytometry analysis of T_{reg} cells.

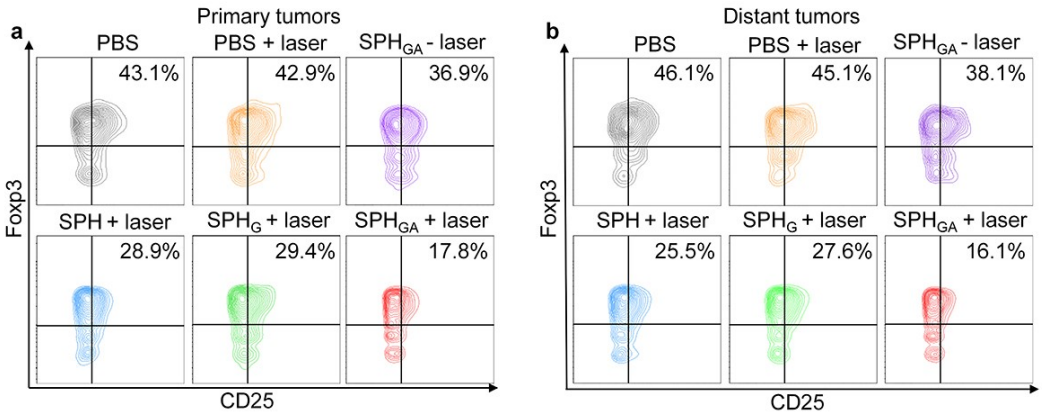

Fig. S31. (a) Flow cytometer analysis of T_{reg} cells in (a) primary tumors and (b) distant tumors after treatments with SPH, SPH_G and SPH_{GA} (250 μg/mL, 200 μL) and laser irradiation (1.0 W/cm^2, 10 min).

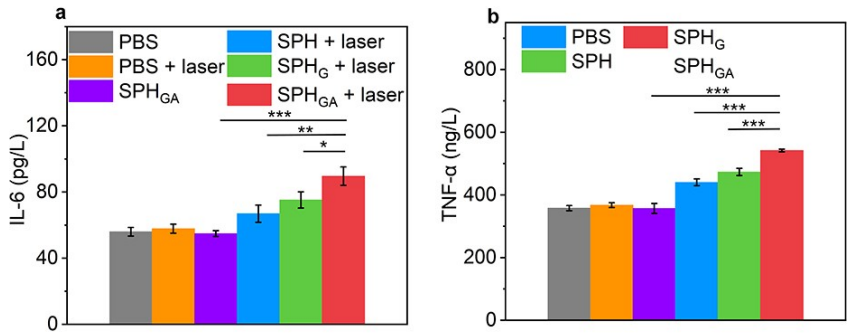

Fig. S32. The levels of (a) IL-6 and (b) TNF-α in serum of tumor-bearing mice after treatments with SPH, SPH_G and SPH_{GA} (250 μg/mL, 200 μL) and laser irradiation (1.0 W/cm^2, 10 min) (n = 5).

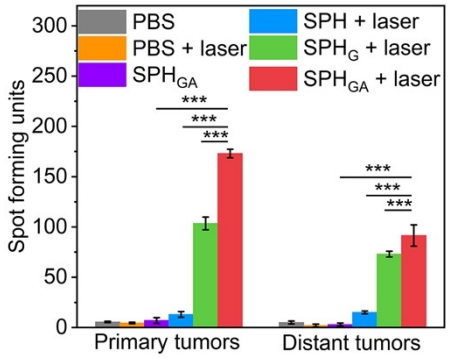

Fig. S33. Units of IFN-γ spot in primary tumors and distant tumors after treatments with SPH, SPH_G and SPH_{GA} (250 μg/mL, 200 μL) and laser irradiation (1.0 W/cm^2, 10 min) (n = 3).