

Electronic Supplementary Material

Integrating Au and ZnO nanoparticles onto graphene nanosheet for enhanced sonodynamic therapy

Fei Wang¹, Boyu Wang², Wei You², Guang Chen² (✉), and Ye-Zi You² (✉)

¹ Neurosurgical Department, the First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei 230001, China

² Department of Polymer Science and Engineering, University of Science and Technology of China, Hefei 230026, China

Supporting information to <https://doi.org/10.1007/s12274-022-4599-5>

Table S1 The quality percent of Au and ZnO in Au-rGO-ZnO@PVP.

Nanomaterials	Au (%)	ZnO (%)
Au-rGO-ZnO@PVP	5.93	32.20

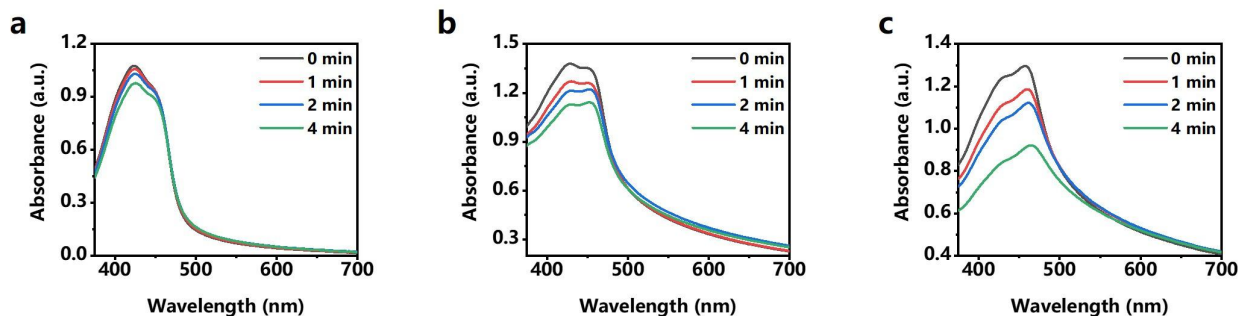


Figure S1 UV-vis-NIR absorption spectra of the DPBF in (a) PBS, (b) ZnO, and (c) ZnO-rGO@PVP under US irradiation.

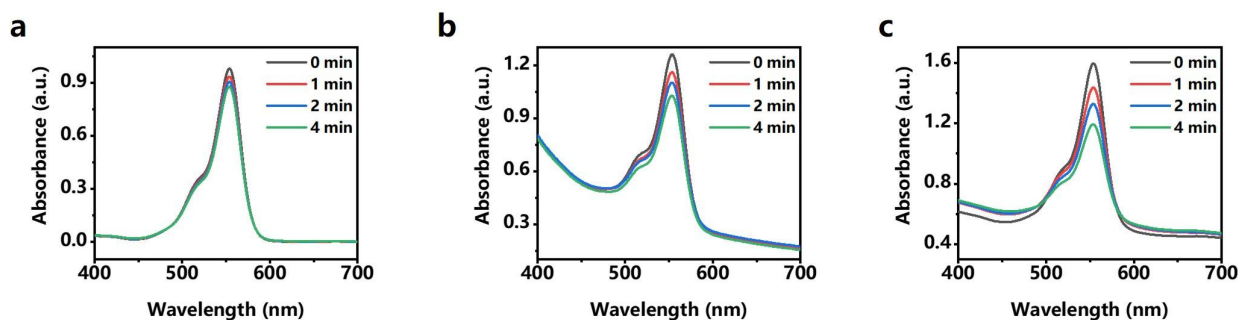


Figure S2 UV-vis-NIR absorption spectra of the RhB in (a) PBS, (b) ZnO, and (c) ZnO-rGO@PVP under US irradiation.

Table S2 The mass ration of Au and ZnO in different nanomaterials.

Nanomaterials	The mass ratio of Au: ZnO
Au-rGO-ZnO@PVP	0.19: 1.00
Au _{0.5} -rGO-ZnO@PVP	0.09: 1.00
Au-rGO-ZnO _{0.5} @PVP	0.19: 0.50

Address correspondence to [Guang Chen, cg1995@mail.ustc.edu.cn](mailto:Guang.Chen, cg1995@mail.ustc.edu.cn); [Ye-Zi You, zyyou@ustc.edu.cn](mailto:Ye-Zi.You, zyyou@ustc.edu.cn)

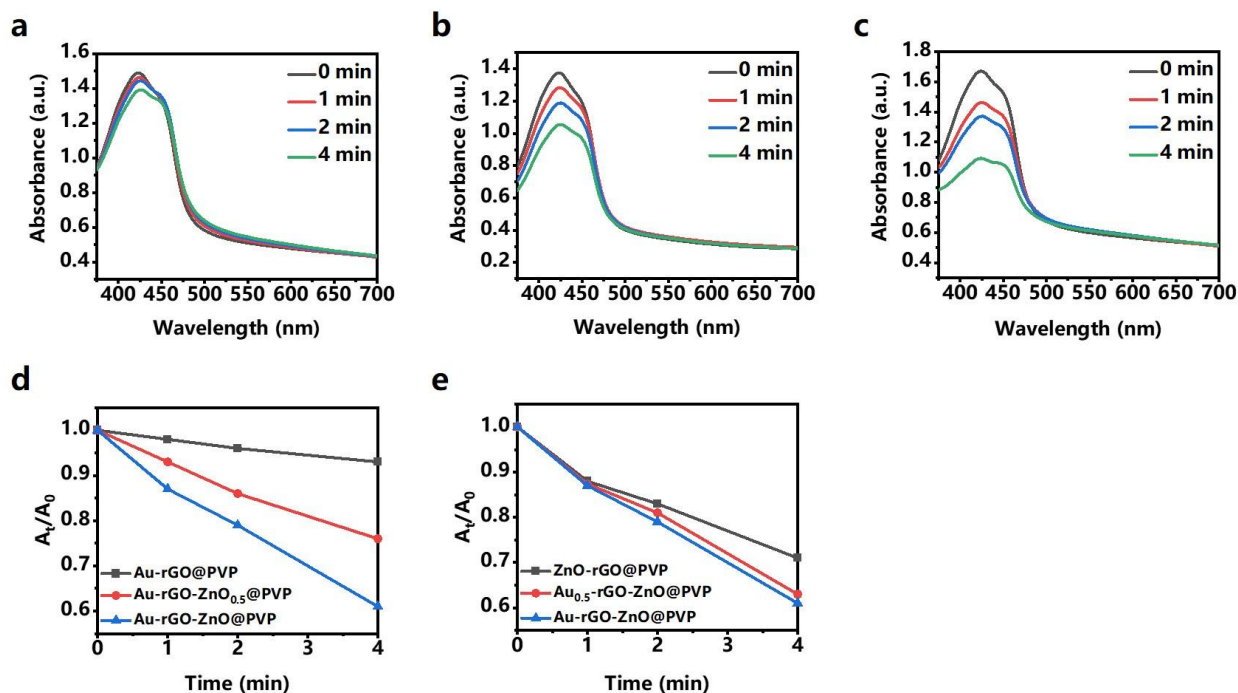


Figure S3 UV-vis-NIR absorption spectra of the DPBF in (a) Au-rGO@PVP, (b) Au-rGO-ZnO_{0.5}@PVP, and (c) Au_{0.5}-rGO-ZnO@PVP under US irradiation. (d) Relative absorption of DPBF at 420 nm after coincubation with Au-rGO@PVP, Au-rGO-ZnO_{0.5}@PVP, and Au-rGO-ZnO@PVP under US irradiation. (e) Relative absorption of DPBF at 420 nm after coincubation with ZnO-rGO@PVP, Au_{0.5}-rGO-ZnO@PVP, and Au-rGO-ZnO@PVP under US irradiation. US irradiation: 1.0 MHz, 1.0 W/cm², 50% duty cycle.

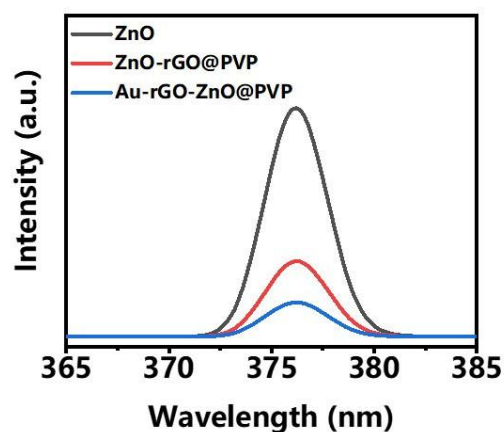


Figure S4 Photoluminescence (PL) of ZnO, ZnO-rGO@PVP and Au-rGO-ZnO@PVP.

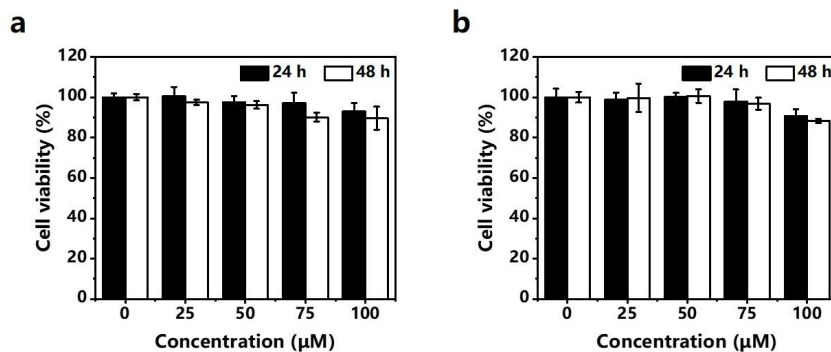


Figure S5 The viabilities of (a) HeLa cells and (b) CT26 cells after being co-incubated with different concentrations of H₂O₂ for 24 h and 48h.

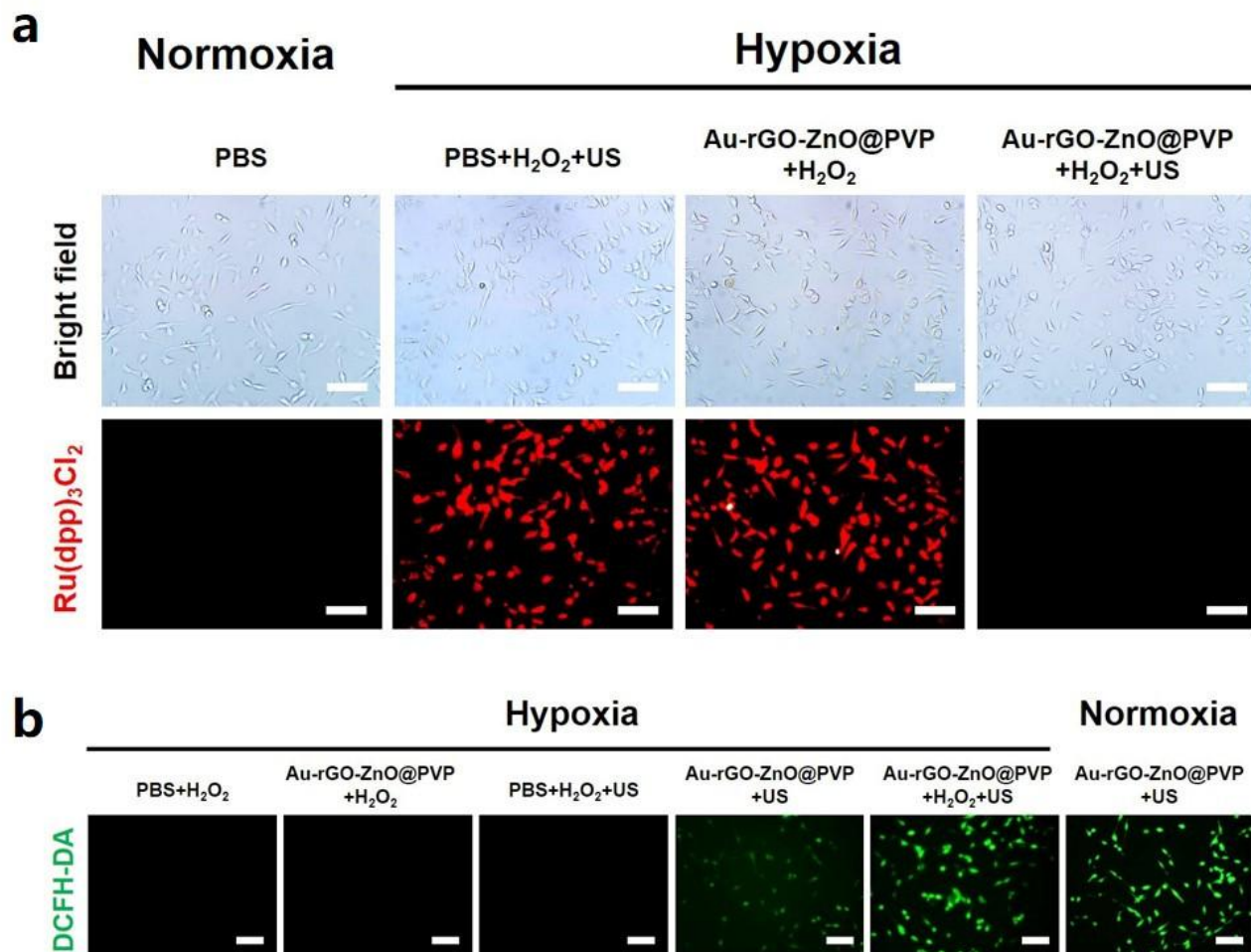


Figure S6 Hypoxia tumor microenvironment (TME) mitigated by Au-rGO-ZnO@PVP. (a) Fluorescence images of Ru(dpp)₃Cl₂-stained CT26 cells after different treatments. (b) Fluorescence images of CT26 cells stained by DCFH-DA after various treatments in hypoxic and normoxic environment. All scale bars represent 200 μ m. The concentration of H₂O₂ was 50 μ M. US irradiation: 1.0 MHz, 1.0 W/cm², 50% duty cycle.

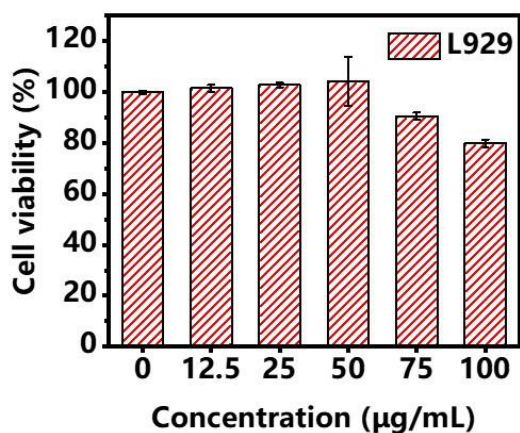


Figure S7 Relative viabilities of normal cells (L929 cells) after treated with Au-rGO-ZnO@PVP nanomaterials for 24 h.

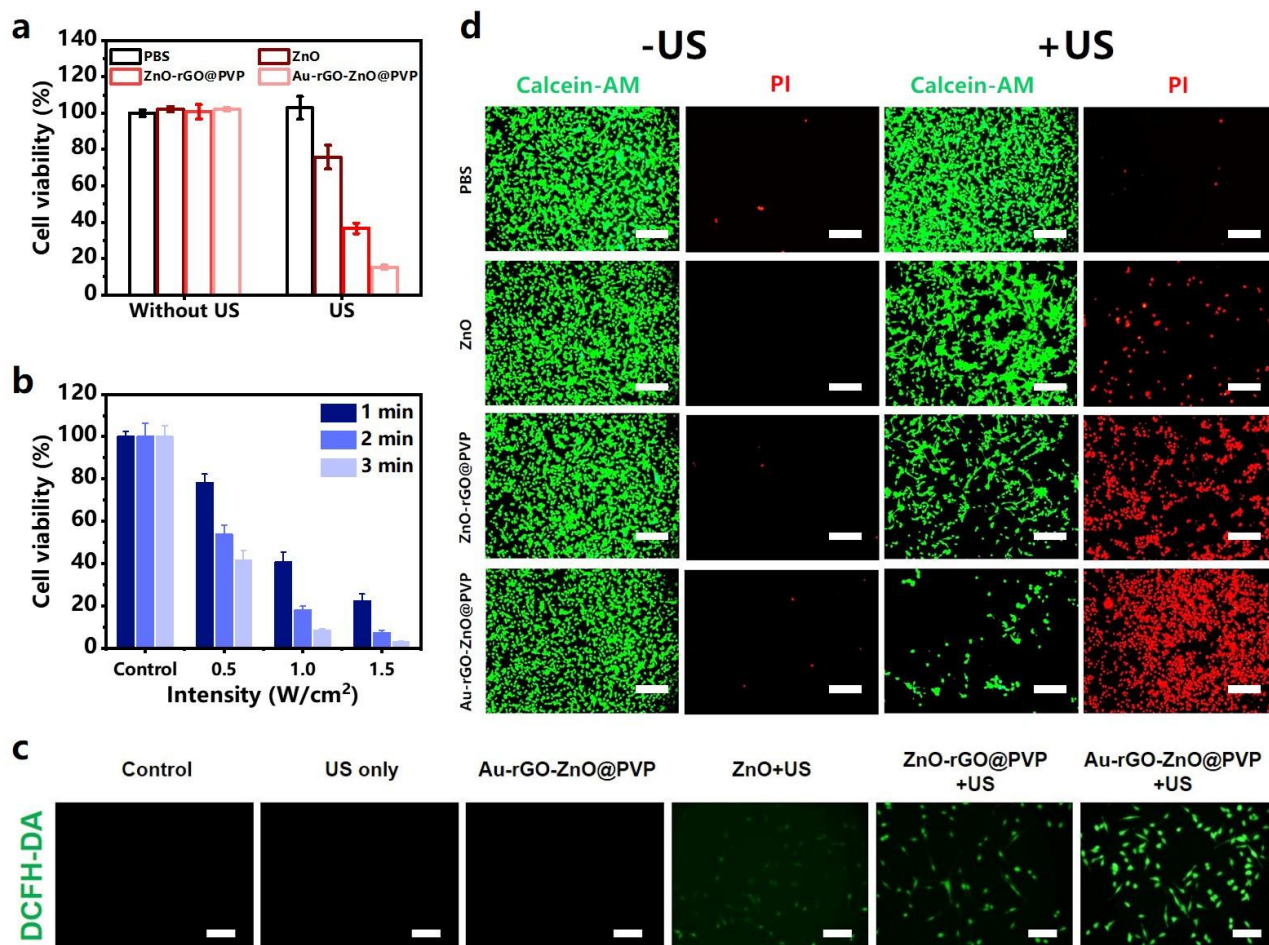


Figure S8 *In vitro* SDT using Au-rGO-ZnO@PVP. (a) The viabilities of CT26 cells after different treatments, including PBS only, ZnO, ZnO-rGO@PVP, and Au-rGO-ZnO@PVP with or without US irradiation. (b) The viabilities of CT26 cells after incubation with Au-rGO-ZnO@PVP under different conditions, including elevated US power densities, and prolonged US irradiation durations. (c) Fluorescence images of CT26 cells stained by DCFH-DA after different treatments, scale bars represent 200 μm . (d) Fluorescence images of CT26 cells stained by Calcein AM/PI after different treatments. Green signal represents live cells; red signal represents dead cells, scale bars represent 500 μm . US irradiation: 1.0 MHz, 1.0 W/cm², 50% duty cycle.

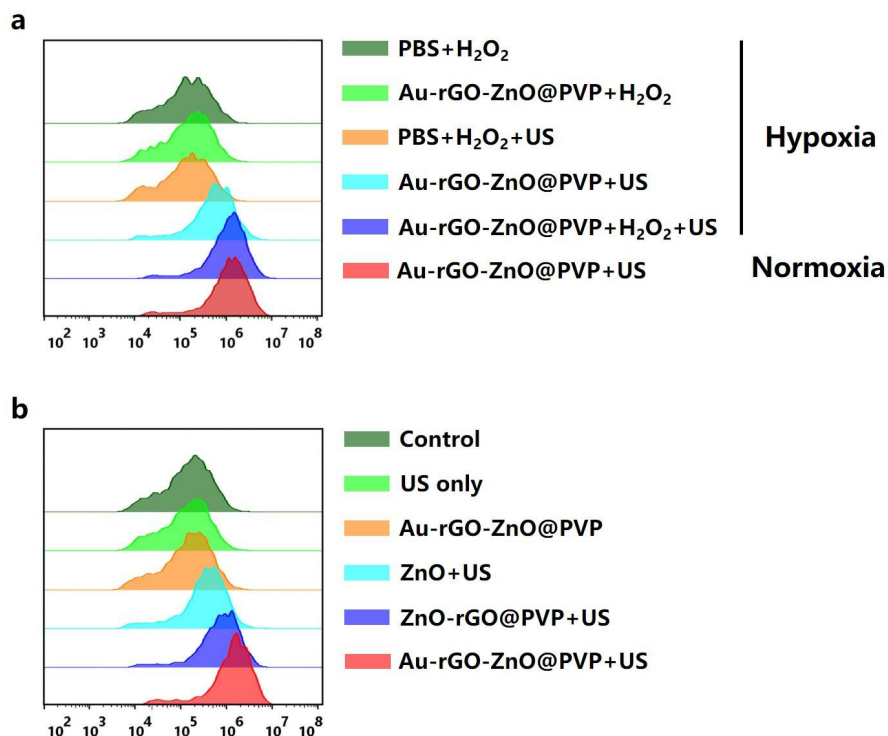


Figure S9 (a) ROS level of HeLa cells stained by DCFH-DA after different treatments in hypoxic and normoxic environment. (b) ROS level of HeLa cells stained by DCFH-DA after different treatments, including Control, US only, Au-rGO-ZnO@PVP, ZnO+US, ZnO-rGO@PVP+US, and Au-rGO-ZnO@PVP+US.

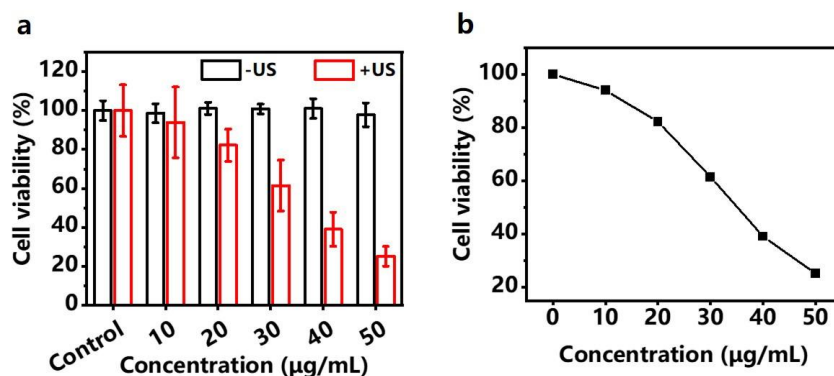


Figure S10 (a) Relative viabilities of U373MG cells after co-incubated with various concentrations of Au-rGO-ZnO@PVP nanomaterials with or without US irradiation. (b) Dose-response curves for Au-rGO-ZnO@PVP nanomaterials against U373MG cells under US irradiation. US irradiation: 1.0 MHz, 1.0 W/cm², 50% duty cycle.

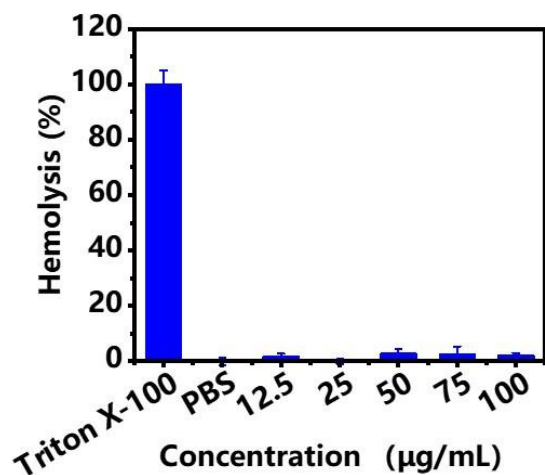


Figure S11 Hemolysis assay of Au-rGO-ZnO@PVP nanomaterials at different concentrations.

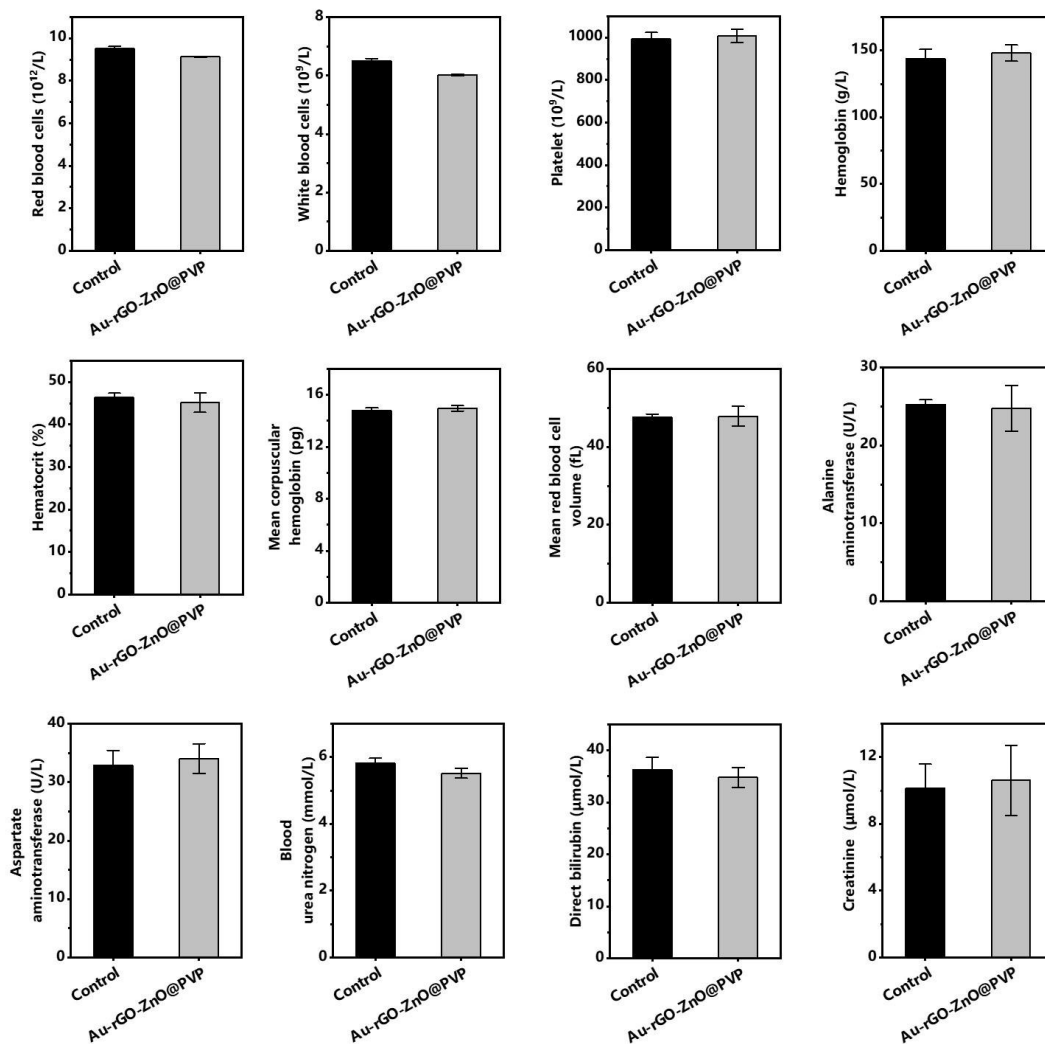


Figure S12 Hematology and blood biochemistry analysis of mice after intravenous injection.

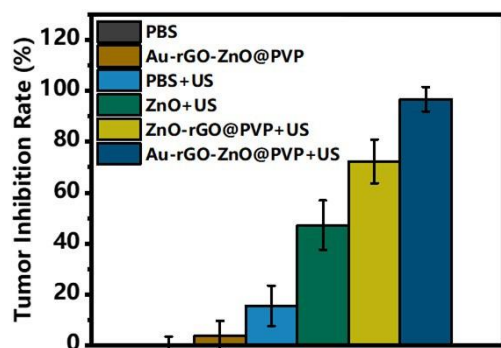


Figure S13 Tumor inhibition rate of different groups.

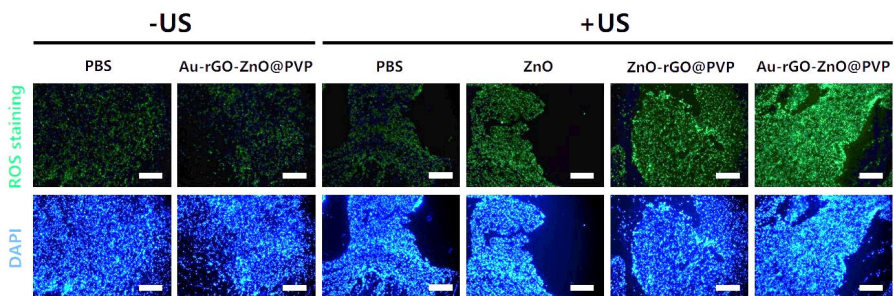


Figure S14 Representative ROS fluorescence images of tumor slices, scale bars represent 500 μm.

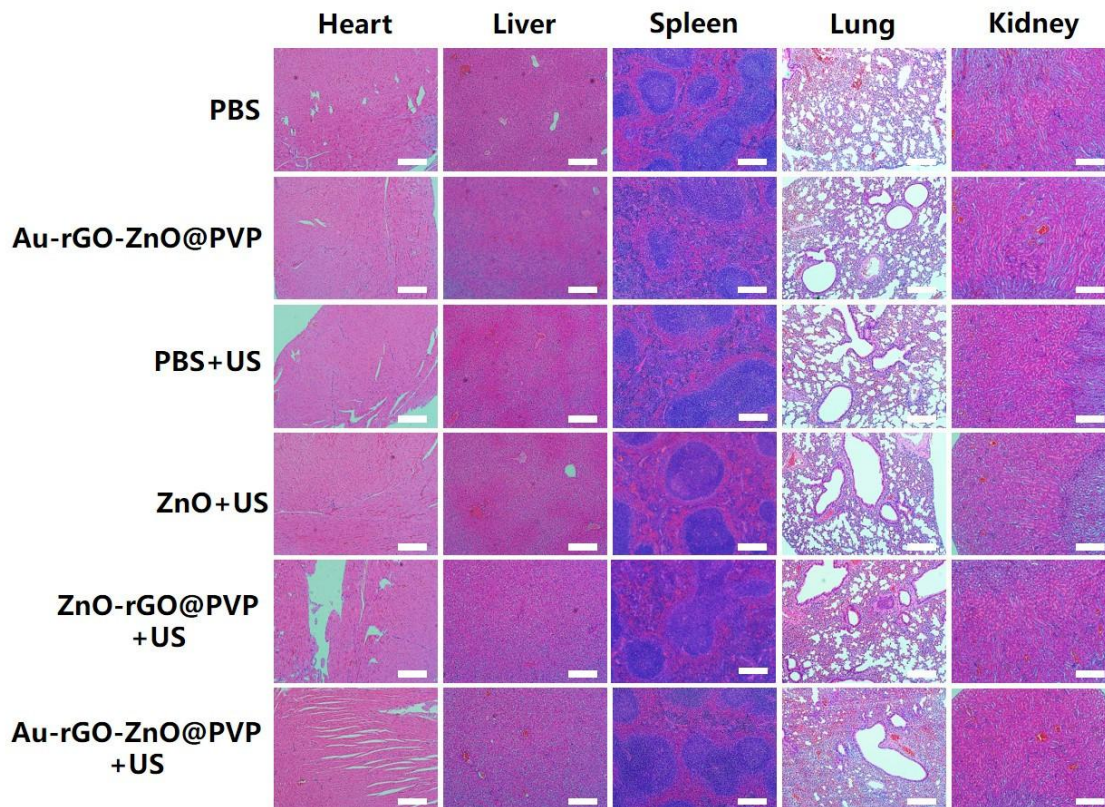


Figure S15 H&E staining of organs after different treatment, scale bars represent 500 μm.