Electronic Supplementary Information
for

Erbium oxide nanoplate as a sonosensitizer devoid of photosensitivity

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S1. Materials and Instruments

S1.1 Materials

Oleic acid (OA, >90%) and 1-octadecene (ODE, >90%) were purchased from Sigma-Aldrich. Cyclohexane (99.5%), N-hexane (97%) and ethanol (99.7%) chloroform (>99.0%) were purchased from General-Reagent. Sodium oleate (NaOL, 98%) and Poly (acrylic acid) (PAA, Mw= 2000) were purchased from Macklin. Erbium chloride hexahydrate ($ErCl_3 \cdot 6H_2O$, 99.99%), N, N-dimethylformamide (DMF, 99.8%) and Oleylamine (OM, 80-90%) were purchased from Aladdin. Diphenylisobenzofuran (DPBF, 97%) were purchased from Adamas. 3,3',5,5'-Tetramethyl-[1,1'-biphenyl]-4,4'-diamine hydrochloride hydrate(1:2:x) was purchased from Bide Pharmatech Ltd. Cell counting kit-8 (CCK-8), were purchased from Beyotime Biotechnology. Calcein AM/PI double stain kit was purchased from Shanghai Maokang Biotechnology Co., LTD. The dialysis membrane (MWCO=3500) was purchased from Viskase. 2,7-dichlorofluorescein diacetate (DCFH-DA) was purchased from Thermo Fisher.

S1.2 Instruments

The morphology of nanoparticles was obtained by transmission electron microscope (120keV, Talos L120C G2). The FT-IR spectra were measured by Fourier transform infrared spectrometer (Nicolet 6700, Thermo-fisher, USA). The X-ray diffraction pattern was measured by X-ray diffractometer (D8 Advance Da Vinci, Bruker Co.). The X-ray photoelectron spectra was measured by X-ray Photoelectron Spectrometer (AXIS UltraDLD, Shimazu). The absorption spectra were measured by UV-Visible spectrophotometer (UV-2600i, Shimadzu, Japan). The electron spin resonance spectra were recorded by Electron Paramagnetic Resonance Spectrometer (Bruker EMXplus). The Xenon lamp was that equipped on a Shimazu RF-6000 fluorescence spectrophotometer, white light was generated by Ocean Optics HL-2000-FHSA (electric power of the bulb=7 W, and output light power=6.7 mW, see the emission spectrum in Figure S10). 254nm and 365nm UV light were generated by Portable Ultraviolet Analyzer (ZF-7A). Ultrasound was generated by Portable Sonodynamic Therapy System (SXUltrasonic).

S1.3 The synthesis and surface modification of Er_2O_3 nanoplates

Er_2O_3 nanoparticles were synthesized via modifying the thermolysis method. We prepared erbium-oleate precursor at first. In brief, erbium chloride hexahydrate (8 mmol) and NaOL (24 mmol) were added to a mixture of ethanol (32 mL), deionized water (24 mL), and hexane (56 mL). The mixture was stirred at 70 °C for four hours and then transferred to a separatory funnel. After the layer separation, the lower organic orange viscous layer was collected, followed by evaporating the solvent. A waxy solid Er-oleate precursor was consequently obtained.

The precursor Er-oleate (0.399g) and a mixed solvent of OA (6 mL), OM (18 mL), and ODE (16mL) were added in a three-neck round-bottom flask and heated to 100 °C for 1 h under vacuum. After bubbling in a nitrogen atmosphere for 10 min, the reaction solution was then quickly heated to 320 °C for 1 h under nitrogen atmosphere. After being washed by ethanol for three times, the Er_2O_3 nanoparticles were then freeze-dried.

In the surface modification, a chloroform solution(3mL) of PAA (170mg) and a chloroform solution (9mL) of as-prepared Er_2O_3 (40mg) were prepared separately and mixed. The mixture was stirred at room temperature over 48h. The solution was transferred to centrifuge tubes and mixed with hexane in a ratio around 1:2. The PAA coated Er_2O_3 nanoparticles were precipitated at the speed of 10000r/min for 5 min. Then, the nanoparticles were dissolved in deionized water and dialysized (MWCO=3500) for 12h with changing water 3 times.

S1.4 The calculation of bandgap structure

The electronic structure of Er_2O_3 nanoparticles was calculated by the UV-visible diffuse reflection spectrum and X-ray photoelectron valence band spectrum. First, the UV-Vis diffuse reflection spectrum of Er_2O_3 nanoparticles were measured by UV-VIS spectrophotometer, and the absorption value and wavelength data were substituted into the formula $(\alpha hv)^2=A(hv-E_g)$, in which α is the optical absorption index, h is Planck's constant, v is the frequency, A is a constant, and E_g is the bandgap of the semiconductor. Taking $(\alpha hv)^2$ as the vertical coordinate as hv as the horizontal coordinate, the cross intercept of the straight part of the plot is the value of bandgap. The cross intercept of the straight part of the X-ray photoelectron spectroscopy valence band spectrum represents the value of $E_{VB,XPS}$. And $E_{VB,NHE}$ is calculated according to $E_{VB,\,NHE}=\varphi+E_{VB,\,XPS}-4.44$, where φ represent the work function of equipment. Finally, E_{CB} is calculated according to $E_{CB}=E_{VB,NHE}-E_g$.

S1.5 ROS detection

Two chemical indicators, DPBF and TMB, were used to test the production of reactive oxygen species. The DMF solutions of 2.5mg/mL DPBF and 8mg/mL TMB, respectively, were configured and stored in dark. A 15uL DPBF probe (or 30 uL TMB probe) and 1ml Er_2O_3 aqueous solution (0.4 mg/mL) were then mixed in a 12-well plate. The mixture was irradiated by ultrasound (1 MHz, 0.75 W/cm^2) or light (254 nm, 265 nm, 379 nm, 521 nm, and white light, respectively). The absorption at 420 nm for DPBF (652 nm for TMB) were recorded by UV-Visible spectrophotometer.

Electron spin resonance technology was applied to distinguish the species of ROS. TEMP was used as the capture agent for $^1O_2$ (deionized water as solvent). DMPO was used as the capture agent for $\cdot O_2^-$ production (methanol as solvent). Four groups of samples for each ROS species were tested: untreated pure solvent, US-treated solvent, untreated Er_2O_3 solution and US-treated Er_2O_3 solution. The concentration of Er_2O_3 solution was 0.4 mg/mL.

Intracellular ROS generation were characterized by 2,7-dichlorofluorescein diacetate (DCFH-DA). B16F10 cells were incubated with materials (PBS, 200 ug/mL Er_2O_3 solution or 200 ug/mL PpIX solution) in dark for 2h and washed by PBS solution. Then the US group was sonicated for 5min (0.75 W/cm^2), and the white light group was irradiated for 40min. After stained with DCFH-DA (20 μM) for 30 min, the cells were observed by laser scanning confocal microscope (LSCM).

S1.6 Cytotoxicity, phototoxicity, and SDT

The toxicity under different conditions was evaluated by hemolysis ratio of erythrocyte. Dark group, white light group and US group were set to characterize cytotoxicity, phototoxicity, and sonodynamic effect, respectively. In each group, a series of concentration (50, 100, 150, 200, 250, 300 ug/mL) of Er_2O_3 or PpIX was chosen. 200 uL erythrocyte suspension was incubated with 800uL Er_2O_3 nanoparticles or PpIX solutions in dark for 6 hours. Then, the dark group was kept in dark, the US group was sonicated for 30 s (0.75 W/cm^2), and the white light group was irradiated for 1.5 h. Negative control group added 800uL PBS solution, positive control group added 800uL deionized water. The treated suspension was centrifuged at 2500 r/min for 5 min and the absorption values of the supernatant at 570nm were measured by UV-vis spectrophotometer.

The toxicity of under US was also evaluated by cell viability of B16F10 melanoma cell. Dark group and US group were set to characterize cytotoxicity and sonodynamic effect of materials, respectively. B16F10 cells were incubated with the materials in dark for 2h. Then, the dark group was remained in dark, and the US group was sonicated for 5min (0.75 W/cm^2). The cell viability was measured by CCK-8 assays, in which a 10 μL CCK-8 solution was added into each well for another 4h incubation.

The absorbance at 450 nm was measured by a microplate reader (Multiskan GO, Thermo scientific). Calcein AM/PI double stain kit was used to label B16F10 cells under different conditions. Dark group, white light group and US group were set to characterize cytotoxicity, phototoxicity and sonodynamic effect of materials, respectively. Each group consisted of blank, Er_2O_3 (200 ug/mL) or PpIX (200 ug/mL). B16F10 cells were incubated with materials in dark for 2h. Then the dark group was kept in dark, the US group was sonicated for 5min (0.75 W/cm^2), and the white light group was irradiated for 40min. After adding Calcein AM (5 μM in DMEM) and PI (10 μM in DMEM), the cells were observed by a laser scanning confocal microscope (LSCM).

S2. Supplementary Figures

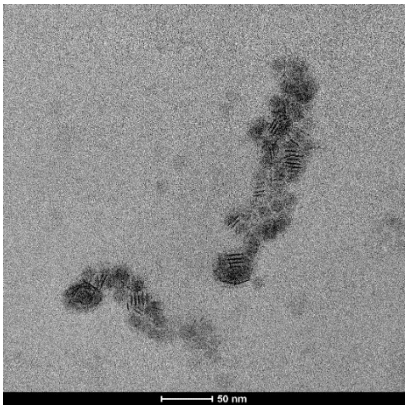

Figure S1. TEM image Er_2O_3 after PAA modification

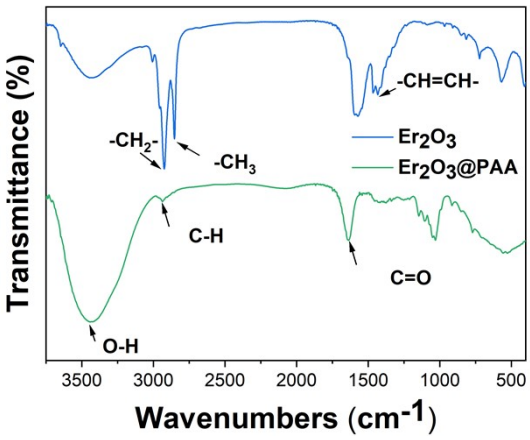

Figure S2. FT-IR spectra of Er_2O_3 and Er_2O_3@PAA

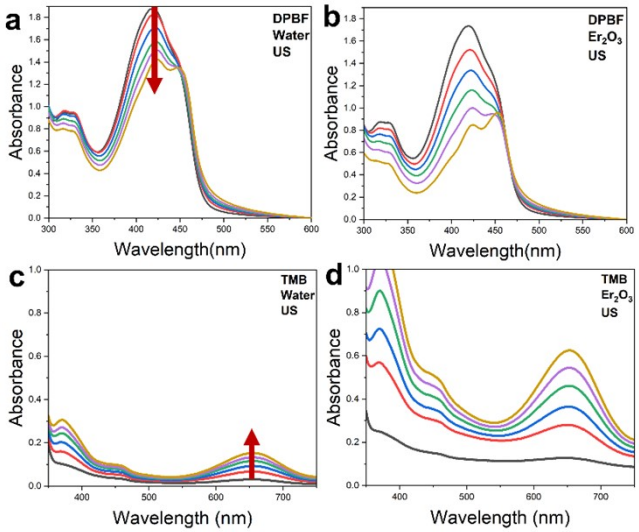

Figure S3. UV-vis absorption spectra of (a, b) DPBF and (c, d) TMB in (a, c) water and (b, d) Er_2O_3 soluion under ultrasonic treatment (measured once per minute).

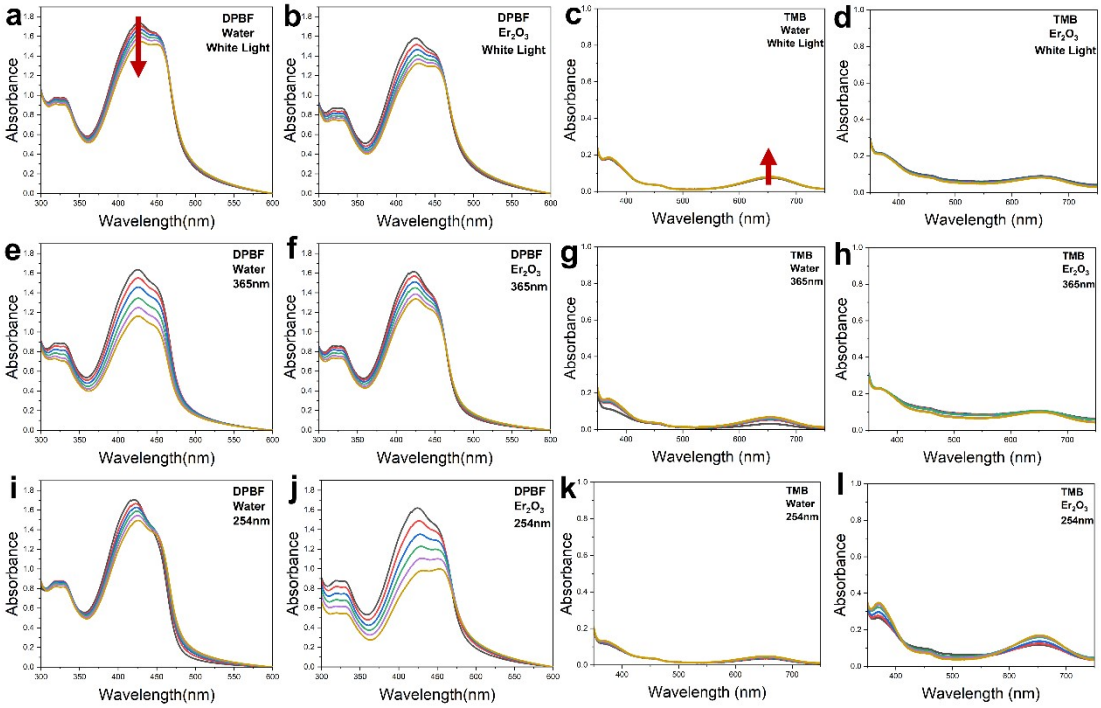

Figure S4. UV-vis absorption spectra of (a, b, e, f, i, j) DPBF and (c, d, g, h, k, l) TMB in (a, c, e, g, i, k) water and (b, d, f, h, j, l) Er_2O_3 soluion under (a-d) white light, (e-f) 365nm irradiation and (i-l) 254nm irradiation (measured once per minute).

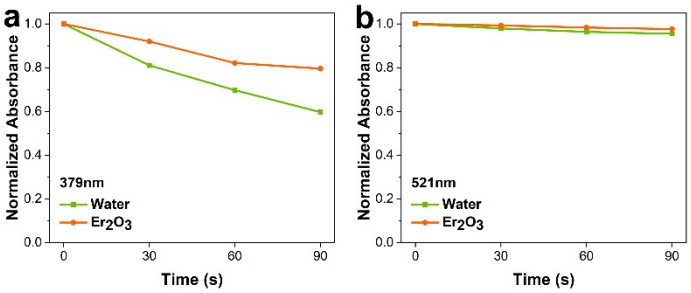

Figure S5. ROS generation of Er_2O_3 nanoplates detected by DPBF under (a) 379nm and (b) 521nm irradiation.

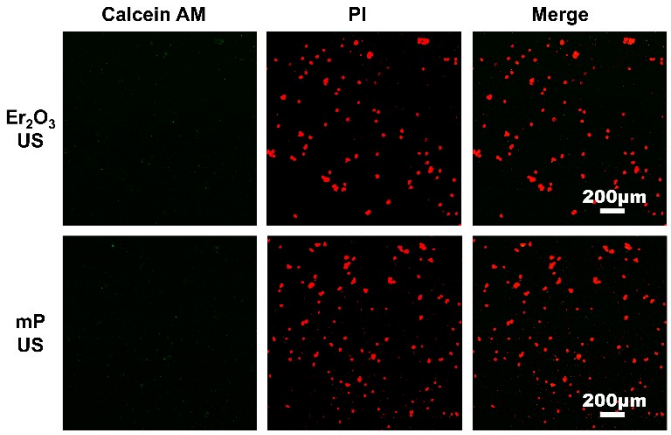

Figure S6. Confocal fluorescence microscopy images of B16F10 cells under US irradiation in the presence of Er_2O_3 or PpIX.

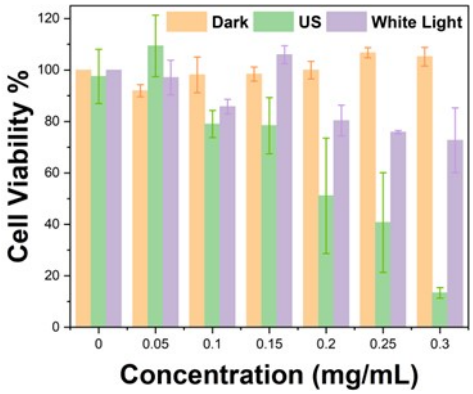

Figure S7. Cell viability of B16F10 cells under ultrasounic treatment, darkness and white light in the presence of Er_2O_3 nanoparticles.

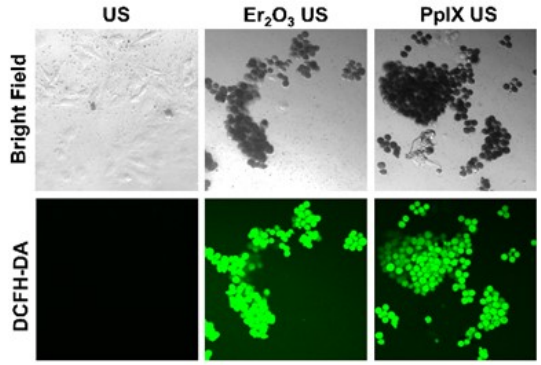

Figure S8. Bright field and confocal images of B16F10 cells stained with DCFH-DA after ultrasound exposure.

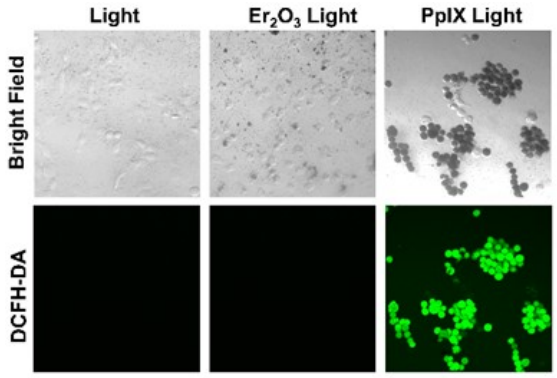

Figure S9. Bright field and confocal images of B16F10 cells stained with DCFH-DA after light exposure.

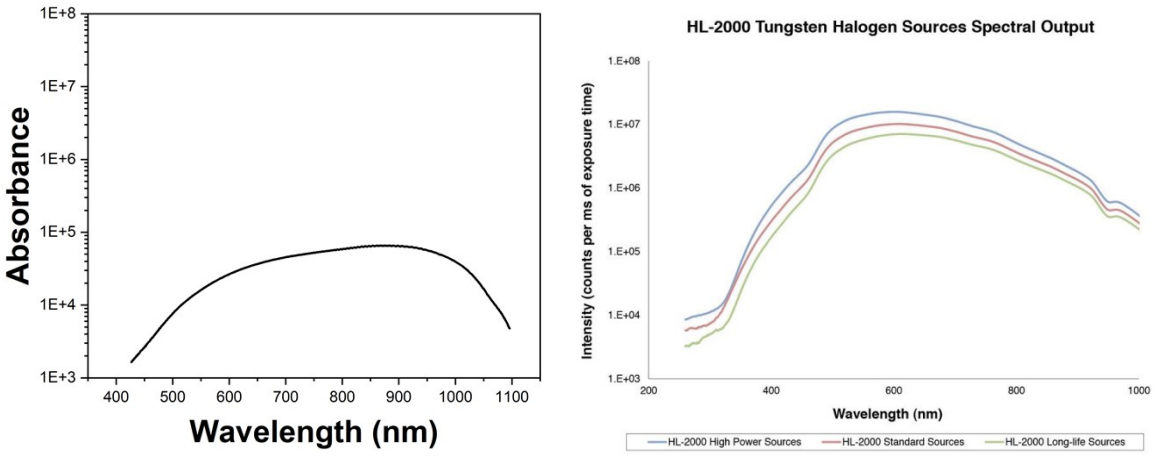

Figure S10. The emission spectrum of the white light, Ocean Optics HL-2000-FHSA. (Left) tested by a fiber spectrometer (OceanOptics UV-VIS-NIR) and (right) provided by supplier.