Supporting Information for

Nanoscintillator-mediated X-ray inducible photodynamic therapy for *in vivo* cancer treatment

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Supplementary Method:

1. SAO nanoparticle synthesis, surface modification, and photosensitizer loading.

SAO was synthesized by a carbo-thermal reduction and vapor-phase deposition method, which was published by us previously.[1, 2] To render SAO amenable to bio-related applications, bulk SAO was ground into particles with diameters of ~ 150 nm. These bare SAO nanoparticles were coated with a layer of solid silica by following a previously published protocol.[3] The resulting nanoparticles were subsequently coated with a layer of mesoporous silica using an established method[4], except that 3-aminopropyltriethoxysilane (5%) (Sigma Aldrich) was mixed with tetraethyl orthosilicate (TEOS) (Sigma Aldrich) as silane precursors.

2. Characterizations of SAO nanoparticles.

UV-Vis absorption spectra were recorded on a Shimdzu 2450 UV-Vis spectrometer. Photoluminescence measurements were performed on a Hitachi F-7000 fluorometer. X-ray excited optical luminescence (XEOL) was measured on Horiba JobinYvon FL3-2iHR fluorescence spectrometer using an emission filter of 285 nm and an emission slit of 3 nm. The recorded spectrum was smoothed by a Savitzky-Golay method of 5 points. A mini-X X-ray tube (Amptek Inc.) was used as the X-ray source, and was set at 50 kV and 70 μA for all the experiments in this study. TEM and HR-TEM samples were prepared by dripping sample solutions onto carbon-coated copper grids and evaporating the solvent. TEM/HR-TEM images were taken on an FEI Tecnai 20 transmission electron microscope operating at 200 kV. SEM images were taken on an FEI Inspect F field emission gun

scanning electron microscope at 20 kV. Dynamic light scattering (DLS) analysis was performed using a Zetasizer Nano S90 size analyzer (Malvern Corp, U.K.).

3. Loading MC540 onto SAO@SiO_2 nanoparticles.

For MC540 loading, MC540 (Invitrogen) in ethanol was added to an aqueous solution of SAO@SiO_2 nanoparticles, and the incubation went on overnight at room temperature[5]. The mixture was then centrifuged and the supernatant removed. The collected nanoparticles were resuspended in PBS (Thermo Scientific). The MC540 content in the supernatant was quantified by UV-Vis analysis and compared to a pre-determined standard curve. The yielded MC540 quantity was deducted from the mass of MC540 added at the beginning to arrive at the amount of MC540 that was loaded onto SAO@SiO_2 nanoparticles. The loading efficiency in wt% was computed using **Equation 1**:

$$\text{Photosensitizer loading (\%)} = \frac{\text{Mass of photosensitizers incorporated into particles}}{\text{Mass of particles}} \times 100 \quad (1)$$

4. $^1O_2$ production in solutions.

1 mL of 0.05 mg M-SAO@SiO_2/mL solution was added into a quartz cuvette (equilibrated with air at room temperature) containing 1 μM of SOSG (Life Technologies). For controls, SAO and MC540 solutions, and water were analyzed. The solutions were irradiated by X-ray at rate of 1 Gy/h for 20 min, with a 1-min intermission after each 5-min irradiation cycle. Fluorescence intensities (ex/em: 504/525 nm) were measured on a Hitachi F-7000 fluorescence spectrophotometer.

5. Cell imaging.

U87-MG cells were incubated with 30 μg/mL of SAO@SiO_2 nanoparticles for 1 h. The cells were washed three times with PBS to remove unbound nanoparticles. The nuclei were counterstained with DAPI (Vector) and the slide was mounted by a glass cover slip. Images were taken on an Olympus X71 fluorescence microscope (ex/em: 360/460 nm).

To monitor $^1O_2$ generation in live cells, SOSG was added to the incubation medium. Briefly, U87MG cells were seeded in a petri dish and grown for 24 h. The medium was then replenished with fresh medium containing 1 μM SOSG. The incubation went on for 30 min and the cells were washed by PBS to remove excess SOSG. Subsequently, the cells were incubated with M-SAO@SiO_2 nanoparticles (50 μg/mL) for 4 h and then washed with PBS for three times. X-ray irradiation was applied to cells at a dose rate of 1 Gy/h for 30 min. Fluorescence images were acquired on an Olympus X71 fluorescence microscope (ex/em: 504/525 nm).

6. *In vitro* X-PDT using pork as model tissue

M-SAO@SiO_2nanoparticles (50μg/mL) were incubated with U87MG cells in petri dishes for 1 h at 37 °C, and the cells were washed with PBS. A stack of pork slices (a total thickness of 4.5 cm) was placed between the X-ray source and the U87MG cells. Cells were exposed to X-ray for 30 min (dose rate of 1 Gy/h), and then cultured for another 24 h. Cell viability was determined by MTT assay. As a comparison, cells treated with X-PDT but without the pork stack were also studied.

7. Measurement of blood circulation half-life

Normal athymic nude mice were intravenously injected with M-SAO@SiO_2 nanoparticles (2.5 mg M-SAO@SiO_2/ml, or 1.7 mg SAO/m in 50 μL of PBS). At different time points (5 min, 15 min, 0.5 h, 1 h, 2 h, 4 h, 24 h and 48 h after the injection), 10-20 μL blood sample was drawn from the tail vein of the moice, and then dissolved in heparin solutions. The concentration of Sr of each obtained blood sample was measured by ICP. The curve fitting and half-life analysis were performed based on a biphasic exponential decay model using WinNonlin® (version 5.3).

Supplementary Figures:

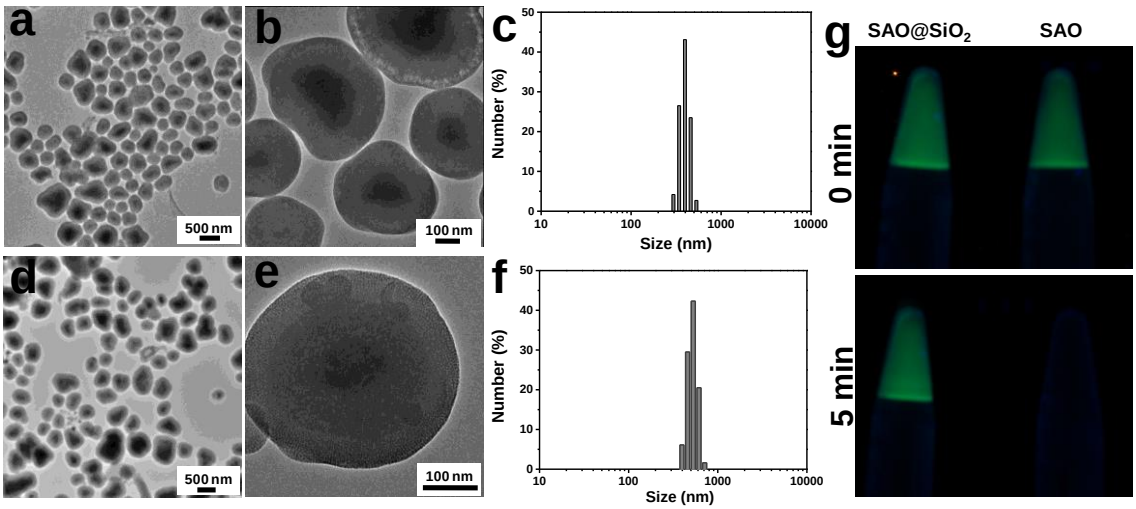

Figure S1. Size distribution and stability of silica coated SAO nanoparticles. (a,b) TEM images at relatively low (a) and high (b) magnifications for SAO nanoparticles coated with one layer of solid silica. (c) Size distribution of the particles in (a) and (b), analyzed by DLS (PDI is 0.442 and Z-average diameter is 365.1 nm). (d,e) TEM images at relatively low (d) and high (e) magnifications for SAO nanoparticles coated with two layers of silica (i.e. SAO@SiO_2 nanoparticles). (f) Size distribution of SAO@SiO_2 nanoparticles, analyzed by DLS (PDI is 0.301 and Z-average diameter is 417.3 nm). (g) Without silica coating, photoluminescence of SAO nanoparticles vanished within 5 min in water.

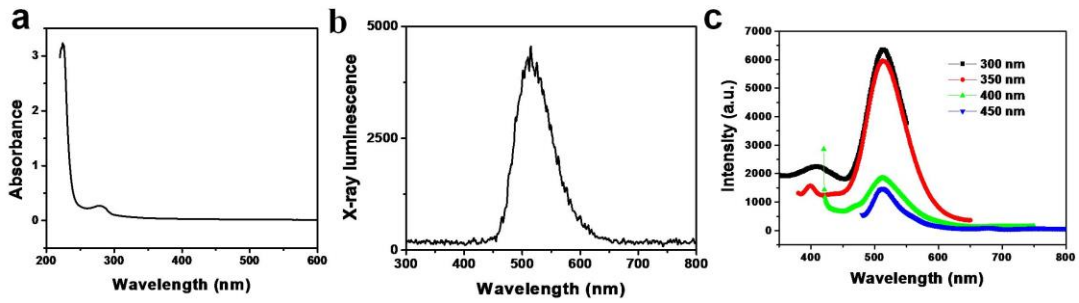

Figure S2. Optical properties of as-synthesized SAO. (a) Absorbance spectrum. (b) X-ray excited luminescent spectrum under X-ray excitation. The spectrometer was coupled with a 285-nm emission filter and the emission slit was set at 3 nm. X-ray tube was operated with a tube voltage of 50 KV and a tube current of 70 μA. (c) Photoluminescence spectra of SAO under excitation by light of different wavelengths.

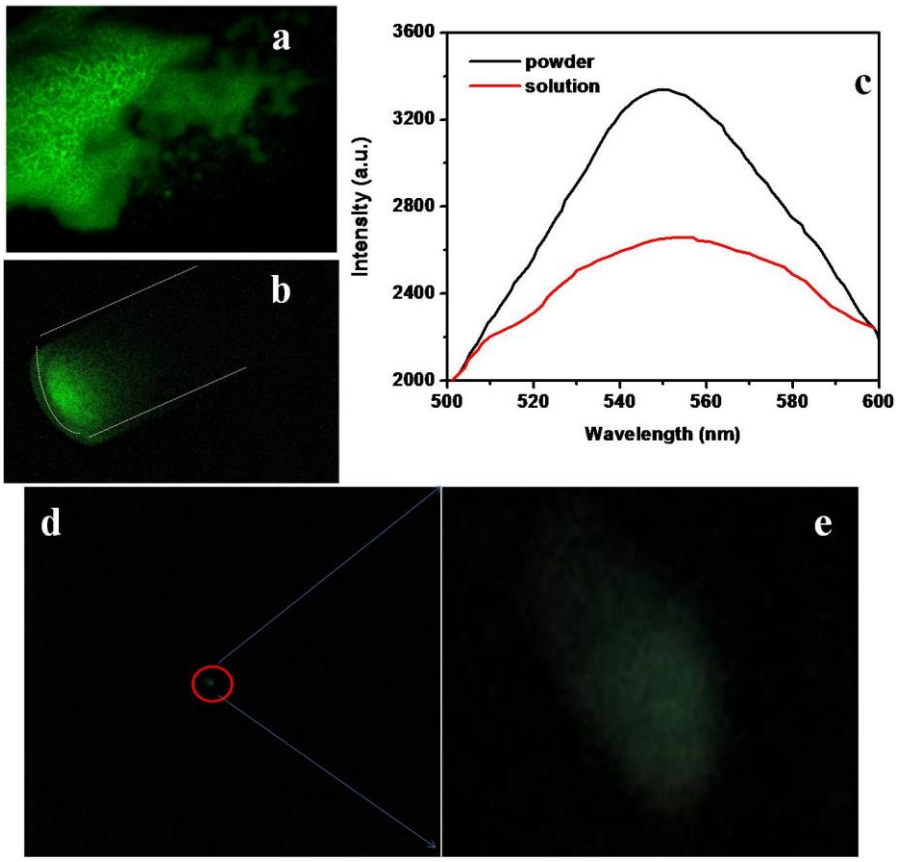

Figure S3. X-ray excited optical luminescence of SAO@SiO_2 nanoparticles. (a,b) Photographs of SAO in powder (a) and aqueous solutions (b, 1 mg/mL) under X-ray irradiation in the dark. Images were taken on a Mastro small animal imager. A mini-X X-ray tube was set up in the chamber of the imager as the excitation source. (c) X-ray excited optical luminescence spectra of (a) and (b), taken by the imager. (d,e) Photographs of M-SAO@SiO_2 nanoparticle powder under X-ray irradiation, taken by an iPhone 4s. X-ray tube was operated with a tube voltage of 50 KV and a tube current of 70 μA.

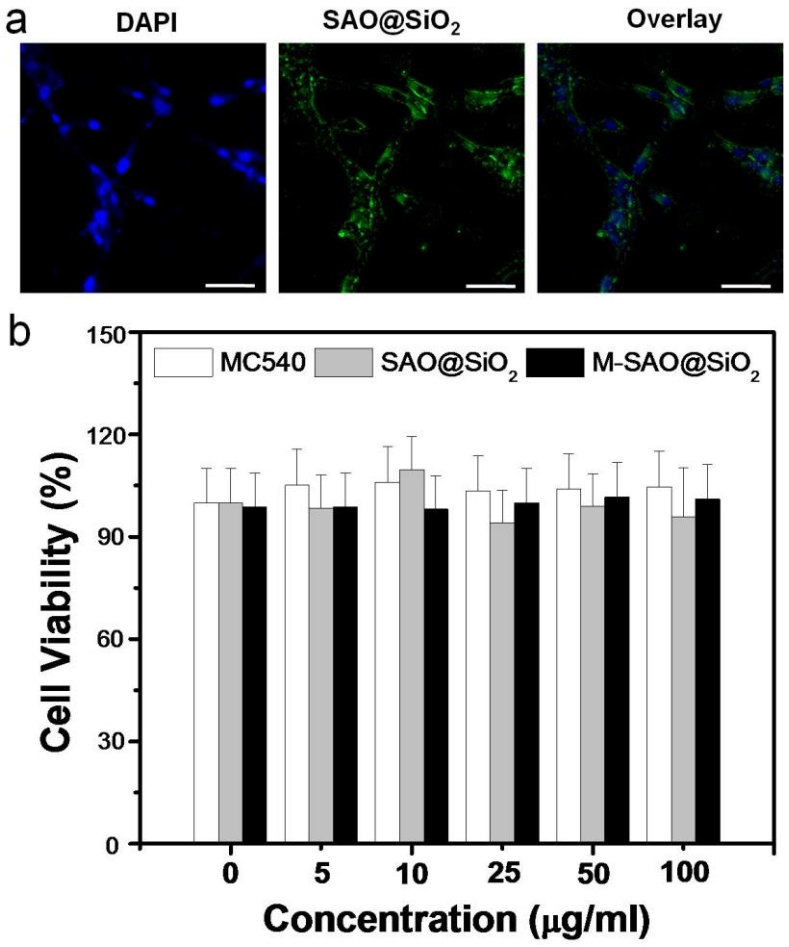

Figure S4. (a) Uptake of $SAO@SiO_2$ nanoparticles by U87MG cells (scale bars: 50 μm). Blue, DAPI (ex/em: 360/460 nm). Green, $SAO@SiO_2$ nanoparticles (ex/em: 360/520nm). (b) MTT assay results with MC540, $SAO@SiO_2$, and M-$SAO@SiO_2$ nanoparticles after 24 h incubation. The error bars represent ± s.e.m. (n = 5 per group).

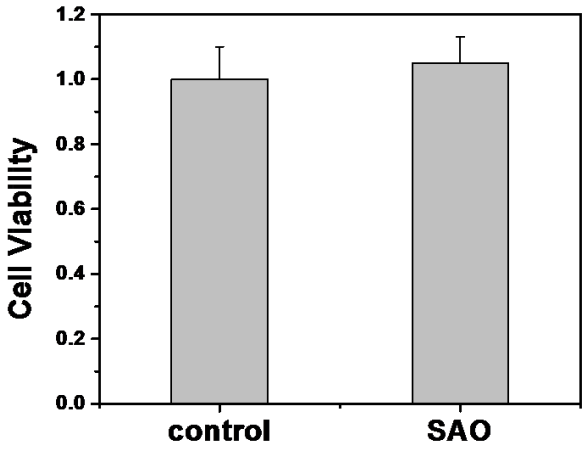

Figure S5. MTT assay results, studied using hydrolytes of bare SAO nanoparticles (0.05 mg/mL). No sign of cytotoxicity was observed. The error bars represent ± s.e.m. (n=5 per group).

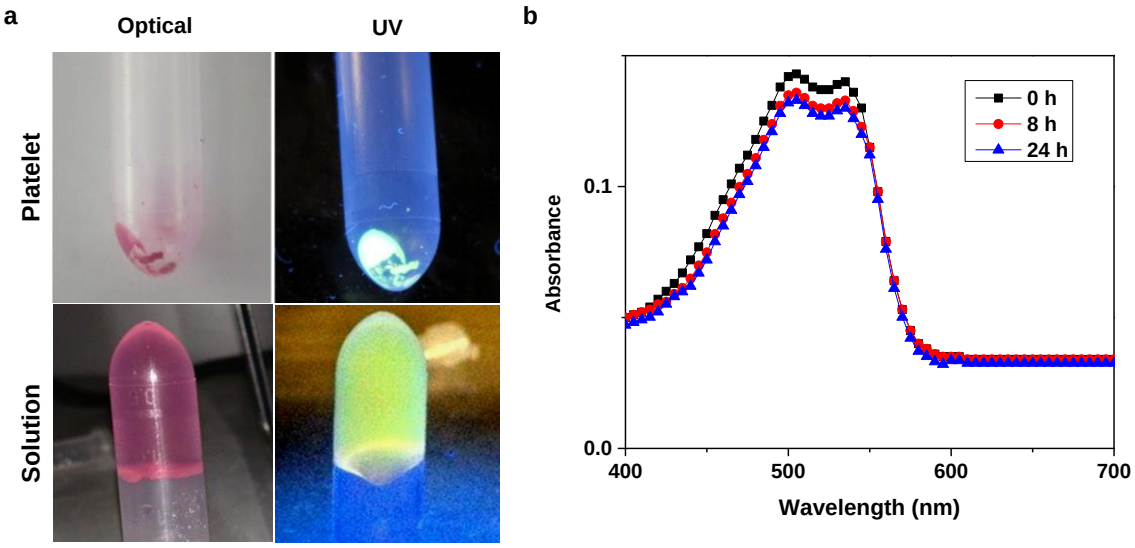

Figure S6. (a) Photographs of M-SAO@SiO_2 in powder (upper panel) and solution (lower panel) when irradiated in the dark by 365-nm UV light. (b) Release of MC540 from M-SAO@SiO_2, investigated by analyzing the change of absorbance over time. Compared to the initial time point (0 h), the release of MC540 at 8 h and 24 h is minimal.

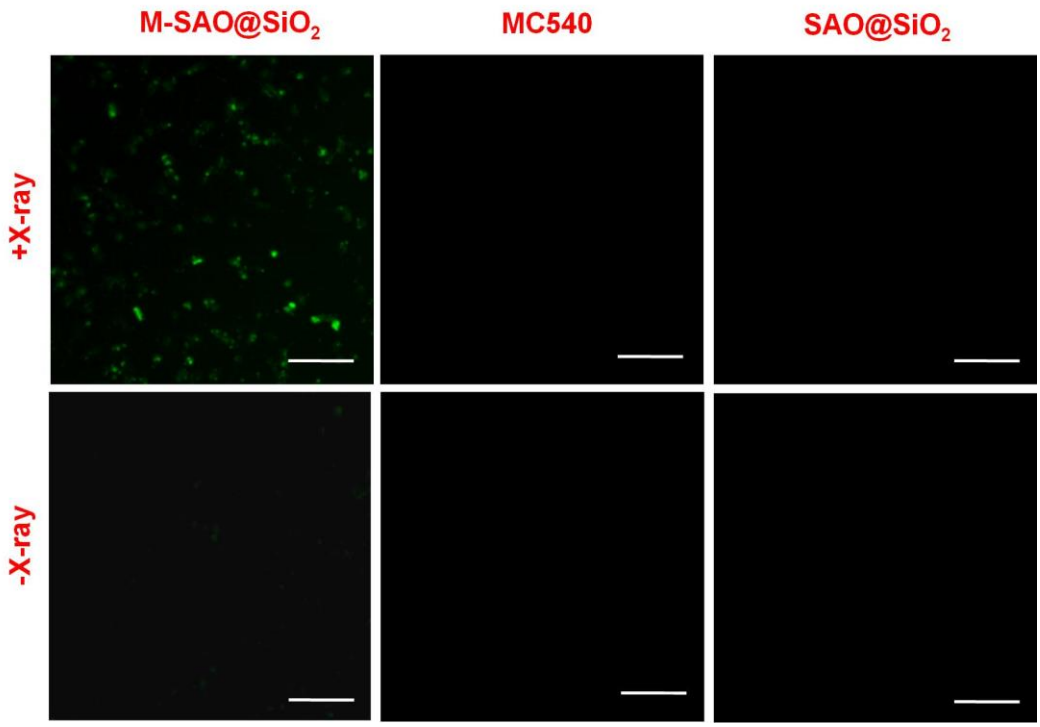

Figure S7. $^1O_2$ production in cells. U87MG cells were incubated with M-SAO@SiO_2 nanoparticles, SAO@SiO_2 nanoparticles, or MC540, with and without subsequent X-ray irradiation. X-ray tube was operated with a tube voltage of 50 KV and a tube current of 70 μA. SOSG was used as a $^1O_2$ indicator. Enhanced fluorescence (ex/em:504/525 nm) was only observed with cells treated with a combination of M-SAO@SiO_2 nanoparticles and X-rays. Scale bars, 100 μm.

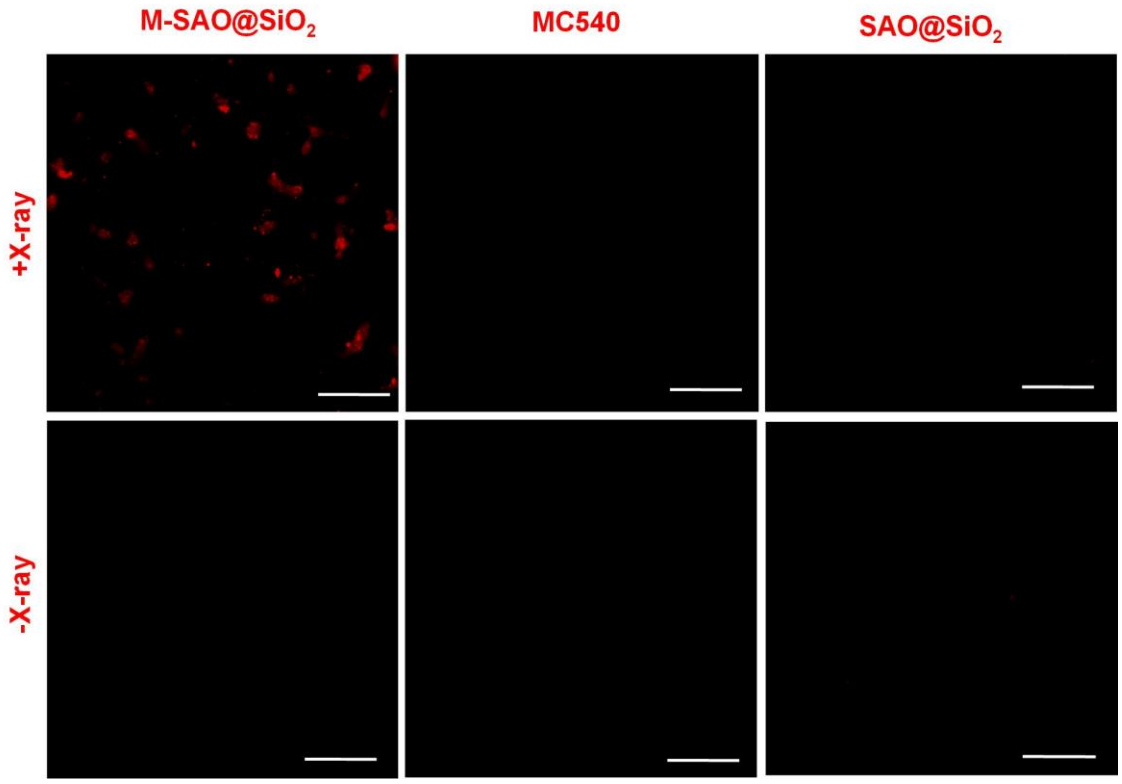

Figure S8. Cytotoxicity induced by X-PDT, studied by ethidium homodimer-1 assay. M-SAO@SiO_2 nanoparticles (0.05 mg/mL) were incubated with U87MG cells for 1 h before X-ray irradiation. X-ray tube was operated with a tube voltage of 50 KV and a tube current of 70 μA. Consistent with the observations made in **Fig. S7**, toxicity was only found with cells treated with the M-SAO@SiO_2 nanoparticle and X-ray combination. Ex/em: 517/617 nm. Scale bars, 100 μm.

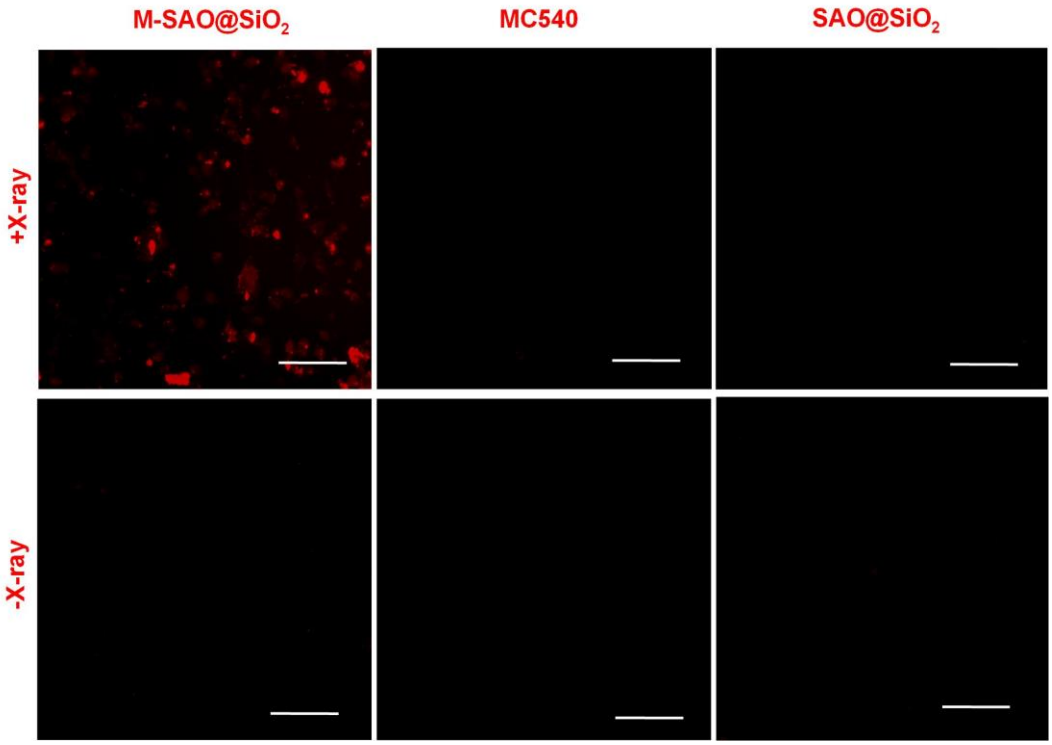

Figure S9. Cytotoxicity induced by X-PDT, studied by ethidium homodimer-1 assay. X-ray tube was operated with a tube voltage of 50 KV and a tube current of 70 μA. M-SAO@SiO_2 nanoparticles (0.05 mg/mL) were incubated with U87MG cells for 24 h before X-ray irradiation. Ex/em: 517/617 nm. Scale bars, 100 μm.

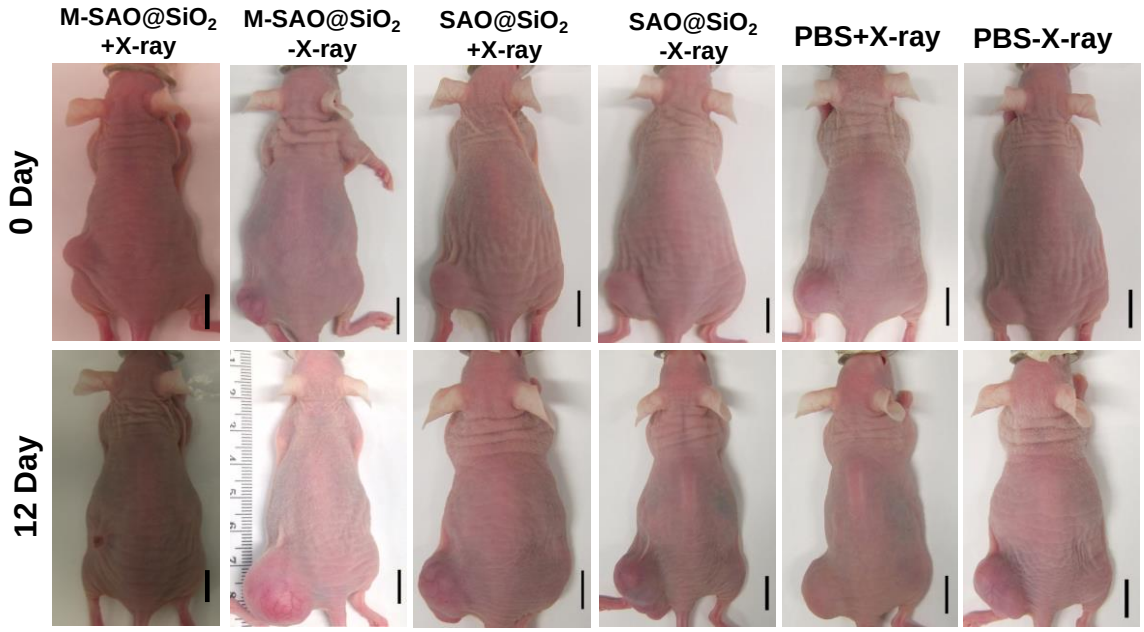

Figure S10. Representative photographs of mice from Groups 1-6 on day 12 (scale bar: 1 cm).

Figure S11. Photographs of tumors taken from Groups 1 (i.e. therapy group).

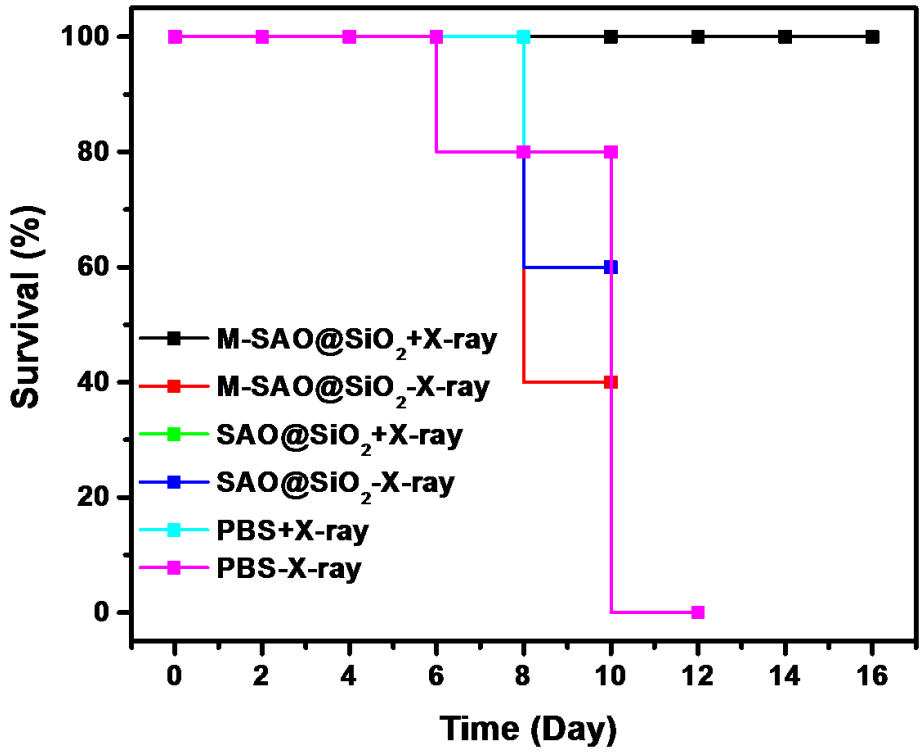

Figure S12. Survival curves for animals from Groups 1-6.

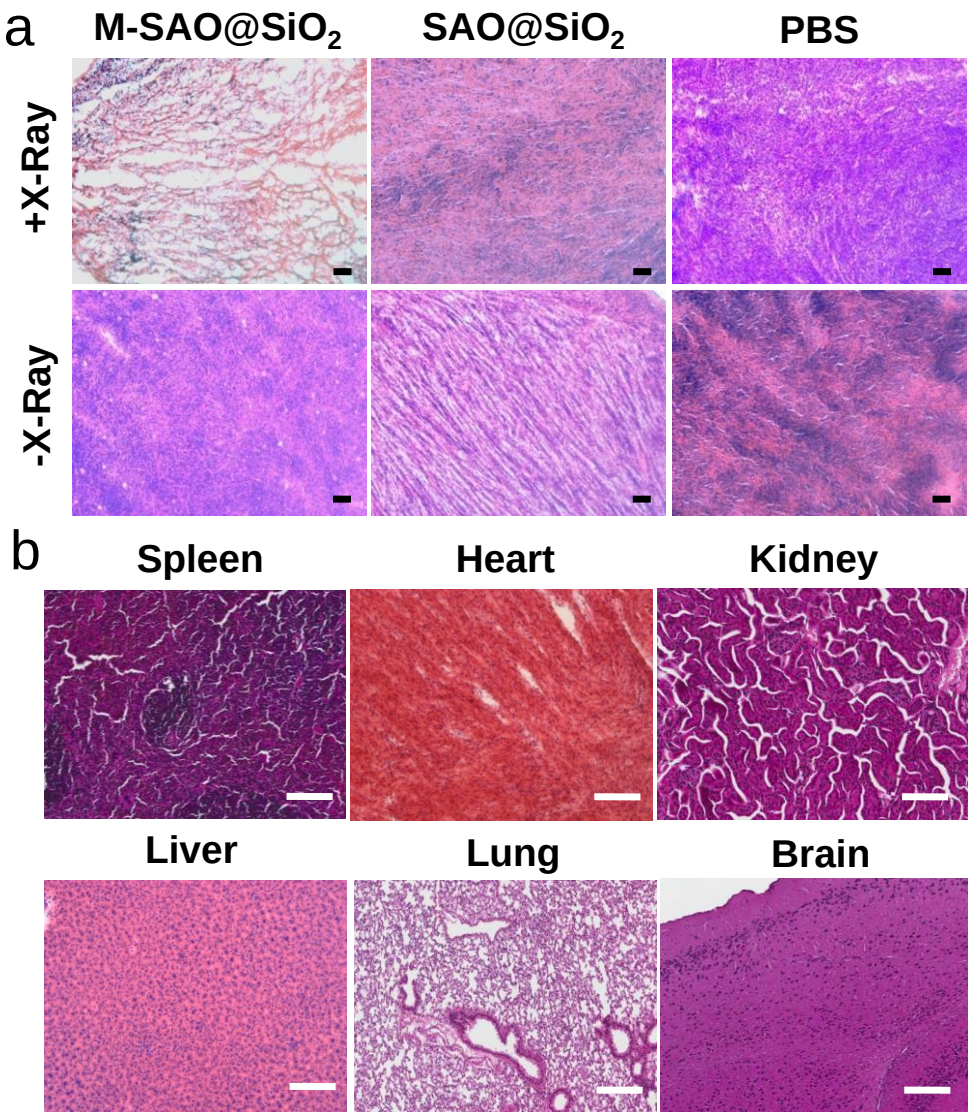

Figure S13. H&E staining results. (a) H&E staining with tumor tissues from different treatment groups. Scale bars, 100 μm. (b) H&E staining with normal tissues taken from Group 1. Scale bars, 100 μm.

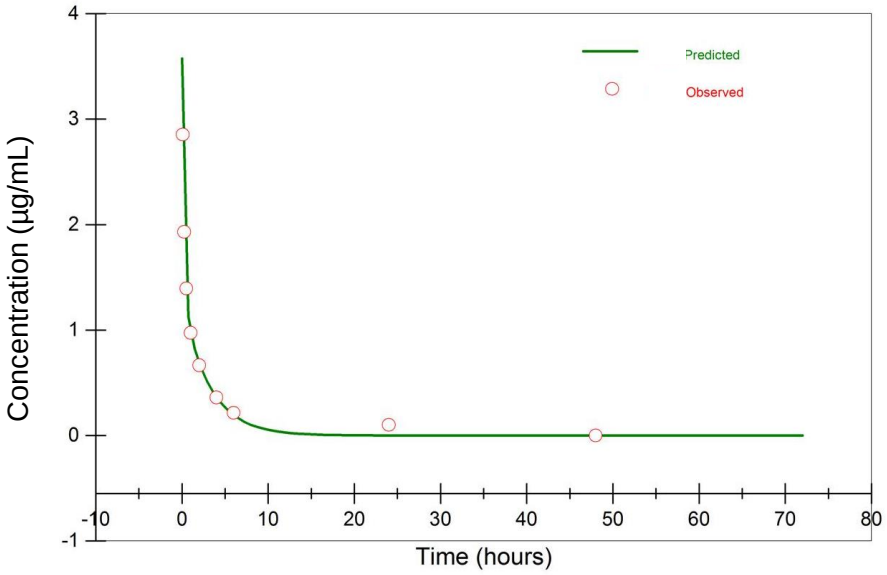

Figure S14. Blood circulation half-life ($t_{1/2}$) of M-SAO@SiO_2 nanoparticles. The plasma concentration of SAO nanoparticles (based on ICP data on Sr) follows a biphasic exponential decay model, with the second phase $t_{1/2}$ of 131 minutes. The data analysis was performed with WinNonlin® (version 5.3).

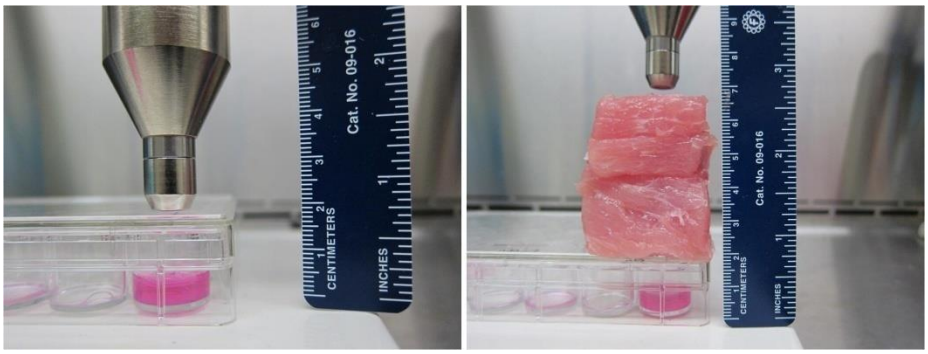

Figure S15. Photographs showing the experimental setup for assessing *in vitro* toxicity induced by X-PDT without (left) and with (right) pork as an X-ray blocker. X-ray tube was operated with a tube voltage of 50 KV and a tube current of 70 μA.

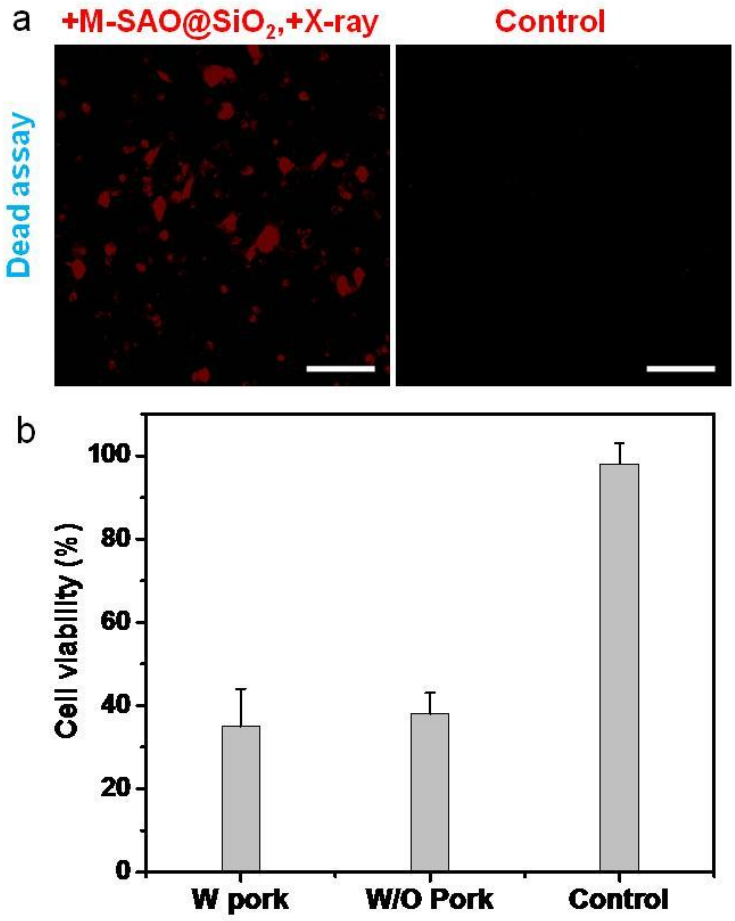

Figure S16. ***In vitro*** **cytotoxicity study with X-PDT, with 4.5-cm pork blocked between the X-ray source and the cells.** (a) Ethidium homodimer-1 assay results. Despite of the thick pork as a blocker, X-rays can effectively activate X-PDT to cause cell death, manifested by enhanced red fluorescence (ex/em: 517/617 nm). Scale bar: 100 μm. (b) Comparison of cytotoxicity, with and without the use of pork as an X-ray blocker. X-ray tube was operated with a tube voltage of 50 KV and a tube current of 70 μA. The error bars represent ± s.e.m. (n = 4 per group).

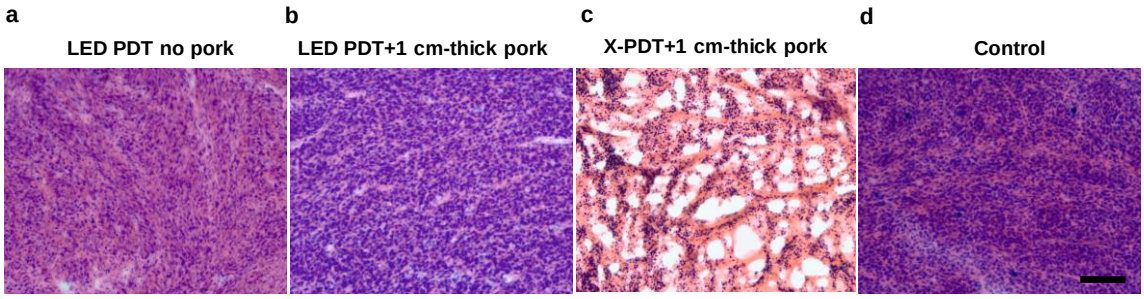

Figure S17. **H&E staining results.** (a) UG87 tumor xenografts were intratumorally injected with M-SAO@SiO_2 nanoparticles (6.25 mg/kg) and irradiated by 520 nm LED light (0.1 W/cm^2) for 30 min. The tumors were excised 14 days after the treatment. (b) A 1-cm-thick pork slice was laid on top of the tumors during the irradiation; otherwise the conditions were the same as those in **a**. No detectable damage was observed for animals from (a) and (b). (c) Animals were injected with M-SAO@SiO_2 nanoparticles but were irradiated by X-ray (1 Gy/h for 30 min); similar to b, a 1-cm-thick pork was laid between the X-ray tube and the tumors. There was a significant decrease in cancer cell density. (d) Animals were injected with PBS and received no irradiation. Scale bar: 100 μm.

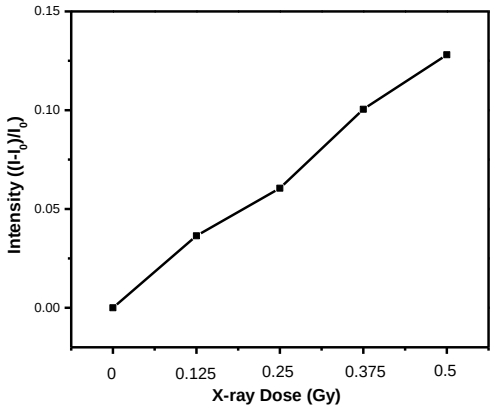

Figure S18. An elevated X-ray dose corresponds with an increased $^1O_2$ production, performed with a 6.25 mg/mL M-SAO@SiO_2 solution. X-ray was operated at 1 Gy/h, 50 kV.

References:

1. Liu, F.; Budai, J. D.; Li, X. F.; Tischler, J. Z.; Howe, J. Y.; Sun, C. J.; Meltzer, R. S.; Pan, Z. W. *Adv. Funct. Mater.* **2013,** 23, 1998-2006.
2. Li, X. F.; Budai, J. D.; Liu, F.; Howe, J. Y.; Zhang, J. H.; Wang, X. J.; Gu, Z. J.; Sun, C. J.; Meltzer, R. S.; Pan, Z. W. *Light-Sci. Appl.* **2013,** 2, e50.
3. Taylor, K. M.; Rieter, W. J.; Lin, W. *J. Am. Chem. Soc.* **2008,** 130, 14358-14359.
4. Kim, J.; Kim, H. S.; Lee, N.; Kim, T.; Kim, H.; Yu, T.; Song, I. C.; Moon, W. K.; Hyeon, T. *Angew. Chem. Int. Ed.* **2008,** 47, 8438-8441.
5. Idris, N. M.; Gnanasammandhan, M. K.; Zhang, J.; Ho, P. C.; Mahendran, R.; Zhang, Y. *Nat. Med.* **2012,** 18, 1580-1585.