Electronic Supplementary Information (ESI) for

Radiation-Induced Photodynamic Therapy Using Calcium Tungstate Nanoparticles and 5-Aminolevulinic acid Prodrug

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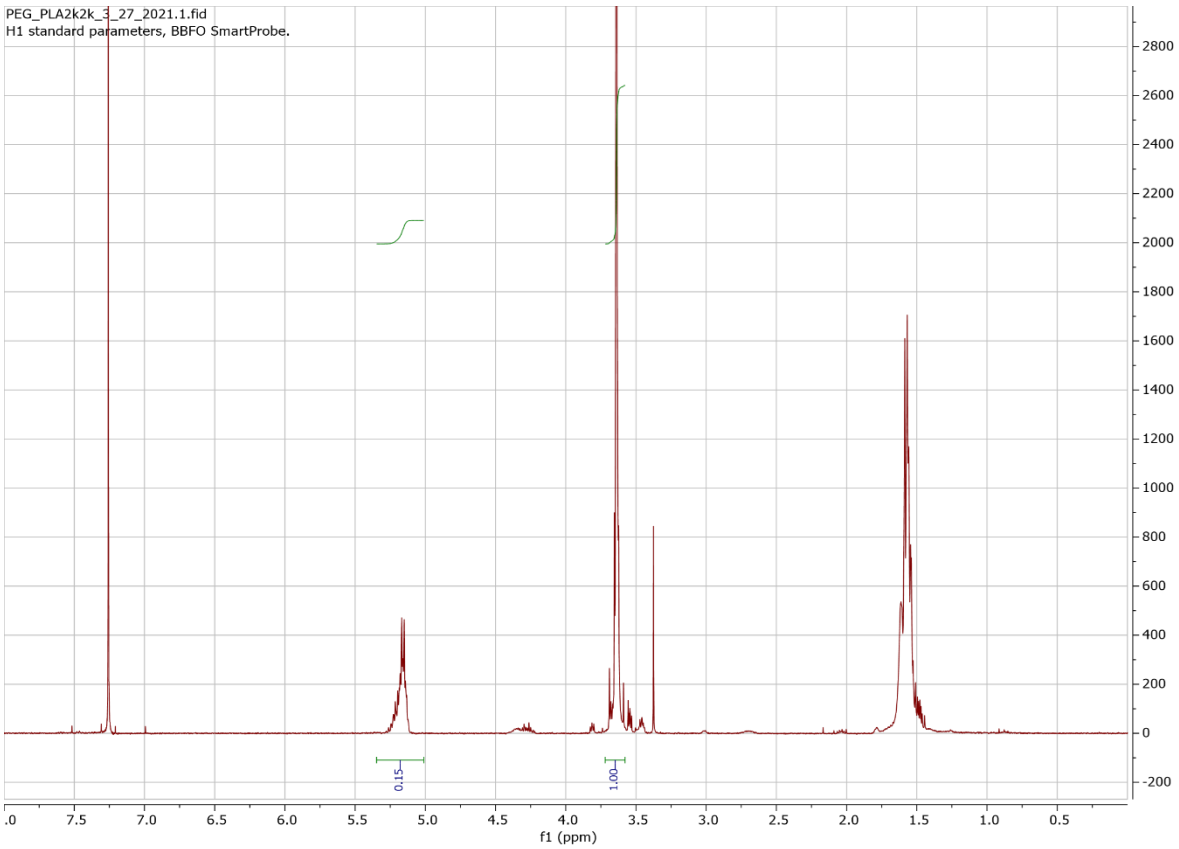
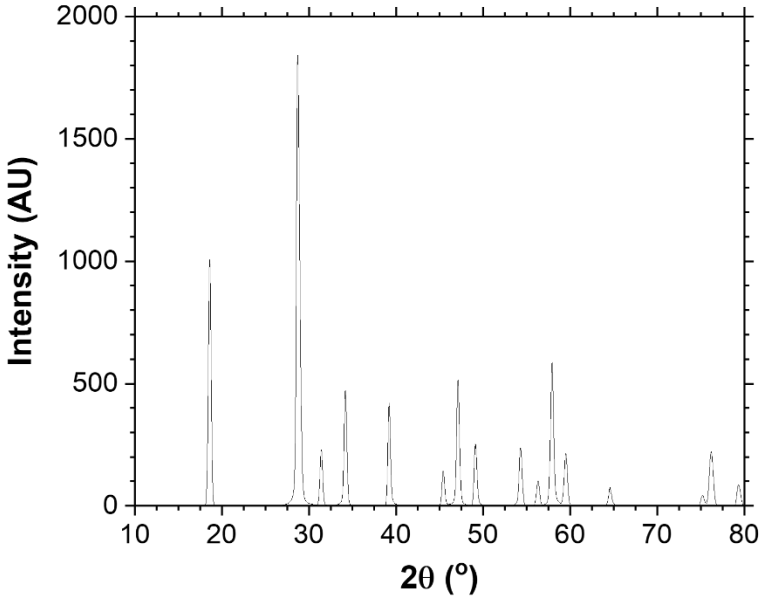


Figure S1. ^{1}H NMR spectra for the PEG-PLA in $CDCl_3$. The M_n of the PLA block was calculated to be 1,932 Da from the area ratio of the peaks corresponding to the methyl protons ($-CH_3$) in the lactate units of the PLA block (5.01 – 5.35 ppm) and the methylene protons ($–CH_2–$) in the ethylene glycol units of the PEG block (3.58 – 3.72 ppm), assuming that the M_n of the PEG block is equal to 2,000 Da (information provided by the vendor).



FWHM (°)	0.4288
Shape Factor	0.9
Wavelength (Å)	1.54
Bragg Angle (°)	28.72
Grain Size (nm)	21.13

Figure S2. X-ray diffraction (XRD) pattern of CWO NPs obtained in the parallel beam (PB) mode. The average crystallite (grain) size (presented in the accompanying table) was estimated from the XRD pattern using the Debye-Scherrer equation.

CWO NPs

PEG-PLA/CWO NPs

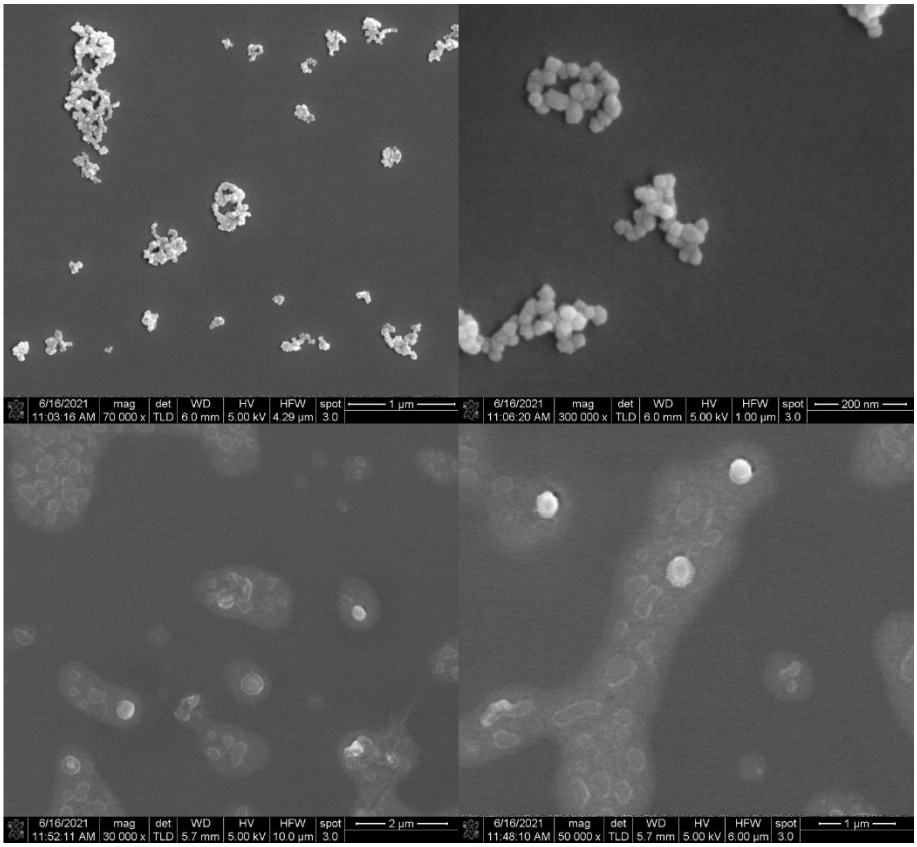


Figure S3. Representative SEM images of (A) pristine (uncoated) CWO NPs and (B) PEG-PLA-encapsulated CWO NPs. NPs were dried on a silicon substrate for SEM imaging.

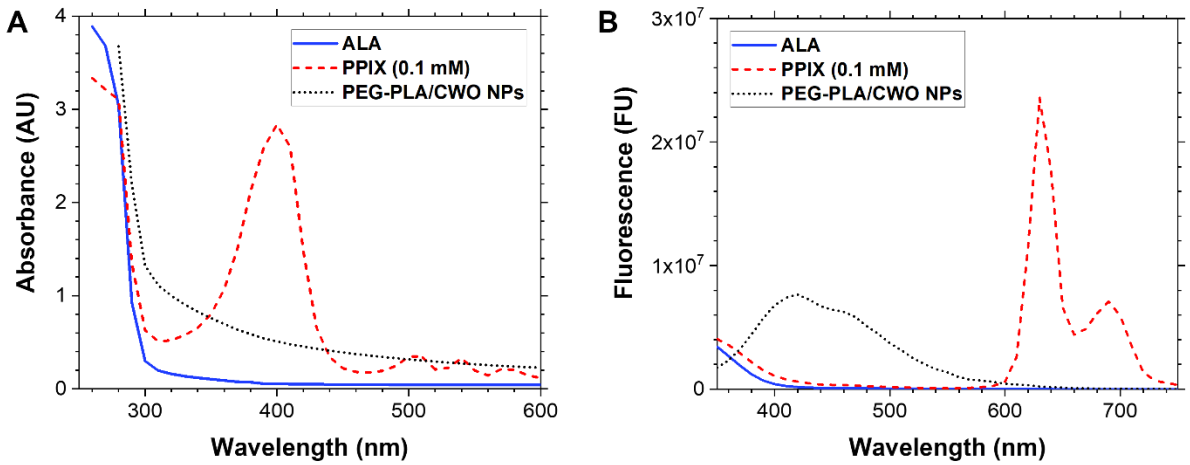


Figure S4. (A) Absorbance and (B) fluorescence spectra of ALA (1.0 mM in a 50:50 by volume mixture of DMF and water), PPIX (0.1 mM in a 50:50 by volume mixture of DMF and water), and PEG-PLA/CWO NPs (0.1 mg/mL (based on CWO mass) in water). Step size for wavelength scanning = 10 nm. Excitation wavelength for fluorescence measurements = 250 nm.

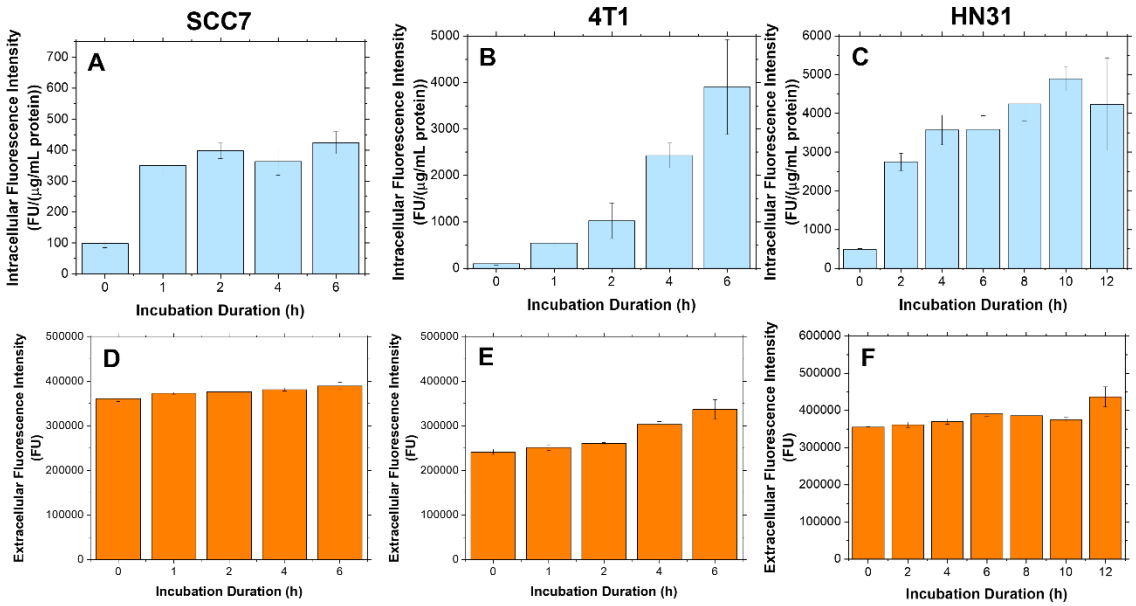


Figure S5. (A – C) Intracellular and (D – F) extracellular PPIX fluorescence intensities in three different cell lines. The fluorescences were measured after varying durations of incubation with 1.0 mM ALA. The intracellular fluorescence intensity was normalized by the total protein concentration determined via BCA assay. Error bars represent standard deviations (N = 4).

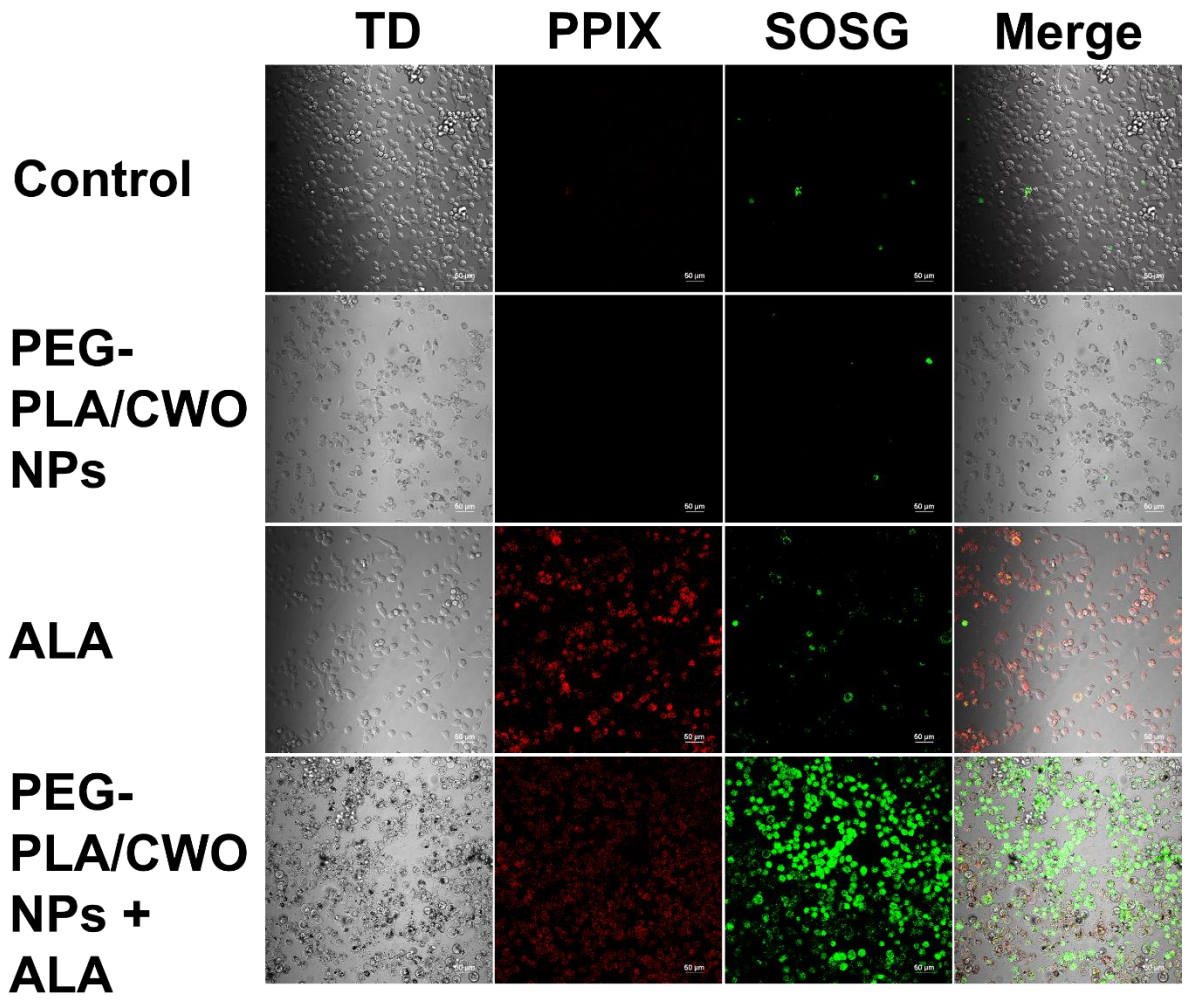
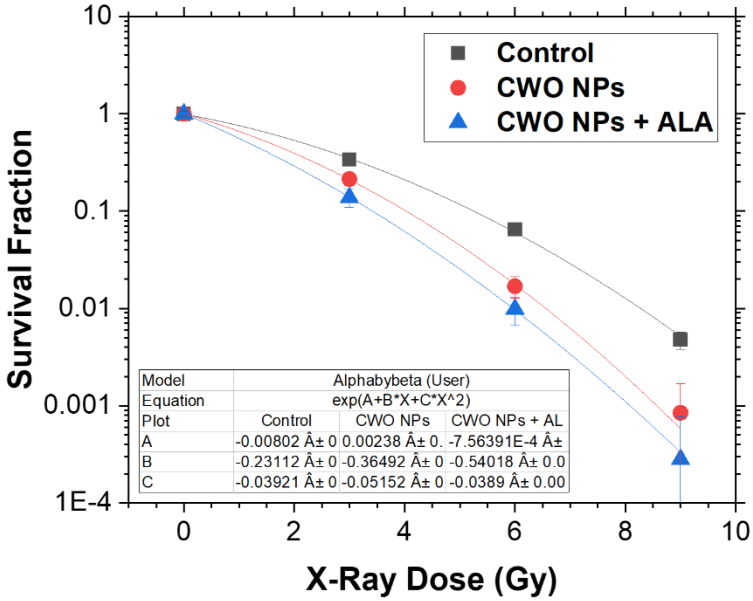
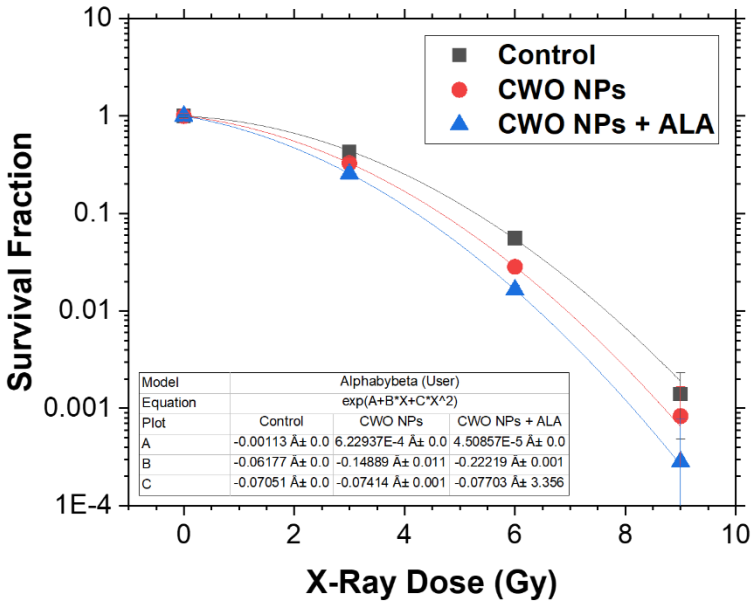


Figure S6. Low magnification confocal microscopy images of SCC7 cells treated with PEG-PLA/CWO NPs (0.2 mg/mL based on CWO mass), ALA (1mM) or a combination of the two. After overnight incubation, all samples were stained with SOSG and irradiated with 8 Gy X-rays. PPIX and SOSG were excited using 405 and 488 nm lasers, respectively. All images were acquired at identical laser settings and displayed with identical lookup table (LUT) values for direct comparison. Scale bar = 50 µm.



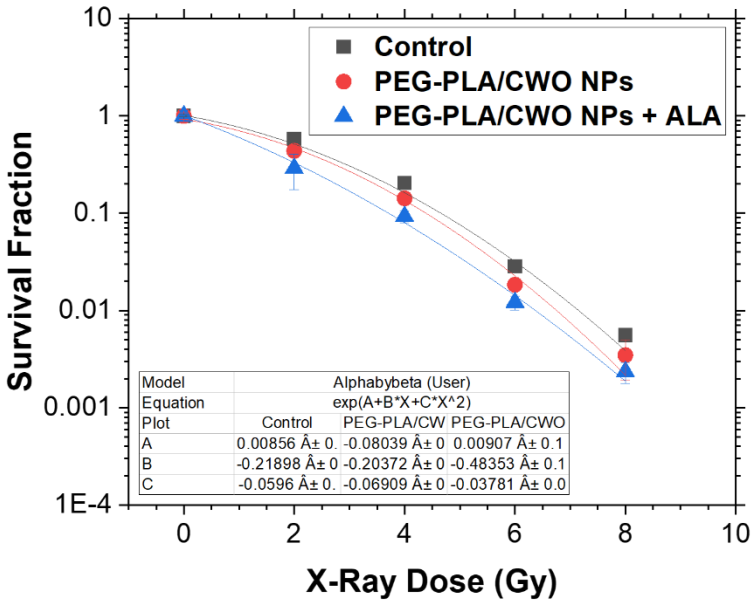
	α	β	α/β	SER
Control	-0.231	-0.039	5.894	1.000
CWO NPs	-0.365	-0.052	7.083	1.304
CWO NPs + ALA	-0.540	-0.039	13.886	1.534

Figure S7. Clonogenic survival of HN31 cells post RT-PDT. HN31 cells were plated in 6-well plates at densities of 0.2 × 10^3, 1.0 × 10^3, 2.0 × 10^3 and 5.0 × 10^3 cells/well for 0, 3, 6 and 9 Gy, respectively, incubated with CWO NPs (0.2 mg/mL), ALA (1.0 mM), or both, for 4 hours, and irradiated with varying doses of X-rays. Surviving colonies were counted after 6 cell division cycles (~ 14 – 15 days). Error bars represent standard deviations (N = 3). The survival curves were fit to the exponential quadratic decay formula, *SF* = exp($\alpha D + \beta D^2$) where *SF* = survival fraction, and *D* = X-ray dose (Gy), using α and β as fitting parameters. The resulting α/β and SER values (calculated at 10% cell survival) are given in the accompanying table.



	α	β	α/β	SER
Control	-0.062	-0.071	0.876	1.000
CWO NPs	-0.149	-0.074	2.008	1.136
CWO NPs + ALA	-0.222	-0.077	2.884	1.256

Figure S8. Clonogenic survival of SCC7 cells post RT-PDT. SCC7 cells were plated in 6-well plates at densities of 0.4 × 10^3, 2.0 × 10^3, 5.0 × 10^3 and 10.0 × 10^3 cells/well for 0, 3, 6 and 9 Gy, respectively, incubated with CWO NPs (0.1 mg/mL), ALA (1.0 mM), or both, for 4 hours, and irradiated with varying doses of X-rays. Surviving colonies were counted after 6 cell division cycles (~ 6 – 7 days). Error bars represent standard deviations (N = 3). The survival curves were fit to the exponential quadratic decay formula, $SF = \exp(\alpha D + \beta D^2)$ where SF = survival fraction, and D = X-ray dose (Gy), using α and β as fitting parameters. The resulting α/β and SER values (calculated at 10% cell survival) are given in the accompanying table.



	α	β	α/β	SER
Control	-0.219	-0.060	3.674	1.000
PEG-PLA/CWO NPs	-0.204	-0.069	2.952	1.062
PEG-PLA/CWO NPs + ALA	-0.484	-0.038	12.804	1.257

Figure S9. Clonogenic survival of SCC7 cells post RT-PDT. SCC7 cells were plated in 6-well plates at densities of 0.4×10^3, 1.0×10^3, 2.0×10^3, 5.0×10^3 and 10.0×10^3 cells/well for 0, 2, 4, 6 and 8 Gy, respectively, incubated with PEG-PLA/CWO NPs (0.1 mg/mL based on CWO mass), ALA (1.0 mM), or both, for 4 hours, and irradiated with varying doses of X-rays. Surviving colonies were counted after 6 cell division cycles (~ 6 – 7 days). Error bars represent standard deviations (N = 3). The survival curves were fit to the exponential quadratic decay formula, $SF = \exp(\alpha D + \beta D^2)$ where SF = survival fraction, and D = X-ray dose (Gy), using α and β as fitting parameters. The resulting α/β and SER values (calculated at 10% cell survival) are given in the accompanying table.

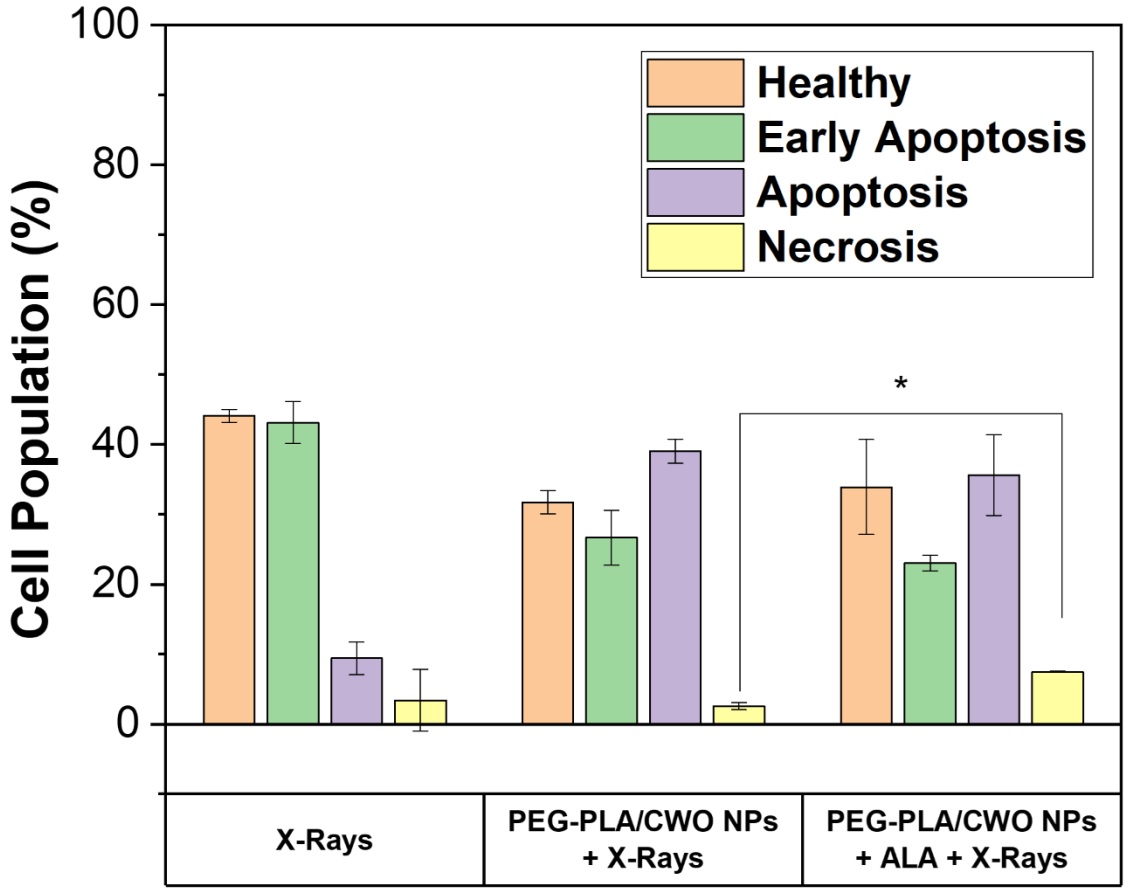


Figure S10. Mode of cell death in 4T1 cells. 4T1 cells were seeded in glass bottom dishes, treated with PEG-PLA/CWO NPs ± ALA for 4 hours, irradiated with 8 Gy X-rays, incubated for 24 hours, and stained with FITC-Annexin V and Ethidium Homodimer-III for confocal microscopy analysis; see the Methods section for details. Brightfield images were segmented to isolate individual cells. The mean fluorescence intensity was calculated for each cell. Fluorescence intensities were gated against untreated 4T1 cells (control) and sorted as positive for FITC (early apoptosis), Ethidium Homodimer-III (necrosis), both (apoptosis), or neither (healthy).

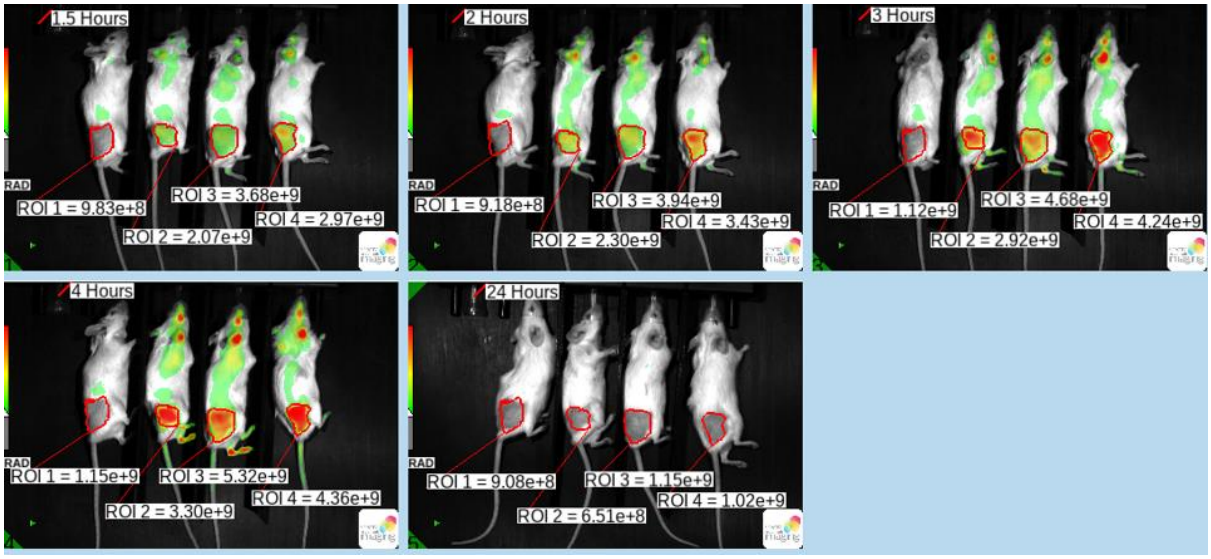


Figure S11. Processed AMI images of tumor-bearing mice treated with ALA demonstrating intratumoral PPIX pharmacodynamics. 4T1 allografts were established in BALB/c mice as described in the Methods section. Once tumors reached 100 mm^3, ALA (100 µL, 50 mg/mL dissolved in PBS containing 0.5 % methyl cellulose) was administered to each mouse via oral gavage. PPIX production by ALA metabolization in tumor was tracked as a function of time using Spectral AMI (λ_{ex} = 430 nm, λ_{em} = 650 nm). The region of interest (ROI, tumor) is marked with a red circle. In each panel, the leftmost animal was untreated (control), and the 3 animals on the right were treated with ALA.

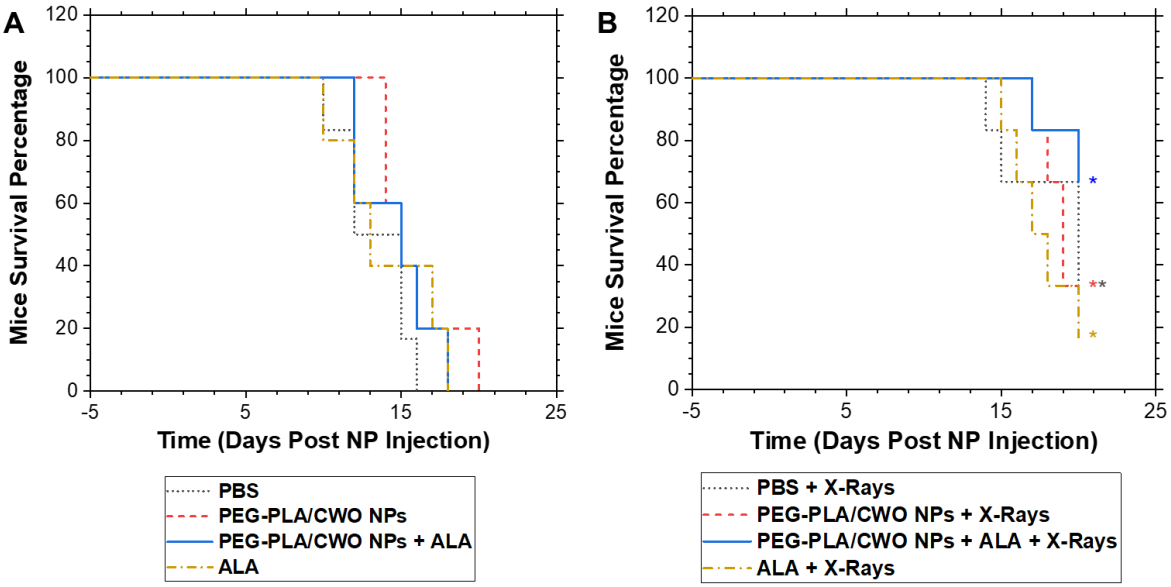


Figure S12. Kaplan-Meier curves for survival of BALB/c mice bearing 4T1 allografts treated with PEG-PLA/CWO NPs ± ALA in the (A) absence and (B) presence of X-rays. 4T1 allograft tumor growth in BALB/c mice. 4T1 cells (5×10^6 cells/mL in PBS, 100 µL) were subcutaneously implanted into the right flanks of BALB/c mice on Day -5. Once tumors reached ~ 75 – 100 mm^3, blank PBS or NP suspensions in PBS (50 mg/mL based on CWO mass, 10 µL per injection) were intratumorally injected at 0 and 1 days post 4T1 implantation. An ALA solution in PBS (50 mg/mL, 100 µL per administration) containing 0.5% methyl cellulose was administered via oral gavage on Days 1 and 2. On each of those days, tumors were irradiated with 4 Gy X-rays at 4 hours post ALA administration. The study was concluded on Day 20 post NP Injection due to the significant ulceration observed in the tumors beyond that point; ulceration causes erroneous tumor volume measurements. A statistical analysis was performed on survival percentage data on Day 20 using log-rank analysis. See Table S1 for details of the analysis results. Asterisks have been used to denote the final survival percentages on Day 20.

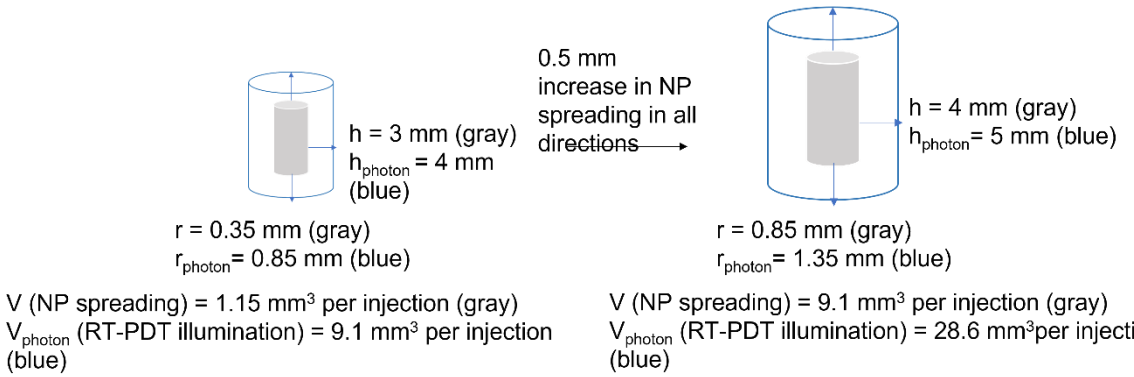


Figure S13. Schematic explaining the calculating of the RT-PDT illumination volume within tumor.

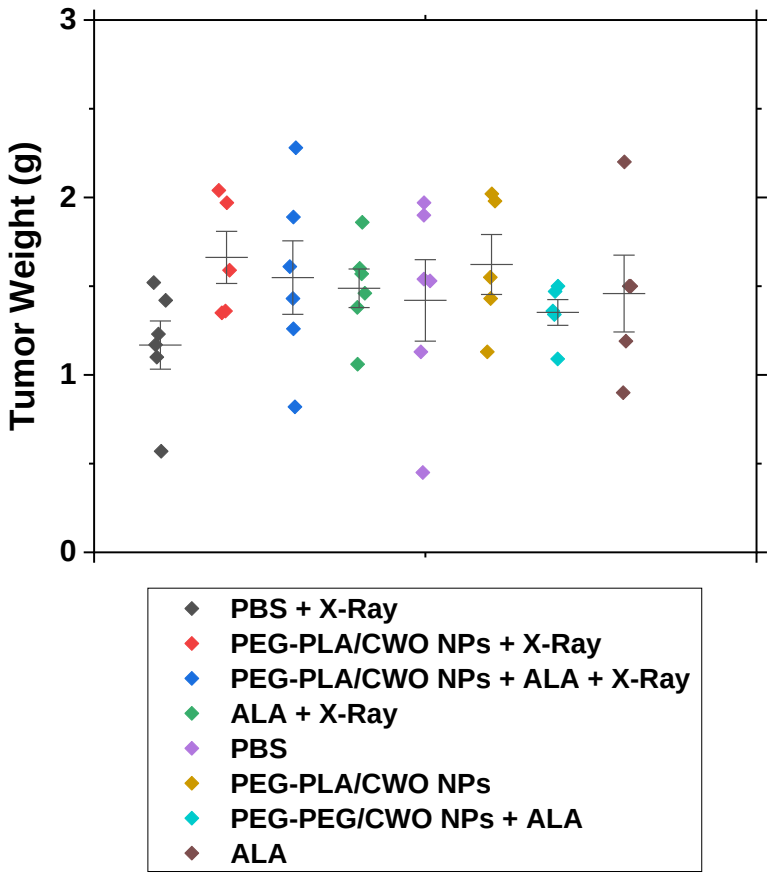


Figure S14. Tumor weights were measured after excision. It is important to note that the tumors were excised at different time points, and therefore direct comparisons between the groups do not accurately represent therapeutic efficacy.

Table S1. Log-rank pairwise analysis of the mouse survival data presented in Figure S12. Pairs with statistically significant ($p < 0.05$) and highly statistically significant ($p < 0.01$) differences are highlighted in yellow and green, respectively.

		No X-Rays			X-Rays			
		PEG-PLA/CWO NPs	PEG-PLA/CWO NPs + ALA	ALA	PBS	PEG-PLA/CWO NPs	PEG-PLA/CWO NPs + ALA	ALA
No X-Rays	PBS	0.257	0.455	0.507	0.031	0.002	0.002	0.016
	PEG-PLA/CWO NPs	-	0.853	0.704	0.340	0.265	0.059	0.464
	PEG-PLA/CWO NPs + ALA	-	-	0.764	0.101	0.021	0.011	0.189
	ALA	-	-	-	0.066	0.027	0.014	0.218
X-Rays	PBS	-	-	-	-	0.801	0.866	0.723
	PEG-PLA/CWO NPs	-	-	-	-	-	0.297	0.800
	PEG-PLA/CWO NPs + ALA	-	-	-	-	-	-	0.203