Supplementary data

EGFR targeted nanobody functionalized polymeric micelles loaded with mTHPC for selective photodynamic therapy

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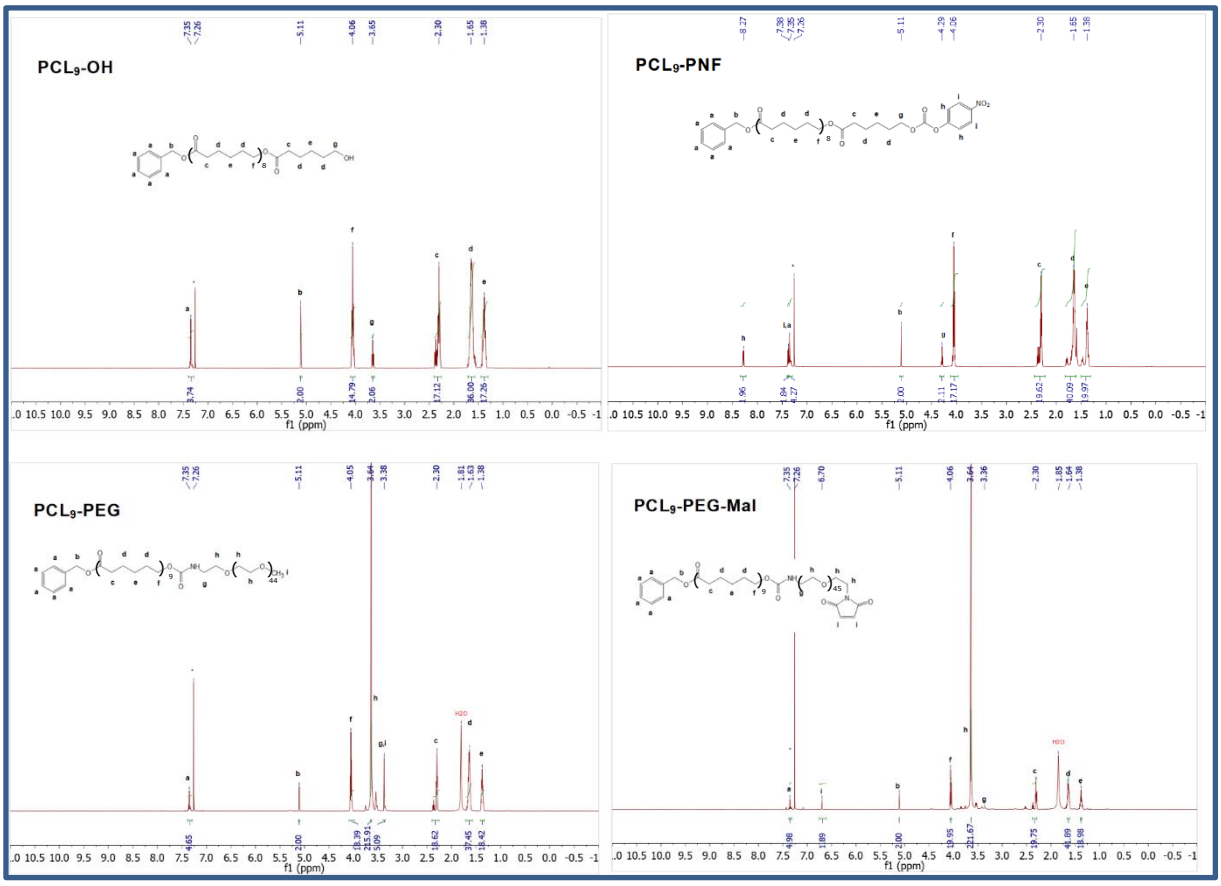
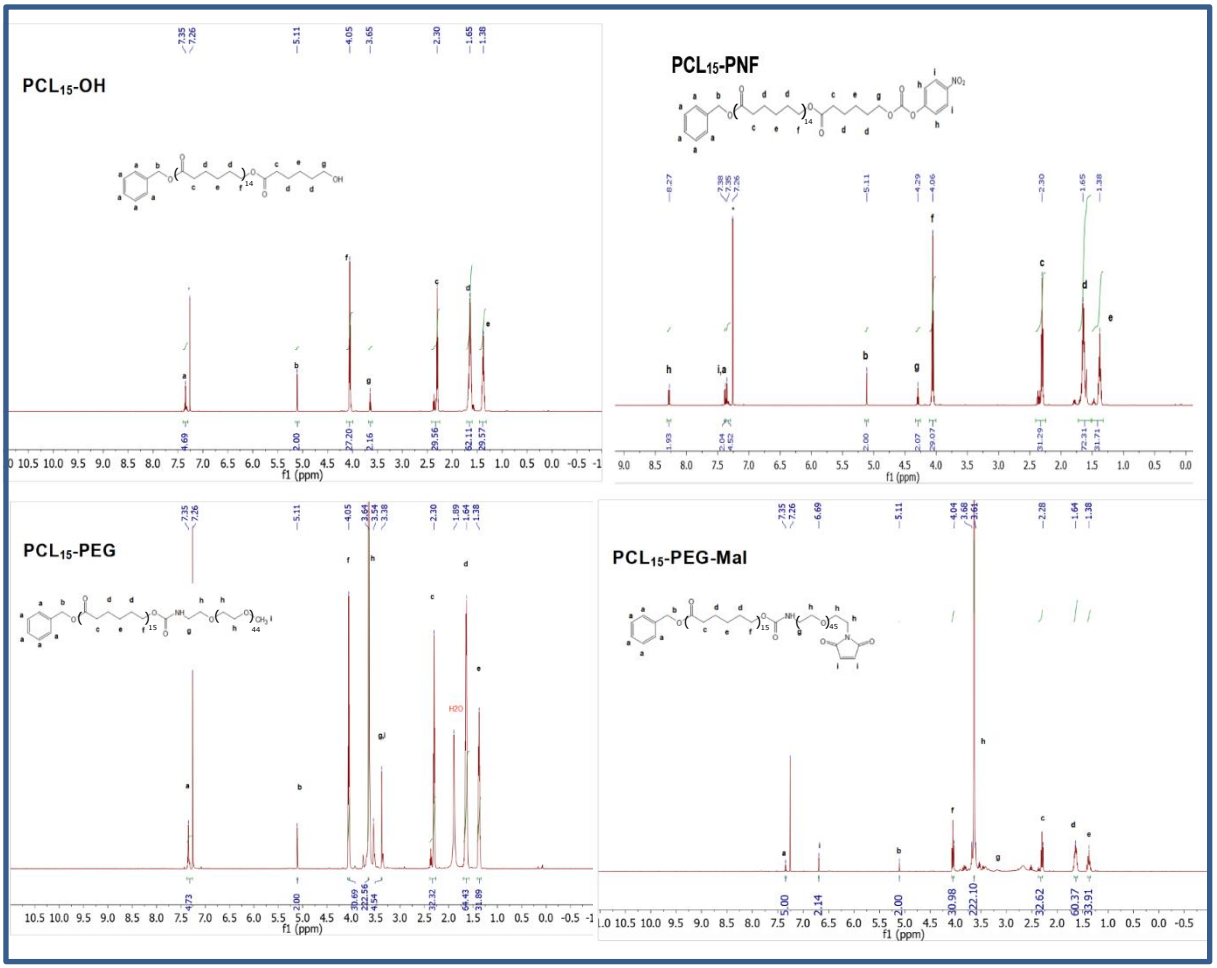

PCL9-OH
PCL9-PNF
PCL9-PEG
PCL9-PEG-Mal
f1 (ppm)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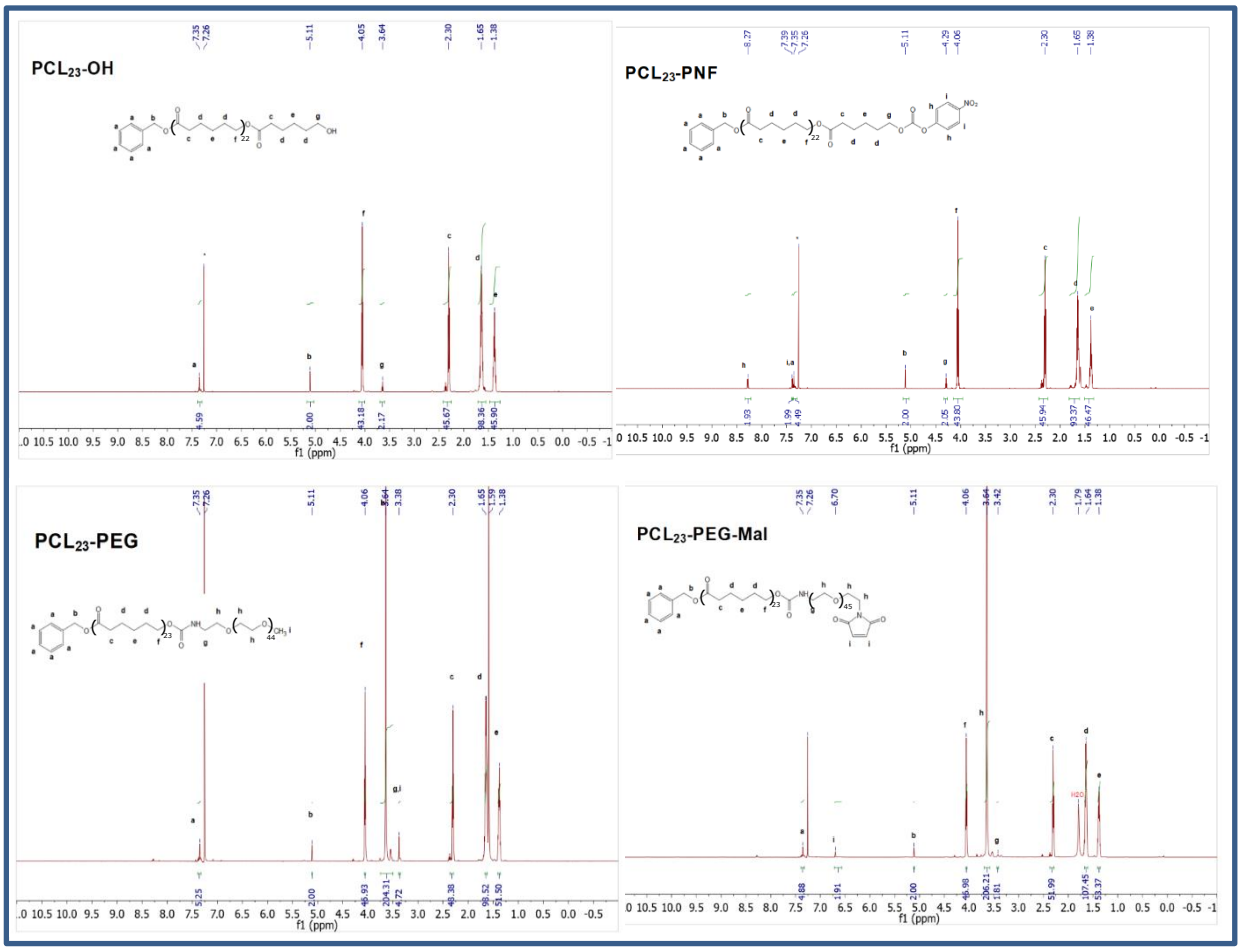
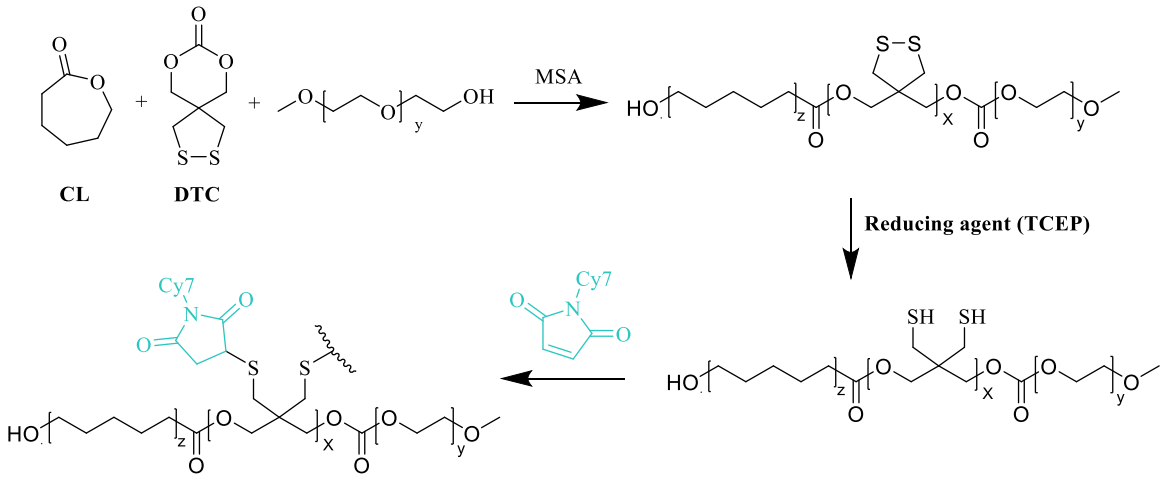


Figure S1. ^{1}H NMR spectra of the PCL_n oligomers, intermediate products and final PCL_n-PEG/PCL_n-PEG-Mal block copolymers (n=9, 15 and 23).



Scheme S1. Synthesis and Cy7 labeling of PCL-PDTC-PEG.

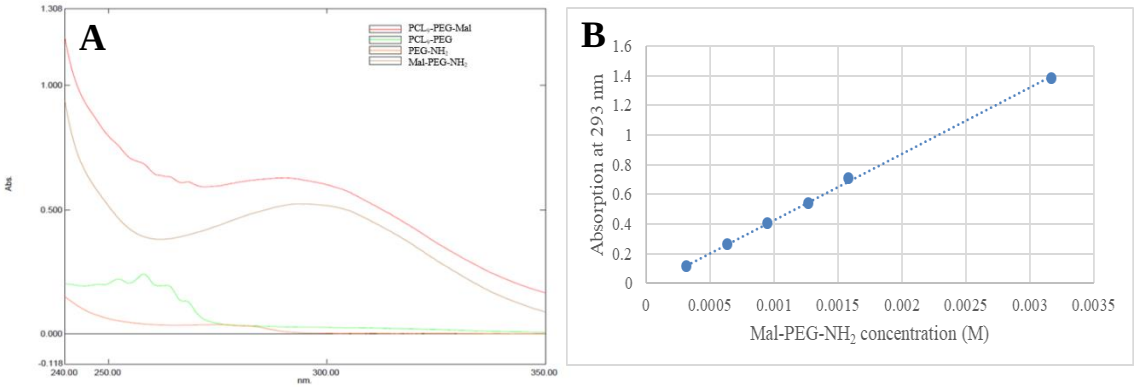


Figure S2. (A) UV-Vis spectra of PCL_9-PEG-Mal, Mal-PEG-NH_2, PCL_9-PEG, PEG-NH_2 in DCM at a polymer concentration of 5 mg/mL and (B) calibration curve of Mal-PEG-NH_2 in DCM.

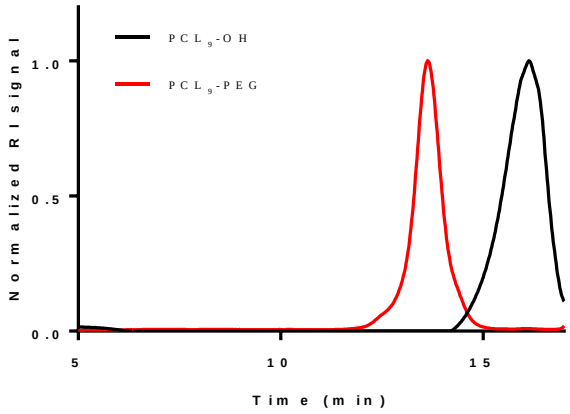


Figure S3. GPC chromatograms of PCL_9-OH and PCL_9-PEG recorded by refractive index (RI) detector.

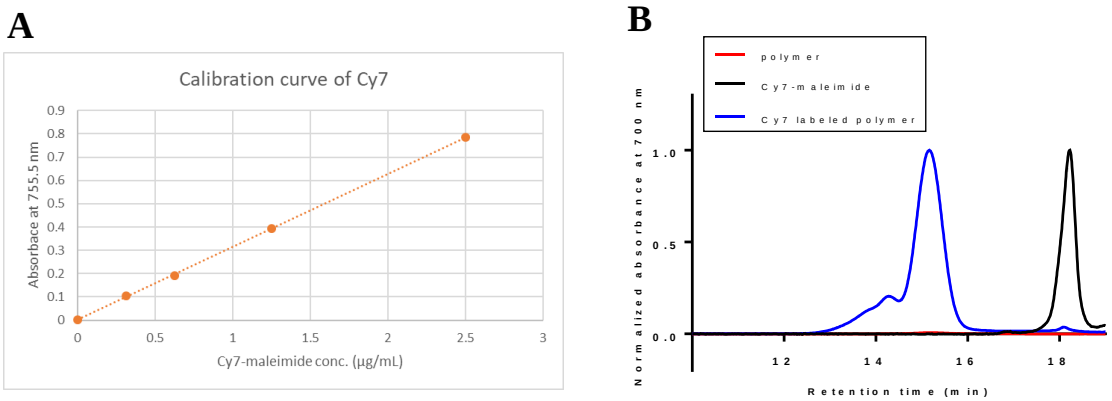
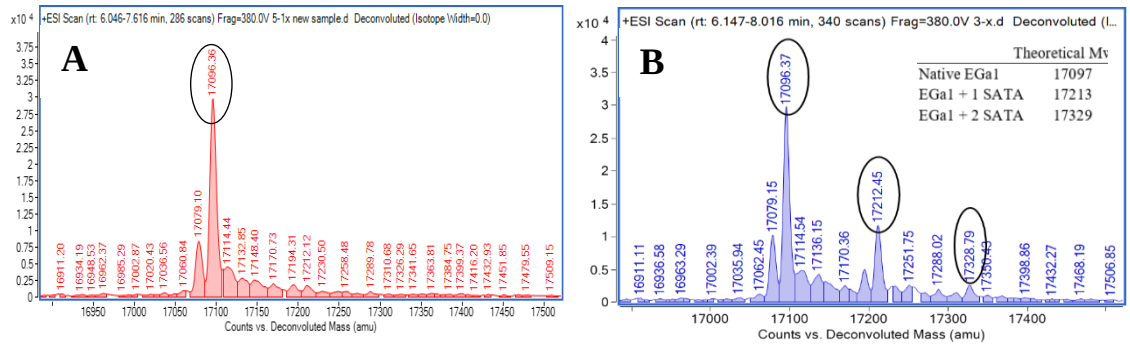


Figure S4. (A) Calibration curve of Cy7-maleimide in DCM, recorded at 755.5 nm and (B) GPC curves of Cy7-maleimide, PCL_{18}-$PDTC_{7.5}$-PEG polymer and Cy7-labeled PCL_{18}-$PDTC_{7.5}$-PEG polymer, recorded at 700 nm by UV/vis detector.



C

	Native EGa1	Reduced EGa1	EGa1-SATA
Average number of sulfhydryls	0.19±0.01	1.93±0.13	1.84±0.06

Figure S5. (A-B) LC-ESI-TOF-MS spectra of native EGa1 (A) and SATA modified EGa1 (B) at a 1:5 molar ratio of EGa1 to SATA, respectively. The inset of theoretical mass in (B) correlated with the molecular weight of 0 to 2 SATA molecules conjugated to one EGa1 molecule. (C) the number of sulfhydryls (-SH) per native EGa1, reduced EGa1 and SATA modified EGa1 after 1:5 molar incubation ratio of EGa1 to SATA based on Ellman's assay.

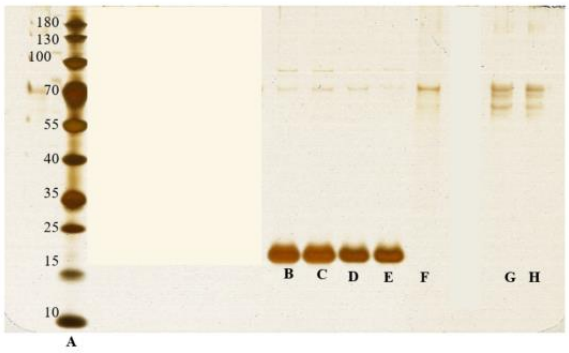


Figure S6. SDS-PAGE silver staining of SATA-EGa1 and micelles composed of 9:1 mixtures of PCL_n-PEG and PCL_n-PEG-Mal incubated with/without (protected) SATA-EGa1. A is the protein ladder; lane B represents SATA-modified EGa1; lanes C-E are P_n micelles incubated with protected EGa1-SATA (n=9 (C), n=15 (D), n=23 (E)); lanes F-H display P_n micelles alone (n=9 (F), n=15 (G), n=23 (H)).

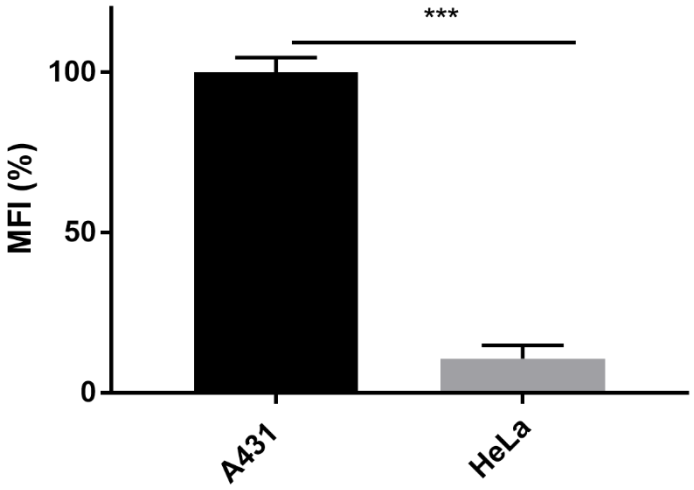


Figure S7. EGFR expression in A431 and HeLa cells as determined by flow cytometry, using cells that were incubated with an antibody against the EGFR receptor. *** represents $p < 0.001$.

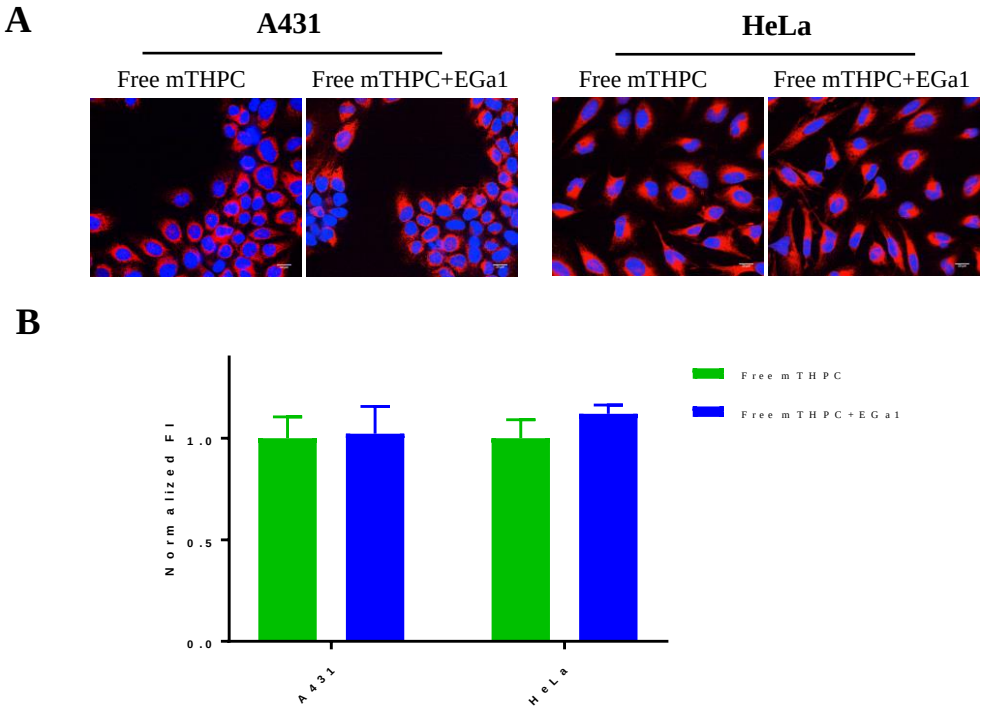


Figure S8. (A) Representative confocal fluorescence microscopic images of A431 and HeLa cells incubated with free mTHPC and mTHPC containing a 9 fold excess of free EGa1 (named free mTHPC+EGa1) for 7 h at 37 °C. mTHPC concentration was 38 µg/mL, corresponding to a concentration using 5wt% mTHPC-loaded micelles as used in Figure 2. Cell nuclei are stained in blue with Hoechst, while fluorescence of mTHPC is represented in red. Scale bars indicate 20 µm. The images were taken using the same parameters in both cells (excitation times requied

for images were 25 msec). (B) Quantification of fluorescence intensity of mTHPC (λ_{ex} 405 nm, λ_{em} 676 nm) in A431 and HeLa cells by ImageJ. The quantified fluorescence intensity was normalized by the intensity of mTHPC in A431 cells and by the number of cells.

S1 Cytotoxicity of empty micelles

EGa1-P_n micelles without mTHPC loading and their corresponding non-targeted micelles at a fixed polymer concentration of 10 mg/mL in PBS (prepared as described in Section 2.4 and 2.5) were employed to assess the cytotoxicity of the empty micelles on A431 and HeLa cells, following the similar protocol in photo-cytotoxicity study (described in section 2.9) with a slight adjustment of the volume ratio between the medium and the micellar dispersions, depending on the predetermined polymer concentration. For instance, to get a final polymer concentration of 4 mg/mL, 80 µL of micellar dispersions was added to 120 µL medium and the cells were cultured for 24 h at 37 ℃. Cell viability was determined by MTS assay.

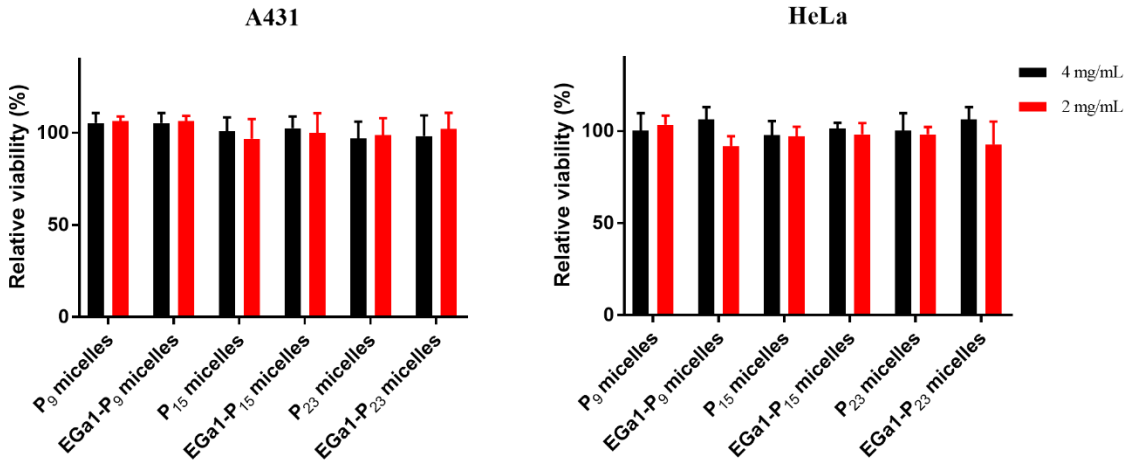


Figure S9. Cytotoxicity assessed by MTS assay of empty targeted EGa1-P_n and non-targeted P_n micelles composed of 2 and 4 mg/mL PCL_n-PEG/PCL_n-PEG-Mal (9:1) polymer on A431 and HeLa cells after 24 h incubation at 37 ℃ (n = 9, 15 and 23, respectively). N=3.

Table S1. Loading efficiency (LE) and loading capacity (LC) of mTHPC loaded in P_{23} micelles decorated with EGa1 nanobody (targeted) or cysteines only (non-targeted).

Feed ratio of mTHPC to polymer(wt%)	P_{23} micelles (non-targeted)		EGa1-P_{23} micelles (targeted)	
	LE%	LC%	LE%	LC%
1%	65	0.3	65	0.3
2%	94	1.8	93	1.8
4%	94	3.6	95	3.6
6%	83	4.7	83	4.7
8%	72	5.4	72	5.4
10%	76	7.1	76	7.1

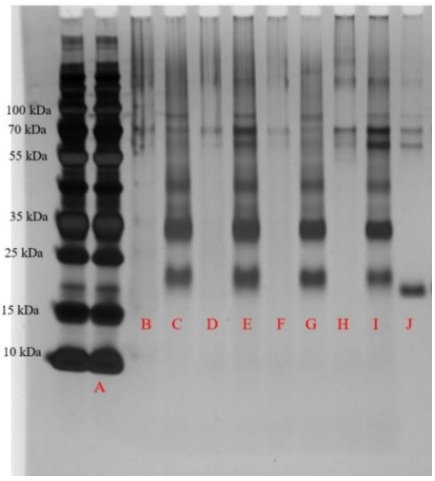


Figure S10. SDS-PAGE silver staining of mTHPC-loaded EGa1-conjugated and unconjugated micelles (*i.e.* EGa1-P_{23} micelles (lanes I, G, E, C) and P_{23} micelles (lanes H, F, D, B), respectively) with 2, 4, 6, 8 wt% mTHPC, respectively, after 10 washings with PBS using centrifugation with Vivaspin-6 tubes along with native EGa1 control (J). Lane A represents a protein ladder.

Table S2. EC_{50} of free mTHPC and mTHPC loaded in micelles (1 mg/mL polymer) on A431 and HeLa cells after 7 h incubation.

	EC_{50} (μg/mL)	
	A431	**HeLa**
P_{23} micelles	38.3±1.9	29.2±2.6
EGa1-P_{23} micelles	10.4±0.7	28.5±1.8
Competition	48.3±3.1	32.7±1.0
Free mTHPC	1.6 ±0.1	1.2[a]
Free mTHPC+EGa1	1.7±0.1	1.3[a]

[a] SD < 0.1.

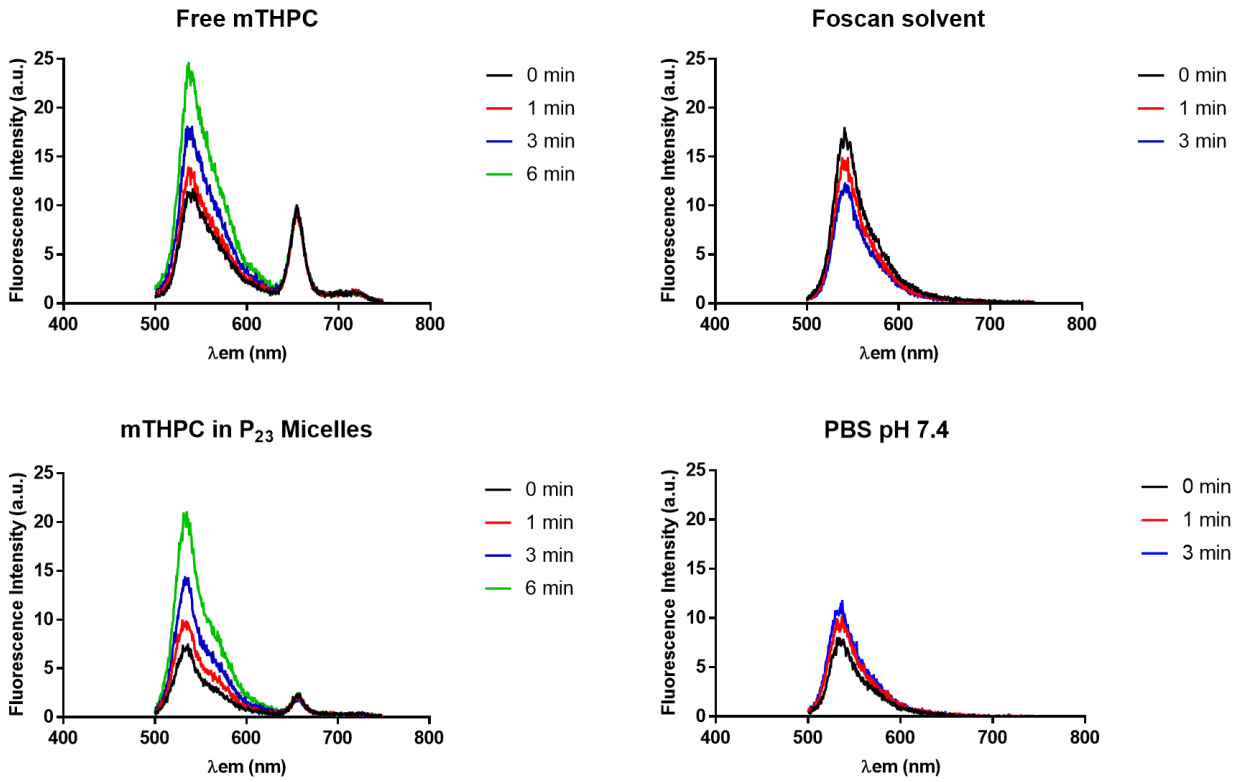


Figure S11. Fluorescence emission spectrum of singlet oxygen sensor green reagent in free mTHPC solution or its solvent only; in mTHPC in P_{23} micelles, or in PBS pH 7.4. Measurements performed after 0, 1, 3 or 6 min of illumination with a filtered white light source at 645-665 nm at a fluence rate of 5 mW/cm^2.

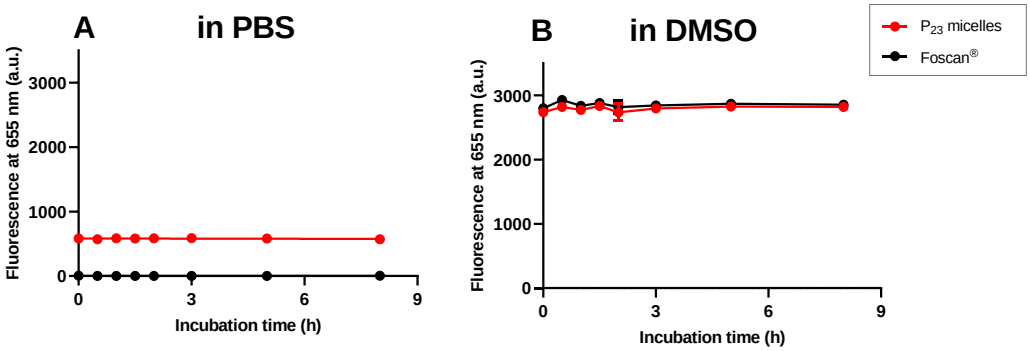


Figure S12. Fluorescence intensity (λ_{ex} 420 nm, λ_{em} 655 nm) as a function of time at 37 °C in PBS (A) and DMSO (B); Foscan® and micelles of 10 mg/mL with 5wt% loading amounts were prepared and diluted 10× in PBS or DMSO, to obtain the final mTHPC concentration of 40 μg/mL.

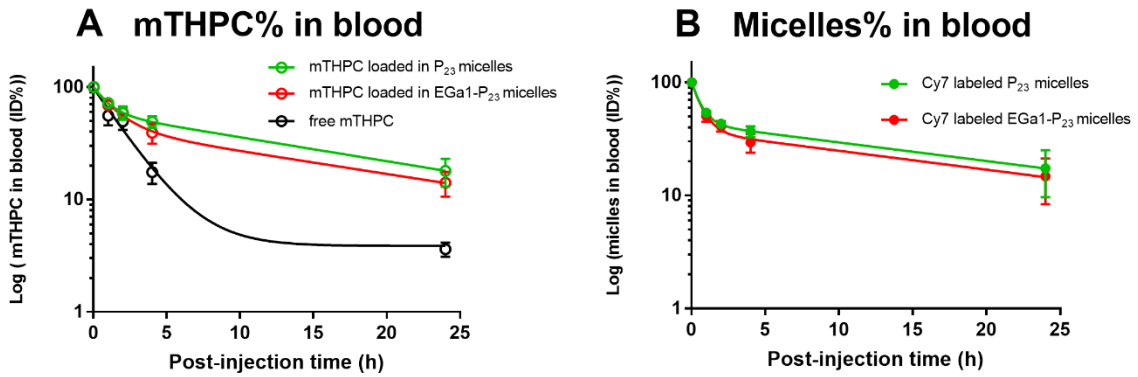


Figure S13. (A-B) The log-concentration of free mTHPC (A) and Cy7 labeled (EGa1)-P_{23} micelles (B) loaded with mTHPC (A) in blood overtime upon tail vein administration in A431 tumor-bearing Balb/c mice (0.3 mg mTHPC per kg bodyweight of the mouse, *i.e.*, ~6 µg mTHPC). Blood samples taken at different time points were used to quantify the percentage of mTHPC and the corresponding Cy7 labeled micelles of the injected dose (%ID) present in systemic circulation. Data are presented as mean ± SD, N= 4.