Supporting Information

FAP Targeting of Photosensitizer-Loaded Polymersomes for Increased Light-Activated Cell Killing

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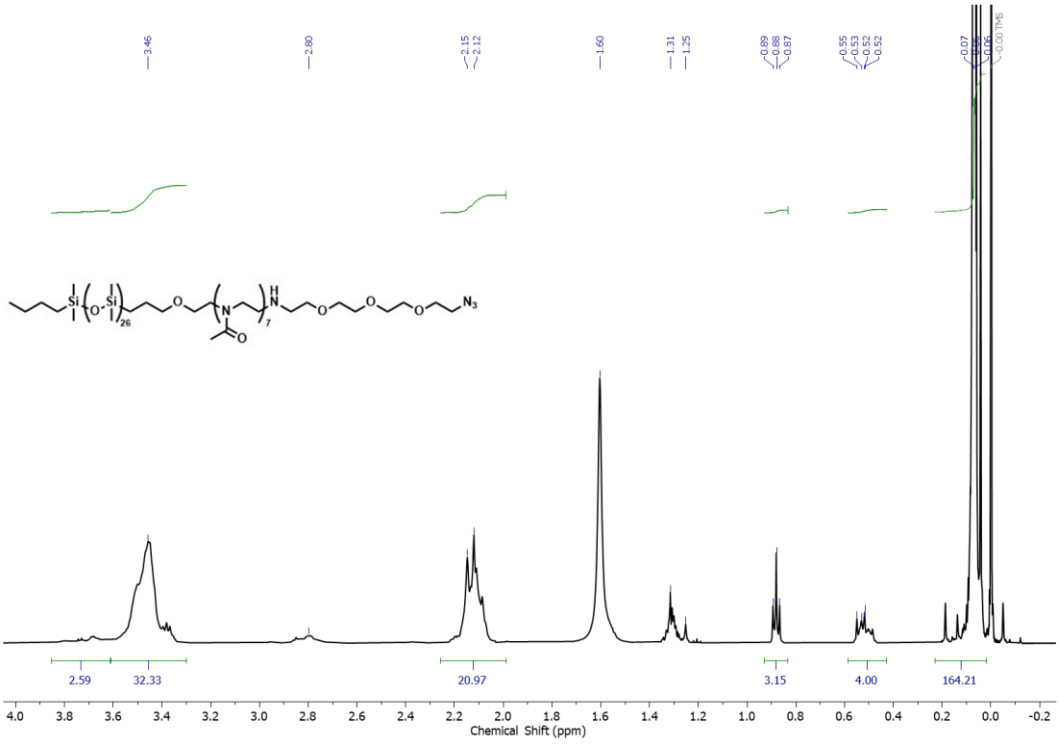

Figure S1. ^{1}H NMR of $PDMS_{27}$-$PMOXA_{7}$-PEG_{3}-N_{3}

Table S1. Determination of the size and concentration of polymersomes by nanoparticle tracking analysis (NTA). Samples were diluted 1:4000 – 1:2000 in PBS prior measurements. The obtained results were multiplied by the dilution factor.

	Concentration (NPs/mL)	**Hydrodynamic radius R_h (nm)**
Pol-Atto633	2.0×10^{12} +/- 3.5×10^{10}	55 ± 3
FAPi-Pol-Atto633	$7.0 \times 10^{11} \pm 1.2 \times 10^{10}$	58 ± 5
Pol-RB	$9.1 \times 10^{11} \pm 2.0 \times 10^{10}$	54 ± 4
FAPi-pol-RB	$9.2 \times 10^{10} \pm 2.8 \times 10^{9}$	58 ± 7

Table S2. Determination of the size of polymersomes by dynamic light scattering (DLS) measured at fixed angle (angle of 173°) and Zeta-potential measurements.

	R_h (nm)	**Zeta potential (mV)**
Pol-Atto633	68 ± 5	−4.0 ± 0.5 mV
FAPi-Pol-Atto633	73 ± 7	−6.2 ± 0.5 mV

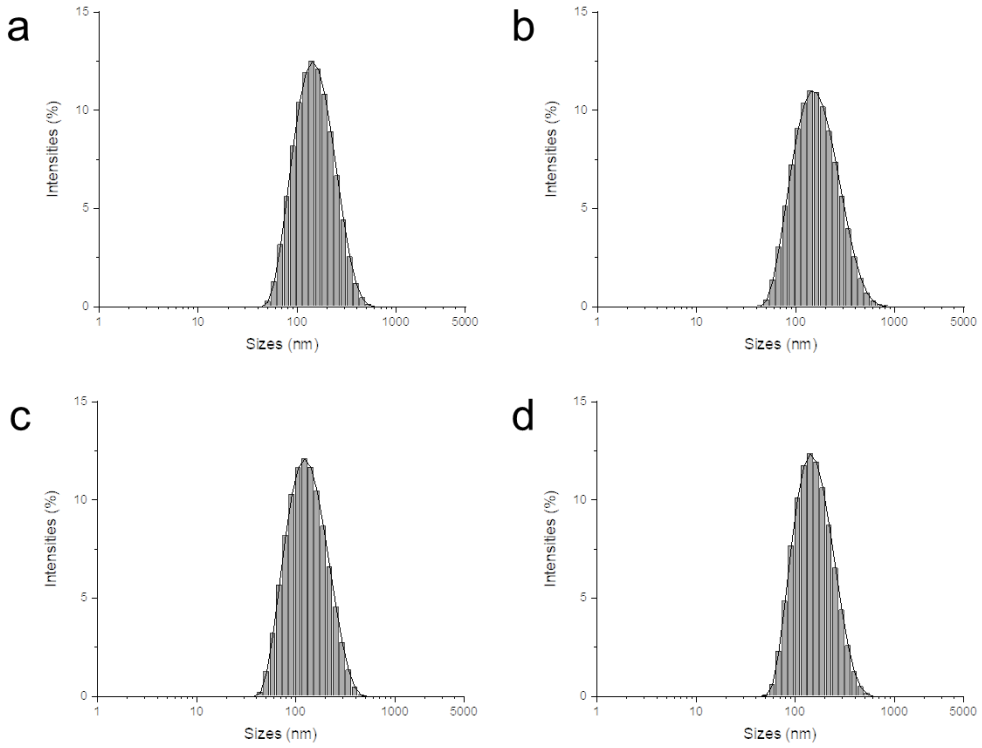

Figure S2. Polymersome size distribution measured by DLS at fixed angle (angle of 173°). (a) Pol-Atto633, (b) FAPi-Pol-Atto633, (c) Pol-RB, (d) FAPi-Pol-RB. Samples were diluted 1:500 – 1:1000 in PBS prior measurements.

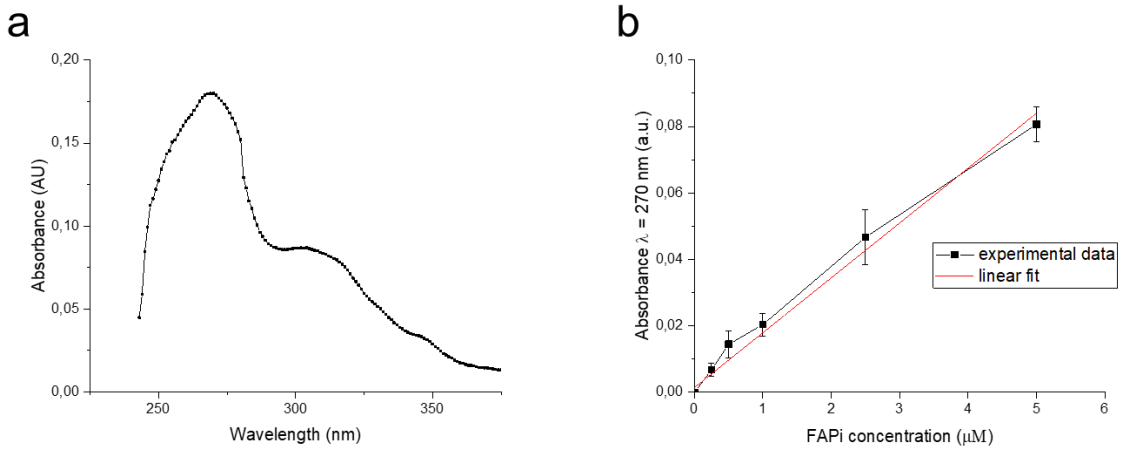

Figure S3. UV absorbance spectra of DIBO-PEG$_3$-FAPi. (a) Absorbance spectrum of 8 μM DIBO-PEG$_3$-FAPi in PBS containing 2% DMF. Absorbance spectrum has been recorded at range of λ = 243 nm - 375 nm and the background absorbance of the solution (2% DMF in PBS) has been subtracted. (b) linear calibration curve of absorbance (λ = 270 nm) vs. concentration of DIBO-PEG$_3$-FAPi (0.25 – 5 μM).

Table S3. Determination of the size of polymersomes by DLS measured at scattering angles from 20° to 150°.

	R_h	R_g	R_g/R_h
Pol-RB	60 ± 2	62 ± 3 nm	1.03
FAPi-pol-RB	67 ± 4	69 ± 4 nm	1.03

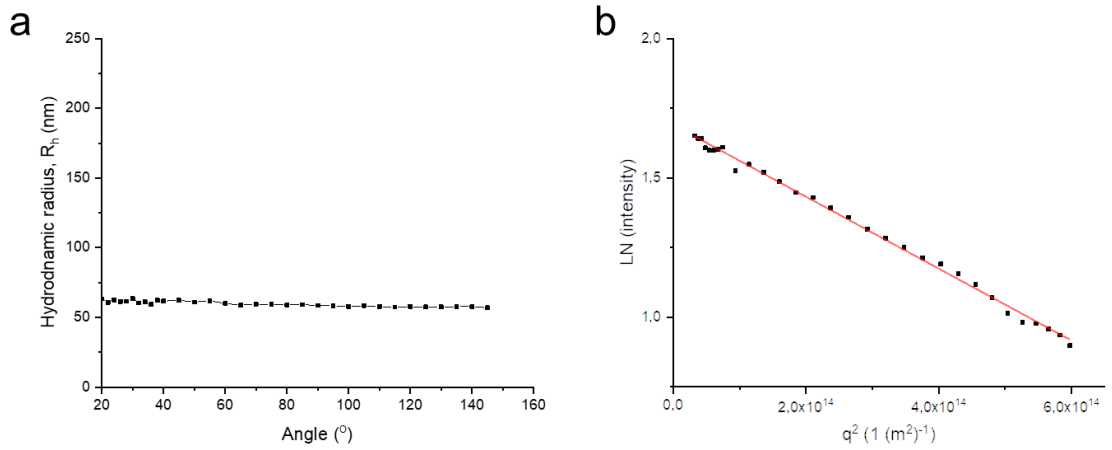

Figure S4. Static light scattering (SLS) measurement of RB-loaded polymersomes. (a) DLS profile showing the mean hydrodynamic radius, Rh; (b) SLS data and linear fit to the Guinier equation.

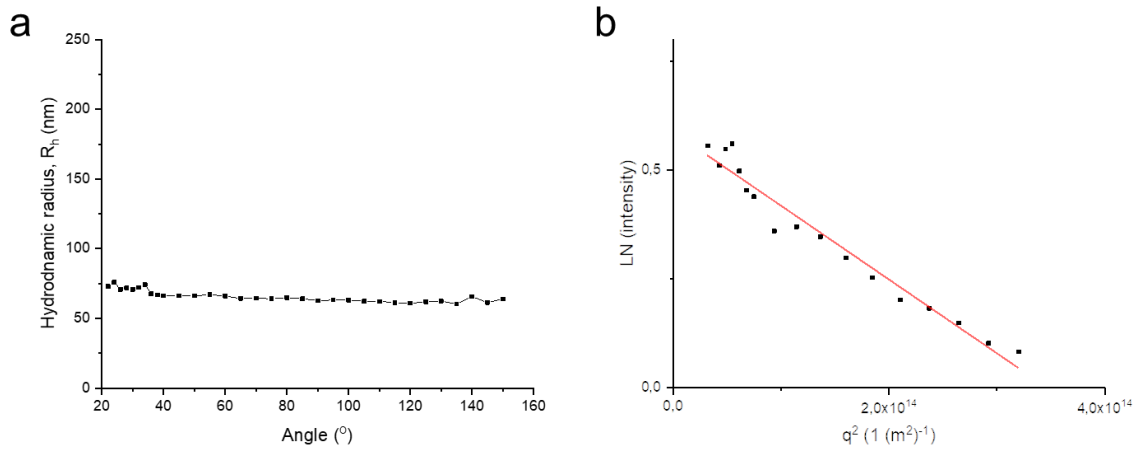

Figure S5. SLS measurement of RB-loaded FAPi-functionalized polymersomes. (a) DLS profile showing the mean hydrodynamic radius, Rh; (b) SLS data and linear fit to the Guinier equation.

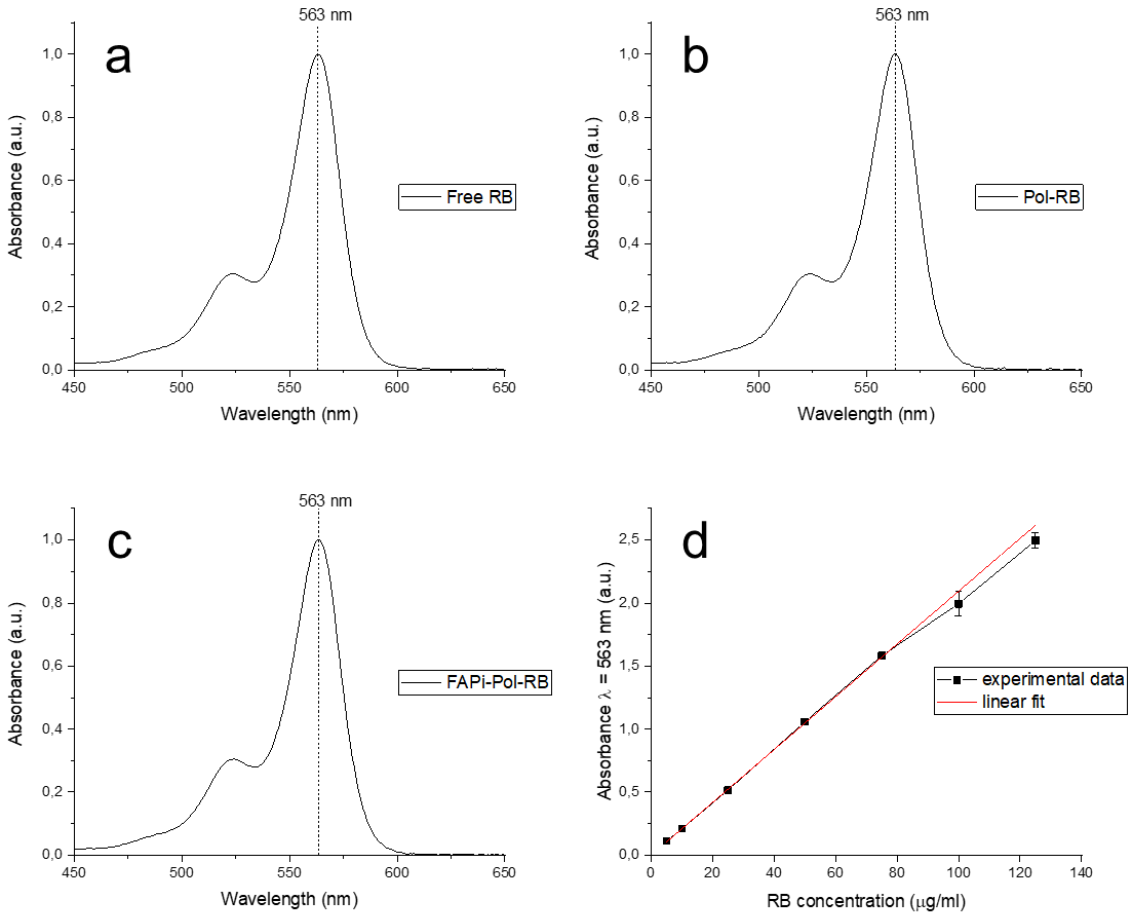

Figure S6. RB concentration measurement. Normalized absorbance spectra of samples containing: (a) free RB, (b) Pol-RB, (c) FAPi-Pol-RB after incubation at 95 °C for 15 min in the presence of 1% Triton X-100. (d) linear calibration curve of absorbance (λ = 563 nm) vs. concentration of RB (5 – 125 μg/ml) after incubation at 95 °C for 15 min in the presence of 1% Triton X-100.

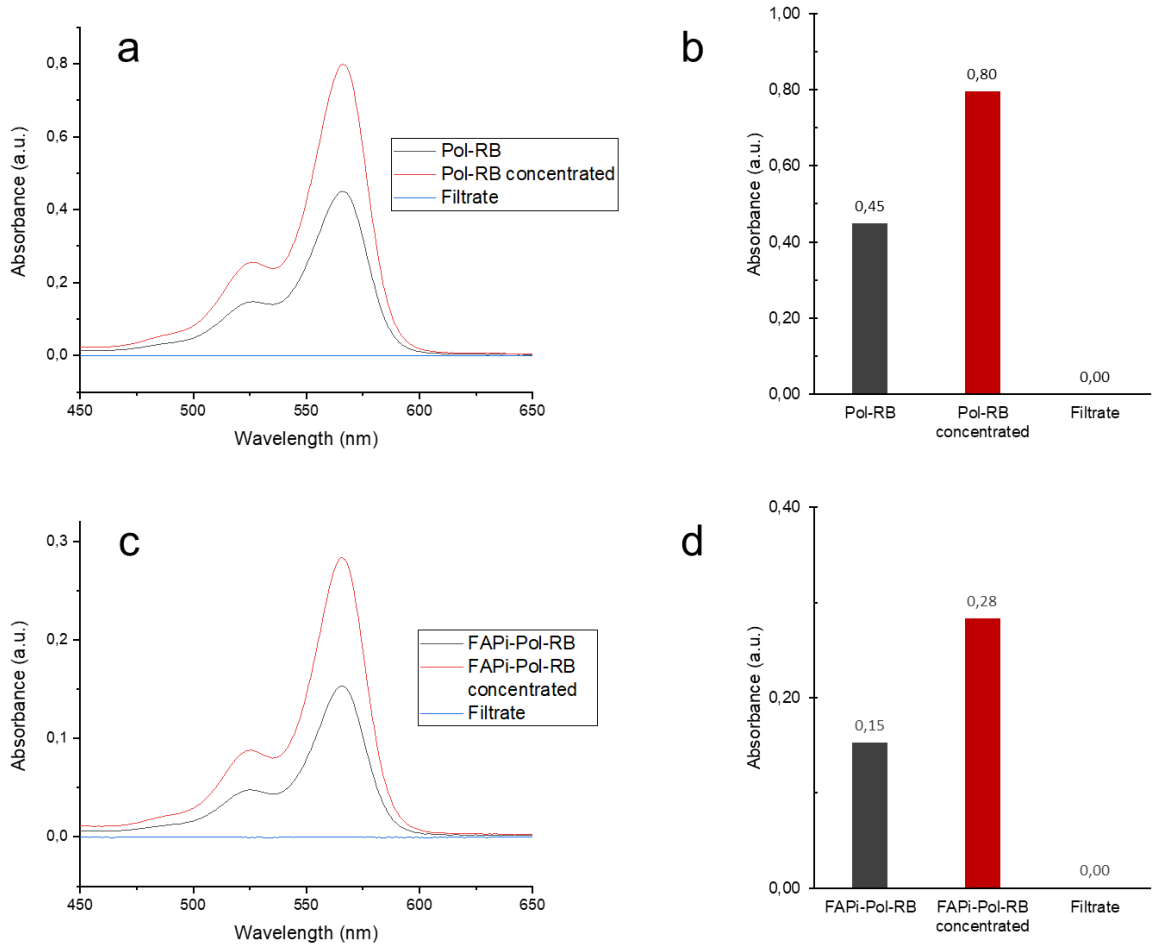

Figure S7. Stability measurement of RB-loaded polymersomes upon 4-month storage at 4 °C. PBS suspensions of: Pol-RB (≈20 µg RB/ml) (a, b) and FAPi-Pol-RB (≈7.5 µg RB/ml) (c, d) were filtered through centrifugal regenerated cellulose filters of 3000 Da MWCO. (a, c) absorbance spectra of input polymersome samples (black), polymersome concentrate (red) and filtrate (blue) for Pol-RB (a) and FAPi-Pol-RB (c). (b, d) histograms representing absorbance values of each sample of Pol-RB (b) and FAPi-Pol-RB (d) measured at the λ_{max} (λ = 565 nm for polymersomes, λ = 549 nm for free RB).

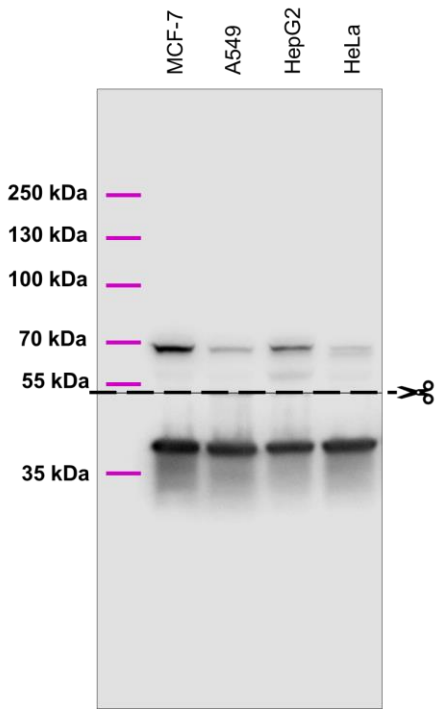

Figure S8. Comparison of FAPα expression between different cell lines. Western blot analysis of FAP protein expression in protein extracts from MCF-7, A549, HepG2 and HeLa cell lines (equivalent of 40 µg of protein per lane). GAPDH was used as loading control.

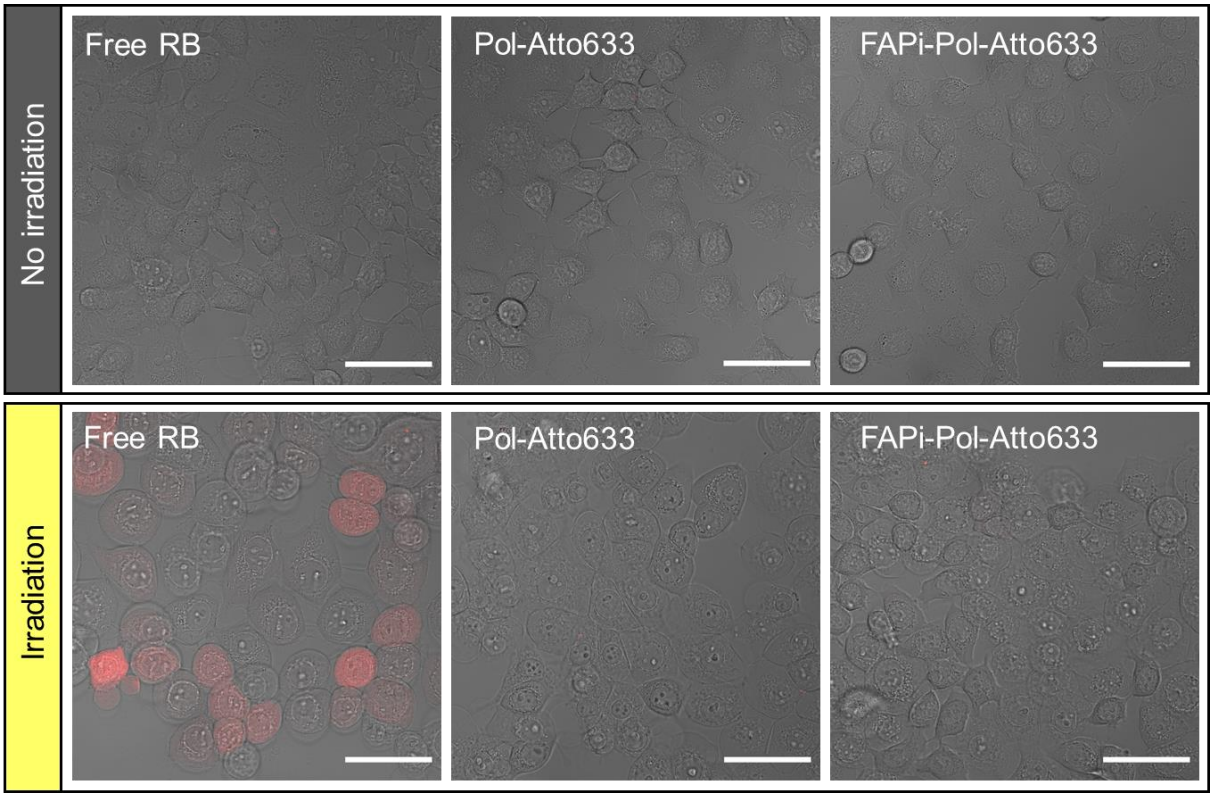

Figure S9. Reactive oxygen species detection. CLSM images of MCF-7 cells after 24 h incubation with free RB, Pol and FAPi-Pol samples with and without irradiation. Images show transmitted light channel merged with fluorescence of the ROS detection agent (DCF, red). Scale bars, 50 μm.