ARTICLE

Conjugating M13 bacteriophage targeting folate receptor alpha with multiple photosensitizers: a flexible phototheranostic platform against ovarian cancer

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Supplementary Information

ScFv MORAB-003:VH-(GGGGS)$_3$-VL**, **pIII-Linker**, **pIII C-terminal domain

EVQLVESGGGVVQPGRSLRLSCSASGFTFSGYGLSWVRQAPGKGLEWVAMISSGGSYTYYADSVKGRFAISRDNAKNTLFLQMDSLRPEDTGVYFCARHGDDPA
WFAYWGQGTPVTVSS-GGGGSGGGGSGGGGS-DIQLTQSPSSLSASVGDRVTITCSVSSSISSNNLHWYQQKPGKAPKPWIYGTSNLASGVPSRFSGSGSGTDYT
*FTISSLQPEDIATYYCQQWSSYPYMYTFGQGTKVEIK**GSPSGGSRLEEELRRRLTEPRSEGGGSEGGGSEGGGSEGGGSGGGSGSGDFDYEK**MANANKGAMTEN*
ADENVLQSDAKGKLDSVATDYGAAIDGFIGDVSGLANGNGATGDFAGSNSQMAQVGDGDNSPLMNNFRQYLPSLPQSVECRPFVFGAGKPYEFSIDCDKINLF
RGVFAFLLYVATFMYVFSTFANILRNKES

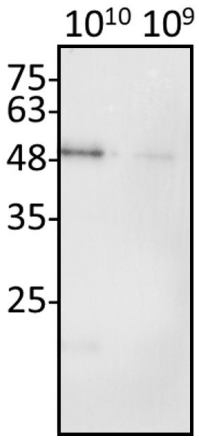

Figure S1. Western blot analysis performed on M13$_{FR\alpha}$ using anti-pIII antibody. Experiment was performed by loading a total amount of 10^{10} or 10^9 phage virions. A single band was visible around 48 kDa which corresponds to the expected molecular weight of engineered pIII in fusion with MORAB003-derived scFv.

In vitro models

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Supplementary Information available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

Cell line	Origin site	TP53 mutation	Aa change	Classification
OVSAHO	Abdomen (solid metastasis)	c.1024C>T	p.R342*	nonsense
SKOV3	Ascites (liquid metastasis)	c.267delC	p.S90Pfs*33	frameshift del.
CAOV3	Ovary (primary tumour)	c.406C>T	p.Q136*	nonsense
OC314	Ascites (liquid metastasis)	c.818G>A	p.R273H	missense
OV90	Ascites (liquid metastasis)	c.643A>C	p.S215R	missense

Table S1 - Ovarian cancer cell models used in the study, specific origin site and TP53 driver mutations as reported in Cellosaurus. The presence of these driver mutations was previously and recently confirmed in our models through gene sequencing[1]

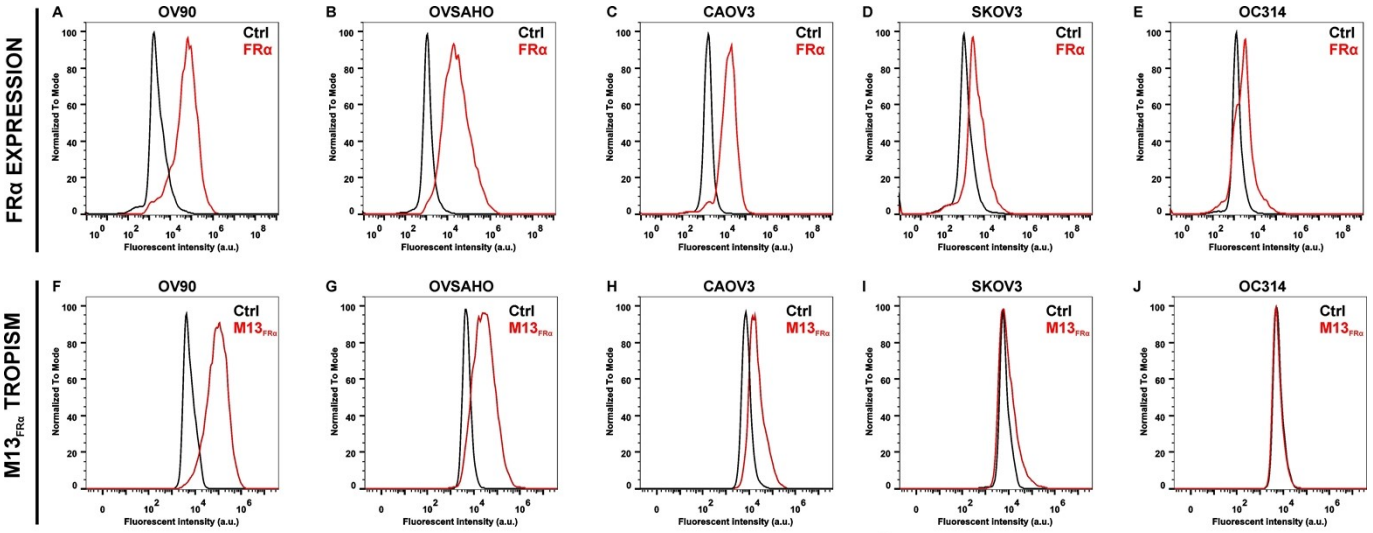

Figure S2. Flow cytometry peak profiles relative to Figure 1. Flow cytometry analysis was performed on OV90 (A, F), OVSAHO (B, G), CAOV3 (C, H), SKOV3 (D, I) and OC314 (E, J) cell lines. Experiments were conducted in immunohistechemistry by using anti-FRα monoclonal antibody (A-E) or $M13_{FR\alpha}$ (F-J). Mean Fluorescence Intensity (MFI) and percentage of fluorescent events relative to this experiment are shown in Figure 1.

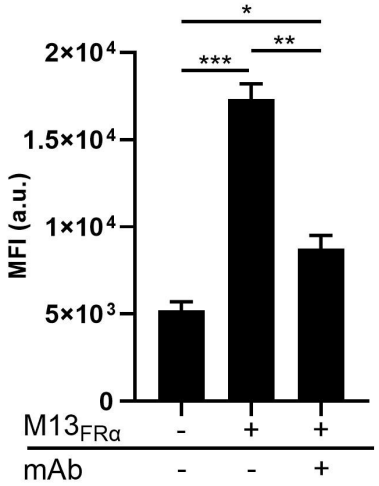

Figure S3. Competition assay demonstrating $M13_{FR\alpha}$ specificity for FRα. OV90 cells were pre-incubated with an anti-FRα monoclonal antibody (mAb clone 548908) or left untreated prior to incubation with engineered phage. Phage binding was then assessed by immunofluorescence in flow cytometry using a FITC-conjugated anti-pVIII antibody. Results are expressed as mean fluorescence intensity (MFI) measured in flow cytometry. Statistical significance was determined by one-way ANOVA with multiple comparisons (*p < 0.05, **p < 0.01, ***p < 0.001). A

significant reduction in $M13_{FR\alpha}$ tropism towards OV90 cell line was observed in cells pre-incubated with anti-FRα mAb compared to non-pretreated cells, demonstrating that engineered phage and mAb compete overlapping epitopes on FRα.

UV-Vis spectra

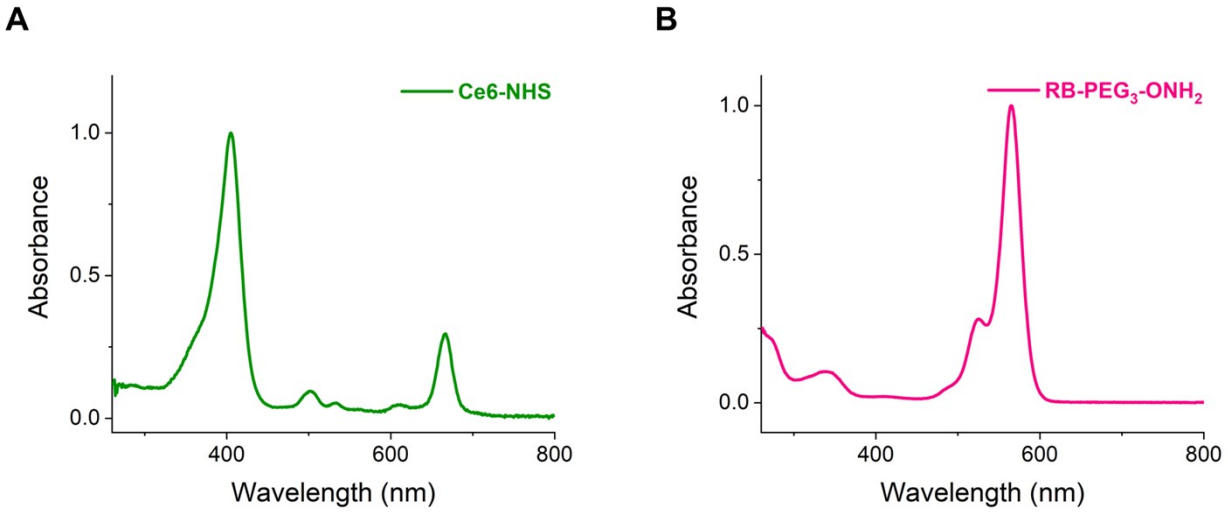

Figure S4. Normalized UV-Vis spectra of A) Ce6-NHS solution in DMSO. The most intense band (λ_{max} 408 nm) belongs to the Soret band, while Q-bands are visible in the region between 500-700 nm; B) RB-PEG_3-ONH_2 solution in DMSO (λ_{max} 565 nm).

Emission spectra

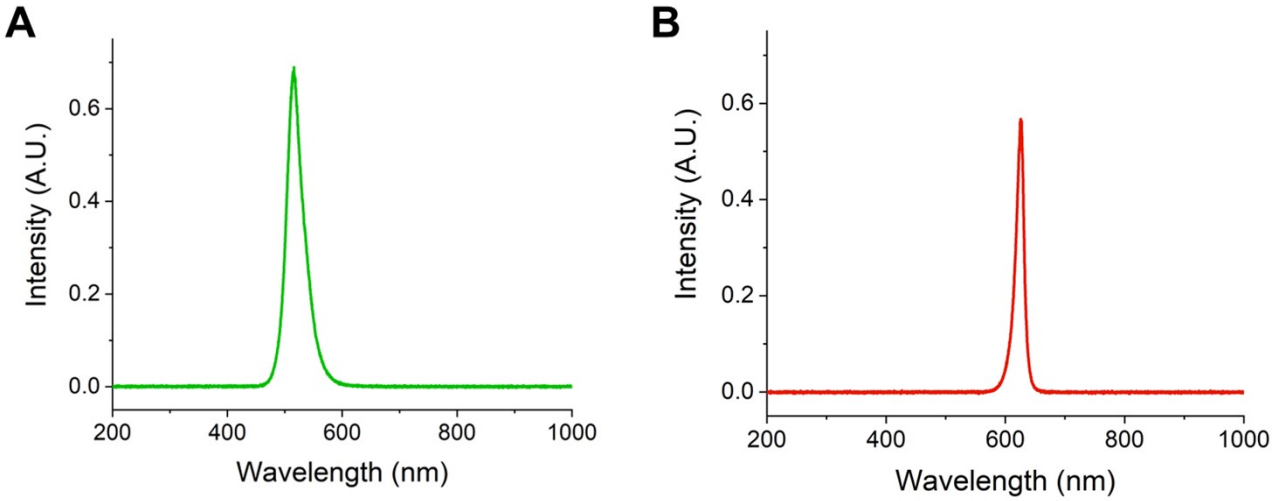

Figure S5. Emission spectra of the green and red LED sources (V-TAC 50 W VT-4752 SKU5691 RGB SMD Floodlight): green light λ_{max} 515 nm (FWMH 32nm), red light λ_{max} 633 nm (FWHM 15 nm).

Singlet oxygen generation assay

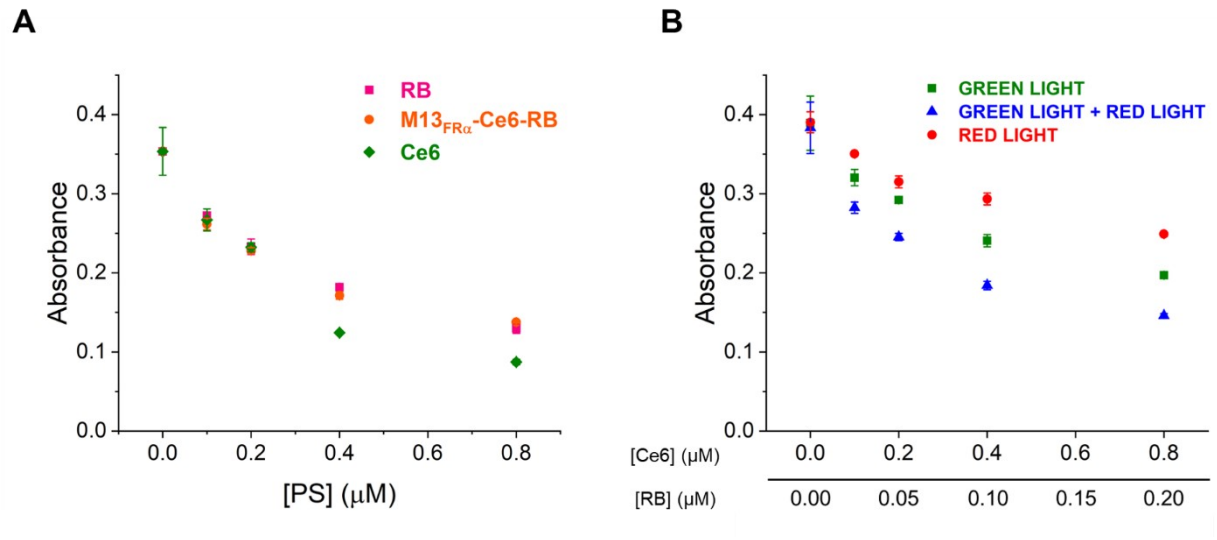

Figure S6. Absorbance decay, monitored at 401 nm, related to the reaction between ABMDMA with singlet oxygen A) at different concentrations of Ce6, RB and $M13_{FR\alpha}$-Ce6-RB upon combined irradiation with green and red light, and B) under three different irradiation modalities: green light, red light and combined green and red light.

ESI-MS (Ce6-NHS)

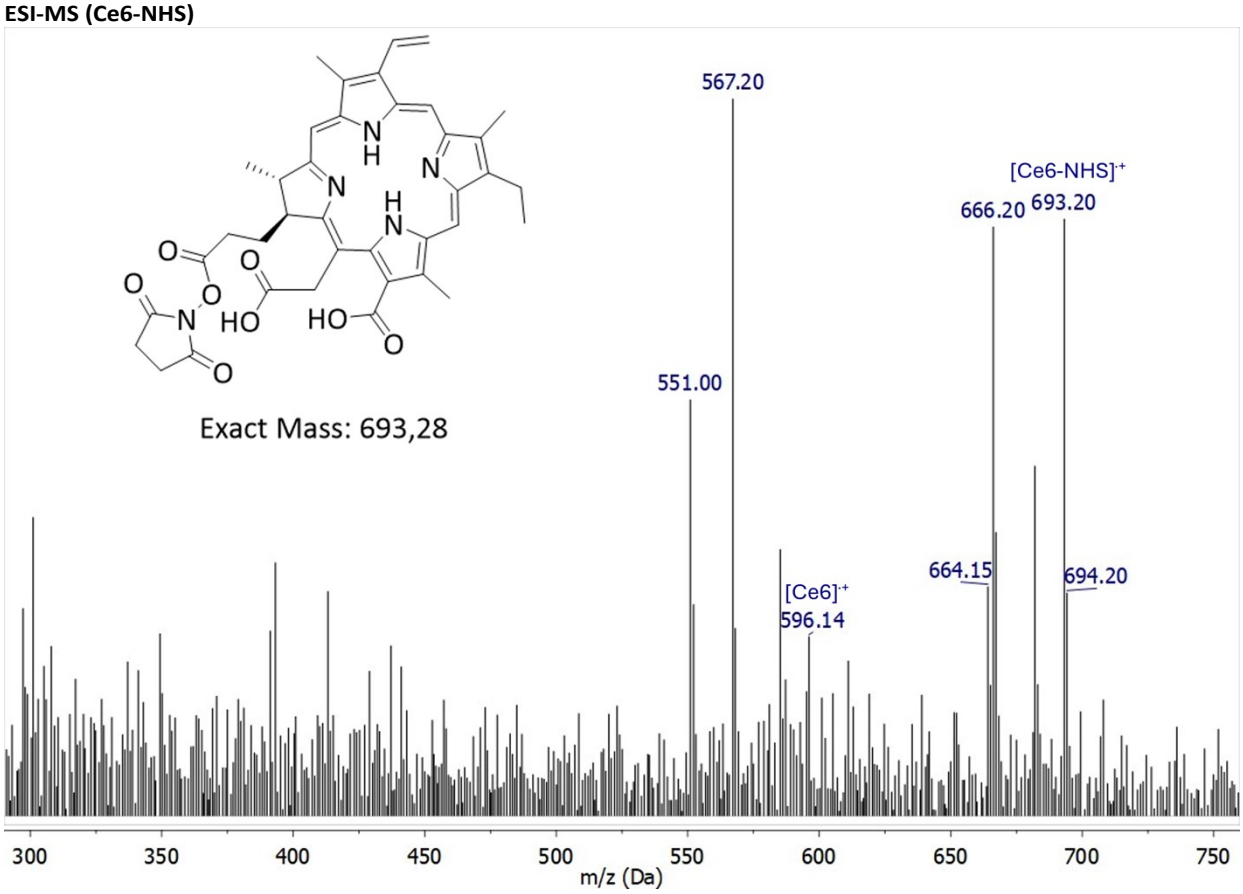

Figure S7. Positive ion mode ESI-MS spectrum of Ce6-NHS after work-up. MS spectrum acquired with an Agilent Technologies HP1260 instrument.

NMR

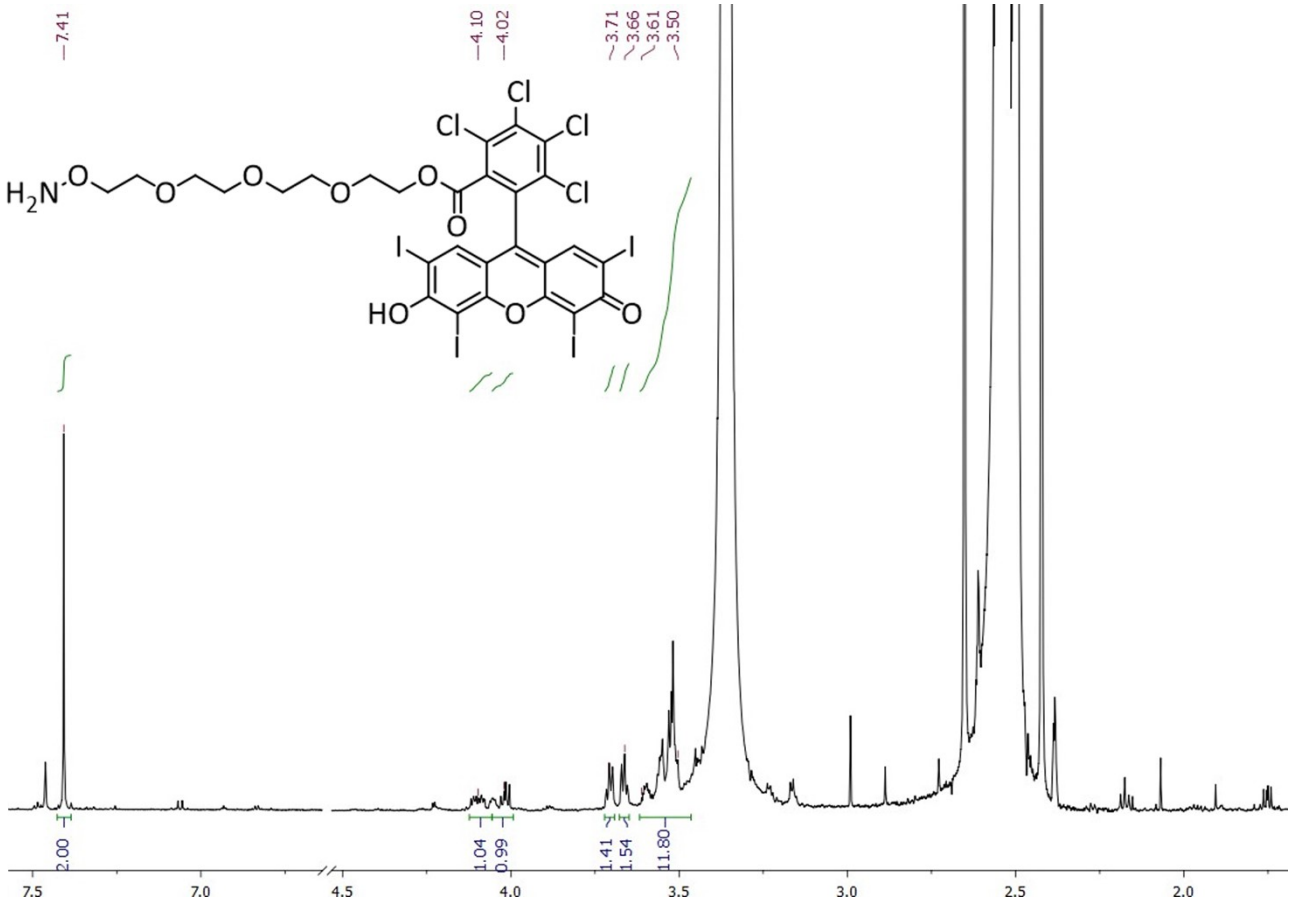

Figure S8. ^{1}H NMR spectrum of RB-PEG$_3$-ONH$_2$ in DMSO-d$_6$ after work-up**.** ^{1}H NMR spectrum recorded on a Bruker Ascend 600 MHz. with a 5mm probe; chemical shifts (δ) are reported in ppm and coupling constants in Hertz (Hz).

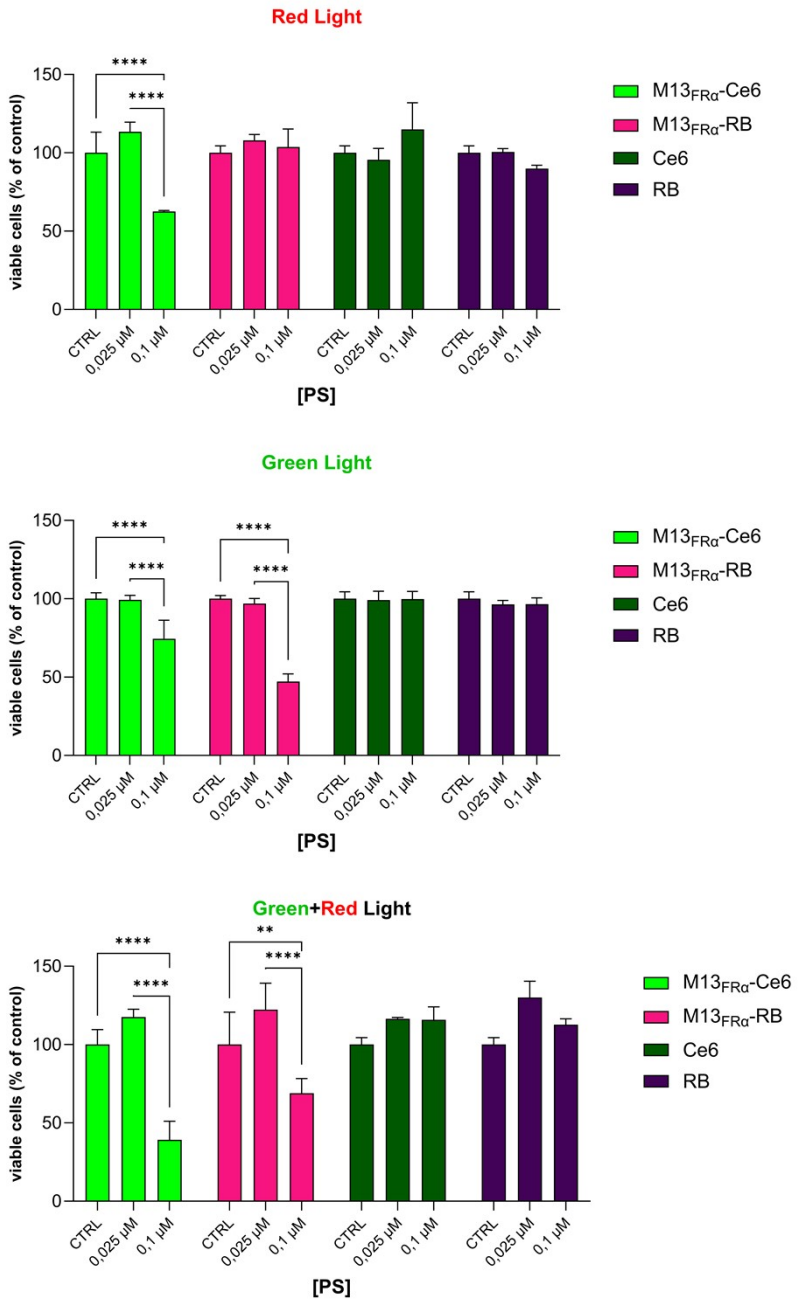

Figure S9. PDT on OV90 cell line performed with $M13_{FR\alpha}$, conjugated with Ce6 (in light green) and RB (in magenta), and the two photosensitizers alone (Ce6 in dark green and RB in purple). OV90 cells were irradiated for 15 minutes with red light (A), green light (B) or with the combination of green and red lights (C). Statistical significance was determined by two-way ANOVA with multiple comparisons (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

References

1 K. Filipek, D. Pollutri, I. Kurelac, G. Gasparre and M. Penzo, *Noncoding RNA Res*, 2026, 16, 104–116.

