Photoimmunotherapy retains its anti-tumor efficacy with increasing stromal content in heterotypic pancreatic cancer spheroids

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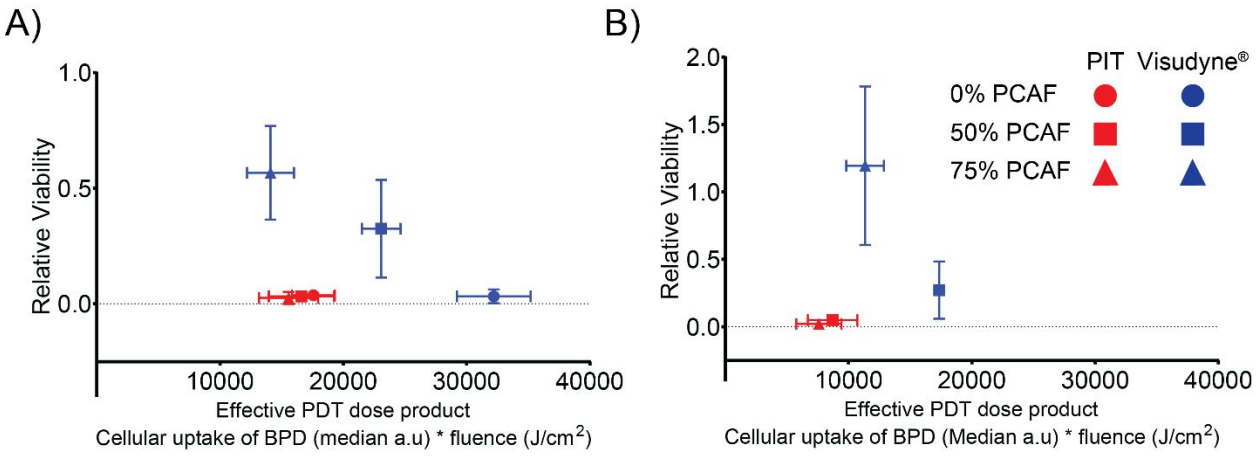
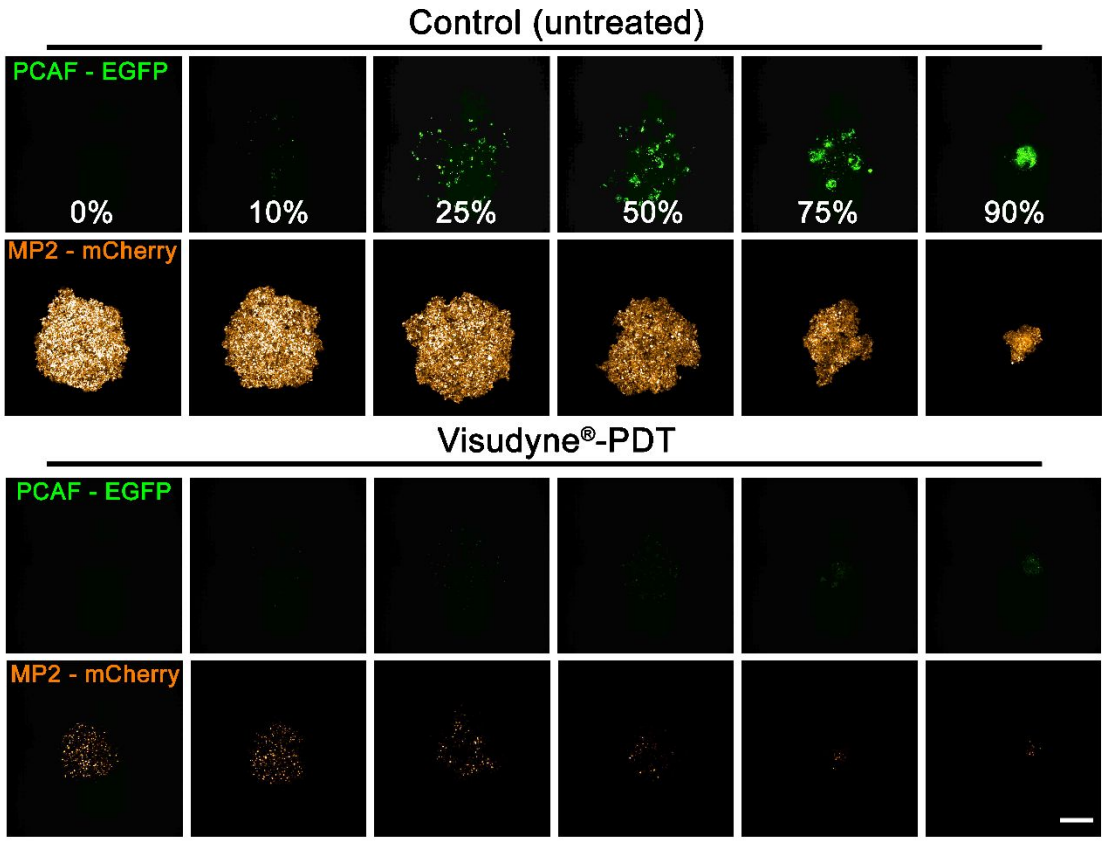
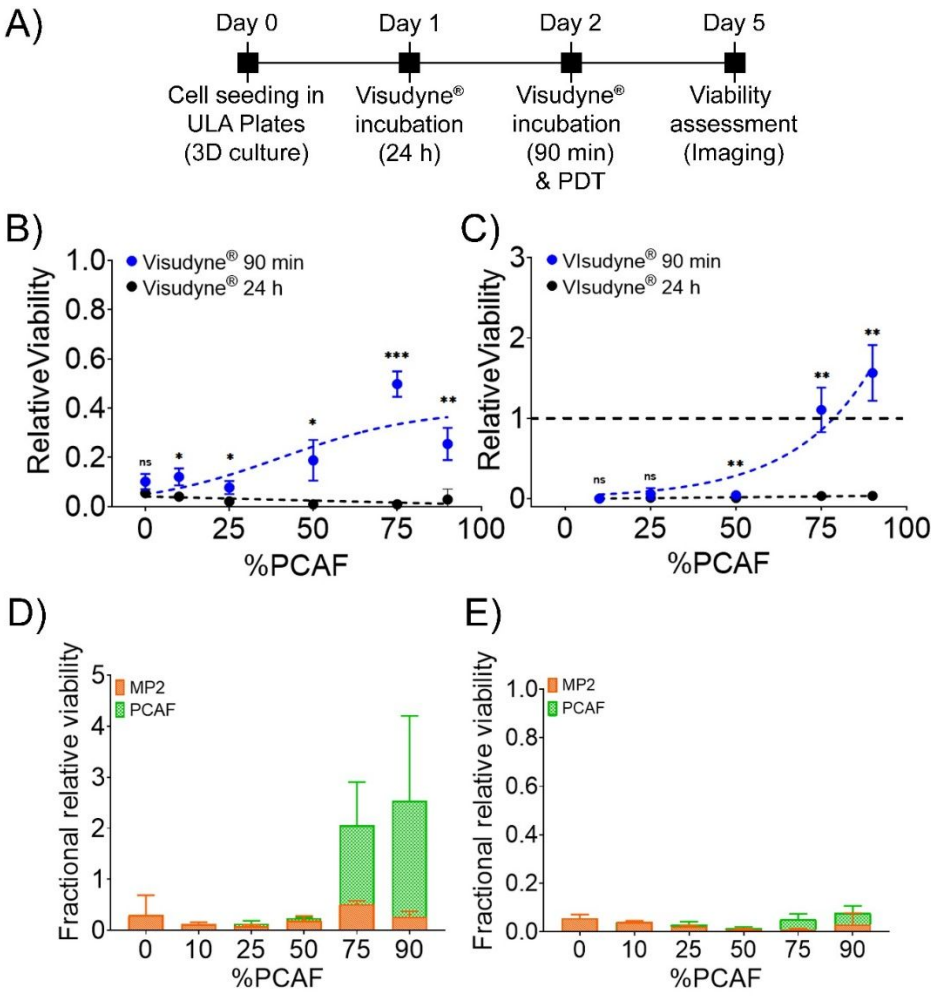


Supplementary figure S1: Relative viability of individual MIA PaCa-2 (**A**) and PCAF (**B**) populations in spheroids (formed with 0% (circles), 50% (squares) and 75% (triangles) PCAFs treated with either PIT (red shapes) or Visudyne®-PDT (blue shapes). Relative viabilities are plotted as a function of effective PDT dose (Cellular uptake of BPD (median a.u) * fluence (J/cm^2)). Although Visudyne®-PDT and PIT were performed with fluence of 5 J/cm^2 and 20 J/cm^2, respectively, the PDT dose was significantly higher for Visudyne®-PDT. However, even with lower PDT doses, PIT was more phototoxic than Visudyne®-PDT. The PDT doses for MIA PaCa-2 cells were similar for spheroids formed with 75% PCAF, however their relative viabilities were significantly different with PIT being more phototoxic.

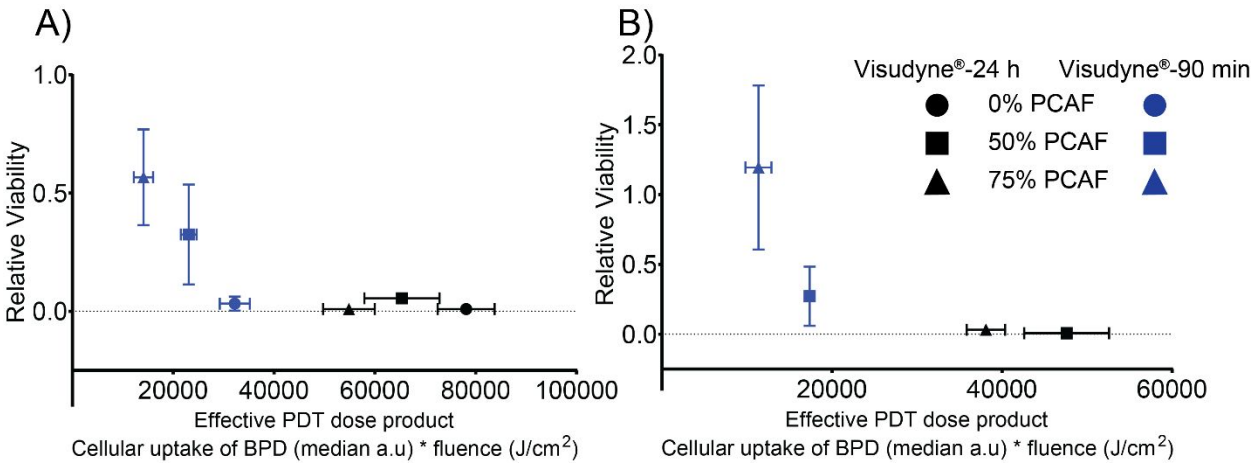


Supplementary figure S2: Response of heterotypic MIA PaCa-2-PCAF 3D cultures to Visudyne®-PDT with a 24 h drug-light interval (DLI). Maximum intensity projection (MIP) of fluorescence signals from MIA PaCa-2-PCAF spheroids with different PCAF percentage on day 3 post-PDT. PCAF percentage is mentioned in the top panel (control spheroids PCAF-EGFP panel). Scale bar corresponds to 500 µm.

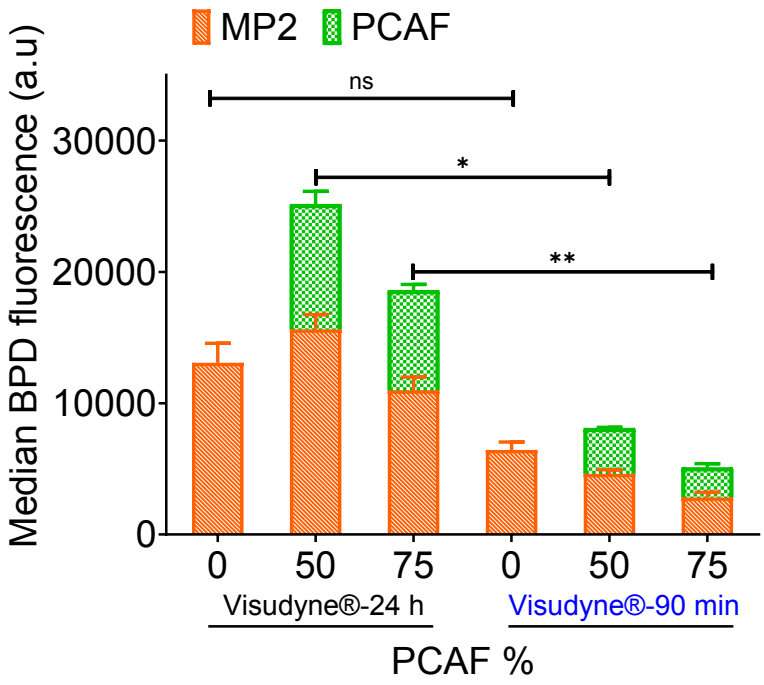


Supplementary figure S3: Quantitation of cell viability in heterotypic MIA PaCa-2-PCAF 3D cultures in response to Visudyne®-PDT with drug-light intervals of 90 min and 24 h. (**A**) Experimental schedule for Visudyne®-PDT of heterotypic MIA PaCa-2-PCAF spheroids. Cells were seeded in CellCarrier Spheroid ULA 96-well Microplates followed by incubation with Visudyne® for 24 h and 90 min, respectively. The spheroids were then irradiated with a light dose of 5 J/cm^2 followed by imaging for 3 days. Relative viability of individual MIA PaCa-2 (**B**) and PCAF (**C**) populations in spheroids (formed with different PCAF percentage) treated with either Visudyne® (90 min DLI) (blue circles) or Visudyne®-PDT (24 h DLI) (black circles). Data are presented as mean ± S.D (n ≥3), analyzed using Welch's t-test analysis. P-values < 0.05 were considered to be significant and are indicated by asterisks as follows: ^{ns}P>0.05, *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001. (**C**) Fractional relative viability of MIA PaCa-2 and PCAFs in spheroids treated with Visudyne®-PDT with a 90 min DLI (**D**) and Visudyne®-PDT with a 24 h DLI (**E**)

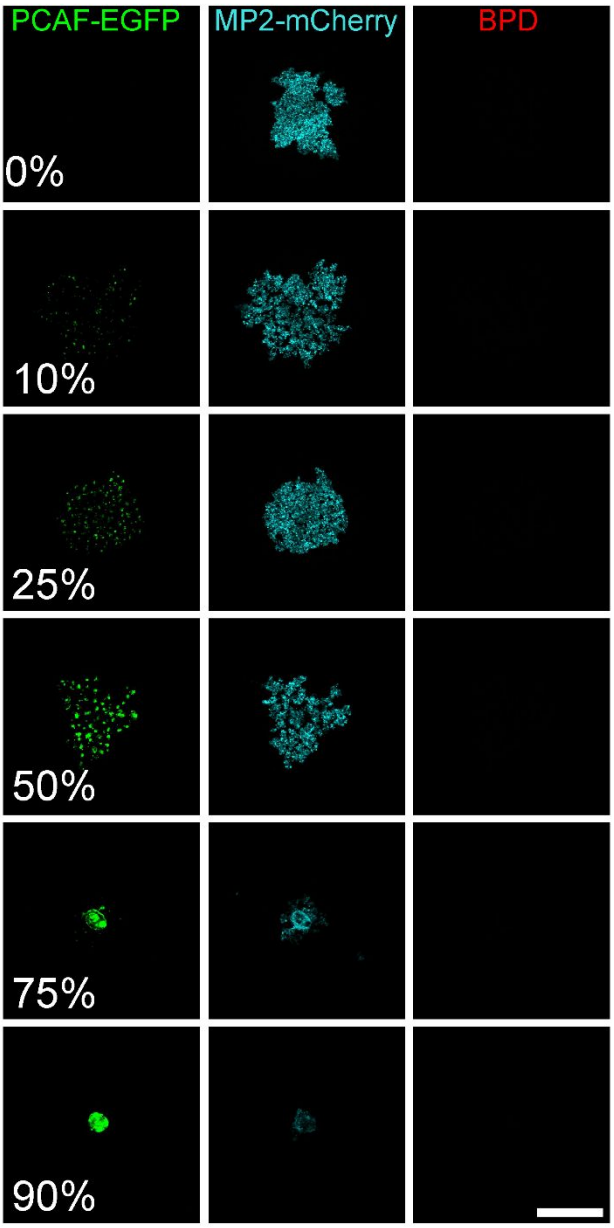
calculated as described in the methods. MIA PaCa-2 are represented as orange bars and PCAFs are represented as green bars. Data are presented as mean ± S.D (n ≥3).



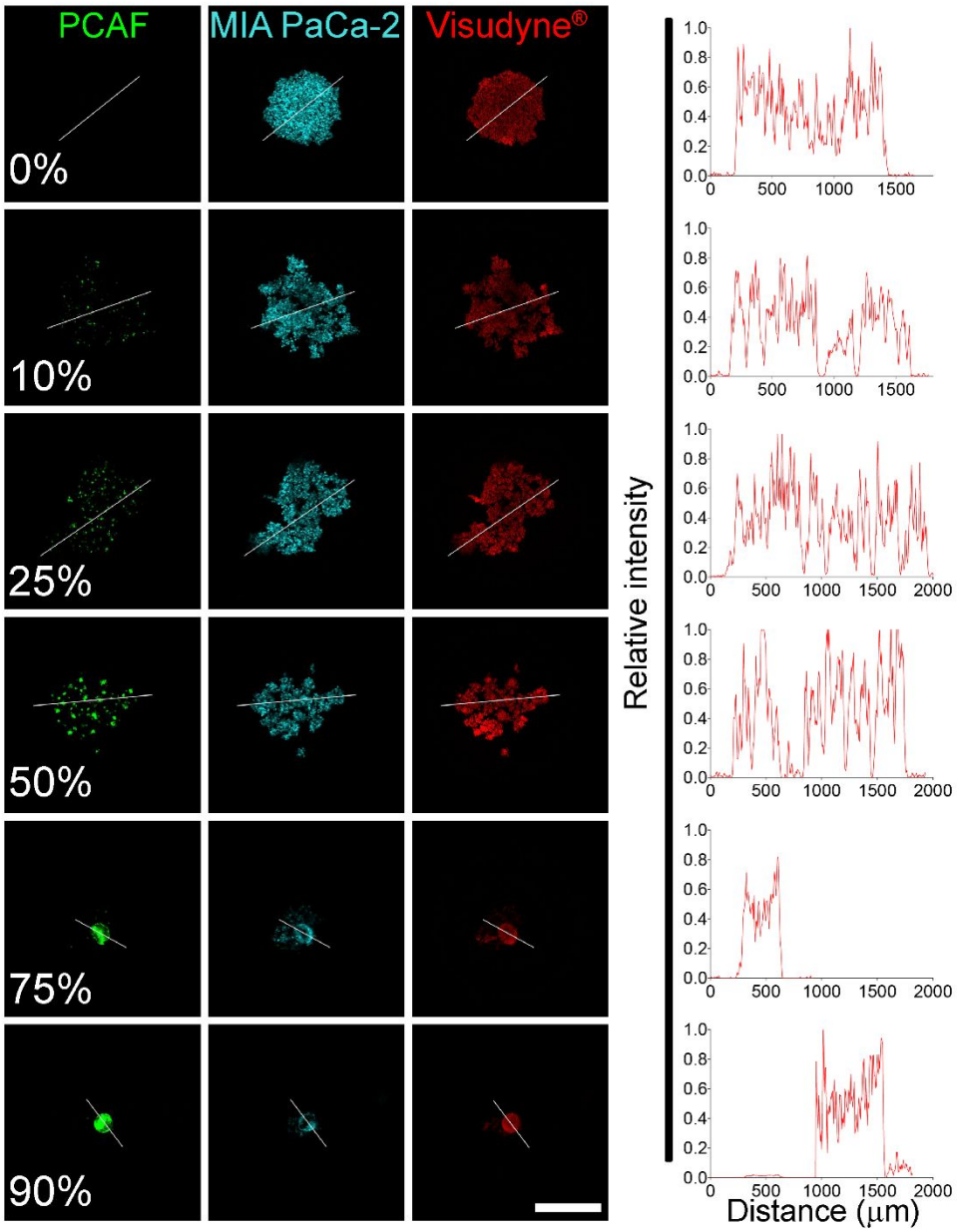
Supplementary figure S4: Relative viability of individual MIA PaCa-2 (**A**) and PCAF (**B**) populations in spheroids (formed with 0% (circles), 50% (squares) and 75% (triangles) PCAFs treated with Visudyne®-PDT after a 24 h (black shapes) or 90 min (blue shapes) drug light interval. Relative viabilities are plotted as a function of effective PDT dose (Cellular uptake of BPD (median a.u) * fluence (J/cm^2)). Although PDT was performed with fluence of 5 J/cm^2 the effective PDT dose product was significantly higher for Visudyne®-PDT performed after a 24 h incubation due to the significantly higher cellular BPD uptake. Therefore, irrespective of the spheroid composition, Visudyne®-PDT performed after a 24 h drug light interval was significantly more phototoxic than Visudyne®-PDT performed after a 90 min incubation.



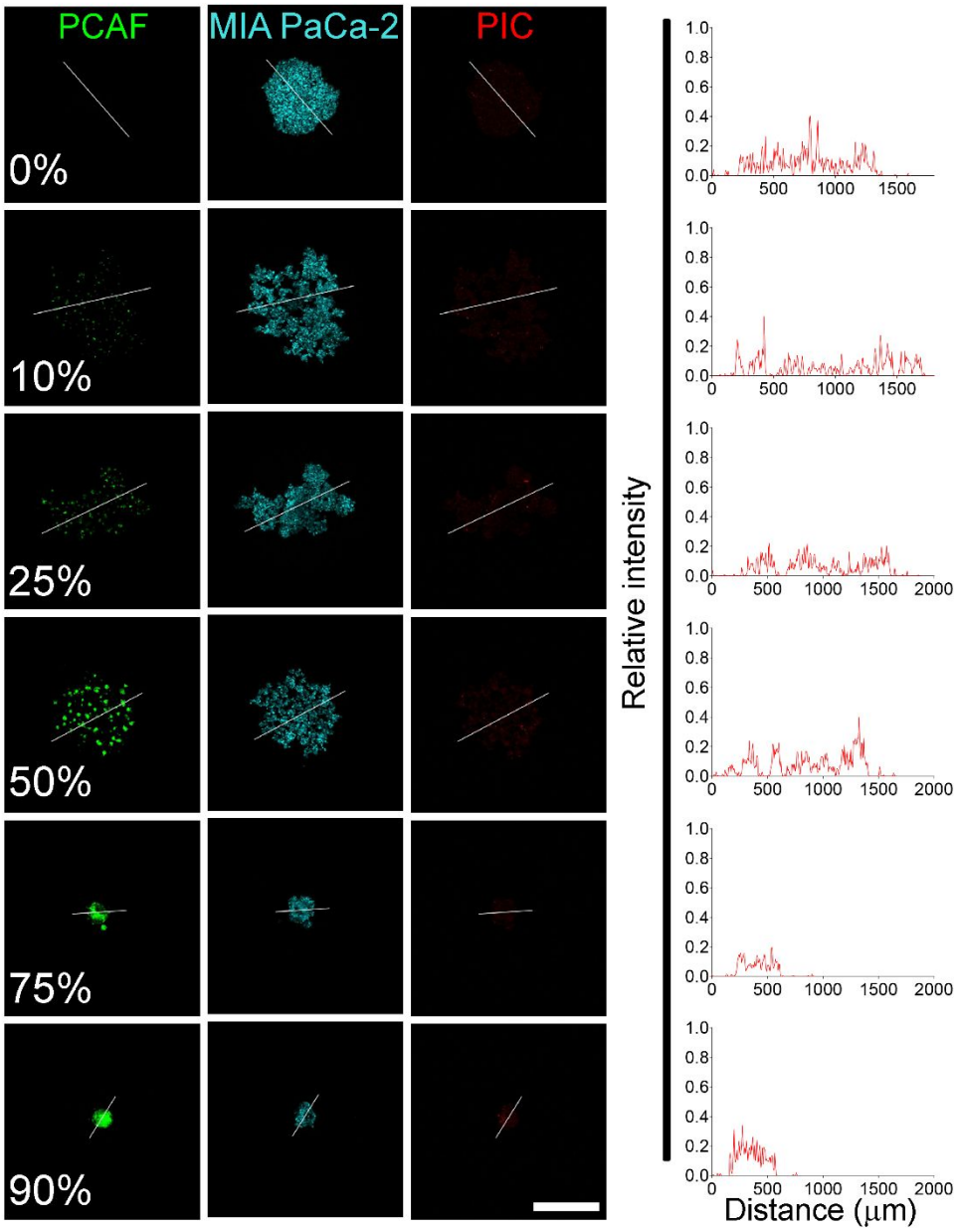
Supplementary figure S5: Photosensitizer uptake in heterotypic 3D spheroids. Median BPD fluorescence of spheroids treated with Visudyne® for either 90 min or 24 h. Green bars represent PCAFs while orange bars represent MIA PaCa-2 cells. BPD uptake in the spheroids increased with increasing incubation time. Data are presented as mean ± S.D (n ≥3), analyzed using Welch's t-test analysis. P-values < 0.05 were considered to be significant and are indicated by asterisks as follows: ^{ns}P>0.05, *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001.



Supplementary figure S6: Representative confocal fluorescence images of the central optical section of untreated 3D spheroids. MIA PaCa-2 cells and PCAFs are pseudo-colored as cyan and green. No signal was observed in the BPD channel suggesting minimal contribution of EGFP and mCherry fluorescence in the BPD channel (scale bar = 500 µm).



Supplementary figure S7: Photosensitizer distribution in heterotypic 3D spheroids treated with Visudyne® for 24 h. Representative confocal images of the central optical section of the 3D spheroids. MIA PaCa-2 cells and PCAFs are pseudo-colored as cyan and green. BPD is shown in pseudo-colored as red. Profile plots were generated using image J to monitor BPD distribution across the central plane. As suggested by the profile-plots, the distribution of BPD was homogeneous across the central section irrespective of the spheroid composition (scale bar = 500 μm).



Supplementary figure S8: Photosensitizer distribution in heterotypic 3D spheroids treated with PIC for 90 min. Representative confocal images of the central optical section of the 3D spheroids. MIA PaCa-2 cells and PCAFs are pseudo-colored as cyan and green. BPD is pseudo-colored as red. Profile plots were generated using image J to monitor

BPD distribution across the central plane. PICs are taken up by receptor-mediated endocytosis and attains maximum fluorescence intensity in 8 – 24 h. A 90 min incubation period was thus too low to achieve efficient PIC uptake, accounting for the low BPD signal. However, profile-plots suggest a homogeneous distribution of BPD (PIC) across the central section irrespective of the spheroid composition (scale bar = 500 μm).