SUPPORTING INFORMATION

Dual Function Antibody Conjugates for Multimodal Imaging and Photoimmunotherapy of Cancer Cells

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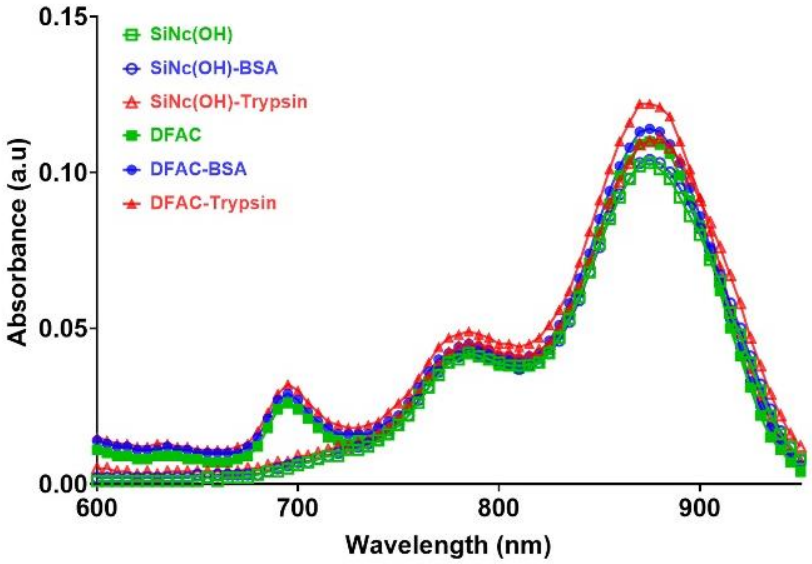

Figure S1. Comparison of absorption spectra of SiNc(OH) and DFAC, at 1 µM (SiNc(OH) equivalent), performed in solutions containing 0.1% BSA (blue curves) and 0.1% Trypsin (red curves). The absorption spectra of SiNc(OH) and DFAC were not affected by the presence of BSA and Trypsin.

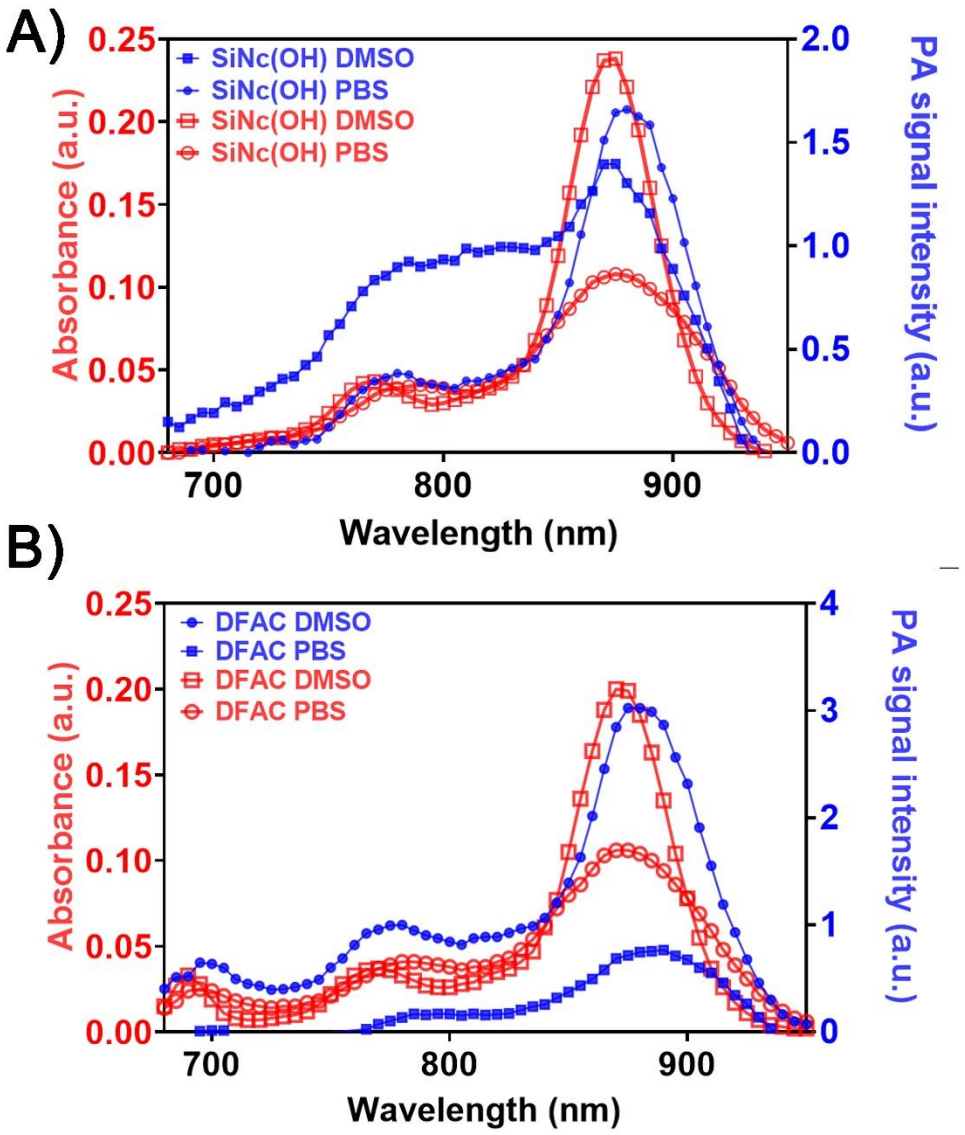

Figure S2. (A) Comparison of UV-Vis (red) and Photoacoustic (blue) spectra of SiNc(OH) dye in PBS (circles) and DMSO (squares). **(B)** Comparison of UV-Vis (red) and Photoacoustic (blue) spectra of DFAC in PBS (circles) and DMSO (squares). The absorption of SiNc(OH) dye at the λmax (865 nm) is higher in DMSO as compared to that in PBS. The PA spectra for DFAC showed a significantly enhanced intensity at λmax (865 nm) in DMSO.

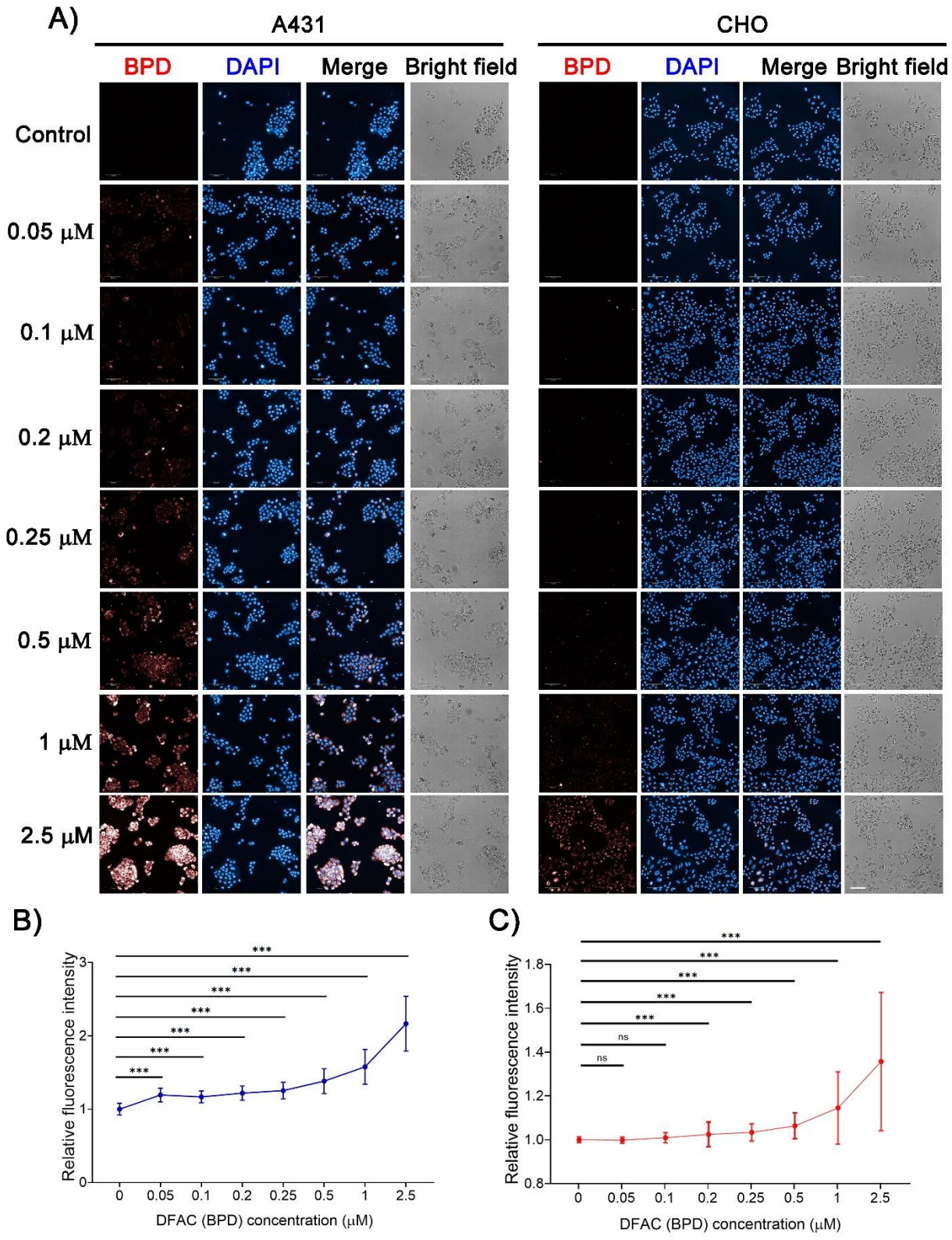
A)
A431
CHO
BPD
DAPI
Merge
Bright field
Control
0.05 μM
0.1 μM
0.2 μM
0.25 μM
0.5 μM
1 μM
2.5 μM
B)
Relative fluorescence intensity
DFAC (BPD) concentration (μM)
C)
Relative fluorescence intensity
DFAC (BPD) concentration (μM)
***
ns

Figure S3. (A) Fluorescence images of A431 and CHO cells incubated with increasing concentrations (BPD equivalent) of DFAC for 24 h. Cells were washed with PBS, stained with Hoechst nuclear stain and imaged using an Operetta high content imaging system (Scale bar = 100 µm). **(B and C)** Quantification of the fluorescence images indicate significantly increased fluorescence intensity in A431 cells incubated with 0.05 µM of DFAC (BPD equivalent). A significantly increased fluorescence signal was observed for CHO cells incubated with 0.2 µM (BPD equivalent) DFAC. Data are presented as mean ± S.D (n ≥600 cells across 5 different imaging fields), analyzed using one-way ANOVA with Tukey's test for post-hoc 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.

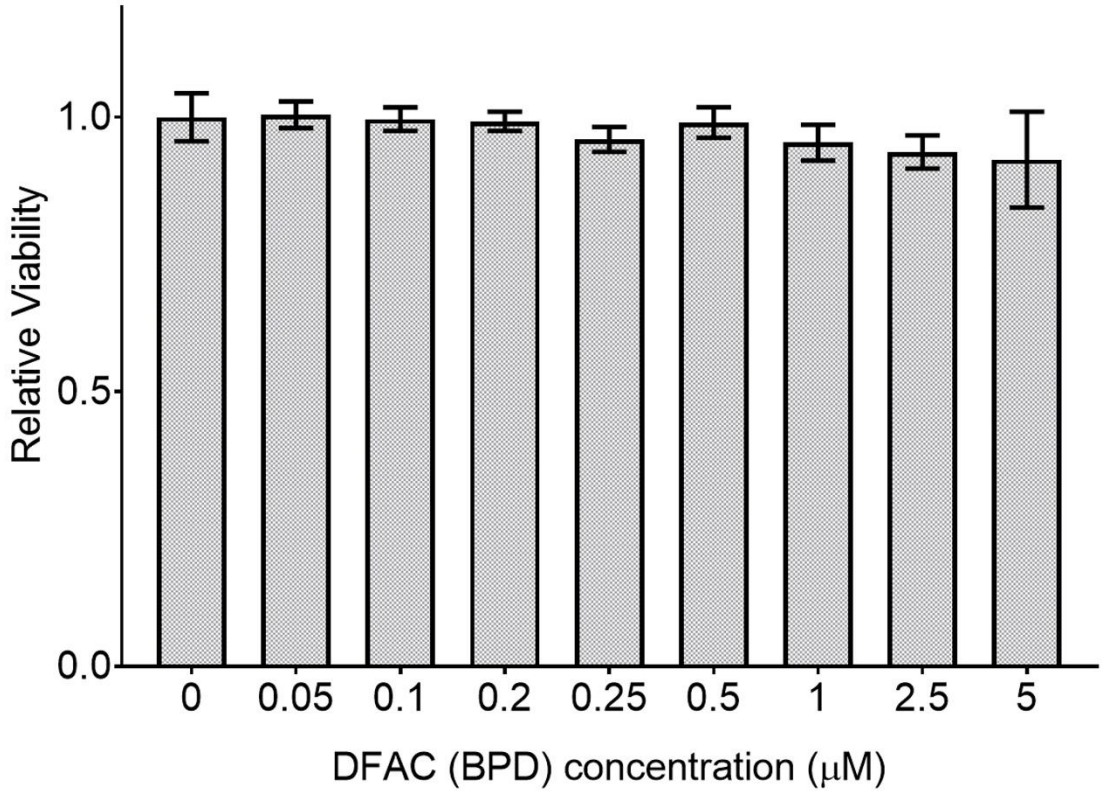

Figure S4. Analysis of dark toxicity of DFAC on A431 cells. A431 cells were incubated with different concentrations of DFAC (BPD equivalent) for 24 h. Cell viability was then measured by MTT assay. No significant toxicity was observed even at the highest dose of DFAC. Data are presented as mean ± S.D (n ≥4).

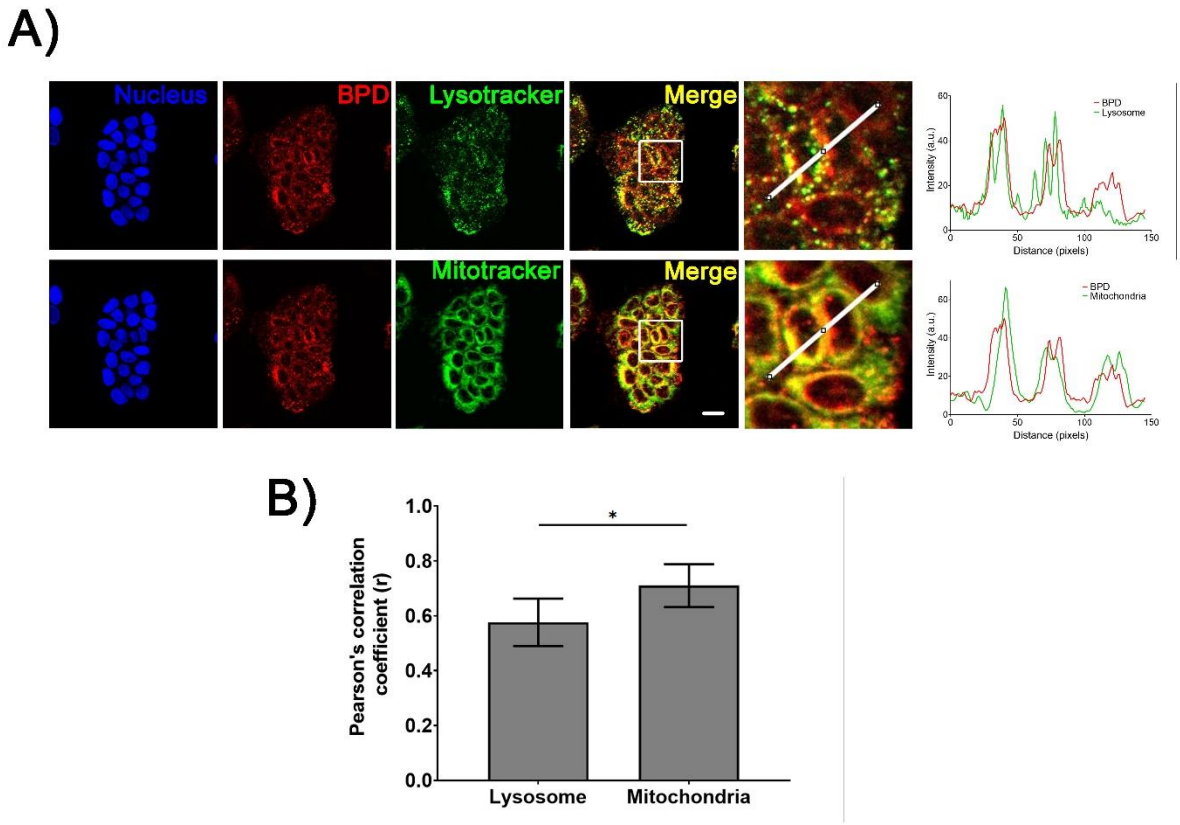

Figure S5. Subcellular localization of DFAC in A431 cells upon 24 h incubation. (A) Representative confocal fluorescence images of A431 cells obtained after 24 h of incubation with DFAC (1 μM BPD equivalent). BPD signal is pseudo-colored in red. Mitochondria and Lysosomes were stained with MitoTracker™ Deep Red FM and LysoTracker™ Red DND-99

(both pseudo-colored in green), respectively. Merged images with yellow color indicate colocalization of BPD (red) with mitochondria and lysosomes (green), respectively. Nuclear staining (Hoechst; pseudo-colored in blue). Scale bar = 20 μm. Profile plots were created using ImageJ software showing co-localization of BPD in mitochondria and Lysosomes. (B) Pearson's correlation coefficient suggests significantly higher colocalization of BPD in the mitochondria (p value 0.033) as compared to the lysosomes. Data are presented as mean ± S.D (n = 5 different imaging fields), analyzed using Welch's t-test.

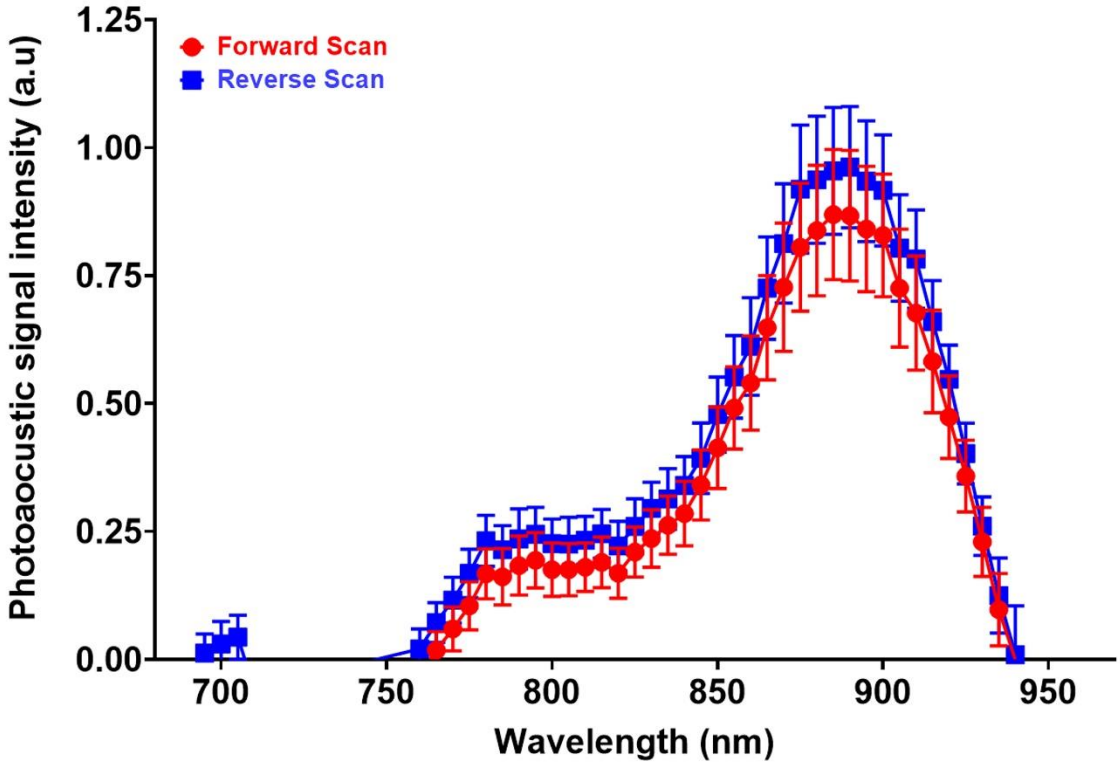

Figure S6. Comparison of PA spectra of DFAC performed as a forward scan (red circles) (680 to 970 nm) and reverse scan (blue squares) (970 to 680 nm). All measurements were performed in PBS at SiNc(OH) equivalent concentration of 5 μM. Although the photoacoustic signal intensity for the forward scan was slightly lower than the reverse scan, the difference was not significant. Data are presented as mean ± S.D ($n \geq 4$).