Supplementary Material

A Novel Tumor Targeting Drug Carrier for Optical Imaging and Therapy

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Supplemental experiments

Stability of ATF-HSA:CPZ

5 μM ATF-HSA:CPZ was mixed with human plasma at a volume ratio of 1:9 and incubated at 37℃ for various periods of time (15 min, 30 min, 1 h, 2 h, 6 h, 12 h, 24 h). The fluorescence intensity of ATF-HSA:CPZ at each time point was measured on a multimodal microplate reader (Synergy 4, BioTek Instruments, Winooski, VT, USA) with λ_{ex} = 610 nm and λ_{em} = 680 nm. Three replicates at each time point were tested and the experiment was repeated three times. In the pH stability measurements of ATF-HSA:CPZ in saline (pH 7.2) and in acetate buffer (pH 4.5), ATF-HSA:CPZ in these two buffers were recorded from 500 to 800 nm using a quartz cuvette with 1 cm path length on a Lambda-35 UV/Vis spectrometer (PerkinElmer, Waltham, MA, USA).

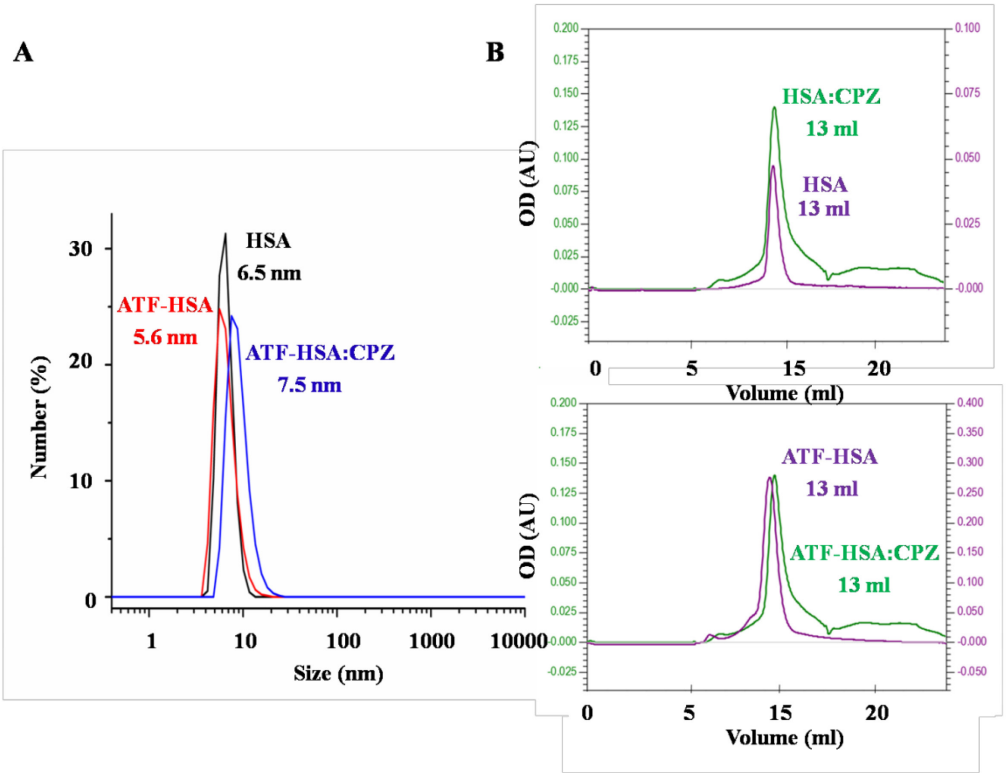

Figure S1. Dynamic light scattering (DLS) measurement (A) and gel filtration chromatography of ATF-HSA:CPZ (B). (A) The hydrodynamic molecular radii of ATF-HSA and ATF-HSA:CPZ in saline by DLS are 5.6 nm and 7.5 nm, respectively. These values are compatible with the hydrodynamic radius of HSA (6.5 nm). (B) Gel filtration chromatography of ATF-HSA:CPZ or HSA:CPZ after being purified by the DIP method is similar to that of ATF-HSA or HSA, respectively (the peaks at 13 ml). The purified ATF-HSA:CPZ or HSA:CPZ is monomeric.

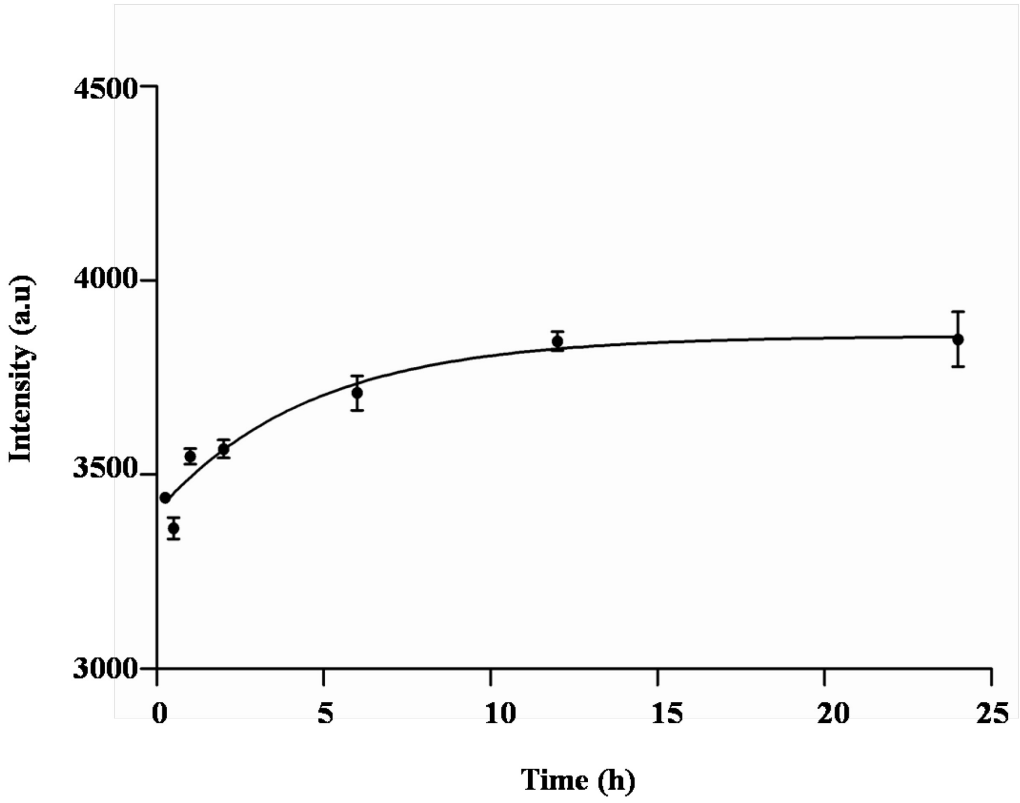

Figure S2. The plasma stability of ATF-HSA:CPZ at 37℃ for 24 h. In this assay, the fluorescence intensity of ATF-HSA:CPZ does not change a lot and ATF-HSA:CPZ is stable in human plasma for at least 24 h. 5 μM ATF-HSA:CPZ and human plasma were mixed at a volume ratio of 1:9 and incubated at 37 ℃ for various periods of time (15 min, 30 min, 1 h, 2 h, 6 h, 12 h, 24 h). Then the fluorescence intensity of ATF-HSA:CPZ at each time point was measured on a multimodal microplate reader (Synergy 4, BioTek Instruments, Winooski, VT, USA) with λ_{ex} = 610 nm and λ_{em} = 680 nm. Three replicates at each time point were tested and the experiment was repeated three times. Values represent the mean of three separate experiments; bars represent standard error of the mean (SEM).

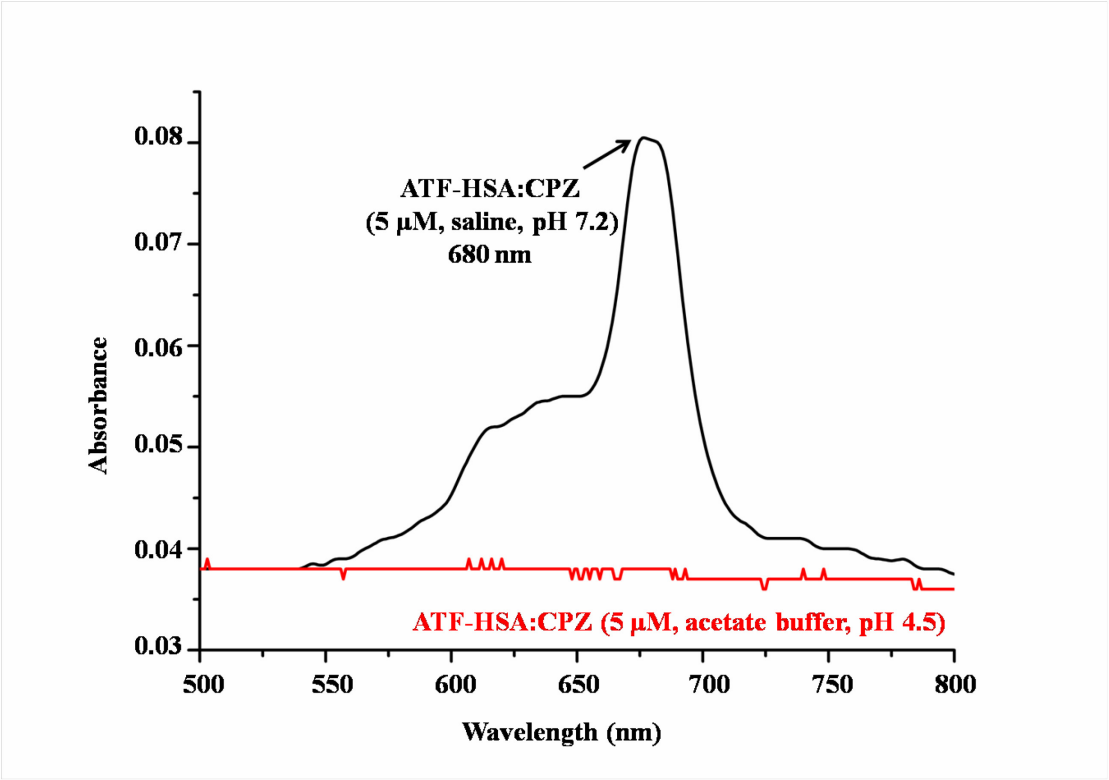

Figure S3. The UV/Vis absorption spectra of ATF-HSA:CPZ in saline (pH 7.2) and in acetate buffer (pH 4.5) were recorded from 500 to 800 nm using a quartz cuvette with 1 cm path length on a Lambda-35 UV/Vis spectrometer (PerkinElmer, Waltham, MA, USA). The UV/Vis absorption spectrum of ATF-HSA:CPZ in acetate buffer, pH4.5, shows the complete vanishment of the UV/Vis absorption at 600–700 nm region due to the discharge of the loaded CPZ at the low pH which causes HSA to adopt an open conformation.

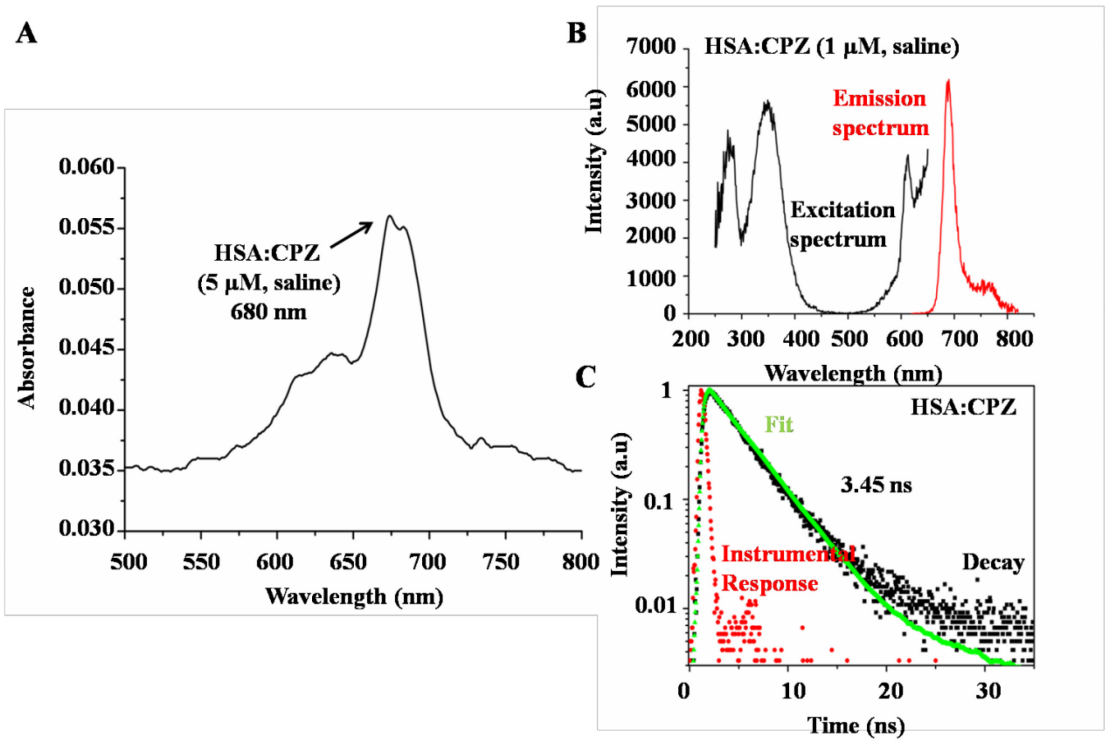

Figure S4. Remarkable optical and photophysical properties of HSA:CPZ.

A. The UV/Vis spectrum of HSA:CPZ in saline shows that CPZ is mainly in monomeric state with the maximum absorption at 680 nm, which is similar to ATF-HSA:CPZ's.

B. The excitation and emission fluorescence spectra of HSA:CPZ are similar to ATF-HSA:CPZ's.

C. The fluorescence decay time of HSA:CPZ in saline is similar to ATF-HSA:CPZ's.

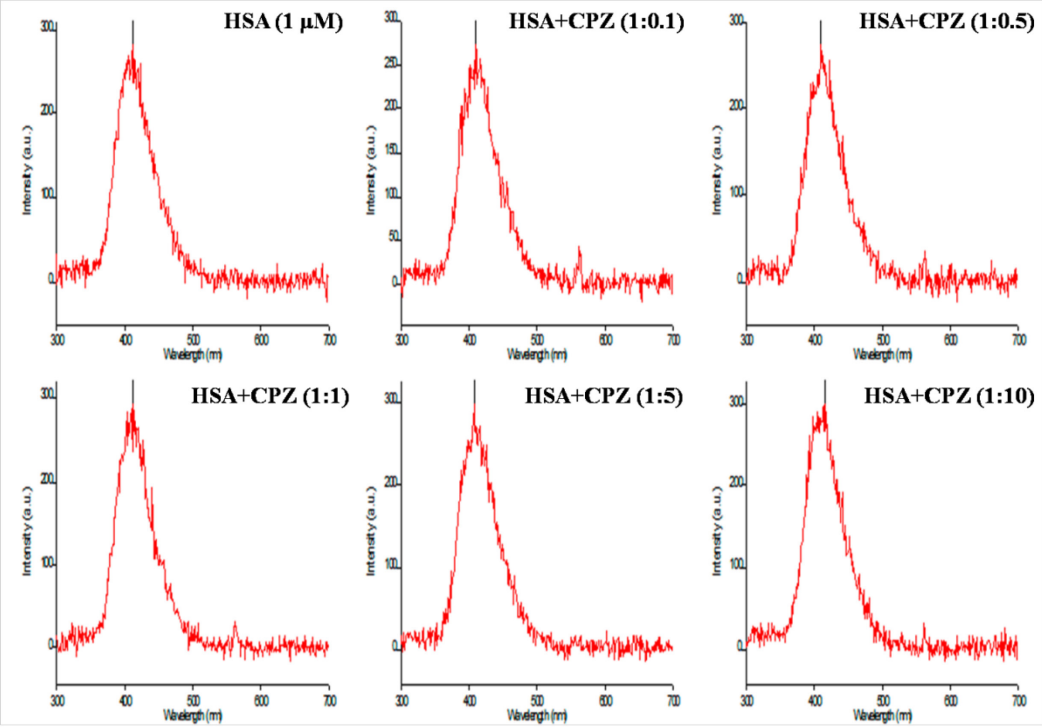

Figure S5. The tryptophan fluorescence quenching assay of HSA:CPZ. CPZ does not bind at the fatty acid binding site 7 (FA7) located at the sub-domain IIA of HSA, where the sole tryptophan residue of HSA is located. In this assay, the tryptophan fluorescence spectra of HSA:CPZ in the presence of increasing amounts of CPZ did not change at all, even at a high molar excess of 10-fold CPZ.

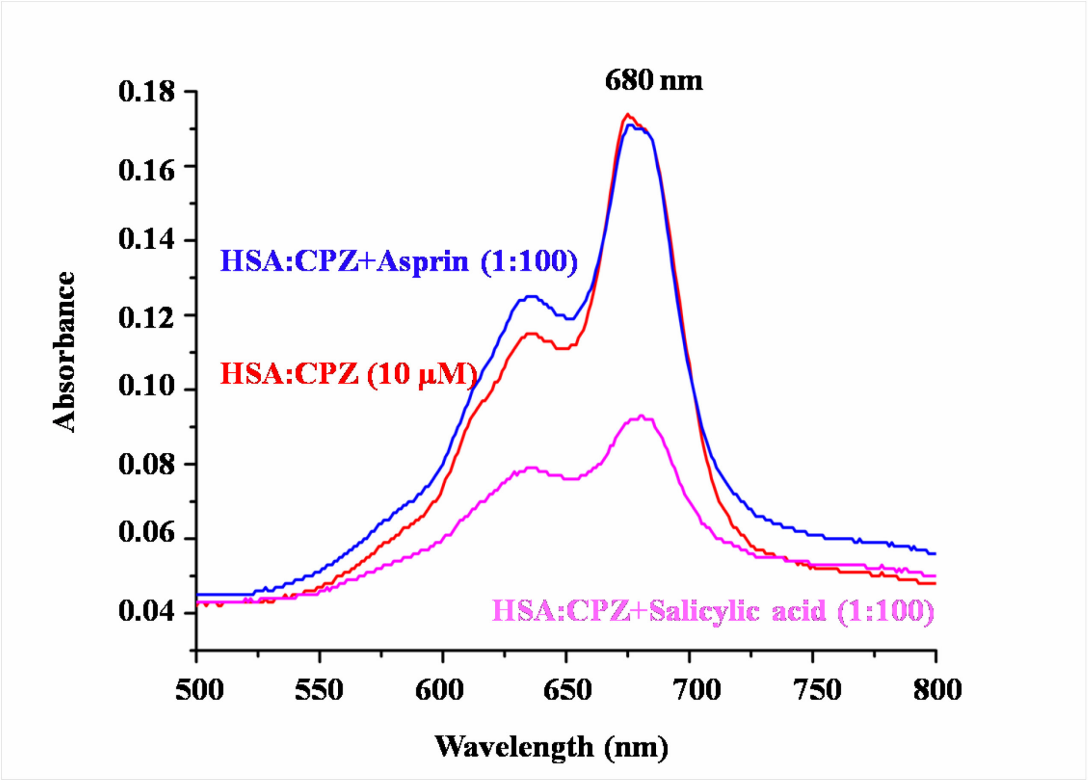

Figure S6. The probe competition assay of HSA:CPZ. CPZ likely binds at the fatty acid binding site 1 (FA1) located at the sub-domain IB of HSA instead of the FA7 located at the sub-domain IIA. Asprin binds at the FA7 site, and salicylic acid binds at the FA1 and FA7 sites. In this assay, the maximum absorption of HSA:CPZ at 680 nm in the presence of 100-fold asprin did not change at all, while it decreased dramatically in the presence of 100-fold salicylic acid.

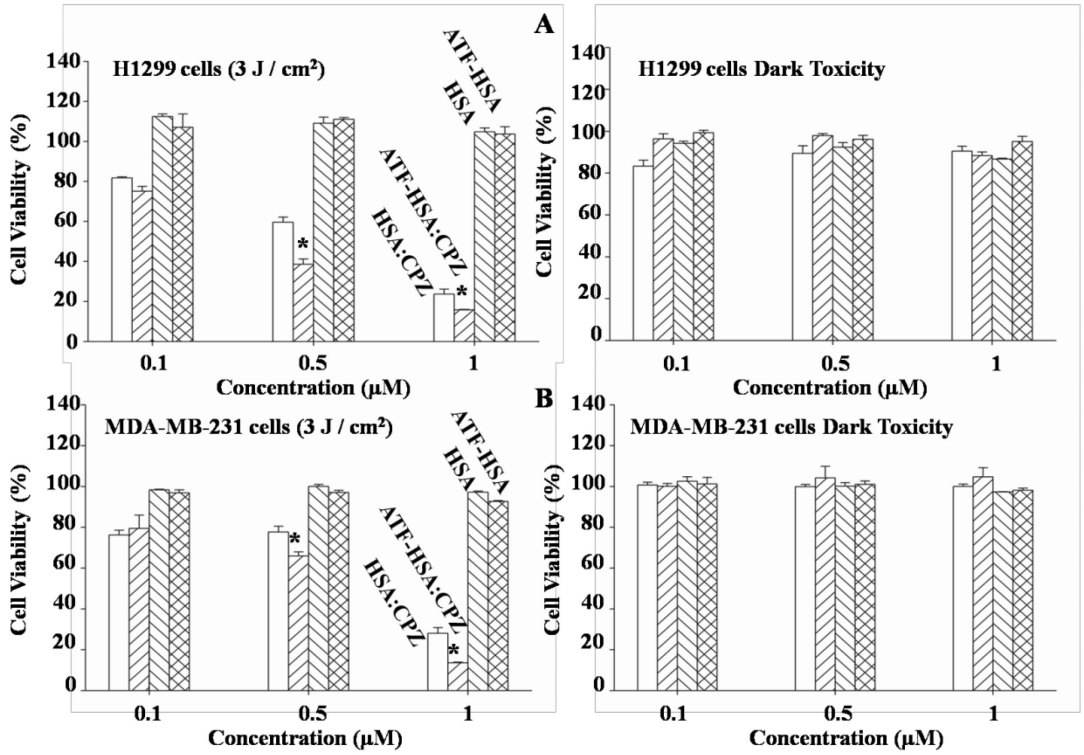

Figure S7. ATF-HSA:CPZ shows enhanced phototoxicities on H1299 cells and MDA-MB-231 cells (A and B left panels) compared to HSA:CPZ (~2 fold). Both ATF-HSA:CPZ and HSA:CPZ show low dark toxicities on H1299 cells and MDA-MB-231 cells (A and B right panels). We incubated these agents with the cells for 24 h to allow their sufficient cellular uptakes. We then washed out the unbound agents, illuminated the cells with or without a 680 nm LED light source (100 mW, Sundynamic Inc., Qingdao, Shandong, China) for 2 min to a light dose of 3 J / cm^2 and finally used a MTT colorimetric assay to measure the cell viability. Four replicates in each dosage were tested for each cell line and each experiment was repeated three times. Values represent the mean of three separate experiments; bars represent standard error of the mean (SEM). The unpaired, 2-tailed Student t test was used to analyze data; *, $p < 0.05$.

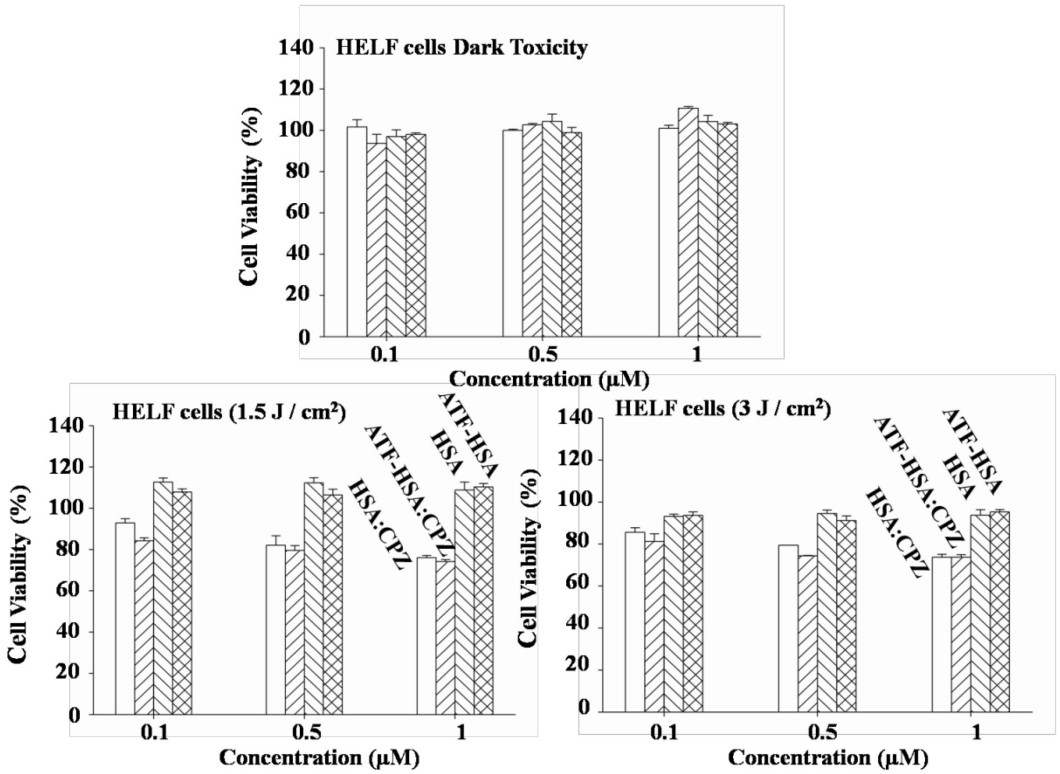

Figure S8. Both ATF-HSA:CPZ and HSA:CPZ show low phototoxicities (bottom panels) and no dark toxicities (top panel) on HELF cells. We incubated these agents with the cells for 24 h to allow their sufficient cellular uptakes. We then washed out the unbound agents, illuminated the cells with or without a 680 nm LED light source (100 mW, Sundynamic Inc., Qingdao, Shandong, China) for 1 or 2 min to a light dose of 1.5 or 3 J / cm^2 and finally used a MTT colorimetric assay to measure the cell viability. Four replicates in each dosage were tested for each cell line and each experiment was repeated three times. Values represent the mean of three separate experiments; bars represent standard error of the mean (SEM). The unpaired, 2-tailed Student t test was used to analyze data.

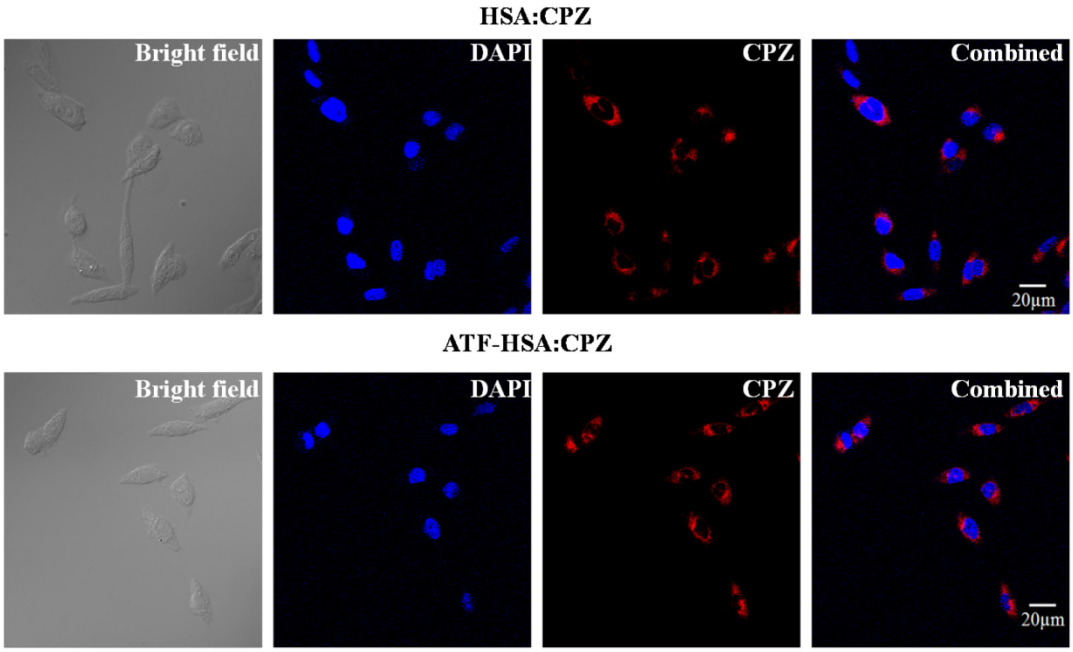

Figure S9. There is no significant difference of confocal laser scanning microscopic imaging between ATF-HSA:CPZ and HSA:CPZ in HELF cells. 5×10^4 / ml HELF cells were seeded in confocal chamber slides for 24 h. Then the cells were incubated with 2 μM ATF-HSA:CPZ or HSA:CPZ for 24 h and washed with PBS buffer, pH 7.2. The cells were finally incubated with DAPI and prepared for scanning according to the confocal laser scanning microscopy manual.

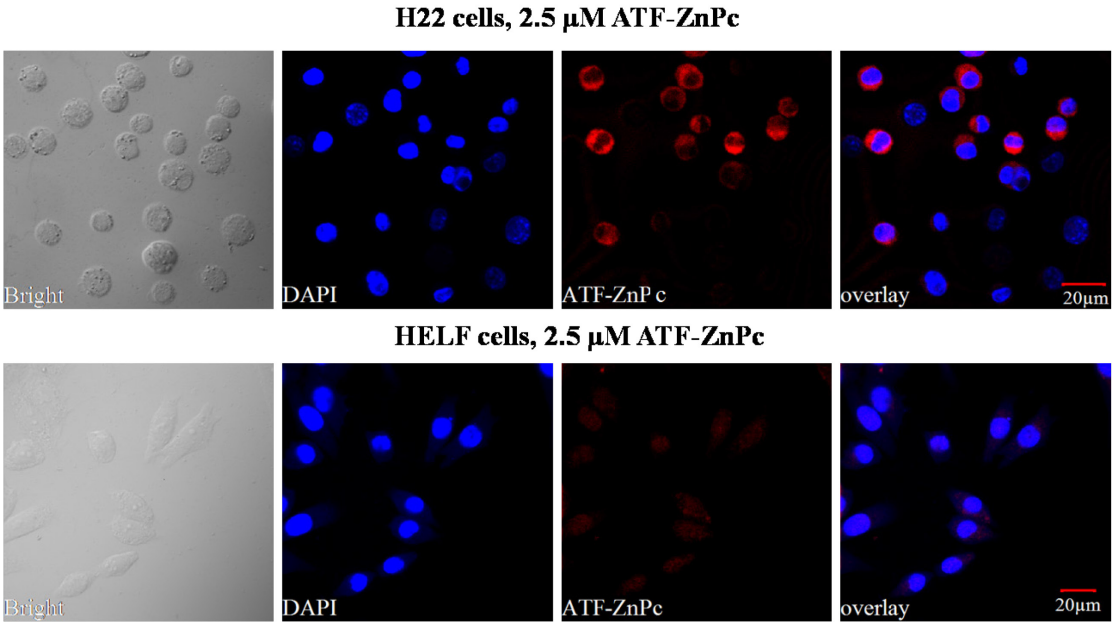

Figure S10. H22 cells have surface mouse uPAR and bind to human ATF. Confocal laser scanning microscopic images show the obvious accumulation of human ATF conjugated with fluorescent ZnPc in hepatocarcinoma H22 cells (upper panels) compared to that in HELF cells (lower panels) after 2 h incubation.

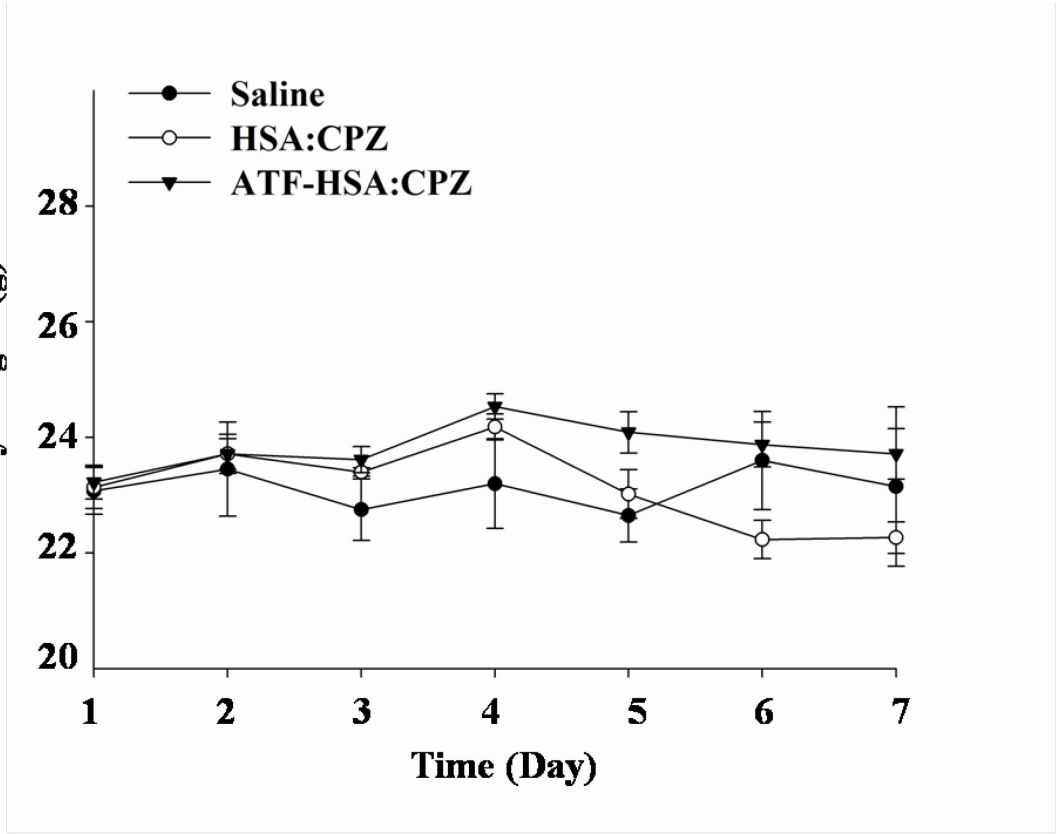

Figure S11. Body weight measurements of H22 tumor-bearing Kunming mice during the 7-day PDT treatment (8 mice per group). ATF-HSA:CPZ or HSA:CPZ (0.080 μmol / kg, or 0.050 mg CPZ / kg of mouse body weight) or saline was injected intravenously via tail vein followed by a daily light illumination (50 J / cm^2) for continuous 7 days. The data were averaged from 8 mice in each group daily; bars represent standard error of the mean (SEM). The unpaired, 2-tailed Student t test was used to analyze data.