Nanoparticle binding to urokinase receptor on cancer cell surface triggers nanoparticle disintegration and cargo release

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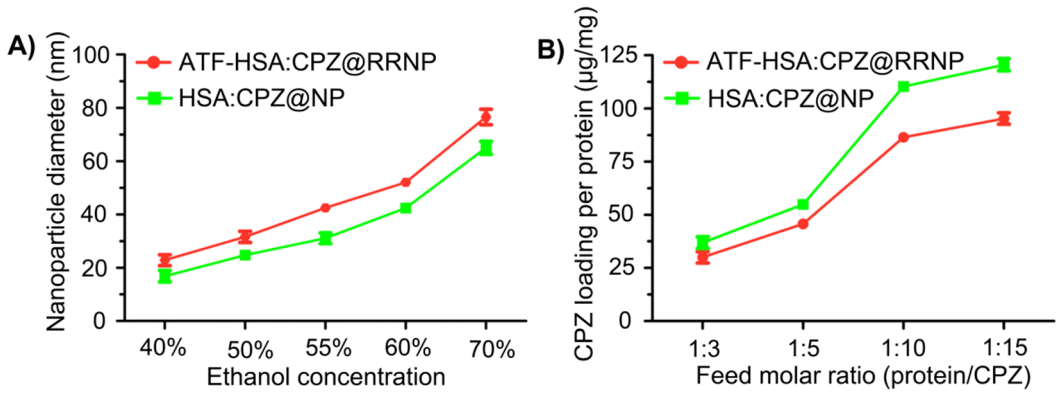

Figure S1. A) Study on the optimal ethanol concentration for the ATF-HSA:CPZ@RRNP or HSA:CPZ@NP preparation. The diameters of nanoparticles can be tuned by ethanol concentration. **B)** Effect of the starting protein/CPZ feed molar ratio on the amount of drug loaded per protein. The feed molar ratio of 1:10 was used for both nanoparticles.

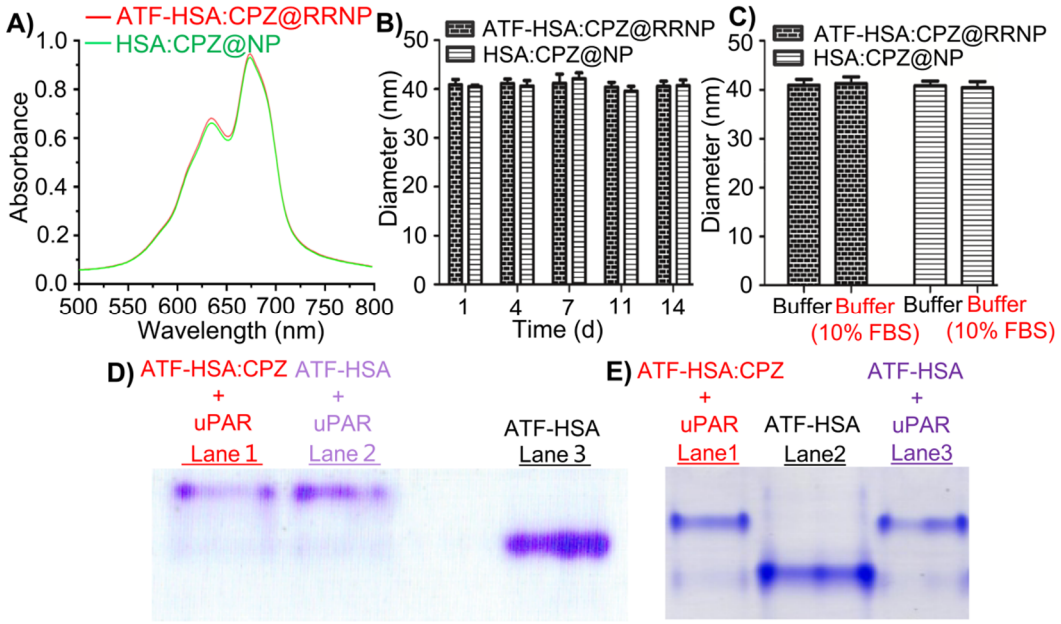

Figure S2. A) UV-Vis spectra of ATF-HSA:CPZ@RRNP (red) and HSA:CPZ@NP (green) at pH 8.0 showed characteristic absorption of CPZ. **B)** Both ATF-HSA:CPZ@RRNP and HSA:CPZ@NP were quite stable for at least 14 days based on dynamic light scattering (DLS) in buffer solution (20 mM Tris-HCl, 50 mM NaCl, pH 8.0). **C)** Both ATF-HSA:CPZ@RRNP and HSA:CPZ@NP were stable in fetal bovine serum (FBS) for at least 2 days at 37 °C. 5 μM ATF-HSA:CPZ@RRNP or HSA:CPZ@NP was mixed with FBS at a volume ratio of 9:1 and incubated at 37 °C for 2 days. Then the size of ATF-HSA:CPZ@RRNP or HSA:CPZ@NP was measured by DLS. The result showed the size of both nanoparticles did not change after adding 10% FBS. **D)** Gel shift experiment showed that addition of recombinant soluble uPAR to monomer ATF-HSA:CPZ (lane 1) from disintegrated ATF-HSA:CPZ@RRNP or to ATF-HSA (lane 2, ATF-HSA expressed by *Pichia pastoris*, control) caused the ATF-HSA:CPZ or ATF-HSA band to shift, demonstrating the direct binding of ATF-HSA:CPZ to uPAR protein, the only ATF-HSA (lane 3) as a control. **E)** Native gel shift assay demonstrated ATF-HSA:CPZ@RRNP disintegrated into small aparts after incubation with uPAR receptor for 2.5 h (lane 1). Only expressed ATF-HSA from *Pichia Pastoris* as a negative control (lane 2) and another sample containing recombinant uPAR and ATF-HSA (1:1) from *Pichia Pastoris* as a positive control (lane 3).

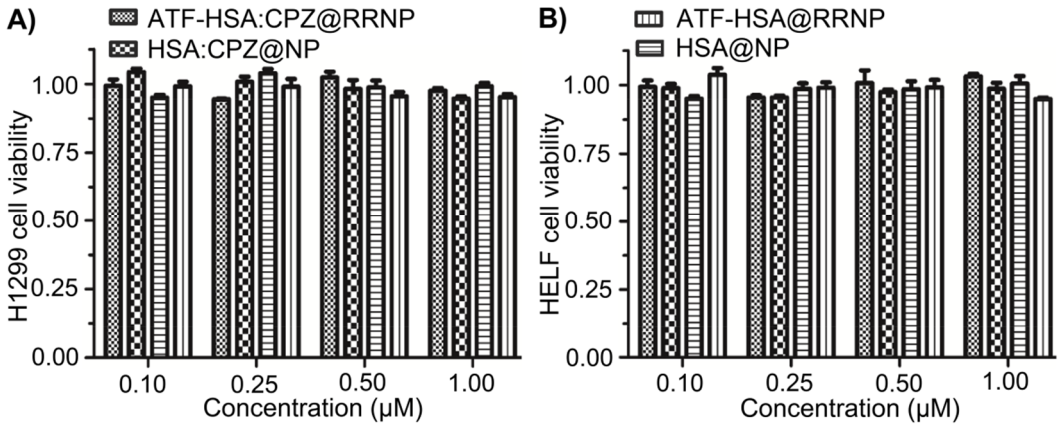

Figure S3. Both ATF-HSA:CPZ@RRNP and HSA:CPZ@NP showed no phototoxicity on H1299 cells **(A)** and HELF cells **(B)** in the absence of light.

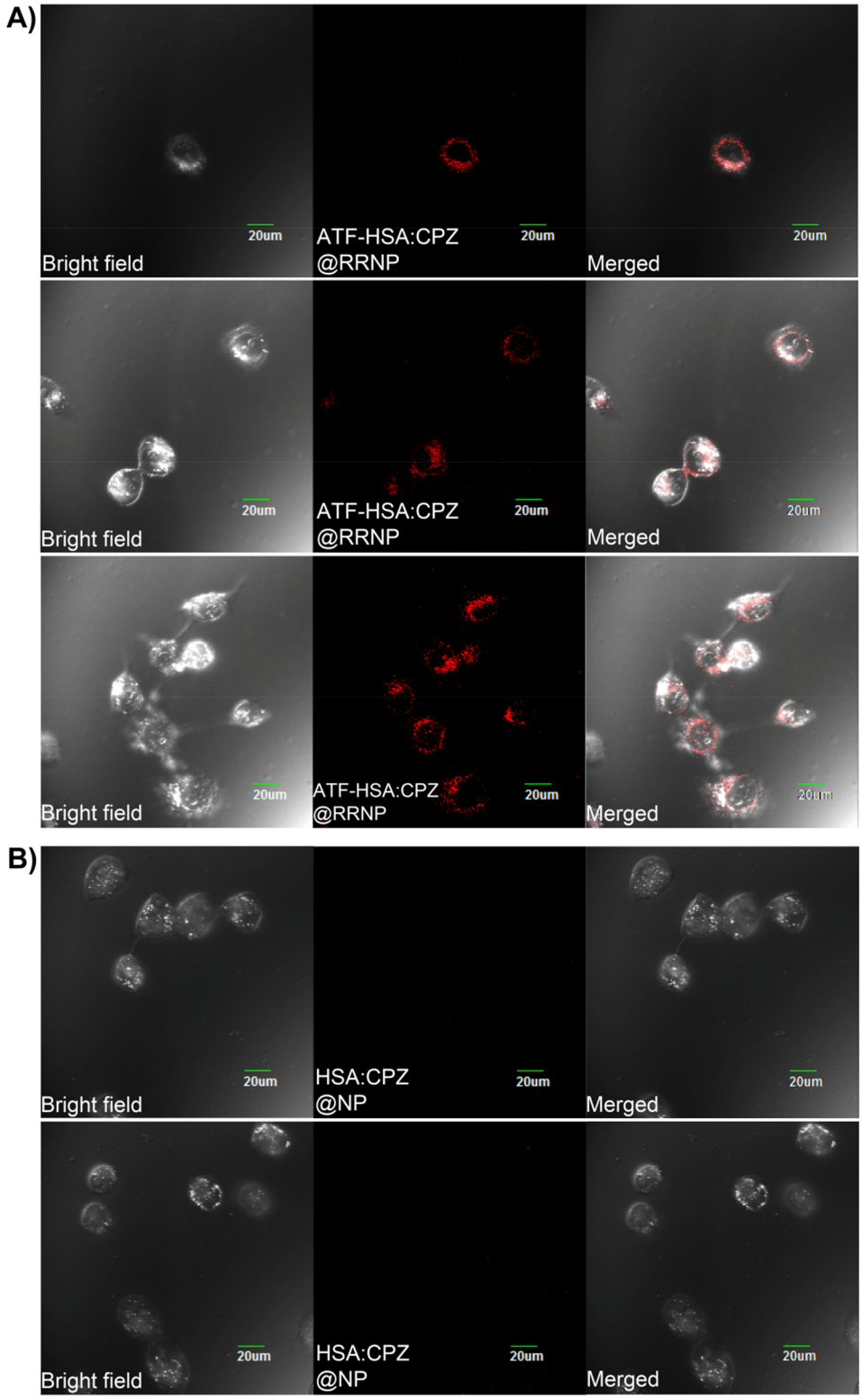

Figure S4: Representative images of laser scanning confocal microscope of the nanoparticles incubated with H1299 for 8 min. **(A)** targeting nanoparticle ATF-HSA:CPZ@RRNP, **(B)** non-targeting nanoparticle HSA:CPZ@NP.

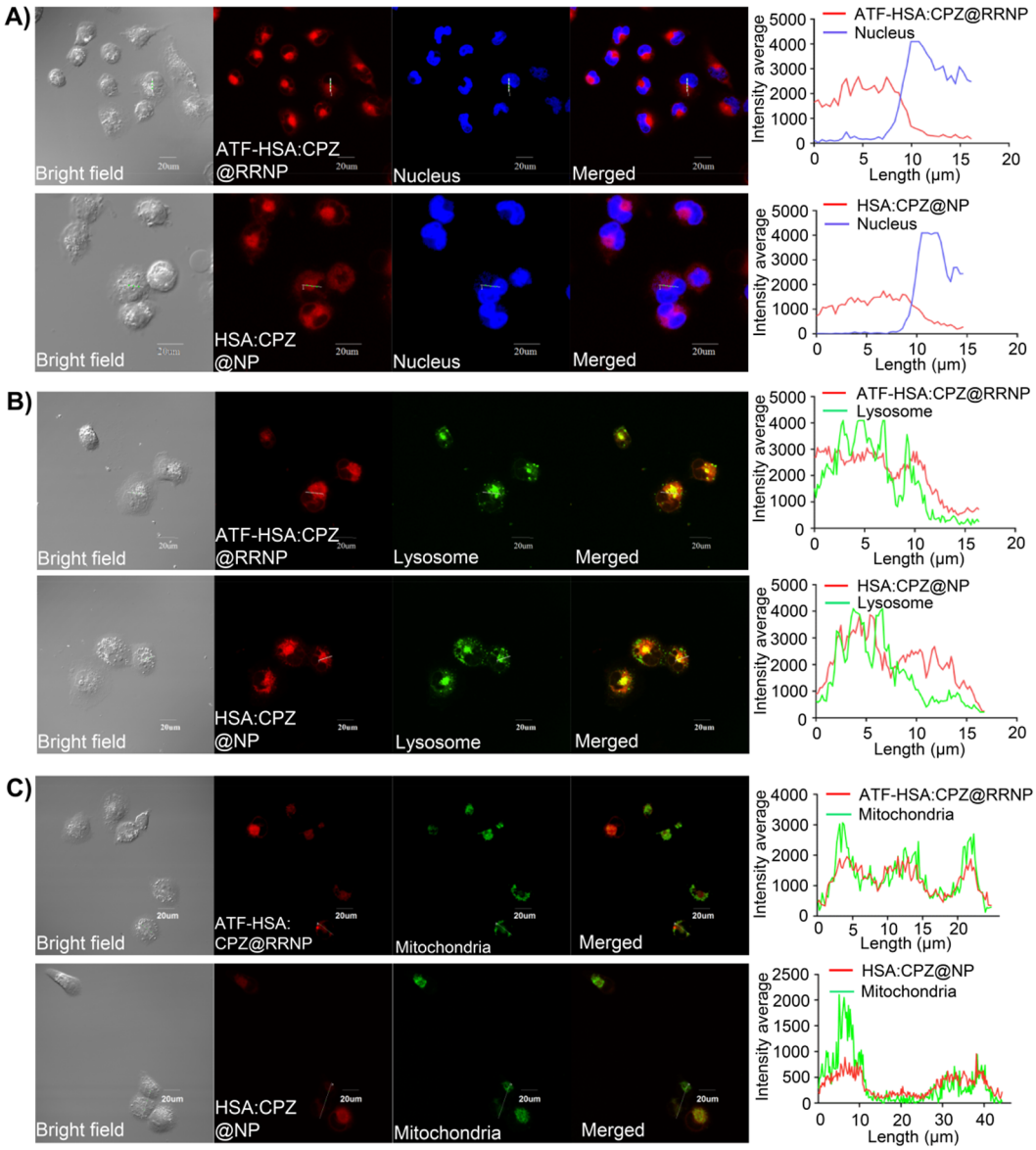

Figure S5. Intracellular localization of nanoparticles by laser scanning confocal microscope. **A)** Both nanoparticles (ATF-HSA:CPZ@RRNP and HSA:CPZ@NP) were localized in cytoplasm of H1299 cell, not in nucleus (gray, red and blue colors represents bright field, nanoparticles and DAPI-stained nucleus, respectively). **B)** Both nanoparticles were co-localized with lysosome (gray, red, green colors represent bright field, nanoparticles, LysoTracker-stained lysosomes, respectively). **C)**. Both nanoparticles were co-localized with mitochondria (gray, red, green colors represent bright field, nanoparticles and MitoTrack-stained mitochondria, respectively).

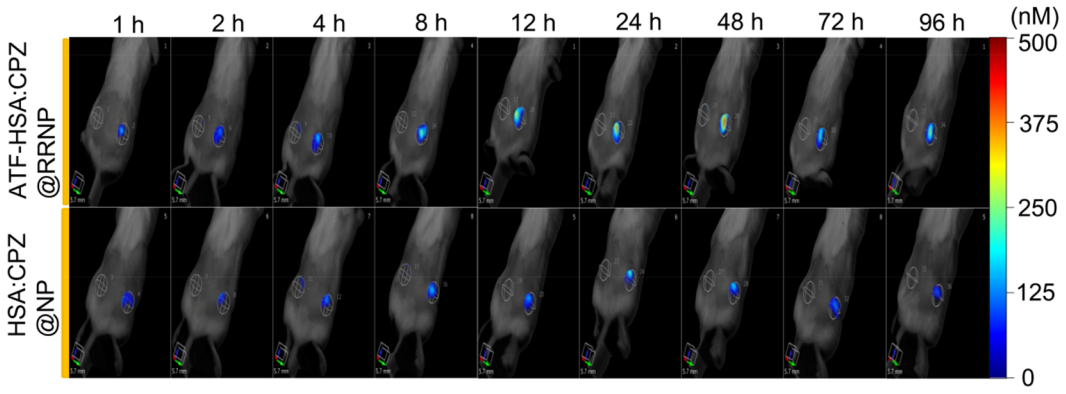

Figure S6. Representative fluorescence images of H22 tumor-bearing mice taken at different time points (1, 2, 4, 8, 12, 24, 48, 72, 96 h) post intravenous injection of ATF-HSA:CPZ@RRNP (top) and HSA:CPZ@NP (bottom).

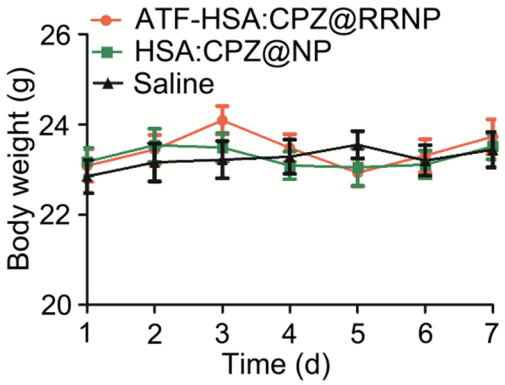

Figure S7. Body weight of H22 tumor-bearing Kunming mice during the 7-day photodynamic therapy (10 mice per group).