Supplementary Materials

Drug-delivering-drug platform-mediated potent protein therapeutics *via* a non-endo-lysosomal route

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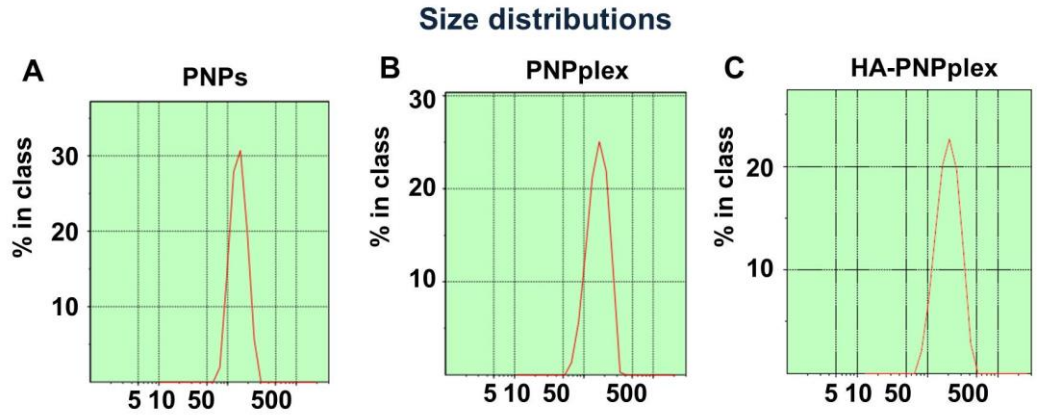

Figure S1. Size distributions of **(A)** PNPs, **(B)** PNPplex and **(C)** HA-PNPplex.

A

CLG:Caspase 3:HA	32:1:3	32:1:1.5	32:1:1	32:1:0.3	32:1:0.15	32:1:0
Particle size (nm)	202.6 ± 1.2	197.5± 0.7	153.9± 3.1	133.9± 2.0	127.8 ± 2.4	148.0± 1.3
PDI	0.176	0.156	0.132	0.152	0.151	0.110
Zeta potential (mv)	6.99 ± 0.30	7.34 ± 0.92	9.41± 0.68	9.66 ± 1.39	15.00 ± 3.73	16.10± 1.07

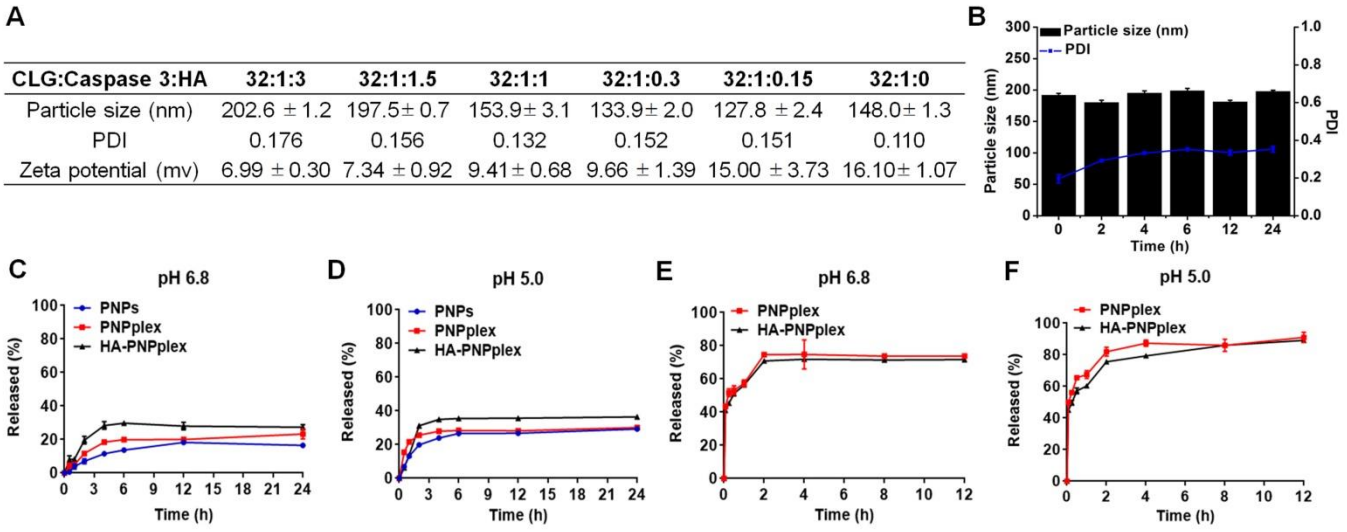

Figure S2. (A) Particle size, PDI and zeta potential of HA-PNPplex at different mass ratio of CLG/caspase 3/HA. **(B)** The stability of HA-PNPplex from optimized formulation stored in a 10% serum at 37 ℃ for 24 h. Release profiles of **(C, D)** PTX and **(E, F)** RITC-BSA from PNPs, PNPplex and HA-PNPplex in PBS solutions containing 1 M sodium salicylate at 37 ℃.

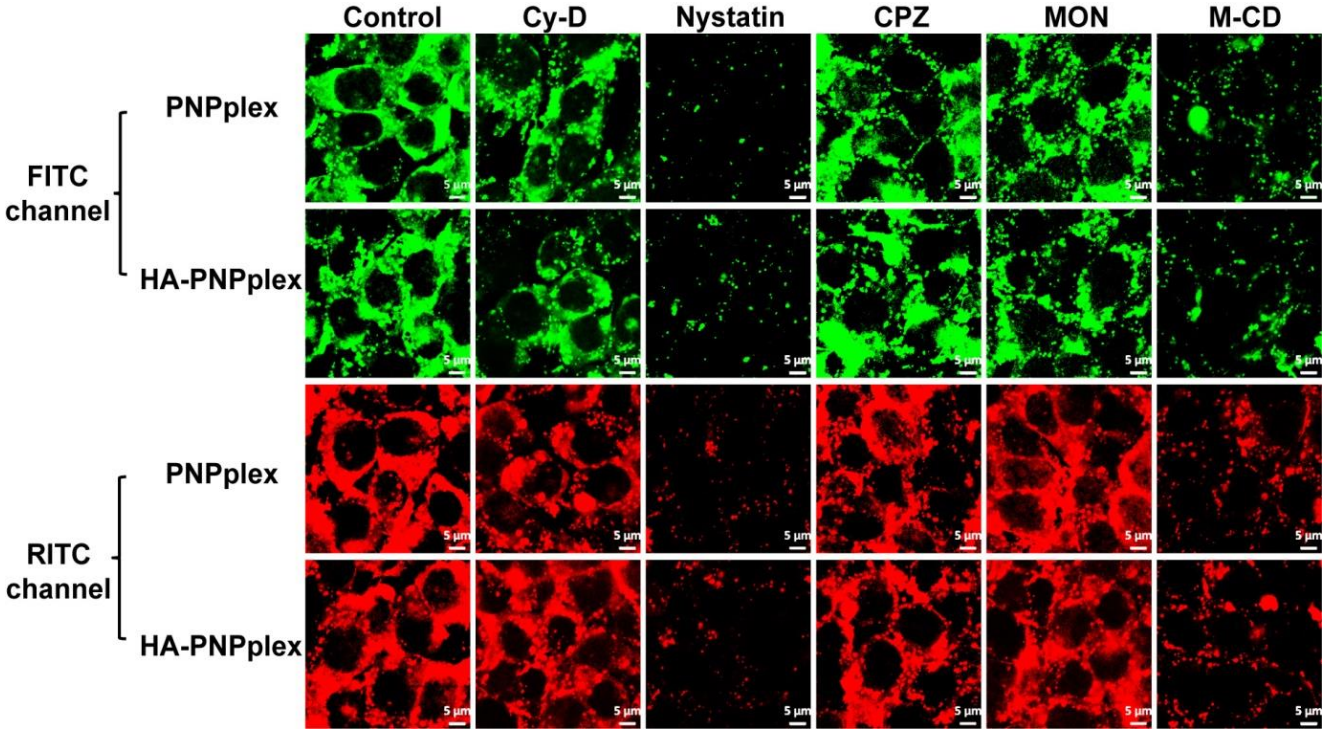

Figure S3. CLSM observation of intracellular accumulation of FITC-PNPplex or HA-PNPplex and RITC-PNPplex or HA-PNPplex in MCF-7 cells pretreated with different inhibitors for 0.5 h at 37 ℃ after 4 h of incubation with an FITC of 2.5 μg/mL or RITC of 500 ng/mL. The scale bar is 5 μm.

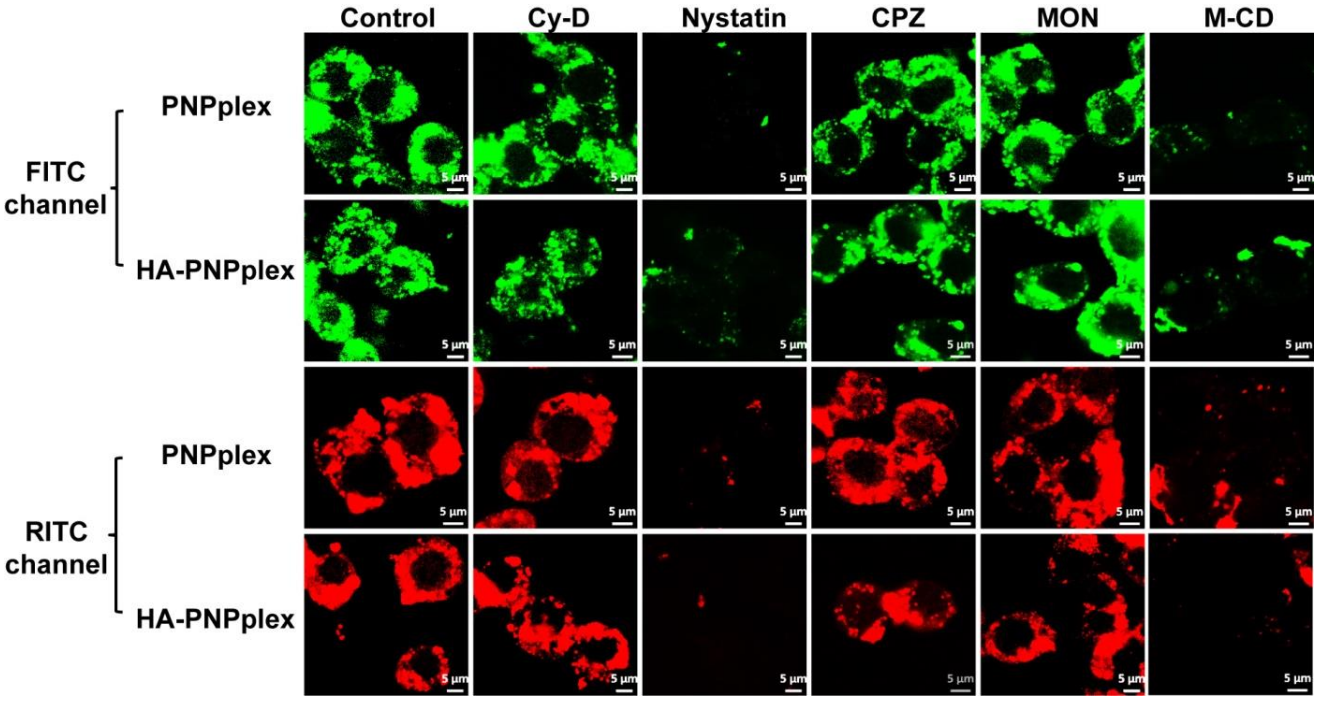

Figure S4. CLSM observation of intracellular accumulation of FITC-PNPplex or HA-PNPplex and RITC-PNPplex or HA-PNPplex in Caco-2 cells pretreated with different inhibitors for 0.5 h at 37 ℃ after 4 h of incubation with an FITC of 2.5 μg/mL or RITC of 500 ng/mL. The scale bar is 5 μm.

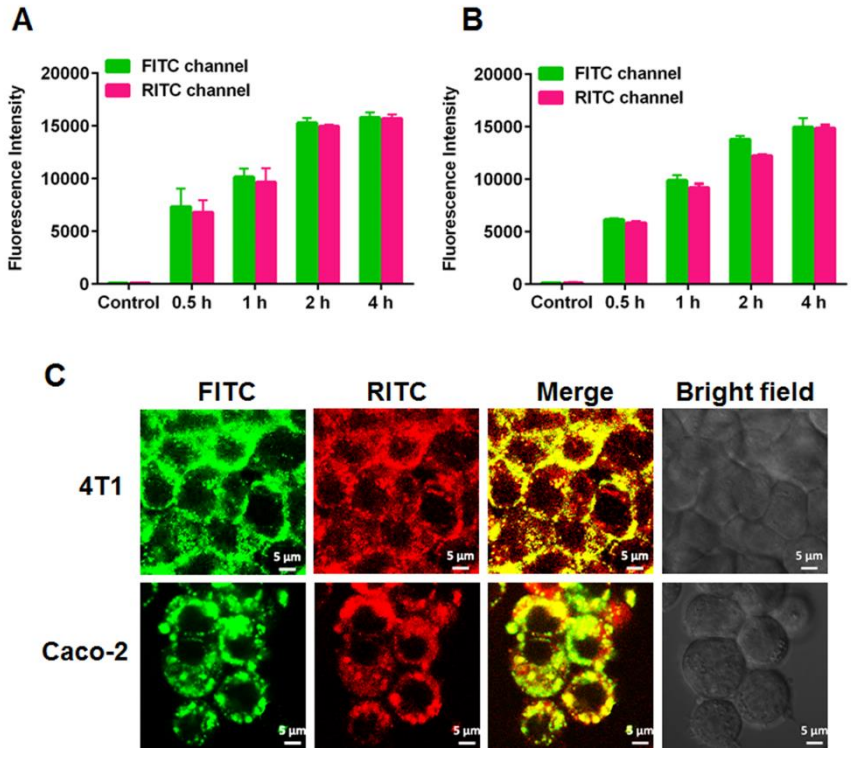

Figure S5. Time-related uptake of dual-labeled HA-PNPplex in **(A)** 4T1 and **(B**) Caco-2 cells after incubation at 37 °C with an FITC of 2.5 μg/mL or RITC of 500 ng/mL. **(C)** CLSM observation of intracellular delivery of dual-labeled HA-PNPplex in 4T1 and Caco-2 cells after incubation at 37 ℃ for 4 h. Yellow spots indicate the integrity of HA-PNPplex.

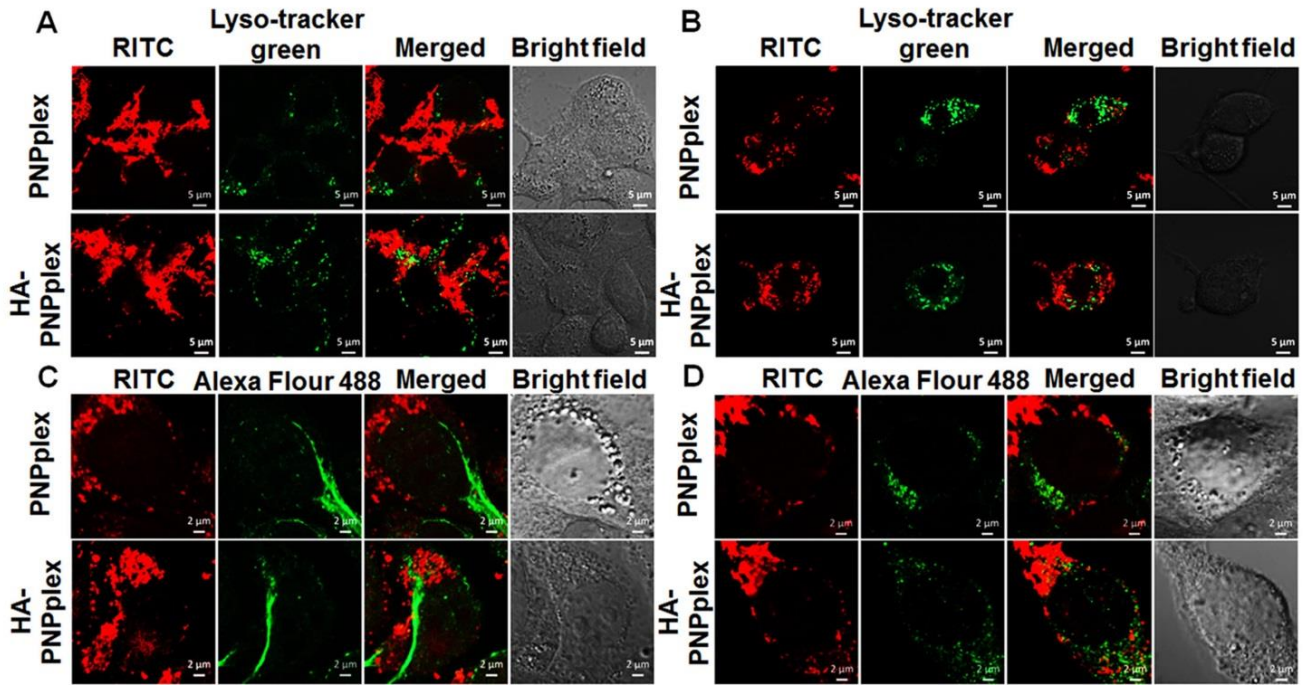

Figure S6. Co-localization of RITC-labeled nanoparticles with lysosomes in **(A)** MCF-7 and **(B)** Caco-2 cells upon incubation with a RITC of 500 ng/mL at 37 °C for 4 h. The scale bar is 5 μm. Colocation of the RITC-labeled nanoparticles with early (C) or late (D) endosomes in MCF-7 cells after a 4-h incubation with a RITC of 500 ng/mL at 37 ℃. The scale bar is 2 μm.

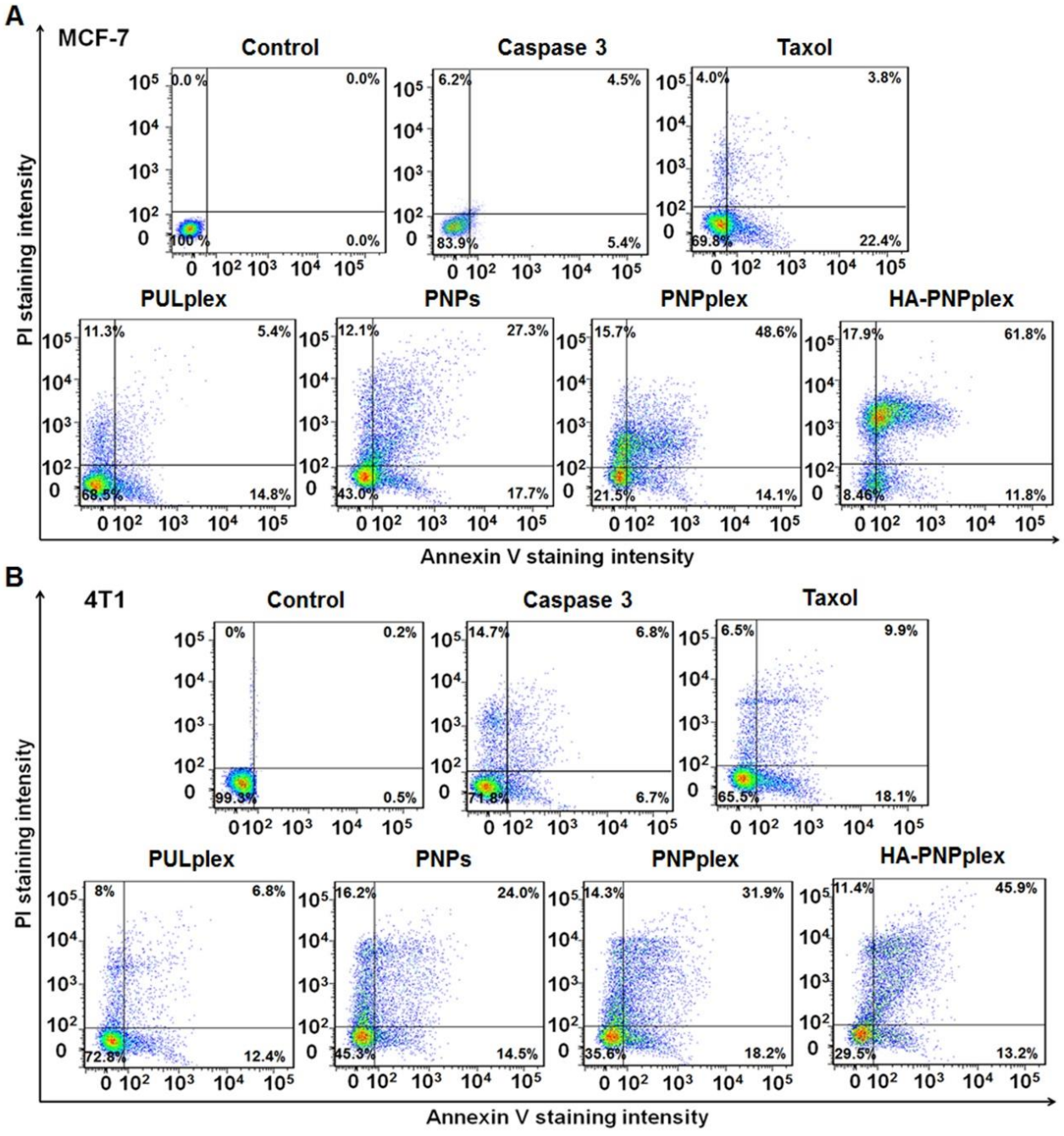

Figure S7. FCM analysis of apoptosis in **(A)** MCF-7 without the expression of endogenous caspase 3 and **(B)** 4T1 cells induced by different preparations after 48 h of incubation at a fixed PTX concentration of 10 μg/mL and caspase-3 concentration of 76 nM. The cells were stained with FITC-Annexin V and PI.

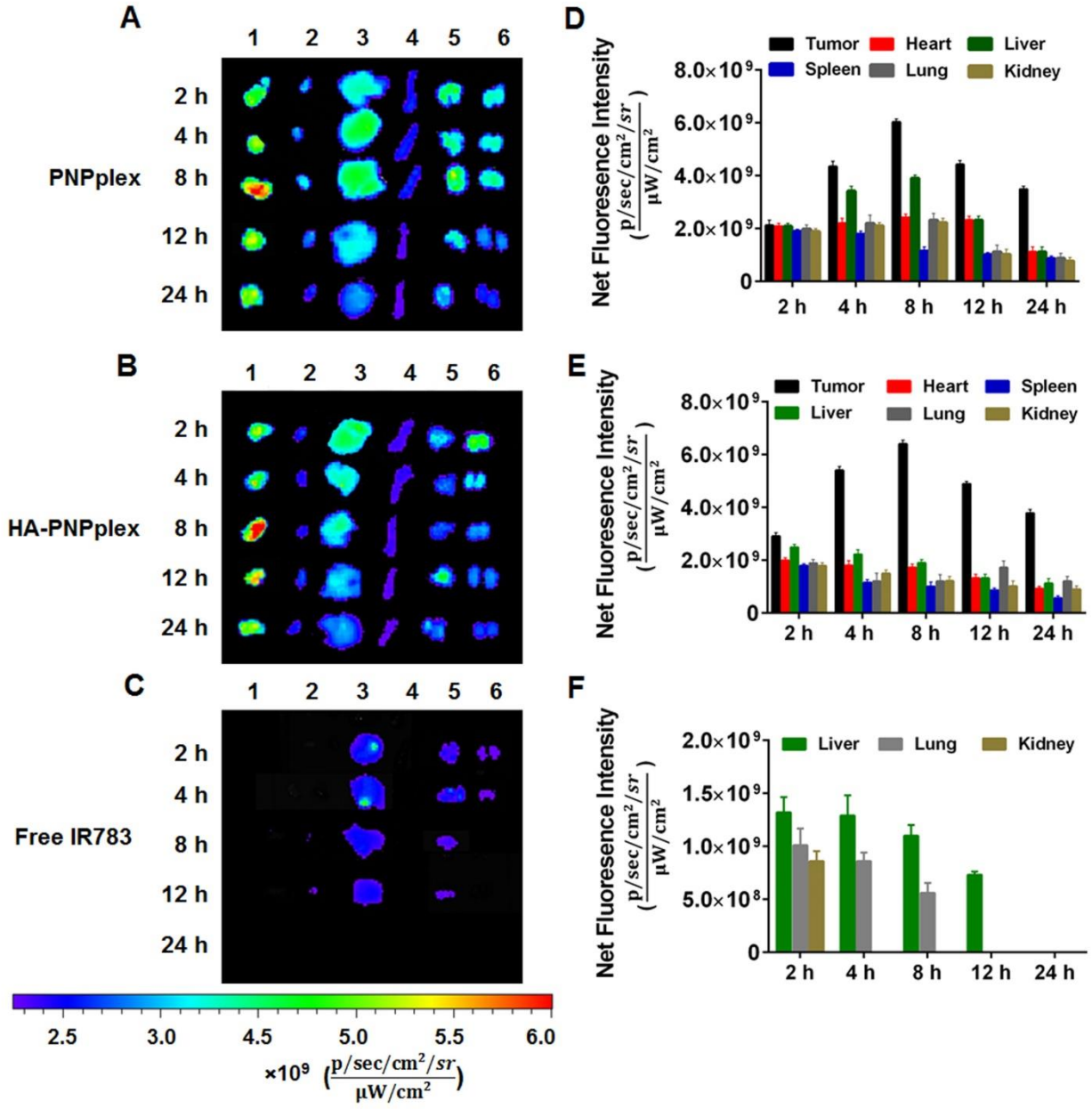

Figure S8. (A–C) *Ex vivo* fluorescence images of tissues including tumor (1), heart (2), liver (3), spleen (4), lung (5) and kidneys (6) collected at 2 h, 4 h, 8 h, 12 h and 24 h post injection of free IR 783 or IR783-labeled nanoparticles at an IR783 dose of 2.5 mg/kg based on animal's body weight. Quantified accumulation of **(D)** IR783-PNPplex, **(E)** IR783-HA-PNPplex and **(F)** free IR783 in tissues at 2 h, 4 h, 8 h, 12 h and 24 h post injection.