Electronic Supplementary Information

Tumor acidity-responsive polymeric nanoparticles to promote intracellular delivery of zoledronic acid by PEG detachment and positive charge exposure for enhanced antitumor potency

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Preparation of IR780-labeled ZA-carrying hybrid polymeric nanoparticles

The IR780-labeled ZA/PEI@PBCTPNs were prepared as follows. First, ZA (3.2 mg) and branched PEI (4.6 mg) were dissolved in tris buffer of pH 8.5 and 6.0 (0.8 mL), respectively. Subsequently, The ZA solution was added into PEI solution and stirred for 30 min to obtain the ZA/PEI mixtures. The ratio of the number of amine from PEI to the number of phosphonates from ZA (N/P ratio) was fixed at 9.0. TPGS (1.0 mg) in pH 7.4 tris buffer (0.1 mL) was added dropwise to the ZA/PEI mixtures under stirring. Then PLGA (6.0 mg), IR780 (0.6 mg) and mPEG-b-C18 (3.0 mg) dissolved in DMSO (0.3 mL) was added to the ZA/PEI/TPGS-containing aqueous solution (1.7 mL) under stirring. The mixed solution was mildly stirred at 25 ^{o}C for 60 min and then equilibrated for 1 h. Through dialysis (Cellu Sep MWCO 12000~14000) of the ZA/PEI@PBCTPNs suspension against pH 8.0 phosphate buffer (10 mM) at 4 ^{o}C, DMSO and unloaded ZA

were eliminated. For comparison, the IR780-labeled ZA/PEI@PCTPNs with an N/P ratio of 9.0 were also obtained in a similar way. For quantitation of IR780 entrapped within hybrid nanoparticles, a prescribed volume of the purified IR780-containing nanoparticle solution was lyophilized and then dissolved into DMSO to disrupt colloidal structure. The absorbance of IR780 at 775 nm was determined by a Hitachi U2900 UV/Vis spectrophotometer.

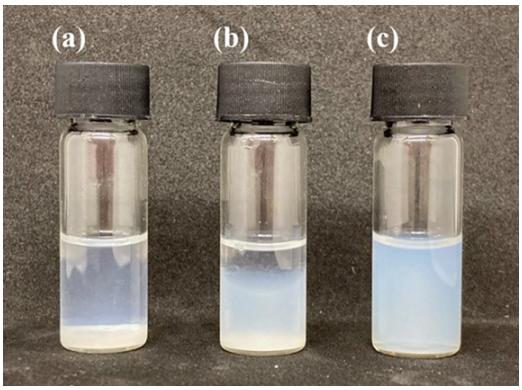

Fig. S1. Photographs of aqueous solutions of (a) ZA/PEI@TPNs (with TPGS alone), (b) ZA/PEI@PBCPNs (with mPEG-b-C18 alone) and (c) ZA/PEI@PBCTPNs (with mPEG-b-C18 and TPGS).

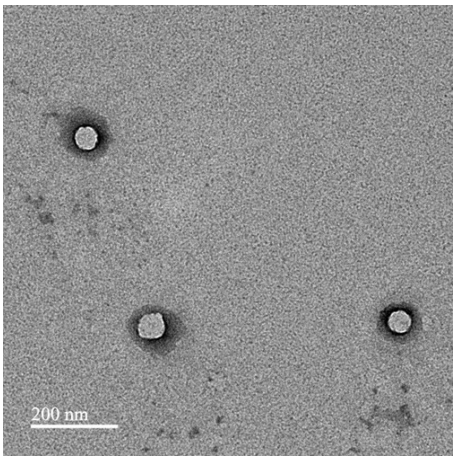

Fig. S2. TEM images of ZA/PEI@PCTPNs.

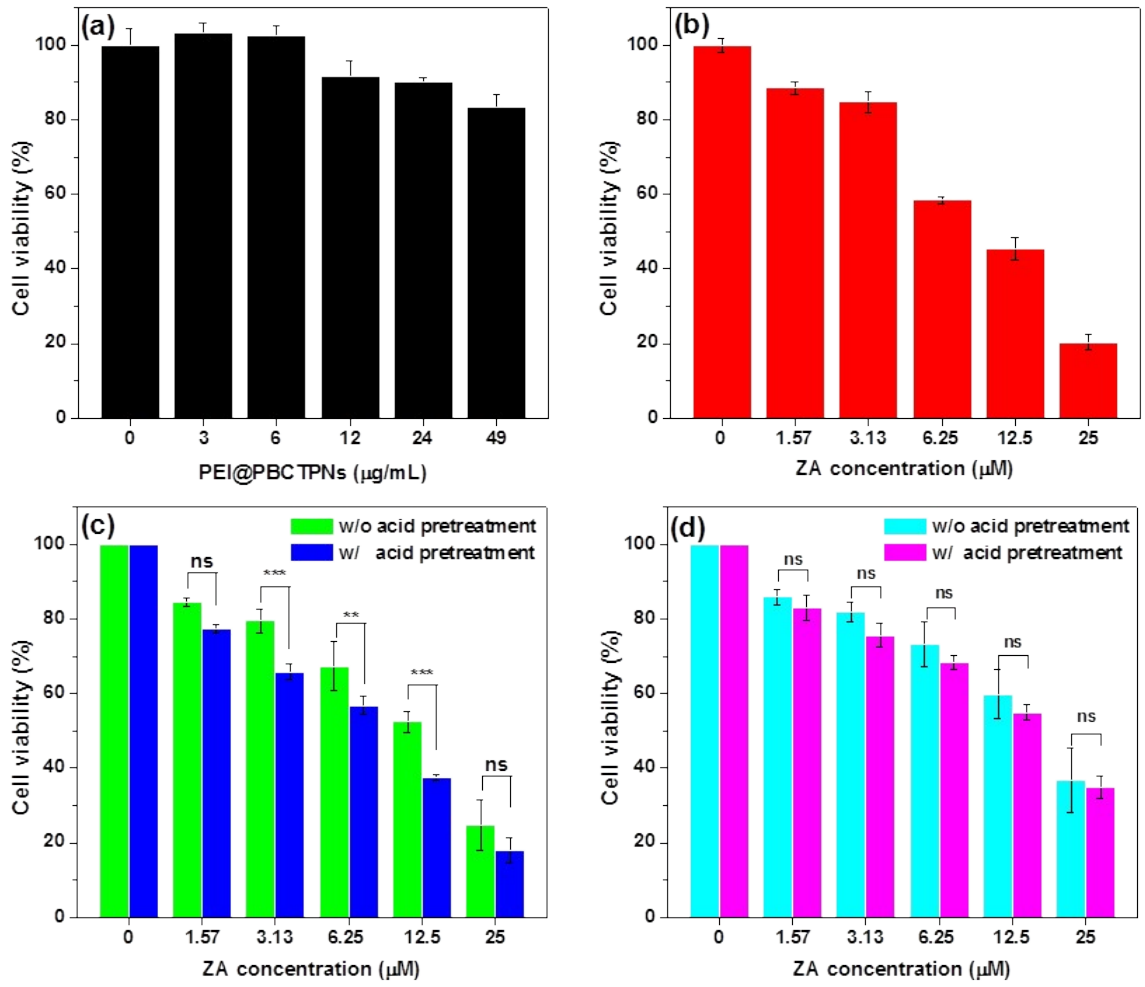

Fig. S3. Cell viability of HeLa cells incubated with (a) ZA-free PEI@PBCTPNs and (b) free ZA at 37 °C for 48 h. Cell viability of HeLa cells treated with (c) ZA/PEI@PBCTPNs and (d) ZA/PEI@PCTPNs with and without acid pretreatment at 37 °C for 48 h. n.s. = statistically insignificant, *P < 0.05, **P < 0.005, ***P < 0.001.

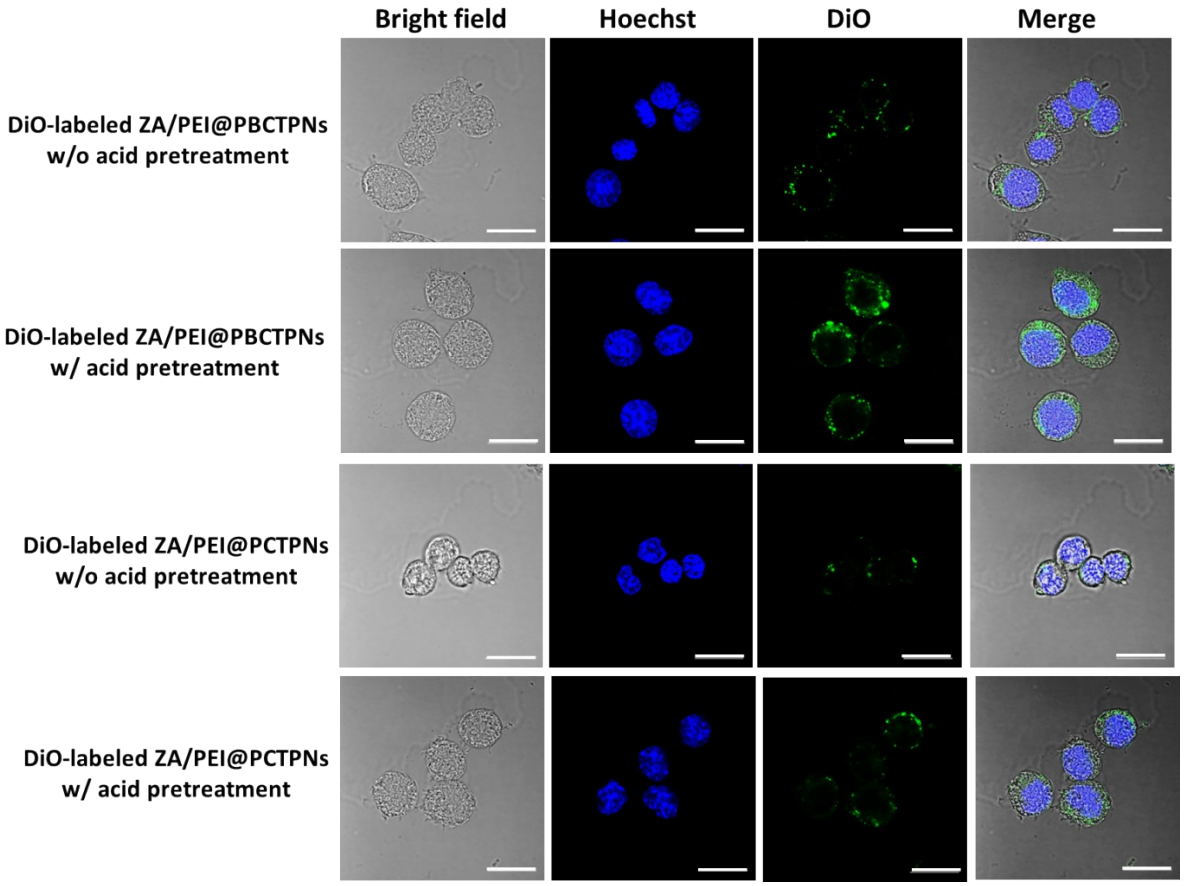

Fig. S4. CLSM images of RAW 264.7 cells incubated with DiO-labeled ZA/PEI@PBCTPNs and ZA/PEI@PCTPNs with and without acid pretreatment for 1 h at 37 °C, respectively. The scale bars are 20 μm.

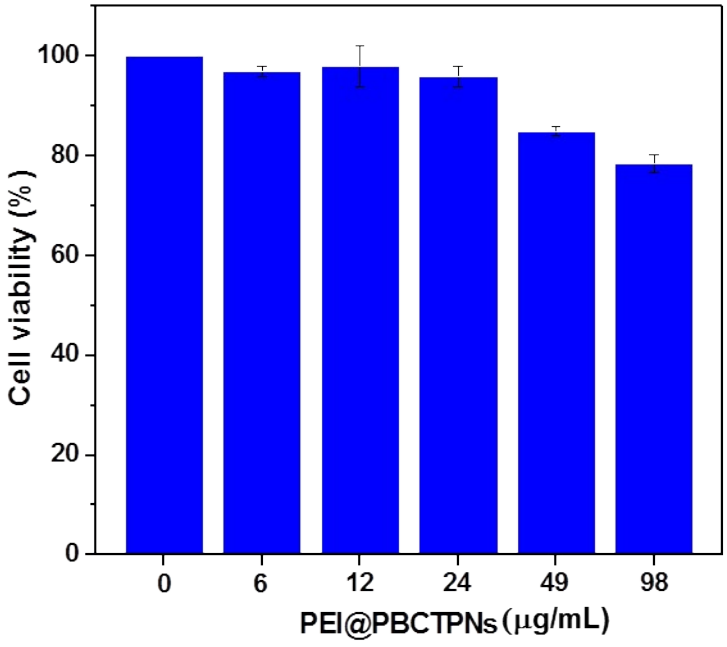

Fig. S5. Cell viability of RAW 264.7 cells incubated with PEI@PBCTPNs at 37 °C for 48 h.

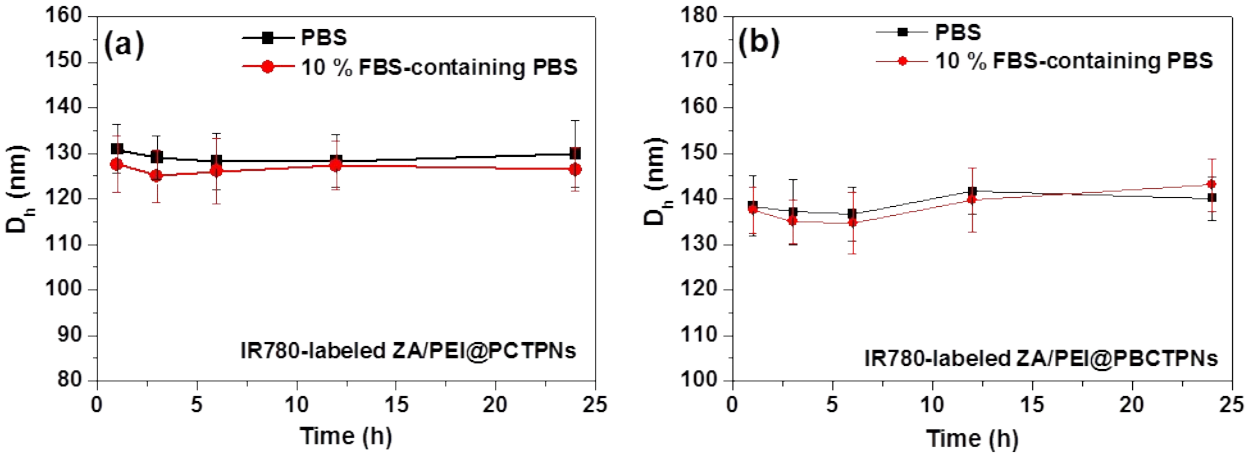

Fig. S6. Colloidal stability of (a) IR780-labeled ZA/PEI@PCTPNs and (b) IR780-labeled ZA/PEI@PBCTPNs dispersed in PBS with and without 10 % FBS at 37 ºC.

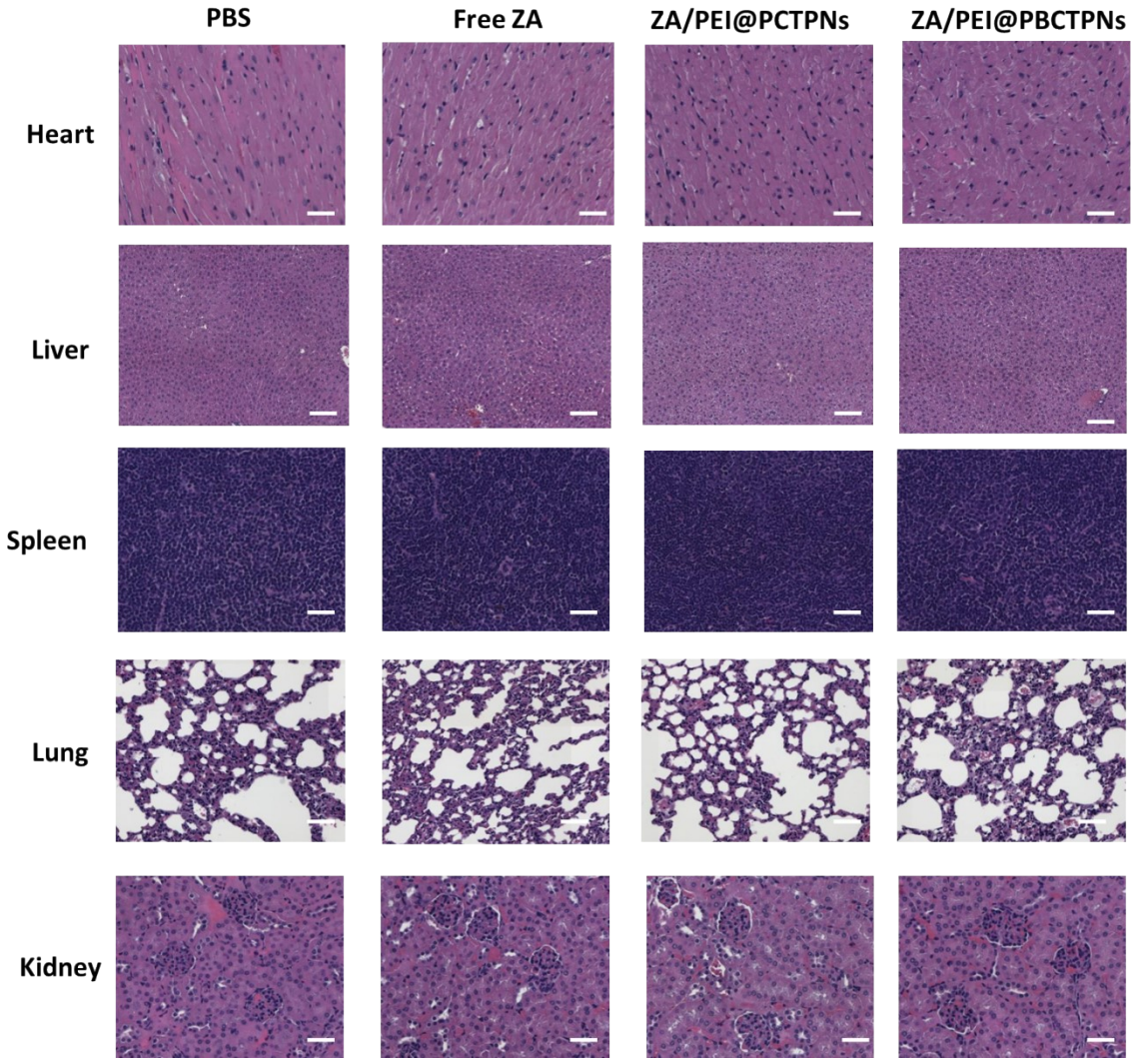

Fig. S7. H&E-stained images of major organs from the TRAMP-C1 tumor-bearing mice receiving different formulations. Scale bars are 50 μm.