Electronic Supplementary Material (ESI) for Biomaterials Science.

Electronic Supplementary Information

Co-delivery of proanthocyanidin and mitoxantrone induce synergistic immunogenic cell death to potentiate cancer immunotherapy

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1.1 Abbreviations

ICD: immunogenic cell death; DMP@NPs: tumor microenvironment-responsive deformable nanoparticle; ^{D}PPA-1: the polypeptide checkpoint inhibitor, the amino acid sequence is NYSKPTDRGYHF; PEG-DMA-^{D}PPA-1: tumor acidity responsive polypeptide checkpoint inhibitor polymer; MITX: mitoxantrone; PC: proanthocyanidins; HMGB-1: high mobility group box-1; CRT: calreticulin; CRC: colorectal cancer; MSS: microsatellite stability; DAMPs: damage-related molecular patterns ; PEG-OH: methoxypolyethylene glycol; DMA: 2,5-dihydro-4-methyl-2,5-dioxo-3-furanpropanoic acid; SDS: sodium dodecyl sulfate

1.2 Instruments

High performance liquid chromatograph (LC-2010 AHT, Shimadzu Company, Japan), Transmission electron microscopy (TEM) (Hitachi, Japan)Preparative liquid chromatography (Prep 150, Waters, US), Matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS, Bruker, Switzerland), Nuclear magnetic resonance spectrometer (NMR, 500MH, Bruker, Switzerland), Laser particle size analyzer (Zetasizer Nano ZS90, Malvern, UK), Microplate reader (Synergy H1, Bio Tek, US), Inverted fluorescence microscope (Axio Vert A1, Zeiss, Germany), Flow cytometer (BD FACSCalibur, Becton-Dickinson, US)

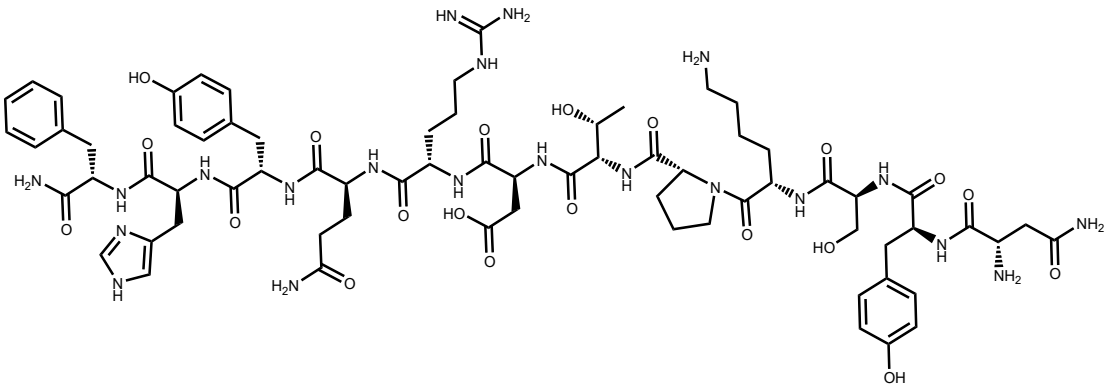

Figure S1. The structure of ^{D}PPA-1 (amino acid sequence NYSKPTDRGYHF).

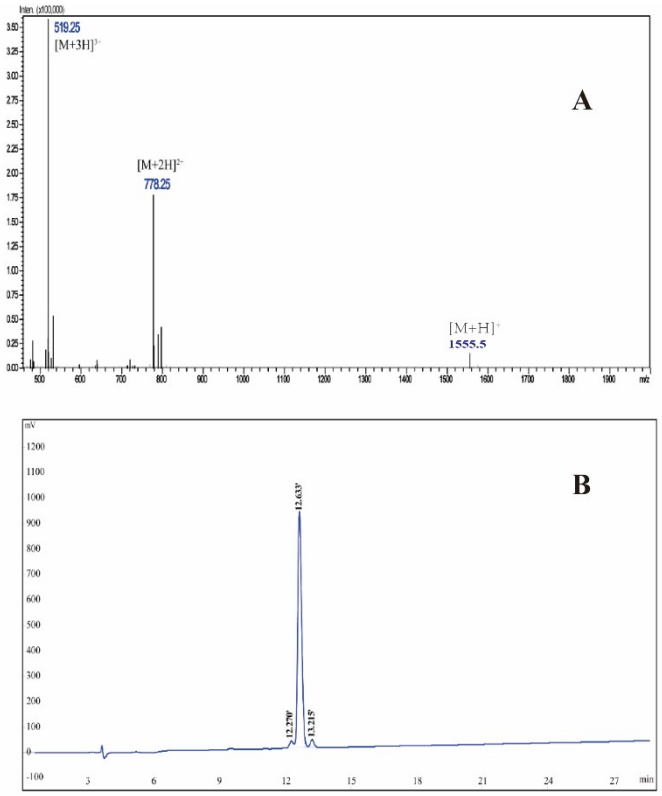

Figure S2. MS (A) and HPLC (B) spectra of ^{D}PPA-1.

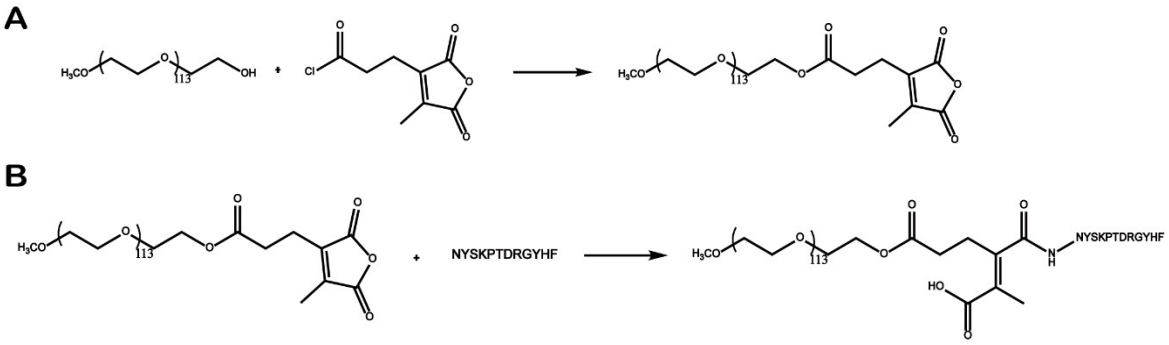

Figure S3. The synthesis route of PEG-DMA (A) and PEG-DMA-^{D}PPA-1 (B).

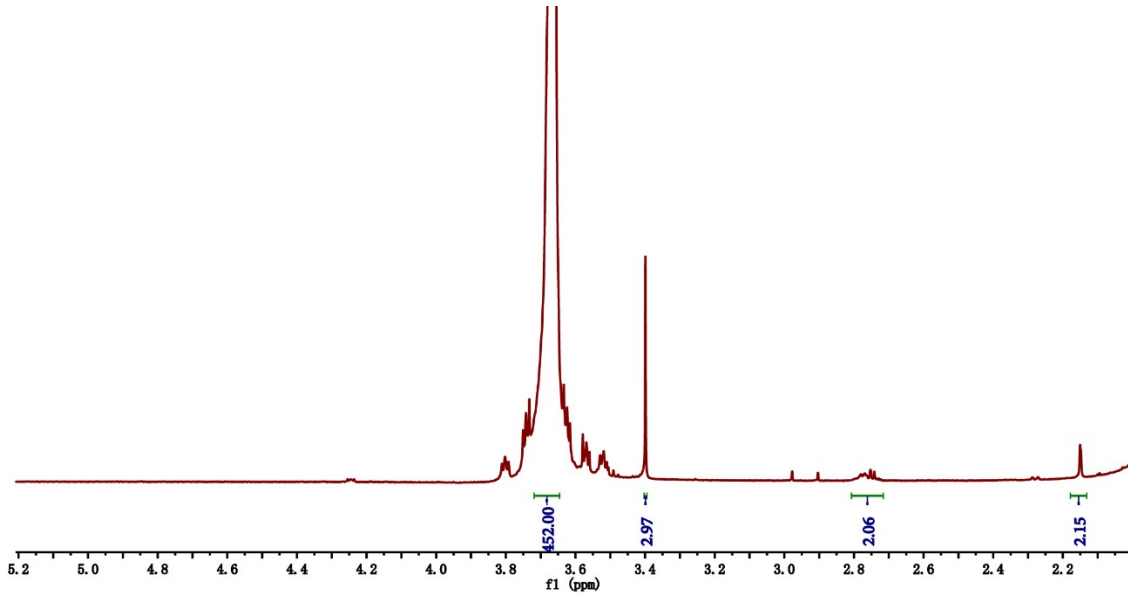

Figure S4. The ^{1}H NMR spectrum of PEG-DMA.

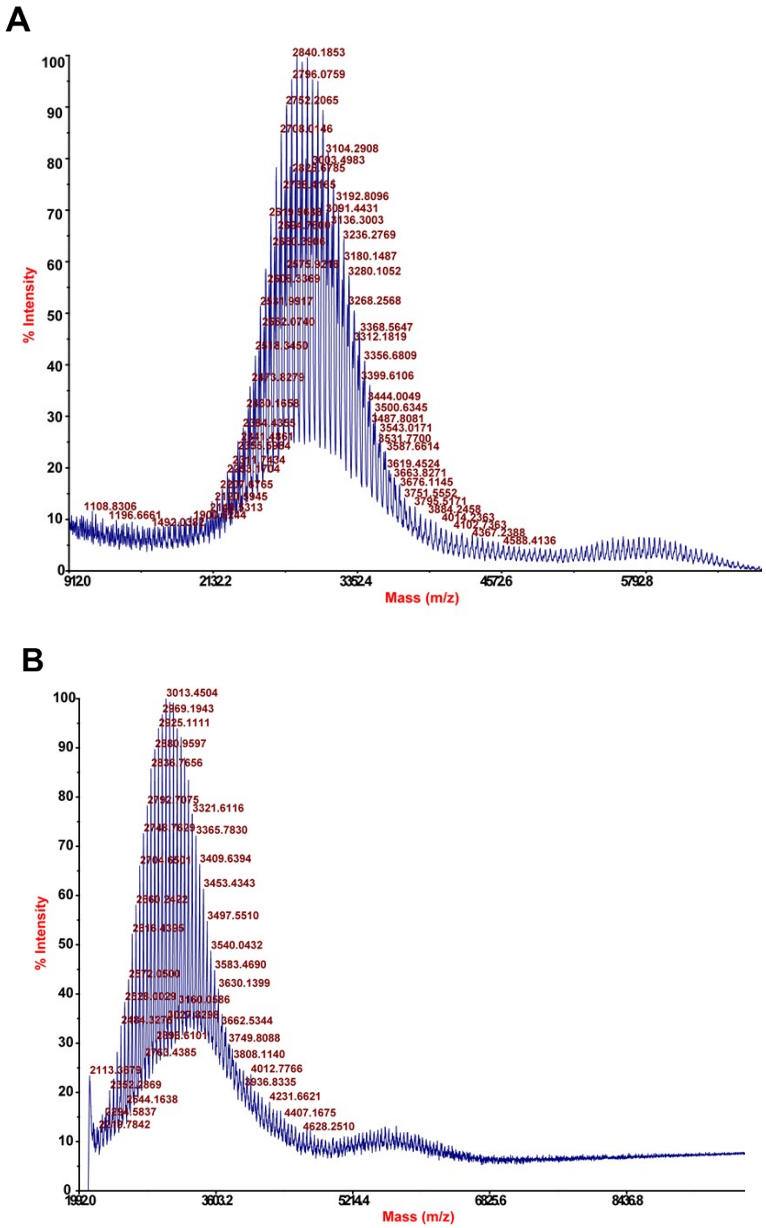

Figure S5. The Maldi-TOF spectra of (A) PEG-OH and (B) PEG-DMA.

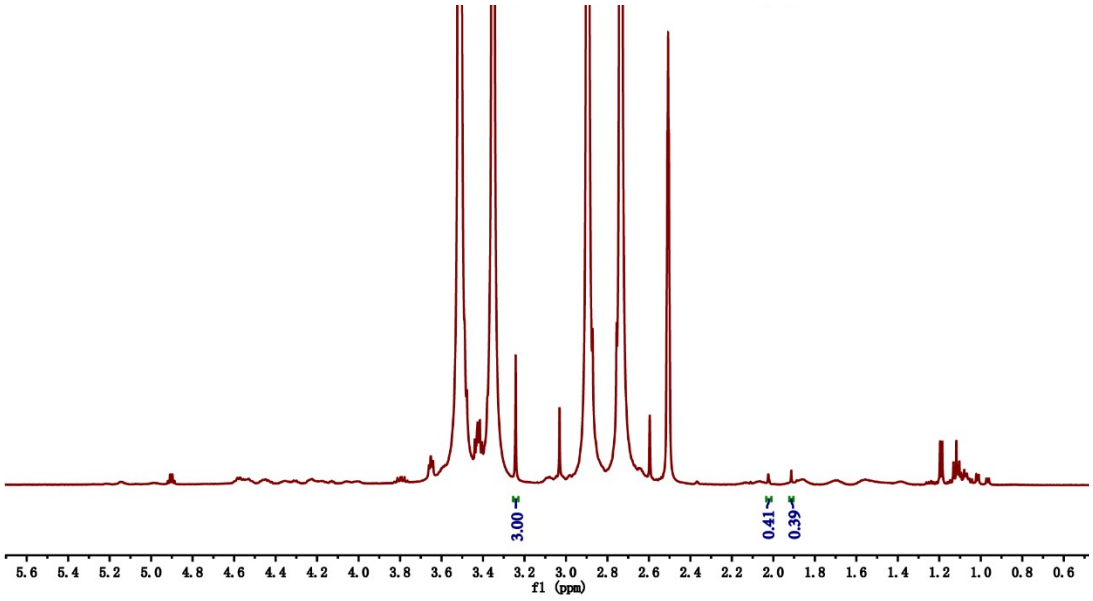

Figure S6. The ^{1}H NMR spectra of PEG-DMA-^{D}PPA-1.

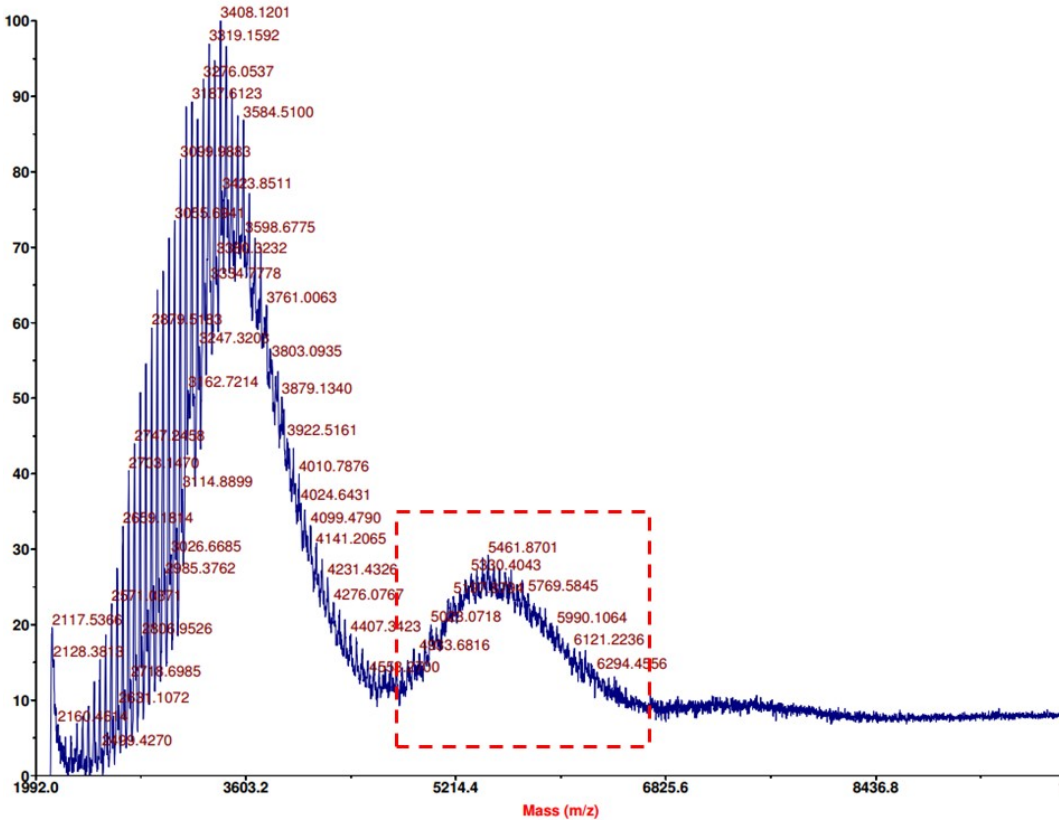

Figure S7. The Maldi-TOF spectra of PEG-DMA-^{D}PPA-1.

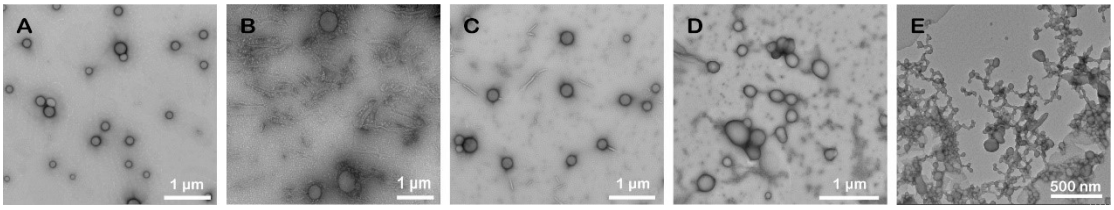

Figure S8. TEM pictures of different proportions of PEG-DMA-^{D}PPA-1:MITX:PC (w/w) at 2:1:0.5 (A), 2:1:1 (B), 2:1:2 (C), 2:1:4 (D) and 0:1:4 (E).

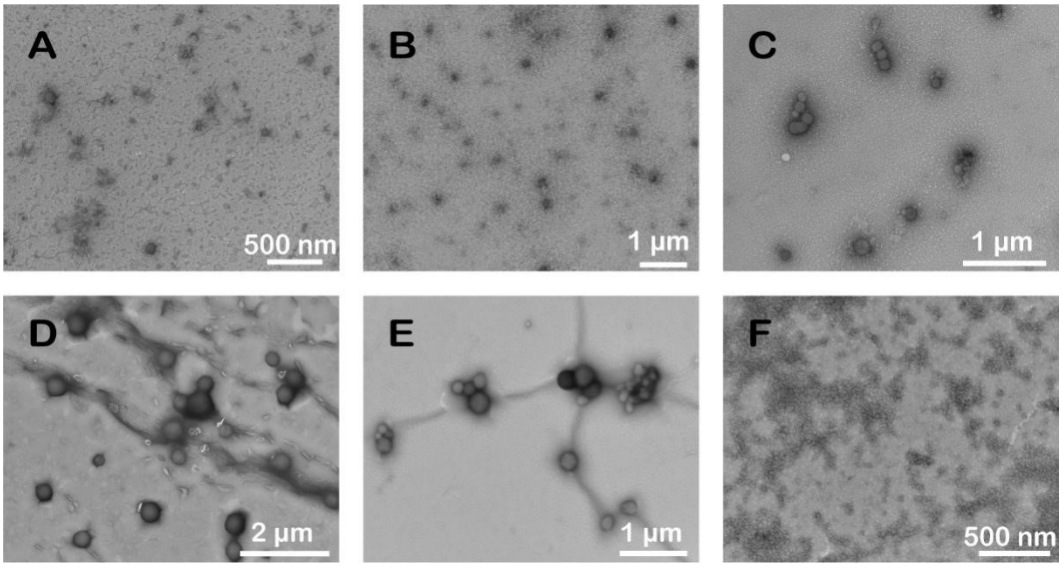

Figure S9. TEM pictures of PEG-DMA-^{D}PPA-1:MITX:PC (w/w) at 2:1:4 in SDS (50 mM) at 0 h (A), 12 h (B), and 24 h (C) and in urea (500 mM) at 0 h (D), 12 h (E), and 24 h (F).

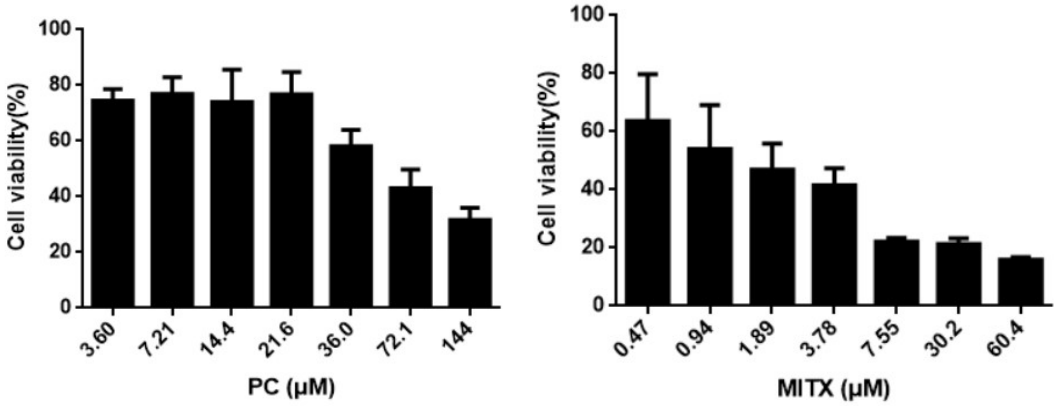

Figure S10. MTT of MITX and PC. (IC_{50} of PC was 57.26 μM, IC_{50} of MITX was 1.378 μM)

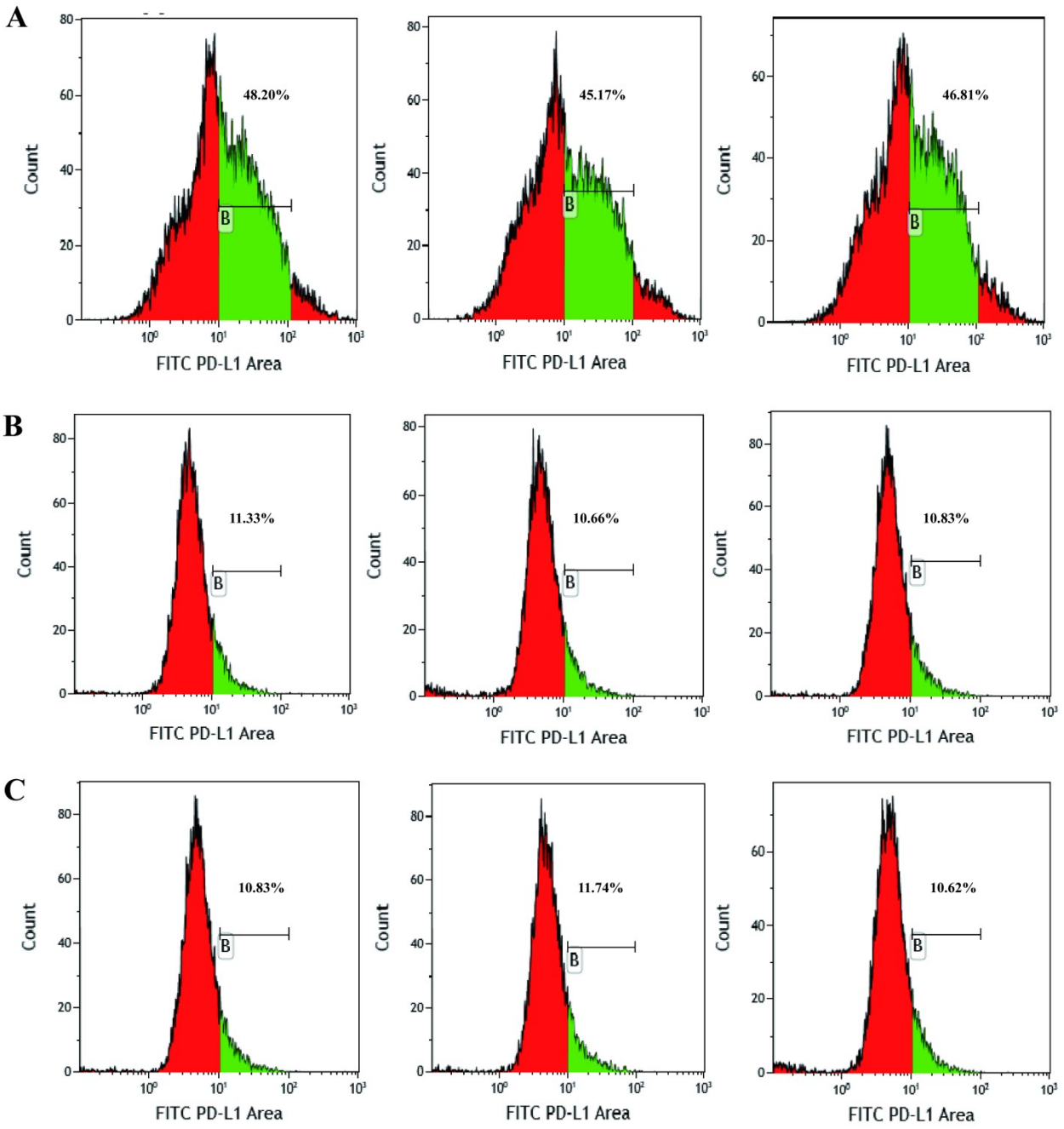

Figure S11. PD-L1 blocking efficacy in CT26 cells after treatment with (A) PBS, (B) free ^{D}PPA-1 (2 mg/mL), (C) pretreated PEG-DMA-^{D}PPA-1 (contain ^{D}PPA-1, 2 mg/mL). Green: the α-PD-L1-FITC positive rate.

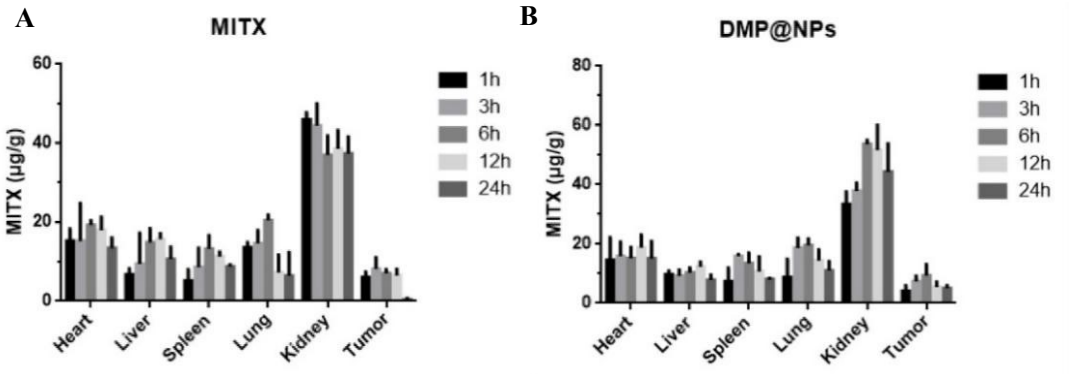

Figure S12. Biodistribution of MITX of free MITX (A) and DMP@NPs (B) in tumor-bearing Balb/c mice. (n=3)

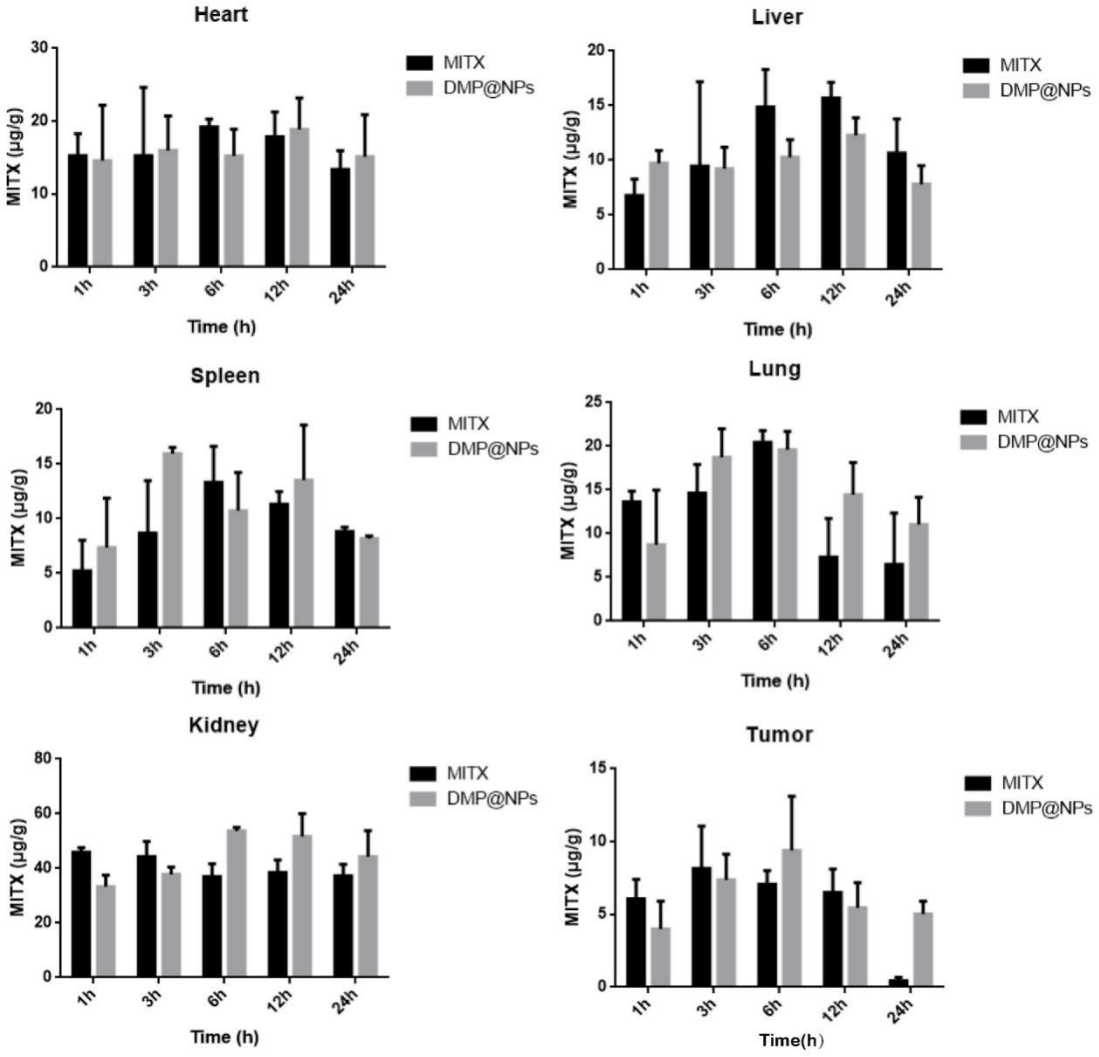

Figure S13. Biodistribution of MITX of free MITX and DMP@NPs in tumor-bearing Balb/c mouse organs at 1 h, 3 h, 6 h, 12 h and 24 h. (n=3)

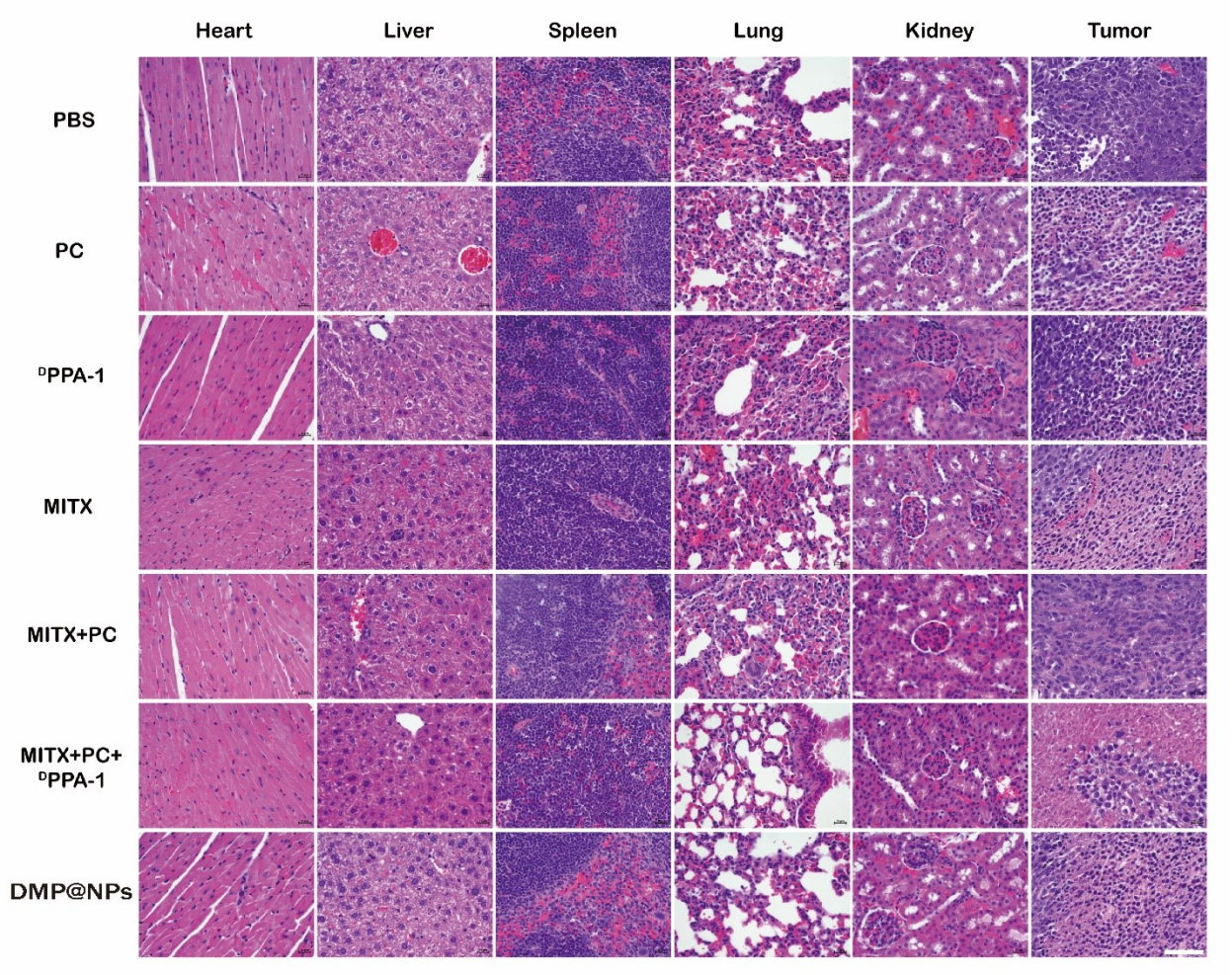

Figure S14. Histological examination of major organs and tumors of the PBS, PC, MITX, ^{D}PPA-1, MITX+PC, MITX+PC+^{D}PPA-1, and DMP@NPs groups. (Scale bar = 40 μm)

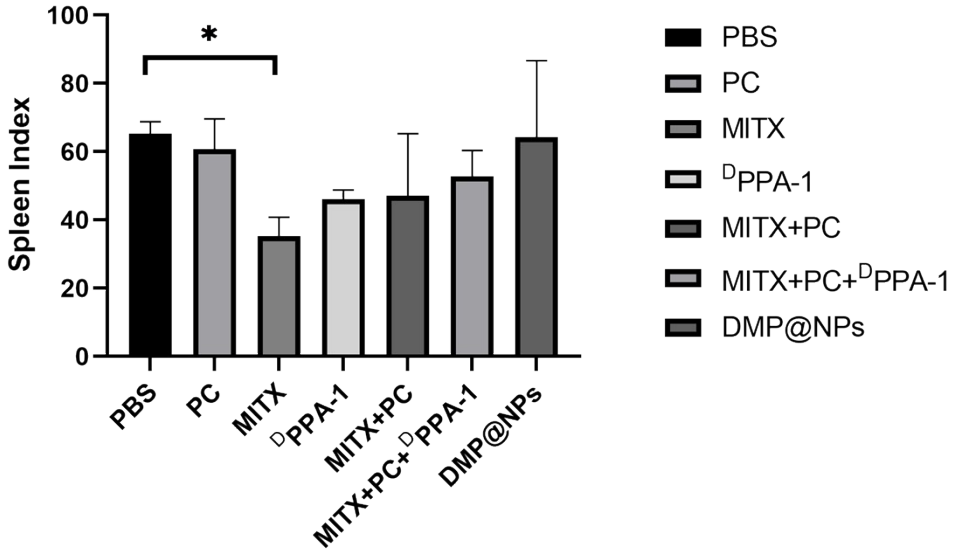

Figure S15. Spleen index of the PBS, PC, MITX, ^{D}PPA-1, MITX+PC, MITX+PC+^{D}PPA-1, and DMP@NPs groups. (Compared with the PBS group, $^{*}P<0.05$).