

Supporting Information

Surface modification of polymer nanoparticles with native albumin for enhancing drug delivery to solid tumors

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4 Supporting Tables

16 Supporting Figures

Table S1. Size and zeta potential of NPs^a

NP type	z-average (d.nm)	Polydispersity index (PI)	Zeta potential (mV)
NP	181 ± 18	0.08 ± 0.04	-2.4 ± 0.7
NP-pD	188 ± 28	0.09 ± 0.08	-3 ± 1
NP/Al	174 ± 13	0.08 ± 0.06	-3 ± 1
NPxAl	173 ± 36	0.12 ± 0.06	-15 ± 4
NP-pD-Al	185 ± 11	0.06 ± 0.02	-6 ± 3

^a PLGA NPs made of unlabeled PLGA.

n = 5 identically and independently prepared samples (mean ± standard deviation)

Table S2. Size of rhodamine-labeled NPs in 50% FBS

NP type	z-average (d.nm)	Polydispersity index (PI)
NP	105	0.54
NP-pD	116	0.42
NP/Al	113	0.47
NPxAl	100	0.32
NP-pD-Al	107	0.38
NP-pD-PEG	111	0.54

NPs were suspended in 50% FBS to 0.1 mg/mL. The size and PI the NPs were measured by a Malvern Zetasizer Nano ZS90.

Table S3. Size, zeta potential and loading efficiency of PTX loaded NPs^a

name	z-average (d.nm)	Polydispersity index (PI)	Zeta potential (mV)	PTX loading efficiency (LE %)
PTX@NPxAl	169 ± 5	0.12 ± 0.02	-13 ± 1	7.9 ± 1.1
PTX@NP-pD-Al	183 ± 4	0.10 ± 0.02	-3.6 ± 0.3	3.4 ± 0.3

^a PLGA NPs made of unlabeled PLGA.

n = 6 identically and independently prepared samples (mean ± s.d.)

Table S4. Serum chemistry of B16F10 tumor bearing mice at 24 h after the last treatment of PBS, PTX@NPxAl, or PTX@NP-pD-Al

Groups	Parameters (Ref. Range)				
	GLU (90-192 mg/dL)	CREA (0.20-0.80 mg/dL)	ALT (28-132 IU/L)	ALKP (62-209 IU/L)	GGT (IU/L)
PBS	185	0.2	450	69	54
	202	0.1	44	113	19
PTX@NPxAl	255	0.3	>2000	68	37
	178	0.2	898	76	17
	265	0.3	2923	98	37
	211	0.2	781	88	16
	166	0.1	1069	107	47
PTX@NP-pD-Al	224	0.2	1147	84	29
	181	0.1	62	72	16
	158	0.1	88	85	73
	170	0.2	70	86	28

Mice were treated with PBS (n = 2), PTX@NPxAl (n = 5), or PTX@NP-pD-Al (n = 4) at 15 mg/kg q3d × 2. One day after the second dose, mice were sacrificed for the analysis of serum chemistry. GLU, glucose; CREA, creatinine; ALT, alanine aminotransferase; ALKP, alkaline phosphatase; GGT, gamma glutamyl transferase.

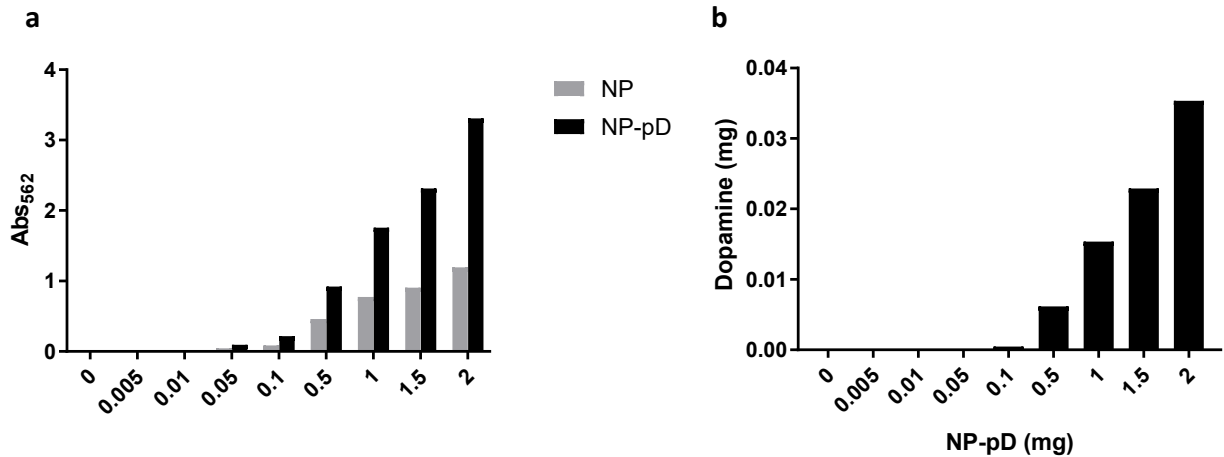


Fig. S1. Micro BCA assay of NP and NP-pD. (a) Absorbance (562 nm) difference between NP and NP-pD at different concentrations indicating the presence of pD. (b) The pD content in the NP-pD, estimated based on the absorbance difference between NP-pD and bare NP. Dopamine, NP, or NP-pD at different concentrations was incubated in the BCA working reagent for 2 h at 37 °C. A supernatant was separated from the NP suspension by centrifugation, and its absorbance was read at 562 nm. The pD content in NP-pD was estimated based on the absorbance difference between NP-pD and bare NP and a calibration curve drawn with dopamine solutions of known concentrations.

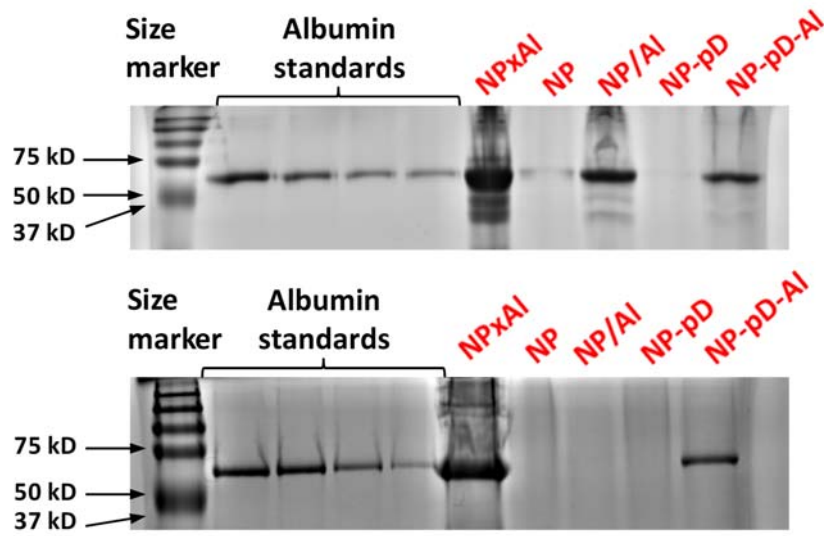


Fig. S2. Two representative SDS-PAGE gels for determination of albumin contents in NPs. Albumin standards were prepared as (Top) 0.0625, 0.03125, 0.015625 and 0.0125 mg/mL and (Bottom) 0.1, 0.08, 0.04 and 0.02 mg/mL.

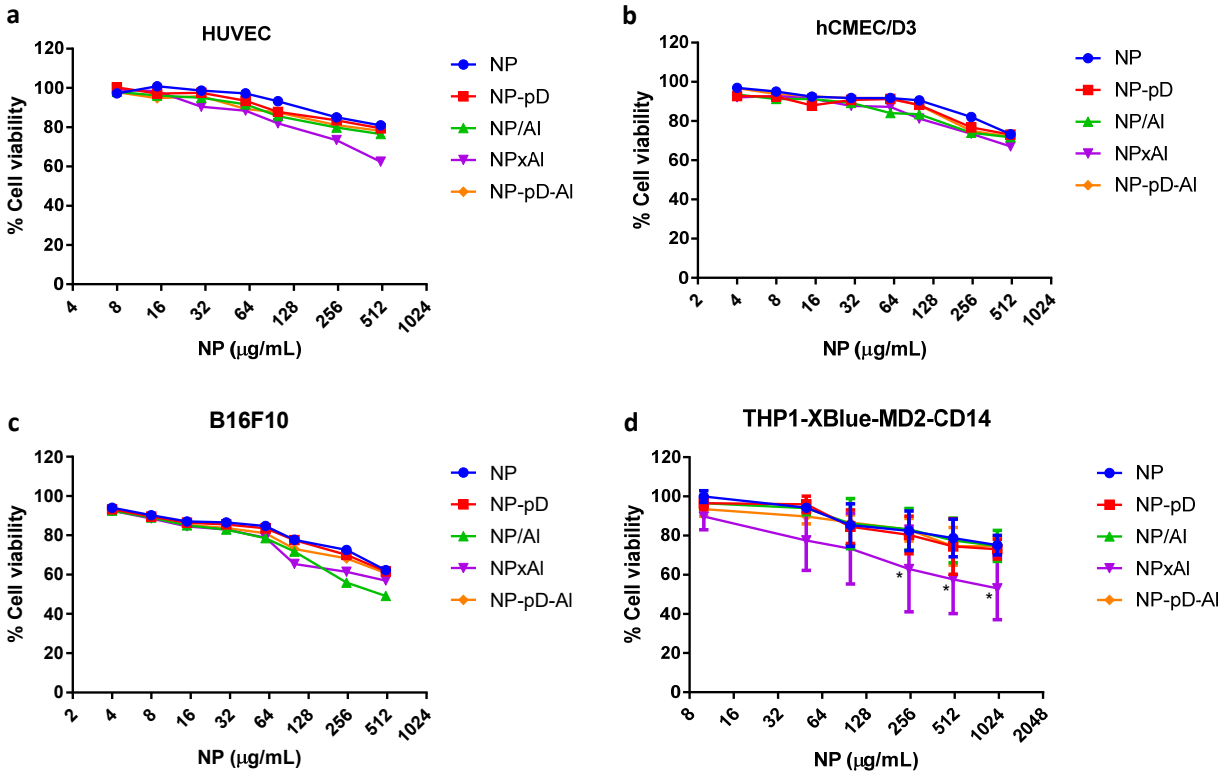


Fig. S3. Cell viability of (a) HUVEC and (b) hCMEC/D3 cells incubated with NPs (0-0.5 mg/mL) for 6 h; (c) B16F10 melanoma cells incubated with NPs (0-0.5 mg/mL) for 24 h; and (d) THP1-XBlue-MD2-CD14 cells incubated with NPs (0-1 mg/mL) for 24 h, in media supplemented with 2% (HUVEC and hCMEC/D3 cells) or 10% (B16F10 and THP1-XBlue-MD2-CD14 cells) FBS, determined by MTT assay ($n = 3-4$ identically and independently prepared samples, mean \pm s.d.). *: $p < 0.05$ vs. NP by Dunnett's multiple comparisons test following two-way ANOVA.

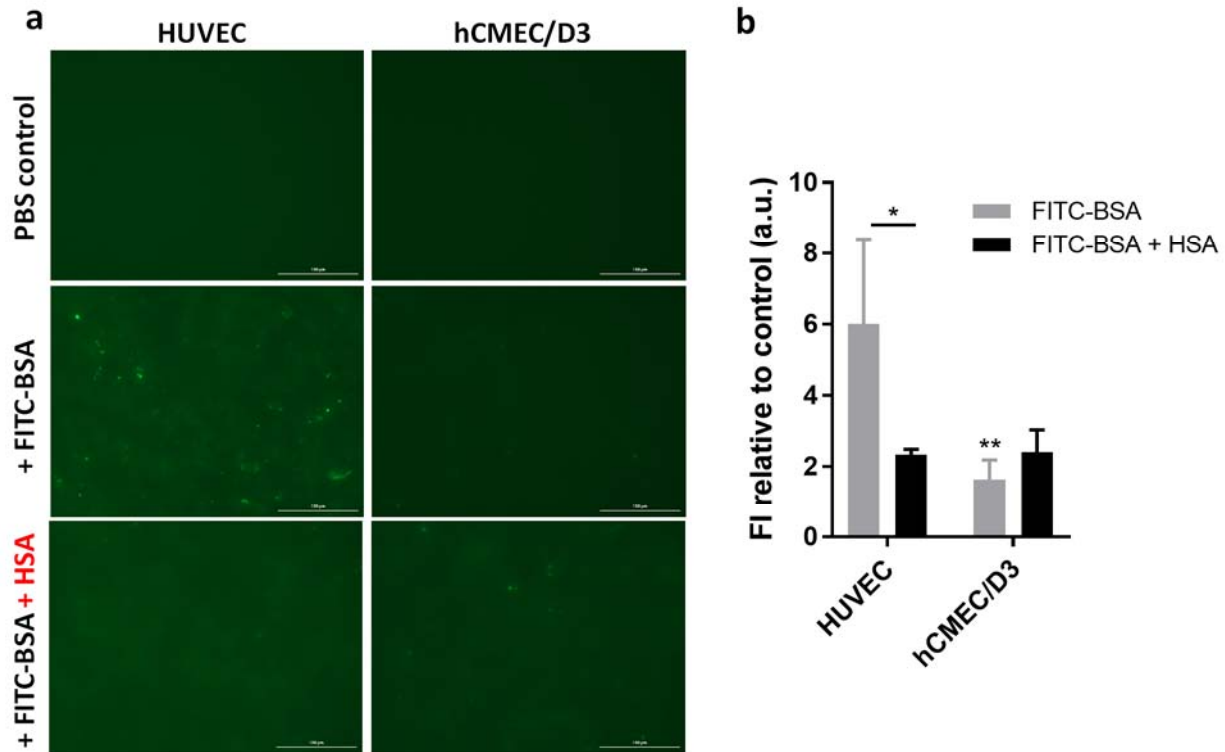


Fig. S4. Cells were incubated with PBS (control), FITC-BSA (0.8 mg/mL) alone or co-incubated with FITC-BSA and HSA (1.6 mg/mL) in the 10% serum-supplemented medium for 30 min. (a) Cells were imaged without fixation by a Biotek Cytation 3 cell imaging multimode reader. Scale bars = 100 μ m. (b) FITC-BSA interactions with HUVEC or hCMEC/D3 were determined by flow cytometry. $n = 3$ identically and independently prepared samples (mean \pm s.d.) *: $p < 0.05$; **: $p < 0.01$ vs. HUVEC with a corresponding treatment, by Sidak's multiple comparisons test following two-way ANOVA.

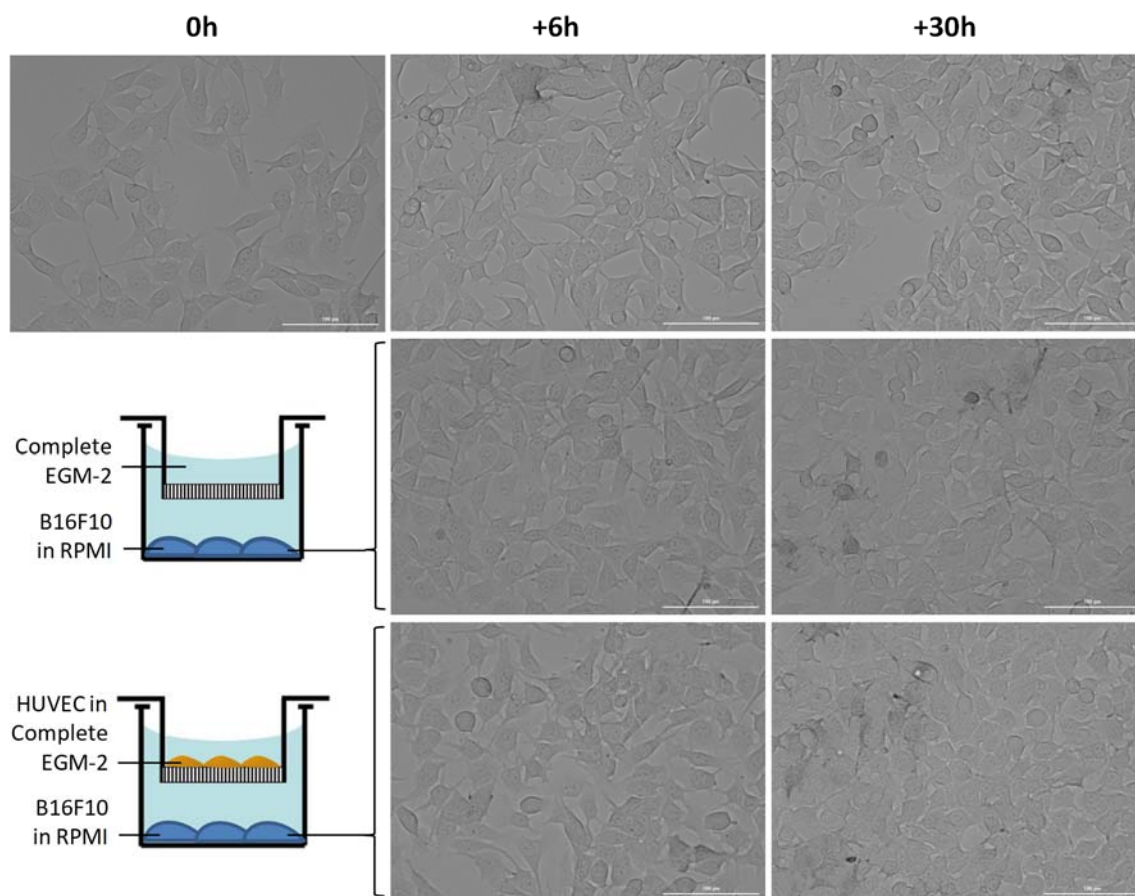


Fig. S5. Images of B16F10 cells after 6 and 30 h (6+24 h) incubation with complete EGM-2 media \pm HUVEC seeded on the Transwell insert. Scale bar: 100 μ m.

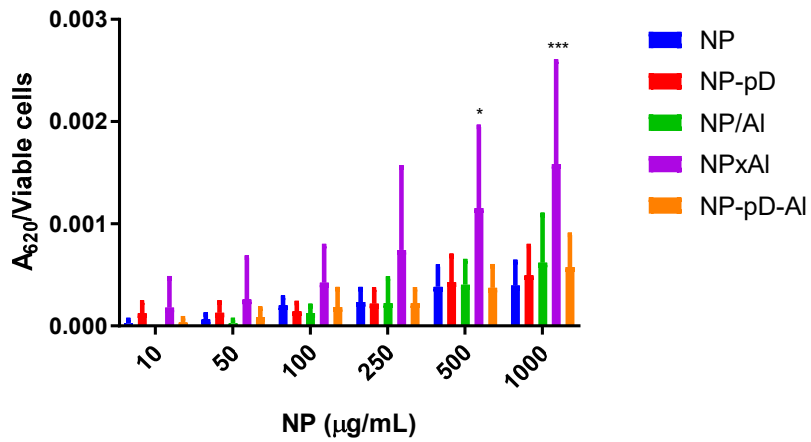


Fig. S6. SEAP levels normalized to cell viability. THP1-XBlue-MD2-CD14 cells incubated with NPs (0.1 mg/mL) for 24 h, and the supernatants were analyzed for the production of SEAP. $n = 3$ identically and independently prepared samples (mean \pm s.d.). *: $p < 0.05$; ***: $p < 0.001$ vs. NP by Dunnett's multiple comparisons test following two-way ANOVA.

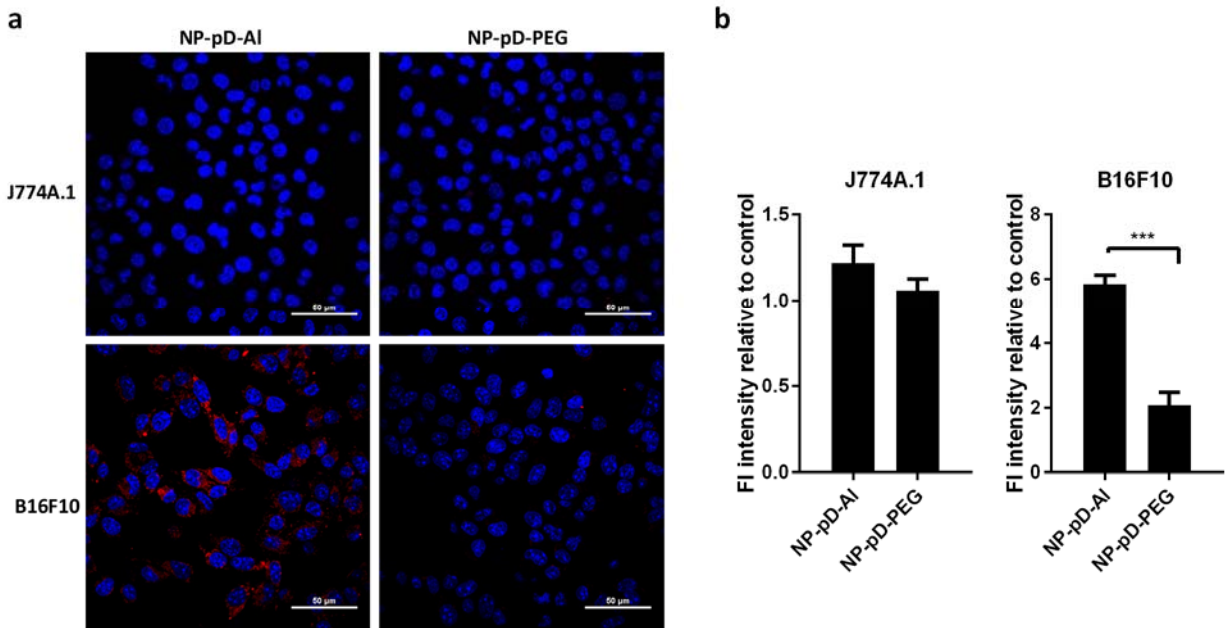


Fig. S7. Cellular uptake of NP-pD-Al and NP-pD-PEG (0.1 mg/mL) by J774A.1 macrophages after 30 min incubation and B16F10 cells after 1 h incubation in media supplemented with 10% serum. (a) Imaged by confocal microscopy (Red: rhodamine-labeled NPs; Blue: nuclei stained with Hoechst 33342). Scale bar: 50 μm. (b) Quantified by flow cytometry. n = 3 repeated tests of the same batch NPs. NP-pD-PEG was produced by incubating NP-pD with methoxyl polyethylene glycol 2000 Da (mPEG; Nanocs, New York, NY) at an mPEG-to-NP weight ratio of 2/1 for 30 min in Tris buffer (10 mM, pH 8.5). The NP-pD-PEG was collected by centrifugation at 13,600 rcf for 20 min at 4 °C and washed twice with DI water.

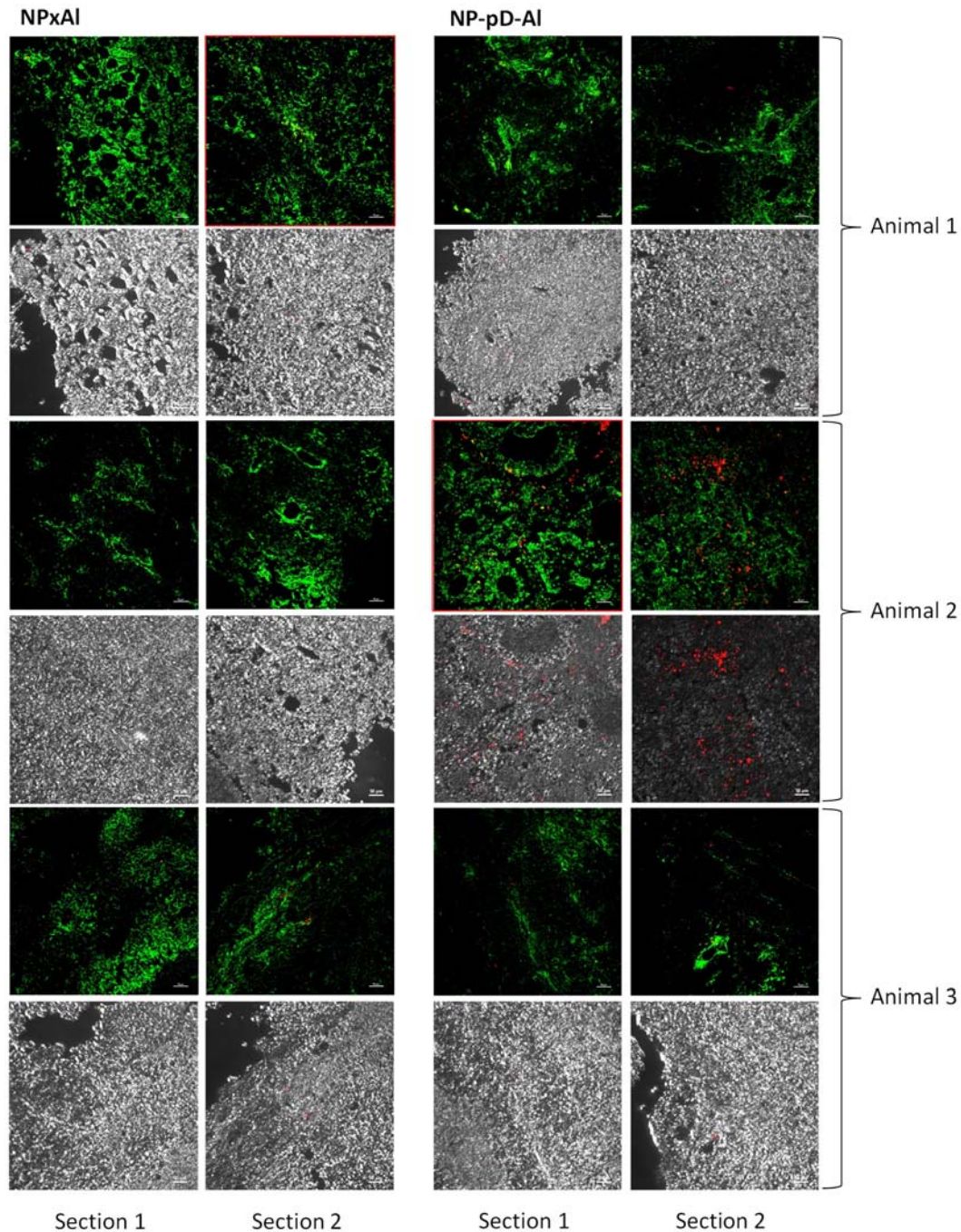


Fig. S8. All images of fluorescently labeled NPs (red) in FITC-lectin stained **tumor** blood vessels (green). A series of z-stack images were collected from two randomly selected fields per slide (16- μ m cryostat section) at 0.5 μ m intervals and presented as projection images. Mice (n=3 per group) were given a single IV injection of fluorescently labeled NPxAl or NP-pD-Al at 300 mg NP/kg. After 24 h, mice were injected IV with lectin-FITC (100 μ L, 1 mg/mL in saline), perfused with saline 5 min later and sacrificed. Scale bars = 50 μ m. Images used in the main text are highlighted with red outline.

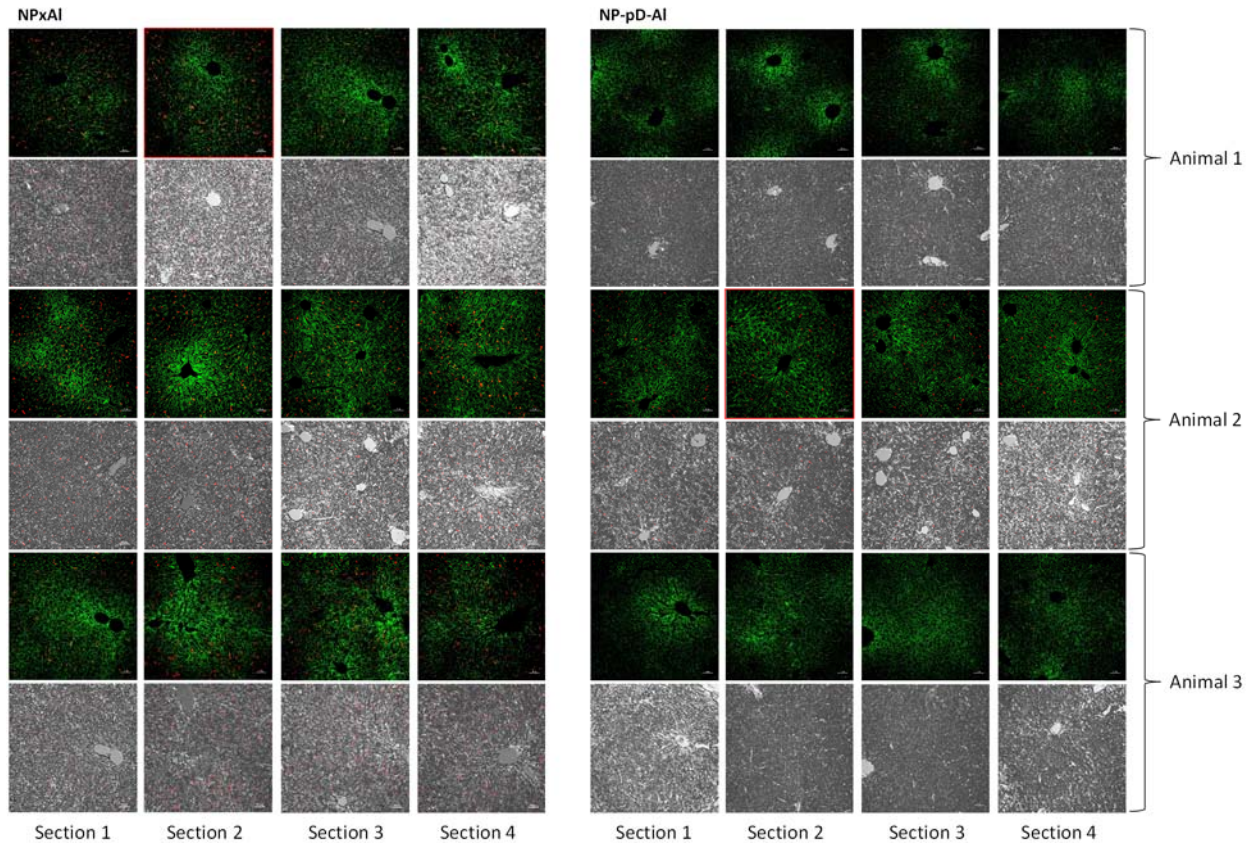


Fig. S9. All images of fluorescently labeled NPs (red) in FITC-lectin stained **liver** blood vessels (green). A series of z-stack images were collected from four randomly selected fields per slide (16- μm cryostat section) at 0.5 μm intervals and presented as projection images. Mice (n=3 per group) were given a single IV injection of fluorescently labeled NPxAl or NP-pD-Al at 300 mg NP/kg. After 24 h, mice were injected IV with lectin-FITC (100 μl , 1 mg/mL in saline), perfused with saline 5 min later and sacrificed. Scale bars = 50 μm . Images used in the main text are highlighted with red outline.

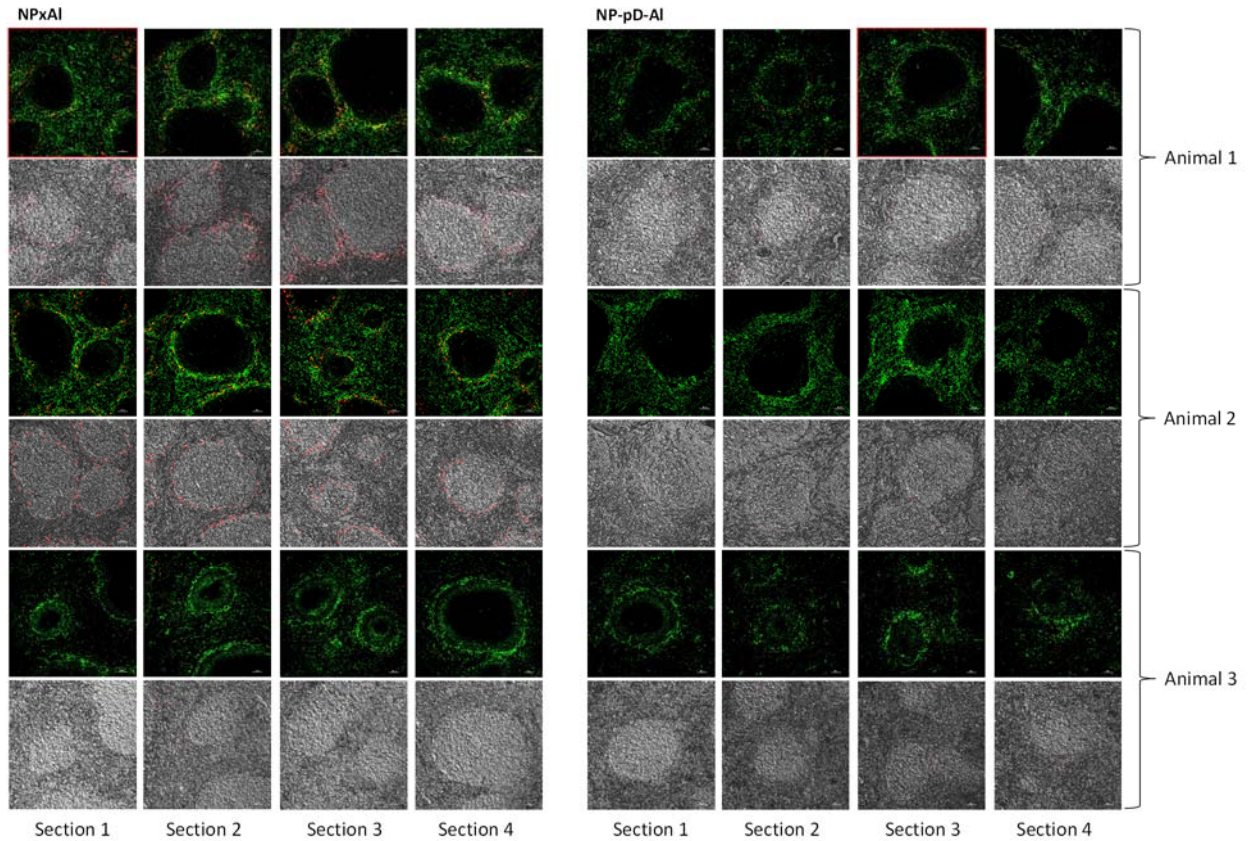


Fig. S10. All images of fluorescently labeled NPs (red) in FITC-lectin stained **spleen** blood vessels (green). A series of z-stack images were collected from four randomly selected fields per slide (16- μm cryostat section) at 0.5 μm intervals and presented as projection images. Mice ($n=3$ per group) were given a single IV injection of fluorescently labeled NPxAl or NP-pD-Al at 300 mg NP/kg. After 24 h, mice were injected IV with lectin-FITC (100 μl , 1 mg/mL in saline), perfused with saline 5 min later and sacrificed. Scale bars = 50 μm . Images used in the main text are highlighted with red outline.

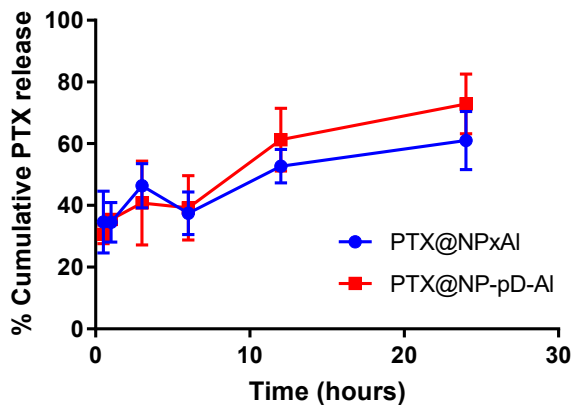


Fig. S11. Release kinetics of PTX/NPs in PBST (PBS containing 0.2% Tween 80). PTX@NPxAl or PTX@NP-pD-Al were suspended in PBS containing 0.2% Tween 80 (PBST) to a concentration equivalent to PTX 4 $\mu\text{g}/\text{mL}$. The NP suspensions were divided into multiple 1 mL aliquots and incubated at 37 $^{\circ}\text{C}$ with constant agitation. At each time point, the aliquots were centrifuged to separate NP pellets and supernatants. The supernatant was analyzed using HPLC. n=3 tests with representative batches (mean \pm s.d.).

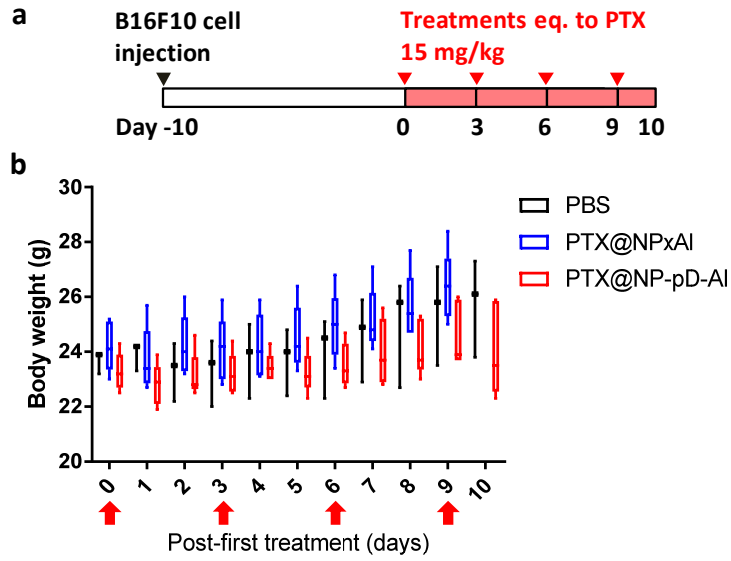


Fig. S12. (a) Dosing schedule of PTX-loaded NPs and (b) body weight change of animals. PBS (black; n = 3); PTX@NPxAl (blue; n = 5); PTX@NP-pD-Al (red; n = 5). Arrows indicate treatment times.

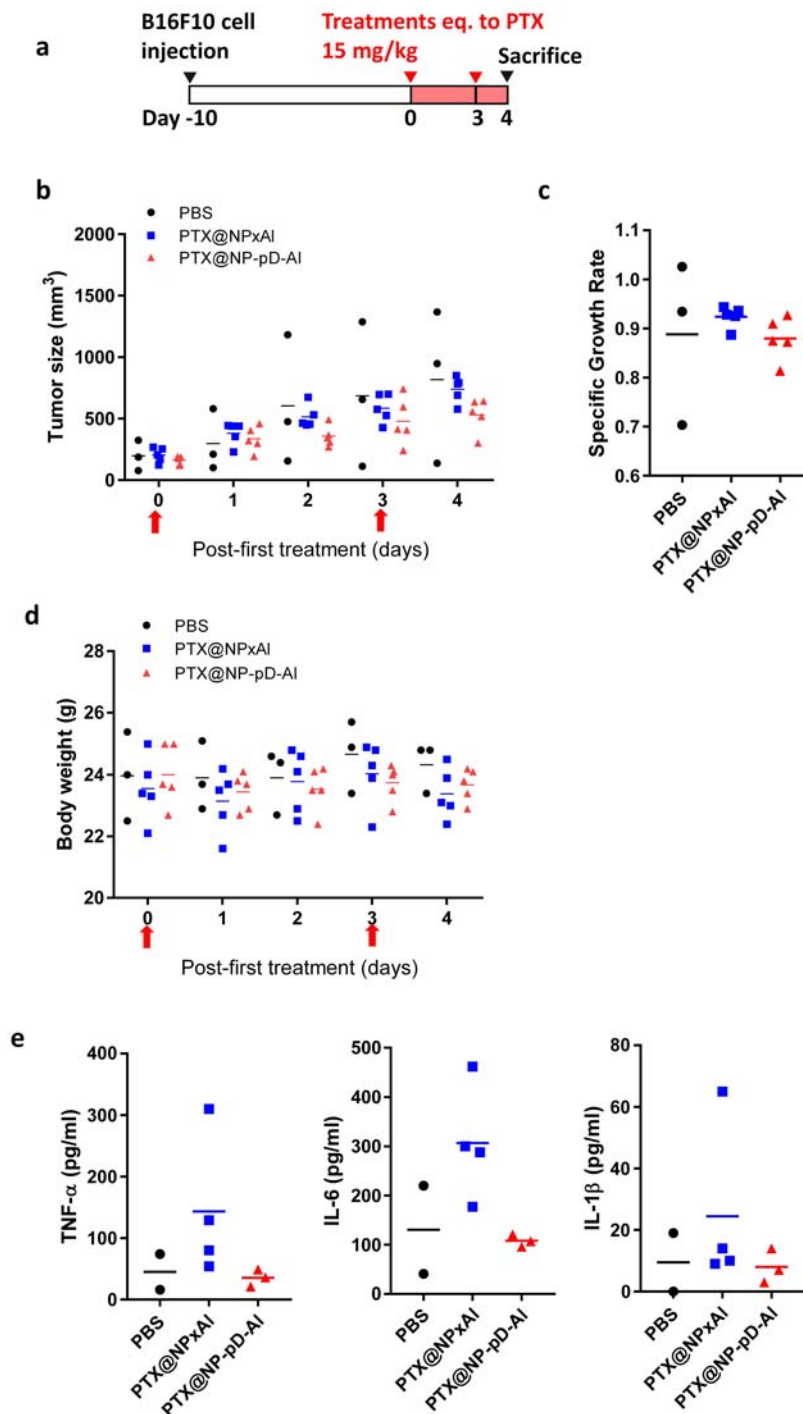


Fig. S13. In vivo activity of PTX@NPxAl and @NP-pD-Al in C57BL/6 mice. Mice were treated with PBS (n = 3), PTX@NPxAl (n = 5), or PTX@NP-pD-Al (n = 5) at 15 mg/kg q3d \times 2. Arrows indicate treatment times. (a) Dosing schedule of PTX-loaded NPs, (b) tumor size (mm³), (c) specific growth rate of B16F10 tumor = $\Delta\log V/\Delta t$ (V: tumor volumes; t: time in days), (d) body weight change of animals, and (e) serum levels of TNF- α , IL-6, and IL-1 β in B16F10-tumor bearing mice treated with PBS (n = 2), PTX@NPxAl (n = 4) or PTX@NP-pD-Al (n = 3).

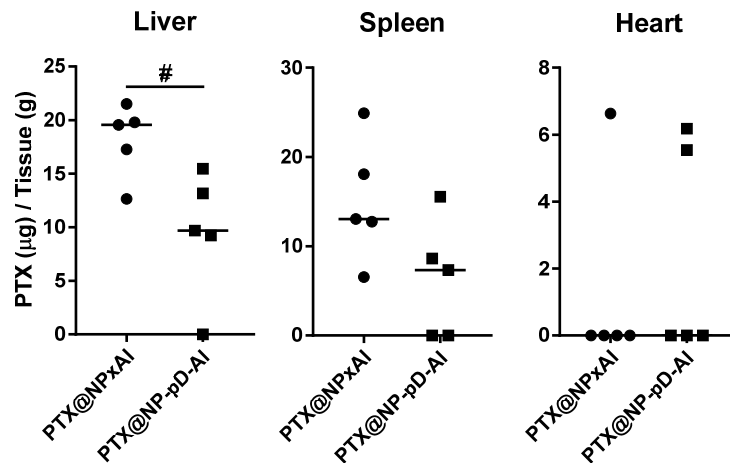


Fig. S14. PTX content in liver, spleen, and heart of B16F10 tumor-bearing mice. Mice were treated with PBS ($n = 3$), PTX@NPxAl ($n = 5$), or PTX@NP-pD-Al ($n = 5$) at a dose equivalent to PTX 15 mg/kg q3d \times 2. One day after the second dose, mice were sacrificed for the analysis. #: $p < 0.05$ by non-parametric Mann-Whitney test.

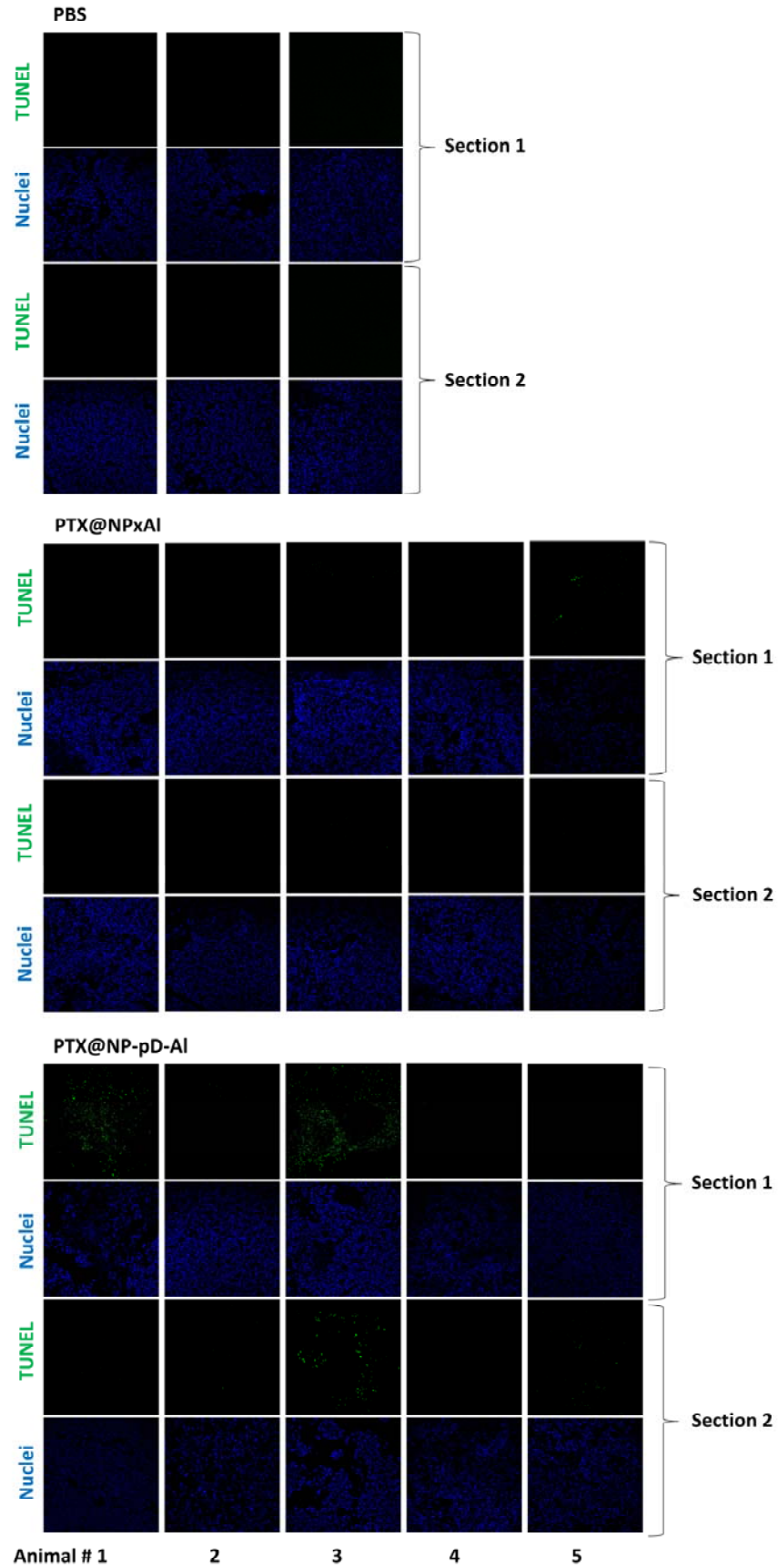


Fig. S15. All images of TUNEL-stained B16F10 tumor sections. Two randomly selected fields were imaged for each animal. Mice were treated with PBS (n = 3), PTX@NPxAl (n = 5), or PTX@NP-pD-Al (n = 5) at a dose equivalent to PTX 15 mg/kg q3d × 2. One day after the second dose, mice were sacrificed for the analysis. Scale bars = 50 μ m.

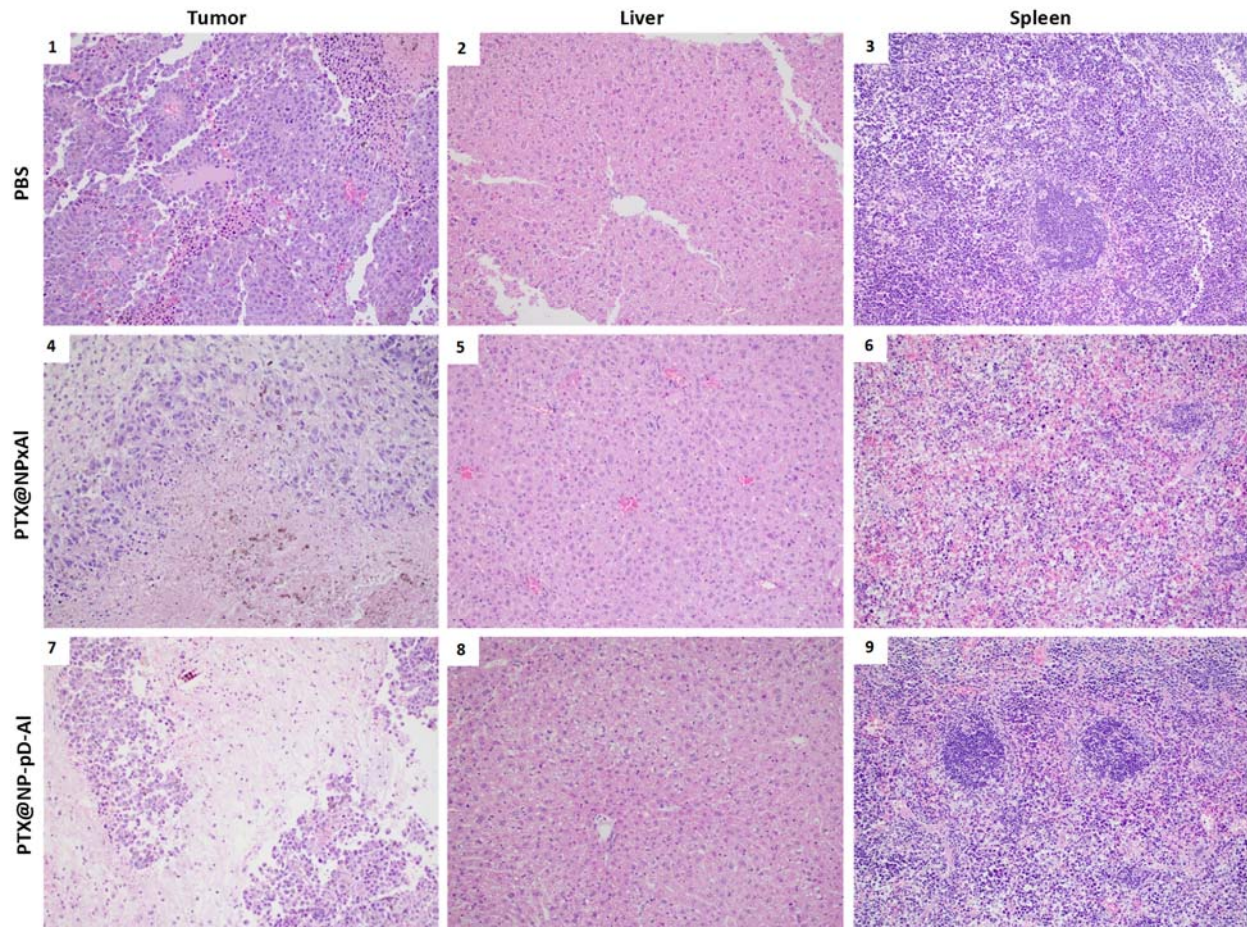


Fig. S16. Photomicrographs (20 \times objective) of PBS treated (1-3) animals with rare tumor hemorrhage, rare vacuolar change within the liver, and normal spleen pathology. PTX@NPxAl treated animals (4-6) demonstrating multifocal tumor necrosis, multifocal vacuolar change within the liver, and lymphoid depletion within the spleen. PTX@NP-pD-Al treated animals (7-9) demonstrating marked tumor necrosis, multifocal vacuolar change within the liver, and lymphoid depletion within the spleen.