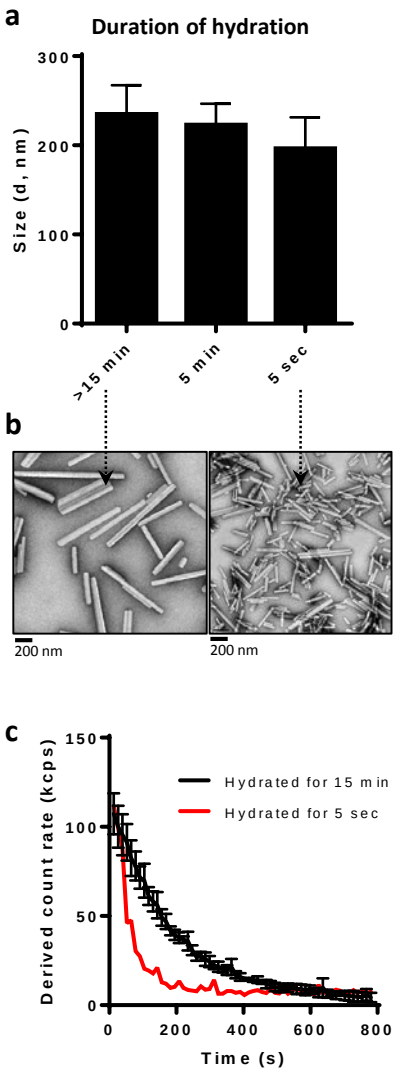
Supporting Information

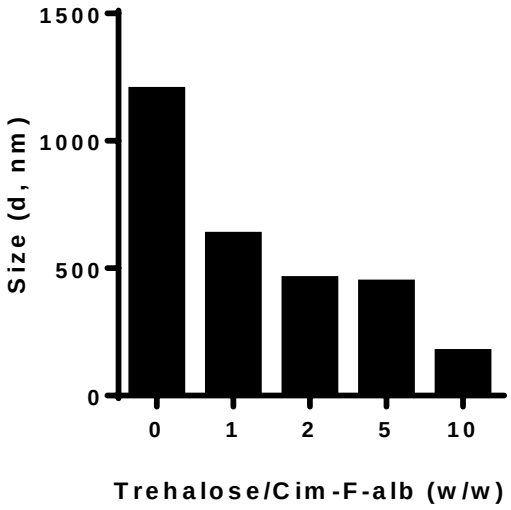
Supporting Table 1. IC_{50} values of free PTX, Abraxane, and Cim-F-alb

Cell lines	Treatment conditions	Assay methods	Free PTX (nM)	Abraxane (nM)	Cim-F-alb (nM)	Comparison*
SKOV3	24h treatment + 48h post-treatment incubation	MTT assay	34.21	38.11	37.20	No statistical difference (p= 0.8508)
B16F10	24h treatment	PI staining + flow cytometry	423.1	548.3	454.3	No statistical difference (p=0.8842)
B16F10	24h treatment + 12h post-treatment incubation	MTT assay	91.30	76.58	94.34	No statistical difference (p=0.7708)
B16F10	24h treatment + 24h post-treatment incubation	MTT assay	213.3	280.0	245.2	No statistical difference (p=0.7235)
B16F10	24h treatment + 48h post-treatment incubation	MTT assay	765.1	806.0	926.7	No statistical difference (p=0.7548)

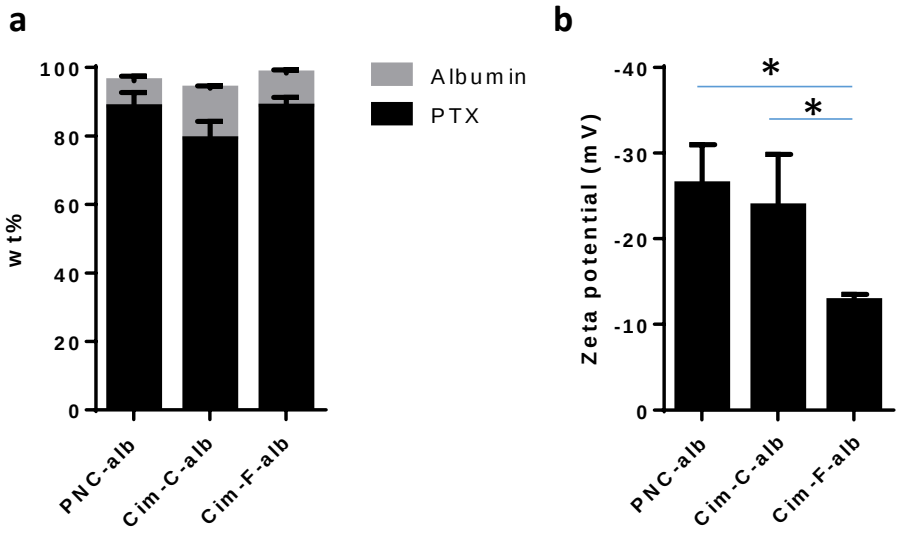
* Extra sum-of-squares F test (Prism 6.0)



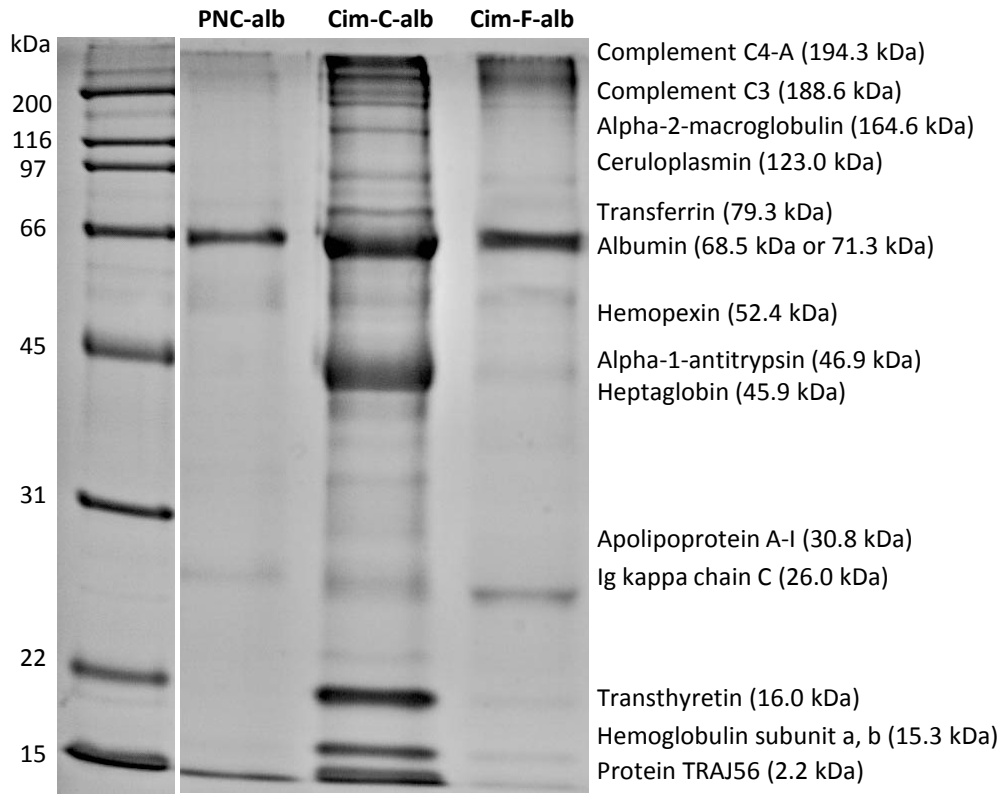
Supporting Figure 1. (a) Size of Cim-F-alb as a function of hydration time (n=9 for >15 min hydration and n=3 for 5 min and 5 sec hydration). (b) TEM images of Cim-F-alb prepared with >15 min hydration (left) or 5 sec hydration (right). (c) Dissolution rate of Cim-F-alb (eq. to PTX 2 μg/mL) prepared with 15 min hydration (black) or 5 sec (red) in PBS containing 0.2% Tween80.



Supporting Figure 2. Particle size of Cim-F-alb freeze dried with different trehalose/Cim-F-alb w/w ratio and reconstituted in water.

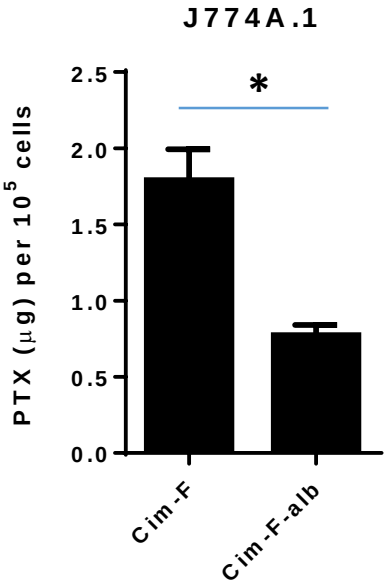


Supporting Figure 3. (a) PTX and protein contents in each PTX NC formulation, determined by HPLC and SDS-PAGE, respectively. n = 4 independently and identically performed experiments. (b) Zeta potential of NC, measured in phosphate buffer (pH 7.4, 1 mM). n = 3 independently and identically performed experiments. *: $p<0.05$ by Tukey's multiple comparisons test.

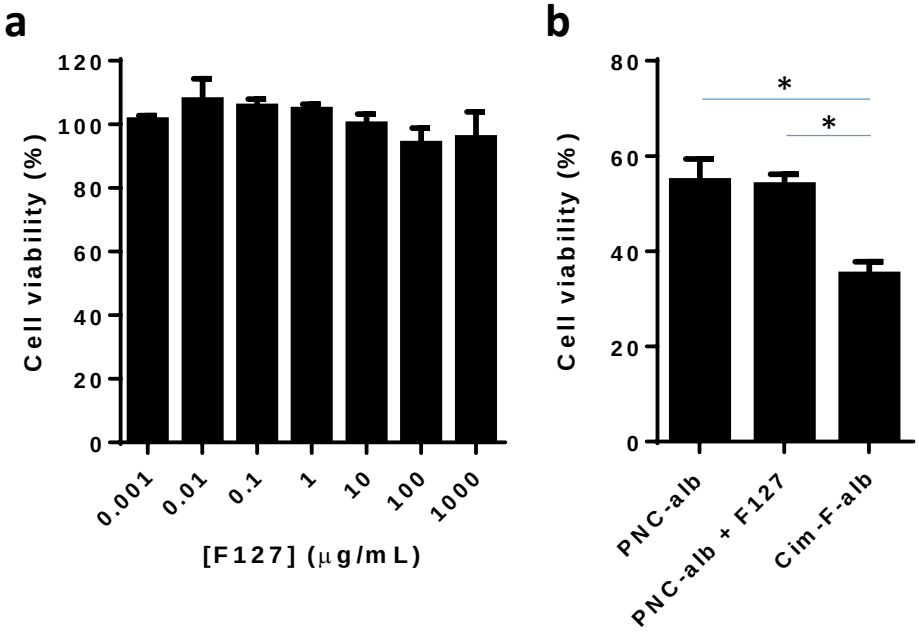


Supporting Figure 4. Proteins bound to NC. The NC were analyzed by SDS-PAGE as described in Section 2.3.5, and the protein bands were identified by LC-MS/MS. Briefly, the Coomassie stained bands were excised from the gel and destained by washing with a 50:50 mixture of 25 mM ammonium bicarbonate (ABC; pH 8.5) and ACN for 4 times. The gel was dried with vacuum centrifuge and treated with 10 mM dithiothreitol (DTT) for 1 h at 55˚C to reduce cysteines. After DTT was removed, 55 mM iodoacetamide (IAA) was added to alkylate cysteines for 45 min, followed by washing with a 50:50 mixture of 25 Mm ABC and ACN for 3 times. Lys-C/trypsin was added to cover each gel piece and incubated in barocycler at 50˚C (120 cycles of 20 kpsi for 50 seconds and atmospheric pressure for 10 sec) for digestion. Peptides from gel pieces were extracted by adding 60% ACN/5% trifluoroacetic acid (TFA) with sonication, and the dry peptide pellet was resuspended in 94.9% water, 3% ACN, 0.1% formic acid (FA). The samples were run on a nano Eksigent 425 HPLC system coupled to the Triple TOF 5600 plus (Sciex, Framingham, MA). The peptides were loaded onto a Sciex NanoLC Chrom XP C18 trap column (350 μm x 0.5 mm) for concentration, and this enrichment column was switched to the nano flow path after 5 min. Peptides were separated using the reversed phase 3C18-CL C18 analytical column (75 μm x 150 mm) from Sciex. The sample was injected into the Triple TOF 5600 plus through the Nanospray III source fitted with an emission tip from New Objective. Peptides from the digestion were eluted from the columns using a mobile phase A (0.1% formic acid in H_2O) and a mobile phase B (0.1% formic acid in ACN). With a flow rate of 300 nL/min, the method began at 95% A for 1 min followed by a gradient of 5% B to 35% B in 61 min and from 35% B to 80% B in 1 min. 80% B was held for 6 min, then brought to 5% B, and held for 20 min. Data acquisition was performed monitoring 50 precursors at an

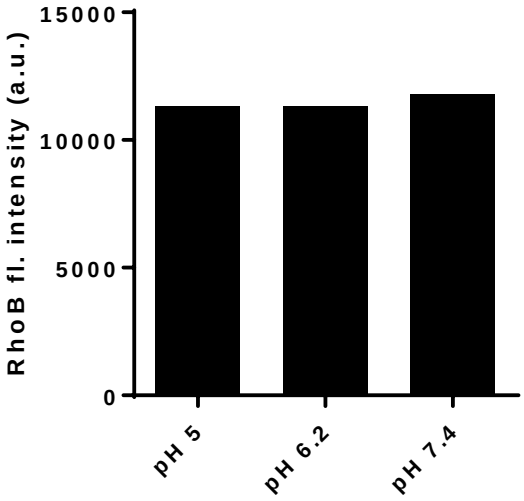
accumulation time of 250 ms/scan. Database searches of the UniProt_human proteins were performed using Mascot Daemon v.2.5.1 (Matrix Science) with peptide mass tolerance of 0.05 Da and fragment mass tolerance of 0.2 Da. The false discovery rate (FDR) was adjusted to 5%. Among the identified proteins, proteins with (i) no relevance (e.g. keratin), (ii) molecular weight with much discrepancy with one estimated from gel, (iii) Mascot protein score below the 5% confidence threshold, and (iv) exponentially modified protein abundance index (emPAI), an estimation of protein abundance, less than 1.0 were excluded. The selected proteins were matched to the identified proteins from albumin stock solution.



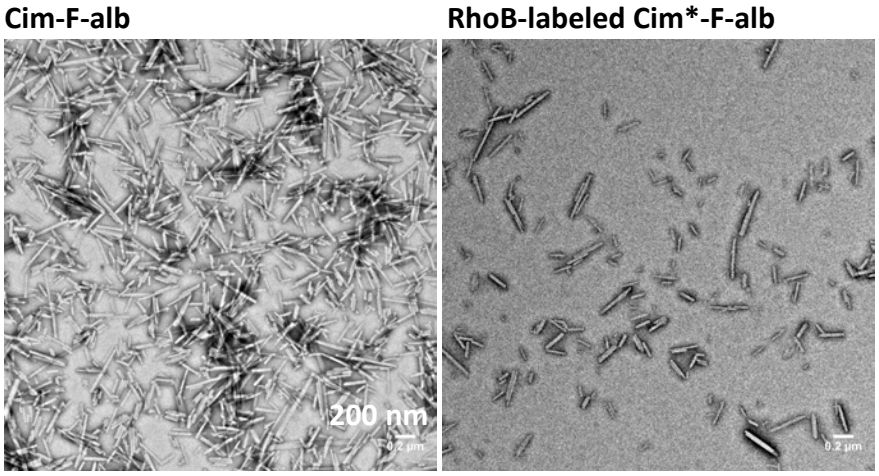
Supporting Figure 5. PTX uptake by J774A.1 macrophages after 3h incubation at 37 °C with Cim-F and Cim-F-alb (equivalent to 30 μg/mL PTX) in serum containing media. n= 3 replicates. *: $p<0.01$ by two-tailed unpaired t-test.



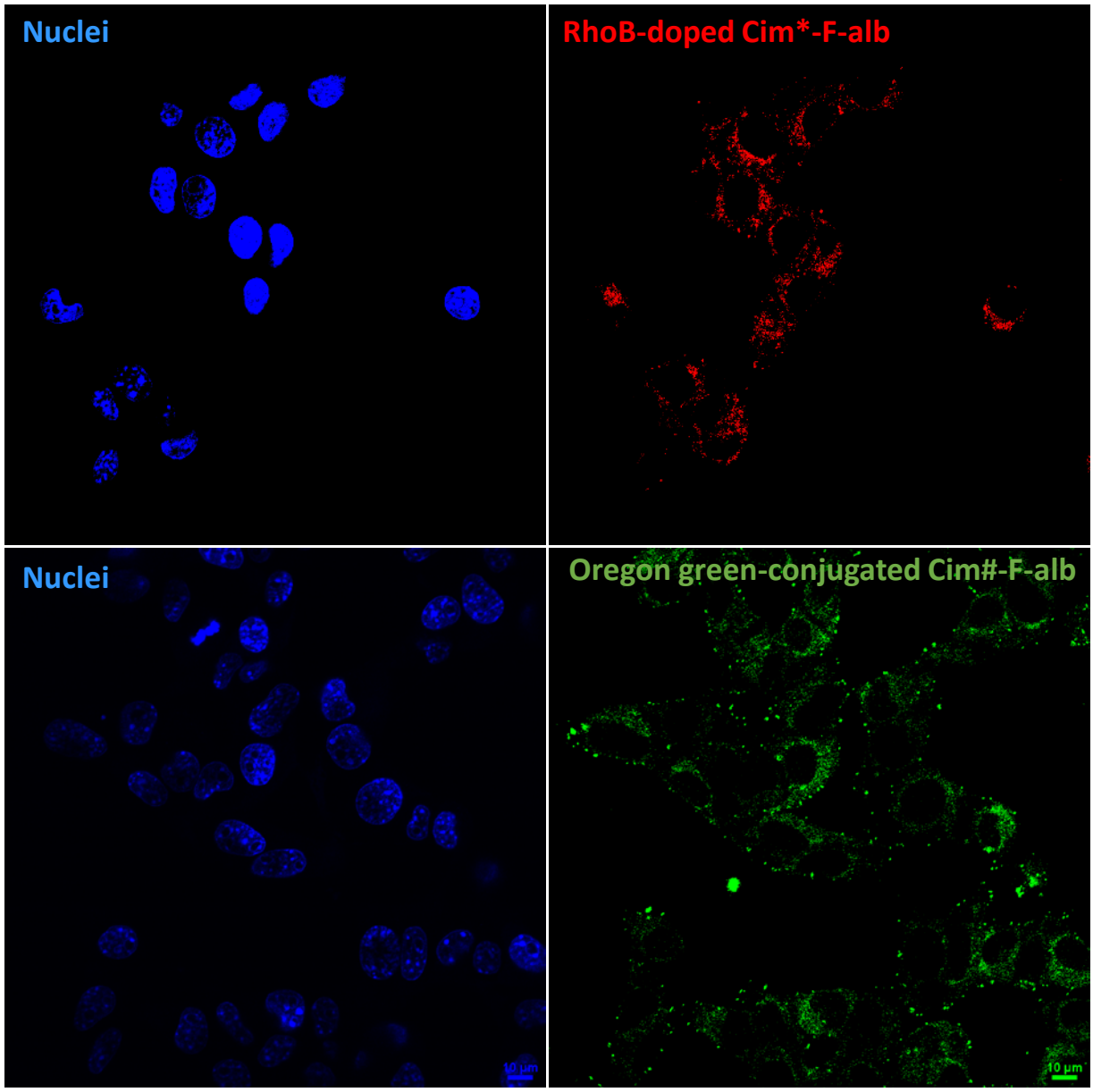
Supporting Figure 6. (a) Cytotoxicity of Pluronic F127 in B16F10 cells after 3h exposure, measured by MTT assay (n=3 measurements). (b) Cytotoxicity of PNC-alb, Cim-F-alb, and a mixture of PNC-alb and 10 μg/mL F127 in B16F10 cells after 3 h exposure, measured by MTT assay (n=3 measurements). *: $p<0.01$ by Tukey's multiple comparisons test.



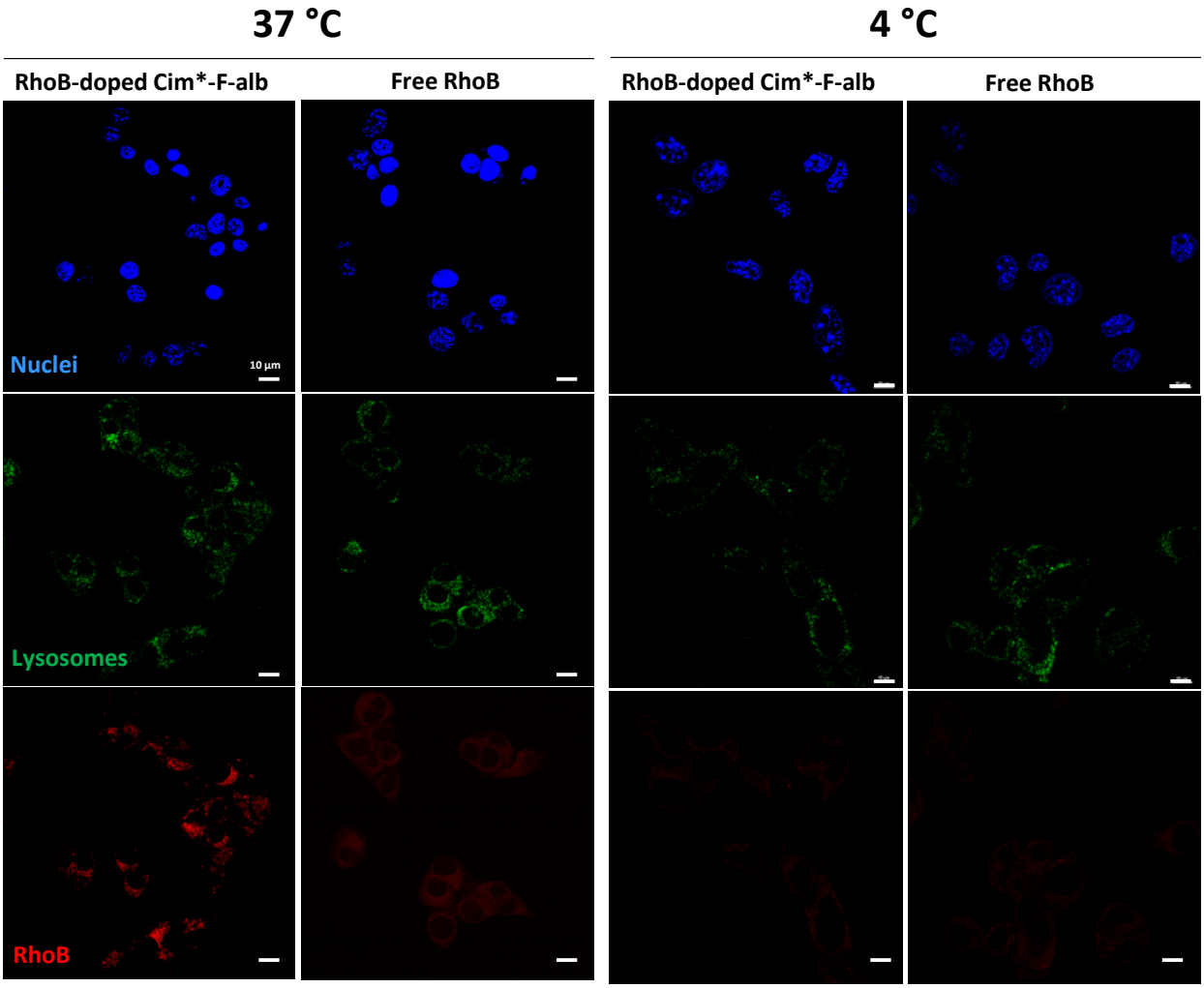
Supporting Figure 7. Fluorescence intensity of Rhodamine B at different pH's.



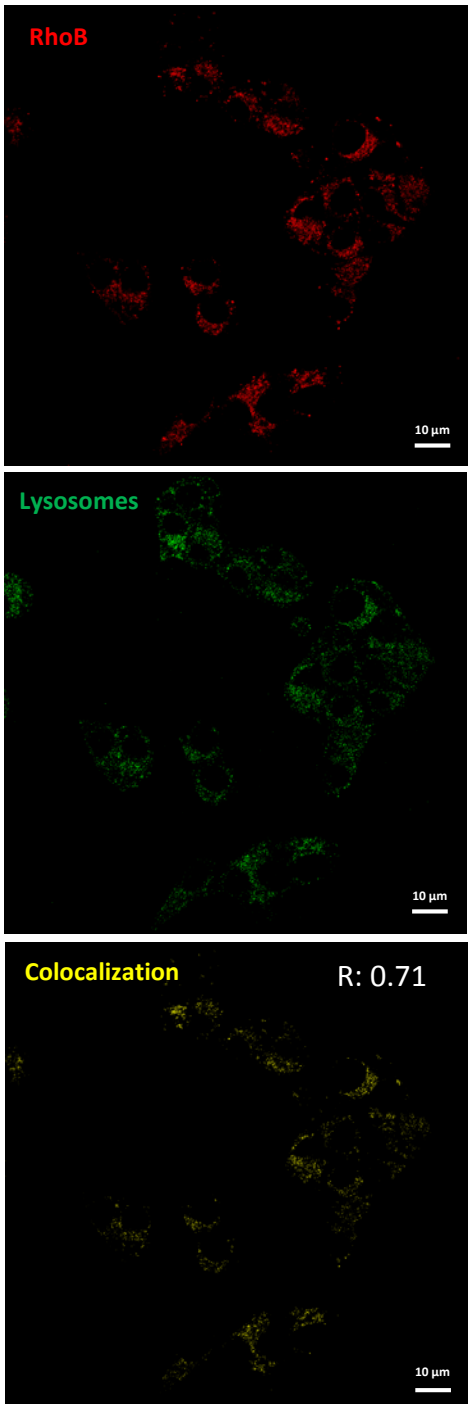
Supporting Figure 8. TEM images of Cim-F-alb and RhoB-labeled Cim*-F-alb.



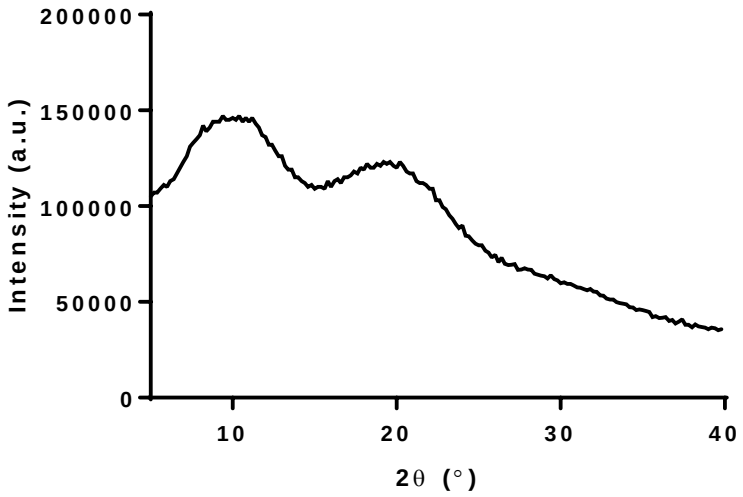
Supporting Fig. 9. Intracellular localization of rhodamine B doped Cim*-F-alb (Top) or Oregon green-conjugated Cim#-F-alb (bottom) in B16F10 cells after 30 min incubation.



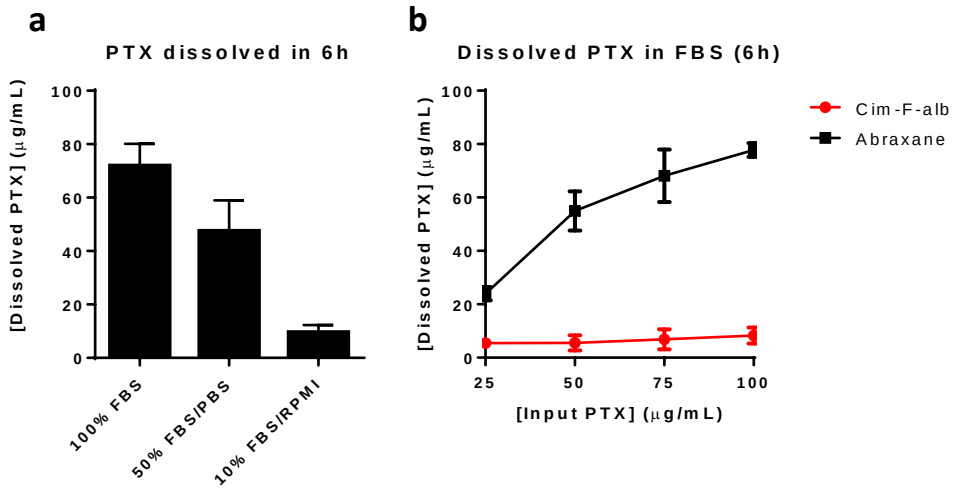
Supporting Figure 10. Intracellular localization of RhoB doped Cim*-F-alb in B16F10 cells after 30 min incubation at 37 ˚C or 4 ˚C. Scale bar: 10 μm.



Supporting Figure 11. Assessment of colocalization of Cim*-F-alb with Lysotracker. The degree of colocalization was analyzed by using Coloc 2 plugin of ImageJ. Pearson's correlation coefficient (R) represents the degree of colocalization: R=1 (perfect colocalization), R=0 (no colocalization), R=-1 (perfect exclusion).



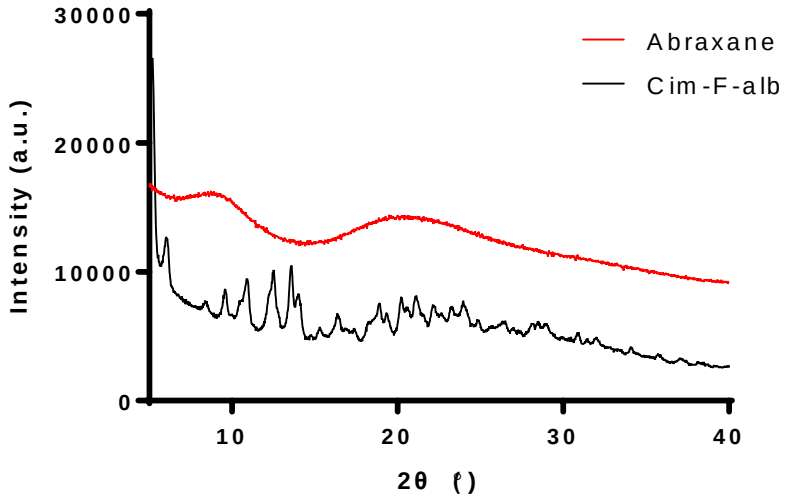
Supporting Figure 12. X-ray diffraction patterns of as-received PTX.



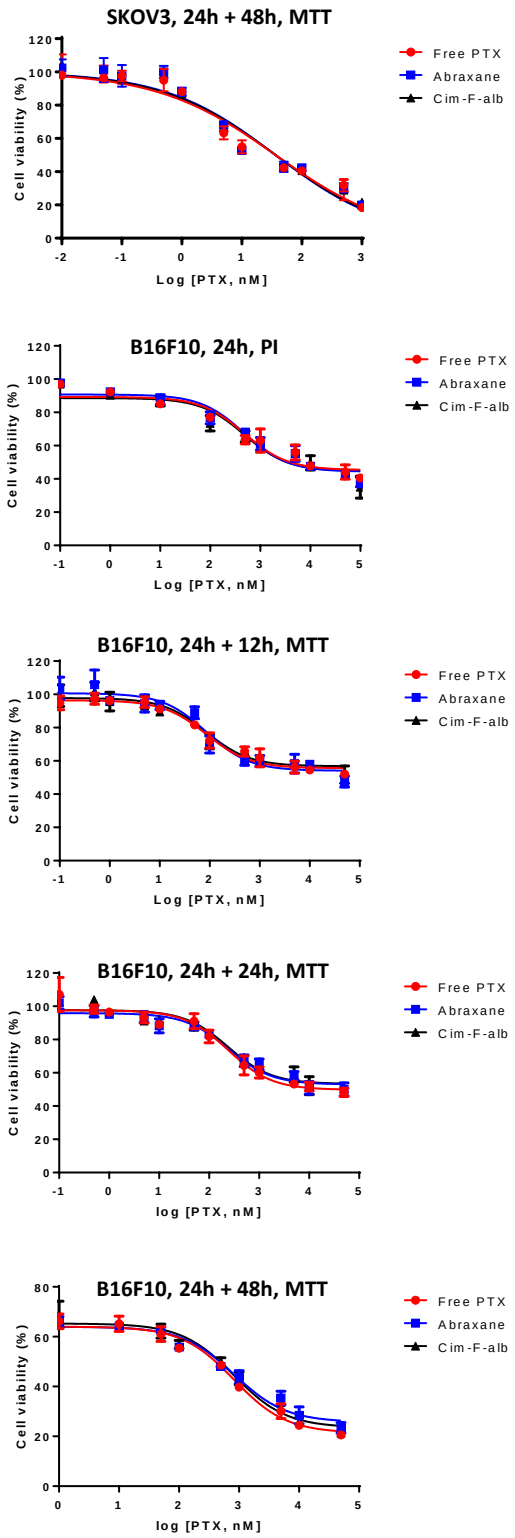
Supporting Figure 13. (a) Amorphous as-received PTX dissolved in undiluted FBS, 50% FBS/PBS, and 10% FBS/RPMI after 6 h incubation. One milligram of PTX was added to 1 mL of each medium and incubated for 6 h with agitation. A supernatant was separated from the undissolved PTX by centrifugation at 135,700 rcf for 10 min, extracted with 3 mL of ethyl acetate, which was evaporated and reconstituted in 50% ACN for HPLC analysis. (b) Abraxane and Cim-F-alb dissolved in undiluted FBS after 6 h incubation. The FBS suspension was sampled at 6 h to quantify dissolved PTX by that time. A supernatant was separated from the sample by 10 min centrifugation at 135,700 rcf and analyzed in the same way. The incubation time was limited to 6 h due to the instability of PTX in serum [1, 2]. Since the extraction method cannot differentiate protein-bound PTX from free PTX, the measured values represent the sum of serum protein-bound and free PTX.

[1] G. Bajaj, M.R. Kim, S.I. Mohammed, Y. Yeo, Hyaluronic acid-based hydrogel for regional delivery of paclitaxel to intraperitoneal tumors, J. Control. Release 158 (2012) 386-392.

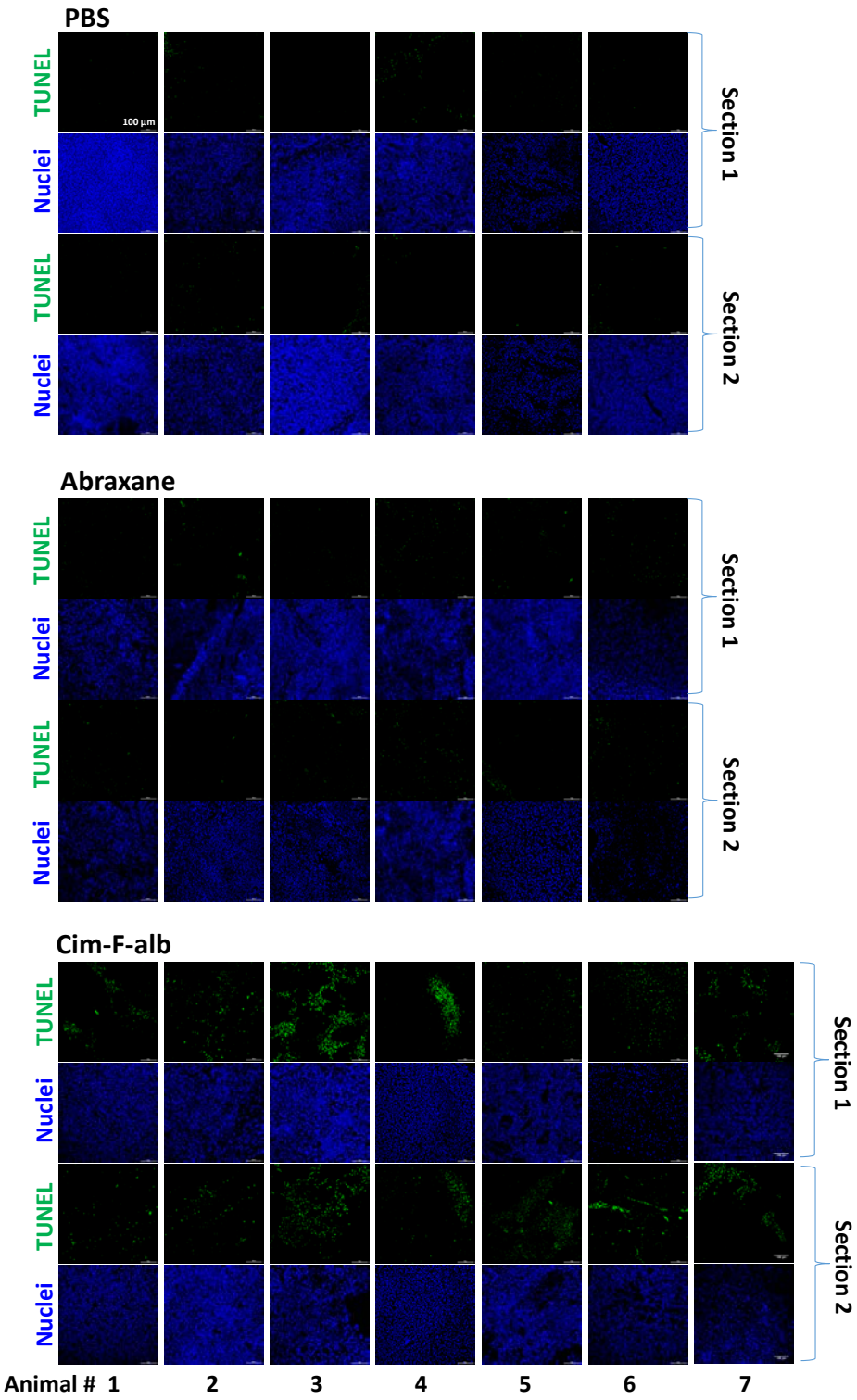
[2] I. Ringel, S.B. Horwitz, Taxol is converted to 7-epitaxol, a biologically active isomer, in cell culture medium, J. Pharmacol. Exp. Ther. 242 (1987) 692-698.



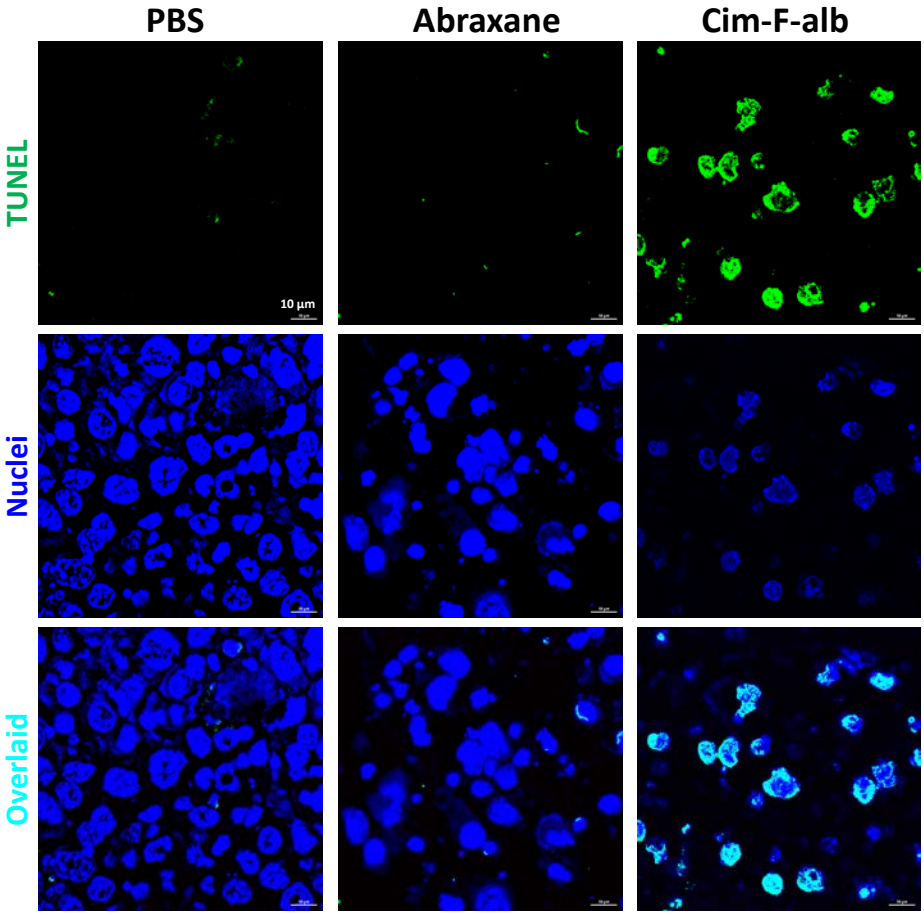
Supporting Figure 14. X-ray powder diffraction patterns of Abraxane and Cim-F-alb.



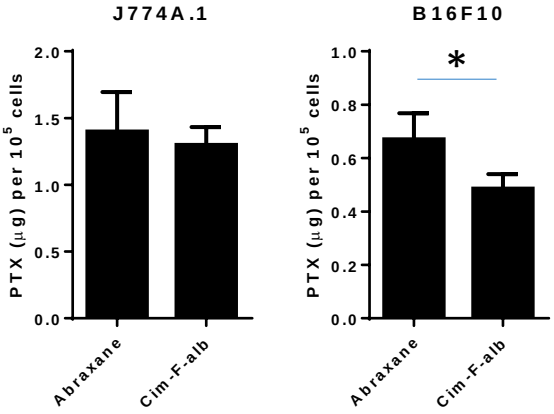
Supporting Figure 15. Cytotoxicity of Cim-F-alb, Abraxane, and free PTX in SKOV-3 human ovarian cancer cells measured by MTT assay (n=4 measurements) and B16F10 melanoma cells measured by PI staining and flow cytometry (n=2 independently and identically performed experiments) and MTT assay (n=3 measurements) varying the post-treatment incubation time.



Supporting Figure 16. All images of TUNEL-stained B16F10 tumor sections. Two randomly selected fields were imaged for each animal in the PBS, Abraxane and Cim-F-alb treatment groups (n=6 for PBS, Abraxane; n=7 for Cim-F-alb).



Supporting Figure 17. TUNEL stained B16F10 tumor section at high magnification indicating co-localization of TUNEL signal and nuclei stain.



Supporting Figure 18. Uptake of Abraxane and Cim-F-alb by (a) J774A.1 macrophages and (b) B16F10 melanoma cells. Cim-F-alb and Abraxane (eq. to 30 µg PTX/mL) were incubated with J774A.1 cells for 30 min or with B16F10 cells for 3 h at 37 °C. Intracellular PTX content was determined by HPLC and normalized with the number of analyzed cells. Data are presented as averages and standard deviations of 3 independently and identically performed experiments. *: $p<0.05$ by unpaired t-test.