Supporting Information for

ORIGINAL ARTICLE

Alleviating experimental pulmonary hypertension *via* co-delivering FoxO1 stimulus and apoptosis activator to hyperproliferating pulmonary arteries

Bingbing Li[b,a,†], Chao Teng[a,†], Huiling Yu[b,e,†], Xiaohong Jiang[a,†], Xuyang Xing[a], Qi Jiang[a], Chenshi Lin[a], Zongmin Zhao[c], Ruifeng Zhang[d,*], Wei He[a,*]

[a]*School of Pharmacy, China Pharmaceutical University, Nanjing 211198, China*

[b]*Department of Anesthesiology, Affiliated Drum Tower of Nanjing University, Nanjing 210008, China*

[c]*Department of Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, USA*

[d]*Department of Respiratory Medicine, Zhongda Hospital of Southeast University, Nanjing 210009, China*

[e]*Department of Anesthesiology, Women's Hospital of Nanjing Medical University, Nanjing Maternity and Child Health Care Hospital, Nanjing 210004, China*

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[*]Corresponding authors.

E-mail addresses: zrf2000@163.com (Ruifeng Zhang), weihe@cpu.edu.cn (Wei He).

[†]These authors made equal contributions to this work.

Supporting Figures S1–S14

Supporting Table S1

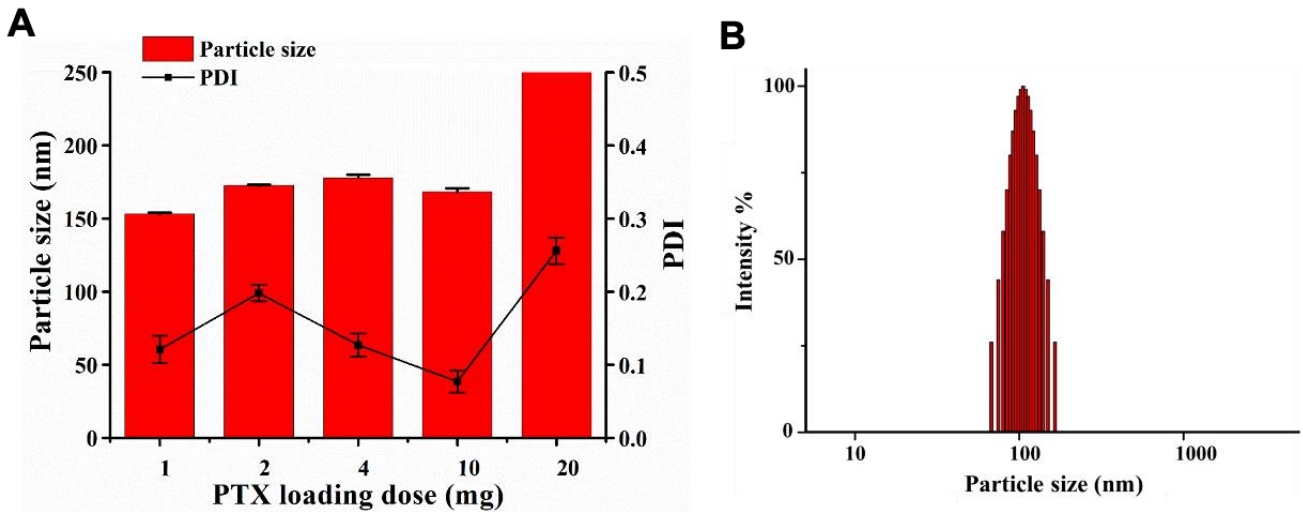

Figure S1 (A) Effect of PTX loading on particle size and PDI of MPN-PTX NPs. (B) Particle size distribution of MPN-PTX NPs from selected formulation measured by DLS. A 250 μL of ethanol solution of PTX was mixed with 9 mL of water, followed by ultrasonication and mixing with 0.5 mL of TA solution (2 mg/mL) and 0.25 mL of $FeCl_3$ $6H_2O$ solution (Fe^{3+} 0.25 mg/mL) in sequence. Data are presented as mean ± SD, n = 3.

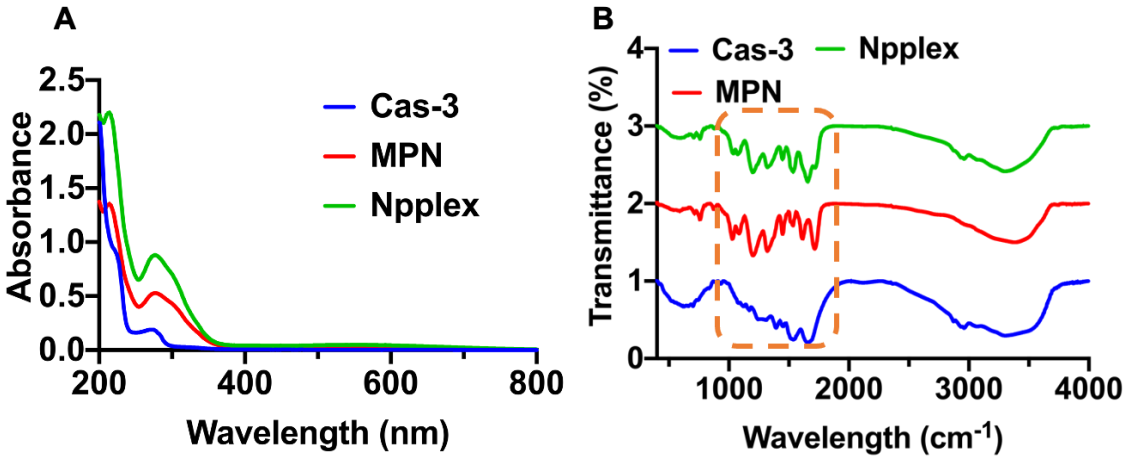

Figure S2 (A) UV–Vis and (B) FTIR spectra of different preparations. For UV–Vis spectra, Cas-3 displays two absorption peaks at around 240 and 260 nm, while MPN has peaked at 210 and 290 nm; Complexing the two allows redshift and absorbance enhancement nearby 290 nm. For FTIR spectra, NPplex reveals changes at around 1,000, 1,300 and 1,700 nm, corresponding to the vibration bands of C–O, OH, and C=O, respectively, compared to Cas-3 and MPN.

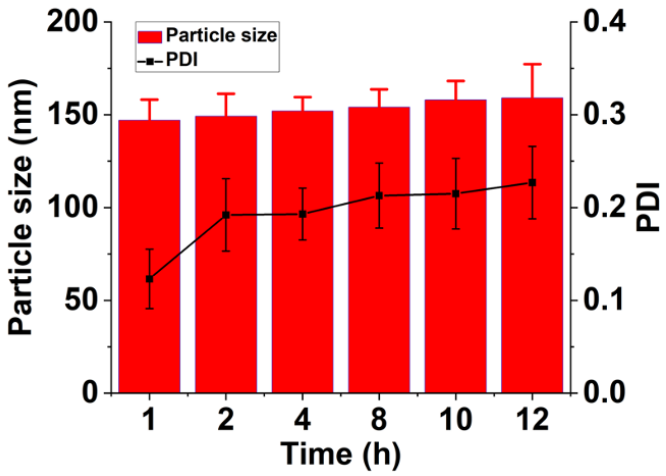

Figure S3 Serum stability of MPN-PTX NPs after incubation in 10% FBS at 37 ^{o}C. Data are presented as mean ± SD, n = 3.

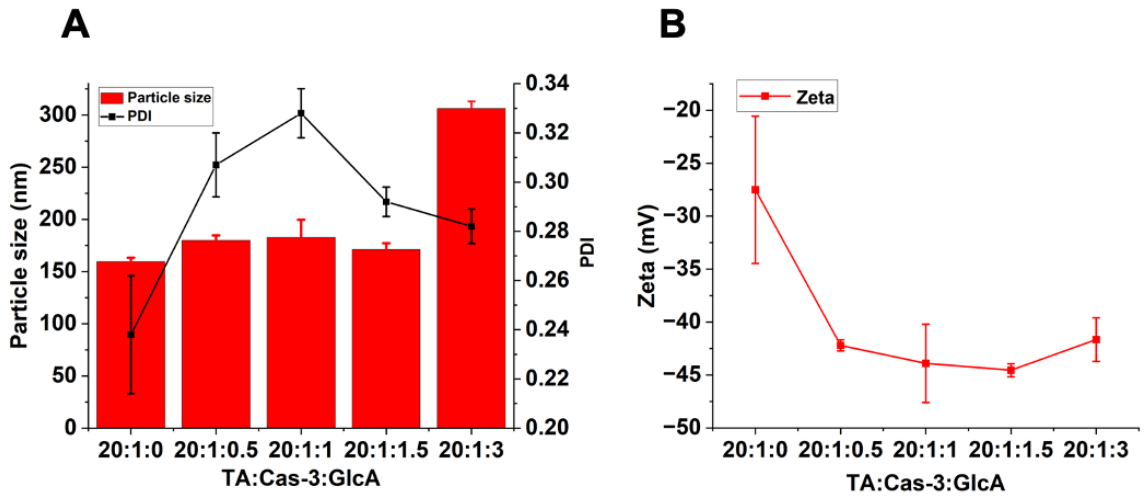

Figure S4 Influence of GlcA coating on (A) particle size, PDI and (B) zeta potential of GlcA-NPplex with the fixed mass ratio of TA/Cas-3 (20:1). Data are presented as mean ± SD, *n* = 3.

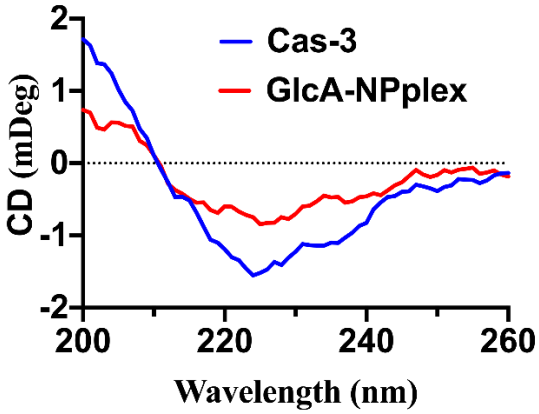

Figure S5 CD spectra of naked and released protein. The measurement was performed using parameters: bandwidth, 1 nm; response, 1 s; cell length, 0.1 cm; temperature, 25 ℃; protein concentration, 0.1 mg/mL.

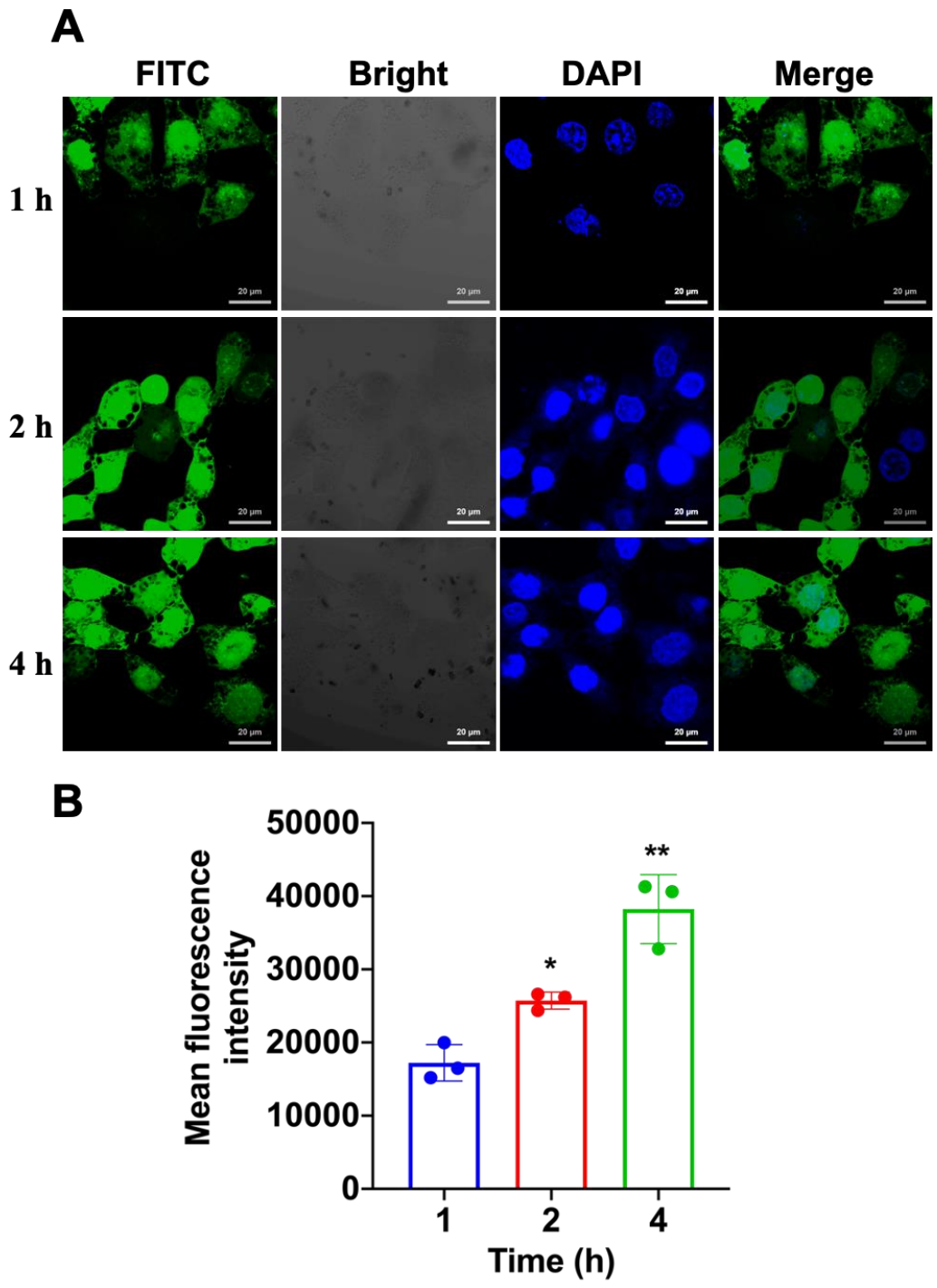

Figure S6 Time-dependent uptake of FITC-labeled GlcA-NPplex in PASMCs. (A) Confocal imaging and (B) quantitative analysis. The cells were incubated with the nanoparticles at 5 μg/mL FITC at 37 ℃. Data are presented as mean ± SD, $n = 3$, $^{*}P < 0.05$, $^{**}P < 0.01$ compared to 1 h. The scale bar is 20 μm.

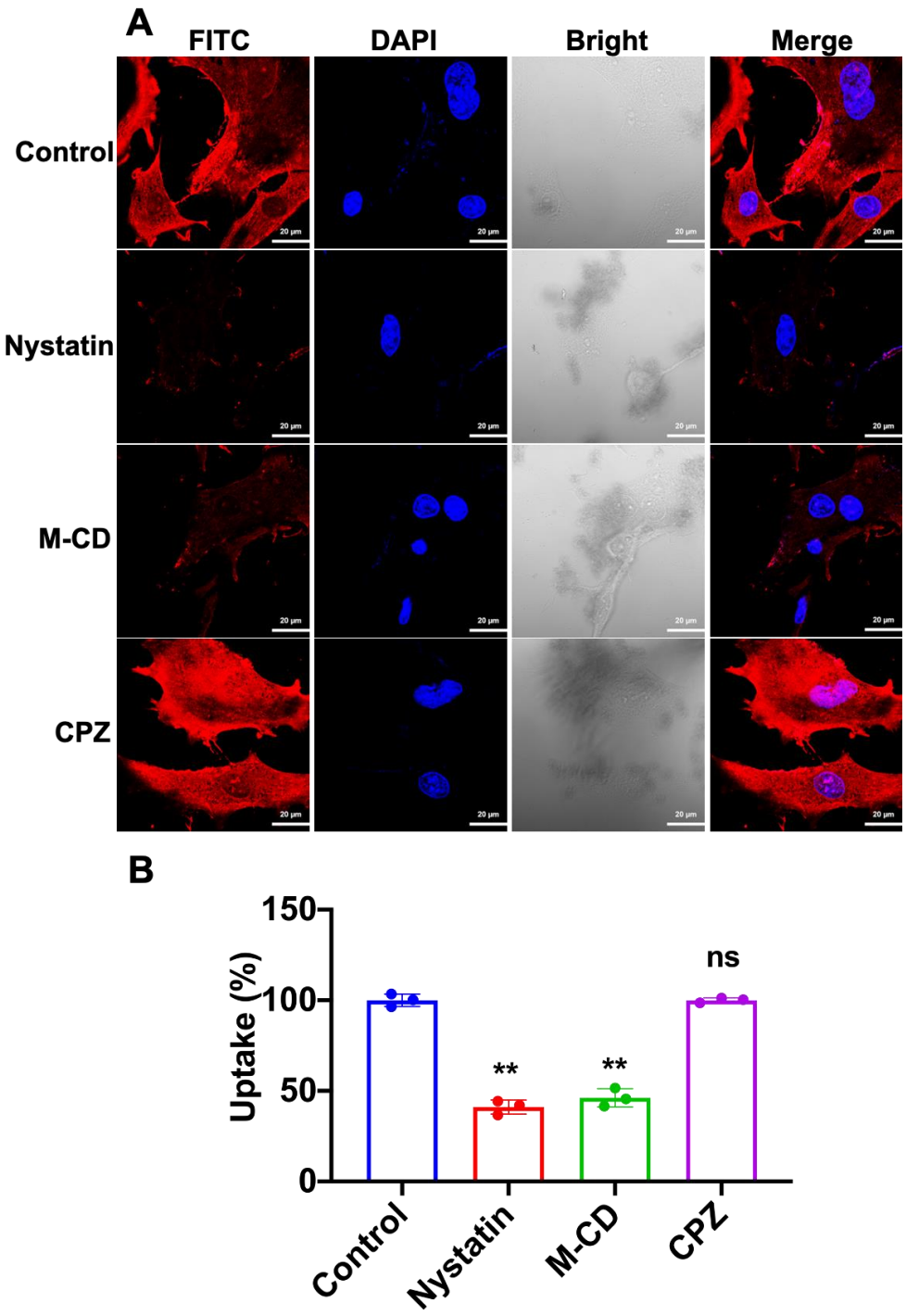

Figure S7 Cellular uptake mechanism. (A) Qualitative and (B) quantitative analysis of FITC-labeled GlcA-NPplex uptake in PASMCs pretreated with different caveolae-pathway inhibitors (nystatin and M-CD) or endocytosis inhibitor chlorpromazine (CPZ) for 0.5 h at 37 ℃. Cellular uptake was detected using FCM. The scale bar is 20 μm. Data are presented as mean ± SD, n = 3, $^{**}P<0.01$ compared with the control group. ns, not significant

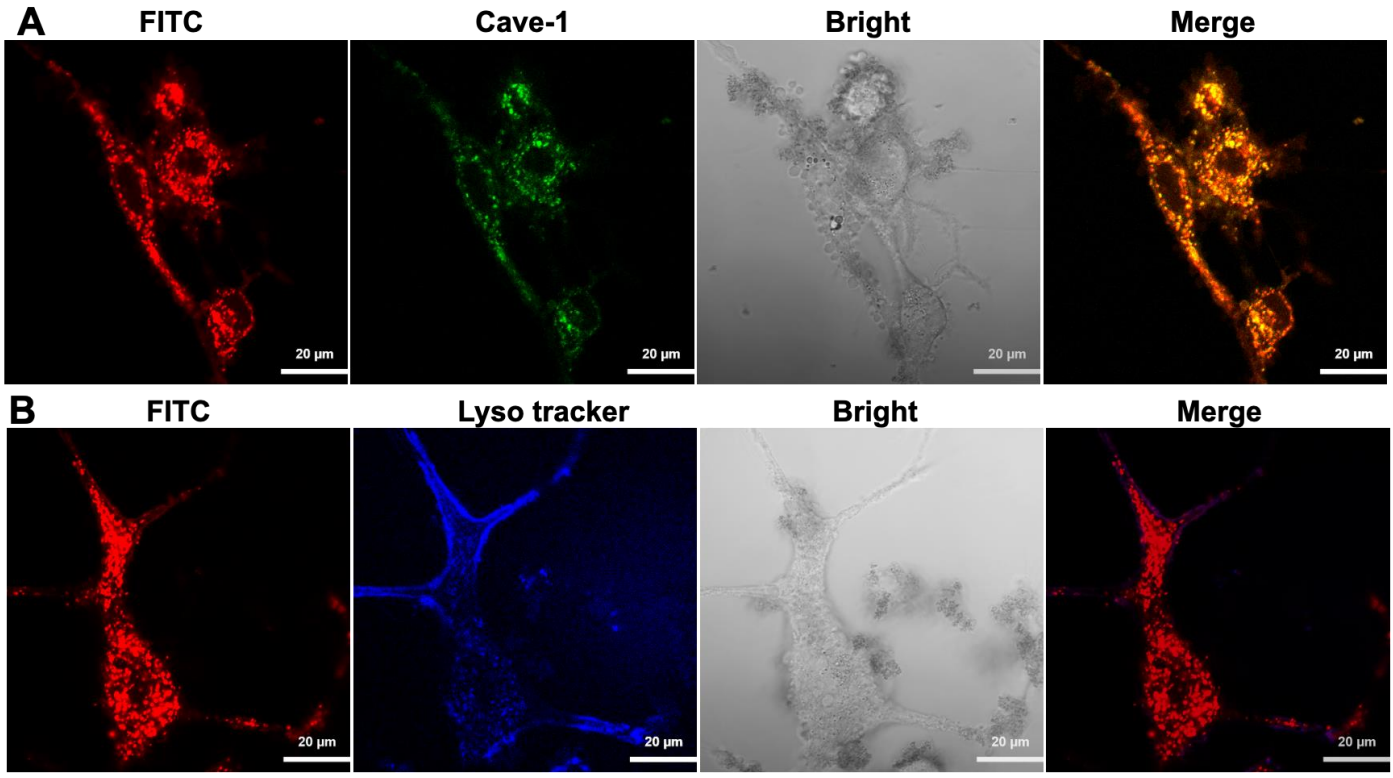

Figure S8 Colocalization of FITC-labeled nanoparticles (red) with (A) caveolae protein marked with Alexa Fluor 488-labeled Cave-1 (green) and (B) lysosomes stained using LysoTracker Red (blue). Cells were cultured with FITC-labeled nanoparticles with 5 μg/mL FITC for 3 h at 37 ℃, followed by staining with 1 mL of LysoTracker Red for 1 h and with the caveolae marker, Alexa Fluor 488-labeled Cave-1, for 3 h.

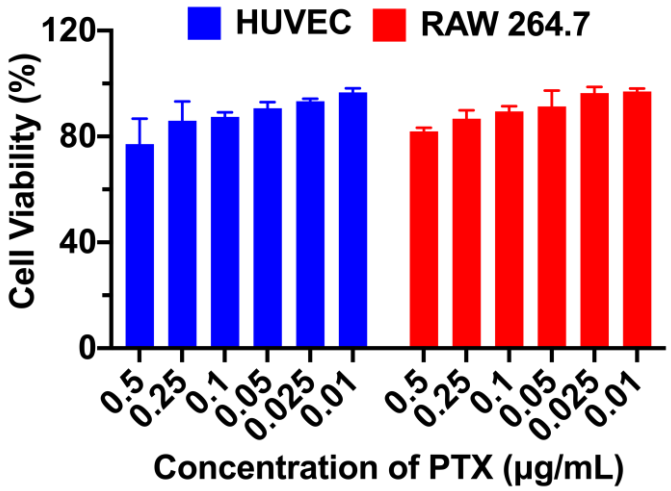

Figure S9 GlcA-NPplex cytotoxicity to HUVEC and RAW 264.7 cell lines after 48-h incubation with 0.01–0.5 μg/mL PTX. The data are expressed as mean ± SD, *n* = 3.

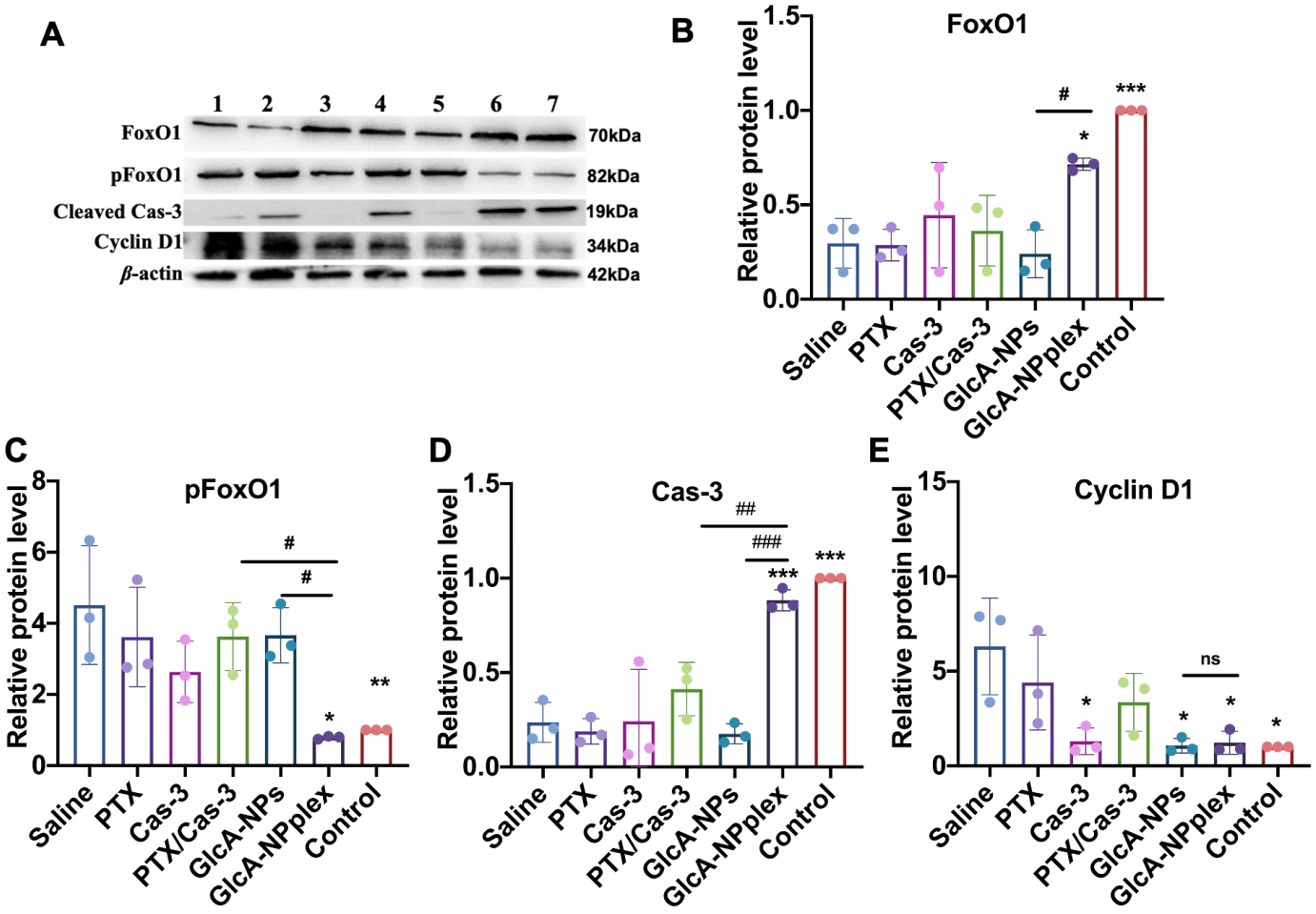

Figure S10 Determination of apoptosis-related pathway in PASMCs isolated from MCT-PH rats. (A) FoxO1/pFoxO1/Ca-3/Cyclin D1 expression examined by Western blot analysis. 1: Saline; 2: PTX; 3: Cas-3; 4: PTX/Cas-3 mixture; 5: GlcA-NPs; 6: GlcA-NPplex; 7: normal PASMCs treated with saline. The internal control for normalizing protein expression is *β*-actin. (B–E) Quantitative analysis. Data are represented as mean ± SD, $n = 3$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to PASMCs-Saline group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ compared to GlcA-NPplex group； ns, no significance. Control: PASMCs isolated from normal rats.

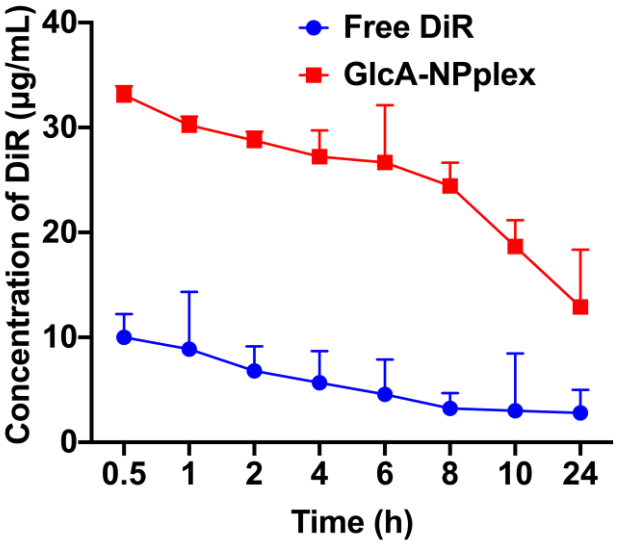

Figure S11 Prolonged blood circulation. Mean plasma concentration-time curves of DiR-labeled preparations. Data are presented as mean ±SD, n = 3. DiR-labeled preparations were dosed *via* intravenous injection at 0.5 mg/kg DiR, according to the body weight.

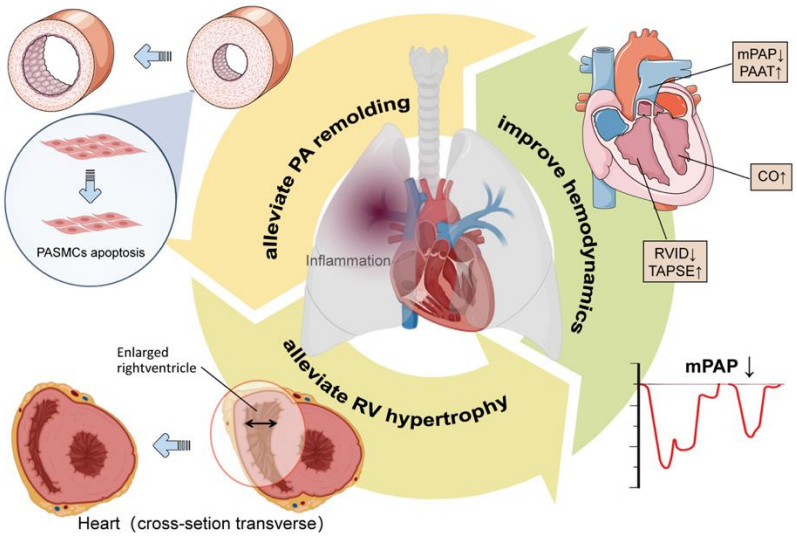

Figure S12 A schematic illustration of the relationship among the treatment index. Inhibiting PASMC hyperproliferation alleviates PA remodeling and improves the RV function and hemodynamics.

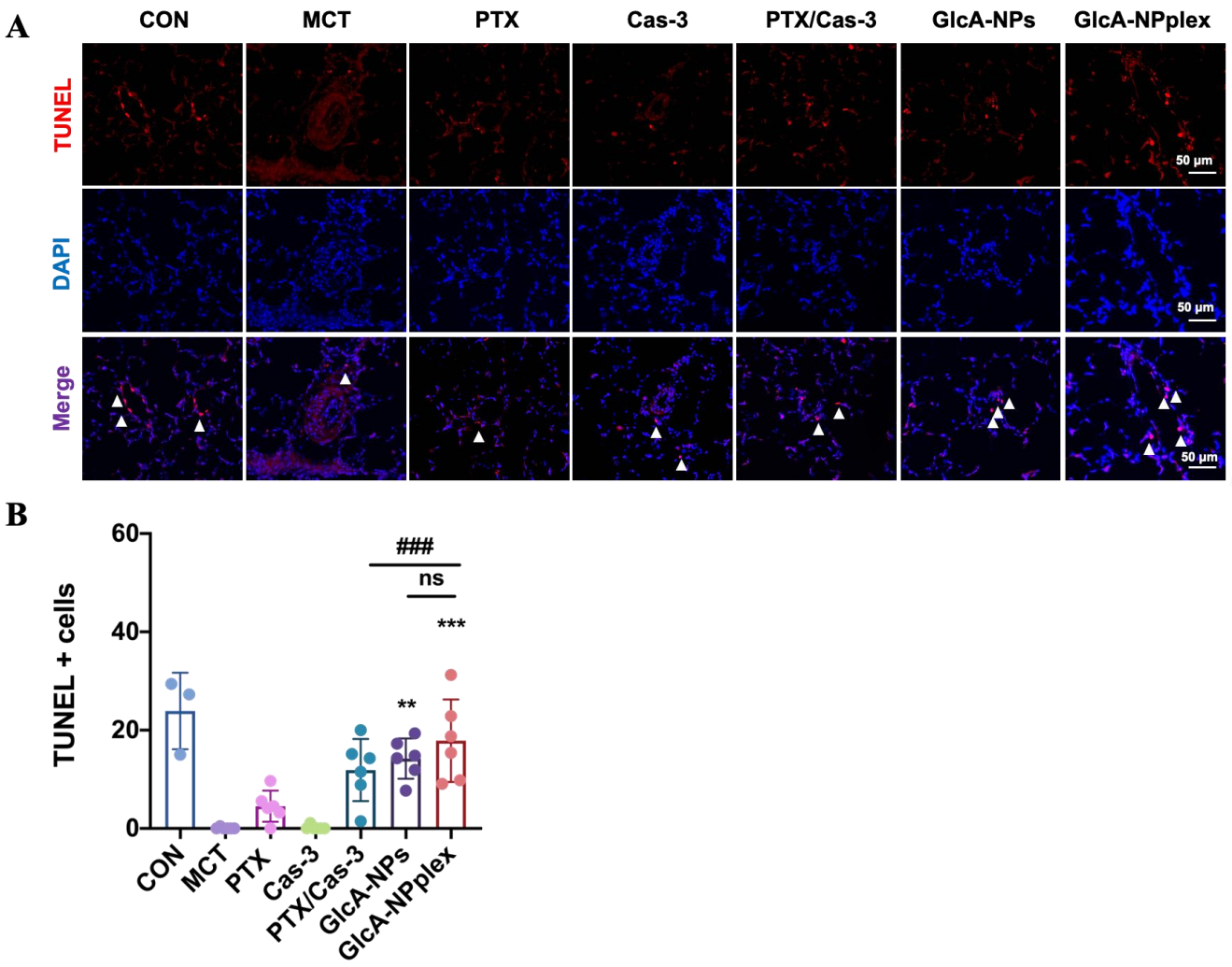

Figure S13 Efficacy of the different formulations on the media tunica PASMC apoptosis in MCT-PH rats. (A) The apoptotic cells in the media tunica of PAs were detected *via* TUNEL assay. The representative immunofluorescent images were shown by the colocalization of the apoptotic cells (Red) with the nucleus (DAPI) and merged images (purple). (B) Semi-quantification of the apoptotic cells in the media tunica of PAs. Data are represented as mean ± SD. n = 6 rats/group, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to MCT group; $^{###}P < 0.001$ compared to GlcA-NPplex group; ns means no significance. The scale bar is 50 μm. CON: controlled rats received 3 intravenous injections of 0.9% saline; MCT: rats received MCT treatment; PTX/Cas-3: the combination of PTX and Cas-3. The MCT-induced rats were dosed with preparations at 0.4 mg/kg PTX and 0.04 mg/kg Cas-3 *via* the tail vein.

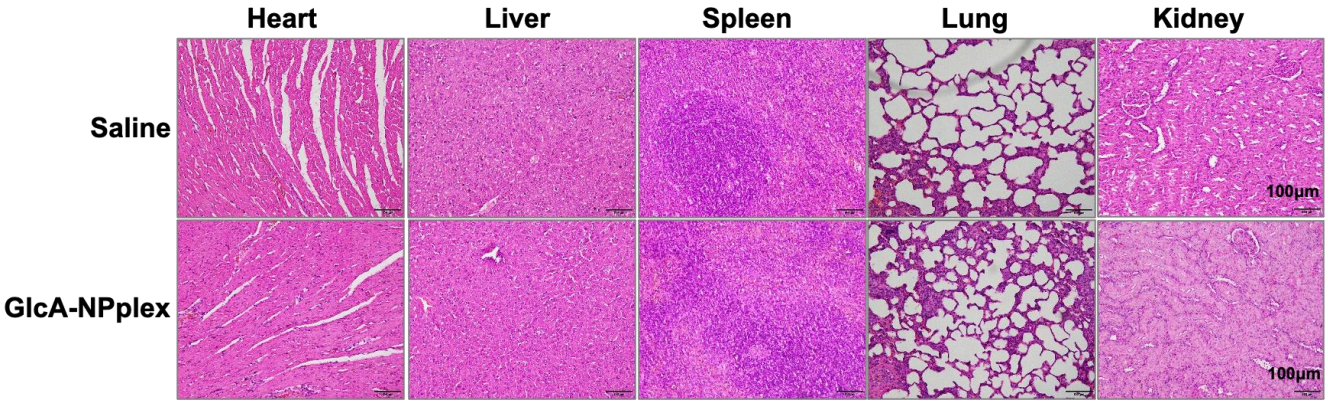

Figure S14 The histological changes of vital organs in MCT-PH rats after receiving GlcA-NPplex for 14 Days. The rats received 3 intravenous injections of 0.9% saline or GlcA-NPplex at 0.4 mg/kg PTX and 0.04 mg/kg Cas-3 *via* the tail vein at predetermined time points according to the experiment protocol. At the end of treatment, the rats were sacrificed, and the vital organs were harvested and fixed in 10% neutral formalin. The histopathological changes of vital organs were assessed with H&E staining. The GlcA-NPplex administration demonstrates little locomotor impairment, dehydration, muscle loss, or other symptoms coupled with systemic toxicity.

Table S1 Pharmacokinetic parameters of DiR-labeled nanoparticles after intravenous injection at a DiR dose of 0.5 mg/kg (n = 3)

Formulations	C_{max} (mg/L)	T_{max} (h)	AUC^{0-t} (mg/L*h)	$t_{1/2}$ (h)	CL ($L \cdot h^{-1} \cdot kg^{-1}$)	MRT (h)
Free DiR	10.01 ± 2.20	0.50 ± 0.00	101.59 ± 4.92	2.46 ± 0.36	0.03 ± 0.00	17.41 ± 2.93
GlcA-NPplex	33.10 ± 0.83	0.50 ± 0.00	807.74 ± 5.52	17.58 ± 0.26	0.00 ± 0.00	25.23 ± 5.54

C_{max}, the maximum plasma concentration of drug; T_{max}, the time of peak concentration; AUC^{0-t}, area under the time-concentration curve; $t_{1/2}$, elimination half-life in the blood; CL, clearance rate; MRT, mean residence time.