Supplementary Materials

Targeting intracellular MMPs efficiently inhibits tumour metastasis and angiogenesis

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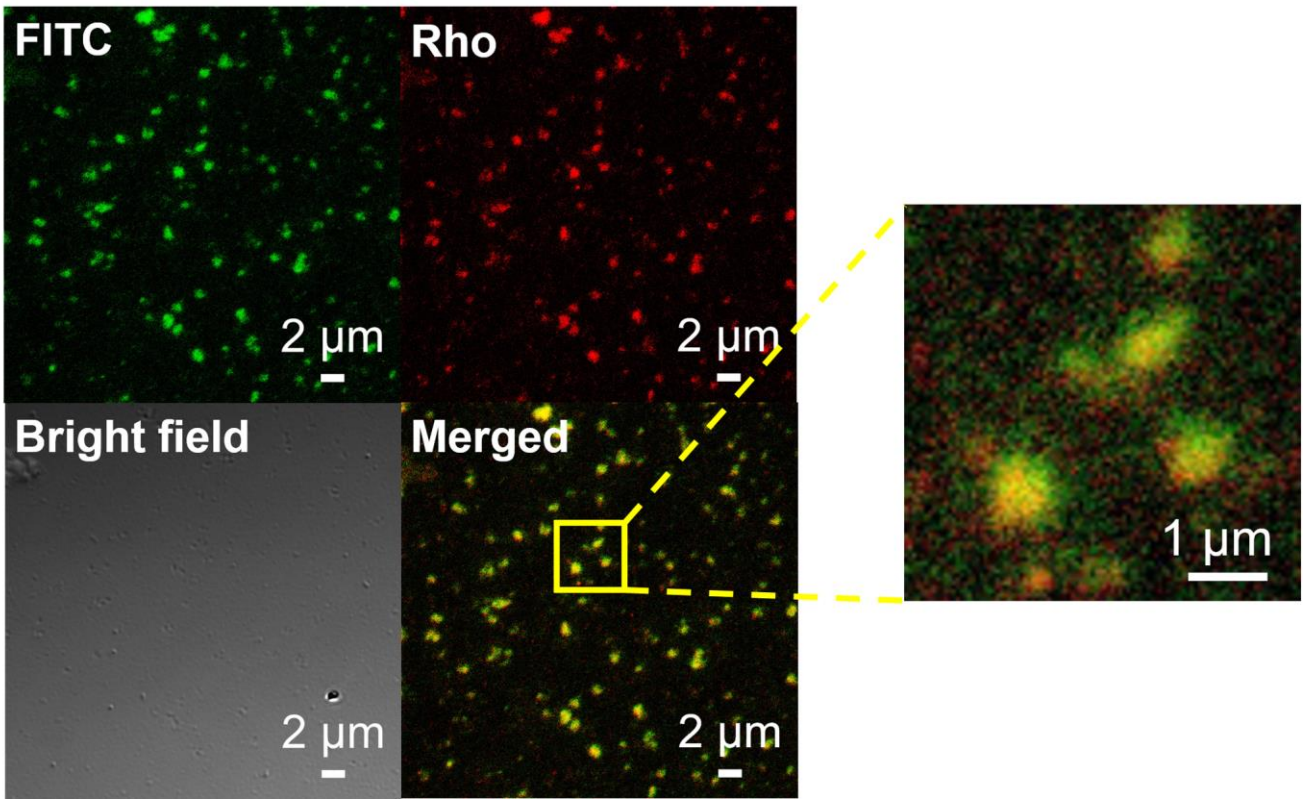

Figure S1. CLSM images of HPMC NPs at a FITC-HA-PTX/Rho-MATT/CN mass ratio of 2:1. The FITC and Rho concentrations were both 800 ng/mL. Yellow fluorescence indicates the assembly of FITC-HA-PTX and Rho-MATT/CN.

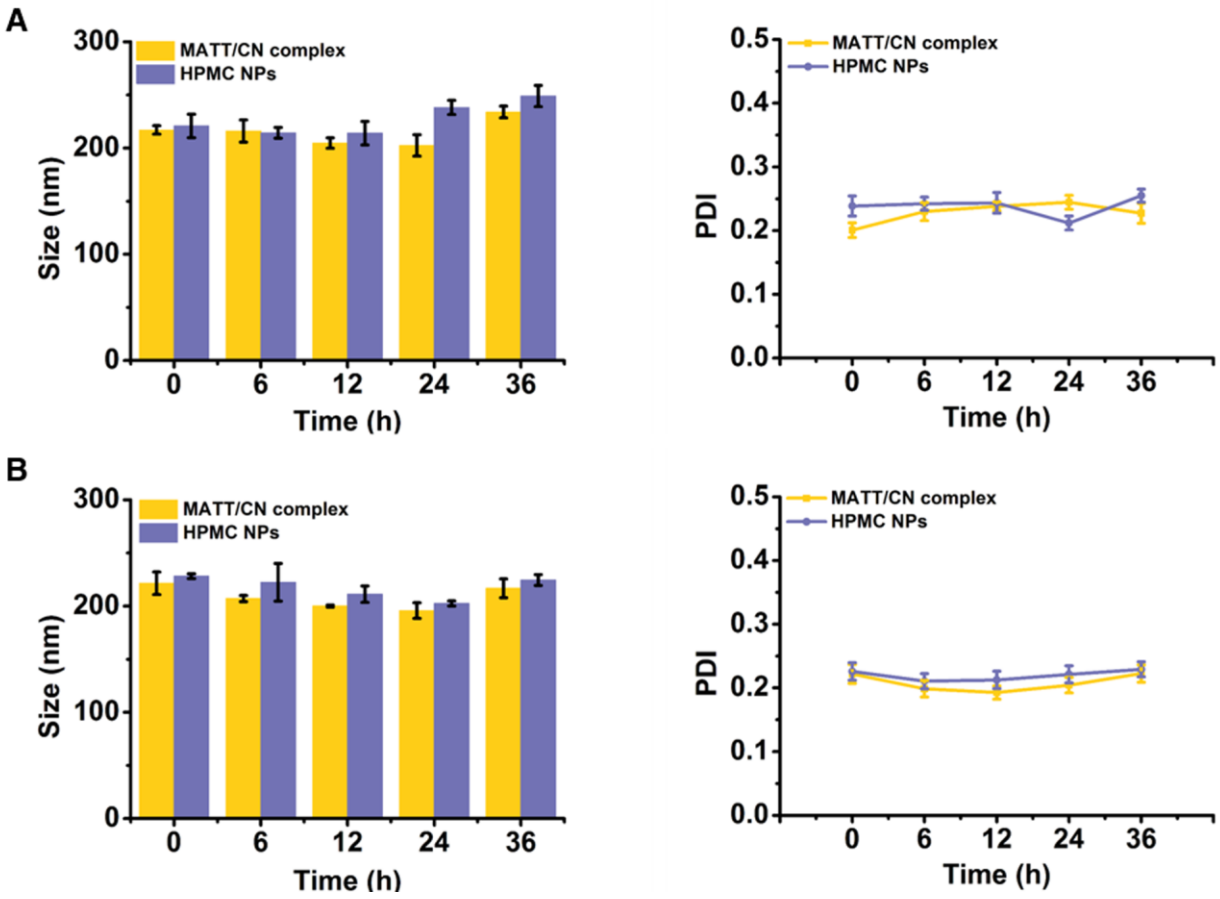

Figure S2. Stability study of MATT/CN complex or HPMC NPs in (A) PBS (pH 7.4) and (B) RPMI1640 containing 10% FBS in terms of particle size and PI at 37 ℃.

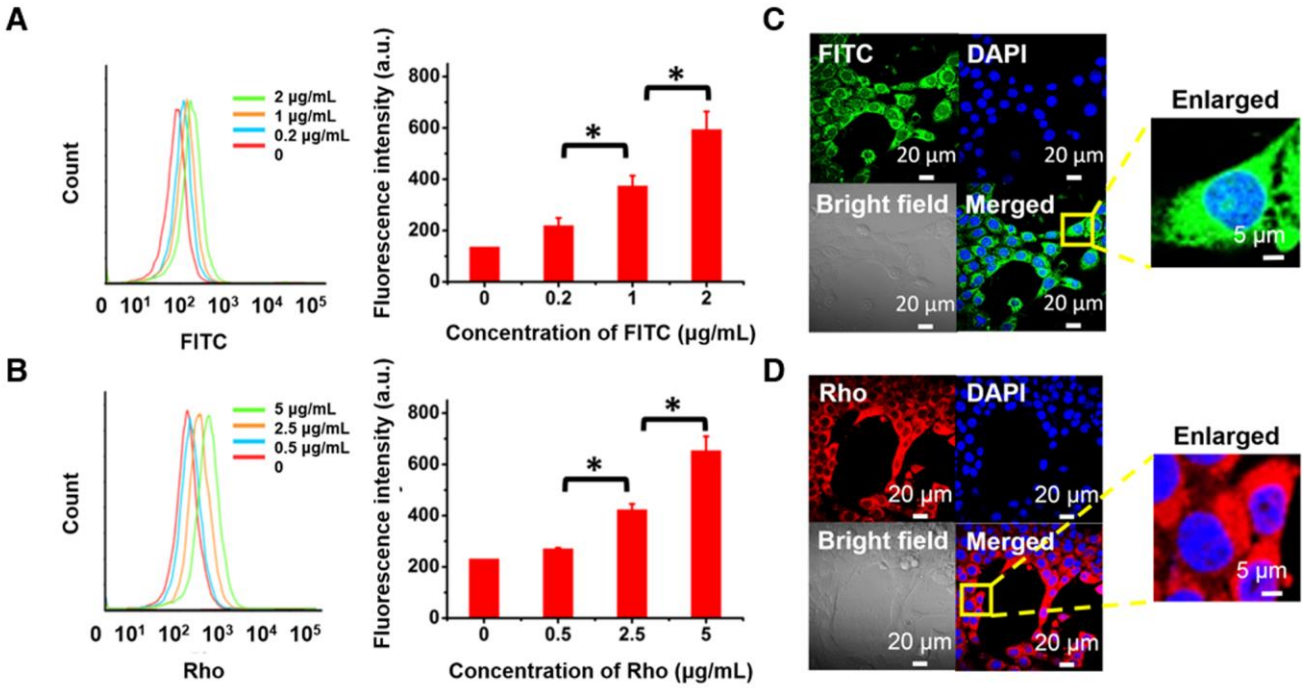

Figure S3. Cellular uptake. Concentration-related uptake of (A) FITC-HPMC NPs and (B) Rho-HPMC NPs measured by flow cytometry after incubation with 4T1 cells for 4 h at 37 ℃. CLSM images of (C) FITC-HPMC NPs (green) and (D) Rho-HPMC NPs (red) after incubation for 4 h at 37 ℃ at FITC or Rho concentrations of 800 ng/mL. The nuclei were stained with DAPI (blue).

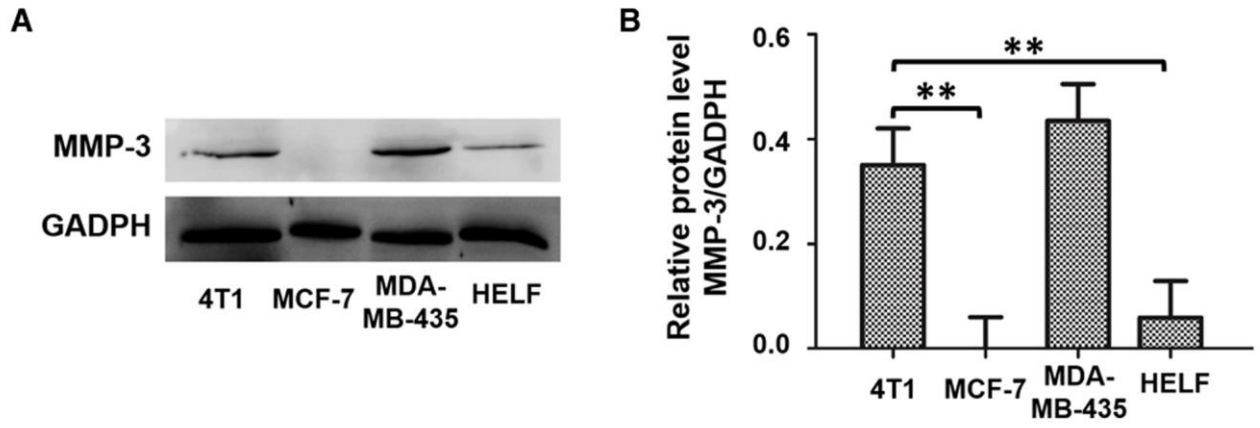

Figure S4. Western blotting analysis of MMP-3 expression. (A) MMP-3 expression in different cell lines. GADPH was used as a loading control. Dark bands indicate MMP-3 expression. (B) Quantitative analysis of the MMP-3 expression. (mean ± S.D., $n = 3$, **P < 0.01).

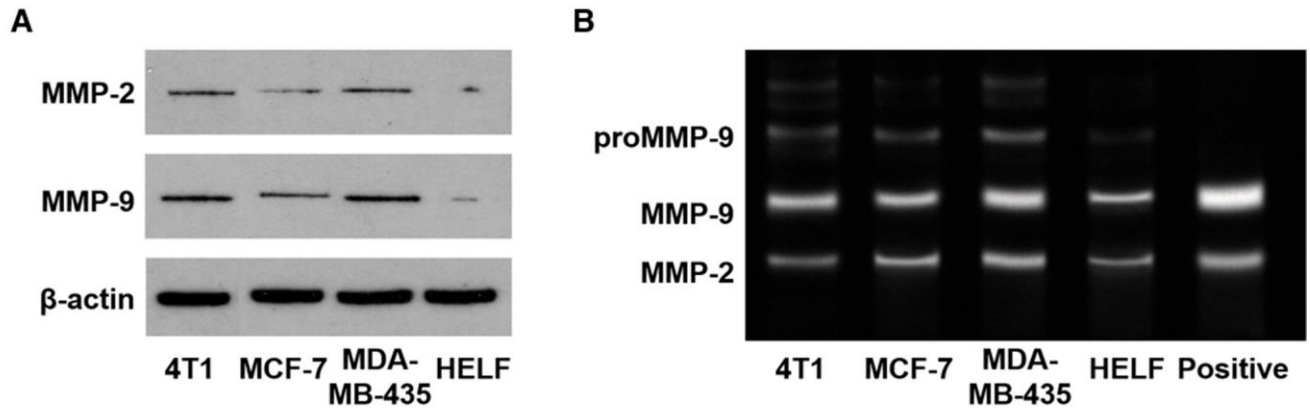

Figure S5. MMP expression and activities in different cell lines. **(A)** Western blotting analysis of MMP-2 and MMP-9 expression. β-Actin was used as a loading control. Dark bands indicate MMP expression. **(B)** Activities of MMP-2 and MMP-9 analysed by gelatine zymography. Bright bands on the dark background indicate MMP activity.

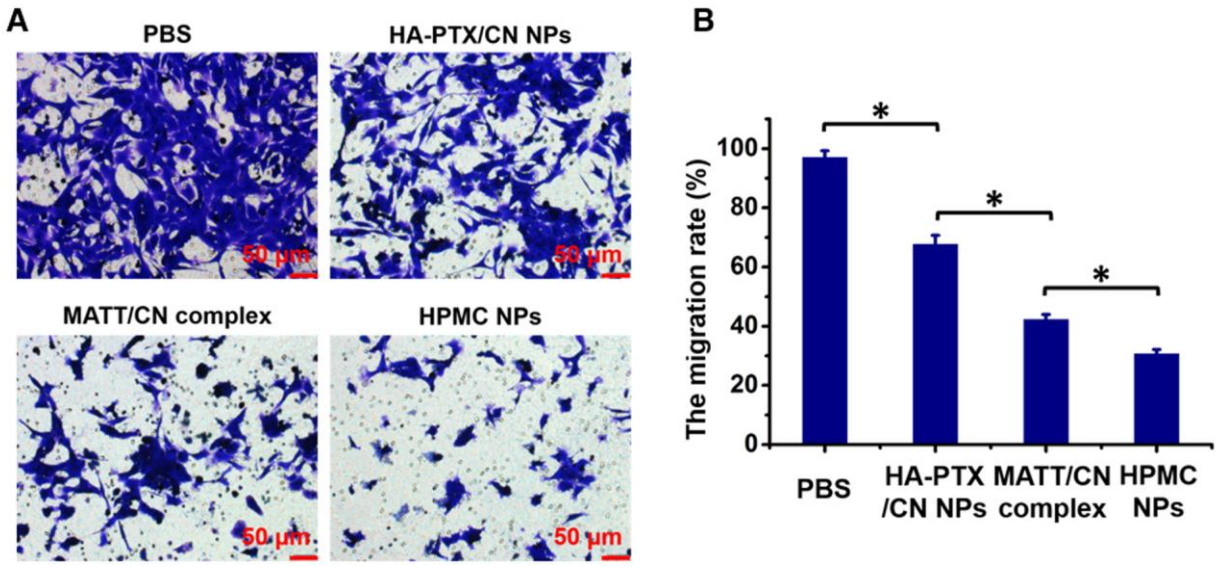

Figure S6. Anti-metastasis study. **(A)** Microscopy images of cell invasion using a Matrigel® transwell assay after incubation with HPMC NPs at PTX and MATT concentrations of 2 μg/mL and 5 μg/mL, respectively, for 24 h at 37 ℃. The migrated cells were stained with 0.1% crystal violet at 25 °C. The scale bar is 50 μm. **(B)** Quantitative analysis of the migrated cells by determining the optical density (OD) ratio of stained crystal violet with a microplate reader at 570 nm. The data was presented as the mean ± s.e.m. (n = 3, *P < 0.05, **P < 0.01).

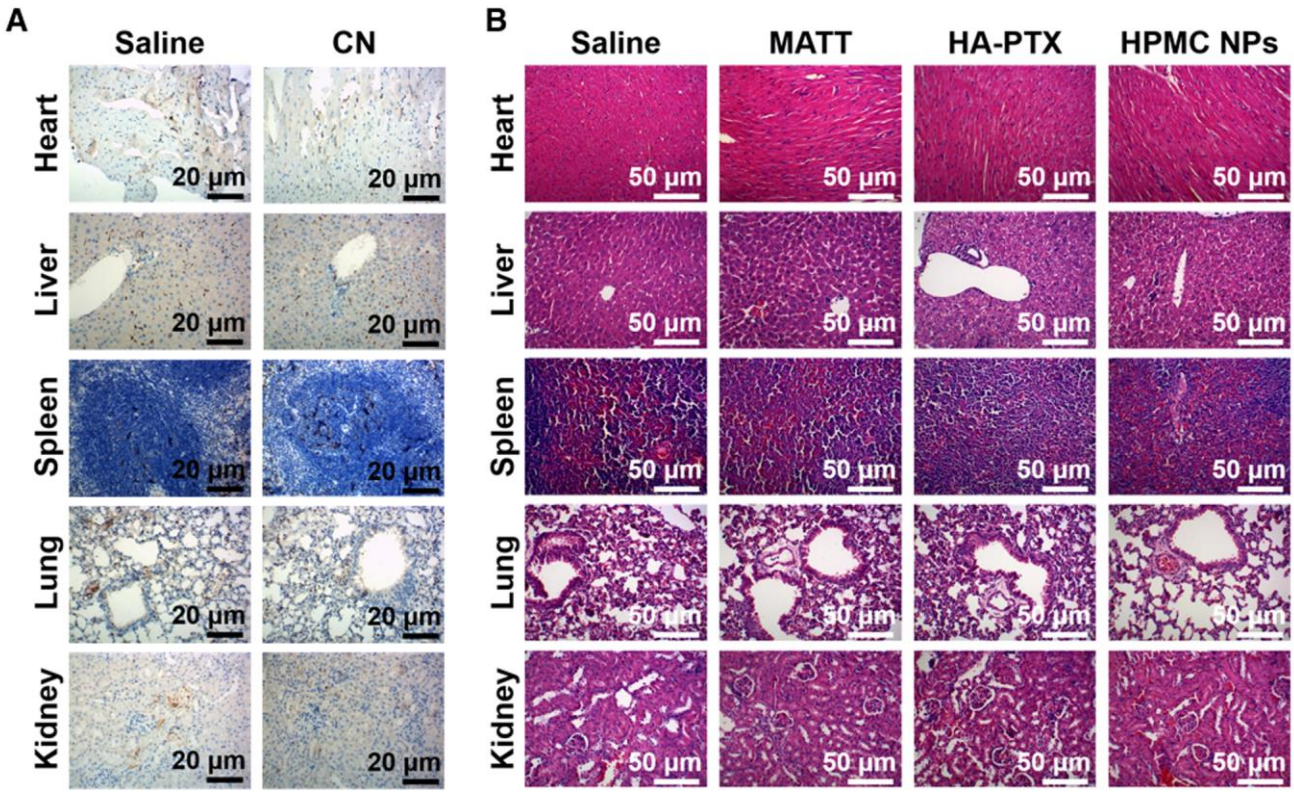

Figure S7. Safety examination. Normal mice were injected with blank β-CN, MATT, HA-PTX, or HPMC NPs via a tail vein injection every 3 days at doses of 2 mg/kg for PTX and 5 mg/kg for MATT, respectively, for 5 times. The injection volume was 0.2 mL. CN were injected at the same dose as HPMC NPs. The main organs (i.e., heart, liver, spleen, lung, and kidney) were collected on post-injection day 16 and (**A**) CD68 and (**B**) H&E analyses were conducted. Saline was used as a control.

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Table S1. Size, PDI and zeta potential of nanoparticles.

Formulations	Size	PDI	Zeta potential
β-CN	204.83 ±4.74	0.041 ±0.009	-27.32 ±1.27
MATT/CN complex	192.77 ±4.89	0.097 ±0.021	-6.85 ±3.08
HA-PTX/CN NPs	250.83 ±1.74	0.137 ±0.015	-8.98 ±1.96
HPMC NPs	233.87 ±6.13	0.133 ±0.015	-8.53 ±1.77

Table S2. Stern–Volmer quenching rate constant (K_{sv}), fluorescence quenching rate constant (K_q), and binding constant (K_a) at different temperatures.

Formulations	***T* (K)**	K_{sv} **(mg/mL^{-1})**	K_q **(mg/mL^{-1}s^{-1})**	***n***	K_a
MATT/CN complex	288	0.1461	1.46×10^{7}	1.2	0.0107
	298	0.2956	2.96×10^{7}	0.8	0.296
	310	0.2664	2.66×10^{7}	0.5	0.257
HA-PTX/CN complex	288	0.1133	1.13×10^{7}	0.5	0.057
	298	0.2119	2.12×10^{7}	0.8	0.234
	310	0.0925	9.25×10^{6}	1.1	0.0868
HPMC NPs	288	0.3363	3.36×10^{7}	0.1	0.399
	298	0.8786	8.79×10^{7}	0.2	1.15
	310	0.4783	4.78×10^{7}	0.5	0.497

Table S3. Hematologic analysis of mice after five *i.v.* injections of different formulations (n = 3)

Formulations	**WBC** (10^9/L)	**RBC** (10^{12}/L)	**PLT** (10^9/L)	**HGB** g/L	**HCT** %
Normal ranges	5.1-11.6	7.7-12.5	100-1000	122-162	42-44
Saline	7.26±3.69	8.34±0.47	701.0±36.78	138.0±7.07	43.55±1.34
β-CN	6.07±1.40	7.82±0.50	594.5±221.3	129.5±2.12	43.05±0.35
MATT	5.84±0.66	8.40±0.22	543.0±106.1	132.5±3.54	43.30±0.57
HA-PTX	6.40±2.03	7.83±0.44	548.0±230.5	131.0±9.90	42.95±2.62
HPMC NPs	7.12±0.35	8.35±0.64	665.0±100.4	140.0±2.83	45.45±0.92

white blood cell, WBC; red blood cell, RBC; platelet, PLT; hemoglobin, HGB; hematocrit, HCT