

Supplementary Information

Smart design of therapeutic nanoplatform for mitochondria-targeted copper-depletion therapy combined with chemotherapy

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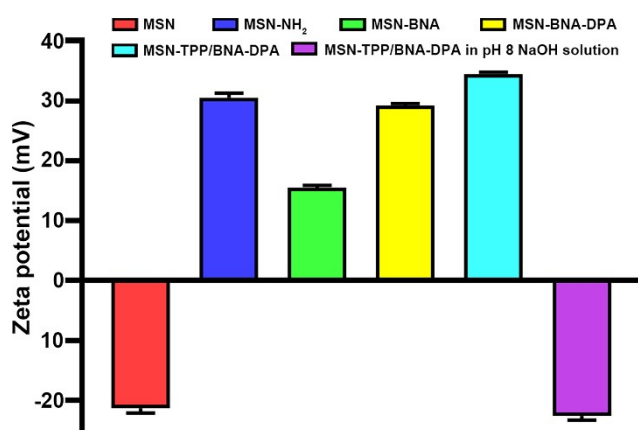


Fig. S1. Zeta potential of MSN, MSN-NH₂, MSN-BNA, MSN-BNA-DPA and MSN-TPP/BNA-DPA in deionized water and MSN-TPP/BNA-DPA in pH 8 NaOH solution.

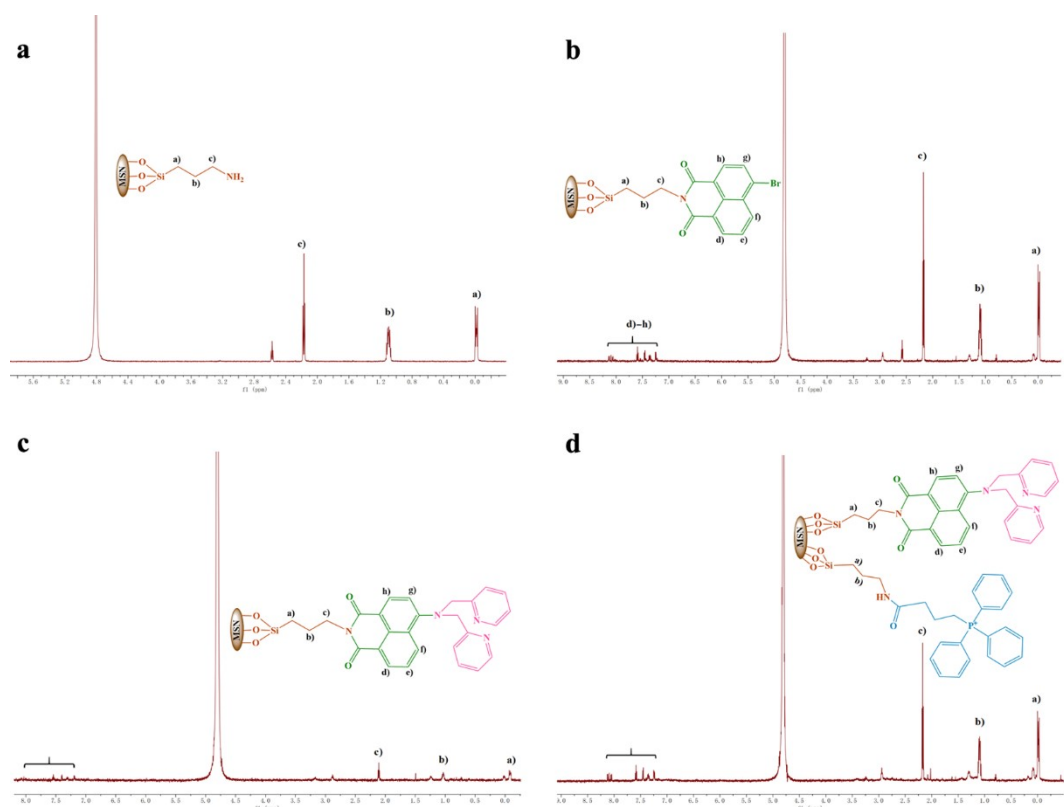


Fig. S2. Solution ^1H NMR (pH=13) with peaks assigned for the inset molecules. ^1H NMR spectra of (a) MSN-NH₂, (b) MSN-BNA, (c) MSN-BNA-DPA and (d) MSN-TPP/BNA-DPA.

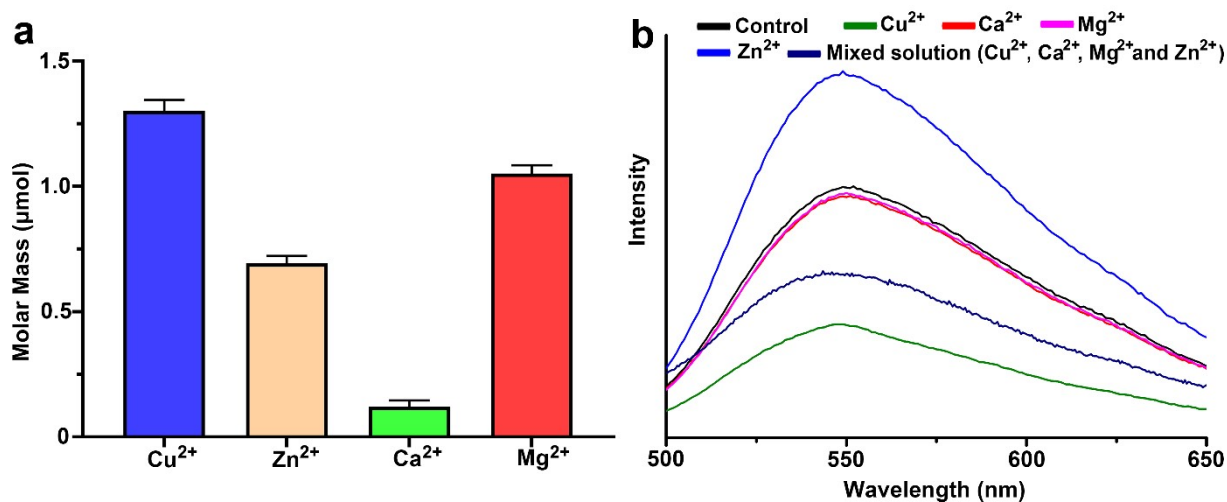


Fig. S3. (a) Depleting amount of various divalent metal cations by MSN-TPP/BNA-DPA (3 mg) after 24 h incubation with a mixed solution simultaneously containing 2 μmol of various divalent cations. (b) Fluorescence spectra of MSN-TPP/BNA-DPA suspension after mixing with various metal cations.

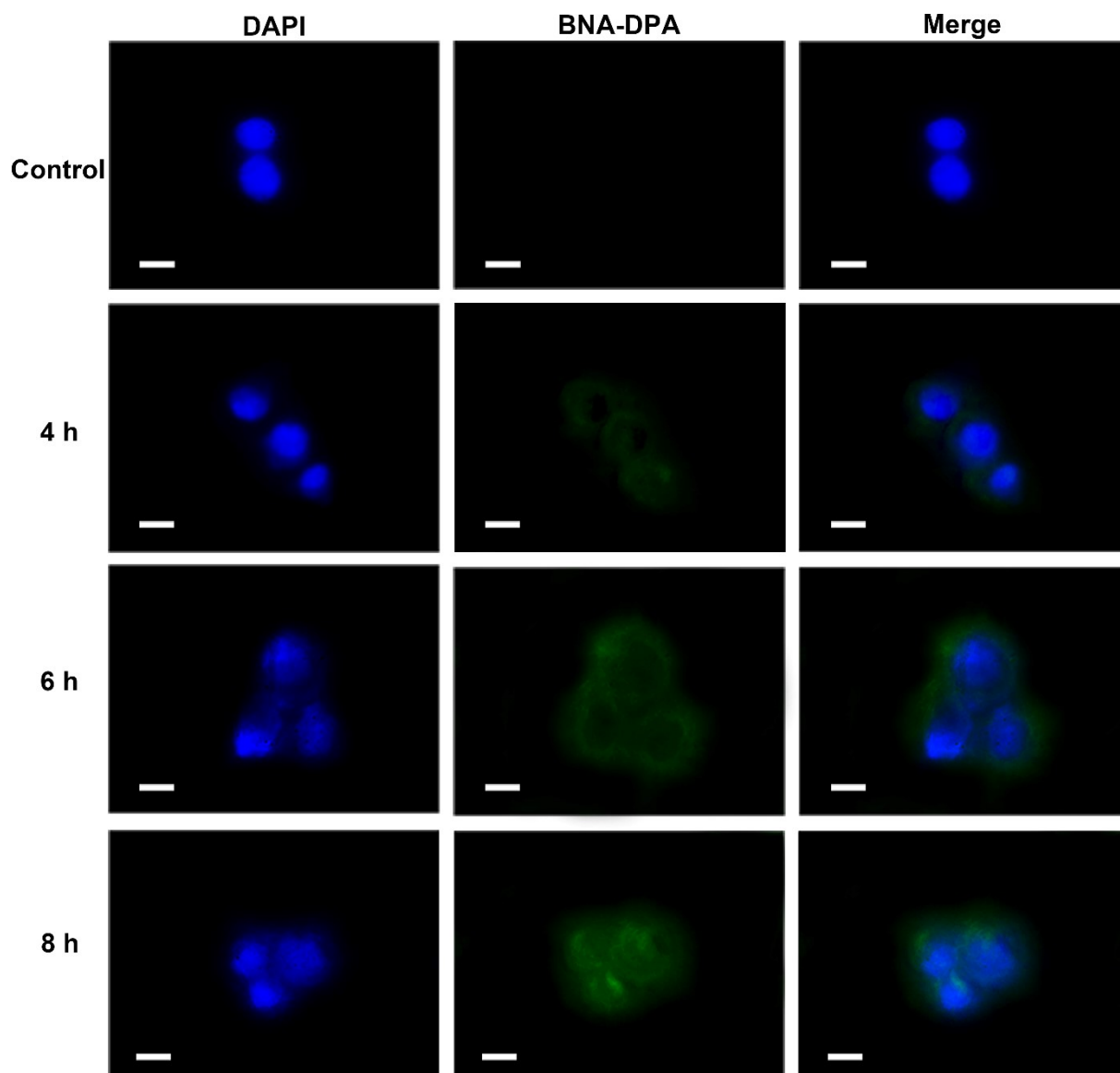


Fig. S4. Cellular uptake of MSN-TPP/BNA-DPA in 4T1 cells. Scale bar 20 μm .

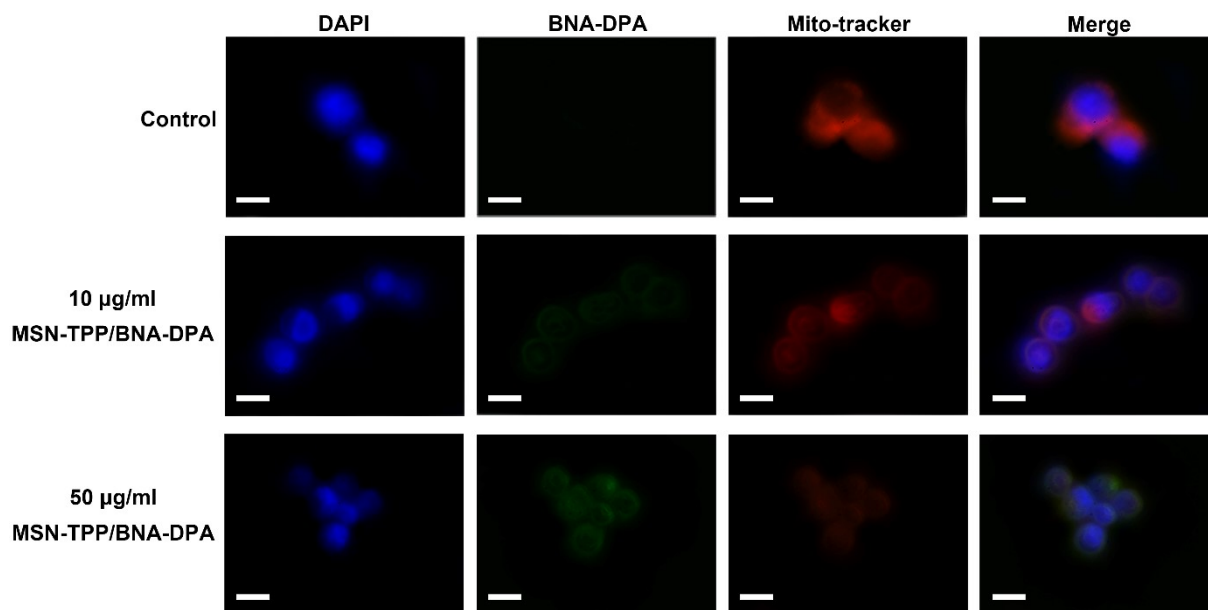


Fig. S5. Representative images of 4T1 cells after treatment with different concentrations of MSN-TPP/BNA-DPA to evaluate dose-dependent compromised mitochondrial membrane integrity. Scale bar 50 µm.

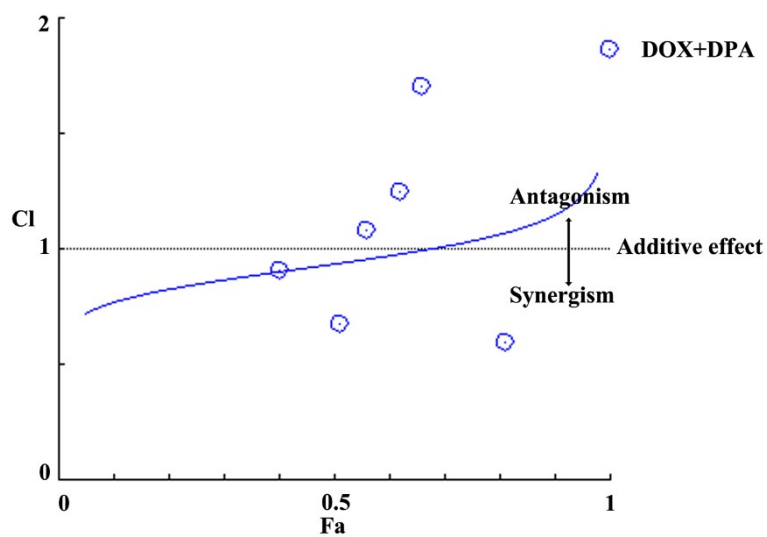


Fig. S6. Fa-CI plot (Chou-Talalay plot).

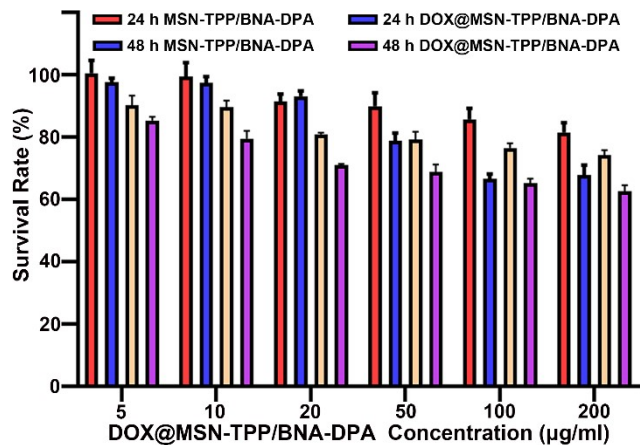


Fig. S7. Cell viability of movas cells after 24 and 48 h treatment with different concentration of MSN-TPP/BNA-DPA and DOX@MSN-TPP/BNA-DPA.

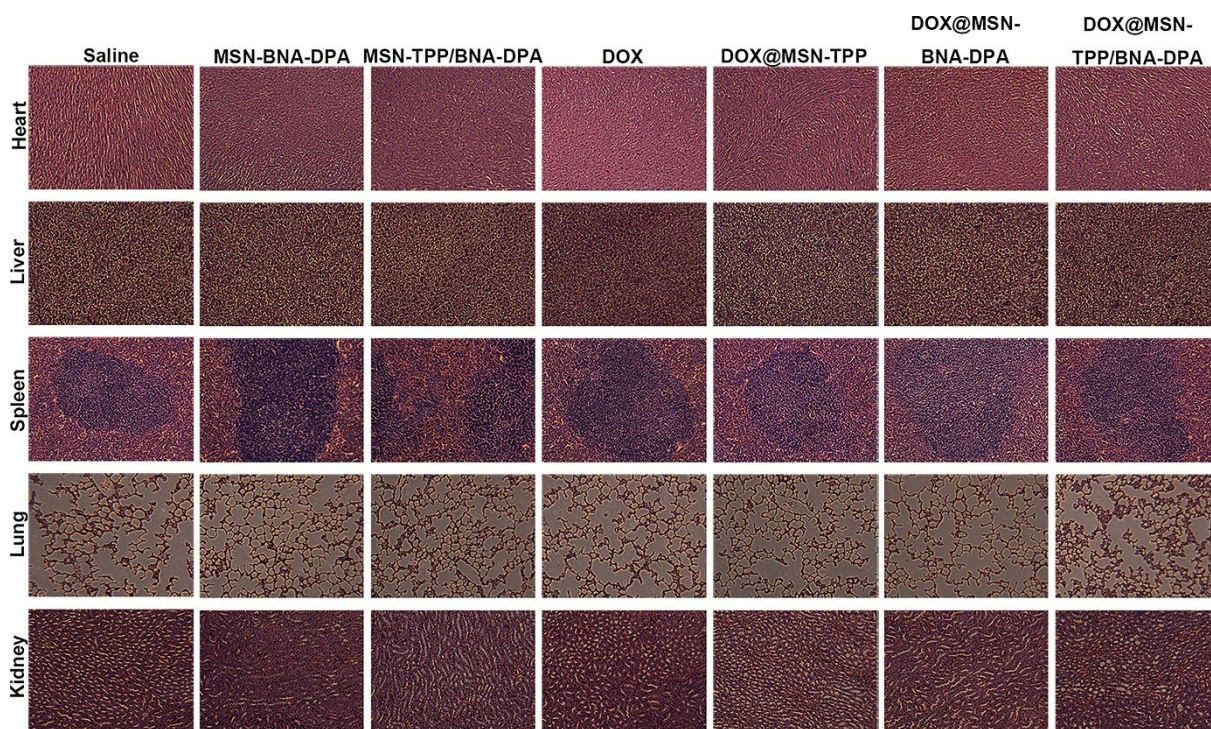


Fig. S8. H&E images of the major organs taken from 4T1 tumor-bearing mice on day 13 post-treatment with different formulations.

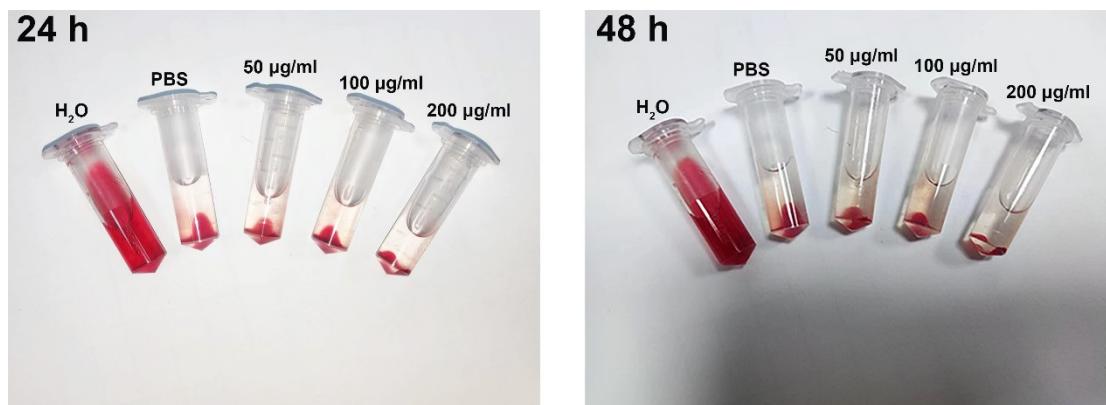


Fig. S9. Hemolysis assay of DOX@MSN-TPP/BNA-DPA after incubation with RBC suspension for 24 and 48 h.

Table S1. Quantitative measurement of intracellular copper levels of 4T1 cells after treatment with different nanoparticles (100 µg/ml) for 24 h.

4T1 cells	Copper content (ng/10 ⁵ cells)
Control	3.12 ± 0.88
MSN-TPP treatment	2.88 ± 0.88
MSN-BNA-DPA treatment	< 1
MSN-TPP/BNA-DPA treatment	< 1

Table S2. Quantitative measurement of intracellular copper levels of 4T1 cells after Cu²⁺ supplement and subsequent treatment with MSN-TPP/BNA-DPA (100 µg/ml) for 12 h.

4T1 cells	Copper content (µg/10 ⁶ cells)
2 µg/ml Cu ²⁺ supplement	2.92 ± 0.14
2 µg/ml Cu ²⁺ supplement + MSN-TPP/BNA-DPA treatment	2.82 ± 0.06
4 µg/ml Cu ²⁺ supplement	3.32 ± 0.30
4 µg/ml Cu ²⁺ supplement + MSN-TPP/BNA-DPA treatment	3.07 ± 0.15

Table S3. Dose-effect and combinational index (CI) values of DOX and DPA in DOX@MSN-TPP/BNA-DPA. CI < 1 shows the synergistic effects of the drugs whereas CI=1 shows additive and CI > 1 shows the antagonistic effect.

Drug	Dose ($\mu\text{g/ml}$)	Effect (Inhibition %)	CI value
DOX	0.45	0.33	
	0.90	0.45	
	2.24	0.51	
	4.48	0.6	
	8.96	0.64	
	17.92	0.76	
DPA	0.18	0.28	
	0.36	0.37	
	0.91	0.44	
	1.81	0.53	
	3.62	0.62	
	7.24	0.66	
DOX+DPA	0.45+0.18	0.40	0.91
	0.89+0.36	0.51	0.68
	2.24+0.91	0.56	1.08
	4.48+1.81	0.62	1.25
	8.96+3.62	0.66	1.70
	17.92+7.24	0.81	0.60

Table S4. Routine blood test data of mice on day 14 after intravenous injection of DOX@MSN-TPP/BNA-DPA (20 mg/kg) and saline.

Name	Control	20 mg/kg MSN-TPP/BNA-DPA	Unit
WBC	7.8 ± 0.7	7.1 ± 0.6	10 ⁹ /L
Lymph#	5.83 ± 0.97	5.43 ± 0.57	10 ⁹ /L
Mon#	0.23 ± 0.07	0.2 ± 0.01	10 ⁹ /L
Gran#	1.73 ± 0.23	1.47 ± 0.07	10 ⁹ /L
Lymph%	74.27 ± 5.53	76.77 ± 1.13	%
Mon%	3.07 ± 0.57	2.73 ± 0.23	%
Gran%	22.67 ± 4.97	20.5 ± 0.9	%
RBC	8.93 ± 0.02	9.30 ± 0.20	10 ¹² /L
HGB	130 ± 2	135.33 ± 4.3	g/L
HCT	45.7 ± 0.6	47.23 ± 1.23	%
MCV	51.23 ± 0.53	50.83 ± 0.23	fL
MCH	14.5 ± 0.2	14.5 ± 0.2	pg
MCHC	284 ± 2	285.67 ± 1.67	g/L
RDW	15.07 ± 0.57	15.1 ± 0.2	%
PLT	1043.67 ± 82.67	939.33 ± 181.33	10 ⁹ /L
MPV	5.2 ± 0.2	5.27 ± 0.13	fL
PDW	16.27 ± 0.17	16.37 ± 0.07	/
PCT	0.54 ± 0.03	0.495 ± 0.08	%