Supplementary Information

Enhance cellular uptake by non-endocytic pathway for tumor

therapy

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Figure S1. Hemolytic activity of various concentrations of P-DOX and P-DOX-CLIP6 on red blood cells after incubation for 3 h. (n=3)



Figure S2. Confocal laser scanning microscopy images of HeLa cells after incubation with P-DOX (A) and P-DOX-CLIP6 (B) for 4 h. Red: DOX. Blue: DAPI. A-B bottom: Cellular fluorescence intensity measured within the cytoplasm or nucleus.



Figure S3. Lactate dehydrogenase (LDH) released from HeLa cells exposure to various concentrations of free CLIP6 peptide solution for 24 h, and cells incubated with blank culture medium used as control (n=6). LDH assay was performed to analyze the cell membrane integrity of cells incubated with various concentrations of free CLIP6 peptide. HeLa cells were seeded into 96-well plates at a seeding intensity of 5×10^3 cells/well, and then incubated with various concentrations of free CLIP6 peptide. Then the LDH assay was carried out with LDH cytotoxicity assay kit (Beyotime institute of Biotechnology) according to the manufacturer's instruction. Cells treated with 0.1% (w/v) Triton X-100 was set as 100% LDH release control. The individual LDH release is the ratio of LDH release to the control. It was defined that LDH release lower than 10% was non-toxic.



Figure S4. *In vitro* cytotoxicity of various concentrations of free CLIP6 on HeLa cells for 24h.



Figure S5. Cell viability of HeLa cells after treatment for (A)48 h and (B) 72 h with P-DOX and P-DOX-CLIP6. (n=6)



Figure S6. Inhibition of HeLa tumor spheroids growth after the administration of free DOX, P-DOX and P-DOX-CLIP6 (DOX equivalent concentration 10 μ g/mL) (n=10).



Figure S7. Histological evaluation of main organs (heart, liver, spleen, lung and kidney) from HeLa xenograft model nude mice after treatment with saline, DOX, P-DOX and P-DOX-CLIP6. Tissues were stained with hematoxylin and eosin (200×).

Groups	WBC (10º/L)	RBC (10 ¹² /L)	PLT (10 ⁹ /L)	LY (%)	MO (%)	GR (%)
Saline	4.30 ± 0.61	8.40 ± 0.85	574.50 ± 98.69	43.75 ± 9.01	18.23 ± 4.38	44.50 ± 9.69
DOX	4.08 ± 0.61	8.56 ± 0.72	604.20 ± 95.17	39.43 ± 10.15	23.80 ± 3.48	36.77 ± 10.01
P-DOX	4.80 ± 1.28	8.60 ± 0.77	608.86 ± 251.05	39.89 ± 10.67	15.50 ± 2.34	44.61 ± 9.71
P-DOX-CLIP6	4.57 ± 0.76	8.60 ± 0.84	670.29 ± 124.27	39.38 ± 17.87	14.28 ± 2.79	50.48 ± 14.73

Table S1. Blood cell levels in BALB/c nude mice after treatment with different DOX formulations (n=6). Results are means \pm SD.

Abbreviations: RBC, red blood cells; WBC, white blood cells; PLT, platelets; MO, monocytes; LY, lymphocytes; GR, neutrophils.

Table S2. Serum biomarkers in BALB/c nude mice after treat	tment with different DOX
formulations (n=6). Results are means ± SD, * indicated P<0.	.05 versus other groups.

Groups	ALT(IU/L)	AST(IU/L)	BUN(mmol/L)	CREA(mmol/L)	CK(IU/L)	LDH(IU/L)
Saline	35.67 ± 3.70	141.17 ± 22.84	8.16 ± 0.75	16.83 ± 0.75	930.80 ± 121.71	1335.20 ± 189.38
DOX	43.00 ± 7.14	185.14 ± 23.22	6.71 ± 1.04	17.14 ± 2.91	1295.33 ± 94.52	1411.40 ± 123.63
P-DOX	34.57 ± 9.78	145.57 ± 35.55	7.20 ± 0.69	14.00 ± 1.29	1124.25 ± 114.48	1269.29 ± 230.85
P-DOX-	33.86 ± 2.41	109.43 ± 15.05	7.54 ± 1.07	14.29 ± 1.38	526.80±155.00*	899.20 ± 27.72*
CLIP6						

Abbreviations: ALT, alanine aminotransferase; AST, aspartate transaminase; BUN, urea nitrogen; CREA, creatinine; CK, creatine kinase; LDH, lactate dehydrogenase.

Groups	Body	Heart	Liver	Spleen	Lung	Double kidneys
Saline	21.77 ± 1.28	0.11 ± 0.01	1.49 ± 0.21	0.34 ± 0.06	0.15 ± 0.02	0.37 ± 0.04
DOX	20.57 ± 0.91	0.11 ± 0.01	1.44 ± 0.12	0.31 ± 0.06	0.16 ± 0.02	0.35 ± 0.02
P-DOX	19.64 ± 0.56	0.11 ± 0.01	1.40 ± 0.11	0.30 ± 0.07	0.14 ± 0.02	0.34 ± 0.02
P-DOX-CLIP6	19.21 ± 0.63	0.12 ± 0.02	1.40 ± 0.04	0.28 ± 0.03	0.14 ± 0.01	0.31 ± 0.01

Table S3. Main organ weights of BALB/c nude mice after treatment with different DOX formulations (n=6). Results are means \pm SD.

Groups	Heart	Liver	Spleen	Lung	Double kidneys
Saline	0.51 ± 0.07	6.85 ± 0.72	1.57 ± 0.29	0.70 ± 0.05	1.71 ± 0.14
DOX	0.51 ± 0.05	7.00 ± 0.50	1.53 ± 0.30	0.77 ± 0.09	1.72 ± 0.11
P-DOX	0.57 ± 0.06	7.12 ± 0.66	1.53 ± 0.40	0.71 ± 0.08	1.72 ± 0.14
P-CLIP6-DOX	0.60 ± 0.05	7.05 ± 0.20	1.50 ± 0.13	0.71 ± 0.08	1.71 ± 0.04

Table S4. Main organ coefficient of BALB/c nude mice after treatment with different DOX formulations (n=6). Results are means \pm SD.