Supplementary Information

Fusion peptide functionalized hybrid nanoparticles for synergistic drug delivery to reverse cancer drug resistance

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Determination of drug encapsulation efficiency and drug loading content

The concentration of non-encapsulated DOX in water was determined by a fluorescence spectrophotometer (Shimadzu RF-5301PC) with an excitation wavelength at 485 nm. The concentration of encapsulated CXB (dissolved in methanol) was determined by the absorbance at 255 nm using a UV−vis spectrophotometer (PerkinElmer Lambda Bio 40). The drug loading content and encapsulation efficiency were calculated as follows:

loading content = $(W_T - W_F) / W_{NP} \times 100\%$

where W_T is the total weight of drug fed, W_F is the weight of non-encapsulated free drug, and W_{NP} is the weight of nanoparticles.

encapsulation efficiency = $(W_T - W_F) / W_T \times 100\%$

where W_T is the total weight of drug fed and W_F is the weight of non-encapsulated free drug.

Evaluation of targeting property of drug loaded nanoparticles

MCF-7 cells in 1 ml of culture medium containing 10% FBS were seeded in the well (1×10^5 cells per well) of a 6-well plate and incubated at 37 °C for 24 h. For the cells without pretreatment, the cells were then co-incubated with DOX loaded nanoparticles (DOX@NP or DOX@PNP with a DOX concentration of 1 μg/ml). After co-incubation for 4 h at 37 °C, the medium was removed, and the cells were washed several times by PBS. The cell nuclei were stained with Hoechst 33258 solution for 20 min at 37 °C. Subsequently, the cells were washed with PBS for three times, and then incubated in 1 ml of PBS and observed by a confocal laser scanning microscope (PerkinElmer UltraVIEW VoX). For the cell with the pretreatment by free

biotin or free peptide, the cells were treated by free biotin or free peptide with a concentration of 100 μg/ml for 1 h and then the medium was removed. After that, the cells were co-incubated with DOX loaded nanoparticles for 4 h and then observed by the confocal laser scanning microscope.

Table S1. Drug encapsulation efficiency and drug loading content of mono-drug loaded nanoparticles and dual-drug loaded nanoparticles.

Sample	Drug	Encapsulation efficiency (%)	Drug loading content (wt %)
DOX@NP	DOX	93.5	1.4
DOX@PNP	DOX	93.9	1.4
DOX/CXB@PNP	DOX	91.7	1.3
DOX/CXB@PNP	CXB	87.7	5.0

For the drug loaded nanoparticles, the DOX fed amount was 1 μg, and the CXB fed amount was 4 μg.

Table S2. IC_{50} (half maximal inhibitory concentration) of DOX for different treatments.

Agent	IC_{50} of DOX (μg/ml)	
	MCF-7/ADR	MCF-7
Free DOX	~100	0.6
Free DOX+CXB	~10	0.1-0.2
DOX@NP	8	<0.1
DOX@PNP	4	<0.1
DOX/CXB@PNP	2	<0.1

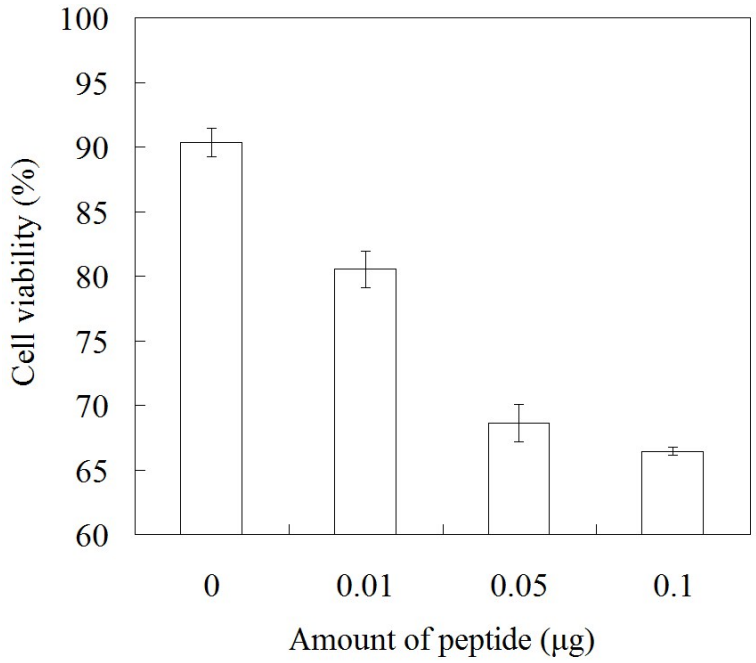

Figure S1. Cell viability of MCF-7/ADR cells after being treated by DOX@PNP with different peptide amounts for 48 h. The DOX concentration was 1 μg/ml.

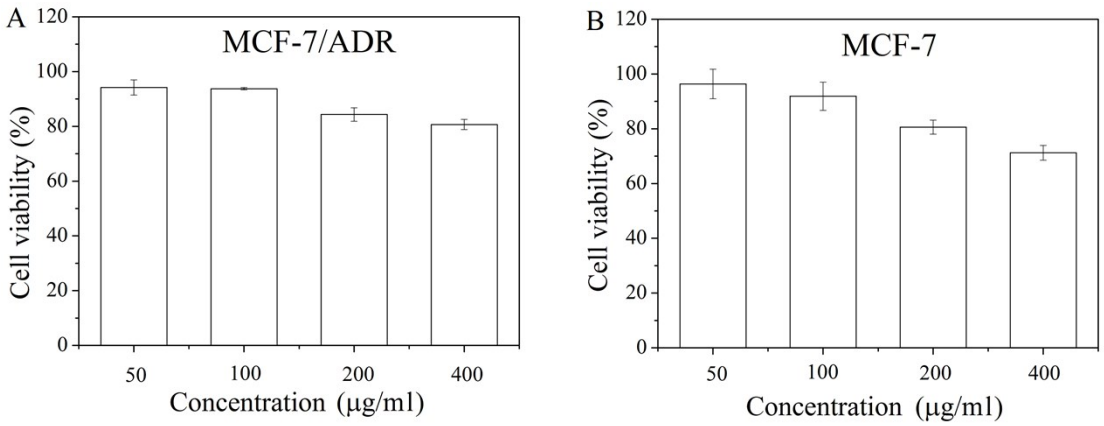

Figure S2. Cell viability of (A) MCF-7/ADR cells and (B) MCF-7 cells after being treated by blank PNP for 48 h.

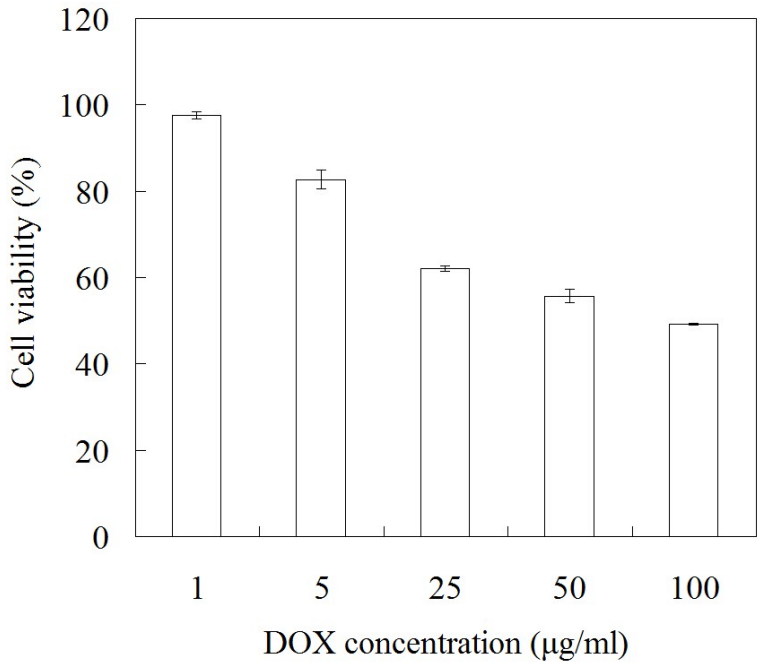

Figure S3. Cell viability of MCF-7/ADR cells after being treated by free DOX for 48 h.

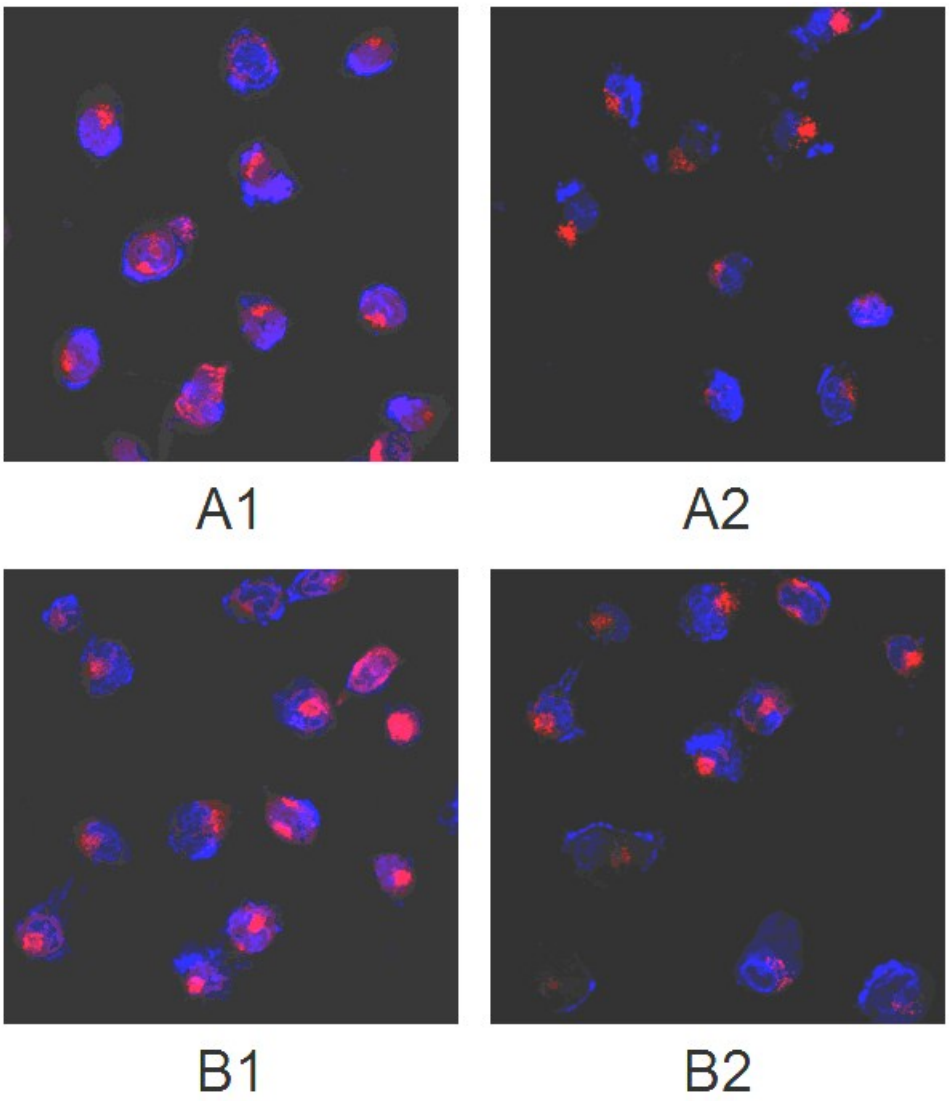

Figure S4. Confical images of MCF-7 cells after being treated by drug loaded nanoparticles for 4 h. (A1) DOX@NP without pretreatment, (A2) DOX@NP with the pretreatment of free biotin, (B1) DOX@PNP without pretreatment, (B2) DOX@PNP with the pretreatment of free peptide.

As shown in Figure S4, the enhanced cell uptake mediated the targeting groups can be obviously suppressed after the addition of free biotin (by comparing A1 and A2) and free peptide (by comparing B1 and B2) due to the pre-saturation of biotin and peptide receptors, respectively.