Supporting Information

A drug-self-gated and tumor microenvironment-responsive mesoporous silica vehicle: "four-in-one" versatile nanomedicine for targeted multidrug-resistant cancer therapy

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Materials

Tetraethylorthosilicate (TEOS), hexadecyltrimethyl ammonium bromide (C_{16}TAB), 3-aminopropyltriethoxysilane (APTES), 4-formylbenzoic acid, 4-methylbenzoic acid, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl), N-hydroxysuccinimide (NHS), amino-terminated poly(ethylene glycol) (PEG-NH_2), amino-terminated poly (ethylene glycol)-folic acid (NH_2-PEG-FA), MTT, dimethyl sulfoxide (DMSO), and methanol were all purchased from Sigma-Aldrich (St.Louis,MO,USA). Doxorubicin hydrochloride (DOX) was bought from Dalian Meilun Biology Technology Co., Ltd. (Dalian, China). Sodium hydroxide (NaOH) was purchased from Aladdin Industrial Co., Ltd. (Shanghai, China). All other chemicals and reagents of the highest quality were commercially available and used as received. Human breast cancer cell line MCF-7 and drug-resistant human breast cancer cell line MCF-7/ADR were from American Type Culture Collection (ATCC, Rockville, MD). P-gp siRNA (5'-CGGAAGGCCUAAUGCCGAATT-3'), scrambled control for P-gp siRNA and fluorescence FAM labeled siRNA (FAM-siRNA) were obtained from GenePharma Co., Ltd. (Suzhou, Jiangsu, China). Anti-Pgp antibody was purchased from Abcam (Cambridge, MA).

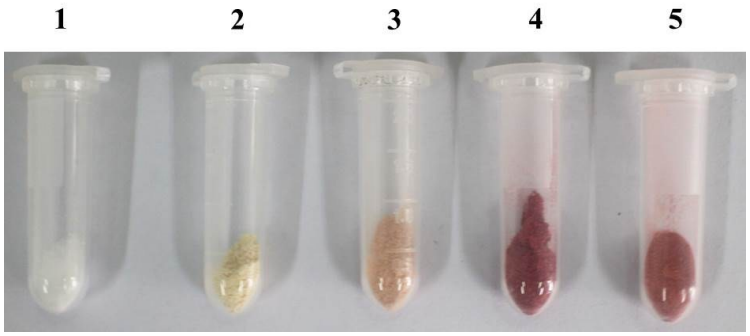

Figure S1. Photos of various samples. 1: MSNs, 2: MSNs-CHO, 3: MSNs-DOX (MSNs-CHO:DOX = 1:0.5, m/m), 4: MSNs@DOX (MSNs-CHO:DOX = 1:0.5, m/m), 5: MSNs@DOX-PEG-FA.

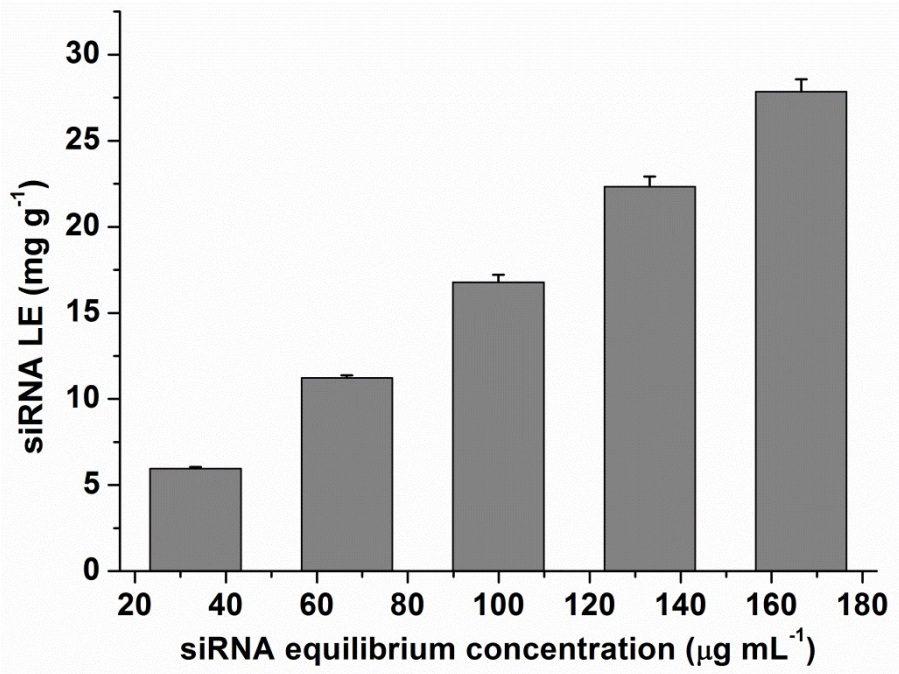

Figure S2. The siRNA adsorption capacity of MSNs-CHO without template (CTAB) in pores under various siRNA concentrations, LE = loading efficiency, n=3, the concentration of MSNs-CHO was 5 mg mL^{-1}.

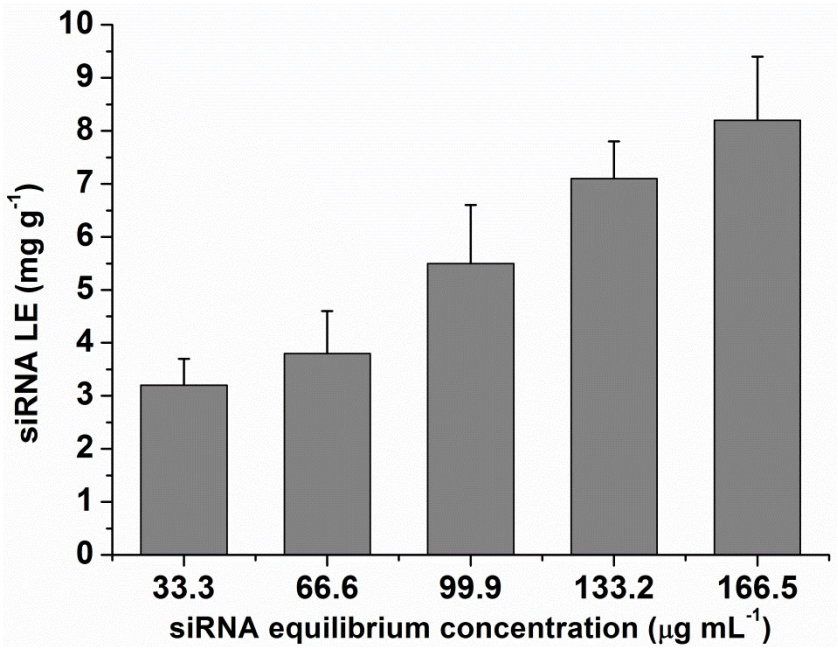

Figure S3. The siRNA adsorption capacity of MSNs-CHO with template (CTAB) in pores under various siRNA concentrations, LE = loading efficiency, n=3, the concentration of MSNs-CHO was 5 mg mL^{-1}.

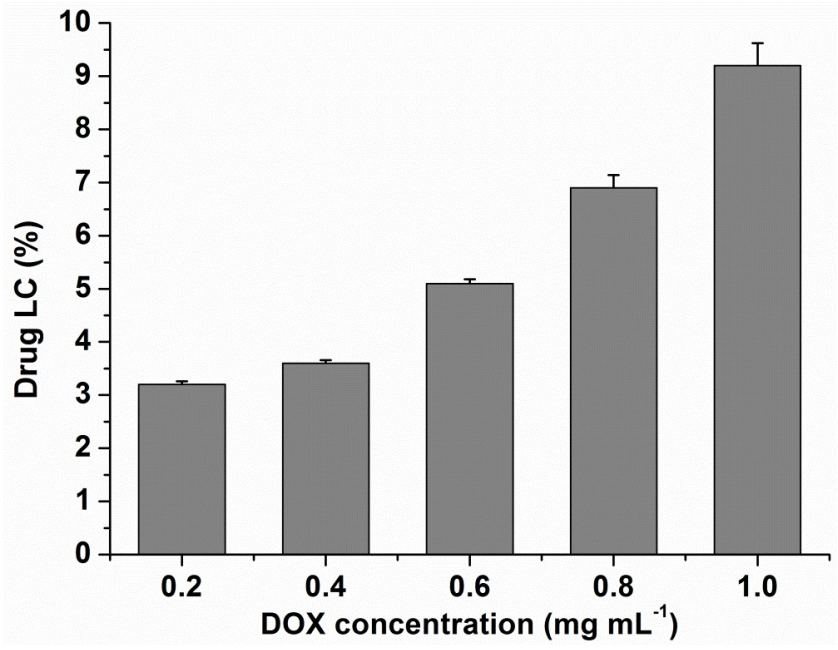

Figure S4. The DOX loading capacity of MSNs-CHO under various DOX concentrations, LC = loading content, n=3, the concentration of MSNs-CHO was 5 mg mL^{-1}.

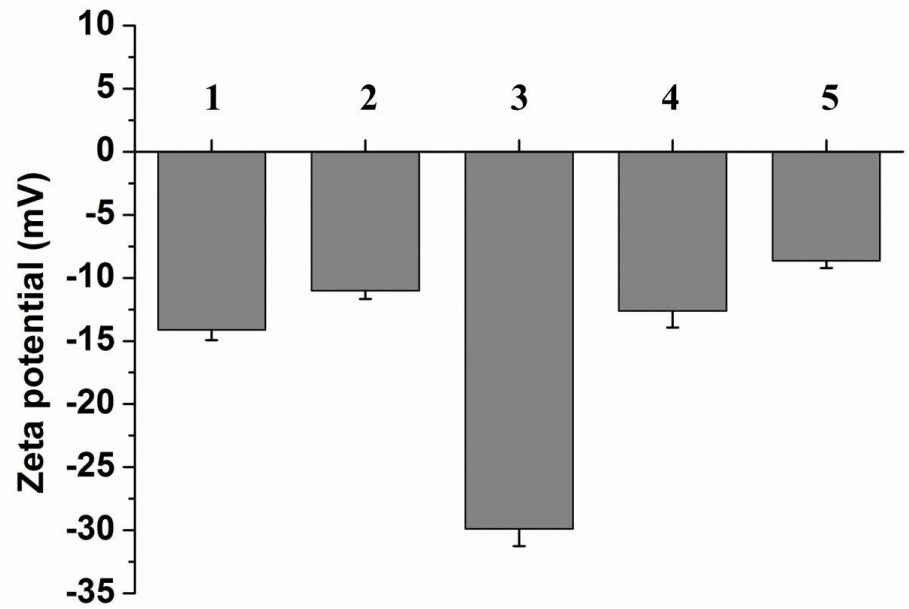

Figure S5. Zeta potential of various samples. 1: MSNs, 2: MSNs-CHO, 3: MSNs-siRNA 4: MSNs-siRNA@DOX and 5: MSNs-siRNA@DOX-PEG-FA.

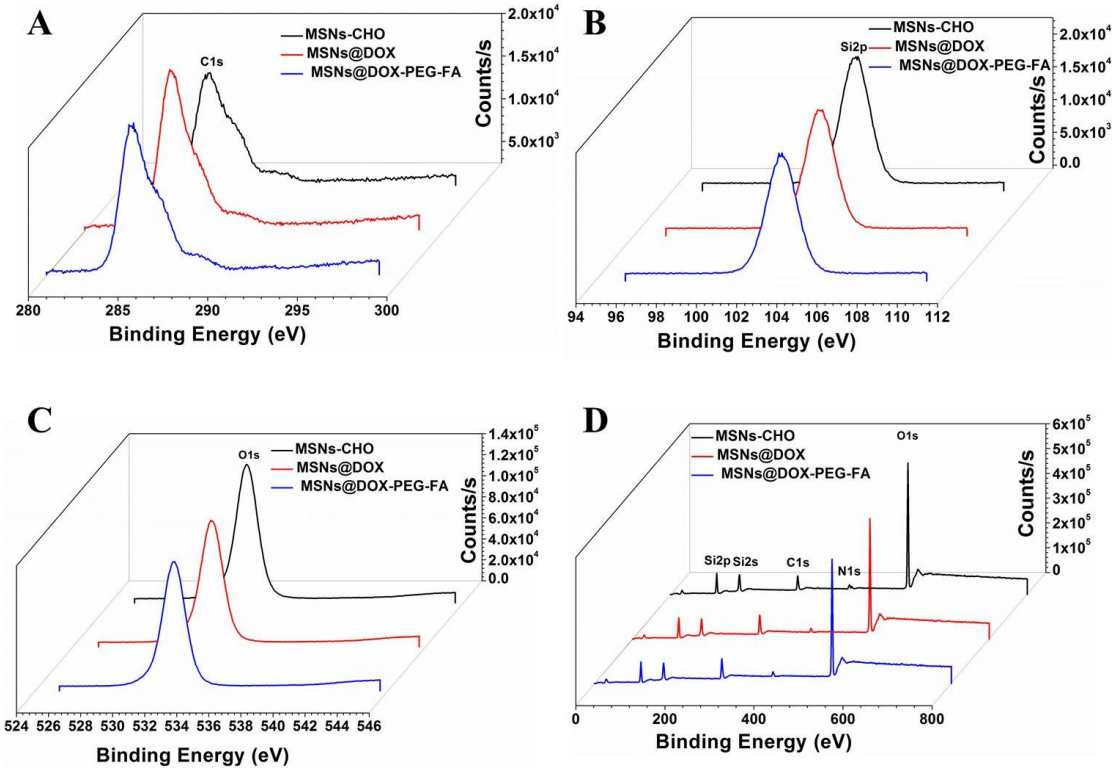

Figure S6. XPS spectra of MSNs-CHO, MSNs@DOX, and MSNs@DOX-PEG-FA: (A) Si2p spectrum, (B) O1s spectrum, (C) C1s spectrum, and (D) survey.

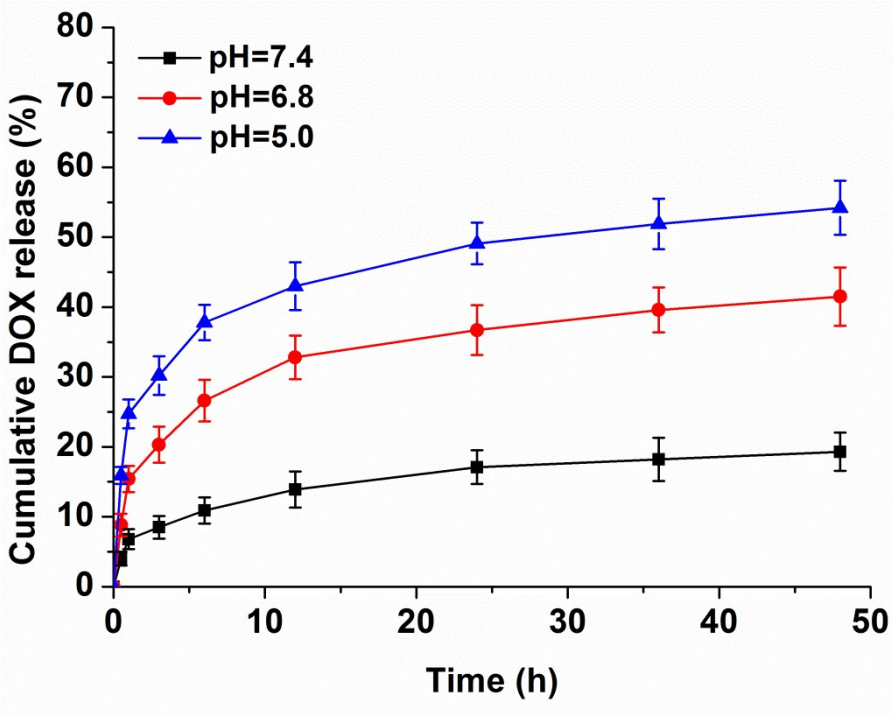

Figure S7. Drug release profile of MSNs-siRNA@DOX under different pH values.

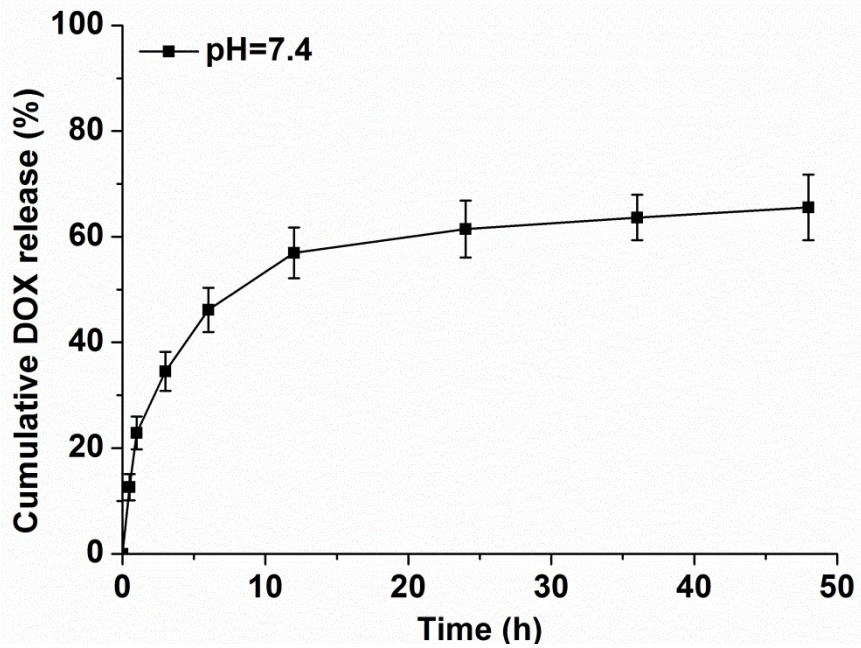

Figure S8. Drug release profile of MSNs-CH_3-siRNA@DOX at pH 7.4.

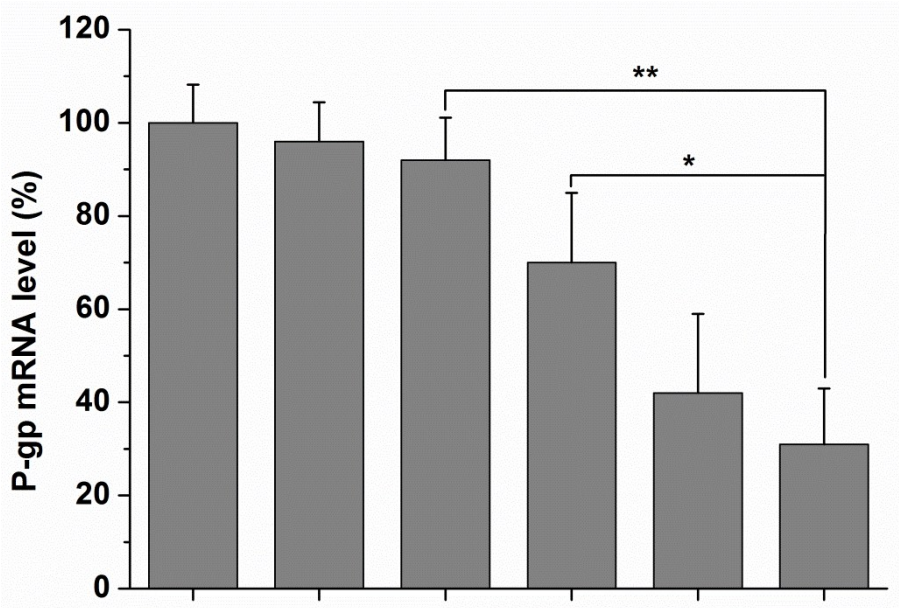

Figure S9. The relative expression level of P-gp mRNA determined by real-time PCR

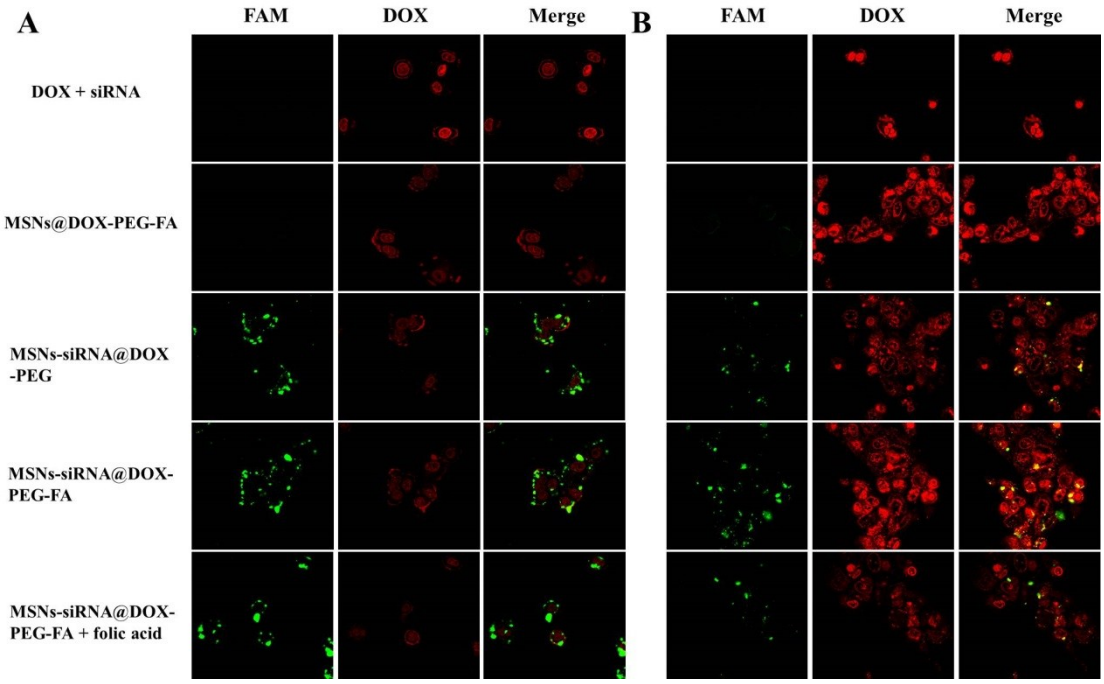

Figure S10. Confocal laser scanning microscopy images of MCF-7 cells after (A) 2 h and (B) 36 h-incubation. Green color: FAM-labeled siRNA. Red color: DOX.

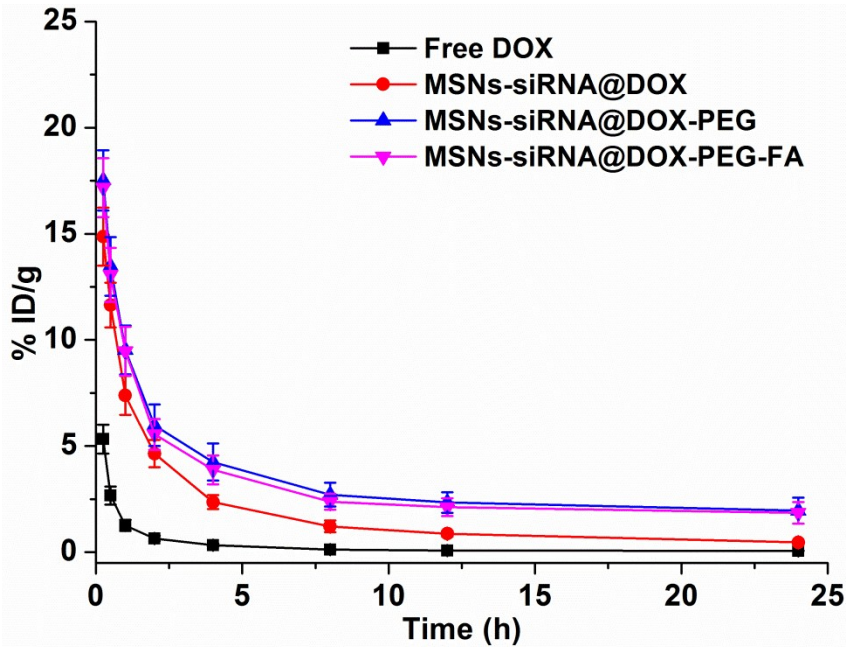

Figure S11. In vivo pharmacokinetics of DOX in blood after Free DOX, MSNs-siRNA@DOX, MSNs-siRNA@DOX-PEG and MSNs-siRNA@DOX-PEG-FA were intravenously injected into the MCF-7/ADR tumor-bearing mice through the tail vein at a DOX dose of 5 mg/kg.

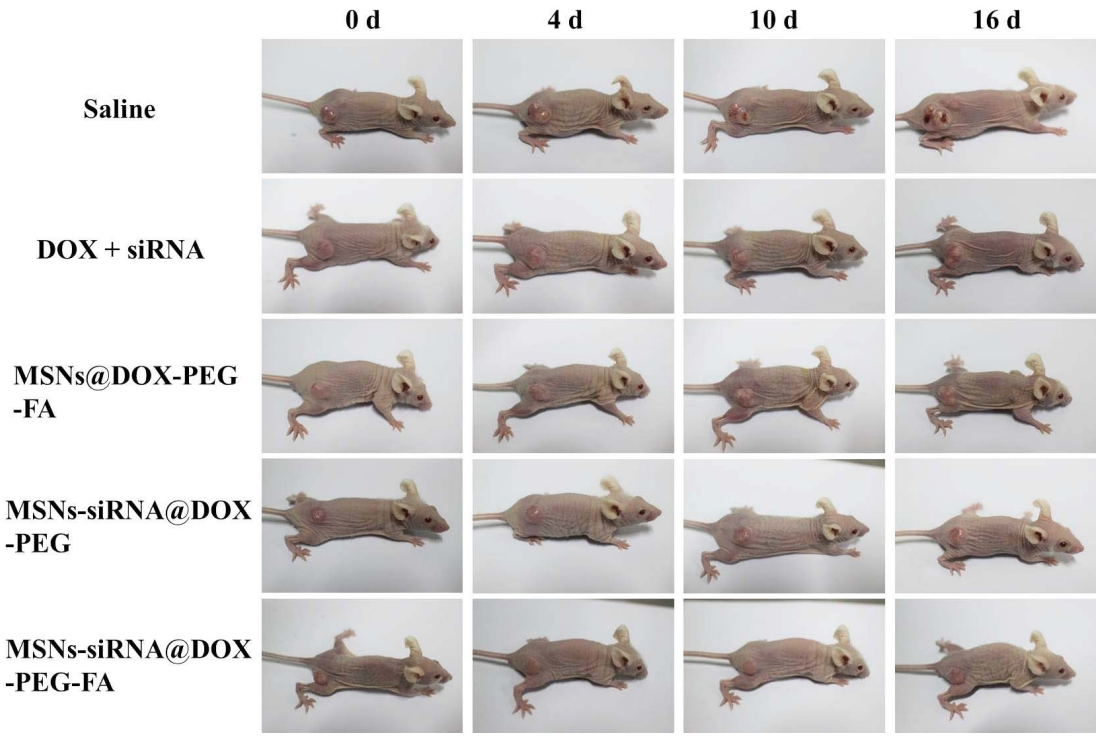

Figure S12. Representative images of mice bearing MCF-7/ADR tumors after different treatments for varied time periods.

Table S1. Size and zeta potential of prepared nanoparticles

Polymer	Size(nm)	PDI	ZP(mV)
MSNs	142.8 ± 8.6	0.180	-14.1 ± 0.82
MSNs-CHO	149.1 ± 7.3	0.227	-11.0 ± 0.66
MSNs-siRNA	145.4 ± 6.6	0.205	-29.9 ± 1.35
MSNs-siRNA@DOX	161.1 ± 9.5	0.198	-12.6 ± 1.32
MSNs-siRNA@DOX-PEG-FA	169.6 ± 11.2	0.233	-8.65 ± 0.57

PDI = polydispersity index, ZP = zeta potential, LC = loading content, n=3

Table S2. Relative contents of elements in various NPs

Polymer	Relative contents of elements (%)			
	C1s	N1s	O1s	Si2p
MSNs-CHO	19.65	5.33	52.34	22.67
MSNs-siRNA@DOX	25.03	4.43	48.73	21.81
MSNs-siRNA@DOX-PEG-FA	25.02	4.33	48.95	21.69

Table S3. BET and BJH measurements of prepared NPs

Polymer	BET surface area (m^2/g)	Pore volume[a] (cm^3/g)	Pore size[b] (nm)
MSNs-CHO	526.82	0.78	2.26
MSNs-siRNA	364.17	0.42	1.97
MSNs-siRNA@DOX	69.48	0.33	N/A
MSNs-siRNA@DOX-PEG-FA	37.18	0.29	N/A

[a] BJH cumulative pore volume for pores between 1.7 and 300 nm in width.

[b] The most probable pore size.

N/A = Not Applicable

Table S4 IC_{50} values of various samples on MCF-7 cells after 24 h- and 48 h-incubation

Incubation time (h)	IC_{50} (μg/ml)					
	DOX	siRNA	DOX + siRNA	MSNs@DOX-PEG -FA	MSNs-siRNA@DOX -FA	MSNs-siRNA@DOX -PEG-FA
24	4.65 ± 0.82	N/A	4.87 ± 0.66	4.02 ± 0.75	4.96 ± 0.80	3.76 ± 0.48
48	1.72 ± 0.23	N/A	1.87 ± 0.35	1.78 ± 0.27	2.31 ± 0.33	1.81 ± 0.21

N/A = Not Applicable

Table S5 IC_{50} values of various samples on MCF-7/ADR cells after 24 h- and 48 h-incubation

Incubation time (h)	IC_{50} (μg/ml)					
	DOX	siRNA	DOX + siRNA	MSNs@DOX-PEG -FA	MSNs-siRNA@DOX -FA	MSNs-siRNA@DOX -PEG-FA
24	>500	N/A	>500	25.1 ± 2.25	31.4 ± 2.86	21.0 ± 1.98
48	>500	N/A	92.4 ± 7.56	23.5 ± 1.97	15.9 ± 1.66	5.48 ± 0.32

N/A = Not Applicable