Electronic supplementary information (ESI)

Polymeric nanovesicle as simultaneous delivery platform with Doxorubicin conjugation and Elacridar encapsulation for enhanced treatment of multidrug-resistant breast cancer

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Synthesis of methoxy-poly(ethylene glycol)-poly(β-benzyl-L-aspartate) (mPEG-PBLA)

Firstly, ε-benzyloxycarbonyl-L-aspartic acid N-carboxyanhydride (BLA-NCA) was synthesized as previously reported.[1] Then the diblock polymer mPEG-PBLA was prepared by ring-opening polymerization of BLA-NCA according to our previous literature.[2] Briefly, mPEG-NH_2 (0.33 g, 0.33 mmol) was vacuum dried at 70 ℃ for 2 h and dissolved in 50 mL of anhydrous CH_2Cl_2. Then, BLA-NCA (2.49 g, 10 mmol) dissolved in anhydrous DMF (5.0 mL) was added into the solution under N_2 atmosphere and the mixture was stirred at 35 ℃ for 48 h. mPEG-PBLA was purified by precipitation in diethyl ether (1 L) for three times. The product was obtained as a white sticky solid after vacuum drying. (Yield: 85.64%, Mn = 7150 Da calculated from ^{1}H NMR analysis)

Synthesis of methoxy-poly(ethylene glycol)-poly(N-(6-hydroxyhexyl)-L-aspartamide-β-benzyl-L-aspartate) (mPEG-P[Asp(HPA)-BLA])

Ammonolysis reaction of mPEG-PBLA was conducted as reported before.[3] Briefly, mPEG-PBLA (0.80 g, 0.12 mmol) was dissolved in anhydrous DMF (10 mL), then

5-amino-1-pentanol (0.5 equiv. to the residual benzyl ester groups in mPEG-PBLA) was added into the solution and the reaction was kept at 35 ℃ for 24 h. The mixture solution was dialyzed (molecular weight cut off (MWCO): 3.5 kDa) against methanol for 24 h and precipitated in diethyl ether twice to obtain the polymer mPEG-P[Asp(HPA)-BLA]. (Yield: 54.28%, Mn = 7075 Da calculated from ^{1}H NMR analysis)

Synthesis of 3-(4-methyl-2,5-dioxo-2,5-dihydrofuran-3-yl) propanoic acid (CDM) conjugated polymer (mPEG-P[Asp(HPA-*g*-CDM)-BLA])

The chlorine-substituted CDM was synthesized according to reported methods.[4-6] Briefly, 3-(4-methyl-2,5-dioxo-2,5-dihydrofuran-3-yl) propanoic acid (0.276 g, 1.5 mmol) was dissolved in 10 mL dry dichloromethane, and then oxalyl chloride (0.378 g, 3 mmol) and a catalytic amount of DMF (40 μL) were added on an ice-cold bath under N_2 protection. The solution was stirred for 15 minutes at 0 ℃, and then transferred to room temperature for an additional 1 h. The solvent was rotary evaporated and then the residue was re-dissolved in 20 mL CH_2Cl_2 and repeat the evaporation procedure for three times to remove excess oxalyl chloride. The chlorine-substituted CDM was obtained as a yellowish oil and used directly without further purification.

Then the chlorine-substituted CDM (200 mg, 0.99 mmol) was reacted with mPEG-P[Asp(HPA)-BLA] (400 mg, 5.7 μmol) in 5 mL dry dichloromethane with 30 μL of fresh distilled TEA as acid-capturer. The reaction was proceeded at 35 ℃ for 24 h under N_2 atmosphere and the solution was then precipitated in diethyl ether twice. The product was collected by centrifugation and vacuum dried to obtain mPEG-P[Asp(HPA-*g*-CDM)-BLA]. (Yield: 82.86%, Mn = 9560 Da calculated from ^{1}H NMR analysis)

Conjugation of DOX to the polymer

50 mg DOX·HCl, 10 μL fresh distilled TEA and 200 mg polymer were dissolved in 4 mL anhydrous DMF and added into a 10 mL Schlenk-tube under N_2 atmosphere. The

mixture solution was stirred at 35 ℃ for 24 h under dark condition. Subsequently, the mixture solution was dialyzed (MWCO: 3.5 kDa) against DMF to remove excess DOX and TEA, followed by precipitated in diethyl ether. The final product was obtained as a dark-red solid by centrifugation and vacuum drying. (Yield: 63.57%, Mn = 13600 Da calculated from ^{1}H NMR analysis)

Polymer Characterization

^{1}H NMR spectra were carried out on a Bruker 400 MHz spectrometer and DMSO-d_6 was used as solvent. UV-Vis spectral measurements were recorded using Unico UV-2000 UV-Vis spectrophotometer. The fluorescence spectra were measured on a spectrofluorophotometer (Perkin Elmer Ltd., United Kingdom). The molecular weight distribution of polymers was analyzed using a gel permeation chromatography (GPC) system consisting of a Waters 1515 pump, an Ultrahydrogel^{TM} 500 column, an Ultrahydrogel^{TM} 250 column, and a Waters 2417 differential refractive index detector with PEG as a calibration standard. DMF containing LiBr (1.0 g/L) was used as an eluent at a flow rate of 1.0 mL/min.

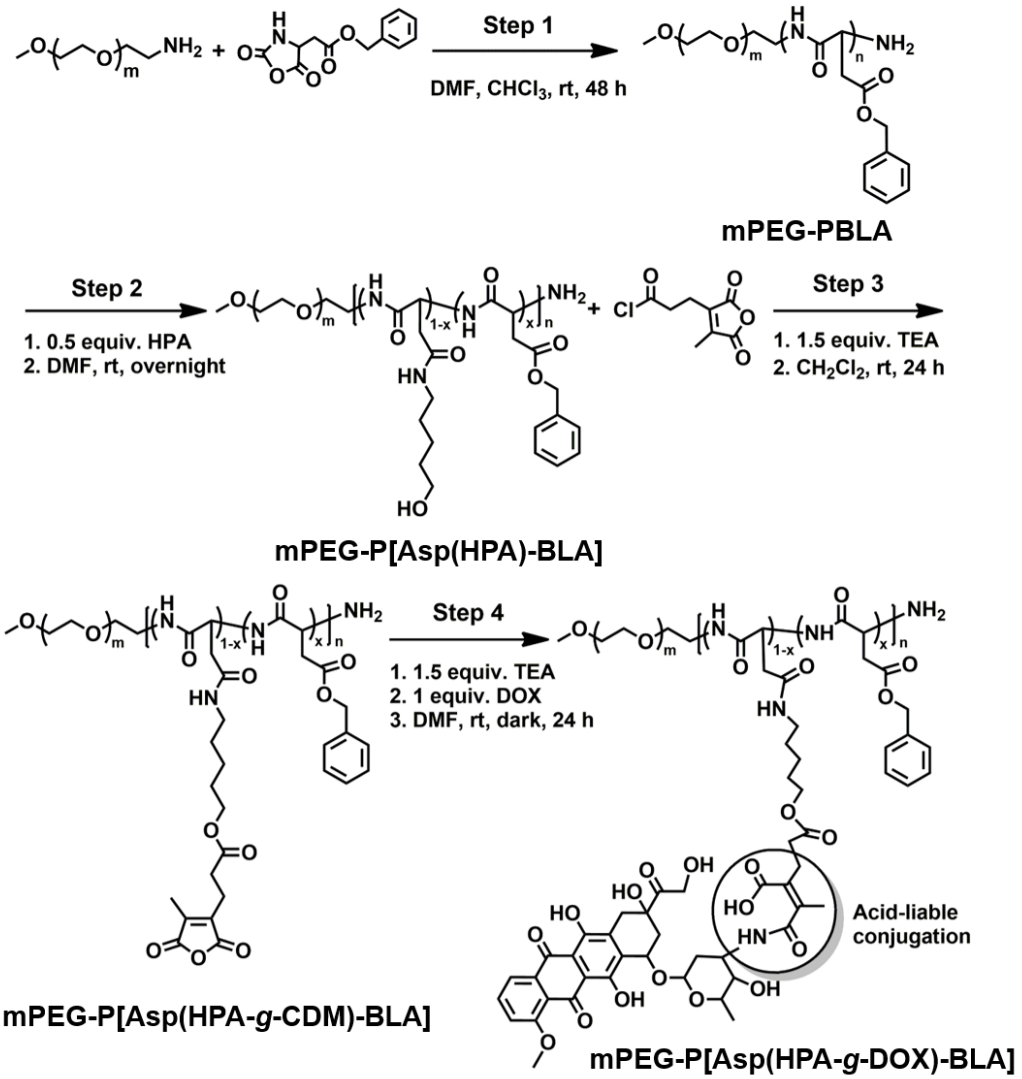

Fig. S1 Schematic illustration of the synthetic approaches for the acid-liable DOX-conjugated polymer.

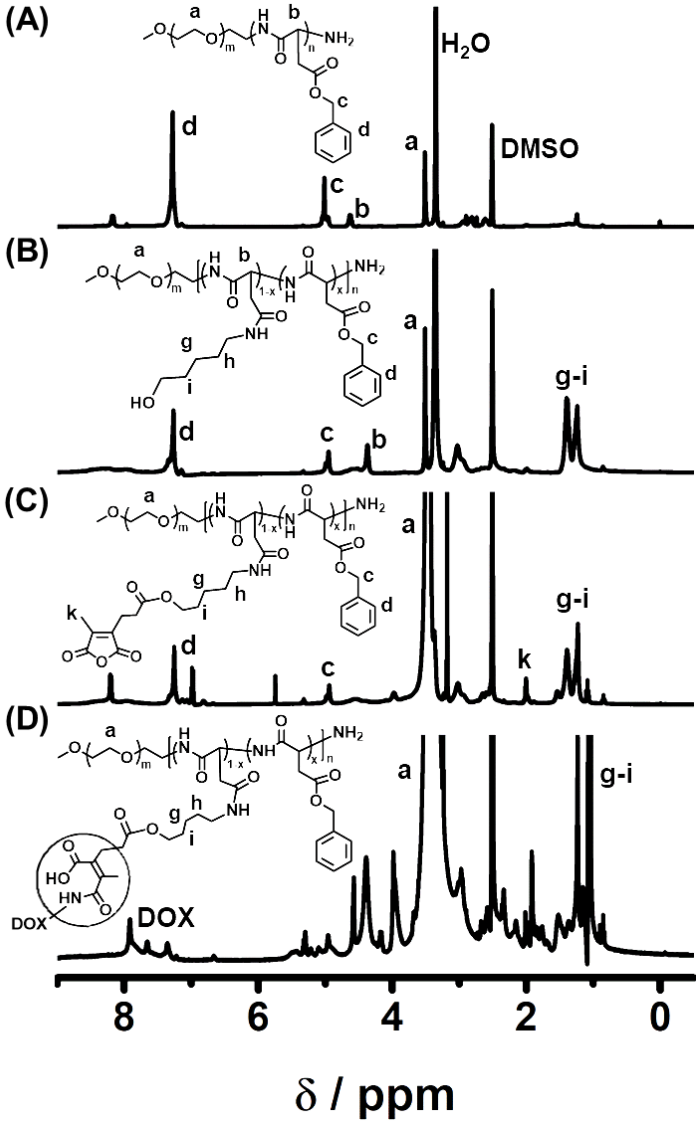

Fig. S2 ^{1}H NMR spectra of the block polymers in DMSO-d_6: (A) PEG-PBLA, (B) mPEG-P[Asp(HPA)-BLA], (C) mPEG-P[Asp(HPA-*g*-CDM)-BLA], (D) mPEG-P[Asp(HPA-*g*-DOX)-BLA].

Table S1. Characteristics of block copolymers

Polymer	M_w[a] (kDa)	M_w[b] (kDa)	M_w/M_n[b]
mPEG-P[Asp(HPA)-BLA]	7.0	8.5	1.21
mPEG-P[Asp(HPA-*g*-DOX)-BLA]	13.6	19.2	1.41

[a] calculated by ^{1}H NMR; [b] calculated by GPC.

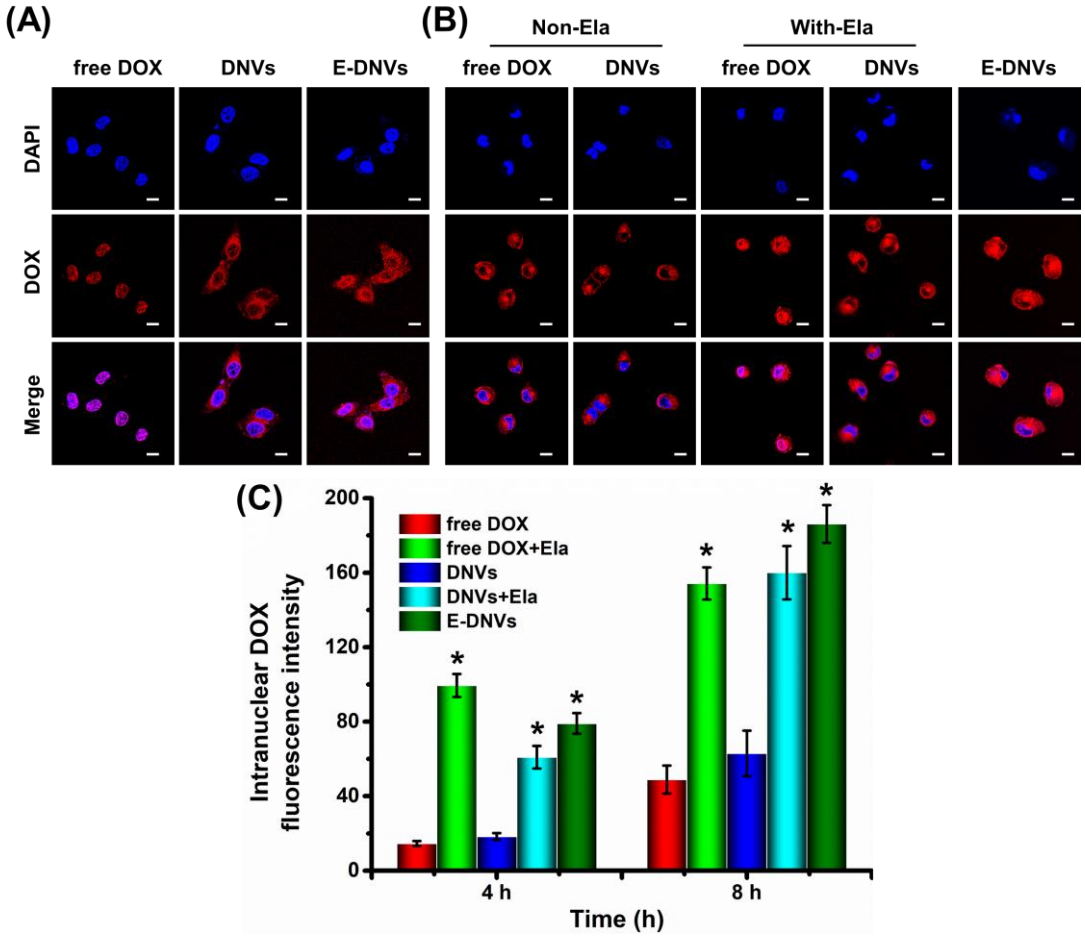

Fig. S3 (A) Confocal laser scanning microscopic (CLSM) images of MCF-7 (A) and MCF-7/ADR (B) cells incubated with free DOX, DNVs and E-DNVs at 10 μg/mL DOX concentration for 4 h. MCF-7/ADR cells were pre-treated with Ela for 12 h before transfection in With-Ela groups. Red fluorescence: DOX; blue fluorescence: nuclei stained with DAPI. The scale bars represent 20 μm. DOX concentration if applied: 10 μg/mL; Ela concentration if applied: 0.5 μg/mL. (C) Statistical data of intranuclear DOX fluorescence intensity in MCF-7/ADR cells after receiving the treatment of free DOX, free DOX + Ela, DNVs, DNVs + Ela and E-DNVs for 4 h and 8 h, respectively. *$P < 0.05$ *vs.* free DOX and DNVs at the same incubation time.

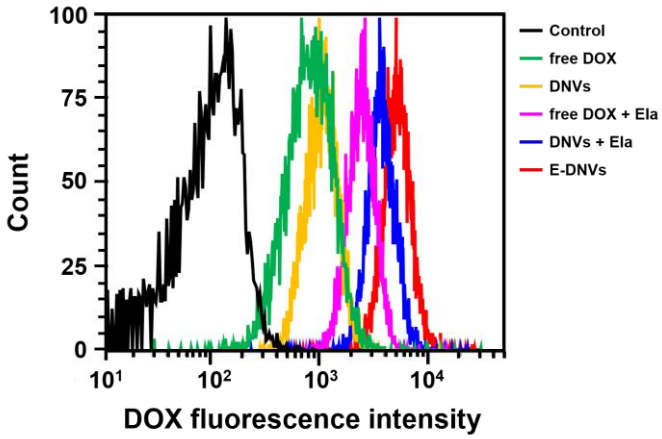

Fig. S4 The cell uptake of DOX in MCF-7/ADR cells detected using flow cytometry assay. MCF-7/ADR cells were co-cultured with free DOX, D-NVs, free DOX + Ela, DNVs + Ela and E-DNVs for 8 h. MCF-7/ADR cells were pre-treated with Ela for 12 h before transfection in free DOX + Ela and DNVs + Ela groups. DOX concentration if applied: 10 μg/mL, Ela concentration if applied: 0.5 μg/mL.

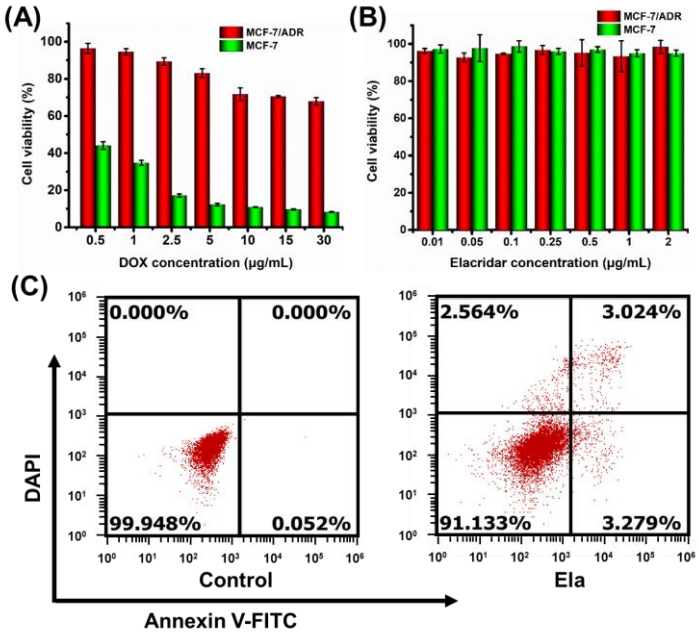

Fig. S5 The viability of MCF-7 and MCF-7/ADR cells incubated with free DOX (A) and free Ela (B) on various concentration. (C) Detection of apoptotic MCF-7/ADR cells using Annexin V-FITC and DAPI flow cytometry assay. MCF-7/ADR cells were co-cultured with free Ela for 36 h. Ela concentration was 0.5 μg/mL.

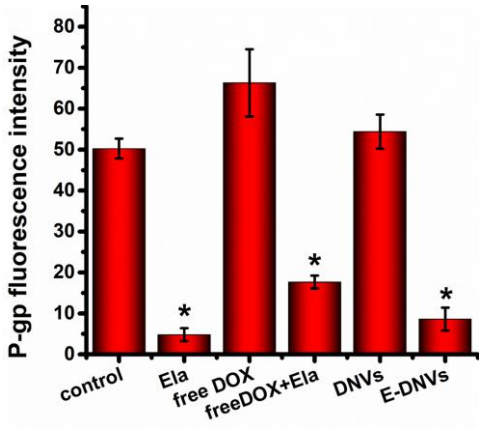

Fig. S6 The P-gp fluorescence intensity was calculated from the immunofluorescent images of MCF-7/ADR cells incubated with different samples. *P < 0.05 *vs.* free DOX and DNVs.

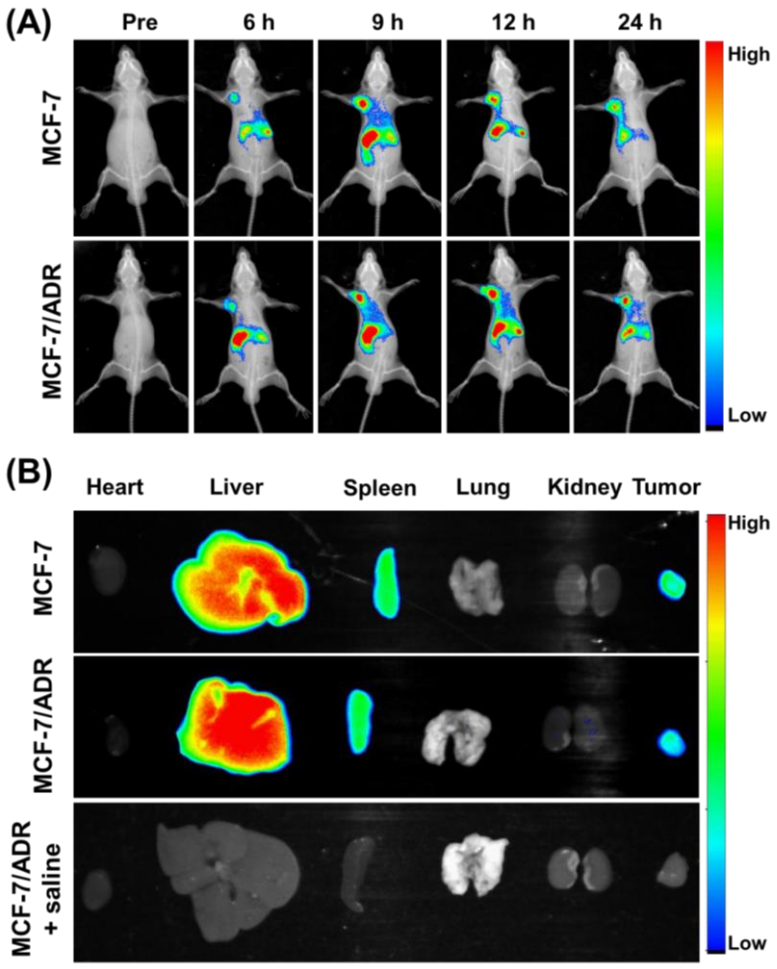

Fig. S7 *In vivo* ICG fluorescence images of tumor bearing mice at different times after tail vein injection (A) and *ex vivo* ICG fluorescence images of the organs from the

same mouse (B). The tumors were excised at 24 h post-injection time. The animal models were the mice bearing MCF-7 tumor and MCF-7/ADR tumor. The images of the organs from the mouse bearing MCF-7/ADR tumor receiving equivalent saline solution showed no fluorescence in organs and tumor.

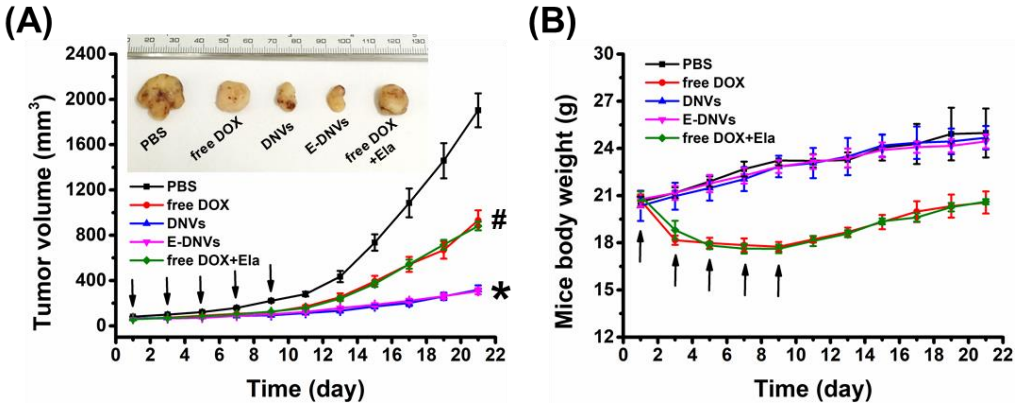

Fig. S8 Tumor growth inhibition of various treatments in MCF-7 tumors (A). Tumor sizes were measured every 2 days (n = 6). Means and standard errors were shown. The body weight of animals bearing MCF-7 tumors (B). The arrows pointed the drug administration time. $^{*}P < 0.05$ compared with PBS and free DOX, $^{\#}P < 0.05$ compared with PBS for MCF-7 tumor. DOX dose: 2.5 mg/kg body weight; Ela dose: 0.25 mg/kg body weight.

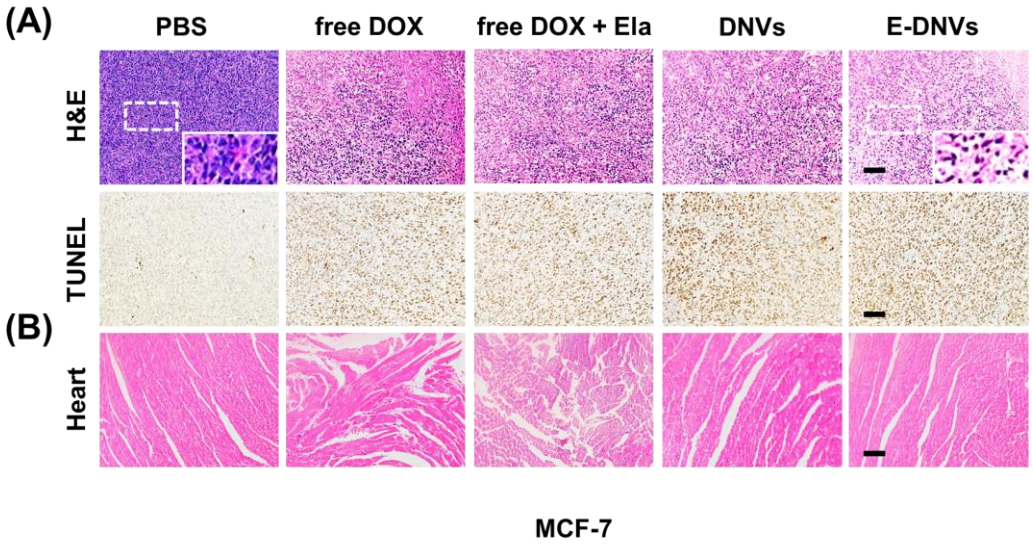

Fig. S9 *Ex vivo* histological analyses of tumor sections excised from mice bearing

MCF-7 after various treatments. Nuclei were stained blue, and extracellular matrix and cytoplasm were stained red in H&E staining. In TUNEL assay, brown and green stains indicated apoptotic and normal cells, respectively (A). H&E staining images of the heart of tumor-bearing mice after various treatments (B). DOX dose: 2.5 mg/kg body weight; Ela dose: 0.25 mg/kg body weight. Scale bars were 50 μm.

Notes and references

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