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Electronic Supplementary Information (ESI)

Multifunctional polyethylenimine-based nanoplatform for targeted anticancer drug delivery to tumors *in vivo*†

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Part of the experimental details

Materials

Branched PEI (Mw = 25 000) was obtained from Sigma-Aldrich (St. Louis, MO). PEG monomethyl ether with one end of carboxyl group (*m*PEG-COOH, Mw = 2 000) and dual functional PEG with one end of carboxyl group and the other end of amine group (NH_2-PEG-COOH, Mw = 2 000) were from Shanghai Yanyi Biotechnology Corporation (Shanghai, China). FI, FA, 1-ethyl-3-(3-dimethylaminopropy) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were supplied by J&K Chemical Ltd. (Shanghai, China). FI-phalloidin was purchased from Thermo Fisher Scientific Ltd. (Shanghai, China). 4',6-Diamidino-2-phenylindole (DAPI) and Hoescht 33342 were from Bestbio Biotechnology Corporation (Shanghai, China). DOX·HCl was from Beijing Huangfeng Pharmaceutical Co., Ltd. (Beijing, China). Dimethyl sulfoxide (DMSO), acetic anhydride, triethylamine and all the other chemicals and solvents were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Cell counting kit-8 (CCK-8) was acquired from 7sea Biotech. Co., Ltd. (Shanghai, China). HeLa cells (a human cervical carcinoma cell line) were from Institute of Biochemistry and Cell Biology (the Chinese Academy of Sciences, Shanghai, China). Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS), penicillin, and streptomycin were purchased from HyClone Lab., Inc. (Logan, UT). All chemicals and materials were used as received. Water used in all experiments was purified using a Milli Q Plus 185 water purification system (Millipore, Bedford, MA) with a resistivity higher than 18.2 MΩ·cm. Regenerated cellulose dialysis membranes with molecular weight cut-off (MWCO) of 1 000 or 14 000 were acquired from Fisher (Pittsburgh, PA).

Loading DOX within the FA-mPEI

Briefly, DOX·HCl with 10 molar equiv. of the FA-mPEI was dissolved in 300 μL methanol, and neutralized by addition of 10 μL of triethylamine to form DOX. The DOX solution was then added into an aqueous FA-mPEI solution (10 mg, 1.5 mL in water) and the mixture was vigorously stirred overnight to evaporate the methanol solvent. Then, the FA-mPEI /DOX mixture solution was

centrifuged (8 000 rpm) for 5 min to remove the precipitate, which is associated with the noncomplexed free DOX. The supernatant solution was collected and lyophilized to obtain the FA-mPEI/DOX complexes.

Characterization techniques

^{1}H NMR spectra were recorded on a Bruker AV400 nuclear magnetic resonance spectrometer (Karlsruhe, Germany). Samples were dissolved into D_2O before NMR measurements. UV-vis spectroscopy was carried out using a Lambda 25 UV-vis spectrophotometer (PerkinElmer, Waltham, MA). Dynamic light scattering (DLS) and zeta potential were conducted under a Malvern Zetasizer Nano ZS model ZEN3600 (Worcestershire, UK) with a standard 633 nm laser. The size and morphology of the FA-mPEI/DOX complexes were characterized by atomic force microscopy (AFM, Veeco DI-NanoScopy IV, Santa Barbara, CA) by tapping mode using a rotated monlithic silicon probe. The parameters were set as following: Imaging Mode (AC mode), scan lines (256), scan points (256), scan rate (1.00Hz), and scan size (3.00 μm).

***In vitro* drug release**

The FA-mPEI/DOX complexes (3 mg) was dispersed into 1 mL of PBS (pH 7.4) or acetate buffer (pH 5.0) and placed in a dialysis bag (MWCO = 14 000). The dialysis bag was then immersed in the corresponding buffer medium with a volume of 9 mL and kept in a vapor-bathing constant temperature vibrator at 37 ^{o}C with a vibraing speed of 200 rpm. At each time point, 1 mL of outer phase medium was taken out and the same volume of fresh corresponding buffer medium was replenished. The DOX release was quantified by UV-vis spectroscopy.

Cytotoxicity assay

HeLa cells at a density of 8 000 cells/well were seeded in 96-well plates with 200 μL of fresh DMEM in each well. After overnight culture to lead to cell confluence, the cell medium was substituted with fresh medium containing FA-mPEI, FA-mPEI/DOX complexes, or free DOX with different final concentrations. Followed by incubation for another 24 h, the cells were analyzed using CCK-8 assay according to the manufacturer's instructions.

Likewise, after HeLa cells were treated with the those samples at different final concentrations

for 24 h, the cells were also observed using a Leica DM IL LED inverted phase contrast microscope (Wetzlar, Germany) with a magnification of 100 × for each sample.

To check the impact of the materials on the cytoskeleton, 5×10^4 HeLa cells were seeded onto each cover slip in each well of a 12-well plate with 1 mL of fresh DMEM. After 24 h, the medium was replaced with fresh medium containing FA-mPEI with equivilent concentration of the FA-mPEI/DOX complexes, FA-mPEI/DOX complexes, or free DOX at the DOX concentration of 5 μg/mL for 24 h. Then the cells were washed three times with PBS and fixed using 4.0% paraformaldehyde for 30 min at room temperature. Then, the cells were washed two times with PBS, and permeabilized with Triton X-100 (0.1%, in PBS) for 10 min. Afterwards, the cells were two times washed with PBS and blocked with 1% bovine serum albumin for 30 min. The cells were washed two times with PBS and F-actin staining was carried out using Alexa Fluor 568-conjugated phalloidin for 30 min. Then the cells were stained with DAPI for 5 min, and two times washed with PBS before confocal microscopic imaging.

Statistical analysis

One way analysis of variance (ANOVA) statistical analysis was used to assess the significance of the experimental data. Accordingly, the significance levels were indicated with $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$, respectively.

Table S1. Zeta potentials and hydrodynamic sizes of the different samples (data are provided as mean ± S.D., n = 3)

Samples	Zeta potential (mV)	hydrodynamic size (nm)
mPEI	2.65 ± 0.43	80.8 ± 16.0
FA-mPEI	1.33 ± 0.14	167.6 ± 0.93
mPEI/DOX	12.33 ± 0.32	172.2 ± 1.0
FA-mPEI/DOX	7.56 ± 0.21	225.3 ± 3.3

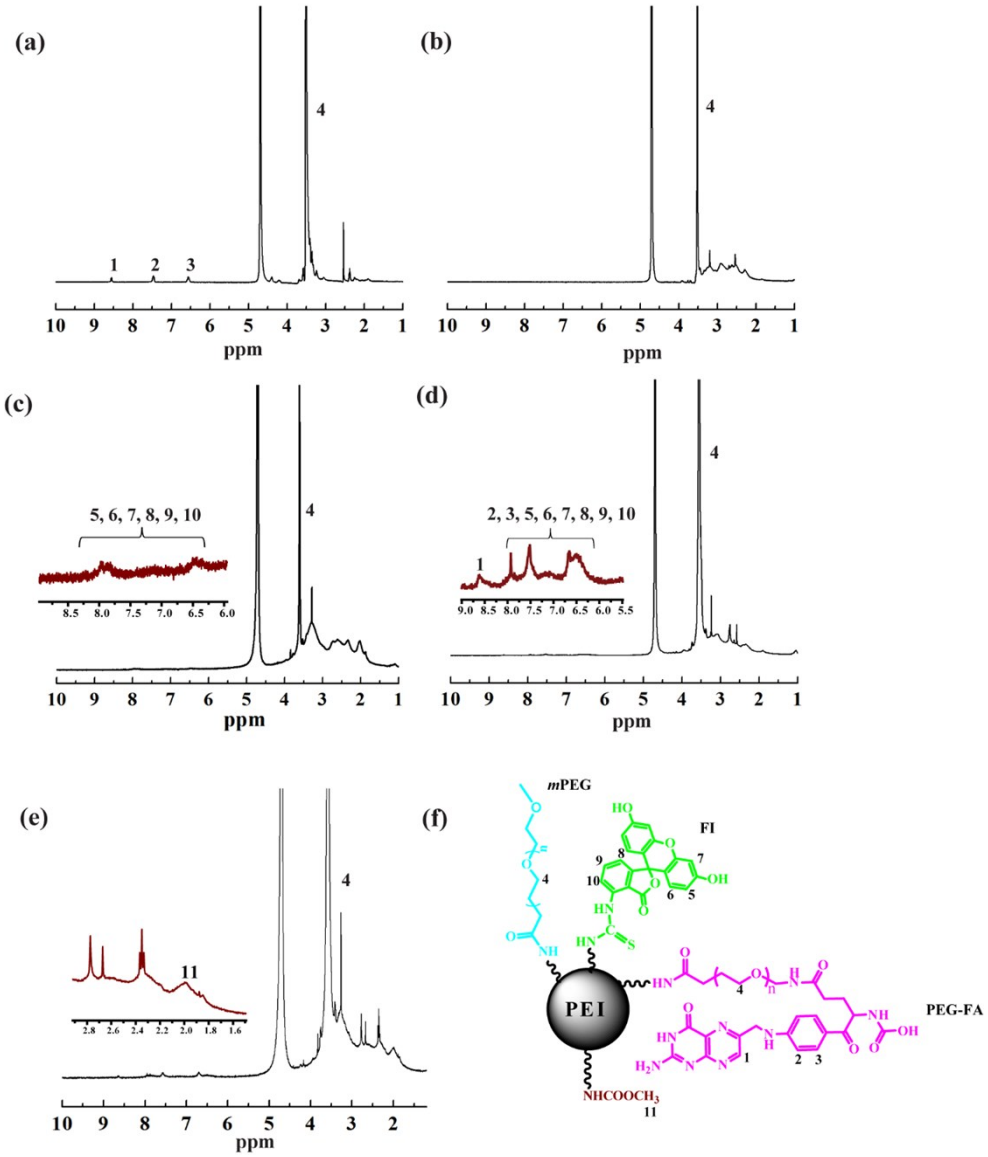

Figure S1. ^{1}H NMR spectra of FA-PEG-COOH (a), PEI-*m*PEG (b), PEI-Ac-FI-*m*PEG (mPEI) (c), PEI-FI-*m*PEG-(PEG-FA) (d), and PEI-Ac-FI-*m*PEG-(PEG-FA) (FA-mPEI) (e), respectively. The schematic illustration of the structure of FA-mPEI is shown in (f).

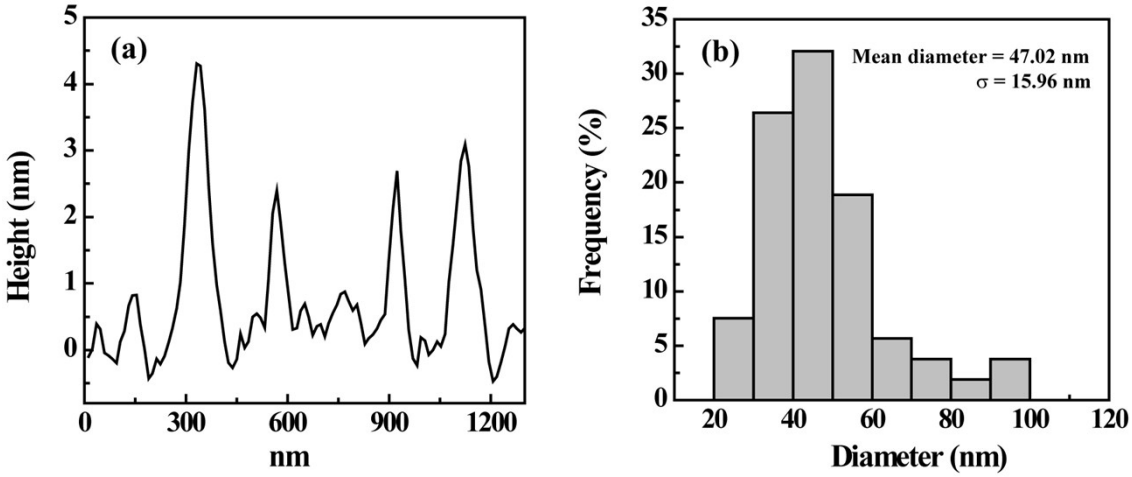

Figure S2. Section analysis (height, a), and size distribution histogram (b) of the AFM image of the FA-mPEI/DOX complexes.

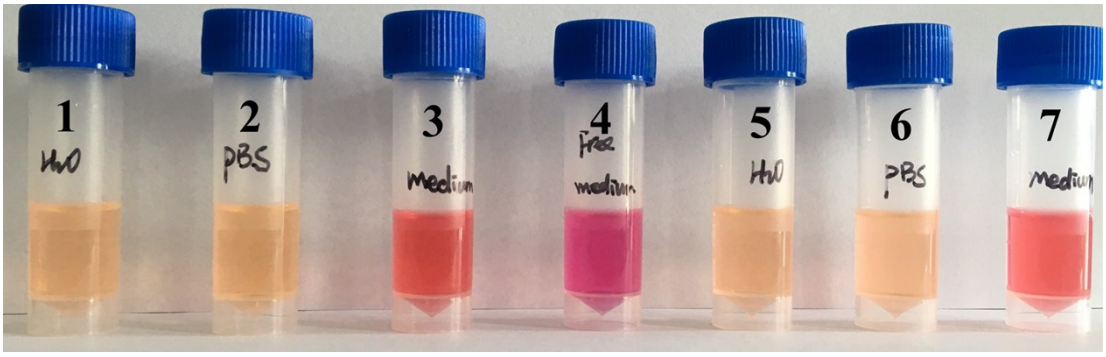

Figure S3. Photos of FA-mPEI conjugate dispersed in water (1), PBS (2), and cell culture medium (3), and FA-mPEI/DOX complexes dispersed in water (5), PBS (6), and cell culture medium (7). Blank cell culture medium is shown in (4).

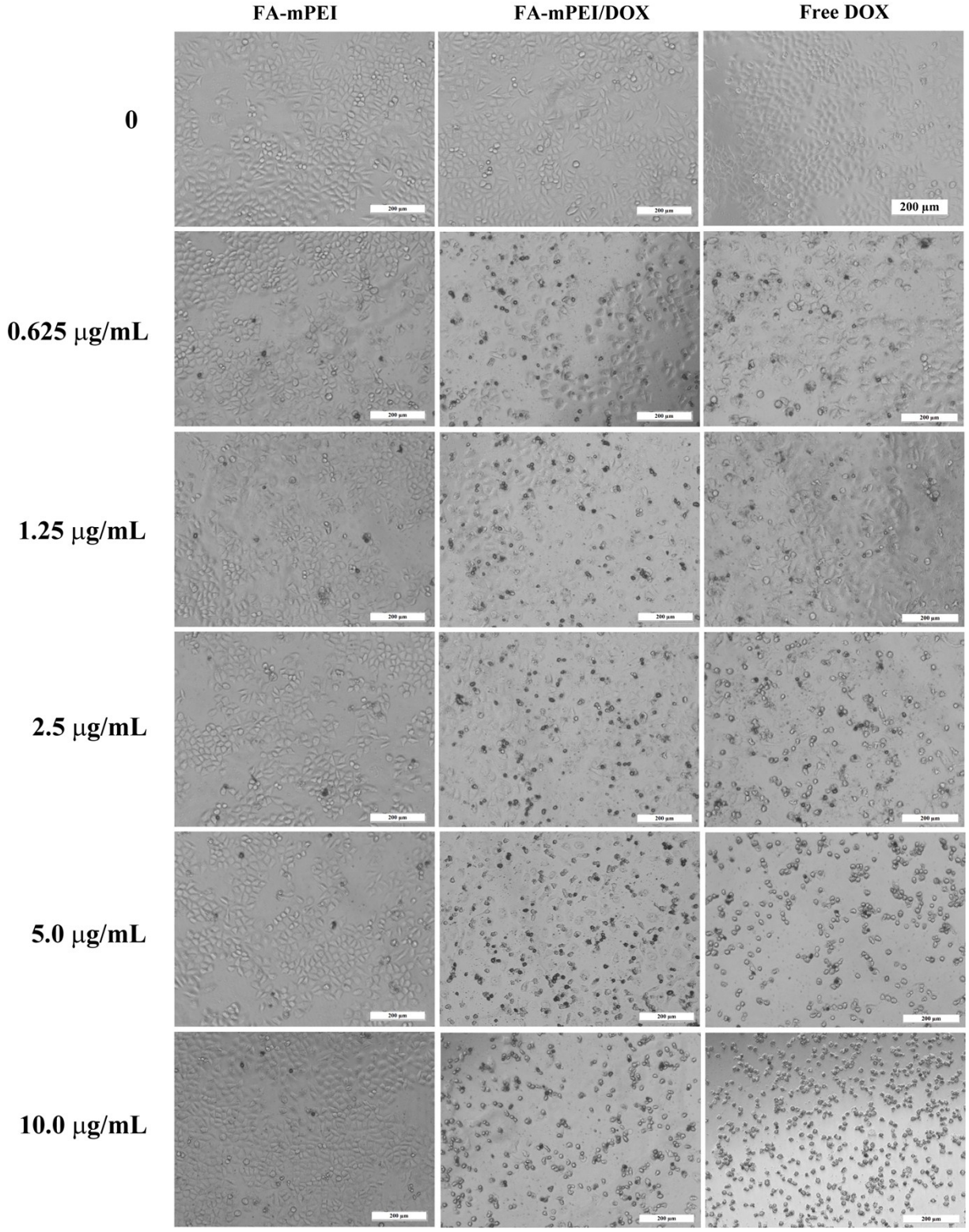

Figure S4. The micrographs of HeLa cells treated with FA-mPEI, FA-mPEI/DOX complexes, and free DOX at different DOX concentrations for 24 h. The concentrations of FA-mPEI were similar to those of the corresponding FA-mPEI/DOX complexes.

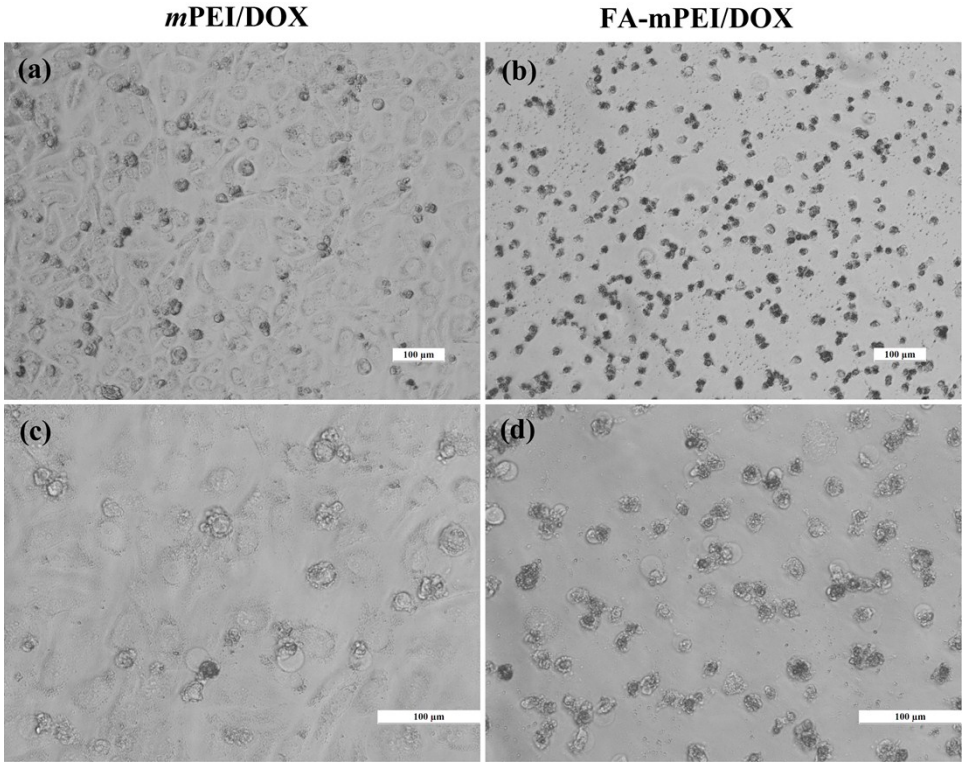

Figure S5. Phase contrast photomicrographs of HeLa cells treated with mPEI/DOX complexes (a, c) and FA-mPEI/DOX complexes (b, d) at a DOX concentration of 10 μg/mL for 4 h, followed by incubation with DOX-free fresh medium for another 48 h. The samples were scanned using a 10× objective lens for (a) and (b), 20 × objective lens for (c) and (d).

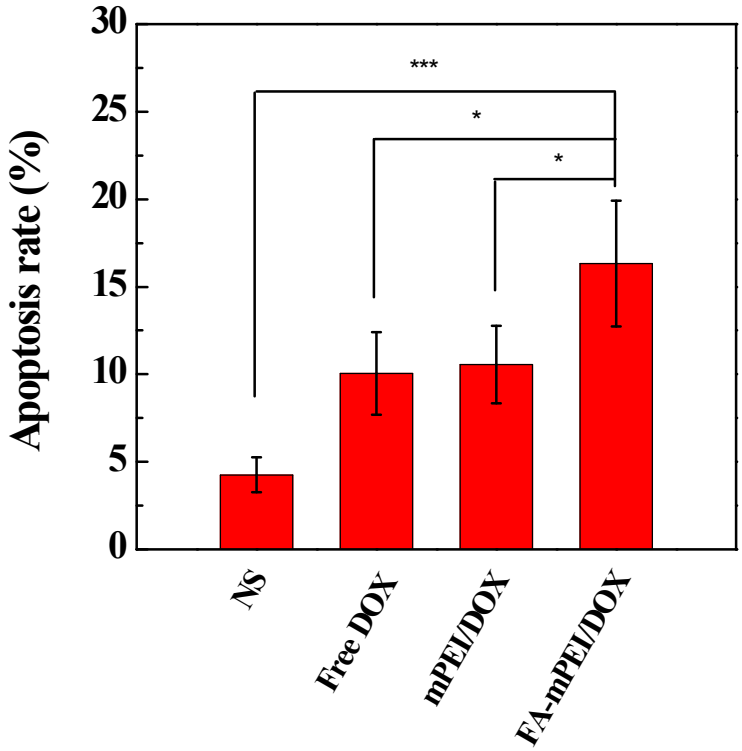

Figure S6. Cell apoptosis rate of tumors treated with NS, free DOX, mPEI/DOX complexes, and FA-mPEI/DOX complexes, respectively at 30 days postinjection.