Supplementary Material

Synergistic effects of A-B-C type amphiphilic copolymer on reversal of drug resistance in MCF-7/ADR breast carcinoma

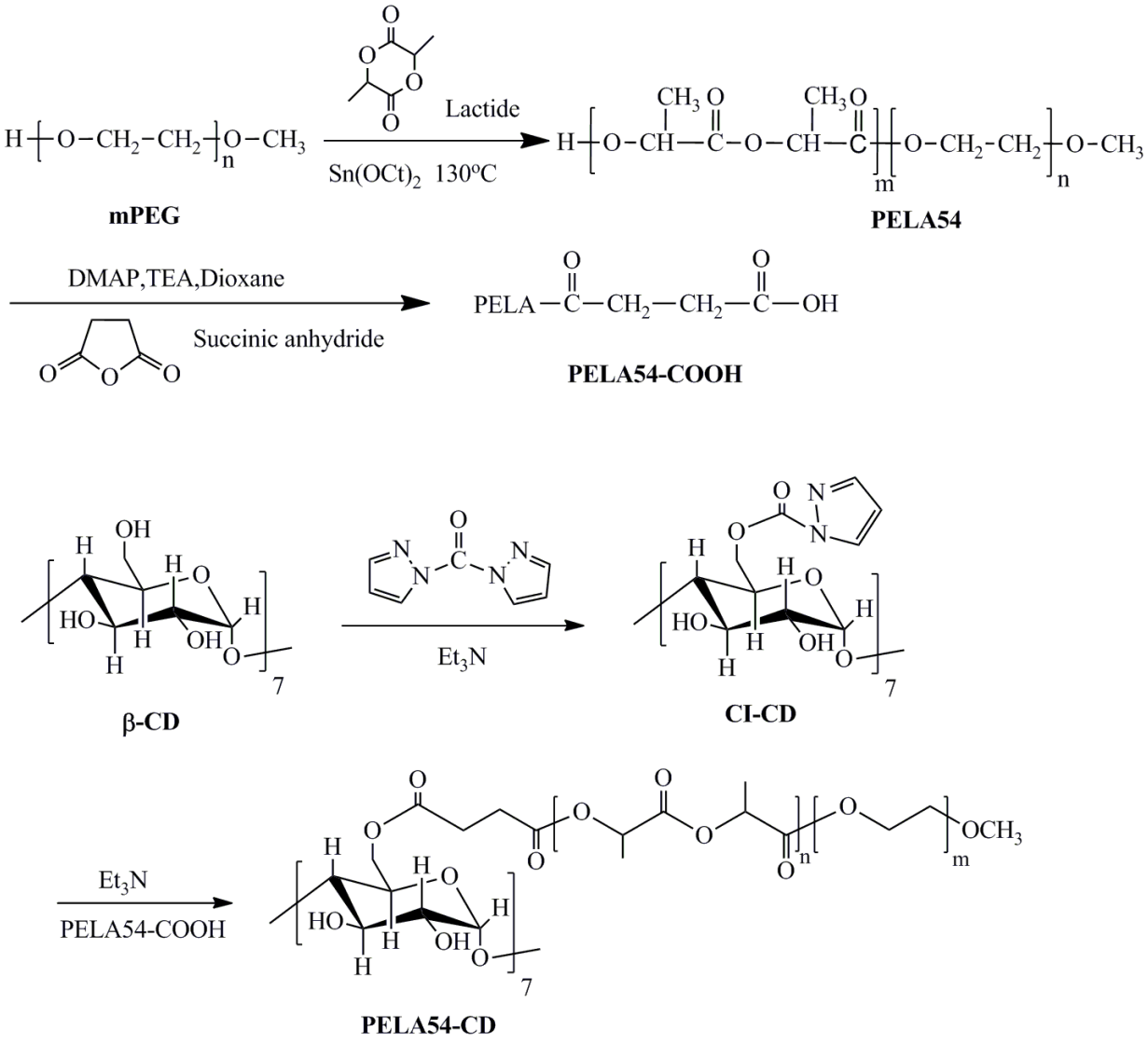


Figure S1 Synthesis route of PELA54-CD.
Abbreviations: β-CD, β-cyclodextrin; PEG5000-CD, PEGylated β-CD derivants; PELA54, copolymer with a feed ratio of mPEG5000 to LA: 5/4; PELA54-CD, a linear amphiphilic copolymer by linking β-CD at the end of hydrophobic block of PEGylated poly(D,L-lactide) at 1:1 mole ratio; $Sn(Oct)_2$, stannous octoate; Et_3N, triethylamine; CI-CD, carbonyldiimidazole activated β-CD.

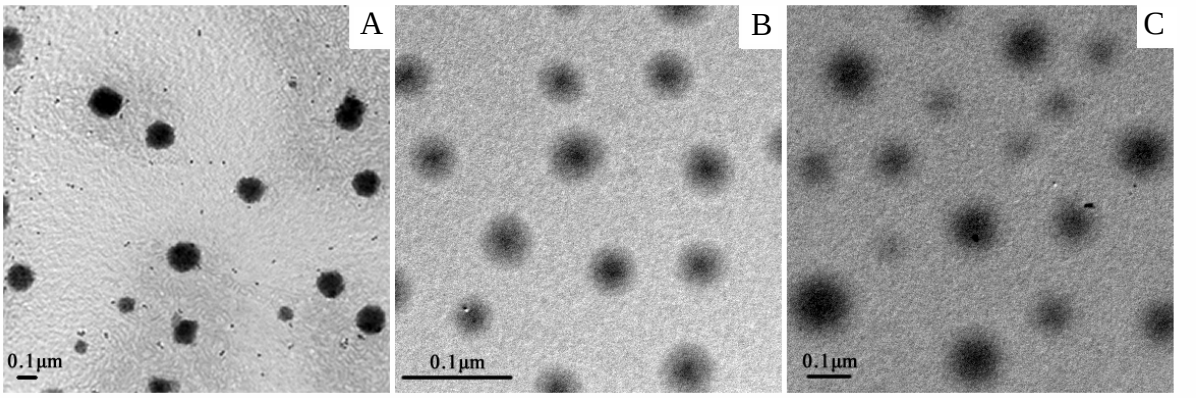


Figure S2 TEM micrographs of polymeric micelles.
Notes: **(A)** PELA54-CD. **(B)** PELA54. **(C)** PEG5000-CD. Bar=0.1 μm.
Abbreviations: PEG5000-CD, PEGylated β-CD derivants; PELA54, copolymer with a feed ratio of mPEG5000 to LA: 5/4; PELA54-CD, a linear amphiphilic copolymer by linking β-CD at the end of hydrophobic block of PEGylated poly(D,L-lactide) at 1:1 mole ratio; TEM, Transmission Electron Microscope.

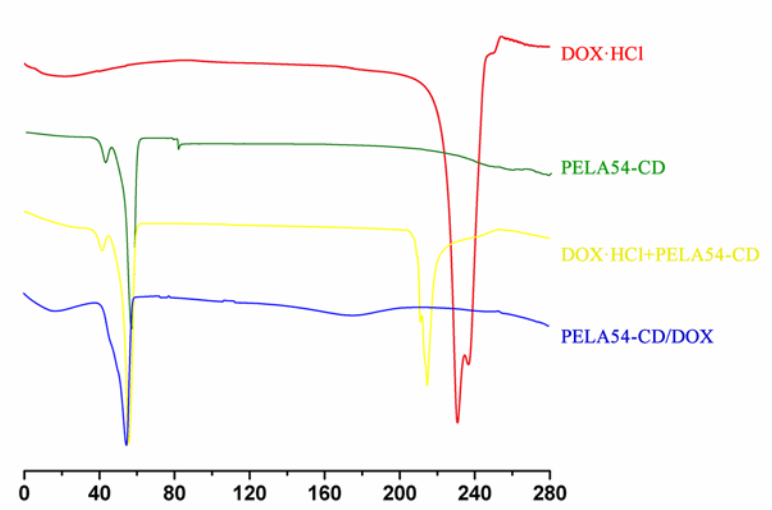


Figure S3 DSC curves of DOX·HCl, blank PELA54-CD micelles, physical mixture of DOX·HCl and blank PELA54-CD micelles, and lyophilized PELA54-CD/DOX micelles.
Abbreviations: DOX, doxorubicin; DOX·HCl, free doxorubicin hydrochloride; PELA54-CD, a linear amphiphilic copolymer by linking β-CD at the end of hydrophobic block of PEGylated poly(D,L-lactide) at 1:1 mole ratio; PELA54-CD/DOX, DOX-loaded micelles; DSC, differential scanning calorimetry.

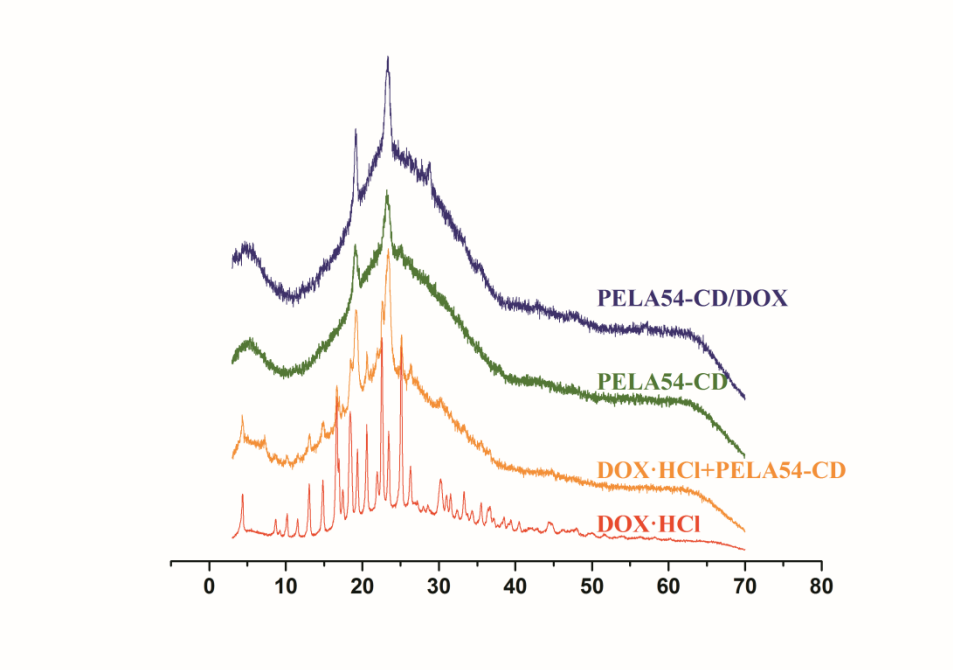


Figure S4 Evolution of powder X-ray diffraction patterns of DOX·HCl powder, PELA54-CD lyophilized powder, physical mixture of DOX·HCl and blank PELA54-CD and PELA54-CD/DOX lyophilized powder.

Abbreviations: DOX, doxorubicin; DOX·HCl, free doxorubicin hydrochloride; PELA54-CD, a linear amphiphilic copolymer by linking β-CD at the end of hydrophobic block of PEGylated poly(D,L-lactide) at 1:1 mole ratio; PELA54-CD/DOX, DOX-loaded micelles; XRD, X Ray diffraction.

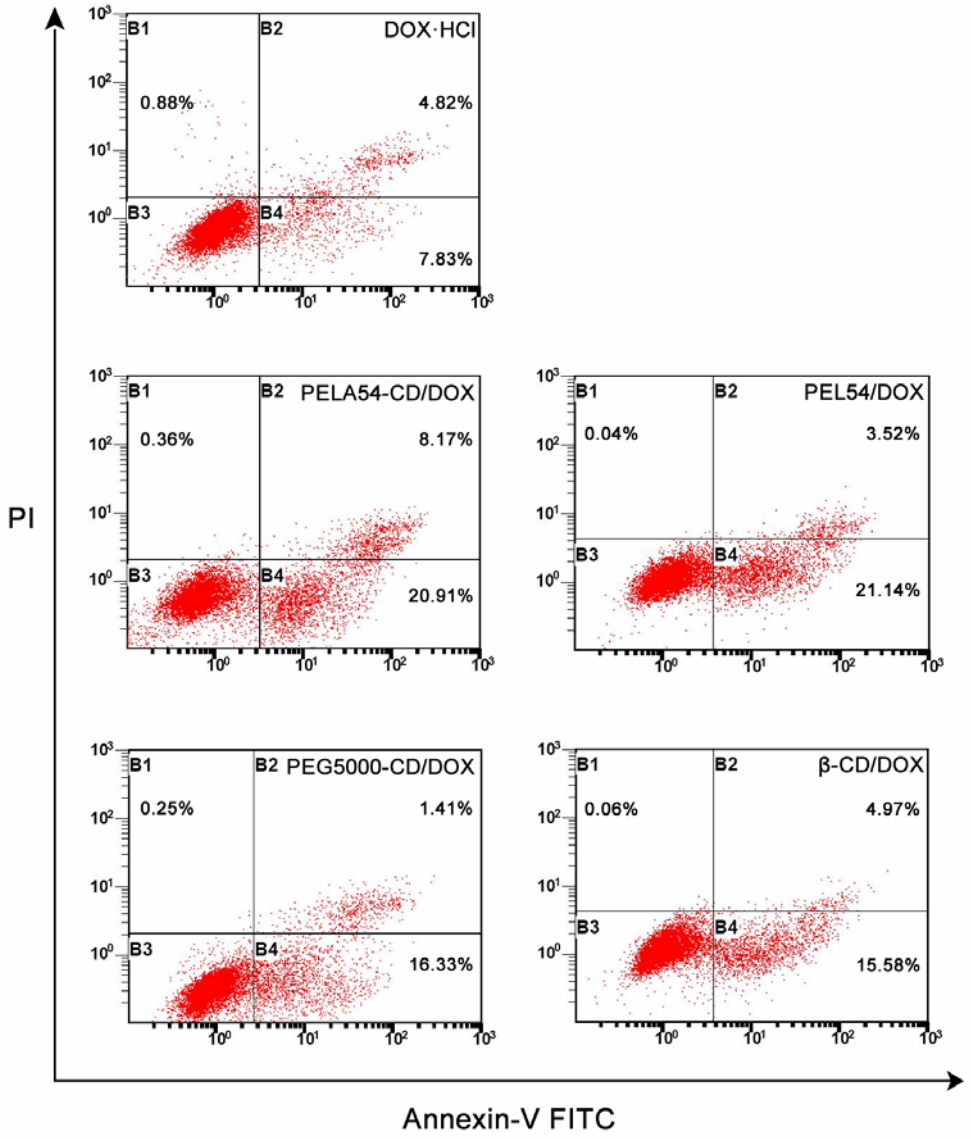


Figure S5 Percentage of DOX-induced apoptosis in MCF-7/ADR cells detected by flow cytometry.

Notes: Cells were treated with DOX·HCl solution or in DOX-loaded micelles containing total DOX concentration of 2 μg/mL for 48 h. The histogram shows the percentage of apoptosis represented as mean ± S.D. (n=3), statistical significance ($p < 0.01$) was evaluated by using SPSS software when compared with DOX·HCl group.

Abbreviations: DOX·HCl, free doxorubicin hydrochloride; PELA54-CD/DOX, DOX-loaded micelles; PELA54/DOX, DOX-loaded micelles; PEG5000-CD/DOX, DOX-loaded cyclodextrin complex; β-CD/DOX, cyclodextrin-doxorubicin inclusion compound; Annexin-V FITC/PI, fluorescent dye used in flow cytometry.

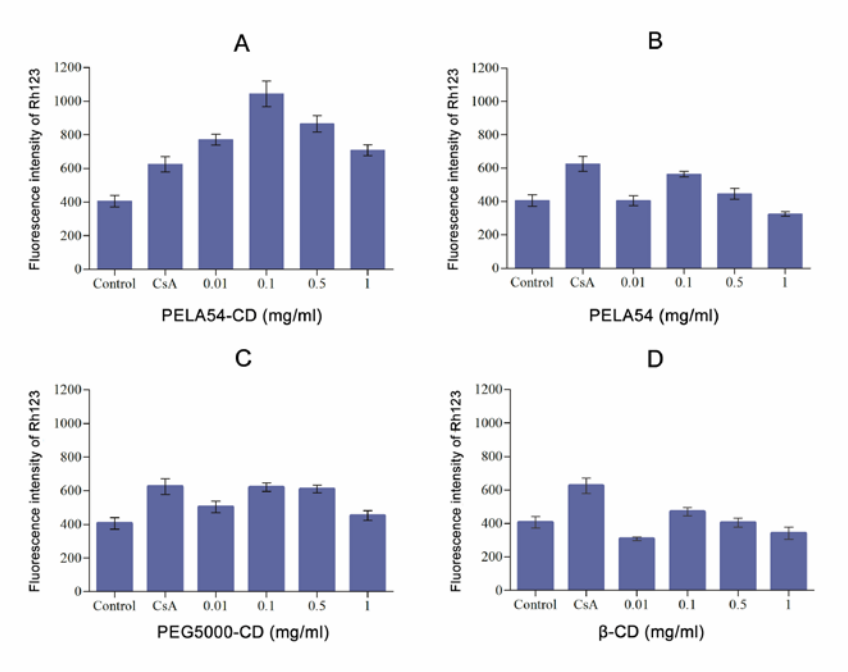


Figure S6 The cellular accumulation of Rh123 in MCF-7/ADR cells in the presence of different polymers.
Notes: (A) PELA54-CD. **(B)** PELA54. **(C)** PEG5000-CD. **(D)** β-CD. The results are represented as mean ± S.D. from three independent experiments (n=3).
Abbreviations: β-CD, β-cyclodextrin; PEG5000-CD, PEGylated β-CD derivants; PELA54, copolymer with a feed ratio of mPEG5000 to LA: 5/4; PELA54-CD, a linear amphiphilic copolymer by linking β-CD at the end of hydrophobic block of PEGylated poly(D,L-lactide) at 1:1 mole ratio; Rh123, Rhodamine 123.

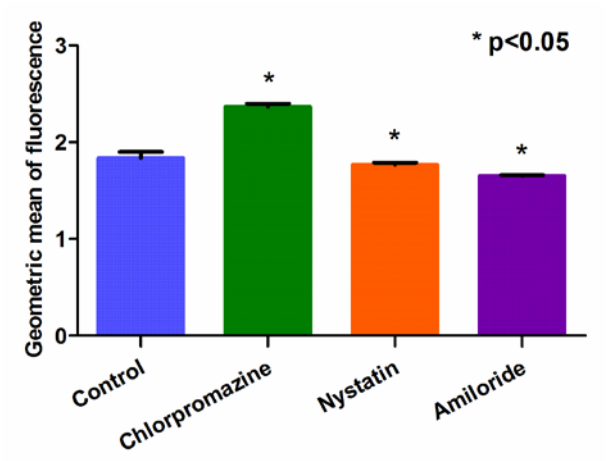


Figure S7 Cellular uptake of DOX in MCF-7/ADR cells in the presence of one of the endocytosis inhibitors (chlorpromazine, nystatin or amiloride) and PELA-CD/DOX with 10 μg/mL DOX concentration for 2 h at 37 °C.
Notes: The results are represented as mean ± SD (n=3), statistical significance ($p < 0.05$) was evaluated by using SPSS software when compared with control.
Abbreviations: PELA54-CD/DOX, DOX-loaded micelles; DOX, doxorubicin.

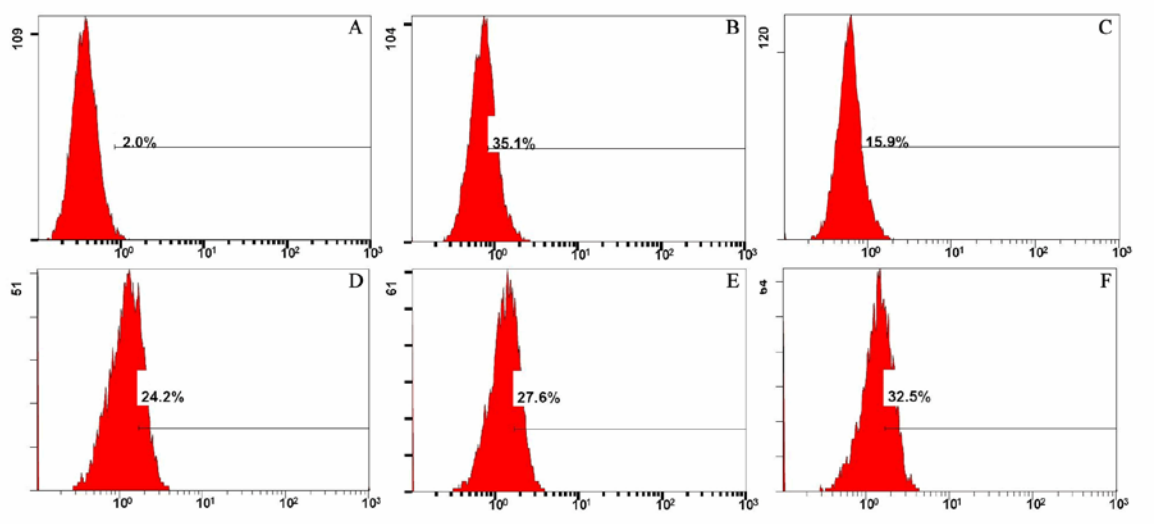
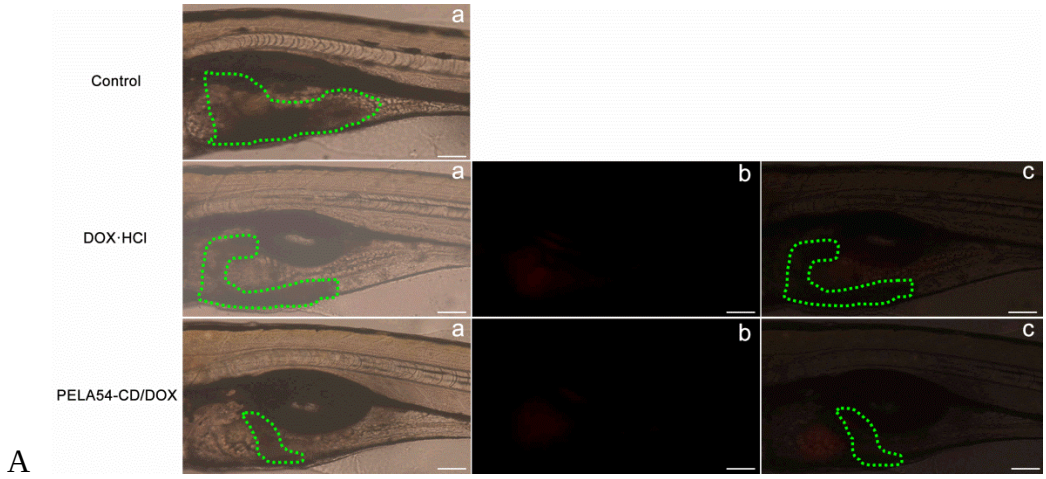


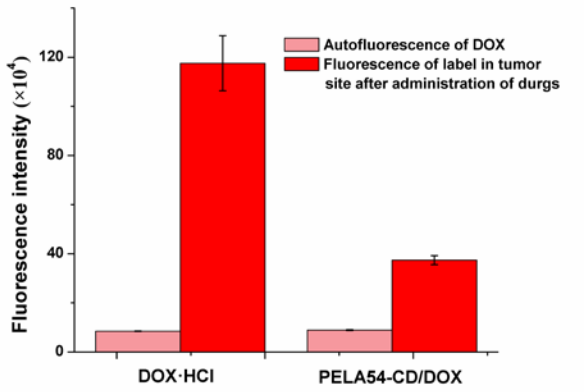
Figure S8 P-gp expression in MCF-7 cells and MCF-7/ADR cells treated with different polymers.

Notes: (A) the untreated MCF-7 cells. **(B)** the untreated MCF-7/ADR cells. **(C-F)** MCF-7/ADR cells treated with different micelles (PELA54-CD, PELA54, PEG5000-CD, β-CD) at 50 μg/mL for 24 h. P-gp expression was determined by flow cytometry using R-PE-conjugated mouse anti-human monoclonal antibody against P-gp. The percentage of area in the right frame indicated the relative quantity of P-gp expression.

Abbreviations: β-CD, β-cyclodextrin; PEG5000-CD, PEGylated β-CD derivants; PELA54, copolymer with a feed ratio of mPEG5000 to LA: 5/4; PELA54-CD, a linear amphiphilic copolymer by linking β-CD at the end of hydrophobic block of PEGylated poly(D,L-lactide) at 1:1 mole ratio; P-gp, P-glycoprotein.



A



B

Figure S9 Zebrafish xenografts by microinjection of MCF-7/ADR cells without fluorescence labeling.

Notes: (A) The green area represent of tumor cells, red fluorescence belongs to natural fluorescence of DOX (20 ng of PELA54-CD/DOX or DOX·HCl was microinjected into the yolk sac 24 h after xenografts of MCF-7/ADR cells). Scale bar=100 μm. **(B)** Fluorescence of CM-Dil-labeled tumor cell and autofluorescence of DOX in zebrafish, which showed that fluorescence signal of CM-Dil were not interferenced by the natural fluorescence of DOX.

Abbreviations: DOX, doxorubicin; DOX·HCl, free doxorubicin hydrochloride; PELA54-CD/DOX, DOX-loaded micelles.