

## Electronic Supplementary Information

### **A multifunctional liposomal nanoplatform co-delivering hydrophobic and hydrophilic doxorubicin for complete eradication of xenografted tumors**

Huili Sun,<sup>a,b</sup> Xing Guo,<sup>a</sup> Si Zeng,<sup>b</sup> Yi Wang,<sup>a,b</sup> Jianwen Hou,<sup>a</sup> Donghua Yang<sup>c</sup> and Shaobing Zhou, <sup>\*a,b</sup>

<sup>a</sup> Key Laboratory of Advanced Technologies of Materials Ministry of Education, School of Materials Science and Engineering, Southwest Jiaotong University, Chengdu 610031, P.R. China

<sup>b</sup> School of Life Science and Engineering, Southwest Jiaotong University, Chengdu 610031, P.R. China

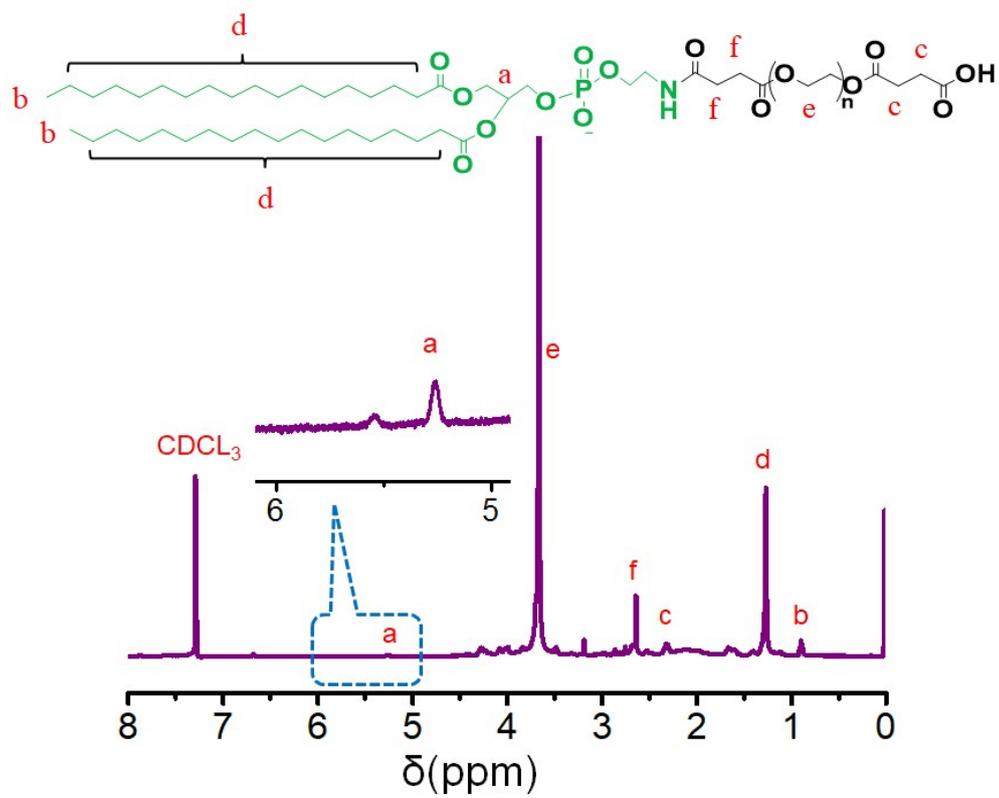
<sup>c</sup> Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences, ST.John's University, Queens, NY 11439, USA

\*Corresponding author: [shaobingzhou@swjtu.edu.cn](mailto:shaobingzhou@swjtu.edu.cn)

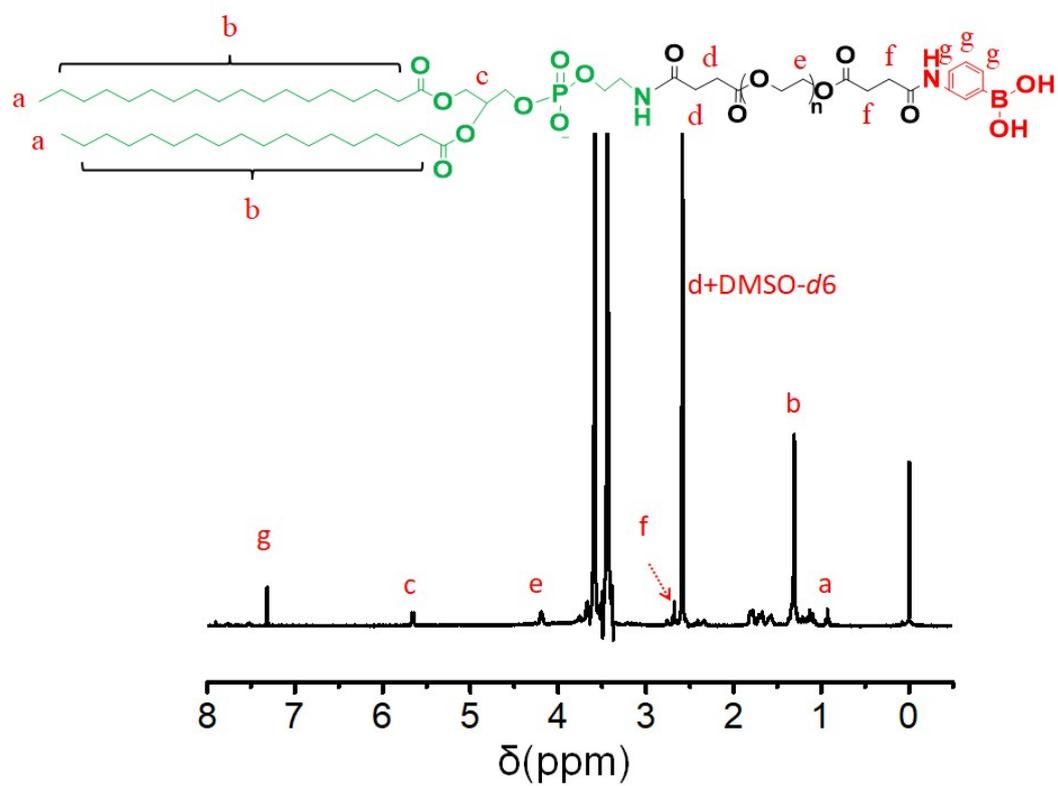
#### **Table of Contents**

<b>Supplementary Figures.....</b>	<b>S2</b>
<b>Supplementary Tables.....</b>	<b>S9</b>
<b>Supplementary Movie.....</b>	<b>S11</b>

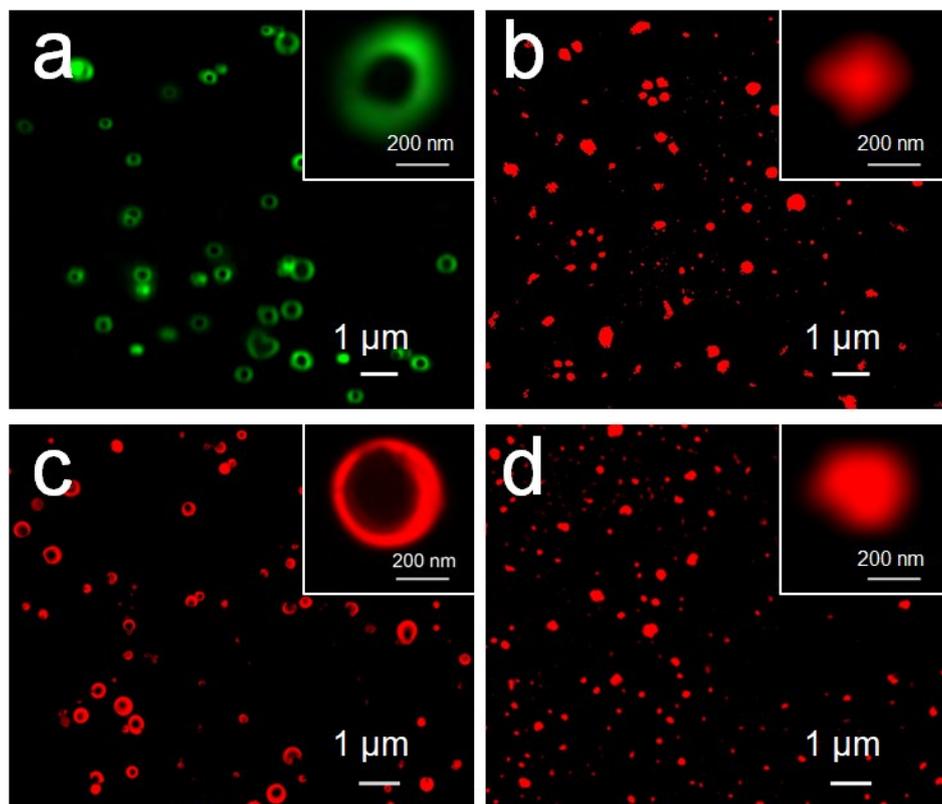
## 1. Supplementary Figures:



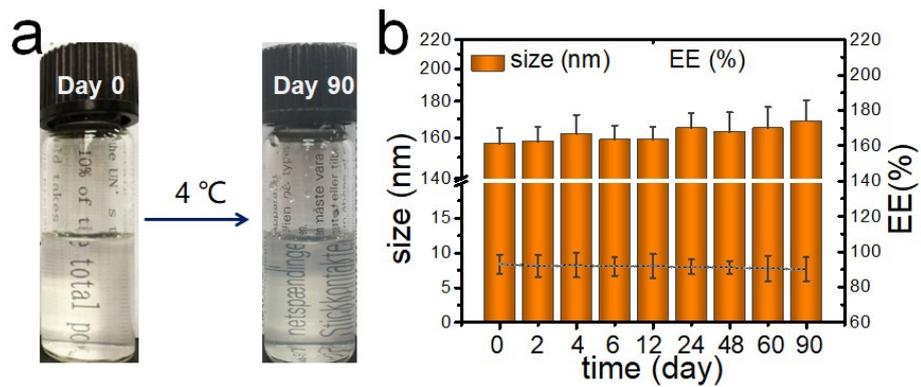
**Figure S1.  $^1\text{H}$  NMR spectra.**  $^1\text{H}$  NMR spectra of DSEG in  $\text{CDCl}_3$ .



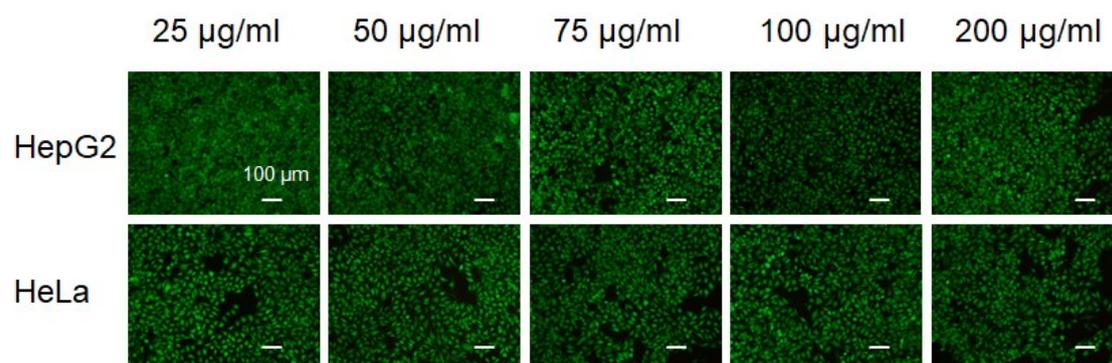
**Figure S2.  $^1\text{H}$  NMR spectra.**  $^1\text{H}$  NMR spectra of DSEG-PBA in  $\text{DMSO-}d_6$ .



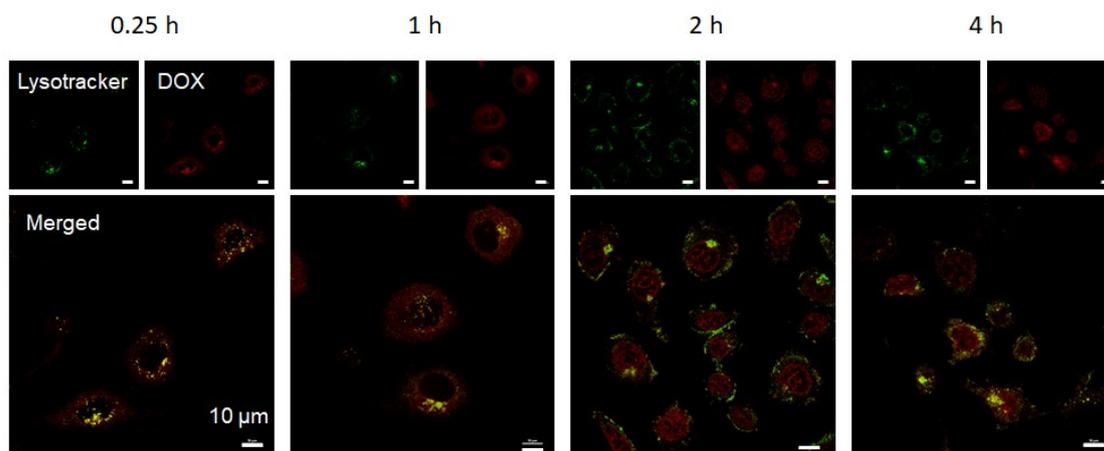
**Figure S3. CLSM images.** CLSM images of DSEG-PBA liposomes loaded with Coumarin-6 (a) and Rhodamine B (b), respectively. CLSM images of DSEG-PBA liposomes loaded with hydrophobic doxorubicin (c) and hydrophilic doxorubicin (d), respectively.



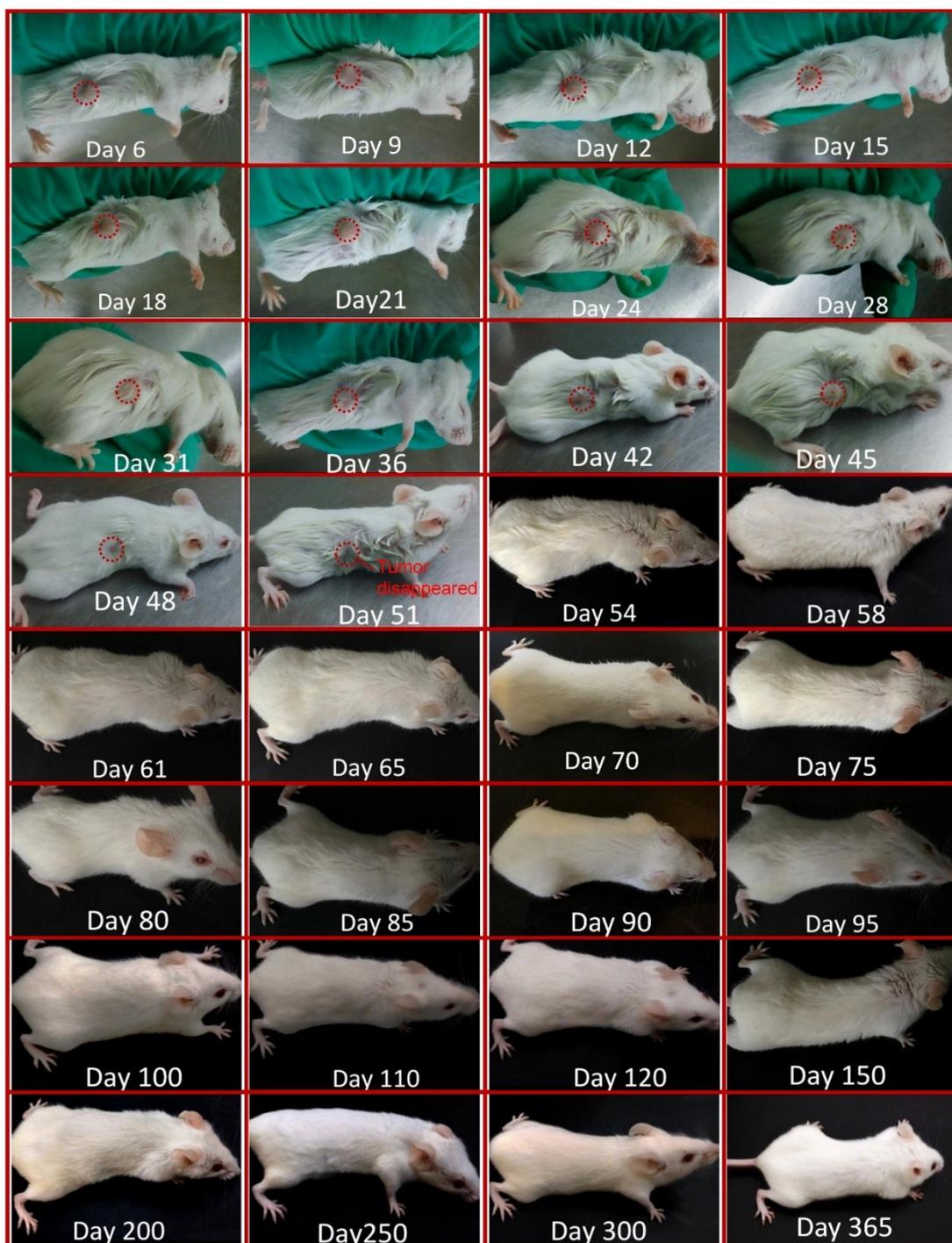
**Figure S4. The stability.** Photographs of the blank DSEG-PBA liposome solution at day 0 and day 90, respectively. (a) The mean sizes characterized by DLS and EE(%) of the drug combination loaded DSEG-PBA liposome as a function of time in the 4 °C environment within 90 days (b).



**Figure S5. Fluorescence images.** Fluorescence images of HepG2 cells and HeLa cells incubated with the blank DSEG-PBA liposome with different concentrations for 24 h. Green calcein fluorescence indicates live cells.



**Figure S6. Lysosomal escape of the liposomes.** Representative color split (upper left and upper right) and merged (below) CLSM images of the HepG2 cell incubated with dual DOX-loaded DSEG-PBA liposome for different time respectively. Then, the cells were stained with LysoTracker green. Green, LysoTracker green-stained lysosomes; red, DOX fluorescence in the liposomes (n = 2). The scale bars are 10  $\mu\text{m}$ .



**Figure S7.** Tumor growth, scarring, disappearance of tumor-bearing mice and long-term healthy survival of mice treatment with the dual DOX-loaded DSEG-PBA liposome in 365 days.

## 2. Supplementary Tables:

**Table S1.** Compose molar ratio, size and  $\zeta$ -potential characterizations of different formulation liposomes (n=3).

Sample	Compose ratio(mol%) ePC:Chol:DSEG:DSEG-PBA	Average size <sup>a</sup> (nm)	PDI <sup>a</sup>	$\zeta$ -Potential <sup>b</sup> (-mv)
PC	65:35:0:0	131.2 $\pm$ 2.07	0.109 $\pm$ 0.021	-10.77 $\pm$ 1.32
DSEG	60:35:5:0	152.7 $\pm$ 1.21	0.089 $\pm$ 0.016	-9.18 $\pm$ 0.82
DSEG-PBA	60:35:4.2:0.8	158.3 $\pm$ 0.96	0.163 $\pm$ 0.014	-8.96 $\pm$ 1.16
core-DOX	60:35:4.2:0.8	147.5 $\pm$ 1.60	0.126 $\pm$ 0.026	-12.54 $\pm$ 1.03
shell-DOX	60:35:4.2:0.8	139.3 $\pm$ 1.37	0.148 $\pm$ 0.023	-13.92 $\pm$ 1.16

<sup>a</sup> Measured by DLS, a mean  $\pm$  SD of 3 measurements.

<sup>b</sup> Estimated at pH 7.4 at 25 °C, a mean  $\pm$  SD of 3 measurements.

**Table S2.** Physicochemical characteristics of drug-loaded DSEG-PBA liposomes.<sup>a</sup>

Sample	Feed ratio (%)		LC(%)		EE(%) <sup>d</sup>	
	DOX·HCl	DOX	DOX·HCl	DOX	DOX·HCl	DOX
DSPE-PBA <sup>b</sup>	10	10	9.2±1.73		93.4±2.65	
core-DOX <sup>c</sup>	10	—	8.36±0.52	—	93.1±4.47	—
shell-DOX <sup>c</sup>	—	10	—	3.4±0.31	—	42.1±1.92

<sup>a</sup> In all cases, drug-loaded DSEG-PBA liposome size (nm) = (150~180) ± 1.52 , PDI = 0.122 ± 0.017, n = 3.

<sup>b</sup> DSEG-PBA, binary-drug loaded DSEG-PBA liposomes.

<sup>c</sup> core-DOX & shell-DOX, single drug-loaded DSEG-PBA liposomes.

<sup>d</sup> Encapsulation efficiency (% loaded from amount supplied) and mass loading ratio (mg/mg ,drug/lipid) as determined by UV-VIS spectrophotometer.

### **3. Supplementary Movie:**

**Video S1.** 3-dimensional images at different time points in CLSM images of DSEG-PBA liposomes loaded Coumarin-6 (green).