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Supplemental Information

A Novel Therapeutic Strategy for Cancer Using Phosphatidylserine Targeting Stearylamine-Bearing Cationic Liposomes

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SUPPLEMENTARY FIGURES

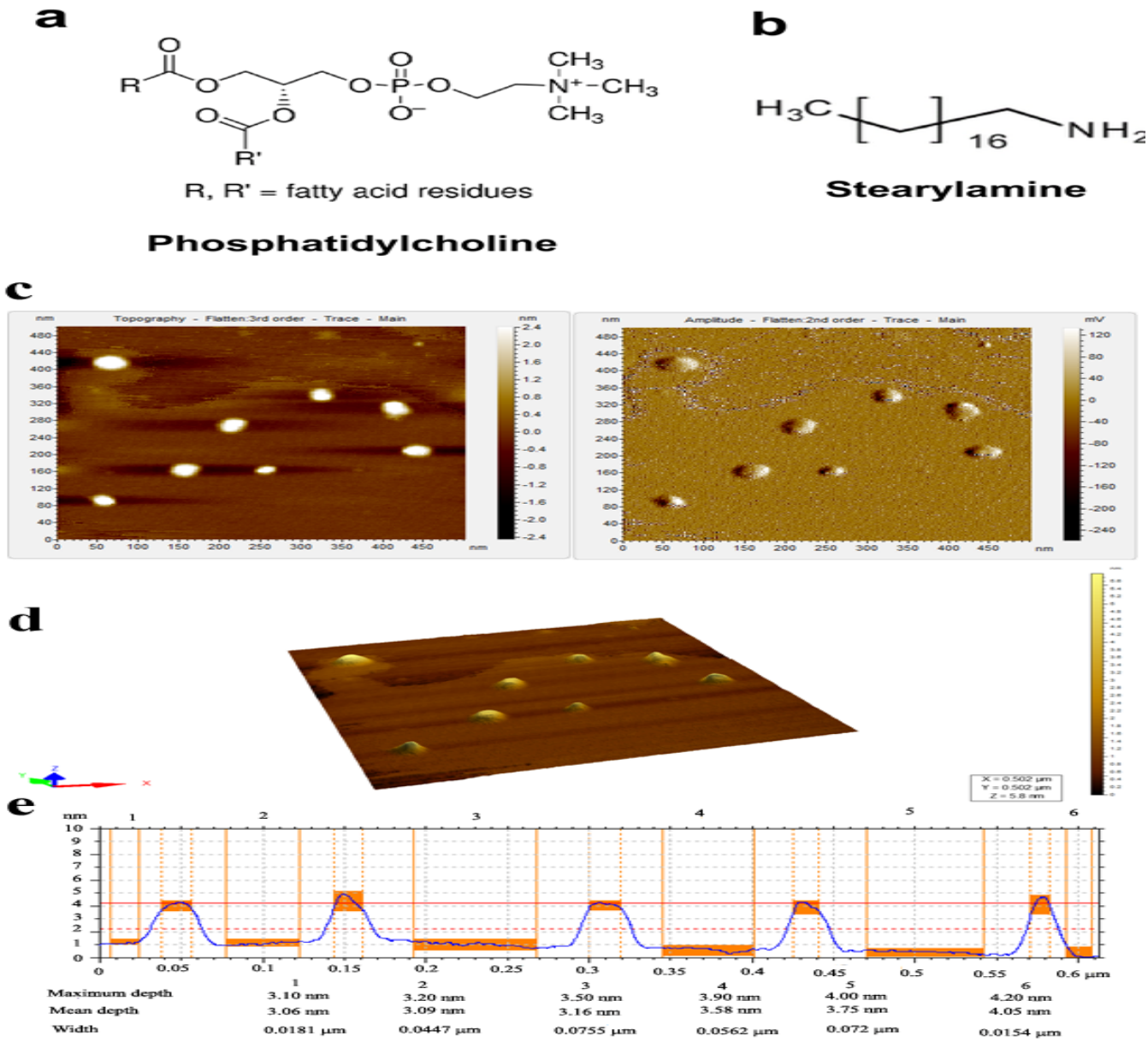

Figure S 1: Chemical structure of PC and SA, AFM analysis of PC-SA liposome (a) Chemical structure of phosphatidylcholine (b) and stearylamine. (c) AFM images presented as two-dimensional graphics showing the clean spherical shaped PC-SA liposome. Topography flattened and amplitude-flattened views of liposomes are shown. (d) 3D image of PC-SA liposome by AFM studies. (e) Horizontal cross sections indicating the height of the liposome from the substratum, i.e., the mica sheet.

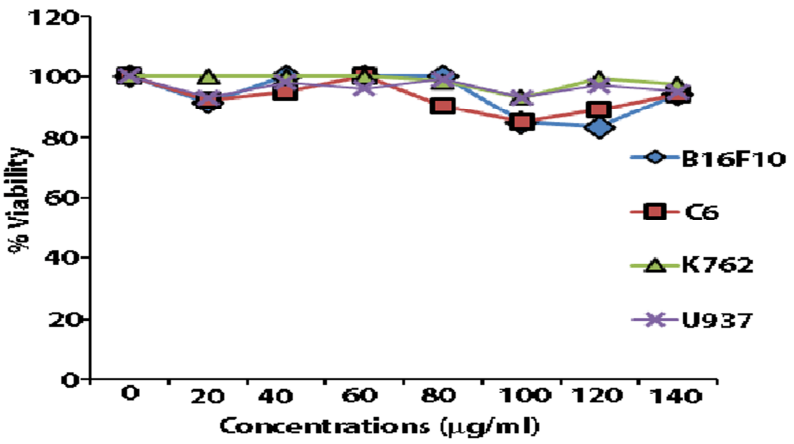

Figure S 2: No killing effect of neutral liposome, PC-Chol, on cancer cell lines. Cell viability of B16F10, rat C6 glioma, K562 and U937 cells following incubation with increasing concentrations (20-140 µg/ml) of PC-Chol liposomes with respect to PC for 2 h. All data represent the mean of three separate experiments with error bars indicating the standard error of the mean.

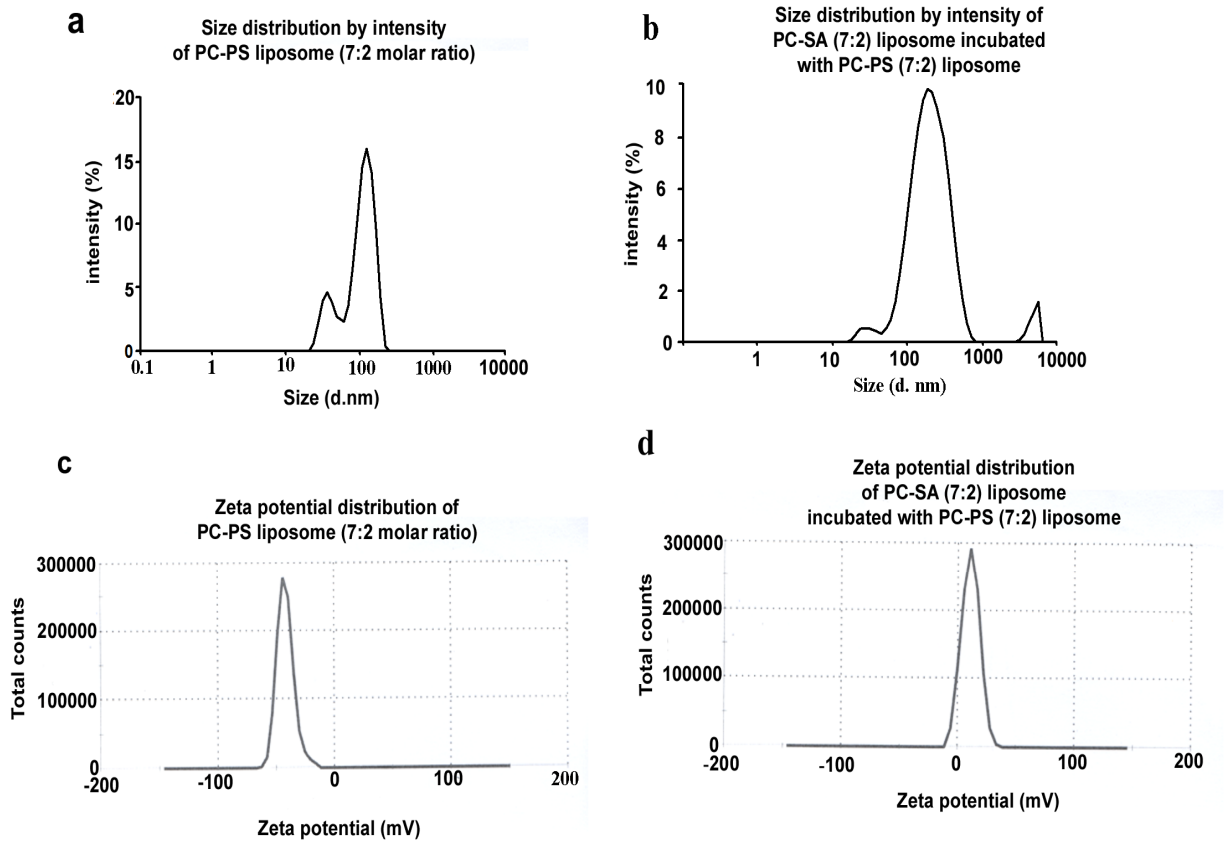

Figure S 3: Size distribution and zeta potential of PC-SA liposomes following incubation with PC-PS liposomes for 30 mins. (a) Size distribution of negatively charge PC-PS liposomes (7:2 molar ratio). (b) Size distribution of PC-SA liposome (7:2 molar ratio) incubated with PC-PS liposome (7:2 molar ratio). (c) Zeta potential of PC-PS liposome. (d) Zeta potential of PC-SA liposome incubated with PC-PS liposome.

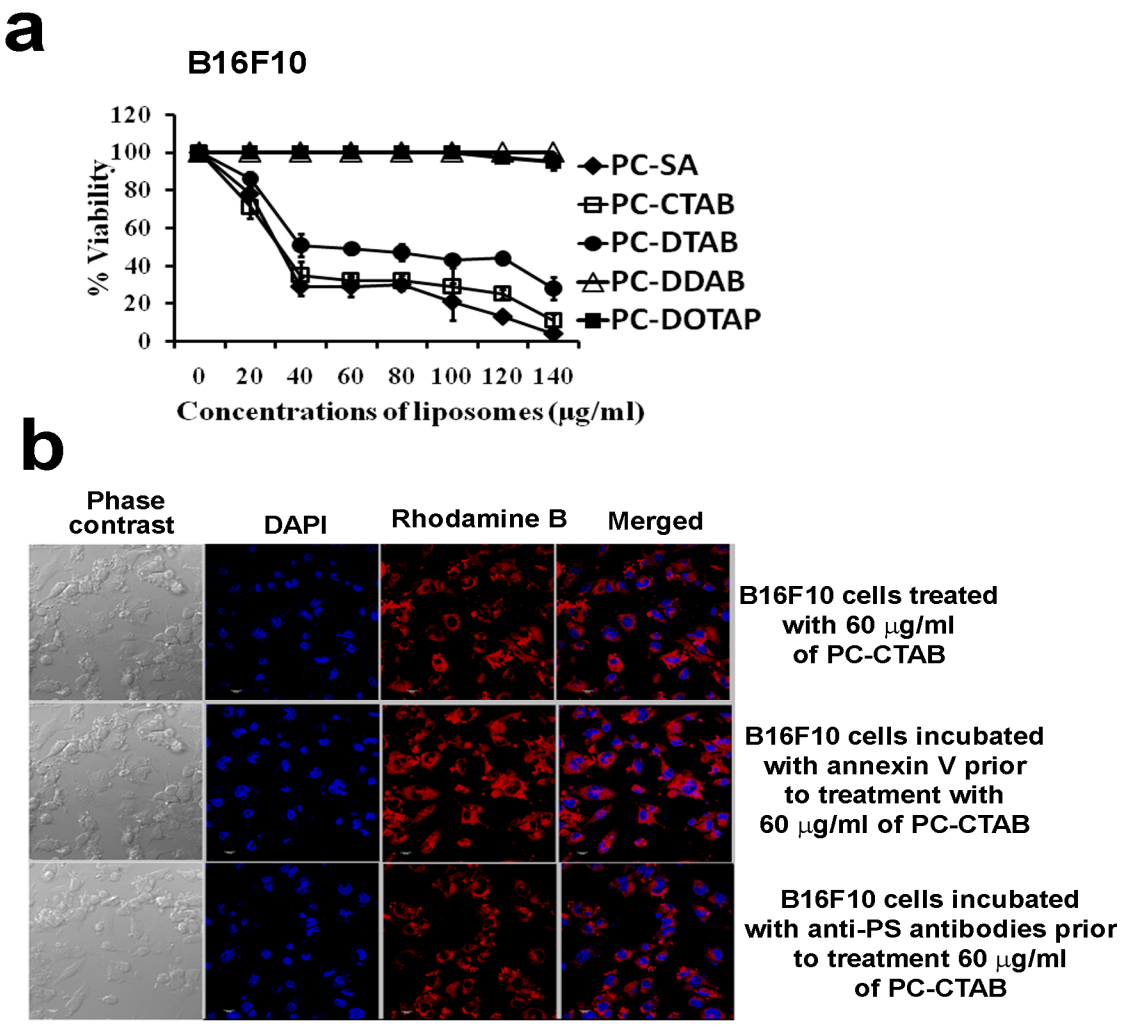

Figure S 4: Effect of different cationic liposomes on B16F10 cells. (a) Viability of B16F10 cells after treatment with graded concentrations of 7:2 molar ratio of PC-SA, PC-CTAB, PC-DTAB, PC-DDAB and PC-DOTAP liposomes with respect to PC for 2 h. Data represent the mean of three separate experiments with error bars indicating the standard error of the mean. (b) Interaction of Rhodamine B PC-CTAB liposomes for 2 h with cancer cell line B16F10 in presence or absence of annexin V or anti-PS antibodies. Cells were visualized under confocal microscope (Leica TCS SP8, software LAS-X), Scale bar: 10 µm.

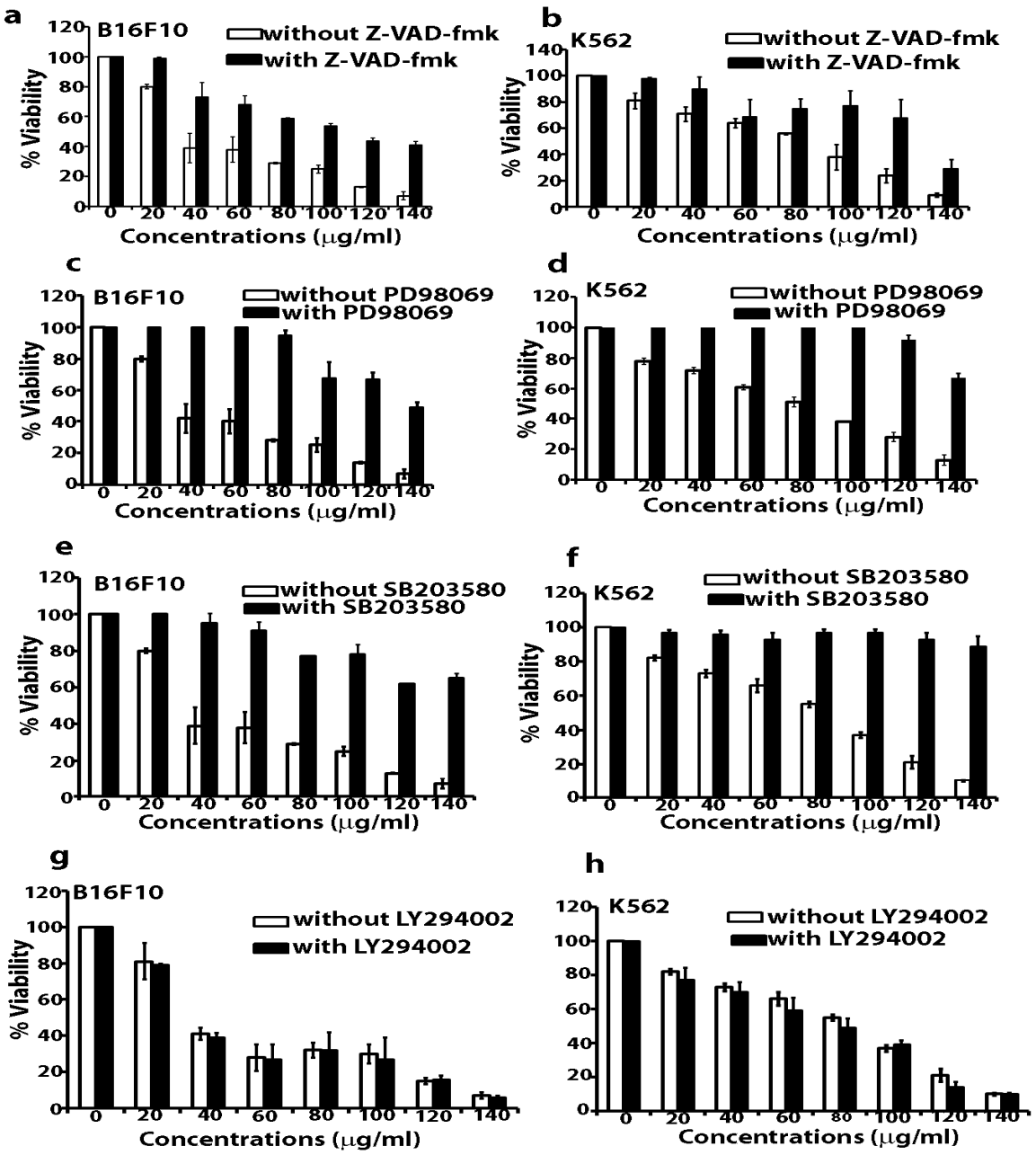

Figure S 5: PC-SA killing activity is Caspase, MAPK and PI3K dependent. (a,c, e, and g) B16F10 and (b, d, f and h) K562 cells were treated with graded doses of PC-SA liposomes (20-140 μg/ml) with respect to PC in the presence or absence of 10 μM of pancaspace inhibitor Z-VAD-fmk, ERK inhibitor PD98069, p38 inhibitor SB203680 and AKT inhibitor LY294002. All data represent the mean of triplicate experiments, with error bars indicating the s.e.m.

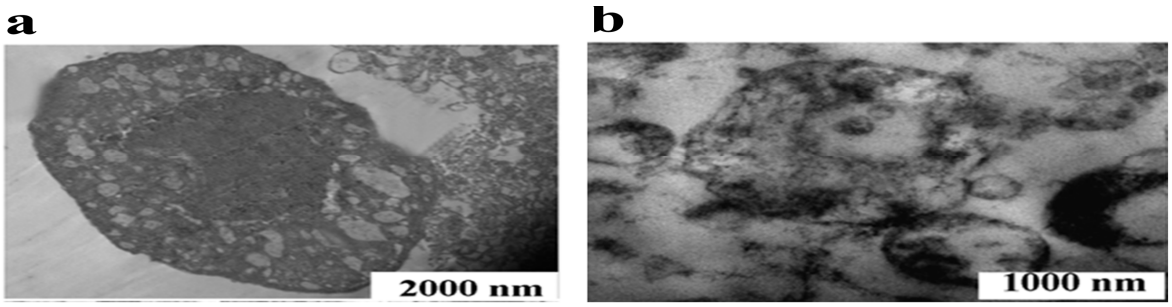

Figure S 6: Transmission electron microscopy of U937 cells treated with PC-SA liposomes for 4 h. U937 cells were incubated for 4 h under standard conditions with medium alone (a), and 140 µg/ml (b) of PC-SA liposomes with respect to PC. Representative of electron micrograph of 140 µg/ml treated cells revealed extensive vacuolization and membrane breakage as well as depletion of electron-dense cytoplasmic material indicating that cell death is in process. Scale bars: 2000 nm (a), 1000 nm (b).

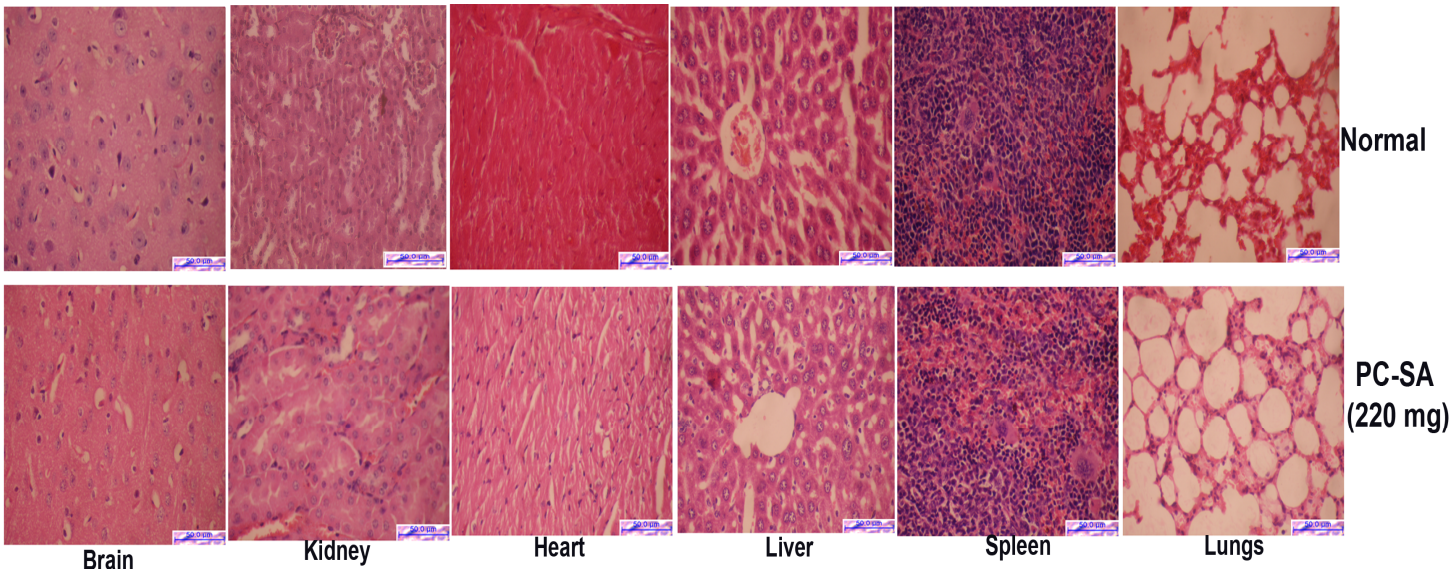

Figure S7: Acute toxicity study. Histopathology of vital organs (brain, kidney, heart, liver, spleen and lungs) in normal and 220 mg of PC-SA liposome treated (within 24 h) Swiss albino mice 15 days after i.v. administration. Scale bar 50 µm and magnification 40x.

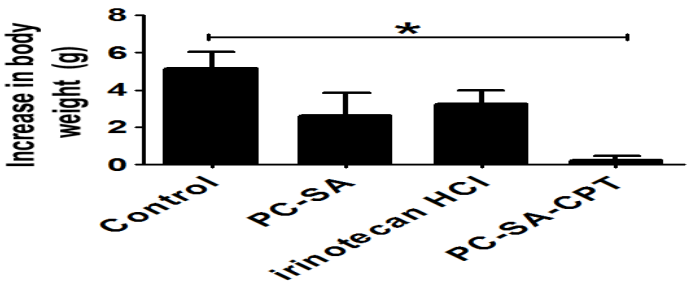

Figure S 8: No increase in body weight between day 0 and 21 of EAC innoculated Swiss albino mice treated with PC-SA-CPT. Mice were innoculated with EAC cells i.p on day 0 and on day 3. Mice were treated with 22 mg of PC-SA, 20 mg/kg body weight of irinotecan HCl and 20 mg/kg body weight of CPT entrapped in 22 mg of PC-SA. Body weights of the mice were recorded on day 0 and 21 and increase in body weights were calculated. All data represent the mean of triplicate experiments, with error bars indicating the s.e.m.

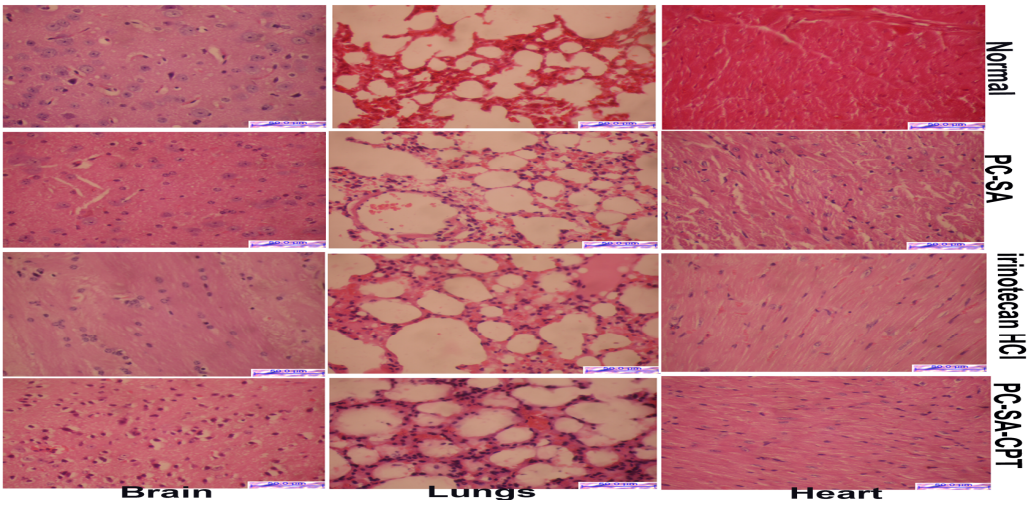

Figure S 9: Toxicity study of brain, lungs and heart in normal Swiss albino mice. Histopathological examinations of the tissues of brain, lungs and heart in normal, 22 mg PC-SA liposome, 20 mg/kg irinotecan HCl and 20 mg/kg CPT entrapped in 22 mg of PC-SA liposome treated Swiss albino mice 15 days after i.v. administration. Scale bar 50 μm and magnification 40x.

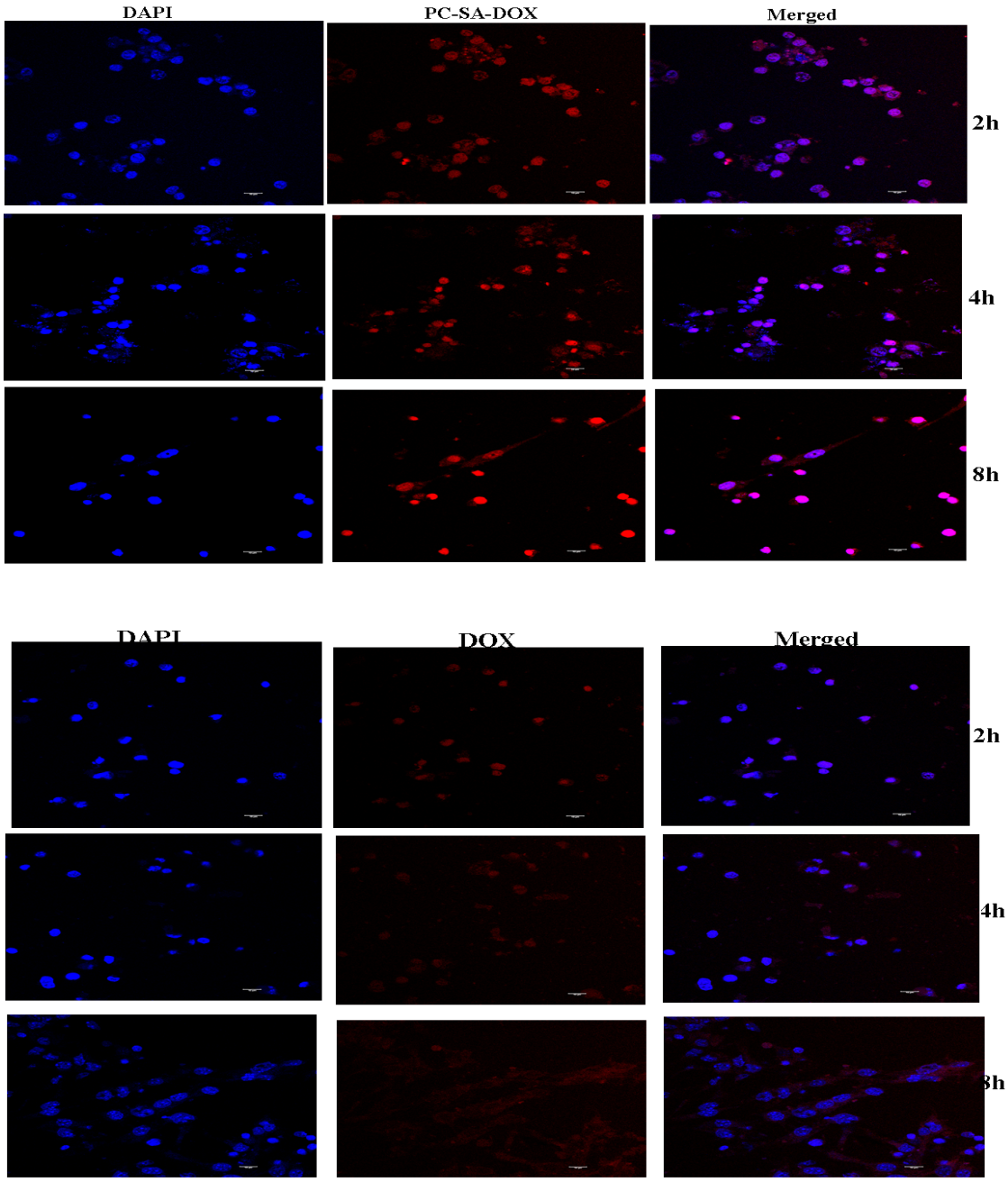

Figure S 10: Uptake of PC-SA-DOX and free DOX by B16F10 tumor cells: Confocal microscopy (Leica TCS SP8, software LAS-X) of B16F10 tumor cells taken out at 2, 4 and 8 h time points from B16F10 tumor induced C57BL6 mice injected s.c. with 4 mg/kg of free DOX or 4 mg/kg of DOX entrapped in 11 mg of PC-SA liposome. Scale bar 10 µm.

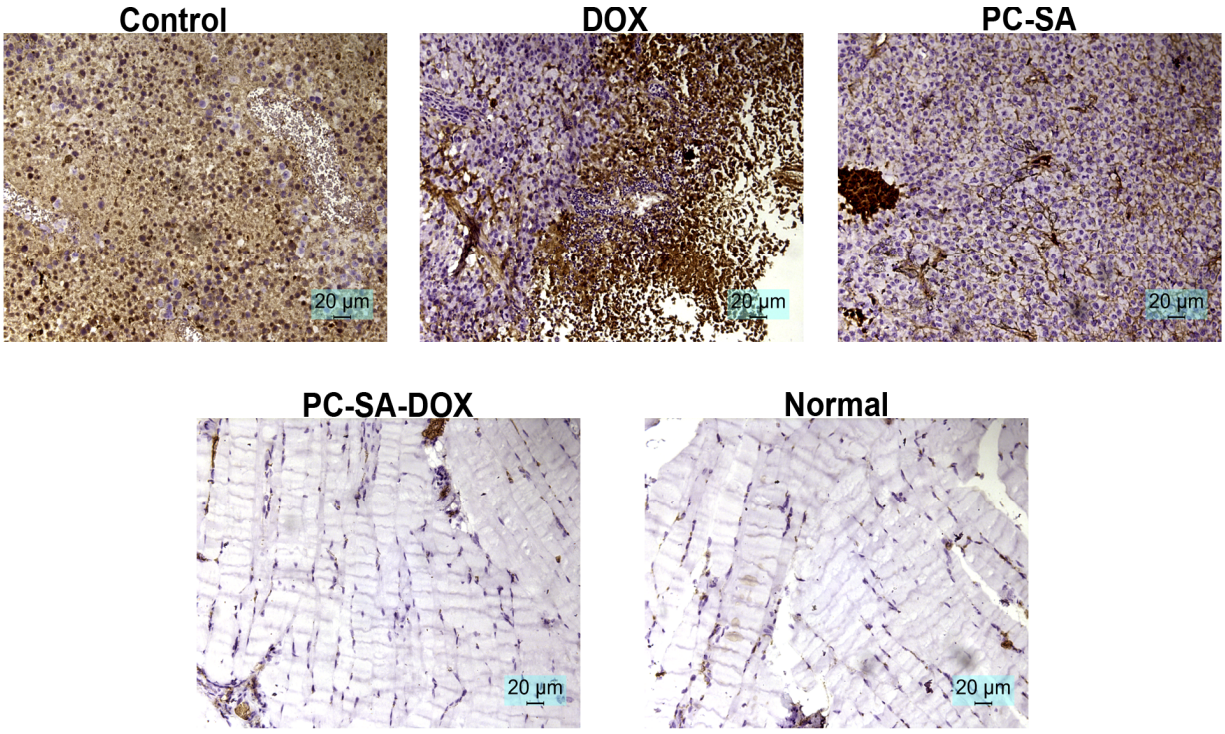

Figure S 11: Immunohistochemical analysis of tumor and normal tissue sections for expression of CD31 (marker used in vascular tumor). Tumor tissue sections obtained from B16F10 tumor induced C57BL6 mice treated on days 7 and 16 of tumor induction with 60 mg/kg of PC-SA, 2.8 mg/kg of DOX and 2.8 mg/kg of DOX entrapped in 60 mg/kg of PC-SA mice and normal tissue section underwent immunohistochemical analysis for the expression of CD31 and visualized under microscope (magnification, 40x, Scale bar: 20 µm).

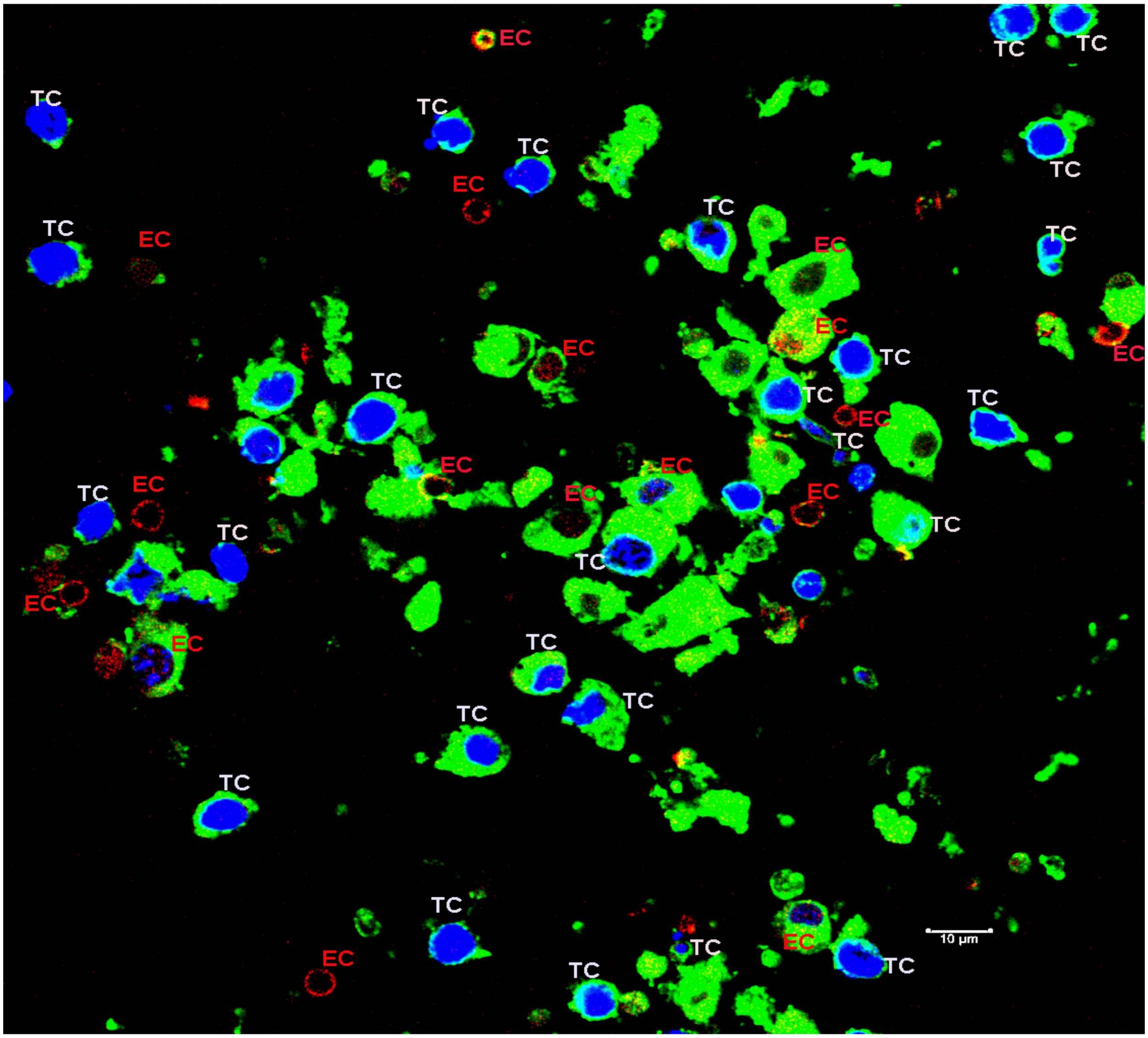

Figure S 12: Uptake of PC-SA liposome by endothelial cells and tumor cells: Confocal microscopy (Leica TCS SP8, software LAS-X) of endothelial cells (marked red EC) and tumor cells (marked TC) isolated from B16F10 tumor induced in C57BL6 mice and injected at the tumor site with PC-SA-FITC (green) liposome. Nuclei are stained with DAPI (blue). Scale bar: 10µm.

Supplementary Table 1

Time (hour)	**^{99m}Tc-PC-SA liposome Labelling Efficiency (%)**
1	**96.41 ± 0.18**
2	**95.53 ± 0.29**
3	**93.81 ± 0.34**
4	**90.66 ± 0.27**

Supplementary Table 1. Stability of ^{99m}Tc-PC-SA lipsome at room temperature at different time points, values represent mean ± Standard Error (SE) (n = 3).