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Supporting Information

Choline Phosphate Lipid Insert and Rigidify Cell Membrane for Targeted Cancer Chemo-immunotherapy

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1 Materials and characterization

1.1 Materials

Palmitic acid (PA), 3-diisopropylcarbodiimide (DIC), dimethylaminopyridine (DMAP), 2-chloro-2-oxo-1,3,2-dioxaphospholane, 1-butyn-4-ol, 3-azidopropylamine, 3-(dimethylamino)-1,2-propanediol, 1,2-dipalmioyl-sn-glycero-3-phosphocholine (PC-Lip) and dipalmitonyl phosphatidyl ethanolamine (DPPE) were purchased from Aladdin (Beijing, China). Dichloromethane, acetonitrile, and ethanol were dried over CaH_2 for 48h and distilled under vacuum before used. Azide-polyethylene glycol-carboxyl and DSPE-PEG2000 were purchased from Ponsure Biological (Shanghai, China). PDL1 monoclonal antibody was purchased from Wuhan Sanying (Wuhan, China). Alexa fluor 488, Alexa fluor 633 dyes and Wheat Germ Agglutinin-633 dyes were purchased from Thermo Fisher (Shanghai, China). Fetal bovine serum (FBS), cell culture media (DMEM), trypsin, Celltiter-Blue were purchased from Powertek Biotechnology (Beijing, China). Female C57 mice (5-6 weeks old; weight, 18-22g) were purchased from Vital River Company (Beijing, China) and kept at an SPF-level laboratory (Northeast Normal University).

1.2 Characterization

The ^{1}H and ^{31}P NMR spectra were recorded on the 400 NMR (AV-400 Bruker) and 500 NMR (AVANCEⅢ 500HD, Bruker). The molecular weight of materials were performed on ESI-MS. The charge and the size distribution of the liposome in PBS medium were measured via DLS on Zetasizer Nano-ZS (Malvern). The morphology of these nanoparticles were measured by transmission electron microscopy (TEM) using JEOL JEM-1011 electron microscope (Bruker), scanning electron microscope (SEM) (Hitachi S4700, Japan) and optical microscope. The fluorescence labelled liposome trace was monitored by confocal laser scanning microscopy (LSM 700 Carl Zeiss Microscopy). Isothermal Titration Calorimetry was performed by using the VP-ITC high-sensitivity titration calorimeter (MicroCal). The synergy microplate reader (Synergy H1, from Bio Tek) was used to detect the call viability. The phase transition temperature (Tm) was determined via differential scanning calorimetry (DSC) (MC-DSC4100, Calorimetry Sciences Corp.). The Dox content loaded in the liposomes was determined by UV-vis spectroscopy (NanoDrop 2000c, Thermo Scientific). The internalization efficiency and immunofluorescence analysis were obtained via flow cytometry (Guava easyCyte 6-2L, Milipore).

2 Synthetic procedures

All the chemicals schematics shown in Figure S1 have been synthesized by the

following procedures.

2.1 The synthesis of 1

1 was synthesized based on our previous method.[1] In brief, all glassware were flam dried and protected by argon before using. ethanol (0.1mol, 4.6g) prepared via distilling CaH_2 mixed solution, 2-chloro-2-oxo-1,3,2-dioxaphospholane (0.1mol, 14.21g), and THF (150mL, dried via Na and distilled freshly before using) were added to 250mL schelenk flask under the argon protection. The solution was stirred and cooled to -78 °C. Then, the trimethylamine (0.11 mol, 11.11g, dried via CaH_2 and distilled freshly before using) was added dropwise about 2h, and the solution was reacted at room temperature for another 4h. After that, the solution was cooled to -40 °C to filter off the white precipitate, the filtrate was directly distilled at Schlenk flask, where the whole process was conducted under argon atmosphere. Finally, the obtained liquid was purified via vacuum distillation, and the **1** was collected with yield 76.4%.^{1}H NMR (500MHz, $CDCl_3$): 4.44～4.35 (m, -OC***H***$_2$C***H***$_2$O-), 4.15 (m, -OC***H***$_2$CH$_3$), 0.93 (t, -OCH$_2$C***H$_3$***); ^{31}P NMR (500MHz, $CDCl_3$): δ (ppm) 17.60 (s).

2.2 The synthesis of 2

2 was synthesized based on our previous method.[1] In brief, all glassware were flam dried and protected by argon before using. 1-butyn-4-ol (0.1mol, 7.00g) prepared via distilling CaH_2 mixed solution, 2-chloro-2-oxo-1,3,2-dioxaphospholane (0.1mol, 14.21g), and THF (150mL, dried via Na and distilled freshly before using) were added to 250mL Schlenk flask under the argon protection. The solution was stirred and cooled to -78 °C. Then, the trimethylamine (0.11 mol, 11.11g, dried via CaH_2 and distilled freshly before using) was added dropwise about 2h, and the solution was reacted at room temperature for another 4h. After that, the solution was cooled to -40 °C to filter off the white precipitate, the filtrate was directly distilled at Schlenk flask, where the whole process was conducted under argon atmosphere. Finally, the obtained liquid was purified via vacuum distillation, and the **2** was collected with yield 84.9%. ^{1}H NMR (500MHz, $CDCl_3$): 4.47 (m, -OC***H***$_2$C***H***$_2$O-), 4.26 (m, -OC***H***$_2$CH$_2$C≡CH), 2.45 (m, -OCH$_2$C***H$_2$***C≡CH), 2.02 (s, -OCH$_2$CH$_2$C≡C***H***), ^{31}P NMR (500MHz, $CDCl_3$): δ(ppm) 17.35 (s).

2.3 The synthesis of 3

Palmitic acid (23 mmol, 6.0g), 3-(dimethylamino)-1,2-propanediol (10 mmol, 1.19g), DMAP (2.0 mmol, 250mg) and DIC (23 mmol, 2.9g) were dissolved in dichloromethane (150mL, dried via CaH_2 before use). After stirred at room temperature for 24h, the solution was filtered to remove the precipitate, and the filtrate was dried by rotary evaporation. Finally, the residue was purified by silica column chromatography with a mixture of petroleum ether/ethyl acetate (3/1, v/v). The product was obtained via removing the solution with yield 94%. ^{1}H-NMR result and ^{31}P-NMR result of **3** were

shown in Figure S2. ^{1}H NMR (500MHz, $CDCl_3$): δ (ppm) 5.19 (m, -OC***H***(CH_2O-)CH_2N-), 4.35, 4.11 (m, -OOC***H₂***CH-), 2.45 (m, -N-C***H₂***-CH-), 2.30(m, -CH_2C***H₂***COO-), 2.26 (s, -CH_2-N(C***H₃***)$_2$), 1.61 (m, -C***H₂***CH_2COO-), 1.26 (m, CH_3C***H₂***-), 0.88 (m, C***H₃***CH_2-).

2.4 The synthesis of 4 (CP-Lip)

4 was synthesized via ring-opening reaction. All glassware were flame dried and protected by argon before using. Briefly, **1** (0.012mol, 1.82g) and **3** (0.01 mol, 5.96g) were added to 50mL acetonitrile (dried via Na and distilled freshly before using). The reaction was conducted at 70 °C for 48h, and then the solution was precipitated three times via THF, after removed the THF, the **4** was obtained with yield 63%. ^{1}H-NMR result, ^{13}C-NMR result and ^{31}P-NMR result of **4** were shown in Figure S3. ESI-MS result of **4** was shown in Figure S4. ^{1}H NMR (500MHz, $CDCl_3$): 5.67 (s, -$COOCH_2$C***H***OOC-), 4.50, 4.12 (d, -COOC***H***$_2$CH(OOC-), 4.35 (m, -PO_4^--C***H***$_2CH_3$-), 4.12 (t, -CH_2C***H***$_2$-PO_4^--), 3.90 (t, -N^+C***H***$_2CH_2$-), 3.79 (d, -COOC***H₂***CH-), 3.32 (d, N^+-(C***H***$_3$)$_2$), 2.28 (t, -CH_2C***H***$_2$COO-), 1.58 (t, -C***H***$_2CH_2$COO-), 1.26 (t, CH_3(C***H***$_2$)$_{12}CH_2$-, -PO-CH_2C***H***$_3$), 0.88 (t, C***H***$_3$(CH_2)$_{12}CH_2$-); ^{13}C NMR (500MHz, $CDCl_3$): 65.89 (-$COOCH_2$***C***HOOC-), 65.35-58.35 (-COO***C***H_2CHOOC-, -PO_4-***C***H_2CH_3-, -CH_2***C***H_2-PO_4, -N^+***C***H_2CH_2-, -COO***C***H_2CH-), 52.55 (N^+-(***C***H_3)$_2$), 34.21-22.69 (-CH_2CH_2***C***OO-, -CH_2***C***H_2COO-, -***C***H_2CH_2COO-, CH_3(***C***H_2)$_{12}CH_2$-), 16.76 (-PO-CH_2***C***H_3), 14.12 (***C***H_3(CH_2)$_{12}CH_2$-); ^{31}P NMR (500MHz, $CDCl_3$): δ (ppm) -0.65 (s). ESI-mass spectrum found 748.7 (M+H), 770.7 (M+Na), 1496.6 (M+M+H).

2.5 The synthesis of 5

5 was synthesized via ring-opening reaction. All glassware were flame dried and protected by argon before using. Briefly, **2** (0.012mol, 2.11g) and **3** (0.01 mol, 5.96g) were added to 50mL acetonitrile (dried via Na and distilled freshly before using). The reaction was conducted at 70 °C for 48h, and then the solution was precipitated three times via THF, after removed the THF, the **5** was obtained with yield 57%. ^{1}H-NMR result ^{13}C-NMR result and ^{31}P-NMR result of **5** were shown in Figure S5. ESI-MS result of **5** was shown in Figure S6. ^{1}H NMR (500MHz, $CDCl_3$): 5.56 (s, -$COOCH_2$C***H***(OOC-), 4.41, 4.05 (d, -COOC***H***$_2$CH(OOC-), 4.25 (m, -PO_4^--C***H***$_2CH_3$-), 4.02 (t, -CH_2C***H***$_2$-PO_4^--), 3.87 (t, -N^+C***H***$_2CH_2$-), 3.71 (d, -COOC***H₂***CH-), 3.25 (d, N^+-(C***H***$_3$)$_2$), 2.43 (t, -CH_2C***H₂***-C≡CH-), 2.19 (t, -CH_2C***H***$_2$COO-), 1.99 (-C≡***CH***-), 1.50 (t, -C***H***$_2CH_2$COO-), 1.16 (t, CH_3(C***H***$_2$)$_{12}CH_2$-), 0.77 (t, C***H***$_3$(CH_2)$_{12}CH_2$-); ^{13}C NMR (500MHz, $CDCl_3$): 76.72 (-C≡***C***H), 64.85 (-$COOCH_2$***C***HOOC-), 64.62-58.02 (-COO***C***H_2CHOOC-, -PO_4-***C***H_2CH_2-, -CH_2***C***H_2-PO_4, -N^+***C***H_2CH_2-, -COO***C***H_2CH-), 51.66 (N^+-(***C***H_3)$_2$), 33.22-22.05 (-CH_2CH_2***C***OO-, -CH_2***C***H_2COO-, -***C***H_2CH_2COO-, CH_3(***C***H_2)$_{12}CH_2$-), 13.10 (-PO-CH_2***C***H_3); ^{31}P NMR (500MHz, $CDCl_3$): δ (ppm) -1.04

(s). ESI-mass spectrum found 794.5 (M+Na), 1566.4 (M+M+Na).

2.6 The synthesis of 6

Azide-polyethylene glycol-carboxyl (1.2 mmol, 1.2g), **5** (1 mmol, 772mg), copper sulfate pentahydrate (12.5mg), sodium ascorbate (20mg) and mixed solvent (5mL, chloroform: methanol=4:1) were transferred to a 20mL one-necked flask. The reaction mixture was stirred for 24h at room temperature. Finally, dialysis was used to remove the excessive azide-polyethylene glycol-carboxyl and the copper catalyst. After freeze-drying, **6** was collected with yield 35%. ^{1}H-NMR result and ^{31}P-NMR result of **6** were shown in Figure S7. ^{1}H NMR (500MHz, $CDCl_3$): 8.11 (s, -C-C***H***-N_3-), 5.68 (s, -$COOCH_2C$***H***(OOC-), 5.35 (t, -CH_2C***H$_2$***-C-N_3-), 4.52 (d, -$COOC$***H$_2$***CH(OOC-), 4.13 (m, -PO_4^--C***H$_2$***CH_3-, -CH_2C***H$_2$***-PO_4^--, -N^+C***H$_2$***CH_2-, -$COOC$***H$_2$***CH-), 3.65 (m, -C***H$_2$C H$_2$***O-), 3.33 (s, N^+-(C***H$_3$***)$_2$), 2.27 (t, -CH_2C***H$_2$***COO-), 1.58 (t, -C***H$_2$***CH_2COO-), 1.26 (t, $CH_3(C$***H$_2$***$)_{12}CH_2$-), 0.88 (t, C***H$_3$***$(CH_2)_{12}CH_2$-); ^{31}P NMR (500MHz, $CDCl_3$): δ (ppm) -0.39 (s).

2.7 The synthesis of 7 (CP-αPDL)

6 (20mg), NHS (5mg) and DIC (5mg) were dissolved in chloroform then stirred at room temperature for 12h. Chloroform was removed under vacuum, then PDL1 monoclonal antibody (20mg) in water solution was added to the mixture and stirred for 5h. Dialysis was used to remove the NHS and DIC to get the solution of **7**. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) result of **7** was shown in Figure S11.

2.8 The synthesis of 8

3-azidopropylamine (1.2 mmol, 120mg), **5** (1 mmol, 772mg), copper sulfate pentahydrate (12.5mg), sodium ascorbate (20mg) and mixed solvent (5mL, chloroform: methanol=4:1) were transferred to a 20mL one-necked flask. The reaction mixture was stirred for 24h at room temperature. Finally, dialysis was used to remove the excessive azide-polyethylene glycol-carboxyl and the copper catalyst. After freeze-drying, **8** was collected with yield 85%. ^{1}H-NMR result and ^{31}P-NMR result of **8** were shown in Figure S8. ^{1}H NMR (500MHz, $CDCl_3$): 8.59 (s, -C-C***H***-N_3-), 7.91 (t, -CH_2C***H$_2$***-C-N_3-), 5.65 (s, -$COOCH_2C$***H***(OOC-), 4.55 (d, -$COOC$***H$_2$***CH(OOC-), 4.13-3.62 (m, -PO_4^--C***H$_2$***CH_3-, -CH_2C***H$_2$***-PO_4^--, -N^+C***H$_2$***CH_2-, -$COOC$***H$_2$***CH-), 3.31 (s, N^+-(C***H$_3$***)$_2$), 2.30 (t, -C***H$_2$C H$_2$***N***H$_2$***-, -CH_2C***H$_2$***COO-), 1.57 (t, -C***H$_2$***CH_2COO-), 1.26 (t, $CH_3(C$***H$_2$***$)_{12}CH_2$-, -CH_2C***H$_2$***CH_2NH_2-), 0.88 (t, C***H$_3$***$(CH_2)_{12}CH_2$-); ^{31}P NMR (500MHz, $CDCl_3$): δ (ppm) -0.41 (s).

2.9 The synthesis of 9 (CP-488)

Alexa fluor 488-NHS dye (1mg) was added to the chloroform solution of **8** (10mg),

then stirred for 12h. Then chloroform was removed under vacuum and dialysis was used to remove the excessive AF488 dyes to get the solution of **9**. ^{1}H-NMR result and ^{31}P-NMR result of **9** were shown in Figure S9. ^{1}H NMR (500MHz, $CDCl_3$): 7.77 (s, -C-C***H***-N_3-), 6.79 (t, -CH_2C***H*****$_2$**-C-N_3-), 5.55 (s, -$COOCH_2C$***H***(OOC-), 4.45 (d, -$COOC$***H*****$_2$**CH(OOC-), 4.17-3.56 (m, -PO_4^--C***H*****$_2CH_3$**-, -CH_2C***H*****$_2$**-PO_4^--, -N^+C***H*****$_2CH_2$**-, -$COOC$***H*****$_2$**CH-), 3.16 (s, N^+-(C***H*****$_3$**)$_2$), 2.26 (t, -CH_2C***H*****$_2NH_2$**-, -CH_2C***H*****$_2$**COO-), 1.51 (t, -C***H*****$_2CH_2COO$**-), 1.18 (t, $CH_3(C$***H*****$_2)_{12}CH_2$**-, -CH_2C***H*****$_2CH_2NH_2$**-), 0.81 (t, C***H*****$_3(CH_2)_{12}CH_2$**-); ^{31}P NMR (500MHz, $CDCl_3$): δ (ppm) -1.27 (s).

2.10 The synthesis of 10 (PC-αPDL)

DSPE-PEG2000 (20mg), NHS (5mg) and DIC (5mg) were dissolved in chloroform then stirred at room temperature for 12h. Chloroform was removed under vacuum, then PDL1 monoclonal antibody (20mg) in water solution was added to the mixture and stirred for 5h. Dialysis was used to remove the NHS and DIC to get the solution of **10**. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) result of **10** was shown in Figure S11.

2.11 The synthesis of 11 (PC-488)

Alexa fluor 488-NHS dye (1mg) was added to the chloroform solution of DPPE (10mg), then stirred for 12h. Then chloroform was removed under vacuum and dialysis was used to remove the excessive AF488 dyes to get the solution of **11**. ^{1}H-NMR result and ^{31}P-NMR result of **11** were shown in Figure S10. ^{1}H NMR (500MHz, $CDCl_3$): 5.23 (s, -$COOCH_2C$***H***(OOC-), 4.42 (d, -$COOC$***H*****$_2$**CH(OOC-), 4.16 (t, -CH_2C***H*****$_2$**-PO_4-), 3.69 (t, -PO_4-C***H*****$_2CH_2$**-), 2.32 (t, -PO_4-CH_2C***H*****$_2$**NH-), 2.04 (t, -CH_2C***H*****$_2$**COO-), 1.40 (t, -C***H*****$_2CH_2COO$**-), 1.30 (t, $CH_3(C$***H*****$_2)_{12}CH_2$**-), 0.87 (t, C***H*****$_3(CH_2)_{12}CH_2$**-); ^{31}P NMR (500MHz, $CDCl_3$): δ (ppm) -1.27 (s).

3 Isothermal Titration Calorimetry (ITC)

Typically, 17 consecutive injections of 2.5 μL each (PC-Lips or CP-Lips at a concentration of 13 mM) were made into the chamber (200 μL) filled with 1.625 mM PC-Lips solution. Both solutions in the syringe and chamber were degassed under vacuum for 30min immediately before use. Injections were made at 300-sec intervals. A constant stirring speed of 400 rpm was maintained during the experiments to ensure sufficient mixing after each injection. For calculation of the binding curve, the heat of dilution was measured in separate titrations and used as background to be subtracted.

4 Preparation of the micelles of liposomes

Micelles of liposomes were prepared via the thin film hydration. Briefly, the PC-Lips and different amount of CP-Lips were dissolved in 5mL chloroform solution. After

completely dissolved, the solution was dried via vacuum distillation using rotary evaporator at 40℃ for 1h to make thin film in 100mL flask. Then, 5mL PBS was added. The solution was sonicated at 60℃ for 15 minutes, and filtered through polycarbonate membrane (pore size: 220 nm) to prepare micelles of PC-Lips, CP-Lips and their mixture. The liposomes solution were determined via dynamic light scattering (DLS).

5 Differential scanning calorimetry (DSC) tests

The T_m for PC-Lip, CP-Lip and their mixture (PC-Lips and different amount of CP-Lips were mixed together in chloroform then removed chloroform under vacuum) were determined via DSC. Data were obtained over a range of 25-100℃ at 5℃/min with a heat-cool-heat cycle, and last heat cycle data were recorded.

6 Cell culture

Mouse melanoma cancer (B16-F10) cell line, human cervical carcinoma (Hela) cell line, mouse embryonic fibroblast (NIH-3T3) cell line, and human lung cancer (A549) cell line were obtained from American Type Culture Collection (ATCC). B16-F10, Hela, A549 and NIH-3T3 cells were incubated with high-glucose DMEM medium supplemented with 10% (v/v) fetal bovine serum (FBS, Gibco) and 1% (w/v) penicillin (100 U/mL)/streptomycin (100 μg/mL) at 37 °C under a 5% CO_2 atmosphere.

7 Confocal Laser Scanning Microscopy (CLSM)

B16-F10 cells were incubated in confocal dishes with the density of 1×10^5 cells. After incubation for 24 h, removed the medium and added fresh medium with micelles of tCP-Lipos-488 (CP-Lip/CP-αPDL/CP-488=6/1/1, molar ratio, the concentration of liposomes were 10μg/mL) and tPC-Lipos-488 (PC-Lip/PC-αPDL/PC-488=6/1/1, molar ratio, the concentration of liposomes were 10μg/mL). Incubated another 0.5h, 12h and 48h, the medium was removed, and washed the cells with PBS three times. Then, 4% formaldehyde was added to fix cells at room temperature for 30min. After that, removed the medium, and added Wheat Germ Agglutinin-633 (WGA-633, 10 μg/mL) to stain cell membrane for 10min at room temperature. Finally, the cells were washed three times with PBS and observed. The NIH-3T3 cells were added with micelles of tCP-Lipos-488, and the steps were done as previously mentioned. For Pearson correlation coefficient analysis, three separate images for the tCP-Lipos-488 and tPC-Lipos-488 experiments at different time were used for colocalization test in Image J to determine the results.

8 Scanning Electron Microscopy (SEM) and optical microscope analysis

B16-F10 cells morphology after incubation with PC-Lips or CP-Lips were assessed

using optical microscope and SEM analysis. B16-F10 cells were equilibrated with micelles of tPC-Lipos (PC-Lip/PC-αPDL=6/1, molar ratio, 100μg/mL) and tCP-Lipos (CP-Lip/CP-αPDL=6/1, molar ratio, 100μg/mL) for 24h in glass dish, respectively. The cell morphology was observed by optical microscope. After that, the cells were washed with PBS for three times, then fixed overnight at room temperature using 2.5 % glutaraldehyde in isotonic saline. The bottom of the glass dish was removed and cut to suitable size. The cells were dehydrated by immersing the glass sheet in 70, 85, 95, 100 % (v/v) ethanol for 10min, respectively. Finally, the cells were dried overnight at room temperature. The cells were coated with gold and examined by SEM.

9 Tumor suppression ratio

B16, NIH-3T3, A549 and Hela cells were incubated in 96 well plates with the density of 1×10^4 cells. After incubation for 12h, the medium was replaced of the fresh containing different amounts of micelles of CP-Lipos (CP-Lip, 10, 50, 100, 200μg/mL), tPC-Lipos (PC-Lip/PC-αPDL=6/1, molar ratio, 10, 50, 100, 200μg/mL) and tCP-Lipos (CP-Lip/CP-αPDL=6/1, molar ratio, 10, 50, 100, 200μg/mL), respectively. Then, the cells were incubated for 24 h, 10 μL Celltiter-Blue reagent was added to each well. After incubated for another 4 h, the cell viability was detected via microplate reader ($\lambda_{ex=}$560 nm, λ_{em}=590 nm). The cell suppression ratio was calculated via following equation:

$$suppression\ ratio\ (\%) = \frac{Fluorescent\ intensity(control) - Fluorescent\ intensity(sample)}{Fluorescent\ intensity(control)} \times 100\%$$

10 Cellular uptake detection via flow cytometry

The cellular uptake of different amount of PC-Lips and CP-Lips was determined via flow cytometry. B16-F10 cells and NIH-3T3 cells were incubated in DMEM medium with 10% FBS, respectively. Then, the cells were seeded in 12 well plates with the density of 2×10^5 cells per well. After incubation for 1 day, the medium was replaced of fresh medium containing micelles of tPC-Lipos (PC-Lip/PC-αPDL=6/1, molar ratio, 10, 50, 100, 200μg/mL) and tCP-Lipos (CP-Lip/CP-αPDL=6/1, molar ratio, 10, 50, 100, 200μg/mL), respectively. Incubation for another 12 h, discarded the medium, and fresh medium with Alexa fluor 633 dyes (5 μg/mL) was added to cells then incubated for 1h. The cells were washed three times with PBS. Then, cells were digested and collected for flow cytometry tests.

11 Preparation of drug loaded liposomes and characterization

Doxorubicin (Dox)-loaded liposomes were prepared via $(NH_4)_2SO_4$ concentration method.[2] Briefly, the PC-Lip/PC-αPDL (molar ratio=6/1) and CP-Lip/CP-αPDL (molar

ratio=6/1) thin film were prepared according to above-mentioned method. Then, 5mL $(NH_4)_2SO_4$ solution (200 mM) was added, and sonicated at 60 ℃ for 15 minutes. The liposomes containing solution was filtered through polycarbonate membrane (pore size: 220 nm). After that, the solution was dialyzed (MCW: 3500) against PBS for 4h. Subsequently, these liposomes were incubation with Dox at room temperature for 30min. Finally, the unloaded Dox was removed via dialyzed (MCW: 3500) against 200mL PBS for 6h, the dialysate was replaced every 3h. Amount of solution was taken out lyophilizing to detect the drug loading content (DLC) and drug loading efficiency (DLE). In brief, after lyophilization, 1mg powder was dissolved in 1mL water, and then, the concentration of Dox was measured via UV-vis spectra according the Dox calibration curve. The size and charge of Dox@tCP-Lipos and Dox@tPC-Lipos were determined by DLS and TEM.

12 In vivo drug release behavior

The Dox release behaviors from liposomes were conducted via dialysis method. In brief, 1mL Dox-loading liposomes (Dox@tCP-Lipos or Dox@tPC-Lipos) was transferred into dialysis bag (MCW:3500), and immersed in 9mL PBS with continuous stirring at 37 ºC. At fixed time intervals, 1mL dialysis solution outside the bag was collected to determine the Dox concentration via UV-Vis spectra, and 1mL fresh PBS was added after determination.

13 Animals and tumor model

C57 female mice (18-21g, 4-6 weeks, purchased from Center for Experimental Animals, Jilin University) were used. All animal procedures were in accordance with the Animal Care and Use Committee of Northeast Normal University. Melanoma was established via subcutaneous injection of B16-F10 cells. The tumor volume was calculated via the following equation: $Volume=0.5\times a\times b^2$, where a and b were represented for length and width, respectively. The length and width of tumor were measured via Vernier caliper.

14 In vivo imaging

The accumulation of liposomes at tumor and main organs was real-time determined via *in vivo* imaging method in Maestro In Vivo Imaging System (Cambridge Research & Instrumentation, Inc., USA). Dox@tCP-Lipos was injected into melanoma bearing mice via tail vein at the Dox dose of 5mg/kg body weight. At the time points of 6, 24, 48h, mice were sacrificed, and the tumor and main organs (heart, liver, spleen, lung, kidney) were excised for NIR fluorescence imaging. Dox was shown in red.

15 In vivo antitumor efficacy

The melanoma-bearing mice were randomly divided into five groups and treated with different samples respective: 1. PBS; 2.tPC-Lipos; 3. Dox@tPC-Lipos; 4. tCP-Lipos; 5. Dox@tCP-Lipos (Dox-equivalent dose of 5mg/kg body weight). These mice were treated at 1^{st}, 3^{nd}, 5^{th} day. The mice body weight and tumor volume were measured every other day. The treatment efficacy could be monitored via determine the relative changes of body weight and tumor volume. After 16 days, all mice were sacrificed, and the tumor and main organs (heart, liver, spleen, lung, kidney) were excised and analysed. Then stained via hematoxylin-eosin (H&E) for pathological analysis.

16 In Vivo Immune Response Analysis

Tumor tissues were harvested on 7 days after treatments and cut into small pieces, followed by digestion using a tumor dissociation kit (miltenyi biotec, Germany) in a benchtop incubating shaker at 37 °C for 1h. The digested tissues were filtered through a 70 μm cell strainer, centrifuged, and the cell pellets were suspended in 1mL ACK lysis buffer for 1min to lyse red blood cells. After centrifugation and washing twice with cold PBS, the single-cell suspension was incubated with antimouse CD3-FITC, CD4-PE and CD8a-APC (Biolegend) for 15min on ice to block nonspecific binding. The cell suspension was fully mixed and incubated for 30min on ice, followed by PBS washing prior to conducting flow cytometry analysis.

17 Statistics

All of the measurements presented are expressed as mean ± standard deviation (S.D.). Student's t-test was used to compare the statistical significance (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

Figures

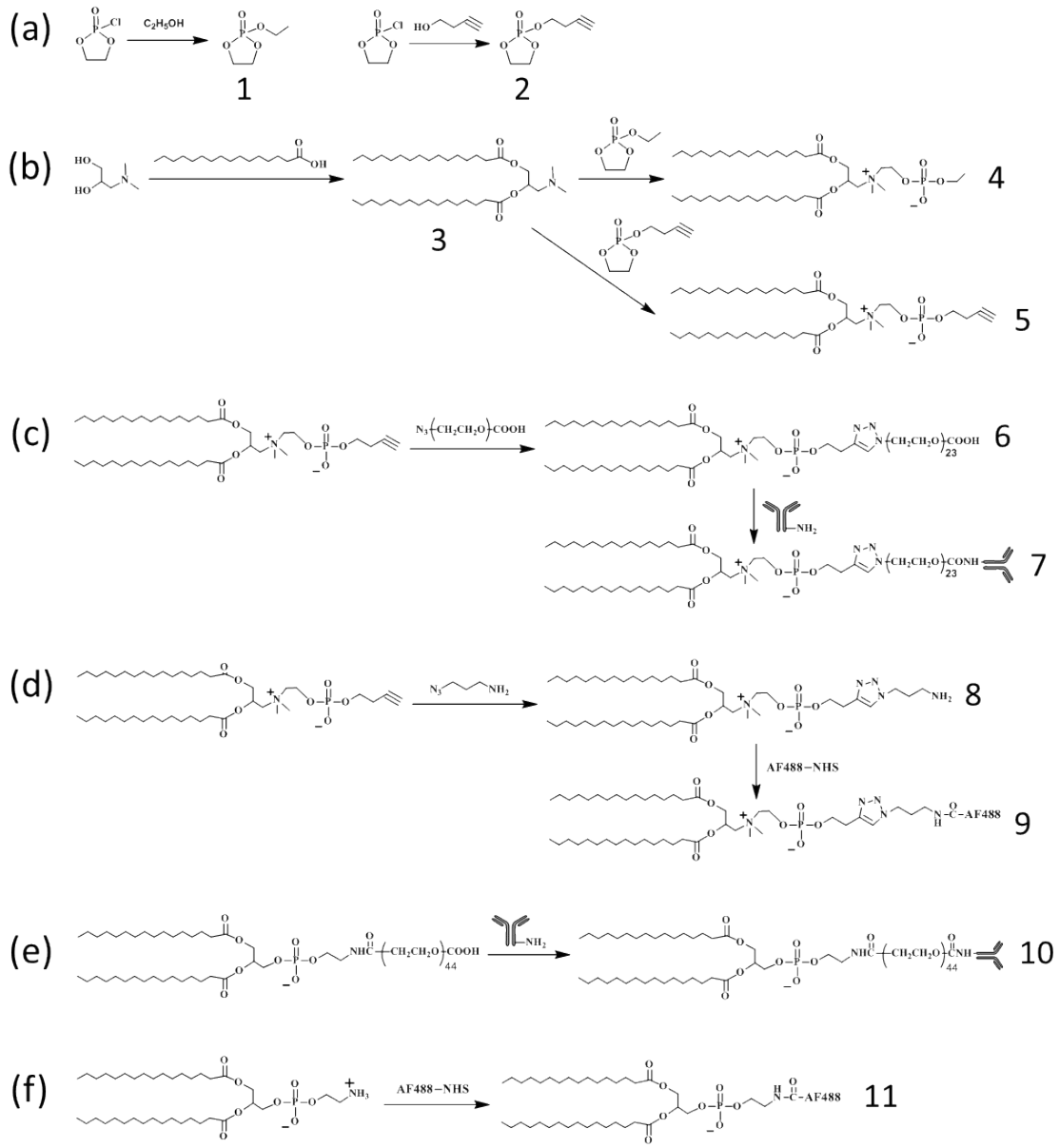

Figure S1. The synthetic procedure of (a) phosphorus heterocyclic; (b) CP-Lip; (c) CP-αPDL; (d) CP-488; (e) PC-αPDL and (f) PC-488.

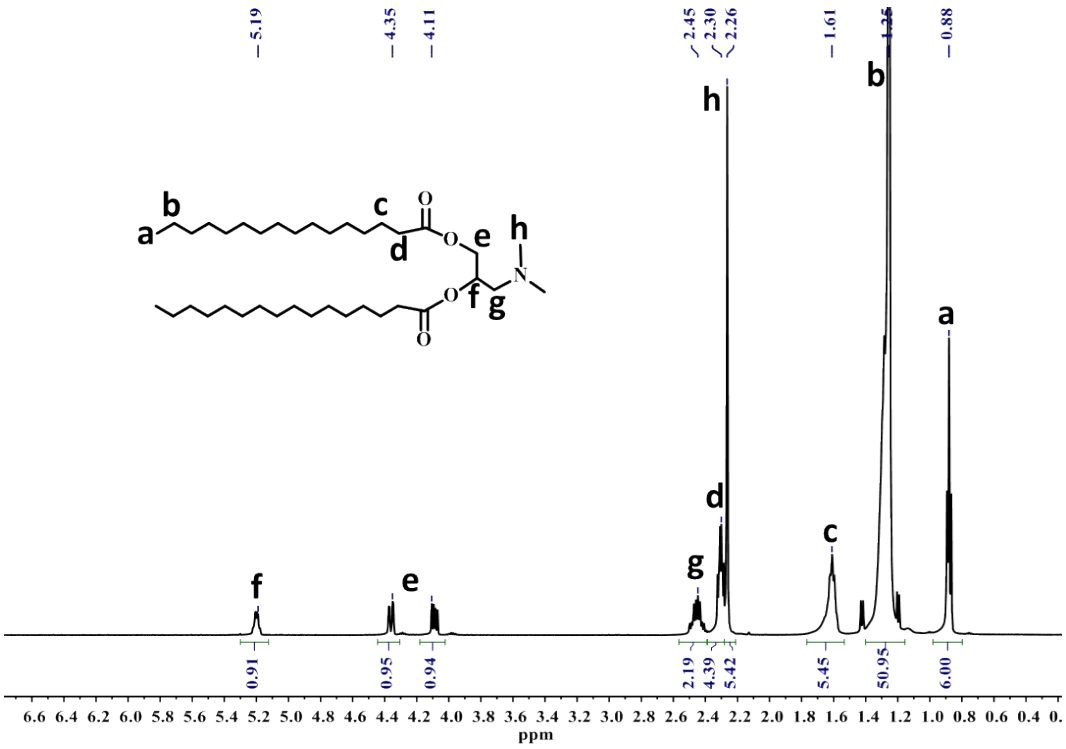

Figure S2. ^{1}H-NMR spectrum of **3** in $CDCl_3$.

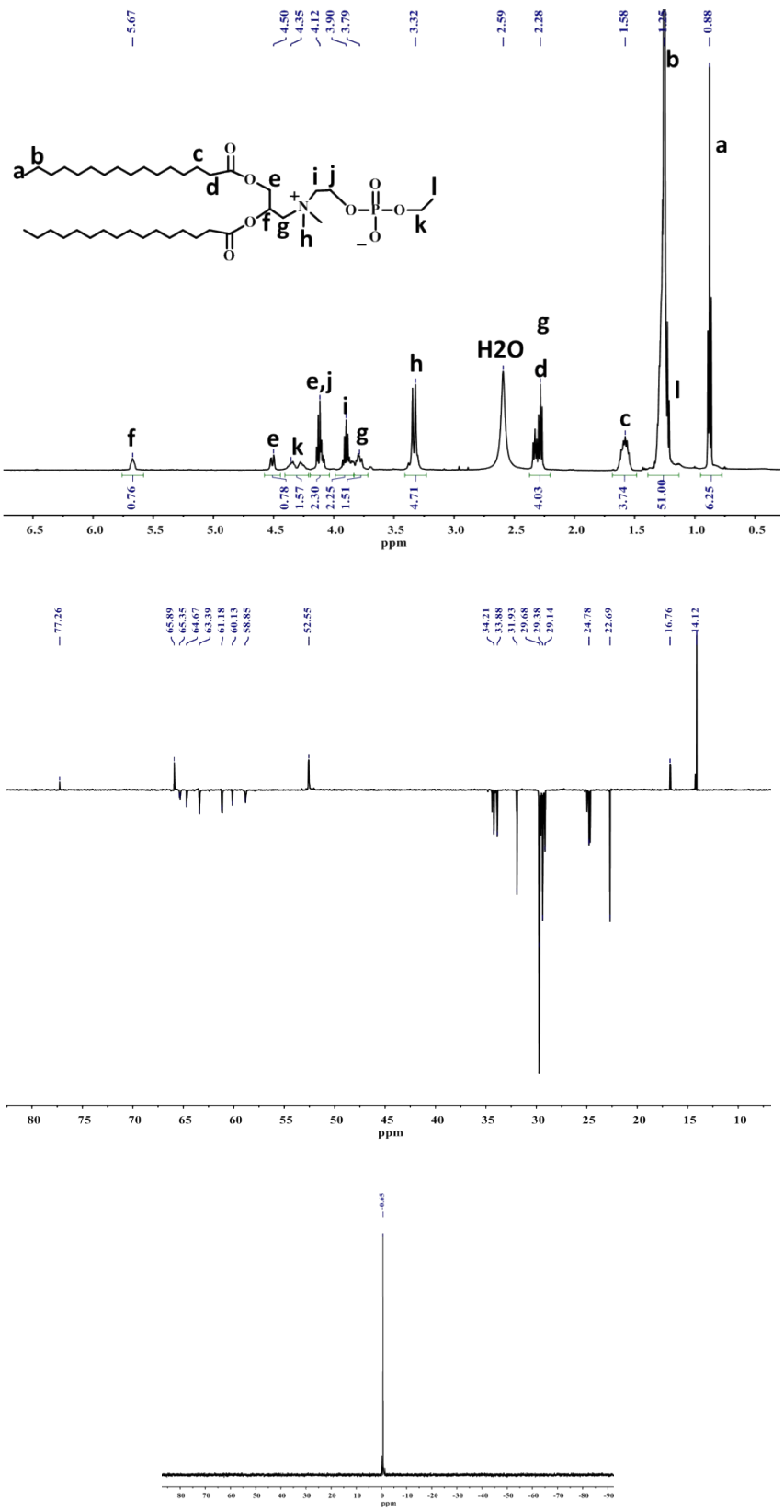

Figure S3. (top) ^{1}H-NMR spectrum of **4** in $CDCl_3$; (medium) ^{13}C-NMR spectrum of **4** in $CDCl_3$; (bottom) ^{31}P-NMR spectrum of **4** in $CDCl_3$.

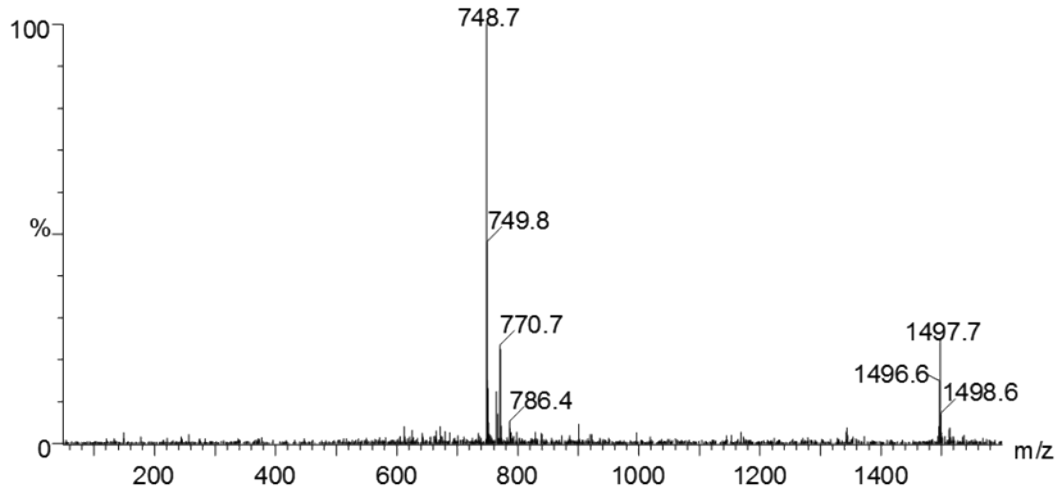

Figure S4. ESI-MS spectrum of **4** (CP-Lip).

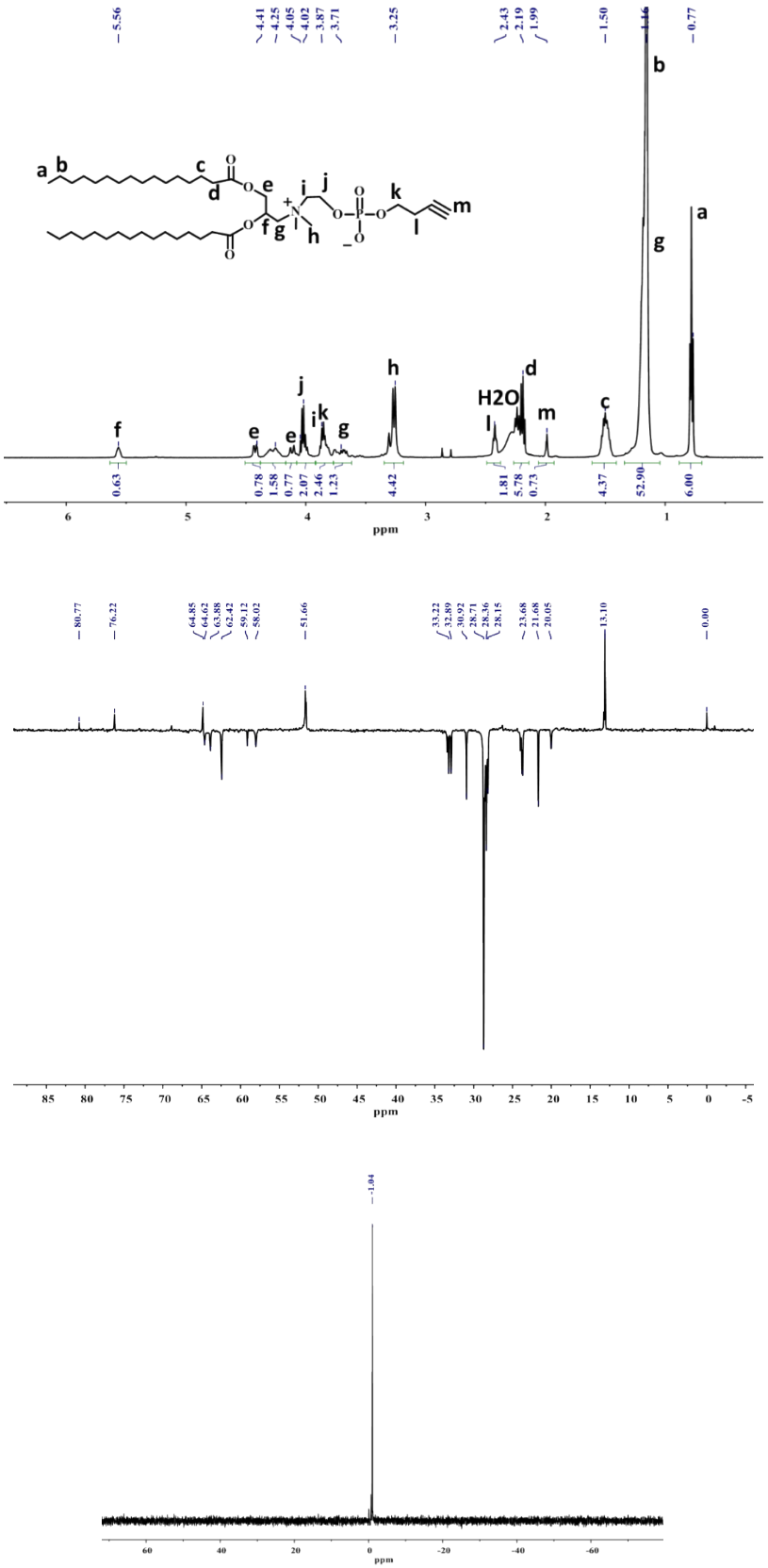

Figure S5. (top) ^{1}H-NMR spectrum of **5** in $CDCl_3$; (medium) ^{13}C-NMR spectrum of **5** in $CDCl_3$; (bottom) ^{31}P-NMR spectrum of **5** in $CDCl_3$.

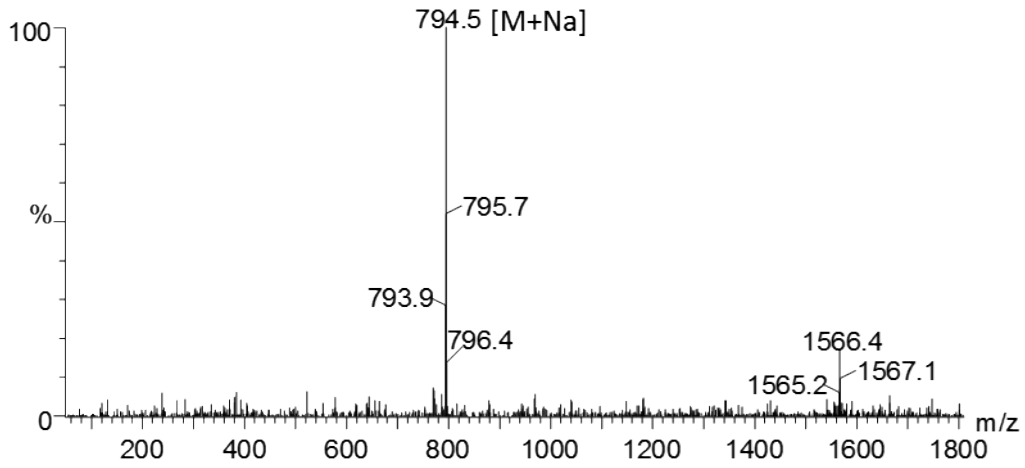

Figure S6. ESI-MS spectrum of **5**.

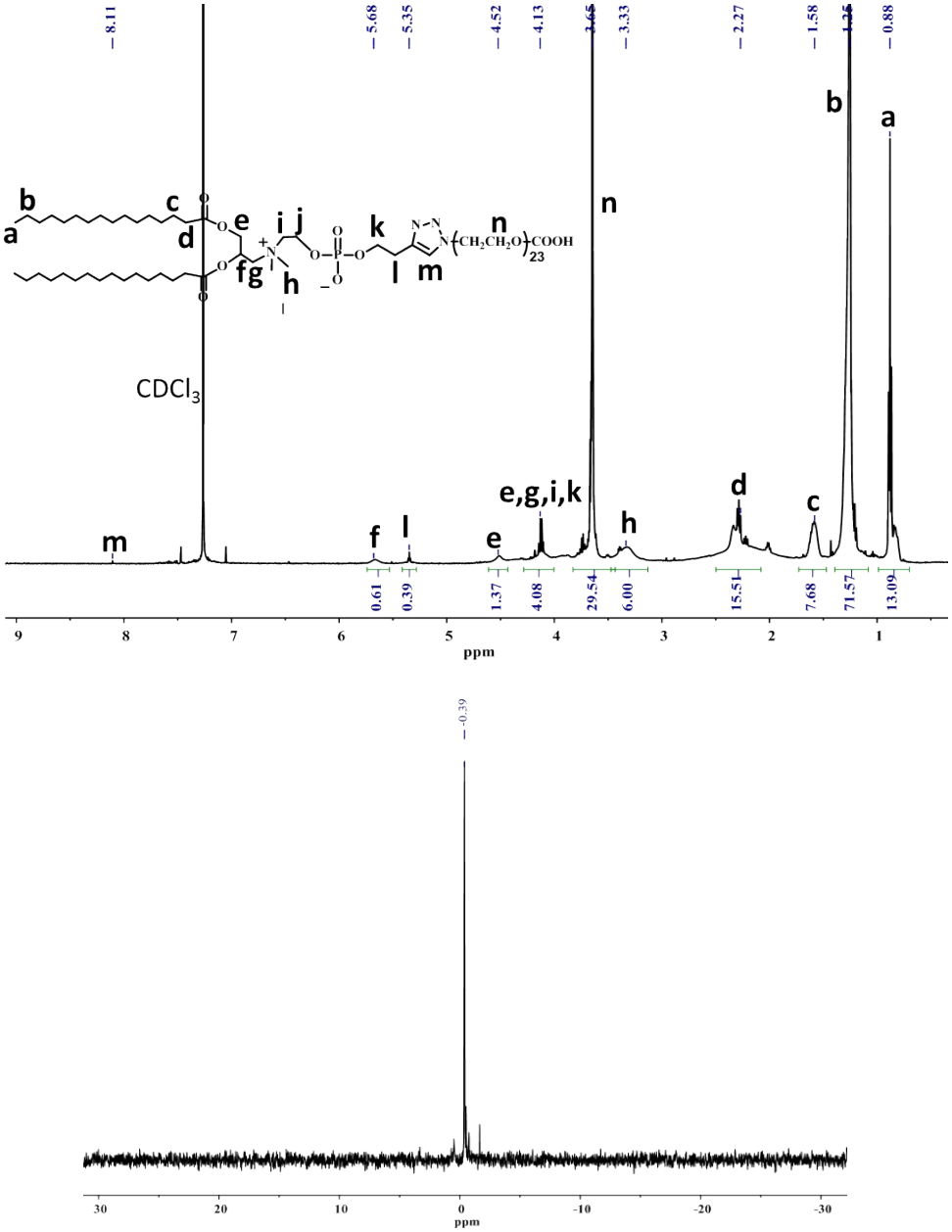

Figure S7. (top) ^{1}H-NMR spectrum of **6** in $CDCl_3$; (bottom) ^{31}P-NMR spectrum of **6** in $CDCl_3$.

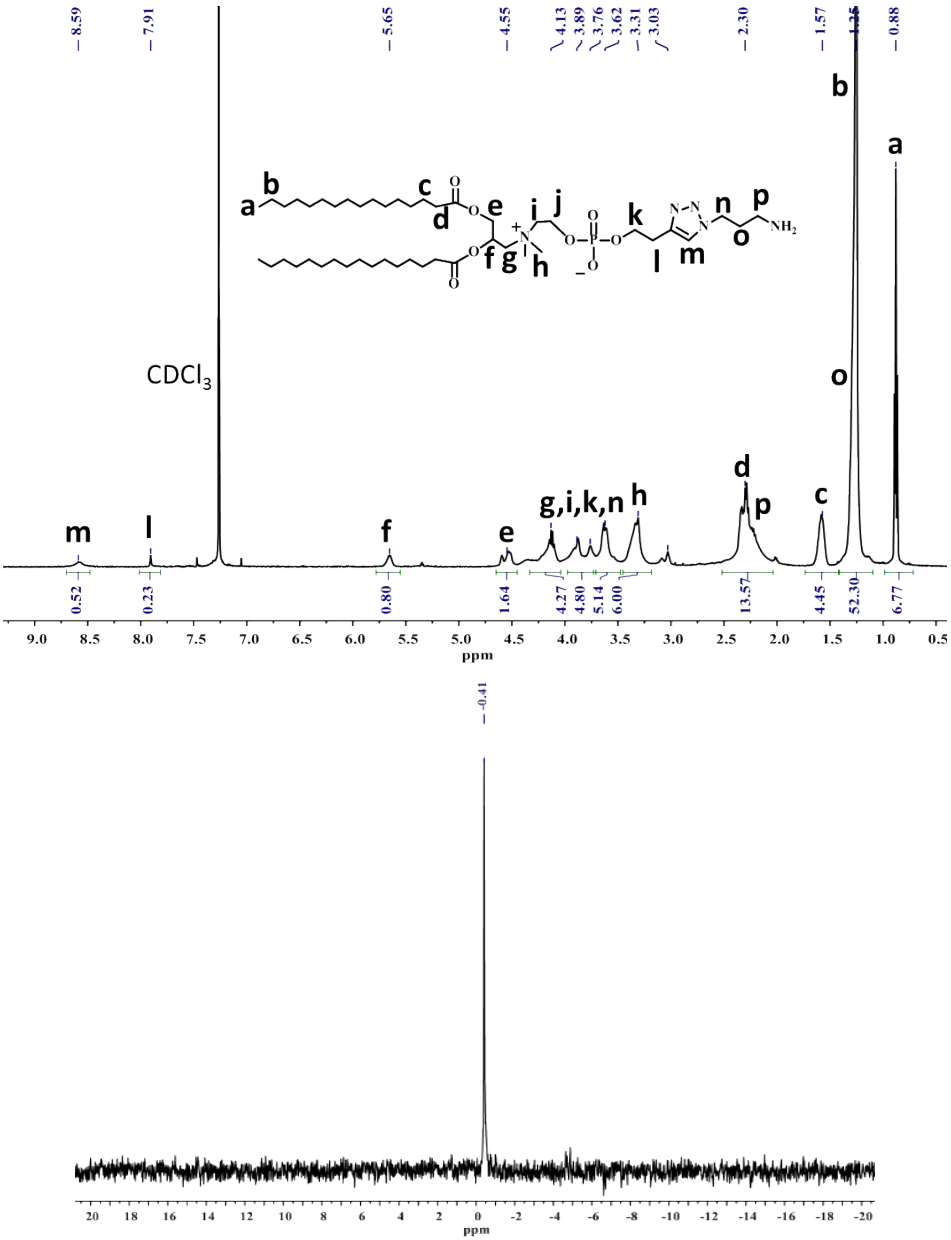

Figure S8. (top) ^{1}H-NMR spectrum of **8** in $CDCl_3$; (bottom) ^{31}P-NMR spectrum of **8** in $CDCl_3$.

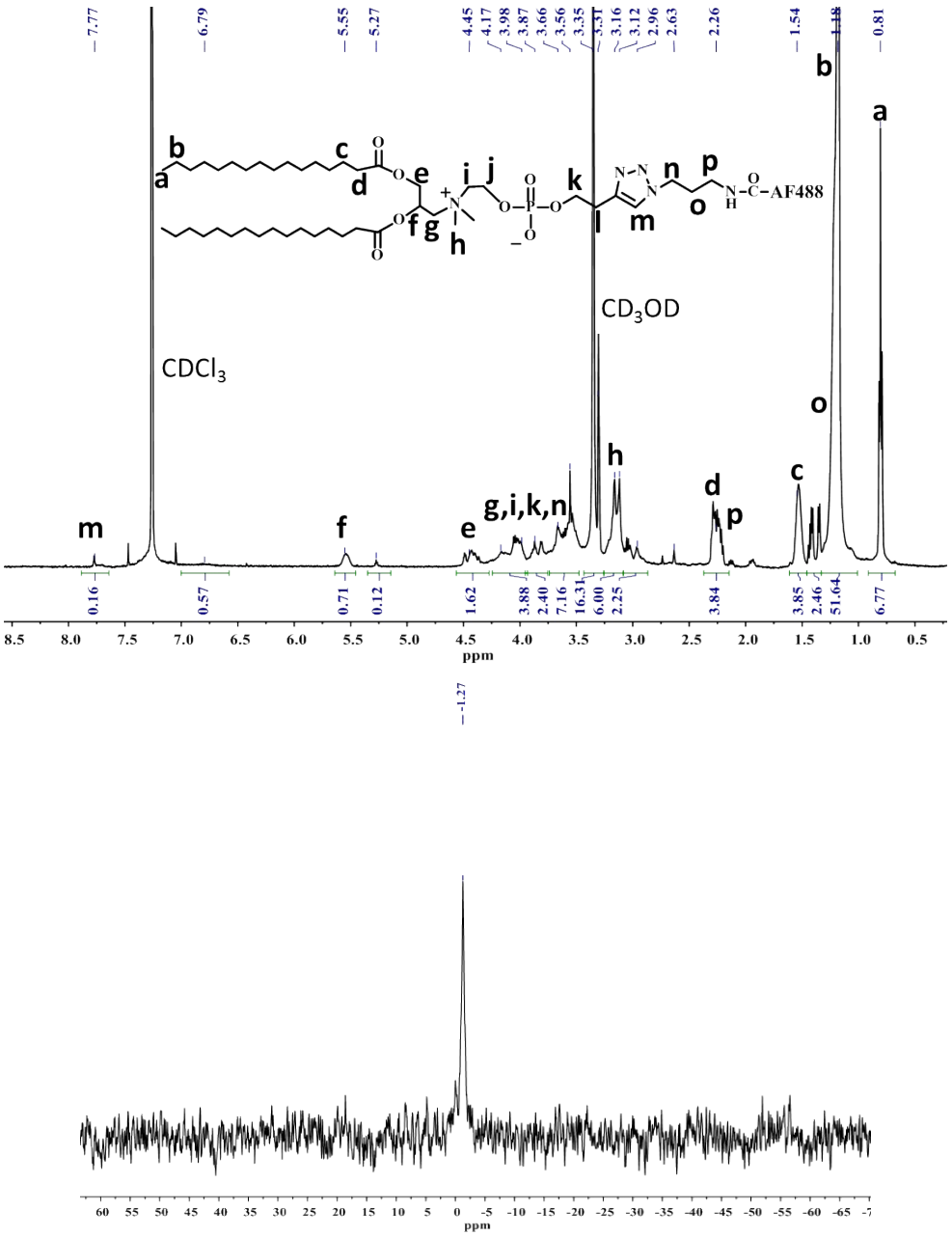

Figure S9. (top) ^{1}H-NMR spectrum of **9** in $CDCl_3$; (bottom) ^{31}P-NMR spectrum of **9** in $CDCl_3$.

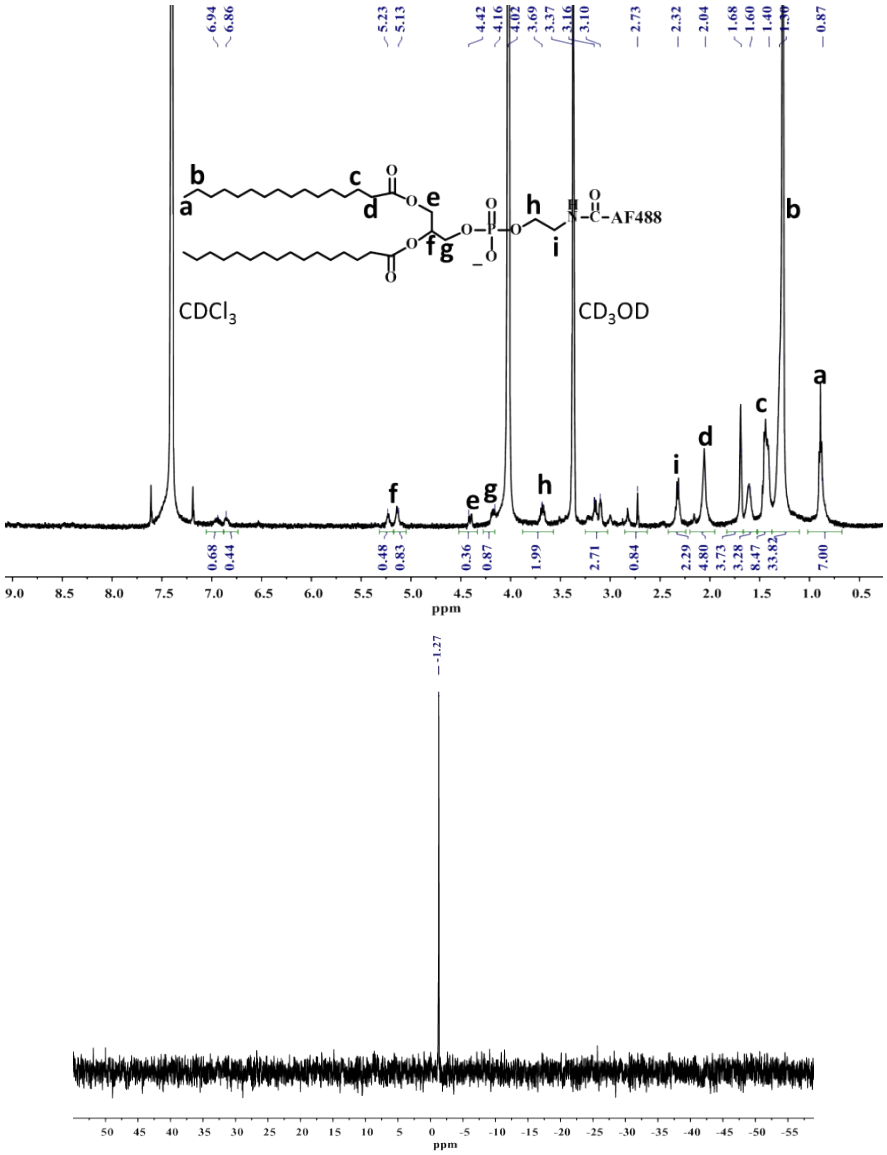

Figure S10. (top) ^{1}H-NMR spectrum of **11** in $CDCl_3$; (bottom) ^{31}P-NMR spectrum of **11** in $CDCl_3$.

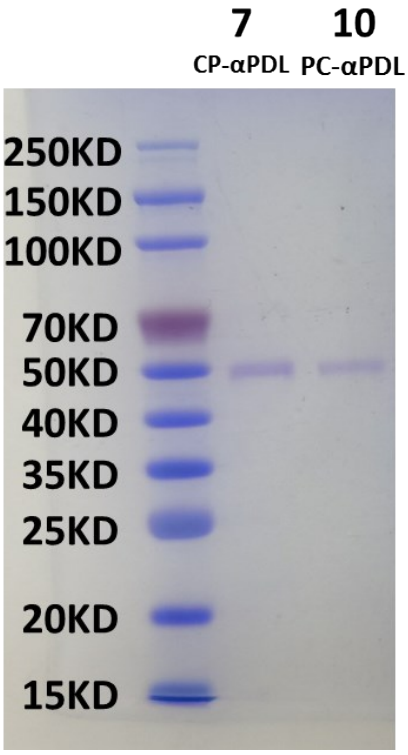

Figure S11. SDS-PAGE analysis of **7** (CP-αPDL) and **10** (PC-αPDL).

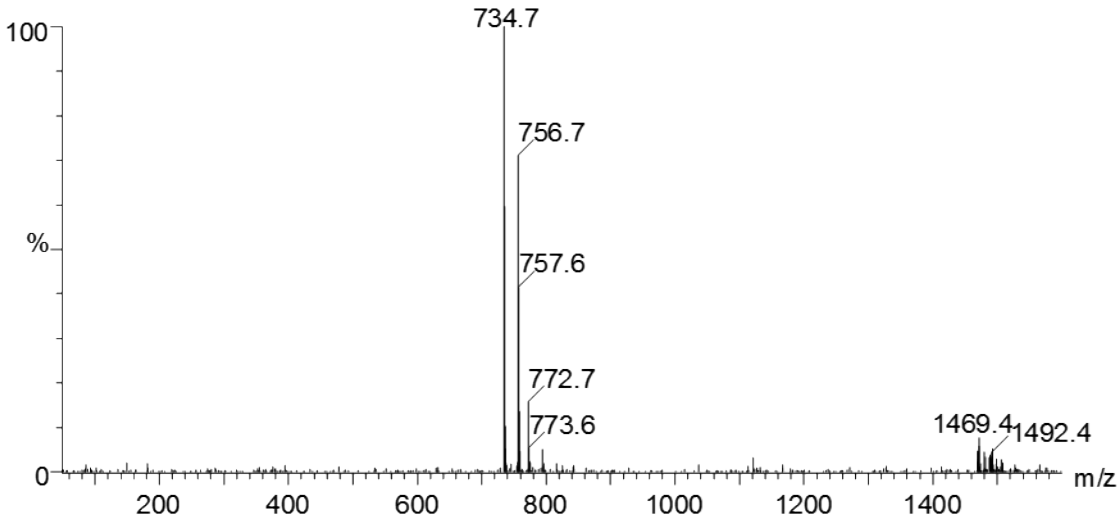

Figure S12. ESI-MS spectrum of PC-Lip.

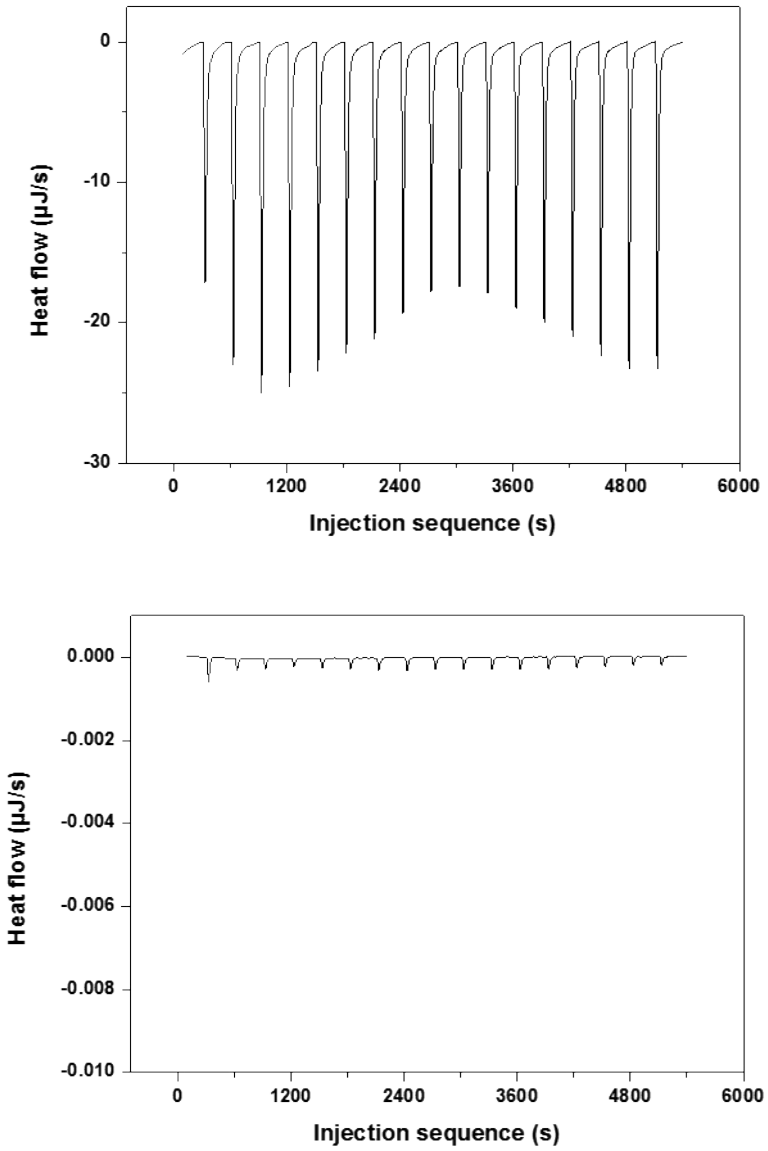

Figure S13. (top) ITC of different amount of CP-Lips added to PC-Lips; (bottom) ITC of different amount of PC-Lips added to PC-Lips. They were all dissolved in chloroform.

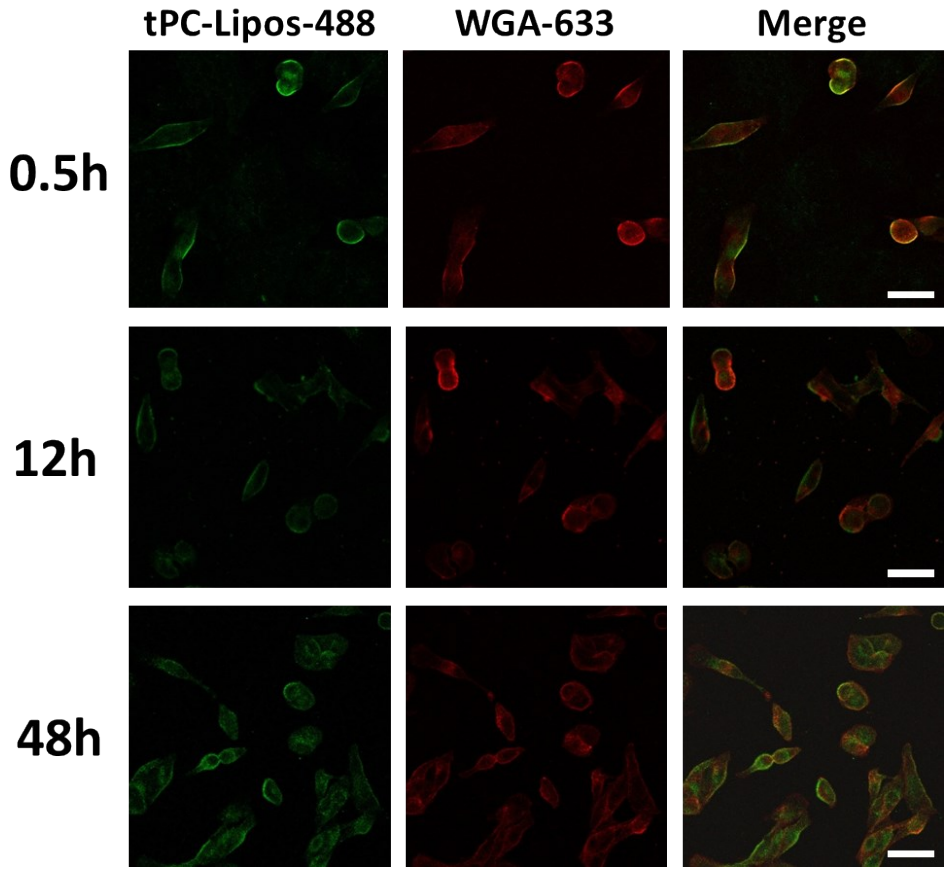

Figure S14. CLSM images of B16-F10 cells cultured with tPC-Lipos-488 (PC-Lip/PC-αPDL/PC-488=6/1/1, molar ratio) for 0.5 h, 12 h and 48 h; cell membrane stained with WGA-633 are shown in red; scale bars: 20μm.

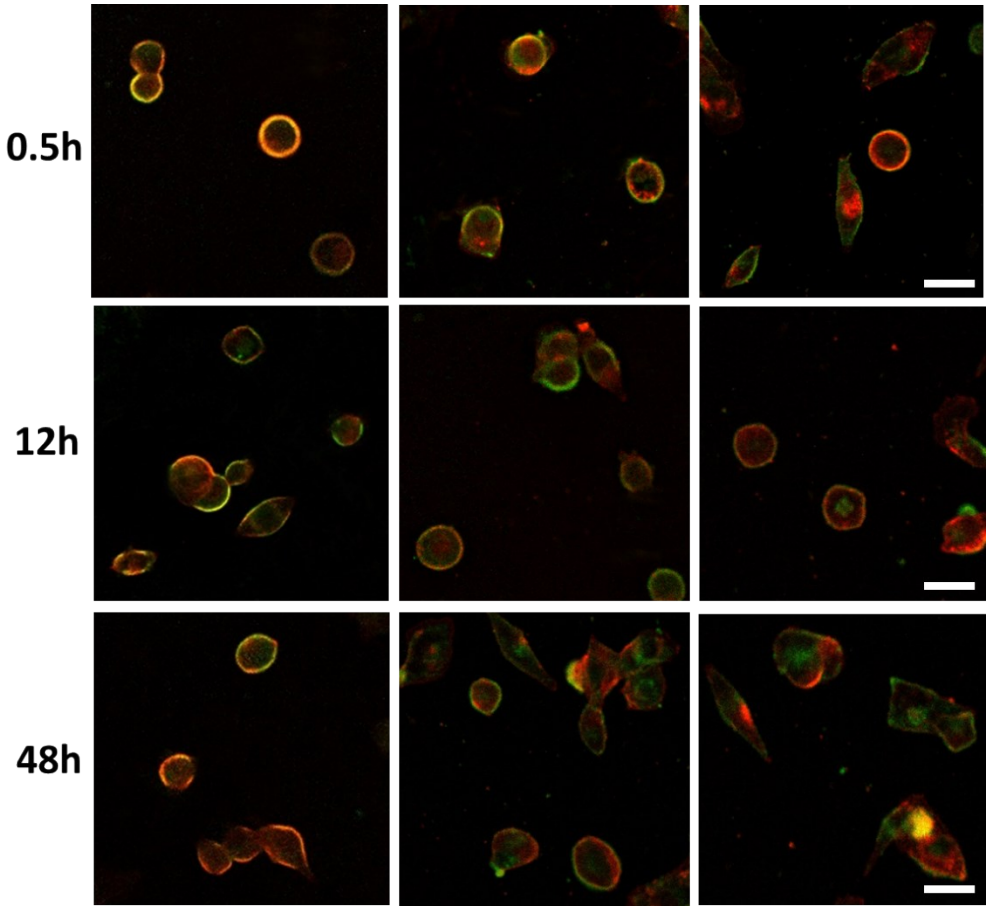

Figure S15. Three separate CLSM images of B16-F10 cells cultured with tCP-Lipos-488 (CP-Lip/CP-αPDL/CP-488=6/1/1, molar ratio) for 0.5 h, 12 h and 48 h; Green channel is tCP-Lipos-488 and red channel is cell membrane; scale bars: 20μm.

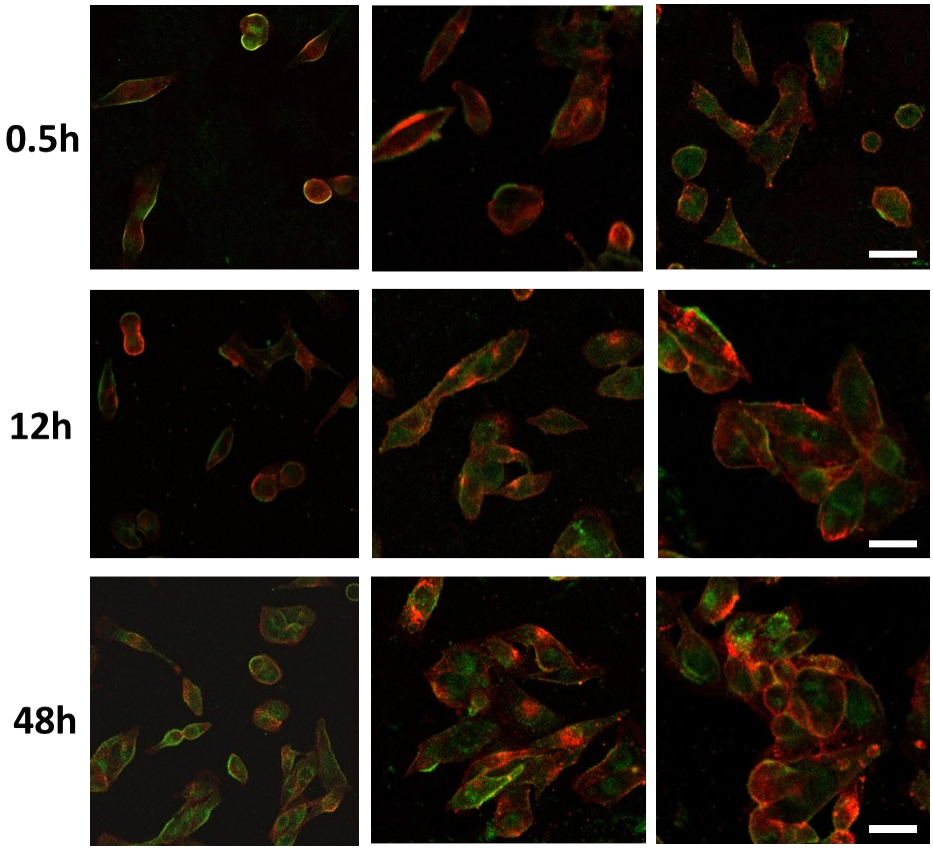

Figure S16. Three separate CLSM images of B16-F10 cells cultured with tPC-Lipos-488 (PC-Lip/PC-αPDL/PC-488=6/1/1, molar ratio) for 0.5 h, 12 h and 48 h; Green channel is tPC-Lipos-488 and red channel is cell membrane; scale bars: 20μm.

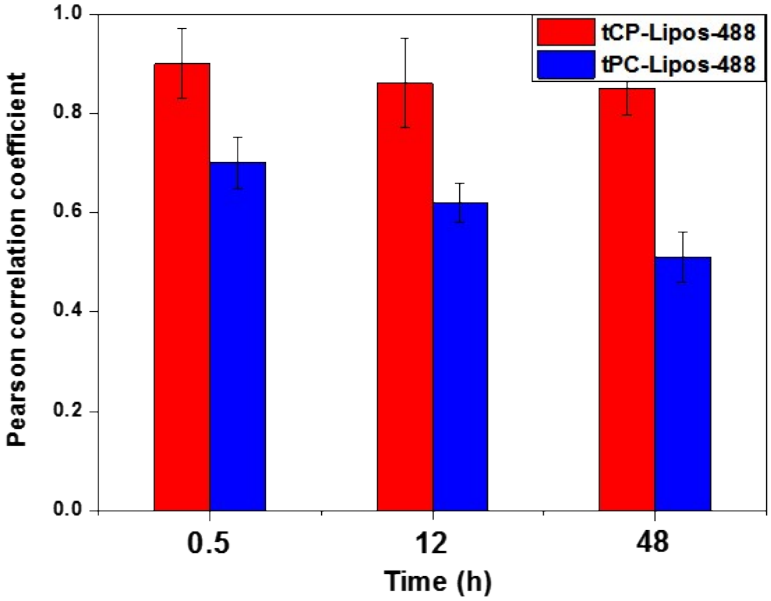

Figure S17. Pearson correlation coefficient for the colocalization of tCP-Lipos-488 / cell membrane and tPC-Lipos-488 / cell membrane.

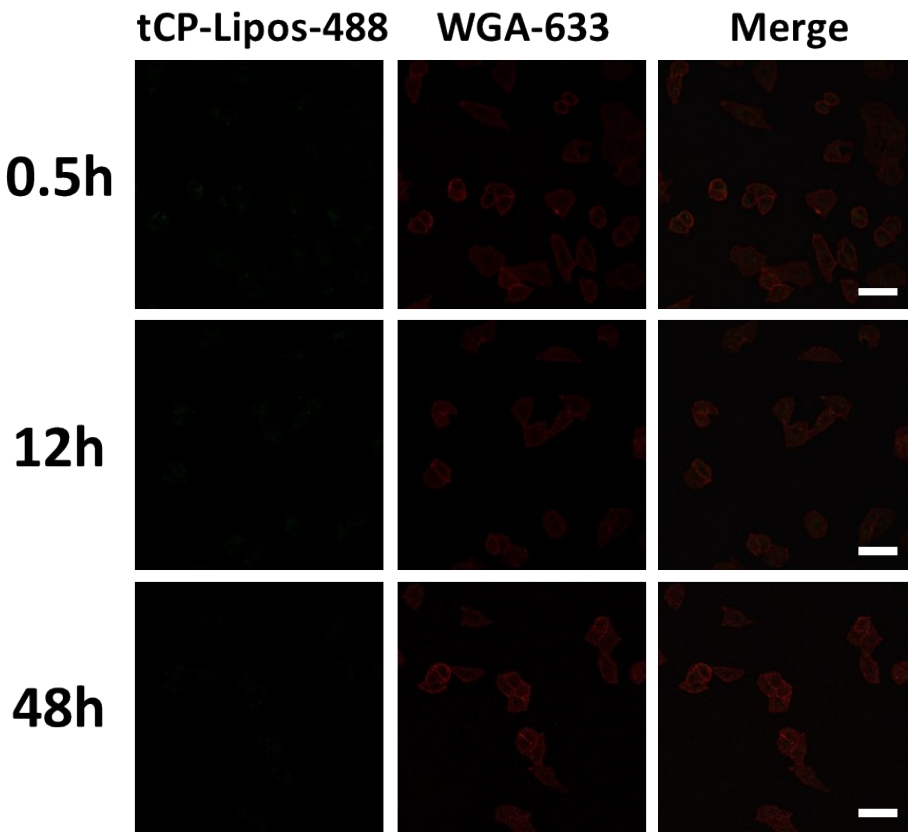

Figure S18. CLSM images of NIH-3T3 cells cultured with tCP-Lipos-488 (CP-Lip/CP-αPDL/CP-488=6/1/1, molar ratio) for 0.5 h, 12 h and 48 h; cell membrane stained with WGA-633 are shown in red; scale bars: 20μm.

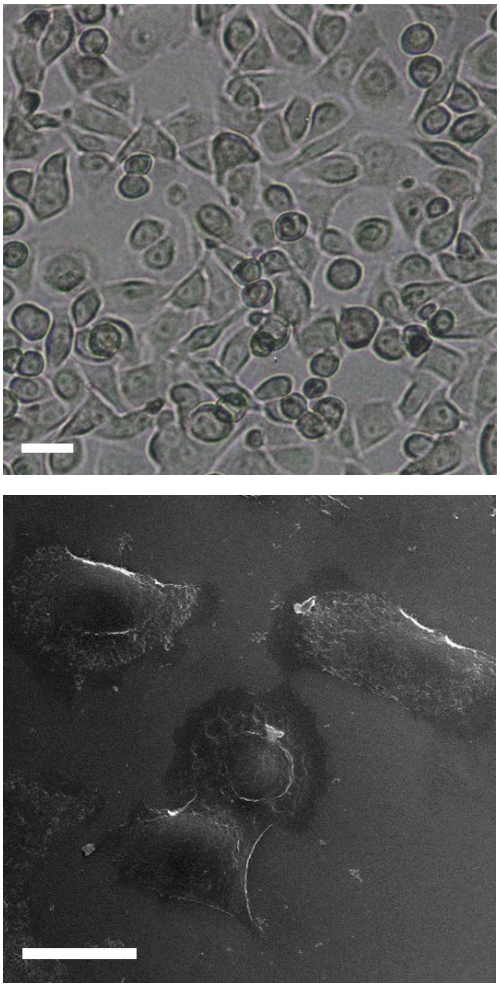

Figure S19. Optical microscope (top) and SEM (bottom) images of untreated B16-F10 cells. scale bars: 20 μm.

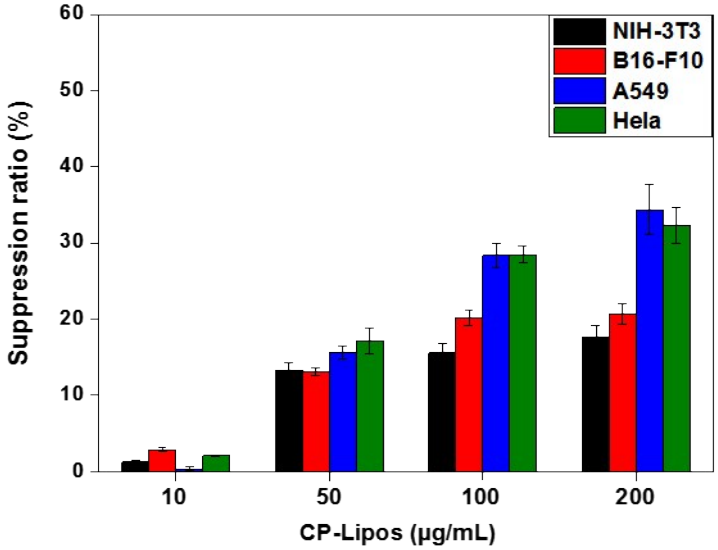

Figure S20. The suppression ratio of different types of cells cultured with different concentrations of CP-Lipos for 24 h.

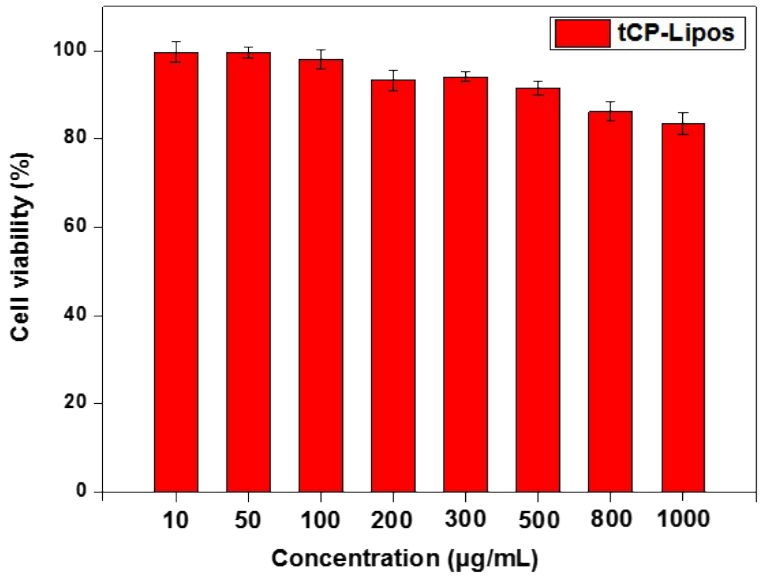

Figure S21. Cell viability of NIH-3T3 cells treated by different amount of tCP-Lipos (CP-Lip/CP-αPDL=6/1, molar ratio) via Celltiter-Blue assay.

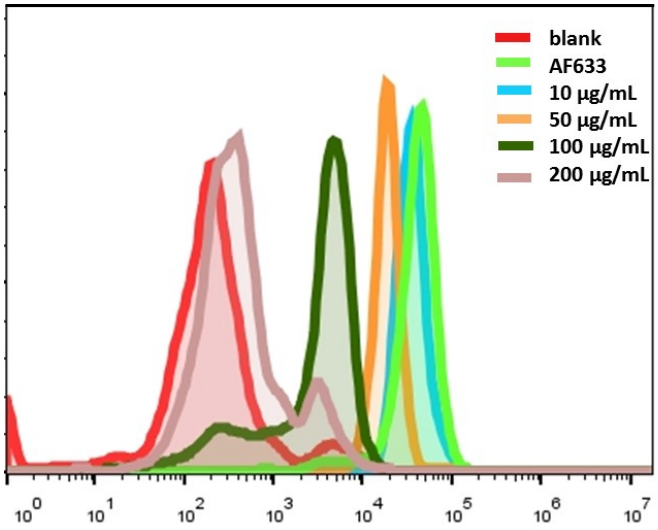

Figure S22. Cell endocytosis of AF633 after B16-F10 cells treated by different amount of tCP-Lipos (CP-Lip/CP-αPDL=6/1, molar ratio) detected by flow cytometry.

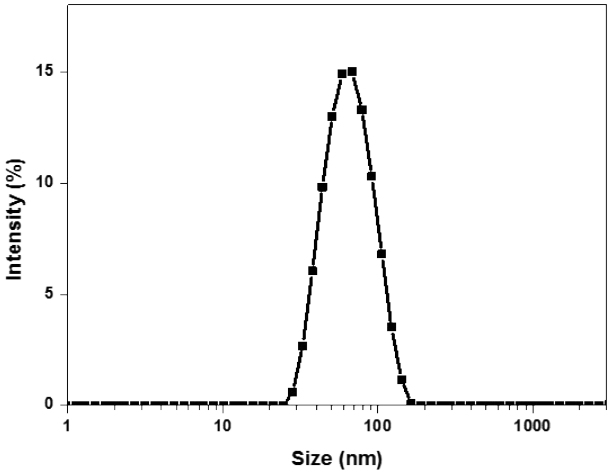

Figure S23. The size distribution of the nano-drug Dox@tCP-Lipos.

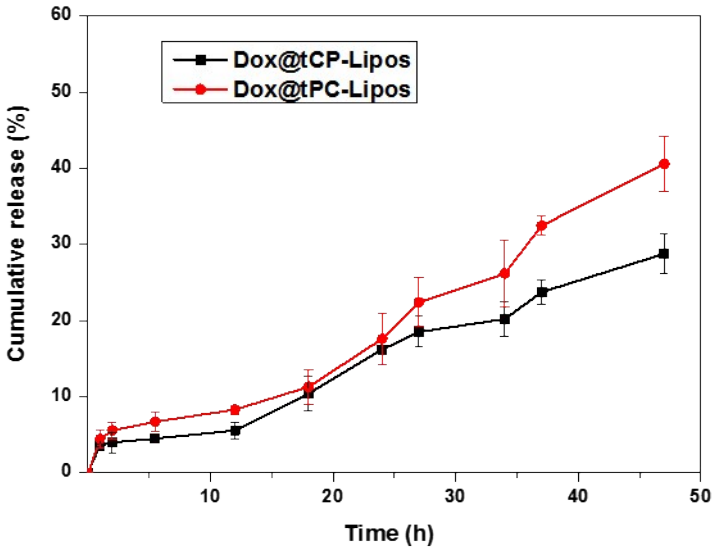

Figure S24. Drug release curves of Dox@tPC-Lipos and Dox@tCP-Lipos at 37℃ in PBS.

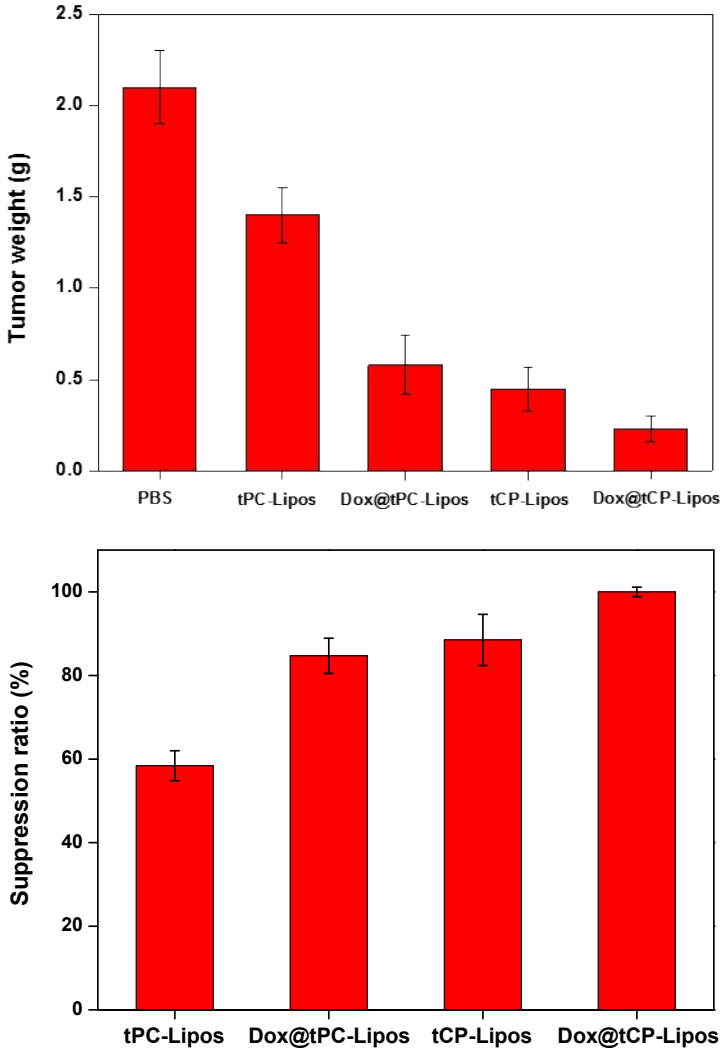

Figure S25. (top) Tumor weights of the mice treated with different samples; (bottom) The tumor suppression ratio of different treatment groups.

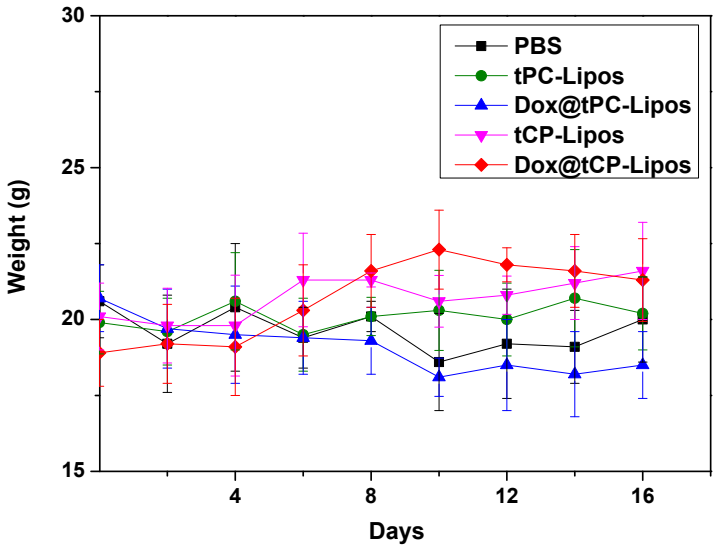

Figure S26. Body weight variation of the mice during the treatment.

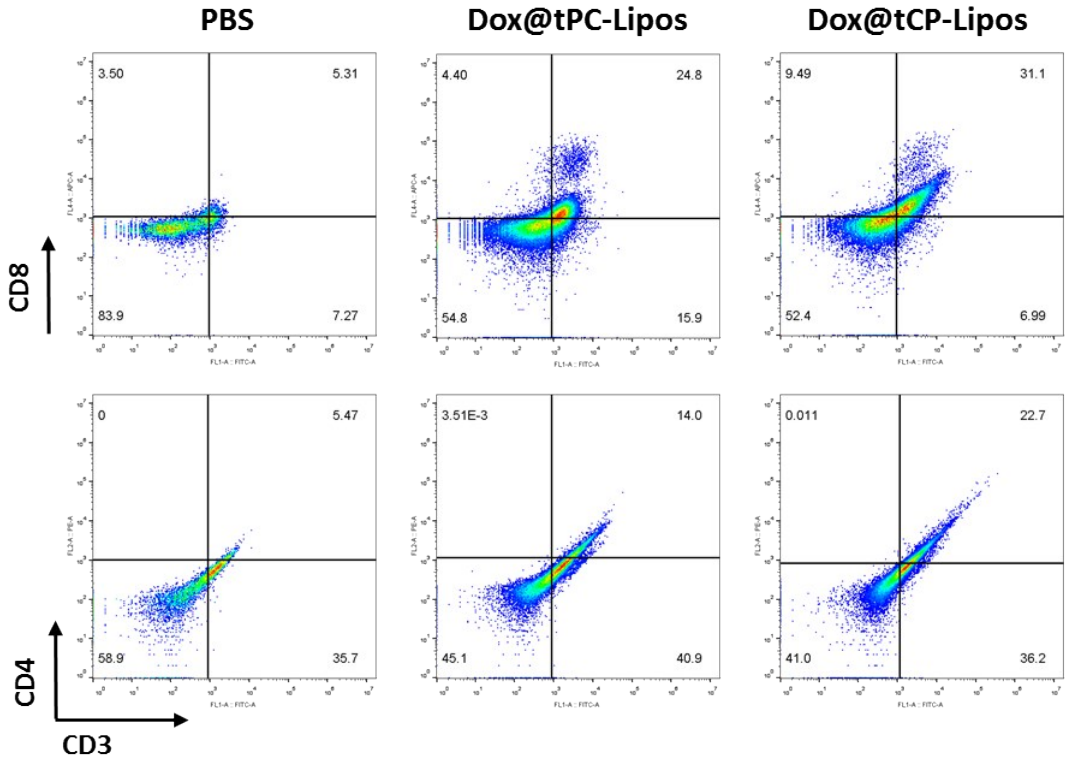

Figure S27. Flow cytometry analysis images and the relative quantification of $CD4^+$ cells (PE dyes) and $CD8^+$ cells (APC dyes) gating on $CD3^+$ cells (FITC dyes) after treatment.

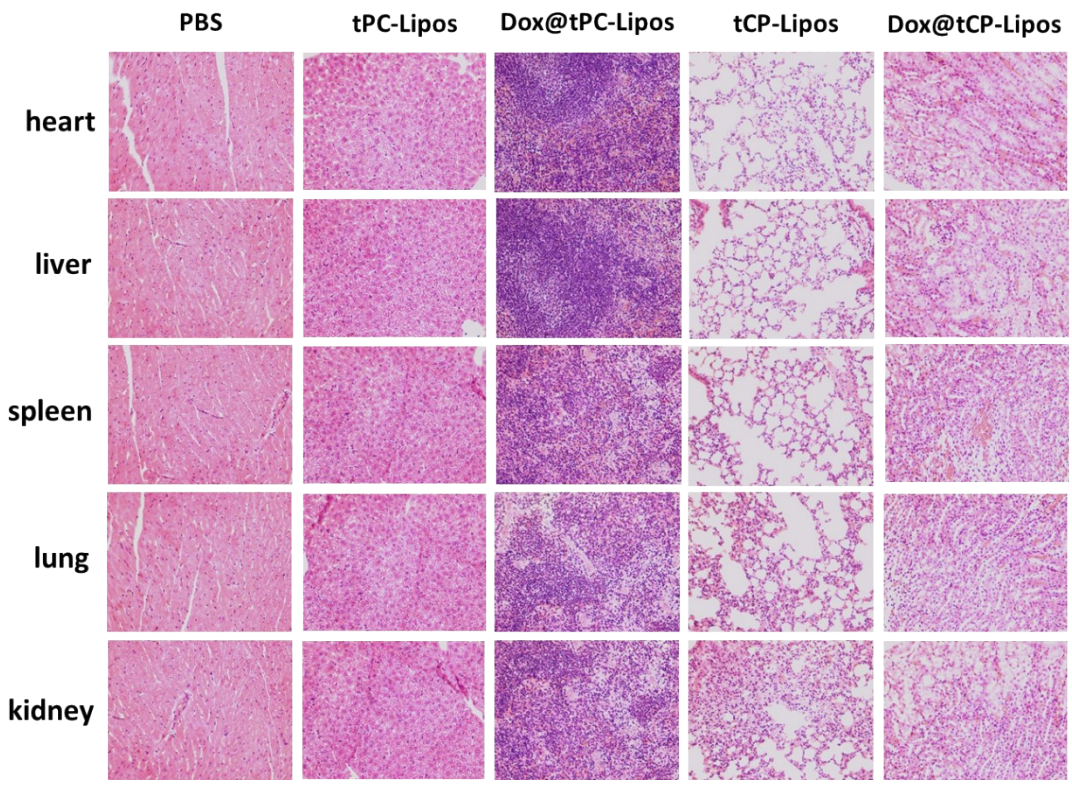

Figure S28. Histological analysis of normal organs treated via various formulations after 16 days.

Tables

Abbreviation	Full name	Components
CP-Lip	Choline Phosphate (CP) Lipid	/
PC-Lip	Phosphatidyl Choline (PC) Lipid	/
CP-αPDL	CP-Lipid conjugated PD-L1 antibody	/
PC-αPDL	PC-Lipid conjugated PD-L1 antibody	/
CP-488	CP-Lipid conjugated Alexa Fluor 488 dye	/
PC-488	PC-Lipid conjugated Alexa Fluor 488 dye	/
CP-Lipos	CP-Liposome	CP-Lip
PC-Lipos	PC-Liposome	PC-Lip
tCP-Lipos	targeted CP-Liposome	CP-Lip + CP-αPDL
tPC-Lipos	targeted PC-Liposome	PC-Lip + PC-αPDL
tCP-Lipos-488	targeted CP-Liposome conjugated AF488 dye	CP-Lip + CP-αPDL + AF488
tPC-Lipos-488	targeted PC-Liposome conjugated AF488 dye	PC-Lip + PC-αPDL + AF488
Dox@tCP-Lipos	Doxorubicin loaded targeted CP-Liposome	CP-Lip + CP-αPDL + Dox
Dox@tPC-Lipos	Doxorubicin loaded targeted PC-Liposome	PC-Lip + PC-αPDL + Dox

Table S1. Abbreviation and full name of all the samples used in the work.

	Size (nm)	Charge (mV)
CP-Lipos	72±1.5	-7.54
PC-Lipos	97±0.8	-6.50
tCP-Lipos	71±1.2	-7.51
tPC-Lipos	105±1.1	-3.37
tCP-Lipos-488	94±1.7	-6.47
tPC-Lipos-488	128±2.2	-5.27

Table S2. The size and charge of nanoparticles of different samples.

	Size (nm)	Charge (mV)	DLC (%)	DLE (%)
Dox@tPC-Lipos	95±2	-7.58	7.6	92
Dox@tCP-Lipos	60±2	-5.56	15.2	96

Table S3. The size, charge, drug loading content (DLC) and drug loading efficiency (DLE) of Dox@tPC-Lipos and Dox@tCP-Lipos.

References

1. Wang, W.; Liu, S.; Yan, X.; Li, S.; Ma, X.; Yu, X. DNA-Inspired Adhesive Hydrogels Based on the Biodegradable Polyphosphoesters Tackified by a Nucleobase. *Biomacromolecules* **2019,** *20*, 3672−3683.

2. Feng, Y.; Wang, G.; Chang, Y.; Cheng, Y.; Sun, B.; Wang, L.; Chen, C.; Zhang, H., Electron Compensation Effect Suppressed Silver Ion Release and Contributed Safety of Au@Ag Core-Shell Nanoparticles. *Nano Letters* **2019,** *19* (7), 4478-4489.