Supporting Information

for

Triple-Layered pH-Responsive Micelleplexes Loaded with siRNA and Cisplatin Prodrug for NF-Kappa B Targeted Treatment of Metastatic Breast Cancer

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Materials and Methods

Methoxy poly(ethylene glycol)$_{225}$ (PEG_{225}-OH), copper bromide (CuBr, 99.99%), N,N,N',N'',N''-Pentamethyldiethylenetriamine (PMDETA), sulforhodamine B (SRB) were all purchased from Sigma-Aldrich (Shanghai, China). N,N-Dimethylacetamide (DMAC), trimethylamine (TEA), 2-bromoisobutyryl bromide and nile red (NR) were ordered from J&K Chemical Ltd (Shanghai, China). Glycidyl methacrylate (GA), octanoic anhydride and 2-(diisopropylamino) ethyl methacrylate (DPA) were obtained from TCI Inc. (Shanghai, China). Diethyl pyrocarbonate (DEPC), 2-Hydroxyethyl methacrylate (HEMA), oligoamines (ethylenediamine (EDA), diethylenetriamine (DETA), triethylenetetramine (TETA) and tetraethylenepentamine (TEPA)) were obtained from Sigma-Aldrich (Shanghai, China). Cisplatin (CDDP) was purchased from Platinum Energy. Co. Ltd (Shandong, China). Ultracentrifugation tubes with molecular weight cut-off (MWCO) of 10 kDa or 3 kDa were obtained from Millipore (Shanghai, China). Tetramethylrhodamine-5-carbonyl-azide (TMR-azide) was ordered from Life Technologies (Shanghai, China). Chlorin e6 (Ce6) was obtained from Frontier Scientific, Inc., (UT, USA). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl), 4-Dimethylaminopyridine (DMAP) and N-hydroxy succinimide (NHS) were all ordered TCI Chemical. Inc. (Shanghai, China).

Trypsin-EDTA and fetal bovine serum (FBS) were obtained from Gibco-BRL (Burlington, Canada). The RPMI 1640 medium, antibiotics, DNA loading buffer, Hoechst 33342 and Lysotracker Red were purchased from Invitrogen (Oregon, USA). Trypan Blue was purchased from TianGen (Beijing, China). Apoptosis Kit was obtained from BD Biosciences (USA). Crystal violet staining solution and antifade mounting media was purchased from Biyotime (Shanghai, China). RIPA lysis buffer was obtained from Roche (Basel, Switzerland). PVDF membrane was obtained from Millipore (Shanghai, China). All other reagents were of analytical grade and obtained from Sinopharm Group Chemical Reagent Co. Ltd (Shanghai. China).

The siRNA duplexes targeting specific sequences of green fluorescence protein (siRNA-GFP) (5'-GGCTACGTCCAGGAGCGCACC-3' (sense)), NF-κB subunit p65

(siRNA-p65) (5'-GCCCUAUCCCUUUACGUCATT-3' (sense)), scrambled siRNA non-specific to any human gene (siRNA-NC) [5'-CGG UGA GCC AGG CGU GCA AUU-3' (sense) (with or without FAM label at 5' end)] were all purchased from GenePharm Co. Ltd. (Shanghai, China). The siRNA stock solution was prepared by dissolving siRNA duplexes in DEPC-treated Milli-Q water.

Synthesis and characterization of PEG-*b*-PAGA-*b*-PDPA triblock copolymer

Triblock copolymer PEG_{225}-*b*-PGA_{45}-*b*-$PDPA_{50}$ was synthesized by the sequential atom transfer radical polymerization (ATRP) method. Firstly, PEG_{225}-Br macroinitiator was prepared according to the procedure as described previously.[1] Afterwards, PEG_{225}-Br 1.0 g (0.010 mmol), GA (0.59 mL, 0.45 mmol) and PMDETA (25 μL, 0.010 mmol) were dissolved in 3 mL of anhydrous DMAC, degassed by three freezing-thawing cycle under vacuum condition, added with 12 mg of CuBr (0.010 mmol) under argon protection. The polymerization was conducted overnight at 40 ℃. The reaction product was purified by passing through a neutral alumina column and dialyzing against DI water. The final product was obtained after lyophilizing (yield 63%). The chemical composition of the diblock copolymer was determined to be $mPEG_{225}$-*b*-PGA_{45}-Br using ^{1}H-NMR spectra examination.

Next, PEG_{225}-*b*-PGA_{45}-Br 1.0 g (0.0061 mmol), DPA 0.75 mL (0.31 mmol), and PMDETA 15.2 μL (0.0061 mmol) were dissolved in 3 mL of DMAC. The solution was degassed by three freezing-thawing cycles. After added with 7.3 mg of CuBr (0.0061 mmol) under argon protection, the reaction was conducted at 40 ℃ overnight. The reaction product was diluted with THF and went through a neutral alumina column to remove copper catalyst. The THF solution was condensed, dialyzed against DI water and lyophilized to obtain final product of $mPEG_{225}$-*b*-PGA_{45}-*b*-$PDPA_{50}$ as determined by ^{1}H-NMR spectrum (yield 98%).

To synthesize aminolated PEG_{225}-*b*-$PAGA_{45}$-*b*-$PDPA_{50}$ triblock copolymer, 100 mg of as-synthesized PEG_{225}-*b*-PGA_{45}-*b*-$PDPA_{50}$ copolymer was dissolved in 3 mL of DMAC. The polymer solution was slowly added with 2 mL DMAC solution of EDA, DETA, TETA or TEPA at an oligoamine to GA molar ratio of 50:1. The reaction was continued at 40 ℃ for 12 h. The reaction product was purified by dialyzing against DI water and lyophilizing to

obtain a set of $mPEG_{225}$-*b*-$PAGA_{45}$-*b*-$PDPA_{50}$ triblock copolymer (termed as PEDA, PDETA, PTETA and PTEPA respectively, according to the oligoamine used for aminolation).

Synthesis of TMR or Ce6-labeled mPEG-b-P(DPA-co-HEMA) diblock copolymer

The $mPEG_{225}$-*b*-P(DPA_{50}-co-$HEMA_3$) diblock copolymer was synthesized by ATRP method following the procedure described in our previous study.[2] The resulting diblock copolymer was then covalently labeled with TMR-azide or Ce6 according to our previously published procedures.[2,3] The reaction product was purified by dialyzing against Dimethyl sulfoxide (DMSO) and water, respectively. Any trace TMR or Ce6 dye was removed by multiple ultracentrifugation (Milli-pore, molecular weight cut-off 100 kDa). The TMR or Ce6 conjugation percentage was determined by UV-Vis spectroscopic examination. The complete removal of free Ce6 from PEG-*b*-PDPA-Ce6 diblock copolymer was verified by high performance liquid chromatography (HPLC) examination using acetonitrile as the eluent.

Synthesis of cisplatin prodrug (Pt(IV)-OC)

Hydrophobic CDDP prodrug Pt(IV)-OC was synthesized following a procedure similar to a literature method.[4] Briefly, 0.35 g of Pt(IV)-OH (1.03 mmol) was dissolved in 5 mL of DMSO, and 0.56 g octanoic anhydride (2.10 mmol) was added. The mixture was then stirred at room temperature for 48 h. After that, Milli-Q water was added to precipitate the product. The resulting yellow solid product was dissolved in acetonitrile and precipitated into ethyl ether and washed twice with ethyl ether, and dried under reduced pressure to get 0.48 g of pale yellow product with a yield of 80%. The chemical structure of final product was examined by ESI-MS measurement. The calculated molecular mass was 586.4, and that found by ESI-MS was 585.0 [$M-H^+$].

Preparation and characteristics of PEG-*b*-PAGA-*b*-PDPA micelles

Micelle stock solution (5.0 $mg \cdot mL^{-1}$) in Hepes buffer solution (20 mM, pH 7.4) was prepared by a solvent precipitation method. Typically, 15.0 mg of PEG_{225}-*b*-$PAGA_{45}$-*b*-$PDPA_{50}$ copolymer was dissolved in 600 μL of DMAC. The solution was added to 6 mL of DI water under ultrasonication. The organic solvent was removed by dialyzing against deionized water overnight. The solution was concentrated by ultrafiltration

(MWCO 10 kDa) and re-dispersed in 3 mL of Hepes buffer. PEDA micelles with 1% (w/w) Pt(IV)-OC loading (denoted M/Pt(IV)-OC) were prepared by following a similar procedure that applied for blank micelle preparation.

The particle size and zeta-potential of the micelles or siRNA-loaded micelleplexes were determined by dynamic light scattering (DLS) with a Zetasizer Nano ZS (Malvern Instruments, U.K.). All the measurements were conducted at 25 ℃ using a He-Ne laser (λ = 633 nm). The zeta-potential of the micelles and siRNA-loaded micelleplexes was measured using a folded capillary cell (Malvern Instruments, Herrenberg, Germany). The presented data were averaged from three independent measurements.

The buffering capacity of the aminolated triblock copolymers was determined using acid-base titration assay at a copolymer concentration of 20 $mg \cdot mL^{-1}$. Aqueous solutions of 0.1 M HCL and 1 M NaOH were used as titrants.

siRNA binding ability analysis

Gel shift assay was employed to determine the siRNA binding ability of the as-prepared cationic micelles. The micelleplexes were prepared at different micelle to siRNA-NC weight ratios. Agarose gel with concentration of 1.0% (w/v) was prepared with TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0). The amount of siRNA loaded in each lane was 300 ng and the electrophoresis was performed at 80 V for 20 min. The gels were imaged with a ChemiDoc MP System (Bio-Rad, USA). The amount of siRNA unpackaged with the micelles was determined using the ImageJ software (NIH, USA).

Hemolytic activity assay

The erythrocyte lysis assay was conducted following a method as described in our previous study.(1) Erythrocytes were isolated from fresh mouse blood by centrifugation (1000 g/min) for 3 min, thoroughly washed with phosphorus buffer saline (PBS) solution. The erythrocyte pellets were then diluted with 10-fold lysis assay buffers (20 mM Hepes buffer, 0.9% NaCl, pH 7.4 or 6.2). A certain amount of cationic micelle solution was added into 135 μL of lysis assay buffer in a 96-well tissue culture plate, mixed with 15 μL of erythrocyte suspension. The tissue culture plates were then incubated at 37 ℃ for 1 h under constant

shaking. The hemoglobin release was determined using an UV-Vis microplate reader (Abs_{450}). 10% Triton X-100-treated erythrocyte solution was set as positive control.

Cell lines and cell culture

Murine breast cancer cell line of 4T1 was obtained from cell bank of Chinese Academy of Sciences, Shanghai, China. 4T1 cells with stable luciferase expression (4T1-Luc) were produced by lectiviral vector (encoded with a pGL3 luciferase promoter) mediated transfection. Both 4T1 and 4T1-Luc cells were maintained in RPMI 1640 medium containing 10% FBS, 100 μg/mL streptomycin sulfate and 100 U/mL penicillin G sodium. Human A549 lung cancer cells with stable green fluorescence protein expression (A549-GFP) were obtained from UTSouthwestern Medical Center at Dallas (TX, USA). A549-GFP cells were cultured in DMEM cell culture medium supplemented with 10% of FBS, 100 $\mu g \cdot mL^{-1}$ of streptomycin sulfate, and 100 $U \cdot mL^{-1}$ of penicillin G sodium. All cells used in this study were tested to be mycoplasma free and cultured in humidified incubator at 37 ℃ with 5% CO_2 supply.

GFP knockdown *in vitro*

To screen mostly optimized copolymer composition, the GFP knockdown studies were performed in A549-GFP cells. A549-GFP cells were seeded in 24-well tissue culture plates at a density of 5.0×10^4 cells for 24 h, the medium was then replaced with fresh one containing siRNA-loaded micelleplexes. The micelleplexes were prepared at varied micelle to siRNA weight ratios and the siRNA final concentrations was fixed at 1.0 $\mu g \cdot mL^{-1}$. The medium was replaced with fresh one after 24 h incubation. After additional 24 h incubation, the cells were analyzed for GFP expression using flow cytometric examination (FACS Caliber, BD Biosciences, USA). The cell viability was measured by the sulforhodamine B (SRB) staining assay using a microplate reader (Enspire multimode plate reader, PerkinElmer, USA).

Cellular uptake and distribution of micelleplexes *in vitro*

To examine the cellular uptake and distribution of siRNA-loaded micelleplexes, 15 wt.% of TMR-labeled PEG-*b*-PDPA diblock copolymer was loaded into PEDA micelles at a final TMR loading percentage of 1.0 wt.%. A549 cells were seeded on 10 mm^2 glass coverslips in

24-well tissue culture plates at a density of 5.0×10^4 cells per well. After 24 h pre-incubation, the cells were treated with TMR-labeled micelleplexes loading siRNA-FAM at a siRNA final concentration of 1.0 $\mu g \cdot mL^{-1}$. The micelleplexes were prepared at a micelle to siRNA weight ratio of 20. The cells were stained with Hoechst-33342 after 12 h micelleplex incubation. The extracellular fluorescence was quenched by trypan blue. The cells were then thoroughly washed with PBS, fixed with 4% (v/v) paraformaldehyde and examined using a confocal laser scanning microscope (CLSM, Fluoview FV-1000, Olympus).

Western blotting and flow cytometric assay

To determine the target gene knockdown efficiency of siRNA-loaded micelleplexes, 3.0×10^5 4T1 cells were pre-incubated in 6-well plates for 24 h, transfected with siRNA-loaded micelleplexes for 24 h at a siRNA concentration of 0.5, 1.0 or 2.0 μg per well. After additional 24 h incubation, the cells were harvested and lysed for western blotting analysis. Typically, 50 mg of protein per lane was separated by SDS-PAGE gel under reducing conditions, transferred onto a PVDF membrane and blocked with 5% milk. The membrane was firstly probed with first antibodies of p65 (Beyotime), matrix metalloprotease-9 (MMP-9) or Cyclin-D1 (Abcam, Cambridge, UK), then with secondary anti-mouse or anti-rabbit-HRP antibody (Beyotime, Shanghai, China). The detection was performed using the enhanced Chemiluminescence system (Pierce, Rockford, IL, USA). β-actin was probed as the loading control for each sample. The expression of target protein was semi-quantitatively analyzed with the ImageJ software (NIH, Bethesda, MD).

Micelleplex-treatment induced cellular apoptosis and proliferation inhibition was examined by flow cytometric analysis. To do that, 1.5×10^5 4T1 cells were pre-seeded in 12-well cell culture plate for 24 h. The cells were then treated with siRNA-loaded micelleplexes with or without Pt prodrug encapsulation for 24 h at a final siRNA or Pt prodrug concentrations of 1.0 $\mu g \cdot mL^{-1}$ or 0.32 $\mu g \cdot mL^{-1}$, respectively. After additional 24 h incubation, the cells were collected, washed with cold PBS, stained with Annexin V-FITC and PI. The cell populations in early apoptosis, later apoptosis and necrosis stage were determined using flow cytometric examination.

For cell cycle analysis, 4T1 cells seeded in 6-well tissue culture plate were transfected with siRNA-loaded micelleplexes for 24 h. After additional 24 h incubation, the cells were collected, washed twice with cold PBS, fixed with 70% cold ethanol and stored at 4 °C overnight and washed it again. The cells were then incubated with RNase A (0.1 $mg \cdot mL^{-1}$) for 30 min, stained with PI for 30 min. The siRNA-transfection induced cell cycle change and apoptosis were then analyzed using a flow cytometric method.

Cell cycle and apoptosis analysis

4T1 cells were seeded in 24-well plates at a density of 5.0×10^4 cells for 24h, then the medium was changed with fresh medium containing micelleplexes siRNA-p65 or siRNA-NC loaded micelleplexes prepared at a polymer to siRNA weight ratio of 20 (i.e. M/p65 and M/NC, the final siRNA concentrations was 150 nM). After 24h incubation, the medium was changed with fresh one and continually cultured for another 24h. The cells were collected and stained with Annexin V-FITC apoptosis detection kit and detected by flow cytometry. Cells without treatment were used as blank control.

Wound healing, migration and invasion assay

To investigate the influence of p65 knockdown on the wound healing ability of 4T1 cells, the cells were seeded in 12-well plates at a density of 2.0×10^5 cells to create a confluent monolayer. Scratch wounds were produced with pipette tips and the cells were washed with PBS, added with fresh medium. The cells were then incubated with siRNA-loaded micelleplexes for 24 h at a siRNA concentration of 75 nM. The cells were then imaged after 24 h continued culture to quantify the wound healing ratio.

To examine the migration inhibition efficacy by silencing p65, 4T1 cells transfected with siRNA-p65 or siRNA-NC-loaded micelleplexes (w/w 20, the final siRNA concentrations was 75 nM) for 24 h were collected and counted. A density of 1.5×10^5 cells was suspended in serum-free medium in each upper chamber, and 500 μL of medium was added to the lower chamber. After incubation for 24 h, the cells on upper surface were removed with a cotton swab. The cells passing through the filters on the lower surface were fixed with 90% ethanol, stained with crystal violet and photographed. For quantification, cells were counted under a

microscope in five predetermined fields. Data were expressed as the percentage of passed through the membrane relative to the number of non-transfected cells through the membrane.

To evaluate the influence of p65 knockdown on the invasion ability of 4T1 cells, each upper chamber was pre-coated with the matrigel and seeded with 2.0×10^5 cells. The cells passing through the filters on the lower surface were fixed with 90% ethanol, stained with crystal violet and photographed. For quantification, cells were counted under a microscope in five predetermined fields. Data were expressed as the percentage of passed through the membrane relative to the number of non-transfected cells through the membrane.

Cytotoxicity assay *in vitro*

To evaluate the cytotoxicity of Pt(IV)-OC prodrug and siRNA-p65 co-loaded PEDA micelleplexes (denoted as M/p65/Pt(IV)-OC), 4T1 cells were seeded in 96-well culture plates at a density of 3.5×10^3 cells per well for 24 h. Then the medium was changed with fresh culture media containing various concentrations of M/p65/Pt(IV)-OC micelleplexes loading 1.0 %(wt) Pt(IV)-OC and siRNA-p65, the medium was changed after 24 h micelleplex incubation and the cells were continually cultured for additional 24 h. Cells without treatment were used as control. The cell viability was measured by the SRB staining assay.

Pharmacokinetics and biodistribution of PEDA micelleplexes *in vivo*

To investigate the pharmacokinetics of prodrug-loaded micelleplexes, female nude mice (6-week old, 18~22 g) were purchased from Shanghai experimental animal center (Shanghai, China). All animal procedures were performed under the guideline approved by the Institutional Animal Care and Use Committee (IACUC) of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences. Nude mice were randomly divided into 2 groups (n = 3), and i.v. injected with Pt(IV)-OC or Pt(IV)-OC /siRNA-NC co-loaded PEDA micelleplexes at an equal Pt(IV)-OC dose of 0.6 mg/kg. After injection, 0.5 mL of blood sample was collected in a heparinized tube from orbital venous plexus at 0.083, 0.5, 1.0, 2.0, 8.0 and 24 h time points. The blood Pt concentration was determined using inductively coupled plasma mass spectrometry (ICP-MS) measurement. The pharmacokinetic parameters were calculated with Winnonlin software in two-component model.

To investigate the biodistribution of siRNA/Pt(IV)-OC co-loaded micelleplexes, PEDA micelles were loaded with 20 wt.% Ce6-labeled PEG-*b*-PDPA diblock copolymer at a final Ce6 loading concentration of 1.5 wt.%. The Ce6-labled and Pt(IV)-OC-encapsulated PEDA micelles were then loaded with siRNA-FAM at a micelle to siRNA weight ratio of 20. Two nude mouse groups bearing 100 ~ 200 mm^3 orthotopic 4T1 tumor were i.v. injected with siRNA and prodrug co-loaded PEDA micelles or Pt-prodrug at an equal Pt-prodrug dose of 0.6 mg/kg. In each group, the mice were sacrificed at 1, 4 or 24 h post micelleplex injection. The major organs (i.e. heart, liver, spleen, lung, kidney and tumor) were collected and the organ concentration of Pt was determined by ICP-MS measurement.

Anti-tumor effect *in vivo*

The anti-tumor effect experiment was conducted using an orthotopic 4T1 tumor bearing nude mouse model. The mice were randomly divided into 6 groups (n = 6) when the tumor volume reaching 50 mm^3. The mice were intravenously injected with PBS, cisplatin, or PEDA micelleplexes loading siRNA-NC (NC), siRNA-p65 (p65), siRNA-NC/Pt(IV)-OC or siRNA-p65/Pt(IV)-OC. The doses for siRNA and Pt(IV)-OC injection were fixed at 3.0 and 0.60 mg/kg, respectively. The injection was repeated for 6 times at an interval of 2 days. The tumor growth was monitored by measuring tumor volume. The tumor volume was calculated by using a formula $V = (L \times W \times W)/2$ (L, the longest diameter; W, the shortest diameter). All mice were sacrificed at the 24^{th} day post the initial treatment. The tumors, lungs, liver and kidney of all mice were collected, imaged, fixed and sliced for hematoxylin-eosin (H&E) or Tunnel staining assays.

Liver function examination

To investigate the biosafety of PBS, cisplatin, siRNA-NC loaded PEDA micelles (M/NC), Pt(IV)-OC prodrug and siRNA-NC co-loaded PEDA micelles (M/Pt(IV)-OC/NC), Balb/c mice (n = 3) were intravenously injected with PBS, cisplatin, M/NC, or M/Pt(IV)-OC/NC at the same dose applied for anti-tumor studies. Blood samples were collected 24 h later after injection and evaluated using blood biochemical analysis. The liver function was evaluated with serum levels of alanine aminotransferase (ALT), aspartate

aminotransferase (AST), total bilirubin level (T-BIL), total protein (TP), albumin (ALB), globulin (GLOB), alkaline phosphatase (ALP), ALB/GLOB and AST/ALT.

Statistical analysis

Results were presented as mean or means ± standard deviation. The statistical significance was determined using the analysis of variance (ANOVA) and two-tail Student's test. $p<0.05$ was considered statistical significant.

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Table S1. Pharmacokinetic parameters of Pt(IV)-OC prodrug and Pt(IV)-OC-loaded PEDA micelles (M/Pt(IV)-OC) in rats injected at a Pt(IV)-OC dose of 0.6 mg/kg.

Parameters	Pt(IV)-OC	M/Pt(IV)-OC
C_{max} (ng/mL)	323.4	1263.8
$AUC_{(0-t)}$ (ng/mL*h)	407.0	7153.7
Relative bioavailability*	---	17.6

*Determined by normalizing the $AUC_{(0-t)}$ value of M/Pt(IV)-OC with that of Pt(IV)-OC.

Table S2. The influence of PBS, cisplatin, siRNA-NC loaded PEDA micelles (M/NC), Pt(IV)-OC prodrug and siRNA-NC co-loaded PEDA micelles (M/Pt(IV)-OC/NC) on the liver function of the Balb/c mice. The serum levels of albumin (ALB), globulin (GLOB), and ALB/GLOB (A/G), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin level (TBIL) and total protein (TP) were examined using blood biochemical analysis, respectively.

Formulations	PBS	M/NC	Cisplatin	M/Pt(IV)-OC/NC
A/G	0.65±0.003	0.51±0.002	0.55±0.01	0.63±0.02
GLOB (g/L)	24.0±5.4	17.5±0.04	16.5±0.02	17.0±1.5
ALB (g/L)	14.2±3.3	13.0±0.2	10.4±0.07	13.5±0.45
ALP (U/L)	4.7±0.25	4.4±4.0	11.3±1.6	4.8±3.1
ALT (U/L)	25.6±10.7	19.6±5.0	21.2±6.2	15.6±2.1
AST (U/L)	71.4±5.1	41.1±4.1	80.6±10.3	65.9±2.3
T-BIL (μmol/L)	3.5±0.4	2.1±0.3	3.0±0.001	1.6±0.02
TP (g/L)	37.8±10.0	35.4±0.5	26.9±0.70	35.0±0.30

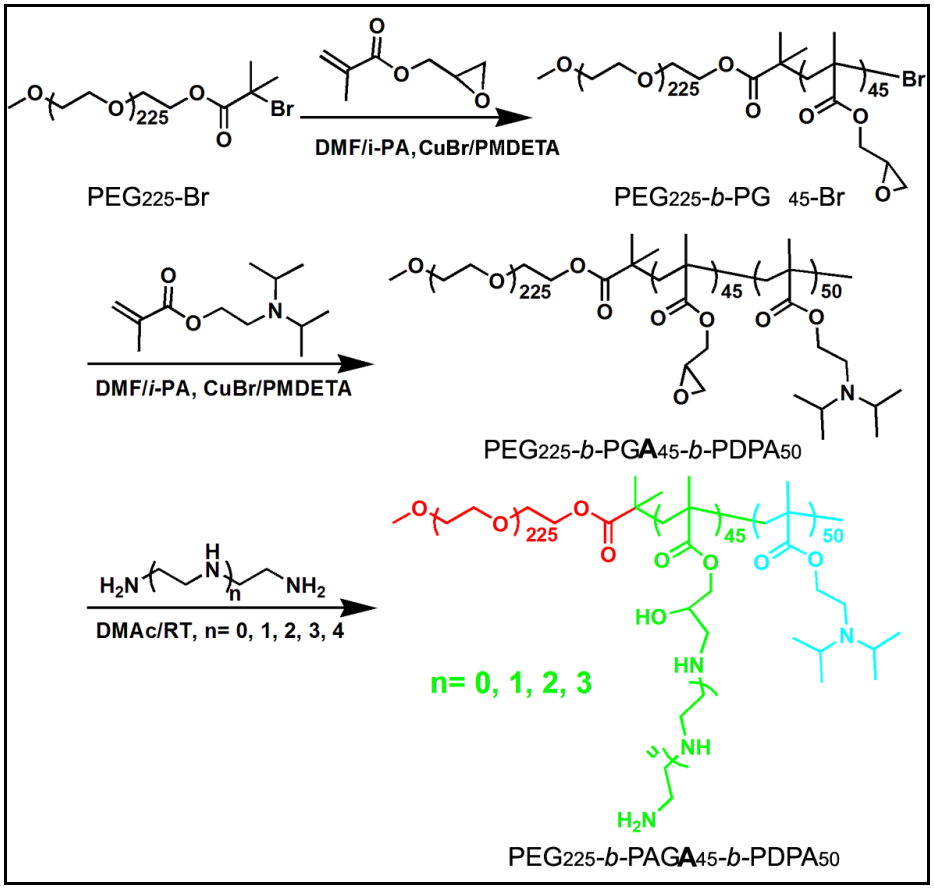

Figure S1. Synthetic route for PEG-*b*-PAGA-*b*-PDPA triblock copolymer bearing different oligoamine side chains.

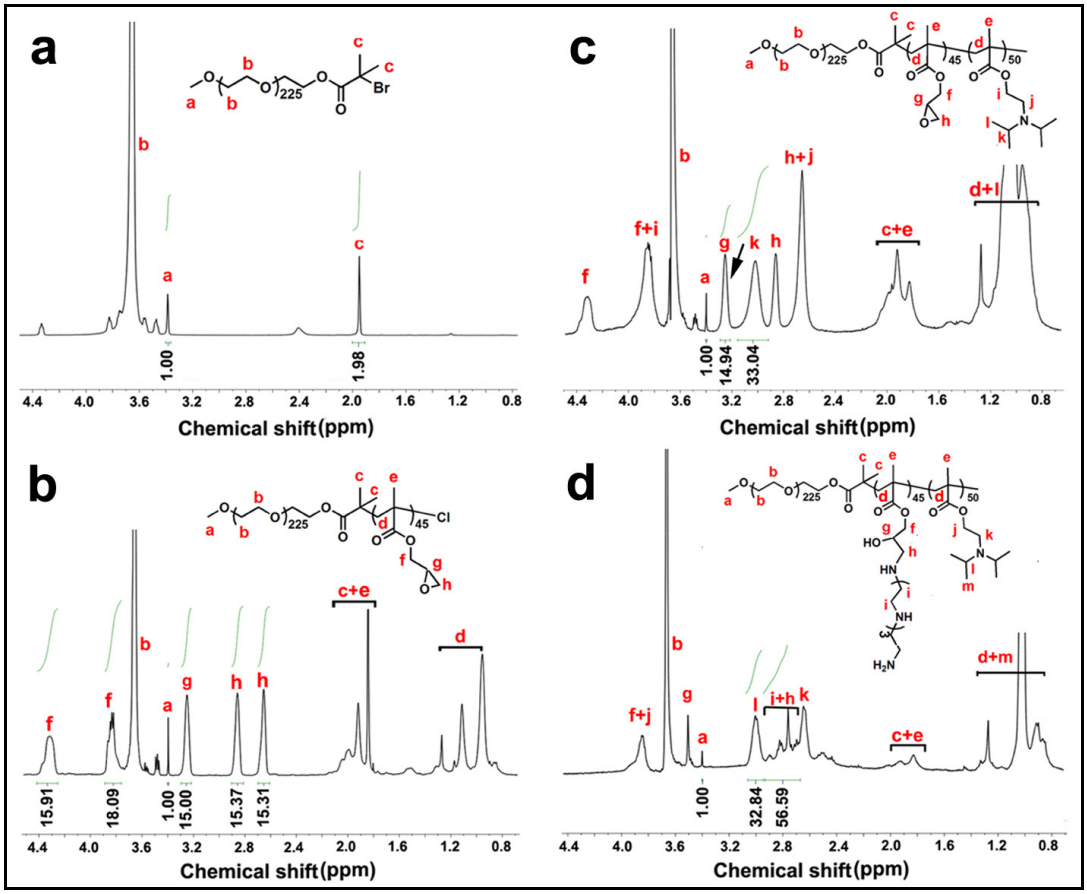

Figure S2. Representative ^{1}H-NMR spectrums of PEG_{225}-Br, PEG_{225}-*b*-PGA_{45}-Br, PEG_{225}-*b*-PGA_{45}-*b*-$PDPA_{50}$ and PEG_{225}-*b*-$PEDA_{45}$-*b*-$PDPA_{50}$.

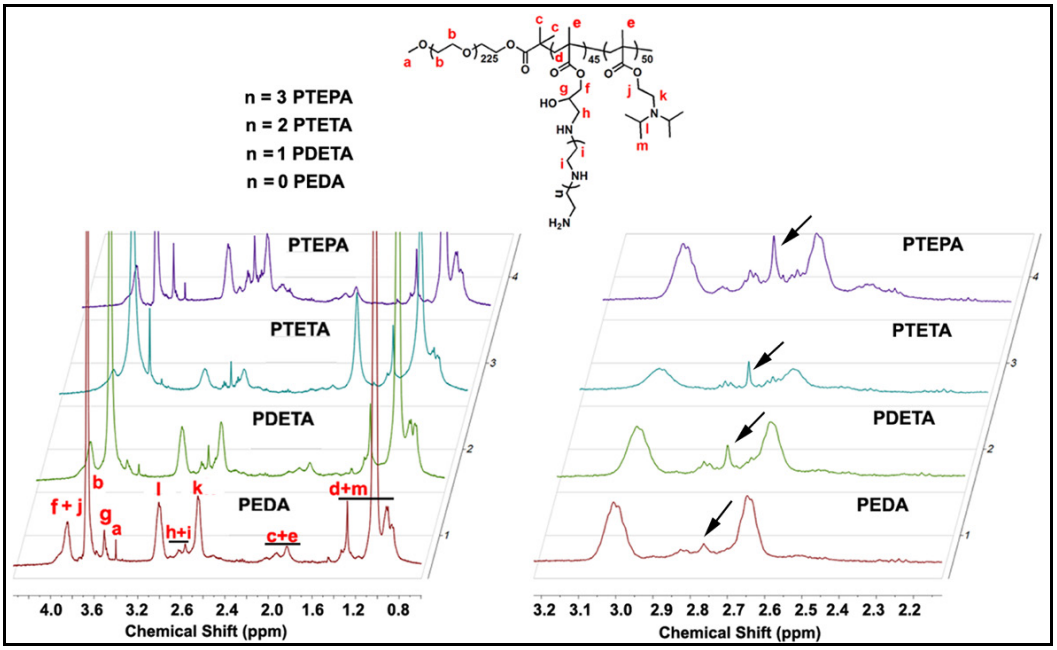

Figure S3. Representative ^{1}H-NMR spectrums of PEDA, PDETA, PTETA and PTEPA triblock copolymers.

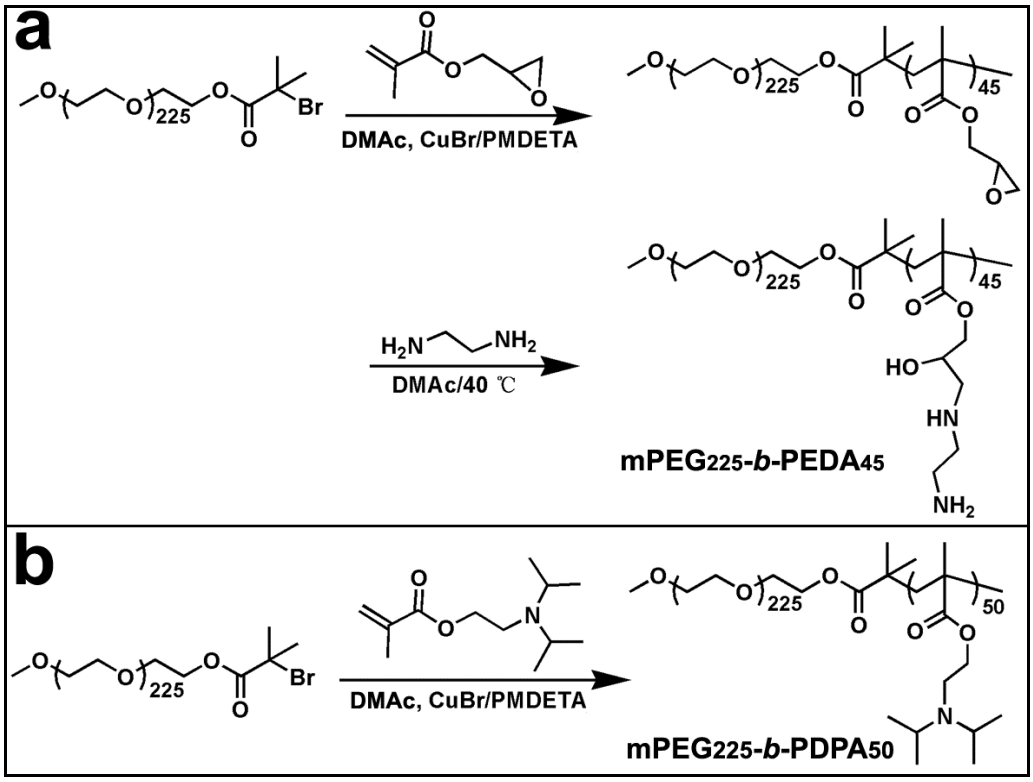

Figure S4. Synthetic route for (a) aminolated PEG-*b*-PEDA and (b) PEG-*b*-PDPA diblock copolymer.

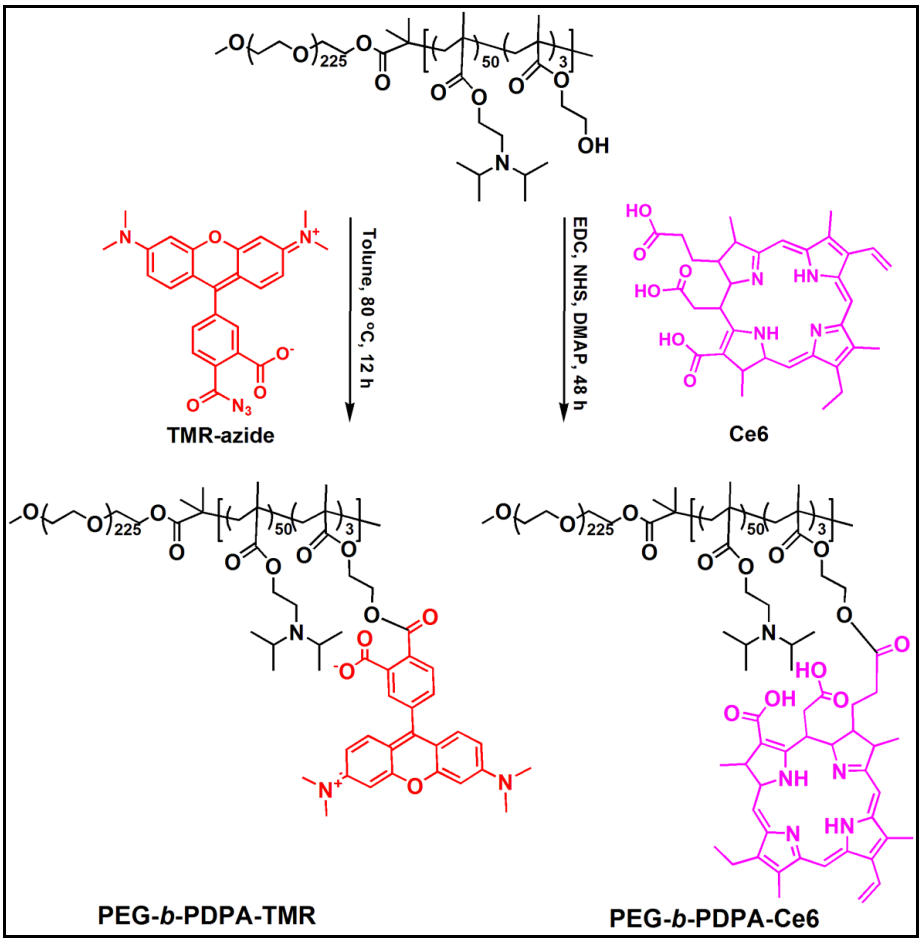

Figure S5. Synthetic route for PEG-*b*-PDPA diblock copolymer labeled with TMR or Ce6.

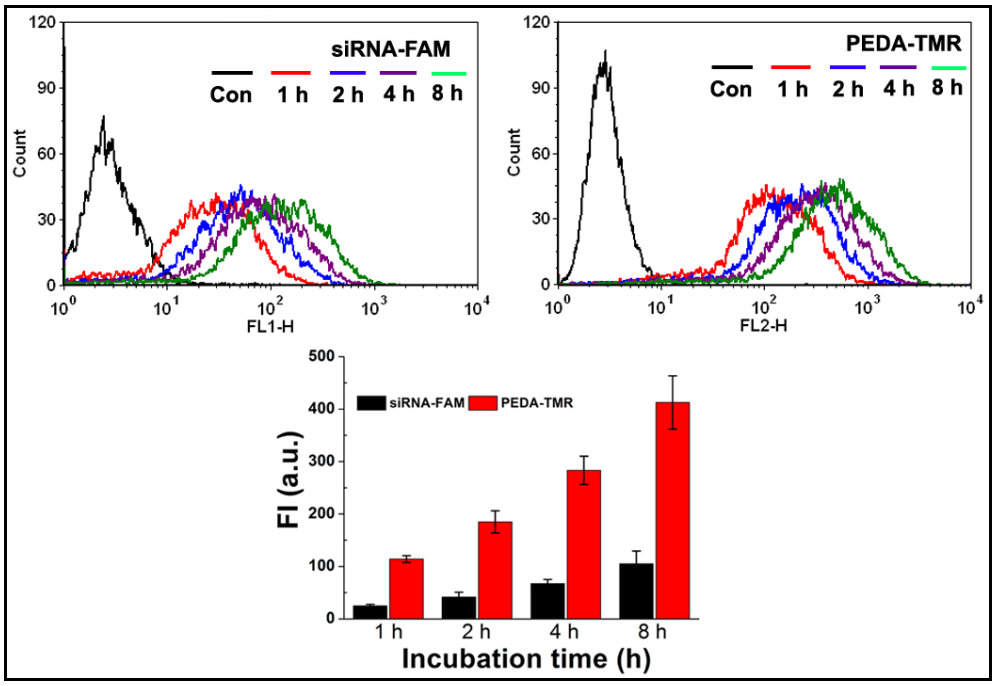

Figure S6. Flow cytometric measurement determined intracellular uptake efficiency of siRNA-loaded PEDA micelleplexes in A549 cells. The siRNA and PEDA was covalently labeled with FAM and TMR, respectively.

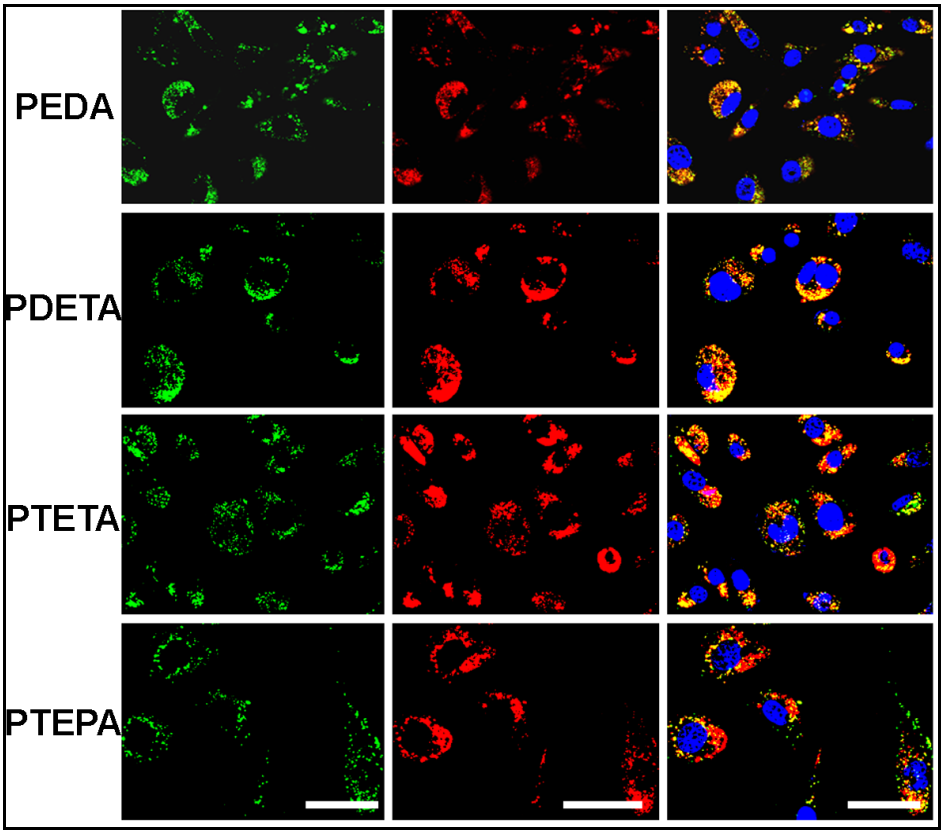

Figure S7. Representative CLSM images of intracellular distribution of siRNA-loaded PEDA micelleplexes in A549 cells. The siRNA and PEDA was covalently labeled with FAM (Green) and TMR (Red), respectively. The cellular nucleus was stained with DAPI (blue) (scale bar 100 μm).

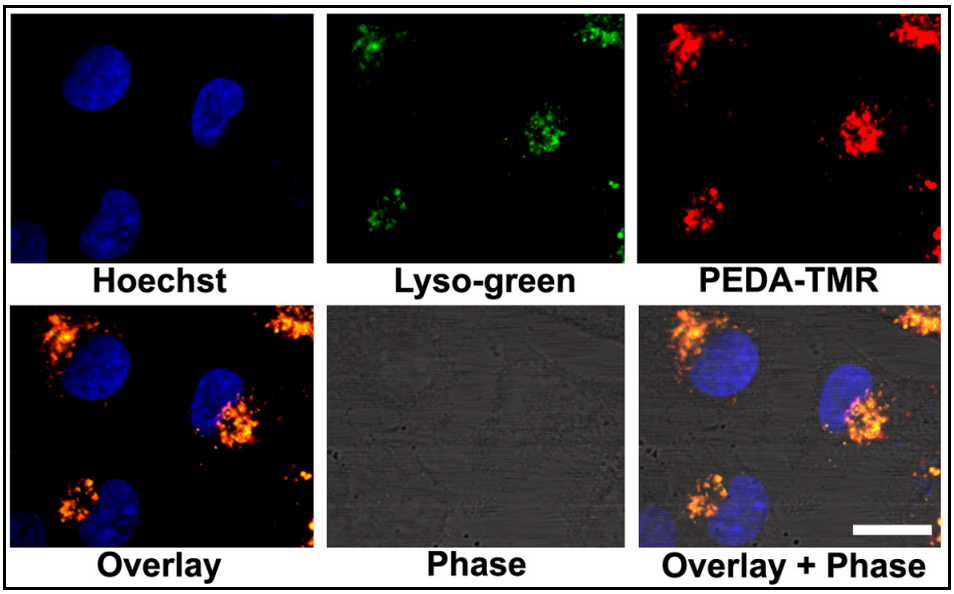

Figure S8. Representative CLSM images of colocalization of siRNA-loaded PEDA micelleplexes with lysosome vesicles in A549 cells. The PEDA micelles were covalently labeled with TMR (Red). The nuclei and lysosome vesicles were stained with Hoechst and lysotracker green, respectively (scale bar 50 μm).

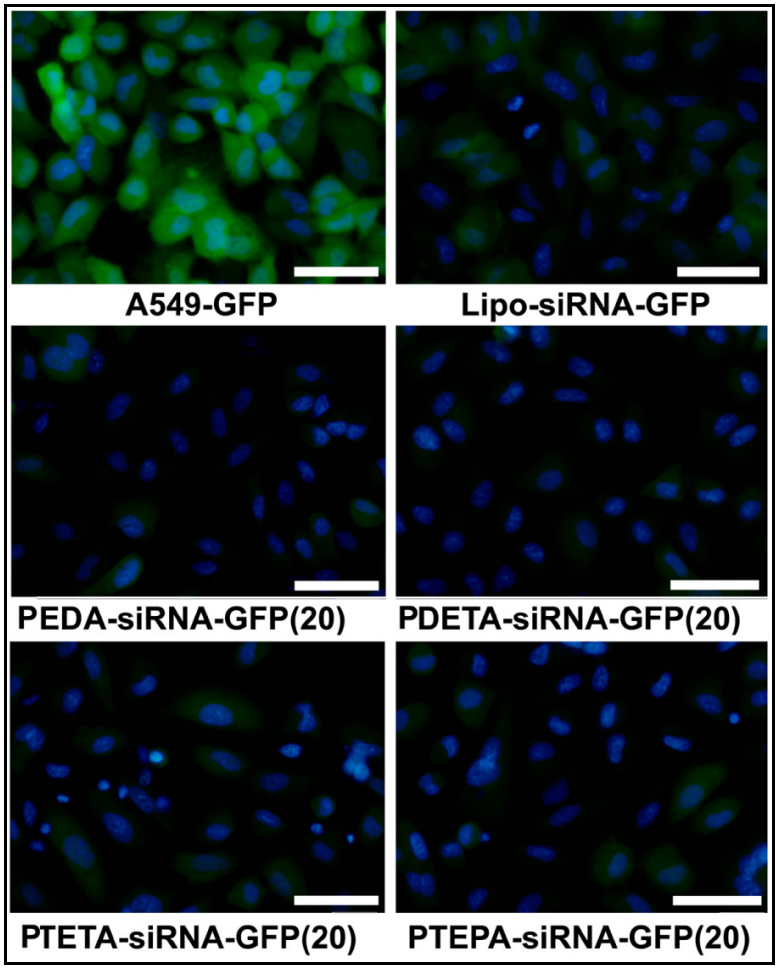

Figure S9. Representative fluorescence images of A549-GFP cells transfected by siRNA-GFP loaded micelleplexes or Lipoplexes. The micelleplexes were prepared at a micelle to siRNA weight ratio of 20. A siRNA concentration of 150 nM was applied for the transfection studies (scale bar 100 μm).

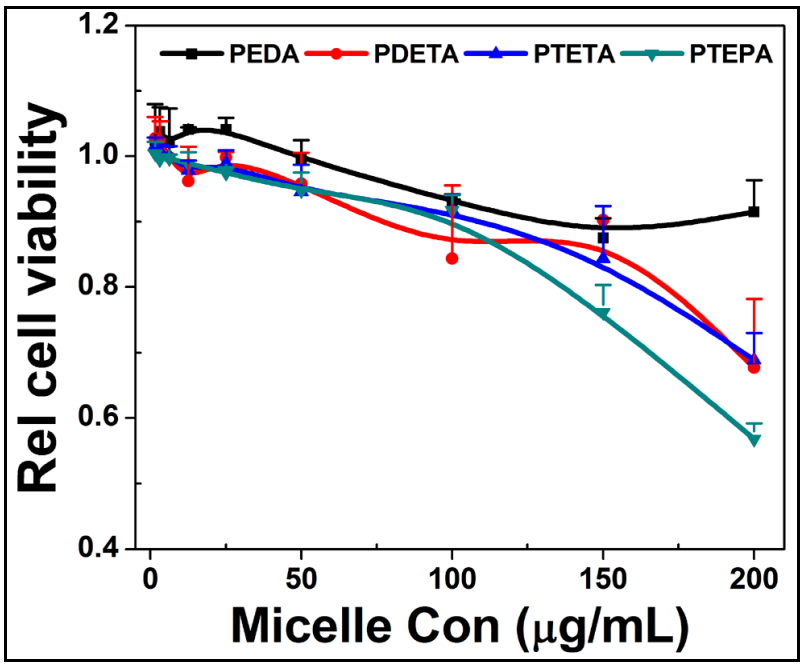

Figure S10. Cytotoxicity of PEDA, PDETA, PTETA and PTEPA micelles in A549 cells. The cells were incubated with the micelles for 24 h and then exposed to SRB assay.

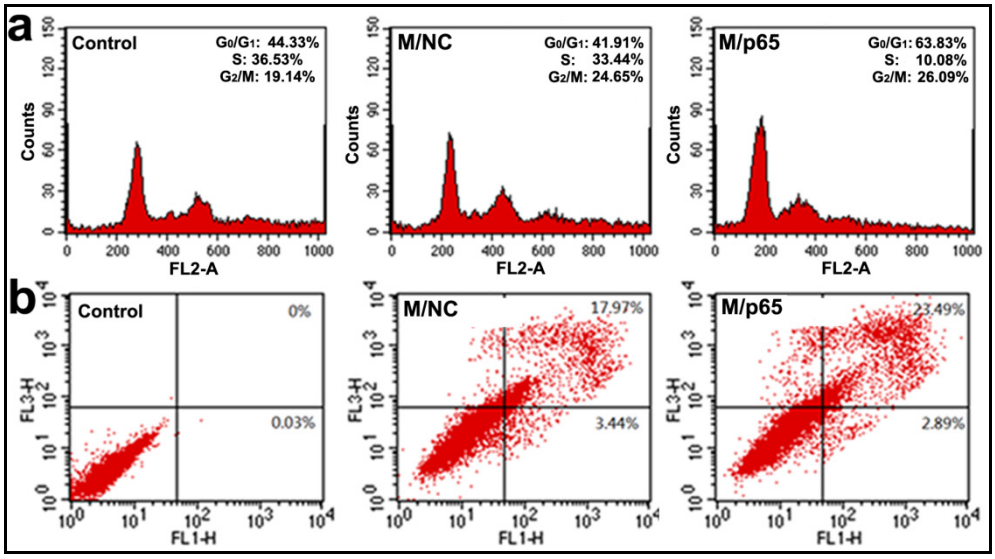

Figure S11. Influence of p65 knockdown on the proliferation and viability of 4T1 cells: (a) Cell cycle change; (b) apoptosis induction in 4T1 cells after transfected with siRNA-p65 loaded PEDA micelleplexes. A siRNA dose of 150 nM was applied for all the transfection studies.

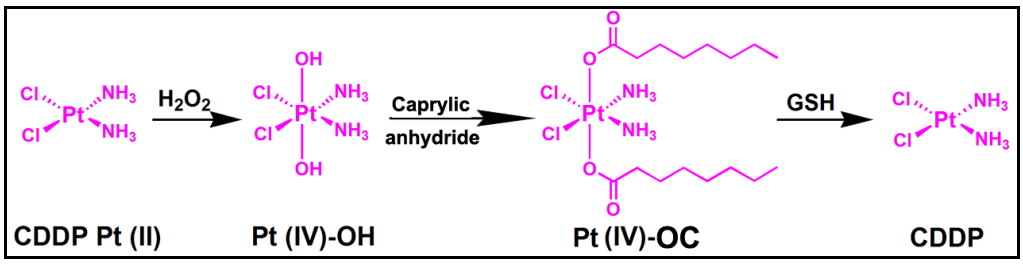

Figure S12. Synthetic route for hydrophobic Pt(IV)-OC prodrug.

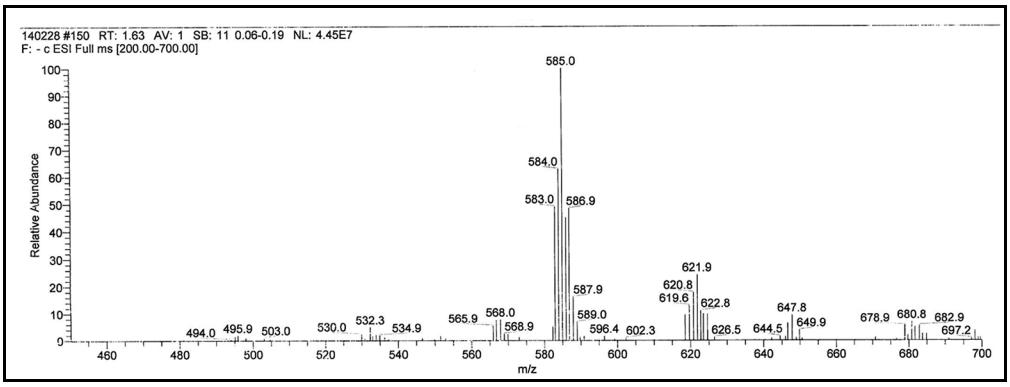

Figure S13. ESI-MS spectrum for Pt(IV)-OC prodrug.

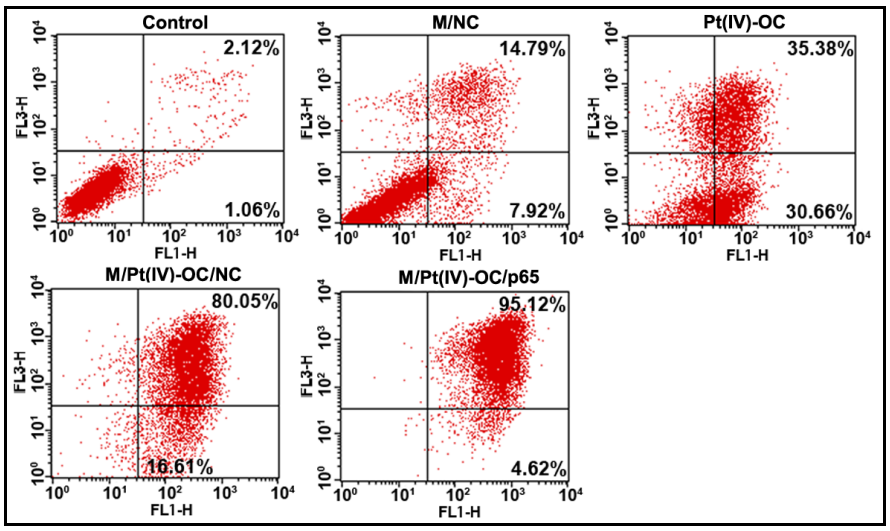

Figure S14. Apoptosis induction in 4T1 cells treated with siRNA-p65 and Pt(IV)-OC co-loaded PEDA micelleplexes at a siRNA concentration of 75 nM.

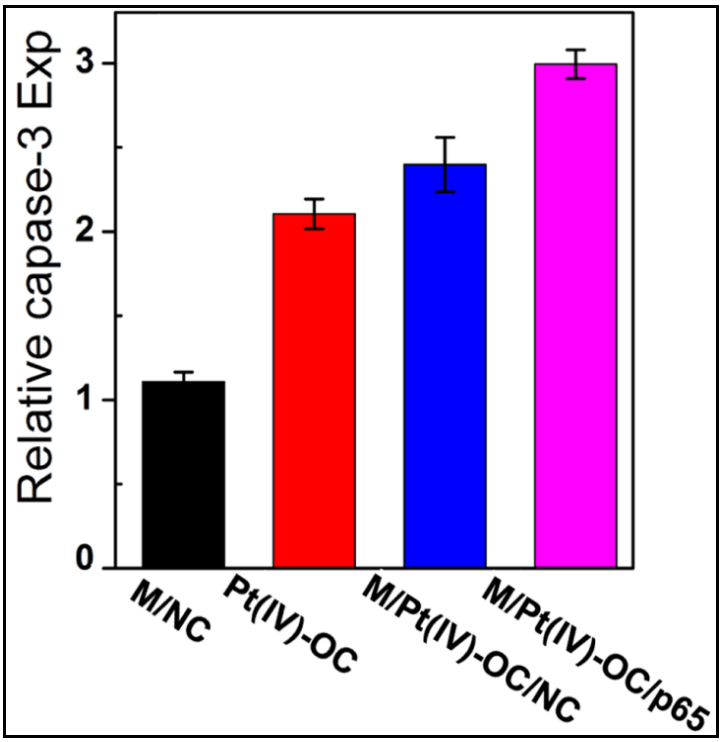

Figure S15. Colorimetric assay determined caspase-3 activation in 4T1 cells treated with Pt(IV)-OC and siRNA-p65 co-loaded PEDA micelleplexes at a siRNA concentration of 75 nM.

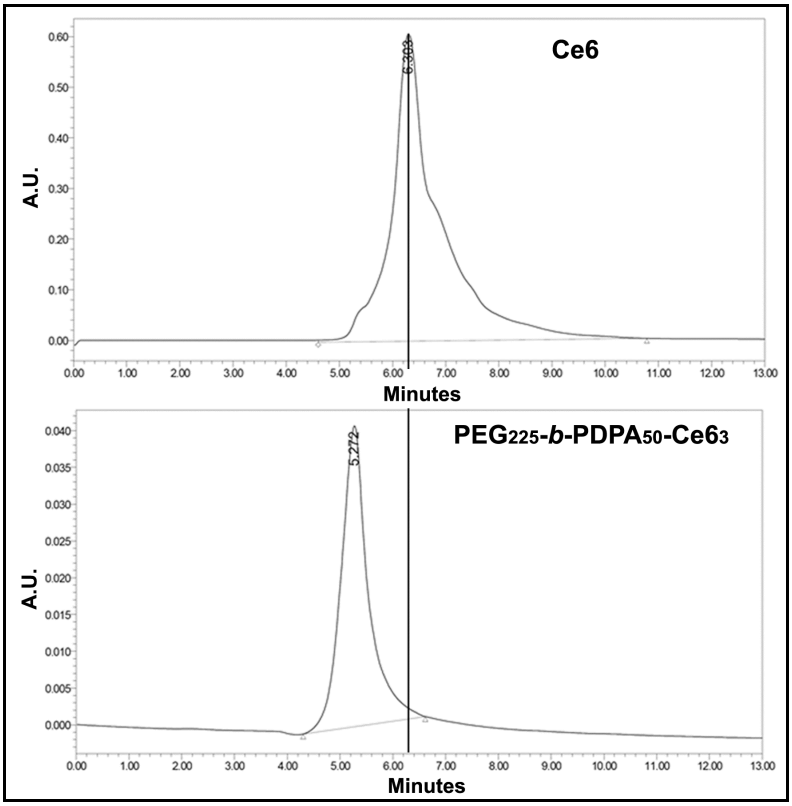

Figure S16. HPLC curves for free Ce6 and PEG-*b*-PDPA-Ce6 diblock copolymer. A narrow elution peak of PEG-*b*-PDPA-Ce6 was found, indicating the complete removal of free Ce6 by dialysis purification.

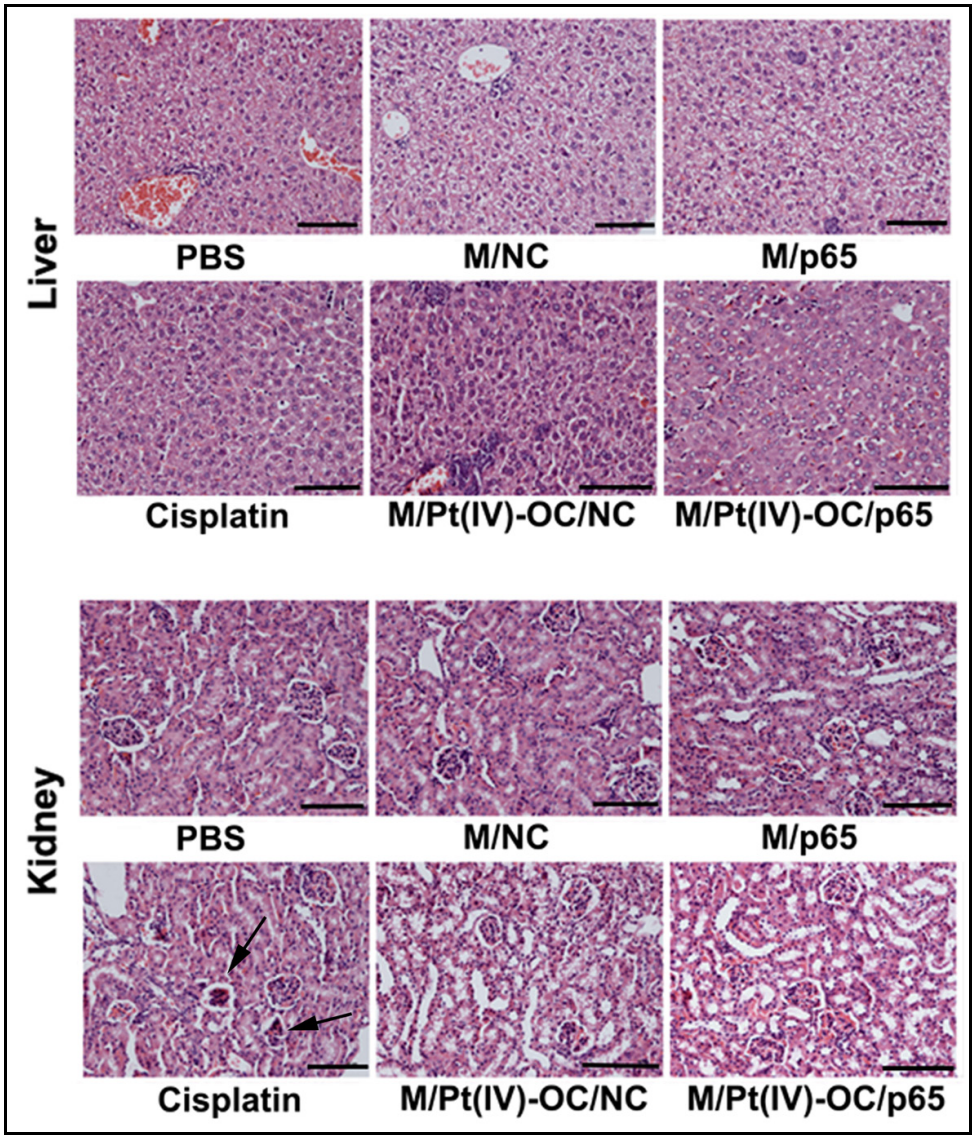

Figure S17. H&E examination of the liver and kidney collected at the end of anti-tumor studies (scale bar = 200 μm). The black arrows indicated the presence of tubular atrophy in the kidneys of cisplatin-injected mouse.