Supporting Information

Homologous-Targeting "Nanoconverter" with Variable Size for Tumor Deep Penetration and Immunotherapy

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Materials and animals

Dopamine hydrochloride (DA) and paclitaxel (PTX) were obtained from Aladdin Industrial Corporation (Shanghai, China). C6-ceramide was purchased from Cayman Chemical (Shanghai, China). 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) and 3,3′-dioctadecyloxacarbocyanine perchlorate (DIO) were purchased from Shanghai Beyotime Biological Technology Co. Ltd. Fluorescein diacetate (FDA) and propidium iodide (PI) were purchased from Shanghai Aladdin Bio-Chem Technology Co., LTD.

HeLa cells (cervical cancer cell line), U14 cells (mouse uterine cervix cancer cell) and 293T cells (human embryonic kidney cells line) were purchased from Shanghai Tianjing Biological Technology Co. Ltd. (Shanghai, China). The Lsgc-7801 cells (gastric cancer cell line) and HepG2 cells (human liver cancer cell line) were purchased from Cell Bank of the Chinese Academy of Sciences (Shanghai, China). The ELISA kits of CD4, CD8, interleukin-10 (IL-10), interleukin-12 (IL-12), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), 2′,7′-Dichlorofluorescin diacetate (DCFH-DA), IFN-γ, CD25, Blood urea nitrogen (BUN), alanine aminotransferase (ALT) and aspartate transaminase (AST) were purchased from MSKBIO (Wuhan, China). Fluorochrome-conjugated antibodies against of CD80/B7-1, CD44, G3 and CD62L/L-Selectin/SELL were purchased from Sino Biological (Beijing, China).

Male Kunming mice (19-22 g, 5-7 weeks old) were purchased from Vital River Laboratory Animal Technology Co., Ltd (Beijing, China).

Methods

Synthesis of PTX/PDA

5 mg dopamine hydrochloride (DA) was added to 10 mL Tris-HCl buffer solution (pH 8.5) under magnetic stirring to obtain black polydopamine (PDA) solution after 2 h. The PDA precipitation was

collected by centrifuging (10000 rpm, 15 min), and PDA was then resuspended in phosphate buffer (PBS pH 7.4). Subsequently, 0.1 mg paclitaxel (PTX) was dissolved in 1 mL of C_2H_5OH. The mixed solution was then added drop by drop into the PDA solution under stirring for 10 min. The mixture was centrifugated (10000 rpm, 15 min) and collected under room temperature to obtain the PTX/PDA.

Synthesis C6-ceramide-loaded Cytomembrane

Firstly, U14 cells (mouse uterine cervix cancer cell) were collected and washed with PBS three times, the cells were resuspended in PBS containing 10 mM Tris, 10 mM KCl, 2 mM $MgCl_2$ and 1×EDTA-free protease inhibitor at 4 °C for 1 h , and then the cell was centrifuged in 5000 rpm for 15 min at 4 °C, and the cells homogenate were disrupted under the ice with Ultrasonic Cell Disruption System with the mode of working for 2 s and resting for 2 s (300 W, 10 min). Followed, the cell homogenate was centrifuged in 14000 rpm for 1 h. Finally, the cytomembrane precipitation was resuspended with PBS under room temperature. Afterwards, the C6-ceramide was added into U14 cytomembrane solution drop by drop under magnetic stirring for 30 minutes at 40 °C, then the mixture was washed three times with fresh PBS to obtain C6-ceramide-loaded cytomembrane (M-C6) containing C6-ceramide 30 mol%.

Synthesis of PTX/PDA@M-C6

The mixture of PTX/PDA and M-C6 were shaken overnight in the air baths at 40 °C. Subsequently, the obtained solution was centrifuged in 3000 rpm for 10 min, then the supernatant solution was extruded for 15 times by Liposome Extruder with 100 nm filter and further centrifuged at 8000 rpm for 10 min. Lastly, the resulting PTX/PDA@M-C6 nanoparticles were resuspended in PBS.

Cell Culture and Animal Experiment

In all cell experiments, the cytomembrane of PTX/PDA@M-C6 was taken from Hela cells, and the cytomembrane was driven from U14 cells in animal experiments, which ensured the homologous targeting of the nanodrugs.

Analysis of Homologous targeting to Hela cells. The content of CD44 and G3 on the extracted cytomembrane, tumor cells and PTX/PDA@M-C6 was detected by Elisa kit. The HeLa cells, Lsgc-8701 cells and HepG2 cells were incubated with same concentration of DIO-dyed DOX/PDA@M-C6 for 2 h (The fluorescent drug DOX was used to replace the non-fluorescent drug PTX, DIO was used for M-C6 staining), and then observed under an inverted fluorescence microscope and quantitative analysis by Flow Cytometry.

Antitumor effect in vitro. HeLa cells were treated with 100 μL of 1: PTX, 2: PDA, 3: C6-ceramide 4: PTX/PDA, 5: PTX/PDA with laser irradiation, 6: PTX/PDA@M, 7: PTX/PDA@M with laser irradiation, 8: PTX/PDA@M-C6, 9: PTX/PDA@M-C6 with laser irradiation respectively, and evaluated the antitumor effect by MTT. Apoptosis rates of Hela cells detected by flow cytometry after treating with 100 μL of PTX/PDA@M+Laser, PTX/PDA@M-C6+Laser, PBS, PTX, C6-ceramide and PTX/C6-ceramide (the contercention of PTX/PDA@M-C6 was 1 mg/mL). Fluorescent microscopy images of live/dead staining of Hela cells cultured with 100 μL of saline, PTX, C6-ceramide, PTX/C6-ceramide, PDA+Laser, PTX/PDA+Laser, PTX/PDA@M+Laser and PTX/PDA@M-C6+Laser (808 nm, 1.5 W/cm^{-2}).

Sensitization of C6-ceramide to chemotherapy. 100 μL of PTX, C6-ceramide, and C6-PTX (Group 1: PTX: 0.00625 mg/mL, C6-ceramide:5% mmol; Group 2: PTX: 0.0125 mg/mL, C6-ceramide: 10% mmol; Group 3: PTX: 0.025 mg/mL, C6-ceramide: 15% mmol; Group 4: PTX: 0.05 mg/mL, C6-ceramide: 20% mmol; Group 5: PTX: 0.1 mg/mL, C6-ceramide: 25% mmol; Group 6:

PTX: 0.2 mg/mL, C6-ceramide: 30% mmol;) were added into Hela cells respectively, and antitumor effects were monitored by MTT assay.

Detection of intracellular ROS consumption by C6-ceramide. The content of ROS in RAW264.7 cells was detected by 2′, 7′-dichlorofluorescin diacetate (DCFH-DA) ROS assay kit. RAW264.7 cells were inoculated into the 12-well plates for 24 h. Different samples (1 mL of saline and 1mL of PTX/PDA@M-C6) were added to each well respectively. After 2 h, Dulbecco's Modified Eagle Medium (DMEM) was removed and the wells were washed twice with PBS, and then 1 mL DCFH-DA (0.01 mM) was added to each well and incubated for 20 min. DCFH-DA was removed and washed with PBS for three times. Then, fluorescence intensity of the RAW264.7 cells were tested by flow cytometer.

Accumulation and penetration of the nanodrugs in tumor.

The two group of tumor-bearing mice treated with PTX/PDA@M-C6 and PTX/PDA@M-C6 (+) were sacrifice after 12 h and tumors were collected. The tumors were weighed and freeze-dried. The fresh tumor tissue was cut and placed into RPMI-1640 culture medium with sterile operation. The tumor tissue was first cut into pieces with scissors and gently ground to obtain small cell masses. Then add 5 mL of enzymolysis liquid (collagen enzyme 0.2%, 0.01% hyaluronate enzyme and 0.002% hyaluronidase DNA enzymes in the RPMI-1640 culture medium), then mixed solution was kept warm in a water bath at 37 °C to digest 60 min, mixed the cells with a pipette once every 5 to 10 min. Subsequently, RPMI-1640 culture medium containing serum was added to termination of digestion, repeatedly mixed with a pipette for single cell suspension. Then, 100 mesh sieve filtration was used to collect filtrate twice, 1000 rpm centrifugal for 5 min, and then the cells were collected by serum free medium after 1 to 2 times. And the cells were then ablated with aqua regia and supernatant solution was taken. Amount of PTX/PDA@M-C6 in supernatant solution was analyzed

using absorbance at λ = 231 nm by UV-vis. Drug accumulation in all tumor tissue were calculated by measuring the content of PTX.

The two groups of tumor-bearing mice with similar tumor volumes (about 4 mm in radius) were treated with PTX/PDA@M-C6 (+) and PTX/PDA@M-C6. After 12 h, the tumors were carefully removed from mice. At 0 and 3 mm away from the tumor center (in radius), 1 mm^3 tissue block was taken, respectively. The fresh tumor tissue was cut and placed into RPMI-1640 culture medium with sterile operation. The tumor tissue was first cut into pieces with scissors and gently ground to obtain small cell masses. Then add 5 mL of enzymolysis liquid (collagen enzyme 0.2%, 0.01% hyaluronate enzyme and 0.002% hyaluronidase DNA enzymes in the RPMI-1640 culture medium), then mixed solution was kept warm in a water bath at 37 °C to digest 60 min, mixed the cells with a pipette once every 5 to 10 min. Subsequently, RPMI-1640 culture medium containing serum was added to termination of digestion, repeatedly mixed with a pipette for single cell suspension. Then, 100 mesh sieve filtration was used to collect filtrate twice, 1000 rpm centrifugal for 5 min, and then the cells were collected by serum free medium after 1 to 2 times. And the cells were then ablated with aqua regia and supernatant solution was taken. Finally, amount of PTX/PDA@M-C6 was analyzed using absorbance at λ = 231 nm by UV-vis.

All experiments with mice were performed in accordance with the statute of Experimental Animal Ethics Committee of Department of Bioengineering, School of Environment and Chemical Engineering, Yanshan University.

Immune Factors Analyses

Fresh mouse eye peripheral blood was added with anticoagulant, and mononuclear cells were isolated by erythrocyte lysate. Ex *vivo* staining of lymphocytes with fluorochrome-labeled antibodies was performed on single-cell suspension and analyzing. Besides, after the mice were injected the

different samples (saline, PTX/PDA@M, PTX/PDA@M-C6 and PTX/PDA2M-C6+Laser), their blood serum cytokines including CD4, CD8, IL-10, IL-12, IL-6, TNF-α, IFN-γ, CD80, CD62L and CD25 levels were determined by ELISA method.

Monitoring for Tumors Recurrence

The other tumor-bearing mice treated with saline, PTX/PDA@M+Laser, PTX/PDA@M-C6+Laser were continuously tracked of body weights and tumor volume after treatment for 10 d (The mice were treated in exactly the same conditions as the corresponding treatment groups). Meanwhile, their blood serum cytokines levels were determined by ELISA method.

Loading Capacity of PTX

Amount of PTX on PTX/PDA@M-C6 was analyzed using absorbance at λ = 231 nm according to the corresponding standard calibration curve by subtracting the absorbance of PTX/PDA@M-C6 at that wavelength (λ = 231). The loading capacity was calculated as follows:

$$Loading\ capacity\ (\%W/W) = \frac{mass\ of\ drug\ loaded}{mass\ of\ nanoparticles} \times 100\ \%$$

Characterization

The hydrodynamic diameters and zeta potentials of M-C6, PTX/PDA or PTX/PDA@M-C6 nanoparticles were measured using dynamic light scattering examination (Malvern Instruments, UK). Surface morphology of the samples were characterized by transmission electron microscope (TEM) operated at 80 kV. The thermal images were recorded by thermal imaging camera (InfraTec VarioCAM, Germany). The chemical composition of PTX/PDA@M-C6 was analyzed using Energy dispersive spectroscopy (EDS) on JEM-2010 instrument (accelerating voltage of 200 kV). At the same time, SHIMADZU UV2550 Ultraviolet–visible spectrophotometer (UV–Vis) was used to observe the absorption spectra of the samples. Cell apoptosis was tested by Flow Cytometry (FCM, BD FACS-Calibur, USA).

Stability Studies of PTX/PDA@M-C6.

To evaluate the serum stability of PTX/PDA@M-C6, the sizes of nanodrug were determined after incubation in DMEM with different concentration (0, 10%, 20% and 30%) of fetal bovine serum (FBS).

Photothermal Effects

Photothermal Conversion Capacity and Photothermal Stability. The photothermal effects of PTX/PDA@M-C6 with various concentrations (1 mg/mL, 5 mg/mL and 10 mg/mL) were investigated under 808 nm laser irradiation at a power of 1.5 W cm^{-2} for 5 min, and the temperature was measured one time per 30 s. The thermal images were recorded by thermal imaging camera to examine the photothermal effects in tube irradiated by near-infrared light (NIR) every 1 min.

Drug Release Controlled by NIR. The PTX release was studied by dialysis the nanodrug under moderate shaking. At two points of time (the 1st and 4th hour), the prepared-solutions were irradiated by 808 nm laser (1.5 W cm^{-2}, 5 min), and the amounts of PTX were analyzed by UV–Vis at 231 nm.

Cell Culture and Animal Environment

Culture Environment of Cells. The Hela cells, 293T cells, Lsgc-7801 cells and HepG2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum and 1% penicillin/streptomycin at 37 °C containing 5% CO_2.

Animal Modeling The mice acclimated to standard laboratory environment for a week, and then the U14 cells (2.0×10^6 cells) were injected subcutaneously into the right buttock of the mice. The tumor-bearing mice were randomly divided into 8 groups (6 animals in each group) and treated differently when the average tumor size reached about 100 mm^3. 200 μL samples were injected into the tail vein of each mouse every 2 d, and recording the tumor volume and body weights of mice during the following 14 d. Relative tumor volumes (V) were calculated with the following formula:

$$V = \frac{V_n}{V_0}$$

V_0 was the initial tumor volume, V_n was the real-time tumor volume.

Potential Toxic Effects of PTX/PDA@M-C6. Fresh mouse eye peripheral blood with anticoagulant was obtained from different groups after treatment (saline and PTX/PDA@M-C6+Laser), and the levels of BUN, ALT, AST were determined by ELISA method to explore the biological toxicity of PTX/PDA@M-C6. AST level can be sensitive to detect liver damage. BUN level is an important indicator of normal renal function. ALT level can be used to detect lesions in liver and heart tissue.

Macrophages infiltration and polarization. Tumor tissues were collected with different treatment and the macrophages were processed with Cell Separation Media. For flow cytometry assay of macrophages, single cell suspensions were obtained by abrading of tissues. Follow the kit instructions, the single-cell solution were co-stained with anit-Ly6C and anti-F4/80 antibodies and analyzed by flow cytometry for analysis M1-like macrophages. In addition, the single-cell solution were co-stained with anti-F4/80 and anti-RELMα antibodies and analyzed by flow cytometry for M2-like macrophages.

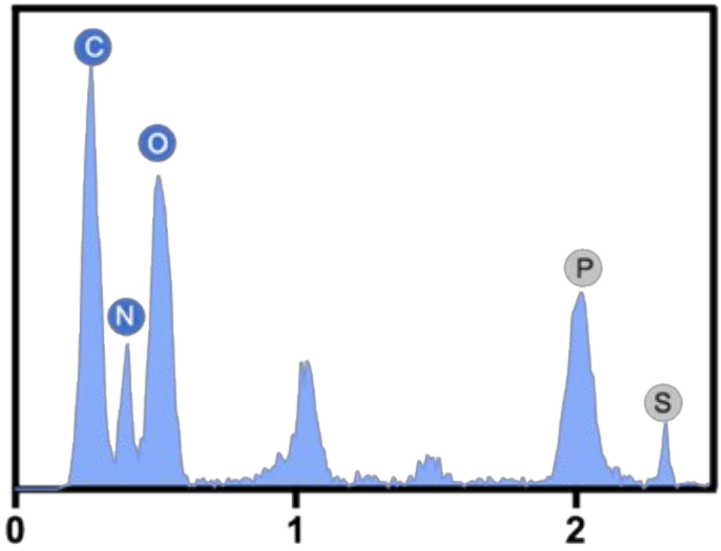

Figure S1. EDS analysis of the various components of PTX/PDA@M-C6.

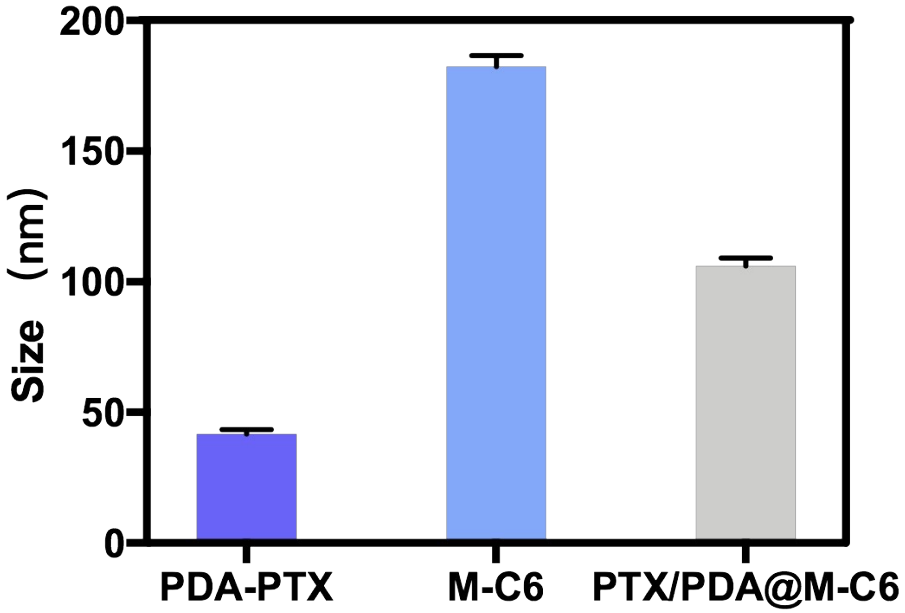

Figure S2. Particle sizes of PTX/PDA, M-C6 and PTX/PDA@M-C6.

Figure S3. Encapsulation efficiency of C6-Ceramide, PTX and CM in PTX/PDA@M-C6.

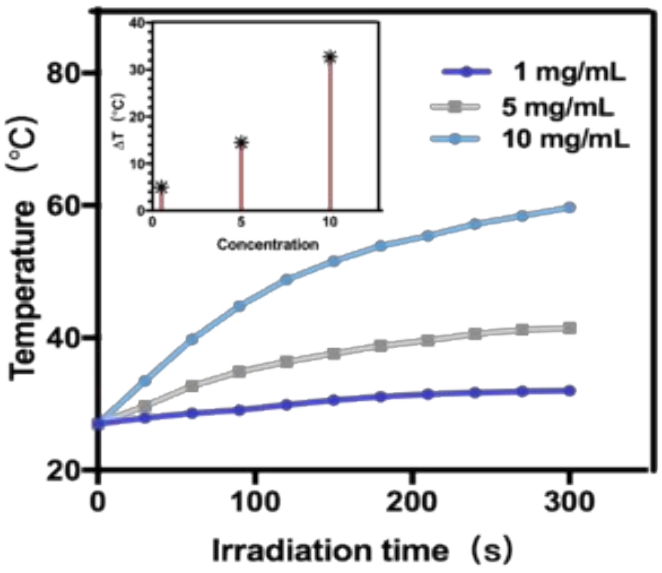

Figure S4. Temperature elevation profiles of PTX/PDA@M-C6 with different PDA concentrations (1, 5, 10 mg/mL).

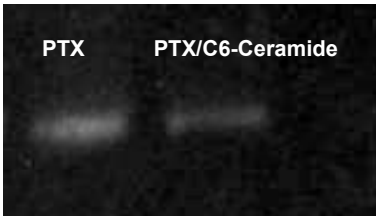

Figure S5. Bcl-2 expression in HeLa cells treated with PTX and PTX/PTX/C6-ceramide detected by western blot.

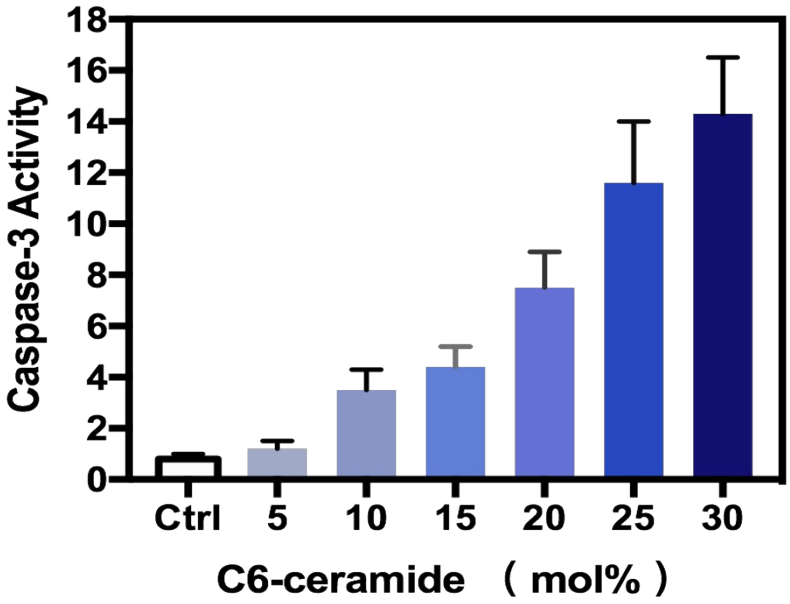

Figure S6. Levels of cleaved caspase-3 in HeLa cells treated with the PTX/C6-creamide with different C6-ceramide concentrations.

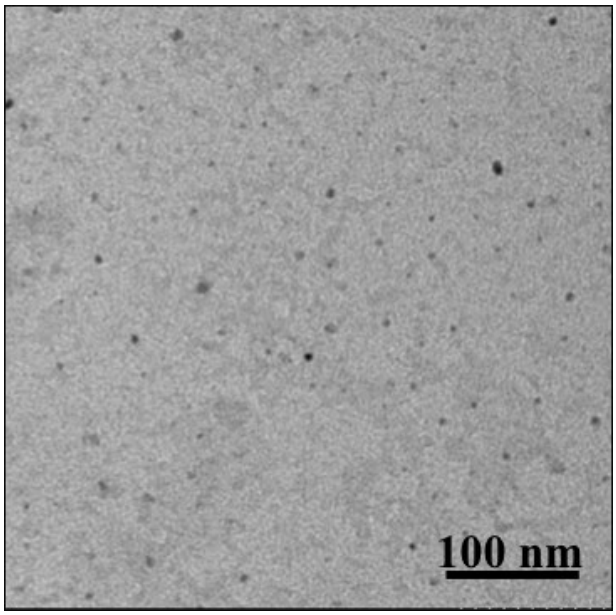

Figure S7. TEM of morphology and size change of PTX/PDA @M-C6 after NIR irradiation.

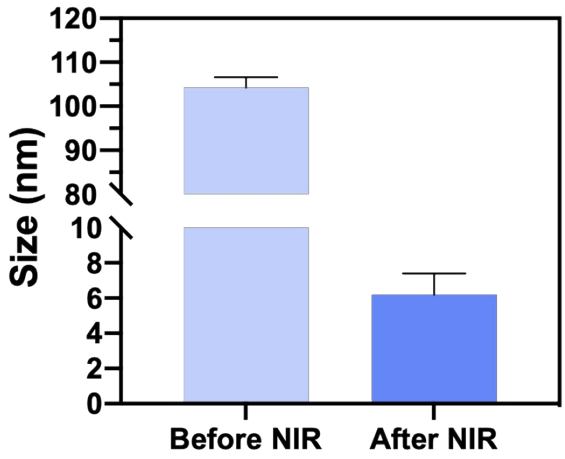

Figure S8. Size of PTX/PDA@M-C6 before and after NIR irradiation.

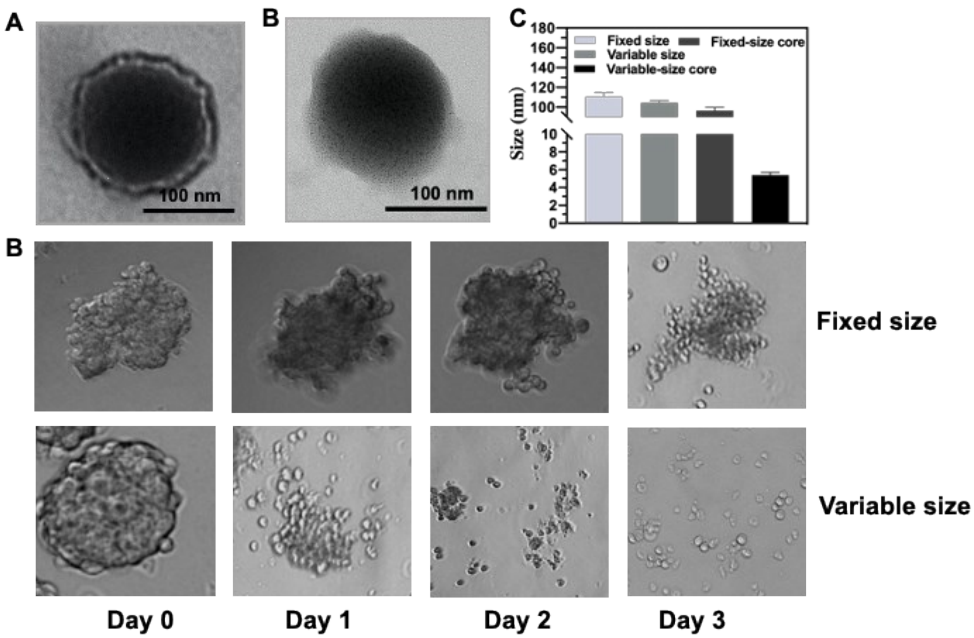

Figure S9. TEM images of **(A)** fixed-size nanomaterials of PTX/PDA@M-C6 and **(B)** variable-size nanoconverters of PTX/PDA@M-C6. **(C)** Size of fixed-size nanomaterials, variable-size nanoconverters, fixed-size PTX/PDA core and variable-size PTX/PDA core. **(D)** 3D cell spheroids treated with fixed-size nanomaterials and variable-size nanoconverters at 0, 1, 2 and 3 d.

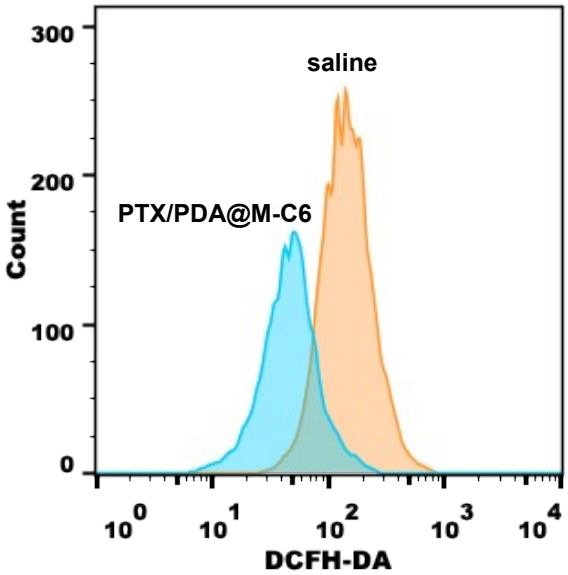

Figure S10. Fluorescence intensity of DCFH-DA in macrophages (RAW264.7) treated with saline and PTX/PDA@M-C6.

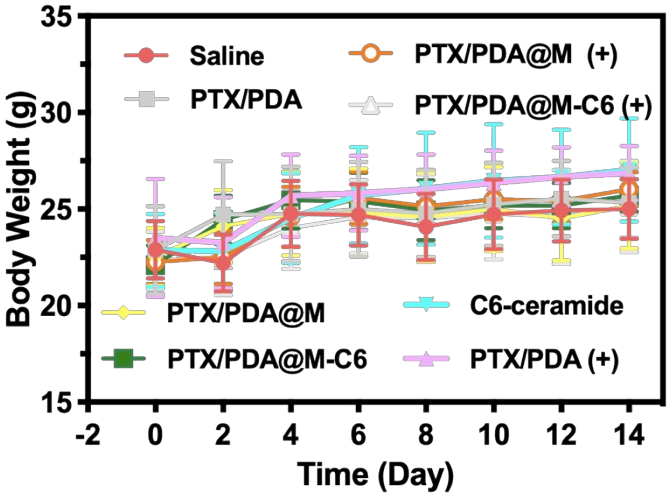

Figure S11. The body weight of mice in different treatment groups.