

Supporting Information

A dendritic polymer-based nanosystem mediates drug penetration and irreversible endoplasmic reticulum stresses in tumor via neighboring effect

*Xiuli Zheng, Dayi Pan, Guonian Zhu, Lu Zhang, Apanpreet Bhamra, Rongjun Chen, Hu Zhang, Qiyong Gong, Zhongwei Gu, Kui Luo**

Dr. X. L. Zheng, Dr. D. Y. Pan, G.N. Zhu, Prof. Q. Y. Gong, Prof. Z. W. Gu, Prof. K. Luo
Huaxi MR Research Center (HMRRC), Department of Radiology, National Clinical Research Center for Geriatrics, Frontiers Science Center for Disease-Related Molecular Network, State Key Laboratory of Biotherapy, West China Hospital, Sichuan University, Chengdu, 610041, China

*E-mail: luokui@scu.edu.cn (Prof. Luo)

G.N. Zhu, L. Zhang

Laboratory of Clinical Proteomics and Metabolomics, Institutes for Systems Genetics, and Core Facility of West China Hospital, West China Hospital, Sichuan University, Chengdu, 610041, China

A. Bhamra, Dr. R. Chen

Department of Chemical Engineering, Imperial College London, South Kensington Campus, London, SW7 2AZ, United Kingdom

Prof. H. Zhang

Amgen Bioprocessing Centre, Keck Graduate Institute, Claremont, CA 91711, USA

Prof. Q. Y. Gong, Prof. K. Luo

Functional and molecular imaging Key Laboratory of Sichuan Province, and Research Unit of Psychoradiology, Chinese Academy of Medical Sciences, Chengdu, 610041, China

1. Experimental Section

1.1 Materials

Amino acids (H-Glu(OtBu)-OtBu·HCl, Fmoc-Lys(Fmoc)-OH) and condensation agents (1-hydroxybenzotriazole (HOBt), 1-Hydroxy-7-azabenzotriazole (HOAt), O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluorophosphate (HBTU), O-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU)) were purchased from GL Biochem Ltd. (Shanghai, China). N, N-Diisopropylethylamine (DIPEA) and trifluoroacetic acid (TFA) were purchased from Asta Tech Pharmaceutical Co. Ltd. (Chengdu, China). Boc-cystamine hydrochloride was purchased from Shanghai Bide Pharmaceutical Technology Co. Ltd. (Shanghai, China). Methoxy PEG succinimidylcarboxymethyl ester (mPEG-NHS, MW=2000) was purchased from Shanghai Ponsure Biotechnology Co. Ltd and Pyropheophorbide-a (Ppa) from Shanghai Xianhui Pharmaceutical Co. Ltd. (Shanghai, China). Dimethyl sulfoxide-*d*₆ (DMSO-*d*₆), deuterium oxide (D₂O), 9, 10-dimethylanthracene (DMA) and doxorubicin hydrochloride (DOX·HCl) were purchased from Sigma-Aldrich (Shanghai, China). Glutathione (GSH) and Pyrene were purchased from Aladdin Reagents Company (Shanghai, China). Sodium Chloride (NaCl), Urea and Triton@X-100 were purchased from Guangzhou saiguo biotech Co. Ltd. (Guangzhou, China). The CCK-8 kit was purchased from Dojindo Molecular Technologies, Inc., Japan.

1.2 DPD simulations method

In DPD simulations, individual atoms or molecules are coarse-grained into beads and the canonical ensemble simulations are performed in a three-dimensional box of size 30 x 30 x 30. The bead number density ρ is 3, and the integration time step Δt is 0.02. The interaction parameter α_{ij} is a constant which describes the maximum repulsions between interacting beads, which are shown in Table S1. The interaction parameter α_{ij} between beads can be mapped to the Flory-Huggins parameter χ through $\Delta\alpha_{ij} = 3.27\chi_{ij}$. The parameter χ can be calculated by the following equation:

$$\chi_{ij} = v_{ij} / RT (\delta_i - \delta_j)^2$$

Here, V_{ij} is the average molar volume of the bead, R is the gas constant, T is the absolute temperature, δ_i and δ_j are the solubility parameters for the chemical entities *i* and *j*, which can be calculated with Materials Studio Program (Accelrys Inc.)

To capture the essential features of the drug-loaded system and PDPP, we constructed a coarse-grained model of PDPP and DOX, which are typically shown in Scheme S1. There are different beads in this coarse-grained model, i.e., “G” for PEG, “K” for lysine, “E” for glutamic

acid, “S” for disulfide bond, “P” for Ppa, and “D” for DOX, and three molecules of water are represented as one bead “W”. Water beads are not shown for the sake of clarity.

1.3 Syntheses and characterizations of PDPP@D

Synthesis of (Fmoc)₄-K₂E₂-SS-(Boc)₄

Firstly, (Fmoc)₄-K₂E₂-(OtBu)₄ was prepared via a synthesis route from previously reported studies.^[1] Tert-butyl groups of (Fmoc)₄-K₂E₂-(OtBu)₄ were removed after treatment with 50% TFA in DCM for 6 h. Boc-cystamine hydrochloride was reacted with (Fmoc)₄-K₂E₂-(COOH)₄ to generate (Fmoc)₄-K₂E₂-SS-(Boc)₄. (Fmoc)₄-K₂E₂-(COOH)₄ (1 equiv), Fmoc-Lys(Fmoc)-OH (6 equiv), HOBT (6 equiv), HBTU (6 equiv) were combined in 20 mL of anhydrous DMF, and DIPEA (16 equiv) was added in an ice bath. The reaction mixture was stirred at room temperature under nitrogen for 48 h. The solvent was evaporated and the residue was dissolved in EtOAc. The mixture was washed with saturated NaHCO₃, HCl (1 M) and brine. The solution was dried with MgSO₄ and concentrated using a rotary evaporator. The residue was recrystallized in cold EtOAc to give a final yield of 61.4 %. MALDI-TOF MS: m/z= 2639.22 ([M+Na]⁺). ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.20 (s, 4H), 7.96 (d, *J* = 20.5 Hz, 5H), 7.94 – 7.84 (m, 8H), 7.68 (dd, *J* = 15.8, 7.4 Hz, 8H), 7.41 (m, 10H), 7.30 (t, *J* = 7.0 Hz, 8H), 6.96 (d, *J* = 3.9 Hz, 4H), 4.24 (dd, *J* = 23.5, 10.3 Hz, 18H), 3.18 (d, *J* = 6.3 Hz, 8H), 2.92 (d, *J* = 32.4 Hz, 14H), 2.73 (d, *J* = 6.2 Hz, 16H), 2.13 (d, *J* = 21.6 Hz, 12H), 1.86 (s, 6H), 1.57 (d, *J* = 47.7 Hz, 6H), 1.33 (d, *J* = 18.1 Hz, 36H), 1.23 (s, 6H).

Synthesis of (PEG)₄-K₂E₂-SS-(Boc)₄

(Fluoren-9-ylmethoxy) carbonyl groups of (Fmoc)₄-K₂E₂-SS-(Boc)₄ were removed after treatment with 50% DEA in DMF for 30 min. mPEG2k-NHS was reacted with (NH₂)₄-K₂E₂-SS-(Boc)₄ to generate (PEG)₄-K₂E₂-SS-(Boc)₄. (NH₂)₄-K₂E₂-SS-(Boc)₄ (1 equiv) and mPEG2k-NHS (9 equiv) were combined in 10 mL of anhydrous DMF, and DIPEA (12 equiv) was added in an ice-bath. The reaction mixture was stirred at room temperature under argon in the dark for 48 h. The mixture was dialyzed in the dark with Milli-Q water, and further fractionated/purified by size-exclusion chromatography using a Superose 12 HR/16/30 column on an ÄKTA FPLC system (GE Healthcare) with sodium acetate buffer containing 30% acetonitrile (pH 6.5) as a mobile phase. The mixture was dialyzed again in the dark with Milli-Q water and lyophilized to give a final yield of 72.5 %. MALDI-TOF MS: m/z= 9749.05 ([M+Na]⁺).

Synthesis of PEG-Dendritic peptide-SS-Ppa (PDPP)

t-Butyloxy carbonyl groups of (PEG)₄-K₂E₂-SS-(Boc)₄ were removed after treatment with 50% TFA in DCM for 6 h. Pyropheophorbide a was reacted with (PEG)₄-K₂E₂-SS-(NH₂)₄ to generate (PEG)₄-K₂E₂-SS-(Ppa)₄. (PEG)₄-K₂E₂-SS-(NH₂)₄ (1 equiv), pyropheophorbide a (6

equiv), HOAt (6 equiv), HATU (6 equiv) were combined in 15 mL of anhydrous DMF, and DIPEA (16 equiv) was added in an ice-bath. The reaction mixture was stirred at room temperature under nitrogen in the dark for 48 h. The mixture was dialyzed in the dark with Milli-Q water, and further fractionated/purified by size-exclusion chromatography using a Superose 12 HR/16/30 column on an ÄKTA FPLC system (GE Healthcare) with sodium acetate buffer containing 30% acetonitrile (pH 6.5) as a mobile phase. The mixture was dialyzed again in the dark with Milli-Q water and lyophilized to give a final yield of 49.7 %. MALDI-TOF MS: $m/z = 11414.82$ ($[M+Na]^+$).

Preparation of DOX-loaded nanoparticles

Typically, 40 mg PDPP was dissolved in 1 mL DMF, followed by adding a predetermined amount of DOX·HCl and three molar equivalents of triethylamine. The mixture was stirred at room temperature for 1 h. The mixture was slowly added into 30 mL Milli-Q water and stirred for 24 h. Subsequently, the mixture was dialyzed in the dark with Milli-Q water and lyophilized to give a black solid product. Finally, the drug loading content (DLC) of PDPP@D was determined by a UV-Vis spectrophotometer (Specord 200, Analytik Jena, Germany). The DLC was calculated according to the following equation:

$$\text{DLC (wt\%)} = (\text{weight of drug in NPs} / \text{weight of NPs}) \times 100\%$$

Differential scanning calorimetry (DSC)

Thermal analysis was conducted on a differential scanning calorimeter device (TADSC Q2000, USA) to evaluate chemical bonding of DOX in PDPP@D. Samples, PDP, PDPP@D, DOX·HCl, physical mixtures of DOX·HCl and PDPP were sealed into aluminum pans with DSC sample press and heated from 40 °C to 300°C at a heating rate of 10 °C·min⁻¹ under a nitrogen atmosphere, respectively. A blank aluminum cell was used as a reference, and the heat change of the sample during the heating process was recorded, and the DSC curve of each sample was plotted.

Critical assembly concentration (CAC)

The CAC of PDPP was determined by using pyrene as a fluorescence probe. Typically, 20 µL of an acetone solution of pyrene (5×10^{-5} mol L⁻¹) was transferred into a 5 mL vial. After acetone was evaporated, 2 mL of an aqueous solution of PDPP was added into the vial to obtain the pyrene concentration of 5×10^{-7} mol L⁻¹. The excitation spectra of pyrene with different nanoparticle concentrations were acquired by a fluorescence spectrophotometer (F-7000, Hitachi, Japan, Emission: 668 nm, Excitation: 320-360 nm, slit width: 5 nm).

Photophysical properties

UV-Vis absorption spectrum: PDPP, PDPP@D, Ppa and DOX were dissolved in anhydrous DMF (1 mg mL⁻¹) and then diluted 10 folds in different solutions (DMSO, H₂O, H₂O +0.1%SDS). The absorption spectra of PDPP, PDPP@D, and Ppa at 300-800 nm, and PDPP@D and DOX at 500-750 nm were recorded.

Fluorescence emission spectrum: To assess the self-quenching and dequenching effect of PDPP and PDPP@D, the fluorescence emission spectra of PDPP, PDPP@D, Ppa and DOX in different solutions (DMSO, H₂O, H₂O+0.1%SDS) were obtained (Excitation: 405 nm or 480 nm, Emission: 600-750 nm or 500-750 nm, slit width: 5 nm).

Photostability: In a 96-well plate, 100 μ L aliquots of PDPP, PDPP@D and Ppa in different solutions (DMSO and H₂O) at a Ppa concentration of 10 μ g mL⁻¹ were arrayed in triplicate. The absorbance at 667 nm was read on a microplate reader (ThermoFisher SCIENTIFIC). Each well was then irradiated with a 660 nm laser at 5 mW·cm⁻² at 4 min intervals for 52 min, and the absorbance value was read at 8 min intervals. The absorbance values at 667 nm were then normalized to the pre-irradiation values and the normalized absorbance values were plotted versus the cumulative irradiation time.

Molar extinction coefficients (ϵ): The molar extinction coefficients of PDPP and PDPP@D were measured by a UV/Vis spectrophotometer. PDPP and PDPP@D were dissolved in anhydrous DMSO and then diluted to different concentrations (0.01875, 0.0375, 0.075, 0.15, 0.3, 0.6, and 1.2 $\times 10^{-6}$ mol L⁻¹). The absorbance values of samples at 668 nm were read and the extinction coefficient was calculated according to the Beer-Lambert law.

Fluorescent quantum yield (FQ): PDPP, PDPP@D and Ppa were dissolved in anhydrous toluene (6 $\times 10^{-6}$ mol L⁻¹). The peak area of the fluorescence emission spectrum of PDPP, PDPP@D and Ppa was calculated (Excitation: 660 nm, Emission: 600-750 nm, slit width: 5 nm). The absorbance value of PDPP, PDPP@D and Ppa at 600 nm was read. The fluorescent quantum yield was calculated from the following equation:

$$\Phi_{\Delta} = \frac{P_S}{P_R} \times \frac{A_R}{A_S} \times \text{ref. } \Phi_{\Delta}$$

Where A was the absorbance at an excitation wavelength of 660 nm, and P was the peak area of the fluorescence emission spectrum. S referred to the sample and R to the reference. The FQ (Φ_{Δ}) of each sample was calculated from the reference Φ_{Δ} value, 0.30 for Ppa.

Singlet oxygen quantum yield (SOQ): The amount of singlet oxygen generated from PDPP, PDPP@D and Ppa was detected using 9, 10-dimethylanthracene (DMA) as a singlet oxygen sensor. PDPP, PDPP@D and Ppa were dissolved in DMSO and added to a DMA stock solution

to obtain a final concentration of 25 μM DMA. Each sample was added into a 96-well plate, followed by laser irradiation (80 mW cm^{-2}) at a wavelength of 660 nm. The fluorescence intensity of DMA (excitation at 360 nm; emission 440 nm) was measured over time. The singlet oxygen quantum yield was calculated from the equation:

$$\Phi_{\Delta} = \frac{K_S}{K_R} \times \frac{A_R}{A_S} \times \text{ref. } \Phi_{\Delta}$$

Where A was absorbance at 660 nm (the excitation wavelength), and K was the gradient of the DMA intensity curve. S referred to the sample and R to the reference. The SOQ (Φ_{Δ}) of each sample was calculated from the reference Φ_{Δ} value, 0.50 for Ppa.

Size, zeta potential and morphology

The size, size distribution and zeta potential of NPs were measured by NanoBrook Omni (Brookhaven Instruments, New York, USA) via dynamic light scattering (DLS) methods, and the morphologic and particle size characterizations of NPs were further conducted by a transmission electron microscope (TEM, FEI Tecnai GF20S-TWIN, USA). Briefly, PDPP and PDPP@D were dissolved in Milli-Q water and ultrasonically dispersed to prepare NPs solutions ($100 \mu\text{g mL}^{-1}$) for DLS and TEM measurements. Furthermore, the effects of hydrophobic force, ionic interaction, and hydrogen bonding on the self-assembly of PDPP and DOX loading of PDPP@D were also explored. Tween 20, Triton X-100, NaCl, and urea were added to the PDPP and PDPP@D solutions to reach the final concentrations of 1, 5, 10, 20, 50 and 100 mM, respectively. After incubation for 1 h, the size changes of samples were recorded by DLS.

Colloidal stability

The colloidal stability of PDPP and PDPP@D was assessed by monitoring the change in particle size and polydispersity index (PDI) as a function of time. PDPP and PDPP@D were added in PBS containing 10% of fetal bovine serum (FBS), and these mixtures were incubated at 37°C in a humidified chamber, and their sizes and PDI were and monitored by DLS at a pre-determined time.

In vitro drug release and size evolutionary study

DOX release: PDPP@D (1 mL) in dialysis bag (MWCO = 2000 Da) was immersed in 45 mL PBS (pH 7.4 with 10 μM GSH, pH 7.4 with 10 mM GSH, pH 5.2 with 10 μM GSH and pH 5.2 with 10 mM GSH respectively) in triplicate. To mimic the conditions similar to the *in vivo* delivery, the samples were incubated at 37°C under a mild shaking condition (170 r/min). The external solution was collected at predetermined times and replaced with the same amount of fresh medium until 60 h. The concentration of DOX was measured by fluorescence spectrum

at an excitation wavelength at 480 nm and an emission wavelength at 550 nm. Cumulative release profiles were plotted verse the release time.

Ppa release: The release of Ppa from PDPP@D was monitored in PBS (pH 7.4 with 10 μ M GSH, pH 7.4 with 10 mM GSH, respectively). To mimic the conditions similar to the *in vivo* delivery, the samples were incubated at 37 °C under a mild shaking condition (170 r/min). At predetermined time intervals, 200 μ L of sample solution was removed and diluted with four times volume of methanol. The concentration of Ppa was measured via High Performance Liquid Chromatography (HPLC, SHIMADZU, Japan) with the following conditions. Column name: Shim-pack GLST, C18, 5 μ m; Size: 250 $\times$ 4.6 mm I.D.; Temperature: 35 °C; Flow rate: 1 mL min⁻¹; Injection volume: 20 μ L; Detector: UV light, 405 nm for Ppa; Mobile phase: a mixture of methanol and water (v: v = 9:1). Cumulative release profiles were plotted verse the release time.

1.4 Cell culture and Animals

The 4T1 cell line (mouse mammary carcinoma) and the L02 cell line (human normal hepatocytes) were selected for this study. Both cell lines were purchased from Chinese Academy of Science Cell Bank for Type Culture Collection (Shanghai, China) and cultured in RPMI-1640 medium, supplemented with 10% fetal bovine serum (FBS), and incubated in 5% CO₂ atmosphere at 37 °C.

Female BALB/c mice (18-22 g) were purchased from Chengdu Dashuo Biological Technology (Chengdu, China). All animal studies were carried out in compliance with the protocol of the care and use of laboratory animals, approved by the Animal Ethics Committee of West China Hospital, Sichuan University (Approval No. 2019292A). Mice were inoculated with 5 $\times$ 10⁵ 4T1 cells subcutaneously on the right flank. About 2 weeks after implantation, mice with a tumor volume size of 50 mm³ were used for biodistribution, imaging and antitumor studies.

1.5 Cellular studies

In vitro cellular uptake

To evaluate cellular uptake and intracellular distribution of PDPP and PDPP@D, 4T1 cells (2 $\times$ 10⁴) were seeded on each glass coverslip to allow attachment, and incubated with Ppa, DOX, DPP and PDPP@D for 1 h, 3 h and 6 h, while Ppa was used as a control. After nuclear staining by Hoechst 33258, cells were washed by PBS and then observed under a confocal laser scanning microscopy (CLSM) (excitation and emission wavelengths: 630 nm and 678 nm), and images were captured by Leica Confocal Software. For further characterize uptake of PDPP and

PDPP@D by cells, 4T1 cells were seeded in 6 well plates at 1×10^5 cells per well and incubated for 6 h, 12 h and 24 h. After the spent medium was replaced with fresh one, cells were treated with Ppa, PDPP and PDPP@D at an equivalent concentration of $1 \mu\text{g Ppa mL}^{-1}$ for pre-specified times. Cells were then washed with PBS twice, harvested, and suspended in $100 \mu\text{L}$ of PBS for flow cytometer analyses. 20,000 events were collected and Flowjo software was used for generating each histogram.

Endocytosis mechanism

To unveil the endocytosis mechanism of PDPP and PDPP@D, inhibition of the endocytosis pathways was applied to 4T1 cells incubated with NPs, and non-treated cells were used as a control. 4T1 cells were planted at a density of 2.0×10^5 cells per well in the 12-well plates and incubated in the complete medium for 24 h. The cells were washed with PBS twice, and incubated at 37°C for 1 h with one of the following endocytosis inhibitors dissolved in serum-free RPMI medium 1640: chlorpromazine (an endocytosis inhibitor of clathrin-mediated endocytosis, $10.0 \mu\text{g mL}^{-1}$), amiloride (an endocytosis inhibitor of macropinocytosis-mediated endocytosis, $20.0 \mu\text{g mL}^{-1}$) and methyl- β -cyclodextrin (an endocytosis inhibitor of caveolae-mediated endocytosis, $500 \mu\text{g mL}^{-1}$). After replacing the spent medium with complete RPMI medium 1640 containing PDPP and PDPP@D at the same concentration of $2 \mu\text{g Ppa mL}^{-1}$ and cells were incubated with the above inhibitors for another 15 min. The medium was removed and the cells were washed twice with cold PBS solution. The cells were collected and analyzed by flow cytometry. All experiments were carried out in triplicate.

In vitro ROS generation

Intracellular ROS generation was assessed by using a peroxide-sensitive fluorescent probe, 2', 7'- dichlorofluorescein-diacetate (DCFH-DA) (Beyotime, Beijing, China). According to the manufacturer's instruction, cells (1×10^6 cells per well) were treated with Ppa, PDPP and PDPP@D at the same concentration of $1 \mu\text{g Ppa mL}^{-1}$. After 24 h incubation, the spent medium was discarded and fresh one added, and each well was then irradiated. After incubating with $1.0 \mu\text{M}$ DCFH-DA at 37°C for 40 min, cells were harvested and suspended in $100 \mu\text{L}$ of PBS for flow cytometer analyses. 20,000 events were collected and Flowjo software was used for quantifying the intensity of fluorescence.

Cytotoxicity

The CCK-8 assay was utilized to detect the effect of PDPP and PDPP@D on cell viability against various cell lines. 4×10^3 4T1 cells per well were seeded in a 96-well plate, and then incubated overnight. The cells were treated with Ppa, DOX, Ppa and DOX, PDPP, PDPP and DOX, PDPP@D at different concentrations but at an equivalent Ppa or DOX dose. After 24 h

incubation, the medium in each well was replaced and then irradiated (1 J cm^{-2}). 24 h later, the cell viability was assessed using the CCK-8 reagent. The absorbance at 480 nm of each well was recorded on a microplate reader. Untreated cells were served as a control. Results were presented as the average cell viability by following the manufacturer's instruction.

1.6 *In vivo* pharmacokinetics

Healthy pathogen-free BALB/c mice (5-6 weeks, female) were used to determine the pharmacokinetics of PDPP and PDPP@D ($n = 5$). The mice were intravenously injected with Ppa, DOX, PDPP and PDPP@D (5 mg Ppa kg^{-1}), respectively. Blood was collected at set time points (5 min, 15 min, 30 min, 2 h, 4 h, 6 h, 8 h, 12 h, 24 h, 48 h, 36 h and 72 h). Plasma was diluted to 50-fold with DMSO for fluorescence measurements using a BioTek plate reader. The pharmacokinetic parameters were calculated using a two-compartment model by WinNonlin 5.2 software.

1.7 *In vivo* fluorescence imaging

After BALB/c mice developed established tumors (around 100 mm^3), saline, Ppa, PDPP and PDPP@D were intravenously injected into mice ($n = 3$). Signals were detected at 1 h, 6 h, 12h, 24 h, 3 d, 5 d, 7 d, 9 d, 11 d, and 14 d post intravenous injection via a small-animal *in vivo* fluorescence imaging system (CRi Maestro EX, USA). The mean fluorescence intensity (MFI) was quantitated by CRi Maestro EX 3.0.

1.8 *In vitro* and *in vivo* penetration

***In vitro* penetration**

Multicellular tumor spheroids (MTS) from 4T1 cells were obtained using a liquid overlay method in our previous report.^[2] Cells were seeded in pretreated plates and cultured for ~7 days in custom condition. When growing to a diameter of ~200 μm , MTS were properly transferred to confocal dishes and treated by Ppa, DOX, Ppa and DOX, PDPP and PDPP@D for 12 h. Optical sections of MTS were imaged under a CLSM (Nikon TiA1-N-STORM). Topological 3D views of images were analyzed by ImageJ software.

***In vivo* penetration**

The BALB/c mice bearing 4T1 tumors were intravenously injected with Ppa, DOX, Ppa and DOX, PDPP and PDPP@D (5 mg Ppa kg^{-1} , $2.5 \text{ mg DOX kg}^{-1}$). 72 h later, solid tumors were removed and frozen sections were prepared after the mice were sacrificed. The slides of tumor tissues were then incubated with Alex Fluor 594 labeled anti-CD31 antibody (Abcam, UK) and DAPI (Dojindo, Japan). A CLSM (Nikon TiA1-N-STORM) was used to observe the drug penetration in the collected solid tumor.

Blood vessel extravasation and tumor penetration

4T1-eGFP cells were inoculated in the ears of BALB/c mice to establish the tumor model. The tumor-bearing mice were then anesthetized after intravenous injection of PDPP@D (5 mg Ppa kg⁻¹, 2.5 mg DOX kg⁻¹). The tumor regions were observed via a confocal microscope system (Nikon A1RMP+) to investigate real-time extravasation and tumor penetration of PDPP@D.

1.9 Neighboring effects

Neighboring effect on drug release from dying tumor cells and cytotoxicity

4T1 cells (1×10^5 cells/well) were incubated with culture medium containing PDPP@D at the concentration of 1 $\mu\text{g Ppa mL}^{-1}$ and 0.5 $\mu\text{g DOX mL}^{-1}$. All samples were in triplicate. After incubation for 2 h, 8 h, and 24 h, cells were subsequently subjected to laser irradiation (1 J cm^{-2}), and then the medium was aspirated and collected to detect the drugs pumped out from treated 4T1 cells. 50 μL of each medium sample was mixed with 250 μL of methanol to precipitate proteins. Then the centrifuged supernatant was dried for HILIC-Q-Orbitrap-HRMS analysis. Xcalibur (Version 4.0, Thermo Fisher Scientific) was used to obtain MS and MS/MS spectrum. Qualitatively analysis was performed by comparing the mass spectrum data and the retention time of samples to standards. Quantitative analysis was performed comparing the integrated peak areas of samples with the external calibration curves obtained from the standards.

Parameters of HILIC-Q-Orbitrap-HRMS analysis: HILIC-Q-Orbitrap-HRMS was performed using an Ultimate 3000 rapid separation liquid chromatography coupled with Q Exactive Plus Q-Orbitrap HRMS (Thermo Fisher Scientific, Waltham, MA, USA). Chromatographic separation was performed on a BEH C18 column ($2.1 \times 50 \text{ mm}$, $1.7 \mu\text{m}$) (Waters, Milford, MA, USA) with a flow rate of 0.4 mL/min. The mobile phase was composed of water with 0.1% formic acid and acetonitrile. The gradient was linearly changed as follows: 0-1.5 min, 20% B; 1.5-5 min from 20% B to 95% B; 5-9 min, 90% B for column cleaning; 9-12 min, 100% B for column re-equilibration. The Q Exactive Plus Q-Orbitrap HRMS was equipped with heated electrospray ionization (HESI) source in positive mode. Full scan parameters: m/z range from 100 to 1200; mass resolution, 70000; AGC target, 3×10^6 ; maximum IT, 100 ms. PRM parameters: mass resolution, 17500; AGC target, 1×10^5 ; maximum IT, 100 ms; loop count, 12; m/z isolation window within 1.

The aspirated and collected medium in the above procedure was also tested to evaluate the toxicity of secreted products from 4T1 cells. These mediums were transferred and incubated with untreated cells seeded in 96-well plates. Cells were subjected to laser irradiation (1 J cm^{-2}) after 24 h. Cell viability was measured by a CCK-8 kit (MCE) according to the manufacturer's

protocols.

Neighboring effect on tumor penetration

When growing to a diameter of $\sim 150\ \mu\text{m}$, MTS were properly transferred to a chamber cover glass and incubated with PDPP@D for 12 h, and then subjected to laser irradiation ($1\ \text{J cm}^{-2}$). The medium was replaced with fresh PBS buffer after 3 h and a Hoechst 33258 staining buffer was added for 10 min. After staining, the MTS were carefully washed with PBS buffer twice and imaged under a CLSM (Nikon TiA1-N-STORM). Topological 3D views of images were analyzed by ImageJ software.

1.10 *In vivo* photodynamic therapy

The therapeutic efficacy of PDPP@D was evaluated and compared to Ppa, DOX and saline controls. When the tumor volume reached about $50\ \text{mm}^3$, the mice were divided into 6 groups ($n = 5$) and injected intravenously with saline, Ppa, DOX, Ppa and DOX, PDPP, and PDPP@D ($5\ \text{mg Ppa kg}^{-1}$, $2.5\ \text{mg DOX kg}^{-1}$) every four days 4 times. After 24 h post-injection, the tumors of anesthetized mice were subjected to laser irradiation ($200\ \text{mW cm}^{-2}$) for 8 min.

The mice were monitored daily to identify any signs of toxicity, while the tumor volumetric size and the body weight were measured every 2 days. Tumor volumes were calculated using the following formula: tumor volume = length $\times$ width² $\times$ 0.5. A normalized tumor volume was calculated as V/V_0 , where V_0 was the original tumor volume before the treatment. Mice were euthanized at a defined end point or when the tumor diameter reached 10 mm.

To evaluate therapeutic effect, the main organs (heart, liver, spleen, lung and kidney) of the sacrificed mice on day 17 were harvested for Hematoxylin-Eosin (H&E) staining. The tumors harvested from all groups were weighed and stained by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), CD31 and H&E for histology studies.

1.11 Transcriptome and signaling analysis

4T1 cells at a density of 10^6 cells per well were treated by PDPP@D and a combined formulation (Ppa+DOX) with or without laser irradiation. Untreated cells were set as a control. Total RNA per sample was extracted (TRIzol Reagent, Thermo Fisher Scientific, USA), and analyzed using the DNBSEQ platform, with an average yield of 6.71G data per sample. The average alignment ratio of the sample in comparison with the mouse genome was 93.28%, which was comparative. The average alignment of the gene set was 75.52%. Reference Genome Version GCF_000001635.26_GRCm38.p6 were used for gene annotations. An absolute value of fold changes > 1.5 and a P value of < 0.05 , which were obtained by comparing each treated sample to the control, were applied for selecting significant DEGs. Functional comparison analyses were performed using IPA analysis. Enrichment analyses were performed according

to the KEGG database. All gene sets were analyzed by using the default parameters of gene set enrichment analysis (GSEA) software. Presentation of the data was performed using R and RStudio with R packages dplyr, ggplot2, and pheatmap. Experiments were performed in biological replicates ($n = 3$).

1.12 Western blotting

After treatments, 4T1 cells were lysed in a lysis buffer containing 1× radioimmunoprecipitation assay (RIPA) buffer (Solarbio®) and 1× Protease inhibitor cocktail (bimake). Centrifuged cell lysates were collected for western blotting. The Pierce BCA Protein Assay Kit (Thermo Scientific) was used to assess the protein content. Using a 4-20% gel, 30 g of denatured proteins was subjected to SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) for separation. The separated proteins in the gel were transferred to a PVDF membrane (Millipore) for incubation with a primary antibody and subsequently a secondary antibody. For signal detection, a chemiluminescence imaging system (Bio-rad, USA) was used.

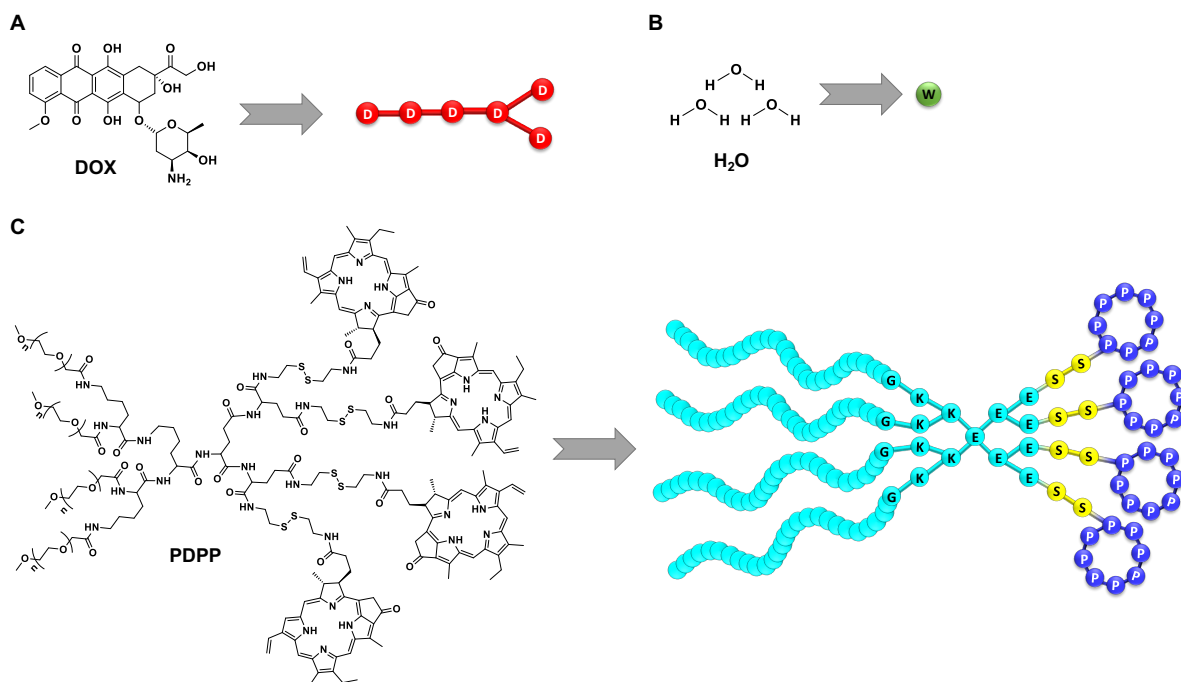
1.13 Quantitative PCR

After treatments, total RNA of 4T1 cells from each group was extracted with an RNA Extraction Kit (TRIzol Reagent, Thermo Fisher Scientific, USA), purified following the manufacturer's instruction, and then reversely transcribed by a RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific). PCR amplification was performed using the FastStart Universal SYBR Green Master (Rox). The $\Delta\Delta Ct$ values were calculated by relating each individual CT value to its internal beta-actin control, and then normalizing to the vehicle-treatment control. The forward primer of mouse beta-actin was: CACTGTCGAGTCGCGTCC, reverse primer of mouse beta-actin: TCATCCATGGCGAACTGGTG; forward primer of mouse tnfsf10: CCTCAGAAAGTGGCAGCTCA, reverse primer of mouse beta-actin: GTCTTTCCATCCTTGGAGATTGG.

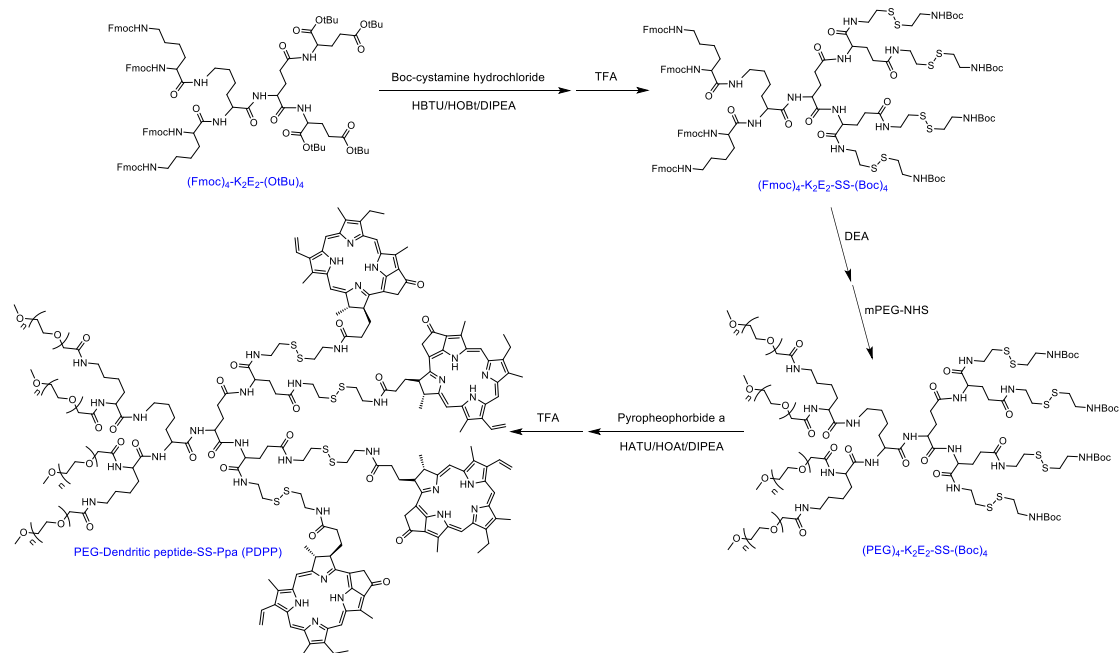
1.14 Statistical analysis

Unless otherwise noted, data was provided as mean $\pm$ s.d. If the variance was equal, the student's t-test (two-tailed) was used for comparison between two independent samples, or the Welch's Two Sample t-test was used for adjusted analysis. ANOVA was used to compare multiple samples, and Welch's ANOVA was employed if the variance was not equal. R was used to conduct the statistical analyses by using the R packages multcomp, PMCMRplus, and agricolae. If P values were less than 0.05, significant differences were considered: * for $P < 0.05$, ** for $P < 0.01$, *** for $P < 0.001$ and **** for $P < 0.0001$. ns denotes non-significant.

2. Results



Scheme S1. Schematic illustration of molecular structures and corresponding coarse-grained models of DOX (A), H₂O (B) and PDPP (C). Color scheme in coarse-grained model: PEG is coarse-grained into 28 Cambridge Blue G beads, lysine into 6 Cambridge Blue K beads, glutamic acid into 7 Cambridge Blue E beads, disulfide bond into 2 yellow S beads, Ppa into 8 dark blue P beads, DOX into 6 red D beads, and three molecules of water into a green W bead.



Scheme S2. Synthetic route of PEG-Dendritic peptide-SS-Ppa (PDPP).

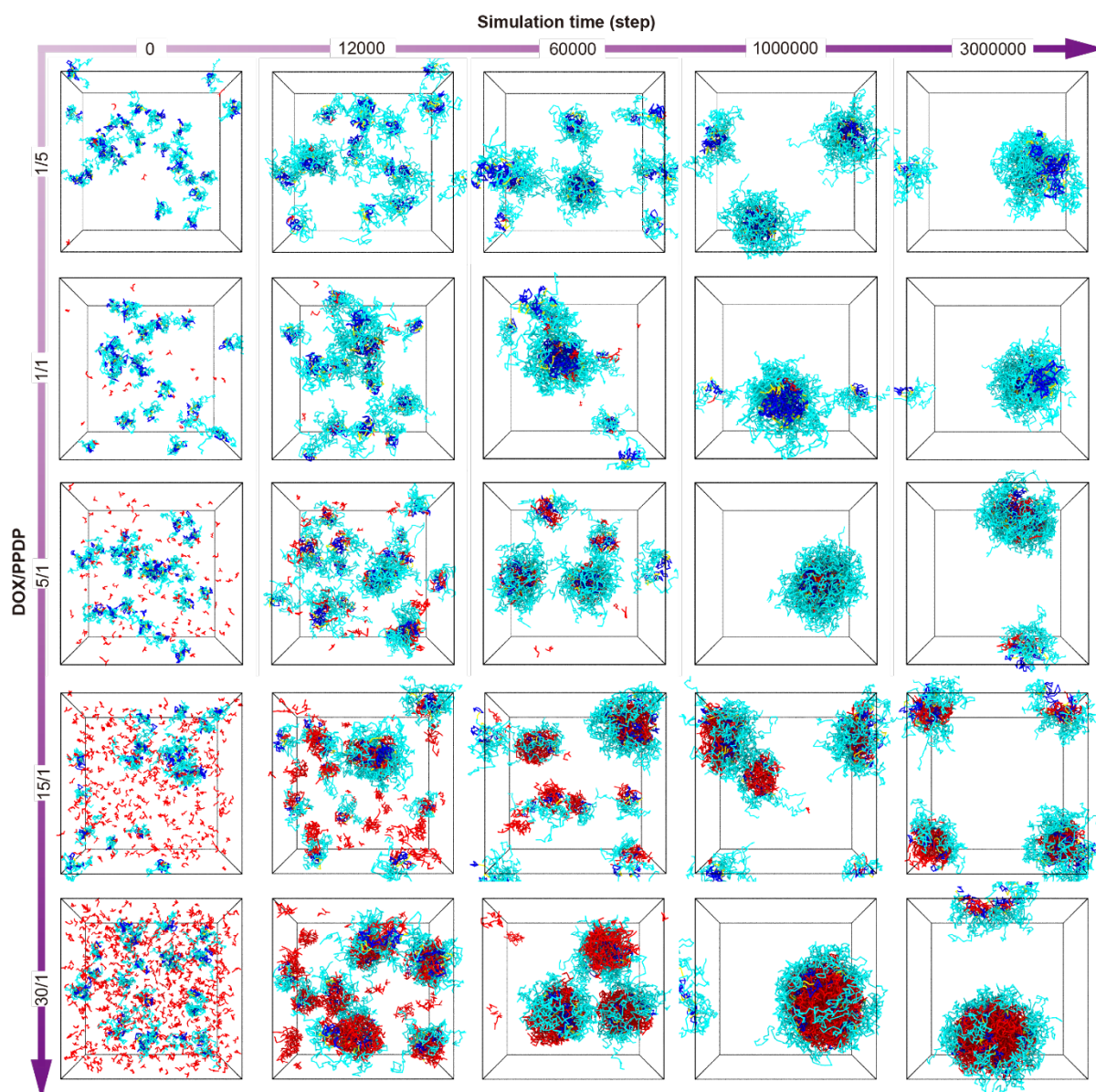


Figure S1. Formation processes of PDPP@D at different ratios of DOX/PDPP from 1/5 to 30/1. Coarse-grained Cambridge blue beads for PDP, dark blue beads for Ppa, red beads for DOX, and yellow beads for disulfide bond. Water beads are not shown for the sake of clarity.

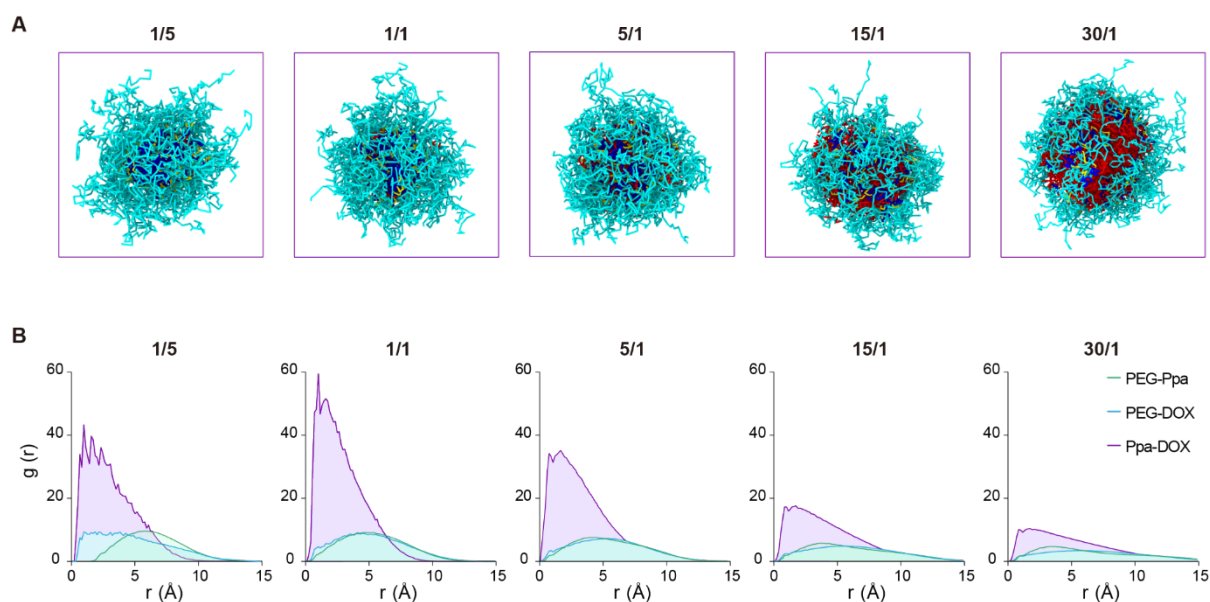


Figure S2. (A) Structures of DOX-loaded PDPP@D at different ratios of DOX/PDPP from 1/5 to 30/1. Water beads are not shown for the sake of clarity. (B) Radial distribution functions of interaction between PEG-Ppa, PEG-DOX, and Ppa-DOX in PDPP@D at different ratios of DOX/PDPP from 1/5 to 30/1.

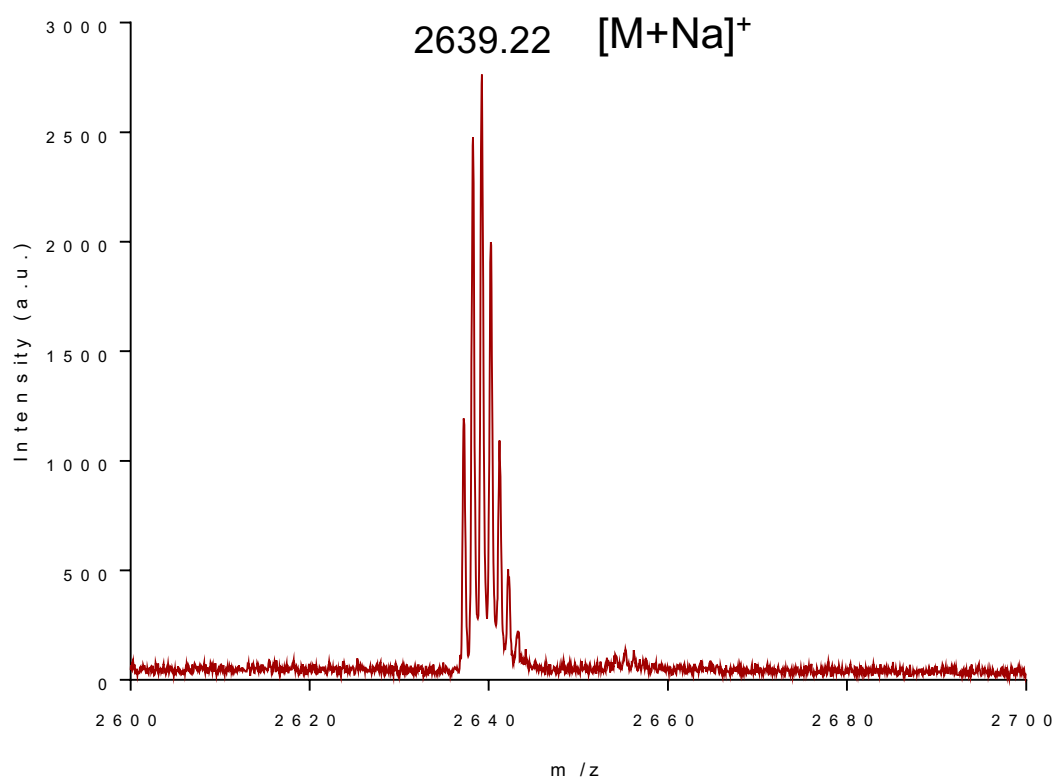


Figure S3. MALDI-TOF MS of $(Fmoc)_4\text{-K}_2\text{E}_2\text{-SS-(Boc)}_4$ (m/z , $[M+Na]^+$): 2639.36 (calculated), 2639.22(observed).

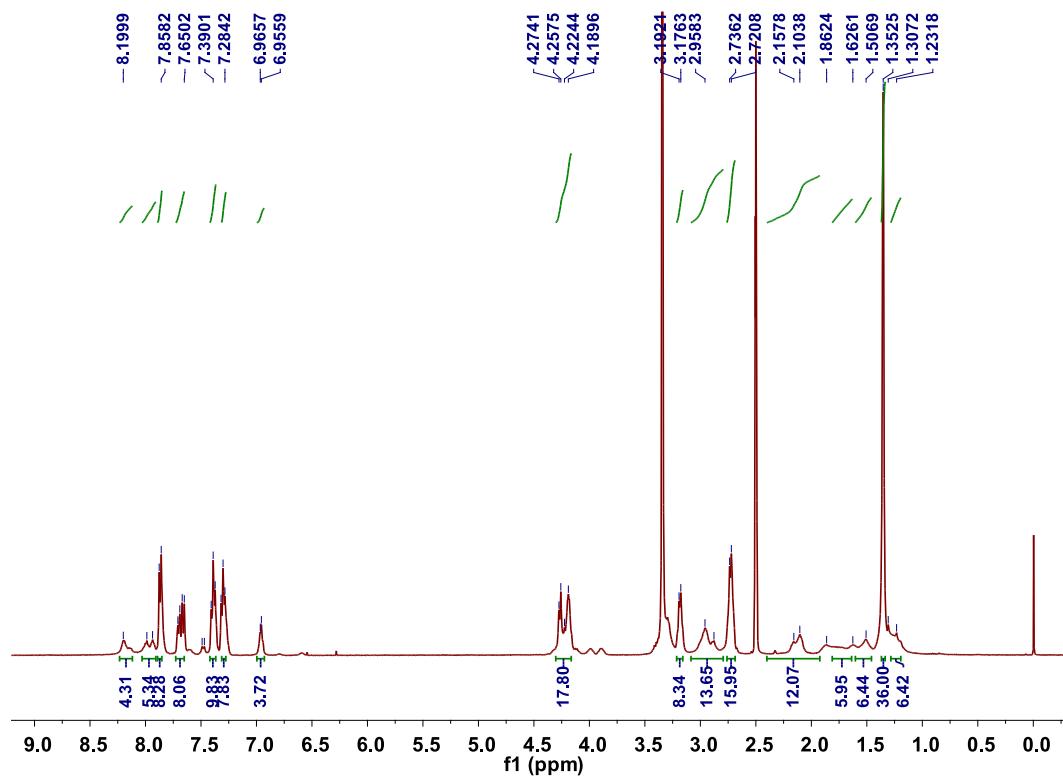


Figure S4. ^1H -NMR spectra of $(\text{Fmoc})_4\text{-K}_2\text{E}_2\text{-SS-(Boc)}_4$ in DMSO-d_6 .

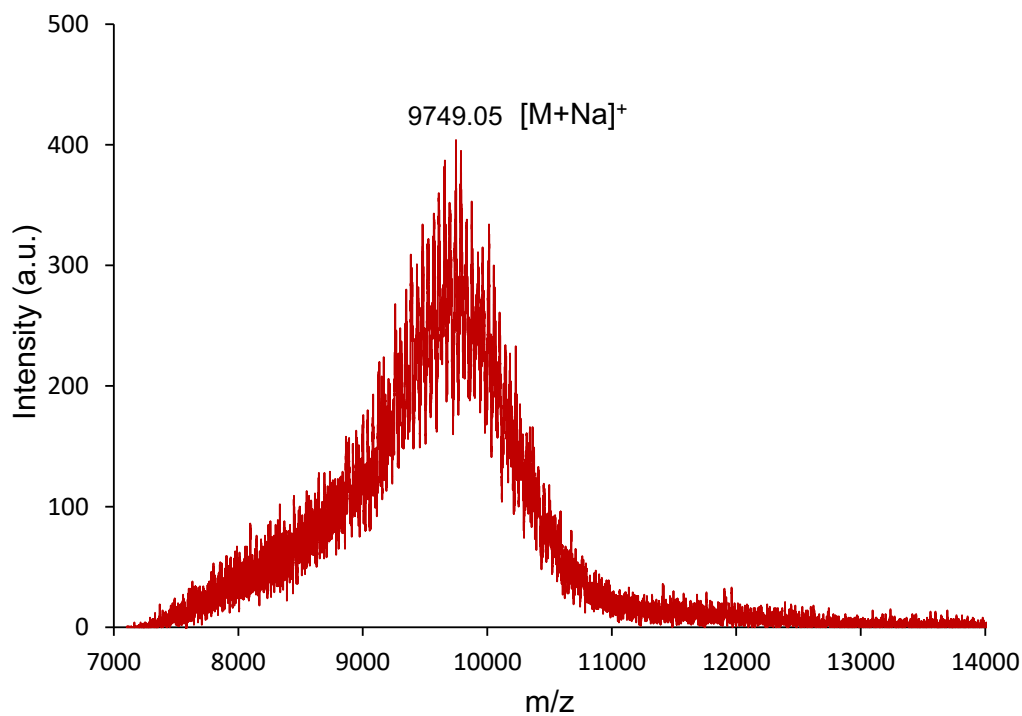


Figure S5. MALDI-TOF MS of $(\text{PEG})_4\text{-K}_2\text{E}_2\text{-SS-(Boc)}_4$ (m/z , $[\text{M}+\text{Na}]^+$): 9748.77(calculated), 9749.05(observed).

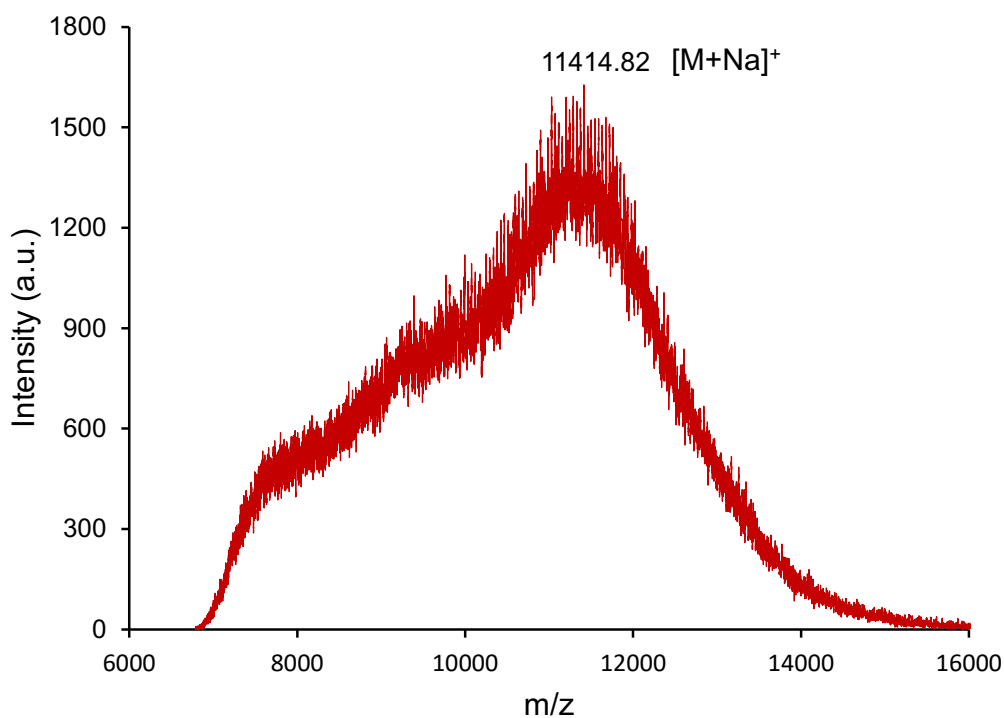


Figure S6. MALDI-TOF MS of PEG-Dendritic peptide-SS-Ppa (PDPP) (m/z , $[M+Na]^+$): 11415.16 (calculated), 11414.82(observed).

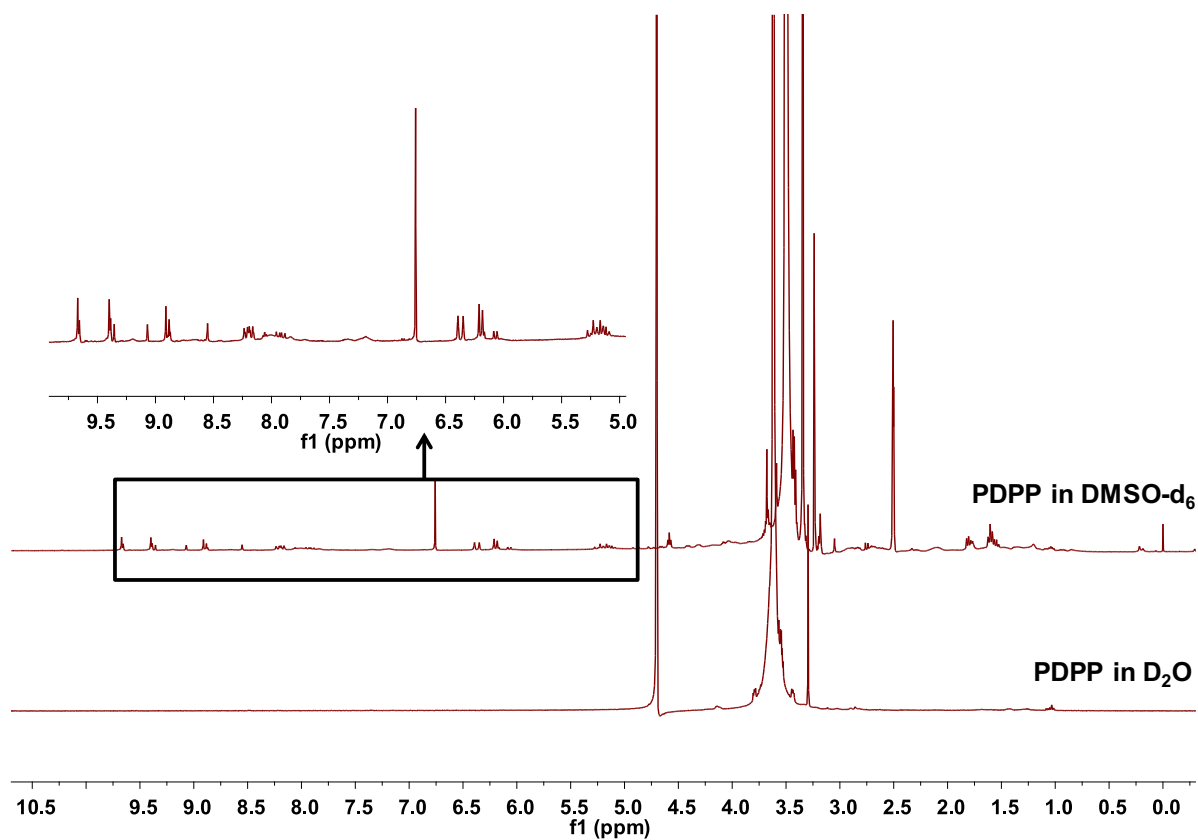


Figure S7. ^1H NMR spectrum of PEG-Dendritic peptide-SS-Ppa (PDPP) in $\text{DMSO-}d_6$ and D_2O . After PDPP was dissolved in D_2O , the proton signals of Ppa completely disappeared due to a self-assembly process of PDPP in D_2O .

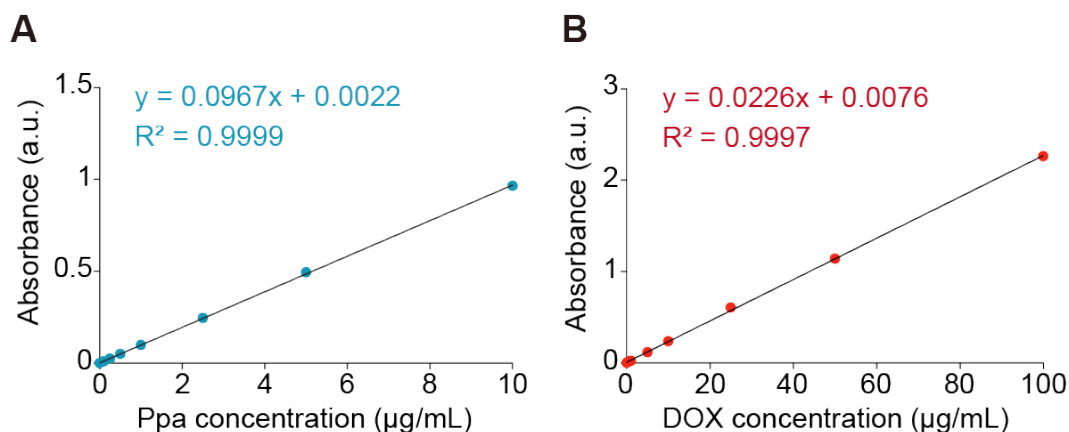


Figure S8. Standard absorption curves of Ppa (A) and DOX (B) in DMSO.

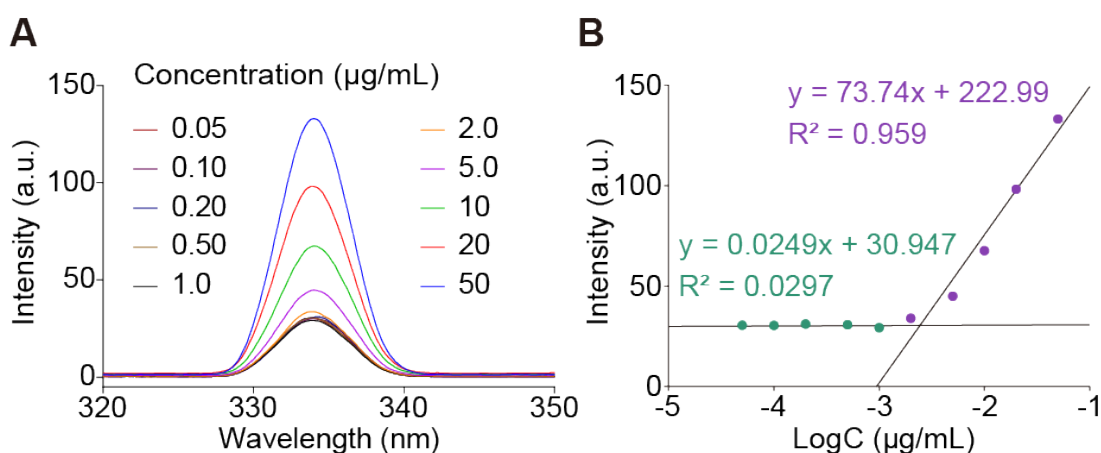


Figure S9. The fluorescence intensity of the fluorescent probe pyrene in PDPP at different concentrations (A) to determine the CAC of PDPP through a linear regression curve (B).

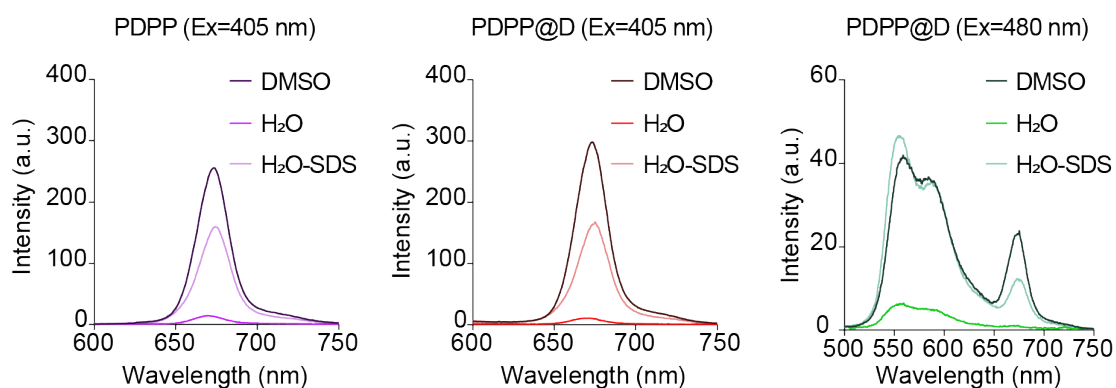


Figure S10. Fluorescence emission spectra of PDPP and PDPP@D in different solvents.

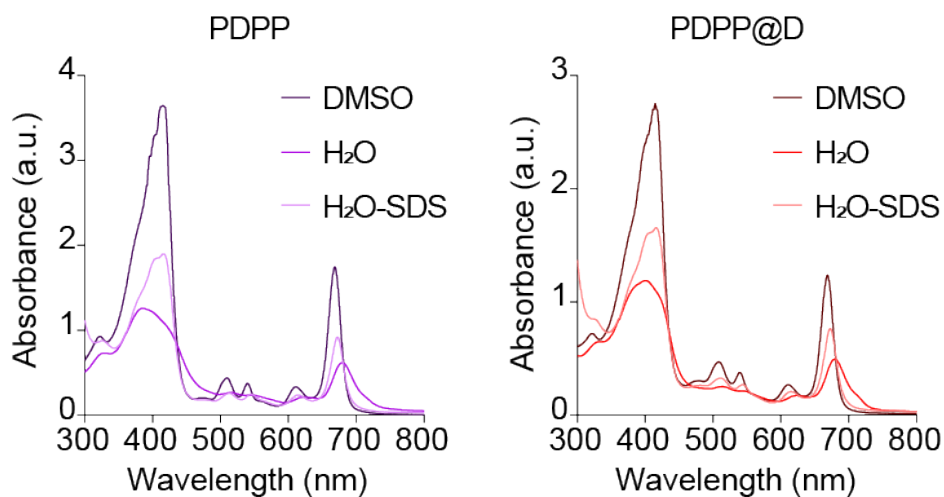


Figure S11. UV-vis absorbance spectra of PDPP and PDPP@D in different solvents.

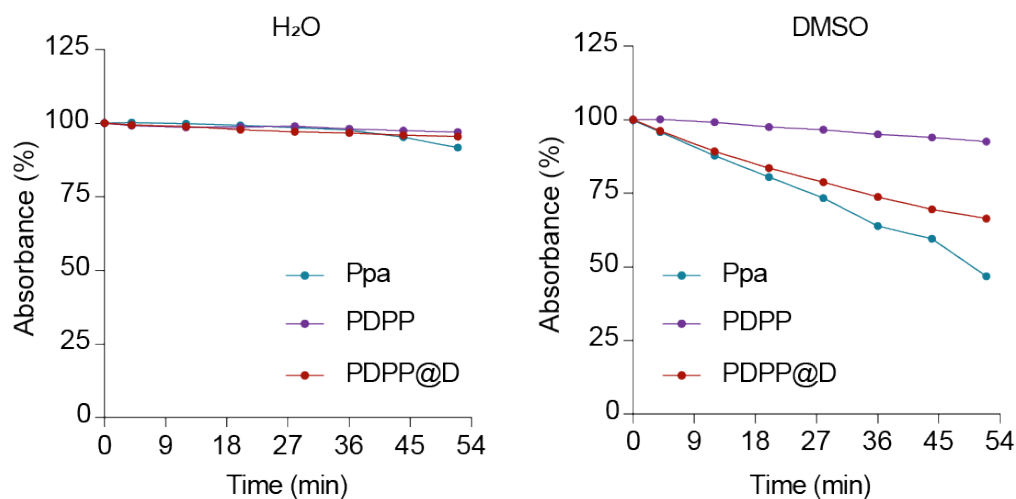


Figure S12. Decay curves of absorption at 667 nm for Ppa, PDPP and PDPP@D in H₂O or DMSO, as a function of irradiation time (660 nm, 5 mW cm⁻²).

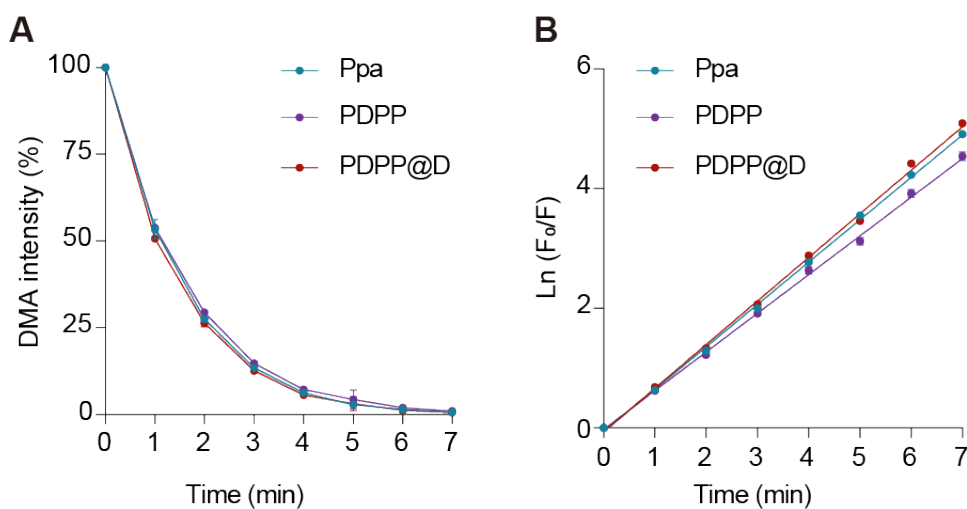


Figure S13. Singlet oxygen generation by Ppa, PDPP and PDPP@D measured by DMA.

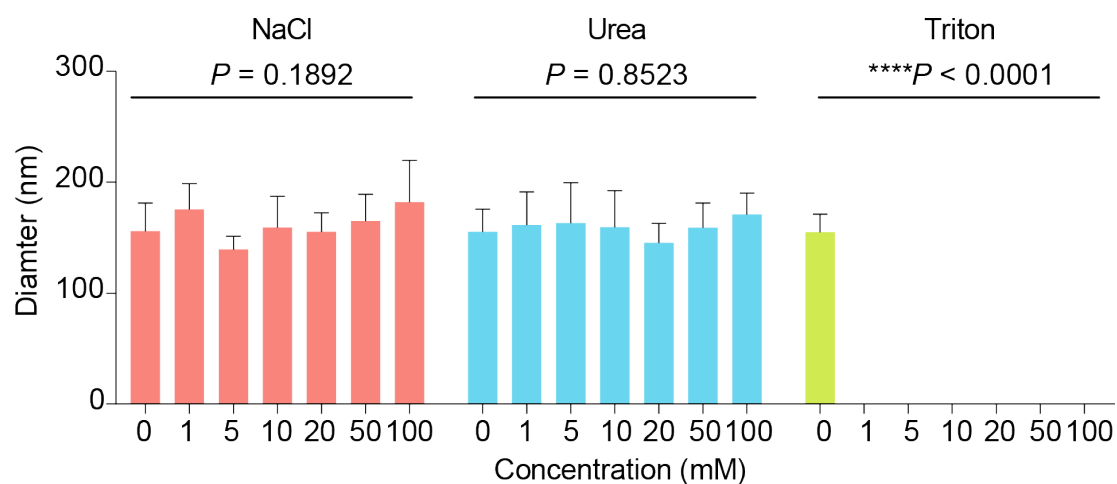


Figure S14. Size variation of PDPP after dispersion in NaCl, urea or Triton solutions ($n = 5$).

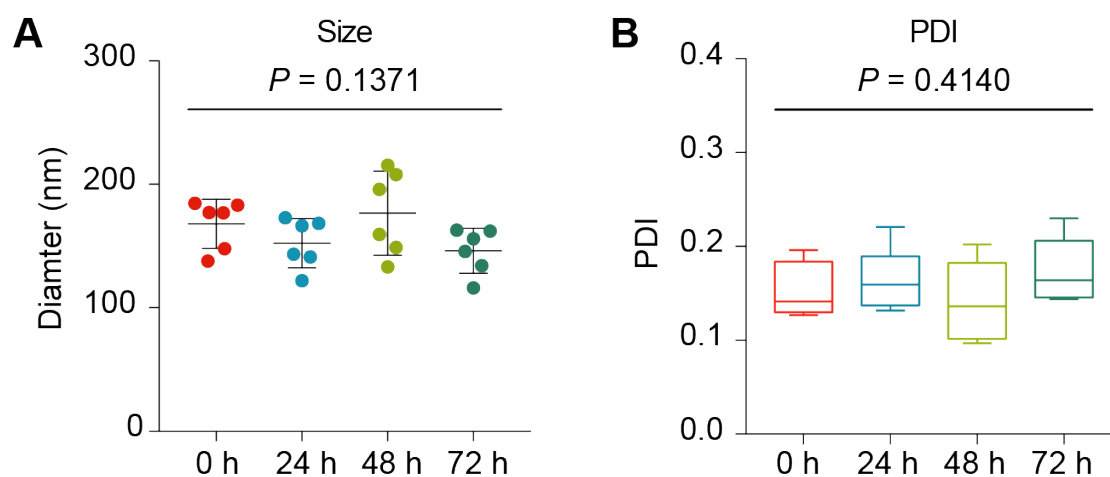


Figure S15. Colloidal stability of PDPP in PBS with 10% FBS at 37°C ($n = 6$) from 0 h to 72 h: (A) size variation, (B) PDI variation.

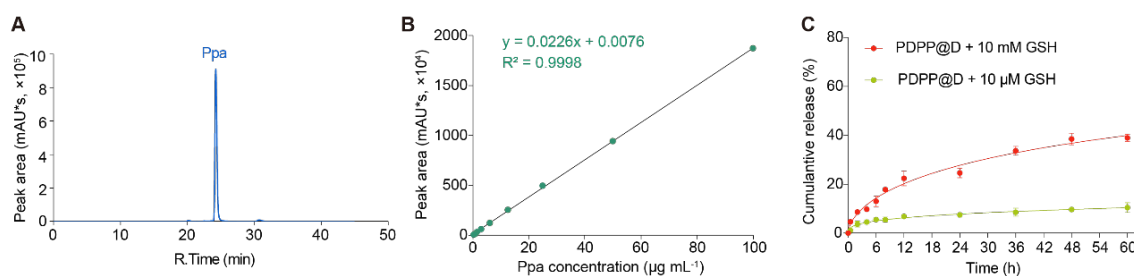


Figure S16. A) HPLC chromatograms of Ppa (MeOH/H₂O at 90/10 as the mobile phase). B) Standard absorption curves of Ppa determined by HPLC. C) The cumulative Ppa-release profile of PDPP@D under two release conditions: (i) pH 7.4 with 10 mM GSH, (ii) pH 7.4 with 10 μM GSH. Three independent samples per group at each time point ($n = 3$).

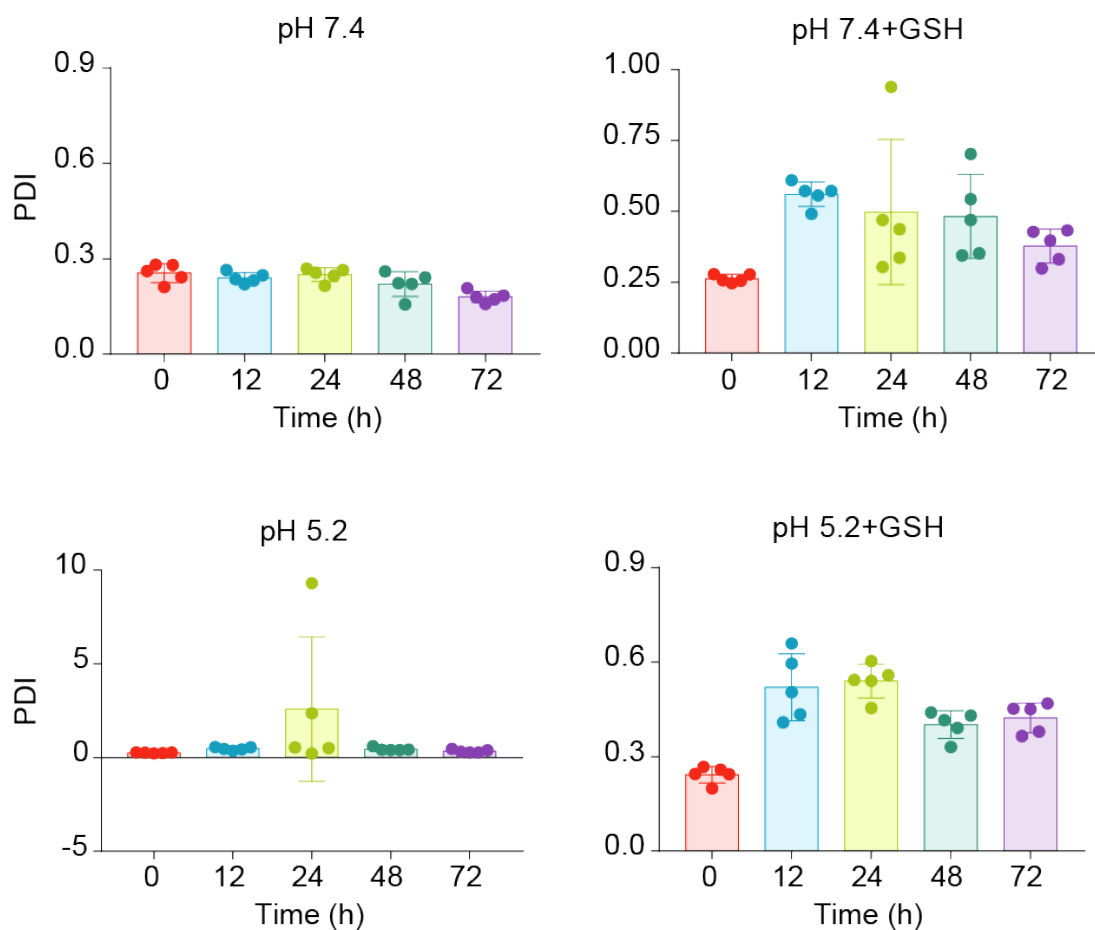


Figure S17. Size variation of PDPP under different release conditions for different durations ($n = 5$).

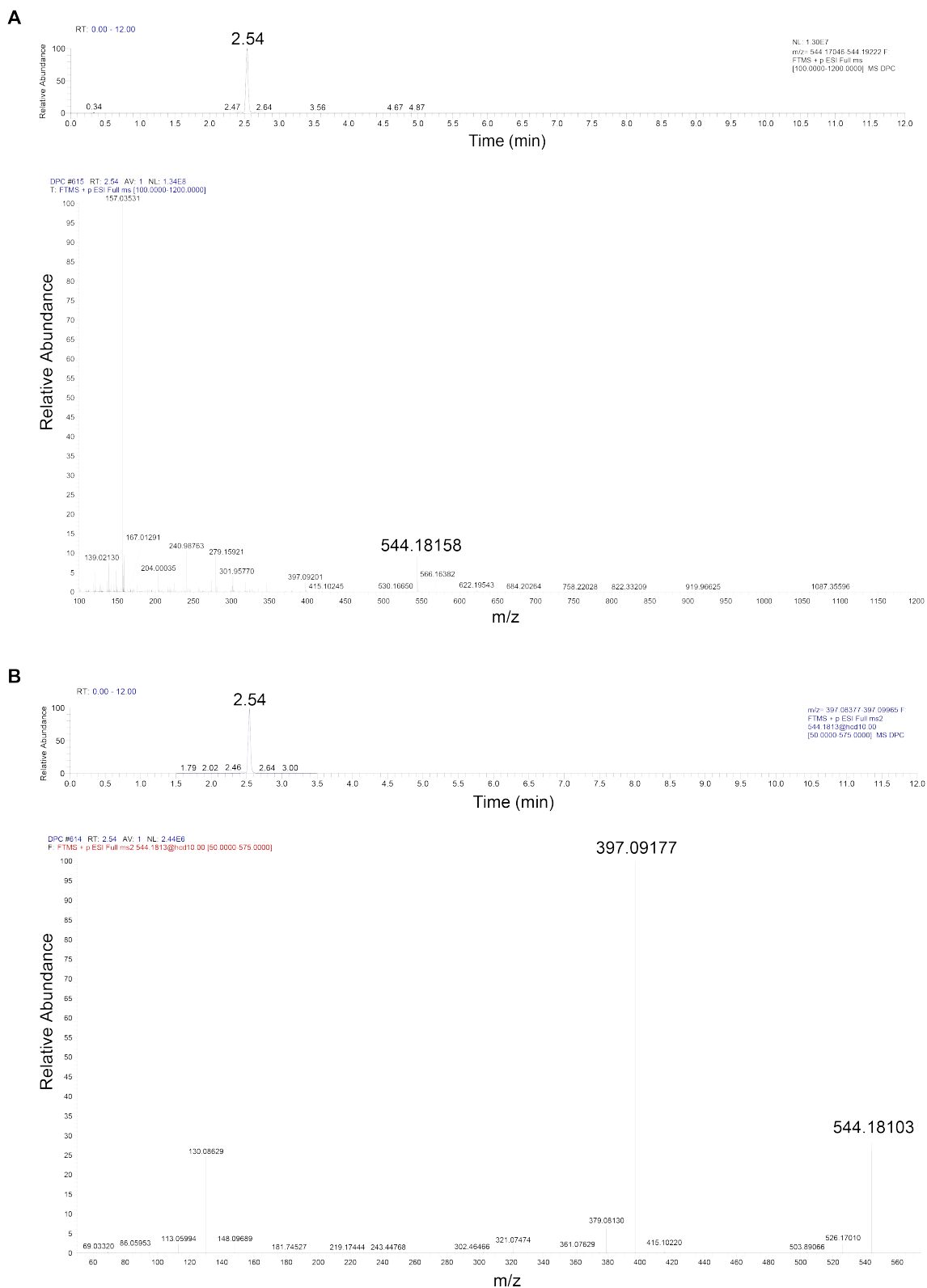


Figure S18. LC-MS spectra of standard samples (free DOX). A) The retention time and the mass of DOX analyzed by mass spectrometry (MS1). B). The retention time and the mass of DOX analyzed by tandem mass spectrometry (MS2).

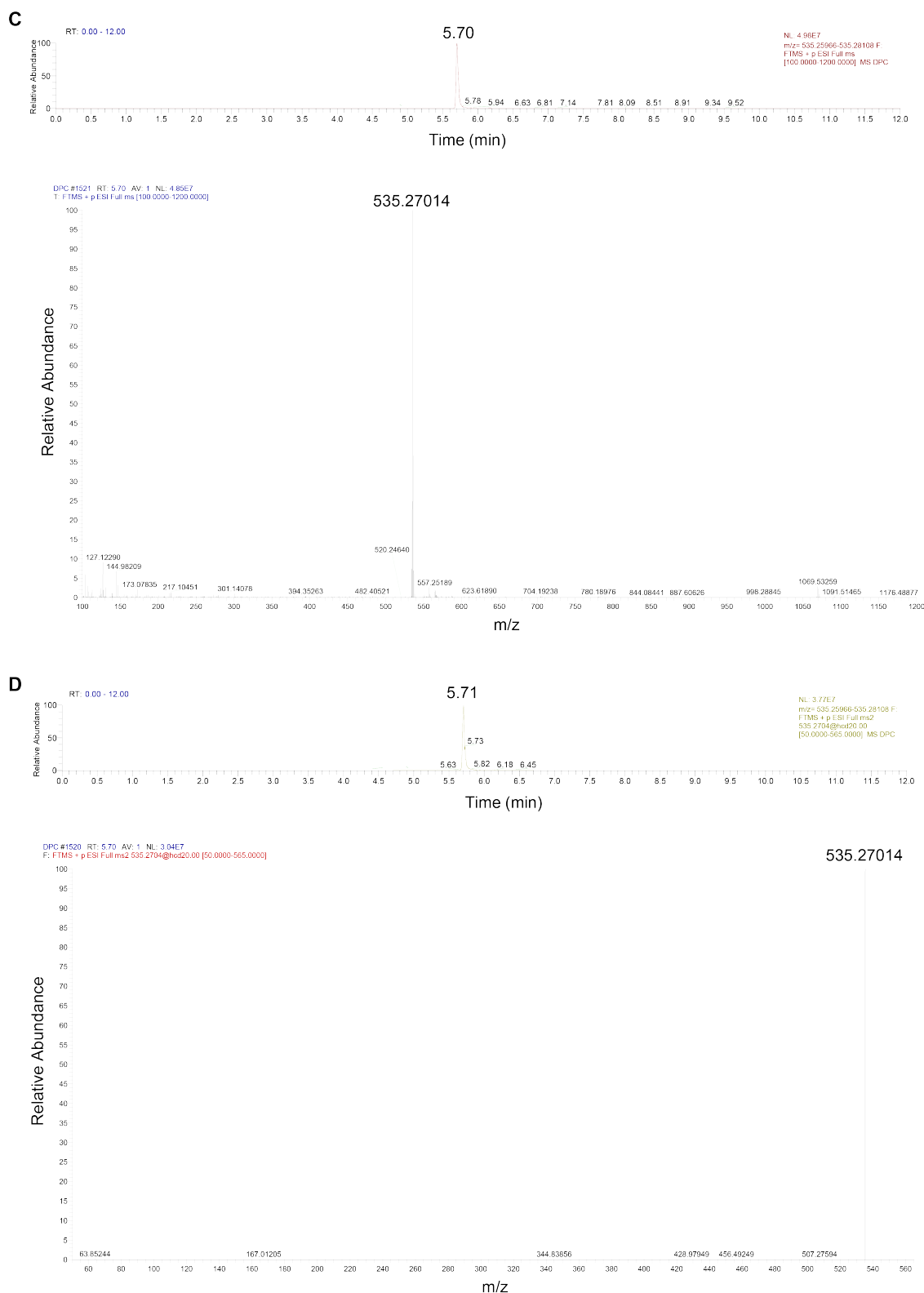


Figure S19. LC-MS spectra of standard samples (free Ppa). A) The retention time and the mass of Ppa analyzed by mass spectrometry (MS1). B). The retention time and the mass of Ppa analyzed by tandem mass spectrometry (MS2).

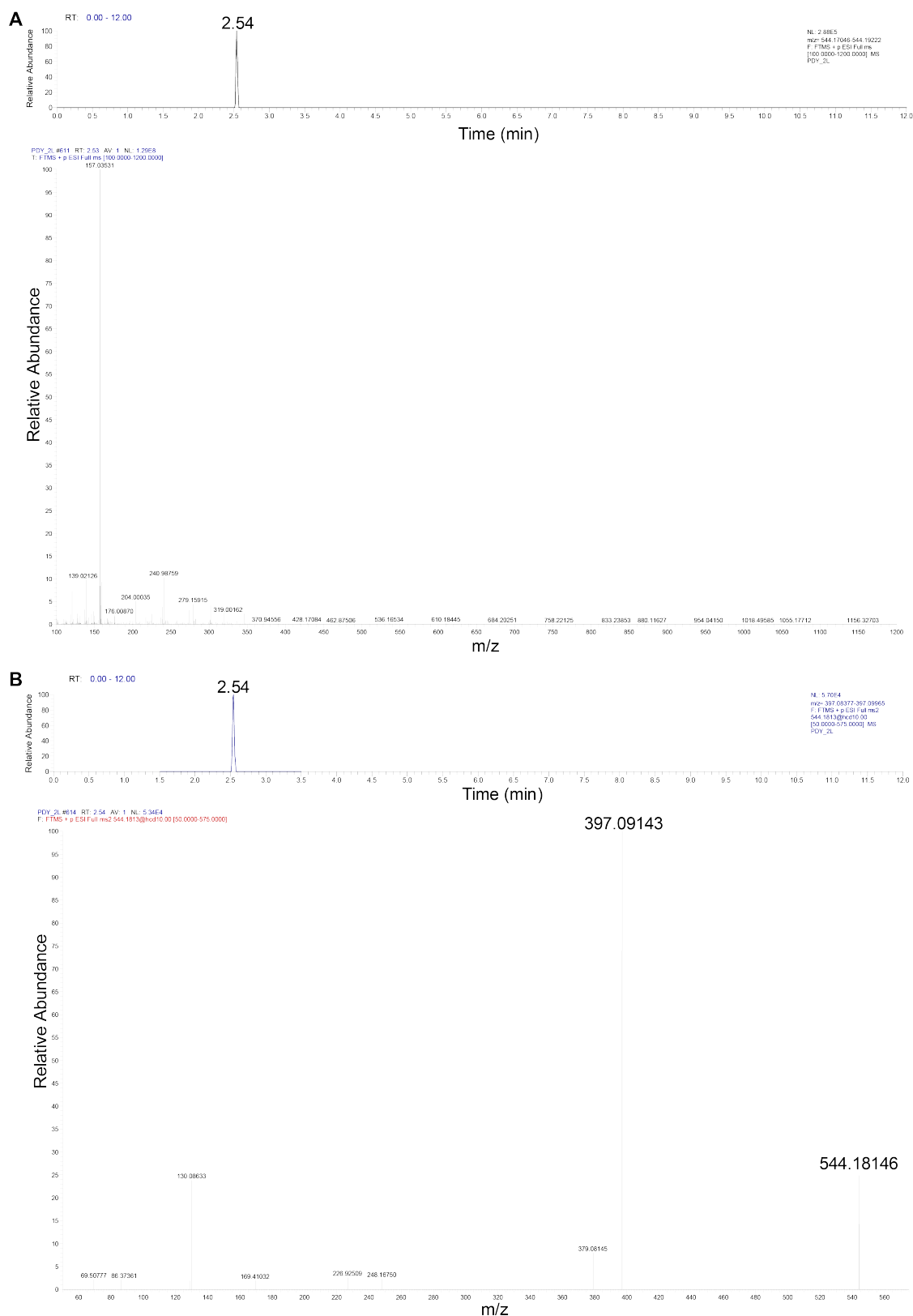


Figure S20. LC-MS spectra of PDPP@D released DOX from 4T1 cells incubated with PDPP@D for 2 h with laser irradiation. A) The retention time and the mass of DOX analyzed by mass spectrometry (MS). B). The retention time and the mass of DOX analyzed by tandem mass spectrometry (MS/MS, MS2).

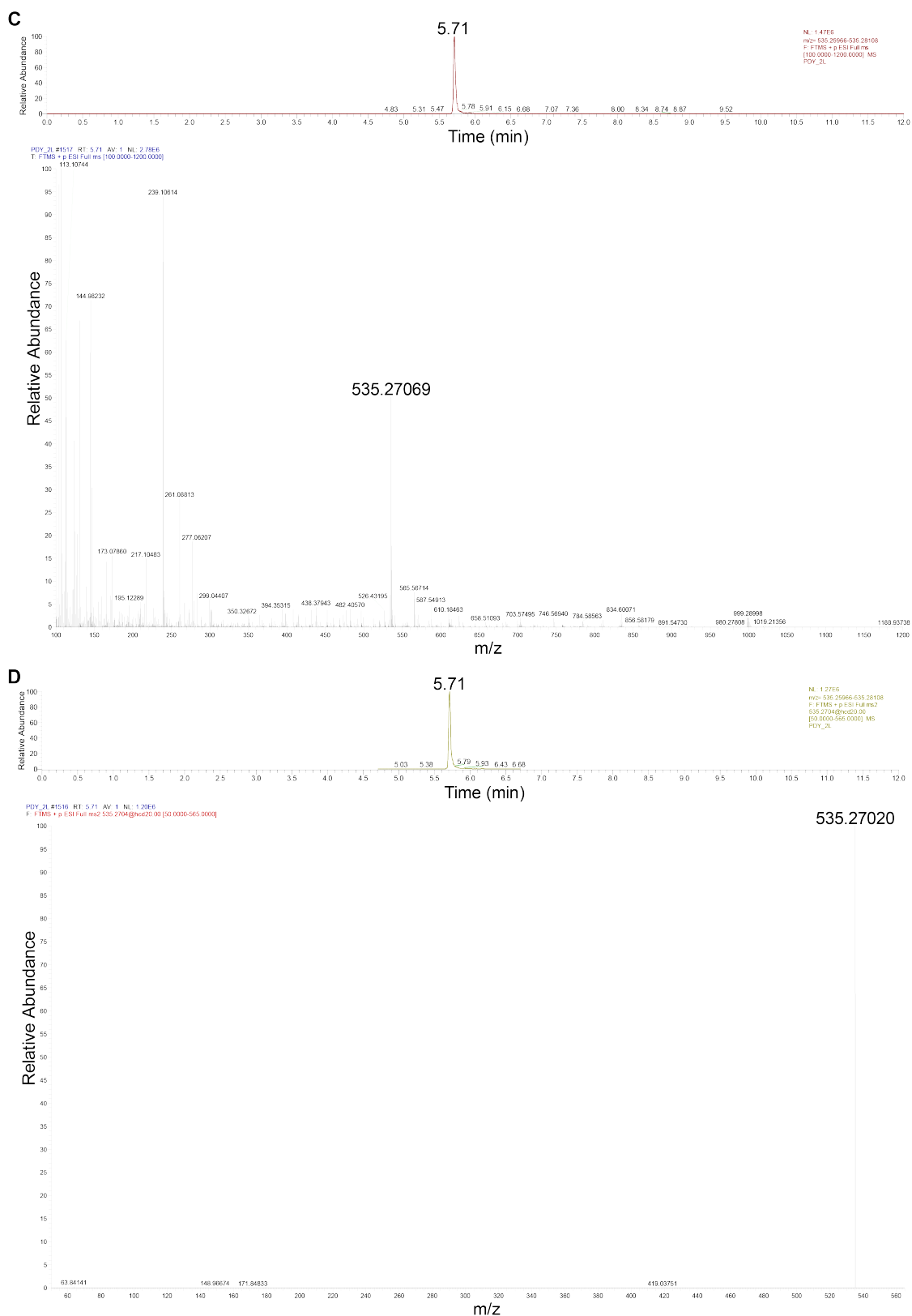


Figure S21. LC-MS spectra of PDPP@D released Ppa from 4T1 cells incubated with PDPP@D for 2 h with laser irradiation. A) The retention time and the mass of Ppa analyzed by mass spectrometry (MS). B). The retention time and the mass of Ppa analyzed by tandem mass spectrometry (MS/MS, MS2).

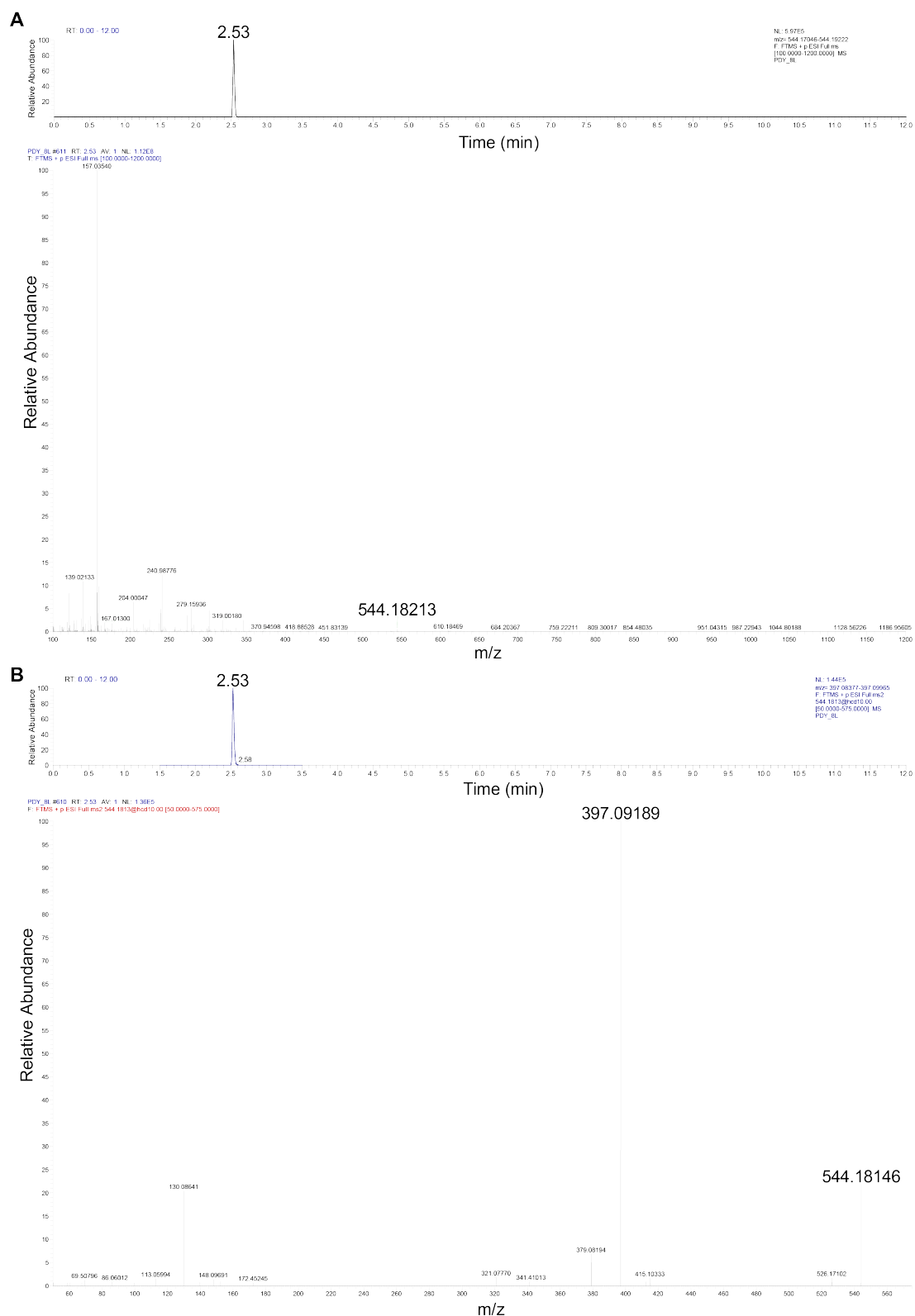


Figure S22. LC-MS spectra of PDPP@D released DOX from 4T1 cells incubated with PDPP@D for 8 h with laser irradiation. A) The retention time and the mass of DOX analyzed by mass spectrometry (MS). B). The retention time and the mass of DOX analyzed by tandem mass spectrometry (MS/MS, MS2).

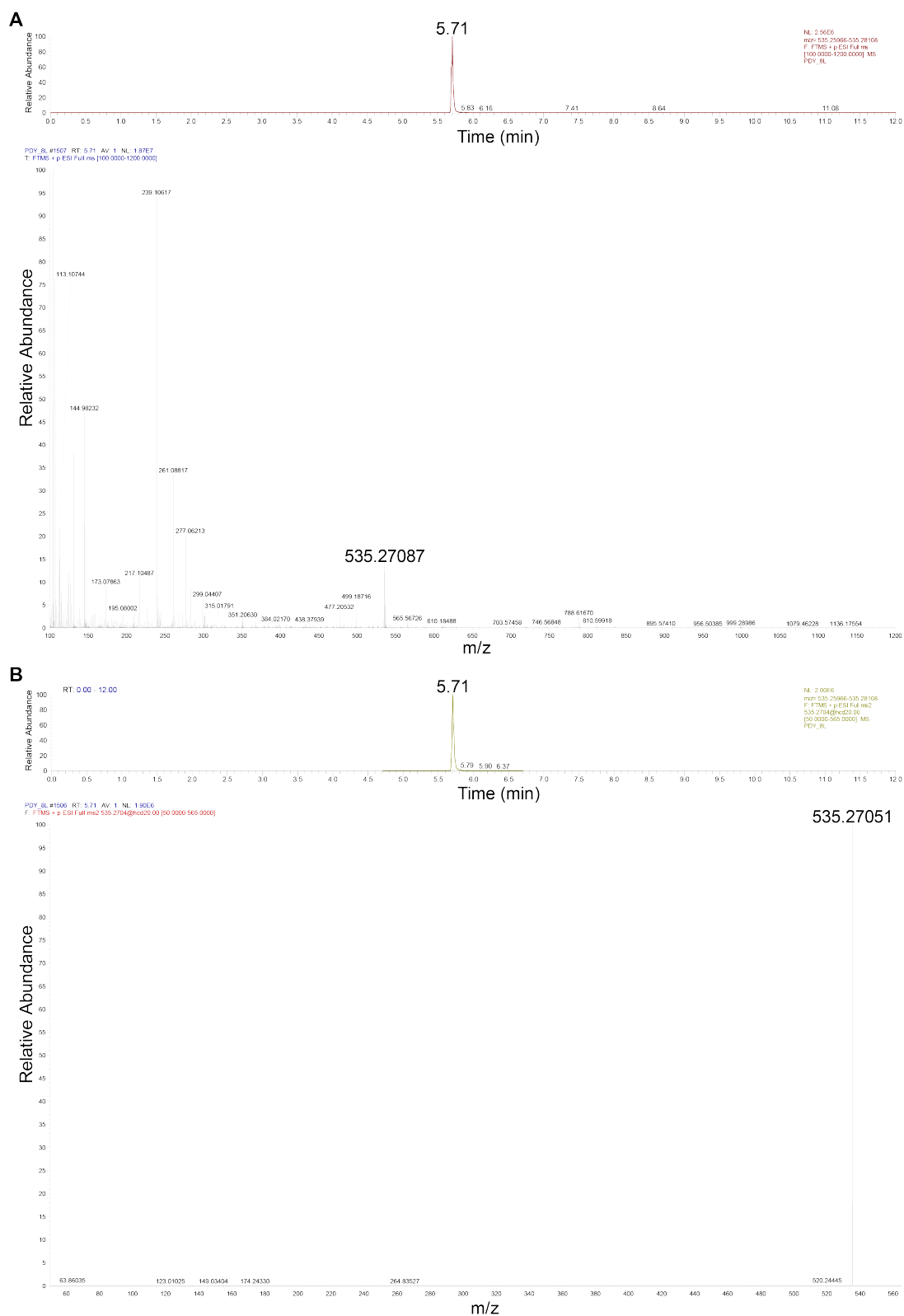


Figure S23. LC-MS spectra of PDPP@D released Ppa from 4T1 cells incubated with PDPP@D for 8 h with laser irradiation. A) The retention time and the mass of Ppa analyzed by mass spectrometry (MS). B). The retention time and the mass of Ppa analyzed by tandem mass spectrometry (MS/MS, MS2).

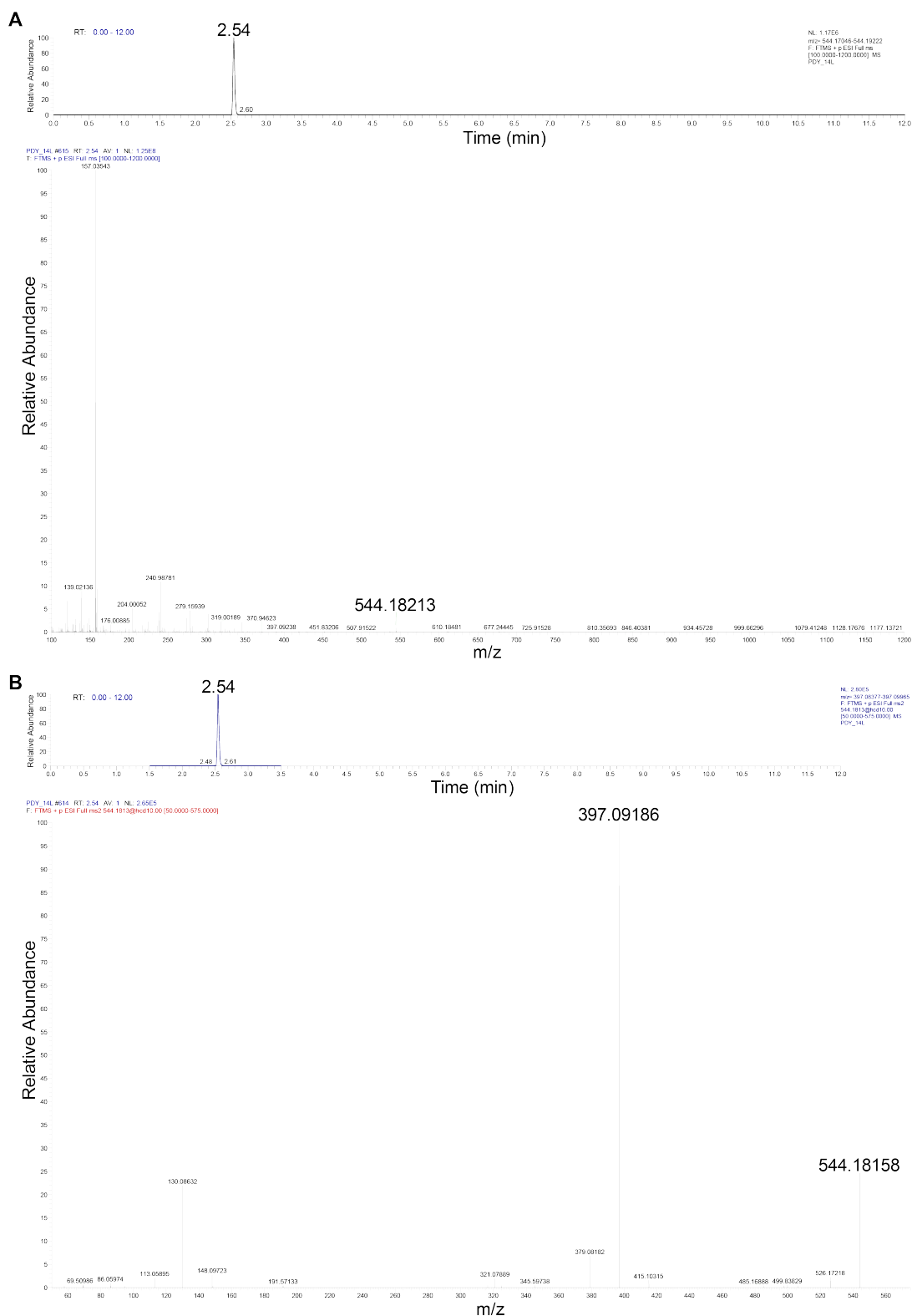


Figure S24. LC-MS spectra of PDPP@D released DOX from 4T1 cells incubated with PDPP@D for 24 h with laser irradiation. A) The retention time and the mass of DOX analyzed by mass spectrometry (MS). B). The retention time and the mass of DOX analyzed by tandem mass spectrometry (MS/MS, MS2).

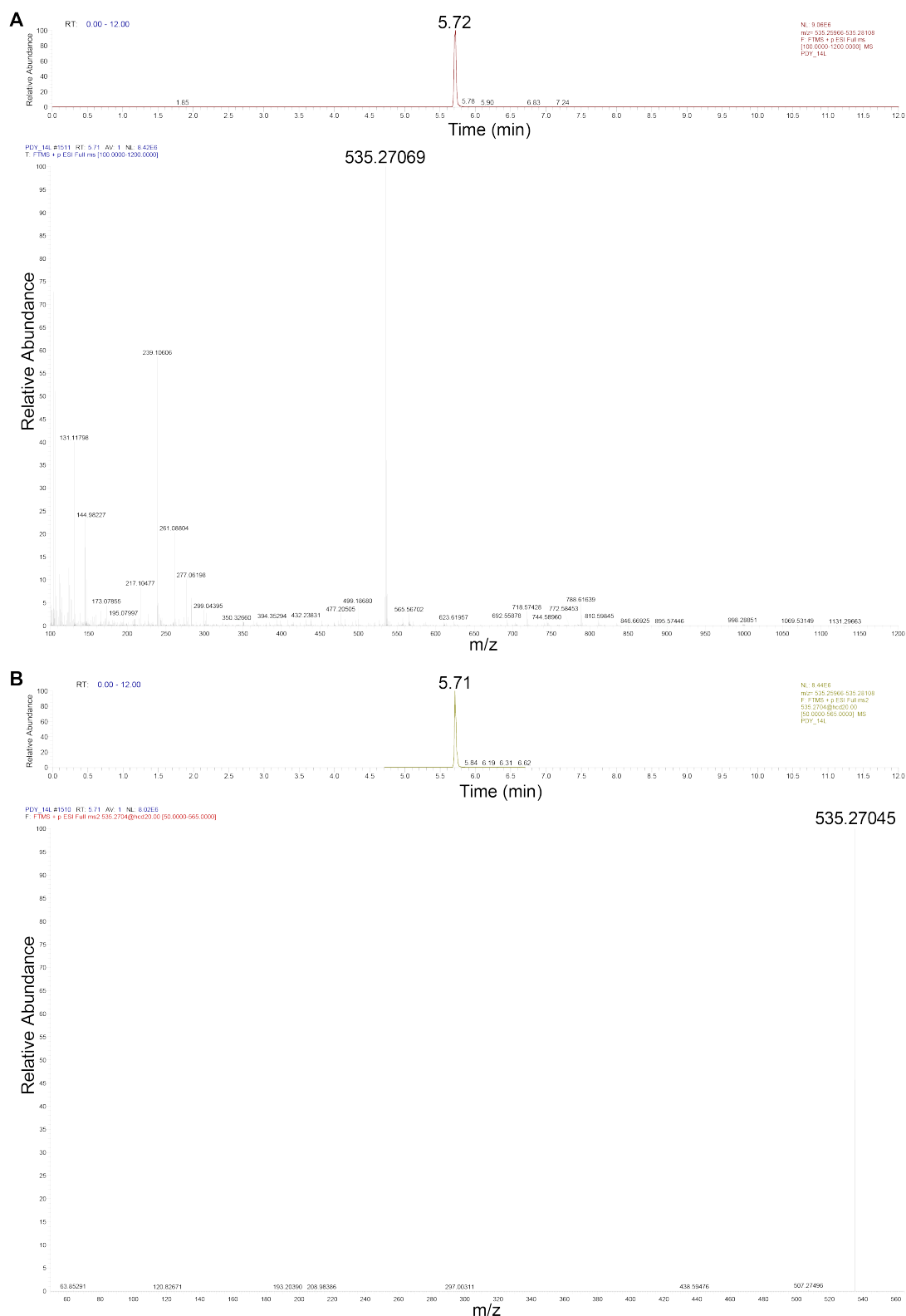


Figure S25. LC-MS spectra of PDPP@D released Ppa from 4T1 cells incubated with PDPP@D for 24 h with laser irradiation. A) The retention time and the mass of Ppa analyzed by mass spectrometry (MS). B). The retention time and the mass of Ppa analyzed by tandem mass spectrometry (MS/MS, MS2).

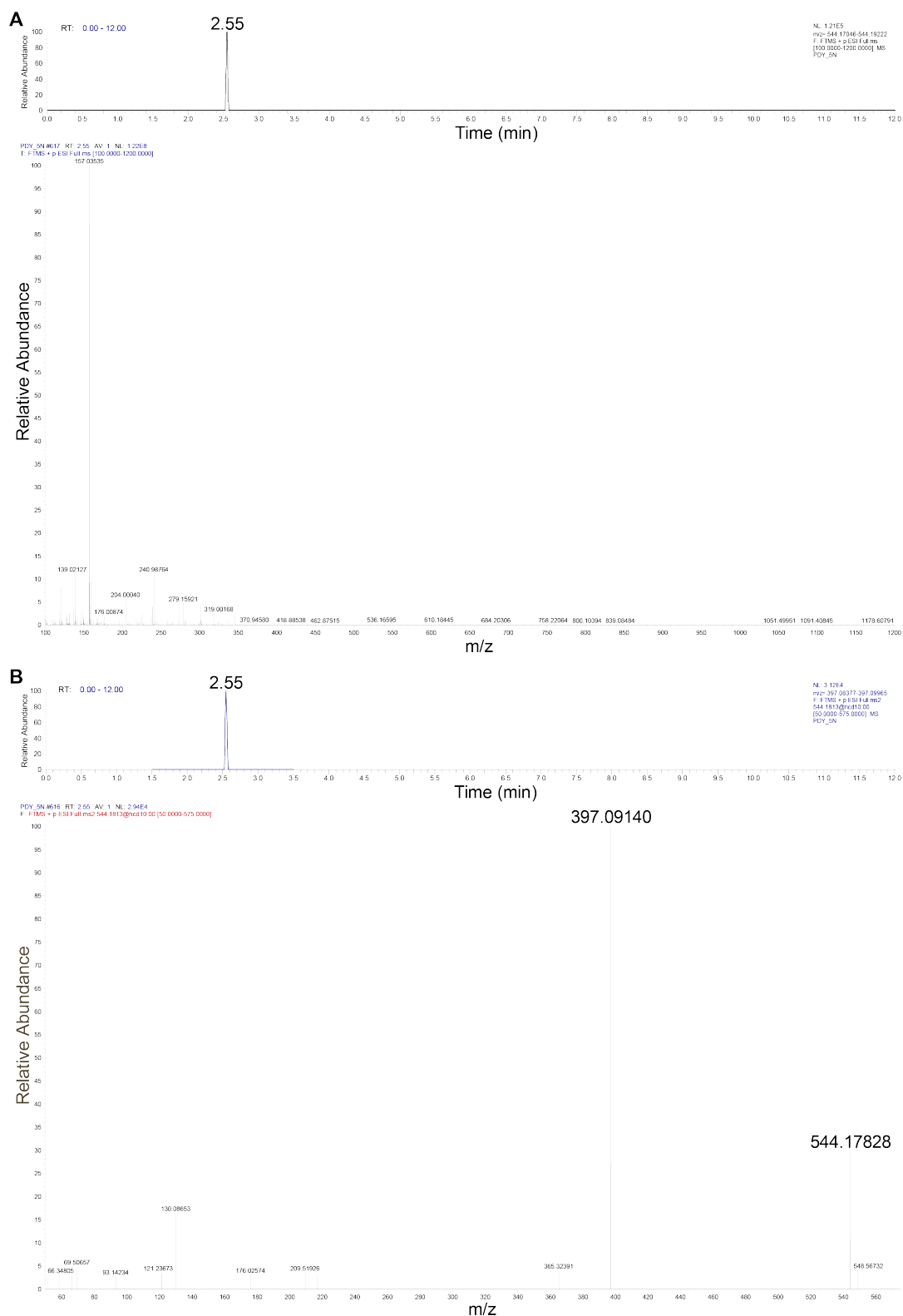


Figure S26. LC-MS spectra of PDPP@D released DOX from 4T1 cells incubated with PDPP@D for 2 h without laser irradiation. A) The retention time and the mass of DOX analyzed by mass spectrometry (MS). B). The retention time and the mass of DOX analyzed by tandem mass spectrometry (MS/MS, MS2).

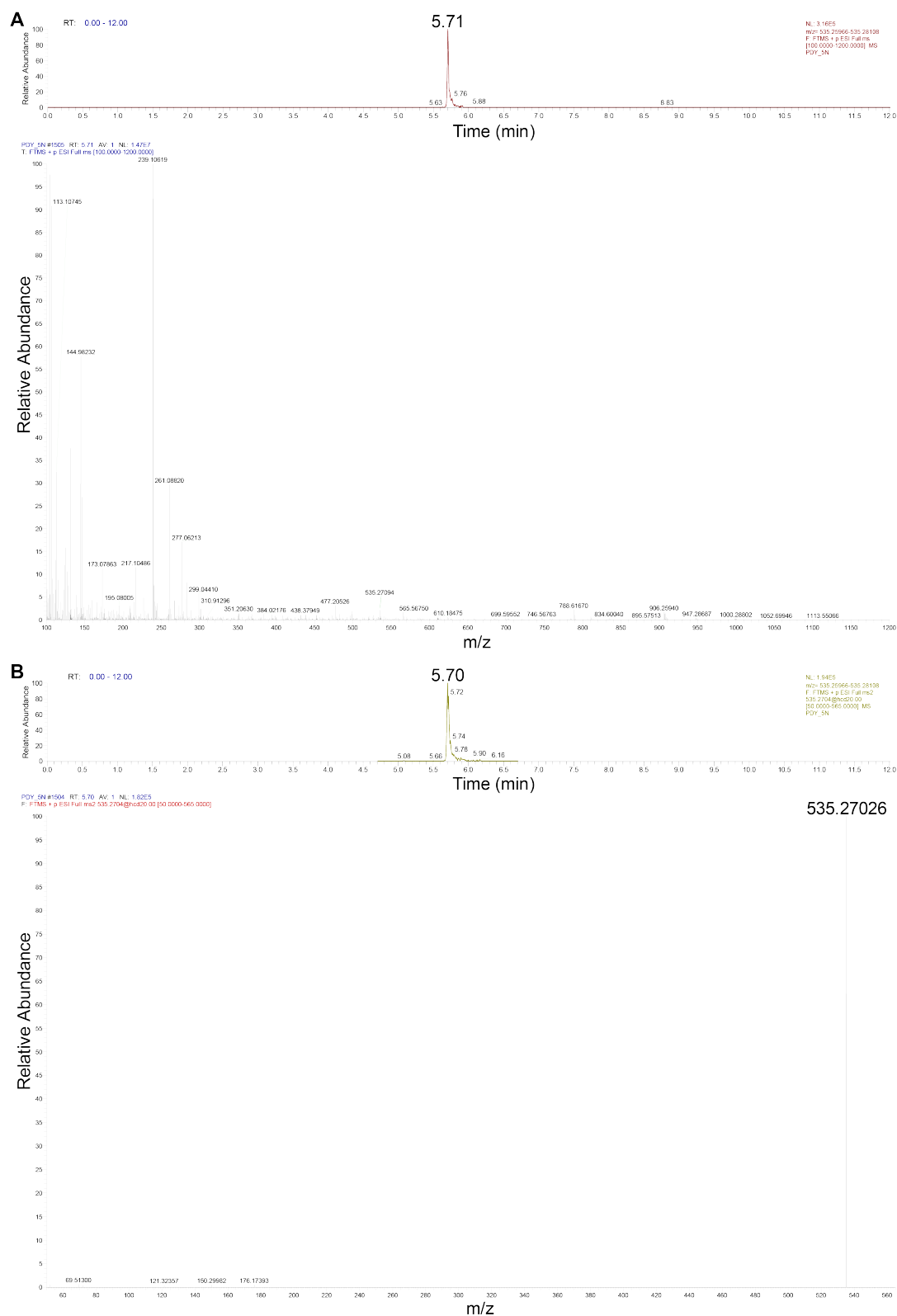


Figure S27. LC-MS spectra of PDPP@D released Ppa from 4T1 cells incubated with PDPP@D for 2 h without laser irradiation. A) The retention time and the mass of Ppa analyzed by mass spectrometry (MS). B). The retention time and the mass of Ppa analyzed by tandem mass spectrometry (MS/MS, MS2).

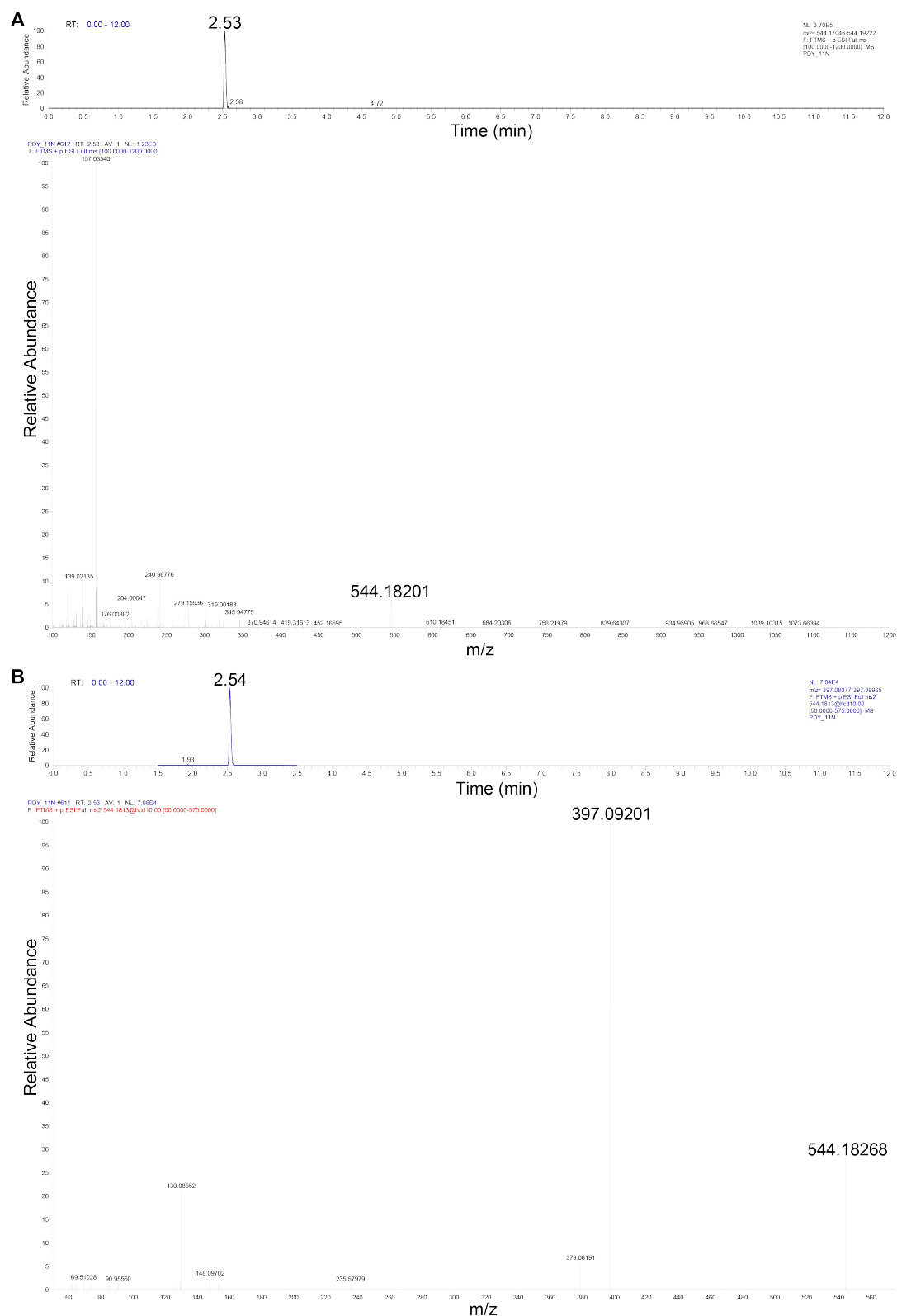


Figure S28. LC-MS spectra of PDPP@D released DOX from 4T1 cells incubated with PDPP@D for 8 h without laser irradiation. A) The retention time and the mass of DOX analyzed by mass spectrometry (MS). B). The retention time and the mass of DOX analyzed by tandem mass spectrometry (MS/MS, MS2).

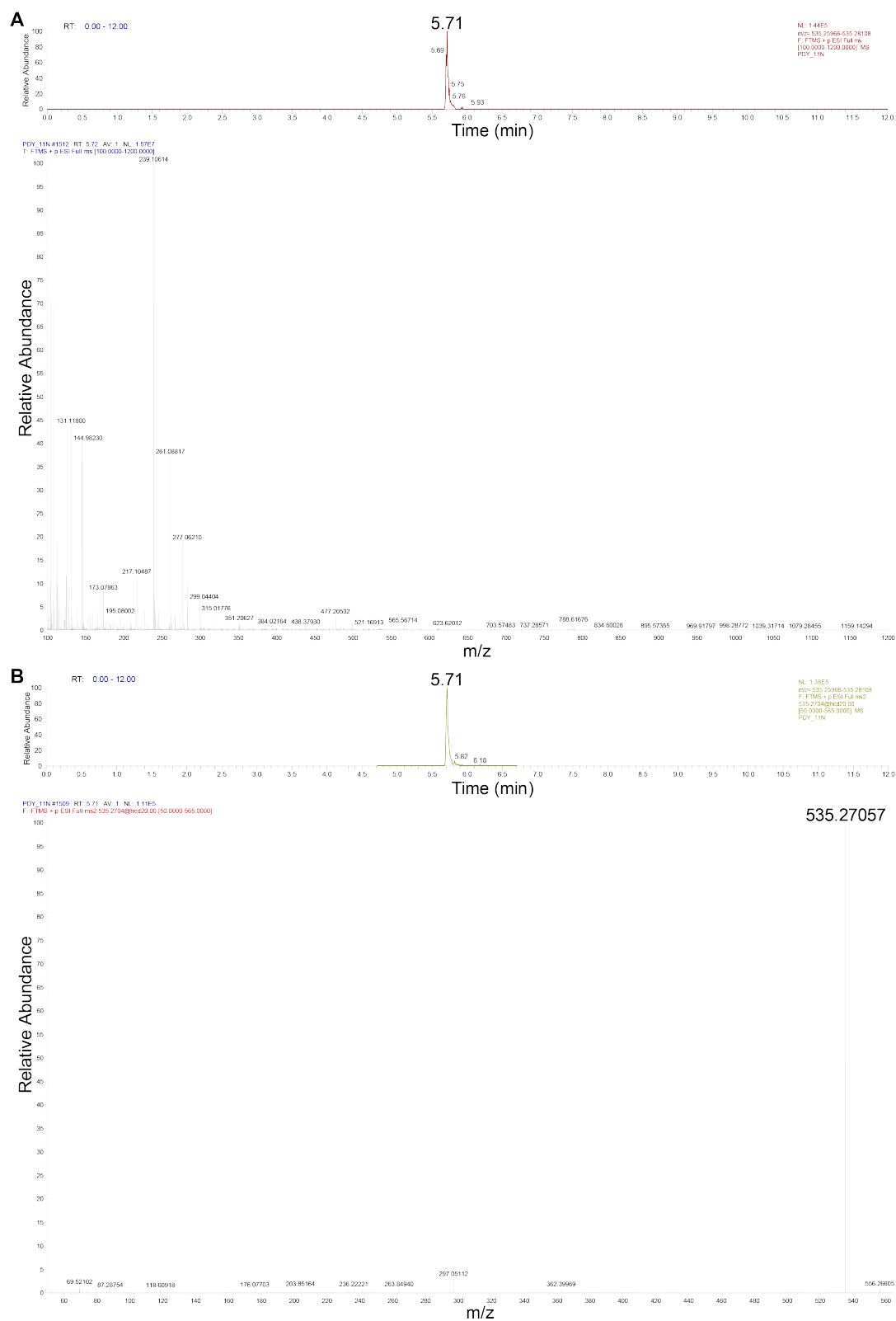


Figure S29. LC-MS spectra of PDPP@D released Ppa from 4T1 cells incubated with PDPP@D for 8 h without laser irradiation. A) The retention time and the mass of Ppa analyzed by mass spectrometry (MS). B). The retention time and the mass of Ppa analyzed by tandem mass spectrometry (MS/MS, MS2).

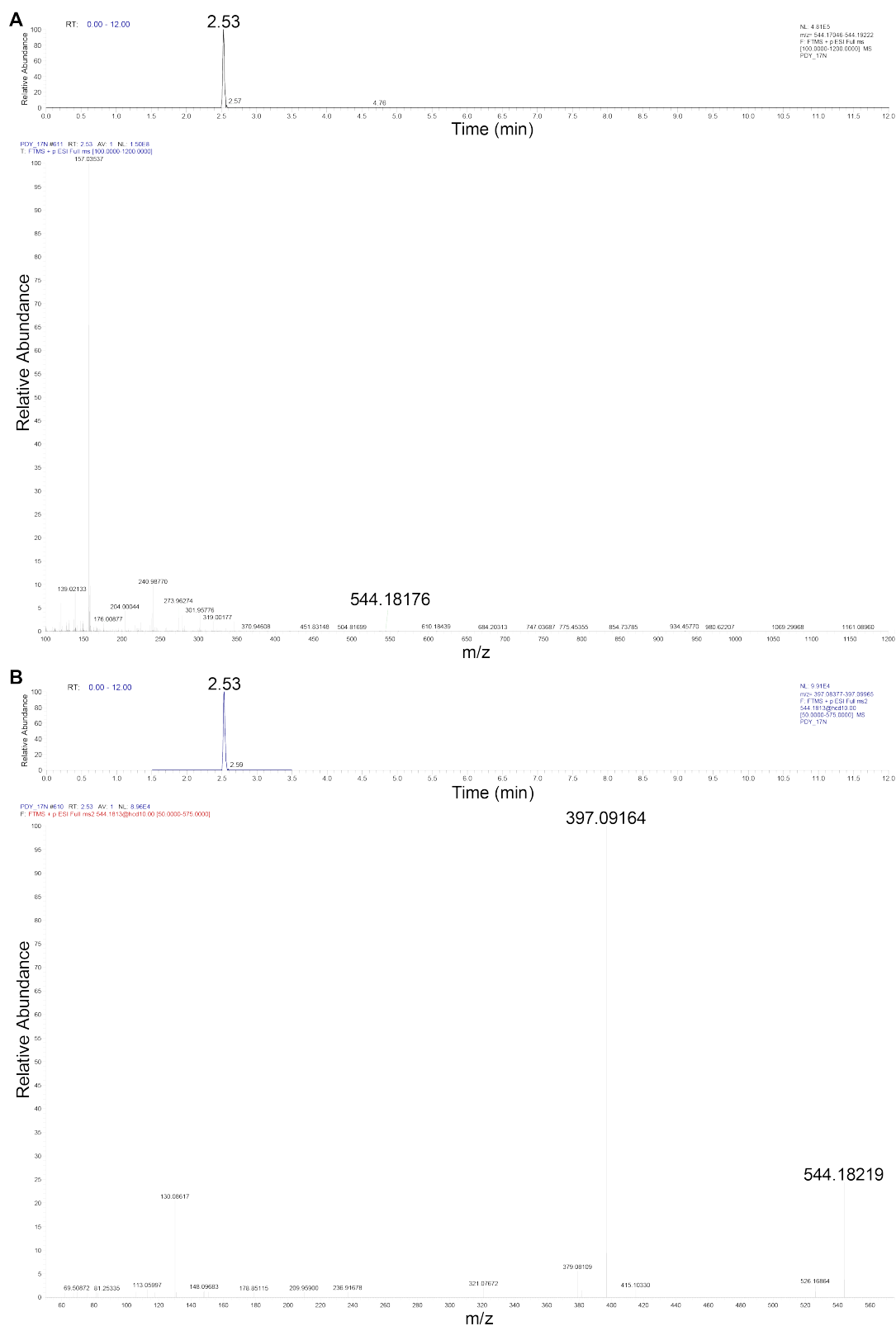


Figure S30. LC-MS spectra of PDPP@D released DOX from 4T1 cells incubated with PDPP@D for 24 h without laser irradiation. A) The retention time and the mass of DOX analyzed by mass spectrometry (MS). B). The retention time and the mass of DOX analyzed by tandem mass spectrometry (MS/MS, MS2).

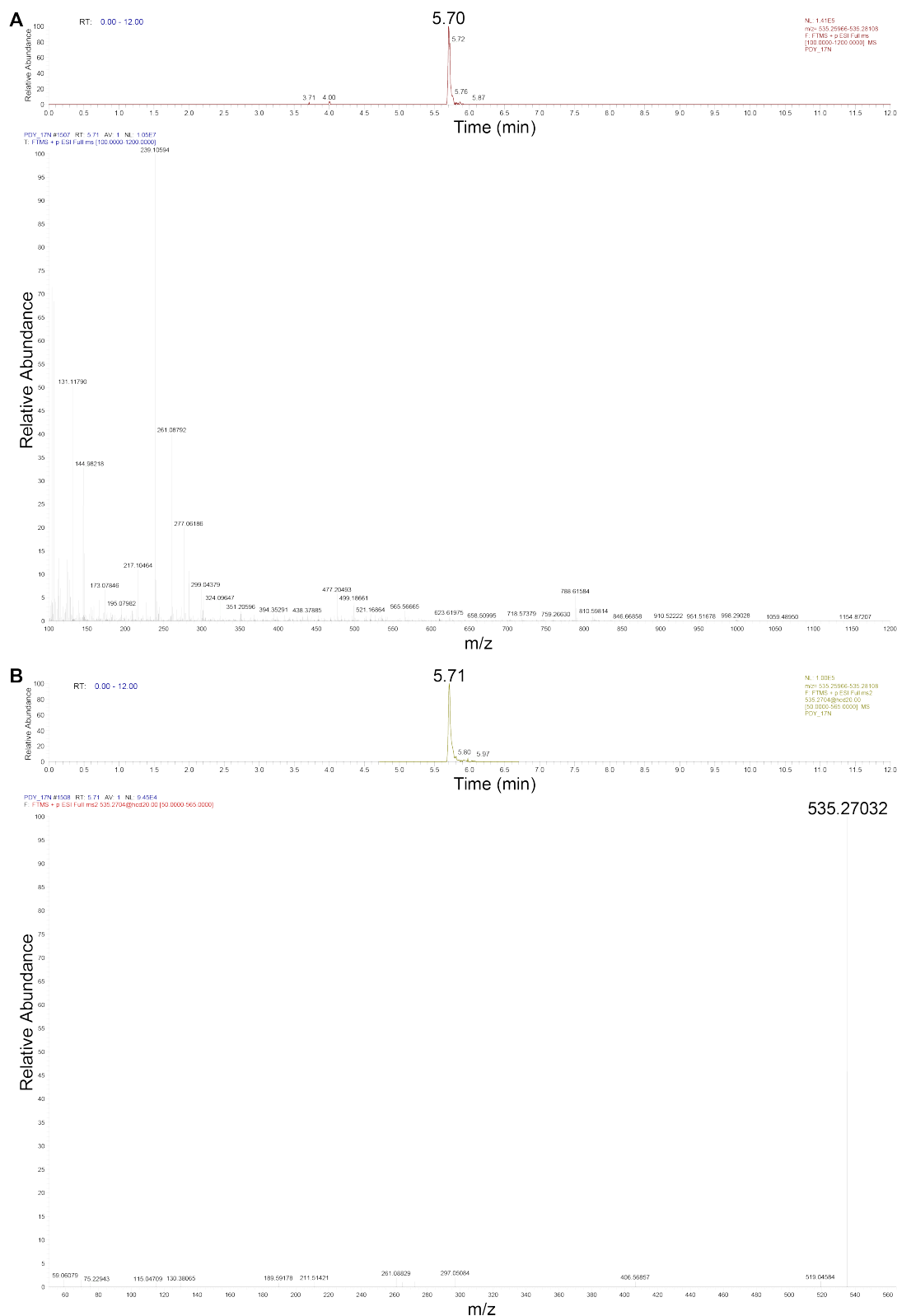


Figure S31. LC-MS spectra of PDPP@D released Ppa from 4T1 cells incubated with PDPP@D for 24 h without laser irradiation. A) The retention time and the mass of Ppa analyzed by mass spectrometry (MS). B). The retention time and the mass of Ppa analyzed by tandem mass spectrometry (MS/MS, MS2).

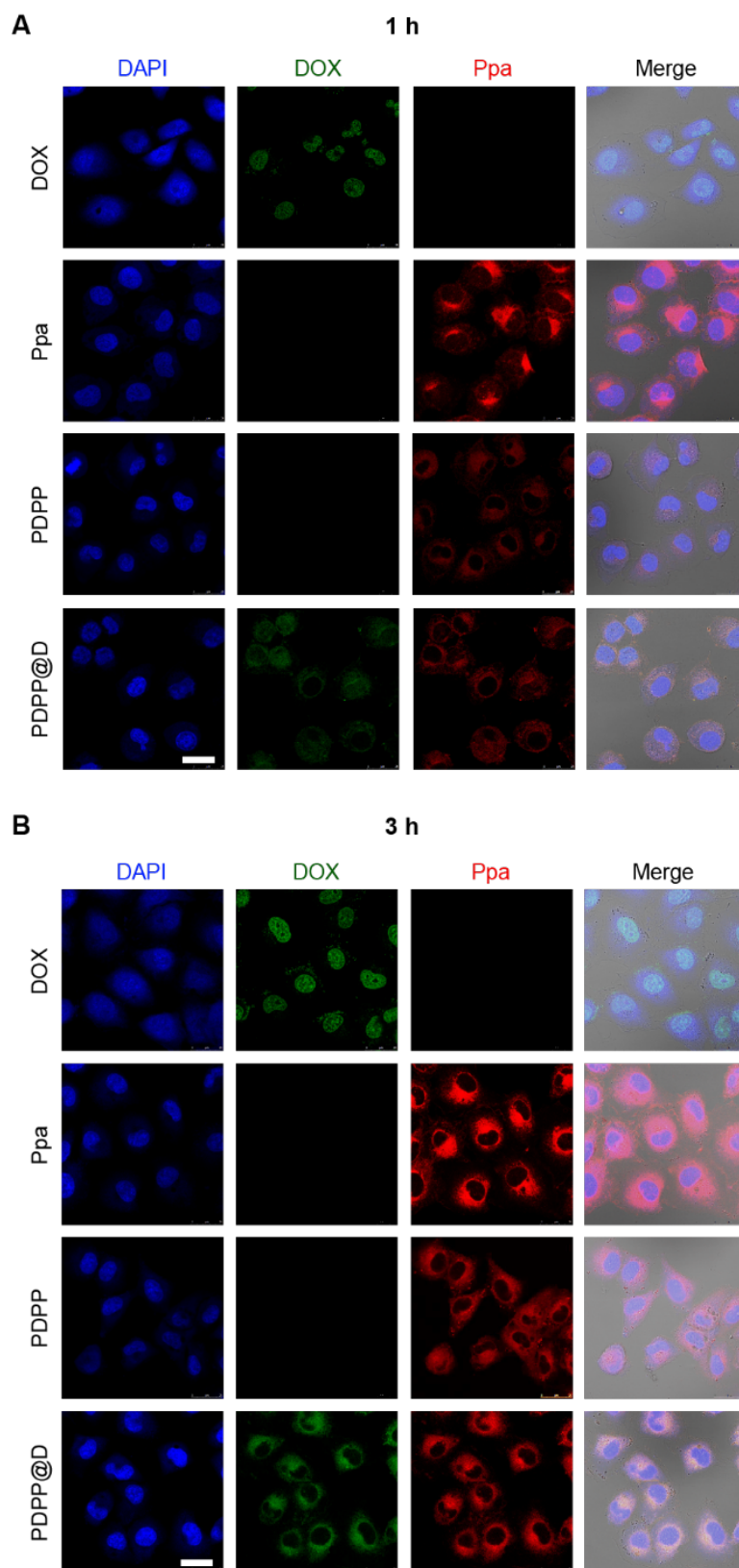


Figure S32. Cellular uptake images for cells cultured with DOX, Ppa, PDPP or PDPP@D for 1 h (A) or 3 h (B) observed by CLSM. The fluorescent signals include DOX (green), Ppa (red) and nuclear staining by DAPI (blue).

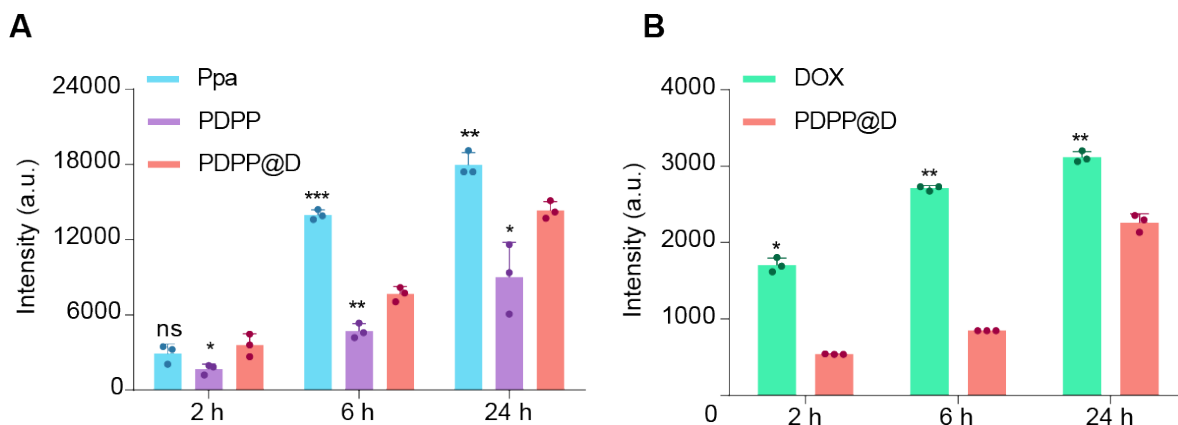


Figure S33. Cellular uptakes of Ppa, PDP, PDPP@D by cells cultured with DOX, Ppa, PDPP or PDPP@D measured by FACS in terms of the intensity of Ppa and DOX signals in 4T1 cells after 2 h, 6 h or 24 h culture. Statistical analysis in comparison with PDPP@D treated cells.

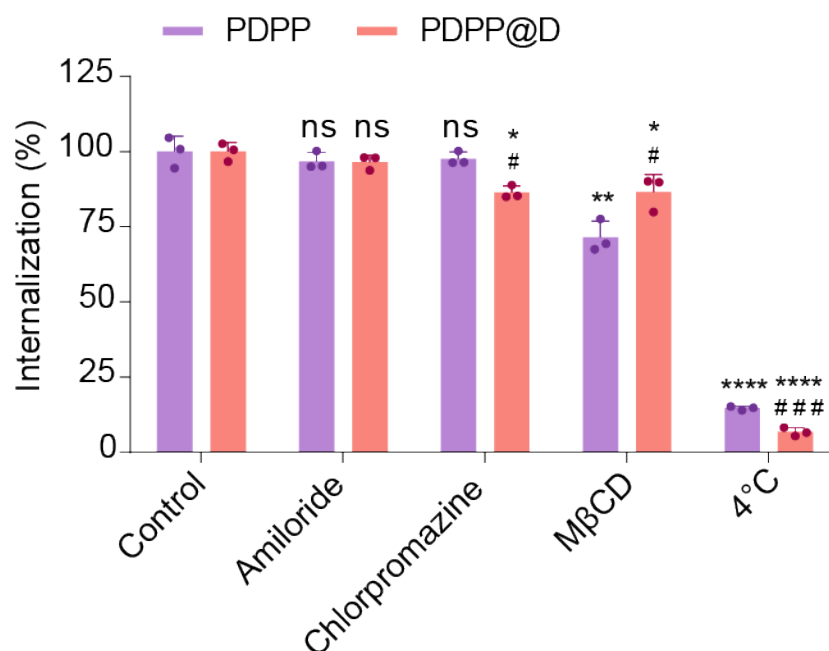


Figure S34. The cellular internalization process of PDPP and PDPP@D in 4T1 cells. The normalized drug internalization of PDPP and PDPP@D at a Ppa concentration of $2 \mu\text{g}\cdot\text{mL}^{-1}$ by 4T1 cells after pretreatment with endocytosis inhibitors (Amiloride to inhibit macropinocytosis-mediated endocytosis; chlorpromazine to inhibit clathrin-mediated endocytosis; methyl- β -cyclodextrin to inhibit caveolae-mediated endocytosis) and a low temperature (4°C) analyzed by flow cytometry ($n = 3$). Cellular internalization after different treatments was compared to that by 4T1 cells without pretreatment by endocytosis inhibitors. Cellular internalization of PDPP and PDPP@D by 4T1 cells without pretreatment by endocytosis inhibitors was set as a control of 100%. Statistical analysis in comparison with control cells, * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$; PDPP@D treatment in comparison with

PDPP treatment, # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$.

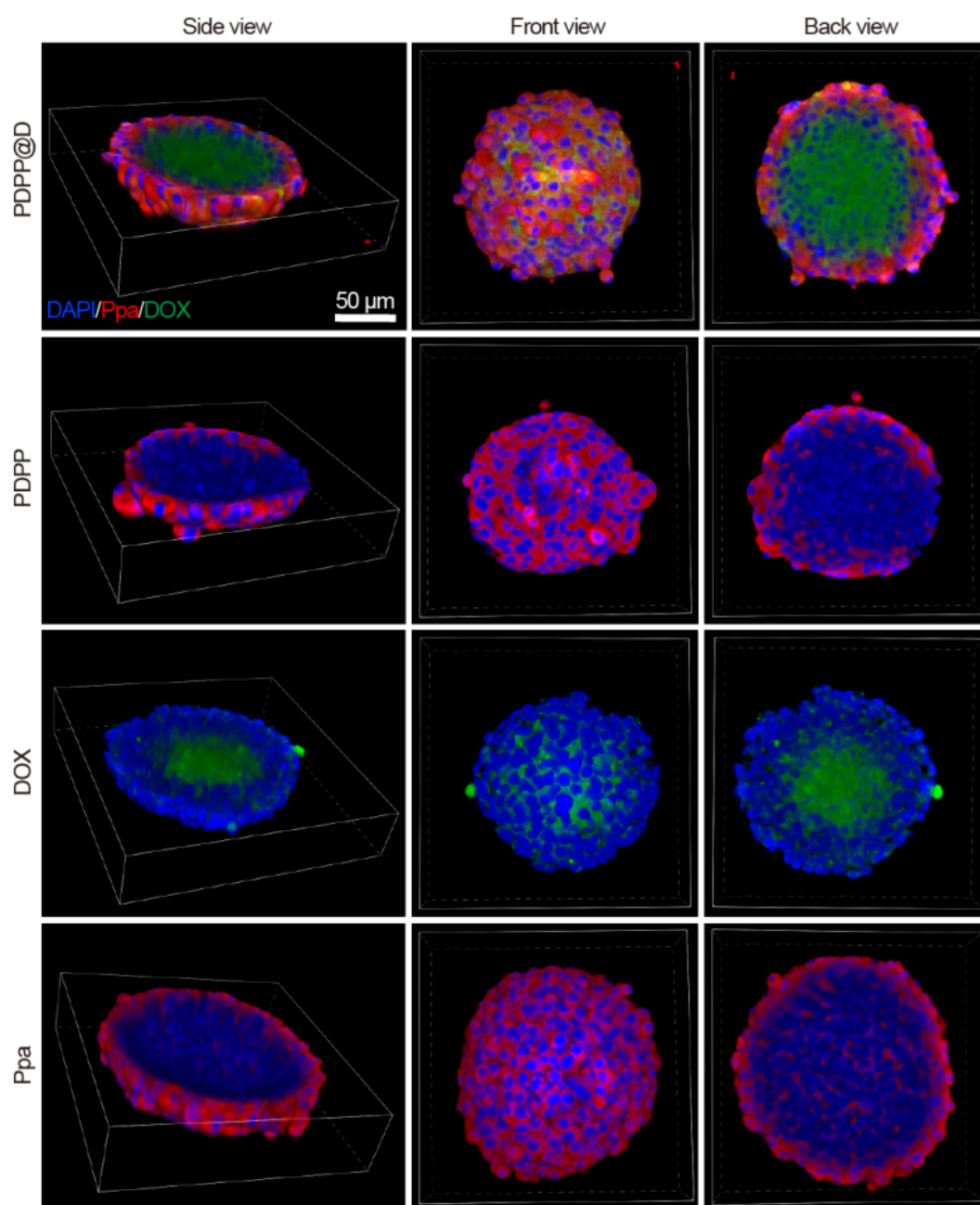


Figure S35. 3D-reconstructed images for MTS penetration of PDPP@D, PDPP, DOX and Ppa in one 4T1 MTS after 3 h incubation. Cell nuclei staining via Hoechst 33258, blue; Ppa, red; and DOX, green.

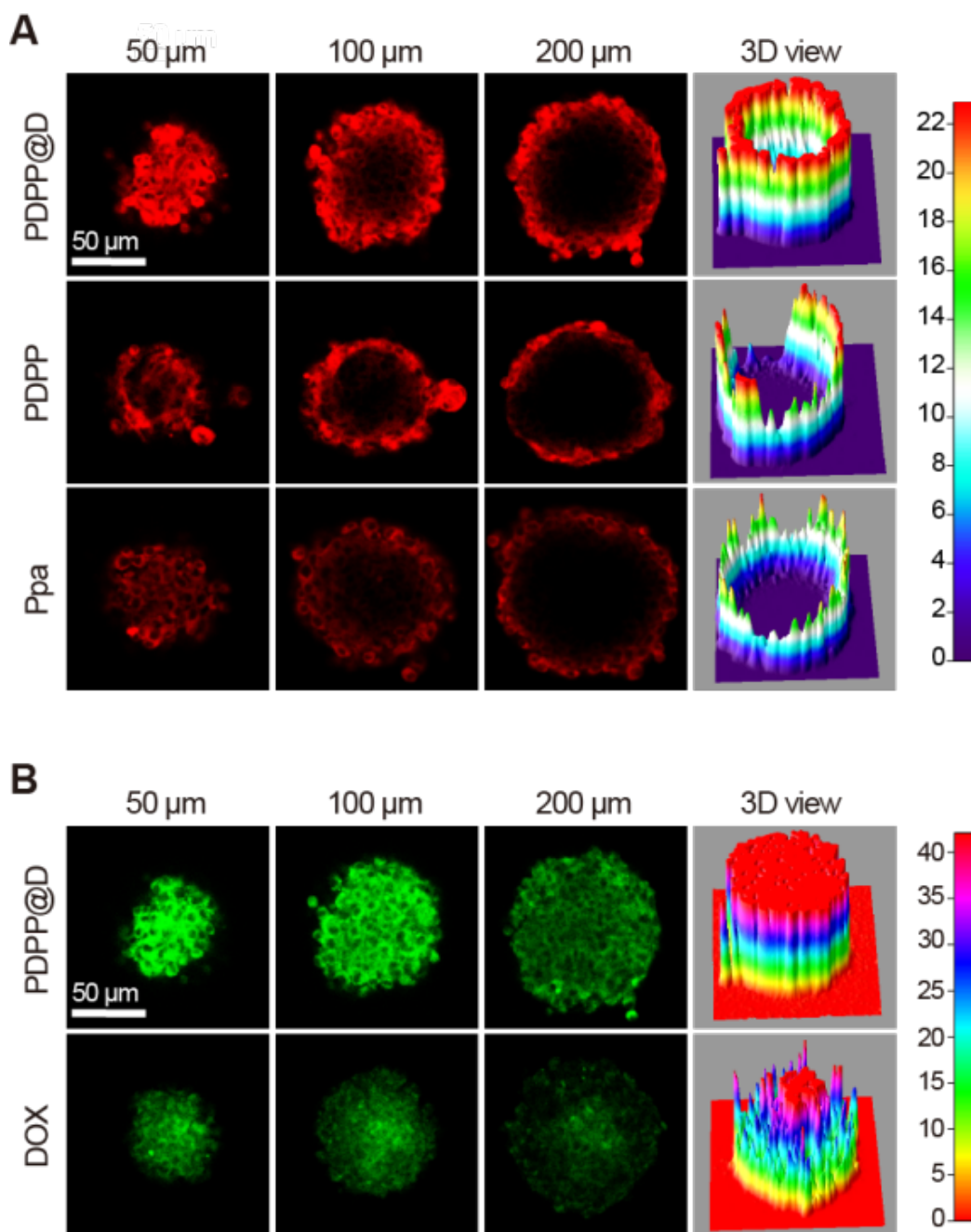


Figure S36. A) CLSM images for *in vitro* penetration of PDPP@D, PDPP and Ppa in 3D-cultured 4T1 MTS via the Ppa channel. B) CLSM images for *in vitro* penetration of PDPP@D and DOX in 3D-cultured 4T1 MTS via the DOX channel. The MTS were incubated with PDPP@D, PDPP, DOX and Ppa for 3 h and observed under a CLSM from the top to bottom with 10 μm thickness per section. The corresponding 2.5D diagrams for 200 μm depth sections of MTS reconstructed by Image J.

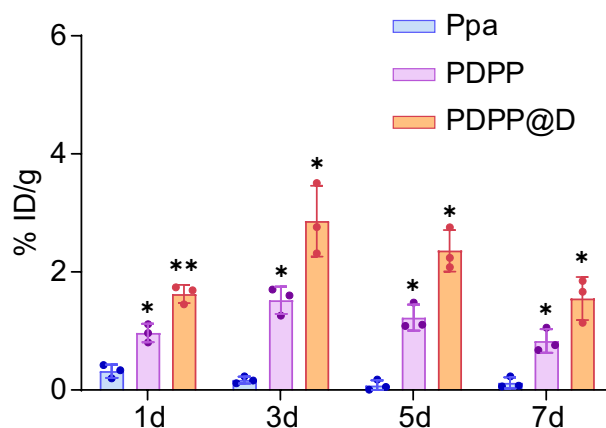


Figure S37. Quantitative measurements of accumulation of PDPP and PDPP@D in the tumor tissues compared with that of Ppa. The accumulation rate was calculated as %ID/g through a fluorescence standard curve.

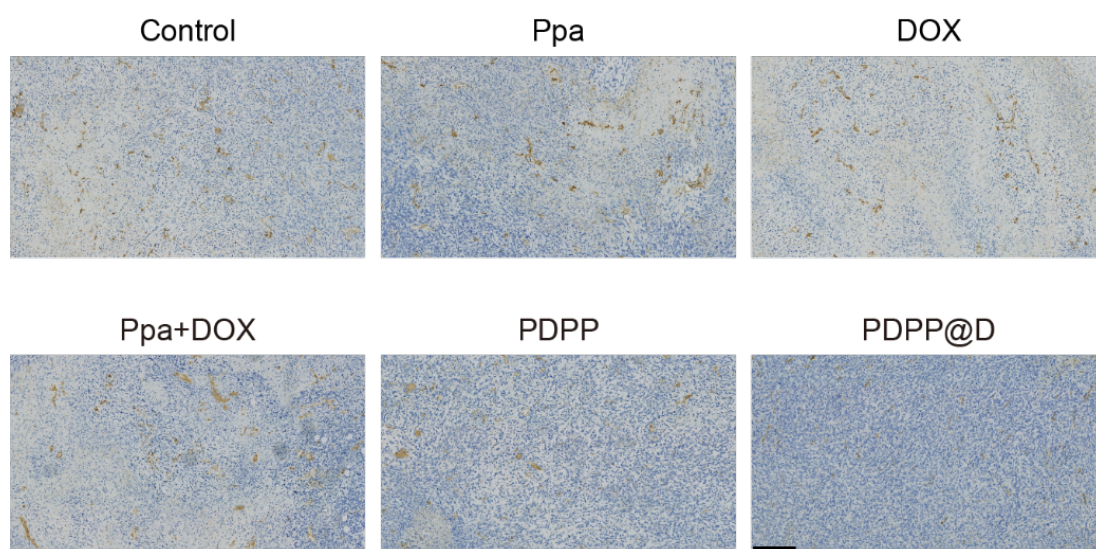


Figure S38. Images for CD31 immunohistochemical staining of tumors from mice treated with different formulations at the experimental endpoint. CD31 is a prognostic marker for the microvessel density of tumors. The stained brown color indicated positive expression of CD31 in treated tumor tissues. The cell nuclei were stained blue. Treatment of PDPP@D significantly decreased the number of CD31 positive cells in tumor tissues. Scale bars: 200 μ m.

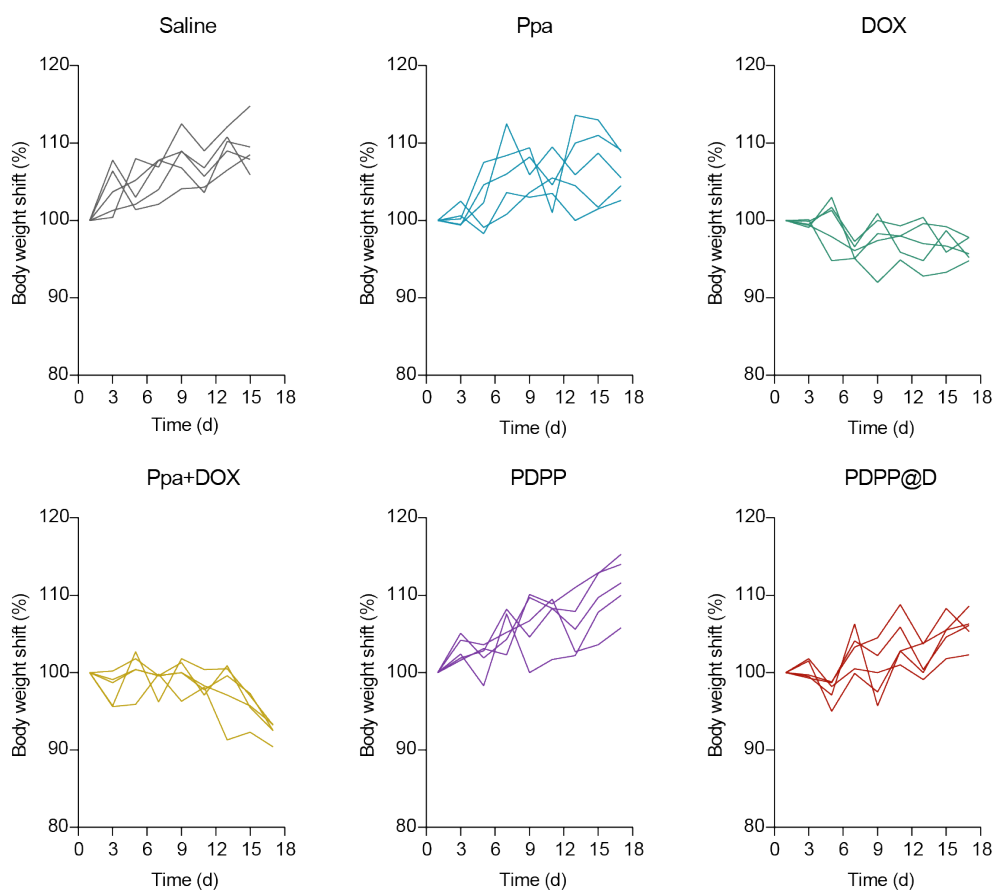


Figure S39. Body weight changes of BALB/c mice treated with different formulations during the treatment period.

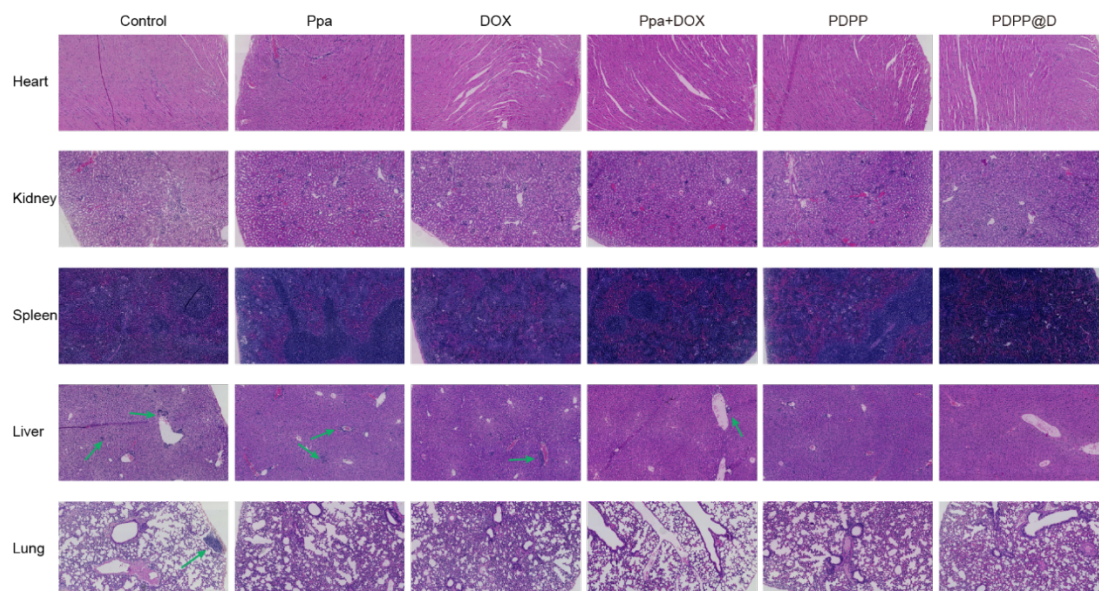


Figure S40. H&E images for histological analysis of tumors extracted from mice treated with different formulations at the experimental endpoint. Arrows indicate the metastatic lesions of tumors. Scale bars: 200 μ m.

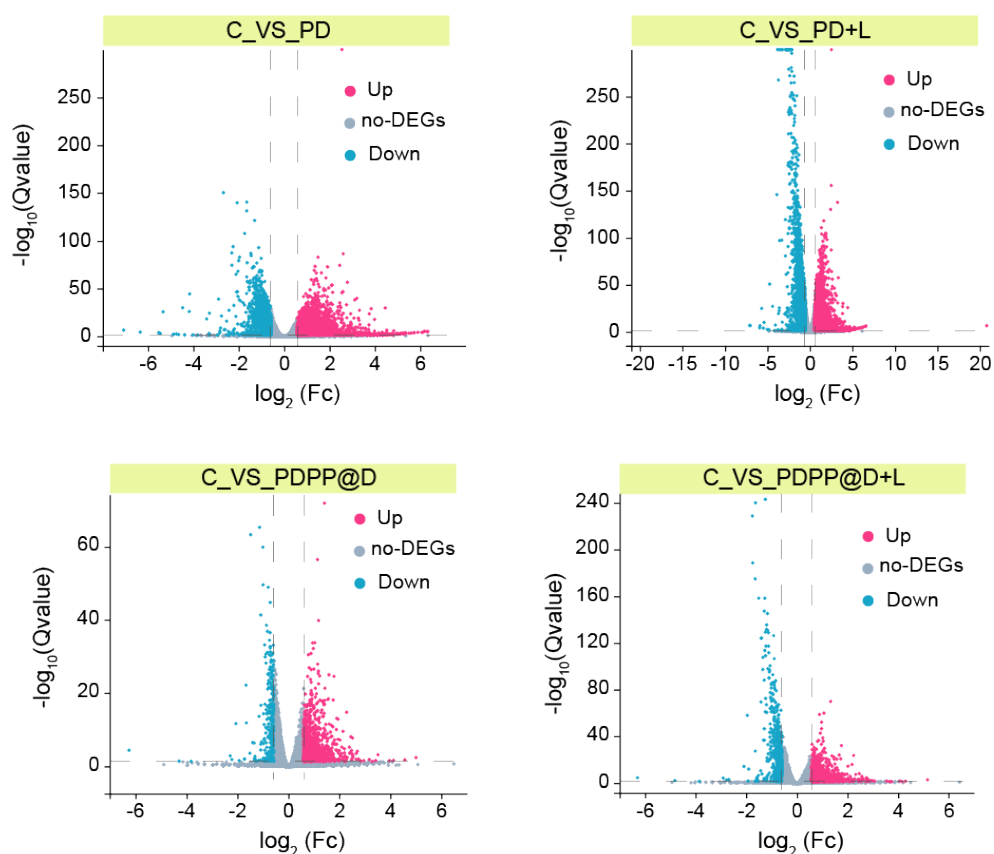


Figure S41. Volcano plots of DEGs in 4T1 cells after different treatments versus the control. Significance was indicated with a fold change in the absolute value > 1.5 and a P value of < 0.05 after analysis of variance (ANOVA) between genes from treatments versus those from the control ($n = 3$).

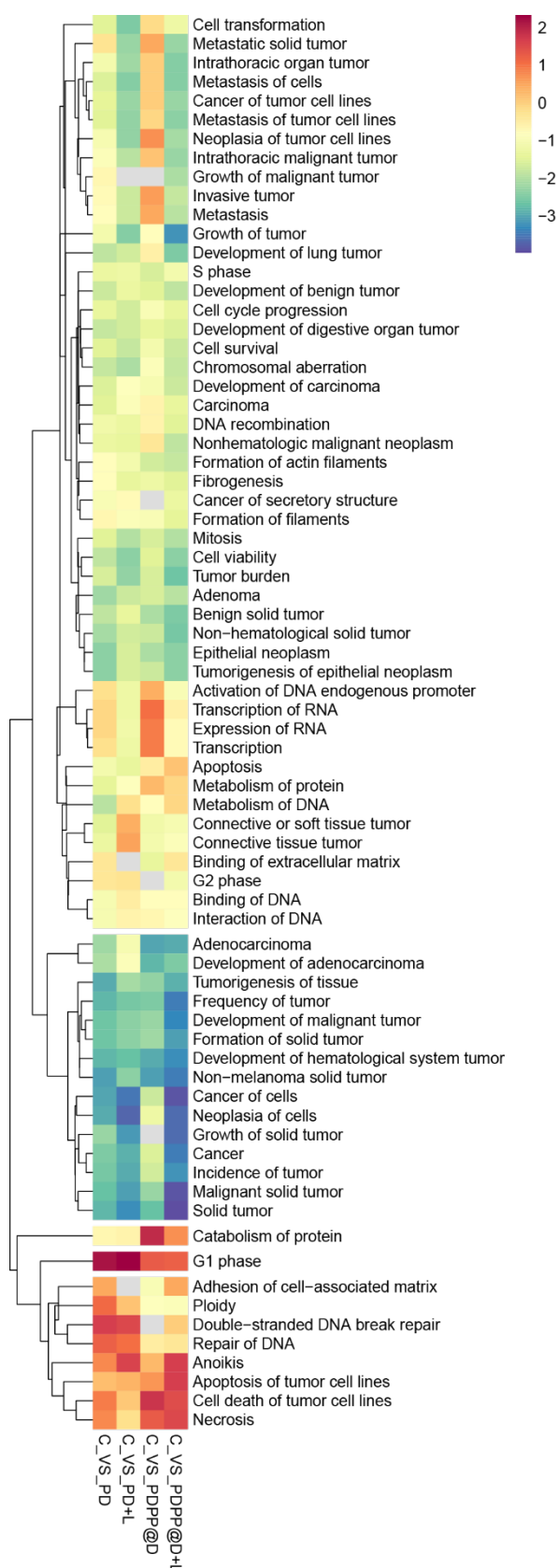
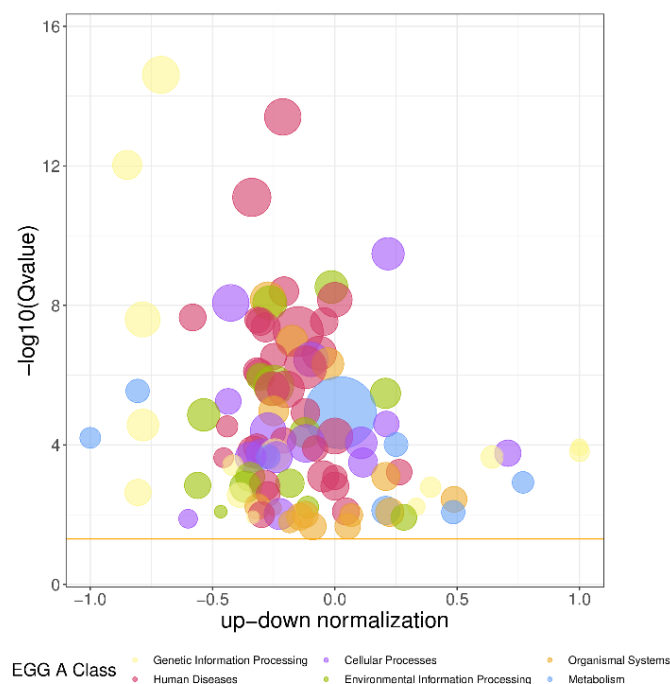


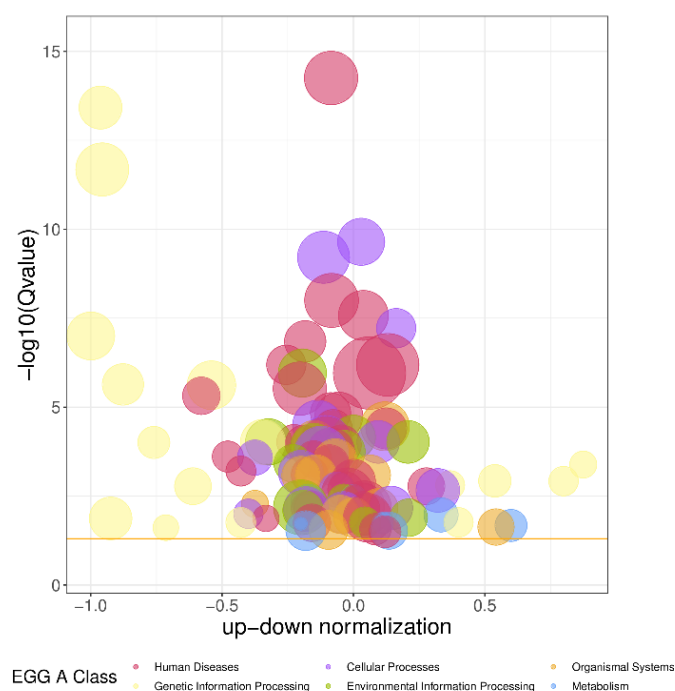
Figure S42. Heatmap of differentially influenced biofunction ($P < 0.05$) after different treatments by Ingenuity Pathway Analysis (IPA).



Pathway Top 20

ID	Description
ko04068	FoxO signaling pathway
ko04150	mTOR signaling pathway
ko04141	Protein processing in endoplasmic reticulum
ko03008	Ribosome biogenesis in eukaryotes
ko03013	RNA transport
ko04110	Cell cycle
ko04510	Focal adhesion
ko05206	MicroRNAs in cancer
ko05205	Proteoglycans in cancer
ko05210	Colorectal cancer
ko05161	Hepatitis B
ko05211	Renal cell carcinoma
ko05213	Endometrial cancer
ko05212	Pancreatic cancer
ko05220	Chronic myeloid leukemia
ko05222	Small cell lung cancer
ko05200	Pathways in cancer
ko05160	Hepatitis C
ko04360	Axon guidance
ko04722	Neurotrophin signaling pathway

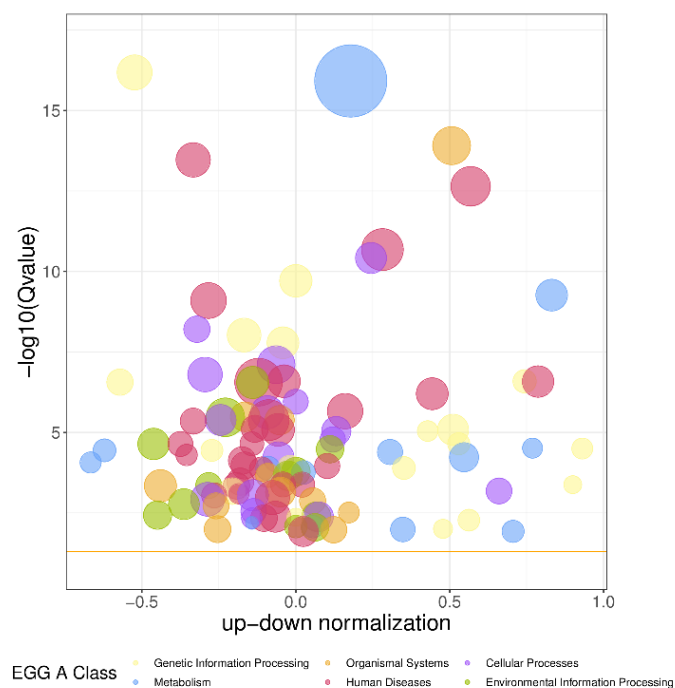
Figure S43. KEGG class of DEGs after treatment with PDPP@D+L compared to the control.



Pathway Top 20

ID	Description
ko04150	mTOR signaling pathway
ko03008	Ribosome biogenesis in eukaryotes
ko03013	RNA transport
ko03040	Spliceosome
ko03015	mRNA surveillance pathway
ko04141	Protein processing in endoplasmic reticulum
ko04110	Cell cycle
ko04218	Cellular senescence
ko04115	p53 signaling pathway
ko05206	MicroRNAs in cancer
ko05205	Proteoglycans in cancer
ko05161	Hepatitis B
ko05210	Colorectal cancer
ko05220	Chronic myeloid leukemia
ko05165	Human papillomavirus infection
ko05200	Pathways in cancer
ko05203	Viral carcinogenesis
ko05211	Renal cell carcinoma
ko01522	Endocrine resistance
ko05160	Hepatitis C

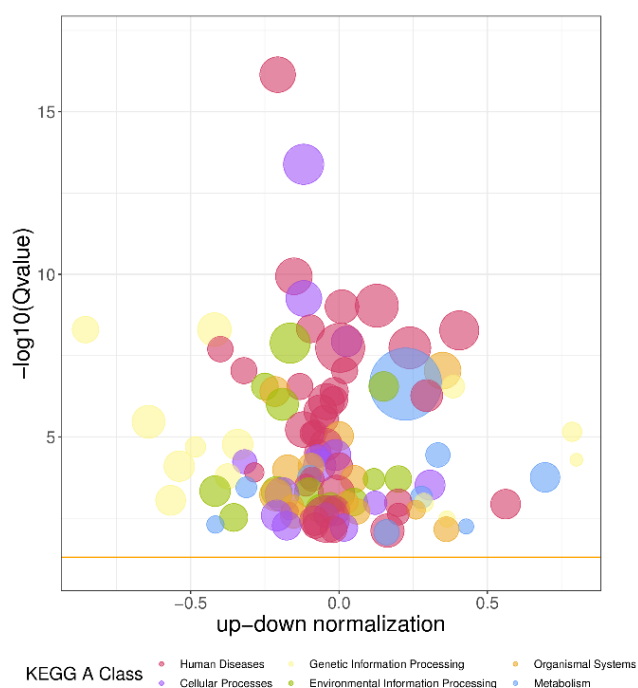
Figure S44. KEGG class of DEGs after treatment with PDPP@D compared to the control.



Pathway Top 20

ID	Description
ko01100	Metabolic pathways
ko00190	Oxidative phosphorylation
ko04141	Protein processing in endoplasmic reticulum
ko03040	Spliceosome
ko03013	RNA transport
ko04120	Ubiquitin mediated proteolysis
ko03050	Proteasome
ko03008	Ribosome biogenesis in eukaryotes
ko04110	Cell cycle
ko04520	Adherens junction
ko04144	Endocytosis
ko04510	Focal adhesion
ko05206	MicroRNAs in cancer
ko05016	Huntington disease
ko05010	Alzheimer disease
ko05205	Proteoglycans in cancer
ko05161	Hepatitis B
ko05012	Parkinson disease
ko05200	Pathways in cancer
ko04714	Thermogenesis

Figure S45. KEGG class of DEGs after treatment with PD+L compared to the control.



Pathway Top 20

ID	Description
ko01100	Metabolic pathways
ko04010	MAPK signaling pathway
ko03008	Ribosome biogenesis in eukaryotes
ko04141	Protein processing in endoplasmic reticulum
ko03460	Fanconi anemia pathway
ko04144	Endocytosis
ko04510	Focal adhesion
ko04110	Cell cycle
ko05206	MicroRNAs in cancer
ko05205	Proteoglycans in cancer
ko05165	Human papillomavirus infection
ko05161	Hepatitis B
ko05210	Colorectal cancer
ko05016	Huntington disease
ko05010	Alzheimer disease
ko05200	Pathways in cancer
ko05211	Renal cell carcinoma
ko05212	Pancreatic cancer
ko05220	Chronic myeloid leukemia
ko04714	Thermogenesis

Figure S46. KEGG class of DEGs after treatment with PD compared to the control.

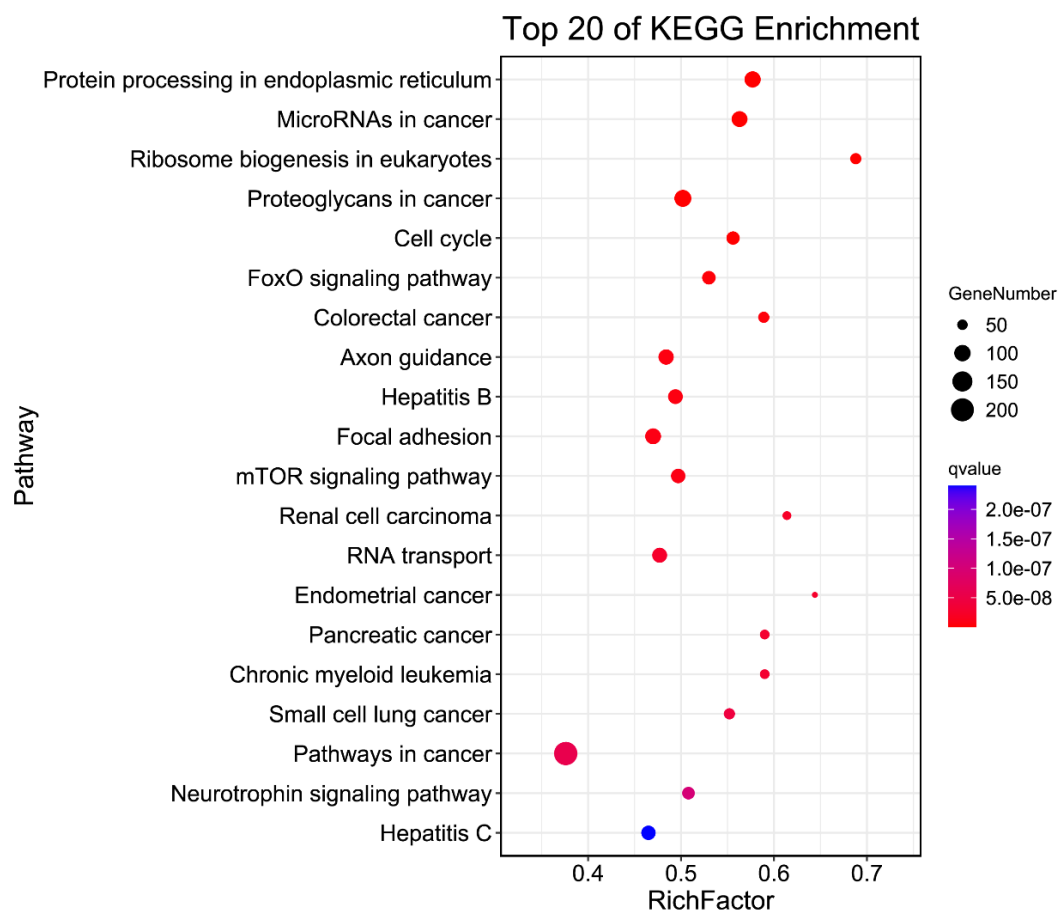


Figure S47. Top 20 enriched pathways from DEGs after treatment with PDPP@D+L compared to the control.

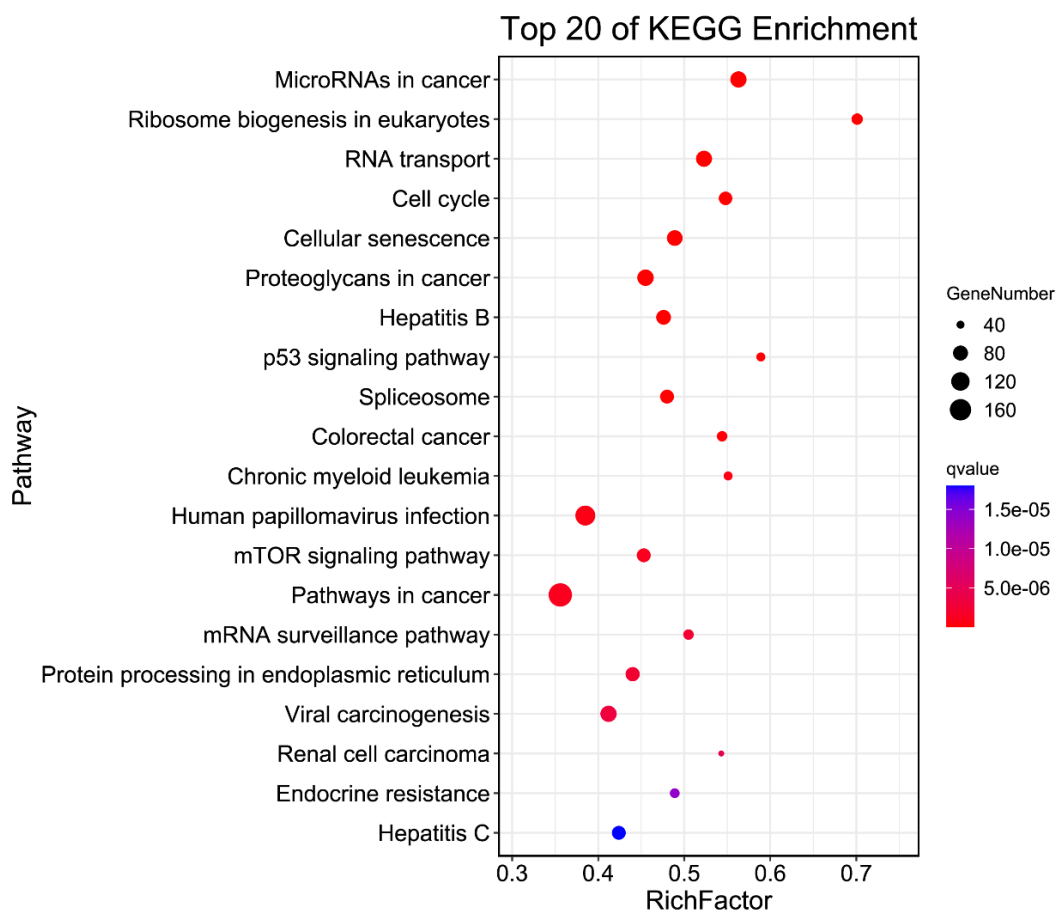


Figure S48. Top 20 enriched pathways from DEGs after treatment with PDPP@D compared to the control.

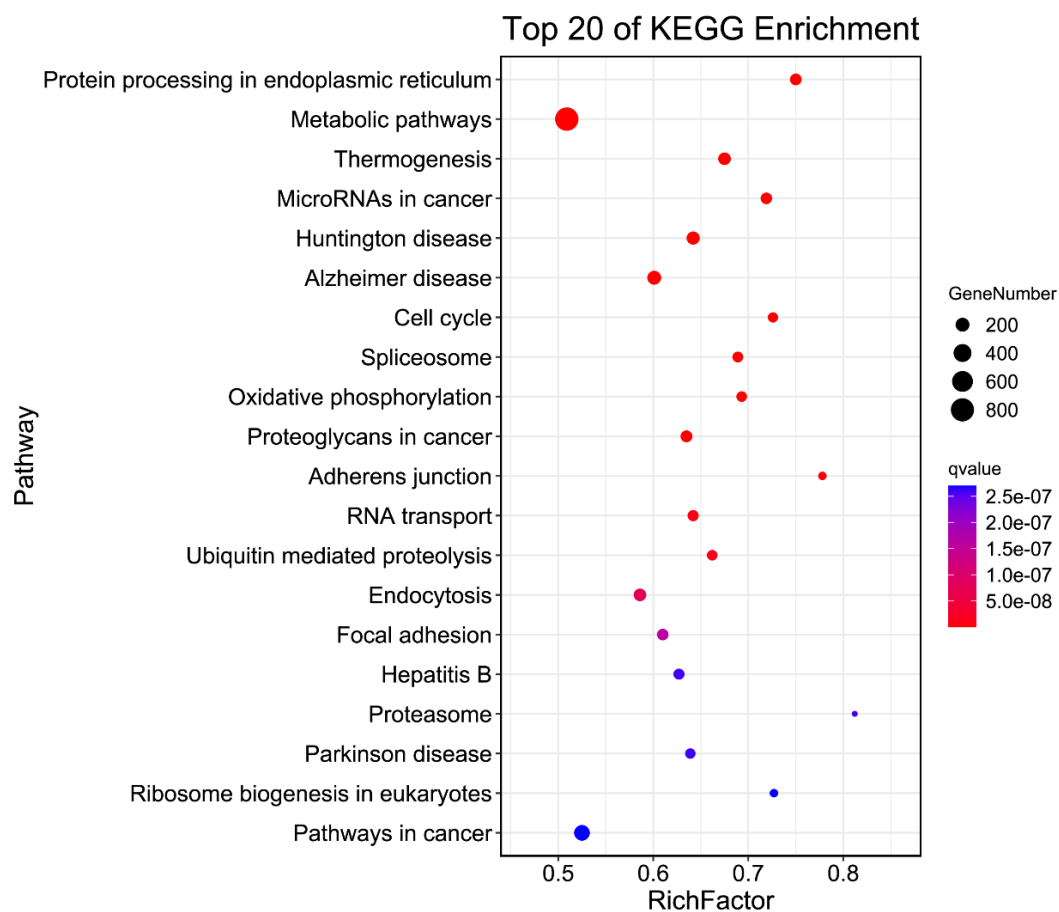


Figure S49. Top 20 enriched pathways from DEGs after treatment with PD+L compared to the control.

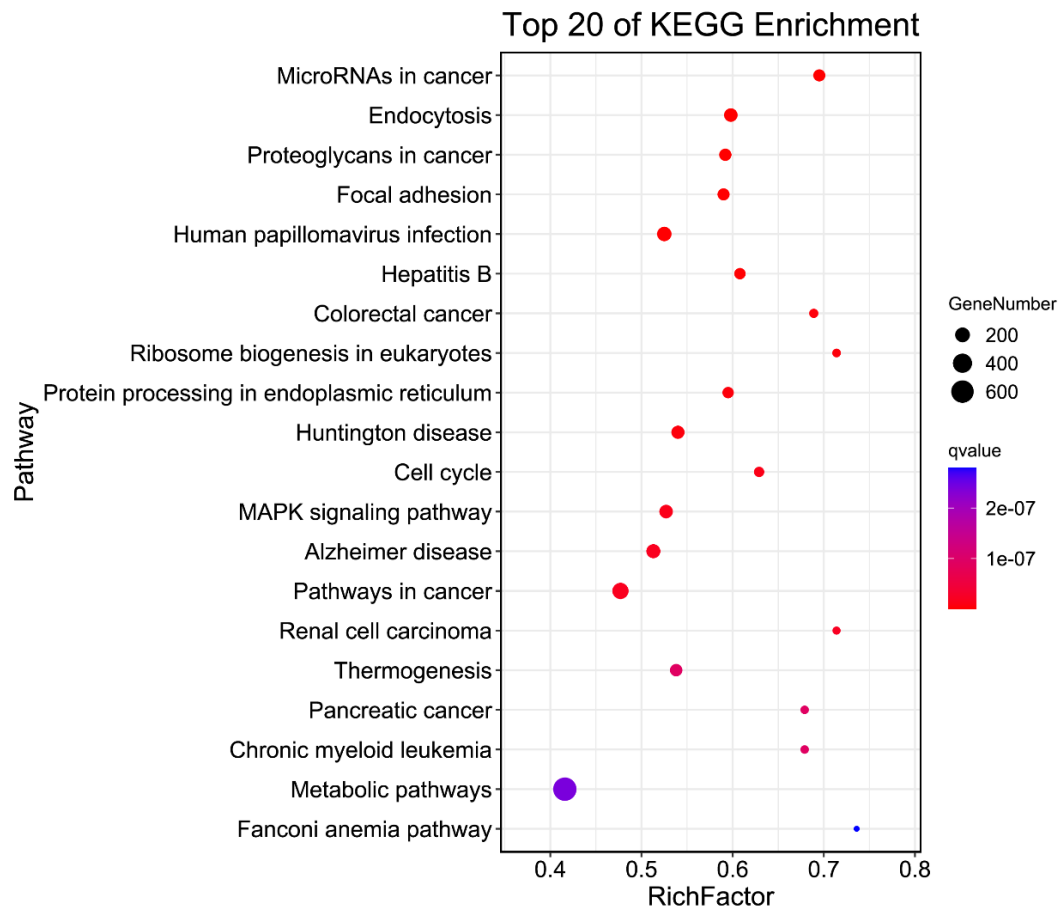


Figure S50. Top 20 enriched pathways from DEGs after treatment with PD compared to the control.

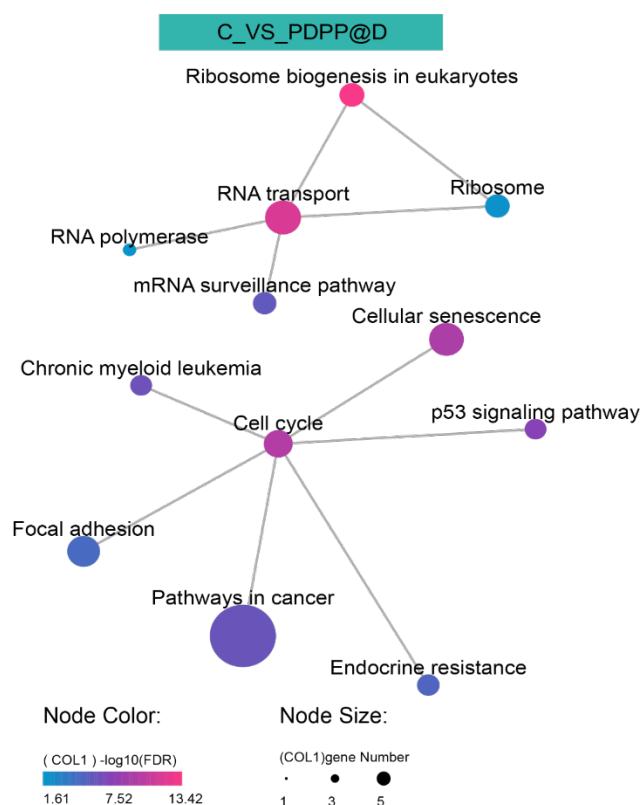


Figure S51. Interaction networks of the enriched KEGG pathways in cells treated with PDPP@D.

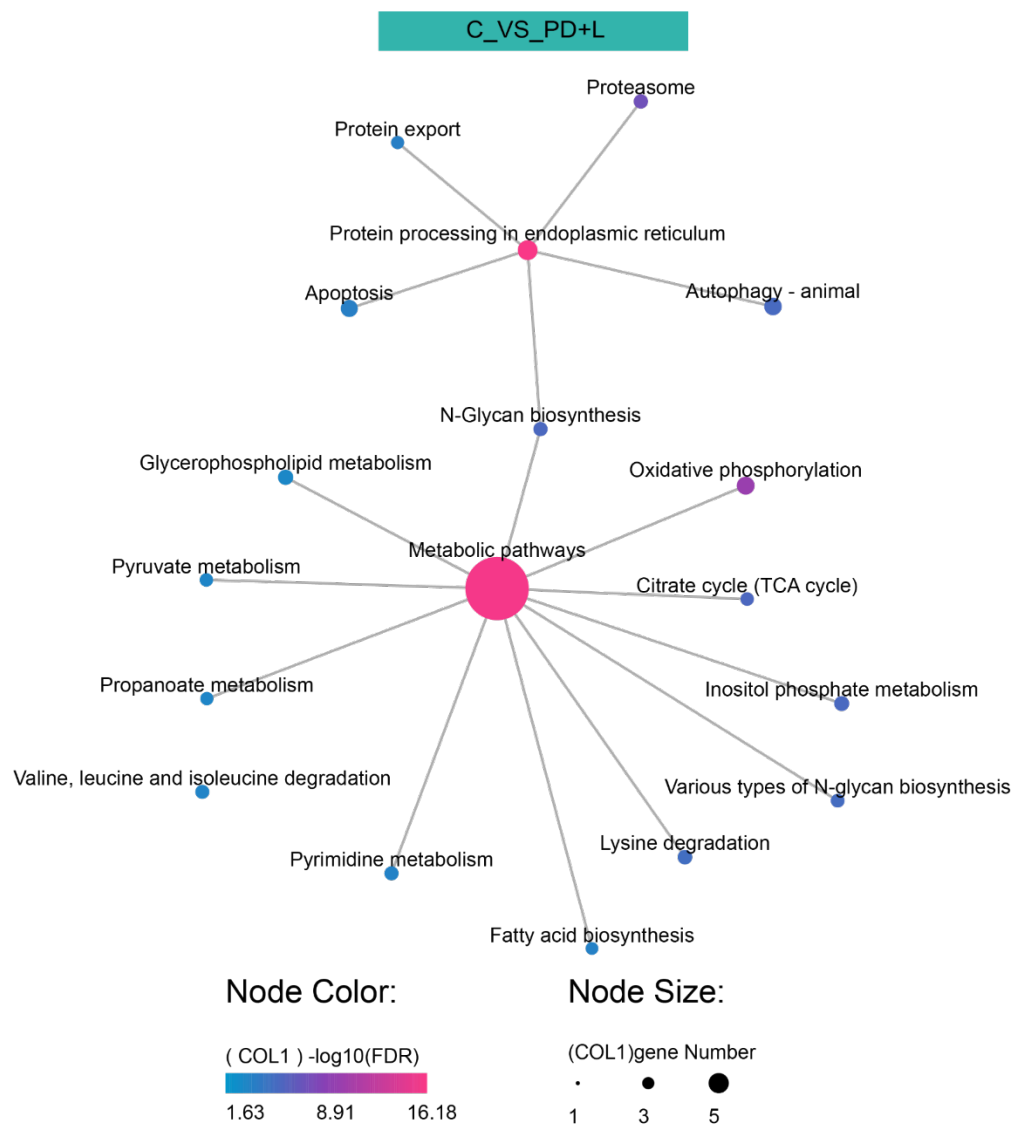


Figure S52. Interaction networks of the enriched KEGG pathways in cells treated with PD+L.

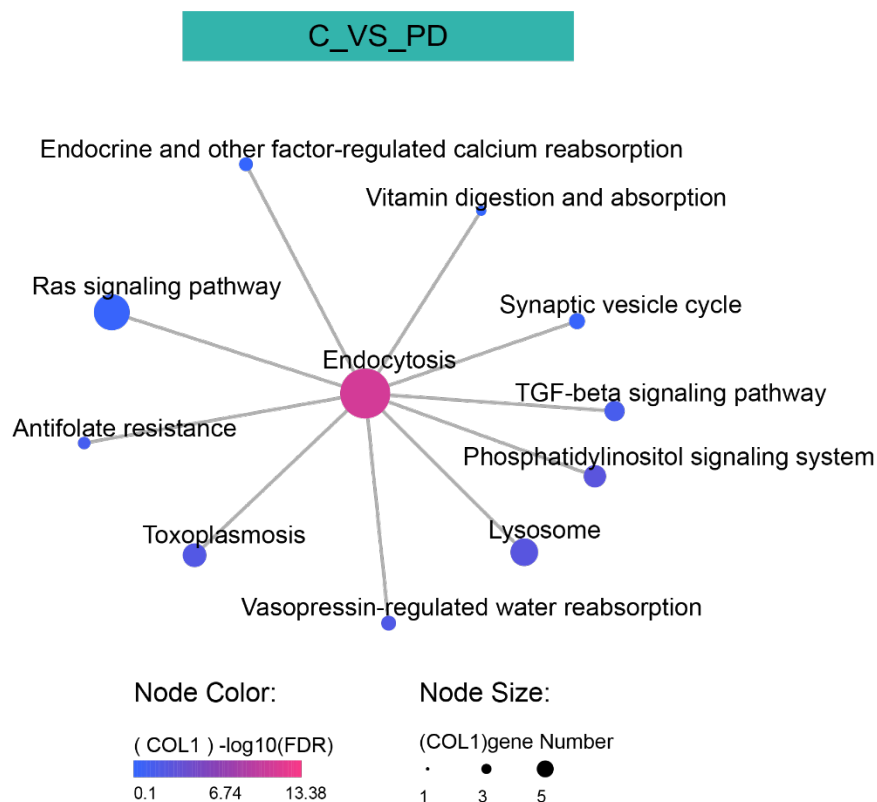


Figure S53. Interaction networks of the enriched KEGG pathways in cells treated with PD.

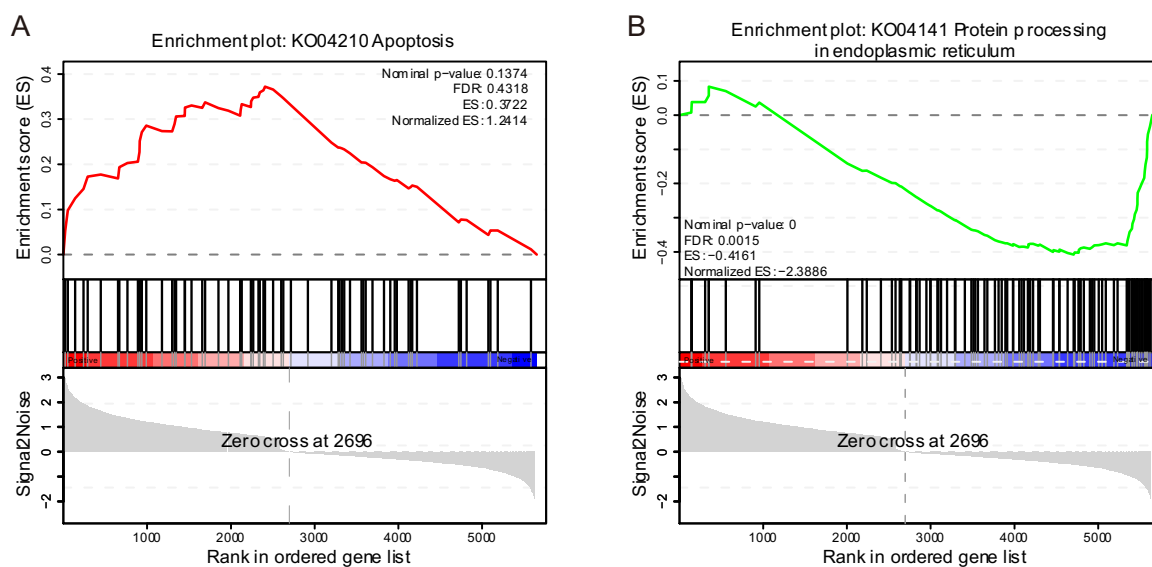


Figure S54. GSEA of the pathways in cells treated by PDPP@D+L based on the KEGG database.

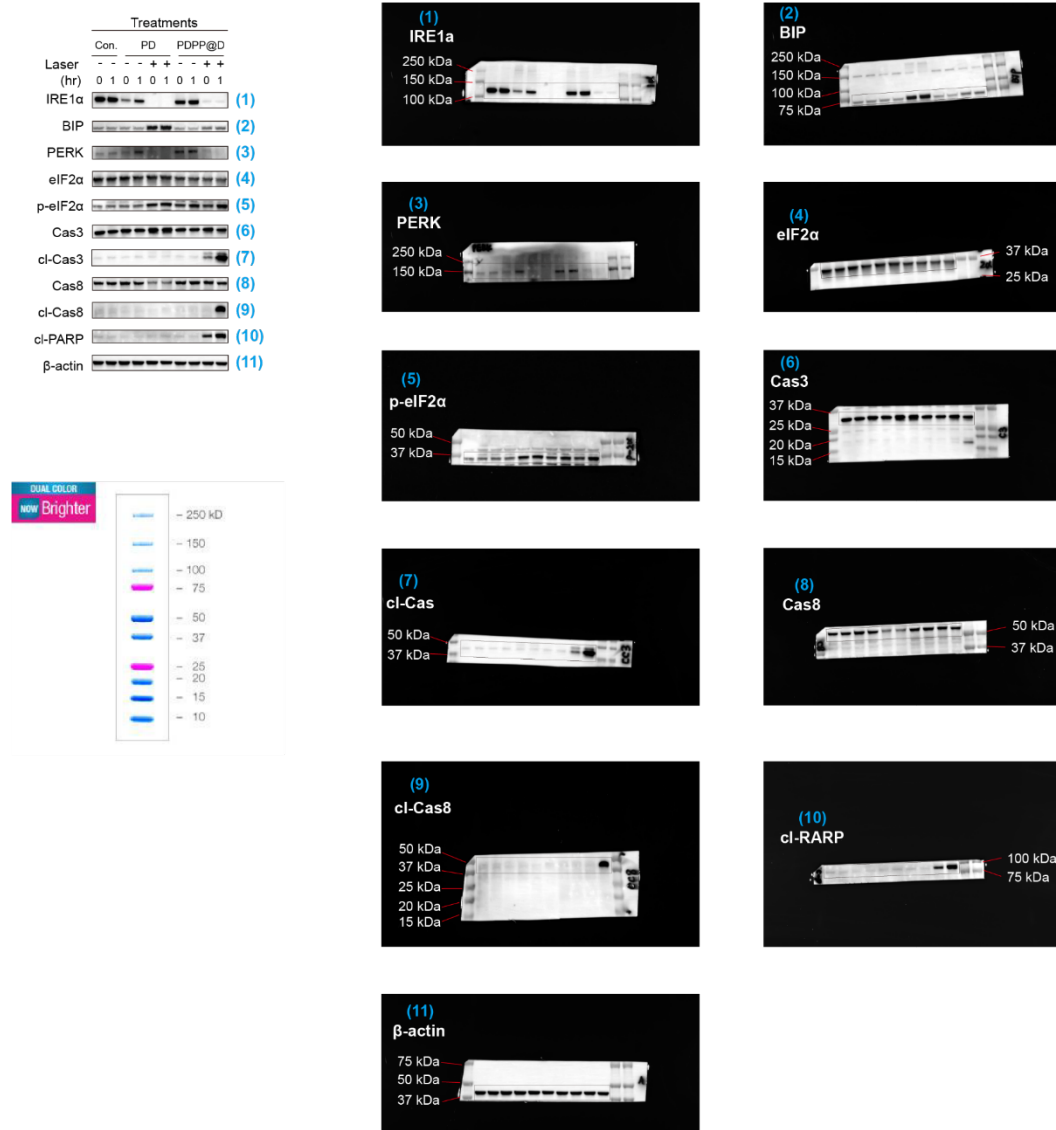


Figure S55. Western Blots for Figure 6H. Lanes indicated by black rectangles were used. Precision Plus Protein Dual Color Standards (#1610374) were used for molecular weight estimation on SDS-PAGE gels and western blots.

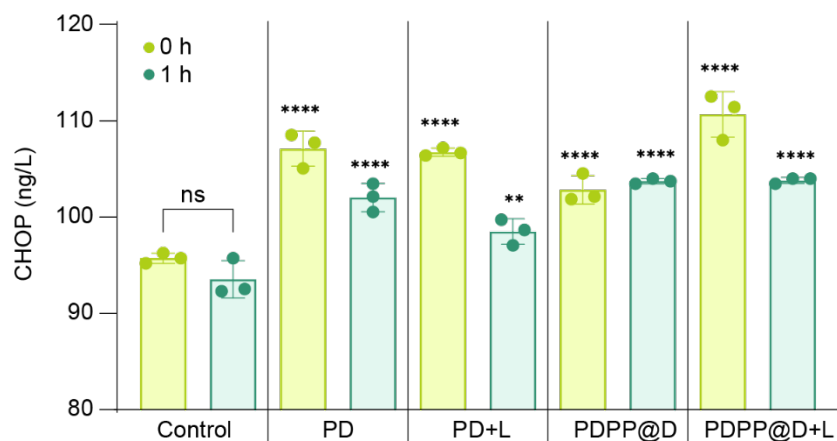


Figure S56. Intracellular CHOP levels after different treatments compared with untreated cells as a control at the same time after laser irradiation, which were measured by ELISA. PD: Ppa+DOX treatment without laser irradiation; PD+L: Ppa+DOX treatment with laser irradiation; PDPP@D: PDPP@D treatment without laser irradiation; PDPP@D+L: PDPP@D treatment with laser irradiation.

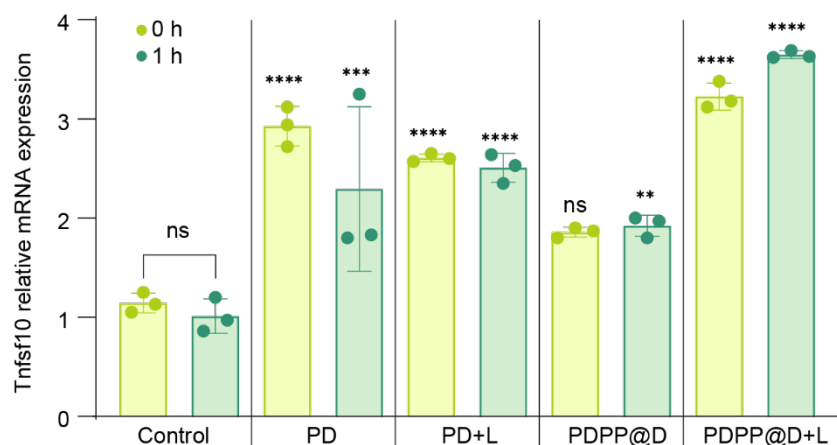


Figure S57. Normalized expression levels of *TNFSF10* gene in 4T1 cells after treatment with different formulations compared with the untreated cells as a control at the same time after laser irradiation, which were measured through RT-PCR analyses. PD: Ppa+DOX treatment without laser irradiation; PD+L: Ppa+DOX treatment with laser irradiation; PDPP@D: PDPP@D treatment without laser irradiation; PDPP@D+L: PDPP@D treatment with laser irradiation.

Table S1. The interaction parameters α_{ij} between different beads in DPD simulations (unit: kT).

α_{ij}	G	K	E	P	D	S	W
G	25	25	26	31	28	25	28
K		25	25	30	27	25	30
E			25	28	25	25	32
P				25	26	30	43
D					25	27	36
S						25	29
W							25

“G” for PEG, “K” for lysine, “E” for glutamic acid, “S” for disulfide bond, “P” for Ppa, and “D” for DOX, and three molecules of water are represented as one bead “W”.

Table S2. Photophysical characterizations of PDPP, PDPP@D and Ppa.

Groups	$\epsilon^a) / 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$	FQ ^{b)}	SOQ ^{c)}
Ppa	3.49	0.30	0.50
PDPP	3.16	0.27	0.47
PDPP@D	3.11	0.26	0.47

^{a)} Molar extinction coefficients (ϵ) in DMSO; ^{b)} FQ (Fluorescent quantum yield (Φ_Δ)) in toluene; and ^{c)} SOQ (singlet oxygen quantum yield (Φ_Δ)) in DMSO.

Table S3. Size, zeta potential and PDI of PDPP and PDPP@D in different solutions.

Groups	Size (d.nm)	Zeta Potential (mV)	PDI
PDPP-H ₂ O	150.00±9.09	-14.87±1.93	0.163±0.012
PDPP-PBS	163.91±18.70	-2.81±4.86	0.155±0.023
PDPP@D-H ₂ O	56.49±6.89	-8.16±1.13	0.258±0.035
PDPP@D-PBS	57.29±2.58	-6.62±3.75	0.255±0.012

Table S4. Pharmacokinetic parameters of PDPP, PDPP@D and Ppa.

Groups	$t_{1/2}^a$ (h)	AUC ^{b)} (mg/L·h)	CL ^{c)} (L/h)	MRT ^{d)} (h)	V _{ss} ^{e)} (L)
PDPP	17.54±5.81	112.96±12.31	0.41±0.11	16.95±6.71	16.95±4.72
PDPP@D	22.90±7.12	126.66±14.74	0.35±0.12	17.09±6.89	11.64±2.78
Ppa	0.52±0.01	2.07±0.02	9.66±0.12	5.51±0.10	53.23±0.37

^{a)} $t_{1/2}$: Half-life of the drug; ^{b)} AUC: Area under the drug concentration; ^{c)} CL: Clearance rate; ^{d)} MRT: Mean residence time; ^{e)} V_{ss}: Steady-state apparent volume of distribution.

Table S5. Pharmacokinetic parameters of PDPP@D and DOX PDPP@D

Groups	$t_{1/2}^a$ (h)	AUC ^{b)} (mg/L·h)	CL ^{c)} (L/h)	MRT ^{d)} (h)	V _{ss} ^{e)} (L)
PDPP@D	25.53±8.79	498.72±36.13	0.06±0.01	25.59±6.56	5.06±1.52
DOX	4.05±0.80	13.46±2.34	1.49±0.26	11.73±2.91	17.44±1.68

^{a)} $t_{1/2}$: Half-life of the drug; ^{b)} AUC: Area under the drug concentration; ^{c)} CL: Clearance rate; ^{d)} MRT: Mean residence time; ^{e)} V_{ss}: Steady-state apparent volume of distribution.

Table S6. Combination indexes (CI) and IC50 values of different formulations.

Groups (Ppa:DOX=2:1)	CI (at IC50)	IC50 of DOX (μg/mL)	IC50 of Ppa (μg/mL)
Ppa+DOX	0.93	0.045	0.09
PDPP+DOX	1.03	0.08	0.16
PDPP@D	0.77	0.06	0.12

Reference

- [1] X. Zheng, D. Pan, X. Chen, L. Wu, M. Chen, W. Wang, H. Zhang, Q. Gong, Z. Gu, K. Luo, *Advanced Science* **2021**, 8, 2102741.
- [2] X. Zheng, D. Pan, M. Chen, X. Dai, H. Cai, H. Zhang, Q. Gong, Z. Gu, K. Luo, *Adv. Mater.* **2019**, 31, 1901586.