Supplementary information

A highly effective self-supplying photosensitizer drug for deep-tissue metastatic tumors treatment

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Experimental Section

Materials

Unless otherwise noted, all commercial reagents and solvents were obtained from the commercial provider and used without further purification. All reagents used in experiments were purchased from Aladdin Industrial Corporation (Beijing, China). DSPE-PEG_{2000} and DSPE-PEG_{2000}-FA were purchased from Ponsure Biological Technology Co., Ltd. (Shanghai, China). DSPE-SS-PEG_{2000} was purchased from Qiyue Biological Technology Co., Ltd. (Xi'an, China). Singlet Oxygen Sensor Green Fluorescent Probe (SOSG) was purchased from Maokang Biological Technology Co., Ltd. (Shanghai, China). A naphthalene-2,3-dicarboxaldehyde (NDA) were purchased from Heowns Biochem Technologies, LLC, (Tianjin, China). Calcein-AM/PI Double staining Kit, Mitochondrial Membrane Potential Detection Kit (JC-1) was purchased from G-Clone Biotechnology Co., LTD (Beijing China). 4T1 cells and 4T1-luc cells were purchased from Procell Life Science&Technology Co., Ltd. (Wuhan, China). The mouse normal liver cell line (AML 12) was purchased from Ubigene Biosciences (Guangzhou, China). The water used was Mill-Q secondary ultrapure water (18.2MΩ/cm).

Instruments

TEM images were taken by a HT7700 electron microscope. Zeta potentials were measured by a Malvern Zeta Sizer Nano (Malvern Instruments). The absorbance of the 96-well plate was recorded with a microplate reader (Synergy 2, Biotek, USA). Flow cytometry was taken by CytoFLEX S. A confocal laser scanning microscope (CLSM, Nikon AIR HD25) was used to take the fluorescence images. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance 300, 400 or 600 MHz spectrometers. Fluorescence spectra were recorded on F-4700 Portable Hitachi F-4700 Fluorescence Spectrophotometer, while the UV-Vis spectra were obtained by a pharmaspec UV-1700 UV-visible spectrophotometer (Shimadzu, Japan). High-resolution mass spectra (HR-MS) were obtained on a Bruker ultrafleXtreme™ mass spectrometer system operated in matrix-assisted laser desorption and ionization–time-of-flight (MALDI-TOF) mode. All chemiluminescent images were performed by the PerkinElmer IVIS Lumina III.

Synthesis of Cy7-EOM

Synthesis of Compound 1

2,3,-trimethyl-3H indole (5.0 g, 31.5 mmol) and 2-iodoethanol (7.5 g, 44 mmol) was dissolved in MeCN (50 mL). The solution was refluxed under nitrogen for 24 hours. The reaction mixture was cooled to room temperature and product precipitated by the addition of hexane. The purple solid was filtered and dried without further purification. Yield: 90%.

Synthesis of Compound 2

To a solution of DMF (20 mL, 273 mmol) in ice-bathed anhydrous DCM (20 mL) under Ar, $POCl_3$ (17.5 mL, 115 mmol) in anhydrous DCM (5 mL) was added dropwise within 0.5 h. Then, cyclohexanone (5.0 g, 50 mmol) was injected slowly into the above solution. The resulting mixture was stirred vigorously at 80 °C for 3 h, and poured into ice-cold water under stirring to obtain a yellowish precipitation. The solid was filtered off, washed with water, and dried under vacuum to give compound 2 (7.9 g, 91.9%) as a yellowish solid with a fine purity.

Synthesis of Cy7

Compound 1 (2.9 mg, 11.0 mmol), Compound 2 (860 mg, 5.0 mmol) and CH_3COOK(1.1 mg, 11.0 mmol) was dissolved in dry ethanol under nitrogen overnight. After that, the reaction mixture was cooled and a large amount of green precipitation was collected by filtration. The crude precipitation was further purified by column chromatography using CH_2Cl_2/MeOH (20:1, v/v) to provide compound Cy7 (2.23 mg, 82%) as a dark green solid. The ^{1}H NMR spectrum is shown in Figure S1. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.36-8.36(d, 2H), 7.40-7.34(m, 4H), 7.25-7.20(m, 4H), 6.51-6.48(d, 2H), 4.37-4.35(t, 4H), 4.07-4.04(t, 4H), 2.83-2.80(tH), 2.09(s, 1H), 1.97-1.94(t, 2H), 1.73(s, 12H). The ^{13}C NMR spectrum is shown in Figure S2. ^{13}C NMR (400 MHz, Chloroform-d) δ 173.31, 144.68, 142.66, 141.07, 128.86, 127.94, 125.19, 122.09, 111.39, 102.26, 77.36, 77.15, 76.94, 58.76, 49.38, 47.26, 46.12, 28.34, 27.08, 20.87, 8.79, 0.07.

Synthesis of Cy7-EOM

Cy7 (2.72 mg, 5.0 mmol) and Ethyl oxalyl monochloride (2.3 mg, 15.0 mmol) were mixed in 20 mL of anhydrous DCM and then triethylamine (1 mL) was added to the solution in an ice bath. The mixture was stirred under an argon (Ar) atmosphere for 6 h at room temperature. The solvent was removed by vacuum rotary evaporation and the residue was purified by column

chromatography to obtain rquired products using CH_2Cl_2/MeOH (20:1, v/v) to provide compound Cy7-EOM. The ^{1}H NMR spectrum is shown in Figure S4. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.26-8.22(d, 2H), 7.62-7.60(d, 2H), 7.45-7.39(, 4H), 7.29-7.25(2H), 6.43-6.40(2H), 4.29(4H), 3.79(4H), 3.09-3.02(4H), 2.70-3.67(4H), 1.85-1.82(2H), 1.68(12H), 1.21-1.17(6H). The ^{13}C NMR spectrum is shown in Figure S5. ^{13}C NMR (101 MHz, Chloroform-d) δ 173.21, 150.48, 144.63, 142.52, 140.95, 128.82, 127.95, 125.17, 122.06, 111.37, 102.14, 77.42, 77.10, 76.78, 58.59, 49.34, 47.20, 46.51, 29.71, 28.31, 27.25, 20.67, 14.06, 8.83.

Preparation of various Nano-micelles (NMs)

NMs were fabricated through a thin-film hydration method.

For Cy7/CPPO NMs, Cy7 (1.0 mg), CPPO (2.5 mg), DSPE-PEG_{2000} (4.0 mg) and DSPE-PEG_{2000}-FA (2.0 mg) were dissolved at 1 mL DCM under ultrasound for 10 min, and then DCM was removed by evaporation. 1 mL of deionized water was added and was vigorously stirred overnight at room temperature to afford an aqueous solution of nano-micelles. The solution was placed in dialysis membrane (3500) and dialyzed in deionized water for 24 h. Almost no Cy7 and CPPO were detected in the dialysate by measuring the absorption, indicating a nearly 100% encapsulation rate. The resulting solution was stored at 4 °C for further use.

The same procedure was applied for Cy7 NMs, Cy7-EOM NMs and Cy7-EOM SS NMs. For Cy7 NMs, Cy7 (1.0 mg), DSPE-PEG_{2000} (4.0 mg) and DSPE-PEG_{2000}-FA (2.0 mg) were used. For Cy7-EOM NMs, Cy7-EOM (1.0 mg), DSPE-PEG_{2000} (4.0 mg) and DSPE-PEG_{2000}-FA (2.0 mg) were used. For Cy7-EOM NMs, Cy7-EOM (1 mg), DSPE-PEG_{2000} (4.0 mg) and DSPE-PEG_{2000}-FA (2.0 mg) were used. For Cy7-EOM SS NMs, Cy7-EOM (1.0 mg), DSPE-SS-PEG_{2000} (4.0 mg) and DSPE-PEG_{2000}-FA (2.0 mg) were used. The remaining steps are the same as those described above. In subsequent experiments, the amount of Cy7 or Cy7-EOM in the nano-micelles in each group is equivalent, and all the concentration values referred to Cy7 or Cy7-EOM.

The chemiluminescent properties of the Cy7-EOM

To assess the response of Cy7-EOM to endogenous H_2O_2 levels, CL imaging of Cy7-EOM (10 μM) was carried out with low-concentration H_2O_2 (0, 6.25, 12.5 25, 37.5 and 50 μM). The image was acquired immediately after the H_2O_2 addition.

To better demonstrate the shielding of nano-micelles against ROS, different concentration of H_2O_2 (final concentration: 0.1, 1.0, 5.0, 10, 25 and 50 mM) were added to Cy7-EOM and Cy7-EOM SS NMs (25 μM), respectively. Time dependent chemiluminescent images were obtained by PerkinElmer IVIS Lumina III.

Detection of $^1O_2$ by ESR

H_2O_2 (10 mM) was added to Cy7-EOM (10 μM) or Cy7-EOM SS NMs (10 μM) solutions containing 2,2,6,6-Tetramethyl-4-piperidone hydrochloride (TEMP, 100mM), respectively. After incubation for 15 min, the produced singlet oxygen was measured by ESR.

Cell culture

4T1 cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium containing 10% fetal bovine serum and 1% penicillin/streptomycin. 4T1-luc cells were treated with high-glucose Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum and 1% penicillin/streptomycin. AML 12 cells were cultured in DMEM medium containing 10% fetal bovine serum, 0.5% Insulin-Transferrin-Selenium (ITS-G, 100X), dexamethasone (40 ng/mL) and 1% penicillin/streptomycin. All the cells were incubated at 37 °C in a humidified atmosphere with 5% CO_2.

Cell viability assays

4T1 cells or AML 12 cells were seeded onto the 96-well microtiter plate and cultured for 24 h in a cell culture incubator, followed by incubation with different concentrations of Cy7-EOM SS NMs (0, 10, 20, 30, 40, 50, 60 μg/mL) for 12 hours. Then, the cell culture medium was removed and replaced by 150 μL of MTT solution (0.5 mg/mL). After 4 hours incubation, the MTT solution was removed, and 150 μL of DMSO was added into each well. Subsequently, the 96-well microtiter plate was shaken slightly in the dark to fully dissolve the formazan, and the absorbance

at a wavelength of 490 nm was measured. Cell viability is estimated based on the following formula: Cell viability (%) = (OD treatment/OD control) × 100%.

Then, the same step was repeated to evaluate the toxicity of different groups (PBS, Cy7 NMs, Cy7/CPPO NMs, Cy7-EOM NMs, Cy7-EOM SS NMs) against 4T1 cells at the same concentration of 50 μg/mL.

In vitro chemiluminescent imaging of 4T1 cells

Different amounts of 4T1 cells were seeded into a 48-well plate and incubated in 200 μL 1640 medium for 6 h. Then, the medium was replaced with 1 mL fresh medium containing Cy7-EOM SS NMs or Cy7-EOM NMs (25 μM). After incubation for 2 h, the old medium was removed and washed twice with PBS buffer (10 mM, pH 7.4), followed by chemiluminescent imaging.

Detection of $^1O_2$ by SOSG

The $^1O_2$ generation was measured using SOSG as an indicator (λex=488 nm, λem=525 nm).

For Cy7-EOM, H_2O_2 (10 mM) was added to a mixed solution of SOSG (1.0 μM) and Cy7-EOM (10 μM). After incubation for 15 min, the fluorescence intensity of SOSG was measured with excitation at 488 nm. A group without H_2O_2 was set as the control.

For Cy7-EOM NMs and Cy7/CPPO NMs, H_2O_2 (10 mM) was added to a mixed solution of SOSG (1 μM) and Cy7-EOM NMs (or Cy7/CPPO NMs) (10 μM). After incubation for 15 min, the fluorescence intensity of SOSG was measured with excitation at 488 nm.

For Cy7-EOM SS NMs, Cy7-EOM SS NMs (10 μM) was pre-treated with GSH (10 μM) for 3 h to destroy the nano-micelles. And the SOSG (1 μM) was added to the above solution followed by adding H_2O_2 (10 mM). After incubation for 15 min, the fluorescence intensity of SOSG was measured with excitation at 488 nm.

Intracellular uptake of Cy7-EOM SS NMs by 4T1 cells

4T1 cells were seeded onto a 6-well plate and incubated in a cell incubator for 24 hours. Then, the culture medium was replaced with 1.5 mL medium containing Cy7-EOM SS NMs (50 μg/mL). After incubation for different times (2, 4, 6, 8 h), the cells were washed three times with PBS and

then stained with hoechst33342 at 37 °C for 10 minutes. Finally, the fluorescence images were acquired using CLSM (λex=638 nm, λem=760-860 nm).

Verify the promoting effect of folate receptor mediated cell uptake

4T1 cells were seeded onto two confocal dishes and incubated in a cell incubator. Then, the culture medium in one group was replaced with 300 μL medium containing folate (100 μg/mL). After incubation for 6 h, Cy7-EOM SS NMs was added to two groups. After further incubating for 6 h, the cells were washed three times with PBS and then stained with Hoechst 33342 at 37 °C for 10 minutes. Finally, the cells were washed three times with PBS and fluorescence images were acquired using CLSM (λex=638 nm, λem=760-860 nm).

Cell uptake of folate modified nano-micelles by 4T1 cells and AML 12 cells

4T1 cells were seeded onto a 6-well plate and incubated in a cell incubator for 24 hours. Then, the culture medium was replaced with 1.5 mL medium containing Cy7-EOM NMs (50 μg/mL). After incubation for 6 h, the cells were washed three times with PBS and then stained with Hoechst 33342 at 37 °C for 10 minutes. Finally, the fluorescence images were acquired using CLSM (λex=638 nm, λem=760-860 nm).

Intracellular GSH consumption

The level of intracellular GSH level was measured by using the fluorescent probe 2,3-Naphthalenedicarbaldehyde (NDA). 4T1 cells were seeded onto a 6-well plate and incubated in for 24 hours. Then, the incubate was replaced with 1.5 mL medium containing PBS, Cy7 NMs, Cy7/CPPO NMs, Cy7-EOM NMs, Cy7-EOM SS NMs (50 μg/mL). Then, the cells were incubated for another 8 h, followed by washed three times with PBS and stained with NDA for 20 minutes. Finally, fluorescence images were acquired by CLSM (NDA: λex = 405 nm, λem = 470-510 nm.).

Intracellular $^1O_2$ generation

The level of intracellular $^1O_2$ was measured by the fluorescent probe SOSG. 4T1 cells were seeded onto a 6-well plate and incubated in a cell incubator for 24 hours. Then, the culture medium was replaced with 1.5 mL medium containing PBS, Cy7 NMs, Cy7/CPPO NMs, Cy7-EOM NMs,

Cy7-EOM SS NMs (50 μg/mL), and the cells were incubated for an additional 8 h. After incubation, the cells were washed three times with PBS and then stained with SOSG (1 μM) at 37 °C for 30 minutes. Finally, cells were washed three times with PBS and fluorescence images were acquired using CLSM (λex=488 nm, λem=500-560 nm).

Detection of mitochondrial transmembrane potential (ΔΨm)

JC-1 fluorescent probe was used to detect the changes of mitochondrial membrane potential. 4T1 cells were seeded onto a confocal dish and incubated for 24 hours. Then, the culture medium was replaced with 1.5 mL medium containing PBS, Cy7 NMs, Cy7/CPPO NMs, Cy7-EOM NMs, Cy7-EOM SS NMs (50 μg/mL), and the cells were incubated for an additional 8 h. After that, the cells were washed with PBS and stained with JC-1 at 37 °C for 20 minutes. Then the cells were washed with buffer solution, and subsequently the fluorescence intensity was observed by CLSM. (JC-1 monomer: λex= 488 nm and λem= 515-555 nm; JC-1 aggregates: λex= 561 nm and λem= 580-620 nm).

Intracellular ROS burst

The level of intracellular ROS was measured by using the fluorescent probe 2',7'-dichlorofluorescein diacetate (DCFH-DA). 4T1 cells were seeded onto a 6-well plate and incubated in a cell incubator for 24 hours. Then, the culture medium was replaced with 1.5 mL medium containing PBS, Cy7 NMs, Cy7/CPPO NMs, Cy7-EOM NMs, Cy7-EOM SS NMs (50 μg/mL), and the cells were incubated for an additional 12 h. Next, the cells were washed three times with PBS and stained with DCFH-DA (10 μM) at 37 °C for 30 minutes. Finally, cells were washed three times with PBS and fluorescence images were acquired using CLSM (λex=488 nm, λem=510-550 nm).

Live-dead cell staining experiments

4T1 cells were seeded onto a 6-well plate and incubated in a cell incubator for 24 hours. Then, the culture medium was replaced with 1.5 mL medium containing PBS, Cy7 NMs, Cy7/CPPO NMs, Cy7-EOM NMs, Cy7-EOM SS NMs (50 μg/mL). After incubation for 12 h, the cells were washed three times with PBS and then stained with Calcein-AM and PI for 20 mins. Finally, cells were

washed three times with PBS and the fluorescence images were acquired by CLSM (Calcein-AM: λex=488 nm, λem=500-550 nm and PI: λex=535 nm, λem=590-660 nm).

Apoptosis assay

4T1 cells were seeded onto a 6-well plate and incubated in a cell incubator for 24 hours. Then, the culture medium was replaced with 1.5 mL medium containing PBS, Cy7 NMs, Cy7/CPPO NMs, Cy7-EOM NMs, Cy7-EOM SS NMs (50 μg/mL). After incubating for 12 h, cells were collected and stained with Annexin V-FITC/PI. Finally, the cells were analyzed by flow cytometry.

Animal Models

All animal experiments were performed in compliance with the relevant guidelines and regulations of the People's Republic of China for the care and use of experimental animals (Regulations on the Administration of Laboratory Animals of the People's Republic of China). All animal experiments were approved by Ethics Committee of Medical College of Qingdao University (QDU-AEC-2025550). For all animal experiments, Female BALB/c mice (4-5 weeks old) were housed under normal conditions with 12 hours light and dark cycles and given access to food and water ad libitum. To establish the tumor lung metastasis mouse model, 4T1-luc cells (5×10^5 cells per mouse) were injected intravenously into female BALB/c mice. After 4 days, the growth of tumors in mice was monitored by *in vivo* imaging system. After the successful establishment of the mouse model was confirmed by using the *in vivo* imaging system, follow-up experiments were performed.

Efficacy of Cy7-EOM SS NMs in Lung Metastatic Tumor Model

4T1-luc tumor-bearing mice were randomly divided into five groups (n = 5 per group): (1) PBS alone, (2) Cy7 NMs, (3) Cy7/CPPO NMs, (4) Cy7-EOM NMs, (5) Cy7-EOM SS NMs. The mice were injected intravenously with various drugs with the dosage of 2.5 mg/kg per mouse at 1, 6 and 11 day post injection. Tumors in mice were monitored using an *in vivo* imaging system (Perkin Elmer) at 0, 5, 10, and 15 days. As for body weight and survival rate monitoring, five groups of mice (n=10) with same treatments above were prepared. The survival rate was recorded every other days and the body weight was recorded every three days. After 16 days treatments, all mice

were euthanized and the lungs were isolated. The lungs were treated with 4% paraformaldehyde for bouin fixative staining or H&E immunohistochemical analysis.

In Vivo Chemiluminescence Imaging of Tumor

4T1-luc tumor-bearing mice were injected intravenously with Cy7-EOM SS NMs (3 mg/mL, 1ml), and luminescence imaging of the mouse tumors was monitored using an *in vivo* imaging system (Perkin Elmer).

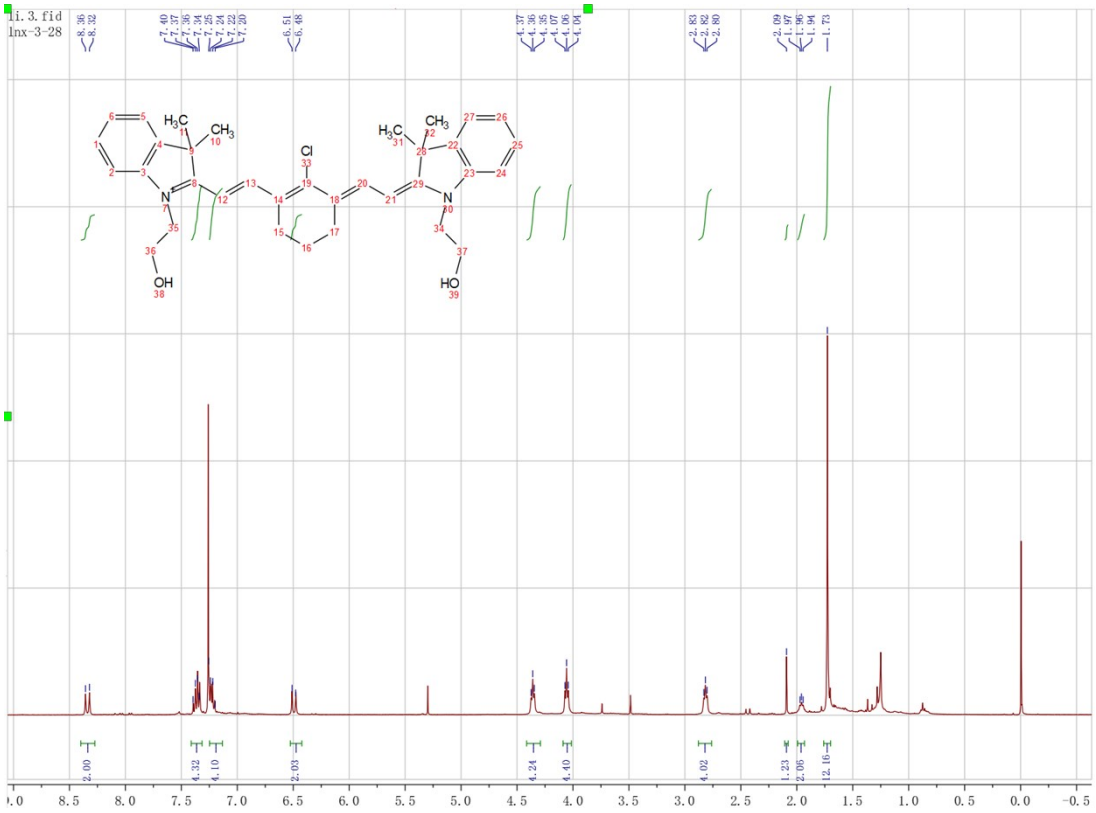

Figure S1. ^{1}H-NMR spectrum of Cy7 in $CDCl_3$.

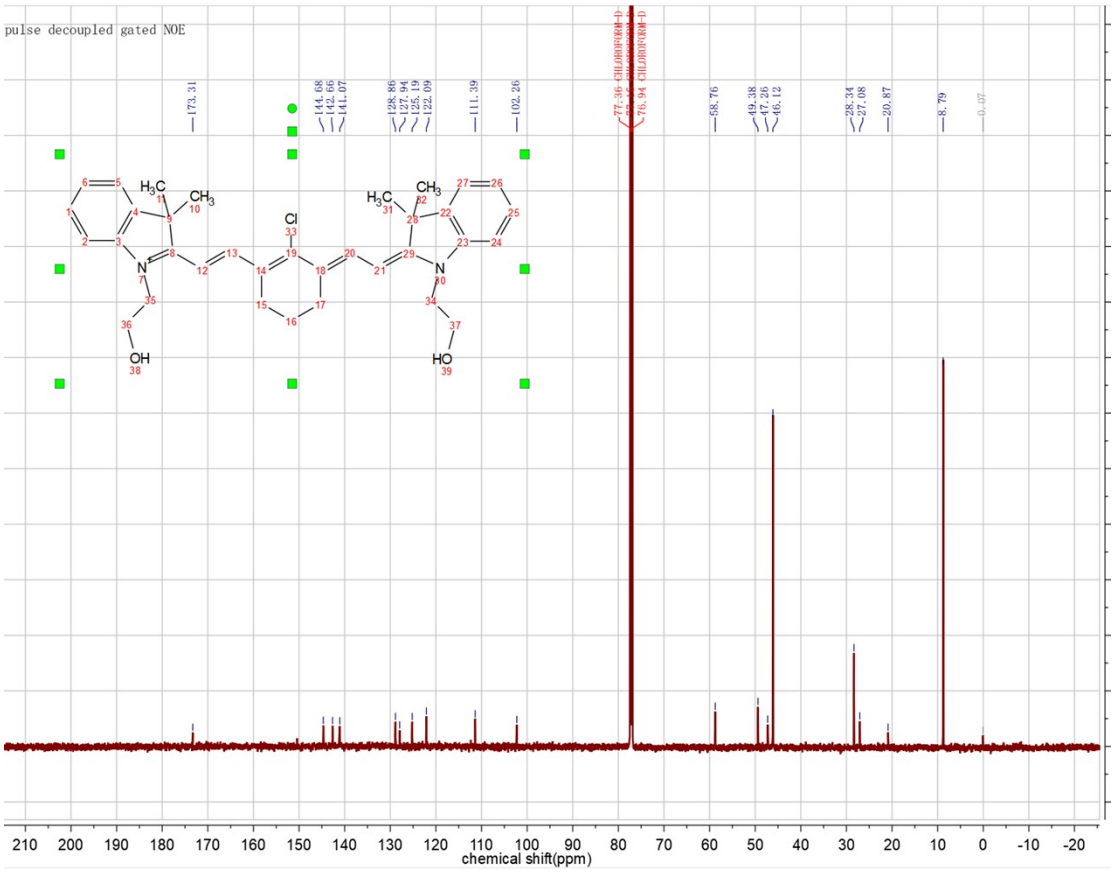

Figure S2. ^{13}C-NMR spectrum of Cy7 in $CDCl_3$.

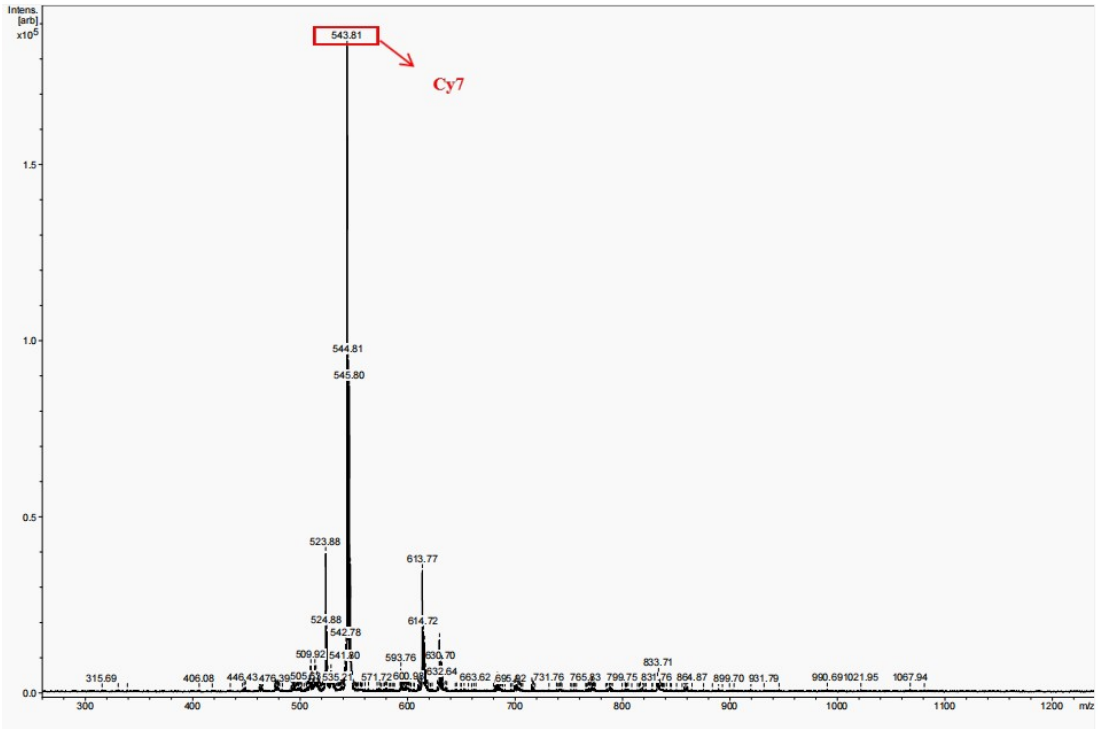

Figure S3. HR-MS of Cy7.

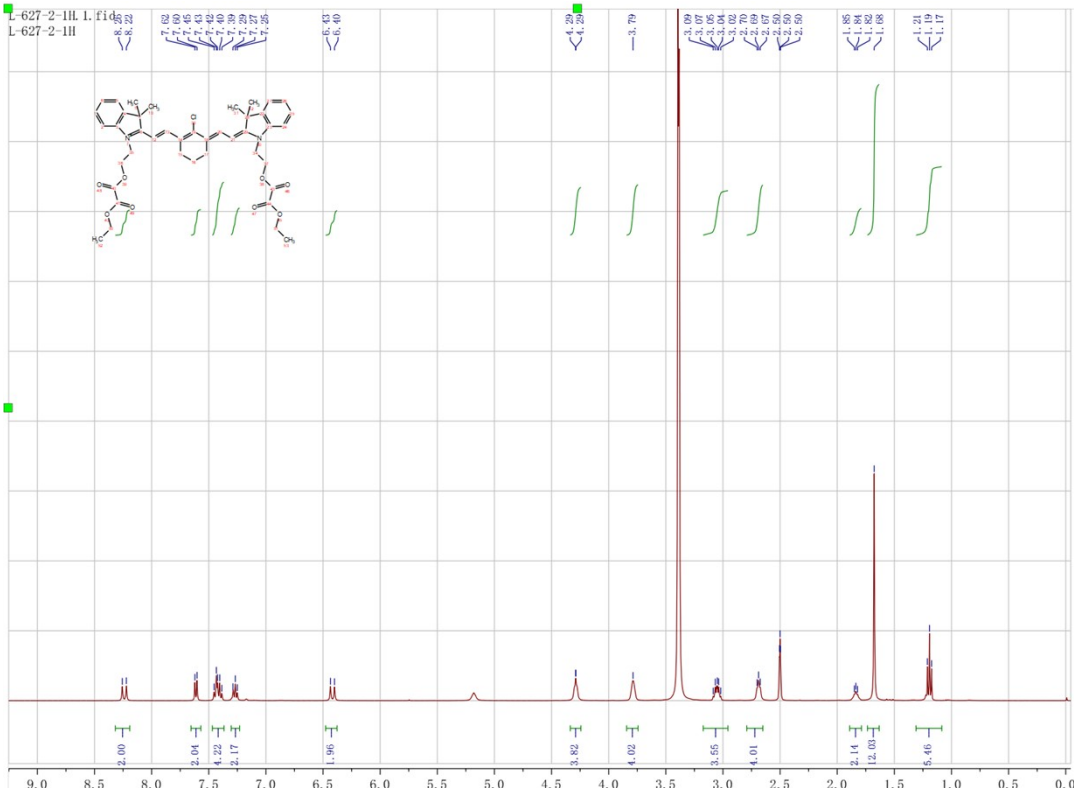

Figure S4. ^{1}H-NMR spectrum of Cy7-EOM in d_6-DMSO.

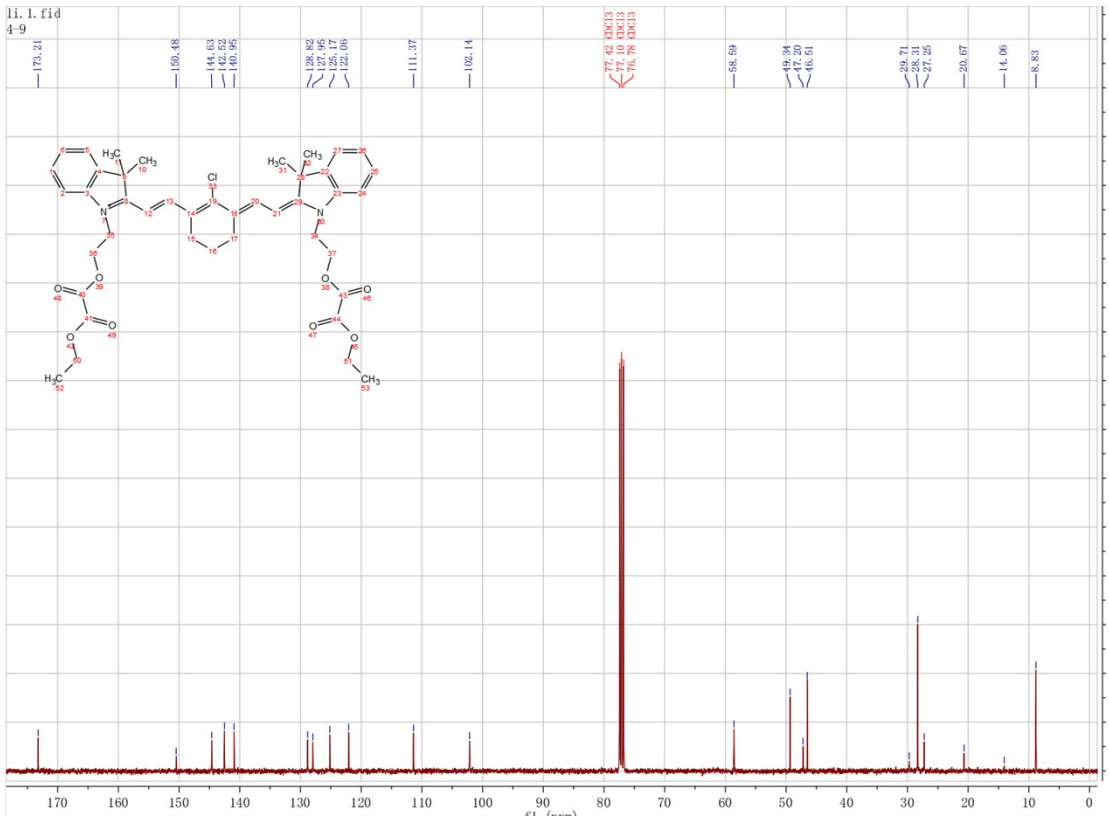

Figure S5. ^{13}C-NMR spectrum of Cy7-EOM in $CDCl_3$.

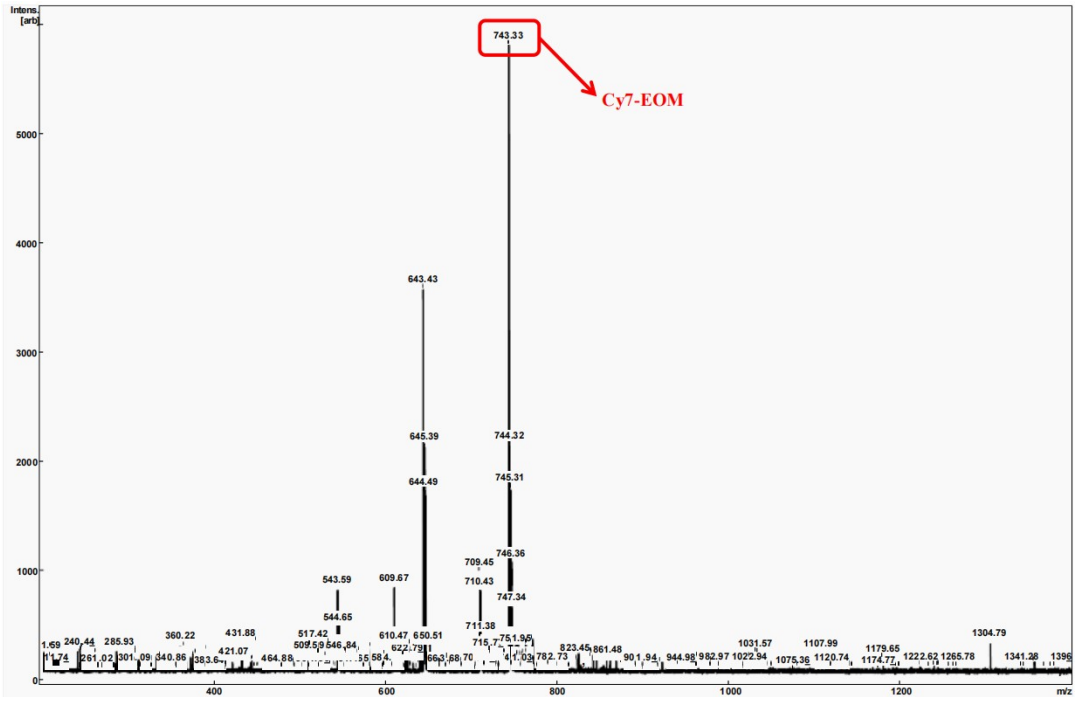

Figure S6. HR-MS of Cy7-EOM.

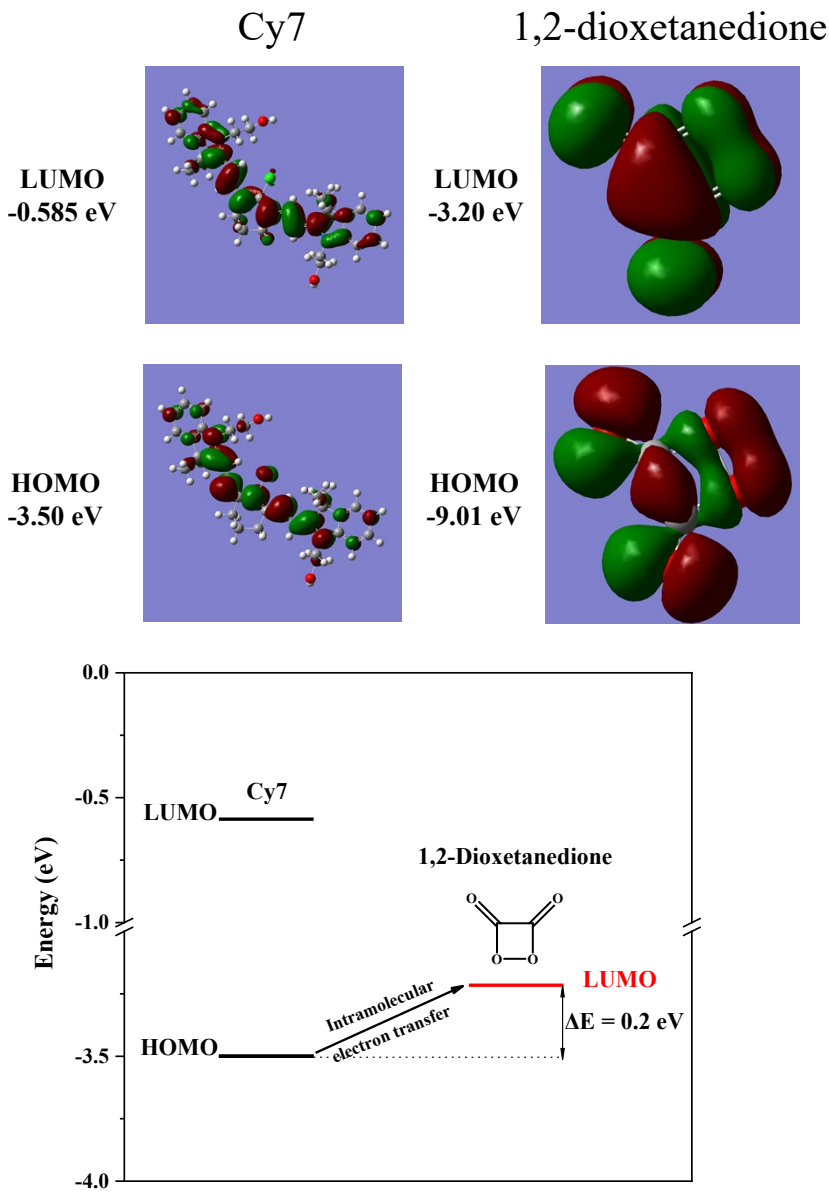

Figure S7. HOMO/LUMO energy levels of Cy7 and 1,2-dioxetanedione.

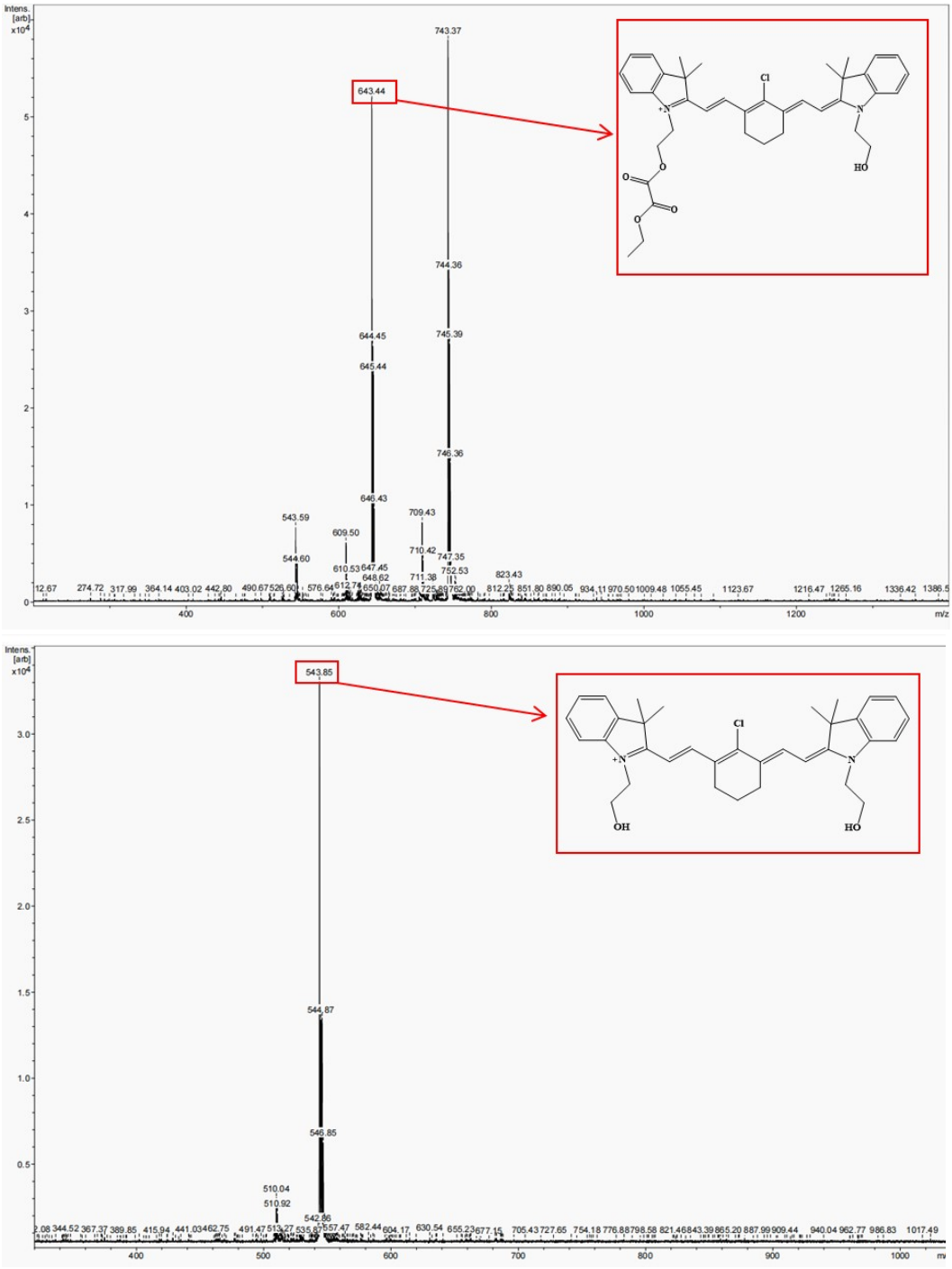

Figure S8. HR-MS of Cy7-EOM after reacted with insufficient H_2O_2 (top) and sufficient H_2O_2 (bottom).

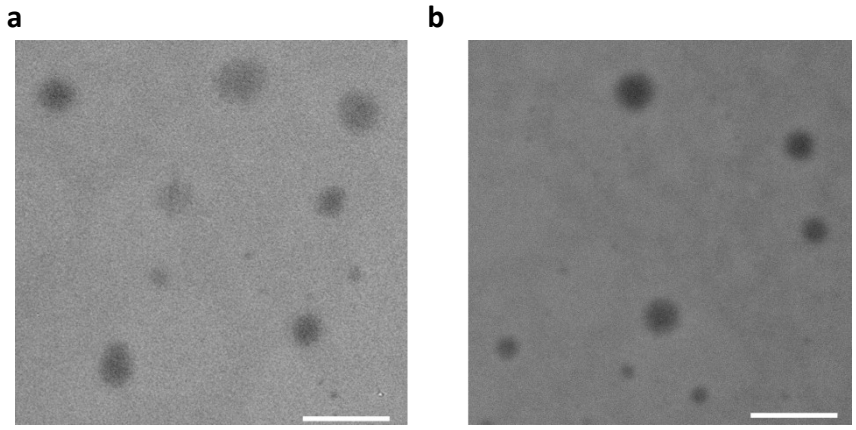

Figure S9. TEM images of Cy7-EOM NMs before (a) and after (b) treated with GSH (10 mM) for 3h. Scale bars are 400 nm.

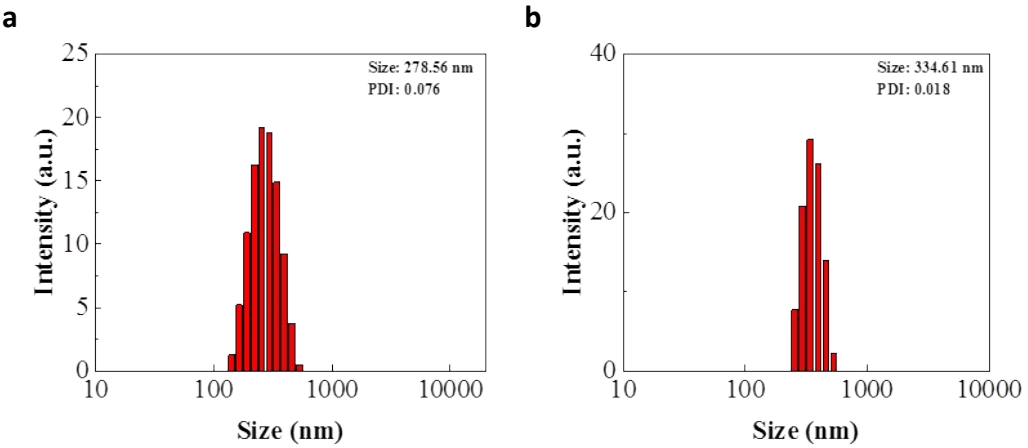

Figure S10. Size distribution of Cy7-EOM SS NMs (a) and Cy7-EOM NMs (b) by DLS measurement.

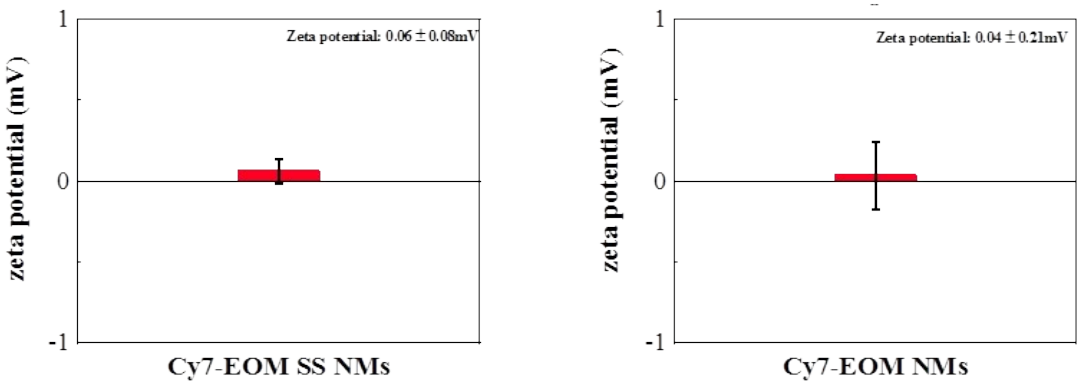

Figure S11. Zeta potentials of Cy7-EOM SS NMs and Cy7-EOM NMs.

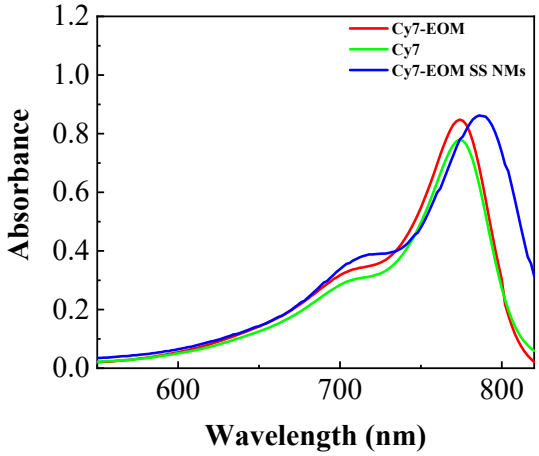

Figure S12. UV-Vis absorption spectra of Cy7, Cy7-EOM and Cy7-EOM SS NMs.

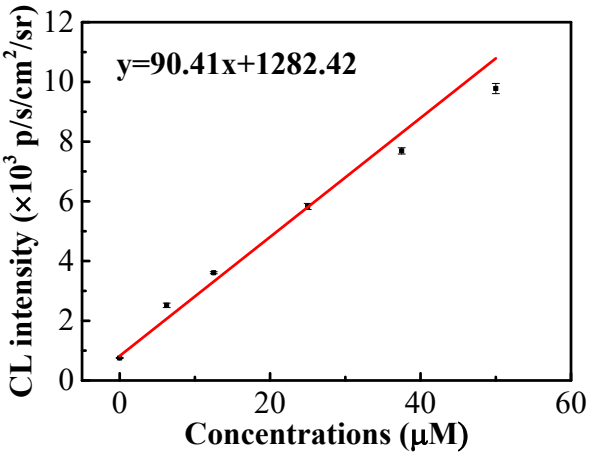

Figure S13. The linear relationship between the CL intensity of Cy7-EOM and various H_2O_2 levels.

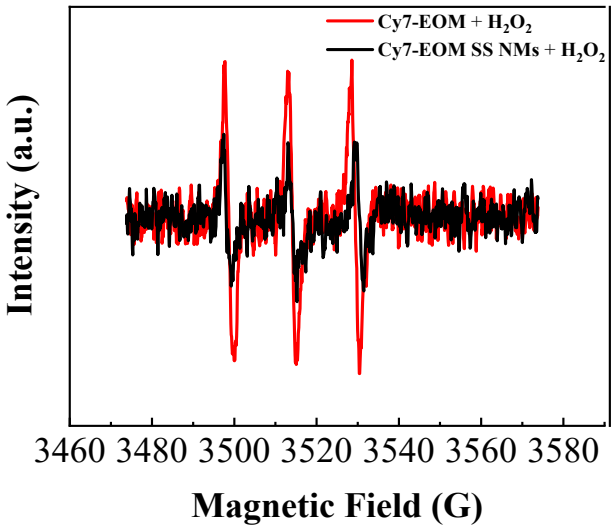

Figure S14. ESR spectra of Cy7-EOM and Cy7-EOM SS NMs with the incubation of H_2O_2 for the detection of $^1O_2$ generation by using TEMP.

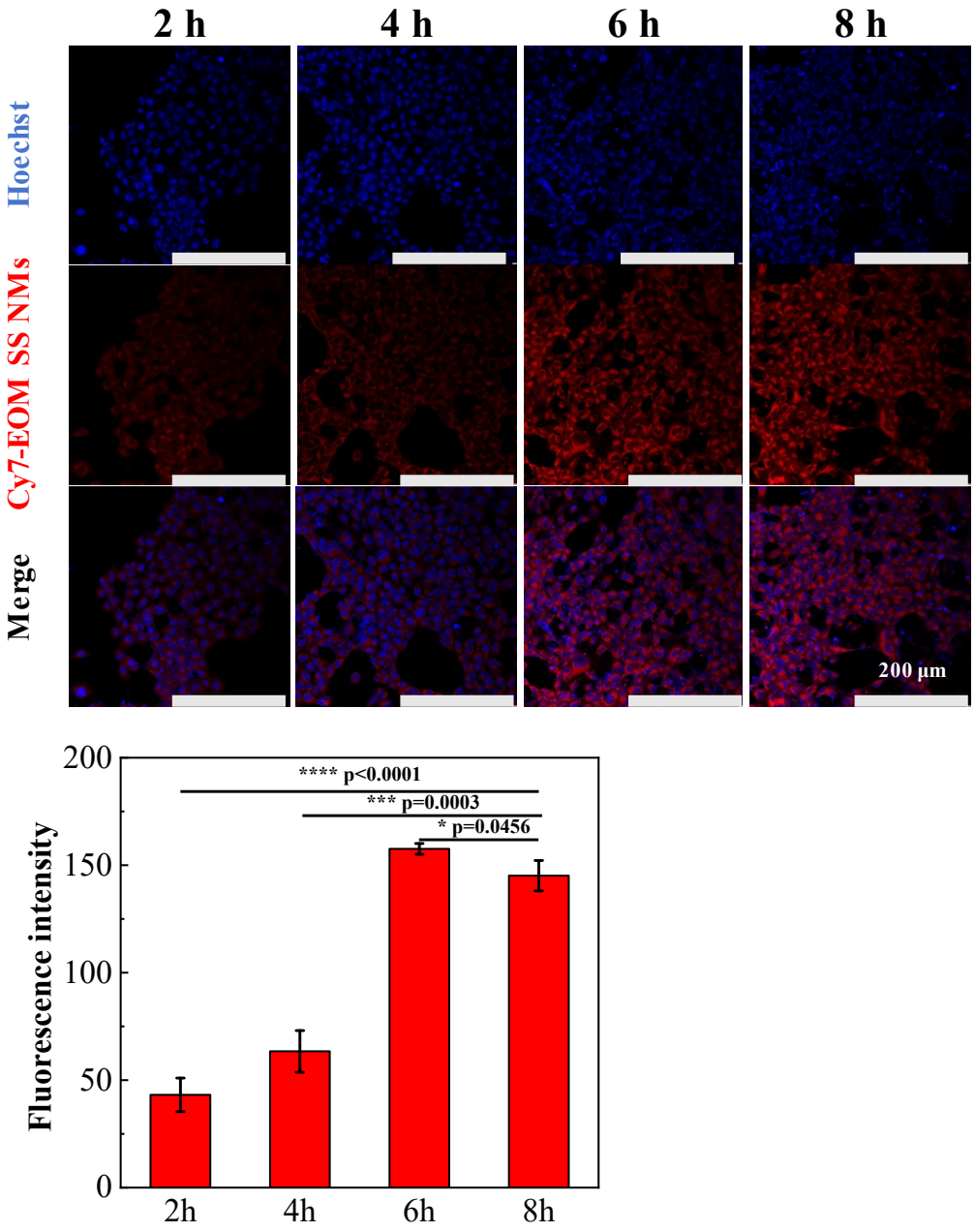

Figure S15. CLSM images of 4T1 cells were incubated with Cy7-EOM SS NMs (50 μg/mL) for 2, 4, 6 and 8h, respectively, and the corresponding statistical analysis of fluorescence intensity. The cell nucleus was stained with Hoechst. Scale bars are 200 μm. Data are reported as the means ± S.D. and analyzed by two-tailed Student's t-test.

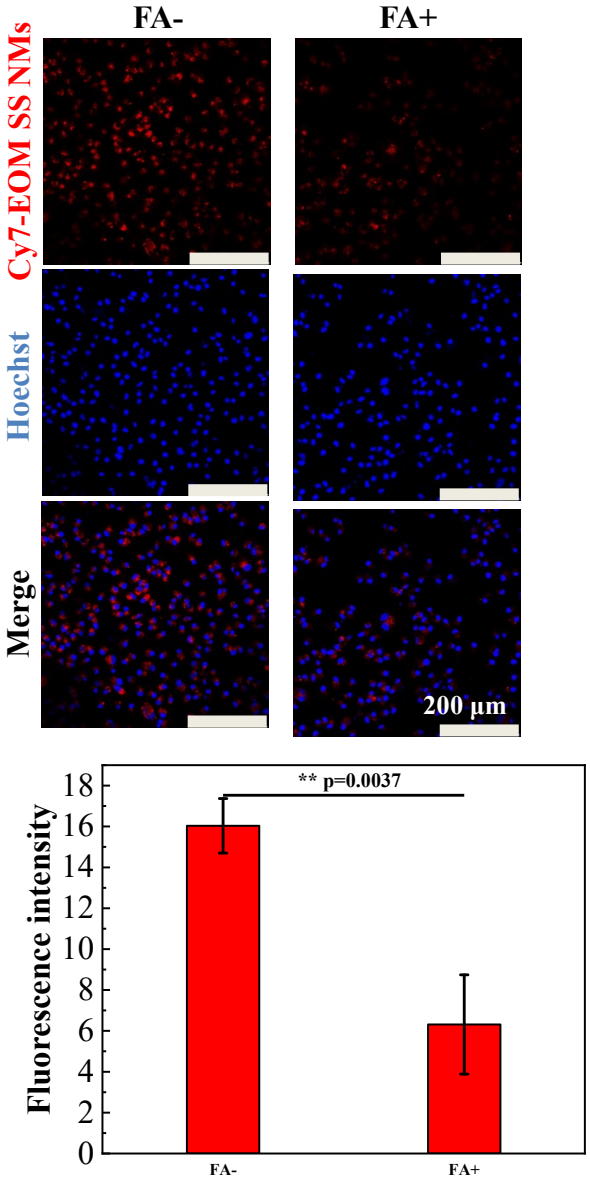

Figure S16. CLSM images of FA pre-treated 4T1 cells or 4T1 cells without pre-treatment after incubation with Cy7-EOM SS NMs for 6h, and the corresponding statistical analysis of fluorescence intensity. Scale bars are 200 μm. Data are reported as the means ± S.D. and analyzed by two-tailed Student's t-test.

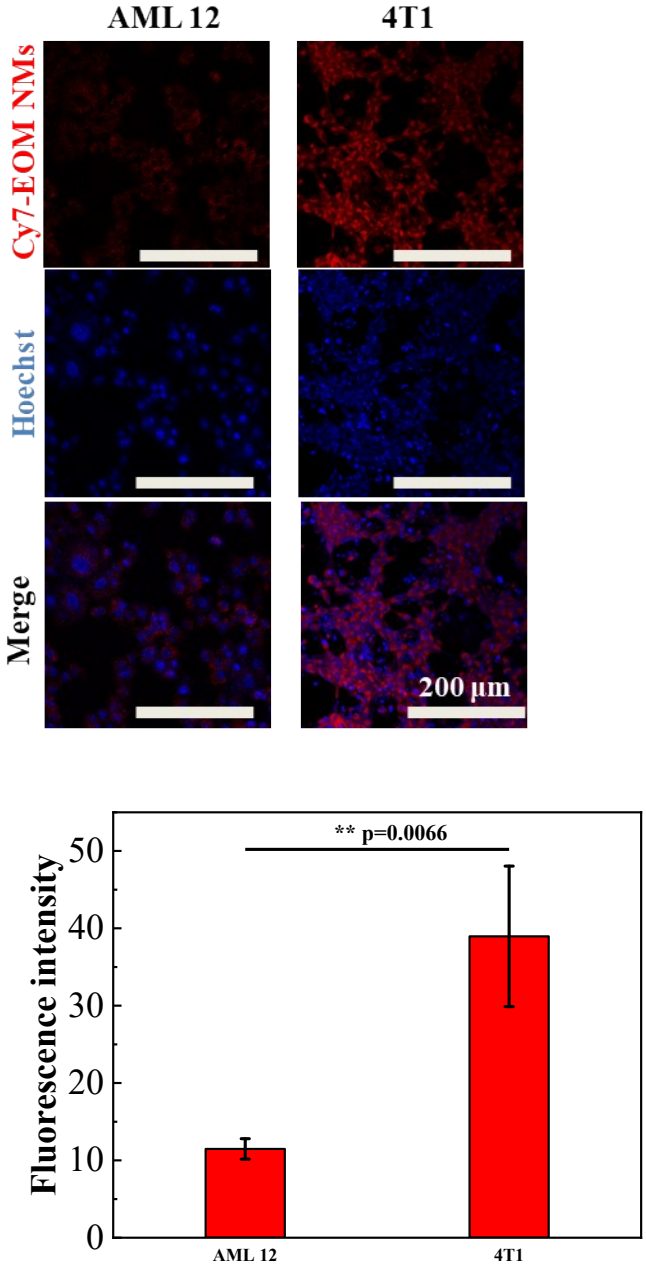

Figure S17. CLSM images of AML 12 cells and 4T1 cells incubated with Cy7-EOM NMs (50 μg / mL) for 6h and the corresponding statistical analysis of fluorescence intensity. Scale bars are 200 μm. Data are reported as the means ± S.D. and analyzed by two-tailed Student's t-test.

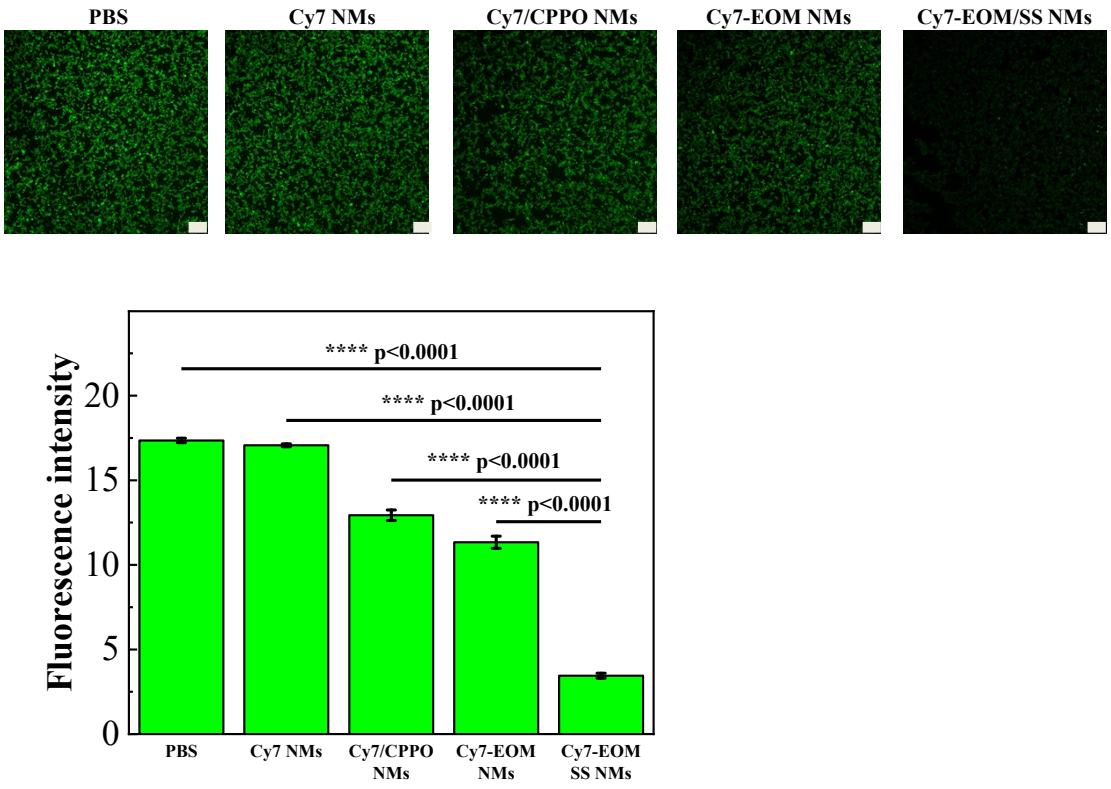

Figure S18. CLSM images of 4T1 cells stained with NDA for the detection of GSH consumption and the corresponding statistical analysis of fluorescence intensity after different treatments. Scale bars are 200 μm. Data are reported as the means ± S.D. and analyzed by two-tailed Student's t-test.

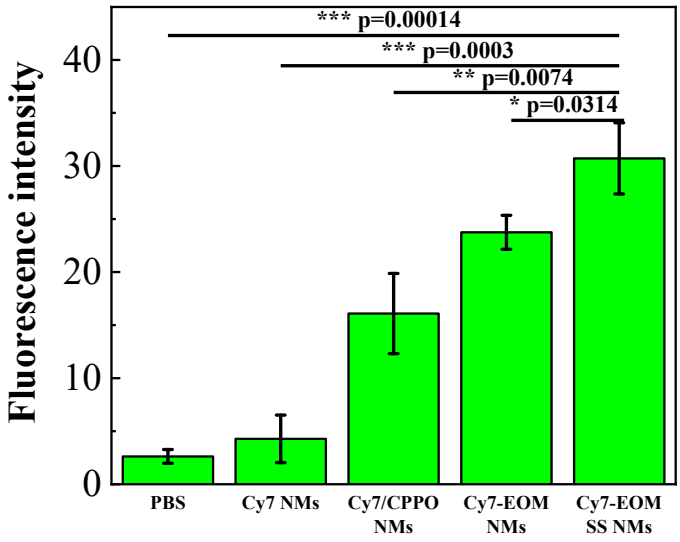

Figure S19. Statistical analysis of the fluorescence intensity in CLSM images of 4T1 cells stained with SOSG after different treatments. Data are reported as the means ± S.D. and analyzed by two-tailed Student's t-test.

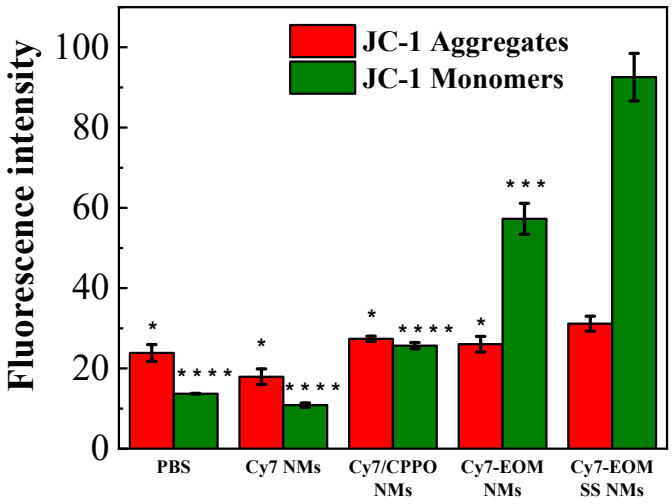

Figure S20. Statistical analysis of the fluorescence intensity in CLSM images of 4T1 cells stained with JC-1 after different treatments. $^{*}p<0.05$, $^{***}p<0.001$, $^{****}p<0.0001$. Data are reported as the means ± S.D. and analyzed by two-tailed Student's t-test.

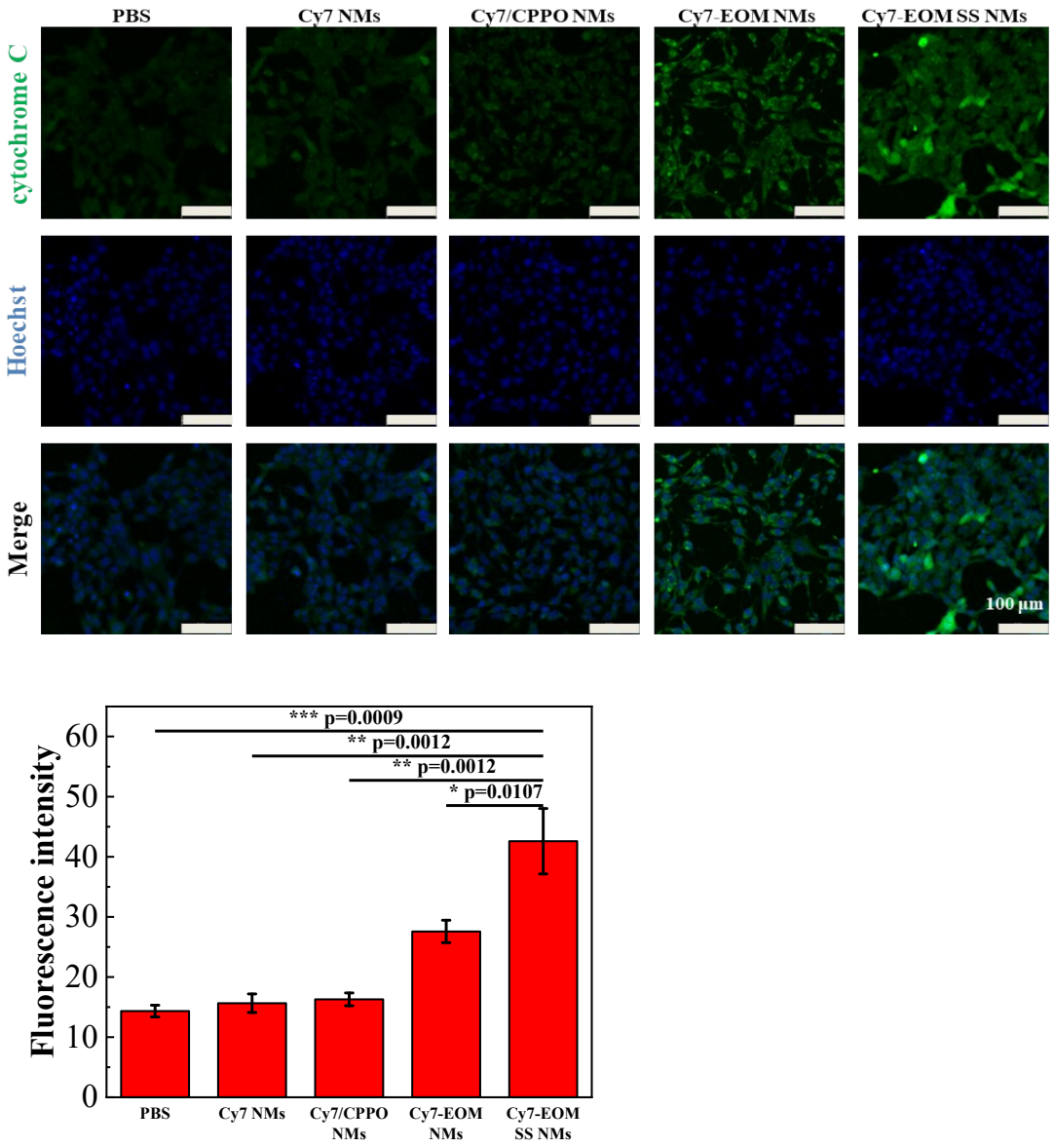

Figure S21. Immunofluorescent staining images and the corresponding fluorescence intensity analysis of cytochrome C in 4T1 cells after different treatments. Data are reported as the means ± S.D. and analyzed by two-tailed Student's t-test.

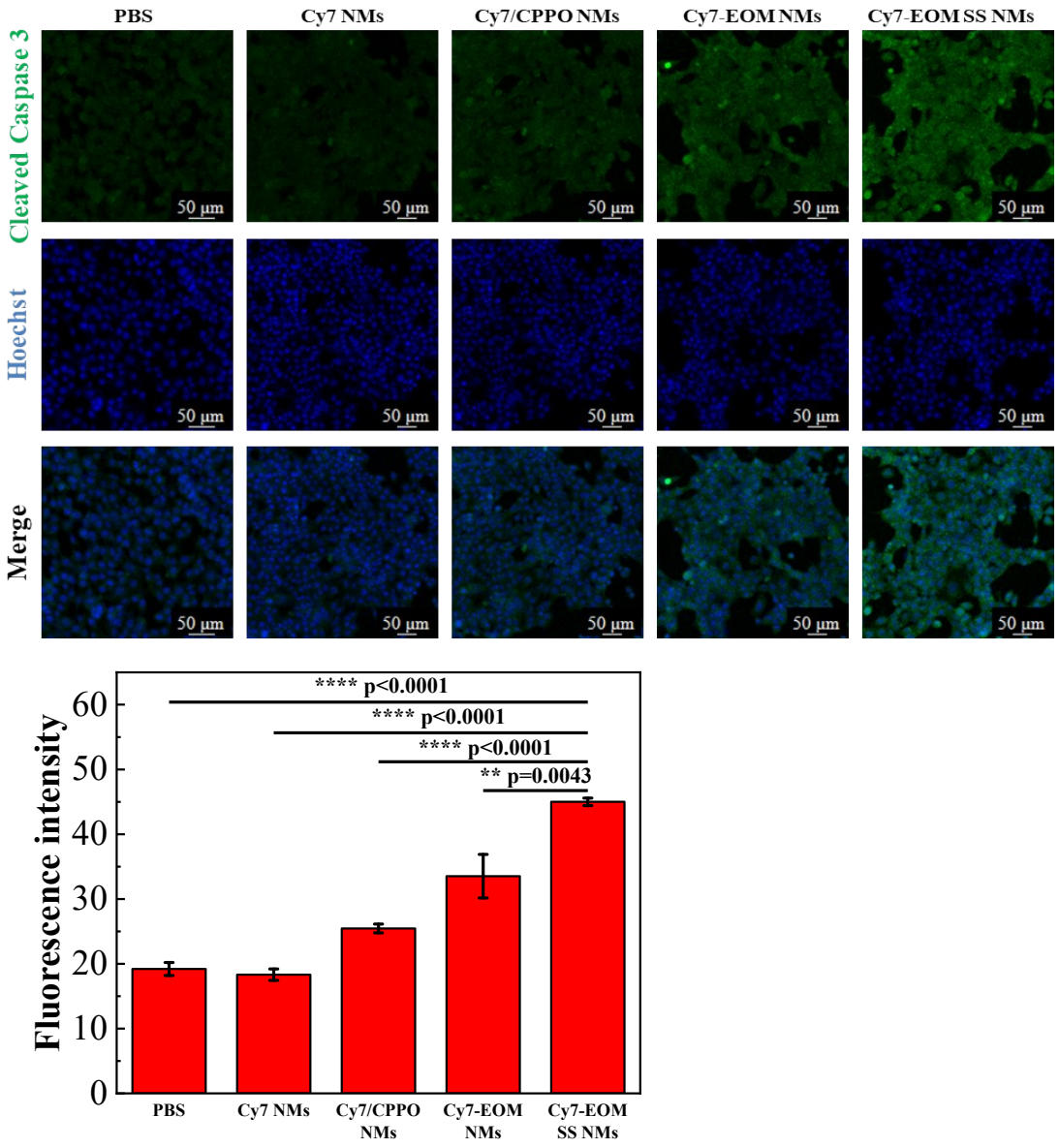

Figure S22. Immunofluorescent staining images and the corresponding fluorescence intensity analysis of cleaved caspase-3 in 4T1 cells after different treatments. Data are reported as the means ± S.D. and analyzed by two-tailed Student's t-test.

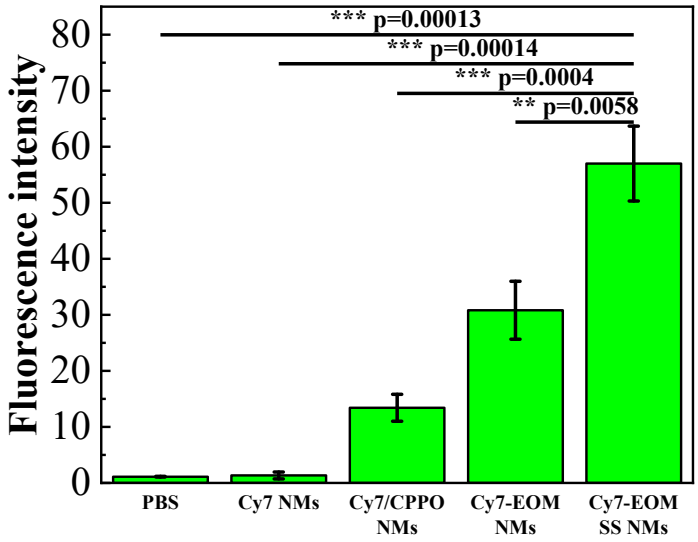

Figure S23. Statistical analysis of the fluorescence intensity in 4T1 cells stained with DCFH-DA after different treatments. Data are reported as the means ± S.D. and analyzed by two-tailed Student's t-test.

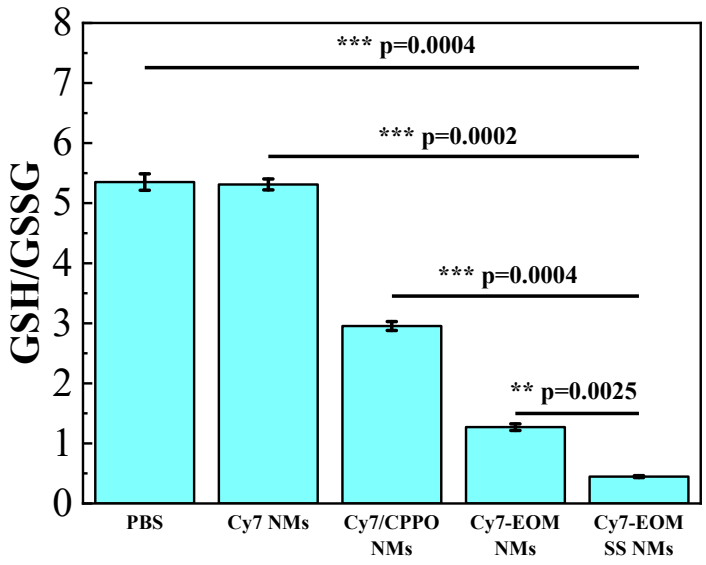

Figure S24. Intracellular GSH/GSSG ratio of 4T1 cells with different treatments. Data are reported as the means ± S.D. and analyzed by two-tailed Student's t-test.

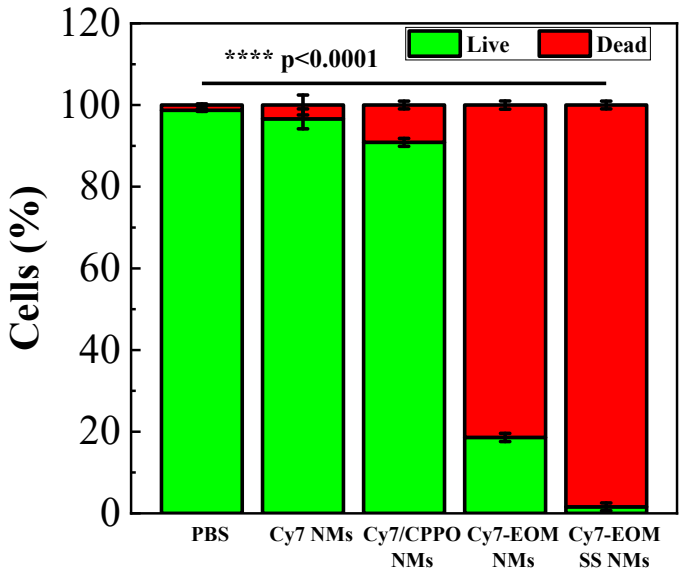

Figure S25. Statistical analysis of percentage of live/dead cells in Figure 4a. Data are reported as the means ± S.D. and analyzed by two-tailed Student's t-test.

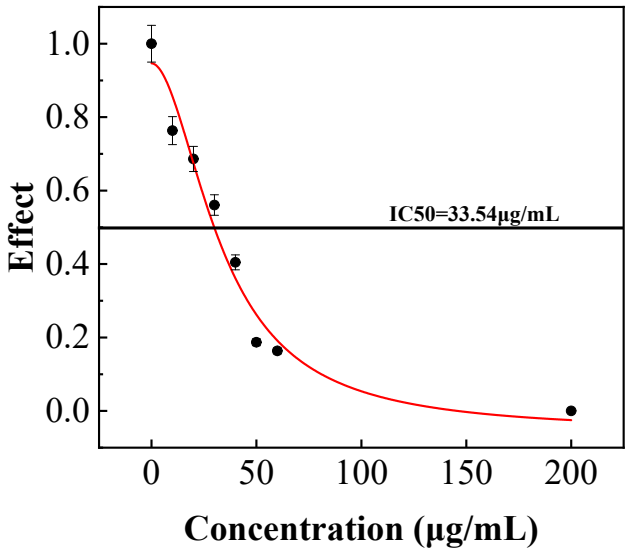

Figure S26. Quantitative analysis of half maximal inhibitory concentration (IC50) of the designed photosensitive drug on cancer cells.

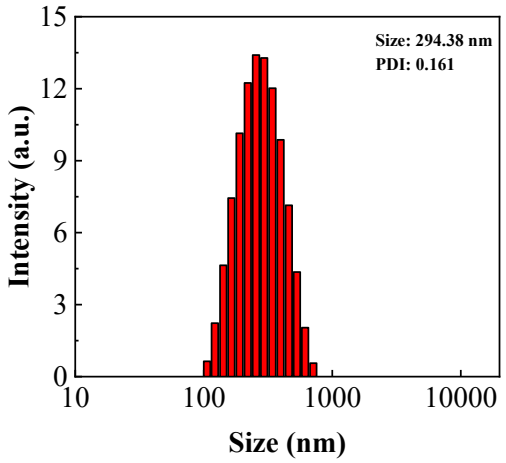

Figure 27. DLS analysis of the hydrodynamic diameter of Cy7-EOM SS NMs after incubation with GSH (10 μM) for 12 h.

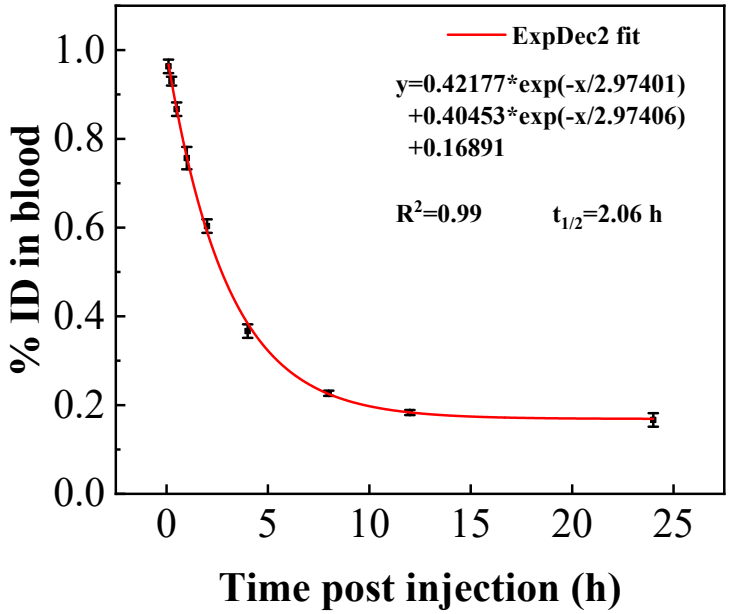

Figure S28. Pharmacokinetic profile of Cy7-EOM SS NMs. The data were determined by fluorescence analysis and presented as percent of injected dose (%ID). Data are presented as mean values ± s.d.

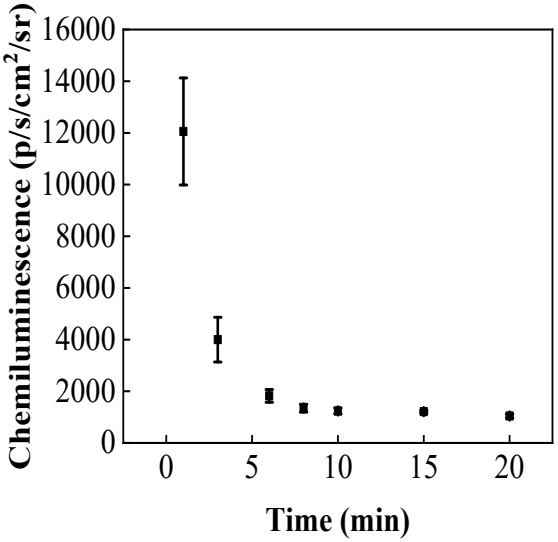

Figure S29. Quantitative analysis of the chemiluminescence intensity signals in mice. Data are presented as mean values ± s.d.

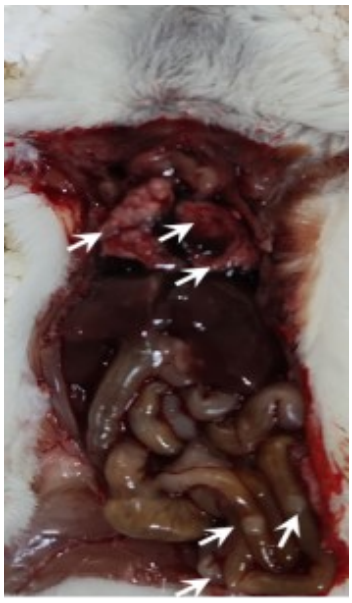

Figure S30. Photograph of the mouse with metastatic tumors in the lung and secondary metastasis sites in intestine (white arrows).

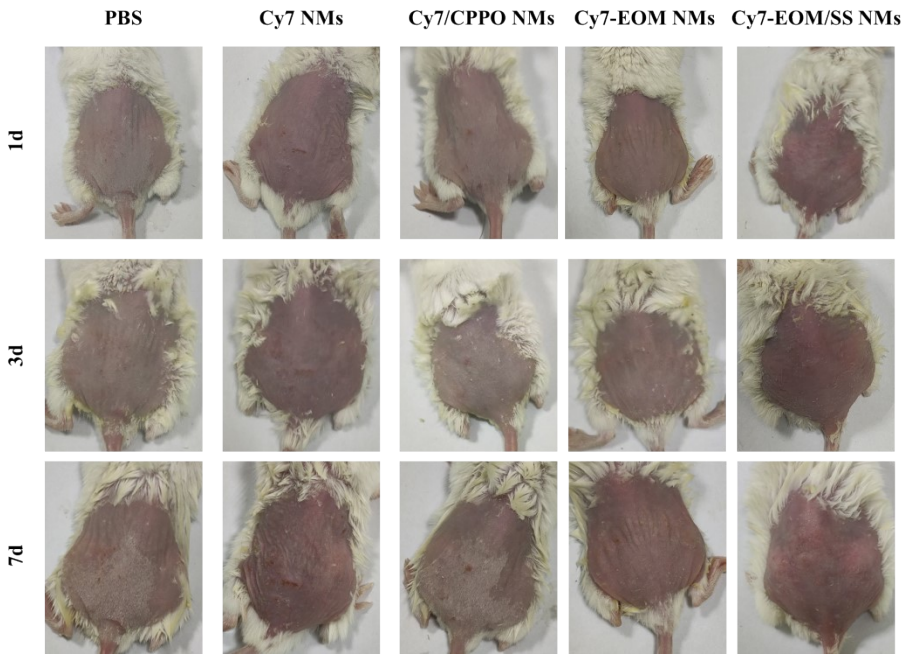

Figure S31. Photographs of dorsal skin of mice with different treatments.

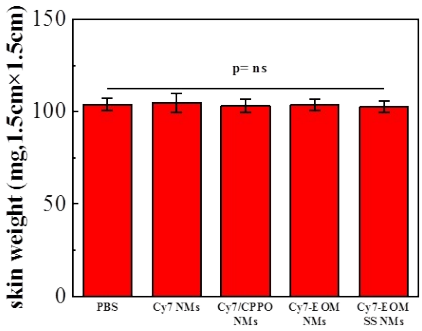

Figure S32. Weights of skins with a fixed size at 1.5 cm * 1.5 cm collected from the sacrificed mice at 7 days post injection.

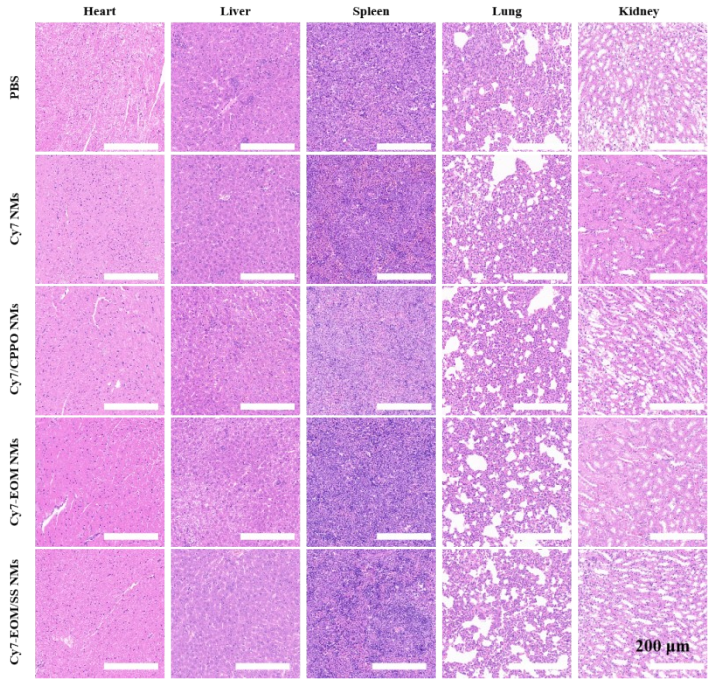

Figure S33. H&E staining images of five major organs (heart, liver, spleen, lung, and kidney).

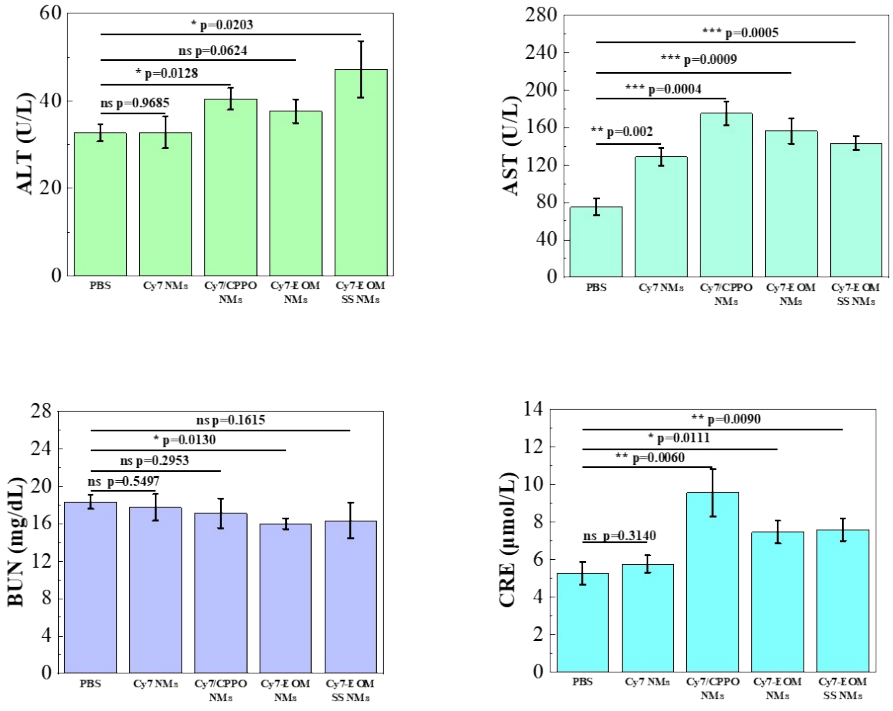

Figure S34. The levels of ALT, AST, BUN and CRE in serum of mice after different treatments. Data are presented as mean values ± s.d. and analyzed by two-tailed Student's t-test.

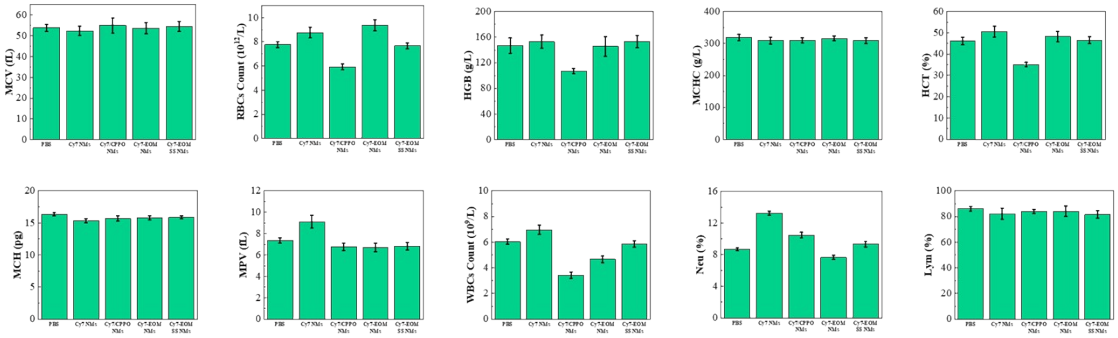

Figure S35. In vivo toxicity analysis of each sample studied by representative indicators of blood routine. Data are presented as mean values ± s.d.