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Electronic Supplementary Information

Macrophage membrane coated persistent luminescence nanoparticle@MOF-derived mesoporous carbon core-shell nanocomposites for autofluorescence-free imaging-guided chemotherapy

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Table of Contents

1. Supplementary Methods

1.1 Chemicals and Materials

$Ga(NO_3)_3 \cdot XH_2O$ (99.9%), $Zn(NO_3)_2 \cdot 6H_2O$ (AR), $Cr(NO_3)_3 \cdot 9H_2O$ (AR), $ZrCl_4$ (99.9%), p-phthalic acid (PTA, 99%), oleic acid (85%), doxycycline hydrochloride, acetylsalicylic acid, N-hydroxysuccinimide (NHS)，1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) were purchased from Aladdin (Shanghai, China). GeO_2 (99.999%) was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). NH_2-PEG-COOH was purchased from Yarebio Co., Ltd (Shanghai, China). Tumor necrosis factor-alpha (TNF-α) mouse recombinant protein (Rockland) was obtained from MULTISCIENCES (LIANKE) Biotech, Co., LTD (Hangzhou, China). Paclitaxel (PTX) was purchased from MedChemExpress LLC. The HUVECs, SCC-7 squamous epithelial cancer cells and J774A.1 macrophages were purchased from Procell Life Science&Technology Co., Ltd (Wuhan, China). Human serum sample (SL010-5 mL) was purchased from Beijing Solarbio Science & Technology Co., Ltd (Beijing, China). The company stated that the informed consent was obtained for using human serum just for scientific research. The ultrapure water used throughout the experiments was purchased from Hangzhou Wahaha Group Co., Ltd (Hangzhou, China).

1.2 Characterization

X-ray diffraction (XRD) pattern was acquired on a D2 PHASER diffractometer (Bruker, Germany). Transmission electron microscopy (TEM) and high-angle annular dark-field scanning TEM imaging combined with energy dispersive spectroscopy (EDS) images were collected from a JEM-2100 transmission electron microscope (JEOL, Japan). Fourier transform infrared (FT-IR) spectra were obtained from a Nicolet Is10 spectrometer (Thermo Scientific, USA). Hydrodynamic size distribution and Zeta potential were recorded on a Nano-ZSE Zetasizer (Malvern, U.K.). Absorbance spectra were recorded on obtained on a

UV-3600 plus spectrophotometer (Shimadzu, Japan). Phosphorescence spectra and luminescence decay curves were acquired on an F-7000 spectrofluorometer (Hitachi, Japan). Himac CP 70ME preparative ultracentrifuge (Hitachi, Japan) was used for ultracentrifugation. In vivo images were captured on an IVIS Lumina XRMS Series Ⅲ imaging system (PerkinElmer, USA). Images were captured in the luminescent imaging mode while the excitation filter was blocked and the emission filter was open. Cell imaging was performed on a Fluoview FV3000 laser scanning confocal microscope (Olympus, Japan). Quantification measurments of cytofluorometry were obtained from FACSCalibular Flow Cytometer (BD, USA). MTT assay was performed on a Synergy H1 microplate reader (Bio Tek, USA). N_2 adsorption experiments were performed on a TriStar II 3020 micropore physisorption analyzer (Micromeritics, USA) using N_2 adsorption at 77 K.

1.3 Preparation of PLMC-PEG.

The PEGlyated PLMC-PEG was obtained using a carbodiimide method. Briefly, EDC·HCl (80 mg) and NHS (32 mg) were quickly added to the carboxylic acid-capped PLNP (PLNP-COOH) dispersion (30 mg in 30 mL water). The mixture was stirred for 2 h and then centrifuged to remove the supernatant. The precipitant was redispersed in 30 mL of water and then the NH_2-PEG-COOH (20 mg) was added. The mixed solution was adjusted to pH 8.0 with 1 mol L^{-1} NaOH and stirred overnight in the dark. The generated PLMC-PEG was collected and washed with water and ethanol for several times.

1.4 Drug Loading and in Vitro Release Properties

To harvest macrophage membranes, 1×10^7 of J774A.1 macrophages were collected and processed with the previous literature.[1] The obtained macrophage membranes were dispersed in 1 mL of PBS and stored at –20 °C for further use.

Assembly of Different Drug Loaded Nanocomposites. For drug loading, doxycycline hydrochloride (Doxy, 6 mg) in 2 mL of water was introduced to PLNP@UiO-66 or PLMC

(10 mg) in 8 mL of water; acetylsalicylic acid (ASA, 6 mg) in 2 mL of ethanol was introduced to PLNP@UiO-66 or PLMC (10 mg) in 8 mL of ethanol; and paclitaxel (PTX, 1 mg) in 2 mL of methanol was introduced to PLNP@UiO-66 or PLMC (10 mg) in 8 mL of methanol; respectively. Six pieces of the solutions were fully dispersed under sonication and the shaken at 140 rpm for overnight. Then, all the solvents were evaporated using a rotary evaporator and the six copies of the prepared 500 μL of membrane solution in 8 mL of PBS were added in these six pieces of the solutions, respectively. After sonication for 10 min, six pieces of mixtures were centrifuged at 8000 rpm for 5 min to obtain different drug-loaded nanocomposites (MPLNP@UiO-66-Doxy, MPLMC-Doxy, MPLNP@UiO-66-ASA, MPLMC-ASA, MPLNP@UiO-66-PTX, MPLMC-PTX). The supernatants were diluted with solvents for twenty times and free drugs were quantified. Then the drug loading (wt%) and entrapment efficiency were calculated according to the formula as follows:

$$Drug_{loading} = (Drug_{total}\ weight - Drug_{free}\ weight)/vehicle\ weight \times 100\% \quad (1)$$

$$Entrapment\ efficiency = (Drug_{total}\ weight - Drug_{free}\ weight)/Drug_{total}\ weight \times 100\% \quad (2)$$

The quantification of free Doxy, ASA from MPLNP@UiO-66 and MPLMC was analyzed by a Shimazu UV3600 plus UV-Vis spectrophotometer. The absorbances of Doxy at 276 nm and ASA at 226 nm were calculated. The quantification of free PTX from MPLNP@UiO-66 and MPLMC was analyzed by HPLC (Waters e2695, USA) combined with a UV detector. The samples were injected into a C18 guard column (methanol: water, 80: 20) after filtration through a 0.22 μm filter membrane. The column temperature was 30 °C. The flow rate was 1.0 mL min^{-1} and the detection wavelength was set at 227 nm.

In vitro Release Properties of Nanocomposites. Freshly prepared drug-loaded MPLNP@UiO-66 or MPLMC were divided into two equal parts and were centrifugated to remove the supernatants. Each solution was equally divided into eight vials and each vial containing 600 μL of sample which were set in two different environment (standing in 10

mmol L^{-1} PBS, pH 7.4, 4 °C; shaken in human serum at 100 rpm, 37 °C; respectively). One vial in each medium was taken out at regular intervals. For Doxy and PTX analysis, samples were added with equal volume of mixture (acetonitrile: methanol = 1:1) whereas ASA-loaded samples were added with equal volume of mixture (acetonitrile: ethanol = 1:1) to precipitate cell membranes. The samples were sonicated thoroughly and then centrifuged to remove the precipitates. Finally, the filtered samples were analyzed as well as the free drug quantification steps.

1.5 *In Vitro* Cytotoxicity

The cytotoxicity of MPLMC or MPLMC-PTX were evaluated with a cell counting kit-8 (CCK-8) assay. In brief, SCC-7 and HUVECs were seeded in 96-well plates (5×10^3 cells per well) and incubated with different concentrations (0, 10, 50, 100, 200, and 500 μg mL^{-1}) of MPLMC or MPLMC-PTX (calculated as PLMC) for 24 h. Then, the supernatants were discarded and 100 μL of fresh medium containing 10 μL of CCK-8 was introduced. After incubation for 4 h, the 96-well plates were softly shaken and the absorbance at 450 nm were recorded.

Cell viabilities of SCC-7 after incubation with MPLMC-PTX were further studied with propidium iodide (PI) staining. SCC-7 cells were cultured in confocal dishes (1×10^6 cells per dish) and incubated with different concentrations of MPLMC-PTX (0, 10, 500 μg mL^{-1}) for 12 h. Then, cells were washed with PBS and stained with PI (100 μg mL^{-1}) before imaging.

2. Supplementary Figures

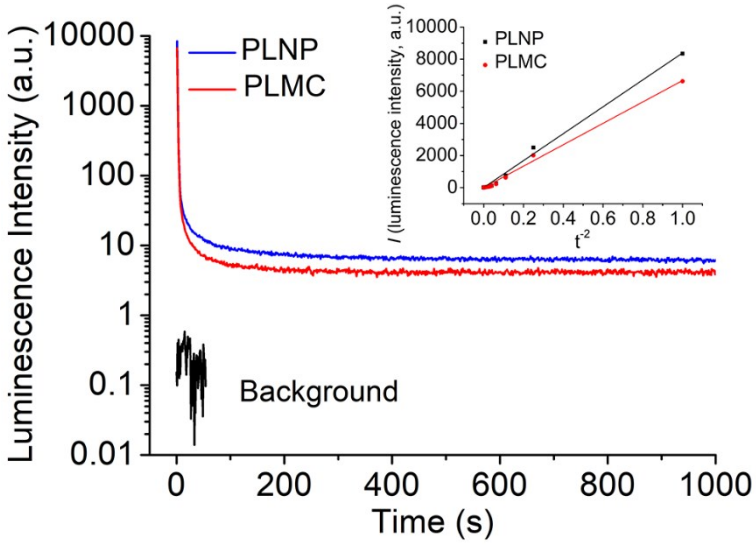

Figure S1. Persistent luminescence decay curves of PLNP and the resultant PLMC powders obtained after 1-h calcination at 500 °C monitored at 700 nm after 10 min pre-excitation by a UV lamp (254 nm, 6 w), and the afterglow emission intensities at 1000 s after stopping excitation were estimated to be ~11 mW m^{-2} for PLNP, ~8 mW m^{-2} for PLMC (100 mg in pellet, respectively). The inset shows the plot of I versus t^{-2} for PLNP and PLMC, and the persistent luminescence intensity (I) linearly depended on the inverse square of time (t^{-2}) (I = 8381 t^{-2} + 5.68 for PLNP, I = 6665.89t^{-2} + 3.42 for PLMC, respectively), indicating the persistent luminescence emission of the PLNP and PLMC both occurred though tunneling-related process.[2]

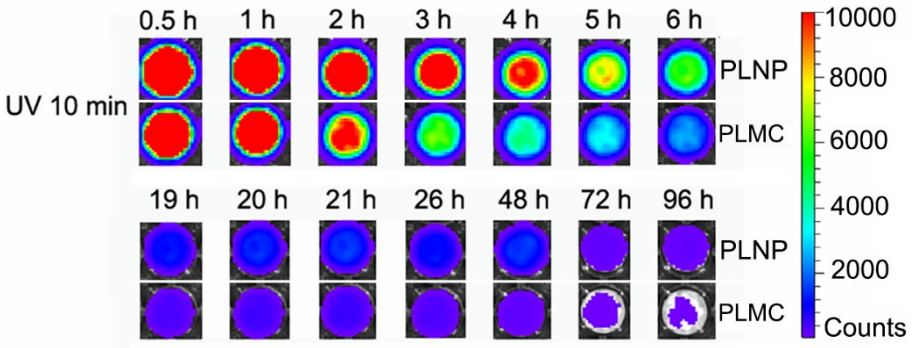

Figure S2. UV pre-excited afterglow images of the PLNP and the resultant PLMC powders obtained after 1-h calcination at 500 °C recorded on IVIS Lumina II imaging system.

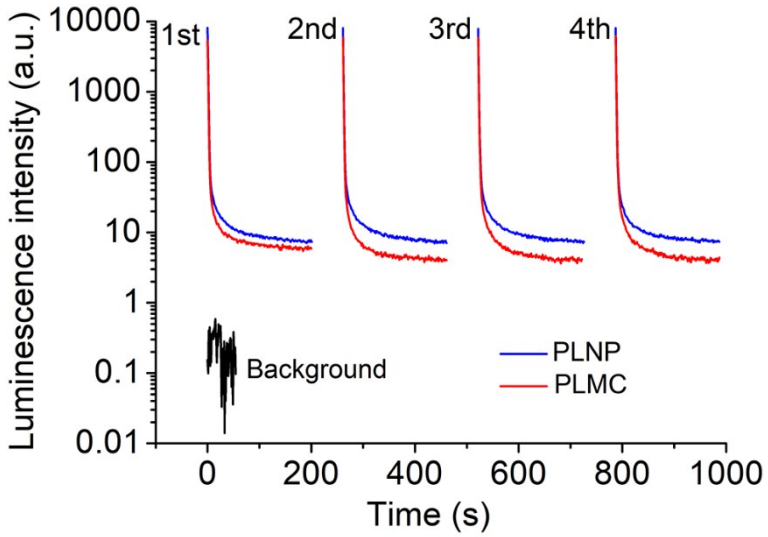

Figure S3. Persistent luminescence decay curves of PLNP and the resultant PLMC powders obtained after 1-h calcination at 500 °C monitored at 700 nm reactivated by 2-min LED illumination (650 ± 10 nm, 5000 lm) for 4 cycles.

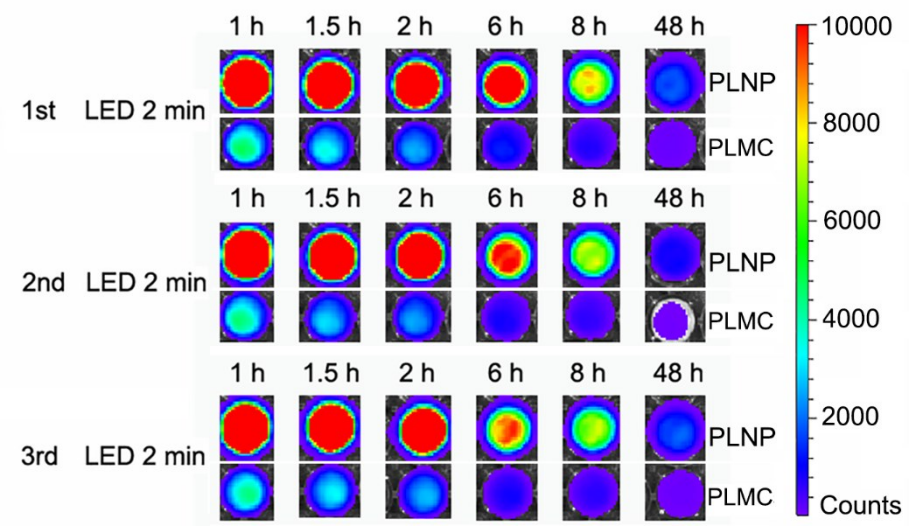

Figure S4. Afterglow images of PLNP and the resultant PLMC powders obtained after 1-h calcination at 500 °C pre-excited with 2-min LED illumination recorded on IVIS Lumina II imaging system for 3 cycles.

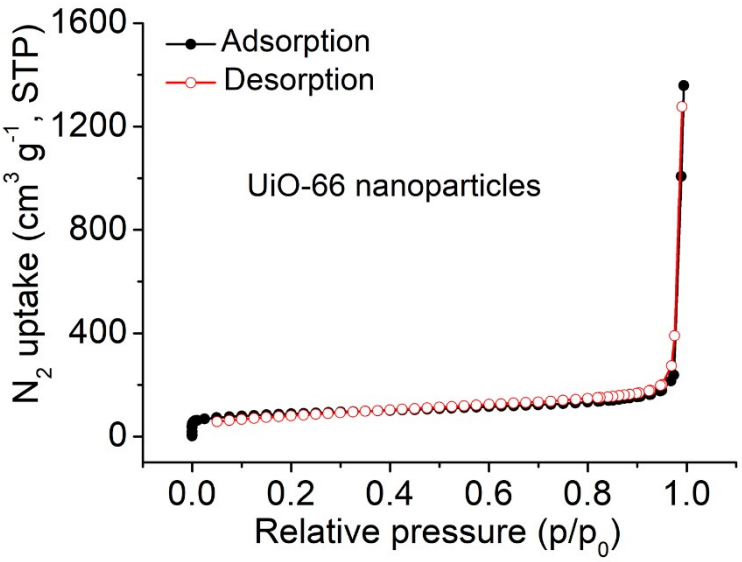

Figure S5. N_2 adsorption–desorption isotherms of the prepared UiO-66 nanoparticles.

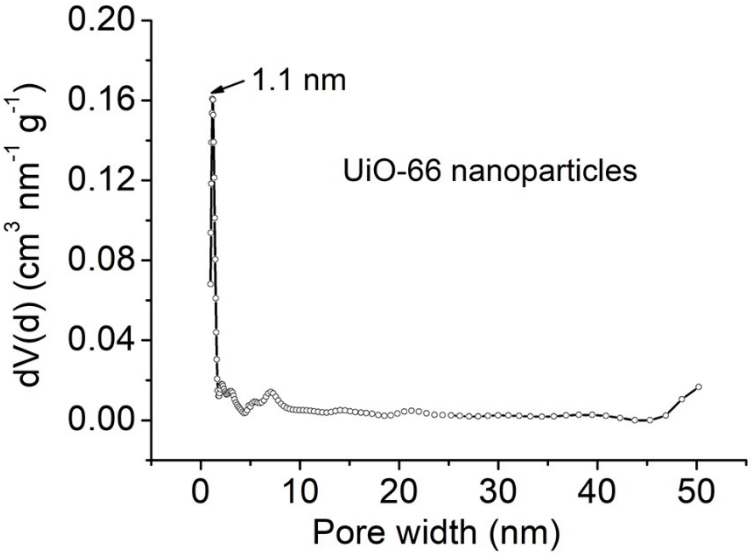

Figure S6. Pore size distribution of the prepared UiO-66 nanoparticles.

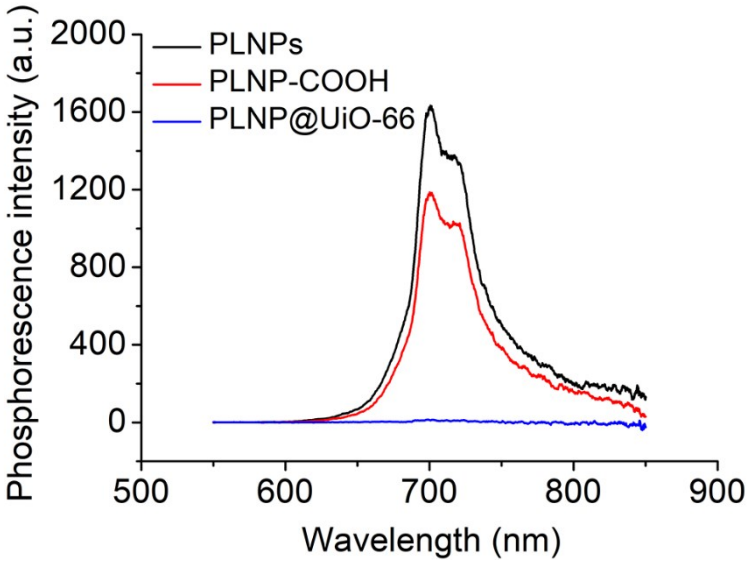

Figure S7. Phosphorescence emission spectra of PLNP, PLNP-COOH and PLNP@UiO-66 in 1 mg mL^{-1} aqeuous solution.

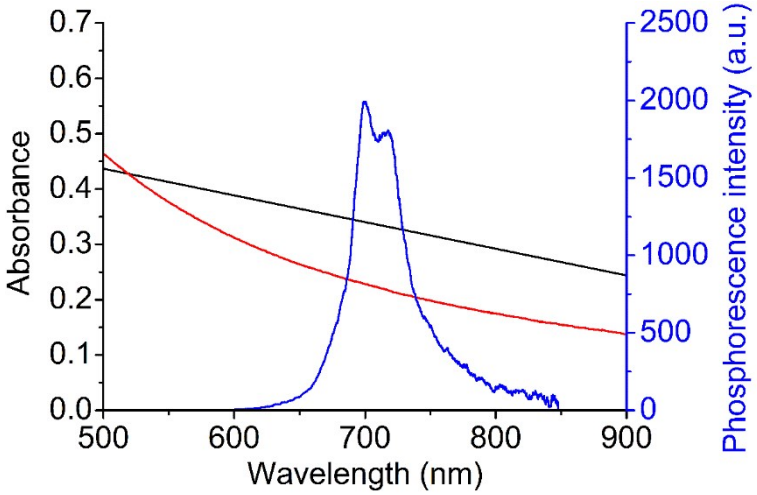

Figure S8. vis-NIR absorption spectra of the prepared UiO-66 nanoparticles (0.25 mg mL^{-1}, black line), UiO-66-derived MC nanoparticles obtained after calcination at 500 °C for 1 h (0.25 mg mL^{-1}, red line), and phosphorescence emission spectrum (blue line) of PLNP (1 mg mL^{-1}) under 254 nm excitation.

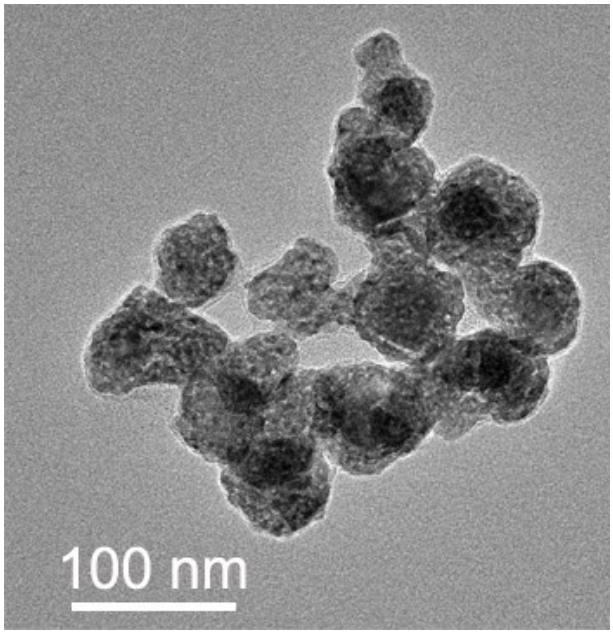

Figure S9. TEM image of resultant PLMC after calcination at 500 °C for 2 h.

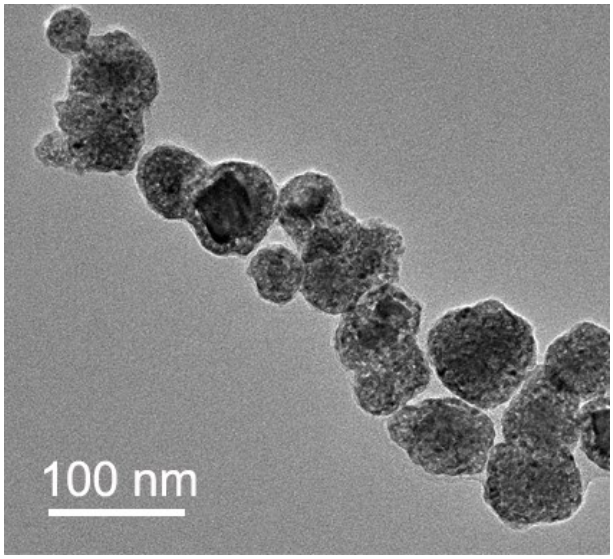

Figure S10. TEM image of resultant PLMC after calcination at 500 °C for 3 h.

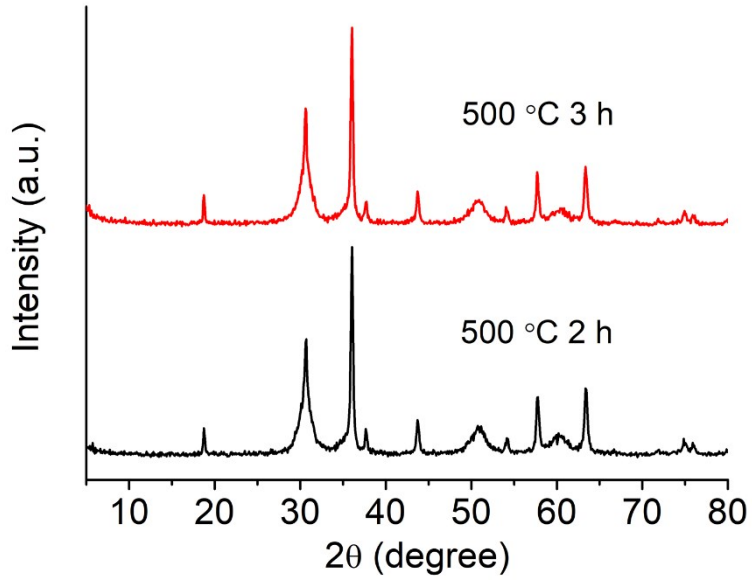

Figure S11. XRD patterns of resultant PLMC after calcination at 500 °C for 2 h and 3 h.

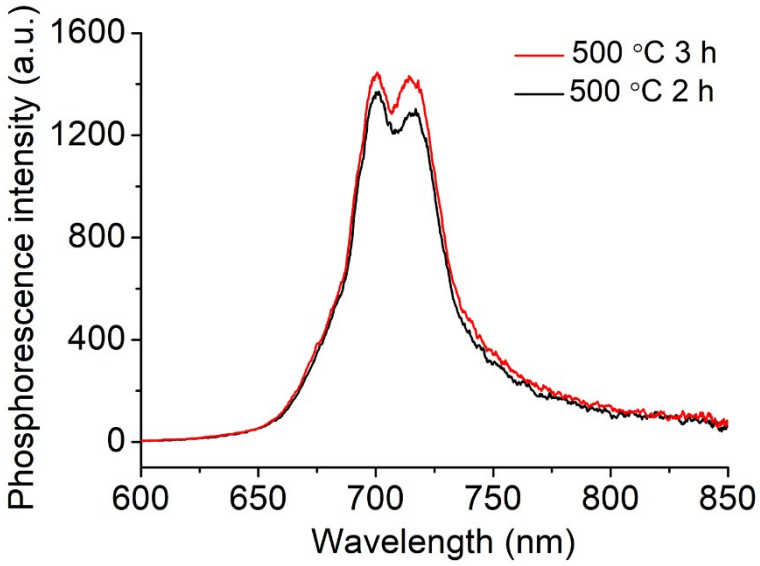

Figure S12. Phosphorescence emission spectra of resultant PLMC after calcination at 500 °C for 2 h and 3 h.

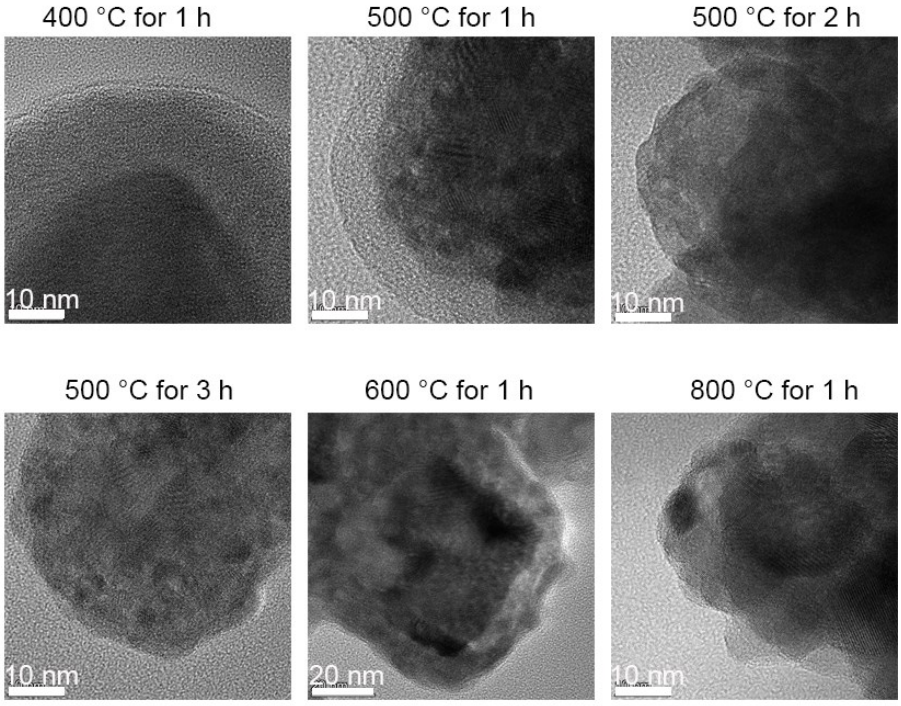

Figure S13. HRTEM images of PLNP-UiO-66-derived nanocomposites after different calcinations.

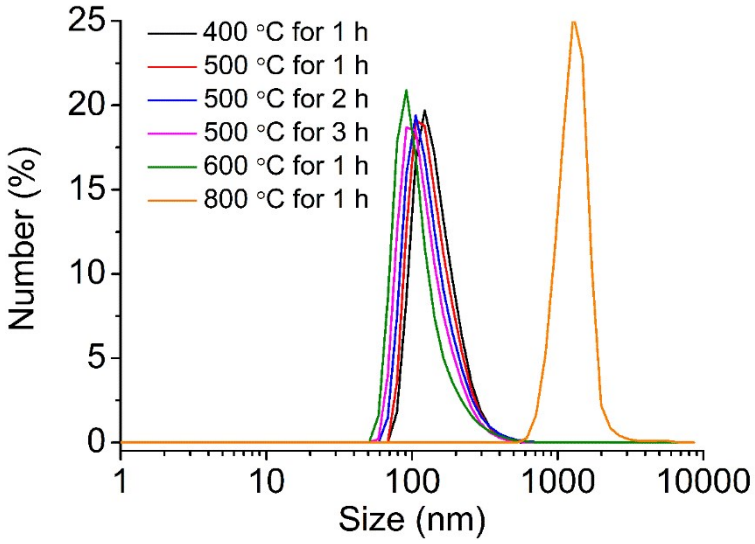

Figure S14. Hydrodynamic size distribution of PLNP@UiO-66-derived nanoparticles after different calcination conditions.

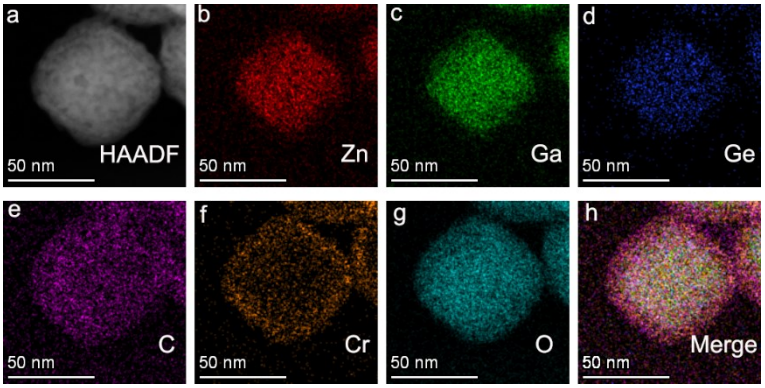

Figure S15. (a) HAADF imaging, (b) Zn element mapping, (c) Ga element mapping, (d) Ge element mapping, (e) C element mapping, (f) Zr element mapping, (g) O element mapping, (h) Zn, Ga, Ge, C and Zr composite element mapping of the resultant PLMC obtained after calcination at 500 °C for 1 h.

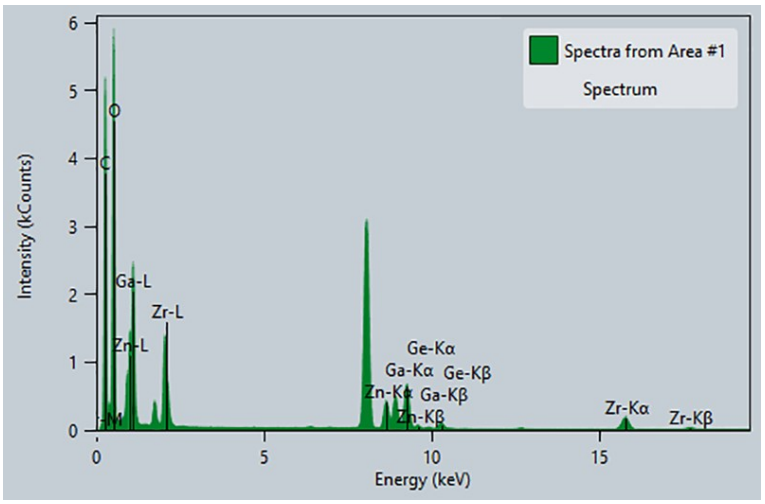

Figure S16. Energy dispersive X-ray spectrum of PLNP@UiO-66.

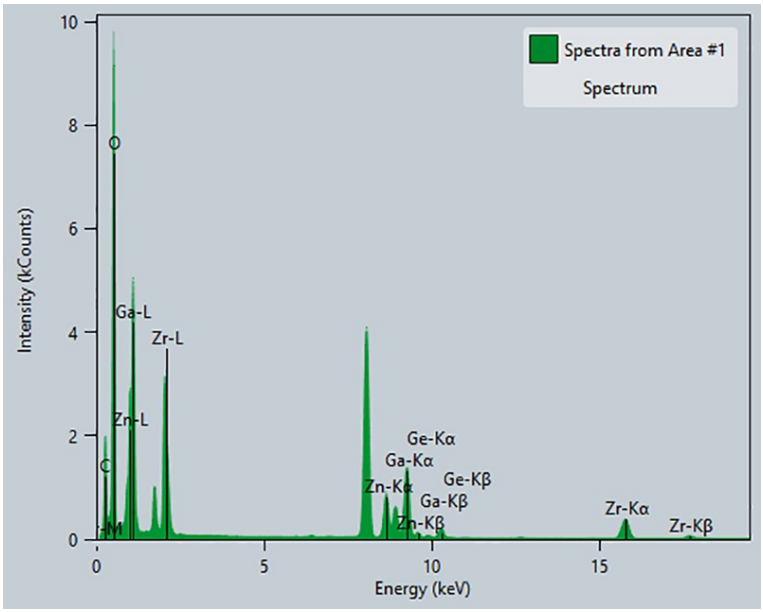

Figure S17. Energy dispersive X-ray spectrum of the resultant PLMC obtained after calcination at 500 °C for 1 h.

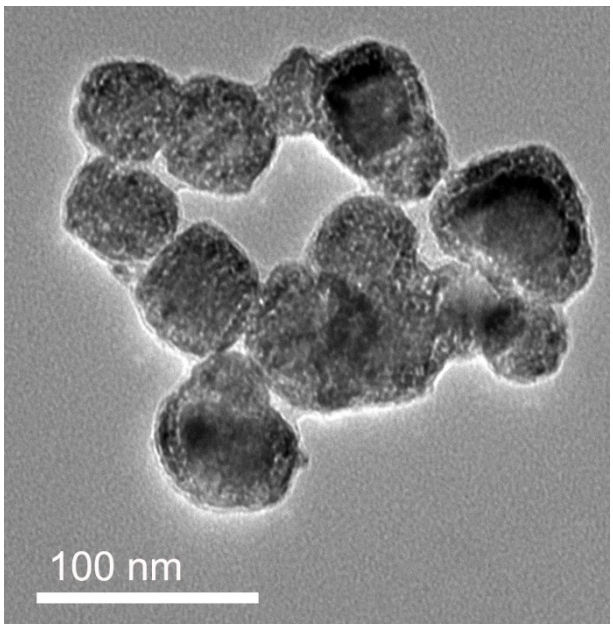

Figure S18. TEM image of J774A.1 macrophage membrane coated PLMC (MPLMC).

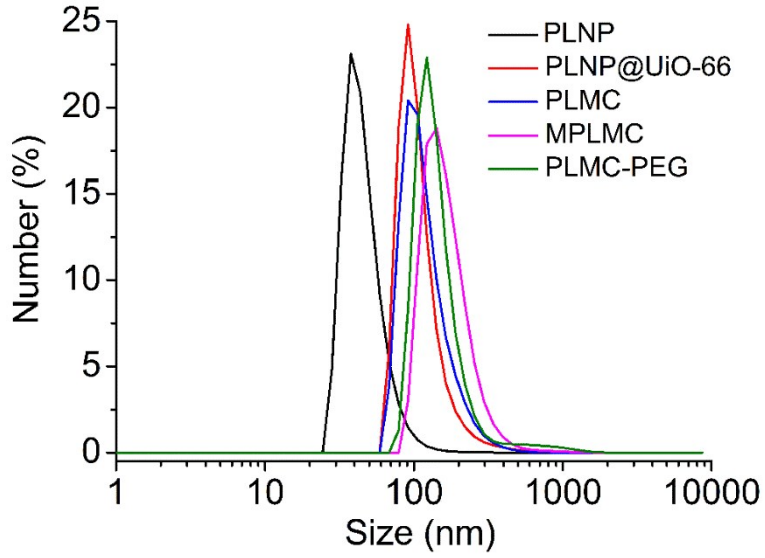

Figure S19. Hydrodynamic size distribution of PLNP, PLNP@UiO-66, PLMC, MPLMC, PLMC-PEG nanoparticles.

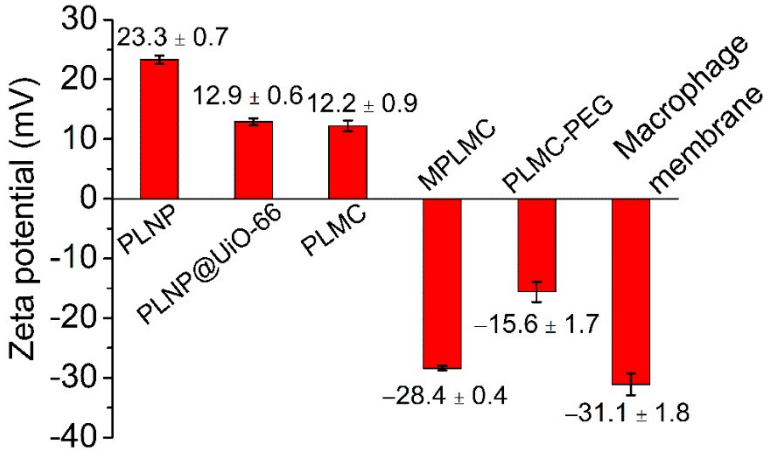

Figure S20. Zeta-potential of PLNP, PLNP@UiO-66, PLMC, MPLMC, PLMC-PEG nanoparticles and J774A.1 macrophage membrane.

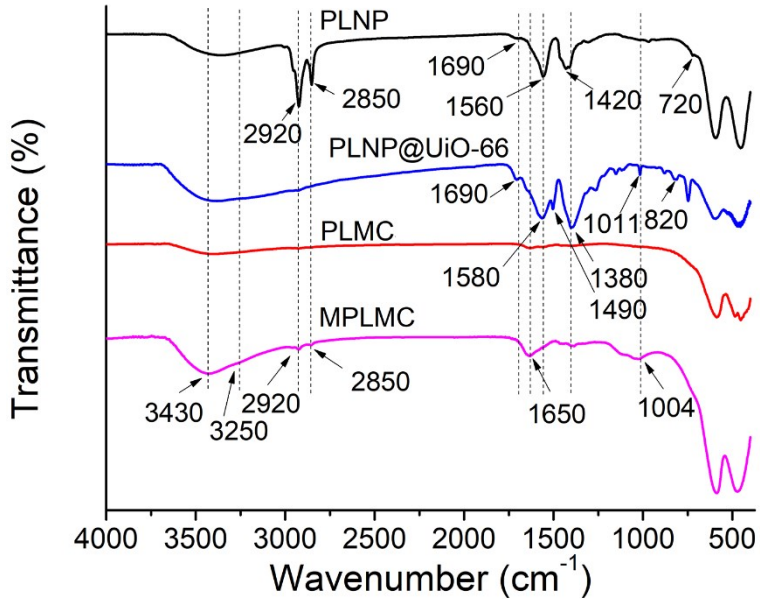

Figure S21. FT-IR spectra of PLNP, PLNP@UiO-66, PLMC, and MPLMC nanoparticles. The FT-IR spectrum of PLNP displays strong absorption bands of asymmetric and symmetric $-CH_2-$ stretching vibration at 2920 and 2850 cm^{-1}, C−C stretching vibration at 1560 cm-1, C=O stretching vibration at 1690 cm^{-1}, $-CH_2-$ bending vibration at 1420 cm^{-1}, and $-(CH_2)_n-$ bending vibration at 720 cm^{-1} representing the long carbon chains. These absorption bands all derive from oleic acid groups on the PLNP. The FT-IR spectrum of PLNP@UiO-66 shows absorption bands of the C=O stretching at 1690 cm^{-1}, C=C stretching vibration of aromatic rings at 1560 and 1490 cm^{-1}, C−O stretching vibration at 1011 cm^{-1}, and bending vibration of disubstituted aromatic rings at 820 cm^{-1}, which originates from the organic linker p-phthalic acid in the framework of UiO-66 shell.

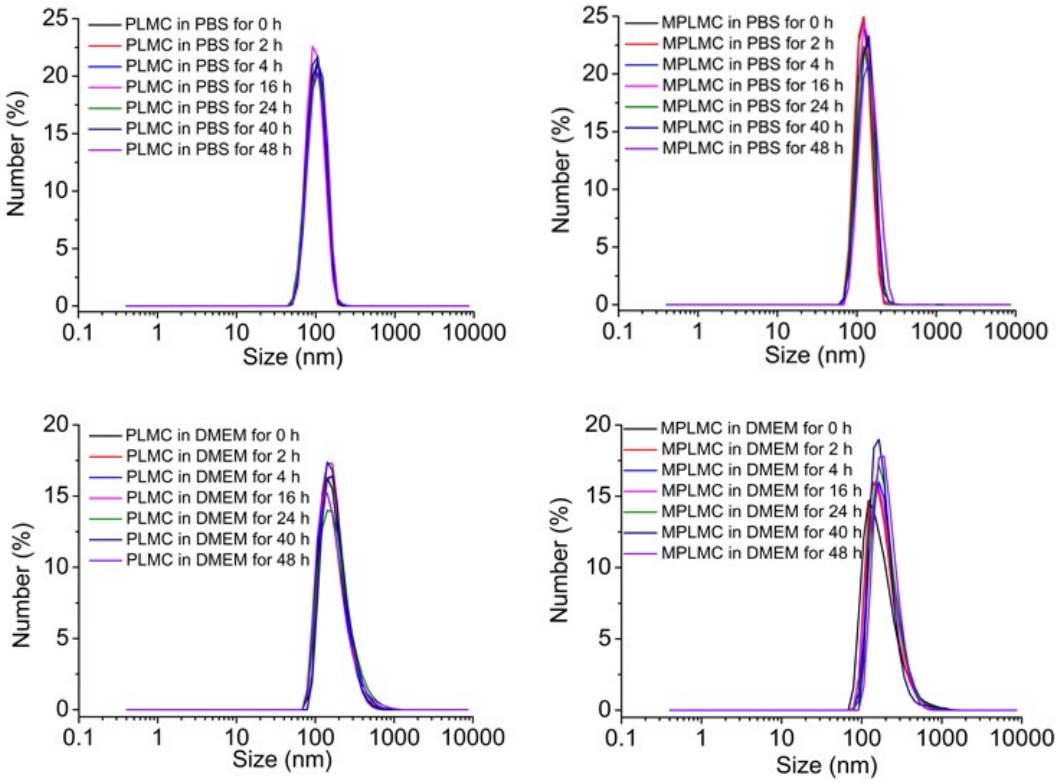

Figure S22. Hydrodynamic size distribution of PLMC and MPLMC in PBS (10 mM, pH 7.4) and DMEM cell culture medium for different hours.

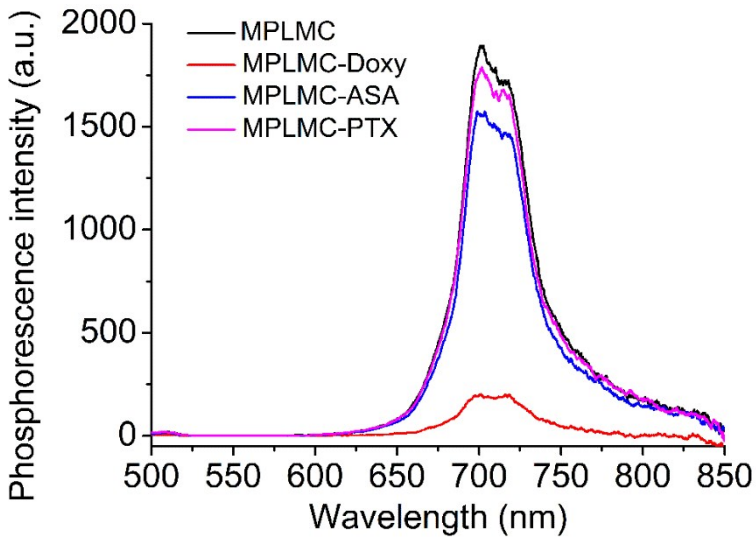

Figure S23. Phosphorescence emission spectra of MPLMC (1 mg mL^{-1}) and different drug-loaded dispersions (1 mg mL^{-1} calculated as PLMC).

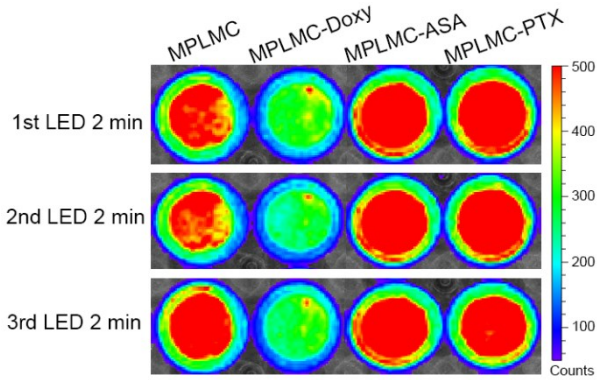

Figure S24. Afterglow images of MPLMC (1 mg mL^{-1}) and different drug-loaded MPLMC dispersions (1 mg mL^{-1} calculated as PLMC) pre-excited with 2-min LED illumination recorded on IVIS Lumina Ⅲ imaging system for 3 cycles.

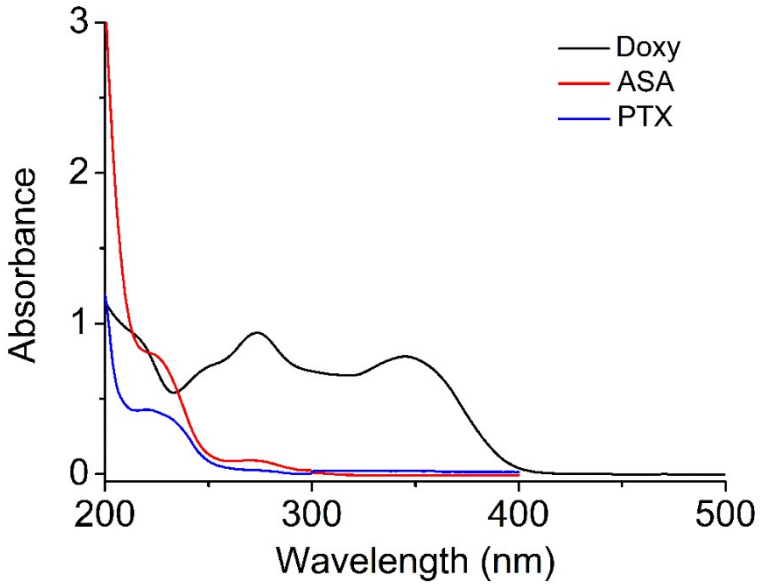

Figure S25. UV-vis absorption spectra of Doxy (0.03 mg ml^{-1}), ASA (0.03 mg ml^{-1}), PTX (0.01 mg ml^{-1}).

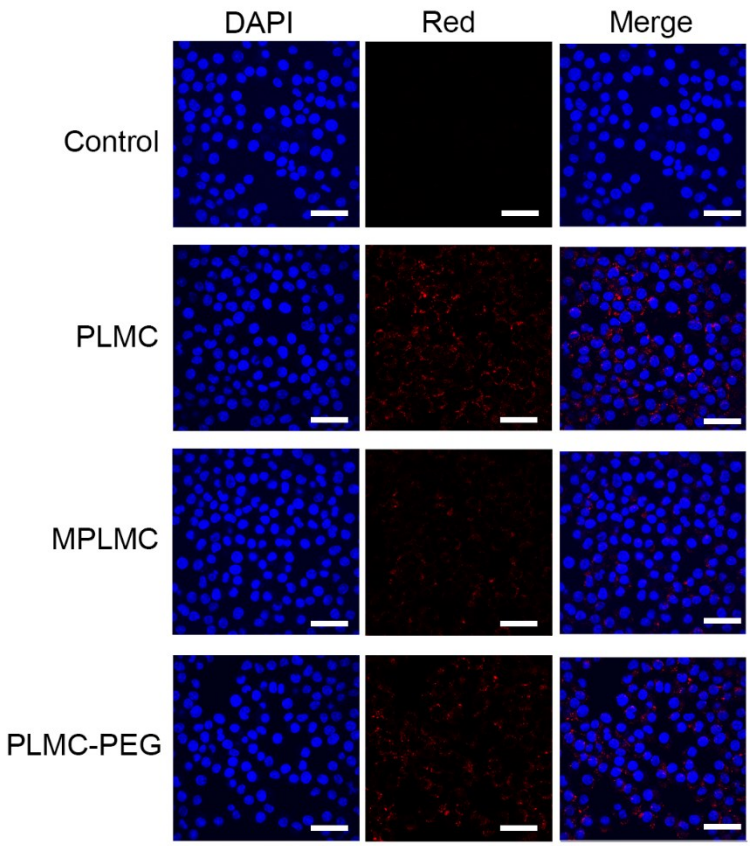

Figure S26. Representative confocal microscopy images of J774A.1 macrophages incubated with PLMC, MPLMC and PLMC-PEG. Scale bar: 40 μm.

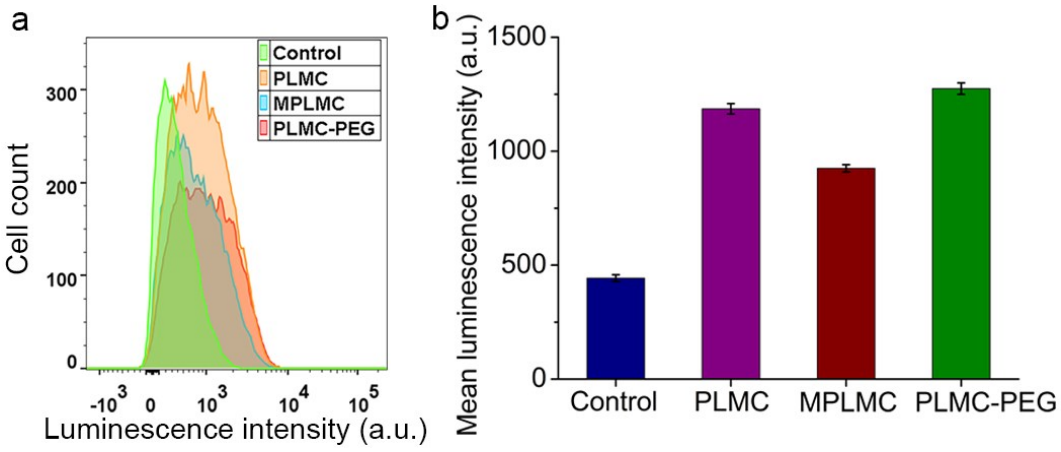

Figure S27. Analysis of luminescence intensity obtained from flow cytometry of J774A.1 macrophages after incubation with different nanoparticles (10000 cells /flow tube). (a)

Statistics histogram of J774A.1 macrophages with red luminescence endowed by different nanoparticles after incubation. (b) Mean luminescence intensity of J774A.1 macrophages after different treatments.

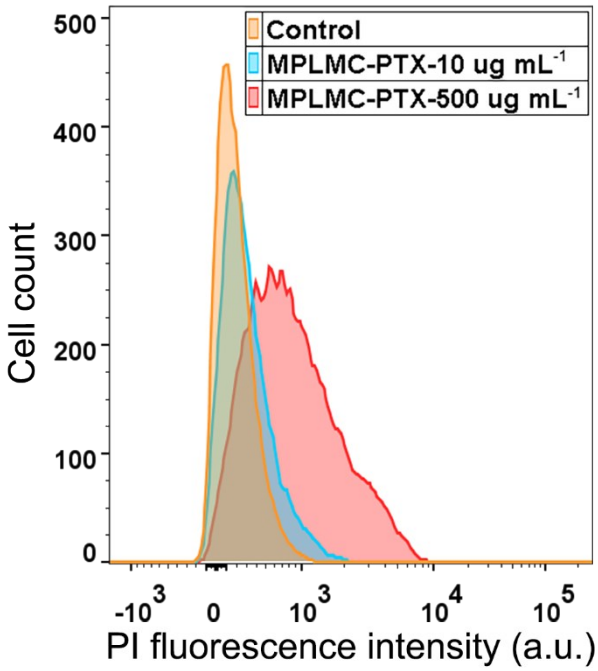

Figure S28. Statistics histogram of J774A.1 macrophages stained by PI after incubation with different concentrations of MPLMC-PTX measured by flow cytometry (10000 cells /flow tube).

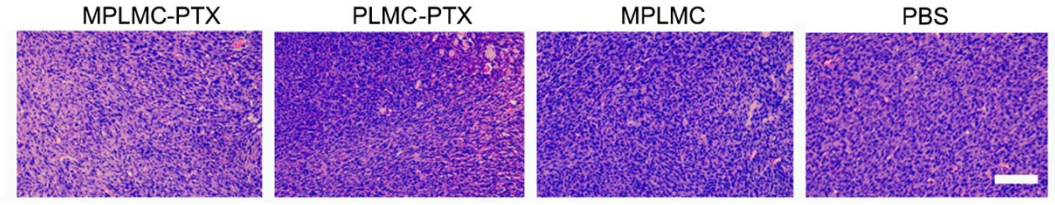

Figure S29. Histological staining of SCC-7 tumor tissues from various treated mice groups. Scale bar: 50 μm.

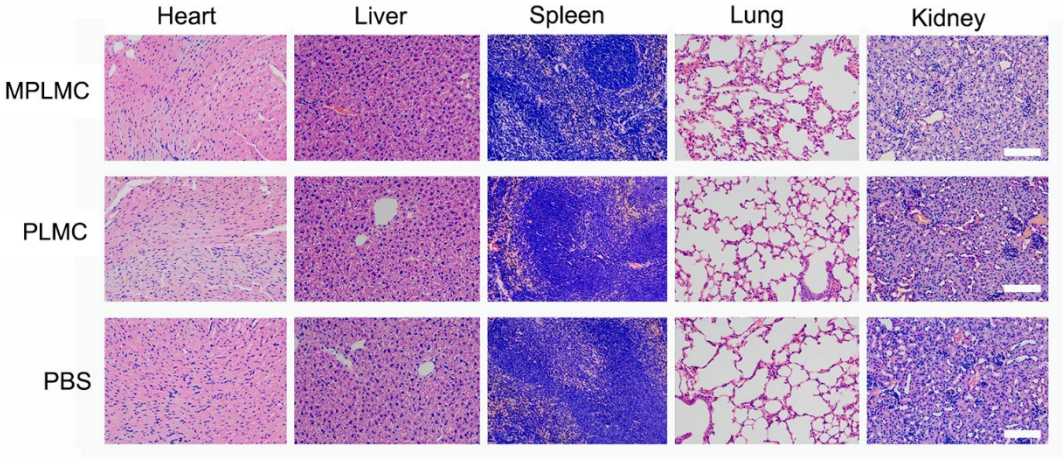

Figure S30. Histological staining of major organs from three healthy mice groups after different treatments. Scale bar: 50 μm.

3. Supplementary Tables

Table S1. Element contents of PLNP@UiO-66 and the resultant PLMC obtained after calcination at 500 °C for 1 h determined by EDS analysis.

Sample	C (%)	O (%)	Zn (%)	Ga (%)	Zr (%)
PLNP@UiO-66	31.6	28.0	8.1	14.8	17.3
PLMC	10.0	33.3	10.2	19.1	27.1

4. References

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2. J. Trojan-Piegza, J. Niittykoski, J. Hölsä and E. Zych, *Chem. Mater.*, 2008, **20**, 2252-2261.