

# Irradiation pretreatment enhances the therapeutic efficacy of platelet-membrane- camouflaged antitumor nanoparticles

**Yin Chen**

Army Medical University

**Xue Shen**

Chengdu University

**Songling Han**

Army Medical University

**Tao Wang**

Army Medical University

**Jianqi Zhao**

Army Medical University

**Yongwu He**

Army Medical University

**Shilei Chen**

Army Medical University

**Shengqi Deng**

Chengdu University

**Cheng Wang** (✉ [wangctmmu@126.com](mailto:wangctmmu@126.com))

Army Medical University <https://orcid.org/0000-0001-5850-4533>

**Junping Wang**

Army Medical University

---

## Research

**Keywords:** irradiation pretreatment, platelet-membrane camouflage, tumor

**Posted Date:** December 30th, 2019

**DOI:** <https://doi.org/10.21203/rs.2.19617/v1>

**License:**   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

**Version of Record:** A version of this preprint was published on July 20th, 2020. See the published version at <https://doi.org/10.1186/s12951-020-00660-z>.

# Abstract

**Background:** Cell membrane-based nanocarriers are promising candidates for delivering antitumor agents. The employment of a simple and feasible method to improve the tumor-targeting abilities of these systems is appealing for further application. Herein, we prepared a platelet membrane (PM)-camouflaged antitumor nanoparticle. The effects of irradiation pretreatment on tumor targeting of the nanomaterial and on its antitumor action were evaluated.

**Results:** The biomimetic nanomaterial constructed by indocyanine green, poly(d,l-lactide-co-glycolide), and PM is termed PINPs@PM. A 4-Gy X-ray irradiation enabled the endocytic enhancement of PINPs@PM by 4T1 breast cancer cells. PINPs@PM produced hyperthermia and reactive oxygen species upon excitation by near-infrared irradiation, which were detrimental to the cytoplasmic lysosome and resulted in cell death. Irradiation pretreatment thus strengthened the antitumor activity of PINPs@PM *in vitro*. Mice experiments revealed that irradiation enhanced the tumor targeting capability of PINPs@PM *in vivo*. When the same dose of PINPs@PM was intravenously administered, irradiated mice had a better outcome than did mice without X-ray pretreatment.

**Conclusion:** The study demonstrates an effective strategy combining irradiation pretreatment and PM camouflage to deliver antitumor nanoparticles, which may be instrumental for targeted tumor therapy.

## Background

Cancer is a global threat to human health [1]. Surgery, radiotherapy, and chemotherapy are conventional methods to treat cancers, but they all have inherent limitations in clinical applications, such as invasiveness, drug resistance, and severe side effects [2]. Phototherapy, including photothermal therapy (PTT) and photodynamic therapy (PDT), is a noninvasive and effective antineoplastic strategy and is considered a promising alternative to classical oncotherapy [3]. PTT and PDT eliminate cancer cells based on the fact that after excitation with light of a specific wavelength, photothermal agents and photosensitizers generate hyperthermia and reactive oxygen species (ROS), respectively, which are detrimental to cancer cells [4, 5].

Indocyanine green (ICG), one of the near-infrared dyes approved by the US Food and Drug Administration for clinical imaging and diagnosis, is a photothermal agent as well as a photosensitizer and thus attracts considerable attention. Because ICG lacks a tumor-targeting ability and tends to be rapidly cleared *in vivo*, many nanocarriers have been developed to deliver ICG [6]. Nevertheless, provided that the carrier is not endowed with antiphagocytic ability against the mononuclear phagocyte system *in vivo*, the bioavailability of ICG is still limited [7]. Cell membrane-based nanoparticles (CMBNPs) represent promising materials to overcome this shortcoming, as the functional molecules on the membrane, such as CD47 [8], CD45, and glycans, can send a "don't eat me" signal to the immune system [9]. The biomimetic strategy is plausibly beneficial for ICG delivery.

Intensifying the tumor-targeting ability of nanocarriers is also instrumental for ICG delivery. Passive and active targeting strategies are employed by therapeutic nanoparticles (TNPs) to reach the tumor site. Compared with the passive targeting phenomenon, which is mostly based on the enhanced permeability and retention effect and is limited by tumor types [10, 11], the active targeting method, such as modifying TNPs with peptides and antibodies [12, 13], is more efficient to promote drug accumulation in tumors. Notably, due to the presence of functional molecules with high affinity to cancer cells on certain cell membranes, P-selectin on the platelet membrane (PM) [4], for example, some CMBNPs possess an active tumor targeting capability. Further decoration of the cell membrane with tumor necrosis factor-related apoptosis-inducing ligand can strengthen the targeting ability [14], but the process is somewhat complex. The employment of simple and feasible methods that can help to target these CMBNPs to the tumor site is appealing for future clinical use.

In this study, we employed a PM-camouflaged poly(D,L-lactide-co-glycolide) (PLGA) nanocarrier to deliver ICG, obtaining a composite termed PINPs@PM (Fig. 1). The effect of X-ray irradiation on the antitumor activity of PINPs@PM in the presence of near-infrared irradiation (NIR) *in vitro* and *in vivo* was evaluated. The combined strategy using irradiation pretreatment and cell membrane camouflage is efficient for delivering drugs to tumors and thus has wide prospects in tumor diagnosis and therapy.

## Results And Discussion

### Preparation and characterization of PINPs@PM

The ICG-loaded PLGA nanoparticles fabricated using a double emulsion method were termed PINPs (Fig. 1). The encapsulation efficiency of ICG was 60.6%, calculated by ultraviolet spectrophotometry as recently described [15]. PINPs were spherical with a good dispersion ability (Additional file 1). After decoration with a PM, the nanoparticles became PINPs@PM which exhibited white halos by transmission electron microscopy (TEM, Fig. 2a). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) confirmed the successful coating of PM (Fig. 2b) [16]. The zeta potential determination revealed that the PM camouflage increased the surface charge of the PINPs (averagely -31 mV) to approximately -20 mV, which was similar to the charge of free platelets (Fig. 2c). The hydrodynamic diameter of the PINPs@PM measured by dynamic laser scattering (DLS) was slightly larger than that of the PINPs (Fig. 2d), which was consistent with the influence of red blood cell membrane cloaking on PLGA nanoparticles encapsulating perfluorocarbons [17].

### *In vitro* and *in vivo* toxicological evaluation

To assess the material toxicity, we incubated PINPs@PM with human umbilical vein endothelial cell (HUVEC) and mouse 4T1 cells for 24 h. PINPs@PM had less of an effect on the cell survival at concentrations up to 50 µg/ml (based on the concentration of ICG, Fig. 3a). The marginal haemolytic property of PINPs@PM further confirmed its biosafety *in vitro* (Fig. 3b). Animal experiments showed that intravenous injection of 60 µg of PINPs@PM (300 µg/ml, based on the dose of ICG) was nonlethal to BALB/c mice. The vital organs including the heart, liver, spleen, lung, and kidney were obtained after three

weeks. In line with the nontoxicity of CMBNPs [18], there were no pathological changes in the tissue revealed by haematoxylin and eosin (HE) staining (Fig. 3c), indicative of the biocompatibility of PINPs@PM *in vivo*.

### **Effect of irradiation on the endocytosis of PINPs@PM by cancer cells**

Platelets have pathophysiological affinity with tumors [19]. The interaction between P-selectin and CD44 partially contributes to the binding of PM-camouflaged nanomaterials to the surface of breast cancer cells [4, 20]. After aggregation on the cell membrane, our data showed that PINPs@PM was endocytosed by 4T1 cells in a time-dependent manner (Fig. 4a). Notably, pretreatment of the cells with a 4-Gy X-ray irradiation (1.0 Gy/min) enhanced the endocytosis. Flow cytometry revealed that the fluorescence intensity of 4T1 cells phagocytosing 30 µg/ml PINPs@PM after 12 h averaged 12332, which was significantly higher than that of cells without irradiation (average of 10696, Fig. 4b). Ionizing radiation can alter the cell cycle and enhance the caveolin-dependent endocytosis pathway of cancer cells [21]. The changes in cellular phagocytic ability can explain the enhancement effect of X-ray irradiation on the uptake of PINPs@PM by 4T1 cells.

### **Antitumor evaluation *in vitro***

ICG is a photosensitizer and a photothermal agent. After the endocytosis by 4T1 cells, PINPs@PM produced intracellular ROS that could be stained by 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) probes upon excitation by NIR at 808 nm (Fig. 5a). The green fluorescence of X-ray-treated cells was visually stronger than that of cells without irradiation, which also indirectly indicated that irradiation pretreatment could strengthen the endocytosis of PINPs@PM by cancer cells. ROS are closely related to the growth and death of cancer cells [22]. On the one hand, ROS can induce the DNA mutation and genomic instability, accelerating the proliferation, immune tolerance, and metastasis of cancer cells [23, 24]. In addition, high ROS levels enhance the cellular oxidative stress, which is detrimental to DNA, proteins, and lipids and thus causes cell death [25]. Ample studies have exploited the modulation of oxidative stress by delivering photosensitizers to the tumor site as the therapeutic strategy [26-28].

Otherwise, the temperature of PINPs@PM increased with increasing exposure time to NIR (Fig. 5b). After 10 min of NIR (808 nm, 1 W/cm<sup>2</sup>), the temperature of 30 µg/ml PINPs@PM reached 56.8 °C, which is sufficient to damage proteins and inactivate cancer cells [29]. Accordingly, with the actions of excess ROS and hyperthermia, the 4T1 cells were eliminated in a dose-dependent manner by PINPs@PM (Fig. 5c). Similar to the endocytic tendency shown in Fig. 4b, X-ray irradiation pretreatment significantly intensified the antitumor activity of PINPs@PM *in vitro*.

The lysosome is a membrane-coated acidic organelle that participates in the degradation and recycling of cellular waste, energy metabolism, and cell signaling transduction. Lysosomes are considered therapeutic targets to treat many diseases including lupus, rheumatoid arthritis, Alzheimer's disease, Parkinson's disease, and cancers [30, 31]. Inhibiting lysosome function enhances the antitumor activity of chemotherapeutic drugs and increases the sensitivity of tumors to radiotherapy [32, 33]. We employed the

acridine orange (AO) dye to stain 4T1 cells. Normally, the intact acidic lysosome exhibits red fluorescence, while the cytosol and nuclei exhibit green fluorescence upon excitation at 488 nm. Fluorescence microscopy showed that the red fluorescence in cells treated with X-ray/PINPs@PM/NIR was dramatically decreased compared with that in PBS-treated cells (Fig. 5d), suggesting that the lysosome is severely injured by the combined treatment. Due to the photochemical internalization effect of ROS on the membrane of lysosomes and the photothermal effect of ICG, the ROS and hyperthermia generated by NIR-treated PINPs@PM may be responsible for the lysosome disruption [34], which partially contributes to cell death.

### **Tumor targeting and therapeutic assessment *in vivo***

We obtained the fluorescent images of 4T1 tumor-bearing mice intravenously administered with 60  $\mu$ g of PINPs@PM 24 h post irradiation (Fig. 6a). Because irradiation not only enhances the material endocytosis by cancer cells but also enables the alteration of the tumor microenvironment to accumulate nanoparticles [35], PINPs@PM accumulated more at the tumor site with the assistance of X-ray than did the group without irradiation pretreatment, which was also evidenced by the *in vitro* tissue imaging (Fig. 6b). The infrared thermal imaging supported that the maximal tumor temperature of PINPs@PM-treated mice reached 41.2 °C after 10 min of NIR treatment (Fig. 6c), which was much higher than that of NaCl-treated mice (30.3 °C, Additional file 2) but lower than that of X-ray-pretreated mice (46.9 °C). Additionally, in contrast to the PINPs@PM/NIR group whose temperature tended to balance after 8 min, the X-ray/PINPs@PM/NIR group exerted an overt time-dependent temperature increase (Fig. 6d).

After NIR treatment, mice were monitored for 21 days and sacrificed, and the tumors were obtained by surgery (Fig. 7a). PINPs@PM was superior to PINPs at decreasing the tumor volumes (Fig. 7b), which was consistent with that PM **cloaking** can improve the efficacy of TNPs [36, 37]. Due to the enhancement to tumor targeting, irradiation pretreatment further improved the curative effect of PINPs@PM. Terminal deoxynucleotidyl transferase-mediated deoxyuridinetriphosphate nick end labeling (TUNEL) assay showed that the dead cells in X-ray/PINPs@PM/NIR-treated tumors are visibly more than those in tumors without irradiation pretreatment (Fig. 7c). HE staining indicated that the structure of tumor tissue was severely destroyed after X-ray/PINPs@PM/NIR treatment.

## **Conclusions**

ICG-based phototherapy is an effective strategy to treat tumors, whereas the circulation time of free ICG is short. In this study, we prepared a PM-camouflaged PLGA nanoparticle to deliver ICG, considering that PM camouflage not only lowers the immune clearance of nanocarriers but strengthens their tumor targeting capabilities. For further targeting enhancement, we optimized the therapeutic regimen by adding a irradiation pretreatment. The X-ray irradiation increased endocytosis of the nanomaterial by cancer cells and enhanced its targeting efficiency, thus intensifying its antitumor action *in vitro* and *in vivo*. The study demonstrating a combined use of irradiation pretreatment and PM camouflage to improve the targeting ability of antitumor nanoparticles is useful for further tumor therapy.

# Methods

## Materials

PLGA (50:50, inherent viscosity 0.20 dL/g, MW = 15,000) was obtained from the Shandong Institute of Medical Instruments (Jinan, Shandong Province, CHN). Poly(vinyl alcohol) (PVA, MW = 30,000 - 70,000, 78-90% hydrolysed) and ICG were purchased from Sigma-Aldrich (St Louis, MO, US). RPMI 1640 cell culture medium and foetal bovine serum (FBS) were obtained from Gibco (Thermo Fisher Scientific, Shanghai, CHN). The SDS-PAGE, DCFH-DA, and CCK-8 kits were obtained from Beyotime (Shanghai, CHN). The formaldehyde solution (4%), HE staining solution, and AO were purchased from Solarbio (Beijing, CHN). Deionized water was obtained from a Milli-Q water purification system.

## Preparation of PINPs and PINPs@PM

The ICG-loaded PLGA nanoparticles (PINPs) were prepared using a reported water-in-oil-in-water double emulsion method with modifications [15, 38]. Briefly, PLGA (120 mg) was dissolved in 2.5 ml of methylene chloride. ICG solution (7.5 mg/ml, 200  $\mu$ l) was added, and the mixture was emulsified by sonication (25% amplitude, 2 min) using a Digital Sonifier S-250D (Branson Ultrasonic, CT, US) in an ice bath. The primary emulsion (first emulsion) was poured into 10 ml of PVA solution (3%, w/v) and sonicated for another 3 min, obtaining a double emulsion (second emulsion). The obtained product was stirred to evaporate the organic solvent and was then centrifuged at 1,200 rpm for 12 min to remove the non-encapsulated ICG.

PM camouflage was performed as we recently described [4]. Mouse platelets were collected by gradient centrifugation, frozen at -80 °C and thawed at room temperature. After three freeze-thaw cycles, the membrane was obtained by centrifugation at 8,000 rpm for 10 min, washed with PBS containing protease inhibitor and sonicated for 5 min. PINPs (300  $\mu$ g, based on the content of ICG) dissolved in PBS (1 ml) were added to equal volumes of PM. The mixture was then sonicated on ice for 2 min and maintained at 4 °C overnight. The PINPs@PM was collected by high-speed centrifugation at 10,000 rpm for 15 min. Animals were cared for and treated in accordance with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals (NIH Publication No. 85e23 Rev. 1985) as approved by the Animal Experimental Ethics Committee of TMMU.

## Characterization analysis

The morphologies of the PINPs and PINPs@PM were obtained by TEM (Tecnai G2 F20 U-TWIN, FEI, Hillsboro, OR, US). To confirm the PM camouflage, PINPs@PM was denatured and resolved via 10% SDS-PAGE. The protein bands were visualized by Coomassie blue staining. The DLS and zeta potential experiments were determined by a Nano-ZS (Malvern, Worcestershire, UK) at room temperature. The temperature alteration of PINPs@PM exposed to NIR at 808 nm for different times (1.0 W/cm<sup>2</sup>, Laserwave, Beijing, CHN) was recorded with a thermoelectric thermometer (HH806W, Omega, US).

## Toxicological evaluation

HUVECs and 4T1 cells obtained from the Chinese Academy of Sciences (Shanghai, CHN) were cultured in RPMI 1640 medium containing 10% FBS. Ten thousand cells cultured to the logarithmic phase were incubated with different concentrations of PINPs@PM (0, 10, 20, 30, 40, and 50 mg/ml, based on the dose of ICG) for 24 h. The cell viability was determined by CCK-8 assay. Mouse erythrocytes diluted in saline solution (2%, v/v, 300 ml) were incubated with 1.2 ml of PINPs@PM at 37 °C for 2 h. The absorbance of the supernatant was determined at 405 nm. These experiments were conducted in triplicate and repeated twice. The toxicity of PINPs@PM *in vivo* was evaluated by a mouse experiment. Animals were cared for and treated as demonstrated in the preparation of PINPs@PM. PINPs@PM (60 µg, based on the content of ICG) was intravenously injected. The mice were sacrificed after 7 days, and their hearts, livers, spleens, lungs, and kidneys were obtained by surgery. The pathological changes were observed with an Olympus DX51 optical microscope (Tokyo, JPN) after HE staining.

## Flow cytometry-based endocytic assay

4T1 cells were seeded in a 6-well plate at a density of  $2 \times 10^5$  CFU per well and cultured overnight in RPMI 1640 medium containing 10% FBS. PINPs@PM (30 µg/ml, based on the content of ICG) was co-incubated with 4T1 cells for 6 and 12 h in the presence and absence of a 4-Gy X-ray irradiation pretreatment with an RS2000 X-ray irradiator (1.0 Gy/min, Rad Source, Suwanee, GA). The counts of fluorescent cells uptaking ICG-loaded PINPs@PM excited at 780 nm and the fluorescence intensity were determined with a BD flow cytometry system (Franklin Lakes, NJ, US), in which 15,000 events per sample were obtained. The experiment was repeated three times, and the data were processed using FlowJo software (version 7.6.1).

## Antitumor cell experiment

4T1 cells seeded in a 96-well plate at a density of  $1 \times 10^4$  CFU per well were cultured overnight and incubated with 30 µg/ml of PINPs@PM (based on the dose of ICG) in the presence and absence of a 4-Gy X-ray irradiation pretreatment. NIR (808 nm,  $1.0 \text{ W/cm}^2$ , 10 min) was performed after 12 h of co-incubation. Cells were then washed with sterile PBS, stained with 100 µl of DCFH-DA (10 µM) and AO dyes (5 µg/ml) according to the manufacturer's instructions and observed with an Olympus IX70 inverted fluorescence microscope. Otherwise, the cells were further incubated for 16 h followed by NIR treatment and the survival was detected by a CCK-8 assay. The experiment was conducted in duplicate and repeated three times.

## Tumor targeting evaluation

Female 8-week-old BALB/c mice were subcutaneously injected with  $2 \times 10^9$  CFU 4T1 cells in the right hind leg. Animals were cared for and treated as demonstrated in the preparation of PINPs@PM. A 4-Gy X-ray local irradiation was performed when the tumor reached approximately  $75 \text{ mm}^3$ . PINPs@PM (60 µg, based on the content of ICG) was intravenously administered 6 h later. The accumulation of PINPs@PM

in the tumor-bearing mice was observed with an IVIS spectrum *in vivo* imaging system (Perkin Elmer, Shanghai, CHN) at 24 h post-treatment. A Fortric 226s thermal imager (Shanghai, CHN) was used to obtain the infrared thermal images and record the temperature variations of the tumors along with the increasing NIR exposure times.

### **Antitumor assay *in vivo***

The tumor-bearing mice (n=5) were prepared and cared for as described in the tumor targeting evaluation. PINPs@PM (60 µg, based on the content of ICG) was given by tail vein injection in the presence and absence of a 4-Gy X-ray local irradiation. NIR treatment was continued for 10 min after 24 h. The tumor volumes were monitored for three weeks. The tumor tissues were obtained by surgery for TUNEL and HE staining.

### **Statistical analysis**

The significant differences (P) were calculated by SPSS 17.0 using the Student's two-tailed t test and LSD multiple-comparison test. A P value lower than 0.05 was defined as statistically significant.

## **Declarations**

### **Availability of data and materials**

All data generated or analyzed during this study are included in this published article.

### **Supplementary information**

Supplementary information accompanies this paper at

Additional file 1: TEM image of PINPs.

Additional file 2: Thermal images of NaCl-treated tumor-bearing mice.

### **Acknowledgements**

We appreciate Dr. Changhong Du (College of Preventive Medicine, Third Military Medical University) for the assistance in flow cytometry analysis.

### **Authors' contributions**

CY and SX performed the experiments. WC, WJ designed the experiments and drafted the manuscript. HS and WT contributed to the data analysis. CS and DS contributed to the manuscript preparation and review. ZJ and HY participated in the animal experiments. All authors approved the final manuscript.

### **Funding**

This work was supported by grants from the National Natural Science Fund of China (Nos. 81725019, 81930090, and 81703395), the Fund from PLA (No. AWS16J014), and the Natural Science Funds of Chongqing City (Nos. cstc2019jcyj-msxmX0011 and cstc2019jcyjqqX0005).

### **Ethics approval and consent to participate**

The study was approved by Ethics Committee of TMMU.

### **Consent for publication**

All the authors have approved the manuscript and agree with submission to your esteemed journal.

### **Competing interests**

The authors declare that they have no competing interests.

### **Author details**

<sup>1</sup> State Key Laboratory of Trauma, Burns and Combined Injury, Institute of Combined Injury of PLA, Chongqing Engineering Research Center for Nanomedicine, College of Preventive Medicine, Third Military Medical University, Chongqing, 400038, China. <sup>2</sup> Sichuan Industrial Institute of Antibiotics, Chengdu University, Chengdu, 610106, China. <sup>3</sup> College of Materials Science and Engineering, Hebei University of Engineering, Handan, 056038, China

## **References**

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin.* 2015;65:87-108.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin.* 2016; 66:7-30.
3. Wan G, Chen B, Li L, Wang D, Shi S, Zhang T, et al. Nanoscaled red blood cells facilitate breast cancer treatment by combining photothermal/photodynamic therapy and chemotherapy. *Biomaterials.* 2018; 155:25-40.
4. Chen Y, Zhao G, Wang S, He Y, Han S, Du C, et al. Platelet-membrane-camouflaged bismuth sulfide nanorods for synergistic radio-photothermal therapy against cancer. *Biomater Sci.* 2019;7:3450-3459.
5. Yuan Z, Tao B, He Y, Mu C, Liu G, Zhang J, et al. Remote eradication of biofilm on titanium implant via near-infrared light triggered photothermal/photodynamic therapy strategy. *Biomaterials.* 2019;223:119479.
6. Liu Y, Chen S, Sun J, Zhu S, Chen C, Xie W, et al. Folate-Targeted and Oxygen/Indocyanine Green-Loaded Lipid Nanoparticles for Dual-Mode Imaging and Photo-sonodynamic/Photothermal Therapy

- of Ovarian Cancer in Vitro and in Vivo. *Mol Pharm*. 2019; 16:4104-4120.
7. Jing L, Qu H, Wu D, Zhu C, Yang Y, Jin X, et al. Platelet-camouflaged nanococktail: Simultaneous inhibition of drug-resistant tumor growth and metastasis via a cancer cells and tumor vasculature dual-targeting strategy. *Theranostics*. 2018; 8:2683-2695.
  8. Oldenborg P-A, Zheleznyak A, Fang Y-F, Lagenaur CF, Gresham HD, Lindberg FP. Role of CD47 as a Marker of Self on Red Blood Cells. *Science*. 2000;288:2051-2054.
  9. Parodi A, Quattrocchi N, van de Ven AL, Chiappini C, Evangelopoulos M, Martinez JO, et al. Synthetic nanoparticles functionalized with biomimetic leukocyte membranes possess cell-like functions. *Nature Nanotechnology*. 2012;8:61-68.
  10. Yang H, Chen Y, Chen Z, Geng Y, Xie X, Shen X, et al. Chemo-photodynamic combined gene therapy and dual-modal cancer imaging achieved by pH-responsive alginate/chitosan multilayer-modified magnetic mesoporous silica nanocomposites. *Biomater Sci*. 2017; 5:1001-1013.
  11. Prabhakar U, Maeda H, Jain RK, Sevick-Muraca EM, Zamboni W, Farokhzad OC, et al. Challenges and key considerations of the enhanced permeability and retention effect for nanomedicine drug delivery in oncology. *Cancer Res*. 2013;73:2412-2417.
  12. Chai Z, Hu X, Wei X, Zhan C, Lu L, Jiang K, et al. A facile approach to functionalizing cell membrane-coated nanoparticles with neurotoxin-derived peptide for brain-targeted drug delivery. *J Control Release*. 2017; 264:102-111.
  13. Li L, Lu Y, Jiang C, Zhu Y, Yang X, Hu X, et al. Actively Targeted Deep Tissue Imaging and Photothermal-Chemo Therapy of Breast Cancer by Antibody-Functionalized Drug-Loaded X-Ray-Responsive Bismuth Sulfide@Mesoporous Silica Core-Shell Nanoparticles. *Adv Funct Mater*. 2018; 28:1704623.
  14. Hu Q, Sun W, Qian C, Wang C, Bomba HN, Gu Z. Anticancer Platelet-Mimicking Nanovehicles. *Adv Mater*. 2015; 27:7043-7050.
  15. Shen X, Li T, Chen Z, Xie X, Zhang H, Feng Y, et al. NIR-Light-Triggered Anticancer Strategy for Dual-Modality Imaging-Guided Combination Therapy via a Bioinspired Hybrid PLGA Nanoplatform. *Mol Pharm*. 2019; 16:1367-1384.
  16. Rao L, Bu L-L, Meng Q-F, Cai B, Deng W-W, et al. Antitumor Platelet-Mimicking Magnetic Nanoparticles. *Advanced Functional Materials*. 2017; 27:1604774.
  17. Gao M, Liang C, Song X, Chen Q, Jin Q, Wang C, et al. Erythrocyte-Membrane-Enveloped Perfluorocarbon as Nanoscale Artificial Red Blood Cells to Relieve Tumor Hypoxia and Enhance Cancer Radiotherapy. *Advanced Materials*. 2017; 29:1701429.
  18. Li R, He Y, Zhang S, Qin J, Wang J. Cell membrane-based nanoparticles: a new biomimetic platform for tumor diagnosis and treatment. *Acta Pharm Sin B*. 2018; 8:14-22.
  19. Borsig L. The role of platelet activation in tumor metastasis. *Expert Rev Anticancer Ther*. 2008;8:1247-1255.
  20. Shang Y, Wang Q, Wu B, Zhao Q, Li J, Huang X, et al. Platelet-Membrane-Camouflaged Black Phosphorus Quantum Dots Enhance Anticancer Effect Mediated by Apoptosis and Autophagy. *ACS*

- Appl Mater Interfaces. 2019; 11:28254-28266.
21. Yi X, Chen L, Chen J, Maiti D, Chai Z, Liu Z, et al. Biomimetic Copper Sulfide for Chemo-Radiotherapy: Enhanced Uptake and Reduced Efflux of Nanoparticles for Tumor Cells under Ionizing Radiation. *Advanced Functional Materials*. 2018; 28:1705161.
  22. Sabharwal SS, Schumacker PT. Mitochondrial ROS in cancer: initiators, amplifiers or an Achilles' heel? *Nature Reviews Cancer*. 2014; 14:709-721.
  23. Piskounova E, Agathocleous M, Murphy MM, Hu Z, Huddlestun SE, Zhao Z, et al. Oxidative stress inhibits distant metastasis by human melanoma cells. *Nature*. 2015; 527:186-191.
  24. Yu X, Lao Y, Teng X-L, Li S, Zhou Y, Wang F, et al. SENP3 maintains the stability and function of regulatory T cells via BACH2 deSUMOylation. *Nature Communications*. 2018; 9:3157.
  25. Gorrini C, Harris IS, Mak TW. Modulation of oxidative stress as an anticancer strategy. *Nature Reviews Drug Discovery*. 2013; 12:931-947.
  26. Ren H, Liu J, Li Y, Wang H, Ge S, Yuan A, et al. Oxygen self-enriched nanoparticles functionalized with erythrocyte membranes for long circulation and enhanced phototherapy. *Acta Biomaterialia*. 2017; 59:269-282.
  27. Wang H, Li X, Tse WC, Yang H, Thorling CA, Liu Y, et al. Indocyanine green-incorporating nanoparticles for cancer theranostics. *Theranostics*. 2018; 8:1227-1242.
  28. Wang D, Niu L, Qiao Z-Y, Cheng D-B, Wang J, Zhong Y, et al. Synthesis of Self-Assembled Porphyrin Nanoparticle Photosensitizers. *ACS Nano*. 2018; 12:3796-3803.
  29. Kaur P, Hurwitz MD, Krishnan S, Asea A. Combined hyperthermia and radiotherapy for the treatment of cancer. *Cancers (Basel)*. 2011; 3:3799-3823.
  30. Bonam SR, Wang F, Muller S. Lysosomes as a therapeutic target. *Nat Rev Drug Discov*. 2019;18:923-948.
  31. Mai TT, Hamai A, Hienzsch A, Caneque T, Muller S, Wicinski J, et al. Salinomycin kills cancer stem cells by sequestering iron in lysosomes. *Nat Chem*. 2017; 9:1025-1033.
  32. Shao M, Zhu W, Lv X, Yang Q, Liu X, Xie Y, et al. Encapsulation of chloroquine and doxorubicin by MPEG-PLA to enhance anticancer effects by lysosomes inhibition in ovarian cancer. *Int J Nanomedicine*. 2018; 13:8231-8245.
  33. Zhou W, Guo Y, Zhang X, Jiang Z. Lys05 induces lysosomal membrane permeabilization and increases radiosensitivity in glioblastoma. *J Cell Biochem*. 2019;121: 2027-2037.
  34. Guo M, Mao H, Li Y, Zhu A, He H, Yang H, et al. Dual imaging-guided photothermal/photodynamic therapy using micelles. *Biomaterials*. 2014; 35:4656-4666.
  35. Miller MA, Chandra R, Cuccarese MF, Pfirschke C, Engblom C, Stapleton S, et al. Radiation therapy primes tumors for nanotherapeutic delivery via macrophage-mediated vascular bursts. *Sci Transl Med*. 2017; 9: eaal0225.
  36. Hu C-MJ, Fang RH, Wang K-C, Luk BT, Thamphiwatana S, Dehaini D, et al. Nanoparticle biointerfacing by platelet membrane cloaking. *Nature*. 2015; 526:118-121.

37. Wang H, Liu Y, He R, Xu D, Zang J, Weeranoppanant N, et al. Cell membrane biomimetic nanoparticles for inflammation and cancer targeting in drug delivery. *Biomaterials Science*. 2020;DOI: 10.1039/C9BM01392J.
38. Shen X, Li T, Chen Z, Geng Y, Xie X, Li S, et al. Luminescent/magnetic PLGA-based hybrid nanocomposites: a smart nanocarrier system for targeted codelivery and dual-modality imaging in cancer theranostics. *Int J Nanomedicine*. 2017; 12:4299-4322.

## Figures

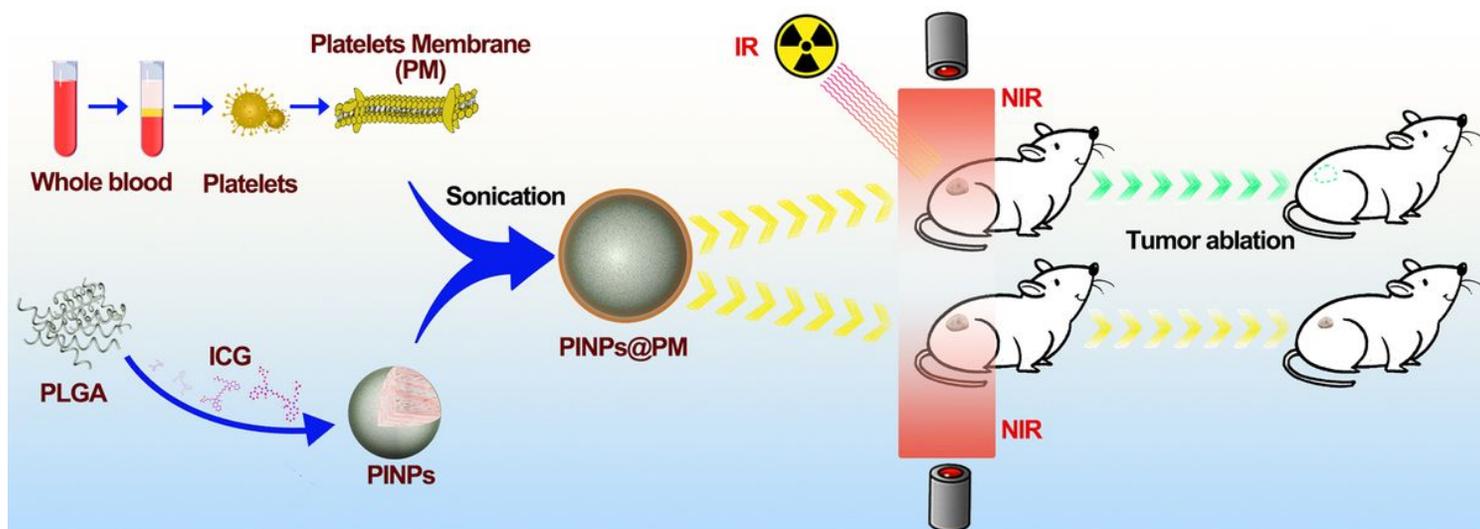
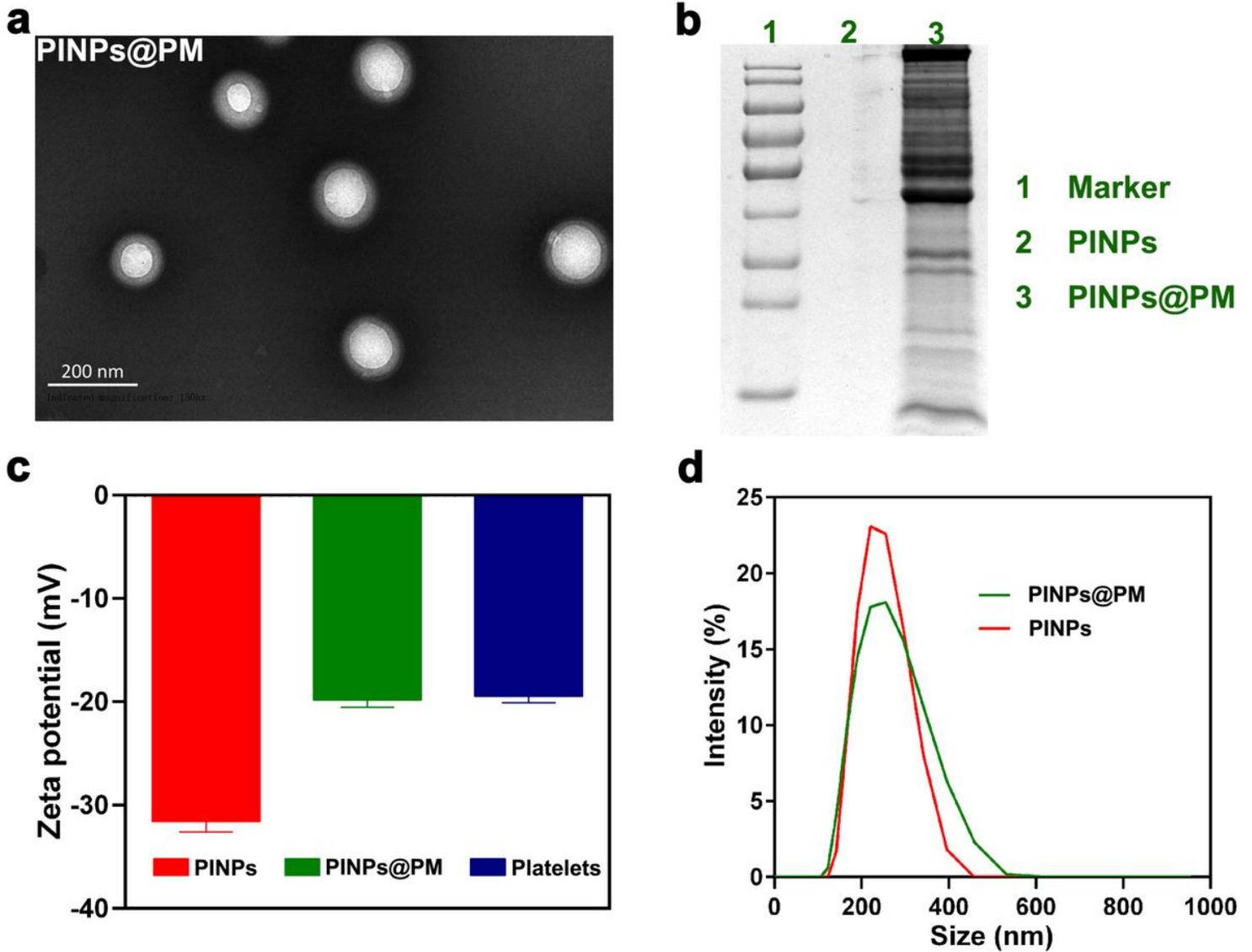


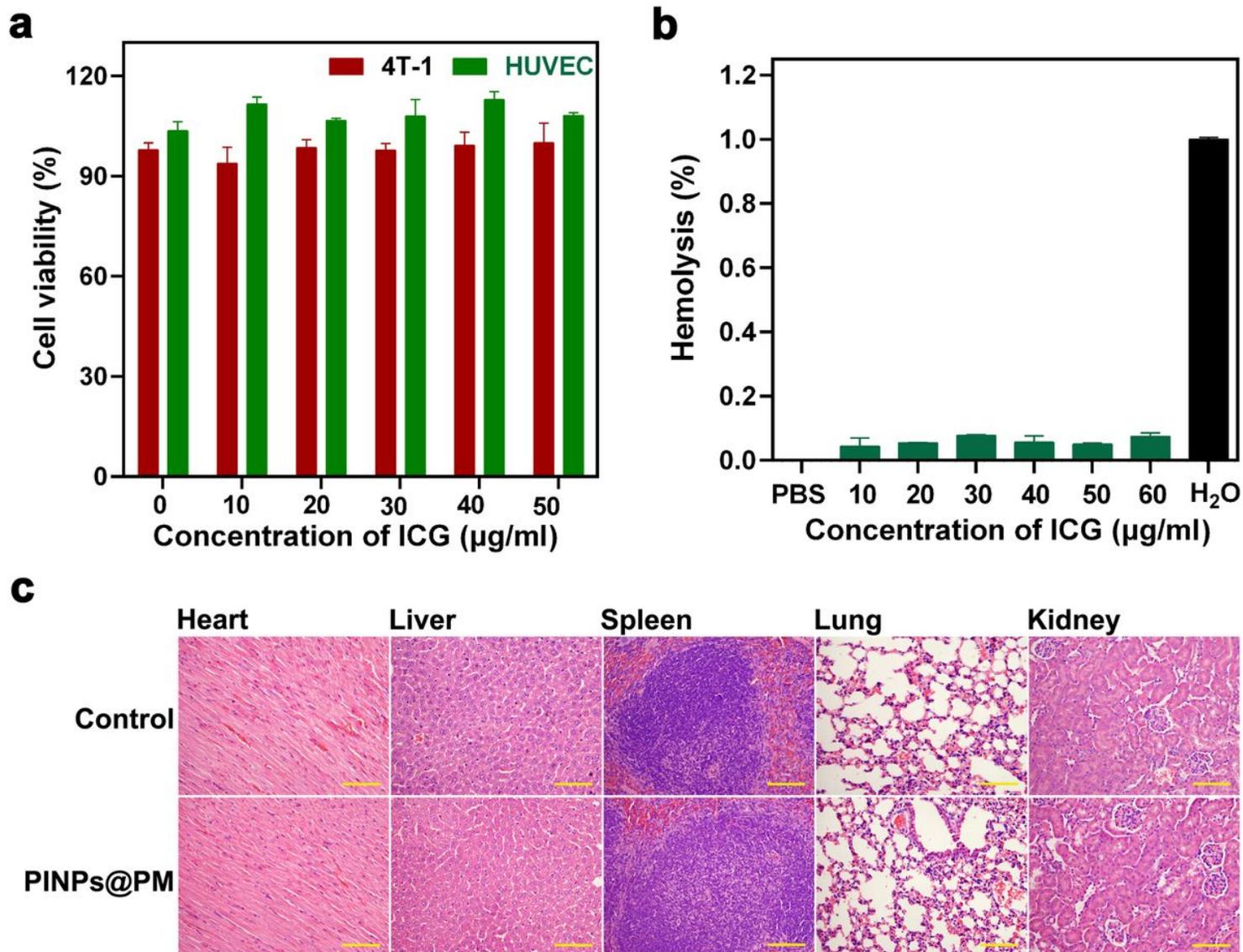
Figure 1

Diagrammatic drawing depicting the preparation and evaluation of PINPs@PM.



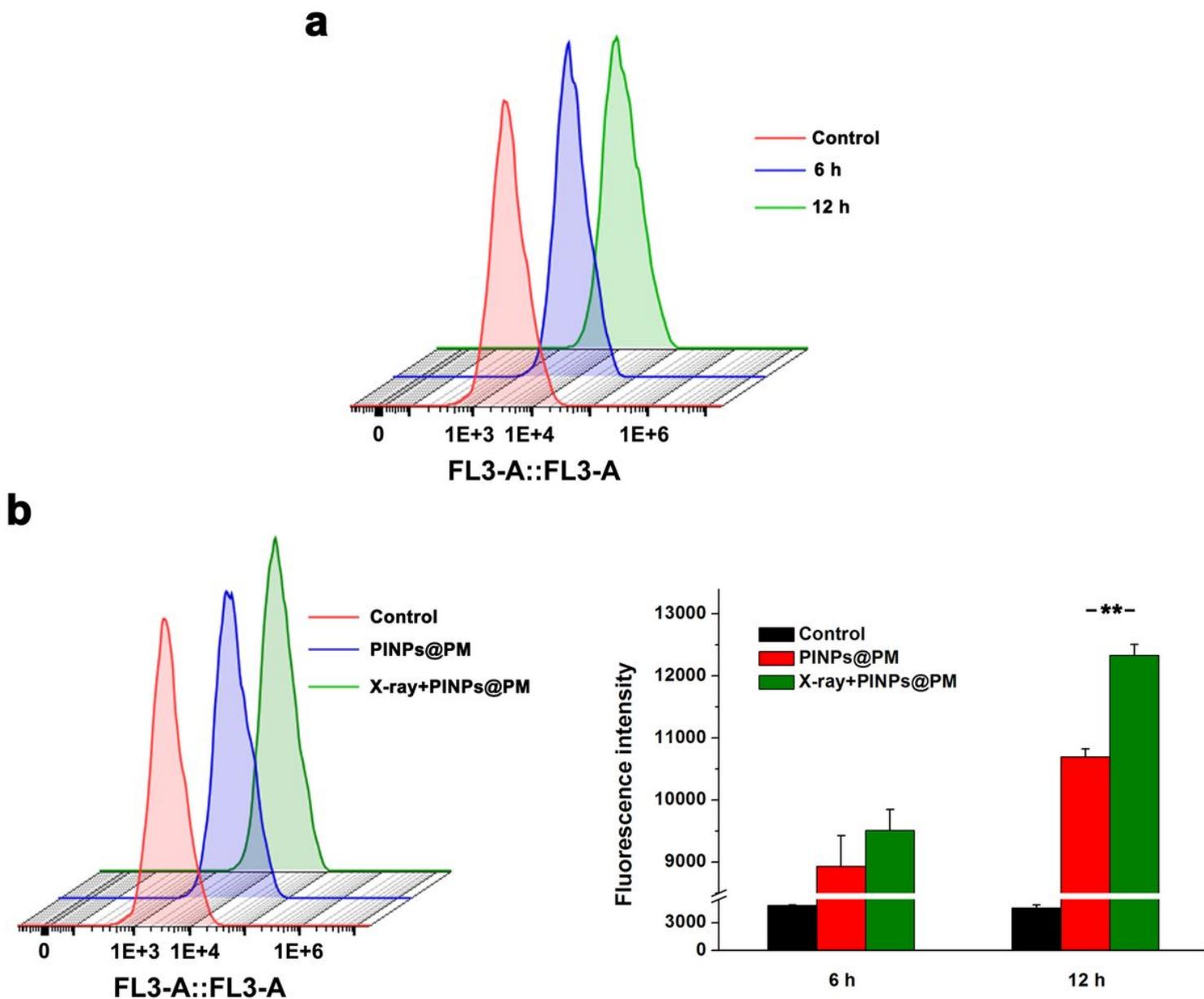
**Figure 2**

Characterization of PINPs@PM. a TEM image of PINPs@PM. The scale is 200 nm. b SDS-PAGE analysis. The PM protein (20  $\mu$ g) was resolved by 10% SDS-PAGE. c Surface charge determination. The results are shown as the means  $\pm$  SD. d DLS detection of the hydrodynamic diameters.



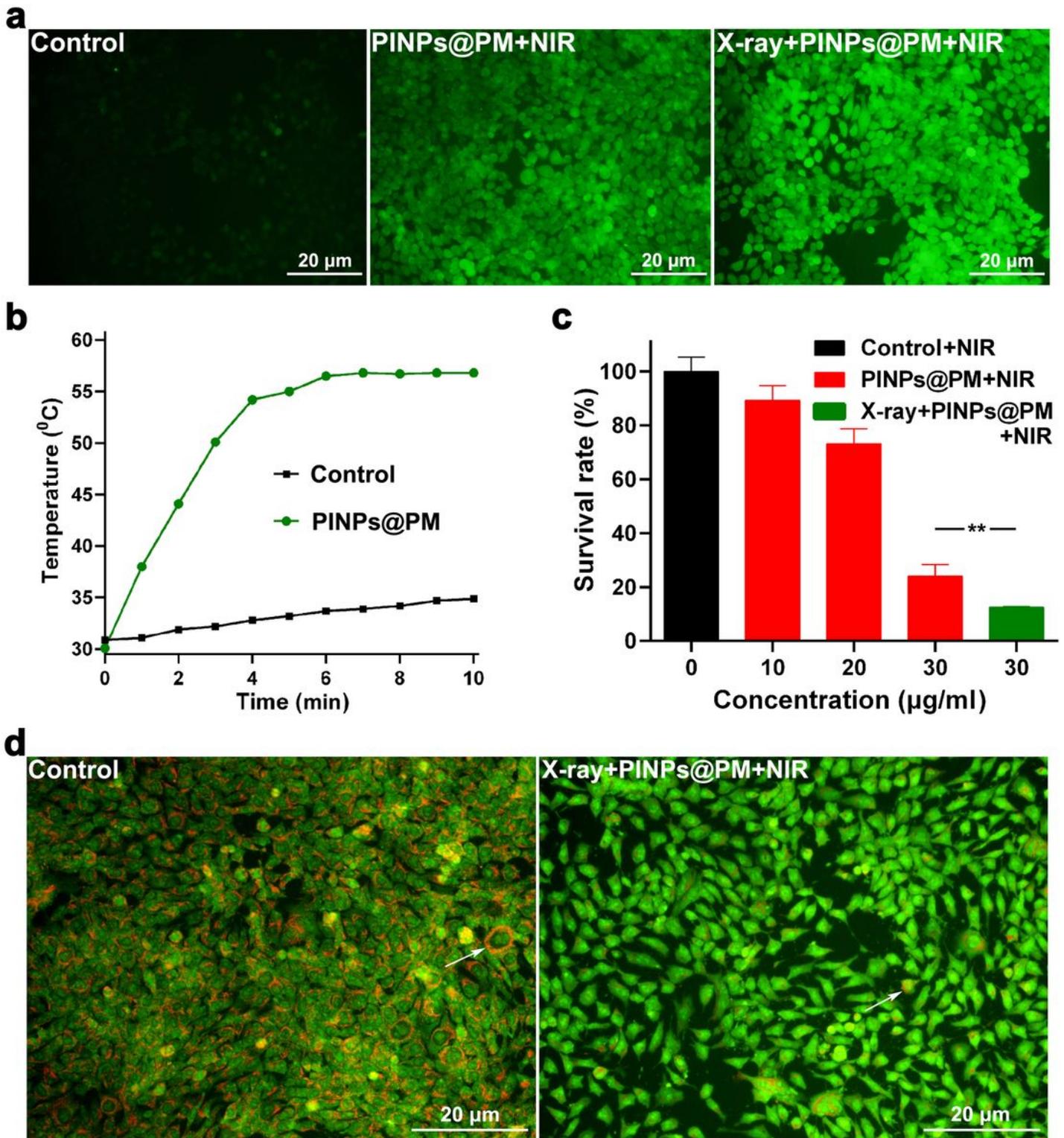
**Figure 3**

Toxicity evaluation of PINPs@PM. a The survival of HUVECs and 4T1 cells exposed to different concentrations of PINPs@PM for 24 h. The results are shown as the means  $\pm$  SD. b Haemolysis of different concentrations of PINPs@PM. c HE staining of the organs of mice treated with NaCl and PINPs@PM. The scale is 100  $\mu$ m.



**Figure 4**

Cellular uptake of PINPs@PM. a Flow cytometry of 4T1 cells treated with 30 µg/ml of PINPs@PM for 6 and 12 h. b Flow cytometry of 4T1 cells treated with PINPs@PM for 12 h in the presence and absence of irradiation pretreatment. The fluorescence intensity is presented as the mean  $\pm$  SD. \*\*,  $P < 0.01$ .



**Figure 5**

Irradiation pretreatment strengthens the antitumor activity of PINPs@PM in vitro. **a** ROS staining of 4T1 cells phagocytosing PINPs@PM in the presence and absence of X-ray irradiation. The scale is 20  $\mu\text{m}$ . **b** The temperature of PINPs@PM (30  $\mu\text{g/ml}$ ) upon excitation by NIR for different times. **c** The survival of 4T1 cells treated with PINPs@PM in the presence and absence of irradiation pretreatment. \*\*,  $P < 0.01$ . **d**

AO staining showing the damage of lysosome in 4T1 cells. White arrows indicate the representative cytoplasmic lysosome. The scale is 20  $\mu\text{m}$ .

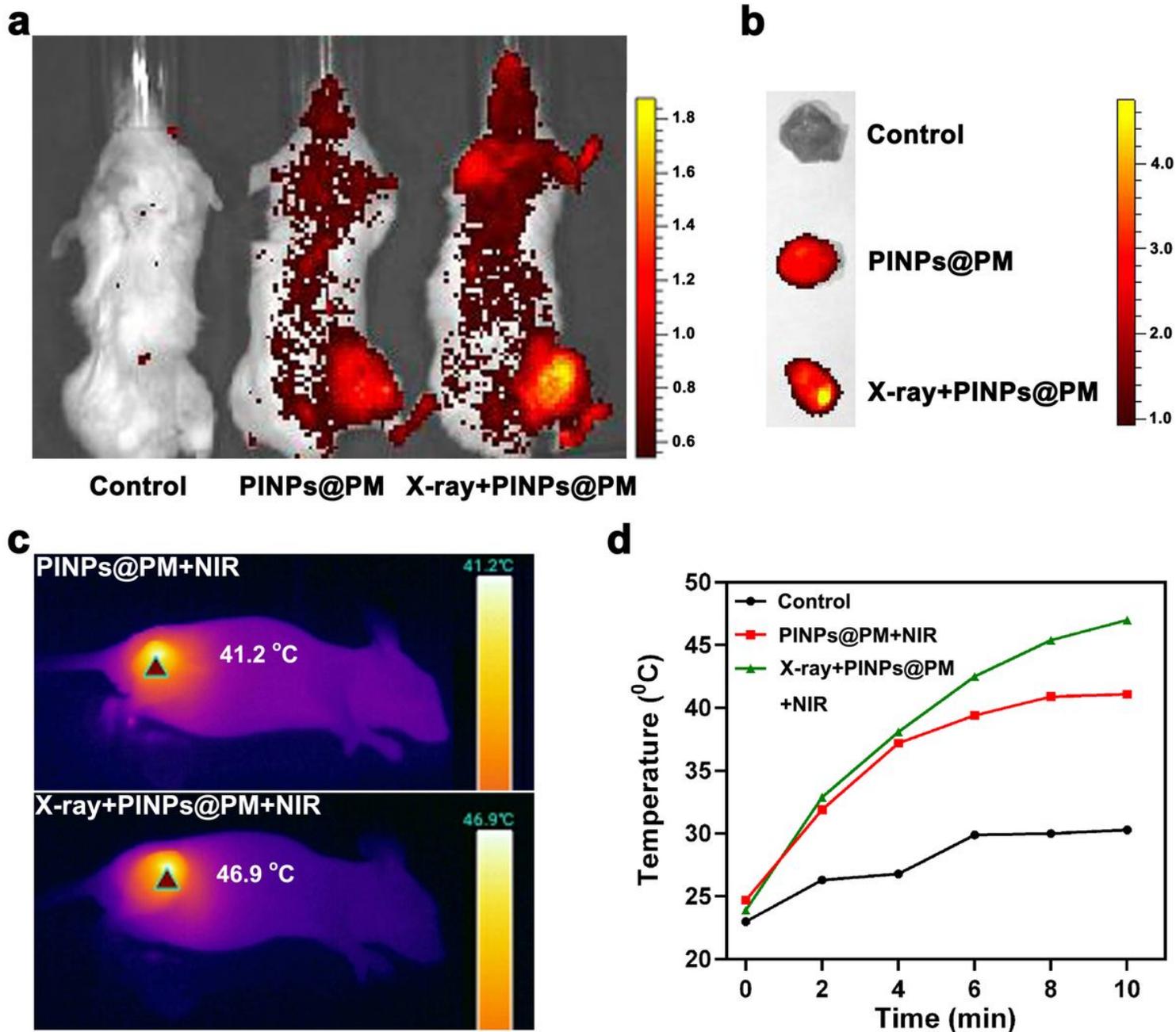
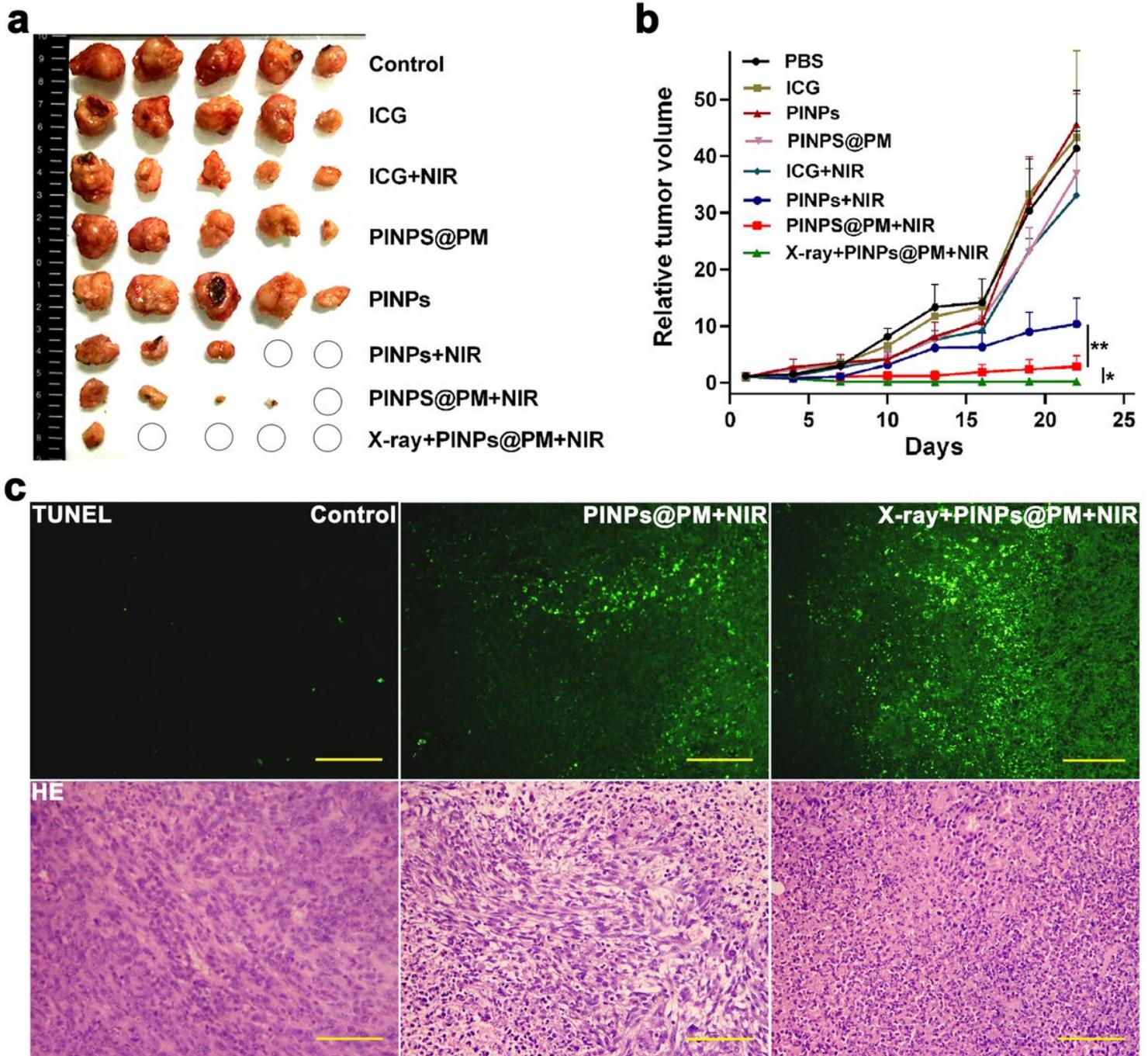


Figure 6

Irradiation pretreatment enhances the accumulation of PINPs@PM in the tumor site. a In vivo imaging of tumor-bearing mice at 24 h post injection of PINPs@PM. b In vitro imaging of PINPs@PM in tumors. c Infrared thermal images of tumor-bearing mice treated with PINPs@PM in the presence and absence of X-ray irradiation. d The maximal tumor temperature of PINPs@PM-treated mice after NIR treatment for different times.



**Figure 7**

Irradiation pretreatment improves the antitumor ability of PINPS@PM in vivo. a Photograph of mice tumors. The circles indicate that the tumors are visually eliminated. b Dynamic alterations of tumor volumes. \*\*,  $P < 0.01$ ; \*,  $P < 0.05$ . c TUNEL and HE staining showing the pathological changes of tumor tissues. The scale is 100  $\mu\text{m}$ .

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Additionalfile1.docx](#)
- [Additionalfile2.docx](#)