

Cascade-amplified cuproptosis in hepatocellular carcinoma via a smart hollow mesoporous CuS nanoplatform enabling synergistic phototherapy/chemodynamic therapy/starvation therapy

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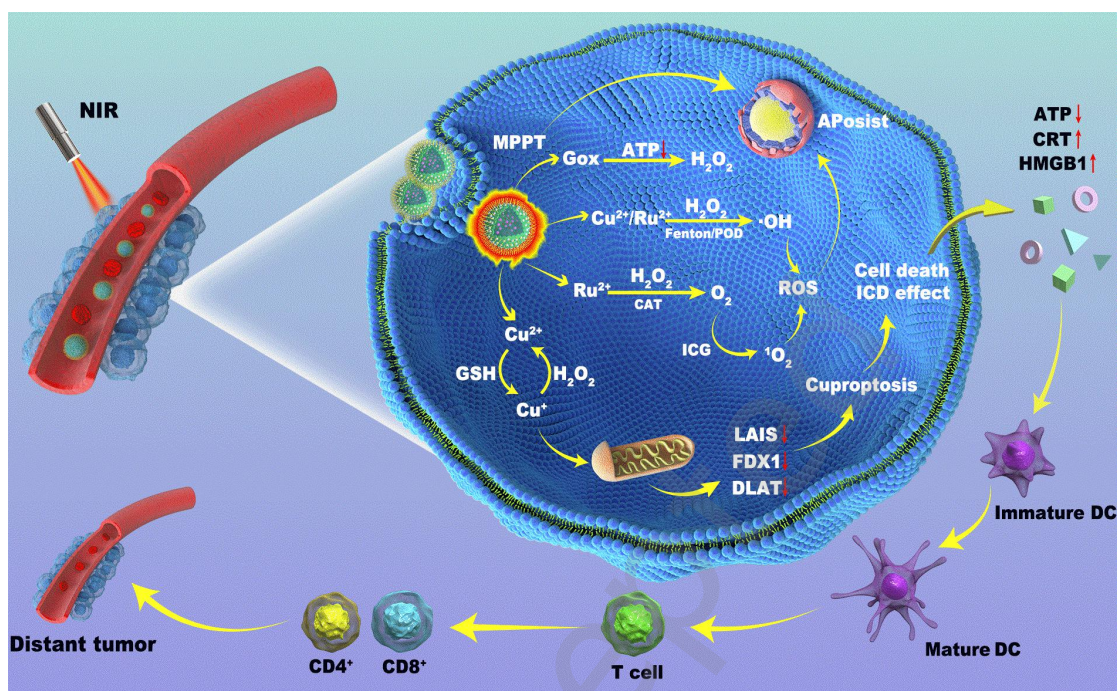
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TABLE OF CONTENTS (TOC)



A cascade-reaction nanoplatform (HMCuS/Ru/ICG/GOx@9R-P201Ti, HRIG9R) was developed to potentiate cancer immunotherapy. Guided by the 9R-P201 peptide, the nanoplatform enables precise tumor targeting and efficient delivery of a multifunctional nanocarrier. Intratumoral H_2O_2 is then catalytically converted into cytotoxic hydroxyl radicals ($\cdot\text{OH}$) via Ru/HMCuS bimetallic enzyme-mimetic activity, synergistically integrating starvation therapy with chemodynamic therapy (CDT). This cascade process potentiates cuproptosis induction and amplifies immunogenic cell death (ICD), thereby enhancing antitumor immune responses.

Cascade-amplified cuproptosis in hepatocellular carcinoma via a smart hollow mesoporous CuS nanoplatform enabling synergistic phototherapy/chemodynamic therapy/starvation therapy

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ABSTRACT: Emerging copper-mediated lethal mechanisms provide cancer therapies, with current research emphasizing precise copper regulation to enhance its dual role in tumor pathology. Here, we presented a bimetallic nanoplatform, HMCuS/Ru/ICG/GOx@9R-P201Ti (HRIG9R), designed to amplify cuproptosis for hepatocellular carcinoma (HCC) therapy. Upon 808 nm near-infrared (NIR) irradiation, the mild photothermal effect enhanced cytotoxic T-cell differentiation. Concurrently, glucose oxidase (GOx) depletes glucose and generated hydrogen peroxide (H₂O₂), fueling Ru-mediated chemodynamic therapy (CDT) and reducing Cu²⁺ to toxic Cu⁺ to potentiate cuproptosis. This synergistic cascade — integrating starvation, CDT, and adenosine triphosphate (ATP) depletion — reprogramed the tumor immune microenvironment and induced immunogenic cell death (ICD). These findings highlight the HMCuS/Ru/ICG/GOx@9R-P201Ti nanoplatform as a potent strategy to enhance cuproptosis-based anticancer immunotherapy.

KEYWORDS: immunotherapy; cuproptosis; cascade reaction; nanozyme; mild photothermal therapy

1. Introduction

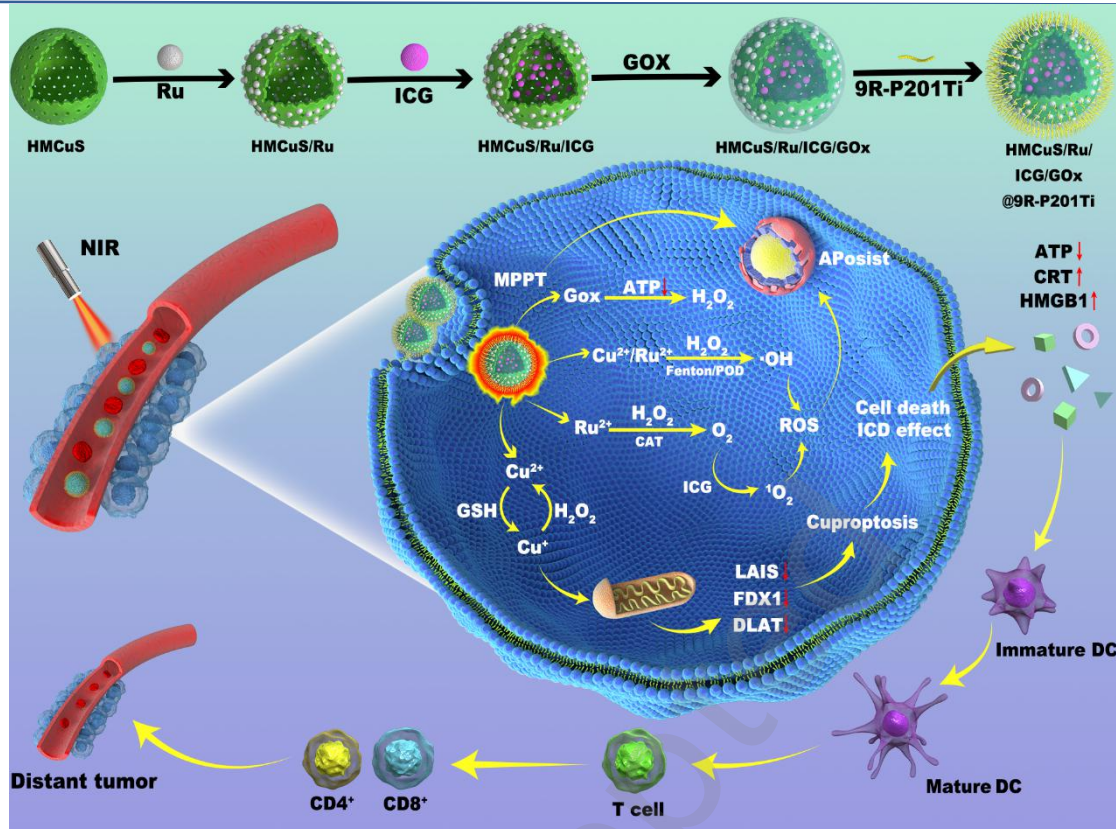
Hepatocellular carcinoma (HCC), ranking as the sixth most prevalent malignancy and the third leading cause of cancer-related mortality globally, is burdened by a dismal five-year survival rate of merely 18% [1-7]. The therapeutic efficacy of conventional chemotherapy is severely hampered by intrinsic and acquired drug resistance, necessitating the exploration of novel modalities [8-10]. Given the liver's pivotal role in systemic copper homeostasis, HCC cells exhibit exquisite sensitivity to copper dysregulation, rendering them exceptionally susceptible to copper-induced cytotoxicity (cuproptosis) [11-13].

First described by Tsvetkov et al. in 2022 [14], cuproptosis represents a paradigm shift in regulated cell death, mechanistically distinct from apoptosis, ferroptosis, or necroptosis. This process is initiated by the direct binding of excess copper to lipoylated components of the tricarboxylic acid (TCA) cycle, specifically dihydrolipoamide S-acetyltransferase (DLAT). This interaction triggers toxic protein oligomerization and subsequent loss of iron-sulfur (Fe-S) cluster proteins (e.g., LIAS), culminating in proteotoxic stress and irreversible cell death [15-19]. Despite its therapeutic potential, the clinical translation of cuproptosis is impeded by two primary challenges: the inability to achieve tumor-specific copper overload and the pervasive glutathione (GSH)-rich, hypoxic tumor

microenvironment (TME), which chelates free copper ions and attenuates therapeutic efficacy [20,21].

To surmount these barriers, we engineered a nucleus-targeting bimetallic nanoplatform, HMCuS/Ru/ICG/GOx@9R-P201Ti (HRIG9R). This system integrates hollow mesoporous copper sulfide (HMCuS) ruthenium (Ru) complexes, capitalizing on Ru intrinsic peroxidase (POD)- and catalase (CAT)- like activities [22-24]. Notably, Ru²⁺ forms stable coordination complexes with GSH ([Ru(GSH)₂]), effectively depleting this antioxidant reservoir and creating a pro-oxidative milieu conducive to cuproptosis [25-27].

As illustrated in Scheme 1, the nanoplatform orchestrates a cascaded synergistic therapy. Indocyanine green (ICG) facilitates real-time fluorescence tracking and generates singlet oxygen (¹O₂) under near-infrared (NIR) irradiation, initiating mild photothermal therapy (MPTT) [28-30]. Concurrently, GOx catalyzes intratumoral glucose oxidation, depriving tumor cells of nutrients (starvation therapy) while generating H₂O₂ to fuel Ru-mediated chemodynamic therapy (CDT) [31-36]. This self-reinforcing cycle reduces Cu²⁺ to the more toxic Cu⁺ species, amplifying cuproptosis. Functionalization with the 9R-P201Ti peptide ensures precise HCC targeting. Ultimately, this multimodal assault induces potent immunogenic cell death (ICD), promoting the release of tumor-associated antigens (TAAs) and damage-associated molecular patterns (DAMPs), thereby remodeling



Scheme 1. Illustration of the preparation process of the HMCuS/Ru/ICG/GOx@9R-P201Ti (HRIG9R) nanoplatform and its mechanism for multimodal synergistic therapy against HCC.

the immunosuppressive TME into an immunostimulatory phenotype capable of eradicating primary tumors and inhibiting distant metastasis [37–42].

2. Materials and methods

2.1 Materials

Chemicals including $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (AR), $\text{RuCl}_3 \cdot 3\text{H}_2\text{O}$ (AR), polyvinylpyrrolidone (PVP, MW ≈ 8000), $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$ (50%) were purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Thiazolyl blue tetrazolium bromide (MTT, $\geq 98\%$), trypsin, penicillin-streptomycin solution (100 $\times$), and Hoechst 33342 were obtained from Servicebio Biotechnology Co., Ltd (Wuhan, China). Assay kits for adenosine triphosphate (ATP), H_2O_2 , and GSH assay kits were sourced from Solarbio Science & Technology Co., Ltd. (Beijing, China). All reagents were of analytical grade and used without further purification.

2.2 Synthesis of HMCuS

HMCuS NPs were synthesized via a sacrificial template method with slight modifications[43]. Briefly, CuCl_2 (2 mL, 0.5 M) and PVP (4 g) were dissolved in ultrapure water, followed by the addition of hydrazine hydrate (2 mL). Subsequently, $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ (2 mL, 280 mg/mL) was introduced dropwise under vigorous stirring. The reaction was maintained at 60 $^\circ\text{C}$ for 2 h, after which the product was isolated by centrifugation (12,000 rpm, 10 min), washed sequentially with deionized water and ethanol, and redispersed in deionized water for storage.

2.3 Synthesis of HMCuS@Ru

The HMCuS@Ru was constructed via a galvanic replacement reaction. Briefly, HMCuS (20 mg) and $\text{RuCl}_3 \cdot 3\text{H}_2\text{O}$ (48 mg) were dispersed in 50 mL of ethylene glycol and sonicated. Then, 1.5 mL of methyl benzoate (0.1 M) as a reducing agent and PVP

(2 g) as a stabilizer were added, respectively. The mixture was transferred to a Teflon-lined autoclave and reacted at 180 $^\circ\text{C}$ for 3 h. The resultant nanocomposites were purified by centrifugation (14,000 rpm, 10 min) using an ethanol/acetone mixture (1:10, v/v).

2.4 Synthesis of HMCuS@Ru/ICG

ICG was loaded onto the HMCuS@Ru via physical adsorption. Briefly, HMCuS@Ru (2 mL, 1 mg/mL) was mixed with ICG (1 mg) in phosphate-buffered saline (PBS, pH 7.4) and stirred in the dark for 24 h. The conjugate was collected by centrifugation (12,000 rpm, 10 min), washed twice with PBS to remove free dye, and stored at 4 $^\circ\text{C}$ in the dark.

2.5 Synthesis of HMCuS@Ru/ICG/GOx

GOx was conjugated to HMCuS@Ru/ICG via electrostatic adsorption leveraging under acidic conditions (pH 4.0). HMCuS@Ru/ICG (1.5 mg/mL) was incubated with GOx (1 mg/mL) at a 1.5:1 volume ratio under gentle stirring at 4 $^\circ\text{C}$ for 3 h. The resulting HMCuS@Ru/ICG/GOx composite was isolated by centrifugation (12,000 rpm, 10 min), washed three times with ice-cold PBS (pH 7.4) to eliminate any unbound enzyme, and stored at 4 $^\circ\text{C}$.

2.6 Synthesis of HMCuS@Ru/ICG/GOx/9R-P201Ti (HRIG9R)

The nanoplatform was functionalized with the HCC-targeting peptide 9R-P201Ti. HMCuS@Ru/ICG/GOx (3 mg) was incubated with an equal mass of the peptide in 5 mL PBS (pH 7.4), sonicated (200 W, 5 min), and stirred in the dark for 12 h. The final product (HRIG9R) was purified by repeated centrifugation/washing cycles and lyophilized for long-term storage at 4 $^\circ\text{C}$.

2.7 Characterization

The ultraviolet-visible-near infrared (UV-Vis-NIR) absorption

spectra of the samples were recorded on a Shimadzu UV-3600 Plus spectrophotometer. The zeta potential and hydrodynamic diameter were determined using a Malvern Nano ZS90 analyzer via dynamic light scattering (DLS). Fourier-transform infrared (FT-IR) spectra were acquired on a Bruker VERTEX 70 spectrometer. Morphological and microstructural analysis was performed using a JEOL JEM-F200 field emission transmission electron microscopy (FE-TEM) operating at 200 kV.

2.8 Photothermal performance

The photothermal conversion efficiency of the HRIG9R was systematically investigated under 808 nm laser irradiation at varying concentrations (0, 20, 50, 100, 150, 200 $\mu\text{g/mL}$) and power densities (0.5 to 1.5 W/cm^2). Photothermal stability was assessed over five on/off cycles. Temperature variations were monitored in real-time using an infrared thermal camera (TiS75⁺), with measurements taken at one-minute intervals for the control, free ICG, and HRIG9R groups. All experiments were performed in triplicate.

2.9 Production of reactive oxygen species (ROS) and oxygen (O_2) in vitro

The $\cdot\text{OH}$ generation was monitored via the oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) at 652 nm. O_2 production was quantified using a JPB-607A dissolved oxygen meter under a liquid paraffin seal. $^1\text{O}_2$ production was determined using 9,10-anthracenediyl-bis(methylene)dimalonic acid (ABDA) as a probe.

2.10 GSH depletion

The GSH depletion was evaluated using the 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) method. The reduction of DTNB to 2-nitro-5-thiobenzoic acid (TNB) ($\lambda=412\text{ nm}$) was monitored under two sets of conditions: (1) varying HRIG9R concentration (0–200 $\mu\text{g/mL}$) at a fixed GSH level, and (2) varying GSH concentrations at a fixed HRIG9R concentration. The depletion efficiency was quantified by measuring the decrease in absorbance at 412 nm using a microplate reader.

2.11 Detection of H_2O_2 production

GOx-mediated H_2O_2 production was quantified using a commercial assay kit. Fluorescence intensity was measured at Ex/Em: 571/585 nm using a microplate reader.

2.12 Cell culture

Human HCC (HepG2, SNU-739), cervical carcinoma (HeLa), and normal hepatocytes (HL-7702) cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (100 $\times$) at 37 $^\circ\text{C}$ in a 5% CO_2 humidified incubator.

2.13 In vitro cytotoxicity

The cytotoxicity was evaluated via the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were treated with serial concentrations (10, 20, 50, 100, and 200 $\mu\text{g/mL}$) of samples for 24 h. Formazan crystals were dissolved in dimethyl sulfoxide (DMSO), and absorbance was measured at 490 nm using a microplate reader to determine cell viability.

2.14 Cellular uptake and intracellular localization

HepG2 and HL-7702 cells were incubated with HRIG9R for different time periods (0, 2, 4, 6, 8, 12 h). Nuclei were counterstained with Hoechst 33342, and cellular uptake was visualized using confocal laser scanning microscopy (CLSM).

2.15 Live/dead cell staining

Post-treatment with HRIG9R $\pm$ 808 nm laser irradiation, cells were stained with Calcein-AM/propidium iodide (PI). Live

(green) and dead (red) cells were imaged using a fluorescence microscope.

2.16 Intracellular ROS detection

Intracellular ROS levels were detected using the 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) probe. Cells were incubated with 10 μM DCFH-DA for 30 min post-treatment, and fluorescence were imaged using an inverted fluorescence microscope.

2.17 Intracellular GSH and H_2O_2

HepG2 cells were treated with various formulations (saline, HR, HRIG, HRIG9R, ICG+NIR, HRIG+NIR, HRIG9R+NIR) for 24 h. Intracellular GSH and H_2O_2 levels were quantified using commercial assay kits according to the manufacturer's protocol.

2.18 Detection of mitochondrial membrane potential (MMP)

MMP disruption was evaluated using the JC-1 fluorescent probe. HepG2 cells were incubated with JC-1 at 37 $^\circ\text{C}$ in the dark for 20 min, and the red/green fluorescence ratio was analyzed by CLSM.

2.19 Induction of ICD in vitro

ICD marker expression (calreticulin (CRT) and high-mobility group box 1 (HMGB1)) was evaluated by immunofluorescence. Intracellular ATP levels were quantified using a commercial ATP assay kit according to the manufacturer's protocol.

2.20 In vivo biodistribution

H22 tumor-bearing mice received HRIG9R (5 mg/kg) via tail vein injection. Fluorescence imaging was performed at predetermined time points (0–36 h) using a small-animal imaging system. Major organs and tumors were harvested for ex vivo imaging.

2.21 In vivo antitumor efficacy

Mice with established tumors ($\sim 100\text{ mm}^3$) were randomized into seven groups ($n=7$): Saline, HR, HRIG, HRIG+NIR, ICG+NIR, HRIG9R, and HRIG9R+NIR. Treatments were administered every other day for 14 days. Tumor volume ($V = L \times W^2/2$) and body weight were monitored. Tumors and organs were harvested for H&E, Ki67, TUNEL, and flow cytometry analyses.

2.22 In vivo and ex vivo biodistribution

H22 tumor-bearing mice received tail-vein injections of HRIG9R (5 mg/kg). Longitudinal fluorescence imaging was performed at preset intervals (0, 1, 2, 4, 8, 12, 24, and 36 h) using a small animal imaging system. Upon sacrifice, major organs (heart, liver, spleen, lung, kidney) and tumors were excised for ex vivo fluorescence imaging.

2.23 In vivo therapeutic efficacy and immune profiling

Mice with tumors ($\sim 100\text{ mm}^3$) were randomized into seven groups ($n=7$ per group): Saline, HR, HRIG, HRIG+NIR, ICG+NIR, HRIG9R, and HRIG9R+NIR. Formulations (200 μL) were administered intravenously, and phototherapy groups received 808 nm NIR irradiation 24 h later. Body weights and tumor volumes ($V = (L \times W^2)/2$) were monitored for 14 days. Tumors, blood, and major organs were harvested for hematoxylin and eosin (H&E), Ki67 immunohistochemistry, TUNEL, and flow cytometry analyses. Single-cell suspensions from tumors, liver, and lymph nodes were stained with CD86-FITC, CD80-APC, CD11c-PE, CD3-FITC, CD4-APC, CD8-APC, and CD38-PE (Sev $\acute{e}$ r Biotechnology) to profile immune cell populations.

2.24 Systemic antitumor immunity (distant effect)

A bilateral H22 tumor model was established. The primary tumor was treated, while the distal tumor remained untreated.

Growth of the distal tumor was monitored for 14 days to assess abscopal effects. Upon termination, serum, tumor (both primary and distal), and major organs were collected for further analysis.

2.25 Serum cytokine analysis

Serum concentrations of tumor necrosis factor-alpha (TNF- α), interferon-gamma (IFN- γ), and interleukin-6 (IL-6) were quantified using enzyme-linked immunosorbent assay (ELISA) kits [44].

2.26 Western blot analysis

Protein expression related to cuproptosis (e.g., DLAT, FDX1, LIAS), cell cycle regulation (e.g., FOXM1, Skp2, Cks1, P27Kip1), and apoptosis (e.g., Bak, PARP-1, Bcl-2, Caspase-8) were analyzed by Western blotting.

2.27 Statistical analysis

All quantitative data are represented as mean $\pm$ standard deviation (SD). Statistical significance was determined using one-way analysis of variance (ANOVA), Student's t-test, or two-way ANOVA, as appropriate. A P-value of less than 0.05 was considered statistically significant (*P < 0.05, **P < 0.01, ***P < 0.001).

3. Results and discussion

3.1 Fabrication and characterization of HRIG9R

The HMCuS/Ru/ICG/GOx@9R-P201Ti (HRIG9R)

nanoplateform was constructed via a sequential modular strategy (Scheme 1). Initially, HMCuS NPs served as a bimetallic scaffold. Ru was deposited via galvanic replacement, leveraging the higher reduction potential of Ru³⁺/Ru over Cu²⁺/Cu. ICG was physically adsorbed for theranostic capabilities, while GOx was immobilized to initiate starvation therapy and generate H₂O₂. Finally, the HCC-targeting nuclear localization peptide 9R-P201Ti was conjugated to confer nuclear targeting specificity.

TEM confirmed the evolution of morphology (Figure 1A-D). HMCuS possessed a well-defined hollow structure, uniform size, and good dispersity. Post-Ru modification, distinct nanoparticle attachments were visible on the CuS matrix (Figure 1B). GOx coating resulted in a discernible surface corona (Figure 1C), and the final peptide functionalization yielded an amorphous outer shell (Figure 1D). DLS revealed that HRIG9R had a hydrodynamic diameter of approximately 160 nm (Figure 1I), optimal for enhanced permeability and retention (EPR) effect. Elemental mapping (Figure 1E) confirmed the homogeneous distribution of phosphorus (P) (from GOx) and the colocalization of Cu, S, and Ru, validating successful integration. Energy-dispersive X-ray spectroscopy (EDS) further corroborated the elemental composition (Figure S1).

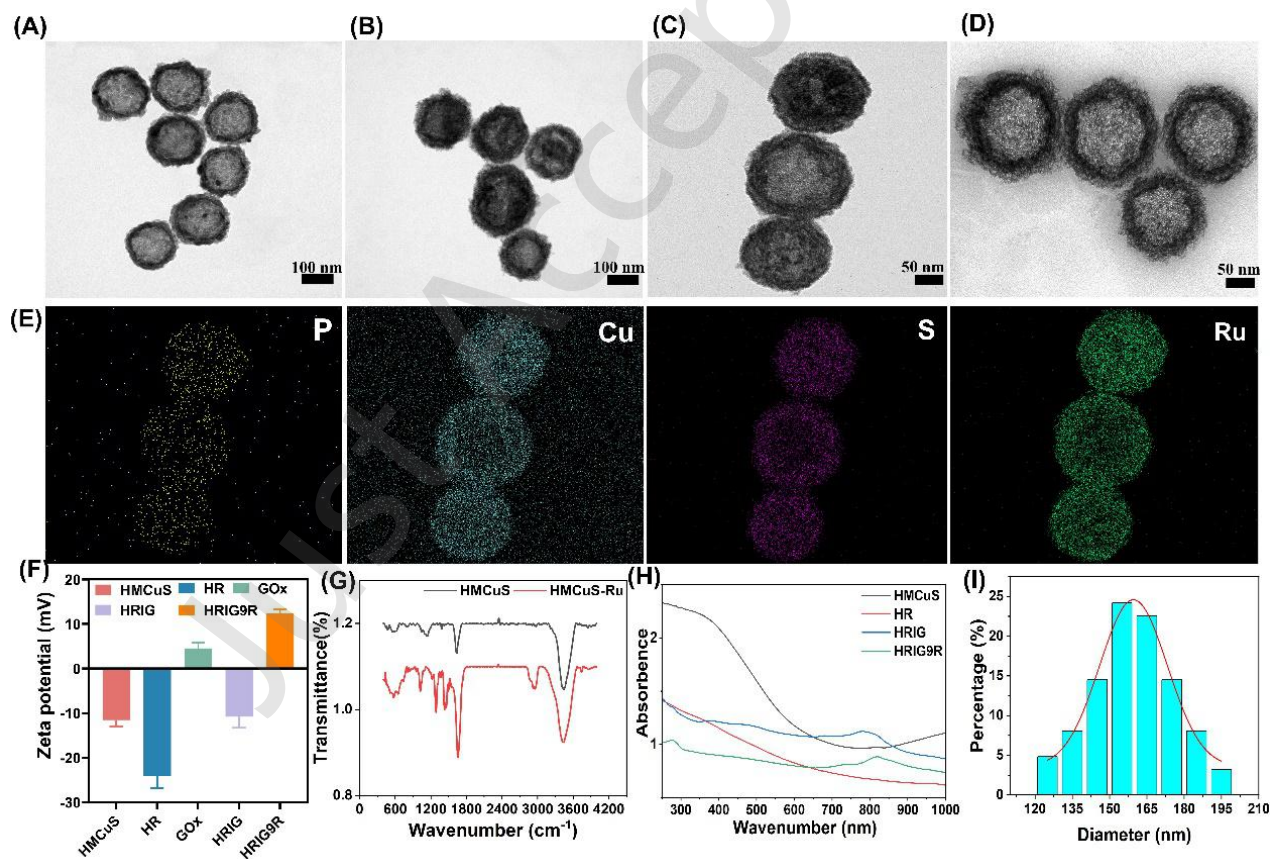


Figure 1 Physicochemical characterization of HRIG9R nanoplateform. (A-D) TEM images of (A) HMCuS, (B) HR, (C) HRIG, and (D) HRIG9R nanoplateform. (E) Elemental mapping image of HRIG showing the distribution of Cu, S, Ru, and P. (F) Zeta potential of HMCuS, HR, free GOx, HRIG, and HRIG9R. (G) FTIR spectra of HMCuS and HR. (H) UV-Vis-NIR absorption spectra of HMCuS, HR, HRIG and HRIG9R. (I) Hydrodynamic size distribution of HRIG9R measured by DLS.

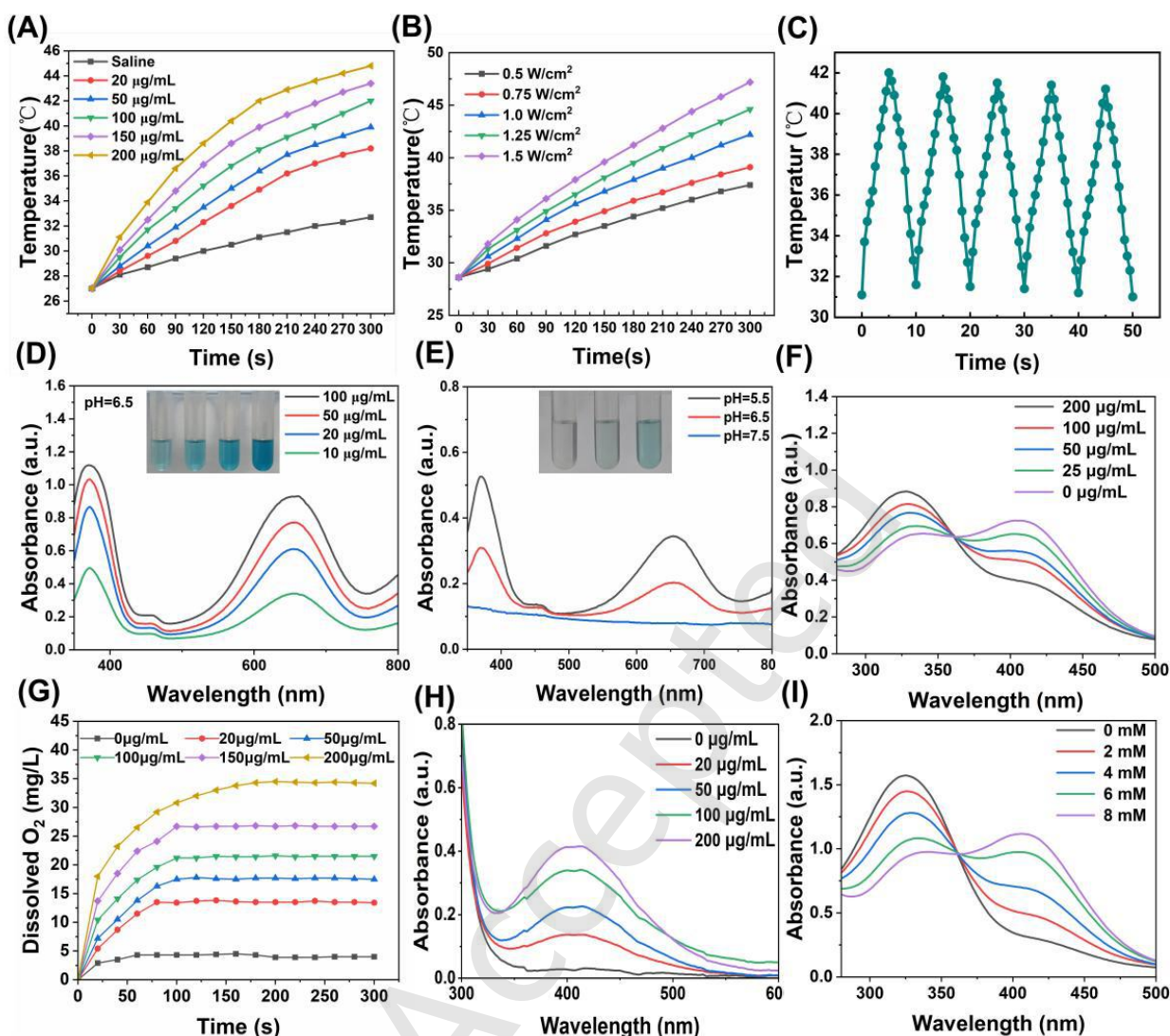


Figure 2 In vitro assessment of PTT and CDT performance. (A) Concentration-dependent photothermal heating curves of HRIG9R under 808 nm laser irradiation (1.0 W/cm^2). (B) Temperature profiles of HRIG9R (fixed concentration) under varying laser power densities. (C) Photothermal stability of HRIG9R evaluated over five laser on/off cycles. (D) Concentration-dependent $\cdot\text{OH}$ generation, quantified via the TMB probe. (E) pH-responsive $\cdot\text{OH}$ generation by HRIG9R under simulated TME acidity. (F) Concentration-depletion of GSH, monitored using the DTNB assay. (G) The ability of different drug concentrations to produce O_2 . (H) Concentration-dependent H_2O_2 production, confirming GOx enzymatic activity. (I) Time-dependent consumption of GSH by HRIG9R at fixed concentrations

Zeta potential measurements tracked the surface charge evolution (Figure 1F). The potential shifted from -11 mV (HMCuS) to -25 mV (post-Ru), then to -10 mV (post-GOx), and finally reversed to $+12 \text{ mV}$ after 9R-P201Ti conjugation, confirming stepwise assembly. FT-IR spectroscopy (Figure 1G) identified a new band near 3000 cm^{-1} corresponding to Cu–S–Ru bond. UV-Vis spectroscopy (Figure 1H) showed attenuated HMCuS absorption at 800 nm post-modification, indicating successful cargo loading.

3.2 Stability of materials

The physicochemical stability of the material constitutes a critical prerequisite for the subsequent experiments. To assess storage stability, the hydrodynamic diameter (D_h) was monitored over a continuous 7-day period under physiologically relevant storage conditions (37°C , protected from light). As presented in Figure S2, the D_h remained within $[80 \pm 5 \text{ nm}]$ (mean $\pm$ SD, $n=5$) with no statistically significant deviation over the entire observation window ($p > 0.05$, one-way ANOVA), indicating that the as-prepared nanoplateform exhibits exceptional colloidal

stability and is amenable to routine handling without appreciable aggregation or degradation.

3.3 ICG loading efficiency

ICG loading efficiency was quantified using a standard calibration curve (Figure S3A). Under varying mass feed ratios (1:1 to 1:4), the encapsulation efficiency were determined to be 21.9%, 47.5%, 62.3%, and 80.1%, respectively (Figure S3B), demonstrating tunable drug loading capacity.

3.4 MPTT performance

HRIG9R exhibited strong NIR absorption at 800 nm (Figure 2A). Under 808 nm laser irradiation (1.0 W/cm^2), the HRIG9R group demonstrated a rapid temperature surge compared to saline and free ICG controls (Figure 2B, Figure S4). Crucially, the nanoplateform maintained excellent photostability over five on/off laser cycles (Figure 2C), confirming its robustness for repeated photothermal applications.

3.5 CDT and enzyme-mimetic activities

HRIG9R catalyzes $\cdot\text{OH}$ generation via dual pathways: a

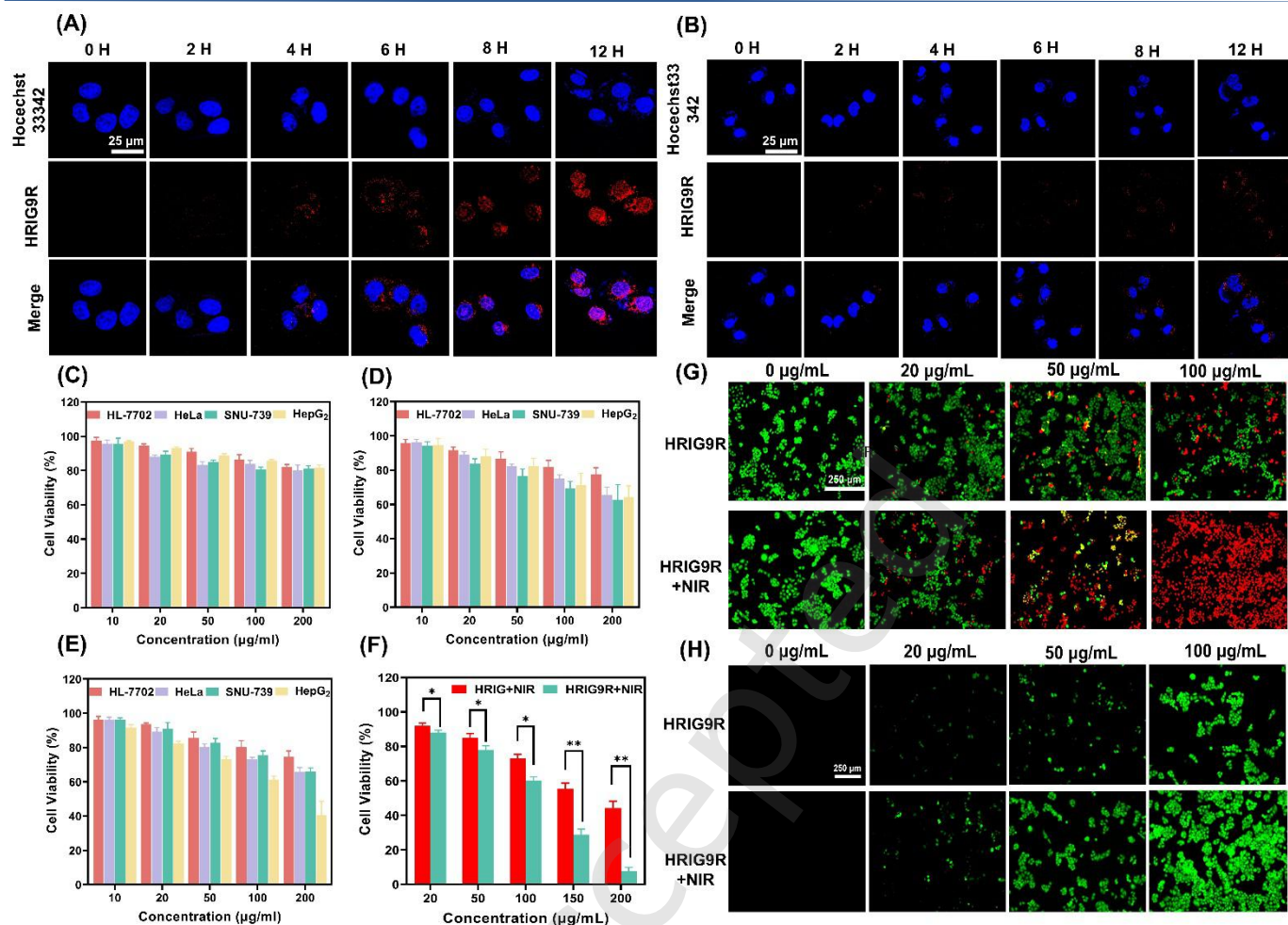


Figure 3 In vitro cellular internalization, cytotoxicity, and ROS generation. (A,B) Time-dependent cellular uptake of HRIG9R by (A) HepG2 and (B) HL-7702 cells, visualized via CLSM at indicated time points (0, 2, 4, 6, 8, 12 h). (C-F) Comparative cytotoxicity profiles against HL-7702, HeLa, SNU-739, and HepG2 following treatment with (C) HR, (D) HRIG, (E) HRIG9R, and (F) HRIG or HRIG9R combined with 808 nm laser irradiation (HRIG+NIR, HRIG9R+NIR). (G) Live/dead cell staining (Calcein-AM/PI) of HepG2 cells post-treatment, illustrating differential cytotoxic efficacy (scale bar: 250 μ m). (H) Quantitative assessment of intracellular ROS levels in HepG2 cells under varied treatment conditions, probed using the DCFH-DA fluorescent probe.

Cu^{2+} -mediated Fenton-like reaction and Ru-based POD-mimetic activity. The characteristic $\cdot\text{OH}$ absorbance increased dose-dependently with HRIG9R concentrations (Figure 2D) and was significantly enhanced under acidic pH (Figure 2E), confirming pH-responsive catalytic behavior. Additionally, the CAT-like activity was evidenced by O_2 evolution (Figure 2G), suggesting the potential to alleviate tumor hypoxic.

3.6 H_2O_2 generation

GOx-mediated H_2O_2 production was confirmed via a commercial assay. Absorbance at 415 nm increased with escalating HRIG9R concentration (Figure 2H), verifying the nanoplatform's capacity to fuel subsequent Fenton-like reactions.

3.7 GSH depletion

The GSH-scavenging capability of HRIG9R was evaluated using the DTNB assay. HRIG9R induced a concentration-dependent decrease in GSH levels (Figure 2F, I). Notably, at a fixed concentration of HRIG9R, higher initial GSH levels resulted in more pronounced depletion, indicating efficient GSH consumption. Depletion of this natural copper chelator promotes the release of free Cu^{2+} , exacerbating mitochondrial damage and priming the system for cuproptosis.

3.8 Generation of $^1\text{O}_2$

$^1\text{O}_2$ generation under 808 nm irradiation was probed using the

ABDA. A marked decrease in ABDA absorbance at 398 nm confirmed efficient $^1\text{O}_2$ production (Figure S5) underscoring the nanoplatform's PDT potential.

3.9 Cellular uptake and cytotoxicity

Cellular uptake behavior of HRIG9R was investigated using HepG2 cells and normal human hepatocytes (HL-7702). CLSM revealed that HRIG9R, guided by 9R-P201Ti, achieved progressive nuclear accumulation in HepG2 cells by 12 h (Figure 3A). In contrast, non-targeted HL-7702 cells showed minimal cytoplasmic distribution (Figure 3B), highlighting high selectivity of HRIG9R for malignant cells and its potential to minimize off-target toxicity.

In vitro cytotoxicity assays against HepG2, HeLa, SNU-739, and HL-7702 cells demonstrated that the unmodified HR nanomaterial showed high biocompatibility, with over 80% cell viability after 12 h (Figure 3C). While the HRIG group (Figure 3D) induced moderate tumor cell inhibition, the incorporation of the targeting peptide 9R-P201Ti in HRIG9R significantly enhanced its cytotoxic selectivity toward HepG2 cells, while maintaining low toxicity toward HL-7702 cells (Figure 3E). Upon 808 nm laser irradiation, the cytotoxicity of the HRIG+NIR group was further enhanced. Most notably,

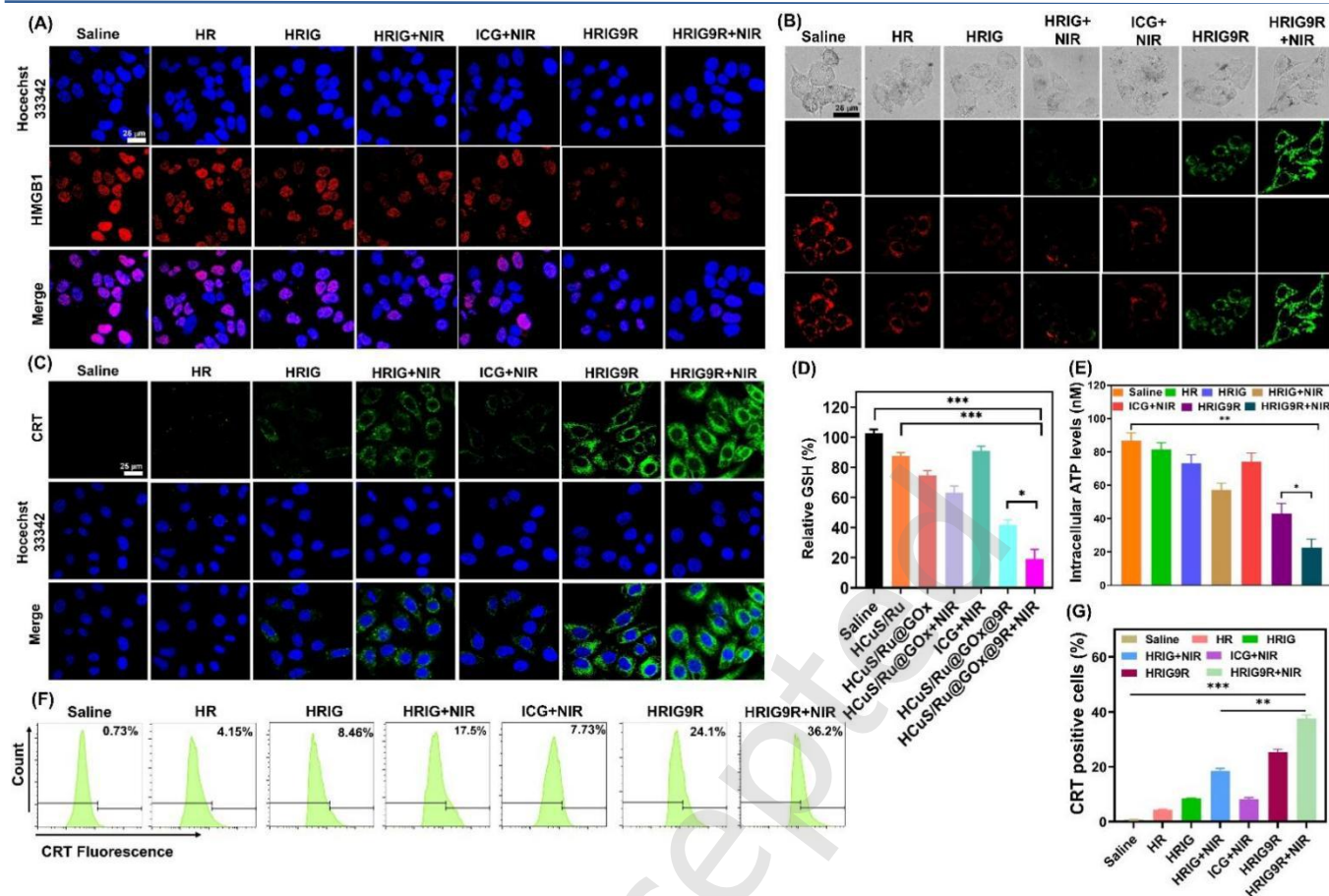


Figure 4 Mechanistic analysis of mitochondrial dysfunction and ICD markers. (A) Immunofluorescence imaging of HMGB1 translocation from the nucleus to the cytoplasm in HepG2 cells following various treatments. (B) MMP ($\Delta\Psi_m$) assessed via JC-1 staining (red: high $\Delta\Psi_m$; green: low $\Delta\Psi_m$). (C) Surface exposure of CRT on HepG2 cells, detected by immunofluorescence staining. (D) Quantitative analysis of intracellular GSH depletion post-treatment. (E) Intracellular ATP levels following exposure to the indicated formulations. (F) Flow cytometry quantification of CRT-positive cell populations. (G) Statistical analysis of mean CRT fluorescence intensity, confirming enhanced ecto-CRT exposure.

the HRIG9R+NIR achieved the highest inhibition rate (89%) (Figure 3F), indicative of a potent photo-enhanced synergistic effect. Live/dead staining visually confirmed extensive cell death in the HRIG9R+NIR group (Figure 3G). Notably, HRIG9R exhibited minimal toxicity toward HL-7702 cells, confirming a favorable therapeutic window.

3.10 Intracellular ROS generation

DCFH-DA probing revealed a dose-dependent ROS production, which was markedly amplified under 808 nm irradiation (Figure 3H). Flow cytometry quantification (Figure S6 and S7) showed a approximately 50% increase in ROS levels in the HRIG9R+NIR group versus controls, validating the cascaded catalytic amplification.

3.11 Dual ROS Mechanism ($^1O_2$ and $\cdot OH$)

SOSG and coumarin probes confirmed simultaneous $^1O_2$ and $\cdot OH$ generation. $^1O_2$ production was strictly NIR-dependent (Figure S8), whereas $\cdot OH$ was generated constitutively and further enhanced by irradiation (Figure S9). This dual-mode ROS generation ensures robust oxidative damage.

3.12 MMP disruption

JC-1 staining demonstrated a shift from red (aggregates, high MMP) to green (monomers, low MMP) fluorescence in the HRIG9R+NIR group (Figure 4B), indicating severe mitochondrial depolarization. This aligns with cuproptosis-associated mitochondrial dysfunction.

3.13 In vitro induction of ICD

HRIG9R+NIR effectively induced ICD, characterized by the release of TAAs and DAMPs, thereby promoting an anti-tumor immune response. ICD induction was assessed by detecting the expression and release of classic DAMPs, including CRT, high mobility group box 1 (HMGB1), and ATP. CLSM revealed a marked reduction in HMGB1 fluorescence in the HRIG9R+NIR group, indicative of nuclear-to-cytoplasmic translocation and subsequent extracellular release—a key hallmark of ICD (Figure 4A). Concurrently, prominent ectopic exposure of CRT on the plasma membrane was observed (Figure 4C), with flow cytometric quantification confirming significant CRT translocation (Figure 4F, G), thereby emitting a potent "eat-me" signal to recruit antigen-presenting cells. Additionally, intracellular ATP levels were most profoundly depleted in the HRIG9R+NIR group (Figure 4E). Collectively, these findings demonstrate that NIR-triggered HRIG9R orchestrates a robust ICD cascade, effectively converting immunologically "cold" tumors into "hot" ones.

3.14 Intracellular GSH depletion

Consistent with in vitro assays, intracellular GSH levels were most profoundly depleted in the HRIG9R+NIR group (Figure 4D), reinforcing the nanoplatform's redox-modulatory capacity.

3.15 In vivo antitumor effects and biosafety

Prior to efficacy evaluation, the hemocompatibility of HRIG9R was evaluated via hemolysis assay. Compared to the

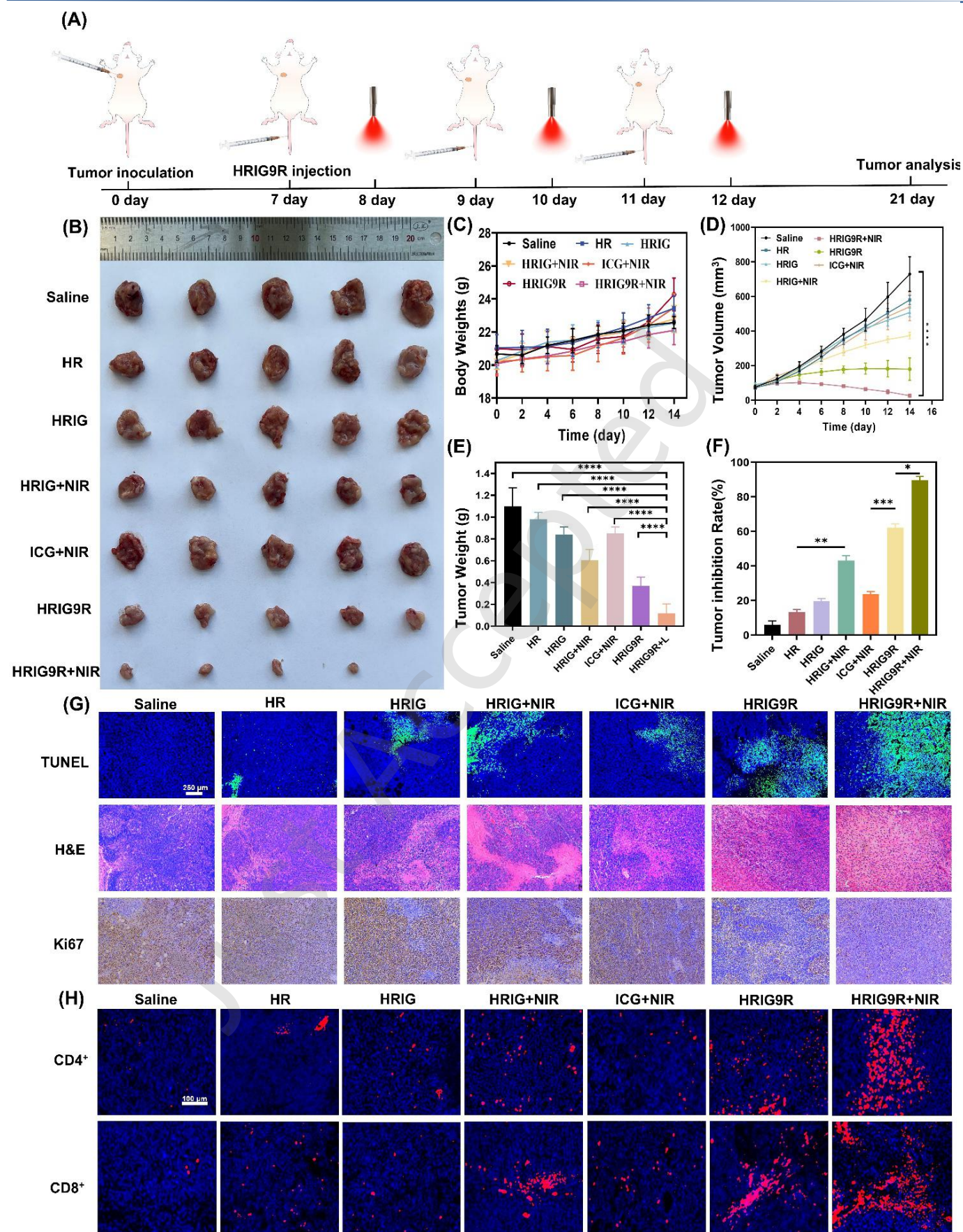


Figure 5. In vivo antitumor efficacy and biosafety evaluation. (A) Schematic representation of the experimental timeline and treatment regimen. (B) Representative photographs of excised tumors from each group at the experimental endpoint. (C) Temporal body weight change of mice throughout the treatment period (mean $\pm$ SD, n=5). (D) Tumor growth curves for the different groups (mean $\pm$ SD, n=5; *p < 0.05, p < 0.01). (E) Quantitative analysis of final tumor weights per group (mean $\pm$ SD, n=5; p < 0.01). (F) Statistical comparison of tumor inhibition rates across treatment cohorts. (G) Representative histopathological images of tumor sections stained with TUNEL (apoptosis assay), H&E, Ki67 (proliferation marker). (H) Immunofluorescence analysis of tumor-infiltrating CD4⁺ and CD8⁺ T cells, indicating immune cell recruitment.

positive control, HRIG9R treatment induced only a gradual, concentration-dependent increase in hemolysis, remaining below the 5% safety threshold even at 600 $\mu\text{g/mL}$ (Figure S10A, B), indicating favorable hemocompatibility for systemic administration.

In vivo fluorescence imaging demonstrated that while free ICG group lacked tumor specificity, HRIG9R showed time-dependent tumor accumulation, peaking at 12 h post-injection (Figures S11A, S12A). Ex vivo imaging confirmed predominant tumor-specific distribution, outperforming the diffuse biodistribution of free ICG (Figure S11B, S12B). Based on this pharmacokinetic profile, 808 nm laser irradiation was applied at 12 h post-injection. Real-time infrared thermal imaging confirmed efficient photothermal conversion at the tumor site (Figure S13), validating HRIG9R as a potent photothermal agent.

The therapeutic efficacy of HRIG9R was systematically evaluated in H22 tumor-bearing Balb/c mice across seven treatment groups (Saline, HR, HRIG, HRIG+NIR, ICG+NIR, HRIG9R, HRIG9R+NIR). HRIG9R+NIR achieved the most profound tumor growth inhibition (91.2% inhibition rate) (Figure 5A-F), with stable body weights indicating minimal systemic toxicity (Figure 5C). Histopathological analysis (H&E,

TUNEL, and Ki67) revealed extensive tumor necrosis, apoptosis, and suppressed proliferation in the HRIG9R+NIR group (Figure 5G). Comprehensive biosafety assessments, including major organs (heart, liver, spleen, lung, and kidney) histology and hematological analyses, revealed no significant abnormalities (Figure S14-S16). Immunofluorescence staining revealed robust infiltration CD4⁺ helper and CD8⁺ cytotoxic T cells into tumor beds (Figure 5H), linking therapeutic efficacy to immune activation.

3.16 Immune cell differentiation and cytokine profiling

To delineate the systemic immune response, immune cell subsets in tumors, spleens, and lymph nodes were profiled via flow cytometry. HRIG9R+NIR induced the highest proportion of CD3⁺CD4⁺ helper T cells (31.1%), CD3⁺CD8⁺ cytotoxic T cells (21.2%), and CD38⁺ activated T cells (21.3%) (Figure 6A-C). DC maturation in lymph nodes was markedly enhanced, exhibiting a 3.43-fold increase in maturation markers relative to the saline controls (Figure 6D). These results demonstrate that the NIR-triggered cascade reactions potentially stimulate systemic anti-tumor immunity, highlighting the potential of this combinatory strategy for cancer immunotherapy.

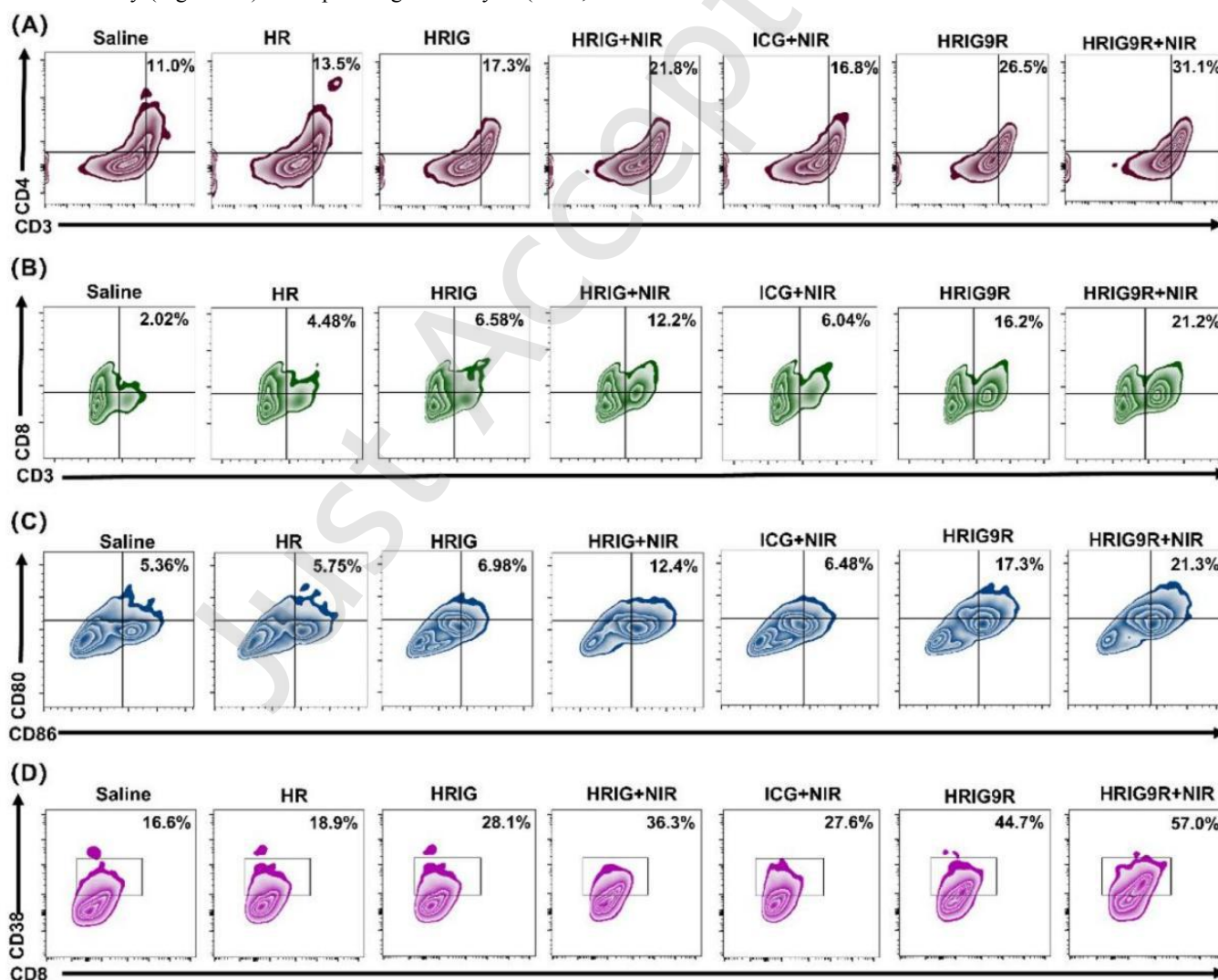


Figure 6. In vivo profiling of immune cell maturation and activation. (A-C) Flow cytometric analysis of T cell subsets infiltrating tumor tissues: (A) helper T cells (CD3⁺, CD4⁺), (B) cytotoxic T cells (CD3⁺, CD8⁺), and (C) activated cytotoxic T cells (CD80⁺, CD86⁺). (D) Quantitative assessment of DCs maturation in the lymph nodes, based on the expression of activation markers (CD8, CD38).

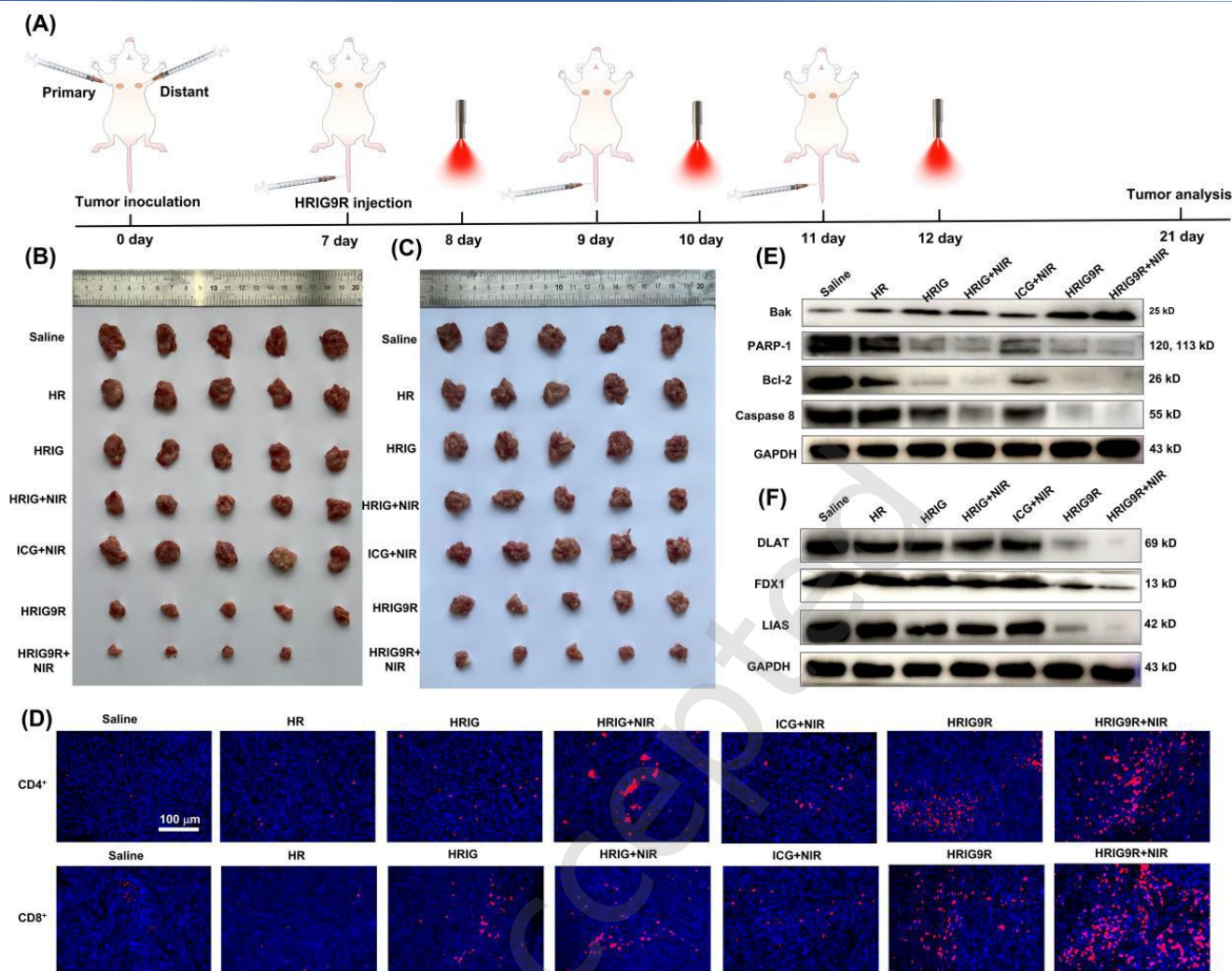


Figure 7. Evaluation of the abscopal effect and underlying molecular mechanisms in a bilateral tumor model. (A) Schematic representation of the treatment protocol for the bilateral tumor model. (B,C) Representative photographs of excised (B) primary and (C) distant (non-irradiated) tumors from each group at the experimental endpoint. (D) Immunofluorescence staining of CD4⁺ and CD8⁺ T cell infiltration in distant tumor sections. (E) Western blot analysis of apoptosis-related protein expression (Bak, PARP-1, Bcl-2, Caspase-8) in tumor tissues. (F) Western blot analysis of cuproptosis-related proteins expression (DLAT, FDX1, LIAS), elucidating the mechanistic basis of cell death.

3.17 Suppression of distant metastasis

To evaluate the abscopal effect, a bilateral tumour model was established (Figure 7A). HRIG9R+NIR significantly suppressed the tumour growth of both primary and distal tumors (Figures 7B, 7C, S17, S18). Histological analysis confirmed extensive necrosis and reduced proliferation in both in situ and distant tumors (Figure S19). Immunofluorescence staining and flow cytometry analysis revealed that the infiltration of CD4⁺/CD8⁺ T cell at the distal sites was significantly increased, while the maturation level of DC was markedly elevated (Figures 7D, S20). Concurrently, tissue and serum levels of pro-inflammatory cytokines (TNF- α , IL-6, and IFN- β) were significantly elevated (Figures S21, S22), indicating the establishment of a systemic pro-inflammatory environment that inhibits metastatic progression.

3.18 Mechanistic elucidation via western blotting

Western blot analysis elucidated the multi-pathway cell death mechanisms induced by HRIG9R. The nanoplateform triggered apoptosis via upregulated the expression of the pro-apoptotic protein Bak, activation of Caspase-8, and cleavage of PARP-1, accompanied by downregulation of the anti-apoptotic protein Bcl-2 (Figure 7E). Concurrently, hallmarks of cuproptosis were observed, including upregulation of FDX1 (facilitating Cu²⁺ to

Cu⁺ reduction), aberrant oligomerization of DLAT, and downregulation of LIAS, leading to disrupted mitochondrial metabolism (Figure 7F, Figure S23). Furthermore, HRIG9R+NIR modulates the FOXM1 signaling axis, suppressing Skp2 and Cks1 suppressing reducing p27Kip1 levels (Figure S24). This multi-pronged intervention synergistically disrupts redox homeostasis, energy metabolism, and cell cycle progression, culminating in irreversible tumor cell demise.

4. Conclusions

In summary, we engineered a nucleus-targeting multifunctional nanoplateform, HRIG9R, that integrated starvation therapy, MPTT, PDT, and CDT within a single entity. By exploiting the 9R-P201Ti peptide, HRIG9R achieved precise nuclear delivery. The acidic TME triggered Ru²⁺-mediated GSH depletion and H₂O₂ decomposition, synergizing with Cu²⁺ to generate toxic $\cdot$ OH for enhanced CDT. GOx established a self-reinforcing cycle by depleting glucose and supplying H₂O₂. Under NIR irradiation, the platform amplified ICD, activating DCs and cytotoxic T cells to eradicate both local and distant tumors. This work presents a transformative strategy that converges cuproptosis induction with systemic immunotherapy,

offering a potent and clinically translatable paradigm for HCC treatment.

Electronic Supplementary Material: Supplementary material (please provide the brief detail of the ESM) is available in the online version of this article at <https://doi.org/10.26599/NR.2026.94909207>.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article. The supporting data include experimental procedures and analytical data (NMR and HRMS), which can be found in the ESI.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contribution statement

Wen-Qi Zhu: Data curation, Formal analysis, Investigation, Methodology, Writing - original draft. **Lin-Song Li:** Formal analysis, Visualization, Supervision. **Bang-Bang Liu:** Validation, Visualization. **Dong Cheng:** Data curation. **Mei-Xia Zhao:** Investigation, Supervision, Project administration, Funding acquisition, Writing - review & editing.

Ethics statement

All animal experiments conducted were granted approval by the Animal Management and Ethics Committee of Henan University (Nos: HUSOM-2024-066).

References

- [1] E.Y. Tan, P. Danpanichkul, J.N. Yong, Z. Yu, D.J.H. Tan, W.H. Lim, B. Koh, R.Y.Z. Lim, E.K.J. Tham, K. Mitra, A. Morishita, Y.-C. Hsu, J.D. Yang, H. Takahashi, M.-H. Zheng, A. Nakajima, C.H. Ng, K. Wijarnpreecha, M.D. Muthiah, A.G. Singal, D.Q. Huang, Liver cancer in 2021: Global burden of disease study, *J. Hepatol.* **2025**, 82, 851-860.
- [2] P. Danpanichkul, M.B. Aboona, B. Sukphutanan, S. Kongarin, K. Duangsonk, C.H. Ng, M.D. Muthiah, D.Q. Huang, Y. Seko, L.A. Diaz, J.P. Arab, J.D. Yang, V.L. Chen, D. Kim, M. Nouredin, S. Liangpunsakul, K. Wijarnpreecha, Incidence of liver cancer in young adults according to the global burden of disease database 2019, *Hepatology.* **2024**, 80, 828-843.
- [3] B.-B. Liu, L.-S. Li, P.-W. Chen, D. Cheng, W.-Q. Zhu, T. Qiao, M.-X. Zhao, A photothermally triggered cascade nanodelivery platform for on-demand nitric oxide release in targeted hepatocellular carcinoma therapy, *Small* **2026**, 22, e73927.
- [4] L.A. Kondili, J.V. Lazarus, P. Jepsen, F. Murray, J.M. Schattenberg, M. Korenjak, L. Craxi, M. Buti, Inequities in primary liver cancer in Europe: the state of play, *J. Hepatol.* **2024**, 80, 645-660.
- [5] Z. Wu, F. Xia, W. Wang, K. Zhang, M. Fan, R. Lin, Worldwide burden of liver cancer across childhood and adolescence, 2000-2021: a systematic analysis of the global burden of disease study 2021, *eClinicalMedicine.* **2024**, 75, 102765.
- [6] J. Huang, M.K. Yim, C.C. Zhong, J. Hang, M.C.S. Wong, Tu1576 global premature mortality and temporal trends for liver cancer: A population-based worldwide study, *Gastroenterology* **2024**, 166, S-1737.
- [7] C. La Vecchia, C. Santucci, Liver cancer in young adults: Validity of global data sets, *Hepatology.* **2024**, 80, 766-769.
- [8] M. Müller, S. May, H. Hall, T.J. Kendall, L. McGarry, L. Blukacz, S. Nuciforo, A. Georgakopoulou, T. Jamieson, N. Phinichkousolchit, S. Dhayade, T. Suzuki, J. Huguet-Pradell, I.R. Powley, L. Officer-Jones, R.L.

- Pennie, R. Esteban-Fabro, A. Gris-Oliver, R. Pinyol, G.L. Skalka, J. Leslie, M. Hoare, J. Sprangers, G. Malviya, A. Mackintosh, E. Johnson, M. McCain, J. Halpin, C. Kiourtis, C. Nixon, G. Clark, W. Clark, R. Shaw, A. Hedley, T.M. Drake, E.H. Tan, M. Neilson, D.J. Murphy, D.Y. Lewis, H.L. Reeves, J. Le Quesne, D.A. Mann, L.M. Carlin, K. Blyth, J.M. Llovet, M.H. Heim, O.J. Sansom, C.J. Miller, T.G. Bird, Human-correlated genetic models identify precision therapy for liver cancer, *Nature.* **2025**, 639, 754-764.
- [9] P.-W. Chen, Y.-M. Zhang, L.-S. Li, B.-B. Liu, H.-L. Sun, L. Chen, X. Yang, M.-X. Zhao, Oxaliplatin/norcantharidin prodrug co-loaded nanoparticles: Synergistic DNA-repair blockade plus PD-L1 downregulation for inhibit tumor growth and metastasis, *Adv. Healthcare Mater.* **2025**, 15, e05173.
- [10] W. Fan, K. Adebawale, L. Vancza, Y. Li, M.F. Rabbi, K. Kunitomo, D. Chen, G. Mozes, D.K.-C. Chiu, Y. Li, J. Tao, Y. Wei, N. Adeniji, R.L. Brunsing, R. Dhanasekaran, A. Singhi, D. Geller, S.H. Lo, L. Hodgson, E.G. Engleman, G.W. Charville, V. Charu, S.P. Monga, T. Kim, R.G. Wells, O. Chaudhuri, N.J. Török, Matrix viscoelasticity promotes liver cancer progression in the pre-cirrhotic liver, *Nature.* **2024**, 626, 635-642.
- [11] X. Han, X. Zhang, Z. Liu, H. Liu, D. Wu, Y. He, K. Yuan, Y. Lyu, X. Liu, Copper-based nanotubes that enhance starvation therapy through cuproptosis for synergistic cancer treatment, *Adv. Sci.* **2025**, 12, e04121.
- [12] W. Li, H. Wang, Y. Liu, B. Li, F. Wang, P. Ye, Y. Xu, Y. Lai, T. Yang, "trinity" comprehensively regulates the tumor microenvironment of lipid-coated $\text{CaCO}_3/\text{CuO}_2$ nanoparticles induces "cuproptosis" in HCC, *ACS Appl. Mater. Interfaces.* **2024**, 16, 58203-58216.
- [13] D. Cheng, L.-S. Li, X.-J. Zhao, B.-B. Liu, W.-Q. Zhu, P.-W. Chen, B. Ren, M.-X. Zhao, Accurate intranuclear delivery of smart nanoplateforms synergistically enhances hepatocellular carcinoma therapy by remodeling tumor microenvironment, *Colloids Surf. A-Physicochem. Eng. Asp.* **2026**, 735, 139528.
- [14] M.A. Kahlson, S.J. Dixon, Copper-induced cell death, *Science.* **2022**, 375, 1231-1232.
- [15] M. Zhang, H. Xu, X. Wu, B. Chen, X. Gong, Y. He, Engineering dual-responsive nanoplateform achieves copper metabolism disruption and glutathione consumption to provoke cuproptosis/ferroptosis/apoptosis for cancer therapy, *ACS Appl. Mater. Interfaces.* **2025**, 17, 20726-20740.
- [16] W. Yin, J. Chang, H. Zhi, S. Chen, L. Xue, X. Zhang, H. Dong, Y. Li, Copper deposited metal-phenolic networks enabled crosstalk between cuproptosis and ferroptosis for triple-negative breast cancer immunotherapy, *Chem. Eng. J.* **2025**, 515, 163577.
- [17] J. Lee, B.S. Cha, D. Kim, Beyond apoptosis: Navigating cancer therapy with Cu/Fe-ligand nano-complexes through cuproptosis and ferroptosis, *Coord. Chem. Rev.* **2026**, 548, 217164.
- [18] Y. Li, J. Liu, Y. Chen, R.R. Weichselbaum, W. Lin, Nanoparticles synergize ferroptosis and cuproptosis to potentiate cancer immunotherapy, *Adv. Sci.* **2024**, 11, 2310309.
- [19] J. Wang, Y. Xie, G. Zhu, Y. Qian, Q. Sun, H. Li, C. Li, Acidity-unlocked glucose oxidase as drug vector to boost intratumor copper homeostatic imbalance-enhanced cuproptosis for metastasis inhibition and anti-tumor immunity, *Biomaterials.* **2025**, 319, 123207.
- [20] Y. Zhang, X. Du, X. Gao, Q. Feng, Z. He, L. Wang, H. Zhou, Q. Hu, C. Wu, W. Wang, Y. Chen, L. Ye, G. Lu, J. Ji, Y. Xi, X. Yang, G. Zhai, A metabolic intervention strategy for enhanced ferroptosis/cuproptosis activation and boosted anti-tumor immunity, *Chem. Eng. J.* **2024**, 500, 156732.
- [21] X. Liang, S. Fang, Y. Xin, J. Lei, W. Wang, Y. Wei, W. Li, C. Li, H. Tang, X. Wei, Y. Huang, L. Zheng, Y. Shentu, X. Meng, X. Liu, Cascade-targeting copper homeostasis nano-regulators for mild-photothermal boosted cuproptosis/ferroptosis mediated breast cancer therapy, *J. Nanobiotechnology.* **2025**, 23, 651.
- [22] X. Zeng, Y. Wang, J. Han, W. Sun, H.-J. Butt, X.-J. Liang, S. Wu, Fighting against drug-resistant tumors using a dual-responsive Pt(IV)/Ru(II) bimetallic polymer, *Adv. Mater.* **2020**, 32, 2004766.
- [23] Y. Shi, Y. Xia, M. Zhou, Y. Wang, J. Bao, Y. Zhang, J. Cheng, Facile synthesis of Gd/Ru-doped fluorescent carbon dots for fluorescent/MR bimodal imaging and tumor therapy, *J. Nanobiotechnology.* **2024**, 22, 88.
- [24] P. Vishwa, M. Nagaraj, S. Palanivel, T. Annaiah, S. Prakash, R.V. Kumar, V.R. Sarojamma, S. Kandaiah, Incorporation of ruthenium ions into nanometer-thick films of copper sulfide by anodization for proton-coupled electron transfer reactions, *ACS Appl. Nano Mater.* **2024**, 7, 4453-4464.
- [25] A. Dao, S. Chen, L. Pan, Q. Ren, X. Wang, H. Wu, Q. Gong, Z.

- Chen, S. Ji, J. Ru, H. Zhu, C. Liang, P. Zhang, H. Xia, H. Huang, A 700 nm LED light activated Ru(II) complex destroys tumor cytoskeleton via photosensitization and photocatalysis, *Adv. Healthc. Mater.* **2024**, 13, 2400956.
- [26] N. Zheng, X. Hu, L. Yan, L. Ding, J. Feng, D. Li, T. Ji, F. Ai, K. Yu, J. Hu, Bimetallic Cu@Ru core-shell structures with ligand effects for endo-exogenous stimulation-mediated dynamic oncotherapy, *Nano Lett.* **2024**, 24, 6165-6173.
- [27] M. Xiao, Y. Zhang, J. Xing, K. Qiao, Y. Ba, X. Wang, S. Gao, Z. Yuan, Ru-ph nanozyme-based hydrogels for tumor chemodynamic therapy by enhancing enzyme catalytic efficiency through multiple pathways, *Adv. Healthc. Mater.* **2024**, 14, 2403868.
- [28] S. Liu, Y. Wang, S. Yao, Q. Li, C. Sun, Y. Chen, J. Ye, W. Li, D. Wu, J. Zhou, H. Cheng, Y. Ding, Combinative dual-nanoparticle assembly improves oxygen-autarky PDT efficacy in hypoxic tumors, *Chem. Eng. J.* **2025**, 515, 163836.
- [29] Z. Chen, L. Huang, D. Gao, Z. Bao, D. Hu, W. Zheng, J. Chen, J. Liao, H. Zheng, Z. Sheng, High spatiotemporal near-infrared II fluorescence lifetime imaging for quantitative detection of clinical tumor margins, *Adv. Sci.* **2024**, 12, 2411272.
- [30] W. Gu, W. Guo, Z. Ren, Y. Zhang, M. Han, Q. Zhao, Y. Gao, Y. Mao, S. Wang, A bioactive nanocomposite integrated specific TAMs target and synergistic TAMs repolarization for effective cancer immunotherapy, *Bioact. Mater.* **2024**, 38 (2024) 472-485.
- [31] Y. Sun, S. Xiong, X. Jiang, J. Jin, T. Song, F. Wu, F. Chen, D. Wu, Y. Wang, X. Meng, W. Zou, Z. Liao, W. Bu, Polymorphic reactive oxygen species (ROS) storm for enhanced tumor therapy, *Rare Met.* **44** (2025) 8732-8743.
- [32] X. Xie, J. Liu, Z. Liu, H. Wei, M. Lin, G. Chen, Z. Liu, M. He, X. Huang, S. Huang, Y. Wang, J. Wang, H. Chen, Q. Chen, X. Xie, X. Shuai, Real-time ROS monitoring-guided tumor electrodynamic therapy using a metal microneedle array system, *Nano Today*. **2025**, 63, 102731.
- [33] Z. Zhao, K. Ling, J. Yan, Z. Wang, C. Chen, D. Sun, J. Liu, Nano-carriers mediate reciprocally chained promotion between ROS and mitochondrial calcium overload for enhanced antitumor therapy, *Acta Biomater.* **2025**, 201, 545-558.
- [34] X. Hu, M. Zhang, C. Quan, S. Ren, W. Chen, J. Wang, ROS-responsive and triple-synergistic mitochondria-targeted polymer micelles for efficient induction of ICD in tumor therapeutics, *Bioact. Mater.* **2024**, 36, 490-507.
- [35] J. Liu, Z. Zhou, C. Li, W. Xiong, H. Ding, S. Wu, Z. Wang, L. Wang, J. Shen, A self-immunoregulatory nanosensitizer for sonodynamic cancer therapy, *Adv. Mater.* **2026**, 38, e73775.
- [36] K. Li, Z. Wang, Z. Zhou, H. Ding, S. Wu, Y. Li, J. Shen, Mitochondria-targeting nanocuproptosis breaks immune resistance, *Adv. Funct. Mater.* **2026**, 36, e28750.
- [37] X. Song, M. Wang, S. Liu, H. Liu, A. Jiang, Y. Zou, Y. Deng, Q. Qin, Y. Song, Y. Zheng, A sequential scheme including PTT and 2'3'-cGAMP/CQ-LP reveals the antitumor immune function of PTT through the type I interferon pathway, *Pharmacol. Res.* **2023**, 196, 106939.
- [38] S. Wang, S. Yao, Q. Hu, Z. Wang, Y. Zhao, L. Li, A metal-quercetin-histidine co-coordinated nanoparticle enhances cancer photothermal therapy via reshaping immunosuppressive tumor microenvironment, *Chem. Eng. J.* **2024**, 499, 156027.
- [39] T. Li, X. Zhang, C. Shi, Q. Liu, Y. Zhao, Biomimetic nanodrug blocks CD73 to inhibit adenosine and boosts antitumor immune response synergically with photothermal stimulation, *J. Nanobiotechnology*. **2024**, 22, 214.
- [40] Z. Zhao, Y. He, C. Shi, J. Li, Q. Liu, Y. Zhong, J. Zhong, T. Li, Y. Deng, X. Zhang, N. Wang, Y. Zhao, Immunotherapy augmentation through photothermal nanomaterial-mediated tumor microenvironment modulation, *Coord. Chem. Rev.* **2026**, 547, 217118.
- [41] Z. Hu, X. Zhou, W. Zhang, L. Zhang, L. Li, Y. Gao, C. Wang, Photothermal amplified multizyme activity for synergistic photothermal catalytic tumor therapy, *J. Colloid Interface Sci.* **2025**, 679, 375-383.
- [42] J. Ma, N. Li, J. Wang, Z. Liu, Y. Han, Y. Zeng, In vivo synergistic tumor therapies based on copper sulfide photothermal therapeutic nanoplateforms, *Exploration*. **2023**, 3, 20220161.
- [43] L.-S. Li, P.-W. Chen, X.-J. Zhao, D. Cheng, B.-B. Liu, X.-J. Tang, W.-Q. Zhu, X. Yang, M.-X. Zhao, Nuclear-targeted smart nanoplateforms featuring double-shell hollow mesoporous copper sulfide coated with manganese dioxide synergistically potentiate chemotherapy and immunotherapy in hepatocellular carcinoma cells, *J. Colloid Interface Sci.* **2025**, 680, 202-214.
- [44] M. Guan, K. Cheng, X.-T. Xie, Y. Li, M.-W. Ma, B. Zhang, S. Chen, W. Chen, B. Liu, J.-X. Fan, Y.-D. Zhao, Regulating copper homeostasis of tumor cells to promote cuproptosis for enhancing breast cancer immunotherapy, *Nat. Commun.* **2024**, 15, 10060.

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Electronic Supplementary Material

Cascade-amplified cuproptosis in hepatocellular carcinoma via a smart hollow mesoporous CuS nanoplatform enabling synergistic phototherapy/chemodynamic therapy/starvation therapy

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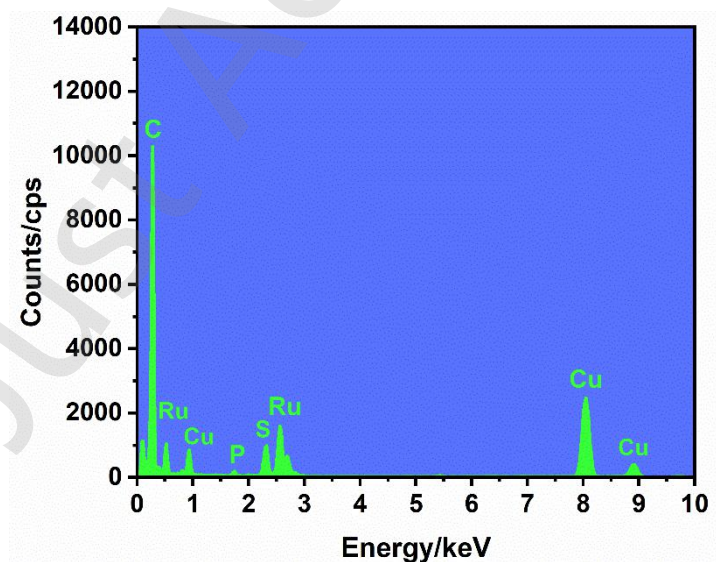


Figure S1. EDS spectra of HRIG9R NPs.

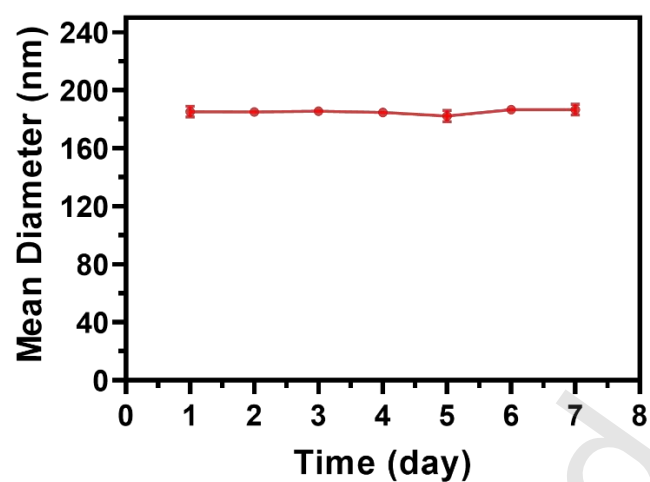


Figure S2. Changes in particle size of materials at different time points.

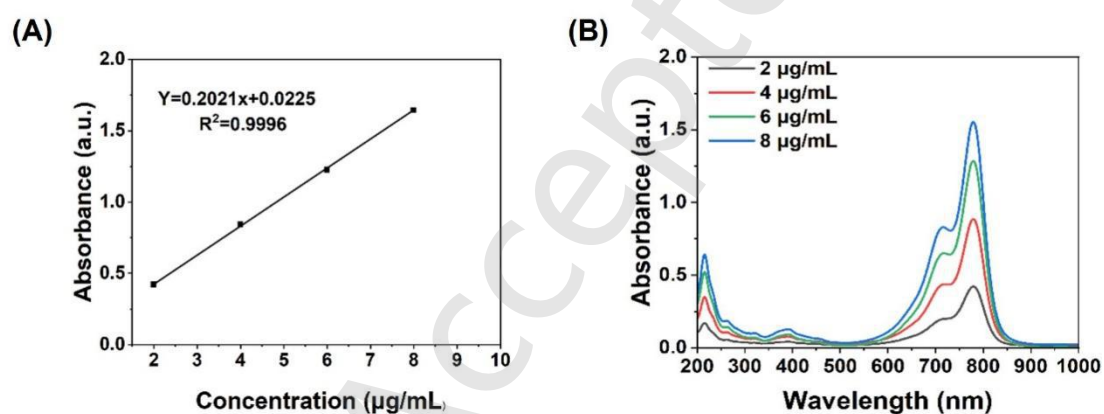


Figure S3. (A) Standard curve of of ICG used to quantitatively determine the drug loading efficiency. (B) UV absorption of ICG under different feeding ratios.

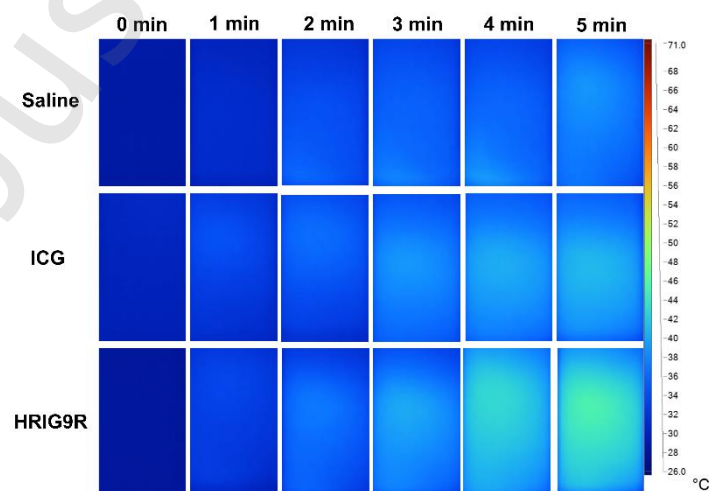


Figure S4. In-vitro near-infrared imaging of different materials (saline, ICG, HRIG9R)

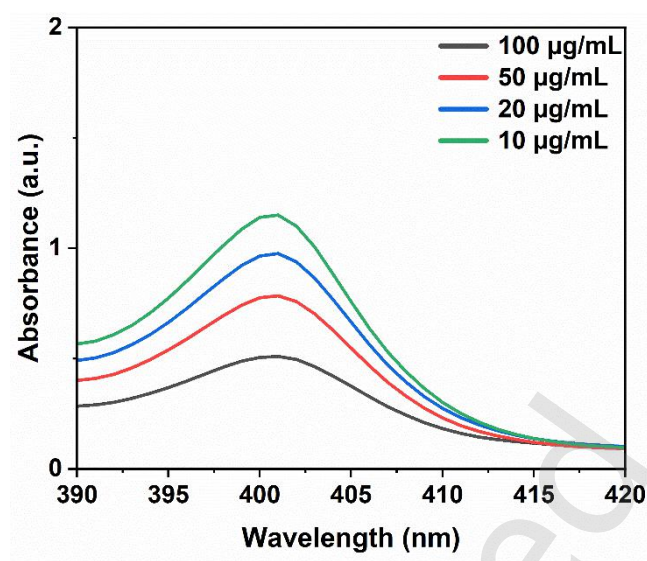


Figure S5. UV-Vis spectra demonstrating the photocatalytic generation of singlet oxygen ($^1\text{O}_2$).

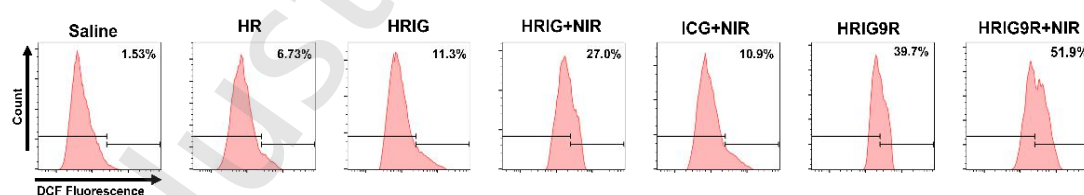


Figure S6. Flow cytometric analysis of intracellular ROS levels.

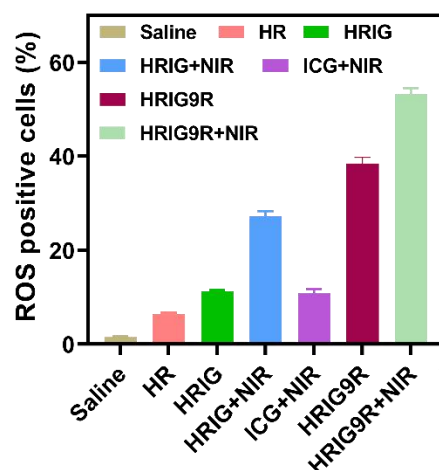


Figure S7. Bar chart of intracellular ROS levels.

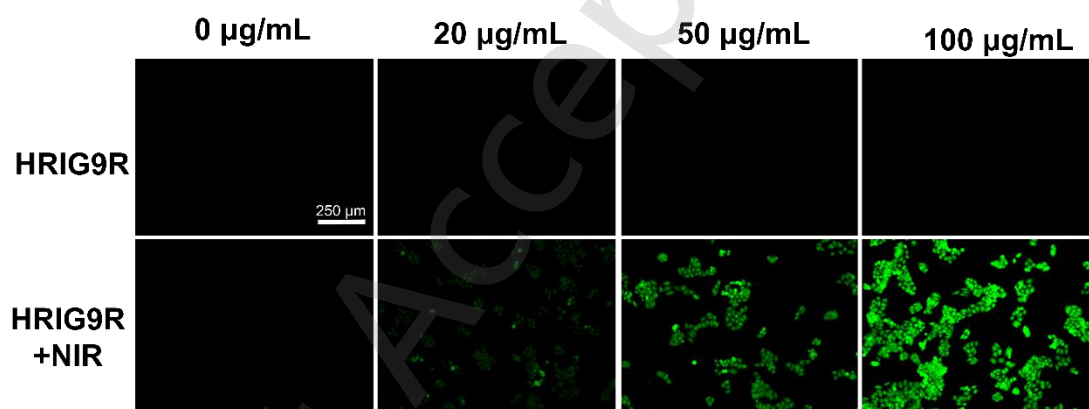


Figure S8. Intracellular $^1\text{O}_2$ generation.

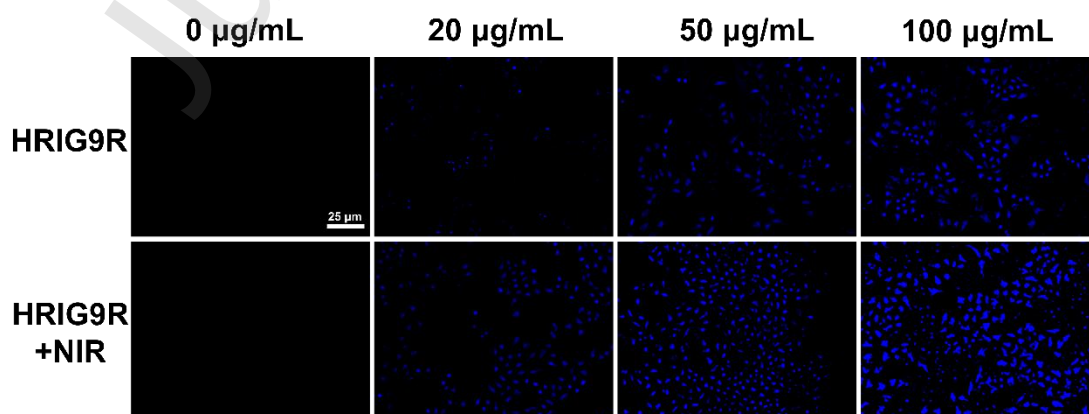


Figure S9. Intracellular $\cdot\text{OH}$ production.

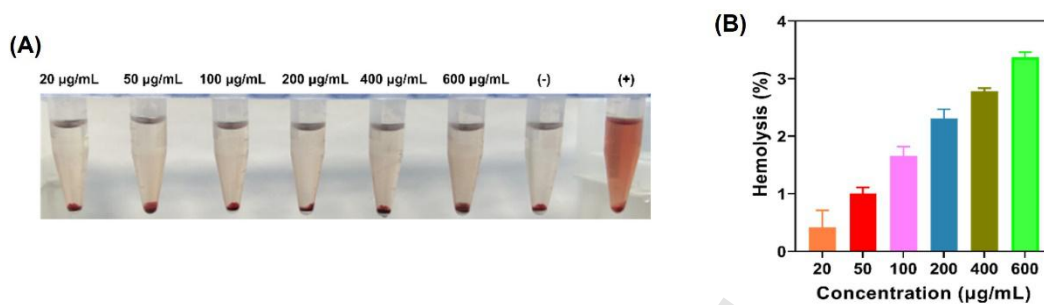


Figure S10. (A) Numerical camera diagram of HRIG9R hemolytic effects at different drug concentrations. (B) Enzyme-linked immunosorbent assay for quantitative analysis of hemolysis rate.

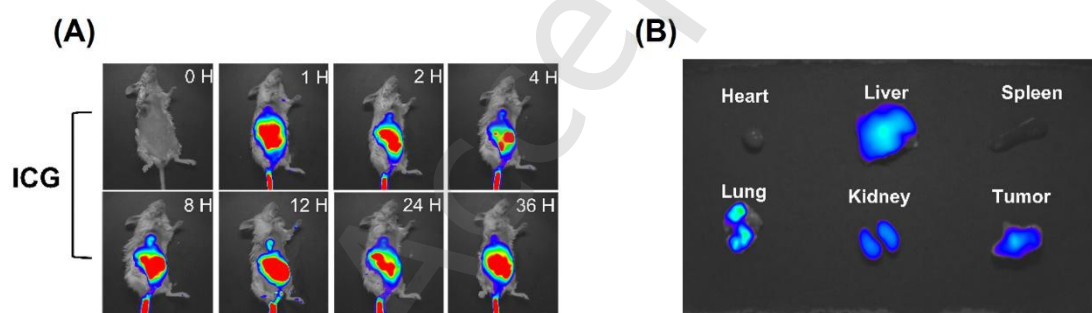


Figure S11. (A) In vivo fluorescence images of mice administered with free ICG. (B) Ex vivo fluorescence images of major organs and tumors from the ICG group.

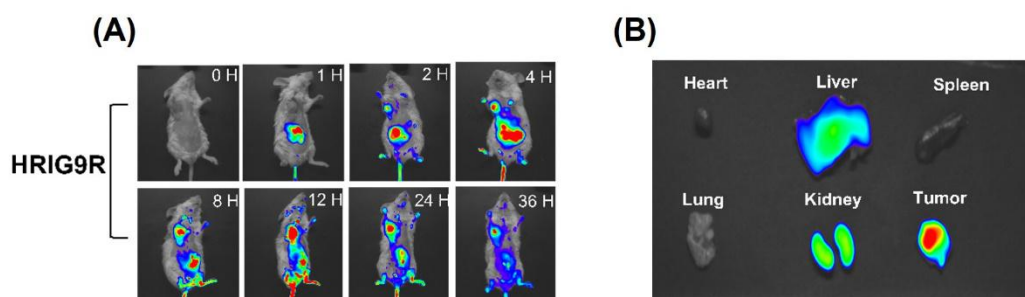


Figure S12. (A) In vivo fluorescence images of mice administered with HRIG9R. (B)

Ex vivo fluorescence images of major organs and tumors from the HRIG9R group.

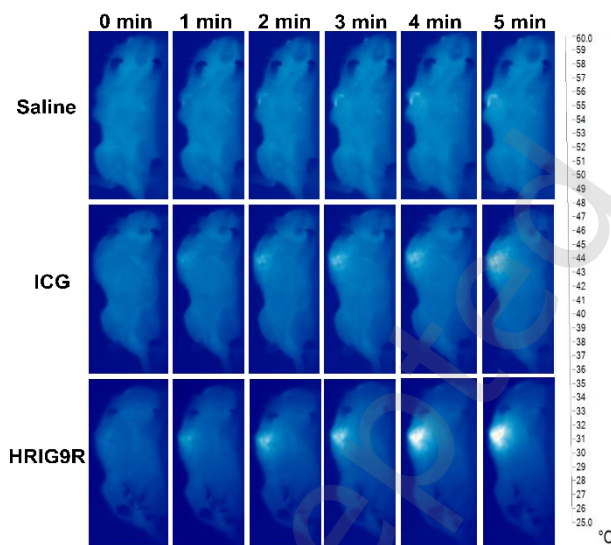


Figure S13. In vivo infrared thermal imaging of mice.

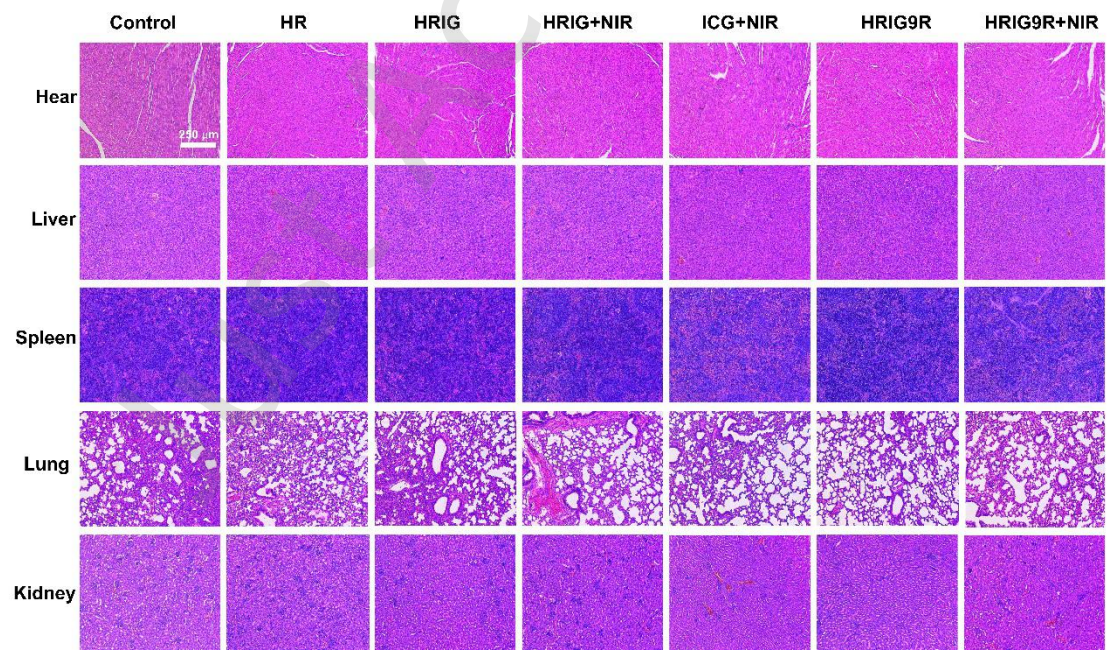


Figure S14. Representative H&E-stained images of major organ sections.

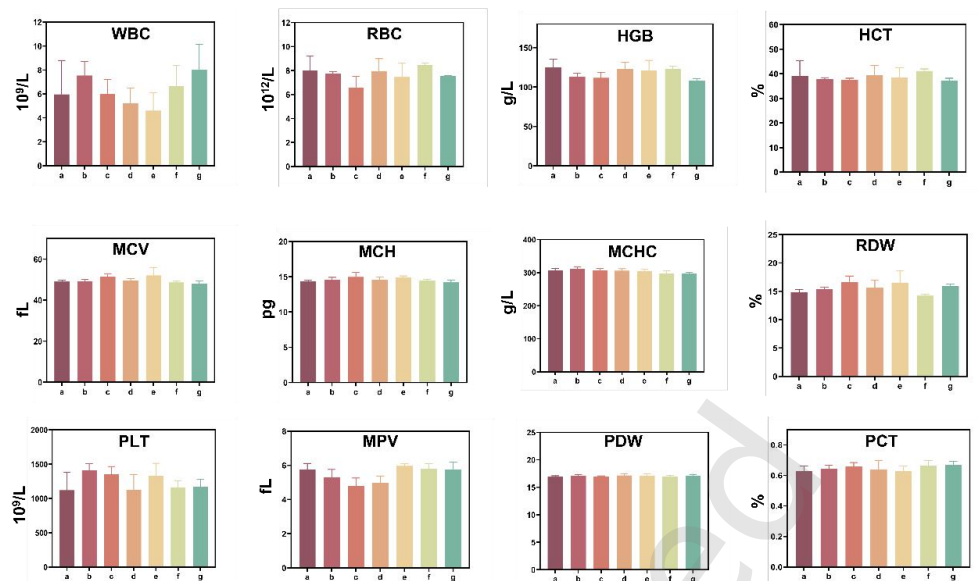


Figure S15. Blood biochemical analysis of mice after treatment.

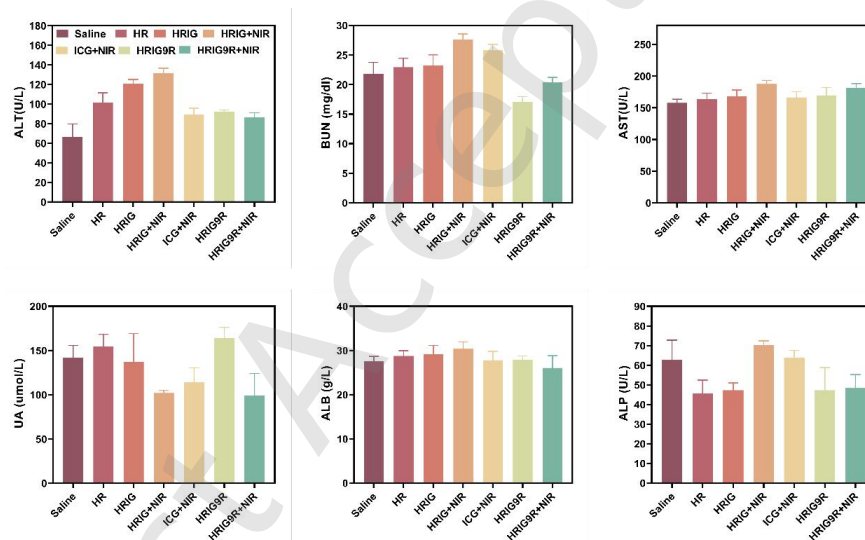


Figure S16. Complete blood count analysis of mice after treatment.

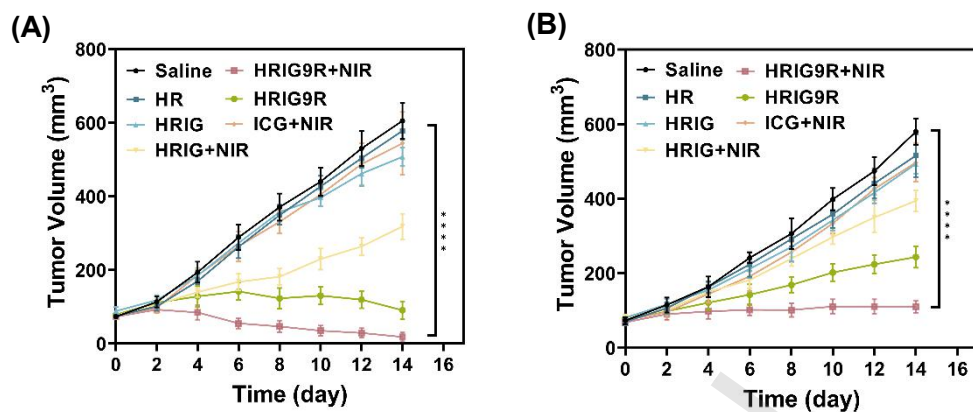


Figure S17. Tumor volume measurements: (A) primary and (B) distal tumor.

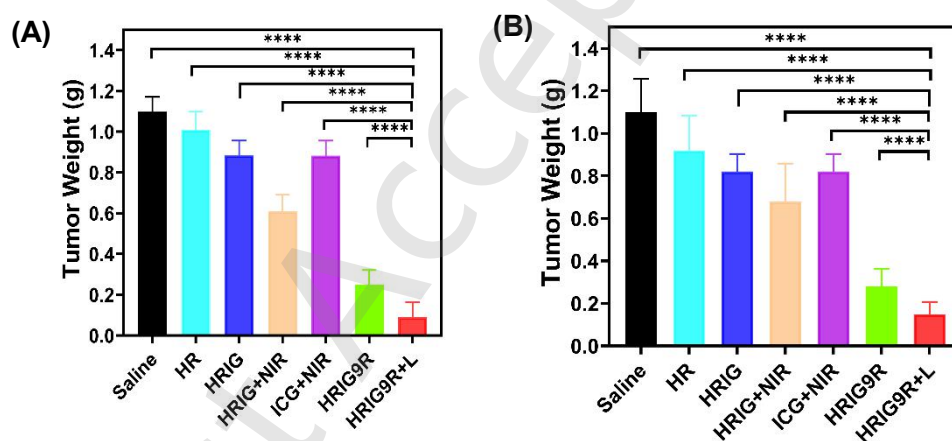


Figure S18. Weights of excised tumors at the end of the treatment: (A) primary tumor and (B) distal tumor in the bilateral tumor model.

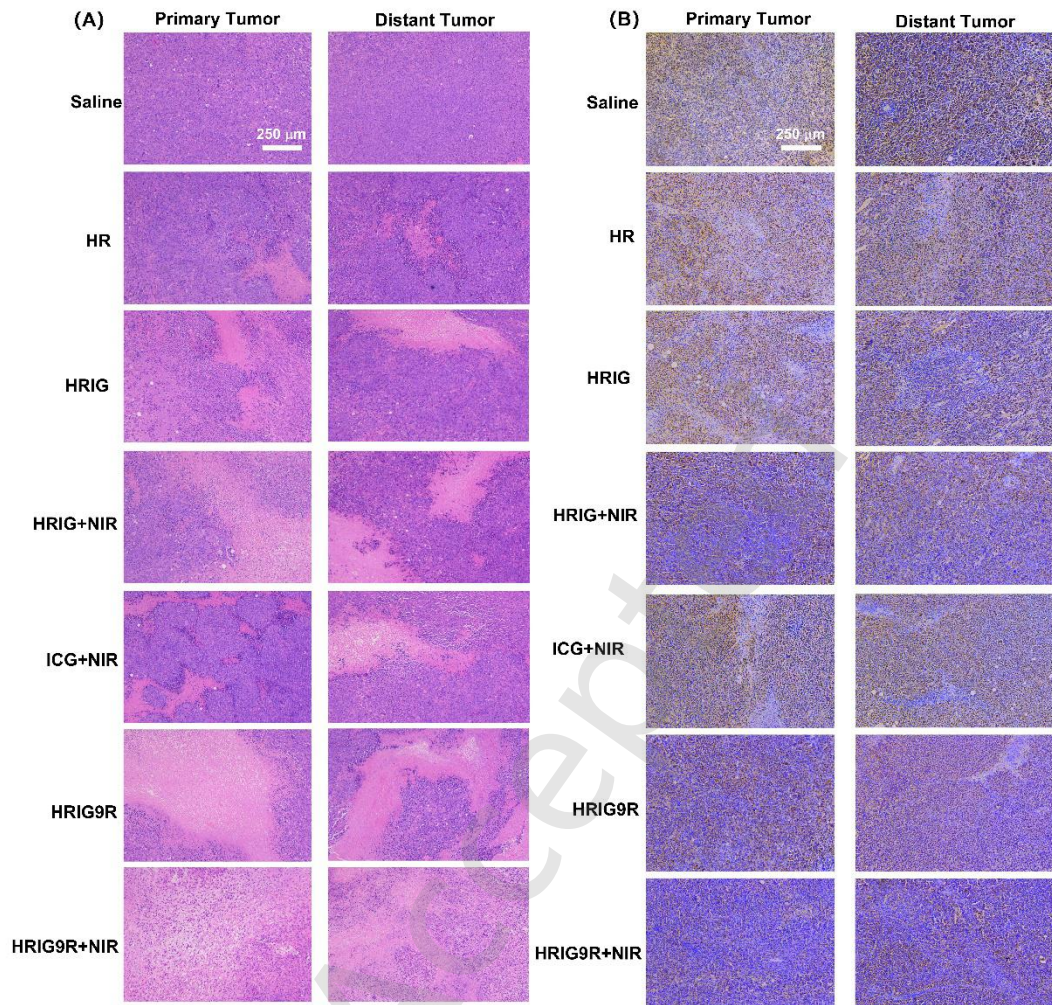


Figure S19. Histopathological analysis of tumor tissues from the bilateral model. (A) H&E-stained and (B) Ki67-immunostained sections of primary and distal tumors.

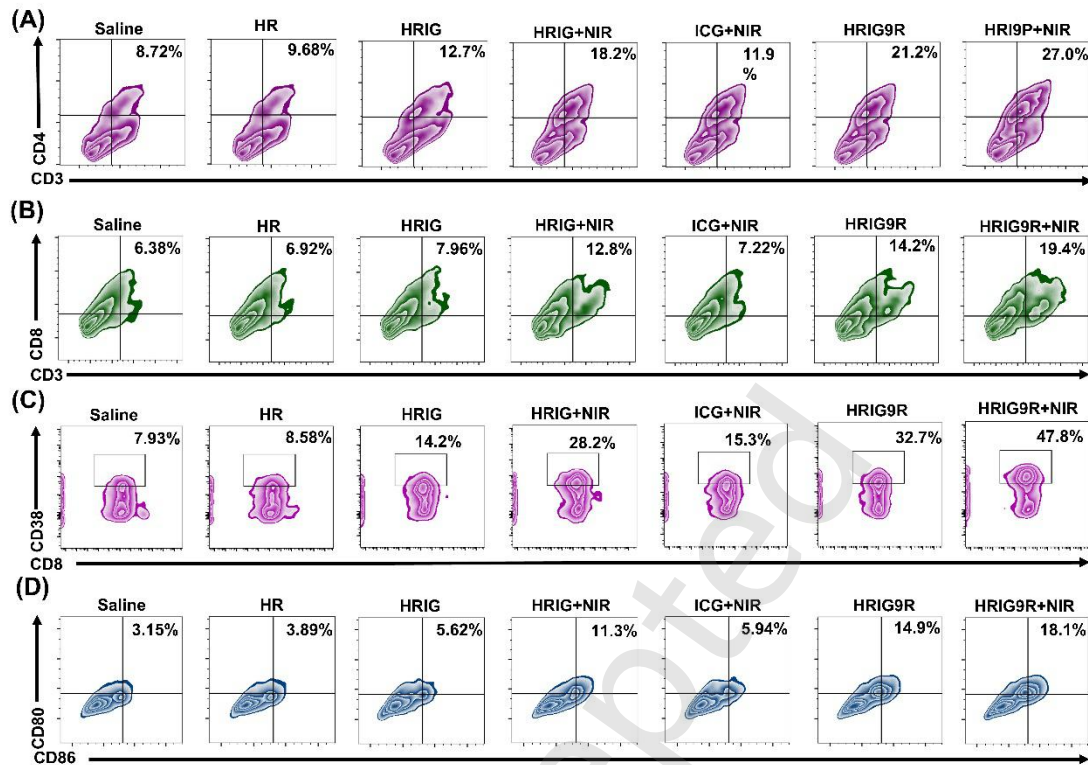


Figure S20. Flow cytometric quantification of immune cell maturation: (A) Helper T cell (CD3⁺ CD4⁺), (B) Cytotoxic T cell (CD3⁺ CD8⁺), (C) Activated cytotoxic T cell (CD8⁺ CD38⁺), and (D) DC cell maturation (CD80⁺ CD86⁺ on CD11c⁺ cells).

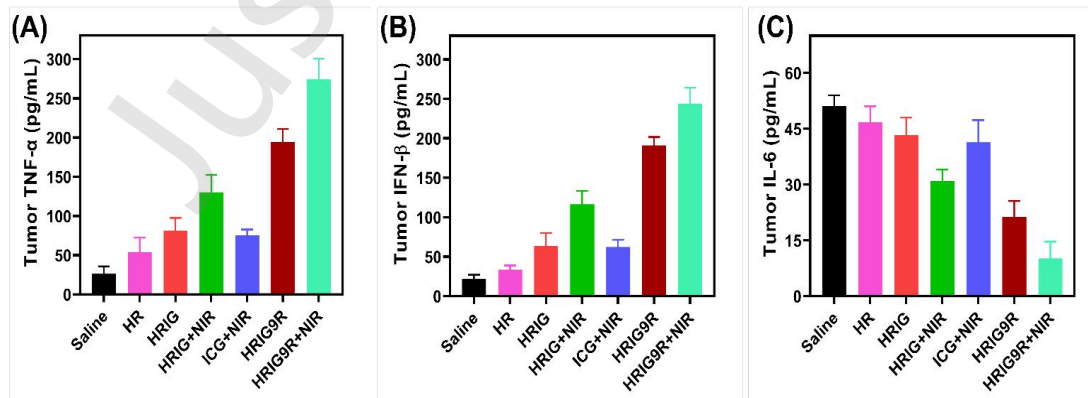


Figure S21. Cytokine levels in tumor tissues measured by ELISA: (A) TNF-α, (B) IFN-β, (C) IL-6.

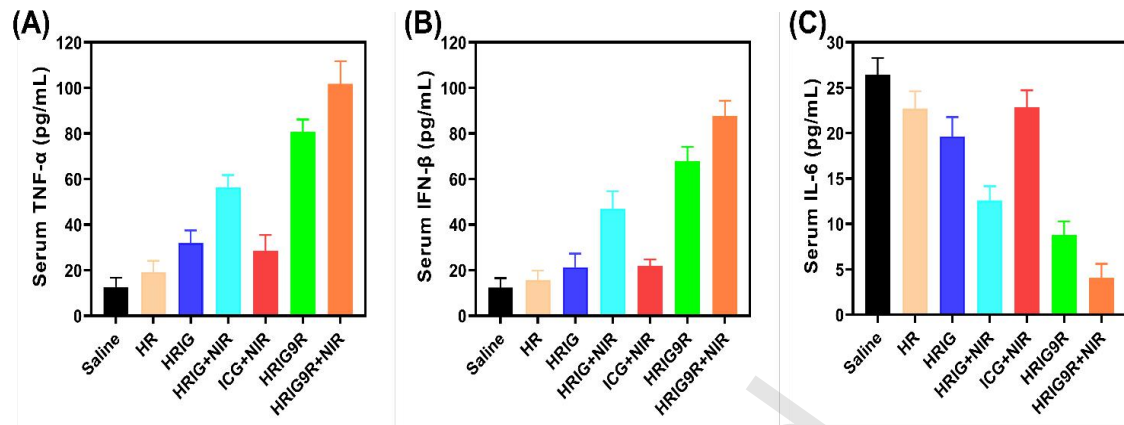


Figure S22. Serum cytokine levels measured by ELISA: (A) TNF- α , (B) IFN- β , (C) IL-6.

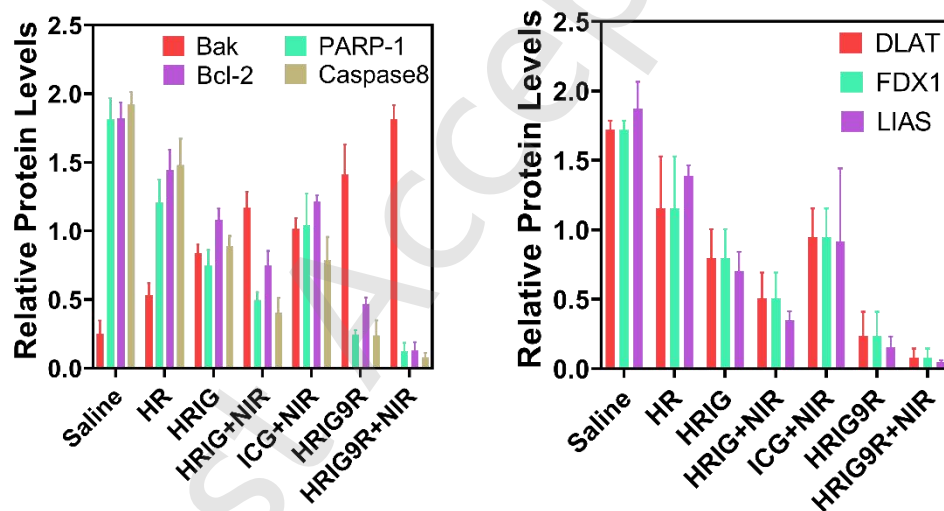


Figure S23. (A) Quantitative statistics of apoptosis-related proteins; (B) Quantitative statistics of cuproptosis-related proteins.

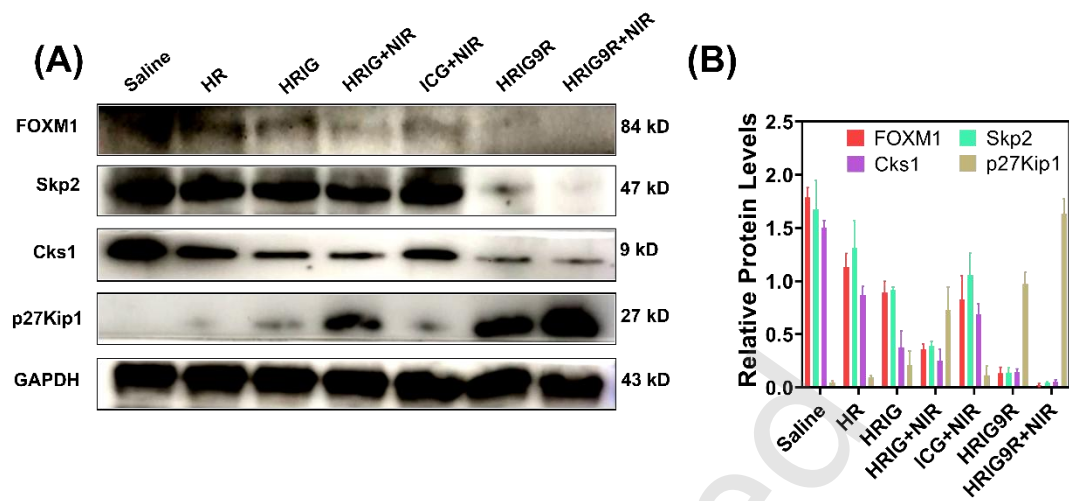


Figure S24. (A) 9R-P201 peptide receptor protein blotting;(B) Quantitative analysis of 9R-P201 peptide receptor protein.