



Ruthenium-doped carbon dots with “three-in-one” chemodynamic, photodynamic, and photothermal activity induce panoptosis for tumor therapy

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ABSTRACT

Combined nanocatalytic therapy has showed potential feasibility in cancer treatment. However, designing nanocatalytic particles with good biocompatibility, high catalytic activity, and precise targeted delivery remains a challenge. In this study, ingenious Ru-doped carbon dots (Ru-CDs) leveraging “three-in-one” photothermal, photodynamic, and chemodynamic modalities were synthesized without using additional photosensitizer or photothermal agent for synergistic tumor treatment. The Ru-CDs efficiently generated type I (hydroxyl radicals, $\cdot\text{OH}$) and type II (singlet oxygen, $^1\text{O}_2$) reactive oxygen species (ROS), which was rarely reported in Ru complexes. Subsequently, glucose oxidase (GOx) and hyaluronic acid (HA) were conjugated to Ru-CDs to construct Ru-CDs-GOx@HA nanocomposites that had tumor cell targeting, starvation effects, as well as supplied the substrate (H_2O_2) for $\cdot\text{OH}$. The photothermal, photodynamic, and chemodynamic activities of the CDs were markedly enhanced by Ru-doping. Furthermore, the immune adjuvants (cytosine-phosphate-guanine sequence, CpG) was conjugated to Ru-CDs-GOx@HA to produce an immunotherapeutic nanocomposite. Ru-CDs-GOx@HA inhibited the proliferation of cancer cells, and the inhibition further enhanced under near-infrared (NIR) radiation. Ru-CDs-GOx@HA suppressed tumor growth and metastasis and thus prolonged the survival of tumor-bearing nude mice under NIR irradiation. Interestingly, for the first time, we found that Ru-CDs-GOx@HA treatment elicited PANoptosis, including pyroptosis, apoptosis, necroptosis, and ferroptosis in cancer cells under NIR, which increased the immune response and potentially prevented tumor metastasis, thus enhancing the therapeutic effect. This study highlighted Ru doping as an efficient approach for enhancing the chemodynamic, photodynamic, and photodynamic effects of CDs and therefore offered a promising strategy for efficient tumor therapy.

1. Introduction

Malignant tumors are very common diseases that seriously threaten people's health, and they have become the dominant cause of disease-related death worldwide [1]. Several conventional therapeutic modalities, including radiotherapy, chemotherapy, surgery operation, and, more recently, immunotherapy, have been widely applied to treating tumors [2]. However, the drawbacks of low efficiency, nonselective side

effects in the body, and tumor metastasis associated with these therapies still must be solved [3]. Chemodynamic therapy (CDT) is an emerging strategy for cancer treatment based on Fenton-reaction-induced cellular oxidative stress elicited by generating hydroxyl radicals ($\cdot\text{OH}$) in the tumor region. Although some progress has been made in CDT tumor treatment, several characteristics of the tumor microenvironment (TME), including low levels of intracellular hydrogen peroxide (H_2O_2) (10 and 50 $\mu\text{mol/L}$), and low pH values, low pH values, and high levels

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of the reducing substance glutathione (GSH), still hinder the efficient elimination of malignant tumors [4]. Photodynamic therapy (PDT) and photothermal therapy (PTT) have attracted considerable attention in tumor treatment due to their minimal invasiveness, high selectivity, and few side effects. Both therapies utilize distinct mechanisms to target cancer cells: PDT induces localized intracellular chemical damage through the generation of reactive oxygen species (ROS), while PTT causes localized thermal damage by converting light energy into heat [5]. However, individually, PDT and PTT may have inefficient anticancer effects, due to low levels of H_2O_2 in the TME [6]. Additionally, resistance can develop in the hypoxic environment of tumors during PDT, which hinders therapeutic efficacy. Although PTT and PDT modalities have been combined and applied in tumor therapy, the PTT and PDT conversion reagents, having crucial functions in a phototherapeutic efficacy [7], in the nanoparticles were independent [8], incurring the preparation process of the nanoparticles was complex. Therefore, highly effective, multifunctional, and integrated nanocarriers that can overcome these systemic hurdles and optimize anticancer efficacy are urgently needed [9].

Carbon dots (CDs) are promising phototherapy agents as they are simple to prepare and easily modified, demonstrating excellent electron-transfer characteristics and significant photothermal conversion effects, and have high biocompatibility [10]. However, not all CDs show superior activity in tumor treatment due to the different precursors used during synthesis. Metal doping is an available strategy and has gained increased attention for its ability to improve the photothermal activities of CDs [11,12]. The synergistic action between active metals and CDs may improve the stability of metal materials and enhance the activity of metal sites [13]. Importantly, the metal-doping may increase the CDT, PTT, and PDT performance of CDs for more efficient tumor therapy [14,15]. Ruthenium (Ru) is a transition metal that belongs to the platinum group of metals with redox activity. Compared with Pt, Ru is inexpensive ($\sim 1/25$ the price of Pt), and it has gained widespread attention because of its unique electronic structure and redox activity [16]. Therefore, Ru doping may be a viable approach for improving CDT, PTT, and PDT in tumor treatment. Although Ru-doped CDs (Ru-CDs) have been used to treat tumors [17,18], the mechanisms underlying their anticancer effects, such as the cell death pathway triggered in cancer cells and the immune effect of Ru-CDs, have not been

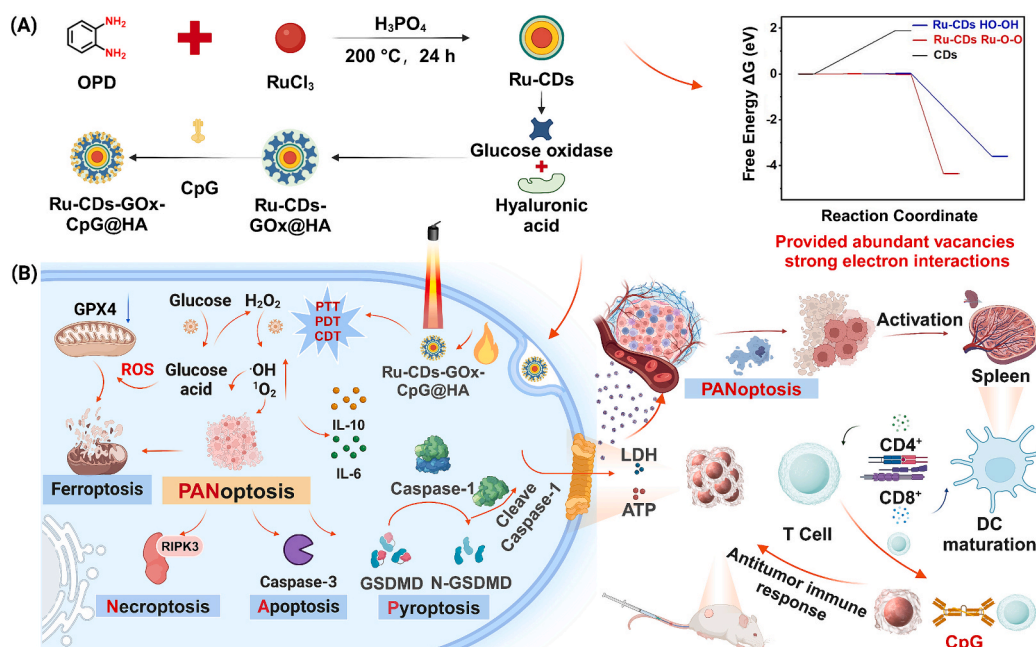
investigated to date.

In this study, Ru-CDs were synthesized via a straightforward hydrothermal method. Ru-CDs exhibited notable absorption and emission properties. Glucose oxidase (GOx), hyaluronic acid (HA), and immune adjuvants (cytosine-phosphate-guanine sequence, CpG) were conjugated to Ru-CDs to create the Ru-CDs-GOx-CpG@HA nanocomposites. This system effectively used “starvation therapy” to deplete glucose and generate H_2O_2 in the tumor for targeted cancer treatment. Notably, incorporating Ru combined with 808 nm irradiation significantly enhanced the photothermal conversion efficiency to 66.21 %. We revealed for the first time that Ru-CDs triggered PANoptosis (pyroptosis, apoptosis, necroptosis, and ferroptosis) in cancer cells, releasing immunoadjuvants into the TME. These, in turn, reprogrammed M2-like macrophages into the M1 phenotype, promoted dendritic cell (DC) maturation, and activated the immune system. These overall effects of the Ru-CDs inhibited cancer cell proliferation, and eliminated the tumor and tumor metastases, establishing a novel and effective synergistic strategy for tumor therapy (Scheme 1).

2. Results and discussion

2.1. Characterization of the functionalized Ru-CDs

The Ru-CDs were synthesized via a conventional hydrothermal method using o-phenylenediamine and $RuCl_3$ as precursors [19]. The Ru-CDs were highly stable and dispersible even stored in DW for 7 d (Fig. S1). Subsequently, GOx and HA were loaded onto the Ru-CDs, forming Ru-CDs-GOx@HA nanocomposites. transmission electron microscopy (TEM) analysis revealed that the Ru-CDs were uniform, monodisperse spheres with an average diameter of approximately 3.19 nm (Fig. 1A and B). After loading GOx and HA, the average diameter increased to approximately 7.77 nm, indicating that the Ru-CDs were successfully modified with GOx and HA (Fig. 1C and D). This result was corroborated by atomic force microscope (AFM), which revealed average particle sizes of approximately 4.33 and 7.69 nm for the Ru-CDs and Ru-CDs-GOx@HA, respectively (Fig. 1E and F), which aligned with the TEM results. The Ru content of the Ru-CDs was 6.85 % (Table S2). Energy-dispersive X-ray (EDX) mapping confirmed the uniform distribution of C, N, O, P, and Ru elements within the Ru-CDs (Fig. 1G). The



Scheme 1. Illustration of the nanomaterial synthesis and therapeutic process. (A) Preparation of Ru-CDs-GOx-CpG@HA and (B) inducing cell PANoptosis through CDT, PDT, and PTT therapies, combined with immunotherapy for tumor treatment and inhibition of lung metastasis.

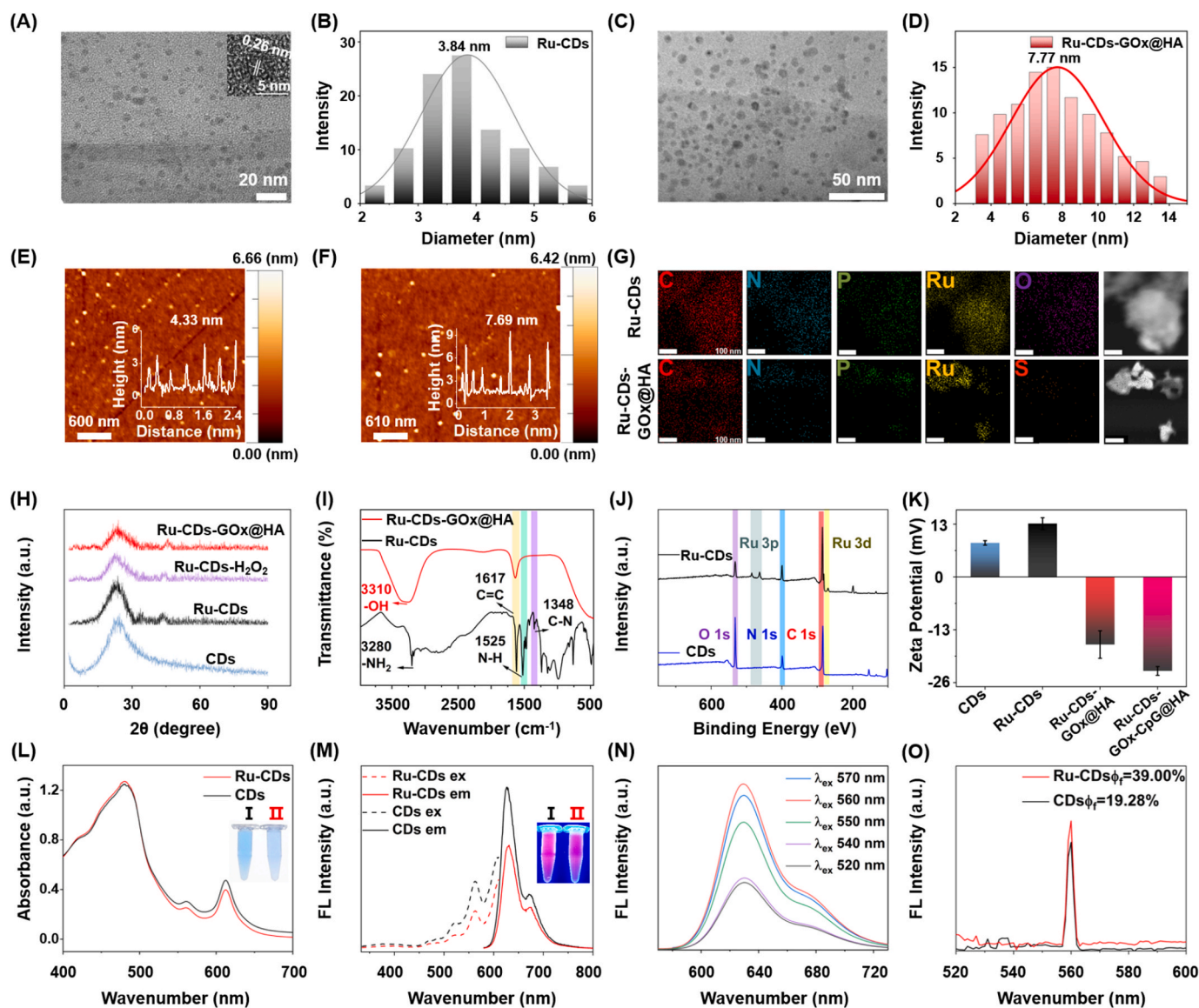


Fig. 1. Composition and characterization of materials. (A, C) TEM images. (B, D) Size distribution. (E, F) AFM imaging. (G) Elemental mapping. (H) XRD patterns of the CDs and Ru-CDs before and after reaction with H_2O_2 . (I) FT-IR spectra. (J) XPS spectra. (K) Zeta potential. (L) UV-vis spectra, (M) NIR fluorescence spectra, (N) emission spectra showing varying excitation wavelengths from 490 to 720 nm and (O) quantum yield assessments of CDs and Ru-CDs. Insets: corresponding optical photographs.

loading efficiency of GOx onto Ru-CDs-GOx@HA was 41.15 % (Fig. S2). Similarly, EDX mapping confirmed a uniform distribution of C, N, O, P, Ru, and S in Ru-CDs-GOx@HA, further verifying that the nanomaterials were successfully synthesized. The crystalline phase and crystallinity were analyzed using X-ray diffraction (XRD). The broad peak near 23° , as shown in Fig. 1H, corresponded to the graphite (002) diffraction peak of Ru-CDs and Ru-CDs-GOx@HA, suggesting that GOx and HA loading did not significantly alter the crystallinity of the Ru-CDs in the presence of H_2O_2 . Fourier transform infrared (FTIR) spectroscopy identified the functional groups on the surface of the nanomaterials. The peaks at 3280, 1617, 1525, and 1348 cm^{-1} (Fig. 1I) corresponded to the stretching vibrations of $\text{N}-\text{H}_2$, $\text{C}=\text{C}$, $\text{N}-\text{H}$, and $\text{C}-\text{N}$, respectively, indicating the presence of hydrophilic groups. X-ray photoelectron spectroscopy (XPS) confirmed the chemical composition of the Ru-CDs, which showed five characteristic XPS binding energy peaks corresponding to C 1s, N 1s, O 1s, Ru 3p, and Ru 3d, confirming that Ru doping was successful (Fig. 1J). The TEM and XPS analyses indicated that the Ru-CDs were composed of substrate-anchored CDs and positively charged Ru atoms. Further analysis of the Ru 3p XPS spectrum

revealed distinct chemical states Ru^0 3p (450.5 and 480.3 eV) and $\text{Ru}^{\delta+}$ 3p (465.7 and 488.57 eV) within the Ru-CDs (Fig. S3). Given the strong affinity between nitrogen and metals, as well as the high nitrogen content, we proposed that Ru was stabilized in the CDs substrate through Ru-N bonding. This hypothesis was supported by the high-resolution N 1s XPS spectra (Fig. S4), which displayed a prominent peak at approximately 398.1 eV, attributed to Ru-N bonding [20]. Deconvolution of the N 1s peak revealed two nitrogen species in both CDs and Ru-CDs: pyridinic N (sp^2 -hybridized nitrogen) at around 399.0 eV and graphitic N at 401.1 eV (Fig. S4). The zeta potential of the CDs was $8.38 \pm 0.55\text{ mV}$ (Fig. 1K). Upon Ru doping, the zeta potential increased to $13.12 \pm 1.44\text{ mV}$, suggesting that positively charged Ru had been incorporated. Following the conjugation of negatively charged GOx and HA, the zeta potential of the Ru-CDs-GOx@HA composite decreased to $-16.64 \pm 3.38\text{ mV}$. Following CpG oligodeoxynucleotides loading (bearing negatively charged phosphate backbones) further increased the negative zeta potential of Ru-CDs-GOx-CpG@HA to $-23.13 \pm 1.08\text{ mV}$. The CpG loading efficiency of this final composite was calculated to be approximately 42 % (Fig. S5), confirming the successful preparation of

Ru-CDs-GOx-CpG@HA. The absorption peaks of the CDs and Ru-CDs were 480 and 610 nm, respectively, corresponding to the $N \rightarrow \pi^*$ transitions of the carbon core (Fig. 1L) [21]. The excitation and emission wavelengths of the CDs and Ru-CDs are 560 nm and 630 nm, respectively (Fig. 1M), suggesting that Ru doping minimally affected the fluorescence characteristics. Interestingly, the emission wavelength of the Ru-CDs aligned closely with the biological window in the near-infrared I region (NIR-I), thus enhancing the potential for deep-tissue fluorescence imaging. The Ru-CDs also exhibited excitation-independent behavior (Fig. 1N). The quantum yield of CDs increased from 19.28 % to 39.00 % after Ru doping (Fig. 1O), suggesting the formation of a unique emission cluster [22]. Based on these results, Ru doping effectively could minimize interference from tissue autofluorescence, underscoring the potential of Ru-CDs in biological fluorescence imaging.

2.2. Time-dependent internalization of Ru-CDs-GOx@HA by tumor cells

The NIR-I region, with wavelengths greater than 600 nm, is well-known for its robust emission, minimal tissue absorption, and substantial tissue penetration [23]. The Ru-CDs showed excellent photostability and a high quantum yield of $\sim 39.00\%$ at an emission wavelength of 630 nm (Fig. 1M-O). 4T1 cells (mouse breast carcinoma cells) incubated with Ru-CDs-GOx@HA and evaluated using confocal microscopy revealed a marked increase in red fluorescence intensity over time. This increase was linked to the targeting of CD44 receptors, an over-expressing protein on tumor stem cells by HA (Fig. S6) [24]. The small size of Ru-CDs-GOx@HA (~ 7.7 nm) enhanced its cellular uptake. Its strongest fluorescence signal was observed 8 h post uptake, demonstrating its potential in cancer imaging applications.

2.3. CDT, PDT, and PTT effects of Ru-CDs-GOx@HA

ROS have emerged as critical agents in CDT and PDT for cancer treatment, with singlet oxygen (1O_2) and $\cdot OH$ being the most effective. CDT uses the Fenton reaction to convert intracellular H_2O_2 into ROS, particularly $\cdot OH$, facilitating targeted destruction of cancer cells. Peroxidase (POD)-like enzyme activity is vital in this process, as it catalyzes the oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) by H_2O_2 , leading to ROS production, which underlies the efficacy of CDT. As shown in Fig. 2A, a significant absorbance at 652 nm was observed and a noticeable blue color change occurs only when Ru-CDs, TMB, and H_2O_2 coexist. This result indicated that the Ru-CDs effectively catalyzed H_2O_2 to generate ROS, converting TMB to its oxidized form. In contrast, the CDs did not exhibit this catalytic activity, underscoring the critical role of Ru for POD activity. Further analysis of the POD-like activity of the Ru-CDs revealed a corresponding increase of absorbance at 652 nm with the increase of Ru-CDs ranging from 0 $\sim$ 100 $\mu g/mL$ (Fig. S7). Steady-state kinetic studies revealed that H_2O_2 followed the Michaelis-Menten model under optimal conditions (Fig. 2B, C), with a maximum initial velocity (V_{max}) of 3.26×10^{-6} mM/s and a Michaelis constant (K_m) of 1.63 mM. A lower K_m value indicated greater affinity, and a higher V_{max} value indicated a faster reaction for Ru-CDs than for the native enzyme horseradish peroxidase or for other CDs previously reported (Table S3).

Terephthalic acid was employed as a fluorescence probe, as it reacts specifically with $\cdot OH$ to produce highly fluorescent 2-hydroxyterephthalic acid. Fig. 2D showed a fluorescence peak at 427 nm when the Ru-CDs were combined with H_2O_2 , suggesting that the Ru-CDs enhanced the O-O bond cleavage of H_2O_2 and generated $\cdot OH$. Neither the CDs nor Ru alone catalyzed this reaction. The production of $\cdot OH$ was further evaluated using methylene blue (MB) degradation. As shown in Fig. 2E, the absorbance at 665 nm was high with H_2O_2 alone but it decreased significantly when Ru-CDs were added, demonstrating their excellent catalytic oxidation of MB by generated $\cdot OH$. Electron spin resonance (ESR) spectroscopy, which used DMPO as a trapping agent, further

confirmed the generation of $\cdot OH$ (Fig. 2F). The characteristic 1:2:2:1 quartet signal in the Ru-CDs + H_2O_2 group provided a robust evidence of $\cdot OH$ formation, validating the effects of CDT. Additionally, GOx oxidizes glucose to generate H_2O_2 in the presence of oxygen. Thus, the toxic $\cdot OH$ may be produced through a cascade reaction facilitated by Ru-CDs-GOx@HA. Consequently, the catalytic reaction of GOx in Ru-CDs-GOx@HA with endogenous glucose further enhanced H_2O_2 generation in the TME, which may increase the efficacy of CDT (Fig. S8).

PDT combines the interactions between photosensitizers, specific wavelengths of light, and molecular oxygen to selectively destroy target tissues [25]. Here, we used 1,3-diphenylisobenzofuran (DPBF) as an 1O_2 probe to investigate the efficacy of the Ru-CDs. A significant decrease in DPBF absorption at 410 nm was observed in the presence of Ru-CDs compared to that observed in the presence of the CDs and other control materials, indicating superior PDT activity and 1O_2 generation by the Ru-CDs (Fig. 2G). Meanwhile, irradiation at 808 nm further enhanced 1O_2 production by the Ru-CDs. The 1O_2 production increased as the Ru-CDs concentration increased (Fig. 2H). ESR spectroscopy confirmed the generation of both 1O_2 and $\cdot OH$ after 808 nm laser irradiation, indicating that the Ru-CDs efficiently produced ROS through both type I (electron transfer generating $\cdot OH$) and type II (energy transfer generating 1O_2) mechanisms (Fig. 2I; Fig. S9). Interestingly, this dual-mechanism capability of ROS generation is rare among Ru complexes, underscoring the unique potential of Ru-CDs for applications in chemotherapeutic and photodynamic therapies.

Ru-based nanomaterials are well known for their high catalytic activity in multielectron reactions involving oxygen intermediates [26]. This activity has been attributed to their rich unoccupied d-electron orbitals and excellent redox stability [27]. The POD enzyme activity of Ru-CDs is closely related to their size, with smaller particle diameters (~ 3.19 nm) leading to enhanced enzyme-like activity. Furthermore, the incorporating Ru into CDs was shown to improve the external electrical responsiveness by reducing the electron density of the nanozyme and shifting the d-band center closer to the Fermi level [28]. This modification enhanced substrate adsorption, lowers energy barriers, ensures adequate exposure of reactive atoms, and promoted mass transfer during reactions, thereby significantly increasing the catalytic activity.

PTT uses light-absorbing materials to thermally destroy cancer cells and therefore is minimally invasive while still providing precise spatial and temporal selectivity [29]. The photothermal properties of the Ru-CDs, including their photostability and photothermal conversion efficiency, were thoroughly investigated. Compared with the CDs, the Ru-CDs shows significantly greater absorbance in the NIR-I region, suggesting comparatively enhanced photothermal performance (Fig. S10). As shown in Fig. 2J, the CDs exhibited weak photothermal activity ($<30^\circ C$), but the Ru-CDs showed substantial activity ($\sim 64^\circ C$). Importantly, the addition of GOx or HA (Ru-CDs@HA or Ru-CDs-GOx@HA) had little effect on the photothermal activity of the Ru-CDs. The photothermal conversion efficiency (η) of the Ru-CDs was determined to be 66.21 % (Fig. 2K), indicating superior efficiency than the reported values of the CDs (Table S4). Similar results were obtained from the NIR thermal images of the PBS, CDs and Ru-CDs under continuous irradiation of 808 nm (Fig. 2L). Although the loading of HA and GOx slightly decreased the conversion efficiency, a relatively high efficiency (49.31 %) was still maintained under NIR irradiation (Fig. S11A). The temperature of the Ru-CDs solutions under 808 nm irradiation increased from ~ 26.7 to $\sim 57.7^\circ C$ as the concentrations of Ru-CDs increased (Fig. 2M and N), indicating the suitability of phototherapy for tumor ablation. Photostability is a crucial requirement of photothermal agents. The photothermal activity of the Ru-CDs and Ru-CDs-GOx@HA remained consistent after four cycles of 808 nm laser irradiation, demonstrating outstanding stability (Fig. 2O; Fig. S11B). Considering that the rise of temperature of Ru-CDs-GOx@HA to $\sim 50^\circ C$ after irradiation of 808 nm, which may affect the activity of GOx, we evaluated the GOx activity of Ru-CDs-GOx@HA after incubation at $50^\circ C$ for 5 min. As shown in Fig. S12, the GOx activity of Ru-CDs-GOx@HA

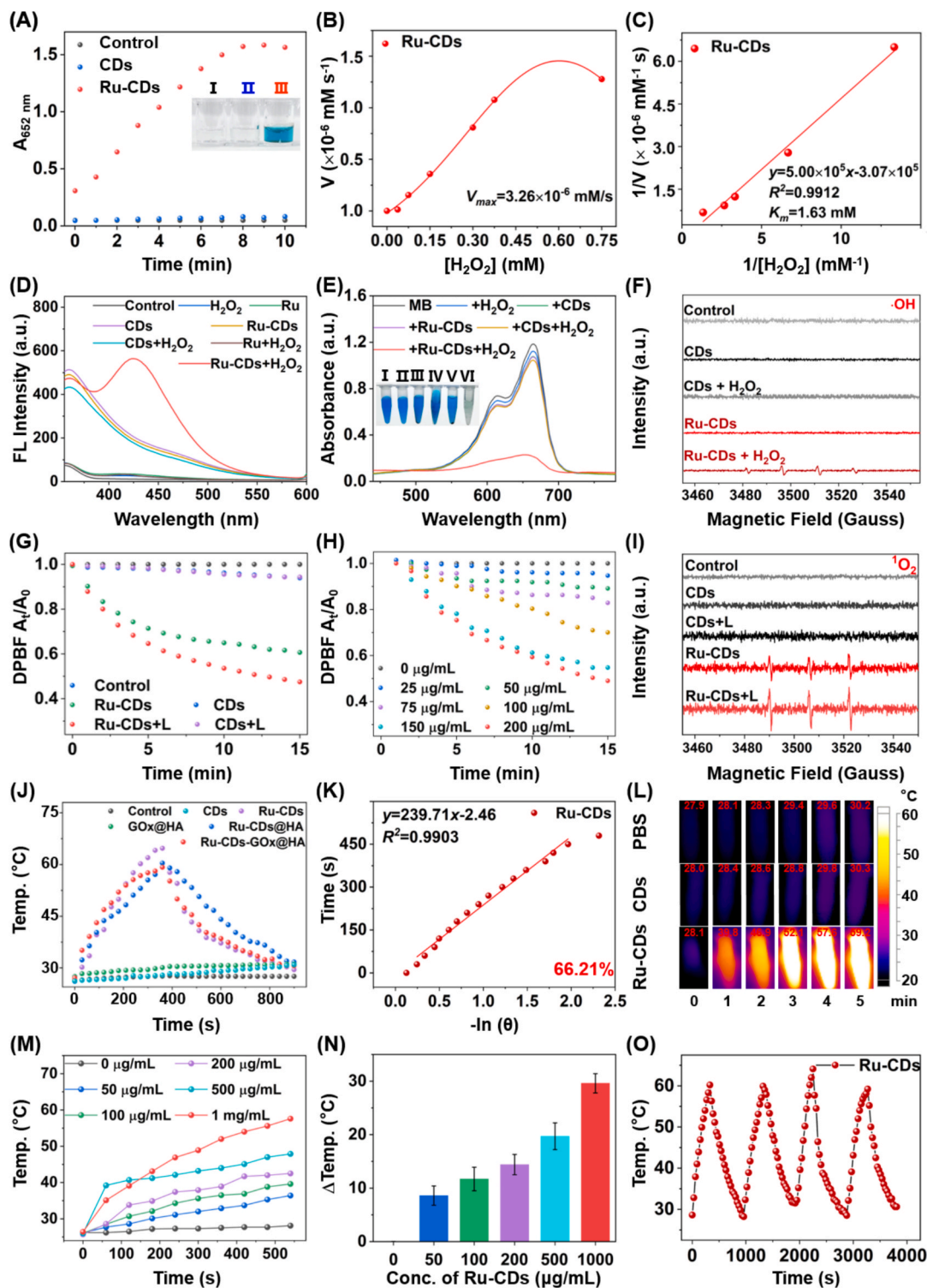


Fig. 2. Chemical kinetics, photodynamic and photothermal properties of the materials. (A) UV-vis absorption spectra of the CDs and Ru-CDs during TMB oxidation. Insets: corresponding optical photographs of the control, CDs, and Ru-CDs. (B) Steady-state kinetic analyses and (C) Lineweaver-Burk plots of Ru-CDs using H₂O₂ as the substrate. (D) Fluorescence emission spectra of the terephthalic acid probe in the presence of the nanomaterials to evaluate hydroxyl free radicals (excitation at 315 nm). (E) UV-vis absorption spectra of MB in different nanomaterials to evaluate MB degradation. Insets: corresponding optical photographs of MB (I), +H₂O₂ (II), +CDs (III), +Ru-CDs (IV), +CDs + H₂O₂ (V), and +Ru-CDs + H₂O₂ (VI). (F) ESR spectra for detecting $\cdot OH$ generation. 1O_2 generation by different nanomaterials (CDs and Ru-CDs) (G) and (H) Various concentrations of Ru-CDs under 808 nm laser irradiation (1 W/cm², 15 min) at different time intervals with DPBF was used as the probe. (I) ESR spectra for detecting 1O_2 generation. (J) Temperature versus time profiles of PBS, CDs, Ru-CDs, Ru-CDs@HA, GOx@HA, and Ru-CDs-GOx@HA under 808 nm irradiation at 1 W/cm² for 5 min. (K) Plot of cooling time data versus $-\ln \theta$ and the corresponding linear fitting curve of Ru-CDs under 808 nm irradiance at 1 W/cm² for 5 min. (L) NIR thermal images of PBS, CDs, and Ru-CDs under continuous 808 nm irradiation (1.0 W/cm²) for 5 min. Data are presented as the means $\pm$ SDs. (M, N) Concentration-dependent photothermal response of the Ru-CDs under 808 nm laser irradiation for 5 min (1 W/cm²). (O) Temperature variation of the Ru-CDs dispersion with 1 W/cm² for 5 min on-and-off for four cycles.

retained 95 % of its original activity, indicating that the activity of GOx was hardly affected under photothermal conditions in our study (50 °C, 5 min). Next, in the power gradient experiments, the temperature of the Ru-CDs solution exceeded the effective therapeutic threshold (~ 62 °C) within 5 min (1 W/cm^2) (Fig. S13), confirming the potential of the Ru-CDs for use in PTT.

2.4. Density functional theory (DFT) calculations

To better understand the catalytic performance and enhanced photothermal efficiency of the Ru-CDs, DFT calculations were performed to explore the underlying microscopic mechanisms [30]. Fig. 3A shows the geometric configurations and optimal adsorption models for CDs and Ru-CDs, which revealed that vacancy-rich CDs effectively anchored Ru, thereby preventing aggregation and enhancing reaction kinetics. Vertical excitation simulations indicated that the absorption peak of the CDs was at $\sim 1163 \text{ nm}$, whereas the main peak for the Ru-CDs was at $\sim 1460 \text{ nm}$ (Fig. 3B). This band aligned with the absorption band of liquid water in the NIR range ($\sim 1470 \text{ nm}$) and effectively generated heat generation upon light exposure [22]. Therefore, exposure to light within the

resonance energy range of the Ru-CDs led to synchronized oscillation of the conduction band electrons in the Ru-CDs, generating heat generation within the system. This confirmed that the Ru-CDs exhibited strong optical absorption and high surface activity in the NRI range. Using POD-like activity as an example, the adsorption of H_2O_2 at the active sites of the Ru-CDs marked the initial step of the catalytic process (I) (Fig. 3C). The adsorption energy of H_2O_2 to the Ru-CDs (-0.01 eV) was significantly lower than that to the CDs (1.89 eV), indicating a stronger binding affinity between the Ru-CDs and H_2O_2 molecules. We then investigated two possible decomposition pathways: homolytic cleavage (red line) and heterolytic cleavage (blue line) of the O–O bond in H_2O_2 by the Ru-CDs (Fig. 3D). The reaction energy for homolytic cleavage (-4.34 eV) was markedly lower than that for heterolytic cleavage (-3.59 eV), demonstrating that Ru-CDs significantly accelerated the homogeneous dissociation of H_2O_2 molecules adsorbed at the active sites to form two $\cdot\text{OH}$ radicals (II and III). This step was a spontaneous reaction; CDs, which lacked active center, could not facilitate this process. These results indicated that Ru-CDs promoted $\cdot\text{OH}$ generation. The intrinsic reaction coordinate results further confirmed that the energy barrier of the catalytic homolytic cleavage of H_2O_2 by Ru-CDs was

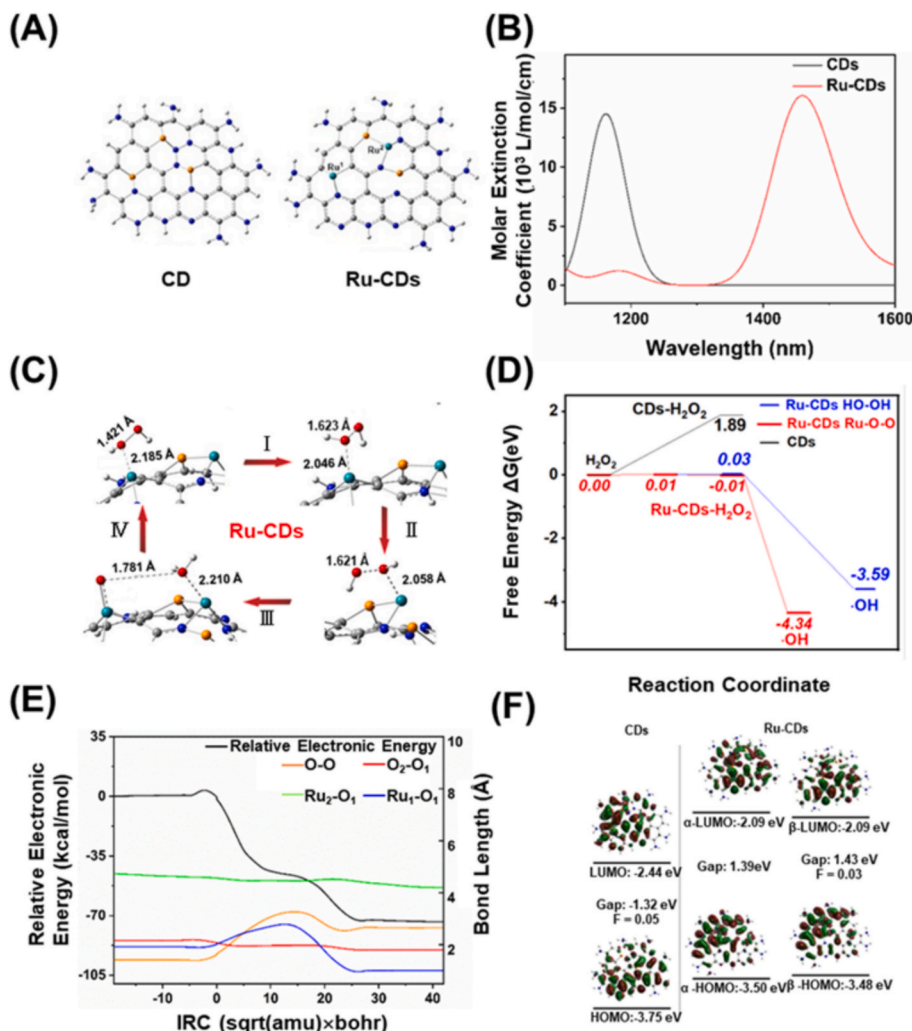


Fig. 3. Geometric structures and steady-state kinetics of the materials based on DFT calculations. (A) Geometric structures of the various states of CDs and Ru-CDs. (B) Simulated UV/Vis spectra of CDs, and Ru-CDs. Calculated at TD-PBE1PBE-D3BJ/def2-TZVP//PBE1PBE-D3BJ/def2-SV (P) level with SMD model (solvent = water). (C) The proposed catalytic mechanism of Ru-CDs in the POD-like reaction, including intermediates and transition states involved in Ru-CDs-assisted H_2O_2 homolysis (side view and focused view). The red, grey, blue, white, and yellow spheres represent O, C, N, H, and Ru atoms, respectively. (D) Calculated free energy diagram comparing the energy barriers of Ru-CDs (HO-OH homolysis and heterolytic cleavage formation) and CDs. (E) IRC calculation results for CDs and Ru-CDs. (F) DFT calculations of the energy band differences between CDs and Ru-CDs. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

substantially lower than that of heterolytic cleavage (activation energy $\Delta G \neq 45.0$ kcal/mol) and therefore produced a much faster catalytic reaction (Fig. 3E). Finally, following the complete desorption of water molecules, the Ru-CDs generated new active Ru=O species. These, in turn, significantly enhanced the subsequent oxidation process (IV), presenting a unique feature not typically observed in other catalytic materials. Furthermore, band structure calculations for both the CDs and Ru-CDs revealed that, although Ru-CDs had slightly higher band energy, both materials maintained relatively low band energies and narrow band gaps, which could be advantageous for specific catalytic applications. (Fig. 3F) [31]. In summary, compared with planar and symmetrical CDs, Ru doping endowed the active sites of CDs with a stronger electron transfer capability. This effectively optimized the free energy of each reaction step, reduced the energy barrier of the rate-limiting step, and accelerated the overall reaction.

2.5. Tumor cell killing effect of Ru-CDs-GOx@HA in vitro

To assess the cytotoxicity of Ru-CDs-GOx@HA, Human breast cancer cells (MCF-7) and normal human breast epithelial cells (MCF-10A) were cultured with various concentrations of Ru-CDs-GOx@HA (Fig. 4A). Even after treatment with a high concentration of Ru-CDs-GOx@HA (500 $\mu\text{g/mL}$), the MCF-10A cells maintained over 95 % viability, demonstrating good biosafety of Ru-CDs-GOx@HA to normal cells. In contrast, the viability of MCF-7 cancer cells decreased to 75 % at a concentration of 500 $\mu\text{g/mL}$, indicating the selective cytotoxicity of Ru-CDs-GOx@HA to cancer cells. We further explored the inhibitory effect of Ru-CDs on 4T1 cells. Under the same concentrations of 500 $\mu\text{g/mL}$, cells treated with Ru-CDs alone maintained over 90 % cell viability, whereas treatment with Ru-CDs-GOx@HA reduced viability to ~ 70 % (Fig. 4B). This result suggested that the incorporating GOx and HA into the nanoparticles effectively resolved the issue of low H_2O_2 levels in the TME. We then investigated whether combining Ru-CDs-GOx@HA with CDT, PDT, and PTT could further inhibited cancer cell proliferation. Combining Ru-CDs-GOx@HA with H_2O_2 (100 mM) reduced the viability of 4T1 cells to 35 % and that of MCF-7 cells to 46 %. To evaluate the effects of PDT and PTT, 4T1 or MCF-7 cells were cultured with Ru-CDs-GOx@HA and 808 nm laser irradiation was applied. The cell viability of the 4T1 cell decreased to 29 % and that of MCF-7 cell decreased to 17 % (Fig. 4C and D). The enhanced cytotoxicity was attributed to the generation of cytotoxic radicals and the higher temperature increase, which efficiently induced cell death. Summarizing, Ru-CDs-GOx@HA combined with H_2O_2 and NIR laser irradiation exerted a powerful cytotoxic effect, with MCF-7 and 4T1 cell viability reduced to 22 % and 10 % (Fig. 4C and D), respectively, rendering this strategy highly inhibited the cancer cell proliferation.

Next, the 2',7'-dichlorofluorescein diacetate (DCFH-DA) probe was used to measure the intracellular ROS generation in the presence or absence of H_2O_2 . As shown in Fig. 4E and H, the Ru-CDs + H_2O_2 and Ru-CDs-GOx@HA + H_2O_2 groups exhibited significantly stronger green fluorescence than the control group, indicating massive ROS (primarily $\cdot\text{OH}$) generation by the Ru-CDs. The Ru-CDs-GOx@HA group exhibited slight fluorescence even without H_2O_2 , which was attributed to H_2O_2 production from glucose oxidation by GOx of Ru-CDs-GOx@HA. ROS generation usually reduces the mitochondrial membrane potential, leading to apoptosis [32]. The 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide (JC-1) probe was used to assess the impact of $\cdot\text{OH}$ on the mitochondrial membrane potential [33]. Fig. 4F shows that the Ru-CDs-GOx@HA group exhibited strong green fluorescence of JC-1 monomers and weak red fluorescence of J-aggregates, indicating a substantial reduction in the mitochondrial membrane potential, likely due to $\cdot\text{OH}$ generated by Ru-CDs-GOx@HA in the presence of H_2O_2 . To assess cell viability, calcein-AM/propidium iodide (PI) staining was performed. As shown in Fig. 4G and I, in the groups treated with H_2O_2 (G2) or laser irradiation alone (G3), no significant cell death was observed, indicating that these treatments alone had no

inhibitory effect on cell growth. However, in the groups (G6 and G7) treated with Ru-CDs-GOx@HA and H_2O_2 or the NIR, there was a significant increase in cell death, demonstrating the synergistic catalytic therapeutic effects of CDT, PDT, and PTT. In the combination treatment group (G8), nearly all the 4T1 cells were dead, indicating high levels of oxidative stress within the cancer cells. Accordingly, the Ru-CDs-GOx@HA nanoparticles effectively targeted cancer cells. The combination of multiple treatments further enhanced intracellular oxidative stress, leading to sustained glucose depletion and an increase in endogenous ROS.

2.6. RNA sequencing (RNA-seq) analysis

To further elucidate the potential mechanisms of the nanomaterial at the cellular level, RNA sequencing analysis was performed on cells treated with phosphate-buffered saline (PBS, control group) and Ru-CDs-GOx@HA + H_2O_2 + L. Principal component analysis revealed significant transcriptomic differences between the control and treatment groups (Fig. 5A). The tight clustering within each group, and the clear separation between them, confirmed the high quality and reliability of the data. This result was further supported by a correlation heatmap, which showed strong intragroup correlations and pronounced intergroup differences (Fig. 5B). A volcano plot further highlighted 1253 upregulated and 1811 differentially expressed genes (DEGs) (Fig. 5C). Gene set enrichment analysis identified the involvement of apoptosis and ferroptosis pathways (Fig. S14, Fig. 5D). Further analysis revealed that apoptosis-related genes, including *Bid*, *Parp2*, and *Ddit3*, were upregulated. Additionally, there was a notable activation of *caspase-3* expression, indicating the induction of apoptosis through *caspase-3* activation and mitochondrial damage. (Fig. 5E). Recently, the concept of PANoptosis has emerged, which describes an inflammatory form of programmed cell death that combines several key characteristics of known forms of cell death, pyroptosis, necroptosis, apoptosis, and/or ferroptosis [34]. Interestingly, we found that our observed DEGs were involved in the pyroptosis pathway, characterizing the up-regulation of key signaling genes (*Cxcl2*, *Tnf*, and *Rela* (Fig. 5F). Additionally, functional genes associated with necroptosis, including *Adams7*, *Brca1*, and *Gata3*, were found to be generally upregulated (Fig. 5G). Additionally, ferroptosis-related genes were upregulated, including *Tfrc*, *Sat1*, and the lipid peroxidation gene *Ptgs2*, while the ferroptosis-inhibiting genes *Acs11* and *Hmox1* were downregulated, further confirming the occurrence of ferroptosis in the treatment group (Fig. 5H). Collectively, these findings demonstrated that Ru-CDs-GOx@HA + H_2O_2 + L may induce the combined actions of programmed cell death involving the PANoptosis pathway.

2.7. Verification of PANoptosis

Because RNA-seq analysis suggested that the programmed cell death potentially involved PANoptosis, including pyroptosis, apoptosis, necroptosis, and ferroptosis [35], this encouraged us to confirm whether the mechanism of cell death involved PANoptosis after Ru-CDs-GOx@HA + H_2O_2 + L treatment. Apoptosis is a key mechanism in cancer cell death and the process can be further amplified through upregulated proapoptotic protein, such as Bax and cleaved caspase-3 (C-Cas-3) [36]. Flow cytometry analysis revealed that Ru-CDs-GOx@HA + H_2O_2 + L treatment induced an apoptosis rate of ~ 83.02 %, which was significantly greater than that of the control (PBS) (~ 0.00 %), Ru-CDs-GOx@HA (~ 25.71 %), Ru-CDs-GOx@HA + H_2O_2 (~ 48.71 %), and Ru-CDs-GOx@HA + L (~ 65.81 %) groups (Fig. 6A). Western blot (WB) analysis was performed to evaluate the expression of proapoptotic proteins. Compared with the control group, the Bax and C-Cas-3 levels in the Ru-CDs-GOx@HA + H_2O_2 + L group were 3.27- and 2.39-fold higher, respectively (Fig. 6B-D). This result indicated that the combined effects of CDT, PDT, and PTT synergistically enhanced the apoptosis of the tumor cells.

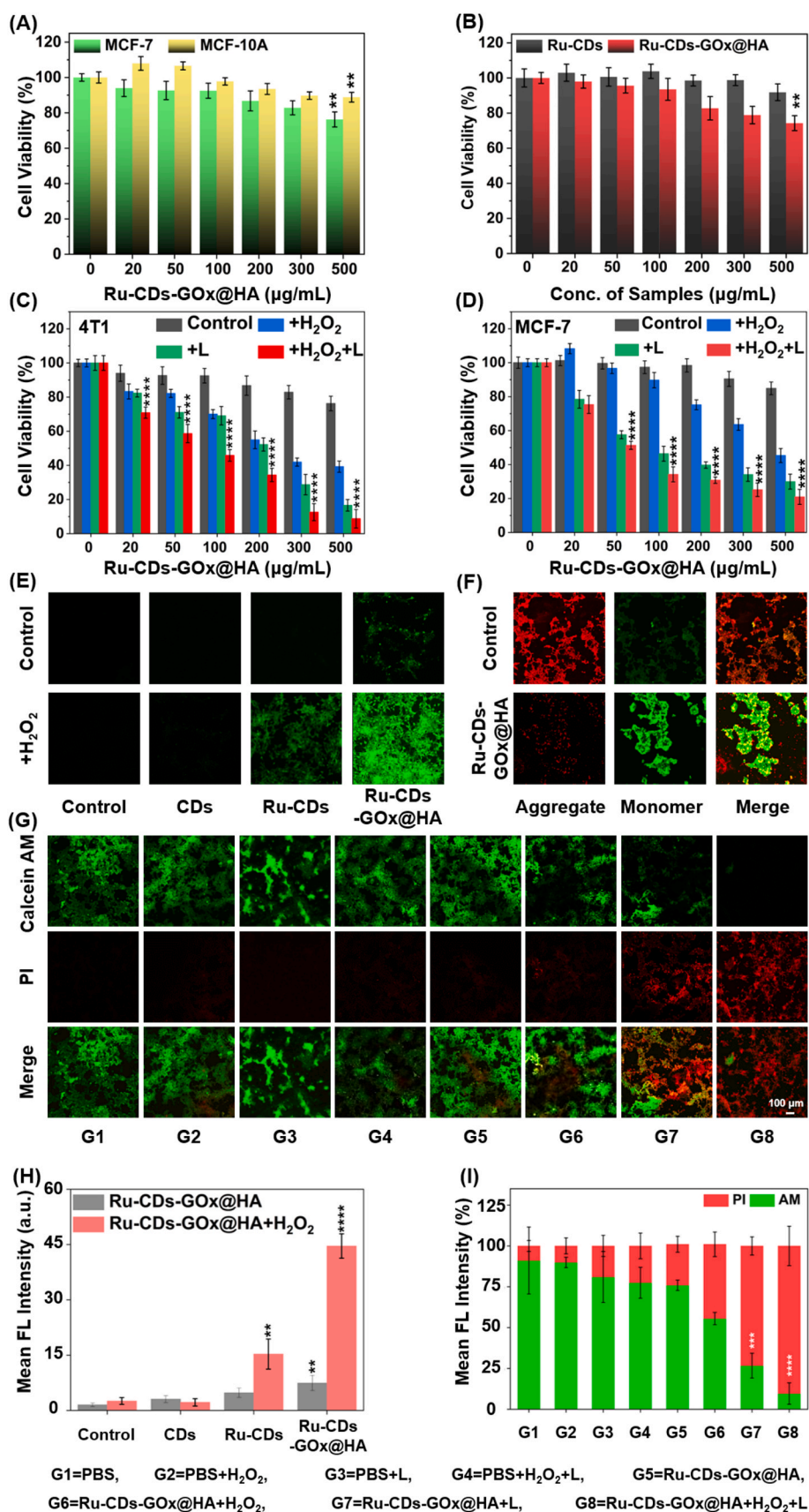


Fig. 4. Assessment of in vivo antitumor efficacy. (A) Cytotoxic effects of different concentrations of Ru-CDs-GOx@HA on MCF-7 and MCF-10A cells after 24 h of incubation. (B) Cytotoxicity of Ru-CDs and Ru-CDs-GOx@HA on 4T1 cells. (C, D) Cytotoxicity of Ru-CDs-GOx@HA combined with or without H₂O₂ and with or without 808 nm irradiation in 4T1 and MCF-7 cells. (E, H) CDT-induced cytotoxic effects of different nanomaterials on 4T1 cells and the corresponding fluorescence intensities. (F) Mitochondrial membrane potential in 4T1 cells treated with H₂O₂. (G, I) Live/dead cell imaging of 4T1 cells stained with calcein-AM/PI and the corresponding fluorescence intensity. **p* < 0.05, ***p* < 0.01, ****p* < 0.001, and *****p* < 0.0001.

In addition to apoptosis, substantial ROS release induced by CDT, PDT, and PTT can trigger pyroptosis. Pyroptosis is regulated by gasdermin proteins, particularly gasdermin D (GSDMD). GSDMD is cleaved by caspase-1 to release N-terminal gasdermin D fragment (N-GSDMD), which facilitates the formation of bubble-like protrusions (Fig. 6E) [37]. To confirm whether Ru-CDs-GOx@HA induced pyroptosis in an ROS- and temperature-dependent manner, activation of the caspase-1-mediated pathway and adenosine triphosphate (ATP) and lactate dehydrogenase (LDH) releases were determined. As shown in Fig. 6F and G, the results revealed a significant increase in extracellular ATP and LDH in 4T1 cells treated with Ru-CDs-GOx@HA, particularly when H₂O₂ was combined with 808 nm laser irradiation, whereas no significant changes were observed in the PBS, PBS + L, or Ru-CDs-GOx@HA groups. TEM analysis further confirmed the typical features of pyroptosis, including swelling and bubble formation, in 4T1 cells treated with Ru-CDs-GOx@HA + H₂O₂ + L (Fig. 6H). WB analysis also revealed significant increases of cleaved caspase-1 (C-Cas-1) and N-GSDMD expression, which were 1.94- and 6.57-fold higher than those of control group, respectively (Fig. 6I-K). These findings clearly indicated that the CDT, PDT, and PTT effects of Ru-CDs-GOx@HA amplified ROS production and heat generation, leading to GSDMD-dependent pyroptosis. This process induces oxidative stress, recruits inflammasomes, and activates the caspase-1 pathway, resulting in cell membrane rupture and subsequent cell death. In addition to apoptosis and pyroptosis, previous studies have demonstrated that mitochondrial ROS can also induce necroptosis, particularly through the phosphorylation of MLKL (p-MLKL) [38]. To investigate this, we further measured the protein expression levels of Receptor-Interacting Protein Kinase 3 (RIPK3), and p-MLKL. As shown in Fig. 6L-N, Ru-CDs-GOx@HA, combined with H₂O₂ or irradiation, induced higher expression of endogenous RIPK3 and p-MLKL compared with that to the control group. In particular, in the Ru-CDs-GOx@HA + H₂O₂ + L group, the expression of RIPK3 and p-MLKL was significantly upregulated, confirming the tangible occurrence of necroptosis.

We subsequently investigated ferroptosis induced by excessive ROS, which leads to lipid peroxidation, GSH depletion, and the down-regulation of Glutathione Peroxidase 4 (GPX4) [39]. Notably, the mitochondrial morphology is significantly altered with the onset of ferroptosis. The mechanism of ferroptosis is illustrated in Fig. 7A. To confirm the ferroptosis, we measured intracellular GSH levels. As shown in Fig. 7B, the Ru-CDs-GOx@HA + H₂O₂ + L treatment group presented the greatest GSH depletion, which was attributed to the increased ROS generated by the combination treatment. The depleted GSH levels further promoted ferroptosis. Next, we assessed human soluble transferrin receptor 1 (sTfR1), a biomarker for the progression of ferroptosis. Compared with the control group, sTfR1 expression significantly increased in the Ru-CDs-GOx@HA + H₂O₂ + L group in 4T1 cells (Fig. 7C), indicating increased ferroptosis. Moreover, WB analysis revealed that GPX4 and solute carrier family 7 member 11 (xCT) levels of 4T1 cells significantly decreased, whereas transferrin receptor (TFRC) level expression increased treated with Ru-CDs-GOx@HA + H₂O₂ + L (Fig. 7E-G), highlighting the importance of the synergistic effects of CDT, PDT, and PTT in driving ferroptosis (Fig. 7D-G). Additionally, both normal 4T1 cells or exposed to laser irradiation, after staining with C11-BODIPY, exhibited bright red fluorescence and weak green fluorescence, suggesting the absence of lipid peroxidation. However, treatment with Ru-CDs-GOx@HA + L/H₂O₂ induced the generation of green fluorescence. Notably, 4T1 cells treated with Ru-CDs-GOx@HA + H₂O₂ + L displayed the strongest green fluorescence (Fig. 7H), indicating the

highest level of lipid peroxidation. This further confirms the potent ferroptosis-inducing ability of Ru-CDs-GOx@HA. TEM analysis revealed substantial mitochondrial alterations, including condensation and a reduced volume, further confirming the occurrence of ferroptosis events (Fig. 7I). These results clearly indicated that Ru-CDs-GOx@HA + H₂O₂ + L effectively induced ferroptosis when combined with CDT, PDT, and PTT by disrupting the redox balance and causing mitochondrial dysfunction. Overall, there was a significant association between the cell death pathways upon combined treatment with Ru-CDs-GOx@HA and NIR irradiation. All four modes of cell death were observed in the tumor cells, enabling PANoptosis and thereby enhancing the efficacy of the combination treatment.

2.8. In vivo antitumor effects

Considering that Ru-CDs-GOx@HA exhibited excellent inhibition of tumor cell proliferation, the antitumor efficacy of Ru-CDs-GOx@HA was then evaluated in vivo in BALB/c-nu mice xenografted with 4T1 cells. Mice with tumors measuring approximately 100 mm³ were randomly assigned to four groups (*n* = 5): the PBS, PBS + L, Ru-CDs-GOx@HA, and Ru-CDs-GOx@HA + L. The tumor-bearing mice received intravenous injections of the respective treatments every 2 d, followed by NIR laser irradiation (808 nm, 1 W/cm²) for 5 min at 12 h post-injection (Fig. 8A). As shown in Fig. 8B and C, compared with the PBS group, the PBS + L group exhibited a similar therapy effect, suggesting that NIR irradiation alone had little effect on the tumor therapy. The Ru-CDs-GOx@HA group exhibited rapid growth of tumor, which was attributed to the limited catalytic activity of GOx in the Ru-CD-GOx@HA. In contrast, the Ru-CDs-GOx@HA + L group showed extraordinarily effective tumor suppression, which aligned with the results in vitro cytotoxicity. All mice maintained stable body weights throughout the 14-d observation period, indicating the good biosafety of Ru-CDs-GOx@HA + L (Fig. 8D). At the conclusion of the treatment, Fig. S15 showed that the Ru-CDs-GOx@HA + L 1.0 mg/kg dosage exhibited weaker tumor inhibition compared to the 2.5 mg/kg dosage. Interestingly, tumor weight results revealed an 88.12 % tumor suppression in the Ru-CDs-GOx@HA + L (2.5 mg/kg) group (Fig. S16), with complete tumor disappearance in two mice (Fig. 8E). This significant tumor inhibition was attributed to the increased ROS generation and high photothermal conversion of nanomaterial induced by laser irradiation, which triggered PANoptosis, thereby increasing the antitumor treatment efficacy. The potential of Ru-CDs-GOx@HA in tumor imaging in breast cancer-bearing mice was also assessed. As shown in Fig. 8F, negligible NIR-I fluorescence was observed in untreated mice at 0 h, whereas a distinct fluorescence signal appeared at the tumor site after Ru-CDs-GOx@HA administration at 6 h. The signal intensity at the tumor site increased gradually at ~ 24 h post-injection, offering clear visualization of Ru-CDs-GOx@HA tumor targeting. Additionally, laser irradiation (808 nm, 5 min) caused a significant increase in temperature at the tumor site (Fig. 8G). With continued irradiation, the temperature at the tumor site reached approximately 56.4°C, which was consistent with the NIR photothermal conversion observed in vitro (Fig. 2L). The tumor growth curves of each mouse in the different treatment groups further demonstrated that single Ru-CDs-GOx@HA exhibited mild anti-tumor efficacy. However, the combination of Ru-CDs-GOx@HA and laser treatment resulted in the most significant tumor inhibition (Fig. 8H).

To evaluate the extent of apoptosis and necrosis in the tumor tissues after treatment, histological analyses, including hematoxylin-eosin (H&E) staining and terminal deoxynucleotidyl transferase dUTP nick

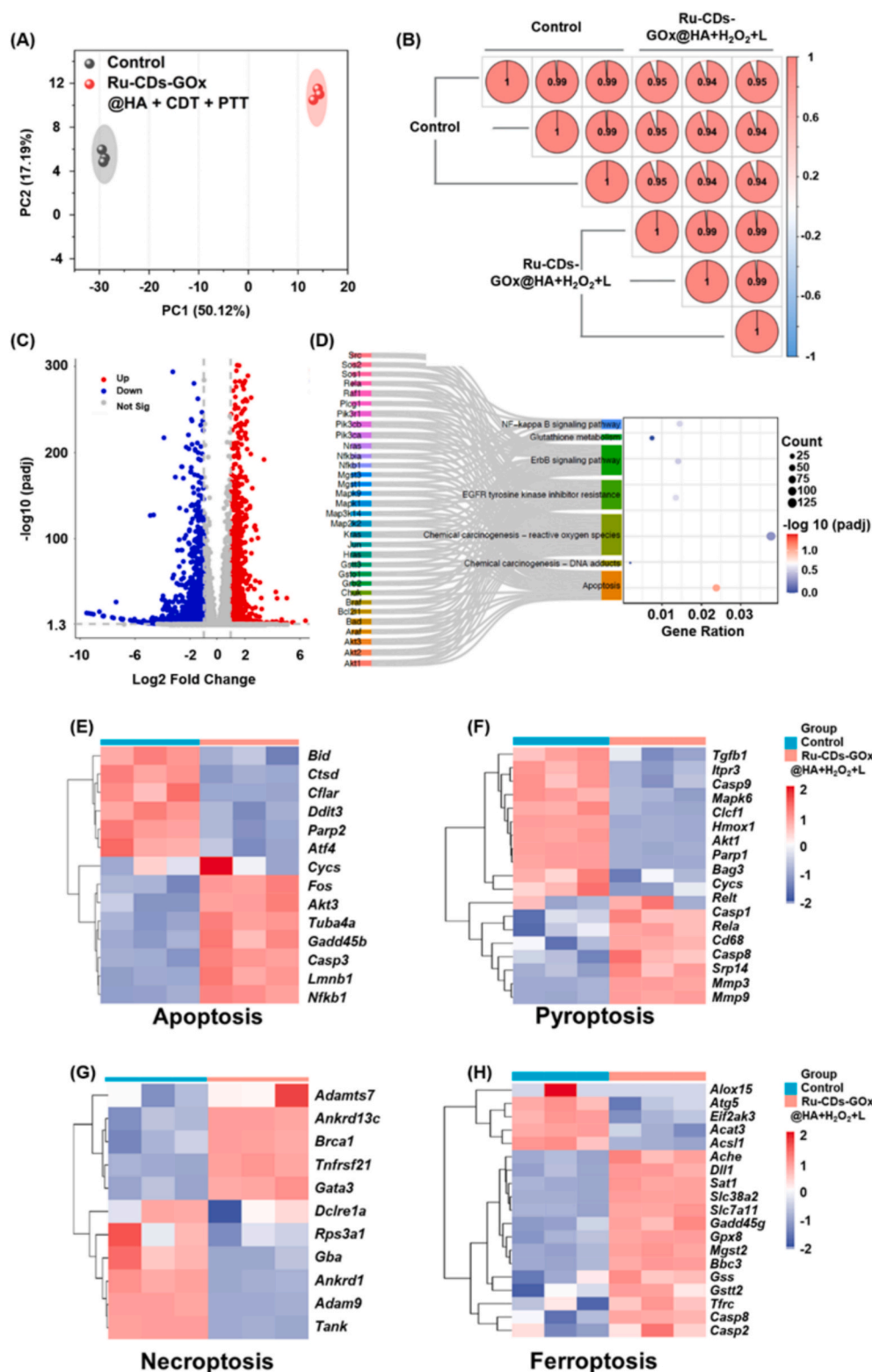


Fig. 5. RNA sequencing (RNA-Seq) analysis of 4T1 cells treated with the treatment group (Ru-CDs-GOx@HA + H₂O₂ + L) and control group (PBS). (A) Principal Component Analysis (PCA) plots of the treatment group and the control group. Each dot represents a cell sample from the respective groups. PC1 and PC2 denote the first and second principal components, respectively. (B) Heatmap of sample-to-sample correlation, showing the squared correlation coefficients calculated by Spearman's rank correlation method, displayed as circles on both horizontal and vertical axes. (C) Volcano plot illustrating differentially expressed genes (DEGs) between the treatment and control groups. Up-regulated genes are indicated by red points, while down-regulated genes are shown as blue points. (D) Sankey dot pathway enrichment diagram highlighting the enrichment of PANoptosis pathways among different cell populations. The dot plot shows the numbers of GSEs between the treatment group and the control group, along with the major genes involved in each enriched pathway. (E-H) Clustering heatmaps of DEGs related to PANoptosis pathways, comparing the treatment group to the control group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

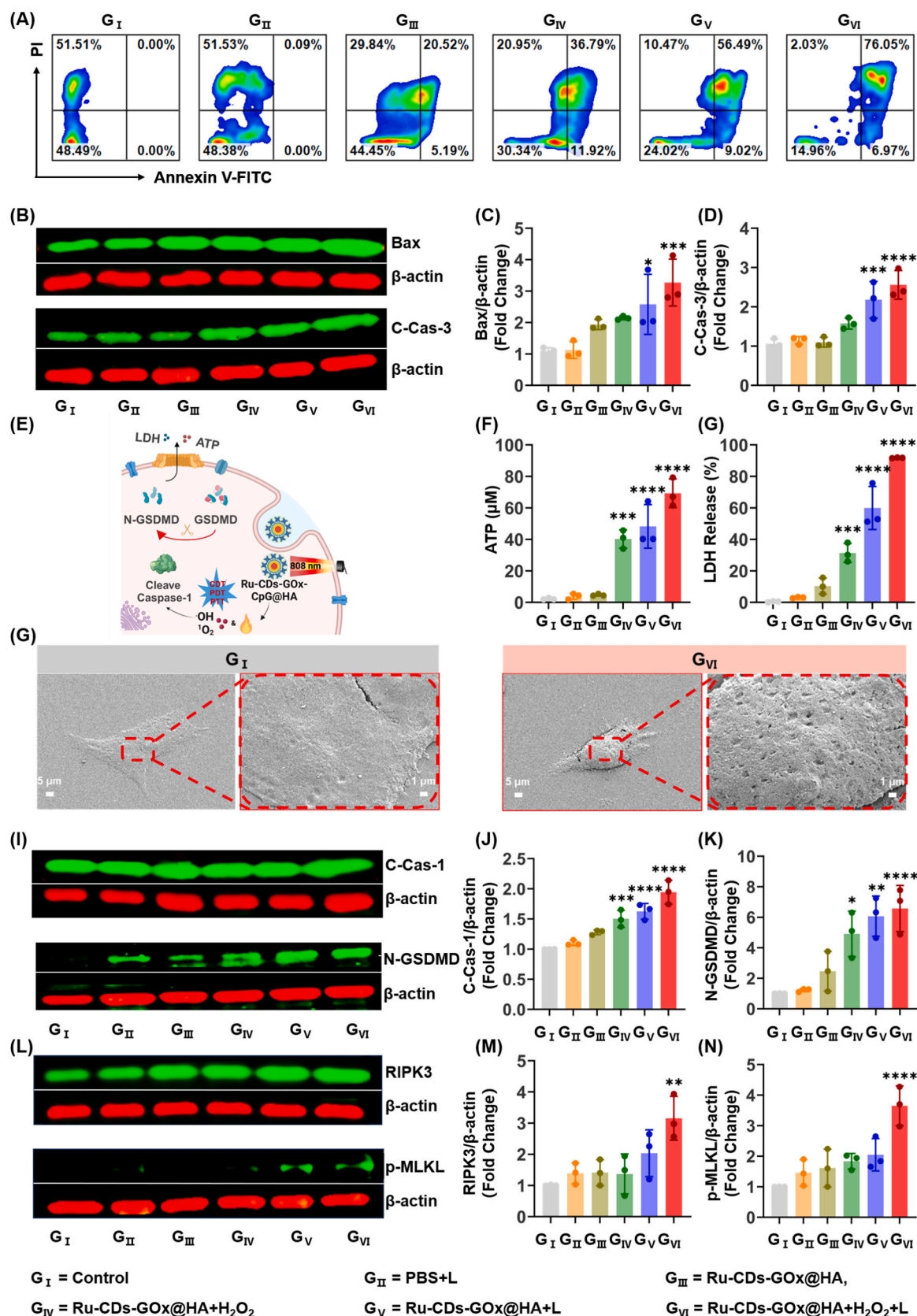


Fig. 6. Investigation of the mechanisms underlying apoptosis, pyroptosis, and necroptosis by the material. (A) Annexin V-FITC and PI staining assays were conducted on 4T1 cells. (B–D) The expression levels of C-Cas-3 and Bax were evaluated using WB analysis. (E) Role of Ru-CDs in inducing GSDMD-dependent pyroptosis. (F, G) Quantitative assessment of the thermonecrosis factors ATP and LDH. (H) Morphological changes in treated 4T1 cells. WB results for (I–K) C-Cas-1 and N-GSDMD and for (L–N) RIPK3 and p-MLKL.

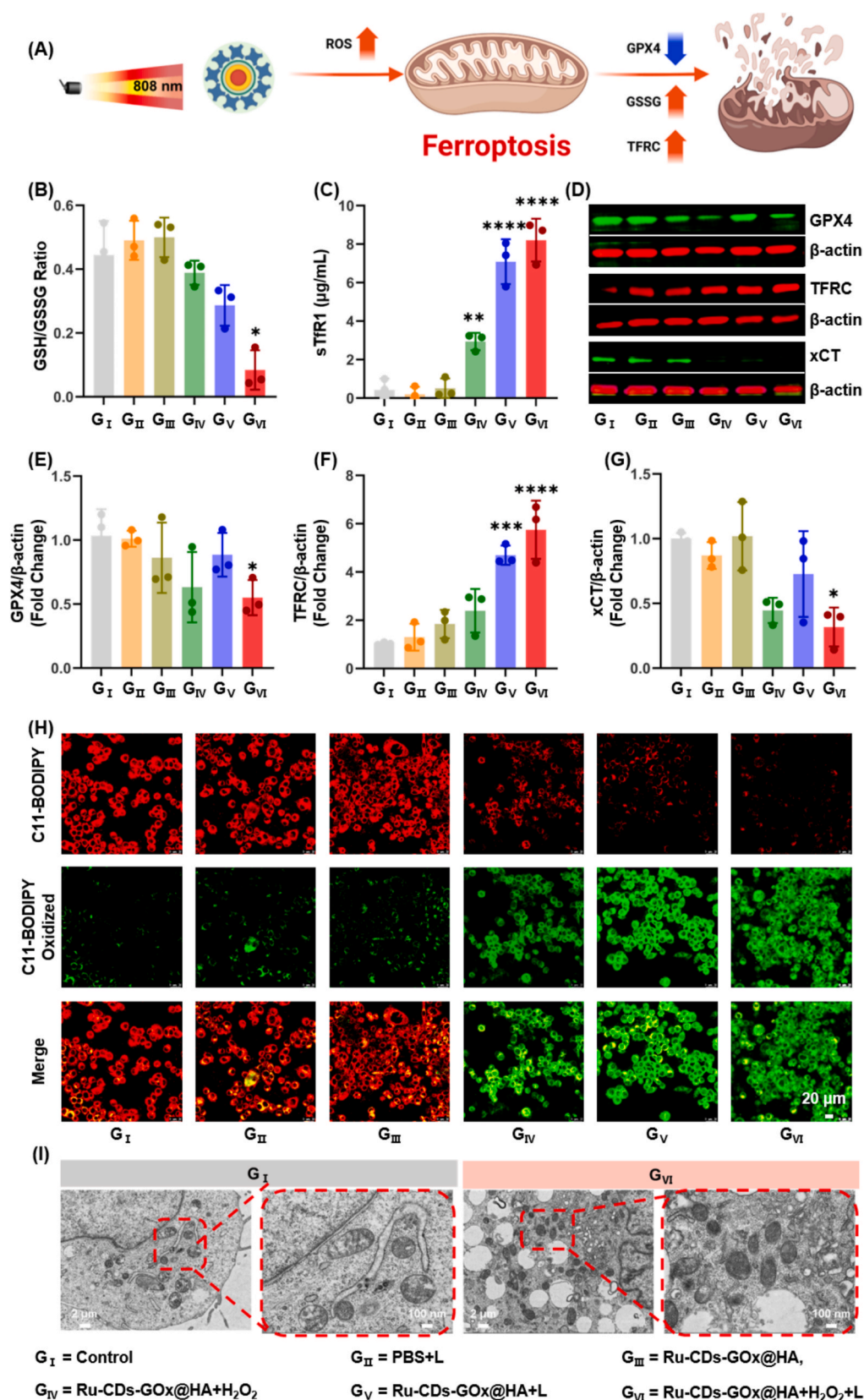


Fig. 7. Mechanisms of ferroptosis induced by the material. (A) Illustration of the possible ferroptosis mechanism induced by Ru-CDs-GOx@HA through ROS generation. (B, C) Changes in the GSH/GSSG ratio and transferrin receptor levels. (D-G) WB analysis of the ferroptosis-related proteins GPX4, TFRC, and xCT. (H) Confocal images highlighting lipid peroxidation. (I) Bio-TEM images of mitochondria in treated cells.

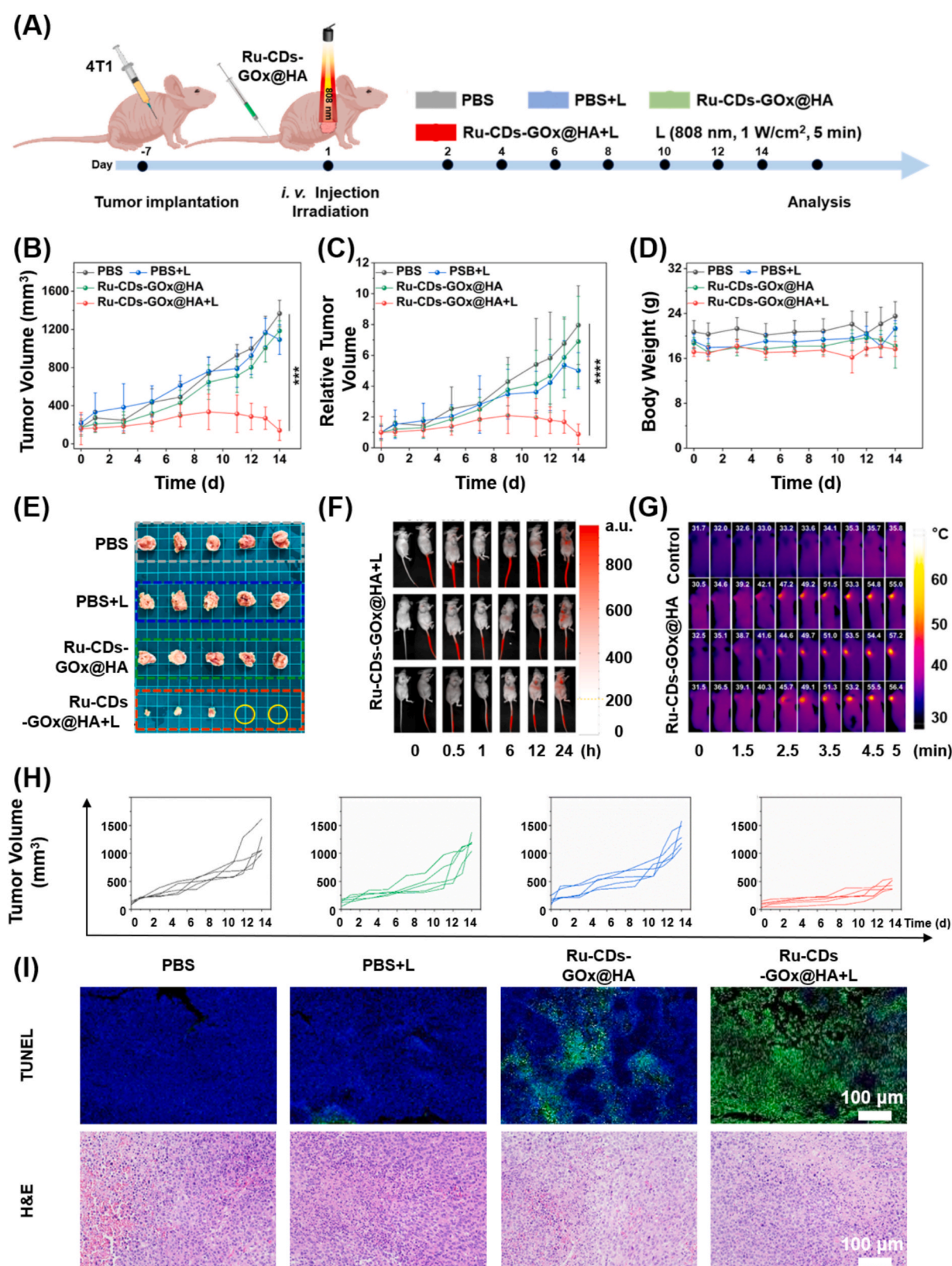


Fig. 8. In vivo therapeutic efficacy evaluation in tumor-bearing mice model. (A) Schematic illustration of the Ru-CDs-GOx@HA treatment protocol. (B, C) Tumor growth and (D) body weight changes. (E) Tumor photographs and (F) NIR-1 imaging results. (G) Thermographic images of the tumor-bearing mice. (H) Tumor growth curves at day 14. (I) TUNEL and H&E-stained images collected from tumor-bearing mice following various treatments.

end labeling (TUNEL) staining, were performed. The results revealed extensive necrotic regions and increased levels of apoptosis in the tumor tissue of mice treated with the Ru-CDs-GOx@HA + L group (green fluorescence) (Fig. 8I). After a 14-d treatment, the indices of blood biochemistry, liver and kidney functions remained within their respective reference ranges (Fig. S17 and S18). These results indicated that Ru-CDs-GOx@HA-treated mice did not experience any hematological, hepatic, or renal damage. Additionally, H&E staining of the major organs of the treated mice revealed no significant physiological or morphological changes (Fig. S19), underscoring the biosafety of Ru-CDs-GOx@HA even when combined with NIR irradiation; this was attributed to the low cytotoxicity of Ru-CDs-GOx@HA to normal cells (Fig. 4A).

2.9. Tumor immunotherapy for metastasis inhibition

The exceptional antitumor efficacy of Ru-CDs-GOx@HA + L motivated us to further investigate the potential of Ru-CDs-GOx-CpG@HA in activating immune responses and inhibiting tumor metastasis in BALB/c mice xenografted with 4T1 tumors. When the tumor diameter reached approximately 100 mm³, various treatments were administered, and tumor sizes were recorded (Fig. 9A). During the treatment period, the tumor growth curves revealed that tumors in the PBS, CpG, and Ru-CDs-GOx-CpG@HA groups grew rapidly, whereas those in the Ru-CDs-GOx@HA + L treatment group were inhibited, indicating significant antitumor activity. Interestingly, the Ru-CDs-GOx-CpG@HA + L treatment group exhibited more pronounced tumor suppression (Fig. 9B and Fig. S20). One week after treatment, following the initiation of the immune response, tumors from different groups were photographed and weighed to visually demonstrate the differences in anticancer efficacy. The tumor inhibition rates for the Ru-CDs-GOx@HA + L and Ru-CDs-GOx-CpG@HA + L groups were 55.6 % and 91.9 %, respectively, compared to the PBS-treated group. Overall, the combination of Ru-CDs-GOx-CpG@HA and laser treatment significantly enhanced anticancer efficacy. (Fig. 9C and D). As shown in Fig. S21, the median survival times of the mice treated with PBS and CpG (control groups) were 31 and 36 d, respectively, whereas that of the mice treated with Ru-CDs-GOx@HA + L was 48 d, which was significantly longer than that of the control groups. Notably, 60 % of mice in the Ru-CDs-GOx-CpG@HA + L group were still alive at the conclusion of treatment (120 d) (Fig. S22).

Additionally, a breast cancer lung metastasis model was established using 4T1 cells to evaluate the therapeutic effect of the nanomaterials on metastatic tumors. Eighteen days after the start of treatment, 1×10^4 4T1 cells were injected into the tail vein, and tumor metastasis was evaluated 3 weeks later. Ru-CDs-GOx@HA + L treatment inhibited the primary tumor, but it was unable to prevent the growth of all the metastatic tumors (Fig. 9E). Contrastingly, mice treated with Ru-CDs-GOx-CpG@HA + L exhibited almost complete suppression of metastatic 4T1 growth in the lungs. Histological analysis further revealed that lung infiltration by 4T1 cells was entirely suppressed in the mice treated with Ru-CDs-GOx-CpG@HA + L, whereas significant tumor masses were detected in the other groups (Fig. 9E). H&E staining of the heart, liver, spleen, and kidney revealed no significant damage to any organs (Fig. S23) during the experiment. Moreover, as shown in Fig. 9F, the TUNEL staining results indicated that no notable apoptosis in either the PBS or the CpG group. In contrast, the Ru-CDs-GOx@HA + L group showed marked nuclear shrinkage and tissue damage, whereas the Ru-CDs-GOx-CpG@HA + L group exhibited the highest apoptosis signals. Moreover, the Ru-CDs-GOx-CpG@HA + L group had the lowest level of Ki67 staining compared with the other groups, indicating significant inhibition of tumor cell proliferation. These findings highlight the strong therapeutic potential of Ru-CDs-GOx-CpG@HA + L as an immunotherapeutic agent, given its ability to inhibit tumors and prevent metastasis.

Next, we also investigated the antitumor immune effects of the nanomaterials in vivo. We first quantitatively assessed CD4⁺ and CD8⁺ T-cell infiltration within the tumor tissue of different treatment groups.

As shown in Fig. 10A-D, CD4⁺ (~3.85 %) and CD8⁺ (~4.59 %) T-cell infiltration increased following Ru-CDs-GOx@HA + L treatment compared with that of the control group. The tumors treated with Ru-CDs-GOx-CpG@HA + L presented the highest levels of CD4⁺ (~14.95 %) and CD8⁺ (~16.84 %) T-cell infiltration, indicating that PANoptosis effectively induced DC maturation. Given the crucial role of DC maturation in T-cell activation and polarization, we also quantified the expression of CD86⁺ and CD80⁺, which are biomarkers of DC maturation, in the tumor tissue from different treatment groups. As shown in Fig. 10E and F, the percentage of CD86⁺ and CD80⁺ cells in the Ru-CDs-GOx-CpG@HA + L group significantly increased from approximately 0.02 % to 0.60 %, confirming that Ru-CDs-GOx-CpG@HA + L effectively stimulated DC maturation. These findings suggested that the Ru-CDs-GOx-CpG@HA nanoplateform effectively integrated pyroptosis, apoptosis, necroptosis, and ferroptosis, triggering the release of immune-stimulatory factors and functioning as an in situ tumor vaccine that elicited robust immune responses and potent tumoricidal effects. This was validated by flow cytometric analysis of T-cell from the tumor tissue. In summary, the Ru-CDs-GOx-CpG@HA system, designed to incorporate immune-stimulatory molecules, strongly induced DC maturation. PANoptosis (pyroptosis, apoptosis, necroptosis, and ferroptosis) further enhanced this maturation, leading to the activation of cytotoxic T lymphocytes and a reduction of regulatory T cells, ultimately increasing tumor immunogenicity in vivo.

2.10. Activation of the immune response and remodeling of the immune microenvironment

We then explored the role of the nanomaterials in the remodeling of the immunosuppressive TME. Activated macrophages consist primarily of proinflammatory M1 and anti-inflammatory M2 macrophages. The M2 subtype, with immunosuppressive activity, inhibits the proliferation of effector T cells [40]. Redirecting macrophage polarization from the M2 to the M1 subtype is critical to reversing tumor-associated immunosuppression [25]. Because multiple programmed cell death pathways, including apoptosis, pyroptosis, ferroptosis and necroptosis, work in concert to cause tumor cell death, we hypothesized that the induced immune response may also be amplified. As shown in Fig. 10G-J, an analysis of spleen tissue from the treated mice revealed that, compared with the group treated with PBS, the groups treated with CpG, Ru-CDs-GOx-CpG@HA, and Ru-CDs-GOx@HA + L presented increased CD86⁺ expression (~1.44 %) in F4/80⁺ M1-like macrophages and decreased CD206⁺ expression (1.33 %) in F4/80⁺ M2-like macrophages. The most significant changes were observed in the Ru-CDs-GOx-CpG@HA + L-treated group, in which the percentage of M1-like macrophages increased to ~2.36 % and that of M2-like macrophages decreased to ~0.13 %. These results suggested that Ru-CDs-GOx-CpG@HA effectively stimulated the conversion of M2-like macrophages to M1-like macrophages through the combined effects of PANoptosis and immunostimulation. Furthermore, as depicted in Fig. 10K-N, compared with the other control groups, the Ru-CDs-GOx-CpG@HA + L group secreted the highest levels of secretion of interleukin-6 (IL-6), interleukin-12 (IL-12), and tumor necrosis factor- α (TNF- α), indicating robust immune activation. These findings confirmed that Ru-CDs-GOx-CpG@HA stimulated the conversion of M2 to M1 macrophages (Fig. 10O). Overall, these results suggest that Ru-CDs-GOx-CpG@HA under NIR irradiation, has the potential to effectively remodel the suppressive TME, thereby aiding in the establishment of a more robust antitumor immune response [32] and enhancing antitumor efficacy.

In this study, we successfully synthesized a novel type of Ru-CDs, which exhibited significant NIR absorption and emission properties, along with excellent biocompatibility. Ru doping not only significantly enhanced the fluorescence quantum yield of the Ru-CDs but also effectively promoted the generation of ROS, thereby improving their thermal response under NIR irradiation. These superior properties were primarily attributed to the Ru-induced redshift in the absorption peak of

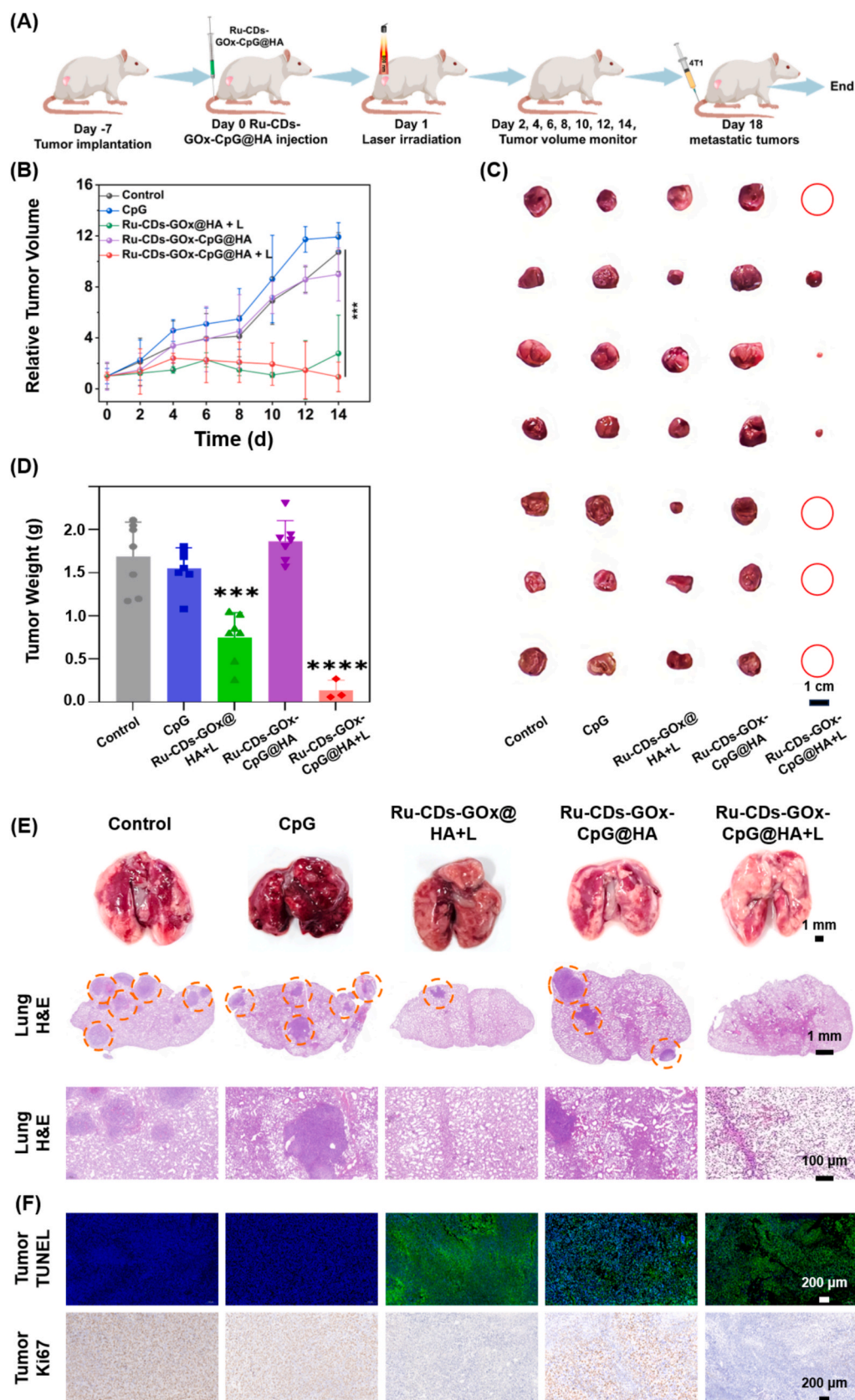


Fig. 9. Establishment of durable immune memory to prevent tumor metastasis by the material. (A) Schematic representation of the in vivo experimental design of the treatments. (B) Tumor growth profiles after different treatments (n = 7). One week after the conclusion of treatment, the tumors were photographed (C) and weighed (D) following the initiation of the immune response. (E) Therapeutic effect on lung metastasis, as illustrated by H&E-stained lung images. (F) TUNEL and Ki67-stained images of tumors collected from tumor-bearing mice following various treatments. *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.

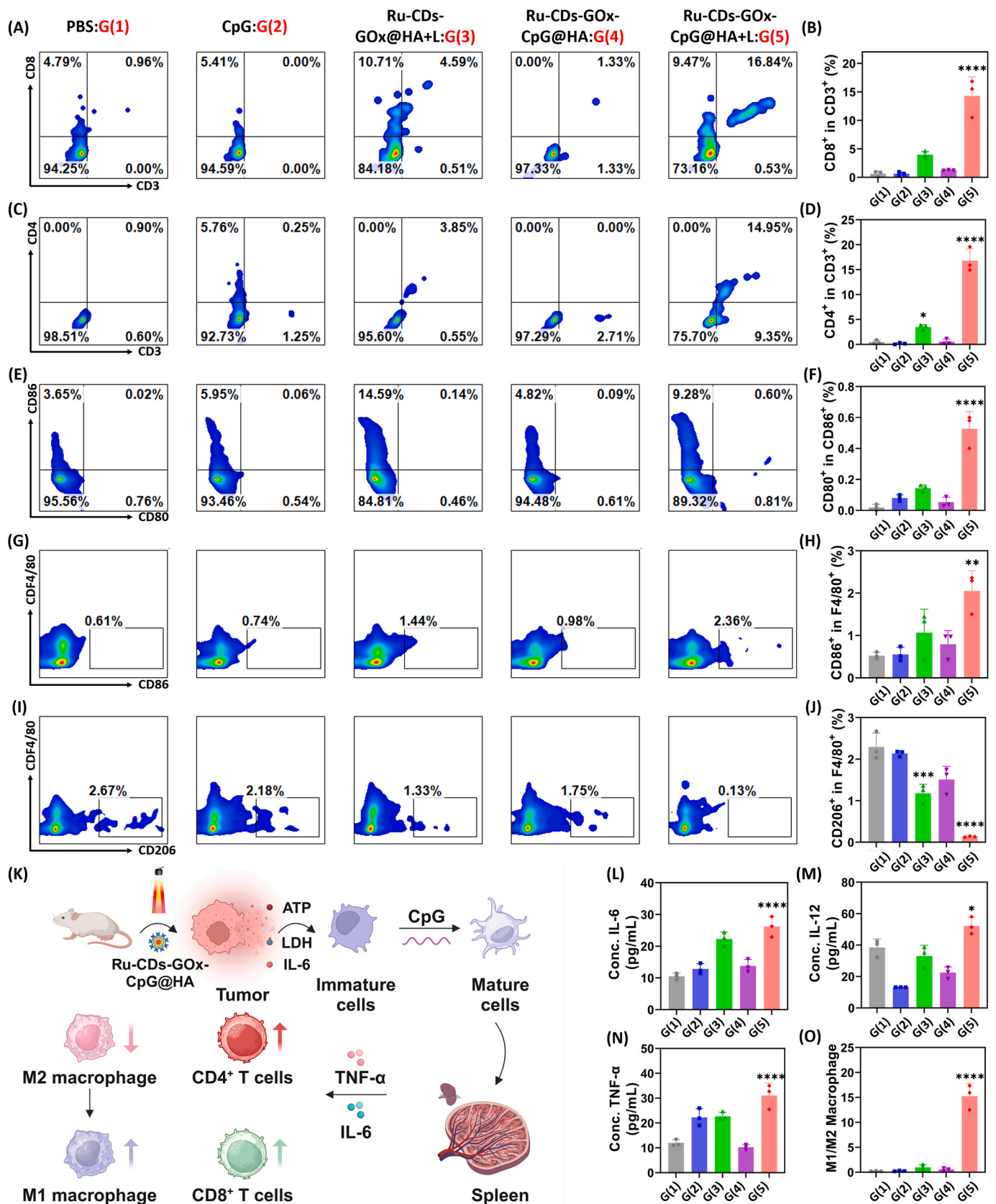


Fig. 10. Immune response activation and remodeling of the immune microenvironment by the materials. (A, B) Representative flow cytometry plots of CD8⁺ T cells. (C, D) Representative flow cytometry plots of CD4⁺ T cells. (E, F) Representative flow cytometry plots of CD80⁺ and CD86⁺ T cells. (G–J) Representative flow cytometry plots of M2 and M1 macrophages. (K) Schematic representation of the damage-associated molecular pattern pathway that induces immune responses. (L–O) Evaluation of immune-related cytokine secretion levels in different treatment groups after treatment. The data are presented as the means $\pm$ SD ($n = 3$). Statistical significance is indicated as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

the CDs to 1460 nm, which overlapped with the NIR absorption band of water. Under the laser irradiation, conduction band electrons in the Ru-CDs oscillate synchronously, generating heat within the system. Moreover, the introduction of Ru significantly lowered the activation energy for catalyzing H_2O_2 decomposition, leading to the formation of a new active species, $\text{Ru}=\text{O}$, which substantially enhanced the efficiency of subsequent oxidation reactions. To the best of our knowledge, this process was difficult to be achieved with other materials. For example, Xu et al. synthesized LaCoO_3 nanostructures, which returned to their ground state after catalyzing H_2O_2 to form dihydroxy structures [40], while Bao et al. synthesized PtCNPs, which generated $\text{HO}_2^\cdot$ free radicals through Pt (II)-catalyzed H_2O_2 decomposition but failed to generate new oxidative active centers [11]. In contrast, in our work, Ru-CDs significantly enhanced the generation of ROS, lowered the energy barrier of the reaction, and effectively promoted photothermal energy conversion. These unique advantages endowed Ru-CDs with substantial potential for cancer therapy.

For the treatment of tumor, individual therapy such as CDT, PDT, or PTT is often limited by factors such as low H_2O_2 levels, oxygen dependency, and cellular thermotolerance in the TME, all of which hinder their therapeutic efficacy [41]. In this work, we proposed a novel “three-in-one” integrated therapeutic system that combined CDT, PDT, and PTT modalities. This multifunctional therapeutic system was realized through Ru-CDs-GOx-CpG@HA nanoparticles, which harnessed the photothermal and photodynamic properties of Ru-CDs in combination with the synergistic effects of GOx, CpG immunoadjuvants, and HA. This combination significantly enhanced tumor targeting and immune responses, while overcoming the limitations of individual therapies. The synergistic effects of these therapies provided complementary enhancements, resulting in improved overall antitumor efficacy.

For the mechanism of programmed cell death, few studies have focused on the PANoptosis using nanoparticles [42,43]. In this work, we are the first to innovatively employed Ru-CDs-GOx-CpG@HA to induce the PANoptosis as a therapeutic strategy to enhance the antitumor effect by activating multiple programmed cell death pathways, including pyroptosis, apoptosis, necrosis, and ferroptosis. PANoptosis is an innate immune-driven, lytic, inflammatory cell death mechanism. Recently, Zhou et al. reported a PANoptosis-inducing ultrasmall $\text{Bi}_2\text{Sn}_2\text{O}_7$ nanozyme for ultrasound-enhanced enzyme therapy against hepatocellular carcinoma, demonstrating efficacy in activating pyroptosis, apoptosis, and necroptosis both in vitro and in vivo [42]. However, their study did not include ferroptosis, another cell death pathway that plays a crucial role in PANoptosis. In our study, under the NIR irradiation and H_2O_2 , Ru-CDs-GOx@HA modulated the interactions between protein domains of the PANoptosome complex, regulating the interactions of catalytic effector proteins such as GSDMD, RIPK3, caspase-1, xCT [44]. This modulation significantly enhanced the antitumor effects. Herein, we employed PANoptosis-guided multimodal synergistic therapy by CDs, which was validated through a series of in vitro experiments and in vivo models using 4T1 tumor-bearing mice and metastasis models. These remarkable findings broadened the application of multifunctional nanotechnology in cancer therapy and opened avenues for exploring multi-pathway cell death mechanisms. The system not only demonstrated potent antitumor activity in both in vitro and in vivo models but also offers a novel approach and strategy for cancer treatment. This system holds great promise for applications in precision cancer therapy, offering substantial clinical potential. Despite these promising results, several limitations should be noted. First, although PANoptosis was involved in cell death of cancer cells and deeply included in cancer immune and played an important role in innate immune cells [40], the accurate molecular detail of PANoptosis were unclear, therefore it is needed to be further investigated. Second, our findings are currently limited to cell and animal (mouse) models, necessitating further investigation in more complex biological systems to assess potential applications in human patients. Third, refining control over laser parameters and dosing would enhance both reproducibility and safety. Evaluating

the system's efficacy across different cancer models and further elucidating the molecular regulation of PANoptosis may provide valuable insights in developing comprehensive cancer therapies.

3. Conclusion

In summary, we developed an Ru-CDs nanocarrier combined with GOx, HA, and the immune adjuvant CpG that effectively targeted tumor HA and enabled both tumor imaging (fluorescence) and therapy through a cascade of biocatalytic reactions under NIR irradiation. The Ru-CDs synergistically integrated CDT, PDT, and PTT, successfully evoking multi-programmed cell death mechanisms through PANoptosis. Our experiments demonstrated that Ru-CDs-GOx-CpG@HA under NIR irradiation triggered multiple cell death processes, released immune adjuvants into the TME that effectively converted M2-like macrophages into an M1 phenotype, promoted DC maturation, and activated the immune system through the release of tumor-associated antigens. This multimodality treatment significantly enhanced tumor cytotoxicity, achieved a high cure efficacy and prevented metastasis with minimal side effects. Therefore, this synergistic therapeutic treatment represents a promising approach for cancer diagnosis and treatment.

CRediT authorship contribution statement

Xiaokang Lu: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Weijie Sun:** Visualization, Methodology, Investigation. **Xingye Zheng:** Visualization, Methodology, Investigation. **Lishen Yang:** Visualization, Methodology, Investigation. **Tan Feng:** Visualization, Methodology, Investigation. **Xiaoqin Deng:** Visualization, Methodology, Investigation. **Fengqi Zhou:** Visualization, Methodology. **Hongmin Zhu:** Visualization, Methodology. **Ding Ma:** Visualization, Methodology. **Bilian Li:** Visualization, Methodology. **Guobao Ning:** Visualization, Methodology. **Hang Chen:** Visualization, Methodology. **Yan Yang:** Visualization, Methodology. **Hui Zhao:** Writing – review & editing, Supervision, Methodology, Investigation. **Can-Peng Li:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Can-Peng Li reports was provided by Yunnan University. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cej.2025.161355>.

Data availability

The authors are unable or have chosen not to specify which data has been used.

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