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ORIGINAL ARTICLE

Binary pyroptosis-amplified self-assembling prodrug nanomedicine enhances immunogenicity and inhibits abdominal metastasis in ovarian cancer



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Abstract Ovarian cancer remains a formidable therapeutic challenge due to its high propensity for abdominal metastasis, recurrence, and the presence of an immunosuppressive tumor microenvironment. To overcome these obstacles, we developed a self-assembled nanoplateform (OSN) by integrating a near-infrared semiconducting polymer with an oxaliplatin(IV) prodrug. This multifunctional design enables a synergistic triple-modality therapy—photothermal therapy (PTT), photodynamic therapy (PDT), and

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Oxaliplatin;
Semiconducting polymer;
Abdominal metastasis;
Multimodal therapy

chemotherapy—within a single nanoparticle, effectively enhancing immunogenic cell death (ICD) and systemic antitumor immunity. Upon laser irradiation, OSN generates localized hyperthermia and reactive oxygen species. These effects synergistically enhance oxaliplatin activation and tumor penetration while triggering pyroptosis through dual caspase-1-mediated and caspase-3-dependent pathways. This robust pyroptotic response amplifies the release of damage-associated molecular patterns (*e.g.*, ATP, HMGB1) and pro-inflammatory cytokines (*e.g.*, IL-18, IL-1 β), thereby remodeling the immunosuppressive microenvironment, promoting dendritic cell maturation, and facilitating cytotoxic T-cell infiltration. In murine ovarian cancer models, OSN achieved over 90% tumor suppression, significantly outperforming monotherapies. Notably, this nanoplatform establishes long-term immune memory, effectively reducing the risk of tumor relapse. By concurrently targeting immunogenic barriers and metastatic progression through multimodal mechanisms, OSN represents a paradigm-shifting strategy with high clinical translatability for the treatment of aggressive ovarian malignancies.

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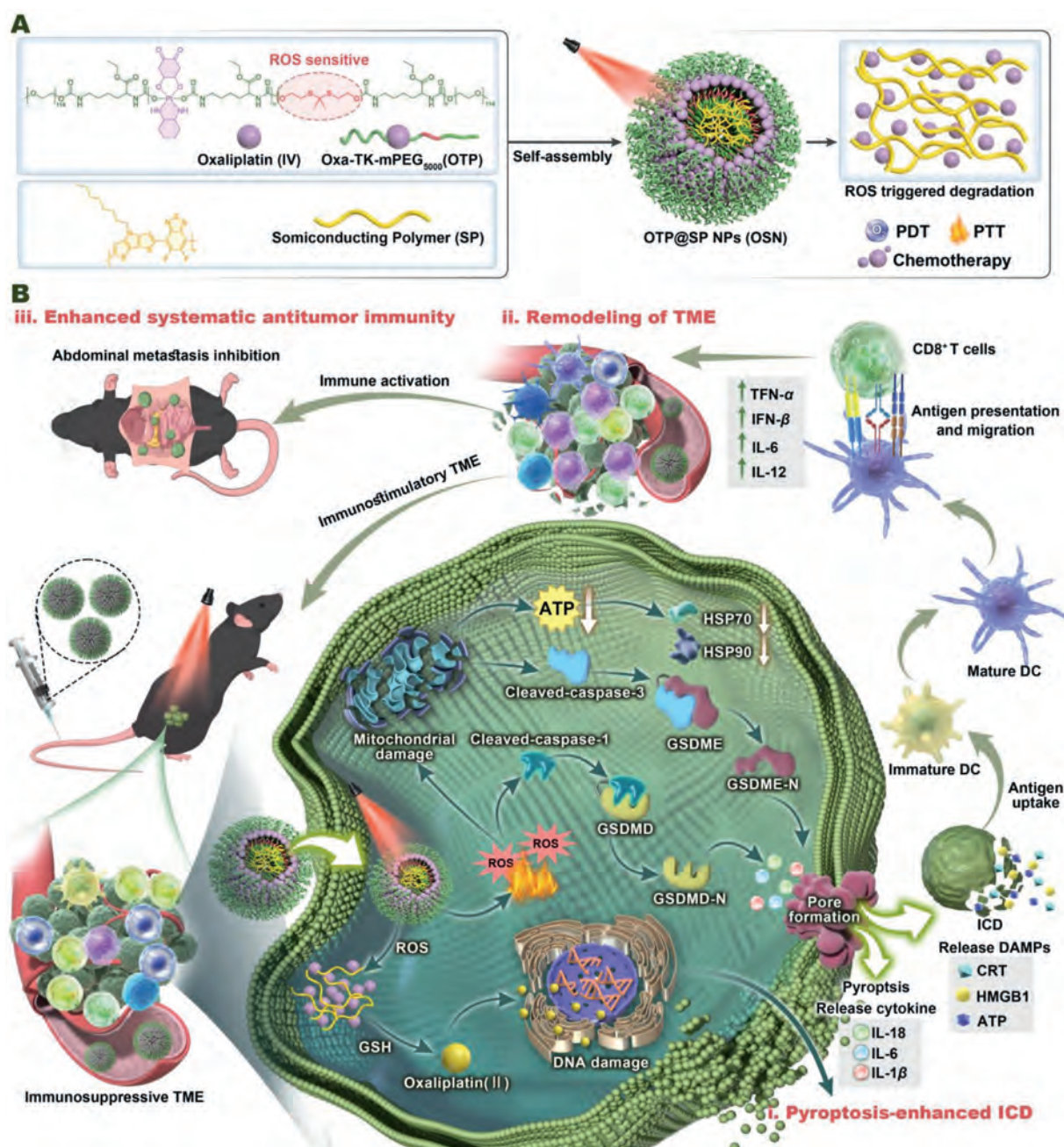
1. Introduction

Ovarian cancer remains the most lethal gynecologic malignancy worldwide, with over 300,000 new cases annually and the highest mortality rate among female reproductive cancers. Approximately 70% of patients are diagnosed at advanced stages (FIGO III/IV), and despite standard treatments—cytoreductive surgery combined with platinum-based chemotherapy—the 5-year survival rate for advanced-stage patients remains below 30%^{1,2}. This poor prognosis is attributed to three key biological hallmarks: (1) trans-coelomic metastasis, leading to widespread peritoneal dissemination across the omentum, mesentery, and abdominal organs; (2) an immunosuppressive tumor microenvironment (TME) dominated by regulatory T cells (Treg) infiltration and myeloid-derived suppressor cells (MDSCs) expansion; and (3) chemotherapy-resistant cancer stem cells and metastatic niches that drive recurrence and disease progression^{3,4}. Although emerging therapies such as immune checkpoint inhibitors (ICIs) and poly(ADP-ribose) polymerase (PARP) inhibitors have demonstrated clinical potential, response rates remain below 20%, and effective strategies targeting abdominal metastases are still lacking^{5,6}. Consequently, dismantling immunosuppressive barriers, eradicating metastatic lesions, and establishing durable immune memory remain critical challenges in ovarian cancer treatment.

First-line therapy for ovarian cancer still relies on cytoreductive surgery and platinum-based chemotherapy; however, this treatment paradigm has significant limitations⁷. Surgical resection is effective for localized tumors but fails to eliminate disseminated peritoneal metastases^{8,9}. While oxaliplatin (Oxa) can induce immunogenic cell death (ICD), its insufficient immunogenicity leads to rapid degradation of damage-associated molecular patterns (DAMPs; *e.g.*, ATP, HMGB1) by enzymatic systems or neutralization by immunosuppressive cells in the TME^{10,11}. As a result, dendritic cells (DCs)-mediated antigen presentation and cytotoxic T lymphocyte (CTL) infiltration are severely compromised. Furthermore, residual tumor cells post-chemotherapy secrete immunosuppressive factors such as TGF- β and IL-10, reinforcing the "cold tumor" phenotype and facilitating immune evasion^{12–14}. Photothermal therapy (PTT) and photodynamic therapy (PDT) offer localized precision but are limited by metastatic progression and persistent immunosuppression¹⁵.

Recent advances in gasdermin-mediated pyroptosis provide a promising alternative strategy¹⁶. Unlike apoptosis, pyroptosis—mediated by gasdermin proteins (*e.g.*, GSDMD, GSDME)—induces pore formation in the plasma membrane, leading to the explosive release of DAMPs and pro-inflammatory cytokines (*e.g.*, IL-18, IL-1 β)^{17,18}. These signals strongly enhance DCs maturation, recruit CTLs and natural killer (NK) cells, and convert immunologically "cold" tumors into "hot" phenotypes. However, the therapeutic potential of pyroptosis in ovarian cancer is hindered by epigenetic silencing or mutations in gasdermin proteins and the existence of compensatory resistance mechanisms that counteract single-pathway pyroptotic induction^{19,20}. Thus, the development of a multifunctional platform capable of activating dual pyroptotic pathways and synergistically enhancing tumor immunogenicity is imperative. Nanotechnology offers a transformative strategy for multimodal therapy integration. Rational nanocarrier design enables spatiotemporal coordination of chemotherapy, PTT, and PDT. For instance, near-infrared semiconducting polymers serve as dual photothermal and photodynamic agents, generating reactive oxygen species (ROS) to directly kill tumor cells while enhancing drug permeability *via* the enhanced permeability and retention (EPR) effect^{21,22}. However, existing nanoplatforms primarily focus on single therapeutic mechanisms and fail to simultaneously address immunogenic enhancement, metastasis suppression, and TME reprogramming^{23,24}.

To overcome these challenges, we developed a multifunctional nanoplatform (OSN) through the molecular engineering of an oxaliplatin(IV) prodrug and a near-infrared semiconducting polymer (SP). This platform enables laser-triggered drug release to achieve triple-modality therapy (PTT/PDT/chemotherapy), while simultaneously activating dual pyroptotic pathways, effectively combating immunosuppression and metastasis in ovarian cancer (Scheme 1). Mechanistically, localized hyperthermia and ROS generated by PTT/PDT synergistically orchestrate two distinct pyroptotic cascades: NLRP3 inflammasome activation *via* the caspase-1/GSDMD canonical pathway, and mitochondrial damage-driven caspase-3/GSDME non-canonical pathway. The resultant gasdermin pore formation induces the explosive release of DAMPs and pro-inflammatory cytokines, converting immunologically "cold" tumors into "hot" phenotypes. Notably, this therapeutic strategy effectively suppressed ovarian cancer abdominal metastasis by establishing a long-term immune



Scheme 1 Schematic illustration of the design and therapeutic mechanisms of semiconducting polymer (SP) nanoparticles loaded with oxaliplatin(IV) polyprodrug (OTP) for phototherapy and chemoimmunotherapy. (A) Design and formation of OSN nanoparticles: The chemical structures of SP and OTP are shown, which self-assemble to form OTP@SP nanoparticles (OSN) for combined phototherapy and chemotherapy. (B) Mechanisms of OSN-mediated chemo-immunotherapy in ovarian cancer: (i) OSN nanoparticles accumulate within tumor tissues *via* the EPR effect. Upon laser irradiation, the nanoparticles generate ROS and release the oxaliplatin(IV) prodrug, which is reduced to active oxaliplatin(II) by intracellular GSH, leading to DNA damage and the induction of immunogenic cell death (ICD). Concurrently, SP mediates PTT and PDT, which synergistically enhance ICD effects and induce pyroptosis through both caspase-1-dependent and caspase-3-mediated pathways. (ii) The ICD effect triggers a robust adaptive anti-tumor immune response, transforming the immunosuppressive TME into an immunostimulatory one. (iii) The immune activation establishes durable immune memory, enhancing systemic anti-tumor immunity and effectively preventing tumor recurrence and metastasis.

memory. This work pioneers a "multimodal therapy—immunogenicity enhancement—metastasis suppression" paradigm, offering a clinically actionable strategy to overcome key barriers in ovarian cancer management.

2. Materials and methods

2.1. Materials and reagents

Oxaliplatin, methoxypoly(ethylene glycol)hydroxyl-5000 (mPEG₅₀₀₀-OH), L-lysine diisocyanate, 4,7-dibromo-5,6-difluorobenzo[c][1,2,5]thiadiazole and D-Luciferin potassium salt were purchased from Macklin Biochemical Co., Ltd. (Shanghai, China). 4-Octyl-2,6-bis(trimethylstannyl)-4*H*-dithieno[3,2-*b*:2',3'-*d*]pyrrole and 2,2'-(propane-2,2-diylbis(sulfaneydiyl)) diethanol was purchased from Bidepharm Co., Ltd. (Shanghai, China). YF[®] dye phalloidin conjugates (YP0059S) were purchased from Uelandy Co., Ltd. (Suzhou, China). Thiazolyl blue tetrazolium bromide (MTT) and ATP content assay kit were purchased from Solarbio Co., Ltd. (Beijing, China). The live & dead assay kit was sourced from Keygen BioTECH Co., Ltd. (Jiangsu, China). Annexin V-FITC/PI Apoptosis Kit was purchased from Elabscience Biotechnology Co., Ltd. (Wuhan, China). Anti- γ -H2A.X, anti-cleaved-caspase-1, anti-GSDME-N, and anti-GSDME-N antibodies were purchased from Abcam (Cambridge, UK). Anti-cleaved-caspase-3 and anti-cytochrome *c* were purchased from Affinity Biosciences Co., Ltd. (Jiangsu, China). Anti-HSP90 (E05-06), anti-HSP70 (SA0379), anti-CRT (EM1701-60), and anti-HMGB1 (SA39-03) antibody were purchased from HUABIO Co., Ltd. (Zhejiang, China). Anti-GAPDH antibody was purchased from Servicebio Co., Ltd. (Wuhan, China). Immunoflow antibodies were obtained from BioLegend Co., Ltd. (California, USA) and Elabscience. Cell-culture dishes and plates were bought from Bioland Biotechnology Co., Ltd. (Zhejiang, China). Fetal bovine serum (FBS, FSP500) was purchased from ExCell Bio Co., Ltd. (Suzhou, China). DMEM, 0.25% trypsin-EDTA, and penicillin–streptomycin solution were obtained from Pricella Co., Ltd. (Wuhan, China). All the other reagents and solvents were sourced from Macklin and used as received.

2.2. Measurements

¹H NMR spectra were measured by a 400 MHz NMR spectrometer (Bruker, Switzerland). Gel permeation chromatography (GPC) was performed on Agilent 1260 Infinity II (USA) at Shiyanjia Lab (www.Shiyanjia.com). The concentrations of SP and Pt were analyzed by UV–Vis photometer (UV-5800PC, China) and inductively coupled plasma mass spectrometer (ICP-MS, Thermo Scientific, USA), respectively. The particle size and Zeta potential of the nanoparticles were analyzed by a nanoparticle size and Zeta potential analyzer (Brookhaven, USA), and the morphology was further measured by transmission electron microscopy Talos L120C (ThermoFisher, USA). Flow cytometry (FCM) analysis was performed on a NovoCyteQuanteon flow cytometer instrument (Agilent Technologies, USA). Confocal laser scanning microscopy (CLSM) images were recorded on a LSM-900 with an Airyscan2 microscope (ZEISS, Germany).

2.3. Preparation and characterization of OTP@SP NPs (OSN)

The OSN was prepared by the nanoprecipitation method. OTP (20 mg) and SP (1 mg) were dissolved in a THF/DMF (2/1, *v/v*) solvent mixture and slowly injected into ultrapure water under sonication to self-assemble to form nanoparticles. It is mixed thoroughly under sonication conditions to form a colloidal suspension. Then the solution was transferred to a dialysis bag (MWCO: 3500 Da) and dialyzed for 8 h to remove the organic solvent to obtain OSN. The OTP NPs were obtained by the same method without the addition of SP. The concentration of Pt in OSN and OTP was assessed *via* ICP-MS. The concentration of SP was analyzed by a UV–Vis photometer. The hydrodynamic size was detected *via* a dynamic light scattering (DLS) device. The morphology and shape of OSN were visualized with a transmission electron microscope (TEM) and the elemental composition of nanoparticles was analyzed using a scanning electron microscope (SEM). The stability of OSN was examined by continuous detection of particle size and polydispersity index (PDI) in phosphate-buffered saline (PBS, Bioland, China) and 10% FBS solution for 7 consecutive days.

2.4. Detection of ROS generation

Photo-triggered singlet oxygen generation (¹O₂) of the OSN was determined by using the chemical trapping method. Briefly, the absorbance of 1,3-diphenylisobenzofuran (DPBF) at 415 nm was adjusted to about 0.8 in water, and the absorbance of SP in THF was adjusted to about 0.3. Then, the cuvette was irradiated with 660 nm monochromatic light at various times, and absorption spectra were measured immediately. The absorbance changes of DPBF at 415 nm were used to quantify the decomposition rate.

2.5. Cell culture

Mouse ovarian epithelial carcinoma cells (ID8 cells) and Luciferase labeling of ID8 cells (ID8-Luc cells) were obtained from the cell bank of the School of Pharmacy, Southern Medical University (Guangdong, China). Cells were frozen using a Serum-Free Cell Freezing medium (Shanghai ZhongQiaoXinZhou Biotechnology Co., Ltd.; CSP042-100). ID8 cells were cultured in DMEM medium containing 10% FBS (JYK-FBS-301, Inner Mongolia Jinyuankang Biotechnology Co., Ltd.) and 1% penicillin/streptomycin. ID8-Luc cells were cultured using a DMEM complete medium containing 1 μ g/mL puromycin (Solarbio; Beijing, China). All the cell lines were cultured in an incubator at 37 °C containing 5% (*v/v*) CO₂. When the degree of cell fusion reached 80%–90%, the cells were digested with 0.25% trypsin, and then sub-cultured or inoculated in cell plates (Bioland, China) for subsequent experiments.

2.6. LDH release assay

ID8 cells were seeded in 6-well plates (Bioland, China) at a density of 5×10^5 cells per well and cultured for 12 h. Subsequently, the cells were treated with PBS, Oxa, OSN, SP + L, Oxa + SP + L, or OSN + L (Pt at 1.25 μ g/mL, SP at 1.25 μ g/mL) for 12 h and then irradiated with a 660 nm laser (0.5 W/cm², 5 min). Afterward, ID8 cells were further cultured for 12 h, collected and centrifuged at 1000 rpm for 3 min (Centrifuge,

CF1524R, Scilogex, Connecticut, USA) to obtain the cell cultural medium samples. The amount of LDH in the medium was assessed with a CheKine™ Micro Lactate Dehydrogenase (LDH) Assay Kit (Abbkine, Wuhan, China) according to the manufacturer's instructions. The absorbance of each sample was measured *via* a microplate reader at 450 nm. The caspase inhibition experiments were performed using specific inhibitors (Z-YVAD-FMK and Z-DEVD-FMK) to pre-treat the cells, and the LDH of the culture medium was detected by the same method as described above.

2.7. Western blot analysis

To quantify protein expression, ID8 cells were incubated with PBS, Oxa, OSN, SP + L, Oxa + SP + L, or OSN + L (Pt: 1.25 µg/mL; SP: 1.25 µg/mL) for 12 h and then irradiated with a 660 nm laser (0.5 W/cm², 5 min). Subsequently, the cells were further cultured for 12 h, and total cellular proteins were extracted. Protein content was determined using a BCA protein assay kit (Bioswamp Life Science Lab; Wuhan, China). Proteins (25 µg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under constant voltage, followed by membrane transfer under constant current conditions. The membranes were then incubated with Sparkjade ECL Star (Shandong Sparkjade Biotechnology Co., Ltd.) for chemiluminescent detection. Lastly, proteins were analyzed *via* Image J software.

2.8. Dendritic cells activation *in vitro*

To evaluate DCs activation *in vitro*, bone marrow-derived dendritic cells (BMDCs) were obtained from the bone marrow of 6-week-old healthy female C57BL/6 mice and cultured in RPMI 1640 medium supplement with 10% FBS, granulocyte-macrophage colony-stimulating factor (GM-CSF, 20 ng/mL), and interleukin-4 (IL-4, 10 ng/mL) at 37 °C with 5% (v/v) CO₂. After 5 days of culturing, pretreated ID8 cells were co-cultured with BMDCs in transwell chamber culture plates (NEST Biotechnology, Co., Ltd.; Wuxi, China) for 24 h. After that, the BMDCs were collected and stained with anti-CD11c, anti-CD80, and anti-CD86 antibodies (BioLegend) for 1 h at room temperature. Finally, the activation of DCs was assayed by using FCM measurement.

2.9. Immunofluorescence staining of ICD markers *in vitro*

ID8 cells were seeded in 6-well plates and incubated with different formulations, including PBS, Oxa, OSN, SP + L, Oxa + SP + L, or OSN + L (Pt at 1.25 µg/mL, SP at 1.25 µg/mL). After 12 h incubation, cells in the SP + L, Oxa + SP + L, and OSN + L groups were irradiated with a 660 nm laser (0.5 W/cm², 5 min) and then continued to incubate for another 12 h as the other groups. Next, ID8 cells were fixed with 4% paraformaldehyde solution and incubated with rabbit anti-HMGB1/AF488 (bs-0664R-AF488) conjugated antibody and rabbit anti-calreticulin/AF488 (bs-0664R-AF488) conjugated antibody (Bioss; Beijing, China). Finally, cells were stained with DAPI for observation by CLSM.

2.10. RNA-sequencing analysis

Firstly, ID8 cells were seeded in 6-well plates (1 × 10⁶) and cultured overnight. Next, ID8 cells were treated with PBS, Oxa,

OSN + L (Pt at 1.25 µg/mL, SP at 1.25 µg/mL) for 12 h and then irradiated with a 660 nm laser (0.5 W/cm², 5 min). Afterward, ID8 cells were further cultured for 12 h and gathered for BGISEQ-500-based RNA-sequencing analysis. The transcription levels were quantified by RSEM. When the fold change was ≥2 or the q-value was ≤0.001, differentially expressed genes were identified. Advanced volcano plots, advanced heatmap plots, and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment scatter plots were conducted by the OmicStudio tools at <https://www.omicstudio.cn/tool>.

2.11. ELISA assay

The peripheral blood of ovarian cancer subcutaneous transplantation tumor model mice treated with different drugs was collected and centrifuged at 8000 rpm (Centrifuge, CF1524R, Scilogex, Connecticut, USA) for 10 min at 4 °C, and the supernatant was collected and stored at −80 °C. Ascites from ovarian cancer intraperitoneal metastasis model mice treated with different drugs were collected and centrifuged at 3000 rpm (Centrifuge, 3-18 KS, Sigma, Lower Saxony, Germany) for 10 min at 4 °C, and the supernatant was collected and stored at −80 °C. The above samples were strictly tested according to the manufacturer's instructions of Mouse IFN-γ, TNF-α, IL-18, IL-1β, IL-6, and IL-12 ELISA Kit (Servicebio; Wuhan, China).

2.12. Construction of C57BL/6 mice model of ovarian cancer

The 4–5 weeks female C57BL/6 mice were purchased from Guangdong Medical Laboratory Animal Centre and raised in SPF animal rooms. All experimental procedures were executed according to the protocols approved by the Ethics Committee of the Tenth Affiliated Hospital of Southern Medical University (Dongguan People's Hospital) under approval number IACUC-AWEC-202501003. The ID8 subcutaneous tumor mouse model was established by injecting 5 × 10⁷ ID8 cells suspended in 100 µL PBS into the right dorsal side of the mouse near the root of the thigh. The mice were treated when the tumor volumes approached 140–160 mm³. The ID8-Luc intraperitoneal metastasis mouse model was established by intraperitoneally injecting 5 × 10⁶ ID8-Luc cells into subcutaneous ID8 tumor-bearing mice.

2.13. Biodistribution of OSN

The biodistribution of OSN was evaluated in ID8 subcutaneous tumor mice. At predesigned time intervals (4, 8, 12, 24, and 48 h), the fluorescence imaging (Ex: 640 nm, Em: 840 nm) of the mice was performed with an IVIS system (PerkinElmer, MA, USA). At the end of the *in vivo* imaging, the mice were sacrificed, and the hearts, livers, spleens, lungs, kidneys, and tumors of each group of mice were collected for *ex vivo* fluorescence imaging.

2.14. In vivo platinum distribution

Platinum distribution was measured by ICP-MS. Oxa and OSN (Pt at 2 mg/kg) were injected intravenously into mice, respectively. After 24 h, the Pt content of the heart, liver, spleen, lungs, kidney, and tumor were analyzed by ICP-MS.

2.15. *In vivo photothermal imaging*

SP and OSN (100 μ L, SP and Pt at 2 mg/kg) were injected intravenously into ID8 subcutaneous tumor mice ($n = 3$), respectively. At 12 h post-injection, tumor regions of living mice were irradiated with 660 nm laser at 0.5 W/cm². FLIR thermal camera was used to monitor the temperature of the tumor region every 1 min for 4 min during laser irradiation. Mice injected with PBS were evaluated as a control.

2.16. *In vivo antitumor efficacy*

ID8 subcutaneous tumor mice models were used to evaluate antitumor efficacy *in vivo*. Similarly, when the tumor grew to about 140–160 mm³, mice were randomly divided into six groups: (G1) PBS, (G2) Oxa, (G3) OSN, (G4) SP + Laser, (G5) Oxa + SP + Laser, (G6) OSN + Laser. The tumor-bearing mice were injected with PBS, Oxa, SP, or OSN (Pt or SP = 2 mg/kg) *via* tail vein on Days 1, 3, and 5, and the laser-treated groups were performed by a 660 nm laser (2 min, 0.5 W/cm²) after 12 h post-injection. To evaluate tumor growth, the tumor volume and body weight of mice in each group were recorded every two days. Tumor volume was calculated as Eq. (1):

$$\text{Tumor volume} = (\text{Length} \times \text{Width} \times \text{Width})/2 \quad (1)$$

At the end of treatment, the mice were euthanized and blood samples were collected for ELISA of IFN- γ , TNF- α , IL-6, IL-12, and blood biochemical indexes (alanine transaminase (ALT), aspartate transaminase (AST), creatinine (CREA) and blood urea nitrogen (BUN)). Correspondingly, tumors and major organs were collected for immunofluorescence and hematoxylin and eosin (H&E) staining after the experiment. In addition, tumors, lymph nodes, and spleens were collected for the detection of relevant immune cells.

2.17. *ID8-Luc intraperitoneal metastasis mice model*

The ID8 subcutaneous tumor model was established and treated according to a detailed schedule. At the end of the third treatment, about 5×10^6 ID8-Luc cells were injected into the abdominal cavity of these mice. The body weight and abdominal perimeter of the mice were measured every two days. Bioluminescence detection was performed every seven days to monitor tumor growth. Mice were injected intraperitoneally with D-luciferin potassium salt solution (10 mg/mL, 200 μ L) and then tumors were observed for luciferase bioluminescence using the IVIS spectral imaging system. At the end of the study, all mice were euthanized and ascites were collected for determination of functional cytokine (TNF- α , IFN- γ , and IL-6) levels using ELISA kits. In addition, relevant immune cells in the ascites were detected by FCM analysis, and metastatic foci in the abdominal cavity of the mice were captured.

2.18. *Flow cytometric analysis of immune cells*

To evaluate the immune response in TME, surgical resection of tumor-draining lymph nodes (TDLNs), tumor, and spleen tissues was performed on ID8 subcutaneous tumor models from different treatment groups, and the relevant immune cells were detected by FCM. Briefly, TDLNs and tumor tissues were processed using dissociation buffer (0.4 mg/mL collagenase I and 0.1 mg/mL collagenase II), and spleens were processed using erythrocyte lysis

buffer (Solabio; Beijing, China). Single-cell suspensions were collected by 70 μ m mesh filtration and stained for 1 h at room temperature. Maturation of DCs in TDLNs was detected with anti-mouse CD11c (VF450), CD80 (FITC), and CD86 (APC-Fire750). Tumor cell suspensions were incubated with CD45 (FITC), CD3 (PerCP-Cy5.5), CD4 (PE-Cy7), CD8a (Pacific Blue), CD49b (PE), Gr-1 (APC) and CD11b (ER780), F4/80 (BV450), CD86 (PerCP-Cy5.5), CD206 (PE) and CD25 (APC) antibody following the manufacturer's instructions. After surface staining, the cell suspension was fixed and permeabilized with the commercial buffer Flow Cytometry Permeabilization/Wash Buffer I (BD Biosciences), then stained intracellularly with antibody against FOXP3 (PE). For spleen tissue, Treg cells were identified as for tumor tissue; memory T cells were identified with anti-mouse CD3 (PerCP-Cy5.5), CD4 (PE-Cy7), CD8a (Pacific Blue), CD44 (FITC), and CD62L (APC). T cells from ascites cells were identified with anti-mouse CD3 (PerCP-Cy5.5), CD4 (PE-Cy7), and CD8a (Pacific Blue). VF, violet fluor; FITC, fluorescein isothiocyanate; PerCP, peridinin chlorophyll protein; Cy, cyanine; PE, phycoerythrin; APC, allophycocyanin; BV, brilliant violet; ER, elab fluor red.

2.19. *Statistical analysis*

All analyses were performed by GraphPad Prism 9.0 software. The quantitative data are described as mean $\pm$ standard deviation (SD). Comparative analyses between two groups were conducted *via* Student's *t*-test and comparative analyses between three and more than three groups were conducted *via* one-way ANOVA. The levels of * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$ are statistically significant.

3. Results and discussion

3.1. *Preparation and theoretical calculation of SP*

The near-infrared (NIR)-emissive SP was synthesized *via* Stille polymerization, using difluorobenzothiadiazole as the electron acceptor and octyldithienopyrrole as the electron donor (Supporting Information Scheme S1). The successful synthesis was confirmed by ¹H NMR spectroscopy (Supporting Information Fig. S1). The photophysical properties of SP were investigated using density functional theory (DFT) and time-dependent DFT (TD-DFT) calculations at the B3LYP level. To facilitate computational analysis, model compounds with different repeating units ($n = 1, 2, 3$) were structurally optimized (Fig. 1A). As the number of repeating units increased, structural twisting was reduced, leading to enhanced planar rigidity. This improvement strengthened intermolecular interactions and facilitated electron transport. Additionally, the incorporation of alkyl chains mitigated molecular rigidity, imparting flexibility to the polymer backbone. As illustrated in Fig. 1B, the lowest unoccupied molecular orbital (LUMO) was primarily localized on the difluorobenzothiadiazole core, while the highest occupied molecular orbital (HOMO) was mainly distributed on the dithienopyrrole donor, with minor contributions from the acceptor moiety. A slight overlap between HOMO and LUMO was observed. The energy gap progressively decreased with increasing repeating units, reaching 1.71 eV at $n = 3$, which aligns with the emergence of NIR emission. TD-DFT calculations under singlet and triplet-state excitations revealed that the energies of higher excited states (S_n and T_n)

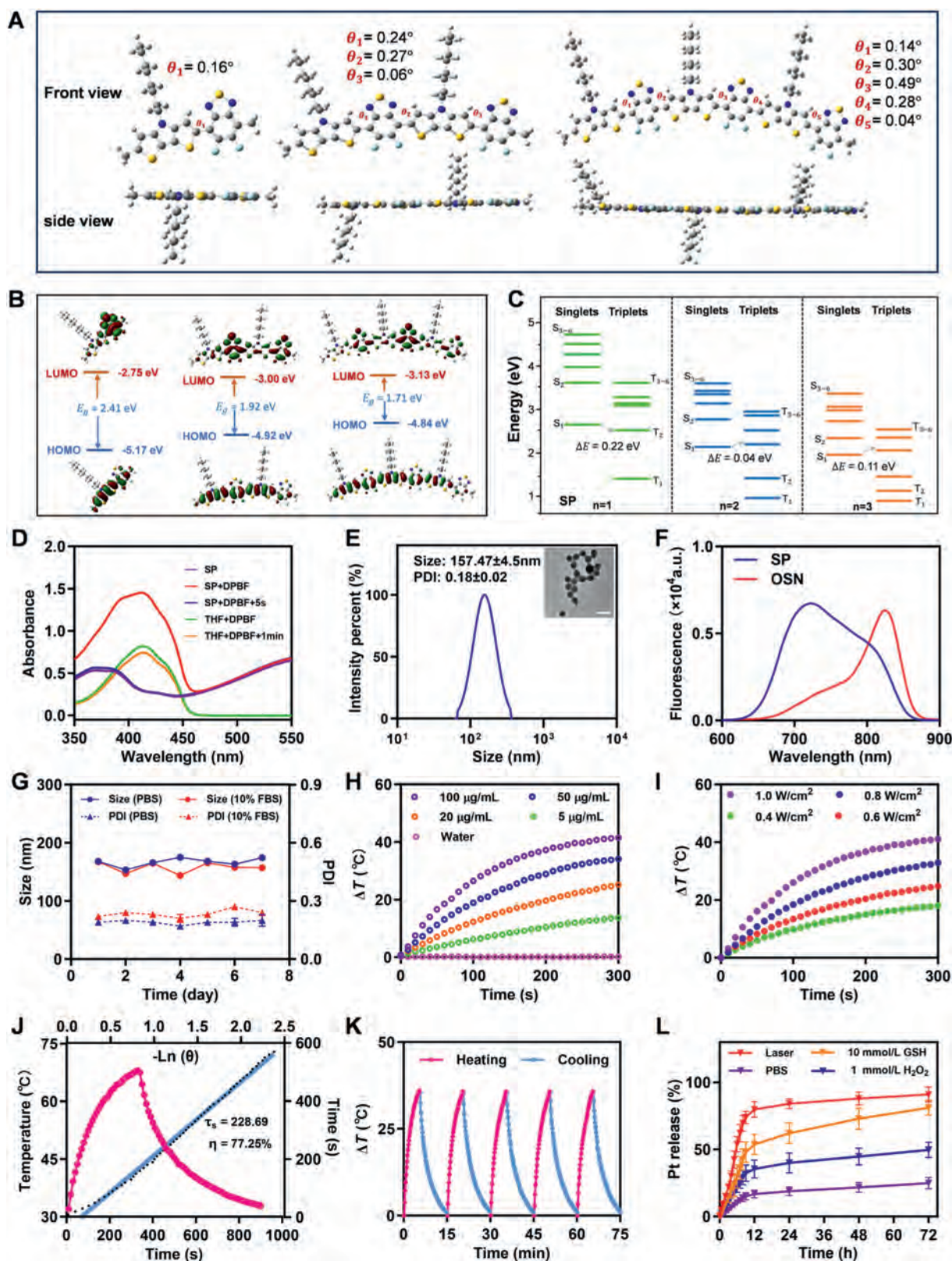


Figure 1 Theoretical calculations of SP and characterization of OSN. (A) DFT-optimized structures, (B) energy gap and HOMO/LUMO distribution of SP models with varying repeating units ($n = 1, 2, 3$). (C) Calculated energy levels and ISC channels for SP compounds ($n = 1, 2, 3$). S and T denote singlet and triplet excited states, respectively; arrows indicate ISC transitions. (D) $^1\text{O}_2$ generation efficiency of SP evaluated via DPBF degradation under 660 nm laser irradiation (1.0 W/cm²). (E) DLS measurement of OSN and TEM morphology analysis (scale bar: 200 nm). (F) Emission spectra of SP in THF and OSN in aqueous solution. (G) Nanoparticle size and PDI changes of OSN in PBS solution and 10% FBS for 7 consecutive days. (H) Photothermal heating curves of OSN (5, 20, 50, 100 μg/mL, SP equivalent) and PBS under 660 nm laser

converged toward the lowest excited states (S_1 and T_1) as the number of repeating units increased (Fig. 1C). This dense energy-level distribution strongly favors the intersystem crossing (ISC) process, thereby enhancing singlet oxygen (1O_2) generation. Notably, for $n = 1$, the energy gap between S_1 and T_1 was calculated to be 0.22 eV, which is below the 0.3 eV threshold required for efficient ISC. For $n = 3$, the T_1 – T_3 energy levels were positioned below S_1 , and the S_1 – T_4 energy gap was less than 0.3 eV, further promoting ISC efficiency. Building on these theoretical insights, we further evaluated the 1O_2 generation capability of SP. This was assessed using DPBF, a probe that undergoes oxidative degradation in the presence of 1O_2 ²⁵. As shown in Fig. 1D, DPBF exhibited no significant degradation after 1 min of laser irradiation alone. However, upon the addition of SP, complete degradation of DPBF was observed within 5 s of irradiation, demonstrating the robust ROS generation of SP under laser excitation. This rapid and efficient ROS production highlights the potential of SP for PDT.

3.2. Preparation and characterization of OSN

The oxaliplatin(IV) polyprodrug (Oxa-TK-mPEG₅₀₀₀, OTP) was synthesized *via* polycondensation, as illustrated in Supporting Information Scheme S2. Successful polymerization was confirmed by 1H NMR analysis (Supporting Information Fig. S2). GPC determined a number-average molecular weight (M_n) of 18,230 g/mol, a weight-average molecular weight (M_w) of 28,586 g/mol, and a PDI of 1.59 (Supporting Information Fig. S3 and Table S1). ICP-MS analysis revealed a platinum mass percentage of 4.75% in OTP. OTP@SP nanoparticles (OSN) were subsequently prepared *via* nanoprecipitation, achieved by co-assembling OTP with SP. Systematic optimization of the OTP/SP mass ratio identified 20:1 as the optimal formulation, yielding nanoparticles with ideal size uniformity and similar drug-loading (Supporting Information Table S2). Successful co-assembly was confirmed through absorption spectral analysis and SEM elemental mapping (Supporting Information Figs. S4 and S5). DLS measurements demonstrated that OSN exhibited a hydrodynamic diameter of 157.47 ± 4.5 nm and a zeta potential of -13.48 ± 0.79 mV (Fig. 1E). TEM analysis revealed that the nanoparticles possessed a uniform spherical morphology and a monodisperse distribution. Photophysical characterization showed a red-shifted emission maximum for OSN (840 nm) compared to free SP (720 nm, Fig. 1F), which can be attributed to nanoparticle aggregation in aqueous media. Stability assessments over 7 days in PBS and 10% FBS indicated negligible changes in particle size and PDI (Fig. 1G), demonstrating excellent colloidal stability, which is essential for potential *in vivo* applications.

3.3. Photothermal effect of OSN

The photothermal properties and photostability of OSN are critical for achieving robust antitumor efficacy both *in vitro* and *in vivo* applications²⁶. Photothermal performance was evaluated in aqueous solutions under 660 nm laser irradiation (Supporting Information Fig. S6). The solution temperature increased proportionally with

OSN concentration, reaching a 41.5 °C rise at 100 μ g/mL (SP equivalent) after 5 min of irradiation, whereas PBS exhibited negligible heating under the same conditions (Fig. 1H). Temperature elevation also showed a positive correlation with laser power at a fixed OSN concentration (Fig. 1I). The photothermal conversion efficiency (η), calculated from cooling kinetics (Fig. 1J), was determined to be 77.25%, significantly surpassing most reported photothermal agents ($\eta < 40\%$). Notably, OSN maintained stable temperature modulation over five consecutive heating/cooling cycles, confirming exceptional photostability (Fig. 1K). These results establish OSN as a potent and reliable photothermal transducer for precision PTT. Leveraging the dual responsiveness of OTP to ROS and GSH *via* its oxaliplatin(IV) prodrug and thioketal (TK) linkages, drug release kinetics were evaluated under tumor-mimetic redox conditions. OSN exhibited $>40\%$ cumulative Pt release in 10 mmol/L H_2O_2 and $>80\%$ release in 10 mmol/L GSH over 72 h, compared to only $24.8 \pm 4.1\%$ release in PBS (Fig. 1L). The greater Pt release from OSN under 10 mmol/L GSH conditions compared to H_2O_2 may be a potential correlation with the TK bond content of OTP. Furthermore, laser irradiation (5 min) dramatically accelerated Pt release in PBS, achieving $>80\%$ release at 24 h and $90.9 \pm 5.5\%$ at 72 h. This enhanced drug release is likely attributed to the ROS surge induced by laser exposure. TEM revealed nanoparticle disintegration and amorphous aggregation after 24 h incubation with 10 mmol/L GSH or 5 min laser exposure (Supporting Information Fig. S7), further validating the redox-responsive behavior of OSN.

3.4. *In vitro* anti-tumor efficacy of OSN + L

Nanoscale drug delivery systems have been widely explored to enhance intracellular drug uptake²⁷. The cellular internalization of OSN was assessed *via* CLSM images by tracking the fluorescence signal of the SP. A progressive increase in red fluorescence was observed in ID8 cells over time, indicating time-dependent cellular uptake (Fig. 2A). As shown in Supporting Information Fig. S8, the uptake efficiency of free SP was lower than OSN at the same treatment time. FCM analysis further quantified this trend, revealing a 2.3- and 4.3-fold increase in OSN internalization at 5 h compared to 3 and 1 h, respectively (Fig. 2B and Supporting Information Fig. S9). Further, 3D tumor spheres were constructed to study the drug penetration ability (Fig. 2C). Under laser irradiation, the tumor penetration ability of OSN was significantly enhanced, which may be related to phototherapy. Notably, pre-treatment with chlorpromazine (CPZ)—a clathrin-mediated endocytosis inhibitor—or incubation at 4 °C significantly reduced OSN uptake (Fig. 2D), confirming that internalization primarily occurs *via* energy-dependent micropinocytosis and clathrin-mediated endocytosis²⁸.

The antitumor efficacy of OSN was evaluated in ID8 cells using the MTT assay. OSN exhibited a semi-inhibitory concentration (IC_{50}) of 8.71 μ g/mL, slightly lower than free oxaliplatin (Oxa, $IC_{50} = 9.68$ μ g/mL), while free SP and OTP displayed minimal cytotoxicity ($IC_{50} > 20$ μ g/mL; Supporting Information Fig. S10). Remarkably, laser irradiation significantly enhanced cytotoxicity (Fig. 2E), reducing IC_{50} values to 4.92 μ g/mL

irradiation (1.0 W/cm²). (I) Temperature elevation of OSN (100 μ g/mL) under varying laser intensities (0.4, 0.6, 0.8, 1.0 W/cm²). (J) Photothermal heating/cooling cycles of OSN (100 μ g/mL) with time constant (τ_s) derived from cooling kinetics. (K) Photothermal stability of OSN over five laser on/off cycles (660 nm, 1.0 W/cm²); ΔT represents temperature change. (L) Cumulative Pt release from OSN in PBS, 1 mmol/L H_2O_2 , 10 mmol/L GSH, and under laser irradiation at 37 °C.

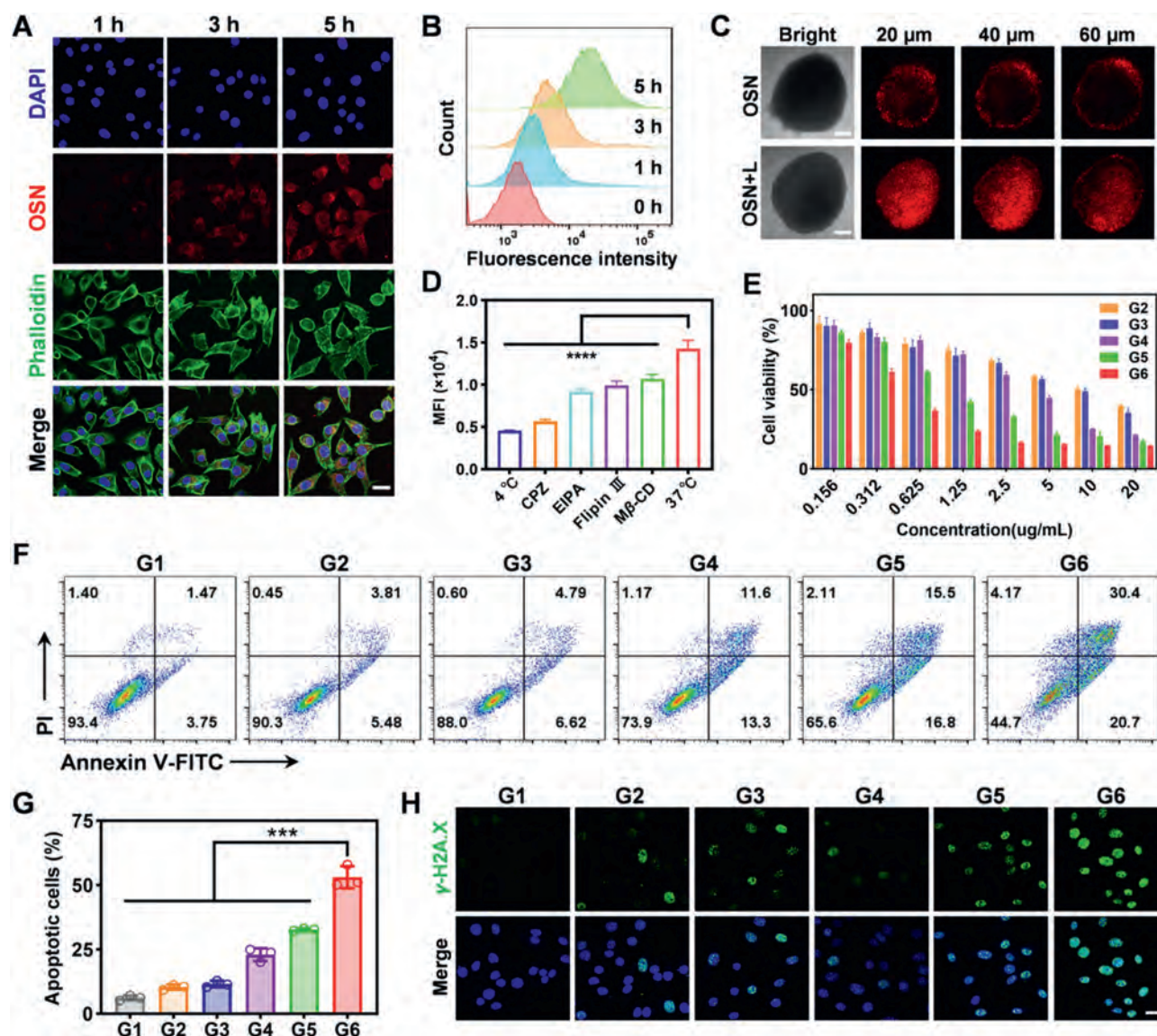


Figure 2 *In vitro* anti-tumor efficacy of OSN + L. (A) CLSM images showing the intracellular uptake of OSN in ID8 cells over time (scale bar: 25 μm). (B) FCM analysis of OSN uptake kinetics. (C) CLSM images showing the penetration of OSN and OSN + L into tumor spheroids (Scale bar: 150 μm). (D) Inhibition of OSN internalization in ID8 cells using endocytosis inhibitors (CPZ, EIPA, Filipin III, and M-β-CD) at 37 °C. (E) Cell viability of ID8 cells post-treatment, determined by MTT assay. (F) Apoptosis analysis via Annexin V-FITC/PI staining and (G) quantitative apoptotic rates. (H) CLSM images of γ-H2A.X expression (green fluorescence) in ID8 cells (scale bar: 25 μm). G1, PBS; G2, Oxa; G3, OSN; G4, SP + L; G5, Oxa + SP + L; G6, OSN + L. Data are presented as mean ± SD (*n* = 3), **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

(SP + L) and 0.47 μg/mL (OSN + L). Notably, OSN + L demonstrated superior potency compared to the Oxa + SP + L group (IC₅₀ = 1.172 μg/mL). Annexin V-FITC/PI staining revealed that OSN + L induced the highest apoptosis rate (53.1 ± 4.3%) at 1.25 μg/mL, whereas non-irradiated OSN caused only 11.5 ± 1.3% apoptosis (Fig. 2F and G). Live/dead staining (Calcein-AM/PI) further confirmed extensive cell death in laser-treated groups, with OSN + L exhibiting the most pronounced tumor suppression (Supporting Information Fig. S11). To elucidate the underlying mechanism, γ-H2A.X expression, a biomarker of platinum-induced DNA damage^{29,30}, was assessed via CLSM images. Elevated green fluorescence (γ-H2A.X) was observed in all treatment groups, with the OSN + L group exhibiting the strongest signal (Fig. 2H), indicating synergistic DNA damage from combined chemo-phototherapy.

3.5. OSN + L enhances tumor phototherapy by modulating mitochondrial energy and amplifying oxidative stress

We hypothesized that the synergistic enhancement of DNA damage is linked to OSN-mediated amplification of intracellular oxidative stress through photoconversion of triplet oxygen (³O₂) to ¹O₂. To test this hypothesis, intracellular ROS levels were measured using 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA), a fluorescent indicator of ROS. Upon cellular internalization, DCFH-DA is oxidized by ROS to generate the green-fluorescent dichlorofluorescein (DCF)³¹. As shown in Fig. 3A, the SP, Oxa + SP mixtures, and OSN treatments significantly increased DCF fluorescence intensity upon laser irradiation, confirming that photoactivated SP induces ROS generation. FCM further confirmed substantial ROS accumulation in

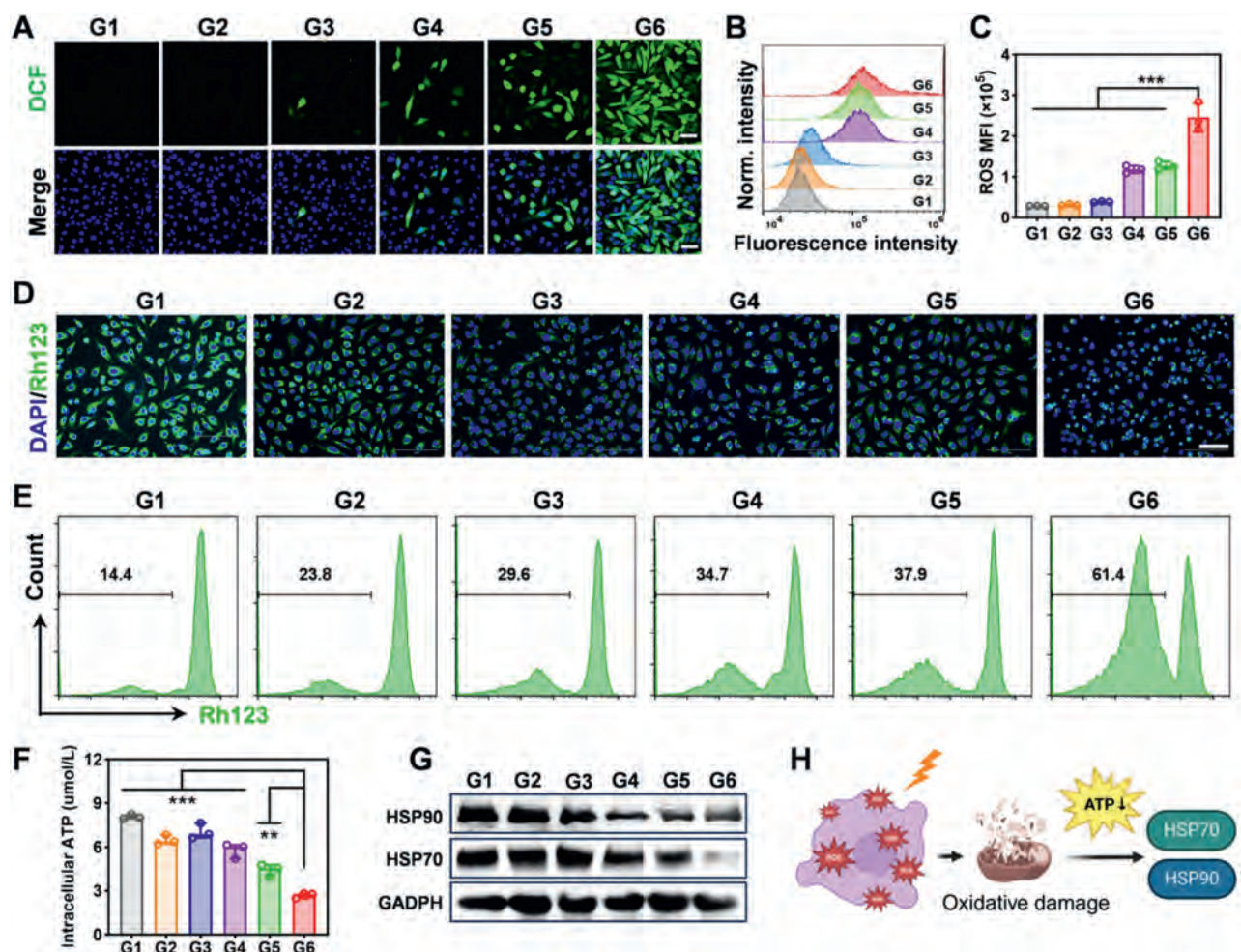


Figure 3 Enhanced tumor phototherapy by OSN + L *via* mitochondrial energy modulation and oxidative stress amplification. (A) CLSM images showing intracellular ROS production in ID8 cells post-treatment (scale bar: 50 μm). (B) FCM analysis and (C) quantitative measurement of ROS levels across treatment groups. (D) CLSM images (scale bar: 100 μm) and (E) FCM analysis of mitochondrial membrane potential using Rh123. (F) Quantification of intracellular ATP levels post-treatment. (G) Western blot analysis of HSP70/HSP90 expression. (H) Schematic illustrating oxidative stress-mediated suppression of HSPs in tumor cells. G1, PBS; G2, Oxa; G3, OSN; G4, SP + L; G5, Oxa + SP + L; G6, OSN + L. Data are presented as mean $\pm$ SD ($n = 3$), $^{*}P < 0.01$, $^{***}P < 0.001$.

the OSN + L group (Fig. 3B and C), validating the amplification of oxidative stress. Excessive ROS typically leads to mitochondrial damage and functional impairment. Mitochondrial integrity was assessed using Rh123, a fluorescent probe for mitochondrial membrane potential³². In the PBS group, normal mitochondria with high membrane potential retained strong Rh123 fluorescence. However, all treatment groups showed reduced fluorescence signals (Fig. 3D), with the OSN + L group displaying the most pronounced decrease due to ROS overproduction under laser irradiation. FCM analysis similarly confirmed severe mitochondrial damage in the OSN + L group (Fig. 3E). Mitochondria, the primary sites of cellular bio-oxidation and energy conversion, produce over 90% of cellular ATP *via* oxidative phosphorylation³³. Intracellular ATP levels were significantly reduced in the OSN + L group under laser irradiation (Fig. 3F). Heat shock proteins (HSP70/HSP90), ATP-dependent molecular chaperones critical for thermoresistance³⁴, were downregulated in the OSN + L group, as evidenced by western blotting (Fig. 3G, Supporting Information Fig. S12A and S12B). Together, these findings indicate that OSN + L induces: (1) a surge in ROS,

causing mitochondrial damage and ATP depletion; (2) suppression of HSPs, impairing the heat-shock response; (3) synergistic PTT enhancement through reduced tumor thermotolerance (Fig. 3H).

3.6. OSN + L enhances anti-tumor immune response by inducing cellular pyroptosis

A surge in ROS has been reported to induce cellular pyroptosis³⁵. As shown in Supporting Information Fig. S13, ID8 cells treated with OSN + L exhibited pronounced cellular swelling and membrane blebbing (red arrows), characteristic features of pyroptosis. We hypothesized that ROS-mediated mitochondrial damage drives pyroptotic cell death. Bio-TEM analysis revealed hallmark features of pyroptosis in OSN + L-treated cells, including membrane rupture, vesicle formation, and cytoplasmic content release (Fig. 4A). During the pyroptosis process, holes are formed in the cell membrane, eventually leading to the cell rupture and the release of intracellular contents and resulting in elevated levels of LDH from tumor cells²⁵. The supernatant-released LDH from ID8 cells after various treatments was further measured. As

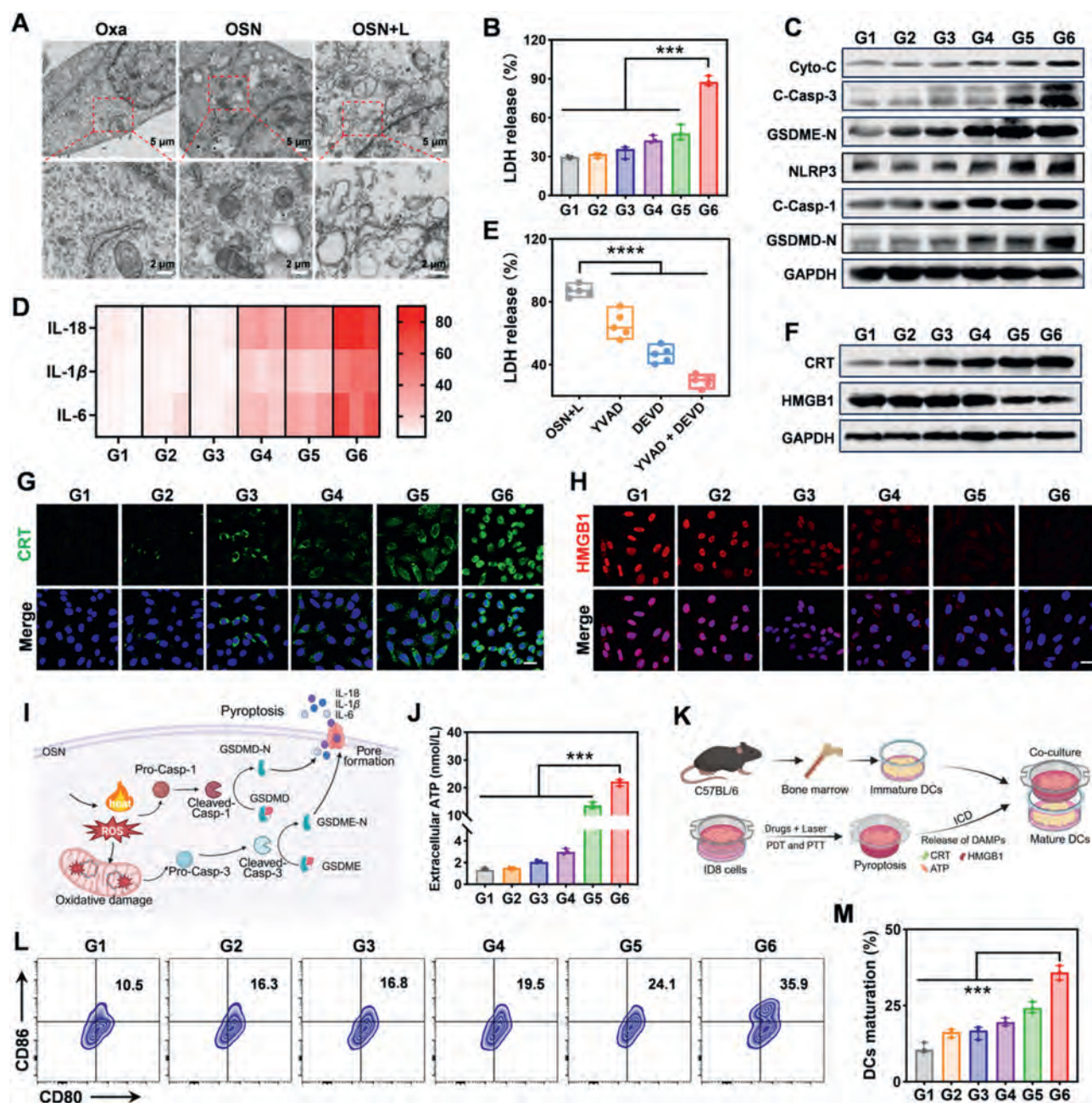


Figure 4 *In vitro* induction of pyroptosis and enhanced ICD effect by OSN + L. (A) Bio-TEM images showing pyroptotic morphology in ID8 cells treated with Oxa, OSN, and OSN + L. (B) Proportions of released LDH after various treatments ($n = 3$). (C) Western blot analysis of pyroptosis-associated proteins: cytochrome *c* (Cyto-C), cleaved caspase-3 (C-Casp-3), GSDME-N, NLRP3, cleaved caspase-1 (C-Casp-1), GSDMD-N, and GAPDH. (D) Heatmap for ELISA analysis of inflammatory cytokines. (E) Proportions of released LDH after pretreatment with specific inhibitors (YVAD, Z-YVAD-FMK; DEVD, Z-DEVD-FMK; $n = 5$). (F) Western blot analysis of CRT and HMGB1 expression. (G, H) CLSM images of CRT exposure (green fluorescence) and HMGB1 release (red fluorescence) in ID8 cells (scale bar: 25 μ m). (I) Schematic illustrating OSN + L-mediated amplification of pyroptosis *via* ROS generation and dual caspase activation. (J) Extracellular ATP levels in ID8 cell cultures ($n = 3$). (K) Schematic illustrating DAMPs release promoting BMDCs maturation. (L, M) Quantification and FCM analysis of BMDCs maturation (CD11c⁺CD80⁺CD86⁺; $n = 3$). G1, PBS; G2, Oxa; G3, OSN; G4, SP + L; G5, Oxa + SP + L; G6, OSN + L. Data are presented as mean $\pm$ SD, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

depicted in Fig. 4B, the OSN + L group exhibited significantly higher levels of LDH release than the other treatment groups, indicating that OSN + L treatment induced strong cell pyroptosis. Western blot analysis demonstrated significant upregulation of pyroptosis-associated proteins in the OSN + L group, including

cytochrome *c* (Cyto-C), cleaved caspase-3 (C-Casp-3), GSDME-N, NLRP3, cleaved caspase-1 (C-Casp-1), and GSDMD-N (Fig. 4C and Fig. S12C–S12H). These results suggest that OSN + L induces mitochondrial damage *via* ROS overproduction, triggering Cyto-C release and NLRP3 inflammasome activation.

To clarify the individual contributions of caspase-1 and caspase-3, we further performed inhibitor studies using Z-YVAD-FMK (caspase-1 inhibitor) and Z-DEVD-FMK (caspase-3 inhibitor). The inhibition of pyroptosis was indicated by detecting LDH release. As shown in Fig. 4E, LDH release in the OSN + L group amounted to 86.9%, which was reduced to 65.5% after pretreatment with Z-YVAD-FMK, whereas LDH release was reduced to 46.3% after pretreatment with Z-DEVD-FMK. Notably, dual inhibition (YVAD + DEVD) resulted in a reduction of LDH release to 29.6%, confirming the activation of the cooperative pathway. These results validate caspase-3/GSDME as the primary cleavage pathway and caspase-1/GSDMD as the secondary activation pathway. This cascade activates caspase-3 and caspase-1, leading to the cleavage of GSDME and GSDMD into N-terminal fragments that perforate cell membranes, releasing pro-inflammatory mediators (Fig. 4I).

Pyroptosis is a pro-inflammatory programmed cell death mechanism characterized by the massive release of DAMPs and cytokines³⁶. ELISA results revealed that OSN + L treatment significantly elevated inflammatory mediators: a 7.3-fold increase in IL-18, a 5.6-fold increase in IL-1 β , and a 7.2-fold increase in IL-6 (Fig. 4D and Supporting Information Fig. S14). Immunofluorescence confirmed the pyroptosis-mediated ICD effect, showing calreticulin (CRT) translocation (green fluorescence; Fig. 4G) and HMGB1 extracellular release (red fluorescence; Fig. 4H). Western blot analysis further validated these findings (Fig. 4F, Fig. S12I and S12J). ATP, a critical "find-me" signal for DCs maturation³⁷, peaked at 41.7 ± 1.9 nmol/L in the OSN + L group (Fig. 4J). To assess immune activation, BMDCs were co-cultured with treated ID8 cells (Fig. 4K). FCM analysis revealed that OSN + L induced the highest BMDCs maturation rate ($32.8 \pm 3.9\%$, 3.02-fold increase compared to PBS; Fig. 4L and M). Collectively, these results demonstrate that OSN + L amplifies oxidative stress to drive pyroptosis, leading to the release of DAMPs and cytokines that potentiate anti-tumor immunity.

3.7. RNA-sequencing analysis reveals potential anti-tumor mechanisms

To elucidate the cytotoxic mechanisms of OSN + L treatment, we performed genome-wide RNA sequencing (RNA-seq) on ID8 cells across different treatment groups. Fig. 5A shows the gene correlation patterns, with 230 and 194 transcripts uniquely expressed in Oxa- and OSN + L-treated cells, respectively. Comparative analysis identified 709 upregulated and 2802 downregulated genes in OSN + L-treated cells compared to Oxa-treated cells (Fig. 5B). In the differentially expressed genes between the OSN + L group and the PBS/Oxa groups, pyroptosis-related genes were identified, including upregulated expression of *GSDMD*, *GSDME*, *NLRP3*, *CASP1*, *CASP3*, *IL-1 β* and *IL-18*. Pathway enrichment analysis revealed significant alterations in key signaling pathways, including the TNF signaling pathway, MAPK signaling pathway, PI3K–Akt signaling pathway, T cell receptor signaling pathway, Toll-like receptor signaling pathway, and chemokine signaling pathway (Fig. 5C). Notably, mitochondrial-associated genes were dysregulated in OSN + L-treated cells (Fig. 5D), likely due to ROS-induced mitochondrial damage. Transcriptional changes in immunomodulatory genes—such as *CXCL8*, *CCL2*, *CCL5*, *IL12A*, *CXCL2*, *TNF*, *Hspa1a*, and *Hspa1b*—suggested a robust ICD effect and subsequent immune activation, consistent with the observed tumor cell destruction. Gene Set Enrichment Analysis (GSEA) further confirmed the activation of pyroptosis-mediated

immune responses, highlighting upregulated pathways including NK cell activation in immune response, T cell activation in immune response, cytokine receptor binding, B cell proliferation, and cytochrome *c* oxidase activity (Fig. 5E). Collectively, these findings demonstrate that OSN + L triggers pyroptosis-driven immune activation through ROS-mediated mitochondrial dysfunction and inflammatory cytokine release, synergizing with enhanced anti-tumor immunity.

3.8. In vivo biodistribution, antitumor efficacy, and biosafety evaluation of OSN + L

Hemocompatibility assessment of OSN *via* hemolytic assay demonstrated concentration-dependent hemolytic activity below 10% (Supporting Information Fig. S15), confirming its blood compatibility and suggesting prolonged systemic circulation. Subsequent pharmacokinetic analyses in SD rats demonstrated distinct pharmacokinetic profiles between free Oxa and OSN. Free Oxa exhibited rapid systemic clearance, whereas OSN markedly prolonged blood circulation time, as supported by a 2.1-fold elevation in maximum plasma concentration and a 6.4-fold reduction in total clearance (Supporting Information Fig. S16 and Table S3). These enhancements were further corroborated by extended elimination half-life and significantly augmented area under the concentration-time curve, underscoring the superior pharmacokinetic performance of the nanoformulation. Given the critical role of biodistribution in therapeutic efficacy and biocompatibility, an ID8 tumor-bearing murine model was developed to evaluate the spatial-temporal trafficking and anti-tumor performance of OSN. Comparative real-time fluorescence imaging following caudal vein administration of SP *versus* OSN revealed enhanced tumor-specific accumulation and retention kinetics in the OSN-treated group (Fig. 6A). *Ex vivo* fluorescence imaging of harvested organs 48 h post-injection exhibited characteristic nanoparticle hepatic deposition, with marked OSN localization in tumor tissues contrasting SP's predominant hepatic sequestration. Semi-quantitative fluorescence intensity analyses corroborated these distribution patterns, validating tumor-targeting specificity of OSN (Fig. 6B). ICP-MS quantification of Pt biodistribution identified significantly elevated tumor Pt levels in OSN-treated subjects compared to Oxa controls, while Oxa demonstrated preferential hepatic and renal accumulation, highlighting OSN's tumor-focused pharmacokinetic advantage (Fig. 6C). Photothermal profiling under laser irradiation (660 nm, 0.5 W/cm²) in the ID8 model revealed superior energy transduction capacity of OSN. While PBS and SP controls exhibited marginal thermal elevations ($\Delta 3$ and $\Delta 9$ °C, respectively), OSN induced rapid tumor hyperthermia ($\Delta 20$ °C within 4 min; Fig. 6D and E), confirming its target-specific photothermal conversion efficiency.

Leveraging *in vitro* synergistic observations, the antitumor potency of OSN + L was assessed through triplicate intravenous dosing (Days 1/3/5) with subsequent localized laser exposure (Fig. 6F). Longitudinal monitoring revealed moderate tumor suppression in Oxa/OSN monotherapy groups compared to profound regression in OSN + L groups (Fig. 6G and H). Treatment groups maintained stable somatometric parameters without distress indicators (Supporting Information Fig. S17). Terminal tumor mass analysis and gross morphological assessment confirmed superior growth inhibition in the OSN + L group (Fig. 6I and J). Histopathological evaluation *via* H&E staining revealed extensive tumor necrosis with nuclear condensation and

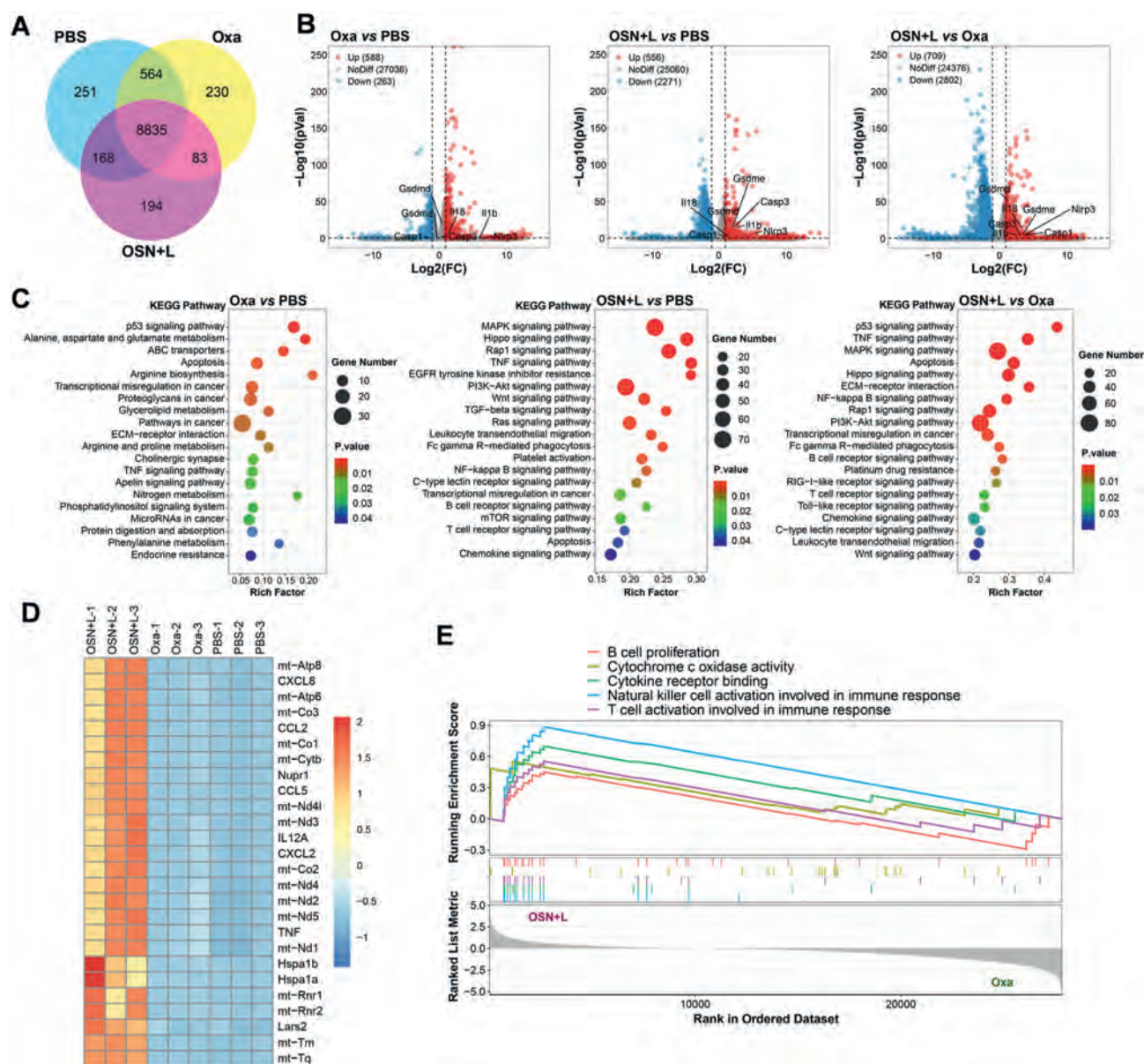


Figure 5 Transcriptomic profiling of ID8 Cells *via* RNA-seq Post-treatment. (A) Venn diagram illustrating unique and overlapping gene transcripts across treatment groups. (B) Volcano plots of differentially expressed genes between: Oxa vs. PBS, OSN + L vs. PBS, OSN + L vs. Oxa. (C) KEGG pathway enrichment analysis of differentially expressed genes for Oxa vs. PBS, OSN + L vs. PBS, and OSN + L vs. Oxa. (D) Heatmap of the specific gene expressions of concern in ID8 cells treated with PBS, Oxa and OSN + L. (E) GSEA highlighting immune-related pathways upregulated in OSN + L-treated cells.

architectural disintegration in OSN + L-treated specimens, while TUNEL assays demonstrated amplified apoptotic activity, collectively evidencing enhanced *in vivo* cytotoxicity (Fig. 6K). Comprehensive biosafety evaluation showed transient ALT/CREA elevation exclusively in Oxa-containing groups, with OSN-treated groups maintaining physiological hepatic/renal function (Supporting Information Fig. S18). Histopathological examination of major organs revealed no treatment-related abnormalities, affirming the biocompatibility of OSN (Supporting Information Fig. S19). These multimodal analyses establish OSN + L as a precision nanotherapeutic platform with optimized biodistribution, potent antitumor efficacy, and favorable biosafety.

3.9. OSN + L orchestrates systemic immune activation through TME remodeling

Pyroptosis-mediated programmed cell death plays a crucial role in immune system activation. The substantial release of pro-inflammatory cytokines and DAMPs triggered by pyroptosis induces robust inflammatory responses and immunological activation. These cytokines facilitate the recruitment and activation of immune cells (*e.g.*, macrophages, T cells), thereby initiating both innate and adaptive immune responses. Concurrently, released DAMPs are recognized by pattern recognition receptors, which subsequently enhance immune cell activation and amplify inflammatory signaling cascades^{38–40}. Therapeutic analysis of

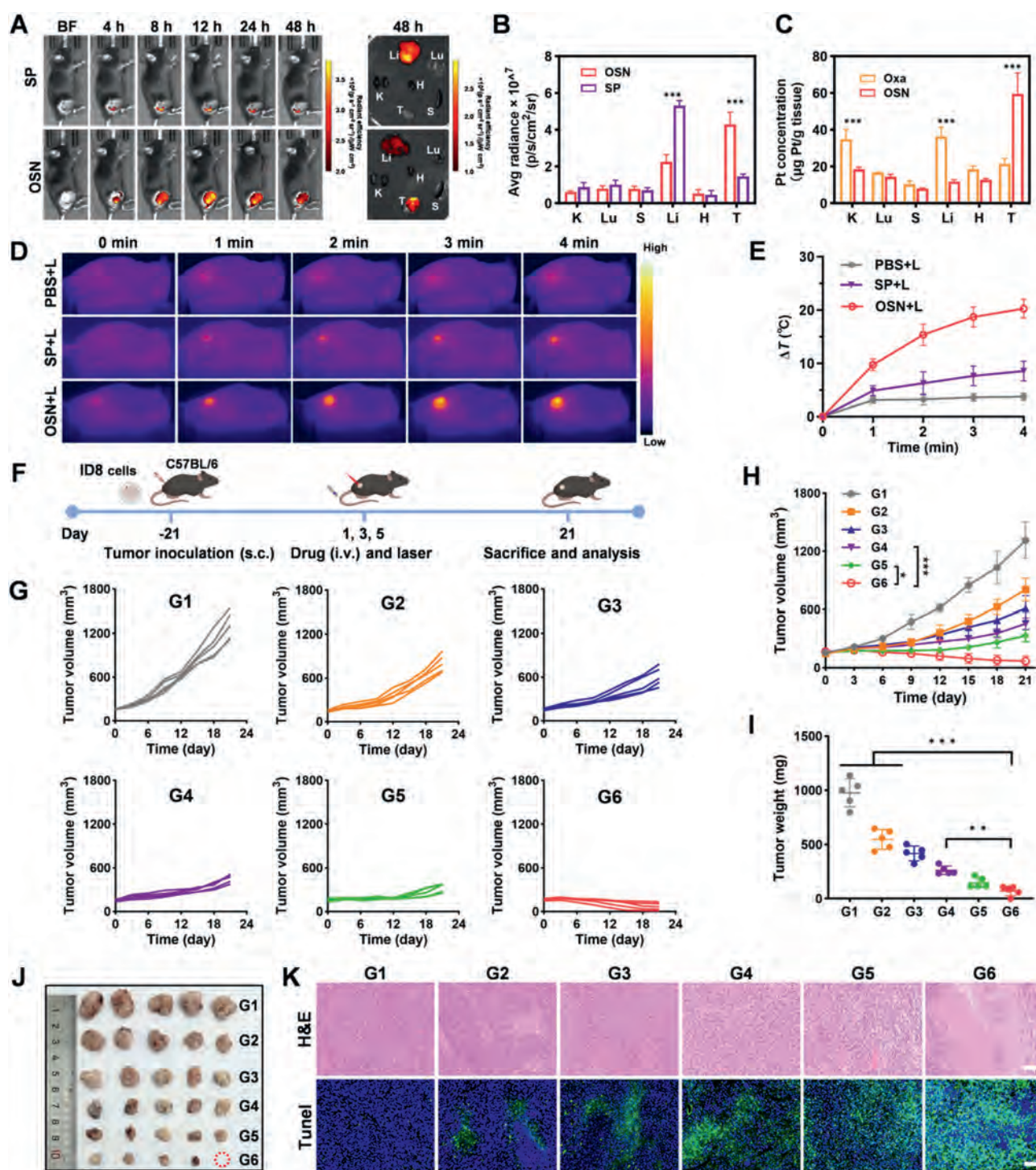


Figure 6 Biodistribution and antitumor efficacy of OSN + L in subcutaneous ID8 tumor-bearing mice. (A) *In vivo* fluorescence tracking of tumor-targeted accumulation following systemic administration of SP or OSN at designated intervals. *Ex vivo* organ fluorescence mapping 48 h post-injection: Heart (H), Liver (Li), Spleen (S), Lung (Lu), Kidneys (K), Tumor (T). (B) Semi-quantitative biodistribution profiles of fluorescent signals across major organs ($n = 3$). (C) Pt deposition quantitation *via* ICP-MS 24 h post-Oxa or OSN administration ($n = 3$). (D, E) Thermographic images and temporal temperature elevation profiles during laser irradiation (660 nm, 0.5 W/cm²) 12 h post-PBS, SP, or OSN injection. (F) Schematic of the therapeutic protocol for ID8 tumor-bearing cohorts. (G) Longitudinal tumor volume kinetics across treatment groups ($n = 5$). (H) Comparative tumor growth inhibition trajectories. (I, J) Terminal tumor mass quantification and macroscopic tumor morphology after 21 days of treatment ($n = 5$). (K) Histomorphological analysis *via* H&E staining and apoptotic assessment by TUNEL assay (scale bar: 100 µm). G1, PBS; G2, Oxa; G3, OSN; G4, SP + L; G5, Oxa + SP + L; G6, OSN + L. Data are presented as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

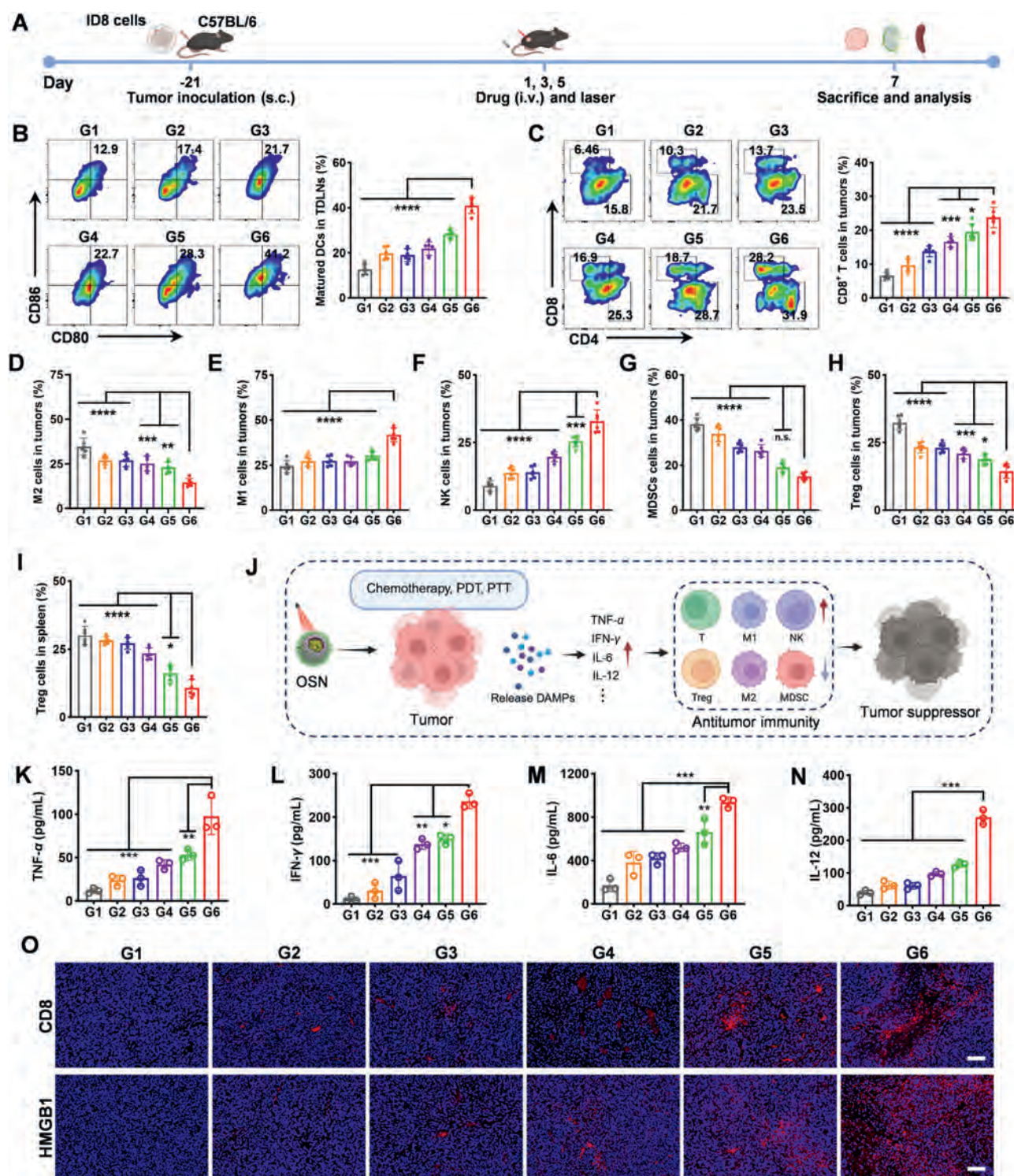


Figure 7 Systemic immunomodulatory cascade and activation of antitumor immunity. (A) Therapeutic protocol schematic for ID8 tumor-bearing mice. (B) FCM profiling and quantitative analysis of CD80⁺CD86⁺ mature DCs (CD11c⁺ gated) in tumor-draining lymph nodes ($n = 6$). (C) Tumor-infiltrating lymphocyte characterization: CD3⁺-gated distribution of CD4⁺/CD8⁺ T cells and quantitative assessment of CD8⁺ T cells infiltration ($n = 6$). (D–H) Quantification of the immunosuppressive niche: M2-polarized (CD11b⁺F4/80⁺CD206⁺) and M1-polarized (CD11b⁺F4/80⁺CD86⁺) macrophage ratios, NK cells (CD45⁺CD49b⁺) infiltration, MDSC (CD11b⁺Gr-1⁺) prevalence, and Treg cells (CD45⁺CD3⁺CD4⁺CD25⁺FOXP3⁺) within the tumor ($n = 6$). (I) Quantification of splenic Treg cells ($n = 6$). (J) Mechanistic schematic illustrating the OSN + L-induced antitumor immune cascade. (K–N) Systemic elevation of cytokines (TNF- α , IFN- γ , IL-6, IL-12) quantified *via* ELISA in serum ($n = 3$). (O) Immunofluorescence images showing CD8⁺ T cells infiltration and HMGB1 translocation (scale bar: 100 μ m). G1, PBS; G2, Oxa; G3, OSN; G4, SP + L; G5, Oxa + SP + L; G6, OSN + L. Data are presented as mean $\pm$ SD (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; **** $P < 0.0001$; one-way ANOVA with Bonferroni correction).

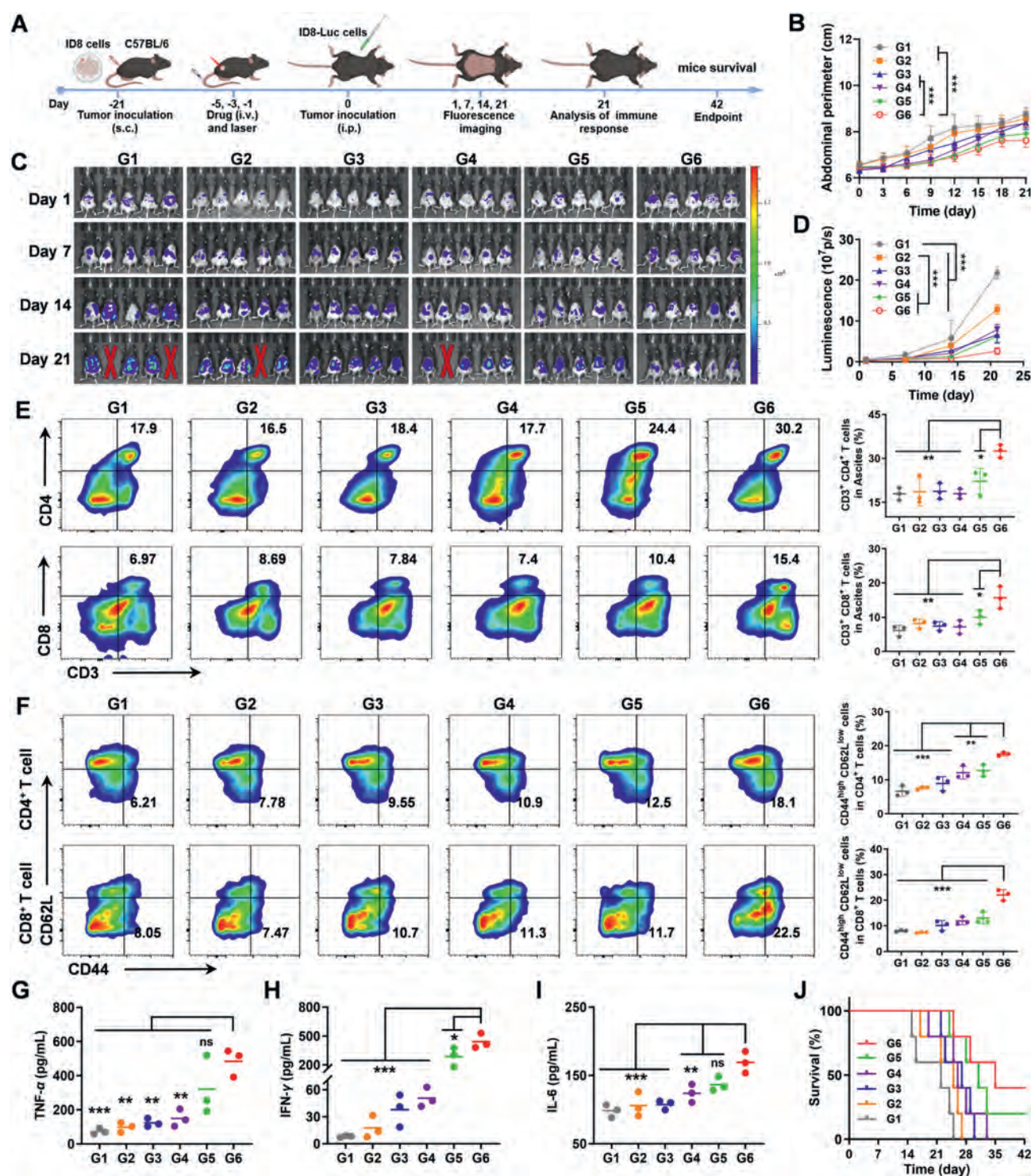


Figure 8 OSN + L-mediated immunostimulation suppresses abdominal metastasis in high-grade serous ovarian cancer models. (A) Schematic showing the experimental protocol for intraperitoneal inoculation of ID8-Luc cells in HGSO models and triple-cycle OSN + L treatment of subcutaneous tumors. (B) Therapeutic intervention with OSN + L attenuates pathological abdominal distension ($n = 5$). (C, D) Bioluminescent imaging and quantitative bioluminescent flux analysis of metastatic progression in OSN + L-treated versus control groups ($n = 5$). (E) Immunophenotypic analysis of CD3⁺CD8⁺ cytotoxic T lymphocytes and CD3⁺CD4⁺ helper T cells in ascitic fluid ($n = 3$). (F) Splenic memory T cell pools (CD44^{high}CD62L^{low}) in CD4⁺ and CD8⁺ populations ($n = 3$). (G–I) Proinflammatory cytokine (IFN-γ, TNF-α, IL-6) in ascitic fluid ($n = 3$). (J) Survival curves following intraperitoneal ID8-Luc challenge. G1, PBS; G2, Oxa; G3, OSN; G4, SP + L; G5, Oxa + SP + L; G6, OSN + L. Data are presented mean ± SD (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; one-way ANOVA with Bonferroni correction).

OSN + L-mediated immunomodulation revealed significant remodeling of the tumor immune landscape in ID8-bearing models. Following tripartite intravenous dosing with adjunctive laser phototherapy (Fig. 7A), FCM profiling showed progressive DCs maturation in tumor-draining lymph nodes of the OSN + L group, indicated by a 3.2-fold increase in CD86⁺CD80⁺ DCs population compared to PBS control (Fig. 7B and Supporting Information Fig. S20), demonstrating enhanced antigen-presenting capacity for T cell priming. Concurrently, tumor-infiltrating lymphocyte analysis revealed substantial expansion of cytotoxic CD8⁺ T cell effectors ($23.78 \pm 2.70\%$; 3.6-fold increase *vs.* PBS) and CD4⁺ helper populations ($33.92 \pm 5.41\%$; 2.1-fold increase *vs.* PBS) in OSN + L group, indicative of robust adaptive immune engagement (Fig. 7C, Supporting Information Fig. S21 and S22). Notably, the immunosuppressive networks of the OSN + L group were profoundly reconfigured, with coordinated depletion of protumoral elements: M2-polarized macrophages (CD11b⁺F4/80⁺CD206⁺) decreased 2.6-fold while proinflammatory M1 (CD11b⁺F4/80⁺CD86⁺) increased by 1.7-fold (Fig. 7D and E and Supporting Information Fig. S23); infiltration of MDSCs (CD11b⁺Gr-1⁺) diminished by 2.9-fold (Fig. 7G and Supporting Information Fig. S24), and Treg cells (CD45⁺CD3⁺CD4⁺CD25⁺FOXP3⁺) decreased by 2.6-fold in tumor and 3.5-fold in spleen (Fig. 7H and I and Supporting Information Fig. S25). Simultaneously, innate immune surveillance was restored with a 3.9-fold increase in NK cell (CD45⁺CD49b⁺) infiltration, reaching $32.85 \pm 4.22\%$ (Fig. 7F and Supporting Information Fig. S26). Immunofluorescence confirmed CD8⁺ T cell penetration and HMGB1 translocation, markers of immunogenic cell death (Fig. 7O). Systemic immune activation was further validated by elevated serum cytokines levels: TNF- α (8.2-fold), IFN- γ (22.1-fold), IL-6 (5.5-fold), and IL-12 (6.8-fold) compared to PBS controls (Fig. 7K–N). Together, these findings demonstrate that OSN + L synergizes pyroptosis-driven DAMP release, DCs-mediated antigen cross-presentation, cytotoxic lymphocyte expansion, and immunosuppressive niche ablation (Fig. 7J). This comprehensive immunoregulatory cascade transforms immunologically inert tumors into inflamed microenvironments, establishing durable systemic antitumor immunity through coordinated innate and adaptive immune activation.

3.10. OSN + L-mediated immunostimulation suppresses abdominal metastasis in high-grade serous ovarian cancer models

Pyroptotic tumor cells release tumor-associated antigens and DAMPs, which drive the maturation of DCs. This cascade profoundly enhances T cell-mediated antitumor immunity, promotes the differentiation of memory T cells, and establishes durable immunological memory^{41–43}. To assess the translational potential of OSN + L-induced systemic immunity against aggressive malignancies, we established a high-grade serous ovarian cancer (HGSOC) model—characterized by peritoneal dissemination and malignant ascites—*via* intraperitoneal inoculation of ID8-Luc cells following triple-cycle OSN + L treatment of subcutaneous tumors (Fig. 8A). While the model does not fully recapitulate the entire metastatic cascade (*e.g.*, detachment from primary tumors, stromal invasion, or angiogenesis), it reliably mimics the later stages of transcoelomic spread and peritoneal niche colonization. OSN + L therapy significantly alleviated pathological abdominal distension (Fig. 8B) and body weight gain (Supporting Information Fig. S27), while markedly delaying metastatic progression, as

evidenced by bioluminescent monitoring (Fig. 8C and D). Terminal necropsy revealed a substantial reduction in peritoneal metastatic nodules and ascitic fluid volume in OSN + L-treated mice compared to controls, indicating effective immunomodulatory disruption of the ascites-mediated protumoral niche (Supporting Information Fig. S28). To investigate the presence of extraperitoneal metastases, H&E staining was performed on the major organs (liver, lung) of abdominal metastasis mice. As shown in Supporting Information Fig. S29, no metastatic lesions were detected in these organs, confirming the model's specificity to peritoneal dissemination. Immunophenotypic analysis of ascitic fluid further demonstrated robust immune remodeling: CD3⁺CD8⁺ cytotoxic T lymphocytes increased 2.5-fold and CD3⁺CD4⁺ helper T cells expanded 1.8-fold relative to PBS controls (Fig. 8E), accompanied by significantly elevated proinflammatory cytokines (TNF- α : 6.6-fold, IFN- γ : 54.7-fold, IL-6: 1.7-fold; Fig. 8G–I). Additionally, splenic memory T cell pools (CD44^{high}CD62L^{low}) expanded 2.6-fold for CD4⁺ and 2.8-fold for CD8⁺ T cells in OSN+L-treated mice (Fig. 8F and Supporting Information Fig. S30), indicative of sustained immunological memory formation. This adaptive immune priming conferred a significant survival benefit, with OSN + L-treated cohorts achieving a 40% long-term survival rate at the endpoint (Day 42) whereas all PBS control mice succumbed by Day 25 (Fig. 8J). These findings establish OSN + L as a powerful multimodal immunotherapeutic strategy capable of overcoming the immunosuppressive peritoneal microenvironment. By orchestrating a coordinated immune response—activating cytotoxic effector cells, inducing proinflammatory cytokine surges, and expanding memory T cell populations—OSN + L provides robust and sustained protection against metastatic HGSOC progression.

4. Conclusions

This study introduces a pioneering molecular engineering strategy by integrating oxaliplatin(IV) prodrugs with near-infrared semi-conducting polymers to construct self-assembled multifunctional nanoparticles (OSN), enabling triple-modality therapy—PTT, PDT, and chemotherapy—through synergistic effects. For the first time, dual activation of pyroptosis pathways and remodeling of the immune microenvironment were strategically combined to overcome the challenges of immunosuppression and metastasis in ovarian cancer. The rationally designed OSN nanoparticles demonstrated superior photothermal conversion efficiency and ROS generation capacity while achieving tumor-specific redox activation of oxaliplatin through GSH/ROS dual-responsive release, thereby synchronizing chemotherapeutic and phototherapeutic effects with precise spatiotemporal control. Under laser irradiation, OSN activated both caspase-1-dependent canonical pyroptosis and caspase-3-mediated non-canonical pyroptosis pathways, triggering a "bystander effect" through gasdermin pore formation and initiating a cascade of DAMPs. This immunogenic cascade orchestrated a coordinated immune response, including DCs maturation, CD8⁺ T cells, and NK cells recruitment, M2 macrophage polarization to the M1 phenotype, and suppression of Tregs and MDSCs, collectively remodeling the immunosuppressive TME into an immunologically active state. In ID8 ovarian cancer models, OSN + L treatment resulted in over 90% regression of primary tumors and specific suppression of peritoneal metastasis, along with a significant expansion of splenic memory T cells. This work establishes a novel multimodal

therapeutic paradigm that overcomes the limitations of conventional chemoimmunotherapy by amplifying the ICD effect through photon energy. The ability to concurrently eradicate primary tumors, inhibit metastatic spread and induce systemic anti-tumor immunity positions OSN + L as a promising clinical candidate for treating advanced ovarian cancer and therapy-resistant metastases.

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Author contributions

Feng Fang: Writing – original draft, Investigation, Methodology, Formal analysis, Conceptualization. Min Su: Investigation, Formal analysis. Xue Liu: Methodology, Formal analysis, Software, Data curation. Jing Chen: Formal analysis, Data curation. Qiwen Liu: Methodology, Data curation. Xinran Li: Methodology, Data curation. Xuanbo Zhang: Writing – review & editing, Methodology. Yuanyuan Chen: Resources, Investigation. Huijiao Fu: Methodology, Data curation. Zhengchai Chen: Formal analysis, Data curation. Xuzi Cai: Resources, Investigation. Cao Zhou: Methodology, Investigation. Zhengfen Li: Supervision. Zhiqiang Yu: Writing – review & editing, Supervision. Xuefeng Wang: Supervision, Project administration, Funding acquisition, Conceptualization. All of the authors have read and approved the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest. National Natural Science Foundation of China.

Appendix A. Supporting information

Supporting information to this article can be found online at <https://doi.org/10.1016/j.apsb.2025.10.042>.

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