

Peer Review File

Intermetallics triggering pyroptosis and disulfidptosis in cancer cells promote anti-tumor immunity

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REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author): with expertise in nanomedicine for cancer therapy

This work reported the preparation of Pd₂Sn intermetallics and then integrated with GOx for self-reinforced ROS storm and thus Caspase-1-dependent pyroptosis and cystine-mediated disulfidptosis. The procedure also reprogrammed the immune microenvironment for cancer multiple-modality therapy and immunotherapy. Pd₂Sn exhibited the peroxidase- and oxidase-mimic activities, in combination with the activity of GOx, so cascade biocatalysis was realized to improve the therapy efficiency. The composites were well prepared and characterized with abundant data and profound analysis, while their bioactivity was illustrated carefully. I suggested major revision for the paper before publication in Nature Communications.

1. The full name of "SP" in (Pd₂Sn@GOx-SP) should be added at its first time appearance.
2. As illustrated with the XPS result, Pd₂Sn contains Pd²⁺, Sn⁴⁺, and Sn²⁺. What is the conter-anion and how to connect between the cations.
3. Less references were cited in some parts of Result section abnormally.
4. "As a noble metal, palladium exhibits the peculiar localized surface plasmon resonance (LSPR) effect and various enzyme catalytic activity, which has made a profound study" and others: some references should be cited.
5. Page 6-7 and 7-8: the paragraph is too long within two pages.
6. Similarly, each paragraph in Result section is too long.
7. "both Pd₂Sn-SP and Pd₂Sn@GOx-SP were cytotoxic to CT26 cells": How to the safety of the composites to the normal cells and why non-cytotoxic is to the normal cells as Pd and Sn are heavy metals.
8. DFT should be defined as "density functional theory" when appearing for the first time. OXD should be defined as "oxidase" when appearing for the first time.
9. Please add the indentation for the first line. The format other paragraph in SI needs to be also readjust.
10. In page 4, line 10, Please add "nm" after 0.195.
11. It is better to correct the abbreviation of "blood oxygen saturation" as acknowledged "SaO₂".

12. In page 14, line 34, "in vivo behavior" should be replaced by "biodistribution assessment", since in vivo behavior refers to sophisticated animal ethology method such as rotarod test, open field test, etc.
13. There are two figure 4c in Fig. 4, the lower one should be figure 4e, please carefully check.
14. In Fig. 4, Supplementary Fig. 16 and other similar figures, the corresponding treatments of G1-G5 should be clearly identified in figure caption to make it easy to read.
15. In Supplementary Fig. 19f, there is a scale mistake in color bar, red should mean the high temperature, while blue means low temperature.
16. I feel confused for Supplementary Fig. 19e, the tumor of control group located in the left side, while the tumor of 3, 6, 12, 24 h located in the right side with different morphology and size. Apparently, the CT imaging of mouse injected with Pd2Sn-SP at different timepoints should be performed on a same tumor-bearing mouse. Meanwhile, please check the i.v. injection is Pd2Sn@GOx-SP or Pd2Sn-SP, there is description contradict in your figure caption of Supplementary Fig. 19. Another suggestion: using circle to highlight the tumor region.

Reviewer #2 (Remarks to the Author): with expertise in nanomedicine for cancer therapy

Feng, Zhou, Yang et al. reported in this work about the intermetallic Pd2Sn modified with glucose oxidase (Pd2Sn@GOx-SP), and utilized them as pyroptosis promoter to induce the pyroptosis by cascade biocatalysis for remodeling tumor microenvironment, facilitating tumor cell immunogenicity, and further triggering disulfidptosis mediated by cystine accumulation. They combined the good properties of photothermal and various enzyme-like catalytic activities of the intermetallic Pd2Sn@GOx-SP, and demonstrated promising applications in the field of antitumor immunotherapy. The idea of evaluating and tuning intermetallic nanomaterials in biological antitumor therapy is new, and the work is well-presented in general, their experiments mostly support their conclusions. However, there are still some places that need to be further improved. Thus, I would like to suggest it publish after they answering the following questions:

- 1) During the synthesis of intermetallic Pd2Sn, TOP is used as surfactant to stabilize the nanoparticles. The phosphorus atom in TOP is well-known to form very strong bond

interaction with Pd atoms (Pd-P bond), thus it is very difficult to completely remove the TOP on nanoparticle surface. If TOP is still on surface, there will be two issues: i) the bio-toxicity of P from TOP; ii) The TOP will greatly hamper the following surface modifications of SP and GOx. So, I suggest the authors give more details about how they effectively remove the TOP (give experimental evidences). If TOP is still on surface, please evaluate the toxicity of TOP.

2) Generally speaking, though the intermetallic nanostructure is more stable than the alloyed ones, the authors should provide direct experimental evidence showing that the alloyed Pd₂Sn is indeed less active and less stable than the intermetallic Pd₂Sn. Maybe the alloyed Pd₂Sn is already good enough in such biological antitumor therapy application.

3) It is very weird that there is more Pd²⁺ than metallic Pd⁰ in Pd₂Sn, and no metallic Sn⁰ in Pd₂Sn at all in their XPS measurement. This XPS result means their Pd₂Sn NPs and NRs are almost oxidized and very unstable in air, which is contradictory to the statement that intermetallic Pd₂Sn is stable.

4) The pH became lower when the GOx catalyzing glucose into gluconic acid. So, will the more acidic microenvironment cause the etching and leaking of Sn ions? If yes, will the Sn ions leaking cause the severe biotoxicity? Will the GOx be malfunctioned in a more acidic environment.

5) I don't think the DFT calculations support their conclusion. From their DFT calculations, they conclude that the (001) crystal facet possess higher catalytic activity than the (011) crystal facet. But how this can be used to explain why the NRs with moderate length show the best catalytic performance, longer or shorter NRs both decrease the catalytic activity? From their current DFT results, the longer NRs with more (001) facets should have the best catalytic activity, which is inconsistent with their experimental results.

6) The migration ability of CT26 cells is suggested to be evaluated by transwell migration assay.

7) The images of CT26 cells are suggested to be provided to illustrate the morphology change after the treatment.

8) The staining images of GSDMD in vivo is suggested, which is essential to confirm the pyroptosis process in vivo.

Reviewer #3 (Remarks to the Author): with expertise in cancer cell death, disulfidptosis

This manuscript proposes an interesting and novel approach to activate tumor immunogenicity and reprogram the immunosuppressive microenvironment for cancer immunotherapy by targeting pyroptosis and disulfidptosis. The idea of utilizing intermetallics modified with glucose oxidase (Pd2Sn@GOx-SP) to induce pyroptosis and further promote disulfidptosis through cystine accumulation is novel and hold the potential to enhance the therapeutic effectiveness. They showed that Pd2Sn@GOx-SP generated abundant ROS and subsequently pyroptotic cell death. Additionally, the authors proved that Pd2Sn@GOx-SP promotes the consumption of glucose, leading to decreased NADPH production (likely through the pentose phosphate pathway), which impairs the reduction of cystine imported by SLC7A11, disulfide stress and eventually a newly identified form of programmed cell death called "disulfidptosis" in tumor cells. They also demonstrated that Pd2Sn@GOx-SP-induced pyroptosis and disulfidptosis significantly suppress tumor growth and recurrence. Overall, this work introduces an interesting strategy for designing antitumor agents by inducing pyroptosis and disulfidptosis. I have a few comments and suggestions:

1, Since oxidative stress might also trigger ferroptosis, I'm wondering if Pd2Sn@GOx-SP generated abundant ROS could induce the occurrence of ferroptosis. Interestingly, glucose deprivation-mediated AMPK was shown to inhibit ferroptosis (PMID: 32029897). Based on this, Pd2Sn@GOx-SP-mediated glucose consumption might suppress ferroptosis (if any). Ferroptosis might not be shown in this context then. Treatment with ferroptosis inhibitors, such as deferoxamine mesylate (DFO), ferrostatin-1, or liproxstatin-1 would give insight on the role of ferroptosis in the context of this story.

2, SLC7A11-mediated cystine uptake is a critical factor that induces disulfide stress. The author might need to test if Pd2Sn@GOx-SP showed limited or impaired ability of inducing cell death in SLC7A11 low cells or SLC7A11 KD/KO cells. Alternatively, removing cystine from the culture medium or treatment of SLC7A11 inhibitor (erastin, sulfasalazine) will also provide solid evidence to their conclusion.

3, There is a typo in the text. In line 31 under Discussion section, cysteine should be cystine.

“to the abundant accumulation of cysteine in CT26 cells with high SLC7A11 expression”

Reviewer #4 (Remarks to the Author): with expertise in immunology, cancer cell death, pyroptosis

The authors developed Pd2Sn nanorods that exhibit photothermal and enzyme catalytic activities, including peroxidase-mimic and catalase-mimic activity. The nanorods were further loaded with soybean phospholipid and glucose oxidase (Pd2Sn@GOx-SP). The authors speculate that Pd2Sn@GOx-SP induce pyroptosis and disulfidptosis through reactive oxygen species storms and glucose starvation. Cell death was induced by Pd2Sn@GOx-SP plus laser irradiation in CT26 colon cancer cells, but not L929 cells used as normal cells. Pd2Sn@GOx-SP plus laser markedly reduced CT26 xenografts in vivo and promoted anticancer immunity.

I reviewed this manuscript as an expert in immunology and cell death. It is interesting to note that Pd2Sn@GOx-SP plus laser has a very high tumor therapeutic effect. However, I have concerns with some of the authors' conclusions, especially the type of cell death induced by the Pd2Sn@GOx-SP plus laser. The authors designed Pd2Sn@GOx-SP to induce pyroptosis and disulfidptosis, because these necrotic forms of cell death can be immunogenic. The experimental results presented in this manuscript are insufficient to prove that Pd2Sn@GOx-SP plus laser triggered pyroptosis and disulfidptosis.

The best way to confirm that Pd2Sn@GOx-SP plus laser-induced cell death is pyroptosis is to examine whether it is GSDMD-dependent. It is necessary to determine whether knocking out and/or knocking down the expression of GSDMD in CT26 reduce Pd2Sn@GOx-SP plus laser-induced cell death. It is also necessary to determine whether Pd2Sn@GOx-SP plus laser-induced cell death is dependent on the NLRP3 inflammasome as the authors assumed.

In disulfidptosis, disulfide bonds between actin proteins are formed, which can be detected by non-reducing SDS-PAGE and subsequent Western blotting for actin cytoskeleton proteins (Nature Cell Biology 25:404–414 2023). In addition, shrinkage of the actin cytoskeleton can be observed by immunofluorescence staining. it is necessary to investigate whether the

Pd2Sn@GOx-SP plus laser induces disulfide bond formation between actin proteins.

Disulfidptosis is inhibited by reducing agents, such as dithiothreitol (DTT), β -mercaptoethanol (2ME), and tris-(2-carboxyethyl)-phosphine (TCEP). Is the Pd2Sn@GOx-SP plus laser-induced cell death reduced by these reducing agents?

Selective toxicity is very important in cancer drugs. In this respect, it is interesting that Pd2Sn@GOx-SP plus laser killed CT26 cells but not L929 cells. However, the mechanism of this selective toxicity is not clear. Is the selective toxicity of Pd2Sn@GOx-SP plus laser observed with other cancer cell lines and normal cells?

If the authors believe that high expression of SLC7A11 determines the induction of cell death by Pd2Sn@GOx-SP plus laser, this needs to be experimentally demonstrated. The sensitivity of multiple cancer cell lines and normal cells, including L929 cells, to Pd2Sn@GOx-SP plus laser should be tested, and the expression levels of SLC7A11 should be compared between these cells to see if there is a correlation between SLC7A11 expression levels and cell death induction.

The authors should provide the source data for Fig. 4k. Please show uncropped scans of these immunoblots along with molecular weight markers. This is the standard way to rigorously evaluate the results of immunoblots, especially in high impact journals. GSDMD and GSDMD-N should be shown in the same slit, and if the exposure times are different between them, then both uncropped images should be presented; likewise for Caspase-1 and C-Caspase-1. Typically, when GSDMD and caspase-1 are proteolytically activated, their proforms are reduced, but this is not the case in Fig. 4k. The authors must address this comment.

Does Pd2Sn@GOx-SP plus laser have a therapeutic effect in tumor models other than CT26?

The expression of IFN- γ in CD8 T cells is examined in Fig. 6, but the expression level of granzyme B in CD8 T cells is also a good marker of the activation of anti-cancer immunity. Also, if depletion of CD8 T cells reduces the therapeutic effect of Pd2Sn@GOx-SP plus laser,

it will be more clearly shown that this treatment involves the activation of anti-cancer immunity.

This manuscript does not fully explain the laser used in the experiments. In Fig. 2, the laser power is mentioned. However, there is no description of the laser power in the in vivo experiments. This manuscript also lacks a description of what equipment was used to generate the laser in each experiment and what wavelength was used.

Fig. 4 shows G1-5, but these are only explained in Fig. 5 (page 15 lines 13-14).

Fig. 4b, which hides values around 0, is not appropriate for a scientific paper.

Reviewer #5 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports as part of the Nature Communications initiative to facilitate training in peer review and appropriate recognition for co-reviewers.

The reviewers gave very valuable comments on our manuscript, and we would like to take this opportunity to express our great appreciation for him/her as well as the comments. The following are the changes made in the new version together with our responses to the reviewers' comments.

REVIEWER COMMENTS:

Reviewer #1:

(Remarks to the Author): with expertise in nanomedicine for cancer therapy

Comments:

This work reported the preparation of Pd₂Sn intermetallics and then integrated with GOx for self-reinforced ROS storm and thus Caspase-1-dependent pyroptosis and cystine-mediated disulfidptosis. The procedure also reprogrammed the immune microenvironment for cancer multiple-modality therapy and immunotherapy. Pd₂Sn exhibited the peroxidase- and oxidase-mimic activities, in combination with the activity of GOx, so cascade biocatalysis was realized to improve the therapy efficiency. The composites were well prepared and characterized with abundant data and profound analysis, while their bioactivity was illustrated carefully. I suggested major revision for the paper before publication in Nature Communications.

1. The full name of “SP” in (Pd₂Sn@GOx-SP) should be added at its first time appearance.

Answer: Firstly, we would like to express our great appreciation for you as well as your comments. We have added the full name of “SP” at its first time appearance. (For details please see page 1, line 18, and page 3, line 13 in the revised manuscript)

2. As illustrated with the XPS result, Pd₂Sn contains Pd²⁺, Sn⁴⁺, and Sn²⁺. What is the counter-anion and how to connect between the cations.

Answer: We are grateful for this good question. The existence of Pd²⁺, Sn⁴⁺, and Sn²⁺ could be attributed to the oxidation of surface atoms upon exposure in air for relatively long period, which is agreement with previous studies of intermetallic compounds (*Nat. Commun.* **10**, 4514 (2019); *Science* **348**, 1230 (2015); *Nat. Commun.* **14**, 7414 (2023); *Angew. Chem., Int. Ed.* **60**, 10942 (2021); *Science* **343**, 1339 (2014)). The Pd₂Sn intermetallics contain both metallic and

ionic bonds. The XRD results suggest that the as-prepared product is an ordered intermetallic Pd_2Sn phase structure as a whole. To further investigate the compositional evolution, we analyzed the Pd_2Sn intermetallics with short storage time by XPS analysis. As shown in **Fig. 1i,j**, the majority of the surface Sn was in the oxidized state and the surface Pd was mainly in the metallic state, indicating Sn atoms on the surface are more susceptible to be oxidized than the Pd atoms. In addition, Pd in Pd_2Sn intermetallics would exhibit a certain degree of electropositivity because of the higher electronegativity of Sn (*Nat. Commun.* **15**, 1626 (2024)). Therefore, the Pd_2Sn intermetallics contain Pd^{2+} , Pd^0 , Sn^{4+} , Sn^{2+} , and Sn^0 , which is beneficial for generating more ROS through the redox reaction. (For details please see page 4, lines 21-34, and Fig. 1i,j in the revised manuscript)

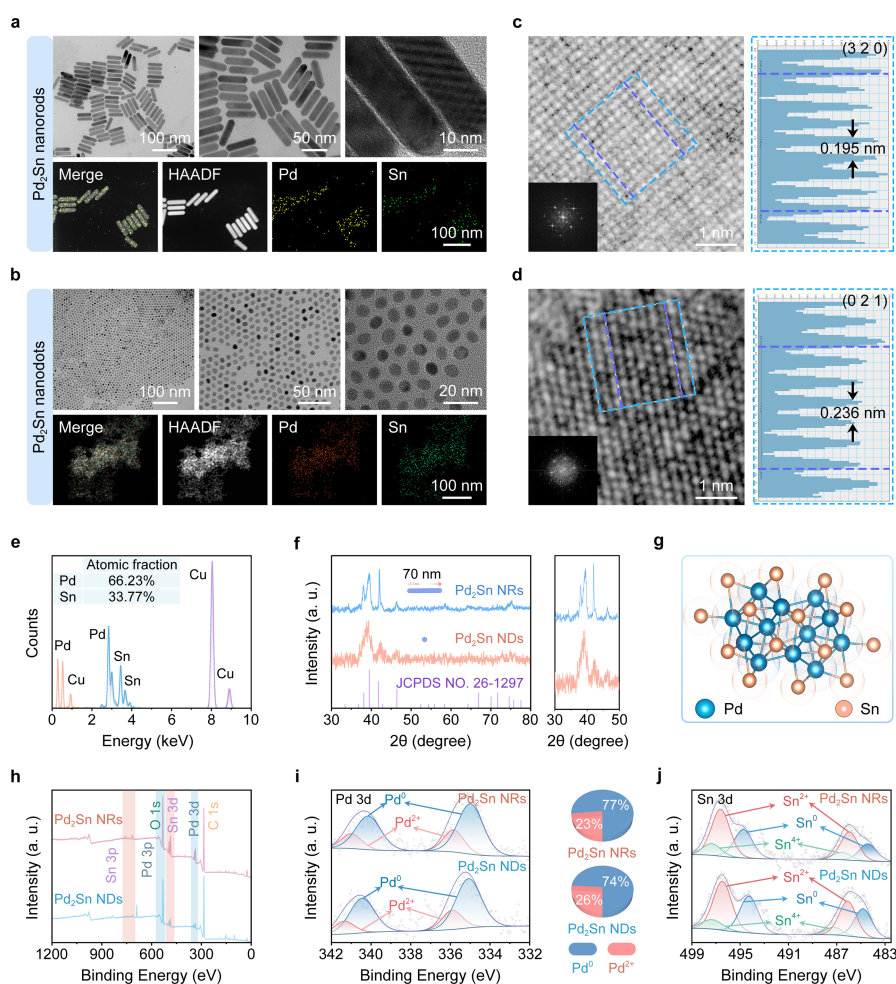


Fig. 1 Structural and Compositional Characterization. TEM images at different magnifications and corresponding elemental mapping of **a** Pd_2Sn NRs and **b** Pd_2Sn NDs. High-resolution TEM images and lattice spacing measurement of **c** Pd_2Sn NRs and **d** Pd_2Sn NDs. **e**

EDS spectrum of Pd₂Sn NRs. **f** PXRD patterns, **g** crystal structure, **h** XPS survey spectra, and **i, j** high-resolution XPS spectra of Pd 3d and Sn 3d for Pd₂Sn NRs and Pd₂Sn NDs.

3. Less references were cited in some parts of Result section abnormally.

Answer: Great thanks for your kind comments. We have added some relevant references in the parts of the Result section to support our conclusion. For example, palladium exhibits the peculiar localized surface plasmon resonance (LSPR) effect and various enzyme catalytic activity, which has made a profound study (*Science* **352**, 797 (2016); *Angew. Chem., Int. Ed.* **58**, 8794-8798 (2019); *J. Am. Chem. Soc.* **142**, 5649-5656 (2020); *Adv. Mater.* **34**, 2202609 (2022)); the tumor cells exhibited high levels of SLC7A11, which could import cystine to promote GSH synthesis and resist pyroptosis (*Sci. Adv.* **9**, eadi5192 (2023)). (For details please see references 27-32, 35-37, 41-45, 50, 56, 59, 60, and 63 in the revised manuscript)

4. “As a noble metal, palladium exhibits the peculiar localized surface plasmon resonance (LSPR) effect and various enzyme catalytic activity, which has made a profound study” and others: some references should be cited.

Answer: According to your suggestion, we added some relevant references (*Science* **352**, 797 (2016); *Angew. Chem., Int. Ed.* **58**, 8794-8798 (2019); *J. Am. Chem. Soc.* **142**, 5649-5656 (2020); *Adv. Mater.* **34**, 2202609 (2022)) to support the statement for “As a noble metal, palladium exhibits the peculiar localized surface plasmon resonance (LSPR) effect and various enzyme catalytic activity, which has made a profound study” in the section of photothermal and multi-enzymatic catalytic activity evaluation. (For details please see page 5, lines 18-19, references 29-32, and others in the revised manuscript)

5. Page 6-7 and 7-8: the paragraph is too long within two pages.

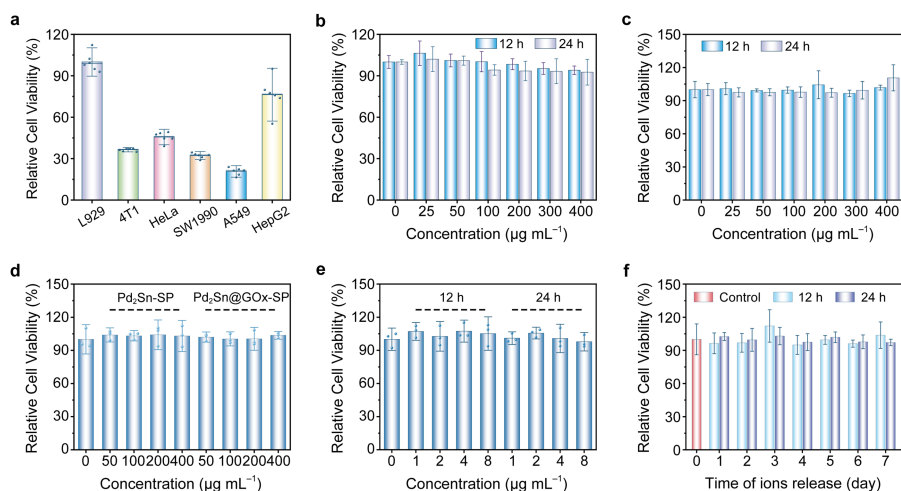
Answer: We have made appropriate adjustments to the length of the mentioned paragraph to make it easier for readers to read and understand. (For details please see pages 6-9 in the revised manuscript)

6. Similarly, each paragraph in Result section is too long.

Answer: We have made appropriate adjustments to the length of the paragraph in the Result section to make it easier for readers to read and understand. (For details please see the Result section in the revised manuscript)

7. “both Pd₂Sn-SP and Pd₂Sn@GOx-SP were cytotoxic to CT26 cells”: How to the safety of the composites to the normal cells and why non-cytotoxic is to the normal cells as Pd and Sn are heavy metals.

Answer: Thank you for your professional question. To begin with, the tumor microenvironment has many distinctive characteristics that are different from normal cells, such as hypoxia, mild acidity, and overproduced hydrogen peroxide (H₂O₂). On the one hand, H₂O₂ could be decomposed into toxic reactive oxygen species (ROS) for killing tumor cells, realizing tumor growth inhibition. On the other hand, H₂O₂ also could be decomposed into O₂ to relieve tumor hypoxia, inhibiting tumor proliferation and enhancing the therapeutic effects. Thus, the Pd₂Sn-SP and Pd₂Sn@GOx-SP nanocomposites could catalyze H₂O₂ to generate ROS and O₂ after endocytosis into tumor cells, achieving significant tumor cell killing. On the contrary, when engulfed by normal cells, the Pd₂Sn-SP and Pd₂Sn@GOx-SP nanocomposites have no toxicity and could be eliminated by cell metabolism (*Nat. Commun.* **12**, 745 (2021); *Nat. Commun.* **15**, 1626 (2024)). To prove the selective cytotoxicity of nanocomposites on tumor cells and normal cells, we employed L929 cells as normal cells and compared their response to the cytotoxic effect of nanocomposites. Our findings revealed that the nanocomposites showed concentration-dependent cytotoxic effects only on CT26 cells, while no significant toxic side effect on L929 cells (**Fig. 4b** and Supplementary Fig. 20b,d). Furthermore, we also used 3T3 cells (mouse embryonic fibroblasts) as another normal cell to measure the bio-compatibility of Pd₂Sn@GOx-SP by standard MTT assay. Similarly, Pd₂Sn@GOx-SP exhibited almost non-cytotoxicity on 3T3 cells after co-incubation of 12 and 24 h, showing good biocompatibility even at higher concentrations (Supplementary Fig. 20c). Meanwhile, the influence of Sn and Pd ions leaking on L929 cells was also investigated through MTT assay by co-culturing the released Sn and Pd ions with L929 cells. The results showed that there was no obvious biotoxicity on L929 cells (Supplementary Fig. 20f), indicating the high biosafety of Pd₂Sn@GOx-SP. Thus, the nanocomposites exhibited negligible cytotoxicity on normal cells without laser irradiation, demonstrating high selective toxicity. (For details please see Fig. 4b, page 10, lines 21-33, and page 11, lines 1-3 in the revised manuscript, Supplementary Fig. 20b-d and Supplementary Fig. 20f in Supplementary Information)



Supplementary Fig. 20. *In vitro* biosafety and toxicity analysis. **a** Relative cell viability of multiple cell lines after being treated with Pd₂Sn@GOx-SP plus laser. Data are expressed as mean $\pm$ S.D. (n = 6). Relative cell viability of **b** L929 and **c** 3T3 cells after incubation with Pd₂Sn@GOx-SP for 12 and 24 h. Data are expressed as mean $\pm$ S.D. (n = 3). **d** Relative cell viability of L929 cells incubated with Pd₂Sn-SP and Pd₂Sn@GOx-SP at various concentrations for 24 h. Data are expressed as mean $\pm$ S.D. (n = 3). **e** Relative cell viability of L929 cells after incubation with TOP at different concentrations for 12 and 24 h. Data are expressed as mean $\pm$ S.D. (n = 3). **f** Relative cell viability of L929 cells after incubation with Sn and Pd ions released from Pd₂Sn@GOx-SP. Data are expressed as mean $\pm$ S.D. (n = 3).

8. DFT should be defined as “density functional theory” when appearing for the first time. OXD should be defined as “oxidase” when appearing for the first time.

Answer: Great thanks for your kind suggestion. We have added the definitions of DFT and OXD as “density functional theory” and “oxidase” when appearing for the first time. (For details please see page 1, line 21, page 3, line 16, and page 7, line 9 in the revised manuscript) 9. Please add the indentation for the first line. The format other paragraph in SI needs to be also readjust.

Answer: Great thanks for your kind comments. We have added the indentation for the first line and the format other paragraph in the Supplementary Information have been readjusted. (For details please see the revised manuscript and Supplementary Information)

10. In page 4, line 10, Please add “nm” after 0.195.

Answer: We have added “nm” after 0.195. (For details please see page 4, line 10 in the revised manuscript)

11. It is better to correct the abbreviation of “blood oxygen saturation” as acknowledged “SaO₂”.

Answer: We have corrected the abbreviation of “blood oxygen saturation” as acknowledged “SaO₂”. (For details please see page 16, line 11 in the revised manuscript, Supplementary Fig. 24b, page 6, line 25, and page 33, line 3 in Supplementary Information)

12. In page 14, line 34, “in vivo behavior” should be replaced by “biodistribution assessment”, since in vivo behavior refers to sophisticated animal ethology method such as rotarod test, open field test, etc.

Answer: Based on your suggestion, we have replaced “in vivo behavior” with “biodistribution assessment”. (For details please see page 17, line 2 in the revised manuscript)

13. There are two Figure 4c in Fig. 4, the lower one should be Figure 4e, please carefully check.

Answer: We have carefully checked Fig. 4 and found that there was only one Figure 4c. To avoid this misunderstanding, we have adjusted the clarity of the Figure. (For details please see Fig. 4 in the revised manuscript)

14. In Fig. 4, Supplementary Fig. 16 and other similar Figures, the corresponding treatments of G1-G5 should be clearly identified in Figure caption to make it easy to read.

Answer: As suggested, we have clearly identified the G1-G5 with the corresponding treatments in Figure caption to make it easy to read. For example, CLSM images of intracellular e O₂ generation and f ROS level after various treatments (G1, control; G2, laser; G3, Pd₂Sn-SP; G4, Pd₂Sn@GOx-SP; G5, Pd₂Sn@GOx-SP + laser). (For details please see Fig. 4, Fig. 5, and Fig. 6 in the revised manuscript, Supplementary Fig. 21-23, and Supplementary Fig. 27 in Supplementary Information)

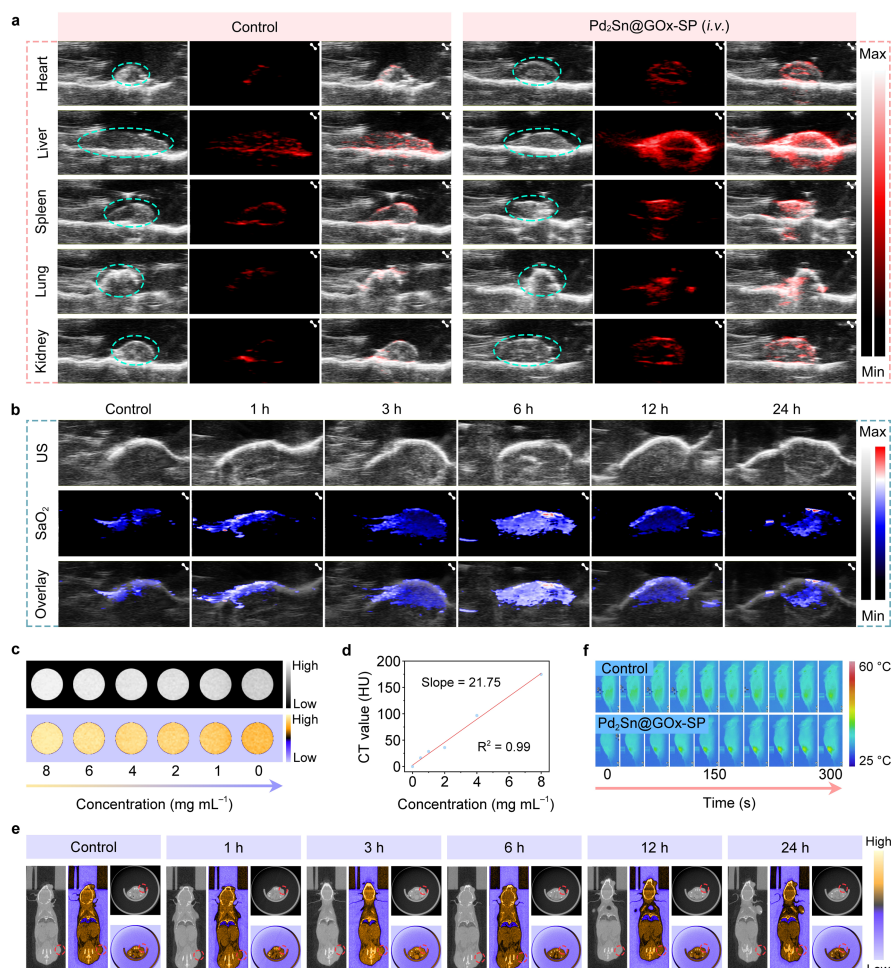
15. In Supplementary Fig. 19f, there is a scale mistake in color bar, red should mean the high temperature, while blue means low temperature.

Answer: Great thanks for your kind comments. We have modified the color bar in Supplementary Fig. 24f. (For details please see Supplementary Fig. 24f in Supplementary Information)

16. I feel confused for Supplementary Fig. 19e, the tumor of control group located in the left side, while the tumor of 3, 6, 12, 24 h located in the right side with different morphology and size. Apparently, the CT imaging of mouse injected with Pd₂Sn-SP at different timepoints should be performed on a same tumor-bearing mouse. Meanwhile, please check the i.v.

[injection is Pd₂Sn@GOx-SP or Pd₂Sn-SP, there is description contradict in your Figure caption of Supplementary Fig. 19. Another suggestion: using circle to highlight the tumor region.](#)

Answer: We are grateful for this good question. We performed CT imaging on the same tumor-bearing mouse at different time points after being injected with Pd₂Sn@GOx-SP. Within 6 h, the CT density in the tumor region of the mice *i.v.* injected with Pd₂Sn@GOx-SP were markedly enhanced with the injection time extension (Supplementary Fig. 24e), which showed the same trend as PA imaging, suggesting the efficient tumor accumulation of Pd₂Sn@GOx-SP. The administration of Pd₂Sn@GOx-SP intravenously into the tumor-bearing mouse to perform CT imaging, and we further modified the figure caption of Supplementary Fig. 24. Meanwhile, the tumor regions were circled to highlight the tumor. (For details please see Supplementary Fig. 24 in Supplementary Information)



Supplementary Fig. 24. Imaging performance evaluation of Pd₂Sn@GOx-SP. **a** PA images of various organs collected from the representative mice before and after 6 h of injection. **b** *In vivo* PA images of SaO₂ in the tumor region after *i.v.* injected with Pd₂Sn@GOx-SP at different

time intervals. **c** *In vitro* CT pseudocolor images of Pd₂Sn@GOx-SP at different concentrations. **d** CT values of Pd₂Sn@GOx-SP at different concentrations. **e** *In vivo* coronal and transverse sections of tumor area after *i.v.* injected with Pd₂Sn@GOx-SP at different time intervals. The left and right columns show pseudo-color images and grayscale images, respectively. **f** Time-dependent *in vivo* thermal imaging of mice at the tumor region after *i.v.* injected with PBS or Pd₂Sn@GOx-SP.

Reviewer #2:

(Remarks to the Author): with expertise in nanomedicine for cancer therapy

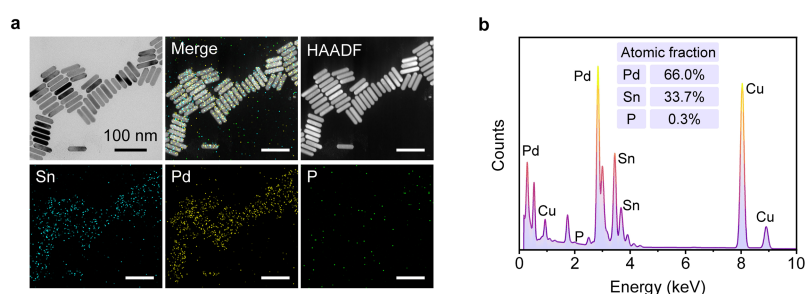
Comments:

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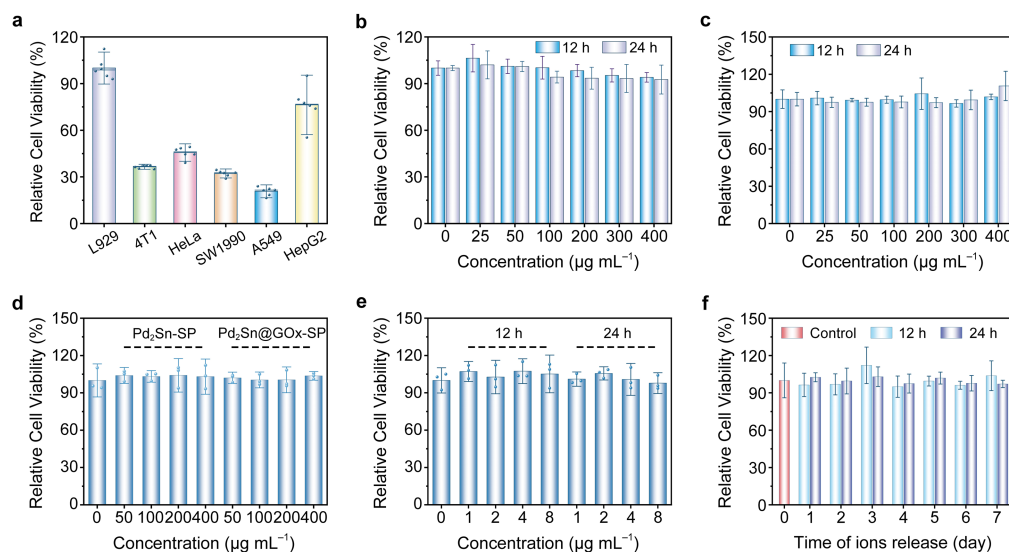
1. During the synthesis of intermetallic Pd₂Sn, TOP is used as surfactant to stabilize the nanoparticles. The phosphorus atom in TOP is well-known to form very strong bond interaction with Pd atoms (Pd-P bond), thus it is very difficult to completely remove the TOP on nanoparticle surface. If TOP is still on surface, there will be two issues: i) the bio-toxicity of P from TOP; ii) The TOP will greatly hamper the following surface modifications of SP and GOx. So, I suggest the authors give more details about how they effectively remove the TOP (give experimental evidences). If TOP is still on surface, please evaluate the toxicity of TOP.

Answer: Firstly, we would like to express our great appreciation for you as well as your

comments. As mentioned above, when TOP was used as a surfactant, the TOP would coordinate on the surface of nanoparticles due to the coordination potency of phosphine ligands (*Angew. Chem.* **124**, 11927 (2012)). The corresponding elemental mapping, EDS spectrum, and Fourier transform infrared spectrum (FT-IR) showed that the surface of Pd₂Sn intermetallics interacted with TOP due to the coordination potency of phosphine ligands (Supplementary Fig. 4,5a). Significantly, the content of TOP on the surface is very low compared with the Pd and Sn. The bio-toxicity of Pd₂Sn-SP and Pd₂Sn@GOx-SP on normal cells was evaluated by standard MTT assay. Our findings revealed that the nanocomposites have no significant toxic effect on L929 cells (Supplementary Fig. 20b,d). Furthermore, we also used 3T3 cells as another normal cell to measure the bio-compatibility of Pd₂Sn@GOx-SP by standard MTT assay. Similarly, Pd₂Sn@GOx-SP exhibited almost non-cytotoxicity on 3T3 cells after co-incubation of 12 and 24 h, showing good biocompatibility even at higher concentrations (Supplementary Fig. 20c). To further evaluate the bio-toxicity of P from TOP, we co-cultured the L929 cells with TOP at different concentrations. As shown in Supplementary Fig. 20e, the TOP exhibited no significant toxic side effect on L929 cells in the concentration range of 0 – 8 µg/mL, demonstrating the negligible bio-toxicity of P from TOP. The successful functionalization of SP and modification of GOx were confirmed by the Fourier transform infrared spectrum (Supplementary Fig. 5b). Thermogravimetric analysis (TGA) confirmed that the proportion of SP on Pd₂Sn-SP nanocomposites was approximately 20.81% (Supplementary Fig. 6). In addition, the zeta potentials showed obvious changes from 0.13 mV to –29.93 mV after the functionalization of SP, and the zeta potential of Pd₂Sn@GOx-SP was further changed to –23.0 mV (Supplementary Fig. 7a). Therefore, the bio-toxicity of P from TOP could be ignored and the surface modifications of SP and GOx could be achieved on the Pd₂Sn intermetallics. (For details please see page 4, line 34, page 5, lines 1-15, and page 10, lines 27-32 in the revised manuscript, Supplementary Fig. 4-7 and Supplementary Fig. 20b-e in Supplementary Information)



Supplementary Fig. 4. Characterization of Pd₂Sn NRs. **a** TEM image and corresponding elemental mapping of Pd₂Sn NRs. **b** EDS spectrum of Pd₂Sn NRs.



Supplementary Fig. 20. *In vitro* biosafety and toxicity analysis. **a** Relative cell viability of multiple cell lines after being treated with Pd₂Sn@GOx-SP plus laser. Data are expressed as mean ± S.D. (n = 6). Relative cell viability of **b** L929 and **c** 3T3 cells after incubation with Pd₂Sn@GOx-SP for 12 and 24 h. Data are expressed as mean ± S.D. (n = 3). **d** Relative cell viability of L929 cells incubated with Pd₂Sn-SP and Pd₂Sn@GOx-SP at various concentrations for 24 h. Data are expressed as mean ± S.D. (n = 3). **e** Relative cell viability of L929 cells after incubation with TOP at different concentrations for 12 and 24 h. Data are expressed as mean ± S.D. (n = 3). **f** Relative cell viability of L929 cells after incubation with Sn and Pd ions released from Pd₂Sn@GOx-SP. Data are expressed as mean ± S.D. (n = 3).

2. Generally speaking, though the intermetallic nanostructure is more stable than the alloyed ones, the authors should provide direct experimental evidence showing that the alloyed Pd₂Sn is indeed less active and less stable than the intermetallic Pd₂Sn. Maybe the alloyed Pd₂Sn is already good enough in such biological antitumor therapy application.

Answer: Great thanks for your kind comments. To begin with, the intermetallics have well-defined atomic structures. Unlike random alloys whose atomic arrangements are largely unpredictable, intermetallics have precisely determined crystallographic structures that lead to uniform active sites (*ACS Catal.* **8**, 3237 (2018)). In addition, the structure transformation from disordered to ordered structures at high temperatures is spontaneous, and the change in entropy for ordering is negative. Thus, the formation of intermetallic nanostructure is driven by enthalpy.

Hence, from a thermodynamic perspective, the ordered intermetallics may be more stable than disordered alloys. Compared with disordered alloys, the ordered intermetallics have stronger interaction and thus exhibit stronger electronic or strain effects, which may be conducive to the enhancement of catalytic performance. Significantly, the intermetallics have higher stability and catalytic activity than disordered alloys, which have been confirmed in many works (*RSC Adv.* **4**, 27510 (2014); *Nano Lett.* **16**, 2560 (2016); *Angew. Chem., Int. Ed.* **55**, 9030 (2016); *Chem. Mater.* **27**, 7538 (2015)). Compared with the disordered phase, the ordered PdCu (B2-type) phases exhibited a lower overpotential and a higher oxygen reduction/evolution kinetic rate, which contributed to the remarkable enhancement of cyclic stability and a superior rate capability in Li–O₂ battery (*Energy Environ. Sci.* **7**, 1362 (2014)). Furthermore, ordered Pd₃Fe intermetallic has been reported to exhibit significantly enhanced activity and durability toward ORR and Li-air full-cell cycling performance (*J. Am. Chem. Soc.* **137**, 7278 (2015)). Based on the unique performance of intermetallics, we developed Pd₂Sn intermetallics to elicit potent antitumor immune responses by pyroptosis and disulfidptosis. As for the mentioned alloyed Pd₂Sn, the random distribution of the atoms makes its catalytic efficiency low, and it is difficult to calculate by density functional theory calculations. Maybe the alloyed Pd₂Sn could also be used for biological antitumor therapy applications, but the antitumor efficiency is also worth considering. In this work, our main objective is to compare the performance of intermetallics with different morphologies. Such direct comparisons between alloy and intermetallic of the same composition are rare in the literature, and it is much more common to compare alloy/intermetallic with different sizes, element ratios, or shapes (*Chem. Rev.* **121**, 736 (2021)). Therefore, Pd₂Sn intermetallic compounds with various morphologies (including NDs and NRs with different lengths) were constructed and the properties were further contrasted by both *in vitro* experiments and DFT calculations, rather than compared with alloyed Pd₂Sn.

3. It is very wired that there is more Pd²⁺ than metallic Pd⁰ in Pd₂Sn, and no metallic Sn⁰ in Pd₂Sn at all in their XPS measurement. This XPS result means their Pd₂Sn NPs and NRs are almost oxidized and very unstable in air, which is contradictory to the statement that intermetallic Pd₂Sn is stable.

Answer: We are grateful for this good question. The existence of Pd²⁺, Sn⁴⁺, and Sn²⁺ could be attributed to the oxidation of surface atoms upon exposure to air for relatively long period,

which is in agreement with previous studies of intermetallic compounds (*Nat. Commun.* **10**, 4514 (2019); *Science* **348**, 1230 (2015); *Nat. Commun.* **14**, 7414 (2023); *Angew. Chem., Int. Ed.* **60**, 10942 (2021); *Science* **343**, 1339 (2014)). To further investigate the compositional evolution, we analyzed the Pd₂Sn intermetallics with short storage time by XPS analysis. As shown in **Fig. 1i,j**, the majority of the surface Sn was in the oxidized state and the surface Pd was mainly in the metallic state, indicating Sn atoms on the surface are more susceptible to be oxidized than the Pd atoms. In addition, Pd in Pd₂Sn intermetallics would exhibit a certain degree of electropositivity because of the higher electronegativity of Sn (*Nat. Commun.* **15**, 1626 (2024)). The XRD results suggest that the as-prepared product is an ordered intermetallic Pd₂Sn phase structure as a whole. It is worth noting that the stability of intermetallic compounds is higher than that of alloys, and the stability of the intermetallics depends on the particular bimetallic combination (*ACS Catal.* **7**, 735 (2017)). The difference in the stability of intermetallics may be attributed to the intrinsic oxygen philicity of the second metal. Therefore, intermetallics have achieved excellent stability compared with alloys, but they are still far from practical applications. In future work, we will further investigate how to improve the stability of intermetallic compounds, including increasing the interaction between the carrier/coating layer and the ordered intermetallic, forming core-shell structure, and doping modification. (For details please see page 4, lines 21-34, and Fig. 1i,j in the revised manuscript, Supplementary Fig. 3 in Supplementary Information)

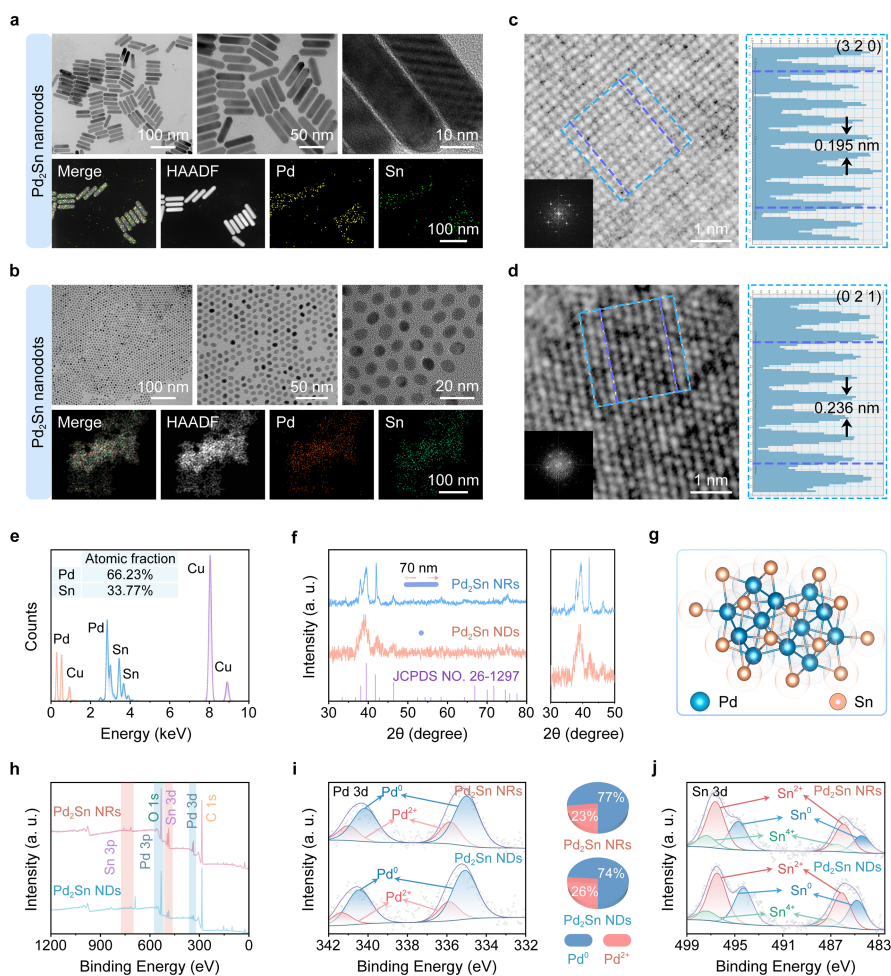
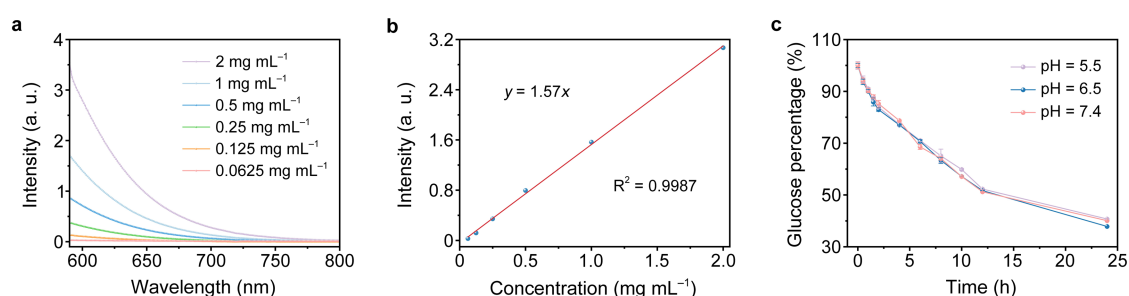


Fig. 1 Structural and Compositional Characterization. TEM images at different magnifications and corresponding elemental mapping of **a** Pd₂Sn NRs and **b** Pd₂Sn NDs. High-resolution TEM images and lattice spacing measurement of **c** Pd₂Sn NRs and **d** Pd₂Sn NDs. **e** EDS spectrum of Pd₂Sn NRs. **f** PXRD patterns, **g** crystal structure, **h** XPS survey spectra, and **i, j** high-resolution XPS spectra of Pd 3d and Sn 3d for Pd₂Sn NRs and Pd₂Sn NDs.

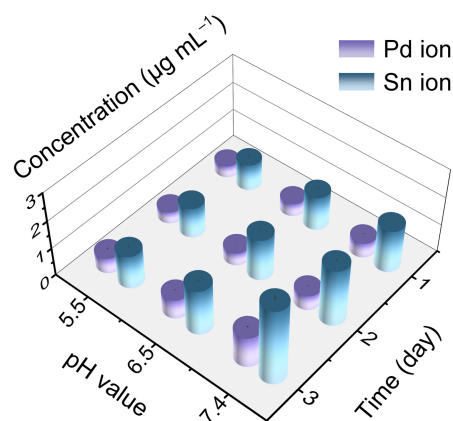
4. The pH became lower when the GOx catalyzing glucose into gluconic acid. So, will the more acidic microenvironment cause the etching and leaking of Sn ions? If yes, will the Sn ions leaking cause the severe biotoxicity? Will the GOx be malfunctioned in a more acidic environment.

Answer: Thank you for your professional question. According to your comment, we further evaluated the influence of pH value on the etching and leaking of Sn and Pd ions by using ICP-OES. As shown in Supplementary Fig. 18, the Sn and Pd ions could be released from Pd₂Sn@GOx-SP, while the amount of ion release was negligible even during 3 days of incubation, which was only 2.73 and 1.10 $\mu\text{g mL}^{-1}$, respectively. To investigate the biotoxicity

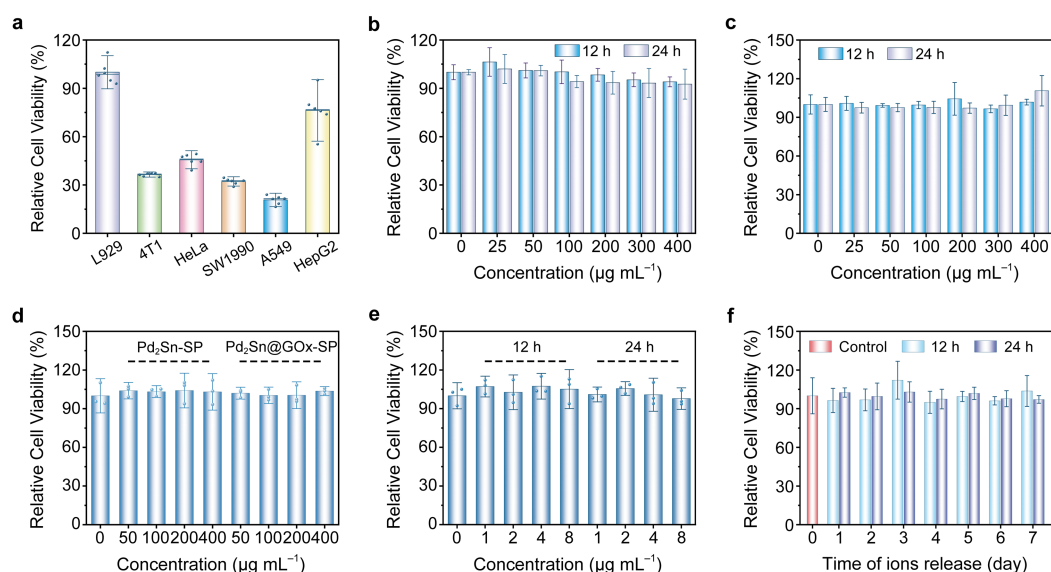
caused by the Sn and Pd ions leaking, we performed the MTT assay by co-culturing the released Sn and Pd ions with L929 cells, respectively. The results showed that there was no obvious biotoxicity on L929 cells (Supplementary Fig. 20f), indicating the high biosafety of Pd₂Sn@GOx-SP. Thus, the nanocomposites exhibited negligible cytotoxicity on normal cells without laser irradiation, demonstrating high selective toxicity. Furthermore, the catalytic activity of GOx was also evaluated in PBS with different pH values to investigate whether the GOx would malfunction in a more acidic environment. The results showed that there was no obvious change in the catalytic performance of GOx in a more acidic environment, demonstrating that the GOx was able to effectively degrade glucose and afford H₂O₂ for the cascade catalytic reaction, and inhibit NADPH supply to induce disulfidptosis (Supplementary Fig. 17). (For details please see page 7, lines 27-34, page 8, lines 1-3, page 10, lines 32-33, and page 11, lines 1-3 in the revised manuscript, Supplementary Fig. 17, Supplementary Fig. 18, and Supplementary Fig. 20f in Supplementary Information)



Supplementary Fig. 17. Glucose oxidase activity. **a** UV-vis-NIR absorption spectra of glucose at different concentrations after being reacted with dinitrosalicylic acid reagent. **b** The fitting curve of glucose concentration and absorbance at 595 nm. **c** Glucose consumption with the change of time at different pH values.



Supplementary Fig. 18. Evaluation of ions release performance. The release of Pd and Sn ions from Pd₂Sn@GOx-SP in PBS with various pH values during the incubation process. Data are expressed as mean $\pm$ S.D. (n = 3).



Supplementary Fig. 20. *In vitro* biosafety and toxicity analysis. **a** Relative cell viability of multiple cell lines after being treated with Pd₂Sn@GOx-SP plus laser. Data are expressed as mean $\pm$ S.D. (n = 6). Relative cell viability of **b** L929 and **c** 3T3 cells after incubation with Pd₂Sn@GOx-SP for 12 and 24 h. Data are expressed as mean $\pm$ S.D. (n = 3). **d** Relative cell viability of L929 cells incubated with Pd₂Sn-SP and Pd₂Sn@GOx-SP at various concentrations for 24 h. Data are expressed as mean $\pm$ S.D. (n = 3). **e** Relative cell viability of L929 cells after incubation with TOP at different concentrations for 12 and 24 h. Data are expressed as mean $\pm$ S.D. (n = 3). **f** Relative cell viability of L929 cells after incubation with Sn and Pd ions released from Pd₂Sn@GOx-SP. Data are expressed as mean $\pm$ S.D. (n = 3).

5. I don't think the DFT calculations support their conclusion. From their DFT calculations, they conclude that the (001) crystal facet possess higher catalytic activity than the (011) crystal facet. But how this can be used to explain why the NRs with moderate length show the best catalytic performance, longer or shorter NRs both decrease the catalytic activity? From their current DFT results, the longer NRs with more (001) facets should have the best catalytic activity, which is inconsistent with their experimental results.

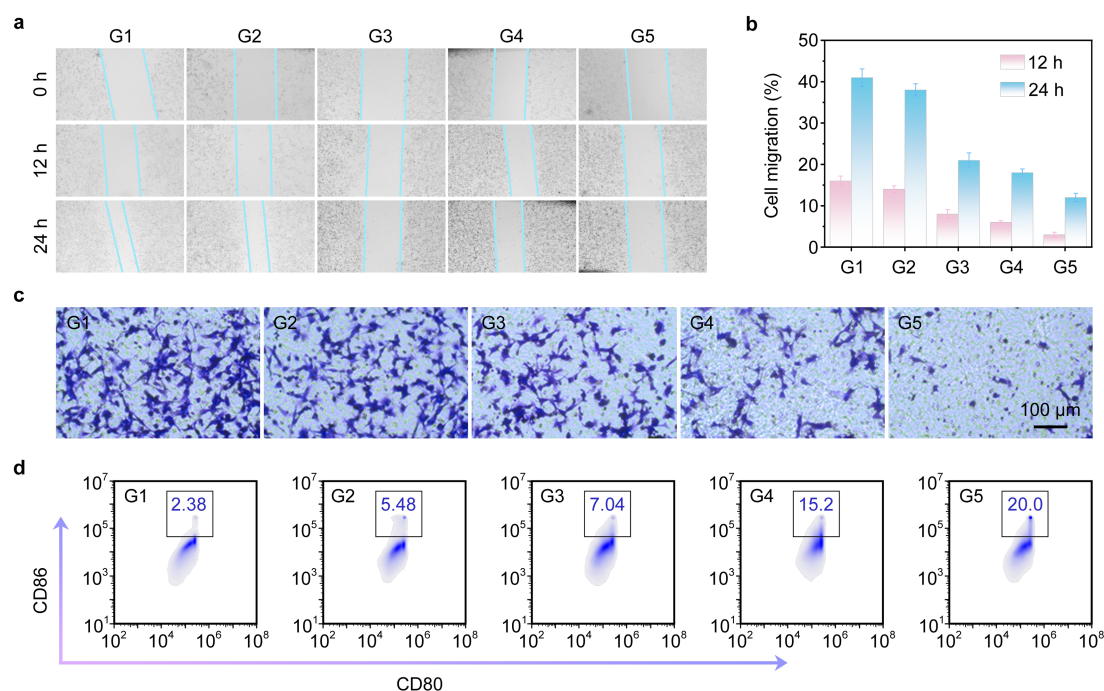
Answer: Great thanks for your kind comments. To begin with, DFT calculations were conducted to confirm the higher catalytic activity of the (001) crystal facet than the (011) crystal facet, and investigate the role of Pd and Sn sites in various crystal facets for CAT-mimic and

POD-mimic activities. DFT results demonstrated that the Pd₂Sn NRs with exposed (001) facets possessed higher catalytic activity than Pd₂Sn NDs with exposed (011) facets, which was consistent with experimental results. Thus, the Pd₂Sn NRs were better at achieving CAT-mimic and POD-mimic activities than Pd₂Sn NDs. The catalytic activity is related to multiple factors, including crystal facet, specific surface area, number of exposed catalytic sites, and *et al* (*J. Am. Chem. Soc.* **145**, 7352 (2023); *Adv. Energy Mater.*, **6**, 1600458 (2016); *Chem. Rev.* **119**, 4357 (2019)). Although the longer NRs possess more (001) facets, the catalytic activity is not proportional to the number of (001) facets. To compare the catalytic activity of Pd₂Sn NRs with various lengths, steady-state kinetic analyses were performed. The Pd₂Sn-SP nanocomposites were mixed with H₂O₂ at various concentrations (1, 2, 4, 8, and 16 mM), and the absorbance change of oxTMB was recorded with time (Supplementary Fig. 13), all of which could align well with the typical Michaelis-Menten kinetics (**Fig. 2j**). Based on the Lineweaver-Burk plots (**Fig. 2k**), the maximum reaction velocity ($V_{\max}$) and Michaelis-Menten constants (K_m) were acquired and summarized in **Fig. 2l**. Generally, a higher $V_{\max}$ and lower K_m lead to better catalytic performance. The results showed that the catalytic efficiency was relevant to the length of Pd₂Sn-SP NRs, neither too long nor too short is beneficial for the POD-mimic activity. Additionally, Pd₂Sn-SP nanocomposites with diverse lengths were employed to further evaluate the catalytic activity by altering the concentrations of Pd₂Sn-SP nanocomposites and detecting the absorption intensity of oxTMB (Supplementary Fig. 14a-d). The specific activity values of Pd₂Sn-SP nanocomposites were calculated (Supplementary Fig. 14e-h) and summarized in Supplementary Fig. 14i. Similarly, the Pd₂Sn-SP NRs showed the supreme POD-mimic catalytic activity than the others. Therefore, Pd₂Sn-SP NRs with moderate length show the best catalytic performance, longer or shorter NRs both decrease the catalytic activity.

6. The migration ability of CT26 cells is suggested to be evaluated by transwell migration assay.

Answer: According to your suggestion, the transwell migration assay of CT26 cells after diverse treatments was performed to evaluate the migration ability of CT26 cells. The cells treated with Pd₂Sn@GOx-SP + laser were greatly repressed (Supplementary Fig. 23c), demonstrating that Pd₂Sn@GOx-SP had a significant inhibitory effect on cell migration. Furthermore, we also examined whether the Pd₂Sn@GOx-SP + laser treatment could alleviate cell migration by wound-healing assays (Supplementary Fig. 23a). Indeed, cell migration was

largely inhibited after treatment with Pd₂Sn@GOx-SP + laser for 12 and 24 h, while the control or only laser group had minimal effect on the cell migration (Supplementary Fig. 23b). Therefore, GOx-induced disulfidptosis combined with Pd₂Sn@GOx-SP-induced pyroptosis greatly promoted tumor cell death and inhibited its migration. (For details please page 15, lines 20-28 in the revised manuscript, Supplementary Fig. 23a-c in Supplementary Information)



Supplementary Fig. 23. Cell migration and immune stimulation analysis. **a** Wound-healing assays and **b** the corresponding quantitative analysis of CT26 cells treated with various formulations (G1, control; G2, laser; G3, Pd₂Sn-SP; G4, Pd₂Sn@GOx-SP; G5, Pd₂Sn@GOx-SP + laser) at different times. **c** The transwell migration assay of CT26 cells after diverse treatments. **d** Flow cytometry profiles of DCs maturation (CD80⁺ and CD86⁺ cells) after different treatments by *in vitro* stimulation maturation experiment. Data are expressed as mean ± S.D. (n = 3).

[7. The images of CT26 cells are suggested to be provided to illustrate the morphology change after the treatment.](#)

Answer: The images of CT26 cells have been provided by the bright-field cell morphology (Supplementary Fig. 22h) and bio-TEM images (**Fig. 4I**) to illustrate the morphology change after diverse treatments. As can be seen in Supplementary Fig. 22h, CT26 cells with the treatment of Pd₂Sn@GOx-SP possessed more distinct swelling (bubbling) features compared with those in the control group, indicating the occurrence of pyroptosis. Meanwhile, Bio-TEM

was also performed to monitor the cells with the different treatments (**Fig. 4l**). It can be observed that the cell membrane of the cells treated with Pd₂Sn@GOx-SP exhibited extensive vacuolization and incomplete cell membranes, and mitochondria were deformed and swollen. These results provide concrete evidence for Pd₂Sn@GOx-SP-induced pyroptosis due to a large amount of ROS generation. (For details please see page 14, lines 9-15, and Fig. 4l in the revised manuscript, Supplementary Fig. 22h in Supplementary Information)

8. The staining images of GSDMD *in vivo* is suggested, which is essential to confirm the pyroptosis process *in vivo*.

Answer: According to your suggestion, the staining images of GSDMD were performed on the slices of tumor tissue collected from the mice with various treatments to confirm the pyroptosis process *in vivo*. Similar to the western blot analysis, the higher expression of GSDMD proteins could be observed in the Pd₂Sn@GOx-SP-related group compared with the control group (**Fig. 5o**), which could support the drilled pores in the cell membrane, demonstrating the occurrence of pyroptosis process *in vivo*. Furthermore, the underlying therapeutic mechanism induced by Pd₂Sn@GOx-SP *via* pyroptosis was also confirmed through the immunohistochemical investigation of NLRP3 and C-Caspase-1. All of them were visibly elevated in the tumor tissues extracted from the mice treated with Pd₂Sn@GOx-SP + laser (Fig. 5o), evidencing the occurrence of pyroptosis of tumor cells. (For details please see page 18, lines 5-8, and Fig. 5o in the revised manuscript)

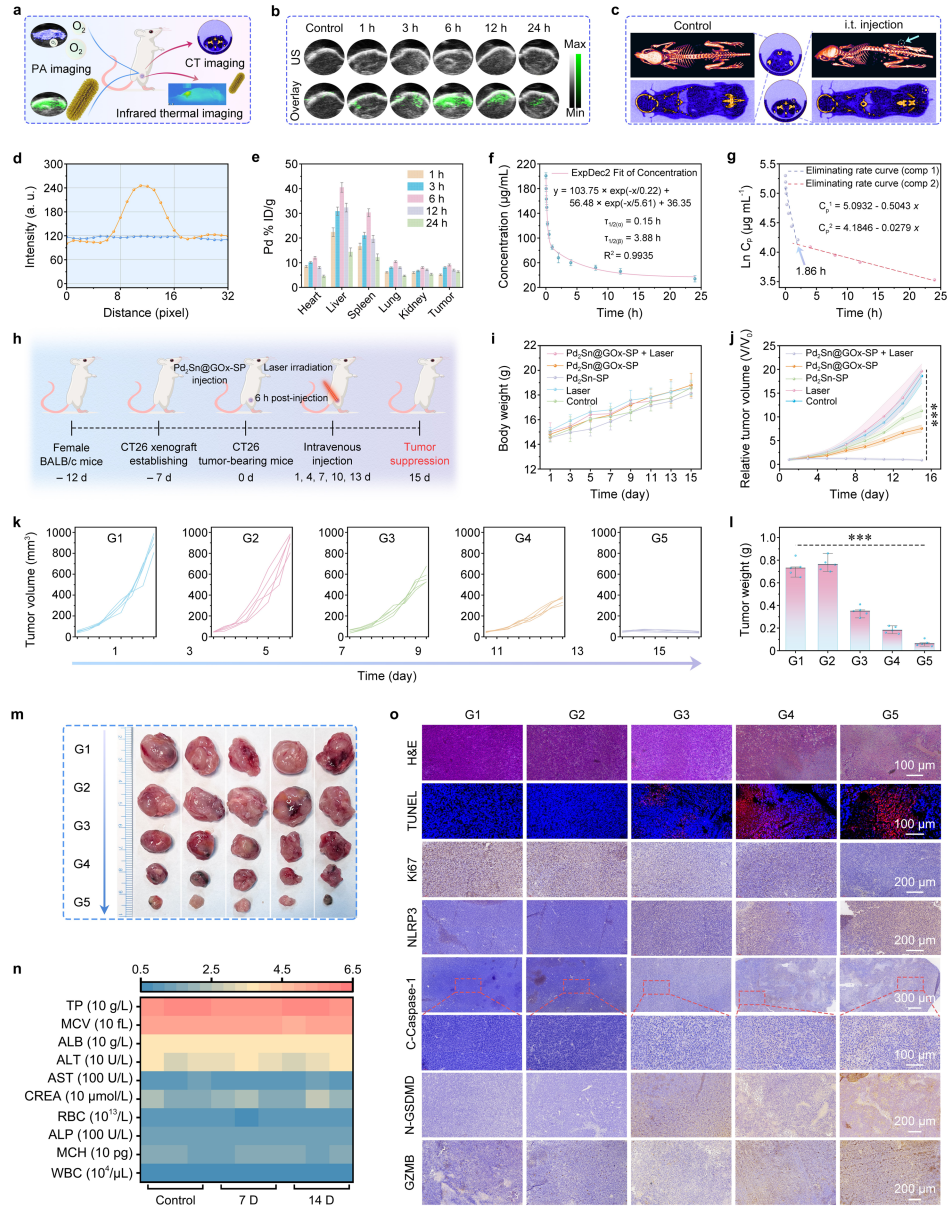


Fig. 5 *In vivo* PA/CT imaging-guided synergistic antitumor therapy. **a** Schematic illustration of the PA, CT, and infrared thermal imaging. Representative *in vivo* **b** PA images after being *i.v.* injected with Pd₂Sn@GOx-SP and **c** CT images after *i.t.* injected without or with Pd₂Sn@GOx-SP. **d** The corresponding line profiles of CT values. **e** *In vivo* biodistribution of Pd₂Sn@GOx-SP in main organs and tumors at various time intervals. **f** The blood circulation curve and **g** the eliminating rate curve after *i.v.* injected with Pd₂Sn@GOx-SP. Data are expressed as mean ± S.D. (n = 3). **h** Therapeutic schedule of Pd₂Sn@GOx-SP for CT26 tumor-bearing mice. **i** Body weight, **j** relative tumor volume, **k** tumor volume, **l** tumor weight, and **m** the photographs of excised tumors from representative mice in different treatment groups (G1, control; G2, laser; G3, Pd₂Sn-SP; G4, Pd₂Sn@GOx-SP; G5, Pd₂Sn@GOx-SP + laser). **n**

Hematological indexes and biochemical data of mice after *i.v.* injection with PBS and Pd₂Sn@GOx-SP. **o** Staining images of H&E, TUNEL, Ki67, NLRP3, C-Caspase-1, GSDMD, and GZMB for tumor slices from different groups. Data are expressed as mean $\pm$ S.D. (n = 5). Statistical significance is assessed by the unpaired Student's two-sided t-test: *** $p < 0.001$.

Reviewer #3:

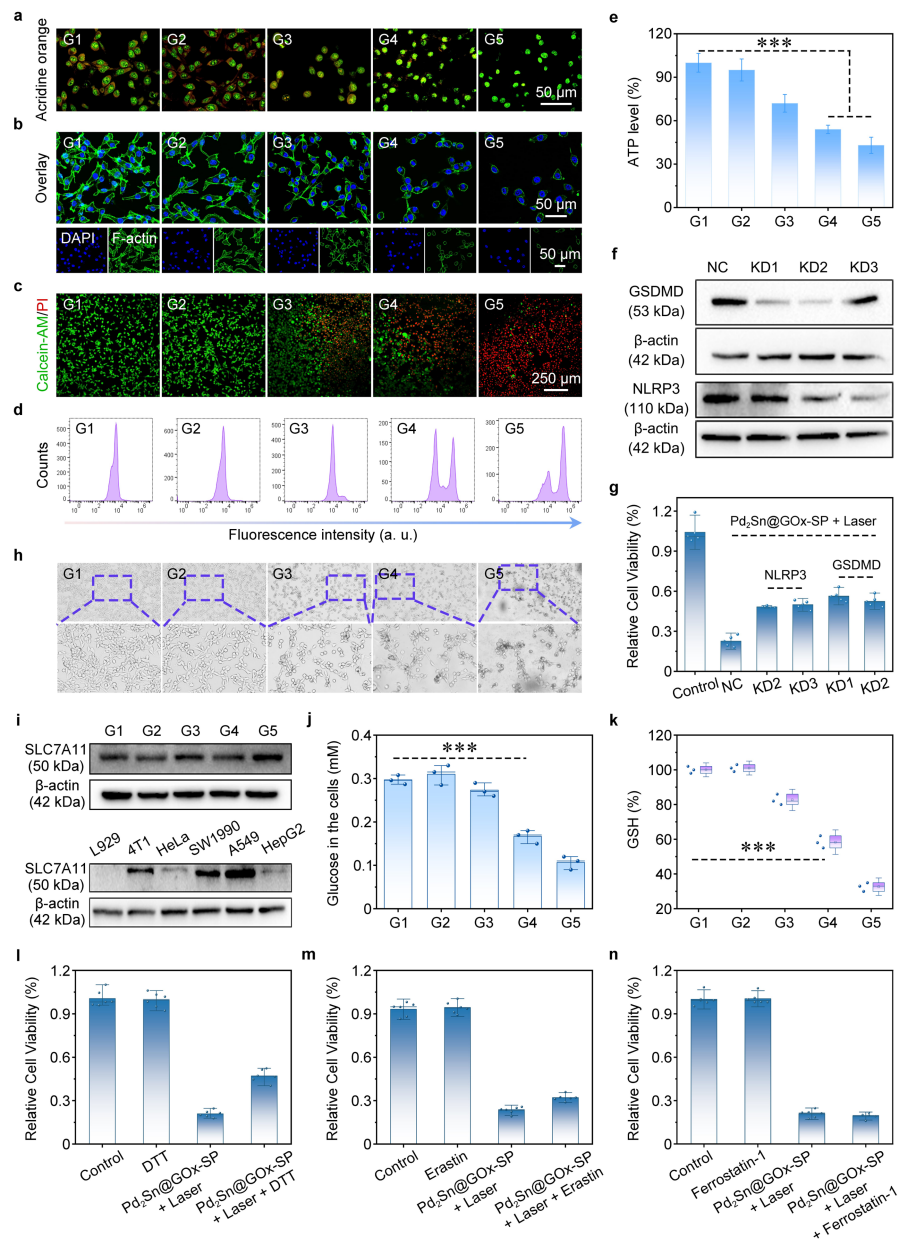
(Remarks to the Author): with expertise in cancer cell death, disulfidptosis

Comments:

This manuscript proposes an interesting and novel approach to activate tumor immunogenicity and reprogram the immunosuppressive microenvironment for cancer immunotherapy by targeting pyroptosis and disulfidptosis. The idea of utilizing intermetallics modified with glucose oxidase (Pd₂Sn@GOx-SP) to induce pyroptosis and further promote disulfidptosis through cystine accumulation is novel and holds the potential to enhance therapeutic effectiveness. They showed that Pd₂Sn@GOx-SP generated abundant ROS and subsequently pyroptotic cell death. Additionally, the authors proved that Pd₂Sn@GOx-SP promotes the consumption of glucose, leading to decreased NADPH production (likely through the pentose phosphate pathway), which impairs the reduction of cystine imported by SLC7A11, disulfide stress and eventually a newly identified form of programmed cell death called “disulfidptosis” in tumor cells. They also demonstrated that Pd₂Sn@GOx-SP-induced pyroptosis and disulfidptosis significantly suppress tumor growth and recurrence. Overall, this work introduces an interesting strategy for designing antitumor agents by inducing pyroptosis and disulfidptosis. I have a few comments and suggestions:

1. Since oxidative stress might also trigger ferroptosis, I'm wondering if Pd₂Sn@GOx-SP generated abundant ROS could induce the occurrence of ferroptosis. Interestingly, glucose deprivation-mediated AMPK was shown to inhibit ferroptosis (PMID: 32029897). Based on this, Pd₂Sn@GOx-SP-mediated glucose consumption might suppress ferroptosis (if any). Ferroptosis might not be shown in this context then. Treatment with ferroptosis inhibitors, such as deferoxamine mesylate (DFO), ferrostatin-1, or liproxstatin-1 would give insight on the role of ferroptosis in the context of this story.

Answer: Firstly, we would like to express our great appreciation for you as well as your comments. To give insight into the role of ferroptosis in this work, the CT26 cells were treated with a ferroptosis inhibitor (Ferrostatin-1), and the inhibitory effects were evaluated after various treatments. The result showed that the CT26 cells treated with Pd₂Sn@GOx-SP + laser also exhibited significant inhibitory effect after the addition of Ferrostatin-1, which was similar to that without addition of Ferrostatin-1, demonstrating the effect of ferroptosis could be ignored (Supplementary Fig. 22n). Therefore, GOx-induced disulfidptosis combined with Pd₂Sn@GOx-SP-induced pyroptosis are the main manner for promoting tumor cell death. (For details please see page 15, lines 15-20 in the revised manuscript, Supplementary Fig. 22n in Supplementary Information)

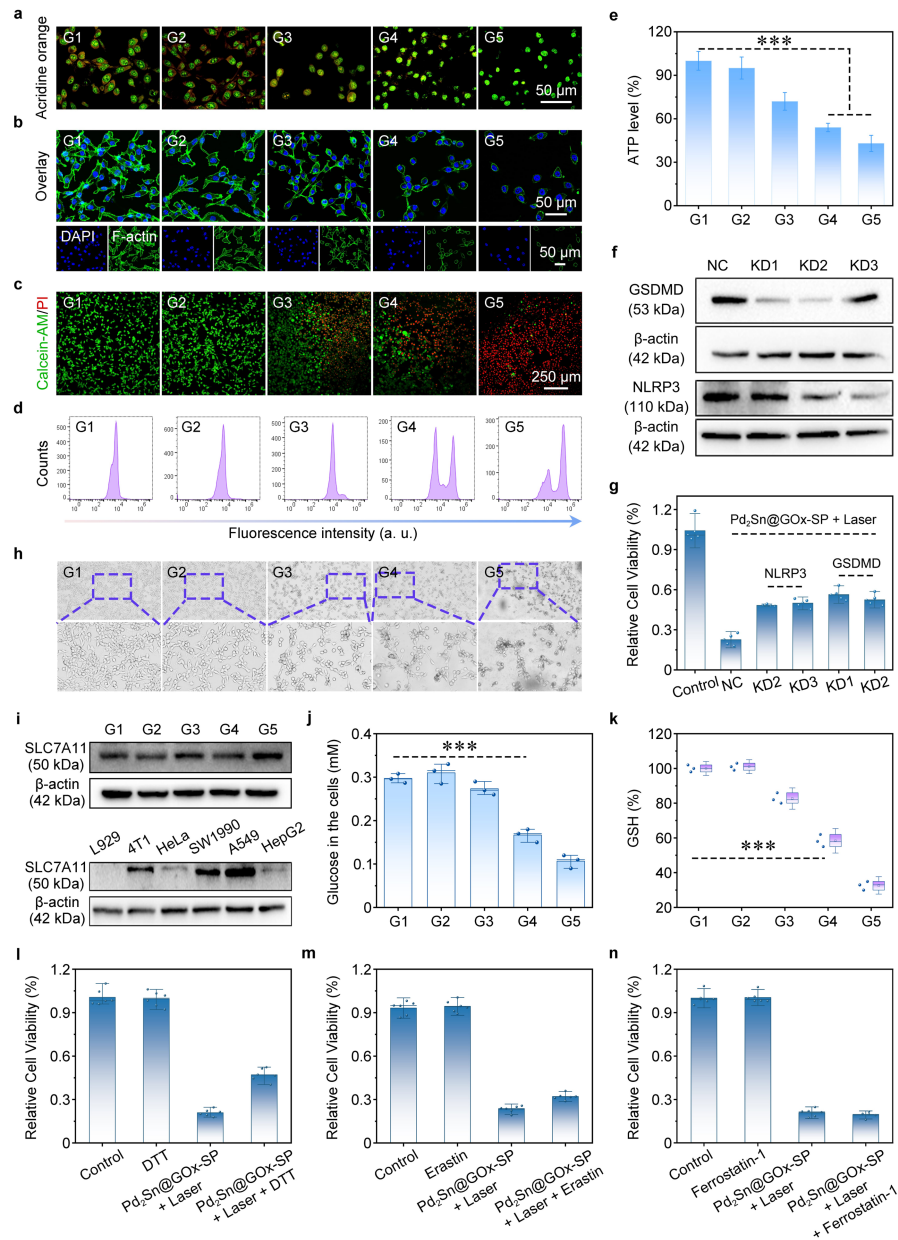


Supplementary Fig. 22. *In vitro* anticancer performance of Pd₂Sn@GOx-SP. **a** Acridine orange staining, **b** F-actin morphology, and **c** Calcein-AM/PI co-staining images of CT26 cells with various treatments (G1, control; G2, laser; G3, Pd₂Sn-SP; G4, Pd₂Sn@GOx-SP; G5, Pd₂Sn@GOx-SP + laser). **d** Flow cytometry analysis of the living and dead cells proportion after various treatments. **e** Intracellular ATP levels after diverse treatments. Data are expressed as mean $\pm$ S.D. (n = 3). **f** Protein levels of NLRP3 and GSDMD in NLRP3- or GSDMD-knockdown CT26 cells by using western bolt analysis. **g** Relative cell viability of NLRP3-knockdown or GSDMD-knockdown CT26 cells treated with Pd₂Sn@GOx-SP + laser. Data are expressed as mean $\pm$ S.D. (n = 5). **h** Bright-field cell morphology after diverse treatments. **i** Protein levels of SLC7A11 in CT26 cells (upper panel) and multiple cell lines (bottom panel). **j** Glucose content and **k** GSH depletion in different treatment groups. Data are expressed as mean $\pm$ S.D. (n = 3). Relative cell viability of CT26 cells upon Pd₂Sn@GOx-SP + laser treatment with the addition of **l** DTT, **m** Erastin, and **n** Ferrostatin-1. Data are expressed as mean $\pm$ S.D. (n = 6). Statistical significance is assessed by the unpaired Student's two-sided t-test: *** $p < 0.001$.

[2. SLC7A11-mediated cystine uptake is a critical factor that induces disulfide stress. The author might need to test if Pd₂Sn@GOx-SP showed limited or impaired ability of inducing cell death in SLC7A11 low cells or SLC7A11 KD/KO cells. Alternatively, removing cystine from the culture medium or treatment of SLC7A11 inhibitor \(erastin, sulfasalazine\) will also provide solid evidence to their conclusion.](#)

Answer: Great thanks for your kind suggestion. To confirm the critical role of SLC7A11-mediated cystine uptake for disulfide stress, the CT26 cells were incubated with Pd₂Sn@GOx-SP plus laser after the treatment of SLC7A11 inhibitor (Erastin). Compared with that without the treatment of Erastin, the cell death rate of CT26 cells exhibited an obvious decrease due to the inhibition of SLC7A11-mediated cystine uptake (Supplementary Fig. 22m), indicating that the disulfide stress originated from the accumulation of cystine mediated by SLC7A11 in SLC7A11 overexpression cancer cells. In addition, the expression levels of SLC7A11 in multiple cancer cells (4T1, HeLa, SW1990, A549, and HepG2 cells) and normal cells (L929 cells) were evaluated by western blot analysis (Supplementary Fig. 22i). The results showed the higher expression level of SLC7A11 in these cancer cells than that in normal cells, leading

to higher cell mortality in cancer cells compared to normal cells with low SLC7A11 expression. (For details please see page 15, lines 5-14 in the revised manuscript, Supplementary Fig. 22i and Supplementary Fig. 22m in Supplementary Information)



Supplementary Fig. 22. *In vitro* anticancer performance of Pd₂Sn@GOx-SP. **a** Acridine orange staining, **b** F-actin morphology, and **c** Calcein-AM/PI co-staining images of CT26 cells with various treatments (G1, control; G2, laser; G3, Pd₂Sn-SP; G4, Pd₂Sn@GOx-SP; G5, Pd₂Sn@GOx-SP + laser). **d** Flow cytometry analysis of the living and dead cells proportion after various treatments. **e** Intracellular ATP levels after diverse treatments. Data are expressed as mean $\pm$ S.D. (n = 3). **f** Protein levels of NLRP3 and GSDMD in NLRP3- or GSDMD-knockdown CT26 cells by using western bolt analysis. **g** Relative cell viability of NLRP3-

knockdown or GSDMD-knockdown CT26 cells treated with Pd₂Sn@GOx-SP + laser. Data are expressed as mean ± S.D. (n = 5). **h** Bright-field cell morphology after diverse treatments. **i** Protein levels of SLC7A11 in CT26 cells (upper panel) and multiple cell lines (bottom panel). **j** Glucose content and **k** GSH depletion in different treatment groups. Data are expressed as mean ± S.D. (n = 3). Relative cell viability of CT26 cells upon Pd₂Sn@GOx-SP + laser treatment with the addition of **l** DTT, **m** Erastin, and **n** Ferrostatin-1. Data are expressed as mean ± S.D. (n = 6). Statistical significance is assessed by the unpaired Student's two-sided t-test: ****p* < 0.001.

[3. There is a typo in the text. In line 31 under Discussion section, cysteine should be cystine. “to the abundant accumulation of cysteine in CT26 cells with high SLC7A11 expression”](#)

Answer: Great thanks for your kind comments. We have modified the cysteine to cystine, which was described as “leading to the abundant accumulation of cystine in CT26 cells with high SLC7A11 expression, achieving cystine-related disulfidptosis”. (For details please see page 21, lines 23-24 in the revised manuscript)

Reviewer #4:

(Remarks to the Author): with expertise in immunology, cancer cell death, pyroptosis

Comments:

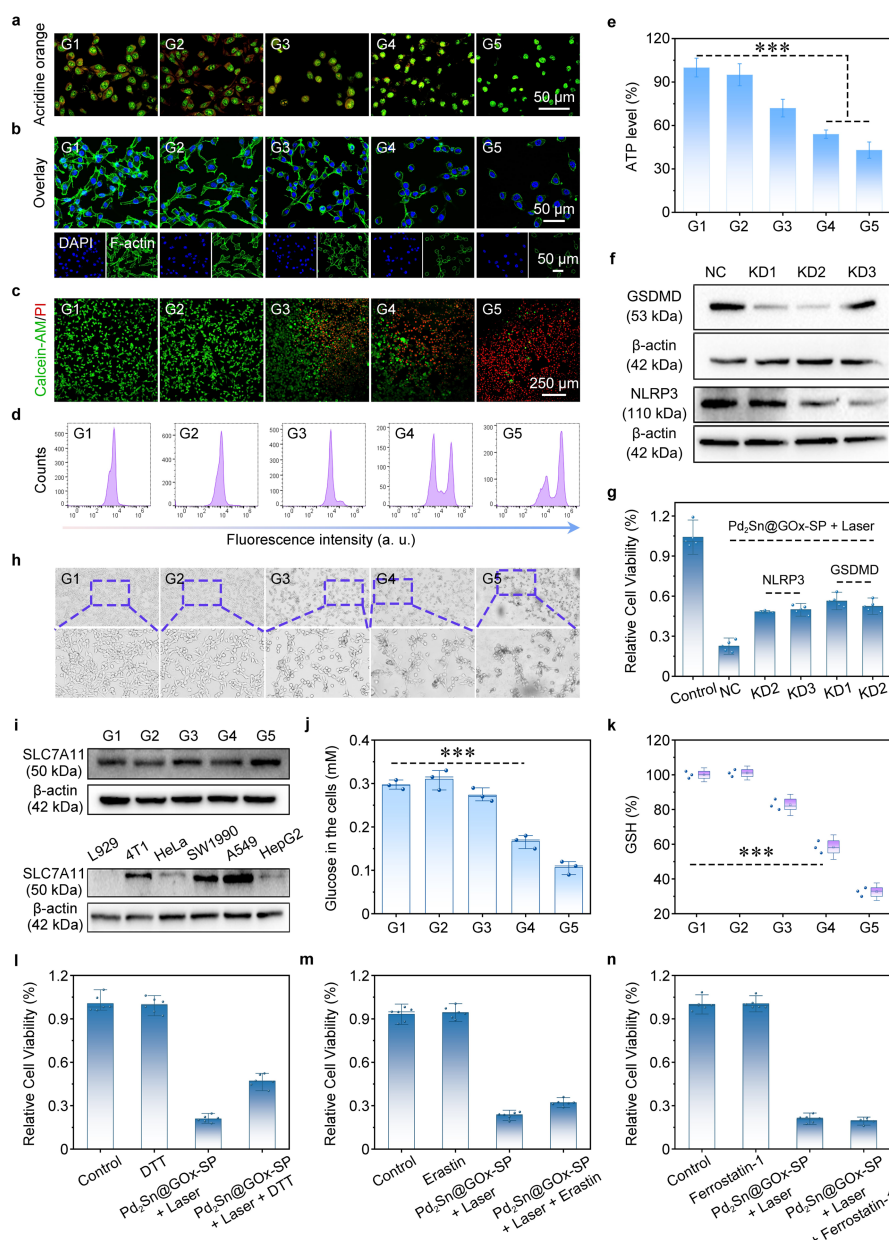
The authors developed Pd₂Sn nanorods that exhibit photothermal and enzyme catalytic activities, including peroxidase-mimic and catalase-mimic activity. The nanorods were further loaded with soybean phospholipid and glucose oxidase (Pd₂Sn@GOx-SP). The authors speculate that Pd₂Sn@GOx-SP induce pyroptosis and disulfidptosis through reactive oxygen species storms and glucose starvation. Cell death was induced by Pd₂Sn@GOx-SP plus laser irradiation in CT26 colon cancer cells, but not L929 cells used as normal cells. Pd₂Sn@GOx-SP plus laser markedly reduced CT26 xenografts *in vivo* and promoted anticancer immunity.

I reviewed this manuscript as an expert in immunology and cell death. It is interesting to note that Pd₂Sn@GOx-SP plus laser has a very high tumor therapeutic effect. However, I have concerns with some of the authors' conclusions, especially the type of cell death induced by the Pd₂Sn@GOx-SP plus laser. The authors designed Pd₂Sn@GOx-SP to induce pyroptosis and

disulfidptosis, because these necrotic forms of cell death can be immunogenic. The experimental results presented in this manuscript are insufficient to prove that Pd₂Sn@GOx-SP plus laser triggered pyroptosis and disulfidptosis.

1. The best way to confirm that Pd₂Sn@GOx-SP plus laser-induced cell death is pyroptosis is to examine whether it is GSDMD-dependent. It is necessary to determine whether knocking out and/or knocking down the expression of GSDMD in CT26 reduce Pd₂Sn@GOx-SP plus laser-induced cell death. It is also necessary to determine whether Pd₂Sn@GOx-SP plus laser-induced cell death is dependent on the NLRP3 inflammasome as the authors assumed.

Answer: Firstly, we would like to express our great appreciation for you as well as your comments. Based on the suggestions, we have knocked down the expression of GSDMD and NLRP3 in CT26 cells, then the cytotoxicity of Pd₂Sn@GOx-SP plus laser on the treated CT26 cells was measured by using the Cell Counting Kit-8 (CCK-8) assay. To begin with, transient transfection was performed to establish GSDMD or NLRP3 knockdown CT26 cells, respectively. The transfection efficiency was verified by western blot analysis (Supplementary Fig. 22f). We next verified by CCK-8 assay that two cell lines with low expression of GSDMD and NLRP3 had higher cell viability after Pd₂Sn@GOx-SP + laser induction than the control group, respectively (Supplementary Fig. 22g). Thus, the pyroptosis could be achieved by the treatment of Pd₂Sn@GOx-SP plus laser, which is dependent on the GSDMD and NLRP3. In addition, western blot analysis was also initiated to assess protein expression during the pyroptosis process (**Fig. 4k**). The high expression of NLRP3 could be observed in the group treated with Pd₂Sn@GOx-SP due to the “two-pronged” strategy. In addition, the Pd₂Sn@GOx-SP-related group exhibited high expression of Cleaved Caspase-1 (C-Caspase-1) and N-GSDMD proteins, which could underpin the drilled pores in the cell membrane, leading to the secretion of damage-associated molecular patterns. Furthermore, the underlying therapeutic mechanism induced by Pd₂Sn@GOx-SP *via* pyroptosis was also confirmed through the immunohistochemical investigation of NLRP3, C-Caspase-1, and GSDMD. All of them were visibly elevated in the tumor tissues extracted from the mice treated with Pd₂Sn@GOx-SP + laser (Fig. 5o), evidencing the occurrence of pyroptosis in tumor cells. Therefore, Pd₂Sn@GOx-SP plus laser-induced pyroptosis was dependent on GSDMD and NLRP3. (For details please see page 13, lines 33-34, page 14, lines 1-7, page 18, lines 5-8, Fig. 4k, and Fig. 5o in the revised



Supplementary Fig. 22. *In vitro* anticancer performance of Pd₂Sn@GOx-SP. **a** Acridine orange staining, **b** F-actin morphology, and **c** Calcein-AM/PI co-staining images of CT26 cells with various treatments (G1, control; G2, laser; G3, Pd₂Sn-SP; G4, Pd₂Sn@GOx-SP; G5, Pd₂Sn@GOx-SP + laser). **d** Flow cytometry analysis of the living and dead cells proportion after various treatments. **e** Intracellular ATP levels after diverse treatments. Data are expressed as mean $\pm$ S.D. (n = 3). **f** Protein levels of NLRP3 and GSDMD in NLRP3- or GSDMD-knockdown CT26 cells by using western bolt analysis. **g** Relative cell viability of NLRP3-knockdown or GSDMD-knockdown CT26 cells treated with Pd₂Sn@GOx-SP + laser. Data are expressed as mean $\pm$ S.D. (n = 5). **h** Bright-field cell morphology after diverse treatments. **i**

Protein levels of SLC7A11 in CT26 cells (upper panel) and multiple cell lines (bottom panel). **j** Glucose content and **k** GSH depletion in different treatment groups. Data are expressed as mean $\pm$ S.D. (n = 3). Relative cell viability of CT26 cells upon Pd₂Sn@GOx-SP + laser treatment with the addition of **l** DTT, **m** Erastin, and **n** Ferrostatin-1. Data are expressed as mean $\pm$ S.D. (n = 6). Statistical significance is assessed by the unpaired Student's two-sided t-test: *** $p < 0.001$.

2. In disulfidptosis, disulfide bonds between actin proteins are formed, which can be detected by non-reducing SDS-PAGE and subsequent Western blotting for actin cytoskeleton proteins (Nature Cell Biology 25:404–414 2023). In addition, shrinkage of the actin cytoskeleton can be observed by immunofluorescence staining. it is necessary to investigate whether the Pd₂Sn@GOx-SP plus laser induces disulfide bond formation between actin proteins.

Answer: Great thanks for your kind suggestion. Based on your suggestion, we have performed non-reducing SDS-PAGE and subsequent Western blotting for actin cytoskeleton proteins to further validate glucose starvation could induce disulfide-bond formation in the actin cytoskeleton proteins. Disulfide-bond formation is known to affect the electrophoretic mobility of proteins under non-reducing conditions. As shown in **Fig. 4m**, the actin cytoskeleton proteins (FLNA, TLN1, and MYH9) in CT26 cells exhibited slower migration on the smears following treatment with Pd₂Sn@GOx-SP. Some of these proteins displayed exceptionally high-molecular-weight bands near the stacking layer, whereas the control or laser-only groups did not show similar migration patterns, indicating that the actin cytoskeleton proteins formed multiple intermolecular disulfide bonds under the treatment of Pd₂Sn@GOx-SP. Furthermore, the destruction of Pd₂Sn@GOx-SP on CT26 cells was also confirmed by observing the morphological changes of actin filaments (F-actin). The cytoskeletal disruption could be seen in the cells after being treated with Pd₂Sn@GOx-SP, while the F-actin of the cells in the group of control or only laser were highly elongated and well-organized (Supplementary Fig. 22b), demonstrating the cell injury induced by Pd₂Sn@GOx-SP. Therefore, all the results confirmed the aberrant disulfide bonds in the actin cytoskeleton proteins induced by Pd₂Sn@GOx-SP, which could cause the subsequent F-actin contraction. (For details please see page 12, lines 27-31, page 14, lines 23-29, and Fig. 4m in the revised manuscript, Supplementary Fig. 22b in Supplementary Information)

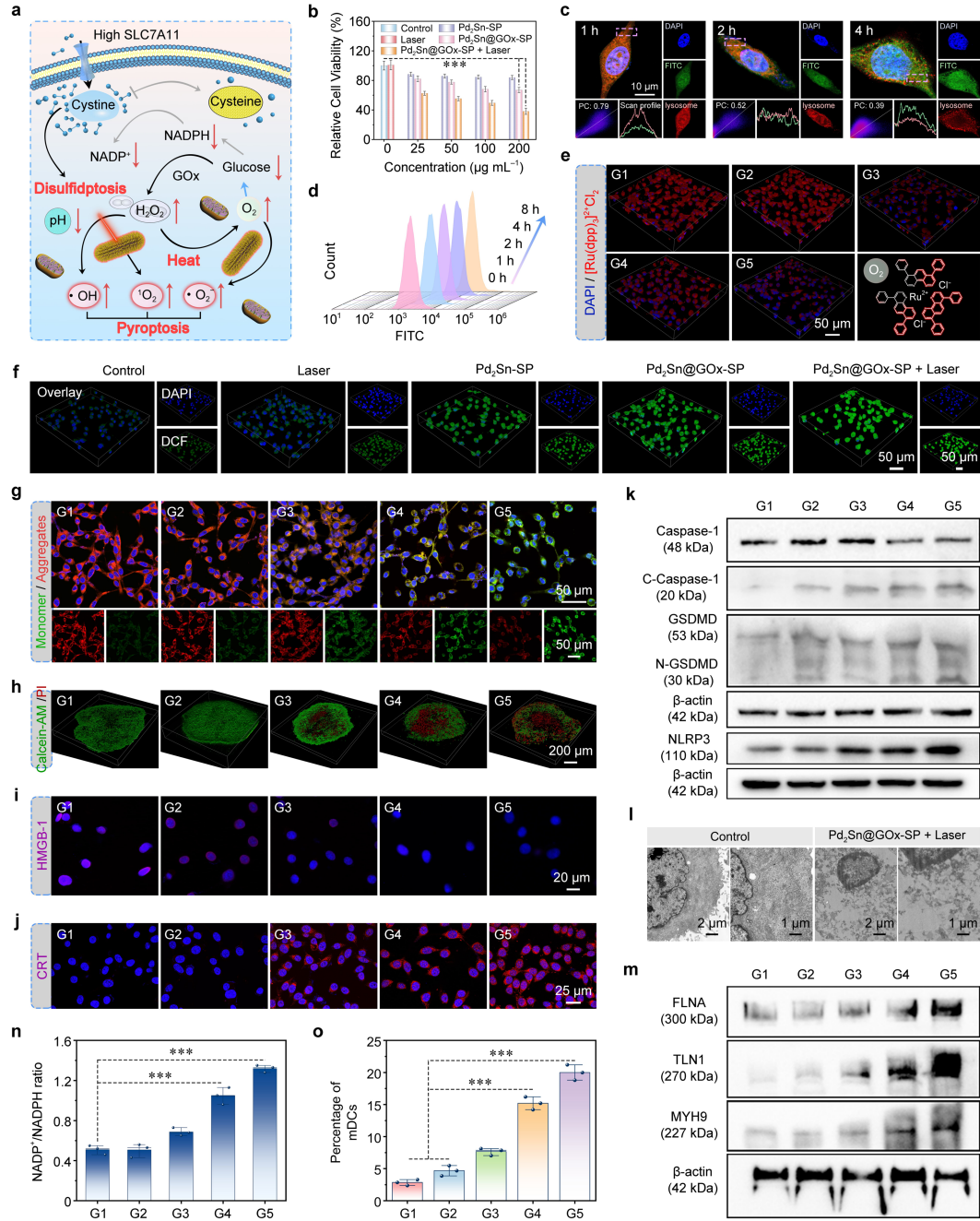
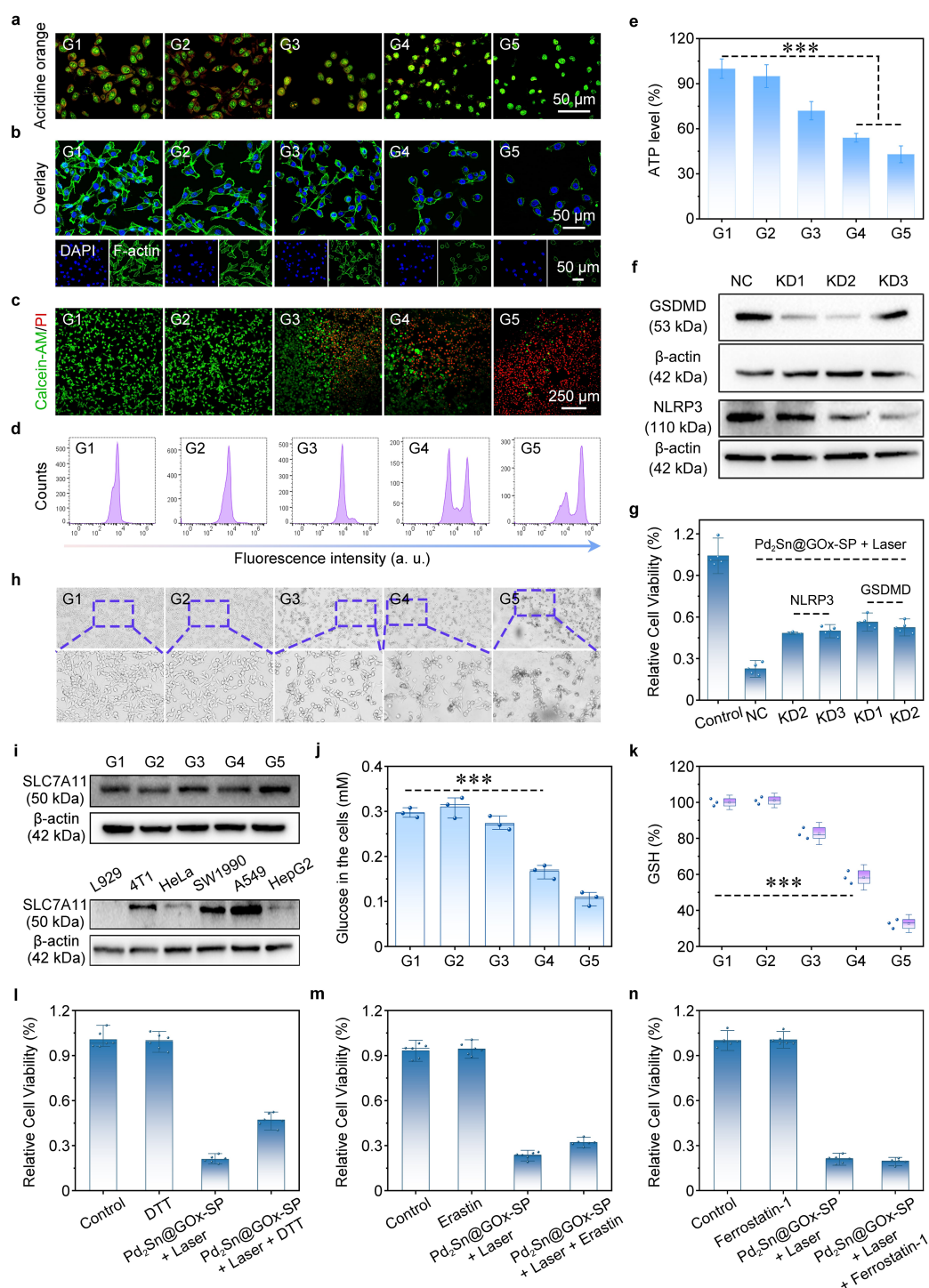


Fig. 4 *In vitro* synergistic pyroptosis and disulfidptosis. **a** Schematic illustration of the synergistic pyroptosis and disulfidptosis mediated by Pd₂Sn@GOx-SP. **b** Cytotoxicity assays of CT26 cells with different treatments. **c** Representative CLSM images of the co-localization of FITC-labeled Pd₂Sn@GOx-SP with the lysosome. **d** Flow cytometry profile of FITC-labeled Pd₂Sn@GOx-SP in CT26 cells. CLSM images of intracellular **e** O₂ generation and **f** ROS level after various treatments (G1, control; G2, laser; G3, Pd₂Sn-SP; G4, Pd₂Sn@GOx-SP; G5, Pd₂Sn@GOx-SP + laser). **g** JC-1 staining of CT26 cells with diverse treatments. **h** Calcein-AM/PI staining of CT26 multicellular spheroids. Fluorescence image of **i** HMGB1 migration and **j** CRT exposure of CT26 cells with diverse treatments. **k** Western blot analysis of Caspase-

l, C-Caspase-1, GSDMD, N-GSDMD, and NLRP3 in CT26 cells after diverse treatments. **l** Bio-TEM images of CT26 cells with diverse treatments. **m** Western blot analysis of FLNA, TLN1, and MYH9 in CT26 cells after diverse treatments. **n** Measurement of the NADP⁺/NADPH ratio. **o** The quantitative data of matured DCs after diverse treatments by *in vitro* stimulation maturation experiment. Data are expressed as mean ± S.D. (n = 3). Statistical significance is assessed by the unpaired Student's two-sided t-test: ****p* < 0.001.

3. Disulfidptosis is inhibited by reducing agents, such as dithiothreitol (DTT), β-mercaptoethanol (2ME), and tris-(2-carboxyethyl)-phosphine (TCEP). Is the Pd₂Sn@GOx-SP plus laser-induced cell death reduced by these reducing agents?

Answer: Thank you for your professional question. To further confirm the disulfidptosis induced by Pd₂Sn@GOx-SP plus laser, the dithiothreitol (DTT) as disulfidptosis inhibitor was added to the cells, and the cell viability was analyzed by CCK-8 assay. The results showed that the cell viability of the cells added with DTT was obviously higher than that in the absence of DTT after being treated with Pd₂Sn@GOx-SP plus laser (Supplementary Fig. 22l), demonstrating the disulfidptosis could be triggered by Pd₂Sn@GOx-SP. Meanwhile, to confirm the critical role of SLC7A11-mediated cystine uptake for disulfide stress, the CT26 cells were incubated with Pd₂Sn@GOx-SP plus laser after the treatment of SLC7A11 inhibitor (Erastin). Compared with that without the treatment of Erastin, the cell death rate of CT26 cells exhibited an obvious decrease due to the inhibition of SLC7A11-mediated cystine uptake (Supplementary Fig. 22m), indicating that the disulfide stress originated from the accumulation of cystine mediated by SLC7A11. (For details please see page 15, lines 1-10 in the revised manuscript, Supplementary Fig. 22l,m in Supplementary Information)



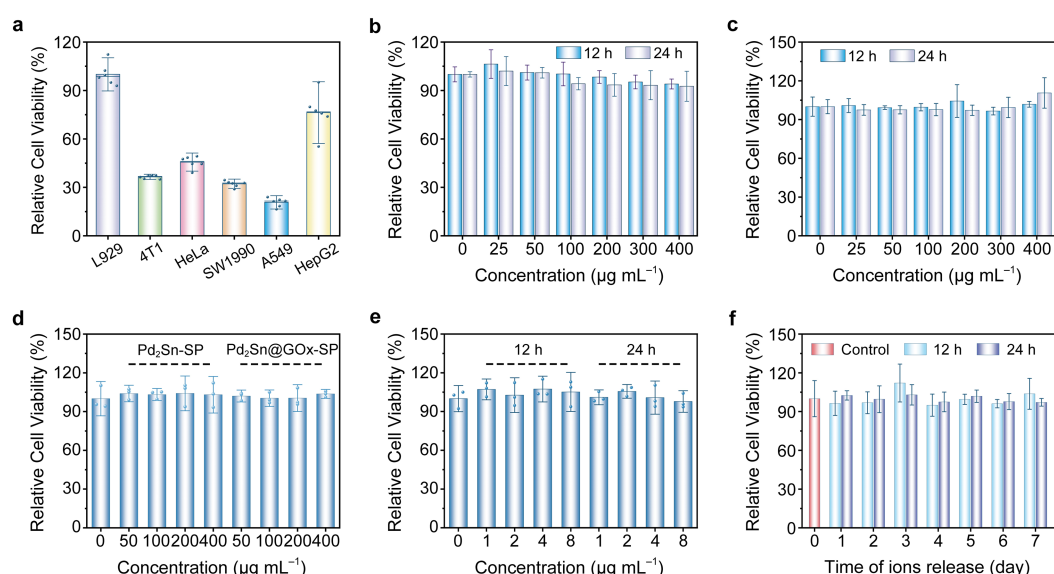
Supplementary Fig. 22. *In vitro* anticancer performance of $\text{Pd}_2\text{Sn@GOx-SP}$. **a** Acridine orange staining, **b** F-actin morphology, and **c** Calcein-AM/PI co-staining images of CT26 cells with various treatments (G1, control; G2, laser; G3, $\text{Pd}_2\text{Sn-SP}$; G4, $\text{Pd}_2\text{Sn@GOx-SP}$; G5, $\text{Pd}_2\text{Sn@GOx-SP} + \text{laser}$). **d** Flow cytometry analysis of the living and dead cells proportion after various treatments. **e** Intracellular ATP levels after diverse treatments. Data are expressed as mean $\pm$ S.D. (n = 3). **f** Protein levels of NLRP3 and GSDMD in NLRP3- or GSDMD-knockdown CT26 cells by using western bolt analysis. **g** Relative cell viability of NLRP3-

knockdown or GSDMD-knockdown CT26 cells treated with Pd₂Sn@GOx-SP + laser. Data are expressed as mean $\pm$ S.D. (n = 5). **h** Bright-field cell morphology after diverse treatments. **i** Protein levels of SLC7A11 in CT26 cells (upper panel) and multiple cell lines (bottom panel). **j** Glucose content and **k** GSH depletion in different treatment groups. Data are expressed as mean $\pm$ S.D. (n = 3). Relative cell viability of CT26 cells upon Pd₂Sn@GOx-SP + laser treatment with the addition of **l** DTT, **m** Erastin, and **n** Ferrostatin-1. Data are expressed as mean $\pm$ S.D. (n = 6). Statistical significance is assessed by the unpaired Student's two-sided t-test: *** $p < 0.001$.

[4. Selective toxicity is very important in cancer drugs. In this respect, it is interesting that Pd₂Sn@GOx-SP plus laser killed CT26 cells but not L929 cells. However, the mechanism of this selective toxicity is not clear. Is the selective toxicity of Pd₂Sn@GOx-SP plus laser observed with other cancer cell lines and normal cells?](#)

Answer: We are grateful for this good question. On the one hand, the tumor microenvironment has many distinctive characteristics that are different from normal cells, such as hypoxia, mild acidity, and overproduced H₂O₂. Pd₂Sn@GOx-SP nanocomposites could catalyze H₂O₂ to generate ROS and O₂ after endocytosis into tumor cells, achieving tumor cell killing. On the contrary, when engulfed by normal cells, the Pd₂Sn-SP and Pd₂Sn@GOx-SP nanocomposites have no toxicity and could be eliminated by cell metabolism (*Nat. Commun.* **12**, 745 (2021); *Nat. Commun.* **15**, 1626 (2024)). On the other hand, most cancer cells usually exhibit overexpressed SLC7A11 compared with normal cells. The overexpressed SLC7A11 in cancer cells (Supplementary Fig. 22i) could import extracellular cystine, which could accumulate in tumor cells to induce cystine-related disulfidptosis under glucose starvation. Moreover, the 808 nm laser could be used to irradiate the specific areas to achieve specific treatment, which is controllable and selective. For *in vivo* experiments, the 808 nm laser was used to irradiate the tumor area, resulting in hyperthermia concentrated only in the tumor area instead of in normal cells. Thus, the 808 nm laser was only used for irradiating tumor cells instead of normal cells. To prove the selective cytotoxicity of nanocomposites on tumor cells and normal cells, the cell viability of multiple cancer cell lines (4T1, HeLa, SW1990, A549, and HepG2 cells) and normal cells (L929 and 3T3 cells) was further evaluated and compared. Our findings revealed that the nanocomposites showed varying degrees of inhibitory effects on cancer cells, while no

significant toxic side effect on normal cells even at higher concentrations (**Fig. 4b** and Supplementary Fig. 20a-d). Therefore, the nanocomposites exhibited negligible cytotoxicity on normal cells without laser irradiation, demonstrating high selective toxicity. (For details please see page 10, lines 18-29, and Fig. 4b in the revised manuscript, Supplementary Fig. 20a-d in Supplementary Information)

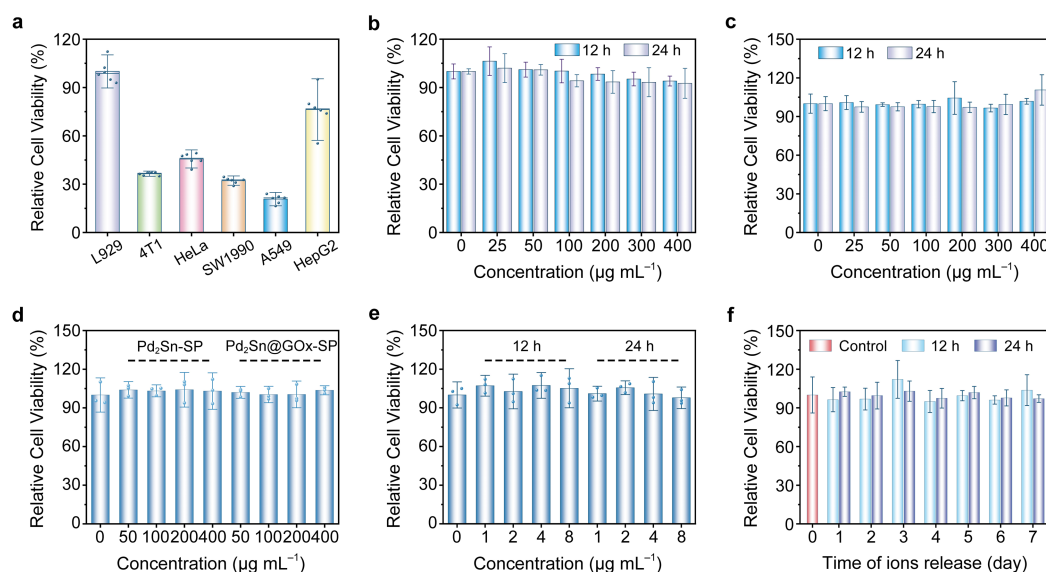


Supplementary Fig. 20. *In vitro* biosafety and toxicity analysis. **a** Relative cell viability of multiple cell lines after being treated with Pd₂Sn@GOx-SP plus laser. Data are expressed as mean $\pm$ S.D. (n = 6). Relative cell viability of **b** L929 and **c** 3T3 cells after incubation with Pd₂Sn@GOx-SP for 12 and 24 h. Data are expressed as mean $\pm$ S.D. (n = 3). **d** Relative cell viability of L929 cells incubated with Pd₂Sn-SP and Pd₂Sn@GOx-SP at various concentrations for 24 h. Data are expressed as mean $\pm$ S.D. (n = 3). **e** Relative cell viability of L929 cells after incubation with TOP at different concentrations for 12 and 24 h. Data are expressed as mean $\pm$ S.D. (n = 3). **f** Relative cell viability of L929 cells after incubation with Sn and Pd ions released from Pd₂Sn@GOx-SP. Data are expressed as mean $\pm$ S.D. (n = 3).

5. If the authors believe that high expression of SLC7A11 determines the induction of cell death by Pd₂Sn@GOx-SP plus laser, this needs to be experimentally demonstrated. The sensitivity of multiple cancer cell lines and normal cells, including L929 cells, to Pd₂Sn@GOx-SP plus laser should be tested, and the expression levels of SLC7A11 should be compared between these cells to see if there is a correlation between SLC7A11 expression levels and cell death induction.

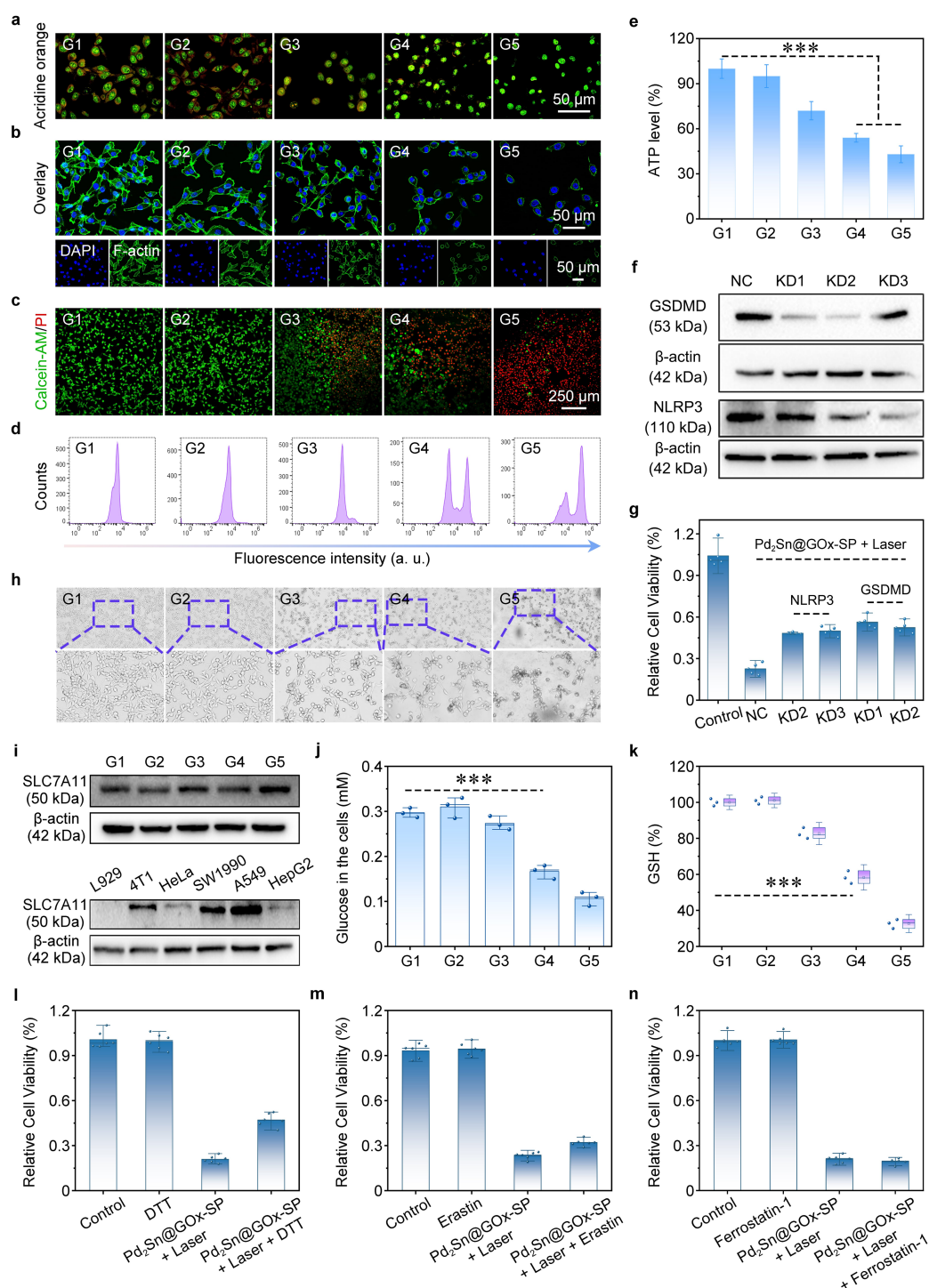
Answer: Great thanks for your kind suggestions. The consumption of glucose induced by

Pd₂Sn@GOx-SP could increase the NADP⁺/NADPH ratio, leading to the abundant accumulation of cystine in CT26 cells with high SLC7A11 expression, achieving cystine-related disulfidptosis. To explore the relationship between SLC7A11 expression levels and cell death induction, we evaluated cell viability after the treatment of Pd₂Sn@GOx-SP plus laser of multiple cancer cell lines (4T1, HeLa, SW1990, A549, and HepG2 cells) and normal cells (L929 cells), which possess different expression levels of SLC7A11. To begin with, the expression levels of SLC7A11 in these cells were evaluated by western blot analysis (Supplementary Fig. 22i). The results showed a higher expression level of SLC7A11 in these cancer cells than that in normal cells, leading to higher cell mortality in cancer cells compared to normal cells with low SLC7A11 expression (Supplementary Fig. 20a). Meanwhile, to further confirm the critical role of SLC7A11-mediated cystine uptake for disulfide stress, the CT26 cells were incubated with Pd₂Sn@GOx-SP plus laser after the treatment of SLC7A11 inhibitor (Erastin). Compared with that without the treatment of Erastin, the cell death rate of CT26 cells exhibited an obvious decrease due to the inhibition of SLC7A11-mediated cystine uptake (Supplementary Fig. 22m), indicating that the disulfide stress originated from the accumulation of cystine mediated by SLC7A11. Therefore, the high expression of SLC7A11 in cancer cells could import extracellular cystine, and promote cystine-related disulfidptosis under the condition of glucose starvation. (For details please see page 10, lines 21-29, and page 15, lines 5-14 in the revised manuscript, Supplementary Fig. 20a, Supplementary Fig. 22i, and Supplementary Fig. 22m in Supplementary Information)



Supplementary Fig. 20. *In vitro* biosafety and toxicity analysis. a Relative cell viability of

multiple cell lines after being treated with Pd₂Sn@GOx-SP plus laser. Data are expressed as mean $\pm$ S.D. (n = 6). Relative cell viability of **b** L929 and **c** 3T3 cells after incubation with Pd₂Sn@GOx-SP for 12 and 24 h. Data are expressed as mean $\pm$ S.D. (n = 3). **d** Relative cell viability of L929 cells incubated with Pd₂Sn-SP and Pd₂Sn@GOx-SP at various concentrations for 24 h. Data are expressed as mean $\pm$ S.D. (n = 3). **e** Relative cell viability of L929 cells after incubation with TOP at different concentrations for 12 and 24 h. Data are expressed as mean $\pm$ S.D. (n = 3). **f** Relative cell viability of L929 cells after incubation with Sn and Pd ions released from Pd₂Sn@GOx-SP. Data are expressed as mean $\pm$ S.D. (n = 3).



Supplementary Fig. 22. *In vitro* anticancer performance of $\text{Pd}_2\text{Sn@GOx-SP}$. **a** Acridine orange staining, **b** F-actin morphology, and **c** Calcein-AM/PI co-staining images of CT26 cells with various treatments (G1, control; G2, laser; G3, $\text{Pd}_2\text{Sn-SP}$; G4, $\text{Pd}_2\text{Sn@GOx-SP}$; G5, $\text{Pd}_2\text{Sn@GOx-SP}$ + laser). **d** Flow cytometry analysis of the living and dead cells proportion after various treatments. **e** Intracellular ATP levels after diverse treatments. Data are expressed as mean $\pm$ S.D. (n = 3). **f** Protein levels of NLRP3 and GSDMD in NLRP3- or GSDMD-knockdown CT26 cells by using western bolt analysis. **g** Relative cell viability of NLRP3-

knockdown or GSDMD-knockdown CT26 cells treated with Pd₂Sn@GOx-SP + laser. Data are expressed as mean ± S.D. (n = 5). **h** Bright-field cell morphology after diverse treatments. **i** Protein levels of SLC7A11 in CT26 cells (upper panel) and multiple cell lines (bottom panel). **j** Glucose content and **k** GSH depletion in different treatment groups. Data are expressed as mean ± S.D. (n = 3). Relative cell viability of CT26 cells upon Pd₂Sn@GOx-SP + laser treatment with the addition of **l** DTT, **m** Erastin, and **n** Ferrostatin-1. Data are expressed as mean ± S.D. (n = 6). Statistical significance is assessed by the unpaired Student's two-sided t-test: *** $p < 0.001$.

[6. The authors should provide the source data for Fig. 4k. Please show uncropped scans of these immunoblots along with molecular weight markers. This is the standard way to rigorously evaluate the results of immunoblots, especially in high impact journals. GSDMD and GSDMD-N should be shown in the same slit, and if the exposure times are different between them, then both uncropped images should be presented; likewise for Caspase-1 and C-Caspase-1. Typically, when GSDMD and caspase-1 are proteolytically activated, their proforms are reduced, but this is not the case in Fig. 4k. The authors must address this comment.](#)

Answer: Great thanks for your kind comments. We have re-conducted the western blot analysis and provided the source data for **Fig. 4k**, showing uncropped scans of these immunoblots along with molecular weight markers. As shown in **Fig. 4k**, the high expression of NLRP3 could be observed in the group treated with Pd₂Sn@GOx-SP due to the “two-pronged” strategy. In addition, the Pd₂Sn@GOx-SP-related group exhibited high expression of Cleaved Caspase-1 (C-Caspase-1) and N-GSDMD proteins, which could underpin the drilled pores in the cell membrane, leading to the secretion of damage-associated molecular patterns. (For details please see page 13, lines 26-33, and Fig. 4k in the revised manuscript)

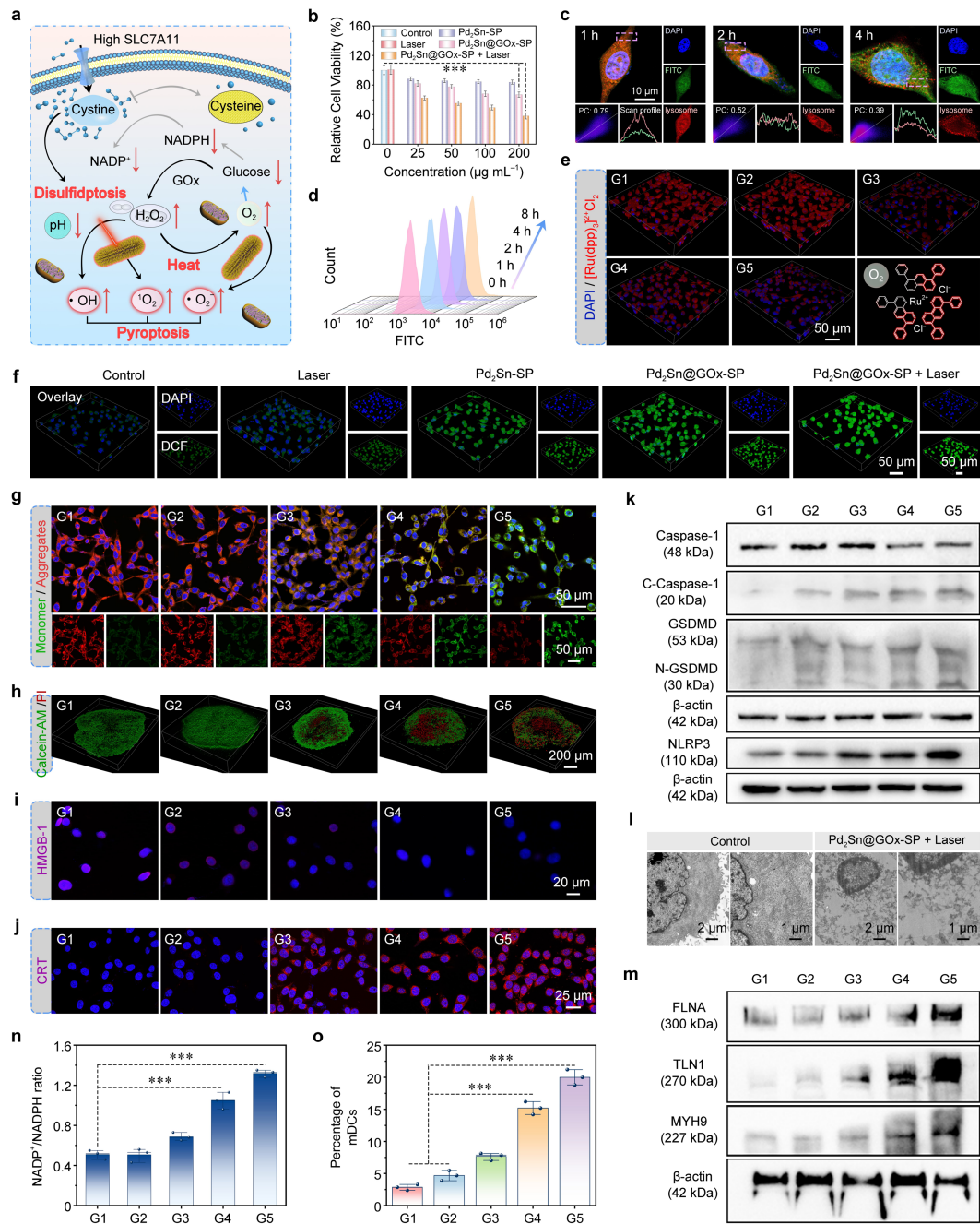
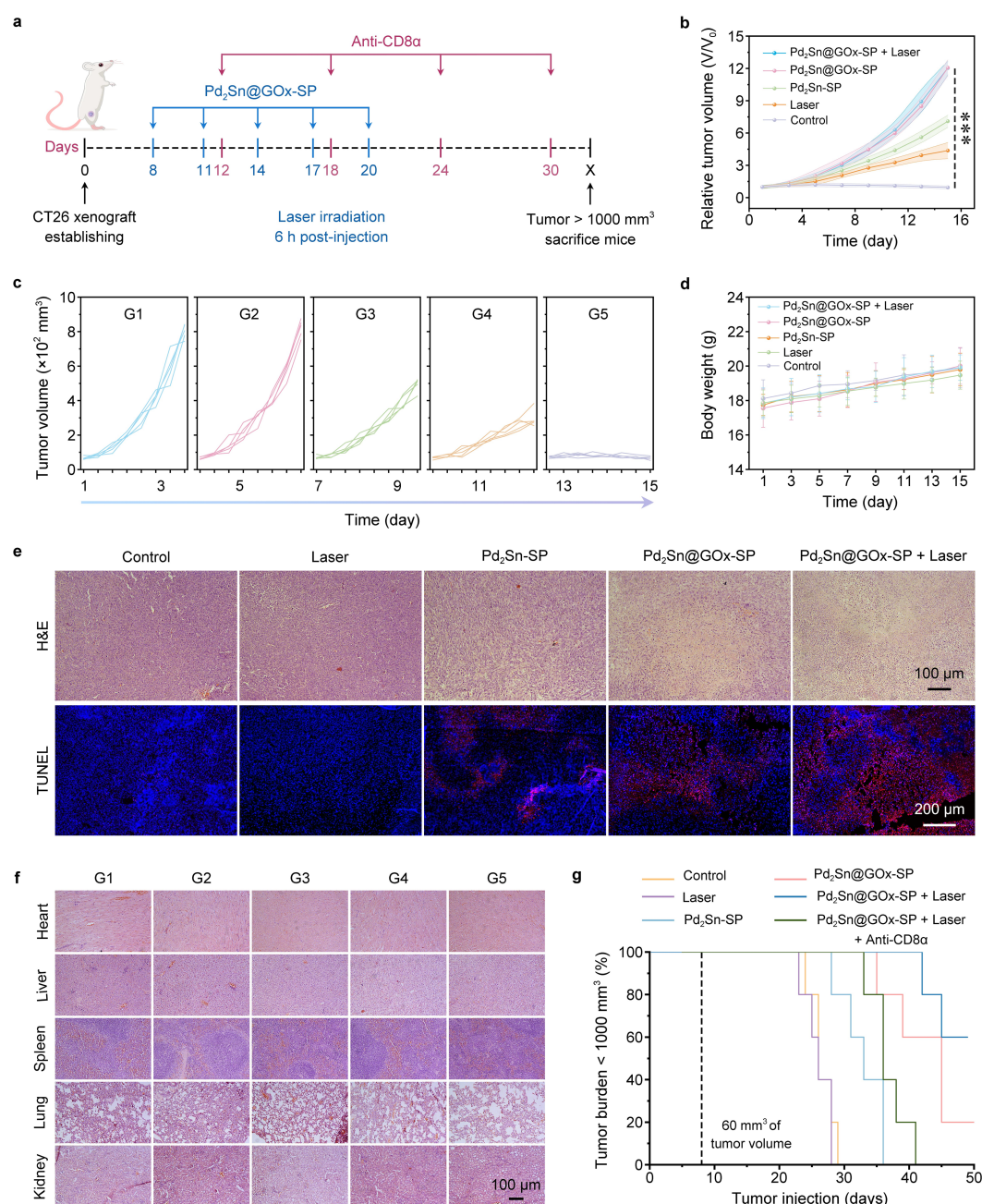


Fig. 4 *In vitro* synergistic pyroptosis and disulfidptosis. **a** Schematic illustration of the synergistic pyroptosis and disulfidptosis mediated by Pd₂Sn@GOx-SP. **b** Cytotoxicity assays of CT26 cells with different treatments. **c** Representative CLSM images of the co-localization of FITC-labeled Pd₂Sn@GOx-SP with the lysosome. **d** Flow cytometry profile of FITC-labeled Pd₂Sn@GOx-SP in CT26 cells. CLSM images of intracellular **e** O₂ generation and **f** ROS level after various treatments (G1, control; G2, laser; G3, Pd₂Sn-SP; G4, Pd₂Sn@GOx-SP; G5, Pd₂Sn@GOx-SP + laser). **g** JC-1 staining of CT26 cells with diverse treatments. **h** Calcein-AM/PI staining of CT26 multicellular spheroids. Fluorescence image of **i** HMGB1 migration and **j** CRT exposure of CT26 cells with diverse treatments. **k** Western blot analysis of Caspase-

l, C-Caspase-1, GSDMD, N-GSDMD, and NLRP3 in CT26 cells after diverse treatments. **l** Bio-TEM images of CT26 cells with diverse treatments. **m** Western blot analysis of FLNA, TLN1, and MYH9 in CT26 cells after diverse treatments. **n** Measurement of the NADP⁺/NADPH ratio. **o** The quantitative data of matured DCs after diverse treatments by *in vitro* stimulation maturation experiment. Data are expressed as mean ± S.D. (n = 3). Statistical significance is assessed by the unpaired Student's two-sided t-test: ****p* < 0.001.

7. Does Pd₂Sn@GOx-SP plus laser have a therapeutic effect in tumor models other than CT26?

Answer: We are grateful for this good question. To further validate the excellent antitumor therapeutic effect of Pd₂Sn@GOx-SP on different types of tumors, we constructed another tumor xenograft model using 4T1 mammary cancer cells, which are also known to exhibit the high expression of SLC7A11 (*Protein Cell* **12**, 599 (2021)). The results demonstrated that the growth of 4T1 tumors was significantly inhibited by the treatment of Pd₂Sn@GOx-SP plus laser (Supplementary Fig. 27b,c). Meanwhile, the body weight of mice in each treatment group exhibited no significant abnormality (Supplementary Fig. 27d), confirming the biosafety of Pd₂Sn@GOx-SP. The excellent tumor suppression effect could also be observed based on the H&E and TUNEL staining images of tumor tissue slices (Supplementary Fig. 27e). Moreover, the H&E staining images of the major organs collected from the mice with diverse treatments showed no distinct inflammatory or pathological changes, which further validated the excellent biosafety of Pd₂Sn@GOx-SP (Supplementary Fig. 27f). These results highlighted the therapeutic potential of Pd₂Sn@GOx-SP, which could induce both pyroptosis and disulfidptosis, making it a promising candidate for treating various types of tumors. (For details please see page 18, lines 11-22 in the revised manuscript, Supplementary Fig. 27b-f in Supplementary Information)



Supplementary Fig. 27. *In vivo* synergistic antitumor therapy on 4T1 tumor-bearing mice.

a Treatment schedule for mice receiving nanocomposites or CD8-depleting antibodies (Anti-CD8α). **b** Relative tumor volume, **c** tumor volume, and **d** body weight of the mice in different treatment groups (G1, control; G2, laser; G3, Pd₂Sn-SP; G4, Pd₂Sn@GOx-SP; G5, Pd₂Sn@GOx-SP + laser). **e** H&E and TUNEL staining images of tumor tissue slices from the representative mice in different treatment groups. **f** H&E staining images of heart, liver, spleen, lung, and kidney collected from the representative mice in different treatment groups. **g** Kaplan-Meier survival graph of mice receiving various formulations. Data are expressed as mean ± S.D. (n = 5). Statistical significance is assessed by the unpaired Student's two-sided t-test: ***p <

0.001.

8. The expression of IFN- γ in CD8 T cells is examined in Fig. 6, but the expression level of granzyme B in CD8 T cells is also a good marker of the activation of anti-cancer immunity. Also, if depletion of CD8 T cells reduces the therapeutic effect of Pd₂Sn@GOx-SP plus laser, it will be more clearly shown that this treatment involves the activation of anti-cancer immunity.

Answer: Great thanks for your kind suggestions. As you mentioned, the expression level of granzyme B (GZMB) in CD8⁺ T cells is also a good marker of the activation of anti-cancer immunity. Thus, the expression level of GZMB in tumors was evaluated by immunohistochemical analysis to confirm the activation of anti-cancer immunity. As can be seen in Fig. 5o, the GZMB levels were significantly up-regulated in the tumors of the mice treated with Pd₂Sn@GOx-SP + laser compared with other groups, demonstrating the effective activation of CD8⁺ T cells to achieve anti-cancer immunity. Furthermore, depleting antibodies against CD8 (anti-CD8 α) were injected into mice to deplete CD8⁺ T cells, and the tumor growth was monitored to confirm that the treatment involves the activation of anti-cancer immunity (Supplementary Fig. 27a). After the depletion of CD8⁺ T cells by the administration of anti-CD8 α , the therapeutic efficacy of Pd₂Sn@GOx-SP plus laser decreased significantly (Supplementary Fig. 27g). All these data provide convincing evidence that Pd₂Sn@GOx-SP could recruit immune cells to tumors, thereby inducing a specific anti-tumor immune response. (For details please see page 19, lines 6-11, page 20, lines 1-7, and Fig. 5o in the revised manuscript, Supplementary Fig. 27g in Supplementary Information)

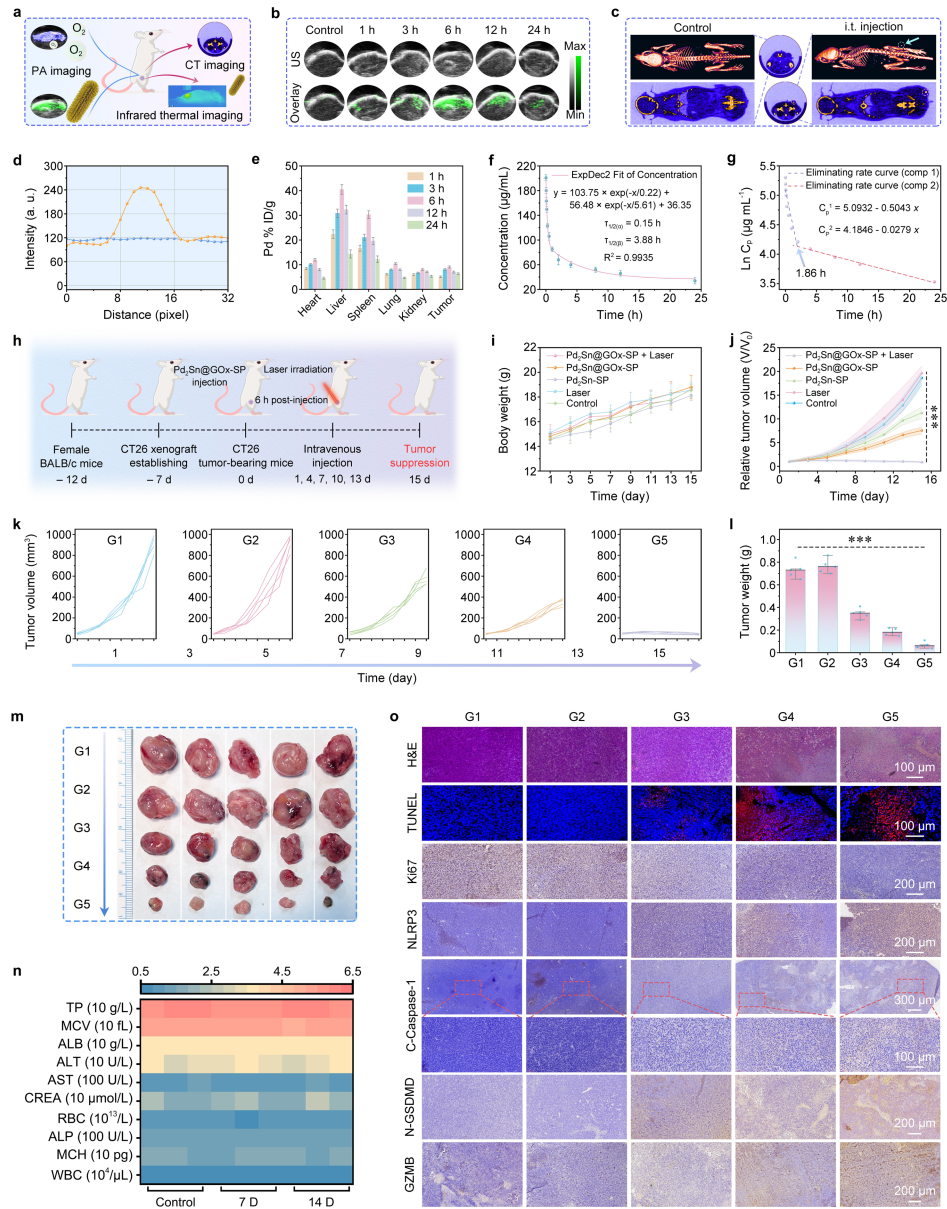
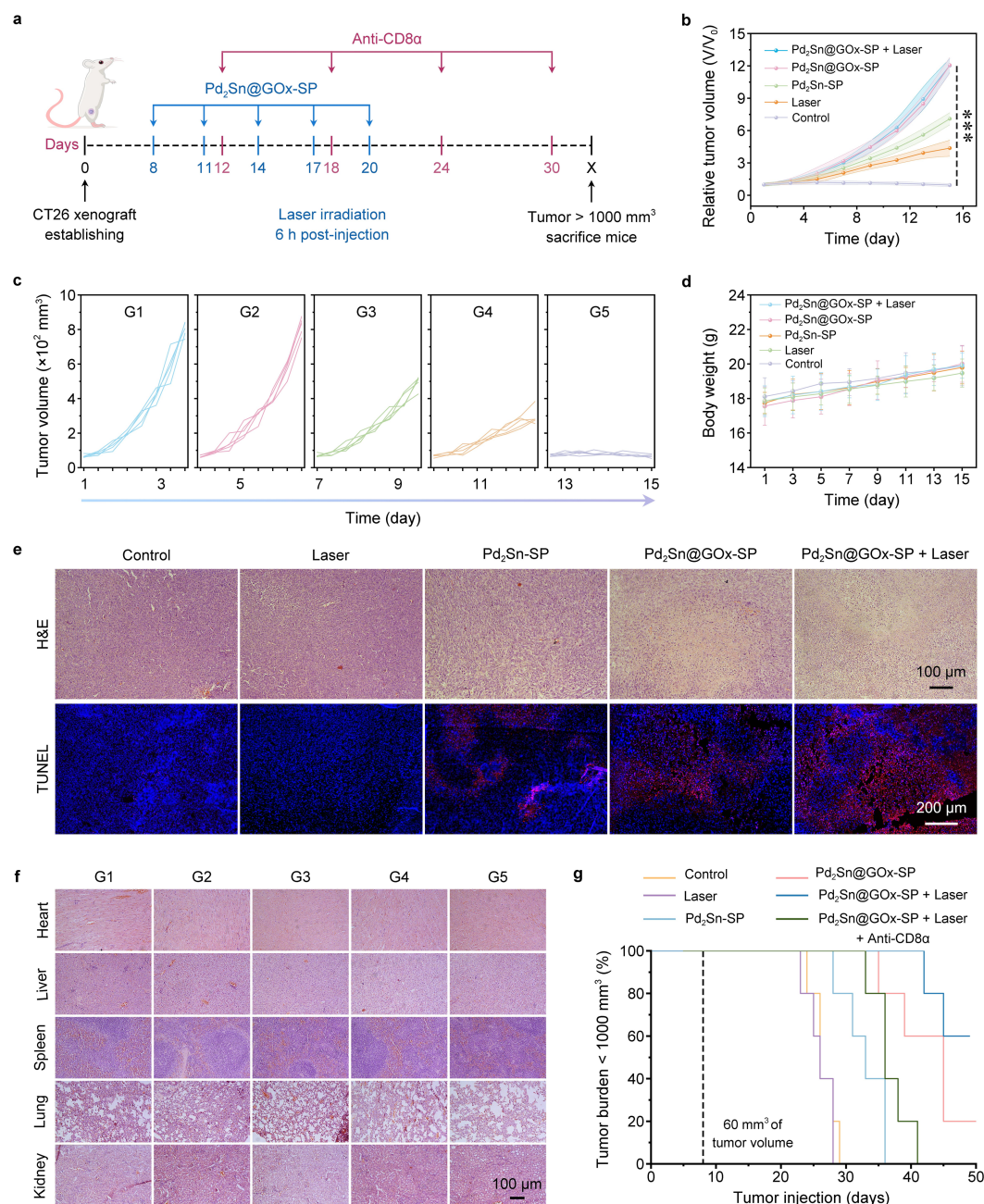


Fig. 5 *In vivo* PA/CT imaging-guided synergistic antitumor therapy. **a** Schematic illustration of the PA, CT, and infrared thermal imaging. Representative *in vivo* **b** PA images after being *i.v.* injected with Pd₂Sn@GOx-SP and **c** CT images after *i.t.* injected without or with Pd₂Sn@GOx-SP. **d** The corresponding line profiles of CT values. **e** *In vivo* biodistribution of Pd₂Sn@GOx-SP in main organs and tumors at various time intervals. **f** The blood circulation curve and **g** the eliminating rate curve after *i.v.* injected with Pd₂Sn@GOx-SP. Data are expressed as mean ± S.D. (n = 3). **h** Therapeutic schedule of Pd₂Sn@GOx-SP for CT26 tumor-bearing mice. **i** Body weight, **j** relative tumor volume, **k** tumor volume, **l** tumor weight, and **m** the photographs of excised tumors from representative mice in different treatment groups (G1, control; G2, laser; G3, Pd₂Sn-SP; G4, Pd₂Sn@GOx-SP; G5, Pd₂Sn@GOx-SP + laser). **n**

Hematological indexes and biochemical data of mice after *i.v.* injection with PBS and Pd₂Sn@GOx-SP. **o** Staining images of H&E, TUNEL, Ki67, NLRP3, C-Caspase-1, GSDMD, and GZMB for tumor slices from different groups. Data are expressed as mean $\pm$ S.D. (n = 5). Statistical significance is assessed by the unpaired Student's two-sided t-test: ****p* < 0.001.



Supplementary Fig. 27. *In vivo* synergistic antitumor therapy on 4T1 tumor-bearing mice.

a Treatment schedule for mice receiving nanocomposites or CD8-depleting antibodies (Anti-CD8 α). **b** Relative tumor volume, **c** tumor volume, and **d** body weight of the mice in different treatment groups (G1, control; G2, laser; G3, Pd₂Sn-SP; G4, Pd₂Sn@GOx-SP; G5, Pd₂Sn@GOx-SP + laser). **e** H&E and TUNEL staining images of tumor tissue slices from the

representative mice in different treatment groups. **f** H&E staining images of heart, liver, spleen, lung, and kidney collected from the representative mice in different treatment groups. **g** Kaplan-Meier survival graph of mice receiving various formulations. Data are expressed as mean $\pm$ S.D. (n = 5). Statistical significance is assessed by the unpaired Student's two-sided t-test: *** p < 0.001.

9. This manuscript does not fully explain the laser used in the experiments. In Fig. 2, the laser power is mentioned. However, there is no description of the laser power in the *in vivo* experiments. This manuscript also lacks a description of what equipment was used to generate the laser in each experiment and what wavelength was used.

Answer: Great thanks for your kind comments. The laser wavelength used in this manuscript was 808 nm, which was generated by the 808nm multimode fiber coupled laser purchased from Changchun Laser Optoelectronics Technology Co., Ltd. The power density of the 808 nm laser used in the *in vivo* experiments was 0.8 W cm⁻², which is similar to the *in vitro* experiments. All relevant descriptions have been supplemented in the text to make the manuscript more rigorous. (For details please see page 22, lines 20-22 in the revised manuscript)

10. Fig. 4 shows G1-5, but these are only explained in Fig. 5 (page 15 lines 13-14).

Answer: Great thanks for your kind comments. The explanation of G1-G5 has been added in the caption of Fig. 4, the corresponding content was also added to the text and figure caption to make it easier for readers to understand. (For details please see Fig. 4, Fig. 5, and Fig. 6 in the revised manuscript, Supplementary Fig. 21-23, and Supplementary Fig. 27 in Supplementary Information)

11. Fig. 4b, which hides values around 0, is not appropriate for a scientific paper.

Answer: Great thanks for your kind comments. We have adjusted Fig. 4b to show the values around 0, making it more appropriate for a scientific paper. (For details please see Fig. 4b in the revised manuscript)

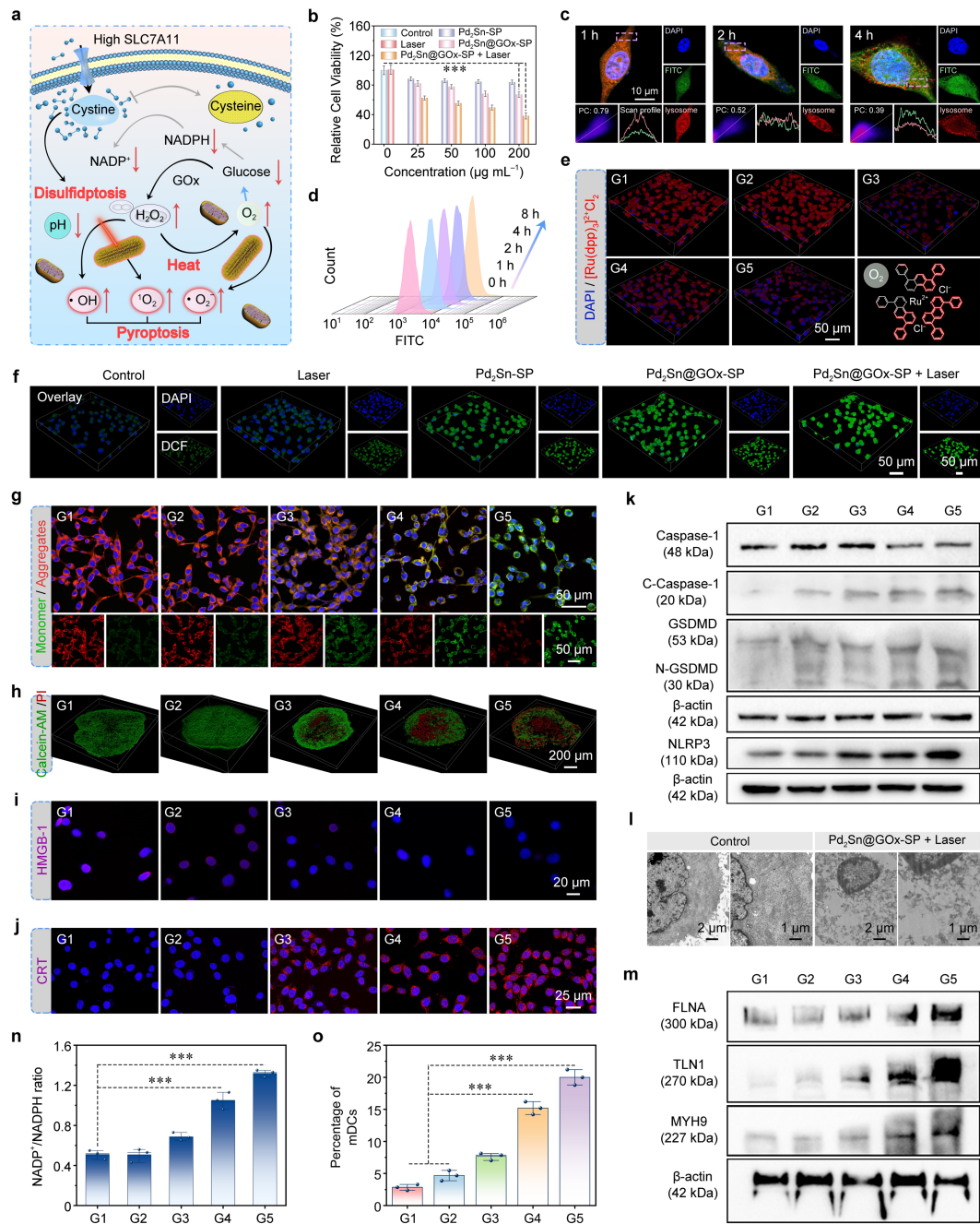


Fig. 4 *In vitro* synergistic pyroptosis and disulfidptosis. **a** Schematic illustration of the synergistic pyroptosis and disulfidptosis mediated by Pd₂Sn@GOx-SP. **b** Cytotoxicity assays of CT26 cells with different treatments. **c** Representative CLSM images of the co-localization of FITC-labeled Pd₂Sn@GOx-SP with the lysosome. **d** Flow cytometry profile of FITC-labeled Pd₂Sn@GOx-SP in CT26 cells. CLSM images of intracellular **e** O₂ generation and **f** ROS level after various treatments (G1, control; G2, laser; G3, Pd₂Sn-SP; G4, Pd₂Sn@GOx-SP; G5, Pd₂Sn@GOx-SP + laser). **g** JC-1 staining of CT26 cells with diverse treatments. **h** Calcein-AM/PI staining of CT26 multicellular spheroids. Fluorescence image of **i** HMGB1 migration and **j** CRT exposure of CT26 cells with diverse treatments. **k** Western blot analysis of Caspase-

l, C-Caspase-1, GSDMD, N-GSDMD, and NLRP3 in CT26 cells after diverse treatments. **l** Bio-TEM images of CT26 cells with diverse treatments. **m** Western blot analysis of FLNA, TLN1, and MYH9 in CT26 cells after diverse treatments. **n** Measurement of the NADP⁺/NADPH ratio. **o** The quantitative data of matured DCs after diverse treatments by *in vitro* stimulation maturation experiment. Data are expressed as mean ± S.D. (n = 3). Statistical significance is assessed by the unpaired Student's two-sided t-test: *** $p < 0.001$.

Reviewer #5:

(Remarks to the Author):

Comments:

I co-reviewed this manuscript with one of the reviewers who provided the listed reports as part of the Nature Communications initiative to facilitate training in peer review and appropriate recognition for co-reviewers.

Answer: We would like to express our great appreciation for you as well as your comments. All the comments and suggestions have been replied to and modified in the revised manuscript, which has been highlighted in red for your easy review.

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

I and my co-reviewer carefully checked the revised manuscript and response of the authors to the comments from the reviewers. We considered that the authors addressed all the concerns from the reviewers and recommended the publication of the manuscript.

Reviewer #2 (Remarks to the Author):

All my concerns have been addressed and therefore the revised manuscript can be accepted for publication in NC.

Reviewer #3 (Remarks to the Author):

My concerns were satisfactorily addressed. I find this manuscript acceptable for publication.

Reviewer #4 (Remarks to the Author):

The authors have adequately addressed all my comments. The authors have demonstrated that their Pd2Sn@GOx-SP has a high tumour effect by activating anti-cancer immunity by inducing pyroptosis and disulfidptosis in SLC7A11 high-expressing cancer cells. I consider the current manuscript to be worthy of publication.

In the Supplementary Information PDF file, some figures are not displayed correctly. They appear as white boxes on both Macintosh and Windows PCs. On the other hand, all figures in the Supplementary Information source file (DOCX file) are displayed correctly. The Supplementary Information PDF file needs to be corrected.

Reviewer #5 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

The reviewers gave very valuable comments on our manuscript, and we would like to take this opportunity to express our great appreciation for him/her as well as the comments. The following are the changes made in the new version together with our responses to the reviewers' comments.

REVIEWER COMMENTS:

Reviewer #1:

Comments:

I and my co-reviewer carefully checked the revised manuscript and response of the authors to the comments from the reviewers. We considered that the authors addressed all the concerns from the reviewers and recommended the publication of the manuscript.

Answer: We would like to express our great appreciation for you as well as your comments.

Reviewer #2:

Comments:

All my concerns have been addressed and therefore the revised manuscript can be accepted for publication in NC.

Answer: We would like to express our great appreciation for you as well as your comments.

Reviewer #3:

Comments:

My concerns were satisfactorily addressed. I find this manuscript acceptable for publication.

Answer: We would like to express our great appreciation for you as well as your comments.

Reviewer #4:

Comments:

The authors have adequately addressed all my comments. The authors have demonstrated that their Pd₂Sn@GOx-SP has a high tumour effect by activating anti-cancer immunity by inducing pyroptosis and disulfidptosis in SLC7A11 high-expressing cancer cells. I consider the current manuscript to be worthy of publication.

In the Supplementary Information PDF file, some figures are not displayed correctly. They appear as white boxes on both Macintosh and Windows PCs. On the other hand, all figures in the Supplementary Information source file (DOCX file) are displayed correctly. The Supplementary Information PDF file needs to be corrected.

Answer: We would like to express our great appreciation for you as well as your comments. Based on your suggestions, we have corrected the Supplementary Information PDF file to make all figures display correctly.

Reviewer #5:

Comments:

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Answer: We would like to express our great appreciation for you as well as your comments.