Supplementary Materials

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We further conducted preliminary investigations into the underlying mechanism of the therapy. JC-1 staining, a widely used method for assessing mitochondrial membrane potential, was employed to evaluate mitochondrial damage in different cell types during thermo-hydrogen therapy. In healthy mitochondria, the JC-1 probe accumulates in the mitochondrial matrix and forms J-aggregates, emitting strong red fluorescence. In contrast, in damaged or depolarized mitochondria, the loss or reduction of membrane potential prevents aggregation, and JC-1 remains in its monomeric form in the cytoplasm, resulting in green fluorescence. The preliminary results are presented as follows.

As shown in the figure, thermo-hydrogen therapy induces significant mitochondrial damage in both cancer cell lines, which may further lead to an imbalance in intracellular ROS homeostasis and trigger a burst of ROS. A more systematic and in-depth investigation of the underlying anticancer mechanisms is currently underway in our group, and the detailed findings will be shared with the community at an appropriate stage. We sincerely thank the reviewer for their attention and valuable comments.

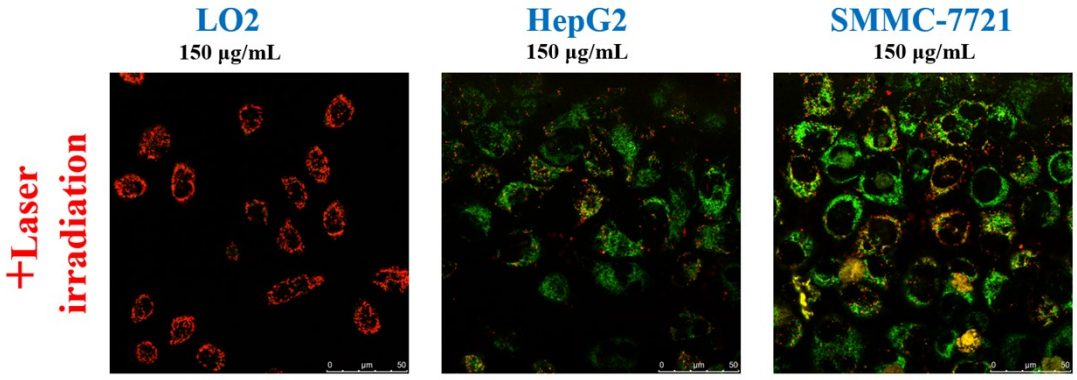

Figure S1 Preliminary Results of Mitochondrial Damage