Supplementary Information

Impairing antioxidant protection by diminishing hyaluronic acid using nanoliposome for tumor therapy

Hegang Lu[a], *Yunjian Yu*[a], *Shengke Zhao*[a], *Youtao Xin*[a], *Hongyu Liu*[a], *Qinghua Feng*[a], *Mahmoud Elsabahy*[b], *Hui Gao*[a]*

a. National Key Laboratory of Advanced Separation Membrane Materials & Key Laboratory of Hollow Fiber Membrane Materials and Membrane Processes (MOE) & Tianjin Key Laboratory of Hollow Fiber Membrane Materials and Processes, School of Materials Science and Engineering, Tiangong University, Tianjin 300387, P. R. China

b. School of Biotechnology, Badr University in Cairo, Badr City, Cairo, 11829, Egypt.

* Corresponding author

General materials and methods

Sodium hyaluronate (MW = 150-250 kDa) and hyaluronidase (HAse, PH20) was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. A 30% H_2O_2 solution and sulfuric acid were gained from Tianjin Fengchuan Chemical Reagent Co. Ribavirin, carbazole, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), $Ti(SO_4)_2$, Cy7 DiC18 (DIR), MTT, and TUNEL kit (green) were obtained from Aladdin (Shanghai, China). Alcian Blue Stain Kit was purchased from Beijing Solarbio Science & Technology Co., Ltd. DSPE-PEG_{2000}, DPPC, and cholesterol were sourced from Bide Pharmatech Ltd. (Shanghai, China). CT26 cells were obtained from Procell (CL-0071, Wuhan, China). RPMI Medium 1640, fetal calf serum, and penicillin/streptomycin were sourced from ThermoFisher Scientific. Catalase kit was purchased from G-clone Biotechnology Co., Ltd. (SH196W, Beijing, China). Human HA ELISA kit was sourced from Lunchangshuo Biotechnology Co., Ltd. (Xiamen, China). Human CD44 ELISA kit was obtained from Ruixin Biotech. (Quanzhou, China). DCFH-DA, calcein-AM/propidium iodide (Calcein AM/PI), and ROS Assay Kit was were acquired from Beyotime Biotechnology. Anti CD44-FITC were purchased from BioLegend (USA).

Results

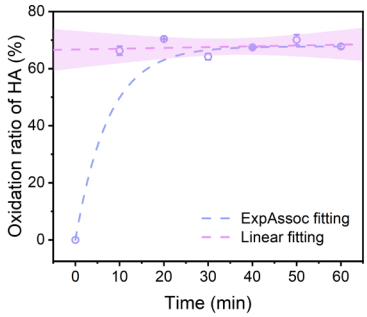

Figure s1. Antioxidant activity of sodium hyaluronate against H_2O_2 at various time intervals (0-60, n = 3).

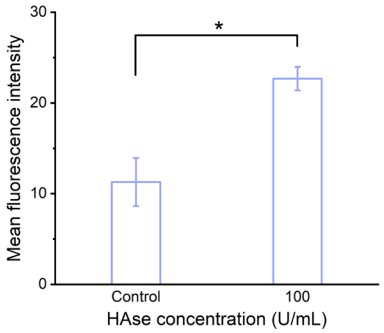

Figure s2. Mean fluorescence intensity of LSCM images analyzed using ImageJ.

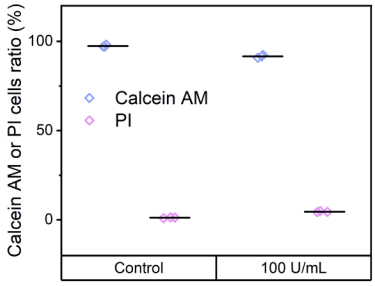

Figure s3. Calcein AM/PI analysis of CT26 cells with the addition of exogenous HAse (n = 3).

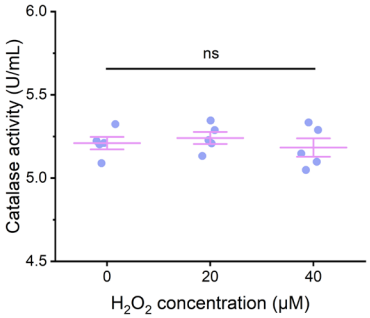

Figure s4. Catalase activity of tumor cells with varying amounts of H_2O_2 added (n = 5).

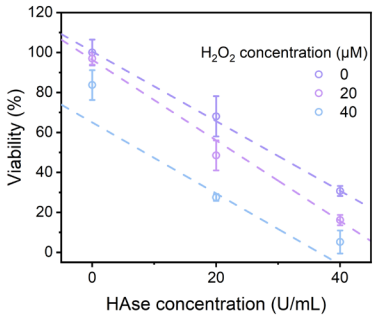

Figure s5. HAse enhances the H_2O_2 cytotoxicity using MTT method (n = 6).

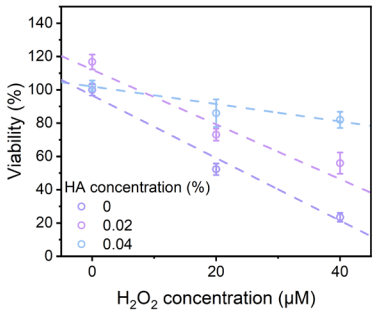

Figure s6. Cell viability of CT26 cells with H_2O_2 and HA treatment (n = 6).

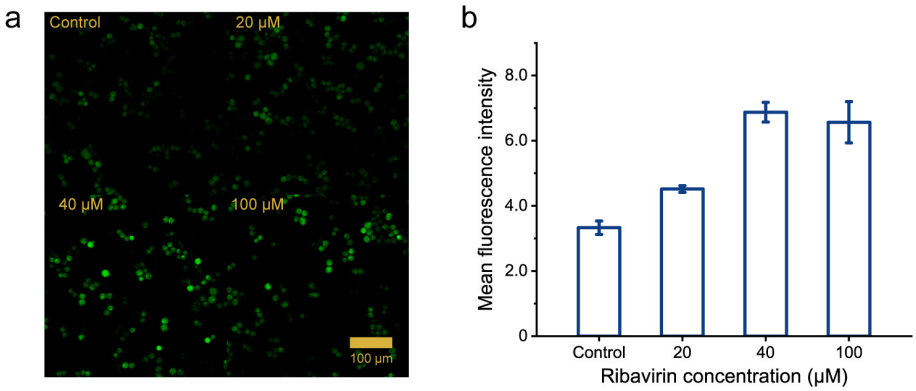

Figure s7. LSCM images and quantification with exogenous ribavirin addition after DCFH-DA staining.

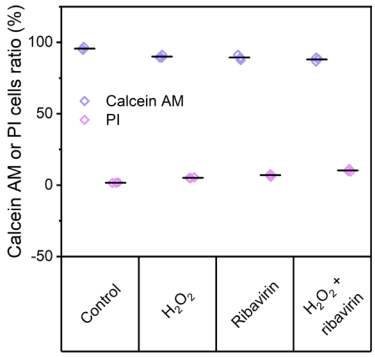

Figure s8. Calcein AM/P analysis of CT26 cells with ribavirin and H_2O_2 added (n = 3).

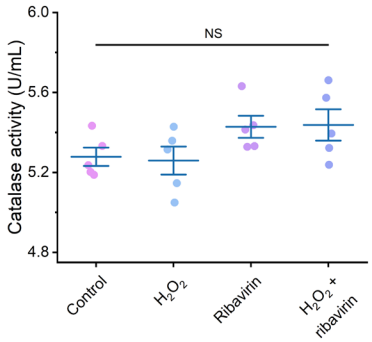

Figure. s9. Catalase activity of CT26 cells after H_2O_2 and ribavirin treatment.

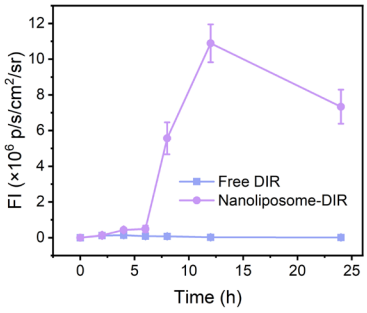

Figure s10. Quantification of PA FIs in tumors as a function of post-injection time (n = 3).

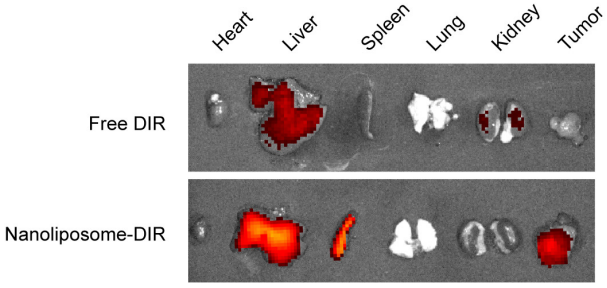

Figure s11. *Ex vivo* fluorescence images of tumor tissue and major organs at 24 h postinjection.

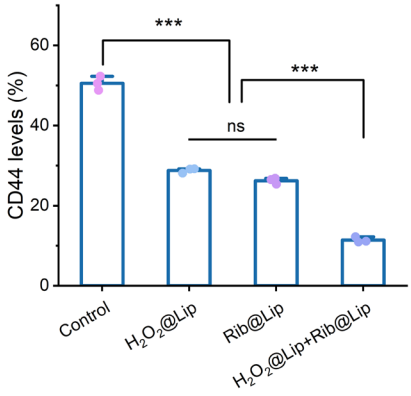

Figure s12. CD44 levels in tumor tissues quantized from flow cytometer after anti-CD44-FITC incubated (n = 3).

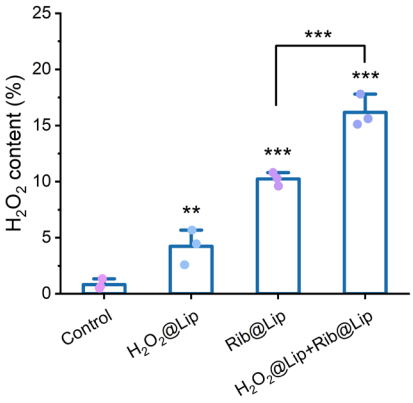

Figure s13. H_2O_2 levels in tumor tissues quantized from flow cytometer after DCFH-DA incubated (n = 3).

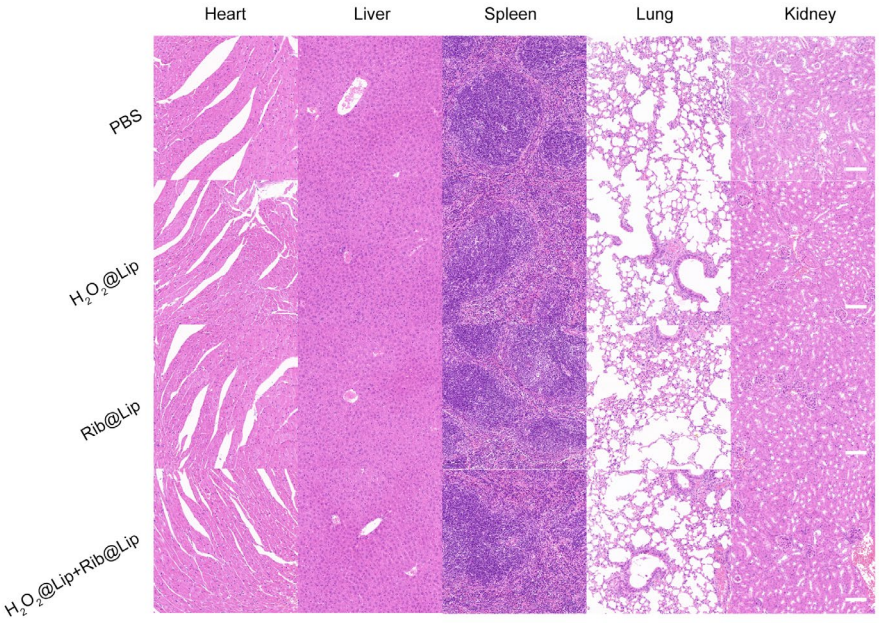

Figure s14. H&E staining of the five major organs (heart, liver, spleen, lung, and kidney) with different nanoliposome treatments.

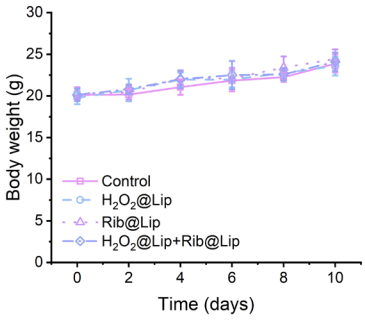

Figure s15. Body weights of CT26 tumor-bearing mice with different treatment (n = 5).

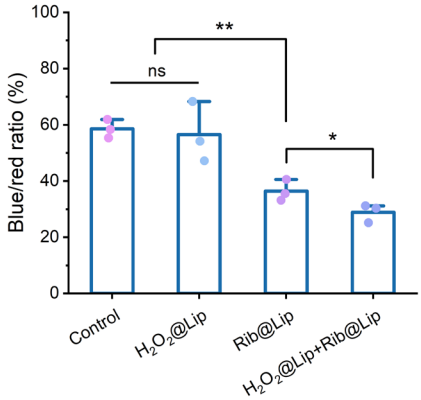

Figure s16. HA content in tumor tissues quantized from AB&NFR staining using ImageJ (n = 3).

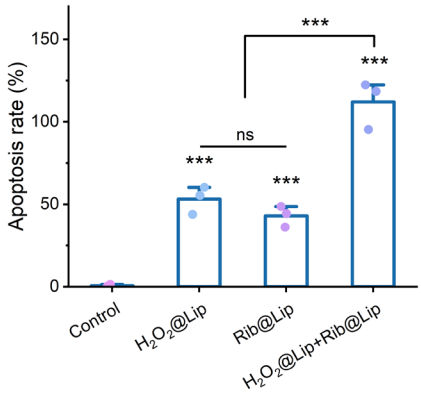

Figure s17. Apoptosis of tumor tissues quantized from TUNEL staining using ImageJ (n = 3).