Experimental Procedures

Materials and reagents.

Ethylene glycol (EG), polyvinyl pyrrolidone (MW: 10 000), iron (III) chloride hexahydrate (FeCl3·6H2O, 99%) anhydrous sodium acetate (NaAc, 99%) were purchased from Sigma-Aldrich. NaOH, ethylene glycol and ammonium hydroxide solution were purchased from Sinopharm Chemical Reagent Co., Ltd. Agarose was purchased from Yare Shanghai. The reduced GSH assay kit was purchased from Nanjing Jiancheng Bioengineering Institute. Sulfur powder (S, 99%) was obtained from Shanghai Rongtai Pharmatech Co. Gibco RPMI 1640 medium were purchased from Invitrogen (Shanghai) Trading Co. The other reagents used in this work were purchased from Sinopharm Chemical Reagent (China) and Aladdin-Reagent (China).

Cell culture

4T1 mouse breast cancer cell line was obtained from the Cell Bank of the Chinese Academy of Sciences and incubated in RPMI-1640 medium supplemented with 10% FBS in a humidified atmosphere at 37℃.

Preparation of pyrite nanozymes (FeS_2).

The pyrite nanozymes with a size of 148 nm were prepared by a one-pot solvothermal method. In a typical synthesis, 0.7 g of PVP was dispersed into 30 mL of EG solution, and then 0.5 g of FeCl3·6H2O was added under constant magnetic stirring. Next, 3.6 g of NaAc was added into above mixture under vigorous stirring. After that, 0.4 g of S powder was added, and the resultant mixture was ultrasonicated for 1 h to form a homogeneous dispersion. Then, the reaction mixture was transferred into a 40 mL Teflon-lined stainless-steel autoclave, which was sealed and maintained at 473 K for 12 h. After the reaction was cooled to room temperature naturally, the resultant black precipitates were collected by centrifugation at 10 000 rpm for 10 min. Then, the collected black products were washed with $CHCl_3$ to remove the excess S and using absolute alcohol and ultrapure water several times to remove impurities. After centrifugation, the products were dried in a vacuum lyophilizer overnight for further

characterization. After centrifugation, the products were dried in a vacuum lyophilizer overnight for further characterization. For preparing pyrite nanozymes with a size of 280 nm, 0.8 g of $FeCl_3 \cdot 6H_2O$ was used, while the other conditions were kept constant as above. Bare pyrite nanozymes with a size of 687 nm were also synthesized following a similar procedure without PVP.

Preparation and characterization of AFH

The general protocol for the hydrogel preparation is as follows. The prepared FeS_2 (10 mg/mL in PBS) and AIPH (1 mg/mL in a mixed solvent of DMSO and water (8: 2)) were added into 2% agarose solution to form AFH (FeS_2 concentration: 1.67 mM). The prepared FeS_2 (10 mg/mL in PBS) was added into 2% agarose solution to form FH. Scanning electron microscopy (SEM) images were captured on a Hitachi FE-SEM S4800 instrument with an acceleration voltage of 3 kV. The morphology structures of FeS_2 were detected by the TEM (JEOL-2100). UV-vis spectra of samples were recorded by the UV-vis spectrophotometry Lambda 35 (Perkin-Elmer).

Rheological Test

Rheology experiments were performed on an Anton Paar rheometer. Hydrogel samples of different temperatures were prepared and gently placed on the middle of a 15 mm diameter parallel plate with a proper gap. Dynamic oscillatory frequency sweep measurements were conducted at a 1% strain amplitude. To prevent the evaporation of water, a lid was prepared on the top.

Photothermal Conversion Ability

An 808 nm NIR laser (Changchun New Industries Tech.Co., Ltd., Changchun, China) with irradiation powers was used to stimulate the concentrations of FeS_2 (0, 50, 100, 200 ug/mL) in an aqueous medium. The photothermal images of the FeS_2-based suspensions during laser irradiation were recorded every 30 s using an infrared thermal imaging system. The NIR laser source was equipped with a 4 mm diameter laser module with an adjustable power.

***In vitro* anti-cancer effect of AFH**

Typically, 4T1 cells were incubated in six-well plates at 37 °C with 5% CO_2 for 24 h; afterward, the culture medium was replaced by new culture medium and the Afterwards, cells were incubated for 5 different groups: (1) PBS + NIR; (2) AFH; (3) AIPH; (4) FH + NIR; (5) AFH + NIR. The FeS_2 concentration was 200 μg/mL in group 2, 4, and 5. The AIPH concentration was 20 μg/mL in group 2 and 5. Then, cells in group 1, 4 and 5 were exposed to 808 nm laser radiation (0.5 W/cm^2) for 5 min. After incubation for another 6 h, both fluorescein diacetate and propidium iodide (live cells, green fluorescence; dead cells, red fluorescence) were used to costain the cells to determine the effect of AFH, and detected under a fluorescent microscope (IX81, Olympus, Japan). To quantitatively analyze the therapy effect of AFH, cells were plated and incubated in 96-well plates at 37 °C in an atmosphere of 5% CO_2 and 95% air for 24 h. Above-mentioned Different formulations were added and the cells continued incubating for another 6 h. Finally, the viability of 4T1 cells was determined by a CCK-8 cell cytotoxicity assay. The cell viability was normalized by control group without any treatment.

***In vitro* ROS generation**

ROS generation was also assessed in vitro on 4T1 cells. The intracellular generation of ROS was detected utilizing 2′,7′-dichlorofluorescein diacetate (DCFH-DA) as a fluorescent probe. Using confocal laser scanning microscope (CLSM). Afterwards, cells were incubated for 5 different groups: (1) PBS + NIR; (2) AFH; (3) AIPH; (4) FH + NIR; (5) AFH + NIR. The AIPH concentration was 20 μg/mL in group 2 and 5. Then, cells in group 1, 4 and 5 were exposed to 808 nm laser radiation (0.5 W/cm^2) for 5 min. Then, DCFH-DA fluorescent probe were used to detect different ROS level.

Detection of Intracellular GSH.

The commercially available GSH assay kit was used to detect the depletion of GSH. 4T1 cells were incubated with 5 different group: (1) PBS + NIR; (2) AFH; (3) AIPH; (4) FH + NIR; (5) AFH + NIR. The AIPH concentration was 20 μg/mL in group 2 and 5. Then, cells in group 1, 4 and 5 were exposed to 808 nm laser radiation (0.5 W/cm2)

for 5 min. After 12 hours of incubation, the GSH content was measured by employing a commercial colorimetric GSH assay kit from Beyotime Biotechnology, Shanghai, China. The assay was carried out according to the manufacturer's instructions. The absorbance of 340 nm was measured by a microplate reader.

Animal tumor models

Female BALB/c nude mice aged 4-5 week were purchased from Vital River Company (Beijing, China). 100 μL of 4T1 cell suspension (1×10^6 cells per mL) were subcutaneous injected into each mouse to establish the tumor models. The animal experiments were carried out according to the protocol approved by the Ministry of Health in People's Republic of PR China and were approved by the Administrative Committee on Animal Research of the Wuhan University.

***In vivo* infrared thermography**

To monitor the in vivo photothermal effect, AFH was intratumorally injected into the tumor-bearing mice, and then the tumors suffered from 0.5 W/cm^2 irradiation for 10 min at 1 h post-injection. PBS injection used as control group. Meanwhile, the temperature at the tumor was monitored using an infrared camera (Fotric 225).

***In vivo* antitumor study**

The mice were firstly divided randomly into 5 groups (each group included 5 mice): (1) PBS + NIR; (2) AFH; (3) AIPH; (4) FH + NIR; (5) AFH + NIR. The AIPH concentration was 10 mg/kg in group 2, 4, and 5. Then, cells in group 1, 4 and 5 were exposed to 808 nm laser radiation (0.5 W/cm^2) for 20 min. The injection method is intratumoral injection. NIR was conducted 1h after the injection. Mice body weight and tumor volume in all groups were monitored every 2 days. A caliper was employed to measure the tumor length and tumor width and the tumor volume was calculated according to following formula. Tumor volume = tumor length × tumor width^2 / 2. After 15 days treatment, mice were sacrificed. Five main organs (heart, liver, spleen, lung and kidney) of all mice were harvested, washed with PBS, and fixed with paraformaldehyde for histology analysis. The blood samples from these mice (≈1 mL) were collected for blood biochemistry analysis. And the tumor tissues were weighed, and fixed in 4% neutral buffered formalin, processed routinely into paraffin, and

sectioned at 4 μm. Then the sections were stained with ROS, Ki-7 and TUNEL and finally examined by using an optical microscope (BX51, Olympus, Japan).

Statistical analysis

Data analyses were conducted using the GraphPad Prism 5.0 software. Significance between every two groups was calculated by the Student's t-test. *P < 0.05, **P < 0.01, ***P < 0.005.

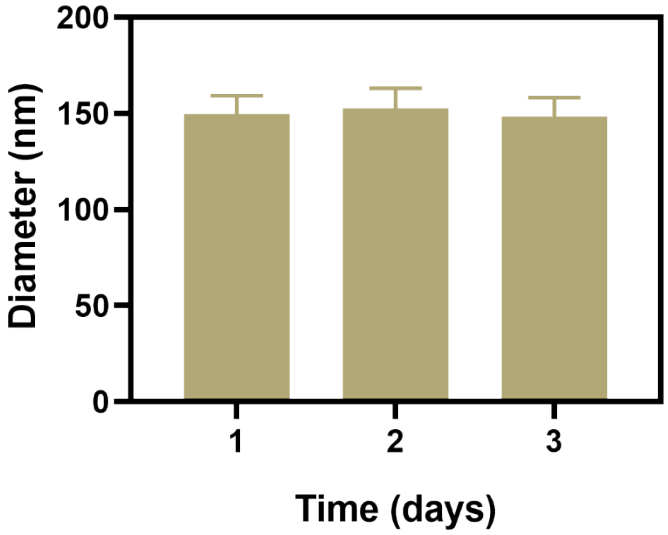

Fig. S1. Statistical graph of measured diameter size of FeS_2.

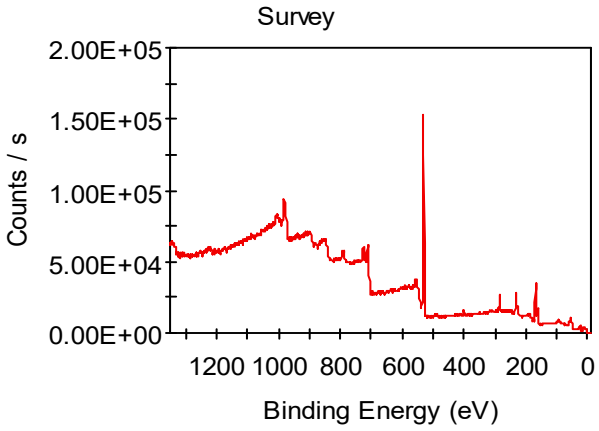

Fig. S2. high-resolution XPS spectra of FeS_2.

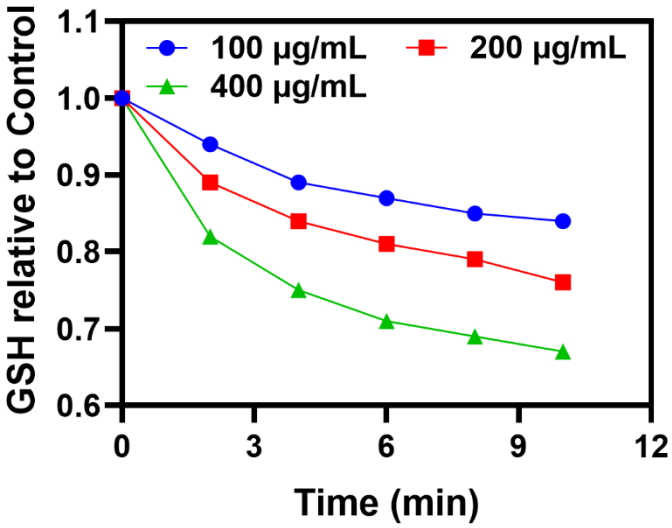

Fig. S3. Time-dependent reduction of GSH after incubating with the indicated concentrations of FeS_2.

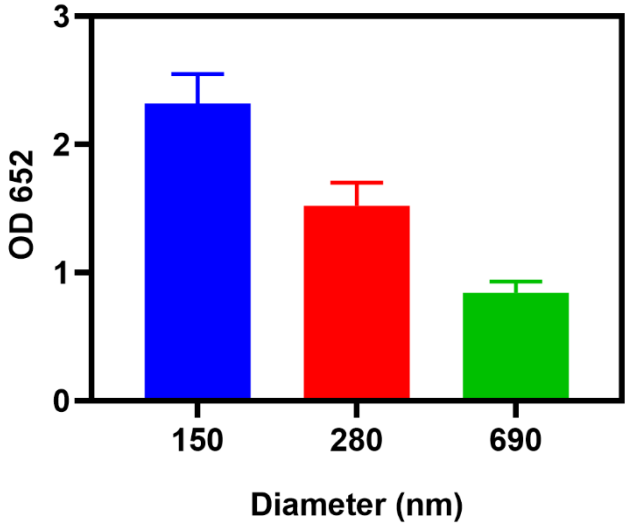

Fig. S4. The size dependency of the POD-like activity of pyrite nanozymes.

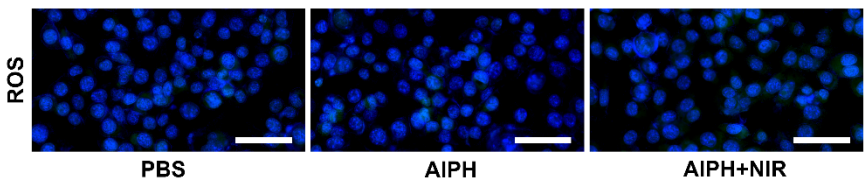

Fig. S5. Tumor cells DCFH-DA fluorescence images were observed after the indicated treatments. Scale bars = 50 μm.

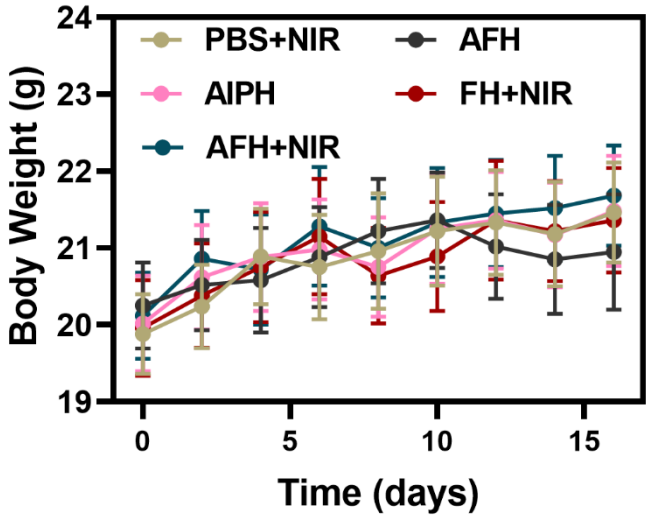

Fig. S6. Changes in body weight in response to the indicated treatments. liver function markers: BUN, (C) CRE and (D) ALT, ALP, and AST after various treatments.

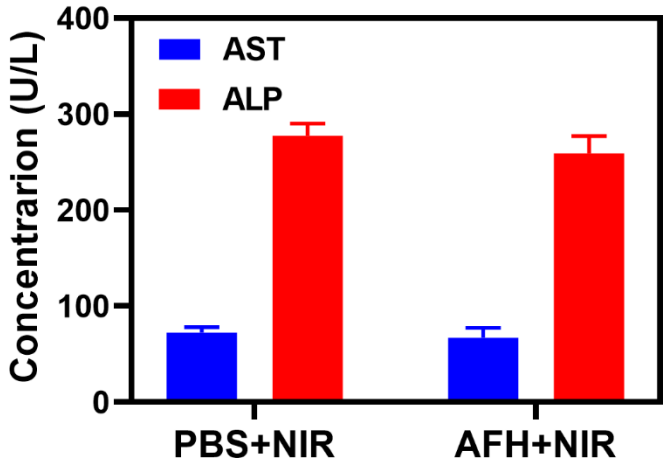

Fig. S7. Liver function markers: ALT and ALP after various treatments.