Supporting Information

Degradable Silver-Based Nanoplatform for Synergistic Cancer Starving-Like/Metal Ion Therapy

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Materials.

Silver trifluoroacetate (CF_3COOAg), Ethylene glycol (EG), Thioctic acid (TA), and acetone were purchased from JK Chemical. Glucose oxidase (GOx), poly(vinyl pyrrolidone) (PVP, MW ≈ 55 000) and H_2O_2 Assay Kit was purchased from Sigma-Aldrich. All reagents were of analytical grade and used without any purification.

Characterization.

Transmission electron microscopy (TEM) images were taken on a JEM-1230 TEM (JEOL, Tokyo, Japan). UV-Vis absorption spectra were measured on a Cary 60 UV–vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). Fluorescence spectra were measured on a Thermo Scientific Lumina fluorescence spectrophotometer (Thermo Fisher Scientific Co., USA. Zeta potential was measured on a Malven model Zetasizer 2000 zeta potential analyzer. FT-IR spectra were collected on an attenuated total reflectance FTIR spectrometer (Spectrum Two™,

PerkinElmer). The H_2O_2 concentration in solutions were detected by a H_2O_2 Assay Kit (Biyotime, Shanghai, China). The H_2O_2 concentration in the cells was measured by a H_2O_2 Assay Kit (Sigma, Shanghai, China). PA/US imaging was performed on a VisualSonics Vevo LAZR system (VisualSonics Inc. New York, NY). Fluorescence imaging was performed on an IVIS Spectrum system (Caliper Life Sciences, Hopkinton, MA).

Synthesis of Ag nanocube.

Ag nanocube (AgNC) were prepared according to the method reported previously. In detail, we used ethylene glycol (EG) as the solvent and silver trifluoroacetate (CF_3COOAg) as the precursor. After 0.06 mL NaHS (3 mM in EG), 0.5 mL of HCl (3 mM in EG) and 1.25 mL of poly(vinyl pyrrolidone) (PVP, 20 mg/mL in EG, MW ≈ 55 000) were added orderly, 0.4 mL CF_3COOAg (282 mM in EG) were injected to the mixture to initialize the reaction. The reaction was kept stirring at 150 °C for 20 min to get a brownish solution. The resulting product was collected by centrifugation (9000 g, 10 min) and dispersed in 5 mL deionized water for further use.

Preparation of AgNC-TA.

To couple AgNC with thioctic acid (TA), we injected 34 μL NaOH (0.5 M) into 5 mL AgNC solution prepared above under stirring to adjust pH value to 11. Then we slowly added 500 μL thioctic acid (15 mM in ethanol) to the solution. The solution was stirred for 2 h to produce AgNC-TA and the final product was centrifuged at 9000 g for 10 min and dispersed in 2 mL deionized water for further use.

Preparation of AgNC-GOx.

2 mg NHS and 3 mg EDC were dissolved into 1 mL deionized water, respectively. 10 μL of each were injected into 2 mL AgNC-TA solution prepared above and kept the system static for 0.5 h. Then 3 mg of glucose oxidase (GOx) was dissolved into 1 mL deionized water and injected into the solution prepared above under stirring for 1 h. The final product was collected by centrifugation (9000 g 10 min) and dispersed in deionized water for further use.

Preparation of AgNC-GOx-IR800.

AgNC-GOx (200 μL，5 μM) was pipetted into the microcentrifuge tube. Deionized water was added to adjust the reaction volume to 2.0 ml. pH was adjusted to 8.5–8.7 using 50 mM Na_2CO_3. IRDye800CW NHS ester-stock solution was diluted to 1.5 mM in DMSO and immediately added to AgNC-GOx solution for a 2.0-fold molar excess of the dye over AgNC-GOx. The mixture was incubated for 2 h at room temperature (300 rpm). The final product was collected by centrifugation (9000 g, 10 min) and dispersed in deionized water for further use.

Measurement of intracellular H_2O_2 concentration.

Each 4T1 tumor-bearing mouse was intratumorally injected with 100 μL of AgNC-GOx, and the tumor was unclamped after 1 hour. Tumor tissue was homogenized by adding 100 μL of cell lysate per 5 mg of tissue. Centrifuged at about 12000g at 4˚C for 3 minutes and taken the supernatant for detection. Taken 50 μL of supernatant and added 100 μL of hydrogen peroxide detector. After 30 minutes at room temperature, measure A560. Hydrogen peroxide concentration was calculated from the standard curve.

In vitro toxicity of AgNC against tumor cells.

A375 cells were cultured in DMEM (10% fetus bovine serum), while 4T1 cells were cultured in 1640 (10% fetus bovine serum). Cells were seeded into 96-well plates at a density of 1×10^4 cells per well. After incubation for 24 h in the incubator, cells were washed once with PBS. Different concentrations (2, 5, 10, 20, 100, 200, 500, 1000 nM) of AgNC in media were added into the wells and co-incubated for 24 h. To evaluate the cytotoxicity, the cells of each group were rinsed twice with PBS. The standard MTT assay was carried out to evaluate the cell viability.

In vitro toxicity of H_2O_2 against tumor cells.

A375 cells were cultured in DMEM (10% fetus bovine serum), while 4T1 cells were cultured in 1640 (10% fetus bovine serum). Different concentrations (0.3, 0.6, 1.5, 2, 3, 4 mM) of H_2O_2 in media were added into the wells and co-incubated for 24 h. To

evaluate the cytotoxicity, the cells of each group were rinsed twice with PBS. The standard MTT assay was carried out to evaluate the cell viability.

***In vitro* promotion of glucose for A375 cell proliferation.**

A375 cells were cultured in DMEM (10% fetus bovine serum). Different concentrations (0.3, 0.6, 1.2, 2.4, 6 mM) of glucose in DMEM media were added into the wells and co-incubated for 24 h. To evaluate the promotion, the cells of each group were rinsed twice with PBS. The standard MTT assay was carried out to evaluate the cell viability.

***In vitro* evaluation of synergistic starving-like/metal ion therapy.**

A375 cells were cultured in DMEM (10% fetus bovine serum), while 4T1 cells were cultured in 1640 (10% fetus bovine serum). To stain live and dead cells, the cells of each group were incubated with calcein AM (4 μM) and propidium iodide (4 μM) for 30 min, respectively. Then the fluorescence of cells was examined using a Nikon Eclipse Ti inverted fluorescence microscope (Nikon Canada, Mississauga, Canada).

Intratumoral blood oxygen saturation assessment.

The intratumoral oxygen saturation (sO_2) was monitored and measured on a VisualSonic Vevo LAZR instrument. The corresponding PA/US coregistered images were acquired using 'Oxyhemo' mode, which collects PA data at 750 and 850 nm and creates a parametric map of estimated oxygen saturation at a rate of 1 Hz. The 4T1 tumor-bearing nude mice were anesthetized using 2% isoflurane in oxygen. After intratumoral injection of AgNC-GOx (50 nM, in 50 μL saline), the PA/US coregistered images were collected in 0.5, 1 and 4 h to monitor the change of the intratumoral sO_2. The PA signal of the region of interest (ROI) was measured by using the Vevo LAZR imaging system software package.

***In vivo* evaluation of synergistic starving-like/Ag^+ therapy.**

The mice were divided into 4 groups. The first group was injected with 50 μL PBS as control group; the second group was injected with pure AgNC (2μM, 50 μL) as AgNC group; the third group was injected with pure GOx (0.9 g mL^{-1}, 50 μL) as GOx

group; the fourth group was injected with AgNC-GOx (2 μM, 50 μL) as AgNC-GOx group. During 15 days after the corresponding treatments, the volume of tumors was measured every other day and calculated by the following equation: volume = width^2 × (length/2). Besides, the tumors were sectioned into slices and Hematoxylin and eosin (H&E) staining were performed for histological analysis.

Evaluation of mice survival.

All experiments with live animals were conducted in accordance with a protocol approved by the National Institutes of Health Clinical Center Animal Care and Use Committee (NIH CC/ACUC). In general, the mice must be euthanized when the tumor size reaches 2 cm, so the mice survival was evaluated based on the life span from the date when the tumor received treatment to the date when the tumor size reached 2 cm. For each group subjected to the corresponding treatment, the survival rate was calculated by dividing the number of surviving mice at different days of post-treatment with the total number of mice before treatment.

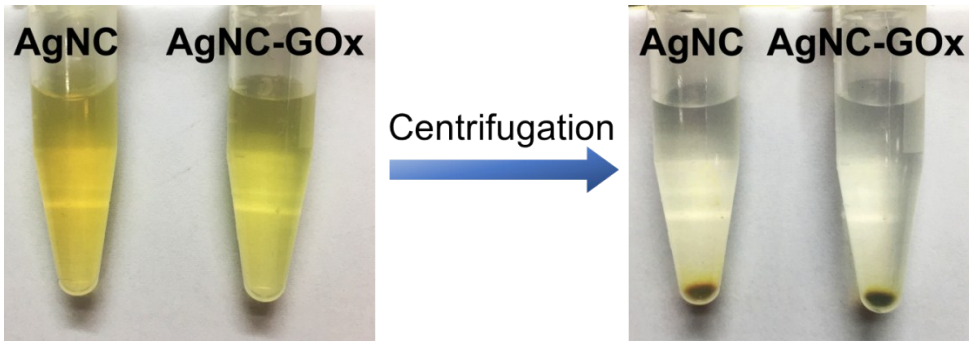

Figure S1. Digital photos of AgNC and AgNC-GOx in solutions before (left) and after (right) centrifugation. Importantly, the colorless supernatant after centrifugation confirms the covalent conjugation of GOx onto the surface of AgNC without obvious leakage.

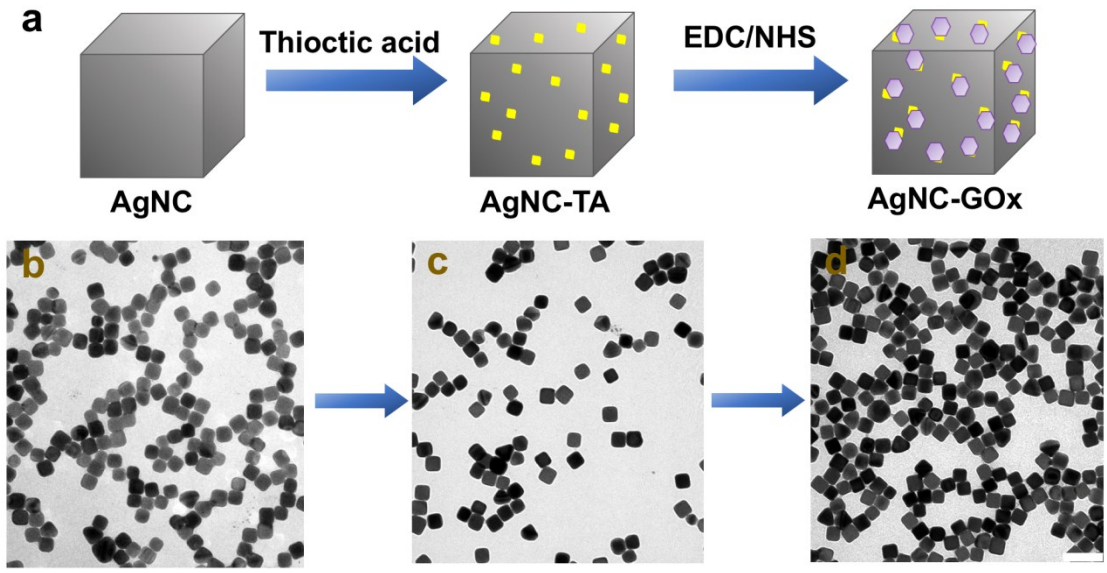

Figure S2. a) Schematic illustration of AgNC-GOx synthesis process. b-d) TEM images of (b) AgNC, (c) AgNC-TA, (d) AgNC-GOx. Scale bar: 100 nm.

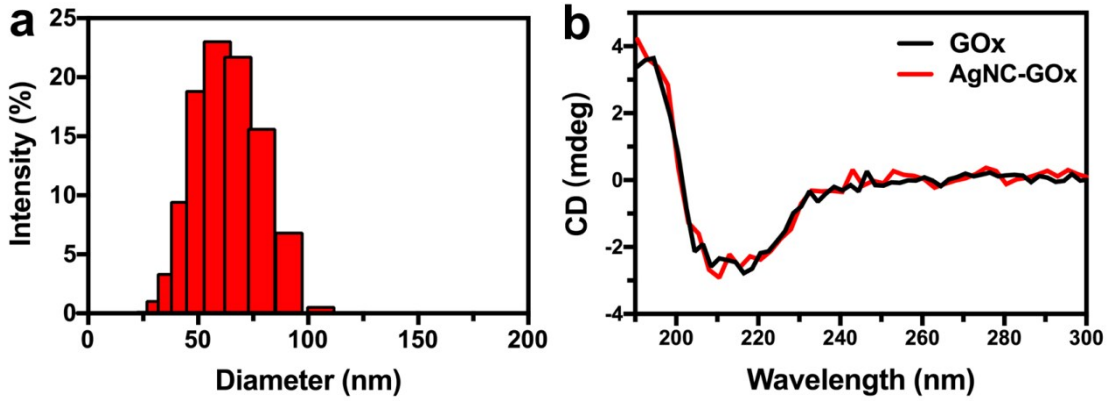

Figure S3. a) Hydrodynamic diameter distribution of AgNC-GOx that measured by DLS. b) CD spectra of AgNC-GOx and GOx, demonstrated that the conjugation of AgNC and GOx did not cause obvious second structure changes of GOx.

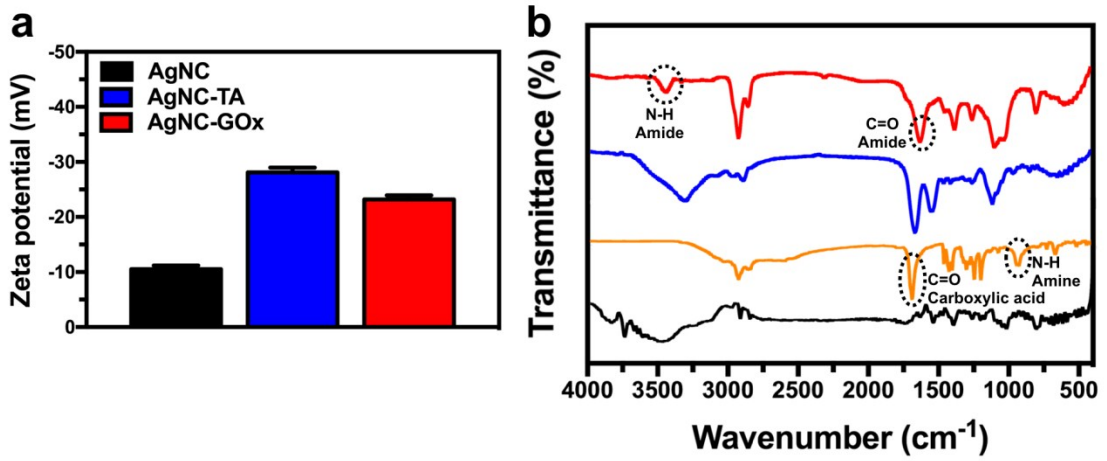

Figure S4. a) Zeta potential of AgNC, AgNC-TA and AgNC-GOx solutions. b) FTIR spectra of AgNC (black), AgNC-TA (yellow), GOx (blue) and AgNC-GOx (red).

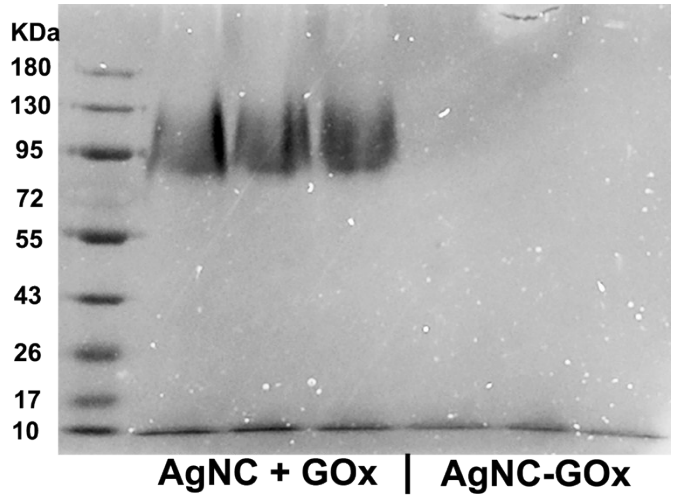

Figure S5. SDS-PAGE of GOx in AgNC-GOx or AgNC/GOx mixture (three parallel groups).

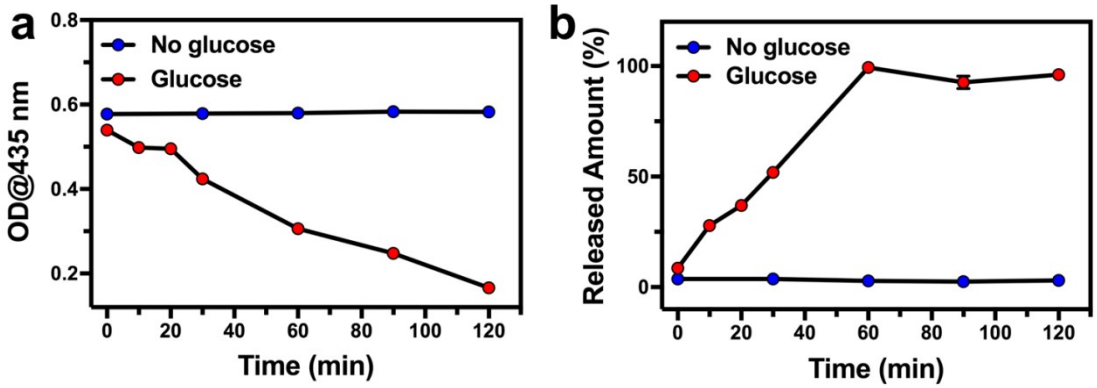

Figure S6. a) Absorbance changes of AgNC-GOx at 435 nm with or without glucose. b) The release profile of GOx from AgNC-GOx with or without glucose (10 mM).

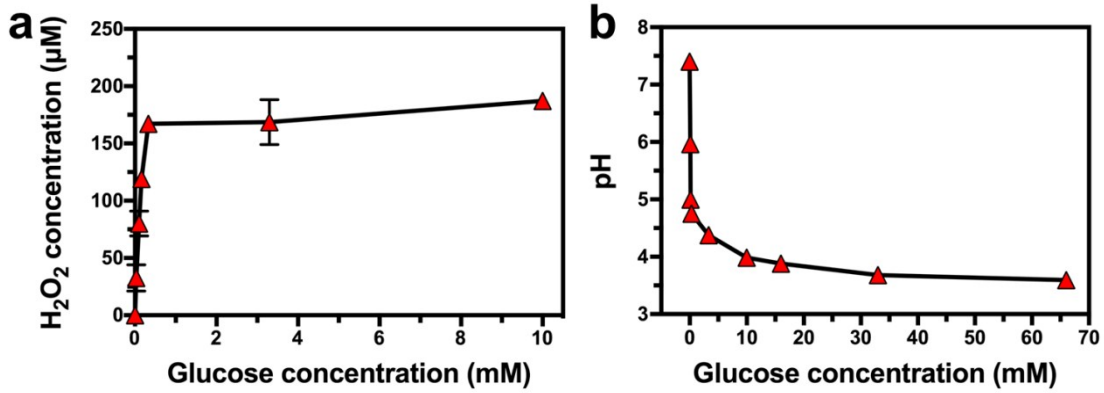

Figure S7. a) The concentrations of generated H_2O_2 and b) pH values at different concentrations of glucose arising from the reaction between GOx and glucose.

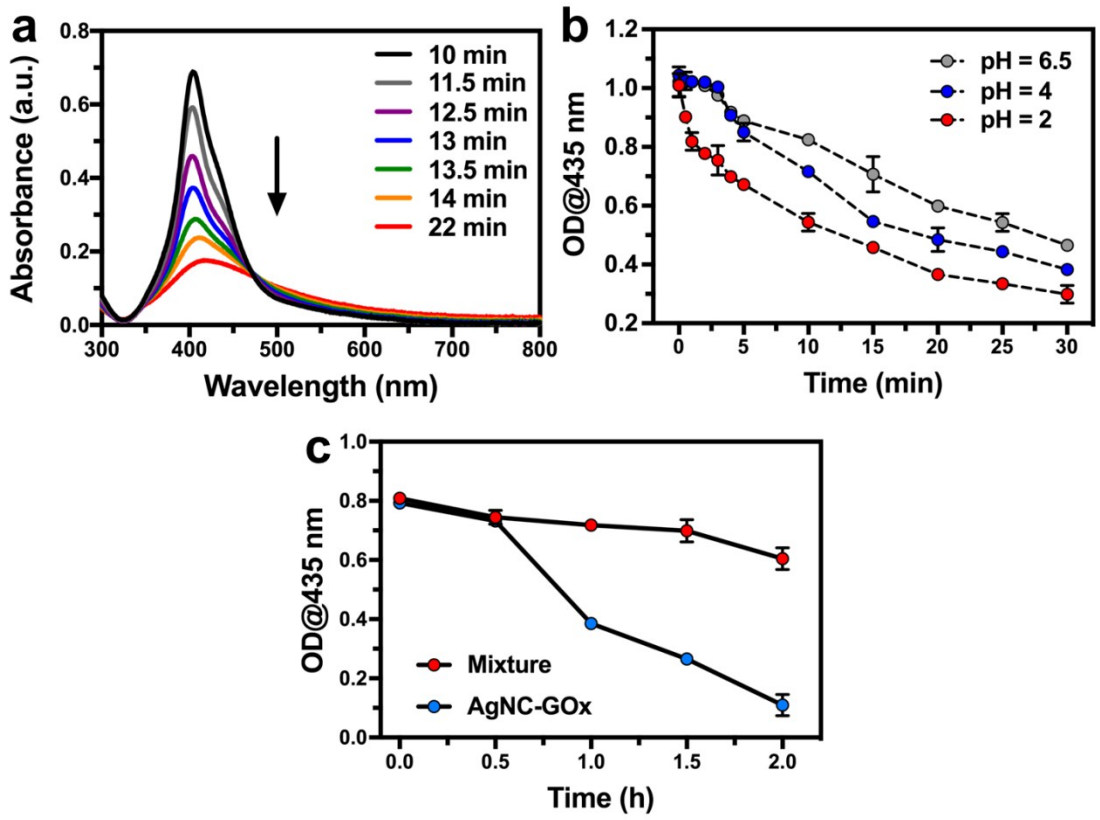

Figure S8. a) UV spectra of AgNC in H_2O_2 solutions. The absorption of AgNC in 0.1 mM H_2O_2 solutions was decreased as reaction time was prolonged, which indicated that AgNC could be degraded into Ag^+ ions in H_2O_2 solutions. b) The decrease curve of absorbance at 435 nm of AgNC in H_2O_2 solutions with time (pH = 2, 4 or 6.5). c)

The decrease curve of absorbance at 435 nm of AgNC with time in the AgNC/GOx mixture solution or AgNC-GOx solution combined with glucose.

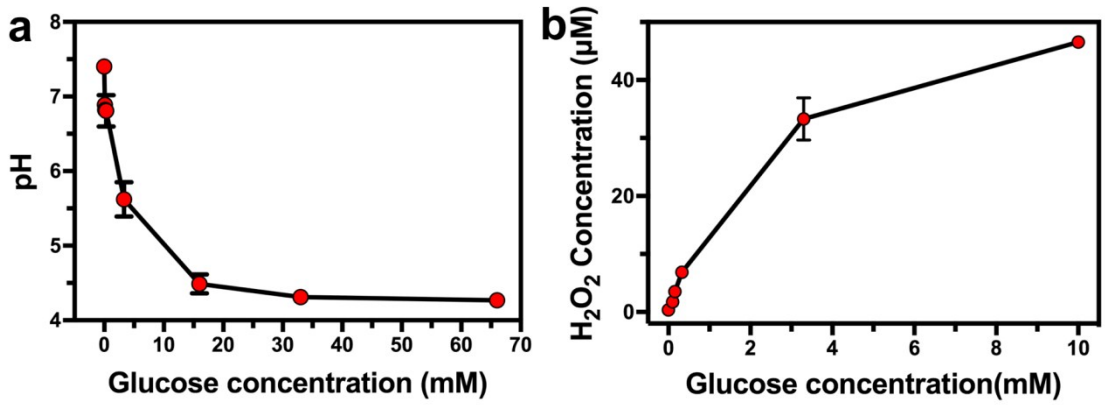

Figure S9. a) pH values and b) H_2O_2 concentration of AgNC-GOx solution after 1 h incubation with different concentrations of glucose.

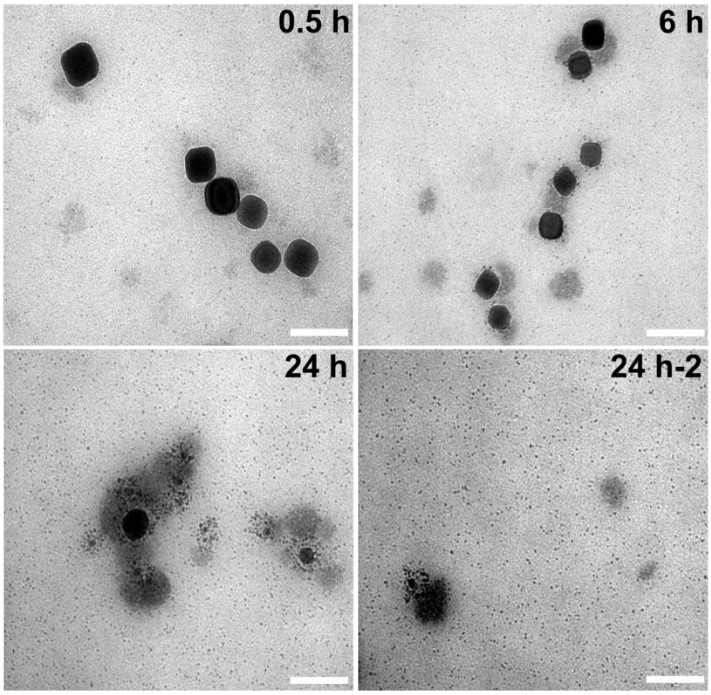

Figure S10. TEM images of AgNC-GOx inside 4T1 cells incubated with glucose (2 mM) for different time periods. Scale bars: 100 nm.

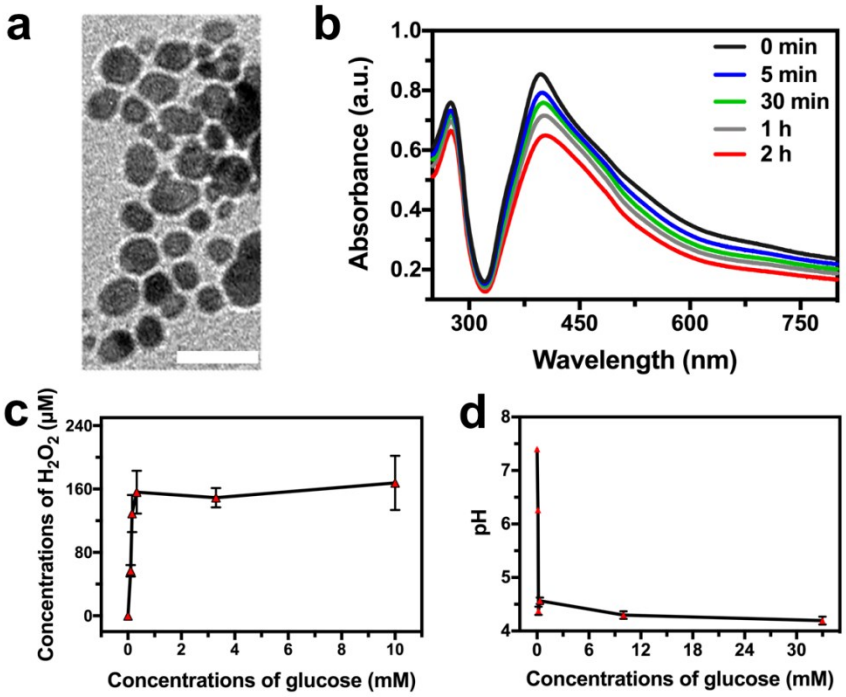

Figure S11. (a) TEM image of round silver nanoparticles (scale bar: 50 nm). (b) Absorbance changes of AgNC-GOx at 435 nm during 2 h incubation with glucose (2 mM). (c) H_2O_2 concentrations and (d) pH values of AgNC-GOx solution after 1 h incubation with different concentrations of glucose.

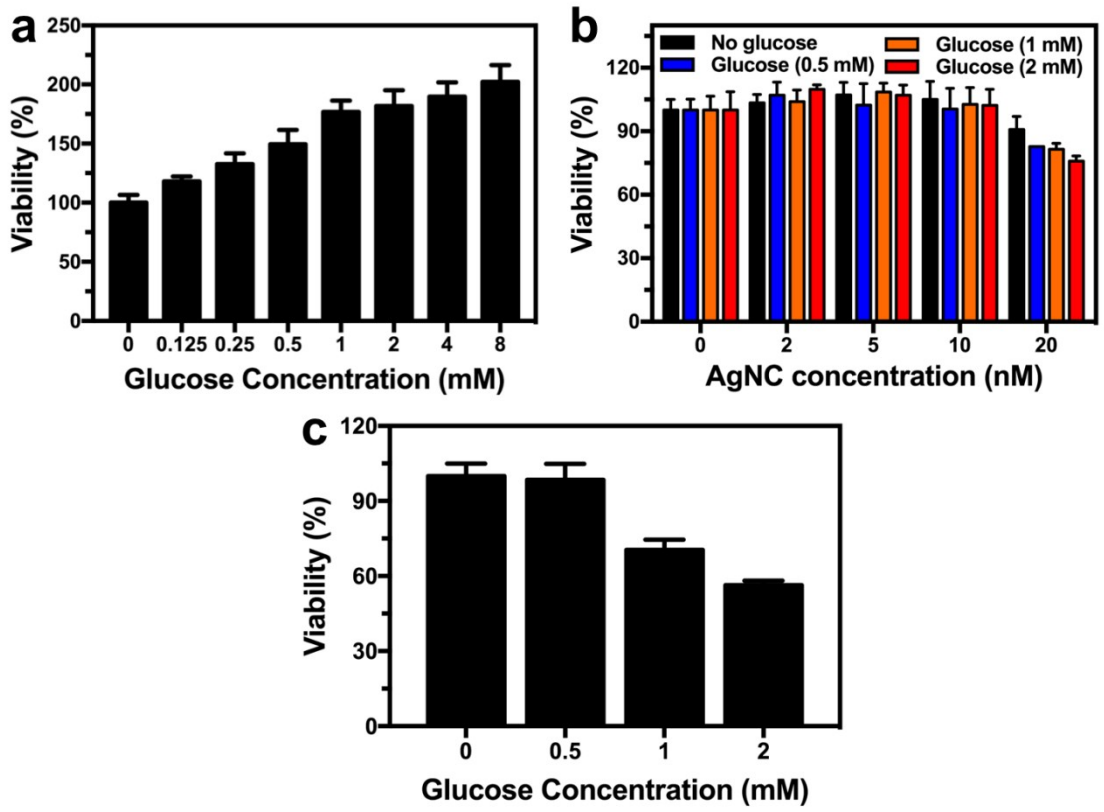

Figure S12. a) Viability of 4T1 cells in different concentrations of glucose-containing 1640 media. b) Viability of 4T1 cells after 24 h of incubation with AgNC in different concentrations of glucose-containing 1640 media. c) Viability of 4T1 cells after 24 h of incubation with the mixture of AgNC and GOx in different concentrations of glucose-containing 1640 media ([AgNC] = 5 nM, [GOx] = 67 pM).

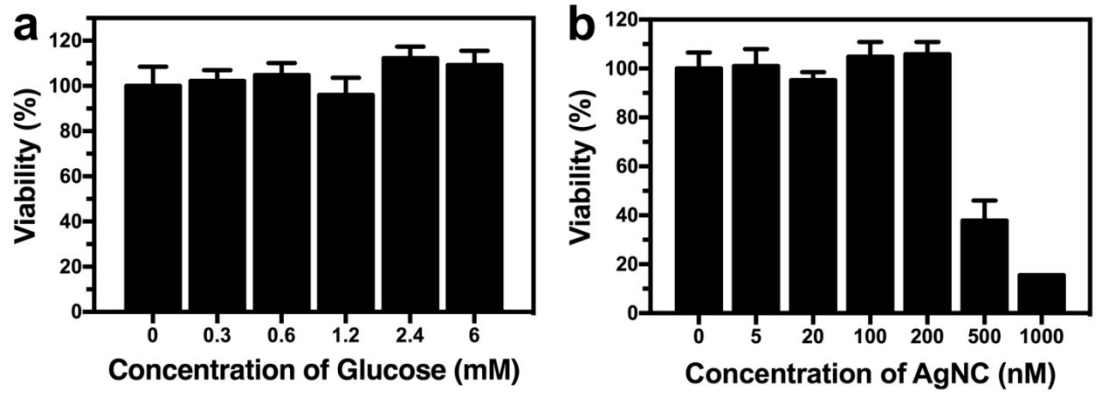

Figure S13. a) Viability of A375 cells in different concentrations of glucose-containing DMEM media. b) Viability of A375 cells after 24 h of incubation with AgNC in different concentrations of DMEM media ([glucose] = 2 mM).

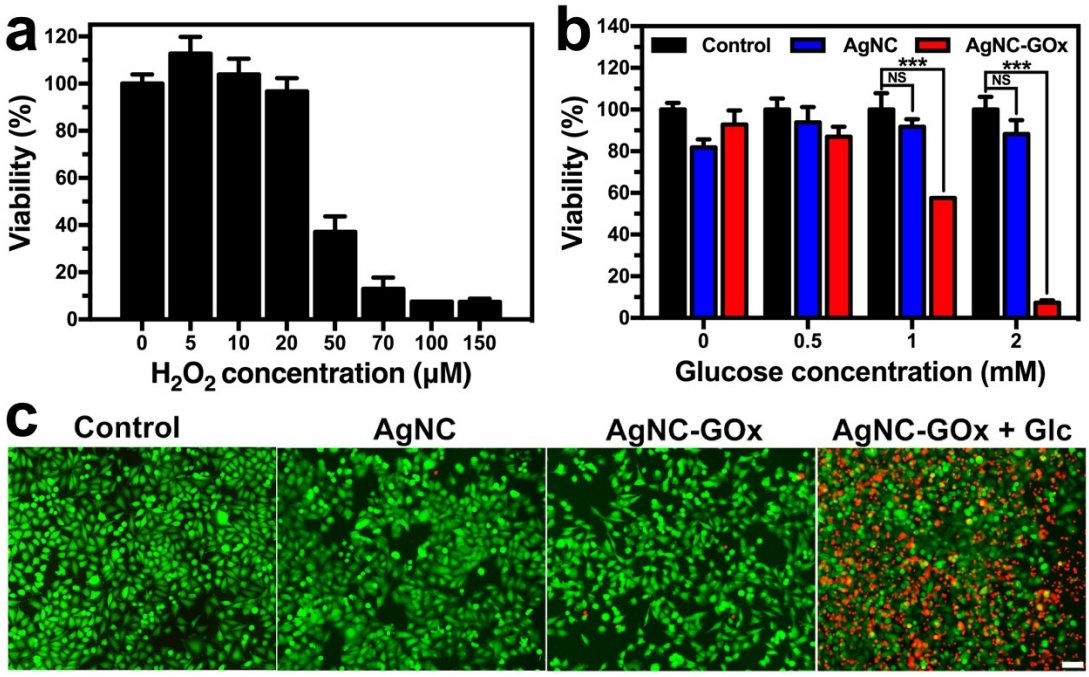

Figure S14. a) Viability of A375 cells after 24 h of incubation with H_2O_2 at different concentrations. b) Viability of A375 cells after 24 h of incubation with AgNC or AgNC-GOx in different concentrations of glucose-containing DMEM media. c) Fluorescence images of CA/PI stained A375 tumour cells after 24 h incubation with fresh medium, AgNC or AgNC-GOx.

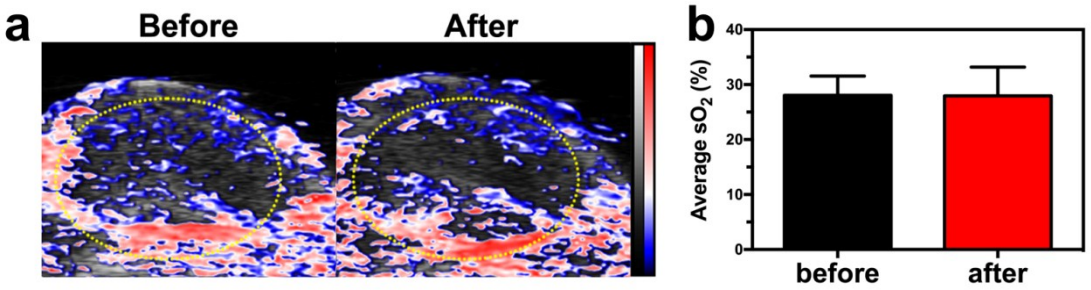

Figure S15. a) PA oxygen saturation mapping and US coregistered imaging of 4T1 tumors before and after 1 h of *i.t.* injection of PBS. b) The corresponding quantitative analysis of the sO_2 levels in 4T1 tumors before and after *i.t.* injection of PBS.

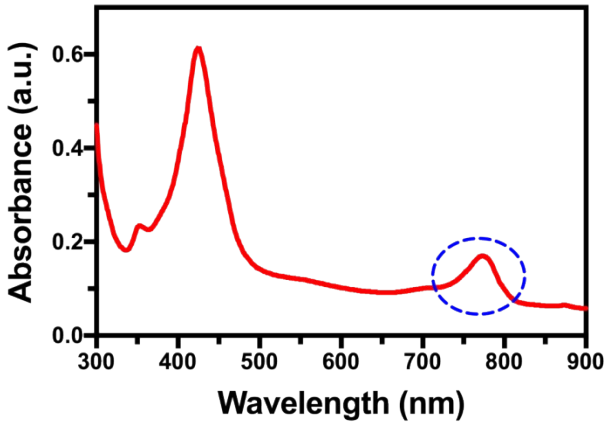

Figure S16. UV-Vis-NIR spectra of AgNC-GOx-IR800. The blue circles show the absorption peak of IR800, which confirms the successful connecting of IR800 to AgNC-GOx.

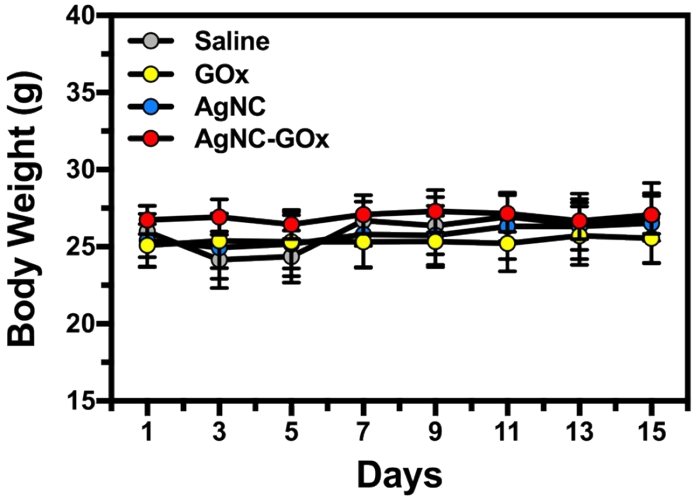

Figure S17. The body weight change of 4T1 tumor-bearing mice with different treatments.

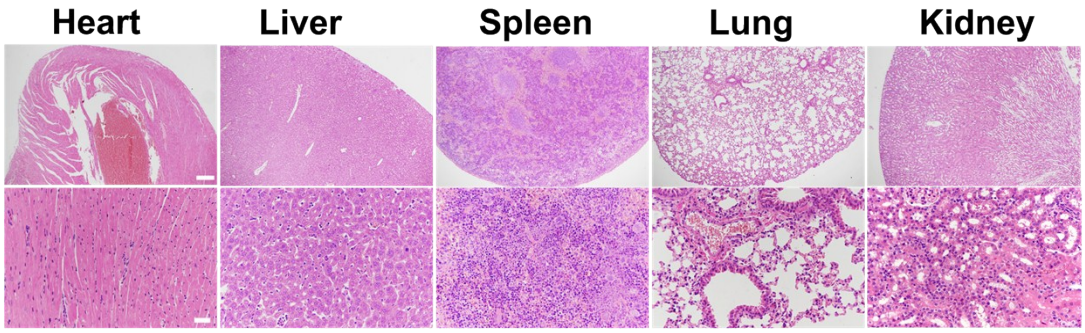

Figure S18. Representative H&E staining images for cellular morphology from major organs of healthy nude mice. The mice were intratumoral injected with AgNC-GOx ([AgNC] = 2 μM) and sacrificed 15 days after injection. Scale bar: 600 μm and 100 μm, respectively.