Supporting Information

Hydrogen-producing Hyperthermophilic Bacteria Synthesized Size-controllable Fine Gold Nanoparticles with Excellence for Eradicating Biofilm and Antibacterial Applications

Experimental setiction

Materials. Hydrogen tetrachloroaurate (III) ($HAuCl_4 \cdot 3H_2O$) were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). H_2O_2 was obtained from Beijing Chemicals (Beijing, China). 2,2'-Azinobis (3-ethylbenzthia-zoline-6-sulfonate) (ABTS) and crystal violet were obtained from Aladdin Chemistry Co., Ltd. (Shanghai, China). *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) bacterial strains were obtained from Chuanxiang Biotechnology, Ltd. (Shanghai, China). The type strain is CBS-Z^T (=DSM 26941^T=CGMCC 1.5180^T) was a kind gift provided by professor Zuoming Zhang, Jilin University. Ultra-pure water (18.2 MΩ; Millpore Co., USA) was used throughout the experiment. All reagents were used as received without any further purification.

Measurements and characterizations. Fluorescence measurements were measured using a JASCO FP-6500 spectrofluorometer (JASCO International Co., Japan). The UV/Visible absorption spectra were carried out on a JASCO V550 UV/Visible spectrophotometer (JASCO International Co., LTD., Tokyo, Japan), equipped with a Peltier temperature control accessory. Scanning electron microscope (SEM) images were recorded using a HITACHI S-4500 instrument. The SEM samples were prepared by depositing a dilute aqueous dispersion of the as-prepared samples onto a silicon wafer. The transmission electron microscopy (TEM) images were recorded using a FEI TECNAI G2 20 high resolution transmission electron microscope operating at 200 kV. X-ray diffraction (XRD) spectra were obtained from a D8 Focus diffractometer (Bruker) at a scanning rate of 0.2° min^{-1} by using Cu-Kμ radiation (λ=0.15406 nm). The operation voltage and current were kept at 40 kV and 40 mA. The X-ray photoelectron spectra (XPS) measurements were carried out using an ESCALab220i-XL electron spectrometer from VG scientific using 300 W AlKα radiations.

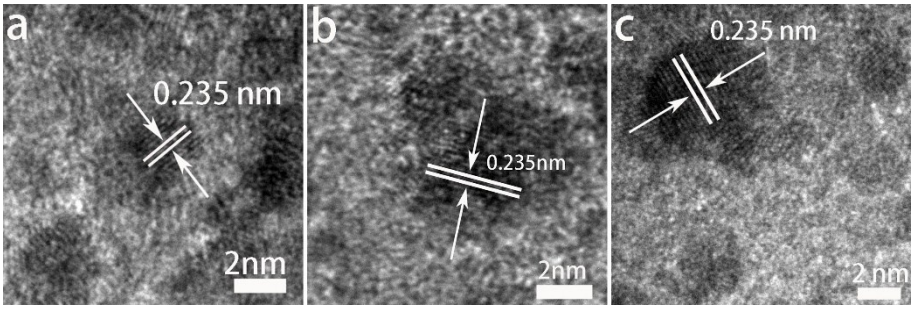

Figure S1. HRTEM images of representative images of individual (a)AuNPs-small, (b) AuNPs-moderate and (c) AuNPs-big.

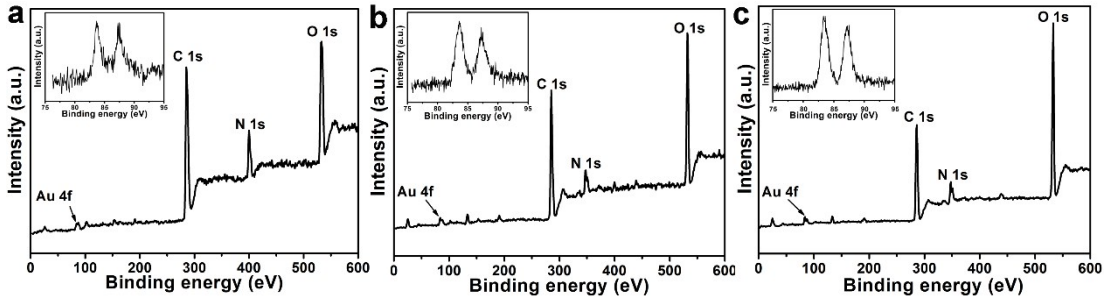

Figure S2. XPS analysis surveys of (a) B-AuNPs-small, (b) B-AuNPs-moderate and (c) B-AuNPs-big.

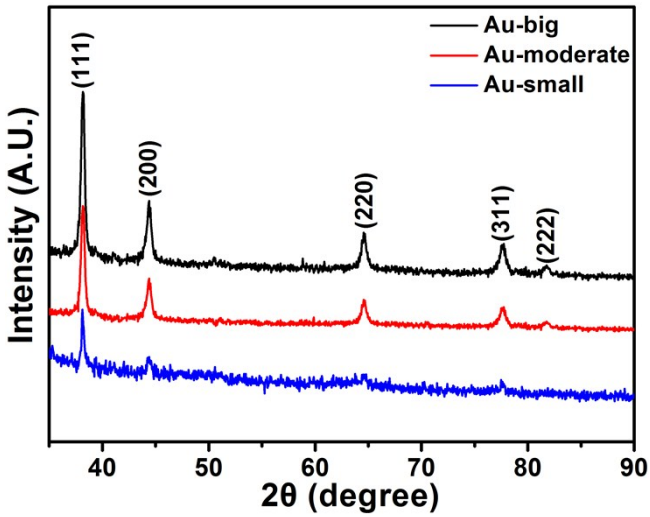

Figure S3. Wide-angle powder XRD pattern of B-AuNPs nanocomposites. The wide-angle X-ray diffraction pattern exhibits five peaks, which could be indexed as the (111), (200), (220), (311) and (222) reflections of the face centered cubic structure of crystalline Au^0.

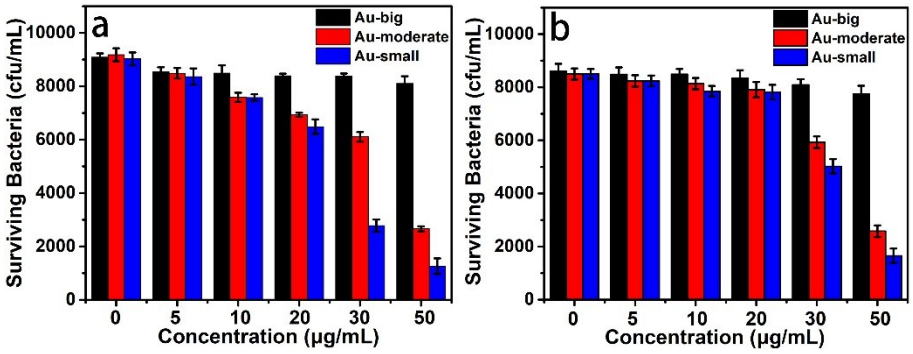

Figure S4. Antibacterial activity studies of the B-AuNPs on both (a) *E. coli* and (b) *S. aureus.*

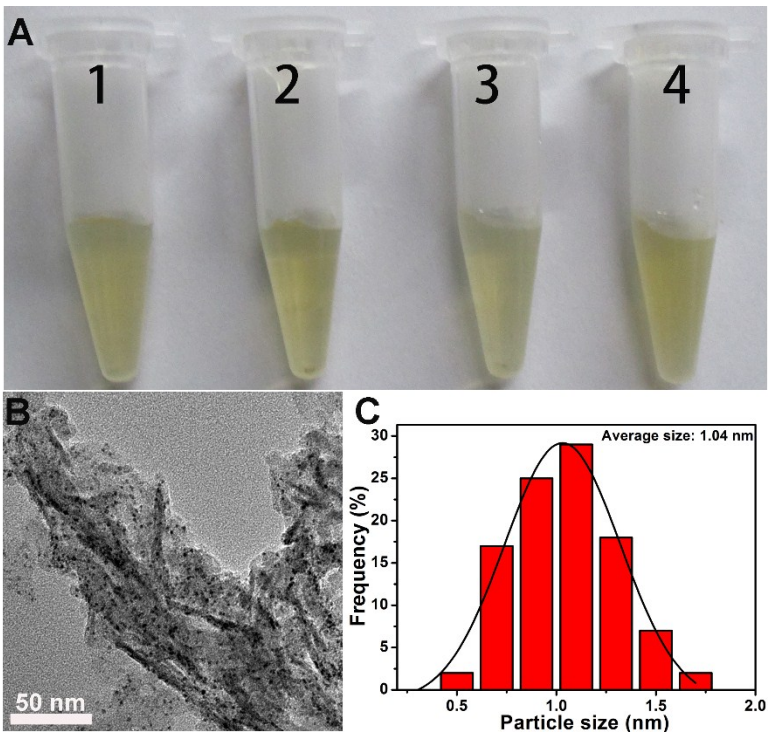

Figure S5. Typical photographs of A: (1) B-AuNPs in 0 M NaCl; (2) B-AuNPs in 0.1 M NaCl; (3) B-AuNPs in 0.5 M NaCl; (4) B-AuNPs in 1 M NaCl. B: TEM of B-AuNPs-small after 1 M NaCl treated. C: Particle size distributions of B-AuNPs-small after 1 M NaCl treated.

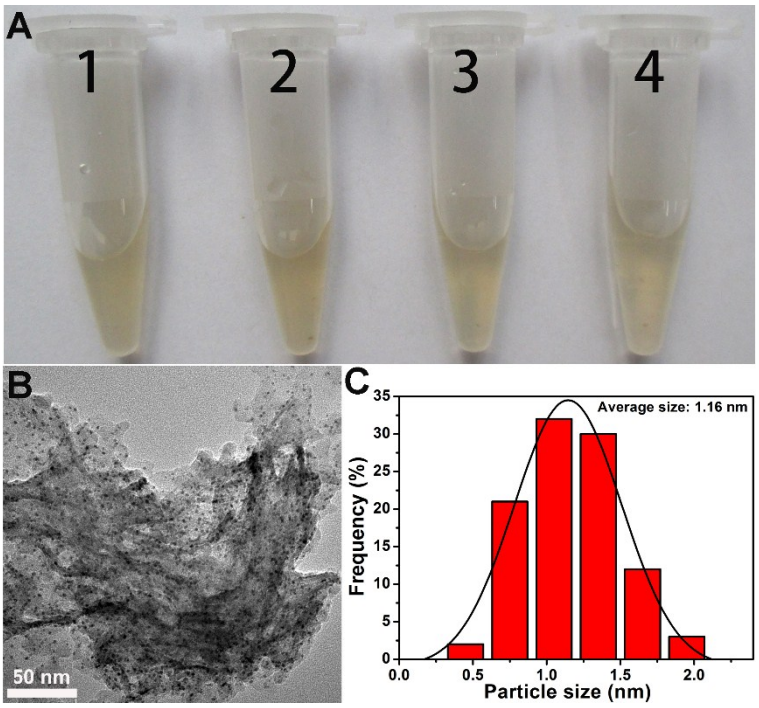

Figure S6. Typical photographs of A: (1) B-AuNPs in 0 M HCl; (2) B-AuNPs in 0.1 M HCl; (3) B-AuNPs in 0.5 M HCl; (4) B-AuNPs in 1 M HCl. B: TEM of B-AuNPs-small after 1 M HCl treated. C: Particle size distributions of B-AuNPs-small after 1 M HCl treated.

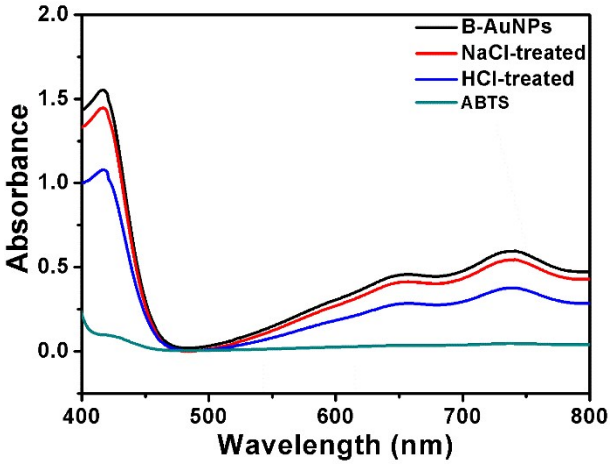

Figure S7. The peroxidase-like activities of the B-AuNPs after 1 M NaCl or 1 M HCl treated for 12 h.

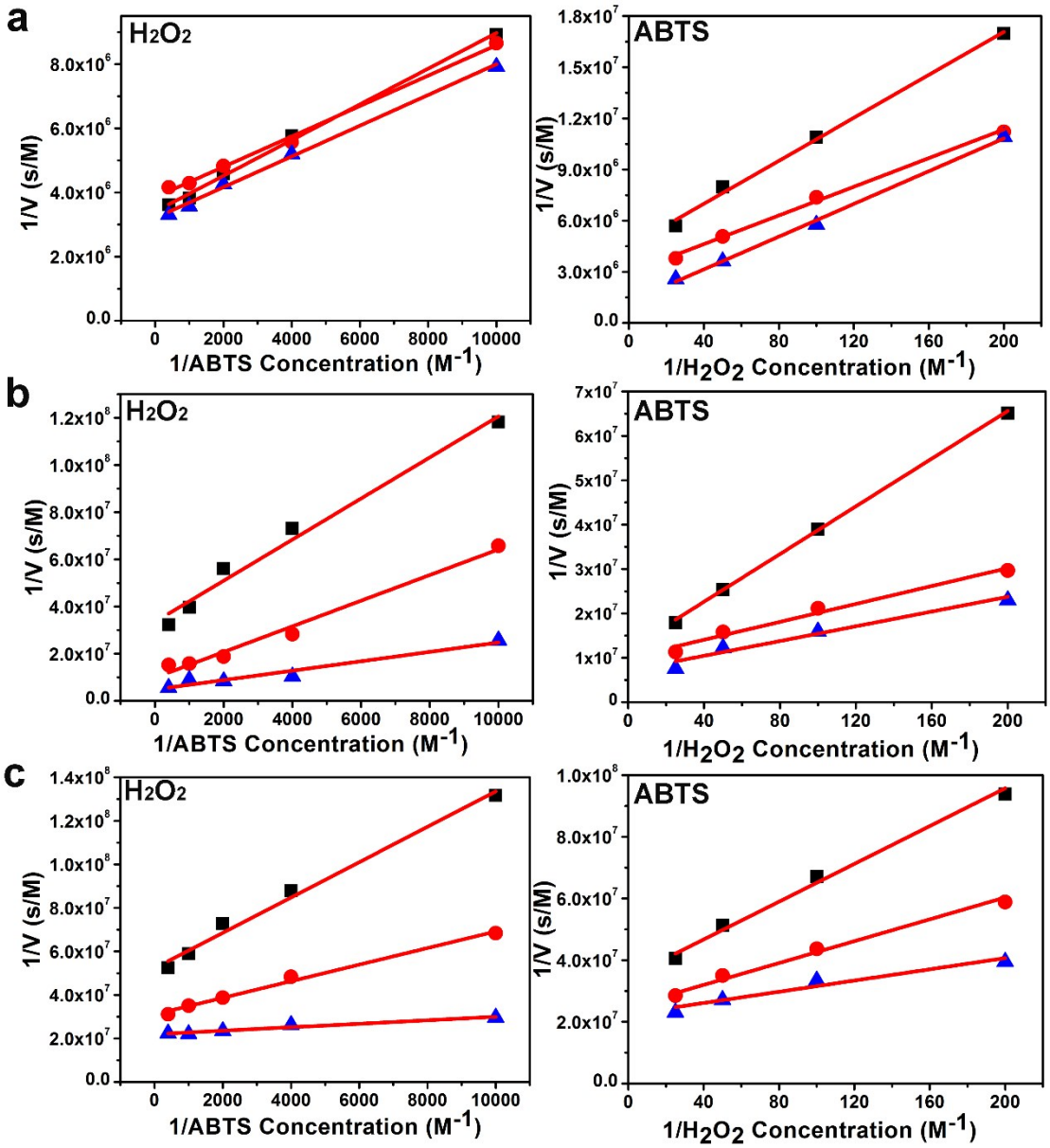

Figure S8. Steady-state kinetic assay and catalytic mechanism of B-AuNPs. The velocity (*v*) of the reaction was measured using B-AuNPs (5 μg/mL, Au content) in 500 μL of 25 mM PB buffer pH 4.0 at 37 °C. Double-reciprocal plots of activity of (a) B-AuNPs-small, (b) B-AuNPs-moderate, c) B-AuNPs-big at a fixed concentration of one substrate versus different concentration of the second substrate for H_2O_2 or ABTS. Details were described in experimental section.

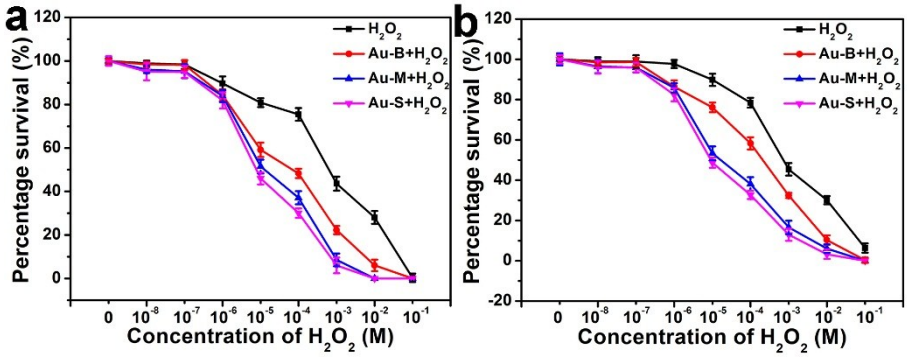

Figure S9. Survival rate of (a) *E. coli* and (b) *S. aureus* treated with H_2O_2 at different concentration with or without different size of B-AuNPs nanocomposites (5 μg/mL, Au content).

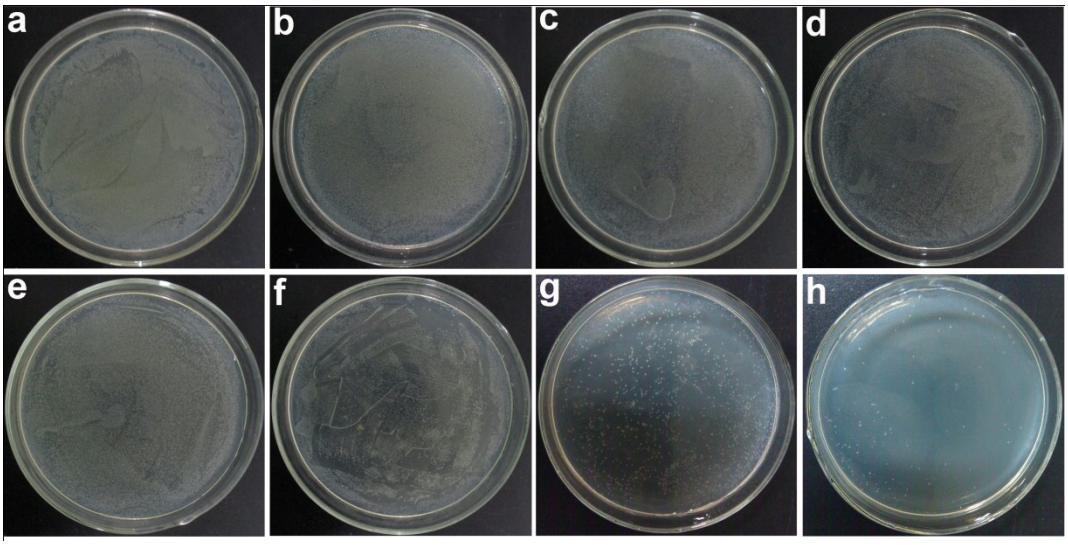

Figure S10. The plate samples showing colonies of (a) *E. coli*; (b) *E. coli* treated with B-AuNPs-big; (c) *E. coli* treated with B-AuNPs-moderate; (d) *E. coli* treated with B-AuNPs-small; (e) *E. coli* treated with H_2O_2; (f) *E. coli* treated with B-AuNPs-big and H_2O_2; (g) *E. coli* treated with B-AuNPs-moderate and H_2O_2; (h) *E. coli* treated with B-AuNPs-small and H_2O_2.

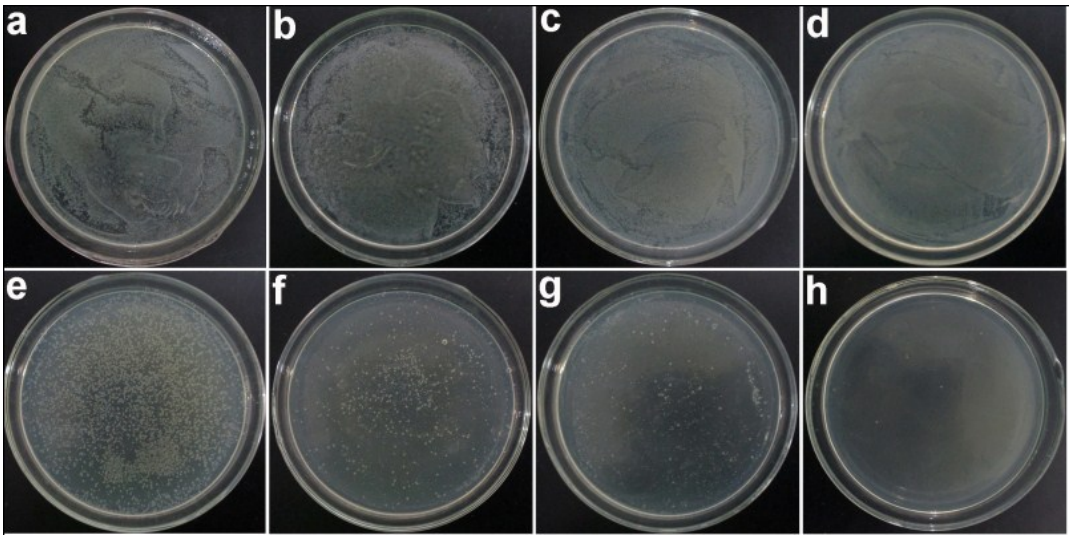

Figure S11. The plate samples showing colonies of (a) *S. aureus*; (b) *S. aureus* treated with B-AuNPs-big; (c) *S. aureus* treated with B-AuNPs-moderate; (d) *S. aureus* treated with B-AuNPs-small; (e) *S. aureus* treated with H_2O_2; (f) *S. aureus* treated with B-AuNPs-big and H_2O_2; (g) *S. aureus* treated with B-AuNPs-moderate and H_2O_2; (h) *S. aureus* treated with B-AuNPs-small and H_2O_2.

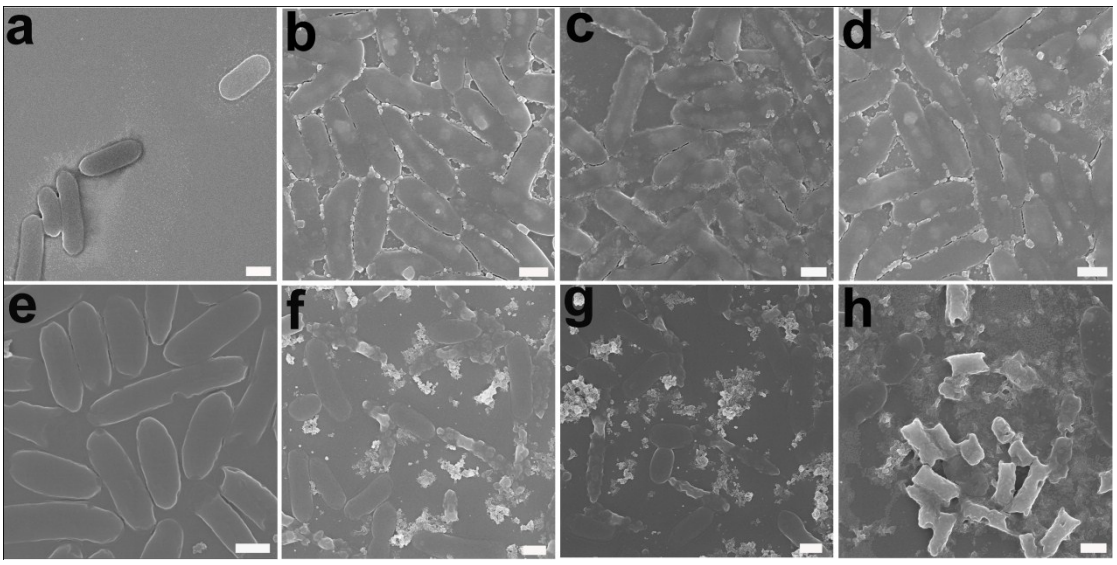

Figure S12. Typical SEM images of (a) *E. coli*; (b) *E. coli* treated with B-AuNPs-big; (c) *E. coli* treated with B-AuNPs-moderate; (d) *E. coli* treated with B-AuNPs-small; (e) *E. coli* treated with H_2O_2; (f) *E. coli* treated with B-AuNPs-big and H_2O_2; (g) *E. coli* treated with B-AuNPs-moderate and H_2O_2; (h) *E. coli* treated with B-AuNPs-small and H_2O_2. The scale bars are 1 μm.

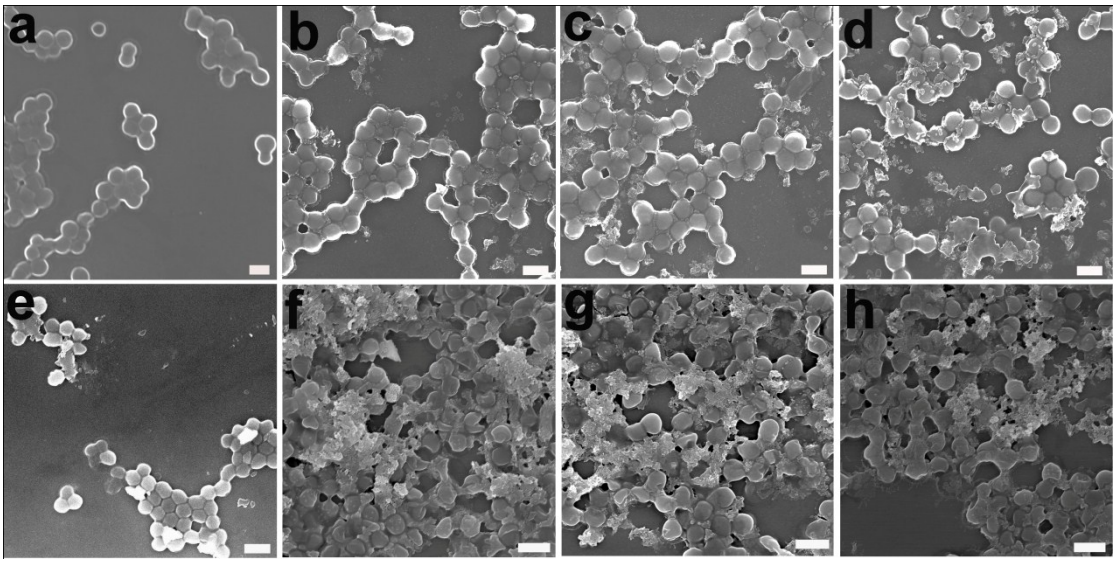

Figure S13. Typical SEM images of (a) *S. aureus*; (b) *S. aureus* treated with B-AuNPs-big; (c) *S. aureus* treated with B-AuNPs-moderate; (d) *S. aureus* treated with B-AuNPs-small; (e) *S. aureus* treated with H_2O_2; (f) *S. aureus* treated with B-AuNPs-big and H_2O_2; (g) *S. aureus* treated with B-AuNPs-moderate and H_2O_2; (h) *S. aureus* treated with B-AuNPs-small and H_2O_2. The scale bars are 500 nm.

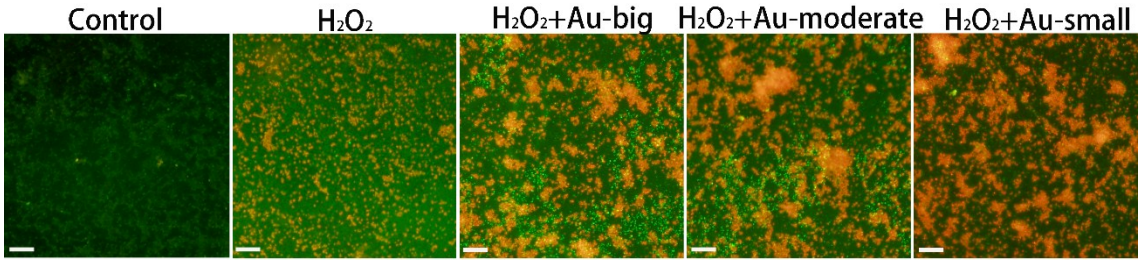

Figure S14. Biofilm dispersal study of *S. aureus* after treatment with different size of B-AuNPs (50 μg/mL, Au content) and 1 mM H_2O_2. Under fluorescence microscopy green and red stains indicate viable and dead cells, respectively, scale bar = 25 μm.

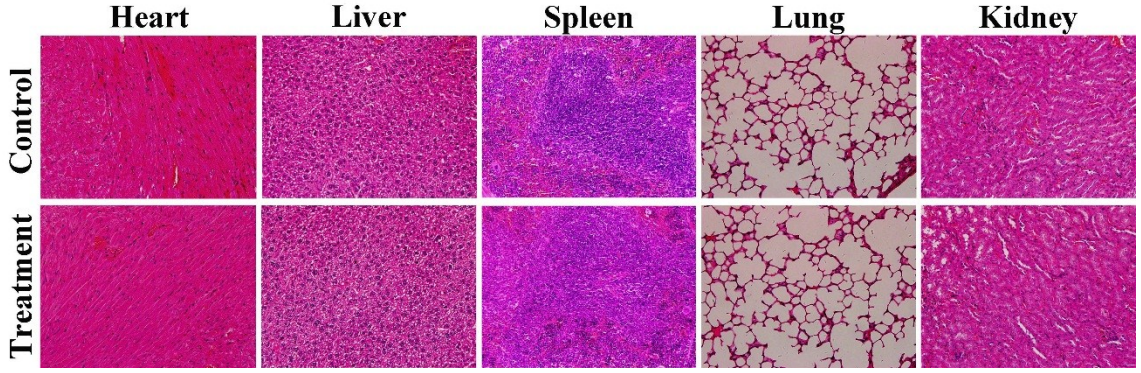

Figure S15. Histologic evaluation of tissues from mice treated with normal saline (Control) and B-AuNPs (Treatment).

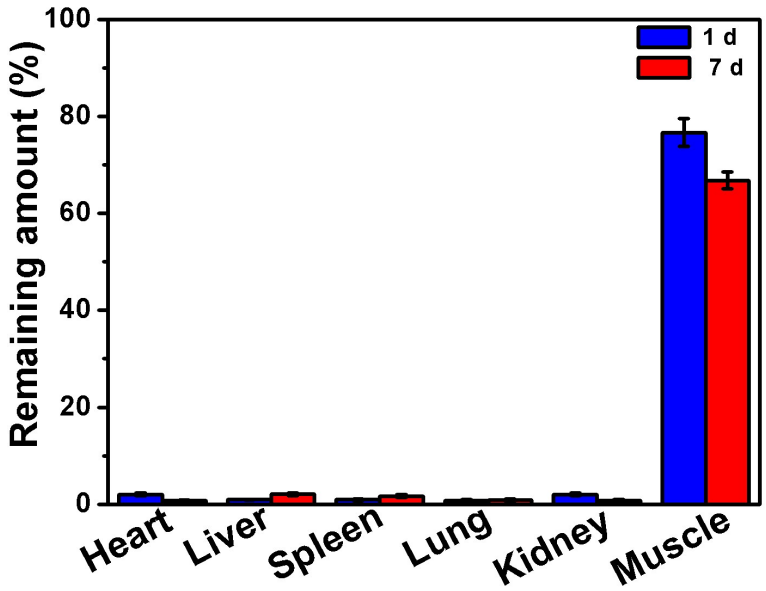

Figure S16. Time-dependent biodistribution of B-AuNPs after subcutaneous injection.

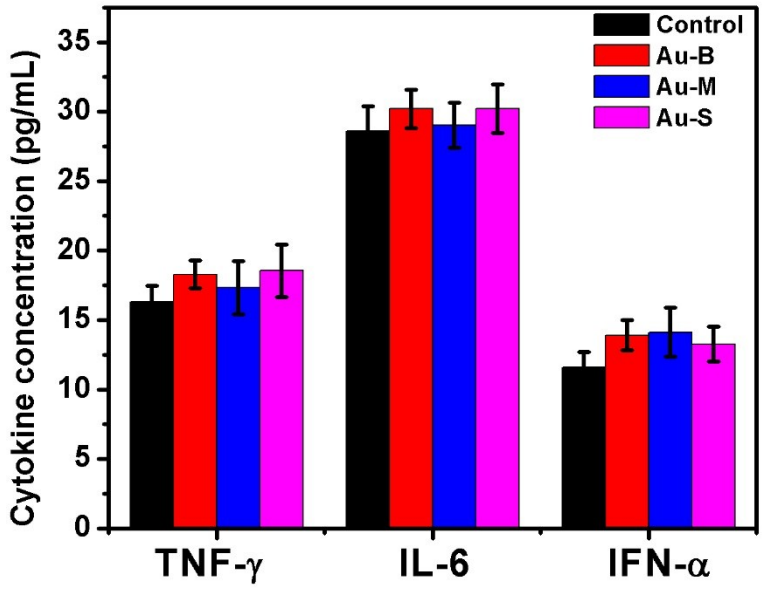

Figure S17. Immunogenic response of mice treated with normal saline and B-AuNPs