Supplementary Information

Photothermal Therapy may be a Double-edge Sword by Inducing the Formation of Bacterial Antibiotic Tolerance

Methods

Preparation of AuNCs

To synthesize AuNCs, silver nanoparticles were firstly prepared. Briefly, 50 mL of ethylene glycol (EG, Aladdin Reagent Co., Ltd, China) was heated at 150 °C for 30 min. Then 600 μL sodium hydrosulfide (Alfa Aesar, USA) solution in EG (3.0 mM) was added with magnetic stirring. 12.5 mL Polyvinylpyrrolidone (PVP, Mw = 55 kDa, Sigma Aldrich, USA) in EG (20 mg mL-1) and 5 mL hydrochloric acid solution in EG (3.0 μM mL-1) was added into the flask after 2 min stirring. After stirred for another 2 min, 4 mL silver trifluoroacetate (CF3COOAg, Sigma Aldrich, USA) solution in EG (282 mM) was added and then waited for about 45 min until the whole synthesis period was done. Silver nanoparticles were collected by centrifugation and washed with acetone and ultrapure water to remove excess EG and PVP. 10 mL silver nanoparticles (720 μg mL-1) synthesized above, 150 mg PVP and 90 mL of ultrapure water were added into a flask using an oil bath at 90 °C under stirring for 10 min. Then Chloroauric acid hydrate (HAuCl4, Sinopharm Chemical Reagent Co., Ltd, China) aqueous solution was added by using a syringe pump at a constant injecting rate of 0.7 mL min-1. By measuring the ultraviolet UV-vis absorption spectrum of aliquots sampled from the solution, the extent of replacement could be monitored until the absorption peaks are approximately 808 nm. The AuNCs were preserved in 4 °C for further use.

Characterization of AuNCs

Transmission electron microscope (TEM, JEOL, JEM-2100) was used to show the morphology of AuNCs. The hydrodynamic diameters and the zeta potentials were examined by Zetasizer Nano ZS90 at 25 °C. The UV-vis absorption spectra of AuNCs dispersions were recorded by a dual beam spectrophotometer (TU-1901, Beijing Purkinje General Instrument, China) with the wavelength range of 500-900 nm at room temperature.

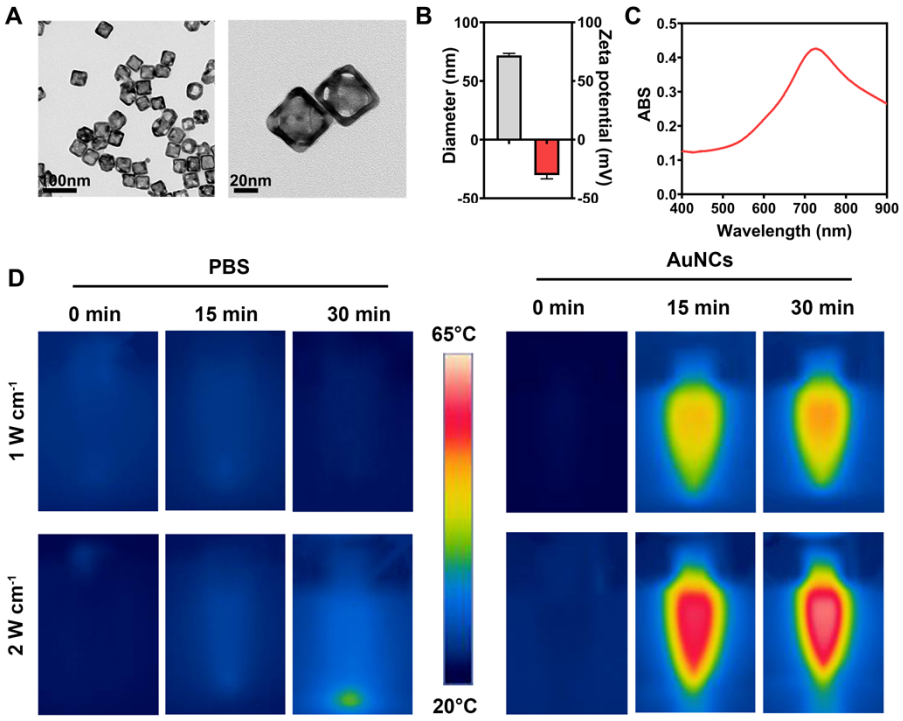

Fig. S1 (A) Transmission electron microscopy (TEM) images of AuNCs. (B) Size and zeta-potential data of AuNCs. (C) UV-vis spectra of AuNCs solution. (D) Temperature variation of PBS and AuNCs solution (10 μg mL^{-1}) under NIR laser irradiation (1 W cm^{-2} or 2 W cm^{-2}). Images were captured by thermal imaging camera.

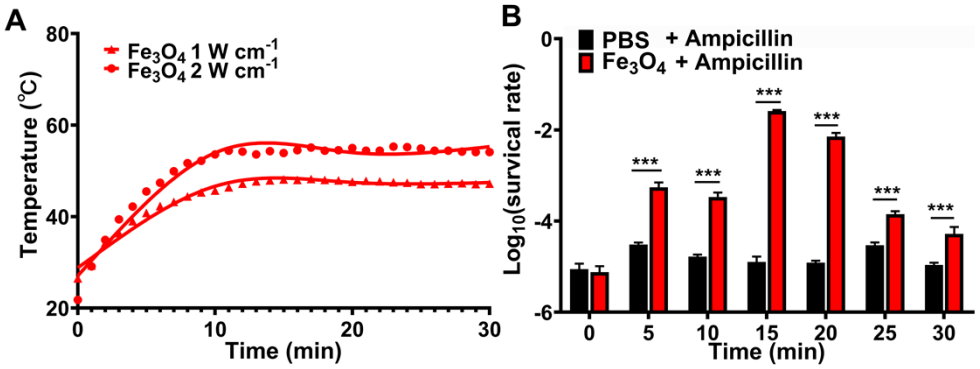

Fig. S2 A) Temperature variation of Fe_3O_4 solution (100 μg mL^{-1}) under NIR laser irradiation (1 W cm^{-2} or 2 W cm^{-2}). B) Frequency of persister formation for *E. coli* treated with PBS or Fe_3O_4 (100 μg mL^{-1}) under 1 W cm^{-2} NIR laser irradiation and 150 μg mL^{-1} ampicillin.

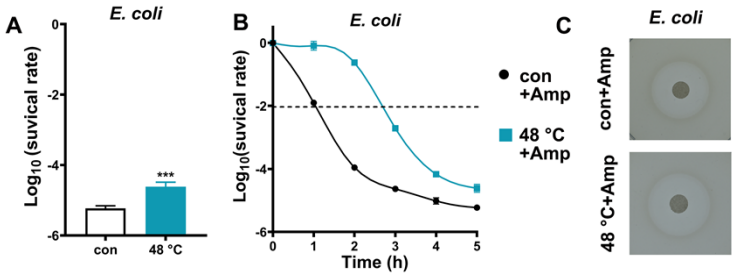

Fig. S3 Heat treatment increase bacterial antibiotic tolerance. (A) Frequency of survived cells for *E. coli* quantified by CFU countings. Bacteria were treated with PBS at 37 °C or 48 °C and then treated with 150 μg mL^{-1} ampicillin. (B) Kinetics of bacterial killing under ampicillin treatment (150 μg mL^{-1}) for *E. coli* at 37 °C or 48 °C. (C) Bacteriostatic ring of ampicillin for *E. coli* at 37 °C or 48 °C.

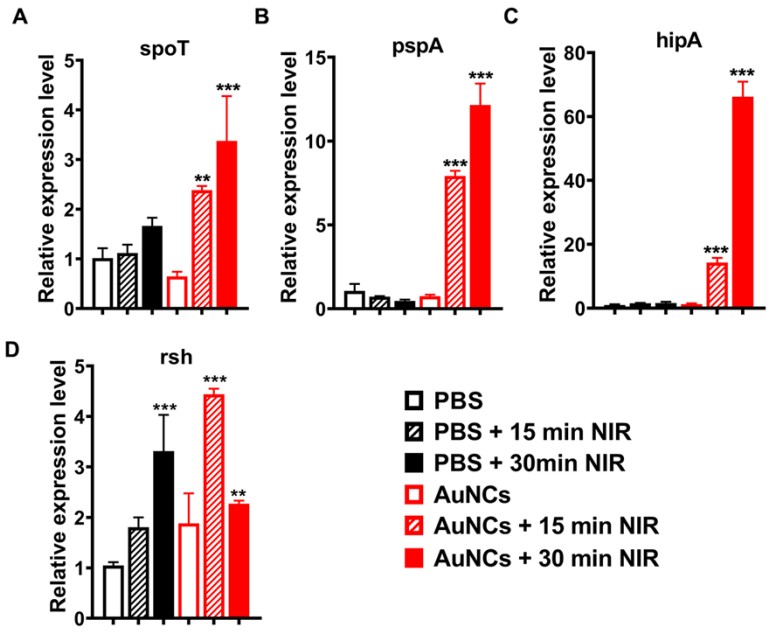

Fig. S4 QPCR showed the relative mRNA expression levels of antibiotic tolerance related genes.

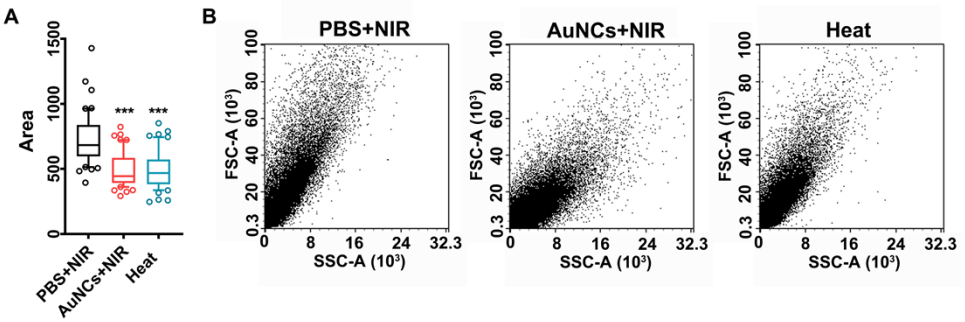

Fig. S5 (A) Volume of bacterial cells determined by ImageJ. (B) Scatter plots of flow cytometry.

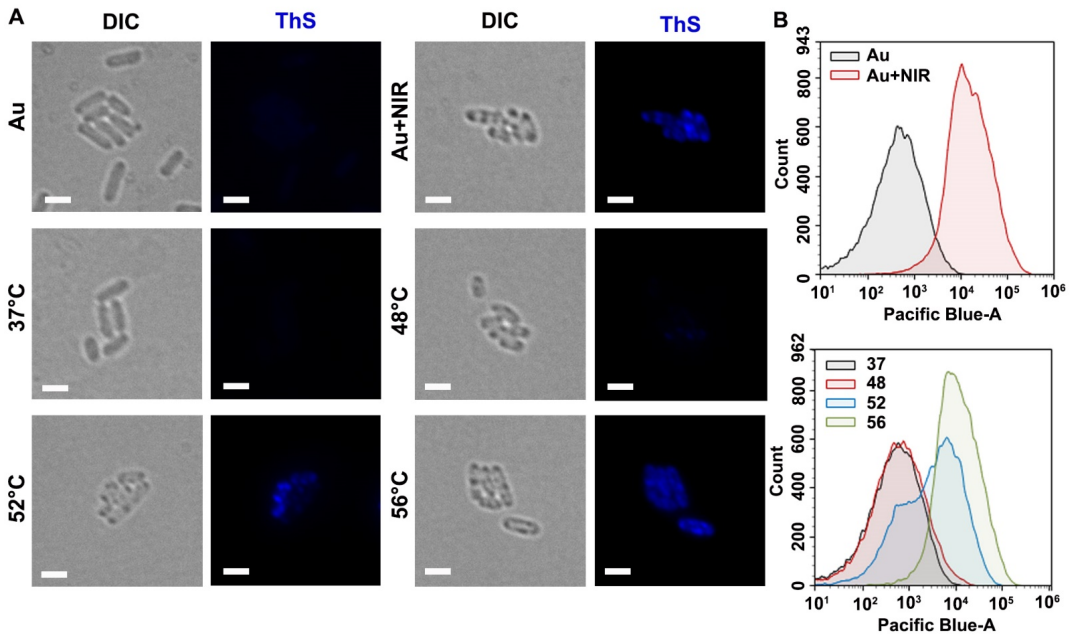

Figure S6. ThS labeled protein aggregates in *E. coli*. A) Bright field and fluorescence images of treated bacteria. B) Flow cytometry results distinguished the staining positive bacteria.

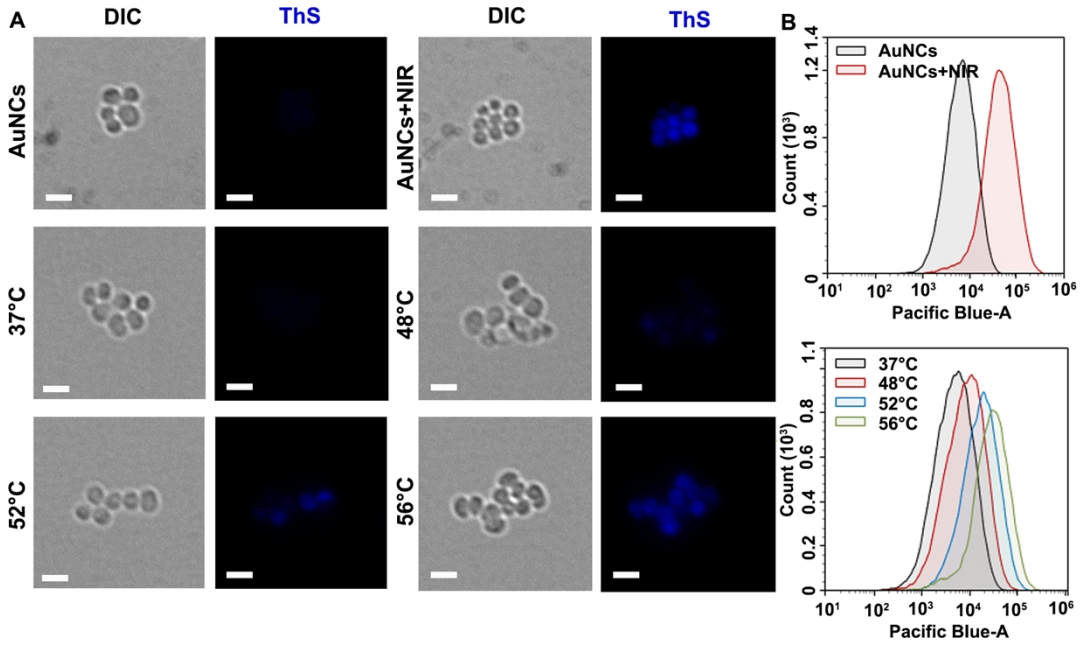

Figure S7. ThS labeled protein aggregates in *S. aurues*. A) Bright field and fluorescence images of treated bacteria. B) Flow cytometry results distinguished the staining positive bacteria.

Supplementary Table

Table 1. The primers sequences used in qPCR of *E. coli* and *S. aureus*.

Strain	**Gene**	**Primer sequence (5'-3')**
Both	16s F	AGTACGGCCGCAAGGTTAAA
	16s R	CTCCAATCCGGACTACGACG
E. coli	spoT F	CTGCCGTAATATCCGTGGCT
	spoT R	AGCGCACCCTGATGATTGAA
	pspA F	CCGACATCGTGAATGCCAAC
	pspA R	CGCTTGTTCAATACGGCGAG
	hipA F	CGCCAGCAGCAGACAGTAAT
	hipA R	CGATTGGCGAAATCAGGCAG
	dnaK F	ATGACCTGGGTGGTGGTACT
	dnaK R	CGTTGGTTGCCAGAACTTCG
	ropH F	GCGCAAACTGTTCTTCAACC
	ropH R	GAATCGTCGTCGGAAGACAG
	dnaJ F	ACCTGTATTGCGAAGTCCCG
	dnaJ R	GCGAGTCGGTCAGAACTTCA
S. aurues	rsh F	CCCCAGCGAGTGATGTTATT
	rsh R	AATTTTGCCATTCACCTTGG
	dnaK F	AGGTGGCGGTACATTTGACG
	dnaK R	AGTTTGTTGTCACCGGCTGT
	groEL F	CCGGGTGTTGGTTTTAACGC
	groEL R	TGCCACCCATGTTAGGTTGG