Supporting Information for

Impact of nano-TiO2 on horizontal transfer of resistance genes mediated by filamentous phage transduction

Xue Han[a,†], Peng Lv[a,†], Lu-Guang Wang[b], Fei Long[b], Xiao-Lin Ma[a], Yu-Jie Feng[c,*], Ming-Feng Yang[a], Xiang Xiao[a, c*], Chang Liu

[a] School of The Environment and Safety Engineering, Jiangsu University, Zhenjiang, 212013, China

[b] Department of Biological and Ecological Engineering, Oregon State University, Corvallis, OR 97333, USA

[c] State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, Harbin 150090, China

[†] These authors contributed equally to this work.

*** Corresponding authors:**

Ph.D. Xiang Xiao, Fax: +86-511-88790955; E-mail: xiaox@ustc.edu.cn

Prof. Yu-Jie Feng, Fax: +86-451-86283068; E-mail: yujief@hit.edu.cn

Table S1 Target primers used in this study

Target primers	Primer sequences (5′-3′)	Length (bp)
rpoA-F	GGAAACCAACGGCACAATC	193
rpoA-R	GCAGTTAGCAGAGCGGACAG	
traV-F	GCCTGGCTGAAGGGAACT	176
traV-R	CGGCGTAACTGAACTGACC	
traX-F	GCGGAAGACAGAATGGAAC	117
traX-R	CAGCACCGTCATCACAAGG	
traA-F	AGTGTTCAGGGTGCTTCT	191
traA-R	GCCTTAACCGTGGTGTT	

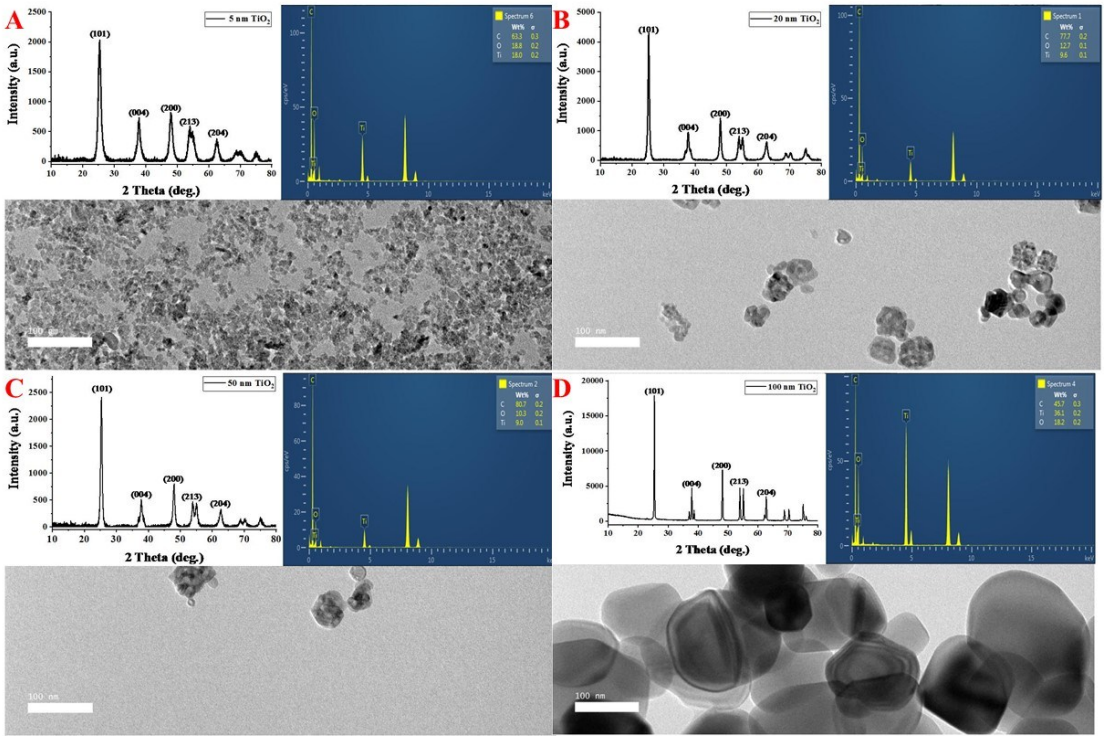

Figure S1 Characterization of nano-TiO_2 using TEM, EDS spectrum and XRD. A, 5 nm nano-TiO_2; B, 20 nm nano-TiO_2; C, 50 nm nano-TiO_2; D, 100 nm nano-TiO_2.

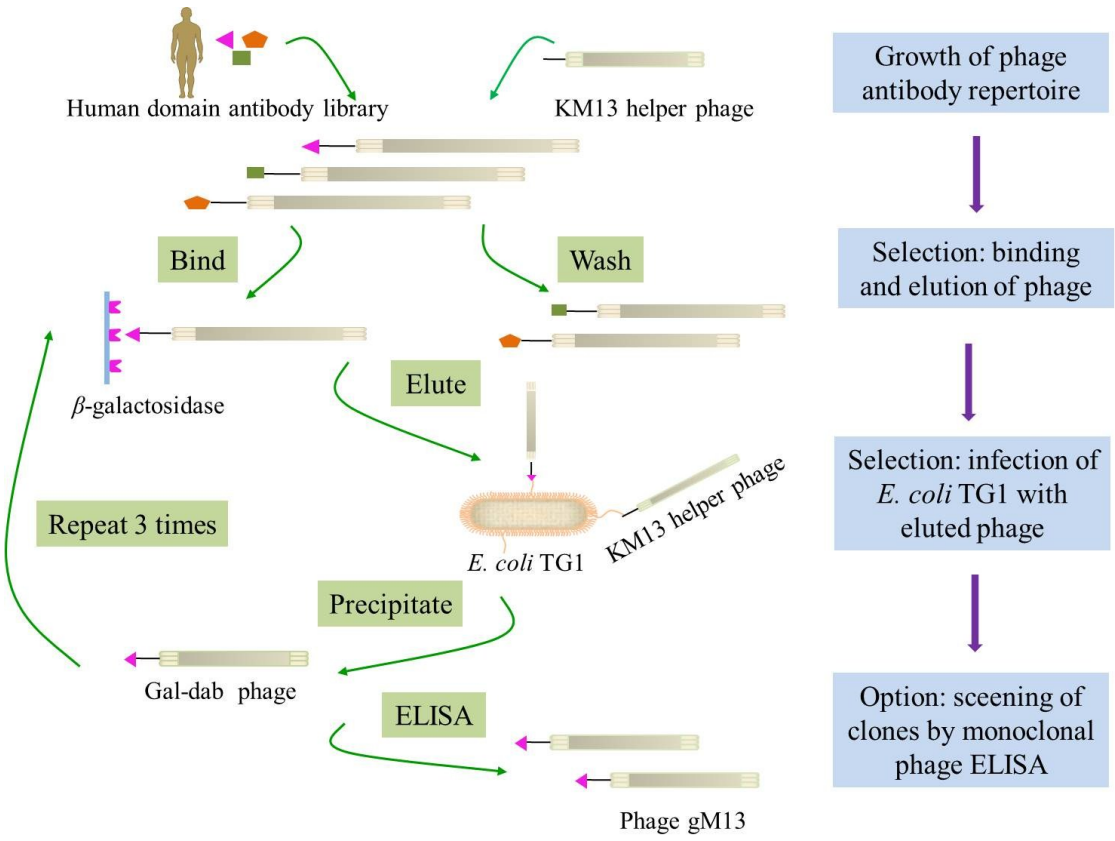

Figure S2 Selection of phage gM13 against *β*-galactosidase used in this study

Briefly, the library phages were produced by infecting of Dab library culture (500 mL) with KM13 helper phage (1×10^{12} PFU/mL), and precipitated by PEG/NaCl (PEG 6000, 20%; NaCl, 1.5M) after overnight production. The biotinylated *β*-galactosidase (Sigma, USA) was used to select the specific domain antibody against galactosidase (Gal-Dab), which was incubating with Dab library phages for 15 min. Then panning was performed by adding 100 μl magnetic streptavidin beads for 15 min. The non-specific phages were removed by washing 10 times with PBST in the magnetic field. The specific phage was eluted with DTT, and used to infect exponentially growing *E. coli* TG1. TG1 colonies from the first round of selection were scraped from 2×TY agar plates containing ampicillin and infected with KM13 helper phages. The Gal-Dab phages were concentrated by PEG/NaCl precipitation and subjected to further rounds of selection.

After three rounds of selection, each clone was grown in 96-well V-bottom plates

containing 200 µl of 2×TY medium supplemented with 100 µg/ml of ampicillin and 40 mg/ml glucose and grown overnight at 37°C in a rotary shaker at 250 rpm. Overnight cultures of 5 µl were sub-cultured in 96-well V-bottom plates containing 200 µl of 2×TY medium with 100 µg/ml of ampicillin and 40 mg/ml glucose. After incubation for 3 h, KM13 helper phages were added to each well in 50 µl 2×TY medium. Plates were mixed by gentle agitation and incubated 30 min at 37°C without shaking. After 1 h, plates were centrifuged at 3200× g for 10 min at room temperature. Supernatants were discarded and 200 µl of 2×TY medium supplemented with 100 µg/ml of ampicillin, 50 µg/ml of kanamycin and 1 mg/ml glucose was added to each well. Phages were produced by overnight incubation at 37°C under gentle agitation. The next day, plates were centrifuged, and the supernatants of each well containing Gal-Dab phage clones were tested in ELISA. A higher response of phage against *β*-galactosidase and lower cross reaction was picked and termed as phage gM13 for further study.

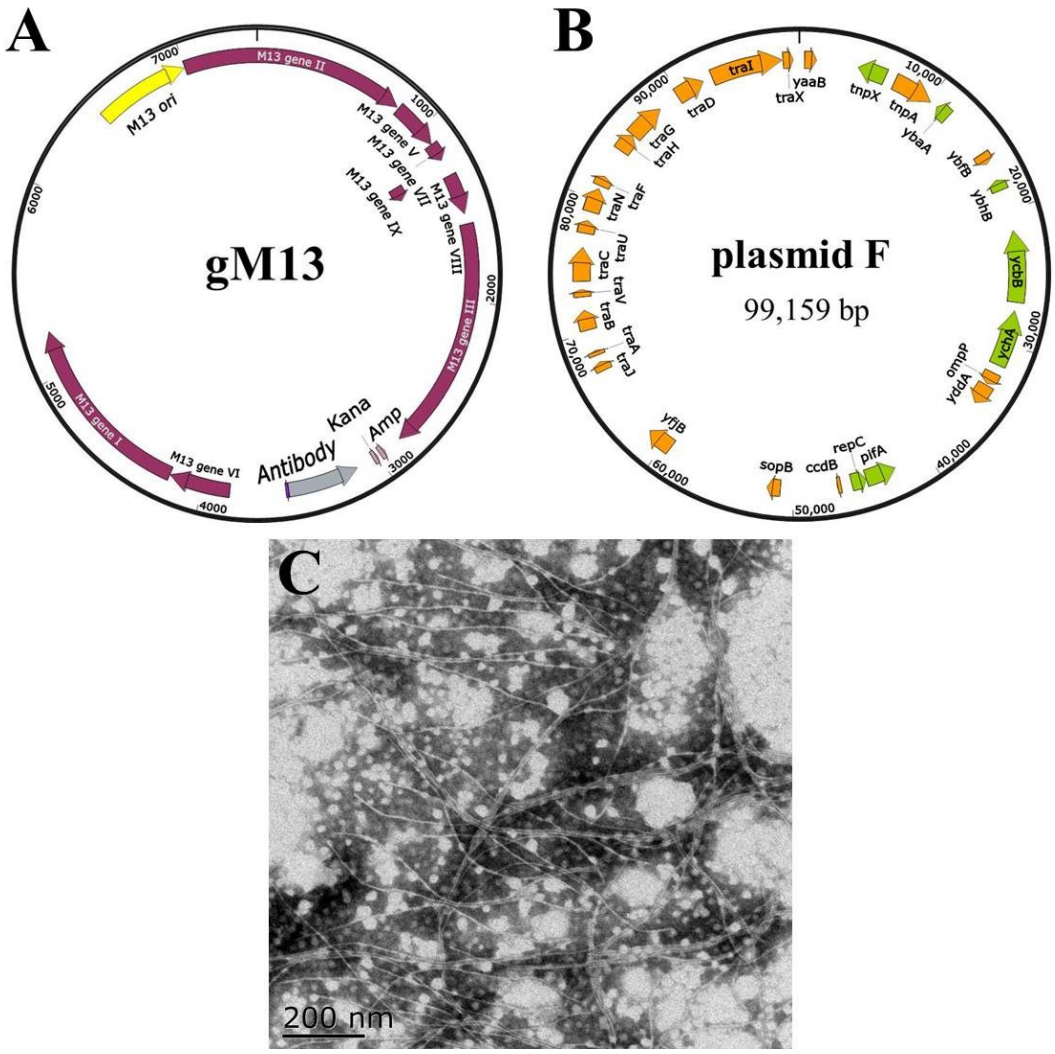

Figure S3 Gene maps of gM13 (A), plasmid F of *E. coli* TG1 (B) and TEM image of phage gM13 (C).

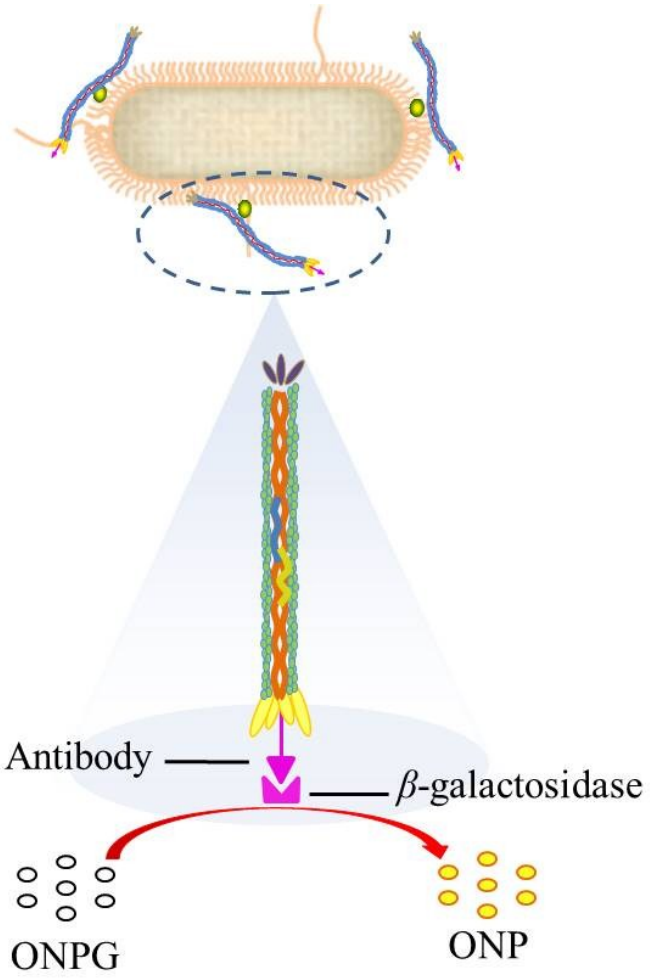

Figure S4 Schematic diagram for the detection of bacteriophage adsorption.

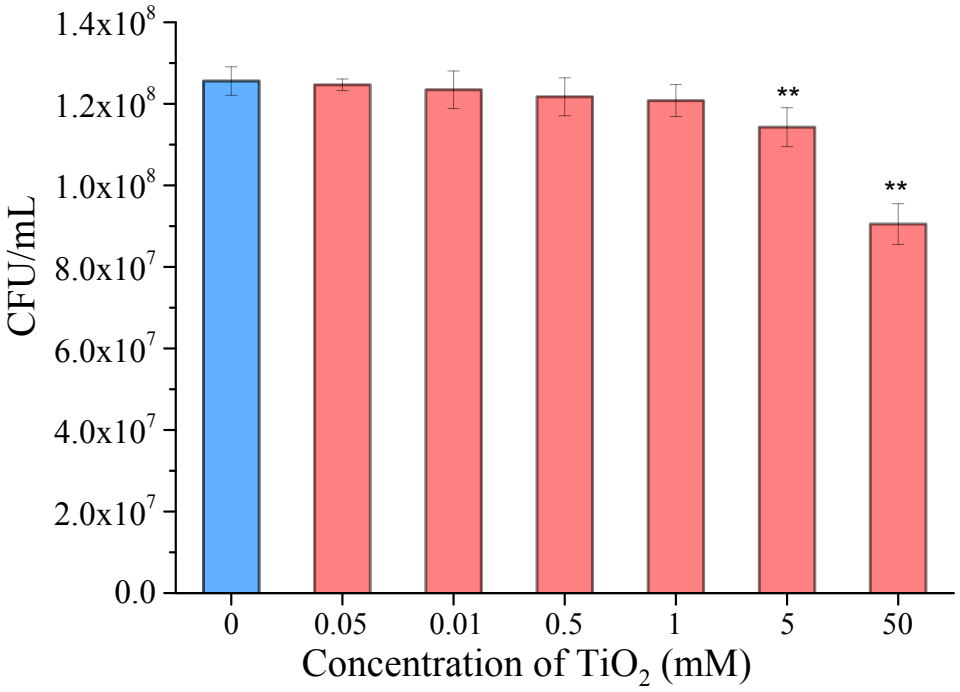

Figure S5 Effect of different nano-TiO_2 concentrations on colony formation of *E. coli* TG1. All values represent mean ± SD. Significant differences between groups were tested with independent-sample t-test and shown with * ($p < 0.05$), ** ($p < 0.01$).

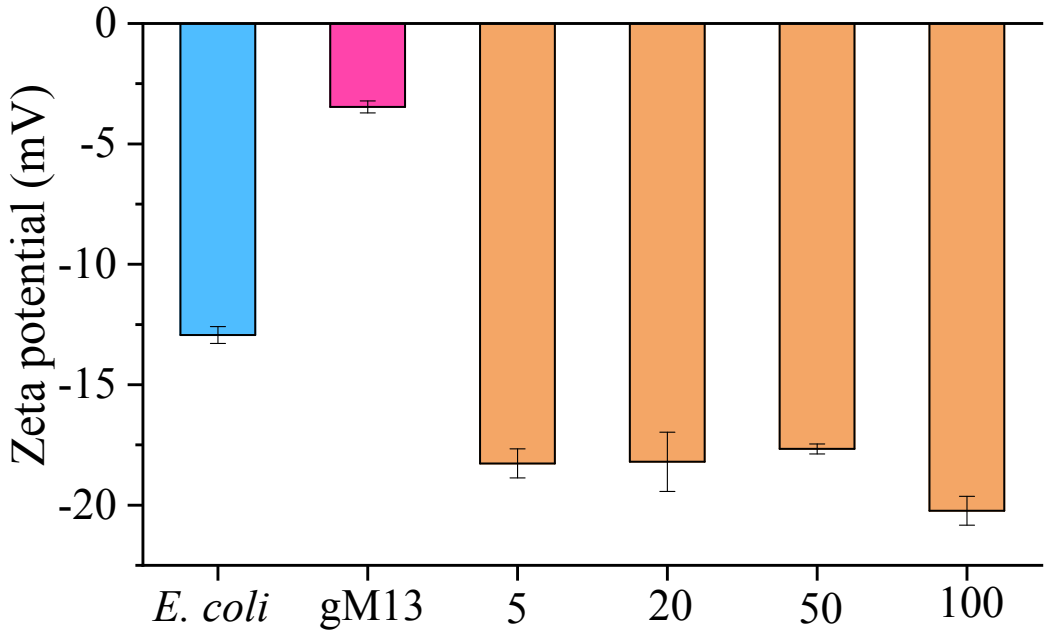

Figure S6 ζ-potentials of *E. coli* TG1 cell, phage gM13 and nano-TiO_2 with different sizes (5, 20, 50, and 100 nm). The concentrations of *E. coli* TG1 cell, phage gM13 and nano-TiO_2 used were 1.0×10^8 CFU/mL, 1.0×10^8 PFU/mL and 0.5 mM, respectively. ζ-potential was measured at 25°C using a nanoparticle size potentiometer (Zetasizer Nano ZS90, Malvern, England). All values represent mean ± SD.

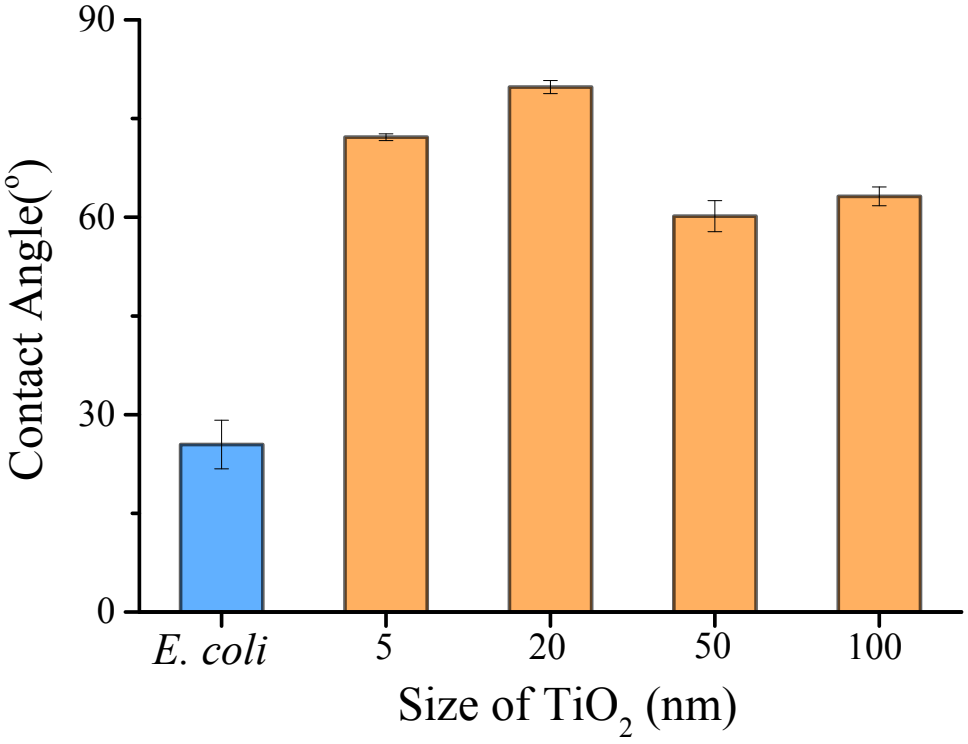

Figure S7 Contact angles of *E. coli* TG1 and nano-TiO_2 with different sizes (5, 20, 50, and 100 nm). All values represent mean ± SD.

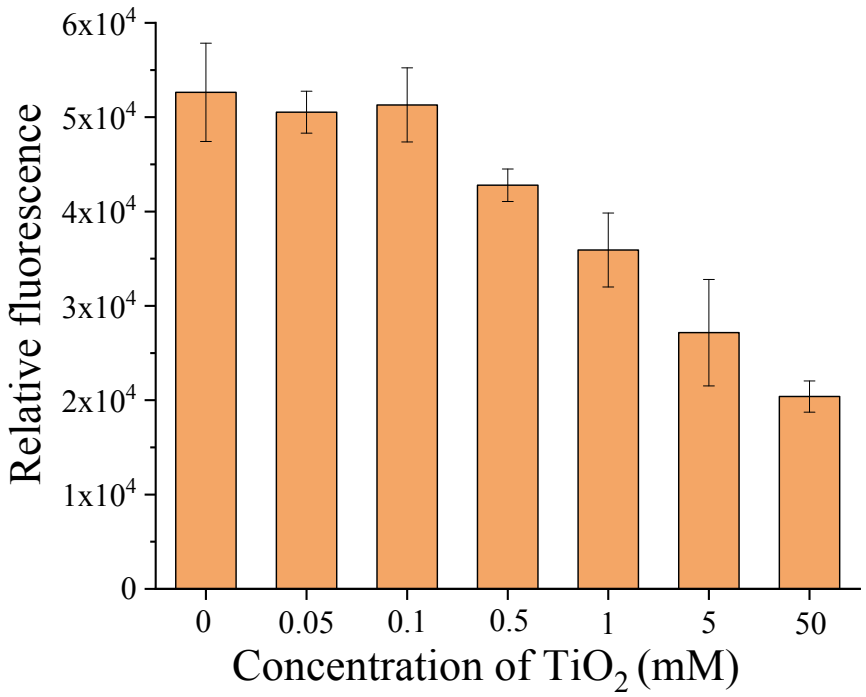

Figure S8 Interference of different concentrations of nano-TiO_2 on fluorescence-based assay. A gradient concentration of nano-TiO_2 (0, 0.05, 0.01, 0.5, 1, 5, and 50 mM) was prepared with phosphate-buffered saline (PBS, pH=7.0) containing 0.5 mg/L rhodamine B. 200 μL of mixed suspension was added into a black 96-well plates and then measured by a fluorescence spectrometer (Infinite M1000 PRO, Tecan, Austria). The excitation and emission wavelengths were set at 556 nm and 580 nm, respectively.

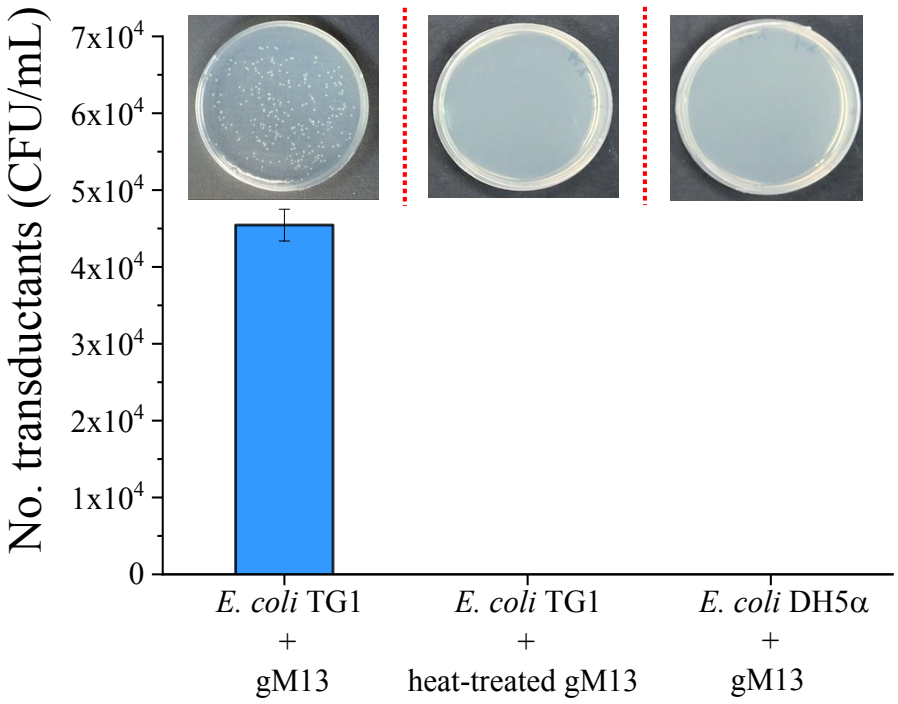

Figure S9 Assessment of infection specificity of gM13 phage. The group of *E. coli* TG1+gM13 represents that transduction of gM13 to *E. coli* TG1 mediated by nano-TiO_2; The group of *E. coli* TG1+heat-treated gM13 represents gM13 was treated at 90°C for 1h before being used for transduction; The group of *E. coli* DH5α+gM13 represents that host cell was *E. coli* DH5α rather than *E. coli* TG1. The concentration of nano-TiO_2 was 0.5 mM. The bacterial suspension was diluted 15 times and then coated on LB plates.

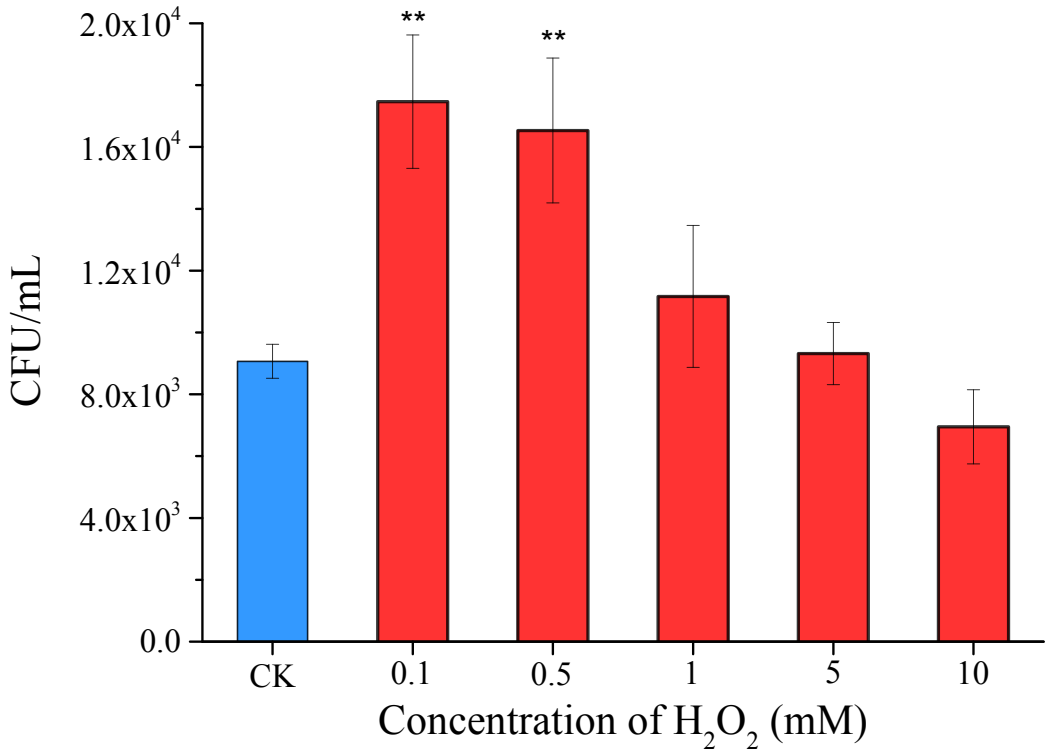

Figure S10 Effect of H_2O_2 on gM13 infection. *E. coli* TG1 cells and gM13 were mixed with a ratio of 1:1 (PFU/CFU). Significant differences between groups were tested with independent-sample t-test and shown with * ($p < 0.05$), ** ($p < 0.01$).