ADVANCED MATERIALS

Supporting Information

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Antimicrobial Microwebs of DNA–Histone Inspired from Neutrophil Extracellular Traps

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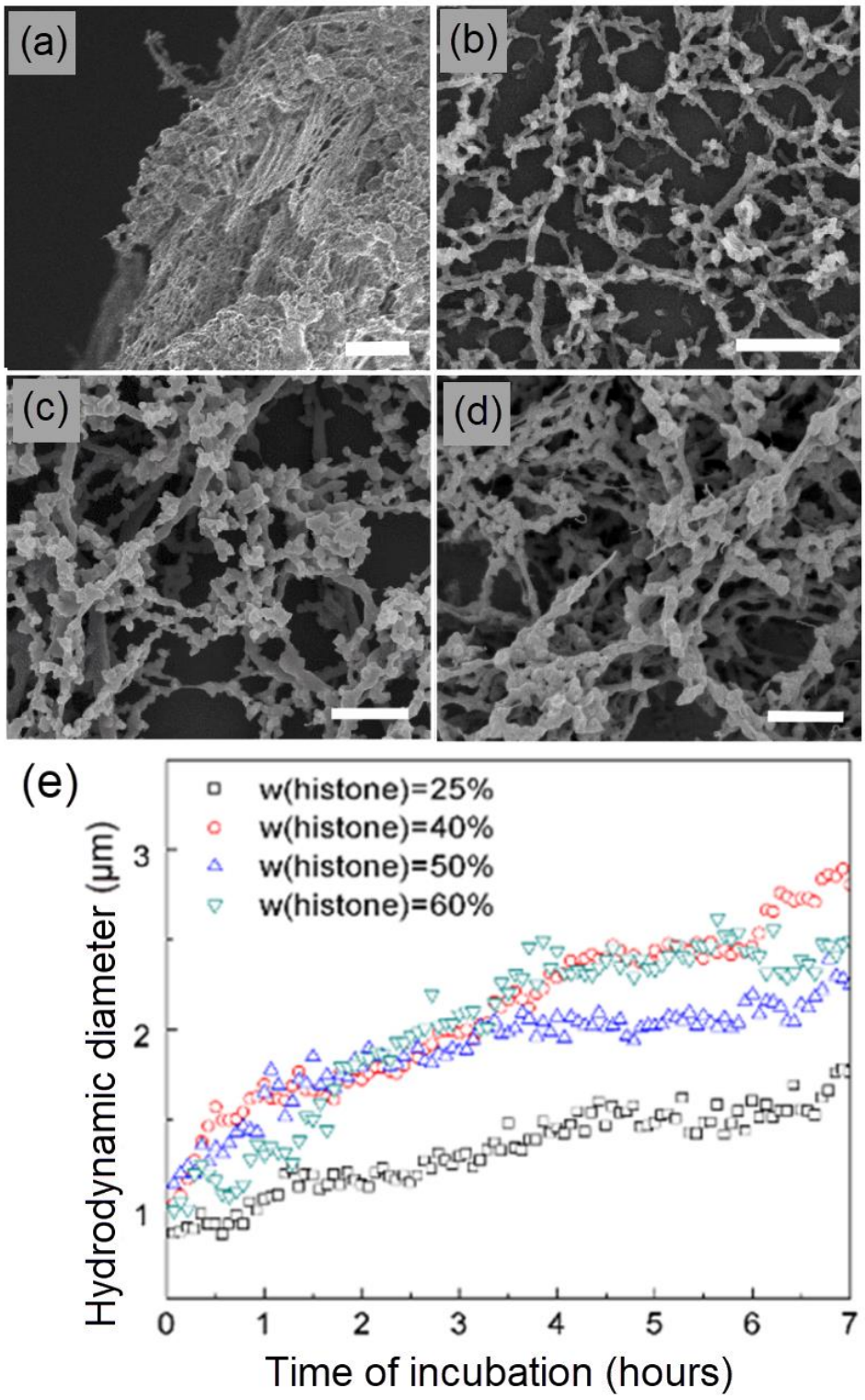

Figure S1. Morphology and colloidal instability of microwebs. SEM images of microwebs (a) before ultrasonication and (b-d) after sonication. Scale bar represent 1 μm. The weight fraction of histone in the microwebs are b) 25%, c) 40% and (d) 60%, respectively. Scale bar represent 1 μm. (f) Hydrodynamic diameter of DNA-histone as measured from dynamic light

scattering.

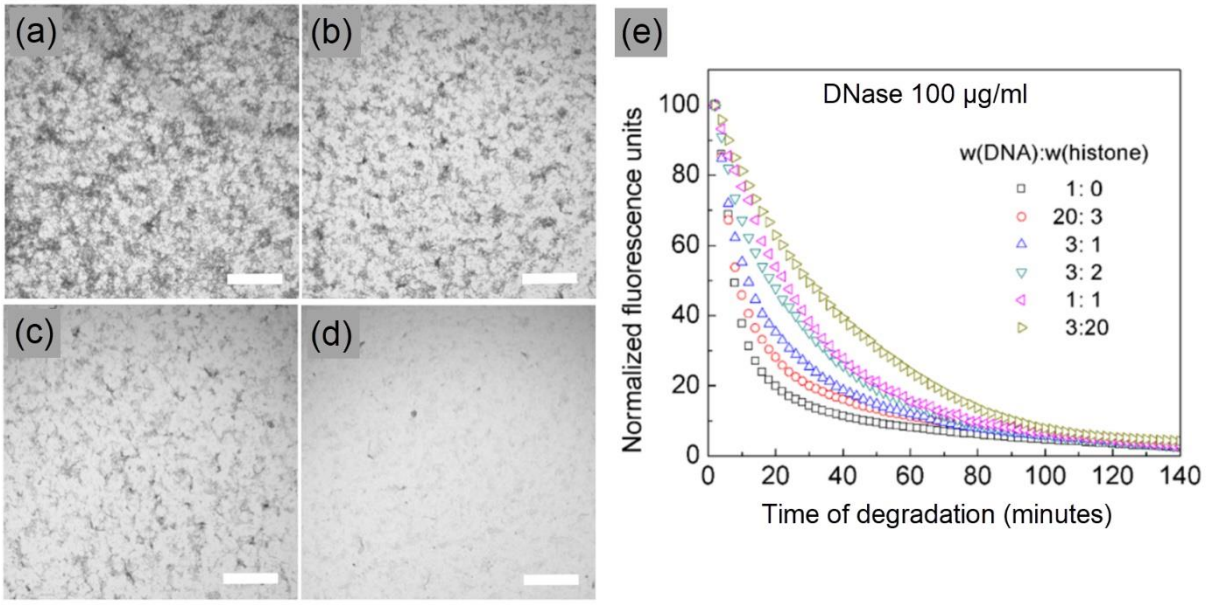

Figure S2. DNase-induced degradation of microwebs in HBSS. (a-d) Morphology of microwebs after degrading in DNase solution for 4 hours, scale bars represent 20 µm. Concentration of DNase, a) 1 µg ml^{-1}, b) 10 µg ml^{-1}; c) 100 µg ml^{-1}; d) 500 µg ml^{-1}. e) Degradation kinetics of SYTOX green stained microwebs as a function of the ratio between ω_{DNA}: ω_{his}.

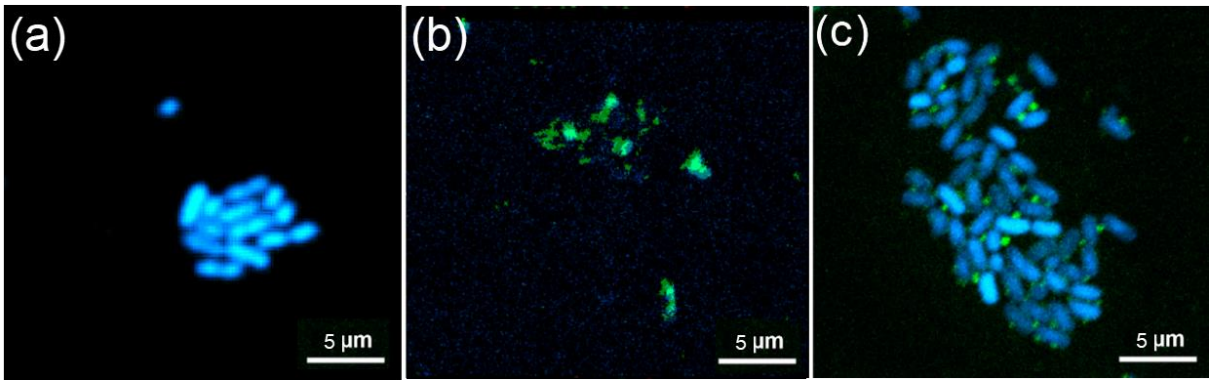

Figure S3. CLSM images of *E. coli* after culture in a) HBSS; b) a FITC-histone solution; and c) suspension of DNA/FITC-histone microwebs for one hour. Nuclear DNA of *E. coli* was

stained blue with DAPI; FITC-histone is labelled green. In b), FITC-histone alone causes lysis of *E. coli*. The cyan color indicates an overlap of FITC-histone with bacteria nuclear DNA. In c), DNA/FITC-histone microwebs were removed from bacteria after co-culture of bacteria *E. coli* with microwebs for 1 hour. Some FITC-histone (green) granules were absorbed on the extracellular matrix of bacteria while the cellular structure remains intact.

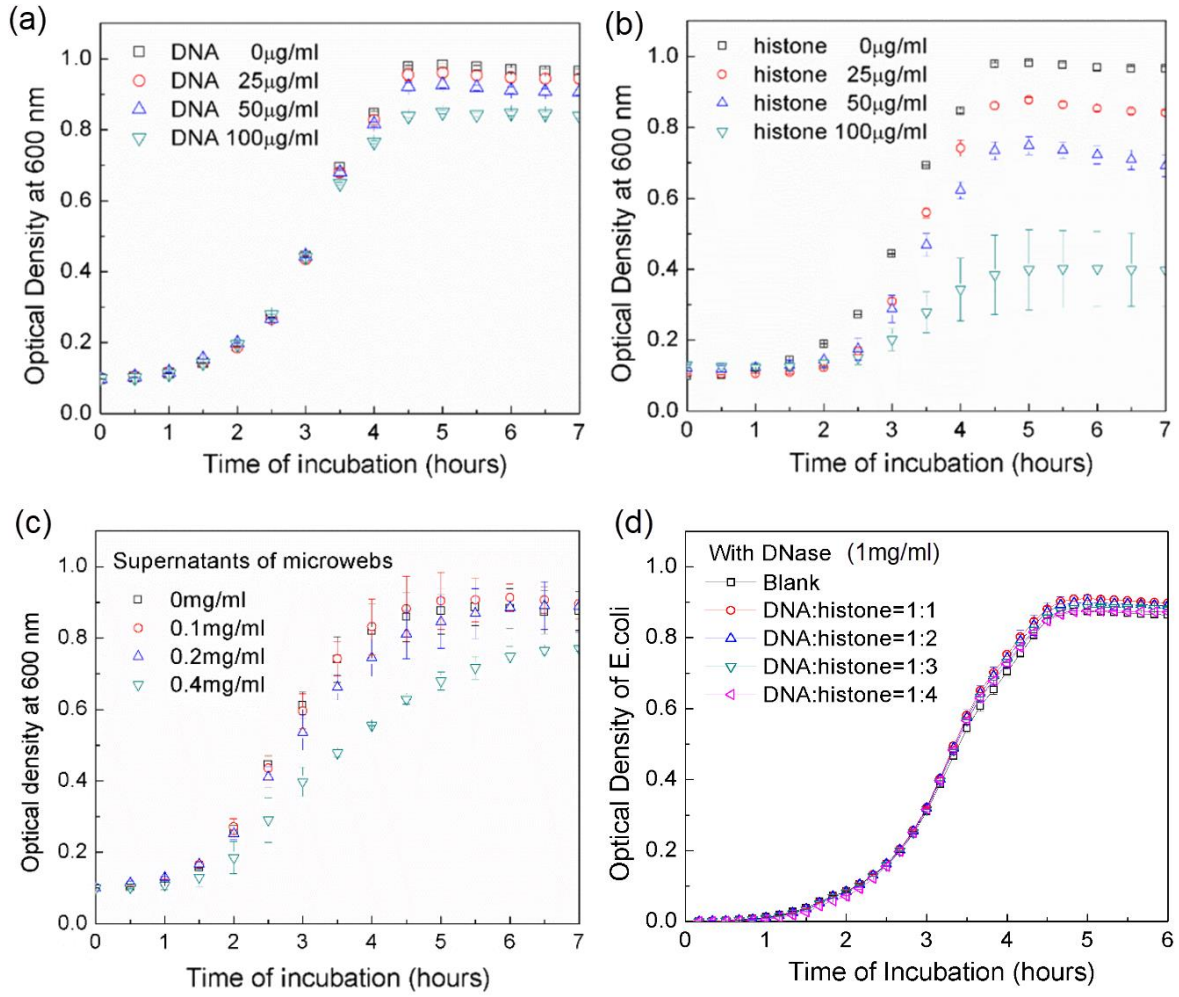

Figure S4. Growth curve of *E. coli* in an equal volume mixture of TSB (100μl) and a) DNA solutions; b) histone solutions; c) microweb supernatants; and d) microwebs with DNase.

To extract the supernatants, DNA/histone mixture was centrifuged (Microfuge® 20R, Beckman) at 14,400rpm for 5 minutes. The supernatant was extracted and mixed with TSB at a volume ratio of 1:1 as culture medium of *E. coli*.

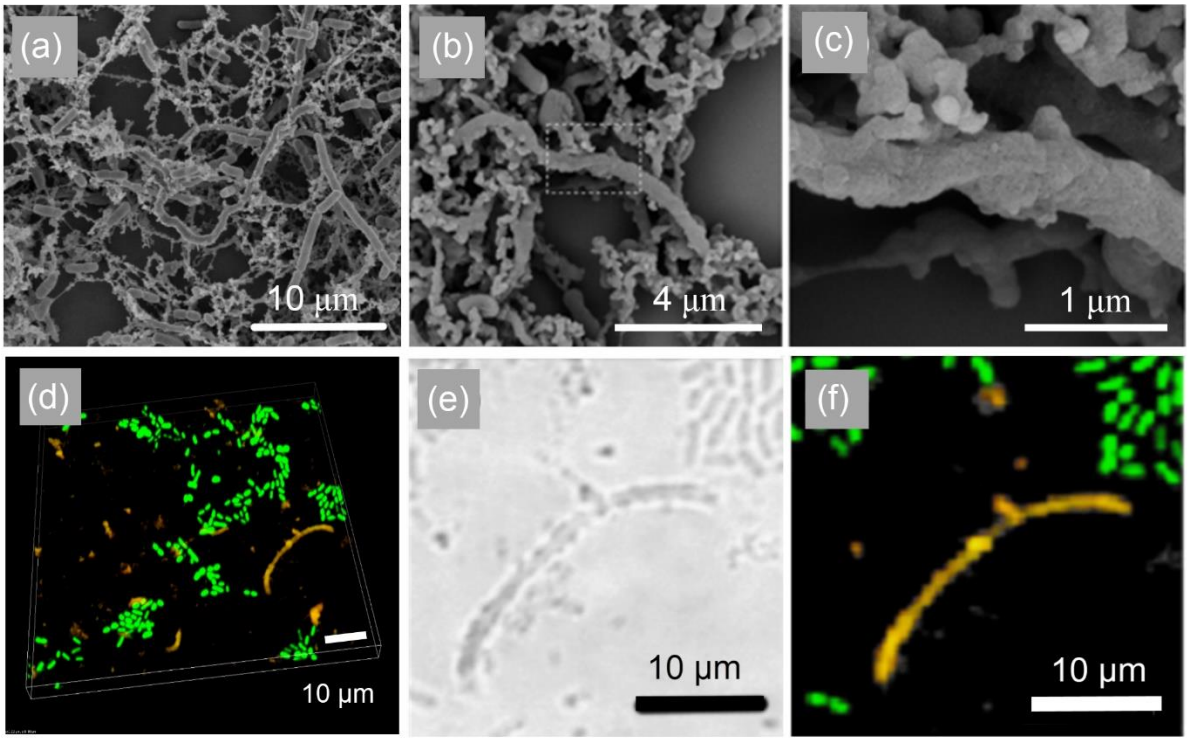

Figure S5 a,b,c) SEM images showing filamentous *E. coli* cells trapped on microwebs have nano-sized pores on their surface. d) low and f) high magnification fluorescent microscopy as well as e) bright field images of the filamentous *E. coli* cells. Live *E. coli* cells were stained green color by SYTO 9 and dead cells were stained yellow color due to co-localization of red propidium iodide. Propidium iodide is typically permeable to filamentous *E. coli* cells, but not to healthy *E. coli* cells.

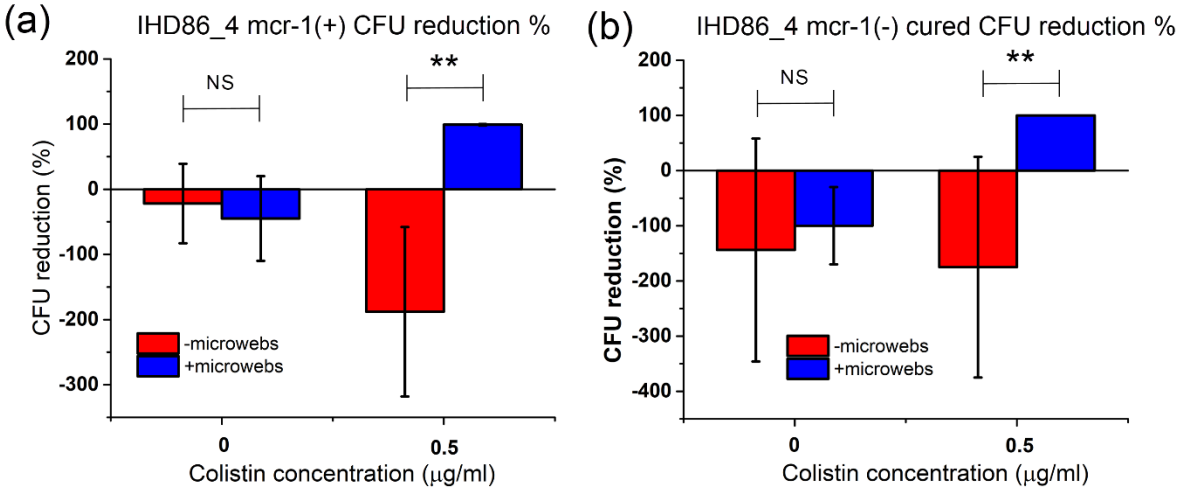

Figure S6. a,b) In HBSS, microwebs works synergistically with colistin and kill colistin-resistant *E. coli* IHD86_4 *mcr-1*(+) and the mcr-1 cured strain *mcr-1*(-), as shown by counting the CFUs after serial dilution, plating and overnight incubation at 37 °C. *mcr-1* is a colistin-resistance gene carried by colistin-resistant *E. coli* IHD86_4. ** P < 0.01. Colistin-resistant *E. coli* strain IHD86_4 which harbors the *mcr-1* gene was originally isolated in Cambodia, see Ref.[S1]. The plasmid encoding *mcr-1* was cured by serial passage in lysogeny broth and its loss was confirmed by colony PCR, see Ref.[S2]. These strains were streaked onto MH agar and after incubation, a single colony of each was inoculated into MH broth and grown shaking at 37^{o}C overnight.

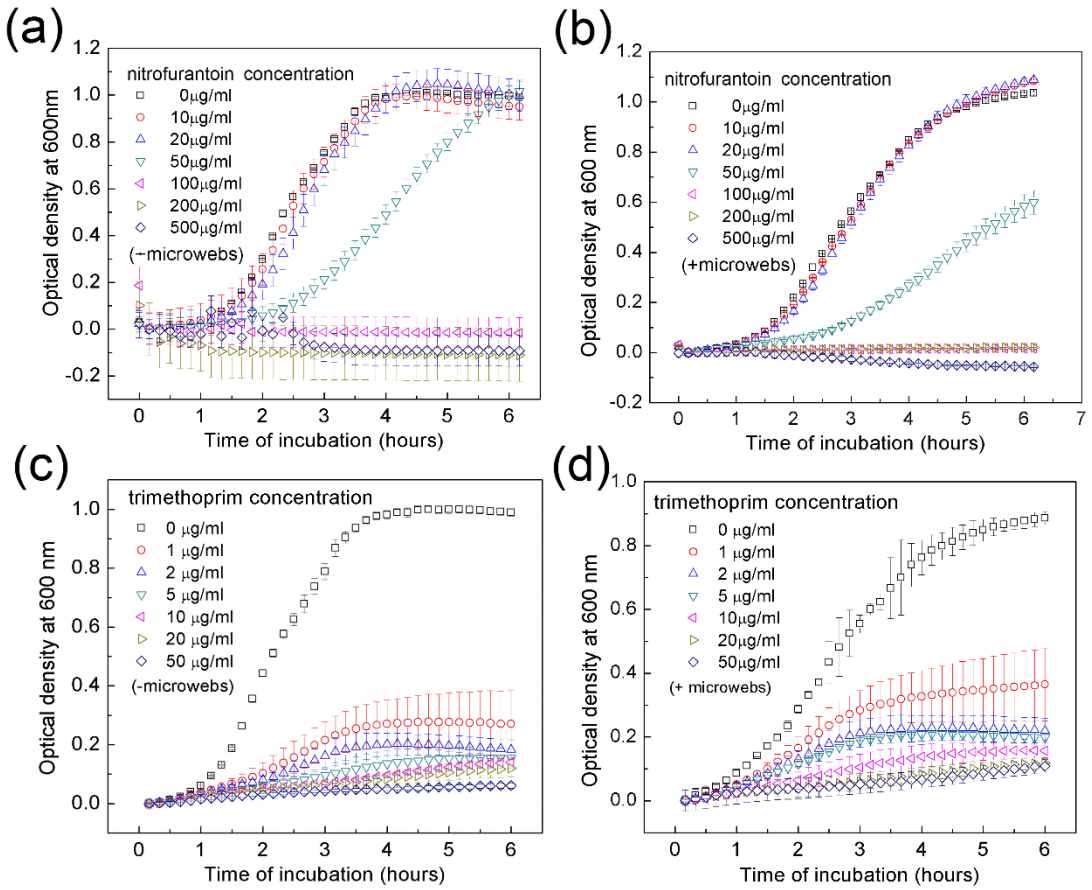

Figure S7 Presence of microwebs does not dramatically change the antimicrobial potency of nitrofurantoin and trimethoprim against *E. coli UTI89*, as shown by the growth curves of *E. coli* in TSB solution. a) +nitrofurantoin, -microwebs; b) +nitrofurantoin, +microwebs; c)

+trimethoprim, -microwebs; d) +trimethoprim, +microwebs.

Supplementary Experimental

Isolation of NETs: Human neutrophils were isolated from the blood of healthy volunteers using a previously described protocol (PMID: 26097119). Briefly, citrated blood was fractionated by centrifugation through Ficoll-Paque PLUS density gradient media (GE Healthcare) to separate neutrophils from mononuclear cells. Neutrophils were then further purified by dextran sedimentation of the red blood cell layer, before lysing residual red blood cells with 0.2% sodium chloride. Purified neutrophils were washed twice in phosphate-bufferred saline, and then cultured in RPMI-1640 media supplemented with L-glutamine and 3% fetal bovine serum. Neutrophils were stimulated with 500 nM phorbol 12-myristate 13-acetate (PMA). After 4 hours, NETs were collected by serial centrifugation exactly as described by Najmeh et al (PMID: 25938591). NET DNA was quantified with a microvolume spectrophotometer, and the quality of the NETs was confirmed by agarose gel electrophoresis.

In vitro bacterial assay of NETs: To make a reasonable comparison between NETs and microwebs, the amount of DNA in NETs is ensured to be equal to that in microwebs. A pellet of NETs collected from neutrophils was dispersed into HBSS using an ultrasonic homogenizer (Qsonica Q125 sonicator, intensity set 20%) for 15 seconds. The resulting NET suspension is further diluted until the DNA concentration is 50μg/ml. In the assays of both bacteria trapping and colony forming units, 100μL NET suspension were used. 10μg of NET DNA corresponds to between 1×10^7 to 1.8×10^7 neutrophils isolated from fresh blood.

References:

[S1] N. Stoesser, A. J. Mathers, C. E. Moore, N. P. Day, and D. W. Crook. *Lancet Infect. Dis.* **2016**, *16*, 285-286.

[S2] E. X. Sherman, D. A. Hufnagel, D. S. Weiss. MCR-1 confers cross-resistance to lysozyme. *Lancet Infect. Dis*., **2016**, *16*, 1226-1227.