Supplementary Information

A microfluidic platform for studying the effect of bacterial interactions on antimicrobial susceptibility testing in polymicrobial cultures

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Standard Two-layer Soft Lithography

We used standard two-layer soft lithography to fabricate the microfluidic devices.[1] A thin layer of PDMS in the ratio 20:1 (monomer to cross-linker) is spin-coated on the fluid layer (~ 35 μm) and a thick layer of PDMS in the ratio 5: 1 is poured over the control layer master. The two layers are then aligned and cured overnight to get an assembled two-layer microfluidic device (**Fig. S1**).

Coverslip Cleaning Protocol

Five 24x50 mm, thickness #1.5 glass coverslips from Ted Pella, Inc. are placed in a cylindrical glass slide holder. The glass slide holder is filled with 100% ethanol and is sonicated to remove dust particles for 10 minutes in Branson 2510 Ultrasonic Cleaner filled with deionized water. Ethanol is then discarded and replaced with deionized water in the glass slide holder, and the coverslips are sonicated for another 10 minutes to eliminate trace contaminants. Finally, coverslips are dried with pressurized N_2 in a cleanroom.

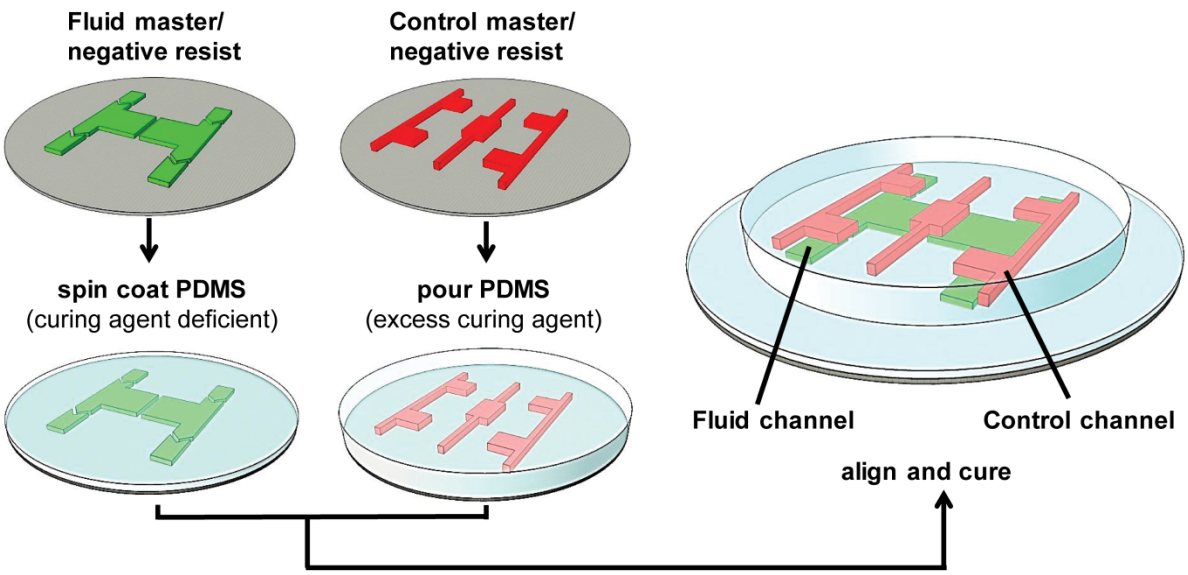

Figure S1. Fabrication of the chip using standard two-layer soft lithography.

Polymicrobial Culture Preparation

Polymicrobial cultures of cell types 1 and 2 are prepared as shown (**Fig. S2**). We prepared a range of cell type 1 and type 2 concentrations along with the diluted cultures of the two types and then mixed to obtain a range of polymicrobial combinations. We ensured that the observations of the competition experiments were solely due to the interaction between bacterial cells by centrifugation and by utilizing LB without antimicrobials for all the dilution steps.

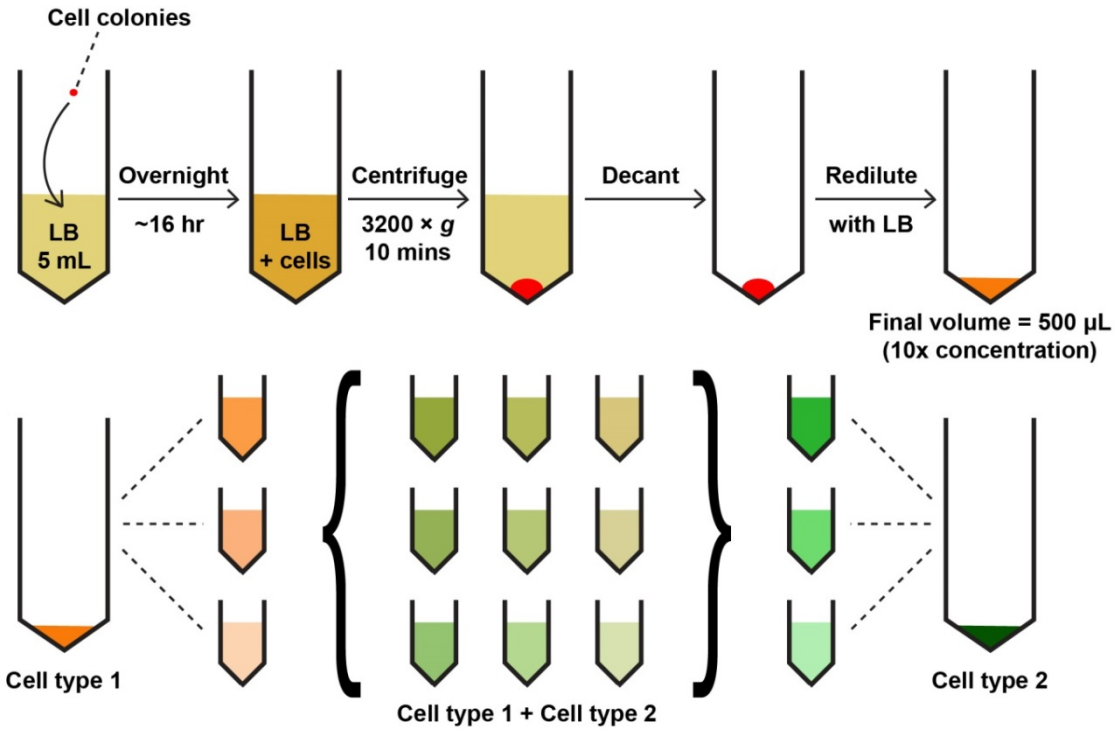

Figure S2. Preparation of mixed cultures of *E. coli*, *P. aeruginosa*, and *K. pneumoniae* in pairs.

Data Analysis

We used ImageJ to count cell numbers in each chamber as shown (**Fig. S3**).[2] Time-lapse fluorescence microscopy was used to capture images every 30 minutes. We used two different filter sets to image red and green cells as shown in **Fig. S3**. The alignment between the two filter sets was manually aligned for all chamber positions at the beginning of the experiment. This alignment did not significantly change over the course of the experiment and mechanical drift was determined to be negligible relative to the image (field) length scales due to the use of Newport optical table that reduced fluctuations. Following acquisition, images are sorted and post processed using ImageJ 1.47c. Using this algorithm, we were able to detect a maximum of ~10,000 cells for *E. coli* and slightly more for *K. pneumoniae*, which are non-motile cells.

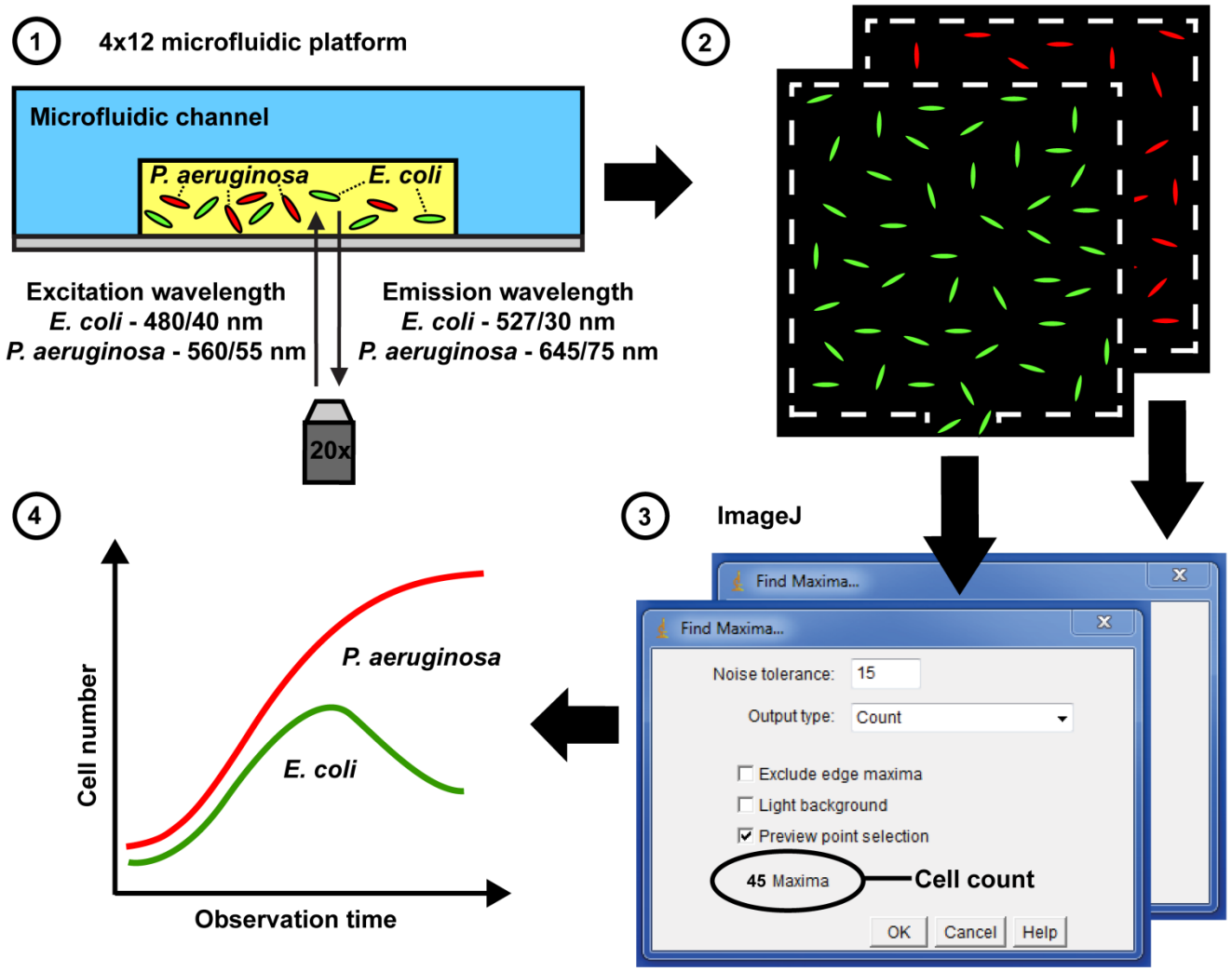

Figure S3. A general schematic showing data analysis procedure for AST in polymicrobial cultures using multiplexed microfluidic platform.

Time-lapse optical micrographs of the experiments

Fig. S4 and **Fig. S5** show the optical micrographs (fluorescent images) at different time intervals for co-cultures of *P. aeruginosa* and *E. coli*, and *P. aeruginosa* and *K. pneumoniae,* respectively. The different rows depict the images for different initial cell numbers.

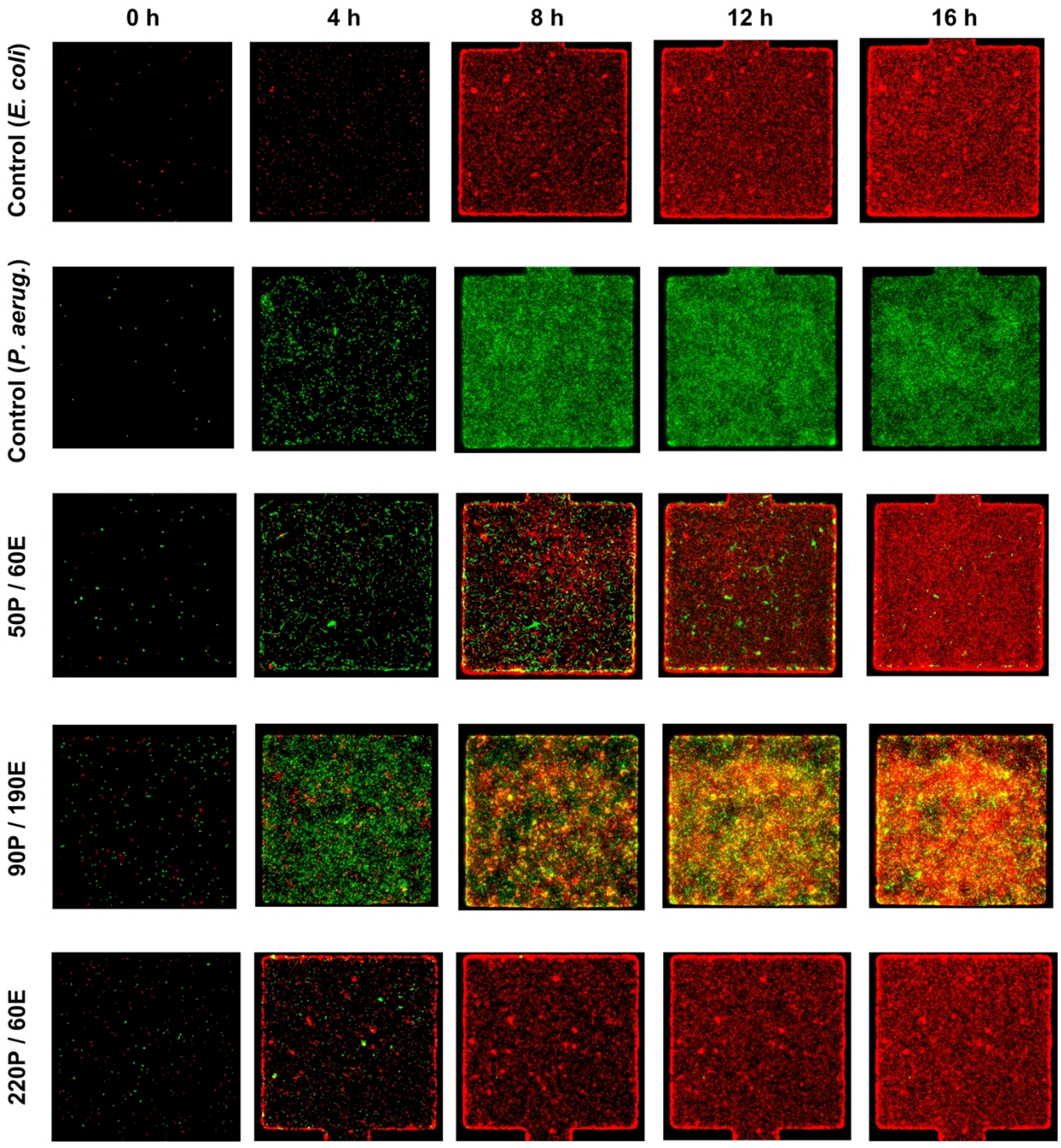

Figure S4. Optical micrographs or fluorescence images of individual microfluidic wells (400 μm by 400 μm) showing changes in cell numbers of *P. aeruginosa* (P), expressing red fluorescent protein or RFP, and *E. coli* (E), expressing green fluorescent protein or GFP in the absence of antimicrobials for different initial conditions, *i.e.*, different initial cell numbers.

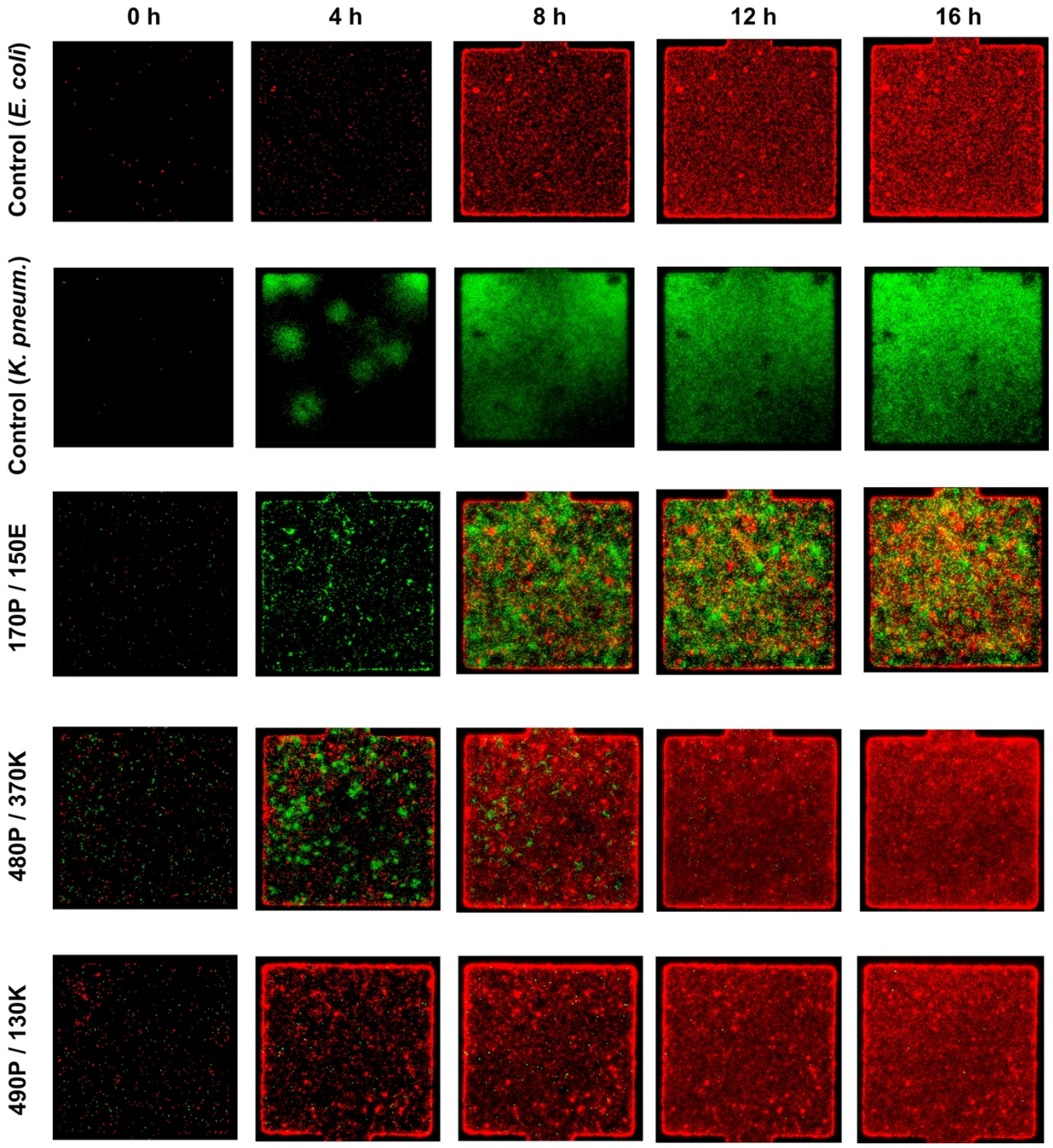

Figure S5. Optical micrographs or fluorescence images of individual microfluidic wells (400 μm by 400 μm) showing changes in cell numbers of *P. aeruginosa* (P), expressing red fluorescent protein or RFP, and *K. pneumoniae* (K), expressing green fluorescent protein or GFP in the absence of antimicrobials for different initial conditions, *i.e.*, different initial cell numbers.

Raw data for growth or time-kill curves

Fig. S6 and **Fig. S7** show examples of raw data for growth or time-kill curves for co-cultures of *P. aeruginosa* and *E. coli*, and *P. aeruginosa* and *K. pneumoniae,* respectively. This type of data was used to generate the plots shown in the main manuscript. Cell growth and death were monitored by counting cells in each well at 30 minute intervals, over a period of 16 h.

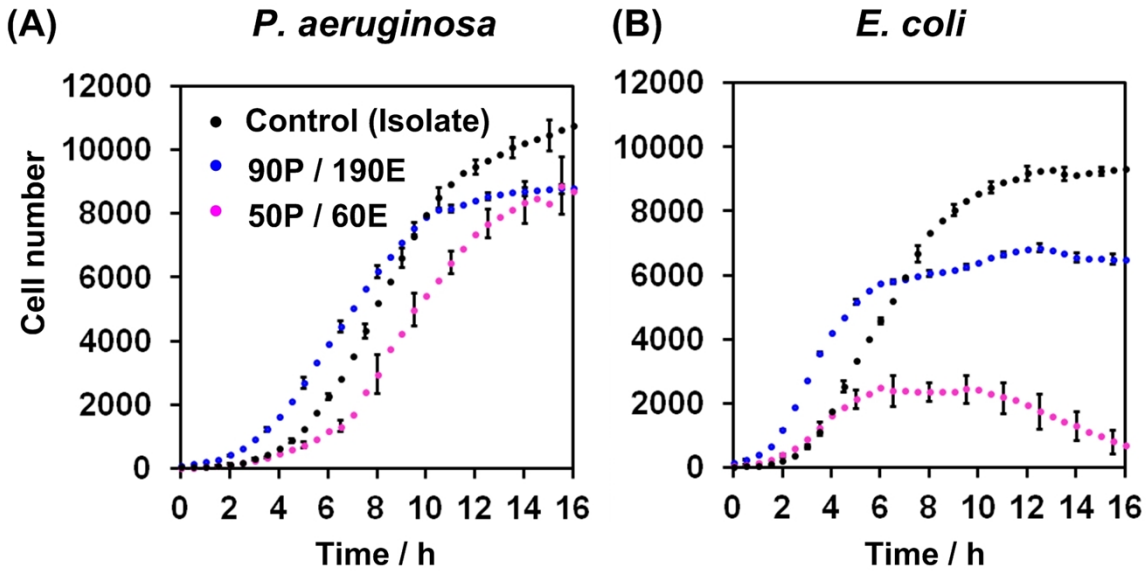

Fig. S6. On-chip real-time monitoring of cell numbers of *P. aeruginosa* (P) and *E. coli* (E) in the absence of antimicrobials for different initial conditions, *i.e.*, different initial cell numbers. Data points represent the mean of the measurements across replicates from three different experiments, and error bars represent the standard error of the mean (depicted for every fourth data point for clarity). The values of initial cell numbers represent the average initial cell numbers across quadruplicate conditions for a single experiment and across three different experiments.

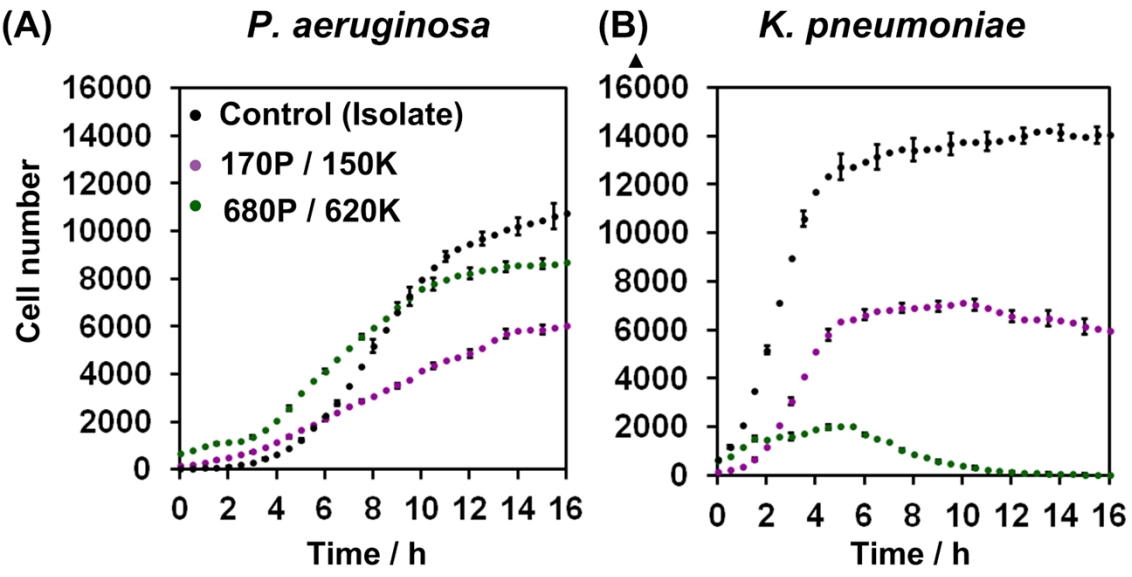

Fig. S7. On-chip real-time monitoring of cell numbers of *P. aeruginosa* (P) and *K. pneumoniae (K)* in the absence of antimicrobials for different initial conditions, *i.e.*, different initial cell numbers. Data points represent the mean of the measurements across replicates from three different experiments, and error bars represent the standard error of the mean (depicted for every fourth data point for clarity). The values of initial cell numbers represent the average initial cell numbers across quadruplicate conditions for a single experiment and across three different experiments.

References:

1. Y. Xia and G. M. Whitesides, *Angewandte Chemie International Edition*, 1998, **37**, 550-575.
2. R. Mohan, A. Mukherjee, S. E. Sevgen, C. Sanpitakseree, J. Lee, C. M. Schroeder and P. J. A. Kenis, *Biosensors and Bioelectronics*, 2013, **49**, 118-125.