Supporting Information

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SI Materials and Methods

Bacterial Strains, Phage, and Chemicals. T4 DNA ligase and all restriction enzymes were purchased from New England Biolabs. PCR reactions were carried out using PCR SuperMix High Fidelity from Invitrogen or Phusion High Fidelity from New England Biolabs. Purification of PCR reactions and restriction digests was carried out with the QIAquick gel extraction or PCR purification kits (Qiagen). Plasmid DNA was isolated using the Qiagen QIAprep Spin Miniprep kit. All other chemicals and materials were purchased from Fisher Scientific, Inc.

Engineering M13mp18 Bacteriophage to Target Genetic Networks. To construct engineered phage, *lexA3*, *soxR*, *csrA*, and *ompF* genes were first placed under the control of the $P_L$tetO promoter in the pZE11G vector (1, 2). Using PCR with primers 5′ ttatca ggtacc atgAAAGCGT TAACGGCC 3′ and 5′ atacat aagctt TTACAGCCA GTCGCCG 3′, *lexA3* was cloned between the KpnI and HindIII sites of pZE11G to form pZE11-*lexA3*. Because *soxR* has an internal KpnI site, we built a synthetic RBS by sequential PCR using 5′ agaggagaaa ggtacc atgGAAAAGA AATTACCCCG 3′ and 5′ atacat aagctt TTAGT TTTGT-TCATC TTCCAG 3′ followed by 5′ agtaga gaattc attaaagag-gagaaa ggtacc atg 3′ and 5′ atacat aagctt TTAGT TTTGT-TCATC TTCCAG 3′. The resulting EcoRI-RBS-*soxR*-HindIII DNA was ligated to an XhoI-$P_L$tetO-EcoRI fragment excised from pZE11G, and the entire DNA fragment was ligated into pZE11G between XhoI and HindIII to form pZE11-*soxR* (2). Primers for *csrA* for cloning into pZE11G between KpnI and HindIII to form pZE11-*csrA* were 5′ agaggagaaa ggtacc atgCT-GATTC TGACTCGT 3′ and 5′ atacat aagctt TTAGTA ACT-GGACTGC TGG 3′; and primes for cloning ompF to form pZE11-*ompF* were 5′ agaggagaaa ggtacc atgATGAAGC GCAATATTCT 3′ and 5′ atacat aagctt TTAGAACTG GTA-AACGATA CC 3′. To express *csrA* and *ompF* simultaneously under the control of $P_L$tetO, we PCR amplified RBS-*ompF* DNA from pZE11-*ompF* using 5′ ccagtc aagctt attaaagaggagaaa ggtacc 3′ and 5′ atacat GGATCC TTAGAACTG GTAAACGATA CC 3′ and cloned the product between HindIII and BamHI in pZE11-*csrA* to form pZE11-*csrA*-*ompF*. The resulting plasmids were transformed into *E. coli* XL-10 cells.

Flow Cytometer Assay of SOS Induction. To monitor φ_{lexA3}'s suppression of the SOS response (Fig. S2), we used a plasmid containing an SOS-response promoter driving *gfp* expression in EMG2 cells ($P_L$lexO-*gfp*) (3). After growing 1:500 dilutions of the overnight cells for 2 h and 15 min at 37 °C and 300 rpm (model G25 incubator shaker, New Brunswick Scientific), we applied ofloxacin and bacteriophage; cells were treated for 6 h at 37 °C and 300 rpm (model G25 incubator shaker; New Brunswick Scientific). Cells then were analyzed for GFP fluorescence using a Becton Dickinson FACScalibur flow cytometer with a 488-nm argon laser and a 515–545 nm emission filter (FL1) at low flow rate. The following photo-multiplier tube settings were used for analysis: E00 (FSC), 275 (SSC), and 700 (FL1). Becton Dickinson Calibrite Beads were used for instrument calibration. For each sample 200,000 cells were collected and processed with MATLAB (Mathworks).

Persister Killing Assay. We performed a persister killing assay to determine whether engineered phage could help kill persister cells in a population that survived initial drug treatment without bacteriophage (Fig. S5). We first grew 1:500 dilutions of overnight EMG2 for 3.5 h at 37 °C and 300 rpm (model G25 incubator shaker; New Brunswick Scientific), followed by treatment with 200 ng/ml ofloxacin for 3 h to create a population of surviving bacteria. Then we added no phage, 10^9 pfu/ml unmodified phage φ_{unmod}, or 10^9 pfu/ml engineered phage φ_{lexA3}. After 3 h of additional treatment, we collected the samples and assayed for viable cell counts as described previously.

Biofilm Killing Assay. Biofilms were grown using *E. coli* EMG2 cells according to a previously reported protocol (4). Briefly, lids containing plastic pegs (MBEC Physiology and Genetics Assay) were placed in 96-well plates containing overnight cells that were diluted 1:200 in 150 μl LB. Plates then were inserted into plastic bags to minimize evaporation and inserted in a Minitron shaker (Infors HT). After 24 h of growth at 35 °C and 150 rpm, lids were moved into new 96-well plates with 200 μl LB with or without 10^8 pfu/ml of bacteriophage. After 12 h of treatment at 35 °C and 150 rpm, lids were removed, washed 3 times in 200 μl of 1X PBS, inserted into Nunc #262162 microtiter plates with 150 μl 1X PBS, and sonicated in an Ultrasonics 5510 sonic water bath (Branson) at 40 kHz for 30 min. Serial dilutions, using the resulting 150 μl 1X PBS, were performed on LB plates, and viable cell counts were determined. Mean killing ($\Delta\log_{10}$(cfu/ml)) was calculated by subtracting mean $\log_{10}$(cfu/ml) after 24 h of growth from mean $\log_{10}$(cfu/ml) after 12 h of treatment (Figs. S6 and S8).

1. Little JW, Harper JE (1979) Identification of the lexA gene product of *Escherichia coli* K-12. *Proc Natl Acad Sci USA* 76:6147–6151.
2. Lutz R, Bujard H (1997) Independent and tight regulation of transcriptional units in *Escherichia coli* via the LacR/O, the TetR/O and AraC/I1–I2 regulatory elements. *Nucleic Acids Res* 25:1203–1210.
3. Dwyer DJ, Kohanski MA, Hayete B, Collins JJ (2007) Gyrase inhibitors induce an oxidative damage cellular death pathway in *Escherichia coli*. *Molecular Systems Biology* 3:91.
4. Lu TK, Collins JJ (2007) Dispersing biofilms with engineered enzymatic bacteriophage. *Proc Natl Acad Sci USA* 104:11197–11202.

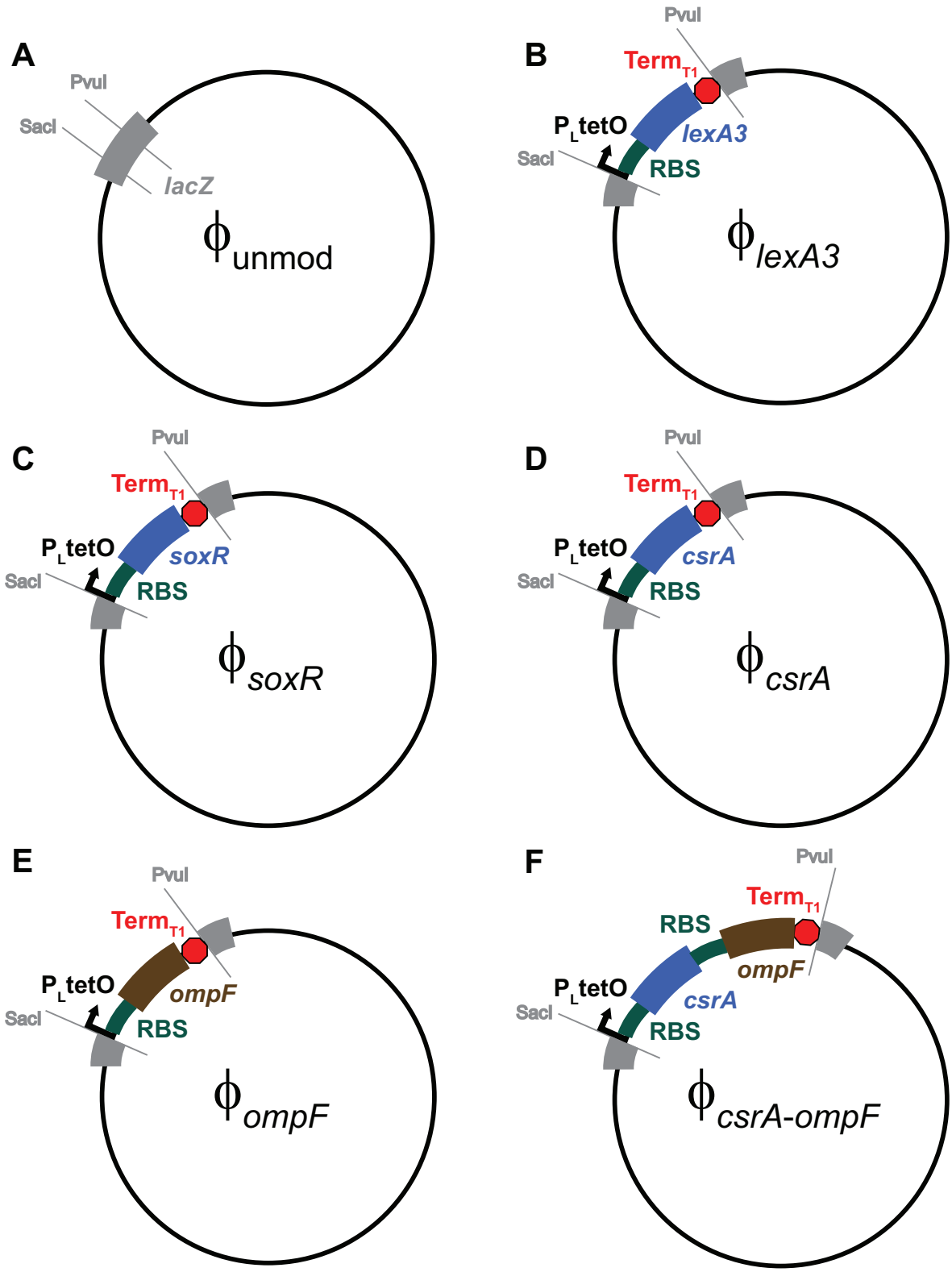

Fig. S1. Genomes of unmodified M13mp18 bacteriophage and engineered bacteriophage. Engineered bacteriophage were constructed by inserting genetic modules under the control of a synthetic promoter ($P_L$tetO) and RBS between SacI and PvuI restriction sites. A terminator ($Term_{T1}$) ends transcription of the respective gene(s). (*A*) Unmodified M13mp18 (φ_{unmod}) contains *lacZ* to allow blue-white screening of engineered bacteriophage. (*B*) Engineered M13mp18 bacteriophage expressing *lexA3* (φ_{lexA3}). (*C*) Engineered M13mp18 bacteriophage expressing *soxR* (φ_{soxR}). (*D*) Engineered M13mp18 bacteriophage expressing *csrA* (φ_{csrA}). (*E*) Engineered M13mp18 bacteriophage expressing *ompF* (φ_{ompF}). (*F*) Engineered M13mp18 bacteriophage expressing *csrA* and *ompF* ($\varphi_{csrA\text{-}ompF}$).

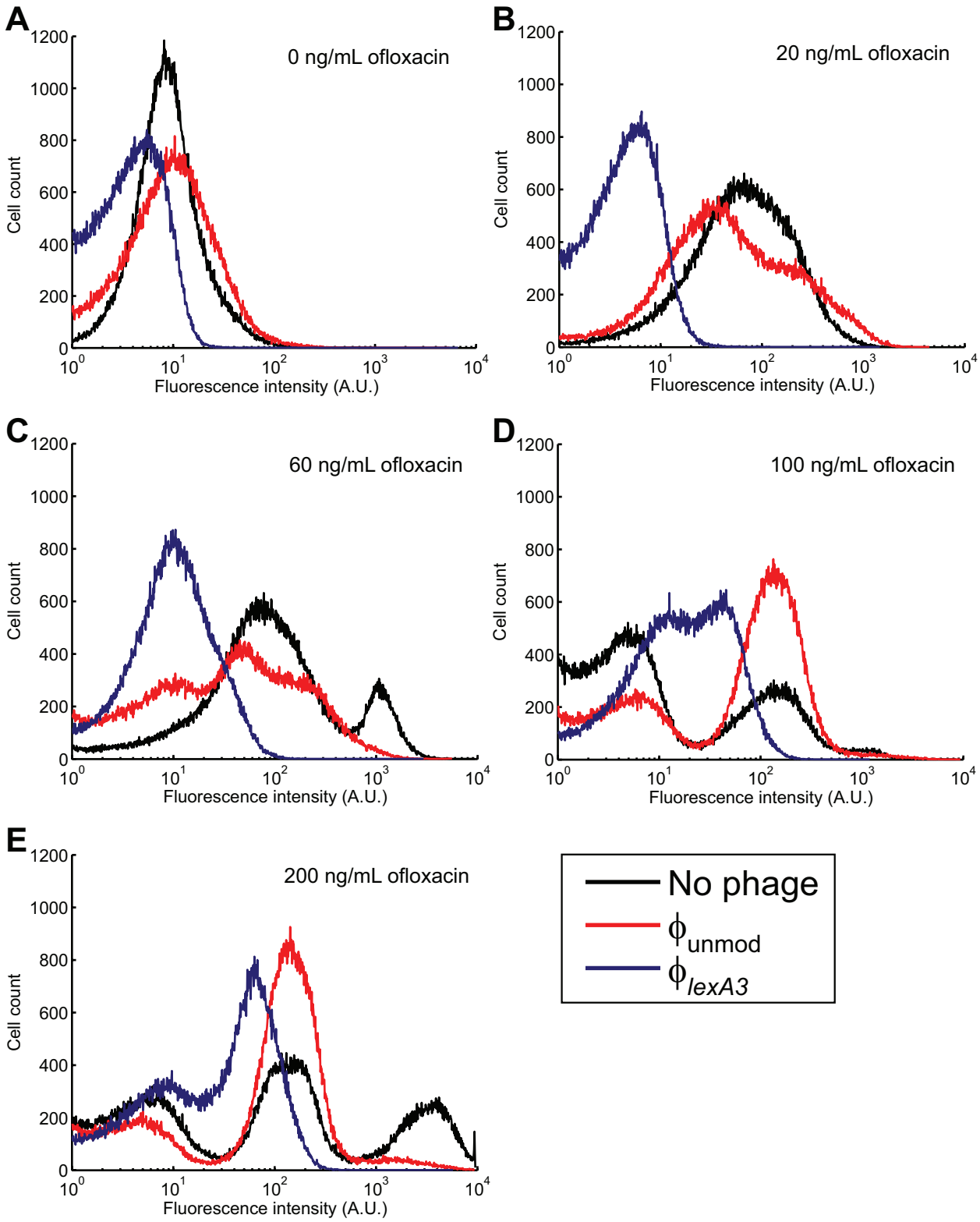

Fig. S2. Flow cytometry of cells with an SOS-responsive GFP plasmid exposed to no phage (*black lines*), unmodified phage φ_{unmod} (*red lines*), or engineered phage φ_{lexA3} (*blue lines*) for 6 h with varying doses of ofloxacin. Where indicated, 10^8 pfu/ml of phage was applied. Cells exposed to no phage or φ_{unmod} showed similar SOS induction profiles, whereas cells with φ_{lexA3} exhibited significantly suppressed SOS responses. (*A*) No ofloxacin treatment. (*B*) Treatment with 20 ng/ml ofloxacin. (*C*) Treatment with 60 ng/ml ofloxacin. (*D*) Treatment with 100 ng/ml ofloxacin. (*E*) Treatment with 200 ng/ml ofloxacin.

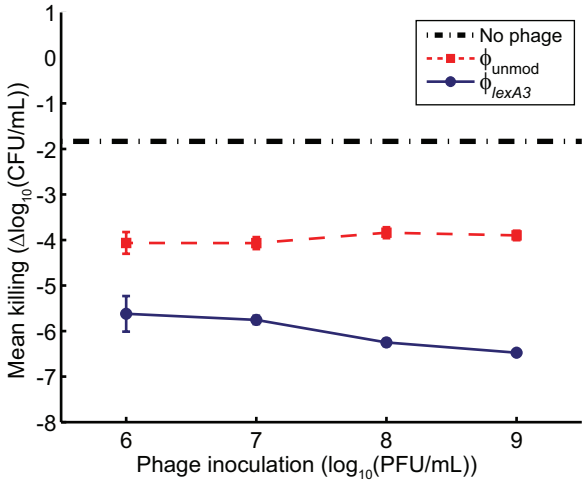

Fig. S3. Engineered φ_{lexA3} bacteriophage enhances killing of wild-type *E. coli* EMG2 bacteria by bactericidal antibiotics. Phage dose–response shows that φ_{lexA3} (*blue circles with solid line*) is a strong adjuvant for ofloxacin (60 ng/ml) over a wide range of initial inoculations compared with no phage (*black dashed/dotted line*) and φ_{unmod} (*red squares with dashed line*). The starting concentration of bacteria was about 10^9 cfu/ml (data not shown).

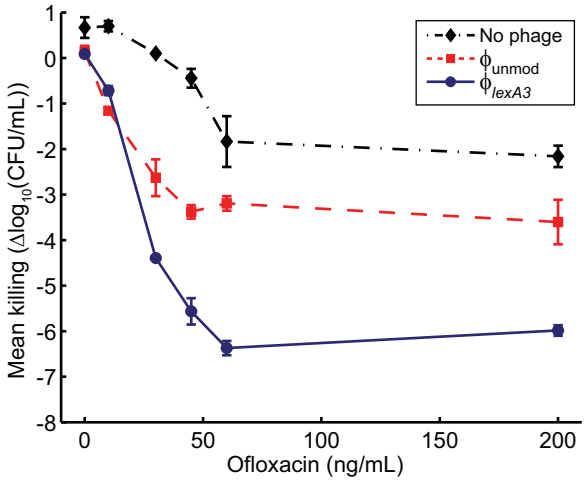

Fig. S4. Engineered φ_{lexA3} bacteriophage enhances killing of wild-type *E. coli* EMG2 bacteria by bactericidal antibiotics. Ofloxacin dose–response shows that φ_{lexA3} (*blue circles, solid line*) increases killing even at low levels of drug compared with no phage (*black diamonds, dash/dotted line*) and φ_{unmod} (*red squares, dashed line*). 10^8 pfu/ml phage was used.

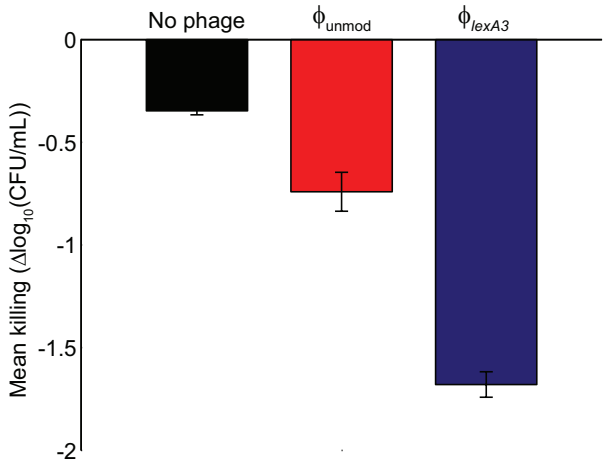

Fig. S5. Persister killing assay demonstrates that engineered bacteriophage can be applied to a previously drug-treated population to increase killing of surviving persister cells. After 3 h of treatment with 200 ng/ml ofloxacin, no phage (*black bar*), 10^9 pfu/ml unmodified phage φ_{unmod} (*red bar*), or 10^9 pfu/ml engineered phage φ_{lexA3} (*blue bar*) was added to the previously drug-treated cultures. Three additional hours later, viable cell counts were obtained and demonstrated that φ_{lexA3} was able to reduce persister cell levels better than no phage or φ_{unmod}.

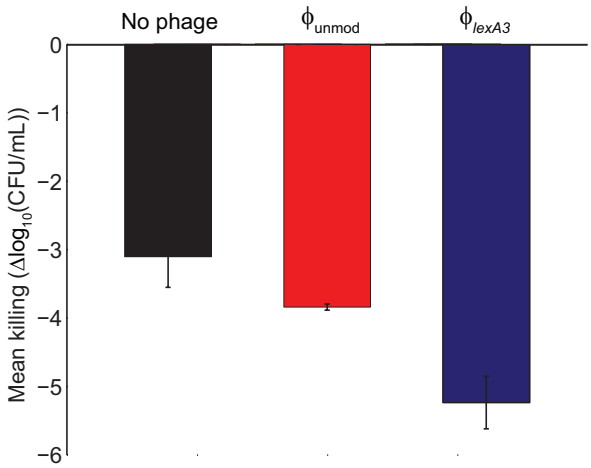

Fig. S6. Mean killing with 60 ng/ml ofloxacin after 12 h of treatment of *E. coli* EMG2 biofilms pregrown for 24 h. Where indicated, 10^8 pfu/ml of φ_{lexA3} bacteriophage was used.

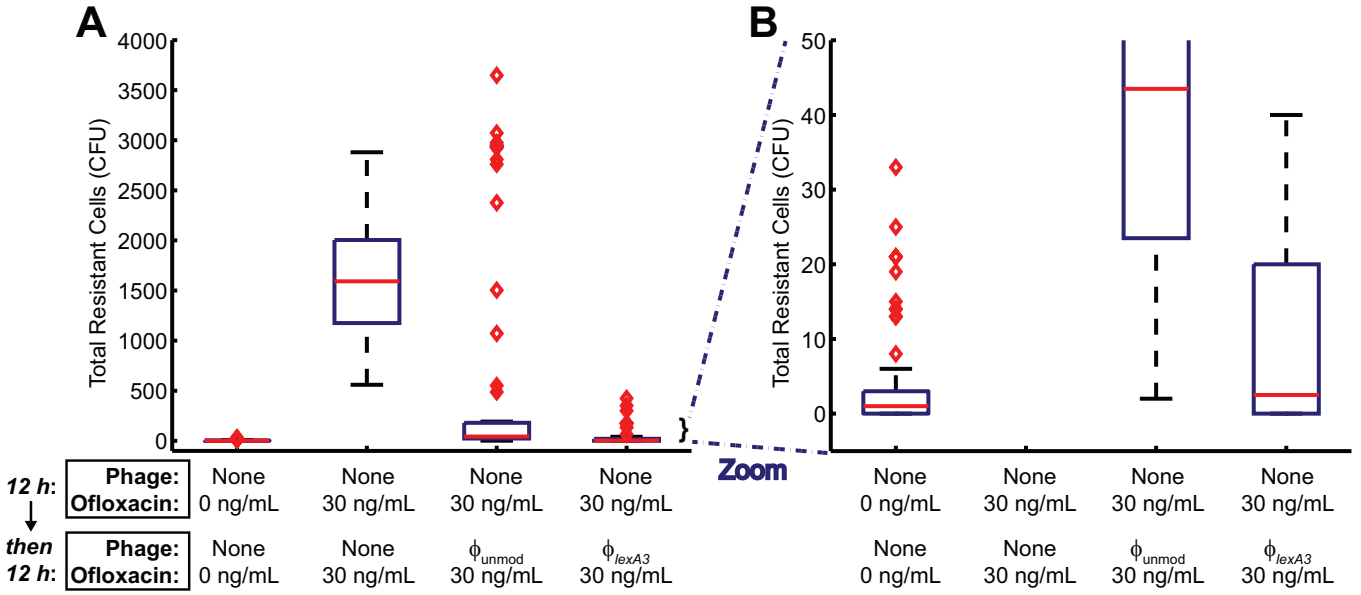

Fig. S7. Box-and-whisker plot of the total number of *E. coli* EMG2 cells in 60 observations that were resistant to 100 ng/ml ofloxacin after growth under various conditions. Red bars indicate medians, red diamonds represent outliers. (*A*) Cells grown with no phage and no ofloxacin for 24 h had very low numbers of antibiotic-resistant cells. Cells grown with no phage and 30 ng/ml ofloxacin for 24 h had high numbers of resistant cells because of growth in subinhibitory drug concentrations. Cells grown with no phage and 30 ng/ml ofloxacin for 12 h followed by 10^9 pfu/ml unmodified phage φ_{unmod} and 30 ng/ml ofloxacin for 12 h exhibited a modest level of antibiotic-resistant bacteria. Cells grown with no phage and 30 ng/ml ofloxacin for 12 h followed by 10^9 pfu/ml φ_{lexA3} and 30 ng/ml ofloxacin for 12 h exhibited a low level of antibiotic-resistant bacteria, close to the numbers seen with no ofloxacin and no phage. (*B*) Zoomed-in version of box-and-whisker plot in (*A*) for increased resolution around low total resistant cell counts confirms that treatment with φ_{lexA3} plus 30 ng/ml ofloxacin reduced the number of resistant cells to levels similar to those seen with no ofloxacin and no phage.

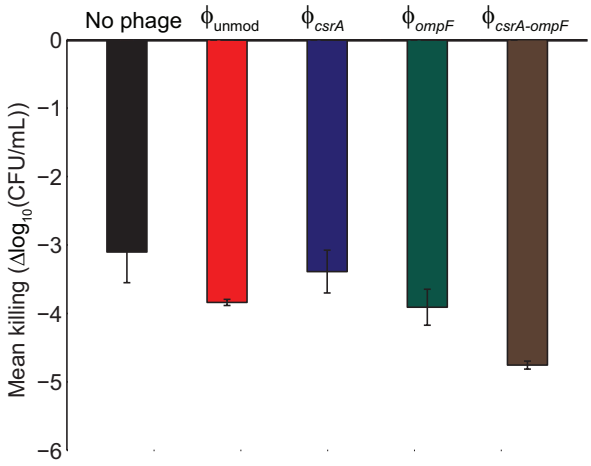

Fig. S8. Mean killing with 60 ng/ml ofloxacin after 12 h of treatment of *E. coli* EMG2 biofilms pregrown for 24 h. Where indicated, 10^8 pfu/ml of φ_{csrA}, φ_{ompF}, or $\varphi_{csrA\text{-}ompF}$ bacteriophage was used.