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Supplementary Information for

Timing of DNA damage responses impacts persistence to fluoroquinolones

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SI MATERIALS AND METHODS

Bacterial Strains and Plasmids

Bacterial strains and plasmids used in this study are provided in Supplemental Table S1. Primers used for strain construction and verification are in Table S1*C*. Construction of strains WM01 (parental strain with mCherry growth reporter and endogenous *mazEF*), WM02 (strain lacking endogenous *mazEF* but bearing inducible *mazE* in place of *araBAD*), and WM03 (strain lacking endogenous *mazEF* but bearing inducible *mazE* and *mazF*) are detailed in our previous work (1). WM03 derivatives with mutations or deletions in SOS response and DNA repair genes were generated using the Datsenko-Wanner method (2) or by P1 transduction (3) of deletions from the Keio collection (4) or *E. coli* MG1655 mutants lacking these genes (5). WM18 (Δ*thyA* mutant) was generated using the Datsenko-Wanner method and selected on LB-kanamycin (KAN) agar with 200 μg/mL thymidine. Where indicated, the kanamycin resistance marker (*kan*) was removed using Flp recombinases expressed from pCP20 (2). Successful deletion was confirmed by PCR using primers indicated in Supplemental Table S1*C*.

To replace *lexA* with the non-inducible *lexA3* mutation (WM15), a *malK* deletion was first introduced into strain WM03 by P1 transduction and selected on LB agar with 50 μg/mL KAN, producing a maltose auxotroph (WM14). *lexA3* was transduced from *E. coli* MG1655*lexA3* (5) into WM14. Due to the proximity of *malK* and *lexA*, transduction of *lexA3* would restore *malK*, thereby allowing these mutants to grow on M9 minimal media with 10 mM maltose as the sole carbon source. Replacement of *lexA* with *lexA3* was confirmed by PCR and sequencing (Genewiz Inc., South Plainfield, NJ).

Chemicals and Growth Media

All media components, inducers, antibiotics, and dyes used in this study were purchased from Sigma-Aldrich (St. Louis, MO) or Fisher Scientific (Waltham, MA) unless otherwise indicated. Growth media were prepared in MilliQ water. LB media (10 g/L tryptone, 5 g/L yeast extract, and 10 g/L NaCl) was prepared from individual components, and LB-agar was prepared with Difco pre-mixed LB Miller broth and 15 g/L agar. Gutnick-glucose media (6) was prepared with 10X Gutnick minimal salts (47 g/L KH_2PO_4, 135 g/L K_2HPO_4, 10 g/L K_2SO_4, and 1 g/L $MgSO_4.7H_2O$), 10 mM NH_4Cl, and 10 mM glucose. For persistence assays carried out under glucose-limited conditions, cells were cultured in Gutnick media with 0.5 mM glucose. Gutnick agar used for post-OFL treatment recovery of persisters was prepared using 1X Gutnick minimal

salts, 10 mM NH_4Cl, and 15 g/L agar. Glucose (10 mM), 5-ethynyl-2-deoxyuridine (EdU; 60 μg/mL), and thymidine (60 μg/mL) were added to Gutnick agar where indicated. M9-maltose agar used for selection of *lexA3* mutants was prepared with 5X M9 salts (33.9 g/L Na_2HPO_4, 15 g/L KH_2PO_4, 5 g/L NH_4Cl, and 2.5 g/L NaCl; Becton Dickinson, Franklin Lakes, NJ), 15 g/L agar, and 10 mM maltose. LB, Gutnick minimal salts, LB-agar, and M9-agar were autoclaved at 121 ^oC for 30 min to achieve sterilization. Carbon and nitrogen sources were filter-sterilized using 0.22 μm filters prior to being added to autoclaved growth media. Gutnick media were sterilized using 0.22 μm filters after they were prepared.

For mutant selection, 100 μg/mL ampicillin (AMP) or 50 μg/mL KAN were used. 300 ng/mL anhydrotetracycline (aTc) was used to induce *mazF* expression, 100 mM L-arabinose (ara) was used to induce *mazE* expression, and 1 mM IPTG was used to induce *recA* expression. For planktonic persistence assays, 5 μg/mL ofloxacin (OFL) and 1 μg/mL ciprofloxacin (CIP) were used. For biofilm persistence assays, 200 μL of a 10 μg/mL OFL solution was delivered onto each biofilm-containing filter. 50 μg/mL chloramphenicol (CM) were used to inhibit translation. Stock solutions of aTc (2 mg/mL) and CM (50 mg/mL) were prepared in ethanol. Stock solutions of AMP (100 mg/mL), KAN (50 mg/mL), OFL (5 mg/mL), ara (1 M), and IPTG (1 M) were dissolved in MilliQ water. OFL stock solution was further titrated with 1 M sodium hydroxide until it was dissolved. To prepare CIP, 5 mg/mL CIP was dissolved in 0.2 N HCl before it was diluted to a 1 mg/mL stock solution in MilliQ water. All antibiotics and inducers prepared in MilliQ water were filter-sterilized with 0.22 μm filters. PicoGreen stock was dissolved in dimethyl sulfoxide (DMSO) and diluted (1:100) in 25% DMSO (diluted in MilliQ water) before being added to cells. EdU was prepared as a 2500 μg/mL stock in DMSO.

Planktonic Persistence Assays

Strains WM01, WM02, WM03, and mutants derived from these strains were inoculated from -80 ^oC stocks stored in 15% glycerol into 2 mL of LB in test tubes. Cells were grown at 37 ^oC with shaking at 250 rpm for 4 h before being diluted (1:100) into 2 mL of Gutnick media with 10 mM glucose in test tubes, where they were grown for 16 h. To mimic changes in gene expression that lead to endogenous MazF accumulation in *E. coli*, where both MazF and MazE are expressed in growing cells and degradation of the labile MazE under stressful conditions enable MazF accumulation, overnight cultures were inoculated in 250 mL baffled flasks to OD_{600} ~0.01 into 25 mL of Gutnick media with 10 mM glucose and 100 mM ara, which was added to induce

mazE expression in WM02 and WM03. After approximately one doubling, aTc was added to the cultures, which in WM03 induced *mazF* expression. With this culturing regimen, MazF was sequestered by MazE as it was expressed, similar to the natural conditions, allowing WM03 to enter exponential phase. When the OD_{600} of the cultures reached 0.1, 5 mL of each culture was pelleted by centrifugation at 4,000 rpm and 37 ^{o}C for 10 min. Following this centrifugation step, 4 mL of supernatant was removed, and the remaining 1 mL of culture was pelleted at 15,000 rpm for 3 min in microcentrifuge tubes. After removing the supernatant, the cells were washed with 1 mL of pre-warmed (37 ^{o}C) Gutnick-glucose (with aTc) and centrifugation at 15,000 rpm for 3 min was repeated. These centrifugation and washing steps were conducted to ensure that all of the arabinose-containing supernatant could be removed. After removal of the supernatant and resuspending the pellet in 300 µL of fresh growth media, all strains were inoculated into 25 mL of Gutnick-glucose with aTc or with both aTc and ara in 250 mL baffled flasks, achieving an OD_{600} ~0.02. Cells were grown at 37 ^{o}C with shaking at 250 rpm. At designated times, 300 µL of each culture was removed for OD_{600} measurements using a BioTek Synergy H1 Multi-mode reader (BioTek Instruments, Inc., Winooski, VT). When toxin-free controls were treated with CM prior to and during OFL treatment, CM was added 2 h following inoculation. It should be noted that CM treatment was carried out with strain WM01, which is the parental strain of WM02 and WM03, as WM02 contains a CM-resistant marker that was used to select for antitoxin integration. OFL was added to cultures 4 h post-inoculation (2 h after CM treatment or ~2 h after MazF-mediated growth arrest). Exponentially growing toxin-free control was cultured to an OD_{600} ~0.2-0.4 at the time of treatment, as this density matched those of MazF persisters and CM-inhibited cells at the time of growth arrest.

Before OFL addition, 500 µL of each culture was collected to enumerate cells. After OFL was added, cultures were incubated at 37 ^{o}C with shaking at 250 rpm. At 1, 3, and 5 h post-treatment, 500 µL of each culture was collected to enumerate persisters. After collecting cells in microcentrifuge tubes, cells were pelleted by centrifugation at 15,000 rpm for 3 min. After removing 450 µL of supernatant, the pellets were resuspended with 450 µL PBS. Centrifugation and resuspension in PBS were repeated to reduce OFL concentration to below its minimal inhibitory concentration (7). Following the final centrifugation, 400 µL of supernatant was removed and cells were resuspended in the remaining supernatant. Cells were serially diluted (10-fold dilutions) in PBS and 10 µL of each dilution was plated on LB-agar supplemented with

arabinose. For Δ*recA*, Δ*recB*, and *lexA3* mutants, all remaining cells were plated. To enumerate persisters, colonies were counted following incubation at 37 ^{o}C for 16 h. For each data point, 10-100 colonies were counted.

To elucidate whether recovery on non-nutritive media following OFL or CIP treatment affects persister levels, *E. coli* MG1655 was inoculated and cultured overnight as described above. Following overnight growth, cells were inoculated to OD_{600} ~0.01 in 25 mL of Gutnick media with 10 mM glucose or 0.5 mM glucose in 250 mL baffled flasks. Cells were grown until cultures propagated in Gutnick media with 0.5 mM glucose had been growth-inhibited for 2 h due to glucose exhaustion (Fig. S7*AB*), 500 μL of culture was collected to enumerate cfus prior to treatment, and the remaining culture was treated with OFL or CIP. At 1, 3, and 5 h, cells were collected and removed of OFL or CIP as described above. Cells were serially diluted (10-fold dilutions) in PBS after removal of OFL or CIP. Dilutions that enabled 10-100 colonies to be counted when 10 μL of samples was plated on LB agar (countable dilutions) were determined from previous assays. Under "delay" conditions, 10 μL of cell suspensions from countable dilutions were plated on 25 mm Supor hydrophilic polyethersulfone (PES) filter discs with 0.2 μm pores (VWR International, South Plainfield, NJ) overlaid on Gutnick agar (without glucose), and then incubated at 37 ^{o}C. At designated times, these filters were transferred to plates with LB agar, where they were then incubated at 37 ^{o}C for 16-20 h. For controls, where cells were not recovered on non-nutritive agar ("no delay" conditions), 10 μL of cell suspensions from countable dilutions were plated on PES filter discs overlaid on LB agar, where they were then incubated at 37 ^{o}C for 20 h. To assess the effect of extended incubation times on cfu counts, we plated 30 μL of countable dilutions of OFL-treated MG1655 on filter discs overlaid on LB agar immediately after OFL removal ("no delay" condition) or on Gutnick agar for 4 h before being transferred to LB agar ("delay" condition). We observed that incubation of both of the "delay" and "no delay" populations on LB agar for 16 h or 48 h did not result in significant differences in cfu counts (Fig. S7*G*). For each replicate, 10-100 colonies were counted.

To evaluate the impact of post-OFL treatment recovery on persisters originating from stationary phase cultures, persistence and recovery experiments were performed with *E. coli* MG1655 following a protocol similar to the one described above. However, following inoculation and growth in LB for 4 h, cells were diluted (1:100) in 25 mL of Gutnick media with

10 mM glucose in a 250-mL baffled flask, where they were cultured at 37 ^{o}C for 16 h prior to OFL treatment and cfu enumeration.

RecA Induction and OFL Persistence

RecA induction experiments were performed with *E. coli* WM16 (Δ*recA* mutant of WM03, which was used to generate MazF persisters) and WM19 (MG1655Δ*recA*). These strains carried IPTG-inducible, plasmid-borne RecA (pKV012) or *gfp*-expressing vector control (pP_{T5lac}*gfp*), and they were cultured and treated with OFL in media containing KAN for plasmid retention following procedures described for persistence assays. For WM16 samples, each plasmid-bearing strain was diluted to OD_{600}~0.02 in two baffled flasks with Gutnick media (10 mM glucose) and 300 ng/mL aTc for MazF induction after cells reached exponential phase (OD_{600}~0.1); one of these flasks contained media with 1 mM IPTG to induce *recA* expression in pKV012-bearing strains (or GFP expression in the control) throughout the course of MazF arrest and OFL treatment, whereas the other culture was not exposed to IPTG before and during treatment. All Δ*recA* mutants of MazF persisters were treated with OFL 4 h post-dilution. After 5 h of OFL treatment, 500 μL aliquots of cells were collected from each culture, transferred to microcentrifuge tubes, and pelleted by centrifugation. Pellets were washed to remove OFL as described for persistence assays.

In Figure 3, condition 1 describes samples that were collected from cultures induced with IPTG throughout the course of MazF-mediated stasis and OFL treatment. These samples were then inoculated into LB-KAN with IPTG and 100 mM ara after treatment. For condition 2, samples were collected from cultures lacking IPTG, but the samples were then inoculated into the same media as cells from condition 1 following OFL removal. Similar to samples for condition 2, samples for conditions 3 and 4 were also collected from cultures lacking IPTG, but these cells were inoculated into LB-KAN lacking IPTG. All samples were incubated in 17x100-mm polypropylene tubes (Thermo Fisher Scientific) at 37 ^{o}C with shaking at 250 rpm for 4 h. Cells were then collected in microcentrifuge tubes, pelleted by centrifugation, and washed once with PBS. Cells were serially diluted in PBS (10-fold dilutions), and 10 μL of each dilution was plated on LB-KAN agar with ara and IPTG (conditions 1, 2, and 3) or ara alone (condition 4). To enumerate persisters, colonies were counted after incubation at 37 ^{o}C for 16 h.

For experiments performed with MG1655Δ*recA*, cells were inoculated into two 250 mL baffled flasks with 25 mL of Gutnick media with 0.5 mM glucose to OD_{600} ~0.01 following

overnight growth. One of these cultures was induced with 1 mM IPTG, whereas the other culture was not induced. These cultures were treated with OFL after growth had been inhibited for 2 h due to glucose exhaustion. After 5 h of treatment, 500 μL aliquots of cells were collected from each culture. Pellets were washed to remove OFL and serially diluted in PBS (10-fold dilutions) as described for persistence assays. In Fig. 4*D*, condition 5 represents cells that were collected from cultures that were induced with IPTG throughout growth, stasis, and OFL treatment, and these cells were spotted on PES filters overlaid on Gutnick-KAN agar pads with IPTG after antibiotic removal. Condition 6 represents cells that were collected from cultures lacking IPTG, and these cells were spotted on PES filters overlaid on Gutnick-KAN agar pads with IPTG. For conditions 7 and 8, cells were collected from cultures lacking IPTG and spotted onto Gutnick-KAN agar pads lacking IPTG. These samples were incubated at 37 ^{o}C for 4 h before the filters were transferred onto LB-KAN agar pads with IPTG (for conditions 5, 6, and 7) or without IPTG (for condition 8), where they were incubated at 37 ^{o}C for 16 h before cfu enumeration. For condition 9 in Fig. 4*D* and S7*F*, samples were collected from cultures induced with IPTG during growth, stasis, and OFL treatment, and cells were spotted onto PES filters on LB-KAN agar pads with IPTG after OFL removal. Samples for condition 10 in Fig. S7*F* were collected from cultures lacking IPTG and cells were spotted onto PES filters on LB-KAN agar pads with IPTG. For condition 11, samples were also collected from cultures lacking IPTG, but these cells were spotted on PES filters on LB-KAN agar pads without IPTG. For samples from conditions 9, 10, and 11, cfus were enumerated after 20 h of incubation at 37 ^{o}C, so as to be consistent with the cumulative time after OFL treatment for conditions 5, 6, 7, and 8 (4 h starvation plus 16 h recovery).

To assess the effect of incubation time on cfu counts of *recA*-null (WM19 bearing pP_{T5lac}*gfp*) and *recA*-complemented (WM19 bearing pKV012) strains, these strains were cultured, treated with OFL, and recovered in the presence of KAN (for plasmid maintenance) and 1 mM IPTG. After 5 h of treatment, the antibiotic was removed with PBS washses as described above. Following serial dilution, 30 μL of countable dilutions were spotted on PES filters overlaid on LB-KAN agar ("no delay" condition) or on Gutnick-KAN agar without carbon source for 4 h before being transferred to LB-KAN agar ("delay" condition). All of these agar pads contained 1 mM IPTG. cfus were enumerated after 16 h and 48 h of incubation at 37 ^{o}C (Fig. S7*HI*). We note that for MG1655Δ*recA* mutant harboring pP_{T5lac}*gfp* (without *recA*

complementation), carbon source starvation after OFL treatment resulted delayed colony formation, as fewer cfus were observed at 16 h compared with samples that were immediately plated on LB agar after treatment. By 48 h, cfu counts for both the "no delay" and "delay" samples were higher but still comparable to each other. Consistent with our observations in Fig. 4*D*, delaying growth of *recA*-null mutants by starvation following OFL treatment did not improve survival.

Colony Biofilm Persistence Assays

To elucidate whether recovery on non-nutritive media following OFL treatment impacts persistence of cells originating from biofilms, colony biofilms were grown as described previously (8). Briefly, *E. coli* MG1655 was inoculated from -80 ^{o}C stocks and cultured overnight as described for planktonic persistence assays. Following overnight growth, cells were diluted to OD_{600} ~0.01 in Gutnick media with 2.5 mM glucose, and 100 μL of this culture was inoculated onto sterile PES filter discs overlaid on 5 mL Gutnick agar pads, which lacked carbon sources (Fig. S8*A*). Colony biofilms were grown at 37 ^{o}C for 16 h. To ensure that cells in the 16 h biofilm were growing as surface-attached communities, a filter disc inoculated with *E. coli* MG1655 expressing GFP (strain WM20) was visualized using fluorescence microscopy (Fig. S8*B*). In comparison to a control, where planktonic cells at the same density were inoculated onto the filter just prior to microscopy (Fig. S8*C*), the colony biofilm is far less uniform and depicts higher-density cell clustering. To ensure that glucose had been exhausted at the time of OFL treatment, we quantified the amount of glucose in the filter discs at inoculation and after 16 h incubation (Fig. S8*D*). For this experiment, filter discs were aseptically removed from the pads and transferred to a 15 mL conical tube containing 1 mL PBS. The samples were vortexed at maximum speed for 1 min to dislodge cells. The resulting cell suspension was filtered using 0.22 μm filters, and glucose was quantified using the Amplex Red Glucose/ Glucose Oxidase Kit (Thermo-Fisher Scientific).

For persister assays, each time point had to be obtained from a different filter disc, since colony count enumeration required dislodging the cells from the filter surface. For the initial time point, a filter disc was aseptically transferred to a 15 mL conical tube prior to OFL treatment. Cells were dislodged by vortexing in PBS for 1 min, pelleted by centrifugation, and washed with PBS. The sample was then serially diluted in PBS, and 10 μL of each dilution was plated on LB agar to enumerate cfus. Additional colony biofilms were treated with 200 μL of 10

μg/mL OFL applied to the top of each biofilm-containing filter disc and incubated at 37 ^{o}C. At designated times, cells from treated biofilms were collected, washed, and serially diluted as described above. 10 μL of each dilution was plated on LB agar, and 10-100 cfus were counted for each time point after 16 h incubation at 37 ^{o}C (Fig. S8*E*).

We further evaluated the impact of post-treatment starvation on OFL persistence in colony biofilms. Following 5 h of OFL treatment, one of the filter discs, which constitutes the $t_{recovery}$=0 sample in Fig. 5*B*, was aseptically removed. Cells were dislodged from the filter by vortexing, collected in microcentrifuge tubes, washed with PBS, serially diluted, and plated. The remaining filters were transferred onto 25 mL Gutnick agar pads lacking carbon sources and incubated at 37 ^{o}C, allowing biofilms to recover on non-nutritive media after treatment. At designated times, filter discs were removed, vortexed, washed, serially diluted, and plated on LB agar as described above. cfus were enumerated after 16-20 h of incubation at 37 ^{o}C. As the antibiotic diffuses into the 5 mL Gutnick agar pads during 5 h of treatment, it is estimated that 0.4 μg/mL OFL remained in the filter disc (assuming that the volume of the filter disc is negligible compared to that of the agar pad). Given that the thickness of the 25 mm PES filter is 145 μm, the volume of a disc is 0.071 mL. Thus, placing the filter on a 25 mL agar pad following treatment would effectively dilute OFL in the disc by approximately 350-fold via diffusion to ~0.001 μg/mL, which is below its MIC for *E. coli* MG1655 (7).

Fluorescence Microscopy of Colony Biofilms

To ensure that cells existed as surface-attached communities following overnight growth on PES membranes overlaid on Gutnick agar, *E. coli* MG1655 expressing GFP (strain WM20) grown on PES membranes were visualized by fluorescence microscopy following a protocol similar to the one described by Amato and Brynildsen (8). *E. coli* MG1655 expressing GFP was inoculated in 2 mL of LB, cultured for 4 h, diluted (1:100) in 2 mL of Gutnick media with 10 mM glucose with 2 mM IPTG to induce GFP expression, and cultured for 16 h. The overnight culture was subsequently diluted in Gutnick media with 2.5 mM glucose and 2 mM IPTG to OD_{600} ~0.01, and 100 μL of this culture was spotted onto a PES membrane overlaid on a Gutnick agar pad with 2 mM IPTG. The membrane was incubated at 37 ^{o}C for 16 h before it was imaged. To prepare the planktonic control sample, the overnight culture was diluted to OD_{600} ~0.01 in 25 mL of Gutnick media with 2.5 mM glucose and 2 mM IPTG in a 250 mL baffled flask and cultured for 16 h at 37 ^{o}C with shaking at 250 rpm. Following overnight growth, 100 μL of this

culture, which contained 10^7 cells (approximately the same number of cells present in the colony biofilm), was spotted onto a PES membrane overlaid on Gutnick agar with 2 mM IPTG. Once the sample had dried, the membrane was prepared for imaging.

Membranes were immobilized on a glass slide, covered with a cover slip, and imaged using a Nikon TE2000 inverted microscope (Nikon, Melville, NY) equipped with a 20X Plan Fluor Nikon objective (0.45 NA). GFP fluorescence was collected with a Nikon FITC HYQ filter cube (Nikon #96320) with 480/40 exication and 535/50 emission filters. A Hamamatsu Orca-Flash 2.8 camera (Hamamatsu, Bridgewater, NJ) and NIS Elements software (Nikon) were used for image acquisition. ImageJ (9) was used for image analysis.

Expression of an SOS Reporter During Recovery from OFL Treatment

The *recA* promoter (P_{recA}) fused to GFP was used as the SOS reporter in strains WM01, WM02, WM03, and WM15. The aforementioned strains harboring the SOS reporter (pKV006) were cultured and treated with OFL in media containing KAN for plasmid retention following the procedures described for persistence assays. Two cultures of each strain were prepared. OFL was added to one of the cultures, while the second culture remained untreated. CM (50 μg/mL) was added to WM01 2 h before OFL administration.

After 5 h of OFL treatment, OD_{600} was measured and volumes of each culture that yielded OD_{600} ~0.05 when the cells were diluted into 20 mL of the recovery media (LB with 100 mM ara and KAN) were collected. Cells from each culture were pelleted by centrifugation at 4,000 rpm for 10 min at 37 ^{o}C. After removing all but 1 mL of supernatant, cells were resuspended in the remaining media and transferred to microcentrifuge tubes. To remove OFL, cells were pelleted by centrifugation at 15,000 rpm for 3 min. After removing all of the supernatant, cell pellets were washed with 1 mL of the recovery media. Following centrifugation, the supernatant was removed and cells were inoculated into 20 mL of recovery media.

Immediately following inoculation and every 2 h thereafter, OD_{600} of each culture was measured, 500 μL of each culture was collected for fixation. Cells were pelleted by centrifugation and pellets were resuspended in 500 μL of 4% paraformaldehyde (PFA) in PBS. Cells were fixed at room temperature for 30 min before being pelleted by centrifugation. After removing the PFA, pellets were resuspended in PBS and stored at 4 ^{o}C until they were used for flow cytometry. For fluorescence measurements, cells were diluted to OD_{600} ~0.01 in 1 mL of PBS and analyzed using an LSRII flow cytometer. Bacteria were identified using forward scatter

(FSC) and side scatter (SSC) parameters determined from an untreated control. GFP fluorescence was excited with a laser emitting at 488 nm and fluorescence emission was collected using a green fluorescene filter (525/50 nm-band-pass filter). Data was acquired and analyzed using the FACSDiVa software and FlowJo (TreeStar, Ashland, OR). For analysis, we used FSC versus SSC gating to to identify single cells, and histograms generated with these gated populations are shown in Fig. S3. For comparison, we also show the histograms of the ungated population in Fig. S4. To set the threshold for fluorescent cells, we used a non-fluorescent negative control (*lexA3* mutant without OFL treatment). This threshold captured 99.9% of the non-fluorescent control, and cells with fluorescence intensities above this threshold were considered fluorescent.

Liquid Chromatography and Mass Spectroscopy (LC-MS)

Strains WM02 and WM03 were cultured and treated with OFL as described above for persistence assays. Cellular metabolites were extracted before OFL addition and 1 h following OFL treatment. The toxin-free control (WM02) was grown in the presence of aTc to ensure that the inducer of MazF expression did not impact the metabolome. Culture densities (OD_{600}) were measured at the time of collection and 4 mL of each culture was vacuum-filtered on a nylon membrane (0.2 μm pore size). Metabolism was quenched, and metabolites were extracted and dried as described previously (1). Metabolites were resuspended in HPLC grade H_2O, and they were analyzed using reversed-phase ion-pairing liquid chromatography coupled to a stand-alone Exactive Orbitrap Mass Spectrometer (Thermo-Fisher Scientific) operated under negative-ion mode electrospray ionization (10). Using the MAVEN software, metabolite peaks that matched mass-to-charge ratios and retention times of authenticated standards were quantified (11).

Metabolite abundances were normalized by culture density (OD_{600}) measured at the time of extraction. For each strain, ratios of the density-normalized metabolite abundance of OFL-treated samples to normalized metabolite abundance of untreated samples were calculated to determine the effects of OFL treatment. Metabolites that were detected in both MazF persisters and toxin-free populations across both time points in at least three of the five experimental replicates performed are depicted in Fig. S2.

Southern Blotting

Chromosomal integrity at the *dnaA-gyrB* locus following OFL treatment was examined following the Southern blotting protocol described by Franco and Drlica (12). Strains WM01,

WM02, and WM03 were cultured as described above for persistence assays. WM01 was treated with CM 2 h before OFL addition. Two cultures were prepared for each strain, and one of the cultures (representing the untreated control) was pelleted by centrifugation at 4,000 rpm, for 10 min at 37 ^{o}C. After removing 24 mL of supernatant, the cells were resuspended in the remaining media, transferred to a microcentrifuge tube, pelleted by centrifugation at 15,000 rpm for 3 min. Meanwhile, cells in the other flasks were treated with OFL for 30 min as quinolones can block DNA synthesis rapidly (13), and cells were collected by centrifugation thereafter. Genomic DNA (gDNA) was collected using the Qiagen Blood and Tissue kit (Qiagen Inc., Germantown, MD) following manufacturer's protocol. Cells were lysed using buffer ATL with 0.5% SDS at 56 ^{o}C for at least 1 h. Following extraction, gDNA was eluted with 30 µL of DI water, and DNA yield was quantified using the BioTek Synergy H1 Multi-mode reader equipped with Take3 Micro-Volume Plate.

Approximately 25 µg of gDNA was digested with 80 units of high-fidelity HindIII (New England Biolabs, Ipswich, MA) at 37 ^{o}C for 16 h. Following digestion, the enzyme was deactivated by heating at 80 ^{o}C for 20 min. gDNA was resolved on a 0.7% agarose gel (15 cm x 20 cm) at 100 V for 2 h. As a marker, a 10 kb sequence encompassing the upstream region of *dnaA* to *gyrB* was amplified by PCR and ~2 µg of the product was resolved on the gel along with the gDNA.

Following gel electrophoresis, DNA on the gel was depurinated, denatured, neutralized, and blotted by capillary transfer onto a Nytran membrane using the GE TurboBlotter system (GE Healthcare Life Sciences, Pittsburgh, PA). The agarose gel was soaked in ~100 mL of 0.2 N HCl for 30 min for DNA depurination. After briefly rinsing the gel with MilliQ water, the gel was incubated in the denaturation buffer (1.5 M NaCl and 0.5 M NaOH) with gentle shaking for 30 min. The gel was rinsed again and incubated with neutralization buffer (1.5 M NaCl, 0.5 M Tris-HCl, pH 7.0) with gentle shaking for 30 min. The gel was then equilibrated in filter-sterilized 20X SSC Transfer buffer (3M NaCl, 0.3 M sodium citrate, pH 7.0) with gentle shaking for 30 min. Meanwhile, the SuPerCharge Nytran membrane was rinsed with purified DI water, and the membrane along with four sheets of 3 MM Chr blotting paper were equilibrated in 20 X SSC for 5 min. The gel and membrane were assembled in the TurboBlotter transfer system following the manufacturer's instructions. DNA was transferred from the gel to the membrane at room temperature for 3 h. Following transfer, the membrane was placed on a piece of filter paper

soaked with 10X SSC and DNA was cross-linked to the nylon matrix of the membrane by UV irradiation using a trans-UV lamp (302 nm) installed in a Bio-Rad Gel Doc XR+ gel documentation system for 1 min. After cross-linking, the membrane was briefly rinsed with purified MilliQ water and allowed to air dry. Excess membrane (sections that did not align with DNA containing lanes on the gel) was excised.

Hybridization and immunodetection were carried out using a Roche DIG High Prime DNA Labeling and Detection Kit (Sigma-Aldrich). Hybridization temperature was determined to be ~46 ^{o}C using the following equations provided in the manufacturer's manual: T_m=49.82+0.41(%G+C)-(600/*l*) and $T_{hybridization}$=T_m- (20 to 25 ^{o}C), where *l* is the length of the hybrid in base pairs. Prior to hybridization, the membrane was incubated in 50 mL of filter-sterilized DIG Easy Hyb Buffer at 46 ^{o}C for 30 min for pre-hybridization. To generate the digoxigenin (DIG)-labeled probe for hybridization, a 1 kb DNA product that is complementary to the 5' end of *dnaA* (downstream of a HindIII restriction site) was amplified by PCR using primers presented in Table S1C. One μg of the purified template was boiled at 100 ^{o}C for 5 min and quickly chilled on ice for denaturation before being labeled with digoxigenin (DIG)-dUTP using DIG-High Prime. The reaction was carried out at 37 ^{o}C for 1 h and the sample was heated at 80 ^{o}C for 10 min to stop the reaction. Before it was used for hybridization, the labeled probe was boiled at 100 ^{o}C for 5 min and chilled on ice for denaturation, and it was diluted 2,500-fold in 50 mL of pre-heated DIG Easy Hyb Buffer. After decanting the pre-hybridization solution, the DIG Easy Hyb Buffer (with probe) was poured onto the membrane in a sealed container, where they were incubated at 46 ^{o}C in a shaker with gentle shaking (50 rpm) overnight. Unbound probes were removed with two 5 min washes with 2X SSC with 0.1% SDS carried out at room temperature followed by two 15 min washes with 0.5X SSC with 0.1% SDS warmed to 65-68 ^{o}C. Washes were carried out with gentle shaking. After these stringency washes, the membrane was rinsed with washing buffer (0.1 M maleic acid, 0.15 M NaCl, 0.3% (v/v) Tween 20) at room temperature with gentle shaking for 5 min.

Blocking, immunodetection, and washes were carried out at room temperature with gentle shaking. Non-specific binding sites on the membrane were blocked with 100 mL of blocking solution (10X blocking solution supplied with the kit diluted in 0.1 M maleic acid and 0.15 M NaCl) for 30 min. For immunodetection, the anti-digoxigenin-alkaline phosphatase antibody was diluted to 75 mU/mL (1:10,000) in 20 mL of blocking solution and the antibody

solution was incubated with the membrane for 30 min. Unbound antibodies were removed with two 15 min washes in 100 mL of washing buffer, and the membrane was equilibrated with 20 mL of detection buffer (0.1 M Tris-HCl, 0.1 M NaCl, pH 9.5) for 5 min. Antibody binding was detected using a chemiluminescent substrate in the CSPD ready-to-use solution, and chemiluminescence was detected after exposing the membrane to a Hyblot CL autoradiography film (Denville Scientific Inc., Holliston, MA). Membrane was exposed to film for 2-4 h depending on signal intensity.

PicoGreen Staining

Strains WM01, WM02, and WM03 were cultured as described above for persistence assays. Four hours following dilution, 10 mL of each culture was pelleted by centrifugation at 4,000 rpm at 37 ^{o}C for 10 min. After removing 9 mL of supernatant, the pellet was resuspended in the remaining media and the cells were fixed with 9 mL of 70% ethanol. Cells were fixed at 4 ^{o}C for at least 3 h.

To determine the chromosome equivalents of fluorescence intensity, stationary phase cultures of *E. coli* MG1655 were collected based on a protocol described by Akerlund and colleagues (14). *E. coli* MG1655 was inoculated from -80 ^{o}C stocks stored in 15% glycerol into 2 mL of LB in test tubes and cultured for 16 h at 37 ^{o}C with shaking at 250 rpm. Following overnight growth, cells were diluted 10^{7}-fold in 25 mL of LB with 0.2% glucose in 250 mL baffled flasks. OD_{600} of the culture was measured and 1 mL of culture was fixed with 9 mL of 70% ethanol 24, 48, 72, and 144 h post-inoculation.

PicoGreen staining was carried out following a protocol by Ferullo and colleagues (15). Prior to staining, fixed cells were pelleted by centrifugation at 4,000 rpm for 10 min at 4 ^{o}C. After removing 9 mL of supernatant, pellets were resuspended and cells were transferred to microcentrifuge tubes. Cells were pelleted by centrifugation at 15,000 rpm for 3 min after which the entire supernatant was removed. After air-drying overnight to ensure removal of ethanol, pellets were resuspended and adjusted to OD_{600} ~0.4 with PBS. 500 µL of each sample was stained with 100 µL of PicoGreen (diluted 100-fold from stock with 25% DMSO) in the dark for 3 h. One-mL of 1000-fold diluted PicoGreen was added to each sample and fluorescence was analyzed using an LSRII flow cytometer. Bacteria were identified using forward and side scatter parameters. PicoGreen fluorescence was excited with a laser emitting at 488 nm and fluorescence emission was collected using a green fluorescene filter (525/50 nm-band-pass

filter). Data were acquired and analyzed using the FACSDiVa software and FlowJo. Stained cells were identified based on comparisons with an unstained control. A PicoGreen-negative gate was assigned based on signal from an unstained population. Fluorescence intensity corresponding to one chromosome was determined from *E. coli* MG1655 stationary phase controls to be in the range of 2-3.25x10^4 fluorescence units, and two chromosomes was found to be in the range 4-6.5x10^4 fluorescence units. Percent of *E. coli* in the PicoGreen-positive gate with more than one chromosome was defined as the percent of cells with fluorescence intensities above 3.25x10^4 fluorescence units, and more than two chromosomes to be cells fluorescence intensities above 6.5x10^4 fluorescence units.

Click-iT EdU Flow Cytometry Assay

Synthesis of new DNA during recovery from OFL treatment was measured using the Click-iT EdU Alexa Fluor 647 Flow Cytometry Assay Kit (Thermo-Fisher Scientific, Waltham, MA, USA). To ensure that thymidine transporters were active prior to introduction of the thymidine analogue EdU and to reduce the expression of biosynthetic enzymes whose products would compete with EdU, cells were cultured in the presence of 50 μg/mL of thymidine. This thymidine concentration was determined by culturing an *E. coli* thymine auxotroph, WM18, in the presence of different concentrations of thymidine in order to elucidate one that mitigated growth defects (Fig. S5*A*). Previously, it was reported that *E. coli* Δ*thyA* mutants could be selected and maintained on LB media with 200 μg/mL thymidine (16). Based on this protocol, *E. coli* MG1655 and WM18 (*E. coli* MG1655Δ*thyA*) were inoculated from -80 ^oC stocks into LB with 200 μg/mL thymidine and were grown in test tubes at 37 ^oC for 4 h before being diluted 1:100 into Gutnick media with 10 mM glucose and 200 μg/mL thymidine, where they were grown for 16 h. Cells were then diluted to OD_{600} ~0.01 in 25 mL of Gutnick-glucose media with 0-200 μg/mL thymidine in 250 mL baffled flasks. OD_{600} of each culture was monitored hourly for 8 h. From these growth assays, it was determined that 50 μg/mL of thymidine was sufficient to support the growth of thymine auxotrophs.

EdU incorporation and click labeling with Alexa Fluor 647 was performed following a protocol that is modified from Ferullo and colleagues (15), where 30 μg/mL EdU was added to cultures for incorporation. To determine the concentration of EdU that would result in the most robust signal under our culture conditions, *E. coli* MG1655 was cultured in Gutnick-glucose with 50 μg/mL thymidine as described above. When the OD_{600} of the culture reached 0.1, 500 μL of

culture was transferred into microcentrifuge tubes. Cells were pelleted, washed with warm (37 ^{o}C) LB, and resuspended in 1 mL of LB with 0, 30, 60, or 120 μg/mL EdU. Cells were transferred into 15 mL Falcon tubes (loosely-capped) and were incubated at 37 ^{o}C with shaking at 250 rpm for 1 h. Cells were fixed with 7 mL of cold 90% methanol (MeOH) at 4 ^{o}C overnight. Analysis of these controls indicated that the incorporation with 60 μg/mL EdU resulted in the highest percentage of fluorescent cells, and 60 μg/mL of thymidine could outcompete EdU incorporation at this concentration (Fig. S5*B*). Based on these observations, 60 μg/mL EdU was used in subsequent experiments.

To compare the resumption of DNA synthesis in MazF persisters and CM-treated toxin-free control, WM01 and WM03 were cultured and treated with OFL as described above for the persistence assays. Following 5 h of OFL treatment, three 500 μL aliquots were collected from each culture. Cells were pelleted and washed twice with PBS to reduce OFL concentration to below its minimal inhibitory concentration. After the second wash, PBS was removed and the pellet was resuspended in 1 mL of warm (37 ^{o}C) LB-ara with 60 μg/mL EdU. One aliquot from each culture was immediately fixed with 7 mL of cold 90% MeOH. The other aliquots were incubated at 37 ^{o}C, shaking at 250 rpm, and were fixed at 2 and 4 h post-recovery.

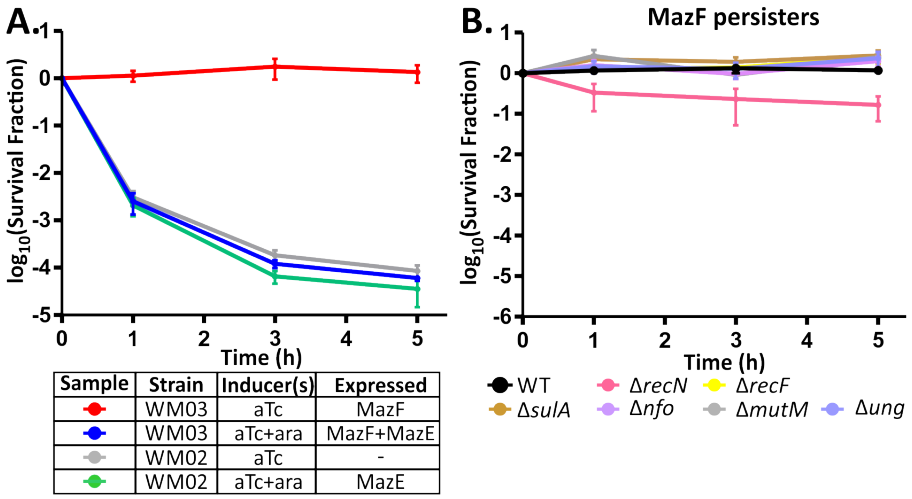
To compare DNA synthesis in WT persisters originating from growth-arrested cultures that were subjected to growth delay with persisters that were not subjected to the delay following OFL treatment, *E. coli* MG1655 was cultured in Gutnick media with 0.5 mM glucose and 50 μg/mL thymidine. Cells were treated with OFL 2 h after arrest brought on by glucose exhaustion (Fig. S7*B*). After 5 h of treatment, four 500 μL aliquots were collected from the culture, and cells were pelleted. Depending on the recovery media on which the cells were to be inoculated, cells were washed with Gutnick media with 10 mM glucose (for cells subjected to "no delay" conditions) or without glucose (for cells subjected to "delay" conditions). Following removal of OFL, pellets were resuspended in 100 μL of Gutnick media with 60 μg/mL EdU or 60 μg/mL thymidine either in the absence ("delay" samples) or presence ("no delay" samples) of glucose, and entire cell suspensions were spotted onto 25 mm PES filters on top of agar pads prepared with Gutnick media and 1.5% agar. These pads were prepared with (for "no delay" samples) or without glucose (for "delay" samples) and either 60 μg/mL EdU or 60 μg/mL thymdine. Gutnick agar was used for both "delay" and "no delay" conditions in this experiment, so that the media used for both conditions are the same except for the presence or absence of glucose. Cells were

incubated at 37 ^{o}C for 4 h. The filters were then transferred into 15 mL Falcon tubes with 2 mL Gutnick media either with or without glucose depending on whether the cells were recovered on nutritive or non-nutritive media. The samples were vortexed for 1 min to dislodge the cells, the filters were removed, and the cells were fixed with 13 mL of cold 90% MeOH overnight (at 4 ^{o}C).

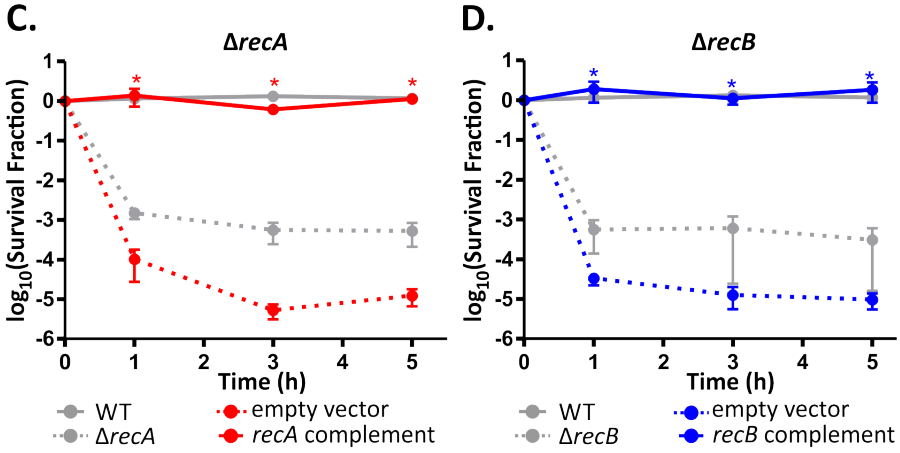
Following fixation, cells were pelleted by centrifugation at 4,000 rpm, 4 ^{o}C, for 10 min. After removing 14 mL of supernatant, the remaining supernatant and cells were transferred into a microcentrifuge tube, where cells were pelleted by centrifugation at 15,000 rpm for 3 min. Residual MeOH was removed and cells were resuspended in 500 µL PBS. After pelleting the cells, the supernatant was removed and pellets were gently resuspended in 100 µL of 0.5% Triton X-100 in PBS. Cells were incubated at room temperature for 30 min. After incubation, cells were pelleted and washed with 500 µL PBS. Pellets were resuspended in 100 µL of Click-iT reaction cocktail, which was prepared following the manufacturer's instructions. Cells were incubated at room temperature, protected from light, for 30 min. After labelling, cells were pelleted by centrifugation, washed with 500 µL PBS, and resuspended in PBS. Fluorescence was analyzed using an LSRII flow cytometer. Bacteria were identified using forward and side scatter parameters. Alexa647 fluorescence was excited with a 640 nm laser and fluorescence emission was collected using a red fluorescene filter (670/30 nm-band-pass). Data were acquired and analyzed using the FACSDiVa software and FlowJo. An Alexa 647-negative gate was assigned based on fluorescence signal from cells that were cultured in the presence of thymidine. Percent of fluorescent cells in each sample was determined from cells with fluorescence intensities above the negative control.

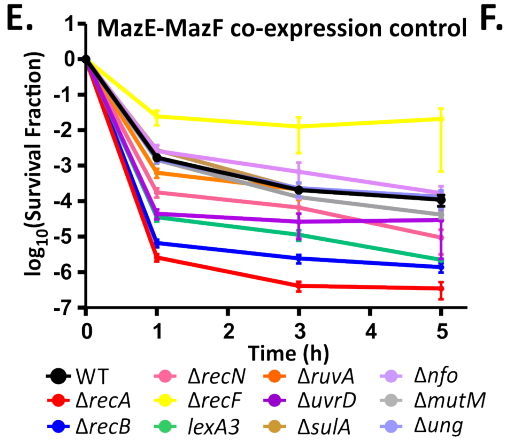
Statistical Analysis

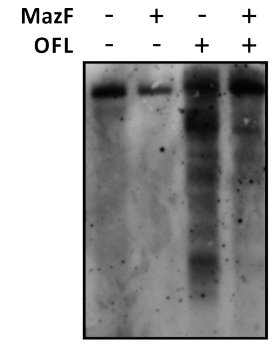
At least three biological replicates were performed for each experiment. Each data point represents the mean value ± standard error. Where indicated, a two-tailed t-test was performed for pairwise comparisons and p-values ≤ 0.05 were considered statistically significant.



Sample	Strain	Inducer(s)	Expressed
●	WM03	aTc	MazF
●	WM03	aTc+ara	MazF+MazE
●	WM02	aTc	-
●	WM02	aTc+ara	MazE







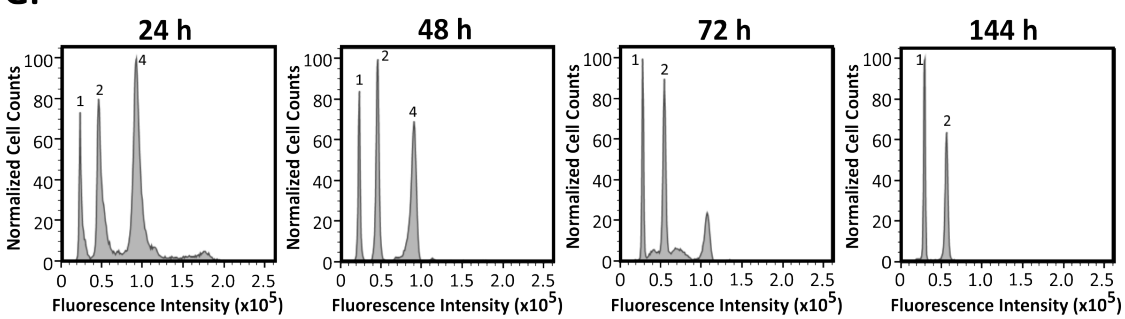


Fig. S1. Control experiments for examining MazF persister response to OFL. (*A*) Compared with the survival of a toxin-free control (induced with the same concentration of aTc as MazF-arrested cells), cells expressing MazE, and cells co-expressing MazE and MazF, survival of MazF persisters following OFL treatment was higher by four orders of magnitude and approached complete tolerance. (*B*) *recN*, *recF*, *sulA*, and genes involved in base excision repair (*e.g., mutM, ung,* and *nfo*) were deleted in WM03. In MazF persisters harboring these deletions, significant changes in survival following OFL treatment were not observed. (*C*) Complementation of Δ*recA* and (*D*) Δ*recB* deletions in MazF mutants with plasmid-borne copies of *recA* and *recB* restored persistence to OFL. Asterisks indicate statistical significance between the *recA*-complemented and empty-vector containing strains. Wild-type, Δ*recA*, and Δ*recB* are provided for context. (*E*) MazE and MazF were co-induced in WM03 derivatives with mutations or deletion of DNA repair enzymes before and during OFL treatment, resulting in growing and OFL-susceptible populations. Similar to MazF persisters, deletion of *recA* and *recB*, as well as mutation of *lexA3* significantly reduced persister levels at all time points following OFL treatment. (*F*) A second representative Southern blot illustrating DNA fragmentation in the *dnaA* locus following 30 min of OFL treatment in the toxin-free control (lane 3) and MazF persisters (lane 4). (*G*) To determine the fluorescence intensities corresponding to unit chromosome numbers with PicoGreen staining, *E. coli* MG1655 was cultured to late stationary phase. As culture time increased, more cells with a single chromosome emerged as reported previously (14). From these measurements, it was determined that a single chromosome corresponds to 2-3.25x10^4 fluorescence units and cells containing two chromosomes corresponds to 4-6.5x10^4 fluorescence units with the cytometer settings used for analysis. At least three biological replicates were performed for each experiment, and error bars represent S.E.M.

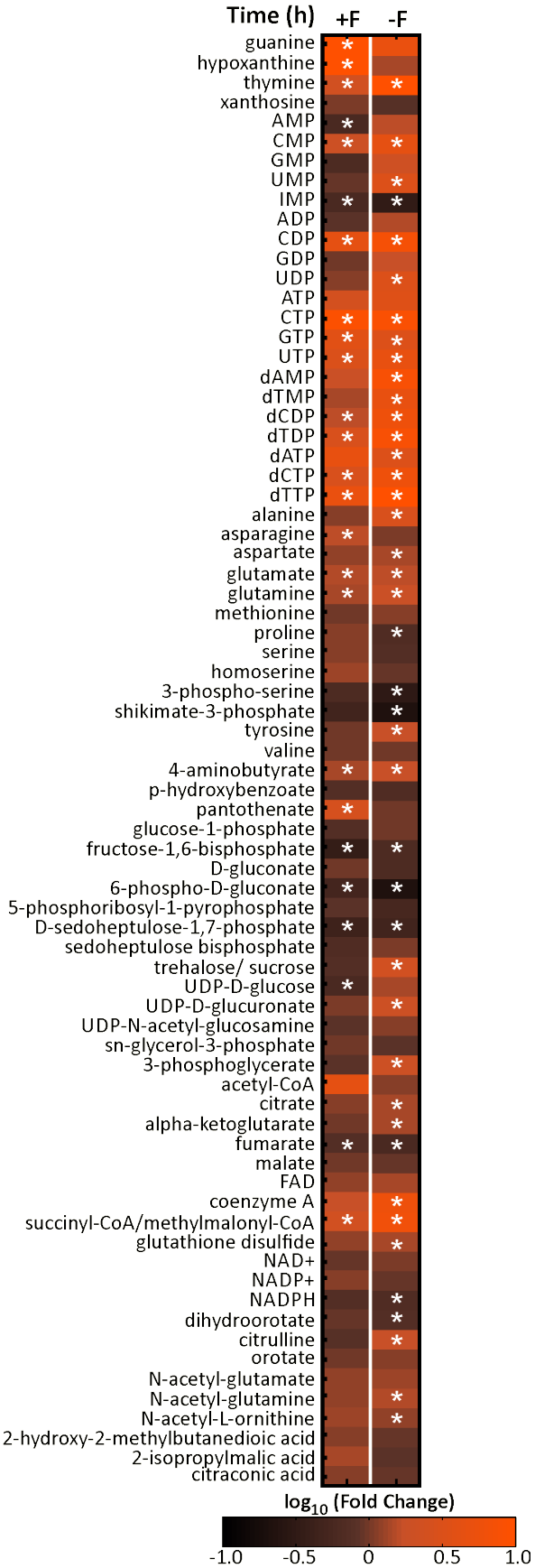

Time (h)
+F
-F
guanine
hypoxanthine
thymine
xanthosine
AMP
CMP
GMP
UMP
IMP
ADP
CDP
GDP
UDP
ATP
CTP
GTP
UTP
dAMP
dTMP
dCDP
dTDP
dATP
dCTP
dTTP
alanine
asparagine
aspartate
glutamate
glutamine
methionine
proline
serine
homoserine
3-phospho-serine
shikimate-3-phosphate
tyrosine
valine
4-aminobutyrate
p-hydroxybenzoate
pantothenate
glucose-1-phosphate
fructose-1,6-bisphosphate
D-gluconate
6-phospho-D-gluconate
5-phosphoribosyl-1-pyrophosphate
D-sedoheptulose-1,7-phosphate
sedoheptulose bisphosphate
trehalose/ sucrose
UDP-D-glucose
UDP-D-glucuronate
UDP-N-acetyl-glucosamine
sn-glycerol-3-phosphate
3-phosphoglycerate
acetyl-CoA
citrate
alpha-ketoglutarate
fumarate
malate
FAD
coenzyme A
succinyl-CoA/methylmalonyl-CoA
glutathione disulfide
NAD+
NADP+
NADPH
dihydroorotate
citrulline
orotate
N-acetyl-glutamate
N-acetyl-glutamine
N-acetyl-L-ornithine
2-hydroxy-2-methylbutanedioic acid
2-isopropylmalic acid
citraconic acid
log10 (Fold Change)
-1.0
-0.5
0
0.5
1.0

Fig. S2. Metabolomic changes resulting from OFL treatment. Ratio of changes in metabolite abundances in MazF persisters (+F) and in the toxin-free control (-F). Both populations were induced with aTc during stasis and OFL treatment. For each strain, ratios were calculated by dividing OD_{600}-normalized metabolite abundances from cells collected 1 h post-treatment by those from cells before treatment. This experiment was repeated five times. Metabolites whose signals were detected in at least three replicates are shown. For these metabolites, * denotes statistically significant difference ($p \leq 0.05$) between log-transformed ratios and 0 [$\log_{10}(1)$], as a ratio of 1 indicates no difference between treated and untreated cultures.

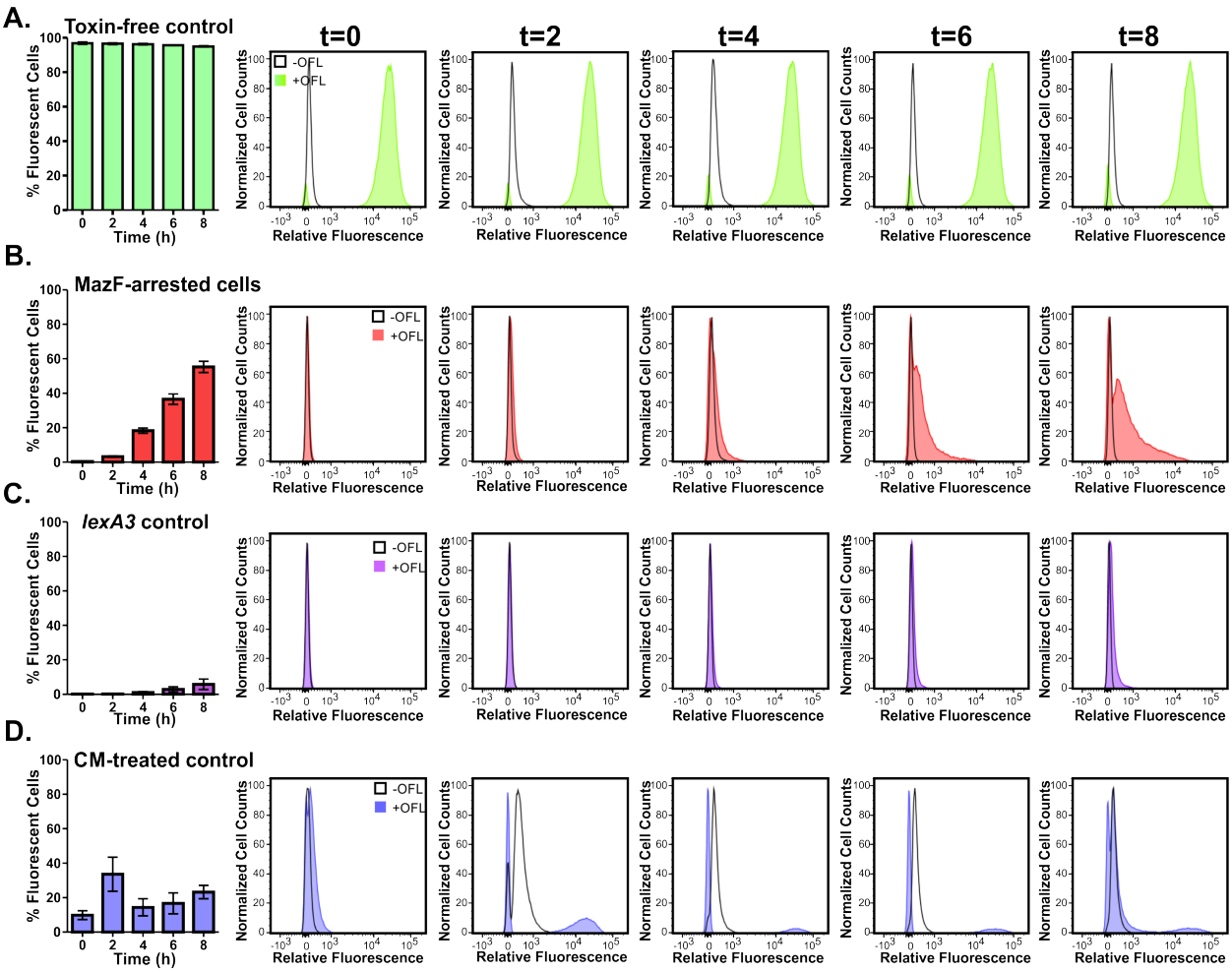


Fig. S3. Induction of SOS response during recovery from OFL treatment. In these analyses and histograms, single cells were gated based on forward and side scattering parameters of an untreated toxin-free population. (*A-D*) Expression of an SOS reporter (P_{recA}-*gfp*) was quantified in the toxin-free control (*A*), MazF persisters (*B*), an SOS response defective *lexA3* mutant of strain WM03 (*C*), and toxin-free cells treated with translation inhibitor chloramphenicol (CM) prior to and during OFL treatment (*D*) by flow cytometry every 2 h during recovery from OFL treatment in LB with arabinose. Average percentage of cells with fluorescence intensities above a non-fluorescent negative control (*lexA3* mutant without OFL treatment) measured at each time in three biological replicates is shown in the bar graphs (error bars depict S.E.M.). The histograms shown are representative of these three replicates.

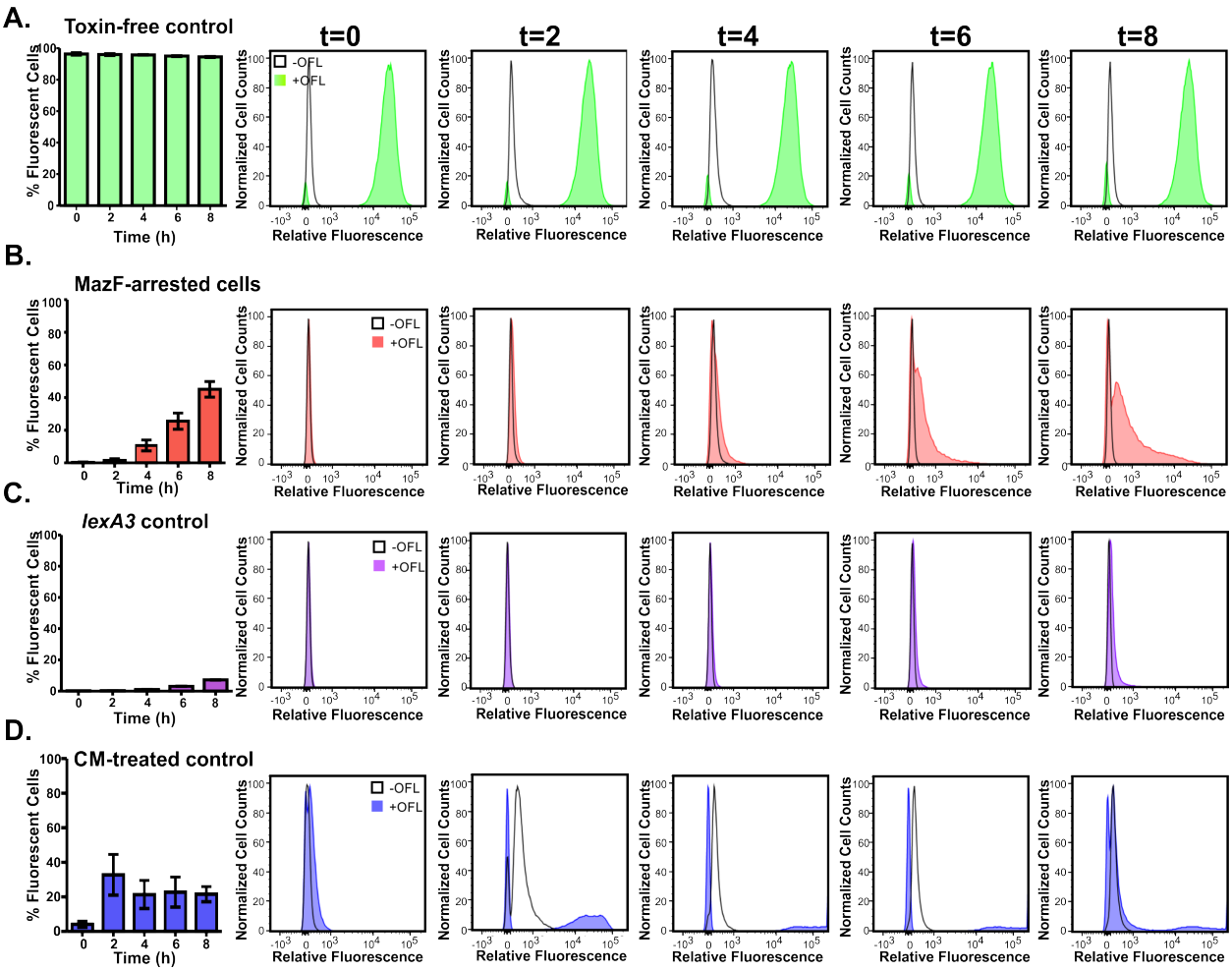
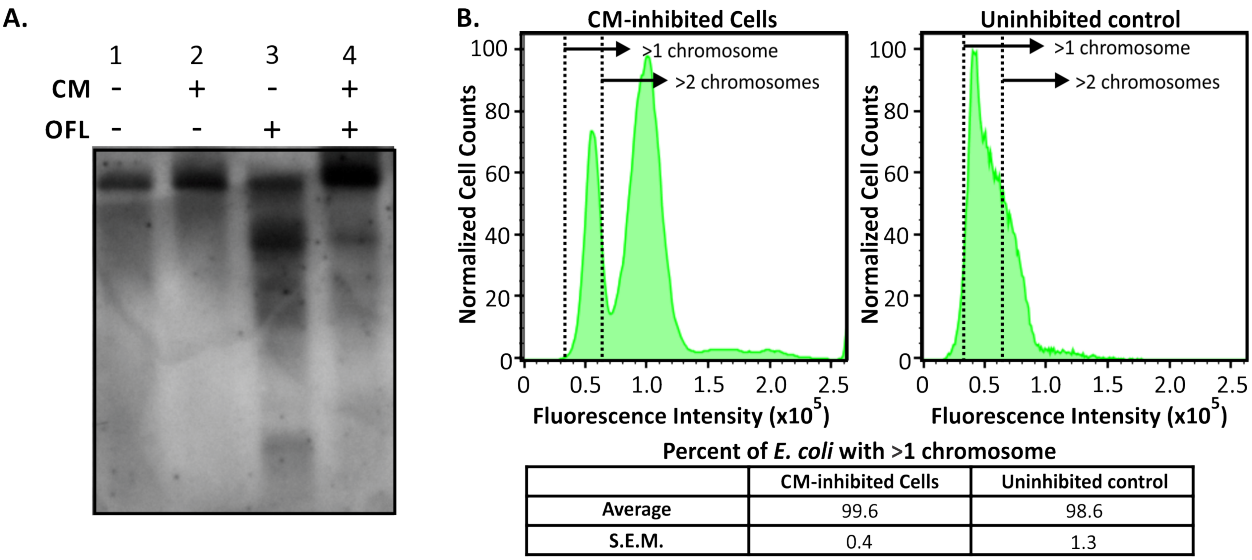


Fig. S4. Induction of SOS response during recovery from OFL treatment. The data shown here are the same as those presented in Fig. S3, but single cells were not gated based on side and forward scattering in these analyses and histograms. Expression of an SOS reporter (P_{recA}-*gfp*) was quantified in the toxin-free control (*A*), MazF persisters (*B*), an SOS response defective *lexA3* mutant of strain WM03 (*C*), and toxin-free cells treated with chloramphenicol (CM) prior to and during OFL treatment (*D*) during recovery in LB with arabinose after 5 h of OFL treatment. Average percentage of cells with fluorescence intensities above a non-fluorescent negative control (*lexA3* mutant without OFL treatment) measured at each time in three biological replicates is shown in the bar graphs (error bars depict S.E.M.). The histograms are representative of these three replicates.



Percent of *E. coli* with >1 chromosome

	CM-inhibited Cells	Uninhibited control
Average	99.6	98.6
S.E.M.	0.4	1.3

Fig. S5. Response of CM-treated toxin-free control to OFL treatment. (*A*) The integrity of the *dnaA* locus before and following 30 min of OFL treatment was examined in untreated and CM-treated cells using Southern blotting. The *dnaA* locus is intact in the untreated (lane 1) and CM-treated (lane 2) toxin-free control before OFL treatment. After 30 min of treatment, fragmentation was observed in both the uninhibited (lane 3) and the CM-inhibited populations (lane 4). (*B*) CM-inhibited and uninhibited toxin-free controls have more than one chromosome and most have more than two chromosomes at the time of OFL treatment. The Southern blot and histograms shown here are representative of three biological replicates.

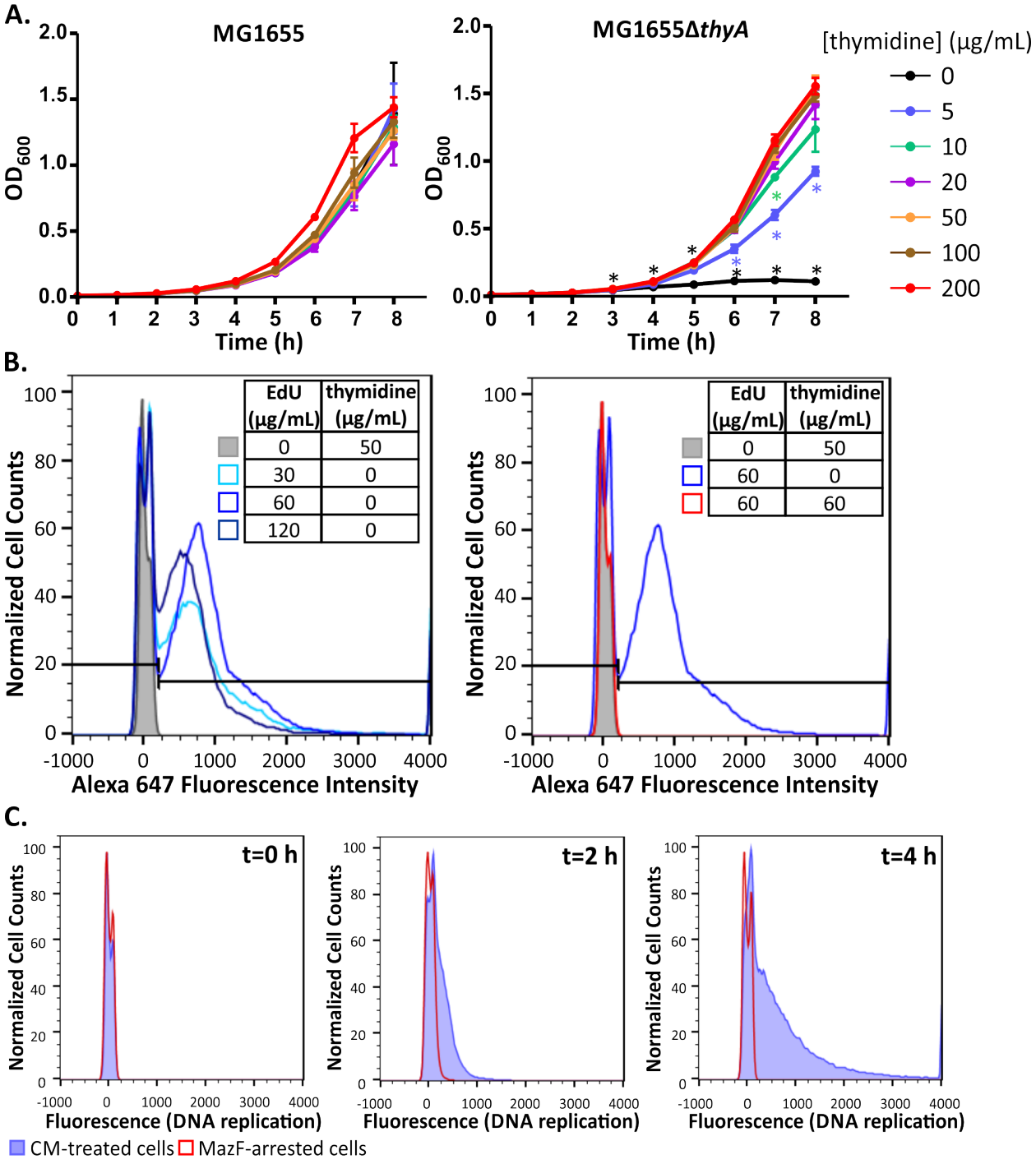


Fig. S6. Controls of EdU incorporation experiments. (*A*) *E. coli* MG1655 and MG1655Δ*thyA* were grown in the Gutnick-glucose media with 0-200 μg/mL thymidine. Growing the Δ*thyA* mutant in the presence of 50 μg/mL thymidine was found to support exponential growth, thus 50 μg/mL thymidine was added to culture media for subsequent experiments to improve EdU

incorporation. n=3. * denotes statistically significant difference ($p \leq 0.05$) in OD_{600} measured in cultures grown in the presence of 200 μg/mL thymidine and those grown in the presence of less thymidine at each time point. (*B*) Exponentially-growing *E. coli* MG1655 was incubated with 0, 30, 60, and 120 μg/mL EdU for 1 h before being fixed and stained. Incorporation with 60 μg/mL EdU was found to result in the highest percentage of labeled cells (left panel), and 60 μg/mL thymidine was able to out-compete EdU at this concentration (right panel). (*C*) EdU incorporation in newly synthesized DNA in MazF persisters and persisters derived from CM-inhibited cells during recovery from OFL treatment in LB with arabinose was quantified every 2 h. Histograms shown are representative of three biological replicates performed for this experiment.

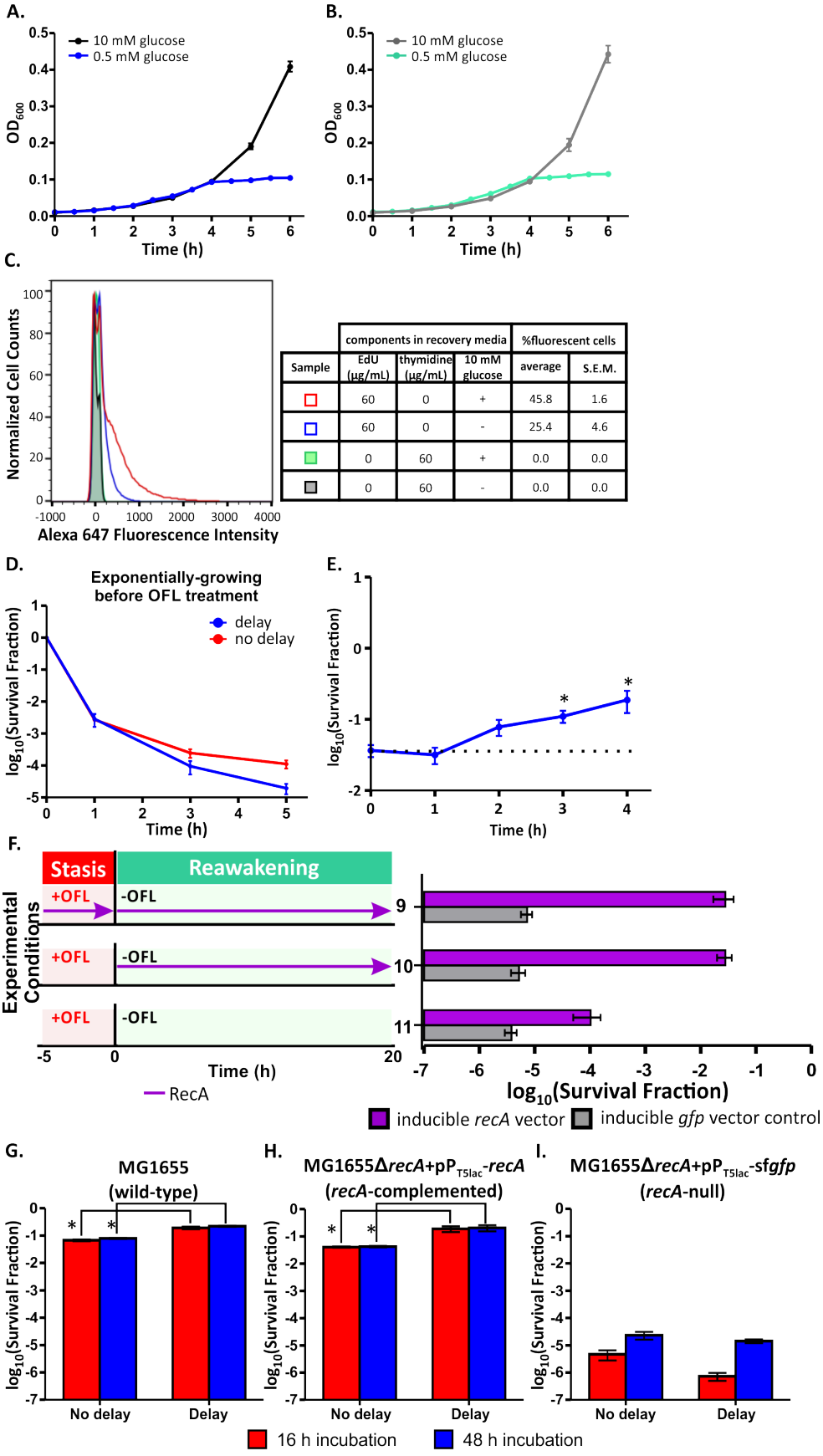

A.
10 mM glucose
0.5 mM glucose
OD600
Time (h)
B.
10 mM glucose
0.5 mM glucose
OD600
Time (h)
C.
Normalized Cell Counts
Alexa 647 Fluorescence Intensity
components in recovery media
%fluorescent cells
Sample
EdU (μg/mL)
thymidine (μg/mL)
10 mM glucose
average
S.E.M.
60 0 + 45.8 1.6
60 0 - 25.4 4.6
0 60 + 0.0 0.0
0 60 - 0.0 0.0
D.
Exponentially-growing before OFL treatment
delay
no delay
log10(Survival Fraction)
Time (h)
E.
log10(Survival Fraction)
Time (h)
F.
Stasis
Reawakening
+OFL
-OFL
Experimental Conditions
Time (h)
RecA
9
10
11
log10(Survival Fraction)
inducible recA vector
inducible gfp vector control
G.
MG1655 (wild-type)
H.
MG1655ΔrecA+pPT5lac-recA (recA-complemented)
I.
MG1655ΔrecA+pPT5lac-sfgfp (recA-null)
log10(Survival Fraction)
No delay
Delay
16 h incubation
48 h incubation

Fig. S7. Controls for nutrient deprivation and persistence experiments. (*A*) *E. coli* MG1655 was cultured in Gutnick media with 10 mM or 0.5 mM of glucose. In media with 0.5 mM glucose, it was observed that growth was inhibited when cultures reached OD_{600} ~0.09. (*B*) *E. coli* MG1655 was cultured in Gutnick media with 10 mM or 0.5 mM glucose with 50 μg/mL of thymidine. In glucose-limiting media with thymidine, growth inhibition was observed at OD_{600} ~0.1. (*C*) Incorporation of EdU into newly-synthesized DNA in cells recovering on non-nutritive and nutritive agar after 5 h of OFL treatment. (*D*) When exponentially-growing cultures were treated with OFL, delaying growth resumption by carbon starvation did not improve persister survival. (*E*) Inoculation of OFL-treated WT cells on non-nutritive media for 3 h was sufficient to significantly increase survival compared with cells that were immediately inoculated onto nutritive media after OFL removal (* denotes statistically significant difference [$p \leq 0.05$] in survival fraction quantified at t=0 and at designated time points). (*F*) In MG1655Δ*recA* bearing an inducible copy of *recA*, inoculating cells on nutritive media immediately after OFL treatment killed ~97% of cells regardless of whether cells expressed RecA throughout the course of treatment and recovery (condition 9, purple bar) or only during recovery (condition 10, purple bar). By comparison, survival of cells bearing plasmids lacking *recA* (grey bars) or populations in which *recA* expression was not induced (condition 11, purple bar) decreased by 4-5 orders of magnitude. (*G*) *E. coli* MG1655, (*H*) MG1655Δ*recA* complemented with *recA*, and (*I*) *E. coli* MG1655Δ*recA* harboring pP_{T5lac}gfp (*recA*-null mutant) were starved of glucose for 2 h (in IPTG-containing media) and treated with OFL for 5 h. After removing OFL, cells were either immediately inoculated onto filter discs on top of LB agar with IPTG ("no delay") or on Gutnick agar with IPTG before being trasferred onto LB-IPTG agar ("delay"). For *E. coli* MG1655 and MG1655Δ*recA* complemented with *recA*, delaying growth by starvation significantly enhanced persister levels (* denotes statistically significant difference [$p \leq 0.05$] in survival between "delay" and "no delay" samples), and incubation of samples for 16 h or 48 h at 37 ^{o}C resulted in similar cfu counts. On the other hand, delaying growth resumption of MG1655Δ*recA* harboring pP_{T5lac}gfp (*recA*-null mutant) following OFL treatment did not improve survival at 16 or 48 hrs of incubation, although we note that colonies counts for this mutant were somewhat higher at 48 h compared to those at 16 h. With the exception of panel *C*, three biological replicates were performed for each experiment, and error bars depict S.E.M. For panel *C*, histograms shown are representative of three biological replicates.

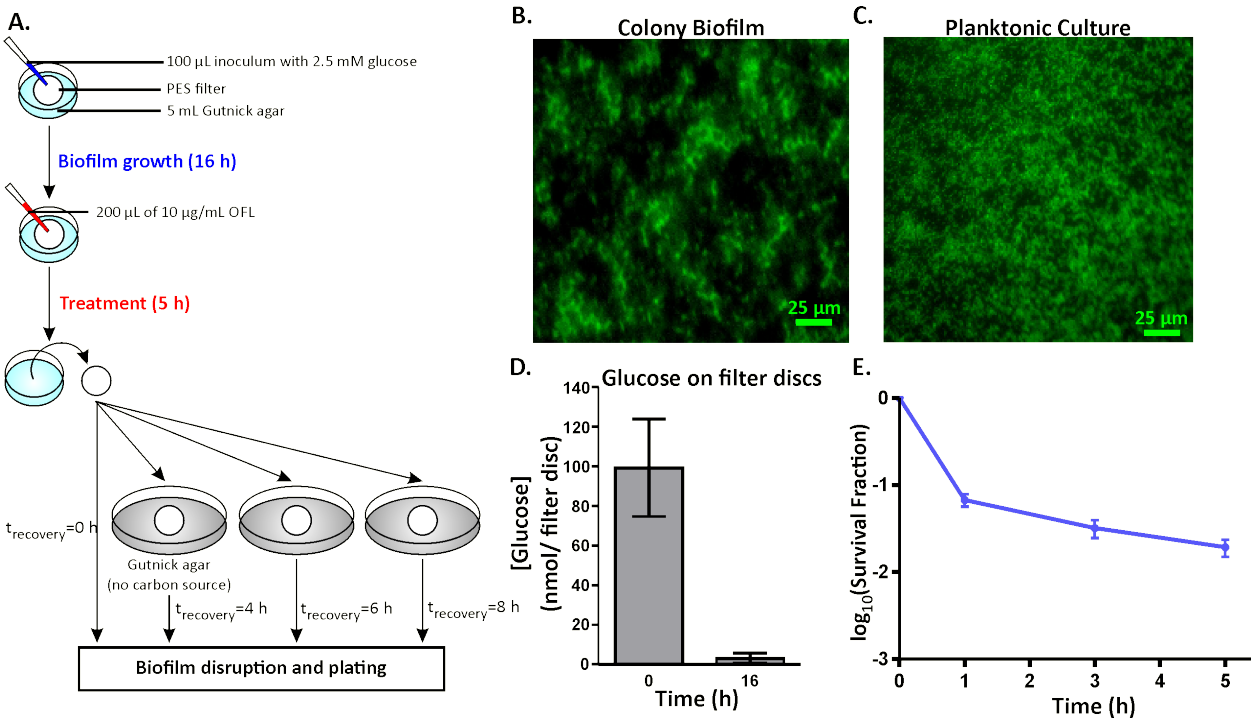


Fig. S8. Controls for colony biofilm experiments. (*A*) Schematic of colony biofilm persister assays. *E. coli* MG1655 biofilms were grown on nutrient-permeable filter discs overlaid on Gutnick agar for 16 h. Following overnight growth, biofilms were treated with OFL for 5 h. After treatment, cells from one filter (representing $t_{recovery}$=0 h) were immediately dislodged from the filter, washed with PBS, and plated. Additional filters were transferred onto Petri dishes with Gutnick agar, which allowed for dilution of OFL on the filter by diffusion. At designated times, cells were dislodged from each filter, washed with PBS, and plated. (*B*) Colony biofilms of *E. coli* MG1655 expressing GFP were grown on PES membranes for 16 h. Membranes were asceptically removed and visualized by fluorescence microscopy. (*C*) A planktonic control, where *E. coli* MG1655 expressing GFP from a 16 h overnight culture was inoculated at the same density (~10^7 cells) as colony biofilms on a PES filter, was visualized by fluorescence microscopy. (*D*) At inoculation, PES filters on which biofilms were cultured contained 99.3±24.6 nmol of glucose. Following 16 h of incubation, the filters contained 3.0±2.7 nmol of glucose, indicating that glucose was depleted in the biofilms at the time of OFL treatment (n=3, error bars represent S.E.M.). (*E*) Biphasic killing was observed when colony biofilms were treated with a 200 µL bolus of 10 µg/mL OFL for 5 h (n=3, error bars represent S.E.M.).

Table S1. Bacterial strains (A), plasmids (B), and primers (C) used in this study.

A. Bacterial strains used in this study.

Strain	Genotype	Description
MG1655	F^- λ^- *ilvG*$^-$ *rfb*-50 *rph*-1	ATCC 700926 (17)
WM01	MG1655 Δ*lacZYA*::P_{lacIQ}-P_{T5}-*mCherry*	(1)
WM02	WM01Δ*mazEF* Δ*araBAD*::P_{BAD}-*mazE-cat*	(1)
WM03	WM02::P_{LtetO1}-*mazF*-P_{N25}-*tetR-gent*	(1)
WM04	WM03Δ*recA*::*kan*	Generated by deletion of *recA* from WM03 using Datsenko and Wanner method (2)
WM05	WM03Δ*recB*::*kan*	Generated by P1 phage transduction of Δ*recB*::*kan* (5) into WM03
WM06	WM03Δ*recF*::*kan*	Generated by P1 phage transduction of Δ*recF*::*kan* (5) into WM03
WM07	WM03Δ*recN*::*kan*	Generated by P1 phage transduction of Δ*recN*::*kan* (5) into WM03
WM08	WM03Δ*sulA*::*kan*	Generated by P1 phage transduction of Δ*sulA*::*kan* (5) into WM03
WM09	WM03Δ*ruvA*::*kan*	Generated by P1 phage transduction of Δ*ruvA*::*kan* (5) into WM03
WM10	WM03Δ*uvrD*::*kan*	Generated by P1 phage transduction of Δ*uvrD*::*kan* from Keio mutant (4) into WM03
WM11	WM03Δ*ung*::*kan*	Generated by P1 phage transduction of Δ*ung*::*kan* from Keio mutant (4) into WM03
WM12	WM03Δ*nfo*::*kan*	Generated by P1 phage transduction of Δ*nfo*::*kan* from Keio mutant (4) into WM03
WM13	WM03Δ*mutM*::*kan*	Generated by P1 phage transduction of Δ*mutM*::*kan* from Keio mutant (4) into WM03
WM14	WM03Δ*malK*::*kan*	Generated by P1 phage transduction of Δ*malK*::*kan* (5) into WM03
WM15	WM03*lexA3*	Generated by P1 phage transduction of *lexA3* (5) into WM14
WM16	WM03Δ*recA*	WM04 cured of *kan* using pCP20 (2)
WM17	WM03Δ*recB*	WM05 cured of *kan* using pCP20 (2)
WM18	*E. coli* MG1655Δ*thyA*	Generated by deletion of *thyA* from MG1655 using Datsenko and Wanner method (2) and cured of *kan* using pCP20 (2)

WM19	*E. coli* MG1655Δ*recA*	Generated by P1 phage transduction of Δ*recA*::*kan* into MG1655 and cured of *kan* using pCP20 (2)
WM20	*E. coli* MG1655Δ*lacZYA*::P_{lacIQ}-P_{T5}-*gfp*::*kan*	Generated by P1 phage transduction of Δ*lacZYA*::P_{lacIQ}-P_{T5}-*gfp*::*kan* from SA034 (8) into the MG1655 strain used in this study.

B. Plasmids used in this study.

Plasmid	**Genotype**	**Source or Reference**
pUA66	Vector, pSC101 ori, *kan*, *gfp*mut2	(18)
pP_{T5lac}*gfp*	pUA66::P_{lacIQ}-*lacI*::P_{T5lac}-*sfgfp*	(5)
pKV006	pUA66::P_{recA}-*gfp*mut2	(5)
pKV012	pUA66::P_{lacIQ}-*lacI*::P_{T5lac}-*recA*	(5)
pP_{recA}-*recA*	pUA66:: P_{recA}-*recA*	Brynildsen Lab (constructed by Theresa Barrett)
pP_{recA}-*recB*	pUA66:: P_{recB}-*recB*	This work

C. Primers used in this study.

Primers for chromosomal gene deletion.

Primer	**Sequence**
*recA*_deletion_F	5'–CAACAGAACATATTGACTATCCGGTATTACCCGGCATGACAGGAGTAAAAATGGTGTAGGCTGGAGCTGCTTCG
*recA*_deletion_R	5'–ATGCGACCCTTGTGTATCAAACAAGACGATTAAAAATCTTCGTTAGTTTCCATATGAATATCCTCCTTAGTTCCTATTC
*thyA*_deletion_F	5'–ATATCGTCGCAGCCCACAGCAACACGTTTCCTGAGGAACCGTGTAGGCTGGAGCTGCTTC
*thyA*_deletion_R	5'-CGACGCACACTGGCGTCGGCTCTGGCAGGATGTTTCGTAACATATGAATATCCTCCTTA

[a]***Primers used to verify gene perturbations.***

Primer	**Sequence**
kanR_R	5'–ATGATGGATACTTTCTCGGCAGGAG
Upstream_*uvrD*_F	5'–TTACTGCCGCATCTGGAAAT
Internal_*uvrD*_F	5'–TAATGACAAACAGCGCGAAG
Internal_*uvrD*_R	5'–CAGACGCCCGTTATTGTTTT
Upstream_*ung*_F	5'–CATCAACTTATGCGGGTGTG
Internal_*ung*_F	5'–GTCAGGGCGTTCTGCTACTC
Internal_*ung*_R	5'–GATGCGGTGCTTTCAGTACA

Upstream_*nfo*_F	5'–AAAGCGTCATCGCATAAACC
Internal_*nfo*_F	5'–TTCACCAAAAACCAACGTCA
Internal_*nfo*_R	5'–GCAACGCTGCATTTCATCTA
Upstream_*mutM*_F	5'–GCCAGCACGTGATCTACAAA
Internal_*mutM*_F	5'–AAGGGCATAATGTGCTGACC
Internal_*mutM*_R	5'–CAGTGATTCGCTGGCATAGA
Upstream_*thyA*_F	5'–CATGGGGCAAATTCTTTCCA
Internal_*thyA*_F	5'–TGATGCAAAAAGTGCTCGAC
Internal_*thyA*_R	5'–CAGCGCCATTTTATCCAGTT

[a] Primers used to verify the deletion/ mutation of *recA, recB, recF, recN, ruvA, malK, and lexA* were the same as the ones described in Völzing and Brynildsen, 2015 (5).

Primers for plasmid construction.

Primer	**Sequence**
F_P$_{recA}$-*recA*-XhoI	5'–GCGCGGCTCGAGAAAACACTTGATACTGTATG
R_P$_{recA}$-*recA*-SbfI	5'–GCGCGGCCTGCAGGTTAAAAATCTTCGTTAGTTT
*recB*_BamHI_FWD	5'– CTAGTAGGGATCCGCTGTTTCGCTTTAATCAGT
*recB*_SbfI_REV	5'– TCATCATCCTGCAGGTTACGCCTCCTCCAGGGTCA

Primers used to generate 10 kb marker and dnaA probe for Southern blotting.

Primer	**Sequence**
dnaA_probe_FWD	5'– ACGCGTCAGACGTTTAATCC
dnaA_probe_REV	5'– TACGTCGAAAAGACCCTGCT
dnaA_10K_REV	5'– TGATCGCAATGCTAAGTTGC

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