Supporting Information

Girgis et al. 10.1073/pnas.1205124109

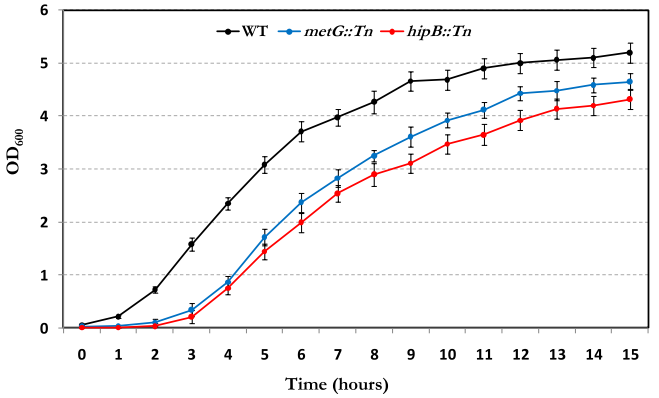

Fig. S1. Growth dynamics of the *metG*::Tn and *hipB*::Tn insertional mutants. Fresh LB media was inoculated with overnight cultures (1% inoculum) and incubated at 37 °C with aeration. OD_{600} readings of each culture were taken every hour for 12 h. Calculated doubling times for the wild type, *metG*::Tn, and *hipB*::Tn strains were 32, 36, and 35 min, respectively.

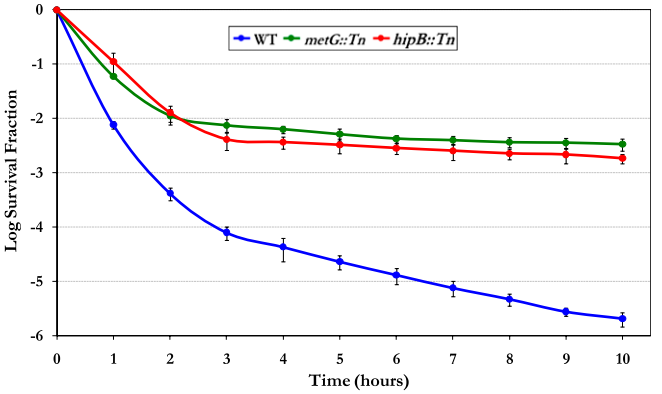

Fig. S2. Kill curves of *metG*::Tn, *hipB*::Tn, and wild type in the presence of ciprofloxacin. Kill curves were generated by inoculating (1% inoculum) fresh LB containing 1 μg/mL ciprofloxacin with overnight cultures. At the indicated time points, aliquots of each culture were washed with fresh LB, diluted, and plated on LB agar. Plates were incubated at 37 °C to permit colony formation. Survival fraction was calculated based on the density of the overnight cultures at the time of inoculation. Average survival fractions and SDs were calculated from triplicate sets of plates at each time point.

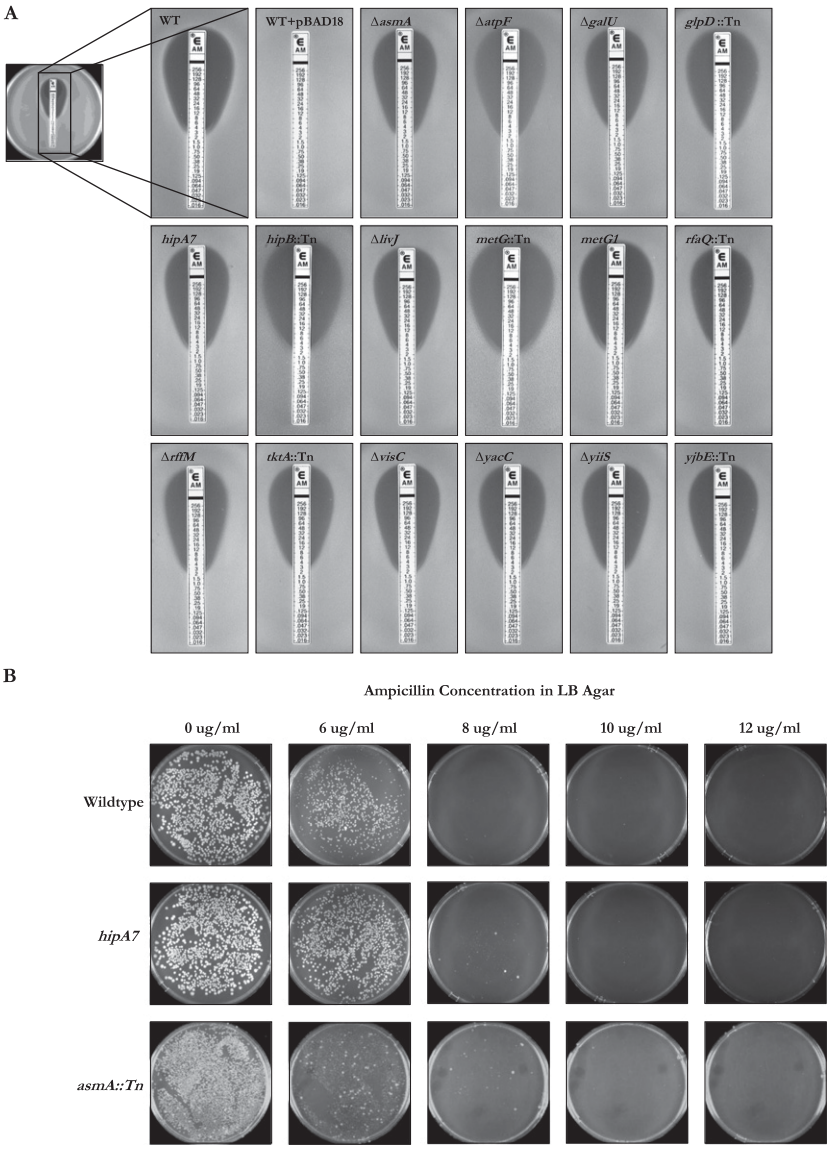

Fig. S3. (Continued)

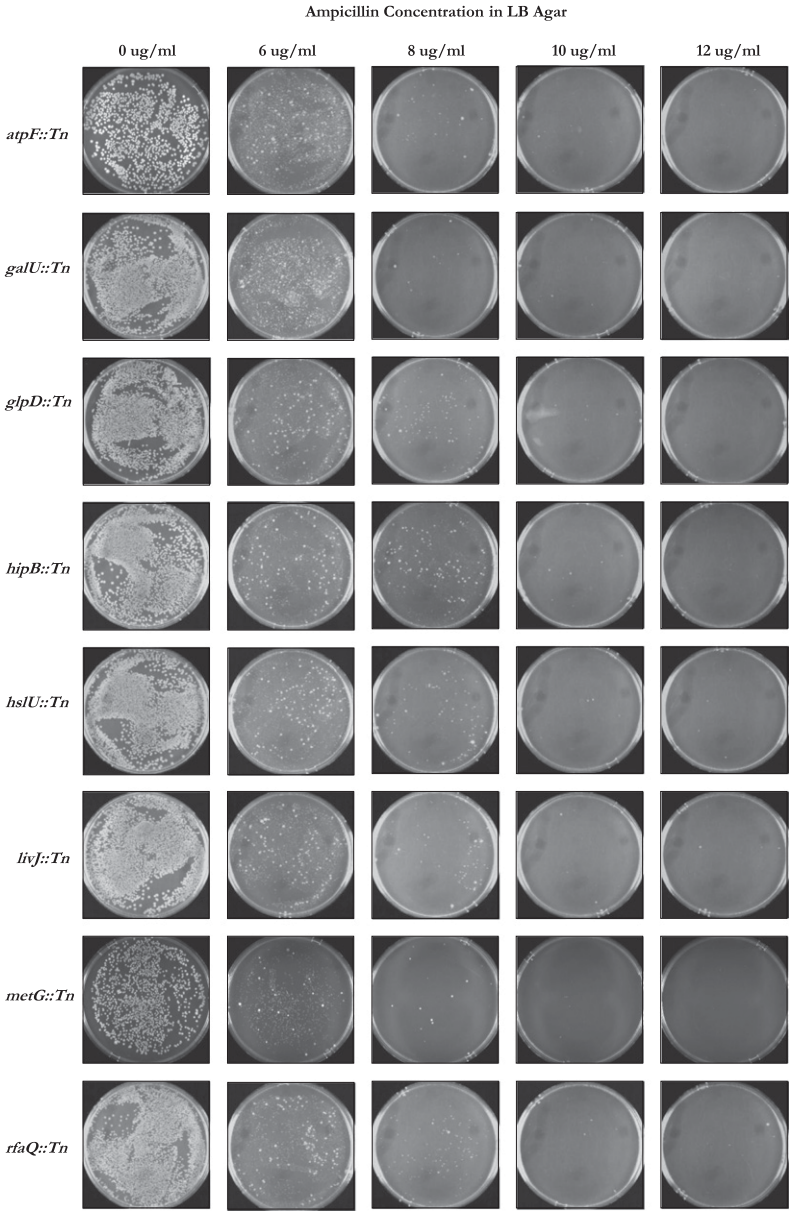

Fig. S3. (Continued)

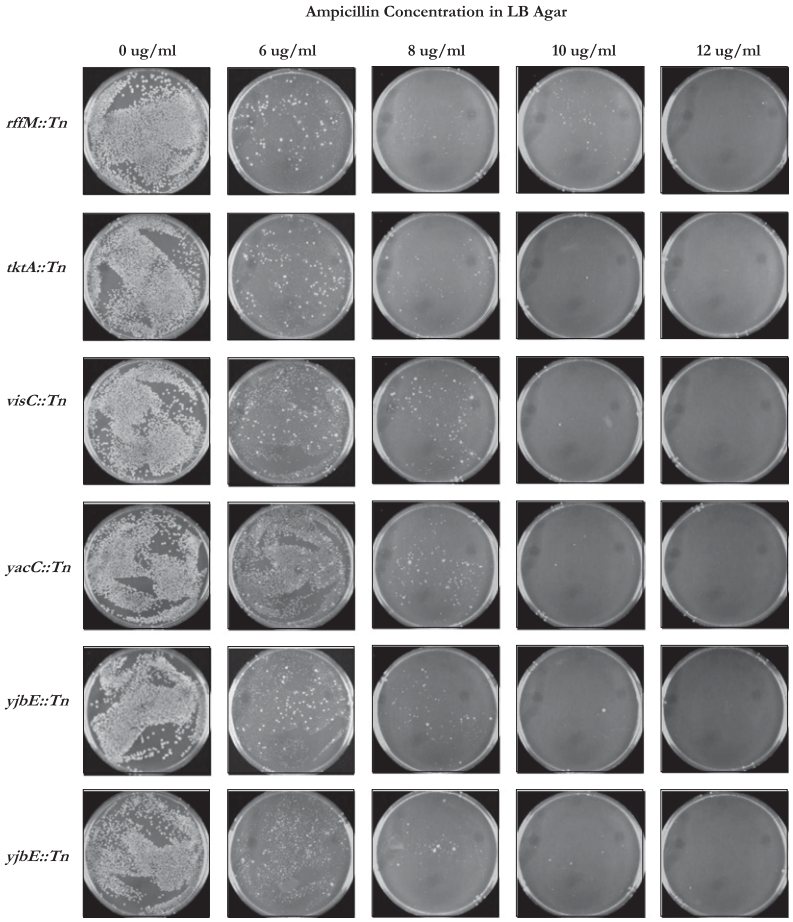

Fig. S3. Antibiotic resistance profiling. (*A*) E-test strips (AB Biodisk) were used to determine the MIC of various strains according to the manufacturer's guidelines. Briefly, 100 μL of an overnight culture was used to seed 3 mL of liquid LB top agar and the mixture was poured over prewarmed LB plates. After the top agar has solidified at room temperature, antibiotic strips were placed on the surface of the agar. Plates were imaged and cell growth was evaluated after 10 h of incubation at 37 °C. The MIC value for each strain was determined to be the point on the edge of the E-strips where the ellipse of growth inhibition intersected with a lawn of cells. Based on the values determined using this method, persister strains did not show a measurable difference in the MIC relative to the wild-type strain. (*B*) A standard plate assay was used to determine the MIC of persister strains. A 1% inoculation of an overnight culture was cultivated to an OD_{600} of 0.5 and then plated on LB agar containing various concentrations of ampicillin. Plates were imaged and cell growth was evaluated after 24 h of incubation at 37 °C. All persister strains identified in this study (Table 1) were subjected to this plate assay to determine their MIC.

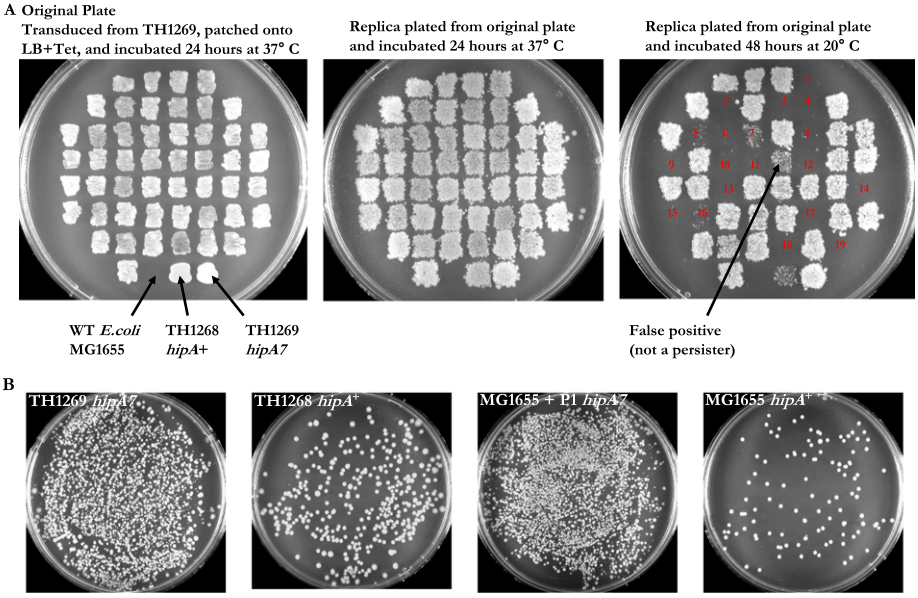

Fig. S4. P1 transduction of the *hipA7* locus to *E. coli* MG1655. (*A*) P1 lysate of the *hipA7* persister mutant (TH1269) was generated and wild-type *E. coli* MG1655 served as a recipient for P1 transduction of the *hipA7* allele. Transductants were selected by plating on LB agar containing tetracycline because the *hipA7* allele was linked to a Tet^R marker. Colonies were patched onto LB agar containing tetracycline and incubated at 37 °C. After an overnight incubation, the cells on this plate were transferred to two other plates via replica plating. One of the plates was incubated at 37 °C for 24 h and the other plate was incubated at 20 °C for 48 h. The *hipA7* allele is known to confer sensitivity to cold (1), so transductants that failed to grow well at 20 °C were tested for persistence. Wild-type *E. coli* MG1655, the *hipA7* strain (TH1269), and the isogenic parent of the *hipA7* strain (TH1268) were used as controls. (*B*) Cold-sensitive transductants were streaked, colony purified, and cultured overnight at 37 °C. The WT strain (MG1655), *hipA7* mutant (TH1269), $hipA^+$ strain (TH1268), and transductants were tested for persistence by inoculating LB broth with 1% of an overnight culture. At midlog (OD_{600} of 0.5), ampicillin was added to a concentration of 100 μg/mL and cultures were incubated for an additional 4 h. Identical dilutions (10^{-2}) of each culture were plated on LB agar and incubated overnight at 37 °C. Shown are representative images of plates of the different strains. Of the 20 transductants that grew poorly at 20 °C, 19 displayed the persister phenotype.

1. Scherrer R, Moyed HS (1988) Conditional impairment of cell division and altered lethality in *hipA* mutants of *Escherichia coli* K-12. *J Bacteriol* 170:3321–3326.

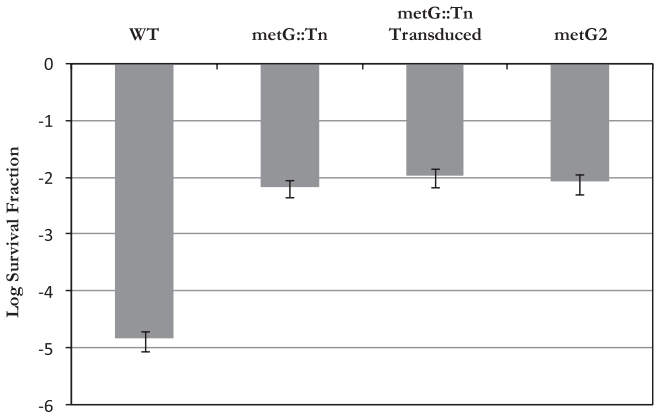

Fig. S5. Persistence frequencies of *metG*::Tn, *metG*::Tn transduced, and *metG2*. Persistence frequencies were determined for the wild-type strain and the following mutants: *metG*::Tn, *metG*::Tn transduced, and *metG2*. The *metG*::Tn-transduced mutant was constructed by transducing the insertion in the *metG*::Tn strain to the wild-type background using P1 phage. The *metG2* mutant was constructed by deleting the 3′ end of the *metG* gene. Persistence assays were performed on each of the mutants as described in the text. Strains were exposed to LB agar plus ampicillin for 24 h before spraying with penicillinase. Data values are the average survival ratios of three repetitions, and error bars represent the SDs.

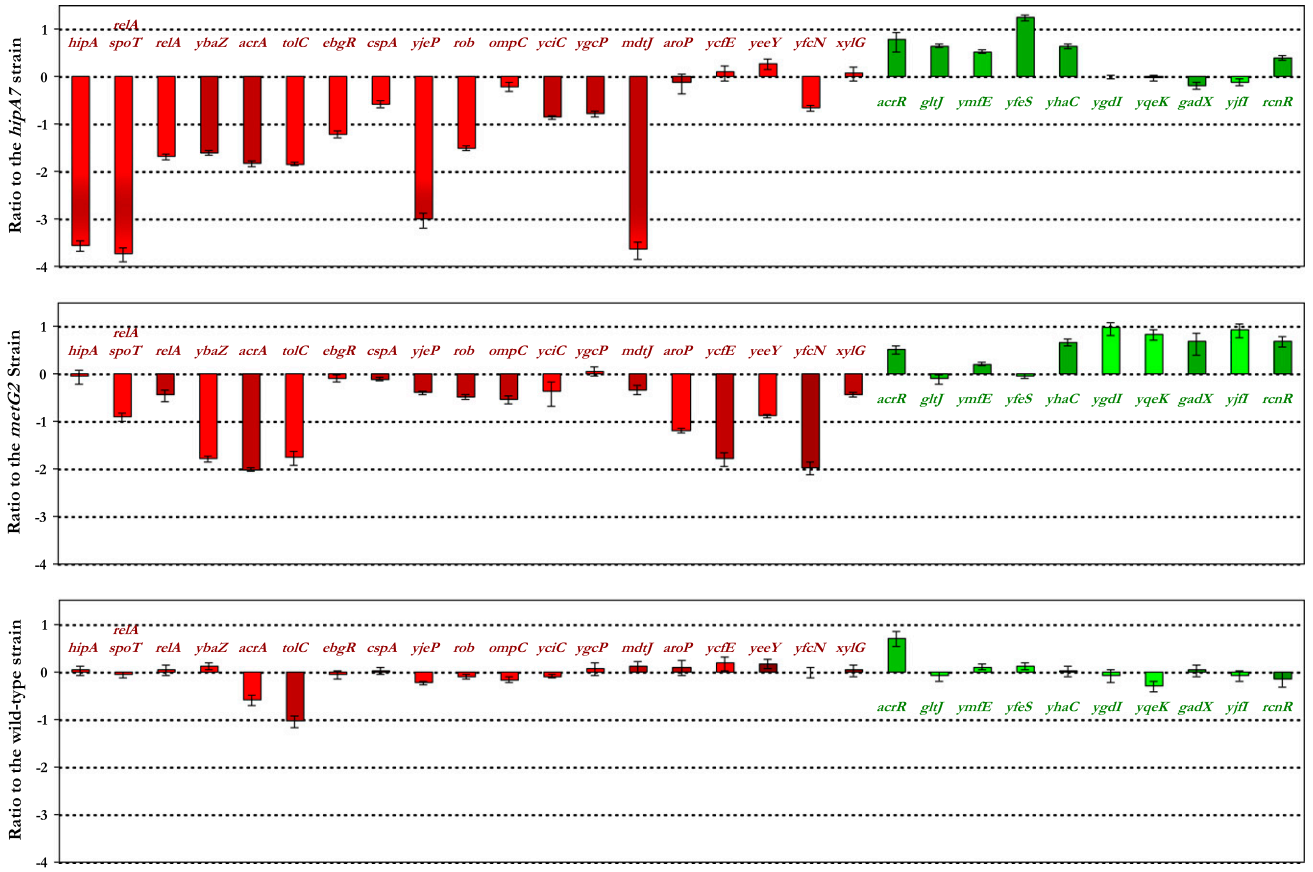

Fig. S6. Systematic epistasis analysis of the *hipA7* and *metG2* alleles. The z-score distributions for the *hipA7* and *metG2* libraries after persister selection guided the identification of genes for further analysis. Genes with very high or low z scores were targeted for deletion in the genetic background of the library in which they were identified. Persistence assays were performed on each of the mutants as described in the text, and survival ratios were calculated relative to the corresponding parent strain. Mutations that caused a half $\log_{10}$ or more change in persistence frequency in either the *hipA7* or *metG2* strains were subsequently transduced to the alternate persister strain for further evaluation. In this manner, all genes found to have a positive or negative genetic interaction with one allele were examined with the alternate genetic locus. Data values are the average survival ratios of three repetitions, and error bars represent the SDs. In addition, null mutations were created and tested in the wild-type strain to determine whether the observed change in persistence frequency was a result of a genetic interaction with the *hipA7* or *metG1* alleles, or rather, a general consequence of the mutations on the physiology of the cells. Of the genes tested, three genes (*tolC*, *acrA*, and *acrR*), known to be involved with multidrug efflux pumps, were found to influence persistence of the wild-type strains and were thus not included in the genetic interaction map displayed in Fig. 3*B*.

Other Supporting Information Files

Table S1 (DOC)
Table S2 (DOC)
Table S3 (DOC)
Table S4 (DOC)