SUPPLEMENTAL MATERIAL for *Functions that protect Escherichia coli from DNA-protein crosslinks*, by Krasich, Wu, Kuo and Kreuzer.

Supplemental Figure Legends:

Supplemental Figure 1: Spot test for quinolone hypersensitivity of strain with insertion in Mu gene 9. Wild-type or the insertion mutant with a transposon in Mu gene 9, both containing pBad33-MEcoRII, were diluted from overnight culture to approximately $4 \times 10^8$ cells/ml. Ten-fold serial dilutions were generated across a 96-well plate and 5 µl of each dilution was spotted onto LB plates with no drug (left panel), ciprofloxacin (Cipro, 0.01 µg/mL, middle panel) or nalidixic acid (Nal, 2 µg/mL, right panel). Plates were photographed after overnight incubation at 37°C.

Supplemental Figure 2: Aza-C sensitivity depends on expression of M.EcoRII. Plate spot tests were performed as described in the Supplemental Figure 1 legend, except that the LB plates contained either glucose (0.2%) or arabinose (0.05%), either with or without Aza-C (5 µg/mL). The wild-type strain is HK22 (see Materials and Methods) while all mutant derivatives are HK22 with the indicated mutation, transduced into HK22 by phage P1-mediated transduction (selecting for the kanamycin-resistance marker). All strains contained plasmid pBAD-MEcoRII, which expresses M.EcoRII from an arabinose-inducible promoter. Each panel (A through E) was derived from one single set of plates so that comparison of the growth of mutant to wild-type within a panel is always from the same plate.

Supplemental Figure 3: Growth tests for slow growing dnaK, rep and dksA strains. Panels A and B are representative growth curves for HK22 (WT) and dnaK mutant, panels C and D are representative growth curves for HK22 (WT) and rep mutant, and panels E and F are representative growth curves for HK22 (WT) and dksA mutant; all strains contained pBad33-MEcoRII. Cells were grown under the same conditions as in Figure 1 (Aza-C hypersensitivity of mutants expressing M.EcoRII), except for the presence of arabinose.

Supplemental Figure 4. Spot tests for aza-C resistance of rep and dnaK strains. Spot tests using dnaK and rep cells were conducted as in Supplemental Figure 1, except the plates contained 0.05% arabinose and aza-C at 5 or 10 µg/mL. Spot tests were conducted to either confirm resistance or to rule out resistance in the microtiter plates due to the slow growing nature of the mutants.

Supplemental Figure 5: Aza-C sensitivity of dnaK and dksA strains measured at late exponential phase. Panels A and B are comparative titration curves for dnaK and dksA strains respectively (based on the growth curves represented in Supplemental Figure 3). The titration curves were calculated similarly to Figure 1 (Aza-C hypersensitivity of mutants expressing M.EcoRII), with the exception that OD$_{630}$ were plotted from the time at which the growth rate of the no-drug culture dropped to 90% of the earlier exponential rate.
Supplemental Figure 6: Isobolic test for synergy with Rho inhibitor bicyclomycin.
Growth curves for the indicated HK22 derivatives were measured in each well of a 96-well microtitre plate, with varying concentrations of bicyclomycin (right to left) and aza-C (top to bottom). A detailed description of the data analysis and processing can be found in Kuo et al (16). Briefly, at each concentration of bicyclomycin (Bcm), the concentration of aza-C necessary to inhibit growth by 95% was estimated. In addition, the concentration of bicyclomycin necessary to inhibit growth by 95% (in the absence of the aza-C) was estimated from the bicyclomycin inhibition curve. The dashed lines connect the average determined MIC value of each drug alone. The linearity of the data indicates no drug interaction, implying that inhibition of Rho by bicyclomycin treatment does not increase sensitivity to aza-C. Another method used to test for synergy was the fractional inhibitory concentration (FIC) index ((83), also see Instructions to Authors for the ASM Journal Antimicrobial Agents and Chemotherapy). For each level of drug A, the FIC is calculated for the first concentration of drug B that gave 95% inhibition with FIC = [(MIC of drug A tested in combination) / (MIC of drug A tested alone)] + [(MIC of drug B-tested in combination) / (MIC of drug B-tested alone)]. The American Society for Microbiology recommends that synergy is supported by FIC index values less than 0.5, while antagonism is supported by FIC index values greater than 4. The FIC values for hepA, greA, and greB were 0.64, 1.01, 0.81, respectively, also indicating no interaction between the drugs. Thus, the FIC values corroborate the isobolic curves.

Supplementary Material References:

**Supplemental Table 1: Gene context of transposon insertions.** The thin black arrows represent the direction of the indicated gene with respect to the *E. coli* genome, and the thick black arrow in the center column represents the direction of the kanamycin-resistance gene of the transposon with respect to the *E. coli* genome sequence.

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<th>Upstream gene 1</th>
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Supplemental Table 2: Sequence summary of the dinD::lacZ fusion construct. The sequence of the dinD::lacZ fusion was determined by deep sequencing of DNA from strain JH39, followed by reconstructing the genome sequence using the sequencing program Geneious and NCBI Blast. The sequence of the fusion is summarized here, and can be reconstructed by accessing the sequences and positions listed in descending order in the Table. The order of the numbers in the “Positions” column represents the direction of each sequence fragment in the fusion. The dinD::lacZ fusion was originally created by infecting cells with the Mud(ApR, lac) phage and isolating strains that induced β-galactosidase activity during mitomycin C treatment (41,84). The Mud(ApR,lac) phage contains a temperature-sensitive mutation in the Mu repressor (cts62) which induces phage gene transcription at high temperatures, making the cells temperature sensitive for growth. Strain JH39 was created using the dinD1::MudI(ApR, lac) fusion, but with selection for derivatives that had lost the temperature sensitivity and the ability of the Mu phage to transpose (41,85). The sequence shows an IS1 insertion in gene A of Mu, which explains the loss of transposition. This IS1 element has a 298-bp deletion, with an area of microhomology near the deletion site that may have been involved in the deletion formation. The Mu sequence still contains the cts62 mutation, and therefore another mutation(s) must suppress the temperature sensitivity. Likely candidates are: (1) the IS1 insertion in gene A, because IS1 (including this deletion derivative) carries a transcriptional terminator (86) and the toxic Mu gene kil (also called gene 5) is downstream; and (2) the IS30 element, which interrupts the late gene region of Mu and might thereby prevent expression of one or more toxic late genes. The Mu sequence in this element reveals an inverted repeat of 43 bp near the C end of Mu, most likely due to non-standard fusion formation during the transposition of the MudI(ApR,lac) element into dinD or the fusion/deletion of Mu and trpB (the latter mutation is called ∆FI by Kenyon and Walker; non-standard fusion formation is described in (87)). The Mu sequence also contains a point mutation in gene T.
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<sup>a</sup> This segment contains mixed reads at these three sites, presumably resulting from distinct DNA sequences in the dinD::lacZ fusion and the native trpAB segment of the chromosome.

<sup>b</sup> Mutation is in gene T

<sup>c</sup> Interrupts Mu at position 16130, which is in gene 30

<sup>d</sup> Interrupts Mu at position 3183, which is in gene A

<sup>d</sup> Some E. coli chromosomes contain IS1 elements (e.g. P12b) that also contain these same substitution mutations that deviate from the canonical IS1 sequence. No examples of the large deletion were evident in the sequence databases.
Supplemental Figure 1
Supplemental Figure 2B
Supplemental Figure 2C
Glucose

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Arabinose

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Supplemental Figure 2D
Glucose 

WT 
xerD 

Glucose + Aza-C 

WT 
xerD 

Arabinose 

WT 
xerD 

Arabinose + Aza-C 

Supplemental Figure 2E
Supplemental Figure 3
Supplemental Figure 5
Supplemental Figure 6

A. *hepA*

B. *greA*

C. *greB*