Supporting Information

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SI Text

Bacterial Growth Conditions. Unless otherwise indicated, LB and minimal A (with 0.2% glucose) were routinely used as rich and defined media (1), respectively, and the growth temperature was 37 °C. Supplementation with antibiotics was at concentrations described previously (2, 3). Growth rates of cultures were determined with the aid of a Bioscreen C apparatus (OY Growth Curves). All images of bacterial colonies and spots shown in the figures are reproductions to size, within approximation.

Routine DNA Methods and Immunoblotting. Procedures for plasmid cloning, PCR, transformations and immunoblotting [using anti-GST antibody to determine intracellular UvsW levels, because the P_{tac}-UvsW construct encodes a GST-tagged UvsW protein (4)] were as described previously (5).

Plasmids. Plasmids described earlier include (relevant features in parentheses): pMU575 (single copy-number unstable replicon with *lacZ*$^+$) (6); pAM34 [isopropyl-β-D-thiogalactopyranoside (IPTG)-dependent for replication] (7); pHYD781 (multicopy *rnhA*$^+$ encoding RNase HI, which is the plasmid referred to as p*rnhA*$^+$ in Table 1) (8); pSK760 and pSK762c (carrying, respectively, *rnhA*$^+$ and a mutated and inactive version of *rnhA*) (9); pKD13 (for use in recombineering experiments) (10); pCP20 (expressing Flp recombinase for site-specific excision between FRT sites) (10); pHYD2556 (low-copy-number P_{ara}-*nusA*$^+$) (2); and pKCK47 and pKCK48 (multicopy P_{tac}-UvsW and P_{tac}-UvsW-K141R, respectively, each with N-terminal GST tag-encoding sequence) (4). Plasmids constructed in this study include pHYD2380 [pAM34 derivative with cloned *Escherichia coli nusA* ORF between genome coordinates (11) 3313815 and 3316994]; and pHYD2381, pHYD2411, and pHYD2412, which are pMU575 derivatives with, respectively, cloned *E. coli nusA*$^+$ (genome coordinates the same as those for pHYD2380), *Salmonella typhimurium rho*$^+$ [genome coordinates (12) 4125279–4126992], and *S. typhimurium nusG*$^+$ (genome coordinates 4361804–4362966).

Construction of Bacterial Strains. Gene transfers across strains were accomplished by P1 transduction (1). Single-copy chromosomal integrations of P_{tac}-UvsW and P_{tac}-UvsW-K141R at the λ *att* site were achieved with the aid of the plasmid-chromosome shuttling procedure using phage λInCh, as previously described (13), and the plasmids from which these constructs were transferred by homologous recombination were pKCK47 and pKCK48, respectively. The construction of strains with *rho*, *nusG*, and *nusA* deletions is described below.

***rho*, *nusG*, and *nusA* Mutations Used in Tests of Lethality and Suppression by UvsW.** With a lone exception described in Fig. S4*B*, all experiments with the lethal *rho*, *nusG*, and *nusA* mutations were performed in strains MDS42Δ(*argF-lac*)*U169* or MG1655Δ*lacIZYA*.

Two alleles of *rho*, three of *nusG*, and four of *nusA* were used in the tests for lethality and their suppression; the mutations were routinely maintained in strains carrying a functional copy of the cognate genes on plasmids pHYD2411, pHYD2412, and pHYD2381, respectively. Three of the mutations (that have been designated for convenience in this study as *rho*::Kan, *nusG*::Kan, and *nusA*::Cm) are those that were used earlier by Cardinale et al. (14), and the mutations represent, respectively, a deletion-insertion allele in which a 360-bp fragment including the initiation codon of *rho* has been replaced by a kanamycin-resistance (Kan)-cassette, a Kan-insertion after codon 43 of *nusG*, and another deletion-insertion allele in which the segment between codons 128 and 304 of *nusA* has been replaced by a chloramphenicol-resistance (Cm)-cassette.

All of the other mutations were those obtained by the PCR-based recombineering protocol of Datsenko and Wanner (10), leading to deletion of specified chromosomal regions and their replacement by an FRT-site flanked Kan cassette from plasmid pKD13. (*i*) Three mutations, designated Δ*rho*, Δ*nusG*, and Δ*nusA*, are near-complete deletions of the corresponding ORFs (between codon 2 and the seventh codon from the last), of which Δ*rho* was sourced from a strain in the Keio knockout strain collection (15), where it is sheltered by *rho*$^+$ on a tandem duplication (16, 17); Δ*nusG* and Δ*nusA* were constructed in this study with primer pairs 5′-TATTCTGGTTCGCCTGGTATC-CTTTATCACTGGCCTGAGGTTCTGAGATGATTCCGGG-GATCCGTCGACC-3′ (forward) and 5′-ATTAAATCGCCG-CTTTTTTGATCGCTGGGTTAGGCTTTTTCAACCTGGCT-TGTAGGCTGGAGCTGCTTCG-3′ (reverse) for the former, and 5′-ACCTGGTTCCCCACTTTTAATAGTCTGGATGAG-GTGAAAAGCCCGCGATGATTCCGGGGATCCGTCGAC-C-3′ (forward) and 5′- GTCATGCTGTTCCTTCCTGCTAC-AGTTTATTACGCTTCGTCACCGAACCATGTAGGCTGG-AGCTGCTTCG-3′ (reverse) for the latter. Because *nusG* is very tightly linked to *rpoB*, a separate recombineering experiment was undertaken to introduce Δ*nusG*::Kan into the *rpoB*35* strain. (*ii*) The remaining mutations comprised one C-terminal deletion (CTD) of *nusG* after codon 118 and two of *nusA* after codons 137 (CTD-1) and 200 (CTD-2), with each of them extending up to the seventh codon from the last as for the near-complete deletion alleles in (*i*), above. The forward primers used in these three instances were, respectively, 5′-CAATCAG-CGATAAAGAAGTCGATGCGATTATGAACCGCCTGCA-GCAGGTTTAAATTCCGGGGATCCGTCGACC-3′; 5′-CC-GAACGTGCGATGGTGGTTGATCAGTTCCGTGAACACG-AAGGTGAAATCTAAATTCCGGGGATCCGTCGACC-3′; and 5′-CCGTTCGCCCGGAAGCGCGTGGCGCGCAACT-GTTCGTCACTCGTTCCAAGTAAATTCCGGGGATCCG-TCGACC-3′; the reverse primers were the same as those that have been described in (*i*), above. The CTD variants of NusG and NusA were constructed and tested in this study in light of earlier suggestions (18–21) for existence of distinct domain-specific functions in the two proteins.

Testing for Presence or Absence of *rac* Prophage in Strains by PCR. The principle of the PCR-based method that was used to establish the presence or absence of the *rac* prophage in different strains is illustrated in Fig. S1*A*. Under routine PCR conditions, the pair of forward and reverse primers E1 and E2 (annealing to chromosomal loci immediately external to the *rac* prophage and flanking its attachment site) is expected to yield an amplicon only in a Δ*rac* strain (because the *rac* prophage itself is around 20-kb long). On the other hand, PCR performed with E1 and the reverse primer I (that is internal to the prophage) would yield an amplicon only in the *rac*$^+$ strain. The sequences of the three primers E1, E2, and I were, respectively, 5′-GGCGAGAACA-CAGT-3′, 5′-TCACCACTTATCTG-3′, and 5′-TGCCGTTT-ACTCAG-3′.

Testing for Effect of UvsW Expression on Relief of Nonsense Polarity in *rho* Mutants. The effect of UvsW expression was tested on two phenotypes of nonsense polarity that are each relieved by the *rho*-

4 missense mutation (Fig. S4*A*). One is the phenotype of sensitivity to supplementation of defined medium with serine, methionine, and glycine (SMG^S) conferred in a *relA* background by the naturally occurring frame-shift mutation in *ilvG* in *E. coli* K-12, for which the isogenic pair of strains GJ3161 (rho^+ SMG^S) and GJ3110 (*rho-4*, SMG^R) was used (8). The other is the phenotype of tryptophan auxotrophy conferred by a *trpE* frameshift mutation, in which anthranilate can substitute for the tryptophan requirement if the strain is *rho-4* (GJ6509) but not rho^+ (GJ6504) (2).

Strain GJ3161 also carries the mutation *galEp3*, conferring nonsense polarity on the *gal* operon (8) to examine whether nonsense polarity is relieved also in a Δ*rho* mutant expressing P_{tac}-UvsW, isogenic P_{tac}-UvsW derivatives of GJ3161 (rho^+) and GJ3161Δ*rho* were examined for their Gal and SMG phenotypes. GJ3161Δ*rho* was viable only on defined medium supplemented with IPTG (as expected), and it was both Gal^+ and SMG^R on IPTG-supplemented plates, whereas its rho^+ parent was Gal^- and SMG^S (Fig. S4*B*). Thus, P_{tac}-UvsW expression does not affect nonsense polarity relief, even in a Δ*rho* strain.

Results from Experiments with *nusA* Mutations. In the case of the *nusA* mutations, three (Δ*nusA*, CTD-1, and CTD-2) yielded no white colonies in the plasmid-segregation assay, even for MDS42 without or with UvsW expression. The fourth (*nusA*::Cm) did yield white colonies for MDS42 on the plasmid-segregation assay (which may explain the earlier report of viability of this strain), but the cells in these colonies could not be subcultured [as has also been reported by Cardinale et al. (14)]; similarly, among derivatives sheltered by plasmid pHYD2380 (which carries $nusA^+$ and is IPTG-dependent for replication), it was only MDS42*nusA*::Cm (but not MDS42Δ*nusA* or MG1655 with either *nusA*::Cm or Δ*nusA*) that yielded colonies on LB plates without IPTG supplementation (Fig. S5*A*), and once again the cells in these colonies could not be revived upon restreaking. Finally, MDS42*nusA*::Cm (but not MG1655*nusA*::Cm) was indefinitely viable if it carried an additional P_{ara}-*nusA* construct even in absence of arabinose supplementation (Fig. S5*B*), supporting the earlier suggestion (14) that MDS42 requires a considerably lower level of NusA expression than does MG1655 for viability.

Protocols for Bisulfite Treatment and Data Analysis. Total nucleic acids were gently isolated by a procedure using cetyl trimethyl ammonium bromide and lysozyme in buffer of high osmolarity. as previously described (22, 23), from 20 mL of LB-grown midlog phase cultures of the MG1655-derived WT strain (GJ6504), its *nusG*-G146D derivative (GJ6511), and the *nusG* strain with multicopy-$rnhA^+$ (GJ6511/pHYD781). Reagents of the Qiagen Epitect Bisulfite kit were used as per the manufacturers' instructions for treatment of the nucleic acid preparations with sodium bisulfite, with the modification that the incubation temperature was 37 °C (to avoid denaturation of DNA-DNA and RNA-DNA duplexes). The bisulfite-treated samples were subjected to whole-genome amplification with reagents from the Qiagen Epitect Whole Bisulfitome kit followed by sequencing on the SOLiD 4 System platform.

The population of reads from each sample was sequentially mapped, with the aid of the Bioscope Alignment Software available with the System platform, to three MG1655-related reference genomes as described in the main text: native, C-to-T converted, and G-to-A converted. The output from each of these alignments for strains GJ6504 and GJ6511 were recovered and stored as .bed and .bam files, and were "binned" to discrete intervals corresponding to the individual genes and intergene regions, as defined in Baba et al. (15), with the aid of a software program developed in-house; in the process, any read that spanned an interval boundary was discounted, the aggregate of which amounted to around 7.8% and 7.9% of the total reads that were mapped in the two strains, respectively.

Following the binning procedure, the following 157 of the 4,359 gene rows listed in Baba et al. (15) were excluded from further analysis: 74 genes labeled "none"; 61 "*ins*" genes; a cluster of 5 genes deleted at the *trpR* locus in both strains (2) (*yjjK*, *slt*, *trpR*, *yjjX*, *ytjC*); and *htgA*, *rzoD*, *dcuC*, *tpr*, *trpL*, *rzoR*, *pheM*, *hisL*, *gatA*, *rcsC*, *ygeL*, *yqgB*, *ivbL*, *tnaC*, *tnaB*, *yzfA*, and *yjgX*.

N_{upper} and N_{lower} values for the individual genes were then calculated as described in the text; these values were converted to rank orders and percentile ranks within each of the four categories of clockwise (C) and counterclockwise (CC) genes: C+, C−, CC+, and CC−. Clusters of genes exhibiting high bisulfite reactivity were identified by the following criteria: (*i*) at least one gene in each cluster was to be above the 97th percentile in N_{upper} or N_{lower} values for genes of that category, in either the WT or *nusG* strains (Dataset S2, sheet 4); (*ii*) clusters were iteratively enlarged by including genes that are above the 90th percentile (in either the WT or *nusG* strain) on the same strand (upper or lower, as appropriate) as, and that are ≤4 genes separated from, the last gene of the previous iteration; (*iii*) at least one gene in each such cluster was also to be above the 90th percentile for the *nusG* strain; and (*iv*) two or more clusters, identified by application of criteria *i* to *iii* above, were merged if they were separated by ≤4 genes (even if the individual clusters represented bisulfite sensitivity on different strands).

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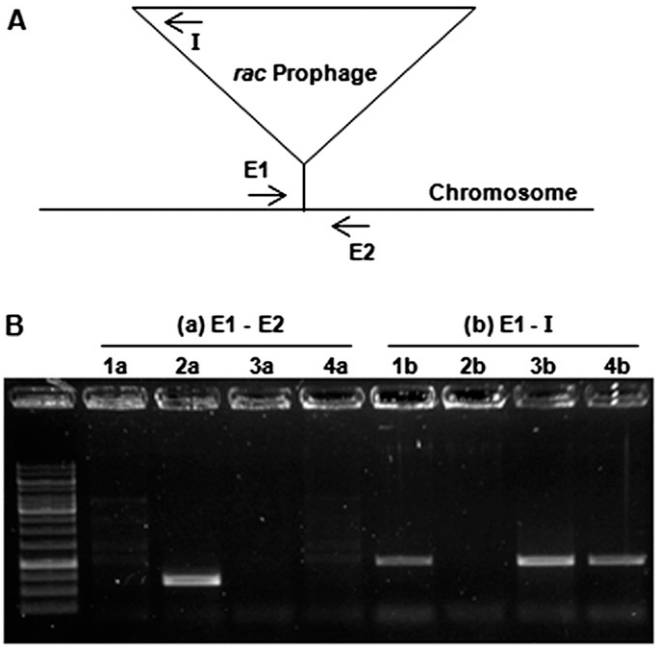

Fig. S1. Testing for presence or absence of *rac* prophage by PCR. (*A*) Schematic depiction of the sites of annealing of primers E1 and E2 to loci flanking the *rac* prophage integration site, and of primer I within the *rac* prophage, as explained in *SI Text*. Successful amplicon generation with E1-E2 signifies a Δ*rac* genotype and that with E1-I a *rac*$^+$ genotype. (*B*) Results of PCR with primer pairs (a) E1-E2 and (b) E1-I for strains GJ6504 (WT strain used in bisulfite experiment; lanes 1a and 1b), GJ6511 (*nusG*-G146D missense mutant used in bisulfite experiment; lanes 2a and 2b), MG1655Δ*rho* P_{tac}-UvsW carrying *rho*$^+$ plasmid pHYD2411, that is, blue colony derivative of strain GJ13492 described in Table S1 (lanes 3a and 3b), and MG1655Δ*rho* P_{tac}-UvsW, that is, white colony derivative of strain GJ13492 described in Table S1 (lanes 4a and 4b). DNA size markers are on lane at extreme left. PCR amplicons of expected lengths are seen in lanes 2a, 1b, 3b, and 4b.

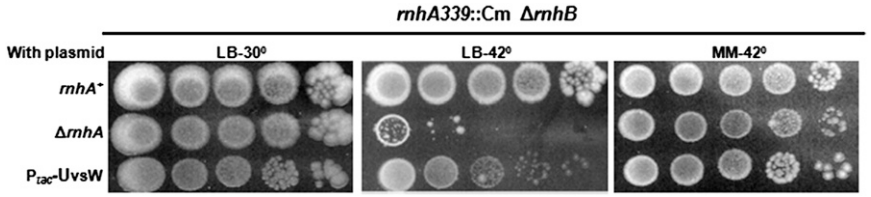

Fig. S2. Rich-medium temperature sensitivity of strains deficient for both RNase HI and RNase HII, and its suppression by UvsW expression. Derivatives of strain GJ13517 (*rnhA339*::Cm Δ*rnhB*::Kan; that is, deficient for RNases HI and HII) carrying one of the following plasmids: pSK760 (*rnhA*$^+$), pSK762c (Δ*rnhA*), or pKCK47 (P_{tac}-UvsW), were spotted at dilutions on LB or glucose-minimal A (MM) plates and incubated at 30 °C or 42 °C, as indicated. The results demonstrate rich-medium–specific temperature sensitivity of the double-mutant strain derivative with the noncomplementing Δ*rnhA* plasmid, successful complementation with *rnhA*$^+$ plasmid, and partial suppression with P_{tac}-UvsW plasmid.

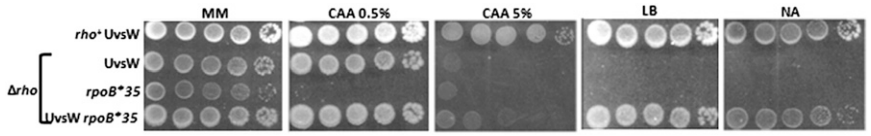

Fig. S3. Rich-medium sensitivity of Δ*rho* derivatives with P_{tac}-UvsW or *rpoB**35. MDS42Δ*lac* derivatives GJ13495 (*rho*$^+$ P_{tac}-UvsW), GJ13493 (Δ*rho* P_{tac}-UvsW), GJ13567 (Δ*rho rpoB**35), and GJ13593 (Δ*rho rpoB**35 P_{tac}-UvsW) were each spotted at dilutions on glucose-minimal A without (MM) or with supplementation with 0.5% or 5% Casamino acids (CAA), LB, and nutrient agar (NA), as indicated. All plates were supplemented with IPTG at 25 μM.

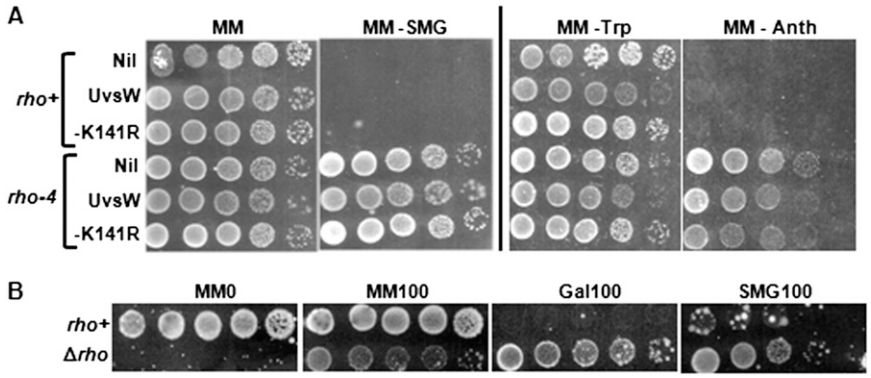

Fig. S4. UvsW expression does not affect relief of nonsense polarity conferred by *rho* mutation. (*A*) Isogenic derivatives of *rho*$^+$ and *rho-4* strains without (Nil) or carrying P_{tac}-UvsW (UvsW) or P_{tac}-UvsW-K141R (-K141R) were spotted at dilutions on glucose-minimal A without (MM) or with supplementation with serine, methionine and glycine (SMG), tryptophan (Trp), or anthranilate (Anth). All plates were supplemented with IPTG at 100 μM. Derivatives of strains GJ3161 (*rho*$^+$) and GJ3110 (*rho-4*) were used for the pair of plates on left, and of strains GJ6504 (*rho*$^+$) and GJ6509 (*rho-4*) for the pair of plates on right (see *SI Text* for details). (*B*) Isogenic derivatives of GJ3161 (*rho*$^+$) and GJ3161Δ*rho* carrying P_{tac}-UvsW were spotted at dilutions on the following plates: glucose-minimal A without (MM0) or with 100 μM IPTG (MM100); galactose-minimal A with 100 μM IPTG (Gal100); and glucose-minimal A wtih serine, methionine and glycine along with 100 μM IPTG (SMG100). Growth of suppressor colonies is visible for low-dilution spots of the *rho*$^+$ strain on the SMG100 plate (because this plate was incubated for three days to compensate for the slow growth-phenotype associated with Δ*rho*).

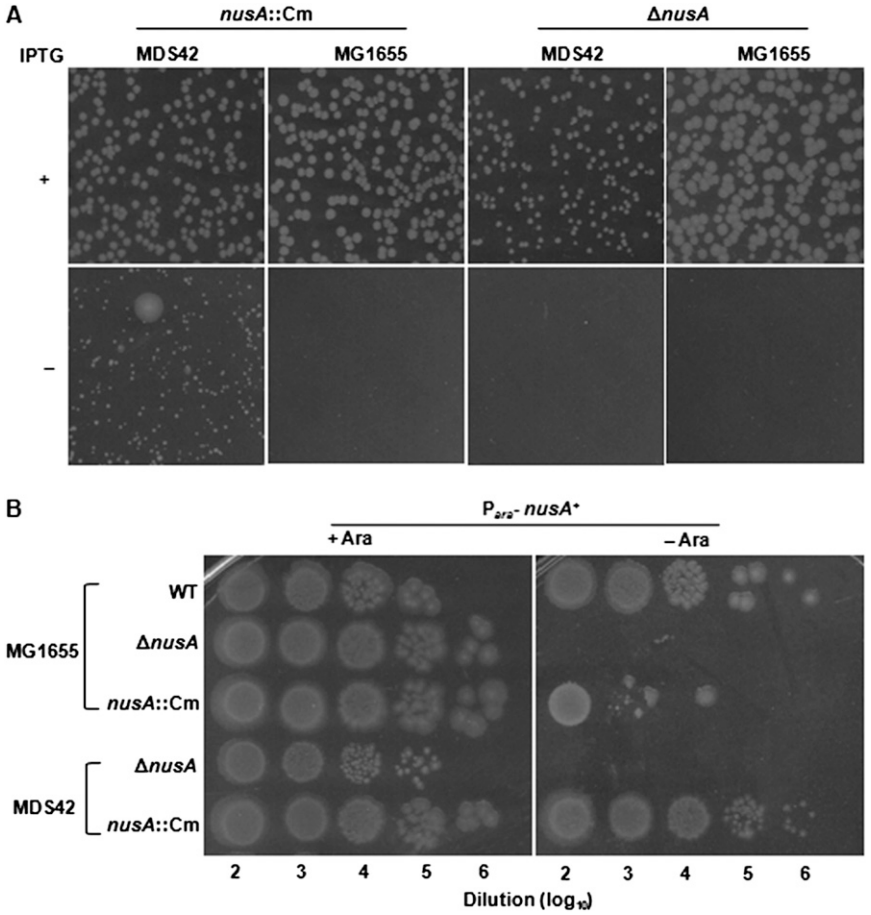

Fig. S5. Phenotypic differences between *nusA* mutants of MDS42 and MG1655, including transient viability of MDS42 *nusA*::Cm and inducer-independent viability of MDS42 *nusA*::Cm bearing P_{ara}-*nusA*$^+$. (*A*) *nusA*::Cm and Δ*nusA* derivatives of MDS42 and MG1655 carrying *nusA*$^+$ on the IPTG-dependent replicon pHYD2380 were each plated at a suitable dilution on a pair of LB plates without (–) and with (+) IPTG supplementation at 500 μM. Strains for the panels were, respectively, from left: GJ13767, GJ13759, GJ13766, and GJ13758. (*B*) Dilution-spotting of indicated MG1655 and MDS42 derivatives carrying plasmid pHYD2556 (with P_{ara}-*nusA*$^+$) on a pair of LB plates without and with supplementation of 0.2% arabinose (–Ara and +Ara, respectively). Strains used were (from top): GJ6504, GJ13742, GJ13743, GJ13754, and GJ13755 respectively.

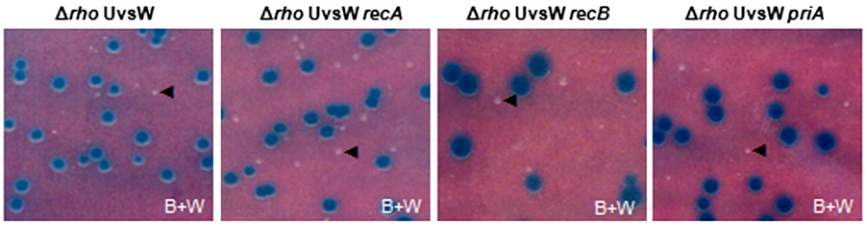

Fig. S6. Viability of MDS42 Δ*rho* expressing UvsW is independent of RecA, RecB, or PriA. Panels show occurrence of mixtures of blue and white (B+W) colonies from the different MDS42 Δ*rho* P_{tac}-UvsW derivatives carrying the *rho*$^+$ *lacZ*$^+$ plasmid pHYD2411 on X-Gal– and IPTG-supplemented minimal medium; representative white colonies are marked by arrowheads. Strains for the panels were, respectively, from left: GJ13493, GJ13561, GJ13553, and GJ13552.

Table S1. Plasmid-segregation assay for viability of *rho*, *nusG* and *nusA* mutants expressing UvsW or UvsW-K141R

Genotype	Nil	With P_{tac}-UvsW	With P_{tac} UvsW-K141R
MDS42Δ*lac* derivatives			
WT	B+W [12134]	B+W [13495]	B+W [13496]
rho::Kan	B [12135]	B+W [12154]	B [12155]
Δ*rho*	B [13551]	B+W [13493]	B [13494]
Δ*nusA*	B [13775]	B [13787]	B [13788]
nusA-CTD1	B [13773]	B [13783]	B [13784]
nusA-CTD2	B [13774]	B [13785]	B [13786]
nusA::Cm	B* [13776]	B* [13789]	B* [13790]
rho::Kan *rpoB**35	B+W [13461]	B+W [13555]	ND
Δ*rho rpoB**35	B+W [13567]	B+W [13593]	ND
Δ*rho*Δ*nusG*	ND	B+W [14227]	ND
MG1655Δ*lac* derivatives			
WT	B+W [12140]	ND	ND
nusG::Kan	B [13477]	B+W [13488]	B [13489]
Δ*nusG*	B [13506]	B+W [13507]	B [13508]
nusG:: CTD	B [13476]	B+W [13486]	B [13487]
Δ*rho*	B [13490]	B+W [13492]	B [13491]
Δ*rho rpoB**35	B [13525]	B+W [13527]	ND
Δ*nusG rpoB**35	B [14232]	ND	ND

Deletion derivatives without (Nil) or with the P_{tac}-UvsW or P_{tac}-UvsW-K141R constructs as indicated, and carrying the cognate *lacZ*$^+$- bearing shelter plasmids, were cultured overnight in glucose-minimal A media and plated on X-Gal– and IPTG-supplemented glucose-minimal A media to determine occurrence of blue (B) or mixture of blue and white (B+W) colonies. Numbers in brackets are GJ-designated strain numbers for the different strains. B* refers to strains that yielded small whites colonies which, however, failed to grow upon restreaking. ND, not determined.

Other Supporting Information Files

Dataset S1 (XLS)
Dataset S2 (XLS)