Supplementary Data for

A new role for *Escherichia coli* Dam DNA methylase in prevention of aberrant chromosomal replication

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Supplementary Text

Details of plasmids constructed

(i) *pHYD2388* (pMU575 derivative carrying *S.enterica dnaA*$^+$): Plasmid pHYD2388 is a derivative of the vector pMU575 (1) and carries between the latter's SalI and HindIII sites the region of *S. enterica* genomic DNA from 4045412 bp (SalI-proximal) to 4043222 bp encompassing *dnaA*$^+$ including its upstream regulatory region and extending into part of the downstream gene *dnaN*, followed on the HindIII-proximal end by 57 bp of DNA from other sources acquired during the steps of PCR and subcloning. The *dnaA*$^+$ region in pHYD2388 was sequence verified.

(ii) *pHYD4805* (pMU575 derivative carrying *S.enterica dnaA*$^+$ and *recG*$^+$): The region of *S. enterica* chromosomal DNA encompassing *recG*$^+$ including its upstream regulatory region was PCR-amplified with a pair of primers 5'-GAGCTCGGTACCAAGCTTCT*TCTAGA*TAAACGTAAAGGTCTG-3' and 5'-TCGATATCGCATGCGGTACC*TCTAGA*ATCTTCTGTTGGCTCTG-3' (XbaI site in each of the two primers italicized), and cloned into the XbaI site of pHYD2388, to generate pHYD4805. This plasmid was used in place of pHYD2388 in all experiments to test for cSDR in *recG* mutants, since we found [as reported previously (2)] a high frequency of suppressors in cultures of the unsheltered *recG* derivatives.

(iii) *pHYD4807* (pBAD18 derivative carrying *E. coli mutS*$^+$): The region of *E. coli* chromosomal DNA encompassing the *mutS* open-reading frame and ribosome-binding site but excluding its promoter region was PCR-amplified with a pair of primers 5'-CG*GAATTC*CACCCCATTTAATATCAGGGAAC-3' and 5'-CC*AAGCTT*GCAAAAGACTATCGGGAATTG-3' (EcoRI and HindIII sites, respectively, in the two primers italicized), and cloned into the EcoRI-HindIII sites of pBAD18, to generate pHYD4807.

(iv) *pHYD5701* (pMU575 derivative carrying *S.enterica recA*$^+$): The region of *S. enterica* chromosomal DNA encompassing *recA*$^+$ including its upstream regulatory region was PCR-amplified with a pair of primers 5'-

CCC*AAGCTT*GTTTGCTTTCGCCAG-3' and 5'-CGG*GGTACC*ATCACCTGATGATTA-3' (HindIII and KpnI sites, respectively, in the two primers italicized), and cloned into the HindIII-KpnI sites of pMU575, to generate pHYD5701. The insert region in pHYD5701 was sequence verified.

Construction of Δ*dnaA*::Kan by recombineering

The Δ*dnaA*::Kan allele was designed on similar principles as those followed for gene knock-outs in the Keio collection (3), with all but the first and last six codons of the *dnaA* open-reading frame substituted by an FRT-flanked Kanʃcassette, and recombineering was performed as described by Datsenko and Wanner (4), into a recipient strain that also carried the *dnaA*$^+$ plasmid pHYD2388. The primers employed for generating the PCR fragment for recombineering from the plasmid pKD13 template (4) were: 5'-TGTTTCAGCCTTAGTCATTATCGACTTTTGTTCGAGTGGAGTCCGCCGTGATT CCGGGGATCCGTCGACC-3' and 5'-GCTCACGTTCTACGGTAAATTTCATAGGTTTACGATGACAATGTTCTGATTGTA GGCTGGAGCTGCTTCG-3'.

Testing for transfer of different alleles in P1 transduction

The *rpoB*35* mutation was introduced either in linkage with *btuB*::Tn*10*, or by virtue of its contransducibility with *argE*. For the latter, the recipient strain was first rendered auxotrophic with φ*argE*::Kan and then transduced to prototrophy with a P1 lysate prepared on an Arg$^+$ *rpoB*35* strain GJ17762. Inheritance of *rpoB*35* was identified by scoring for resistance to rifampicin at 10 μg/ml.

The *priA300* mutation was introduced by virtue of its cotransducibility with *metF*, employing a strategy very similar to that described above for *rpoB*35*. Distinction between *priA*$^+$ and *priA300* transductants was made by a PCR-based approach, in which the common primer was 5'-GCAGATTACTATCATCATCCG-3' and the distinguishing primers (nucleotide differences italicized) were 5'-CTGAGATAAACCTCCGTG*TT*-3' (for *priA*$^+$) and 5'-CTGAGATAAACCTCCGTG*CG*-3' (for *priA300*).

The validation of *polA12*, Δ*dam*::Kan, and ϕ*rdgB*::Kan mutations was done by scoring for synthetic lethality with *recA*, as follows. In the derivative of the *recA lacZ* strain DH5α (5) carrying the *recA*$^+$ *lacZ*$^+$ Tp^R plasmid pHYD5701, introduction of any of these mutations yielded transductants that failed to segregate Tp^S Lac$^-$ colonies (whereas around 10% of colonies from the control strain, not exhibiting synthetic lethality, was Tp^S and Lac$^-$).

Crossing out of phi80 prophage by P1 transduction

The phi80 prophage was crossed out by P1 transduction in two steps as follows. In the first step, the ϕ*trpE* :: Kan mutation from the Keio collection (3), whose chromosomal location is close to the phi80 *att* site, was introduced into the phi80 lysogenic strain, and in the second step the derivative was transduced to Trp$^+$ with a P1 lysate prepared on a phi80-free strain. Both steps were performed at 42°, since the ability of phi80 phage to infect sensitive cells is strongly compromised at this temperature (6). The prophage may be expected to be crossed out in either of the steps above, and Trp$^+$ colonies that were phi80-free or phi80-lysogenic were distinguished both by plaque assays [with supernatants from cultures of phi80 lysogens (6)] and by duplex PCR, with examples of the latter shown in Figure S4A. Although *trp* and the phi80 integration site are expected to be around 80% cotransducible, we observed only around 35% linkage between them which may be indicative of a finite frequency of phi80 phage infection of the sensitive cells even on the plates incubated at 42°.

Analysis of deep sequencing data

Sequence reads were subjected to de novo contig assembly with the aid of Velvet software (version 1.2.10), and were also aligned using Bowtie2 to one of two reference genomes, either strain MG1655 (U00096.2), or MG1655 with integrated phi80 prophage. The latter was generated by assembly of parts of the MG1655 DNA sequence with those of the phi80 DNA sequence (NC_021190.1) in the following order (based on the findings from de novo contig assembly of our own sequence data): 1 to 1308611 (MG1655); 24638 to 46150 (phi80); 1 to 24617 (phi80); and 1308596 to 4639675 (MG1655).

The aggregate base read counts for each culture (after alignment to MG1655) are given in Table S2. Reads were filtered such that those with phred score >30 were taken for further steps.

The *bedcov* function of Samtools software was used to bin the reads to 1kb non-overlapping windows, after expunction of reads erroneously aligned to the *argF-lac* interval (97239 bp, which is deleted in all of the sequenced strains). The base read count for each 1kb interval was normalized to the aggregate of aligned base read counts, and then expressed as an enrichment ratio as described in the text, in comparison with the similar normalized value for wild-type strain GJ13519 grown to stationary phase. These ratio calculations were done separately for the DNA preparations made with the commercial kit and for those made by phenol-chloroform extraction. Graphs were plotted on MS-Excel and smoothening was performed by a moving average approach with a 10-kb window and 4-kb step size.

For detection of SNPs, a custom R script was used to identify positions at which the ratio of mismatches to total was ≥0.8 after alignment; these bases were considered as SNPs if there were at least ten independent reads with that alteration.

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Table S1. *List of E. coli strains*

Strain [a]	Genotype [b]
DH5α	*fhuA2 lac(del)U169 phoA glnV44 Φ80' lacZ(del)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17*
DL2006	BW27784 ΔP_{sbcDC} P_{ara}-*sbcDC lacZ*::pal246 *cynX*::Gm^R
GJ13519	MG1655 Δ(*argF-lac)U169*
GJ17465	DL2006 Δ*dnaA::*FRT *tus::*FRT *rpoB*35 btuB::*Tn*10*
	Derivatives of GJ13519
GJ15830	Ter4::$parS_{pMT1}$
GJ15831	Ter4::parSpMT1 Δ*rnhA*::Kan
GJ15832	Ter4::parSpMT1 Δ*dam*::Kan
GJ15833	Δ*rnhA*::Kan
GJ16427	Δ*dnaA::*FRT Δ*tus::FRT rpoB*35 btuB::*Tn*10* Δ*mutS::*Kan
GJ16437	Δ*dnaA::*FRT Δ*tus::*FRT *rpoB*35 btuB::*Tn*10 dam*::Tn*9* Δ*mutS::*Kan
GJ16438	Δ*dnaA::*FRT Δ*tus::*FRT *rpoB*35 btuB::*Tn*10 dam*::Tn*9* Δ*mutH::*Kan
GJ16445	Δ*dnaA::*FRT Δ*tus::*FRT *rpoB*35 btuB::*Tn*10 dam::*Tn*9*
GJ16449	Δ*dnaA::*FRT
GJ16459	Δ*dnaA::*FRT Δ*tus::FRT rpoB*35 btuB::*Tn*10*
GJ16464	Δ*dnaA::*FRT Δ*tus::*FRT *rpoB*35 btuB::*Tn*10* Δ*recG::*FRT
GJ16467	Δ*dnaA::*FRT Δ*tus::*FRT *rpoB*35 btuB::*Tn*10* Δ*seqA::*FRT
GJ16468	Δ*dnaA*::FRT Δ*tus::*FRT *rpoB*35 btuB::*Tn*10* Δ*rdgB::*FRT
GJ16469	Δ*dnaA::*FRT Δ*tus::*FRT *rpoB*35 btuB::*Tn*10 dam::*Tn*9* Δ*dinG::*Kan
GJ16472	Δ*dnaA::*FRT Δ*tus::*FRT *rpoB*35 btuB::*Tn*10 polA12 zih-3166::*Tn*10*Kan

GJ16475 Δ*dnaA::*FRT Δ*tus::*FRT *rpoB*35 btuB::*Tn*10* Δ*rnhA::*FRT

GJ16476 Δ*dnaA*::FRT *rnhA339*::Cm

GJ16488 Δ*dnaA::*FRT Δ*tus::*FRT *rpoB*35 btuB::*Tn*10* Δ*dam::*Kan

GJ16489 Δ*dnaA::*FRT Δ*tus::*FRT *rpoB*35 btuB::*Tn*10* Δ*recG::*FRT Δ*dinG::*Kan

GJ16490 Δ*dnaA::*FRT Δ*tus::*FRT *rpoB*35 btuB::*Tn*10* Δ*recG::*FRT *att λ:: P_{ara}* -UvsW (Amp^r)

GJ16494 æ*tus rpoB*35 btuB::Tn10* Δ*dam::*Kan

GJ16498 Δ*dnaA::*FRT *rnhA339*::Cm *rpoB*35 btuB*::Tn*10*

GJ16499 Δ*dnaA*::FRT *rnhA339*::Cm *rpoB*35 btuB*::Tn*10* Δ*recA*::Kan

GJ17503 Δ*recG*::Kan

GJ17762 Δ*dnaA*::FRT *rpoB*35*

GJ18603 Δ*dnaA::*FRT Δ*tus::*FRT *rpoB*35 btuB::*Tn*10 dam::*Tn*9* (phi80-free)

GJ18607 Δ*dnaA::FRT* Δ*tus::FRT rpoB*35 btuB::*Tn*10* (phi80-free)

GJ18609 æ*tus rpoB*35 btuB::Tn10*

GJ18611 Δ*dnaA::*FRT Δ*tus::*FRT *rpoB*35 btuB::*Tn*10 dam::*Tn*9* Δ*mutS::*Kan (phi80-free)

GJ18612 Δ*dnaA*::FRT *rnhA339*::Cm Δ*sulA*::FRT

GJ18613 Δ*dnaA::*FRT Δ*tus::*FRT *rpoB*35 btuB::*Tn*10 dam::*Tn*9* Δ*dinG::*Kan *att λ::P_{ara}-rnhA* (Amp^r)

GJ18615 Δ*dnaA::*FRT Δ*tus::*FRT *rpoB*35 btuB::*Tn*10 dam::*Tn*9* Δ*mutL::*Kan (phi80-free)

GJ18617 Δ*dam::*Kan

GJ18618 Δ*dam*::FRT Δ*mutS*::Kan

GJ18619 Δ*dnaA*::FRT *rpoB*35 btuB*::Tn*10* Δ*dam*::Kan

GJ18620 Δd*naA*::FRT *rnhA339*::Cm Δ*dinG::*Kan

GJ18621 Δ*dnaA::*FRT Δ*tus::*FRT *rpoB*35 btuB::*Tn*10* æ*recG::Kan* Δ*dinG::*Kan *att λ::P P_{ara}*-Nil (Amp^r)

GJ18622	Δ*dnaA::*FRT Δ*tus::*FRT *rpoB*35 btuB::*Tn*10* Δ*recG*::Kan Δ*dinG::*Kan *att λ:: P*$_{ara}$*-rnhA* (Amp^r)
GJ18623	Δ*dnaA::*FRT Δ*tus::*FRT *rpoB*35 btuB*::Tn*10* Δ*rnhA::*FRT *att λ:: P*$_{ara}$-UvsW (Amp^r)
GJ18624	Δ*dnaA::*FRT Δ*tus::*FRT *rpoB*35 btuB::*Tn*10 dam::*Tn*9* Δ*seqA*::FRT
GJ18625	Δ*dnaA::*FRT Δ*tus::*FRT *rpoB*35 btuB::*Tn*10 dam::*Tn*9 priA300*
GJ18626	Δ*dnaA::*FRT Δ*tus::*FRT *rpoB*35 btuB::*Tn*10 dam::*Tn*9* Δ*dinG::*Kan att *λ:: P*$_{ara}$-Nil (Amp^r)
GJ18627	Δ*dnaA::*FRT Δ*tus::*FRT *rpoB*35 btuB::*Tn*10 dam::*Tn*9* att *λ:: P*$_{ara}$-UvsW (Amp^r)
GJ18628	Δ*dnaA*::FRT *dam*::Tn*9* (phi80-free)

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[a] Strains DH5α (5), DL2006 (7), and GJ13519 (8) have been described earlier; all other strains were constructed in this study. All Δ*dnaA* strains were routinely maintained as derivatives with the *dnaA*$^+$ shelter plasmid pHYD2388 (or plasmid pHYD4805, in case of strains additionally mutant for *recG*).

[b] The Ter4::parS$_{pMT1}$ allele has been described in Galli et al. (9). GJ13519 and all of its derivatives listed above are phi80 lysogens, unless otherwise indicated.

Table S2. *Deep sequencing data statistics* [a]

Strain	Growth conditions	DNA isolation protocol [b]	Aggregate base read count (in million) after alignment to MG1655	Figure (panel)
GJ13519	LB 37 C log phase	A	424.713	S3A (i)
GJ13519	LB 37 C stationary phase	A	408.585	
GJ13519	LB with phleomycin 37 C log phase	A	1077.499	S3A (ii)
GJ18617	LB 37 C log phase	A	337.939	S3A (iii)
GJ13519	LB 37 C log phase	B	1034.302	4 (i)
GJ13519	LB 37 C stationary phase	B	1247.405	
GJ18617	LB 37 C log phase	B	1054.530	4 (ii); and S3A (iv)
GJ18618	LB 37 C log phase	B	936.746 (Replicate 1)	4 (iii)
GJ18618	LB 37 C log phase	B	922.045 (Replicate 2)	Nil
GJ16488	LB 37 C log phase	B	885.542	4 (iv)
GJ16494	LB 37 C log phase	B	738.344	4 (v)
GJ18619	LB 37 C log phase	B	739.962	4 (vi)

[a] Sequence data for all samples listed in the Table have been deposited in the NCBI database with Bioproject accession number PRJNA513325.

[b] A, with commercial kit; B, by phenol-chloroform extraction.

Table S3. *Comparisons between the different perturbations that confer cSDR* [a]

Attribute	***rnhA***	***recG***	***dam***	**Triple-exonuclease mutant**	***topA***
cSDR (biochemical evidence)	Yes	Yes	Yes	ND [b]	Yes
cSDR (DnaA-independent viability)	Yes	Yes	Yes	Yes	ND
"Mid-terminus peak" in *dnaA*$^+$	Yes	Yes	Yes	Yes	Yes
Loss of *oriC* peak with DnaA deficiency	Yes	Yes	Yes	ND	ND
*tus rpoB*35* requirement in DnaA-independent viability	Partial	Complete	Complete	Complete	ND
PriA helicase requirement in DnaA-independent viability	Partial	Complete	Complete	Complete	ND
RecA requirement in DnaA-independent viability	Partial	Complete	Lethal even in *dnaA*$^+$	Lethal even in *dnaA*$^+$	ND
DinG helicase requirement in DnaA-independent viability	Yes	Yes	Yes	ND	ND
Suppression of Δ*dnaA* viability by UvsW helicase expression	Yes	No	No	ND	ND
Contribution of DSEs to cSDR	No	? Yes [c]	Yes	ND	ND
Reactivity to S9.6 antibody (i.e., presence of R-loops)	Yes	No	No	ND	Yes

Table S3 (continued)

[a] Data for the different mutants are taken from the following sources: *rnhA* (10–12; this study); *recG* (10, 11, 13–15; this study); *dam* (this study); triple-exonuclease (13, 16); and *topA* (17, 18).

[b] ND, not determined.

[c] Azeroglu et al. (15) have proposed that DSEs contribute to reverse replication restart in the *recG* mutant.

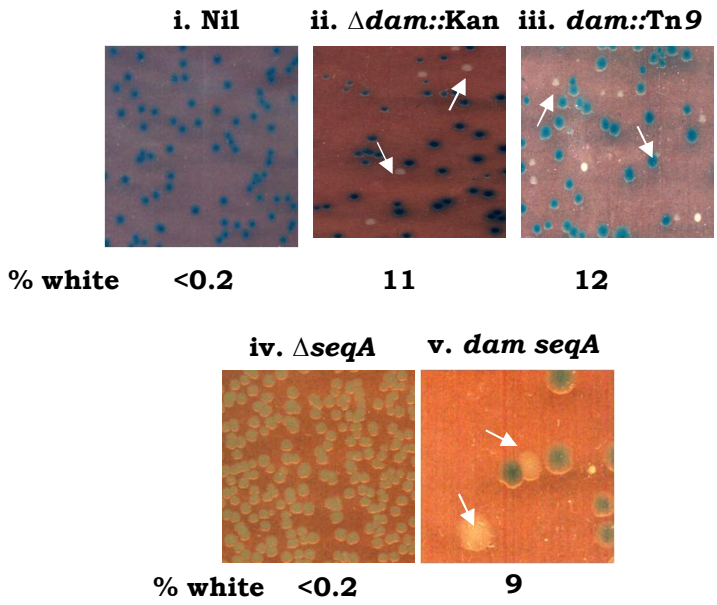

Figure S1: Additional features of rescue of Δ*dnaA* lethality by *dam* mutations. Isogenic derivatives of a Δ*dnaA tus rpoB*35* strain carrying *dnaA*$^+$ shelter plasmid pHYD2388 and the additional genetic perturbation(s) if any as indicated on the top of each panel were plated at 37° on minimal A-Casamino acids (panels i-iii) or LB (panels iv-v) medium, both supplemented with Xgal. Representative images are shown, and the percentage of white colonies to total for each panel is indicated as explained in the legend to Figure 2. Strains employed for the different panels were pHYD2388 derivatives of : i, GJ16459; ii, GJ16445; iii, GJ16488; iv, GJ16467and v, GJ18624.

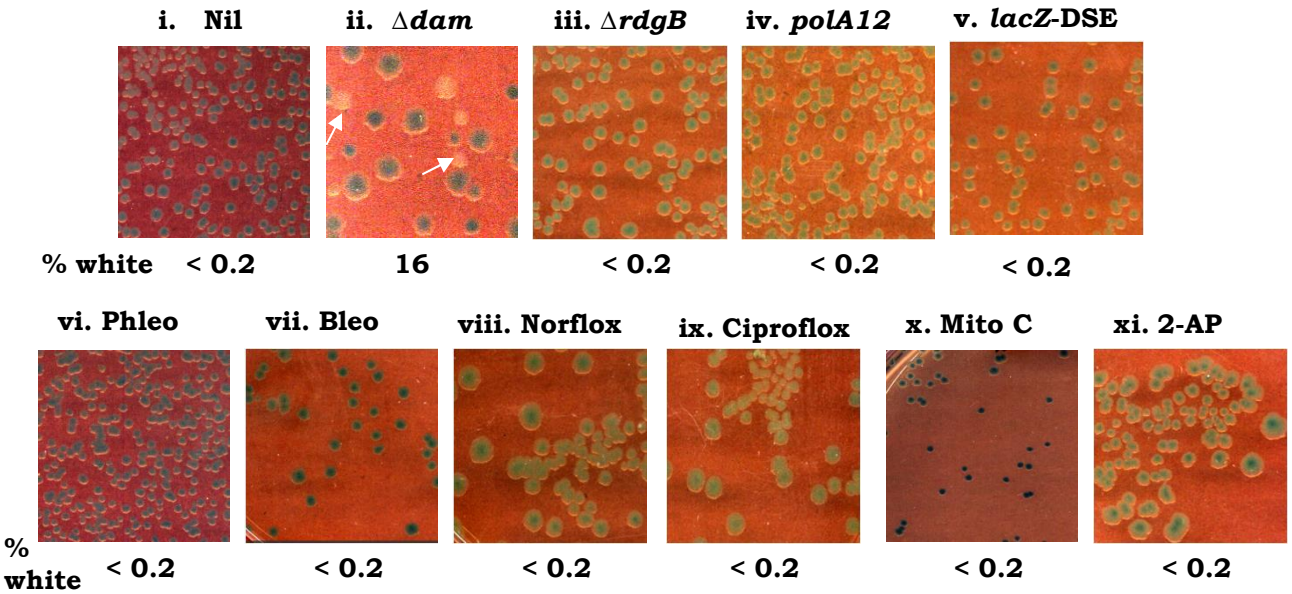

Figure S2: DSEs by themselves are unable to rescue Δ*dnaA* lethality. Derivatives of a Δ*dnaA tus rpoB*35* strain carrying *dnaA*$^+$ shelter plasmid pHYD2388 were plated on LB-Xgal medium at 37°. On top of each panel is shown the additional genetic perturbation if any in the strain (panels i-v) or the genotoxic agent added to the growth medium (panels vi-xi); *lacZ*-DSE, palindrome sequence in *lacZ* which is cleaved by SbcCD to generate DSE The medium was supplemented with Ara at 0.2% for panel v. Representative images are shown, and the percentage of white colonies to total for each panel is indicated as explained in the legend to Figure 2. Strains employed were pHYD2388 derivatives of (panel numbers in parentheses) : GJ16459 (i, and vi-xi); GJ16488 (ii); GJ16468 (iii); GJ16472 (iv); and GJ17465 (v). Genotoxic agents were added at the following concentrations (μg/ml): phleomycin (Phleo), 2; bleomycin (Bleo), 1; norfloxacin (Norflox), 0.02; ciprofloxacin (Ciproflox), 0.005; mitomycin C (Mito C), 1; and 2-aminopurine (2-AP), 20.

Figure S3

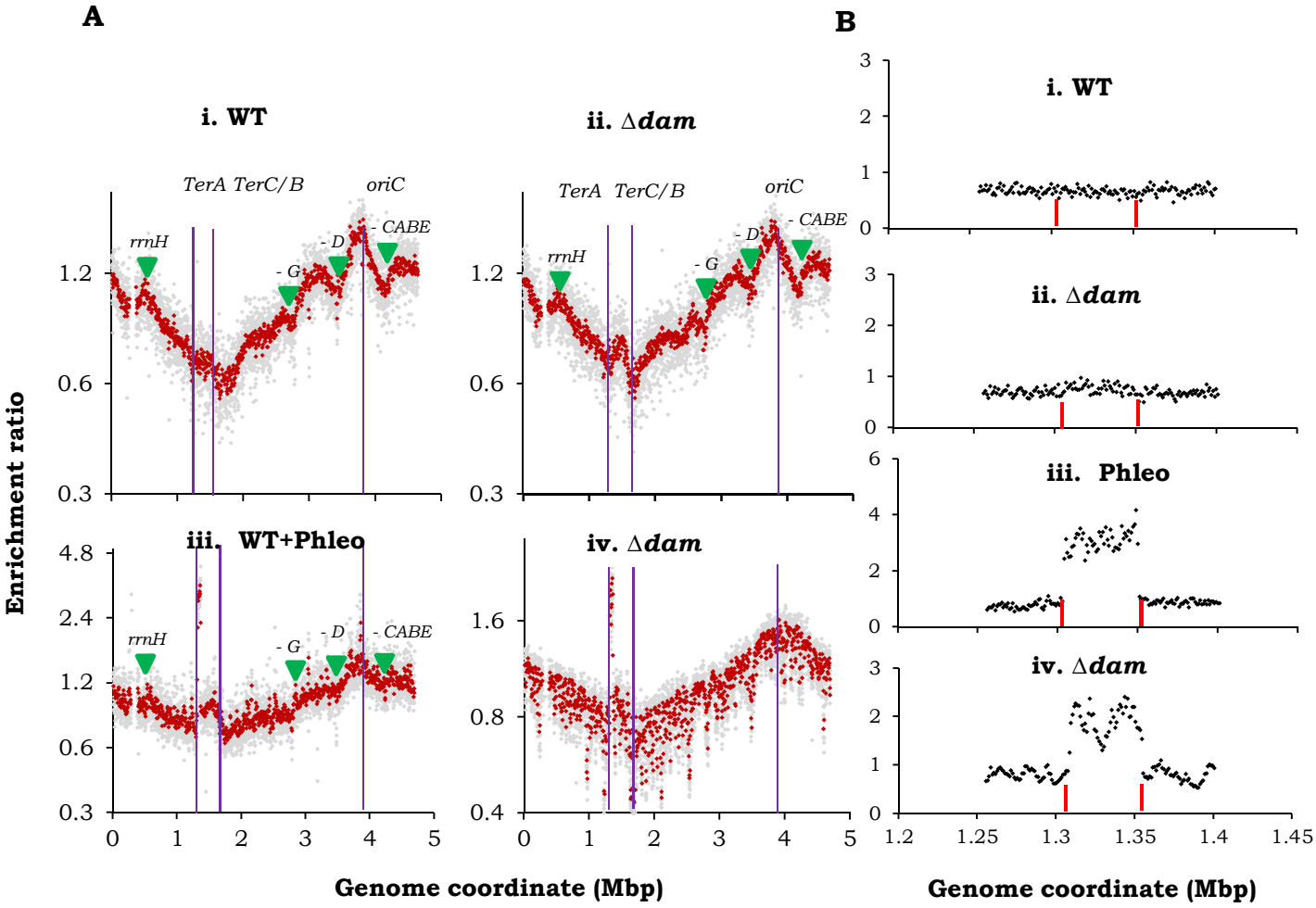

Figure S3: Alignment of deep sequencing reads to reference genome with integrated phi80 prophage. (**A**) Copy number distributions across the genome have been plotted on semi-log graphs as enrichment ratios, as described in the text and *Supplementary Text*, for each of three cultures grown to exponential phase in LB at 37°: i, wild-type (WT) strain GJ13519, ii, *dam* mutant GJ18617, and iii, WT strain with sublethal phleomycin (WT+Phleo) at 2 μg/ml. Genomic DNA preparations were made with the aid of the Invitrogen commercial kit, and the decreased recovery of DNA (19, 20) from the *rrn* operon regions (*rrnH, -G, -D, -CABE*) is indicated by the green arrowheads. In panel iv, copy number distributions in phenol-chloroform extracted genomic DNA preparation of Δ*dam* mutant GJ18617 is shown (that is, the same as that in Fig. 4, panel i, but there it has been aligned to MG1655 reference sequence without phi80 prophage). Positions of *oriC*, *TerA*, and *TerC/B* are marked. The gap at around 0.3 Mbp in each of the distribution plots corresponds to the *argF-lac* deletion present in the strains. (**B**) Close-up view of the genomic region with integrated phi80 prophage. Panel numbering is the same as that in **A**. The pair of red vertical bars demarcate the ends of the integrated phi80 prophage.

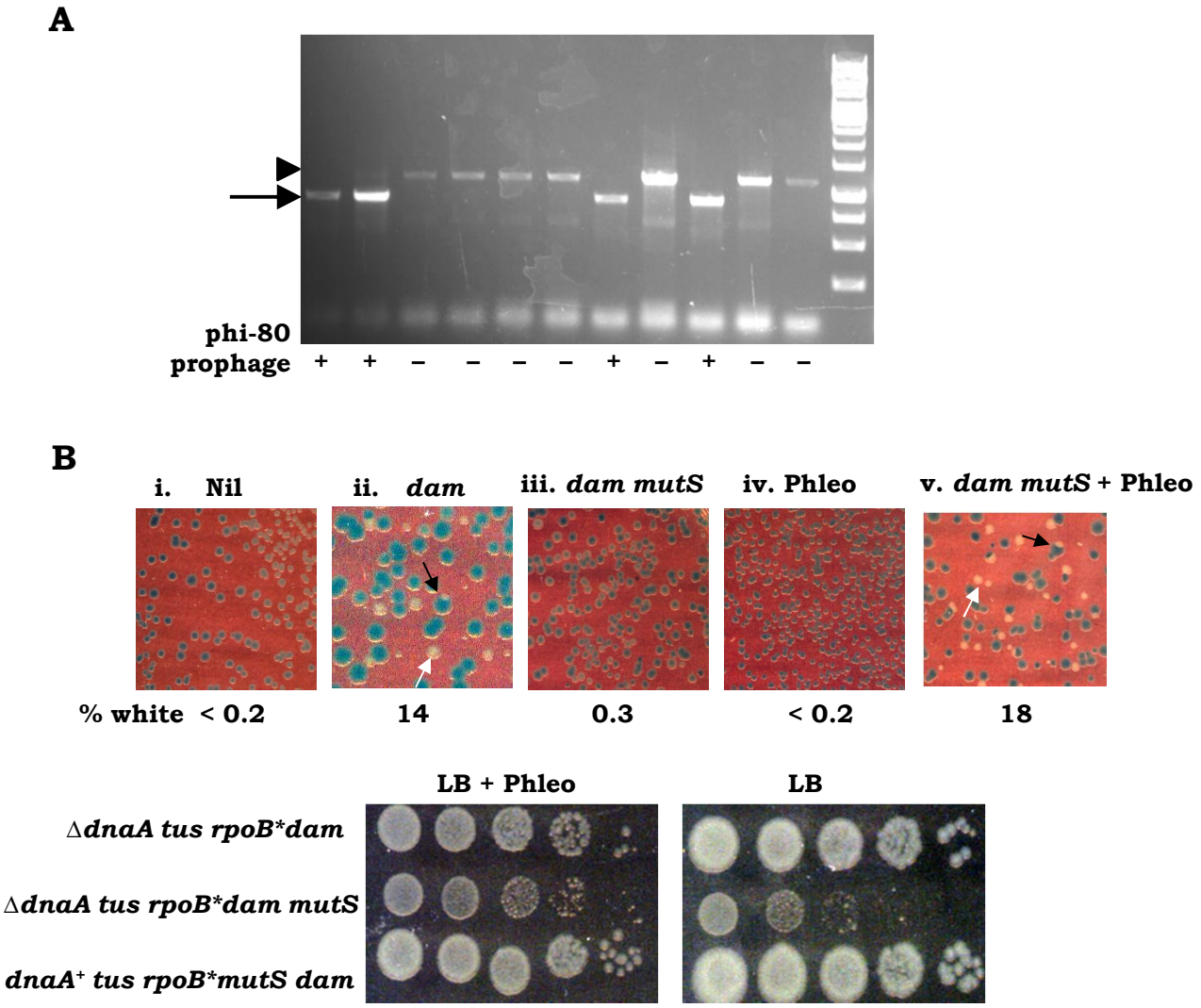

Figure S4: Testing for cSDR in the phi80 prophage-free derivatives. (**A**) Duplex PCR assay [with primers described earlier (9)] to distinguish between transductants that retain and those that are free of phi80 prophage. The primer pair praH1506 and praH1499 amplify the junction region at the site of prophage integration to yield an 875-bp amplicon in a phi80 lysogen, whereas the primer pair praH1506 and praH1521 produce a 1174-bp product in the prophage-free strain. The mutually exclusive occurrence of these amplicons (arrow and arrowhead, respectively) in the duplex PCR assay serves to differentiate the lysogens from non-lysogens, as marked beneath each of the lanes. DNA size markers are shown on lane at extreme right. **(B)** Isogenic derivatives of a phi80-free Δ*dnaA tus rpoB*35* strain carrying *dnaA*$^+$ shelter plasmid pHYD2388 and the additional genetic and/or genotoxic perturbations as indicated on top of each panel were plated on LB-Xgal plates and incubated for 40 hours at 37°; representative images are shown, and the percentage of white colonies to total for each panel is indicated as explained in the legend to Figure 2. Phleomycin (Phleo) supplementation was at 1 μg/ml. Strains employed for the different panels were pHYD2388 derivatives of: i and iv, GJ18607; ii, GJ18603; and iii and v, GJ18611. Examples of white colonies and white-sectored blue colonies are marked by the white and black arrows, respectively.