Supplemental Material for

Topoisomerase I Essentiality, DnaA-independent Chromosomal Replication, and Transcription-Replication Conflict in *Escherichia coli*

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Running Head: Topoisomerase I essentiality in *E. coli*

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Supplementary Methods

Construction of shelter plasmids pHYD2382 and pHYD2390. The pair of primers 5'-CCAAA*CTGCAG*TCGTGCTATAGCGCCTGT-3' and 5'-TTTGTT*AAGCTT*AACCTGACAGAATTAAAGG -3' (*PstI* and *HindIII* sites in them, respectively, italicized) were used with MG1655 genomic DNA to amplify the *topA*$^+$ gene by PCR, and the product was digested with *PstI* and *HindIII* before being cloned into the corresponding sites of plasmid vector pMU575, to generate the *topA*$^+$ shelter plasmid pHYD2382. The two primers are designated, respectively, as b1 and b2 in Supplementary Figure S7.

For constructing plasmid pHYD2390 (*topA*$^+$ *dnaA*$^+$ shelter plasmid), the DNA fragment present in plasmid pHYD2388 (1) carrying the *Salmonella enterica dnaA*$^+$ gene was sub-cloned *via* an intermediate plasmid vector into the *XbaI-KpnI* sites of plasmid pHYD2382.

Generation of *topA* mutations by recombineering. The Datsenko and Wanner method (2), with plasmids pKD13 and pKD46, was used for generating chromosomal *topA* mutations by recombineering in derivatives carrying the shelter plasmids pHYD2382 or pHYD2390. The primer pairs used for PCR with pKD13 template were: for Δ*topA*::Kan, 5'-TCAACGTGCGACGCATTCCTGGAAGAATCAAATTAGGTAAGGTGAATATGATTCCGGGGATCCGTCGACC-3' and 5'-TGTTTATAAAAACCTGACAGAATTAAAGGTTATTTTTTTCCTTCAACCCATGTAGGCTGGAGCTGCTTCG-3' (designated primer Y); for *topA*-Ins480::Kan, 5'-CCAGCACTTTACCAAGCCGCCAGCCCGTTTCAGTGAAGCAATTCCGGGGATCCGTCGACC-3' (designated primer X) and 5'-GACGACCGATACCGCGTTTTTCCAGCTCTTTAACCAGCGATGTAGGCTGGAGCTGCTTCG-3'; and for *topA*-480Δ, primers X and Y.

Given that the recombineering in these derivatives could have occurred on either the chromosome or the shelter plasmid, we employed simple genetic tests on several independent clones to classify them into the two categories. The three *topA*::FRT alleles on the chromosome were generated with the aid of plasmid pCP20, as described (2).

References

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Table S1. List of *E. coli* strains

Strain [a]	Genotype [b]
MG1655	*E. coli* K-12 wild-type
MDS42	MG1655 with 42 engineered deletions in genome
GJ12134	MDS42 æ(*argF-lac*)*U169*
GJ13519	MG1655 æ(*argF-lac*)*U169* [◂80 lysogen]
GJ13531 [c]	GJ13519 ¢*rho*::Kan *att* ə×::P_{tac} ï UvsW (Amp^R)
GJ15603 [d]	GJ13519 ¢*topA*::Kan
GJ15604 [d]	GJ13519 *topA*-Ins480::Kan
GJ15630 [d]	GJ13519 *dnaA177* ¢*dgoT*::FRT *rpoB*35 btuB*::Tn*10 topA*-Ins480::FRT ¢*tus*::FRT
GJ15688 [d]	GJ13519 *topA*-480¢::Kan
GJ15697 [d]	GJ15603 *att* ə×::P_{tac} ï UvsW (Amp^R)
GJ16475 [e]	GJ13519 *rpoB*35 btuB*::Tn*10* ¢*dnaA*:::FRT ¢*tus*::FRT ¢*rnhA*::FRT
GJ16703	GJ13519 *rpoB*35 btuB*::Tn*10*
GJ16813 [d]	GJ16703 ¢*topA*::Kan
GJ16814 [d]	GJ16703 *topA*-Ins480::Kan
GJ16815 [d]	GJ16703 *topA*-480¢::Kan
GJ16816 [d]	GJ12134 ¢*topA*::Kan
GJ16817 [d]	GJ12134 *topA*-Ins480::Kan
GJ16818 [d]	GJ12134 *topA*-480¢::Kan
GJ16819	GJ12134 *rpoB*35 btuB*::Tn*10*
GJ16820 [d]	GJ16819 ¢*topA*::Kan
GJ16821 [d]	GJ16819 *topA*-Ins480::Kan
GJ16822 [d]	GJ16819 *topA*-480¢::Kan
GJ16854 [d]	GJ16703 *topA*-Ins480::FRT
GJ16880 [d]	GJ16854 ¢*uvrD*::Kan
GJ16902 [d]	GJ12134 *rpoB*35 btuB*::Tn*10 dnaA177* ¢*dgoT*::FRT ¢*tus*::FRT ¢*topA*::FRT
GJ16903 [d]	GJ12134 *rpoB*35 btuB*::Tn*10 dnaA177* ¢*dgoT*::FRT ¢*tus*::FRT *topA*-Ins480::FRT
GJ16904 [d]	GJ12134 *rpoB*35 btuB*::Tn*10 dnaA177* ¢*dgoT*::FRT ¢*tus*::FRT *topA*-480¢::FRT
GJ16921 [d]	GJ13519 *topA*-Ins480::FRT
GJ17776 [d]	GJ16819 ¢*topA*::FRT
GJ17777 [d]	GJ16819 *topA*-Ins480::FRT
GJ17778 [d]	GJ16819 *topA*-480¢::FRT

GJ17783 [d]	GJ13519 Δ*tus*::FRT *rpoB*35 btuB*::Tn*10* Δ*topA*::FRT
GJ17784 [d]	GJ13519 Δ*tus*::FRT *rpoB*35 btuB*::Tn*10 topA*-Ins480::FRT
GJ17786 [d]	GJ17783 Δ*dnaA*::Kan
GJ17787 [d]	GJ17784 Δ*dnaA*::Kan
GJ17788 [d]	GJ13519 Δ*tus*::FRT *rpoB*35 btuB*::Tn*10 topA*-480Δ::FRT Δ*dnaA*::Kan
GJ17789 [d]	GJ13519 Δ*tus*::FRT *rpoB*35 btuB*::Tn*10* Δ*dnaA*::FRT
GJ17790 [d]	GJ17789 Δ*topA*::Kan
GJ17791 [d]	GJ17789 *topA*-Ins480::Kan
GJ17792 [d]	GJ17789 *topA*-480Δ::Kan
GJ18601	GJ13519 λ80 free
GJ18904 [d]	GJ17776 Δ*dnaA*::FRT
GJ18905 [d]	GJ17777 Δ*dnaA*::FRT
GJ18906 [d]	GJ17778 Δ*dnaA*::FRT
GJ18907 [d]	GJ18904 Δ*tus*::Kan
GJ18908 [d]	GJ18905 Δ*tus*::Kan
GJ18909 [d]	GJ18906 Δ*tus*::Kan
GJ18910	GJ18601 *rpoB*35 btuB*::Tn*10*
GJ18918 [d]	GJ18910 *topA*-Ins480::FRT
GJ18921 [d]	GJ18918 Δ*dinG*::Kan
GJ18940 [d]	GJ17783 Δ*dnaA*::FRT
GJ18941 [d]	GJ17784 Δ*dnaA*::FRT
GJ18942 [d]	GJ13519 Δ*tus*::FRT *rpoB*35 btuB*::Tn*10 topA*-480Δ::FRT Δ*dnaA*::FRT
GJ18945 [d]	GJ18918 Δ*tus*::Kan
GJ18951 [d]	GJ18941 Δ*rnhA*::Kan
GJ18952 [d]	GJ18941 Δ*dinG*::Kan
GJ18976 [d]	GJ12134 Δ*topA*::FRT
GJ18977 [d]	GJ12134 *topA*-Ins480::FRT
GJ18983 [d]	GJ18940 Δ*rnhA*::Kan
GJ18997 [d]	GJ13519 *dnaA177* Δ*dgoT*::FRT *rpoB*35 btuB*::Tn*10* Δ*tus*::FRT Δ*topA*::FRT
GJ18999 [d]	GJ13519 *dnaA177* Δ*dgoT*::FRT *rpoB*35 btuB*::Tn*10* Δ*tus*::FRT *topA*-480Δ::FRT
GJ19608 [d]	GJ17783 Δ*rnhA*::Kan
GJ19609 [d]	GJ17784 Δ*rnhA*::Kan

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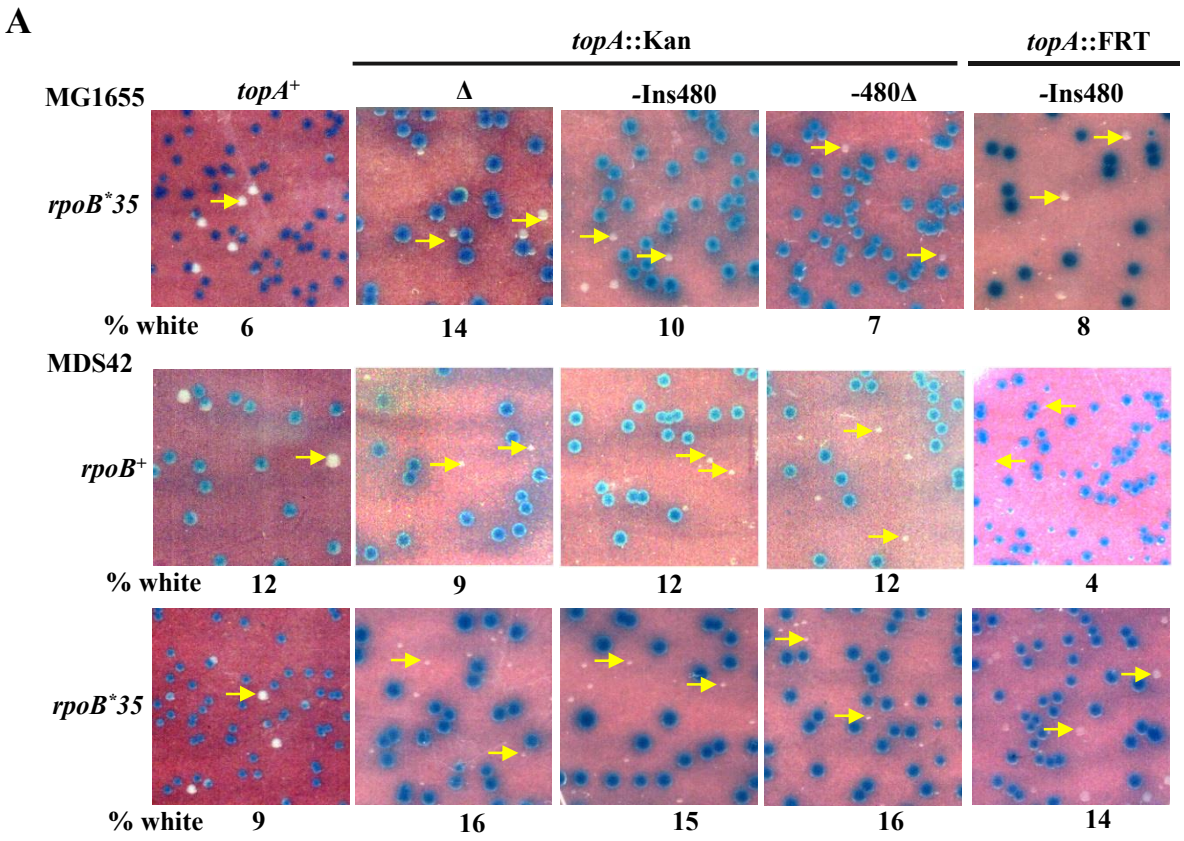
[a] Strain MG1655 was from our laboratory collection. Strains MDS42 (3), GJ12134 (4), GJ13519 (1), GJ13531 (5), and GJ16475 (1) have been described earlier; all other strains were constructed in this study.

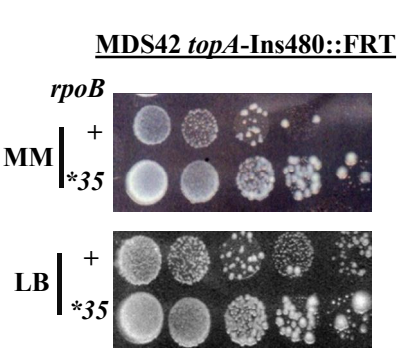
[b] The following alleles and constructs have been described earlier: Δ(*argF-lac)U169* (6); *dnaA177* (7); Keio deletions of *rnhA*, *tus*, *dinG*, *dgoT*, *uvrD*, and *tus* (8); *rpoB*35* and *btuB*::Tn*10* (1); and att λ::P_{tac}–UvsW(Amp^r) (4).

[c] Strain GJ13531 was routinely maintained as derivative with the shelter plasmid pHYD2411.

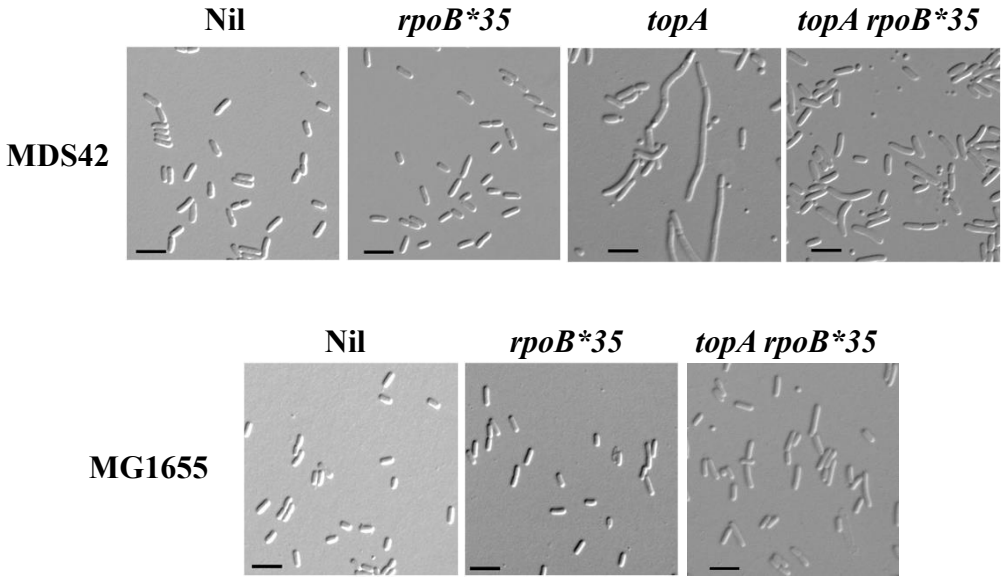
[d] The indicated strains were routinely maintained as derivatives with the shelter plasmid pHYD2390.

[e] Strain GJ16475 was routinely maintained as derivative with the shelter plasmid pHYD2388.



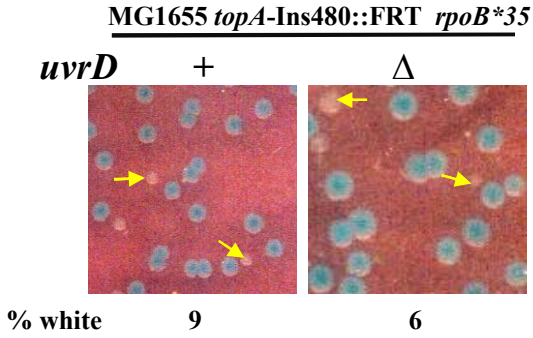


Supplementary Figure S1. (A) Blue-white screening assay on glucose-minimal A medium, of MG1655 or MDS42 strain derivatives with the *topA*$^{+}$ shelter plasmid pHYD2390 and the different *topA* alleles as indicated on the top of each column; the *rpoB* allele status is indicated at left of each row. Examples of white colonies are marked by the yellow arrows. From left to right, strains used for the panels were pHYD2390 derivatives of: row 1, GJ16703, GJ16813, GJ16814, GJ16815 and GJ16854; row 2, GJ12134, GJ16816, GJ16817, GJ16818 and GJ 18977; and row 3, GJ16819, GJ16820, GJ16821, GJ16822 and GJ17777. **(B)** Serial dilution-spotting of *rpoB*$^{+}$ and *rpoB*35* derivatives of MDS42 *topA*-Ins480::FRT (GJ18977 and GJ17777, respectively) on LB and MM.



Supplementary Figure S2. Cell morphology in derivatives of MDS42 and MG1655 (top and bottom rows, respectively), visualized by differential interference contrast microscopy. Scale bar (at bottom left of each panel), 5 μm. Relevant mutations in the strains are indicated on top of the corresponding panels. The *topA* allele in MDS42 derivatives was Δ*topA*::Kan and that in the MG1655 derivative was *topA*-Ins480::Kan. Strains used were (from left to right): top row, GJ12134, GJ16819, GJ16816, and GJ16820; and bottom row, GJ13519, GJ16703, and GJ16814.

A



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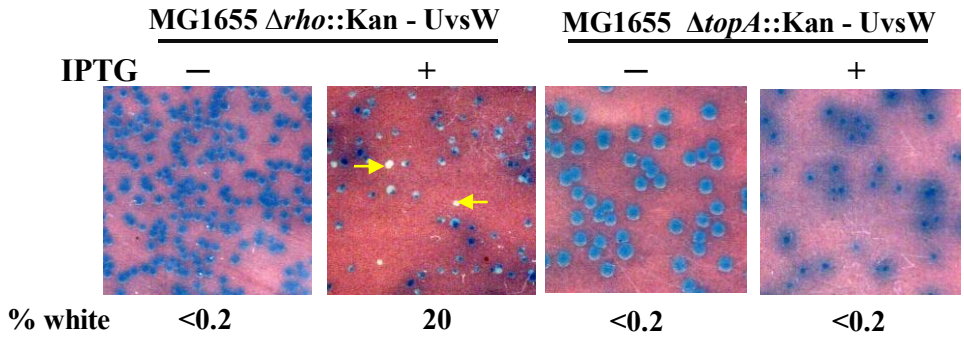
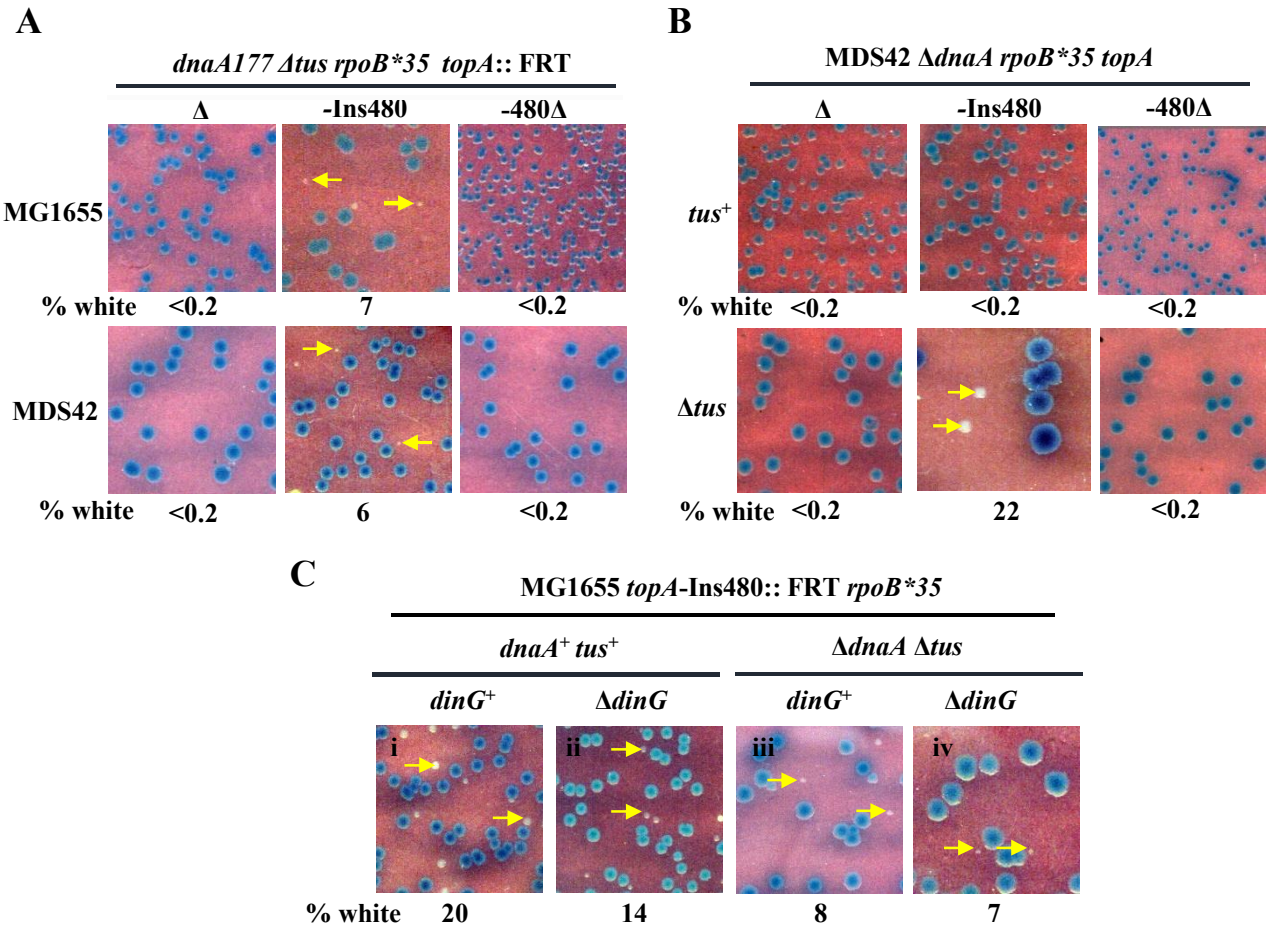
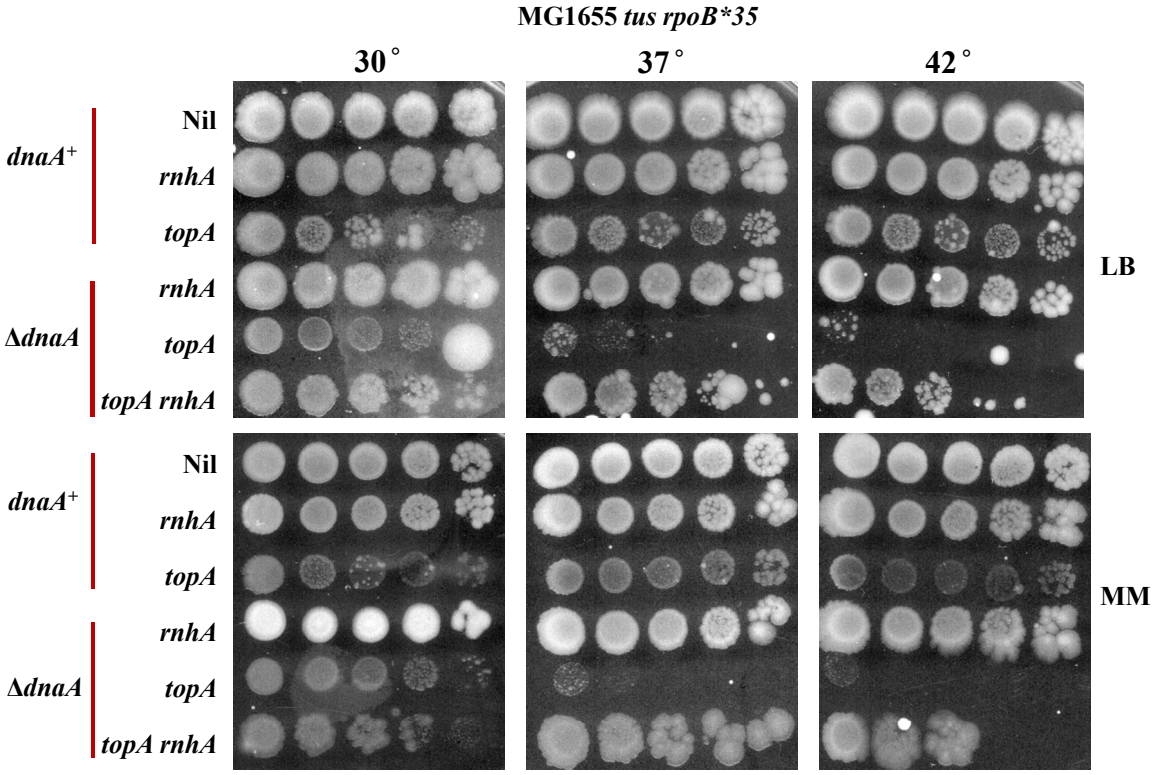


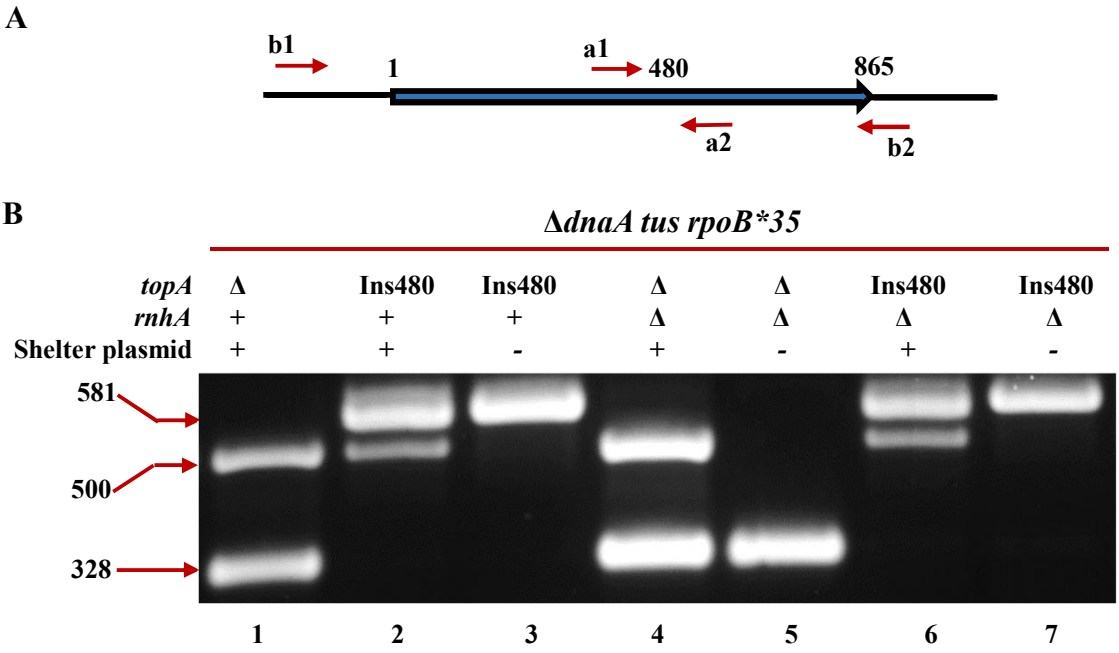
Supplementary Figure S3. Blue-white screening assays with MG1655 derivatives (carrying cognate shelter plasmids) on glucose-minimal A medium to demonstrate that *topA* lethality rescue by *rpoB*35* is UvrD-independent **(A)**, and that UvsW expression rescues Δ*rho* lethality but not Δ*topA* lethality **(B)**. Relevant genotypes are indicated on top of each of the panels. Examples of white colonies are marked by the yellow arrows. UvsW expression from a chromosomal P_{tac}-UvsW construct was regulated by the omission (–) or addition (+) of IPTG (latter at 100 µM and 40 µM, respectively, for the *rho* and *topA* mutants). Strains used were: **(A)** pHYD2390 derivatives of GJ16854 (*uvrD*$^+$) and GJ16880 (Δ*uvrD*); and **(B)** pHYD2411 derivative of GJ13531 (Δ*rho*) and pHYD2390 derivative of GJ15697 (Δ*topA*).



Supplementary Figure S4. Features of *dnaA* lethality rescue by *topA* alleles. **(A-C)** Blue-white screening assays were performed at 30° on glucose-minimal A with *topA*$^+$ *dnaA*$^+$ shelter plasmid pHYD2390 in strain derivatives of MG1655 or MDS42 whose relevant genotypes are indicated on top of each of the panels. Examples of white colonies are marked by the yellow arrows. From left to right, strains employed were pHYD2390 derivatives of: **A** top row, GJ18997, GJ15630 and GJ18999; **A** bottom row, GJ16902, GJ16903 and GJ16904; **B** top row, GJ18904, GJ18905 and GJ18906; **B** bottom row, GJ18907, GJ18908 and GJ18909; and **C**, GJ18918, GJ18921, GJ18941 and GJ18952.



Supplementary Figure S5. Serial dilution-spotting on LB and glucose-minimal A (MM) at indicated temperatures of derivatives of MG1655 Δ*tus rpoB*35* with additional relevant alleles as indicated at left. The Δ*dnaA*, *topA* and *rnhA* alleles were, respectively, Δ*dnaA*::FRT, *topA*-Ins480::FRT, and Δ*rnhA*::Kan. The six strains used were (from top): GJ17784/pHYD2390; GJ16475/pHYD2388; GJ17784; GJ16475; GJ18941; and GJ18951.



Supplementary Figure S6. PCR validation of chromosomal *topA* genotype in Δ*dnaA* derivatives without or with *rnhA* mutation. **(A)** Schematic depiction of *topA* ORF (865 codons); positions of PCR primer pairs a1-a2 (5'-TGTACCAGTTAATCTGGCGTCAG-3' and 5'-TCGTGATTTGCCACCTGGTCGAG-3', respectively) flanking the codon 480 region, and b1-b2 (sequences given in Supplementary Methods above) for the entire ORF, are marked. The former primer pair is expected to yield amplicons of size 500 bp and 581 bp from *topA*$^+$ and *topA*-Ins480::FRT, respectively, and no amplicon from Δ*topA*::FRT. The latter primer pair is expected to yield amplicons of size 2.8 kb and 328 bp from *topA*$^+$ and Δ*topA*::FRT, respectively; however, the conditions used for PCR amplification were such as not to yield the large-size amplicons such as of 2.8 kb length. **(B)** Amplicons detected by gel electrophoresis after PCR with both primer pairs together, on DNA preparations from strains of genotype indicated on top of each lane. The *topA*$^+$ *dnaA*$^+$ shelter plasmid was pHYD2390. Allele designations used for *topA*: Δ, Δ*topA*::FRT and 480, *topA*-Ins480::FRT; and for *rnhA*: Δ, Δ*rnhA*::Kan. Migration positions of fragments of length 328, 500 and 581 bp are marked. Strains employed for the different lanes were: 1, GJ18940/pHYD2390; 2, GJ18941/pHYD2390; 3, GJ18941; 4, GJ18983/pHYD2390; 5, GJ18983; 6, GJ18951/pHYD2390; and 7, GJ18951.