Supplementary Materials: RnlB Antitoxin of the *Escherichia coli* RnlA-RnlB Toxin–Antitoxin Module Requires RNase HI for Inhibition of RnlA Toxin Activity

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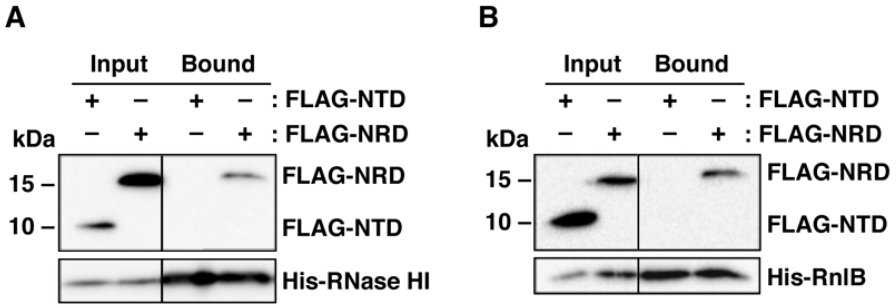

Figure S1. Interaction of NRD with RNase HI or RnlB independently of RNA. (**A**) Δ*rnlAB* Δ*rnhA* cells harboring pQE80L-*rnhA* plus either pBAD33-Flag-NTD or pBAD33-Flag-NRD; or (**B**) Δ*rnlAB* cells harboring pMK19 plus either pBAD33-Flag-NTD or pBAD33-Flag-NRD, were grown in LB medium until the OD_{600} reached 0.4, and treated with 0.06 mM IPTG and 0.05% arabinose for 60 min. Cell extracts were treated with 10 μg/mL of RNase A for 20 min on ice prior to the pull-down analysis. Input and bound fractions were analyzed by western blot with antibodies against FLAG-tag (upper panel) and His-tag (lower panel). The interaction of NRD with RNase HI or RnlB is independent of substrate RNA.

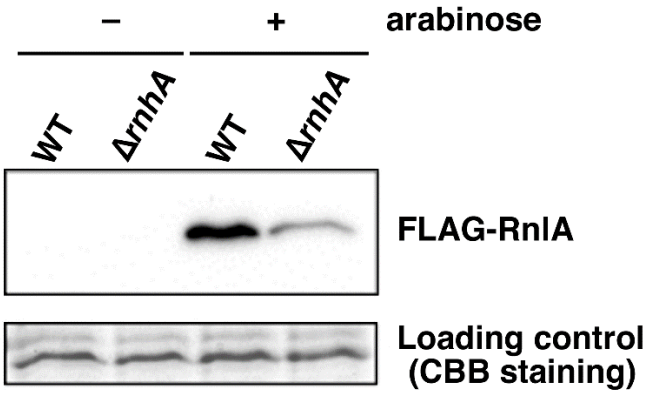

Figure S2. Amount of FLAG-RnlA expressed from a plasmid. Wild-type (WT) or Δ*rnhA* cells harboring pBAD33-Flag-*rnlA* were grown in LB medium until mid-log phase and then treated with (+) or without (−) 0.2% arabinose. Cell extracts were analyzed by western blot with the antibody against FLAG-tag (upper panel) and Coomassie Brilliant Blue staining as a loading control (lower panel). FLAG-RnlA is expressed in both wild-type and Δ*rnhA* cells.

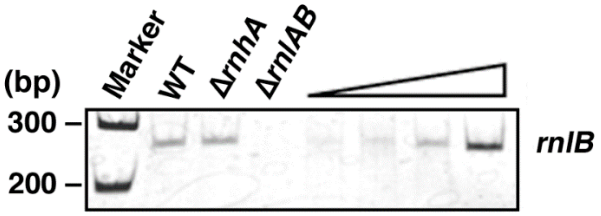

Figure S3. Amount of endogenous *rnlB* transcript. Wild-type (WT), Δ*rnhA* or Δ*rnlAB* cells were grown in LB medium until mid-log phase, and then total RNAs were extracted. RT-PCR analysis for the *rnlB* transcript was performed using the primers, KN-39 (5′-CGGGATCCATGTTTGAAATCACCGG) and KN-28 (5′-GTATCCAGCATGATCCGGCC), as described in [1]. Various amounts (10, 20, 40 and 80 pg) of pBSNO carrying the sequence from *rnlA* to *rnlB* were used as a template to demonstrate a semi-quantitative profile of PCR conditions. RNase HI has no effect on the expression of *rnlB* mRNA.

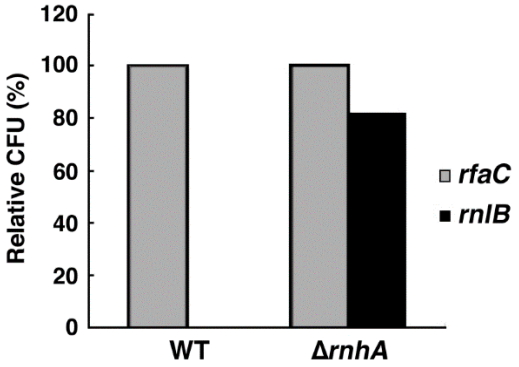

Figure S4. Effect of *rnhA* on the disruption of *rnlB*. Disruption of *rnlB* or a nonessential gene *rfaC* encoding ADP-heptose was performed as described [2]. Briefly, the DNA fragments containing a chloramphenicol acetyltransferase (CAT) cassette flanked with the sequences within *rnlB* or *rfaC* were amplified by PCR with pKD3 as the template using the primers; for *rnlB*, KN-35 (5′-GAGCGTGGGGAATAATCAAGGA CTTATATATTGTTTGAAATCACCGGAATGTGTAGGCTGGAGCTGCTTC) and KN-36 (5′-AAGTTAA TATCATGCCAAAAGGGCGAATTCTATACTGGTTCGTTTAGAAAATGGGAATTAGCCATGGTCC); for *rfaC*, rfaC-Dup (TACTGGAAGAACTCAACGCGCTATTGTTACAAGAGGAAGCCTGACGGATG GTGTAGGCTGGAGCTGCTTC) and rfaC-Ddw (AAGTTTAAAGGATGTTAGCATGTTTTACCTTTAT AATGATGATAACTTTTATGGGAATTAGCCATGGTCC). Each 500 ng of the amplified fragment was introduced into TY0807 or TY0826 cells harboring pKD46, which encodes the λ phage Red recombinase, then cells were spread on LB plates supplemented with chloramphenicol and incubated at 30 °C for 20 h to count colony forming unit (cfu). The number of cfu for disruption of *rfaC* was set to 100% in each recipient. The disruption of *rnlB* in the genome of Δ*rnhA* cells is successful.

References

1. Otsuka, Y.; Miki, K.; Koga, M.; Katayama, N.; Morimoto, W.; Takahashi, Y.; Yonesaki, T. IscR regulates RNase LS activity by repressing *rnlA* transcription. *Genetics* **2010**,*185*, 823–830.
2. Datsenko, K.A.; Wanner, B.L. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 6640–6645.