SUPPLEMENTARY MATERIALS AND METHODS

***E. coli* strains, plasmids, and growth conditions**. *Escherichia coli* strain P90C (1) Δ*dinB*::kan (lab stock) derivative was used as wild-type. MG1655 Δ*alkA tag dinB* (2) is the base excision repair-deficient strain (gracious gift of Ivan Matic, Université Paris Descartes). Plasmids used in this study include: pVector (pWKS30, (3)), pEc-*dinB*^{native} (pYG768, (4)), pEc-*dinB*^{lac} (pYG782, (4)); pAb-*dinB*^{native} and pAb-*dinB*^{lac} were constructed for this work. *E. coli* strains were routinely grown in Luria broth (LB) and supplemented with 200 μg/mL ampicillin (Ap; Sigma) for plasmid maintenance.

Construction of pAb-*dinB*^{lac}. *Acinetobacter baumannii dinB* (gene locus A1S_0186) from strain ATCC 17978 was amplified by PCR using the oligonucleotides 5'-ATG CGC AAA ATC ATT CAT ATC G-3' and 5'-TTA CCA TAA GGA CAA CTG AAA GTC G-3' with Platinum *Taq* DNA polymerase High Fidelity (Life Technologies). The amplification product was purified and ligated into pGEM cloning vector (Promega). The *Pst*I and *Sac*II Ab-*dinB* fragment was subcloned into the low copy number plasmid pWKS30 under the *lac* promoter. The resulting pAb-*dinB*^{lac} plasmid was sequenced with M13 forward and reverse oligonucleotides (Tufts Core Facility).

Construction of pEc-*dinB*^{native}. Site-directed mutagenesis was performed on plasmid pYG768 (contains *E. coli dinB* under its native promoter; (4)) using the Gene-Tailor kit (Life Technologies), according to manufacturer's instructions. Using oligonucleotides 5'-ACC AGT GTT GAG AGG TGA GCT AGC AAT GCG TAA AAT CAT TC-3' and 5'-GCT CA CCT CTC AAC ACT GGT AAA GTA TAC AGT GAT TTC AGG-3', a *Nhe*I restriction site was

inserted between the starting *E. coli dinB* methionine codon and the native promoter region. Resulting plasmid was confirmed by sequencing (Tufts Core Facility) using oligonucleotides 5'-GGG ATA ATT GGC GGT GCT GAT CAC-3' and 5'-CCG GCG CAT TGAG ATT ATG GTG C-3'. The *Nhe*I restriction site was added so that the *A. baumannii dinB* gene could be inserted into the plasmid directly downstream of the *E. coli dinB* promoter.

Construction of pAb-*dinB*^{native}. *A. baumannii dinB* was amplified by PCR with oligonucleotides that introduced restriction site *Nhe*I on the 5' end and *Hind*III on the 3' end of the gene (5' GGG GGC TAG CAA TGC GCA AAA TCA TTC ATA TCG-3', 5'-CTG CAA GCT TTT ACC ATA AGG ACA ACT GAA AGT CG-3'). The amplification product was cloned into the *Nhe*I and *Hind*III sites of pEc-*dinB*^{native}, resulting in Ab-*dinB* directly downstream of the native *E. coli dinB* promoter. The newly constructed plasmid was sequenced (Tufts Core Facility) with 5'-CCG GCG CAT TGA GAT TAT GGT GC-3', 5'-TAA TAC GAC TCA CTA TAG GG-3', 5'-CTC ATG GAC ATG GCA GAG CG-3', and 5'-GCA ACT GAA TGC CCG AGG TG-3'.

***E. coli* Survival Assays and DNA damage treatments**. For survival assays, three independent *E. coli* cultures were grown to saturation. Cultures were serially diluted in SMO and 10 µL spots were deposited on LB-Ap agar with methyl methanesulfonate (MMS; Acros Organics), ethyl methanesulfonate (EMS; Acros Organics), or nitrofurazone (NFZ; Sigma) at the concentrations specified in figure legends. NFZ plates were incubated in the dark for 20 hours and MMS plates were incubated for 20-40 hours depending on the strain and concentration.

Percent survival was determined by calculating the fraction of colony forming units (CFUs) grown with the DNA-damaging agent per total number of CFUs grown on LB.

1. **Cairns, J., and P. L. Foster.** 1991. Adaptive reversion of a frameshift mutation in Escherichia coli. Genetics **128:**695-701.
2. **Bjedov, I., C. N. Dasgupta, D. Slade, S. Le Blastier, M. Selva, and I. Matic.** 2007. Involvement of Escherichia coli DNA polymerase IV in tolerance of cytotoxic alkylating DNA lesions in vivo. Genetics **176:**1431-1440.
3. **Wang, R. F., and S. R. Kushner.** 1991. Construction of versatile low-copy-number vectors for cloning, sequencing and gene expression in Escherichia coli. Gene **100:**195-199.
4. **Kim, S. R., G. Maenhaut-Michel, M. Yamada, Y. Yamamoto, K. Matsui, T. Sofuni, T. Nohmi, and H. Ohmori.** 1997. Multiple pathways for SOS-induced mutagenesis in Escherichia coli: an overexpression of dinB/dinP results in strongly enhancing mutagenesis in the absence of any exogenous treatment to damage DNA. Proc Natl Acad Sci U S A **94:**13792-13797.

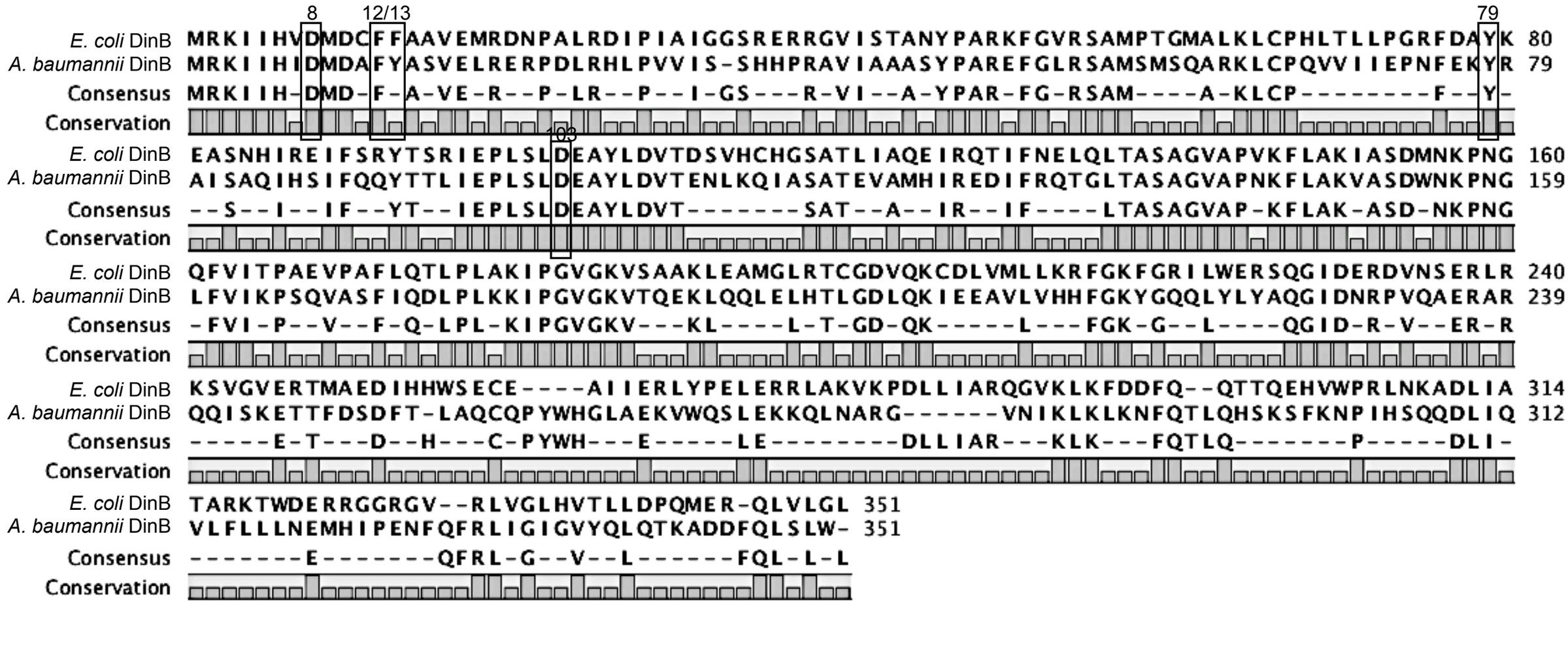


Figure S1. *A. baumannii* DinB shares sequence similarity to *E. coli* DinB. An alignment of *E. coli* DinB and a DinB consensus sequence from 21 strains of *A. baumannii*. Known *E. coli* catalytic residues D8, F12, F13, Y79, and D103 are highlighted in boxes. Bar graph represents conservation with full bars as 100%. Dashes in overall consensus sequence represent ambiguity. Alignment was generated using the CLC Main Workbench (CLC Bio).

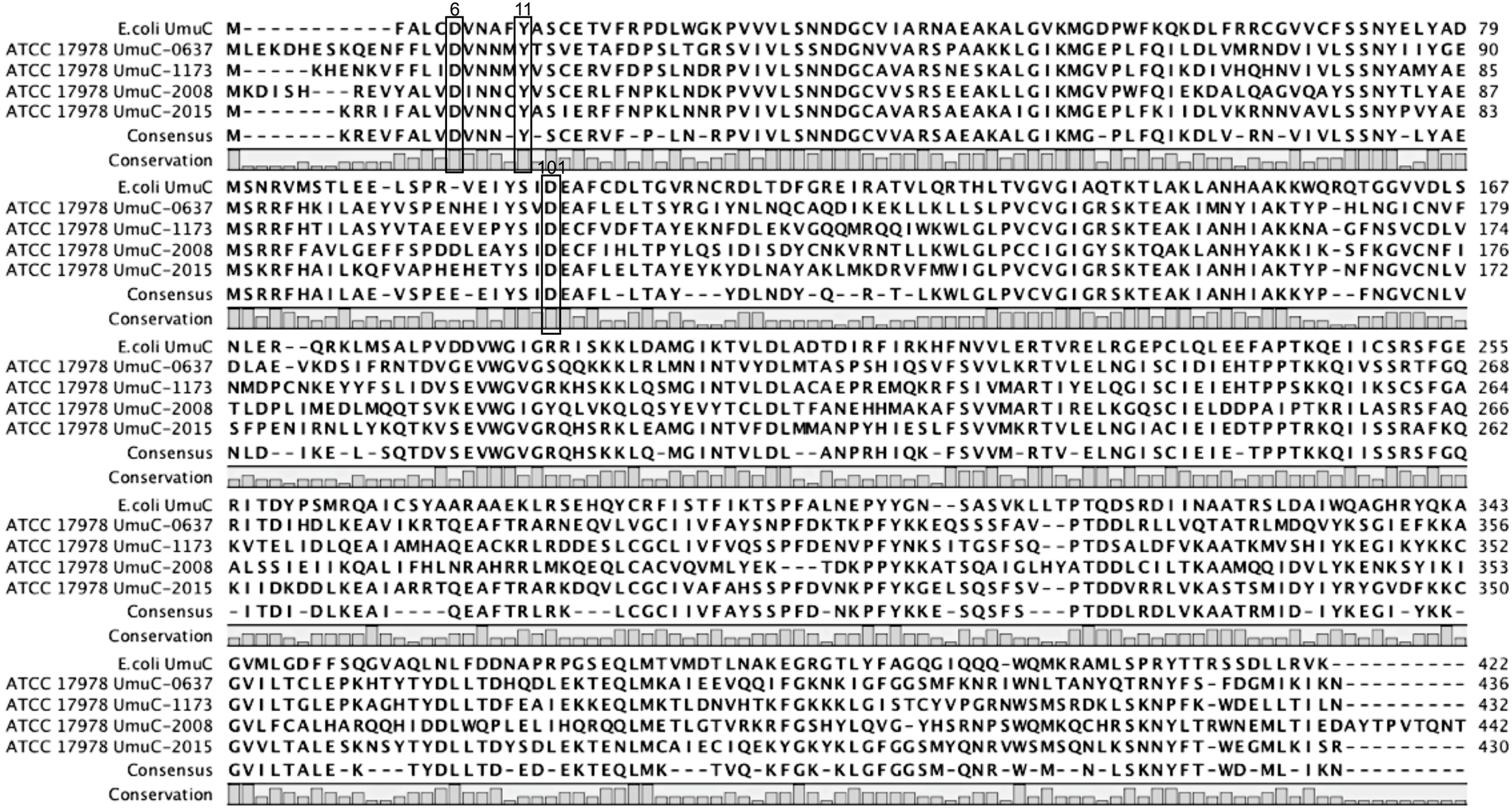
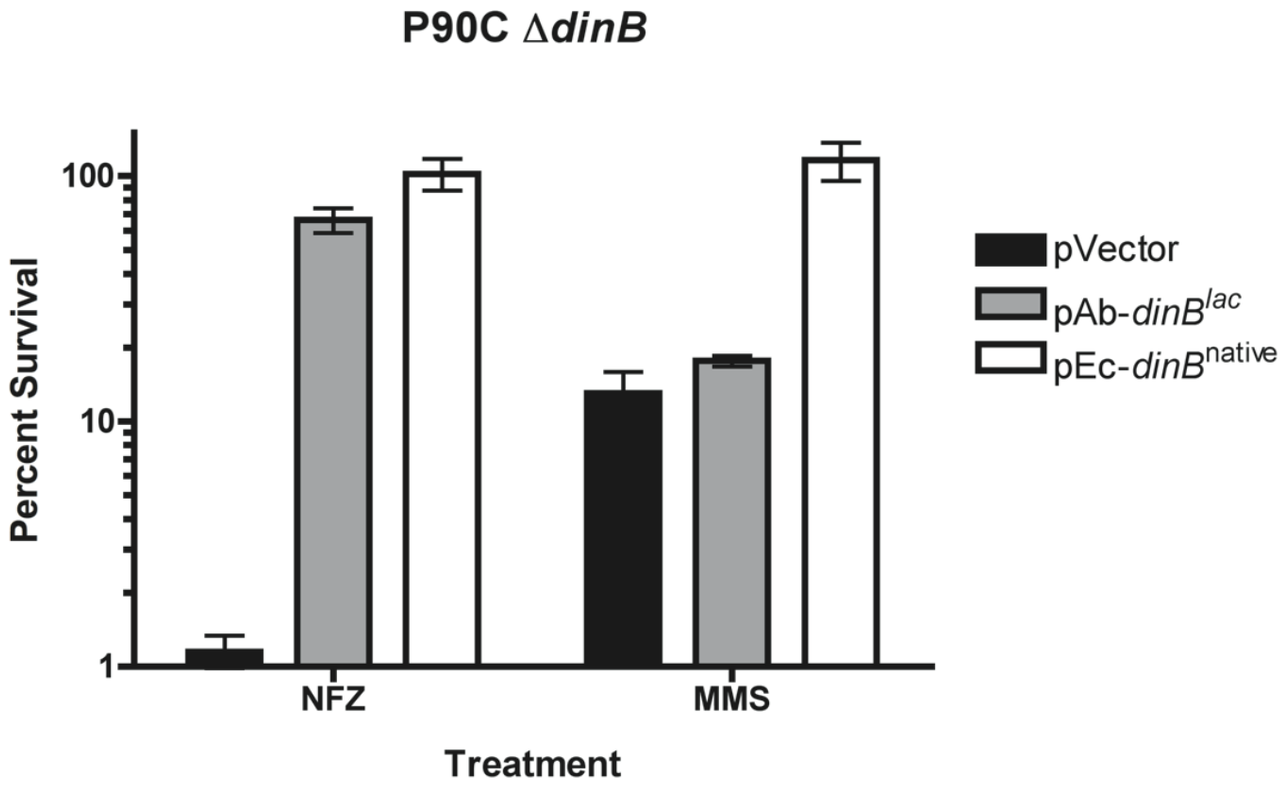


Figure S2. Predicted UmuC proteins from *A. baumannii* 17978 are similar to *E. coli* UmuC. Full alignment of 17978 UmuC sequences with *E. coli* UmuC. Conserved catalytic residues are highlighted in boxes. Bar graph represents conservation with full bars as 100%. Dashes in overall consensus sequence represent ambiguity. E values are all less than or equal to $7x10^{-82}$. Alignment was generated using the CLC Main Workbench (CLC Bio).

A



B

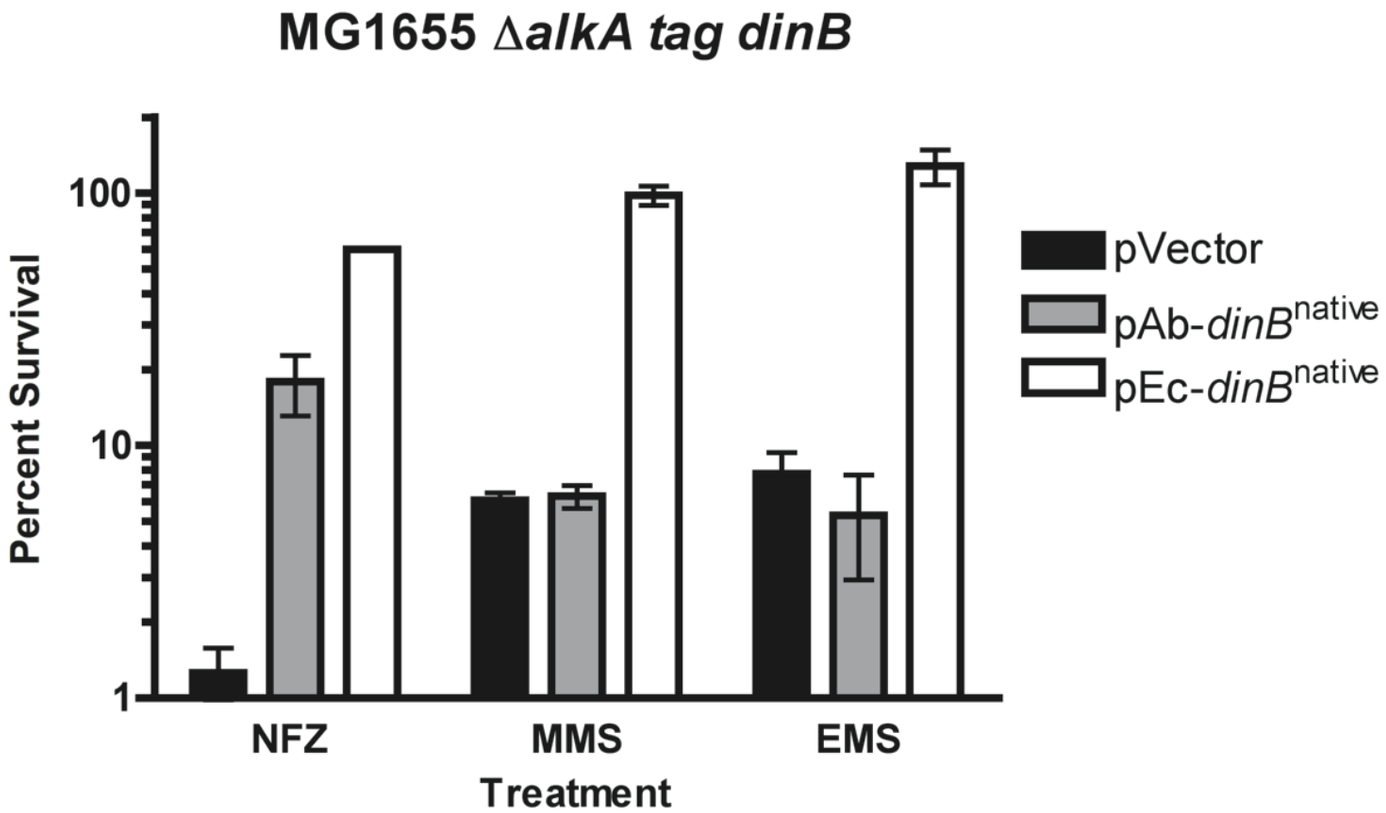


Figure S3. Plasmid-borne *A. baumannii dinB* complements certain phenotypes of *dinB*-deficient *E. coli*. (**A**) Wild-type P90C Δ*dinB* cells bearing *A. baumannii* 17978 *dinB* on a plasmid (pAb-*dinB*^{lac}) are rescued as well as those with *E. coli dinB* (pEc-*dinB*) upon nitrofurazone (NFZ) treatment. There is no rescue of Δ*dinB* strains upon methyl methanesulfonate (MMS) treatment. Ab-*dinB* expression is driven by the *lac* promoter and Ec-*dinB* expression is driven by its native promoter. Percent survival was determined by calculating the fraction of colony forming units (CFUs) that grew on LB medium supplemented with NFZ (7.5 μM) or MMS (7.5 mM) per total number of untreated CFUs. (**B**) Similar results are found using MG1655 Δ*alkA tag dinB*, an *E. coli* strain deficient in base-excision repair, using pAb-*dinB*^{native} (expression driven by the *E. coli dinB* native promoter) and pAb-*dinB*^{lac} (not shown). In addition to MMS, there is no rescue of strains upon ethyl methanesulfonate (EMS) treatment. P90C Δ*dinB* cells were not sensitive to EMS. Percent survival was calculated as described in (A) using NFZ (5 μM), MMS (0.08 mM), or EMS (3.4 mM). Error bars represent the standard deviation of the mean from 3 independent experiments for both graphs.