SUPPLEMENTARY MATERIALS AND METHODS

E. coli strains, plasmids, and growth conditions. *Escherichia coli* strain P90C (1) $\Delta dinB$::kan (lab stock) derivative was used as wild-type. MG1655 $\Delta alkA$ 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).</sup>

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.

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	<u>8</u> 1 <u>2/13</u> <u>79</u>
<i>E. coli</i> DinB	MRKIIHVDMDCFFAAVEMRDNPALRDIPIAIGGSRERRGVISTANYPARKFGVRSAMPTGMALKLCPHLTLLPGRFDAYK 80
<i>A. baumannii</i> DinB	MRKIIHIDMDAFYASVELRERPDLRHLPVVIS-SHHPRAVIAAASYPAREFGLRSAMSMSQARKLCPQVVIIEPNFEKYR 79
Consensus	MRKIIH-DMD-F-A-VE-RP-LRP-I-GSR-VIA-YPAR-FG-RSAMA-KLCPFY-
Conservation	
<i>E. coli</i> DinB	EASNHIREIFSRYTSRIEPLSLDEAYLDVTDSVHCHGSATLIAQEIRQTIFNELQLTASAGVAPVKFLAKIASDMNKPNG 160
<i>A. baumannii</i> DinB	AISAQIHSIFQQYTTLIEPLSLDEAYLDVTENLKQIASATEVAMHIREDIFRQTGLTASAGVAPNKFLAKVASDWNKPNG 159
Consensus	SIIFYTIEPLSLDEAYLDVTSATAIRIFLTASAGVAP-KFLAK-ASD-NKPNG
Conservation	
<i>E. coli</i> DinB	QFVITPAEVPAFLQTLPLAKIPGVGKVSAAKLEAMGLRTCGDVQKCDLVMLLKRFGKFGRILWERSQGIDERDVNSERLR 240
<i>A. baumannii</i> DinB	LFVIKPSQVASFIQDLPLKKIPGVGKVTQEKLQQLELHTLGDLQKIEEAVLVHHFGKYGQQLYLYAQGIDNRPVQAERAR 239
Consensus	-FVI-PVF-Q-LPL-KIPGVGKVKLL-T-GD-QKLFGK-GLQGID-R-VER-R
Conservation	
<i>E. coli</i> DinB	KSVGVERTMAEDIHHWSECEAIIERLYPELERRLAKVKPDLLIARQGVKLKFDDFQQTTQEHVWPRLNKADLIA 314
<i>A. baumannii</i> DinB	QQISKETTFDSDFT-LAQCQPYWHGLAEKVWQSLEKKQLNARGVNIKLKLKNFQTLQHSKSFKNPIHSQQDLIQ 312
Consensus	E-TDHC-PYWHELEDLLIARKLKFQTLQPDLI-
Conservation	
<i>E. coli</i> DinB	TARKTWDERRGGRGVRLVGLHVTLLDPQMER-QLVLGL 351
<i>A. baumannii</i> DinB	VLFLLLNEMHIPENFQFRLIGIGVYQLQTKADDFQLSLW- 351
Consensus	EQFRL-GVLFQL-L-L
Conservation	

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).

	$\underline{6}$ <u>11</u>	
E.coli UmuC	MFALQDVNAFYASCETVFRPDLWGKPVVVLSNNDGCVIARNAEAKALGVKMGDPWFKQKDLFRRCGVVCFSSNYELYAD	79
ATCC 17978 UmuC-0637	MLEKDHESKQENFFLVDVNNMYTSVETAFDPSLTGRSVIVLSSNDGNVVARSPAAKKLGIKMGEPLFQILDLVMRNDVIVLSSNYIIYGE	90
ATCC 17978 UmuC-1173	M – – – – – KHENKVFFLIDVNNMYVSCERVFDPSLNDRPVIVLSNNDGCAVARSNESKALGIKMGVPLFQIKDIVHQHNVIVLSSNYAMYAE	85
ATCC 17978 UmuC-2008	MKDISHREVYALVDINNCYVSCERLFNPKLNDKPVVVLSNNDGCVVSRSEEAKLLGIKMGVPWFQIEKDALQAGVQAYSSNYTLYAE	87
ATCC 17978 UmuC-2015	MKRRIFALVDVNNQYASIERFFNPKLNNRPVIVLSNNDGCAVARSAEAKAIGIKMGEPLFKIIDLVKRNNVAVLSSNYPVYAE	83
Consensus	MKREVFALVDVNN-Y-SCERVF-P-LN-RPVIVLSNNDGCVVARSAEAKALGIKMG-PLFQIKDLV-RN-VIVLSSNY-LYAE	
Conservation		
E.coli UmuC	MSNRVMSTLEE-LSPR-VEIYS IDEAFCDLTGVRNCRDLTDFGREIRATVLORTHLTVGVGIAOTKTLAKLANHAAKKWOROTGGVVDLS	167
ATCC 17978 UmuC-0637	MSRRFHKILAEYVSPENHEIYSVDEAFLELTSYRGIYNLNQCAQDIKEKLLKLLSLPVCVGIGRSKTEAKIMNYIAKTYP-HLNGICNVF	179
ATCC 17978 UmuC-1173	MSRRFHTILASYVTAEEVEPYSIDECFVDFTAYEKNFDLEKVGOOMROOIWKWLGLPVCVGIGRSKTEAKIANHIAKKNA-GFNSVCDLV	174
ATCC 17978 UmuC-2008	MSRRFFAVLGEFFSPDDLEAYS IDECFIHLTPYLOSIDISDYCNKVRNTLLKWLGLPCCIGIGYSKTOAKLANHYAKKIK-SFKGVCNFI	176
ATCC 17978 UmuC-2015	MSKRFHAILKQFVAPHEHETYS IDEAFLELTAYEYKYDLNAYAKLMKDRVFMWIGLPVCVGIGRSKTEAKIANHIAKTYP-NFNGVCNLV	172
Consensus	MSRRFHAILAE-VSPEE-EIYSIDEAFL-LTAYYDLNDY-QR-T-LKWLGLPVCVGIGRSKTEAKIANHIAKKYPFNGVCNLV	
Conservation		
E.coli UmuC	NLERORKLMSALPVDDVWGIGRRISKKLDAMGIKTVLDLADTDIRFIRKHFNVVLERTVRELRGEPCLOLEEFAPTKOEIICSRSFGE	255
ATCC 17978 UmuC-0637	DLAE-VKDSIFRNTDVGEVWGVGSQQKKKLRLMNINTVYDLMTASPSHIQSVFSVVLKRTVLELNGISCIDIEHTPPTKKQIVSSRTFGQ	268
ATCC 17978 UmuC-1173	NMDPCNKEYYFSLIDVSEVWGVGRKHSKKLQSMGINTVLDLACAEPREMQKRFSIVMARTIYELQGISCIEIEHTPPSKKQIIKSCSFGA	264
ATCC 17978 UmuC-2008	TLDPL IMEDLMQQTSVKEVWG I GYQLVKQLQSYEVYTCLDLTFANEHHMAKAFSVVMART I RELKGQSC I ELDDPA I PTKR I LASRSFAQ	266
ATCC 17978 UmuC-2015	SFPENIRNLLYKQTKVSEVWGVGRQHSRKLEAMGINTVFDLMMANPYHIESLFSVVMKRTVLELNGIACIEIEDTPPTRKQIISSRAFKQ	262
Consensus	NLDIKE-L-SQTDVSEVWGVGRQHSKKLQ-MGINTVLDLANPRHIQK-FSVVM-RTV-ELNGISCIEIE-TPPTKKQIISSRSFGQ	
Conservation		
E.coli UmuC	R I TDY P SMRQA I C S YAARAAEK LR SEHQY CR F I ST F I KT S P F ALNE PY YGN – – S A S V K L L T P TQ D S R D I I NAAT R S L D A I WQ AGH R YQ K A	343
ATCC 17978 UmuC-0637	RITDIHDLKEAVIKRTQEAFTRARNEQVLVGCIIVFAYSNPFDKTKPFYKKEQSSSFAVPTDDLRLLVQTATRLMDQVYKSGIEFKKA	356
ATCC 17978 UmuC-1173	KVTELIDLQEAIAMHAQEACKRLRDDESLCGCLIVFVQSSPFDENVPFYNKSITGSFSQPTDSALDFVKAATKMVSHIYKEGIKYKKC	352
ATCC 17978 UmuC-2008	ALSSIEIIKQALIFHLNRAHRRLMKQEQLCACVQVMLYEK TDKPPYKKATSQAIGLHYATDDLCILTKAAMQQIDVLYKENKSYIKI	353
ATCC 17978 UmuC-2015	KIIDKDDLKEAIARRTQEAFTRARKDQVLCGCIVAFAHSSPFDVNKPFYKGELSQSFSVPTDDVRRLVKASTSMIDYIYRYGVDFKKC	350
Consensus	- I TDI -DLKEAIQEAFTRLRKLCGCI IVFAYSSPFD-NKPFYKKE -SQSFS PTDDLRDLVKAATRMID-IYKEGI - YKK -	
Conservation		
E.coli UmuC	GVMLGDFFSQGVAQLNLFDDNAPRPGSEQLMTVMDTLNAKEGRGTLYFAGQGIQQQ-WQMKRAMLSPRYTTRSSDLLRVK	422
ATCC 17978 UmuC-0637	GVILTCLEPKHTYTYDLLTDHQDLEKTEQLMKAIEEVQQIFGKNKIGFGGSMFKNRIWNLTANYQTRNYFS-FDGMIKIKN	436
ATCC 17978 UmuC-1173	GVILTGLEPKAGHTYDLLTDFEAIEKKEQLMKTLDNVHTKFGKKKLGISTCYVPGRNWSMSRDKLSKNPFK-WDELLTILN	432
ATCC 17978 UmuC-2008	GVLFCALHARQQHIDDLWQPLELIHQRQQLMETLGTVRKRFGSHYLQVG-YHSRNPSWQMKQCHRSKNYLTRWNEMLTIEDAYTPVTQNT	442
ATCC 17978 UmuC-2015	GVVLTALESKNSYTYDLLTDYSDLEKTENLMCAIECIQEKYGKYKLGFGGSMYQNRVWSMSQNLKSNNYFT-WEGMLKISR	430
Consensus	GVILTALE-KTYDLLTD-ED-EKTEQLMKTVQ-KFGK-KLGFGGSM-QNR-W-MN-LSKNYFT-WD-ML-IKN	
Conservation		

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).

P90C ∆*dinB*



MMS

Treatment

NFZ

B





Figure S3. Plasmid-borne *A. baumannii dinB* complements certain phenotypes of *dinB*-deficient

E. coli. (A) Wild-type P90C $\Delta dinB$ 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 $\Delta dinB$ 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 μ M) or MMS (7.5 mM) per total number of untreated CFUs. (**B**) Similar results are found using MG1655 $\Delta alkA$ 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 $\Delta dinB$ cells were not sensitive to EMS. Percent survival was calculated as described in (A) using NFZ (5 μ M), 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.