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Supplemental Data

UmuD and RecA Directly Modulate the Mutagenic Potential of the Y Family DNA Polymerase DinB

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Supplemental Figure 1

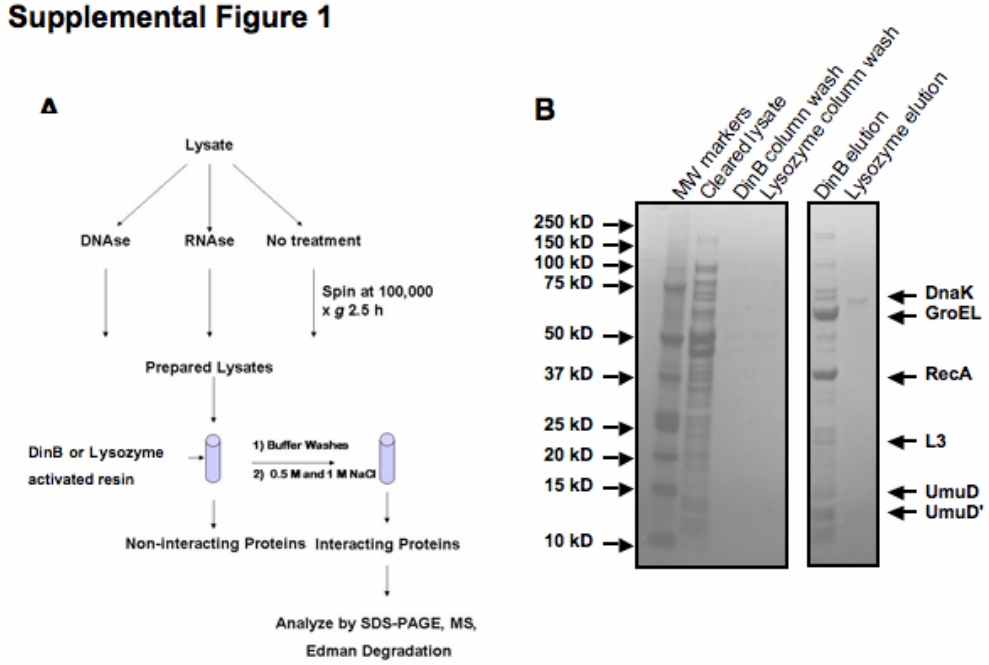

Figure S1. Schematic of the Affinity Chromatography Procedure Followed to Isolate Factors that Bind DinB

(A) DinB affinity resin was generated by coupling 3 mL of Affigel-10 N-hydroxysuccinimide derivatized agarose beads (Bio-RAD) with 30 mg of recombinant DinB (5 mg/mL) at 4^{o}C. The

efficiency of coupling was monitored by determination of the protein concentration remaining in solution, and quantitative conversion was observed after *ca.* 4 h. The resin was blocked with excess 1 M Tris(hydroxymethyl) aminomethane pH 7.8 for 1 h at 4^{o}C and loaded into three 1 mL columns. A control resin was similarly generated using 30 mg hen egg white lysozyme (Sigma) due to its elevated isoelectric point (pI 11.0) and was treated identically with respect to subsequent experimental steps. The DinB affinity columns and lysozyme controls were equilibrated in 50 mM Hepes pH 7.5, 100 mM KCl, 5% glycerol, 1 mM DTT (wash buffer). One liter of *lexA*(Def) *E. coli* cells were grown to fresh saturation in Luria Burtani (LB) broth, harvested by centrifugation, and resuspended in 50 mL wash buffer containing 15 mM $MgCl_2$. A lysate was generated with two passes through a French pressure cell at 14,000 psi, and cleared of ribosomes by ultracentrifugation at 100,000 x *g* for 4 h prior to separation into three equal aliquots. One of these was left untreated and the other two were digested with either DNAse I (1000 units; Sigma) or RNAse A (100 units; Sigma) for 1 h at 4^{o}C. Each aliquot was divided in half and applied to both a DinB affinity column (1 mL) and a lysozyme control column (1mL) at 4^{o}C. Each column was washed with 50 mL of wash buffer and proteins remaining on the resin were identified by removing 100 μL of each resin and boiling with 100 uL SDS-PAGE loading buffer.

(B) The eluates were separated by 4-20% SDS-PAGE and DinB-interacting proteins were identified by Edman degradation.

Supplemental Figure 2

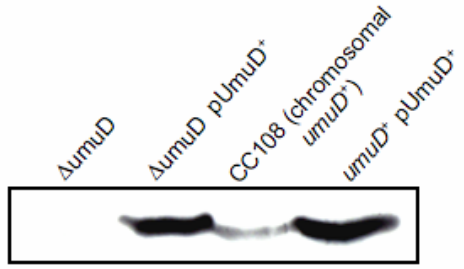

Figure S2. Relative Levels of UmuD Expressed from the Plasmids Employed in This Study

Immunoblot was performed using an antibody against UmuD. Whole cell pellets were obtained from 0.5 mL freshly saturated *E. coli* culture, resuspended and boiled in 100 µL SDS-PAGE loading buffer, and separated on a 4-20% SDS-PAGE before being transferred to a PVDF membrane.

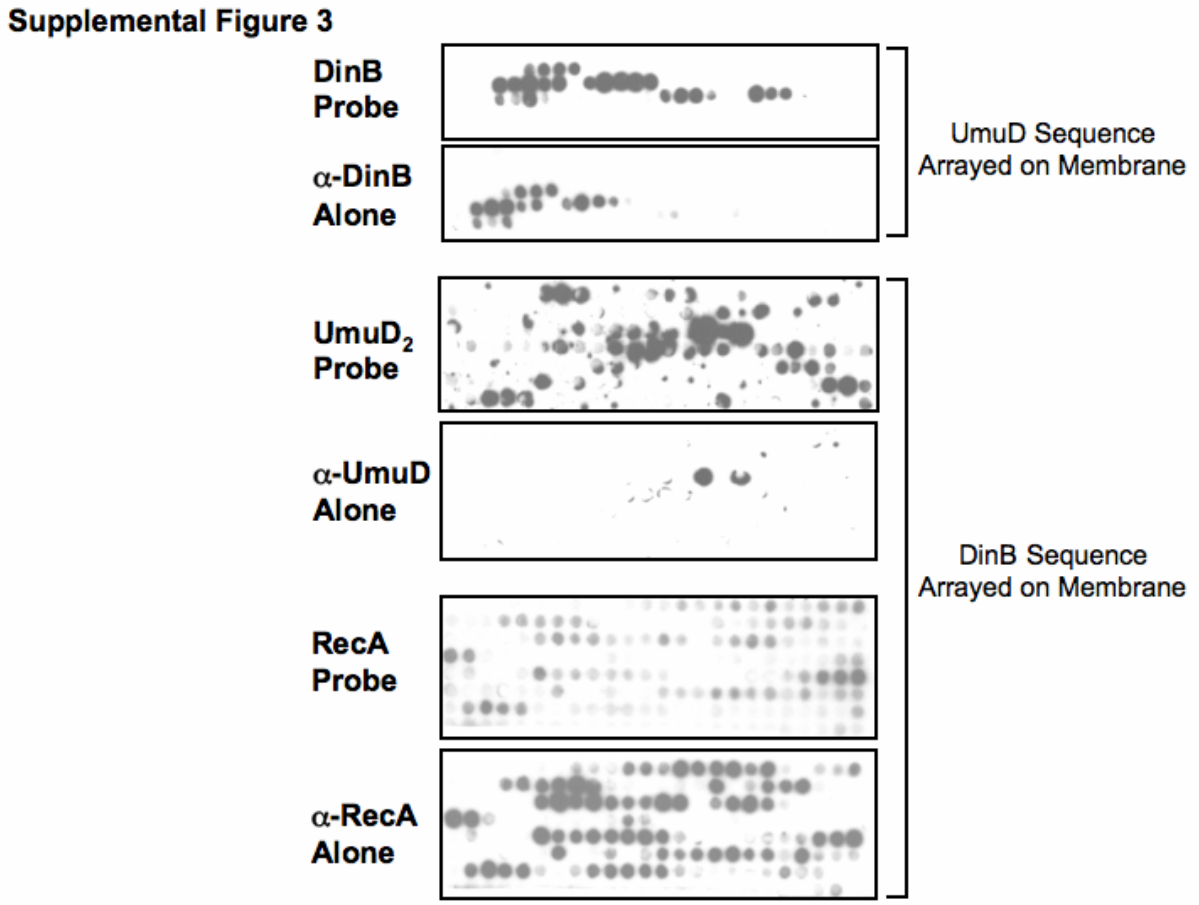

Figure S3. Identification of the Molecular Interfaces among DinB, UmuD, and RecA by Peptide Array Mapping

12-mer peptides scanning the primary sequence of UmuD and DinB, each offset by two residues from the previous peptide, were arrayed consecutively on cellulose filter membranes (from top left to bottom right) at the MIT Biopolymers Core Facility. Arrays were incubated with 100 nM probe ($UmuD_2$, DinB, or RecA) overnight and blocked, washed, and developed as previously described (Niebuhr and Wehland, 1997). As a negative control, arrays were identically treated with the antibody to the probe to determine which signals arose from its spurious interaction with the array.

Supplemental Figure 4

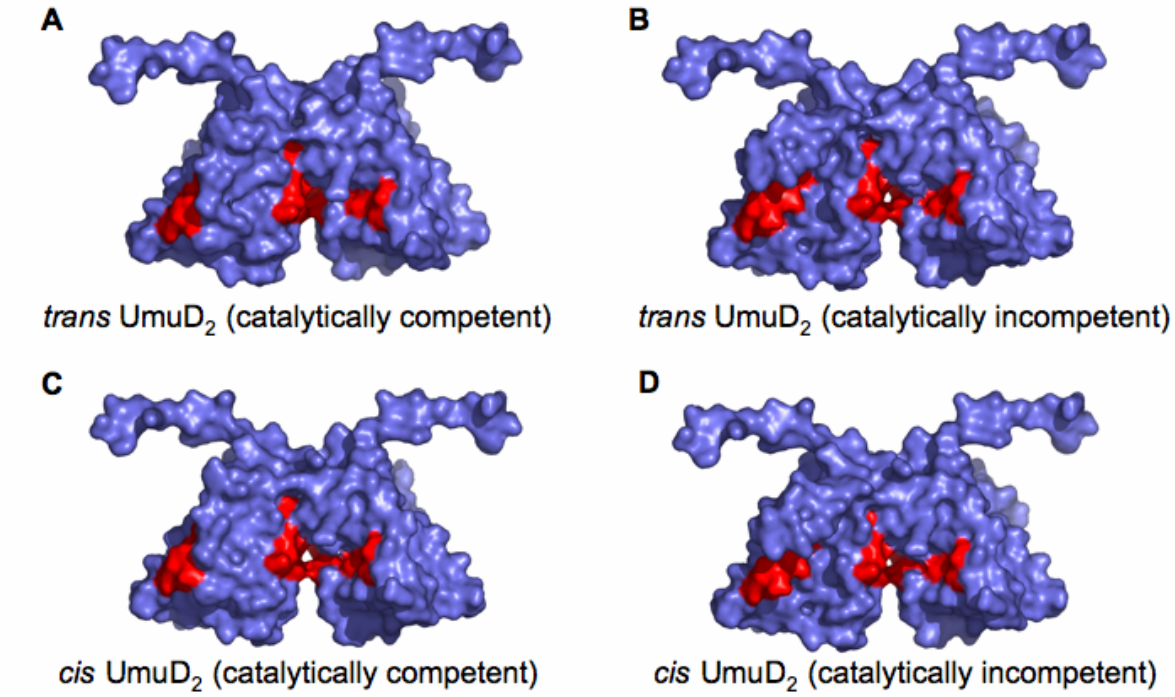

Figure S4. $UmuD_2$ Isoenergetic Conformers

In the *trans* conformations **(A and B)** the UmuD N-terminus crosses to the opposite monomer, while in the *cis* **(C and D)** conformations it remains on the same monomer.

(A) *trans* $UmuD_2$. The structure of this conformer allows RecA-mediated autocleavage, i.e. catalytically competent.

(B) *trans* $UmuD_2$. The structure of this conformer would not allow efficient RecA-mediated autocleavage.

(C and D) Structures of *cis* $UmuD_2$ isoenergetic conformers that would either allow RecA-mediated autocleavage (shown in **C**) or do would not (shown in **D**).

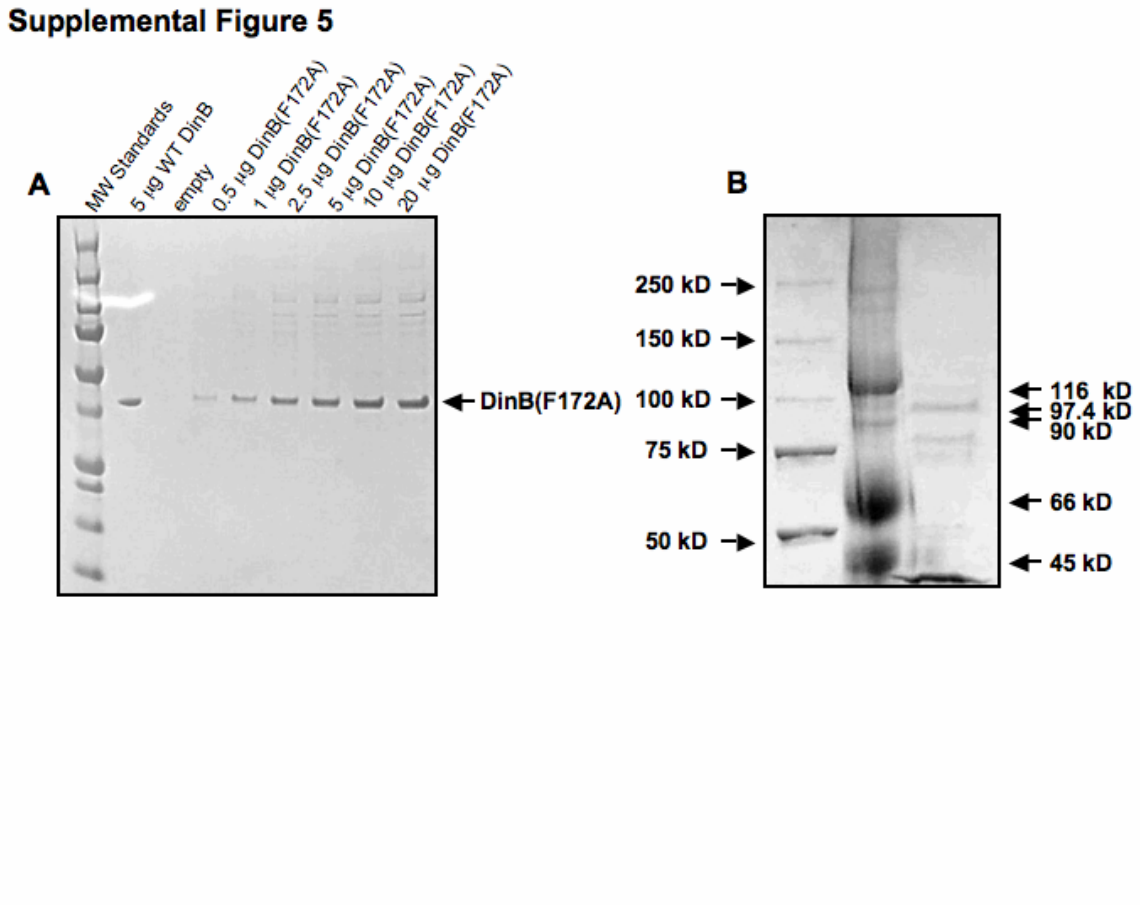

Figure S5. SDS-PAGE of DinB(F172A) Purification

(A) 4-20% SDS-PAGE of purified recombinant DinB(F172A). Precision plus molecular weight standards (BioRad) are shown in the first lane. 5 µg of a wild type DinB protein standard are shown in the second lane and increasing amounts of purified DinB(F172A) are shown in the amounts indicated.

(B) 4-12% SDS-PAGE of purified recombinant DinB(F172A). Lane 1 contains Precision plus molecular weight standards (BioRad). Lane 2 contains BioRad high range molecular weight standards and Pfu Turbo (90 kD; molecular weights indicated to the right of the gel). Lane 3 contains 10 µg of DinB(F172A). Neither of the high molecular weight contaminants in the

DinB(F172A) purification correspond to the size of DNA polymerase I (102.9 kD) or DNA polymerase II (89.9 kD).

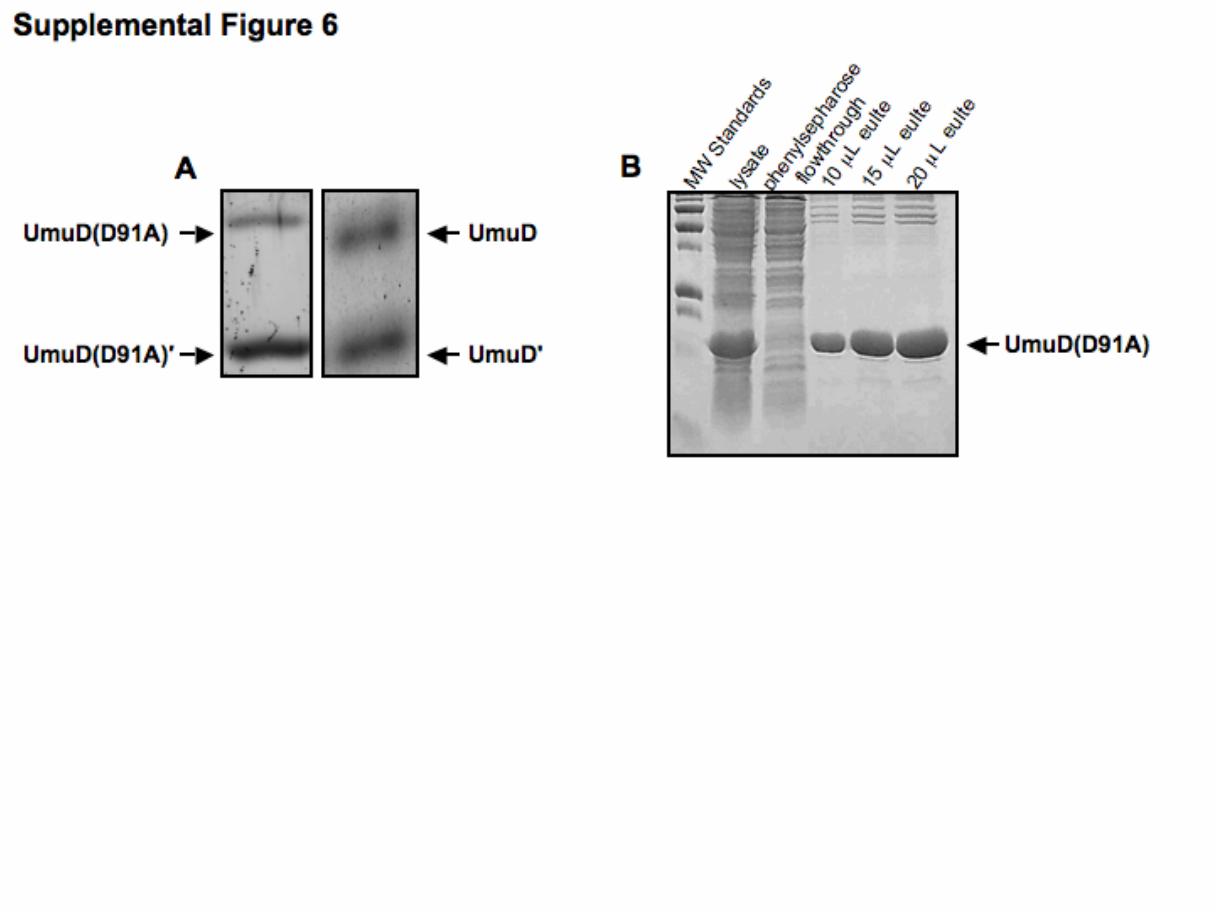

Figure S6. The UmuD(D91A) Protein Behaves as Wild-Type UmuD for RecA-Mediated Cleavage and Purification

(A) RecA-mediated autocleavage of UmuD(D91A) and UmuD after 15 minutes. Reaction conditions are as described in (Beuning et al., 2006b).

(B) 4-20% SDS-PAGE of different stages of purification of the UmuD(D91A) variant protein. Indicated amounts of the purified protein were also run on the gel.

Supplemental Figure 7

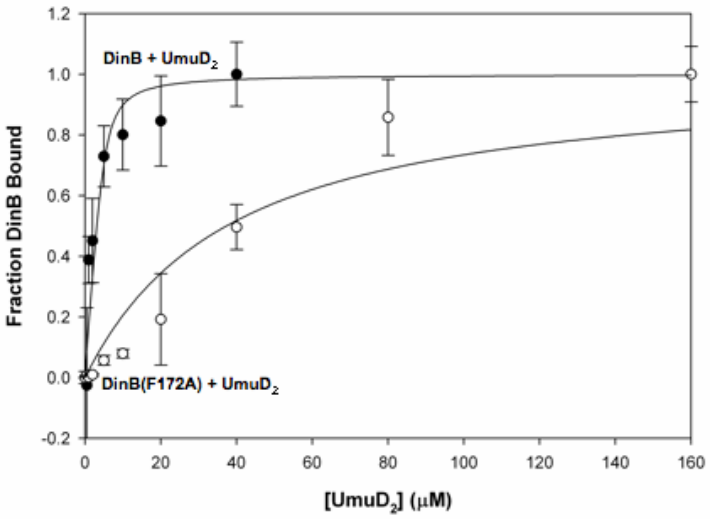

Figure S7. Fluorescence Spectroscopy Data for the Interactions between $UmuD_2$ and DinB

Fraction DinB bound was determined as described in (Beuning et al., 2006b) and is plotted against increasing concentrations of $UmuD_2$. Closed circles represent the interaction of $UmuD_2$ with wild type DinB and open circles represent the interaction with the DinB(F172A) variant. Error bars represent one standard deviation determined from three independent experiments.

Supplemental Figure 8

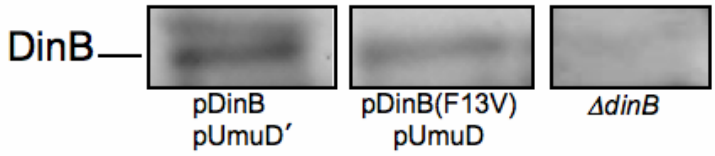

Figure S8. DinB and the Variant DinB(F13V) Are Present at Similar Levels in Whole-Cell Lysates

Bacterial cultures with plasmid-borne copies of wild type *dinB* and of *dinB(F13V)* were prepared by mixing 2 ml of pelleted exponential phase cells with SDS-PAGE loading buffer and boiling for 10 min. Samples were run on an 8-20% SDS-PAGE and transferred to a PVDF membrane. An antibody to DinB was used to detect both DinB and DinB(F13V).

Supplemental Figure 9

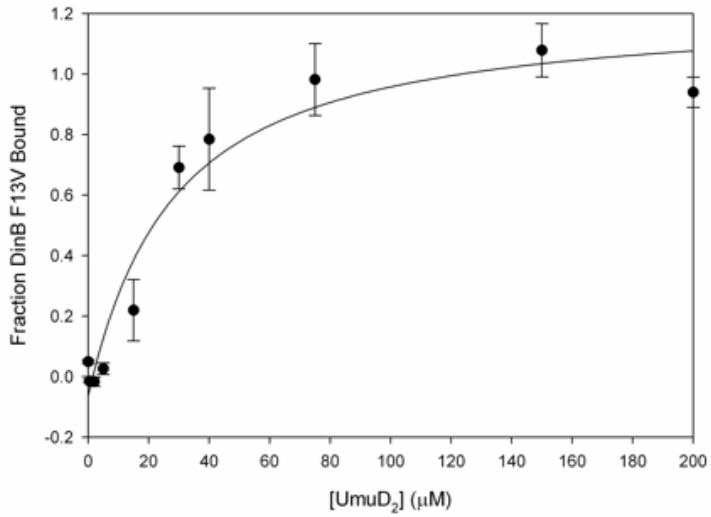

Figure S9. Fluorescence Spectroscopy Data for Interactions between $UmuD_2$ and DinB(F13V)

Fraction DinB bound was determined as described in experimental procedures and is plotted against increasing concentrations of $UmuD_2$. Closed circles represent the interaction of $UmuD_2$ with DinB(F13V). Error bars represent one standard deviation determined from three independent experiments.

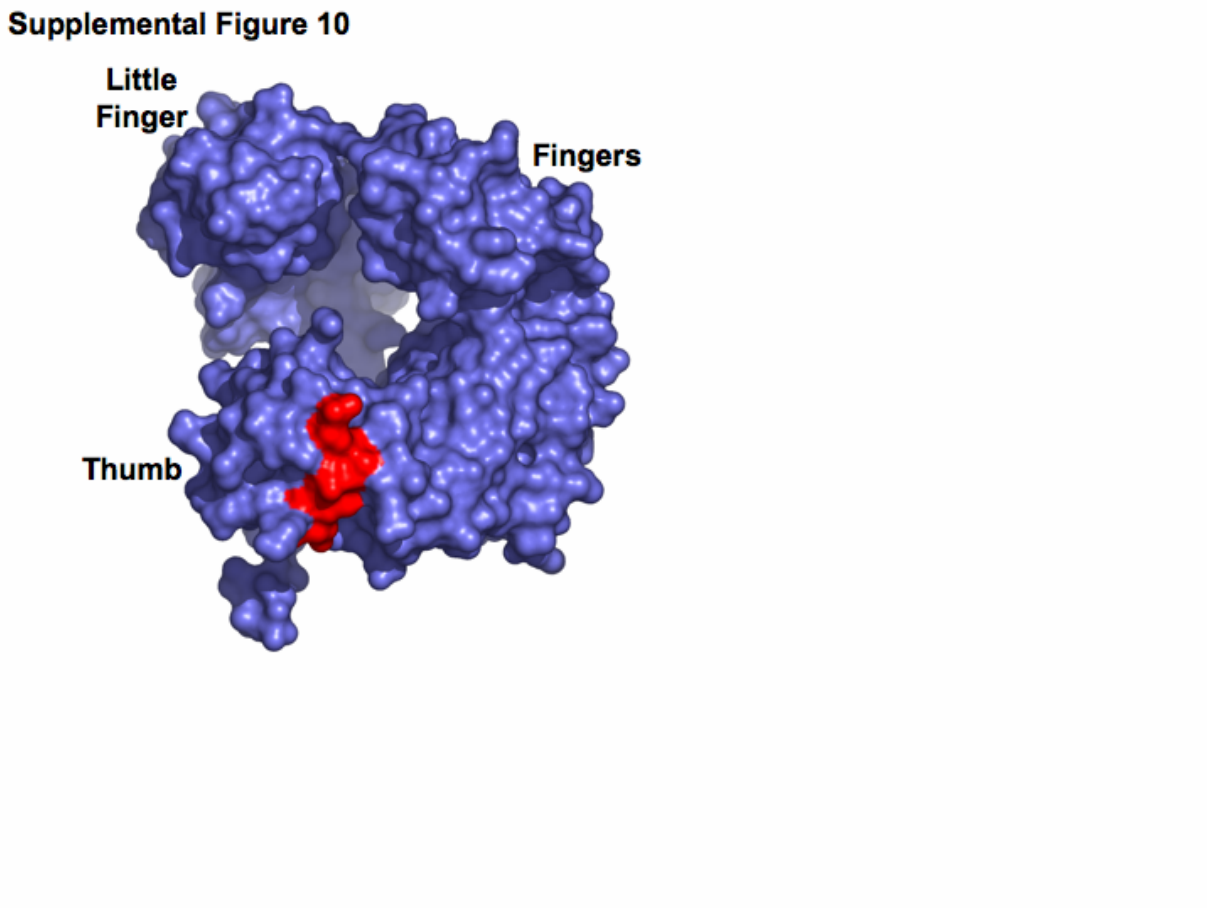

Figure S10. Putative RecA Interaction Site on DinB

DinB model on which RecA interacting residues (colored in red) are mapped based on peptide array analysis.

Table S1. Strains and Plasmids Used in This Study

Strains	Genotype	Reference
Escherichia coli		
BL21 (DE3)	*E.coli* B. Standard strain used for protein overproduction	GCW lab stock
AB1157	*thr-1 araC-14 leuB-6(Am) Δ(gpt-proA)62 lacY1 tsx-33 qsr'-0 glnV44(AS) galK2(Oc) LAM⁻ Rac-0 hisG4(Oc) rfbC1 mgl-51 rpoS396(Am) rpsL31 kdgK51 xylA5 mtl-1 argE3(Oc) thi-1*	GCW lab stock
P90C	$\Delta(lac\text{-}pro)_{XII}$ *ara gal*	Cairns and Foster (1991)
VG187	As P90C but with *recA* gene deletion	This work
VG347	As P90C but with a precise deletion in *dinB*	This work and Jarosz et al. (2006)
P90C *ΔumuD*	As P90C but with a precise deletion of *umuD*	This work; *ΔumuD::kan* allele from Keio collection
FC40	As P90C but bearing a variant of the F'128 episome with the Lac- *LacI33* allele revertible to Lac+	Cairns and Foster (1991)
Plasmids		
CC108	A derivative of the F'128 episome with a Lac- revertible allele	Kim et al. (1997)
pWSK29	pSC101-like replicon with multiple cloning site from pBluescriptSK$^+$II. Amp^R	Jarosz et al (2006)
pYG782	*dinB* gene cloned in the pWSK30 plasmid (Amp^R) which is the same as pWSK29 but MCS cloned in opposite orientation under lac promoter	Kim et al. (1997)
pYG768	Same as pYG768 but the *dinB* gene is cloned under its own promoter	Kim et al. (1997)
pYG768-F172A	Same as pYG768 but the *dinB* gene encodes the F172A mutation.	This work
pYG782-F13V	*dinB* gene encoding the F13V mutation cloned in pWSK30 (Amp^R) under lac promoter	Jarosz et al. (2006). This work
pYG782-F172A	*dinB* gene encoding the F172A mutation cloned in pWSK30 (Amp^R) under lac promoter	This work
pDinB003	*dinB* gene encoding the D103N mutation cloned in pWSK30 (Amp^R) under lac promoter	Wagner et al. (1999) This work
pGW60101	pBR322 (Spec^R) vector	This work
pGW6010	*umuDC* genes cloned in pGW60101	GCW collection
pGW6030	*umuD* gene cloned in pGW60101 plasmid under the lac promoter	GCW collection

pGW6030-D91A	*umuD* gene encoding the D91A mutation cloned in pGW60101 under lac promoter	This work
pGW6030-S60A	*umuD* gene encoding the S60A mutation cloned in pGW60101 under lac promoter	This work
pGW6040	Same as pGW6030 but with the *umuD'* gene.	GCW collection
pSG5-6030-D91A	Same as pGW6030-D91A but in pET11T	Beuning et al. (2006). This work
pET11T-DinB-F172A	Same as pYT782-F172A but in pET11T	Jarosz et al. (2006) This work

Table S2. DinB Suppresses *umuDC*-Dependent UV-Induced Mutagenesis

Supplemental Table 2

Plasmids	-UV	+ UV	Fold change	Fold increase
pWSK29 + pGW60101	$4.0 \pm 1.8 \times 10^{-8}$	$2.4 \pm 1.1 \times 10^{-7}$	6 ± 3.1	
pWSK29 + pUmuDC	$1.2 \pm 0.6 \times 10^{-7}$	$1.7 \pm 0.6 \times 10^{-5}$	142 ± 85	24 ± 18.7
pDinB + pGW60101	$3.9 \pm 2.1 \times 10^{-8}$	$1.3 \pm 0.4 \times 10^{-6}$	33 ± 19	
pDinB + pUmuDC	$5.8 \pm 1.9 \times 10^{-7}$	$1.2 \pm 0.2 \times 10^{-5}$	21 ± 7.2	0.6 ± 0.4
pDinB-F172A + pGW60101	$5.7 \pm 3.1 \times 10^{-8}$	$1.3 \pm 0.7 \times 10^{-7}$	2.0 ± 1.0	
pDinB-F172A + pUmuDC	$5.0 \pm 2.8 \times 10^{-8}$	$1.1 \pm 0.1 \times 10^{-5}$	220 ± 130	110 ± 86

Expression of DinB under its native promoter from a low copy number plasmid suppresses *umuDC* dependent mutagenesis induced by ultraviolet light. Plasmids were maintained with ampicillin [pWSK29 (empty vector); pYG768 (pDinB)] and spectinomycin [pGW60101 (empty vector); pGW6010 (pumuDC)]. Note that pDinB in combination with pGW60101 promotes a

modest increase in the UV-induced mutation frequency, a hitherto unreported property of DinB. Mutation frequency was determined in *E. coli* strain AB1157 by reversion from *argE3* to arg^+ as described in (Beuning *et al.*, 2006b) in the presence (30 J/m^2) and absence of UV-irradiation. Error values represent standard deviations determined from 7 independent experiments.