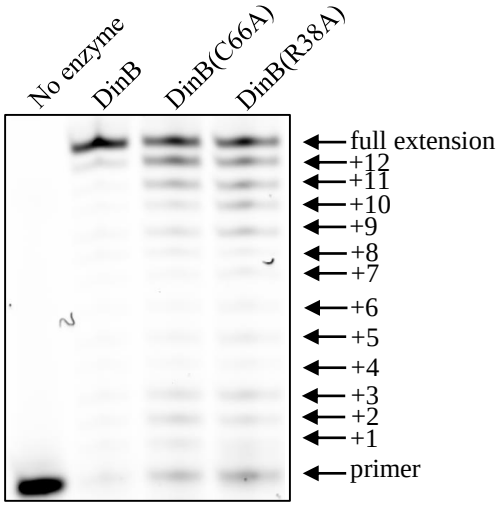
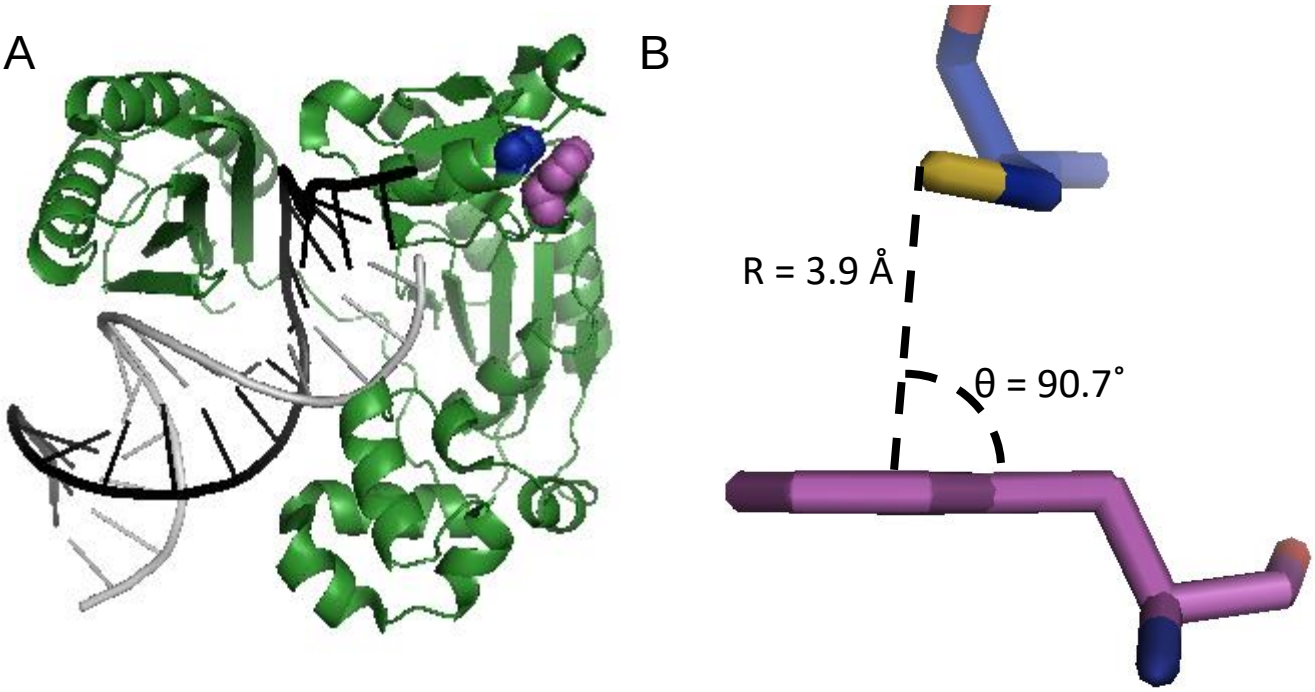
DinB Consensus/ Position	DinB Conservation (%)	DNA polymerase V Consensus/ Position	DNA polymerase V Conservation (%)	DNA polymerase II Consensus/ Position	DNA polymerase II Conservation (%)
R38	86	--	-	V42	81
G39	85	G35	83	A43	65
V40	87	C36	53	F44	92
V41	57	V37	69	I45	57
S42	50	I38	40	P46	73
T43	77	A39	84	A47	41
A44	60	R40	46	E48	26
N45	55	S41	60	Q49	55
Y46	94	P42	37	R50	40
E47	51	E43	55	E51	25
A48	93	A44	79	R52	31
R49	94	K45	89	A53	55
K50	51	-46	29	E54	36
F/Y51		L47	45	A55	23
G52	94	G48	51	G59	37
V53	74	-49	33	E60	62
H54	50	-50	32	R61	31
S55	89	M51	46	G62	28
A56	91	G52	50	V63	33
M57	92	-53	15	E64	48
P58	55	P54	56	L65	63
T59	27	-55	23	R66	53
A60	25	F56	53	P67	59
E61	21	-57	34	L68	83
A62	94	-58	20	A69	23
L63	31	-59	29	L70	83
K64	40	-60	20	K71	26
L65	58	-61	27	D72	77
C66	82	-62	20	F73	92
P67	90	-63	24	-74	-

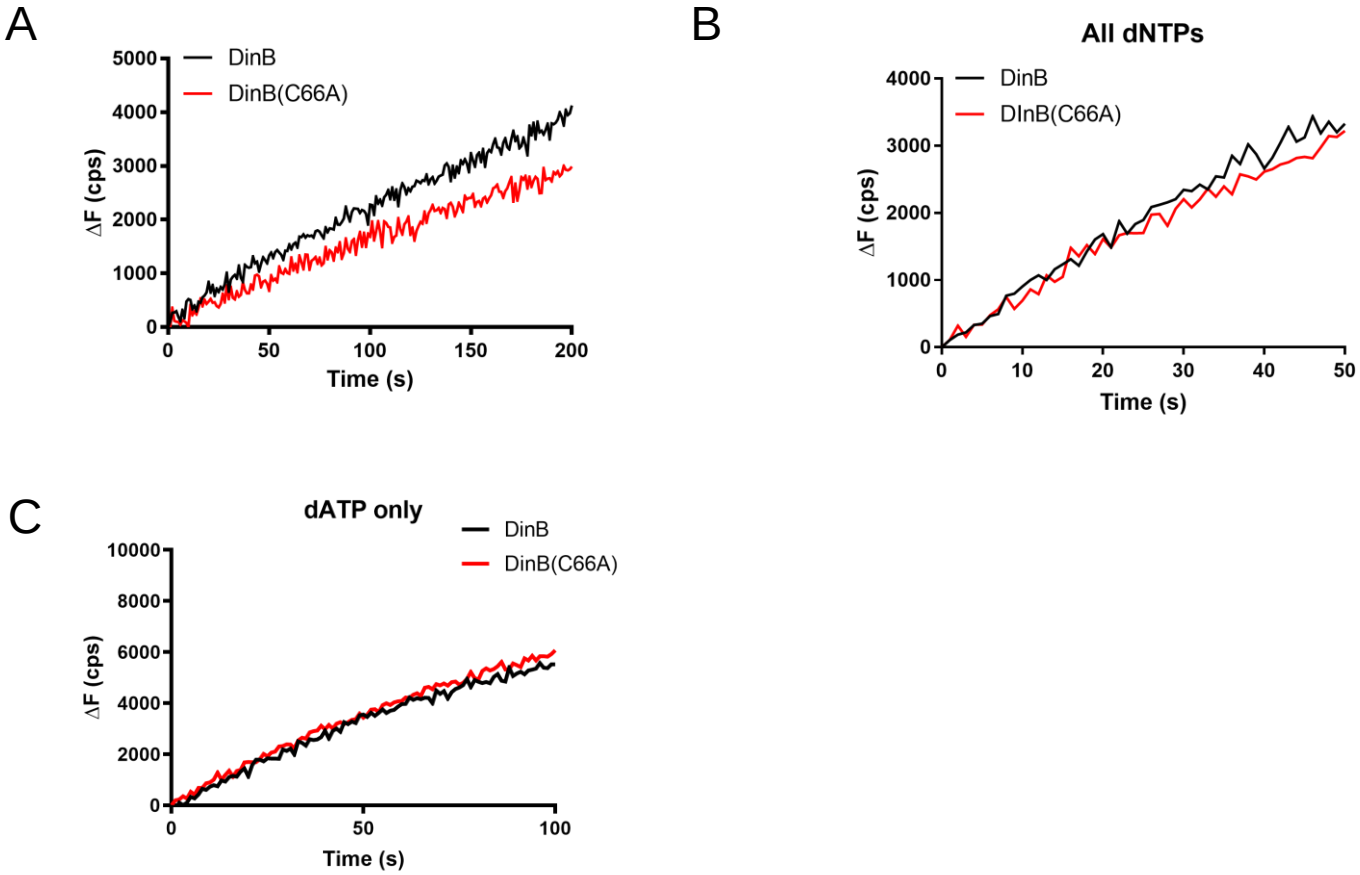
Supplemental Table 1. Several residues of the DinB fingers domain are highly conserved only in DinB-like proteins. This table contains conservation data plotted in Figure 2.



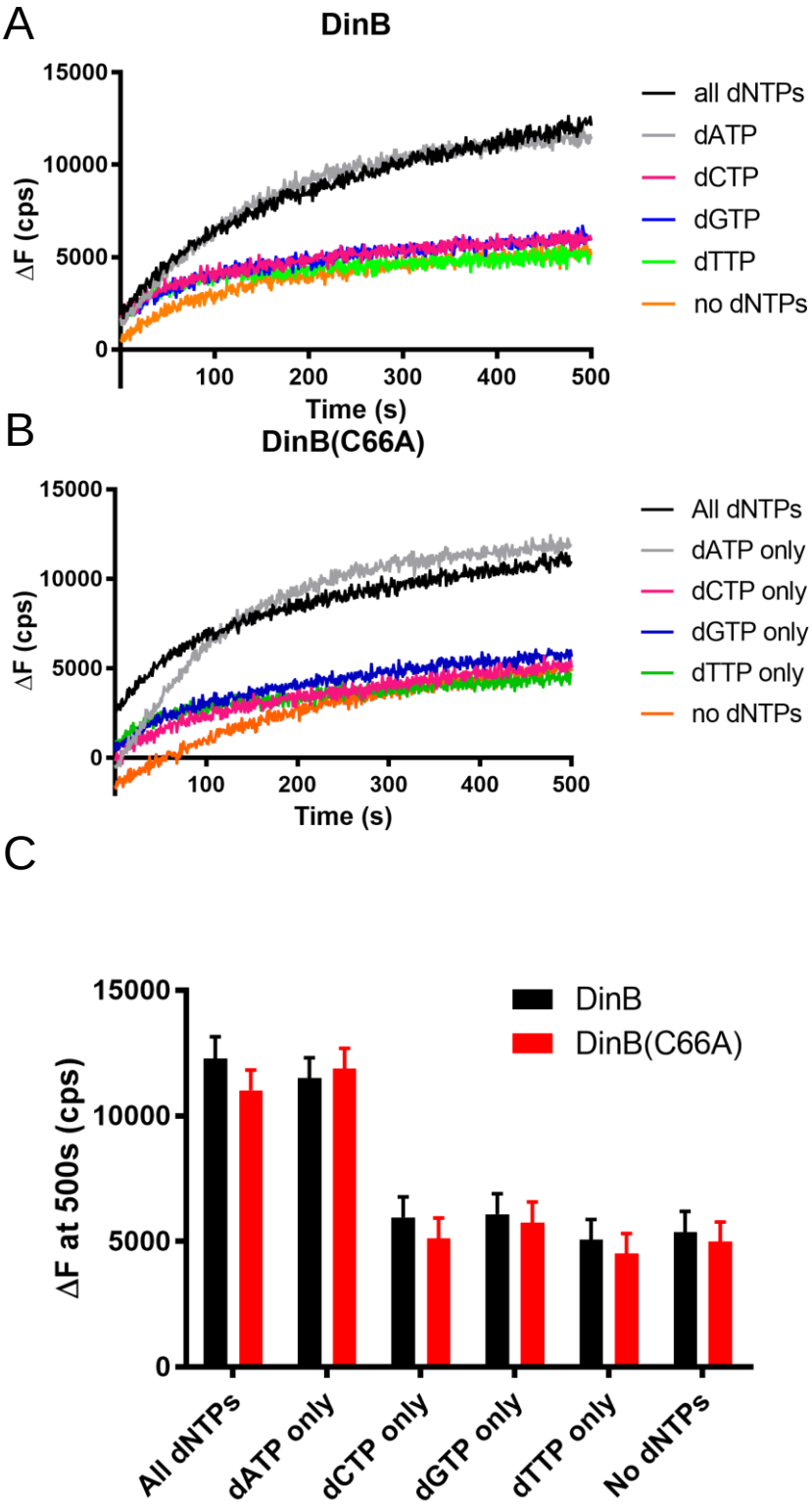
Supplemental Figure 1. DNA synthesis activity of native DinB and DinB(C66A) are similar on undamaged DNA template. DinB activity assay was performed as previously described (1) using a Cy3 labelled DNA primer that was annealed to an unlabeled DNA template. Reactions contained native DinB or DinB variant (1.25 μM), primer:template (0.25 nM), dNTP mix (500 μM), and 1X low salt buffer (20 mM Tris-HCl, pH 8.75, 10 mM KCl, 10 mM (NH4)2SO4, 2 mM MgSO4, 0.1% Triton X-100, 0.1 mg/mL bovine serum albumin). Reactions were performed at 37˚C for 10 minutes. Products were separated on 10% denaturing polyacrylamide gel and imaged for Cy3 fluorescence on a Bio-Rad gel imager. Total DNA synthesis activity was calculated as the sum of the products of quantified bands (normalized to the total band quantification and the number of insertions required for the respective band (i.e. the sum of the quantification of the primer band multiplied by zero, plus the quantification of the +1 band multiplied by one, plus the quantification of the +2 band multiplied by two, etc). Experiments were performed in triplicate. The activity of the DinB(C66A) variant was not significantly different from the native enzyme (as determined by a two-tailed t-test of total activity).



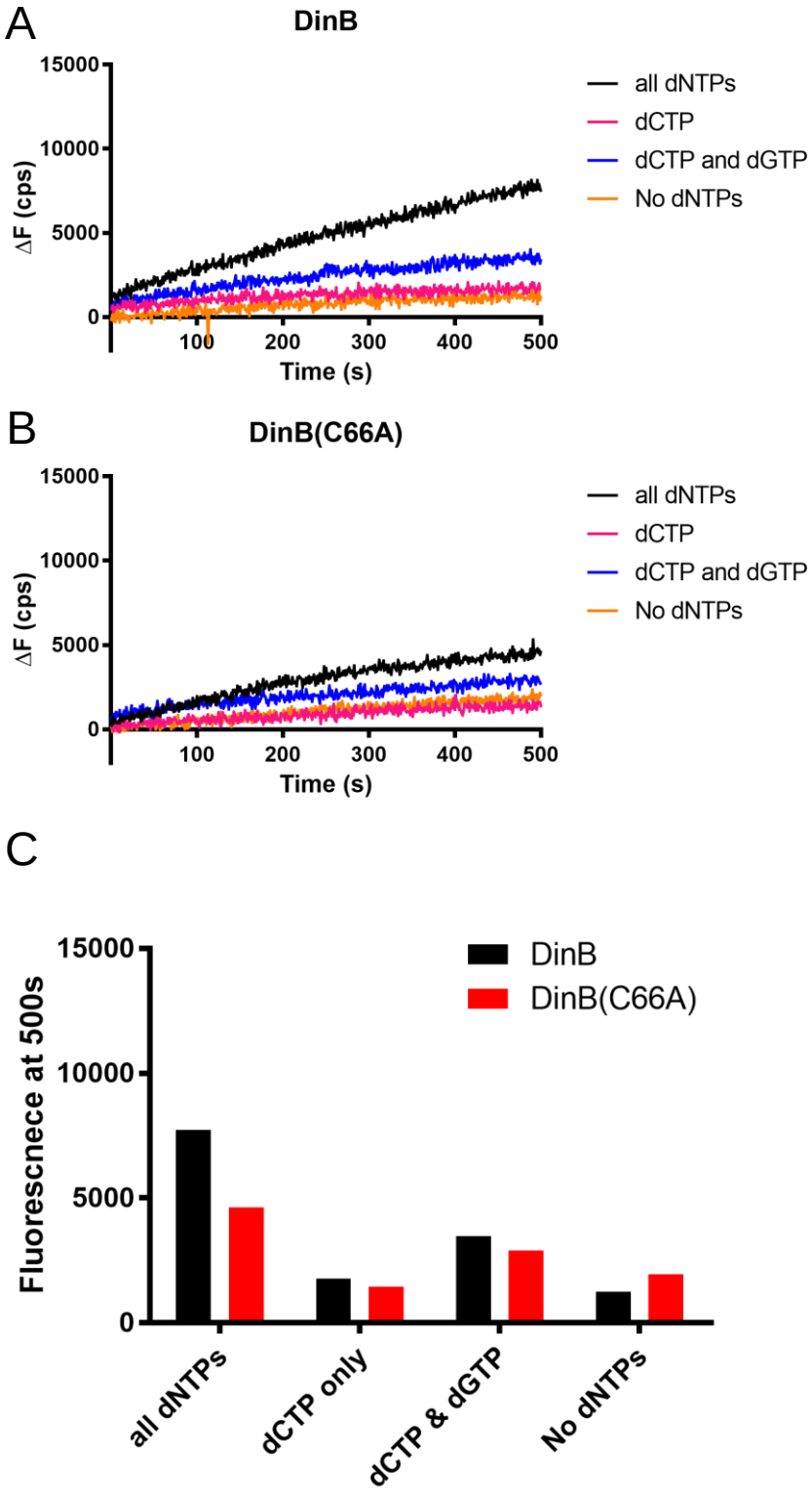
Supplemental Figure 2. The change of cysteine 66 to alanine in the DinB(C66A) variant may eliminate bonding with highly conserved F51 in the fingers domain. (A) Ribbon rendering of DinB crystal structure (PDB code 4IRC; (2)) suggests that an interaction between C66 (blue) and highly conserved F51 (magenta) is likely. (B) The distance between C66's sulfur atom and the center of F51's benzene ring (R) and the angle between C66's sulfur residue (yellow) and the plane of F51's benzene ring (θ) fall within the range necessary (3) to form an aromatic-thiol π-type hydrogen bond. This interaction would be interrupted in the DinB(C66A) variant. Rendering was done using PyMol (4).



Supplemental Figure 3. Enlargements of initial readings for RecA-dependent strand exchange experiments in Figures 5B (A), 6B (B), and 6C (C).



Supplemental Figure 4. DinB and DinB(C66A) show comparable fidelity during strand exchange. Experiments were performed as in Figure 6B using all or individual nucleotides and DinB (A) or DinB(C66A) (B). Experiments were performed in triplicate. Representative data is shown. (C) Fluorescence at 500s is shown for all reactions to compare results of DinB and DinB(C66A). Experiments were performed in triplicate. Mean ± standard deviation is shown. ΔF, change in fluorescence measured in counts per second (cps).



Supplemental Figure 5. DinB and DinB(C66A) show comparable fidelity during strand exchange. Experiments were performed as in Figure 5B using all or individual nucleotides and DinB (A) or DinB(C66A) (B). (C) Fluorescence at 500s is shown for all reactions to compare results of DinB and DinB(C66A). Experiments were performed in triplicate. Representative data is shown. ΔF, change in fluorescence measured in counts per second (cps).

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