Supplementary Information Analysis of conformational changes in the DNA junction-resolving enzyme T7 endonuclease I on binding a four-way junction, using EPR.

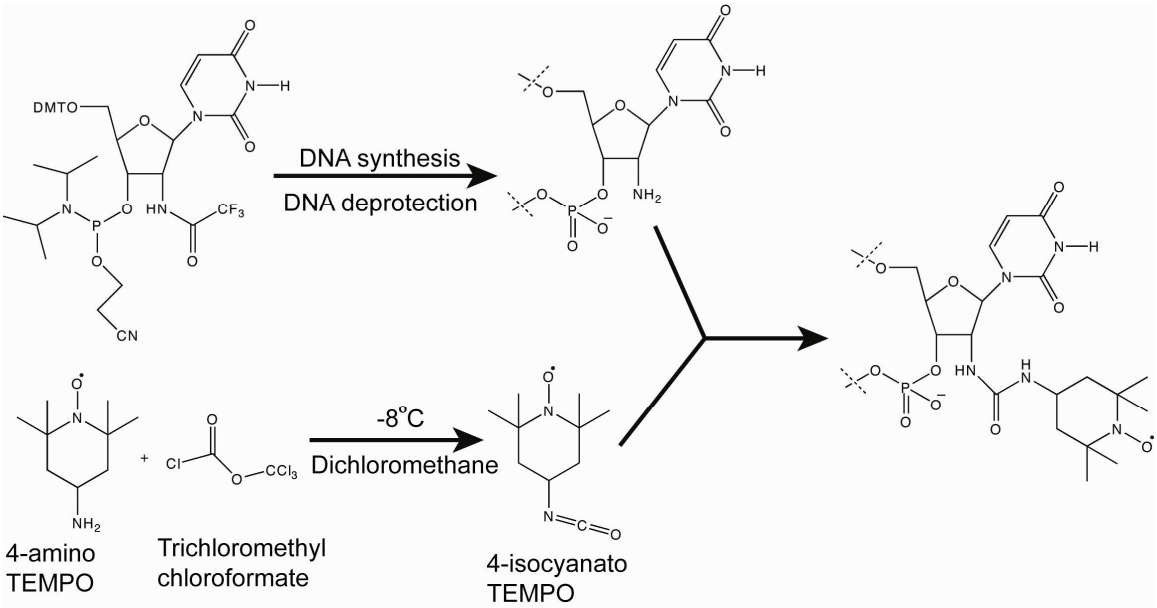

Figure S1: DNA spin labeling scheme. DNA synthesised with a 2'amino deoxyuridine residue at the desired site for the spin label. During DNA deprotection the 2'amino protecting group is also removed. Reaction of the DNA (containing a 2'amino group) with 4 -isocyanato TEMPO yields the spin labeled piece of DNA.

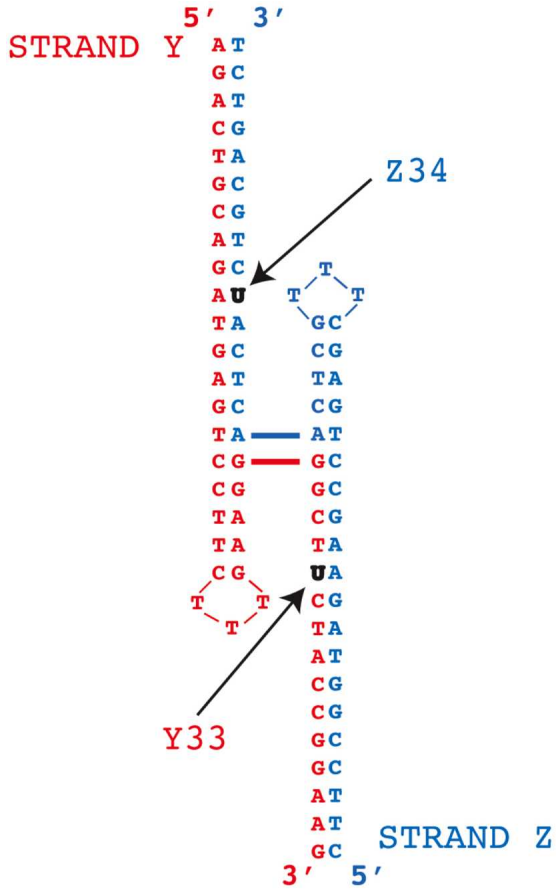

Figure S2: Spin labeling positions on the structure of the DNA four-way junction (DNA-4WJ). Strands Y and Z anneal to form a DNA-4WJ with two long arms and two short hairpin junction arms. The DNA-4WJ was spin labeled at positions Y33 and Z34 by incorporating a 2'amino deoxyuridine (marked as a bold U) at these sites.

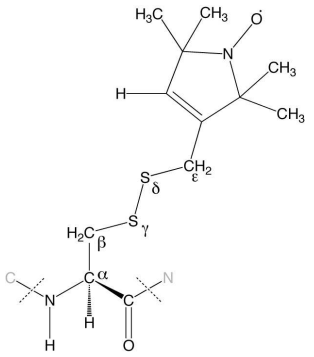

Figure S3 R1 side chain. Cysteine residue reacted with (1-Oxyl-2,2,5,5-tetramethyl-Δ3-pyrroline-3-methyl)-methanethiosulfonate (MTSSL) forms the R1 side chain.

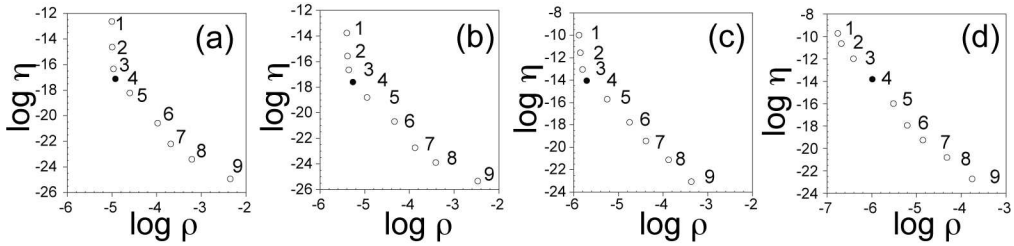

Figure S4 L curve analysis performed in DeerAnalysis2010. The data points 1 to 9 refer to the alpha fitting factor used, which are 0.001 (1), 0.01 (2), 0.1 (3), 1 (4), 10 (5), 100 (6), 1000 (7), 10000 (8), 100000 (9). Black filled data point indicates alpha factor used in this study.(a) T51R1, (b) T51R1 plus DNA-4WJ, (c) T51R1 K67A, (d) T51R1 K67A plus DNA-4WJ.

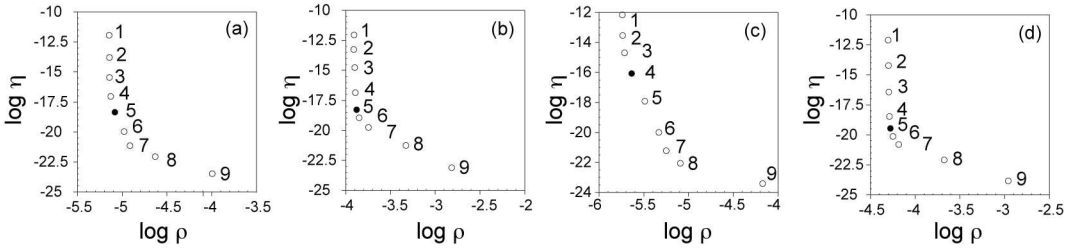

Figure S5 L curve analysis performed in DeerAnalysis2010. The data points 1 to 9 refer to the alpha fitting factor used, which are 0.001 (1), 0.01 (2), 0.1 (3), 1 (4), 10 (5), 100 (6), 1000 (7), 10000 (8), 100000 (9). Black filled data point indicates alpha factor used in this study.(a) S96R1, (b) S96R1 plus DNA-4WJ, (c) S96R1 K67A, (d) S96R1 K67A plus DNA-4WJ.

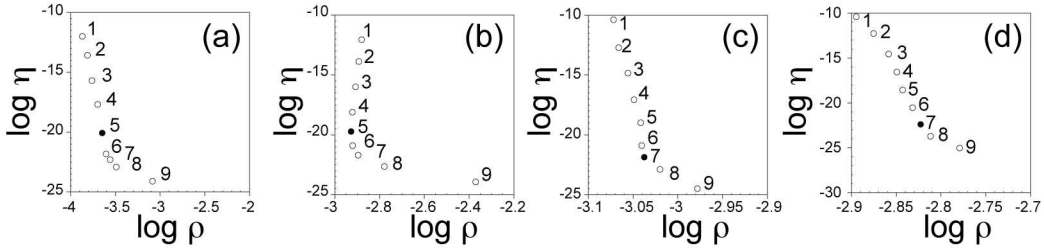

Figure S6: L curve analysis performed in DeerAnalysis2010. The data points 1 to 9 refer to the alpha fitting factor used, which are 0.001 (1), 0.01 (2), 0.1 (3), 1 (4), 10 (5), 100 (6), 1000 (7), 10000 (8), 100000

(9). Black filled data point indicates alpha factor used in this study.(a) K22R1, (b) K22R1 plus DNA-4WJ, (c) K22R1 K67A, (d) K22R1 K67A plus DNA-4WJ.

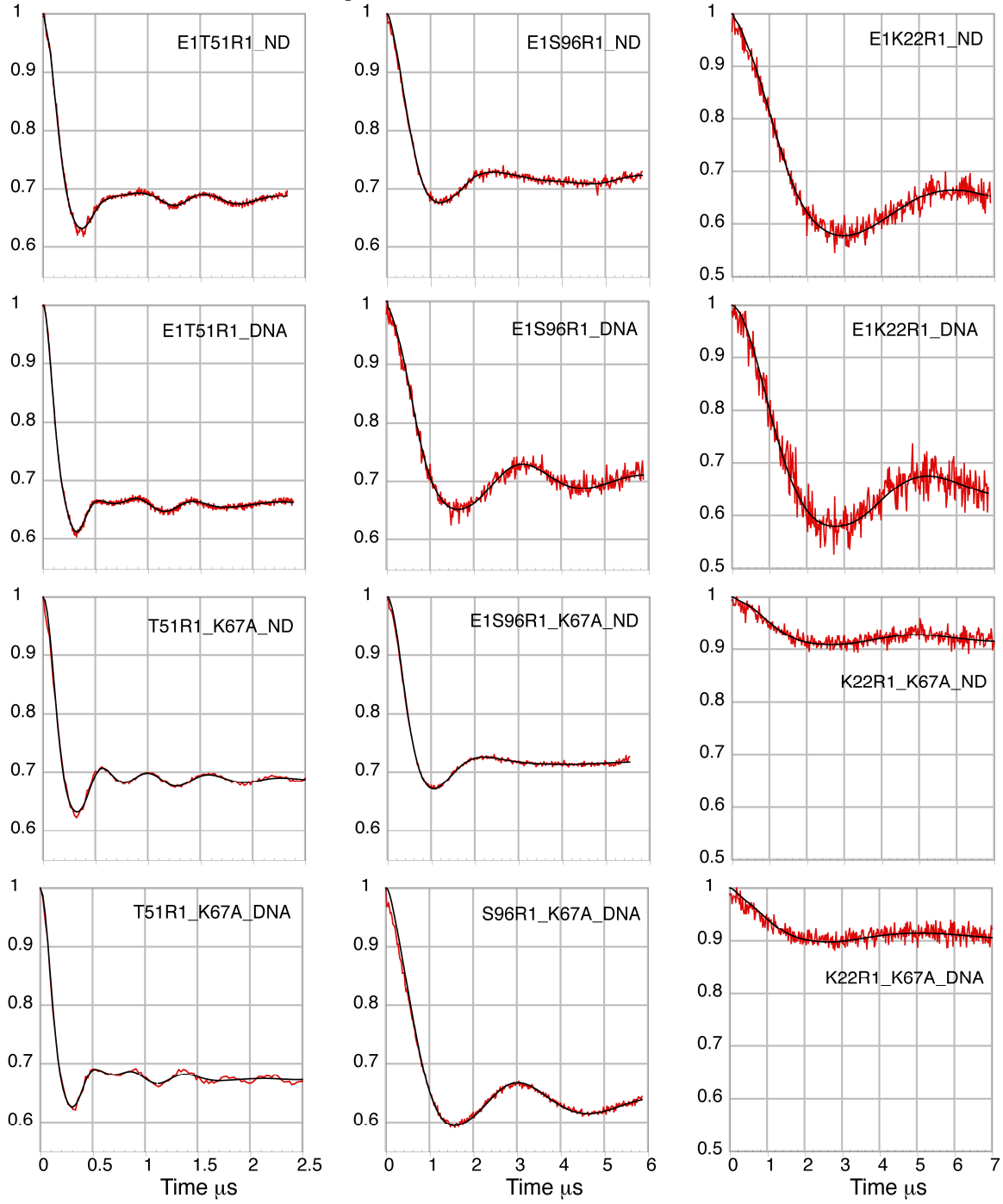

Figure S7
Goodness of fit by the Tikhonov regularization distance distribution, shown as black line on red, background corrected PELDOR data, for the three label positions and results with and without DNA bound and with and without the active site mutation K67A.

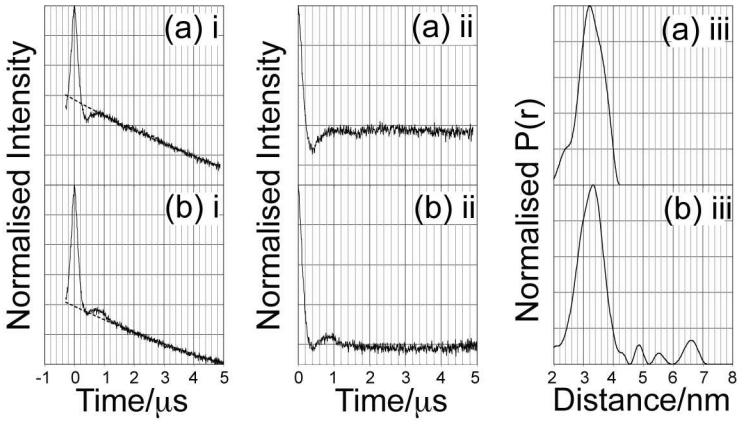

Figure S8. PELDOR data obtained on E83R1 D55A. (a) E83R1 D55A, (b) E83R1 D55A plus DNA-4WJ. i = Experimental PELDOR data, ii = Background corrected PELDOR data, iii = Tikhonov derived distance distribution

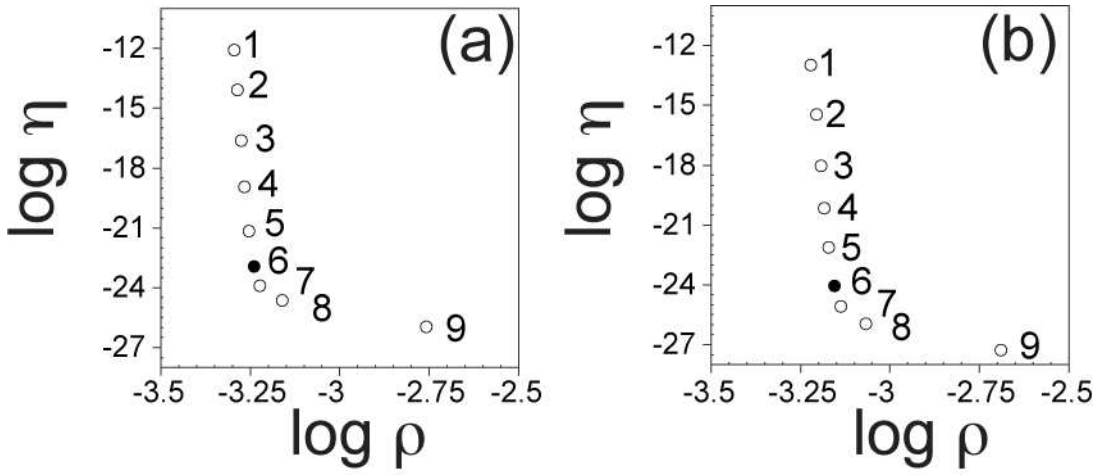

Figure S9. L curve analysis performed in DeerAnalysis2010. The data points 1 to 9 refer to the alpha fitting factor used, which are 0.001 (1), 0.01 (2), 0.1 (3), 1 (4), 10 (5), 100 (6), 1000 (7), 10000 (8), 100000 (9). Black filled data point indicates alpha factor used in this study.(a) E83R1 D55A, (b) E83R1 D55A plus DNA-4WJ.

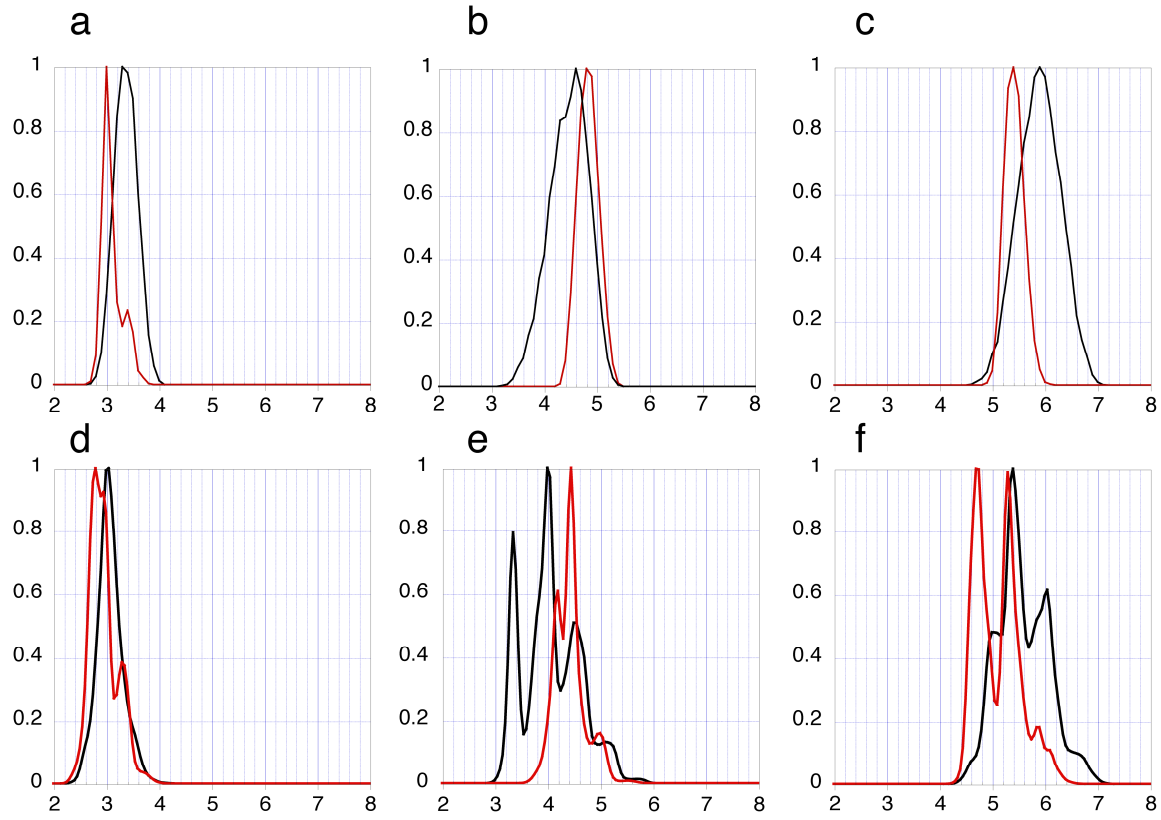

Figure S10 Distance distributions derived from simple molecular dynamics simulation (MDS) and rotamer library searching (RLS) of spin-label conformational space. Red traces derived from DNA bound endonuclease I structure 2PFJ, black traces derived from endonuclease I alone, structure 1MOI. a) T51R1-MDS b) S96R1-MDS. c) K22R1-MDS d) T51R1-RLS e) S96R1-RLS. f) K22R1-RLS.

Mutant of E1	**Time domain/ µs**	**Number of time points**	**Scans**	**Approximate data collection time (hours)**
T51R1_NoDNA	2.33	670	300	12
T51R1_DNA	2.33	670	320	13
T51R1_K67A_NoDNA	2.5	209	798	10
T51R1_K67A_DNA	2.5	209	940	12
S96R1_NoDNA	5.84	309	640	12
S96R1_DNA	5.84	309	726	13
S96R1_K67A_NoDNA	5.84	309	578	11
S96R1_K67A_DNA	5.84	309	695	13
K22R1_NoDNA	6.86	359	564	12
K22R1_DNA	6.86	359	552	12
K22R1_K67A_NoDNA	8.84	459	431	12
K22R1_K67A_DNA	8.84	459	336	9
K22R1_T51R1_NoDNA	3.86	209	915	11
K22R1_T51R1_DNA	3.86	209	940	12

Supplementary table 1 - Data collection parameters for each Endo1 sample.

protein	K_D (nM) E1 in DTT	K_D (nM) E1 with MTSL
E1	1.35 ± 0.17	
E1 K22C	0.2 ± 0.07	2.1 ± 0.5
E1 T51C	0.72 ± 0.14	0.39 ± 0.07
E1 S96C	0.18 ± 0.04	2.1 ± 0.6
E1 K67A	1 ± 0.2	
E1 K22C K67A	0.11 ± 0.02	0.53 ± 0.12
E1T51C K67A	1.2 ± 0.3	1 ± 0.15
E1 S96C K67A	0.76 ± 0.17	3.6 ± 0.5

Supplementary table 2 - Binding of T7 endonuclease (E1) and mutants, to DNA junction 3
Row designations are E1 (wild tyoe T7 endonuclease I) followed by E1 containing the various mutations studied. Column 2 shows Kd values for binding experiments run in the presence of DTT and column 3 showd the Kd values of the various proteins derivitised with MTSSL as appropriate.

Experimental details.
Various quantities of wild type or mutant endonuclease I (typically from 100 nM to 100 pM in serial two fold dilutions) were incubated with 66 pM (5'-^{32}P)-labeled junction 3 (15 base pairs per arm) in a total volume of 12.5 µl in 25 mM Hepes pH7.5, 50 mM NaCl, 1 mM EDTA, 100 µg/ml BSA and 10 mM DTT if indicated for 1 hour at 20ºC. These samples were then mixed with 2.5 µl loading buffer (Promega) and the samples were loaded immediately to a 6% (w/v) polyacrylamide gel in TBE buffer (90 mM Tris-borate pH 8.3, 2 mM EDTA) for 2 hours at 150V. Gels were dried onto 3MM Whatman paper, expose to storage phosphor screens (BAS-IP MP2040, Fuji) and quantified using a Fuji BAS-1500 phosphoimager and Imagegauge v4.2 software. Data were analysed as the fraction of DNA bound (f_{bound}) versus the concentration of protein and were fitted to a two-state model: equation 1 below

$$f_{bound} = \frac{K_D + P_0 + D_0 - \sqrt{(K_D + P_0 + D_0)^2 - 4D_0P_0}}{2D_0} \qquad (1)$$

where total protein and DNA concentration are P_0 and D_0 respectively, and K_D is the dissociation constant.

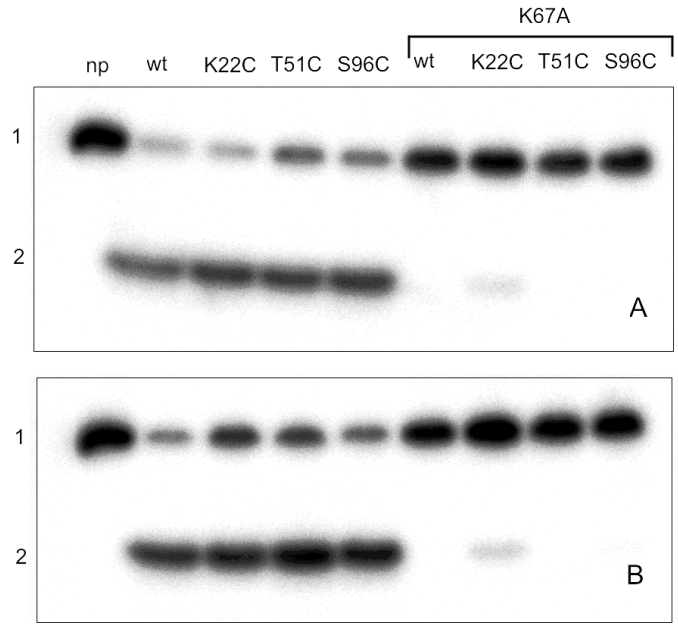

Figure S10 - Analysis of junction cleavage by endonuclease I and the various mutants
Panel A shows the C mutants, panel B shows those same mutants derivitised with MTSSL.
Rows 1 are uncleaved 30mer DNA (X-strand from Junction 3). Rows 2 are cleaved 14mer cleaved DNA.
Columns are; no protein (np); wild type protein (wt); K22C; T51C; S96C. K67A columns are the same as the ones previously described but each protein contains the cleavage inactivating K67A mutation.

Experimental details.
100 nM wild type (wt) or mutant endonuclease I was incubated with 1.6 nM junction 3 radioactively (5'-^{32}P) labeled on the x-strand in 25 mM Hepes pH7.5, 50 mM NaCl, 1mM EDTA, 100 µg/ml BSA and with 10 mM DTT, if indicated, for 1 hour. The reaction was initiated by the addition of 10 mM $MgCl_2$ and carried out at 25ºC for 2 mins. The reaction was terminated by the addition of 50 mM EDTA and 95% formamide. Samples were then heat treated and electrophoresed in a 15% polyacrylamide gel containing 8 M Urea for 45 mins. The gel was exposed to storage phosphor screens (BAS-IP MP2040, Fuji) and phosphoimaged using a BAS-1500 phosphoimager (Fuji).