

Supplementary Figures for

**Molecular Basis of Abasic Site Sensing in Single-Stranded DNA by  
the SRAP domain of *E. coli* yedK**

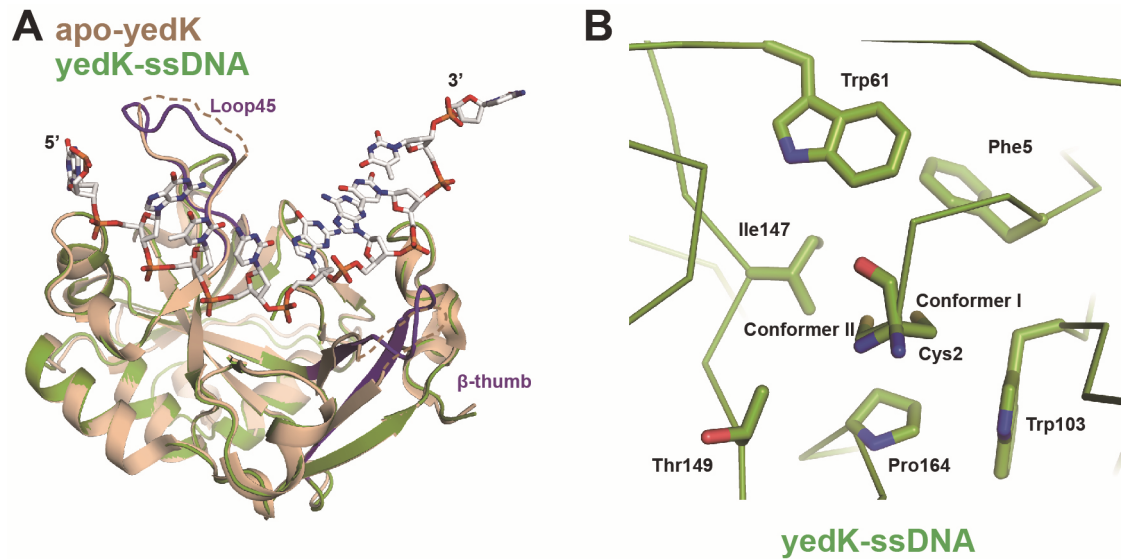
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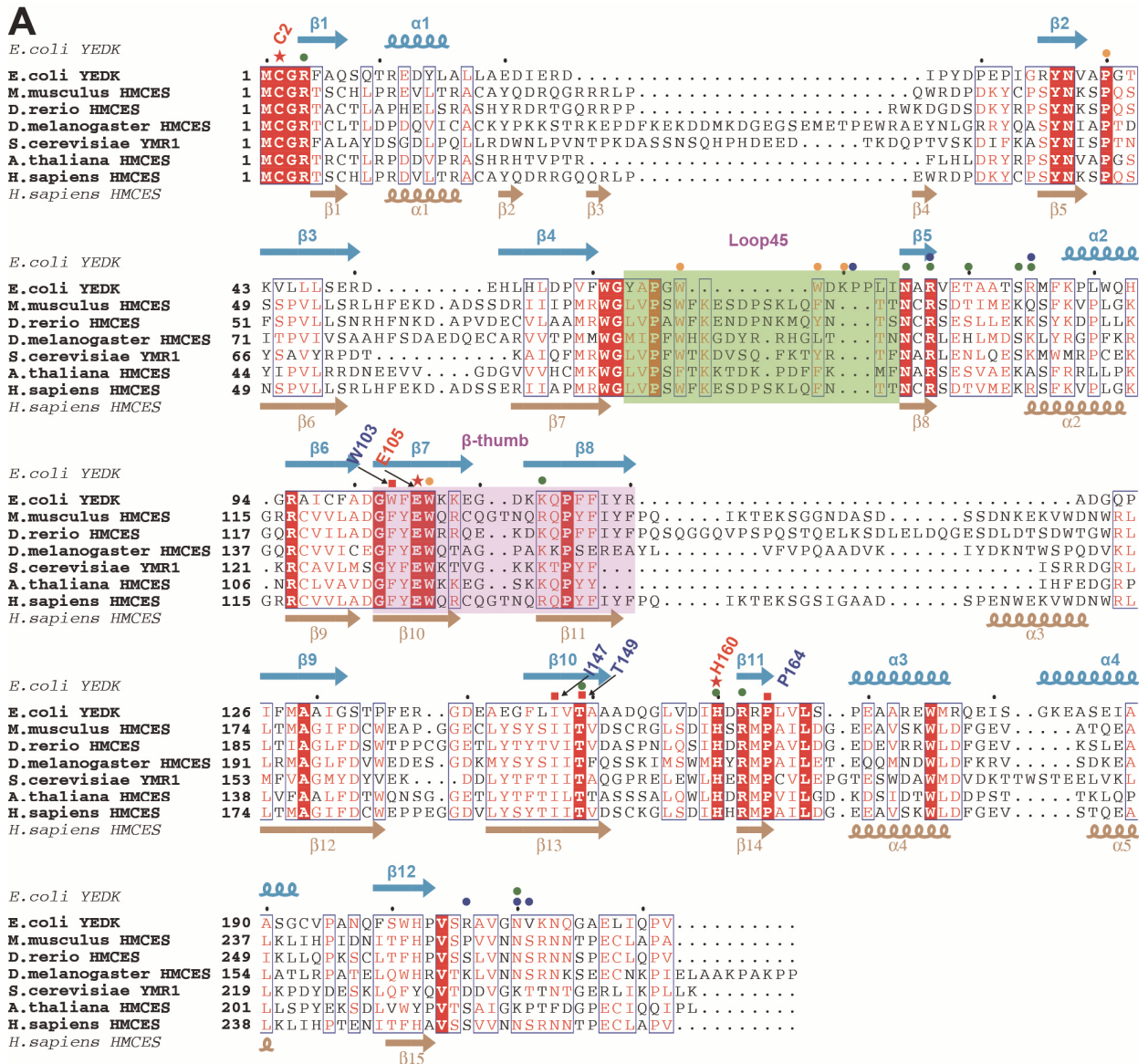
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The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint First Authors.

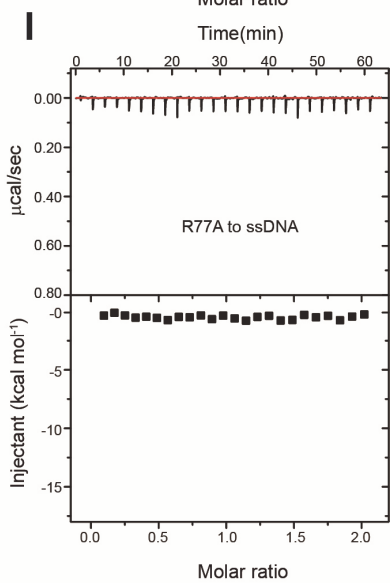
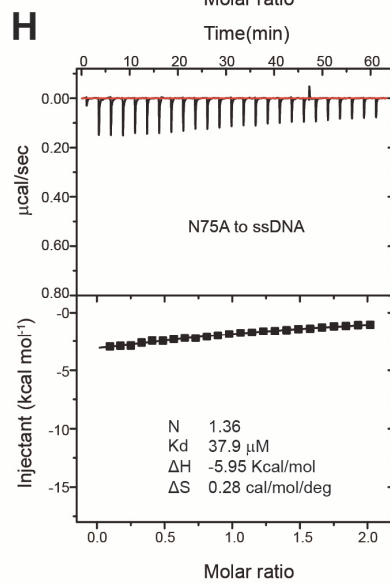
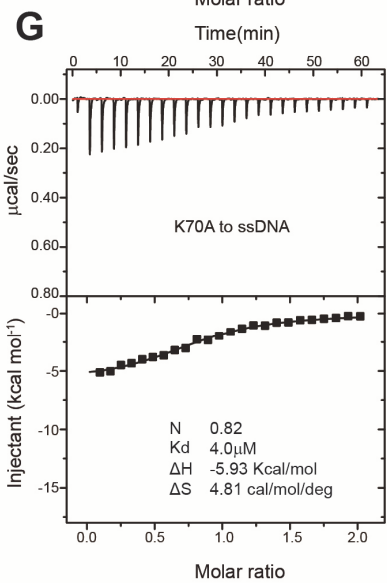
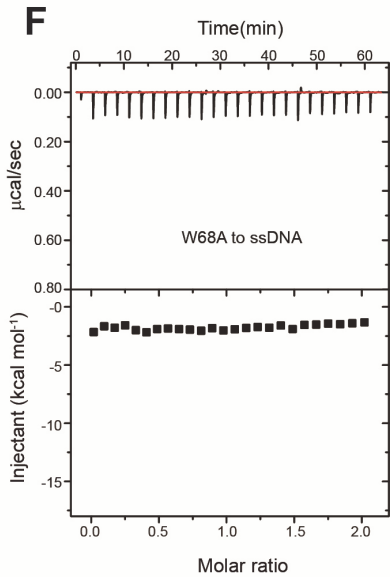
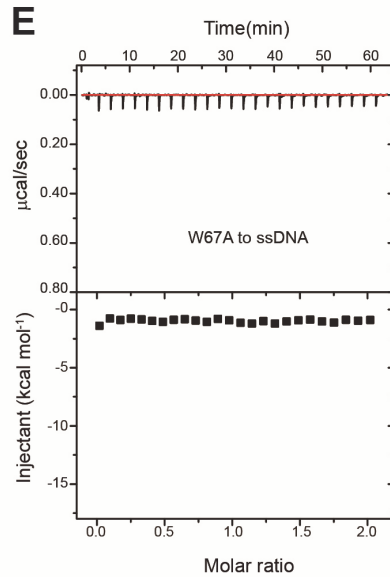
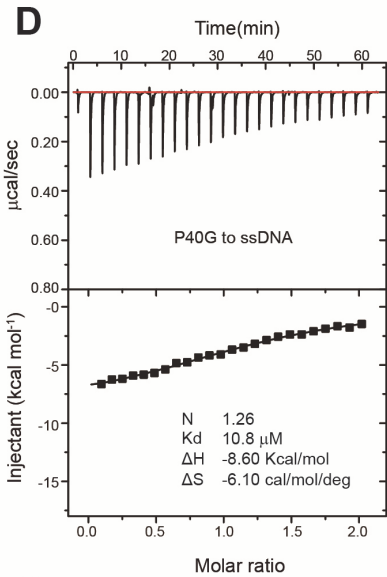
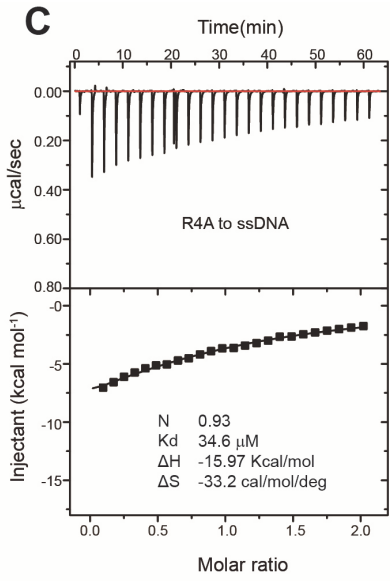
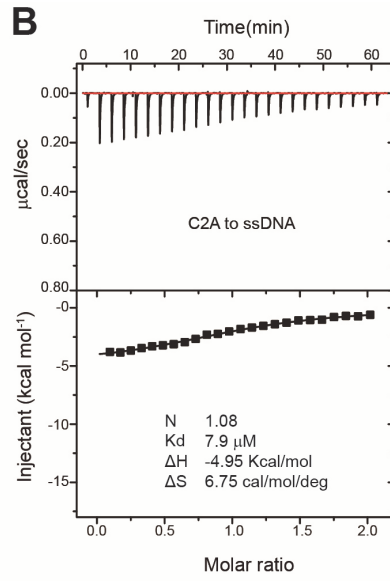
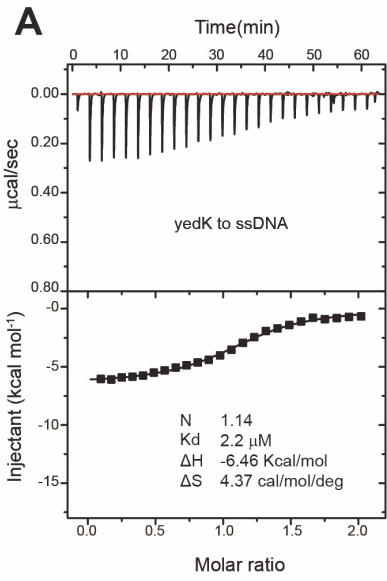


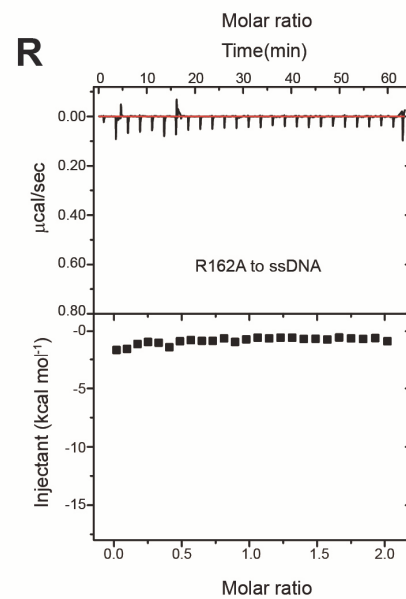
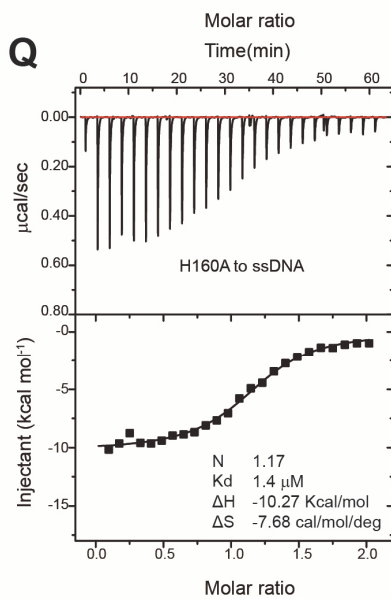
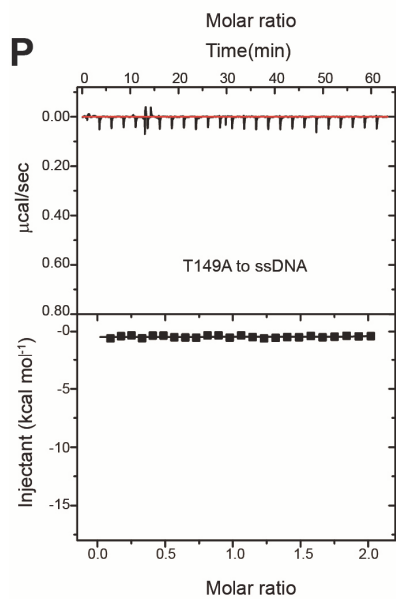
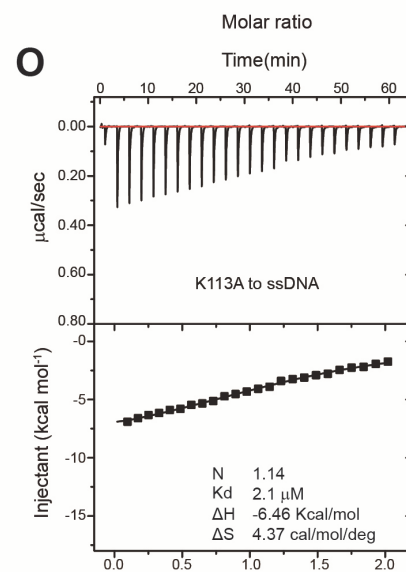
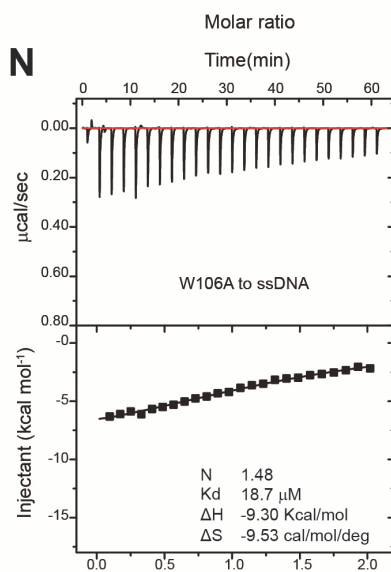
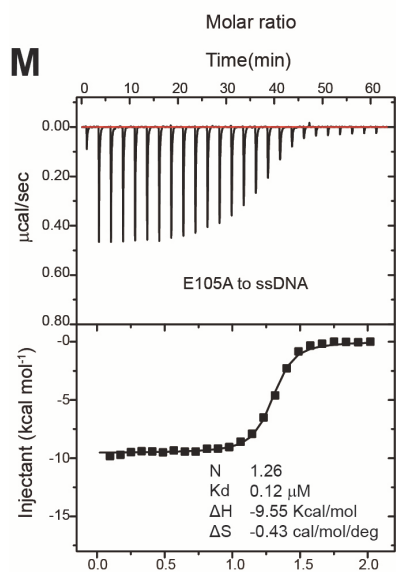
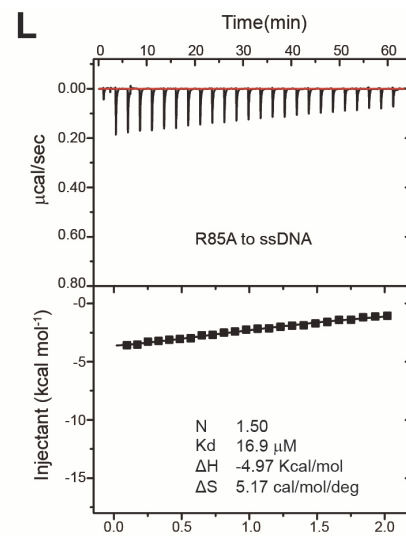
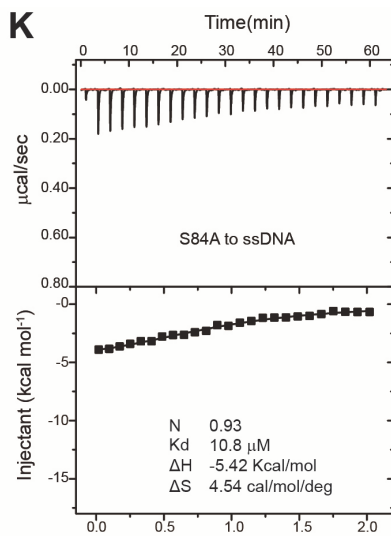
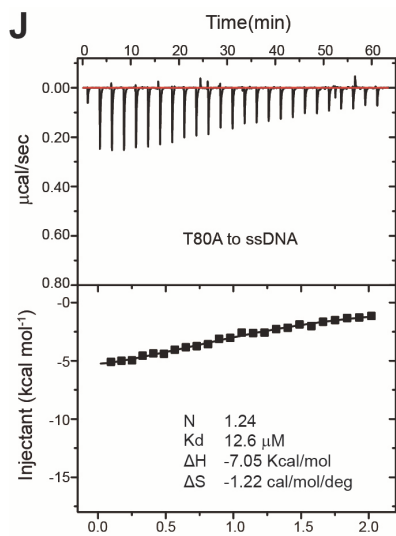
**Supplementary Figure 1.** Structural comparison and characterization of the yedK-ssDNA structure. (A) Superimposition of the apo-yedK and yedK-ssDNA structures, which are shown in wheat and green, respectively. Loop45 and  $\beta$ -thumb of the yedK-ssDNA structure are highlighted in purple, while these two elements of the apo-yedK structure are partially disordered and indicated with dashed lines. (B) The catalytic Cys2 residue of yedK, especially its thiol group, is buried in a hydrophobic pocket. It is noteworthy that the Cys2 residue has two conformers I (major) and II (minor).

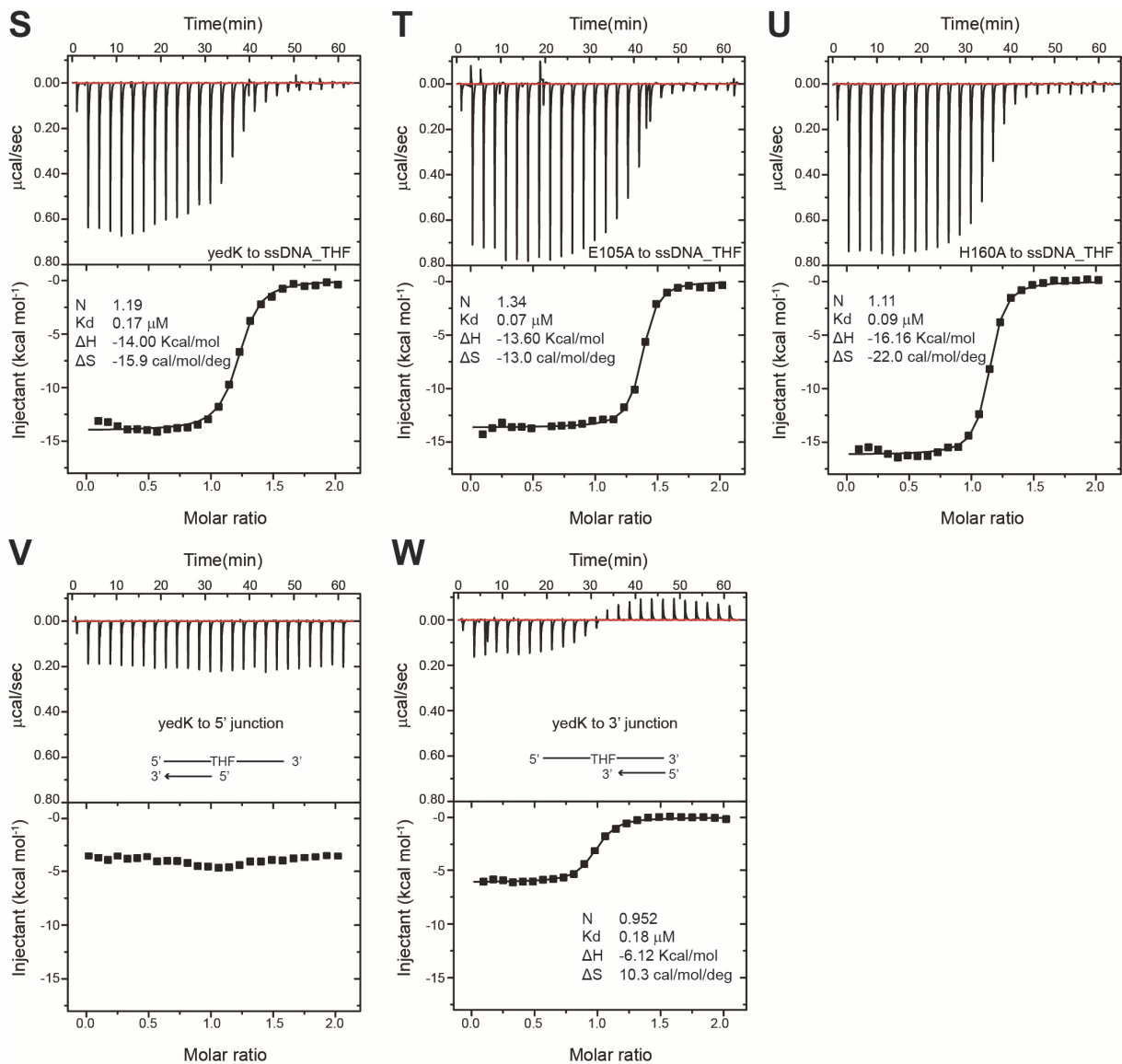


**Supplementary Figure 2.** Sequence alignment of the SRAP domain among different species.

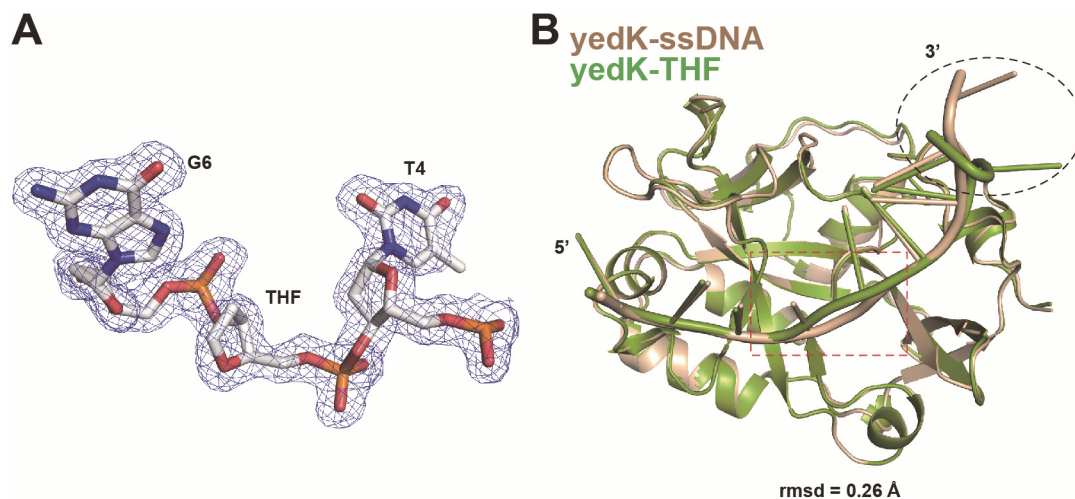
(A) The sequence alignment of the SRAP domain among *E. coli*, mouse, zebrafish, fly, yeast, plant, and human. The secondary structural elements above and below the alignment are derived from the structures of *E. coli* yedK-ssDNA complex (blue; PDB 6KBS) and human HMCES (wheat; PDB 5KO9), respectively. The yedK residues involved in binding ssDNA via side chain and main chain are indicated by green and blue circles, respectively, and the residues interacting via hydrophobic interactions are indicated by yellow circles. The Trp103, Ile147, Thr149, and Pro164 residues forming a hydrophobic pocket for the thiazolidine linkage and the putative catalytic triad are highlighted by red squares and stars, respectively.



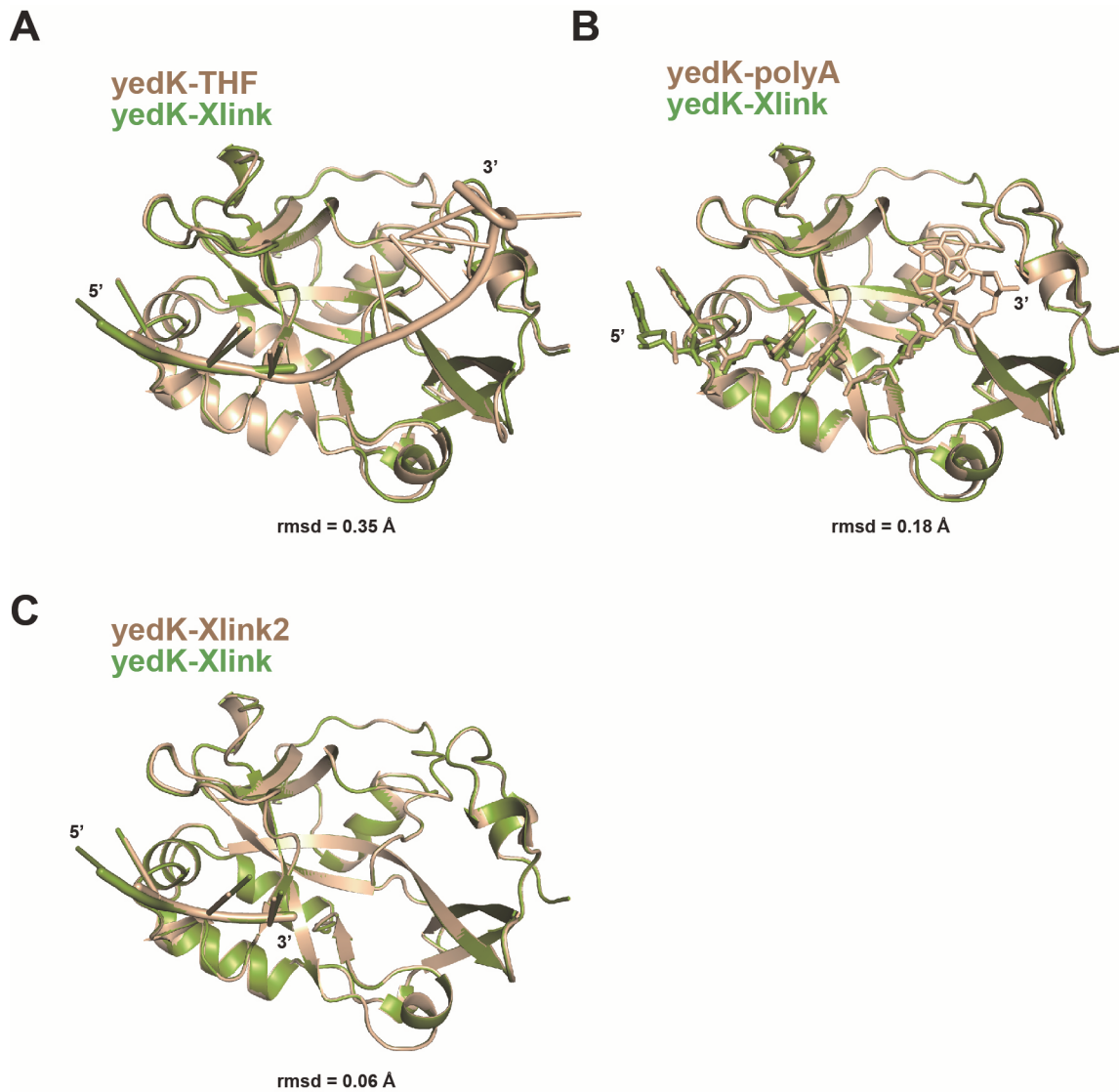




**Supplementary Figure 3.** Results for wild-type yedK and mutants binding different DNA substrates from ITC experiments. (A-W) Upper part of each panel shows the raw data of the heat pulses resulting from each injection. Lower part of each panel shows the integrated heat pulses which are normalized at per mole of injectant and as a function of the molar ratio. Panels A-R, results of binding ssDNA; panels S-U, results of binding ssDNA containing a THF AP site; panels V and W, results of binding 5' and 3' junctions.



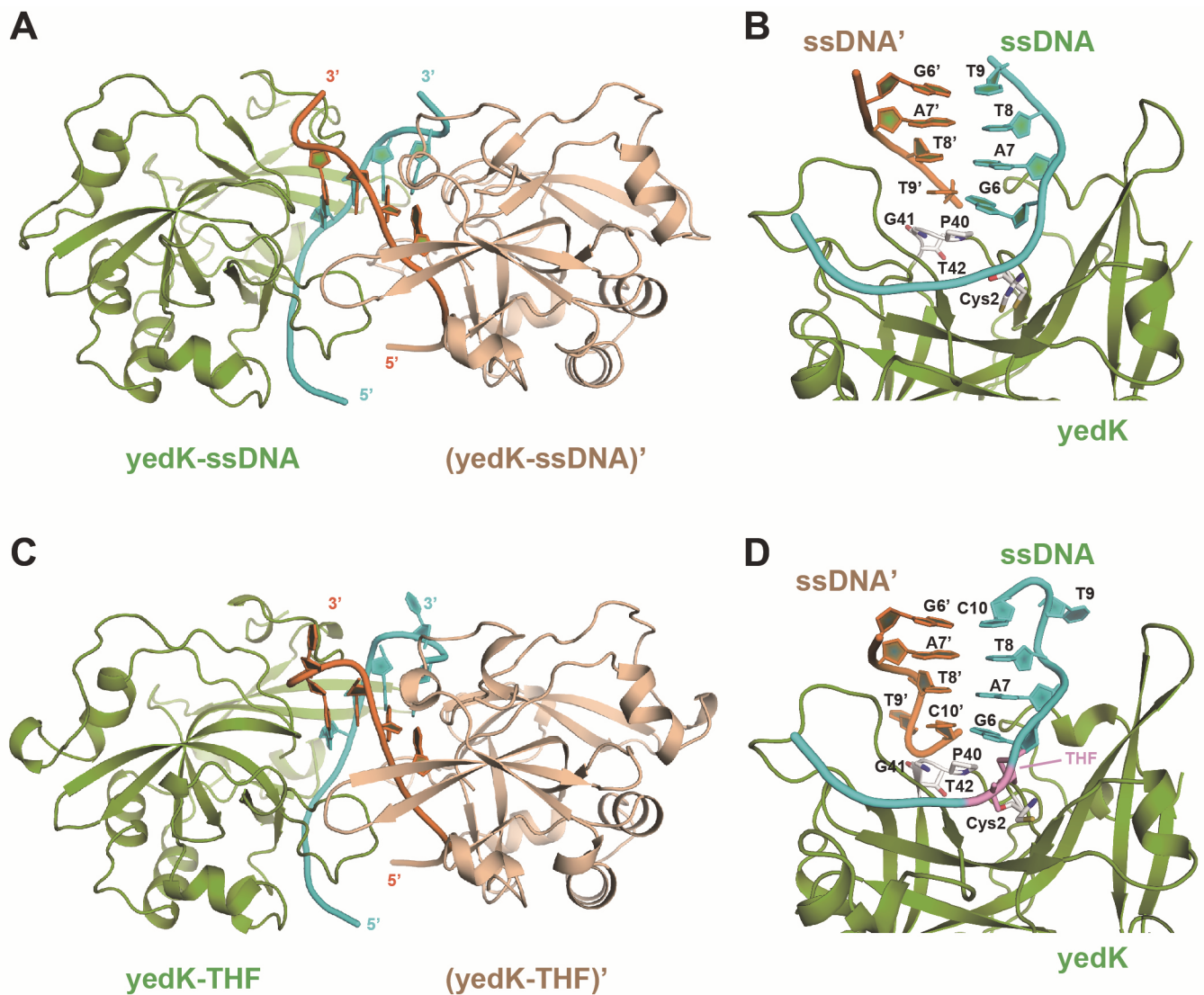
**Supplementary Figure 4.** Structural comparison and characterization of the *yedK*-THF structure. (A) The ssDNA segment including T4-(THF AP site)-G6 is shown in white sticks and their  $2|F_o|-|F_c|$   $\sigma$ -weighted maps are contoured at  $1.0 \sigma$ . The sugar moiety of the THF AP site is highlighted. (B) Superimposition of the *yedK*-THF and *yedK*-ssDNA structures, which are colored in green and wheat, respectively. The difference around the THF AP site is indicated by a red dashed rectangle, and the difference due to the crystal packing is circled by a black dashed line.



**Supplementary Figure 5.** Structural comparisons. (A) Superimposition of the yedK-Xlink and yedK-THF structures, which are colored in green and wheat, respectively. The nucleotides 3' to the thiazolidine linkage are disordered. (B) Superimposition of the yedK-Xlink and yedK-polyA structures, which are colored in green and wheat, respectively. Two more nucleotides that are 3' to the thiazolidine linkage can be modeled in the yedK-polyA structure. (C) Superimposition of the yedK-Xlink and yedK-Xlink2 structures, which are colored in green and wheat, respectively.







**Supplementary Figure 7.** Models of yedK binding to 3' junction. (A) Analysis of the yedK-ssDNA structure reveals that four ssDNA nucleotides (G6-A7-T8-T9) 3' to the AP site are found to form a dsDNA-like structure with G6'-A7'-T8'-T9' in the crystallographic symmetry-related ssDNA' molecule. (B) The model of yedK binding to a 3' ssDNA-dsDNA junction. The G6-T9', A7-T8', T8-A7' and T9-G6' base pairs are observed in the crystal packing in the yedK-ssDNA structure. The conserved residues Pro40, Gly41, and Thr42 of yedK form stacking with the junction. (C) Analysis of the yedK-THF structure reveals that five ssDNA nucleotides (G6-A7-T8-T9-C10) 3' to the AP site are found to form a dsDNA-like structure with G6'-A7'-T8'-T9'-C10' in the crystallographic symmetry-related ssDNA'

molecule. (D) The model of yedK binding to a 3' ssDNA-dsDNA junction. The G6-C10', A7-T8', T8-A7' and C10-G6' base pairs are observed in the crystal packing in the yedK-THF structure, while the T9 and T9' nucleotides are flipped out. The conserved residues Pro40, Gly41, and Thr42 of yedK form stacking with the junction.