Supplemental Information

Identification of one of the AP lyase active sites of topoisomerase V by structural and functional studies

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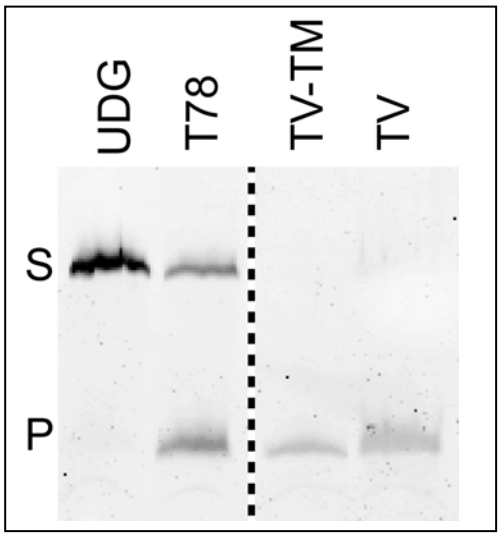
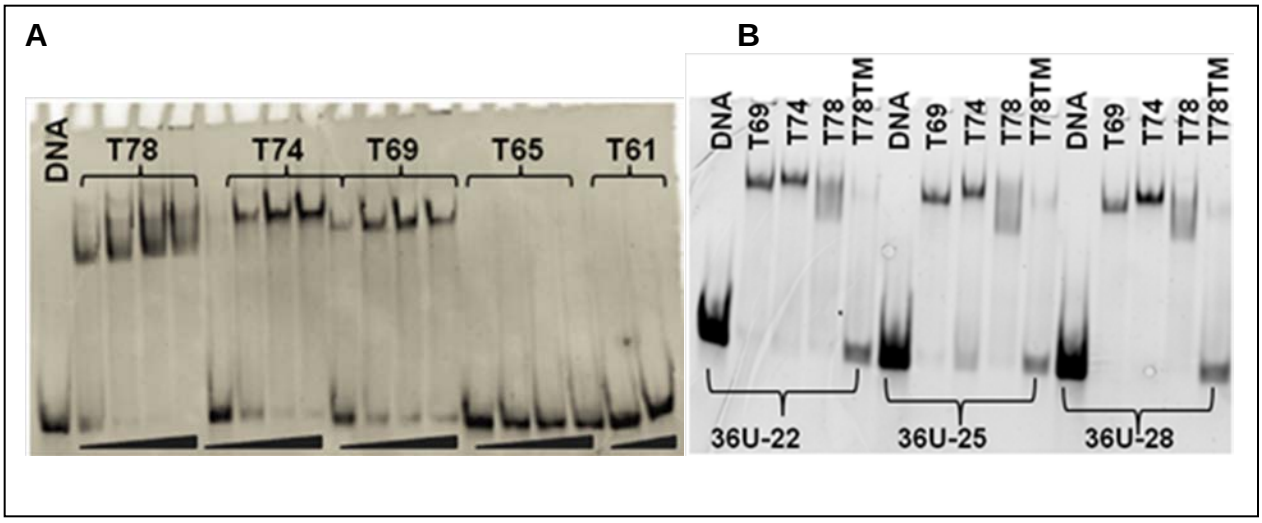
Supplemental Table I. Data collection and refinement statistics

Data Collection	
Space group	$P6_1$
Cell dimensions	a=161.6 Å, b=161.6 Å, c=58.2 Å
Resolution (Å)[a]	29.11 – 2.91 (3.04 – 2.91)
Number of observed reflections	120,493 (14,281)
Number of unique reflections	19,283 (2,264)
Completeness (%)	99.5 (96.5)
Multiplicity	6.2 (6.3)
R_{merge} (%)[b]	6.9 (51.9)
R_{meas} (%)[c]	7.6 (56.6)
$<<I>/\sigma(<I>)>$[d]	20.6 (3.4)
Refinement	
Resolution (Å)	29.11 - 2.91 (2.984 - 2.909)
Number of reflections working/test	18,292/989
R_{work} (%)[e]	20.1(28.7)
R_{free}(%)[f]	26.7 (39.9)
Protein residues/atoms	610/4960
Water molecules	4
Other atoms	1 Zn^{2+}, 3 glycerol molecules
B-factor ($Å^2$)[g]	
Protein atoms (chain)	73.4
Water molecules	22.3
r.m.s. deviations	
bond lengths (Å)	0.008
bond angles (°)	1.04
Ramachandran plot[h]	
Favored regions (%)	95.8
Outliers (%)	0.3

[a]Numbers in parenthesis correspond to highest resolution shell.
[b]$R_{merge}= \sum|I - \langle I\rangle|/\sum I$, where I is the observed intensity and $\langle I\rangle$ the average intensity obtained from multiple measurements.
[c]R_{meas} as described in Diederichs and Karplus (1).
[d]$<<I>/\sigma(<I>)>$ = Mean I_h over the standard deviation of the mean I_h averaged over all reflections in a resolution shell.
[e]$R_{work}= \sum ||F_o| - |F_c||/ \sum|F_o|$, where $|F_o|$ is the observed structure factor amplitude and $|F_c|$ the calculated structure factor amplitude.
[f]$R_{free}= R_{factor}$ based on 5% of the data excluded from refinement.
[g]B-factor= isotropic B-factors without TLS contribution is reported
[h]As reported by Molprobity (2).



Supplemental Figure 1. Topoisomerase V contains two AP lyase sites. AP lyase activity was tested for full length Topo-V with and without the three lysines forming the first AP lyase active site present. The different lanes represent UDG treated DNA control (UDG), wild-type Topo-78 (T78), full length Topo-V containing the K566/570/571A triple mutation (TV-TM) that abolishes the first AP lyase active site, and wild-type Topo-V (TV). The results show clearly the presence of a second repair active site in Topo-V as TV-TM can cleave the abasic DNA whereas the Topo78 triple mutant cannot (Figure 1). P (product) represents the cleaved DNA whereas S (substrate) represents intact, labeled DNA. For the assay, 5'-56FAM modified 49mer DNA with U at the 21st position was annealed to a complementary DNA and then treated with UDG enzyme to create the abasic DNA. This DNA was used as the substrate for the AP lyase experiments. The reaction conditions are the same as in Figure 1, except that the gel was visualized using a GE Healthcare Typhoon scanner. The lanes between T78 and TV-TM were removed as they represent unrelated samples.



Supplemental Figure 2. **DNA binding studies of Topo-V fragments and abasic DNA. A)** Gel shift assay with a 39mer tetrahydrofuran abasic DNA and different Topo-V fragments. DNA was incubated with increasing concentrations of Topo-V fragments at 65°C for 30 minutes and the products were resolved on a 6% native gel following previously described protocols (3). Complex formation is noticed only with those Topo-V fragments (T69, T74, and T78) containing a functional AP lyase active site. **B)** Gel shift assay with a naturally occurring abasic DNA and different Topo-V fragments. A 36mer DNA with U at different positions (22, 25, and 28) was treated with hTDG (human thymine DNA glycosylase) enzyme to create an abasic site. This DNA was incubated with different Topo-V fragments (T69, T74, T78, and T78TM) at 65°C for 30 minutes, followed by 30 mM $NaBH_4$ on ice for 30 minutes. The reaction products were separated on a 6% native gel as described before (3). TM represents the triple mutant (K566/570/571A). The gel clearly shows that the three lysines (K566, K570, and K571) are essential for covalent complex formation.

References

1. Diederichs, K. and Karplus, P.A. (1997) Improved R-factors for diffraction data analysis in macromolecular crystallography. *Nat. Struct. Biol.*, 4, 269-275.
2. Davis, I.W., Murray, L.W., Richardson, J.S. and Richardson, D.C. (2004) MOLPROBITY: structure validation and all-atom contact analysis for nucleic acids and their complexes. *Nucleic Acids Res.*, 32, W615-619.
3. Rajan, R., Taneja, B. and Mondragon, A. (2010) Structures of minimal catalytic fragments of topoisomerase V reveals conformational changes relevant for DNA binding. *Structure*, 18, 829-838.