

## Supplementary Information

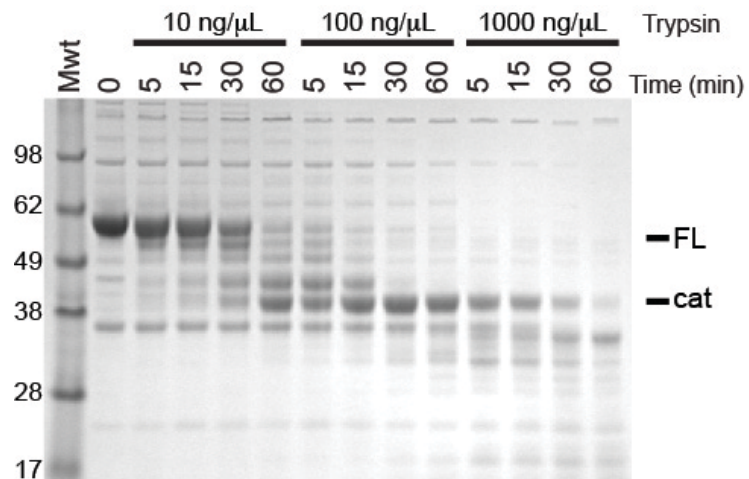
### Mechanism of 5' Topoisomerase II DNA adduct repair by mammalian Tyrosyl DNA phosphodiesterase 2 (Tdp2)

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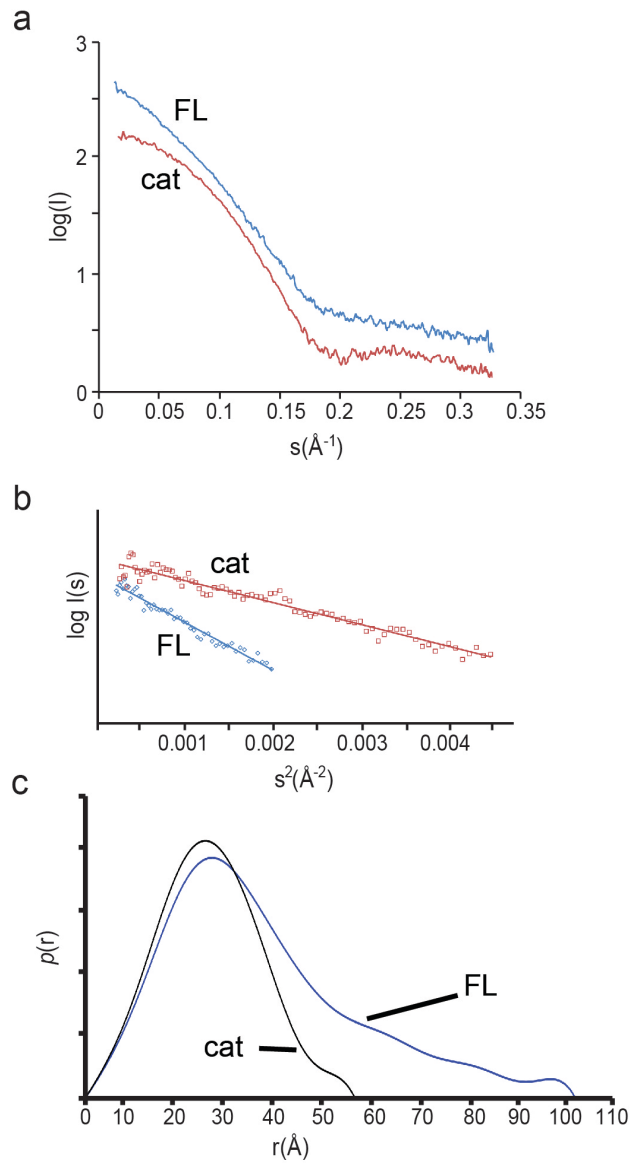
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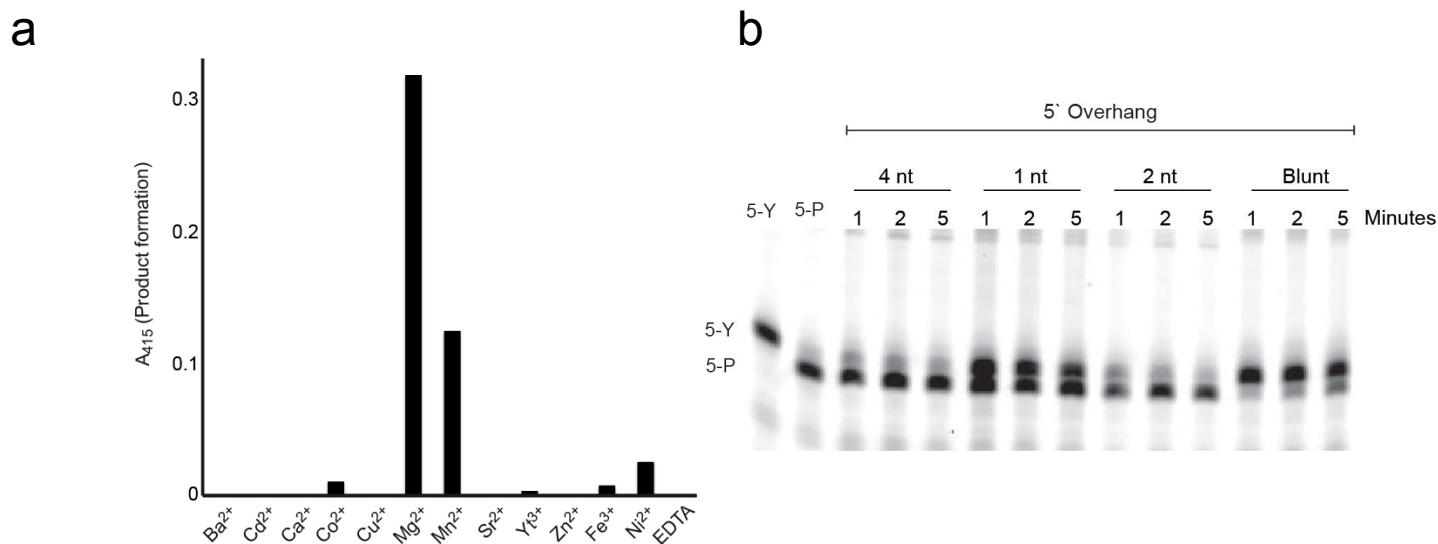


**Supplementary Fig 1. hTdp2 proteolysis.** Full length human Tdp2 protein (FL) was incubated with trypsin, analyzed by SDS-PAGE, and stained with Coomassie blue. The catalytic domain of Tdp2 (cat) is resistant to proteolysis.

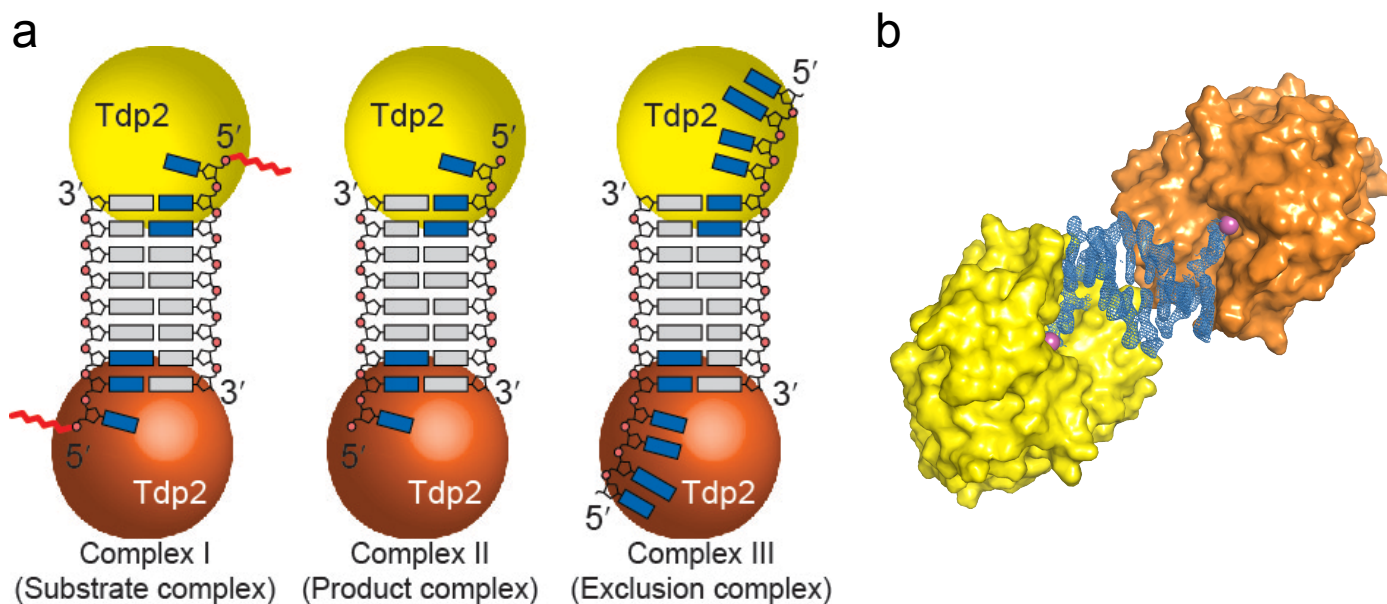




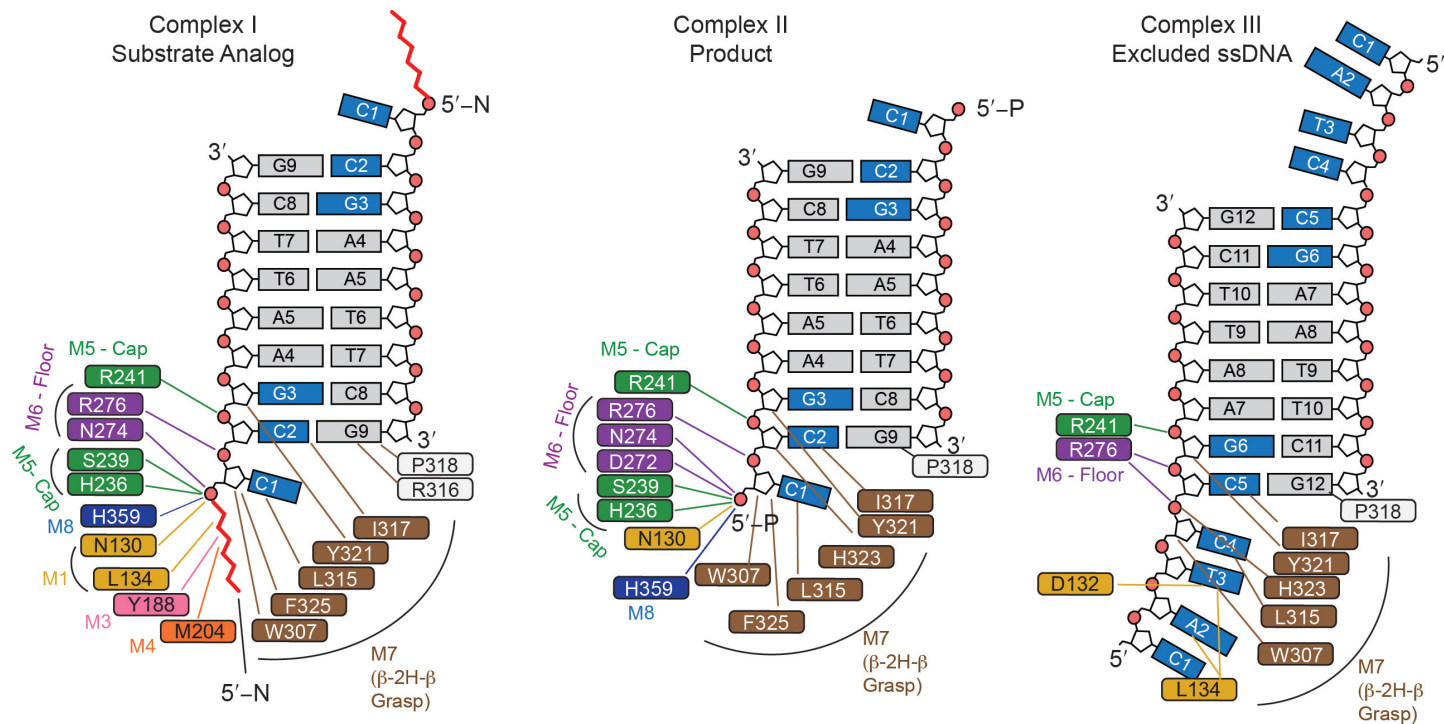
**Supplementary Fig. 3 hTdp2 small angle X-ray scattering SAXS** (a) Merged SAXS profile of full length human Tdp2 (blue) and Tdp2<sup>cat</sup> (red). (b) Guinier plot for full length human Tdp2 (blue) and Tdp2<sup>cat</sup> (red) is linear, indicating the absence of aggregated protein. (c) Small angle X-ray scattering (SAXS) electron pair distribution function calculated from X-ray scattering curve for full length human (FL) and amino acids 108-362 (cat) of human Tdp2.



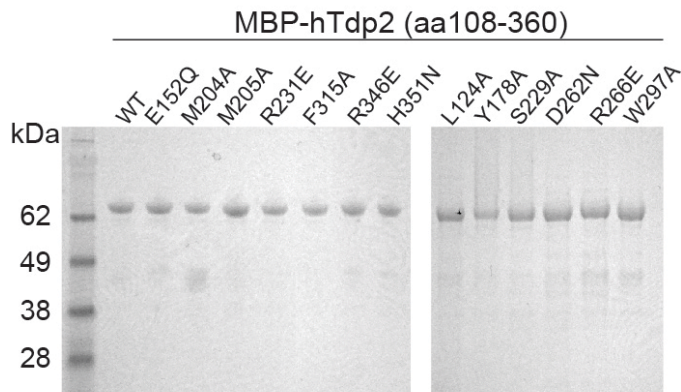
**Supplementary Fig. 4 Tdp2 catalytic activity.** (A) hTdp2 was incubated with the T5PNP substrate and 1 mM of the indicated divalent metal ion for 30 minutes. The reaction product was detected by measuring the absorbance at 415 nm. (b) 1  $\mu$ M substrate DNA (a 16 nucleotide FITC labeled 5'-Y oligo duplexed with a 12, 14, 15, or 16 nucleotide unlabelled strand to form the indicated length of 5' overhang) was incubated with 100nM hTdp2<sup>cat</sup> protein. Products were analyzed on a TBE-urea 15% PAGE.



**Supplementary Fig. 5 mTdp2<sup>cat</sup> DNA complex crystal structures.** (a) mTdp2<sup>cat</sup> was crystallized with self-complementary oligonucleotides. Complex II and complex III contain two Tdp2 molecules bound to opposite ends of the DNA duplex in similar conformations related by non-crystallographic 2-fold symmetry. The asymmetric unit for complex I is analogously arranged, but contains 6 Tdp2 molecules (3 2-ended DNA complexes). (b) The asymmetric unit contains two Tdp2<sup>cat</sup> proteins (yellow and orange) bound to catalytic magnesium ions (purple). The final 2Fo-Fc map (blue) contoured at 1.5 $\sigma$  shows good electron density for the bound product DNA.



**Supplementary Fig. 6. Protein contacts to DNA in complexes I, II and III.** Each mTdp2<sup>cat</sup> protein makes extensive contacts to the 5'-terminus of bound strands (blue DNA bases) from conserved Tdp2 residues. Tdp2 residues are colored according to motif coloring (M1-M8) as in Fig. 3a, 3c and Supplementary Fig. 2.



**Supplementary Fig. 7. SDS PAGE analysis of purified mutant hTdp2-MBP proteins.** Purified MBP-Tdp2<sup>cat</sup> proteins analyzed by SDS-PAGE and stained with Coomassie blue.

### Supplementary Table 1. Small angle X-ray scattering statistics

| Protein                         | Full length hTdp2 | hTdp2 <sup>cat</sup> |
|---------------------------------|-------------------|----------------------|
| Rg - measured from Guinier plot | 29.0 ± 0.4 Å      | 20.2 ± 0.3 Å         |
| Rg - calculated from P(r)       | 29.7 ± 0.2 Å      | 19.7 ± 0.1 Å         |
| Dmax for P(r) plot              | 102 Å             | 56 Å                 |

### Supplementary Table 2. Tdp2 crystallization Oligos

| Oligo Name | 5' modification   | Sequence     | 3' modification |
|------------|-------------------|--------------|-----------------|
| 12SA       |                   | CATCCGAATTCG |                 |
| 5Y-9SA     | phosphotyrosine   | CCGAATTCG    |                 |
| 5N-9SA     | 5'-6-aminohexanol | CCGAATTCG    |                 |

### Supplementary Table 3. Tdp2 duplex DNA substrates

| Figure panel | Substrate name  | Component oligos (sequences in Supp. Table 4) |
|--------------|-----------------|---|
| 1g           | Single-stranded | 5-Y-16  |
| 1g           | 5'-overhang     | 5-Y-16/12R                                    |
| 1g           | Blunt           | 5-Y-16/16R                                    |
| 1g           | 5'-recessed     | 5-Y-16/20R                                    |
| 1f,h,i       | 5'-Y            | 5-Y-16/12R                                    |
| 1f,h,i       | 3'-Y            | 3-Y-16/12R                                    |
| 1f,i         | 5'-N            | 5-N-16/12R                                    |
| 1f,i         | 5'-A            | 5-A-16/12R                                    |
| 2a           |                 | 5N-9SA  |
| 2b           |                 | 5P-9SA - processed by Tdp2                    |
| 2c           |                 | 12SA  |
| 4c           | all             | 5-Y-16/12R                                    |
| Sup. Fig. 4b | 4nt             | 5-Y-16/12R                                    |
| Sup. Fig. 4b | 1nt             | 5-Y-16/15R                                    |
| Sup. Fig. 4b | 2nt             | 5-Y-16/14R                                    |
| Sup. Fig. 4b | Blunt           | 5-Y-16/16R                                    |

### Supplementary Table 4. Tdp2 substrate Oligo sequences

| Oligo Name | 5' modification | Sequence             | 3' modification |
|------------|-----------------|----------------------|-----------------|
| 5-Y-16     | phosphotyrosine | CATCGTTGCCTACCAT     | FITC            |
| 5-N-16     | 6-carbon amine  | CATCGTTGCCTACCAT     | FITC            |
| 5-A-16     | dSpacer         | CATCGTTGCCTACCAT     | FITC            |
| 3-Y-16     | FITC            | ATGGTAGGCAACGATG     | phosphotyrosine |
| 12R        |                 | ATGGTAGGCAAC         |                 |
| 14R        |                 | ATGGTAGGCAACGA       |                 |
| 15R        |                 | ATGGTAGGCAACGAT      |                 |
| 16R        |                 | ATGGTAGGCAACGATG     |                 |
| 20R        |                 | ATGGTAGGCAACGATGTAGT |                 |
| 12         |                 | GTTGCCTACCAT         |                 |