Supplemental Materials for

Enzymatic Excision of Uracil Residues in Nucleosomes Depends on Local DNA Structure and Dynamics[†]

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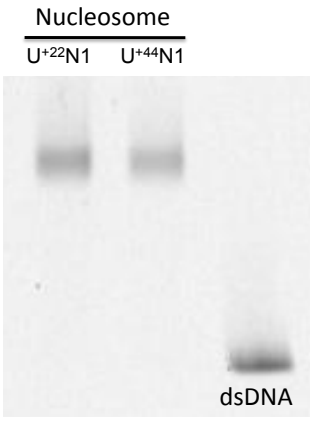

Figure S1. Native 6% polyacrylamide gel electrophoresis of reconstituted nucleosomes containing 147 bp 601 DNA containing site specific uracils ($U^{+22}N1$ and $U^{+44}N1$).

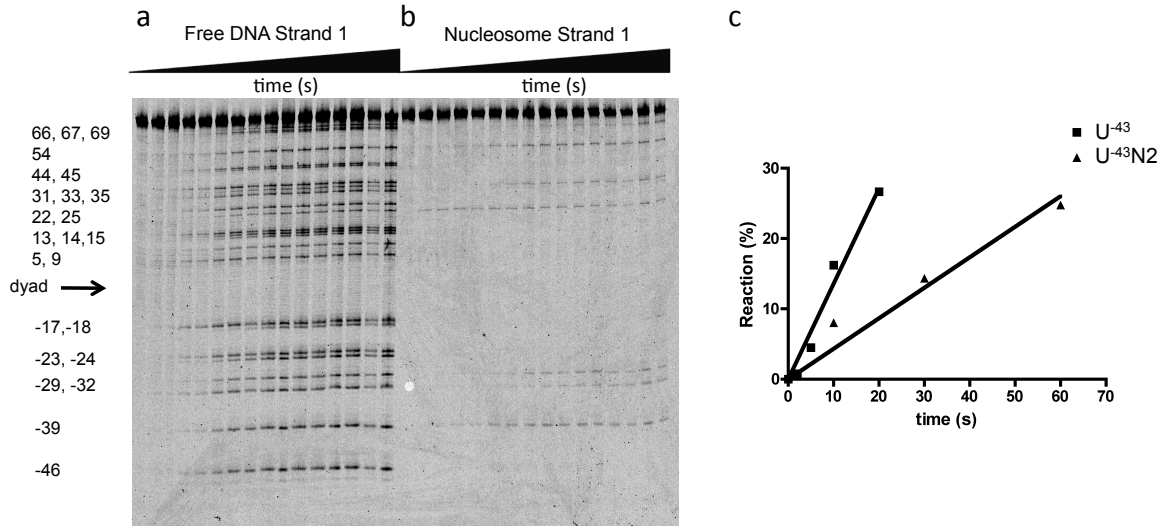

Figure S2. Global analysis of the reactivity of UNG with uracilated 601 DNA in the free state and bound to histones. (a) Denaturing PAGE analysis of the reaction of UNG with free 601 DNA strand 1 with randomly incorporated U/A base pairs (~ 1 U/A base pair per duplex molecule). Strand 1 DNA was enzymatically synthesized using a 5' Fam-labeled DNA primer. Reaction with UNG were quenched at various time points: 0, 5, 10, 20, 30, 60, 150, 300, 600, 900, 1805, 3600 and 5726s (lanes 1-13, left-to-right). (b) The same analysis of NCPs containing 601 DNA that was reacted with UNG for times: 0s, 10s, 32s, 1 min, 2 min, 5 min, 10.9 min, 20 min, 30 min, 45.2 min, 60 min, 90 min, 120 min and 190 min (lanes 1-14, left-to-right). (c) Steady-state initial velocities for sites $U^{-43}N2$ and U^{-43}.

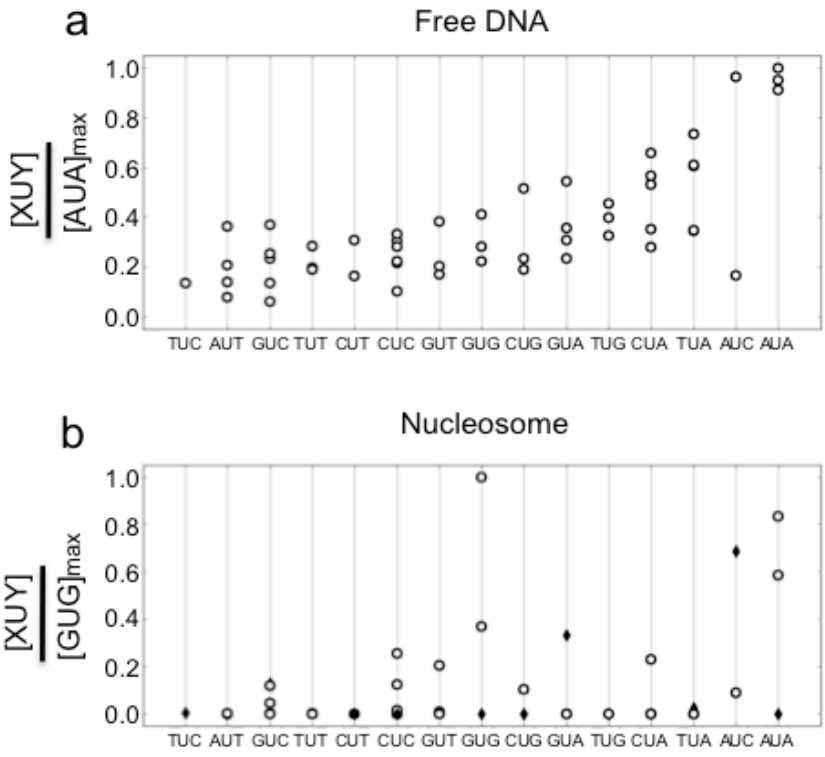

Figure S3. Relative DNA sequence preference of human UNG. (a) Preferences in free 601 DNA. Relative preferences were determined by dividing the reaction rate at each site by the rate at the most reactive site (AUA) (b) Sequence preferences in context of NCPs. Preferences are relative to the most active GUG site. Open circle: exposed uracils; filled diamond: buried uracils.

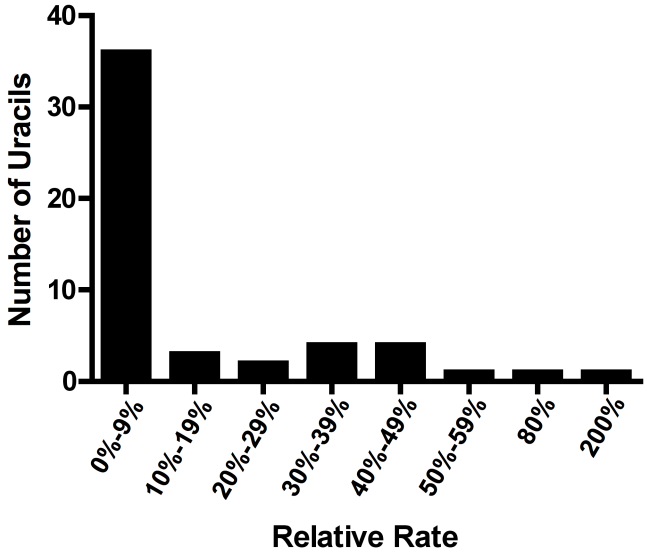

Figure S4. Distribution of relative rates for uracil excision by UNG (relative rate = rate for uracil site in NCP/rate for uracil site free DNA).

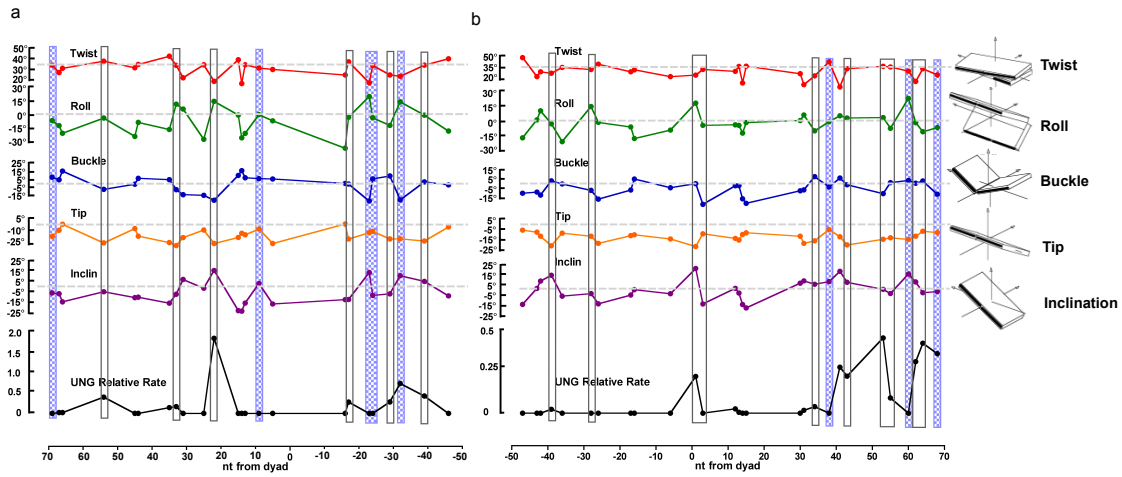

Figure S5. Structural parameters for histone-bound DNA. (a) Strand 1. (b) Strand 2. Vertical open boxes indicate exposed residues and vertical filled boxes indicate buried residues. The dotted lines represent the average values for each parameter in canonical B DNA. The analysis was performed using the program *Curves**.

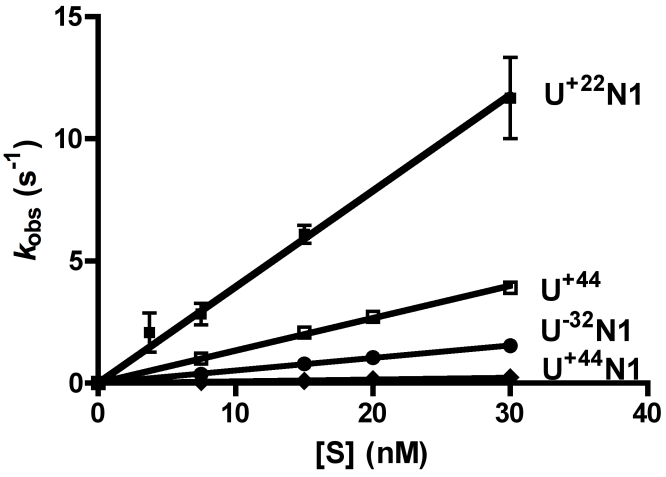

Figure S6. Steady-state kinetic measurements of uracil excision in free 601 DNA (site U^{+44}, strand one), and three sites in the NCP ($U^{+22}N1$, $U^{-32}N1$ and $U^{+44}N1$). The slopes of these plots were used to obtain the second-order rate constants for each reaction (k'^{ss}).

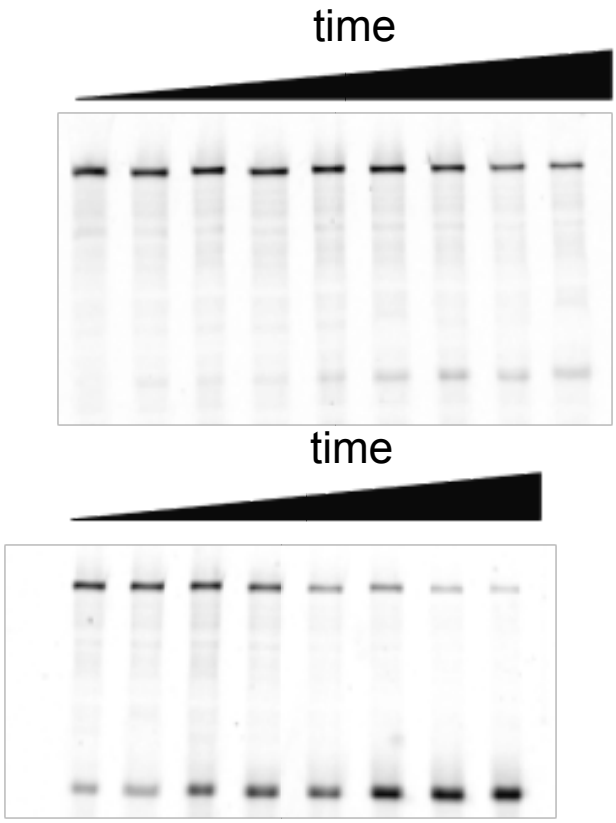

Figure S7. Single-turnover time course for uracil excision at site $U^{+44}N1$ in the NCP. Reaction times (left to right, top to bottom): 0, 2, 5,10, 20, 50, 100, 200, 500 ms; 1, 4, 8, and 40 s; 2, 6, 30 min and 2h. The reaction contained 20 nM $U^{+44}N1$ and 200 nM UNG.

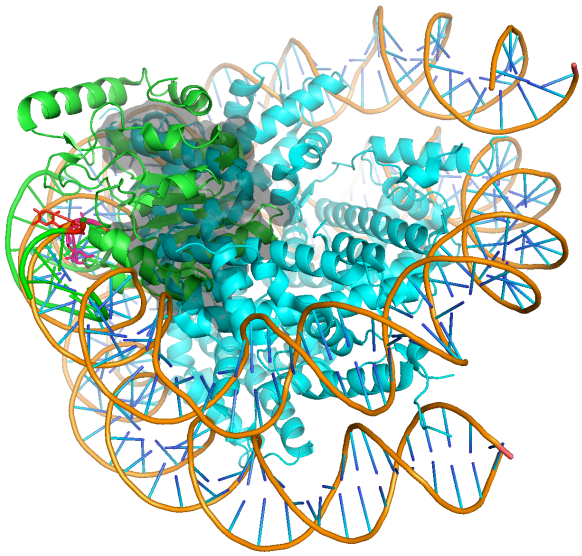

Figure S8: Superposition of the DNA segment of the UNG-DNA complex (PDB2OXM) on the DNA of nucleosome (PDB 3MVD). The flipped base B5 in UNG-DNA complex matches the position of $U^{-32}N1$ on the nucleosome DNA (red stick residues). Major clashes are observed between the enzyme and the histone (transparent black region).

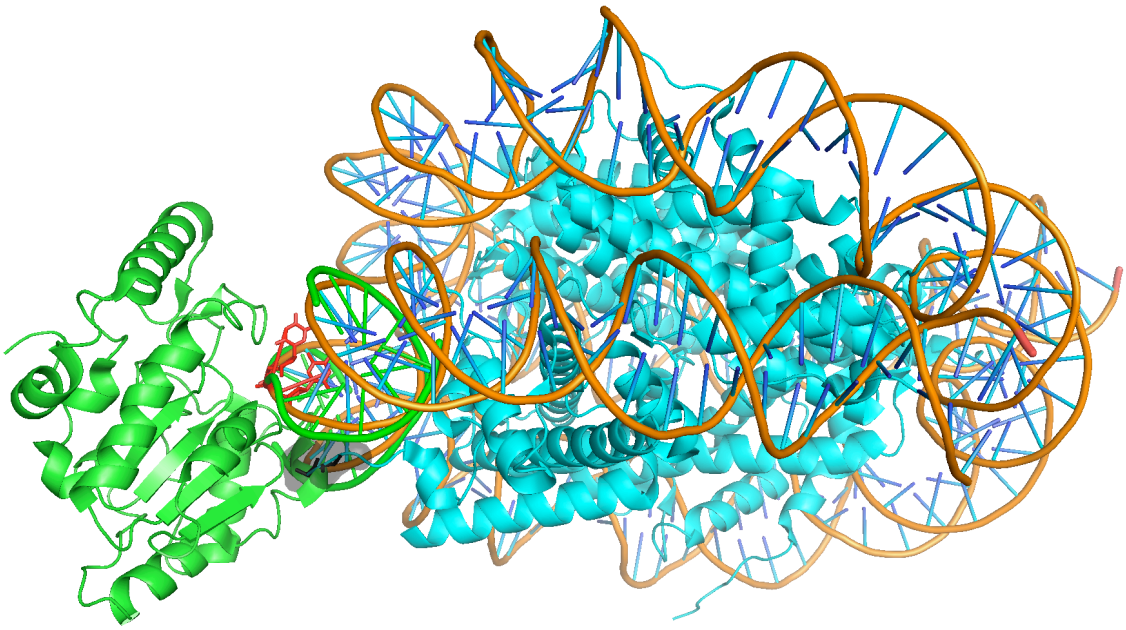

Figure S9: Superposition of the DNA segment of the UNG-DNA complex (PDB 2OXM) on the DNA of nucleosome (PDB 3MVD). The flipped base B5 in UNG-DNA complex matches the position of $U^{+44}N1$ on the nucleosome DNA (red stick residues). Severe clashes are observed between the enzyme residues 270-278 and the N-terminus residues of chain G in the histone (transparent black region).