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Supporting Information

Repair Efficiency of Clustered Abasic Sites by APE1 in Nucleosome Core Particles is Sequence and Position Dependent

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Supplementary Figure S1

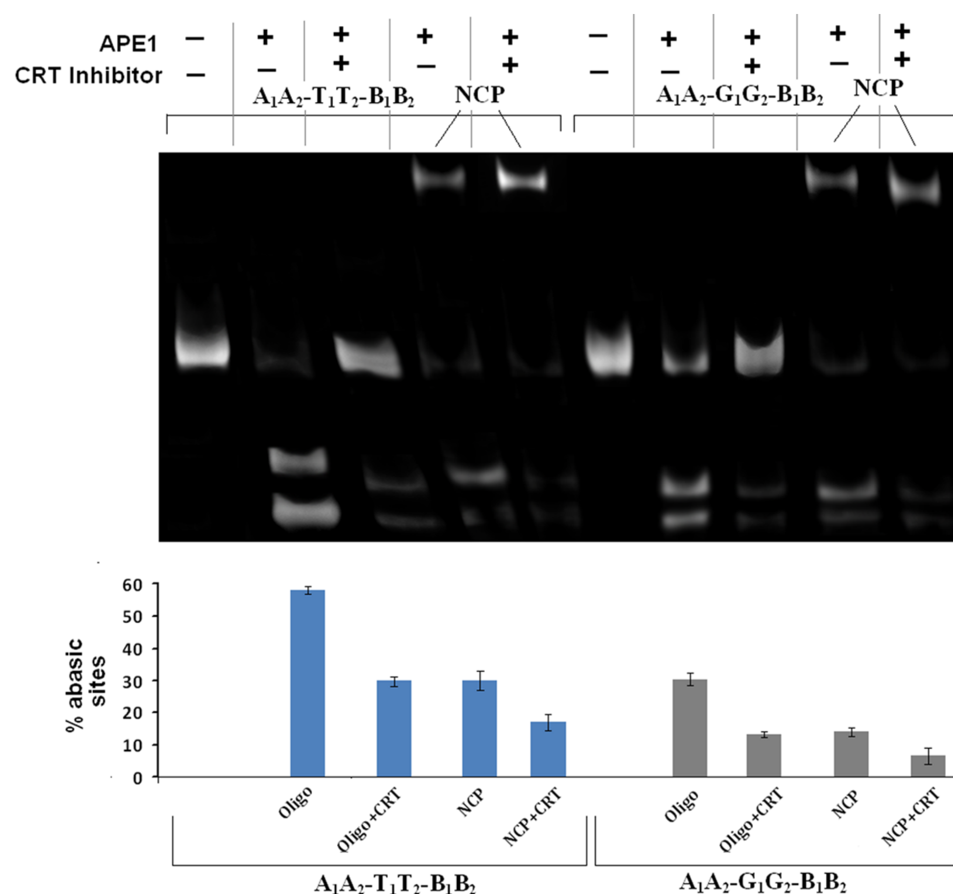

Figure S1 CRT0044876 Mediated APE1 Inhibition in **1)** A_1A_2-T_1T_2-B_1B_2 **2)** A_1A_2-T_1T_2-B_1B_2 + APE1 **3)** A_1A_2-T_1T_2-B_1B_2 + APE1+CRT0044876 **4)** A_1A_2-T_1T_2-B_1B_2 NCP + APE1 **5)** A_1A_2-T_1T_2-B_1B_2 + APE1+CRT0044876 **6)** A_1A_2-G_1G_2-B_1B_2 **7)** A_1A_2-G_1G_2-B_1B_2 + APE1 **8)** A_1A_2-G_1G_2-B_1B_2 + APE1+ CRT0044876 **9)** A_1A_2-G_1G_2-B_1B_2 NCP+APE1 **10)** A_1A_2-G_1G_2-B_1B_2 NCP+APE1+ CRT0044876

Supplementary Figure S2

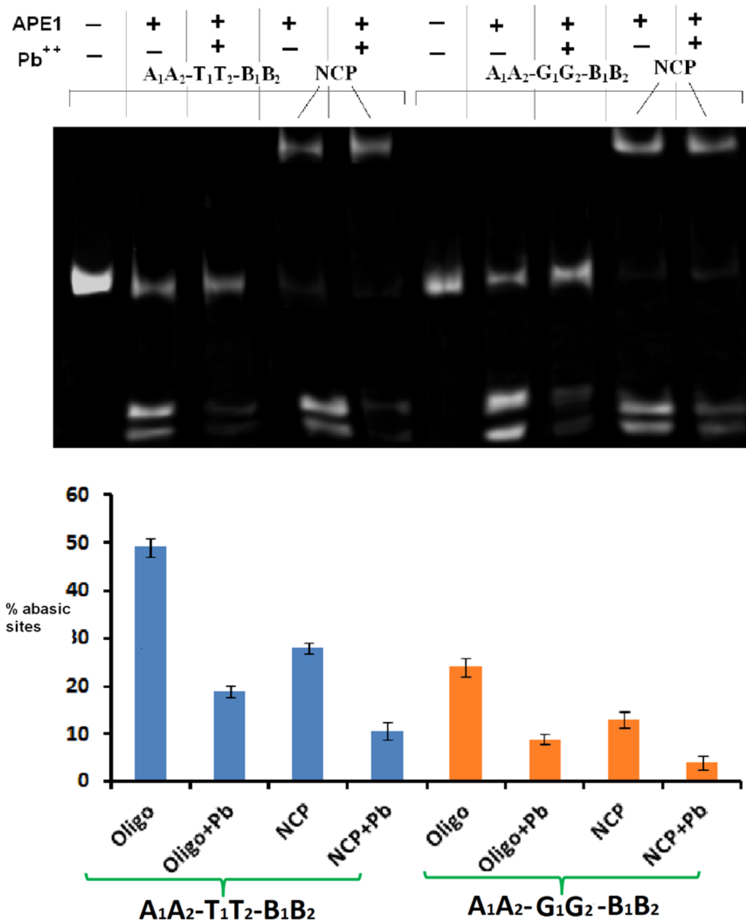

Figure S2 Pb^{2+} Mediated APE1 Inhibition in **1)** A_1A_2-T_1T_2-B_1B_2 **2)** A_1A_2-T_1T_2-B_1B_2 + APE1 **3)** A_1A_2-T_1T_2-B_1B_2 + APE1+ Pb^{2+} **4)** A_1A_2-T_1T_2-B_1B_2 NCP + APE1 **5)** A_1A_2-T_1T_2-B_1B_2 + APE1+ Pb^{2+} **6)** A_1A_2-G_1G_2-B_1B_2 **7)** A_1A_2-G_1G_2-B_1B_2 + APE1 **8)** A_1A_2-G_1G_2-B_1B_2 + APE1+ Pb^{2+} **9)** A_1A_2-G_1G_2-B_1B_2 NCP+APE1 **10)** A_1A_2-G_1G_2-B_1B_2 NCP+APE1+ Pb^{2+}

Supplementary Figure S3

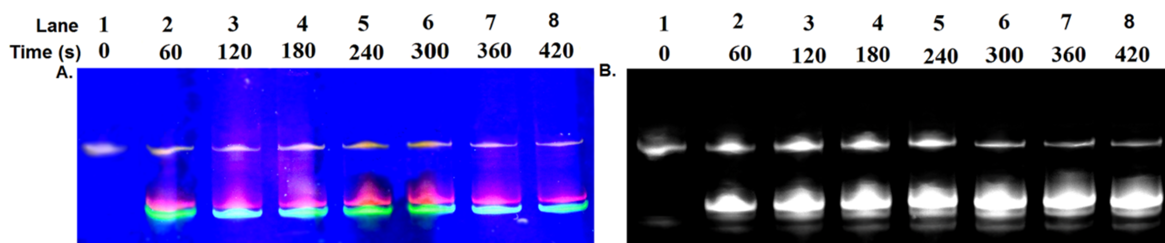

Figure S3 Evaluation of abasic site cleavage with respect to reaction time of linear A_1A_2-T_1T_2-B_1B_2 sequence with APE1 (A) 25 % Denaturing PAGE gel representing true color image of reaction of APE1 enzyme (2 units) with A_1A_2-T_1T_2-B_1B_2 (0.1 μM) in 0- 420(s) (B) SYBR Gold stained gel where the unlabeled lane from right hand side denotes ladder (100 bp), All other lanes are same as A.

Supplementary Figure S4

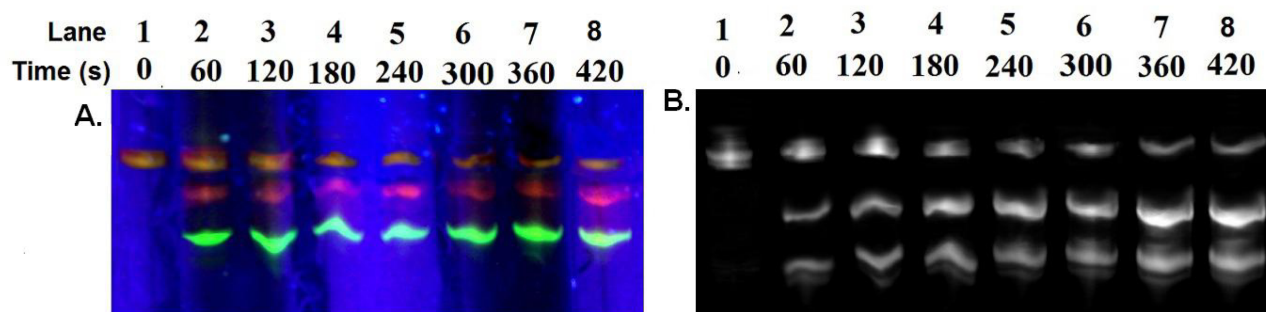

Figure S4 Evaluation of abasic site cleaved in A_1A_2-G_1G_2-B_1B_2 sequence
A) The 25 % Denaturing PAGE gel represents true color image of reaction of APE1 enzyme (2 units) with A_1A_2-G_1G_2-B_1B_2 (0.1 μM) in 0- 420(s) B) SYBR Gold stained gel where the unlabeled lane from right hand side denotes ladder (100 bp), All other lanes are same as A.

Supplementary Figure S5

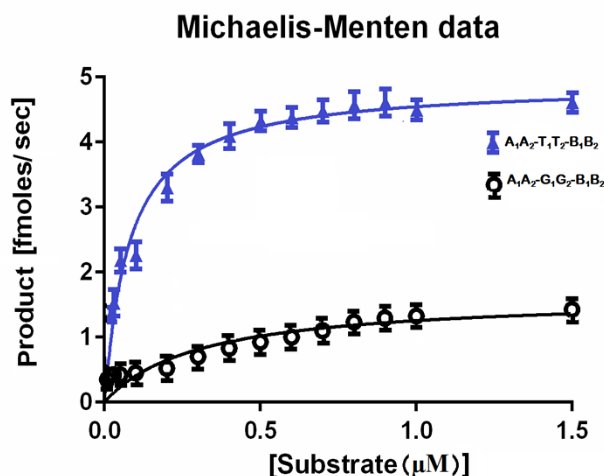

Figure S5 Concentration dependent cleavage activity of APE1 enzyme on linear A_1A_2-T_1T_2-B_1B_2 and A_1A_2-G_1G_2-B_1B_2 sequences with substrate concentration ranging from 0.01 to 1.5 μM.

Enzyme Kinetic Studies

For determination of enzyme kinetics parameters, the concentration of DNA having clustered abasic sites in A_1A_2-T_1T_2-B_1B_2 and A_1A_2-G_1G_2-B_1B_2 was varied from 0.01 μM to 1.5 μM. The APE1 enzyme concentration used was 0.3 nM (0.11 ng) (M.Wt of APE1 35.5 KDa) for a single analysis in a total volume of 10 μL reaction mixture. The reaction mixture having DNA was incubated with APE1 enzyme for time intervals ranging from 30-1800 s. The reaction was quenched using stop buffer (formamide, SDS and EDTA in bromophenol blue) in dry ice. 2 μL of proteinase k (5μg/ μL) was added to dislodge the protein DNA complex and immediately followed by denaturing PAGE analysis. The cleavage products are described in "Calculation of cleavage of abasic sites" under materials and methods section. Initial velocity (V_o) of the reaction was calculated from the percentage cleavage of abasic sites by APE1 enzyme obtained from the PAGE image quantification. Different initial velocity values were obtained for different substrate concentration (0.01 μM to 1.5 μM). Non Linear Fitting method was used to plot the values. Initial velocity values were calculated from reaction where not more than 15 % of the substrate has been converted to product in order to maintain the accuracy of the Michaelis Menten equation. Values of $K_{m,app}$ and $k_{cat,app}$ were subsequently deduced from the plot of initial velocity values vs. substrate

concentration. To avoid the association of one substrate molecule with many protein molecules, low enzyme to substrate ratio was taken. The error bar showed in non-linear curve fitting graph is representative of three different experiments that were conducted on three different days to ensure the reliability of the results.

Supplementary Table ST1

Sl. No.	Name of Sample+ APE1 inhibitor CRT0044876	K (M^{-1})
1	A_1A_2-T_1T_2-B_1B_2	1.35×10^4
2	A_1A_2-T_1T_2-B_1B_2 NCP	3.64×10^3
3	A_1A_2-G_1G_2-B_1B_2	2.50×10^3
4	A_1A_2-G_1G_2-B_1B_2 NCP	1.26×10^3

Table ST1 Thermodynamic parameters for APE1 binding to abasic DNA as determined by ITC. Values represent mean SD determined from at least three independent experiments.

Supplementary Table T2

Sequence	$k_{cat,app}$ **(s−1)**	$K_{m,app}$ **(nM)**	$K_{cat,app}/K_{m,app}$ **(Ms−1)**	$V_{max,app}$ **(fmol/ s)**
A_1A_2-T_1T_2-B_1B_2	1.6	78.1	20.4×10^6	4.927
A_1A_2-G_1G_2-B_1B_2	0.5	305.7	1.73×10^6	1.638

Table ST2 Enzyme kinetic parameters for APE1 enzyme, *i.e.*, $V_{max, app}$, $K_{m, app}$, $k_{cat, app}$, and $k_{cat, app}/K_{m, app}$ for A_1A_2-T_1T_2-B_1B_2 and A_1A_2-G_1G_2-B_1B_2