Human AP-endonuclease (Ape1) activity on telomeric G4 structures is modulated by acetylatable lysine residues in the N-terminal domain

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SUPPLEMENTARY FIGURES

Supplementary figure S1

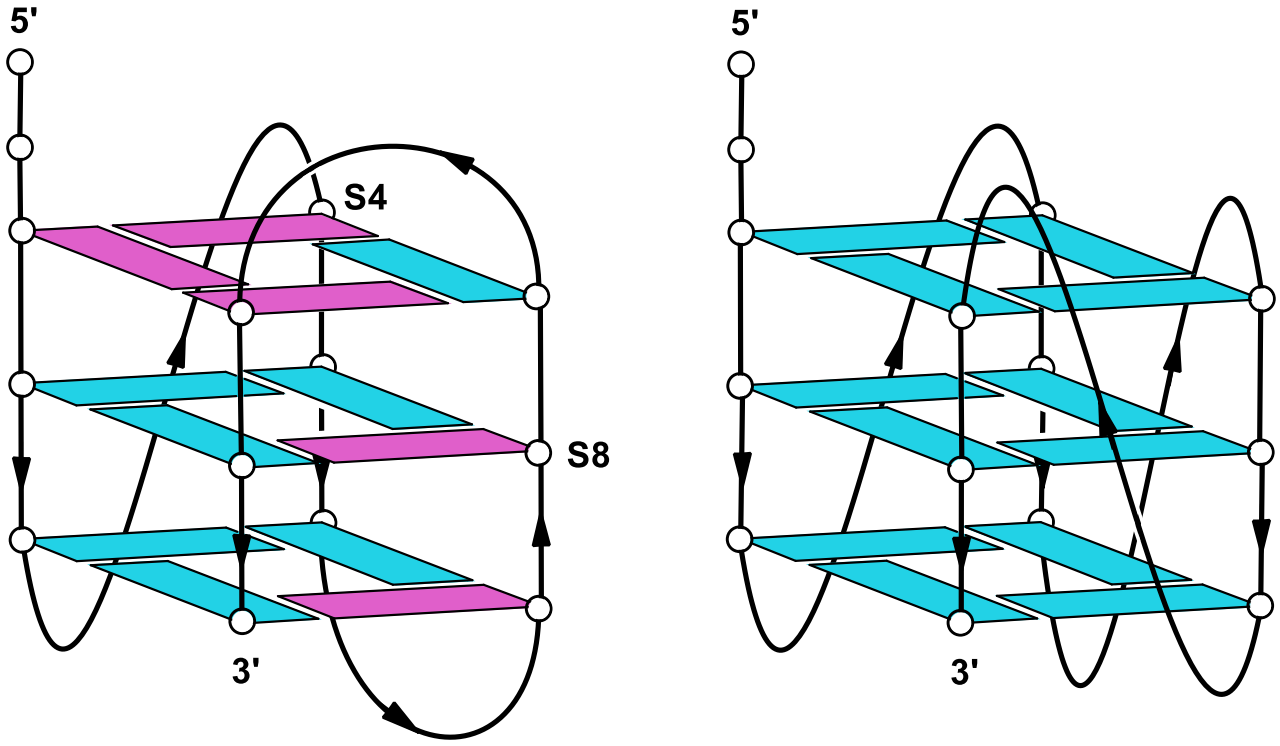

Fig. S1- **Schematic representations of G-quadruplex structures assumed by the oligonucleotides used in this work.**
Hybrid G-quadruplex (left) formed by the original sequence and the parallel conformation (right) adopted in molecular crowding conditions. Guanosines adopting *anti* and *syn* glycosidic conformations are indicated in light blue and purple, respectively. For clarity, the loop residues have been omitted. S4 and S8 indicate the positions in which abasic sites have been introduced. See the main text for detail.

Supplementary figure S2

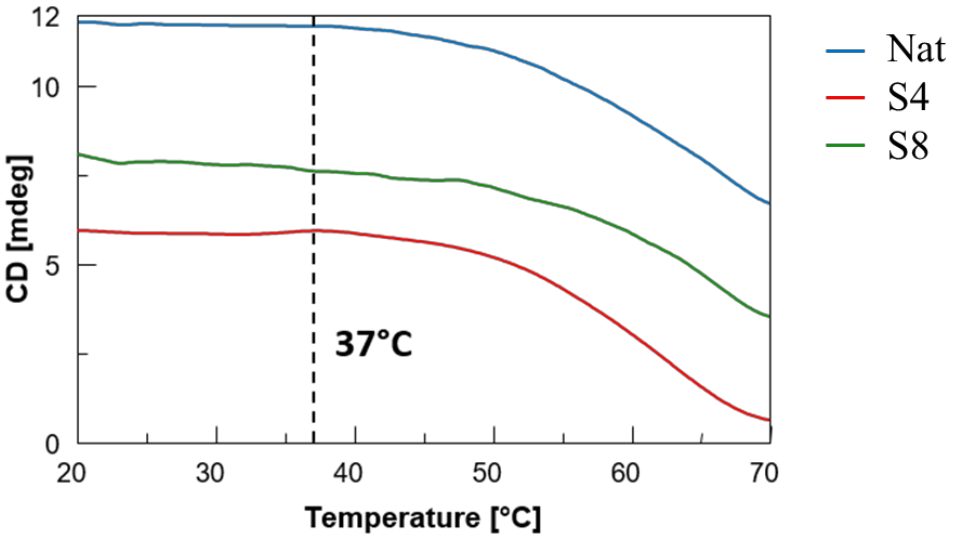

Fig. S2- **Thermal analysis by CD melting for the G4 oligonucleotides used in this study.**
CD melting profiles of each telomeric oligonucleotide acquired at the maximum CD λ values for each sample in a buffer containing KCl 5 mM, Tris 5 mM and $MgCl_2$ 1 mM.

Supplementary figure S3

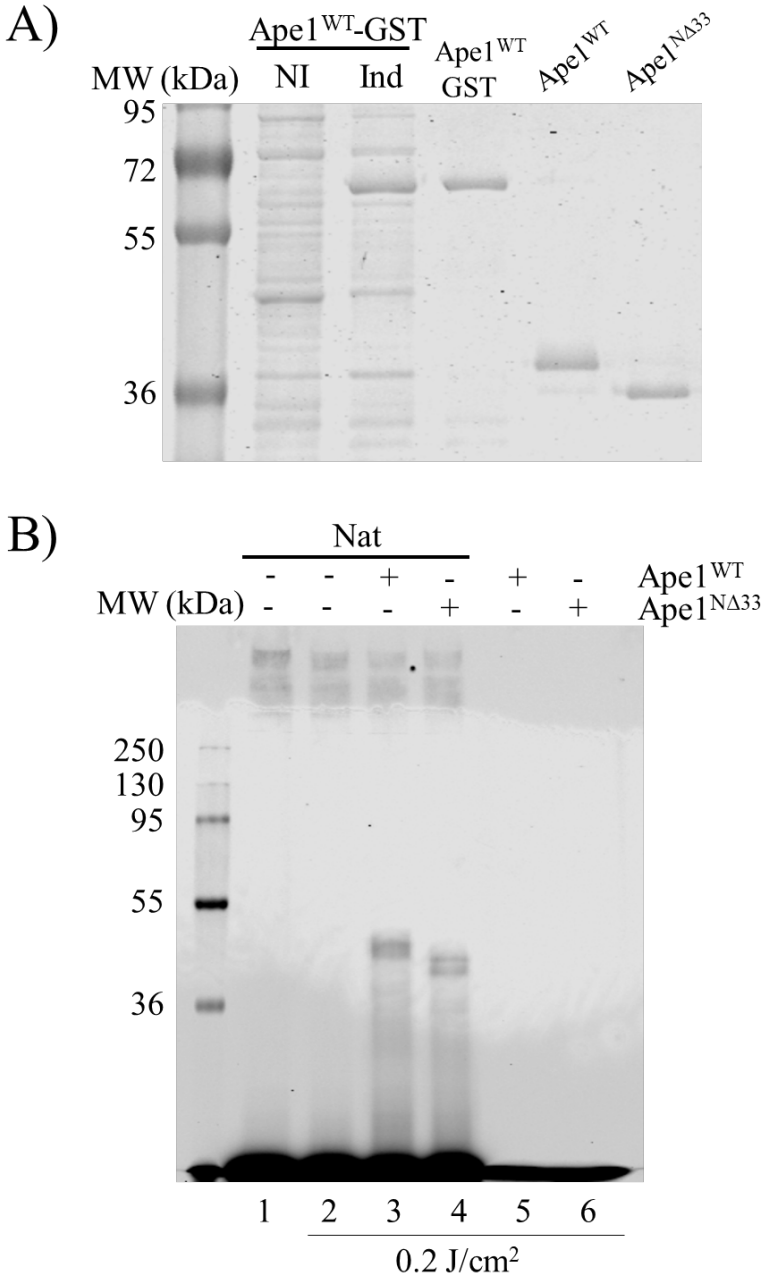

Fig. S3- **Recombinant Ape1 protein check and cross-linking analysis of G4 interaction.**
(A) Gel analysis of human purified recombinant proteins used in this study. Proteins were separated onto 10% SDS-PAGE followed by Coomassie brilliant blue staining. 5 µg loaded of each bacterial lysate ("NI" means not IPTG-induced bacteria and "Ind" means IPTG-Induced bacteria); 0.5 µg Loaded of each purified protein. The molecular weight (MW) is expressed in kilodaltons (kDa).
(B) Cross-linking analysis performed with Nat oligonucleotide.
The substrate was challenged with $Ape1^{WT}$ (lane 3) or $Ape1^{N\Delta33}$ (lane 4) as described in *Materials and methods* section. Reactions were resolved onto SDS-PAGE 10%. Lane 1 and 2: non-crosslinked or crosslinked Nat ODN respectively. Lane 5 and 6: $Ape1^{WT}$ and $Ape1^{N\Delta33}$ respectively crosslinked without Nat.

Supplementary figure S4

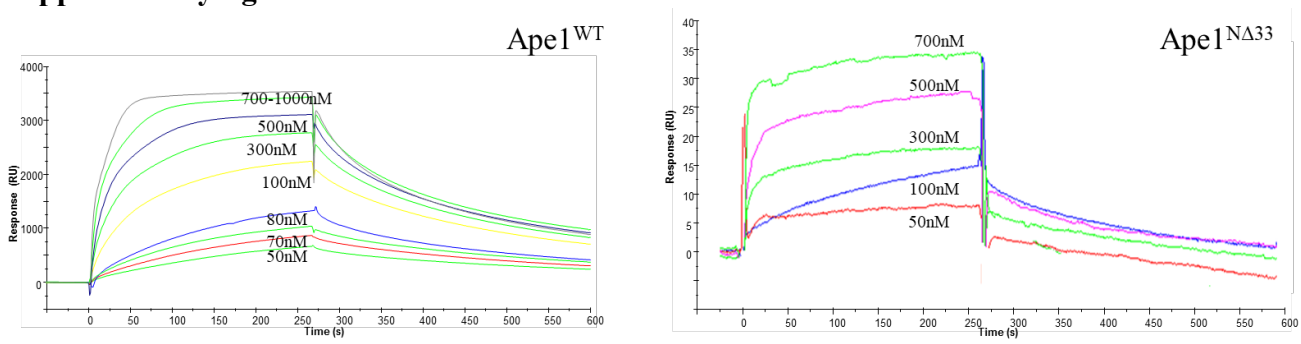

Fig. S4- **SPR sensorgrams for Ape1^{WT} and Ape1$^{N\Delta33}$ with Poly dT oligonucleotide.** Overlay of sensorgrams relative to SPR experiments for the binding to immobilized Biot- Poly dT of Ape1^{WT} (left) and Ape1$^{N\Delta33}$ (right), respectively.

Supplementary figure S5

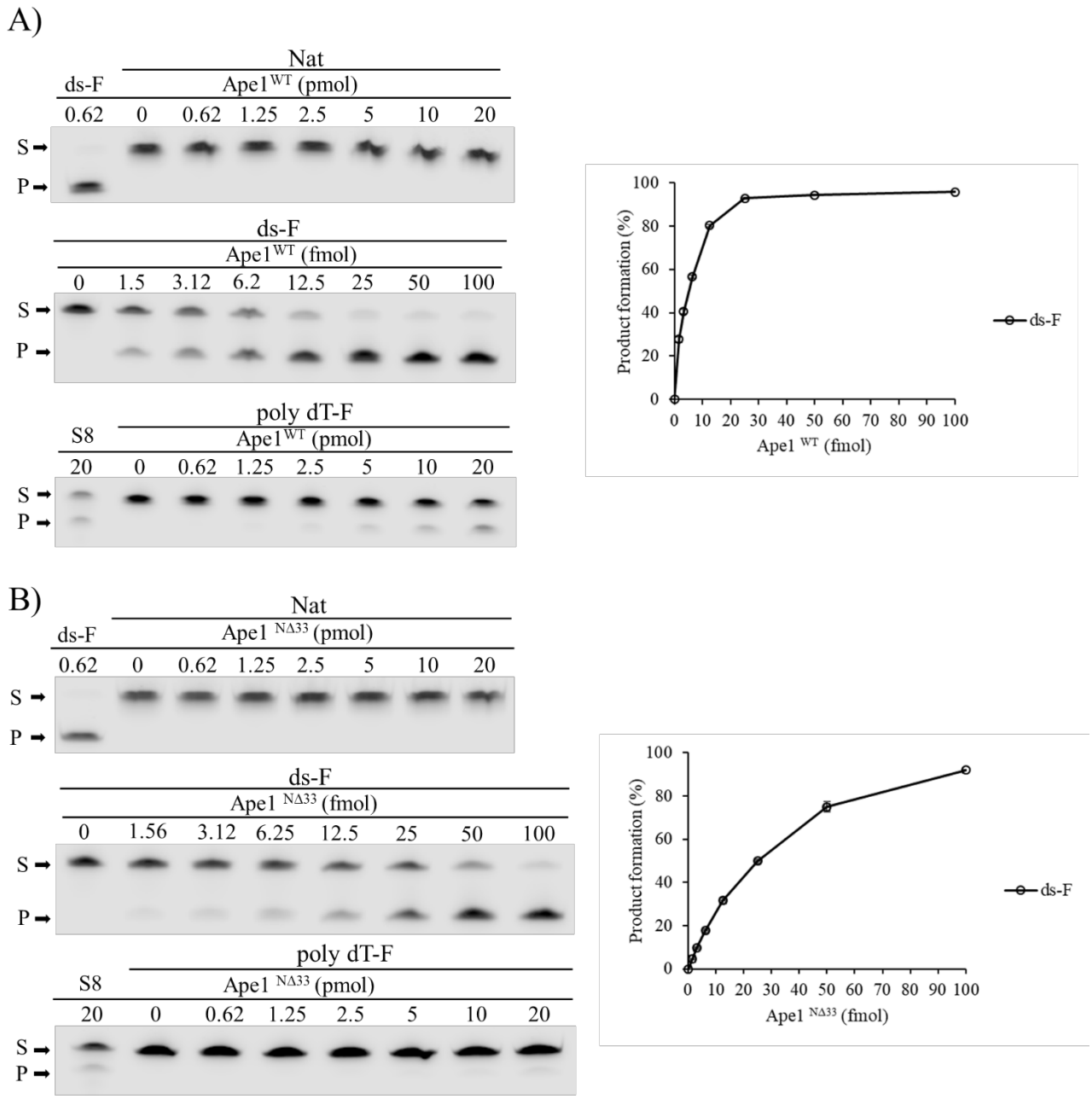

Fig. S5- **Ape1^{WT} and Ape1^{NΔ33} endonuclease activity on control substrates.**
Representative denaturing polyacrylamide gels of AP site incision by Ape1^{WT} (A) and Ape1^{NΔ33} (B) on the indicated oligonucleotides incubated with the specified amount of protein in a solution containing 50 mM KCl as described in *Materials and methods* section (left). Graph describes the percentage of conversion of substrate (S) into product (P) as a function of the dose of protein on the specified substrate (right). ds-F or S8 oligonucleotides were used as positive controls. S denotes the substrate position; the length of the generated products is 1 nt different between S8 (P) and ds-F (P'). Average values are plotted with standard deviations of three loadings of the same experiment. Standard deviation values were always less than 10% of the mean of experimental points.

Supplementary figure S6

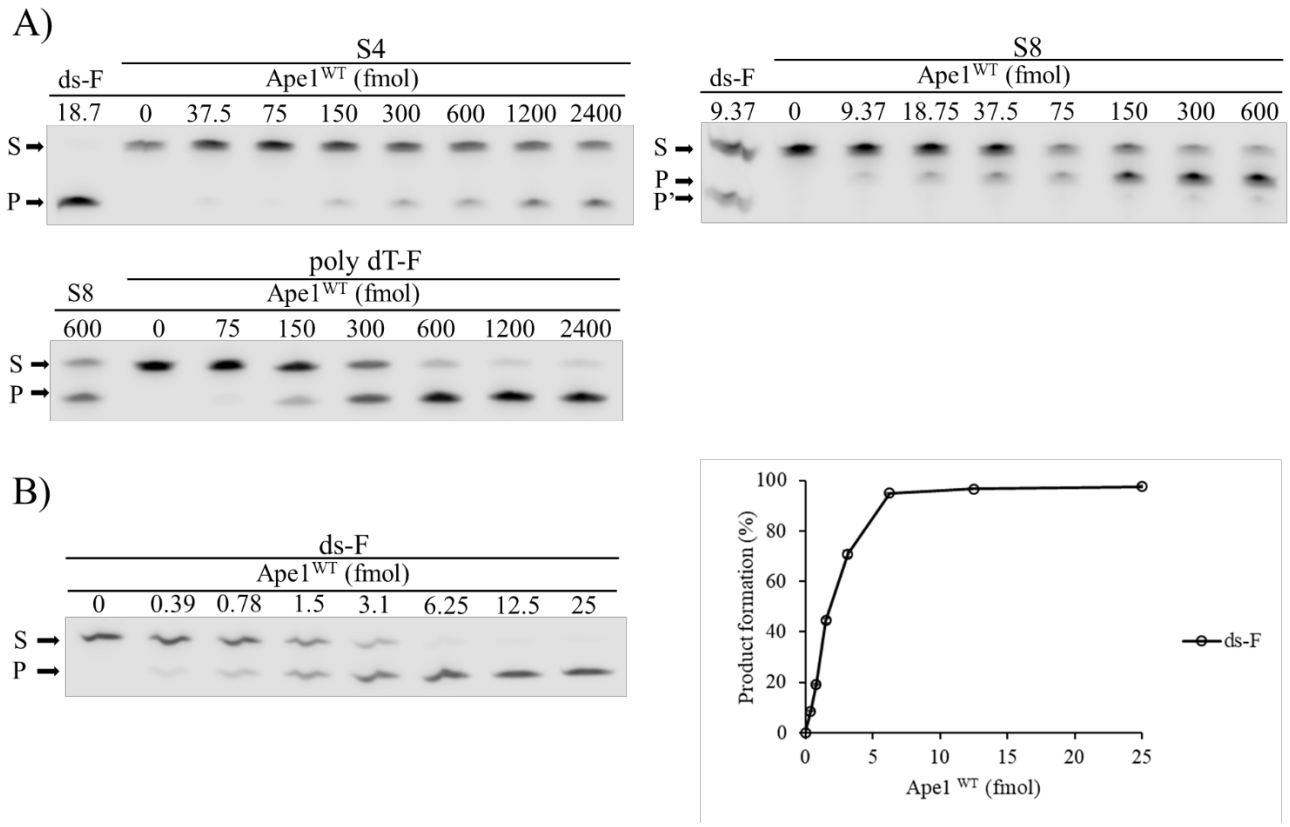

Fig. S6- **Ape1^{WT} cleaves AP sites in G4 substrates more efficiently at lower ionic strength.**
(A) Representative denaturing polyacrylamide gels of AP site incision by Ape1^{WT} on the indicated substrates with the indicated amount of protein in a solution containing 5 mM KCl as described in *Materials and methods* section; ds-F or S8 were used as positive controls.
(B) Representative denaturing polyacrylamide gel of AP site incision by Ape1^{WT} on ds-F substrate (left). The substrate was incubated with the indicated amount of Ape1 in a solution containing 5 mM KCl as described in *Materials and methods* section. S denotes the substrate position; the length of the product is 1 nt different between S8 (P) and ds-F (P'). Graph depicting dose- response experiments with the indicated doses of Ape1^{WT} on ds-F (right). Average values are plotted with standard deviations of three loadings of the same experiment. Standard deviation values were always less than 10% of the mean of experimental points.

Supplementary figure S7

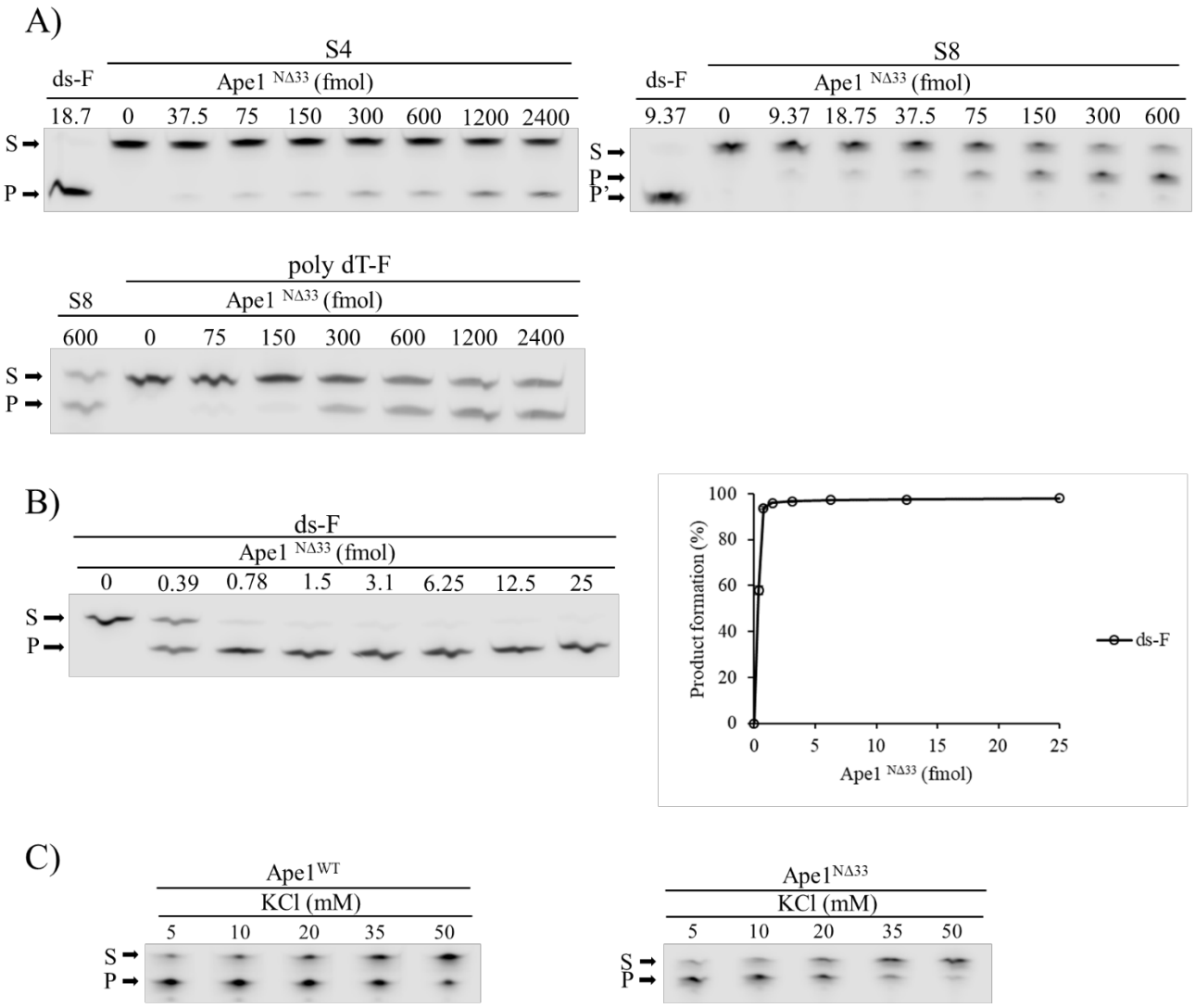

Fig. S7- **Ape1^{NΔ33} cleaves AP sites in of G4 substrates with a higher efficiency, at lower ionic strength.**

(A) Representative denaturing polyacrylamide gels of AP site incision by Ape1^{NΔ33} on the indicated substrates with the indicated amount of protein in a solution containing 5 mM KCl as described in *Materials and methods* section; ds-F or S8 were used as positive controls.

(B) Representative denaturing polyacrylamide gel of AP site incision by Ape1^{NΔ33} on ds-F substrate (left). The substrate was incubated with the indicated amount of Ape1^{NΔ33} in a solution containing 5 mM KCl as described in *Materials and methods* section. S denotes the substrate position; the length of the product is 1 nt different between S8 (P) and ds-F (P'). Graph depicting dose- response experiments with the indicated doses of Ape1^{WT} on ds-F (right). Average values are plotted with standard deviations of three loadings of the same experiment. Standard deviation values were always less than 10% of the mean of experimental points.

(C) Representative denaturing polyacrylamide gels of AP site incision by Ape1^{WT} (left) or Ape1^{NΔ33} (right) on S8 substrate. The substrate was incubated with the indicated amount of Ape1 in a solution containing the indicated concentrations of KCl. S denotes the substrate position; P denotes the product position.

Supplementary figure S8

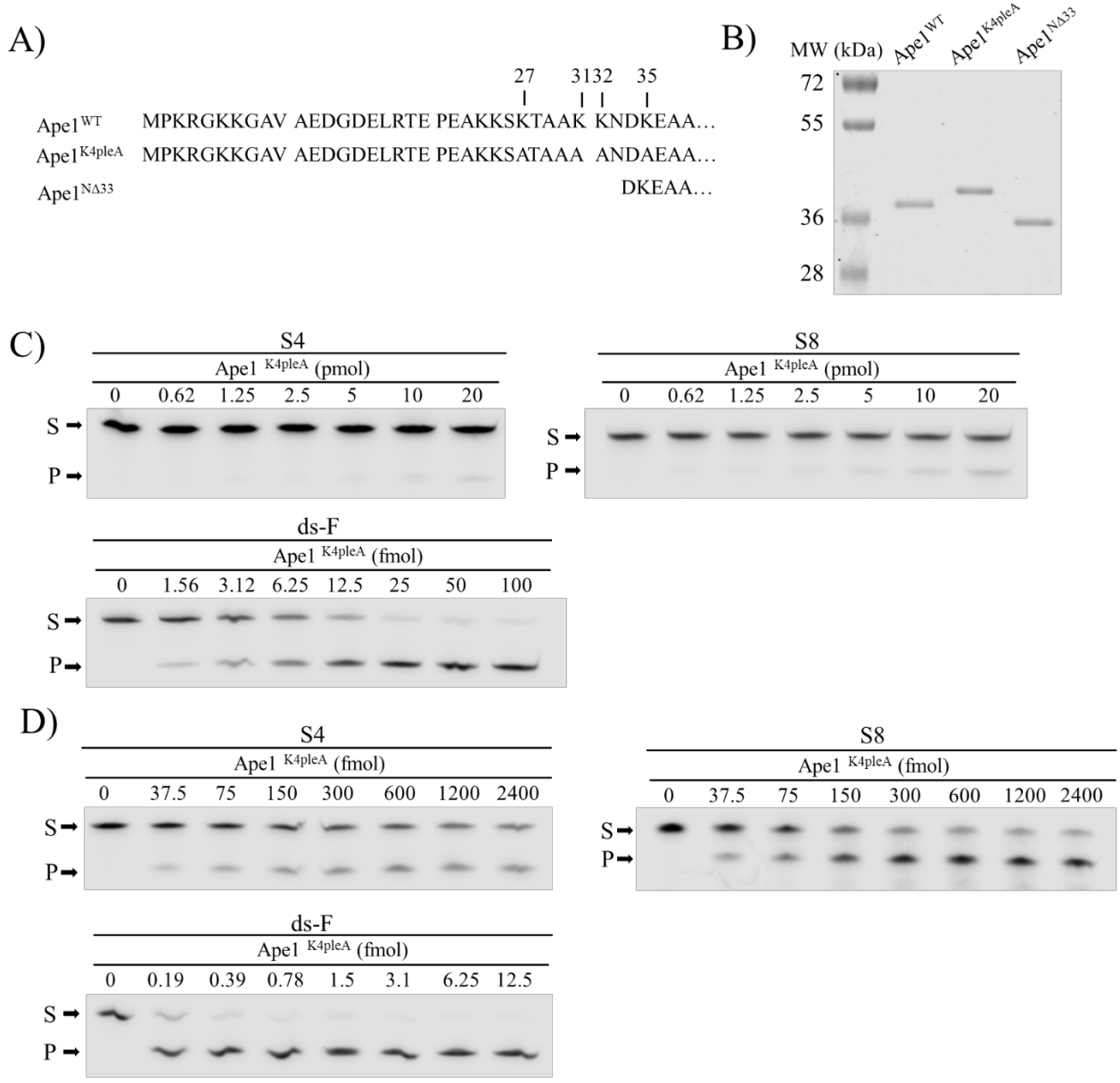

Fig. S8- **Ape1^{K4pleA} protein check and its endonuclease activity on telomeric oligonucleotides and control substrate.**

(A) Schematic representation of the N-terminal amino acidic sequence of each Ape1 protein used in this study.

(B) Gel analysis of human purified recombinant proteins used in this study. 0.5 µg of proteins were loaded and separated onto 10% SDS-PAGE followed by Coomassie brilliant blue staining.

(C) Representative denaturing polyacrylamide gels of AP site incision by Ape1^{K4pleA} on the indicated substrates with the indicated amount of protein in a solution containing 50 mM KCl as described in *Materials and methods* section.

(D) Representative denaturing polyacrylamide gels of AP site incision by Ape1^{K4pleA} on the indicated substrates with the indicated amount of protein in a solution containing 5 mM KCl as described in *Materials and methods* section. S denotes the substrate position and P denotes the product position.

Supplementary figure S9

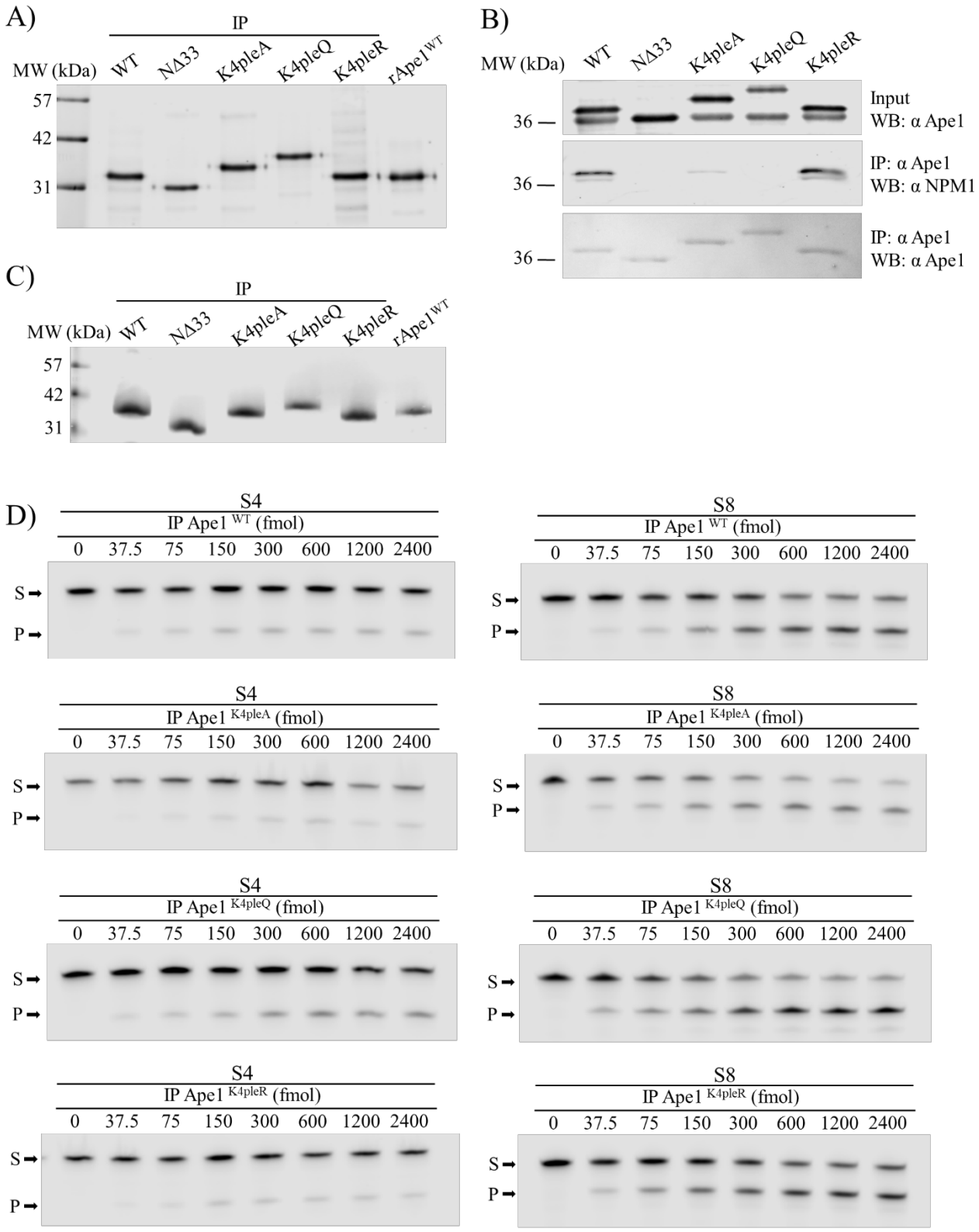

Fig. S9- **Endonuclease activity of immunocaptured Ape1^{WT} and mutants on G4 substrates.**
(A) Evaluation of Ape1 IP recovered through Western blot analysis. Co-IP proteins from U2OS cells overexpressing Ape1 mutants were resolved on 10% SDS-PAGE and immunoprobed with anti Ape1 antibody. rApe1 was included for comparison.
(B) Evaluation of Ape1 IP from U2OS cells overexpressing Ape1 mutants resolved on 10% SDS-PAGE analyzed through Western blot. Total cell extracts were immunoprobed with anti Ape1 antibody (Top, Input); each lane displays a lower band corresponding to the endogenous Ape1 and an upper band corresponds to the exogenous flagged protein. Co-IP proteins were immunoprobed with anti NPM1 antibody (Center, IP NPM1) and Ape1 was detected through Ponceau red staining (Bottom, IP Ape1).
(C) Analysis of gel mobility of Ape1 mutants from IP. The altered electrophoretic mobility observed for the Ape1^{K4pleA} and Ape1^{K4pleQ} mutant is due to an alteration of its overall charge. Samples were separated on a 10% SDS-PAGE containing 7 M urea and immunoprobed with anti-Ape1 antibody.
(D) Representative denaturing polyacrylamide gels of AP site incision by co-IP samples of Ape1^{WT} or the indicated mutants on the specified G4-structured substrates. The substrate was incubated with the indicated amount of Ape1-IP in a solution containing 5 mM KCl as described in *Materials and methods* section. S denotes the substrate position and P denotes the product position.

Supplementary figure S10

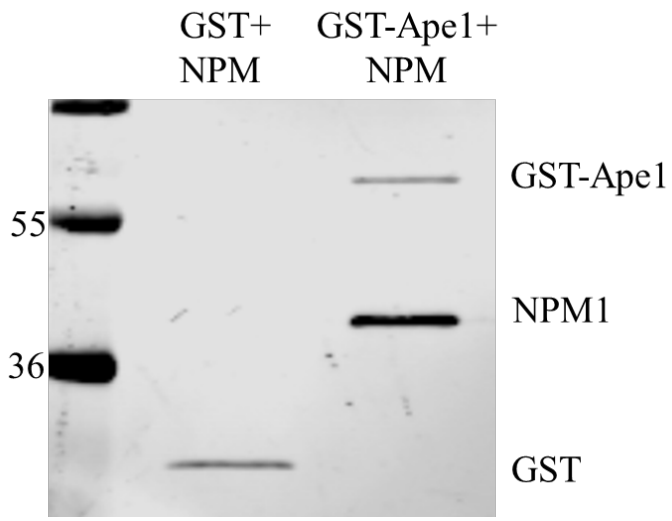

Fig. S10- **GST pull-down experiment showing that NPM1 is able to interact with Ape1 under physiologic conditions.**
GST pull-down assay was performed as described in *Materials and methods* section using GST or GST-tagged recombinant Ape1^{WT} as the bait and NPM1 as the prey. After the incubation, the samples were loaded on a 10% SDS-PAGE and subsequently immunoprobed with anti-GST and anti-NPM1 antibodies.