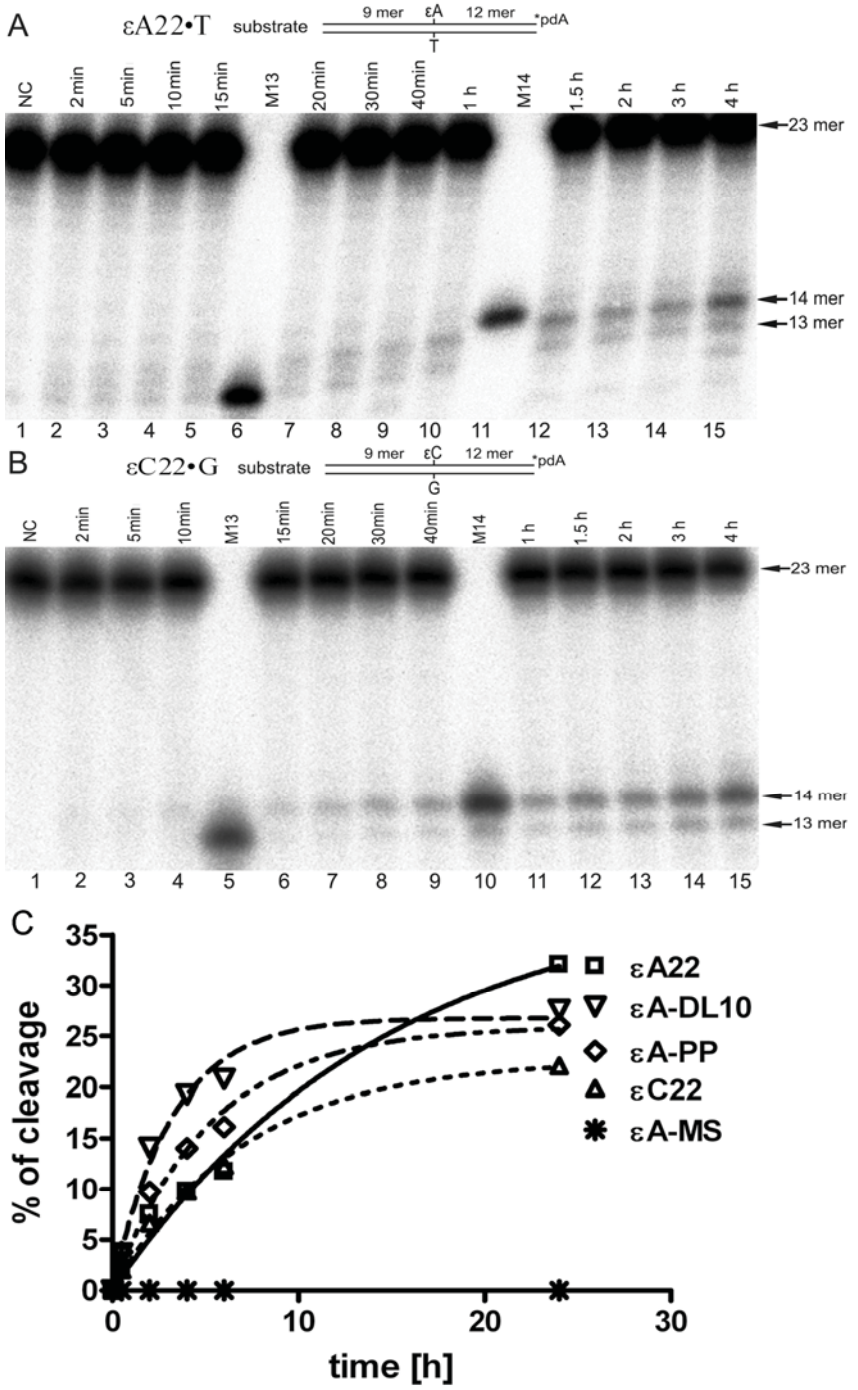


Figure 2. Time dependent kinetics of APE1-catalyzed cleavage activity of various oligonucleotide duplexes containing either single εA or εC residue. 10nM of the 3'-[^{32}P]-labelled (**A**) εA22•T and (**B**) εC22•G oligonucleotide duplexes were incubated with 5 nM APE1 for varying periods of time at 37°C. Lane 1, control, non-treated duplex; lanes 2-4, 6-9 and 11-15, oligonucleotide duplexes incubated with APE1 for 2 min to 4 h; lane 6, 13 mer size marker; lane 11, 14 mer size marker. The arrows and "<" symbol denote the position of the 23-mer, 13-mer and 14-mer fragments, respectively. (**C**) 10nM of the 3'-[^{32}P]-labelled oligonucleotide duplex was incubated with 10 nM APE1 for varying periods of time at 37°C. The formation of cleavage product was quantified and plotted against incubation time. For details see Materials and Methods.

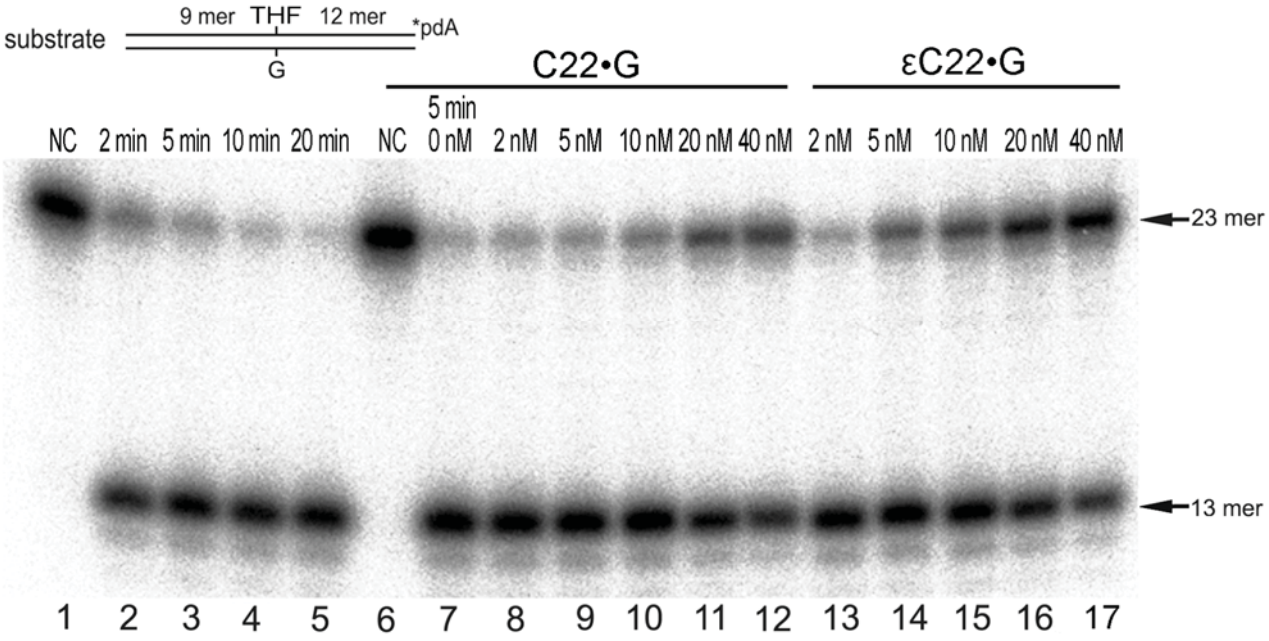


Figure 3. Inhibition of AP site cleavage activity of APE1 by εA22•T and εC22•G oligonucleotide duplexes. 1nM of 3'-[^{32}P]-labelled THF•G oligonucleotide duplex was incubated with 0.5 nM APE1 for 2-20 min at 37°C in the presence or not of increasing amounts of nonlabelled 22 mer C•G or εC22•G duplexes under NIR condition. Lane 1, control, non-treated THF•G duplex; lane 2, as 1 but APE1 for 2 min; lane 3, as 2 but 5 min; lane 4, as 2 but 10 min; lane 5, as 2 but 20 min; lane 7, THF•G duplex with APE1 for 5 min; lane 8, as 7 but 2 nM C•G; lane 9, as 7 but 5 nM C•G; lane 10, as 7 but 10 nM C•G; lane 11, as 7 but 20 nM C•G; lane 12, as 7 but 40 nM C•G; lane 13, as 7 but 2 nM εC22•G; lane 14, as 7 but 5 nM εC22•G; lane 15, as 7 but 10 nM εC22•G; lane 16, as 7 but 20 nM εC22•G; lane 17, as 7 but 40 nM εC22•G. For details see Materials and Methods.

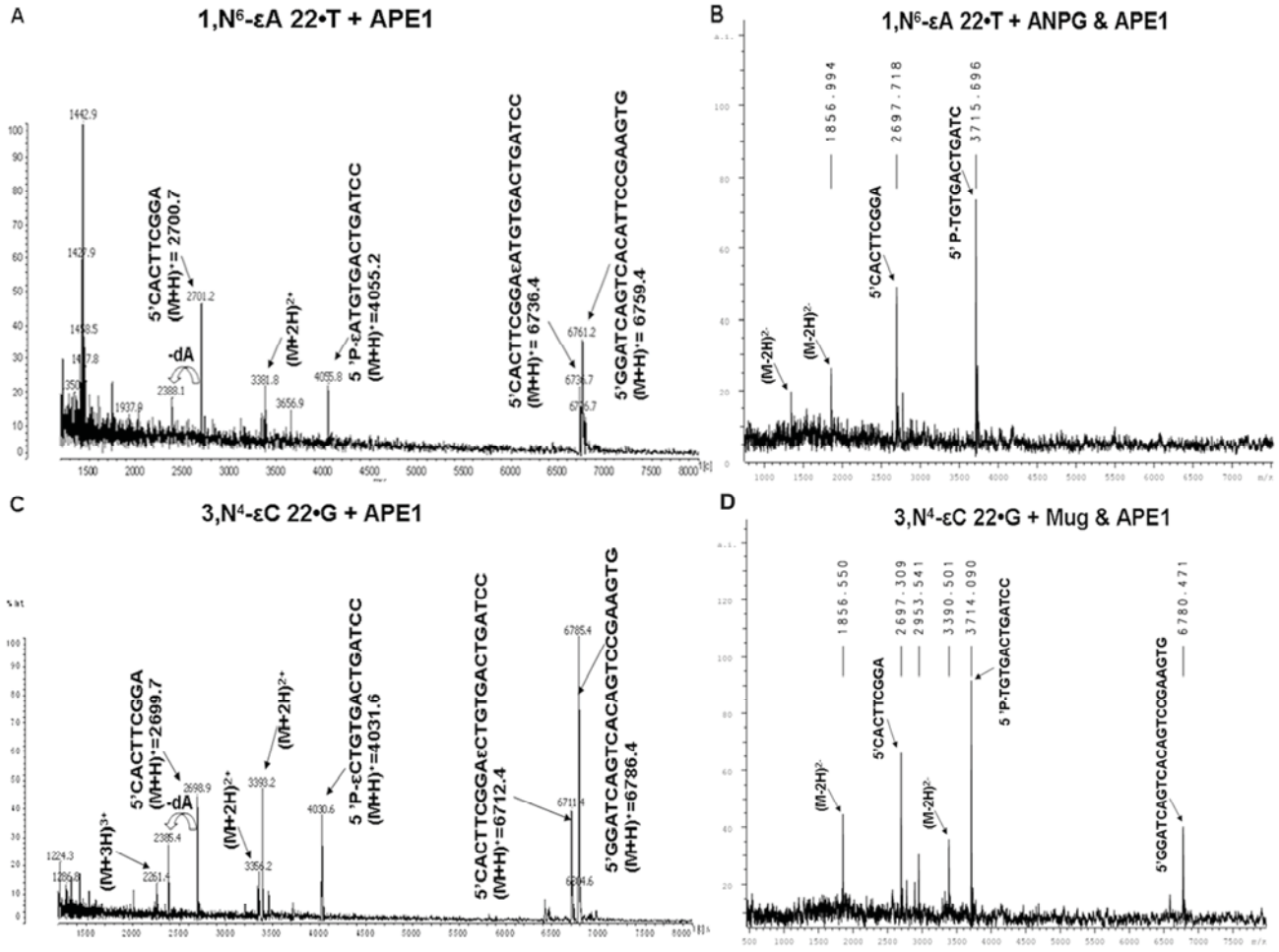


Figure 4. MALDI-TOF MS analysis of the mixture of oligonucleotides arising from the incubation of the 22 mer oligonucleotide duplexes containing single εA or εC residue with APE1 or DNA glycosylases. Typically, 10 pmol of the lesion containing oligonucleotide duplexes were incubated with either 10 nM APE1 under NIR condition at 37°C for 17h or 50 nM ANPG or 50 nM MUG at 37°C for 30 min and subsequently with 10 nM APE1 at 37°C for 30 min under "BER+Mg^{2+}" reaction condition. (**A**) Treatment of εA22•T duplex with APE1; (**B**) Treatment of εC22•G duplex with APE1; (**C**) Treatment of εA22•T duplex with ANPG and APE1; (**D**) Treatment of εC22•G duplex with MUG and APE1. Peaks corresponding to complementary strands are indicated in grey. For details see Materials and Methods.

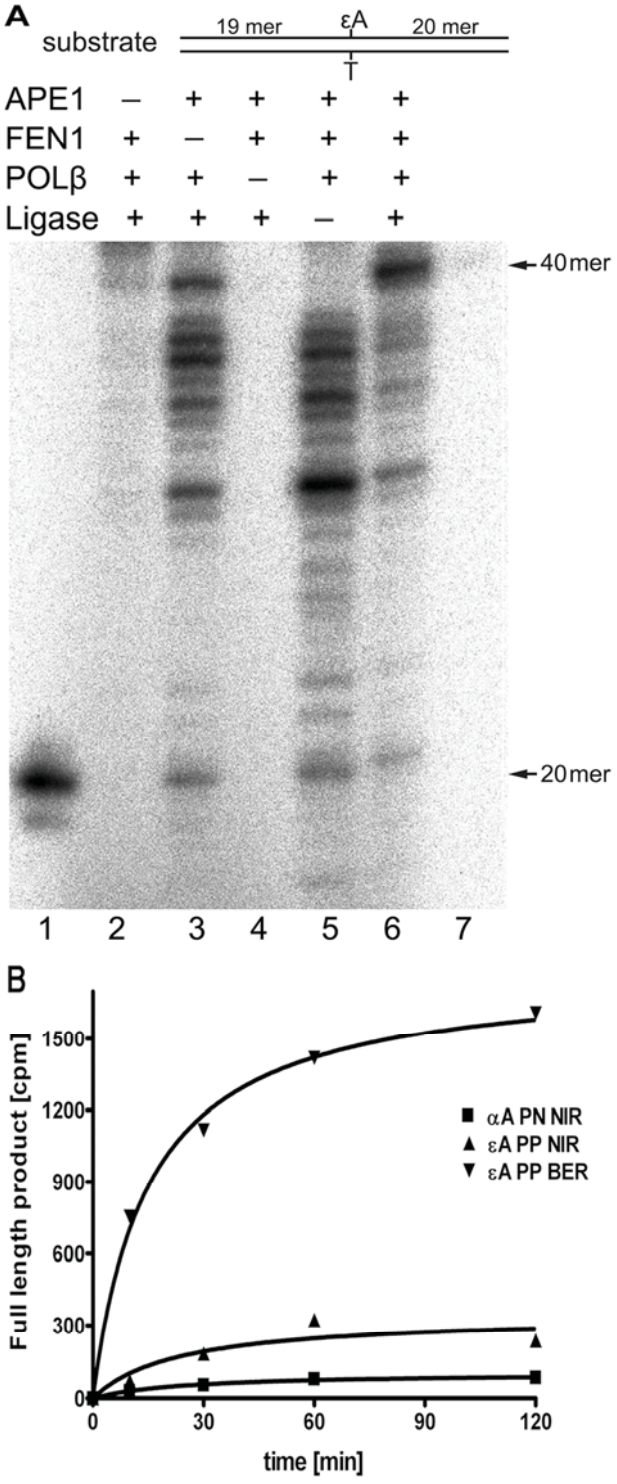


Figure 5. *In vitro* reconstitution of the long-patch NIR pathway using oligonucleotide duplex containing single εA residue. 10 nM of non-labelled 40 mer εA-PP•T oligonucleotide duplex was incubated for 3 h at 37°C in the presence of 10 nM APE1, 2 nM FEN1, 0.02 U POLβ and 20 U T4 DNA ligase in the reaction buffer containing 50 mM HEPES-KOH (pH 7.2), 30 mM NaCl, 3 mM MgCl2, 2 mM ATP, 0.1 mg/ml BSA, 2 mM DTT, 5 mCi of [α-^{32}P]dATP and 50 μM each of dGTP, dCTP and TTP. Lane 1, 20 mer size marker; lane 2, εA-PP•T incubated with all proteins except APE1; lane 3, except FEN1; lane 4, except POLβ; lane 5, except ligase; lane 6, in the presence of all proteins; lane 7, 40 mer size marker.
(**B**) Kinetics of the full length product formation in *in vitro* reconstitution of the long-patch NIR pathway for αdA PN•T and εA-PP•T and of the short-patch BER pathway for εA-PP•T. For details see Materials and Methods.

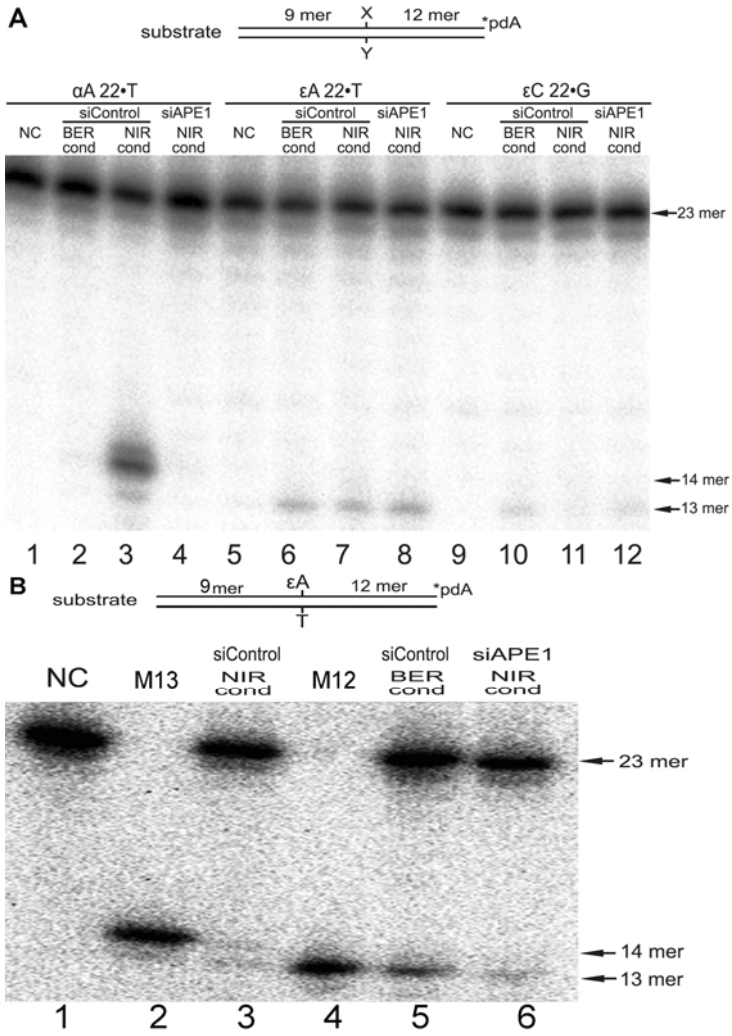


Figure 6. DNA repair activities towards αdA•T, εA22•T and εC22•G oligonucleotide duplexes in HeLa cells extracts. (**A**)10nM of 3'-[^{32}P]-labelled αdA•T, εA22•T and εC22•G oligonucleotide duplexes were incubated for 1 h at 37°C in the presence of 0.5 µg of cell-free extracts from siRNA-treated HeLa cells under either "BER+EDTA" or "NIR+$ZnCl_2$" condition. Cell-free extracts were prepared from HeLa cells treated either with 100 nM of non-specific siRNA (siControl), or with 100 nM of APE1-specific siRNA (siAPE1). Lane 1, control, non-treated αdA•T duplex; lane 2, as 1 but probed with control extract under BER+EDTA condition; lane 3, as 2 but under NIR+$ZnCl_2$ condition; lane 4, as 1 but probed with APE1-silenced extract under NIR+$ZnCl_2$ condition; Lane 5, control, non-treated εA22•T duplex; lane 6, as 5 but probed with control extract under BER+EDTA condition; lane 7, as 6 but under NIR+$ZnCl_2$ condition; lane 8, as 5 but probed with APE1-silenced extract under NIR+$ZnCl_2$ condition; Lane 9, control, non-treated εA22•T duplex; lane 10, as 9 but probed with control extract under BER+EDTA condition; lane 11, as 10 but under NIR+ZnCl2 condition; lane 12, as 9 but probed with APE1-silenced extract under NIR+$ZnCl_2$ condition.
(**B**)10nM of 3'-[^{32}P]-labelled εA22•T oligonucleotide duplexes were incubated for 3 h at 37°C in the presence of 5 µg of cell-free. Lane 1, control, non-treated εA22•T duplex; lane 2, 14 mer size marker, lane 3, as 1 but probed with control extract under NIR+$ZnCl_2$ condition; lane 4, 14 mer size marker; lane 5, as 1 but probed with control extract under BER+EDTA condition; lane 6 as 1 but probed with APE1-silenced extract under NIR+$ZnCl_2$. For details see Materials and Methods.

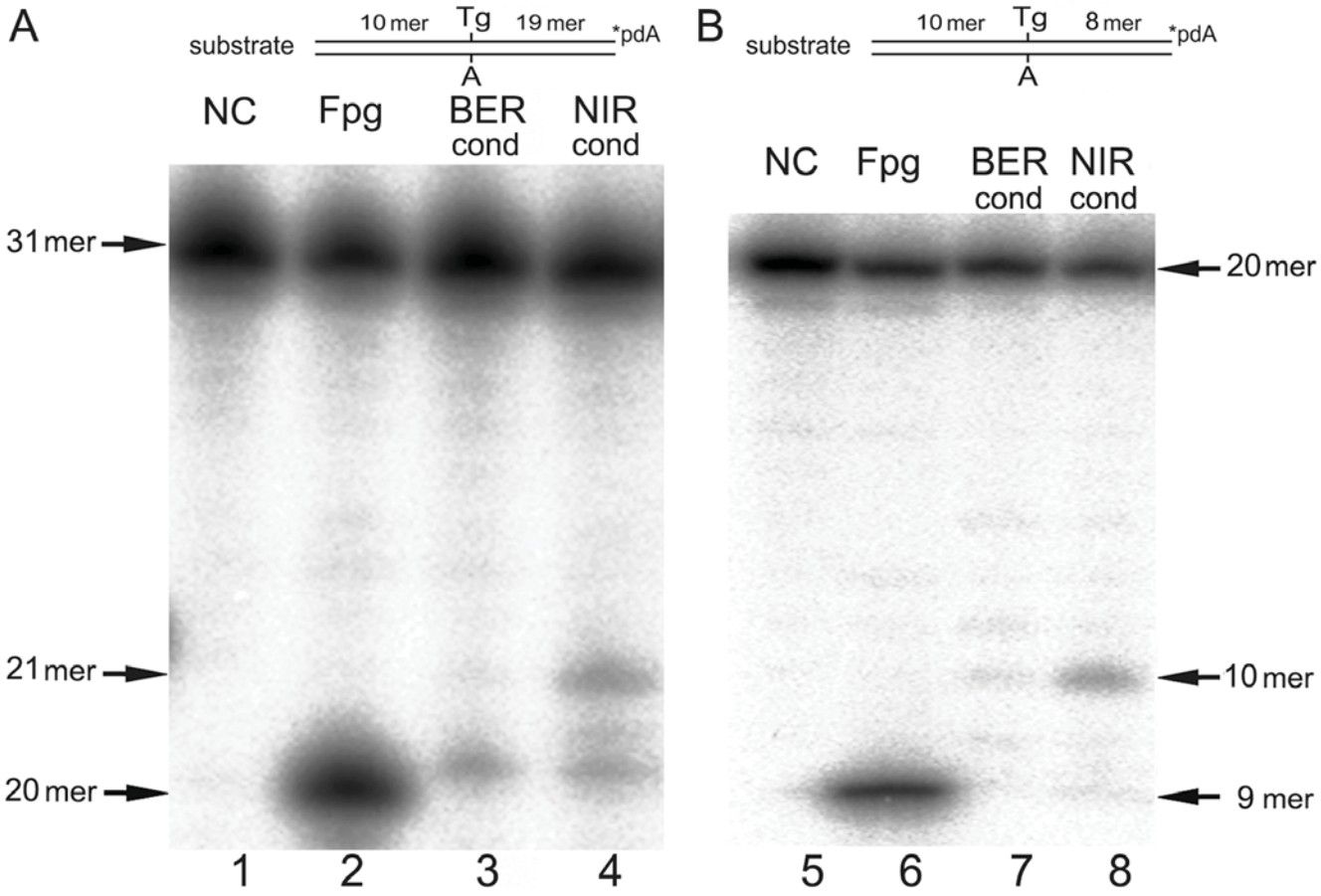
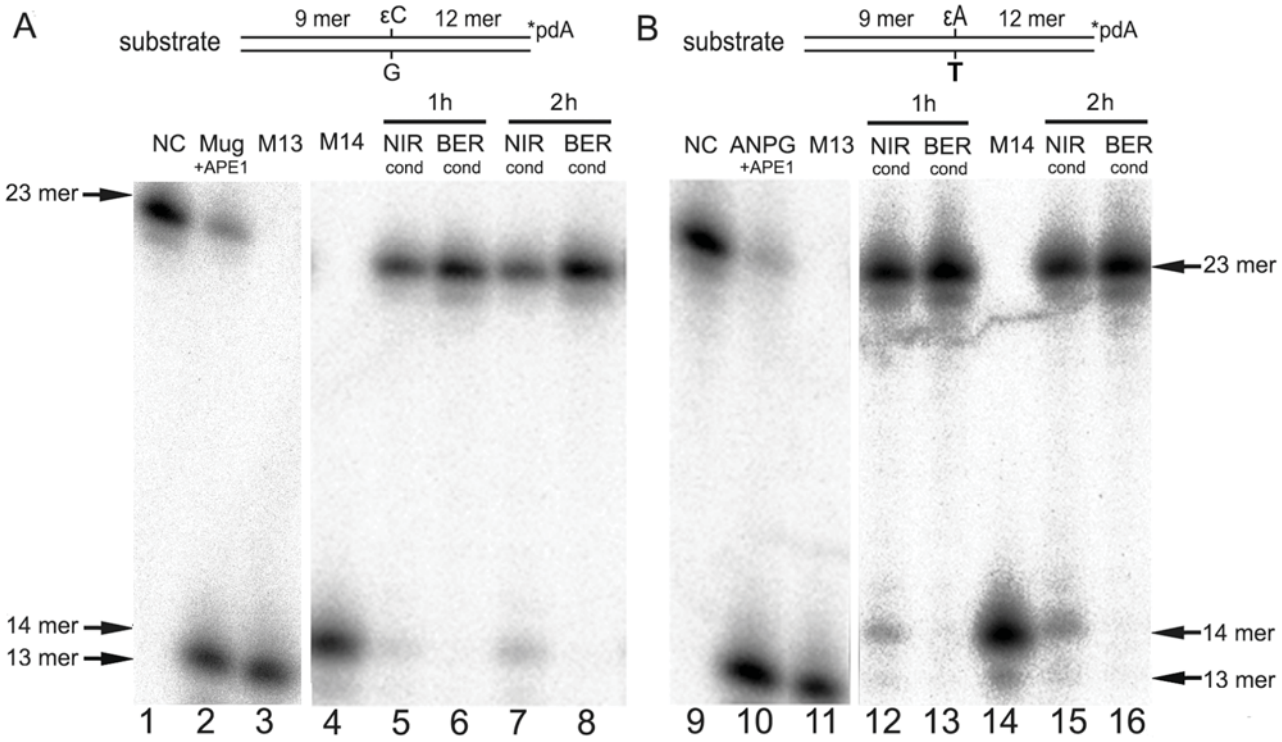
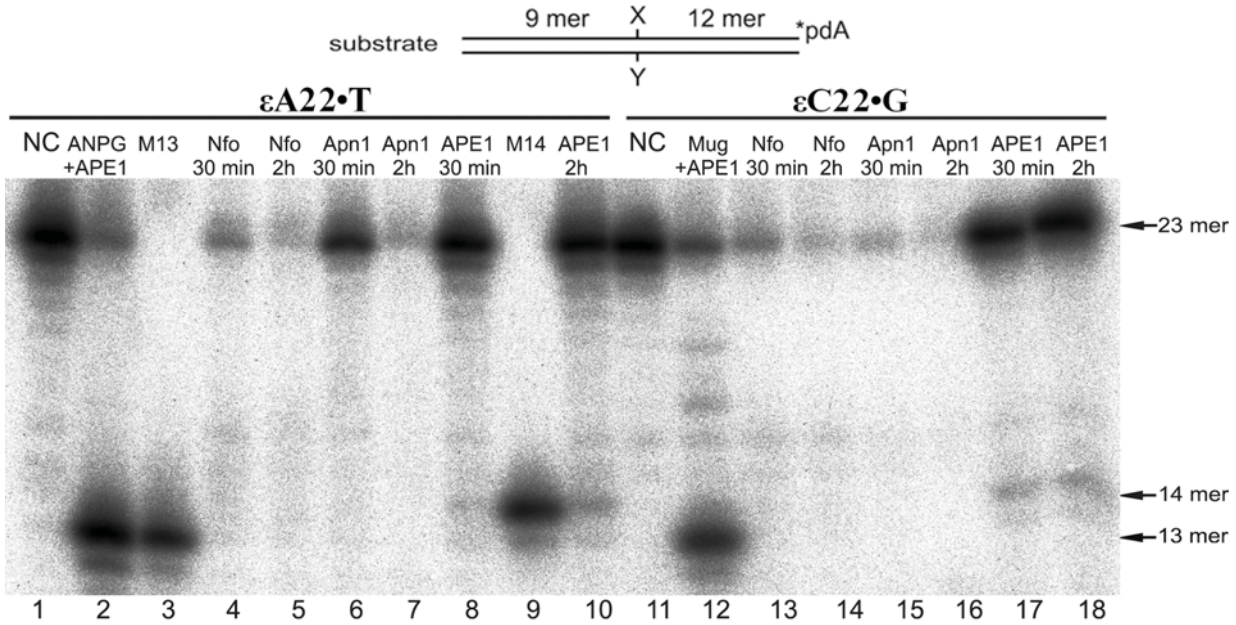


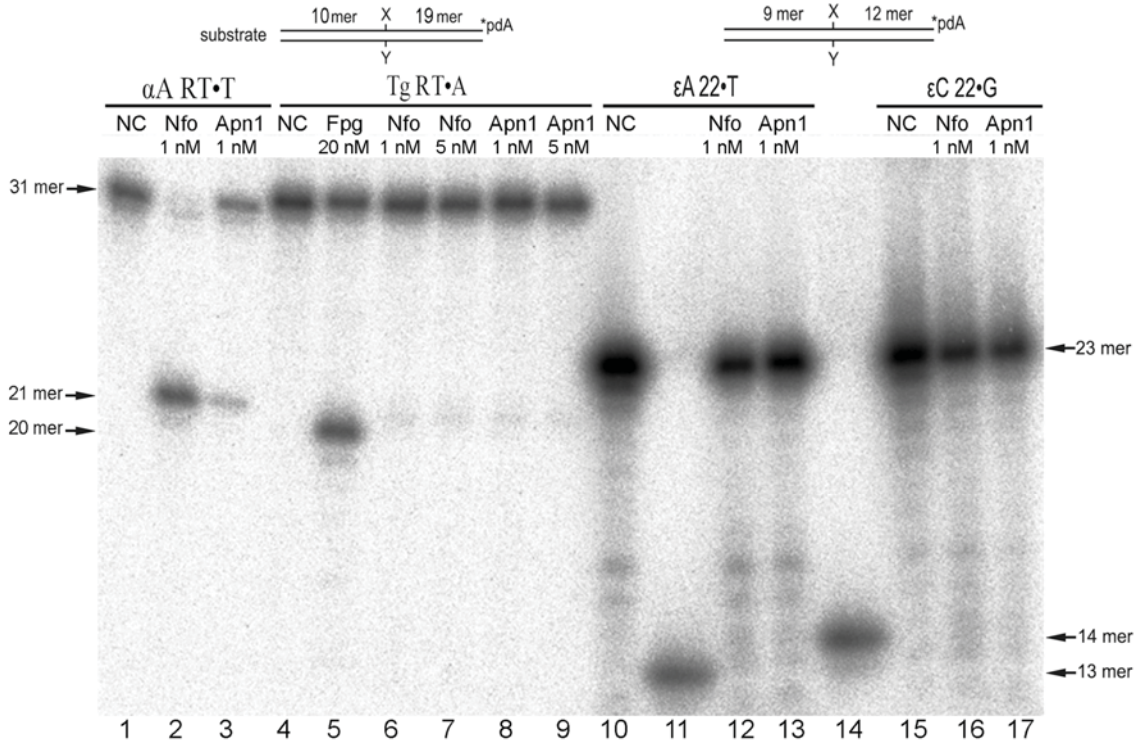
Figure 7. APE1-catalyzed NIR activity towards oligonucleotide duplexes containing single Tg residue. 10nM of 3'-[^{32}P]-labelled 30 mer Tg-RT•A and 19 mer Tg19•A oligonucleotide duplexes were incubated for 30 min or 2 h at 37°C in the presence of 20 nM Fpg or 10 nM APE1 under BER+EDTA or NIR condition. (**A**) Tg-RT•A and (**B**) Tg19•A duplexes. Lane 1, control nontreated Tg-RT•A duplex; lane 2 as 1 but treated with Fpg under BER condition for 30 min at 37°C; lane 3, as 1 but treated with APE1 under BER condition; lane 4, as 3 but under NIR condition; lane 5, control non-treated Tg19•A duplex; lane 6 as 5 but treated with Fpg under BER condition for 30 min at 37°C; lane 7, as 5 but treated with APE1 under BER condition; lane 8, as 7 but under NIR condition. For details see Materials and Methods.



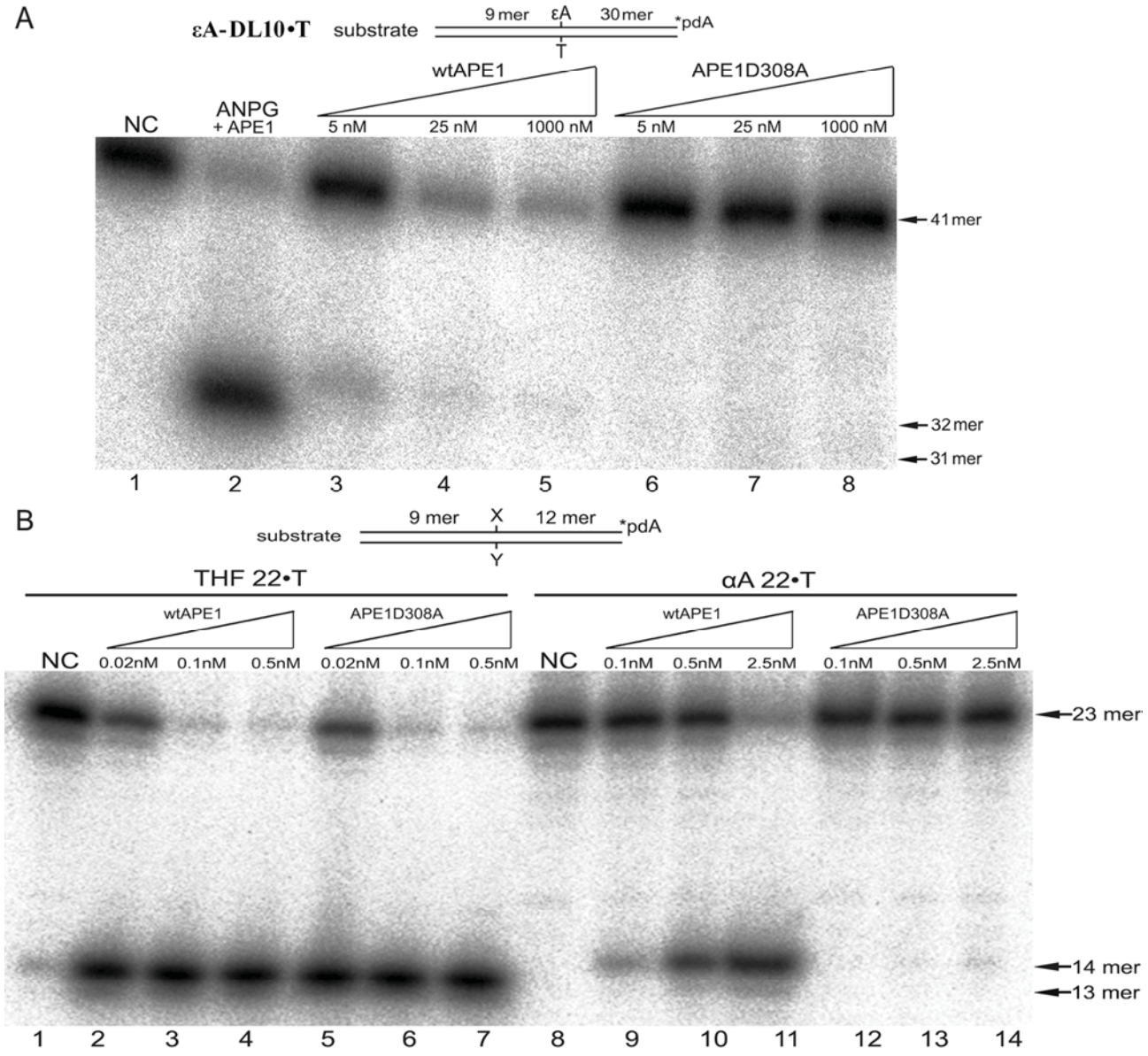
Supporting Information Figure S1. **Dependence of APE1-catalyzed NIR activity on reaction conditions**. 10nM of 3'-[^{32}P]-labelled oligonucleotide duplex containing a single ε-base was incubated for 1-2 h at 37°C with 5 nM APE1 under NIR and BER conditions, unless otherwise stated. (**A**) εC22•G and (**B**) εA22•T duplexes. Lanes 1 and 9, control, non-treated duplex; lanes 2 and 10, duplex incubated with 20 nM MUG and ANPG for 20 min and then with 10 nM APE1 for 20 min under "BER+Mg^{2+}" reaction condition; lanes 3 and 11, 13 mer size marker; lanes 4 and 12, 14 mer size marker; lanes 5 and 13, duplexes incubated with APE1 for 1 h under NIR condition; lanes 6 and 14, duplexes incubated with APE1 for 1 h under BER condition; lanes 7 and 15, duplexes incubated with APE1 for 2 h under NIR condition; lanes 8 and 16, duplexes incubated with APE1 for 2 h under BER condition. For details see Materials and Methods.



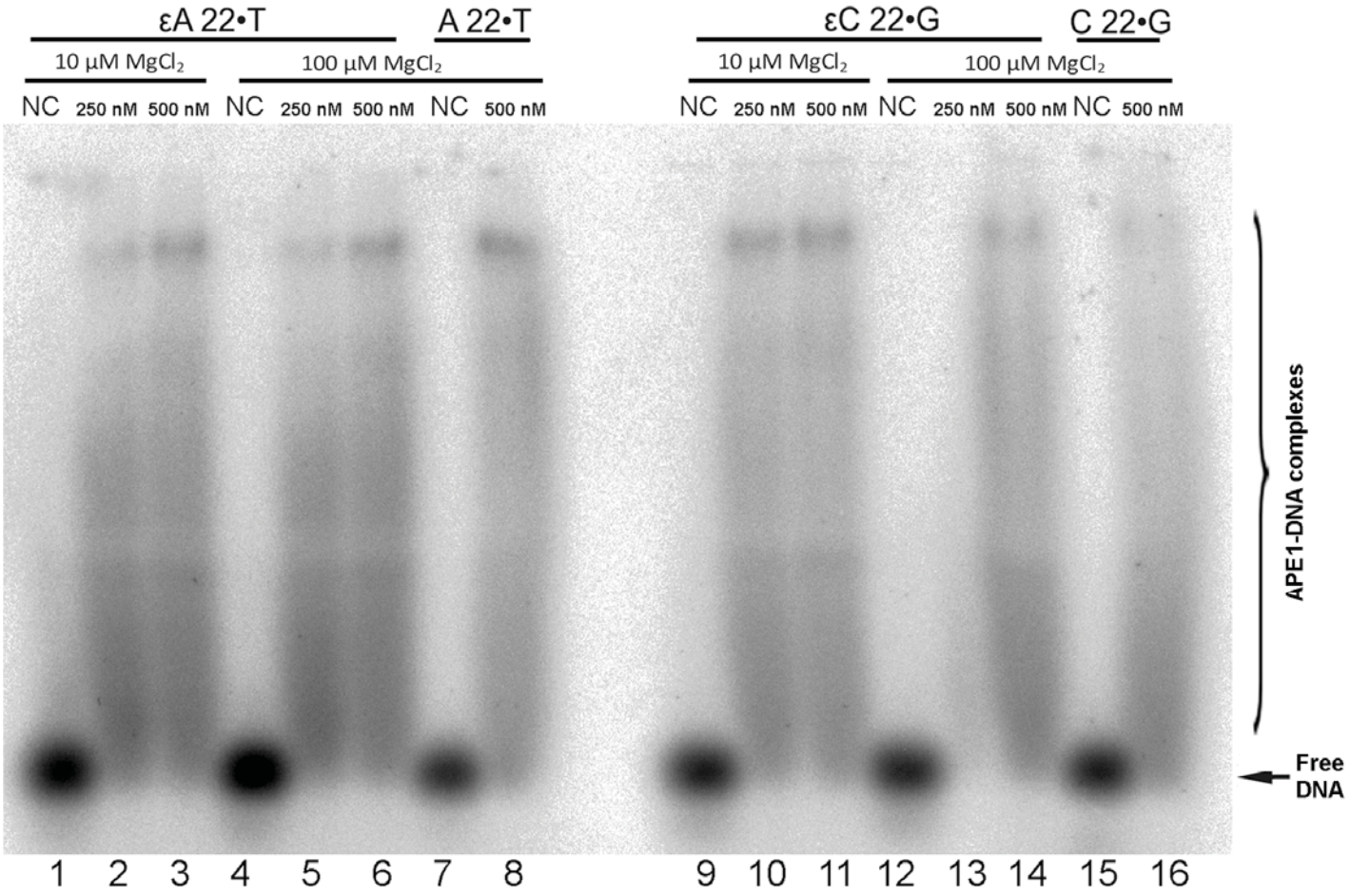
Supporting Information Figure S2A. **Action of various NIR AP endonucleases towards oligonucleotide duplex containing a single ε-base**. 10nM of 22 mer 3'-[^{32}P]-labelled εA22•T and εC22•G oligonucleotide duplexes were incubated with either 10 nM Nfo, or 10 nM Apn1 or 10 nM APE1 for 30 min or 2 h at 37°C, unless otherwise stated. Lane 1, control, non-treated εA22•T; lane 2, as 1 but 20 nM ANPG and 10 nM APE1 under BER+Mg^{2+} condition; lane 3, 13 mer size marker; lane 4, as 1 but Nfo for 30 min; lane 5, as 4 but 2 h; lane 6, as 1 but Apn1 for 30 min; lane 7, as 6 but 2 h; lane 8, as 1 but APE1 for 30 min; lane 9, 14 mer size marker; lane 10, as 8 but 2 h; lane 11, control, non-treated εC22•G; lane 12, as 1 but 20 nM MUG and 10 nM APE1 under BER+Mg^{2+} condition; lane 13, as 11 but Nfo for 30 min; lane 14, as 13 but 2 h; lane 15, as 11 but Apn1 for 30 min; lane 16, as 15 but 2 h; lane 17, as 11 but APE1 for 30 min; lane 18, as 17 but 2 h. For details see Materials and Methods.



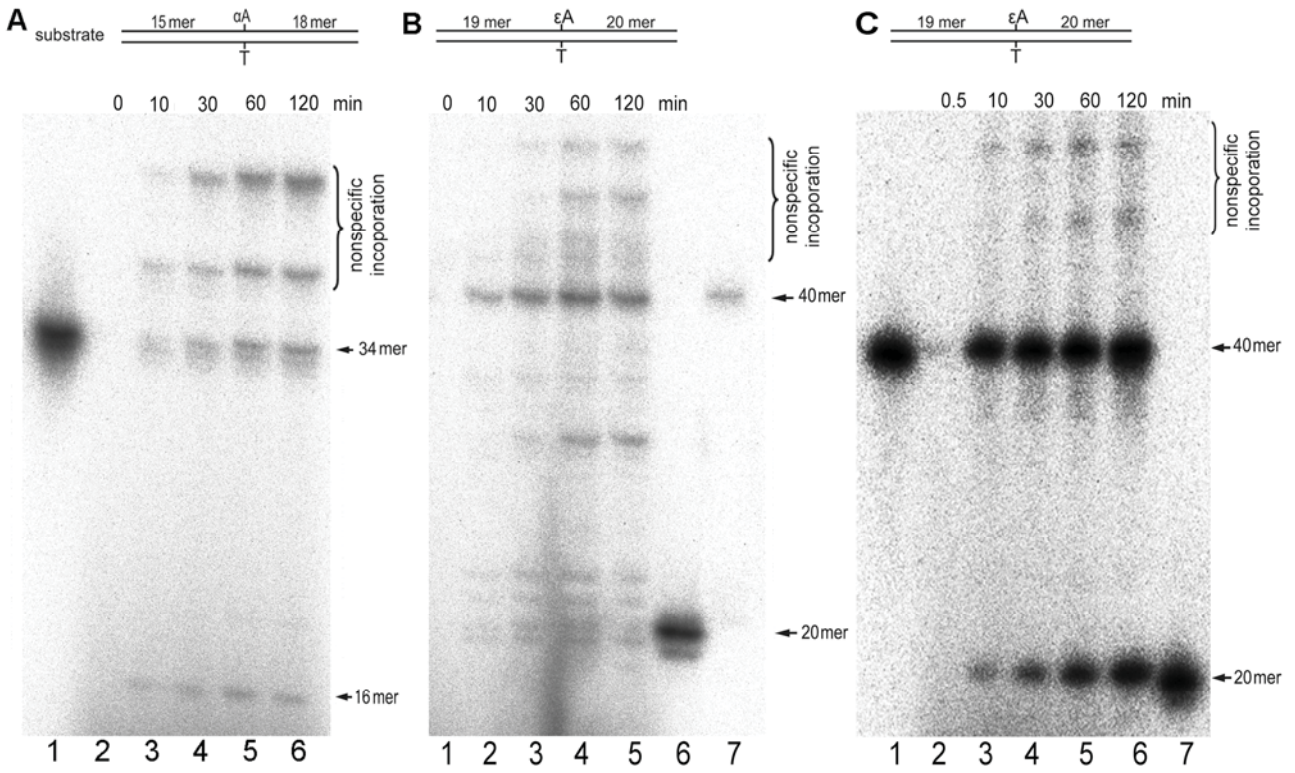
Supporting Information Figure S2B. Action of various NIR AP endonucleases towards oligonucleotide duplex containing a single ε-base. 10nM of 22 mer 3'-[^{32}P]-labelled αdA-RT•T, Tg RT•A, εA22•T and εC22•G oligonucleotide duplexes were incubated with either 1 nM or 5 nM Nfo, or 1 nM or 5 nM Apn1 for 30 min or 2 h at 37°C under NIR condition. Lane 1, control, non-treated αdA-RT•T; lane 2, as 1 but 1 nM Nfo for 30 min; lane 3, as 1 but 1 nM Apn1 for 30 min, lane 4, control, non-treated Tg RT•A; lane 5, as 4 but 20 nM Fpg under BER+Mg^{2+} condition; lane 6, as 4 but 1 nM Nfo for 2h, lane 7, as 4 but 5 nM Nfo for 2h; lane 8, as 4 but 1 nM Apn1 for 2h; lane 9, as 4 but 5 nM Apn1 for 2h, line 10, control, non-treated εA22•T; lane 11, 13 mer size marker; lane 12, as 10 but 1 nM Nfo for 2h; lane 13, as 10 but 1 nM Apn1 for 2h, lane 14, 14 mer size marker, line 15, control, non-treated εC22•T; line 16, as 15 but 1 nM Nfo for 2h; lane 17, as 15 but 1 nM Apn1 for 2h. For details see Materials and Methods.



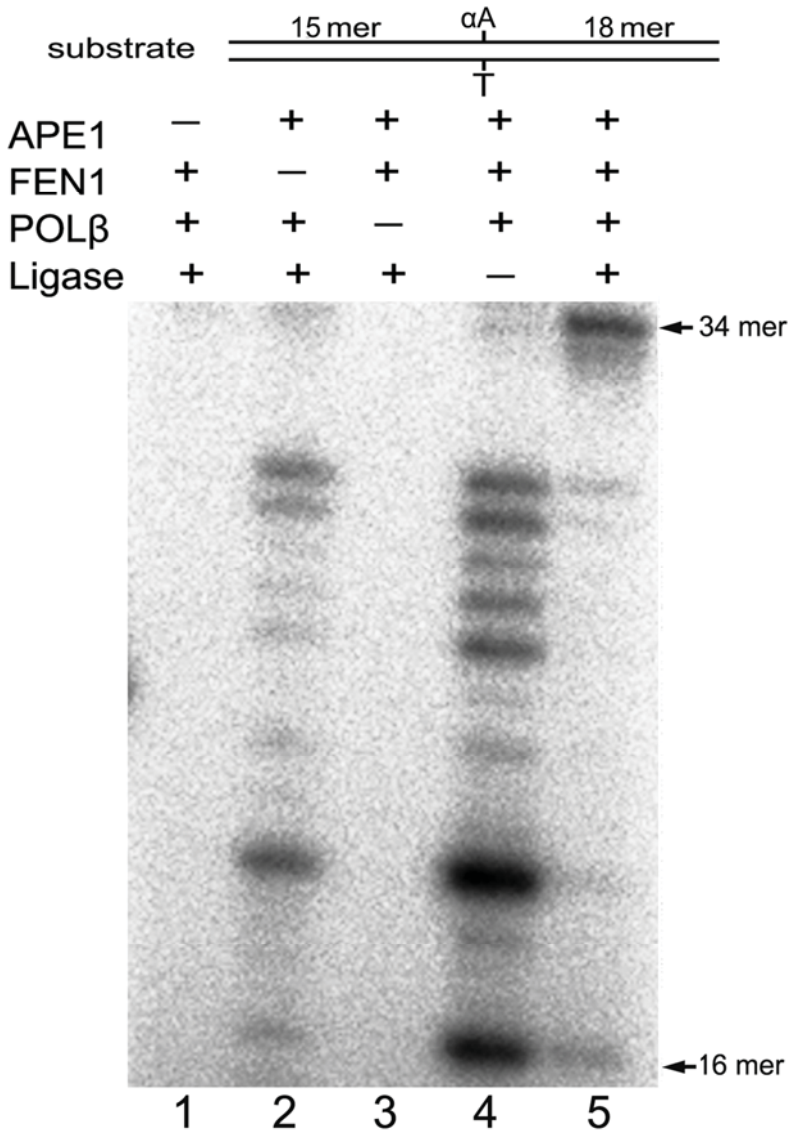
Supporting Information Figure S3. **Comparison of NIR and AP endonuclease activities of APE1-WT and mutant APE1-D308A proteins.** 10nM of 22 mer 3'-[^{32}P]-labelled εA-DL10•T, THF•T and αdA•T oligonucleotide duplexes were incubated with varying amounts of the APE1 proteins under NIR condition, and products of the reaction were analyzed using denaturing PAGE. (**A**) εA-DL10•T duplex was incubated with varying amounts of the APE1-WT and APE1-D308A mutant proteins for 4 h at 37°C. Lane 1, control, non-treated εA-DL10•T; lane 2, 20 nM ANPG and 10 nM APE1 under BER+Mg^{2+} condition; lane 3, 5 nM of APE1-WT; lane 4, 25 nM of APE1-WT; lane 5, 100 nM of APE1-WT; lane 6, 5 nM of APE1-D308A; lane 7, 25 nM of APE1- D308A; lane 8, 100 nM of APE1- D308A. (**B**) THF•T and αdA•T duplexes were incubated with varying amounts of the APE1-WT and APE1-D308A mutant proteins for 5 min at 37°C. Lane 1, control, non-treated THF•T; lane 2, 0.02 nM of APE1-WT; lane 3, 0.1 nM of APE1-WT; lane 4, 0.5 nM of APE1-WT; lane 5, 0.02 nM of APE1-D308A; lane 6, 0.1 nM of APE1- D308A; lane 7, 0.5 nM of APE1-D308A; Lane 8, control, non-treated αdA•T; lane 9, 0.1 nM of APE1-WT; lane 10, 0.5 nM of APE1-WT; lane 11, 2.5 nM of APE1-WT; lane 12, 0.1 nM of APE1-D308A; lane 13, 0.5 nM of APE1- D308A; lane 14, 2.5 nM of APE1- D308A. For details see Materials and Methods.



Supporting Information Figure S4. Electrophoretic Mobility Shift Assay (EMSA) of APE1 endonuclease with oligonucleotide duplexes containing a single ε-base. The standard binding reaction mixture (20 μl) contained 20 mM Hepes-KOH, pH 7.6, 50 mM KCl, 10 μM or 100 μM $MgCl_2$, 10 nM of 22 mer 3'-[^{32}P]-labelled εA22•T, εC22•G, A22•T and C22•G and 250 nM or 500 nM APE1. The mixture was incubated for 10 min on ice, after which an aliquot was analyzed by electrophoresis on a 8% non-denaturing polyacrylamide gel (19:1 acrylamide/bisacrylamide) at 160 V for 14 h at +4 °C. Lane 1, control, εA22•T in 10 μM $MgCl_2$ buffer without APE1, lane 2, as 1 but 250 nM APE1; lane 3, as 1 but 500 nM APE1; lane 4, control, εA22•T in 100 μM $MgCl_2$ buffer without APE1, lane 5, as 4 but 250 nM APE1; lane 6, as 4 but 500 nM APE1; lane 7, control, A22•T in 100 μM $MgCl_2$ buffer without APE1, lane 8, as 7 but 500 nM APE1; lane 9, control, εC22•T in 10 μM $MgCl_2$ buffer without APE1, lane 10, as 9 but 250 nM APE1; lane 11, as 9 but 500 nM APE1; lane 12, control, εC22•T in 100 μM $MgCl_2$ buffer without APE1, lane 13, as 12 but 250 nM APE1; lane 14. as 12 but 500 nM APE1; lane 15, control, C22•G in 100 μM $MgCl_2$ buffer without APE1, lane 16, as 15 but 500 nM APE1. For details see Materials and Methods.



Supporting Information Figure S5. Time kinetics of *in vitro* reconstitution of the long-patch NIR pathway and of the short-patch BER pathway using oligonucleotide duplex containing single αdA or εA residue. 10 nM of non-labelled (**A**) 34 mer αdA-PN•T and (**B**) 40 mer εA-PP•T oligonucleotide duplex was incubated for varied time ranging from 0.5 to 120 min at 37°C in the presence of 10 nM APE1, 2 nM FEN1, 0.01 U POLβ and 4 U T4 DNA ligase in the reaction buffer containing 50 mM HEPES-KOH (pH 7.2), 30 mM NaCl, 3 mM MgCl2, 2 mM ATP, 0.1 mg/ml BSA, 2 mM DTT, 5 mCi of [α-^{32}P]dATP and 50 μM each of dGTP, dCTP and TTP. (**A**)Lane 1, 34 mer size marker; lane 2, product formation after 0 min, lane 3, after 10 min; lane 4, after 30 min; lane 5, after 60 min, lane 6, after 120 min; (**B**) Lane 1, product formation after 0 min, lane 2, after 10 min; lane 3, after 30 min; lane 4, after 60 min, lane 5, after 120 min; lane 6, 20 mer size marker; lane 7, 40 mer size marker. (**C**) 10 nM of non-labelled 40 mer εA-PP•T oligonucleotide duplex was incubated for varied time ranging from 0.5 to 120 min at 37°C in the presence of 40 nM ANPG, 5 nM APE1, 2 nM FEN1, 0.01 U POLβ and 4 U T4 DNA ligase in the reaction buffer containing 20 mM HEPES-KOH (pH 7.6), 50 mM KCl, 5 mM $MgCl_2$, 2 mM ATP, 0.1 mg/ml BSA, 1 mM DTT and 5 mCi of [α-^{32}P]dATP. Lane 1, 40 mer size marker; lane 2, product formation after 0.5 min, lane 3, after 10 min; lane 4, after 30 min; lane 5, after 60 min, lane 6, after 120 min; lane 7, 20 mer size marker. For details see Materials and Methods.



Supporting Information Figure S6. *In vitro* reconstitution of the long-patch NIR pathway using oligonucleotide duplex containing single αdA residue. 10 nM of non-labelled 34 mer αA-PN•T oligonucleotide duplex was incubated for 3 h at 37°C in the presence of 10 nM APE1, 2 nM FEN1, 0.02 U POLβ and 20 U T4 DNA ligase in the reaction buffer containing 50 mM HEPES-KOH (pH 7.2), 30 mM NaCl, 3 mM $MgCl_2$, 2 mM ATP, 0.1 mg/ml BSA, 2 mM DTT, 5 mCi of [α-^{32}P]dATP and 50 μM each of dGTP, dCTP and TTP. Lane 1, αA-PN•T incubated with all proteins except APE1; lane 2, except FEN1; lane 3, except POLβ; lane 4, except ligase; lane 5, in the presence of all proteins. The arrows denote the position of the 16 mer cleavage product and 34 mer full-length product. For details see Materials and Methods.