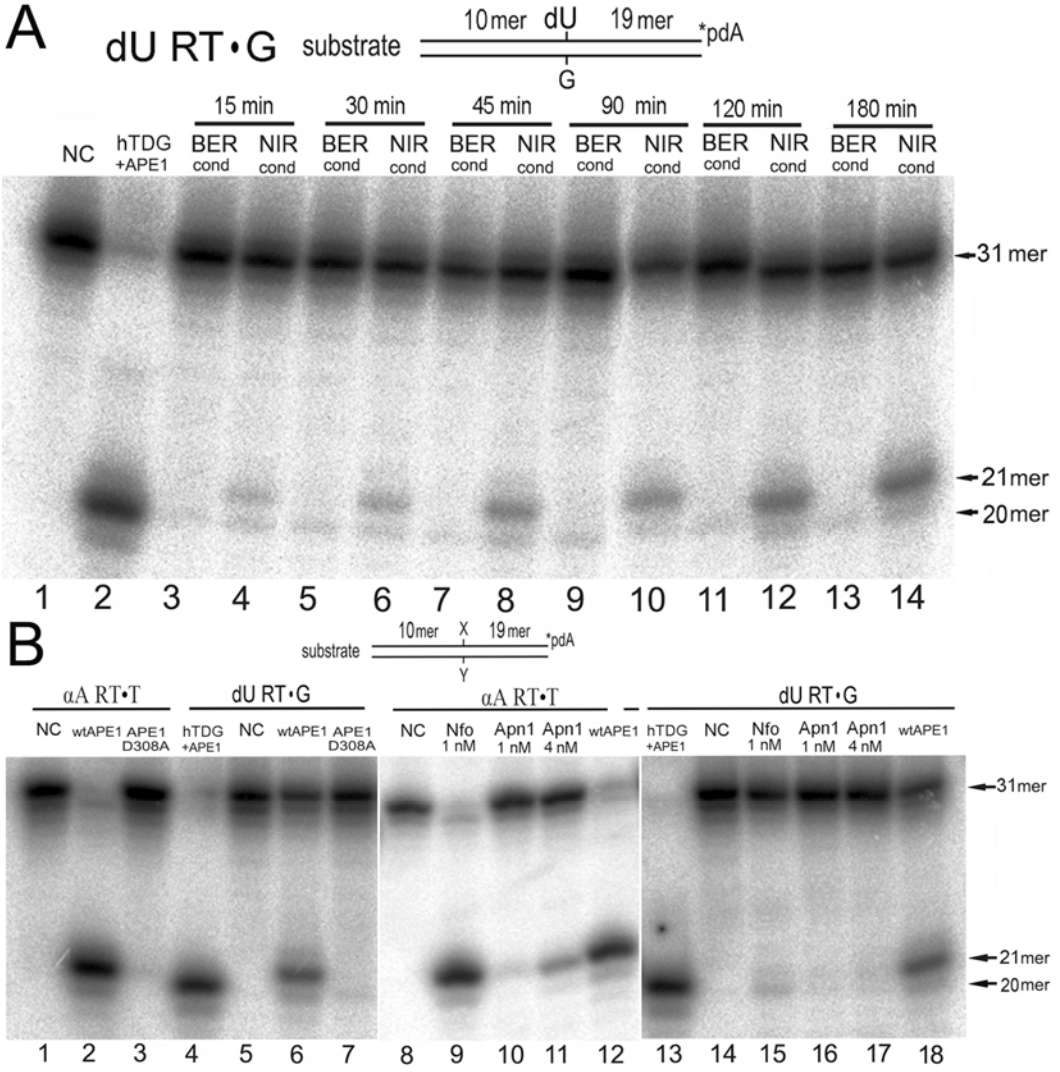


Fig. 1. APE1-catalyzed deoxyuridine endonuclease activity. (**A**) Time- and condition-dependent cleavage of a DNA duplex containing a single uracil residue by APE1. 10 nM of the 3'-[^{32}P]-labelled 30-mer U•G duplex (dU-RT sequence context) was incubated for 15–180 min at 37°C with 10 nM APE1 under the NIR and BER conditions. Lane 1, control non-treated U•G; lane 2, as 1 but incubated with 40 nM hTDG for 20 min under the BER+Mg^{2+} reaction conditions and then treated with 10% piperidine for 15 min to cleave the resulting AP sites. Lanes 3, 5, 7, 9, 11 and 13, as 1 but incubated with APE1 under the BER+Mg^{2+} conditions. Lanes 4, 6, 8, 10,12 and 14, as 1 but incubated with APE1 under the NIR conditions. The arrows denote the position of the intact 31-mer substrate, the position of the 21-mer product of APE1-NIR activity, and the position of the 20-mer product of DNA glycosylase activity. (**B**) Action of various NIR endonucleases and APE1-D308A mutant towards the U•G duplex. 10 nM of the 3'-[^{32}P]-labelled 30 mer U•G, and αdA•T oligonucleotide duplexes (dU-RT sequence context) were incubated with either 10 nM wild-type (WT) APE1, or 10 nM APE1-D308A under the NIR conditions or with a limited amount of Nfo and Apn1 in their respective reaction buffers for 30 min and 2 h at 37°C. Lane 1, non-treated αdA•T; lane 2, as 1 but WT APE1 for 10 min; lane 3, as 1 but APE1D308A for 10 min. Lane 4, U•G incubated with 40 nM hTDG for 30 min and then with 10 nM APE1 for 30 min under the BER+Mg^{2+} conditions; lane 5, non-treated U•G; lane 6, as 5 but WT APE1 for 2 h; lane 7, as 5 but APE1-D308A for 2 h; Lane 8, non-treated αdA•T; lane 9, as 8 but 1 nM Nfo for 30 min; lane 10, as 8 but 1 nM Apn1 for 30 min; lane 11, as 8 but 4 nM Apn1 for 30 min; lane 12, as 8 but WT APE1 for 10 min; lane 13, as 4; lane 14, non-treated U•G; lane 15, as 14 but 1 nM Nfo for 2 h; lane 16, as 14 but 1 nM Apn1 for 2 h; lane 17, as 14 but 4 nM Apn1 for 2h; lane 18, as 14 but WT APE1 for 2 h. The arrows denote the position of the non-cleaved 31-mer substrate, the position of the 21-mer APE1-NIR product, and the position of the 20-mer DNA glycosylase product. For details, see Materials and Methods.

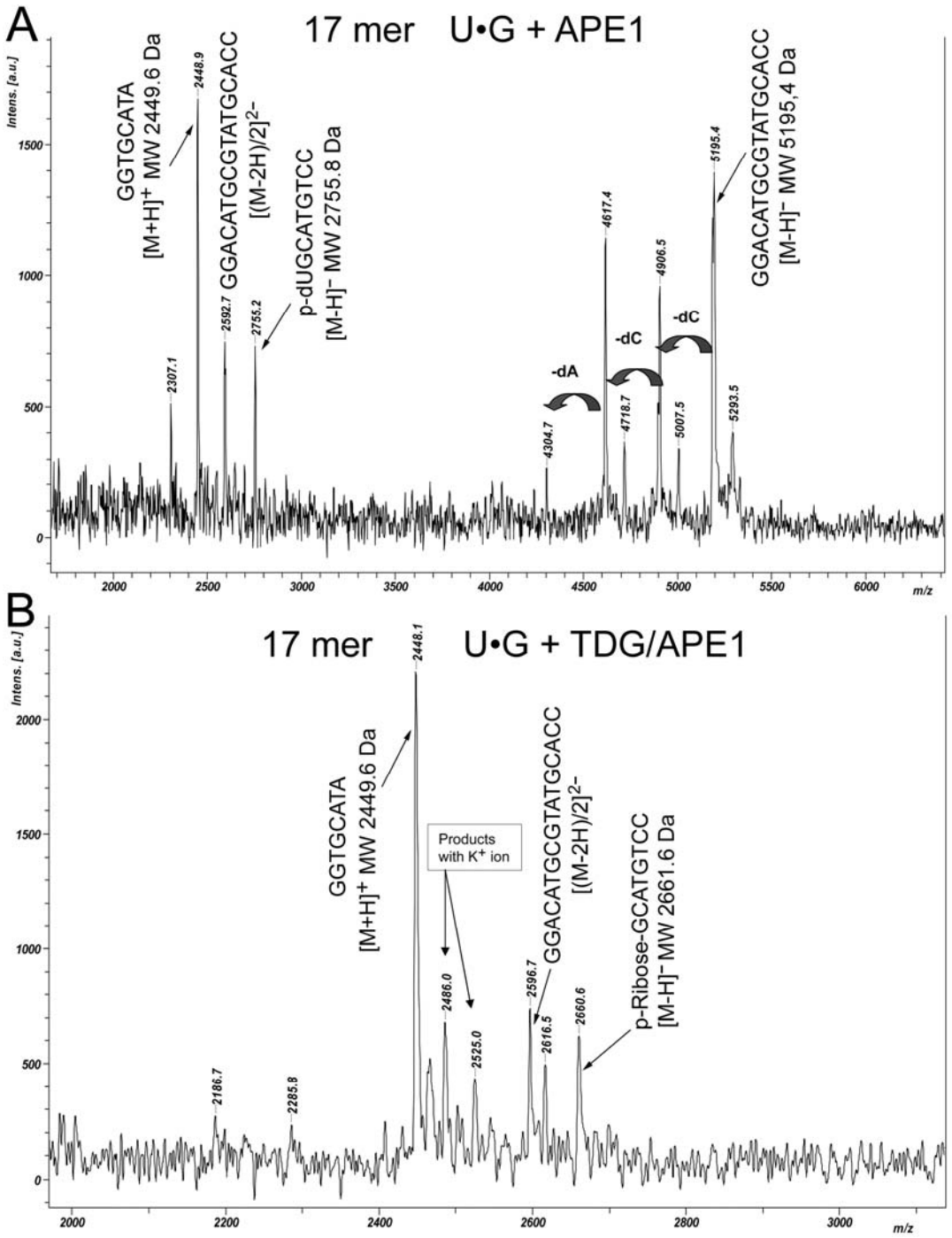


Fig. 2. MALDI-TOF MS analysis of the reaction products generated by the incubation of a 17-mer oligonucleotide duplex containing a single dU residue with APE1 (**A**) and with TDG and APE1 (**B**). Typically, 10 pmol of the 17-mer U•G duplex (dU-RT17 sequence context) was incubated with either 10 nM APE1 under NIR conditions at 37°C for 17 h or with 40 nM hTDG at 37°C for 30 min and then with 10 nM APE1 at 37°C for 30 min under the BER+Mg^{2+} reaction conditions. For details, see Materials and Methods.

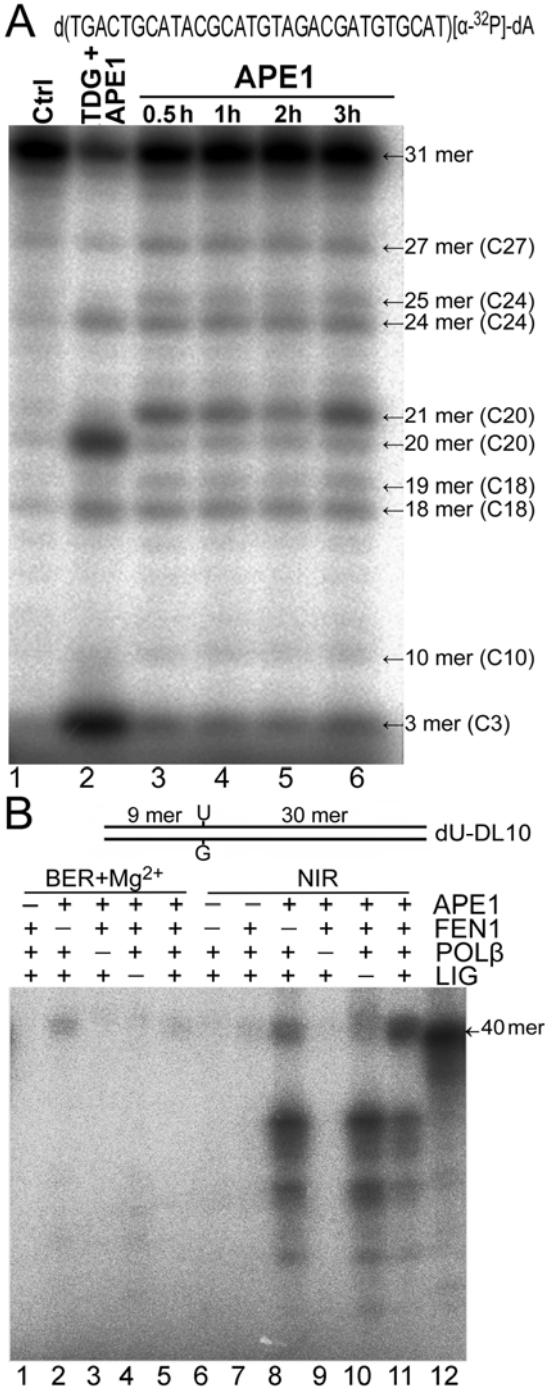


Fig. 3. *In vitro* reconstitution of APE1-catalyzed dU-endonuclease activities. (**A**) Action of APE1 on a sodium bisulfite-treated DNA duplex. $NaHSO_3$-treated and 3'-[^{32}P]-labelled 31-mer C•G duplex was incubated with either hTDG for 30 min and then with APE1 for 30 min at 37°C under the BER+Mg^{2+} condition, or with 10 nM APE1 at 37°C for varying time under the NIR conditions. Lane 1, control $NaHSO_3$-treated C•G duplex; lane 2, as 1 but hTDG/APE1; lane 3, as 1 but APE1 alone for 0.5 h; lanes 4–6, as 3 but 1 h, 2 h, and 3 h, respectively. For details, see Materials and Methods. (**B**) *In vitro* reconstitution of the NIR pathway for uracil residues. 10 nM of the non-labelled 40-mer U•G oligonucleotide duplex (DL10 sequence context) was incubated for 1 h at 37°C in the presence of DNA repair proteins, non-labelled dNTPs, and [α-^{32}P]dCTP. Lane 1, U•G incubated under the BER conditions with all proteins except APE1; lane 2, as 1 but except FEN1; lane 3, as 1 but except POLβ; lane 4, as 1 but except DNA ligase; lane 5, in the presence of all proteins; lanes 6-11, U•G incubated under the NIR conditions with the DNA repair proteins; lane 6, except APE1 and FEN1; lane 7, except APE1; lane 8, except FEN1; lane 9, except POLβ; lane 10, except DNA ligase; lane 11, in the presence of all proteins; lane 12, 40-mer size marker. All incubations were performed in the presence of 1 unit of uracil-DNA glycosylase inhibitor (UGI). For details, see Materials and Methods.

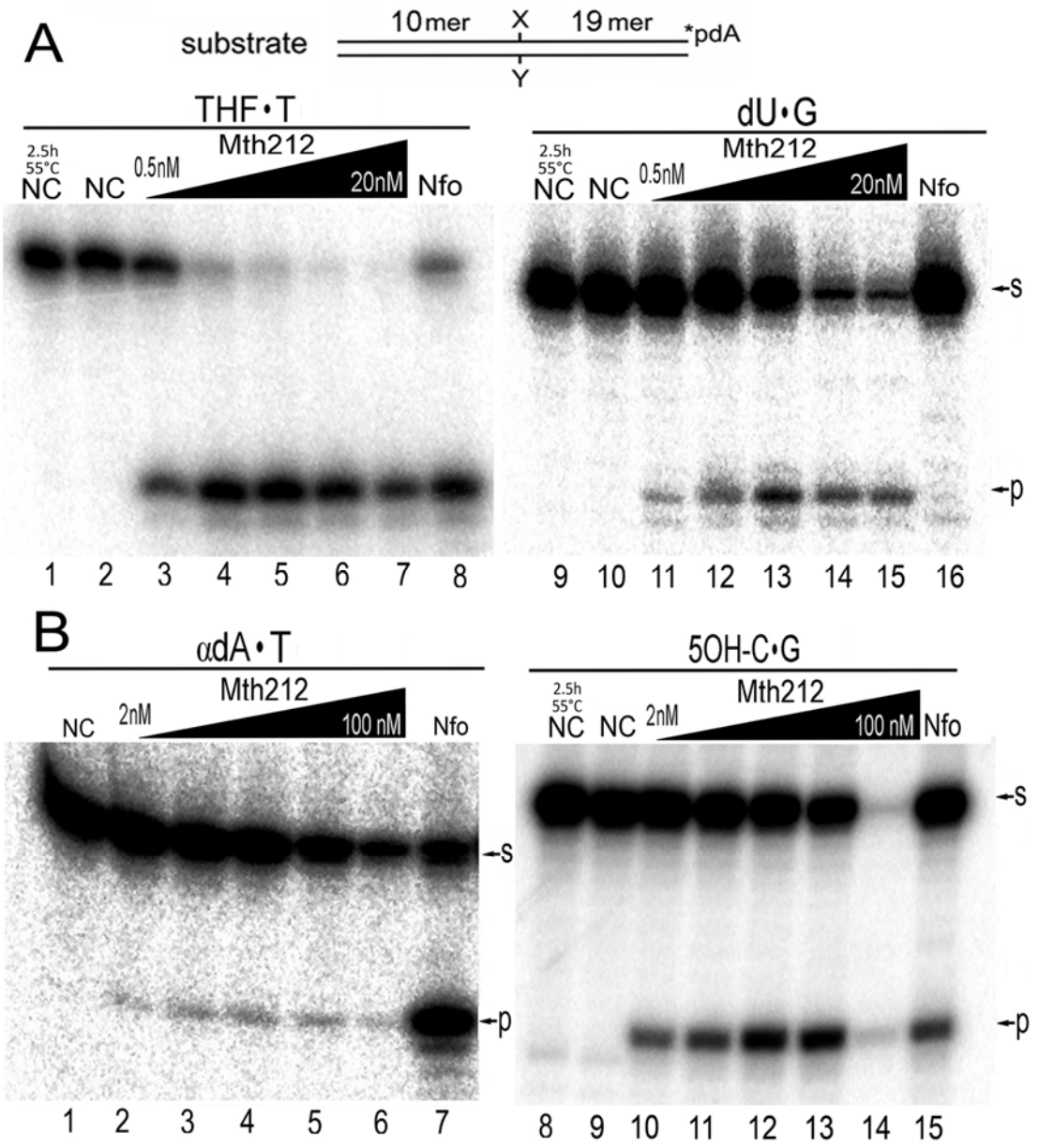
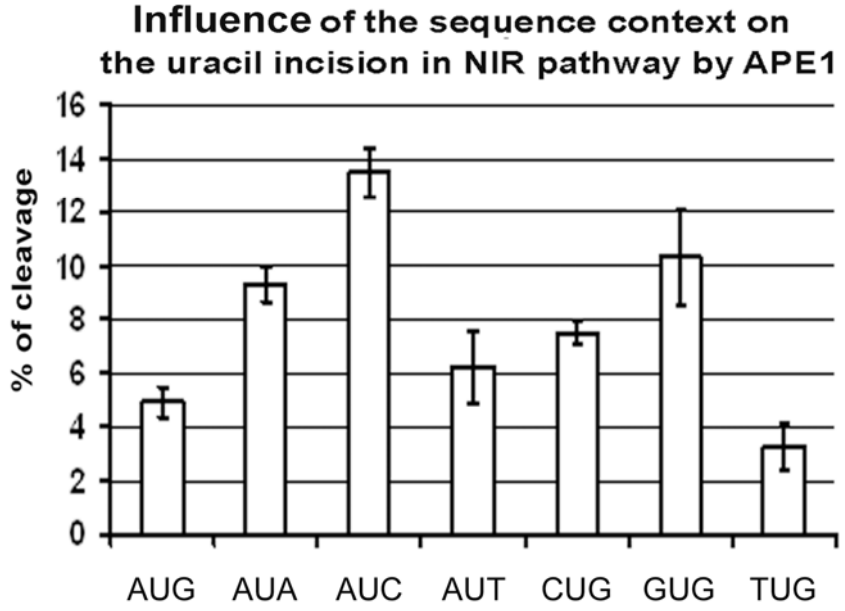


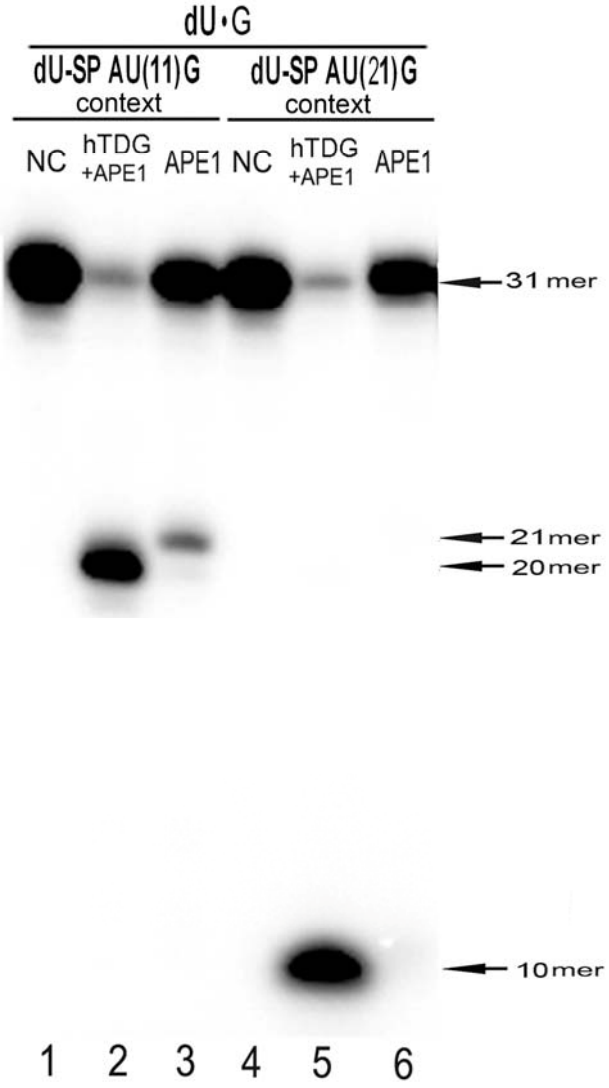
Fig. 4. Characterization of DNA substrate specificity of Mth212. 10 nM of the 3'-[^{32}P]-labelled 30-mer oligonucleotide duplexes (dU-RT sequence context) THF•T, U•G, αdA•T and 5ohC•G were incubated either with Mth212 for 15 min at 55°C, or 1 nM Nfo for 5 min at 37°C, under the respective reaction conditions. (**A**) Action of Mth212 on AP sites and uracil residues. Lane 1, THF•T incubated 2.5h at 55°C; lane 2, non-treated THF•T; lanes 3-7, as 2 but 0.5, 2, 4, 10 and 20 nM Mth212; lane 8, 1 nM Nfo; lane 9, U•G incubated 2.5h at 55°C; lane 10, non-treated U•G; lanes 11-15, as 10 but 0.5, 2, 4, 10 and 20 nM Mth212; lane 16, 1 nM Nfo. (**B**) Action of Mth212 on αdA and 5ohC residues. Lane 1, non-treated αdA•T; lanes 2-6, as 1 but 2, 4, 10, 20 and 100 nM Mth212; lane 7, 1 nM Nfo; lane 8, 5ohC•G incubated 2.5h at 55°C; lane 9, non-treated 5ohC•G; lanes 10-14, as 9 but 2, 4, 10, 20 and 100 nM Mth212; lane 15, 1 nM Nfo. The arrow "s" denotes the position of the non-cleaved 31-mer substrate, the arrow "p" denotes the position of the 21-mer cleavage product. For details, see Materials and Methods.

Supporting Information

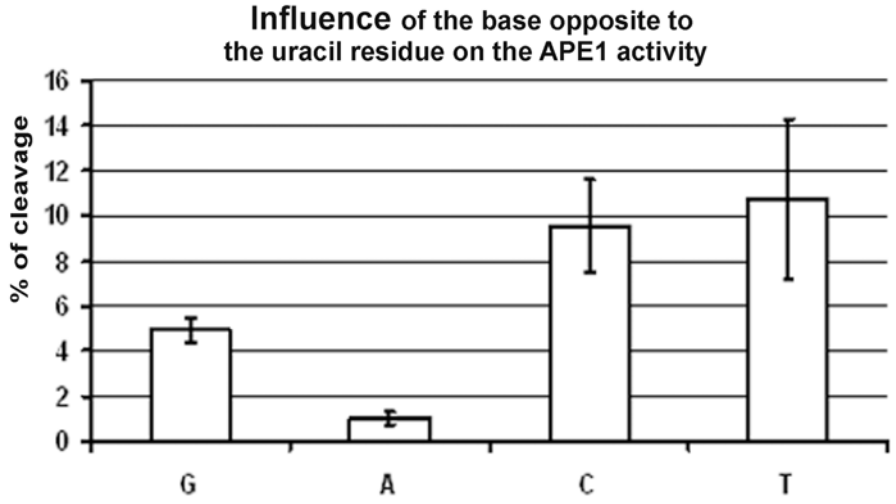
Supporting Information Figure Legends



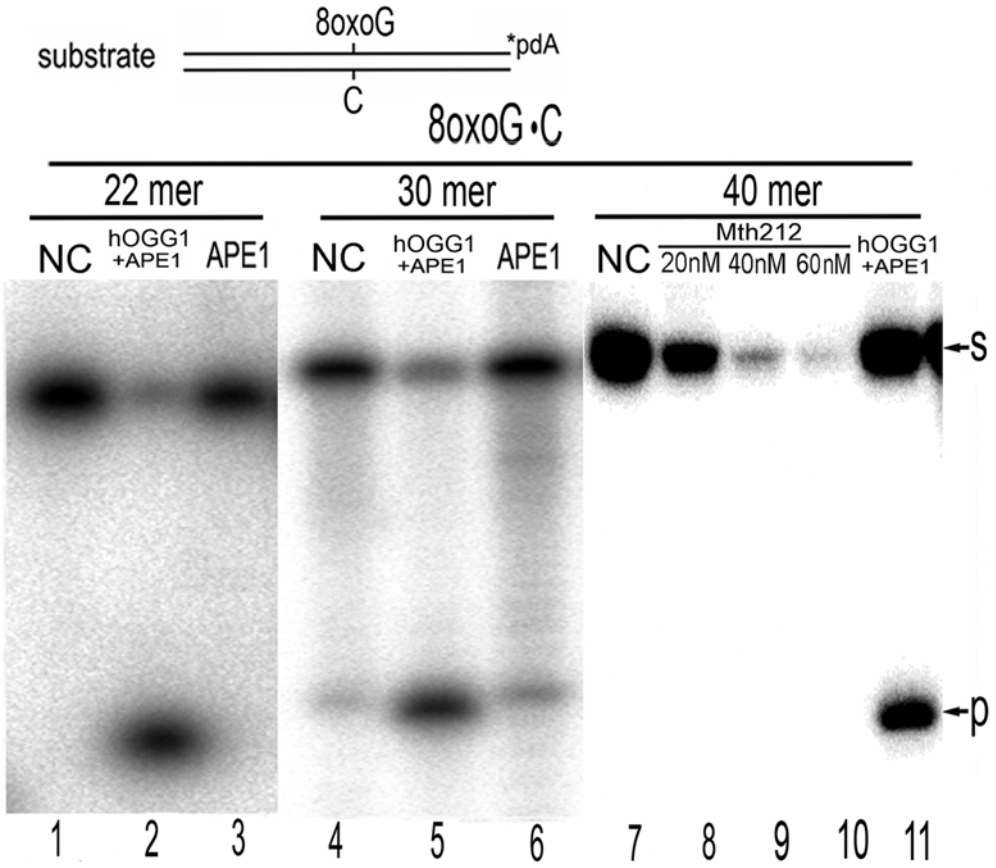
Supporting Information Figure S1. The influence of the nearest neighbouring bases to uracil residue on APE1-catalyzed DNA uridine endonuclease activity. 10 nM of 3'-[^{32}P]-labelled 20 mer oligonucleotide duplex (dU-RT20 sequence context, Table 1) containing a single dU in sequence contexts AUG, AUA, AUC, AUT, CUG, GUG, TUG was incubated for 2h at 37°C in the presence of 10 nM APE1 under NIR condition. All determinations were performed at least three time. For details see Materials and Methods.



Supporting Information Figure S2. Action of APE1 towards deoxyuridine residues when present in varied sequence contexts. Lane 1, non-treated control 10 nM of 3'-[^{32}P]-labelled U•G duplex with the sequence context referred as dU-SP AU(11)G; lane 2, as1 but 50 nM hTDG for 30 min at 37°C and then 10 nM APE1 for 15 min at 37°C; lane 3, as 1 but 10 nM APE1 for 2h at 37°C; lane 4, non-treated control 10 nM of 3'-[^{32}P]-labelled U•G duplex with the sequence context referred as dU-SP AU(21)G; lanes 5, as 4 but TDG + APE1; lane 6, as 4 but 10 nM APE1 for 2h at 37°C.The arrows denotes the position of the non-cleaved 31-mer DNA substrate, the position of the 21-mer NIR cleavage product and the position of the 20-mer and 10-mer BER cleavage product. For details see Materials and Methods.



Supporting Information Figure S3. The influence of the base opposite to dU residue on APE1-catalyzed DNA uridine endonuclease activity. 10 nM of 3'-[^{32}P]-labelled 20 mer oligonucleotide duplex (dU-RT20 sequence context, Table 1) containing a single uracil residue opposite to G, A, C or T was incubated for 2h at 37°C in the presence of 10 nM APE1 under NIR condition. All determinations were performed at least three time. For details see Materials and Methods.



Supporting Information Figure S4. Action of APE1 and Mth212 towards 10 nM of 3'-[^{32}P]-labelled DNA duplexes containing single 8oxoG residue. Lane 1, control non-treated 22 mer 8oxoG•C; lane 2, as1 but 25 nM hOGG1 for 30 min at 37°C and then 10 nM APE1 for 15 min, at 37°C; lane 3, as 1 but 10 nM APE1 for 1h at 37°C; lane 4, control non-treated 30 mer 8oxoG•C (dU-RT context); lanes 5, as 4 but OGG1 and APE1; lane 6, as 4 but APE1; lane 7, control 40 mer 8oxoG•C (dU-DL context); lane 8, as 7 but 20 nM Mth212; lane 9, 40 nM Mth212; lane 10, 60 nM Mth212; lanes 11, hOGG1 and APE1. The arrow "s" denotes the position of the non-cleaved 31 mer DNA substrate, the arrow "p" denotes the position of the 21-mer cleavage product. For details see Materials and Methods.