Supporting Information

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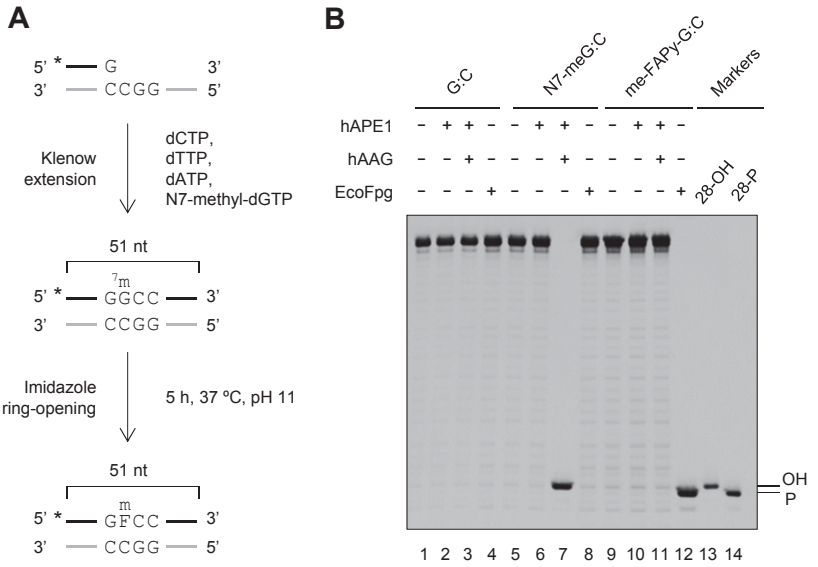

Fig. S1. Generation and characterization of DNA substrates containing N7-meG or me-FAPy-G. (*A*) Scheme of DNA substrate synthesis. A 5′-fluorescein–labeled 28-nt primer annealed to a 51-nt oligonucleotide was extended by *E. coli* DNA polymerase I (Klenow fragment 3′-5′ exonuclease-free) in the presence of dATP, dCTP, dTTP, and 7-methyl-dGTP for 1 h at 37 °C. The N7-meG was converted to the imidazole ring-opened form me-FAPy-G by incubation for 5 h at 37 °C in alkaline buffer (pH 11). (*B*) Validation of DNA substrates. DNA duplexes (20 nM) containing a 5′-end-labeled strand with a single N7-meG or me-FAPy-G opposite C were incubated with human APE1 (1 U), human AAG (2 U), or *E. coli* Fpg (8 U) at 37 °C for 8 h. Reaction products were separated by denaturing PAGE and detected by fluorescence scanning. Asterisks represent the fluorescent label.

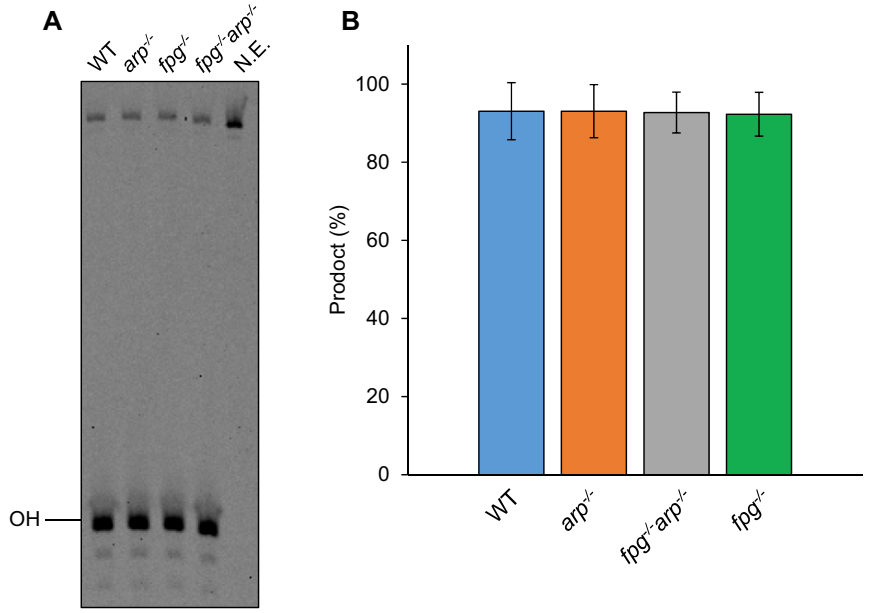

Fig. S2. Uracil DNA glycosylase activity of *Arabidopsis* cell-free extracts. Double-stranded oligonucleotide substrates (20 nM) containing a single U:C mismatch were incubated with WT, *fpg*$^{-/-}$, *arp*$^{-/-}$, or *fpg*$^{-/-}$ *arp*$^{-/-}$ *Arabidopsis* cell-free extracts (8 μg) for 3 h at 37 °C. Then, hAPE1 (10 U) and $MgCl_2$ (2 mM) were added and incubation continued for 1 h. Reaction products were separated by denaturing PAGE and detected by fluorescence scanning (*A*). Values shown in the graph (*B*) are means with SEs from three independent experiments. N.E., nonextract.

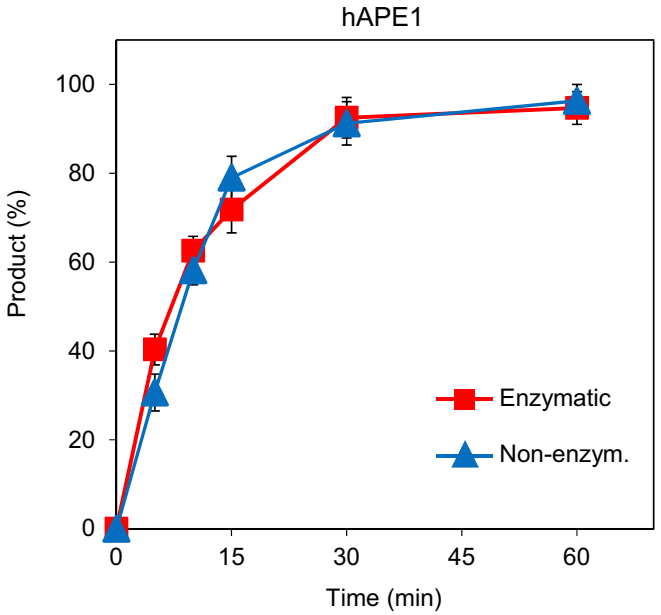

Fig. S3. Activity of hAPE1 on AP sites generated by enzymatic and nonenzymatic release of N7-meG. DNA substrates (20 nM) were a 9:1 mixture of homoduplex G:C and heteroduplex AP:C generated either by spontaneous N7-meG depurination (nonenzymatic, blue triangles) or N7-meG excision by hAAG (enzymatic, red squares). Substrates were incubated with hAPE1 (0.01 U) in the presence of 2 mM $MgCl_2$. Reaction products were separated by denaturing PAGE and detected by fluorescence scanning. Values are means with SEs from three independent experiments.

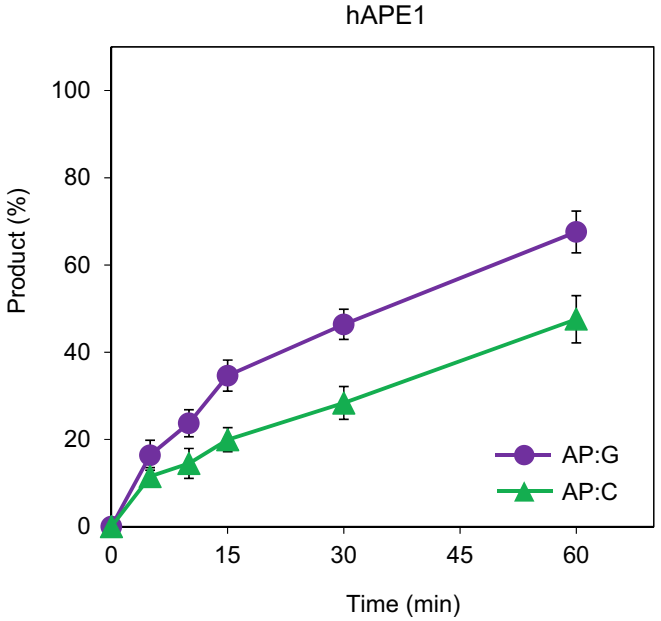

Fig. S4. Activity of hAPE1 on enzymatically generated AP sites opposite C or G. DNA substrates (2 nM) contained either a single AP:G (purple circles) or AP:C (green triangles), both generated by uracil excision. Substrates were incubated with hAPE1 (0.01 U) in the presence of 2 mM $MgCl_2$. Reaction products were separated by denaturing PAGE and detected by fluorescence scanning. Values are means with SEs from three independent experiments.

Table S1. DNA sequence of oligonucleotides used as primers

Name	DNA sequence 5′-3′
FPG_F1	AACGAAGCAATAAAAGGCGC
FPG_R5	CCACTCCTCTGAGTCCTTTACAGC
ARP_F1	GAACTTATCTCAACTTTACGAC
ARP_R1	GCTCTCAAACTTCAACAATCC
LBa1	TGGTTCACGTAGTGGGCCATCG
ZDP_F1	AATGAATCCAACATTGATCGATGGAAG
ZDP_R1	ATACAGCTAAGTCCCTGGCGATGTACTT
LB3	TAGCATCTGAATTTCATAACCAATCTCGATACAC

Table S2. DNA sequence of oligonucleotides used as substrates

Name	DNA sequence 5′-3′	Strand
Fl-28G	TCACGGGATCAATGTGTTCTTTCAGCTG	Upper
GGCCRnoC	GGTATTGATGGTGAGAGTGAGGCCAGCTGAAAGAACACATTGATCCCGTGA	Lower
Fl-GUCCRnoC	TCACGGGATCAATGTGTTCTTTCAGCTGUCCTCACTCTCACCATCAATACC	Upper
GGGCRnoC	GGTATTGATGGTGAGAGTGAGGGCAGCTGAAAGAACACATTGATCCCGTGA	Lower
Fl-GGCC	TCACGGGATCAATGTGTTCTTTCAGCTGGCCTCACGCTGACCAGGAATACC	Upper
GGCC	GGTATTCCTGGTCAGCGTGAGGCCAGCTGAAAGAACACATTGATCCCGTGA	Lower