Supplementary Table S1 Oligonucleotides sequences

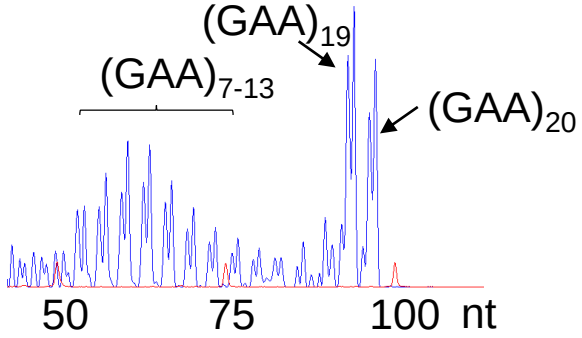
Oligonucleotides	nt	Sequence (5'-3')
Damaged Strands		
D1	**100**	CGA GTC ATC TAG CAT CCG TA GAA GAA GAA GAA GAA GAA GAA GAA GAA **U**AA GAA GAA GAA GAA GAA GAA GAA GAA GAA GAA TA CGT AGA CTT ACT CAT TGC
D2	**100**	CGA GTC ATC TAG CAT CCG TA CAG CAG CAG CAG CAG CAG CAG CAG CAG CA**U** CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG TA CGT AGA CTT ACT CAT TGC
D3	**99**	CGA GTC ATC TAG CAT CCG TA GAA GAA GAA GAA GAA GAA GAA GAA GAA **F**AA GAA GAA GAA GAA GAA GAA GAA GAA GAA GAA TA CGT AGA CTT ACT CAT TGC
D4	**99**	CGA GTC ATC TAG CAT CCG TA CAG CAG CAG CAG CAG CAG CAG CAG CAG CA**F** CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG TA CGT AGA CTT ACT CAT TGC
D5	**100**	CGA GTC ATC TAG CAT CCG TA GAA GAA GAA GAA GAA GAA GAA GAA GAA **U**T**A** GAA GAA GAA GAA GAA GAA GAA GAA GAA GAA TA CGT AGA CTT ACT CAT TGC
D6	**100**	CGA GTC ATC TAG CAT CCG TA CAG CAG CAG CAG CAG CAG CAG CAG CAG CA**U** C**T**G CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG TA CGT AGA CTT ACT CAT TGC
D7	**100**	CGA GTC ATC TAG CAT CCG TA TCG CAC TGT TAT CAT TTC GTG TAC TTC **U**T**G** TGT ATG TGT CAT ATA TTC ATT TGC GCT AAC TA CGT AGA CTT ACT CAT TGC
Upstream Strands		
U1	**47**	CGA GTC ATC TAG CAT CCG TA GAA GAA GAA GAA GAA GAA GAA GAA GAA
U2	**49**	CGA GTC ATC TAG CAT CCG TA CAG CAG CAG CAG CAG CAG CAG CAG CAG CA
Downstream Strands		
D1	**53**	p**U**-AA GAA GAA GAA GAA GAA GAA GAA GAA GAA GAA TA CGT AGA CTT ACT CAT TGC
D2	**51**	p**U**-CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG TA CGT AGA CTT ACT CAT TGC
Template Strands		
T1	**100**	GCA ATG AGT AAG TCT ACG TA TTC TTC TTC TTC TTC TTC TTC TTC TTC TTC TTC TTC TTC TTC TTC TTC TTC TTC TTC TTC TTC TA CGG ATG CTA GAT GAC TCG
T2	**100**	GCA ATG AGT AAG TCT ACG TA CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG TA CGG ATG CTA GAT GAC TCG
T3	**100**	GCA ATG AGT AAG TCT ACG TA TTC TTC TTC TTC TTC TTC TTC TTC TTC TTC TAC TTC T**T**C TTC TTC TTC TTC TTC TTC TTC TA CCG ATG CTA GAT GAC TCG
T4	**100**	GCA ATG AGT AAG TCT ACG TA CTG CTG CTG CTG CTG CTG CTG CTG CTG CAG CTG C**T**G CTG CTG CTG CTG CTG CTG CTG CTG TA CGG ATG CTA GAT GAC TCG
T5	**100**	GCA ATG AGT AAG TCT ACG TA GTT AGC GCA AAT GAA TAT ATG ACA CAT ACA CAC GAA G**T**A CAC GAA ATG ATA ACA GTG CGA TA CGG ATG CTA GAT GAC TCG

[a] The damaged base is in boldface. U, Uracil; F, tetrahydofuran.
[b] The base with internal fluorescence tag is in bold face and underlined. T, Fluorescein dT; **T**, Black Hole Quencher®-1 dT.

Supplementary Figure S1

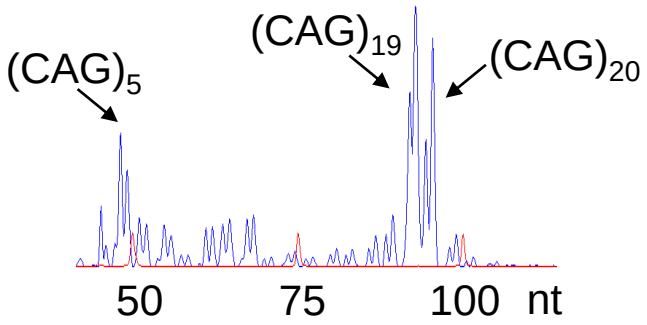
A

Pol β$^{-/-}$ cell extracts
+
pol β 8 kDa domain

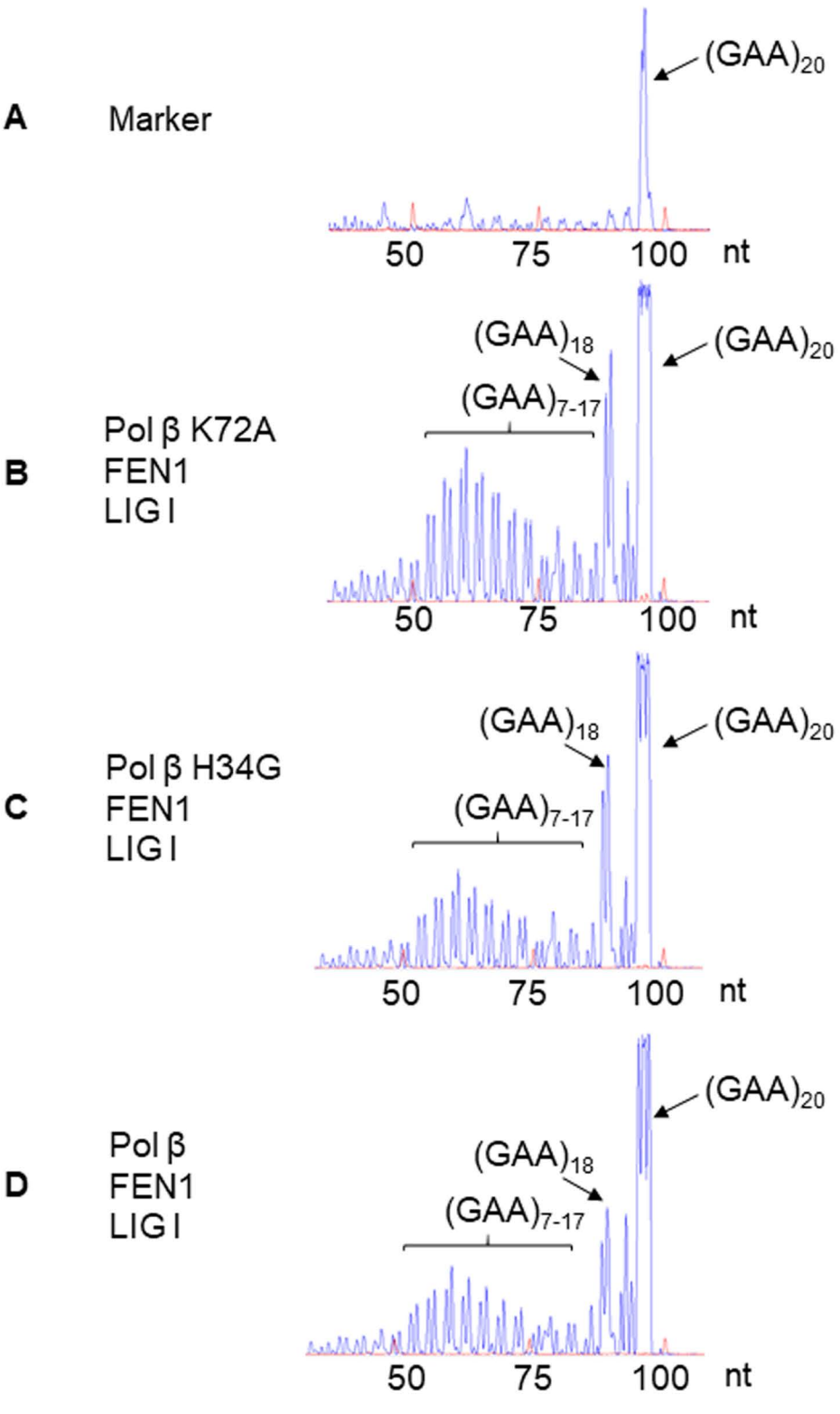


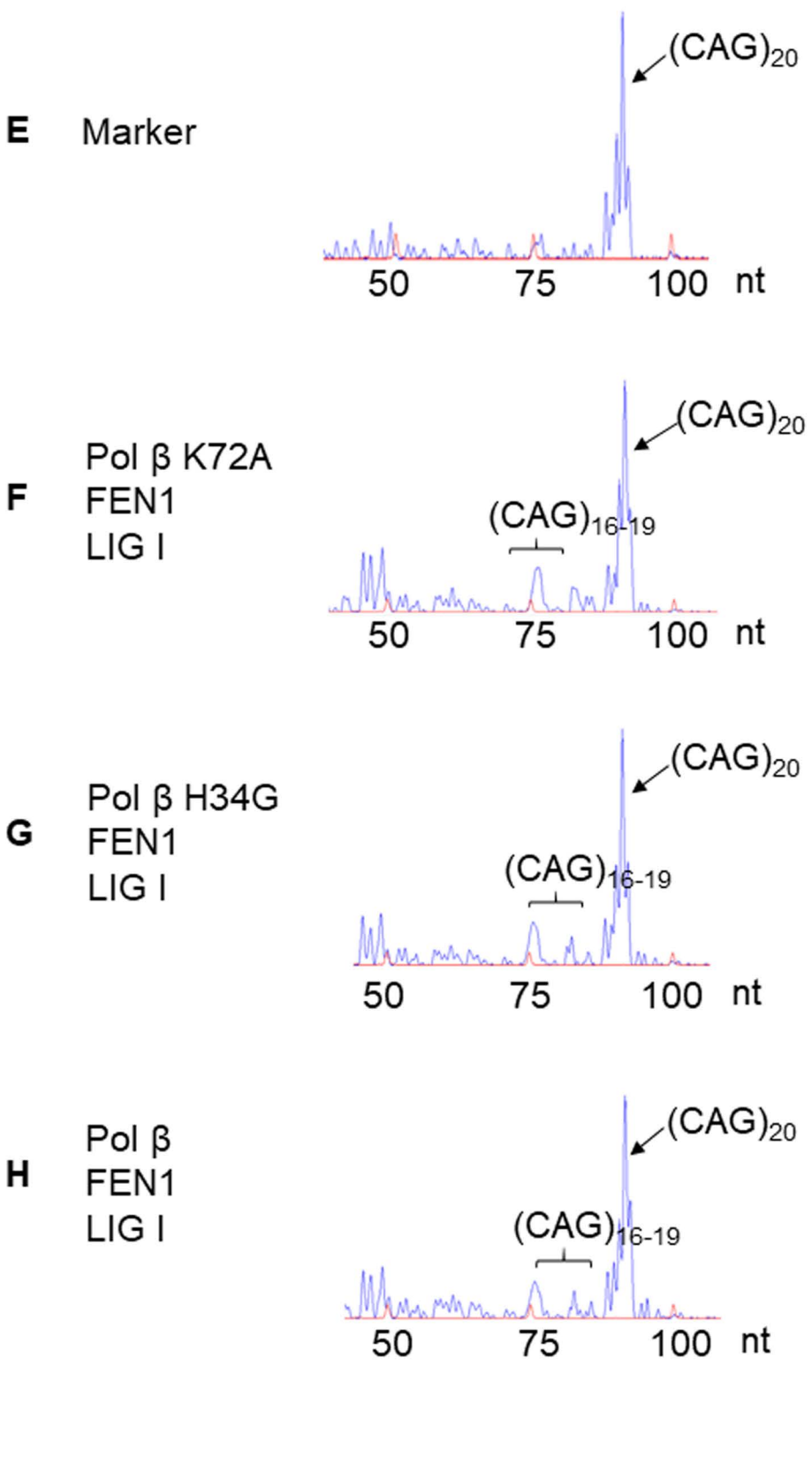
B

Pol β$^{-/-}$ cell extracts
+
pol β 8 kDa domain

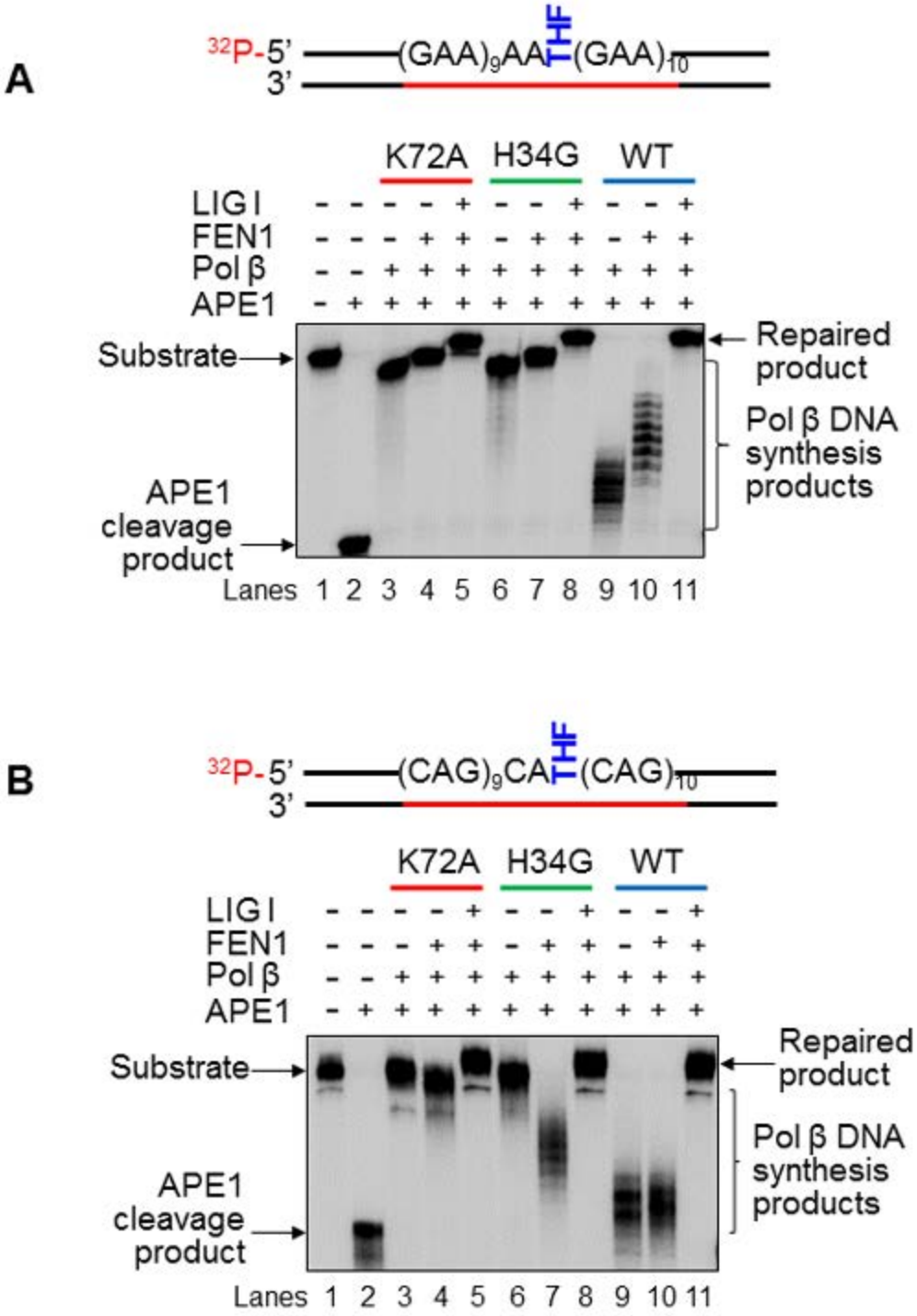


Supplementary Figure S2

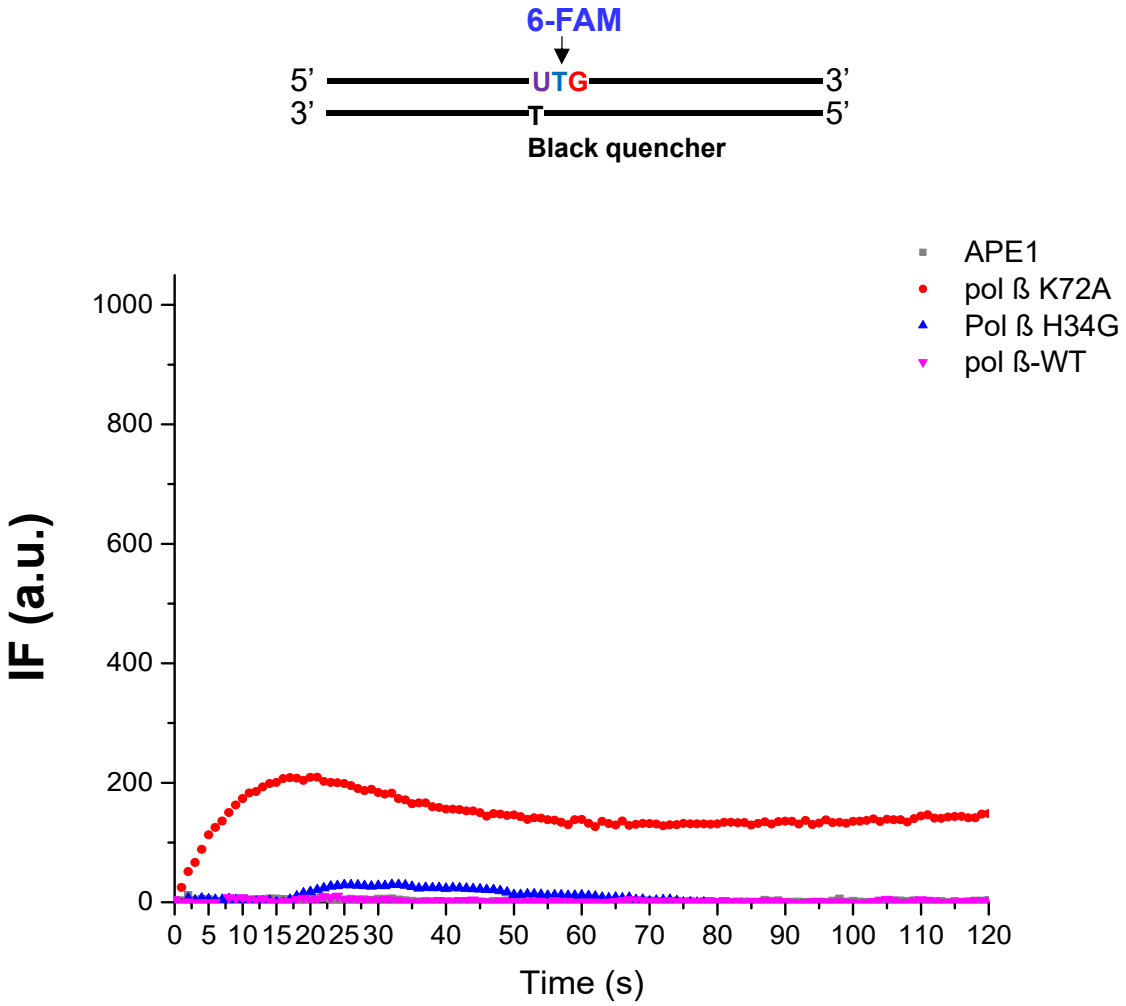




Supplementary Figure S3



Supplementary Figure S4



Supplementary Methods

In vitro BER of an abasic site with a reduced sugar

BER of an abasic site with a reduced sugar was reconstituted by incubating 25 nM APE1, 10 nM pol β K72A mutant protein or 10 nM pol β H34G mutant protein or 10 nM pol β WT protein along with 25 nM FEN1 and 25nM LIG I, with 25 nM $(GAA)_{20}$ or $(CAG)_{20}$ repeat-containing substrates with a THF, an analogue of reduced abasic site. Reaction mixture (20 µl) contained BER buffer with 50 µM dNTPs, 5 mM Mg^{2+}, 2 mM ATP, and indicated concentrations of BER enzymes and substrates. Reaction mixture was assembled on ice, and incubated at 37 °C for 30 min. Reactions were terminated by heating at 95 °C for 10 min in stopping buffer containing 95% formamide and 2 mM EDTA. Repair intermediates and products were separated by 18% urea-denaturing PAGE and detected by the Pharos FX Plus PhosphorImager from Bio-Rad Laboratory (Hercules, CA).To isolate repaired products, the template strand of the substrates was biotinylated at the 5′-end. Repaired strands were separated from the template strand with 0.15 M NaOH and released into the supernatant. Subsequently they were precipitated with ethanol, dissolved in TE buffer, and stored at -20 °C for subsequent repeat sizing analysis

Supplementary Figure Legends

Supplementary Figure S1. Pol β 8 kD domain fails to prevent TNR deletions during BER mediated by pol $\beta^{-/-}$ MEF extracts.

The effect of pol β 8 kD domain on TNR instability was examined by reconstituting BER with $(GAA)_{20}$ or $(CAG)_{20}$ repeat substrates containing a native abasic site and pol $\beta^{-/-}$ MEF extracts in the presence of pol β 8 kD domain as described in the Materials and Methods. (**A**) The DNA fragment analysis results of the repaired products from BER mediated by pol $\beta^{-/-}$ MEF extracts complemented with pol β 8 kD domain with the $(GAA)_{20}$ substrate. (**B**) The DNA fragment analysis results of the repaired products from BER mediated by pol $\beta^{-/-}$ MEF extracts complemented with pol β 8 kDa domain with the $(CAG)_{20}$ substrate. Substrates were illustrated above the graphs. The template strand of the substrates was biotinylated at the 5'-end. Major repaired products are indicated with black arrows. The red peaks are DNA size markers. The sizes of DNA fragments are illustrated as nucleotide, nt. The experiment was conducted in triplicate, and only representative results are illustrated in the figure.

Supplementary Figure S2. Pol β dRP lyase activity fails to prevent TNR deletions during BER of an abasic site with a reduced sugar.

BER of an abasic site with a reduced sugar was reconstituted with the $(GAA)_{20}$ or $(CAG)_{20}$ repeats substrate containing a THF as described in the Supplementary Methods. (**A**) and (**E**) The DNA fragment analysis result of a DNA size marker containing $(GAA)_{20}$ or $(CAG)_{20}$ repeats without a base lesion. (**B**) and (**F**) The DNA fragment analysis results of the repaired products from BER reconstituted with the $(GAA)_{20}$ or $(CAG)_{20}$ repeats substrate in the presence of pol β K72A and FEN1. (**C**) and (**G**) The DNA fragment analysis results of the repaired products from BER reconstituted with the $(GAA)_{20}$ or $(CAG)_{20}$ repeats substrate in the presence of pol β H34G and FEN1. (**D**) and (**H**) The DNA fragment analysis results of the repaired products from BER reconstituted with the $(GAA)_{20}$ or $(CAG)_{20}$ repeat substrate in the presence of wild-type pol β and FEN1. Substrates were illustrated above the graphs. The template strand of the substrates was biotinylated at the 5'-end. Major repaired products are indicated with black arrows. The

red peaks are DNA size markers. The sizes of DNA fragments are illustrated as "nt". Experiments were conducted in triplicate, and only representative results are illustrated in the figure.

Supplementary Figure S3. Pol β DNA synthesis during BER of an abasic site with a reduced sugar in TNR tracts.

DNA synthesis activities of pol β WT and pol β dRP lyase mutant, K72A and H34G during BER were characterized with the $(GAA)_{20}$ (**A**) or $(CAG)_{20}$ (**B**) repeat substrate containing a THF as described in the Supplementary Methods. Lane 1 represents the substrate only. Lane 2 indicates the reaction mixture with 25 nM APE1. Lanes 3 and 4 correspond to the reaction mixture with 10 nM pol β K72A in the absence and presence of 25 nM FEN1. Lane 5 corresponds to the reaction mixture with 10 nM pol β K72A and 25 nM LIG I in the presence of 25 nM FEN1. Lanes 6 and 7 correspond to the reaction mixture with 10 nM pol β H34G in the absence and presence of 25 nM FEN1. Lane 8 corresponds to the reaction mixture with 10 nM pol β H34G and 25 nM LIG I in the presence of 25 nM FEN1. Lanes 9 and 10 correspond to the reaction mixture with 10 nM pol β in the absence and presence of 25 nM FEN1. Lane 11 corresponds to the reaction mixture with 10 nM pol β WT and 25 nM LIG I in the presence of 25 nM FEN1. Substrates were ^{32}P-labeled at the 5'-end of the damaged strand and are illustrated above each gel. The experiments were repeated at least three times. Representative gels are illustrated.

Supplementary Figure S4. The strand displacement synthesis by pol β dRP lyase mutants and WT during BER of a native abasic site in the context of random DNA sequence. The strand displacement synthesis by pol β dRP lyase mutants and WT during BER of a native abasic site in the context of random DNA sequence was examined as described in the Materials and Methods. The intensity of fluorescence (IF) generated from each reaction condition was recorded by the Bio-Rad CFX Connect Real-Time PCR Detection System and plotted against time (s). The IF from each reaction condition was indicated with different color. A schematic diagram of random DNA sequence-containing substrate with a native abasic site, BHQ-1 dT, and 6-FAM-dT was illustrated above the graph. Experiments were repeated in triplicate, and the representative result is shown.