SUPPLEMENTAL MATERIALS

Human Base Excision Repair Creates a Bias Towards -1 Frameshift Mutations*

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Figure S1. Oligonucleotide sequences that were used in this study. 25mer oligonucleotides of the sequence shown were annealed to a complementary 25mer oligonucleotide to form paired or mismatched duplexes (top) or to a complementary 24mer oligonucleotide to form a single nucleotide bulge (bottom). The X position was varied between normal and damaged bases and the X-containing oligonucleotide was labeled at the 5' end with 5-carboxyfluorescein attached by a 6-aminohexyl linker (fam). The Y position was varied among normal bases, or was omitted to create a single nucleotide X-bulge.

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5'fam-CGATAGCATCCTXCCTTCTCTCCAT
   3'-GCTATCGTAGGAYGGAAGAGAGGTA
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                    X
5'fam-CGATAGCATCCT CCTTCTCTCCAT
   3'-GCTATCGTAGGA GGAAGAGAGGTA
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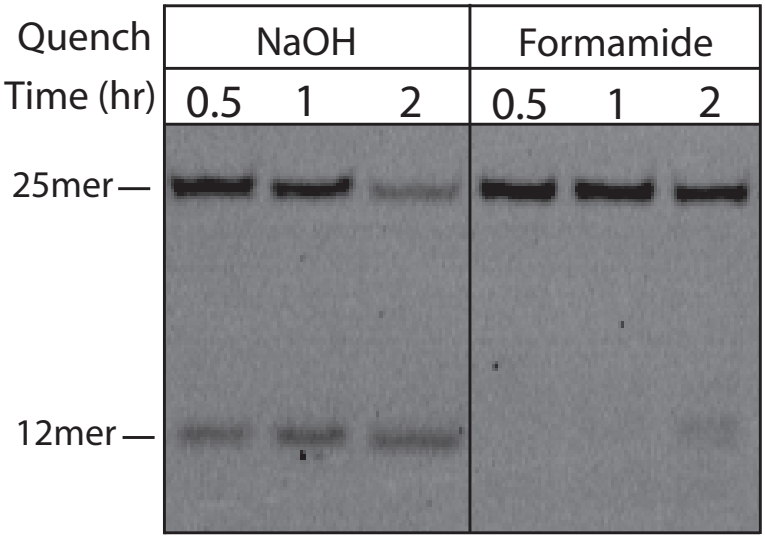
Figure S2. Stability of bulged abasic in HeLa WCE (A) Monitoring of glycosylase activity on 10 nM I-bulge substrate in 0.4 mg/mL HeLa WCE under glycosylase conditions produces a hydroxide cleavable abasic site and the resulting 12mer product. Simultaneous quenching with formamide/EDTA shows that the abasic site largely remains intact under these conditions, producing a small amount of enzymatic cleavage after two hours. This activity is likely due to lyase activity by bifunctional glycosylases. (B) Quantification of nicked intermediate (12mer).

(A)



(B)

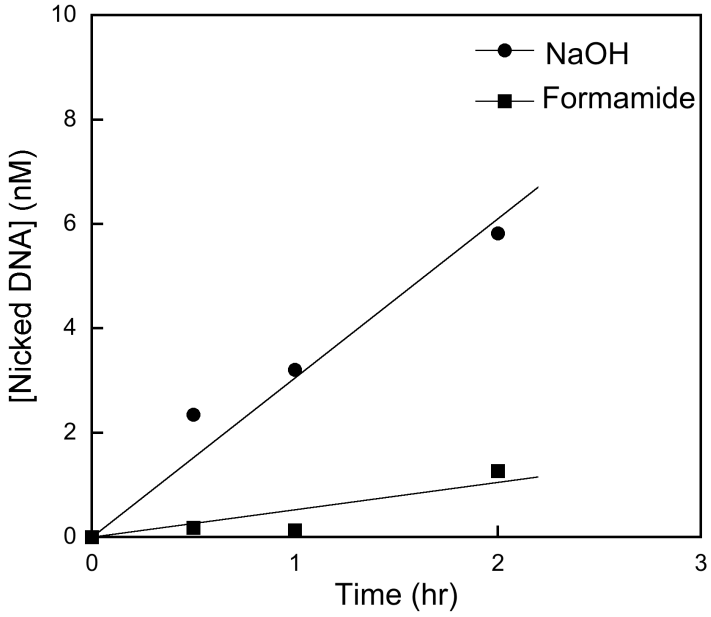


Figure S3. Excision of G-bulge by AAG. Reactions were performed as described in Figure 2 of the text, except that 1 μM recombinant AAG was added. A similar slow rate of excision of guanine is observed in the presence and absence of HeLa WCE (0.2 mg/mL).

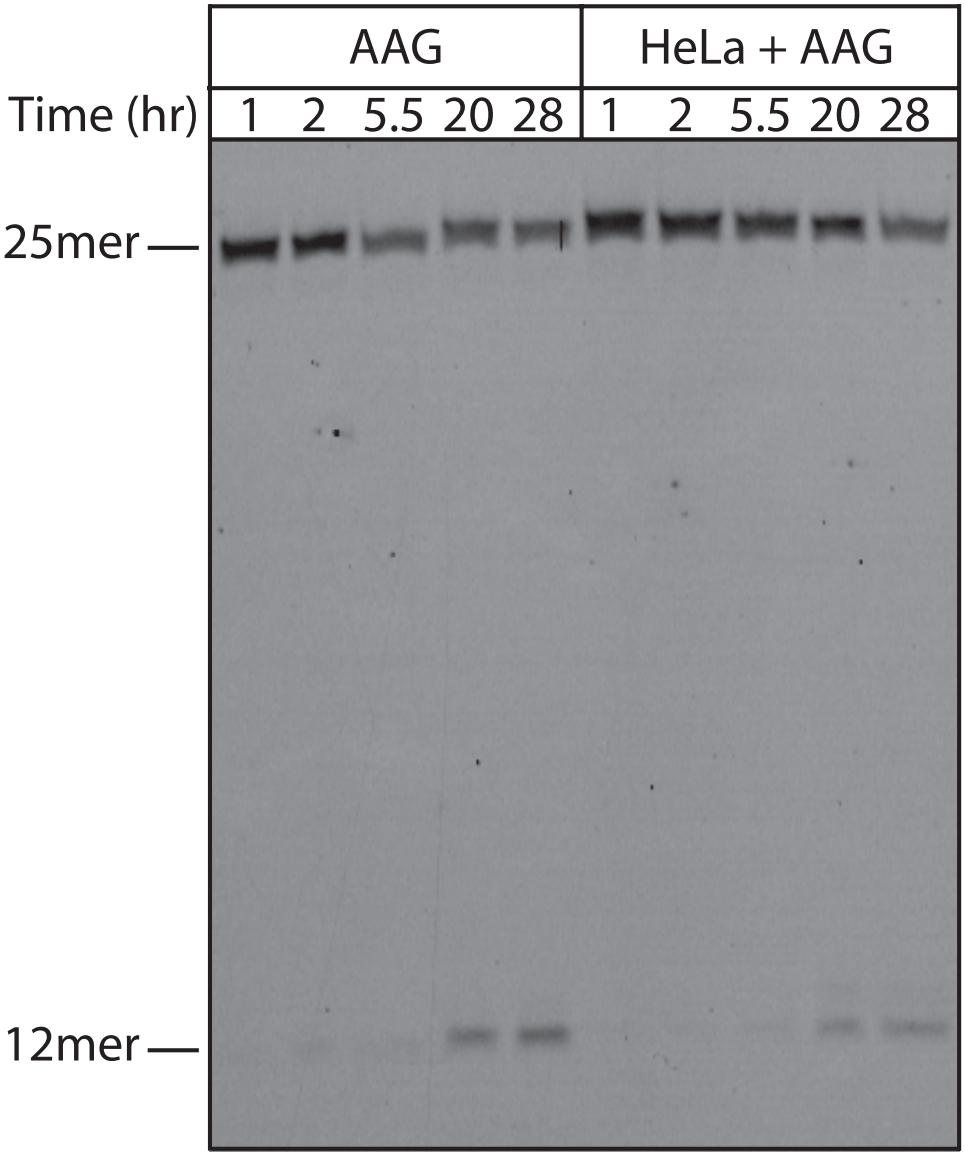


Figure S4. Quantification of DNA alkylation by chloroacetaldehyde. The amount of εA formed upon exposure to chloroacetaldehyde is quantified from Fig. 4 in the text and two additional experiments under the same conditions. The mean ± SD is shown for perfectly paired duplex (A•T) and for the single nucleotide A-bulge. Whereas 18 ± 2 percent of the A-bulge was alkylated and subsequently removed by AAG, no detectable reaction occurred with A•T duplex DNA (≤1%).

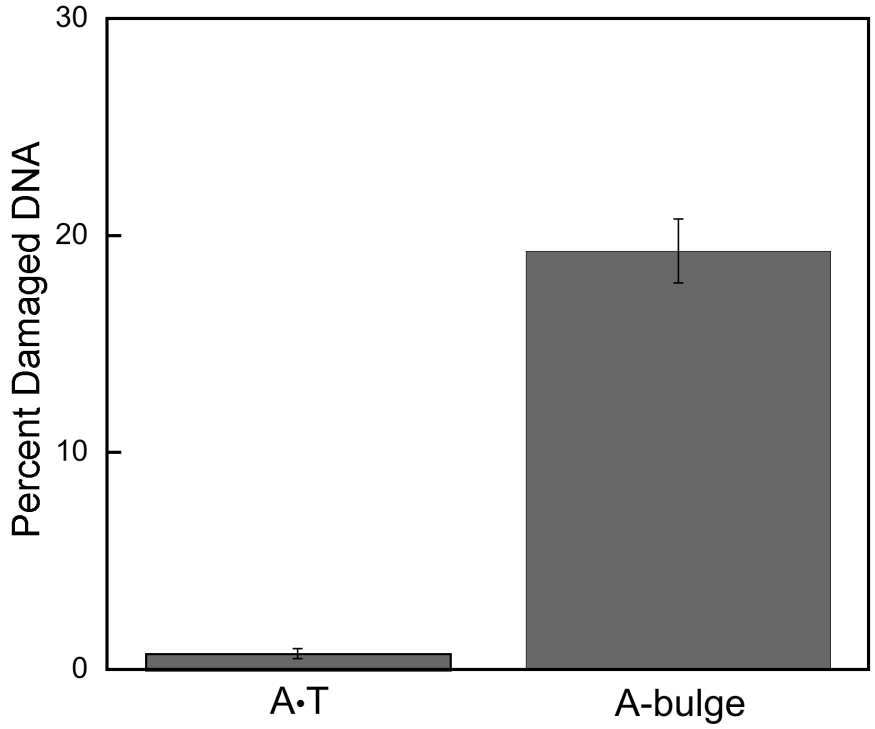


Figure S5. Time and concentration dependence of chloroacetaldehyde modification. This experiment was performed under similar conditions as Fig. 4 in the text. 500nM DNA in a 50 µL reaction volume was incubated with CAA for the time and concentration of chloroacetaldehyde (CAA) indicated. CAA was extracted twice with hydrated ether without a subsequent ethanol precipitation. 4 µL of the damaged DNA was incubated with 2 µM AAG for 30 min at 37 °C in a 20 µL reaction volume. Samples were analyzed according to the standard glycosylase assay.

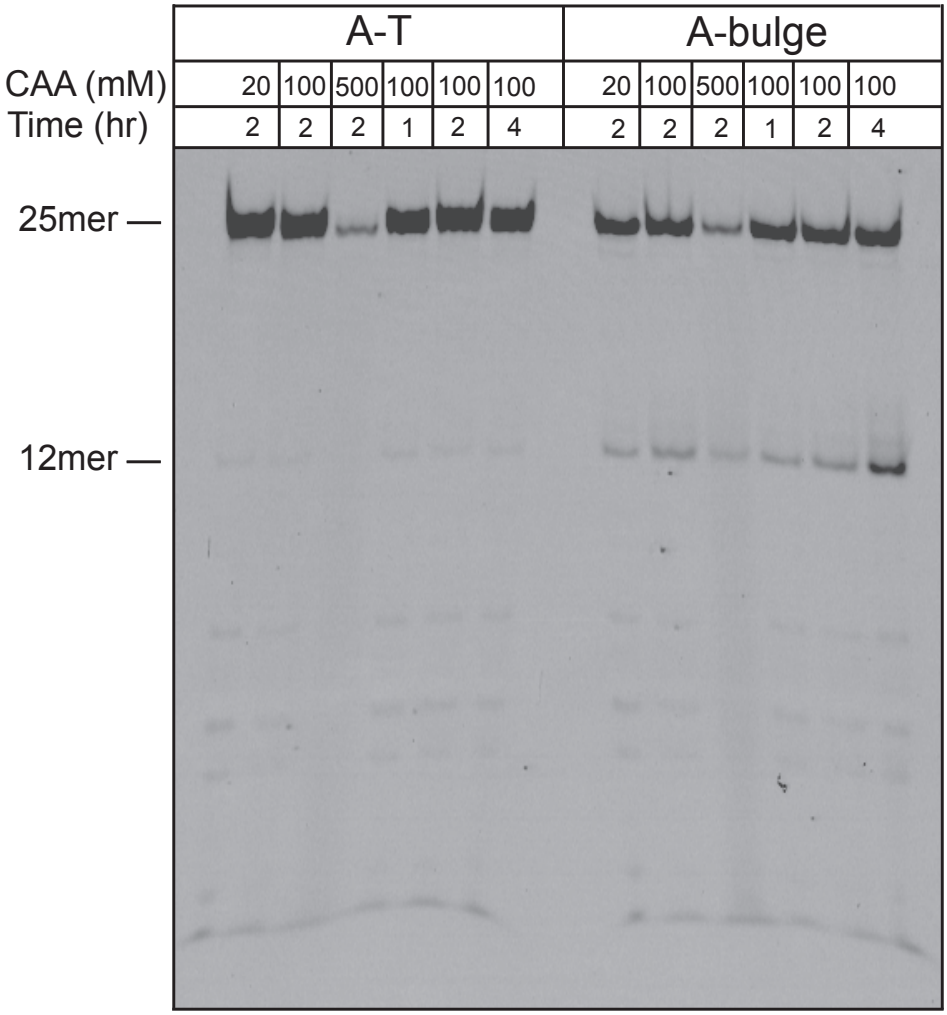
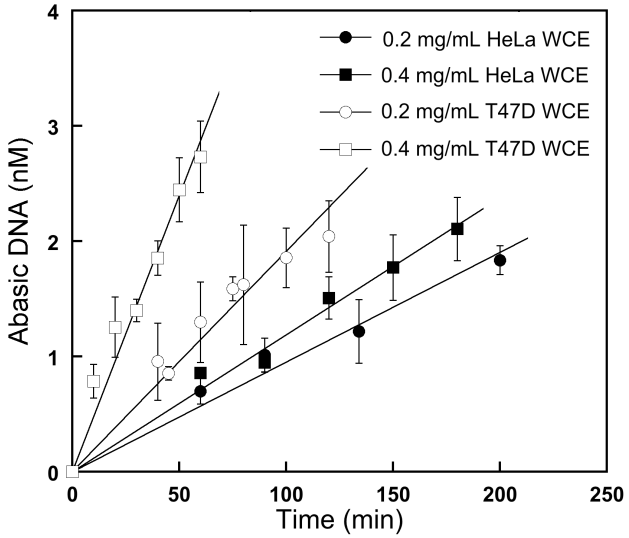


Figure S6. AAG activity on I•T substrate in WCE from HeLa and T47D cells. (A) Initial rates of AAG activity on 10 nM I•T substrate under glycosylase conditions at two concentrations of HeLa and T47D WCE. Each point represents the average of three experiments with error bars representing one standard deviation from the mean. (B) The calculated specific activity for the two extracts is presented as the mean ± SD. This 3-fold greater level of AAG activity in T47D relative to HeLa is calculated from single extract preparations, but are in excellent agreement with the previous finding of 3-fold greater AAG levels in T47D relative to HeLa WCE (*1*).

(A)



(B)

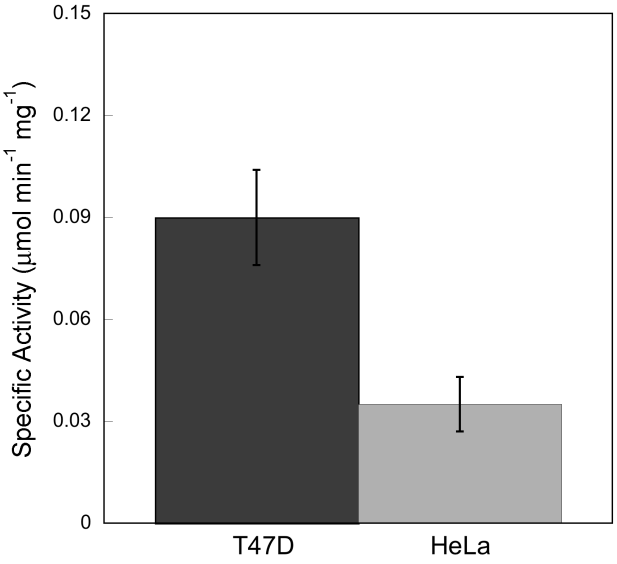


Figure S7. BER activity on U-bulge in WCE from HeLa cells. 10 nM U-bulge fam-labeled DNA and 100 nM A•T unlabeled competitor DNA were incubated in 0.4 mg/mL HeLa WCE for the indicated times in the presence or absence of UGI (0.02 units). In the absence of UGI, the expected nicked intermediate (12mer) formed and appeared to be extended by DNA polymerases (polymerase extension intermediates). No BER intermediates were detected in the presence of UGI, but significant 3'-5' exonuclease degradation was apparent.

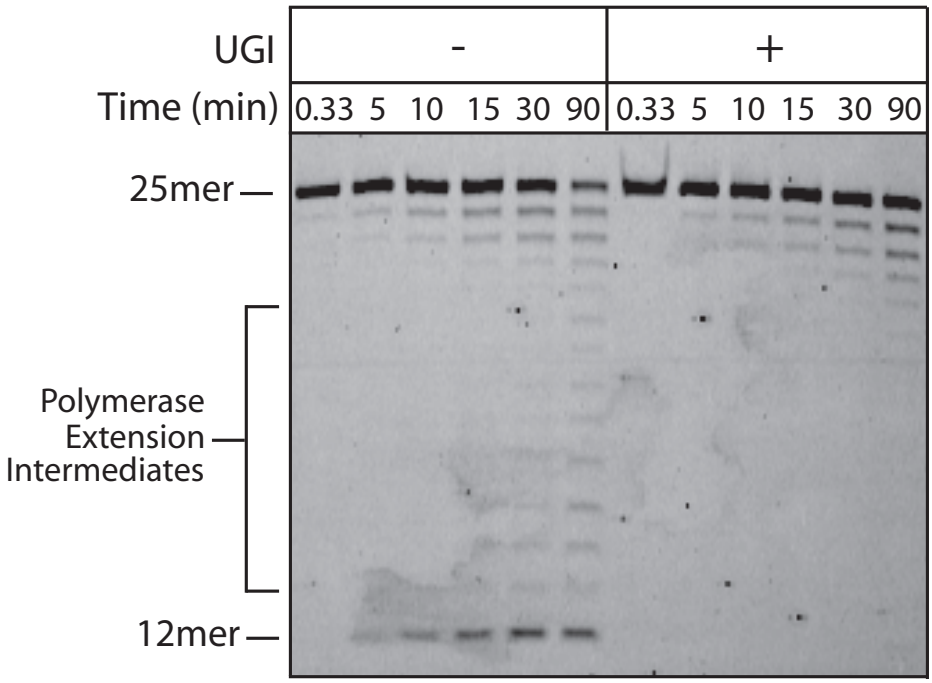


Figure S8. BER activity on I-bulge in nuclear extract from MCF-7 breast cancer cells 10 nM I-bulge DNA was incubated with 100 nM unlabeled A•T competitor DNA in 0.4 mg/mL nuclear extract from MCF-7 cells according the "BER Assays in WCE" in the text. The expected nicked intermediate (12mer) built up more quickly than was observed in HeLa extracts (data not shown), and formed a significant amount of polymerase extension products. The 24mer product that accumulated indicates single nucleotide deletion followed by either short patch or long patch BER.

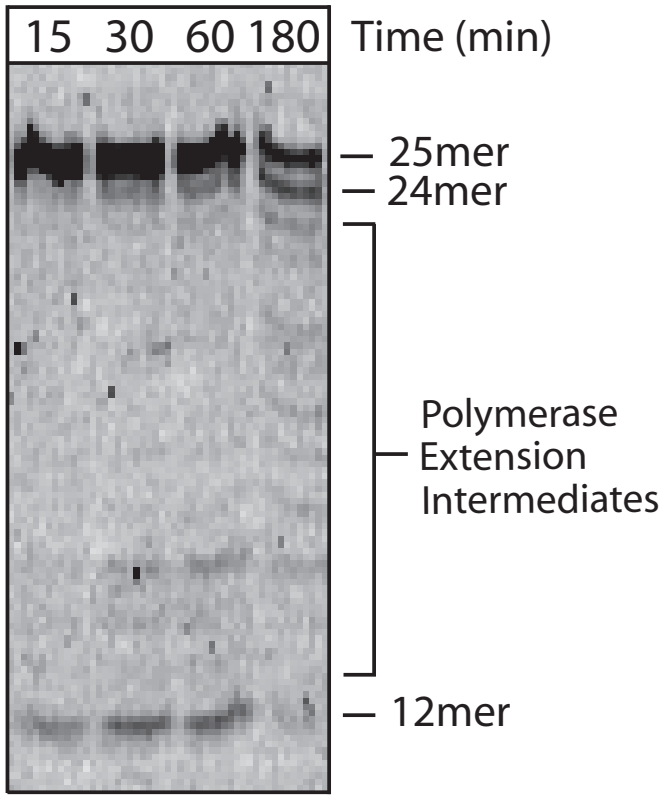


Figure S9. BER activity on I-bulge in nuclear extract from HT-29 colon cancer cells. Conditions were exactly as described in Fig. 6 in the text, except that 0.4 mg/mL nuclear extract from HT-29 cells was used. No BER intermediates were detected in the presence of an εA•T inhibitor, however substantial 3'–5'-exonuclease activity was observed. In the absence of inhibitor the nicked intermediate (12mer) built up, and at later time points polymerase extension intermediates could be detected. The single nucleotide deletion product (24mer) can be readily detected. At all times, there was more 24mer product when AAG was active (A•T competitor) than when AAG was inhibited (εA•T competitor), indicating that the deletion of the I-bulge occurs in HT-29 extracts.

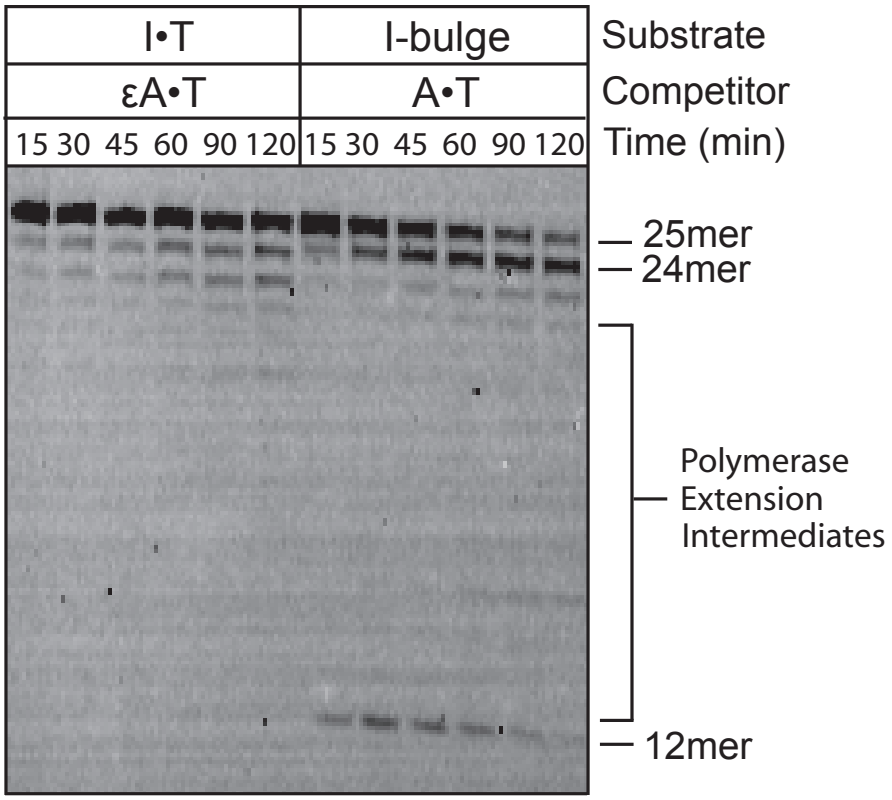


Figure S10. Inhibition of polymerase extension of BER intermediates in T47D WCE with aphidicolin. Aphidicolin is an inhibitor of several replicative polymerases, including pol α, pol δ, and pol ε, but it does not inhibit pol β (2). The I•T mismatch or I-bulge substrate was incubated in T47D WCE as described in "BER Assays in WCE". Aphidicolin (450 μM) was solubilized in DMSO, so the no aphidicolin control reactions also contained the same volume of DMSO to yield 7.5% (v/v) in the reaction. Reactions were quenched at the indicated time in formamide/EDTA and analyzed by denaturing PAGE. Apparently DMSO slightly inhibits either pol β or DNA ligase, because less of the 24mer deletion product was observed relative to reactions without DMSO (see Fig. 5 in the text). However, aphidicolin did not decrease the rate of formation of the deletion product. No 13-15mer products were observed in the presence of 450 μM aphidicolin, suggesting that these were products of an aphidicolin-sensitive polymerase.

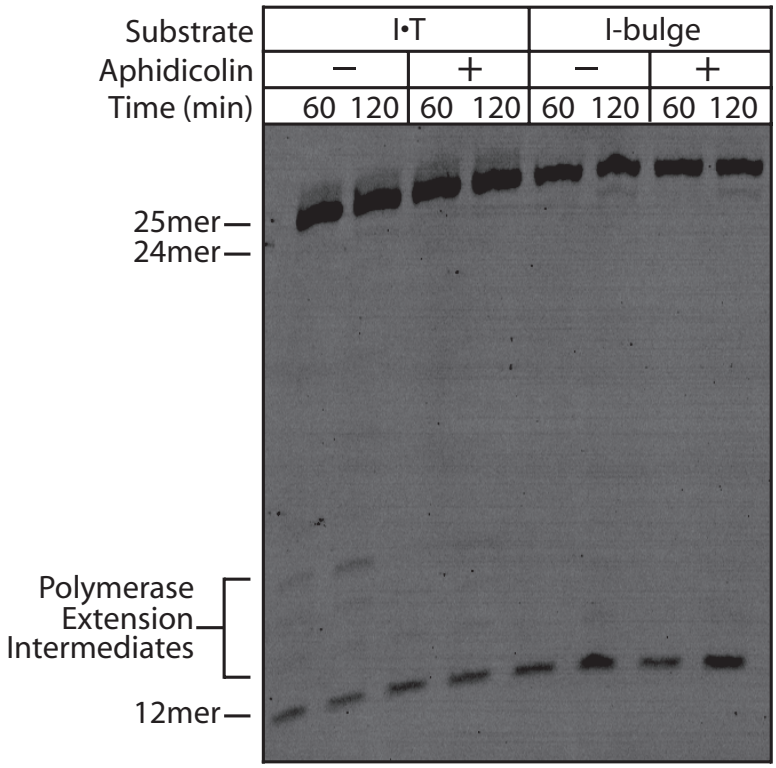


Figure S11. Single nucleotide deletion occurs in T47D WCE in absence of dNTPs. The I•T mismatch or I-bulge substrate was incubated in T47D WCE as described in "BER Assays in WCE", except that the 4 dNTPs were either present at 25 μM or were absent. Reactions were quenched in formamide/EDTA at the indicated times and analyzed be denaturing PAGE. The presence of 13-15mer products in the presence, but not in the absence, of dNTPs is consistent with these resulting from polymerase extension of a nicked 12mer BER intermediate. As predicted in the text (Fig. 7), the formation of the 24mer single nucleotide deletion from an I-bulge occurs even in the absence of dNTPs.

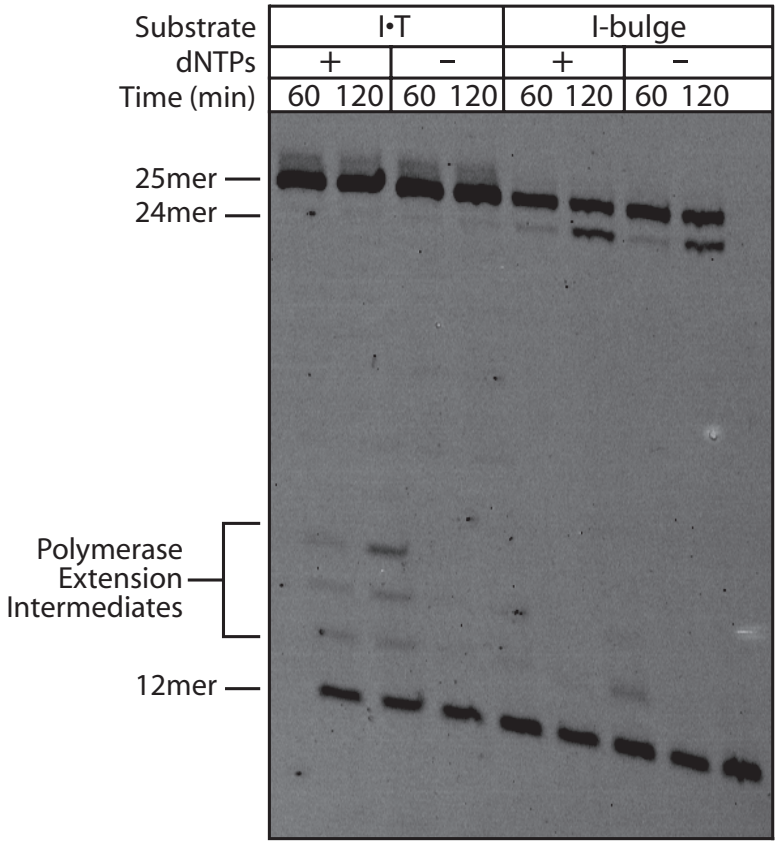
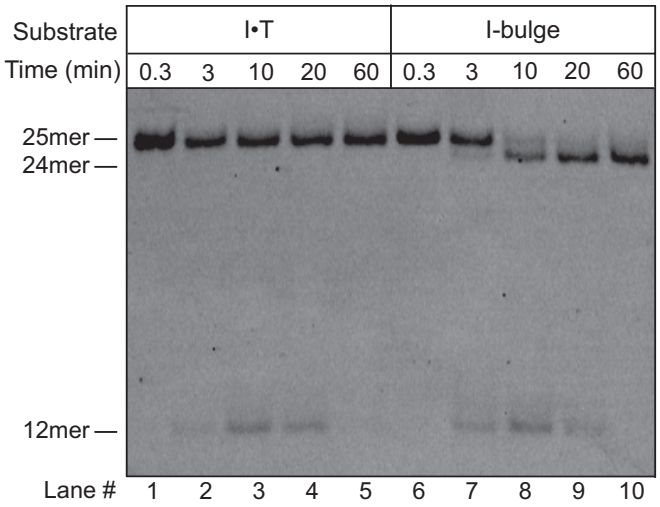


Figure S12. Time dependence for reconstituted repair reactions. Experiments were performed as described in the text for Fig. 7, except that putative physiological concentrations of all recombinant enzymes were used. A, multiple turnover reconstituted BER reactions contained 700 nM I•T mismatch or I-bulge DNA, 70 nM AAG, 120 nM APE1, 20 nM pol β, and 50 nM DNA ligase I and the necessary nucleotide and Mg2+ cofactors (see Methods). Reactions were quenched at the indicated time with formamide/EDTA loading buffer and analyzed by denaturing PAGE. B, the quantification of the formation of the formation of the 24mer deletion product and the formation and breakdown of the 12mer nicked DNA intermediate is from 3 independent experiments and the mean ± SD is shown (see legend for symbols). The similar amounts of intermediates that are formed indicate that the I-bulge and I•T mismatch are processed at similar rates. Little intermediate builds up under these conditions.

A



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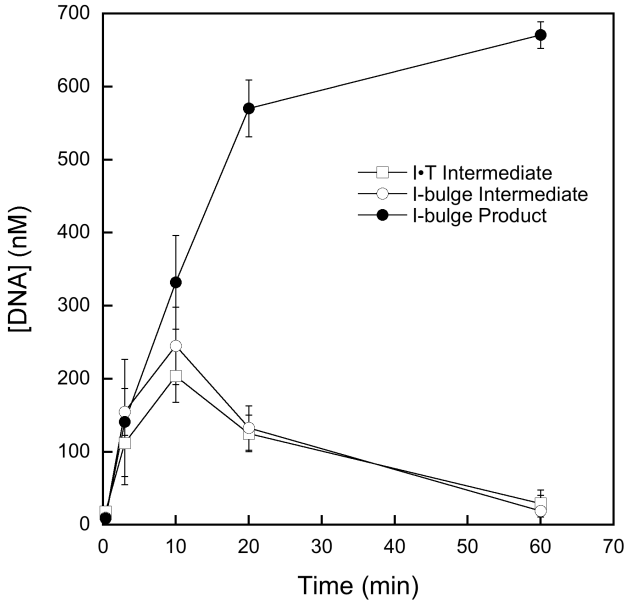

SUPPLEMENTAL REFERENCES

1. Cerda, S. R., Turk, P. W., Thor, A. D., and Weitzman, S. A. (1998) *FEBS Lett.* **431**, 12-18
2. Parlanti, E., Pascucci, B., Terrados, G., Blanco, L., and Dogliotti, E. (2004) *DNA Repair (Amst)* **3**, 703-710