SUPPLEMENTARY MATERIAL FOR

7,8-dihydro-8-oxoadenine, a Highly Mutagenic Adduct, is repaired by *E. coli* and Human Mismatch-Specific Uracil/Thymine-DNA Glycosylases

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This file includes: Supplementary Tables S1 and S2 and Supplementary Figure S1 to Figure S4.

Supplementary Data Tables

Table S1. Pre-steady-state kinetic parameters of hTDG-catalyzed excision of 8oxoA when paired with various bases

	Substrate			
	8oxoA•T	**8oxoA•C**	**8oxoA•G**	**8oxoA•A**
k_{max} **(min^{-1})**[a]	0.35 ± 0.05	0.32 ± 0.04	1.64 ± 0.05	ND

[a]k_{max} constants were calculated by one phase exponential association equation using GraphPad Prism 5.

Table S2. Sequence of the 40 mer oligonucleotides used in this study, where X is 8oxoA.

Name of the oligonucleotide duplex	**DNA sequence of oligonucleotide substrates**
8oxoA•T[a]	5'-AATTGCTATCTAGCTCCG**C**<u>**X**</u>**C**GCTGGTACCCATCTCATGA-3' 3'-TTAACGATAGATCGAGGCG<u>**T**</u>GCGACCATGGGTAGAGTACT-5'
U•G[b]	5'-AATTGCTATCTAGCTCCGC<u>**U**</u>GGCTGGTACCCATCTCATGA-3' 3'-TTAACGATAGATCGAGGCG<u>**G**</u>CCGACCATGGGTAGAGTACT-5'
εC•G[a]	5'-AATTGCTATCTAGCTCCGC<u>**εC**</u>CGCTGGTACCCATCTCATGA-3' 3'-TTAACGATAGATCGAGGCG<u>**G**</u>GCGACCATGGGTAGAGTACT-5'
T•G[b]	5'-AATTGCTATCTAGCTCCGC<u>**T**</u>GGCTGGTACCCATCTCATGA-3' 3'-TTAACGATAGATCGAGGCG<u>**G**</u>CCGACCATGGGTAGAGTACT-5'
CXC (8oxoA•A)	5'-AATTGCTATCTAGCTCCG**C**<u>**X**</u>**C**GCTGGTACCCATCTCATGA-3' 3'-TTAACGATAGATCGAGGCG<u>**A**</u>GCGACCATGGGTAGAGTACT-5'
CXC (8oxoA•C)	5'-AATTGCTATCTAGCTCCG**C**<u>**X**</u>**C**GCTGGTACCCATCTCATGA-3' 3'-TTAACGATAGATCGAGGCG<u>**C**</u>GCGACCATGGGTAGAGTACT-5'
CXC (8oxoA•G)	5'-AATTGCTATCTAGCTCCG**C**<u>**X**</u>**C**GCTGGTACCCATCTCATGA-3' 3'-TTAACGATAGATCGAGGCG<u>**G**</u>GCGACCATGGGTAGAGTACT-5'
GXC (8oxoA•T)	5'-AATTGCTATCTAGCTCCG**G**<u>**X**</u>**C**GCTGGTACCCATCTCATGA-3' 3'-TTAACGATAGATCGAGGCC<u>**T**</u>GCGACCATGGGTAGAGTACT-5'
AXC (8oxoA•T)	5'-AATTGCTATCTAGCTCCG**A**<u>**X**</u>**C**GCTGGTACCCATCTCATGA-3' 3'-TTAACGATAGATCGAGGCT<u>**T**</u>GCGACCATGGGTAGAGTACT-5'
TXC (8oxoA•T)	5'-AATTGCTATCTAGCTCCG**T**<u>**X**</u>**C**GCTGGTACCCATCTCATGA-3' 3'-TTAACGATAGATCGAGGCA<u>**T**</u>GCGACCATGGGTAGAGTACT-5'
CXG (8oxoA•T)	5'-AATTGCTATCTAGCTCCG**C**<u>**X**</u>**G**GCTGGTACCCATCTCATGA-3' 3'-TTAACGATAGATCGAGGCG<u>**T**</u>CCGACCATGGGTAGAGTACT-5'
CXA (8oxoA•T)	5'-AATTGCTATCTAGCTCCG**C**<u>**X**</u>**A**GCTGGTACCCATCTCATGA-3' 3'-TTAACGATAGATCGAGGCG<u>**T**</u>TCGACCATGGGTAGAGTACT-5'
CXT (8oxoA•T)	5'-AATTGCTATCTAGCTCCG**C**<u>**X**</u>**T**GCTGGTACCCATCTCATGA-3' 3'-TTAACGATAGATCGAGGCG<u>**T**</u>ACGACCATGGGTAGAGTACT-5'

[a]The sequence context used in the standard DNA repair reactions and for the kinetic parameters measurements of 8oxoA- and εC-DNA glycosylase activities, unless otherwise stated.
[b]The sequence context used in the standard DNA repair reactions and for the kinetic parameters measurements of mismatched uracil- and thymine-DNA glycosylase activities, unless otherwise stated.

Figures

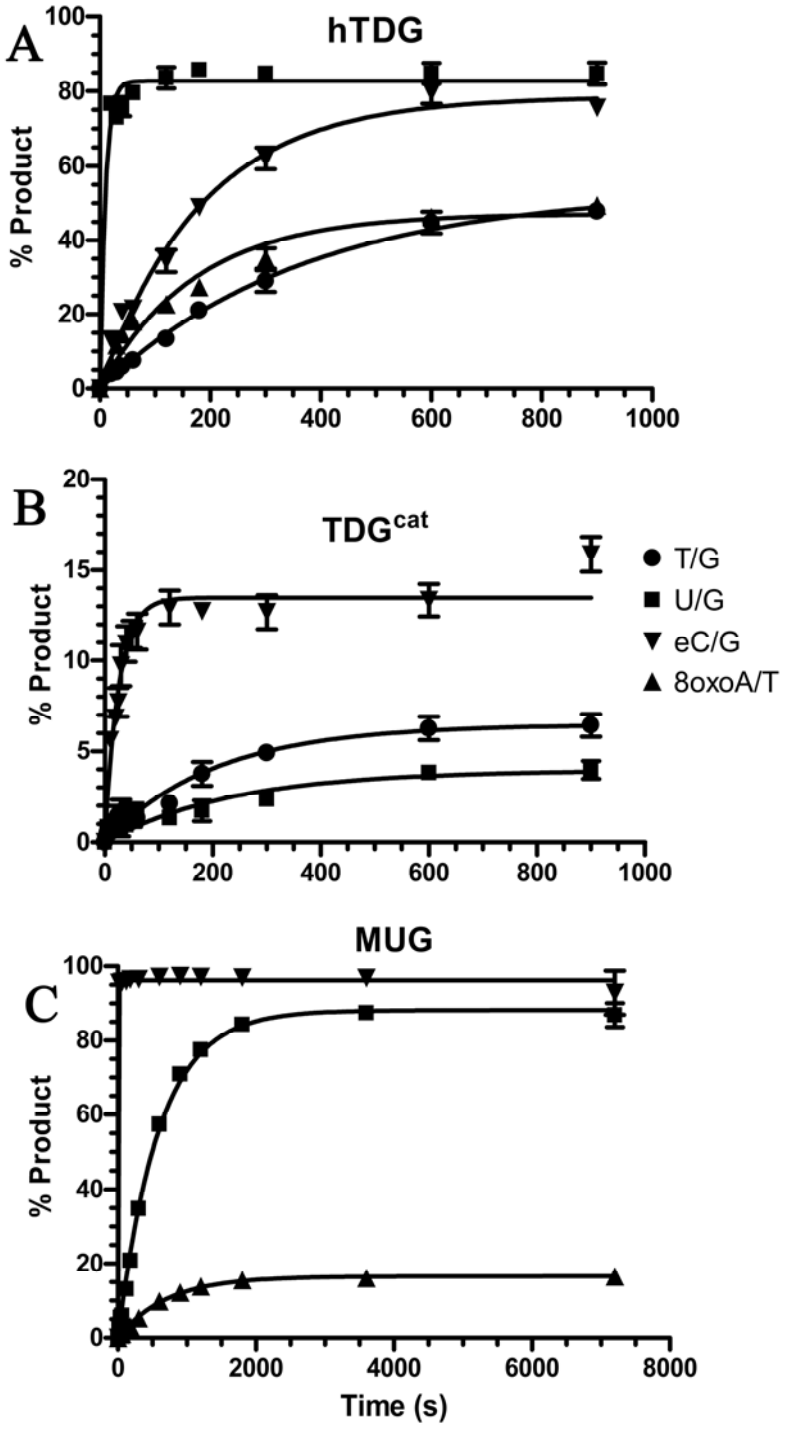

Figure S1. Pre-steady-state single-turnover kinetic of hTDG, hTDG^{cat} and MUG DNA glycosylases. Single-turnover kinetic experiments were performed at 37°C using 500 nM of TDGs or 5µM of MUG in reaction buffer containing 20 mM Tris (pH 8.0), 1 mM EDTA, 1 mM DTT, 100 µg/ml BSA and 100 mM NaCl and 50 nM 5'-[^{32}P]-labelled oligonucleotide duplex U•G (■), εC•G (▼), 8oxoA•T (▲) or T•G (●). (**A**) hTDG. (**B**) TDG^{cat}. (**C**) MUG. The data were fitted to the one phase exponential association equation to calculate k_{max} constant.

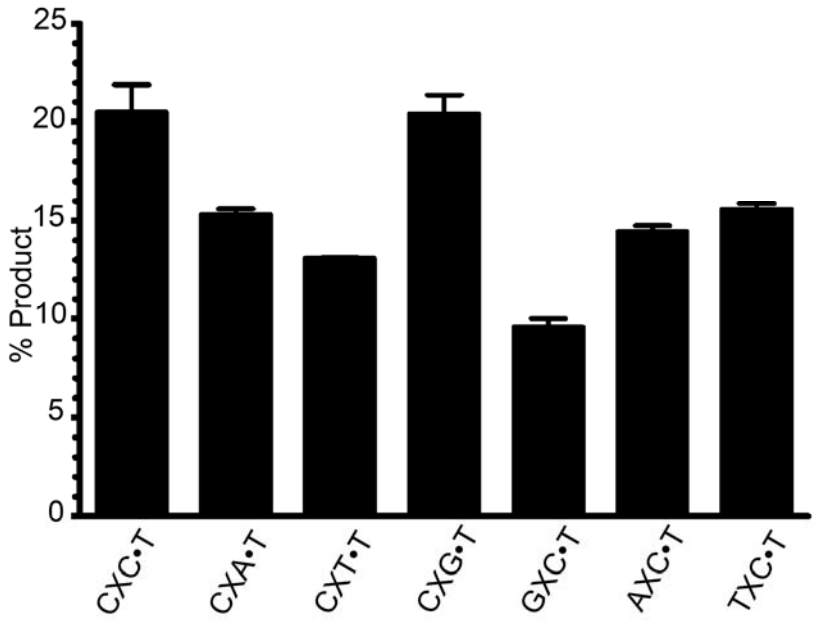

Figure S2. Effects of DNA sequence context on 8oxoA-DNA glycosylase activity of hTDG. 40 mer 8oxoA•T duplex oligonucleotides containing 8oxoA residue placed in different sequence context: CXC, CXA, CXT, CXG, GXC, AXC and TXC, where X is a 8oxoA residue, were used as DNA substrates. 50 nM of 8oxoA•T (5 nM of 5'-[^{32}P]-labelled and 45 nM of non-labelled duplexes) was incubated with 50 nM of hTDG for 15 min at 37°C. Each bar represents the mean values of hTDG activity ± S.D. of three independent experiments. For details see Materials and Methods.

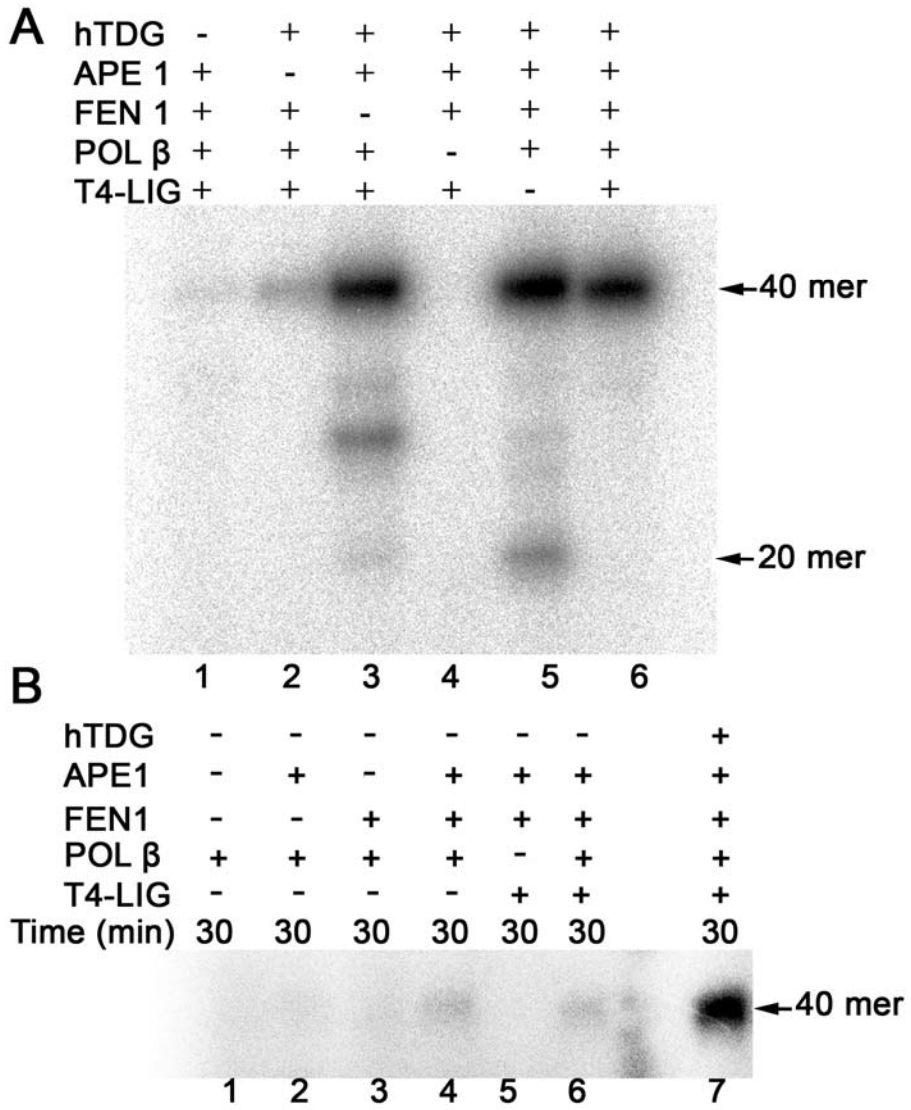

Figure S3. ***In vitro*** **reconstitution of the human BER pathway using 8oxoA•T duplex DNA substrate and purified proteins**. 5 nM of 8oxoA•T oligonucleotide duplex was incubated in the presence of 20 nM hTDG, 5 nM APE1, 2 nM FEN1, 0.1 units POLβ and 5 nM T4 DNA Ligase in buffer containing 20 μCi of [α-^{32}P]dATP, 50 μM dNTPs, 50 mM HEPES-KOH (pH 7.6), 30 mM NaCl, 0.1 mg/mL BSA, 2 mM DTT, 2 mM ATP and 3 mM $MgCl_2$ for 30 min at 37°C, unless otherwise stated. (**A**) Gel analysis of all reaction products in the presence or absence of one of the five DNA repair proteins. Lane 1, no hTDG; lane 2, no APE1; lane 3, no FEN1; lane 4, no POLβ; lane 5, no T4 DNA Ligase; lane 6, all enzymes. (**B**) Generation of full-length repaired product depending on the presence of DNA repair proteins. Lane 1, POLβ; lane 2, POLβ and APE1; lane 3, POLβ and FEN1; lane 4, POLβ, APE1 and FEN1; lane 5, APE1, FEN1 and T4 DNA Ligase; lane 6, all enzymes except hTDG; lane 7, all enzymes. For details see Materials and Methods.

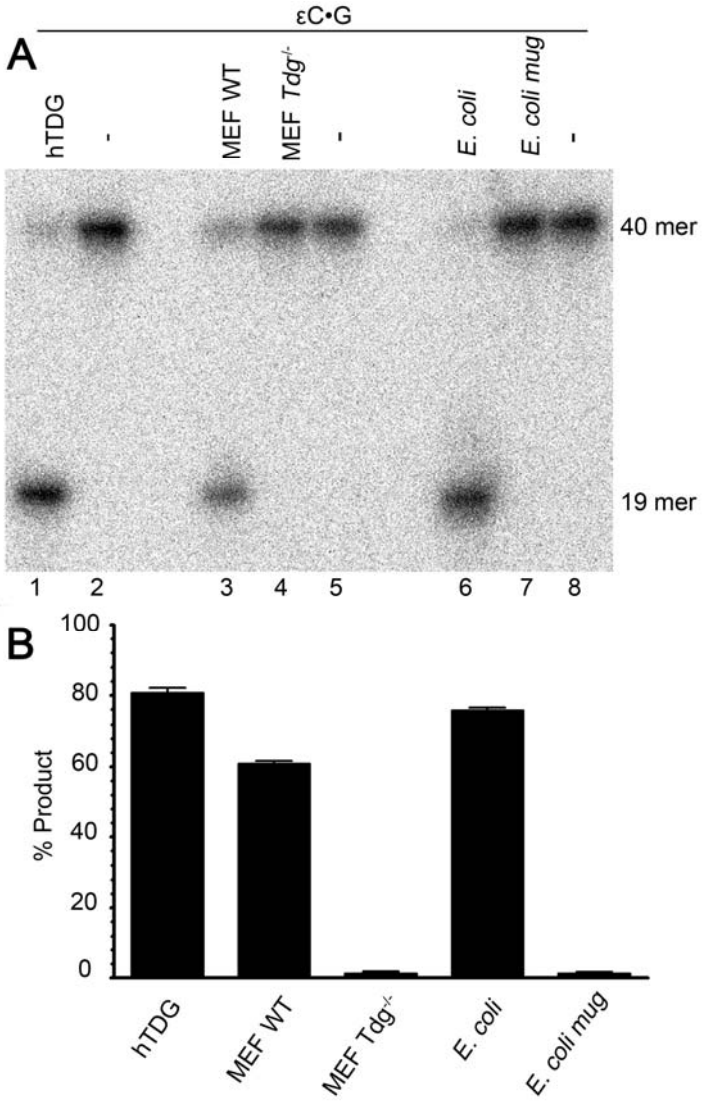

Figure S4. Repair activities toward εC•G duplex oligonucleotide in extracts from *E. coli* and MEF cells. 2.5 nM of 5'-[^{32}P]-labelled εC•G oligonucleotide duplexes were incubated with either 30 µg of MEFs extract, or 20 µg of *E. coli* cell extract or the purified 50 nM hTDG and 20 nM APE1 proteins. The repair assay (volume 100 µl) was performed in BER+EDTA buffer containing 50 mM KCl, 20 mM HEPES-KOH (pH 7.6), 0.1 mg/ml BSA, 1 mM DTT and 1 mM EDTA for 1 h at 37°C. The reactions were stopped by adding SDS and proteinase K. (**A**) Denaturing PAGE analysis of the cleavage products after incubation of εC•G duplexes with *E. coli* cell extracts. (**B**) Graphic representation of the mean values of cleavage activities in *E. coli* and MEF cell extracts. For details see Materials and Methods.