Supporting Information

Markkanen et al. 10.1073/pnas.1211532109

SI Materials and Methods

siRNA Transfection. siRNA transfection was performed with Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer's protocol.

siRNA Against pol λ. The siRNA with sequence sense: CAA AAG UAC UUG CAA AGA UdTdT; antisense: AUC UUU GCA AGU ACU UUU GdTdA was purchased from Microsynth.

Proteins. Proteins were purchased (pol β: Trevigen; pol η: Enzymax) or purified as described here. Recombinant human pol δ was isolated as described (1), with an additional step in which pol δ was loaded onto Hitrap Heparin HP column (1 mL) (GE Healthcare Life Sciences) and eluted with linear gradient of 0.1–1 M KCl in the following buffer: 25 mM Tris·HCl (pH 7.5), 10% (vol/vol) glycerol, 1 mM EDTA (pH 8), 1 mM DTT. Recombinant human pol β was from Trevigen Inc. Recombinant human pol λ and pol λBP were isolated as described (2). Recombinant human pol η was from Enzymax. Recombinant human proliferating cell nuclear antigen (PCNA) was isolated as described (3). Recombinant chemically modified and monoubiquitinated PCNA were isolated as described (4). Recombinant human pol λ D490A was prepared as follows: Site-directed mutagenesis of Codon-optimized pol λ (synthesized by GeneArt and Invitrogen) in the pRSETB vector was performed to obtain pol λ mutated in the residue D490 to alanine using Phusion High-Fidelity pol according to the manufacturer's instructions and the following oligonucleotides (Microsynth) in the indicated order: D490A forward—5′ CGT CGT CAT CGT CGT CTG GCT ATT ATT GTG GTG CCG 3′ and D490A reverse—5′ CGG CAC CAC AAT AAT AGC CAG ACG ACG ATG ACG ACG 3′. Expression and purification was performed as described previously (5).

Whole-Cell Extracts for DNA Polymerase Assays. Preparation of whole-cell extracts for pol assays was performed as described (3). The amount of whole-cell extracts used was normalized by Western blotting for the respective pol δ amounts present in the extracts.

Primer Extension Assays. Reaction mixtures (10 μL) contained 50 mM Tris·HCl (pH 7), 0.25 mg/mL BSA, 1 mM DTT, 2.5 mM $MgCl_2$, 40 μM dNTPs, and 5 nM of 5′ ^{32}P-labeled primer/templates. For single-nucleotide incorporations, the reaction conditions were as outlined in ref. 3. Concentrations of purified proteins and whole-cell extracts are as indicated in the figures and figure legends. The reactions were incubated for 10–30 min at 37 °C and then stopped by the addition of standard denaturing gel loading buffer [95% (vol/vol) formamide, 10 mM EDTA, xylene cyanol, and bromophenol blue], heated at 95 °C for 3 min and loaded onto a 7 M urea/10% (vol/vol) polyacrylamide gel. The percentage of translesion synthesis (TLS) was calculated as the ratio of the intensity of bands present at the position opposite the lesion or beyond to the intensity of these bands plus the one preceding the lesion. The percentage of full-length products was calculated as the ratio of the intensity of bands present at the position of full-length synthesis to the intensity of these bands plus the bands present at the position of TLS.

Gapped Plasmid Assays. The gapped plasmids were designed with help from Zvi Livneh and Sigal Shachar (Weizmann Institute, Rehovot, Israel) as previously established (6, 7). The gap was constructed by inserting oligo 1 [5′ TGC GAC AAC GAA GTG ATT CCC GTC GTG AGC GXG AAA ACC CTG GGC TAC TTG AAC CAG TGC GG 3′, where X indicates the site of 7,8-dihydro-8-oxoguanine (8-oxo-G)] into the pSKSL backbone by the two side oligos (5′ ACC GCC GCA CTG GTT CAA GTA GCC 3′ and 5′ GGA ATC ACT TCG TTG TCG CAA CCG 3′), thus creating a gap of 22 nucleotides in length. The plasmids were transfected into mouse embryonic fibroblasts cells together with a pUC19 carrier plasmid by JetPRIME transfection reagent (Polyplus) and extracted after 5 h of incubation by the PureYield plasmid miniprep system (Promega) and transfected into *muty*$^{-/-}$ *Escherichia coli.* Single colonies were picked, and the sequence of the gapped region was analyzed by sequencing (Microsynth).

Statistical Analysis. For all statistical analysis, the program GraphPad Prism (www.graphpad.com) was used.

1. van Loon B, Ferrari E, Hübscher U (2009) Isolation of recombinant DNA elongation proteins. *Methods Mol Biol* 521:345–359.
2. Ramadan K, Shevelev IV, Maga G, Hübscher U (2004) De novo DNA synthesis by human DNA polymerase lambda, DNA polymerase mu and terminal deoxyribonucleotidyl transferase. *J Mol Biol* 339(2):395–404.
3. Maga G, et al. (2007) 8-oxo-guanine bypass by human DNA polymerases in the presence of auxiliary proteins. *Nature* 447(7144):606–608.
4. Eger S, et al. (2011) Generation of a mono-ubiquitinated PCNA mimic by click chemistry. *Chembiochem* 12(18):2807–2812.
5. Wimmer U, Ferrari E, Hunziker P, Hübscher U (2008) Control of DNA polymerase lambda stability by phosphorylation and ubiquitination during the cell cycle. *EMBO Rep* 9(10):1027–1033.
6. Tomer G, Livneh Z (1999) Analysis of unassisted translesion replication by the DNA polymerase III holoenzyme. *Biochemistry* 38(18):5948–5958.
7. Avkin S, Livneh Z (2002) Efficiency, specificity and DNA polymerase-dependence of translesion replication across the oxidative DNA lesion 8-oxoguanine in human cells. *Mutat Res* 510(1–2):81–90.

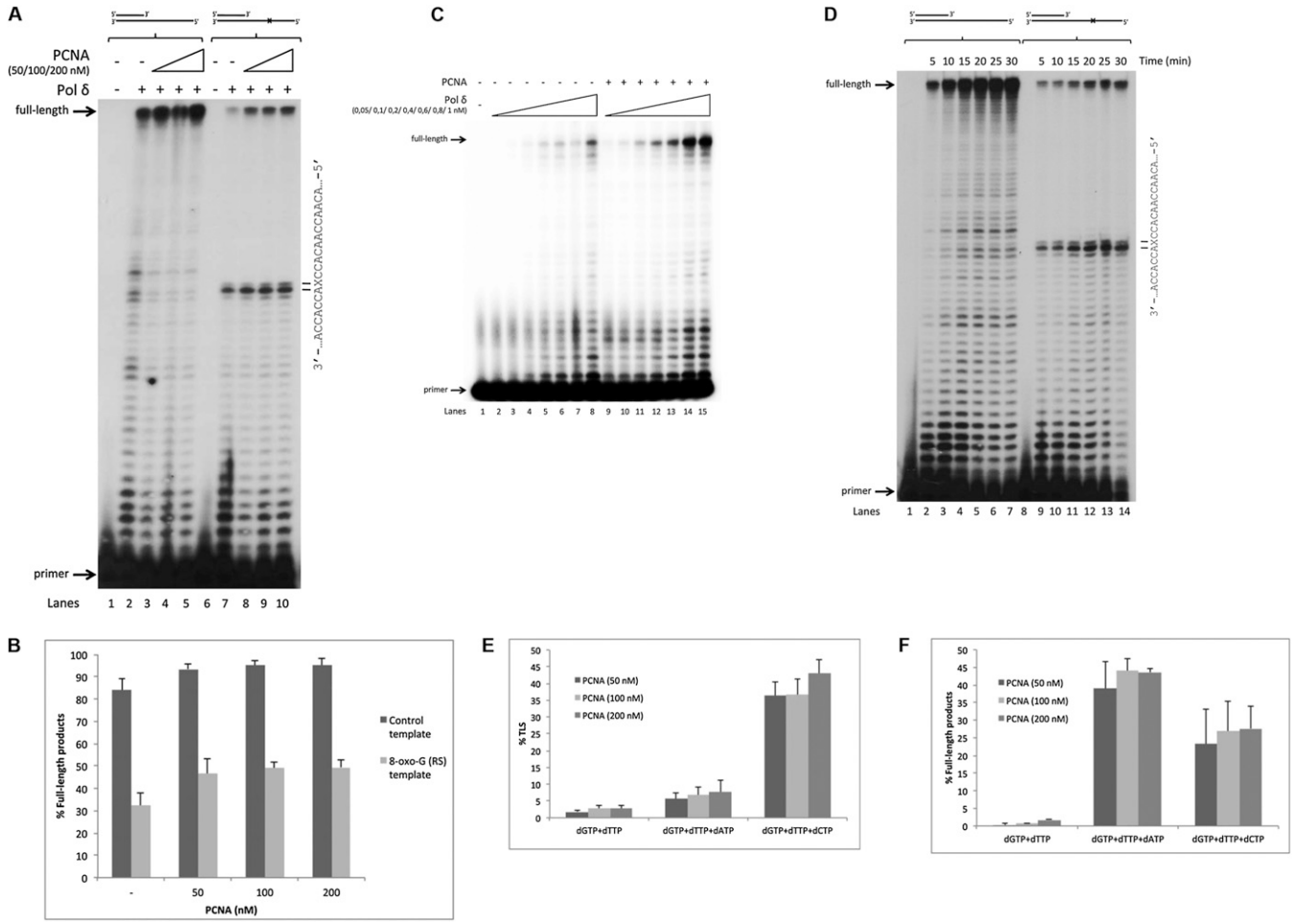

Fig. S1. DNA polymerase δ pauses at an 8-oxo-G lesion before bypassing it. (*A*) PCNA was titrated in the presence of 1 nM pol δ and all four dNTPs on the undamaged template (lanes 1–5) or the 8-oxo-G [running start reaction (RS)] template (lanes 6–10). (*B*) Quantification of full-length products shown in *A*. Values are means of three independent experiments ± SD. (*C*) Pol δ was titrated on the 39/72 primer/template in the absence of PCNA (lanes 2–8) or in the presence of 100 nM of PCNA (lanes 9–15). Lane 1, control reaction in the presence of 100 nM PCNA and in the absence of any pol. (*D*) Time-course experiment on control or damaged DNA templates. Pol δ (1 nM) was incubated in the presence of 100 nM PCNA for the indicated times on control template (lanes 2–7) or on 8-oxo-G (RS) template (lanes 7–14). (*E*) Quantification of TLS products, defined as the incorporation opposite 8-oxo-G and the +1 and +2 nucleotides from Fig. 1*B*. Values are means of three independent experiments ± SD. (*F*) Quantification of full-length products from Fig. 1*B*. Values are means of three independent experiments ±SD.

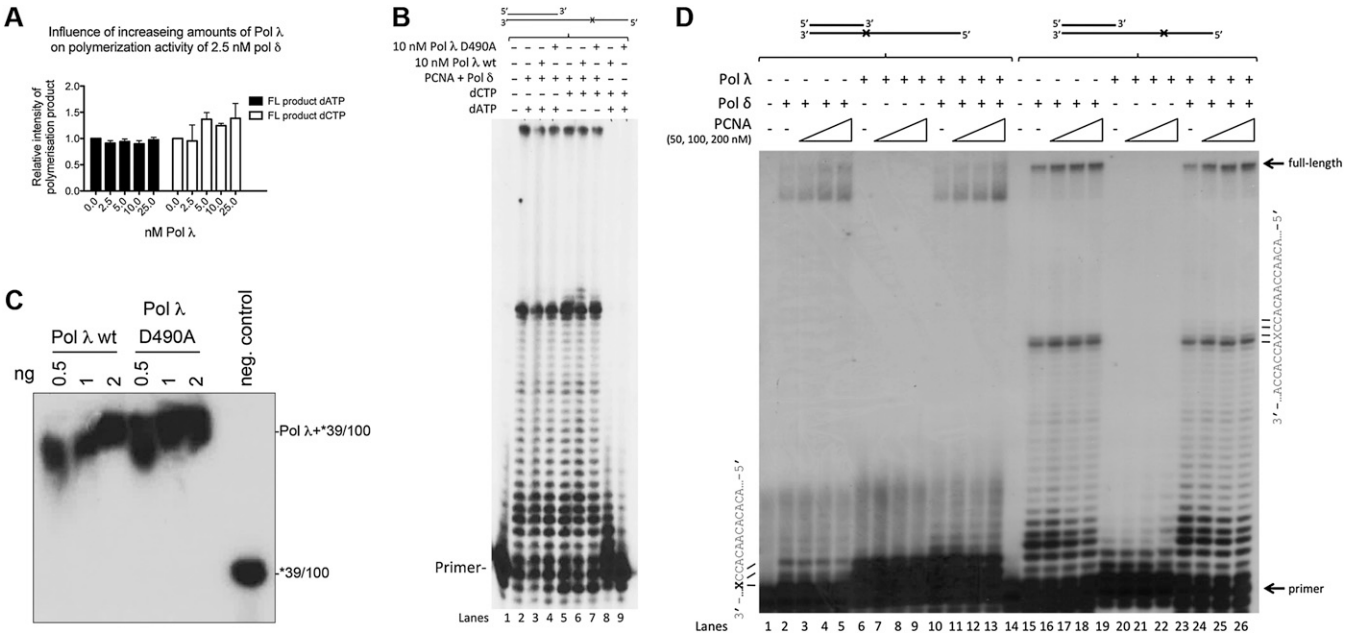

Fig. S2. Influence of the catalytic activity of DNA polymerase λ and PCNA on the TLS reaction. (*A*) Quantification of full-length products from Fig. 2*A*. The signals were normalized to the intensity of the product from pol δ alone, respectively. Values are means of three independent experiments ± SD. (*B*) Comparison of the effect of 10 nM pol λ wild type (wt) or pol λ D490A on TLS in a running start reaction (RS) with 1 nM pol δ and 100 nM PCNA. dATP + dGTP + dTTP (lanes 2–4) or dCTP + dGTP + dTTP (lanes 5–7). (*C*) Electromobility shift assay with the indicated amounts of pol λ wild type or pol λ D490A and the *39/100 mer RS template. (*D*) Titration of PCNA in the presence of 1 nM pol δ, 4 nM pol λ, or both on 8-oxo-G (standing start reaction) and 8-oxo-G (RS) templates, respectively.

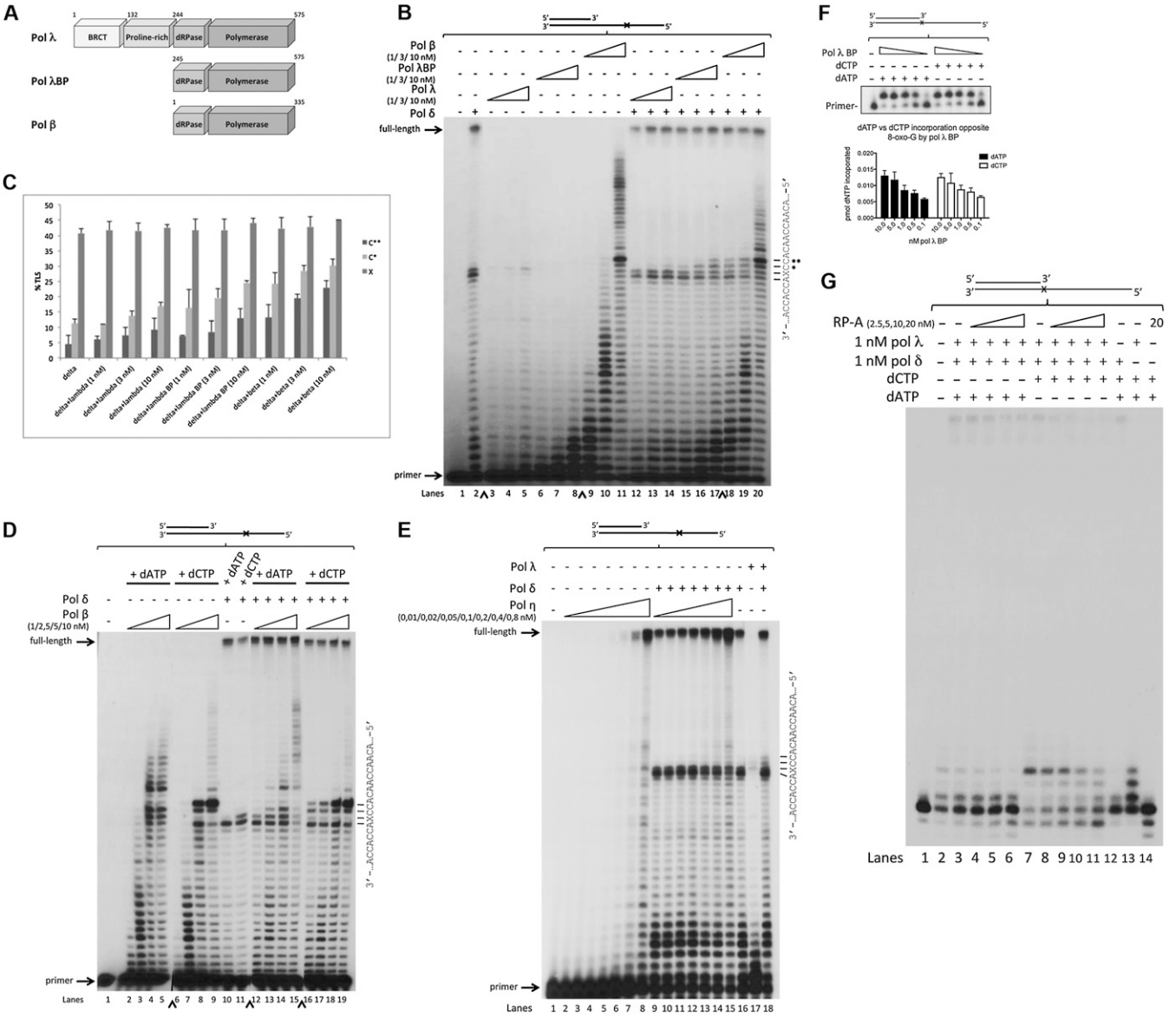

Fig. S3. Influence of DNA polymerases λ BP, β, and η on the TLS by DNA polymerase δ. (*A*) Schematic representations of the full-length pol λ, N-terminally truncated pol λ (pol λ BP), and pol β. (*B*) Pol λ, pol λ BP, and pol β were titrated on the 8-oxo-G [running start reaction (RS)] template with all four dNTPs, in the presence or absence of 1 nM pol δ and in the presence of 100 nM PCNA in all reactions. (*C*) Quantification of TLS, defined as the incorporation opposite 8-oxo-G and the +1 and +2 nucleotides from *B*. Values are means of three independent experiments ± SD. (*D*) Titration of pol β on 8-oxo-G (RS) template in the presence or absence of 1 nM pol δ, with 100 nM PCNA in all reactions. Reactions were performed with dGTP + dTTP and dATP (lanes 2–5) or dCTP (lanes 6–9) or with dGTP + dTTP and dATP (lanes 12–15) or dCTP (lanes 16–19). (*E*) Pol η was titrated on 8-oxo-G [standing start reaction (SS)] template in the presence of 100 nM PCNA and in the absence (lanes 2–8) or in the presence of 1 nM pol δ (lanes 9–15). Lanes 16, 17, and 18, control reactions in the presence of 100 nM PCNA and in the presence of 1 nM pol δ, 4 nM pol λ, or both, respectively. (*F*) Single-nucleotide incorporation specificity by pol λ BP (10, 5, 1, 0.5, and 0.1 nM) in an SS reaction with 0.5 μM C or A, respectively, in the presence of 1 mM $MgCl_2$. (*Lower*) Quantification of four independent experiments showing mean ± SD. (*G*) Effect of RP-A on the pol switch between pols δ and λ on a SS template in the presence of dATP (lanes 2–6) or dCTP (lanes 7–11).

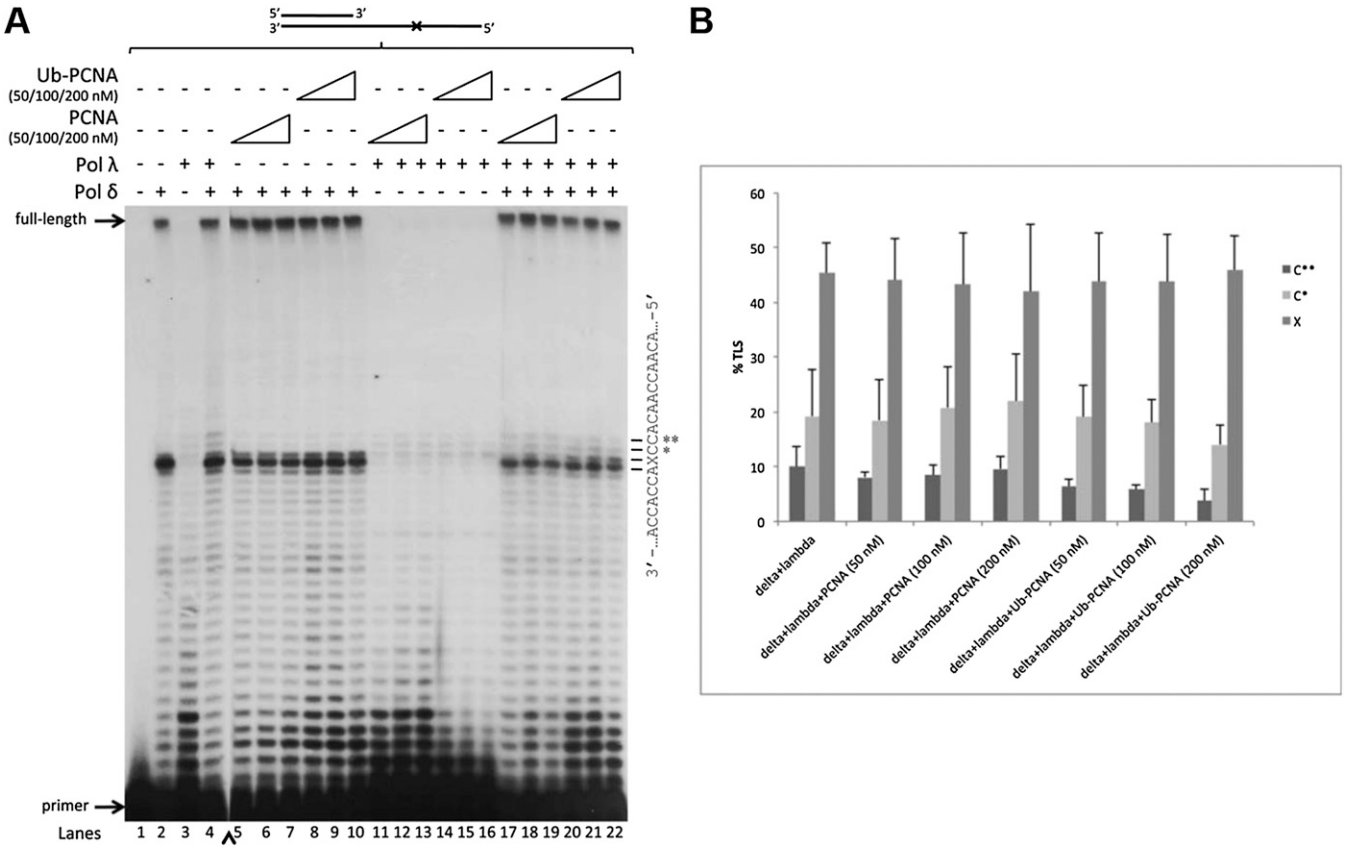

Fig. S4. Monoubiquitination of PCNA does not influence TLS by DNA polymerases λ or β on 8-oxo-G. (*A*) PCNA was titrated on 8-oxo-G (RS) template in the presence of 1 nM pol δ (lanes 5–7), in the presence of 4 nM pol λ (lanes 11–13), or in the presence of 1 nM pol δ and 4 nM pol λ (lanes 17–19). Monoubiquitinated PCNA (Ub-PCNA) was titrated in the presence of 1 nM pol δ (lanes 8–10), in the presence of 4 nM pol λ (lanes 14–16), or in the presence of 1 nM pol δ and 4 nM pol λ (lanes 20–22). Lane 1, control reaction in the absence of PCNA and of any pol. Lane 2, control reaction in the absence of PCNA and in the presence of 1 nM pol δ or 4 nM pol λ, respectively. (*B*) Quantification of TLS in dependence of PCNA and Ub-PCNA from *A*. Values are means of three independent experiments ± SD.

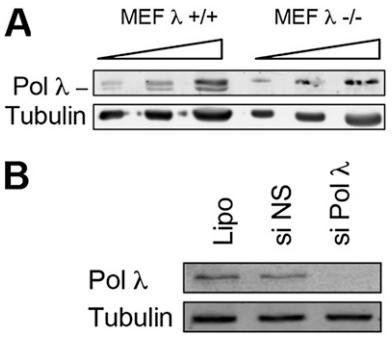

Fig. S5. Verification of pol λ presence/absence in mouse embryonic fibroblast (MEF) and HeLa cell extracts. (*A*) Western blot analysis of whole-cell extracts demonstrates the lack of pol λ in the MEF $\lambda^{-/-}$ cells. The signal for pol λ is the lower band indicated; the upper band is an unspecific band picked up by the antibody. (*B*) Western blot analysis of HeLa whole-cell extracts demonstrates the knockdown efficiency.

Table S1. Primers and templates used in this study

Template	Sequences
Control	5′–TACAACCAAGAGCATACGACGGCCAGTGCCGAATTCACA–3′ 3′–ATGTTGGTTCTCGTATGCTGCCGGTCACGGCTTAAGTGTACCACAACACACAACCAACACCACCAGCCACAACCAACAACCACAACACACACAACCACAC–5′
8-oxo-G (SS)	5′–TACAACCAAGAGCATACGACGGCCAGTGCCGAATTCACA–3′ 3′–ATGTTGGTTCTCGTATGCTGCCGGTCACGGCTTAAGTGT**X**CCACAACACACAACCAACACCACCACAACACACCAACAACCACAACACACACAACCACAC–5′
8-oxo-G (RS)	5′–TACAACCAAGAGCATACGACGGCCAGTGCCGAATTCACA–3′ 3′ ATGTTGGTTCTCGTATGCTGCCGGTCACGGCTTAAGTGTACCACAACACACAACCAACACCACCA**X**CCACAACCAACAACCACAACACACACAACCACAC–5′

RS, running start; SS, standing start; **X**, 8-oxo-G.