Supporting Information

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SI Materials and Methods

Knocking Down the Expression of TLS DNA Polymerase Genes. The expression of specific DNA polymerase genes was silenced in *XPV* or *XPA* cells by transfection with 30 nM of specific siRNA pools, in 6-cm plates for TLS and colony forming ability assays, or in 96-well plates for UV sensitivity assays. All siRNAs were obtained from Dharmacon: *POLK* ON-TARGET*plus SMART*-pool (L-021038), *REV3L* ON-TARGET*plus SMART*pool (L-006302), *REV3L SMART*pool (M-006302), *POLH* ON-TARGET*plus SMART*pool (L-006454), *POLI* ON-TARGET*plus SMART*pool (L-019650), ON-TARGET*plus* Nontargeting Pool (D-001810). Transfection of *XPV* cells was carried out using DharmaFECT-1 (Dharmacon). Transfection of MRC5 and *XPA* cells was carried out using HiPerFect (Qiagen), according to the manufacturers recommendations. siRNA transfection efficiency was measured using siGLO Green transfection indicator (Dharmacon) and was >90% in all cell lines.

RT-PCR and Immunoblot Analysis. Total RNA was extracted from the cells 72 h after transfection with siRNA, using the Perfect-Pure RNA cultured cells kit (5 PRIME). A total of 400 ng total RNA was used for cDNA synthesis and RT-PCR by Access RT-PCR system (Promega) according to the manufacturer recommendations. The following primers were used for the RT-PCRs: 5′-GCTAAGAGGCTGTGCCCACAA-3′ and 5′-TCACTGAATGTCCTCTCAACG-3′ for *POLK*, 5′-AAG-GAGCCACTAAGGAGCAG-3′ and 5′-GATGACGTATG-GCACTCG-3′ for *REV3L*, 5′-GCGGTGACAGCCA-CTAAGAA-3′ and 5′-GCGTTTATTAGTGCAGGCCAA-3′ for *POLH*, 5′-CCTGACCCGCTACAGAGAAA-3′ and 5′-GCAGACACAGCAGGGTTTGA-3′ for *POLI*, 5′-ACCA-CAGTCCATGCCATCAC-3′ and 5′-TCCACCACCCTGTT-GCTGTA-3′ for *GAPDH*. Immunoblot analysis was preformed with a total of 40 μg of whole cell lysate, using a monoclonal anti-polη antibody (17770, Santa Cruz) or a polyclonal anti- polι antibody (NB 100–175A3, Novus Biologicals)

TLS Assay in Cultured Human Cells. The TLS assay is described in refs. 1–3. Briefly, *XPV* cells were subcultured 48 h after siRNA transfection, incubated for additional 16 h, and cotransfected with a mixture containing 50 ng of a plasmid GP-TT-CPD (kan^R), carrying a site-specific CPD (3), 50 ng of a gapped plasmid without a lesion (cm^R), and 5 μg of the carrier plasmid pUC18, using jetPEI transfection reagent (Polyplus-transfection). The cells were incubated for 8 h to allow TLS, and plasmids were extracted using alkaline lysis conditions followed by renaturation, such that only covalently closed plasmids remained nondenatured. A fraction of the purified DNA was used to transform a TLS-defective *E. coli recA* strain, which was then plated on LB-kan and LB-cm plates. The percentage of plasmid repair, of which most occurs by TLS, was calculated by dividing the number of transformants obtained from plasmid GP-TT-CPD (number of kan^R colonies) by the number of transformants obtained from the control gapped-plasmid (number of cm^R colonies). To determine the DNA sequence changes that have occurred during plasmids repair, sequence analysis was carried using the TempliPhi DNA Sequencing Template Amplification Kit (GE Healthcare) and the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems). Reactions were analyzed by capillary electrophoresis on a 3130xl Genetic Analyzer (Applied Biosystems). A small fraction of GP-TT-CPD was repaired by non-TLS events, which involve most likely formation of a double-stranded break as an intermediate. These were observed as plasmid isolates with large deletions or insertions. To obtain precise TLS extents, the plasmid repair extents were multiplied by the fraction of TLS events out of all plasmid repair events, based on the DNA sequence analysis.

UV Sensitivity Assay. *XPV*, *XPA* or MRC5 cells were reverse transfected with siRNA in 96-well plates using the transfection methods described above. At 48 h after transfection, cells were washed twice with Hanks' buffer (Sigma), and irradiated in Hanks' buffer with UVC using a low-pressure mercury lamp (TUV 15w G15T8, Philips) at a dose rate of 0.15 $Jm^{-2}{\cdot}s^{-1}$ for *XPV* and MRC5 cells, or 0.05 $Jm^{-2}{\cdot}s^{-1}$ for *XPA* cells. UV dose rate was measured using an UVX Radiometer (UVP) equipped with a 254-nm detector. After irradiation, Hanks' buffer was removed and the cells were incubated in a fresh growing medium for additional 48 h. For MRC5 cells, 1 mM caffeine (CO750, Sigma) was added immediately after UV irradiation. Viability was determined using the CellTiter-Glo Luminescent Cell Viability Assay (Promega). This assay measures the amount of cellular ATP present, which is rapidly depleted when cells undergo necrosis or apoptosis. Luminescence was measured using a Modulus Microplate Luminometer (Turner Biosystems). Throughout the entire experiment, none of the samples reached cell confluency. Cell viability was determined also by measuring 5-bromo-2-deoxyuridine (BrdU) incorporation. *XPV* or *XPA* cells were transfected with siRNA in 96-well plates and irradiated with UVC as described above. Cells were incubated with BrdU at 40–55 h postirradiation. BrdU incorporation was measured using the BrdU ELISA (chemiluminescence) assay (Roche).

Colony Forming Ability Assay. *XPA* cells were transfected with siRNA against TLS polymerases as described above, and incubated for 48 h. Cells were then trypsinized, counted, and plated in 10-cm Petri dishes (15,000 cells per plate). After incubation of 15 h, cells were UV irradiated as described above, and incubated in fresh medium for 10–12 days. Colonies were fixed and stained with 1% methylene blue (Sigma). Colony forming ability was expressed as the percentage of colony formation in unirradiated control, pretransfected with the same siRNA.

Photoreactivation. *XPA* cells stably expressing CPD photolyase from the rat kangaroo *P. tridactylis* were transfected with siRNA in 96-well plates and irradiated with UVC (at 3 Jm^{-2}) in a dark room as described above. After UVC irradiation, cells were immediately illuminated for 1.5 h with visible light through a perspex surface and a layer of 0.4-cm glass that blocked UV light and prevented overheating of the samples. Photoreactivation for 1.5 h yielded a maximal effect on viability. Nonphotoreactivated control cells were kept in the dark throughout the entire experiment.

ELISA of CPD. *XPA* cells expressing CPD photolyase were UV irradiated at 0–10 J/m^2 in a dark room. CPD photoreactivation was preformed as described above. Nonphotoreactivated control cells were kept in the dark throughout the entire experiment. Genomic DNA was purified using DNeasy Blood and Tissue kit (QIAGEN), diluted to 3 ng/μL and denatured by rapid heating and cooling. 150 ng of DNA was hybridized to a MaxiSorp microplate (NUNC) precoated with 1% Protamine sulfate

(Sigma). The plate was incubated for 1 h at room temperature, first with mouse anti-CPD monoclonal antibody diluted 1:500 (clone KTM53, Kamiya Biomedical Company), and then with horseradish peroxidase (HRP)-Rat anti-Mouse monoclonal antibody diluted 1:5000 (BD PharMingen™). The plate was developed using 3,3′,5,5′ Tetramethylbenzidine (TMB) Liquid Substrate System for ELISA (Sigma-Aldrich). OD at 450 nm was measured using microplate ELISA reader (VersaMax).

1. Avkin S, et al. (2004) Quantitative analysis of translesion DNA synthesis across a benzo[*a*]pyrene-guanine adduct in mammalian cells. The Role of DNA polymerase *k*. *J Biol Chem* 279:53298–53305.
2. Avkin S, et al. (2006) p53 and p21 regulate error-prone DNA repair to yield a lower mutation load. *Mol Cell* 22:407–413.
3. Hendel A, Ziv O, Gueranger Q, Geacintov N, Livneh Z (2008) Reduced fidelity and increased efficiency of translesion DNA synthesis across a TT cyclobutane pyrimidine dimer, but not a TT 6–4 photoproduct, in human cells lacking DNA polymerase eta. *DNA Repair* 7:1636–1646.

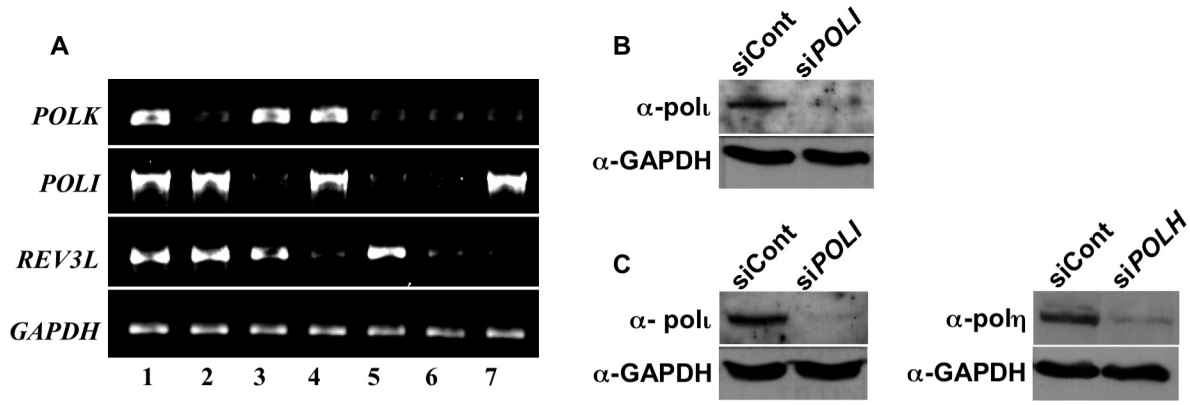

Fig. S1. Knock-down of the expression of TLS polymerases. (*A*) RT-PCR of mRNA from *XPV* cells pretreated with siRNA against human *POLK* (2), *POLI* (3), *REV3L* (4), *POLK&POLI* (5), *POLK&POLI&REV3L* (6), *POLK&REV3L* (7). Nontargeting control siRNA was used in (1). (*B*) Immuno-blot analysis of whole-cell extracts from *XPV* cells pretreated with siRNA against human *POLI* or Nontargeting control siRNA. (*C*) Immuno-blot analysis of whole-cell extracts from *XPA* cells pretreated with siRNA against human *POLI, POLH* or Nontargeting control siRNA.

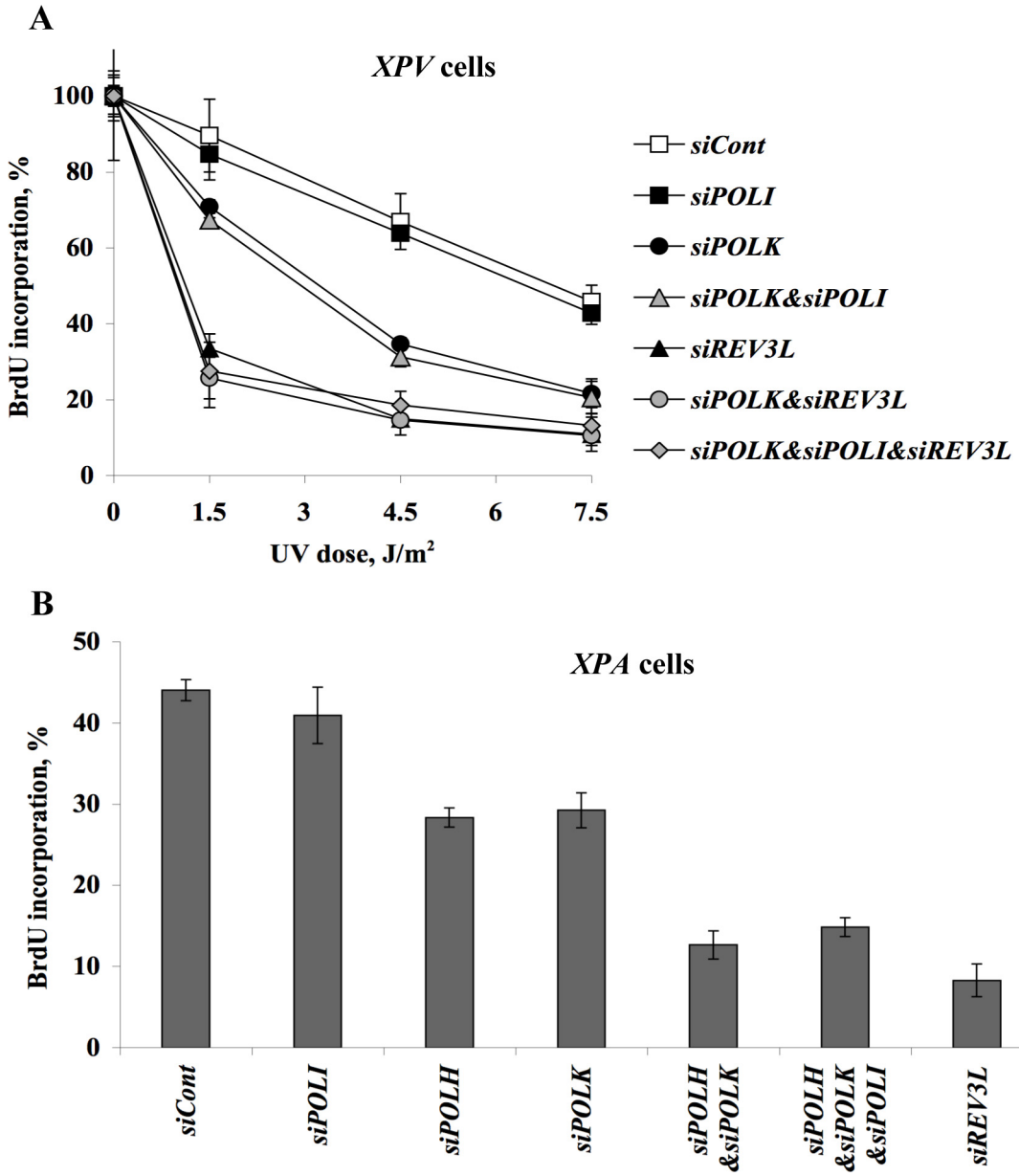

Fig. S2. *XPV* (*A*) or *XPA* (*B*) cells were transfected with siRNA, incubated for 48 h and irradiated with 0–7.5 or 2 J/m^2 UVC, respectively. BrdU was added for 15 h at 40–55 h after irradiation. BrdU incorporation was measured as described under Material and Methods above. Each point represents the average of 3 measurements.

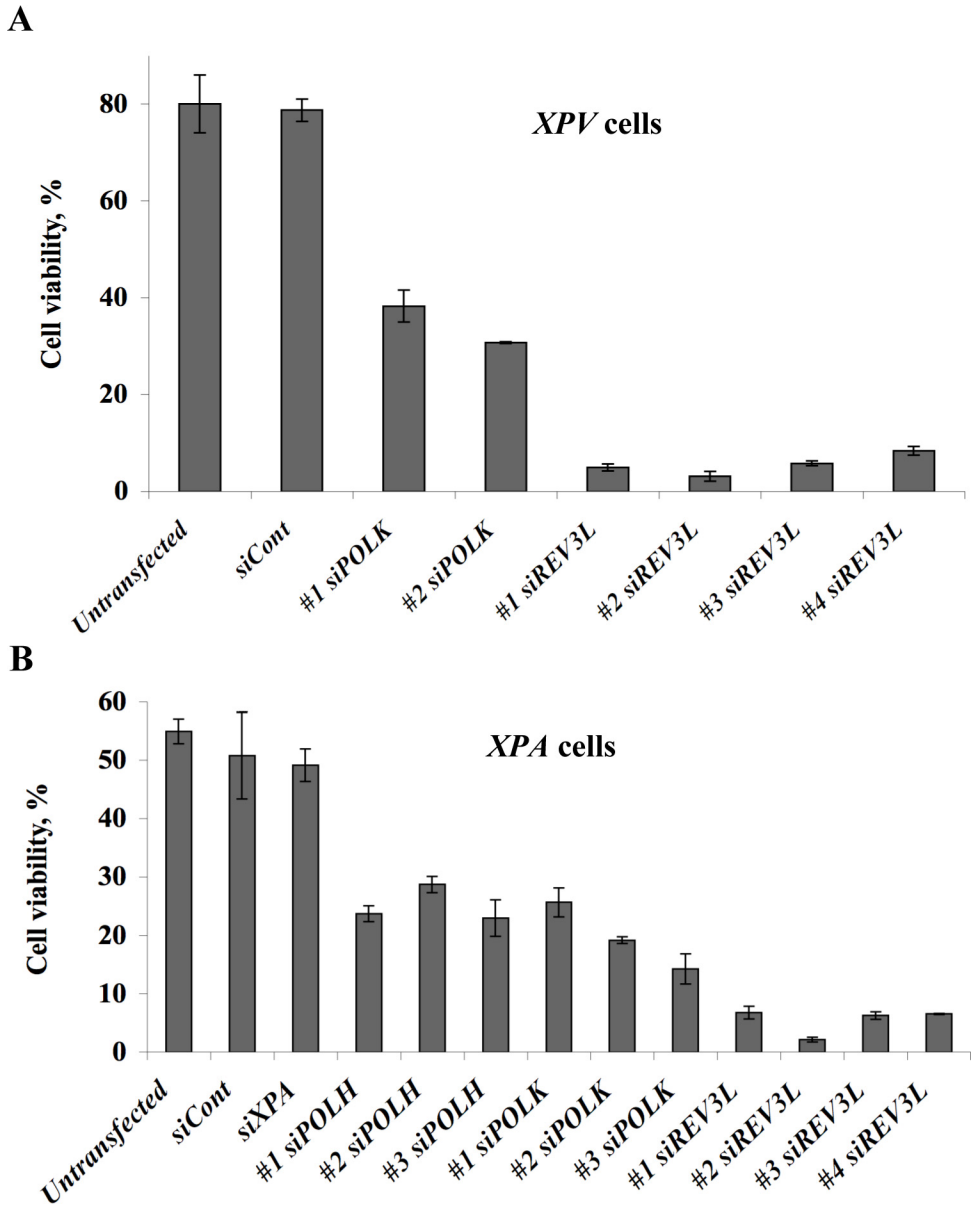

Fig. S3. Validation of the effect of siRNAs on UV sensitivity. *XPV* cells (*A*) or *XPA* cells (*B*) were transfected with siRNA, and UV irradiated after 48 h at 3 or 2 Jm^{-2} respectively. Viability was determined 48 h after UV irradiation by measuring cellular ATP. The siRNAs used were from Dharmacon as follows: 1 si*POLH*: J-006454 09–10; 2 si*POLH*: J-006454 11–12; 3 si*POLH*: D-006454 05–08; 1 si*POLK*: J-021038 05–06; 2 si*POLK*: J-021038 07–08; 3 si*POLK*: D-021038 01–04; 1 si*REV3L*: D-006302 01–02; 2 si*REV3L*: D-006302 03–04; 3 si*REV3L*: J-006302 05–06; 4 si*REV3L*: J-006302 07–08.

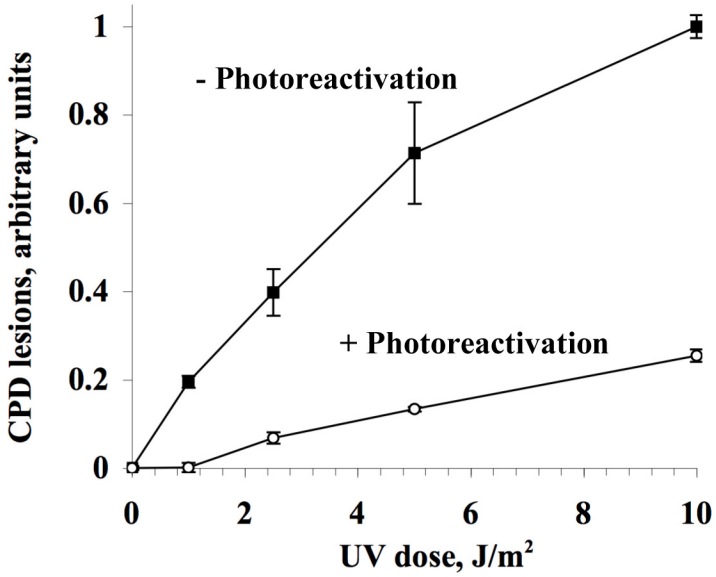

Fig. S4. *XPA* cells expressing CPD photolyase were UV irradiated at 0–10 J/m^2 in a dark room, and CPD photoreactivation was preformed as described above. Nonphotoreactivated control cells were kept in the dark throughout the entire experiment. Genomic DNA was extracted from the cells, denatured, and analyzed for the content of CPD as described under Materials and Methods. Each point represents the average of 5 repeats.

Table S1. TLS extent in *XPV* cells in which the expression of specific TLS DNA polymerases was knocked-down with siRNA

siRNA	Transformants		Plasmid repair, %	TLS, %	Relative TLS, %
	Kan^R	Cm^R			
Control	340	1344	24 ± 1	23 ± 1	100 ± 6
POLK	158	812	20 ± 2	19 ± 2	80 ± 8
POLI	134	728	22 ± 2	21 ± 2	92 ± 7
POLK&POLI	97	1104	9 ± 1	8 ± 1	35 ± 5
REV3L	78	1036	7 ± 1	6 ± 1	26 ± 4
POLK&REV3L	63	968	8 ± 1	7 ± 1	29 ± 2
POLK&POLI&REV3L	71	1032	9 ± 1	8 ± 1	33 ± 4

XPV cells were transiently transfected with siRNA specific for TLS polymerases. After incubation of 64 h the plasmid mixture containing plasmid GP-TT-CPD (kan^R) along with a control (cm^R) plasmid was introduced into the cells. After incubation of 8 h to allow TLS, the DNA was extracted and used to transform an indicator *E. coli* strain. Plasmid repair levels were calculated by the ratio of kan^R/cm^R colonies, which arise mostly due to TLS events. A small fraction of the colonies arise from non-TLS events (large insertions and deletions), and those are easily distinguished by DNA sequence analysis. TLS levels were calculated after subtracting the non-TLS events, determined by DNA sequence analysis (see Table S2). Actual colony counts are presented for a typical experiment. Plasmid repair and plasmid TLS values represent the average level of 6–10 repeats.

Table S2. DNA sequence analysis of TLS across a TT CPD in human *XPV* cells in which the expression of defined TLS DNA polymerases was knocked-down using siRNA.

Event type	siRNA sequence	Fraction of events, % (number of isolates)				
		Control	*POLK*	*POLI*	*POLK*&*POLI*	*REV3L*
Accurate TLS	5′-C-*AA*-C-3′	**68% (65)**	**71% (98)**	**73% (68)**	**71% (66)**	**71% (66)**
Mutagenic TLS		**29% (28)**	**21% (27)**	**22% (21)**	**19% (18)**	**16% (15)**
Targeted base substitution	5′-C-***C***A-C-3′	—	1% (2)	—	1% (1)	—
	5′-C-***G***A-C-3′	6% (6)	4% (5)	4% (4)	5% (5)	5% (5)
	5′-C-***T***A-C-3′	10% (10)	6% (8)	13% (12)	6% (6)	8% (7)
	5′-C-A***C***-C-3′	1% (1)	—	—	—	—
	5′-C-A***G***-C-3′	1% (1)	—	—	—	—
	5′-C-A***T***-C-3′	1% (1)	1% (1)	1% (1)	—	—
	5′-C-***TT***-C-3′	1% (1)	1% (1)	—	—	—
Semitargeted base substitution	5′-**A**-*AA*-C-3′	3% (3)	3% (4)	—	1% (1)	—
	5′-**G**-*AA*-C-3′	—	—	—	2% (2)	—
	5′-**T**-*AA*-C-3′	2% (2)	1% (1)	—	—	—
	5′-C-*AA*-**A**-3′	3% (3)	2% (3)	3% (3)	3% (3)	3% (3)
	5′-C-*AA*-**G**-3′	—	1% (1)	1% (1)	—	—
	5′-C-*AA*-**T**-3′	—	1% (1)	—	—	—
Non-TLS events	Big insertions / deletions	**3% (3)**	**9% (13)**	**4% (4)**	**10% (9)**	**13% (12)**
Total sequences, % (isolates)		**100% (96)**	**100% (138)**	**100% (93)**	**100% (93)**	**100% (93)**

DNA sequence analysis of descendant of repaired plasmid GP-TT-CPD obtained from the TLS experiments described in Table S1. The nucleotides inserted opposite the TT CPD are italicized. Base substitutions are marked in bold. Semitargeted base substitutions are those occurring at the positions flanking the TT CPD.

Table S3. Analysis of accurate and mutagenic TLS across a TT CPD in *XPV* cells, in which the expression of specific DNA polymerases was knocked-down.

siRNA	Plasmid repair, %*	Event type, %†		
		Accurate TLS	Mutagenic TLS	Non-TLS events
Control	24 ± 1	16.3 ± 1	7.0 ± 0.4	0.7 ± 0.1
POLK	20 ± 2	14.2 ± 1.5	4.2 ± 0.4	1.8 ± 0.2
POLI	22 ± 2	16.1 ± 1.2	5.0 ± 0.5	0.9 ± 0.1
POLK&POLI	9 ± 1	6.4 ± 0.9	1.7 ± 0.4	0.9 ± 0.1
REV3L	7 ± 1	5.0 ± 0.8	1.1 ± 0.2	0.9 ± 0.2

*Plasmid repair was obtained from Table S1.

†The extent of each event type (accurate TLS, mutagenic TLS, Non-TLS events) was calculated by multiplying the extent of plasmid repair by the fraction of the corresponding event type taken from Table S2.