

Fig. s1. Expression of endogenous PolN in HeLa cells was suppressed by RNAi. HeLa cells were treated with PolN- siRNA, #1 (lanes 2 and 5) or #2 (lanes 3 and 6) for 24 h and the expression of PolN (lanes 1-3) and PolQ (lanes 4-6) was analyzed by RT-PCR (please see the detailed method in the text). GAPDH was used as a control. The siRNA#2 suppressed the expression of PolN consistently under these conditions ($76\% \pm 6\%$, determined from seven independent experiments). No effect on the expression of PolQ was detected by the siRNAs (lanes 5 and 6).

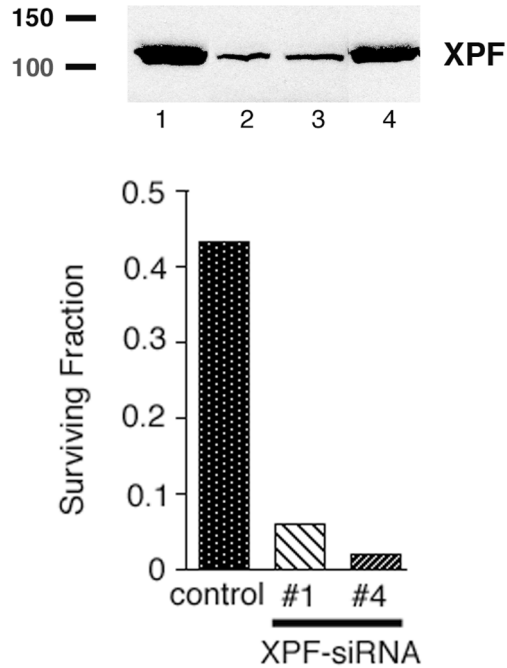


Fig. s2. **Suppression of XPF sensitizes HeLa cells to UV.** (Top) Suppression of XPF by siRNA. HeLa cells were treated with XPF-siRNA, #1 (lane 2) and #4 (lane 3) for one day and the expression of XPF was determined by anti-XPF antibody Ab1 (from NeoMarker). Both siRNA suppressed the expression of XPF by more than 90%. Extracts from mock siRNA-treated cells (lane 1) and control siRNA-treated cells (lane 4) were analyzed as controls. (Bottom) XPF-suppressed HeLa cells are hyper-sensitive to UV irradiation. The XPF-suppressed cells were exposed to UV light at the dose of 2 J/m^2 . The average of the surviving fraction of the siRNA#1 and #4 treated cells after UV irradiation was 0.06 and 0.02, respectively.

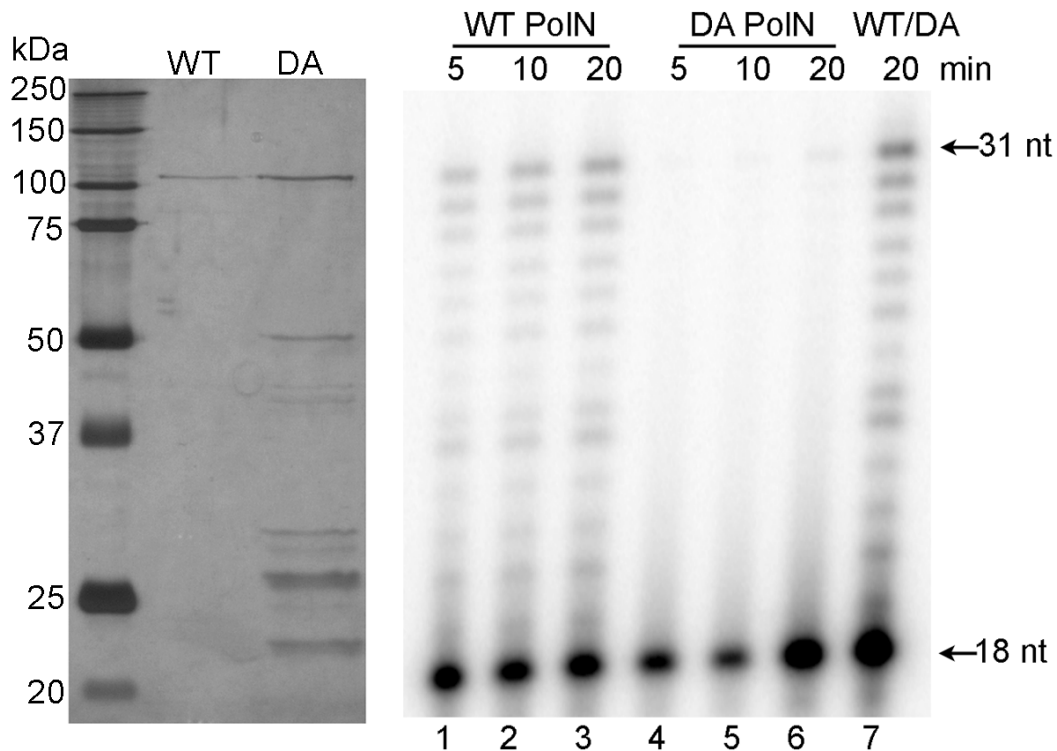


Fig. s3. **Purification of PolN Δ P from *E. coli*.** PolN with a C-terminal 39-residue truncation of the proline-rich region was expressed in *E. coli*. PolN Δ P was His- and FLAG-tagged at the N- and C-terminus, respectively. Cell lysate was subjected to DEAE sepharose. Flow-through of the DEAE was loaded onto a P11. After the extensive wash, the bound proteins were eluted by 1 M NaCl. The P11-bound proteins were incubated with Talon-agarose. PolN Δ P was eluted with 100 mM imidazole. Further processing of the PolN Δ P by immunoaffinity purification using anti-FLAG agarose did not give any advantage in purification. (Left panel) Peak fractions from the TALON column were separated on a 10% SDS-PAGE gel. Proteins were visualized with silver stain to show purity of the 102 kDa WT (10 ng) and PolN Δ P-DA (20 ng as PolN Δ P). Western blot analysis with the anti-FLAG antibody confirmed that the 102 kDa protein is PolN (data not shown). (Right panel) Purified WT PolN has polymerase activity while PolN Δ P-DA is defective in the polymerase activity as reported previously. The polymerase activity was measured by extension of an 18-mer primer annealed to a 31-mer template. WT (2.5 ng; lanes 1-3) and DA (5 ng; lanes 4-6) were incubated for the indicated times at 37°C and separated on a 10% sequencing gel. A mixture of WT and PolN Δ P-DA were incubated to demonstrate that contaminating proteins in the PolN Δ P-DA do not inhibit the polymerase activity of PolN Δ P (lane 7).

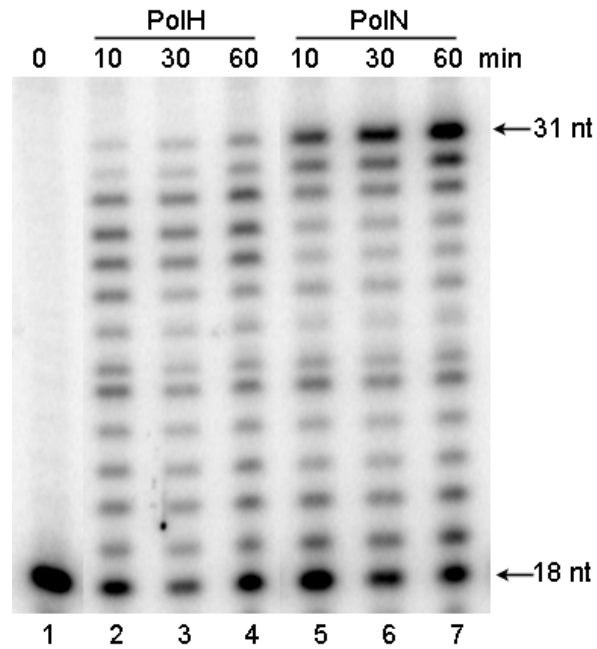


Fig. s4. **DNA polymerase activity of PolN Δ P and PolH on undamaged template.** The concentration of PolN Δ P and PolH that gave similar polymerase activity on an undamaged template was determined. PolH (40 ng) and PolN Δ P (2.5 ng) were incubated with the undamaged template for the given time at 37°C and products were separated on a 10% sequencing gel. The percent extended on an undamaged template in lanes 4 and 7 was 75% and 80%, respectively.

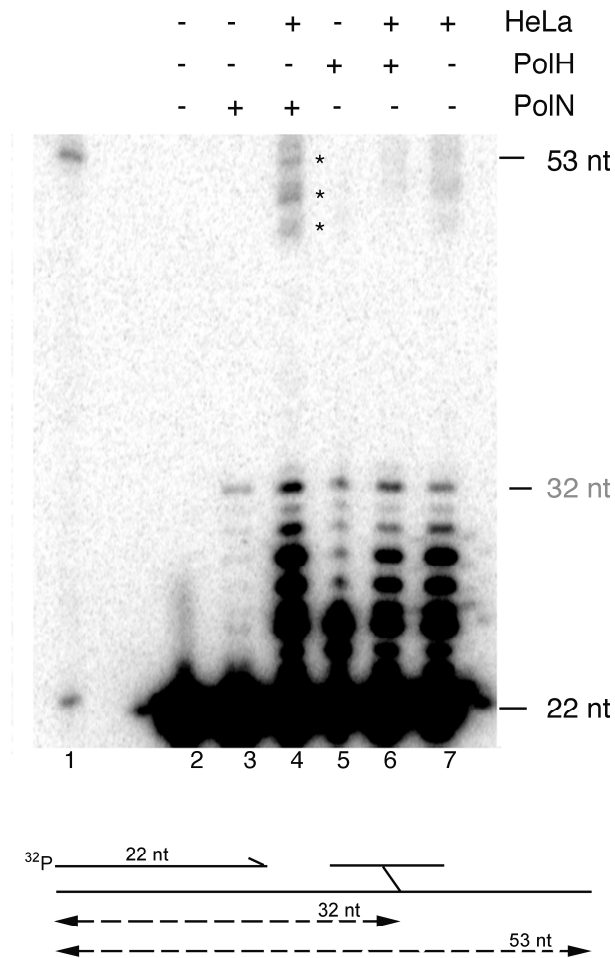


Fig. s5. **The PolN Δ P-mediated TLS of an unhooked psoralen ICL is stimulated by cellular factors in vitro.** A defined primer-template with a 12-mer psoralen ICL (depicted under the gel) contained biotin-moiety both at 3' and 5' end of the template. A 22 nt primer was labeled with ^{32}P . The indicated combination of polymerase or extract was incubated with the primer-template for 15 min at 37°C. The reaction products were analyzed by an 8% sequencing gel. In the presence of nuclear extract, 30~ 50% of the primer were extended (lanes 4, 6, 7). Importantly, nuclear extract greatly stimulated the PolN Δ P-mediated TLS (lane 4). In the presence of PolN Δ P and nuclear extract, ~ 3% of the extended primers were bypassed products (the fragments marked by single asterisks). No significant TLS was detected with nuclear extract alone (lane 7) or in the presence of PolH and nuclear extract (lane 6). Lane 1; 53 nt marker, lane 2; no polymerase, lane 3; 5 ng of PolN Δ P, lane 4; 5 ng of PolN Δ P + 1 μg of nuclear extract, lane 5; 40 ng of PolH, lane 6; 40 ng of PolH + 1 μg of nuclear extract, lane 7; 1 μg of nuclear extract.

Oligo nucleotide sequences used in these experiments:

Template;

5'-Biotin-CCTGCTGCAGCCCAAGCTCGTAGCTTCTGACTGGCGCAGATCTGGCTCGAGGA-Biotin-3'
 (the 12 nt unhooked psoralen ICL is hybridized at the underlined sequence)

Primer;

5'-TCCTCGAGCCAGATCTGCGCCA-3'