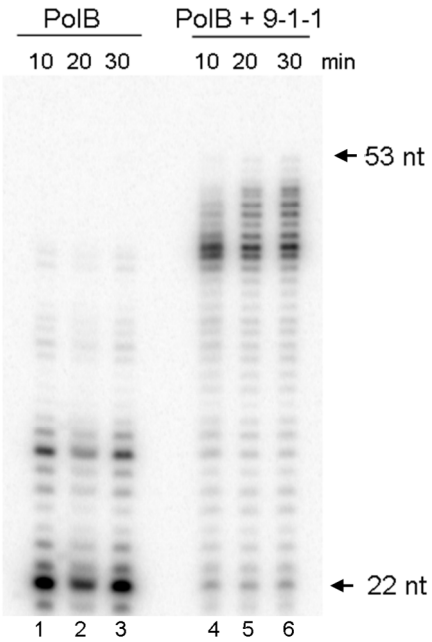
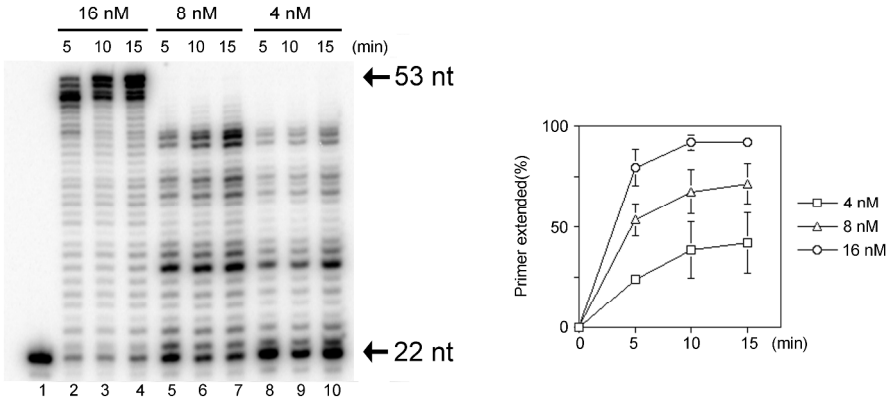
SUPPLEMENTAL INFORMATION



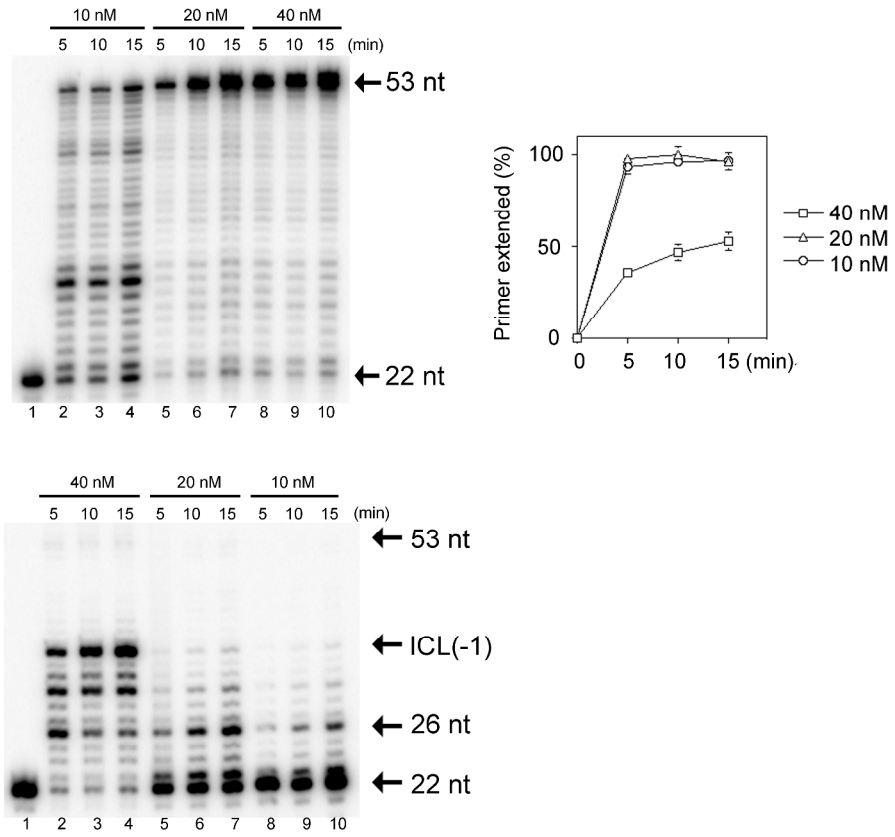
Supplemental Information 1. Stimulation of DNA polymerase beta by the 9-1-1 complex.

The non-damaged substrate was pre-incubated with 25 nM of the 9-1-1 complex on ice for 10 min. DNA polymerase beta (4 nM) was added to the reaction mixture and incubated at 37°C for the indicated time.



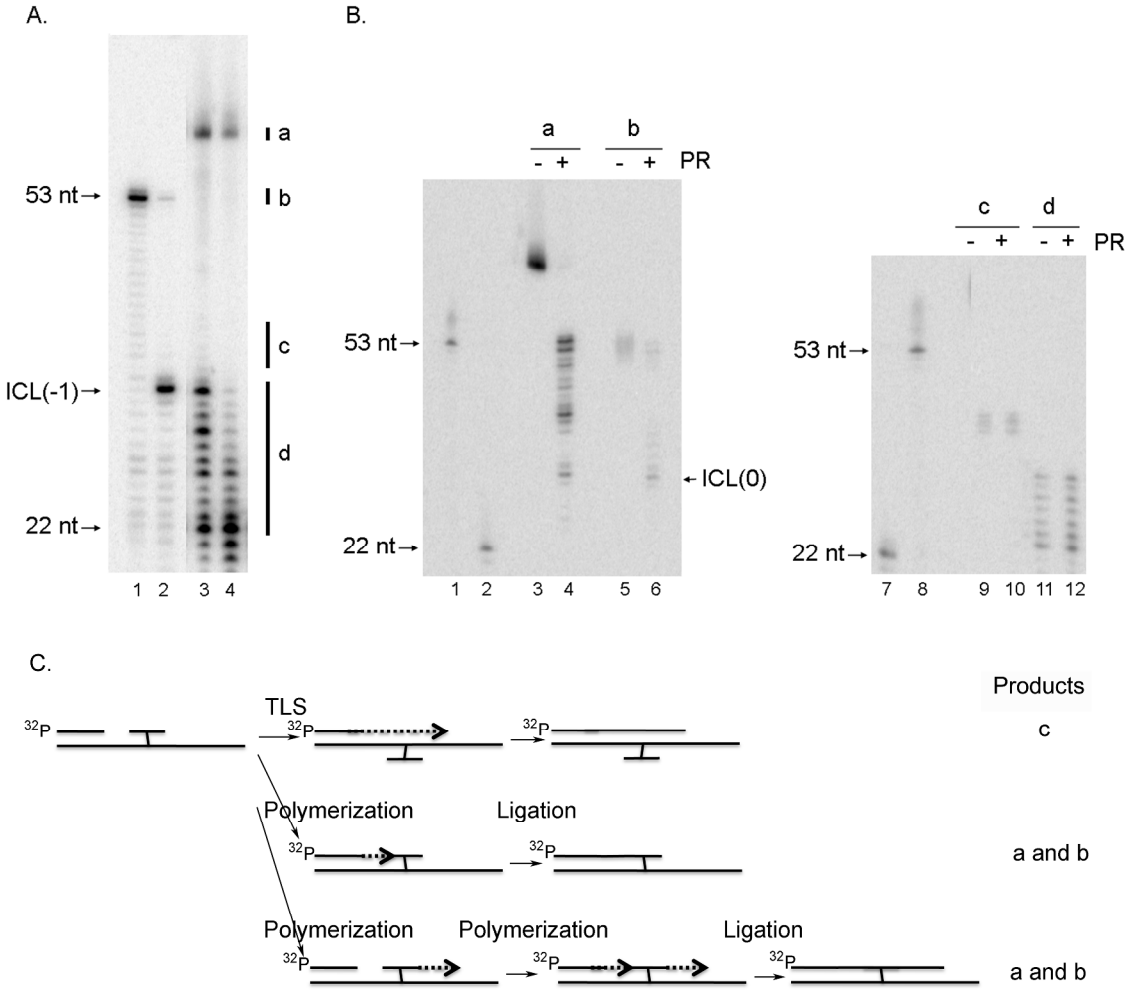
Supplemental Information 2. DNA polymerase activity of DNA polymerase beta

The indicated concentration of DNA polymerase beta was incubated with non-damaged substrate at 37°C for the indicated time. The reaction products were analyzed on an 8% denaturing polyacrlyamide gel. The percentages of primer extended by the polymerase were determined and plotted as a graph next to the gel image.

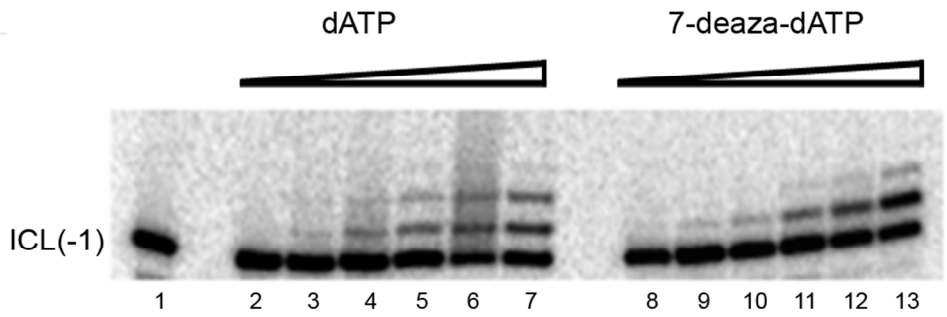


Supplemental Information 3. DNA polymerase activity of DNA polymerase kappa

The indicated concentration of DNA polymerase kappa was incubated with non-damaged substrate or a defined substrate with a single psoralen ICL at 37°C for the indicated time. The reaction products were analyzed on 8% denaturing polyacrlyamide gels. For the reactions with non-damaged substrate, the percentages of primer extended by the polymerase were determined and plotted as a graph next to the gel image. Little bypass products were detected with the defined substrate with a single psoralen ICL.

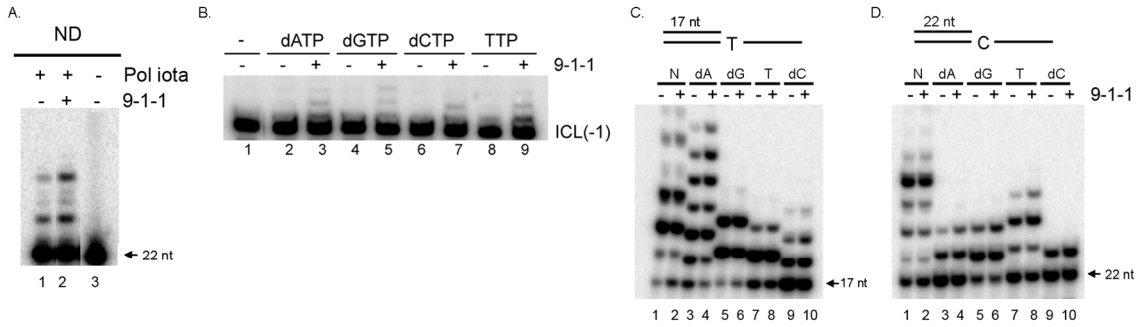


Supplemental Information 4. Analysis of reaction products generated by the incubation of the substrate with nuclear extracts. (A) Nuclear extracts produced reaction products longer than 53 nt in length (lanes 3 and 4). Lane 1, 53 nt marker; lane 2, 32 nt marker, ICL(-1). (B) Photo-reversal experiments revealed that the reaction products longer than 53 nt were cross-linked DNA generated by ligation. Fragments a, b, c, and d (shown in panel A) were isolated from the gel. Half of the isolated products were irradiated with UVA light to reverse psoralen ICL to psoralen mono-adducts + non-damaged thymine. Each product (with or without photo-reversal treatment) was analyzed on 10% denaturing polyacrylamide gels. Products a and b, which were longer than 53 nt, became fragments shorter than 53 nt, but longer than 33 nt (ICL(0)) after photo-reversal treatment (PR). These data demonstrated that products a and b contained psoralen ICL and labeled fragments were longer than 33 nt. The photo-reversal treatment of products c and d did not alter the sizes of the fragments, showing that there was no psoralen ICL in these fragments. Therefore, fragments c are the results of bypass of a psoralen ICL and fragments d are the products of blocked polymerization by a psoralen ICL that might be further degraded by endo/exonucleases. Lane 1, 53 nt marker; lane 2, 22 nt marker; lane 3, products of a; lane 4, products of a with UVA irradiation; lane 5, products of b; lane 6, products of b with UVA irradiation; lane 7, 22 nt marker; lane 8, 53 nt marker; lane 9, products of c; lane 10, products of c with UVA irradiation; lane 11, products of d; lane 12, products of d with UVA irradiation. (C) Possible mechanisms to produce cross-linked products by ligation. Various sizes of cross-linked products will be generated after polymerization, ligation, and/or exo/endonucleolytic actions (products a and b). Products of C represent TLS products.



Supplemental Information 5. DNA polymerase iota incorporates 7-deaza-dATP as efficiently as dATP opposite a psoralen ICL

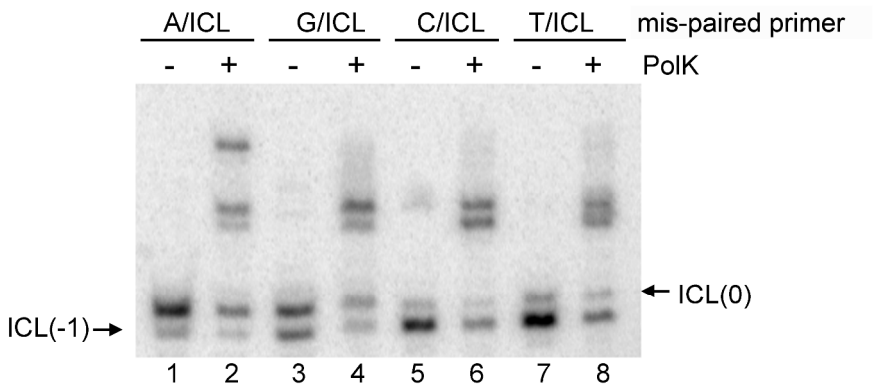
DNA polymerase iota (2 nM) was incubated with 200 pM of the ICL(-1) substrate in the presence of different amounts of dATP (lanes 2-7) or 7-deaza-dATP (lanes 8-13) at 37°C for 6 min. lane 1, no polymerase; lanes 2 and 8, 1 μM dNTP; lanes 3 and 9, 3 μM dNTP; lanes 4 and 10, 10 μM dNTP; lanes 5 and 11, 30 μM dNTP; and lanes 6 and 12, 100 μM dNTP.



Supplemental Information 6. Impact of the 9-1-1 complex on DNA polymerase iota

(A) The 9-1-1 stimulates DNA polymerase iota weakly. The DNA polymerase activity of the DNA polymerase iota was increased slightly with the pre-incubation of the 9-1-1 complex (2 nM) with 200 pM of the non-damaged template (compare lanes 1 and 2). Approximately 8% and 18% of the primer were extended in lanes 1 and 2, respectively. (B) The 9-1-1 complex does not alter the specificity of dNTP incorporation opposite a psoralen ICL. After the 9-1-1 complex (2 nM) was pre-incubated with 0.2 nM of the ICL(-1) substrate on ice for 10 min, DNA polymerase iota (2 nM) was added and incubated in the presence of 1 mM of the indicated dNTP at 37°C for 20 min. (C and D) The 9-1-1 complex marginally stimulates the incorporation of dAMP and dCMP opposite pyrimidines by DNA polymerase iota. After the 9-1-1 complex (2 nM) was pre-incubated with 0.2 nM of non-damaged substrate on ice for 10 min, DNA polymerase iota (2 nM) was added and incubated in the presence of 100 μM of the indicated dNTP at 37°C for 15 min. Nucleotide incorporations opposite thymine (in panel C) and cytosine (in panel D) were examined. The substrate used in the experiments in C was described in Makarova et al[1]. A labeled 17 nt (5'- GGAAGAAGAAGTATGTT -3') was annealed to a 30-mer (5'- CCTTCTTCATTGTAACATACTTCTTCTTCC -3'). The 17-mer is annealed in the underlined region of the 30-mer. A thymine is the first base on the template to be polymerized. Slight increases in the incorporation of dAMP and dCMP were observed with both templates. Percentages of the primer extended were 85% (lane 3), 93% (lane 4), 33% (lane 9), and 47% (lane 10) in the panel C; 29% (lane 3), 41% (lane 4), 30% (lane 9) and 39% (lane 10) in the panel D. N, four dNTPs were included in the reactions.

[1]Makarova, A. V.; Grabow, C.; Gening, L. V.; Tarantul, V. Z.; Tahirov, T. H.; Bessho, T.; Pavlov, Y. I., Inaccurate DNA synthesis in cell extracts of yeast producing active human DNA polymerase iota. PLoS One **2011,** 6, (1), e16612.



Supplemental Information 7. DNA polymerase kappa extends mis-paired primer ends opposite a psoralen ICL

Individual mis-paired primer opposite a psoralen ICL was generated by incubating the ICL(-1) substrate with the exonuclease-defective Klenow(exo-) in the presence of each dNTP (1 mM). Each substrate with a mis-paired primer was incubated with DNA polymerase kappa (20 nM) at 37°C for 10 min in the presence of dNTPs (250 μM). All four primers were extended by DNA polymerase kappa.