Supporting Information

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SI Materials and Methods

DNA Oligonucleotide Sequences. *100 mer Template.* 3′ -ATGTTGGTTCTCGTATGCTGCCGGTCACGGCTTAAGTGT**X**CCACAACACACAACCAACACCACCACAACACACCAACAACCACAACACACACAACCACAC-5′, where X = G or 8-oxo-7,8-dihydroguanine (8-oxo-G) or abasic (AP) site (tetrahydrofurane). Underlining denotes complement to the 39-mer primer.

39 mer Primer. 5′ -TACAACCAAGAGCATACGACGGCCAGTGCCGAATTCACA-3′.

62 mer Template. 5′-ACACACTCGGACTGCACGTGAGCGTCGGCAGG**TT**CCCAAAGGCTGACCAGTGCGGAAGGAGT-3′, where TT = thymidine dimer. The underlined sequence is complementary to the 28-mer primer.

28 mer Primer. 5′-ACTCCTTCCGCACTGGTCAGCCTTTGGG-3′.

Enzymatic Assays. All reactions were in a 10-μL final volume. Polymerase (Pol) λ and β reaction buffer: 50 mM Tris pH 7.5, 1 mM DTT, 0.2mg/mL BSA, and 1 mM $MgCl_2$. Pol η and ι reaction buffer: 40 mM Tris pH 8, 1 mM DTT, 2.5 mg/mL BSA, and 1.25 mM MgCl2. Pol δ reaction buffer: 40 mM Tris pH 8, 1 mM DTT, 2.5 mg/mL BSA, 5 mM MgCl2, and 100 ng proliferating cell nuclear antigen (PCNA).

Enzymes and Proteins. *Cloning Pol δ-interacting protein 2 into expression vector.* The full-length ORF of Pol δ-interacting protein 2 (PolDIP2) was obtained from a testis cDNA library by PCR. PCR conditions: denaturation 94 °C for 3 min; amplification (30 cycles) 94 °C for 3 s, 70 °C for 30 s, and 72 °C for 30 s; extension 72 °C for 7 min. A primer set was designed to amplify the full-length OFR and introducing a NdeI restriction site at the 5′ end as well as a stop codon and a XhoI restriction site at the 3′ end. The forward primer contained a 23-nt homology to the sense strand at the 5′ of PolDIP2 and the reverse primer was 25-nt complementary to the 3′-PolDIP2 sense strand.

The primer set is as follows:

5′ forward: 5′-TATATA**CATatg**gcagcctgtacagcccggcg-3′.

3′ reverse: 5′ TATA**CTCGAG**ctaccagtgaaggcctgagggtggtgtc-3′.

Capital letters indicate sequences not complementary to the target and the underlined letters indicate the restriction site sequence added for cloning purposes. The PCR product was digested by NdeI and XhoI and subcloned using the CloneJET PCR Cloning Kit (Thermo Scientific). Positive clones were identified by colony PCR. PolDIP2 was then cloned using NdeI and XhoI into a modified pETM33 expression vector encoding an N-terminal 6-His tag as well as an N-terminal GST tag and a linker that contains the recognition site for rhinovirus 3C protease.

Purification of recombinant PolDIP2. An overnight culture of PolDIP2 in pETM33 MCS-1 was grown in 100 mL LB medium containing 50 mg/mL kanamycin. Next, 50 mL were inoculated into 1 L LB medium containing 50 mg/mL kanamycin and brought to optical density of ∼0.6 at 30 °C . This was followed by adding IPTG to 100 mM final concentration and incubated overnight (15 h) at 16 °C. The bacteria were centrifuged in a Sorvall H-4000 rotor at 4,500 rpm for 20 min at 4 °C. The resulting pellet was resuspended in 40 mL GST binding puffer [50 mM potassium phosphate (pH 6.5), 200 mM NaCl, 0.05% Nonidet P-40, 1 mM EDTA, 1 mM DTT, 10% glycerol, 1 mM PMSF, and 1 mg/mL each of pepstatin, bestatin, and leupeptin] and the bacteria lysed twice in a French press at 1,500 psi. The extract of 2 L bacterial culture was centrifuged in a Sorvall SS34 rotor at 18,000 rpm for 30 min at 4 °C. Under these conditions 10–20% of PolDIP2 were soluble. The supernatant was bound to a GSTrap colunm (5 mL) equilibrated with 10 column volumes (50 mL) of GST binding buffer. After extensive washing, PolDIP2 was eluted in GST binding buffer containing 40 mM Glutathione. The factions containing PolDIP2 were pooled and loaded onto a 1-mL HiTrap SP HP column equilibrated with 10 column volumes (10 mL) SP binding buffer [50 mM potassium phosphate (pH 6.5), 50 mM NaCl, 0.05% Nonidet P-40, 1 mM EDTA, 1 mM DTT, 10% glycerol, 1 mM PMSF, and 1 mg/mL each of pepstatin, bestatin and leupeptin]. PolDIP2 was eluted after intensive washing with SP binding buffer with a NaCl gradient from 50 to 1,000 mM. The fractions containing PolDIP2 were dialyzed against 50 mM Tris·HCl (pH 8.0), 50 mM NaCl 20% glycerol, 1 mM EDTA, and 1 mM DDT and stored in small aliquots in liquid nitrogen until use. The protein was >95% pure as assessed by SDS/PAGE except for the presence of an extra band which was free GST.

Electronic Image Manipulation. Linear transformations were applied to whole images using Adobe Photoshop CS6 exposure/brightness filters with the sole purpose of reducing excessive background. No masking/enhancement was applied to any specific feature of the images.

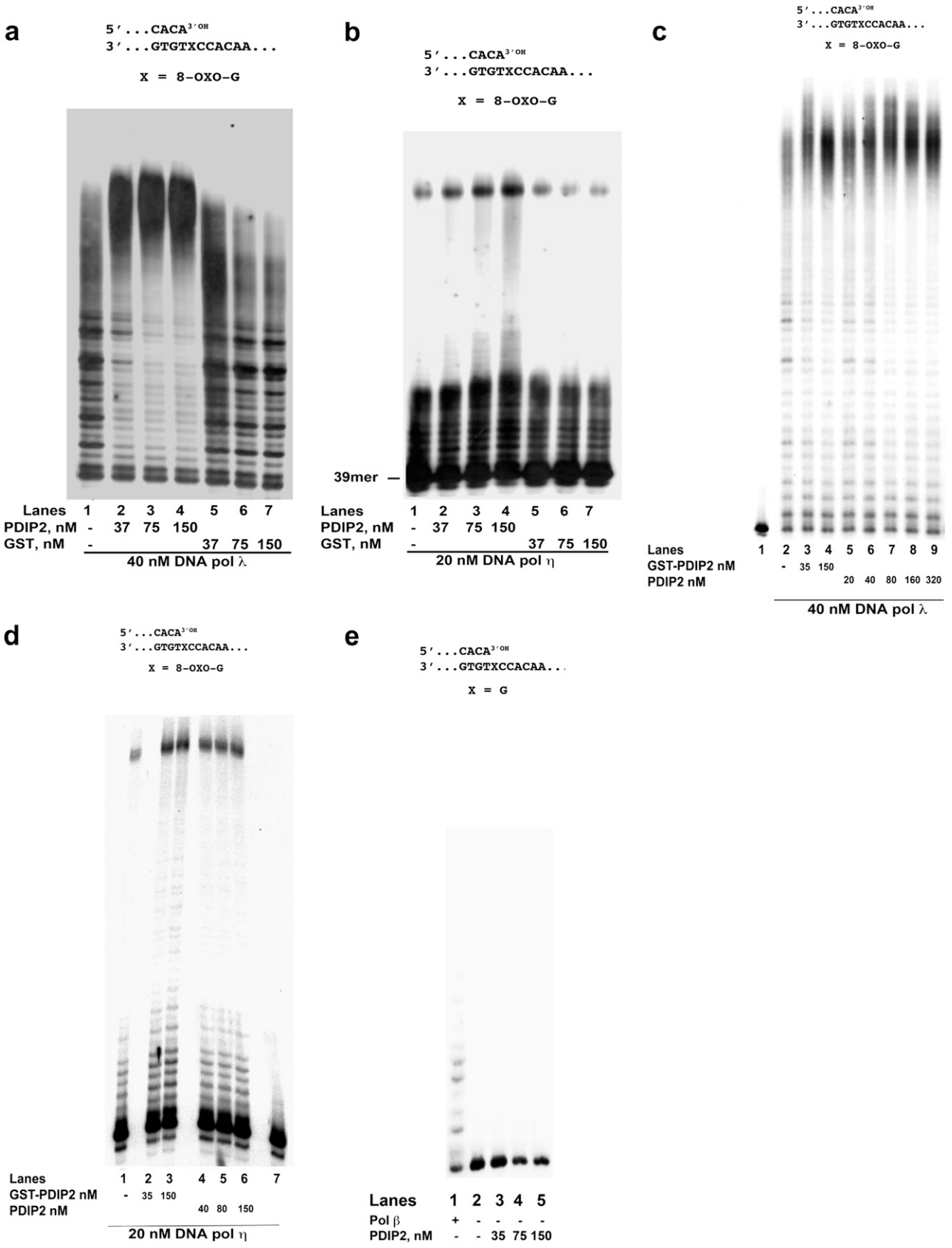

Fig. S1. (*A*) Pol λ was incubated in the presence of the 5′-labeled 8-oxo-G containing 39/100-mer primer/template (p/t) and in the absence (lane 1) or presence of increasing amounts of GST-tagged PolDIP2 (lanes 2–4) or GST (lanes 5–7). (*B*) As in *A* but in the presence of Pol η. (*C*) Pol λ was incubated with the 5′-labeled 8-oxo-G containing 39/100-mer p/t and in the absence (lane 1) or presence of increasing amounts of GST-tagged PolDIP2 (lanes 3–4) or untagged PolDIP2 (lanes

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5–9). (*D*) Pol η was incubated with the 5′-labeled 8-oxo-G containing 39/100-mer p/t and in the absence (lane 1) or presence of increasing amounts of GST-tagged PolDIP2 (lanes 3–4) or untagged PolDIP2 (lanes 4–6). Lane 7 shows control reaction in the absence of enzyme. (*E*) Increasing amounts of PolDIP2 were incubated with the 5′-labeled undamaged 39/100-mer p/t in the presence of dNTPs (lanes 3 and 5). Lane 1 shows reaction in the absence of PolDIP2 and presence of 20 nM Pol β. Lane 2 shows control reaction without enzyme.

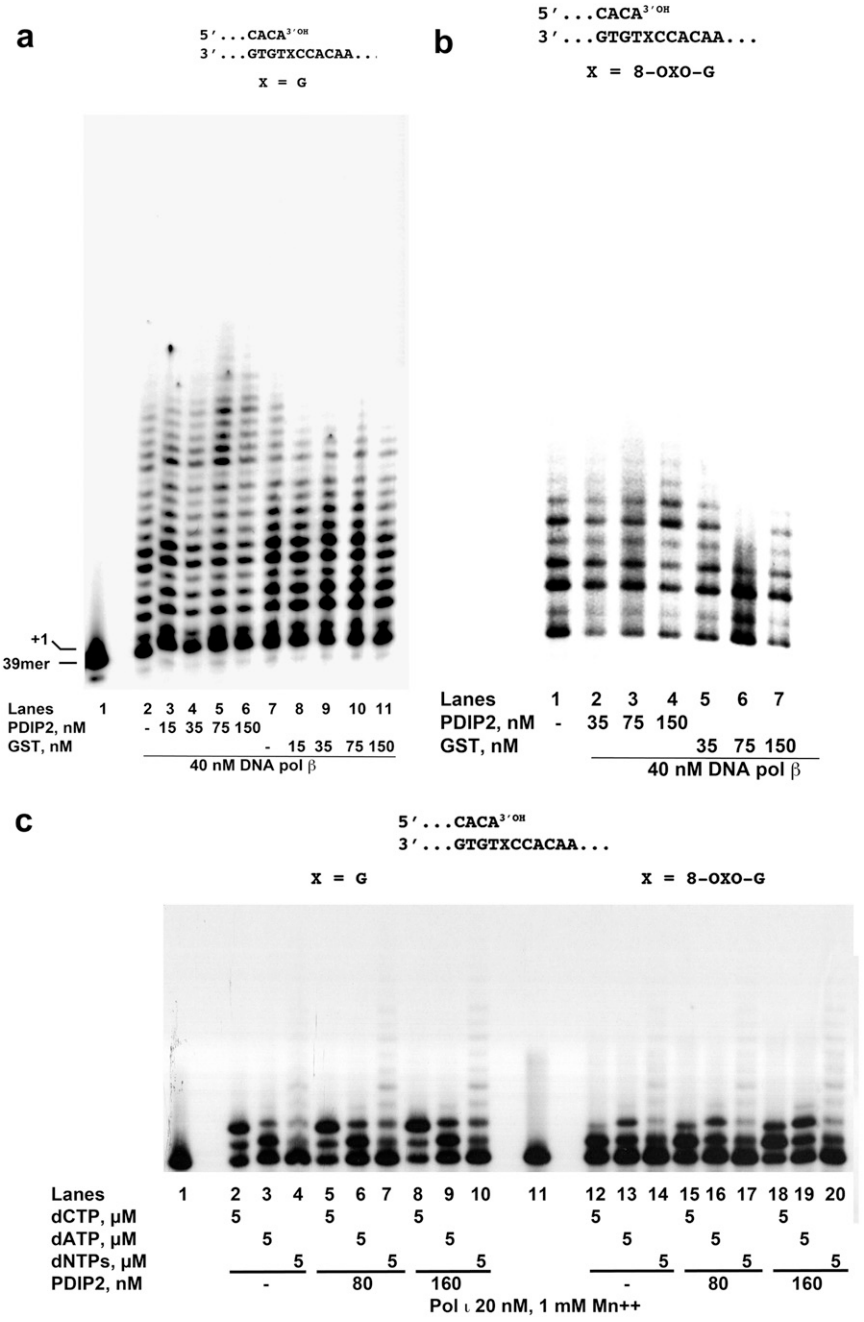

Fig. S2. (*A*) Pol β was incubated with the 5′-labeled undamaged 39/100-mer p/t in the absence (lanes 2 and 7) or presence of increasing amounts of GST-PolDIP2 (lanes 3–6) or GST (lanes 8–11). Lane 1 shows control reaction without enzyme. (*B*) Pol β was incubated with the 5′-labeled 8-oxo-G containing 39/100-mer p/t in the absence (lane 1) or presence of increasing amounts of GST-PolDIP2 (lanes 2–4) or GST (lanes 5–7). (*C*) Pol ι was incubated with the 5′-labeled undamaged 39/100-mer p/t (lanes 2–10) or the 8-oxo-G containing 39/100-mer p/t (lanes 12–20) and various combinations of nucleotides in the absence (lanes 2–4 and 12–14) or presence of increasing amounts of GST-tagged PolDIP2 (lanes 5–10 and 15–20). Lanes 1 and 11 show control reactions without enzyme.

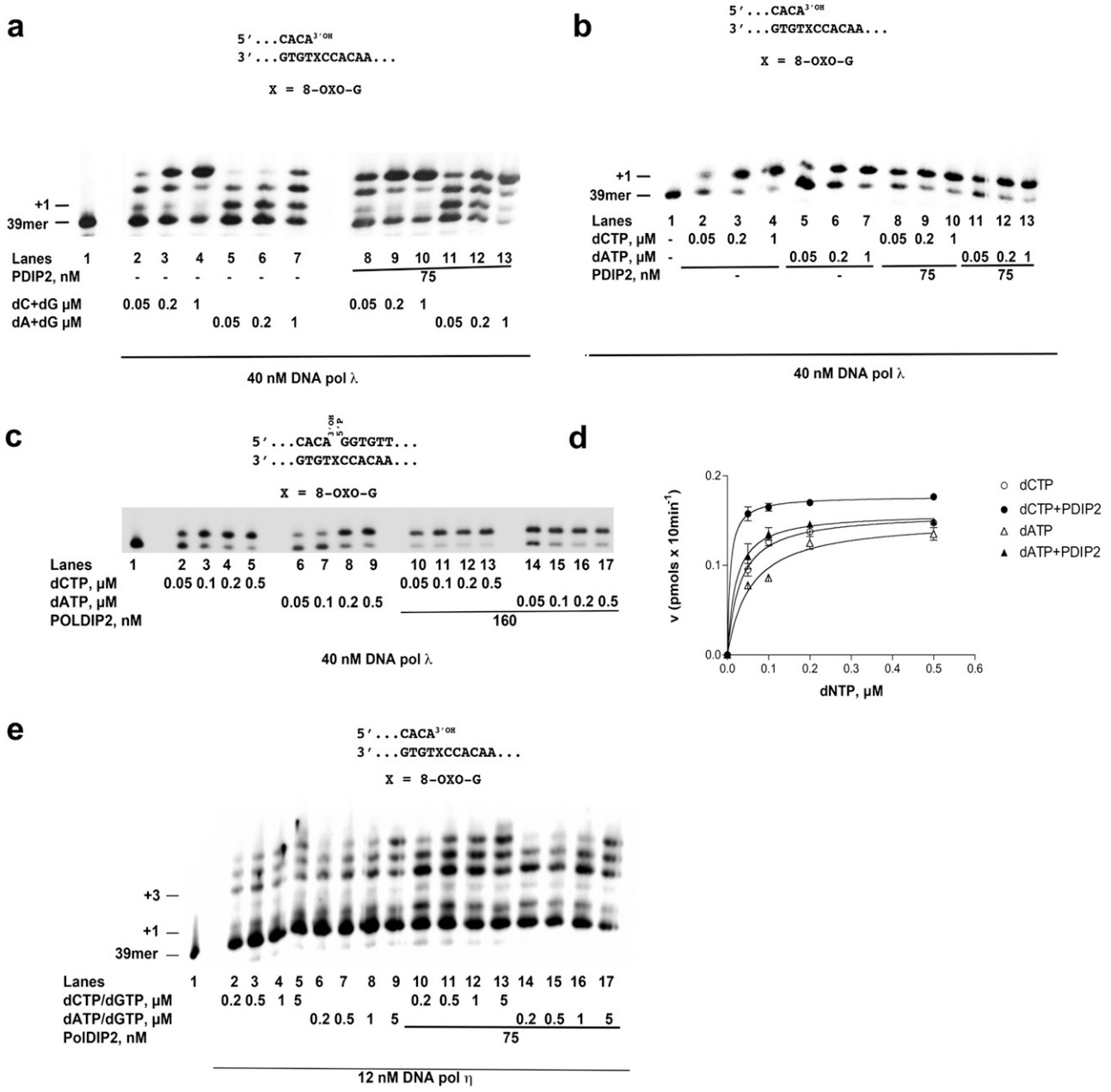

Fig. S3. (*A*) Pol λ was incubated with the 5′-labeled 8-oxo-G containing 39/100-mer p/t in the absence (lanes 2–7) or presence (lanes 8–13) of GST-tagged PolDIP2 with increasing equimolar amounts of dCTP and dGTP (lanes 2–4 and 8–10) or dATP and dGTP (lanes 5–7 and 11–13). Lane 1 shows control reaction in the absence of enzyme. (*B*) Pol λ was incubated with the 5′-labeled 8-oxo-G containing 39/100-mer p/t in the absence (lanes 2–7) or presence (lanes 8–13) of GST-tagged PolDIP2 with increasing amounts of dCTP (lanes 2–4 and 8–10) or dATP (lanes 5–7 and 11–13). Lane 1 shows control reaction in the absence of enzyme. (*C*) Pol λ was incubated with the 5′-labeled 1-nt-gapped p/t containing the 8-oxo-G in the presence of increasing amounts of dCTP (lanes 2–5 and 10–13) or dATP (lanes 6–9 and 14–17) and in the absence (lanes 2–9) or presence (lanes 10–17) of PolDIP2. Lane 1 shows control reaction in the absence of enzyme. (*D*) Variation of the velocity of the reaction catalyzed by Pol λ on the 1-nt-gapped 8-oxo-G substrate as a function of dCTP (circles) or dATP (triangles) concentrations in the absence (open symbols) or presence (filled symbols) of PolDIp2. (*E*) Pol η was incubated with the 5′-labeled 8-oxo-G containing 39/100-mer p/t in the absence (lanes 2–9), or presence (lanes 10–17) of GST-tagged PolDIP2 with increasing equimolar amounts of dCTP and dGTP (lanes 2–5 and 10–13) or dATP and dGTP (lanes 6–9 and 14–17). Lane 1 shows control reaction in the absence of enzyme.

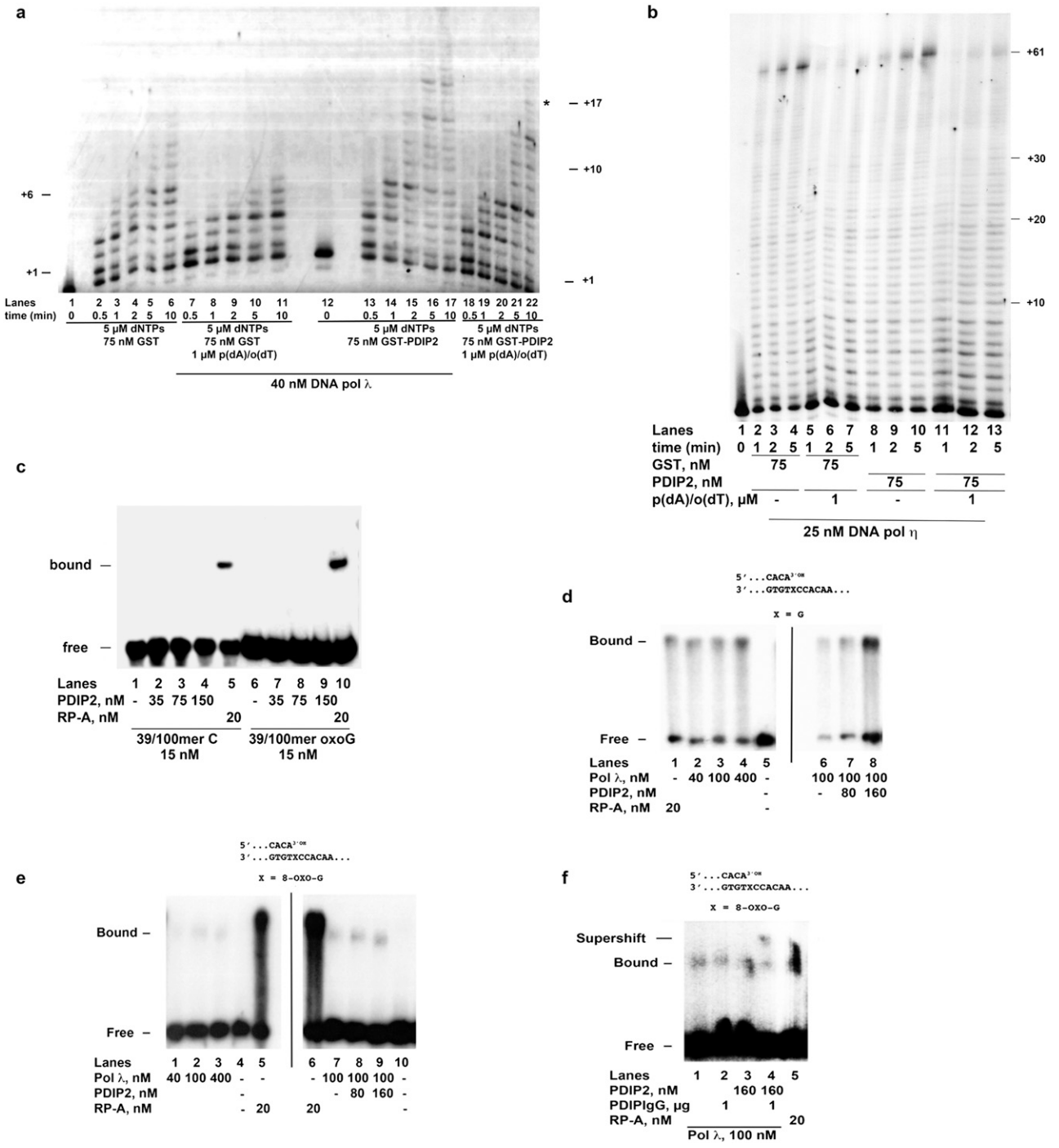

Fig. S4. (*A*) Time course of nucleotide incorporation by Pol λ on the 5′-labeled 8-oxo-G containing 39/100-mer p/t in the presence of GST (lanes 1–11) or GST-tagged PolDIP2 (lanes 12–22) and in the absence (lanes 1–6 and 12–17) or presence (7–11 and 18–22) of a 50-fold molar excess of unlabeled poly(dA)/oligo(dT) over the 39/100-mer p/t as a trapping agent. (*B*) Time course of nucleotide incorporation by Pol η on the 5′-labeled 8-oxo-G containing 39/72-mer p/t in the presence of GST (lanes 1–7) or GST-tagged PolDIP2 (lanes 8–13) and in the absence (lanes 1–4 and 8–10) or presence (lanes 5–7 and 11–13) of a 50-fold molar excess of unlabeled poly(dA)/oligo(dT) over the 39/100-mer p/t as a trapping agent. (*C*) Electrophoretic mobility shift assay (EMSA) with undamaged (lanes 1–4) or 8-oxo-G containing (lanes 6–9) 5′-labeled 39/100-mer p/t in the absence (lanes 1 and 6) or presence of GST-tagged PolDIP2. Lanes 5 and 10 show control reactions in the presence of RP-A. (*D*) EMSA of Pol λ with undamaged 5′-labeled 39/100-mer p/t in the absence (lanes 2–4 and 6) or presence (lanes 7 and 8) of PolDIP2. Lane 1 shows positive control with RP-A. Lane 5 shows negative control in the absence of proteins. (*E*) EMSA of Pol λ with the 8-oxo-G 5′-labeled 39/100-mer p/t in the absence (lanes 1–3 and 7) or presence (lanes 8 and 9) of PolDIP2. Lanes 5 and 6 show positive control with RP-A. Lanes 4 and 10 show negative control in the absence of proteins. (*F*) EMSA of Pol λ with the 8-oxo-G 5′-labeled 39/100-mer p/t in the absence (lane 1) or presence of anti-PolDIP2 IgG (lane 2), PolDIP2 (lane 3), or both (lane 4). Lane 5 shows positive control with RP-A.

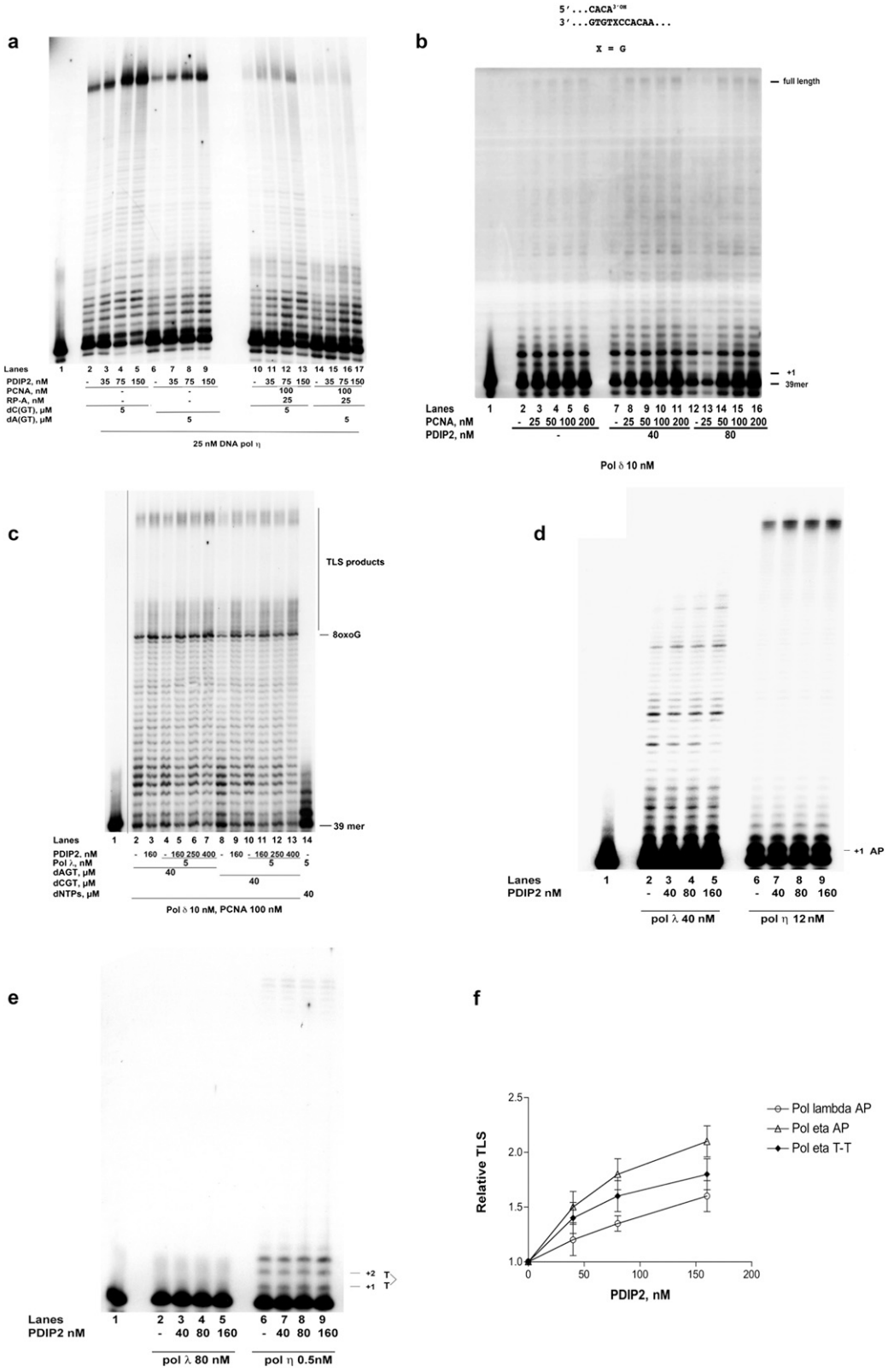

Fig. S5. (*A*) Increasing amounts of GST-tagged PolDIP2 were titrated in reactions containing Pol η with the 8-oxo-G 5′-labeled 39/100-mer p/t in the presence of 5 μM dGTP and dTTP and either 5 μM dCTP (lanes 2–5 and 10–13) or 5 μM dATP (lanes 6–9 and 14–17) and in the absence (lanes 2–9) or presence (lanes 10–17) of PCNA and RP-A. Lane 1 shows control reaction without enzyme. (*B*) Pol δ was incubated with the 5′-labeled undamaged 39/100-mer p/t in the absence (lanes 2, 7, and 12) or presence of increasing amounts of PCNA and in the absence (lanes 2–6) or presence (lanes 7–16) of PolDIP2. (*C*) Pol δ was incubated on the 5′-labeled 39/100-mer p/t containing the 8-oxo-G lesion at position +26 under error-prone (lanes 2–7) or error-free (lanes 8–13) conditions in the absence (lanes 2, 3, 8, and 9) or presence of Pol λ (lanes 4–7 and 10–13), PolDIP2 (lanes 3 and 9), or both (lanes 5–7 and 11–13). Lane 1 shows control reaction in the absence of

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proteins. Lane 14 shows reaction in the presence of Pol λ alone. (*D*) Pol λ (lanes 2–5) or Pol η (lanes 6–9) were incubated with the 5′-labeled 39/100-mer p/t containing an AP site in the absence (lanes 2 and 6) or presence of increasing amounts of PolDIP2. Lane 1 shows control reaction without enzyme. (*E*) As in *A* but in the presence of the 5′-labeled 28/62-mer containing a cyclobutane thymine dimer (T-T). (*F*) Increase in the relative amount of translesion synthesis products synthesized by Pol η in the presence of an AP (triangles) or T-T (filled diamonds) lesion or by Pol λ in the presence of an AP site (circles) as a function of PolDIP2 concentrations. Values are the means of three independent experiments. Error bars are ± SD.