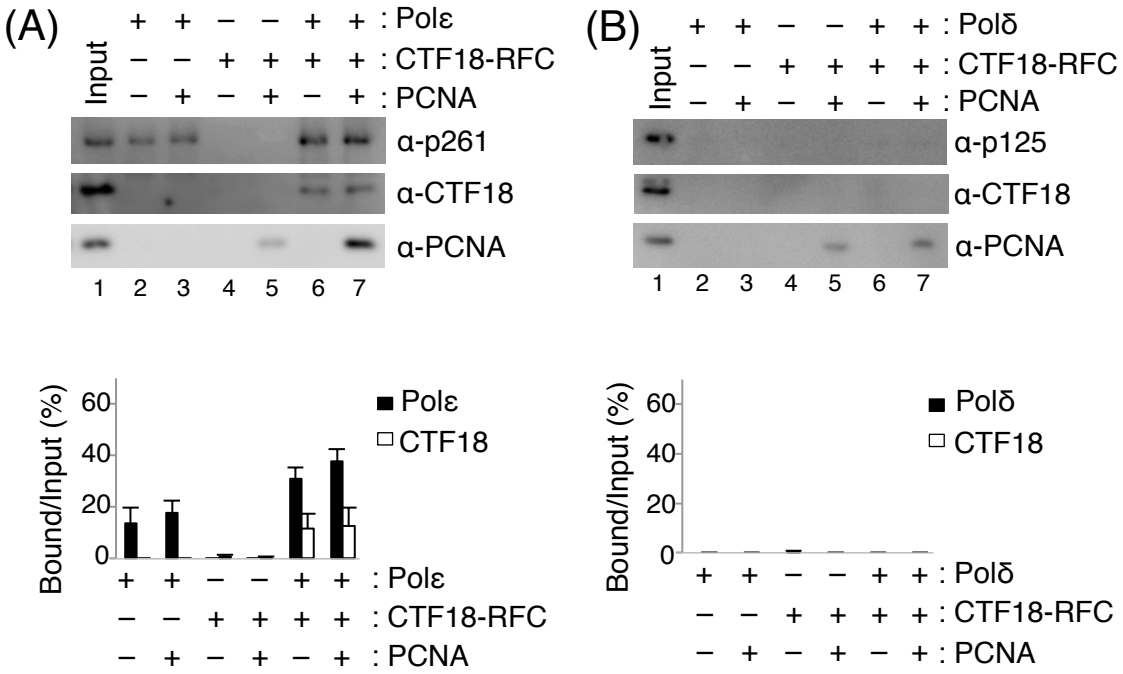

Figure S1. Analyses of loading of PCNA and binding of CTF18-RFC in the presence of Polε or Polδ. Pull down with 30 ng of gapped-DNA beads at 100 mM NaCl using 100 fmol of CTF18-RFC, 6.2 pmol of PCNA and 100 fmol of Polε or Polδ as indicated. Input (10%; Polε, Polδ, CTF18-RFC) and 12 fmol of PCNA (lane 1) and 50% bound fractions (lanes 2–7) were analysed by immunoblotting with indicated antibodies. Bound Polε and CTF18 were graphed with Mean ± S.E. of two experimental replicates (below).

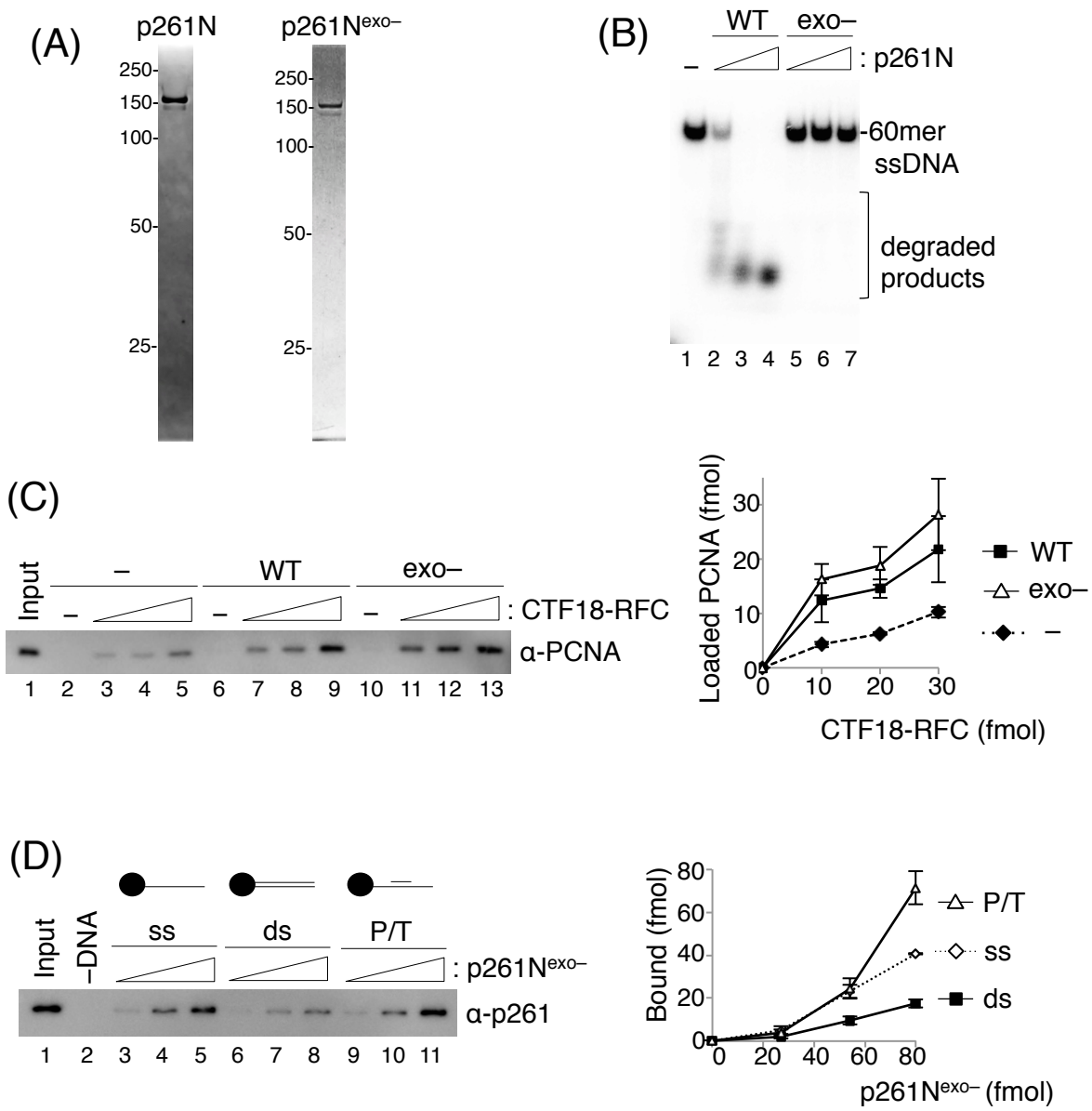

Figure S2. Prepared p261N and the exonuclease-deficient p261N$^{exo–}$ and their stimulation of PCNA loading by CTF18-RFC.

(**A**) Purified p261N and p261N$^{exo–}$ (450 ng each) were separated by electrophoresis and stained with Coomassie Brilliant Blue.

(**B**) Exonuclease assay of p261N (WT) and p261N$^{exo–}$ (exo−). Reaction mixture (5 µl) containing 30 mM NaCl and 80 fmol of 60-mer oligonucleotide (5′-tgaggttcagcaaggtgatgctttag-atactgttgcaggcggtgttaatactgaccgcct) labelled with ^{32}P at its 5′ end was incubated with 15, 30 or 45 fmol of p261N (lanes 2–4) or p261N$^{exo–}$ (lanes 5–7) at 37°C for 10 min. The reaction was stopped by addition of 10× loading solution (50% glycerol, 0.9% SDS, 0.05% bromophenol blue), and the products were separated by electrophoresis in 10% acrylamide gels in TAE and visualised as Figure 6B, E.

(**C**) PCNA-loading assay with 0, 10, 20 or 30 fmol of CTF18-RFC and either 100 fmol of p261N (lanes 6–9) or 100 fmol of p261N^{exo-} (lanes 10–13), or with neither (lanes 2–5), in 10 μl of reaction mixture containing 30 mM NaCl. Input of 12 fmol of PCNA (lane 1) and 50% bound samples (lanes 2–13) were analysed by immunoblotting with anti-PCNA antibody. The loaded PCNA was quantified and graphed at the right with Mean ± S.E. of two experimental replicates.
(**D**) Pull-down assay of p261N^{exo-}. p261N^{exo-} (27, 54 and 80 fmol) was incubated with DNA-magnetic beads conjugated with 250 fmol of an ssDNA (ss; BTN3), a double-stranded DNA (ds; BTN3 annealed with TEMP90-R) or a 5′ and 3′ recessed primer-template DNA (P/T; BTN3 annealed with BTN30) in reaction mixture containing 100 mM NaCl at 32°C for 30 min. The reacted DNA beads were washed with 1× HBS three times, and the bound proteins were analysed as in Figure 3B. p261N^{exo-} (40 fmol; lane 1) and 50% bound fractions (lanes 2–11) were visualised by immunoblotting with anti-p261 antibody and graphed at the right with Mean ± S.E. of two experimental replicates. Lane 2 included magnetic beads only (−DNA).

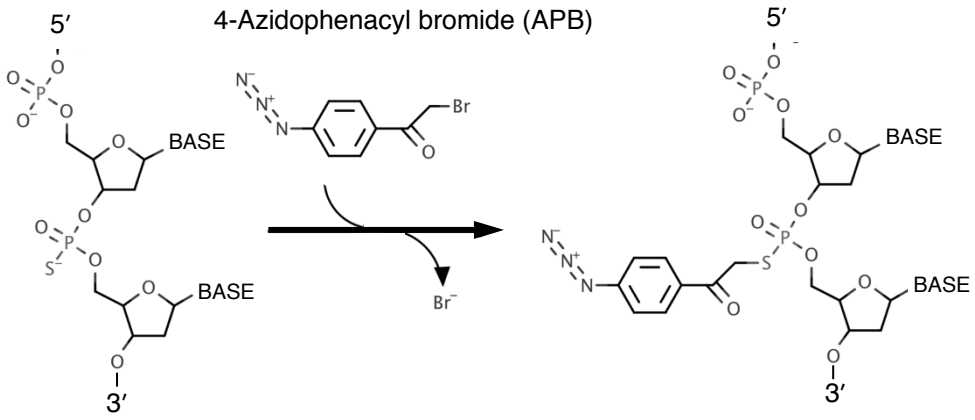

Figure S3. A coupling scheme of APB with S-dNMP. APB couples with the thiol-group at the phosphodiester backbone of a uniquely positioned phosphorothioate nucleotide (S-dNMP) in an oligo DNA.

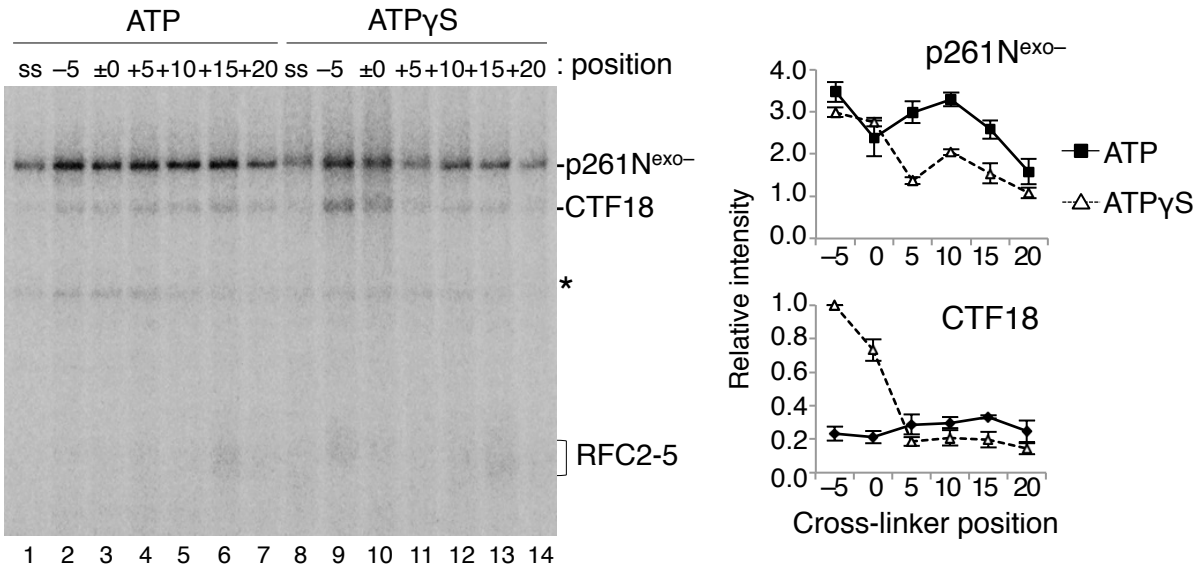

Figure S4. Photo-crosslinking analyses with the CTF18-RFC–p261N complex. Photo-crosslinked bands from 25 fmol of indicated substrate DNAs with 2 mM ATP (lanes 1–7) or 250 μM ATPγS (lanes 8–14) in the presence of 150 fmol of p261N$^{exo–}$, 150 fmol of CTF18-RFC and 500 fmol of PCNA are shown, and quantified band intensities of p261N$^{exo–}$ and CTF18 at each position were graphed as above, using CTF18 in lane 9 as a reference (1.0), with Mean ± S.E. of three experimental replicates.

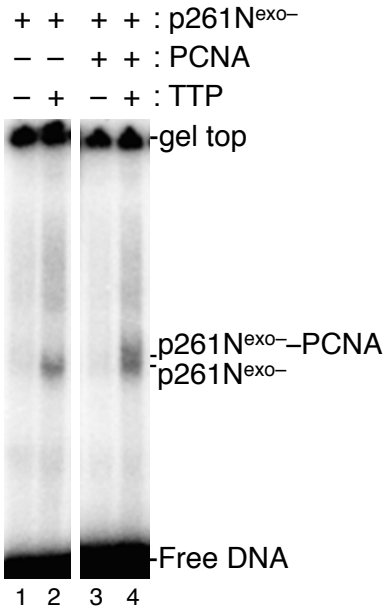

Figure S5. Analyses of DNA binding of p261N in synthesis mode. Assembly of 75 fmol of p261N$^{exo–}$ with (lanes 3, 4) or without (lanes 1, 2) 500 fmol of PCNA to 25 fmol of dd-Junction was analysed by EMSA as shown in Figure 6E at 60 mM NaCl. Addition of 100 μM TTP is indicated by "+" (lanes 2, 4). DNA bandshift positions are indicated at the right.