Supporting Information

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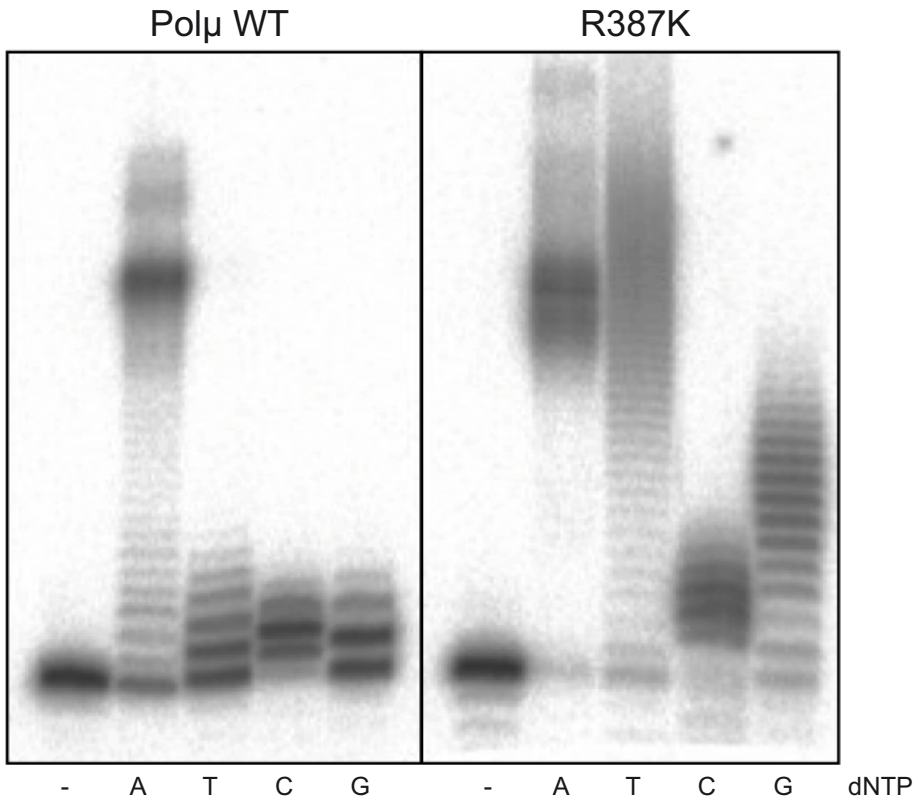

Fig. S1. Mutant R387K has a stronger terminal transferase with any of the four dNTPs. Terminal transferase reaction was carried out using 5 nM of a labeled homopolymer (PolydT) as DNA primer, 1 mM $MnCl_2$, 100 μM of the indicated dNTP, and 600 nM of either Polμ wt or mutant R387K. After incubation for 30 min at 30 °C, extension of the 5′P- labeled polydT was analyzed by 8 M urea-20% PAGE and autoradiography.

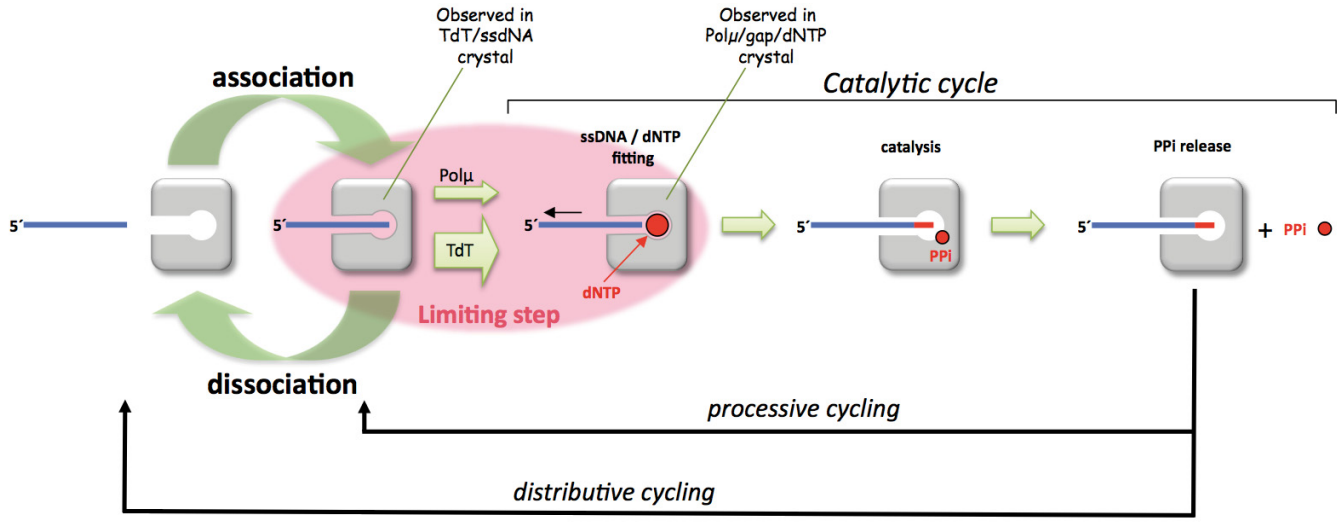

Fig. S2. Limiting step for terminal transferase addition of nucleotides. Untemplated terminal transferase addition of nucleotides on ssDNA requires several steps: 1) Formation of a binary complex between the enzyme and ssDNA. Upon association, that binary complex is stable but unproductive, according to TdT structural information. 2) To obtain a catalytically competent ternary complex, relocation of the primer terminus has to occur, driven by the incoming nucleotide, but in the absence of a templating base. That conformational change imposes a rate-limiting step for untemplated addition of nucleotides, that restricts terminal transferase capacity to specialized enzymes as TdT and Polμ. According to our data, the different terminal transferase efficiency of these two enzymes depends on their intrinsic capacity to overcome this rate-limiting step. 3) Further steps in the catalytic cycle will include catalysis and PPi release. Reiterative synthesis, either being distributive or processive, will always face that rate-limiting step to achieve enzyme/ssDNA/dNTP fitting.

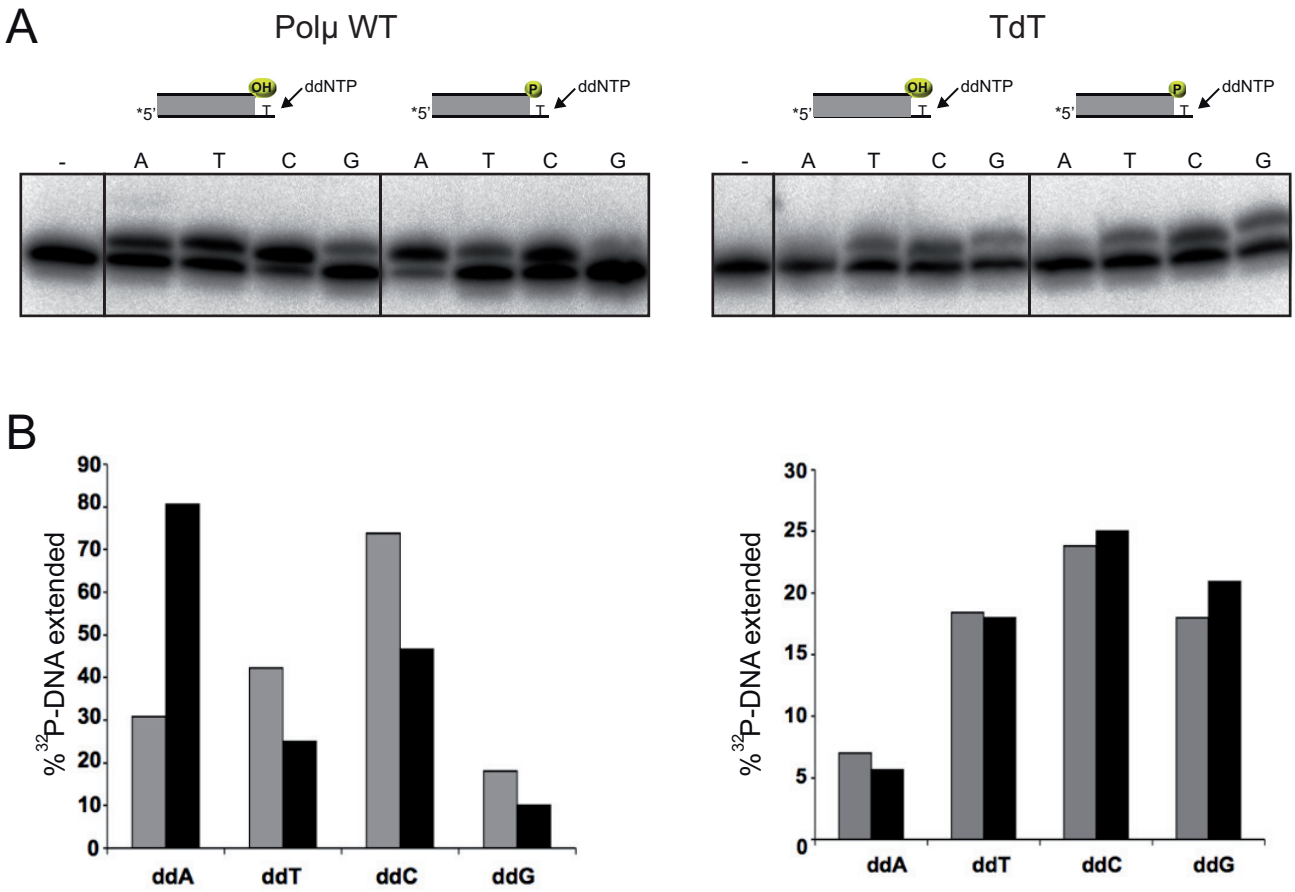

Fig. S3. Importance of the 5′-P group for the terminal transferase of Polμ versus TdT. (*A*) The assay was performed as described in *Materials and Methods*, using a 3′-protruding (1T) dsDNA substrate (200 nM), with or without a recessive 5′P, as indicated, in the presence of 1 mM $MnCl_2$, 100 μM of the indicated ddNTP, and 200 nM of either Polμ wt or TdT. After incubation for 30 min at 30 °C, the + 1 extension of the 5′P- labeled oligonucleotide was analyzed by 8 M urea-20% PAGE and autoradiography. (*B*) Quantification of the + 1 extension catalyzed by Polμ (left diagram) and TdT (right diagram) on 3′-protruding substrates containing (black bars) or lacking (gray bars) a 5′-P group.

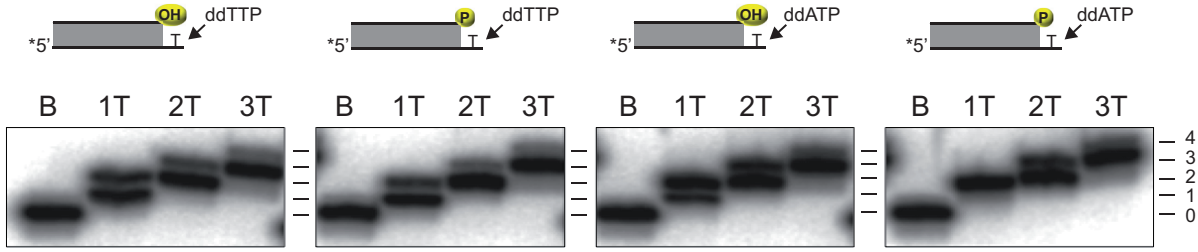

Fig. S4. Switching from terminal transferase to NHEJ mode, dependent on the presence of a 5′P group only occurs with 1nt protruding substrates. The assay was performed as described in *Materials and Methods*, using either blunt or 3′-protruding (1T, 2T, and 3T) dsDNA substrates (200 nM), with or without a recessive 5′P, as indicated, in the presence of 1 mM $MnCl_2$, 100 μM of the indicated ddNTP, and 200 nM of Polμ wt. After incubation for 30 min at 30 °C, the + 1 extension of the 5′P- labeled oligonucleotide was analyzed by 8 M urea-20% PAGE and autoradiography.