Supporting Information

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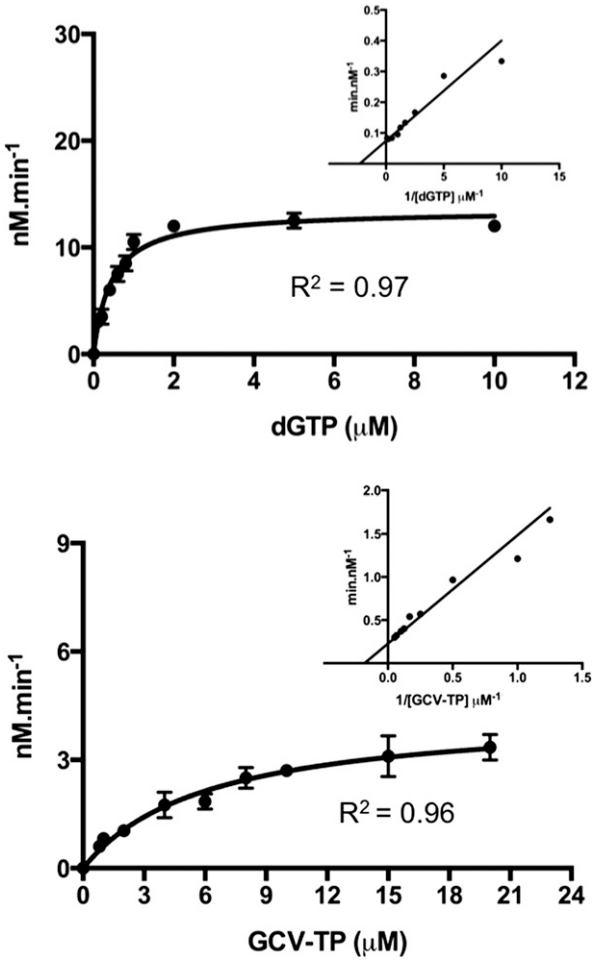

Fig. S1. Saturable rates of incorporation of dGTP (*Top*) and GCV-TP (*Bottom*). A large excess of primer template T1 (Fig. 1) was incubated at 37 °C with WT HCMV Pol for 5 min (*Top*) or 15 min (*Bottom*) and the concentrations of dGTP (*Top*) or GCV-TP (*Bottom*) indicated on the *x* axes. The reactions were analyzed by polyacrylamide gel electrophoresis. The percent of primer templates that were larger (by one nucleotide) in each gel lane was assessed using a phosphorimager, the values converted to rates, and the data were fit to the Michaelis–Menten equation (R^2 values presented under the curves; error bars indicate SEs) and to Lineweaver–Burk plots (*Insets*).

Fig. S2. (*A*) Degradation of primer template by HCMV Pol is more extensive in the presence of UL44. HCMV Pol with (Pol/UL44; filled cycles) or without (filled squares) UL44ΔC290 was incubated with radiolabeled primer template T2 (Fig. 1) at 37 °C for the times indicated on the *x* axis. The reactions were analyzed by polyacrylamide gel electrophoresis, and the percent of products that remained full length in each lane was assessed using a phosphorimager. Error bars show SEs. (*B*) A view of the autoradiogram of the entire polyacrylamide gel from which Fig. 2 was taken. Note that very short products increase with time as the amount of full-length radiolabeled primer template decreases throughout the 30-min time course.

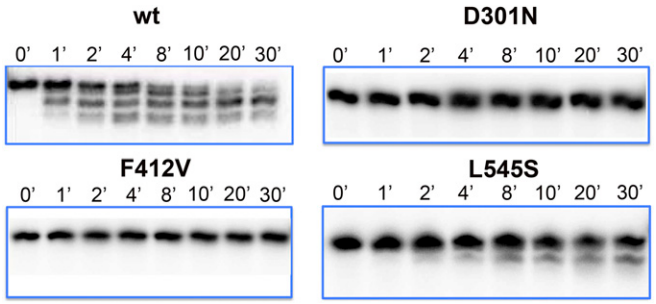

Fig. S3. Exo mutants are defective for degrading a primer template that terminates with dC. Radiolabeled primer template T1 (Fig. 1) was incubated with each WT or mutant Pol in the presence of UL44 at 37 °C for the times indicated above each lane, and the products were analyzed by gel electrophoresis, and autoradiography. The Pol analyzed is indicated at the top of each panel.

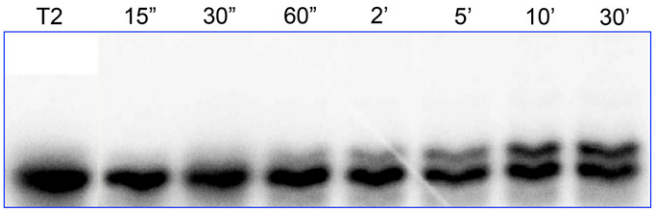

Fig. S4. Extension of GCV-terminated primer template by WT Pol. Radiolabeled primer template T2 (Fig. 1) was incubated with WT Pol, dCTP, dATP, and dTTP in the presence of UL44 at 37 °C for the times indicated at the top of the panel, and analyzed by polyacrylamide gel electrophoresis alongside untreated T2 as a marker and autoradiography. T2, untreated primer template.

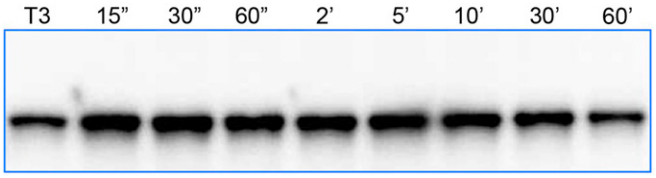

Fig. S5. WT Pol does not detectably extend a GCV plus dC-terminated primer template. Radiolabeled primer template T3 (Fig. 1) was incubated with WT Pol, dATP, and dTTP in the presence of UL44 at 37 °C for the time indicated at the top of the figure and analyzed by polyacrylamide gel electrophoresis alongside untreated T3 as a marker and autoradiography. T3, untreated primer template.

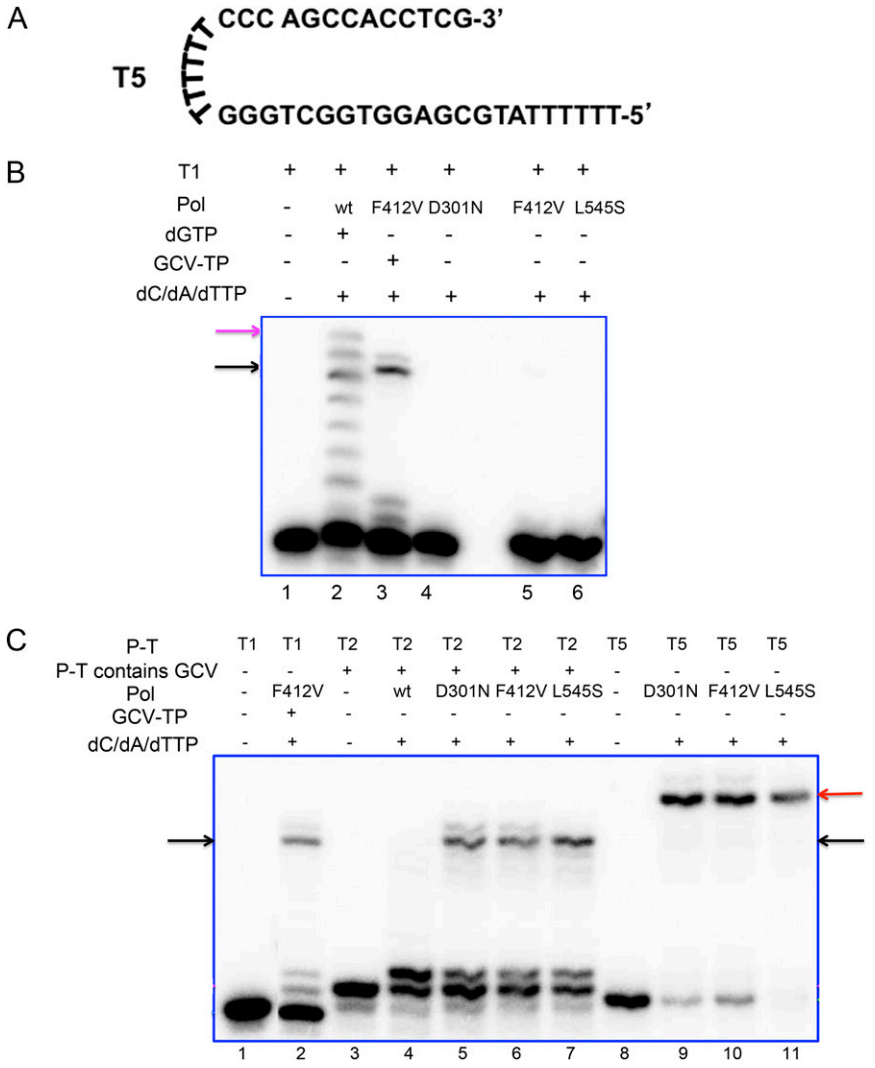

Fig. S6. Full-length products synthesized by mutant polymerases contain GCV. (*A*) Primer template T5, which is identical to primer template T2 (Fig. 1), but with dG instead of GCV at the primer terminus. (*B*) Full-length products require GCV-TP and migrate aberrantly. Radiolabeled primer tempate T1 (Fig. 1) was either loaded directly on a polyacrylamide gel as a marker (lane 1), or incubated with WT Pol and UL44 plus dATP, dCTP, dTTP, and dGTP to generate full-length (indicated by magenta arrow to left of the panel) as well as shorter products, or incubated with mutant Pol F412V and UL44 plus dATP, dCTP, dTTP, and GCV-TP to generate full-length products (black arrow to left of the panel) that are shorter than those in lane 2, or incubated with mutant Pol D301N (lane 4), F412V (lane 5), or L545S (lane 6) and UL44 plus dCTP, dATP, and dTTP (no GCV-TP or dGTP), which results in no products formed. (*C*) Full-length products formed in the presence of GCV-TP comigrate with full-length products formed from a GCV-terminated primer template and migrate faster than products from a dG-terminated primer template. Primer template T1, T2 (Fig. 1), and T5 (*A*) were each loaded directly on a polyacrylamide gel as markers (lanes 1, 3, and 8, respectively). T1 was also incubated with mutant Pol F412V and UL44 plus dATP, dCTP, dTTP, and GCV-TP to generate full-length products (black arrows to the left and right of panel). These products comigrated with products from incubation of GCV-terminated primer template T2 with Pol D301N (lane 5), F412V (lane 6), or L545S (lane 7) and UL44 plus dCTP, dATP, and dTTP (no GCV-TP or dGTP), whereas incubation of T2 with WT Pol and UL44 and the same dNTPs resulted in the addition of only one nucleotide (lane 4), as observed in Fig. S4. Radiolabeled primer template T5 was incubated with mutant Pol D301N (lane 9) or F412V (lane 10) or L545S (lane 11) and UL44 plus dCTP, dATP, and dTTP to generate full-length products that do not contain GCV and migrate more slowly than the reactions with GCV-TP or a GCV-containing primer template (red arrow to right of panel). All incubations were at 37 °C for 10 min and analyzed by denaturing polyacrylamide gel electrophoresis and autoradiography.

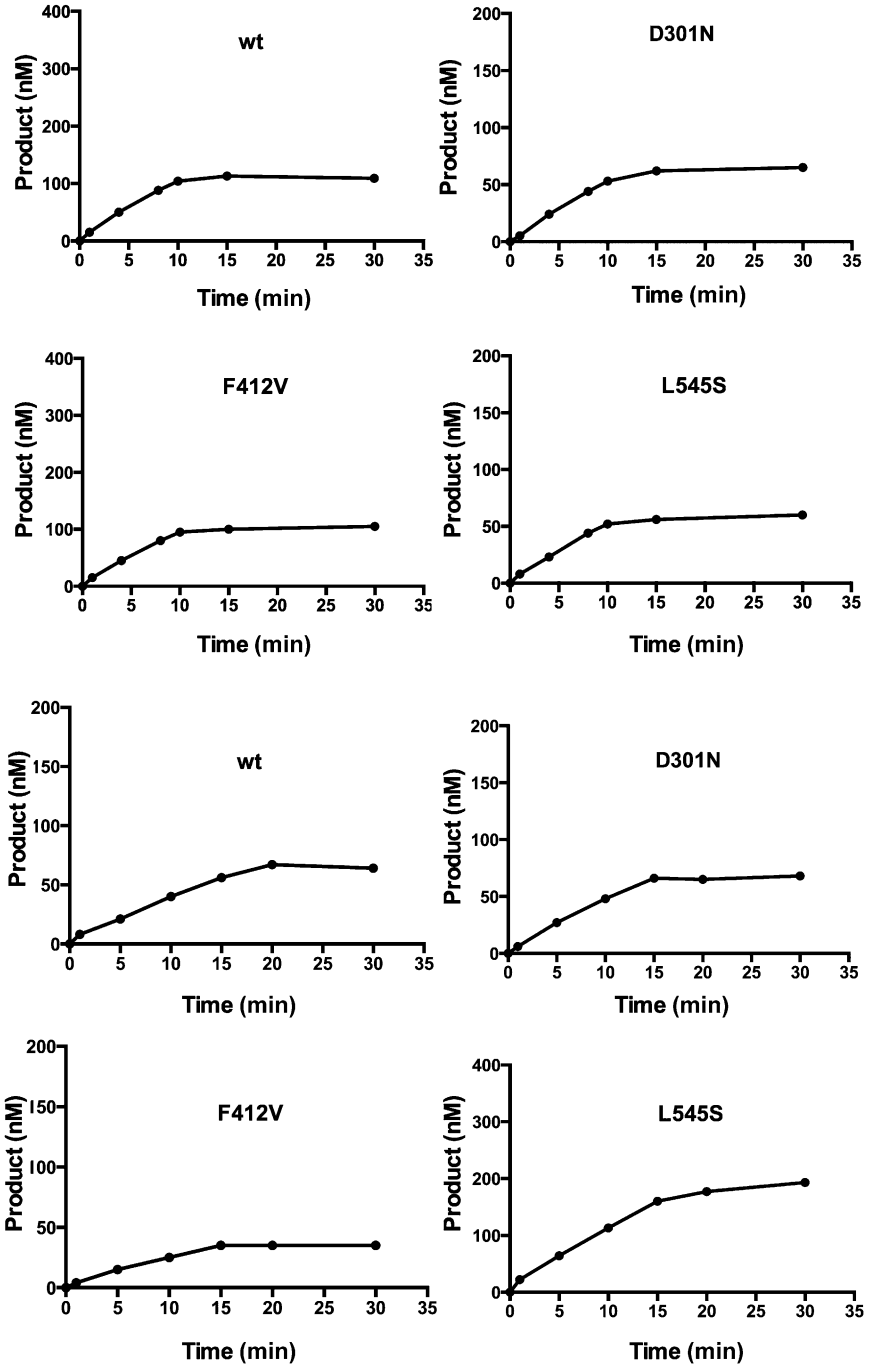

Fig. S7. Polymerase reactions are linear for 10 min for dGTP incorporation (top four panels) and for at least 15 min for GCV-TP incorporation (bottom four panels). Each WT and mutant Pol, as indicated within each panel, was incubated with radiolabeled primer template T1 using 0.3 μM dGTP (top four panels) or 5 μM GCV-TP (bottom four panels) at 37 °C for the times indicated. The products (incorporation of one nucleotide into primer template) were analyzed following polyacrylamide gel electrophoresis by a phosphorimager.

Table S1. Primers used for constructing WT Pol and Pol mutants

Enzyme	Template	Primers
WT Pol	pBAC/AD169 Bacmid	FW, TTTTTTTCGGTCCGATGTTTTTCAACC
		RV, TTTTTTTCGGACCGTCAACAGGATTC
D301N	pGST-WT Pol plasmid	FW, CGATGCCTGTCCTTCAATATCGAGTGCATGAG
		RV, CTCATGCACTCGATATTGAAGGACAGGCATCG
F412V	pGST-WT Pol plasmid	FW, GGTTACAACATCAACTCTGTTGACTTGAAGTACATCC
		RV, GGATGTACTTCAAGTCAACAGAGTTGATGTTGTAACC
L545S	pGST-WT Pol plasmid	FW, CAGGACGCCGTATCGGTGCGCGATCTG
		RV, CAGATCGCGCACCGATACGGCGTCCTG

FW, forward primers; RV, reverse primers.