Supplemental data for

DNA polymerases η and ζ combine to bypass O^2-[4-(3-pyridyl)-4-oxobutyl]thymine, a tobacco-specific nitrosamine derived DNA adduct

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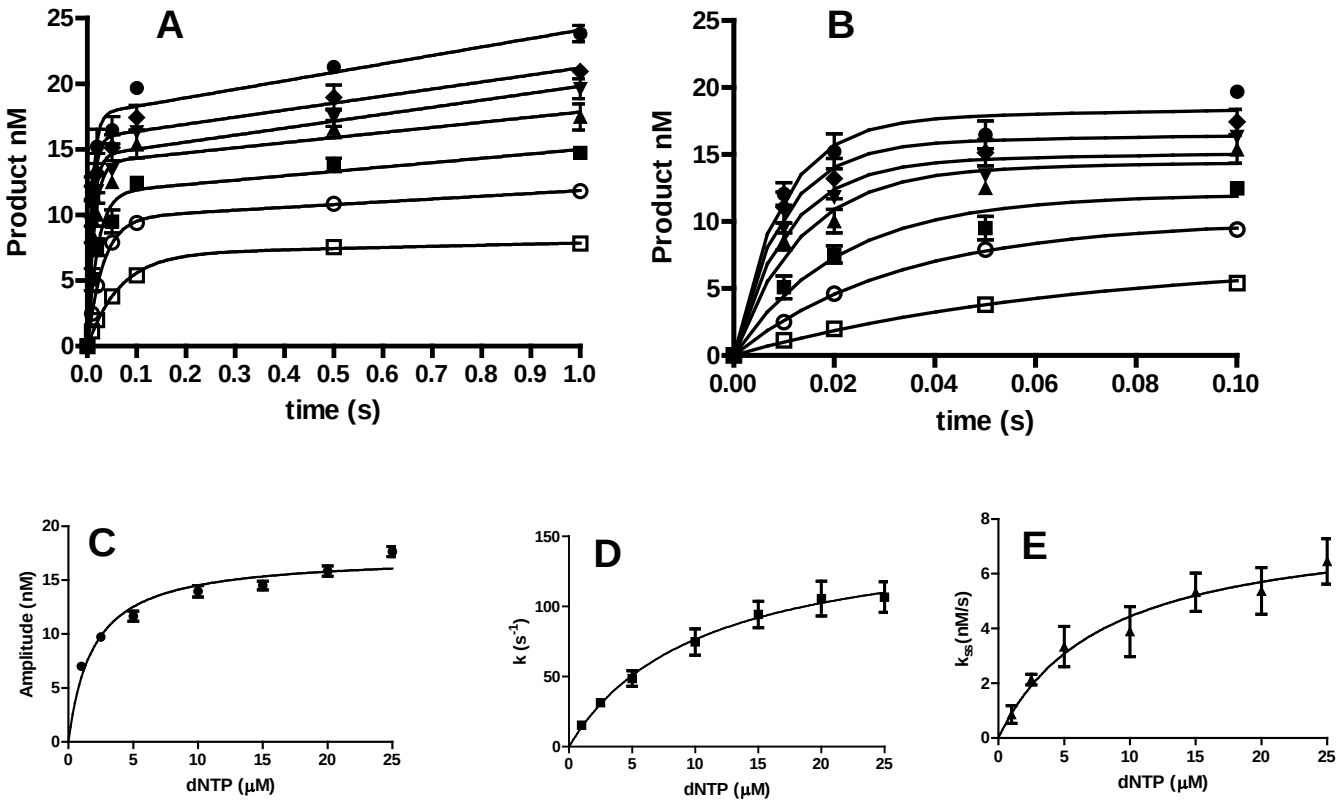

Figure S1. Pol η catalyzed incorporation of dATP opposite dT.

A, The time course was determined with 250 nM pol η, 25 nM DNA, with dT as template base, and 1(open square), 2.5 (open circle), 5 (solid square), 10(up triangle), 15(down triangle), 20(solid diamond), 25 (solid circle) μM dATP. The data points are the mean ± standard deviation of three determinations. The line is the best fit to the burst equation. B Early time points. C, The amplitude determined from panel A was plotted against [dATP]. The line is the best fit to the hyperbolic equation. D, The burst rate constant plotted against [dATP]. The line is the best fit to the hyperbolic equation. E, The steady-state rate constant plotted against [dATP]. The line is the best fit to the hyperbolic equation. The error bars are standard error of the parameter.

Burst equation. $P = A\,(1 - e^{-kt}) + k_{ss}t$

Hyperbolic equation $V = \frac{V_{\max[dNTP]}}{K+[dNTP]}$

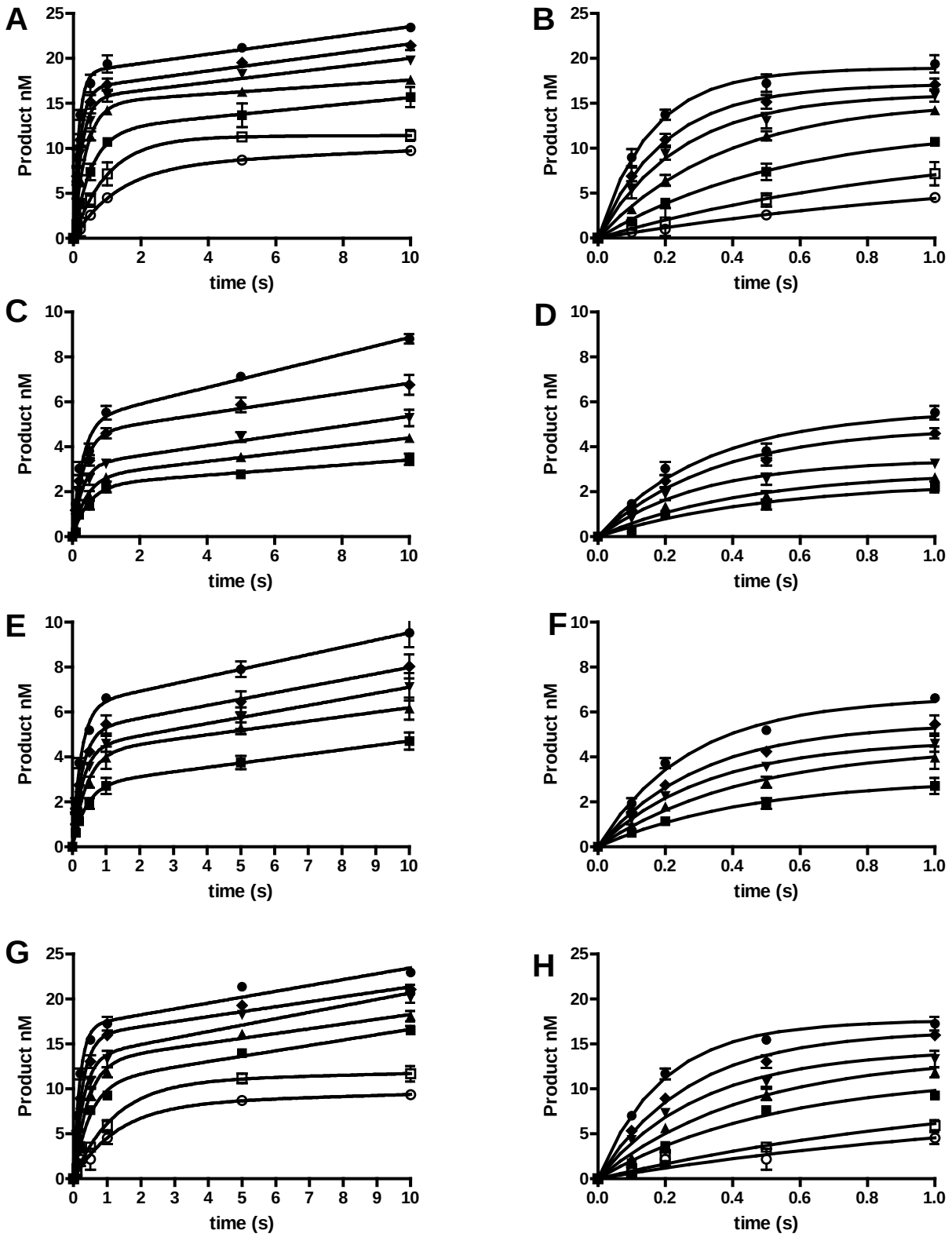

Figure S2. Pol η catalyzed incorporation of dNTPs opposite O^2-Me-dT.
Pol η (250 nM) and O^2-Me-dT DNA (25 nM) were reacted with 20 (open circle), 30 (open square), 50 (black square), 100 (up triangle), 200 (down triangle), 500 (solid diamond), and 1000 μM (solid circle) dATP (A, B), dCTP (C,D), dGTP (E,F), and dTTP (G,H). The data inin B, D, F, and G represent early time points. The lines are the best fit to the burst equation. The data points are the mean of three experiments ± standard deviation.

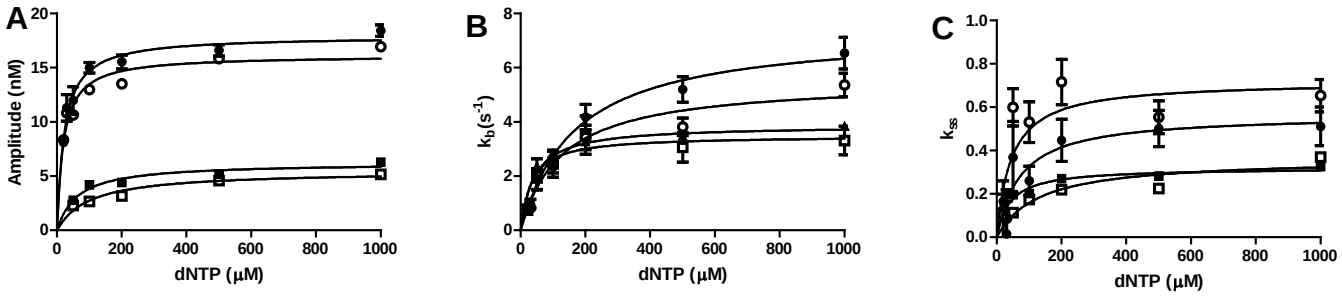

Figure S3 dNTP dependence for the time course parameters of Pol η catalyzed incorporation of dNTPs opposite O^2-Me-dT. The kinetic parameters derived from Figure S2 are plotted against dNTP concentration dATP (solid circle), dCTP (open square), dGTP (solid square), dTTP (open circle). The lines are the best fit to a hyperbolic equation for the purines (solid line) and pyrimidines (dashed line).

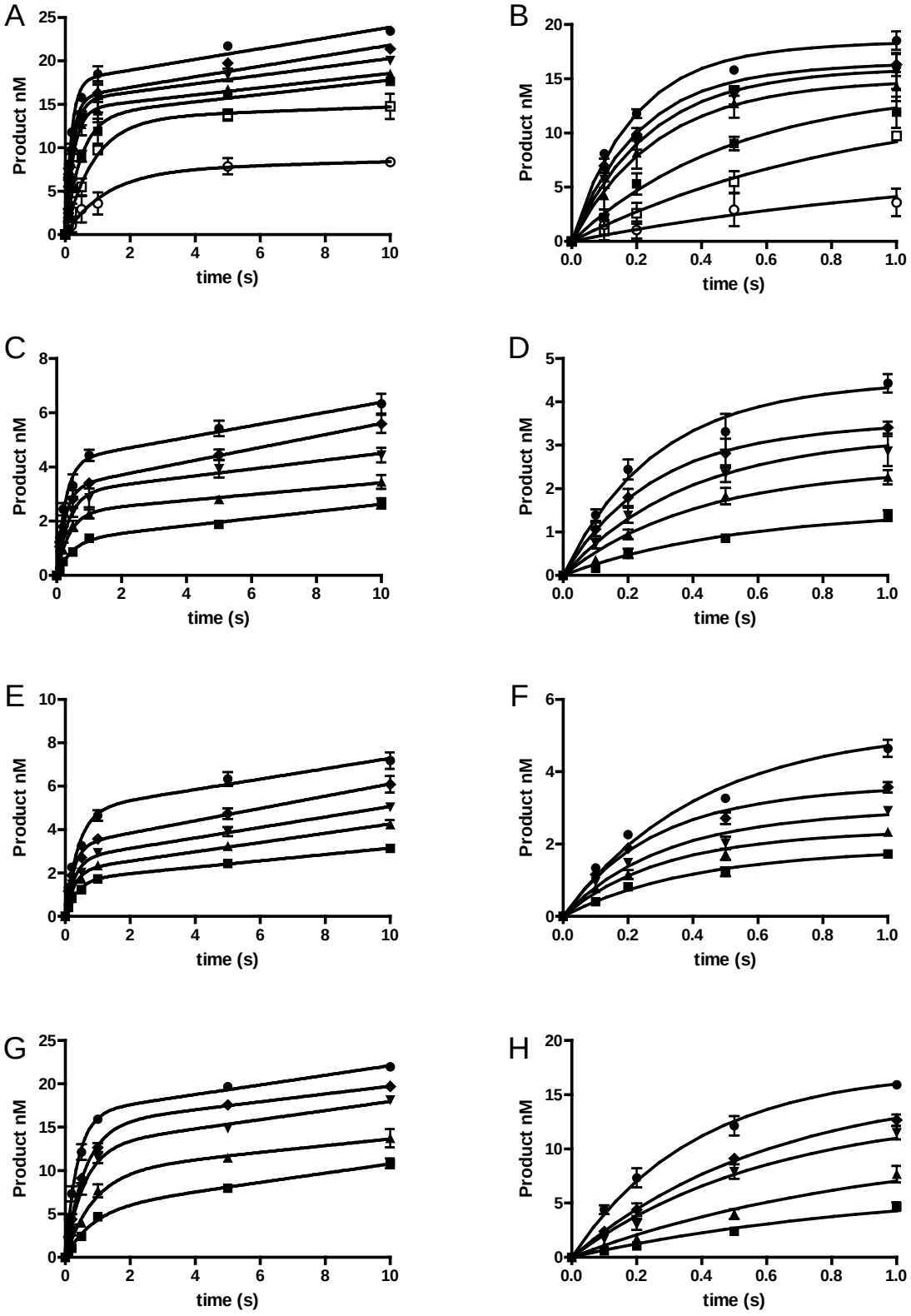

Figure S4. Pol η catalyzed incorporation opposite O^2-POB-dT.
Pol η (250 nM) and O^2-POB-dT DNA (25 nM) were reacted with 10 μM (open circle), 30 μM (open square), 50 μM (solid square), 100 μM (up triangle), 200 μM (down triangle), 500 μM (solid diamond), and 1000 μM (solid crcle) dATP (A, B), dCTP (C,D), dGTP (E,F), and dTTP (G,H). The data in B, D, F, and G represent early time points. The lines are the best fit to the burst equation. The data points are the mean of three experiments ± standard deviation.

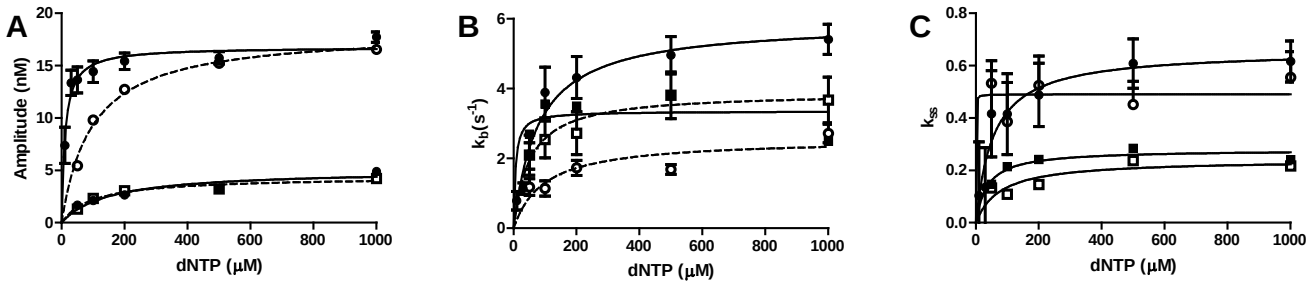

Figure S5. dNTP dependence for the time course parameters of Pol η catalyzed incorporation of dNTPs opposite O^2-POB-dT. The kinetic parameters derived from Figure S2 are plotted against dNTP concentration dATP (solid circle), dCTP (open square), dGTP (solid square), dTTP (open circle). The lines are the best fit to a hyperbolic equation for the purines (solid line) and pyrimidines (dashed line).

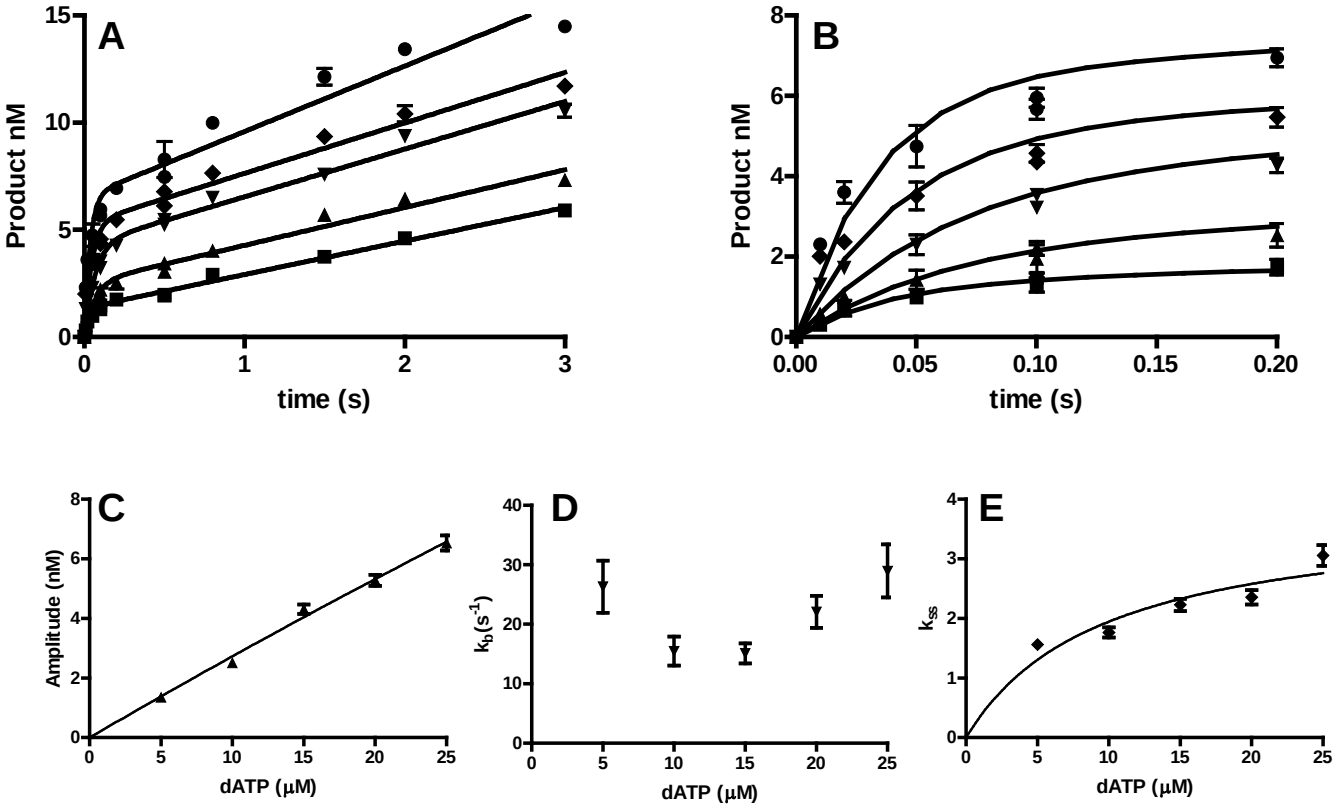

Figure S6. Reaction of pol ι with normal DNA. A, The time course was determined with 150 nM pol ι, 15 nM DNA, with dT as template base, and 5 (square), 10(up triangle), 15(down triangle), 20(diamond), 25 (circle) μM dATP. The data points are the mean ± standard deviation of three determinations. The line is the best fit to the burst equation. B, The early time points from pane A are shown. C, The amplitude determined from panel A was plotted against [dATP]. The line is a linear fit to the data with the slope equal to $3.0 \pm 0.4 \times 10^{-3}$ M. D The burst rate constant plotted against [dATP]. The mean of the values is 22 ± 6 s^{-1}. D, The steady-state rate constant plotted against [dATP]. The line is the best fit to the hyperbolic equation. The error bars are standard error of the parameter.

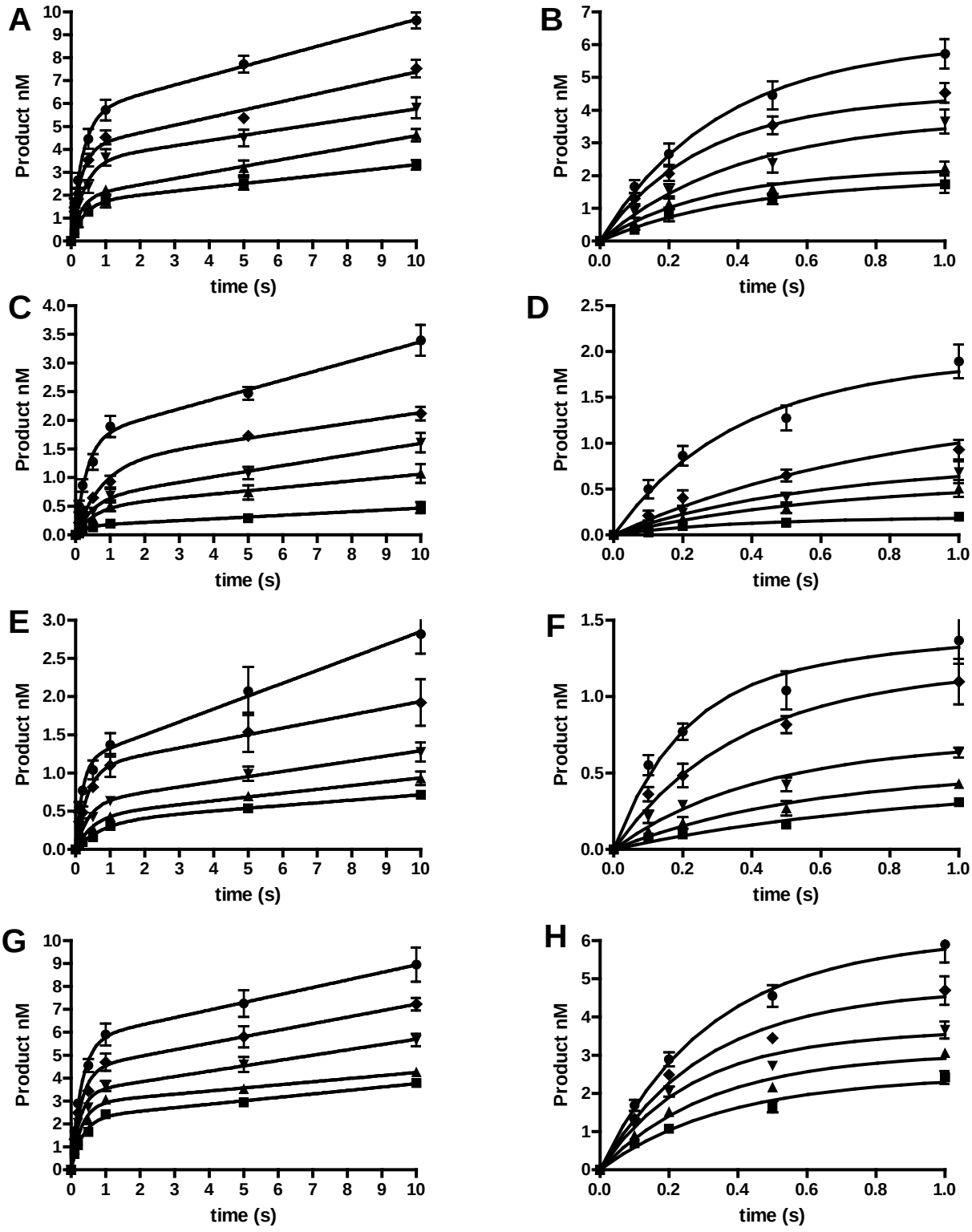

Figure S7 Pol ι catalyzed incorporation opposite O^2-Me-dT. Pol ι (150 nM) and O^2-Me-dT DNA (15 nM) were reacted with 50 μM (square), 100 μM (up triangle), 200 μM (down triangle), 500 μM (diamond), and 1000 μM (circle), dATP (A, B), dCTP (C,D), dGTP (E,F), and dTTP (G,H). The data in B, D, F, and G represent early time points. The lines are the best fit to the burst equation. The data points are the mean of three experiments ± standard deviation.

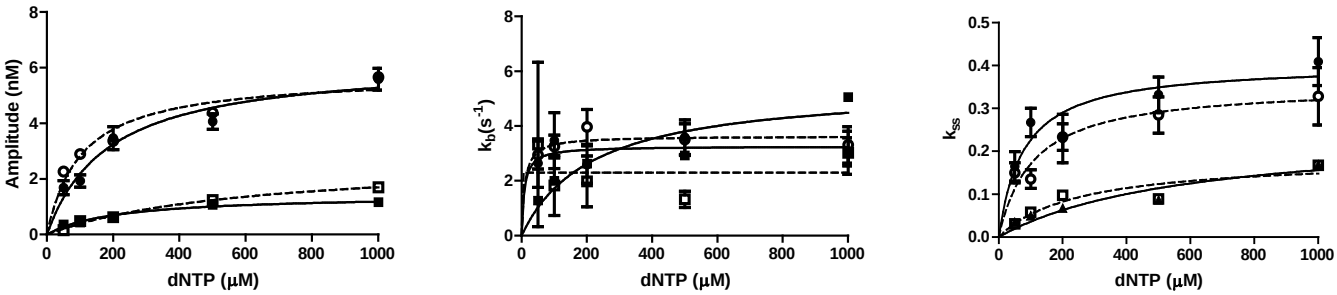

Figure S8 dNTP dependence for pol ι - O^2-Me-dT time course parameters. The kinetic parameters derived from Figure S7 are plotted against dNTP concentration dATP (solid circle), dCTP (open square), dGTP (solid square), dTTP (open circle). The lines are the best fit to a hyperbolic equation for the purines (solid line) and pyrimidines (dashed line). .

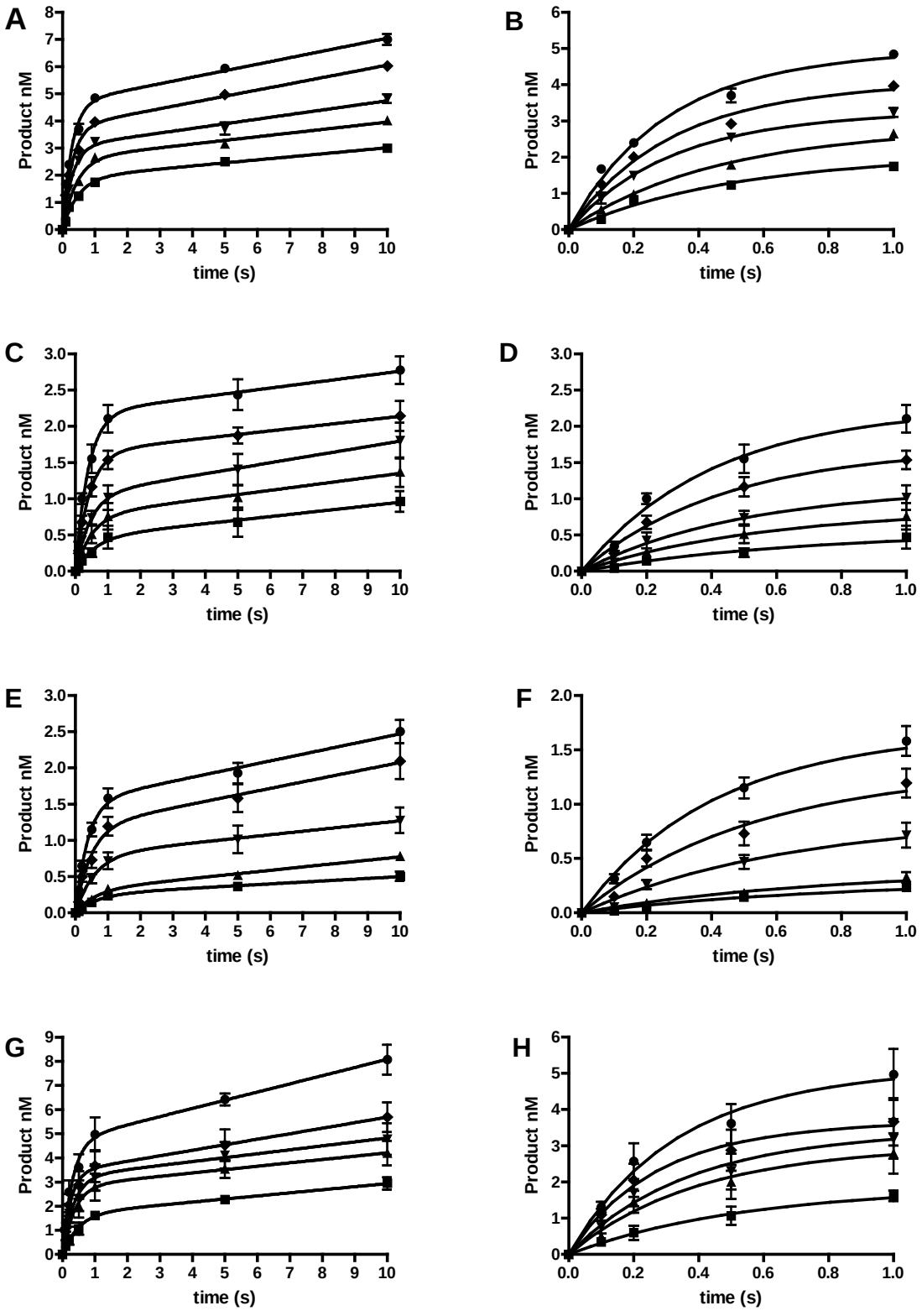

Figure S9 Pol ι catalyzed incorporation opposite O^2-POB-dT. Pol ι (150 nM) and O^2-POB-dT DNA (15 nM) were reacted with 50 μM (square), 100 μM (up triangle), 200 μM (down triangle), 500 μM (diamond), and 1000 μM (circle), dATP (A), dCTP (B), dGTP (C), and dTTP (D). The lines are the best fit to the burst equation. The data points are the mean of three experiments ± standard deviation.

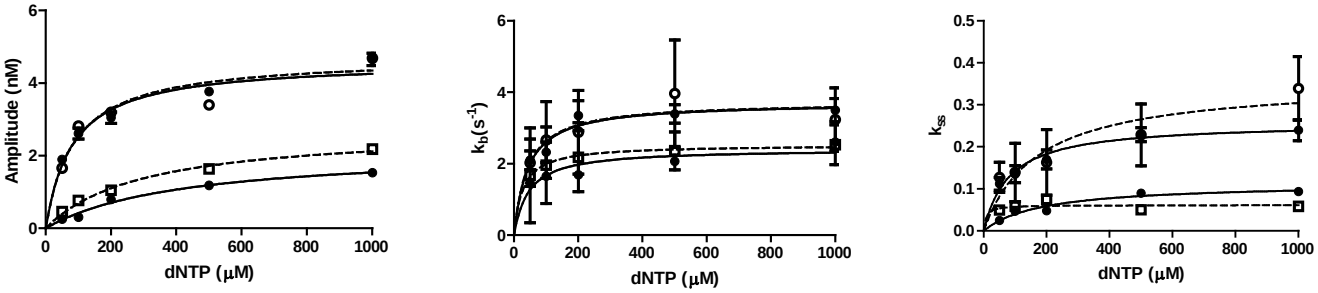

Figure S10 dNTP dependence for pol ι time course parameters. The kinetic parameters derived from Figure S9 are plotted against dNTP concentration dATP (solid circle), dCTP (open square), dGTP (solid square), dTTP (open circle). The lines are the best fit to a hyperbolic equation for the purines (solid line) and pyrimidines (dashed line). .

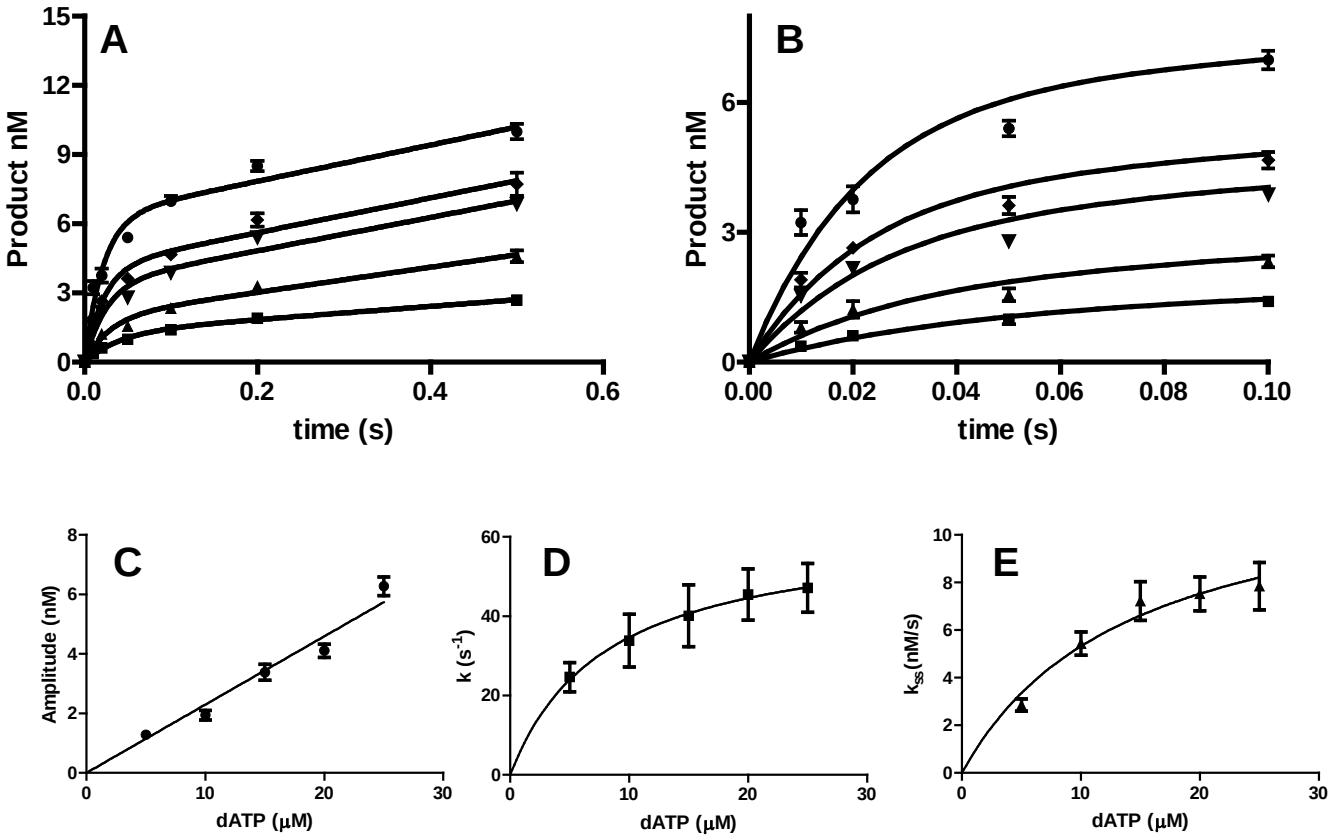

Figure S11. Reaction of pol κ with normal DNA. A, The time course was determined with 150 nM pol κ, 15 nM DNA, with dT as template base, and 5 (square), 10(up triangle), 15(down triangle), 20(diamond), 25 (circle) μM dATP. The data points are the mean ± standard deviation of three determinations. The line is the best fit to the burst equation. B, Short time points. C, The amplitude determined from panel A was plotted against [dATP]. The line is the best fit to a straight line. D, The burst rate constant plotted against [dATP]. The line is the best fit to the hyperbolic equation. E, The steady-state rate constant plotted against [dATP]. The line is the best fit to the hyperbolic equation. The error bars are standard error of the parameter.

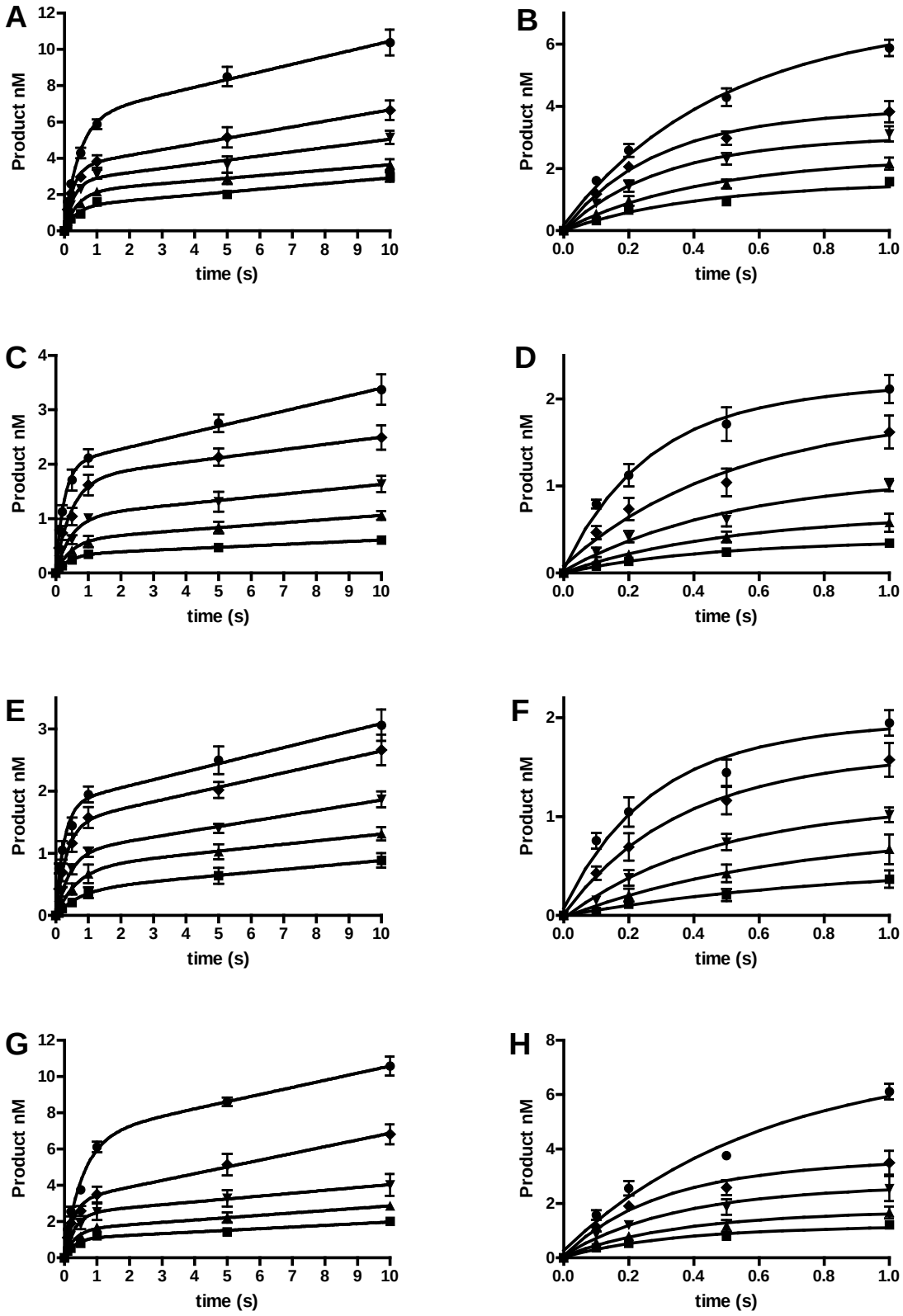

Figure S12 Time course for pol κ catalyzed insertion opposite O^2-Me-dT.
Pol κ (150 nM) and O^2-Me-dT DNA (15 nM) were reacted with 50 μM (square), 100 μM (up triangle), 200 μM (down triangle), 500 μM (diamond), and 1000 μM (circle), dATP (A, B), dCTP (C,D), dGTP (E,F), and dTTP (G,H). The data in B, D, F, and G represent early time points. The lines are the best fit to the burst equation. The data points are the mean of three experiments ± standard deviation.

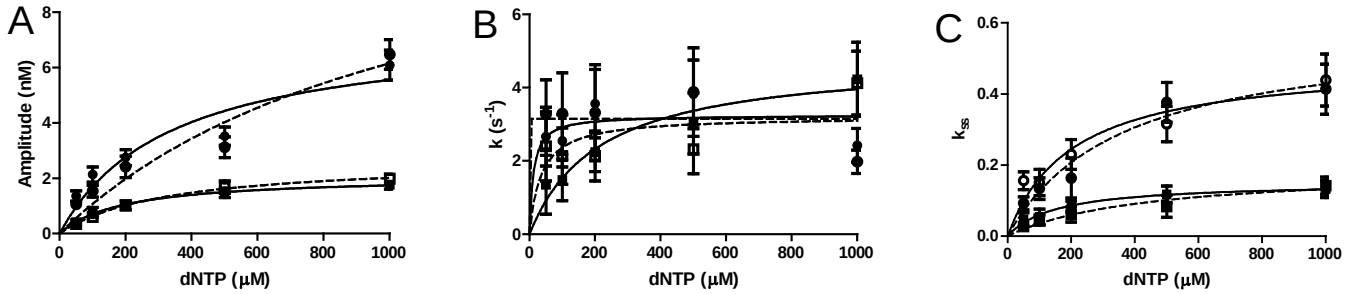

Figure S13 dNTP dependence for Pol κ catalyzed incorporation opposite O^2-Me-dT. The kinetic parameters derived from Figure S11 are plotted against dNTP concentration dATP (solid circle), dCTP (open square), dGTP (solid square), dTTP (open circle). The lines are the best fit to a hyperbolic equation for the purines (solid line) and pyrimidines (dashed line).

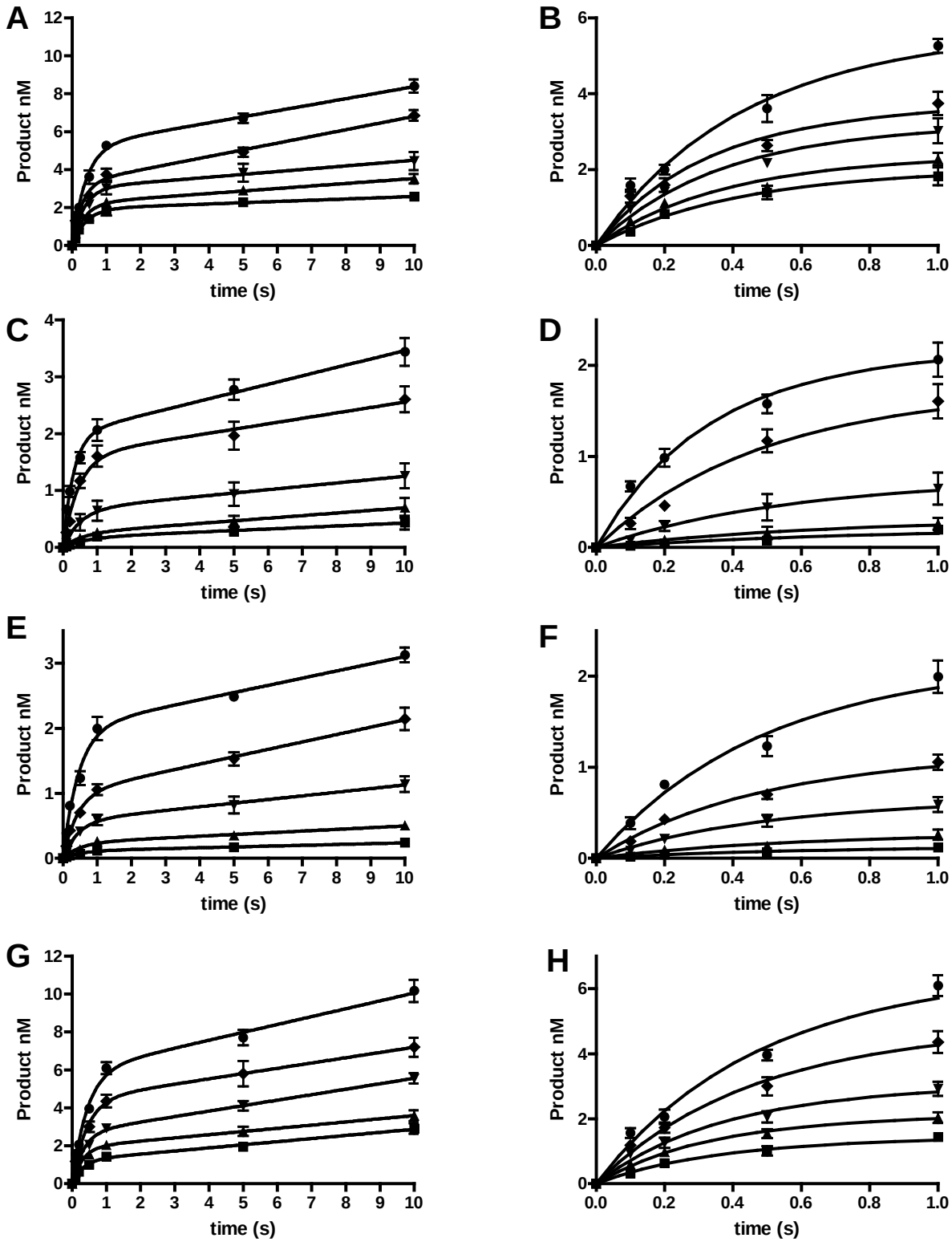

Figure S14 Time course for pol κ catalyzed insertion opposite O^2-POB-dT. Pol κ (150 nM) and O^2-POB-dT DNA (15 nM) were reacted with50 μM (square), 100 μM (up triangle), 200 μM (down triangle), 500 μM (diamond), and 1000 μM (circle), dATP (A, B), dCTP (C,D), dGTP (E,F), and dTTP (G,H). The data in B, D, F, and G represent early time points. The lines are the best fit to the burst equation. The data points are the mean of three experiments ± standard deviation.

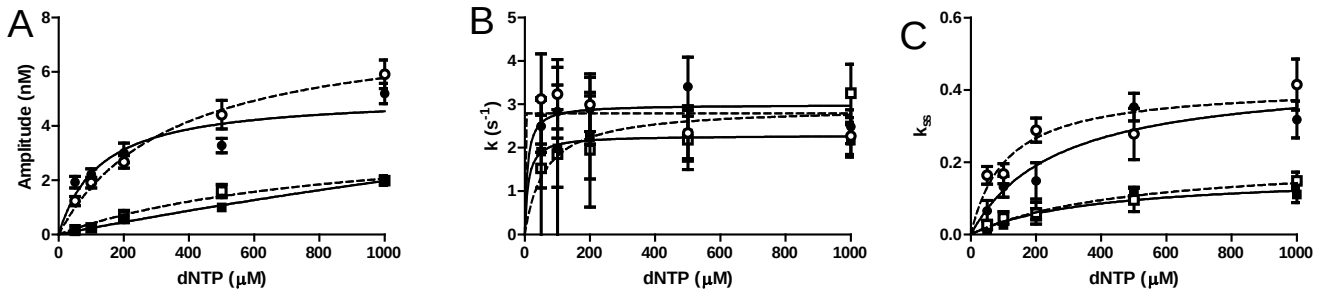

Figure S15 dNTP dependence for Pol κ catalyzed incorporation opposite O^2-POB-dT. dNTP dependence of time course parameters for insertion opposite O^2-POB-dT. The kinetic parameters derived from Figure S14 are plotted against dNTP concentration dATP (solid circle), dCTP (open square), dGTP (solid square), dTTP (open circle). The lines are the best fit to a hyperbolic equation for the purines (solid line) and pyrimidines (dashed line).

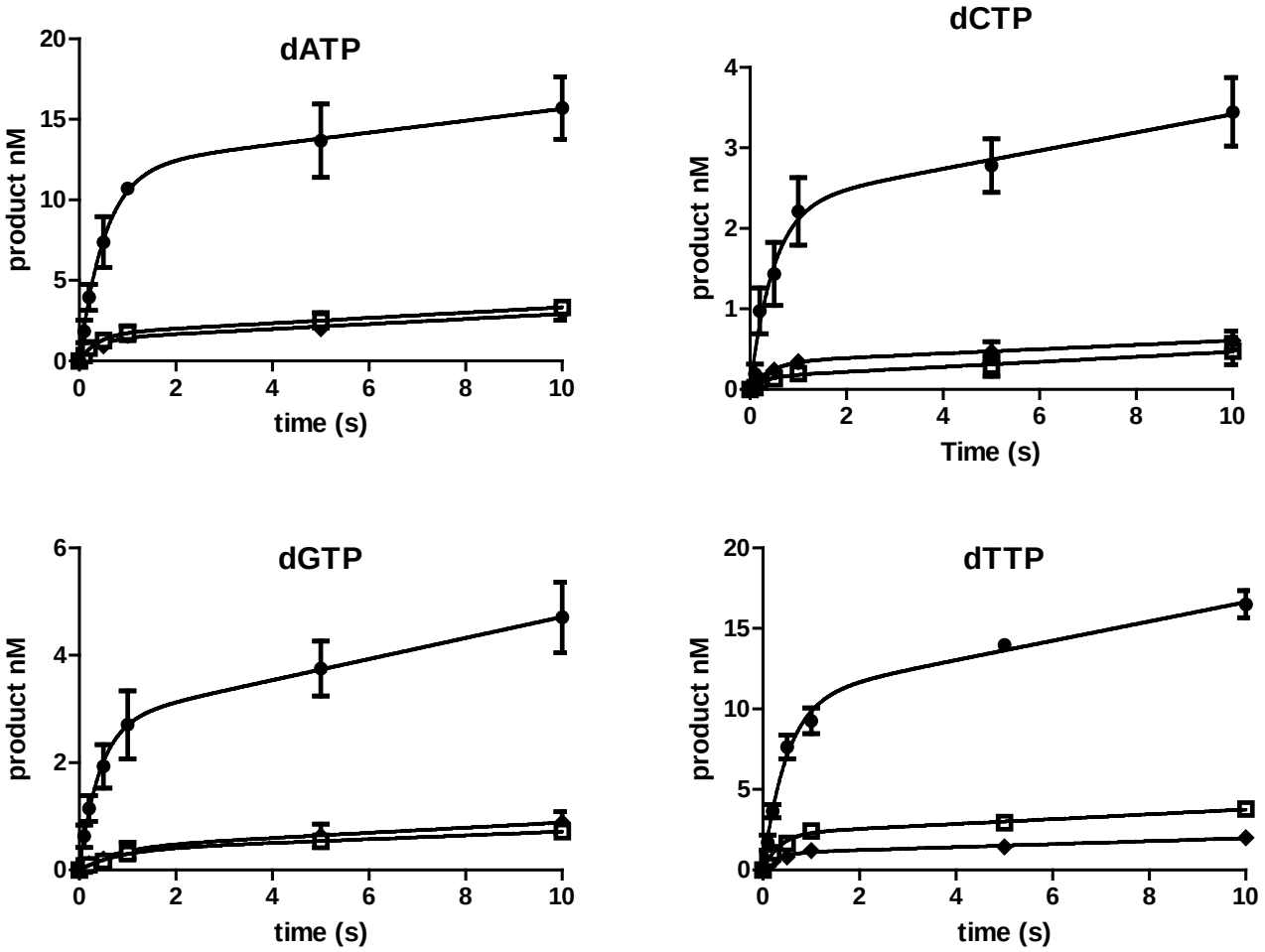

Figure S16. Differences in polymerase reactivity toward O^2-Me-dT. Pol η (solid circle), pol ι(open square), and pol κ (solid diamond) (25 nM) were reacted with 25 nM DNA, containing O^2-Me-dT, and 50 μM dATP(A), dCTP(B), dGTP (C), and dTTP (D).

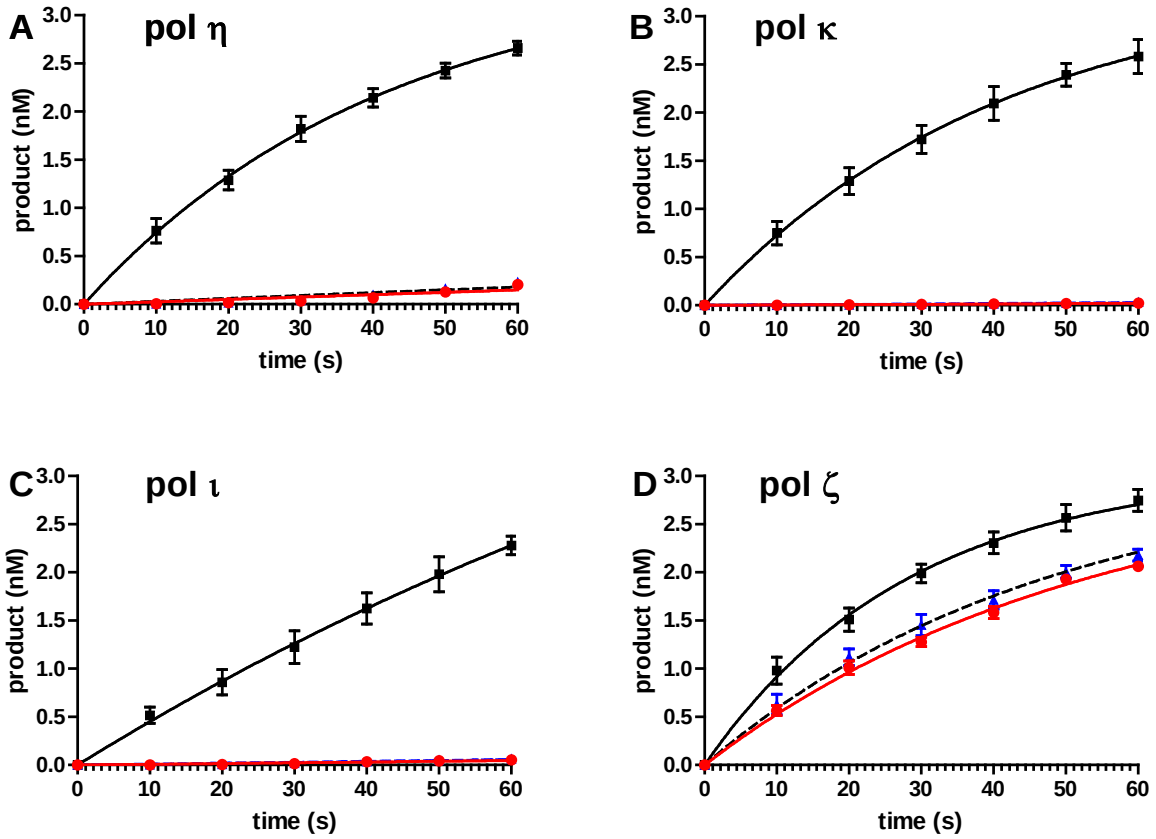

Figure S17. Relative bypass of O^2-alkyl-dT by human (A) pol η, (B) pol κ, (C) pol ι ,and (D) yeast pol ζ. The concentration of the next incoming dNTP (dCTP) was 50 μM, and the concentration of the DNA containing a dA/dT (black square), dA/O^2-Me-dT (blue triangle), and dA/O^2-POB-dT (red circle) base pair was 3 nM. The concentration of pol η was 0.05 nM and the concentrations of pol κ, and ι were 0.1 nM. The active concentration of active pol ζ is unknown, and was set to achieve a similar level of reaction as the other polymerases on the dA/dT DNA substrate. The data points are the mean ± standard deviation of three experiments. The lines are a best fit to a first-order equation.

P16(Y)	5′- G C A C C G C A G A C G C A G Y -3′
T24(X)	3′- C G T G G C G T C T G C G T C **X** G C A G C G T C -5'

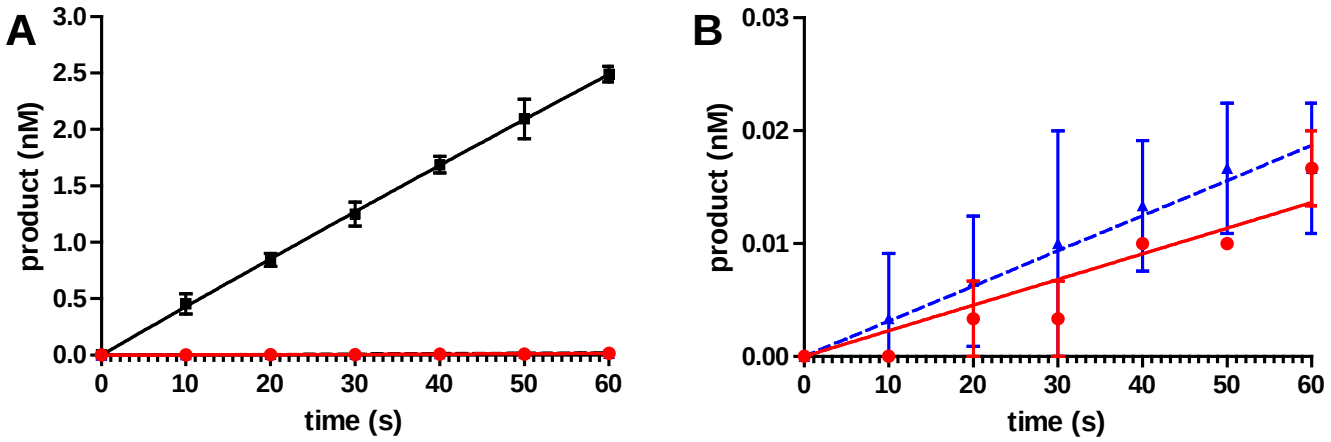

Figure S18. Relative insertion of dATP opposite dT (black square), O^2-Me-dT (blue triangle), and O^2-POB-dT (red circle) by yeast pol ζ. The dATP was 50 μM, and the concentration of the DNA was 3 nM. The data points are the mean ± standard deviation of three experiments. The lines are a best fit to a first-order equation.

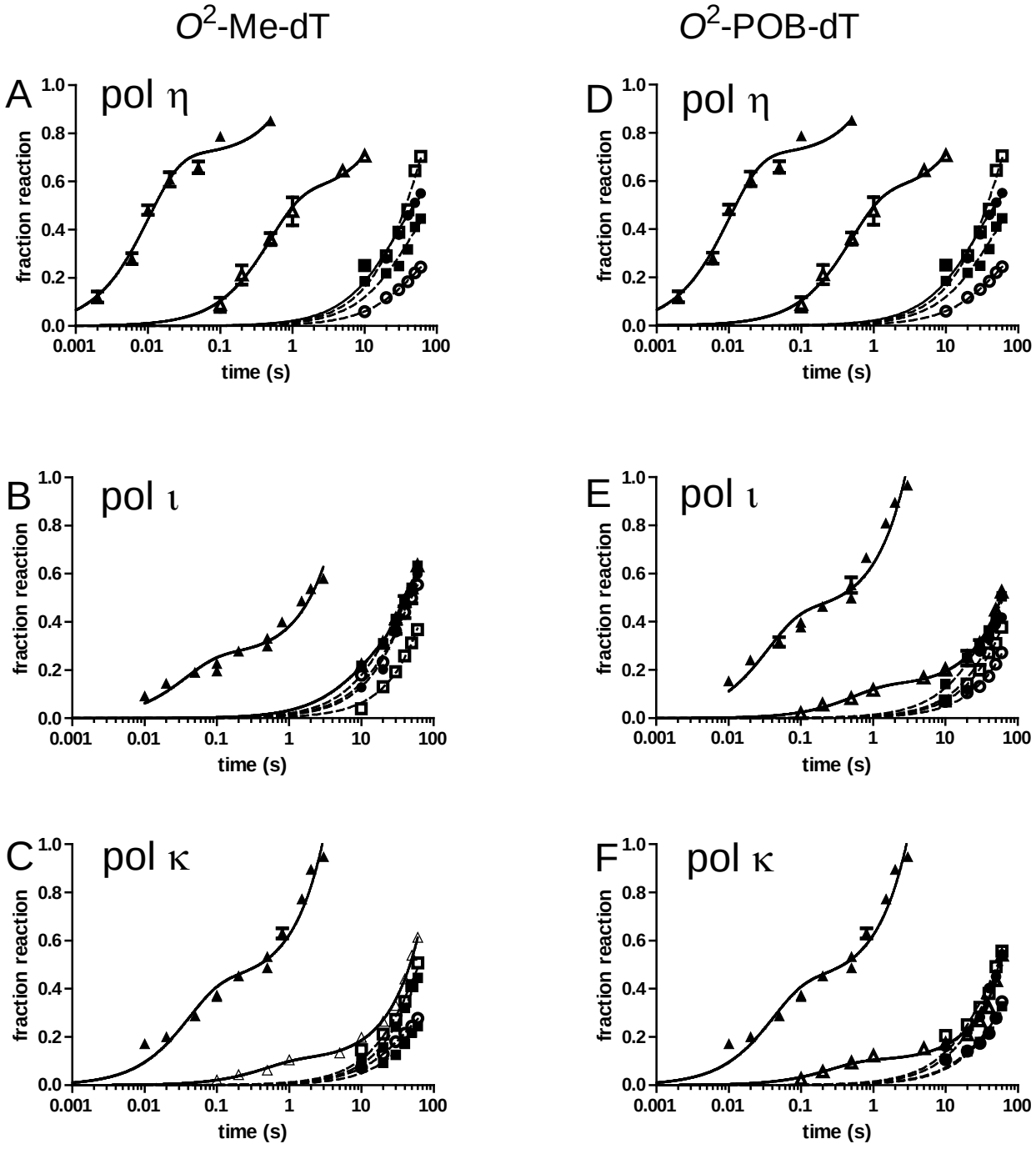

Figure S19. Comparisons of the relative extension reactivities of polymerases. The pol and O^2-alkyl-dT DNA pairing in each panel is as follows: (A), pol η and O^2-Me-dT; (B), pol η and O^2-POB-dT; (C), pol ι and O^2-Me-dT; (D), pol ι and O^2-POB-dT; (E), pol ι and O^2-Me-dT; (F), pol ι and O^2-POB-dT. The polymerase (30 nM) and DNA (3 nM) were reacted with 50 μM dNTP. Each panel shows the insertion of dATP opposite dT (black triangle) and O^2-alkyl-dT (open triangle) using P15/T24 DNA. The extension of dA (black square), dC (open circle), dG (black circle), and dT (open square) opposite O^2-alkyl-dT employ the P16/T24 DNA substrates . The solid lines are the best fit to the burst equation and the dashed lines are fit to a first-order equation.

P15-	5′- G C A C C G C A G A C G C A G -3′
P16(Y)	5′- G C A C C G C A G A C G C A G Y -3′
T24(X)	3′- C G T G G C G T C T G C G T C **X** G C A G C G T C -5'