Figure S1. Kinetic Parameters for POLRMT-Catalyzed Nucleotide Incorporation: Adenosine Analogs. (A) Correct AMP incorporation. POLRMT (0.125 μM) was incubated with 8 bp 2AP scaffold (0.1 μM) for 3 min and then rapidly mixed with ATP (5, 10, 25, 50 or 100 μM) using a stopped-flow. The observed change in fluorescence emission was measured and fit to a single exponential (Eq. 1), yielding $k_{obs}$ values of $4 \pm 1$, $7 \pm 1$, $15 \pm 1$, $20 \pm 1$ and $22 \pm 1 \text{ s}^{-1}$ for 5, 10, 25, 50 or 100 μM ATP, respectively. Values for $k_{obs}$ were plotted as a function of ATP concentration and fit to a hyperbola (Eq. 2), yielding a $k_{pol}$ value of $30 \pm 1 \text{ s}^{-1}$ and a $K_{d,app}$ value of $20 \pm 2 \mu M$. (B) 2′C-methyl-AMP misincorporation. POLRMT (0.5 μM) was incubated with $5^′$-32P-labeled-RNA/DNA 8 bp scaffold (0.1 μM) for 3 min and then rapidly mixed with 2′C-methyl-ATP (250, 500 or 1000 μM). Reactions were quenched at various times with EDTA (300 mM). Quantitated RNA product was plotted as a function of time and fit to a single exponential (Eq. 1) yielding values for $k_{obs}$ of $0.0093 \pm 0.0006$, $0.015 \pm 0.001$ and $0.019 \pm 0.001 \text{ s}^{-1}$ for 250 (●), 500 (○) or 1000 (■) μM 2′C-methyl-ATP, respectively. Values for $k_{obs}$ were plotted as a function of 2′C-methyl-ATP concentration and fit to a hyperbola (Eq. 2), yielding a $k_{pol}$ value of $0.030 \pm 0.010 \text{ s}^{-1}$ and a $K_{d,app}$ value of $530 \pm 100 \mu M$. (C) 7-deaza-AMP misincorporation. POLRMT (0.25 μM) was incubated with 8 bp 2AP scaffold (0.125 μM) for 3 min and then rapidly mixed with 7-deaza-ATP (25, 50, or 100 μM) using a stopped-flow. The observed change in fluorescence emission was measured and fit to a single exponential (Eq. 1), yielding $k_{obs}$ values of $3.91 \pm 0.22$, $5.56 \pm 0.26$, and $8.1 \pm 0.5 \text{ s}^{-1}$ for 25, 50, or 100 μM 7-deaza-ATP, respectively. Values for $k_{obs}$ were plotted as a function of 7-deaza-ATP concentration and fit to a hyperbola (Eq. 2), yielding a $k_{pol}$ value of $15 \pm 2 \text{ s}^{-1}$ and a $K_{d,app}$ value of $80 \pm 20 \mu M$. (D) 3-deaza-AMP misincorporation. POLRMT (0.5 μM) was incubated with $5^′$-32P-labeled-RNA/DNA 8 bp scaffold (0.1 μM) for 3 min and then rapidly mixed with 3-deaza-ATP (5, 50, 200 or 500 μM). Reactions were quenched at various times with EDTA (300 mM). Quantitated RNA product was plotted as a function of time and fit to a single exponential (Eq. 1) yielding values for $k_{obs}$ of $0.0020 \pm 0.0004$, $0.011 \pm 0.001$, $0.036 \pm 0.006$ and $0.057 \pm 0.007 \text{ s}^{-1}$ for 5 (●), 50 (○), 200 (■) or 500 (□) μM 3-deaza-ATP, respectively. Values for $k_{obs}$ were plotted as a function of 3-deaza-ATP concentration and fit to a hyperbola (Eq. 2), yielding a $k_{pol}$ value of $0.10 \pm 0.01 \text{ s}^{-1}$ and a $K_{d,app}$ value of $340 \pm 40 \mu M$. (E) 3′-dAMP misincorporation. POLRMT (0.25 μM) was incubated with 8 bp 2AP scaffold (0.125 μM) for 3 min and then rapidly mixed with 3′-dATP (2.5, 5, 12.5, 25, 50 or 75 μM) plus 1.25 μM single-strand DNA trap using a stopped-flow apparatus. The observed change in fluorescence emission was measured and fit to a single exponential (Eq. 1), yielding $k_{obs}$ values of $0.18 \pm 0.01$, $0.25 \pm 0.02$, $0.38 \pm 0.03$, $0.48 \pm 0.03$, $0.58 \pm 0.04$ and $0.60 \pm 0.04 \text{ s}^{-1}$ for 2.5, 5, 12.5, 25, 50 or 75 μM 3′-dATP, respectively. Values for $k_{obs}$ were plotted as a function of 3′-dATP concentration and fit to a hyperbola (Eq. 2), yielding a $k_{pol}$ of $0.70 \pm 0.02 \text{ s}^{-1}$ and a $K_{d,app}$ value of $8 \pm 1 \mu M$. (F) 6-methylpurine misincorporation. POLRMT (0.25 μM) was incubated with 8 bp 2AP scaffold (0.125 μM) for 3 min and then rapidly mixed with 6-methylpurine-TP (100, 250, 500 or 800 μM) using a stopped-flow. The observed change in fluorescence emission was measured and fit to a single exponential (Eq. 1), yielding $k_{obs}$ values of $8.2 \pm 0.2$, $12.2 \pm 0.5$, $15.7 \pm 0.5$ and $19.5 \pm 0.5 \text{ s}^{-1}$ for 100, 250, 500 or 800 μM 6-methylpurine-TP, respectively. Values for $k_{obs}$ were plotted as a function of 6-methylpurine-TP concentration and fit to a hyperbola (Eq. 2), yielding a $k_{pol}$ value of $30 \pm 5 \text{ s}^{-1}$ and a $K_{d,app}$ value of $280 \pm 10 \mu M$. (G) ribavirin misincorporation. POLRMT (0.5 μM) was incubated with $5^′$-32P-labeled-RNA/DNA 8 bp scaffold (0.1 μM) for 3 min and then rapidly mixed with ribavirin-TP (250, 500, 1000 or 3000 μM). Reactions were quenched at various times with EDTA (300 mM). Quantitated RNA product was plotted as a function of time and fit to a single exponential (Eq. 1) yielding values for $k_{obs}$ of $0.00098 \pm 0.00006$, $0.0017 \pm 0.0004$, $0.0027 \pm 0.0001$ and $0.0036 \pm 0.0001 \text{ s}^{-1}$ for 250 (●), 500 (○), 1000 (■) or 3000 (□) μM ribavirin-TP, respectively. Values for $k_{obs}$ were plotted as a function of ribavirin-TP concentration and fit to a hyperbola (Eq. 2), yielding a $k_{pol}$ value of $0.0050 \pm 0.0010 \text{ s}^{-1}$ and a $K_{d,app}$ value of $800 \pm 100 \mu M$. 