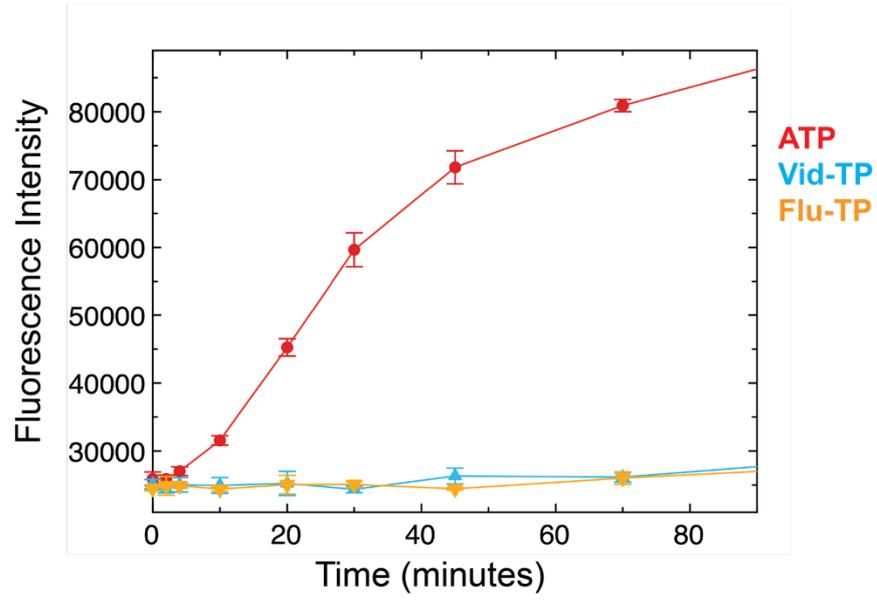
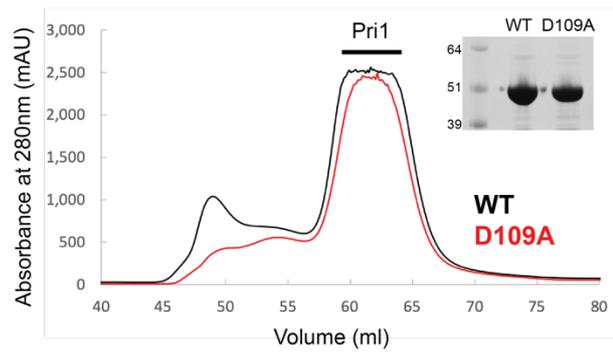
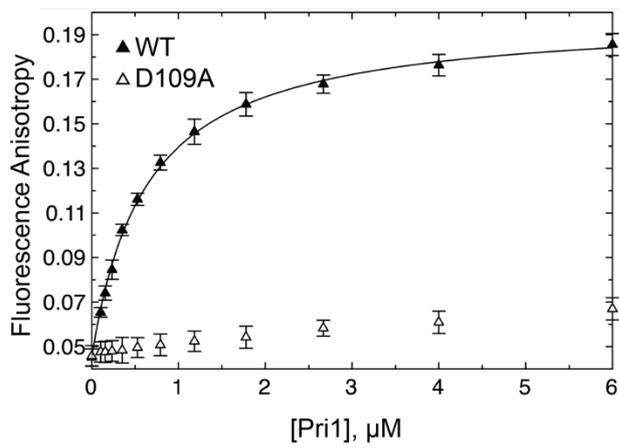
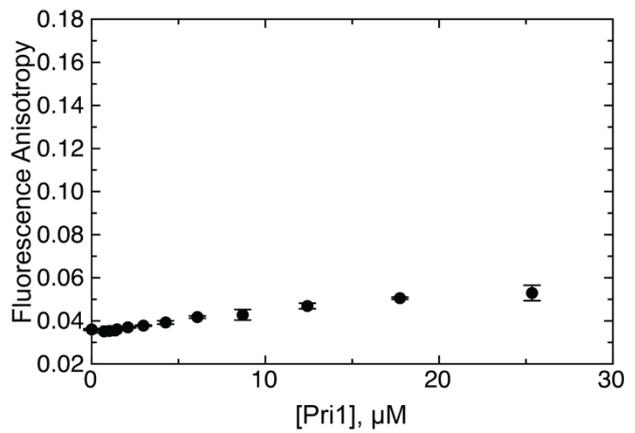
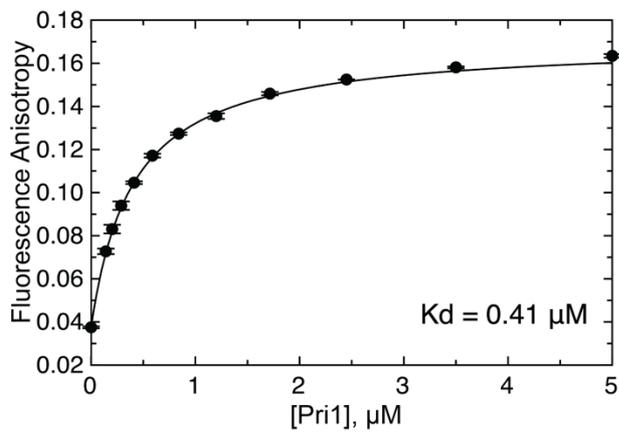


Structural basis for inhibition of human primase by arabinofuranosyl nucleoside analogues fludarabine and vidarabine

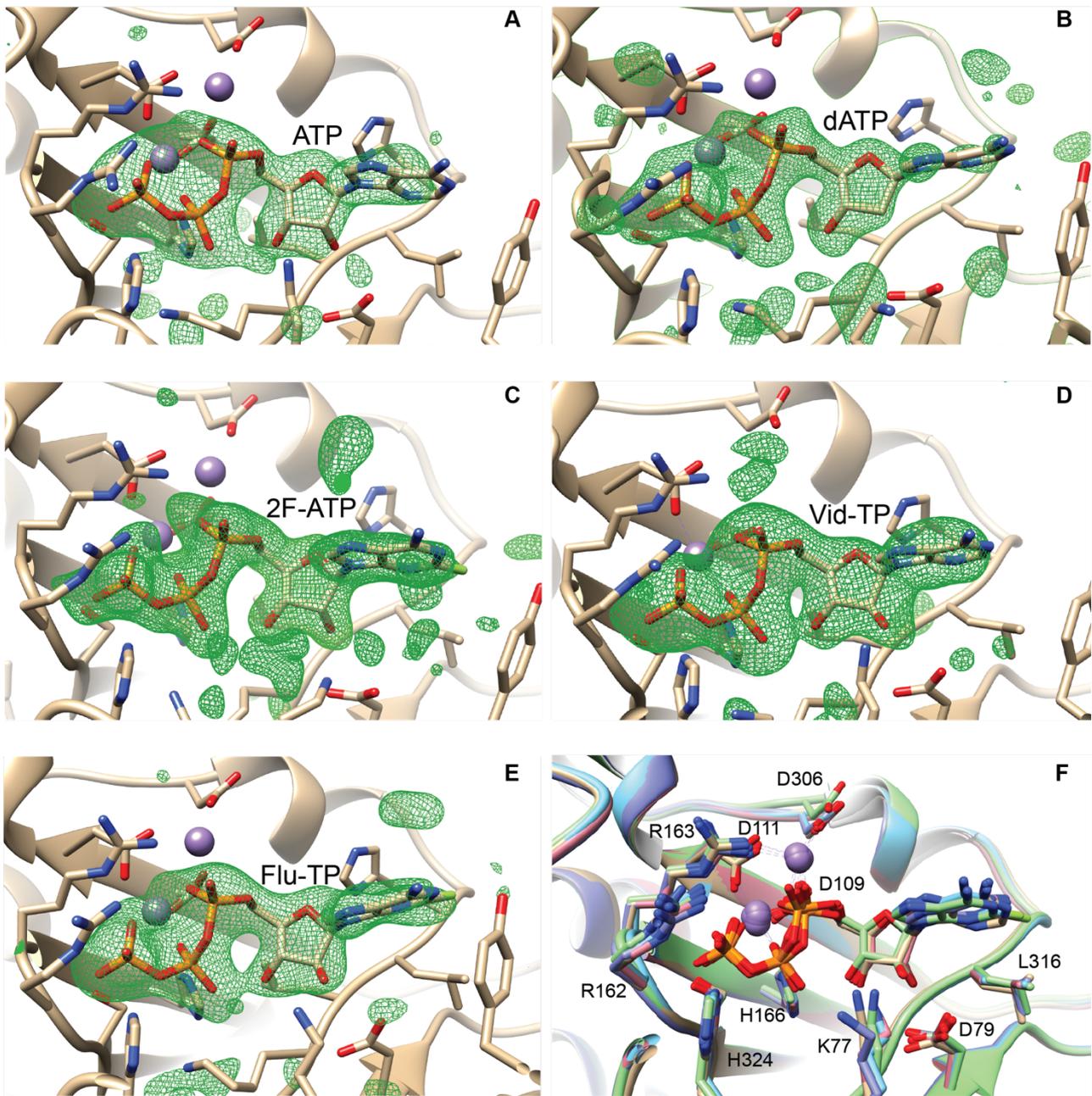
Sandro Holzer¹, Neil J. Rzechorzek¹, Isobel R. Short¹, Michael Jenkyn-Bedford¹, Luca Pellegrini¹, Mairi L. Kilkenny^{1*}

¹ Department of Biochemistry, University of Cambridge, Cambridge, CB2 1GA, UK.

* To whom correspondence should be addressed: Mairi L. Kilkenny, Department of Biochemistry, University of Cambridge, 80 Tennis Court Road, Cambridge, CB2 1GA, U.K.; Phone: +44 1223 333652; E-mail: mlk26@cam.ac.uk

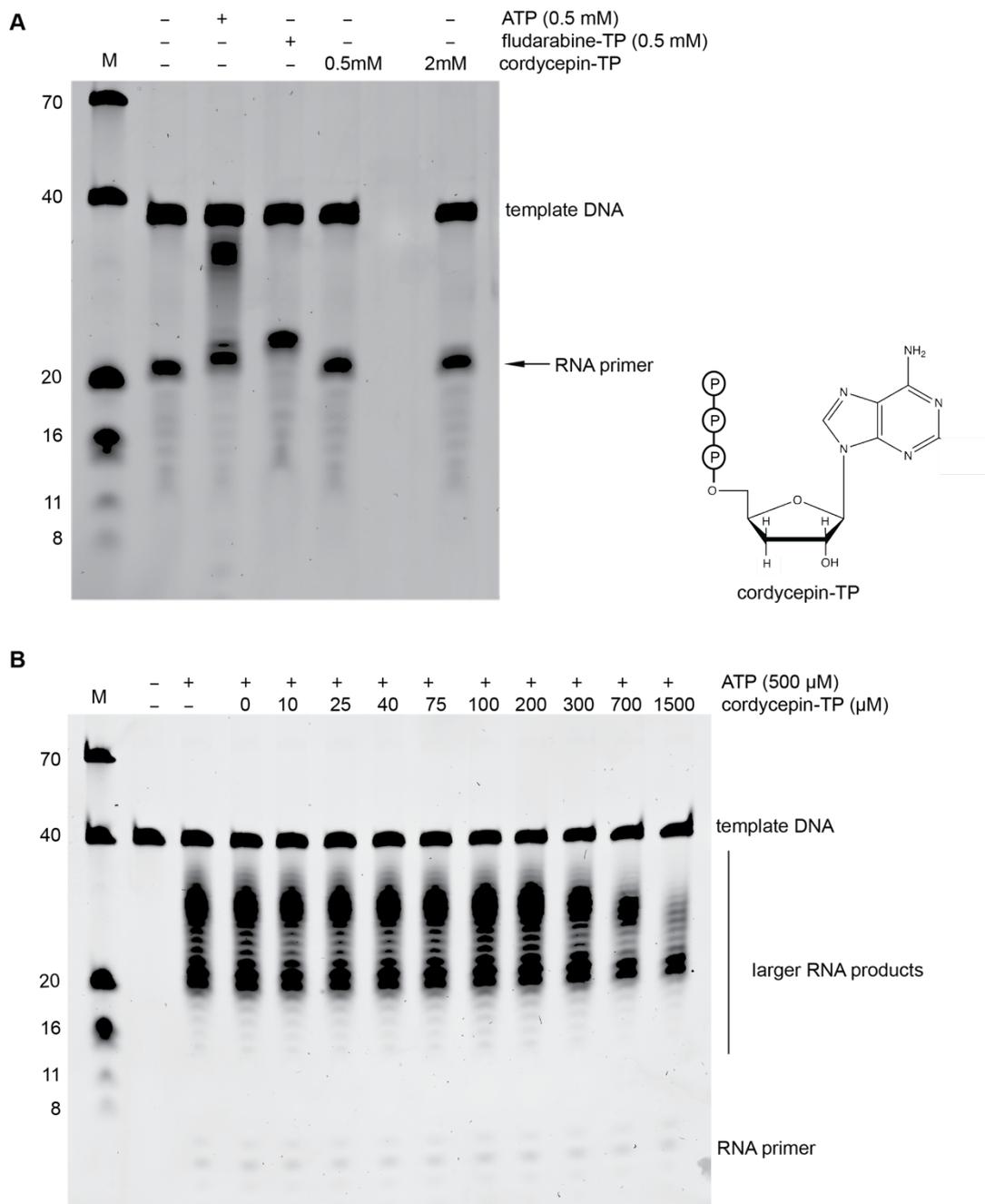
A**B****C**

Supplementary Figure 1 The effect of divalent metals on primase activity and nucleotide binding. a) Fluorescence-based RNA primer synthesis assay on a ssDNA template (5'-GTTGTCCATTATGTCCTACCTCGTGCTCCT) in the presence of 5 mM Mg^{2+} ions and equimolar concentrations of ribonucleotides (20 μ M each rNTP) and the indicated nucleotide analogue (20 μ M). Curves are colored as follows: ATP (red), vidarabine-TP (light blue), fludarabine-TP (orange). b) Fluorescence polarization was used to determine the binding affinity of a Pri1 mutant (WT – black triangles, D109A point mutant – open triangles) for 6FAM-ATP in the presence of 1 mM Mn^{2+} ions (left panel). The D109A mutation removes one of the metal-coordinating side-chains and thus prevents nucleotide binding to the Pri1 elongation site. The fact that this point mutant no longer binds 6FAM-ATP indicates that the binding is specific to the elongation site and the 6FAM label does not mediate non-specific binding to Pri1. Both WT Pri1 and the D109A point mutant eluted at the same volume from a Superdex S75 size exclusion column (GE Healthcare), indicating that the D109A mutant is likely properly folded (right panel). Coomassie-stained SDS-PAGE gel of final, purified proteins is shown as inset panel. c) Fluorescence polarization was used to determine the binding affinity of WT Pri1 for 6FAM-ATP in the presence of 1 mM Mn^{2+} ions ($K_d = 0.41 \mu$ M) (left panel), and 1 mM Mg^{2+} ions (right panel). Each data point represents the mean \pm s.d. (n=3).



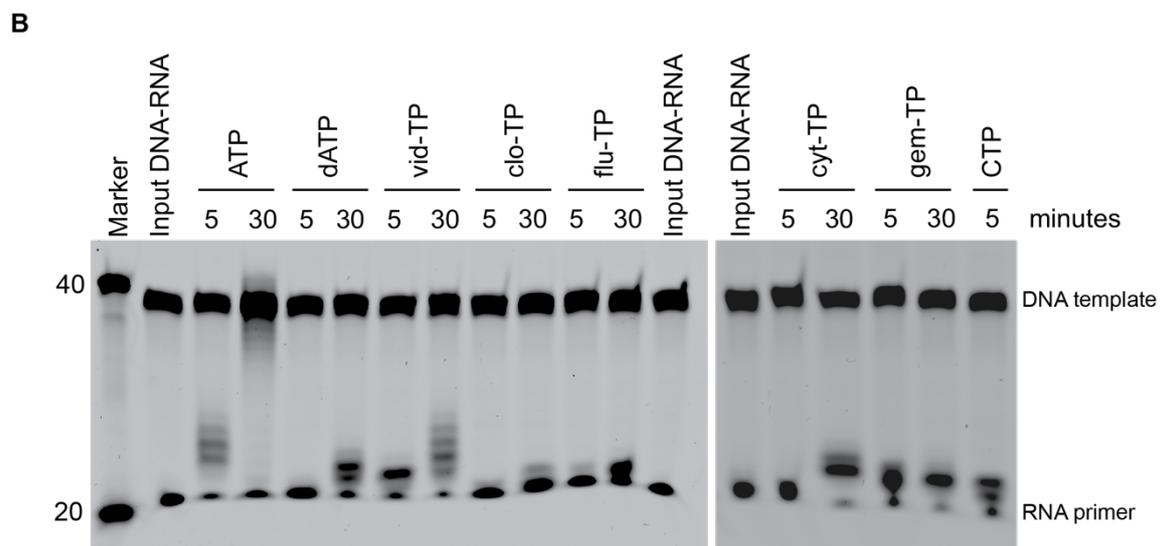
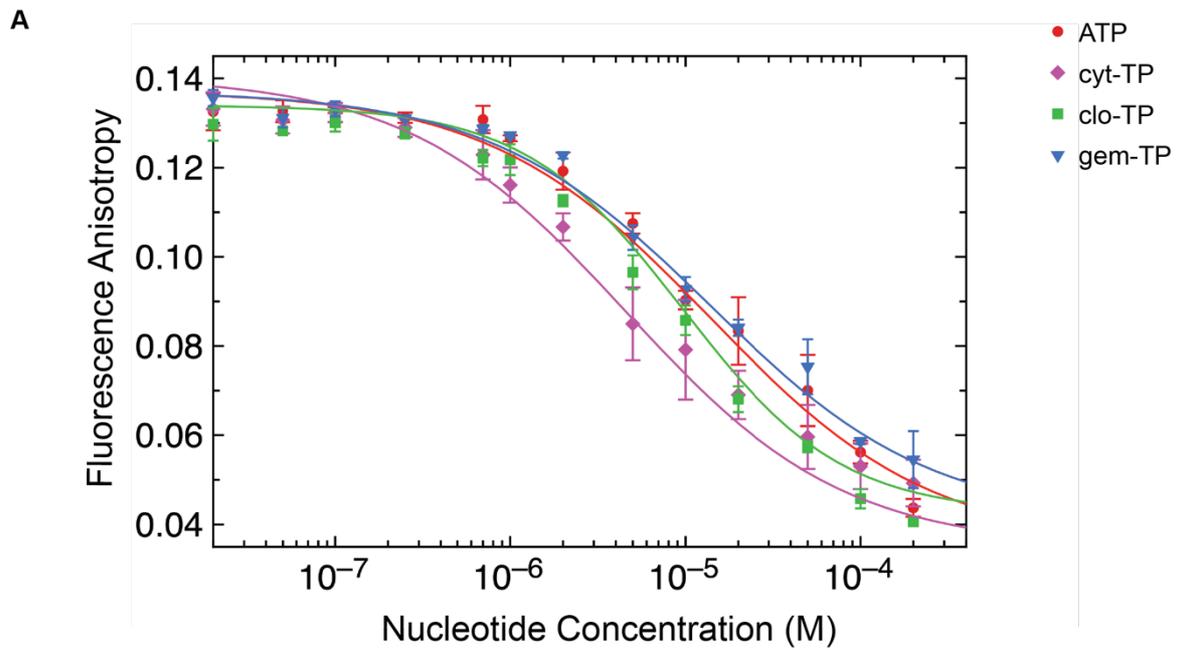
Supplementary Figure 2 Nucleotide binding to the elongation site of Pri1. Fo-Fc omit maps (calculated by omitting the nucleotide) are shown for the (a) ATP, (b) dATP, (c) 2F-ATP, (d) vidarabine-TP and (e) fludarabine-TP structures (Fo-Fc maps are contoured at 3.0 σ). f) Superposition of the crystal structures of Pri1 bound to ATP (beige), dATP (purple), 2F-ATP (pink), vidarabine-TP (green) and fludarabine-TP

(blue). Key nucleotide- and metal-binding side-chains are shown. Mn^{2+} ions are shown as purple spheres. All images were generated using Chimera.⁵³



Supplementary figure 3 Cordycepin-TP is poorly incorporated into an RNA primer and only inhibits primase activity at relatively high concentrations. a) Denaturing gel showing incorporation of cordycepin or fludarabine into an existing RNA primer. The template comprised a 38-mer DNA (5'-T₂₀CCAGAGAGCGCCCAAACG) annealed to an 18-mer RNA (5'-CGUUUGGGCGCUCUCUGG). Reactions contained 0.5 μM

template, 0.5 μM primase, 10 mM $\text{Mg}(\text{OAc})_2$ and 500 μM of either ATP, fludarabine-TP or cordycepin-TP. Reactions were incubated at 37 $^\circ\text{C}$ for 30 minutes, and the products were analyzed by denaturing polyacrylamide gel electrophoresis. b) Denaturing gel showing the effect of cordycepin-TP on RNA primer synthesis. Reactions contained 0.5 μM polydT40 ssDNA template, 0.5 μM primase, 500 μM ATP, 10mM $\text{Mg}(\text{OAc})_2$ and the indicated concentration of cordycepin-TP. Reactions were incubated and analyzed as in (a). All gels were post-stained with Sybr Gold. M = marker.



DNA template: (T)₁₆TTTCCAGAGAGCGCCCAAACG
 RNA primer: GGUCUCUCGCGGUUUGC

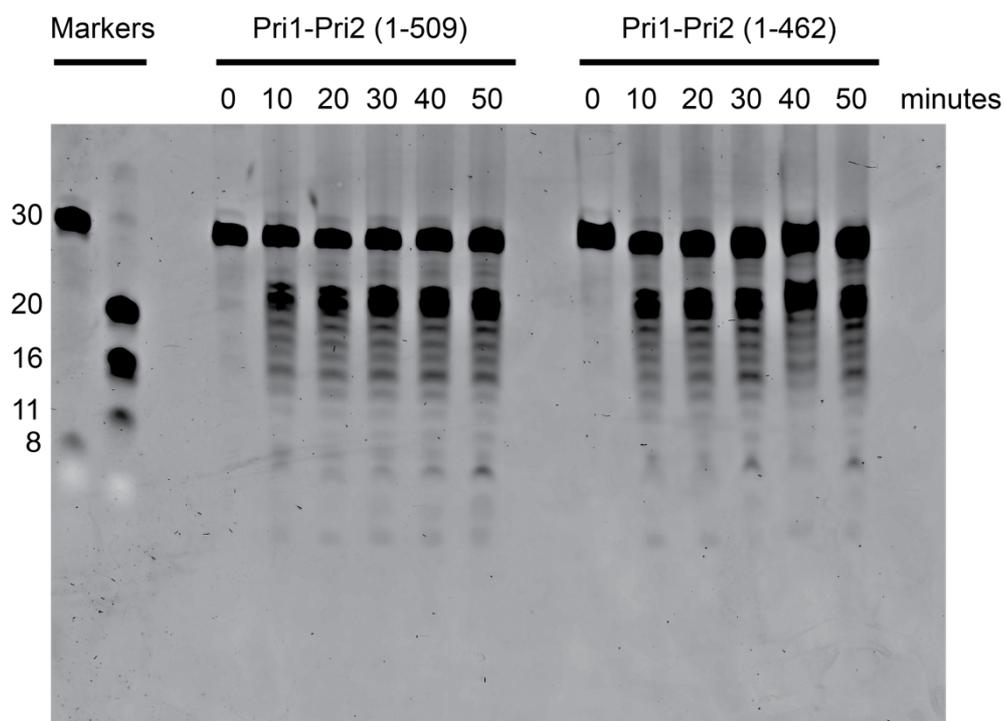
ATP analogues

DNA template: (T)₁₆GGGCCAGAGAGCGCCCAAACG
 RNA primer: GGUCUCUCGCGGUUUGC

CTP analogues

Supplementary figure 4 The effect of cytarabine-TP, clofarabine-TP and gemcitabine-TP on RNA primer synthesis. a) FP-based competition binding experiment in which 6FAM-ATP (30 nM) in the presence of excess Pri1 (1.5 μM) was

challenged with increasing concentrations of the indicated nucleotide (ATP) or nucleotide analogue (cyt-TP: cytarabine triphosphate, gem-TP: gemcitabine triphosphate, clo-TP: clofarabine triphosphate). Each data point represents the mean \pm s.d. (n=3, except cyt-TP for which n=6). b) Denaturing gel showing the incorporation of nucleotide analogues into an existing RNA primer. The template comprised a 38-mer DNA template (5'-T₁₆TTTTCCAGAGAGCGCCCAAACG) annealed to an 18-mer RNA (5'-CGUUUGGGCGCUCUCUGG). For the cytosine analogues (cytarabine-TP and gemcitabine-TP) the DNA template was instead 5'-T₁₆GGGGCCAGAGAGCGCCCAAACG. Reactions contained 0.5 μ M annealed DNA-RNA template, 0.5 μ M primase, 10 mM Mg(OAc)₂, and 500 μ M of either ATP, dATP, vid-TP, clo-TP, flu-TP, cyt-TP or gem-TP. Reactions were allowed to proceed for 5 or 30 minutes at 37 °C before quenching and subsequent separation of the products on an 18% polyacrylamide-urea gel. Gels were post-stained with Sybr Gold. M = marker.



Supplementary figure 5 A C-terminal truncation does not affect the priming activity of human primase. Denaturing gel showing the time-course of RNA primer synthesis on a 30-mer ssDNA template by Pri1(1-420)-Pri2(1-509) and Pri1(1-420)-Pri2(1-462). Primer products were visualized by post-staining the urea-polyacrylamide gel with Sybr Gold.