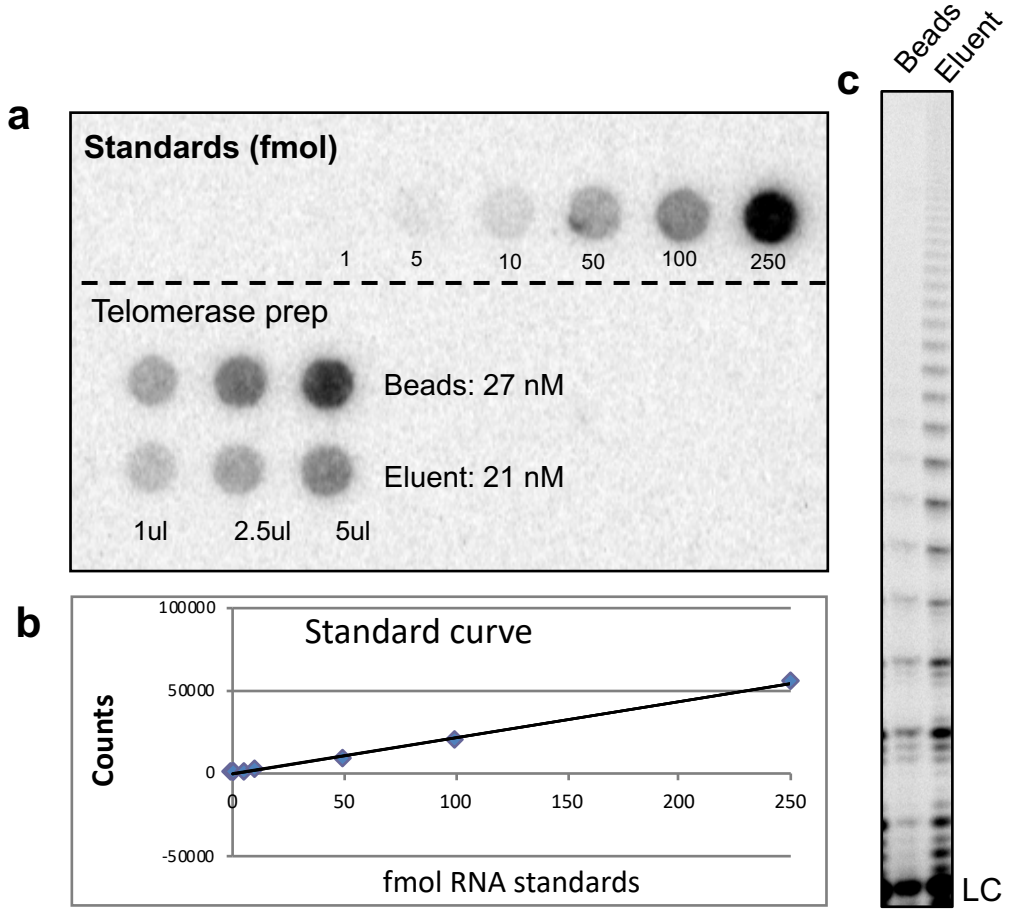
Supplementary Information for

Mechanisms of telomerase inhibition by oxidized and therapeutic dNTPs.

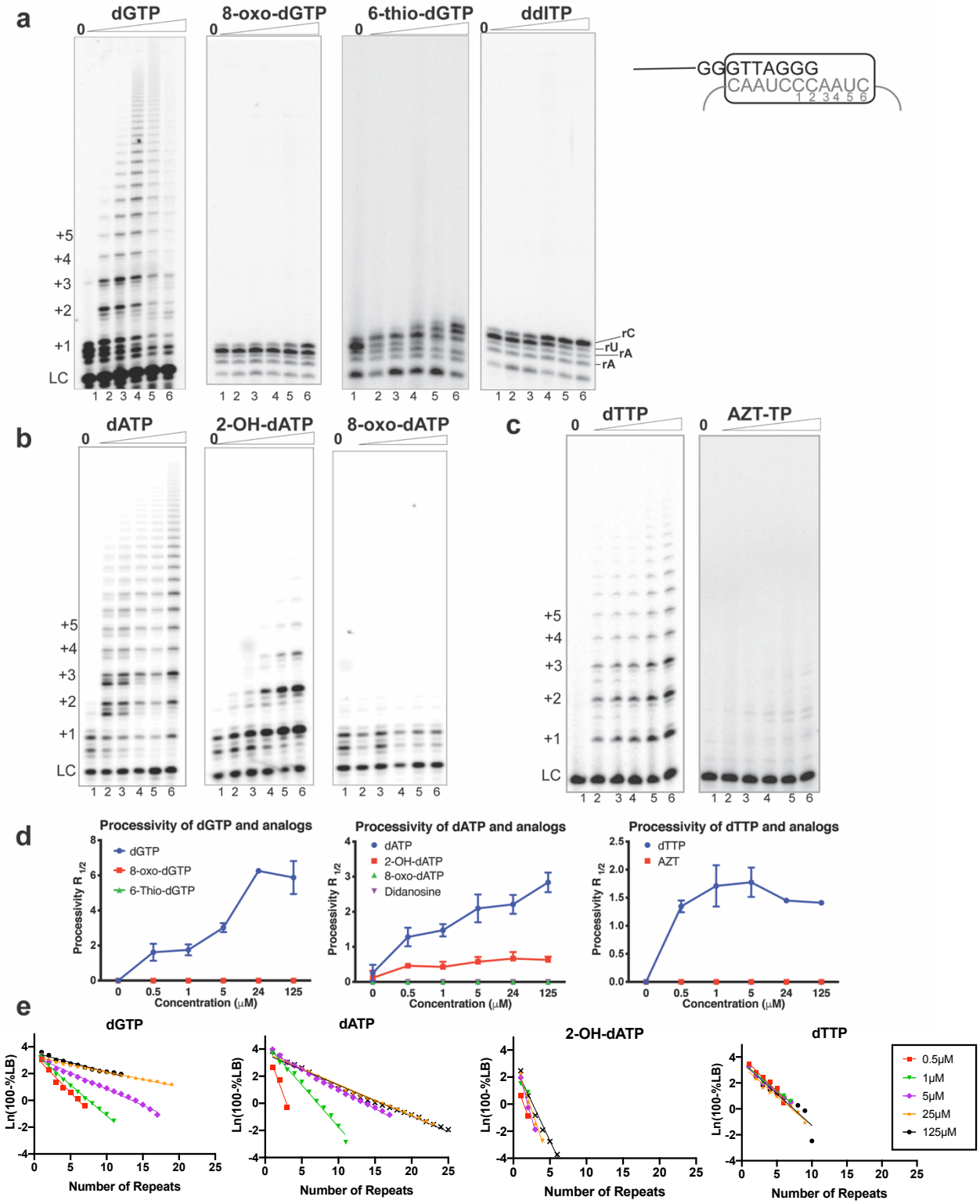
Sanford et al.

*Corresponding author. Email: plo4@pitt.edu

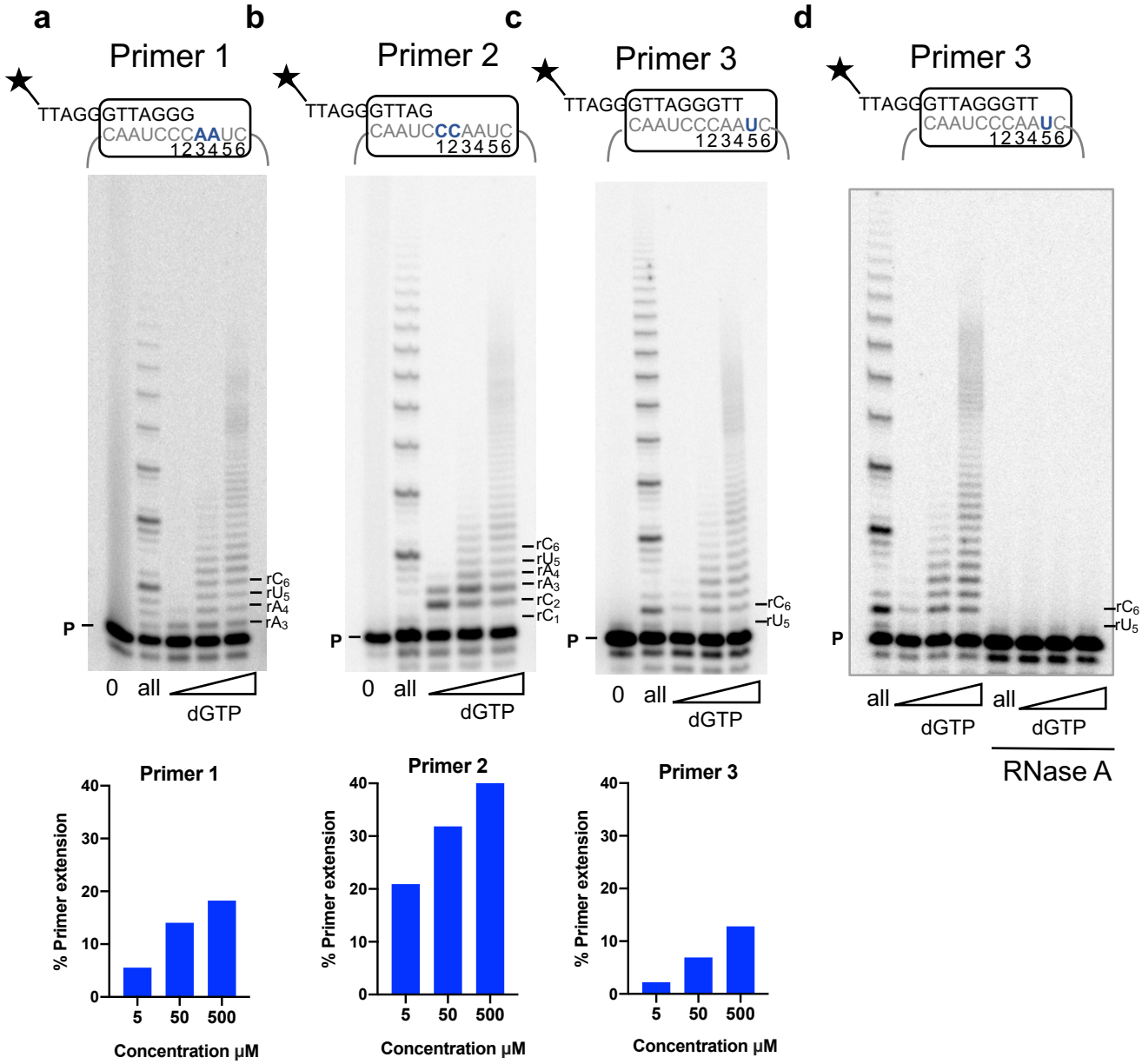
The PDF file includes:



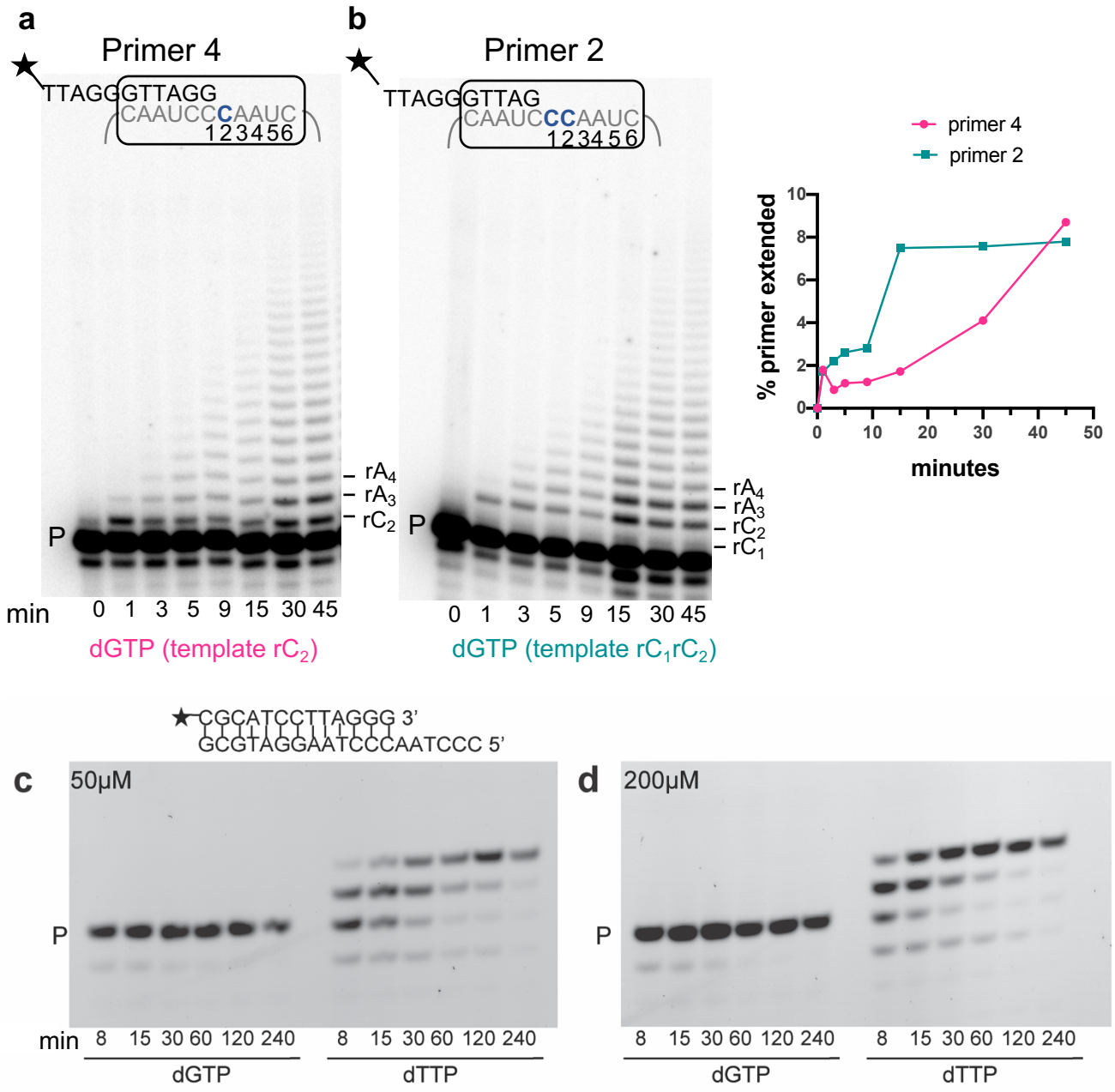
Supplementary Fig 1. Quantification of immunopurified telomerase-protein-RNA complexes. (a) Telomerase was over expressed in HEK293T cells and immunopurified (IP) using a FLAG tag on the N-terminus of TERT. The beads or eluate from IP reactions were spotted on a blot and probed with a ^{32}P labeled probe against hTR to determine the concentrations of RNA that co-purified with TERT. 1, 2.5, and 5 µL aliquots were compared with *in vitro* transcribed hTR standards from 0.5 to 250 fmol. Concentrations are shown. (**b**) Standard curve of hTR standards to determine concentration of telomerase. (**c**) Telomerase primer extension assays. Telomerase activity from beads and eluent preparations were compared using a $(TTAGGG)_3$ primer. LC indicated 18-mer loading control. Images represent dot blot for one telomerase prep. Source data are provided as a Source Data file.



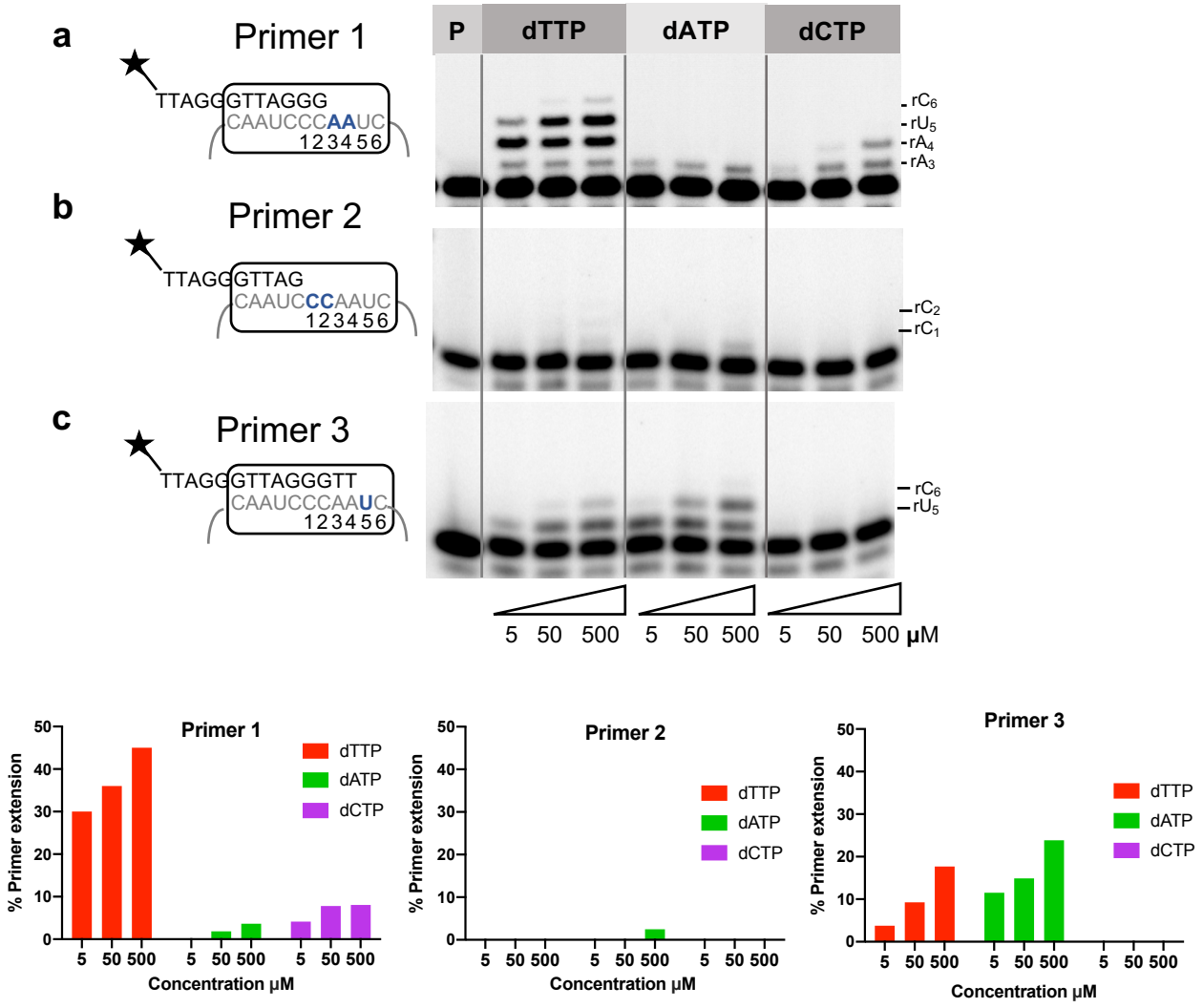
Supplementary Fig 2.Telomerase dNTP titrations and processivity calculations. Telomerase reactions were conducted with $(TTAGGG)_3$ primer 1 and cellular-concentration dNTPs except that the indicated natural or modified dNTP was added at increasing concentrations (0, 0.5, 1, 5, 25, and 125 μM) along with either 0.3 μM [α-^{32}P]dTTP (**a**) or [α-^{32}P] dGTP (**b, c**). The loading control (LC) was a ^{32}P-end labeled 18-mer. Numbers on the left indicate the number of added repeats. **(d)** Processivity was calculated as $R_{1/2}$ (see Methods). (**e**) The natural log of (100-%LB) was plotted vs repeat number. A straight line was fit to the data and the $R_{1/2}$ was calculated by dividing –ln(2) by the slope of each line. Plots shown from one experiment. Images (**a-c**) and plots (**e**) are representative of, and data are mean ± s. d. (**d**) from, three independent experiments. Source data are provided as a Source Data file.



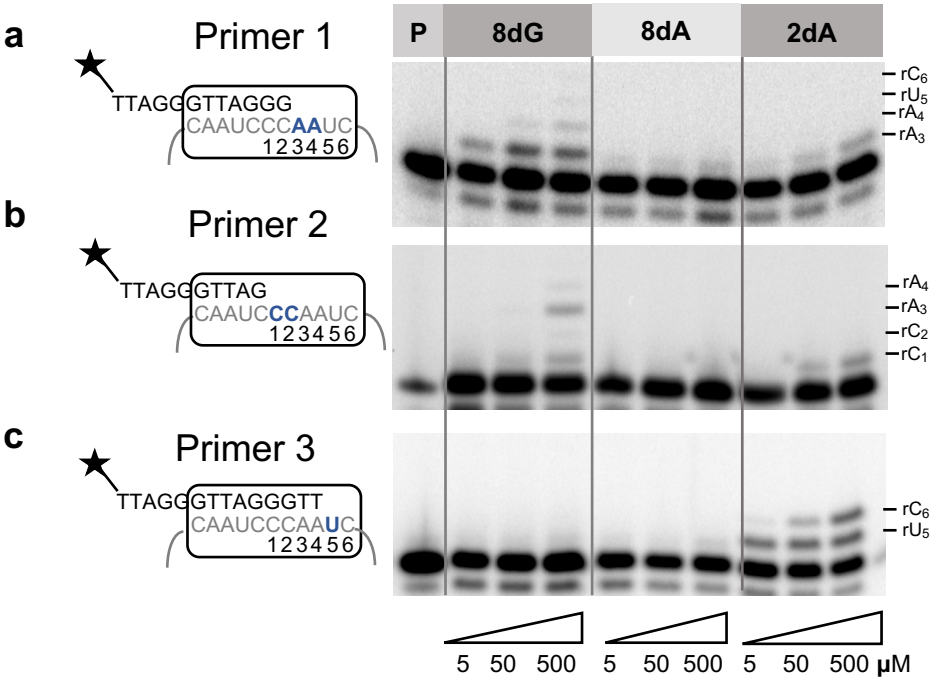
Supplementary Fig 3.Telomerase extension with dGTP. Telomerase reactions were conducted with 5 nM ^{32}P-end labeled primer (**a**) Primer 1 $(TTAGGG)_3$, (**b**) Primer 2 $(GGTTAG)_3$, or (**c** and **d**) Primer 3 $(AGGGTT)_3$. Reactions contained cellular-concentration dNTPs (all) or 5, 50, or 500 µM dGTP. In panel (**d**) reactions contained 3 µg/µl RNase A. Products were separated on denaturing gels. Letters on the right indicate the template base; P indicates unextended 18-mer primer. Graphs represent the percent of total primers extended for the reactions in panels **a** – **c** from one experiment. Panel **d** is a representative image from three independent experiments. Source data are provided as a Source Data file.

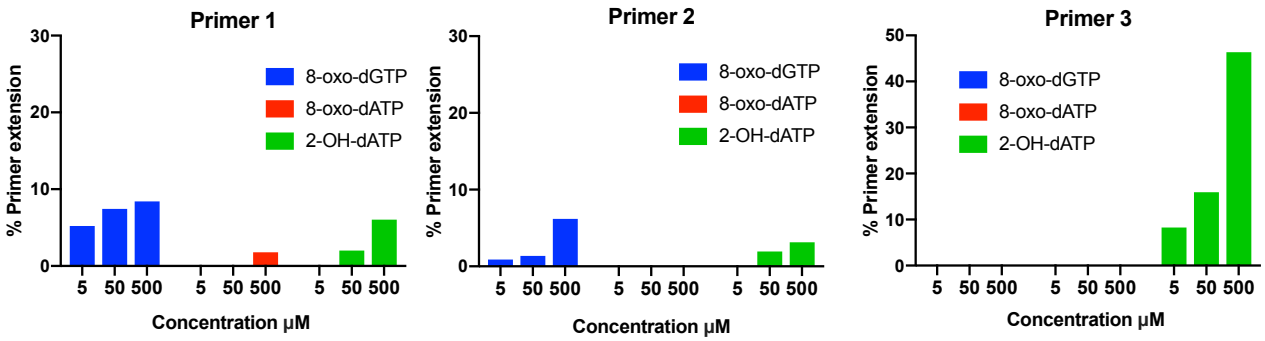


Supplementary Fig 4. Time course reactions with telomerase or pol β. (a, b) Telomerase reactions were conducted with 5 nM ^{32}P-end labeled primer (**a**) Primer 4 $(GTTAGG)_3$ or (**b**) Primer 2 $(GGTTAG)_3$. Reactions contained 50 μM dGTP and were terminated at 0, 1, 3, 5, 9, 15, 30, or 45 minutes as indicated. Products were separated on denaturing gels. Letters on the right indicate the template base; P indicates unextended 18-mer primer. Graphs represent the percent primer extended for the reactions in panels **a** and **b** from one experiment. (**c, d**) Primer extension by human polymerase β. The double stranded 6-FAM labeled primer-template and polymerase β were incubated with either 50 μM dGTP or dTTP (**c**), or 200 μM dGTP or dTTP (**d**) for time ranging from 8-240 minutes as indicated. The products of the reactions were run on denaturing gels and imaged using a Typhoon phosphoimager. P indicates unextended 13-mer primer from three independent experiments. Source data are provided as a Source Data file.

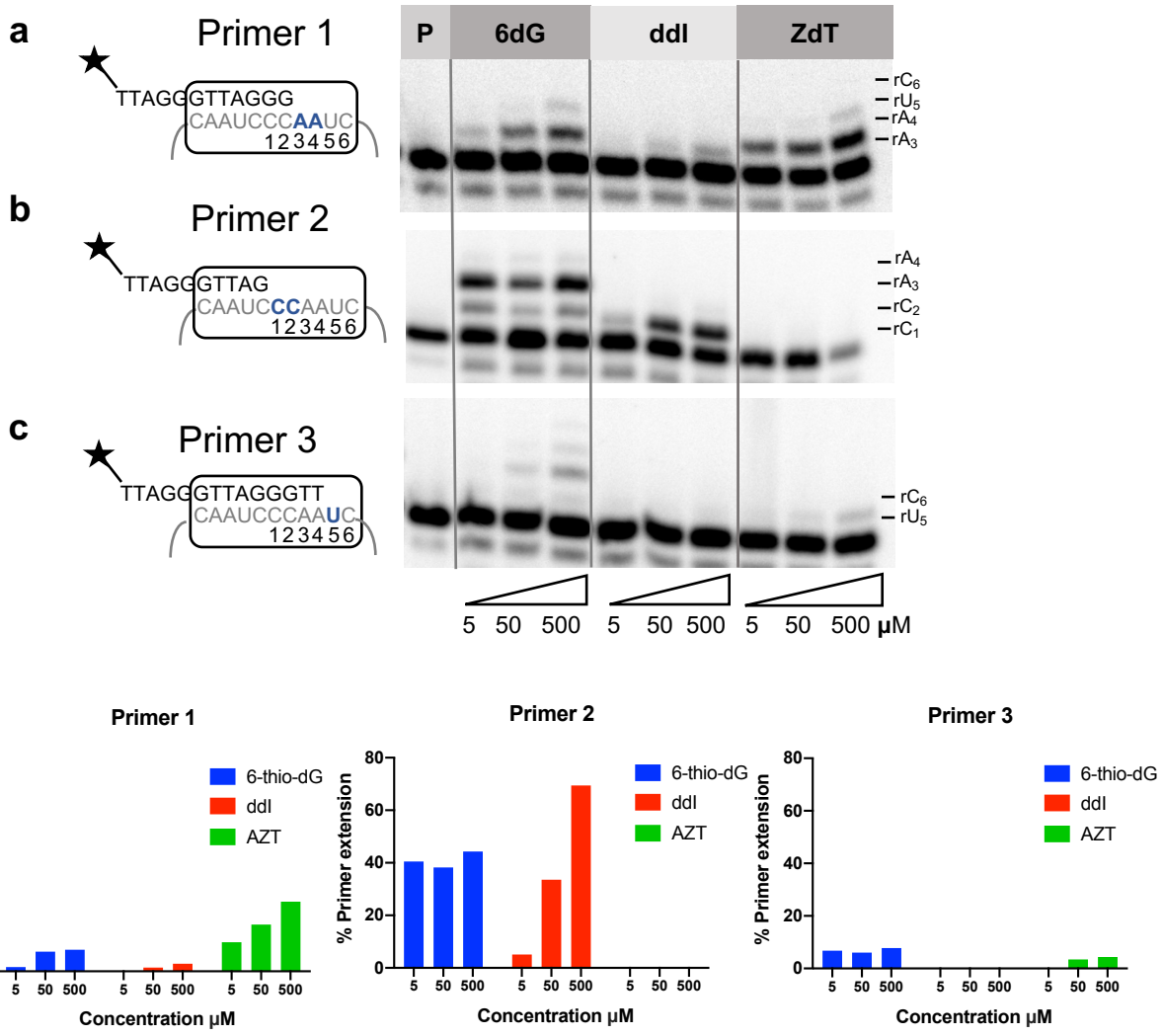


Supplementary Fig 5. Telomerase extension with natural dNTPs. Telomerase reactions were conducted with 5 nM ^{32}P-end labeled primer (**a**) Primer 1 $(TTAGGG)_3$, (the box around dTTP reactions indicates image from a separate gel) (**b**) Primer 2 $(GGTTAG)_3$, or (**c**) Primer 3 $(AGGGTT)_3$. Reactions contained 5, 50, and 500 μM dTTP, dATP, or dCTP as indicated. Products were separated on denaturing gels. Letters on the right indicate the template base; P indicates unextended 18-mer primer. Graphs represent the percent of total primers extended for the reactions in panels **a**, **b**, and **c** from one experiment. Source data are provided as a Source Data file.

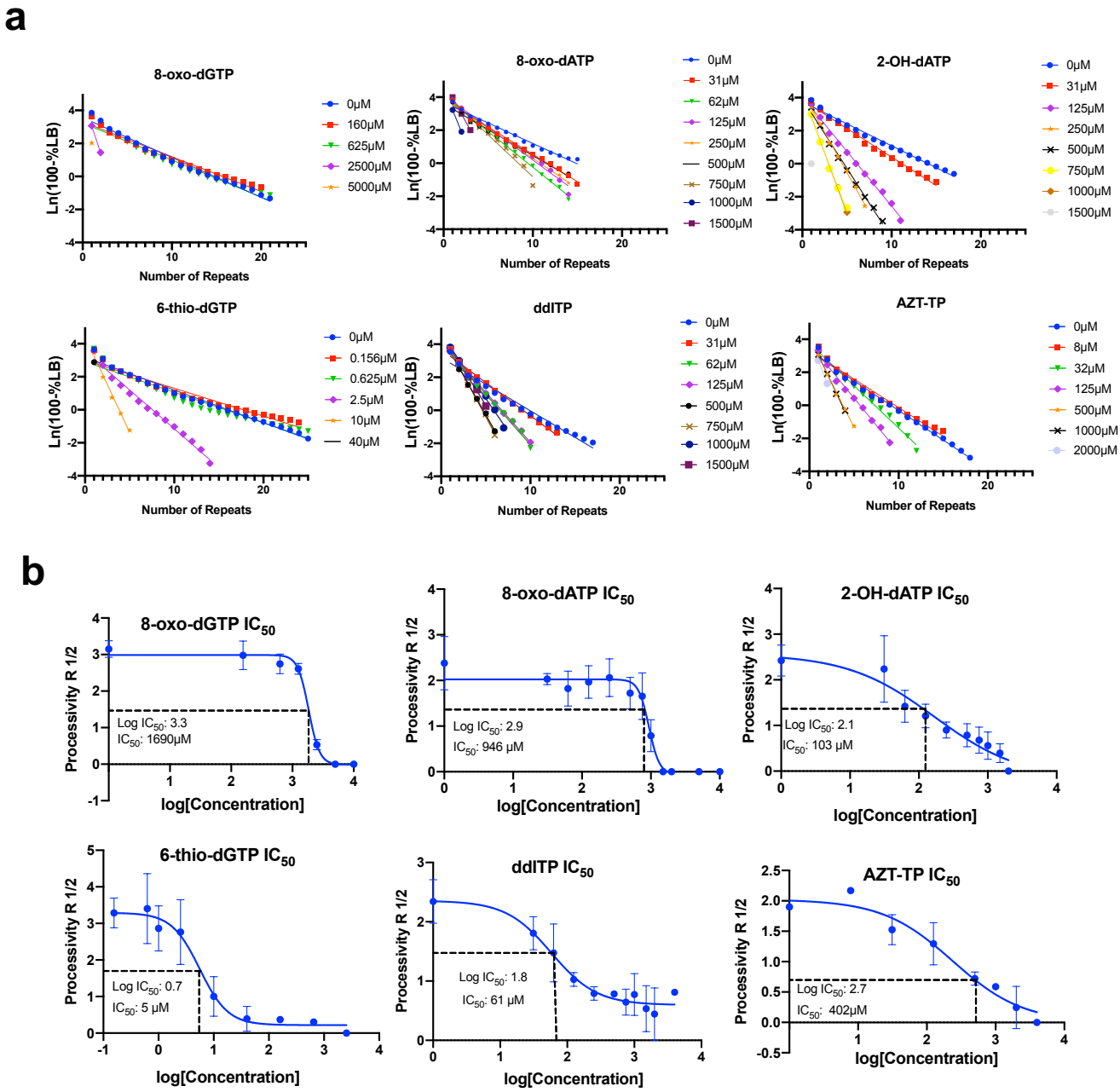




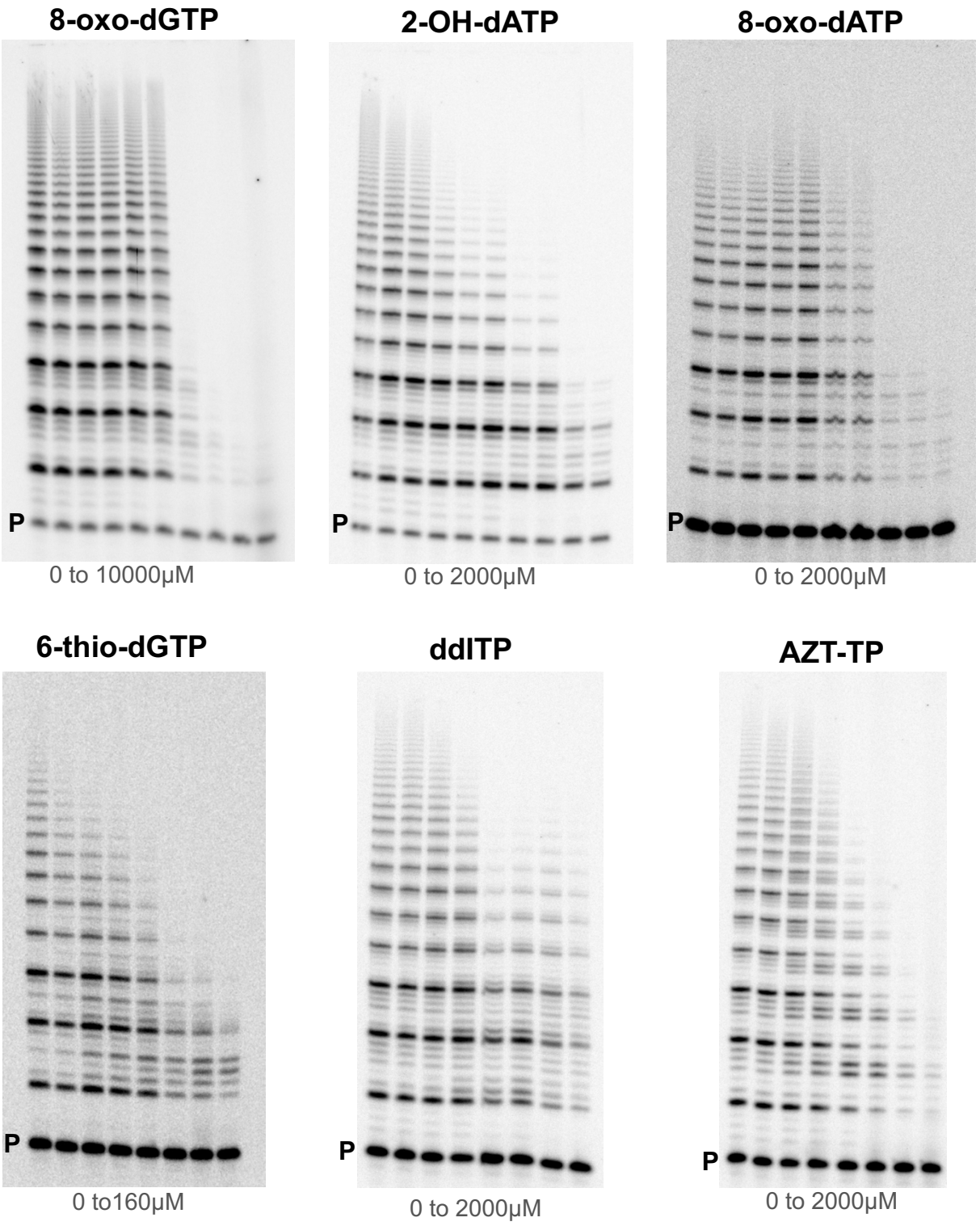
Supplementary Fig 6. Telomerase extension with oxidized dNTPs. Telomerase reactions were conducted with 5 nM ^{32}P-end labeled primer (**a**) Primer 1 $(TTAGGG)_3$, (**b**) Primer 2 $(GGTTAG)_3$, or (**c**) Primer 3 $(AGGGTT)_3$. Reactions contained 5, 50, and 500 µM 8-oxo-dGTP (8dG), 8-oxo-dATP (8dA), or 2-OH-dATP (2dA) as indicated. Products were separated on denaturing gels. Letters on the right indicate the template base; P indicates unextended 18-mer primer. Graphs represent the percent of total primers extended for the reactions in panels **a**, **b**, and **c** from one experiment. Source data are provided as a Source Data file.



Supplementary Fig 7. Telomerase extension with therapeutic dNTPs. Telomerase reactions were conducted with 5 nM ^{32}P-end labeled primer (**a**) Primer 1 $(TTAGGG)_3$, (**b**) Primer 2 $(GGTTAG)_3$, or (**c**) Primer 3 $(AGGGTT)_3$. Reactions contained 5, 50, and 500uM 6dG (6-thio-dGTP), ddI (ddITP) or ZdT (AZT-TP) as indicated. Products were separated on denaturing gels. Letters on the right indicate the template base; P indicates unextended 18-mer primer. Graphs represent the percent of total primers extended for the reactions in panels **a**, **b**, and **c** from one experiment. Source data are provided as a Source Data file.



Supplementary Fig 8. IC_{50} values for telomerase processivity inhibition. Telomerase reactions were conducted with cellular relevant concentrations of all four natural dNTPs and increasing concentrations of the modified dNTP analog from 0 to 10,000 μM to calculate. (**a**) The natural log of (100-%LB) was plotted vs repeat number. A straight line was fit to the data and the $R_{1/2}$ value was calculated by dividing –ln(2) by the slope of each line. Plots shown from one experiment are representative of 3-4 independent experiments, and from 2 experiments for AZT-TP reactions. (**b**) The half maximal inhibitory concentration (IC_{50}) based on telomerase processivity. Means and s.d. are from 3-4 independent experiments, and from 2 experiments for AZT-TP reactions. Source data are provided as a Source Data file.



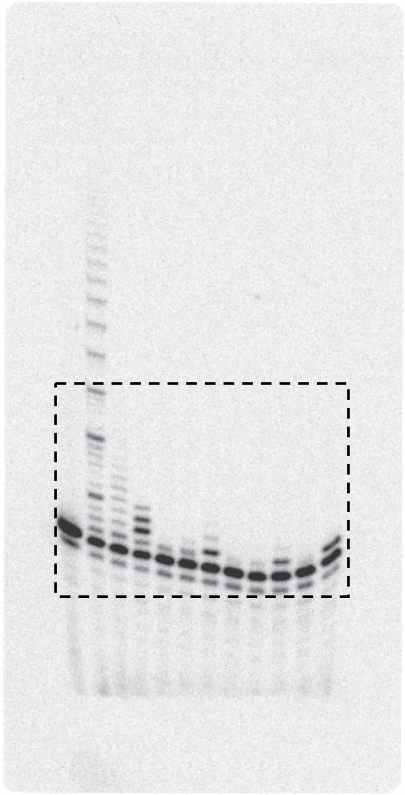
Supplementary Fig 9. Representative titration gels for IC_{50} values. Telomerase reactions were conducted with cellular relevant concentrations of all four natural dNTPs and increasing concentrations of the modified dNTP analog from 0 to 10,000 μM. Products were separated on denaturing gels; P indicates unextended 18-mer primer. Images are representative from 3-4 independent experiments, and from 2 experiments for AZT-TP reactions.

Supplementary Table 1. Oligonucleotide sequences used in this study

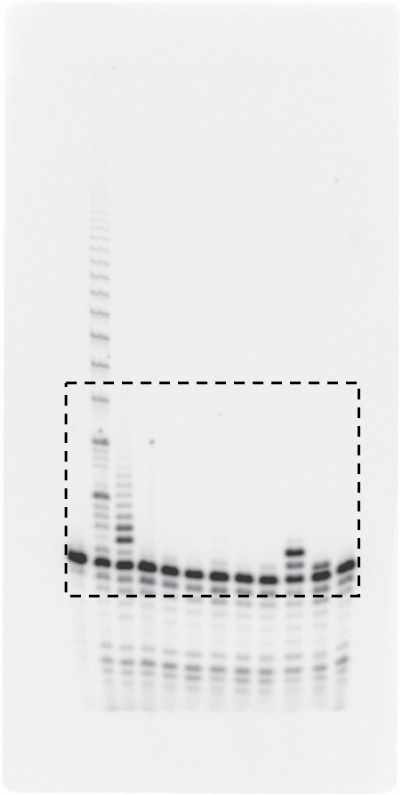
Primer name	Sequence (5' to 3')
Primer 1 (template rA_3rA_4)	TTAGGGTTAGGGTTAGGG
Primer 2 (template rC_1rC_2)	GGTTAGGGTTAGGGTTAG
Primer 3 (template rU_5)	AGGGTTAGGGTTAGGGTT
Primer 4 (template rC_1)	GTTAGGGTTAGGGTTAGG
RNA dot blot probe	CGGTGGAAGGCGGCAGGCCGAGGC
hTR PCR Primer forward	TAATACGACTCACTATAGGGCCATTTTTTGTCTAACCC
hTR PCR Primer reverse	AACGGGCCAGCAGCTG
Primer A5	TTAGGGTTAGCGTTAGGG
PolB Primer 1	/56-FAM/CGCATCCTTAGGG
PolB Primer 2	CCCTAACCCTAAGGATGCG
TcTERT Primer 1	/56-FAM/CCAGCCAGGTCAG
TcTERT Primer 2	GGUCGGUCCAGUCCAGU

Supplementary Figure 10. Uncropped gels from Figure 3

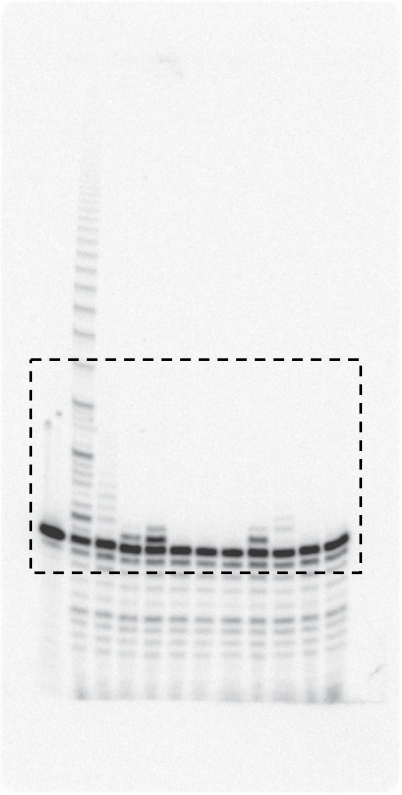
3a



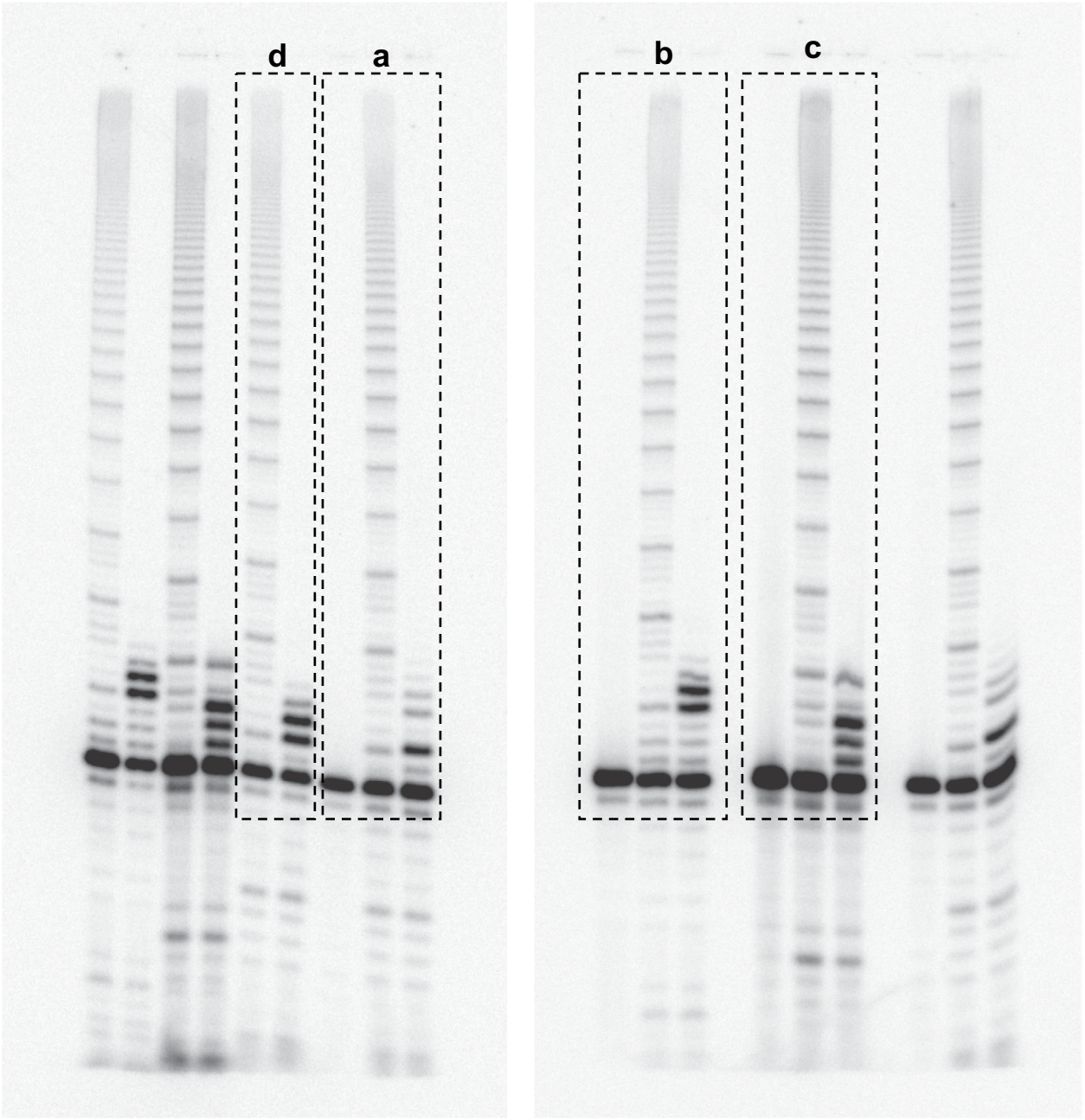
3b



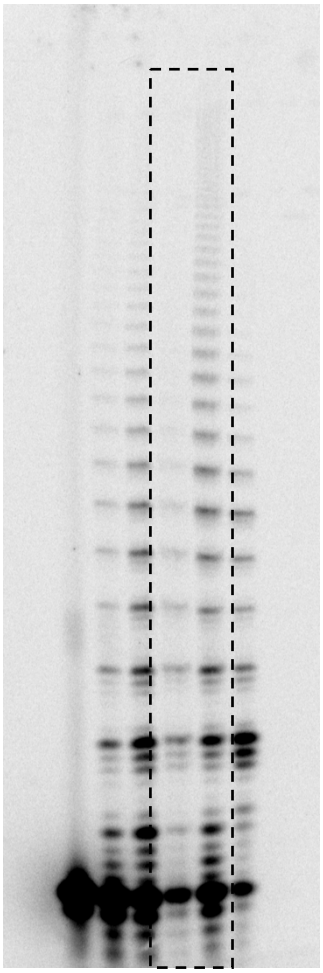
3c



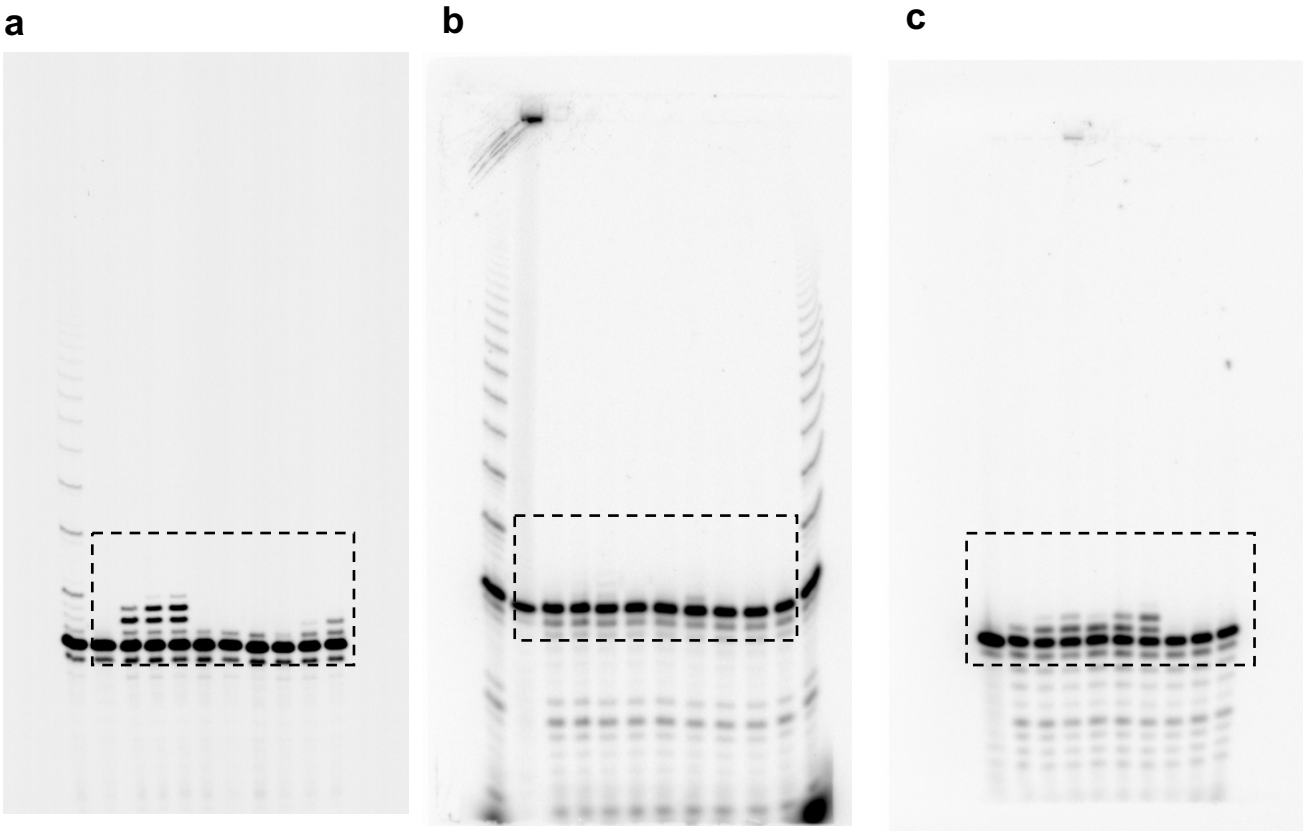
Supplementary Figure 11. Uncropped gels from Figure 4



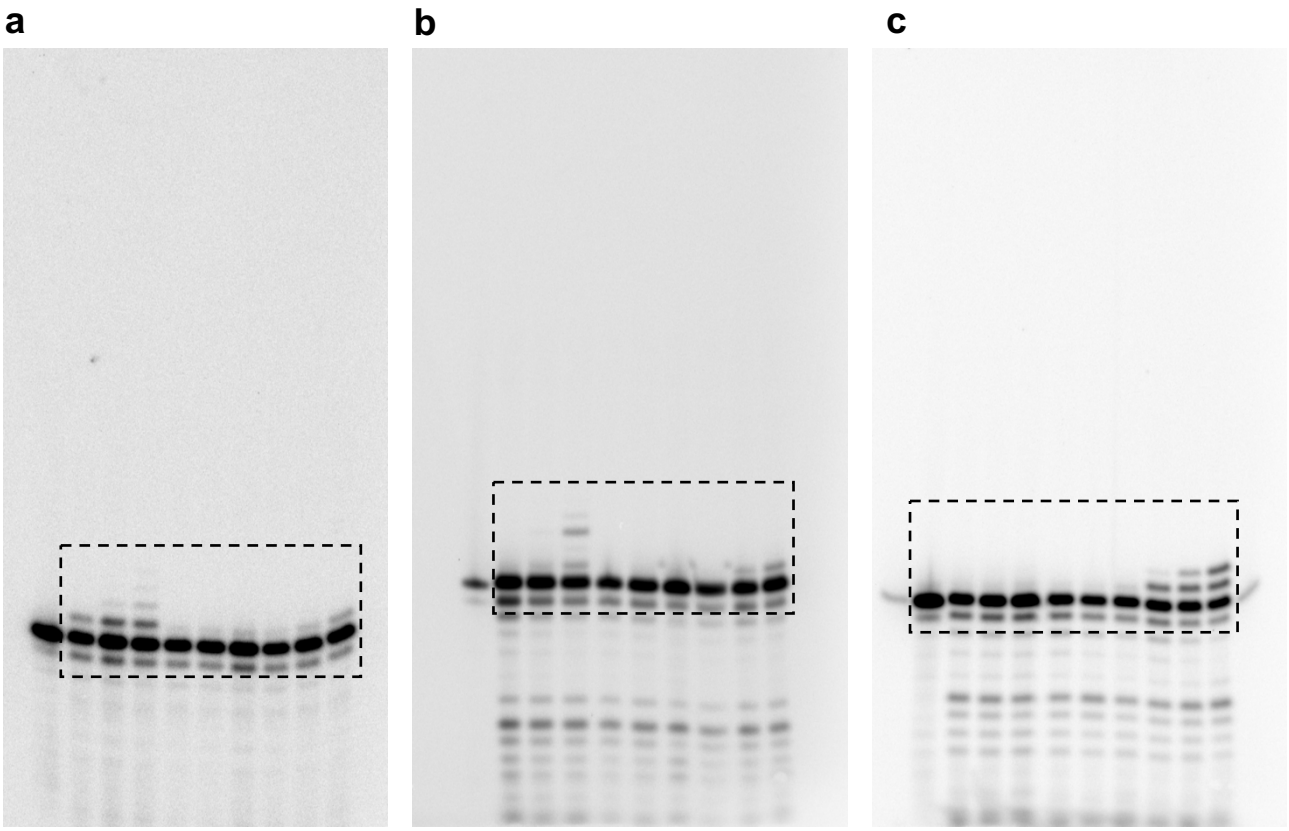
Supplementary Figure 12. Uncropped gel from Supplementary Figure 1c



Supplementary Figure 13. Uncropped gels from Supplementary Figure 5



Supplementary Figure 14. Uncropped gels from Supplementary Figure 6



Supplementary Figure 15. Uncropped gels from Supplementary Figure 7