SUPPLEMENTARY DATA

Differential activities of DNA polymerases in processing ribonucleotides during DNA synthesis in *Archaea*

Mélanie Lemor [1], Ziqing Kong [2], Etienne Henry [3], Raphaël Brizard [1], Sébastien Laurent [1], Audrey Bossé [1], Ghislaine Henneke [1*]

1-Ifremer, Univ Brest, CNRS, Laboratoire de Microbiologie des Environnements Extrêmes, F-29280 Plouzané, France

2-Department of Medical Biochemistry and Biophysics, Umeå University, Umeå, Sweden

3-CNRS, Ifremer, Univ Brest, Laboratoire de Microbiologie des Environnements Extrêmes, F-29280 Plouzané, France

*Correspondence: Ghislaine.Henneke@ifremer.fr, Tel: + 33298224609

Keywords: Archaea; DNA replication and repair; DNA polymerase; Nucleotide pool; Translesion synthesis

Abbreviations used: rNMPs, incorporated ribonucleotides; dNTPs, deoxyribonucleotides; rNTPs, ribonucleotides; DNA Pol, DNA polymerase; HiFi DNA Pol, high-fidelity DNA polymerase; RNase H, ribonuclease H; Top, topoisomerase.

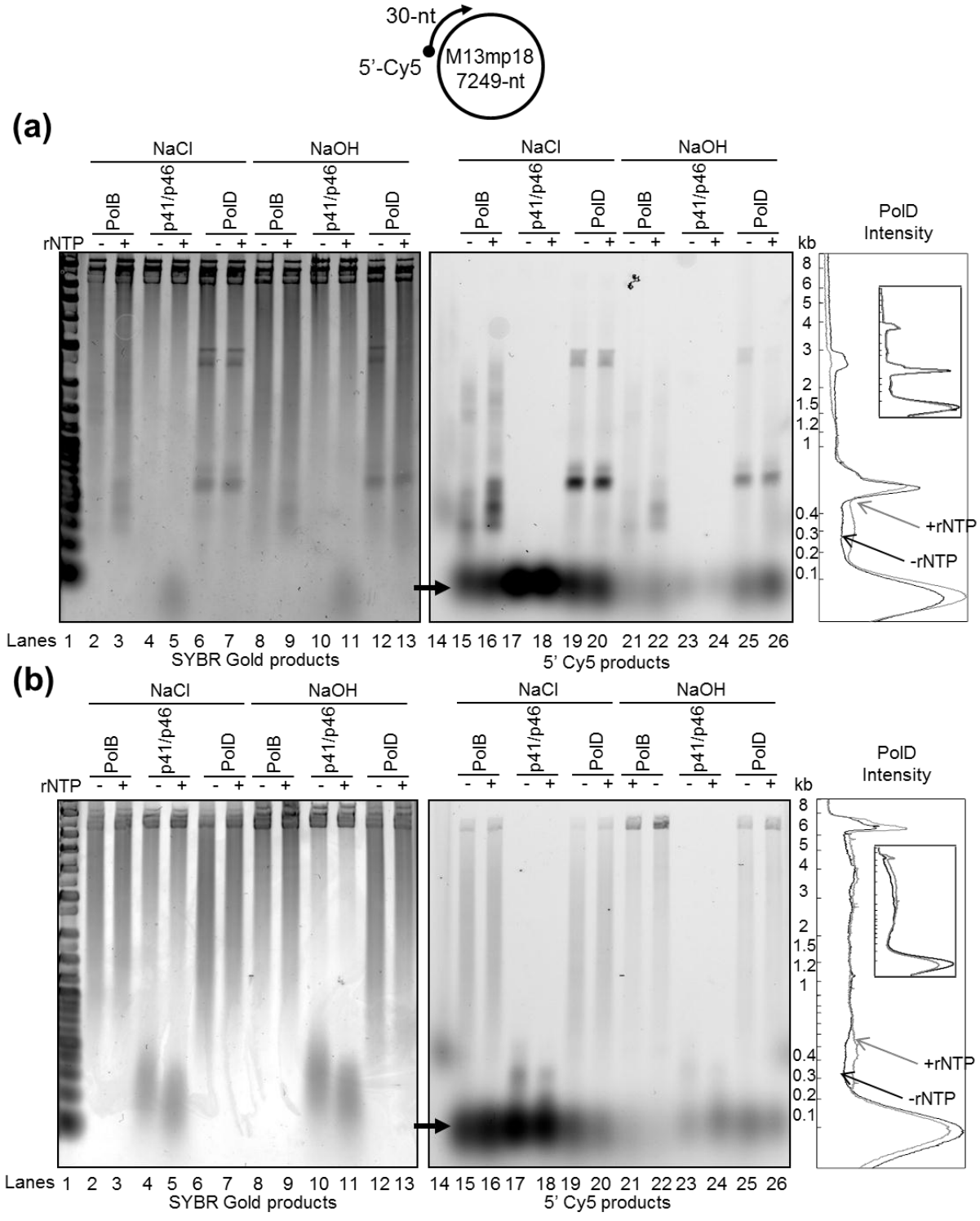


Fig. S1. Incorporation of rNMPs into duplex DNA (M13mp18 DNA primer-template). The structure of the primer-template mimic, consisting of a Cy5-labelled 30-mer primer annealed to the circular M13mp18 DNA template 7249 nucleotides in length, is shown above the

panels. (**a**) Extension of the primed-M13mp18 DNA template by PolB, the p41/p46 complex, and PolD with nucleotide substrates (ratio 2). Reactions contained the four dNTPs at 5.6 µM dATP, 6.1 µM dGTP, 11.9 µM dTTP and 2 µM dCTP with no additional rNTPs (denoted by – rNTP), or all eight nucleotides (denoted by + rNTP) at 5.6 µM dATP, 6.1 µM dGTP, 11.9 µM dTTP, 2 µM dCTP, 200 µM rATP, 128.4 µM rGTP, 112.4 µM rUTP and 58.4 µM rCTP. (**b**) Extension of the primed-M13mp18 DNA template by PolB, the p41/p46 complex and PolD, with nucleotide substrates at equimolar concentrations. Reactions contained 200 µM of each of the four dNTPs with no additional rNTPs (denoted by – rNTP), or 200 µM of each of the eight nucleotides (denoted by + rNTP). In all instances, the samples treated with either 0.25 M NaCl or 0.25 M NaOH are indicated at the top of each gel. The left and right gels were treated with SYBR-Gold and Cy5-labelled products, respectively. The arrow on the left of the Cy5 panel indicates the starting DNA primers. Densitometric traces of the resulting extension products after NaOH and NaCl treatments are shown in Figure supplement 2. Molecular weight markers (Lanes 1 and 14) revealed by gel-staining with SYBR Gold are labelled on the left of each densitometric representation.

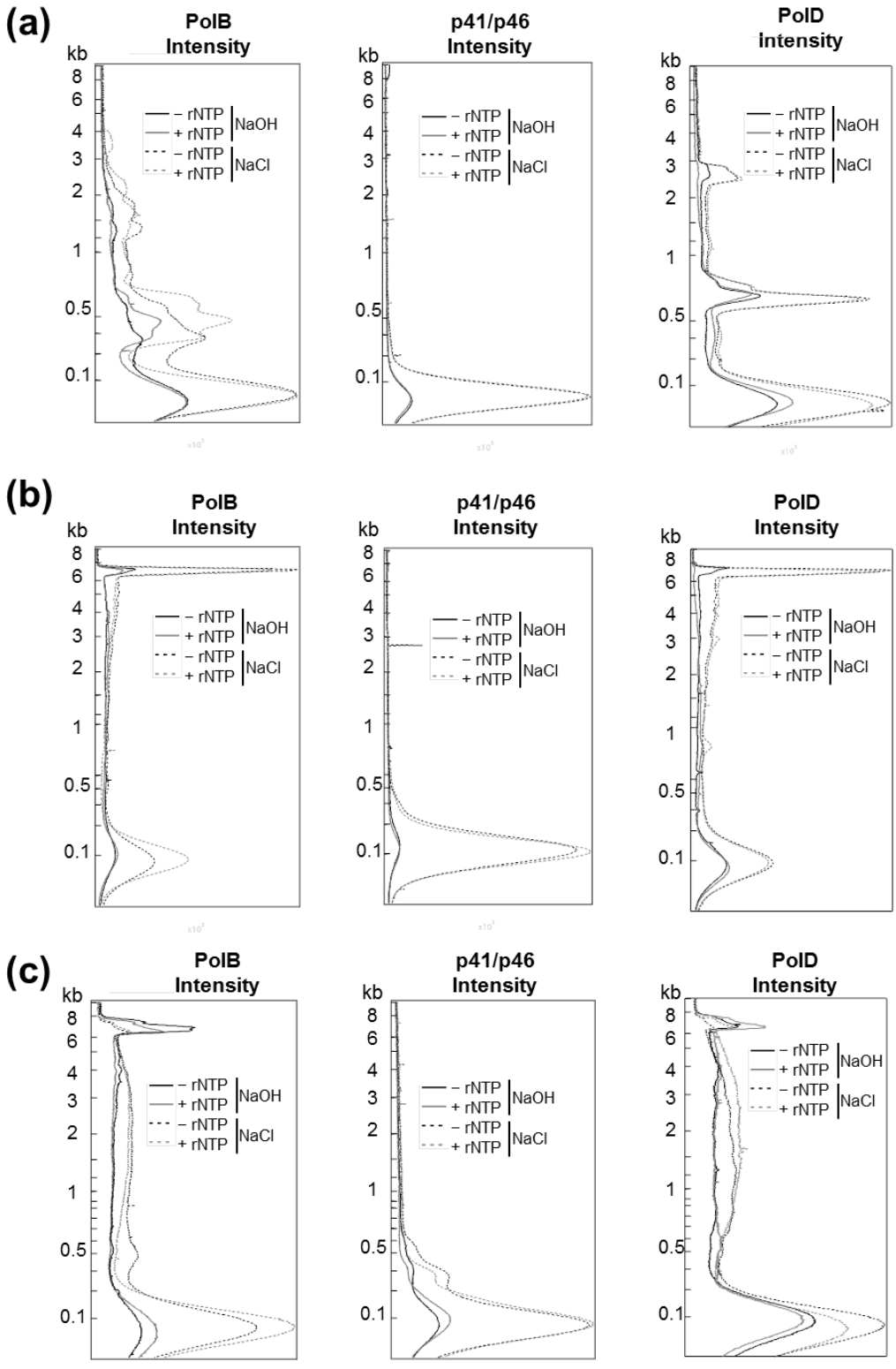


Fig. S2. Densitometric evaluation of polymerised products. Densitometric traces of the resulting PolB, the p41/p46 complex and PolD extension products after NaOH treatment (lanes 21-22 for PolB, lanes 23-24 for p41/p46 and lanes 25-26 for PolD from Fig. 3b and

Fig. S1) with rNTPs (solid grey lane) or without rNTP (solid dark lane). Densitometric traces of the resulting PolB, p41/p46 complex and PolD extension products after NaCl treatment (lanes 15-16 for PolB, lanes 17-18 for p41/p46 and lanes 19-20 for PolD from Fig. 3b and Fig. S1) with rNTPs (dotted grey lane) or without rNTP (dotted dark lane). Molecular weight markers revealed by gel-staining with SYBR Gold are labelled to the left of each densitometric representation. (**a**) Densitometric evaluation of polymerized products generated at ratio 2 from Fig. S1a. (**b**) Densitometric evaluation of polymerized products generated at ratio 1 from Fig. 3b. (**c**) Densitometric evaluation of polymerized products generated at equimolar concentration of nucleotides (200 µM each) from Fig. S1b.

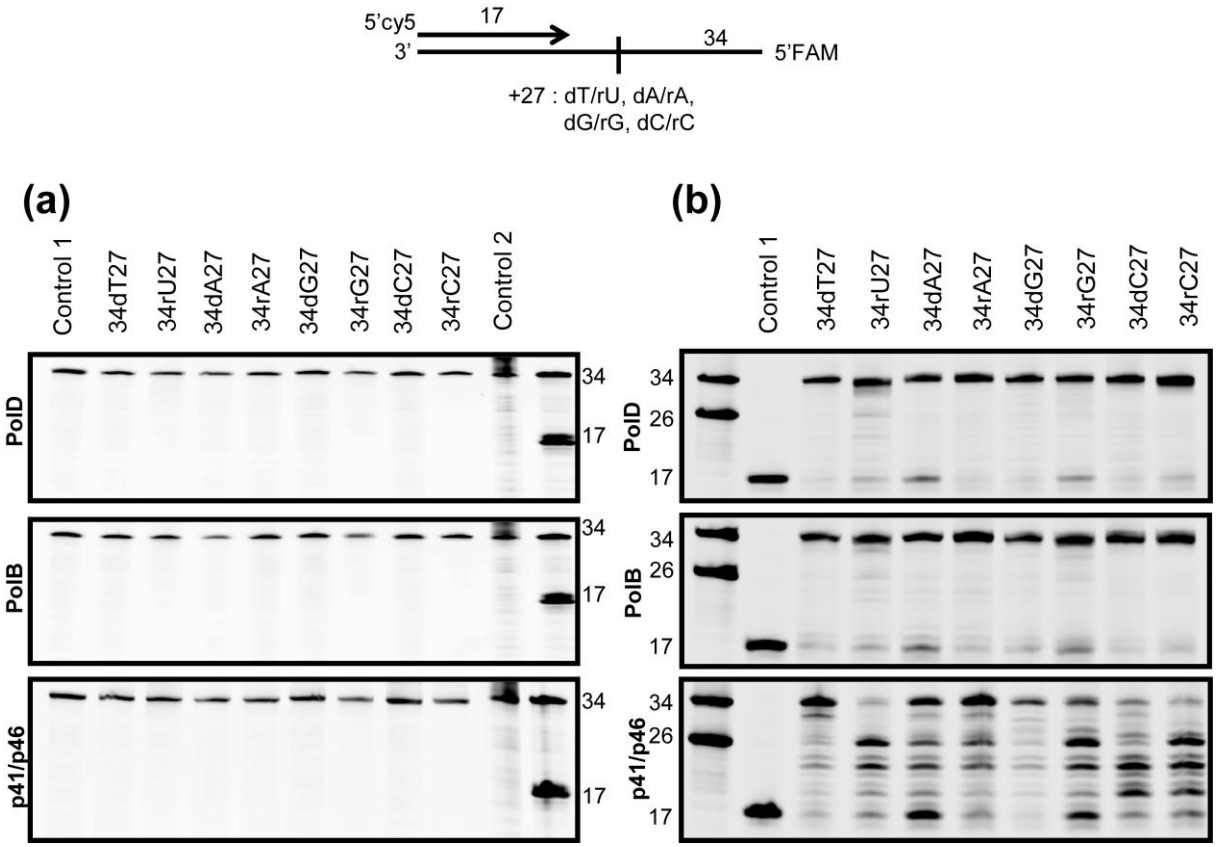


Fig. S3 related to Fig. 5. Primer extension and endonuclease activity by *P. abyssi* cell-free extracts and all three DNA Pols on rNMP-containing primer-templates. The structure of the Cy5 and FAM dual-labelled 17/34 primer-template is represented at the top (for full sequences, see Table S1). Primer-template extension reactions of the 17/34 primer-templates that place either rNMP or dNMP 10 bases ahead of the primer-template junction. (**a**) Gel imaged at 532 nm to observe the effects of cell-free extracts and DNA Pols on the FAM-labelled template strand. Control 1 = no enzyme added; Control 2 = oligodeoxynucleotide 34dT17 in stop buffer with competitor oligonucleotide RC34, an exact complement of the template strand under study. Reference oligodeoxynucleotides of 34 and 17 bases are indicated on the right of each panel. (**b**) Gel imaged at 633 nm to observe the extension of the Cy5-labelled primer. Reference oligodeoxynucleotides of 34, 26 and 17 bases are indicated on the left of each panel. Black lines separate lanes which were not adjacent in the original gel.

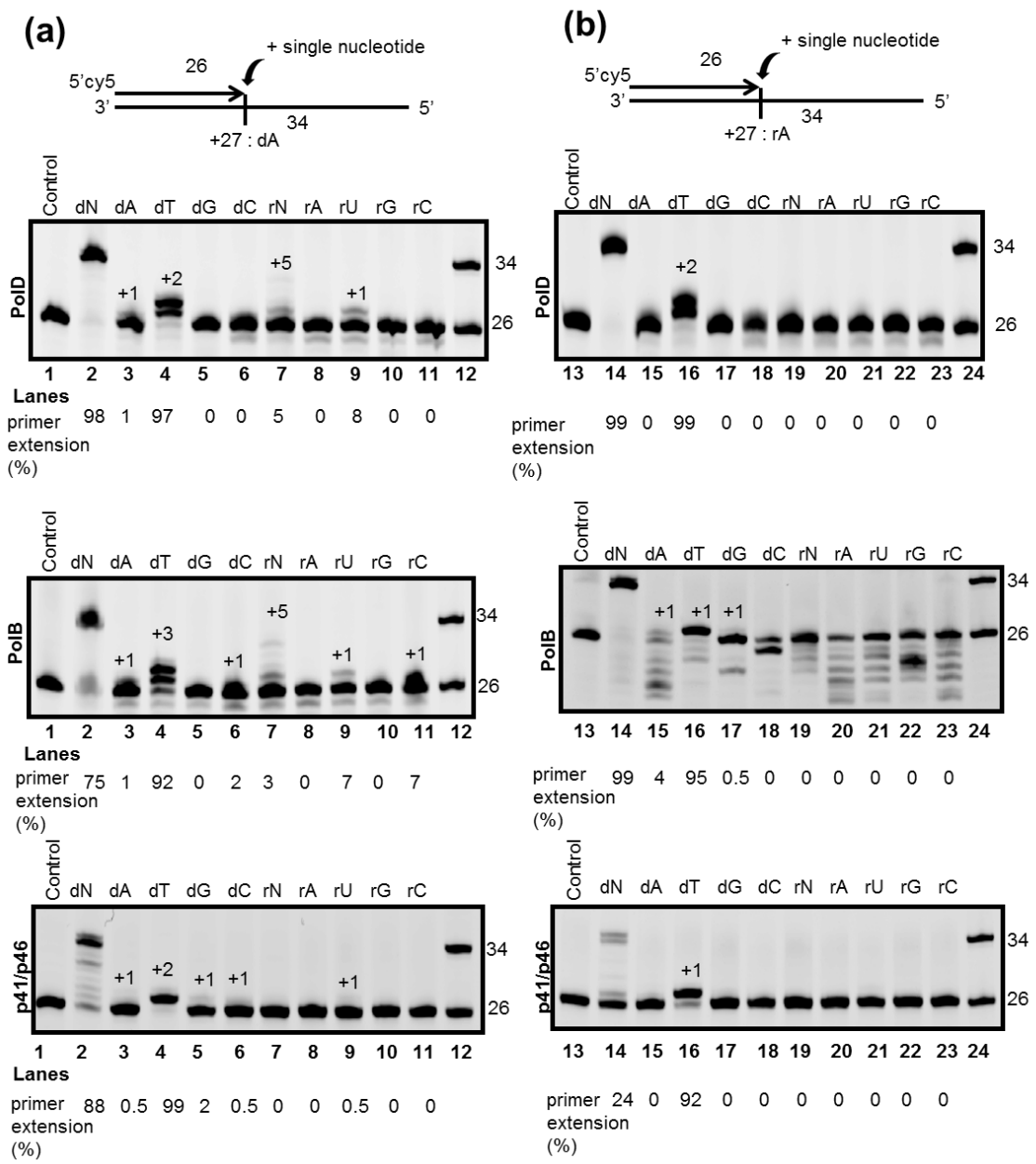
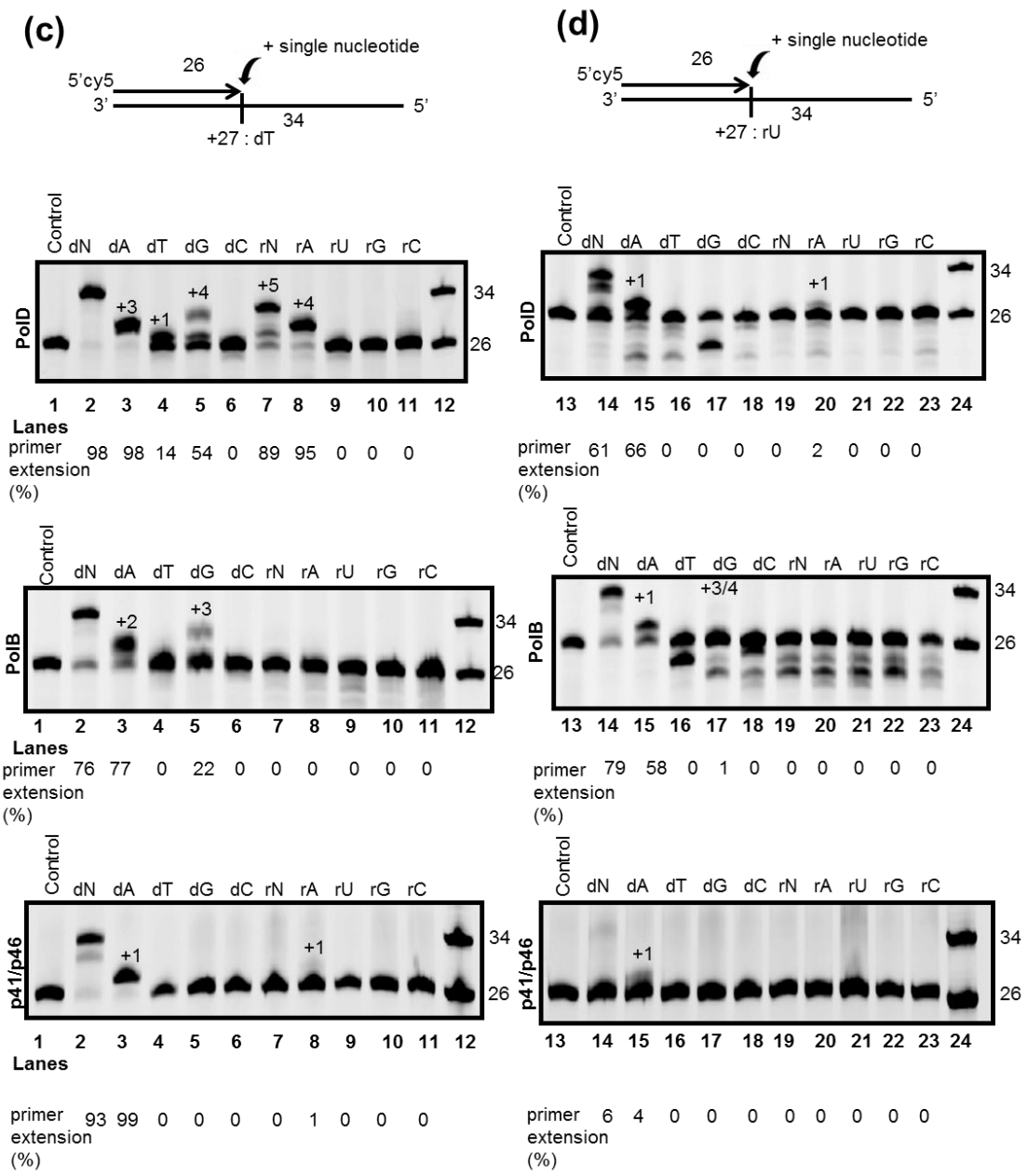


Fig. S4 related to Fig. 6. Single nucleotide incorporation opposite dNMP- or rNMP-containing templates by *Pyrococcus abyssi* DNA polymerases. DNA primer extension reaction of the 5'Cy5-labelled 26/34 primer-templates that locates dNMP or rNMP one base ahead of the primer-template junction. The structure of the primer-templates is represented at the top of each panel (for full sequences, see Table S1). (**a**) Base incorporation opposite template strand dAMP by PolD, PolB and the p41/p46 complex. (**b**) Base incorporation

opposite template strand rAMP by PolD, PolB and the p41/p46 complex. (**c**) Base incorporation opposite template strand dTMP by PolD, PolB and the p41/p46 complex. (**d**) Base incorporation opposite template strand rUMP by PolD, PolB and the p41/p46 complex. (**e**) Base incorporation opposite template strand dGMP by PolD, PolB and the p41/p46 complex. (**f**) Base incorporation opposite template strand rGMP by PolD, PolB and the p41/p46 complex. Each reaction had a running time of 30 min. “Control” = no enzyme added; dN = all four dNTPs added; dA, dT, dG and dC = only dATP or dTTP or dGTP or dCTP added, respectively; rN = all four rNTPs added; rA, rU, rG and rC = only rATP or rUTP or rGTP or rCTP added, respectively; +1, +2, +3, +4 , +5 and +6 represent final products. The extension (%) for selected lanes is shown under the gels. Reference oligodeoxynucleotides of 34 and 26 bases are indicated on the right of each panel.


(c)
+ single nucleotide
26
5'cy5
3'
5'
34
+27 : dT
Control
dN dA dT dG dC rN rA rU rG rC
PolD
+3
+1
+4
+5
+4
34
26
1 2 3 4 5 6 7 8 9 10 11 12
Lanes
primer extension (%) 98 98 14 54 0 89 95 0 0 0
(d)
+ single nucleotide
26
5'cy5
3'
5'
34
+27 : rU
Control
dN dA dT dG dC rN rA rU rG rC
PolD
+1
+1
34
26
13 14 15 16 17 18 19 20 21 22 23 24
primer extension (%) 61 66 0 0 0 0 2 0 0 0
Control
dN dA dT dG dC rN rA rU rG rC
PolB
+2
+3
34
26
1 2 3 4 5 6 7 8 9 10 11 12
Lanes
primer extension (%) 76 77 0 22 0 0 0 0 0 0
Control
dN dA dT dG dC rN rA rU rG rC
PolB
+1
+3/4
34
26
13 14 15 16 17 18 19 20 21 22 23 24
primer extension (%) 79 58 0 1 0 0 0 0 0 0
Control
dN dA dT dG dC rN rA rU rG rC
p41/p46
+1
+1
34
26
1 2 3 4 5 6 7 8 9 10 11 12
Lanes
primer extension (%) 93 99 0 0 0 0 1 0 0 0
Control
dN dA dT dG dC rN rA rU rG rC
p41/p46
+1
34
26
13 14 15 16 17 18 19 20 21 22 23 24
primer extension (%) 6 4 0 0 0 0 0 0 0 0

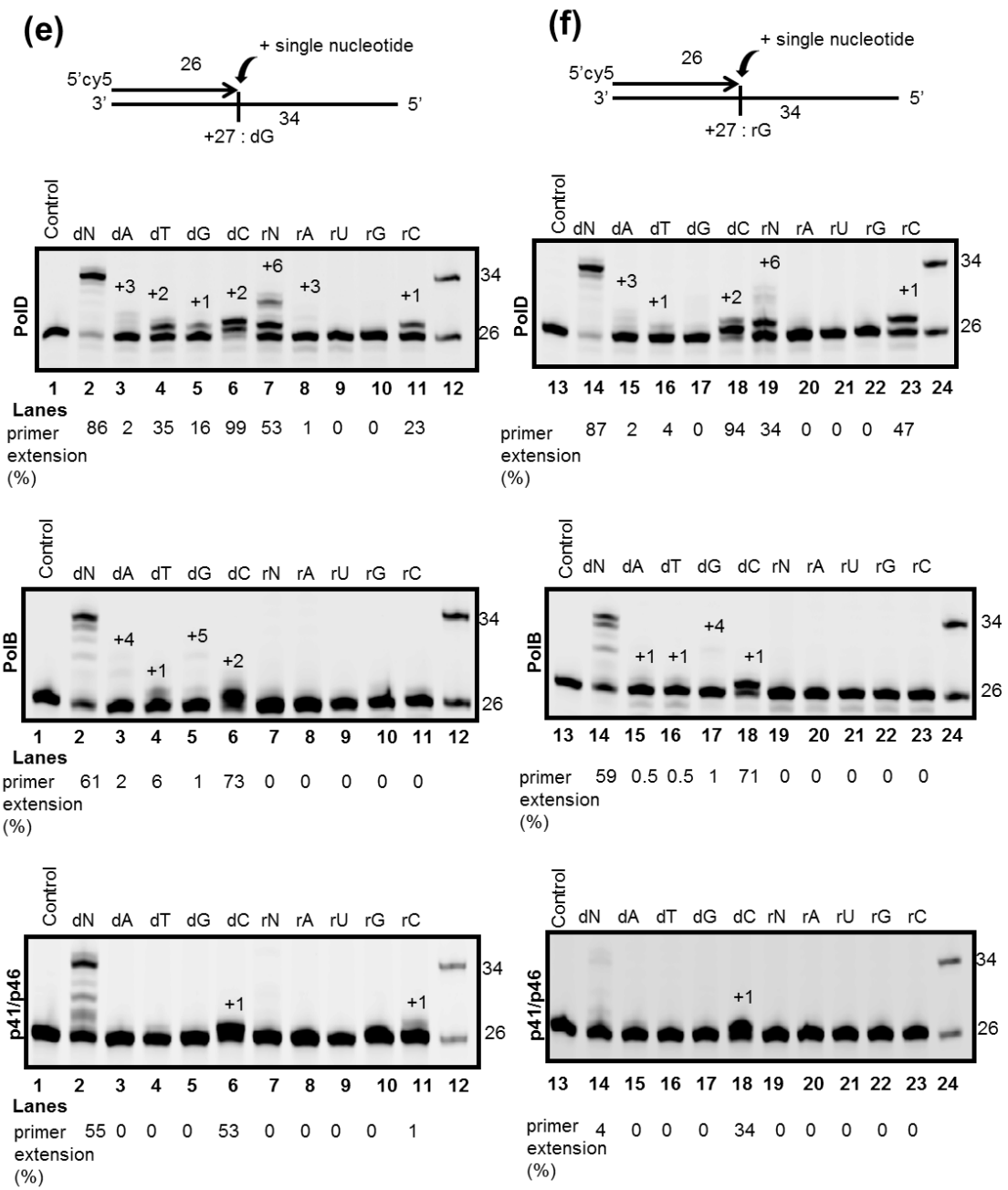

(e)
+ single nucleotide
26
5'cy5
3'
5'
34
+27 : dG
Control
dN dA dT dG dC rN rA rU rG rC
PolD
+3 +2 +1 +2 +6 +3 +1
34
26
1 2 3 4 5 6 7 8 9 10 11 12
Lanes
primer extension (%)
86 2 35 16 99 53 1 0 0 23
(f)
+ single nucleotide
26
5'cy5
3'
5'
34
+27 : rG
Control
dN dA dT dG dC rN rA rU rG rC
PolD
+3 +1 +2 +6 +1
34
26
13 14 15 16 17 18 19 20 21 22 23 24
primer extension (%)
87 2 4 0 94 34 0 0 0 47
Control
dN dA dT dG dC rN rA rU rG rC
PolB
+4 +5 +1 +2
34
26
1 2 3 4 5 6 7 8 9 10 11 12
Lanes
primer extension (%)
61 2 6 1 73 0 0 0 0 0
Control
dN dA dT dG dC rN rA rU rG rC
PolB
+4 +1 +1 +1
34
26
13 14 15 16 17 18 19 20 21 22 23 24
primer extension (%)
59 0.5 0.5 1 71 0 0 0 0 0
Control
dN dA dT dG dC rN rA rU rG rC
p41/p46
+1 +1
34
26
1 2 3 4 5 6 7 8 9 10 11 12
Lanes
primer extension (%)
55 0 0 0 53 0 0 0 0 1
Control
dN dA dT dG dC rN rA rU rG rC
p41/p46
+1
34
26
13 14 15 16 17 18 19 20 21 22 23 24
primer extension (%)
4 0 0 0 34 0 0 0 0 0

Table S1. Oligonucleotides used in this study

Oligodeoxynucleotides	**Length (bases)**	**Sequences (5' to 3')**	**fluorescent label**
Primer	17	TGCCAAGCTTGCATGCC	5'Cy5
Primer	26	TGCCAAGCTTGCATGCCTGCAGGTCG	5'Cy5
Primer	30	TGCCAAGCTTGCATGCCTGCAGGTCGACTC	5'Cy5
87dT33	87	CAGGAAACAGCTATGACCATGATTACGAATTCGAGCT CGGTACCCGGGGATCCT**T**TAGAGTCGACCTGCAGGC ATGCAAGCTTGGCA	/
87rU33	87	CAGGAAACAGCTATGACCATGATTACGAATTCGAGCT CGGTACCCGGGGATCCT**rU**TAGAGTCGACCTGCAGGC ATGCAAGCTTGGCA	/
87dA33	87	CAGGAAACAGCTATGACCATGATTACGAATTCGAGCT CGGTACCCGGGGATCCT**A**TAGAGTCGACCTGCAGGC ATGCAAGCTTGGCA	/
87rA33	87	CAGGAAACAGCTATGACCATGATTACGAATTCGAGCT CGGTACCCGGGGATCCT**rA**TAGAGTCGACCTGCAGGC ATGCAAGCTTGGCA	/
87dC33	87	CAGGAAACAGCTATGACCATGATTACGAATTCGAGCT CGGTACCCGGGGATCCT**C**TAGAGTCGACCTGCAGGC ATGCAAGCTTGGCA	/
87rC33	87	CAGGAAACAGCTATGACCATGATTACGAATTCGAGCT CGGTACCCGGGGATCCT**rC**TAGAGTCGACCTGCAGGC ATGCAAGCTTGGCA	/
87dG33	87	CAGGAAACAGCTATGACCATGATTACGAATTCGAGCT CGGTACCCGGGGATCCT**G**TAGAGTCGACCTGCAGGC	/

		ATGCAAGCTTGGCA	
87rG33	87	CAGGAAACAGCTATGACCATGATTACGAATTCGAGCT CGGTACCCGGGGATCCT**rG**TAGAGTCGACCTGCAGG CATGCAAGCTTGGCA	/
34dT27	34	GGATCCT**T**CGACCTGCAGGCATGCAAGCTTGGCA	5' FAM
34rU27	34	GGATCCT**rU**CGACCTGCAGGCATGCAAGCTTGGCA	5' FAM
34dA27	34	GGATCCT**A**CGACCTGCAGGCATGCAAGCTTGGCA	5' FAM
34rA27	34	GGATCCT**rA**CGACCTGCAGGCATGCAAGCTTGGCA	5' FAM
34dC27	34	GGATCCT**C**CGACCTGCAGGCATGCAAGCTTGGCA	5' FAM
34rC27	34	GGATCCT**rC**CGACCTGCAGGCATGCAAGCTTGGCA	5' FAM
34dG27	34	GGATCCT**G**CGACCTGCAGGCATGCAAGCTTGGCA	5' FAM
34rG27	34	GGATCCT**rG**CGACCTGCAGGCATGCAAGCTTGGCA	5' FAM
RC87	87	TGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGA TCC CCGGGTACCGAGCTCGAATTCGTAATCATGGTCATAG CTG TTTCCTG	
RC34	34	TGCCAAGCTTGCATGCCTGCAGGTCGCAGGATCC	
ladder	17	TGCCAAGCTTGCATGCC	5'FAM

The varying nucleotide in the template strand is shown in bold.

Table S2 related to Fig. 5. Quantitative analyses of stalling events in the presence of rNMP-containing templates**.**

Stalling products (32nt)	87dT17	87rU17	87dA17	87rA17	87dG17	87rG17	87dC17	87rC17
Cell-free extract	0.2%	0.8%	0.1%	0.5%	0.5%	0.7%	0.1%	0.2%
PolD	0.1%	0.2%	0.3%	0.5%	0.6%	2.1%	0.1%	0.4%
PolB	0.2%	0.3%	0.2%	0.5%	0.5%	1.2%	0.3%	0.3%
p41/p46	2%	4.9%	7.3%	33.9%	3.5%	29.8%	0.4%	10.6%

Primer-extension stalling on 17/87 primer-templates. The varying base in the template strand is located at the +33 position. Quantification of stalling one nucleotide upstream of the rNMP template base (32-nt). Stall (%): intensity of the major stall band (32-nt) as a percentage of total lane intensity.