A

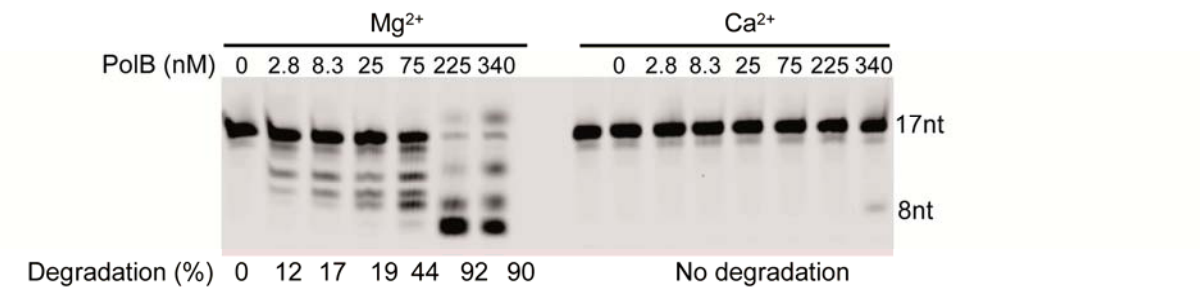
17 base primer

5′-CY5-TGCCAAGCTTGCATGCC-3′

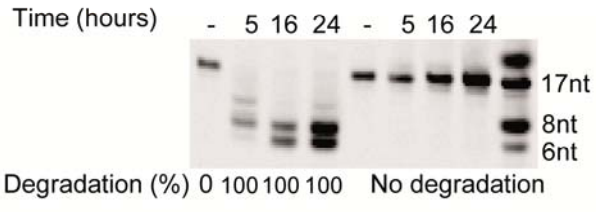
3′-ACGGTTCGAACGTACGGACGTCCAGCTGAGATCTCCTAGGGGCCCATGGCTCGAGCTTAAGCATTAGTACCAGTATCGACAAAGGAC-5′

87 base template

B



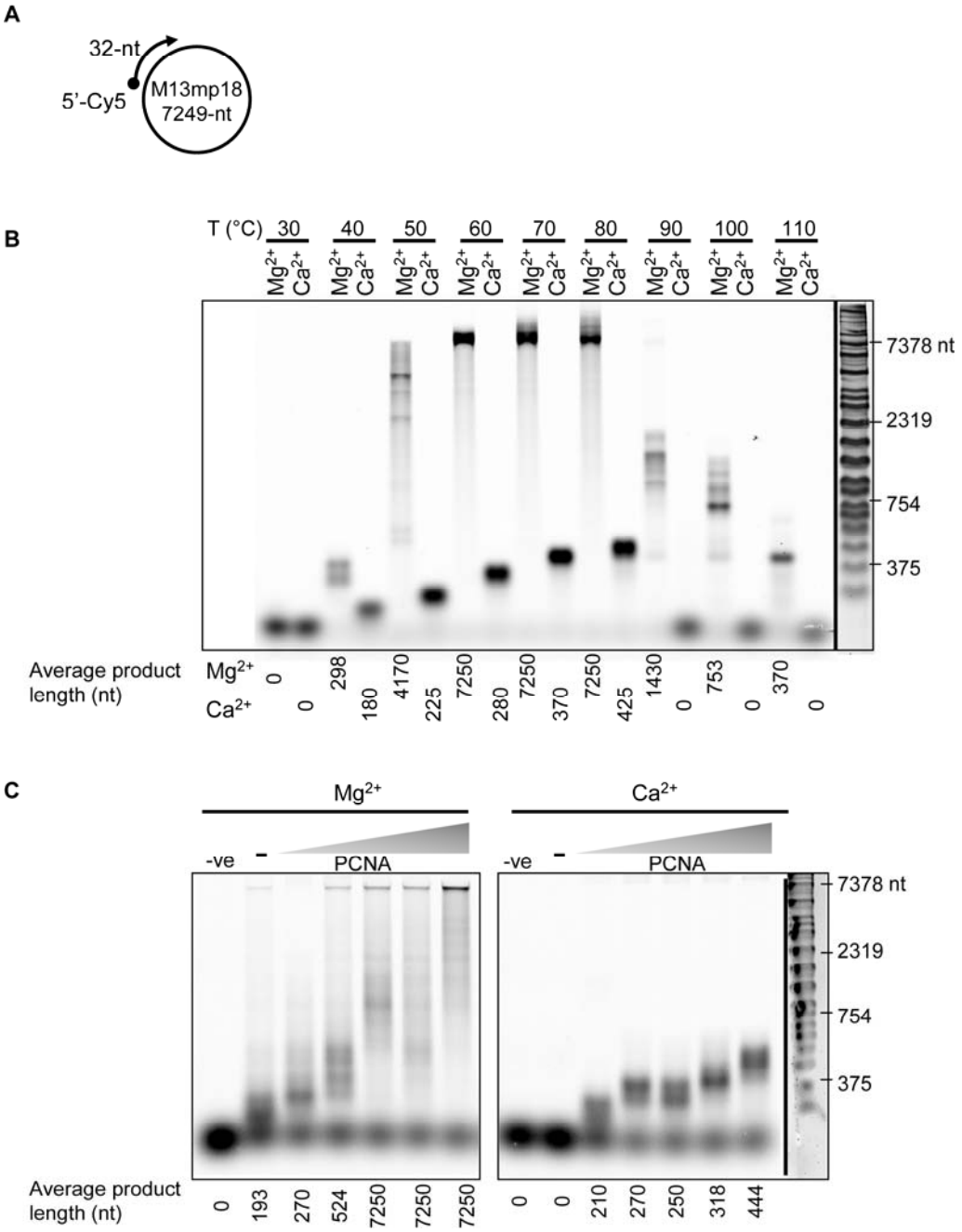
C



D

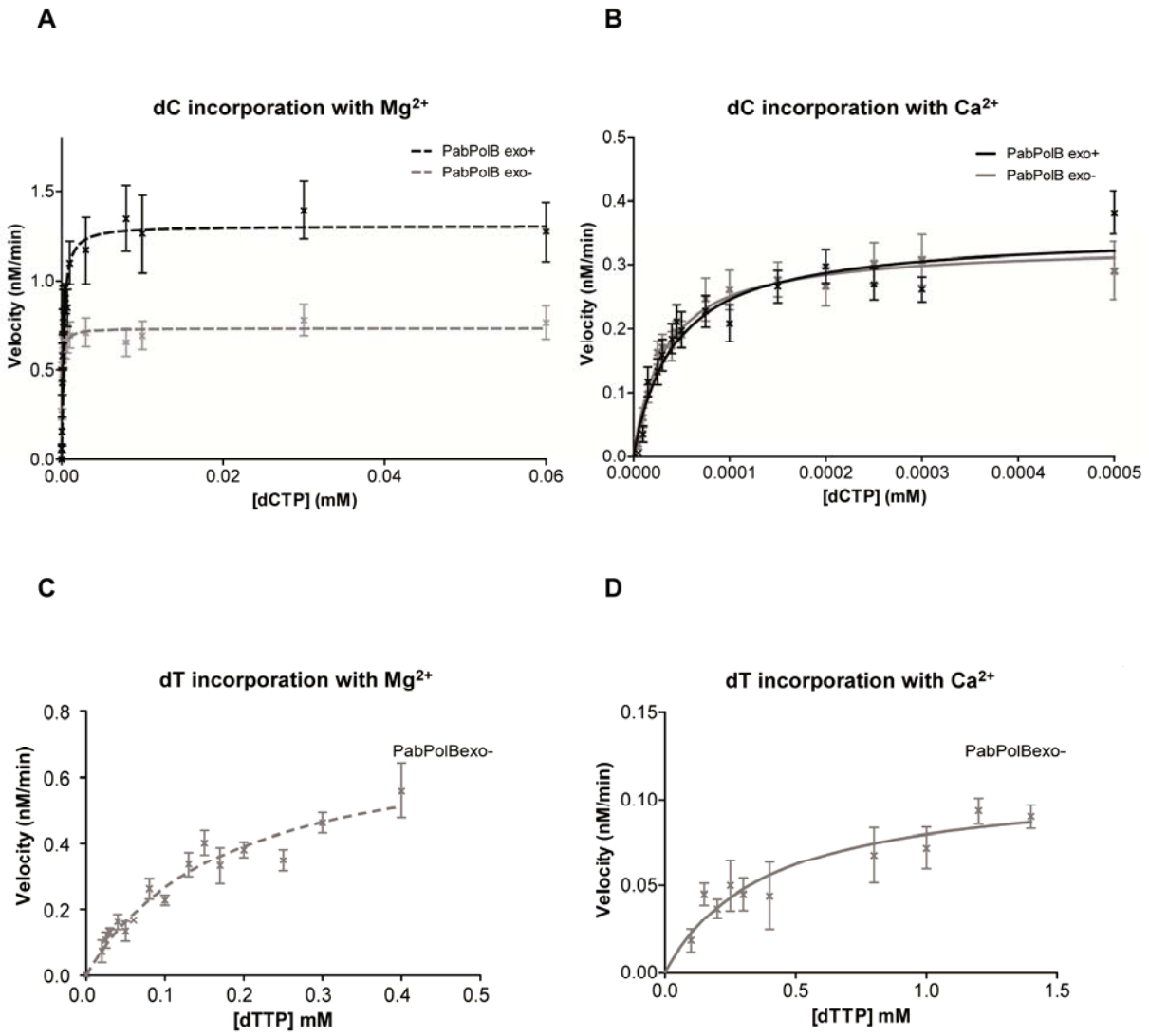
		Primer degradation (%)	
		Mg2+	Ca2+
pH	4.8	-	-
	6.5	-	-
	9	80	-
Counterion	acetate	85	-
	chloride	84	-

Supplementary Figure S1. Catalytic activities of PabPolB mediated by Mg^{2+} or Ca^{2+}. (**A)** Primer-template used for 3'-exonuclease primer degradation experiments. It consists of a Cy5-labelled 17-mer primer annealed to a DNA template of 87-nucleotides in length. (**B**) Proofreading exonucleolysis of the 17/87 primer-template (25 nM) by PabPolB at the indicated concentrations in the presence of either Mg^{2+} or Ca^{2+} (5 mM) for 30 minutes at 55°C. (**C**) Proofreading exonucleolysis of the 17/87 primer-template by PabPolB (75 nM) at fixed Mg^{2+} or Ca^{2+} concentrations (5 mM) in a time course experiment at 55°C. The percentage of degraded primers is depicted under the gels in panels **B**-**C** and reference oligodeoxynucleotides (17, 8 and 6 bases) are indicated on the right. (**D**) Proofreading exonucleolysis of the 17/87 primer-template (25 nM) by PabPolB (75 nM) at varying counterions of Mg^{2+} or Ca^{2+} (5 mM) and in different pH buffers are carried out at 55°C for 30 minutes. The percentage of primer degradation is summarized.



Supplementary Figure S2. Modulation of DNA polymerisation PabPolB with Mg^{2+} or Ca^{2+} on primed-M13mp18 DNA template. (**A**) Structure of the primer-template mimic consisting of a Cy5-labelled 32-mer primer annealed to the circular M13mp18 DNA template of 7250-nucleotides in length. (**B**) Primer extension assay by PabPolB (200 nM) with the 5'-end labelled primed-M13mp18 DNA template (7 nM) in the presence of dNTPs (200 µM) and either 5 mM Mg^{2+} or Ca^{2+}. Reactions were carried out for 30 min at the indicated incubation temperatures. (**C**) Primer extension assay by PabPolB with the 5'-end labelled primed-M13mp18 DNA template (7 nM) in the presence of dNTPs (200 µM) and either 5 mM Mg^{2+} or Ca^{2+} for 30 minutes at 60°C. Pre-incubation is carried out with increased PabPCNA concentrations (from 4 to 64 nM). First lane of the gel (-ve) is the reaction with PabPCNA (64 nM) but without PabPolB. Second lane is the reaction with PabPolB (16 nM) but without PabPCNA. For all gels, molecular weight markers are labelled on the right. Length average products are indicated under the gels.

Figure S3



Supplementary Figure S3. Steady-state kinetics of PabPolB exo+ and PabPolB exo- with either Mg^{2+} or Ca^{2+}. Single nucleotide insertion assays were performed with 5 nM of PabPolB exo+ or PabPolB exo– and 25 nM of 26/34 primer/template in the presence of dCTP or dTTP at the indicated concentration ranges with either Mg^{2+} or Ca^{2+} (5 mM). Reactions were carried out at 55°C, for 4 min for dC incorporation with Mg^{2+}, 8 min for dC incorporation with Ca^{2+}, and 8 min for dT incorporation with Mg^{2+} or Ca^{2+}. Michaelis-Menten plots of PabPolB exo+ (black curve) and PabPolB exo- (grey curve) for dC incorporation with Mg^{2+}(**A**), PabPolB exo+ (black curve) and PabPolB exo- (grey curve) for dC incorporation with Ca^{2+}(**B**), PabPolB exo- for dT incorporation with Mg^{2+}(**C**), PabPolB exo- for dT incorporation with Ca^{2+}(**D**). Kinetics parameters are summarized in **Table 1**.

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5′-CGCCGGGCCGAGCCGTGC
3′-GCGGCCCGGCTCGGCACGTGCTGGA-HEX 5′
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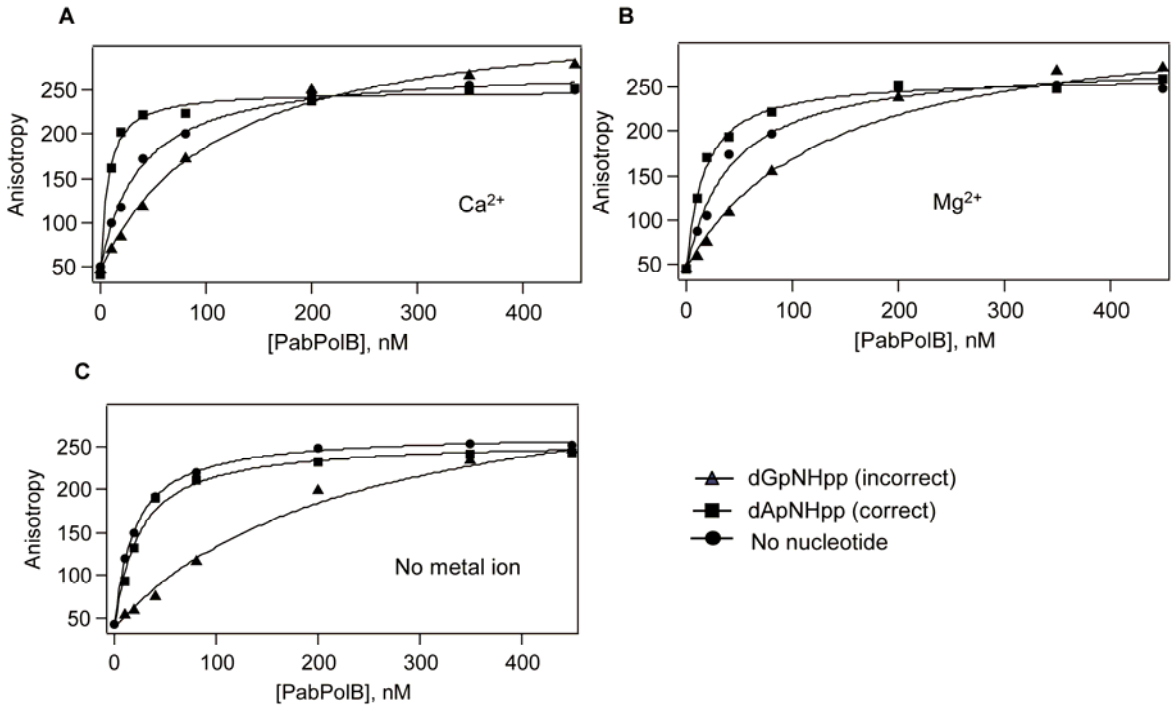

Supplementary Figure S4. Roles of metal ions in primer/template binding by PabPolB. Binding reactions are performed as described in Materials and Methods. The fluorescence anisotropy of the primer/template is plotted as a function of increased PabPolB concentrations. The binding buffer is complemented or not with metallic cofactor (5 mM Ca^{2+}: panel A; 5 mM Mg^{2+}: panel B; no metal ion: panel C) and with (or without) saturating amounts of non-hydrolysable nucleotides (black circle: without nucleotide, black square: 100 µM dApNHpp, black triangle: 1000 µM dGpNHpp). Each curve corresponds to the mean of a triplicate. The equilibrium dissociation constant (K_D) calculated from each fit is reported in **Table 2.**