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Supplemental information

Processing of matched and mismatched rNMPs in DNA by archaeal ribonucleotide excision repair

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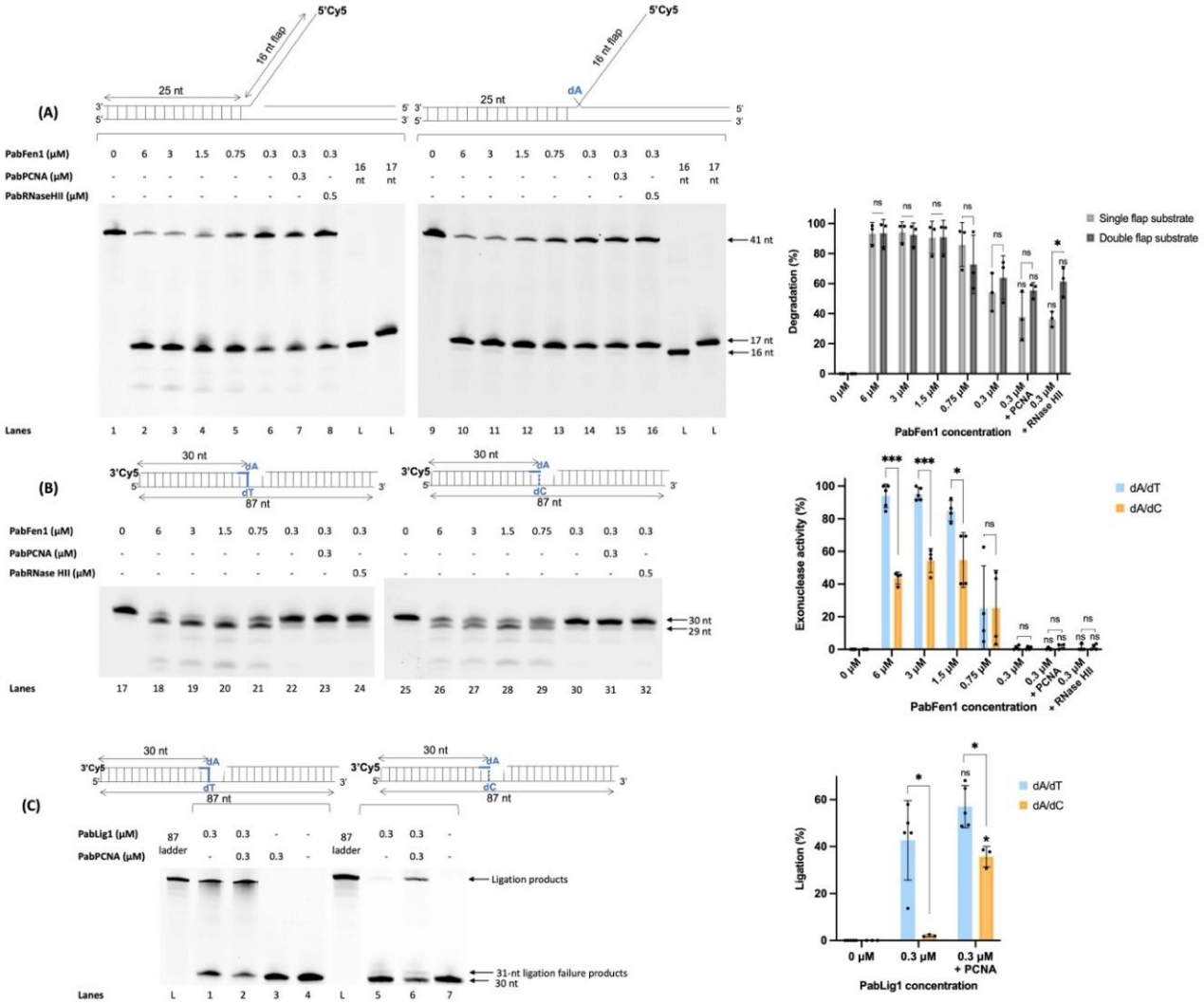

Figure S1. Enzymatic characterization of PabFen1 exonuclease activity and PabLig1 on matched or mismatched rAMP-containing substrates and PabFen1 endonuclease activity on single- and double-flap structures, related to Figure 1.

(A) The structure of single-flap (Flap16:L32/L57, Table S3) or double-flap (Flap16:L33_dA/L57, Table S3) are shown at the top. The indicated amounts of PabFen1 were incubated with single-flap substrate (lanes 2-6) or double-flap substrate (lanes 10-14) without PabPCNA or with 0.3 μM PabPCNA (lanes 7, 15) or 0.5 μM PabRNase HII (lanes 8, 16). Lanes 1 and 9 are negative controls without enzymes. In lanes L, 16-nt and 17-nt correspond to 5'-Cy5 oligonucleotides ladders. Bar graphs of cleavage products are summarized on the right of the gels. Bars are the averages ± standard deviation of at least three independent experiments. (B) The structure of 5'-phosphorylated nicked dsDNA templates containing a matched 5'-dAMP (L30:L57RC/L87, Table S3) or a mismatched (L30:L57RC/L87_dC, Table S3) 5'-dAMP are shown at the top. The indicated amounts of PabFen1 were incubated with matched dA/dT (lanes 18-22) or mismatched dA/dC (lanes 26-30) without PabPCNA or with 0.3 μM PabPCNA (lanes 23, 31) or 0.5 μM PabRNase HII (lanes 24, 32). Lanes 17 and 25 are negative controls without enzymes. Bar graphs of cleavage products are summarized on the right of the gels. Bars are the averages ± standard deviation of at least three independent experiments. Minus (−) denotes the absence of protein. (C) The structure of 5'-phosphorylated nicked dsDNA templates containing a matched 5'-dAMP (L30:L57RC/L87) or a mismatched 5'-dAMP (L30:L57RC/L87_dC) are shown at the top (Table S3). The indicated amounts of PabLig1 were incubated with matched dA/dT in the absence or presence of 0.3 μM PabPCNA (lanes 1-3) or with mismatched dA/dC in the absence or presence of 0.3 μM PabPCNA (lanes 5-6) and 1 mM ATP. Lanes L contains 3'-Cy5 labelled oligonucleotide ladders (87-nt). Lanes 4 and 7 are control without enzyme. Bar graphs represent the percentage of ligation products. Bars are the averages ± standard deviation of at least three independent experiments. p-values were determined

by unpaired t-test and are reported: ***p-value < 0.001, *p-value < 0.05 and non-significant (ns). The p-values represented above the bars when PabPCNA or PabRNaseHII are added to the reactions correspond to the difference observed with the condition of the same enzyme concentration without PabPCNA or PabRNase HII. Minus (−) denotes the absence of protein.

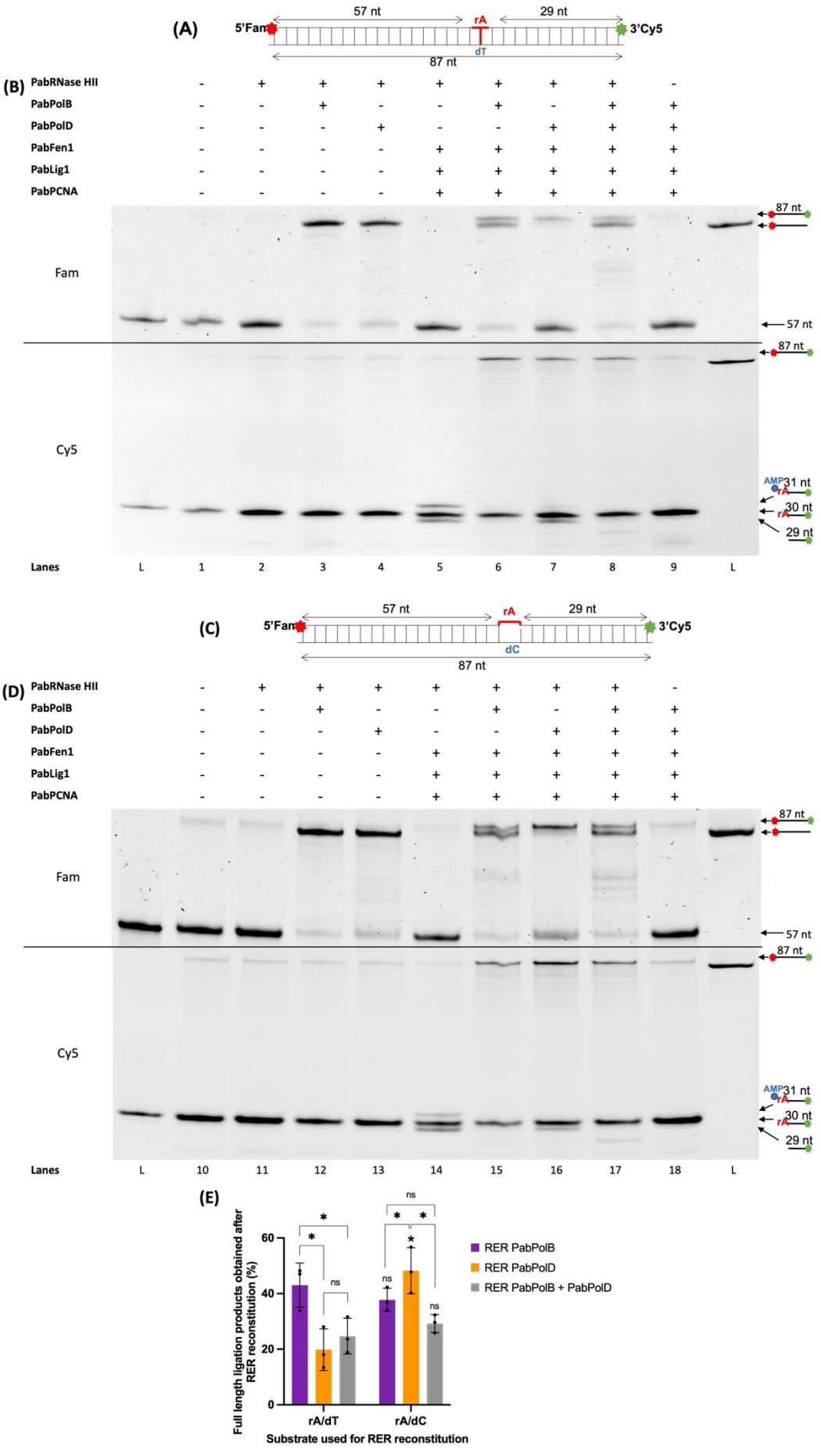
(A)
57 nt
rA
29 nt
5'Fam
3'Cy5
dT
87 nt
(B)
PabRNase HII
PabPolB
PabPolD
PabFen1
PabLig1
PabPCNA
Fam
Cy5
87 nt
57 nt
31 nt
30 nt
29 nt
AMP
Lanes
L 1 2 3 4 5 6 7 8 9 L
(C)
dC
(D)
Lanes
L 10 11 12 13 14 15 16 17 18 L
(E)
Full length ligation products obtained after RER reconstitution (%)
RER PabPolB
RER PabPolD
RER PabPolB + PabPolD
rA/dT
rA/dC
Substrate used for RER reconstitution
ns

Figure S2. Matched and mismatched rNMP correction in PabPolB and/or PabPolD-RER mediated pathway assessed by RNase HII treatment, related to Figure 2.
Lanes 1 and 10 are negative controls without enzyme. (A) Structure of the dual-labelled matched rNMP-containing dsDNA L87_rA/L87_dT (Table S3). (B) Reactions (lanes 2-9) were performed in the presence of various combinations of PabPolB, PabPolD, PabLig1, PabFen1, PabPCNA and PabRNase HII in buffer containing 1 mM ATP and physiological level of dNTPs. After incubation, purified products were submitted to PabRNase HII treatment as described in Material and Methods. In the Fam panel, lanes L contain 5'-Fam labelled oligonucleotide ladders (57- and 87-nt). In the Cy5 panel, lanes L contain 3'-Cy5 labelled oligonucleotide ladders (30- and 87-nt). Plus (+) denotes the presence and minus (−) denotes the absence of protein. (C) Structure of the dual-labelled mismatched rNMP-containing dsDNA template L87_rA/L87_dC (Table S3). (D) Reactions (lanes 11-18) were performed in the presence of various combinations of PabPolB, PabPolD, PabLig1, PabFen1, PabPCNA and PabRNase HII in buffer containing 1 mM ATP and physiological level of dNTPs. After incubation, purified products were submitted to PabRNase HII treatment as described in Material and Methods. In the Fam panel, lanes L contain 5'-Fam labelled oligonucleotide ladders (57- and 87-nt). In the Cy5 panel, lanes L contain 3'-Cy5 labelled oligonucleotide ladders (30- and 87-nt). Plus (+) denotes the presence and minus (−) denotes the absence of protein. (E) Full-length ligation products (%) obtained after RER reconstitution with PabPolB (corresponding to lanes 6 and 15), or PabPolD (corresponding to lanes 7 and 16), or both Pols (corresponding to lanes 8 and 17). Bar graphs represent the averages ± standard deviation of at least three independent experiments. p-values were determined by unpaired t-test and are reported: *p-value < 0.05 and non-significant (ns). The p-values represented above the bars for rA/dC conditions correspond to the difference observed with the same enzyme on the rA/dT substrate. The final reaction products obtained by RER reconstitution were submitted to enzymatic RNase HII treatment to evaluate rNMP correction.

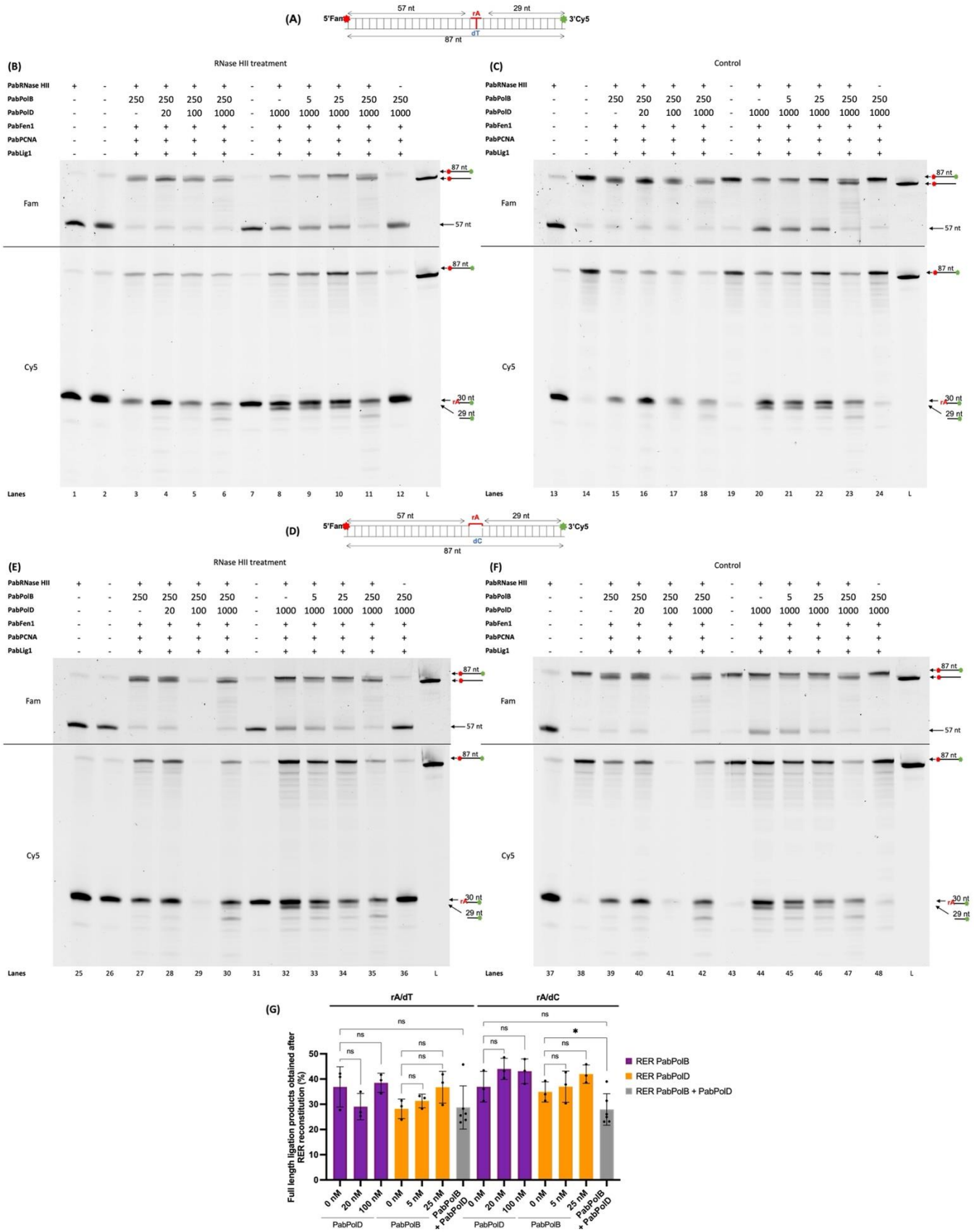

Figure S3. Influence of PabPolB and PabPolD concentrations in RER pathway, related to Figure 2.

Lanes 2, 14, 26 and 38 are negative controls without enzyme. (A) Reactions were performed in presence of a dual-labelled matched rNMP-containing dsDNA template L87_rA/L87_dT (Table S3) (A, B, C) or a mismatched dual-labelled mismatched rNMP-containing dsDNA template L87_rA/L87_dC (Table S3) (D, E, F). Reactions were performed at various combinations of PabPolB, PabPolD in the presence of PabFen1, PabPCNA, PabRNase HII and PabLig1 in buffer containing 1 mM ATP and physiological level of dNTPs with (B and E) or without (C and F) RNase HII treatment as described in Material and Methods. In the Fam panel, lanes L contain 5'-FAM labelled oligonucleotide ladders (57- and 87-nt). In the Cy5 panel, lanes L contain 3'-Cy5 labelled oligonucleotide ladders (30- and 87-nt). Plus (+) denotes the presence and minus (−) denotes the absence of protein. (G) Full-length ligation products (87-nt) obtained after RER reconstitution (%) with various concentrations of PabPolB or PabPolD or both Pols. Bar graphs represent the averages ± standard deviation of at least three independent experiments. p-values were determined by unpaired t-test and are reported: *p-value < 0.05 and non-significant (ns). The p-values represented above the bars for rA/dC conditions correspond to the difference observed with the same enzyme on the rA/dT substrate.

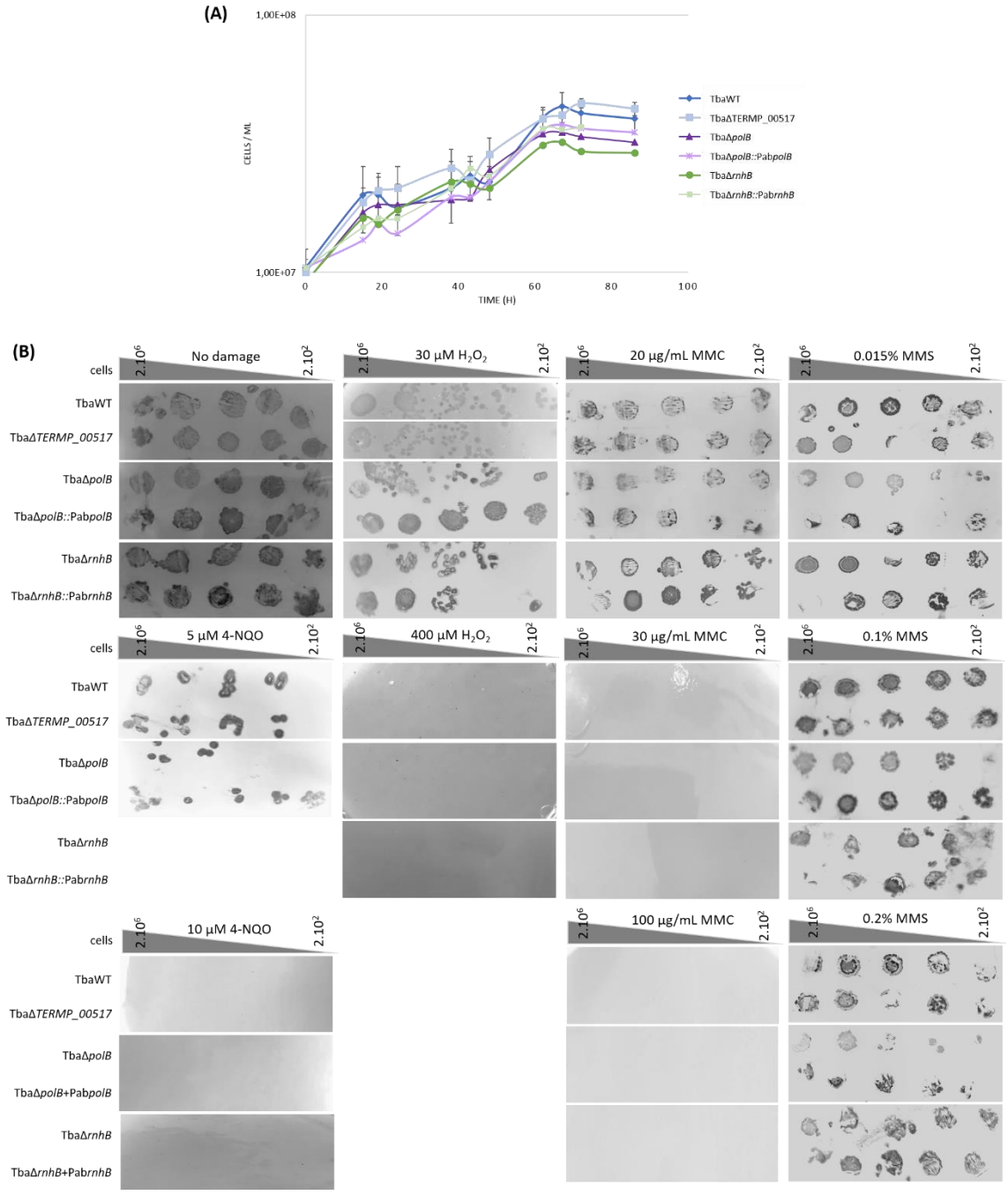

Figure S4. Growth and sensitive phenotypes of TbaWT, TbaΔ*TERMP_00517*, TbaΔ*polB*, TbaΔ*rnhB*, TbaΔ*polB*::Pab*polB* and TbaΔ*rnhB*::Pab*rnhB* strains, related to Figure 3.
(A) The growth curves are the mean of three independent experiments and the error bars represent the standard deviation. (B) Sensitivity of RER mutants and their heterologous complementation after exposition to 4-NQO, H_2O_2, MMC, or MMS. Drop dilution assays were performed using TbaWT, TbaΔ*TERMP_00517*, TbaΔ*polB*, TbaΔ*rnhB*, TbaΔ*polB*::Pab*polB* and TbaΔ*rnhB*::Pab*rnhB* strains. 2 x 10^6 to 2 x 10^2 cells (from left to right) were spotted on TRM medium after treatment with genotoxic agents. Cells are visualized by staining with Coomassie blue after transfer to PVDF membranes.

Table S1. *Thermococcus barophilus* and derivative mutant strains used in this study, related to STAR Methods.

Strains	Genotype	Parent Strain	References
UBOCC-M-3107	Tba wild-type	Tba MP	[S1]
UBOCC-M-3300	TbaΔ517 (TERMP_RS02570)	Tba wild-type	[S2]
UBOCC-M-3302	TbaΔ*polB* (TERMP_RS08040)	UBOCC-M-3300	[S2]
UBOCC-M-3303	TbaΔ*rnhB* (TERMP_RS03345)	UBOCC-M-3300	[S2]
UBOCC-M-3435	TbaΔ*polB* (TERMP_RS08040) complemented by Pab*polB* (PAB_RS09320)	UBOCC-M-3302	This study
UBOCC-M-3436	TbaΔ*rnhB* (TERMP_RS03345) complemented by Pab*rnhB* (PAB_RS02765)	UBOCC-M-3303	This study

Table S2. Not viable mutants in our transformation conditions, related to Figure 3.

Strains	Parent Strain	Nb of transformations	Clones screened
TbaΔ*rnhB*Δ*polB*	TbaΔ*rnhB* (UBOCC-M-3303)	2	39
TbaΔ*polB*Δ*rnhB*	TbaΔ*polB* (UBOCC-M-3302)	2	27

Table S3. Oligonucleotide sequences used in this study, related to STAR Methods.
Deoxyribonucleotides are in capital letters and ribonucleotides are in lower case letters.

Name	Sequence (5'- 3')	Length (nt)	Label	Hybrid Primer (:primer)/template
L34_dA	GGATCCT**A**CGACCTGCAGGCATGCAAGCTTGGCA	34	5'Fam	L34_dA/L34_dT L34_dA/L34_dC
L34_rA	GGATCCT**a**CGACCTGCAGGCATGCAAGCTTGGCA	34	5'Fam	L34_rA/L34_dT L34_rA/L34_dC
L34_dT	TGCCAAGCTTGCATGCCTGCAGGTCG**T**AGGATCC	34	/	L34_dA/L34_dT L34_rA/L34_dT
L34_dC	TGCCAAGCTTGCATGCCTGCAGGTCG**C**AGGATCC	34	/	L34_dA/L34_dC L34_rA/L34_dC
L34_rG	GGATCCT**g**CGACCTGCAGGCATGCAAGCTTGGCA	34	5'Fam	L34_rG/L34_dA_reverse
L34_dA_ reverse	TGCCAAGCTTGCATGCCTGCAGGTCG**A**AGGATCC	34	/	L34_rG/L34_dA_reverse
Flap16	AGATCATCAAGACTGCGAGGATCCCCGGGTACCGAGCTCGA	41	5'Cy5	Flap16:L32/L57 Flap16:L33_dA/L57
L32	TGCCAAGCTTGCATGCCTGCAGGTCGACTCTA	32	/	Flap16:L32/L57
L33_dA	TGCCAAGCTTGCATGCCTGCAGGTCGACTCTA**A**	33	/	Flap16:L33_dA/L57
L57	TCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCA	57	/	Flap16:L32/L57 Flap16:L33_dA/L57
L30_ra	**a**TTCGTAATCATGGTCATAGCTGTTTCCTG	30	3'Cy5	L30_rA:L57RC/L87
L57RC	TGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGA	57	5'Fam	L30_rA:L57RC/L87
L87	CAGGAAACAGCTATGACCATGATTACGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGA CCTGCAGGCATGCAAGCTTGGCA	87	/	L30_rA:L57RC/L87
L30	**A**TTCGTAATCATGGTCATAGCTGTTTCCTG	30	3'Cy5	L30:L57RC/L87
L87_rA	TGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGA**a**TTCGTA ATCATGGTCATAGCTGTTTCCTG	87	5'Fam 3'Cy5	L87_rA/L87_dT L87_rA/L87_dC
L87_dT	CAGGAAACAGCTATGACCATGATTACGAA**T**TCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGA CCTGCAGGCATGCAAGCTTGGCA	87	/	L87_rA/L87_dT
L87_dC	CAGGAAACAGCTATGACCATGATTACGAA**C**TCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGA CCTGCAGGCATGCAAGCTTGGCA	87	/	L87_rA/L87_dC

Table S4. Primers used for mutant strains construction, related to STAR Methods.
Restriction sites used are in bold and underlined.

Name	Sequence 5' → 3'	Tm (°C)	Mutant strain
PabpolB-Up	AAAAAA**GCATGC**AGTCCCGAAATTAAAGTGCGAGGC	59,4	Tba*Δpol*B::Pab*polB*
PabpolB-Do	AAAAAA**CCCGGG**AAGAACTTGTAGTTGACTTAAAGGATCTTCCG	61,3	
PabpromrnhB-Up	AAACGTCGGTAGGGTCTCCCCTTTCTTGAGTTT	59,3	Tba*Δrnh*B::Pab*rnhB*
PabpromrnhB-Do	AAAAAA**CTCGAG**TTATGTTCTCACTTGCGATCAAGTTTATTGTATGG	59,5	
PabrnhB-Up	AAAAAA**CCCGGG**AATCCCACCTGGAGCAGTAGC	61,8	
PabrnhB-Do	GACCCTACCGACGTTTCTAGGTTACTACTTCGACATTC	61,6	

Table S5. qPCR primers used for gene expression, related to Star Methods.

Name	Sequence 5' → 3'	Amplicon (bp)	Target
PCNA-Up	GCATGAGGGCAATGGATCCAA	198	TbaPCNA
PCNA-Do	GGGTTACCTCAAGGAAGTTCTCCTCA		
30S-Up	AGTGGTGGCCGCTATTATTG	156	Tba and Pab 30S
30S-Do	TAGGATTTCACCCCTACCCC		
TbaPolB-Up	AATTGATCTGCCGTATGTTGAGGTTGT	172	TbaPolB
TbaPolB-Do	AATGAACAACCTCAGTCCTAATTTCTGAGC		
TbaRNaseHII-Up	TTCGCCATGCTCTTTGTAGTACTCC	223	TbaRNaseHII
TbaRNaseHII-Do	CGAAATAATTGGGAAAGGCCTGAACTTCT		
PabPolB-Up	GAATACGACATACCCTTTGCGAAGC	170	PabPolB
PabPolB-Do	CCTTCCTCGTCGGCGTAGC		
PabRNaseHII-Up	GAGTTAAGGATTCAAAGCAGTTGACGC	200	PabRNaseHII
PabRNaseHII-Do	GTAGATAACTTCAGGCTTGACCTTCAGG		

REFERENCES

[S1] Marteinsson, V.T., Birrien, J.L., Reysenbach, A.L., Vernet, M., Marie, D., Gambacorta, A., Messner, P., Sleytr, U.B., and Prieur, D. (1999). *Thermococcus barophilus* sp. nov., a new barophilic and hyperthermophilic archaeon isolated under high hydrostatic pressure from a deep-sea hydrothermal vent. Int J Syst Bacteriol *49 Pt 2*, 351-359. http://doi.org/10.1099/00207713-49-2-351.

[S2] Birien, T., Thiel, A., Henneke, G., Flament, D., Moalic, Y., and Jebbar, M. (2018). Development of an Effective 6-Methylpurine Counterselection Marker for Genetic Manipulation in *Thermococcus barophilus*. Genes (Basel) *9*, 77. https://doi.org/10.3390/genes9020077.