Supplementary Information for:

Archaeal DNA polymerase D but not DNA polymerase B is required for genome replication in *Thermococcus kodakarensis*.

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Mutations in TK0664 that Resulted in 6MP^R. At minimum, one hundred independent cultures (3 ml) of *T. kodakarensis* KW128, TR1, TR2, TR3, TR4 and TS744 were grown in ASW-YT-S°, harvested, and dilutions plated on ASW-S° medium containing 100 μM 6MP plus all 20 amino acids. Cells from only two isolated colonies from each plate were used to inoculate cultures from which genomic DNA was isolated. Amplicons containing the TK0664 locus were generated and sequenced. The unique mutations identified in all strains are listed in Table S1 and their positions are shown in Figure S1. The presence or absence of Pol B had no statistically significant effect on the frequency or profile of the mutations that resulted in 6MP^R although deletions and insertions >3 bp occurred more frequently in *T. kodakarensis* TS744 that lacked Pol B. The mutation profiles revealed a very strong strand-bias for C to T transitions. In 79 of 80 mutations, the C to T transition occurred where C was in the template (non-coding) strand. Strand-biased DNA repair is known to occur in Bacteria and Eukaryotes when DNA damage is recognized by a transcribing RNA polymerase (1). Transcription stops and the stalled complex serves as a beacon for DNA repair but the repair occurs predominately on the template strand. The biased

profile of C to T transitions is not therefore likely to be a consequence of transcription-coupled repair, and it has been reported that transcription coupled DNA repair does not occur in Archaea (2, 3).

Complete deletion of TK0001. pBWB1 (Fig. S2) was constructed and transformed into *T. kodakarensis* strain KW128, and from the many colonies recovered, two strains (hereafter TS745-1 and TS745-2) resultant from confirmed double homologous recombination of pBWB1 into the genome of KW128 were retained. Colonies were recovered at the standard frequency, and two independently isolated strains, predicted to be genotypically identical, were phenotypically characterized (Fig. S2). A series of diagnostic PCRs (Fig. S2, panels C, D, and E) and Southern blots (Fig. S2, panel F) demonstrate the complete loss of the entire TK0001 open reading frame from the chromosome, as well as the integration of TK0254 as expected.

TS745-1 and TS745-2 behaved identically to each other as well as to strains TS742 and TS744 with respect of growth in liquid culture (Fig. S3) and on solidified media, response to UV light, and MMS and mitomycin C treatments. The full deletion of TK0001 is thus phenotypically identical, as predicted, to a partial deletion of TK0001 (strain TS744) and a strain containing a stop codon within TK0001 (strain TS742).

References for Supplementary Information

1- Hanawalt PC, Spivak G (2008) Transcription-coupled DNA repair: two decades of progress and surprises. Nat Rev Mol Cell Biol 9:958-970

2- Dorazi R, Gotz D, Munro S, Bernander R, White MF (2007) Equal rates of repair of DNA photoproducts in transcribed and non-transcribed strands in Sulfolobus solfataricus. Mol Microbiol 63:521-529

3- Romano V, Napoli A, Salerno V, Valenti A, Rossi M, Ciaramella M (2006) Lack of strand-specific repair of UV-induced DNA lesions in three genes of the archaeon Sulfolobus solfataricus. J Mol Biol 365:921-929.

Table S1. **Spontaneous mutations in TK0664 that resulted in 6MP^R**

Strain	[a]Unique mutations	[b]Single bp substitutions	[c]A→T T→A	[c]A→C T→G	[c]A→G T→C	[c]G→A C→T	[c]G→C C→G	[c]G→T C→A	[d]Del+Ins	[e]Del+Ins > 3bp
TR1	93	41 (44%)	0	3 (3%)	12 (13%)	20 (22%)	0	6 (6%)	52 (56%)	6 (6%)
TR2	90	42 (47%)	1 (1%)	1 (1%)	11 (12%)	23 (26%)	1 (1%)	5 (6%)	48 (53%)	1 (1%)
TR3	64	37 (58%)	1 (2%)	6 (9%)	4 (6%)	18 (28%)	0	5 (8%)	30 (47%)	3 (5%)
TR4	64	35 (55%)	4 (6%)	3 (5%)	1 (2%)	18 (28%)	0	4 (7%)	29 (45%)	0
TS744	51	8 (16%)	1 (2%)	1 (2%)	0	5 (10%)	0	1 (2%)	43 (84%)	17 (33%)

Footnotes:

[a] The number of unique mutations sequenced in spontaneously 6MP^R clones isolated from that *T. kodakarensis* strain.

[b] The number and percentage of the unique mutations that were single base pair substitutions.

[c] The nature of single base pair substitutions. The paired changes are indistinguishable (e.g. an A to T change in one strand cannot be differentiated from a T

to A change at the same position on the opposite strand).

[d] The number and percentage of the unique mutations that were deletions or insertions.

[e] The number and percentage of the unique mutations that were deletions or insertions longer than 3 bp.

Supplementary Figure legend

Fig. S1. Nucleotide and amino acid substitutions in TK0664 that resulted in $6MP^R$. The nucleotide and amino acid sequence encoded by TK0664 are shown with the mutations identified in $6MP^R$ isolates indicated above and the resulting amino acid substitutions indicated below the sequence. Asterisks identify the positions of nonsense mutations. A region within the TK0664 encoded protein conforms to the consensus for the active site of phosphoribosyltransferases (PRT). This is shown at the bottom of the figure with the missense and in-frame deletions that occurred within this motif highlighted. Two mutations that did not change the TK0664 coding sequence changed the sequence of the presumed promoter, and one changed the sequence of the presumed ribosome binding site (not shown).

Fig. S2. Deletion of the entire TK0001 open reading frame. A) Plasmid pBWB1 was designed to integrate into the KW128 chromosome and restore tryptophan prototrophy while deleting TK0001. B) The entirety of TK0001 was removed in strain TS745. The locations of primers (shown as black arrows, labeled I-X) and

PvuII restriction sites (shown as P) are indicated. C, D, and E) Diagnostic PCR amplifications with the primer pairs shown use genomic DNA isolated from the indicated strains as templates confirm the genomic architecture of strains KW128, and TS745-1 and TS745-2. M = DNA size markers; length in base pairs is indicated. F) Southern blots of PvuII digested total genomic DNA from the indicated strains confirms the loss of TK0001 from strains TS745-1 and TS745-2. An additional PvuII site is located immediately upstream of the TK0254 sequence. DIG-labeled probes were generated with the primer pairs shown above each blot.

Fig. S3. Deletion of TK0001 does not affect growth. Average values for the growth of three or more cultures of *T. kodakarensis* KW128 (□), TS745-1 (Δ), and TS745-2 (O) in ASW-YT-S˚ medium at 85°C are shown, with errors and with moving averages provided as trend lines.