Supplementary Information for

Coordination of multiple enzyme activities by a single PCNA in archaeal Okazaki fragment maturation

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Table S1. Oligonucleotides used to generate *in vitro* substrates

Substrates were assembled from oligonucleotides as described in Materials and Methods.

Ribonucleotides are underlined. All sequences are 5' to 3'.

Oligonucleotide	**Sequence**
Template	GTATAATGTTTATCTTATTTATCTCAATTAGCTAGATTAAGTAATTTCCAGAGG AAATAGATGGGTCCCACTTCGCTTTTAAATGGTATTTTGTGGACTT
Upstream	AAGTCCACAAAATACCATTTAAAAGCGAAG
Downstream_RNA	AUUUCCUCUGGAAATTACTTAATCTAGCTAATTGAGATAAATAAGATAAACATT ATAC
Downstream_DNA	ATTTCCTCTGGAAATTACTTAATCTAGCTAATTGAGATAAATAAGATAAACATT ATAC
Upstream_1nt	AAGTCCACAAAATACCATTTAAAAGCGAAGTGGGACCCATCTATTTCCTCTGGA AATTAC
Downstream_flap	TAAAGGAGACCTTTAATCTTAATCTAGCTAATTGAGATAAATAAGATAAACATT ATAC
Nick_1	GATCGTGGCTATTGTCGCCCTTATTCCGAT
Nick_2	AGTGACTACATTTTTGTAGTCACTATCGGAATAAGGGCGACAATAGCCACGATC
Template_Idling	GTTGTTGGGTTGGTTTTGTTTGGTGGAACCTTTTTTTTTTTTTTTTTTTTTTT TCTCCCTTCTTCTCCTCCCTCTCCCTTCCCT
Upstream_Idling	AGGGAAGGGAGAGGGAGGAGAAGAAGGGAG
Downstream_Idling	GGUUCCACCAAACAAAACCAACCCAACAAC

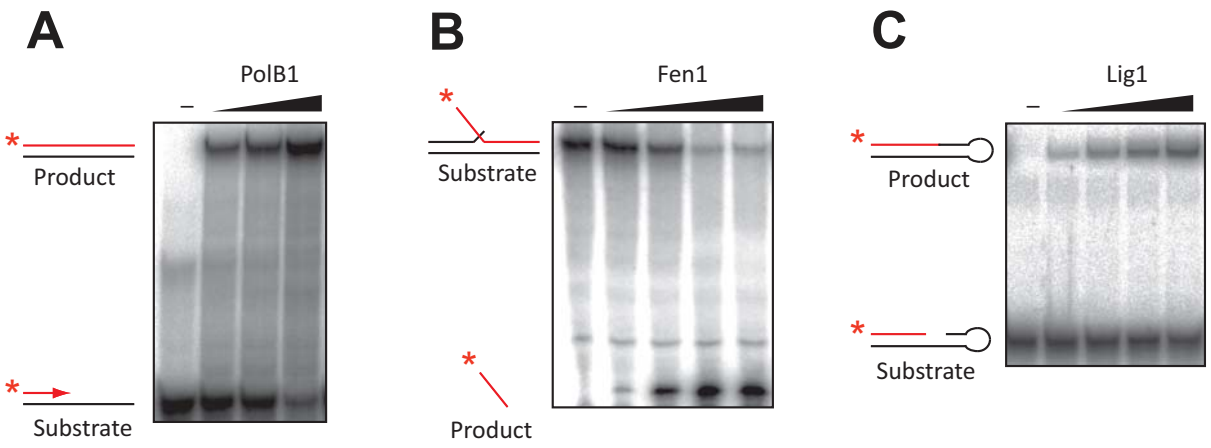

Figure S1. Catalytic activities of PolB1, Fen1 and Lig1 *in vitro*

(A) PolB1 *in vitro* activity on a model primer-template substrate. Substrate and product are indicated on the left with radiolabelled strand indicated in red and label position with a red asterisk. Substrate was incubated with 0, 2.5, 5, 10 fmol PolB1 and reaction products were separated by denaturing PAGE. Assays were performed as described in Materials and Methods with the exception that KCl and $MnCl_2$ were omitted. (B) Fen1 *in vitro* activity on an optimal double flap substrate. Substrate and product are indicated on the left with the radiolabelled strand indicated in red and label position with a red asterisk. Substrate was incubated with 0, 16, 258, 4125, 66000 fmol Fen1 and reaction products were separated by denaturing PAGE. (C) Lig1 *in vitro* activity on a model nicked substrate. Substrate and product are indicated on the left with radiolabelled strand indicated in red and label position with a red asterisk. Substrate was incubated with 0, 3.4, 6.7, 13.5, 27 pmol Lig1 and reaction products were separated by denaturing PAGE.

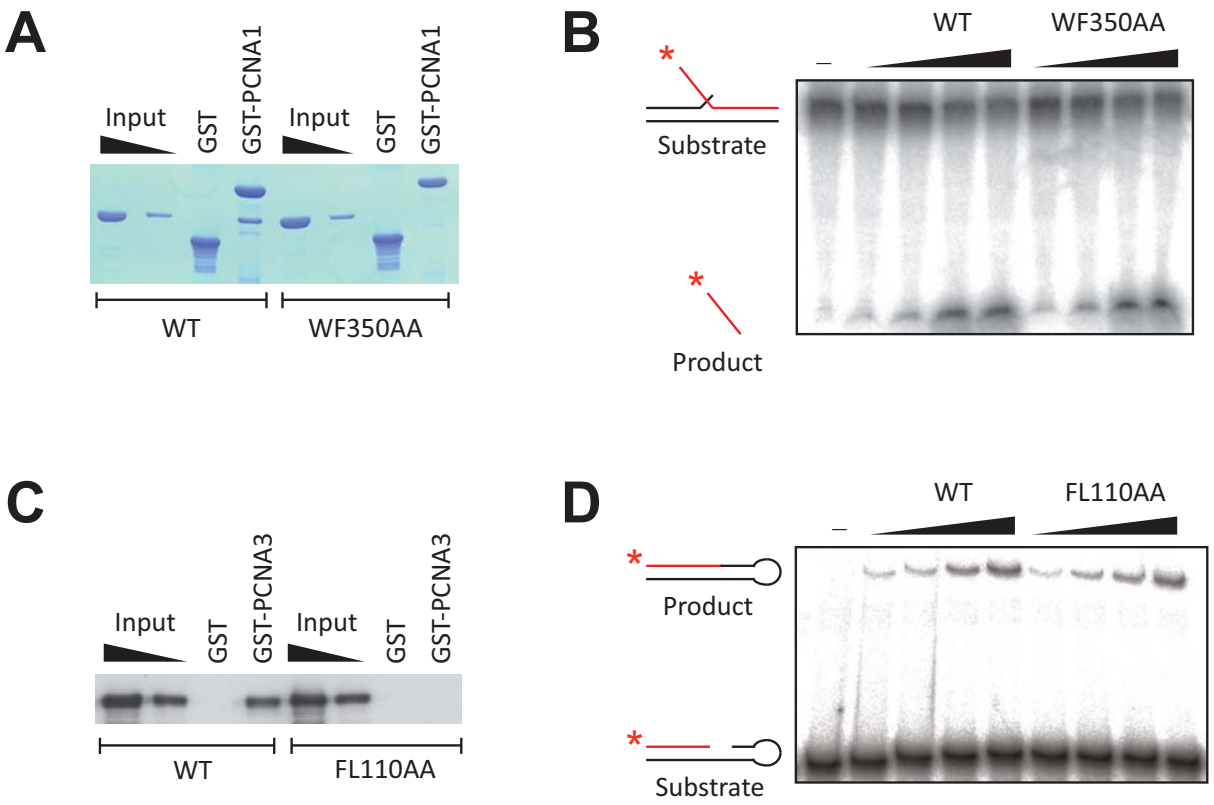

Figure S2. Characterisation of Fen1 and Lig1 PIP mutants

(A) Comparison of PCNA1 binding activity of WT or PIP mutant (WF350AA) Fen1 by GST-pull down. Reactions were analysed by SDS-PAGE and visualised with Coomassie staining. 50% and 10% Fen1 inputs are shown. (B) Comparison of the *in vitro* activity of WT or PIP mutant Fen1 (WF350AA) on an optimal double flap substrate. Substrate and product are indicated on the left with radiolabelled strands indicated in red and label position by a red asterisk. Substrate was incubated with 0, 55, 219, 875, 3500 fmol Fen1 proteins and reaction products were separated by denaturing PAGE. (C) Comparison of PCNA3 binding activity of WT or PIP mutant (FL110AA) Lig1 by GST-pull down. Reactions were analysed by western blotting. 50% and 10% Lig1 inputs are shown. (D) Comparison of the *in vitro* activity of WT or PIP mutant Lig1 (FL110AA) on a model nicked substrate. Substrate and product are indicated on the left with radiolabelled strands indicated in red and label position by a red asterisk. Substrate was incubated with 0, 1.37, 2.75, 5.5, 11 pmol Lig1 proteins and reaction products were separated by denaturing PAGE.

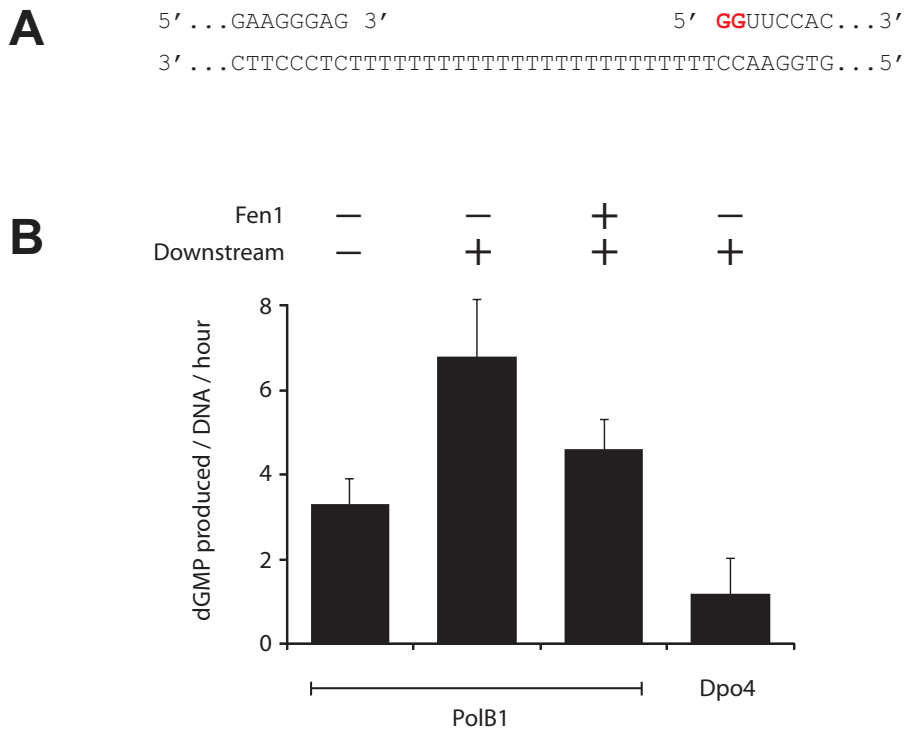

Figure S3. PolB1 undergoes limited idling at a nick in the presence of a downstream Okazaki fragment

(A) Sequence of the central region of substrate used to measure idling activity. Two guanosine residues initiating the downstream Okazaki fragment are highlighted in red, which must be strand displaced to enable dGTP incorporation into nascent DNA by PolB1. As these are the only sites available for dGTP incorporation downstream of the primer, turnover of dGTP to dGMP is indicative of idling at the nick site. (B) dGTP conversion to dGMP by DNA polymerases. Reaction products were separated by thin layer chromatography and conversion of dGTP to dGMP was quantified. Reactions contained 0.1pmol PolB1 or 50fmol Dpo4, 20pmol PCNA, and 0.1pmol Fen1 as indicated. 'Downstream' indicates the presence of a downstream Okazaki fragment. Values are mean ± s.e.m (n=3).

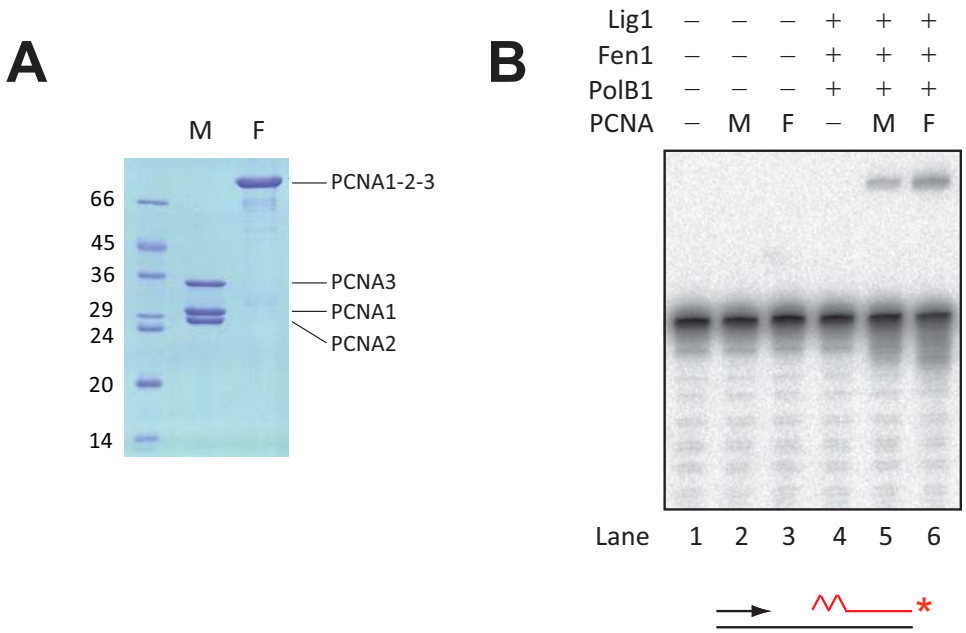

Figure S4. Both non-fused and fused heterotrimeric PCNA support Okazaki fragment maturation *in vitro*

(A) Comparison of purified recombinant PCNA by SDS-PAGE, visualised by Coomassie staining. Molecular weight markers are on the left, sizes are in KDa. M denotes an equimolar mix of PCNA1, PCNA2 and PCNA3, prepared as described in Supplementary Materials and Methods. F denotes a covalent fusion of PCNA1, PCNA2 and PCNA3. Migration of individual subunits is indicated on the right. (B) Comparison of non-fused and fused heterotrimeric PCNA in stimulating Okazaki fragment maturation *in vitro*. Reactions contained 0.1pmol PolB1, 0.1pmol Fen1, 1pmol Lig1 and 20pmol PCNA (total heterotrimer). Reaction products were separated by denaturing PAGE. Substrate is indicated below gel with radiolabelled strand indicated in red and label position with a red asterisk. The jagged region of the substrate denotes a 13 ribonucleotide primer.

Supplementary Materials and Methods

GST pull-down assays

GST-tagged PCNA1 or PCNA3 were prepared as previously (Dionne et al, 2003). Approximately 10µg of GST-fusion protein was incubated with 10µg interaction partner in 100µl TBSTM (10mM Tris pH 8, 150mM NaCl, 5mM $MgCl_2$, 0.1% Tween20) for 30 minutes at room temperature with agitation. Beads were recovered by centrifugation and washed three times with 500µl TBSTM. Beads were resuspended in 1X SDS loading dye and proteins were resolved on a 10 or 12% polyacrylamide gel and visualised with Coomassie staining (Fen1) or western blotting with Lig1 antisera (Lig1) (Dionne et al, 2003).

Idling assays

Assays were adapted from those developed previously (Garg et al, 2004). Substrates were generated by annealing Upstream_Idling to two-fold excess Template_Idling and four-fold excess Downstream_Idling (when present). Reaction mixtures (20µl) contained 50mM Tris pH 7.5, 100mM KCl, 10mM $MgCl_2$, 4mM $MnCl_2$, 1mM ATP, 100µg/ml BSA, 10mM DTT, 0.1pmol substrate, 100µM dATP, dCTP, dTTP, 10µM dGTP, and 50nM [α-^{32}P]dGTP. 0.1pmol PolB1, 50fmol Dpo4, 0.1pmol Fen1, and 20pmol PCNA were added as indicated, and reactions were incubated at 50˚C for 60 minutes before quenching by the addition of 10µl 25mM EDTA, 1% SDS, 5mM dGTP and 5mM dGMP. Reaction products were resolved by thin layer chromatography on PEI/cellulose in 0.5M LiCl, 0.5M formic acid and visualised by autoradiography.

Supplementary References

Dionne I, Nookala RK, Jackson SP, Doherty AJ, Bell SD (2003) A heterotrimeric PCNA in the hyperthermophilic archaeon Sulfolobus solfataricus. *Mol Cell* **11:** 275-282

Garg P, Stith CM, Sabouri N, Johansson E, Burgers PM (2004) Idling by DNA polymerase delta maintains a ligatable nick during lagging-strand DNA replication. *Genes Dev* **18:** 2764-2773