MATERIAL AND METHODS (Supplementary data)

SPR experiments
Data were obtained using a Reichert SR7000DC spectrometer instrument (Reichert Inc., Buffalo, NY). The running buffer was 25 mM HEPES pH 7.0, 150 or 300 mM NaCl, 1 mM DTT, 0.05 % Tween 20, and the flow rate was 25 µl/min. *Pfu*PCNA was immobilized on a mixed self-assembled monolayer (10 % C11-(OEG)6-COOH: 90 % C11-(OEG)3-OH), Reichert Inc.) via classical amine coupling chemistry and the chip was stabilized after serial injections of 100 mM H3PO4 (3 x 30 s). Each curve displayed was double referenced with a set of blank buffer injections. When the interaction with Mre11 PIP-like peptide was analysed, a concentration range from 0.37 µM to 30 µM of peptide was injected on the *Pfu*PCNA chip at 25°C. Acquisitions with *Pfu*MR were performed as described above, with a running buffer containing 25 mM HEPES pH 7.0, 300 mM NaCl, 1 mM DTT, 0.05 % Tween 20 and a concentration range of *Pfu*MR from 1.56 nM to 50 nM. Data were then fitted using a global analysis method with Scrubber 2.0a software (Biologic Software, Australia). Kinetic constants for binding were calculated using global fitting analysis that accounts for both association and dissociation phases as well as the maximal level of complex formation.

Co-immunoprecipitation experiments
Experiments were performed as described in the “materials and methods” section, except for the following steps. In 20 µl reaction, 1µg *Pfu*PCNA alone was incubated 10 min at 4°C with anti-PCNA Dynabeads prepared in 20 mM Tris-HCl pH 8.0, 150 mM NaCl, 1 mM DTT). Beads were washed with binding buffer (25 mM HEPES pH 7.0, 150 mM NaCl, 1 mM DTT, 0.05 % Tween 20). Then in 20 µl reaction, 5 µg *Pfu*MR or *Pfu*MR ΔPIP complex were added to *Pfu*PCNA in binding buffer 150 or 300 mM NaCl. The resulting protein complexes were incubated 15 min at 4°C. Beads were washed 3 times with 100 µl binding buffer 150 mM or 300 mM NaCl before final elution. Fractions bound to the beads were analysed either by Western-blotting (using His-tag antibody) or Coomassie blue staining as indicated in figure legends.

DNA substrates
Labelled dsDNA substrates were constructed with the following sequences. Phosphorothiate bonds are indicated by “s” between nucleotides.

*S50/50:*
5’Cy5-CTGCAGGGTTTTTGTTCCAGTCTGTAGCACTGTGAAGACAGGCGAGATG -3’
5’-CATCTGGCGCT-TCTTACACAGTGCTACAGACTGAACAAAAACCCTGCAG-3’
*Biotine_ S50/50s*
5’Cy5-CT(biotin)GCAGGGTTTTTGTTCCAGTCTGTAGCACTGTGAAGACAGGCGAGATG -3’
5’-CATCTGGCGCT-TCTTACACAGTGCTACAGACTGAACAAAAACCCTGsTsGsAsG-3’.
3’Cy5_S50/50s
5’-CTGCAGGGTTTTTGTTCCAGTCTGTAGCACTGTGAAGACAGGCGAGATG -3’Cy5
5’-CATCTGGCGCT-TCTTACACAGTGCTACAGACTGAACAAAAACCCTGsTsGsAsG-3’.
3’Cy5_S50s/50s
5'-CTGCAGGGTTTTGTCCAGTCTGACTGTGTAAGACAGCCAsGsAsTsG-3'Cy5
5'-CATCTGGGCTGTCTTACACAGTGCTACAGACTGGAACAAAACCCsTsGsCsAsG-3'.

S50/50s:
5'Cy5-CTGCAGGGTTTTGTCCAGTCTGACTGTGTAAGACAGCCAGATG-3'
5'-CATCTGGGCTGTCTTACACAGTGCTACAGACTGGAACAAAACCCsTsGsCsAsG-3'.

S50s/50s:
5'Cy5-CTGCAGGGTTTTGTCCAGTCTGACTGTGTAAGACAGCCAsGsAsTsG-3'
5'-CATCTGGGCTGTCTTACACAGTGCTACAGACTGGAACAAAACCCsTsGsCsAsG-3'.

S87/87s:
5'Cy5-CAGGAAACAGCTATGACCATTACAGAATTCGGGTACCGCTGATCCCGGGGATCTAGAG
TCGACCTGCAAGGCATGAAGCTGCTACTCTAGAGGATTCCCGAATTTCG
TAATCATGATGCTAGCTGTsTsCsTsG-3'

S87s/87s:
5'Cy5-CAGGAAACAGCTATGACCATTACAGAATTCGGGTACCGCTGATCCCGGGGATCTAGAG
TCGACCTGCAAGGCATGAAGCTGCTACTCTAGAGGATTCCCGAATTTCG
TAATCATGATGCTAGCTGTsTsCsTsG-3'

RQ-S87s/87s:
5'FAM-CAGGAAACAGCTATGACCATTACAGAATTCGGGTACCGCTGATCCCGGGGATCTAGAG
GTCGACCTGCAAGGCATGAAGCTGCTACTCTAGAGGATTCCCGAATTTCG
TAAT CATGATGCTAGCTGTsTs(BHQ1)CsCsTsG-3'

RQ23-S87s/87s:
5'CAGGAAACAGCTATGACCATTACAGAATTCGGGTACCGCTGATCCCGGGGATCTAGAG
GTCGACCTGCAAGGCATGAAGCTGCTACTCTAGAGGATTCCCGAATTTCG
TAAT(BHQ1)CATGATGCTAGCTGTsTsCsTsG-3'

The following oligonucleotides were used as reverse complement to avoid re-annealing of DNA product onto the dsDNA substrate.

Trap RC50:
5'-CTGCAGGGTTTTGTCCAGTCTGACTGTGTAAGACAGCCAGATG-3'

Trap RC87:
5'-CATCTGGGCTGTCTTACACAGTGCTACAGACTGGAACAAAACCCsTsGsCsAsG-3'.

Trap 3'87RC:
5'-CAGGAAACAGCTATGACCATTACAGAATTCGGGTACCGCTGATCCCGGGGATCTAGAG
GTCGACCTGCAAGGCATGAAGCTGCTACTCTAGAGGATTCCCGAATTTCG
TAAT(BHQ1)CATGATGCTAGCTGTsTsCsTsG-3'

Electrophoretic mobility shift assays (EMSAs)
In 10 µl reaction, 25 nM DNA substrate S50/50s were pre-incubated with 50 or 200 nM PfuPCNA, when indicated, in a DNA binding buffer (25 mM HEPES pH, 7.0, 150 or 300 mM NaCl, 1 mM DTT, 0.5 mg/ml
BSA, 0.1% Triton-X100) complemented with 1 mM ATP, 5 mM MgCl₂ and 5 mM MnCl₂ at ambient temperature for 5 min. DNA substrate or DNA/PfuPCNA complex were then incubated with a concentration range from 0 nM to 1000 nM of PfuMR at ambient temperature for 15 min. Samples were loaded on a 0.75% agarose gel and run for 4 hrs at 60 V at 4°C with running buffer 1X TBE. Gels were imaged with fluorimager Typhoon 9500 (GE Healthcare) and quantified with Image Quant software.

**ATPase assays**

Pre-incubation of 200 nM PfuMR wt or ΔPIP with 400 nM PfuPCNA (when indicated) was performed at 65°C for 10 min. Proteins were then incubated at 65°C for 30 min in 15 µl buffer containing 25 mM HEPES pH 7.0, 300 mM NaCl, 1 mM DTT, 0.5 mg/ml BSA, 5 mM MgCl₂ complemented with 200 nM dsDNA S50s/50s, when indicated, before adding 1 mM ATP and ATP γ³²P (62.5 nCi/µl) for 60 min. Two microliters of reaction solution were collected at various time points and spotted on TLC plates (PEI-cellulose, Nagel). ATP, ADP and Pi were separated by TLC using 250 mM KH₂PO₄. Products were analysed by autoradiography and level of Pi generated was quantified by MultiGauge (Version 3.0) software to quantify ATPase activity. Three independent experiments were performed to measure ATPase activities.

**Native gel DNA unwinding assays**

Reactions were performed as described in “Real time fluorescence DNA unwinding assays” section of the materials and methods, except that at the end of incubation at 55°C, the samples were loaded onto an electrophoresis gel composed of 10% polyacrylamide 19:1 and 1X TBE in a running buffer (1X TBE) for 3h at 120 V. Labelled fragments were analysed with fluorimager Typhoon 9500 (GE Healthcare) and quantified with Image Quant software.

**FIGURE LEGENDS – SUPPLEMENTAL DATA**

**Figure S1.** (A) Purified recombinant *P. furiosus* PCNA, Mre11-Rad50 and truncated constructs of Mre11 and Rad50. (B) Construct schematics for PfuMre11 and PfuRad50 (adapted from (1)). PfuRad50-link constructs contain Gly-Ser repeat sequences to link Rad50 N and C terminal lobes. In PfuMre11 sequence, dotted arrow indicates a disordered region. In PfuRad50 sequences, black arrows show region binding with Mre11 RBD, the shortened version PfuRad50link₂ lacks several amino acids required for Mre11 binding. (C) Influence of co-factors on the physical interaction between PfuPCNA and PfuMR. Co-immunoprecipitation experiments were performed as described in the “Material and methods” section using beads coated with PCNA antibodies. PfuPCNA and PfuMre11 were revealed through His-tag detection by western-blotting. The experiment was performed in condition containing 150 mM NaCl.

**Figure S2.** Mre11 and Hel308/Hjm proteins of order *Thermococcales* harbour a PIP-like motif. Alignment of selected C-terminal domains from archaeal proteins using ScanProsite with the pattern [PK]-x-[KRNA]-x-[GSPANK]-x(1,3)-[IL]-x(2)-[WFY]-[ILV] as anchor on archaeal proteins. The figure was generated using EsPript server and similar highlighted positions were determined using BLOSUM62 similarity matrix. Abbreviations: *Pfu*, Pyrococcus furiosus; *Pab*, Pyrococcus abyssi; *Pho*, Pyrococcus horikoshii; *PspNA2*, Pyrococcus sp. NA2; *Pya*, Pyrococcus yayanosii; *PspST04*, Pyrococcus sp.
ST04 ; *Pfe*, Palaeococcus ferophilus ; *Ppa*, Palaeococcus pacificus DY20341 ; *Tga*, Thermococcus gammatolerans ; *TspAM4*, Thermococcus sp. AM4 ; *Tna*, Thermococcus nautill ; *Ton*, Thermococcus onnurineus ; *Teu*, Thermococcus eurythermalis ; *Tko*, Thermococcus kodakarensis ; *Tzi*, Thermococcus zilligii ; *Tpa*, Thermococcus paralvinellae ; *Tli*, Thermococcus litoralis ; *TspPK*, Thermococcus sp. PK ; *Tsi*, Thermococcus sibiricus MM 739 ; *Tna*, Thermococcus nautili ; *Tko*, Thermococcus kodakarensis ; *Tzi*, Thermococcus zilligii ; *Tpa*, Thermococcus paralvinellae ; *Tli*, Thermococcus litoralis ; *TspPK*, Thermococcus sp. PK ; *Tsi*, Thermococcus sibiricus MM 739 ; *Tna*, Thermococcus nautili ; *Tko*, Thermococcus kodakarensis ; *Tzi*, Thermococcus zilligii ; *Tpa*, Thermococcus paralvinellae ; *Tli*, Thermococcus litoralis ; *TspPK*, Thermococcus sp. PK ; *Tsi*, Thermococcus sibiricus MM 739 ; *Tna*, Thermococcus nautili ; *Tko*, Thermococcus kodakarensis ; *Tzi*, Thermococcus zilligii ; *Tpa*, Thermococcus paralvinellae ; *Tli*, Thermococcus litoralis ; *TspPK*, Thermococcus sp. PK ; *Tsi*, Thermococcus sibiricus MM 739 ; *Tna*, Thermococcus nautili ; *Tko*, Thermococcus kodakarensis ; *Tzi*, Thermococcus zilligii ; *Tpa*, Thermococcus paralvinellae ; *Tli*, Thermococcus litoralis ; *TspPK*, Thermococcus sp. PK ; *Tsi*, Thermococcus sibiricus MM 739 ;

**Figure S3.** PIP-like motif of *Pfu* MR complex plays a key role in interaction with *Pfu* PCNA (A) Surface plasmon sensograms for binding of Mre11 PIP-like peptide onto immobilised *Pfu* PCNA with a concentration range from 0.37 µM to 30 µM of peptide and 30 µM of non-interacting peptide. The experiment was performed with running buffer containing 150 mM NaCl. (B) Left panel, SDS-PAGE of purified *Pfu* MR and *Pfu* MR ΔPIP proteins, right panel, co-immunoprecipitation assays with *Pfu* MR (wt or ΔPIP and *Pfu* PCNA in binding buffer with 150 or 300 mM NaCl. 1 µg of protein was loaded on SDS-PAGE as Input. IP corresponds to the immunoprecipitation assays in presence of beads coated with PCNA antibodies. Fractions bound to the beads were analysed by Coomassie blue staining. (C) Surface plasmon sensograms for binding of *Pfu* MR complex to immobilised *Pfu* PCNA from a concentration range from 1.56 nM to 50 nM of *Pfu* MR complex. Experimental curves were fitted with a model describing a PCNA:MR stoichiometry of 1:1 and a binding reaction with two events of distinct kinetic constants. Experiments were performed in condition containing 300 mM NaCl.

**Figure S4.** dsDNA end resection by *Pfu* MR nuclease activities. Reactions in (A) and (B) were performed in same conditions as Figure 3B and 3D, except that reaction buffer contained 150 mM NaCl. (C) Time course experiments. Reactions included 25 nM of dsDNA S50/50s incubated with 25 nM *Pfu* MR (left panel) at 70°C from 0 to 90 min and complemented by 50 nM *Pfu* PCNA (right panel). (D) 25 nM of DNA substrate were incubated at 70°C, 30 min with increasing concentrations of *Pfu* MR ΔPIP. (E) 25 nM of DNA substrate were pre-incubated with indicated concentrations of *Pfu* PCNA at room temperature for 5 min before adding 25 nM *Pfu* MR ΔPIP. Reactions were performed for 30 min at 70°C. Reaction buffer contained 300 mM NaCl.

**Figure S5.** *Pfu* PCNA and salinity do not influence DNA binding activity of *Pfu* MR. 25 nM of S50/50s DNA substrate incubated with an increasing concentration of *Pfu* MR complex at ambient temperature for 15 min complemented by 50 or 200 nM *Pfu* PCNA, when indicated, in condition containing 150 or 300 mM NaCl. Experiments were performed in triplicate and error bars correspond to standard deviation.

**Figure S6.** Influence of co-factors on the functional interaction between *Pfu* MR and *Pfu* PCNA. Reactions included 25 nM dsDNA S50/50s incubated 30 min at 70°C with 25 nM *Pfu* MR, complemented with 50 nM *Pfu* PCNA, 1 mM ATP, 5 mM MgCl₂ and 5 mM MnCl₂, when indicated. All experiments were performed in condition containing 300 mM NaCl.

**Figure S7.** Comparison of DNA resection by *Pfu* PCNA/MR wt and ΔPIP on different dsDNA substrates. DNA substrates are illustrated on top of the figures. 25 nM dsDNA substrates were pre-
incubated with 50 nM of PfuPCNA at room temperature for 5 min before adding 25 nM PfuMR. The reactions were performed for 30 min at 70°C.

**Figure S8. DNA unwinding activity of PfuPCNA:MR complex at 55°C.** Reactions included 25 nM RQ-S87s/87s (A) or RQ23-S87s/87s (B) DNA substrates incubated 30 min at 55°C with 25 nM PfuMR wt or ∆PIP, complemented with 50 nM PfuPCNA, 1 mM ATP and 5 mM MgCl₂, 5 mM MnCl₂, when indicated. All experiments were performed in condition containing 300 mM NaCl.

**Figure S9. PfuPCNA has no effect on ATPase activity of PfuMR.** ATPase assays were carried out at 65°C with 200 nM PfuMR wt or ∆PIP in presence of 1 mM ATP, 5 mM MgCl₂, and complemented with 200 nM dsDNA S50s/50s and 400 nM PfuPCNA when indicated. Experiments were performed in triplicate and error bars correspond to standard deviation.

**REFERENCE**

1. Williams, G.J., Williams, R.S., Williams, J.S., Moncalian, G., Arvai, A.S., Limbo, O., Guenther, G., SilDas, S., Hammel, M., Russell, P. *et al.* (2011) ABC ATPase signature helices in Rad50 link nucleotide state to Mre11 interface for DNA repair. *Nature Structural & Molecular Biology*, 18, 423-431.
| Mre11     | PluMre11 (414-425) | K P R D S M T G | PabMre11 (411-422) | K P P D L M A W K | PhoMre11 (400-411) | K P P D L T A M R | PspNA2Mre11 (400-411) | K P P D L M A W R | PyaMre11 (400-411) | K P P D L A M G | PspST04Mre11 (412-423) | K R R D L A M R | PlmMre11 (429-442) | D K S V T D A F K | PpaMre11 (432-443) | K R R D L A M K | TgaMre11 (447-458) | A P P S L A D M R | TspAM4Mre11 (451-462) | A P P S L A D M R | TnaMre11 (451-462) | A P P S L A D M R | TonMre11 (442-453) | K G K D L A M G | TeuMre11 (441-452) | K G K N L A M G | TkoMre11 (446-457) | S G K N L D M G | TziMre11 (438-449) | S G K N L V M G | TpaMre11 (431-442) | K K K D L A M K | TbaMre11 (431-442) | K K K D L A M K | TlmMre11 (427-438) | K K K D L S M K | TspPKMre11 (430-441) | E K K D L S M K | TsiMre11 (429-440) | P V P D L A M G | Tsp4557Mre11 (455-466) | K K K P S L A D M R | TclMre11 (450-461) | A P P S L A D M R |
|----------|-------------------|-----------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| Hel308/   | PluHel308 (708-719) | P K K T D Y R K | PabHel308 (703-714) | P K K T D Y R K | PhoHel308 (703-714) | K P K T D Y R H | PspNA2Hel308 (703-714) | K P K T D Y R S | PyaHel308 (701-712) | K L K T D D R K | PspST04Hel308 (708-719) | K P R T D Y R R | PpaHel308 (713-724) | K A K T D D R K | PlmHel308 (707-718) | K R K T D D R K | TgaHel308 (709-720) | K K K T D D R K | TspAM4Hel308 (709-720) | K K K T D D R K | TnaHel308 (709-720) | K K K T D D R K | TonHel308 (715-726) | K K K T D D R K | TeuHel308 (710-721) | K K K T D D R K | TkoHel308 (1114-1125) | K K K N L Y D R K | TziHel308 (715-726) | K G K T D D R K | TpcHel308 (712-720) | K G K T D D R K | TpaHel308 (712-720) | K G K T D D R K | TbaHel308 (726-737) | K P K T D Y R K | TsiHel308 (713-724) | K V K T D D R K | Tsp4557Hel308 (715-726) | K A K T D D R K | TclHel308 (715-726) | K R K T D D R K |
| Hjm      | (715-726)         | (715-726)       | (715-726)         | (715-726)         | (715-726)         | (715-726)         | (715-726)         | (715-726)         | (715-726)         | (715-726)         | (715-726)         | (715-726)         | (715-726)         | (715-726)         | (715-726)         | (715-726)         | (715-726)         |
Figure S4

A

B

C

Table:

| PtMR (nM) | Ratio DNA/PtMR | PtMR (25 nM) | PtMR (25 nM) + PtNPCNA (50 nM) |
|-----------|----------------|--------------|---------------------------------|
| 0         | 1.0            | -            | -                               |
| 0.5       | 2.0            | -            | -                               |
| 1         | 0.5            | -            | -                               |
| 1.5       | 1.0            | -            | -                               |
| 5         | 2.0            | -            | -                               |
| 10        | 0.5            | -            | -                               |
| 20        | 1.0            | -            | -                               |
| 30        | 2.0            | -            | -                               |
| 60        | 0.5            | -            | -                               |
| 90        | 1.0            | -            | -                               |

Legend:
PtMR (50 nM)
PTMR (25 nM)
PtNPCNA (50 nM)
- (No addition)
Figure S5

A

- **PfuMR + PfuPCNA (50 nM), 150 mM NaCl**
- **PfuMR, 150 mM NaCl**
- **PfuMR + PfuPCNA (200 nM), 300 mM NaCl**
- **PfuMR, 300 mM NaCl**

![Graph showing DNA binding vs. MR concentration](image)

DNA Bound / Total DNA vs. [MR] (nM)
Figure S9

A

B

|        | Hydrolysed ATP % |
|--------|------------------|
| Mg²⁺   | +                |
| Mn²⁺   | -                |
| dsDNA  | -                |
| PfuMR  | +                |

|        | Hydrolysed ATP % |
|--------|------------------|
| Mg²⁺   | +                |
| Mn²⁺   | -                |
| dsDNA  | +                |
| PfuMR  | -                |
| PfuMR ΔPIP | -       |
| PfuPCNA| +                |

