MATERIAL AND METHODS (Supplementary data)

SPR experiments

Data were obtained using a Reichert SR7000DC spectrometer instrument (Reichert Iinc., 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 H_3PO_4 (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-CTGCAGGGTTTTTGTTCCAGTCTGTAGCACTGTGTAAGACAGGCCAGATG -3'

5'-CATCTGGCCTG-TCTTACACAGTGCTACAGACTGGAACAAAAACCCTGCAG-3'

Biotine_S50/50s

5'Cy5-C**T(biotin)**GCAGGGTTTTTGTTCCAGTCTGTAGCACTGTGTAAGACAGGCCAGATG -3'

5'-CATCTGGCCTGTCTTACACAGTGCTACAGACTGGAACAAAAACCCsTsGsCsAsG-3'.

3'Cy5_S50/50s

5'-CTGCAGGGTTTTTGTTCCAGTCTGTAGCACTGTGTAAGACAGGCCAGATG-3'Cy5

5'-CATCTGGCCTGTCTTACACAGTGCTACAGACTGGAACAAAAACCCsTsGsCsAsG-3'.

3'Cy5_S50s/50s

5’-CTGCAGGGTTTTTGTTCCAGTCTGTAGCACTGTGTAAGACAGGCCsAsGsAsTsG-3’Cy5

5’-CATCTGGCCTGTCTTACACAGTGCTACAGACTGGAACAAAAACCCsTsGsCsAsG-3’.

S50/50s:

5’Cy5-CTGCAGGGTTTTTGTTCCAGTCTGTAGCACTGTGTAAGACAGGCCAGATG-3’

5’-CATCTGGCCTGTCTTACACAGTGCTACAGACTGGAACAAAAACCCsTsGsCsAsG-3’.

S50s/50s:

5’Cy5-CTGCAGGGTTTTTGTTCCAGTCTGTAGCACTGTGTAAGACAGGCCsAsGsAsTsG-3’

5’-CATCTGGCCTGTCTTACACAGTGCTACAGACTGGAACAAAAACCCsTsGsCsAsG-3’.

S87/87s:

5’Cy5-CAGGAAACAGCTATGACCATGATTACGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAG
TCGACCTGCAGGCATGCAAGCTTGGCA-3’

5’-TGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCG
TAATCATGGTCATAGCTGTTsTsCsCsTsG-3’

S87s/87s:

5’Cy5-CAGGAAACAGCTATGACCATGATTACGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAG
TCGACCTGCAGGCATGCAAGCTsTsGsGsCsA-3’

5’-TGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCG
TAATCATGGTCATAGCTGTTsTsCsCsTsG-3’

RQ-S87s/87s:

5’FAM- CAGGAAACAGCTATGACCATGATTACGAATTCGAGCTCGGTACCCGGGGATCCTCTAGA
GTCGACCTGCAGGCATGCAAGCTsTsGsGsCsA-3’

5’-TGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAA
TTCGTAATCATGGTCATAGCTGTTs**Ts(BHQ1)**CsCsTsG-3’

RQ23-S87s/87s:

5’CAGGAAACAGCTATGACCATGA**T(FAM)**TACGAATTCGAGCTCGGTACCCGGGGATCCTCTAGA
GTCGACCTGCAGGCATGCAAGCTsTsGsGsCsA-3’

5’- TGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCG
TAA**T(BHQ1)**CATGGTCATAGCTGTTsTsCsCsTsG-3’

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

Trap RC50:

5’-CTGCAGGGTTTTTGTTCCAGTCTGTAGCACTGTGTAAGACAGGCCAGATG-3’

Trap RC87:

5’-CAGGAAACAGCTATGACCATGATTACGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCG
ACCTGCAGGCATGCAAGCTTGGCA-3’

Trap 3’87RC

5’-CAGGAAACAGCTATGACCATGATTACGAAT-3’

Electrophoretic mobility shift assays (EMSAs)

In 10 µl reaction, 25 nM DNA substrate S50/50s were pre-incubated with 50 or 200 nM *Pfu*PCNA, 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 $MgCl_2$ and 5 mM $MnCl_2$ at ambient temperature for 5 min. DNA substrate or DNA/*Pfu*PCNA complex were then incubated with a concentration range from 0 nM to 1000 nM of *Pfu*MR 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 *Pfu*MR wt or ΔPIP with 400 nM *Pfu*PCNA (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 $MgCl_2$ 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 KH_2PO_4. 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 *Pfu*Mre11 and *Pfu*Rad50 (adapted from (1)). *Pfu*Rad50-link constructs contain Gly-Ser repeat sequences to link Rad50 N and C terminal lobes. In *Pfu*Mre11 sequence, dotted arrow indicates a disordered region. In *Pfu*Rad50 sequences, black arrows show region binding with Mre11 RBD, the shortened version *Pfu*Rad50^{link2} lacks several amino acids required for Mre11 binding. (C) Influence of co-factors on the physical interaction between *Pfu*PCNA and *Pfu*MR. Co-immunoprecipitaion experiments were performed as described in the “Material and methods“ section using beads coated with PCNA antibodies. *Pfu*PCNA and *Pfu*Mre11 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-[GSPNK]-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 ferrophilus ; *Ppa*, Palaeococcus pacificus DY20341 ; *Tga*, Thermococcus gammatolerans ; *TspAM4*, Thermococcus sp. AM4 ; *Tna*, Thermococcus nautili ; *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 ; *Tsp4557*, Thermococcus sp. 4557 ; *Tcl*, Thermococcus cleftensis.

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 $MgCl_2$ and 5 mM $MnCl_2$, 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 *Pfu*PCNA at room temperature for 5 min before adding 25 nM *Pfu*MR. The reactions were performed for 30 min at 70°C.

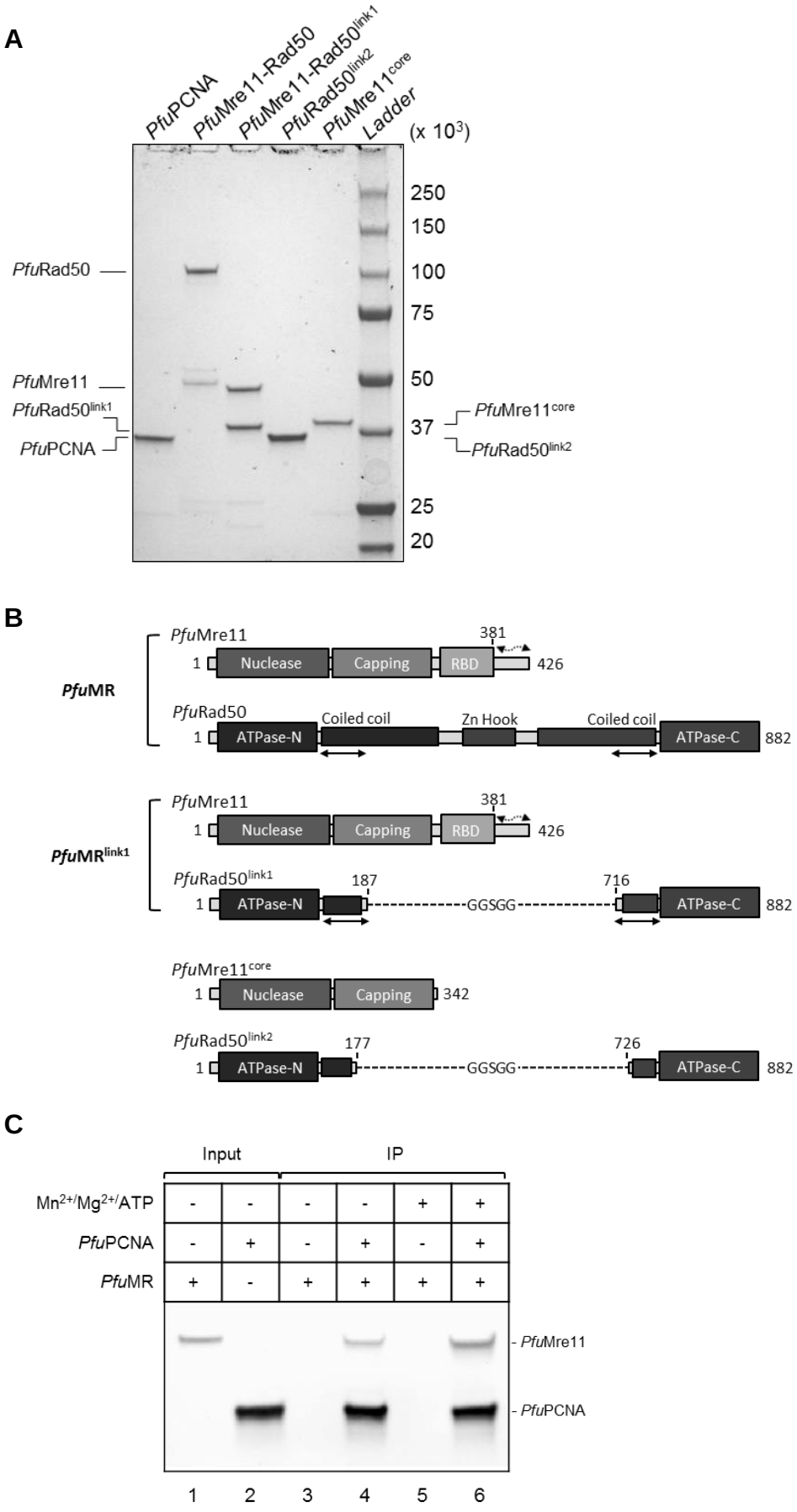
Figure S8. DNA unwinding activity of *Pfu*PCNA/MR complex at 55°C. Reactions included 25 nM RQ-S87s/87s **(A)** or RQ23-S87s/87s **(B)** DNA substrates incubated 30 min at 55°C with 25 nM *Pfu*MR wt or ΔPIP, complemented with 50 nM *Pfu*PCNA, 1 mM ATP and 5 mM $MgCl_2$, 5 mM $MnCl_2$, when indicated. All experiments were performed in condition containing 300 mM NaCl.

Figure S9. *Pfu*PCNA has no effect on ATPase activity of *Pfu*MR. ATPase assays were carried out at 65°C with 200 nM *Pfu*MR wt or ΔPIP in presence of 1 mM ATP, 5 mM $MgCl_2$, and complemented with 200 nM dsDNA S50s/50s and 400 nM *Pfu*PCNA 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.

Figure S1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PfuPCNA
PfuMre11-Rad50
PfuMre11-Rad50link1
PfuRad50link2
PfuMre11core
Ladder
(x 10³)
250
150
100
75
50
37
25
20
PfuRad50
PfuMre11
PfuRad50link1
PfuPCNA
PfuMre11core
PfuRad50link2
B
PfuMR
PfuMre11
381
1
Nuclease
Capping
RBD
426
PfuRad50
Coiled coil
Zn Hook
Coiled coil
ATPase-N
ATPase-C
882
PfuMRlink1
PfuMre11
PfuRad50link1
187
716
GGSGG
PfuMre11core
342
PfuRad50link2
177
726
C
Input
IP
Mn2+/Mg2+/ATP
PfuPCNA
PfuMR
- PfuMre11
- PfuPCNA
1 2 3 4 5 6

Figure S2

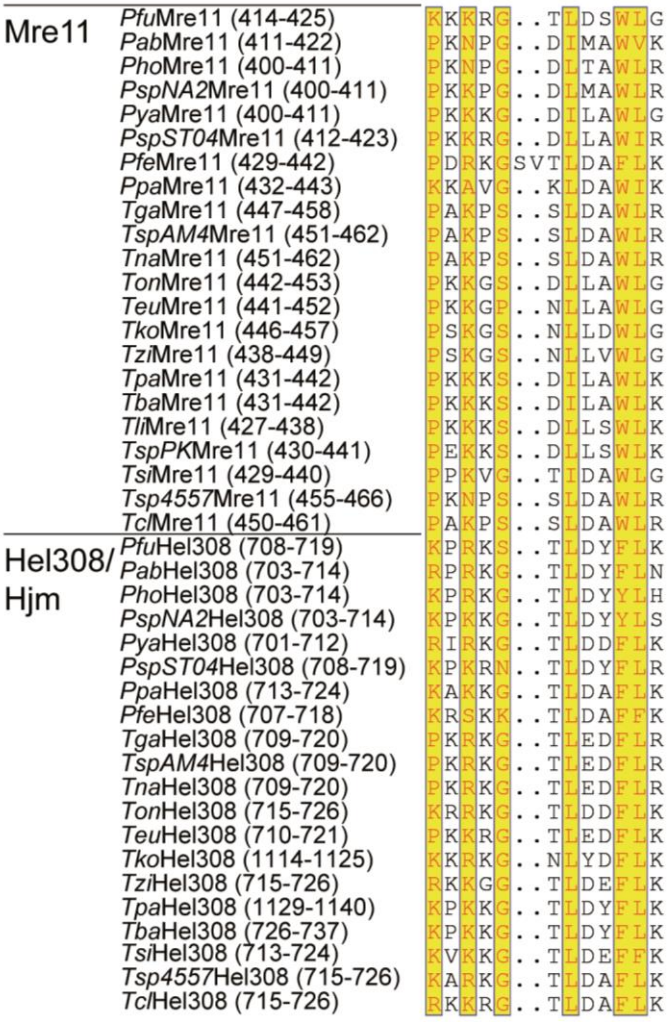
Mre11
PfuMre11 (414-425) KKKRG..TLDSWLG
PabMre11 (411-422) PKNPG..DIMAWVK
PhoMre11 (400-411) PKNPG..DLTAWLR
PspNA2Mre11 (400-411) PKKPG..DLMAWLR
PyaMre11 (400-411) PKKKG..DILAWLG
PspST04Mre11 (412-423) PKKRG..DLLAWIR
PfeMre11 (429-442) PDRKGSVTLDAFLK
PpaMre11 (432-443) KKAVG..KLDAWIK
TgaMre11 (447-458) PAKPS..SLDAWLR
TspAM4Mre11 (451-462) PAKPS..SLDAWLR
TnaMre11 (451-462) PAKPS..SLDAWLR
TonMre11 (442-453) PKKGS..DLLAWLG
TeuMre11 (441-452) PKKGP..NLLAWLG
TkoMre11 (446-457) PSKGS..NLLDWLG
TziMre11 (438-449) PSKGS..NLLVWLG
TpaMre11 (431-442) PKKKS..DILAWLK
TbaMre11 (431-442) PKKKS..DILAWLK
TliMre11 (427-438) PKKKS..DLLSWLK
TspPKMre11 (430-441) PEKKS..DLLSWLK
TsiMre11 (429-440) PPKVG..TIDAWLG
Tsp4557Mre11 (455-466) PKNPS..SLDAWLR
TclMre11 (450-461) PAKPS..SLDAWLR
Hel308/ Hjm
PfuHel308 (708-719) KPRKS..TLDYFLK
PabHel308 (703-714) RPRKG..TLDYFLN
PhoHel308 (703-714) KPRKG..TLDYYLH
PspNA2Hel308 (703-714) KPKKG..TLDYYLS
PyaHel308 (701-712) RIRKG..TLDDFLK
PspST04Hel308 (708-719) KPKRN..TLDYFLR
PpaHel308 (713-724) KAKKG..TLDAFLK
PfeHel308 (707-718) KRSKK..TLDAFFK
TgaHel308 (709-720) PKRKG..TLEDFLR
TspAM4Hel308 (709-720) PKRKG..TLEDFLR
TnaHel308 (709-720) PKRKG..TLEDFLR
TonHel308 (715-726) KRRKG..TLDDFLK
TeuHel308 (710-721) PKKRG..TLEDFLK
TkoHel308 (1114-1125) KKRKG..NLYDFLK
TziHel308 (715-726) RKKGG..TLDEFLK
TpaHel308 (1129-1140) KPKKG..TLDYFLK
TbaHel308 (726-737) KPKKG..TLDYFLK
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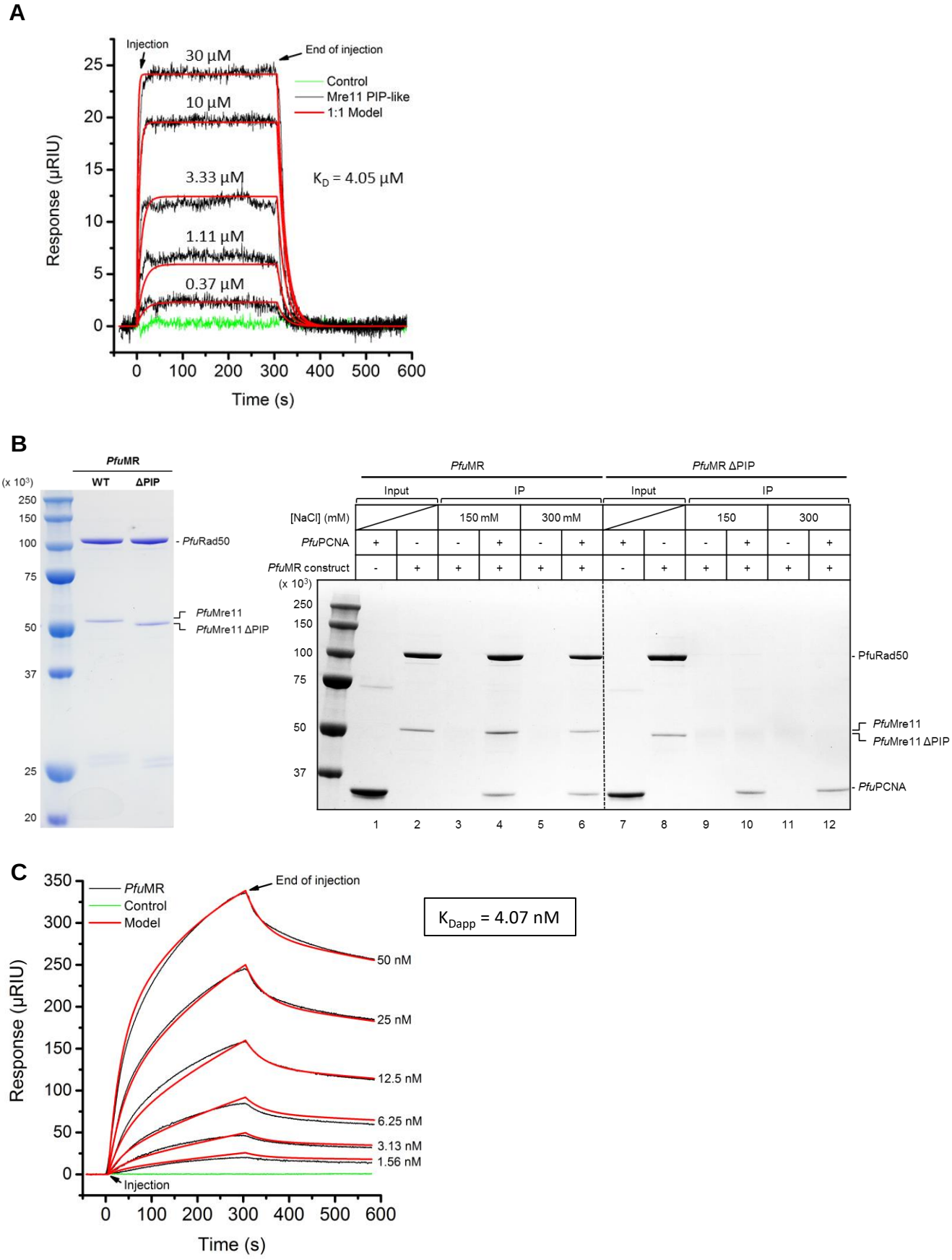
Figure S3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Injection
End of injection
30 µM
10 µM
3.33 µM
1.11 µM
0.37 µM
Control
Mre11 PIP-like
1:1 Model
K_D = 4.05 µM
Response (µRIU)
Time (s)
B
PfuMR
WT
ΔPIP
(x 10^3)
PfuRad50
PfuMre11
PfuMre11 ΔPIP
PfuMR
PfuMR ΔPIP
Input
IP
[NaCl] (mM)
150 mM
300 mM
PfuPCNA
PfuMR construct
PfuPCNA
1 2 3 4 5 6 7 8 9 10 11 12
C
PfuMR
Control
Model
End of injection
50 nM
25 nM
12.5 nM
6.25 nM
3.13 nM
1.56 nM
Injection
K_Dapp = 4.07 nM
Response (µRIU)
Time (s)

Figure S4

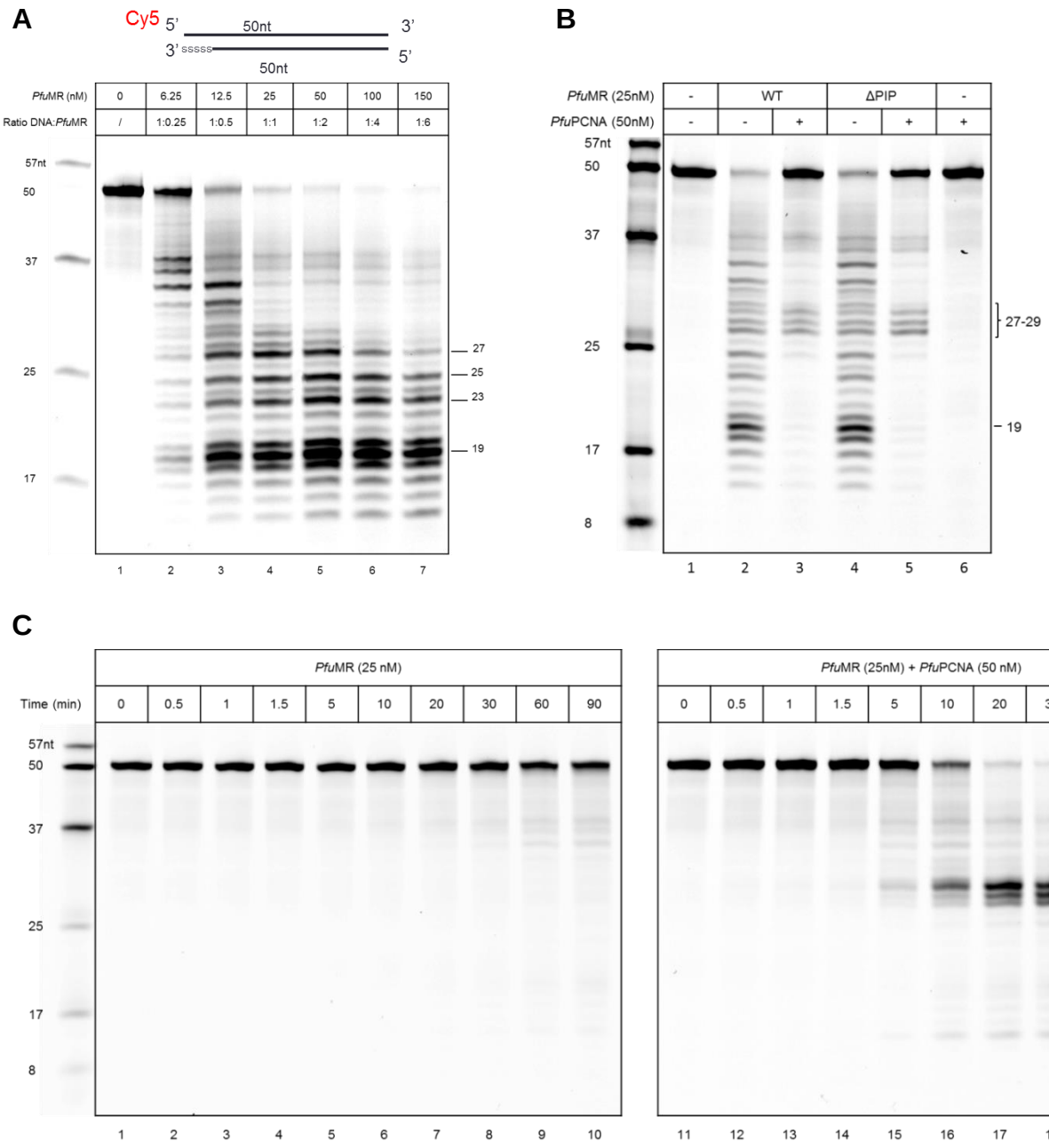

A
Cy5
5'
50nt
3'
3'
sssss
50nt
5'
PfuMR (nM)
0
6.25
12.5
25
50
100
150
Ratio DNA:PfuMR
/
1:0.25
1:0.5
1:1
1:2
1:4
1:6
57nt
50
37
25
17
27
25
23
19
1
2
3
4
5
6
7
B
PfuMR (25nM)
PfuPCNA (50nM)
-
WT
ΔPIP
-
-
-
+
-
+
+
57nt
50
37
25
17
8
27-29
19
1
2
3
4
5
6
C
PfuMR (25 nM)
PfuMR (25nM) + PfuPCNA (50 nM)
Time (min)
0
0.5
1
1.5
5
10
20
30
60
90
57nt
50
37
25
17
8
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

Figure S4

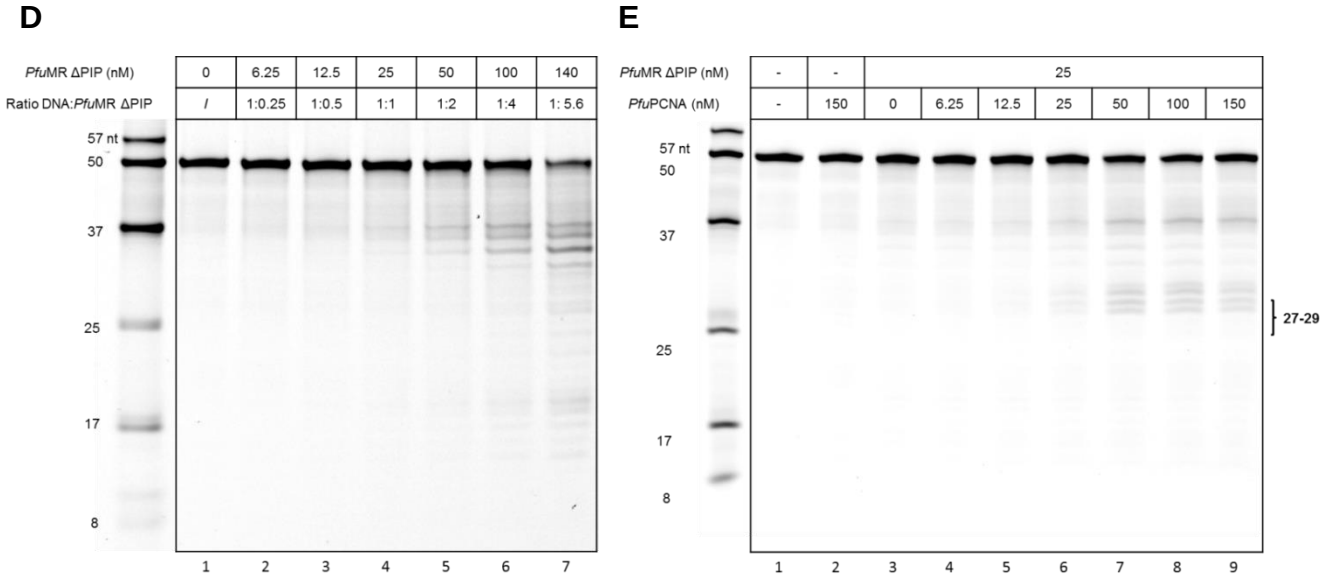

D
PfuMR ΔPIP (nM)
0
6.25
12.5
25
50
100
140
Ratio DNA:PfuMR ΔPIP
/
1:0.25
1:0.5
1:1
1:2
1:4
1: 5.6
57 nt
50
37
25
17
8
1
2
3
4
5
6
7
E
PfuMR ΔPIP (nM)
-
-
25
PfuPCNA (nM)
-
150
0
6.25
12.5
25
50
100
150
57 nt
50
37
25
17
8
27-29
1
2
3
4
5
6
7
8
9

Figure S5

A

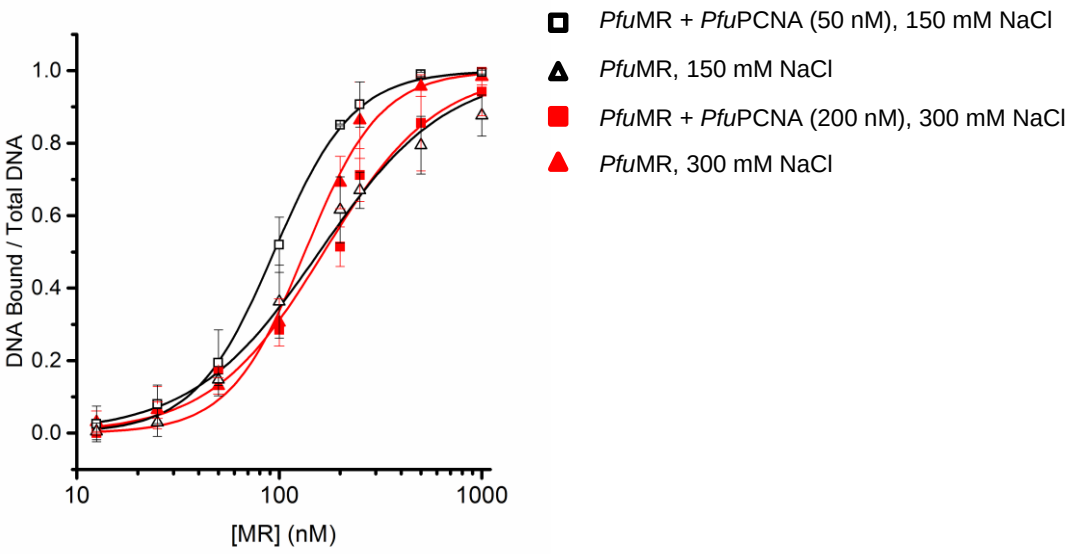

1.0
0.8
0.6
0.4
0.2
0.0
DNA Bound / Total DNA
10
100
1000
[MR] (nM)
*Pfu*MR + *Pfu*PCNA (50 nM), 150 mM NaCl
*Pfu*MR, 150 mM NaCl
*Pfu*MR + *Pfu*PCNA (200 nM), 300 mM NaCl
*Pfu*MR, 300 mM NaCl

Figure S6

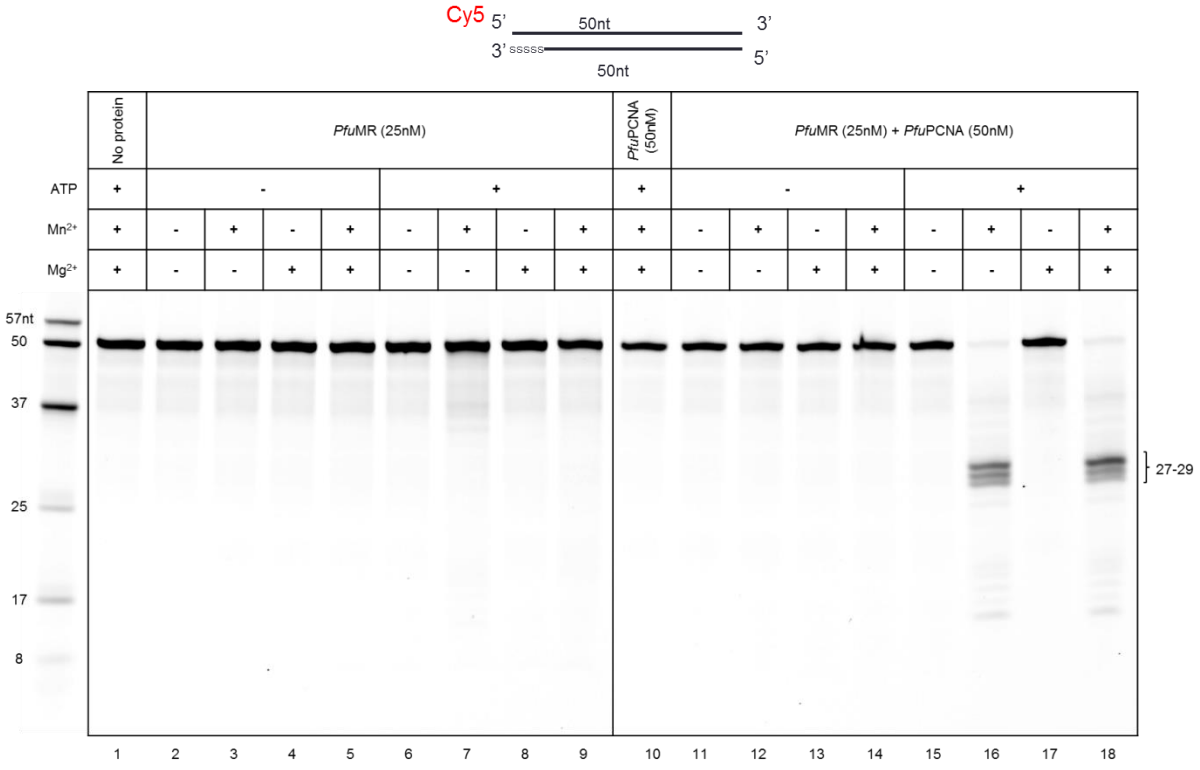
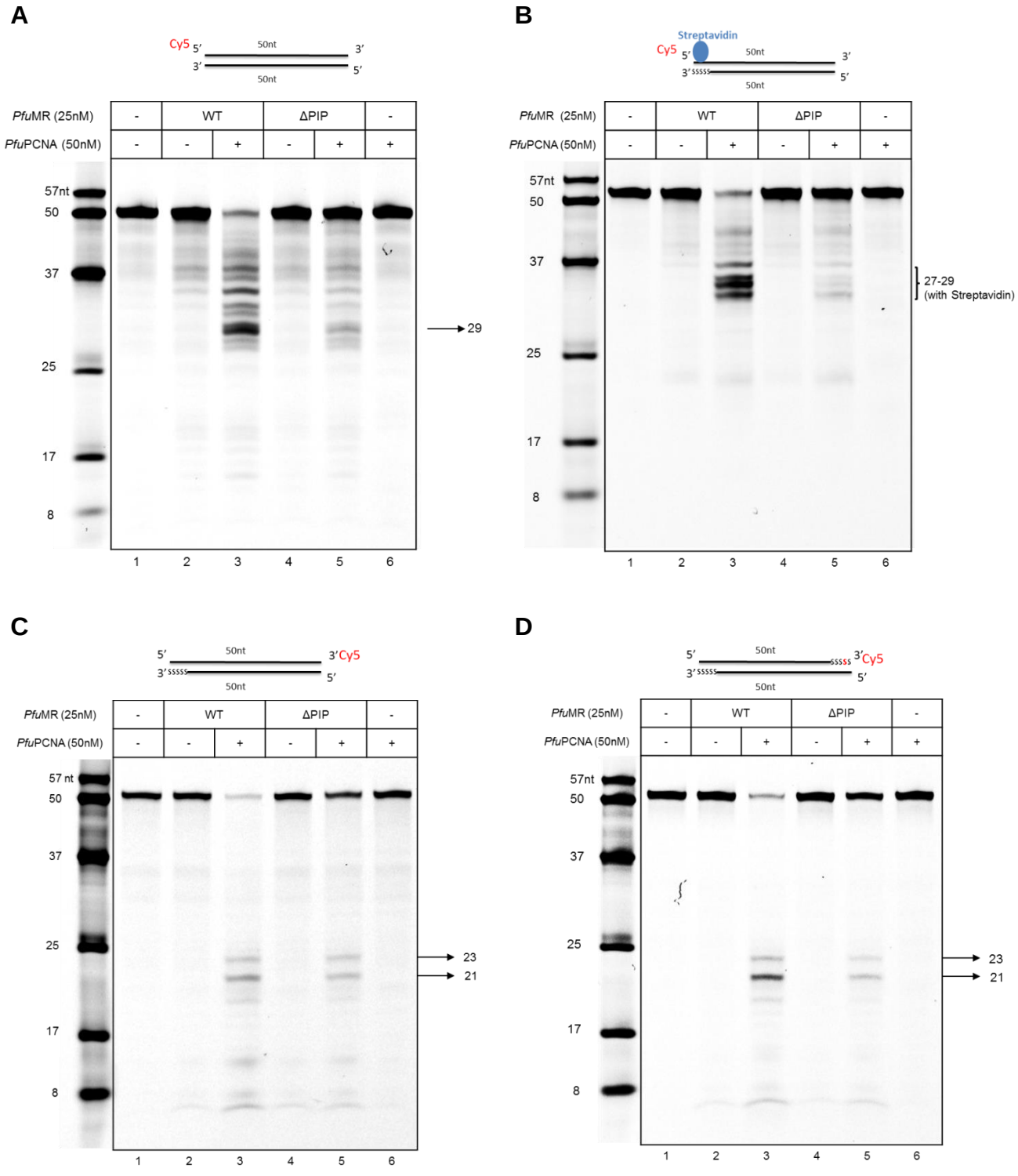

Cy5
5'
50nt
3'
3' sssss
5'
50nt
No protein
PfuMR (25nM)
PfuPCNA (50nM)
PfuMR (25nM) + PfuPCNA (50nM)
ATP
Mn2+
Mg2+
57nt
50
37
25
17
8
27-29
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

Figure S7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Cy5
5′
50nt
3′
3′
5′
50nt
PfuMR (25nM)
PfuPCNA (50nM)
WT
ΔPIP
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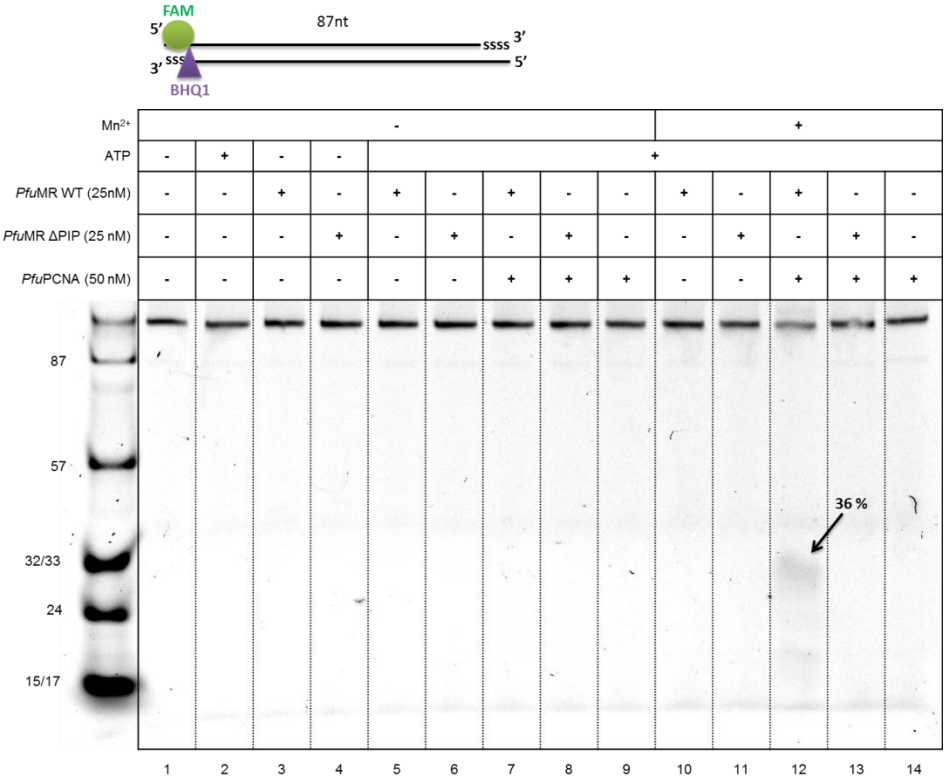
50
37
25
17
8
29
1 2 3 4 5 6
B
Streptavidin
Cy5
27-29
(with Streptavidin)
C
3′Cy5
57 nt
23
21
D

Figure S8

A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87nt
5′
3′
ssss
BHQ1
Mn2+
ATP
PfuMR WT (25nM)
PfuMR ΔPIP (25 nM)
PfuPCNA (50 nM)
87
57
32/33
24
15/17
36 %
1 2 3 4 5 6 7 8 9 10 11 12 13 14

B

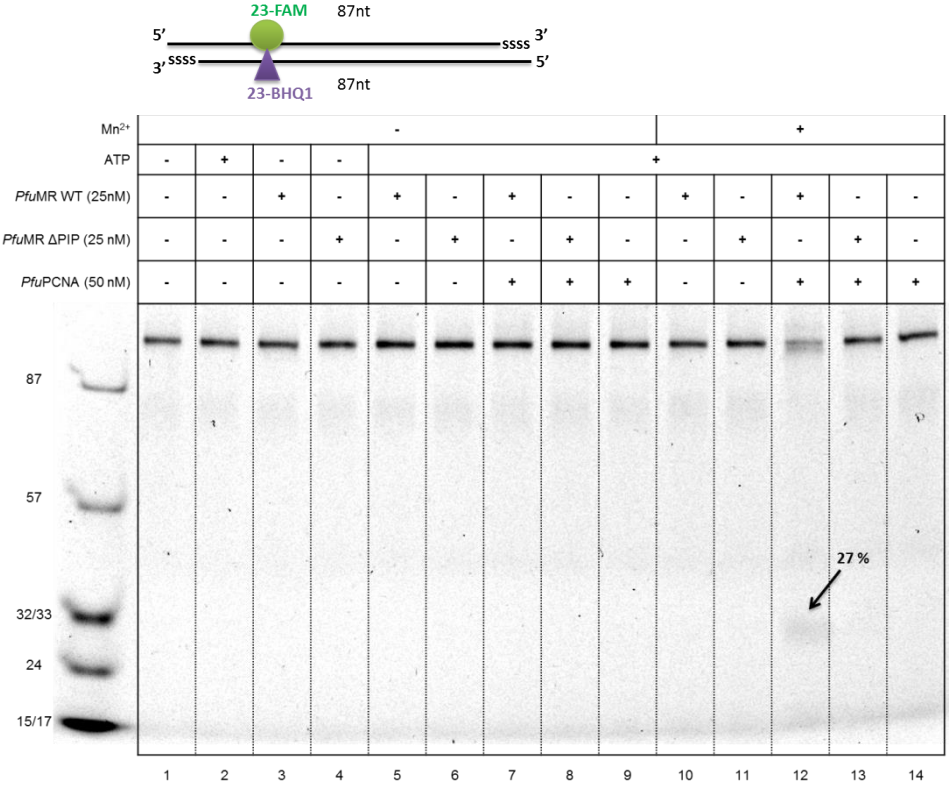
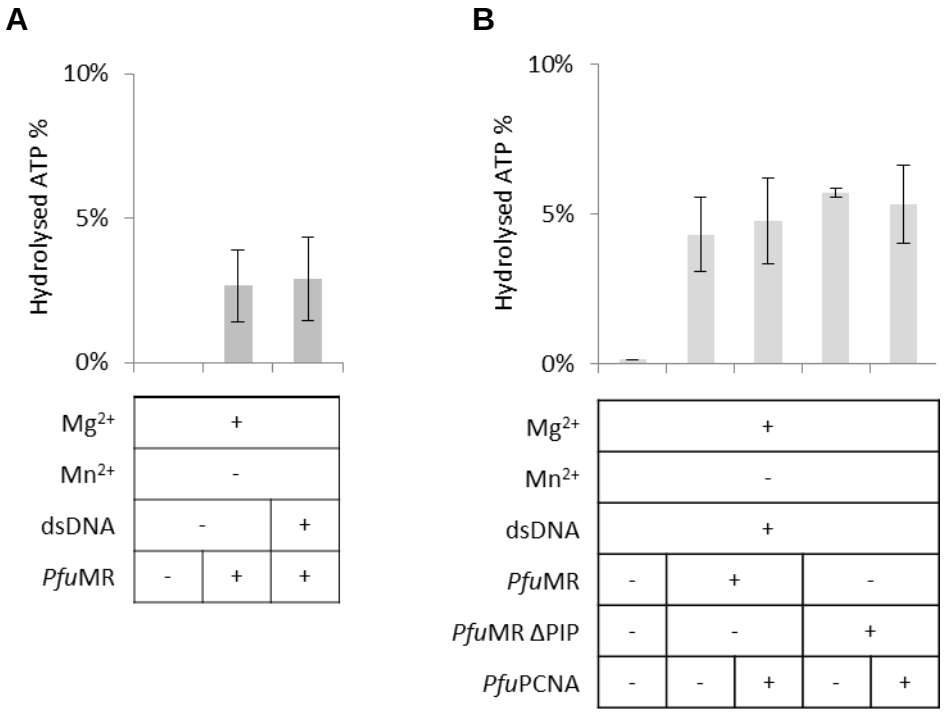

23-FAM
87nt
5′
3′
ssss
23-BHQ1
87nt
Mn2+
ATP
PfuMR WT (25nM)
PfuMR ΔPIP (25 nM)
PfuPCNA (50 nM)
87
57
32/33
24
15/17
27 %
1 2 3 4 5 6 7 8 9 10 11 12 13 14

Figure S9


A
B
10%
5%
0%
Hydrolysed ATP %
Mg2+
Mn2+
dsDNA
PfuMR
PfuMR ΔPIP
PfuPCNA
+
-