

Supplementary Information for

Replisome bypass of a protein-based R-loop block by Pif1

Grant D. Schauer^{a,1,*}, Lianne M. Spengelink^{b,c,1}, Jacob S. Lewis^{b,c,e}, Olga Yurieva^d, Stefan H. Mueller^{b,c}, Antoine M. van Oijen^{b,c,*} and Michael E. O'Donnell^{d*}

*Correspondence should be addressed to:

Grant D. Schauer, grant.schauer@colostate.edu

Antoine M. van Oijen, Email: vanoijen@uow.edu.au

Michael E. O'Donnell, Email: odonnel@rockefeller.edu

This PDF file includes:

Figures S1 to S4

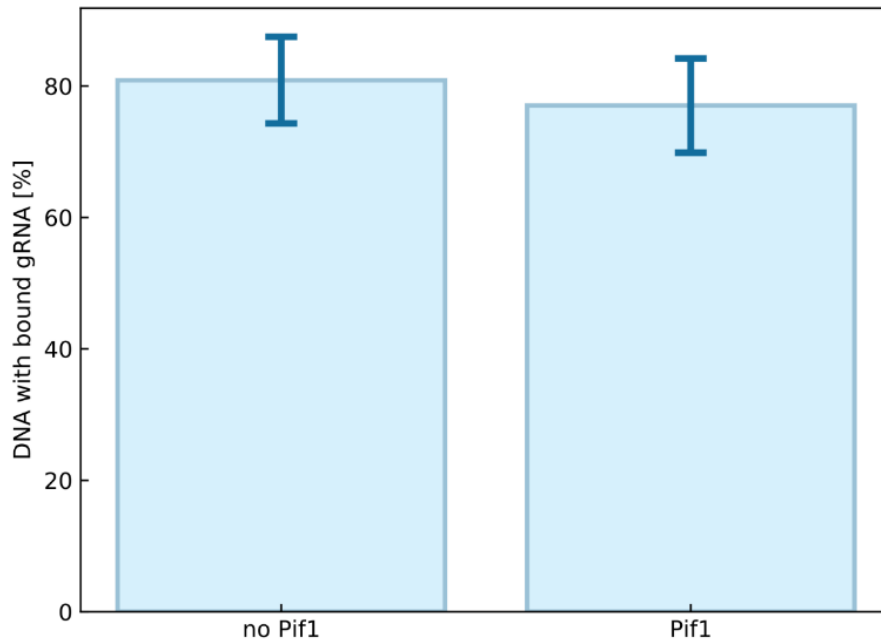


Fig. S1. Pif1 does not displace roadblocks in absence of the replisome. Results are from single-molecule replication studies (see Methods). The amount of DNA molecules with bound dCas9-gRNA was observed before addition of Pif1 and after 30 min of incubation with 80 nM Pif1. Over 4 individual movies the amount of bound dCas9-gRNA stayed constant within uncertainty levels. $(80.9 \pm 6.6)\%$ in absence, $(77.0 \pm 7.2)\%$ in presence of Pif1.

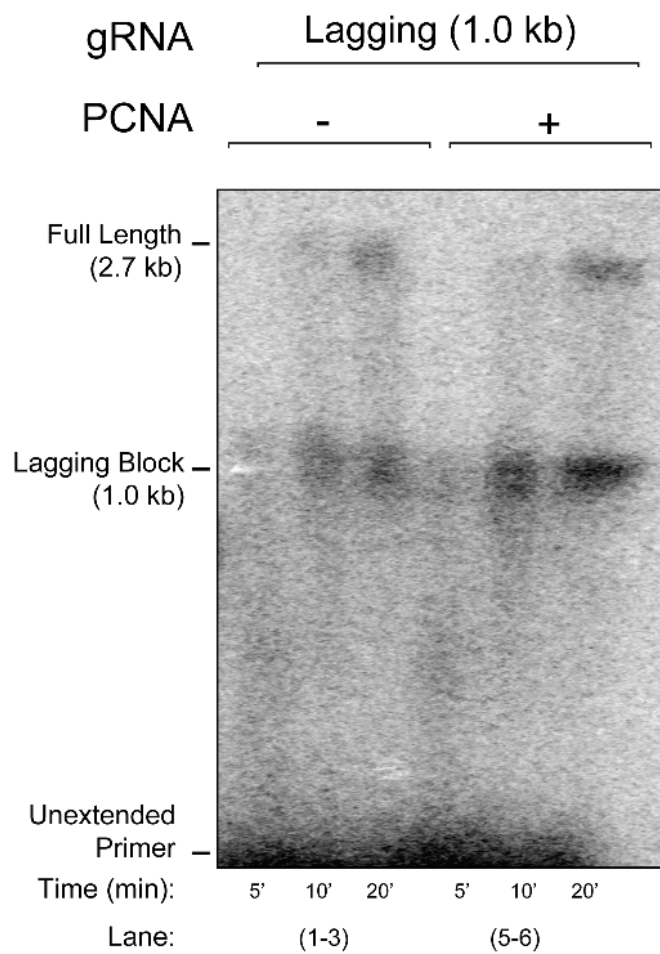


Fig. S2. PCNA is not required for Pif1 mediated bypass of a lagging strand dCas9 block. Alkaline agarose gel of the indicated replication products in the presence of a lagging strand block.

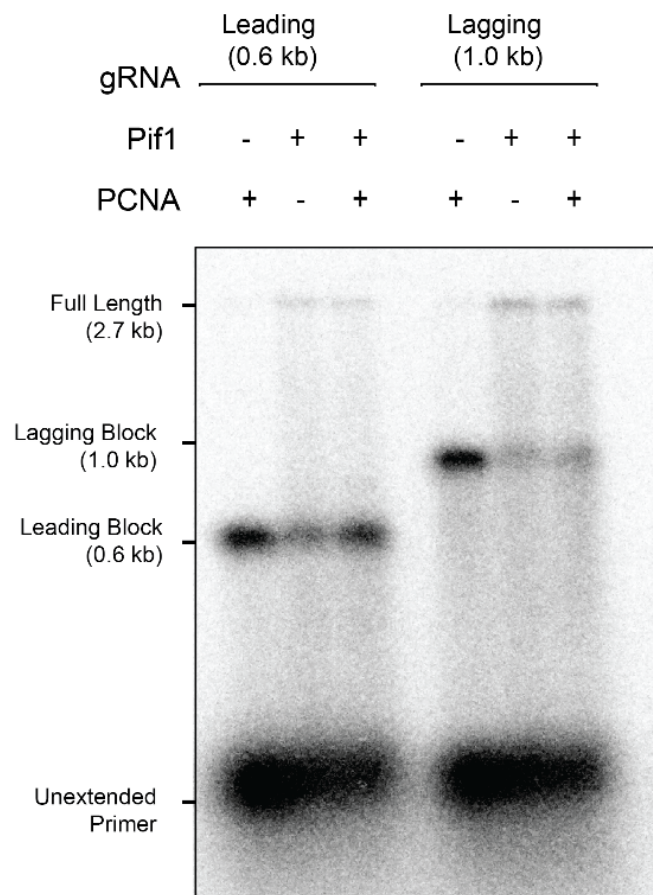


Fig. S3. PCNA does not enhance bypass in the presence of the MTC complex and Mcmc10. Replication products in the presence of the indicated block and the presence or absence of Pif1 and/or PCNA is shown. MTC and Mcm10 were present in all reactions.

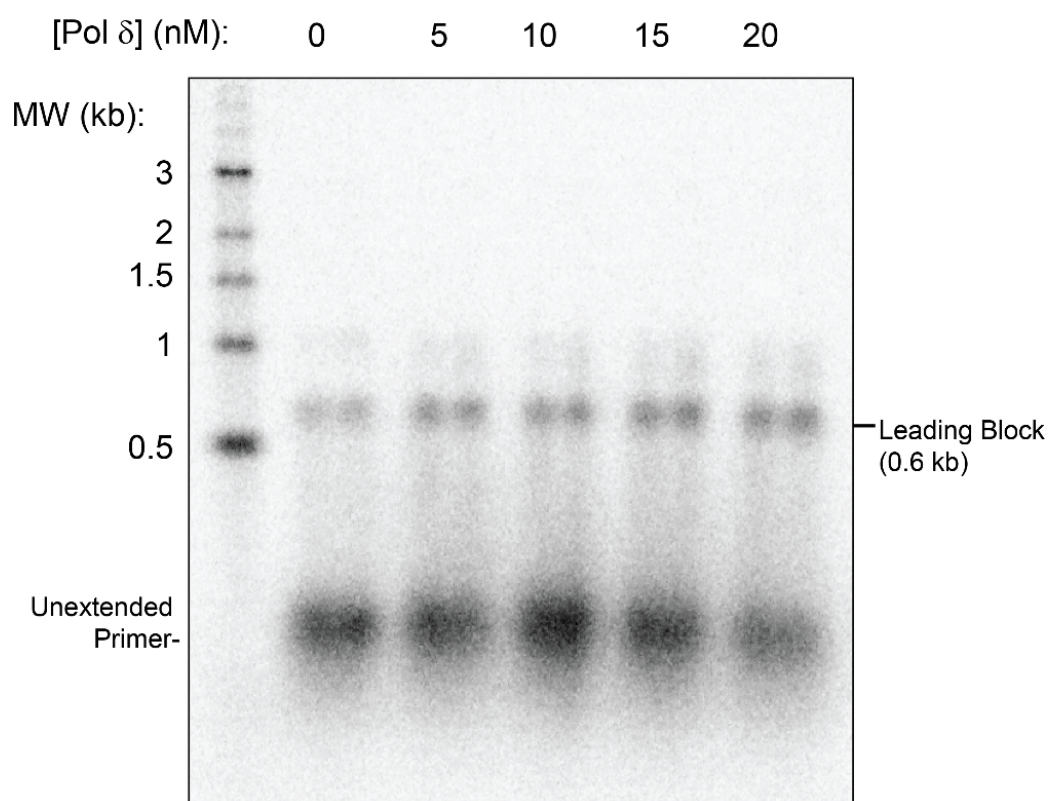


Fig. S4. Addition of Pol delta-PCNA and Pol delta does not permit displacement of the dCas9 R-loop block. Replication products in the presence of the leading strand dCas9 R-loop block at 0.6 kb are shown. In addition to CMG, Pol delta, RFC, PCNA, Mcm10, and MTC, Pol δ were present at 20 nM, and Pol delta was added at the indicated concentrations.