

**Cell, Volume 188**

**Supplemental information**

**Mechanism of DNA capture by the MukBEF**

**SMC complex and its inhibition**

**by a viral DNA mimic**

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## Supplemental Tables

**Table S1.** Bacterial strains. Related to STAR Methods.

Strain ID	Genotype	Figures/Notes
BL21(DE3)	F <sup>-</sup> , <i>lon</i> <sup>-</sup> , <i>ompT</i> <sup>-</sup> , <i>hsdS</i> ( <i>rb</i> <sup>-</sup> <i>mb</i> <sup>-</sup> ), <i>dcm</i> <sup>+</sup> , <i>gal</i> , $\lambda$ (DE3)	
BL21-Gold(DE3)	F <sup>-</sup> , <i>lon</i> <sup>-</sup> , <i>ompT</i> <sup>-</sup> , <i>hsdS</i> ( <i>rb</i> <sup>-</sup> <i>mb</i> <sup>-</sup> ), <i>dcm</i> <sup>+</sup> , <i>tet</i> , <i>gal</i> , $\lambda$ (DE3), <i>endA</i> <sup>-</sup> , <i>Hte</i>	
C41(DE3)	F <sup>-</sup> , <i>ompT</i> , <i>gal</i> , <i>dcm</i> , <i>hsdSB</i> ( <i>rb</i> <sup>-</sup> <i>mb</i> <sup>-</sup> ), $\lambda$ (DE3)	
MG1655	F <sup>-</sup> , $\lambda$ <sup>-</sup> , <i>rph</i> -1, <i>fnr</i> <sup>+</sup>	3A
SFB012	MG1655, <i>mukB</i> :: <i>neoR</i>	S1F
SFB017	MG1655, <i>mukB</i> - <i>HaloTag</i> (C61V, C262A):: <i>neoR</i>	S1F
SFB053	MG1655, $\Delta$ <i>mukFEB</i> :: <i>pheS</i> (T251A, A294G)- <i>hygR</i> , pKW20 Para <i>lambda-red cas9 tet tracrRNA</i>	
SFB065	DH5 $\alpha$ , pJF146 RK24 <i>lux apR bsd</i>	
SFB174	MG1655, <i>mukF</i> (D227C, Q412C) <i>mukE mukB</i> (R143C, R771C, C1118S, K1246C)-TEV- <i>HaloTag</i> (C61V, C262A):: <i>neoR</i>	S1F
SFB208	MG1655, $\Delta$ <i>mukFEB</i> :: <i>Pth mukFEB</i> -TEV- <i>HaloTag</i> (C61V, C262A):: <i>neoR</i>	S1F
SFB209	MG1655, $\Delta$ <i>mukFEB</i> :: <i>Pth mukF</i> (D227C, E412C) <i>mukE mukB</i> (R143C, R771C, C1118S, K1246C)-TEV- <i>HaloTag</i> (C61V, C262A):: <i>neoR</i>	S1F
SFB289	MG1655, <i>mukB</i> - <i>HaloTag</i> (C61V, C262A):: <i>neoR</i> , pBAD322A	S3H
SFB290	MG1655, <i>mukB</i> - <i>HaloTag</i> (C61V, C262A):: <i>neoR</i> , pBAD322A <i>gp5.9</i>	S3H
SFB292	MG1655, $\Delta$ <i>mukFEB</i> :: <i>Pth mukFEB</i> -TEV- <i>HaloTag</i> (C61V, C262A):: <i>neoR</i> , pBAD322A	S3H
SFB293	MG1655, $\Delta$ <i>mukFEB</i> :: <i>Pth mukFEB</i> -TEV- <i>HaloTag</i> (C61V, C262A):: <i>neoR</i> , pBAD322A <i>gp5.9</i>	S3H
MEK1326	MG1655, $\Delta$ <i>recB</i>	3B, gift from Meriem El Karoui

**Table S2.** Plasmids. Related to STAR Methods.

ID	Name	Description	Source
pFB062	pET-Gate2 MukE-CPD-His10	T7 expression plasmid for producing <i>E. coli</i> MukE	This study
pFB069	pET-Gate2 MukF His6-SUMO-MukE	T7 expression plasmid for producing <i>E. coli</i> MukEF	This study
pFB070	pET-Gate2 MukF MukE-CPD-His10	T7 expression plasmid for producing <i>E. coli</i> MukEF	This study
pFB083	pGEX GST-hSENP1	T7 expression plasmid for producing GST-tagged hSENP1	Komander lab
pFB403	pET-Gate2 <i>Pth</i> MukF MukE His6-SUMO-MukB	T7 expression plasmid for producing SUMO-tagged <i>P. thracensis</i> MukBEF	Bürmann et al., 2021
pFB411	pCONEX-Gate4 CRISPR(mukFEB cloDF13) <i>ccdB</i>	Shuttle plasmid for targeting of the <i>mukFEB</i> locus ( <i>Bsal</i> acceptor); crRNA targets pKW20 plasmid	Bürmann et al., 2021
pFB468	pET-Gate2 <i>Pth</i> His6-SUMO-MukB	T7 expression plasmid for producing <i>P. thracensis</i> MukB	Bürmann et al., 2021
pFB478	pET-Gate2 <i>Pth</i> His6-SUMO-ParE	T7 expression plasmid for producing <i>P. thracensis</i> ParE	This study
pFB479	pET-Gate2 <i>Pth</i> His6-SUMO-ParC	T7 expression plasmid for producing <i>P. thracensis</i> ParC	This study
pFB481	pET-Gate2 <i>Pth</i> MukF His6-SUMO-MukE	T7 expression plasmid for producing <i>P. thracensis</i> MukEF	This study
pFB520	pET-Gate2 <i>Pth</i> MukF(D227C, Q412C) MukE His6-SUMO-MukB(R143C, R771C, C1118S, K1246C)	T7 expression plasmid for producing cysteine mutant <i>P. thracensis</i> MukBEF	This study
pFB522	pET-Gate2 <i>Pth</i> MukF(D227C, Q412C) MukE	T7 expression plasmid for producing cysteine mutant <i>P. thracensis</i> MukEF	This study
pFB525	pET-Gate2 <i>Pth</i> His6-SUMO-MukB(R143C, R771C, C1118S, K1246C, E1407Q)	T7 expression plasmid for producing cysteine mutant <i>P. thracensis</i> MukB(EQ)	This study
pFB526	pUC19 matS2	Entrapment assay substrate	This study
pFB527	pUC19 matS2(scrambled)	Entrapment assay substrate	This study
pFB638	pET-Gate2 His6-SUMO-GyrA	T7 expression plasmid for producing <i>E. coli</i> GyrA	This study
pFB639	pET-Gate2 His6-SUMO-GyrB	T7 expression plasmid for producing <i>E. coli</i> GyrB	This study
pFB661	pET-Gate2 MukF(D227C, Q412C) MukE His6-SUMO-MukB(R143C, R771C, C1118S, K1246C)	T7 expression plasmid for producing cysteine mutant <i>E. coli</i> MukBEF	This study
pFB662	pET-Gate2 His6-SUMO-MukB(R143C, R771C, C1118S, K1246C)	T7 expression plasmid for producing cysteine mutant <i>E. coli</i> MukB	This study
	pBAD322K	Arabinose inducible vector, <i>kanR</i>	Cronan, 2006
	pBAD322K-gp5.9	Arabinose inducible gp5.9, <i>kanR</i>	This study
	pBAD322K-gp5.9-FLAG	Arabinose inducible gp5.9-FLAG, <i>kanR</i>	This study
	pBAD322A	Arabinose inducible vector, <i>ampR</i>	Cronan, 2006
	pBAD322A-gp5.9	Arabinose inducible gp5.9, <i>ampR</i>	This study
	pACEBac1 His6-3C-gp5.9	Insect cell expression of gp5.9	Wilkinson et al., 2022
	pET21a MukB-His6	T7 expression plasmid for producing <i>E. coli</i> MukB	Zawadzka et al., 2018
pUC19		Entrapment assay substrate	New England Biolabs
pJF146	RK24 <i>lux apR bsd</i>	RK2 conjugation machinery; NCBI: MK809154.1	Fredens et al., 2019
pKW20	Para <i>lambda-red cas9 tet tracrRNA</i>	REXER helper plasmid; NCBI: MN927219.1	Wang et al., 2016