## **Supplementary Information**

# Spatial coupling between DNA replication and mismatch repair

### in Caulobacter crescentus

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# **Content:**

## **Supplementary Tables** (page 2)

- Table S1 (page 2)
- Table S2 (page 3)
- Table S3 (page 4)

Table S4 (page 5)

### Supplementary Figures with legends (page 6)

- Fig.S1 (page 6)
- Fig.S2 (page 8)
- Fig.S3 (page 9)
- Fig.S4 (page 10)
- Fig.S5 (page 11)
- Fig.S6 (page 12)
- Fig.S7 (page 13)
- Fig.S8 (page 14)

Supplementary Materials and Methods (page 15)

**Supplementary References** (page 16)

# **Supplementary Tables**

# Table S1: Oligonucleotides used in this study. Restriction enzyme recognition sites used

for cloning purposes are underlined.

Name	Sequence $(5' \rightarrow 3')$	Use
CT1	GGT <u>AAGCTT</u> GGTCCGCGTGGGGATCTCTAC (HindIII)	Construction of pNPTS138-∆mutS
CT2	CCC <u>GGATCC</u> GGGCGGCGTTAAGGAATTACAC (BamHI)	
CT3	CCC <u>GGATCC</u> TAGCGGGTACCCATATAGA (BamHI)	
CT4	CGG <u>GCTAGC</u> AACCCAGATCCCACAACGCATAC (Nhel)	
СТ8	CGG <u>GCTAGC</u> CGAGACGATGGATGACAAACACC (Nhel)	Construction of pNPTS138-∆mutL
CT10	GGTAAGCTTATGCCCATCCGCCGCCTGCCG (HindIII)	1
CT11	CCCGGATCCCATGGCCTGGCGCTTGATCTCC (BamHI)	
CT12	CCC <u>GGATCC</u> TGAGGCCAGAGCGCCCCTAC (BamHI)	
TC46	GTCGGATCCGCCTGATCGACAGGGCGAACCA (BamHI)	Construction of pNPTS138-∆uvrD
TC47		1 1
TC63	ATAAAGCTTATGTTTCCCGACACCGACTCGC (HindIII)	
TC64	CGTGGATCCGCGGTTCTTCCAGCCGTCGATG (BamHI)	
TC18	GCGAAGCTTGGTCCGCGTGGGGATCTCTA (HindIII)	Construction of pNPTS138-PmutS-YFP-MutS
TC19	GTG <u>GGATCC</u> CATGACGCCCGGAAACTACAG (BamHI)	1
TC20	GAAGGATCCATGGTGAGCAAGGGCGAGGAG (BamHI)	1
TC21	CTCGAATTCGGACAGCTCGACCGACGCCA (EcoRI)	
TC42	TGACTGACCGCGGCGGCGGCGGCGGCGTCCAGCTTGG	Construction of pNPTS138- MutS(849AAAAA853)
TC43	CCAAGCTGGACGCCGCCGCCGCCGCCGCGGTCAGTCA	and pXYFPN4- MutS( <sub>849</sub> AAAAA <sub>853</sub> )
TC52	AACAAGCTTGGTAAGTCGACCTTCCTGCGCC, HindIII	Construction of pNPTS138- MutS( <sub>849</sub> AAAAA <sub>853</sub> )
TC53	GCG <u>GAATTC</u> GCTTGTAGGGCCGCAGGAACAG, EcoRI	
TC34	CGA <u>AAGCTT</u> CTTTCCCGGCGTCGTTGTGGA (HindIII)	Construction of pNPTS138-DnaQ(G13E)
TC35	TTTGGGTCAAACTCGGTGGTTTCG	
TC36	CGAAACCACCGAGTTTGACCCAAA	
TC37	GTGGGATCCGCCATGGCCCCTGTCGAATGG (BamHI)	
TC14	GGA <u>AAGCTT</u> CCAGCAGGCTCAGAATCCGAA (HindIII)	Construction of pNPTS138-PmutL-YFP-MutL
TC15	GCGGGATCCCATCAAGCGGACTTTCACGGG (BamHI)	
TC16	GACGGATCCATGGTGAGCAAGGGCGAGGA (BamHI)	
TC17	GGCGAATTCGGACTTCATGAATTTCAGGCG (EcoRI)	
JC155	CG <u>GGTACC</u> ATGAACGCCCACGCCACGCCGACC (Kpnl)	Construction of pXYFPN4-MutS, pXYFPN4-
JC156	G <u>GCTAGC</u> CTAGGCCGTGAGCAGACCCTTAAGG (Nhel)	$MutS(_{849}AAAAA_{853}), pXYFPN4-MutS(F44A),$
		pXYFPN4-MutS(K661M), pXYFPN4-MutS(E735A)
TC27	CAGCTCGTAGGCATCGCCCATGC	Construction of pXYFPN4-MutS(F44A)
TC28	GCATGGGCGATGCCTACGAGCTG	7 ' ' '
TC23	GAAGGTCGACATACCGGCCATG	Construction of pXYFPN4-MutS(K661M)
TC24	CATGGCCGGTATGTCGACCTTC	
TC38	ATCCTGGACGCCATCGGCCGGG	Construction of pXYFPN4-MutS(E735A)
TC39	CCCGGCCGATGGCGTCCAGGAT	
CoC-7_1	TCGGTACCATGCCCATCCGCCGCCTGCCG (Kpnl)	Construction of pXYFPN4-MutL, pXYFPN4-
CoC-8_4	GGAGCTCTGGCCTCACCGCCGCCCGAACAGC (Sacl)	MutL( <sub>497</sub> ATLAAP <sub>502</sub> ), pXYFPN4-MutL(D472N) and
		pXYFPN4-MutL(D472N, 497ATLAAP502)
TC40	ACCTCGGGGGCGGCCAGGGTGGCGCGGGTC	Construction of pXYFPN4- MutL(497ATLAAP502)
TC41	GACCCGCGCCACCCTGGCCGCCCCCGAGGT	and pXYFPN4-MutL(D472N, 497ATLAAP502)
TC31	CGCGTGCTGGTTGACAATGAC	Construction of pXYFPN4-MutL(D472N) and
TC32	GGTCATTGTCAACCAGCACGCG	pXYFPN4-MutL(D472N, 497ATLAAP502)
TC54	TAGGTACCATGTTTCCCGACACCGACTCGC (Kpnl)	Construction of pXYFPN4-UvrD
TC55	CCC <u>GCTAGC</u> TCAGGCCTTCTCGACGAAGCTG (NheI)	

Plasmid name	Description	Source
pNPTS138	Suicide vector carrying the <i>sacB</i> gene	D. Alley,
		unpublished
pNPTS138-∆mutS	pNPTS138 derivative used to create the <i>AmutS</i> deletion strain	This study
pNPTS138-∆mutL	pNPTS138 derivative used to create the <i>AmutL</i> deletion strain	This study
pNPTS138-∆uvrD	pNPTS138 derivative used to create the $\Delta uvrD$ deletion strain	This study
pNPTS138-PmutS-YFP-	pNPTS138 derivative used to replace <i>mutS</i> by <i>yfp-mutS</i> (under the	This study
MutS	control of native <i>mutS</i> promoter)	
pNPTS138-DnaQ(G13E)	To replace native <i>dnaQ</i> ( <i>CCNA_00005</i> ) with <i>dnaQ</i> ( <i>G13E</i> ) allele	This study
pNPTS138-	To replace native <i>mutS</i> with the <i>mutS(849AAAAA853)</i> allele	This study
MutS(849AAAAA853)		
pNPTS138-PmutL-YFP-	pNPTS138 derivative used to replace <i>mutL</i> by <i>yfp-mutL</i> (under the	This study
MutL	control of native <i>mutL</i> promoter)	
pXYFPN4	For integrating constructs encoding N-terminal YFP fusions under the	(1)
	control of the native <i>xylX</i> promoter	
pXYFPN4-MutS	mutS cloned into pXYFPN4	This study
pXYFPN4-	<i>mutS(</i> 849AAAAA853) cloned into pXYFPN4	This study
MutS(849AAAAA853)		
pXYFPN4-MutS(F44A)	<i>mutS(F44A)</i> cloned into pXYFPN4	This study
pXYFPN4-MutS(K661M)	<i>mutS(K661M)</i> cloned into pXYFPN4	This study
pXYFPN4-MutS(E735A)	mutS(E735A) cloned into pXYFPN4	This study
pXYFPN4-MutL	mutL cloned into pXYFPN4	This study
pXYFPN4-	<i>mutL(<sub>497</sub>ATLAAP<sub>502</sub>)</i> cloned into pXYFPN4	This study
MutL(497ATLAAP502)		
pXYFPN4-MutL(D472N)	<i>mutL(D472N)</i> cloned into pXYFPN4	This study
pXYFPN4-MutL(D472N,	mutL(D472N, 497ATLAAP502) cloned into pXYFPN4	This study
497ATLAAP502)		
pXYFPN4-UvrD	uvrD cloned into pXYFPN4	This study

# Table S2: Plasmids used in this study.

Strain name	Genotype	Source
Escherichia coli		
TOP10	F- mcrA Δ(mrr-hsdRMS-mcrBC) φ80lacZΔM15 ΔlacX74 nupG recA1	Invitrogen (USA)
	araD139 $\Delta$ (ara-leu)7697 galE15 galK16 rpsL(Str <sup>R</sup> ) endA1 $\lambda$ <sup>-</sup>	
Caulobacter cress	centus	
NA1000	Synchronizable derivative of wild-type strain CB15 (CB15N)	(2)
JC1427	NA1000 <i>AmutS</i>	This study
JC1426	NA1000 AmutL	This study
JC1575	NA1000 AmutS AmutL	This study
JC1847	NA1000 AuvrD	This study
JC1986	NA1000 AuvrD AmutL	This study
JC1784	NA1000 YFP-mutS	This study
JC1433	NA1000 ΔmutS Pxyl::YFP-mutS	This study
JC577	NA1000 dnaN-CFP	(3)
JC1625	NA1000 dnaN-CFP ΔmutS Pxyl::YFP-mutS	This study
JC1770	NA1000 ΔmutS Pxyl::YFP-mutS(849AAAAA503)	This study
JC1799	NA1000 mutS(849AAAAA503)	This study
JC1678	NA1000 dnaQ(G13E)	This study
JC1724	NA1000 ΔmutS Pxyl::YFP-mutS pNPTS138-DnaQ(G13E) integrated	This study
	at dnaQ locus	
JC1666	NA1000 ΔmutS Pxyl::YFP-mutS(F44A)	This study
JC1669	NA1000 dnaN-CFP ΔmutS Pxyl::YFP-mutS(F44A)	This study
JC1665	NA1000 ΔmutS Pxyl::YFP-mutS(K661M)	This study
JC1739	NA1000 ΔmutS Pxyl::YFP-mutS(E735A)	This study
JC1668	NA1000 dnaN-CFP ΔmutS Pxyl::YFP-mutS(K661M)	This study
JC1740	NA1000 dnaN-CFP ΔmutS Pxyl::YFP-mutS(E735A)	This study
JC1769	NA1000 YFP-mutL	This study
JC1825	NA1000 Δ <i>mutL Pxyl::YFP-mutL</i>	This study
JC1812	NA1000 dnaN-CFP ΔmutL Pxyl::YFP-mutL	This study
JC1805	NA1000 dnaN-CFP ΔmutS ΔmutL Pxyl::YFP-mutL	This study
JC1749	NA1000 ΔmutL Pxyl::YFP-mutL(497ATLAAP502)	This study
JC1750	NA1000 dnaN-CFP ΔmutL Pxyl::YFP-mutL(497ATLAAP502)	This study
JC1667	NA1000 ΔmutL Pxy::YFP-mutL(D472N)	This study
JC1670	NA1000 dnaN-CFP $\Delta$ mutL Pxy::YFP-mutL(D472N)	This study
JC1894	NA1000 dnaN-CFP ΔmutS ΔmutL Pxy::YFP-mutL(D472N)	This study
JC1753	NA1000 dnaN-CFP ΔmutL Pxyl::YFP-mutL(D472N, 497ATLAAP502)	This study
JC1870	NA1000 ΔuvrD Pxyl::YFP-uvrD	This study
JC1846	NA1000 Pxyl::YFP-uvrD	This study
JC2211	NA1000 <i>Pxyl::YFP-uvrD</i> pNPTS138-DnaQ(G13E) integrated at	This study
	dnaQ locus	This study
JC1977	NA1000 ΔmutS Pxyl::YFP-uvrD	This study

# Table S3: Strains used in this study.

Table S4: Comparison of spontaneous mutation rates of *C. crescentus* strains used in this study. The symbols \* and # indicate that the mutation rate is statistically significantly different or equivalent, respectively, to that of the NA1000 (WT) reference strain (for Zones A and D), of the JC1433 ( $\Delta mutS Pxyl::YFP-mutS$ ) strain (Zone B) or of the JC1825 ( $\Delta mutL Pxyl::YFP-mutL$ ) strain (Zone C). Student's *t*-tests were used to estimate statistical significance: differences were considered as significant (\*) if P<0.05. The "relative mutation rate" value, facilitating comparisons, was calculated setting the spontaneous mutation rate of the NA1000 (WT) strain to a value of 1 (arbitrary units).

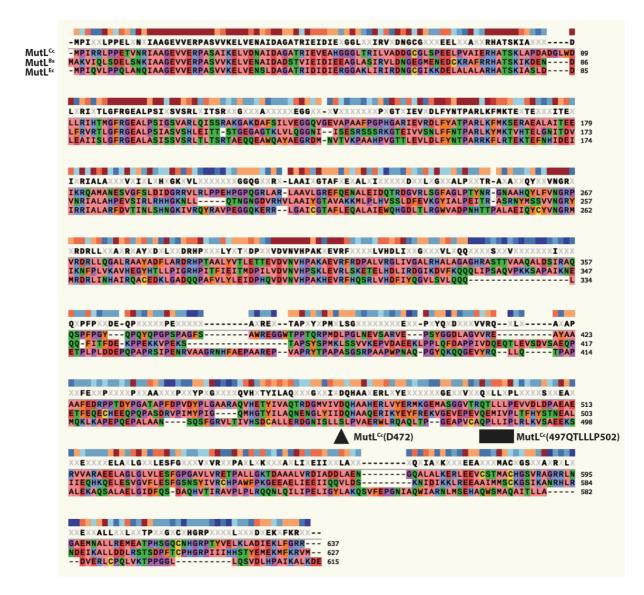
number number rate rate   NA1000 WT status colonies [#standard deviation]   A NA1000 WT status geviation   JC1427 AmutS 998.3 [±212.9]* 120.3 [=0%]   JC1426 AmutL 916.7 [±312.4]* 110.4 [=0%]   JC1847 AuvrD 916.7 [±312.4]* 110.4 [=0%]   JC1847 AuvrD 540.1 [±95.0]* 65.1 [=0%]   JC1786 AuvrD AmutL 1979.3 [±460.0]* 238.5   JC577 dnaN-CFP 55.8 [±33.6]# 6.7   JC1784 YFP-mutS 52.8 [±21.4]# 64.8 [95.5%]   JC1769 MreP-mutL 148.6 [±78.1]# 17.9 [84.6%]   JC1769 YFP-mutL 148.6 [±78.1]# 17.9 [84.6%]   JC1770 AmutS Pxyl::YFP-mutS(staAAAAsss) (β- 45.0 [±21.2]# 5.4 [96.3%]   JC1770 AmutS Pxyl::YFP-mutS(staAAAAsss) (β- 45.0 [±313.2]* 101.6 [15.7%]   JC1666 AmutS Pxyl::YFP-mutS(staAAAAsss) (β- 45.0 [±313.2]* 101.6 [15.7%] <t< th=""><th>Zone</th><th>Strain</th><th>Genotype (disturbed activity)</th><th>Number of Rif<sup>R</sup></th><th>Relative mutation</th></t<>	Zone	Strain	Genotype (disturbed activity)	Number of Rif <sup>R</sup>	Relative mutation
A NA1000 WT activity   A NA1000 WT 8.3 [±2.8] 1 [=100%]   JC1427 AmutS 998.3 [±212.9]* 120.3 [=0%]   JC1426 AmutL 916.7 [±312.4]* 110.4 [=0%]   JC1575 AmutS AmutL 1152.7 [±423.1]* 138.9   JC1847 AuvrD 540.1 [±95.0]* 65.1 [=0%]   JC1786 AuvrD AmutL 1979.3 [±460.0]* 238.5   JC577 dnaN-CFP 55.8 [±33.6]# 6.7   JC1784 YFP-mutS 52.8 [±21.4]# 6.4 [95.5%]   JC1769 mutS[sasAAAAAss3) (β-clamp binding) 56.8 [±24.3]* 6.8 [95.1%]   JC1769 YFP-mutS 3973.3 [±1466.0]* 478.7   JC1769 YFP-mutL 148.6 [±78.1]# 17.9 [84.6%]   JC212 mutL[ayATLAAPso2) (β-clamp binding) 932.7 [±315.6]* 112.4 [~0%]   JC1770 AmutS pxyl::YFP-mutS(sasAAAAAss3) (β- clamp binding) 45.0 [±21.2]# 5.4 [96.3%]   JC1666 AmutS pxyl::YFP-mutS(K661M) 792.3 [±75.0]* 95.5 [20.8%]		number		colonies * 10 <sup>-9</sup>	rate
A NA1000 WT 8.3 [±2.8] 1 [=100%]   JC1427 AmutS 998.3 [±212.9]* 120.3 [=0%]   JC1426 AmutL 916.7 [±312.4]* 110.4 [=0%]   JC1575 AmutS AmutL 1152.7 [±423.1]* 138.9   JC1847 AuvrD 540.1 [±95.0]* 65.1 [=0%]   JC1847 AuvrD 540.1 [±95.0]* 65.1 [=0%]   JC1847 AuvrD 540.1 [±95.0]* 65.1 [=0%]   JC1784 YFP-mutL 1979.3 [±460.0]* 238.5   JC1784 YFP-mutS 52.8 [±21.4]# 6.4 [95.5%]   JC1799 mutS(sagAAAAAss3) (β-clamp binding) 56.8 [±24.3]* 6.8 [95.1%]   JC1678 dnaQ(G13E) 3973.3 [±1466.0]* 478.7   JC1769 YFP-mutL 148.6 [±78.1]# 17.9 [84.6%]   JC212 mutL(sysATLAAPso2) (β-clamp binding) 932.7 [±315.6]* 112.4 [~0%]   JC1665 AmutS Pxyl::YFP-mutS(sagAAAAAS3) (β- clamp binding) 45.0 [±21.2]# 5.4 [96.3%]   JC1770 AmutS Pxyl::YFP-mutS(s61M) 792.3 [±75.0]* 95.5 [20.8%]				total colonies	[% of DNA repair
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$				[±standard	activity]
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $				deviation]	
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	А	NA1000	WT	8.3 [±2.8]	1 [=100%]
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$		JC1427	∆mutS	998.3 [±212.9]*	120.3 [=0%]
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$		JC1426	∆mutL	916.7 [±312.4]*	110.4 [=0%]
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$		JC1575	$\Delta mutS \Delta mutL$	1152.7 [±423.1]*	138.9
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$		JC1847	ΔuvrD	540.1 [±95.0]*	65.1 [=0%]
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$		JC1986	$\Delta uvrD \Delta mutL$	1979.3 [±460.0]*	238.5
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$		JC577	dnaN-CFP	55.8 [±33.6]#	6.7
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $		JC1784	YFP-mutS	52.8 [±21.4]#	6.4 [95.5%]
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$		JC1799	<i>mutS(<sub>849</sub>AAAAA<sub>853</sub>)</i> (β-clamp binding)	56.8 [±24.3]*	6.8 [95.1%]
$\begin{array}{c c c c c c c c c c c c c c c c c c c $		JC1678	dnaQ(G13E)	3973.3 [±1466.0]*	478.7
BJC1433ΔmutS Pxyl::YFP-mutS24.2 [±9.9]2.9 [98.4%]JC1770ΔmutS Pxyl::YFP-mutS( $_{849}$ AAAAA $_{853}$ ) (β- clamp binding)45.0 [±21.2]#5.4 [96.3%]JC1666ΔmutS Pxyl::YFP-mutS(F44A) (mismatch recognition)843.0 [±313.2]*101.6 [15.7%]JC1665ΔmutS Pxyl::YFP-mutS(K661M) (nucleotide binding and ATP hydrolysis)792.3 [±75.0]*95.5 [20.8%]JC1739ΔmutS Pxyl::YFP-mutS(K661M) (ATP hydrolysis)917.0 [±340.0]*110.5 [8.2%]CJC1825ΔmutL Pxyl::YFP-mutL56.3 [±28.5]6.8 [94.7%]JC1749ΔmutL Pxyl::YFP-mutL( $_{497}$ ATLAAP <sub>502</sub> ) (β-clamp binding)751.8 [±71.4]*90.6 [17.2%]JC1667ΔmutL Pxyl::YFP-mutL(D472N) (endonuclease)904.3 [±204.2]*109.0 [0.4%]		JC1769	YFP-mutL	148.6 [±78.1]#	17.9 [84.6%]
$\begin{array}{c c c c c c c c c c c c c c c c c c c $		JC2212	<i>mutL(497ATLAAP502)</i> (β-clamp binding)	932.7 [±315.6]*	112.4 [~0%]
Image: clamp binding) Solution of the second	В	JC1433	ΔmutS Pxyl::YFP-mutS	24.2 [±9.9]	2.9 [98.4%]
Image: Clamp binding) Clamp binding) Image: Clamp binding)   JC1666 ΔmutS Pxyl::YFP-mutS(F44A) 843.0 [±313.2]* 101.6 [15.7%]   JC1665 ΔmutS Pxyl::YFP-mutS(K661M) 792.3 [±75.0]* 95.5 [20.8%]   JC1739 ΔmutS Pxyl::YFP-mutS(K661M) 792.3 [±75.0]* 95.5 [20.8%]   JC1739 ΔmutS Pxyl::YFP-mutS(E735A) 917.0 [±340.0]* 110.5 [8.2%]   C JC1825 ΔmutL Pxyl::YFP-mutL 56.3 [±28.5] 6.8 [94.7%]   JC1749 ΔmutL Pxyl::YFP-mutL(497ATLAAP502) 751.8 [±71.4]* 90.6 [17.2%]   JC1667 ΔmutL Pxyl::YFP-mutL(D472N) 904.3 [±204.2]* 109.0 [0.4%]		JC1770	ΔmutS Pxyl::YFP-mutS( <sub>849</sub> AAAAA <sub>853</sub> ) (β-	45.0 [±21.2]#	E 4 [06 29/]
Image: Market Marke			clamp binding)		5.4 [90.5%]
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$		JC1666	ΔmutS Pxyl::YFP-mutS(F44A)	843.0 [±313.2]*	101.6 [15.7%]
Image: constraint of the state of			(mismatch recognition)		
JC1739 ΔmutS Pxyl::YFP-mutS(E735A) (ATP hydrolysis) 917.0 [±340.0]* 110.5 [8.2%]   C JC1825 ΔmutL Pxyl::YFP-mutL 56.3 [±28.5] 6.8 [94.7%]   JC1749 ΔmutL Pxyl::YFP-mutL(497ATLAAP502) (β-clamp binding) 751.8 [±71.4]* 90.6 [17.2%]   JC1667 ΔmutL Pxyl::YFP-mutL(D472N) (endonuclease) 904.3 [±204.2]* 109.0 [0.4%]		JC1665	∆mutS Pxyl::YFP-mutS(K661M)	792.3 [±75.0]*	95.5 [20.8%]
C JC1825 ΔmutL Pxyl::YFP-mutL 56.3 [±28.5] 6.8 [94.7%]   JC1749 ΔmutL Pxyl::YFP-mutL(497ATLAAP502) 751.8 [±71.4]* 90.6 [17.2%]   JC1667 ΔmutL Pxyl::YFP-mutL(D472N) 904.3 [±204.2]* 109.0 [0.4%]			(nucleotide binding and ATP hydrolysis)		
CJC1825ΔmutL Pxyl::YFP-mutL56.3 [±28.5]6.8 [94.7%]JC1749ΔmutL Pxyl::YFP-mutL( $_{497}$ ATLAAP_{502}) (β-clamp binding)751.8 [±71.4]*90.6 [17.2%]JC1667ΔmutL Pxyl::YFP-mutL(D472N) (endonuclease)904.3 [±204.2]*109.0 [0.4%]		JC1739	ΔmutS Pxyl::YFP-mutS(E735A)	917.0 [±340.0]*	110.5 [8.2%]
JC1749ΔmutL Pxyl::YFP-mutL( $_{497}$ ATLAAP_{502}) (β-clamp binding)751.8 [±71.4]*90.6 [17.2%]JC1667ΔmutL Pxyl::YFP-mutL(D472N) (endonuclease)904.3 [±204.2]*109.0 [0.4%]			(ATP hydrolysis)		
(β-clamp binding) 904.3 [±204.2]* 109.0 [0.4%]   JC1667 ΔmutL Pxyl::YFP-mutL(D472N) 904.3 [±204.2]* 109.0 [0.4%]   (endonuclease) (endonuclease) (endonuclease) (endonuclease)	С	JC1825	ΔmutL Pxyl::YFP-mutL	56.3 [±28.5]	6.8 [94.7%]
JC1667 ΔmutL Pxyl::YFP-mutL(D472N) 904.3 [±204.2]* 109.0 [0.4%]   (endonuclease) 904.3 [±204.2]* 109.0 [0.4%]		JC1749	ΔmutL Pxyl::YFP-mutL(497ATLAAP502)	751.8 [±71.4]*	90.6 [17.2%]
(endonuclease)					
		JC1667	ΔmutL Pxyl::YFP-mutL(D472N)	904.3 [±204.2]*	109.0 [0.4%]
D JC1870 Δ <i>uvrD</i> Pxyl::YFP-uvrD 102.5 [±39.5]# 12.3 [82.3%]			(endonuclease)		
	D	JC1870	ΔuvrD Pxyl::YFP-uvrD	102.5 [±39.5]#	12.3 [82.3%]

# **Supplementary Figures:**

MutS <sup>cc</sup> MutS <sup>Bs</sup> MutS <sup>Ec</sup>	MXAHTPMMQQYLXXKAXHPDALLFFRMGDFYELFFDDAKKASQXLXISLTXRGXXAGEPIPMAGVPYHAA MAAHATPTPAHEIDPTGATPYMAQFFEMKARQPDALIFFRMGDFYELFFDDAYKAAAALGISQTFRGTHNGQPIPMAGVPQHAA MAGYTPMIQQYLKIKAEHQDAFLFFRLGDFYEMFFEDAKKASQELEITLTSRDGGAAEKIPMCGVPYHSA MSAIENFDAHTPMMQQYLRLKAQHPEILLFYRMGDFYELFYDDAKRASQLLDISLTKRGASAGEPIPMAGIPYHAV MSAIENFDAH	70
	EAYLXKLIX.GXKVAICEQXEDPAXSKGVVXRXVVRXVTPGTXDXLLXERXXXXSXGXGXAXADLSTGEXXLXXA EAYLSKLIRLGFKVAVCEQMEDPAEAKKRGSKAVVRRDIVRVTPGTLTEDGLLDARGANRLAAVALRAGQAAVASVELSTGEVEVLAVA SAYIEQLIKKGYKVAICEQTEDPKAAKGVVKREVVQLITPGTVMDGKGIHESENNFIASVACSNGYGLALSDLTTGE-NLAVI ENYLAKLVNQGESVAICEQIGDPATSKGPVERKVVRIVTPGTISDEALLQERQDNLLAAIWQDSKGFGYATLDISSGRFRLSEPA	154
	XR-EVVAXELXXXXPXEXLVAXX-LXXDSLXQXXRGLRXRXXXXLXEXEXDETXXXXLNXXXGTXTLXGFG-XXXPXXXAXG KEgvapilaalapsetlvadrlusdbslsqtLricgglvQpmpsalsepqaseTrvkrlYgvetldgfg-Glspaeigalg Erledviseiysvgareivvsg-Ldbdvag	222
	XLXXYLKXTQXXXLPHLRXXXXXXDXMXIDXATRRNLEITXTX-SGXXXGSLLXXLDXTVTANGXRMLKXWLXRPLXDVXXIXERQXX LIAAHLEMTQAGRLPALRAPRRAADADVMAIDPATRSSLEIDRTQ-EGGDRNGSLLAAIDRTVTAGGARMLASRLARPLLDVAAIDQRLDA -LYTYLKRTQKRSLDHLQPVQVYELEEAMKIDLYSKRNLELTETIRSKNKKGSLLWLLDETKTAMGGRLLKQWIDRPLIRVNQIEERQEM CLLQYAKDTQRTTLPHIRSIIMEREQDSIIMDAATRRNLEITQNL-AGGAENTLASVLDCTVTPMGSRMLKRWLHMPVRDTRVLLERQQI	311
	VEXLXXHXXXRQDLREVLKXVGDLERXLXRLALGXXXPRDLXXLXHAFKQL-PXLXXX VEWFVEHRQLRQRLREVLKGAGDMARALSRLALGRGGPRDLGCIRDTLKVGERLAGMAGGAPDPLSPPPFELEHAFKALTPALHEG VETLMSHFFEREDLREVLKGYDLERLAGRVAFGNVNARDL	368
	L×>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>	458
	GYYIEXTXGXXHXLXXXXYXRRQTLXNAERYITPELKEXEAXILXAXXXALALEXXLFEELREXXXXALQQXAXALQQXAXALAELDVLX Gyfyeatagkadplfqpplnatfihrqtlanqyrftyeladldariaqaaeralamevaafedwreqarlladaiqiasealaridvas GyyieytkanlhlleeGryerkqtltnaeryitpelkekealileaenniceleyelftelrekvkqyiprlqqlakqmseldalq Gyyiqisrgqsh-lapInymrqtlknaeryiipelkeyedkvltskgkalalekqlyeelfdlllphlealqqsasalaeldvlv	544
	×LAE×AEXXXYT×P×F×XKXXXXXEGRHPVVE×VXX>EPY×PN×CXLXXXRRNLIITGPNM×GKSTYMRQ×ALIAIMAQIGCY SLAEMAEDAGAVRPVVDASYAFDAKAARHPVVEAVKRAGEPYTPNDCRLDASGETAARLSIVTGPNMAGKSTFLRQNALLAILAQSGCY CFATISENRHYTKPEF-SKDEVEVIEGRHPVVEKVMDSQEYVPNNCMMGDNRQNLLITGPNMSGKSTYMRQIALISIMAQIGCF NLAERAYTLNYTCPTFIDKPGIRITEGRHPVVEQVLNEPFIANPLNLSPQRRNLIITGPNMGGKSTYMRQTALIALMAYIGSY MutS <sup>CC</sup> (K661)	627
	VPAXKXXLG CORIFTRVGAADDLASGRSTFNVEM ETANIL XNATXXSLVL X DEIGRGTSTYDGLALAWACAE XLHD XIXAXTLFATHY VPAASFRLGVVDRLFSRVGAGDDLARGRSTFNNEMVETASILTQAGPRSLVIIDEIGRGTATYDGLAIAWACAEALHDINRCRALFATHY VPAKKAVLPIFDQIFTRIGAADDLISGQSTFNVEMLEAKNAIVNATKNSLILFDEIGRGTSTYDGMALAQAIEVVHDHIGAKTLFSTHY VPAQKVEIGPIDRIFTRVGAADDLASGRSTFNVEMTETANILHNATEVSLVLMDEIGRGTSTYDGLSLAWACAENLANKIKALTLFATHY MutS <sup>CC</sup> (E735)	717
	HELTXLEXKMXXVXNYHLRAXEXNGTXVFLHXXXXGAADKSYGXXVAXLAGVPXXVIXRARXXLXELES-SXNXXXXV-KXQXXXXXQ HELATLETRMAFVSNLSLRAKEWNGDLVFLHEAAPGPADRSYGVQVAKLAGVPAPVVVRAREVLDRLESKDQSPAKLDD HELTVLEDKLPQLKNYHVRAEFYNGTVVFLHQIKEGAADKSYGIHVAQLAELPGDLIARAQDILKELEH-SGNKPEVPVQRPQVKEEPAQ FELTQLPEKMEGVANVHLDALEHGDTIAFMHSVQDGAASKSYGLAVAALAGVPKEVIKRARQKLRELESISPNAAATQVDGTQ	849 806
	LSLFXVXEAKXXXXXXVE-ALXXLDXDXMTPREALEXXYRLKXLLX- LPLFAVSQAVAVTSAPAKAAPSAVETSLADLDVDGMSPREALEALYRLKGLLTA 903 LSFFDEAEKPAETPKLSKKEKQVID-AFKSLNILDMTPLEAMNEMYKLQKKLH- 858 MSLLSVPEETSPAVE-ALENLDPDSLTPRQALEWIYRLKSLV 853 MutS <sup>cc</sup> (849DLPLF853)	

**Figure S1: Similarities between selected MutS proteins.** Amino-acid sequences of MutS homologs were downloaded from NCBI for *Caulobacter crescentus* NA1000 strain (MutS<sup>Ce</sup>), *Bacillus subtilis* PY79 strain (MutS<sup>Bs</sup>) and *Escherichia coli* MG1655 strain (MutS<sup>Ec</sup>).

Alignments were done using the SnapGene software (from Insightful Science; available at snapgene.com). MutS<sup>Ce</sup> residues/motifs of particular interest are indicated under the three sequences. The F44 residue belongs to a  $_{42}$ GDFYELFFDDA<sub>52</sub> motif where underlined residues are part of the consensus motif for mismatch recognition by MutS proteins (4). The K661 residue belongs to a  $_{655}$ GPNMAGKS<sub>662</sub> Walker A motif supposedly involved in ATP/ADP binding and whose consensus is GxxxxGK[T/S] (x can be any amino acid) (5). The E735 residue belongs to a  $_{689}$ LVLMDE<sub>694</sub> Walker B motif supposedly involved in ATP hydrolysis and whose consensus is hhhhDE (h is a hydrophobic amino acid) (5). The  $_{849}$ DLPFL<sub>853</sub> motif shows similarities with the  $\beta$ -clamp binding motifs MutS<sup>Bs</sup> ( $_{806}$ QLSFF<sub>810</sub>) (6)and MutS<sup>Ec</sup> ( $_{812}$ QMSLL<sub>816</sub>) (7).



**Figure S2: Similarities between selected MutL proteins.** Amino-acid sequences of MutL homologs were downloaded from NCBI for *Caulobacter crescentus* NA1000 strain (MutS<sup>Ce</sup>), *Bacillus subtilis* PY79 strain (MutS<sup>Bs</sup>) and *Escherichia coli* MG1655 strain (MutS<sup>Ee</sup>). Alignments were done using the SnapGene software. MutL<sup>Cc</sup> residues/motifs of particular interest are indicated under the three sequences. The D472 residue belongs to the predicted  $_{472}$ DQHAAHERLVYE483 endonuclease domain (underlined letters indicate conserved residues according to (8)). The  $_{497}$ QTLLLP<sub>502</sub> motif shows similarities with the previously proposed Qxh(L/I)xP consensus β-clamp binding motif for MutL proteins (9).

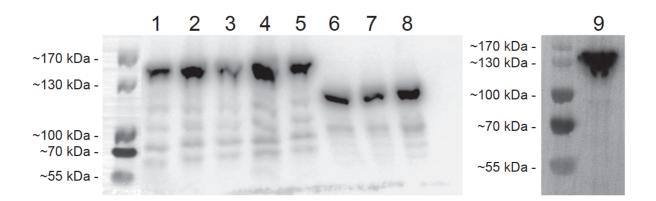


Figure S3: Immunoblot detection of YFP-tagged MutS, MutL and UvrD derivatives from *C. crescentus* cell extracts. Cells from strains JC1433 ( $\Delta mutS Pxyl::YFP-mutS$ ) (Lane 1), JC1665 ( $\Delta mutS Pxyl::YFP-mutS(K661M$ )) (Lane 2), JC1666 ( $\Delta mutS Pxyl::YFP-mutS(F44A$ )) (Lane 3), JC1739 ( $\Delta mutS Pxyl::YFP-mutS(E735A$ )) (Lane 4), JC1770 ( $\Delta mutS Pxyl::YFP-mutS(s_{49}AAAAA_{853})$ ) (Lane 5), JC1825 ( $\Delta mutL Pxyl::YFP-mutL$ ) (Lane 6), JC1667 ( $\Delta mutL Pxyl::YFP-mutL(D472N)$ ) (Lane 7), JC1749 ( $\Delta mutL Pxyl::YFP-mutL(_{497}ATLAAP_{502})$ ) (Lane 8) and JC1870 ( $\Delta uvrD Pxyl::YFP-uvrD$ ) (Lane 9) were cultivated overnight in PYE medium before the culture was diluted into M2G medium. Once the OD<sub>660nm</sub> reached ~0.3 (mid-exponential phase), 0.3% xylose was added. Cell extracts were then prepared when cultures reached an OD<sub>660nm</sub> of ~0.5 (exponential phase) and proteins were separated by SDS-PAGE. YFP-tagged proteins were then detected by immunoblotting using anti-GFP antibodies. MW corresponds to the molecular weight in kDa. This figure shows that the YFP moiety of each fusion protein is not cleaved off.

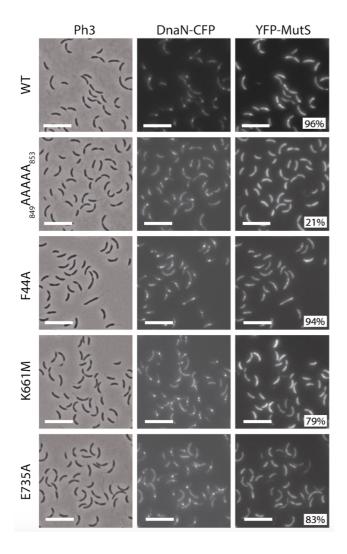


Figure S4: YFP-MutS foci co-localize with the replisome in C. crescentus. Subcellular localization of DnaN-CFP and derivatives of YFP-MutS in AmutS cells. Strains JC1625 dnaN-CFP Pxyl::YFP-mutS),  $(\Delta mutS)$ JC1771  $(\Delta mutS)$ dnaN-CFP Pxyl::YFP $mutS(_{849}AAAAA_{853}))$ , JC1669 ( $\Delta mutS$  dnaN-CFP Pxyl::YFP-mutS(F44A)), JC1668 ( $\Delta mutS$ dnaN-CFP Pxyl::YFP-mutS(K661M)) and JC1740 (\[ \Delta mutS dnaN-CFP Pxyl::YFPmutS(E735A)) were cultivated into PYE medium and then transferred into M2G medium. 0.3% xylose was added to cultures when they reached an  $OD_{660nm} \sim 0.3$ . Cells were then imaged by fluorescence microscopy when the  $OD_{660nm}$  reached ~0.5. The % indicated onto images corresponds to the average proportion of distinct MutS-YFP foci (intensity >2-fold above background) that are co-localized with DnaN-CFP foci (using values obtained from three independent experiments). The white scale bar corresponds to 8µm.

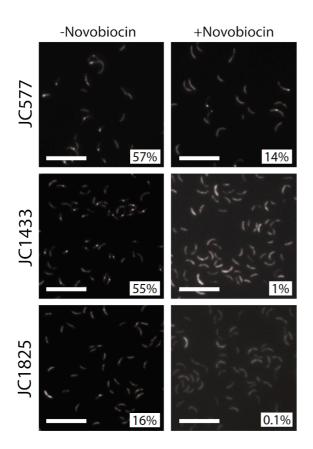


Figure S5: Replication inhibition following novobiocin treatment largely dissociates DnaN-CFP, YFP-MutS and YFP-MutL foci. Strains JC577 (*dnaN-CFP*), JC1433 ( $\Delta mutS$ *Pxyl::YFP-mutS*) and JC1825 ( $\Delta mutL Pxyl::YFP-mutL$ ) were cultivated in PYE medium and then transferred into M2G medium. When the OD<sub>660nm</sub> reached ~0.3, cultures were split into two and 100 µg/mL of novobiocin was added into one of the sub-cultures. 0.3% xylose was added at the same time into each sub-cultures of strains JC1433 and JC1825. Once the OD<sub>660nm</sub> of the sub-culture without novobiocin reached 0.5, cells were imaged by fluorescence microscopy using CFP (for DnaN-CFP) or YFP (for YFP-MutS or YFP-MutL) illumination. Representative images are shown here. % indicated under each image correspond to the average proportion of cells displaying CFP or YFP foci (intensity >2-fold above background) as counted during three independent experiments. The white scale bar corresponds to 11µm.

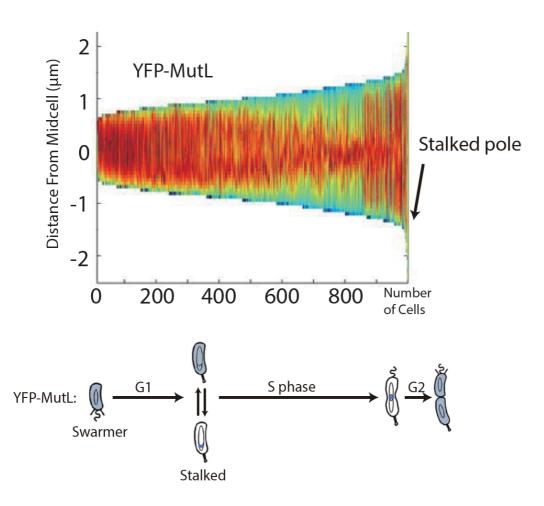


Figure S6: YFP-MutL is enriched at mid-cell towards the end of the S-phase of the C. crescentus cell cycle. Demograph showing the subcellular localization of YFP-MutL in  $\Delta mutL$  cells sorted as a function of their size. JC1825 ( $\Delta mutL Pxyl::YFP-mutL$ ) cells were cultivated and imaged as described for Fig.5A. Short cells correspond to G1/swarmer cells, while intermediate and longer cells correspond to stalked and pre-divisional S-phase cells, respectively. The schematic under the demograph shows the C. crescentus cell cycle and the blue color highlights where YFP-MutL seems to be localized as a function of the cell cycle.

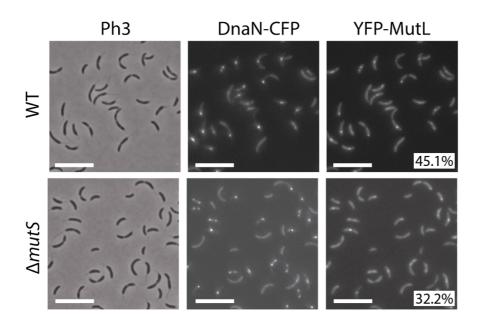


Figure S7: YFP-MutL is recruited to the replisome independently of MutS. Subcellular localization of DnaN-CFP and YFP-MutL in  $\Delta mutL$  cells with or without *mutS*. Strains JC1812 (*dnaN-CFP*  $\Delta mutL$  *Pxyl::YFP-mutL*) labelled "WT" and JC1805 ( $\Delta mutS \Delta mutL$  *dnaN-CFP Pxyl::YFP-mutL*) labelled " $\Delta mutS$ " were cultivated into PYE medium and then transferred into M2G medium. 0.3% xylose was added to cultures when they reached an OD<sub>660nm</sub>~0.3. Cells were then imaged by fluorescence microscopy when the OD<sub>660nm</sub> reached ~0.5. The % indicated onto images corresponds to the average proportion of cells with distinct MutL-YFP foci (intensity >2-fold above average background) using values obtained from three independent experiments. The white scale bar corresponds to 8µm.

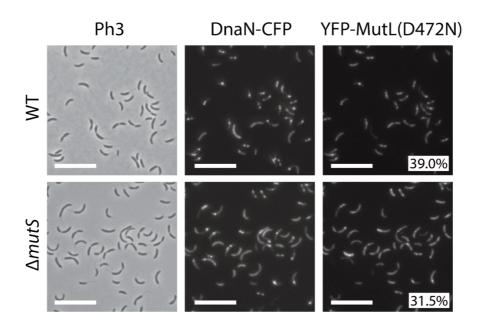


Figure S8: YFP-MutL(D472N) is stabilized at the replisome even in the absence of MutS. Subcellular localization of DnaN-CFP and YFP-MutL(D472N) in  $\Delta mutL$  cells with or without *mutS*. Strains JC1670 ( $\Delta mutL$  *dnaN-CFP Pxyl::YFP-mutL* (D472N)) and JC1894 ( $\Delta mutS$   $\Delta mutL$  *dnaN-CFP Pxyl::YFP-mutL* (D472N)) were cultivated into PYE medium and then transferred into M2G medium. 0.3% xylose was added to cultures when they reached an OD<sub>660nm</sub>~0.3. Cells were then imaged by fluorescence microscopy when the OD<sub>660nm</sub> reached ~0.5. The % indicated onto images corresponds to the average proportion of cells with distinct MutL-YFP(D472N) foci (intensity >2-fold above average background) using values obtained from three independent experiments. The white scale bar corresponds to 11µm.

### **Supplementary Material and Methods**

### **Plasmids constructions**

### Derivatives of pNPTS138:

To construct pNPTS138- $\Delta$ mutS, pNPTS138- $\Delta$ mutL and pNPTS138- $\Delta$ uvrD, ~500 bp sequences upstream and downstream of the corresponding ORF were amplified by PCR from wild-type *C. crescentus* chromosomal DNA using the primer pairs listed in Table S1. The two fragments were subsequently digested by restriction enzymes (as indicated in Table S1) and cloned into the pNPTS138 suicide vector between corresponding restriction sites by triple ligation.

To construct pNPTS138-DnaQ(G13E), pNPTS138-MutS( $_{849}AAAAA_{853}$ ) and pNPTS138-MutL( $_{497}ATLAAP_{503}$ ), overlap extension PCR were used to construct ~1 kb gene fragment with the required mutations in *dnaQ*, *mutS* or *mutL*. The PCR consisted of two consecutive PCR reactions, with four primers (listed in Table S1).

To construct pNPTS138-PmutS-YFP-mutS and pNPTS138-PmutL-YFP-mutL, two primer pairs (listed in Table S1) were used to amplify the ~500 bp promoter regions (PmutS and PmutL, respectively) and the protein fusion regions (YFP-mutS and YFP-mutL, respectively). The two fragments were subsequently digested by the indicated restriction enzymes (Table S1) and cloned into the pNPTS138 suicide vector between corresponding restriction sites by triple ligation.

### Derivatives of pXYFPN4:

To construct pXYFPN4-MutS, pXYFPN4-MutL and pXYFPN4-UvrD, the corresponding ORFs were amplified by PCR from wild-type *C. crescentus* chromosomal DNA using the primer pairs listed in Table S1, digested by the indicated restriction enzymes and cloned into pXYFPN4. To construct pXYFPN4-MutS(F44A), pXYFPN4-MutS(K661M), pXYFPN4-MutS(E735A), pXYFPN4-MutS(<sub>849</sub>AAAAA<sub>853</sub>), pXYFPN4-MutL(D472N), pXYFPN4-MutL(<sub>497</sub>ATLAAP<sub>502</sub>) and pXYFPN4-MutL(D472N, <sub>497</sub>ATLAAP<sub>502</sub>) overlap PCR was used to construct a point mutation in the *mutS* or *mutL* genes. Two consecutive PCR reactions were performed using four primers (listed in Table S1). First, two DNA fragments were amplified from plasmid pXYFPN4-mutS (for *mutS* alleles) or pXYFPN4-mutL (for *mutL* alleles). Then, equimolar amounts of both products were mixed and amplified. The resulting fragments were digested by restriction enzymes (as indicated in Table S1) and cloned into the pXYFPN4 vector.

### **Strains constructions**

Three categories of strains were constructed: (1) gene deletions or gene replacements were constructed using pNPTS138-derived plasmids (listed in Table S2) via two homologous recombination events: first, the suicide vectors were integrated at the desired location into the genome via homologous recombination and selection for kanamycin resistance. Excision of the suicide vector via a second homologous recombination event was then selected by plating on sucrose (0.3%)-containing PYEA plates and the isolated colonies were subsequently selected for kanamycin sensitivity. The mutants were identified by PCR using primer pairs used to construct pNPTS138 derivatives (Table S1) or by DNA sequencing for gene replacements; (2) pXYFPN4 derivatives were integrated at the native *xylX* locus by transformation. Positive clones were screened using RecUni-1 and RecXyl-2 primers (1). These strains were used for the inducible expression of MutS, MutL, UvrD derivatives in the presence of 0.3% xylose; (3) In JC1724, JC1845 and JC2211, the pNPTS138-DnaQ-G13E plasmid was integrated at the native *dnaQ* locus. The resulting strains express the *dnaQ(G13E)* allele together with a truncated and inactive *dnaQ'* allele. Oligos TC34 and M13-F were used to check the integration sites of the plasmid.

### **Immunoblot** analysis

YFP-tagged proteins were resolved on 8% SDS-PAGE gels. Proteins were then transferred to PVDF membranes (Millipore). Two antibodies were used for immunodetection: Anti-GFP antibody (GFP Tag Antibody, GF28R, Mouse IgG, Thermo Fisher) was diluted 1:3000 and Anti-Mouse IgG HRP Conjugate (Promega) was diluted 1:5000. The signal was visualized using a chemiluminescent reagent (Amersham ECL Prime Western Blotting Detection Reagent, GE) and a FUSION FX (VILBER) scanner. Images were processed by Photoshop.

### **Supplementary References**

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