

SUPPLEMENTARY DATA

The human Holliday junction resolvase GEN1 rescues the meiotic phenotype of a *S. pombe mus81* mutant

Alexander LORENZ¹, Stephen C. WEST² & Matthew C. WHITBY^{1,*}

¹Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK

²Cancer Research UK, London Research Institute, Clare Hall Laboratories, South Mimms, Hertfordshire EN6 3LD, UK

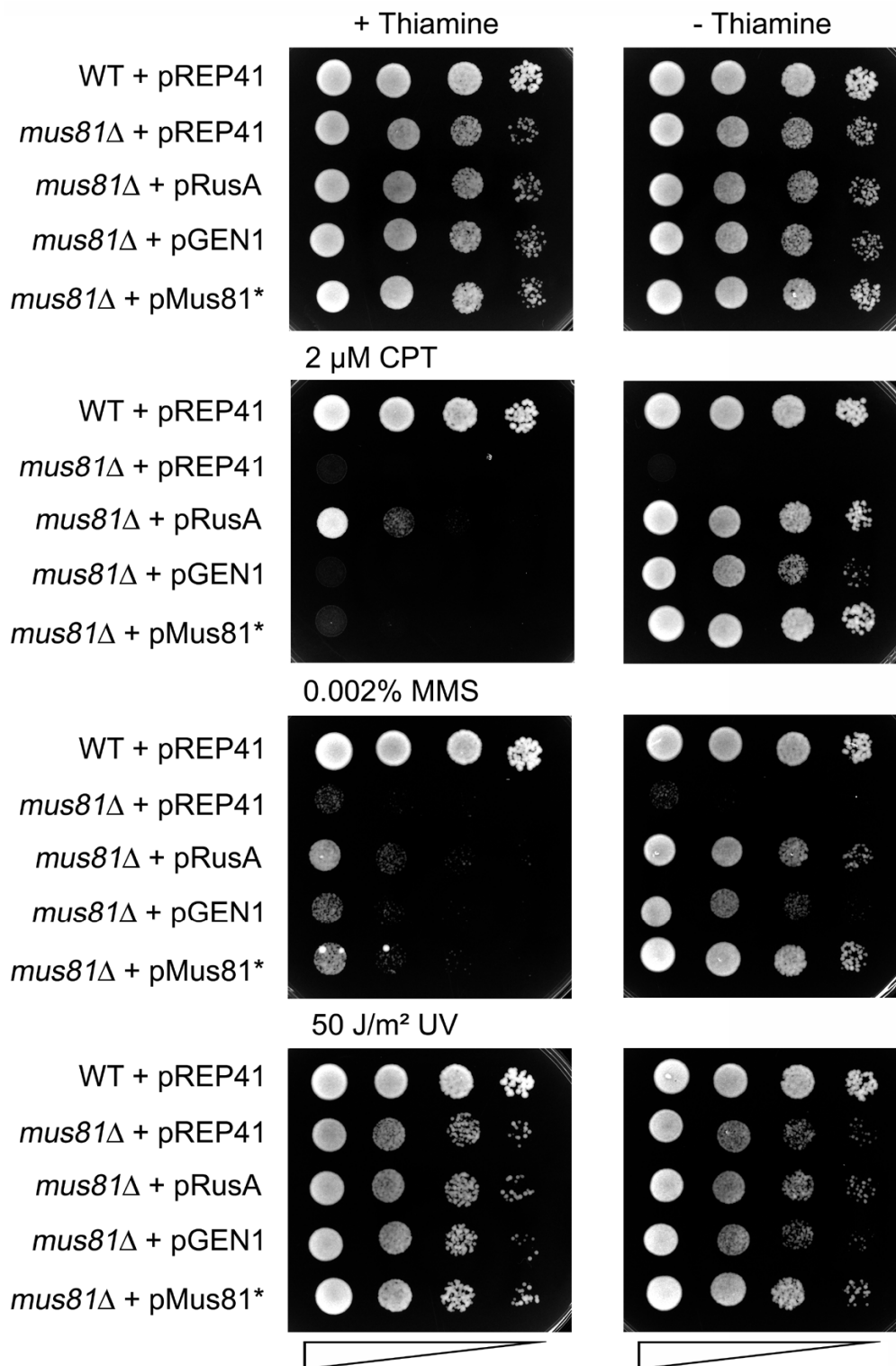


Figure S1. Effect of RusA (expressed from the thiamine-repressible *nmt1*-promotor in pREP1), GEN1 and Mus81-Eme1 (expressed from the thiamine-repressible *nmt1*-promotor in pREP41) on genotoxin sensitivities of a *mus81Δ* (MCW1238) strain. The empty vector pREP41 in the wild-type strain MCW1221 and in MCW1238 serve as controls. The neat spot represents 10^5 cells.

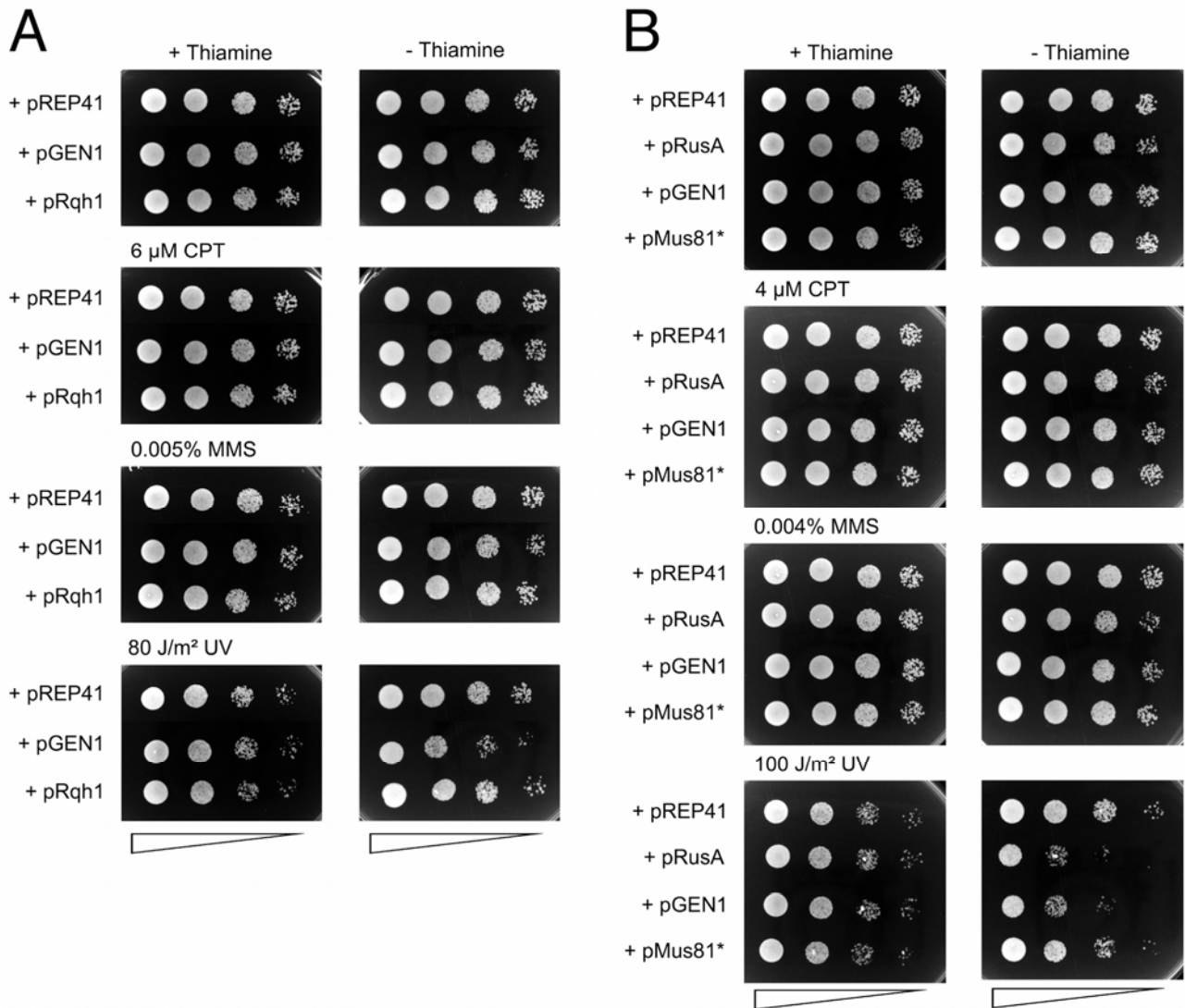


Figure S2. Effect of (A) GEN1 and Rqh1 (expressed from the thiamine-repressible *ntm1*-promotor in pREP41) and (B) RusA (expressed from the thiamine-repressible *ntm1*-promotor in pREP1), GEN1 and Mus81-Eme1 (expressed from the thiamine-repressible *ntm1*-promotor in pREP41) on genotoxin sensitivities of a wild-type strain (MCW1221). pREP41 serves as the empty vector control. The neat spot represents 10^5 cells.

Table S1. Spore viability of crosses of wild-type strains (MCW1221 × FO808) transformed with the plasmid pREP41 and *mus81Δ* strains (MCW1238 × MCW1237) transformed with the plasmids pREP41, pMus81*, pRusA, pGEN1, pGEN1^{EA/EA} and pGEN1^{DA} (see also Figure 4).

strain	plasmid	spore viability in % of plated spores, numbers in brackets are spores plated/experiment ^a					Mean ± s.d. ^a
WT	pREP41	47.57 (885)	58.62 (911)	59.66 (870)	64.65 (843)	83.50 (921)	62.80 ± 13.15 (4,430)
<i>mus81Δ</i>	pREP41	0.80 (19,800)	0.84 (14,700)	1.08 (16,380)	1.20 (16,200)	3.98 (33,000)	1.58 ± 1.35 (100,080)
<i>mus81Δ</i>	pMus81*	22.61 (1,362)	29.71 (912)	36.86 (1,050)	46.55 (885)	52.92 (924)	37.73 ± 12.27 (5,133)
<i>mus81Δ</i>	pRusA	23.04 (1,376)	25.17 (1,029)	25.53 (1,332)	30.45 (972)	34.17 (954)	27.67 ± 4.54 (5,663)
<i>mus81Δ</i>	pGEN1	40.26 (1,950)	42.14 (1,170)	46.77 (789)	47.52 (1,128)	49.91 (1,080)	45.32 ± 3.99 (6,117)
<i>mus81Δ</i>	pGEN1 ^{EA/EA}	1.26 (12,900)	1.56 (13,950)	1.61 (16,200)	1.69 (12,825)	3.67 (15,000)	1.96 ± 0.97 (70,875)
<i>mus81Δ</i>	pGEN1 ^{DA}	0.88 (9,750)	0.94 (8,100)	1.21 (14,550)	1.73 (9,000)	2.05 (9,180)	1.36 ± 0.51 (50,580)

^a numbers in brackets represent total number of plated spores (n).

Table S2. Frequency of gene conversion and crossing over in the *ura4-aim2 – ade6 – his3-aim* interval. The values are the means from *n* independent crosses, the values in brackets are the standard deviations. The number of *ade*⁺ recombinants tested is indicated, as is the total number of viable spores analyzed for crossing over between *ura4-aim2* and *his3-aim*. *ade6-3083* is a known hot spot for recombination and therefore acts predominantly as a recipient of genetic information, which explains the disparity between R1 and R2 classes. CentiMorgan (cM) are calculated from the accumulated data of the independent crosses, not from the mean values.

Cross			% <i>ade</i> ⁺				Crossovers (CO)			cM	
strain	genotype	<i>n</i>	Frequency of <i>ade</i> ⁺ in %	<i>ade</i> ⁺ tested	<i>ura</i> ⁺ <i>his</i> ⁺ (P1)	<i>ura</i> ⁺ <i>his</i> ⁻ (P2)	<i>ura</i> ⁻ <i>his</i> ⁻ (R1)	<i>ura</i> ⁻ <i>his</i> ⁺ (R2)	tested		Frequency of CO in %
MCW3202 × MCW3200	wild type	10	0.906 (0.248)	1,455	4.64 (4.53)	38.54 (8.29)	54.99 (5.52)	1.83 (1.69)	1,647	8.58 (1.77)	9.76
MCW3514 × MCW3589	<i>mus81Δ</i>	10	0.227 ^a (0.085)	46	2.00 ^b (6.32)	94.89 ^b (11.10)	0 ^b	3.11 ^b (9.85)	1,115	1.93 ^c (1.40)	2.06
MCW3202 × FO1267	wild type + pREP41	12	0.803 (0.098)	2,247	2.79 (1.17)	36.02 (4.11)	58.29 (3.86)	2.89 (2.09)	2,374	13.63 (4.95)	15.82
MCW3514 × FO1260	<i>mus81Δ</i> + pMus81 ⁺	10	0.980 ^d (0.216)	1,445	3.26 ^e (1.14)	39.33 ^e (6.90)	53.57 ^e (7.22)	3.84 ^e (2.24)	1,504	12.99 ^f (3.38)	15.91
MCW3514 × FO1260	<i>mus81Δ</i> + pRusA	13	0.836 ^g (0.295)	2,047	8.78 ^h (4.12)	49.36 ^h (7.21)	29.90 ^h (7.24)	11.96 ^h (6.92)	2,088	11.89 ⁱ (4.31)	12.75
MCW3514 × FO1260	<i>mus81Δ</i> + pGEN1	12	0.744 ^j (0.137)	2,054	4.15 ^k (1.50)	53.59 ^k (4.72)	36.18 ^k (6.29)	6.08 ^k (2.36)	2,683	10.61 ^l (5.95)	10.73

^a $p = 5.05 \times 10^{-6}$; two-tailed, heteroskedastic t-test against the data from the wild-type cross MCW3202 × MCW3200; highly significant.

^b $p = 1.89 \times 10^{-5}$; two-sample Kolmogorov-Smirnov test against the data from the wild-type cross MCW3202 × MCW3200; highly significant.

^c $p = 2.59 \times 10^{-8}$; two-tailed, homoskedastic t-test against the data from the wild-type cross MCW3202 × MCW3200; highly significant.

^d $p = 0.03$; two-tailed, heteroskedastic t-test against the data from the wild-type cross MCW3202 × FO1267 + pREP41; not significant.

^e $p = 0.12$; two-tailed, homoskedastic t-test against the data from the wild-type cross MCW3202 × FO1267 + pREP41; not significant.

^f $p = 0.73$; two-tailed, homoskedastic t-test against the data from the wild-type cross MCW3202 × FO1267 + pREP41; not significant.

^g $p = 0.72$; two-tailed, heteroskedastic t-test against the data from the wild-type cross MCW3202 × FO1267 + pREP41; not significant.

^h $p = 7.82 \times 10^{-11}$; two-tailed, homoskedastic t-test against the data from the wild-type cross MCW3202 × FO1267 + pREP41; highly significant.

ⁱ $p = 0.36$; two-tailed, homoskedastic t-test against the data from the wild-type cross MCW3202 × FO1267 + pREP41; not significant.

^j $p = 0.24$; two-tailed, homoskedastic t-test against the data from the wild-type cross MCW3202 × FO1267 + pREP41; not significant.

^k $p = 1.73 \times 10^{-9}$; two-tailed, homoskedastic t-test against the data from the wild-type cross MCW3202 × FO1267 + pREP41; highly significant.

^l $p = 0.19$; two-tailed, homoskedastic t-test against the data from the wild-type cross MCW3202 × FO1267 + pREP41; not significant.