

Supplementary Information for

Meiotic viral attenuation through an ancestral apoptotic pathway

Jie Gao¹, Sabrina Chau¹, Fuad Chowdhury¹, Tina Zhou¹, Saif Hossain¹, G. Angus McQuibban², and Marc D. Meneghini^{1*}

¹Department of Molecular Genetics, University of Toronto, Toronto, ON, M5G 1M1, Canada

²Department of Biochemistry, University of Toronto, Toronto, ON, M5G 1M1, Canada

*Correspondence to: marc.meneghini@utoronto.ca

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Supplementary Materials and Methods

TCA precipitation of secreted Killer toxin. Cells were grown to saturation at 30°C in acidified YPD (pH 4.7) and removed from media by centrifugation. Residual particulates were removed from media using a 0.2 µm filter. Proteins present in the filtrate were precipitated by addition of 100% TCA to a final concentration of 10% (v/v) and incubation at 4°C for 30 minutes, followed by three acetone washes. The resulting protein pellet was resuspended in 2x SDS-PAGE sample buffer and heated at 100°C for 10 minutes for analysis by Western blotting.

pESC plasmids. The *pESC GALpr-NUC1::FLAG HIS3* and *pESC GALpr-NUC1-H138A::FLAG HIS3* plasmids were obtained from Frank Madeo (1). Diploid cells were transformed with the plasmids and selected on synthetic complete medium lacking histidine (SC–HIS) with 2% glucose. For tetrad analysis, diploids were sporulated and tetrads were dissected on SC–HIS with 2% galactose and incubated at 30°C for 3-4 days before genotyping. For Western blotting, cells carrying the plasmids were incubated overnight at 30°C in SC–HIS with 2% raffinose to de-repress the galactose promoter. The overnight raffinose culture was diluted in SC–HIS with 2% galactose and grown at 30°C for 6 hours to induce expression; these cells were then harvested for downstream applications.

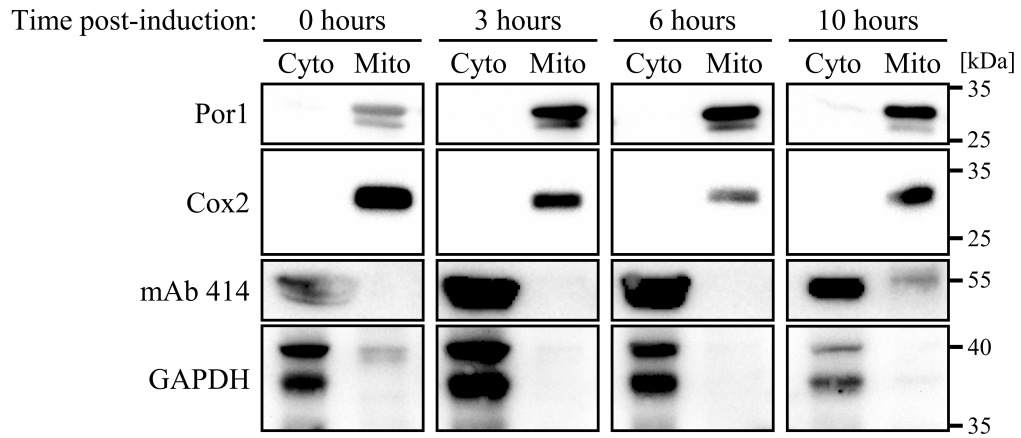


Figure S1. Validation of subcellular fractionation approach. Cell fractions of synchronized meiotic timecourse were probed with antibodies against additional cytosolic (GAPDH) and mitochondrial (Cox2) markers to show appropriate co-fractionation of the selected cell compartment markers. Molecular weight markers on far right. Strain used: MEY322.

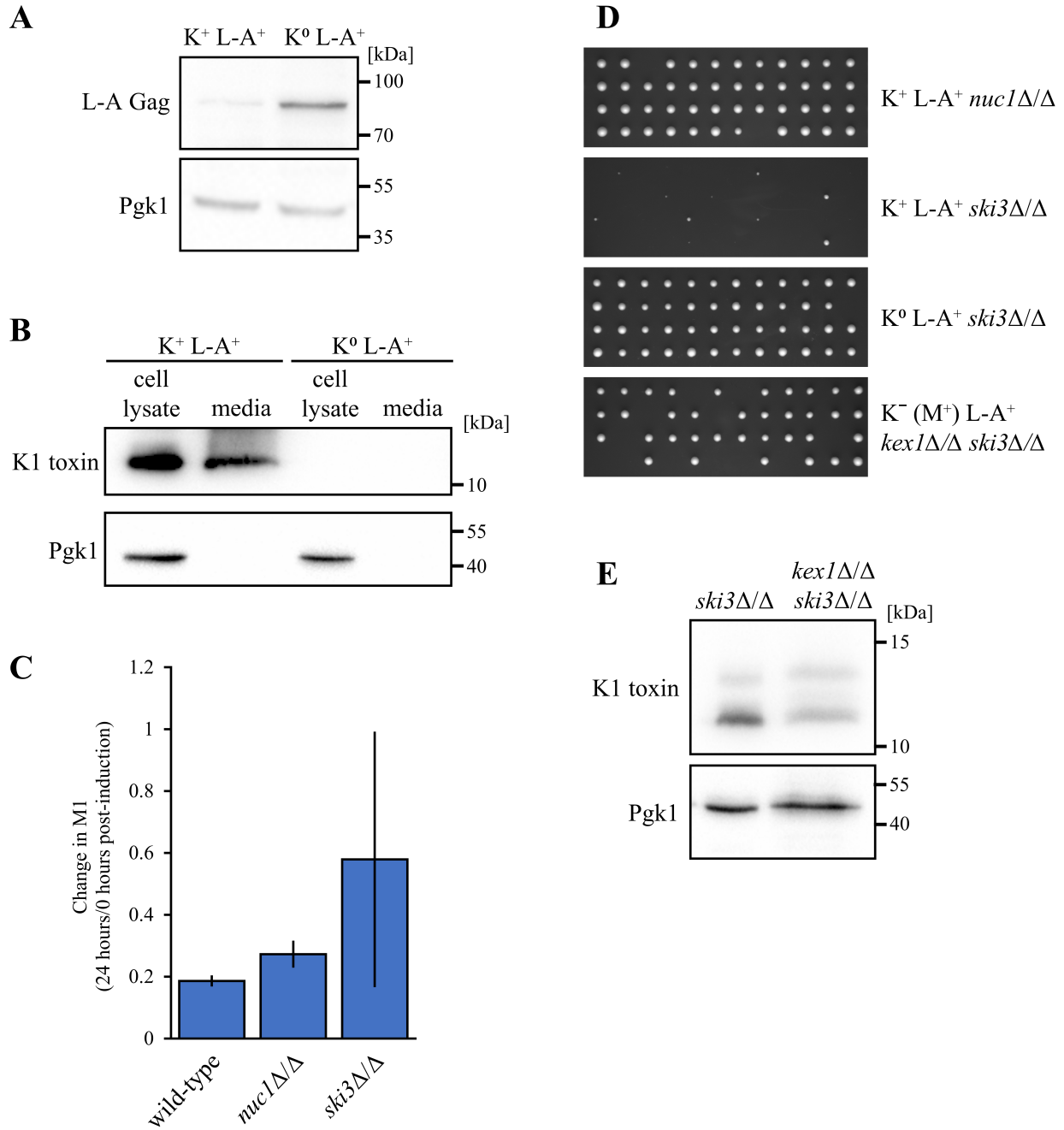


Figure S2. Characterization of M1 and L-A interactions with each other and the host cell.

(A) Anti-L-A Gag Western blot of wild-type K⁺ L-A⁺ and K⁰ L-A⁺ cell lysates sampled from saturated cultures with Pgk1 loading control.

(B) Anti-K1 toxin Western blots of whole-cell lysates and TCA-precipitated media taken from saturated cultures of isogenic K⁺ L-A⁺ and K⁰ L-A⁺ *ski3Δ/Δ* strains. Cells were grown in acidified media to prevent degradation of secreted K1 toxin. Pgk1 was used to confirm the

absence of cellular contamination in the precipitated media.

(C) Bulk *MI* RNA abundance in $K^+ L-A^+$ strains as quantified by qPCR in terminally sporulated (24 hours post-induction) populations shown as fold change over pre-meiotic (0 hours post-induction) wild-type *MI* levels. All samples were normalized to endogenous *ACT1* mRNA. Data points represent mean of biological replicates ($n = 4$) \pm standard deviation.

(D) Homozygous mutant dissections from figure 2B shown in full. Additional panel (bottom) shows partial rescue of Killer-dependent *ski3* Δ/Δ spore lethality by homozygous *KEX1* deletion, which segregate non-Killer spores carrying the M1 genome – annotated as $K^- (M^+) L-A^+$.

(E) Anti-K1 toxin Western blots of whole-cell lysates sampled from isogenic mutants at terminal sporulation (24 hours post-induction) with Pgk1 as loading control.

Strains used: MMY6007, MMY6018, MMY6106, MMY6117, MMY6170, MMY7464, MMY7962, MMY7974, MMY7977, MMY7978, MMY7991, MMY7992, and MMY8680.

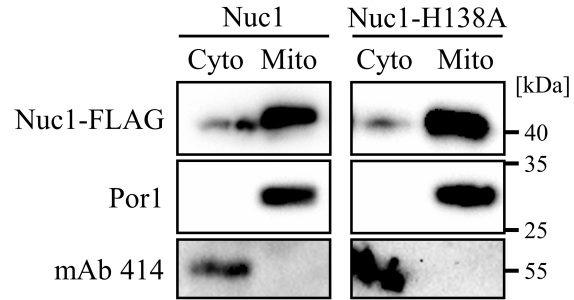


Figure S3. Validation of plasmid-based Nuc1 and Nuc1-H138A constructs. Anti-FLAG Western blot of mitochondrial and cytosolic cell fractions sampled from plasmid-transformed K⁺ *nuc1Δ/+ ski3Δ/+* diploids following induction in SC–HIS+gal media for 6 hours at 30°C to confirm expression of plasmid-based Nuc1 constructs (fractionation controls included). Strains used: HGY432 and HGY433.

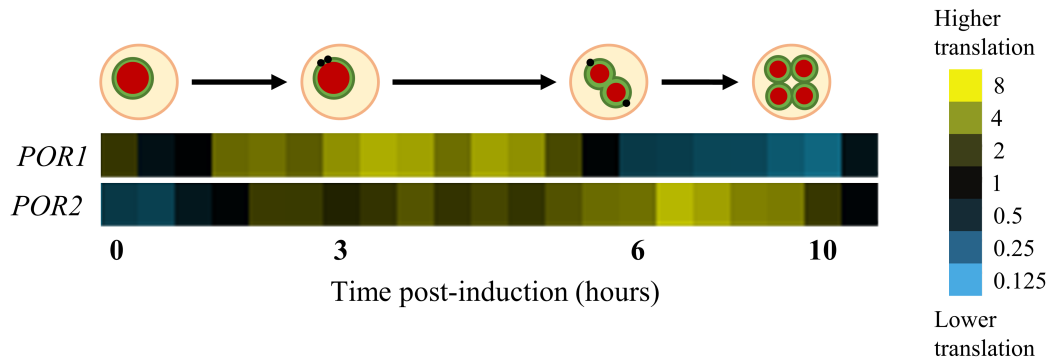


Figure S4. Developmental regulation of *POR1* and *POR2* translation. Meiotic ribosome profiling heatmap of *POR1* and *POR2* derived from the data set provided by Brar et al. (2) with schematic depiction of meiotic events occurring within the timeframes given. Colours indicate translational efficiency, as determined by ribosome footprints mapping to the ORF (reads per kilobase of transcript per million mapped reads, RPKM) normalized over mRNA reads mapping to the ORF (RPKM). Note that the time elapsed between each data point is not proportional to the physical distances depicted here.

Table S1. Nuc1-mediated viral repression requires nuclease activity.

	Nuc1	Nuc1-H138A
K ⁺ L-A ⁺ <i>HIS</i> ⁺ <i>nuc1</i> Δ <i>ski3</i> Δ haploids recovered	27	5
Total K ⁺ L-A ⁺ <i>HIS</i> ⁺ haploids recovered	117	120
Observed frequency of K⁺ L-A⁺ <i>HIS</i>⁺ <i>nuc1</i>Δ <i>ski3</i>Δ haploids	0.23 (.25)	0.04 (.25)

The K⁺ *nuc1*Δ/+ *ski3*Δ/+ parent strain was transformed with plasmids carrying galactose-inducible alleles of FLAG-tagged *NUC1* and dissected on SC–HIS+gal at 30°C. Haploids expressing wild-type Nuc1 or Nuc1-H138A were recovered from multiple independent dissections, genotyped, and counted for the data set. The bottom-most column gives the observed segregation frequency and expected segregation frequency (parenthetical) of the genotype of interest.

Strains used: HGY432 and HGY433.

Table S2. Analysis of *POR1/POR2* genetic interactions.

Parent genotype	Genotypes recovered from dissections								Total	
	<i>por1Δ</i>	<i>por2Δ</i>	<i>por1Δ por2Δ</i>	<i>por1Δ muc1Δ</i>	<i>por1Δ ski3Δ</i>	<i>por2Δ muc1Δ</i>	<i>por2Δ ski3Δ</i>	<i>por1Δ por2Δ muc1Δ</i>		<i>por1Δ por2Δ ski3Δ</i>
$K^+ L-A^+ por1Δ/Δ$ <i>muc1Δ/+</i>	118 0.46 (.5)	–	–	120 0.47 (.5)	–	–	–	–	–	238 64 tetrads
$K^0 L-A^+ por1Δ/Δ$ <i>ski3Δ/+</i>	125 0.48 (.5)	–	–	–	126 0.49 (.5)	–	–	–	–	251 65 tetrads
$K^+ L-A^+ por1Δ/Δ$ <i>ski3Δ/+</i>	92 0.44 (.5)	–	–	–	38 0.18 (.5)	–	–	–	–	130 52 tetrads
$K^+ L-A^+ por1Δ/+$ <i>por2Δ/Δ muc1Δ/+</i>	–	56 0.28 (.25)	39 0.20 (.25)	–	–	41 0.21 (.25)	–	53 0.27 (.25)	–	189 49 tetrads
$K^0 L-A^+ por1Δ/+$ <i>por2Δ/Δ ski3Δ/+</i>	–	47 0.22 (.25)	58 0.27 (.25)	–	–	–	58 0.27 (.25)	–	47 0.22 (.25)	210 53 tetrads
$K^+ L-A^+ por1Δ/+$ <i>por2Δ/Δ ski3Δ/+</i>	–	50 0.23 (.25)	51 0.26 (.25)	–	–	–	54 0.26 (.25)	–	2 0.01 (.25)	157 53 tetrads

Multiple independent tetrad dissections of porin-*NUC1/SKI3* double and triple mutants were assessed for a binary growth/no growth phenotype. The number of haploids of each genotype recovered from dissections of each parental strain is given, with observed segregation frequency (bolded) and expected segregation frequency (bolded, parenthetical) provided below. A dash (–) indicates that no haploids of the given genotype were obtained from dissections of the parent. The rightmost column gives the total number of spores recovered (top). Total number of tetrads analyzed per parent strain (rightmost column, bottom) was used to calculate the observed segregation frequency.

Strains used: SCY1, SCY28, SCY73, SCY164, SCY175, and SCY178.

Table S3. Numerical identifiers and relevant genotypes of yeast strains used in this study.

Strains	Relevant genotype	Plasmids
HGY432	W303 <i>MATa/α K⁺ L-A⁺ his3-11,15/his3-11,15 nuc1Δ::NatMX6/NUC1 ski3Δ::KanMX6/SKI3</i>	pESC <i>NUC1(H138A)-FLAG HIS3</i>
HGY433	W303 <i>MATa/α K⁺ L-A⁺ his3-11,15/his3-11,15 nuc1Δ::NatMX6/NUC1 ski3Δ::KanMX6/SKI3</i>	pESC <i>NUC1-FLAG HIS3</i>
MEY322	SK1 <i>MATa/α K⁰ L-A⁺ NUC1-FLAG::KanMX6/NUC1-FLAG::KanMX6</i>	
MEY783	SK1 <i>MATa/α K⁰ L-A⁺ NUC1-FLAG::KanMX6/NUC1-FLAG::HygMX6</i>	
MMY5914	SK1 <i>MATa/α K⁺ L-A⁺</i>	
MMY5977	SK1 <i>MATa/α K⁺ L-A⁺ nuc1Δ::NatMX6/NUC1 ski3Δ::HygMX6/SKI3</i>	
MMY5994	SK1 <i>MATa/α K⁺ L-A⁺ ura3-1/ura3-1 nuc1Δ::NatMX6/NUC1 ski3Δ::HygMX6/SKI3</i>	p5472 <i>NUC1 CEN/ARS URA3</i>
MMY6007	SK1 <i>MATα K⁰ L-A⁺</i>	
MMY6018	SK1 <i>MATα K⁺ L-A⁺</i>	
MMY6106	SK1 <i>MATa/α K⁺ L-A⁺</i>	
MMY6117	SK1 <i>MATa/α K⁺ L-A⁺ ski3Δ::HygMX6/ski3Δ::HygMX6</i>	
MMY6170	SK1 <i>MATa/α K⁺ L-A⁺ nuc1Δ::NatMX6/nuc1Δ::NatMX6</i>	
MMY6461	SK1 <i>MATa/α K⁰ L-A⁺ nuc1Δ::NatMX6/NUC1 ski3Δ::HygMX6/SKI3</i>	
MMY6469	SK1 <i>MATa K⁰ L-A⁺</i>	
MMY6470	SK1 <i>MATa K⁰ L-A⁺ nuc1Δ::NatMX6</i>	
MMY6471	SK1 <i>MATa K⁰ L-A⁺ ski3Δ::NatMX6</i>	
MMY6472	SK1 <i>MATa K⁰ L-A⁺ nuc1Δ::NatMX6 ski3Δ::HygMX6</i>	
MMY7415	SK1 <i>MATa/α K⁰ L-A⁺ ndt80Δ::HygMX6/ndt80Δ::HygMX6 NUC1-FLAG::KanMX6/NUC1-FLAG::KanMX6</i>	
MMY7464	SK1 <i>MATa/α K⁰ L-A⁺ ski3Δ::HygMX6/ski3Δ::HygMX6</i>	
MMY7506	SK1 <i>MATa/α K⁰ L-A⁺</i>	
MMY7507	SK1 <i>MATa/α K⁰ L-A⁺ nuc1Δ::NatMX6/nuc1Δ::NatMX6</i>	

MMY7571	SK1 MATa/α K ⁰ L-A ⁺ <i>por1Δ::KanMX6/por1Δ::KanMX6</i> <i>NUC1-FLAG::HygMX6/NUC1-FLAG::HygMX6</i>
MMY7572	SK1 MATa/α K ⁰ L-A ⁺ <i>por2Δ::NatMX6/por2Δ::NatMX6</i> <i>NUC1-FLAG::HygMX6/NUC1-FLAG::HygMX6</i>
MMY7599	SK1 MATa/α K ⁺ L-A ⁺ <i>NUC1-FLAG::KanMX6/NUC1-FLAG::KanMX6</i>
MMY7776	SK1 MATa/α K ⁺ L-A ⁺ <i>nuc1Δ::NatMX6/NUC1</i> <i>ski3Δ::HygMX6/SKI3 mak3Δ::KanMX6/MAK3</i>
MMY7827	SK1 MATa K ⁰ L-A ⁰ <i>nuc1Δ::NatMX6</i> <i>mak3Δ::KanMX6 ski3Δ::HygMX6</i>
MMY7829	SK1 MATa K ⁰ L-A ⁰ <i>nuc1Δ::NatMX6</i> <i>mak3Δ::KanMX6 ski3Δ::HygMX6</i>
MMY7839	SK1 MATa/α K ⁺ L-A ⁺ <i>nuc1Δ::NatMX6/nuc1Δ::NatMX6</i> <i>ski3Δ::HygMX6/SKI3 mak3Δ::KanMX6/MAK3</i>
MMY7950	SK1 MATa K ⁺ L-A ⁺
MMY7951	SK1 MATa K ⁺ L-A ⁺
MMY7960	SK1 MATa/α K ⁰ L-A ⁺
MMY7961	SK1 MATa/α K ⁰ L-A ⁺ <i>nuc1Δ::NatMX6/nuc1Δ::NatMX6</i>
MMY7962	SK1 MATa/α K ⁰ L-A ⁺ <i>ski3Δ::HygMX6/ski3Δ::HygMX6</i>
MMY7974	SK1 MATa/α K ⁺ L-A ⁺ <i>nuc1Δ::NatMX6/nuc1Δ::NatMX6</i>
MMY7977	SK1 MATa/α K ⁺ L-A ⁺ <i>ski3Δ::HygMX6/SKI3</i>
MMY7978	SK1 MATa/α K ⁺ L-A ⁺ <i>nuc1Δ::NatMX6/NUC1</i> <i>ski3Δ::HygMX6/SKI3</i>
MMY7991	SK1 MATa/α K ⁺ L-A ⁺ <i>ski3Δ::HygMX6/ski3Δ::HygMX6</i>
MMY7992	SK1 MATa/α K ⁺ L-A ⁺
MMY8226	SK1 MATa/α K ⁰ L-A ⁺ <i>nuc1Δ::NatMX6/NUC1</i> <i>ski3Δ::HygMX6/SKI3</i>
MMY8242	SK1 MATa/α K ⁰ L-A ⁰ <i>nuc1Δ::NatMX6/NUC1</i> <i>ski3Δ::HygMX6/SKI3</i>
MMY8680	SK1 MATa/α K ⁻ (M1 ⁺) L-A ⁺ <i>kex1Δ::KanMX6/kex1Δ::KanMX6</i> <i>ski3Δ::HygMX6/ski3Δ::HygMX6</i>

SCY1	SK1 <i>MATa/α K⁺ L-A⁺ por1Δ::KanMX6/POR1 por2Δ::NatMX6/por2Δ::NatMX6 ski3Δ::HygMX6/SKI3</i>	
SCY28	SK1 <i>MATa/α K⁰ L-A⁺ por1Δ::KanMX6/POR1 por2Δ::NatMX6/por2Δ::NatMX6 ski3Δ::HygMX6/SKI3</i>	
SCY41	SK1 <i>MATa/α K⁺ L-A⁺ his3-1/his3-1 ura3-1/ura3-1 POR1-EGFP::HIS3/POR1 POR2-mCherry::URA3/POR2-mCherry::URA3</i>	
SCY53	SK1 <i>MATa/α ura3-1/ura3-1</i>	pME1 <i>NUC1::EGFP URA3</i> pRS424 <i>ADH-mtRFP</i>
SCY73	SK1 <i>MATa/α K⁺ L-A⁺ por1Δ::KanMX6/POR1 por2Δ::NatMX6/por2Δ::NatMX6 nuc1Δ::NatMX6/NUC1</i>	
SCY164	SK1 <i>MATa/α K⁺ L-A⁺ por1Δ::KanMX6/por1Δ::KanMX6 ski3Δ::HygMX6/SKI3</i>	
SCY175	SK1 <i>MATa/α K⁰ L-A⁺ por1Δ::KanMX6/por1Δ::KanMX6 ski3Δ::HygMX6/SKI3</i>	
SCY178	SK1 <i>MATa/α K⁺ L-A⁺ por1Δ::KanMX6/por1Δ::KanMX6 nuc1Δ::NatMX6/NUC1</i>	

Table S4. Names and sequences of qPCR primers used in this study.

Primer	Sequence
ACT1-Fwd	TGTCACCAACTGGGACGATA
ACT1-Rev	AACCAGCGTAAATTGGAACG
L-A 5-365	ATCCTGGCGTTGGTATGACA
L-A 3-652	ACGGTCAATGGCCTAACATC
M1 killer_564F	ATCAGACACAGCCGAACACA
M1 killer_831R	CCATTGTCCCTCATCCTCAC

Supplementary References

1. S. Büttner *et al.*, Endonuclease G regulates budding yeast life and death. *Mol Cell* **25**, 233-246 (2007).
2. G. A. Brar *et al.*, High-resolution view of the yeast meiotic program revealed by ribosome profiling. *Science* **335**, 552-557 (2012).