Tinline-Purvis et al., Supplementary information

Supplementary Table 1. Rate of minichromosome loss

Genetic background	Description of minichromosome	Rate of minichromosome loss/generation
Wild type	Ch^{16}-RMGAH	$6.68x10^{-4}$
Wild type	$I(Ch^{16L})$	$9.42x10^{-4}$
Wild type	$I(Ch^{16L})$	$5.17x10^{-4}$
Wild type	$I(Ch^{16L})$	$2.75x10^{-4}$

Rate of minichromosome loss per generation calculated as previously described (Murakami et al, 1995).

Supplementary Table 2. Pedigree analysis of *arg$^+$ G418^S ade$^-$ his$^-$* colonies from Ch^{16}-RMGAH.

Daughter 1		Daughter 2		
Phenotype	Genotype	Phenotype	Genotype	Total
arg$^+$ G418^S ade$^-$ his$^-$	$I(Ch^{16L})$	non-viable		17
arg$^+$ G418^S ade$^-$ his$^-$	$I(Ch^{16L})$	$arg^+ G418^S ade^- his^-$	$I(Ch^{16L})$	25
arg$^+$ G418^S ade$^-$ his$^-$	$I(Ch^{16L})$	$arg^- G418^S ade^- his^-$	Absent	40
arg$^+$ G418^S ade$^-$ his$^-$	$I(Ch^{16L})$	$arg^+ G418^S ade^+ his^+$	Ch^{16}	27
arg$^+$ G418^S ade$^-$ his$^-$	$I(Ch^{16L})$	$arg^+ G418^R ade^+ his^+$	Ch^{16}	1

Analysis for sisters of *arg$^+$ G418^S ade$^-$ his$^-$* colonies from Ch^{16}-RMGAH is shown. A total of 800 daughter cells were segregated following HO induction in strain TH2130 on Ye5S plates of which 578 colonies grew. Marker loss was determined for sisters where one daughter was *arg$^+$ G418^S ade$^-$ his$^-$* (Phenotype). 40 $arg^+ G418^s ade^- his^-$ colonies and sister colonies were subject to PFGE analysis and the chromosomal organization determined.

Supplementary Table 3. Pedigree analysis of *Hyg^R ade$^-$ G418^S his$^-$* colonies from Ch^{16}-YAMGH.

Daughter 1		Daughter 2		
Phenotype	Genotype	Phenotype	Genotype	Total
Hyg^R ade$^-$ G418^S his$^-$	$I(Ch^{16L})$	non-viable		36
Hyg^R ade$^-$ G418^S his$^-$	$I(Ch^{16L})$	$Hyg^R ade^- G418^S his^-$	$I(Ch^{16L})$	85
Hyg^R ade$^-$ G418^S his$^-$	$I(Ch^{16L})$	$Hyg^S ade^- G418^S his^-$	Absent	9
Hyg^R ade$^-$ G418^S his$^-$	$I(Ch^{16L})$	$Hyg^R ade^+ G418^S his^+$	Ch^{16}	52
Hyg^R ade$^-$ G418^S his$^-$	$I(Ch^{16L})$	$Hyg^R ade^+ G418^R his^+$	Ch^{16}	2

Analysis for sisters of *Hyg^R ade$^-$ G418^S his$^-$* colonies from Ch^{16}-YAMGH is shown. A total of 800 daughter cells were segregated following HO induction in strain TH3317 on Ye5S plates of which 568 colonies grew and 234 were *Hyg^R ade$^-$ G418^S his$^-$*. Marker loss was determined for sisters where one daughter was *Hyg^R ade$^-$ G418^S his$^-$* (Phenotype).

Supplementary Table 4. Pedigree analysis of *arg$^+$ G418^S ade$^-$ his$^-$* colonies from *rhp51Δ* Ch^{16}-RMGAH.

Daughter 1		Daughter 2		
Phenotype	Genotype	Phenotype	Genotype	Total
arg$^+$ G418^S ade$^-$ his$^-$	$I(Ch^{16L})$	non-viable		149
arg$^+$ G418^S ade$^-$ his$^-$	$I(Ch^{16L})$	$arg^+ G418^S ade^- his^-$	$I(Ch^{16L})$	38
arg$^+$ G418^S ade$^-$ his$^-$	$I(Ch^{16L})$	$arg^- G418^S ade^- his^-$	Absent	40
arg$^+$ G418^S ade$^-$ his$^-$	$I(Ch^{16L})$	$arg^+ G418^S ade^+ his^+$	Ch^{16}	0
arg$^+$ G418^S ade$^-$ his$^-$	$I(Ch^{16L})$	$arg^+ G418^R ade^+ his^+$	Ch^{16}	4

Analysis for sisters of *arg$^+$ G418^S ade$^-$ his$^-$* colonies from *rhp51Δ* Ch^{16}-RMGAH is shown. A total of 1600 daughter cells were segregated following HO induction in strain TH2946 on Ye5S plates of which 526 colonies grew and 266 were *arg$^+$ G418^S ade$^-$ his$^-$*. Marker loss was determined for sisters where one daughter was $arg^+ G418^s$ *ade$^-$ his$^-$* (Phenotype).

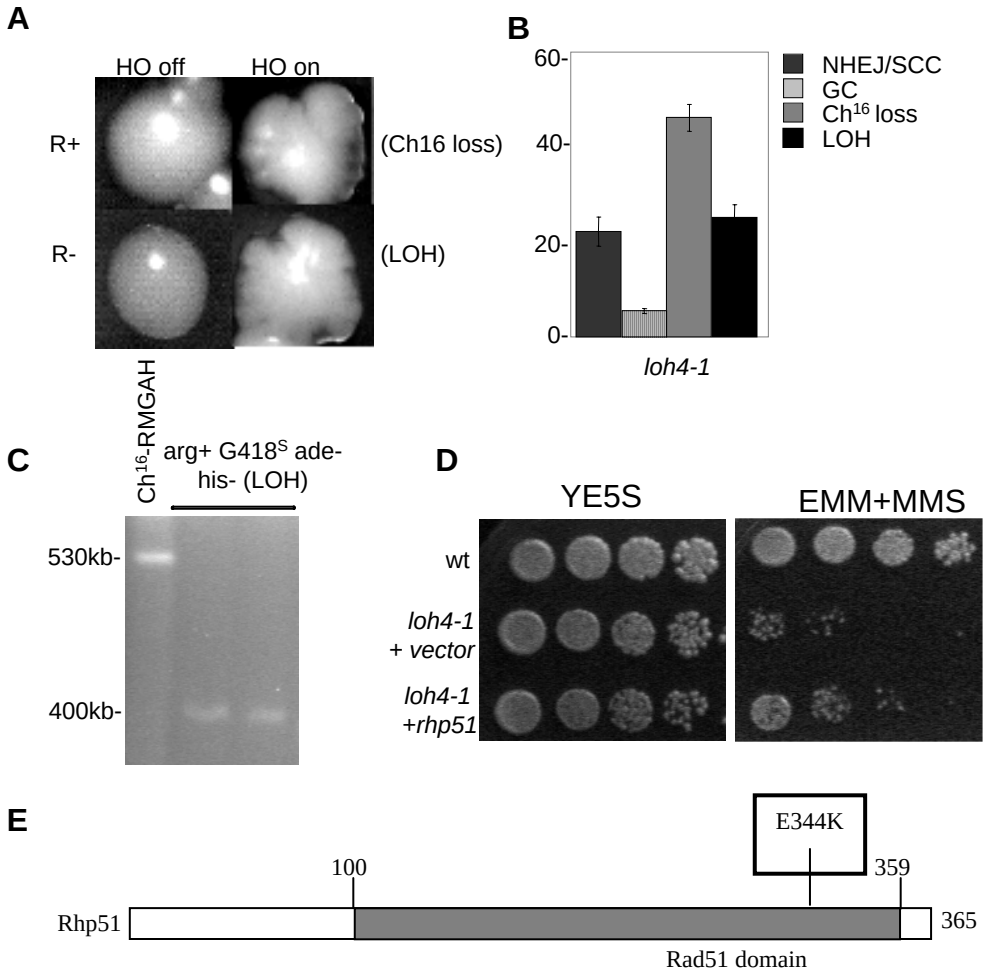
Supplementary Table 5. *S. pombe* strains used in this study.

Strain	Genotype
TH400	*leu1-32 ade6-D1 ura4-D18 his3-D1 h*$^{-}$
TH2125	*leu1-32 arg3-D4 ade6-M210 ura4-D18 his3-D1 Ch*^{16}*-RMGAH h*$^{+}$
TH2130-3	*leu1-32 arg3-D4 ade6-M210 ura4-D18 his3-D1 Ch*^{16}*-RMGAH h*$^{+}$ *pREP81X-HO*
TH2357	*leu1-32 arg3-D4 ade6-M210 ura4-D18 his3-D1 Ch*^{16}*-RMGAH h*$^{+}$ *pREP81X*
TH3315-8	*leu1-32 ade6-M210 ura4-D18 his3-D1* Ch^{16}-YAMGH *h*$^{-}$ pREP81X-HO
TH3317-20	*leu1-32 ade6-M210 ura4-D18 his3-D1* Ch^{16}-YAMGH *h*$^{-}$ pREP81X
TH3896-8*	*rqh1::ura4 leu1-32 ade6-M210 ura4-D18 his3-D1* Ch^{16}-YAMGH pREP81X-HO
TH3899-01*	*rqh1::ura4 leu1-32 ade6-M210 ura4-D18 his3-D1* Ch^{16}-YAMGH pREP81X
TH3980	*rhp51E344K leu1-32 arg3-D4 ade6-M210 ura4-D18 his3-D1* Ch^{16}-RMGAH *h*$^{-}$ pREP81X-HO
TH2942-4	*rhp51::ura4 leu1-32 arg3-D4 ade6-M210 ura4-D18 his3-D1* Ch^{16}-RMGAH *h*$^{+}$ pREP81X
TH2945-7	*rhp51::ura4 leu1-32 arg3-D4 ade6-M210 ura4-D18 his3-D1* Ch^{16}-RMGAH *h*$^{+}$ pREP81X-HO
TH3407	*rhp55::G418 leu1-32 arg3-D4 ade6-M210 ura4-D18 his3-D1* Ch^{16}-RMHAH *h*$^{+}$ pREP81X-HO
TH3847-8	*rhp55::G418 leu1-32 arg3-D4 ade6-M210 ura4-D18 his3-D1* Ch^{16}-RMHAH *h*$^{+}$ pREP81X
TH3243	*rhp57::G418 leu1-32 arg3-D4 ade6-M210 ura4-D18 his3-D1* Ch^{16}-RMHAH *h*$^{-}$ pREP81X-HO
TH3869-70	*rhp57::G418 leu1-32 arg3-D4 ade6-M210 ura4-D18 his3-D1* Ch^{16}-RMHAH *h*$^{-}$ pREP81X
TH3569-70*	*lig4::Ura4 leu1-32 arg3-D4 ade6-M210 ura4-D18 his3-D1* Ch^{16}-RMGAH pREP81X-HO
TH3575-7	*lig4::Ura4 leu1-32 arg3-D4 ade6-M210 ura4-D18 his3-D1* Ch^{16}-RMGAH *h*$^{+}$ pREP81X
TH3918-21*	*nbs1::ura4 leu1-32 arg3-D4 ade6-M210 ura4-D18 his3-D1* Ch^{16}-RMGAH pREP81X-HO
TH3922	*nbs1::ura4 leu1-32 arg3-D4 ade6-M210 ura4-D18 his3-D1* Ch^{16}-RMGAH *h*$^{-}$ pREP81X
TH3966-9*	*rad32::ura4 leu1-32 arg3-D4 ade6-M210 ura4-D18 his3-D1* Ch^{16}-RMGAH pREP81X-HO
TH3971-2	*rad32::ura4 leu1-32 arg3-D4 ade6-M210 ura4-D18 his3-D1* Ch^{16}-RMGAH *h*$^{+}$ pREP81X
TH4135-7	*rad22::ura4 leu1-32 arg3-D4 ade6-M210 ura4-D18 his3-D1* Ch^{16}-RMGAH *h*$^{+}$ pREP81X-HO
TH4138-9	*rad22::ura4 leu1-32 arg3-D4 ade6-M210 ura4-D18 his3-D1* Ch^{16}-RMGAH *h*$^{+}$ pREP81X

TH4156-9*	*rad16::ura4 leu1-32 arg3-D4 ade6-M210 ura4-D18 his3-D1* Ch^{16}-RMGAH pREP81X-HO
TH4161-2*	*rad16::ura4 leu1-32 arg3-D4 ade6-M210 ura4-D18 his3-D1* Ch^{16}-RMGAH pREP81X
TH4313	*leu1-32 arg3-D4 ade6-M210 ura4-D18 his3-D1 I(Ch^{16L}) h^+*
TH4344-7	*rqh1::ura4 exo1::ura4 leu1-32 ade6-M210 ura4-D18 his3-D1* Ch^{16}-YAMGH h^+pREP81X-HO
TH4348-50	*rqh1::ura4 exo1::ura4 leu1-32 ade6-M210 ura4-D18 his3-D1* Ch^{16}-YAMGH h^+ pREP81X
TH4422, 24*	*exo1::ura4 leu1-32 ade6-M210 ura4-D18 his3-D1* Ch^{16}-YAMGH pREP81X-HO
TH4423, 25*	*exo1::ura4 leu1-32 ade6-M210 ura4-D18 his3-D1* Ch^{16}-YAMGH pREP81X
TH4460-1	*cdc27-D1 leu1-32 arg3-D4 ade6-M210 ura4-D18 his3-D1 h-* Ch^{16}-RMGAH pREP81X-HO
TH4463-4	*cdc27-D1 leu1-32 arg3-D4 ade6-M210 ura4-D18 his3-D1 h-* Ch^{16}-RMGAH pREP81X

Ch^{16}-RMGAH (Ch^{16} *yps1::arg3*$^+$ *ert1::MATa-kanMX6 ade6-M216 cid2::his3*$^+$). Ch^{16}-YAMGH (Ch^{16} *chk1::Hph ade6-M216 rad21::MATa-kanMX6 cid2::his3*$^+$). Ch^{16}-RMHAH (Ch^{16} *yps1::arg3*$^+$ *ert1::MATa-Hph ade6-M216 cid2::his3*$^+$). I(Ch^{16L}) indicates isochomosome derived from wild-type Ch^{16}-RMGAH, in which the left arm encoding *yps1::arg3*$^+$ is duplicated. * independent isolates (h^+ or h^-).

Supplementary Figure 1.



Rhp51 suppresses DSB-induced isochromosomes. (A) Isolate identified from the colorimetric screen that had undergone DSB-induced LOH. Representative colonies of *loh4-1* on EMM plus uracil, histidine, low adenine (R-) and EMM plus uracil, histidine, low adenine and arginine (R+) with and without thiamine (DSB off/on). Derepression of pREP81X-HO (not shown) generates a DSB at the *MATa* target site (B) Percentage DSB-induced marker loss in *loh4-1* in comparison to wild-type. The levels of non-homologous end joining/sister chromatid conversion (NHEJ/SCC), gene conversion (GC), minichromosome loss (Ch^{16} loss), and LOH are shown. Standard errors of the mean are indicated. (C) High resolution PFGE analysis from wild-type Ch^{16}-RMGAH (TH2130; lane1) and individual *loh4-1* arg^{+} $G418^{S}$ his^{-} ade^{-} (LOH) strains isolated following DSB induction (lane 2-3). (D) Spot dilutions of wild-type Ch^{16}-RMGAH (TH2130), *loh4-1* containing blank vector and *loh4-1* containing

pREP81X-*rhp51* on Ye5S and 0.005% (v/v) MMS with and without thiamine plates. Derepression of pREP81X-*rhp51* allows expression of *rhp51* (E) Schematic of Rhp51 and where the point mutation occurs in *loh4-1*.

Construction of Ch^{16}-RMGAH.

Using *S. pombe* genomic DNA as a template, 1.5 Kb fragments of SPCC1322.01 (termed '*ert1*') and *yps1* sequences were PCR amplified, (*ert1* forward, TAAAACCCGTAGTTTATTCAC; *ert1* reverse CAATTCAACTGTCGGAGTG; *yps1* forward TTACGCCGTTTCCATTTAC; *yps1* reverse, CTTTTACGTTTTGTCGTTG) and products ligated into pCR2.1. The *MATa-kanMX6* module from pFA6a*MATa-kanMX6* was excised with *Eco*RI and *Bam*HI, klenowed, and ligated into the *Hinc*II site of pCR*ert1* and the *MATa-kanMX6* sequence orientation determined via PCR. Following transformation of *S. pombe* strain TH1999 (Ch^{16} *ade6 - M216, cid 2:: his 3*$^{+}$ Ch3 *ade6 - M210, leu1-32, ura4-D18, his3-D1, arg3-D4 h*$^{+}$) with pCR*ert1*::*MATa-kanMX6* derived PCR product, G418^{R} clones were analysed using PCR, and stable integrants selected. PFGE was performed and Southern blotting analysis performed using a *KanMX6* probe to confirm correct integration into Ch^{16}.

The *arg3*$^{+}$ ORF with its promoter and terminator sequences was amplified from genomic S. pombe DNA (*arg3* forward, TTGATTTCACAAGCATGC; *arg3* reverse, CATTAAGCGGGTAATAATTT and the 2 kb product cloned into pCR2.1. pCR*arg3*$^{+}$ was cut with *Eco*RI to excise the *arg3*$^{+}$ marker, blunt ended with Klenow, and ligated into pCR*yps1* pre-digested with *Mfe*I, also blunt ended with Klenow. The insert orientation of the *arg3*$^{+}$ gene was determined by PCR. The above strain was subsequently transformed with a PCR fragment encoding pCR*yps1*::*arg3*$^{+}$ and arg^{+} colonies selected. PFGE and Southern blot analysis was used to confirm integration at the *yps1* locus on Ch^{16}.

Construction of Ch^{16}-YAMGH.

The *chk1*$^{+}$ ORF with its promoter and terminator sequences was amplified from genomic *S. pombe* DNA (*chk1* forward, TAGAGCTCATGGCTCAAAAATTAGATAAC; *chk1* reverse TACTGCAGTTAATTTTGTGAAACATCTG) and the 2 kb product cloned into pJK210 by digesting the product and insert with *Sac*I and *Pst*I. The *hph* module from pFA6*hyg* was excised with *Bam*HI and *Spe*I, and ligated to pJK210-*chk1* digested with the same enzymes. Following transformation of *S. pombe* strain TH2662 (Ch^{16} *ade6-M216, cid2::his3, rad21::MATa-kanMX6,* Ch3 *chk1::ura4, ade6-M210, leu1-32, ura4-D18, his3-D1 h*$^{-}$) with pJK210*chk1*::*hph* construct, Hyg^{R} clones were analysed using PCR, and stable integrants selected. PFGE was performed and Southern blot analysis was used to confirm integration at the *chk1* locus on Ch^{16}.

Colony Sectoring Assay

Cells mutagenised by 2% (v/v) ethylmethyl sulfonate (Hayles et al, 1986) were plated onto EMM plus uracil and low thiamine (2μM) plates (approximately 500 cells per plate). The cells were incubated at 32°C for 48 hours before replica plating onto EMM plus uracil, histidine, arginine and low adenine (5mg/l) and EMM plus uracil, histidine and low adenine. These plates were incubated at 32°C for 56 hours and stored at 4°C before visually screening for sectoring and further analysis.

References:

Hayles J, Aves S, Nurse P (1986) suc1 is an essential gene involved in both the cell cycle and growth in fission yeast. *EMBO J* **5:** 3373-3379

Murakami S, Yanagida M, Niwa O (1995) A large circular minichromosome of *Schizosaccharomyces pombe* requires a high dose of type II DNA topoisomerase for its stabilization. *Mol Gen Genet* **246:** 671-679